Microbial community composition of the southern Coorong including evaluating seasonal variation and sediment, water column, aquatic macrophytes and filamentous algae as substrates for microbial growth

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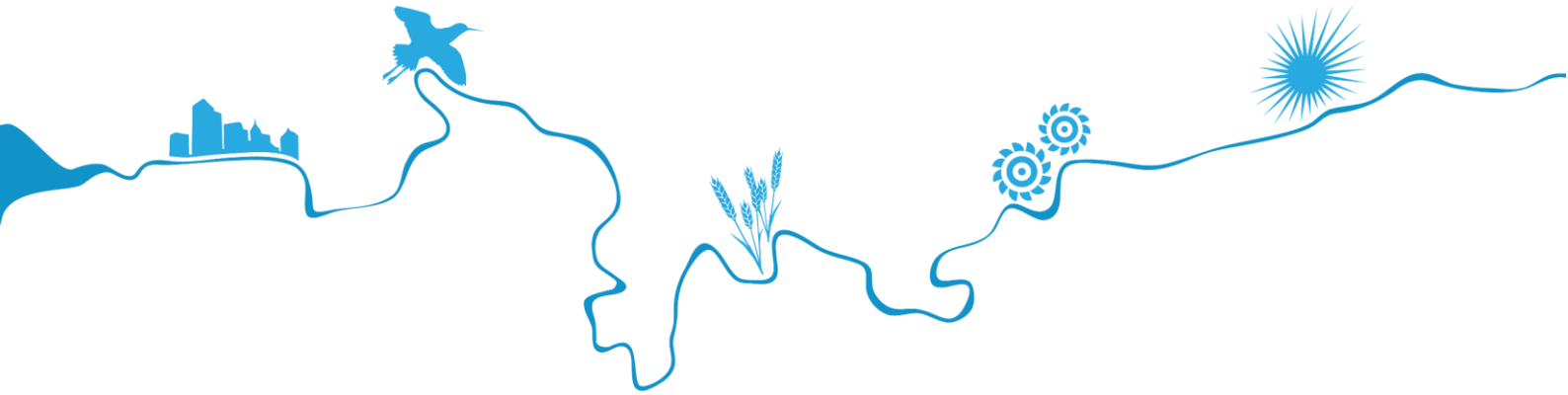

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Respect and reconciliation

Aboriginal people are the First Peoples and Nations of South Australia. The Coorong, connected waters, and surrounding lands have sustained unique First Nations cultures since time immemorial.

The Goyder Institute for Water Research acknowledges the range of First Nations' rights, interests and obligations for the Coorong and connected waterways and the cultural connections that exist between Ngarrindjeri Nations and First Nations of the South East peoples across the region and seeks to support their equitable engagement.

Aboriginal peoples' spiritual, social, cultural, and economic practices come from their lands and waters, and they continue to maintain their cultural heritage, economies, languages, and laws which are of ongoing importance.

Executive Summary

The aquatic macrophyte community of the Coorong consists of mixed populations of *Ruppia tuberosa* and *Althenia cylindrocarpa* and another unresolved species of *Ruppia*. For the purpose of this report, these mixed populations have been termed the '*Ruppia* Community'. An almost complete decline of the *Ruppia* Community as a result of the impact of the Millennium Drought (2001-2010) in the Coorong included the loss of seed banks. The recovery of the *Ruppia* Community has been gradual with the number of sites occupied by plants increasing over the past decade. Recovery of the *Ruppia* Community has been impeded most strongly in the Coorong South Lagoon because of the hyper-eutrophic state of the southern Coorong. The Coorong South Lagoon is dominated by algal blooms, including mat forming filamentous algae that physically disrupt the aquatic macrophytes' ability to flower and set seed. The microbiota associated with algal blooms and the hyper-eutrophic conditions are very poorly understood in the Coorong, the unique set of environmental characteristics of this environment make understanding the microbiota critical to interpretation of the biological status of the system.

The South Australian Government's Healthy Coorong, Healthy Basin (HCHB) program's Trials and Investigations (T&I) project includes studies on the aquatic plants and algae (*Component 2*) undertaken between July 2020 and April 2022. This investigation of the microbial communities present in the Coorong South Lagoon, and in the southern parts of the North Lagoon, improves our understanding of pelagic and benthic microbial dynamics in relation to water quality and promoting (or not) the growth of the *Ruppia* Community. The presence of microbial biofilms at the surface of the plants of *Ruppia* or *Althenia* spp. was investigated for the first time. We observed how they vary during different life cycle stages of members of the *Ruppia* Community. This provides important baseline information to better understand the composition and possible function of these biofilms, and to determine if they can be used as health bio-indicators for the *Ruppia* Community.

This study consisted of the sampling of sediment, water column, aquatic macrophyte and filamentous algal based microbial communities in Spring and Summer of 2020, and in Autumn and Winter of 2021, including analytical, microscopic and molecular analyses. The range of sampling sites extended from Wild Dog Islands near Salt Creek at the southern end of the Coorong South Lagoon to Noonameena at the southern end of the Coorong North Lagoon. These sites are associated with detailed studies of the *Ruppia* dominated community reported separately in *Component 2*. These sampling events documented the composition of microbial communities (including microalgae) in the water column, sediments and on the *Ruppia* and/or *Althenia* plants and filamentous algae mats. We also simultaneously measured the environmental conditions to establish if the microbial communities were measurable health indicators for the ecological condition of the *Ruppia* Community at times of the year critical to the lifecycle. The Spring and Summer sampling events corresponded to the period *Ruppia* or *Althenia* plants were flowering and setting seed (reproductive), while the Autumn sampling event corresponded to aestivation, and the Winter sampling event during the period of vegetative growth.

Differences were observed between sites, with a significantly higher diversity of microalgae at Noonameena compared to the other sites. The community of microalgae present at Noonameena in October 2020, when no plants were observed was distinct from the other sites, i.e. with high abundance of diatoms and Euglenophyta. Over the duration of the study, the composition of the community of microalgae at Noonameena was consistently different from the other sites. The location of the sampling sites within the Coorong, i.e. the environmental conditions present along the north-south axis of the Coorong, is more influential on the microbial communities than seasonal differences in the same environmental parameters.

The dissolved nutrient levels measured throughout the study characterised a strong deficit in nitrogen, i.e. a decrease in the ratio of fixed inorganic nitrogen to phosphorus (N:P) relative to the Redfield ratio (16:1; Redfield et al. 1963). Communities of microalgae can adapt to a wide gradient of light:nutrient ratio conditions but bacterial communities can adjust very fast to different nutrient ratios and can also affect environmental nutrient availability. These bacteria can be an important factor determining the composition and ratios of both the dissolved and particulate pools of nutrients.

The study also highlighted that, throughout the period of study, the microbiota community contributed more than half of the pelagic nutrients (N and P), except at Noonameena. In particular, in Spring, the microbiota

seems to account for up to 85% of N and 90% of P across sites 1 to 4, but only accounts for 69% of N and 1% of P at Noonameena (site 5).

Based on the seasonal survey conducted in this study, the state of the microbial communities across the surveyed region of the Coorong included the following characteristics:

- A total of 47,839 amplicon sequence variants (ASVs) were identified during the study, each of them potentially corresponding to a different strain of bacteria or archaea.
- *Ruppia* was recorded growing at 55% of all sites surveyed.
- All samples showed a low diversity of microalgae, but microbes (particularly bacteria and fungi) are highly diverse, especially in the sediment.
- Local environmental conditions such as salinity, turbidity and concentration of ammonium influenced the composition of the water and associated sediment microbial communities.
- Salinity was the main driver of the microalgae community composition.
- Turbidity was the main driver of the communities of microbes associated with the members of the *Ruppia* Community.

Lagoon systems such as the Coorong can shift between stable states as a result of anthropogenic or natural drivers. Here, we provided a snapshot of some of the stable states that could be encountered within primary producers: submerged, floating and planktonic. Shifts in primary producer dominance affect key supporting, provisioning, regulating, and cultural ecosystem services. However, links between states of the primary producers and services are not currently fully understood in the Coorong. These links should be further explored in order to facilitate future management of the ecosystem as a whole.

During the warmer months, large areas of high biomass filamentous algae mats can form across the southern Coorong and have multiple effects on the *Ruppia* Community. Here, we assessed the microbiota associated to different states of filamentous algae growth, i.e. attached to plants of the *Ruppia* Community and floating mats to compare it with the microbiota associated to *Ruppia* or *Althenia.* No mats or filamentous algae were observed in the South Lagoon of the Coorong but were present at Parnka Point (site 3), North Magrath Flats (site 4) and Noonameena (site 5) in October and December 2020, as well as at North Magrath Flats (site 4) in June 2021. The lack of filamentous algae/mats observed in the South Lagoon may provide an opportunity to understand strategies to manage the presence of algae in the Coorong system.

In the last decade, there has been a loss in the total number of microalgal species from 241 to 81 based on detailed surveys. This indicates the long-term environmental decline of the Coorong system has been accompanied by a loss in microalgal biodiversity. Further examination of the specific Coorong South Lagoon Common Core microbial community, and the types of perturbations that would lead to significant change, for example loss of diversity, would assist the development of a useful monitoring tool in the extreme physico-chemical environment in the Coorong. Identifying potential changes in the microbiota may provide the first evidence of functional changes in sediments, a high priority for proposed intervention options from Phase 1 of the Healthy Coorong, Healthy Basin program

Based on the results of this research, the following key points are highlighted:

- The water column microbial communities were dominated by Actinobacteria, α-proteobacteria, γ-proteobacteria, and Verrucomicrobiae, which are typical of estuarine and marine environments.
- The communities present in the sediment reflect the imbalance of nutrients and the presence of potentially toxic elements (excess of sulfur-based compounds), with the presence of be γ-proteobacteria, α-proteobacteria, Anaerolineae, Bacteroidia, and Desulfobacteria.
- The communities associated with the leaves and roots of plants in the *Ruppia* Community reflect their potential role in sustaining healthy plants and facilitating nutrient absorption while avoiding toxic compounds.
- Bacteria of a group named “Plant Growth Promoting Bacteria (PGPB)” were identified on the leaves and roots of *Ruppia*, but also in the filamentous mats and plant associated aggregates (PAA, which are

associated with the first stages of filamentous algae growth). These bacteria could be promoting the growth of filamentous macroalgae at the same time as promoting the growth of *Ruppia*.

- *Candidatus thiodiazotropha* (*Ca.* Thiodiazotropha) which oxidises sulfides in sediments was observed on the roots and leaves of *Ruppia*, as well as in the PAA and in the sediment. Future work could explore the relationship between *Candidatus thiodiazotropha* (*Ca.* Thiodiazotropha) and *Ruppia*, as well as measuring the impact that *Candidatus thiodiazotropha* (*Ca.* Thiodiazotropha) has on the remediation of the sulfide compounds present in the sediment of the Coorong.

Acknowledgments

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The research team, including our individual researchers from The University of Adelaide, South Australian Research and Development Institute, Flinders University and the Department for Environment and Water wish to thank the members of the Ngarrindjeri community gratefully and respectfully for their support of this research. In particular, we recognise and value the knowledge of Ngarrindjeri community members shared with this program, that has come from the connection that has been maintained by community to the lands and waters of the region and individual experiences.

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1 Introduction

1.1 Background

The Coorong is culturally, environmentally, and economically important at local, national, and international scales but has experienced a long-term decline in its ecological condition due to long term reduction in flows from the River Murray (Krull et al. 2008). In the Coorong, under low water flow conditions, the planktonic food web has been observed previously to be dominated by viruses, bacteria, and nano/pico-phytoplankton interactions (Hemraj et al. 2017a, b). Whilst some elements of the Coorong ecosystem associated with increased inflows have recovered since the Millennium Drought ended in 2010, the South Lagoon has not recovered as expected (Leterme et al. 2015; Brookes et al. 2018). There has been a switch from aquatic macrophytes, specifically a *Ruppia* dominated community (the *Ruppia* Community; Lewis et al. 2022) to a community dominated by microbiota (microalgae, bacteria and archaea) and macroalgae (especially filamentous). The prevalence of fast growing micro- and macro- algal species may be associated with eutrophication (nutrient enrichment). It is now recognised that the Coorong ecosystem has become hyper-eutrophic (Mosely and Hipsey 2019). Subsequent impacts on invertebrates, fish, and waterbirds have also accompanied these ecological changes. The hyper-salinity of the South Lagoon, coupled with hyper-eutrophic conditions will have led to environmental conditions that would be expected to change the structure of the microbial communities and associated ecological functions. Microbiota community composition is typically indicative of system health, which varies in response to environmental conditions that change spatially and temporally. Dramatic changes to microbial communities have been observed with large scale nutrient shifts in other systems (e.g. Cotner and Biddanda 2002; Hemraj et al. 2017 Crossetti et al. 2008; Kiersztyn et al. 2019). Growing awareness of the importance of the microbiota composition and its functional roles in the Coorong are essential to future management plans.

Aquatic ecosystems are rich in microbes, prokaryotic and eukaryotic organisms such as bacteria, archaea, microalgae, protozoa and zooplankton (Hays et al. 2005). Prokaryotes (i.e. bacteria and archaea) make up a major component of the picophytoplankton biomass in aquatic environments and are an integral component of the microbial food web (Kiørboe et al. 1990; Sommer et al. 2002). Microalgal community responses to salinity and nutrient concentrations are well characterised across a wide range of conditions, some studies documenting a decrease in species richness with increasing salinity and increasing with nutrient enrichment (Larson and Belovsky 2013). In the Coorong, it is important to understand the implications of microbial community compositional changes associated with different environmental conditions as the state of the system changes.

The Phase One Trials and Investigations (T&I) project of the Healthy Coorong, Healthy Basin (HCHB) program consists of a series of integrated components that will collectively provide knowledge to inform the future management of the Coorong. *Activity 2.6: Identification of the microbiota communities and partitioning of organic material amongst the primary producer community at macro and micro scales* forms part of *Component 2 – Investigating the drivers and controls of filamentous algae and restoration of aquatic plants in the Coorong* of the T&I project (Figure 1). It aimed to assess the composition of the communities of microorganisms associated with the gradient of conditions that occur across the latitudinal gradient of the Coorong lagoons which has been shown to be a salinity and nutrient gradient (Leterme et al. 2015; Mosley et al. 2020). In addition, it explored the microbial diversity associated with the leaves and roots of Ruppia, and with filamentous algae (Figure 2) identifying the Coorong *Ruppia* Community microbiome.

It is worth noting that the contemporary *Ruppia* dominated aquatic macrophyte community in the Coorong has been determined to include many sites with mixed populations of *Ruppia tuberosa* and *Althenia cylindrocarpa* and another unresolved species of *Ruppia* (Collier et al. 2017; Asanopoulos and Waycott 2020; Waycott et al. 2020, Lewis et al. 2022). The similarity of their simple vegetative form

(i.e. thin, long leaves, fine rhizomes and roots) for these two aquatic plants means that unless flowering they are unable to be differentiated (Waycott et al. 2020, Lewis et al. 2022). Throughout this report we refer to this community of macrophytes as the *Ruppia* Community, but they could be any of the species as described above. DNA markers have been applied to ensure identification of the species of aquatic macrophytes present at any given site (Lewis et al. 2022).

Distinct microbiota can be found inhabiting within and around seagrasses (Tariquinio et al. 2021). Seagrass microbiomes are involved in many processes that benefit the plants (Ugarelli et al. 2017; Tarquinio et al. 2019), including nutrient supply (e.g. nitrogen fixation associated with leaves and roots) and detoxification from harmful compounds (e.g. hydrogen sulfides around roots). Indeed, nitrogen fixing bacteria are estimated to provide up to 50% of the nitrogen required by seagrasses (O'Donohue et al. 1991), while sulfate oxidising bacteria alleviate the roots and rhizomes from the toxic effects of hydrogen sulfide (Martin et al. 2019).

A primary focus of the HCHB T&I project was to support management of the Coorong to return crucial ecological functions. Re-establishing the ecological character as *Ruppia* Community dominated, not a micro- and macro- algal dominated community would be a major contribution to improving water quality in the South Lagoon. Research undertaken as part of Activity 2.6 (microbiota investigations) will fill knowledge gaps about the microbiota present in the Coorong at different times of the year and in more detail those microorganisms which are associated with the *Ruppia* Community (Figure 1). The new knowledge generated around water and sediment microbiota communities will allow for a better understanding of the Coorong health. Additionally, the presence of microbiota biofilms on the leaves and roots of plants in the *Ruppia* Community will provide insight onto the state of the *Ruppia* Community along a gradient of highly variable salinity and nutrient conditions (Figure 2).

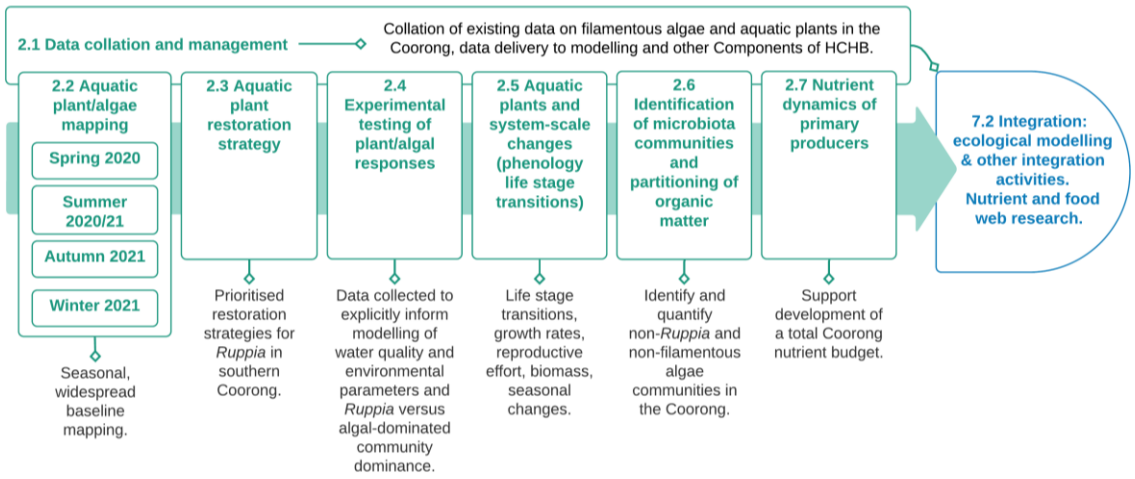

Figure 1. Summary of the main research activities in Trials and Investigations (T&I) *Component 2 Investigating the drivers and controls of filamentous algae and restoration of aquatic plants in the Coorong* and the primary outputs for each activity and a direct linkage to the modelling activities in T&I Component 7 Integration.

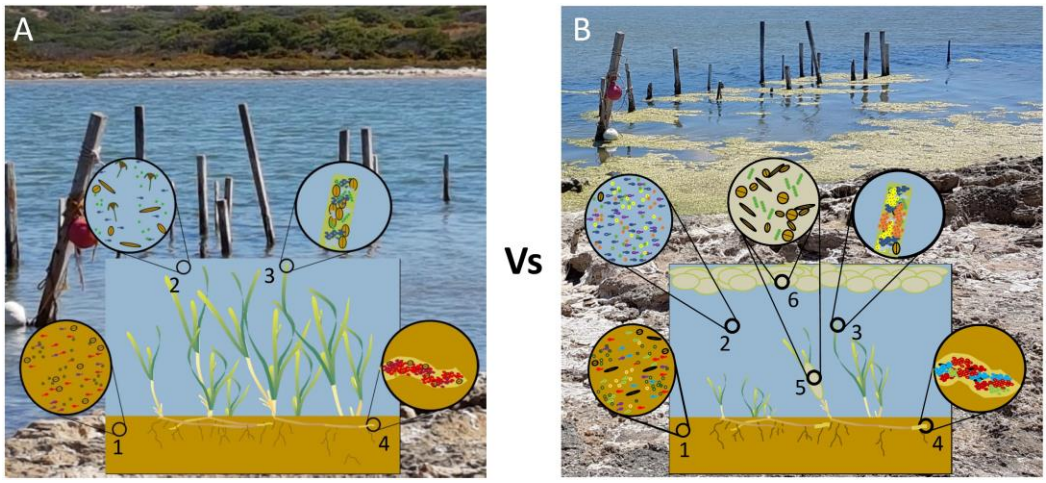

Figure 2. Conceptual diagram summarising alternative states for the southern Coorong based on observed ecological conditions: (A) under optimal water level conditions, (B) under stress. The different communities of microorganisms that have been assessed are indicated on the diagrams. (1) benthic communities, (2) water communities, (3) leaves associated communities (biofilm), (4) root system associated communities (biofilm), (5) communities present in the aggregate of filamentous algae that attaches to the plant and (6) communities present in the filamentous algae floating mats/drifts.

Microbes of all types can form biofilms on most if not all surfaces immersed in water, including the leaves and roots of seagrasses. These microorganisms have the specificity to produce extracellular polysaccharides in the form of Transparent Exopolymer Particles (TEP; Alldredge et al. 1993). In aquatic environments, TEP are "hot spots" of intense microbial and chemical activity within the water column, facilitating the attachment of planktonic TEP to surfaces (Berman et al. 2011). When attached to surfaces (such as seagrasses), TEP become the precursor of biofilm formation.

Biofilms are present in most humid and aquatic environments initially forming through the adhesion of TEP, proteins, eDNA (environmental DNA) and polysaccharides to a surface, followed by the attachment of bacterial cells which modify the surface physicochemical properties (Carvalho et al. 2018). This first phase of adhesion influences the attachment of successive colonizers such as algae, cyanobacteria, and protists determining the structure and function of the mature biofilm (Dang and Lovell, 2016). Bacteria and diatoms have been found to dominate aquatic biofilms (Railkin 2003) and are likely to be present in the biofilms attached to the surface of plants in the *Ruppia* Community in the Coorong.

Microorganisms are important for the fitness, growth, and survival of plants (Vandenkoornhuyse et al. 2015). There are important biochemical processes linked to the interaction between plants (host) and microorganisms (symbionts) involved in the carbon, nitrogen, and sulfur cycles. It has been hypothesised that nitrogen-fixing prokaryotes found both in the phyllosphere (Agawin et al. 2016) and the rhizosphere of seagrasses can provide 30–100% of their nitrogen requirement (Welsh et al. 2000; Cole et al. 2012; Sun et al. 2015). Microbial community composition of the seagrasses and of its environment (water and sediment) could be a useful bio-indicator for environmental changes that may cause stress to plants in the *Ruppia* Community. Here, we identified the microorganisms (prokaryotes and eukaryotes) associated to biofilms at the surface of plants in the *Ruppia* Community to determine the core-microbiome that can be used as a bio-indicator of wider environmental change.

Culturing is the gold standard for microbial characterisation, as it provides large amounts of cells from a clonal population and allows any number of functional tests on bacterial biochemistry, physiology, and genetics to be performed. However, each microbial organism has its own specific growth requirements which cannot be reproduced easily in a laboratory setting. An alternative to culturing is to perform microscopy directly on environmental samples. High-resolution microscopy techniques e.g. scanning electron microscopy (SEM) allow for a number of specific biological questions to be addressed directly from images of live or fixed specimens. However, the taxonomic resolution needed

for the identification of the multitude of microbes typically found in an environmental sample cannot be reached via microscopy alone.

Microbial ecologists have thus established other ways of identifying microbial communities from environmental samples. Due to its broad presence in all organisms, and its adequate level of conservation, the small subunit (SSU) of the ribosomal RNA (rRNA) gene is often used as the golden standard for diversity inventories (Amann et al. 1995). Amplicon-based approaches targeting variable regions of specific markers (e.g. 16S rRNA gene, ITS, or 18S rRNA gene) are widely used to describe the composition of communities of prokaryotes (Woese 1987), and eukaryotes (Lopez-Garcia et al. 2001).

The 16S rRNA genes encode the RNA molecules that form part of the small subunit of the ribosome of prokaryotes. The first step of determining the composition and diversity of a sample using amplicon sequencing is to extract deoxyribonucleic acid (DNA). Once the DNA has been extracted and quantified, polymerase chain reactions (PCR) are required to target the DNA region of interest in the 16S rRNA gene. PCR uses primers (short nucleic acid sequences) to focus on a specific gene region of the DNA helpful in the identification of microorganisms. The region is amplified after the primers bind to the DNA, generating amplicons.

Here, we utilise microscopic techniques and amplicon DNA sequencing to identify the communities of prokaryotes and eukaryotes present in the southern Coorong, a region from Noonameena in the North Lagoon to Salt Creek in the South Lagoon, and specifically the community associated with the plants in the *Ruppia* Community.

1.2 Aims

The aim of this investigation was to document the community composition of microbiota, with an emphasis on the microalgae and bacteria, in the water column, benthic sediments and associated with the *Ruppia* Community in the southern Coorong. We investigated seasonal changes and identified the contribution these communities potentially make to the overall Coorong organic based nutrient budget. Finally, we described the presence of the *Ruppia*-associated microbiota (microbiome), identified its composition, and analysed how they varied in response to water quality. This final technical report presents the results of work completed from October 2020 to June 2022.

2 Methods

2.1 Sample collection and processing

Five sites were chosen along the Coorong, from Wild Dog Islands in the South Lagoon to Noonameena in the North Lagoon (Figure 3) as the focus of our study. These sites were chosen for the presence of plants in the *Ruppia* Community throughout the year based on other ongoing studies as part of HCHB T&I *Component 2 – Investigating the drivers and controls of filamentous algae and restoration of aquatic plants in the Coorong* (e.g. Waycott et al. 2020). Sampling was conducted seasonally over a 12-month period: Spring (27 October 2020), Summer (16 December 2020), Autumn (9 March 2021), and Winter (15 June 2021).

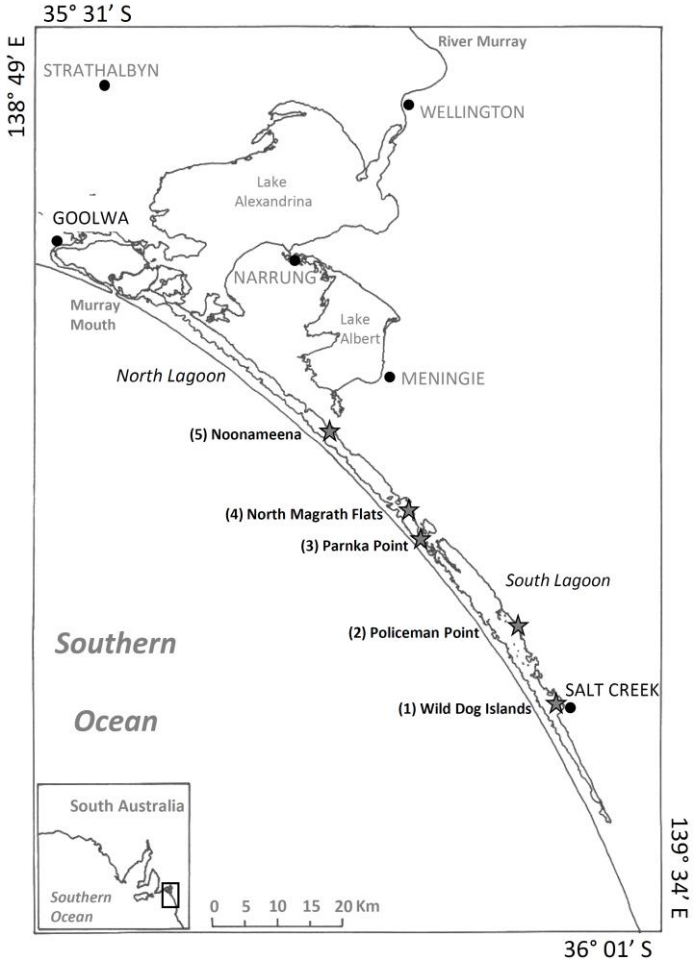

Figure 3. Map of the sampling sites for these Coorong microbiota investigations. Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena.

2.1.1 Water quality

Water sampling was undertaken approximately 10-15 cm under the subsurface. Measurements of temperature (°C), and salinity (Practical Salinity Unit, PSU) were recorded in triplicates using a multi-parameter probe (Hanna, HI 98195). Dissolved Oxygen (DO) was recorded in triplicates using a Handy Polaris (Oxyguard, H01P). Water samples (500 ml) for Turbidity (NTU) were collected in triplicates using a 1.8 metre sampling pole and analysed immediately upon collection using a waterproof Turbidimeter (ThermoScientific EUTech TN-100).

Samples for dissolved nutrient analysis, i.e. ammonium, nitrite, nitrate and phosphate, were collected in triplicates and filtered at 0.45 µm prior to storage at -20°C until analysis. Analyses of all dissolved nutrient concentrations were measured simultaneously and carried out following published methods (Hansen and Koroleff, 2007), using a SKALAR SFA nutrient analyser. Samples were thawed and approximately 10 ml from each replicate was analysed.

The Redfield ratios were calculated for dissolved nitrogen:phosphorus (N:P). Redfield et al. (1963) proposed that growing microalgae take up nutrients from the water column in fixed proportions, namely Carbon:Nitrogen:Phosphorus:Silicon (C:N:P:Si) ratios of 106:16:1:15. Deviations in dissolved nutrient concentrations from these proportions have been used as indicators of the limitation of primary production in pelagic systems. We calculated the dissolved N:P Ratio to identify a possible limitation in dissolved nitrogen or in phosphorus at our sampling sites.

2.1.2 Planktonic microalgae

Plankton samples were obtained by collecting triplicate water samples (1 L) from each of the five sites during each seasonal field trip (Figure 3, Table 1). The water samples were fixed with Lugol iodine (5% final concentration) immediately upon collection in order to preserve the structure of the organisms' chloroplast. Samples were shipped to Microalgal Services (Ormond, Victoria) to be identified and enumerated. The biovolume of the microalgae community was estimated using data provided by the Department for Environment and Water, Healthy Coorong, Healthy Basin Water Quality Monitoring Program (2020-2022). A species, genus or algal group is considered dominant (or forming a bloom) when the total biovolume (or cells.mL^{-1}) of that species accounts for >75% of the total biovolume.

Table 1. Categories and details of sites, site numbers and number of samples collected for each task in this study. Samples were collected in triplicate at each site and time. Data sets; planktonic microalgae (P), water column (W) and sediment microbiome (S); *Ruppia* Community (R for roots and leaves or RR for roots only); filamentous algal mats (F), *Ruppia* associated algal aggregates (A), no collections made for a category (e.g. no plant material), indicated by an X_N (subscript indicates data type).

SITE	LOCATION LATITUDE, LONGITUDE	SPRING 27 OCTOBER 2020	SUMMER 16 DECEMBER 2020	AUTUMN 9 MARCH 2021	WINTER 15 JUNE 2021
COORONG SOUTH LAGOON					
1 Wild Dog Island	-36.119569, 139.639043	P, W, S, R, X_F, X_A	P, W, S, R, X_F, X_A	P, W, S, RR, X_F, X_A	P, W, S, X_R, X_F, X_A
2 Policeman Point	-36.058862, 139.588359	P, W, S, R, X_F, X_A	P, W, S, R, X_F, X_A	P, W, S, RR, X_F, X_A	P, W, S, X_R, X_F, X_A
CENTRAL COORONG					
3 Parnka Point	-35.902271, 139.398209	P, W, S, R, F, A	P, W, S, R, F, X_A	P, W, S, RR, X_F, X_A	P, W, S, R, X_F, X_A
4 North Magrath Flats	-35.852534, 139.384941	P, W, S, R, F, A	P, W, S, R, F, X_A	P, W, S, RR, X_F, X_A	P, W, S, R, F, A
COORONG NORTH LAGOON					
5 Noonameena	-35.754336, 139.262122	P, W, S, R, F, A	P, W, S, R, F, X_A	P, W, S, R, X_F, X_A	P, W, S, R, X_F, X_A

2.1.3 Microbiota and nutrients in the southern Coorong

The contribution of water column microbiota to the nutrient pool (Total Nitrogen, TN, and Total Phosphorous, TP) was estimated using the data provided by the Department for Environment and Water, Healthy Coorong, Healthy Basin Water Quality Monitoring Program (2020-2022) and the measurements of dissolved nutrients concentrations determined in this study. The estimated nutrient data concentrations were compared to the biovolume of planktonic microalgae estimated using the methodology outlined in section 2.1.2.

2.1.4 Water and sediment microbiome

Triplicate 2 L water samples were collected for DNA extraction at all five sampling sites (Figure 3, Table 1) during each seasonal field trip using a 2 m long sampling pole, enabling the collection of water on foot without disturbing the surrounding sediment. The water was filtered on site through a 20 µm sieve and placed in a freezer for transportation. In the laboratory, samples were filtered through 0.22 µm Millipore membrane filters (MF-Millipore™ Membrane Filters HAWP04700). Filters were placed in petri dishes tightly closed using Parafilm and stored at −20°C until DNA extraction.

Triplicate sediment samples for DNA extraction were collected at an undisturbed location along the waterline at all five sampling sites during each seasonal field trip. A 10 mL sterile syringe with the tip cut off was inserted into the sediment to a depth of 2.5 cm, while the piston was being lifted. The

sediment sample was transferred into a 50 mL sterile tube. The samples were placed in the freezer for transportation prior to storage at −20°C until DNA extraction.

