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Drivers of bacterial diversity along a natural transect from freshwater to saline subtropical wetlands

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Keywords

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Abstract

Tropical coastal wetlands provide a range of ecosystem services that are closely associated with microbially-driven biogeochemical processes. Knowledge of the main players and their drivers in those processes can have huge implications on the carbon and nutrient fluxes in wetland soils, and thus on the ecosystems services we derive from them. Here, we collected surface (0-5 cm) and subsurface (20-25 cm) soil samples along a transect from forested freshwater wetlands, to saltmarsh, and mangroves. For each sample, we measured a range of abiotic properties and characterised the diversity of bacterial communities using 16S rRNA gene amplicon sequencing. The alpha diversity of bacterial communities in mangroves exceeded that of freshwater wetlands, which were dominated by members of the Acidobacteria, *Alphaproteobacteria* and Verrucomicrobia, and associated with high soil pore-water concentrations of soluble reactive phosphorous, and nitrogen as nitrate and nitrite ($N\text{-}NO_X^-$). Bacterial communities in the saltmarsh were strongly stratified by depth and included members of the Actinobacteria, Chloroflexi, and *Deltaproteobacteria*. Finally, the mangroves were dominated by representatives of *Deltaproteobacteria*, mainly *Desulfobacteraceae* and *Synthrophobacteraceae*, and were associated with high salinity and soil pore-water concentrations of ammonium ($N\text{-}NH_4^+$). These communities suggest methane consumption in freshwater wetlands, and sulphate reduction in deep soils of marshes and in mangroves. Our work contributes to the important goal of describing reference conditions for specific wetlands in terms of both bacterial communities and their drivers. This information may be used to monitor change and assess wetland health and function.

1. Introduction

Tropical coastal wetlands are among the most valuable ecosystems on the planet (Costanza et al., 1997). They cover less than 8% of the Earth's surface, but provide a range of highly valued ecosystem services, such as habitat provision and coastal protection (Barbier, 2006; Sievers et al., 2019). Coastal wetlands can also mitigate climate change by capturing and storing carbon, and ameliorate pollution by removing excess nutrients (Adame et al., 2019; Duarte et al., 2013). Human activities often impact coastal wetlands with consequences for the provision of their associated ecosystem services. As many of these services are coupled with the activities of soil bacteria, it is important to monitor wetlands using an integrative approach that considers not only abiotic properties, but biotic characteristics as well (Holguin and others 2001; Sims and others 2013).

Soil bacteria play an essential role in carbon and nitrogen cycling in wetlands, which are considered biogeochemical "hotspots" (Cheng and Basu, 2017). For carbon, the ratio between methanotrophs and methanogens indicates whether wetlands are sinks or sources of methane (Conrad, 2007). The abundance and diversity of aerobic soil bacteria can also be associated with the accumulation of organic matter (Wu et al., 2015). For nitrogen, the abundance and diversity of denitrifying bacteria may be associated with a wetland's potential to remove nitrogen pollution (Peralta et al., 2010). However, some biogeochemical processes, such as denitrification, are better explained by environmental parameters, such as nitrate concentration, vegetation, and flooding frequency, rather than by bacterial community composition (Song et al., 2012, 2010). It has been recognised that soil pH, organic carbon content, redox status, moisture, and availability of nitrogen and phosphorus are among the major environmental predictors of

bacterial community structure and composition in soil, with plant identity and other biotic factors being among the least important factors (Fierer, 2017). In coastal wetlands, redox potential plays an important role in controlling bacterial activity and function (Urakawa and Bernhard, 2017). Hence, changes in bacterial communities and the factors that influence them could be used as comprehensive indicators of wetland health (Adame et al., 2012; Hartman et al., 2008; Urakawa and Bernhard, 2017).

Traditionally, wetland health has been assessed based on field observations and monitoring of water quality and chemistry. This further evolved into integrative approaches using chemical, physical and biological indicators, and the development of wetland assessment frameworks, such as the three-level approach of the US Environmental Protection Agency (US EPA, 2006), which consists of habitat inventory and landscape assessment (Level 1), rapid field sampling and wetland assessment (Level 2), and rigorous biological and physicochemical site assessment (Level 3) using indices of biological integrity (IBI) and hydrogeomorphic (HGM) classification. In Australia, a national framework for Assessment of River and Wetland Health (FARWH, 2011) has been standardised based on seven index themes scored 0-1 ("largely unmodified" to "severely modified") with the requirement for selection of locally relevant sub-indicators for each wetland type. Although the relevance of microbial indicators for assessment of wetland health is recognised (van Dam et al., 1998; Sims et al., 2013; Urakawa and Bernhard, 2017), they are not currently listed among the recommended biological indicators for national frameworks. This may be attributed to a lack of practical testing/evaluations and knowledge of key microbial processes and players in non-disturbed systems. The majority of wetland health assessments today are still using wetland area as the primary indicator of condition (Dixon et al., 2016), including remote-sensing techniques to establish a baseline or reference condition (Bunting et

al., 2018). Fewer studies have considered vegetation, vertebrates and macroinvertebrates, and even fewer, bacterial communities and biogeochemical processes (Vovides et al., 2011). The advantage of monitoring microbial communities over traditional, or other bio-, indicators is the sensitivity of bacteria to small fluctuations in their environment due to their high surface-volume ratio (Sims et al., 2013). Thus, microbial indicators could be useful to detect degradation in wetlands at an early-stage (Merkley et al., 2004). Advances in microbial community profiling have resulted in rapid and cost-effective technologies that could be used to routinely track changes in bacterial communities and their associated environmental drivers. Such technologies can be highly standardised and provide an excellent opportunity to describe a reference condition or baseline of a given wetland for further comparison and interpretation of monitoring results based on microbial indicators.

