# Marine microbial communities of the Great Barrier Reef lagoon are influenced by riverine floodwaters and seasonal weather events

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## Abstract

The role of microorganisms in maintaining coral reef health is increasingly recognized. Riverine floodwater containing herbicides and excess nutrients from fertilizers compromises water quality in the inshore Great Barrier Reef (GBR), with unknown consequences for planktonic marine microbial  communities and thus coral reefs. In this baseline study, inshore GBR microbial communities were monitored along a 124 km long transect between 2011 and 2013 using 16S rRNA gene amplicon sequencing. Members of the bacterial orders Rickettsiales (e.g. Pelagibacteraceae) and Synechococcales (e.g. *Prochlorococcus*), and of the archaeal class Marine Group II were prevalent in all samples, exhibiting a clear seasonal dynamics. Microbial communities near the Tully river mouth included a mixture of taxa from offshore marine sites and from the river system. The environmental parameters collected could be summarized into four factors, represented by salinity, rainfall, temperature and water quality, that drove the composition of microbial communities. During the wet season, lower salinity and a lower water quality resulting from higher river discharge corresponded to increases in riverine taxa at sites near the river mouth. Particularly large, transient changes in microbial community structure were seen during the extreme wet season 2010-11, and may be partially attributed to the effects of wind and waves, which resuspend sediments and homogenize the water column in shallow near-shore regions. This work shows that anthropogenic floodwaters and other environmental parameters work in conjunction to drive the spatial distribution of microorganisms in the GBR lagoon, as well as their seasonal and daily dynamics.

## Introduction

Coral reefs are among the most biologically diverse and productive ecosystems on Earth. However, these complex assemblages, often compared to tropical rainforests, are under increasing anthropogenic pressure. Reefs are experiencing a rapid decline due to a combination of local pressures such as overfishing, nutrient enrichment, increased land runoff and sedimentation, and global disturbances such as rises in temperature (Pandolfi et al., 2003; De'ath et al., 2012). The GBR is a World Heritage Area and the largest reef complex in the world, stretching over 2,100 km along the Queensland coast of Australia. Despite being considered one of the best managed marine areas, the GBR is exposed to nutrient, sediments and pollutant inputs from land-based activities  (Schaffelke et al., 2012a, 2013) resulting in a 50.7% decrease in coral cover over the last

47  27 years (De'ath et al., 2012). Given the fundamental socio-economic role coral reefs have in

48  many countries (food production, tourism, coastal protection) and their ecological value (biodi-

49  versity and productivity), it is vital that these ecosystems are better understood and protected.

50  Microorganisms are a diverse group of unicellular organisms that form the base of the marine

51  food chain (Azam et al., 1983), hence indirectly sustaining higher order organisms including in-

52  vertebrates and fish. They are also an essential component of the coral holobiont, and disturbing

53  the balance between the corals and their associated microbiota has been implicated in reduced

54  reef health (Dinsdale et al., 2008; Bruce et al., 2012). In addition, the small size and fast repro-

55  duction rate of microorganisms make them very efficient at cycling nutrients, metabolizing for-

56  eign compounds in marine ecosystems and colonizing new ecological niches (Thurber et al.,

57  2009).

58  In the GBR lagoon, river runoff from agricultural areas introduces sediments, excess nutrients

59  from fertilizers (e.g. phosphate and nitrate) and pesticides (herbicides or insecticides) from the

60  land to the inshore waters (Furnas, 2003; Brodie et al., 2012), predominantly during discrete,

61  short-lived flood events during the 5-month summer monsoonal wet season. Land use changes

62  over the past 200 years (increased agriculture, urbanization) have increased the amounts of sedi-

63  ments, nitrogen, phosphorus and herbicides in these floodwaters (Devlin & Brodie, 2005; Devlin

64  et al., 2012a), with profound impacts on coastal ecosystems (Schaffelke, Mellors & Duke, 2005;

65  Fabricius, 2005; Brodie & Mitchell, 2005; De'ath & Fabricius, 2011; Schaffelke et al., 2013).

66  Particularly high levels of herbicides such as diuron are currently found in the GBR lagoon,

67  which inhibits the photosystem II and damages mangroves, seagrass, corals, and other non-target

68  photosynthetic organisms (Lewis et al., 2009; Shaw et al., 2010). While herbicides can be toxic to

69  some microorganisms (Leboulanger et al., 2008), they can be neutral to others that have dedi-

70  cated enzymes for their degradation (Aislabie & Lloyd-Jones, 1995). To date, microbial commu-

71  nities co-existing with the other macroscopic species on the GBR have not been characterized

72  and it is unclear how anthropogenic compounds found in seasonal runoff affect these communi-

73  ties.

74  In this study, we characterized planktonic microbial communities of seven GBR lagoon sites dif-

75  ferentially exposed to inputs from the rivers of the Wet Tropics catchment. Over three years, we

76  determined water chemistry and characterized microbial communities using 16S rRNA gene am-

77  plicon sequencing. We hypothesized that microbial communities follow seasonal dynamics and

78  respond to riverine input, potentially buffering reef ecosystems against effects of elevated flood-

79  water constituents through nutrient cycling and detoxification.

## Materials & Methods

### Sampling design

82  Sampling was performed in the Wet Tropics Region of the GBR (Figure 1), a well-studied coastal
83  area which is regularly exposed to river runoff and flood events (Devlin & Schaffelke, 2009;
84  Schroeder et al., 2012; Turner et al., 2013). The sites surveyed were located on a transect follow-
85  ing a gradient of river exposure, from the highly-exposed Tully River mouth (TT1) to the fringing
86  coral reefs of Dunk Island (TT3) that are seasonally reached by flood plumes, and the TT4 off-
87  shore location, rarely exposed to river water. Russel (RI) and Fitzroy islands (FI) were additional
88  reef sites with limited exposure to the waters of the Johnstone and Russel rivers, respectively, and
89  a consistently higher coral health index than Dunk Island (Thompson et al., 2014). All sampling
90  sites were classified based on their proximity to the nearest influencing river mouth: 'plume' for
91  <20 km downstream and 'marine' if >20km (Table S1). To characterize the influence of riverine
92  microorganisms on marine communities, a freshwater site located 12 km upstream of the river
93  mouth was also selected (TR).

94  The seven sites were surveyed between January 2011 and October 2013 in the dry season (June),
95  just prior to the wet season (October), and at the end of the wet season (March). At each site and
96  sampling date, a single 2 L seawater sample was taken from a depth of 5 m (just below surface
97  for the TR site, which was a very shallow river bed), passed through a 0.22 µm Sterivex filter,
98  which was stored at -20ºC until further processing. All samples were collected under the auspices
99  of the general permit (G12/35236.1) granted by the Great Barrier Reef Marine Park Authority to
100 the Australian Institute of Marine Science.

