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Research Article

Faecal bacterial communities differ amongst discrete foraging populations of dugongs along the east Australian coast

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Abstract

Gut bacterial communities play a vital role in a host's digestion and fermentation of complex carbohydrates, absorption of nutrients, and energy harvest/storage. Dugongs are obligate seagrass grazers with an expanded hindgut and associated microbiome. Here, we characterised and compared the faecal bacterial communities of dugongs from genetically distinct populations along the east coast of Australia, between subtropical Moreton Bay and tropical Cleveland Bay. Amplicon sequencing of fresh dugong faecal samples (n=47) revealed Firmicutes (62%) dominating the faecal bacterial communities across all populations. Several bacterial genera (*Bacteroides*, *Clostridium sensu stricto* 1, *Blautia* and *Polaribacter*) were detected in samples from all locations, suggesting their importance in seagrass digestion. Principal coordinate analysis showed the three southern-most dugong populations having different faecal bacterial community compositions from northern populations. The relative abundances of the genera *Clostridium sensu stricto* 13 and dgA-11 gut group were higher, but *Bacteroides* was lower, in the southern dugong populations, compared to the northern populations, suggesting potential adaptive changes associated with location. This study contributes to our knowledge of the faecal bacterial communities of dugongs inhabiting Australian coastal waters. Future studies of diet selection in relation to seagrass availability throughout the dugong's range will help to advance our understanding of the roles that seagrass species may play in affecting the dugong's faecal bacterial community composition.

Keywords: 16S rRNA gene amplicon sequencing; dugong; faeces: hindgut fermentation: microbiota bacterial communities; marine mammal; seagrass

Introduction

Bacterial communities in the digestive tracts of marine mammals play important roles in maintaining a host's health, disease and fitness status (Nelson et al. 2015, Bik et al. 2016, Suzuki 2017). Gut bacteria have been linked to immune system development and function, and are involved in protection against pathogens (Nelson et al. 2015). The gut bacterial community is also a critical component of the host's digestive system, aiding in the physical and fermentative breakdown of complex carbohydrates, uptake and utilisation of nutrients and vitamins, as well as energy harvest and storage (Lavery et al. 2012, Smith et al. 2013, Medeiros et al. 2016). This is particularly the case for the herbivorous sirenians in which fermentative digestion, through the action of symbiotic microbes, makes significant contributions towards the animals' energy budgets (manatees: Burn and Odell 1987, Harshaw et al. 2019, dugongs: Murray et al. 1977, Goto et al. 2004). Herbivorous mammals tend to have generally more diverse and complex microbiomes compared to those of carnivores and omnivores in both terrestrial and marine systems (Ley et al. 2008, Nelson et al. 2013, Bik et al. 2016). Factors including age, habitat, and diet (Eigeland et al. 2012, Merson et al. 2014, Suzuki et al. 2019) have been shown to further influence the faecal bacterial community composition of herbivorous sirenians.

The dugong is a marine hindgut-fermenting herbivore with a diet consisting almost exclusively of sub-tropical and tropical seagrasses (Marsh et al. 2011). Seagrass digestion by dugongs includes the release of cell contents by specialised mouthparts and enzymatic digestion (Lanyon and Sanson 2006a,b), followed by slow fibre fermentation in an expanded 30 m long colon (Murray et al. 1977, Lanyon and Marsh 1995). Dugongs have been reported to variably digest seagrass species, both physically and chemically, where more fibrous genera (*Zostera* and *Cymodocea*) have apparent total digestibility of ~60%, whilst less fibrous genera (*Halophila* and *Halodule*) have apparent digestibility of >85% (Murray et al. 1977, Lanyon and Sanson 2006b). Dugongs lack the enamelled dentition that typically allows other mammalian hindgut fermenters to comminute high fibre plant material and instead may preferentially forage on lower fibre *Halophila* and *Halodule* spp. seagrasses, when available (Lanyon and Sanson 2006a,b) The dugong's apparent preference for certain low fibre seagrass species may be

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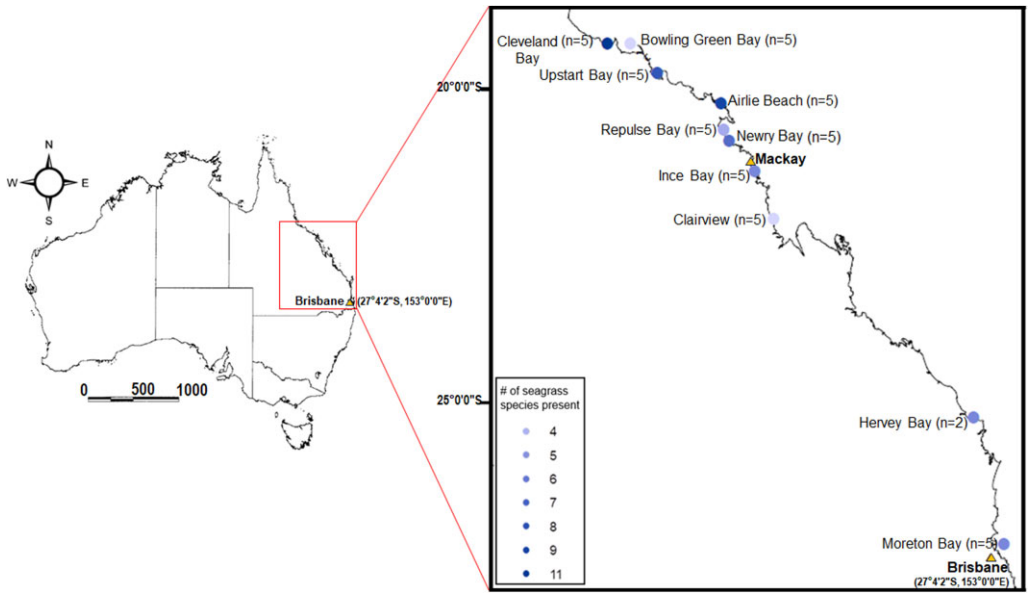

Figure 1. Map of dugong faecal sample collection sites along the east Queensland coast. The number of seagrass species present at each site is shown as a gradient colour scale. n = the number of fresh faecal samples collected at each site.

associated with the evolution of a unique microbiome that facilitates digestion (Eigeland et al. 2012).

Previously, dugong hindgut bacterial communities were investigated by sampling faeces and distal colon contents from wild individuals inhabiting sub-tropical Moreton Bay at the southern limit of the dugong's distribution in eastern Australia, as well as from two individuals held in captivity (Eigeland et al. 2012). Firmicutes and Bacteroidetes were identified as the most abundant bacterial phyla in dugong hindgut and faeces, and faecal bacterial communities varied between milk-fed calves and seagrass-grazing adults, as well as between wild and captive individuals (Eigeland et al. 2012). This previous research indicated that diet might be pivotal in shaping the dugong gut bacterial community.