2.1.5 The *Ruppia* Community

At each sampling site, where possible, three 45 mm diameter sediment cores were taken randomly within a patch of *Ruppia*, to a depth of 10 cm (Table 1) and placed in containers containing site specific water, before being cold-transported to the laboratory. As plants of the *Ruppia* Community were not present at all sampling sites and on all sampling dates, samples were not always collected in triplicate (Table 1). The *Ruppia* Community samples (Figure 4) were dissected to separate the root systems from the leaves of the aquatic plants present. When small filamentous algae aggregates were associated to the plant, they were also isolated and analysed.

Figure 4. Example of *Ruppia* and *Althenia* (flowers visible of the latter, as indicated by white arrows) collected on 27 October 2020.

The plant material (*Ruppia* species*, Althenia* species, filamentous algae aggregates and filamentous algal mats) was processed using the modified protocol of Ugarelli et al. (2019) in which the sonication time was adjusted to 5 minutes for each cycle. The supernatant was sequentially filtered onto 0.22 µm Millipore membrane filters (MF-Millipore™ Membrane Filters HAWP04700). Filters were placed in petri dishes, enclosed using Parafilm and stored at −20°C until DNA extraction.

Various sections of the plant material were fixed and dehydrated as per the protocol of Lee et al. (2010) in preparation for SEM. Prior to examination, the samples were mounted onto carbon-tape coated SEM stubs before being sputter coated with platinum (15 nm) and observed using an Inspect FEI F50 SEM operating at 5-10 kV.

2.1.6 Filamentous algae mats

The presence of significant filamentous algal mats has been well documented across the southern Coorong and the mats have been previously characterised as being comprised of macroalgae *Ulva paradoxa*, *Cladophora* sp. and *Rhizoclonium* sp. (Collier et al. 2017). At sampling sites where filamentous algae mats and/or plant associated aggregates (PAA) were present (Figure 5), the whole surface mat (to a maximum volume of 2 L per replicate) was collected in triplicate (total volume of 6L), where possible, and placed in containers containing water collected from the same site and location. The containers were cold-transported back to the laboratory. Samples could only be collected where

filamentous algae mats were present and analysis of this material was not possible for some sampling sites and dates (Table 1).

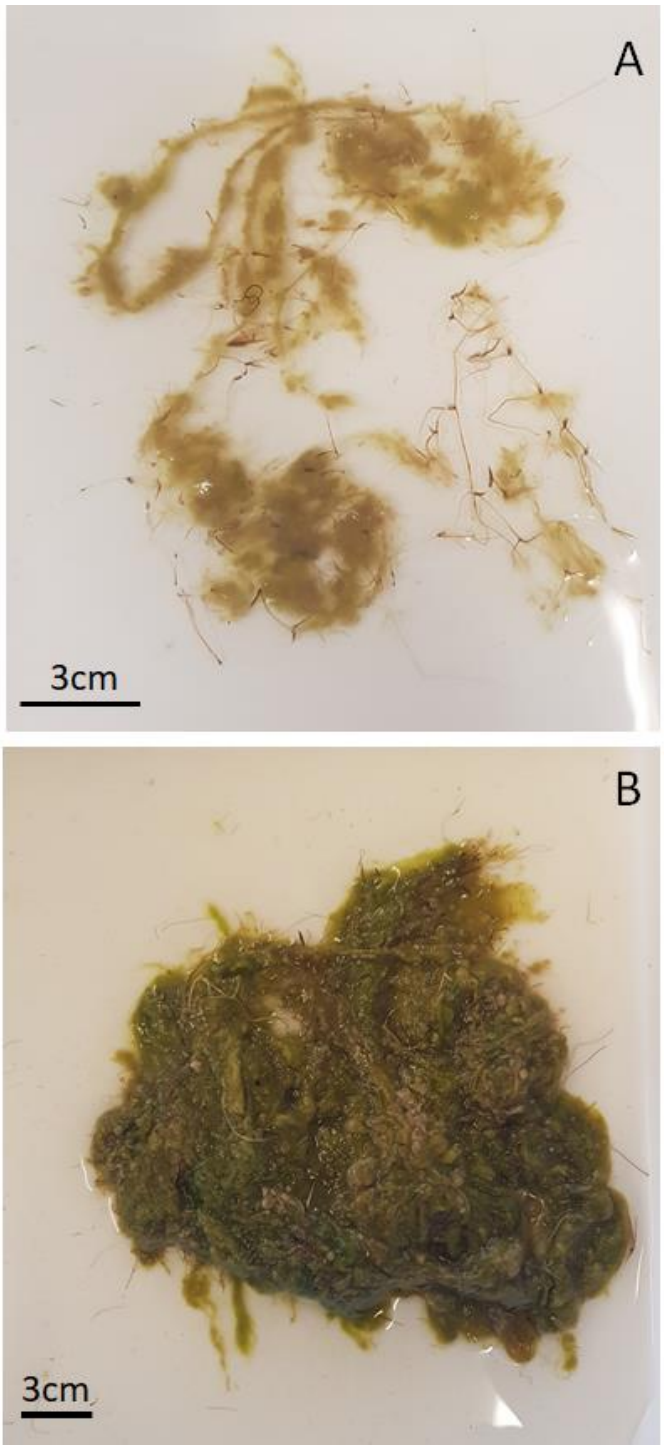

Figure 5. Example of (A) plant associated aggregates (PAA) and (B) filamentous algae mat collected at Parnka Point in October 2020.

2.1.7 Extraction of DNA

For the *Ruppia* (leaves and roots), filamentous algae material (mats, floating mats and PAA) and water samples, DNA was extracted from the filters using Qiagen Dneasy PowerWater Kits. Whereas DNA from the sediment samples (0.25 grams) was extracted using Qiagen Dneasy PowerSoil Kits. Extracted DNA was quantified using a NanoDrop One Spectrophotometer (Thermo Fisher).

2.1.8 Amplicon formation

For each DNA extract, the universal primer pair 515F/806R was used to amplify the prokaryotic V4 region of the 16S rRNA which has a median amplicon length of 292 base pairs (bp), with a range of 290–295 bp (Table 2). The primer pairs also contained Nextera adapter sequences (specific to the sequencing platform) as well as individual barcode sequences for identification of each sample. PCR reactions were performed for each sample, and each reaction included about 1 ng of template DNA, 0.32 µM of each primer, 2 U Q5 Hot Start High-Fidelity DNA Polymerase (New England Biolabs), 1× Q5 reaction buffer (New England Biolabs), 0.8 mM dNTP (Combined; Promega) and MilliQ water for a total volume of 50 µl. The conditions for PCR were as follows: 98°C for 1 min to denature the DNA, with 30 cycles at 98°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, and a final extension of 10 min at 72°C to ensure complete amplification.

Successful DNA amplification was confirmed using gel electrophoresis (Figure 6) and the PCR products were sent to the Australian Genome Research Facility (AGRF, Melbourne, Australia) for sequencing.

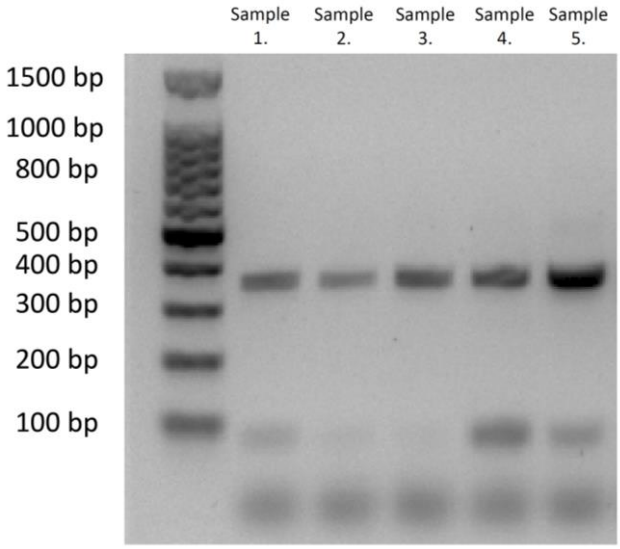

Figure 6. Example of amplicon fragment size (391 base pairs (bp)) obtained after PCR and visulised via gel electrophoresis using a 100 bp ladder (Promega) for reference.

2.1.9 Sequencing and bioinformatic pipeline

DNA sequence data was generated using Illumina MiSeq™ System (2 x 250bp paired-end sequencing) at the Australian Genome Research Facility (AGRF, Melbourne, Australia). A data analysis workflow overview is shown in Figure 7. DADA2 package (version 1.22; Callahan et al. 2016) in R studio v.4.6.1 (R v.4.1.3; https://www.r-project.org/) was used for sequence analysis using amplicon sequence variants (ASVs) which created groups based on sequence similarities. Raw sequences were demultiplexed with barcodes and adaptors removed by AGRF. The paired end 16S rRNA raw sequencing data comprised 228 samples which generated 27,104,165 total reads (see Table 3). The Cutadapt program (Martin, 2011) was used to remove the primers (Figure 7) from the amplicon reads. Sequences were filtered and trimmed following standard filter parameters, with the quality score of the sequence of less than or equal to 2 and with the maximum expected errors for forward reads equalling 2, and 5 for reverse reads. All sequences which matched the phiX genome were discarded. After filtering and trimming, the 228 samples comprised of 24,955,834 sequences (see Table 3). The paired ends sequences were merged followed by denovo chimera removal (see Table 3). Taxonomy was inferred (98% similarity) for the 16S rRNA gene sequences using the SILVA database release 138.1 (https://www.arb-silva.de/documentation/release-1381/; Quast et al. 2013). Singletons and taxon such as Eukaryota, Mitochondria and Chloroplast were removed from the dataset.

Organisms essential to the function of the ecological community they occupy are "common core microorganisms" (Ainsworth et al. 2015). The presence, within all assemblages of a particular ecosystem, of common core microorganism taxa allows for the characterisation of a "healthy" community and consequently the impact of any perturbation (Shade and Handelsman, 2012). We have assessed the common core archaea and bacteria phyla for all sample types and sites across all the trips using the ASV sequencing data obtained.

Table 2. PCR Primer used for the amplification of the v4 region of the 16S rRNA gene (Ugarelli et al. 2019) from DNA extracted from water, sediment, *Ruppia* and filamentous material samples collected from sampled sites (1: Wild Dog Islands, 2: Policeman Point, 3: Parnka Point, 4: North Magrath Flats, 5: Noonameena) in October 2020, December 2020, March 2021, and June 2021.

REGION OF THE GENE	FORWARD PRIMER NAME	FORWARD PRIMER SEQUENCE	REVERSE PRIMER NAME	REVERSE PRIMER SEQUENCE	AMPLICON LENGTH (BP)
V4	515F	GTGYCAGCMGCCGCGGTAA	806R	GGACTACNVGGGTWTCTAAT	292

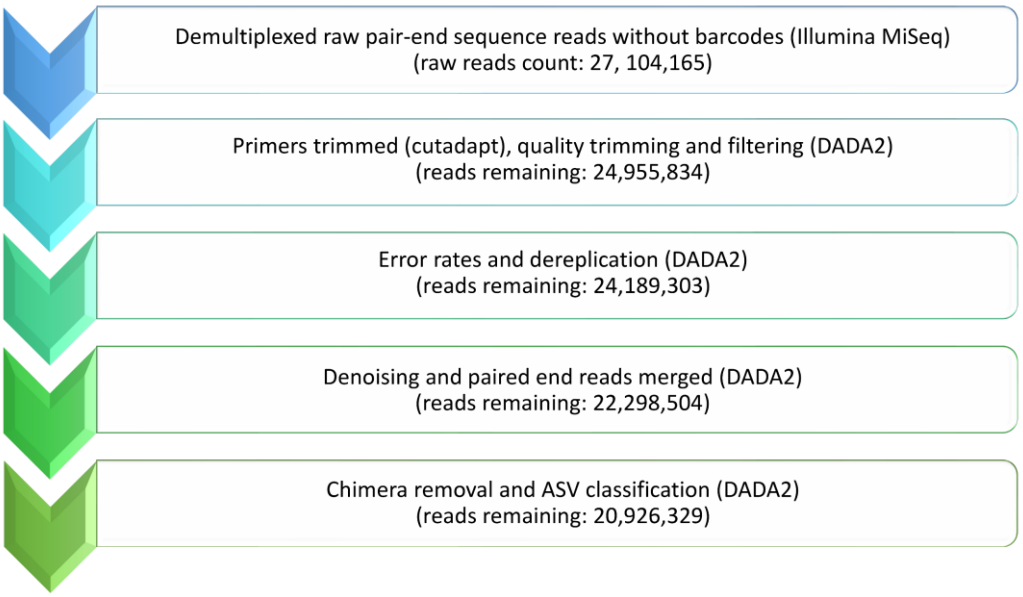

Figure 7. The DADA2 pipeline data analysis workflow, including the tools employed and the changes at each step in the number of processed reads.

Table 3. The number of sequences after each data analysis step for the samples collected at the five sampling sites throughout the four seasonal sampling events.

SAMPLE TYPE	SAMPLING PERIOD #	SITE	INPUT	FILTERED	DENOISED	MERGED	CHIMERA
FLOATING	2	Noonameena	85914	83878	80005	70885	59652
FLOATING	2	North Magrath Flats	73757	70763	65068	54324	48275
LEAVES	1	Wild Dog Island	248111	237998	237326	221309	218886
LEAVES	1	Policeman Pt	266526	257072	255531	232448	230786
LEAVES	1	Parnka Pt	277046	267367	265932	246408	236540
SAMPLE TYPE	**SAMPLING PERIOD #**	**SITE**	**INPUT**	**FILTERED**	**DENOISED**	**MERGED**	**CHIMERA**
LEAVES	1	North Magrath Flats	184630	178670	176098	163609	152614

LEAVES	1	Noonameena	25482	22190	22161	22071	22071
LEAVES	2	Noonameena	169700	163466	163075	155168	153852
LEAVES	4	Parnka Pt	194018	172815	165396	153250	146168
LEAVES	4	North Magrath Flats	170212	150384	143462	129173	125707
LEAVES	4	Noonameena	316950	276137	263454	242491	235747
MATS	1	Parnka Pt	313175	302640	291452	265338	232522
MATS	1	North Magrath Flats	285930	275874	261415	207500	171642
MATS	1	Noonameena	286375	277275	256773	221521	208296
MATS	2	Parnka Pt	295089	284010	275734	257316	246925
MATS	2	North Magrath Flats	299635	290392	284855	266712	255565
MATS	2	Noonameena	373662	358940	343554	291134	210845
MATS	4	North Magrath Flats	214179	191716	179941	163353	161258
PAA	1	Parnka Pt	405756	389859	385310	369730	365399
PAA	1	North Magrath Flats	122785	119125	112383	98924	90059
PAA	2	Parnka Pt	194349	186800	174254	147784	136524
PAA	3	North Magrath Flats	53404	47241	44399	38433	38005
PAA	4	North Magrath Flats	70162	62517	58084	51886	51782
PAA	4	Noonameena	58528	51482	47488	42838	42803
ROOTS	1	Wild Dog Island	336527	323416	320541	301286	288491
ROOTS	1	Policeman Pt	251593	241507	240942	227763	226183
ROOTS	1	Parnka Pt	269333	258664	252179	237638	231036
ROOTS	1	North Magrath Flats	223860	212535	211072	201042	199661
ROOTS	1	Noonameena	335634	325241	316323	297200	291549
ROOTS	2	Wild Dog Island	306934	296567	295311	282103	276841
ROOTS	2	Policeman Pt	232015	218551	217548	197331	194801
ROOTS	2	North Magrath Flats	312589	300706	299556	288698	286219
ROOTS	2	Noonameena	343963	333211	330410	307202	278839
ROOTS	3	Wild Dog Island	290473	280168	278820	256691	252177
ROOTS	3	Policeman Pt	180633	163711	153914	138210	133038
ROOTS	3	Parnka Pt	174402	152322	143619	129748	126230
ROOTS	3	Noonameena	228917	202630	190610	163193	125151
ROOTS	4	Parnka Pt	178826	151607	144280	135663	135433
SAMPLE TYPE	**SAMPLING PERIOD #**	**SITE**	**INPUT**	**FILTERED**	**DENOISED**	**MERGED**	**CHIMERA**
ROOTS	4	North Magrath Flats	227866	202734	192922	178070	172891
ROOTS	4	Noonameena	300799	266264	252391	230015	222340

SAMPLE TYPE	SAMPLING PERIOD #	SITE	INPUT	FILTERED	DENOISED	MERGED	CHIMERA
SEDIMENT	1	Wild Dog Island	455330	402225	389653	360874	357362
SEDIMENT	1	Policeman Pt	431325	379598	364910	327891	325376
SEDIMENT	1	Parnka Pt	369495	327759	309594	275825	271965
SEDIMENT	1	North Magrath Flats	449955	405716	389023	358659	356943
SEDIMENT	1	Noonameena	367211	334036	309182	278755	275136
SEDIMENT	2	Wild Dog Island	342212	307741	296215	273065	271710
SEDIMENT	2	Policeman Pt	365958	327858	318295	297169	295633
SEDIMENT	2	Parnka Pt	383746	336596	316772	276848	273723
SEDIMENT	2	North Magrath Flats	355706	312993	294904	264048	261917
SEDIMENT	2	Noonameena	393986	350674	336219	306640	303923
SEDIMENT	3	Wild Dog Island	290155	262915	255080	239374	238144
SEDIMENT	3	Policeman Pt	381414	346748	340874	328278	326820
SEDIMENT	3	Parnka Pt	457402	406118	376418	332952	324715
SEDIMENT	3	North Magrath Flats	414650	376932	359206	328665	318685
SEDIMENT	3	Noonameena	394380	360539	349368	327731	322971
SEDIMENT	4	Wild Dog Island	264803	254013	229290	197663	193542
SEDIMENT	4	Policeman Pt	257563	238918	228221	196230	189675
SEDIMENT	4	Parnka Pt	263184	245868	226249	181160	176404
SEDIMENT	4	North Magrath Flats	301617	281706	256873	207865	200053
SEDIMENT	4	Noonameena	260178	250681	234107	198310	180203
WATER	1	Wild Dog Island	599074	548394	541968	518077	472421
WATER	1	Policeman Pt	538004	490808	483124	459091	414744
WATER	1	Parnka Pt	540135	504783	502827	494243	484776
WATER	1	North Magrath Flats	555338	515592	511279	494780	473128
WATER	1	Noonameena	394588	369159	367935	363473	363103
WATER	2	Wild Dog Island	532841	491555	481685	453890	388276
WATER	2	Policeman Pt	531229	480790	469574	439101	379532
WATER	2	Parnka Pt	430031	393669	382280	342322	279610
WATER	2	North Magrath Flats	454680	416879	410010	389917	356118
WATER	2	Noonameena	496648	461999	449399	421492	363412
WATER	3	Wild Dog Island	505263	471269	465623	448119	417352
WATER	3	Policeman Pt	435393	404852	401168	385643	355370
WATER	3	Parnka Pt	481102	441661	436474	419852	395475
WATER	3	North Magrath Flats	515881	478974	471730	453902	424689

WATER	3	Noonameena	432268	387247	377358	356128	326967
WATER	4	Wild Dog Island	567502	506637	496912	453847	377323
WATER	4	Policeman Pt	508867	451284	442020	407531	339684
WATER	4	Parnka Pt	318693	279456	268882	244918	212728
WATER	4	North Magrath Flats	344920	301808	291719	265505	221971
WATER	4	Noonameena	858680	757534	727577	652470	547991
Total			**27104165**	**24955834**	**24189303**	**22298504**	**20926329**

2.2 Datasets and statistical analysis

Records from sample collection and processing were compiled and digitally entered into the HCHB T&I *Component 2* Aquatic plants and filamentous algae database. Data entry of information on return included location (GPS cross reference); presence or absence of *Ruppia*; algal mat formation status; aggregate formation status; amount of DNA extracted; and laboratory notes.

To assess species diversity, Pielou's evenness, and species richness, community diversity index was computed using the PRIMER v.7 software +PERMANOVA add on (Clarke and Gorley, 2006; Anderson et al. 2008). Data collected were not normally distributed after applying a Shapiro-Wilk test. To test for significant differences between means of parameters, nonparametric, Kruskal-Wallis tests (Zar, 1996) were calculated using the IBM SPSS 22.0 Software.

Multivariate statistics were performed using PRIMER v.7 software +PERMANOVA add on. Environmental data were examined in draftsman scatter plots to ascertain whether some variables were highly correlated to each other, and if the assumptions made about the data were valid. Draftsman plots were examined before and after log(x+1) transformation and normalisation. A Principal Component Analysis (PCA) was used to explore the water quality parameters contributing to the differentiation between the sampling sites. This ensured the data were approximately multivariate-normally distributed before performing a PERMutational ANalysis Of VAriance (PERMANOVA) using a Euclidean distance resemblance matrix to test for significant differences in overall water quality using a 2-factor design (month – fixed and location – fixed).

The microbial community data were transformed using log(x+1) to meet assumptions of normal distribution and homoscedasticity. Dissimilarity was assessed between pairs of groups (e.g. leaves versus roots, roots versus sediment, leaves versus water) using the SIMPER method (Anderson et al. 2008). A Bray-Curtis similarity matrix was calculated between the communities and the data were then analysed by Principal Coordinate Analysis (PCoA). The six classes responsible for most of the dissimilarities between pairs were used to illustrate differences on the PCoA plot. We then used PERMANOVA to identify whether communities were significantly different between sampling sites and months. A Bray-Curtis similarity-based resemblance matrix was calculated on the communities. Canonical Analysis of Principal coordinates (CAP) based on Bray-Curtis similarities was used to plot the discrimination between the five sites, based on community composition. The relationship between patterns in a resemblance matrix and the environmental variables (i.e. salinity, temperature, turbidity, DO, ammonium, nitrate, nitrite and phosphorus) was observed using a constrained ordination: distance-based redundancy analysis (dbRDA). The BEST procedure was used to identify which environmental variables associated with the differences observed in the community composition. The BVSTEP procedure within BEST searches for high rank Spearman correlations between a similarity matrix of community composition and matrices of normalised environmental variables (Clarke and Gorley, 2006), community compositions are displayed on the dbRDA plot.

Relative abundance plots were created using ggplot2 (Version 3.3.6; Wickham 2016) using R (version 4.1.3). Differential abundance between the microbial communities of Noonameena and South Lagoon sites (Noonameena vs Policeman Point, and Noonameena vs Wild Dog Islands) were compared using the ANCOMBC package (version 1.4.0; Lin and Peddada, 2020) using R (version 4.1.3). The significant differences are expressed as Log-fold changes.

To identify the core, the variable, and the unique taxa among the water samples and the aggregates, Venn diagrams were created with the online tool access through https://bioinfogp.cnb.csic.es/tools/venny/ (Oliveros, 2007).

3 Results

3.1 Water quality

There was both spatial and temporal variation (Figure 8) in the range of temperature, salinity, turbidity, and dissolved oxygen (DO) measured at the five sampling sites (Figure 3, Table 4) during each of the seasonal field trips. The pattern observed in the temperature reflects hourly (as we sample from Wild Dog Islands to Noonameena throughout the day) and seasonal variations expected in the region. The salinity measurements are typical of the salinity gradient observed in the Coorong, with significantly higher salinities observed in the South Lagoon compared to the North Lagoon (ANOVA with Tukey post-hoc test, $p<0.05$). The highest salinity levels were observed in Autumn, except for Noonameena. Turbidity levels showed an increasing trend from Wild Dog Islands to Parnka Point, with significantly higher turbidity at Parnka Point (ANOVA with Tukey post-hoc test, $p<0.05$). The observations made in March 2021 (Autumn) showed the most variability in water quality as reflected by the strongest gradient observed in salinity (from 52.5 ± 0.8 PSU at Site 5; Noonameena to 127.8 ± 1.7 PSU at Site 1; Wild Dog Island), DO (from 56.5 ± 3.5% at Site 2; Policeman Point to 100 ± 0.0% at Site 5; Noonameena) and turbidity (from 5.0 ± 0.7 NTU at Site 5; Noonameena to 10.3 ± 0.1 NTU at Site 1; Wild Dog Island).

Table 4. Water quality data (mean ± standard deviation) collected from the five sampling sites in October 2020, December 2020, March 2021 and June 2021.

DATE	SITE	WATER QUALITY PARAMETERS			
		TEMPERATURE (°C)	SALINITY (PSU)	TURBIDITY (NTU)	DO (%)
Oct-20	Wild Dog Island	19.4 ± 0.2	54.8 ± 0.5	5.5 ± 0.3	N/A
	Policeman Point	25.8 ± 0.7	56.5 ± 1.2	6.6 ± 0.2	N/A
	Parnka Point	23.5 ± 0.4	54.4 ± 0.2	25.7 ± 5.4	N/A
	North Magrath Flats	24.4 ± 0.9	53.2 ± 0.0	10.4 ± 0.5	N/A
	Noonameena	23.2 ± 0.2	45.0 ± 0.0	1.8 ± 0.1	N/A
Dec-20	Wild Dog Island	15.6 ± 0.1	78.6 ± 0.4	10.0 ± 0.2	98.0 ± 0.9
	Policeman Point	16.0 ± 0.2	77.5 ± 0.2	14.3 ± 0.6	97.8 ± 0.6
	Parnka Point	17.6 ± 0.2	79.6 ± 0.7	19.3 ± 0.7	100 ± 0.0
	North Magrath Flats	22.0 ± 0.2	76.5 ± 0.5	12.4 ± 0.1	100 ± 0.0
	Noonameena	22.9 ± 0.1	107.3 ± 2.3	4.1 ± 0.5	100 ± 0.0
Mar-21	Wild Dog Island	16.6 ± 0.1	127.8 ± 1.7	10.3 ± 0.1	81.4 ± 4.2
	Policeman Point	18.8 ± 0.2	118.8 ± 2.7	9.4 ± 0.3	56.5 ± 3.5
	Parnka Point	21.1 ± 0.1	114.3 ± 2.6	13.8 ± 1.0	91.6 ± 1.4
	North Magrath Flats	24.5 ± 0.2	96.4 ± 0.3	22.9 ± 1.2	100 ± 0.0

	Noonameena	26.7 ± 0.1	52.5 ± 0.8	5.0 ± 0.7	100 ± 0.0
Jun-21	Wild Dog Island	11.7 ± 0.0	93.2 ± 1.9	5.7 ± 0.3	99.3 ± 1.3
	Policeman Point	12.8 ± 0.1	93.9 ± 4.8	5.5 ± 0.2	100 ± 0.1
	Parnka Point	12.9 ± 0.0	72.3 ± 4.6	7.6 ± 0.4	100 ± 0.0
	North Magrath Flats	14.4 ± 0.1	81.1 ± 3.6	8.4 ± 0.3	100 ± 0.0
	Noonameena	14.6 ± 0.0	31.3 ± 1.5	1.3 ± 0.1	100 ± 0.0

The dissolved nutrient levels measured throughout the study characterised a strong deficit in nitrogen as per calculation of the Redfield ratio (16:1; Redfield et al. 1963; Table 5). Significant differences were evident between sampling sites (PERMANOVA $p<0.001$) for most of the water quality data. However, differences in temperature between sites were disregarded as the temperature increased during the day as samples were collected. DO was not recorded in October 2020 due to a problem with the probe. No significant differences in the water quality parameters were observed over time at the individual sites. However, based on the Coorong region (South Lagoon: Wild Dog Islands and Policeman Point; Central: Parnka Point and North Magrath Flats; and North Lagoon: Noonameena), significant differences were found between the North and South Lagoon, and North Lagoon and Parnka Point (PERMANOVA $p<0.001$).

A Principal Component Analysis (PCA) was conducted on the water quality data parameters (Figures 9 and 10). DO% (Pearson r = -0.451) and salinity (Pearson r = 0.444) seem to be shaping the differences between sites along the PC1 axis (Figure 9), whereas nutrients (i.e. Phosphate (Pearson r = 0.502) and Nitrite (Pearson r = -0.562)), and temperature (Pearson r = -0.391) are shaping the differences between sites along PC2. Sampling undertaken in March 2021 shows the highest variability between sites (pink line, Figure 9).

When using the type of plant material present at each site as a factor (Figure 10), the relationship between water quality and plant condition becomes more apparent. Plant conditions are characterised by the presence of leaves, roots, plant-associated aggregates, and floating mats of filamentous algae in which the plants get embedded. Indeed, the sampling trip in March 2020 shows the highest salinity (Figure 8B), but also no plant material was observed at any of the five sites sampled during that trip (Figure 10).

Table 5. Water column nutrient data (mean ± standard deviation) collected from the five sampling sites in October 2020, December 2020, March 2021 and June 2021.

DATE	SITE	WATER NUTRIENTS				
		AMMONIUM (NH4; MG N/L)	PHOSPHATE (PO4; MG P/L)	NITRITE (NO2; MG N/L)	NITRATE (NO3; MG N/L)	REDFIELD RATIO (N:P; 16:1)
Oct-20	Wild Dog Island	0.5 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	4.1
	Policeman Point	0.5 ± 0.1	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	2.9
	Parnka Point	0.6 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	3.4
	North Magrath Flats	0.3 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	2.2
	Noonameena	0.4 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	2.6
Dec-20	Wild Dog Island	0.6 ± 0.1	0.4 ± 0.0	0.2 ± 0.2	0.3 ± 0.0	2.6
	Policeman Point	0.6 ± 0.1	0.3 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	2.8
	Parnka Point	0.5 ± 0.0	0.4 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	2.0
	North Magrath Flats	0.5 ± 0.0	0.4 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	1.9
	Noonameena	0.3 ± 0.0	0.5 ± 0.1	0.0 ± 0.0	0.3 ± 0.1	1.4
Mar-21	Wild Dog Island	0.9 ± 0.0	0.8 ± 0.1	0.0 ± 0.0	0.6 ± 0.1	2.0

	Policeman Point	0.8 ± 0.1	0.6 ± 0.3	0.6 ± 0.9	0.6 ± 0.1	5.8
	Parnka Point	0.9 ± 0.1	0.2 ± 0.1	1.4 ± 0.1	0.6 ± 0.3	12.0
	North Magrath Flats	0.8 ± 0.0	0.2 ± 0.1	1.5 ± 0.3	0.3 ± 0.1	10.9
	Noonameena	0.5 ± 0.1	0.4 ± 0.1	1.1 ± 0.1	0.3 ± 0.0	4.8
Jun-21	**Wild Dog Island**	0.8 ± 0.1	0.3 ± 0.1	0.5 ± 0.0	0.3 ± 0.0	5.8
	Policeman Point	0.8 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	0.3 ± 0.1	5.5
	Parnka Point	0.6 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	5.0
	North Magrath Flats	0.7 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	3.8
	Noonameena	0.3 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	2.1

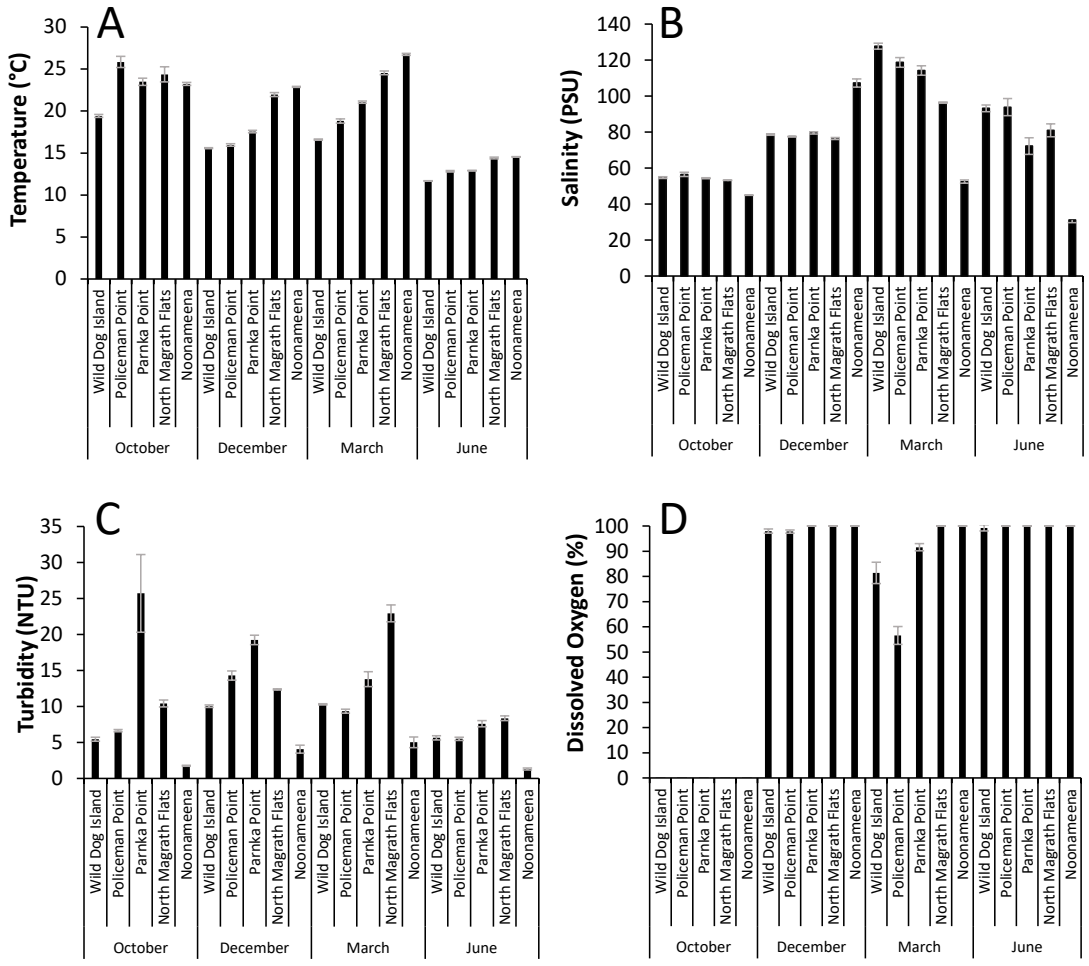

Figure 8. Water quality parameters measured at the five sampling sites in October 2020, December 2020, March 2021 and June 2021. (A) temperature, (B) salinity, (C) turbidity and (D) dissolved oxygen. Error bars indicate the standard deviation to the mean.