### Environmental conditions

102 Water samples collected from 2011 to 2013 were processed according to the long-term GBR Reef
103 Rescue Marine Monitoring Program (Thompson et al., 2013) to assess temperature, salinity, bot-
104 tom depth and water chemistry: concentrations of suspended solids (SS), particulate organic car-
105 bon (POC), particulate phosphorus (PP), particulate nitrogen (PN), dissolved inorganic nitrogen
106 (DIN), silica (Si) and chlorophyll *a* (CHLA). In addition, diuron concentration was determined
107 by collecting 1 L of water in pre-washed bottles, and storing the water at 4ºC until processing by

108 solid phase extraction liquid chromatography tandem mass spectrometry (SPE-LC-MS/MS) at

109 Queensland Health and Forensic Scientific Services, Coopers Plains, Australia. All these mea-

110 sured environmental parameters were deposited as NCBI BioSamples (accession # PR-

111 JNA276058). In addition, meteorological parameters were acquired from public resources: Tully

112 River discharge and water temperature (Department of Natural Resources and Mines, http://wa-

113 termonitoring.derm.qld.gov.au/host.htm), and solar exposure and rainfall (Bureau of Meteorol-

114 ogy, http://www.bom.gov.au/climate/data/index.shtml). An average of these meteorological pa-

115 rameters was calculated for the seven days preceding each sampling date.

116 A river exposure index was calculated using a hydrodynamic model (http://www.bom.gov.au/en-

117 vironment/activities/coastal-info.shtml), based primarily on the Sparse Hydrodynamic Ocean

118 Code (SHOC) hydrodynamic model

119 (http://www.emg.cmar.csiro.au/www/en/emg/software/EMS/hydrodynamics.html). SHOC is a

120 general purpose model, applicable on spatial scales ranging from estuaries to regional ocean do-

121 mains (Herzfeld, 2006). We used outputs from the regional application of SHOC to the GBR us-

122 ing a horizontal spatial resolution of about 4 km, with a model grid size of 180×600 with 48 ver-

123 tical layers with 1 m resolution at the surface. In this context, conservative tracers were used in

124 this study to simulate the transport of unique tracers 'released' from different rivers. In mathemat-

125 ical terms, a conservative substance is represented without terms of sinks or sources of mass in

126 the transport equation. This means that the change in concentration values happens due to physi-

127 cal processes (advection and diffusion). Furthermore, it was stipulated that the tracers used do not

128 affect the hydrodynamics. This technique enables the identification of marine regions influenced

129 by individual catchments, and provides insight into the mixing and retention of river water along

130 various regions in a given domain (Brinkman et al., 2002, 2014; Luick et al., 2007). Model simu-

131 lations of the 3-dimensional distributions of passive tracers were analyzed to produce weekly es-

132 timates of cumulative exposure to tracers above a threshold of 1% of the source concentration.

133 An exposure index was calculated that integrates the tracer concentrations above this threshold,

134 based on a cumulative measurement of the exposure concentration and duration of exposure re-

135 lated to individual river sources, and expressed as Concentration x Days (conc.d). For every loca-

136 tion in the model domain, the cumulative exposure index was calculated as:

137 $$\text{Conc.Days} = \sum_{t=0}^{T} \text{Conc}_{exceed} \times t \quad \text{where} \quad \text{Conc}_{exceed} = \begin{cases} \text{Conc}(t) - \text{Conc}_{thresh}, & \text{if } \text{Conc}(t) > \text{Conc}_{thresh} \\ 0, & \text{if } \text{Conc}(t) \leq \text{Conc}_{thresh} \end{cases}$$

138  and Conc$_{thresh}$ is defined as 1% of the source concentration, Conc($t$) represents the time-varying

139  tracer concentration, and $t$ is the time in days from the beginning of the wet season to the end  (01

140  November – 31 March). Cumulative exposure was calculated for each grid point in the model do-

141  main. Using this representation, the exposure index integrates both concentration above a defined

142  threshold and the duration of exposure. For example, an exposure of 20 days at a concentration of

143  1% above the threshold would produce an index value of 0.2, which is equivalent to 10 days ex-

144  posure at 2% above the concentration threshold. This index provides a consistent approach to as-

145  sess relative differences in exposure of inshore GBR waters to inputs from various rivers. For

146  each of the wet seasons simulated by the model, spatial maps of river exposure indices were cal-

147  culated for the target rivers: Herbert, Tully, Murray, Johnstone, Mulgrave and Russel rivers (Wet

148  Tropics catchment), Burdekin and Haughton rivers (Burdekin catchment, affecting the south of

149  the Wet Tropics catchment).

## 16S rRNA gene amplicon sequencing

151  DNA was extracted from each Sterivex filter using a modified method from Suzuki et al. (2004).

152  In brief, the filters were thawed on ice with Invitrogen's P1 buffer with lysozyme at a final con-

153  centration of 2 mg/mL, and incubated for 30 min at 37 ºC, while rotating at 10 rpm. Proteinase K

154  (0.75 mg/mL final concentration) and 10 % sodium dodecyl sulfate (1 % final concentration)

155  were added and the sample was incubated, with rotation, at 55 ºC for 2 h. DNA was extracted us-

156  ing phenol:choloroform:isoamyl alcohol (25:24:1; pH 8.0) followed by an overnight ethanol pre-

157  cipitation and purified using a MO BIO PowerClean DNA Clean-Up kit (Carlsbad, CA).

158  Amplicons were generated by PCR-amplifying the V6 to V8 variable regions of the 16S rRNA

159  gene using the pyroLSSU926F and pyroLSSU926F universal primers as described in Dove et al.

160  (2013). The resulting DNA amplicons were sequenced on a Roche-454 GS-FLX instrument at the

161  Australian Centre for Ecogenomics and deposited in the NCBI Short Read Archive (accession #

162  PRJNA276058).

## Bioinformatic processing

164  Amplicon reads were processed using Hitman (https://github.com/fangly/hitman), a bioinformatic

165  workflow based around the UPARSE methodology (Edgar, 2013). In brief, Hitman: 1) joins read

166  pairs with PEAR (Zhang et al., 2014), but keeps the forward read when pairs cannot be joined; 2)

167  truncates the 3' end of sequences at the first residue below a threshold quality value ($Q$) using

168  TRIMMOMATIC (Bolger, Lohse & Usadel, 2014); 3) trims the 3' end of all sequences to a target

169  length (*L*) using TRIMMOMATIC, discarding all smaller sequences, 4) removes sequences ex-

170  ceeding the maximum number of expected errors (*E*) using USEARCH's fastq_filter (Edgar &

171  Flyvbjerg, 2015); 5) uses USEARCH's cluster_otus to form operational taxonomic units (OTUs)

172  from high-fidelity sequences (stringent quality processing in steps 2 and 4) that are sorted by de-

173  creasing abundance, occur at least twice in the dataset and meet a minimum percentage of simi-

174  larity (*O*); 6) discards chimeric OTUs using USEARCH's cluster_otus in a reference-independ-

175  dent, and using UCHIME (Edgar et al., 2011) based on a reference database (*C*); 7) assigns regu-

176  lar-fidelity sequences (less stringent quality processing in steps 2 and 4) to each OTU using USE-

177  ARCH's usearch_global (Edgar, 2010); 8) formats the results in BIOM format using Bio-Com-

178  munity's bc_convert_files (Angly, Fields & Tyson, 2014); 9) gives a taxonomic assignment to

179  each OTU by globally aligning their representative sequences against a database (*T*) of reference

180  sequences trimmed to the target region (keeping only the best-matching alignment with a mini-

181  mum required identity percentage (*I*) using USEARCH's usearch_global; 10) removes OTUs be-

182  longing to specific taxa (*W)* using Bio-Community's bc_manage_samples; 11) rarefies the micro-

183  bial profiles at the given depth (*D*) with Bio-Community's bc_accumulate assuming an infinite

184  number of bootstrap replicates; and 12) corrects gene-copy number bias using CopyRighter (An-

185  gly et al., 2014).