The diet of dugongs varies throughout their geographical range and is primarily influenced by the availability and density of local seagrass species (Tol et al. 2016, Marsh et al. 2018). Dugong diet is also affected by temporal (including seasonal) patterns in seagrass species abundance (Lanyon et al. 1989, Lanyon and Marsh 1995, Tol et al. 2016) and presumably by individual foraging behaviour, including diet selection that may be based on palatability and/or nutrient availability (Preen 1995b, Lanyon and Sanson 2006b, Sheppard et al. 2007). Spatial variability in seagrass diversity within the dugong's Australian geographical range is high (Carruthers et al. 2002, Carter et al. 2021). It includes latitudinal variation in seagrass community structure from stands of mixed subtropical species at their high latitude foraging grounds (e.g. Moreton and Shark Bays) through to highly complex and diverse stands of tropical species (e.g. northern Cape York Peninsula and Torres Strait) (Short et al. 2007). Along the eastern Australian coast, dugong distribution follows the linear and mostly nearshore distribution of these seagrass meadows. Recent studies of dugongs along this coastline found a series of largely separate genetic populations associated with sheltered seagrass meadows inshore of the Great Barrier Reef region (Seddon et al. 2014, Cope et al. 2015, McGowan et al. 2023). This significant population structuring suggests that dugongs mostly remain within local foraging grounds, feeding on locally available seagrasses. Consequently, it is likely that dugongs in these separate foraging areas may be feeding in seagrass meadows of variable community composition. If there is a relationship between seagrass dietary composition and hindgut microbes, we might expect gut microbe assemblages to vary spatially or according to host genetics because dugong populations are genetically distinct. Here, we aimed to characterise the faecal bacterial communities of dugongs and investigate variation in these communities across these geographically dispersed and genetically distinct dugong populations (Seddon et al. 2014, McGowan et al. 2023) along more than 1500 km of the east Australian coast. It was hypothesised that faecal bacterial communities would vary amongst discrete foraging populations of dugongs along the Australian coast. If the diversity of hindgut bacterial communities is related to seagrass diversity and availability, this may be informative of the capacity of dugongs to shift their diet in the event of local seagrass degradation or loss that may occur due to environmental stressors, including climate change.

Materials and methods

Study sites and sample collection

Fresh dugong faecal samples (n = 47 dugongs) were collected from across 10 known dugong feeding grounds between tropical Cleveland Bay (19.2 °S, 146.9 °E) and sub-tropical Moreton Bay (27.3 °S, 153.3 °E), Queensland, Australia (Fig. 1), between November 2011 and September 2017. From north to south, these sites were: Cleveland Bay (off Townsville), Upstart Bay, Bowling Green Bay, Airlie Beach (Pioneer Bay, Whitsunday coast), Repulse Bay, Port of Newry Region (also known as Newry Bay), Ince Bay, Clairview, Hervey Bay and Moreton Bay (off Brisbane). Most of these sampling sites were located along mainland Queensland within the Great Barrier Reef World Heritage Region. Five faecal samples were collected from each site, except for Hervey Bay (n = 2). At all field sites, except Hervey and Moreton Bays, fresh dugong faeces (i.e. within 2 h of voiding) found floating on the water surface were collected far enough apart to ensure that these were from different individuals, placed into individual ziplock bags and then frozen at −20 °C. Upon return to the laboratory, approximately 5 g from the centre of each sample (uncontaminated by seawater) was transferred

into 20% glycerol, mixed thoroughly and stored at −80 °C until processed. For Hervey and Moreton Bays, fresh, freely voided faeces were collected into a plastic Frisbee® plate-like disc placed under the dugong during annual out-of-water health assessments (Lanyon et al. 2010) and stored as described above. Dugongs were sampled under The University of Queensland Animal Ethics Permit SBS/181/18, Scientific Purposes Permit WISP14654414, Moreton Bay Marine Parks Permit MPP18-001119, and Great Barrier Reef Marine Park Permit G14/36987.1.

DNA extraction and sequencing

We evaluated the taxonomic profiles of faecal bacterial communities for each of the 47 dugong faecal samples (Table 1) by 16S rRNA gene amplicon sequencing. Genomic DNA was extracted from the thawed faecal samples using the QIAGEN DNeasy PowerLyzer PowerSoil Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. The NanoDrop™ 8000 Spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) was used to determine DNA quality and concentration. Samples with low DNA concentrations underwent ethanol precipitation. Samples with a DNA concentration of ≥ 1.88 µg/µL were submitted for 16S rRNA gene amplicon sequencing to the Australian Genomics Research Facility (AGRF), Adelaide, Australia. The Illumina MiSeq Platform (Illumina, San Diego, CA, USA) was used by AGRF to carry out the 16S rRNA gene amplicon sequencing of the samples, where the V1 to V3 variable region was targeted using the primers 27F (AGAGTTTGATCMTGGCTCAG; Lane 1991) and 519R (GWATTACCGCGGGCKGCTG; Turner et al. 1999).

Bioinformatics and statistical analyses

Generated sequence reads were processed by AGRF for trimming and quality control using their internal "Divpro 2 pipeline", and once the sequence reads were received, they were imported into Quantitative Insights into Microbial Ecology 2 (QIIME 2; Bolyen et al. 2019). The taxonomic classification of representative sequences was obtained using the SILVA rRNA database (Quast et al. 2013), where OTUs were assigned at the threshold of 97% sequence similarity.

The *R* statistical software v4.1.2 (R Core Team 2023), was used to rarefy the data and the depth of sequence reads were visualised after data normalisation to show how well the sequencing reflected the sample diversity. The following packages were used in *R* for faecal bacterial community analysis. Relative abundance data was obtained using *phyloseq* (McMurdie and Holmes 2013), *microbiome* (Lahti and Shetty 2020), *dplyr* (Wickham et al. 2022), *hrbrthemes* (Rudis 2020), *gcookbook* (Chang 2018) and *tidyverse* (Wickham et al. 2019). The packages *ggplot2* (Wickham et al. 2019) and *ggpubr* (Kassambara 2020), in addition to *phyloseq* and *dplyr*, were used to investigate alpha diversity parameters including Observed Richness, Chao1, Shannon Index and Simpson's Index, with respect to each of the 10 locations spanning from north to south along the east Australian (Queensland) coast. Wilcoxon rank-sum tests were conducted to determine the significance of the results obtained for each alpha diversity parameter. The relative abundances of the phyla and the top 20 genera were identified and visualised as stacked bar charts using Excel (Microsoft 365) with respect to geographical location. Beta diversity was explored by principal coordinates analysis (PCoA) using the *phyloseq*, *ggplot2* and *plyr* (Wickham 2011) packages in *R* to determine the bacterial diversity between the dugong faecal samples collected at the various locations along the east Australian coast. To assess whether locations differed significantly ($P < 0.05$) from each other based on Bray and Curtis (1957) dissimilarity, permutation ANOVA was conducted using the R function *adonis2* with 999 permutations, followed by assessing the homogeneity of the dispersion of the locations using the *betadisper* function with 999 permutations, in the package *vegan* (Oksanen et al. 2013). Using the packages *phyloseq*, *ggplot2* and *microbiomeutilities* (Shetty and Lahti) in *R*, heatmaps were generated to display the core faecal bacterial communities for all locations at the genus level, as well as in groupings of the northern (n = 7) versus the southern (n = 3) geographical location along the east Australian coast.

