Extreme storm-induced run-off causes rapid, context-dependent shifts in nearshore subtropical bacterial communities

Ángela Ares[1*], Margaret Mars Brisbin[1*], Kirk N. Sato[1,2], Juan P. Martín[1], Yoshiteru Iinuma[3], Satoshi Mitarai[1]

* contributed equally

[1] Marine Biophysics Unit, Okinawa Institute of Science and Technology (OIST, Okinawa, Japan)
[2] Friday Harbor Laboratories, University of Washington (U.S.A.)
[3] Instrumental Analysis Section, Okinawa Institute of Science and Technology (OIST, Okinawa, Japan)

Abstract

Climate change scenarios predict tropical cyclones will increase in both frequency and intensity, which will escalate the amount of terrestrial runoff entering coastal ecosystems. Prokaryotes are known to respond quickly to environmental change, making them potentially valuable early-warning bioindicators, but relatively little is known about their short-term responses during extreme storms in nearshore subtropical regions. In this study, we combine field observations and mesocosm experiments to assess prokaryotic community dynamics and changes in physicochemical properties during early- and late-season tropical cyclones affecting Okinawa, Japan. Storms caused large and fast influxes of freshwater and terrestrial sediment—locally known as red soil pollution—and caused moderate increases of macronutrients—especially SiO_2 and PO_4. Rather than shifts in marine bacteria, we primarily detected influxes of common soil-derived bacteria, and putative coral and human pathogens that may derive from other sources; mesocosm experiments confirmed that soil input did not differentially affect marine bacteria. The storm effects on bacterial communities were short-lived and baseline assemblages were quickly recovered following disturbances. Early- and late-season storms caused different physicochemical and bacterial community changes. Our results demonstrate rapid and context-dependent shifts in prokaryotic communities due to extreme storm events in a subtropical coastal ecosystem.

Keywords

Tropical cyclones, typhoons, hurricanes, extreme events, bacterioplankton, coastal, nearshore, community dynamics, soil pollution, run-off

Introduction

Extreme storm events, such as tropical cyclones (i.e. tropical storms, hurricanes, and typhoons), can have dramatic consequences on coastal ecosystems, due in part to the effects of terrestrially derived pollution (Hennessy, Gregory and Mitchell 1997; De Jesus Crespo *et al.* 2019). In addition to influencing salinity and turbidity, flood plumes often include elevated concentrations of bacteria (Solo-Gabriele *et al.* 2000), nutrients (i.e. C, N, P) (Chen, Wu and Hong 2012; Gao *et al.* 2014; Chen *et al.* 2018; Paerl *et al.* 2018) and other chemicals, such as herbicides or heavy metals (Lewis *et al.* 2012; Mistri *et al.* 2019), which can act synergistically to

negatively affect coastal ecosystems (Wooldridge 2009; Brodie *et al.* 2012; Lewis *et al.* 2012). Especially in tropical and subtropical coastal ecosystems, which experience severe seasonal storms, huge quantities of terrestrial run-off can enter into coastal waters. Such run-off events can degrade coral reefs directly, through sedimentation or disease (Haapkylä et al. 2011; Voss and Richardson 2006; Philipp and Fabricius 2003; Riegl and Branch 1995; Wilson et al. 2012), and indirectly, through eutrophication, hypoxia (Fabricius 2005; Altieri *et al.* 2017) and decreased water quality. As global climate change is expected to enhance the frequency and intensity of extreme storm events (Groisman *et al.* 2005), it is increasingly important to better understand how such storms impact coastal ecosystem functioning.

Tropical cyclones are most active in the Western North Pacific, where there is an average of 27 named storms per year (Wang *et al.* 2010; Herbeck *et al.* 2011) and where landfalling typhoons are specifically expected to become more common and more destructive (Mei and Xie 2016). Okinawa Island, the largest island of the Ryukyu archipelago at the edge of the Western North Pacific, is an ideal natural laboratory for studying storm effects on coastal ecosystems. Okinawa's coral reefs have experienced significant declines in recent decades, due in part to increased storm induced run-off and sedimentation (Omori 2011; Hongo and Yamano 2013; Harii *et al.* 2014), which is exacerbated by agricultural practices and large coastal development projects (Omija 2004; Masucci and Reimer 2019). The fine-particle, laterite soils with high iron concentrations found in Okinawa and typical to the region are easily suspended and turn coastal waters a deep, cloudy red color during the frequent tropical cyclones (Omija 2004). These events are locally referred to as Red Soil Pollution (RSP) (Omori 2011).