In this study, we collected surface and subsurface soil samples along a transect from forested freshwater wetlands, to saltmarsh and mangroves. For each sample, we measured a range of abiotic properties and characterised the diversity of bacterial communities using 16S rRNA gene amplicon sequencing. Our objectives were: a) to characterise the diversity and composition of bacterial communities of freshwater and saline coastal wetlands of surface and sub-surface soils, and b) to determine the potential environmental drivers associated with the bacterial communities identified. We provide a baseline of natural bacterial communities in wetlands with low anthropogenic impacts and the natural environmental drivers associated with them. With this information, we infer potential biogeochemical pathways that could explain some of the ecosystem services we derive from them.

2. Materials and Methods

2.1. Site description and sampling

Samples were collected from subtropical wetlands on North Stradbroke Island (Minjerribah) in southeast Queensland, Australia (27º5' S, 153º45' E; Fig. 1). The island has a subtropical climate with an annual mean minimum and maximum temperature of 19ºC and 26ºC, respectively (1997-2020, Australian Bureau of Meteorology, ABM, 2020). Mean annual rainfall is 1400 mm, with most of it falling in the summer months (ABM; Station number 040537). The terrestrial vegetation is predominantly eucalypt woodland and heath communities with low bushland and mangroves around the coastal fringes (Clifford and Specht, 1979). The hydrology of the island is dominated by groundwater flows recharged by local rainfall (Cox et al., 2011).

Soil samples were collected during the dry season (August 2012). We sampled three sites along a transect (~130 m) from a freshwater wetland dominated by *Melaleuca quinquenervia* and ferns, to a sedge-like salt marsh dominated by *Juncus* spp., and finally into mangroves dominated by *Avicennia marina* and *Rhizophora stylosa* (Fig. 1). The distance between sampling sites was c. 40-50 m from freshwater to salt marsh, and c. 60-80 m from salt marsh to mangroves. At each site, three 10 m^2 plots separated by about 30 m were randomly selected. At each plot, we took eight cores with a steel corer of 10 cm diameter and 40 cm in depth. The soil cores were divided into surface (0-5 cm) and subsurface (20-25 cm) samples and then mixed to produce a single composite sample per depth for each site. Thus, total of 18 composite samples (3 sites x 3 plots x 2 depth) were prepared and c. 500 g of each sample was set aside for physicochemical analyses. Samples were transported to The University of Queensland on ice and frozen on the same day.

2.2. Physicochemical characterisation

Surface and subsurface soil temperatures were measured *in situ* using a HA145-20 thermometer (Hanna Instruments, USA) equipped with a 300 mm stainless steel probe. Soil pH and electrical conductivity (EC) were measured in 1:2 soil:water (w/v) suspension after 30 min of incubation using a pH meter F-54BW (Horiba, Japan). Salinity was determined using a hand-held refractometer (model 300011 w/ATC, SPER Scientific, Scottsdale, USA) from the soil pore-water extracted by centrifugation at 12,000 rpm for 15 min at 4ºC. The remaining samples were submitted for further physicochemical characterisation at the Forensic and Scientific Services at Queensland Health (Brisbane, Australia). Samples were analysed for moisture content by freeze-drying and for total organic carbon (TOC) by combustion. Total phosphorus (TP) and total Kjeldahl nitrogen (TN) were measured by simultaneous persulfate and Kjeldahl digestion, correspondingly, followed by segmented flow analysis in an AutoAnalyzer II (Bran+Luebbe, Germany). Finally, ammonium (NH_4^+), nitrogen oxides ($N\text{-}NO_x^- = NO_2^- + NO_3^-$), and soluble reactive phosphorous (SRP) were measured from the soil pore-water by flow injection analysis using an automated QuikChem 8500 Flow Injection Analysis system (Lachat, USA).

2.3. Bacterial community characterisation

Total DNA was extracted from 600 mg fresh soil using PowerSoil DNA Isolation kits (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions, except for: an additional initial incubation step at 65ºC for 5 min; a reduction in the vortex time during bead beating to five min; and elution of DNA in 55 µl of sterile DNA-free PCR grade water. DNA concentration and purity were determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The DNA concentration per sample was then normalised to 10 ng μl^{-1}. Bacterial 16S rRNA genes were PCR amplified using the barcoded primers 27F and 519R (Lane, 1991) and then sequenced on a 454 GS FLX Titanium instrument

(Roche) using Titanium XLR70 chemistry at the Australian Genome Research Facility Ltd (AGRF), Brisbane, Australia.