3.2 Planktonic microalgae

Large microalgae (> 5 µm in size) were identified to the lowest taxonomic level possible and grouped as three communities: diatoms, dinoflagellates, and other microalgae (Table 6). The community of the other microalgae included Chrysophytes, Prymnesiophytes, Cryptophytes, Euglenophyta, Chlorophytes, Prasinophytes, and Cyanoprokaryota. Additionally, picophytoplankton cells were also counted and classified according to their size, i.e. 1–4 µm and 4–6 µm (Figure 11). The microalgae community was dominated by picophytoplankton cells, which are recognised as microalgae cells of 2–

6 µm in diameter (Figure 11). The community of picophytoplankton was dominated by very small cells of 1–4 µm in size, possibly Synecchococcus and/or Prochlorococcus.

Microalgal communities were significantly different between sampling sites and seasons (PERMANOVA $p<0.001$) which is explained by the differences in environmental conditions, particularly salinity, over the sampling period (BEST analysis, $p<0.01$). The communities were also significantly different based on the presence/absence of plants and based on the plant health i.e. presence of leaves (PERMNOVA $p<0.001$).

The CAP analysis suggests that the microalgae community composition changes with the condition of the plants (Figure 12). Plant conditions are characterised by the presence of leaves, roots, plant-associated aggregates, and floating mats of filamentous algae in which the plants get embedded. This is illustrated by the clustering of the microalgae communities within three groups based on the plants condition. The vector overlay of the microalgae species based on >0.5 Pearson correlation (Figure 12) revealed the species that were driving the groupings. The species were *Mamiella gilva*, *Ochromonas* sp., *Chlamydomonas/Dunaliella*, *Chrysamoeba*, unidentified flagellates, *Haslea ostrearia*, *Mamiella* sp., *Cryptomonas* sp., *Chroomonas* sp., *Levanderina fissa*, *Diplosalid* sp., *Mastogloia* sp. And *Pseudanabaena* sp.

The relationships between the communities of microalgae and the water quality parameters (used as predictor variables) are presented in Figure 13. The communities of microalgae present at Noonameena in October 2020, with no plants (e.g. indicated by red crosses) were distinct from the other sites, i.e. with high abundance of diatoms and Euglenophyta. Over the duration of the study, the composition of the community of microalgae at Noonameena seems to be consistently different from the other sites (see Figures 14 and 15 for more details).

The large microalgae community (>5µm; Figure 14) was dominated by a consortium consisting of Chrysophytes, Prymnesiophytes, Cryptophytes, Euglenophyta, Chlorophytes, Prasinophytes and Cyanoprokaryota. The most abundant species in the consortium were *Koliella* sp. (chlorophyte), *Scourfieldia* sp. and *Mantoniella squamata* (prasinophyte). Blooms of *Koliella* sp. were observed in December 2020 (except at Noonameena) and in March 2021 (except in the North Lagoon; at North Magrath Flats and Noonameena). The biovolume of the microalgae and picophytoplankton cells was calculated (Figure 14C). The picophytoplankton was 5 orders of magnitude higher than the large microalgae, dominating the biovolume. Indeed, in the March period when the large microalgae were still high, the biovolume decreased.

The composition of the diatom, dinoflagellate, and Harmful Algae Bloom species (HABs) communities, which represented only a minority of the large microalgae community, are presented in Figures 11 - 17. The diversity of microalgae species (Pielou's evenness) was significantly different between sites (Kruskal-Wallis $p<0.05$, Figure 18), with Noonameena showing a significantly higher diversity of species compared to the other sites. This is reflected by the higher number of species (25-28) recorded at Noonameena compared to the other sites (17-20).

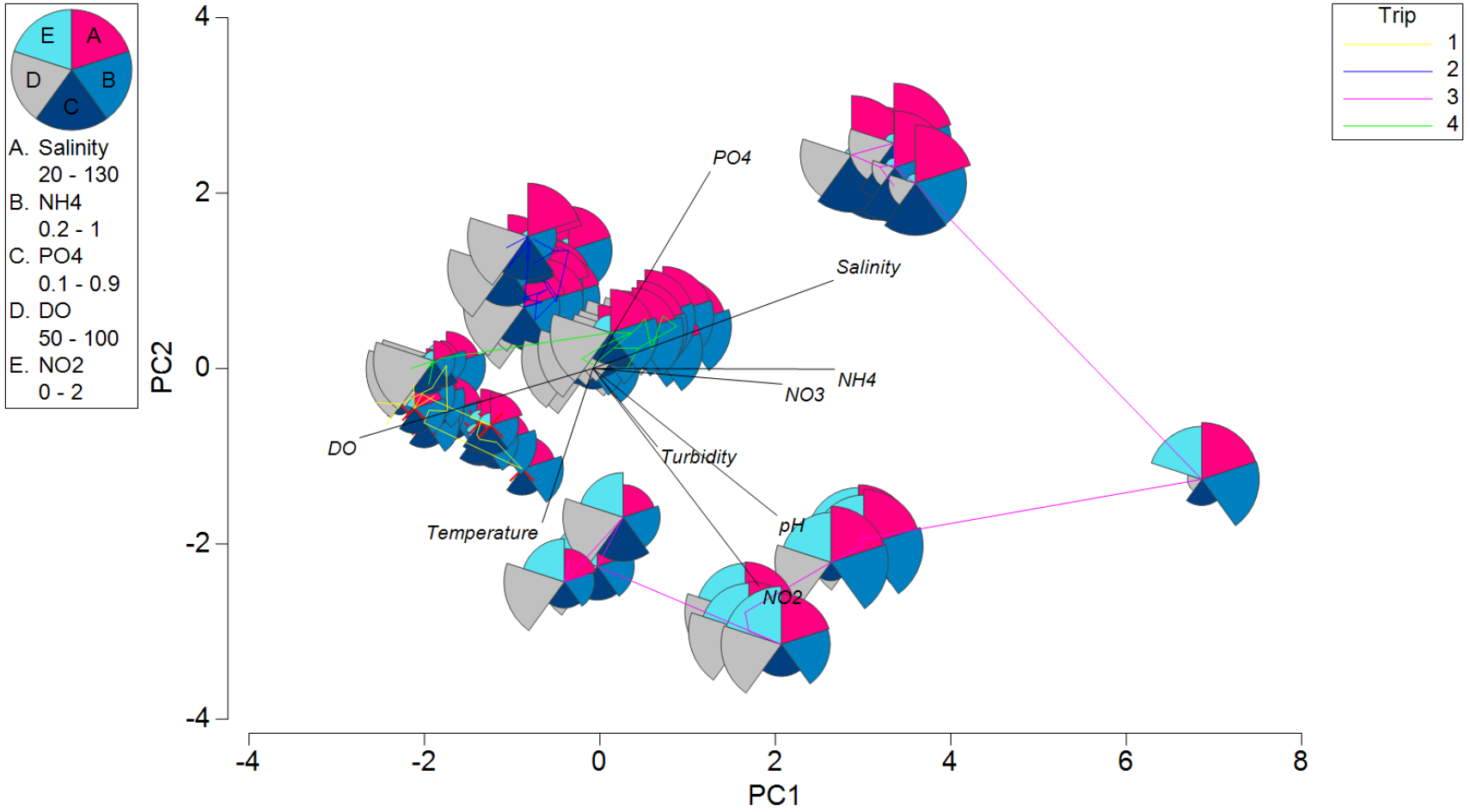

Figure 9. Principal Component Analysis (PCA) plot based on the water quality parameters measured at each of the sampling sites in October 2020 (Trip 1, yellow line), December 2020 (Trip 2, blue line), March 2021 (Trip 3, pink line) and June 2021 (Trip4, green line). PC1 and PC2 explain 42.6% and 20.9% of the total variability, respectively. Pearson correlation vectors ($r > 0.5$) represent the water quality parameters (black vectors) driving the differences between samples. Fluctuations in salinity, DO and nutrient concentrations are indicated by the pie charts.

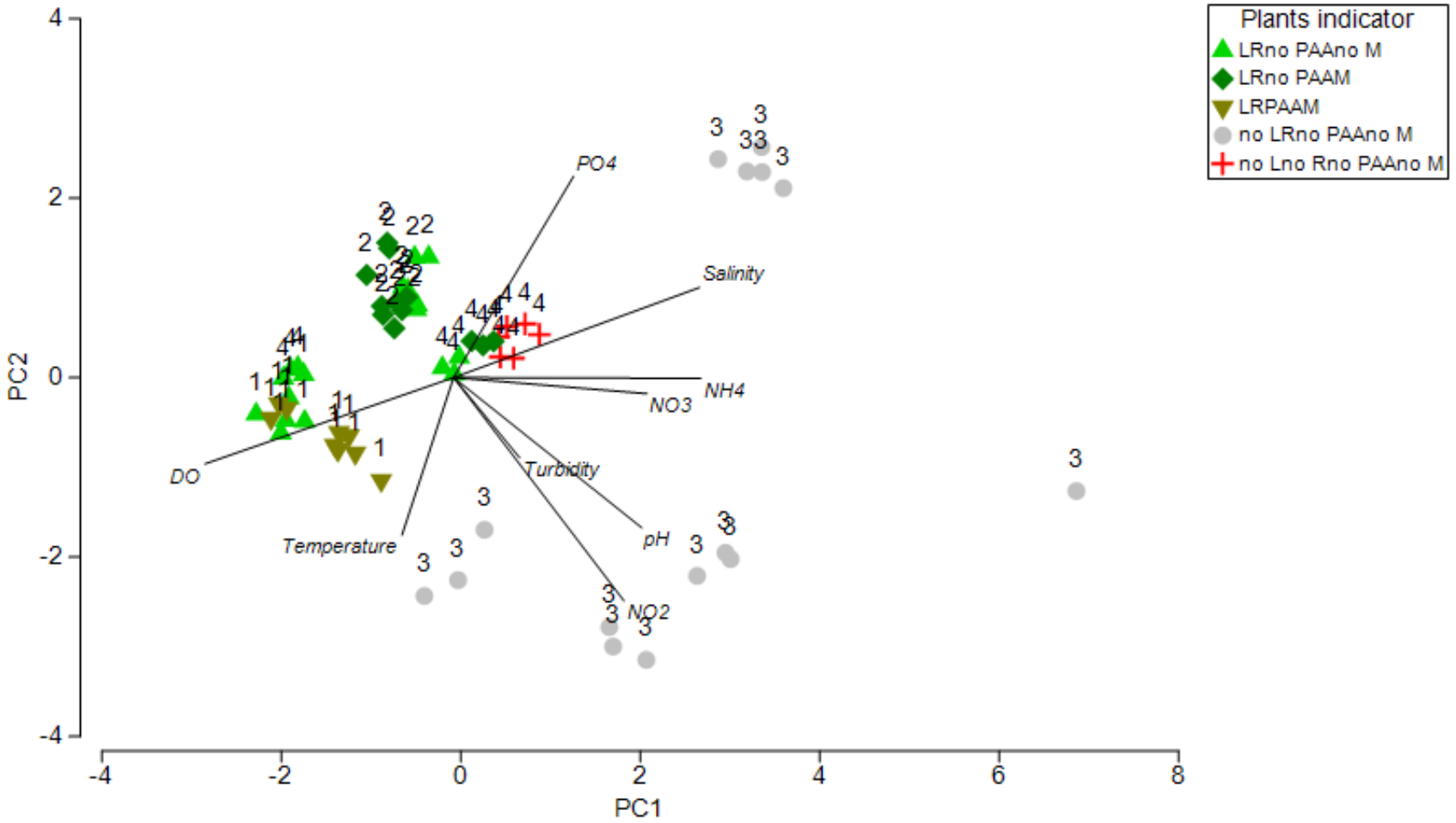

Figure 10. Principal Component Analysis (PCA) plot based on the water quality parameters measured at the five sampling sites in October 2020, December 2020, March 2021 and June 2021. Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. Coorong North Lagoon: site 5, Noonameena. PC1 and PC2 explain 42.6% and 20.9% of the total variability, respectively. Pearson correlation vectors (r > 0.5) represent the water quality parameters (black vectors) driving the differences between samples. Plant conditions are characterised by the presence/absence of leaves and roots (LR/no L no R), plant-associated aggregates (PAA/ no PAA), and floating mats of filamentous algae (M/no M) in which the plants get embedded. When whole plants were present (green symbols), when no plant material was present (red cross), or when only roots/rhizomes were present (grey circles).

Table 6. List of microalgae species identified in the samples throughout the period of study. Harmful Algae Bloom species (HABs) are indicated in bold.

GROUP	GENUS	SPECIES
Chlorophytes	*Chlamydomonas/Dunaliella*	sp.
Chlorophytes	*Koliella*	sp.
Chlorophytes	*Pyrobotris*	sp.
Chrysophytes	*Ochromonas*	sp.
Chrysophytes	*Chrysamoeba*	sp.
Cryptophytes	*Hemiselmis*	sp.
Cryptophytes	*Plagioselmis*	*prolonga*
Cryptophytes	*Rhodomonas*	*salina*
Cryptophytes	*Leucocryptos*	sp.
Cryptophytes	*Goniomonas*	*truncata*
Cryptophytes	*Chroomonas*	sp.
Cryptophytes	*Cryptomonas*	sp.
Cyanoprokaryota	*Geitlerinema*	sp.
Cyanoprokaryota	***Leptolyngbya***	**sp.**
Cyanoprokaryota	***Limnothrix***	**sp.**
Cyanoprokaryota	*Planktolyngbya*	sp.
Cyanoprokaryota	***Pseudanabaena***	**sp.**
Cyanoprokaryota	*Aphanocapsa*	sp.
Cyanoprokaryota	*Merismopedia*	sp.
Cyanoprokaryota	***Pseudanabaena***	***limnetica* (cells)**
Cyanoprokaryota	*Spirulina*	sp.
Cyanoprokaryota	*Phormidium*	sp.
Diatoms	*Amphora*	sp.
Diatoms	*Cocconeis*	sp.
Diatoms	*Cyclotella*	sp.
Diatoms	*Cylindrotheca*	*closterium*
Diatoms	*Cymbella*	sp.
Diatoms	*Dactyliosolen*	*fragilissimus*
Diatoms	*Encyonema*	sp.
Diatoms	*Grammatophora*	sp.
Diatoms	*Gyrosigma*	sp.
Diatoms	*Haslea*	*wawrikae*
Diatoms	*Leptocylindrus*	*minimus*
Diatoms	*Licmophora*	sp.
Diatoms	*Naviculoid*	sp.
Diatoms	***Nitzschia***	**sp.**
Diatoms	***Nitzschia***	**sp. 1 (>50 um)**
Diatoms	*Pleurosigma*	sp.
Diatoms	*Thalassionema*	sp.
Diatoms	*Chaetoceros*	sp.
Diatoms	*Entomoneis*	sp.
Diatoms	*Leptocylindrus*	*danicus*
Diatoms	*Rhizosolenia*	*setigera*

GROUP	GENUS	SPECIES
Diatoms	*Ardissonea*	*crystallina*
Diatoms	*Asteromphalus*	*sarcophagus*
Diatoms	*Bacillaria*	*paillifera*
Diatoms	*Haslea*	*ostrearia*
Diatoms	*Mastogloia*	sp.
Diatoms	*Minidiscus*	*trioculatus*
Diatoms	*Plagiotropis*	sp.
Diatoms	*Fragilaria*	sp.
Diatoms	*Striatella*	*unipunctata*
Dinoflagellates	***Cochlodinium***	**sp.**
Dinoflagellates	*Diplopsalid*	sp.
Dinoflagellates	***Gymnodinioid***	**<20µm**
Dinoflagellates	***Gymnodinioid***	**>20µm**
Dinoflagellates	***Levanderina***	***fissa***
Dinoflagellates	*Oxyrrhis*	*marina*
Dinoflagellates	*Peridinium*	sp.
Dinoflagellates	*Protoperidinium*	sp.
Dinoflagellates	*Scrippsiella*	sp.
Dinoflagellates	*Heterocapsa*	*rotundata*
Dinoflagellates	***Takayama***	**sp.**
Dinoflagellates	*Gyrodinium*	sp.
Euglenophyta	*Eutreptiella*	sp.
Other	*Pedinellid*	sp.
Other	*Mesodinium*	rubrum
Other	*Unidentified*	flagellates
Other	*Codosiga*	sp.
Other	*Unidentified*	amoeba
Picophytoplanktonic cells	*Picophytoplanktonic cells*	(cells 1-4 µm)
Picophytoplanktonic cells	*Picophytoplanktonic cells*	(cells 4-6 µm)
Prasinophytes	*Mamiella*	*gilva*
Prasinophytes	*Mantoniella*	*squamata*
Prasinophytes	*Pyramimonas*	sp.
Prasinophytes	*Scourfieldia*	sp.
Prasinophytes	*Tetraselmis*	sp.
Prasinophytes	*Nephroselmis*	*pyriformis*
Prasinophytes	*Cymbomonas*	*tetramitiformis*
Prasinophytes	*Mamiella*	sp.
Prymnesiophytes	*Chrysochromulina*	sp.
Prymnesiophytes	*Calciopappus*	*caudatus*

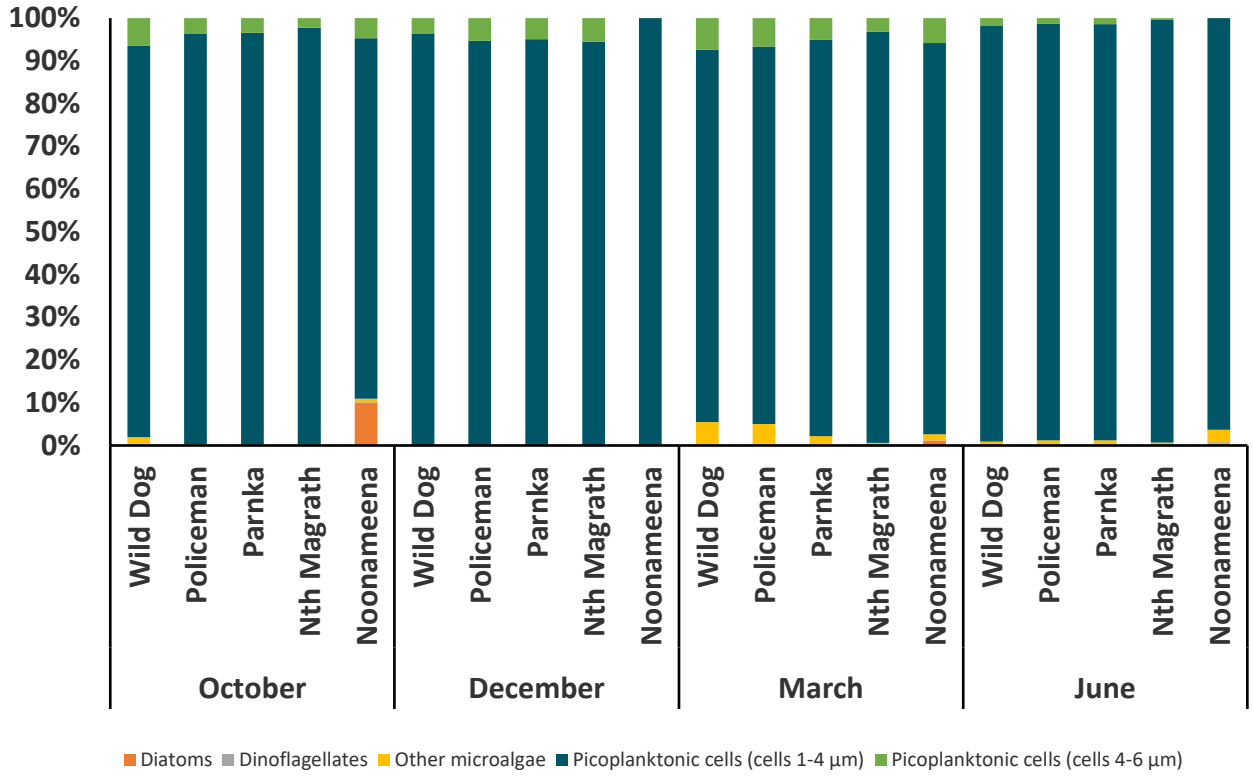

Figure 11. Communities of planktonic microalgae present at the five sampling sites in October 2020, December 2020, March 2021 and June 2021. Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. Diatoms (orange), Dinoflagellates (grey), other microalgae cells (yellow), Picophytoplanktonic cells (4-6µm; green), and Picophytoplanktonic cells (1-4µm; blue).

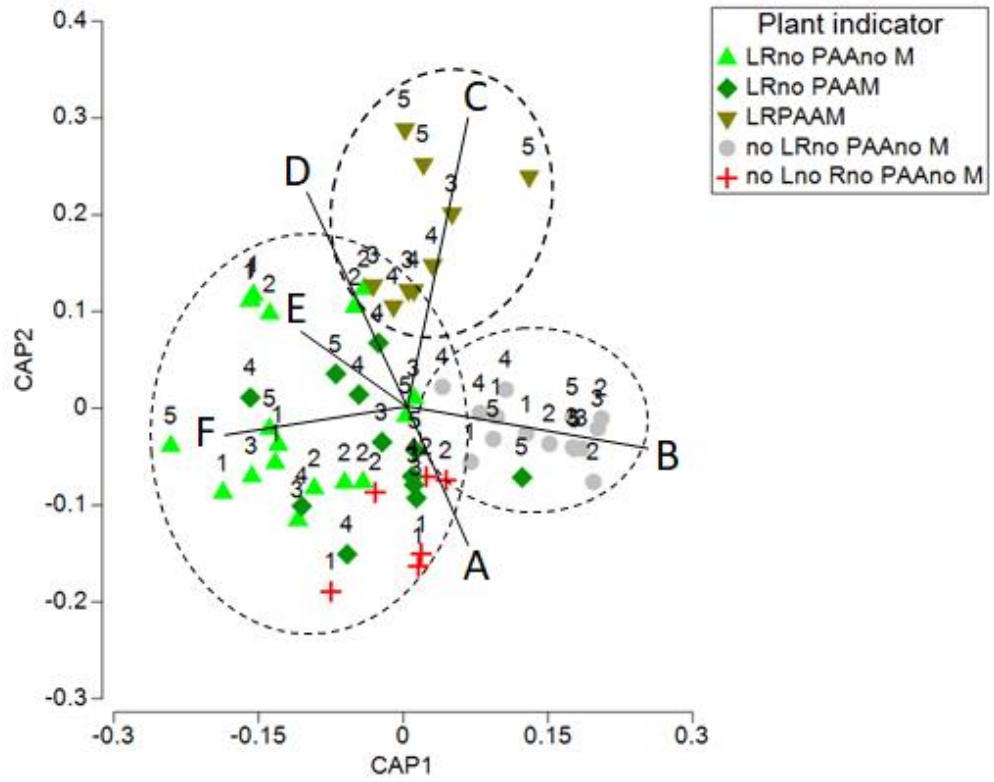

Figure 12. Canonical Analysis of Principal Coordinates (CAP) ordination plot of the communities of planktonic microalgae present at the five sampling sites in October 2020, December 2020, March 2021 and June 2021. Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. Pearson correlation vectors (r > 0.5) represent the microalgae species correlating to the 2 axes and potentially explaining the differences among groups. These are (A) *Mamiella gilva* and *Ochromonas* sp., (B) *Chlamydomonas/Dunaliella*, *Chrysamoeba* and unidentified flagellates, (C) *Haslea ostrearia*, *Mamiella* sp., *Cryptomonas* sp., *Chroomonas* sp., (D) *Levanderina fissa*, I *Diplosalid* sp., and (F) *Mastogloia* sp. and *Pseudanabaena* sp. Plant conditions are characterised by the presence/absence of leaves and roots (LR/no L no R), plant-associated aggregates (PAA/ no PAA), and floating mats of filamentous algae (M/no M) in which the plants get embedded. When whole plants are present (green symbols), when no plant material is present (red cross), or when only roots/rhizomes are present (grey circles).

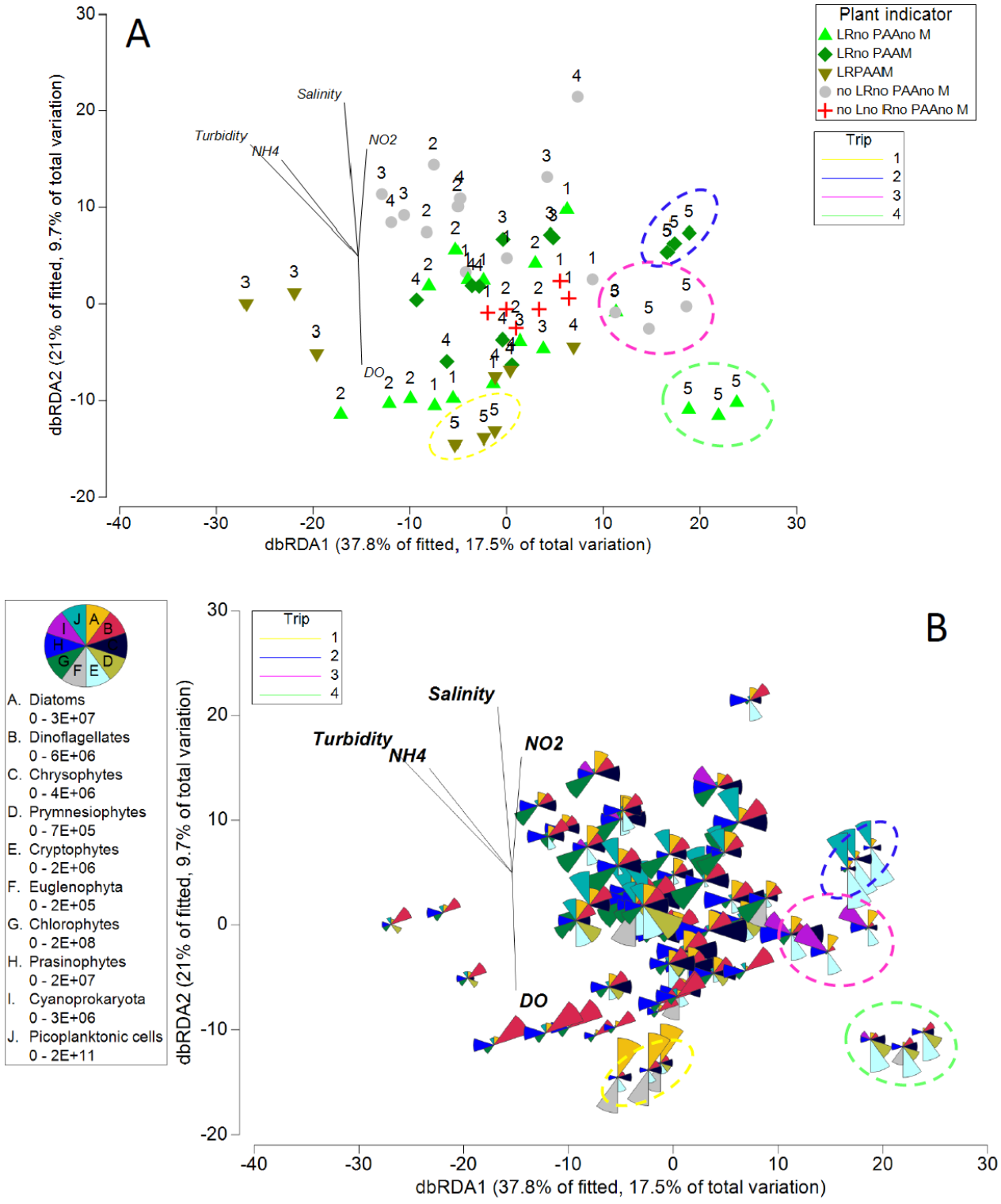

Figure 13. Distance Based Redundancy Analysis (dbRDA) of the communities of planktonic microalgae in relation to water quality changes across the sampling sites over the duration of the study. The vectors (Pearson correlation) represent the water quality parameters correlating to the dbRDA axes (r > 0.5). The temporal sampling for site 5 is indicated by the circles as October 2020 (Trip 1, yellow), December 2020 (Trip 2, blue), March 2021 (Trip 3, pink) and June 2021 (Trip4, green). (A) The ordination of the communities of planktonic microalgae present at each sampling site (1-5) is labelled according to the plant condition at the time of sampling. Plant conditions are characterised by the presence/absence of leaves and roots (LR/no L no R), plant-associated aggregates (PAA/ no PAA), and floating mats of filamentous algae (M/no M) in which the plants get embedded. (B) The different groups of microalgae have been used to illustrate the variability in community composition according to the sampling sites, but also in relation to panel A that shows both the site number and the plant conditions for the ordination.

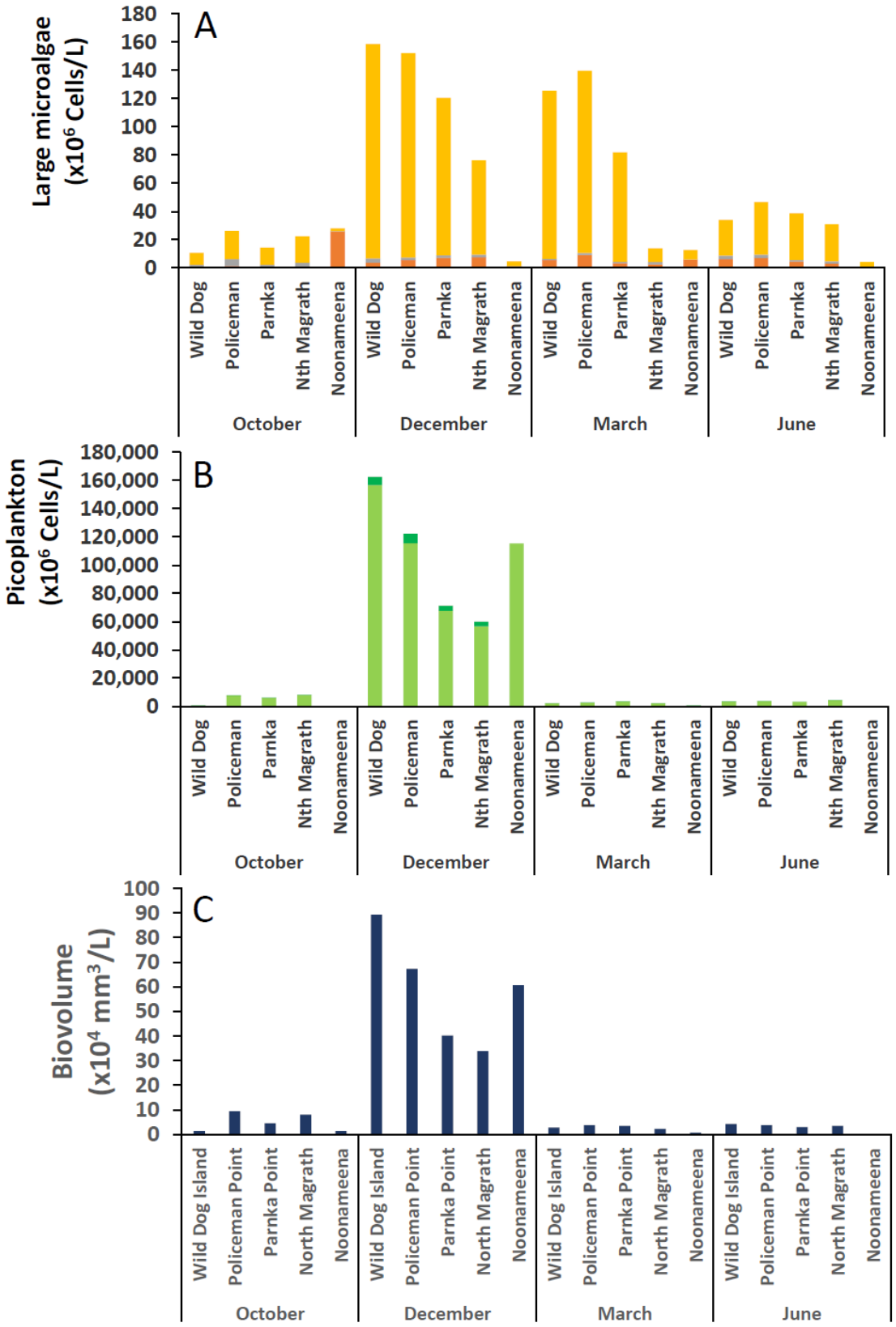

Figure 14. Communities of microalgae and biovolumes at the five sampling sites in October 2020, December 2020, March 2021 and June 2021. Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. (A) Large microalgae (>5µm): Diatoms (orange), Dinoflagellates (grey), and other microalgae cells (yellow). (B) Picophytoplankton: 1-4µm (bright green), and 4-6 µm (dark green). (C) Biovolume equivalent of the whole community of microalgae (picoplankton and large microalgae). Note the differences in y-axis scales.