All sequences generated during and/or analysed in this study are available in the European Nucleotide Archive of the European Bioinformatics Institute (EBI) under the accession number 'PRJEB65915' (https://www.ebi.ac.uk/ena/browser/view/PRJEB65915).

Results

Sequence and alpha diversity analysis

A total of 618422 sequence reads were generated. The depth of sequencing for each dugong faecal sample was assessed by plotting a rarefaction plot, which showed that each sample was sequenced to an adequate depth to capture the microbial diversity present (Fig. S1). The sequence reads ranged from the lowest reads of 1416 obtained for sample NW16005, to the highest of 28634 sequence reads obtained for sample CV16056, across the 47 dugong faecal samples. After rarefying the data to 1 416 reads, the sequence reads per sample were taxonomically classified into 9 phyla, 15 classes, 17 orders, 29 families, and 37 genera, and there were 77 OTUs in total.

Species richness assessed at the OTU level, including observed richness, Chao1, Shannon Index, and Simpson's Index, are presented in Fig. 2. Dugong faecal bacterial diversity was the most significantly different ($P < 0.01$ for observed richness; $P < 0.05$ for Chao1 parameters) between the sampling locations of Cleveland and Moreton Bays. Whilst no significant differences for observed richness were noted between Repulse and Ince Bays (Fig. 2), that was not the case for the Chao1 parameter, where these two sites differed significantly ($P < 0.05$) in their faecal bacterial communities. There were no significant differences in Chao1 when comparing the faecal bacterial communities between the pairs of Bowling Green Bay and Moreton Bay, Upstart Bay and Moreton Bay, as well as Clairview Bay and Moreton Bay (Fig. 2). Furthermore, there were no significant differences in each of Shannon nor Simpson's Indices for bacterial diversity in dugong faecal samples across various locations (Fig. 2).

Bacterial composition of dugong faeces

The bacterial communities identified in the 47 dugong faecal samples were assigned to five main bacterial phyla (Fig. 3). Firmicutes was the most abundant phylum overall, with an average relative abundance of 62%, followed by Bacteroidetes (30%), Actinobacteria (5%), Proteobacteria (2%) and Verrucomicrobia (0.1%). The composition of bacterial phyla (in terms of relative abundance) in faeces collected from the three southern-most Queensland dugong populations (i.e. Clairview, Hervey and Moreton Bays) differed from the overall average composition across the sampled range. Actinobacteria was relatively more abundant (15%) in these three southern 'bacterial communities', with a low abundance of Proteobacteria in some individuals, and Bacteroidetes averaging a lesser relative abundance of ∼15%. From Ince Bay south to Moreton Bay, additional unidentified bacterial phyla grouped as 'other' were detected in most sampled individuals. One

Table 1. Dugong faecal sample collections sites (organised from north to south), number (n) of samples collected and seagrass species previously recorded at each dugong foraging ground along the eastern Australian coast. Each seagrass species has a cell shaded grey when present, and an unshaded cell where absent. * = seagrass species recorded as dominant at the location. Taxonomic notes: *Halophila ovata* is not recognised as separate to *H. ovalis*. *T. ciliatum* = *Thalassodendron ciliatum*. Halodule *univervis* includes both narrow-leaved and wide-leaved forms.

	Zostera muelleri subsp. capricorni	*Halophila ovalis*	*Halodule uninervis* n=narrow w=wide	*Halodule pinifolia*	*Halophila spinulosa*	*Halophila decipiens*	*Syringodium isoetifolium*	*Cymodocea serrulata*	*Cymodocea rotundata*	*Thalassia hemprichii*	*Halophila tricostata*	*T. ciliatum*	*Enhalus acoroides*	Total number species
Cleveland Bay n = 5 [a,b,c,d,e,h,j,k]	*	*	*		*			*						13
Bowling Green Bay n = 5 [a,d,e,k]	*	*	*	*										5
Upstart Bay n = 5 [a,d,e,f,k]	*	*	*											10
Airlie Beach n = 5 [a,d,e,i]	*		*											10
Repulse Bay n = 5 [a,e,i]	*		*											5
Newry Bay n = 5 [a,e,f,i]	*		*											7
Ince Bay n = 5 [a,e,f,i]	*		*											6
Clairview n = 5 [a,e,f,i]		*	*											5
Hervey Bay n = 2 [a,d]	*	*	*		*									6
Moreton Bay n = 5 [c,d,g,h]	*	*	*											7

[a]—Long et al. (1993)
[b]—McKenzie et al. (2018)
[c]—Preen (1995a)
[d]—Seagrass-Watch (http://www.seagrasswatch.org/australia.html)
[e]—https://maps.eatlas.org.au (GBR: seagrass site surveys 1984–2014)
[f]—Coles et al. (2003)
[g]—Young and Kirkman (1975)
[h]—Lanyon (pers. obs)
[i]—McKenzie and Yoshida (2022)
[j]—Unsworth et al. 2009
[k]—Atlas of Living Australia (2023)