While the biological consequences of storm-induced run-off have been investigated for corals and fish species in Okinawa (Hongo and Yamano 2013; Inoue *et al.* 2014; Yamazaki *et al.* 2015; O'Connor *et al.* 2016; Yamano and Watanabe 2016), less is known about how tropical cyclones and red-soil run-off affect coastal microbial communities and especially bacteria (Blanco, Nadaoka and Yamamoto 2008). Microbial communities contribute to marine ecosystems through primary production and by recycling dissolved organic carbon and nutrients through the microbial loop (Azam *et al.* 1983), but can also draw down dissolved oxygen (Anderson and Taylor 2001) and can cause opportunistic infections in marine organisms (Peters 2015; Shinn et al. 2000; Sutherland et al. 2011; Sheridan et al. 2014). Therefore, changes in microbial community compositions in response to storms could precipitate large-scale ecosystem effects. And these can happen extremely quickly; Gammaproteobacteria, Flavobacteriia and many Alphaproteopbacteria can increase in abundance within hours when exposed to high nutrient concentrations, whereas the entire microbial community—including archaea, protists, and viruses—can turn over on the scale of less than one day to about a week (Fuhrman, Cram and Needham 2015). Rapid microbial response times to changing environmental conditions make microbes valuable early-warning bioindicators (Pearman *et al.* 2018), but also hinders their study. Sampling at the scale of microbial response times during tropical cyclones is often dangerous and is further complicated by the poor predictability of storm tracks and event intensities (Zhou *et al.* 2012).

In this study, we characterize nearshore bacterial community dynamics in response to tropical cyclones and storm-induced terrestrial run-off from Okinawa Island. The study included tropical storm Gaemi at the start of the 2018 Okinawa typhoon season (June 16) and successive category 5 super typhoons, Trami and Kong-Rey, on September 30 and October 5, towards the end of the 2018 season. We performed 16S metabarcoding on seawater samples collected before, during/between, and after storms in June and October. Importantly, we combined field observations with concurrent mesocosm experiments in order to isolate the effects of red soil addition from other storm effects, such as wind, waves, and fresh-water input. The specific aims for this study were to: i) assess how bacterial community composition and physicochemical parameters respond in time to tropical cyclones and red soil run-off, ii) evaluate the speed of the responses and recovery, and iii) identify potential ecosystem consequences due to terrestrial run-off from extreme storms.

Materials and Methods

Study setting

Seawater was collected for metabarcoding and physicochemical analysis from four nearshore sampling points approximately 250–500 m apart, along the central west coast of Okinawa Island—a semi-urban region with mixed land-use including agriculture and coastal development projects (Supplemental Figure 2). The sampling points were each at least 1.2 km from the nearest concentrated fresh-water input (e.g. streams or rivers). At the start of the 2018 typhoon season, samples were collected before (June 13), during (June 16), and after (June 19) a red soil pollution event caused by tropical storm Gaemi, which struck Okinawa on June 16, 2018 (Figure 1, Supplemental Figure 3A). Towards the end of the typhoon season, samples were collected before (Sept 28), during (Oct 1 and Oct 3) and after (Oct 8) a red soil pollution event caused by typhoon Trami, which made landfall with Okinawa on September 30, and prolonged by Typhoon Kong-Rey, which approached Okinawa on October 5th (Figure 1, Supplemental Figure 3B–C). These events represented the two largest rain events during the 2018 typhoon season, and typhoon Trami recorded the most extreme sustained and gusting wind speeds in 2018 (Figure 1).

Seawater sampling for DNA and physicochemical analysis

Surface seawater was collected for DNA metabarcoding by submerging clean 500 mL Nalgene bottles just below the sea surface. Seawater for dissolved Fe (dFe) and nutrient analysis was collected in acid-cleaned 50 mL Falcon tubes. Physicochemical properties—dissolved oxygen (DO), salinity, temperature, and turbidity—were measured with a CTD RINKO profiler (JFE Advantech, Japan) at each site. After being immediately transported to the laboratory, seawater samples for metabarcoding were filtered through 0.2 µm pore-size Polytetrafluoroethylene (PTFE) filters (Millipore) under gentle vacuum and filters were stored at -20 ˚C for later DNA extraction. Seawater samples for dFe and nutrient analysis were filtered through 0.45 µm pore-size acid-washed Teflon digiFILTERS (SPC Science, Canada) and filtered water samples were stored at -20 ˚C for later chemical analysis.