Sequences were quality filtered and dereplicated using the QIIME script split_libraries.py with the homopolymer filter deactivated (Caporaso et al., 2010) and then checked for chimeras against the 2013 release of the GreenGenes database (DeSantis et al., 2006) using UCHIME ver. 3.0.617 (Edgar et al., 2011; McKinnon et al., 2003) Homopolymer errors were corrected using Acacia (Bragg et al., 2020). Sequences were then subjected to the following procedures using QIIME: 1) sequences were clustered into Operational Taxonomic Units (OTUs) at 97% similarity using UCLUST (Edgar, 2010) cluster representatives were randomly selected, 3) GreenGenes taxonomy was assigned to the cluster representatives using BLAST, and 4) tables with the abundance of different OTUs and their taxonomic assignments in each sample were generated. The number of reads was then rarefied to 5,800 per sample and numbers of observed OTUs (Sobs; richness), and Simpson's Diversity Index values (composite measure of richness and evenness) were calculated using QIIME to represent the alpha diversity of each sample. The NCBI BioProject accession number for 16S rRNA gene sequences data deposited for this study is PRJNA268784.

2.4. Statistical analyses

Differences in the observed numbers of bacterial OTUs and community composition (Hellinger transformed OTU relative abundances) between sites and depths were investigated using Analysis of Variance (ANOVA) and Permutational Multivariate Analysis of Variance (PERMANOVA), respectively. The influence of metadata parameters on changes in the composition of microbial communities between sites (beta diversity) was assessed using

Permutational Multivariate Analysis of Variance (PERMANOVA). Parsimonious PERMANOVA models were built by forward selection. Associations between OTUs and environmental parameters were investigated by superimposing the metadata as fitted vector on a Principle Component Analysis (PCA) of the Hellinger transformed OTU abundances. All analyses were implemented using R.

3. Results

3.1. Environmental characteristics

Environmental conditions differed between sites and depths along the wetland successional gradient (Tables 1 & 2; Fig. 2A). Differences in physicochemical parameters between depths were most apparent within the salt marsh (Table 1; Fig. 2A). Freshwater wetlands were associated with higher concentrations of soil pore-water SRP and $N\text{-}NO_x^-$ (Table 1; Fig. 2A). Within the saltmarsh, surface samples were associated with higher concentrations of soil TOC, TN and TP. In comparison, subsurface samples had higher concentrations of $N\text{-}NO_x^-$, which increased their similarity to freshwater wetlands (Table 1; Fig. 2A). Mangroves were associated with higher salinity, EC, pH and $N\text{-}NH_4^+$ concentration compared to other wetlands (Table 1; Fig. 2A).

3.2 Bacterial community composition and its relationship with environmental conditions

Bacterial community composition differed significantly between sites and depths, with the largest differences between depths being observed in the saltmarsh (Table 1, Fig. 2B). In addition, the similarities between samples based on bacterial community composition were

significantly correlated with those based on environmental conditions (R^2 = 0.63, P < 0.001, Mantel; P = 0.014, Procrustes).

Mangrove communities were dominated by representatives of the *Deltaproteobacteria*, which were positively associated with salinity, EC, N-NH_4^+, TP and pH (Figs. 2B, 3, and S1). Saltmarsh communities differed considerably between depths, with dominant members of the Actinobacteria, Chlorobi, Gemmatimonadetes, Nitrospirae, Proteobacteria, and Verrucomicrobia at both depths, and more frequent occurrences of an Acidobacteria (OTU 7), a Chlorobi (OTU 12), three Chloroflexi (OTU 13-15), two Nitrospirae (OTU 19-20), a Planctomycetes (OTU 21), two *Deltaproteobacteria* (OTU 38, 40), and two Spirochaetes (OTU 43-44) populations at 20-25 cm depth (Figs. 2B and 3). Lastly, freshwater forested wetlands were dominated by members of the Acidobacteria, *Alphaproteobacteria*, and Verrucomicrobia, with OTUs 4, 6, 8 and 9 (Acidobacteria) and the *Rhodospirillales* (OTU 30-32, *Alphaproteobacteria*) being more abundant in sub-surface than surface soils. Freshwater wetland communities were positively associated with soil pore-water concentrations of SRP and N-NO_x^- (Figs. 2B and 3).

3.3 Variation of bacterial diversity in wetland soil with site and depth

The alpha diversity of bacterial communities also differed significantly between sites and depths (Table 3; Figs. 4 and S2). Mangrove and freshwater forested wetlands hosted the most, and least diverse communities, respectively, while the diversity of saltmarsh bacterial communities lay between the two (Fig. 4). Differences in alpha diversity between depths were only apparent in the saltmarsh communities, which were less diverse at depth (Fig. 4).