186  In this study, Hitman was run using the following parameters: *L*=250 bp, *Q*=7 (16 for HiFi se-

187  quences), *E*=3.0 expected errors (0.5 for HiFi sequences), *O*=97% identity (species-level),

188  *C*=GOLD database (Bernal, Ear & Kyrpides, 2001), *T*=merged Silva (Quast et al., 2012) and

189  Greengenes (McDonald et al., 2012) databases (https://github.com/fangly/merge_gg_silva),

190  *I*=95% identity (genus-level), *W*="Eukaryota* *Chloroplast*" and *D*=279 for Bacteria & Archaea

191  (100 for Eukaryotes). In addition, rarefaction curves were generated using Bio-Community (An-

192  gly, Fields & Tyson, 2014).

193  **Statistical analysis**

194  All statistical analyses were carried out using the R language (R Foundation for Statistical Com-

195  puting, Vienna, Austria). Comparisons of diversity between groups of samples were carried out

196  using the non-parametric, unilateral Mann-Whitney U test (wilcox.test() function). Principal co-

197  ordinates analysis (PCoA) and PERMANOVA were performed using the capscale() and adonis()

198  functions of the vegan package (Dixon, 2003). The indicspecies package (Cáceres & Legendre,

199  2009) was used to determine indicator species with the multipatt() function. Redundancy analysis

200  (RDA) model selection was based on the AIC (Akaike information criterion) and calculated by

201    ordistep() in vegan. Pearson correlations between environment variables were computed using
202    rcorr() from the Hmisc package. The functions fa.parallel(), fa(), target.rot() and fa.diagram()
203    from the psych package were used to conduct exploratory factor analysis (EFA), i.e. to identify
204    groups of co-varying variables. EFA was performed on several subsets of the data including dif-
205    ferent environmental parameters and the results were summarized.

## Results & Discussion

### Sampling and environmental context

208    Seven inshore GBR sites exposed to different levels of river runoff from the Wet Tropics catch-
209    ments were surveyed over three years for water chemistry assessments and determination of mi-
210    crobial community structure. The classification of these sites as plume or marine sites was based
211    on their distance to the nearest influencing river mouth (Table S1) and matched well with their
212    river exposure index as calculated by oceanographic modeling (Figure 1); sites <20 km from a
213    river mouth were more highly exposed to riverine water (>20 conc.d) than sites >20 km away
214    (<15 conc.d).

215    The weather in the Tully catchment from 2010 to 2013 followed the expected seasonal dynamics,
216    dry and cool conditions between the months of May and October, hot and humid with most of the
217    annual rainfall from November to April (Figure 2). However, the 2010–11 wet season was
218    marked by extreme weather (Table S2) and the landfall of category 5 tropical cyclone Yasi (3
219    February 2011), that significantly affected coral reefs (Perry et al., 2014). Annual river discharges
220    reached a record high (Schaffelke et al., 2011), causing elevated exposure to nutrients, PSII herbi-
221    cides and sediments across most inshore GBR regions (Devlin et al., 2012b; Kennedy et al.,
222    2012; Perry et al., 2014).  The following wet season (2011–12) represented a return to typical
223    weather conditions, with river discharge close to the long-term median (Schaffelke et al., 2012a;
224    Wallace et al., 2014). However, coral reef recovery was delayed until 2014, when the coral health
225    index reached pre-2011 levels again (Thompson et al., 2014).

### Microbial diversity

227    The archaeal and bacterial microbial profiles obtained by Roche-454 sequencing of 16S rRNA
228    gene amplicons were rarefied (279 counts per sample) to allow comparison of microbial profiles
229    (Figure S1). A ten times higher sequencing effort would have been needed to sample nearly all

230  the OTUs present in these aquatic samples (richness; Figure S1 A). Nevertheless, the chosen rar-

231  efaction depth recovered the vast majority of archaeal and bacterial diversity (Shannon-Wiener

232  index; Figure S1 B). The microbial diversity of the rarefied profiles was calculated, with a me-

233  dian richness of ~65 OTUs in the river, and ~90–100 at the marine and plume sites (Figure 3 A).

234  At plume sites, richness (Figure 3 A) and evenness (Figure 3 B) were higher in the dry season

235  than in the wet season, which corresponded to a larger overall diversity (Shannon-Wiener index)

236  in the dry season (Figure 3 C) (Mann-Whitney U test; $p < 0.05$).

237  ## Establishing a microbial monitoring baseline for inshore GBR

238  Taxonomic assignments and gene copy number correction were conducted to produce accurate

239  estimates of microbial relative abundance (Angly et al., 2014) (Figure S2) that yield an under-

240  standing of the prevalence of microbial taxa in the GBR lagoon waters. At a coarse taxonomic

241  level, the bacterial orders Sphingobacteriales, Burkholderiales, and Xanthomonadales dominated

242  the TR river site (Figure 4), while the archaeal order E2 and bacterial orders Rickettsiales and

243  Synechococcales were prevalent at the plume and marine sites (Figure 4). On average, Rick-

244  ettsiales and E2 had higher relative abundance at the marine sites (29.6 and 17.7% respectively)

245  compared to the plume sites (20.4 and 10.4% respectively). This distribution was not constant

246  over time, and notably, the plume sites were characterized by a large fraction of Burkholderiales

247  in the January – March 2011 period, i.e. the extreme wet season 2010–11.  Flavobacteriales were

248  found in all sites, riverine, plume and marine. Burkholderiales are Betaproteobacteria commonly

249  found in rivers (Cottrell et al., 2005; Ghai et al., 2011; Liu et al., 2012). Sphingobacteriales have

250  been reported at riverine locations affected by waste water treatment effluent (Drury, Rosi-Mar-

251  shall & Kelly, 2013), where they may degrade complex compounds such as herbicides and antibi-

252  otics (Kämpfer, 2011). Synechococcales such as those found in the plume and marine sites in-

253  clude the *Synechococcus* and *Prochlorococcus* genera, which represent the main photosynthetic

254  bacteria in oceanic waters (Partensky, Blanchot & Vaulot, 1999; Partensky, Hess & Vaulot, 1999),

255  and the small heterotrophic Rickettsiales are also commonly reported in the ocean (Morris et al.,

256  2002; Carlson et al., 2008). More specifically, both orders were previously identified in coral reef

257  waters (Kelly et al., 2012; Lu et al., 2015). However, Thermoplasmata E2 Archaea have only oc-

258  casionally been reported in temperate surface seawater (Massana et al., 1997; Pernthaler et al.,

259  2002). These Archaea are motile photo-heterotrophic cells focused on the degradation of protein

260  and lipids (Iverson et al., 2012) and are found in coral mucus, suggesting a potential role in main-

261  taining coral health (Kellogg, 2004).