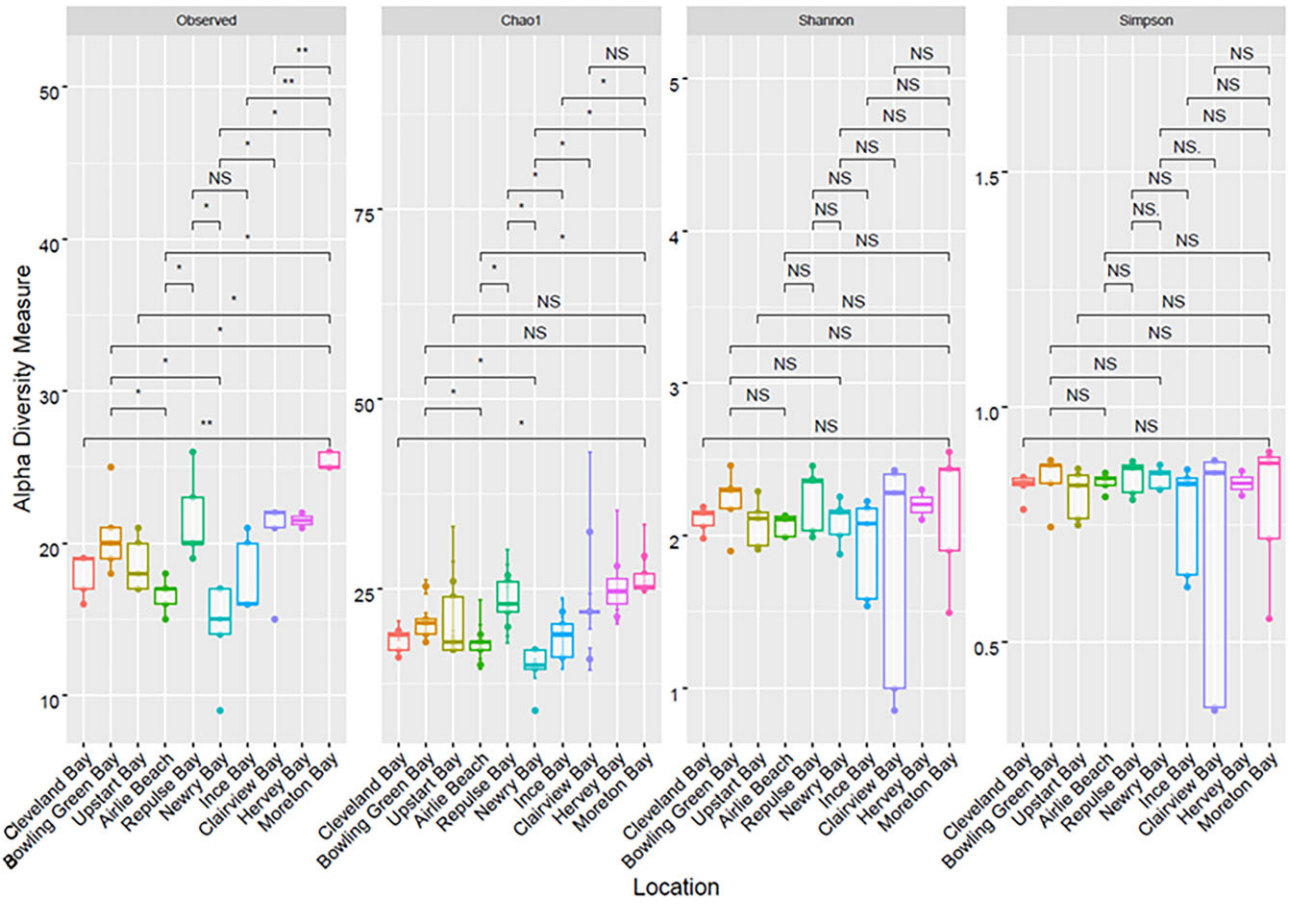

Figure 2. Alpha diversity plots of bacterial communities present in dugong faecal samples per location, along the east Australian coast. Observed richness, Chao1, Shannon Index and Simpson's Index are displayed using Wilcoxon rank-sum test significance (NS = not significant; "*" = $P = 0.05$; "**" = $P = 0.01$). Upper and lower quartiles (box), median (horizontal line) and upper and lower values (whiskers) are shown.

faecal sample from Moreton Bay (Dugong ID# MB16886, adult female 281 cm body length) had a large relative abundance of Proteobacteria (71.9%, Fig. 3).

Bacterial diversity in faeces was also assessed at a finer taxonomic level, identifying a total of 37 genera. Figure 4 presents the % relative abundances of top 20 bacterial genera present per location (Fig. 4A) and per sample (Fig. 4B), in the dugong faecal samples collected from Cleveland Bay in the north to Moreton Bay in the south, along the eastern Australian coast (Fig. 1). Interestingly, the genera *Bacteroides* and *Blautia* were dominant in the faecal samples collected from dugongs in the northern locations, while these genera were present to a much lesser extent in faecal samples collected from dugongs inhabiting the inhabiting the more southern coastal waters, including Clairview, Hervey and Moreton Bays. Instead, the % relative abundance for the genera *Clostridium sensu stricto* 1 and *Clostridium sensu stricto* 13 seemed to increase in the faecal bacterial communities of these dugongs (Fig. 4A). The genus dgA-11 gut group was also noted to be exclusively present in these animals' faecal bacterial communities (Fig. 4A and B).

Variation between samples

Differences in bacterial diversity between faecal samples were assessed using beta diversity analyses. The PCoA analysis at the OTU level, which explained ~55% of the variation, demonstrated dissimilarity in dugong faecal bacterial communities between locations, indicating two major spatial groupings (Fig. 5). Faecal bacterial communities from the three southern-most locations (i.e. Clairview, Hervey and Moreton Bays) clustered together loosely in the right top and bottom-most quadrants of the PCoA plot, whilst the faecal bacterial communities of dugongs inhabiting the more northern coastal waters predominantly clustered towards the left side of the PCoA plot, mostly falling in the bottom left quadrant (Fig. 5). The permutation ANOVA analyses showed significant ($P < 0.001$) compositional differences between the locations based on the distance between points displayed in the PCoA (Fig. 5). However, the permutation test for homogeneity of multivariate dispersions indicated that the variability around those compositions is similar across the locations.

Figure 6 presents the core faecal bacterial communities at the genus level for all locations along the eastern Australian coast (Fig. 6), showing the high prevalence of the genera *Bacteroides*, *Blautia*, *Polaribacter* and Ruminococcaceae UCG-002 in the northern dugong populations. Additionally, the core (shared between all locations), pan (shared between some locations) and unique (not shared) bacterial communities identified at the genus level in dugong faecal samples collected from the 10 locations along the east Australian coast are presented in Table 2. A total of six genera including *Bacteroides*, *Blautia*, *Clostridium sensu stricto* 1, *Clostridium sensu stricto* 13, *Polaribacter* and Ruminococcaceae UCG-002 were identified as being core to the faecal bacterial communities for all 47 samples analyses. Interestingly, while 11 other bacterial genera were shared across varying numbers of locations (i.e. pan bacterial communities), four of these genera including *[Eubacterium] coprostanoligenes* group, *Caproiciproducens*, *Eubacterium*