4. Discussion

Our study highlights that freshwater forested wetlands, saltmarsh and mangroves have distinct bacterial communities that are associated with the environmental conditions that characterise these ecosystems. Freshwater wetlands had the highest SRP and N-NO_X^- concentrations of soil pore-water, and a bacterial community dominated by *Alphaproteobacteria*, Acidobacteria, and Verrucomicrobia. Saltmarsh samples were characterised by bacterial communities strongly stratified by depth, and included members of Actinobacteria, Chlorobi, Chloroflexi, Nitrospirae and *Deltaproteobacteria*. Finally, mangroves were dominated by *Deltaproteobacteria*, mainly *Desulfobacteraceae* and *Synthrophobacteraceae*, which were associated with high salinity, EC and N-NH_4^+ concentrations in the soil pore-water.

Within the freshwater forested wetlands, the most dominant *Alphaproteobacteria* were members of the *Rhizobiales* and *Acidiphilium*, which are frequently observed in temperate and tropical peat swamp forests and lakes (Briée et al., 2007; Kanokratana et al., 2011; Pankratov et al., 2011). The *Rhizobiales* populations within the alpha cluster (OTUs 22 and 23) are closely related to taxa that are putative methanotrophs in sphagnum peat bogs (Pankratov et al., 2011). The presence of such methanotrophs could imply methane oxidation within freshwater wetlands and thus their potentially important role in mitigation of methane emission in these environments. Close relatives of the *Acidiphilium* population (OTU 29) have been shown to reduce ferric iron and are found in acidic habitats, such as wetlands impacted by acid mine drainage, where metal-rich soils can favour growth (Johnson, 1998). The Acidobacteria include many representatives that thrive in acidic soils with high organic matter (Hartman et al., 2008), and this likely explains their prevalence in the freshwater forested wetlands. The composition of acidobacterial assemblages has also been shown to vary with N-N-NO_x^-, N-NH_4^+, TOC, TN, C:N ratio, TP, soil

moisture, and temperature (Naether et al., 2012). Here we found that freshwater forested wetlands were positively associated with $N\text{-}NO_x^-$ and at least one population (OTU 5 *Candidatus* Koribacter) that is known to be capable of reducing nitrate and nitrite, and using a variety of carbon substrates (Ward et al., 2009). Verrucomicrobia, which also appear to thrive in the freshwater forested wetlands, include representatives that are adapted to oligotrophic conditions and may oxidise methane in acidic soils (Dunfield et al., 2007; Kolb and Horn 2012). Overall, the community composition in freshwater wetlands may suggest an aerobic metabolism in a low pH that potentially favours methane consumption.

Saltmarsh bacterial communities included taxa that are associated with freshwater and marine ecosystems. Their composition was similar to other subtropical saltmarshes, where Actinobacteria, Nitrospirae, Chlorflexi, and Proteobacteria have also been found (Gong et al., 2018; Sun et al., 2019). Members of Actinobacteria are associated with plant growth promotion and stress tolerance, hence they may help saltmarsh plants function in saline conditions (Gong et al., 2018). Nitrospirae populations may contribute to nitrogen fixation, and Proteobacteria has been associated with soil carbon stabilisation (Holguin et al., 2001; Sun et al., 2019). Additionally, *Deltaproteobacteria* has been found to be responsible for methanogenesis of choline and consumption of hydrocarbonates in temperate saltmarshes (Jameson et al., 2019; Pearson et al., 2008). The abiotic and biotic characteristics of saltmarsh soils were the most strongly stratified by depth. Microbial communities within deeper horizons included representatives of the Chloroflexi (OTUs 14-16), which are closely related to taxa previously observed in organic, sulphide-rich soils, such as in springs and marine environments (Elshahed et al., 2003; Harrison et al., 2009). Similar stratification has been found in other saltmarshes with deep communities adapted to anoxic conditions (Lambais et al., 2008). For instance the group of

Planctomycetes has a unique anaerobic, autotrophic metabolism that can oxidize ammonium (annamox) (Fuerst and Sagulenko, 2011) and members of the Spirochaeta include obligate and facultative anaerobes, which can consume sulphide and remove oxygen from the saltmarsh soils (Stephens et al., 2008).

For mangroves, we found a bacterial community dominated by members of sulphate reducing *Deltaproteobacteria* such as those of the order *Desulfobacterales* (Sva0081 soil group, SEEP-SRB1) and *Syntrophobacterales* (*Syntrophobacteraceae*). Both orders contain sulphate-reducing, strictly anaerobic bacteria, which are one of the most abundant group in mangrove soils (Andreote et al., 2012; Ikenaga et al., 2010; Taketani et al., 2010). These bacteria are major decomposers of organic matter in mangroves (Holguin et al., 2001). Members of *Syntrophobacterales* were evenly distributed between the surface and subsurface mangrove soils. In contrast, members of *Desulfobacterales* varied with depth, probably reflecting salinity, redox preferences or a decrease in carbon with depth (Taketani et al., 2010).