262   Genomic methods such as 16S rRNA gene amplicon sequencing are effective for marine monitor-

263   ing (Bourlat et al., 2013). The present taxonomic characterization of the microbial communities

264   in inshore GBR waters represents a baseline against which future microbiological studies can be

265   compared. This baseline may prove valuable for assessing future change in this reef ecosystem,

266   be it further degradation or recovery.

267   ## Geographical and temporal distribution of OTUs

268   Non-constrained ordination (PCoA) was applied to get a precise account of the dynamics and dis-

269   tribution of specific microbial taxa in inshore GBR. At the finer operational taxonomic unit

270   (OTU) level (Figure 5 A), significant spatial (distance to river mouth and site type; PER-

271   MANOVA, $p<0.05$) and temporal effects (wet or dry; PERMANOVA, $p<0.05$) were confirmed.

272   Site type (riverine, plume or marine) had the strongest effect (27.8% explained variance along

273   PC1, Figure 5 A), followed by seasons (wet or dry) (10.0% explained variance along PC2, Figure

274   5 A).

275   Marine and plume samples were characterized by indicator OTUs, i.e. OTUs characteristic of the

276   marine and plume areas (indicator species analysis; $p<0.05$). These indicator OTUs belonged to

277   the Rickettsiales (e.g. Pelagibacteraceae), Synechococcales (e.g. *Prochlorococcus*), Acidimicro-

278   biales (e.g. OCS155), and the Archaeal order E2 (e.g. Marine Group II) (Figure 5 A), which is

279   largely consistent with the trends observed at the order level (Figure 4). The main difference be-

280   tween the OTU-  and order-level analyses (Figure 5 and Figure 4 respectively) was the detection

281   of OCS155, an OTU indicator of marine and plume sites (Figure 5 A). OCS155 belongs to the

282   Acidimicrobiales, a group of likely planktonic, free-living microorganisms with a photo-het-

283   erotrophic lifestyle (Mizuno, Rodriguez-Valera & Ghai, 2015) found in all tropical and temperate

284   photic areas (Ghai et al., 2013).

285   Indicator OTUs for the river samples were also identified (indicator species analysis; $p<0.05$), in-

286   cluding members of the orders Burkholderiales (family Comamonadaceae, e.g. *Limnohabitans*,

287   *Acidovorax* and *Rhodoferax*; family Oxalobacteraceae), and Sphingobacteriales (family Flexibac-

288   teraceae, e.g. *Arcicella*; family Chitinophagaceae) (Figure 5 A). These results are congruent with

289   those at the order level (Figure 4). The presence of riverine OTUs belonging to the order

290   Burkholderiales and Sphingobacteriales at some plume sites surveyed in the wet months (Figure

291   4, Figure 5 A), when river discharge is high, is a sign that these sites are affected by riverine wa-

292   ter effluent.

293   When repeating the PCoA with the marine samples only, i.e. restricting the analysis to samples

294   unaffected by river output, a clear partition between dry and wet seasons (Figure 5 B) was seen

295   (PERMANOVA, p<0.05). All samples clustered in one of two season-specific groups arranged

296   along PC1, except for one sample collected at TT4 in December 2011. Several indicator OTUs

297   were identified (indicator species analysis; p<0.05)(Table S3), e.g. Pelagibacteraceae (3 OTUs),

298   Pirellulaceae (2 OTUs) and Marine group II (2 OTUs), which were specific of the dry season.

299   Generally though, Marine group II OTUs seemed to be more abundant in the wet season (Figure

300   5 A and B). Previous studies have shown seasonality in near-shore microbial communities

301   (Treusch et al., 2009; Gilbert et al., 2009, 2012). In particular, *Pelagibacter* is known to exhibit

302   seasonality (Alonso-Sáez et al., 2007; Carlson et al., 2008; Eiler et al., 2009; Fortunato et al.,

303   2013). Overall, the microbial communities of the GBR lagoon seem to respond to seasonal influ-

304   ence, although it is not as pronounced as the influence of geographical location, an observation

305   that was also made in a previous investigation of river to ocean gradient (Fortunato et al., 2012).

306   In addition to a seasonal effects, GBR lagoon samples were susceptible to short term effects of

307   potentially high magnitude. Rapid community changes were evident when looking at the Tully

308   transect samples (TT1–4) collected in the wake of Cyclone Yasi (03 Feb 2011) (Figure S3). The

309   influence of Tully river input was marked, with elevated levels of Sphingobacteriales and

310   Burkholderiales recorded between 13 and 17 February. While these changes were especially pro-

311   nounced at the Tully River mouth, even the offshore site TT4 experienced analogous changes in

312   this period. Especially large changes in community structure were observed between consecutive

313   days, on February 12–13 (Figure 2). These findings add to previous investigations of soil and gut

314   microbiota, which have shown that community composition can change within a few days

315   (Schmidt et al., 2007; Michelland et al., 2011; David et al., 2014). While baselines may be estab-

316   lished using e.g. monthly sampling, it is clear that future research should adopt an intensive daily

317   sampling regimen to better characterize such transient changes in estuarine and marine microbial

318   communities.

### Environmental drivers of coastal microbial dynamics

320   A range of measurements describing the environmental context of the microbial samples were

321   collected in this study, including temperature, salinity, bottom depth and water chemistry vari-

322   ables (concentrations of suspended solids, silica, chlorophyll *a*, the herbicide diuron, dissolved

323   and particulate form of carbon, nitrogen and phosphorus), and complemented with weather con-

324   dition data (Tully River discharge, local rainfall and solar exposure). Many of these environmen-

325    tal parameters were highly correlated (Pearson test; 48.6% of the pairs with r > 0.3), requiring the

326    need for EFA to extract groups of uncorrelated parameters. Following this procedure (Figure S4),

327    four independent factors were identified. Factor MR1 included local rainfall and Tully River dis-

328    charge. Factor MR2 included chlorophyll *a*, suspended solids and other particulates (POC, PN,

329    PP). Factor MR3 included DIN and salinity and can be interpreted as mixing with freshwater.

330    Factor MR4 combined water temperatures and solar exposure. Although larger discharge (factor

331    MR1) can lead to increased suspended solids (factor MR2) and salinity decrease (factor MR3),

332    factor MR1, MR2 and MR3 were not directly correlated because salinity and the amount of sus-

333    pended solids depend not only on site location (proximity to the river mouth) but also on the ac-

334    tion of wind and waves, which homogenize the water column and resuspend sediments (Lar-

335    combe et al., 1995; Orpin & Ridd, 2012; Fabricius et al., 2014). A water quality index based on

336    comparison to water quality guidelines (GBRMPA, 2010) and ranging from -1 for poor quality to

337    +1 for very good quality was previously introduced (Thompson et al., 2013). This index aggre-

338    gates scores given to four indicator concentrations (suspended solids, chlorophyll *a*, particulate

339    nitrogen, particulate phosphorus), which are all part of the suspended material factor (factor

340    MR2) identified by EFA in the present study. Diuron has a strong association with sediments

341    (Stork, Bennett & Bell, 2008; Balakrishnan, Takeda & Sakugawa, 2012; Xu et al., 2013) and is

342    also included in this factor. Overall, this suggests that factor MR2 can be interpreted as the qual-

343    ity of the water.