The identification of microalgae revealed the presence of genera that contains Harmful Algae Bloom species (HABs; Table 7). Due to limited funding, the identification of the microalgae did not have a specific focus on HABs identification to species level. The information presented here is based on the IOC UNESCO Taxonomic Reference List of Harmful micro algae (Lundholm et al. 2009; http://www.marinespecies.org/hab and http://www.algaebase.org) and on the U.S. National Office for Harmful Algal Blooms (Guiry and Guiry 2022; https://hab.whoi.edu/). Among the 81 microalgae identified in this study, 11 genera or species have been listed as potentially harmful: *Cochlodinium* sp., Gymonodinoids, *Leptolyngbya* sp., *Levanderina fissa*, *Limnothrix* sp. (filaments), *Nitzschia* sp., *Pseudanabaena* sp., *Pseudanabaena limnetica*, and *Takayama* sp. (Figure 17). *Pseudanabaena limnetica* was the most abundant HABs recorded and observed at Policeman Point and Parnka Point in March 2021. The only HABs to have formed a bloom during the study was *Nitzschia* sp. at Noonameena in October 2020; *Nitzschia* sp. was present at all sites and during each sampling period. It is worth noting that while the Gymonodinoids did not form a bloom during the period of study, they were present at all sites and during each sampling period and their abundance (20,000 – 2.8 10^6 cells/mL) was always higher than the recommended level of 5,000 cells/L (Table 17).

The Cyanobacteria *Leptolyngbya* sp., *Limnothrix* sp. (filaments), *Pseudanabaena* sp., and *Pseudanabaena limnetica* have been shown to produce Geosmin and 2-methylisoborneol (2-MIB). The presence of metabolites such as Geosmin and 2-methylisoborneol (2-MIB) in water is associated with the muddy or musty odour and taste that can be observed in fish caught in these waters.

Communities of HABs were significantly different between samplings sites and seasons (PERMANOVA $p<0.001$) and based on the presence/absence of plants at each site or based on the plant health, i.e. presence of leaves (PERMANOVA $p<0.001$).

Table 7. List of potentially Harmful Algae Bloom species (HABs) and genera identified in the samples throughout the period of study. Listing is based on information from Lundholm et al. (2009) and Guiry and Guiry (2022). Trigger levels are based on guidelines for recreational waters from the NHMRC (NHMRC Guidelines for Managing Risks in Recreational Water 2008) and Chorus and Walker (2021). Current levels are based on this study.

CLASS	GENUS	SPECIES	TOXICITY	TRIGGER LEVEL	CURRENT LEVEL
DIATOMS					
BACILLARIOPHYCEAE	*Nitzschia*	*species*	Many species are reportedly non-toxic. Some species produce domoic acid, which can cause Amnesic shellfish poisoning	There are no known specific trigger levels	50,000 – $2.3x10^7$ cells/mL
DINOFLAGELLATES					
DINOPHYCEAE	*Cochlodinium*	*species*	Potential toxin producing species; has been associated with fish kills in Australia and overseas	There are no known specific trigger levels	100,000 cells/mL
DINOPHYCEAE	*Gymnodinium*	*species*	Many species are reportedly non-toxic. High abundances of some Gymonodinium species are linked to red tide events that can lead to anoxic conditions. *Gymnodinium catenatum* produces toxins that can lead to paralytic shellfish poisoning.	*Gymnodinium catenatum*: trigger level for flesh testing in mussels is 1,000 cells/L and 2,000 cells/L for other shellfish. Closure of harvesting areas is recommended at 5000 cells/L	20,000 – $2.8x10^6$ cells/mL
DINOPHYCEAE	*Levanderina*	*fissa*	Non-toxic species that causes massive red-tides potentially leading to anoxic conditions	There are no known specific trigger levels	5,000 – $2x10^5$ cells/mL
DINOPHYCEAE	*Takayama*	*species*	Takayama species have been implicated in fish/shellfish mortality, toxin production is unknown	There are no known specific trigger levels	15,000 cells/mL
CYANOBACTERIA					
CYANOPHYCEAE	*Leptolyngbya*	*species*	Some species produce Nodularin	No specific trigger levels except for the prevention of cyanotoxin in recreational waters: 5,000 cells/mL of known toxin producing species (skin irritations) and $1.25x10^5$ cells/mL of total cyanobacteria cells when known toxins are not present but scum is present (ingestion is risk).	50,000 cells/mL
CYANOPHYCEAE	*Limnothrix*	*species*	Some species within these three genera of cyanobacteria have been reported to produce Geosmin and 2-methylisoborneol (2-MIB)		20,000 – 50,000 cells/mL
CYANOPHYCEAE	*Pseudanabaena*	*species*	Some species produce Microcystin		$1.8x10^5$ – $1.2x10^6$ cells/mL

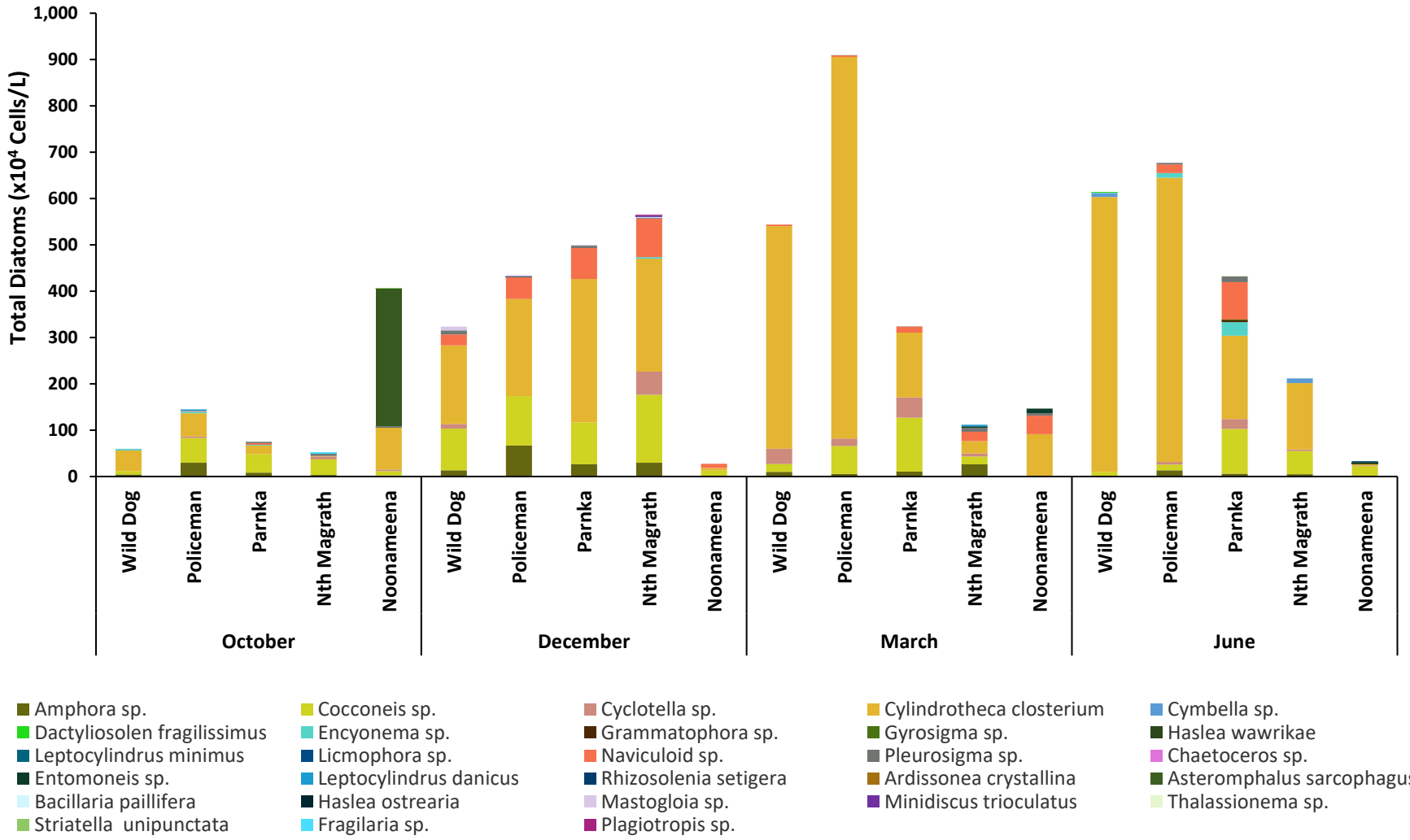

Figure 15. Diatoms present at the five sampling sites in October 2020, December 2020, March 2021 and June 2021. Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena.

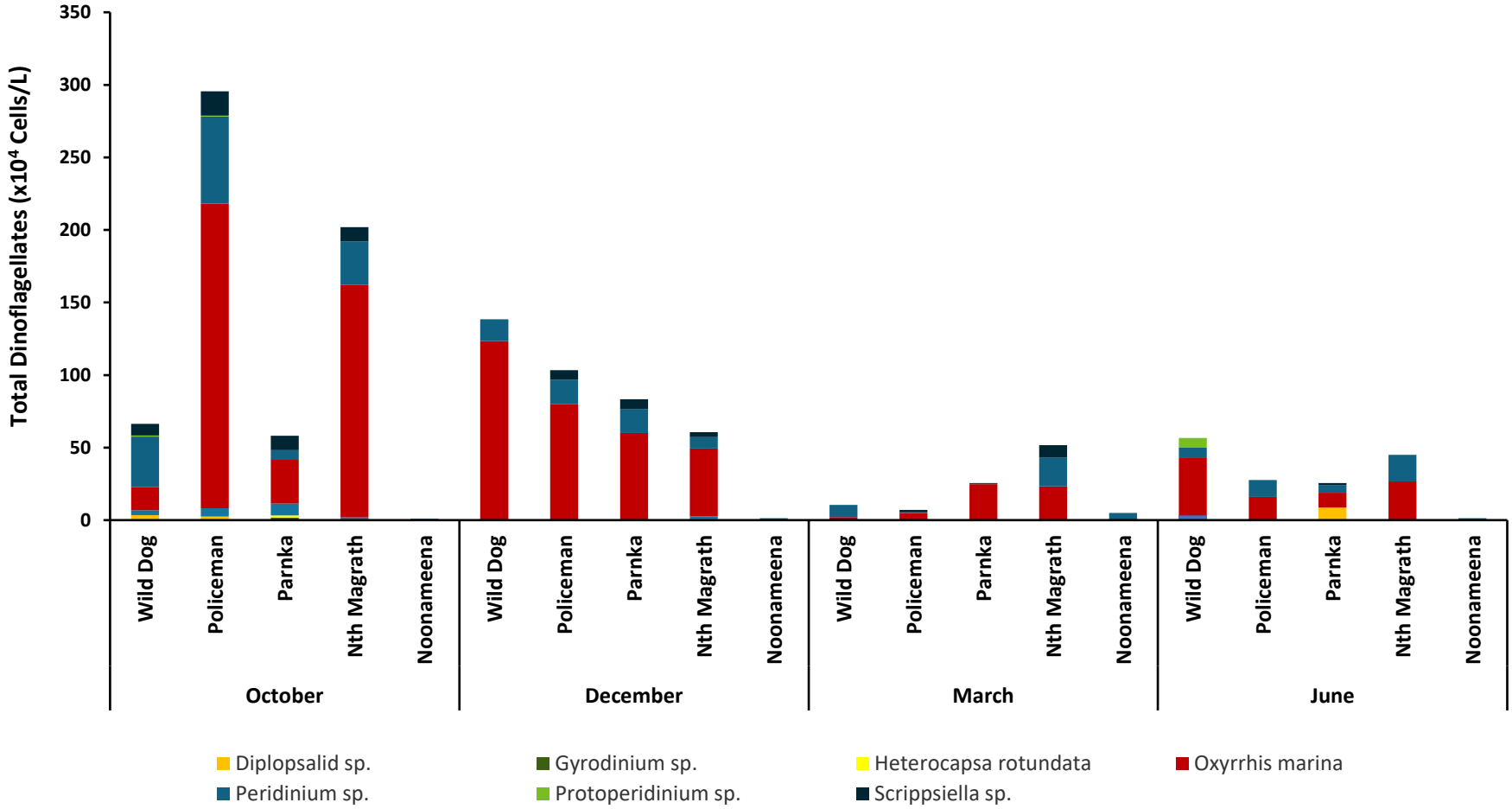

Figure 16. Dinoflagellates present at the five sampling sites in October 2020, December 2020, March 2021 and June 2021. Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena.

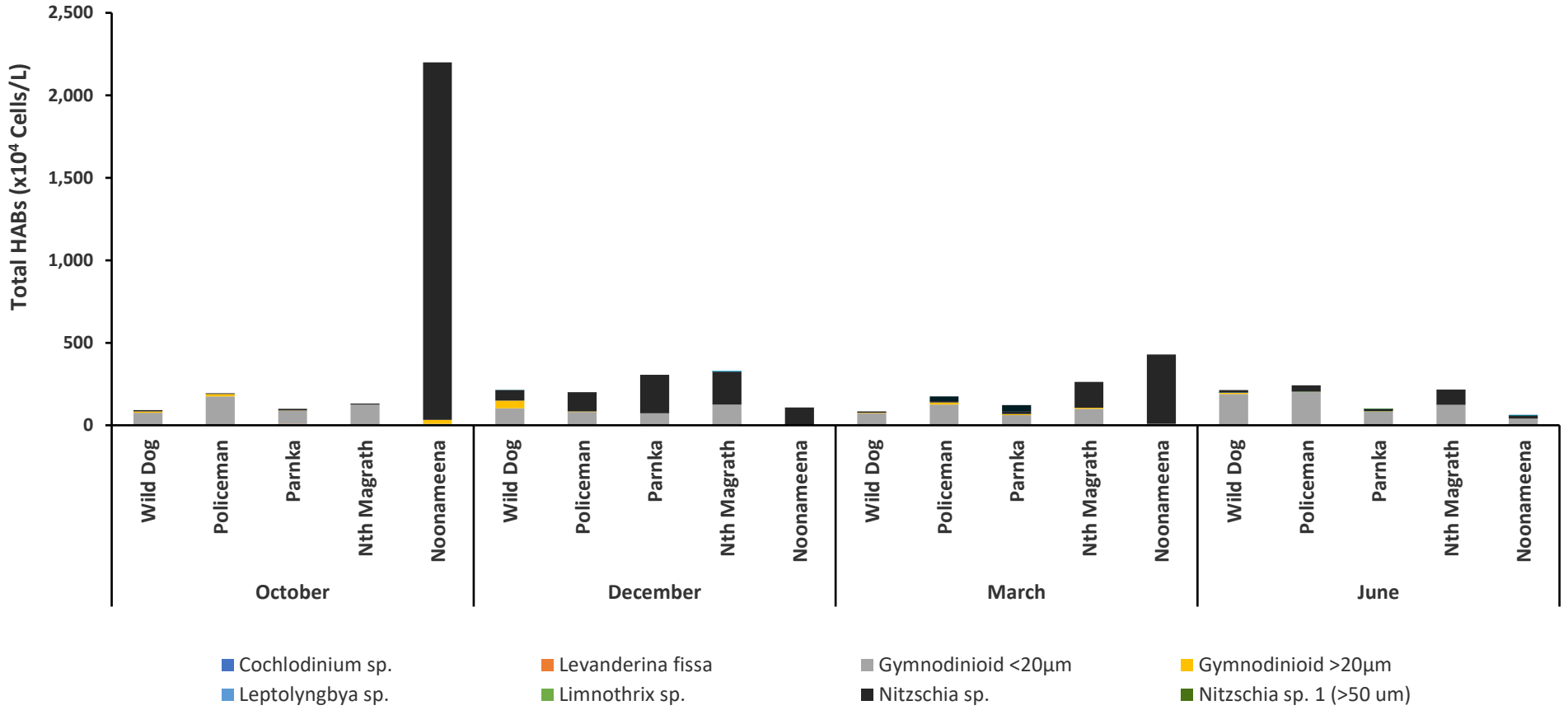

Figure 17. Harmful Algae Bloom species (HABs) present at the five sampling sites in October 2020, December 2020, March 2021 and June 2021. Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena.

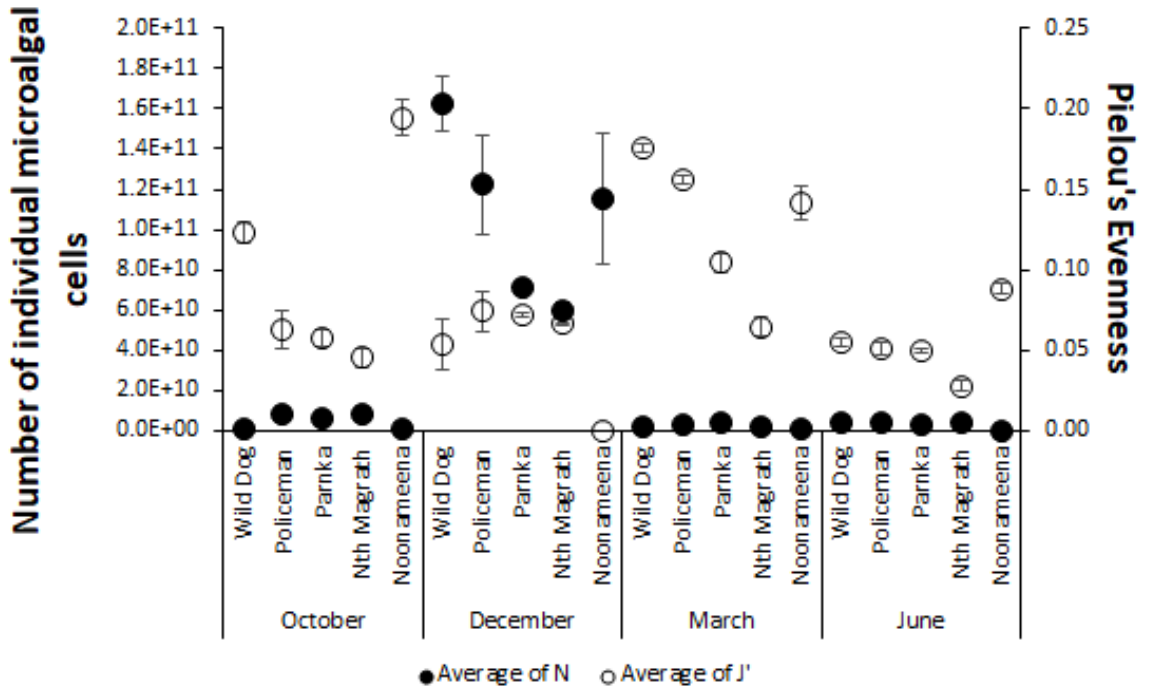

Figure 18. Diversity indices: Total number of individual microalgal cells (N; black circle) and Pielou's evenness (J'; open circle), calculated for the communities of planktonic microalgae present at the five sampling sites in October 2020, December 2020, March 2021 and June 2021. Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. Error bars represent the standard deviation to the mean.

3.3 Microbiota and nutrients in the southern Coorong

The water column microbiota (i.e. Picophytoplankton and large microalgae) highlighted that the microbiota community contributed more than half of the pelagic (water column) nutrients (TN and TP) throughout the period of study, except at Noonameena (Figure 19). However, in Spring (October 2020), the microbiota accounted for up to 85% of water column TN and 90% of water column TP across sites 1 (Wild Dog Island) to 4 (North Magrath Flats), but only accounted for 69% of water column TN and 1% of water column TP at Noonameena.

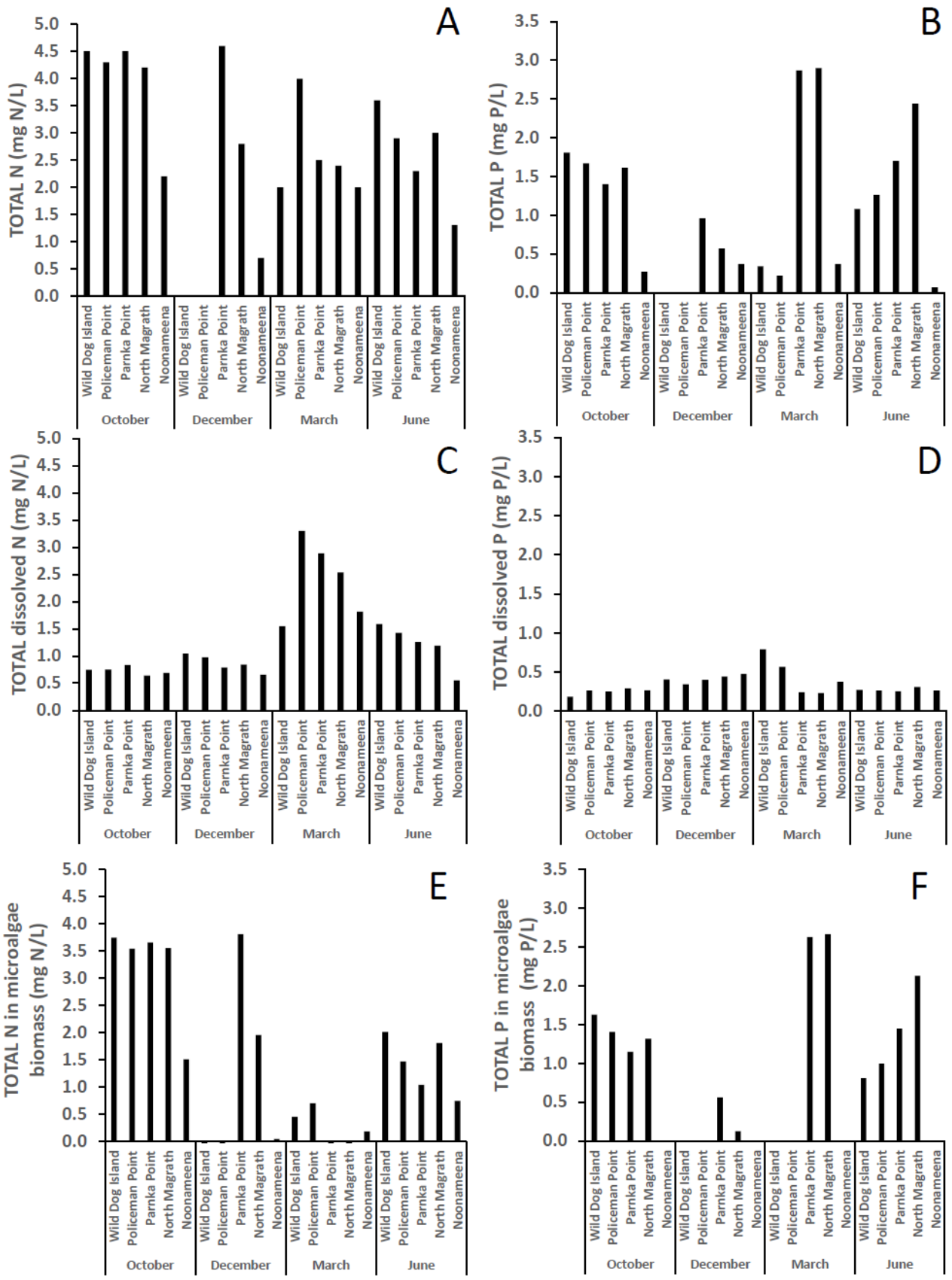

Figure 19. Nutrients (Total N and Total P) associated to the microbiota (i.e. Picophytoplankton and large microalgae) at each sampling site over the period of study. (A,B) Total nutrient concentrations, (C,D) Total dissolved nutrient concentrations, and (E,F) Total nutrient concentration associated to the microalgae community. Data for total N and total P were not available for Wild Dog Island and Policeman Point in December 2020. Note the differences in y-axis scales.

3.4 Microbial communities

Significant differences were observed between most sample types (PERMANOVA p<0.001, Table 8, Figure 20) and sites (PERMANOVA p<0.001, Figure 20).

The results of the SIMPER analysis were used to illustrate the dissimilarities in community composition on the PCoA plot (Figure 20B). SIMPER allowed the identification of 20 classes of Archaea and Bacteria that were driving the dissimilarities between water, sediment, *Ruppia* Community leaves and *Ruppia* roots samples as: Anaerolineae, Bacilli, BD2-11, Calditrichia, Cyanobacteria, Desulfovibrionia, Fusobacteria, Halobacteria (Archaea), Halanaerobiia, Kapabacteria, Latescibacteria, Phycisphareae, Rhodothermia, unclassified NB1-j, unclassified Latescibacteria, unclassified Proteobacteria, unclassified Zixibacteria and Verrrucomicrobiae.

Table 8. Results of the PERMANOVA analysis of microbial communities (Archaea and Bacteria) between sample types (for all sites and sampling times). The results below show P(perm) <0.05 significant differences between water, sediment, *Ruppia* Community (leaves, and roots) and other material such as filamentous algae mats, plant associated aggregates (PAA) and floating filamentous algae mats microbiota (Archaea and Bacteria).

GROUPS	T	P(PERM)
WATER, SEDIMENT	9.9837	0.0001
WATER, LEAVES	3.7298	0.0001
WATER, ROOTS	6.3552	0.0001
WATER, MATS	4.5263	0.0001
WATER, PAA	2.7108	0.0001
WATER, FLOATING	2.297	0.0003
SEDIMENT, LEAVES	3.4497	0.0001
SEDIMENT, ROOTS	5.545	0.0001
SEDIMENT, MATS	3.911	0.0001
SEDIMENT, PAA	2.1019	0.0001
SEDIMENT, FLOATING	2.2885	0.0002
LEAVES, ROOTS	1.7141	0.0001
LEAVES, MATS	2.3728	0.0001
ROOTS, MATS	2.2014	0.0001
ROOTS, FLOATING	1.4766	0.0006
MATS, FLOATING	1.4976	0.0189

The relationship between the identified communities of Archaea and Bacteria and the water quality parameters (used as predictor variables) is presented in Figure 21. This analysis considered the water, sediment and *Ruppia* Community samples collected for 16S rRNA sequencing of the communities of Archaea and Bacteria. The differences observed between sites are explained by the variations in salinity and ammonium (BEST, p<0.01).

The communities of archaea and bacteria associated to the different sample type (Figure 22) showed significant differences in diversity (Pielou's evenness). The diversity in sediment samples was significantly higher (ANOVA, p<0.01) than observed in the water, leaves and root samples. In contrast, the diversity in water samples was significantly lower (ANOVA, p<0.01) than all other sample types.

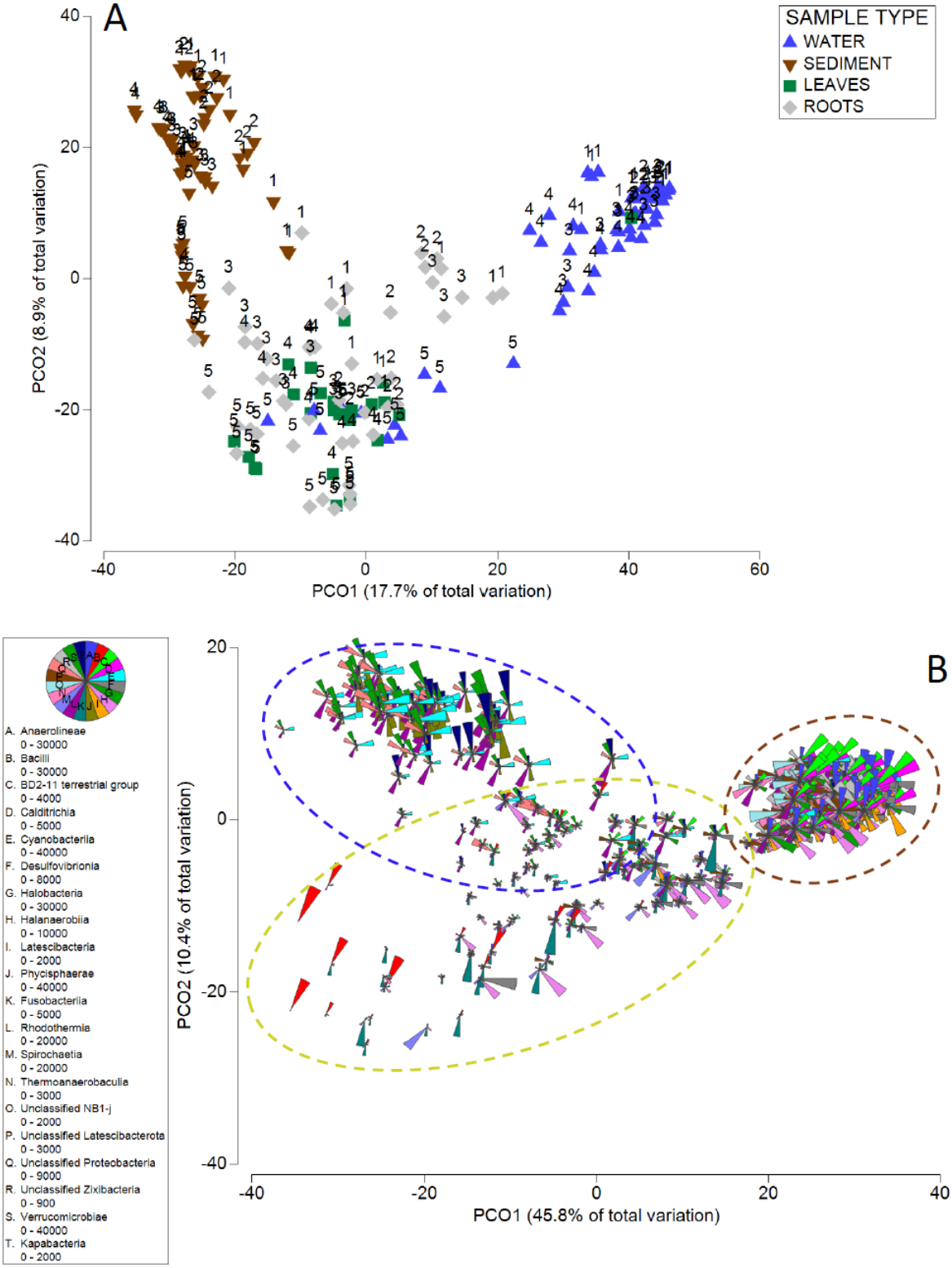

Figure 20. Principal Coordinates analysis (PCoA) of the communities of the kingdoms of Archaea and Bacteria detected in samples of water, sediment, *Ruppia* Community and filamentous algae material (i.e. mats, PAA and floating material) from 16S rRNA sequencing across the sampling sites over the duration of the study. (A) The five sampling sites during the study (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. (B) Communities were analysed at Class level and the classes explaining most of the dissimilarities (SIMPER analysis) between sample types are illustrated. Water column (blue dotted line), sediment (brown dotted line) and *Ruppia* Community (green dotted line) samples are indicated by the circles on the diagram.

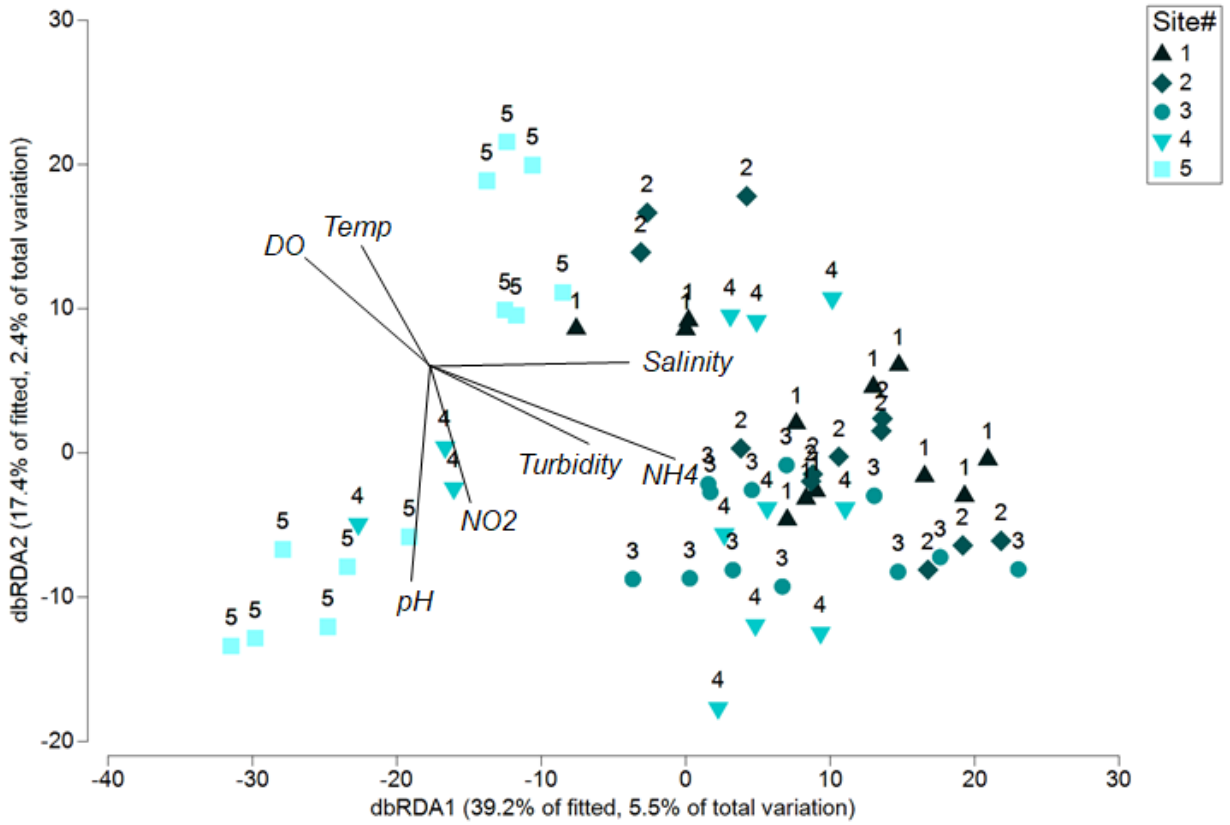

Figure 21. Distance Based Redundancy Analysis (dbRDA) of the communities of the kingdoms of Archaea and Bacteria in relation water quality changes at the five sampling during the study (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. The vectors (Pearson correlation) represent the water quality parameters correlating to the dbRDA axes (r > 0.5).