5. Conclusions

Overall, nutrient concentrations and salinity changes along the natural gradient of wetlands towards the sea were associated with changes in the bacterial communities dominating each wetland. While shifts in bacterial communities may also be attributable to changes in parameter that were not measured, some significant association were identified. Freshwater forested wetlands had high SRP and N-NO_X^- availability, low salinity/conductivity, and low pH, while mangroves had high salinity/conductivity, pH close to neutral and high NH_4^+ in the pore-water. Coastal wetlands such as those studied here are particularly vulnerable to changes due to their

fringing position as they receive the impact from both the land and the sea. Changes in nutrient discharge into these wetlands due to increased land use, increased frequency of fire events or changes in salinity due to sea-level rise or groundwater extraction could result in changes in the wetland composition. As a result, bacterial communities of these wetlands and the biogeochemical pathways could also be altered and thus, the ecosystem services derived from them.

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Declaration of competing interests

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

Fig. 1 Location of sampling sites on North Stradbroke Island (A-D).

Fig. 2 A) Variation in the environmental parameters among wetland types (freshwater forested wetlands, saltmarsh and mangroves) for surface (0-5 cm) and subsurface (20-25 cm) soils, and B) relationship between soil bacterial communities and environmental variables indicated by continuous arrows, dashed arrows (i.e., TN, TOC) are non-significant associations. Numbers on the crosses in panel (B) correspond to the OTUs identifiers on Fig. 3.

Fig. 3 Heatmap summarising the relative abundances of OTUs present at >1% in at least one sample in surface (0-5 cm) and subsurface samples (20-25 cm), by wetland type (mangrove, saltmarsh, freshwater). The numbers in brackets are OTU identifiers and correspond to the OTUs shown in other figures. Taxonomic assignments of OTUs are given at the highest ranks (phylum/proteobacterial classes) at the left and at the lowest taxonomic ranks resolved at the right of the heatmap.

Fig. 4 Differences in the alpha diversity of bacterial communities between sites and depths as represented by A) the numbers of observed OTUs, and B) Simpson's Diversity Index. The letters above the bars indicate Tukey's Honest Significant Difference *post hoc* test results. The error bars are standard errors of the means.

Table 1 Soil and pore water characteristics of subtropical mangrove, saltmarsh and freshwater forested wetlands.

	Mangrove		**Saltmarsh**		**Freshwater**	
Depth	0-5 cm	20-25 cm	0-5 cm	20-25 cm	0-5 cm	20-25 cm
Temperature (°C)	15 ± 0.26	13 ± 0.03	19 ± 0.89	14 ± 0.36	13 ± 1.2	11 ± 0.36
pH	6.5 ± 0.06	6.7 ± 0.06	5.8 ± 0.37	5.5 ± 0.35	3.9 ± 0.04	4.1 ± 0.07
Salinity (ppt)	30 ± 2.3	31 ± 0.7	13 ± 0.7	4.0 ± 3.1	0.0 ± 0.0	0.0 ± 0.0
EC (mS/cm)	11 ± 0.26	14 ± 0.37	6.0 ± 0.36	1.9 ± 1.0	0.11 ± 0.01	0.05 ± 0.01
TOC (%)	3.0 ± 0.34	6.9 ± 1.8	14 ± 1.7	2.0 ± 0.63	3.7 ± 1.1	0.77 ± 0.21
TN (g/kg)	1.8 ± 0.15	3.5 ± 0.90	5.9 ± 0.47	0.74 ± 0.18	1.2 ± 0.17	0.34 ± 0.04
TP (mg/kg)	117 ± 3.3	213 ± 33	250 ± 40	29 ± 3.1	53 ± 4.4	18 ± 1.7
SRP (mg/kg)	2.3 ± 0.57	0.84 ± 0.08	1.3 ± 0.73	0.05 ± 0.02	6.3 ± 2.0	2.6 ± 1.0
$N\text{-}NO_x^-$ (mg/L)	0.03 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.06 ± 0.02	0.09 ± 0.03	0.1 ± 0.01
$N\text{-}NH_4^+$ (mg/L)	4.0 ± 1.2	1.5 ± 0.15	2.1 ± 0.72	0.24 ± 0.01	1.8 ± 0.29	0.75 ± 0.18

Table 2 Data from PERMANOVA models highlighting that Site (i.e. freshwater, saltmarsh, and mangrove), depth, and their interaction are significantly associated with changes in environmental conditions and bacterial community composition.

Response	**Predictors**	R^2 **(%)**	***F* value**	***P* value**	
Environmental conditions	Site	56.7	26.7	<0.001	***
(z-score transformed abiotic variables*)	Depth	12.2	11.5	<0.001	***
	Site : Depth	18.3	7.6	<0.001	***
Bacterial community composition	Site	39.1	5.98	<0.001	***
(Hellinger transformed OTU abundances)	Depth	7.9	2.43	0.011	*
	Site : Depth	13.8	2.11	0.007	**

*abiotic variables include: pH, EC, salinity, TOC, TN, TP, SRP, $N\text{-}NO_x^-$, and $N\text{-}NH_4^+$. Asterisks for *P* values indicate as follows: $P < 0.05$ (*); $P < 0.01$ (**); $P < 0.001$ (***).

Table 3 Data from ANOVA models highlighting the effects of Site (i.e. freshwater, saltmarsh, and mangrove), depth, and their interaction on the alpha diversity of bacterial communities.