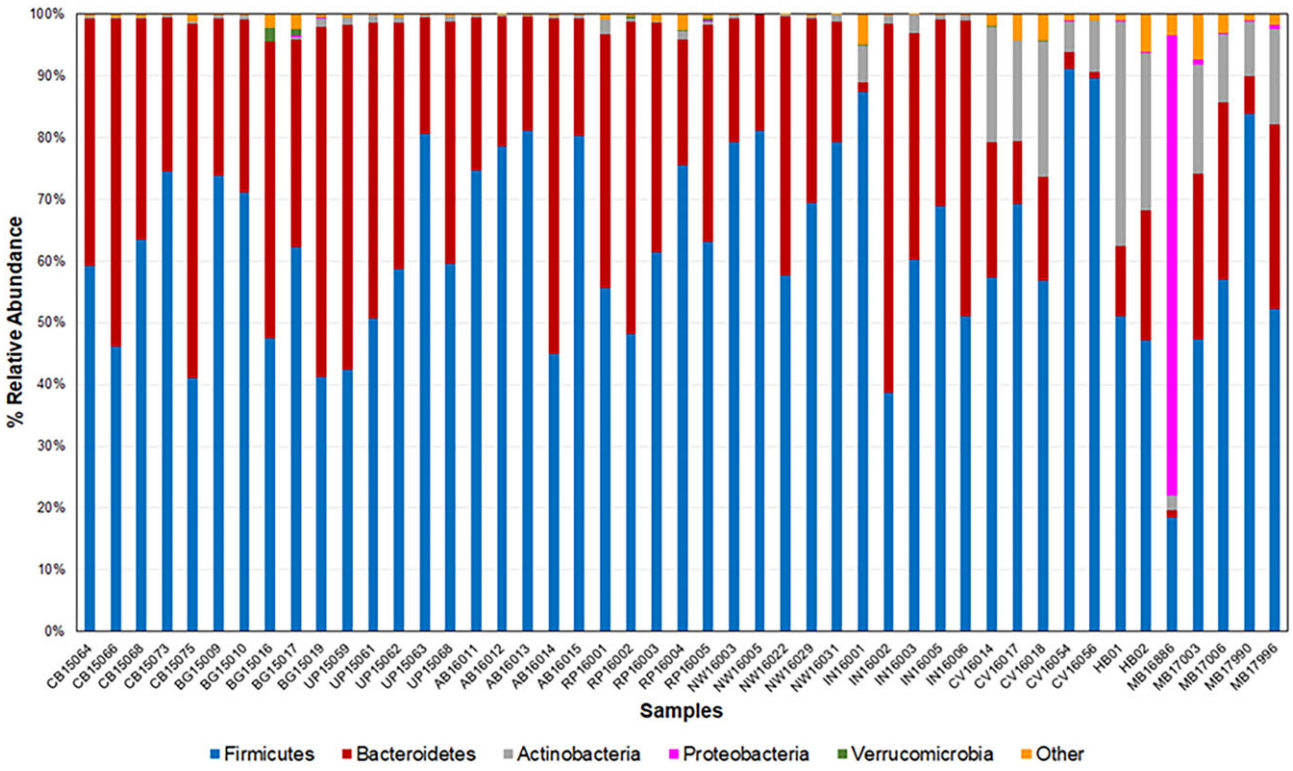

Figure 3. Percentage relative abundance of different bacterial phyla in dugong faecal samples from along the eastern Australian coast. Locations are arranged north to south (left to right). CB = Cleveland Bay; BG = Bowling Green Bay; UP = Upstart Bay; AB = Airlie Beach; RP = Repulse Bay; NW = Newry Bay; IN = Ince Bay; CV = Clairview; HB = Hervey Bay; MB = Moreton Bay.

and *Subdoligranulum* were not identified in the southern locations of Clairview, Hervey and Moreton Bays (Table 2). There were no unique bacterial genera identified from dugong faecal samples collected in the locations of Bowling Green Bay, Airlie Beach, Newry Bay, and Hervey Bay (Table 2), yet unique bacterial genera were identified exclusively within each of Repulse, Clairview and Moreton Bays (Table 2).

Discussion

This study presents the first comparison of faecal bacterial communities of dugongs from multiple foraging grounds along an extensive stretch (~1500 km) of the eastern Australian coast that encompasses the Great Barrier Reef. It provides insights into the likely key bacteria involved in the hindgut digestion of seagrasses across this part of the dugong's geographic range. Using high-throughput sequencing technology, we were able to accurately characterise dugongs' faecal bacterial communities and detect similarities and differences in the bacterial composition of faeces sampled over a broader geographical sampling area than previous studies (e.g. Eigeland et al. 2012).

Diversity profiling of faecal samples from wild dugongs along the coast of Queensland, eastern Australia, identified five main bacterial phyla. The faecal bacterial communities of all dugongs, regardless of sampling location, were dominated by Firmicutes (62% relative abundance), with Bacteroidetes as important contributors (30%). In addition to these core phyla, other phyla present but at significantly lower relative abundance included Actinobacteria (5%), Proteobacteria (2%), and trace amounts of Verrucomicrobia in three individuals only. Previous studies of bacterial communities in the dugong hindgut have been small scale and spatially disparate, however, these studies also found Firmicutes and Bacteroidetes dominating faecal and hindgut samples of dugongs (Tsukinowa et al. 2008, Eigeland et al. 2012). Faecal and hindgut (distal colon) samples from wild dugongs feeding on *Halodule* and *Halophila* spp. in Moreton Bay, Australia, had 76% Firmicutes and 20% Bacteroidetes (Eigeland et al. 2012), whilst faeces from a single captive dugong in Japan feeding on *Zostera marina* had 83% Firmicutes and 15% Bacteroidetes (Tsukinowa et al. 2008). Firmicutes and Bacteroidetes have also been recorded as the most abundant phyla in the hindgut of the related Florida manatee, *Trichechus manatus latirostris* (Merson et al. 2014). The dominance of Firmicutes and Bacteroidetes in herbivorous sirenians is unsurprising since these are the dominant bacterial phyla in most herbivorous mammals (O'Donnell et al. 2017). Seagrass-grazing dugongs also appear to have similar dominant bacterial communities (i.e. Firmicutes and Bacteroidetes), to seagrass-feeding green turtles within the Great Barrier Reef region (Ahasan et al. 2017, 2020). Marine hindgut-fermenting dugongs appear to be similar to terrestrial hindgut fermenters in terms of the composition and abundance of core bacterial phyla in the hindgut, in particular, the dominance of Firmicutes and Bacteroidetes (e.g. white rhinoceros *Ceratotherium simum*, Bian et al. 2013; African elephants *Loxodonta africanus*, Budd et al. 2020; horses *Equus* spp., Edwards et al. 2020; assorted hindgut fermenters, O'Donnell et al. 2017).