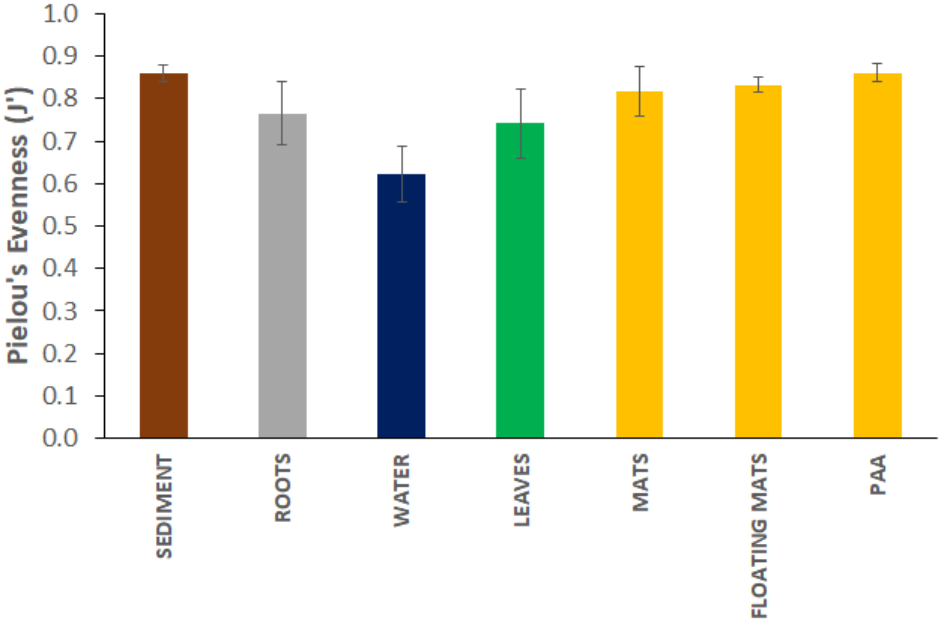

Figure 22. Pielou's evenness calculated for the communities of archae and bacteria present in each sample type (i.e. sediment, *Ruppia* roots, water, *Ruppia* leaves, plant associated mats, floating filamentous algae mats, and plant associated aggregates (PAA)) at each sampling site. Error bars represent the standard deviation to the mean.

A majority of the amplicon sequence variants (ASVs) characterised (for reference https://lifemap-ncbi.univ-lyon1.fr) across all sample types from each site are common in the North Lagoon, Central Coorong, and South Lagoon with only 18 ASVs being lagoon location specific (Figure 23A). The ASVs from archaea and bacterial groups detected in different sampling periods were found to comprise 170 common phyla with only 17 phyla unique to each trip (Figure 23B). Assessing all the samples based on sampling site, 159 archaea and bacterial phyla ASVs are common across all sites and 14 ASVs are unique across the sites (Figure 23C). The common archaea and bacterial phyla across all the sample types were assessed, finding 122 ASVs common and 34 unique ASVs amongst all the sample types (Figure 23D).

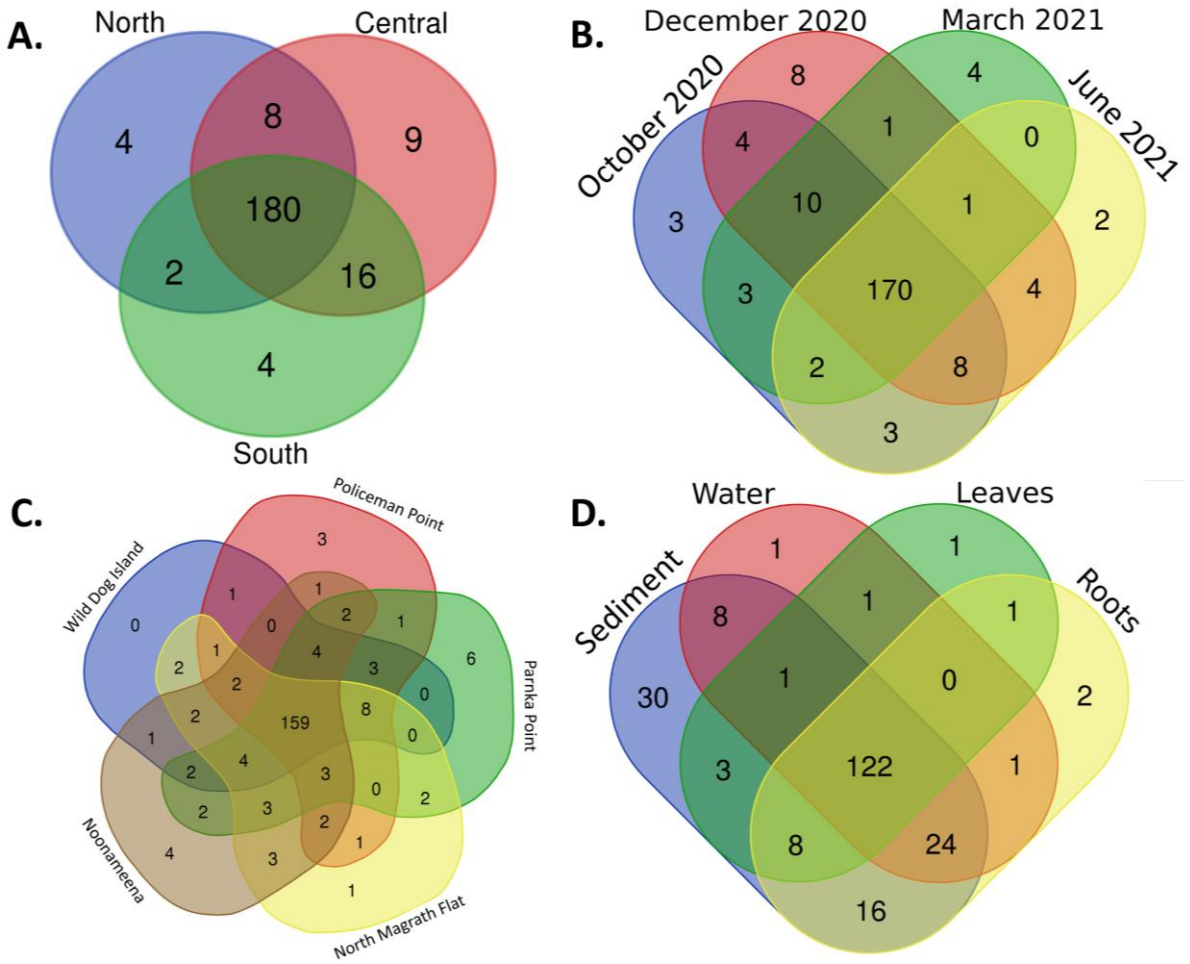

Figure 23. Venn diagrams displaying the overlap between the archaea and bacteria communities in all samples for the (A) lagoon location, (B) trip, (C) sampling site, and (D) sample type during the study period. There were four sampling trips: October 2020 (Trip 1), December 2020 (Trip 2), March 2021 (Trip 3) and June 2021 (Trip 4). There were five sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. The taxa are classified to the order level according to the Silva database (version 138.1).

Across all the sites and sample types (i.e. water, sediment, leaves, roots) the most abundant common classes were identified as γ-proteobacteria, α-proteobacteria, Bacteroidia, and Actinobacteria (Figure 24). Throughout all the sampling, the greatest diversity was present in the sediment. Variation in the archaea and bacterial classes present at the different sites and across the seasons is also evident (Figure 24).

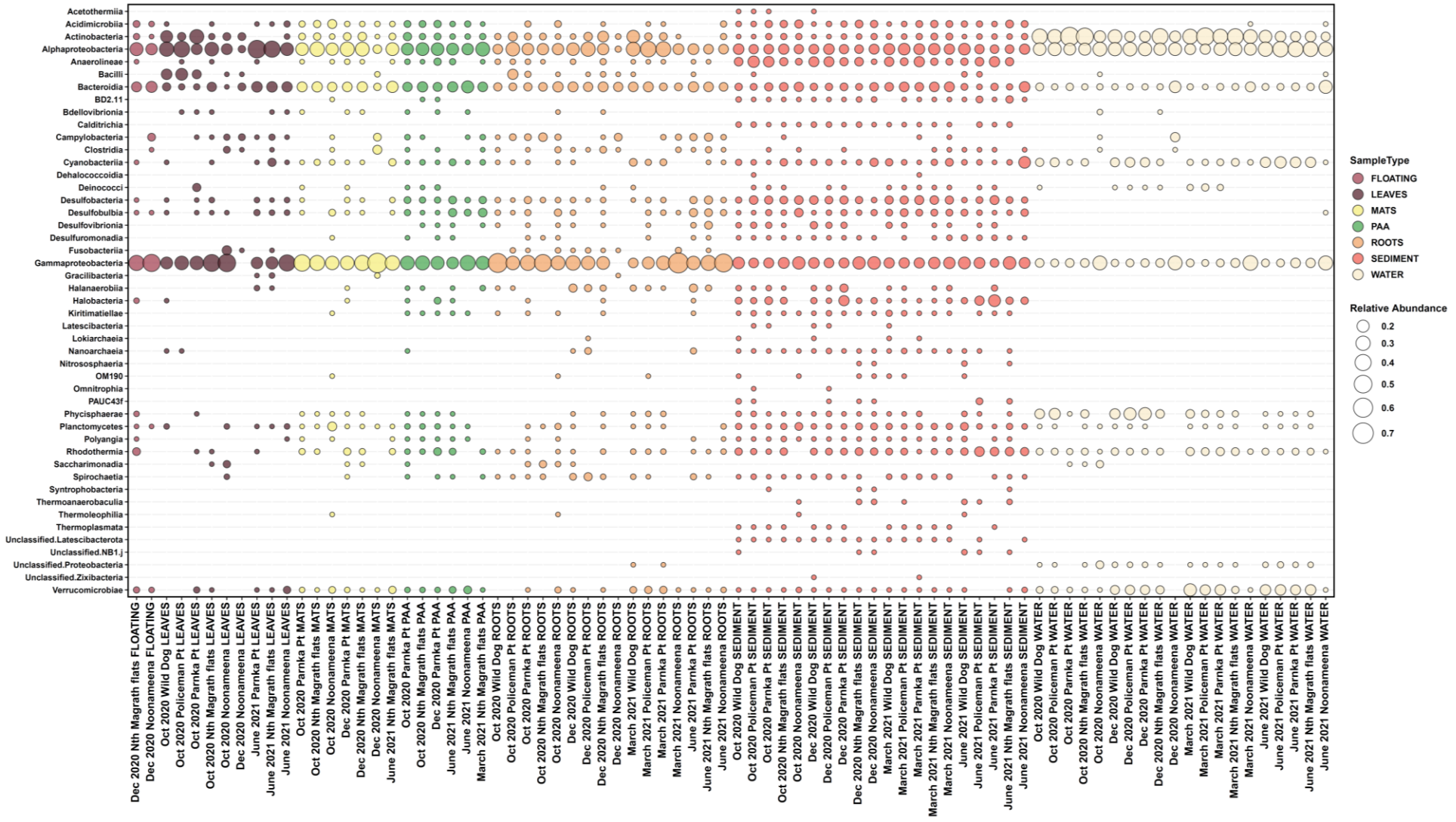

Figure 24. Relative abundance of all the taxa that comprise of more than 1% of the archaea and bacterial sequences in each sample type i.e. water, sediment, *Ruppia* Community and filamentous algae material (i.e. mats, PAA and floating material) from 16S rRNA sequencing at the five sampling sites in October 2020, December 2020, March 2021 and June 2021. Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. The taxa are classified to the class level according to the Silva database (version 138.1).

3.4.1 Microbial communities in water

Samples were collected to analyse the microbiota present in the water column at all sites as identified from the sequencing 16S rRNA gene. Within water samples collected from the five sampling sites, 19 archaea and bacteria classes were identified. The dominant bacterial phyla were Actinobacteria, α-proteobacteria, γ-proteobacteria, and Verrucomicrobiae at all of the sites (Figure 25). With the community structure consistent throughout the study, although some seasonal variation is evident. Seasonal variation is more distinct in the Central Coorong and South Lagoon (Figure 25).

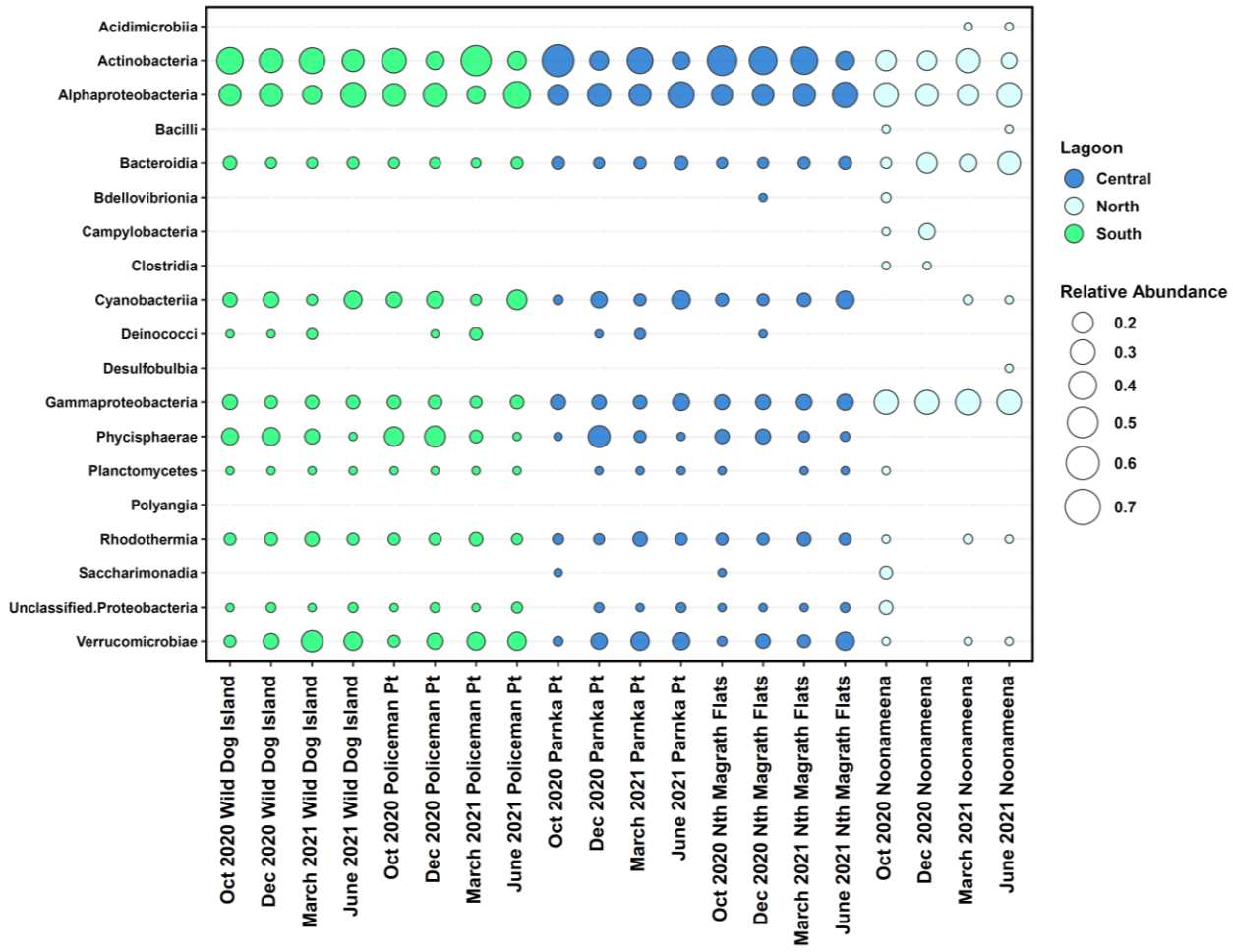

Figure 25. The relative abundance of all the taxa that comprise greater than 1% of the archaea and bacterial sequences in the water at the five sampling sites in October 2020, December 2020, March 2021 and June 2021. Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. The taxa are classified to the class level according to the Silva database (version 138.1).

Proteobacteria, which dominated the communities at Noonameena, are a major phylum of Gram-negative bacteria. In contrast, the phylum of Planctomycetes which was less abundant especially at Noonameena compared to the other sampled sites, is widely distributed throughout both aquatic and terrestrial environments (Figures 25). Members of phylum Verrucomicrobia were present at all the sampled sites except Noonameena in December 2020, which exhibit the ability to adapt to oligotrophic environments (Figure 25).

Canonical analysis of principal coordinates (CAP) based on Bray-Curtis distance ordination was used to plot the discriminations of the 45 samples from the five sites in the Coorong (Figure 26A). Distinctive spatial distributions patterns are evident reflecting the impact of conditions at specific sampling sites on the communities. For example, the CAP1 axis separates site 5 (Noonameena) from the rest of the samples (correlation Δ^2 0.999); whereas CAP2 separates sites 1 and 2 in the South Lagoon (Wild Dog Islands and

Policeman Point) from sites 3 (Parnka Point) (Central Coorong) and 4 (North Magrath Flats) in the North Lagoon (correlation Δ^2 0.947). The differences observed between sites are explained by the variations in temperature, salinity, turbidity, and ammonium (BEST, Spearman ρ=0.475, p<0.01).

A CAP was also used to assess the seasonal changes observed within the microbial communities in the Coorong water (Figure 26B). Distinctive seasonal patterns are evident on the CAP plot, reflecting the impact of seasonal conditions on the communities. For example, the CAP1 axis separates samples collected during the reproductive period of members of the *Ruppia* Community (October and December 2020) from samples collected during the Aestivation period (March and June 2021; (correlation Δ^2 0.989); whereas CAP2 separates trip dates within these periods (correlation Δ^2 0.974). Several ASVs were highly correlated (r>0.7) to the CAP axes i.e. the phylum Bacteroidota and Verrucomicrobiota with CAP1 and the phylum Deionococcei, nanoarchaeia and α-proteobacteria (Rickettsiales) with CAP2.

In this study, the common core of archaea and bacteria within five sites of the Coorong was analysed using the ASV data obtained from sequencing (Figure 27). The core organisms of the order taxa for each site are shown in Table 9. Our results showed that the populations over all the sampling periods are stable over time with some variation associated with seasonality, reflecting changes in water quality.

Table 9. The number of core and seasonal organisms identified in the water at the five sampling sites in October 2020, December 2020, March 2021 and June 2021. Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. The taxa are classified to the class level according to the Silva database (version 138.1).

SITE	CORE ORGANISMS	SEASON SPECIFIC
Wild Dog Island	90	67
Policeman Point	92	80
Parnka Point	84	82
North Mcgrath Flat	80	57
Noonameena	110	74

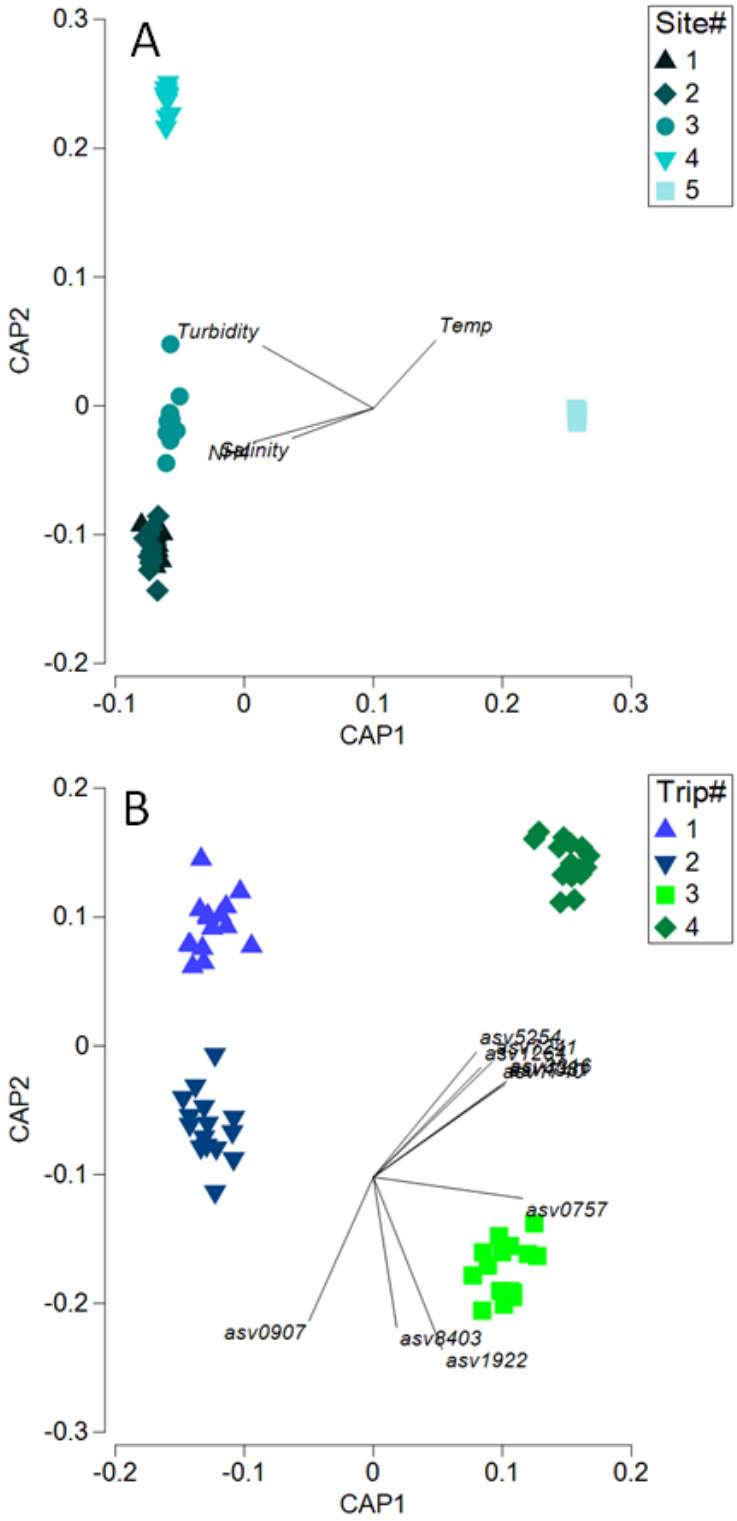

Figure 26. Canonical Analysis of Principal Coordinates (CAP) ordination plot of the communities of the kingdoms of Archaea and Bacteria detected in water samples collected water at the five sampling sites in October 2020 (Trip 1), December 2020 (Trip 2), March 2021 (Trip 3) and June 2021 (Trip 4). Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. (A) The discrimination of communities between sites is clearly visible. Pearson correlation vectors ($r > 0.4$) represent the water quality parameters driving the discrimination between samples. (B) The discrimination of communities between sampling dates is clearly visible. Pearson correlation vectors ($r > 0.7$) represent the main ASVs (i.e. asv0907 Rhodothermia, asv8403 and asv3316 α-proteobacteria, asv1922 γ-proteobacteria, asv0757 and asv1740 Verrucomicrobiota, asv5254 and asv1264 Bacteroidota, asv7241 nanoarchaeota) driving the discrimination between samples.

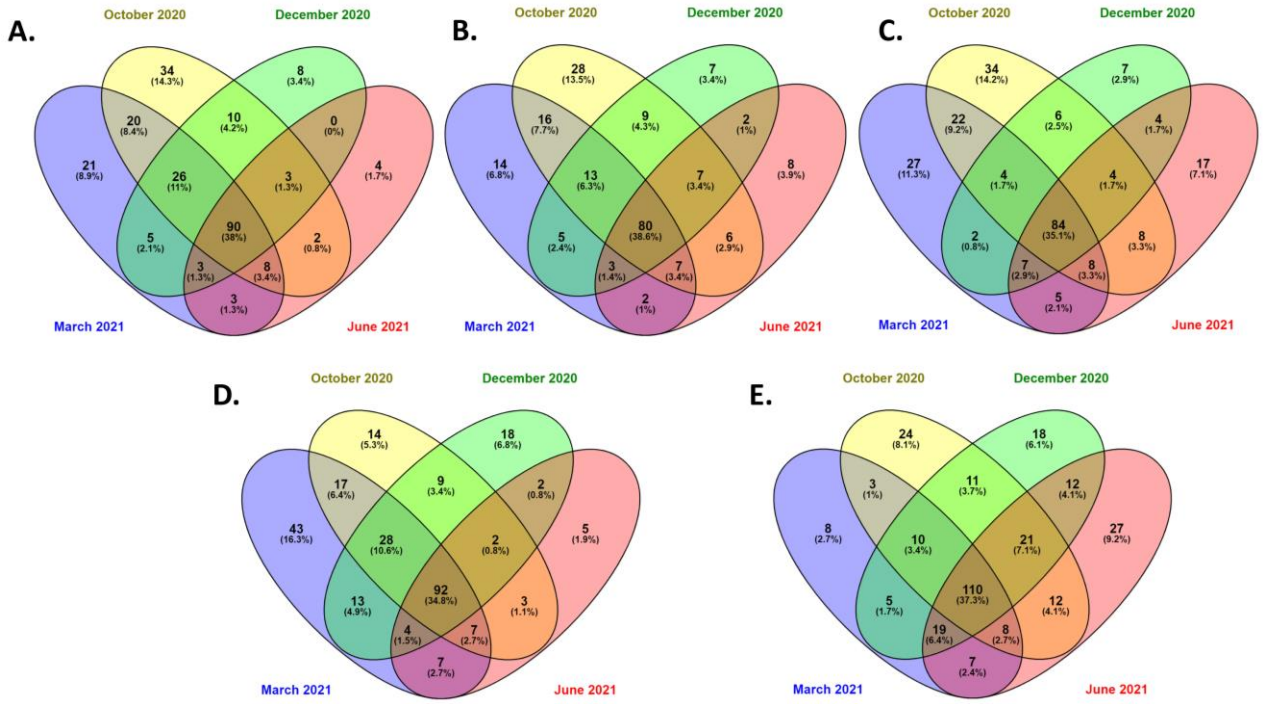

Figure 27. Venn diagrams displaying the overlap between the archaea and bacteria communities in the water at A) Wild Dog Islands, B) Policeman Point, C) Parnka Point, D) North Mcgrath Flat, and E) Noonameena in October 2020 (Trip 1), December 2020 (Trip 2), March 2021 (Trip 3) and June 2021 (Trip 4). The taxa are classified to the class level according to the Silva database (version 138.1).

3.4.2 Microbial communities in sediment

Within the sediment samples collected from the five sampling sites in October 2020, December 2020, March 2021, and June 2021, there were 42 archaea and bacteria taxa identified. At all of the sites the dominant bacterial classes were γ-proteobacteria, α-proteobacteria, Anaerolineae, Bacteroidia, and Desulfobacteria (Figure 28). The dominant communities identified the different sampling sites are consistent throughout the study period. The community composition of Noonameena displayed lower abundance of the dominant classes of Anaerolineae and a higher abundance of Bacteroidia.

While proteobacteria is the most abundant phyla at all sites, some of the other dominant phyla are worth noting, including Desulfobacteria which is capable of growth via sulfate reduction and often isolated from anoxygenic environment. The Anaerolineae phyla have been identified as one of the core populations, responsible for anaerobic digestion by fermentation.

Canonical Analysis of Principal Coordinates (CAP) based on Bray-Curtis distance ordination was used to plot the discriminations of the 60 samples from the five sampling sites in the Coorong (Figure 29). Distinctive spatial distribution patterns are evident reflecting the impact of seasonality within the Coorong on the microbial communities (Figure 29A). For example, the CAP1 axis separates the North Lagoon (Noonameena and North Magrath Flats) and Parnka Point from the South Lagoon (Wild Dog Islands and Policeman Point) samples (eigenvalue 0.9978, correlation Δ^2 0.9957). Whereas CAP2 illustrates the variability between trips at each site (eigenvalue 0.9571, correlation Δ^2 0.9161). The differences observed between sites are explained by the variations in temperature and ammonium (BEST, Spearman ρ=0.265, p<0.01).

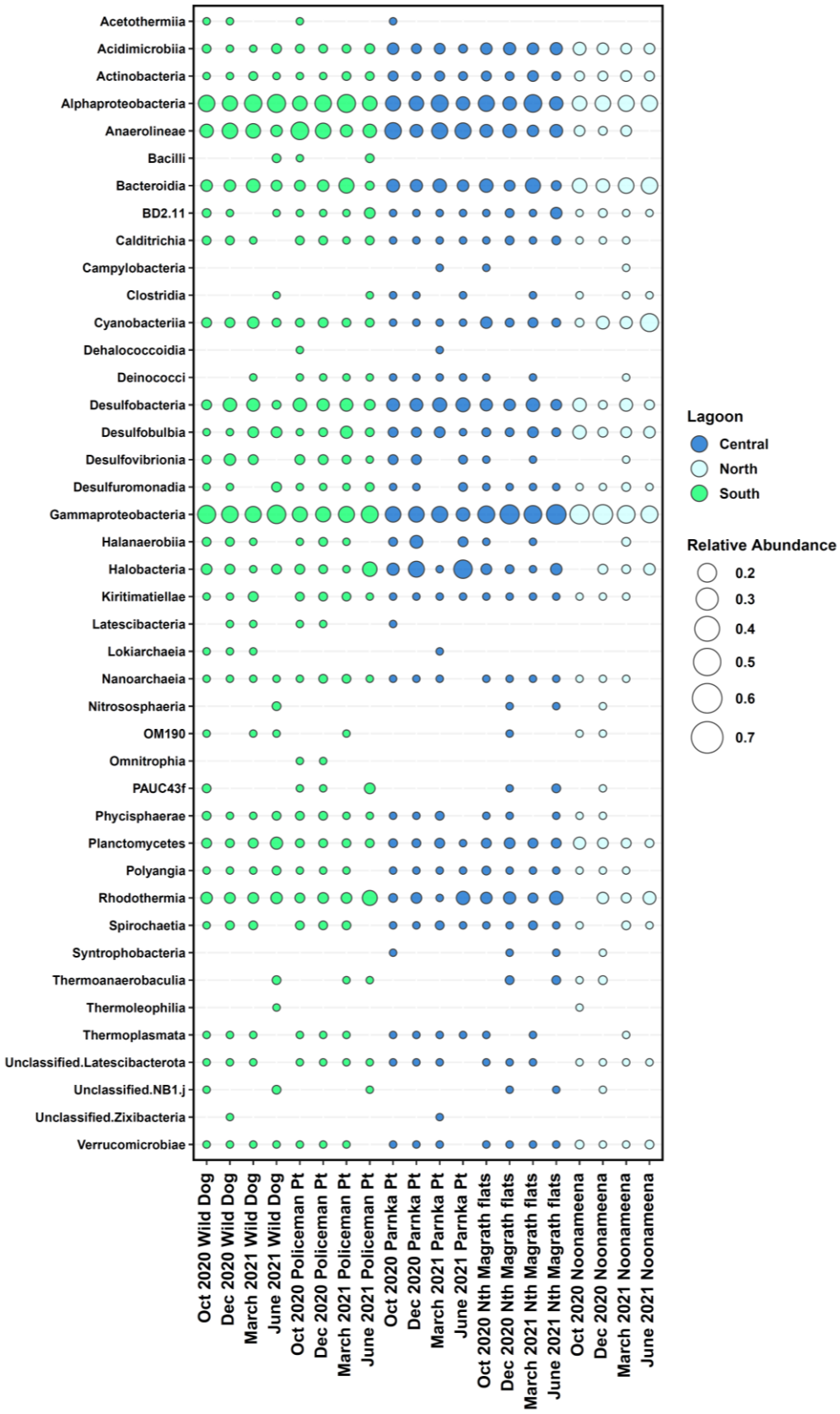

Figure 28. The relative abundance of all the taxa that comprise greater than 1% of the archaea and bacterial sequences in the sediment at the five sampling sites in October 2020 (Trip 1), December 2020 (Trip 2), March 2021 (Trip 3) and June 2021 (Trip 4). Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. The taxa are classified to the class level according to the Silva database (version 138.1).