Response	**Predictors**	***F* value**	***P* value**	
Observed OTUs (Sobs)	Site	26.3	<0.001	***
	Depth	7.5	0.018	*
	Site : Depth	6.9	0.010	*
Simpson's Diversity Index	Site	18.2	<0.001	***
	Depth	7.7	0.017	*
	Site : Depth	2.1	0.160	

Asterisks for *P* values indicate as follows: $P < 0.05$ (*); $P < 0.01$ (**); $P < 0.001$ (***).

Highlights

- Reference conditions for selected coastal wetlands were established.
- Soil bacterial diversity, abiotic properties and their interactions were considered.
- Alpha diversity followed the order Mangroves > Saltmarsh > Freshwater.
- Community composition differed with abiotic conditions between wetlands.
- Communities in the saltmarsh were significantly stratified by depth.

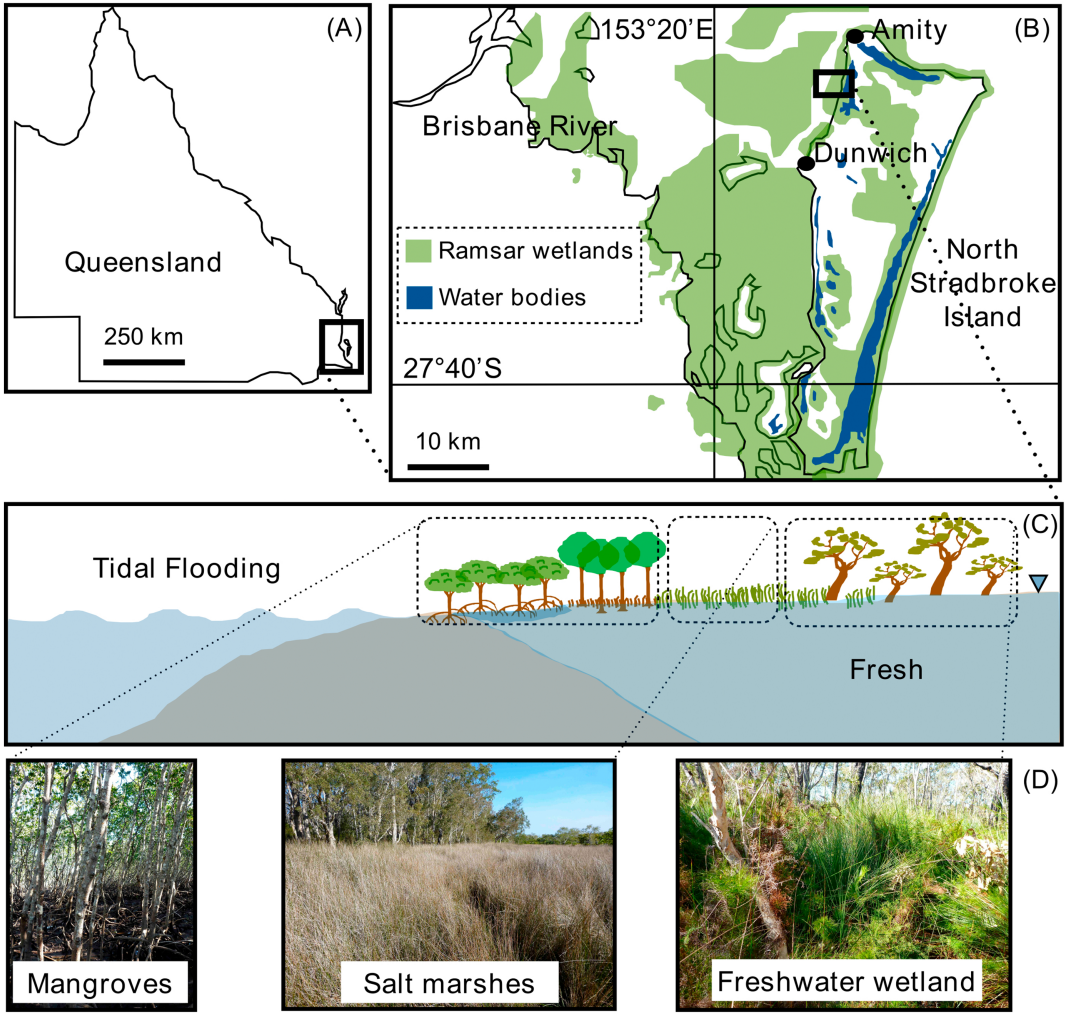
(A)
Queensland
250 km
(B)
153°20'E
Amity
Brisbane River
Dunwich
Ramsar wetlands
Water bodies
North
Stradbroke
Island
27°40'S
10 km
(C)
Tidal Flooding
Fresh
(D)
Mangroves
Salt marshes
Freshwater wetland