344    Constrained ordinations (RDA) were carried out to study the relative importance of the four inde-

345    pendent factors (MR1–4) on microbial community structure. A single environmental parameter

346    was chosen to represent each factor prior to conducting RDA with model selection: rainfall for

347    factor MR1, water quality index for MR2, salinity for MR3, and water temperature for MR4 (Fig-

348    ure 6). These four environmental parameters were significantly associated with microbial com-

349    munity composition (PERMANOVA, p<0.05). Microbial community structure was affected by

350    temperature (Figure 6 A), consistent with previous findings in the Western English Channel

351    (Gilbert et al., 2009), which could explain the seasonality identified in marine sites. Further, sea-

352    sonality can be attributed to the higher rainfall typical of the wet season (Figure 6 A). However,

353    large changes could be attributed to decreases in water quality index and salinity, especially for

354    plume sites (Figure 6 A), with negative consequences for corals (Fabricius et al., 2014). These

355    changes can be interpreted as the effect of riverine floodwater, which was especially pronounced

356    for sites located near the Tully river mouth, and helps explain the geographical pattern seen in mi-

357  crobial communities. The effects of salinity and water quality index were mostly collinear in Fig-
358  ure 6 A, despite the fact that they represent two separate factors (MR2 and MR3). Repeating the
359  RDA analysis with the influence of salinity removed (conditioning term, Figure 6 B), water qual-
360  ity index was still identified as a significant driver of microbial communities (PERMANOVA,
361  p<0.05). This suggests that, as a whole, nutrient, organic compounds and herbicides such as di-
362  uron brought by riverine water into the ocean have an effect on microbial communities.

363  RDA analysis was also conducted to identify the factors driving the large, transient changes de-
364  tected by microbial profiling at the plume sites on 12–13 February 2011 (Figure S5). Being only
365  nine days after the landfall of tropical cyclone Yasi, this period was characterized by extreme
366  weather, e.g. high river discharge (12 Feb: 37.1 ML/d; 13 Feb: 36.2 ML/d; 7–13 Feb mean: 27.5
367  ML/d). While river discharge was similar on the two days, the February 13 microbial communi-
368  ties were characterized by elevated levels of suspended solids and lower salinity (PERMANOVA,
369  p<0.05), suggesting that other environmental factors may contribute to microbial community
370  structure. We speculate that larger wind or waves were present on February 13, which would
371  have decreased the vertical stratification of the water column, resulting in lower salinity and
372  higher concentration of suspended solids. This example illustrates that salinity and suspended
373  solids at inshore locations may be a function of both river flow and wind (Schaffelke et al.,
374  2012b; Fabricius et al., 2014). The 12 February was also marked by an increase in the eukaryotic
375  to prokaryotic reads ratio (EPR) (Figure S6), with a maximum EPR of 1.45 reached on February
376  13 at TT1. The most abundant eukaryal taxon in this sample was assigned to the hydrozoan genus
377  Merona, that includes very small organisms that can spawn eggs (Schuchert, 2004). These data
378  could be interpreted as the potential spawning of hydrozoan or the displacement from their usual
379  habitat (e.g. the benthic zone) and they illustrate that extreme weather may dramatically change
380  environmental conditions, thereby affecting all microbial kingdoms (Archaea, Bacteria and Eu-
381  karyota).

## Conclusions

383  This study is a baseline description of microbial communities in the inshore GBR lagoon. Marine
384  Group II Archaea, Pelagibacteraceae and Rickettsiales were prevalent in all the seawater samples.
385  A seasonal effect of temperature and rainfall on the microbial communities was apparent in the
386  three year sampling period. However spatial effects were more pronounced, with sites located
387  close to the Tully river mouth including many river-specific taxa, particularly during the wet sea-
388  son. Seasonal storms like those that occurred in the wet season 2010–11 caused elevated sus-

389 pended solids and decreased salinity at plume sites, which translated into large, transient changes
390 in microbial community structure. Water quality played a role in driving microbial community
391 structure in the GBR lagoon, but the complex interconnections between environmental parame-
392 ters mean that future research such as experimental manipulations will be needed to precisely elu-
393 cidate how each individual anthropogenic compound shapes microbial community composition
394 and affects coral reefs.

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398 the Australian Centre for Ecogenomics for her assistance with 454 gene amplicon sequencing.

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# 1

*Overview of sampling area in the GBR lagoon*

*Figure 1: Overview of sampling area in the GBR lagoon. The river exposure index is shown for the Wet Tropics river catchments in the 2010–11 wet season, with a color bar indicating clustered cumulative exposure (concentration x days) above 1% of the incoming concentration (capped at 20 conc.d). The direction of the residual coastal current is indicated as a black vector. The location of the sites surveyed for microbial composition in 2011–13 is shown as colored paddles. The sites were classified as marine, plume or riverine, according to their respective distance to the nearest influent river mouth.*