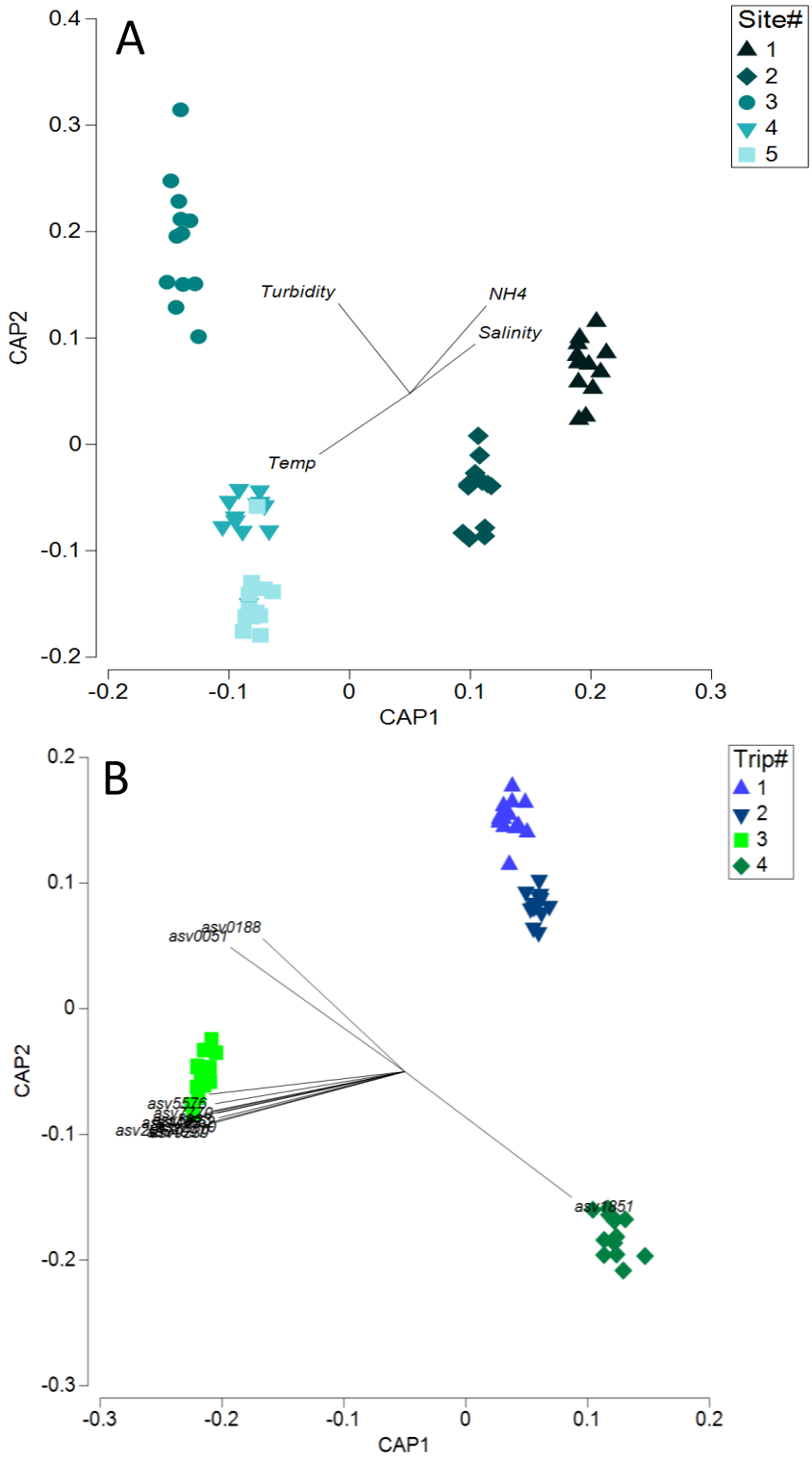

Figure 29. Canonical Analysis of Principal Coordinates (CAP) ordination plot of the communities of phyla in the kingdoms of Archaea and Bacteria detected in sediment samples collected at the five sampling sites in October 2020 (Trip 1), December 2020 (Trip 2), March 2021 (Trip 3) and June 2021 (Trip 4). Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. (A) The discrimination of communities between sites is clearly visible. Pearson correlation vectors (r > 0.3) represent the water quality parameters driving the discrimination between samples. (B) The discrimination of communities between sampling dates is clearly visible. Pearson correlation vectors (r > 0.75) represent the ASVs (i.e. asv0051 and asv1851 Desulfobacterota, asv0188 γ-proteobacteria, asv5576, asv2710, asv3662 and asv2017 Bacteroidota, asv7770 Verrucomicrobiota, asv4663 and asv1440 α-proteobacteria) driving the discrimination between samples.

Similarly, a seasonal pattern is evident in the microbial communities when discriminating samples per sampling trip (Figure 29B). For example, the CAP1 axis separates the sampling undertaken in Summer (trip 3, March 2021) from the other sampling times (correlation Δ^2 0.997). Whereas CAP2 illustrates the variability between trips (correlation Δ^2 0.985). Several ASVs were highly correlated (r>0.75) to the CAP axes i.e. the phylum α-proteobacteria with CAP1 and the phylum Desulfobacterota with CAP2.

In this study, the common core of sediment derived archaea and bacteria within five sites of the Coorong was analysed using the ASV data obtained from sequencing (Figure 30). Table 10 depicts the order taxa that are common core organisms throughout all the sampling times. Here our finding further supports the stability of the community structure within the sediment samples throughout the sampling time period, with seasonality influencing a small fraction of the identified organisms.

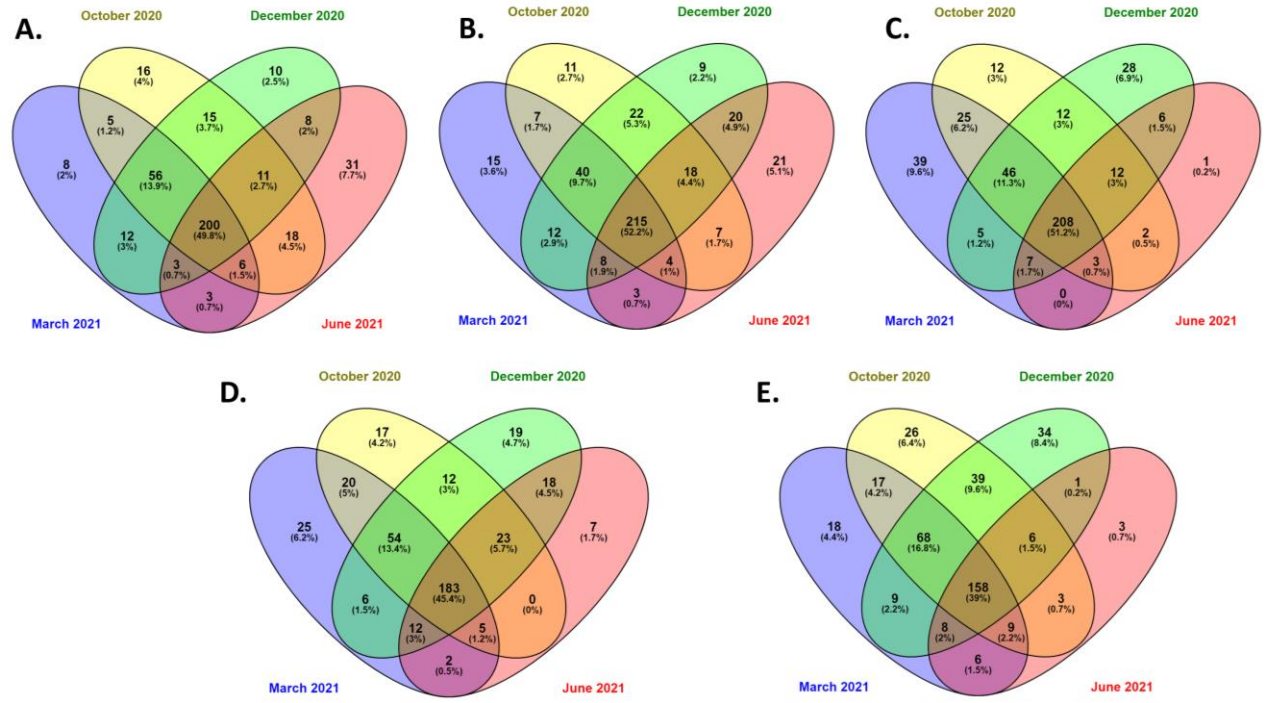

Figure 30. Venn diagrams displaying the overlap between the archaea and bacteria communities in the sediment at A) Wild Dog Island, B) Policeman Point, C) Parnka Point, D) North Mcgrath Flat, and E) Noonameena during the period of study. The taxa are classified to the class level according to the Silva database (version 138.1).

Table 10. The numbers of core and seasonal organisms in the sediment identified at the five sampling sites in October 2020 (Trip 1), December 2020 (Trip 2), March 2021 (Trip 3) and June 2021 (Trip 4). Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. The taxa are classified to the class level according to the Silva database (version 138.1).

SITE	CORE ORGANISMS	SEASON SPECIFIC
Wild Dog Island	200	65
Policeman Point	215	56
Parnka Point	208	80
North Mcgrath Flat	183	68
Noonameena	158	81

3.4.3 Scanning Electron Microscopy trials

Plant samples were prepared and observed with SEM (Figure 31). Filamentous algae aggregates attached to *Ruppia* or *Althenia* plants were also observed under SEM to assess the presence of biofilm at the surface of the plant and/or aggregates (Figure 32).

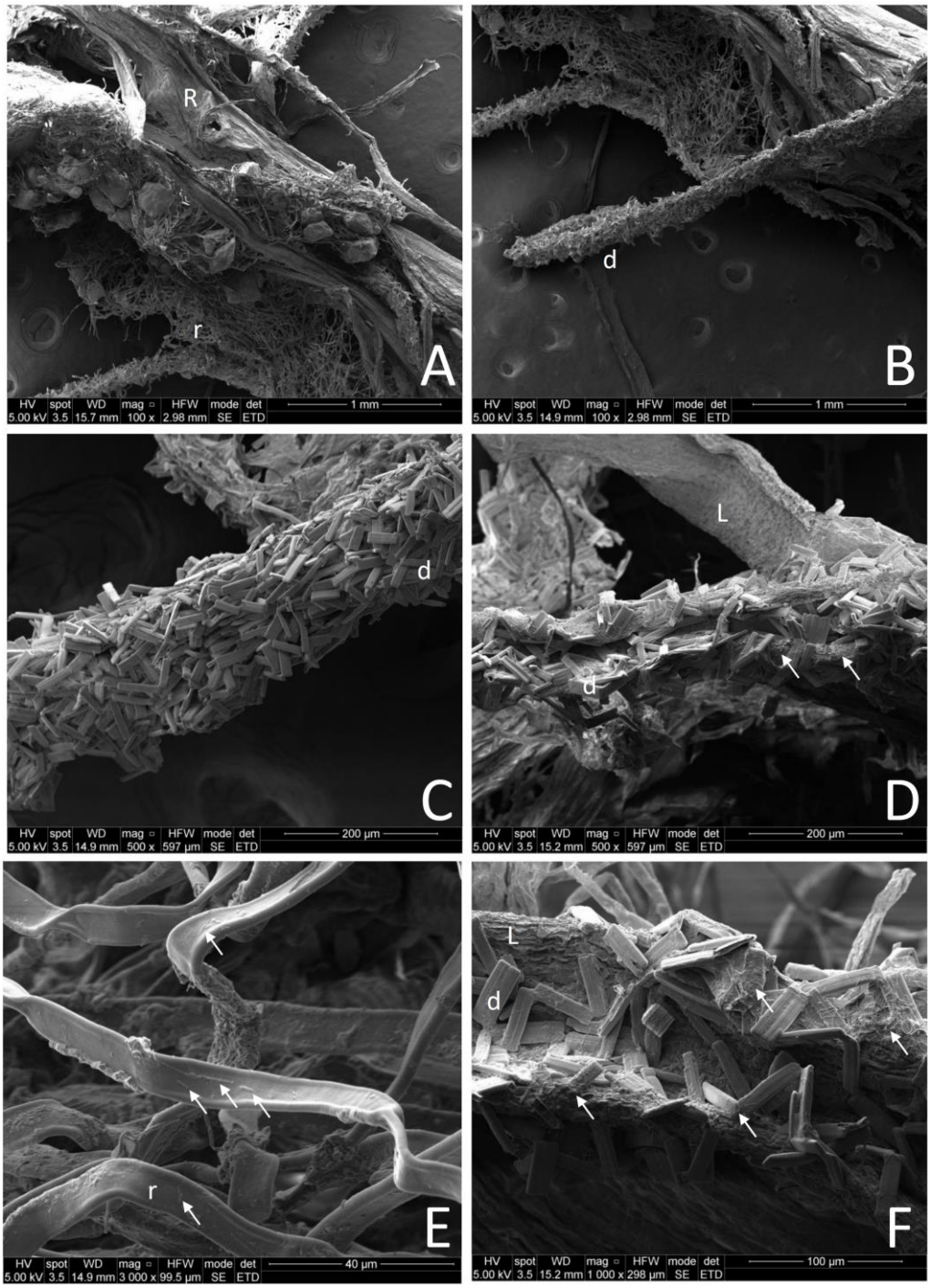

Figure 31. (A-F) Biofilm, including microorganisms, attached on the roots (the rhizoI(R) and leaves (L)) of *Ruppia* or *Althenia* plants. The presence of diatoms (d) within the biofilm is evident. A few bacteria (indicated by arrows in panel E) are also present on the root system. The biofilm matrix is clearly visible in panels D and F (indicated by the arrows in those panels).

While the biofilm observed on *Ruppia* or *Althenia* plants in the absence of filamentous algae (Figure 31) appears to be mainly composed of diatoms, the biofilm present at the surface of the filamentous algae aggregates (Figure 32) appears more complex. Indeed, the presence of bacteria and possibly fungi is evident on the SEM images of the filamentous algae aggregates (Figure 32D). The matrix of the biofilm covering the

surface of the filamentous algae aggregates is also potentially thicker than on *Ruppia* or *Althenia* plants in the absence of filamentous algae, as underlying structures such as diatoms and algae cells are visible under the biofilm matrix.

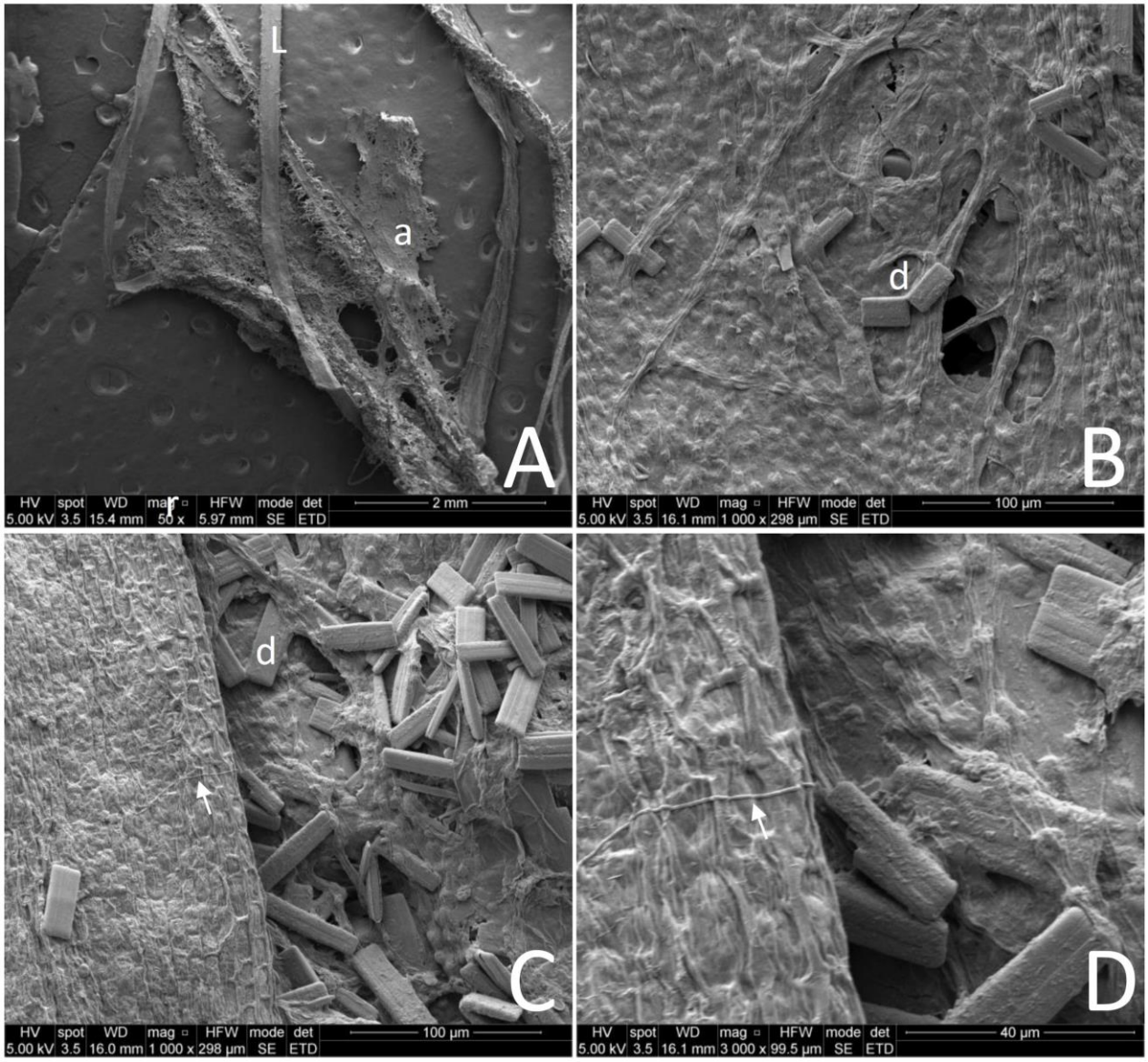

Figure 32. (A-D) Biofilm, including microorganisms and biofilm matrix, attached to filamentous algae aggregates (a) associated to *Ruppia*. (A) Filamentous algae form aggregate that engulf *Ruppia*. (B) Close up imaging of the aggregate structure reveals the presence of diatoms and bacteria within the aggregate. The structure is similar to that of a biofilm with the presence of a thick polysaccharide matrix. (C-D) Diatoms and bacteria are present in the aggregate. The presence of diatoms (d) and bacteria (arrows) within the biofilm is evident.

3.4.4 Microbial communities associated to the leaves and roots of *Ruppia*

Distinctive spatial distributions patterns were evident reflecting the impact that the spatial distance and site conditions within the Coorong have on these biofilm communities (Figure 33A). The CAP1 axis separates site 5 (Noonameena) from the rest of the samples (eigenvalue 0.9974, correlation Δ^2 0.9948); whereas CAP2 separates sites 1 and 2 in the South lagoon (Wild Dog Islands and Policeman Point) from sites 3 (Parnka Point) and 4 (North Magrath Flats) in the North lagoon (eigenvalue 0.9859, correlation Δ^2 0.9721). The pattern observed on this CAP analysis is very similar to the one observed for the water communities of Archaea and Bacteria. The differences observed between sites were explained by the variations in turbidity (BEST, Spearman ρ=0.308, p<0.01).

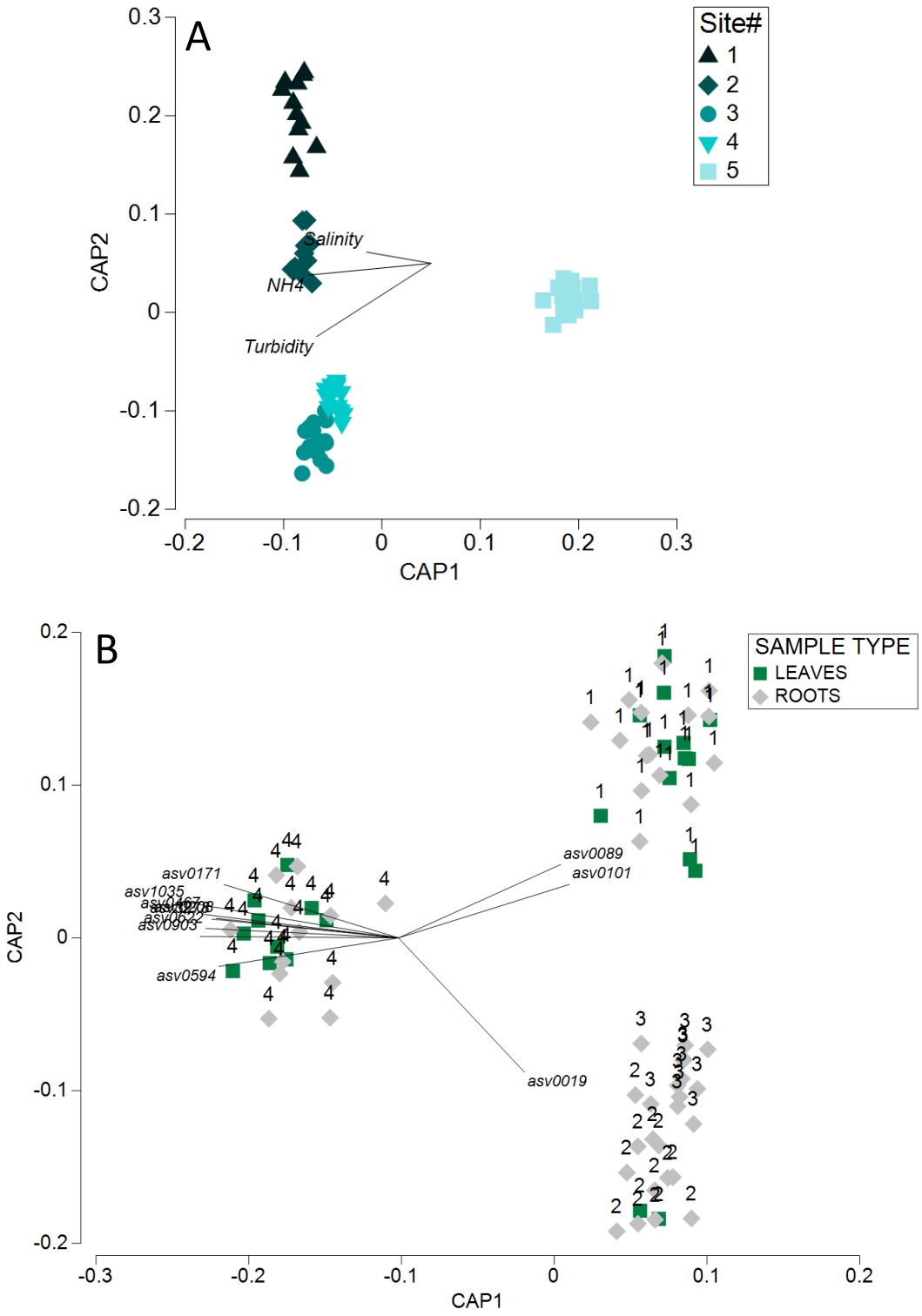

Figure 33. Canonical Analysis of Principal Coordinates (CAP) ordination plot of the communities of Archaea and Bacteria detected in the leaves and root samples collected at the five sampling sites in October 2020 (Trip 1), December 2020 (Trip 2), March 2021 (Trip 3) and June 2021 (Trip 4). Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. (A) The discrimination of communities between sites is clearly visible. Pearson correlation vectors (r > 0.3) represent the water quality parameters driving the discrimination between samples. (B) The discrimination of communities between sampling trips (numbered 1 - 4) is clearly visible. Pearson correlation vectors (r > 0.7) represent the ASVs (i.e. asv1035, asv0019, asv0903, asv0208, asv0594, asv0089, asv0101 and asv0622 γ-proteobacteria, asv0171 α-proteobacteria, asv0467 Bacteroidota, asv03278 Verrucomicrobiota) driving the discrimination between samples.

Similarly, distinctive seasonal patterns are evident reflecting the link between microbial communities and the different stages of the lifecycle of plants in the *Ruppia* Community (i.e. vegetative, reproductive or aestivation stages) and these biofilm communities (Figure 33B). The CAP1 axis separates trip 4 (June 2021) from the rest of the samples (correlation Δ^2 0.971); whereas CAP2 separates trip 1 (October 2020) from trips 2 and 3 (December 2020 and March 2021, respectively; correlation Δ^2 0.933). Several ASVs were highly correlated (r>0.7) to the CAP axes i.e. the phylum α-proteobacteria, γ-proteobacteria (including genus Marinomonas) and Halobacteria (Archaea) with CAP1 and the phylum γ-proteobacteria (including genus Alteromonas) with CAP2.

Ruppia leaves

Ruppia or *Althenia* plants leaves and roots were found at all sites in October and December 2020. However, no leaves were found at any sites except Noonameena in March 2021, and plant Leaves and roots were found at three sites (Parnka Point, North Magrath, Noonameena) in June 2021. However, Archaea and bacteria organisms could be not amplified from all the samples. Of those able to be amplified, 26 archaea and bacteria taxa were identified on the *Ruppia* or *Althenia* plants leaves. The dominant Archaea and Bacteria phyla of the sequenced samples were found to be γ-proteobacteria and, and α-proteobacteria (Figure 34).

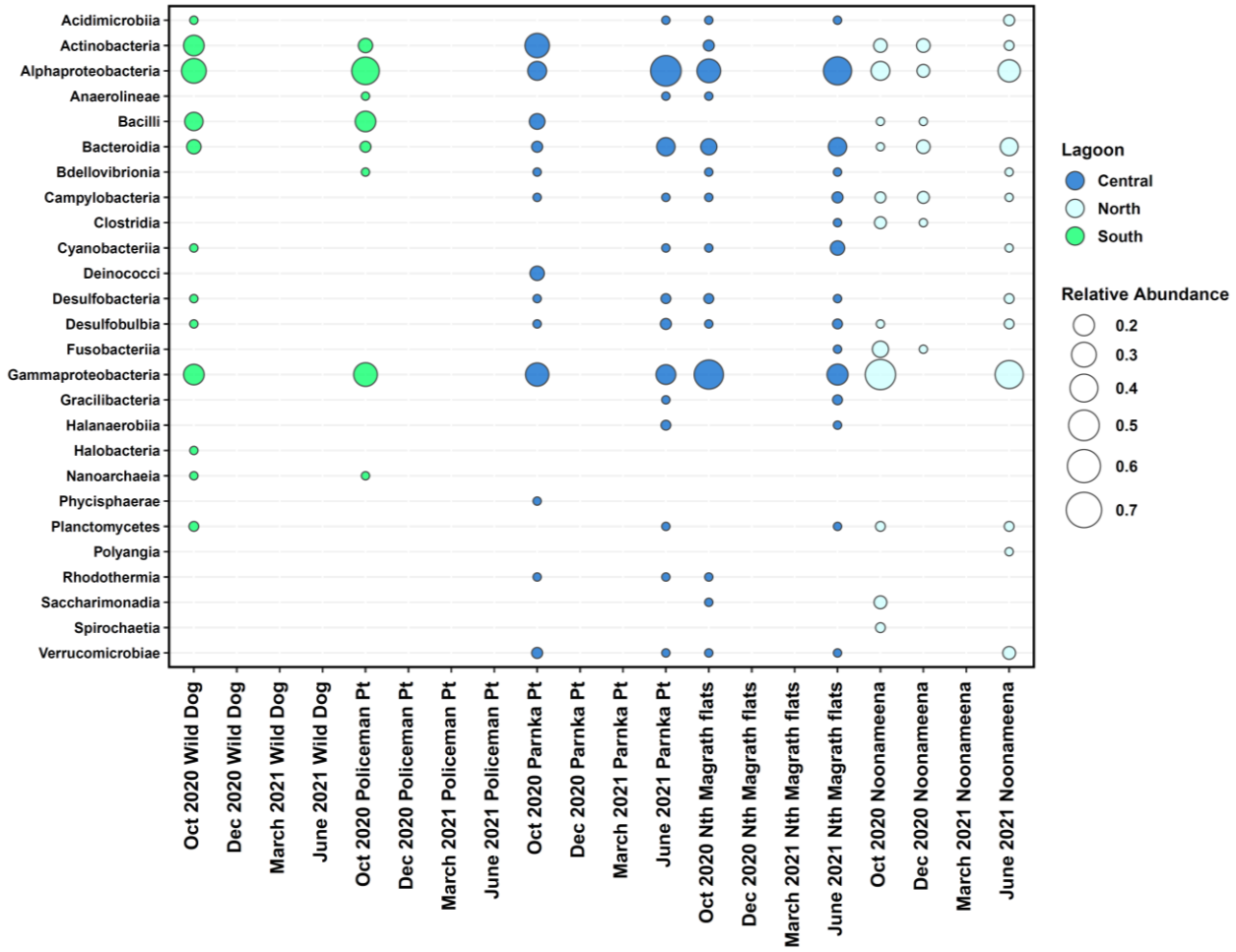

Figure 34. The relative abundance of all the taxa that comprise greater than 1% of the archaea and bacterial sequences associated with the leaves of *Ruppia* at the five sampling sites in October 2020 (Trip 1), December 2020 (Trip 2), March 2021 (Trip 3) and June 2021 (Trip 4). Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. The taxa are classified to the class level according to the Silva database (version 138.1).

Taxa from the genus Marinomonas was commonly associated to the leaves and roots of *Ruppia* or *Althenia*. In this study, the common core of archaea and bacteria within five sites of the Coorong was analysed using

the ASV (taxonomic group) data obtained from sequencing (Figure 35). Table 11 depicts the class taxa that are common core organisms associated with leaves of *Ruppia*.

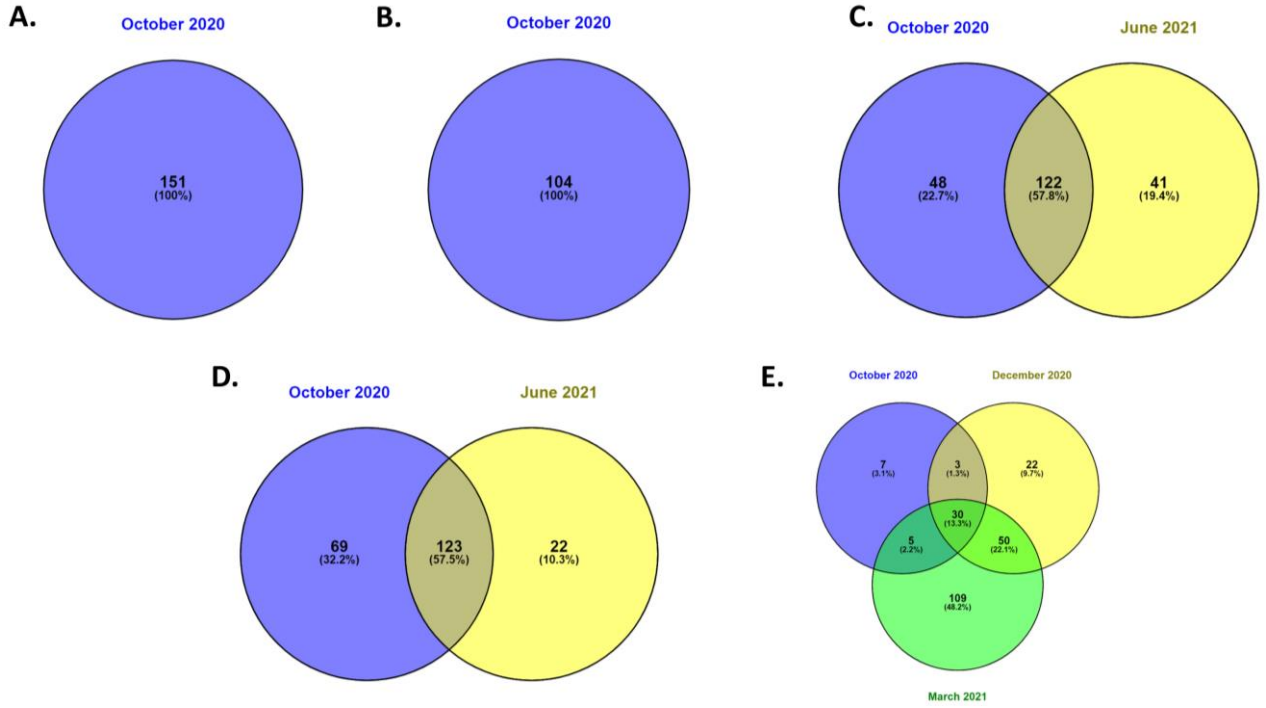

Figure 35. Venn diagrams displaying the overlap in taxa between the common core archaea and bacteria associated with the leaves of the *Ruppia* Community at the sampling sites along the Coorong: A) Wild Dog Islands, B) Policeman Point, C) Parnka Point, D) North Mcgrath Flat, and E) Noonameena during the period of study (October 2020, December 2020, March 2021, June 2021). No archaea and bacteria are shown for those times when *Ruppia* or *Althenia* leaves were absent at sampling sites or where samples could not be amplified. The taxa are classified to the class level according to the Silva database (version 138.1).

Table 11. The number of core and seasonal organisms associated with the leaves of members of the *Ruppia* Community identified at each sampling site in the Coorong during the study period. The taxa are classified to the class level according to the Silva database (version 138.1).

SITE	CORE ORGANISMS	SEASON SPECIFIC
Noonameena	30	138
North Magrath Flats	123	81
Parnka Point	122	89
Policeman Point	104	104
Wild Dog Island	151	151

Log-fold changes in the relative abundance of leaf-associated microbiota were calculated between Noonamena and South Lagoon sites (i.e. Wild Dog Island and Policeman Point). This allowed identifying if differences observed in salinity at these sites could be linked to specific leaf-associated microbiota (Figure 36). There were significant differences in the leaf-associated microbiota between Noonameena and Policeman Point (Figure 36A) and between Noonameena and Wild Dog Island (Figure 36B). In particular, this analysis identified classes of organisms that were significantly less abundant at Noonameena compared to both South Lagoon sites, e.g. Notrosophaeria, Subgroup 26, Thermoleophilia, Kapabacteria, KD4-96, Vampirivibrionia, BD2-11 Terrestrial group, Unclassified NB1-j, ABY1, 028H05-P-BN-P5, VadinHA49, Unclassified SAR324 clade, Synergistia and Unclassified WPS-2. Similarly, this analysis identified classes of organisms that were significantly more abundant at Noonameena compared to both South Lagoon sites, e.g.