Figure 1

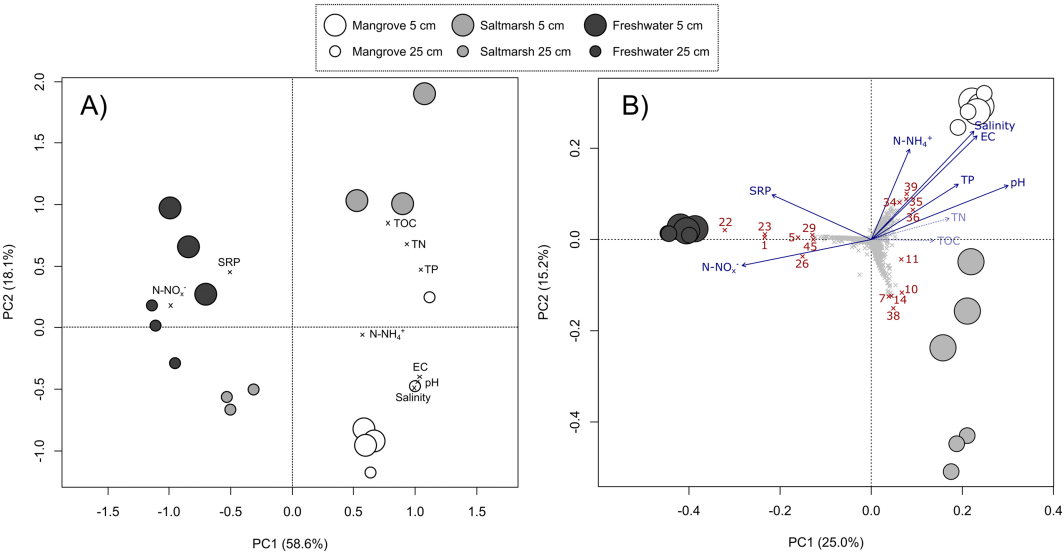
Mangrove 5 cm
Saltmarsh 5 cm
Freshwater 5 cm
Mangrove 25 cm
Saltmarsh 25 cm
Freshwater 25 cm
A)
PC2 (18.1%)
PC1 (58.6%)
SRP
N-NOx-
TOC
TN
TP
N-NH4+
EC
pH
Salinity
B)
PC2 (15.2%)
PC1 (25.0%)
Salinity
EC
N-NH4+
SRP
TP
pH
TN
TOC
N-NOx-

Figure 2

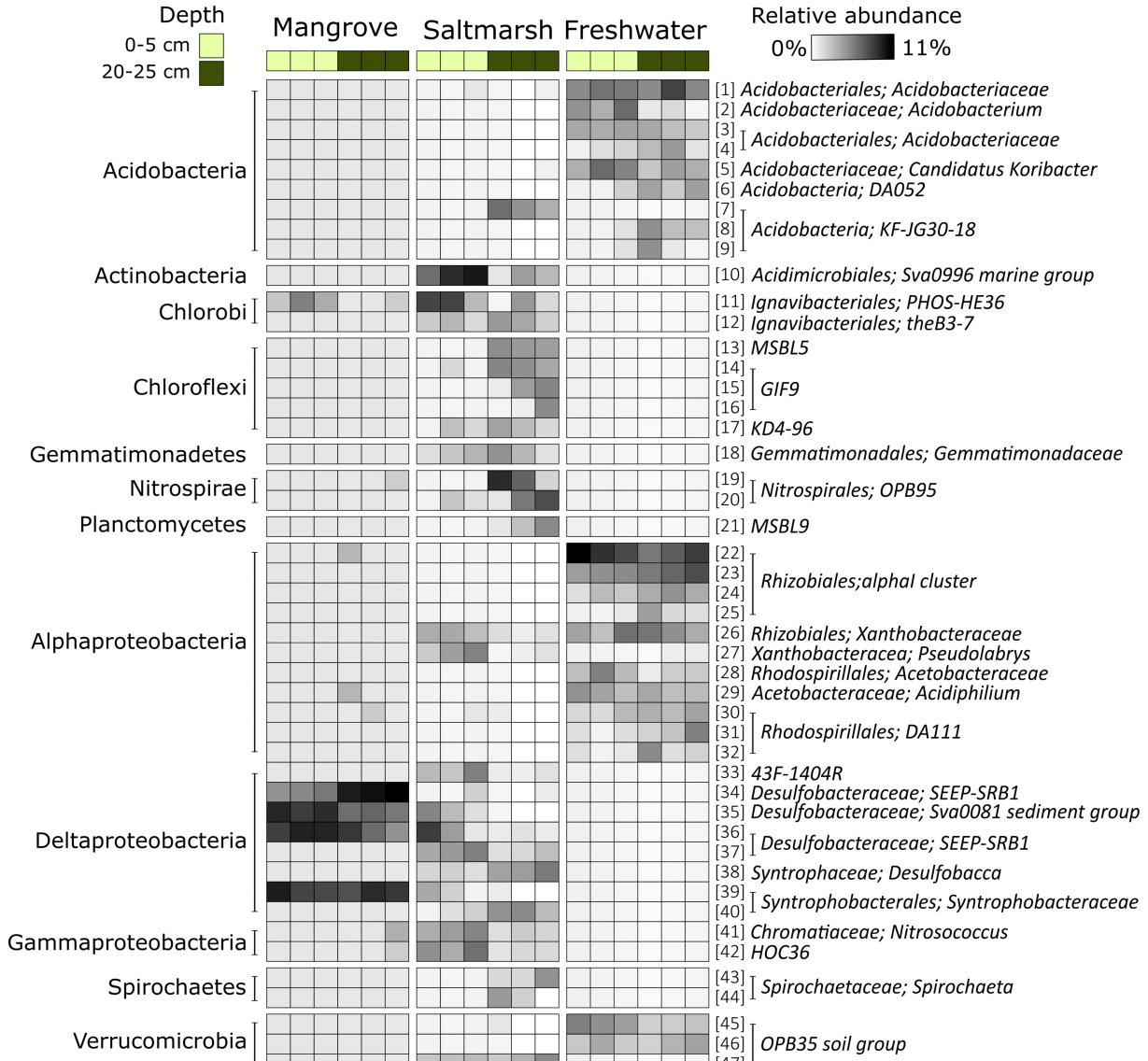
Depth
0-5 cm
20-25 cm
Mangrove
Saltmarsh
Freshwater
Relative abundance
0%
11%
Acidobacteria
Actinobacteria
Chlorobi
Chloroflexi
Gemmatimonadetes
Nitrospirae
Planctomycetes
Alphaproteobacteria
Deltaproteobacteria
Gammaproteobacteria
Spirochaetes
Verrucomicrobia
[1] Acidobacteriales; Acidobacteriaceae
[2] Acidobacteriaceae; Acidobacterium
[3]
[4] Acidobacteriales; Acidobacteriaceae
[5] Acidobacteriaceae; Candidatus Koribacter
[6] Acidobacteria; DA052
[7]
[8]
[9] Acidobacteria; KF-JG30-18
[10] Acidimicrobiales; Sva0996 marine group
[11] Ignavibacteriales; PHOS-HE36
[12] Ignavibacteriales; theB3-7
[13] MSBL5
[14]
[15]
[16] GIF9
[17] KD4-96
[18] Gemmatimonadales; Gemmatimonadaceae
[19]
[20] Nitrospirales; OPB95
[21] MSBL9
[22]
[23]
[24]
[25] Rhizobiales;alphaI cluster
[26] Rhizobiales; Xanthobacteraceae
[27] Xanthobacteracea; Pseudolabrys
[28] Rhodospirillales; Acetobacteraceae
[29] Acetobacteraceae; Acidiphilium
[30]
[31]
[32] Rhodospirillales; DA111
[33] 43F-1404R
[34] Desulfobacteraceae; SEEP-SRB1
[35] Desulfobacteraceae; Sva0081 sediment group
[36]
[37] Desulfobacteraceae; SEEP-SRB1
[38] Syntrophaceae; Desulfobacca
[39]
[40] Syntrophobacterales; Syntrophobacteraceae
[41] Chromatiaceae; Nitrosococcus
[42] HOC36
[43]
[44] Spirochaetaceae; Spirochaeta
[45]
[46]
[47] OPB35 soil group