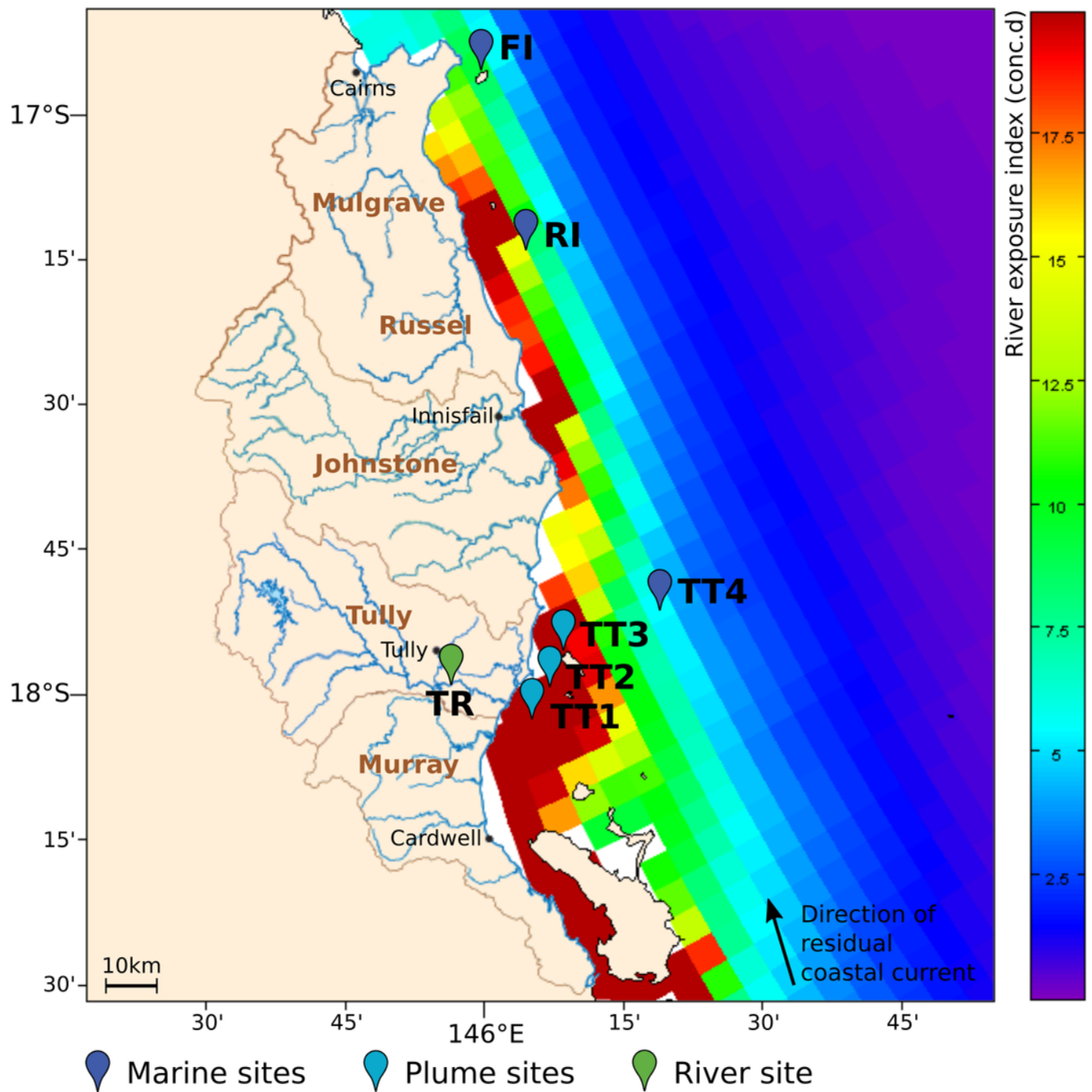
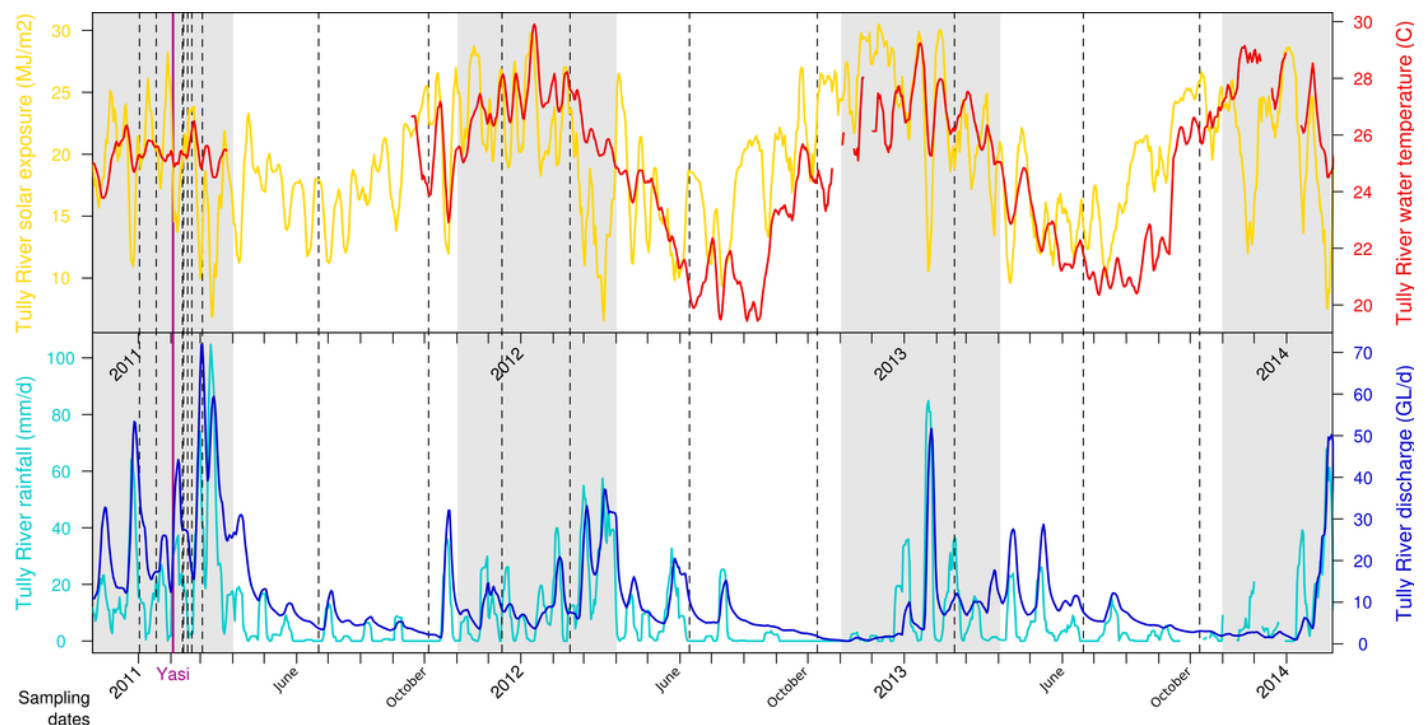
# 2

*Weather in the Tully catchment during the years 2011–13*

*Figure 2: Weather in the Tully catchment during the years 2011–13: A) temperature and solar exposure, and B) rainfall and river discharge. An average value for the previous week is reported for each day. Dashed lines indicate microbial sampling dates, and the purple line the landfall of tropical cyclone Yasi. The shading represents the extent of the wet season. Sources: BOM, DERM.*

# 3

*Seasonal and spatial differences in microbial diversity*

*Figure 3: Boxplot of seasonal (wet or dry) and spatial (three runoff exposure categories) differences in bacterial and archaeal diversity: A) observed OTU richness, B) Shannon-Wiener evenness, C) Shannon-Wiener index. Boxes represent the first quartile, median and third quartile of the data, whiskers the minimum and maximum, and circles the outliers. Blue bars show the statistical comparisons performed and significant differences are represented by a star (Mann-Whitney U test; p<0.05).*