Unclassified Cyanobacteria, babeliae, Chitinivibrionia, Moduliflexia, Alphaproteobacteria, Unclassified Sva0485 and Unclassified Zixibacteria.

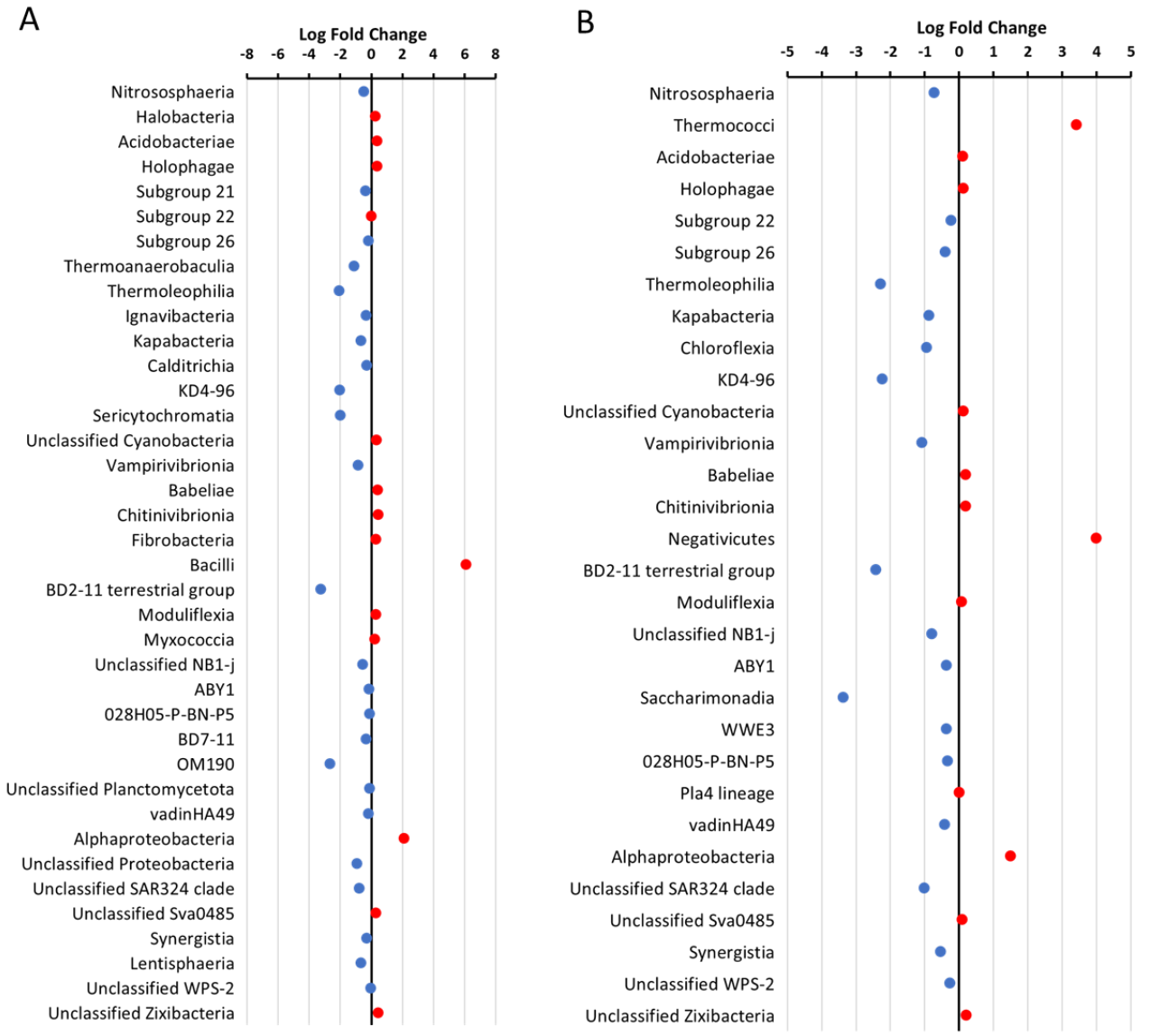

Figure 36. Representation of the significant log fold changes ($p < 0.005$) in the relative abundance of archaea and bacteria associated with the leaves of plants (*Ruppia* or *Althenia* species) between (A) Noonameena and Policeman Point, and (B) Noonameena and Wild Dog Island. Calculations were made at the class level, including the rare taxa. Red dots correspond to positive log fold change while blue dots correspond to negative log fold change. The taxa are classified to the class level according to the Silva database (version 138.1).

Ruppia or *Althenia* roots

Ruppia or *Althenia* roots were found at all sites during October 2020, and only at four sites (Wild Dog, Policeman Point, North Magrath Flats and Noonameena) in December 2020 and March 2021 (Wild Dog, Policeman Point, Parnka Point and Noonamena). Roots were only found at three sites during June 2021 (Parnka Point, North Magrath Flats and Noonamena).

Thirty-three archaea and bacteria taxa were identified on the plant roots. At all of the sites the dominant bacterial class were γ-proteobacteria, α-proteobacteria and Bacteroidia (Figure 37). In this study, the common core of archaea and bacteria within five sites of the Coorong was analysed using the ASV (taxonomic group) data obtained from sequencing (Figure 38). Table 12 depicts the class taxa that are common core organisms associated with roots of members of the *Ruppia* Community.

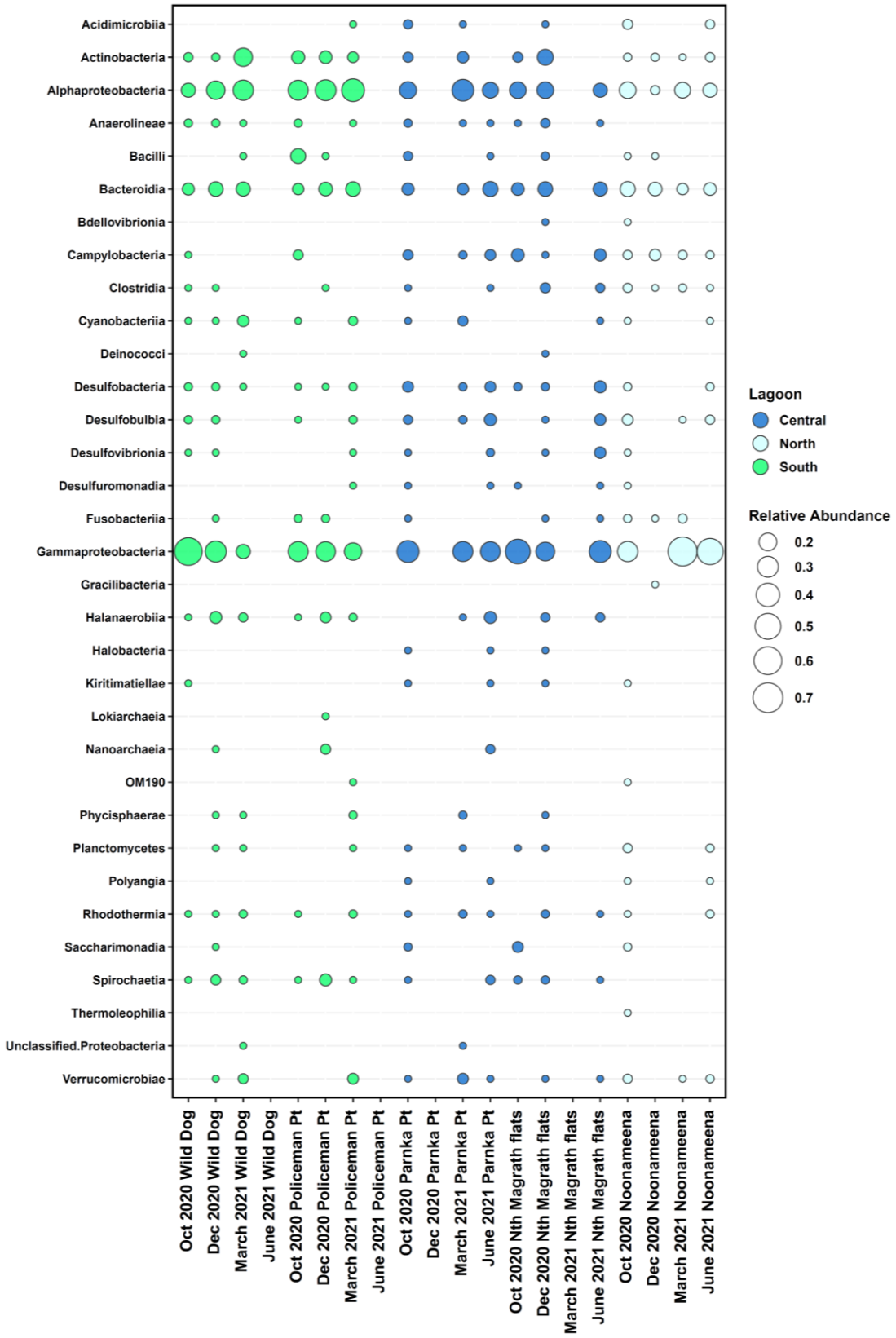

Figure 37. The relative abundance of all the taxa that comprise greater than 1% of the archaea and bacterial sequences associated with the roots of *Ruppia* or *Althenia* at the five sampling sites in October 2020 (Trip 1), December 2020 (Trip 2), March 2021 (Trip 3) and June 2021 (Trip 4). Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. The taxa are classified to the class level according to the Silva database (version 138.1).

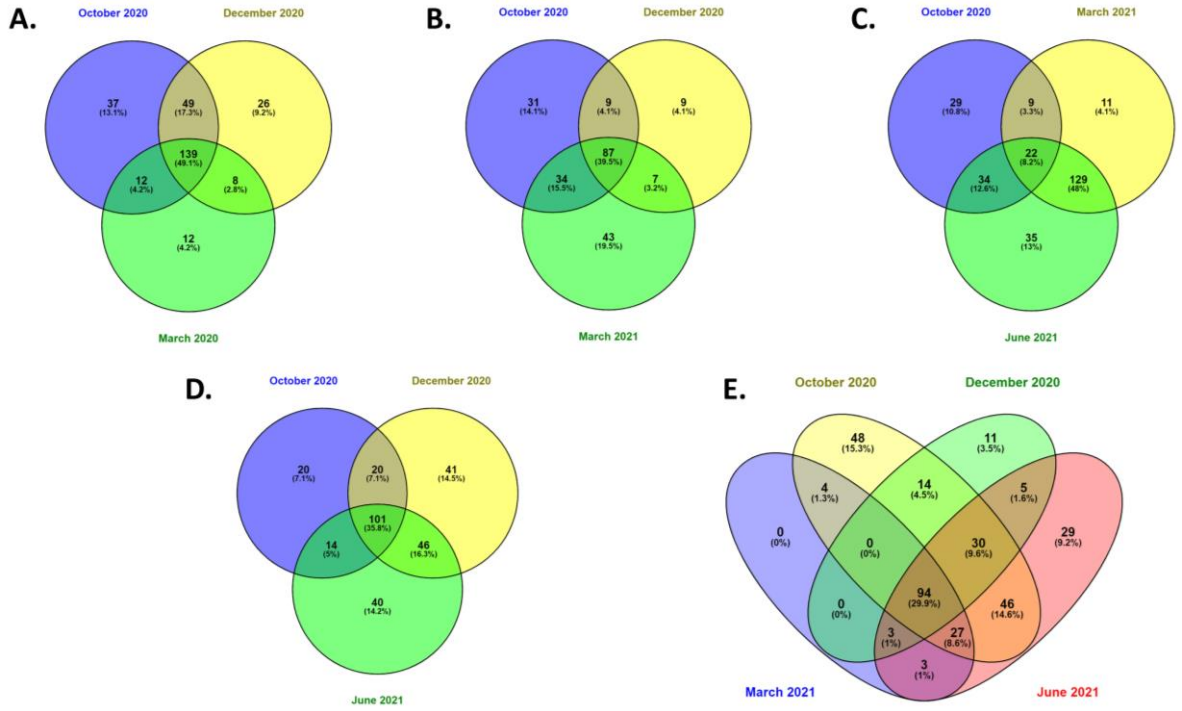

Figure 38. Venn diagrams displaying the overlap between the archaea and bacteria associated with the roots of *Ruppia* or *Althenia* A) Wild Dog Island, B) Policeman Point, C) Parnka Point, D) North Mcgrath Flat, and E) Noonameena over the period of study (October 2020, December 2020, March 2021, June 2021). The taxa are classified to the class level according to the Silva database (version 138.1).

Table 12. The core and seasonal organisms associated with the roots of *Ruppia* or *Althenia* identified at each sampling site in the Coorong during the study period. The taxa are classified to the class level according to the Silva database (version 138.1).

SITE	CORE ORGANISMS	SEASON SPECIFIC
Wild Dog Island	139	75
Policeman Point	87	83
Parnka Point	22	75
North Magrath Flats	101	101
Noonameena	94	88

3.4.5 Microbial communities associated with the filamentous material

At the sites where the *Ruppia* Community was present, filamentous material was sometimes present in the forms of floating mats, ground mats or entangled with the plants (plant associated aggregates, PAA). Filamentous material was found at three sites: Parnka Point, North Magrath Flats, and Noonameena in October and December 2020 (Spring). No filamentous material was found at any sites in March 2021 (Autumn). Whereas in June 2021 (Winter) filamentous material were found at two sites, North Magrath Flats and Noonameena.

Thirty-one archaea and bacteria taxa were identified with the filamentous material. The dominant Archaea and Bacteria phyla of the sequenced samples were γ-proteobacteria, α-proteobacteria and Bacteroidia. (Figure 39). Of note is the absence of filamentous material in the South Lagoon of the Coorong especially when filamentous material is present not only in the North Lagoon but also in the Central Coorong for most of the year.

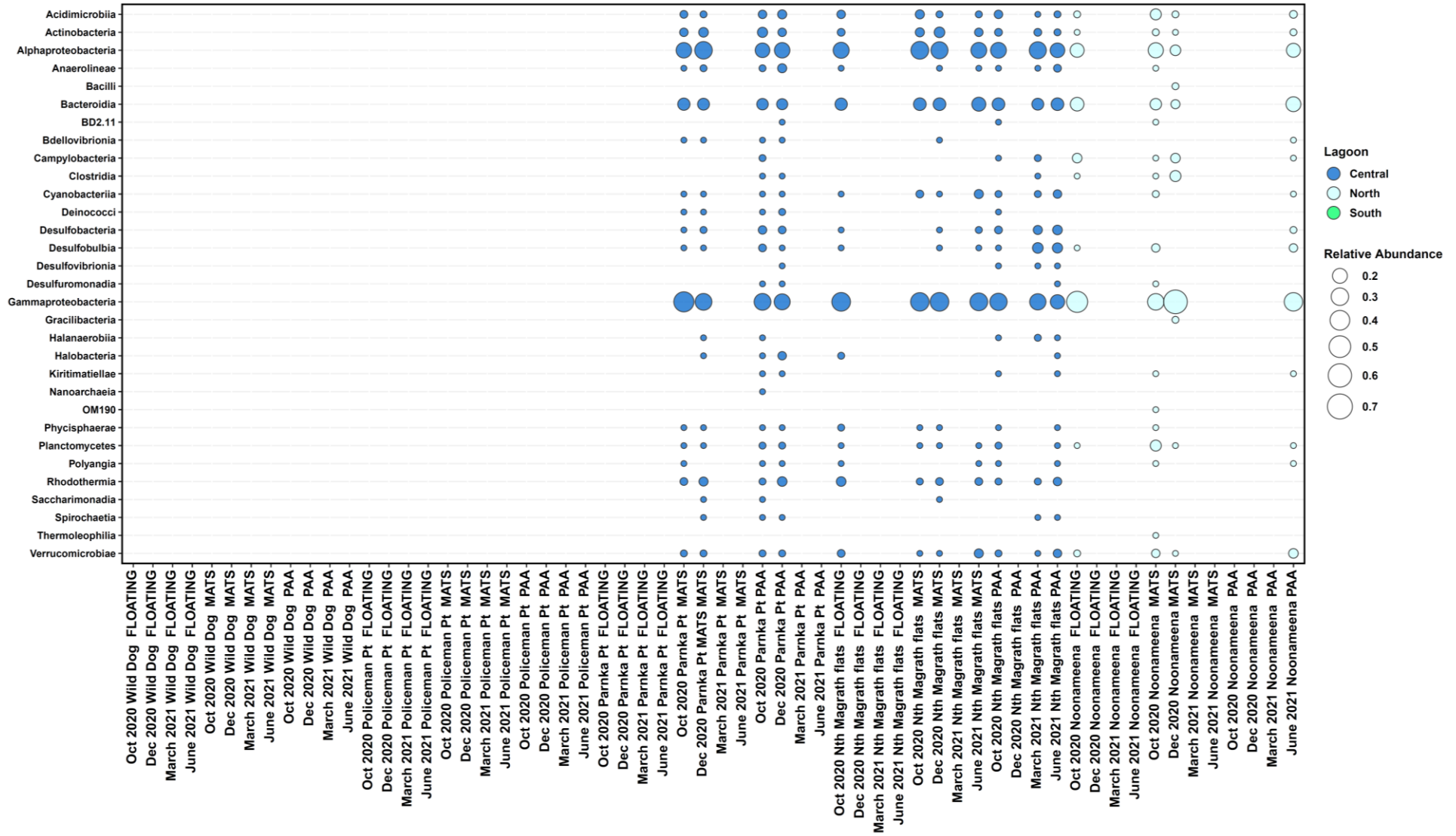

Figure 39. The relative abundance of all the taxa that comprise greater than 1% of the archaea and bacterial sequences associated with the filamentous material at the five sampling sites in October 2020 (Trip 1), December 2020 (Trip 2), March 2021 (Trip 3) and June 2021 (Trip 4). Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. The taxa are classified to the class level according to the Silva database (version 138.1). Floating = Floating Mats, Mats = Ground Mats, PAA = Plant Associated Aggregates.

The common core organisms of archaea and bacteria associated with the leaves and roots of *Ruppia* Community as well as the filamentous material were analysed using the ASV (taxonomic group) data obtained from sequencing (Figure 40).

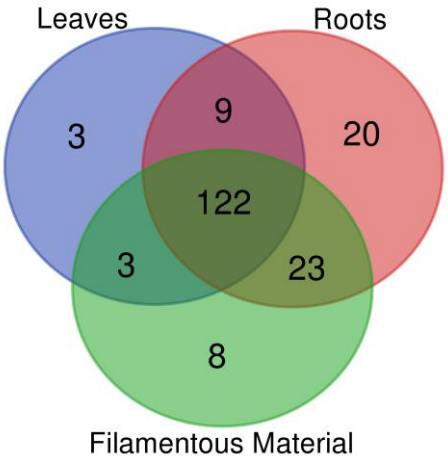

Figure 40. Venn diagrams displaying the overlap between the archaea and bacteria communities associated with the leaves and roots of *Ruppia* Community as well as filamentous material (i.e. plant associated aggregates, ground mats, and floating mats). The taxa are classified to the class level according to the Silva database (version 138.1).

4 Discussion

This study identified the community composition of microorganisms within the southern Coorong ecosystem. It also documented variation across a range of sites and differences in measured water quality and is summarised in Table 13. The research included simultaneously assessing planktonic and benthic microbial communities in the Coorong. It also assessed the presence of biofilm at the surface of the *Ruppia* Community and the communities of microorganisms associated to these biofilms. The results of this study documented a highly complex microbial environment present in the Coorong. We determined distinct microbial communities associated with the water column and the sediment. In addition, specific communities were found to be associated with the *Ruppia* Community. The microalgal communities are bioindicators of the state of the Coorong environment, reflecting its prevailing environmental conditions (in particular salinity), the existing nutrient load, as well as potentially reflecting the current health of the aquatic plants.

Table 13. The microbiota present at all sites or only present at specific sites are listed below for the five sampling sites in October 2020 (Trip 1), December 2020 (Trip 2), March 2021 (Trip 3) and June 2021 (Trip 4). Sampling sites (refer to Table 1 for details) – *Coorong South Lagoon*: site 1, Wild Dog Islands; site 2, Policeman Point. *Central Coorong*: site 3, Parnka Point; site 4, North Magrath Flats. *Coorong North Lagoon*: site 5, Noonameena. The Bacteria and Archae taxa are classified to the class level according to the Silva database (version 138.1).

	ALL SITES	WILD DOG ISLAND	POLICEMAN POINT	PARNKA POINT	NORTH MACGRATH FLAT	NOONAMEENA
Bacteria and Archaea	γ-proteobacteria, α-proteobacteria, Actinobacteria, Bacteroidia	TK10, Unclassified Bdellovibrionota, Longimicrobia	Aquificae, Symbiobacteriia, Leptospirillia	DG-56	Abditibacteria, Methanomicrobia, Syntrophomonadia, Unclassified 10bav-F6, Dojkabacteria, Thermobacteria	Unclassified Fibrobacterota, TK17, Fimbriimonadia, Unclassified Nitrospinota
Harmful Algae	Gymnodinoids, *Nitzschia* sp.	*Cochlodinium* sp., *Leptolyngbya* sp., *Planktolyngbya* sp.	*Limnothrix* sp., *Planktolyngbya* sp.		*Takoyama* sp.	
Microalgae	*Cocconeis* sp., *Cylindrotheca Closterium*, *Koliella* sp., *Mantoniella squamata*, *Scourfieldia* sp., Picoplanktonic cells (cells 1-4 µm)	*Leucocryptos* sp., *Pyrobotris* sp.	*Cymbomonas tetramitiformis*,		*Asteromphalus sarcophagus*, *Bacillaria paillifera*, *Minidiscus trioculatus*, *Plagiotropis* sp., *Goniomonas truncate*, *Fragilaria* sp., *Calciopappus caudatus*,	*Haslea wawrikae*, *Licmophora* sp., *Striatella unipunctata*, Chroomonas sp., *Cryptomonas* sp., *Mamiella* sp.

In addition to the overall observations of community diversity, we determined the presence of microalgae in the system that have the potential to release toxic secondary metabolites that are associated with Harmful Algae Bloom species (HABs). Further work should inform on the presence of HABs and potential HABs (diatoms, dinoflagellates and cyanobacteria) to species level and on the levels of toxins present in the Coorong, if any. Some species of *Leptolyngbya* sp. and of *Pseudanabaena* sp. have been reported to produce toxins such as Nodularin and Microcystin, respectively (Fastner and Humpage 2021). Environmental factors that contribute to cyanobacterial growth are complex but some baseline conditions can be identified to provide a measure of the likelihood of a cyanobacterial bloom occurring in a water body (NHMRC Guidelines

for Managing Risks in Recreational Water 2008). The NHMRC recommends that the baseline condition should include a history of cyanobacteria presence and identification, water temperature, water nutrients and indication of thermal stratification. This is an area for future study in the Coorong. At this stage the presence of the species does not imply they will become harmful. Monitoring and identifying HAB species, and whether toxic metabolites are present, will inform an assessment of potential harm to humans and/or fisheries.

4.1 Water quality

This study illustrates the strong spatial and temporal variation in water quality parameters measured along the Coorong (Figure 8). The location of the sampling sites within the Coorong, i.e. the environmental conditions present along the north-south axis of the Coorong, is more influential to the observed microbial communities than seasonal differences under the same environmental parameters. The observations made in March 2021 showed the most variability in water quality as reflected by the strongest gradient observed in salinity, DO and turbidity. These results are consistent with previous findings (Leterme et al. 2015; Hemraj et al. 2017a and b).

The nutrient levels measured throughout the study characterised a strong deficit in nitrogen, i.e. a decrease in the ratio of fixed inorganic nitrogen to phosphorus (N:P) relative to the Redfield ratio (16:1; Redfield et al. 1963). Low N:P ratios are often associated with a low total algal biomass (Pick 1989) and favour a community of microalgae dominated by cyanobacteria (Xie et al. 2003). It is often assumed that inland waters have large inputs of organic matter from terrestrial environments with typically high carbon:nitrogen:phosphorus ratios (Hessen et al. 2003). However, DO levels in the environment can impact on the nitrogen and phosphorus cycles, with anoxic conditions causing a decrease in the main inorganic nitrogen compounds (i.e. nitrate, nitrite and ammonium), while increasing bioavailable phosphorus (Spilling et al. 2019). The pool of dissolved organic nutrient can buffer fluctuations in the nutrient requirements of microbial communities depending on the rate organic matter input from external environments.

For example, bacteria grow fast and consume primarily dissolved pools of inorganic and organic matter and can thus play an important role in shaping the elemental stoichiometry of nutrients in an ecosystem. Indeed, aquatic microbes play a central role in shaping aquatic ecosystem nutrient ratios (e.g. C, N, and P) and bacteria are the major consumers of phosphorus- and nitrogen-based compounds in both marine and inland waters (Cotner and Biddanda, 2002). The low N:P ratio stimulates N-fixing species like cyanobacteria which could be helping the Coorong ecosystem to stay within a cycle of internal nutrient cycling through water-column N fixation and P releases from sediments. The identification of nutrient sources and maximum allowable inputs of nutrients into the Coorong ecosystem would help guide potential restoration measures. Indeed, the balancing of DO levels and nitrogen levels in the water could assist with re-establishing a diverse community of larger microalgae that benefit planktonic and benthic invertebrates.

4.2 Water and sediment microbial communities

Globally planktonic microorganisms in marine environments dominate not only the biomass but also biogeochemical cycles. The planktonic microorganisms drive the carbon and nitrogen cycles and also key ecosystem functions such as primary production, transformation of organic compounds and remineralisation of organic matter (Falkowski et al. 2008; Worden et al. 2015). Environmental variables are known to affect microorganism communities (Fuhrman et al. 2015; Guidi et al. 2016), however, consensus on how strongly these factors influence and change the microbial population is varied.

Plankton communities are often used as bio-indicators to monitor ecological change in aquatic systems and are natural bio-indicators due to their rapid response to fluctuating environmental conditions (Albaina et al. 2009; Amengual-Morro et al. 2012). In particular, communities of microalgae reflect climate variability and dramatic changes that occur in aquatic ecosystems (Edwards and Richardson, 2004; Richardson and Schoeman, 2004; Leterme et al. 2005). Assessing their temporal and spatial dynamics is, therefore, of particular importance when considering the impact of environmental variability on ecological changes of coastal lagoons (Gönenç and Wolflin, 2005). In the Coorong, the microalgae community was dominated by

Picophytoplankton cells, which are recognised as microalgae cells <2–6 µm in diameter. Unique nutrient recycling control, short (several hours) doubling rates and the relative independence of the picophytoplankton to environmental conditions, including light attenuation and turbidity, result in this class of microbe being the main contributors to primary productivity in oligotrophic marine systems. These picophytoplankton communities are present in nearly all aquatic systems. Picophytoplankton are increasingly being recognized as significant contributors to primary productivity and microalgae biomass in coastal and estuarine systems. Across these ecosystems, they account for ~25% of productivity and biomass, however, in the summer months these communities can account for up to 100% of productivity and biomass. In the Coorong, picophytoplankton contributed to 92-99% of the biovolume of the microalgae community in Summer. The dominance of the picophytoplankton in this study reflected the highly opportunistic nature of these organisms even in a nutrient-limited environment as has been found elsewhere (Barber 2007). In this study, their presence could be associated with the low levels of nutrients observed in the water column, especially the limitation in Nitrogen. The Picophytoplankton low cell biomass might allow them to swiftly exploit the small amount of nutrients present, easily outcompeting larger microalgae cells (Richardson and Jackson 2007; Massana 2011).

Microalgae are usually the first autotrophic level to respond to a reduction in nutrient concentrations. Previous studies have focused on the effect of oligotrophication in lakes and the response of microalgae to a reduction in nutrients, which resulted in considerable changes in microalgae biomass, as well as in the size, structure and diversity of the microalgae, and in the development of mixotrophic organisms (Gaedke, 1998; Ruggiu et al. 1998; Jeppesen et al. 2005). In this study, the total number of large microalgae species decreased from 241 to 81 (Figure 41) study in the Coorong (ARC DP 2011-2014; Leterme et al. 2015, Hemraj et al. 2017b). At the same time, the number of picophytopkankton cells increased by x6 fold.

The microalgae community (i.e. the large microalgae > 5µm) included Chrysophytes, Prymnesiophytes, Cryptophytes, Euglenophyta, Chlorophytes, Prasinophytes and Cyanoprokaryota. Chrysophytes are mixotrophs and long-term drops in nutrients such as Nitrogen could favour their community which can alleviate nutrient limitation through mixotrophy (Krom et al. 2005). Bacteria compete directly with small and large microalgae for N and P (Joint et al. 2002), but can be grazed by mixotrophic microalgae, especially under limited light and increased temperature (Wilken et al. 2018). Monitoring microalgae diversity is essential not only to identify potential harmful taxa, but also to track highly competitive bloom-stabilizing taxa and shifting life strategies.

We have observed a significant change in the composition of the large microalgae community study in the Coorong (ARC DP 2011-2014; Leterme et al. 2015, Hemraj et al. 2017b) with a significant increase in Chlorophytes and Prasinophytes, and a significant decrease in Cyanoprokaryota and in Cryptophytes (Mann-Whitney, $p<0.001$; Figure 41). For example, the Chlorophyte *Koliella* sp. formed blooms in December 2020 (except in Noonameena) and March 2021 (in the South Lagoon and Central Coorong). The abundance of *Koliella* sp. has increased by x40 since the previous study of microalgae in the Coorong (Leterme et al. 2015). *Koliella* sp. is commonly associated with freshwater environments but has been known to readily adapt to the marine environments (Suzuki et al. 2019). Little is known about the *Koliella* sp. found in the Coorong, yet it is not possible to understand the consequences (positive or negative) that this genus can have on the ecosystem. Indeed, most of the literature focuses on *Koliella antarctica* and its adaptation to low light and low salinity environments (Ferroni et al. 2007; Suzuki et al. 2019).

The Prasinophytes *Scourfieldia* sp. and *Mantoniella squamata* had not been reported in the previous study of microalgae in the Coorong (Leterme et al. 2015), but are now the second group dominating the consortium of large microalgae, after *Koliella* sp. (chlorophyte). *Scourfieldia* sp. have been identified as mixotrophic feeders under certain conditions, whereby they feed on dissolved organic carbon (DOC) osmotrophically (Ulrich and Röske 2018). *Mantoniella squamata* is known to initiate resting stages that have the potential for long-term survival thereby remaining viable for several decades in sediments (Lundholm et al. 2011; Ribeiro et al. 2011; Ellegaard et al. 2013). These highly resilient microalgae species are ideally suited to dominating at times the rapidly changing environmental conditions in the Coorong.

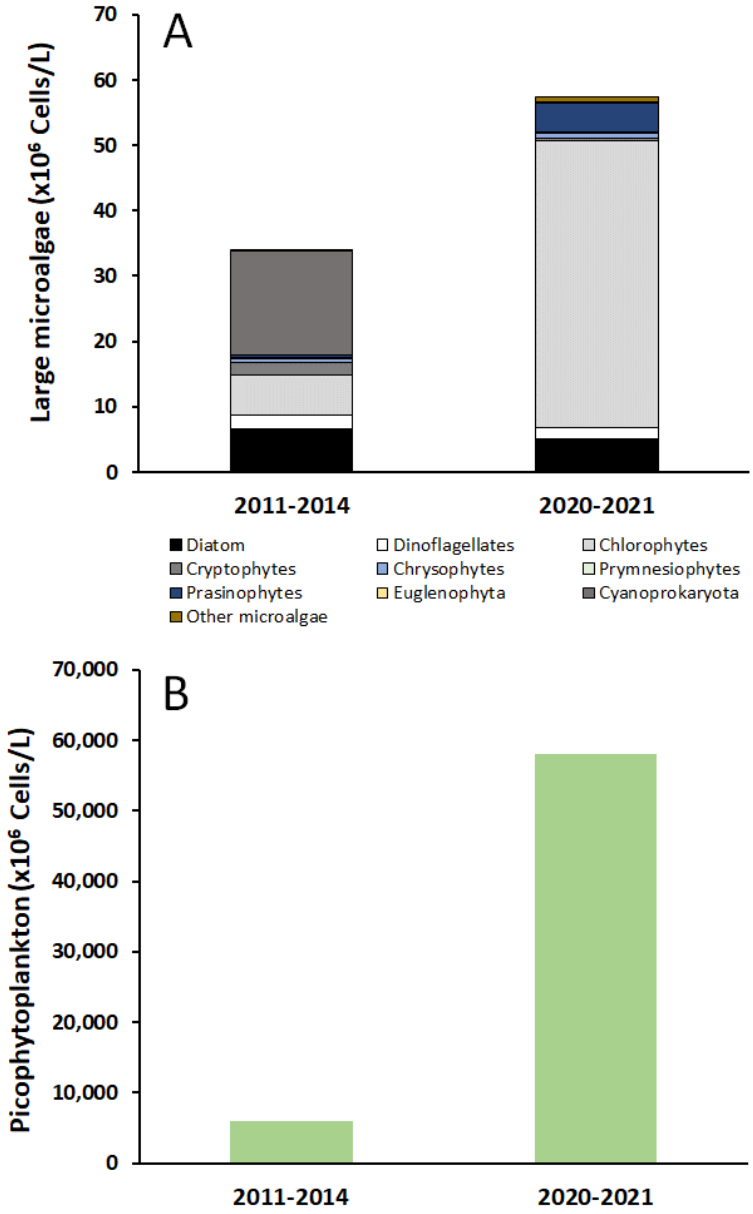

Figure 41. Change in the (A) large microalgae and (B) picophytoplankton community composition between the current period of study and a previous project (ARC DP 2011-2014; Leterme et al. 2015), sampling the same sites in the Coorong.