Blautia, *Clostridium sensu stricto* 1, *Clostridium sensu stricto* 13 and Ruminococcaceae UCG-002 (phylum Firmicutes), and *Bacteroides* (phylum Bacteroidetes) were identified in all dugong populations in this study. These bacterial genera are involved in cellulose fermentation and the digestion of starch and other polysaccharides in other herbivores, including the Florida manatee (Merson et al. 2014) and terrestrial hindgut fermenters (Bian et al. 2013, Dougal et al. 2013, Gamage et al. 2017, O'Donnell et al. 2017). The

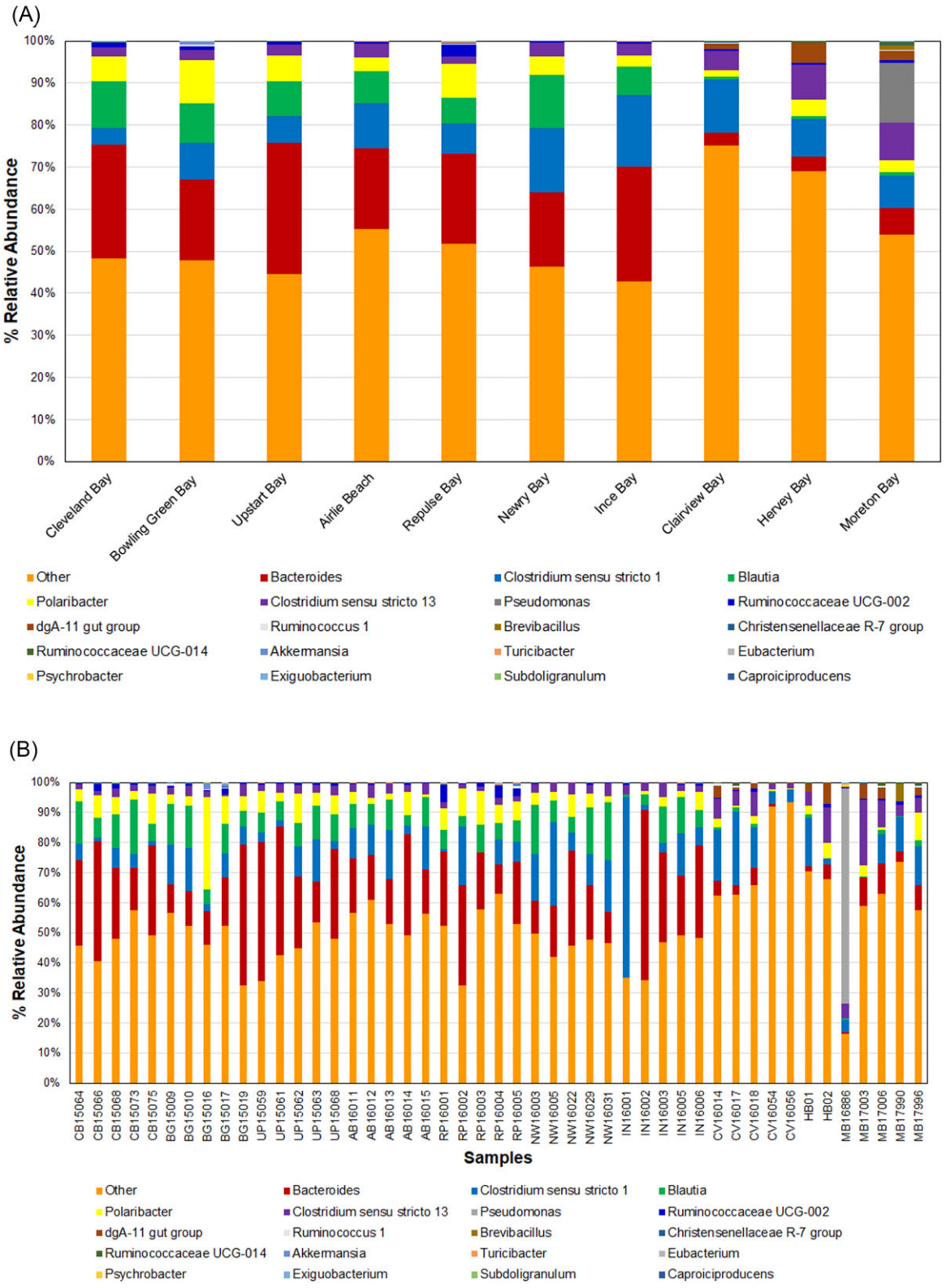

Figure 4. Percentage relative abundance of the 20 most abundant bacterial genera (left to right) identified in dugong faecal samples: A) per location; and B) per sample, collected from sites along the eastern Australian coast. CB = Cleveland Bay; BG = Bowling Green Bay; UP = Upstart Bay; AB = Airlie Beach; RP = Repulse Bay; NW = Newry Bay; IN = Ince Bay; CV = Clairview; HB = Hervey Bay; MB = Moreton Bay.

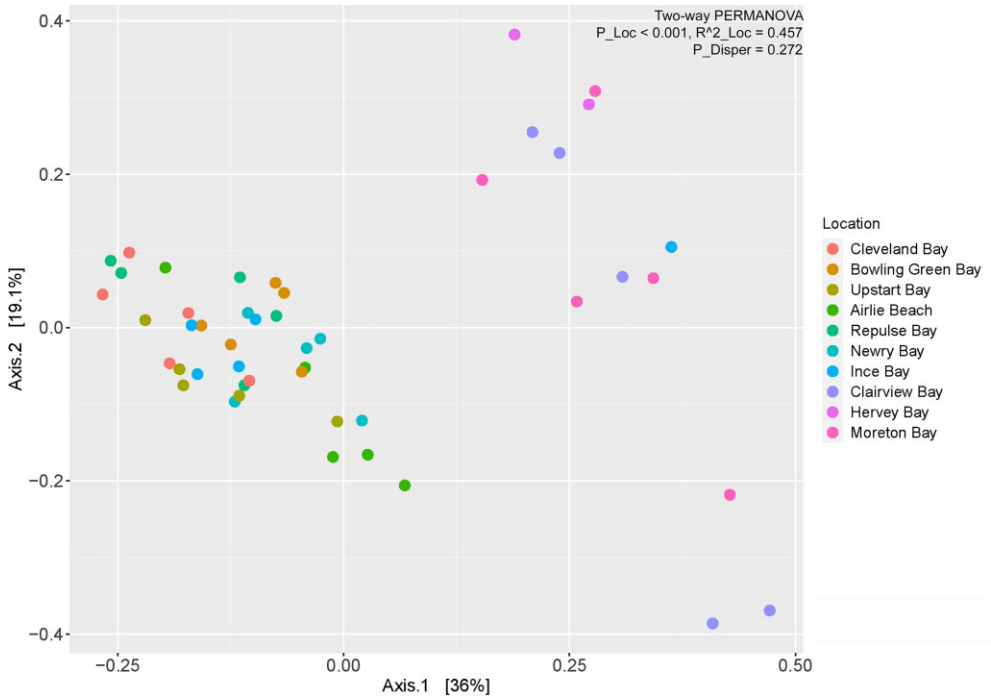

Figure 5. Principal Coordinate Analysis (PCoA) plot showing similarities in dugong faecal bacterial communities at the OTU level in terms of relative abundance amongst the eastern Australian locations, using Bray–Curtis dissimilarity and permutation ANOVA. Loc: Location. Disper: beta diversity dispersion.

large number of shared bacterial families across these hindgut fermenters confirms their likely importance in plant digestion in both terrestrial and marine herbivores. However, several bacterial communities found in dugong faecal samples could not be classified at either the genus- or species-level, which has also been the case in other marine studies (Eigeland et al. 2012, Merson et al. 2014, Ahasan et al. 2017). This indicates that either the V1 to V3 region of the 16S rRNA gene may not be an adequate site to target for taxonomic classification, or perhaps some bacteria in the dugong hindgut may be novel and undescribed or, alternatively, that there are deficits in reference microbial databases.