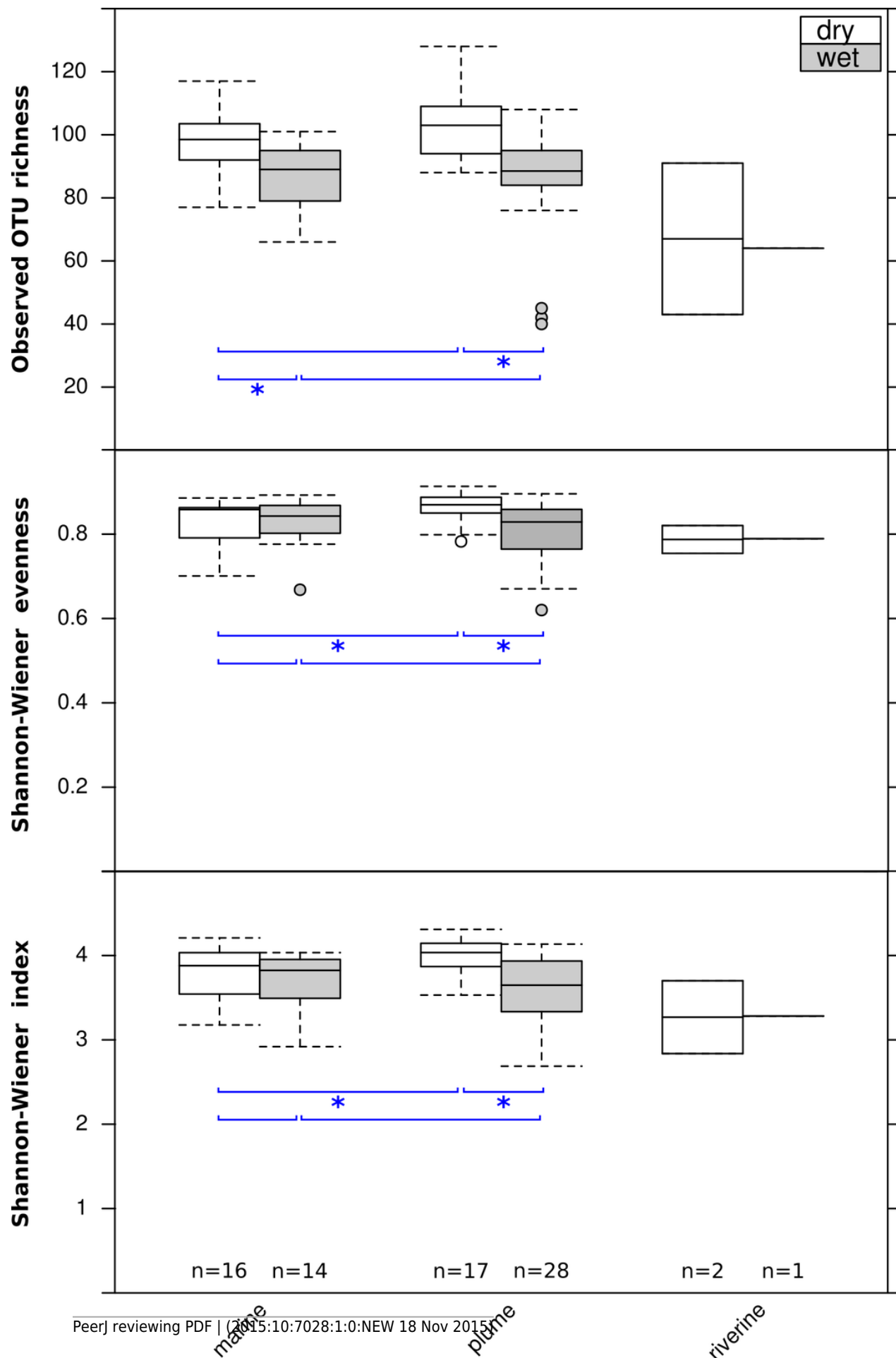
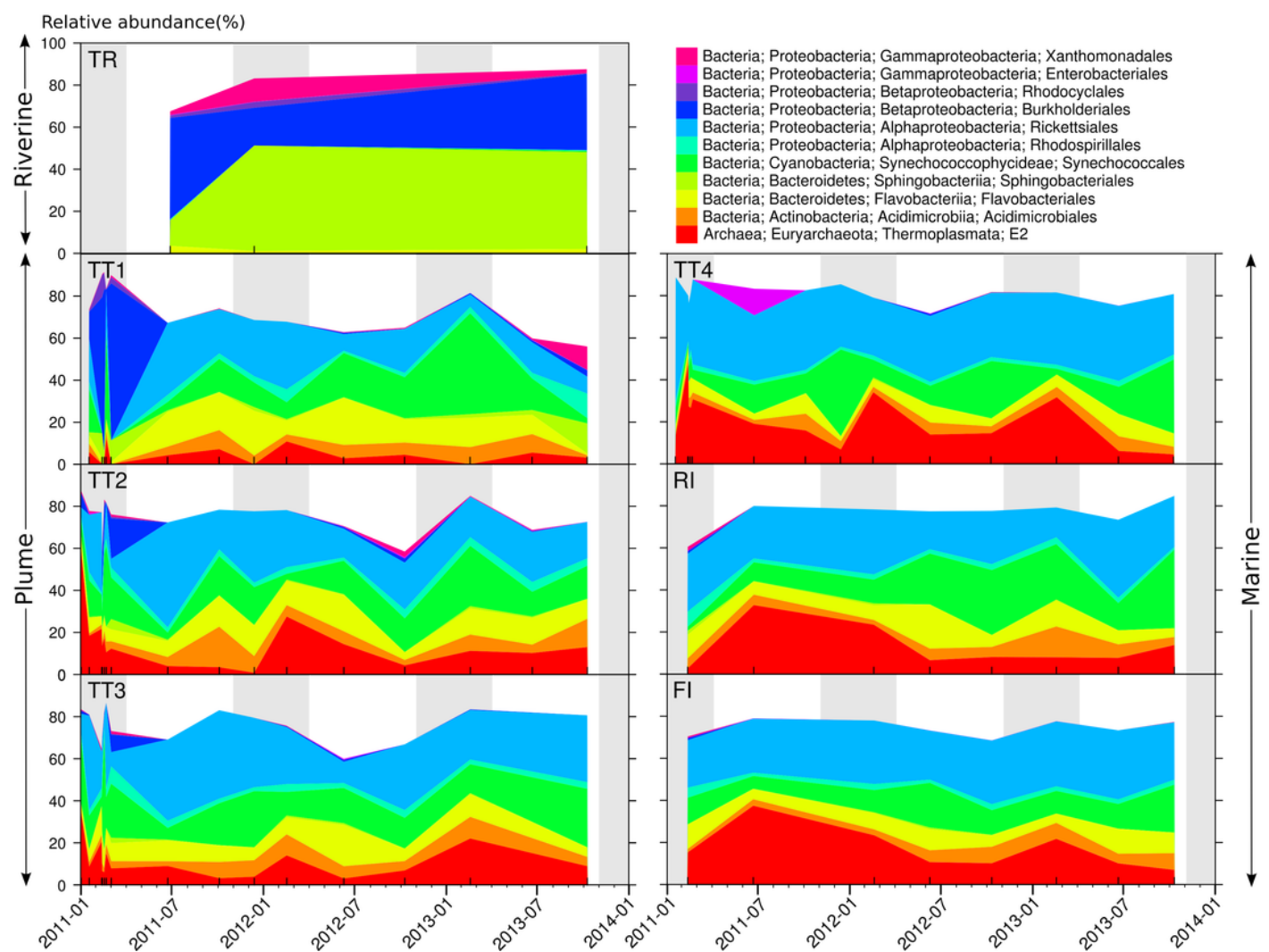
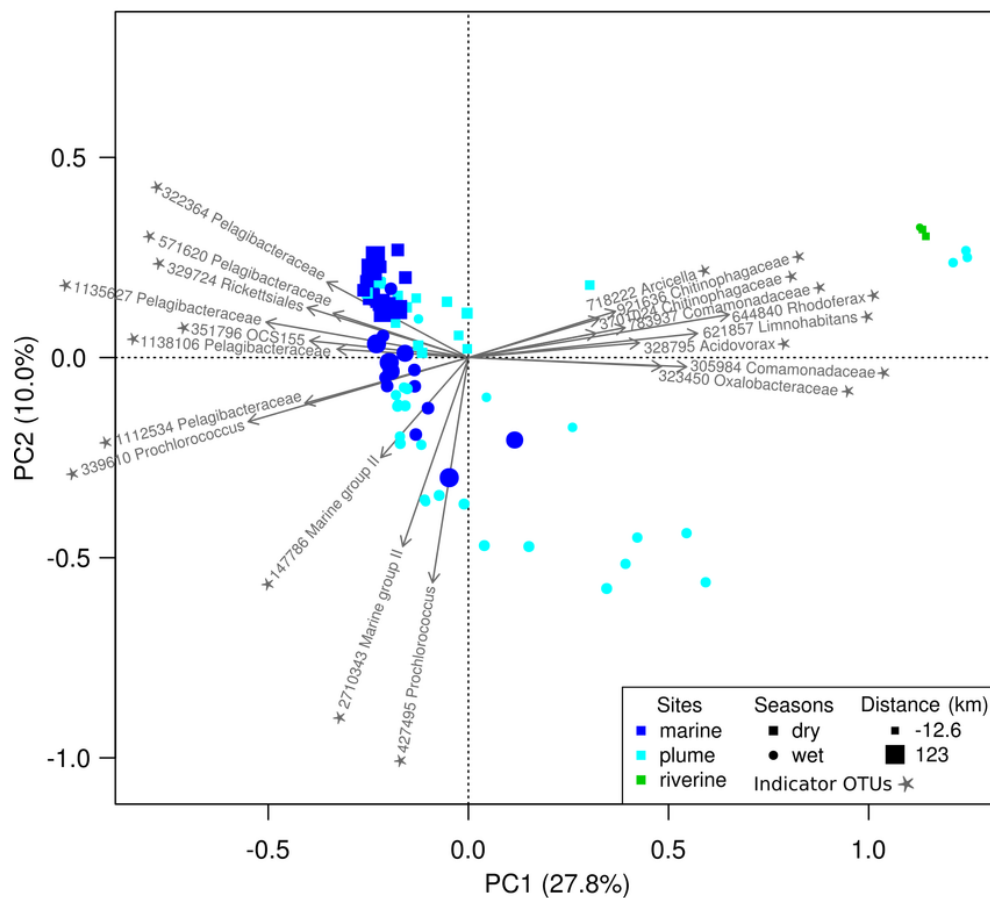
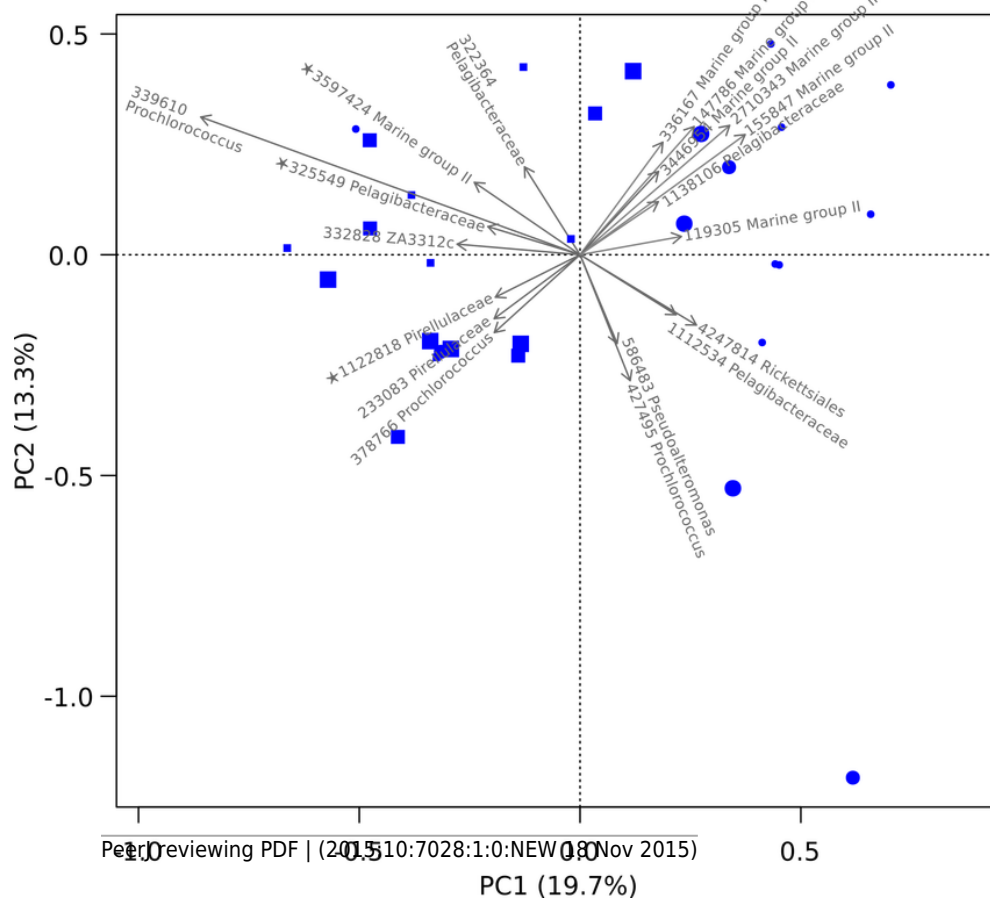
# 4