The microbial communities identified within the water across more than 50 km of the length of the Coorong are reflective of those commonly found in estuarine environments (Xu et al. 2018; Li et al. 2017). The differences observed in all the sites, except Noonameena, reflect the microbial community's response to the seasonal changes and environmental conditions. It is worth noting that the community composition of the bacterial taxa did not change, only the relative abundance of each phylum. For example, the phylum of Planctomycetes, which was less abundant in the water at Noonameena compared to the other sampled sites, contains many species capable of anammox (the anaerobic conversion of Nitrite and Ammonium into Nitrogen gas, N_2, and water). Planctomycetes are also recognised for their role in the global carbon. Here, the calculation of the Redfield ratio indicated an imbalance in nutrients, with a clear deficit in Nitrogen. This deficit could be partly explained by the activity of these anammox bacteria with the conversion of Nitrite into N_2. Anammox bacteria are chemoautotrophic microorganisms (i.e. organisms that obtain their energy from oxidizing inorganic compounds), which generally take inorganic carbon as the only carbon source and have no demand for organic carbon sources (Strous et al. 1999). The presence of organic matter often leads to the inhibition of anammox process (Jin et al. 2012), or even the loss of anammox function (Tang et al. 2010; Chamchoi et al. 2008). Probes based on the 16S rRNA diversity can be used to identify specifically some

anammox bacteria (Woebken et al. 2007) and could be the focus of future work. These probes can provide specific information and insights into this anammox process that could have a devastating impact on the water quality throughout the Coorong. Finally, members of phylum Verrucomicrobia were present at all the sampled sites except Noonameena in December 2020, which exhibit the ability to adapt to oligotrophic environments. A number of species within the phylum Verrucomicrobia have been identified as having symbiotic relationships with eukaryotes, their presence provides an opportunity to follow up in future research to improve our understanding of the relationship between this group and the ability of the *Ruppia* Community to grow in poor conditions.

Another of the dominant core phyla observed in the Coorong was Thermodesulfobacteriota (comprising the classes of Desulfobacteria, Desulfobulbia, Desulfovibrionia and Desulfuromonadia; Figure 20), which was present throughout the study in sediment samples and in some of the *Ruppia* or *Althenia* leaf and root samples. The phylum Thermodesulfobacteriota gets its energy from sulfate reduction and is often isolated from anoxygenic environments. Bacterial sulfate reduction is of great ecological and biogeochemical importance in anoxic hypersaline sediments and in hypersaline photosynthetic microbial mats (Canfield et al. 1993; Caumette et al. 1994). Such bacteria reduce elemental sulphur to hydrogen sulfide (H_2S; Camacho, 2009). Sulfide compounds can reduce photosynthetic performance and block aerobic respiration (Lee et al. 1999). High levels of sulfide have been reported in shallow and deeper sediments of the Coorong, indicative of highly anoxic conditions (Huang et al. 2022). High sulfate concentrations are characteristic for many hypersaline aquatic systems (Oren 2002), favouring the activity of sulfate-reducing bacteria (SRB) in anoxic zones. Finally, the Chloroflexota phyla have been identified as one of the core populations especially in sediment, responsible for anaerobic digestion by fermentation. A by-product of bacterial fermentation especially in anoxia environments is the production of hydrogen (Kessler et al. 2019). While this can be a major lifeline for aerobic bacteria by way of a fuel source, it is also a building block for sulfate-reducing organisms contributing to the production of hydrogen sulfide (Burrow et al. 2014, Greening et al. 2022). Of note is the role that hydrogen sulfide is implicated to have in the decline of seagrasses globally (Greve et al. 2005, Borum et al. 2005, Frederiksen et al. 2007).

It is unclear from our results what role the Desulfobacterota associated to the roots or leaves of *Ruppia* or *Althenia* are playing or what impact they might have on the life cycle of members of the *Ruppia* Community. Future analysis of the microbial community and the plants may lead to further insights into the relationship between sulfate concentrations and the presence of Desulfobacterota in the sampled areas, which would inform on strategies to remediate areas in the Coorong that are showing high levels of sulfide. Fraser and Kendrick (2017) have shown that the bacterium *Candidatus thiodiazotropha* (*Ca.* Thiodiazotropha), which grows on the roots of seagrasses (Martin et al. 2020), oxidizes sulfides in sediments, which can prevent sulfide intrusion, a driver that is known to hinder seagrass recolonization. Its presence on the seagrass roots might also alleviate sulfide stress to the seagrass. *Candidatus thiodiazotropha* (*Ca.* Thiodiazotropha) was also observed on the roots and leaves of members of the *Ruppia* Community during this study, as well as in the PAA and in the sediment. Future work could explore the relationship between *Candidatus thiodiazotropha* (*Ca.* Thiodiazotropha) and Ruppia, as well as measuring the impact that *Candidatus thiodiazotropha* (*Ca.* Thiodiazotropha) has on the sulfide compounds present in the sediment of the Coorong.

The composition of the root-associated microbiota is an important indicator of seagrass health and better understanding these seagrass root-microbial interactions could allow us to optimise the seagrass microbiota in order to increase future restoration chances of success. Work by Celdrán et al. (2012) has demonstrated the efficacy of inoculating seagrass leaves with bacteria that would promote its growth. Similar work could be done on the root-associated microbiota. Potential restoration measures for the sites that are showing high levels of sulfide should consider the synergy between members of the *Ruppia* Community and both the sediment and the root-associated microbiota. This approach could enhance future restoration options for *Ruppia* community under poor sediment quality conditions, indeed the root microbiome may be a critical component of establishing seagrasses in repatriated or rehabilitated sediments. Further experimental and field work in the Coorong could inform optimisation of the microbiota.

4.3 Harmful microalgae

Within the community of microalgae identified in this study, some genera and species are known to produce toxins and/or to contribute to HABs. These genera and species were diatoms (*Nitzschia* sp.), dinoflagellates (Gymonodinoids and *Takayama* sp.) and cyanobacteria (*Leptolyngbya* sp., *Levanderina fissa*, *Limnothrix* sp. (filaments), *Pseudanabaena* sp. and *Pseudanabaena limnetica*). The only HABs to have formed a bloom during the study was *Nitzschia* sp. at Noonameena in October 2020. *Pseudanabaena limnetica* was the second most abundant HABs recorded over the project, after *Nitzschia* sp. *P. limnetica* has been previously reported in shallow oligohaline and hyper-eutrophic lagoons in South America (de la Lanza Espino et al. 2008). To our knowledge, this is the first report of *Leptolyngbya* sp. and *Levanderina fissa* in the Coorong. Flaherty et al. (2007) reported the ability of bacterium SG-3 (*Lysobacter* cf. *brunescens*) to control species of cyanobacteria, suggesting it could be used as a biological control agent in aquatic systems. The genus Lysobacter has been shown to prey on species from the genus Leptolyngbya (Bauer and Forchhammer 2021). Here, the genus Lysobacter was reported in filamentous algae mats at Parnka Point in October 2020 and in the sediment of Policeman Point in December 2020. Some experimental work could be conducted on the ecological impact of Lysobacter in the Coorong.

Gymonodinoids can present different nutritional modes such as autotrophy, heterotrophy or mixotrophy. It is worth noting that while the Gymonodinoids did not form a bloom during the period of study, their abundance (20,000 – 2.8 10^6 cells/mL) was always higher than the recommended level (Table 9; NHMRC (NHMRC Guidelines for Managing Risks in Recreational Water 2008, Chorus and Walker 2021). Since only some Gymnodinoids are potentially toxic future work should involve the identification of the Gymnodinoids to species level to better understand if any action needs to be taken for their management in the Coorong.

The cyanobacteria taxa identified in this study are filamentous cyanobacteria which are successful in turbid mixed water under light deficient conditions (Kurmayer et al. 2006). The cyanobacteria *Pseudanabaena* sp. and *Leptolyngbya* sp. identified in the Coorong have been reported as potentially producing microcystin and Nodularin, respectively (Paerl and Otten 2013; Furtado et al. 2009; Bradt and Villena 2002, Fastner and Humpage 2021). Both genera have previously been reported in the Coorong (Nguyen et al. 2021). *Pseudanabaena* sp. has previously been reported in Lake Alexandrina and Lake Albert (Aldridge et al. 2012) and in the River Murray (https://mdba.ala.org.au). Since only some species from these genera are potentially toxic, future work should involve the identification to species level to better understand if any action needs to be taken for their management in the Coorong. Microcystins are the one of the most frequently detected cyanotoxins within freshwater harmful cyanobacterial blooms (Krausfeldt et al. 2019) and are the most significant public health issue associated with cyanobacterial blooms in southeastern Australia (NHMRC/NRMMC 2004).

Pseudanabaena sp. was present at Wild Dog Islands and North Magrath Flats in December 2020, while *Pseudanabaena limnetica* was recorded at Policeman Point and Parnka Point in March 2021. *Pseudanabaena* spp. are filamentous cyanobacteria. Reports of *Pseudanabaena* sp. in the scientific literature are limited, with little known about their physiology or molecular and metabolic characteristics (Foster et al. 2020). While being associated with bloom events in a range of environments, they are often overlooked (Acinas et al. 2009; Foster et al. 2020). *Pseudanabaena* sp. has been reported to form blooms at high pH ~11.4, but is usually observed at a pH 7-9, it has shown tolerance for low light and Phosphorous deficiency. Under a high N:P ratio, it is an efficient remediator of NO_3-N from wastewater (Liu et al. 2015). In this study no blooms were observed and *Pseudanabaena* sp. was observed at a N:P ratio of 2-2.6, while *Pseudanabaena limnetica* was observed at a N:P ratio of 6-12.

4.4 Microbial communities associated with *Ruppia*

Seagrasses such as *Ruppia* and *Althenia* are critical to the aquatic system as ecosystem engineers that are primary producers as well as a food source and habitat (e.g. Tarquinio et al. 2019). The Millennium Drought, particularly during the period from 2001 to 2010, caused severe reductions in freshwater inflows from the River Murray to the Coorong and resulted in a decline in its ecological condition. This was particularly evident

in the South Lagoon which experienced extreme increases in salinity (Leterme et al. 2015) and a widespread loss of aquatic vegetation and declines in the diversity and abundance of fish, waterbirds and macroinvertebrates (Brookes et al. 2018). In order to improve the ecosystem health of the Coorong South Lagoon, research into the water quality and the microbiota of the system needs to be conducted simultaneously to better understand the microbial communities associated with the species in the *Ruppia* Community. Understanding the structure and dynamics of these microbial communities is likely a key determinant of restoration and conservation success.

Two classes of the phylum Firmicutes (i.e. Bacilli and Clostridia) were mainly associated with *Ruppia* and *Althenia* roots and leaves and observed in the sediment samples (Figure 22). Previous studies have shown that Clostridiales is often part of the microbiome of seagrass species (Cúcio et al. 2016; Garcias-Bonet et al. 2020). Tariquinio (2021) suggested that the association between Clostridiales and seagrasses could benefit the overall seagrass health, since some members of this class can be involved in nitrogen fixation (Minamisawa et al. 2004), fermentation (Cato et al. 1986), as well as sulfate reduction (Widdel, 2006). Although, these functions are highly dependent on the operational group which is in turn dependent on the fermentable substrate(s) available. While Clostridiales were identified in the sediment and on a majority of the root system of *Ruppia* and *Althenia*. Fedorov et al. (2019) determined that the ability of the Clostridiales to reduce sulfite to sulfide was reliant upon a number of factors including population present, temperature and substrate availability. Further studies need to be undertaken to determine if Clostridiales are able to be used or assist in the restoration of high level sulfide sites in the Coorong. In particular, future work could focus on the function that Clostridiales might have during the different stages of the *Ruppia* community life cycle in the Coorong, and could thus potentially be used as a promoter for restoration. While in terrestrial systems microbiomes are manipulated to achieve positive restoration outcomes, this has not yet been attempted in seagrass restoration projects. Considering microbiota in seagrass restoration is essential to ensure that transplants are equipped to adapt to their new environments.

4.4.1 *Ruppia* or *Althenia* roots

The microbial community plays an active part in seagrass growth, development, and resistance to stressors (Liu et al. 2017a; Tarquinio et al. 2019). Studies have shown that different aspects of the seagrass plants have distinctive bacteria communities commonly associated with them. Our findings of the microbiota associated with the *Ruppia* and *Althenia* roots are consistent with previous studies examining associated bacteria with the root system of seagrasses (Liu et al. 2017b, Ferrando et al. 2015, Marques et al. 2015, Tarquinio et al. 2019). Seagrasses are highly selective of the potential colonising organisms with phyla such as Proteobacteria, Actinobacteria, Firmicutes and Bacteroides being commonly associated with roots (Liu et al. 2017b; Bulgarelli et al 2013; Reinhold-Hurek et al. 2015). The phylum Proteobacteria includes species that are essential for ecosystem health, undertaking nitrogen fixation, sulphur, and methane oxidation (Zhou et al. 2020). The selection of the microbiota is partly happening through seagrasses root exudates, such as amino acids, organic acids, sugars, which contribute to microbial growth, thus increasing the microbial abundance of certain phyla (Haichar et al. 2014; Somenahally et al. 2011). Here, the identification of potentially beneficial bacteria associated with the roots of the *Ruppia* community provides avenues for a specific selection of these bacteria to improve seagrass restoration in the Coorong.

In this study, the phylum Campylobacterota was present in *Ruppia* and *Althenia* root and leave samples, with higher relative abundance in the root samples. Some studies have suggested that Campylobacterota associated to seagrass roots may serve as nitrogen fixers (McClung and Patriquin, 1980) and as detoxicant against the presence of Hydrogen sulfide (H_2S) (Tarquinio et al. 2021). Similarly, the class Saccharimonadia was associated to root, leaves and water samples of the South Lagoon and Central Coorong regions in October 2020 but absent from the sediment. Saccharimonadia has been shown to play an important role in resisting and removing toxic pollutants (Shi et al. 2021). During this productive period for *Ruppia* and *Althenia*, the presence of Saccharimonadia on leaves and roots could be an adaptation to reduce toxic conditions and reduce the availability of toxic compounds to the plants.

Finally, the phylum Fusobacteria was present in most of the root samples, but only in a few of the leave samples and in none of the water or sediment samples (Figure 22). Members of this phylum are able to

ferment carbohydrates, amino acids, and other organic acids and benefit from plant-derived carbon sources (Zhang et al. 2022).

The instability or inconsistency of the populations associated with the roots of *Ruppia* and *Althenia* could be impacted by various factors including seasonality, health of the ecosystem, water quality. However, another factor to consider is the influence of *Ruppia* community on the organisms. The release of volatile organic compound by plants to recruit organisms to colonise their roots is well documented (Rudrappa et al. 2008; Liu and Brettell, 2019; Raza and Shen, 2020; Santoyo, 2021; Wen et al. 2021). Interactions with beneficial organisms can provide plants with multiple benefits such as protection, stress relief, and a nutrient source.

4.4.2 *Ruppia* or *Althenia* leaves

The microorganisms associated with the seagrass leaves are highly variable with both generalist and specialist microorganisms, however, they are frequently related to the communities found in the surrounding water (Tarquinio et al. 2019). The communities associated with *Ruppia* and *Althenia* leaves in the Coorong study were consistent with those identified on other seagrass plants (Tarquinio et al. 2019). The main classes of prokaryotes observed on the leaves of *Ruppia* or *Althenia* were Actinobacteria, α-proteobacteria, Bacilli and γ-proteobacteria.

Research has not definitively determined if the communities exhibit differences due to the seagrass species (*Ruppia or Althenia*) or whether specific physico-chemical conditions influence those microbial communities present on the surface (Tarquinio et al. 2019). The proposed 'host effect' has been supported on a number of occasions, with settled microorganisms sharing a broad range of traits for a host-associated life (Tarquinio et al. 2019). Here, significant changes in the relative abundance of leaf-associated microbiota were identified between Noonamena and South Lagoon sites. These differences observed in salinity between these sites could be linked to specific leaf-associated microbiota (Figure 34). This analysis indicated that there were significant differences in the leaf-associated microbiota between Noonameena and Policeman Point (Figure 34 A) and between Noonameena and Wild Dog Island (Figure 34B). However, further work would be needed to decipher the impact that these different microbiotas might have on the health of *Ruppia* and *Althenia* and how useful they might be for future restoration efforts.

The leaf-associated microbiota can have a positive role in the development of seagrass. Indeed, Celdrán et al. (2012) showed through laboratory and in situ experiments that inoculating leaves of the seagrass *Posidonia oceanica* with *Marinomonas posidonica* enhanced leaf growth compared with the controls to which no bacterial strain was added. Likewise, inoculation of leaves with *Marinomonas posidonica* induced changes in the structure of the epiphyte community attached to the leaves. In contrast, inoculation with *Marinomonas mediterranea* had no effect which highlights how specific the effect of the microbiota is. The production of nutrients especially in oligotrophic waters, and their secondary metabolites are known to provide cues for seed germination (Celdrán et al. 2012). Taxa from the genus Marinomonas was commonly associated to the leaves and roots of *Ruppia* and *Althenia*. This genus is recognised as Plant Growth Promoting Bacteria (PGPB), which assist and increase growth and development in macroalgae from the genus Ulva (Singh et al. 2011). It is suggested that the same PGPBs have profound effects and enhance the development rate of seagrass leaves (Celdrán et al. 2012). Here, Marinomonas was also observed in filamentous algae mats and in PAA. Filamentous algal mat forming species *Ulva paradoxa*, *Cladophora* sp. and *Rhizoclonium* sp. have previously been reported in the Coorong (Asanopoulos and Waycott 2020), thus it is possible that members of the genus Marinomonas promotes the growth of the filamentous macroalgae in the Coorong.

4.4.3 Rare microbial taxa

While the majority of published research focuses on dominant microbial communities, there has been an increasing focus on the impact of rare taxa and their ecological relevance (Galand et al. 2009; Logares et al. 2014). Rare taxa have been found to be active members of microbiotic communities, keystone species within groups, as well as supporting biogeochemical functions (Hamasaki et al. 2007; Campbell et al. 2011; Hunt et al. 2013; Pester et al. 2010; Sjöstedt et al. 2012; Sauret et al. 2014). Alonso-Sáez et al. (2015) determined that

rare taxa had a predictable regular pattern of growth within the marine environment suggesting that the rare biosphere functions in parallel with its own seasonal dynamics.

One reason to be a member of the rare biosphere is that environmental conditions are unfavourable for growth (Jones and Lennon, 2010, Gibbons et al. 2013, Alonso-Sáez et al. 2014, Alonso-Sáez et al. 2015). However, favourable conditions existed in the past and will likely appear again in the future. The most obvious instance of this situation is the seasonal cycle (Jones and Lennon, 2010, Gibbons et al. 2013, Alonso-Sáez et al. 2014, Alonso-Sáez et al. 2015). Dormancy builds seed banks, which are reservoirs of inactive individuals that can potentially be resuscitated in the future under a different set of environmental conditions (Jones and Lennon, 2010, Lennon and Jones 2011, Gibbons et al. 2013, Alonso-Sáez et al. 2014, Alonso-Sáez et al. 2015). These studies suggest that, in addition to being an important diversity-maintaining mechanism, dormancy may have important implications for understanding and predicting ecosystem processes (Jones and Lennon, 2010, Lennon and Jones 2011, Gibbons et al. 2013, Alonso-Sáez et al. 2014, Alonso-Sáez et al. 2015). Some bacteria may be adapted to use only a few specific substrates that are present only at certain times, or to episodic situations of high nutrient abundance (Kalenitchenko et al. 2018).

This kind of situation likely occurs constantly for many different bacteria. The rank-abundance distribution must be a dynamic one, with members of the abundant part decreasing toward the rare biosphere and vice versa, as environmental conditions change both regularly (as with seasons) and episodically (as with heavy rains or storms) (Shade et al. 2014, Kurm et al. 2019). Shifts in environmental cues can affect the dormancy of microbial communities, specifically member of the rare biosphere, in ways that maintain biodiversity and influence ecosystem processes (Lawson et al. 201, Liang et al. 2020).

However, some rare groups of bacteria contribute disproportionately to certain biogeochemical processes (Shade et al. 2014, Jousset et al. 2017). Species that are considered functionally non-relevant under a given environmental condition may become important under changing conditions by providing necessary traits or acting as partners in new interspecific interactions (Shade et al. 2014, Jousset et al. 2017). Rare species offer a pool of genetic resources that may be activated under the appropriate conditions (Chen et al. 2020).

Microbial communities play a key role in the degradation of organic compounds, including pollutants and the insurance provided by rare species can therefore contribute to ecosystem resilience to anthropogenic pollution (Jousset et al. 2017, Gaur et al. 2018). Organic pollutant degradation involves complex metabolic pathways shared across different species (Gaur et al. 2018). In marine ecosystems, conditionally rare bacterial taxa tend to be more active when rare. In fluctuating conditions, a rare taxon could be disproportionally active due to continuous regrowth. Indeed, rare species could sustain community activity and could be functionally dissimilar from abundant ones, therefore likely to offer complementary, or unique, metabolic pathways to support community function (Shade et al. 2014, Lawson et al. 2015, Gomez-Alvarez et al. 2016, Kaminsky and Morales 2016, Nyirabuhoro et al. 2020, Wang et al. 2020).

Accordingly, the presence of rare microbes could induce metabolic responses in more abundant microbes, implying that rare microbes have indirect effects on ecosystem functioning. The presence of rare species of microbes in bacterial communities was associated with the maintenance of important ecological functions (Jousset et al. 2017). For example, reduced fungal growth via production of antifungal volatiles has been observed suggesting that rare bacteria either produce or trigger dominant bacteria to synthesize these compounds (Hol et al. 2015).

4.5 Management implications

4.5.1 Microbial community composition

There was a strong Common Core microbial community present across the Coorong South Lagoon sites. However, the North Lagoon site, Noonameena differed significantly in most analyses. This further supports the existence of fundamentally different ecological processes operating in the North Lagoon represented by the Noonameena site. The common core microbes, essential to the function of the communities they occupy and consequently their loss would reflect the impact of any system scale perturbations. Further examination

of the specific Coorong South Lagoon Common Core microbial community, and the types of perturbations that would lead to significant change, for example loss of diversity, would assist the development of a useful monitoring tool in the extreme physico-chemical environment in the Coorong. In the last decade, there has been a loss in the total number of microalgal species from 241 to 81 based on detailed surveys. This indicates the long-term decline of the system has been accompanied by a loss in biodiversity.

The microalgal community composition varied significantly in the southern Coorong and corresponds to changes in chemical composition of the water and sediment, nutrient availability and the form nutrients are in, the presence of a macrophyte community, and the physical environment particularly temperature. As a result, there is sufficient evidence that an evaluation of microbial community composition could provide insights into the recent status of the ecosystem. It is likely that additional data collection could link to ongoing planned water quality monitoring to deliver such information, although evaluation of the steps to do this requires the development of targeted evaluation questions. Critical to analysing the diversity present was the addition of DNA analysis to determine the composition of the microbial community. It would be of benefit to include ongoing monitoring of the community with traditional morphology, jointly with the DNA screening tools. In addition, the detection of rare taxa through these methods is informative to identify microbial groups that may have important ecological roles such as denitrification or potentially cause side effects such as harmful algal bloom forming species.

Seasonal variation in the microbial community varied based on substrate, the least seasonally variable was sediment as substrate (e.g. 23% seasonal specific AVLs). Sampling in this study was conducted in the near-shore littoral zone that experiences annual drying followed by inundation due to changing water availability. As a result, the conditions in sediments are likely to be more stable than in the water column or plants which experience greater change. The microbial communities of deeper waters should be also assessed for seasonal variability to inform community composition changes.

The dominant presence of Desulfobacterota in sediments at particular sites conforms to the observation that in many locations the littoral zone has an annual deposition of black oozes into the sediments from decaying organic matter. The presence of the sulphur reducing bacterial microbes confirms that the nutrient cycling processes in these areas have led to anoxic conditions.

Communities of HAB forming species were significantly different between samplings sites and dates. There were several HAB taxa detected but only one was present in larger numbers during these surveys (*Nitzschia* sp. at Noonameena). One class of HAB forming microbe, the Gymonodinoids did not form a bloom during the period of study, but was present at all sites and during each sampling period. The abundance of Gymonodinoids was always higher than the recommended level for human health. Awareness of these taxon groups through ongoing monitoring would be highly desirable if timely interpretation is possible.

4.5.2 Microbial community and nutrients

The microbiota community contributed more than half of the pelagic nutrients (TN and TP) throughout the period of study (October 2020, December 2020, March 2021 and June 2021), except at Noonameena. In Spring, however, the microbiota accounted for up to 85% of TN and 90% of TP across sites 1 (Wild Dog Island) to 4 (North Magrath Flats), but only accounted for 69% of TN and 1% of TP at Noonameena. At the same time low N:P ratios were observed. Such low ratios can lead to an increase in the N-fixing species like cyanobacteria which could be helping the system to stay within a cycle of internal nutrient cycling through water-column N fixation and P releases from sediments. The dominance of the picophytoplankton in this study reflects their highly opportunistic nature such as in a nutrient-limited environment. Here, their presence could be associated with the low levels of nutrients observed in the water column, especially the limitation in Nitrogen. The identification of nutrient sources and maximum allowable inputs of nutrients into aquatic ecosystems will help guide potential restoration measures.

4.5.3 The *Ruppia* Community holobiont

The microorganisms associated with the leaves of seagrass are recruited from the water column thereby creating site specific communities. Taxa from the genus Marinomonas was commonly associated to the leaves and roots of *Ruppia*. There was partially a different microbial community in the South Lagoon compared to Noonameena associated with the plants. The *Ruppia* Community 'holobiont', or symbiotic relationship between seagrasses and microorganisms, with both the organisms and the seagrass forming an integrated community that survives due to the support provided by each member. Shoots and roots had different microbial communities where it is likely these perform different functions and are supported by the presence of the *Ruppia* Community in different ways. As there are a growing number of sites across the Coorong that despite the presence of a *Ruppia* Community are subject to poor sediment and water quality affecting plant condition.

4.5.4 Knowledge gaps and microbiota associated research supporting management of the Coorong

The link between ecological function and diversity in microbial communities is associated with their fast turnover times and responsiveness to local conditions. Both diversity and abundance change in response to features of the environment that are the focus of environmental management actions including water quality, nutrient loads, turbidity, chemical composition of the water and sediment. This study has developed an understanding of broad scale seasonal changes in the microbial community across the southern Coorong and linked many of these aspects of environmental management to the microbiota present in the Southern Coorong ecosystem. The ecological functions of identified microbiota have been inferred from their taxonomic groups, rather than testing of direct ecological functions. Potential changes in the microbiota may provide the first evidence of functional changes in sediments, a high priority for proposed intervention options from Phase 1 of the Healthy Coorong, Healthy Basin program (Healthy Coorong, Healthy Basin 2022) Such data sets would need to be collected at multiple scales and we suggest they include;

- Documenting changes to the microbiota at a greater frequency to determine the dynamics of the community more closely aligned with changing variables in the Coorong ecosystem, likely monthly.
- Investigating species level microalgae for high priority groups such as Harmful Algal Bloom forming groups.
- Determining the variation in microbiota presence and abundance over longer periods to determine their responses to changing Coorong ecosystem inputs;
    - freshwater inputs from the River Murray through barrage releases,
    - inputs to the South Lagoon via Salt Creek,
    - groundwater inputs where they are identified as significant in affecting local conditions.
- Improving the resolution of community shifts associated with microbiota-driven nutrient cycling.
- Utilising DNA screening such as presented in this study to improve the diversity of taxon groups able to be detected. This study utilised probes based on the widely applied 16S rRNA diversity, future studies could explore a greater range of screening methods cost effectively. This approach can specifically target groups for example, the anammox bacteria that play an important role in nutrient cycling in the Coorong and would provide powerful insights if associated directly with monitoring of water quality and nutrients.
- Improving our understanding of the sediment–water column–plant microbiome to support improved restoration outcomes and inform assessment of ecological condition. The application of community assessment above would be enhanced by;
    - Researching metabolomics, the role of microbiota in growth of *Ruppia* where a food source or by-product changes with colonisation of leaves and roots by specific microbiota. This

would provide insights in the microbiota that is needed for future restoration under specific conditions.

- The 'rare' taxa present in the Coorong could be explored further to investigate how their presence influences growth and survival of the *Ruppia* Community, in particular, their importance in the root-sediment interface and successful colonisation and improving productivity.

List of shortened forms and glossary

AGRF	Australian Genome Research Facility
Amplicon	A piece of DNA that has been amplified via PCR
Anammox	Anaerobic Ammonium Oxidation by which bacteria transform ammonium and nitrogen dioxide into nitrogen gas and water
Anoxygenic	Not reliant on oxygen for the electrons used in photosynthesis
Biofilm	A consortium of bacteria which are surrounded by a matrix of extracellular polymeric substances
Bioindicator	Organisms that can be used to monitor the health of an ecosystem
Bloom	Rapid increase or accumulation in the population of microalgae in aquatic systems
Chimeras	The byproduct of two or more incorrectly joined sequences
DNA	Deoxyribonucleic acid
DO	Dissolved Oxygen
eDNA	Environmental Deoxyribonucleic acid
Eukaryotes	Organisms whose cells contain a nucleus and other membrane-bound organelles
HABs	Harmful algae species
HCHB	Healthy Coorong, Healthy Basin Program
Hyper-eutrophic	A body of water extremely rich in nutrients and minerals
Laboratory detection limits	The smallest amount or concentration of an analyte that can be reliably distinguished from the baseline
Metabolomics	Study of the complete set of metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes
Microbiome	The community of microorganisms that can usually be found living together in any given habitat.
Millennium Drought	An Australian drought which impacted the Murray-Darling Basin over the period 1996-2010, and substantially impacted the Coorong over the period 2001-2010.
Mixotrophy	The ability to assimilate organic compounds as carbon sources while using inorganic compounds as electron donors
Oligotrophic	A body of water having a deficiency of nutrients
PAA	Plant Associated Aggregate
PCR	Polymerase Chain Reaction
Phyllosphere	The surface of a plant which is above ground which microorganisms can colonise
Picophytoplankton	Microalgal cells < 5-6 µm in diameter that are present in nearly all aquatic systems
Prokaryotes	Organisms that lack a distinct nucleus and other organelles due to the absence of internal membranes

Rhizosphere	A section of soil which is influenced directly by the microorganisms associated with plant roots
RNA	Ribonucleic acid
***Ruppia* Community**	The multi species assemblage that has become established across the southern Coorong and includes *Ruppia tuberosa*, *Althenia cylindrocarpa* along with an as yet unresolved species of *Ruppia*.
SEM	Scanning electron microscopy
Stoichiometry	The relationship between the relative quantities of substances taking part in a reaction or forming a compound, typically a ratio of whole integers
T&I	Trials and Investigations project

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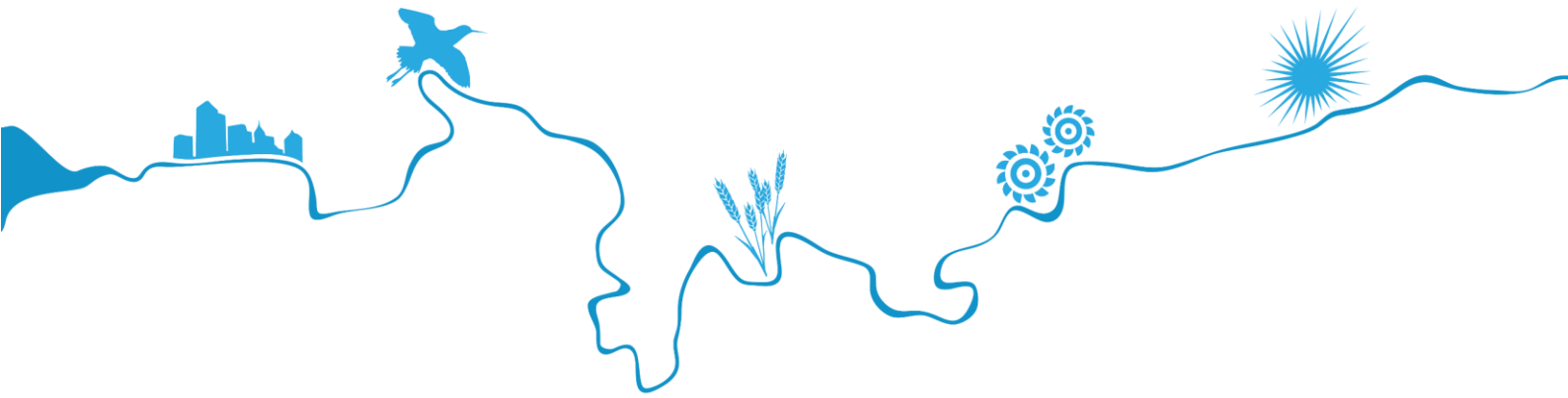

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