Figure 3

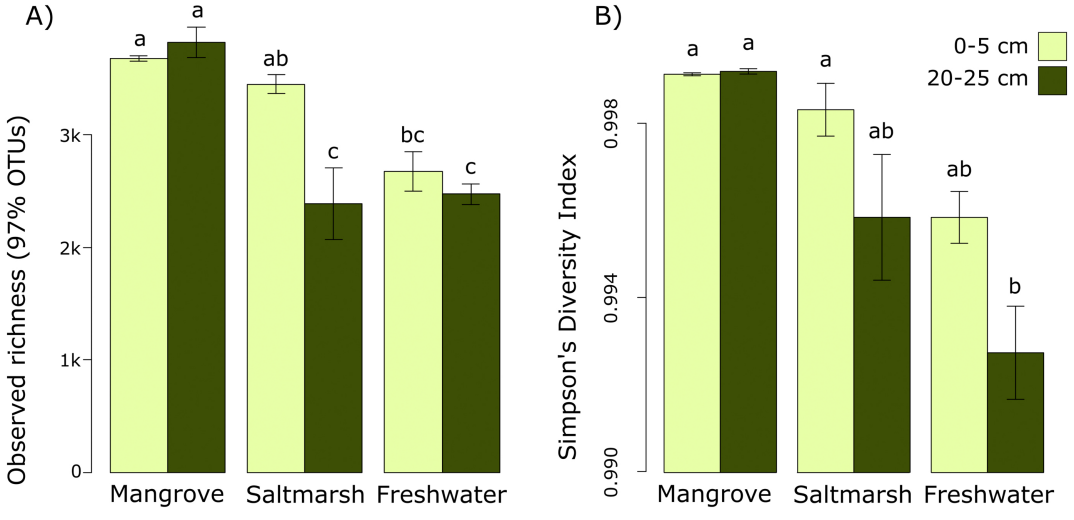
A)
Observed richness (97% OTUs)
3k
2k
1k
0
a
a
ab
c
bc
c
Mangrove
Saltmarsh
Freshwater
B)
Simpson's Diversity Index
0.998
0.994
0.990
0-5 cm
20-25 cm
a
a
a
ab
ab
b
Mangrove
Saltmarsh
Freshwater

Figure 4