*Distribution and dynamics of abundant microbial orders for the seven sites surveyed*

*Figure 4: Distribution and dynamics of abundant microbial orders for the seven sites surveyed in 2011, 2012 and 2013. Microbial orders present at less than 10% relative abundance in all samples were omitted. The shading represents the extent of the wet season.*

# 5

*Spatiotemporal organization of microbial communities*

*Figure 5: PCoA plot showing the spatiotemporal organization of microbial communities based on their Hellinger dissimilarity. Symbol color, shape and size depend on the type of site, season of collection and distance from the Tully River mouth, respectively. The OTUs explaining the largest variation are represented by arrows and their assigned Greengenes ID and genus-level taxonomy is shown. A star indicates indicator OTUs (indicator species analysis; p<0.05).*

# 6

*Relationship between environmental parameters and microbial profiles*

*Figure 6: RDA plot showing the relationship between GBR environmental parameters and Hellinger-transformed microbial profiles: A) for all environmental factors, and B) with the contribution of salinity removed. Disks represent sampling sites and are colored according to the AIMS water quality index (dark green: very good, green: good, yellow: moderate, orange: poor, red: very poor). OTUs are depicted by red crosses and the genus-level Greengenes taxonomy of the most discriminating ones is shown. The factors explaining sample distribution are represented by blue arrows: rainfall in the last 7 days (rain_7d), water temperature (water_temp), water quality index (water_qual_idx) and salinity. Any significant association is indicated by a star (PERMANOVA; p<0.05).*

## A) All factors

## B) Without salinity