Differences in bacterial community composition of dugong faecal samples were identified between regions along the eastern Australian coast. In the southern-most locations (Clairview, Hervey and Moreton Bays), the average relative abundance of bacteria at each phylum- and genus-level was different than the overall averages across the entire coastline. The Bacteroidetes dgA-11 gut group were found in each of the dugong faecal samples collected from animals in Ince, Clairview, Hervey and Moreton Bays only (Table 2). Some bacteria within the Bacteroidetes family are known to function in the modulation of gut permeability by producing enzymes involved in mucin degradation, metabolism, and in the breakdown of dietary carbohydrates including starch (Johnson et al. 2017). Additionally, the increase of the genera *Clostridium sensu stricto* 1 and *Clostridium sensu stricto* 13 were noted in dugong faecal samples from these three southern Queensland locations. These genera are recognised as opportunistic pathogens in monogastrics, causing intestinal inflammation and decreased short chain fatty acid production (Fan et al. 2017). These differences in faecal microbial communities between locations were also captured and shown to be significant ($P < 0.001$) in the beta-diversity analyses. Therefore, it is likely that dugongs inhabiting the most southern Queensland coastal waters have different faecal bacterial communities compared to dugongs located in more northern coastal waters.

It is challenging to directly assess which seagrasses are consumed by wild dugongs. Direct observations of feeding activities are usually impossible since dugongs are shy of people, spend > 98% of their time underwater, and normally forage in turbid nearshore waters. Measuring diet through analysis of gut contents has its own inherent challenges and biases: mouth contents may only be examined if a dugong is captured, stomach contents only from recovered carcasses, and faecal material is informative of undigested foodstuffs only (see below). Since dugongs feed mostly on highly digestible low fibre seagrasses, faeces yield few recognisable food remnants (Lanyon and Sanson 2006b). Other approaches to examine dugong dietary composition could include (i) carrying out DNA metabarcoding of faecal samples to identify seagrass spp. consumed; and/or (ii) observe feeding trails left in the seagrass bed and compare seagrasses consumed in these trails with the relative abundances of seagrasses in the local area. The latter has been informative in some relatively accessible local areas. As a consequence of these issues, we were unable to directly compare faecal bacterial communities to individual diets of dugongs across broad spatial scales, but we can compare bacterial communities to potential foodstuffs, i.e. seagrasses available in their local foraging grounds. Recent genetic studies of dugongs along the same eastern Australian coastline have shown that dugongs show strong site fidelity to local areas throughout most of their lives (Seddon et al. 2014, McGowan et al. 2023) with very low migration rates (Cope et al. 2015), suggesting that locally available seagrasses may be used as a proxy for potential diet.

For herbivores that have a resident microbiome (e.g. in the dugong hindgut), diet is considered one of the primary factors influencing the diversity and composition of the microbial community (Muegge et al. 2011). At both the phylum- and genus-level,

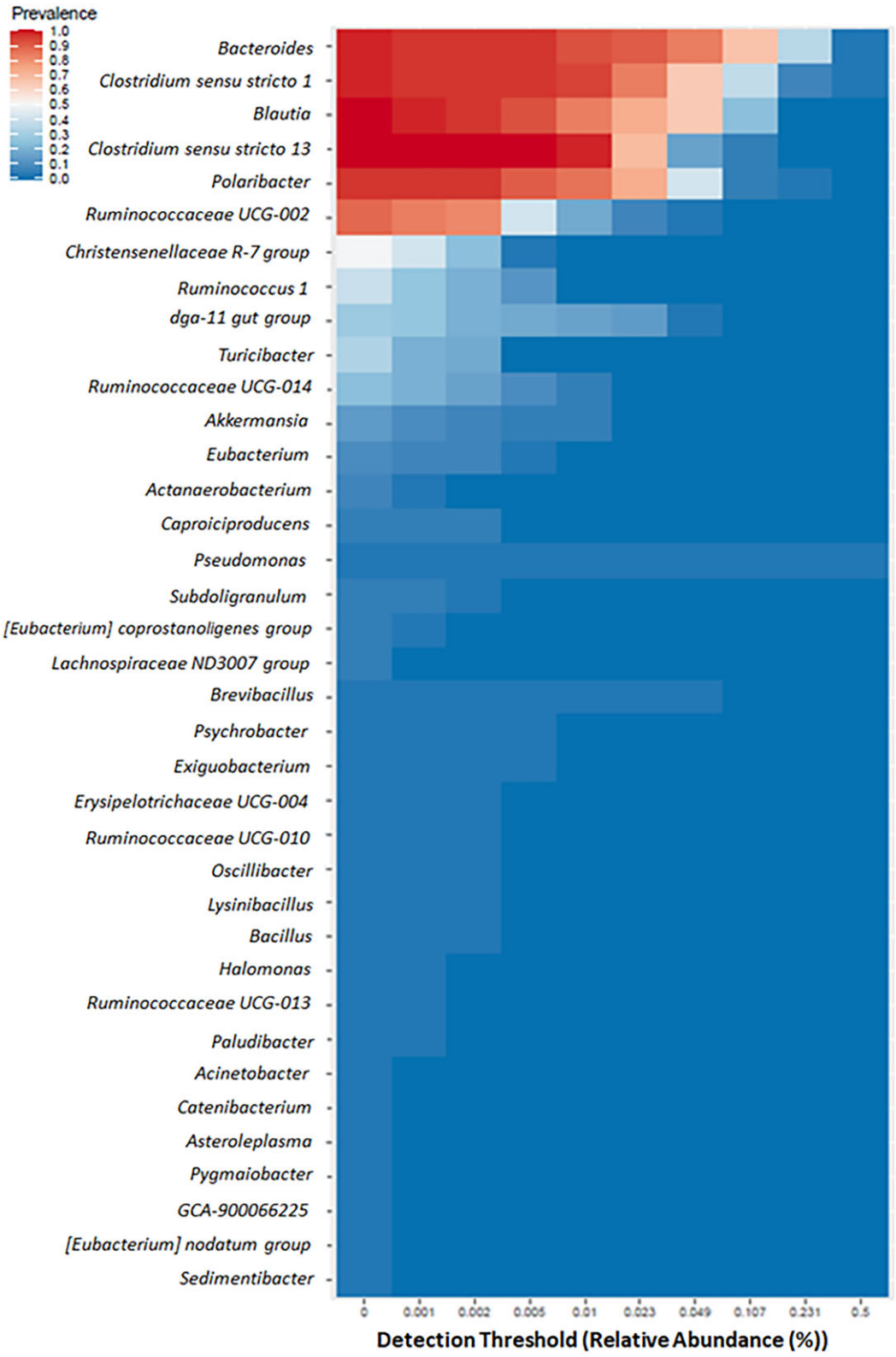

Figure 6. The core faecal bacterial communities at the genus level for all locations along the eastern Australian coast.

dominant faecal bacteria were shared by dugongs in all sampled locations along the eastern Australian coast. Several studies have suggested that if available, dugongs prefer to feed on *Halophila* spp. and *Halodule* spp. throughout their range (see review by Marsh et al. 2011). Since these seagrasses were present at each of the sampling sites and, in some cases, were the predominant seagrasses (Table 1), it is possible that at least some of the dugongs across the study coastline were feeding on these species and thus had similar diets. At the southern-most foraging areas of Moreton, Hervey and Clairview Bays, the predominant seagrasses are *Halophila* and

Table 2. Core (shared between all locations), pan (shared between some locations) and unique (not shared) bacterial communities identified at the genus level in dugong faecal samples collected from 10 locations along the east Australian coast.

Genus	Cleveland Bay	Bowling Green Bay	Upstart Bay	Airlie Beach	Repulse Bay	Newry Bay	Ince Bay	Clairview Bay	Hervey Bay	Moreton Bay	Bacteria Community Type
Bacteroides	1	1	1	1	1	1	1	1	1	1	Core
Blautia	1	1	1	1	1	1	1	1	1	1	Core
Clostridium sensu stricto 1	1	1	1	1	1	1	1	1	1	1	Core
Clostridium sensu stricto 13	1	1	1	1	1	1	1	1	1	1	Core
Polaribacter	1	1	1	1	1	1	1	1	1	1	Core
Ruminococcaceae UCG-002	1	1	1	1	1	1	1	1	1	1	Core
Unknown	1	1	1	1	1	1	1	1	1	1	Core
Christensenellaceae R-7 group	1	1	1	1	1	1	1	1		1	Pan
Ruminococcus 1		1	1	1	1			1	1	1	Pan
Turicibacter		1	1	1	1	1	1				Pan
Ruminococcaceae UCG-014	1		1					1	1	1	Pan
Akkermansia		1			1		1			1	Pan
dgA-11 gut group							1	1	1	1	Pan
Acetanaerobacterium		1			1					1	Pan
[Eubacterium] coprostanoligenes group	1		1								Pan
Caproiciproducens	1						1				Pan
Eubacterium		1			1						Pan
Subdoligranulum	1	1									Pan
[Eubacterium] nodatum group							1				Unique
Acinetobacter										1	Unique
Asteroleplasma								1			Unique
Bacillus										1	Unique
Brevibacillus										1	Unique
Catenibacterium	1										Unique
Erysipelotrichaceae UCG-004										1	Unique
Exiguobacterium										1	Unique
GCA-900 066 225							1				Unique
Halomonas										1	Unique
Lachnospiraceae ND3007 group										1	Unique
Lysinibacillus										1	Unique
Oscillibacter										1	Unique
Paludibacter			1								Unique
Pseudomonas										1	Unique
Psychrobacter										1	Unique
Pygmaiobacter					1						Unique
Ruminococcaceae UCG-010										1	Unique
Ruminococcaceae UCG-013					1						Unique
Sedimentibacter					1						Unique

Halodule genera, so it is unsurprising that these dugongs' faecal bacterial communities are similar. However, differences between these southern dugongs' more diverse bacterial communities versus bacterial communities elsewhere are more difficult to explain and may be related to *actual* rather than *potential* diet. Certainly, simple variation in local seagrass diversity does not necessarily explain this latitudinal pattern in bacterial communities' composition.

Furthermore, although there are more seagrass species in warmer northern waters, these are not necessarily seagrasses that dugongs will choose to consume. For example, some highly fibrous tropical species (*Cymodocea rotundata*, *Thalassodendron ciliatum*, *Enhalus acoroides*, *Zostera muelleri* wide-leaved morph) will never be consumed, or rarely, or only when alternative species are unavailable (Lanyon and Sanson 2006b, Marsh et al. 2011). It does not follow that an increase in the number of available seagrass species leads to an increase in the diversity of diet in dugongs. In this study, we found higher microbial diversity in southern foraging grounds with fewer seagrass species than in northern areas with greater seagrass diversity.

Higher microbial diversity in some individual dugongs in some areas and variation amongst areas (i.e. southern versus northern populations) may also reflect individual feeding behaviour (e.g. diet preference) rather than seagrass community diversity or overall abundance. For example, of 106 live dugongs whose mouth contents were analysed during health assessment in Moreton Bay (Lanyon et al. 2010), the dugong with the anomalous and unique microbial profile (MB16886; Fig. 4) was one of only two to have been eating *Zostera muelleri capricorni*; the other dugong's microbial profile was not examined. This may be evidence of an individual diet resulting in a unique bacterial community, however, the sample size of one dugong indicates that further investigation is warranted.

Our results show noted differences in faecal bacterial communities between dugongs from the northern (n=7) and southern (n=3) locations along the east Australian coast, notwithstanding some differences in collection methods along the coast. Faecal samples from dugongs in Moreton and Hervey Bays were fresh and uncontaminated by seawater, whilst all other samples were apparently fresh floating stools collected immediately after defecation. The fact that bacterial composition from faeces collected at Clairview aligned closely with those in Moreton and Hervey Bays, despite different collection methods, supports the premise of a real regional difference.

Conclusions

This study has identified several core bacterial genera including *Bacteroides*, *Blautia*, *Clostridium sensu stricto* 1, *Clostridium sensu stricto* 13, *Polaribacter* and Ruminococcaceae UCG-002 that were identified across all dugong populations along the eastern Australian coast, implying that these are likely important in seagrass digestion. Interestingly, the three southern-most populations had different faecal bacterial community compositions from northern populations, suggesting that there may be differences in diet, at least at times, between these locations. and/or due to genetic differences between host populations. Future analysis of the diet composition of dugongs across their Australian range will help to advance our understanding of the roles that seagrass species may play in affecting the dugong's faecal bacterial community composition. These studies will also aid in clarifying which seagrass communities and foraging grounds constitute essential dugong habitats and must be protected for the species' survival. Furthermore, the number of unique genera found in this study potentially indicates that dugongs harbour novel bacterial species that may be important to the digestion of seagrass in coastal marine systems, thus warranting culture-dependent studies coupled with 'omics-based investigations.

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Author contributions

Deirdre Mikkelsen (Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing), Alexandra M. McGowan (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft), Justine S. Gibson (Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing), Janet M. Lanyon (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing), Sara Horsman (Formal analysis, Software, Visualization, Writing – review & editing), and Jennifer M. Seddon (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing)

Supplementary data

Supplementary data is available at *FEMSEC Journal* online.

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