Mesocosm experimental design

Seawater was collected for concurrent mesocosm experiments on June 11 (26.51 ˚N, 127.87 ˚E) and October 10 (26.47 ˚N, 127.83 ˚E). Nearshore coastal seawater was pumped from just below the sea surface and filtered through 1 mm and 300 µm nylon mesh sizes to remove debris and larger organisms. Acid-cleaned 22 L clear-plastic carboys were rinsed twice with seawater before filling to 20 L with filtered seawater. Bottles were covered with parafilm and kept shaded during transport to the Okinawa Institute of Science and Technology (OIST) Marine Science Station, where they were submerged in a basin with continuous flow-through seawater to keep conditions within the bottles similar to natural conditions. Mesocosm bottles were topped with silicone sponge stoppers to allow gas exchange, but limit evaporation and prevent dust, water or other contaminants from entering the bottles during the experiment (Supplemental Figure 4). In addition, small pumps were included in each mesocosm to maintain water circulation (2 L min^{-1}). HOBO temperature and light loggers (Onset) were fastened to the pumps and at the same depth in the basin surrounding mesocosms to ensure that mesocosms conditions remained similar to ambient conditions (Supplemental Figure 5). In addition, salinity and DO were measured each time water was sampled from the mesocosms throughout the experiment (Supplemental Figure 6). Mesocosms experiments included 9–10 mesocosms: 4–5 control replicates (4 in June and 5 in October) and 5 treatments replicates with red soil added to an ecologically relevant concentration of 200 mg L^{-1} (O'Connor *et al.* 2016). Mesocosms were sampled (100 mL for metabarcoding, 50 mL for dFe and nutrient concentration) with 50 mL sterile pipettes before the experiment started (t0), and 1, 4, 12, 24, and 48 h following red soil addition to treatment bottles. Water samples were processed as described in the previous section.

Red soil collection for mesocosm experiments and DNA analysis

Soil samples were collected from an open agricultural field with exposed soil, on June 10 and October 9 for addition to red soil treated mesocosms and to evaluate soil microbiomes. Soil samples were sieved through 330 µm mesh and maintained at 4 °C for 24 h until use in the mesocosm experiment. To determine soil moisture content, 10 g subsamples (n=10) were weighed and dried at 100 °C by following the standard method AS 1289.2.1.1-2005 (Standards Association of Australia). Soil moisture content was used to calculate how much wet soil should be added to mesocosms in order to reach the final concentration of 200 g soil (dry weight) per L seawater. In both June (n=4) and October (n=2), additional 50 g aliquots of soil were kept at -20 °C for subsequent metabarcoding analysis.

Chemical analysis: dFe and major nutrients

Dissolved Fe (dFe) concentration was determined following the methodology of Wu and Boyle (1998). This method uses a $Mg(OH)_2$ co-precipitation to pre-concentrate Fe from seawater followed by an isotope dilution method. Seawater dFe was quantified using an internal standard element (^{57}Fe) with inductively coupled plasma mass spectrometry (Element 2, Thermo Scientific). The mass spectrometer was operated in medium-resolution mode with 4000 resolution (FWHM). The mass calibration was performed using a multi-element ICP-MS tune-up solution (Thermo Fisher Scientific). In order to ensure the quality of the ICP-MS analysis, control standards and samples (i.e. analytical replicates, certified reference material and analytical blank) were analyzed once every 12 samples. Recovery of Fe from reference certified material

QC3163 (Sigma-Aldrich, USA) was satisfactory and ranged from 65 to 80%. The overall error associated with the analytical process was typically lower than 5% and never higher than 15%. All measurements were above the instrument LOD (Limit of Detection). Analysis was carried out at the OIST Instrumental Analysis Section mass spectrometry laboratory. Special attention was paid to avoid Fe contamination and an exhaustive cleaning process was carried out following the methods of King and Barbeau (2011).

Nutrient concentrations—including Nitrate (NO_3), Nitrite (NO_2), Ammonium (NH_4), Phosphate (PO_4) and Silica (SiO_2)—were determined on a QuAAtro39 Continuous Segmented Flow Analyzer (SEAL Analytical) following manufacturer guidelines. Final concentrations were calculated through AACE software (SEAL Analytical). Nutrient Analysis was carried out at the Okinawa Prefecture Fisheries and Ocean Technology Center.

Nutrient and dFe statistical analyses

Mesocosm data were found to be normally distributed with a Shapiro-Wilks test and, therefore, a one-way ANOVA was performed to test overall differences between treatments. Post-hoc Tukey HSD analysis was performed to identify which specific groups differed. Field data were not normally distributed, regardless of transformation, so a Kruskal-Wallis test was used to test for significant differences between sampling dates. Analyses were performed within the R statistical environment (R Core Team 2018).

DNA extraction and metabarcode sequencing

DNA was extracted from frozen PTFE filters following the manufacturer protocol for the DNeasy PowerWater Kit (Qiagen), including the optional heating step. DNA was extracted from soil samples by following manufacturer protocol for the DNeasy PowerSoil Kit (Qiagen). Metabarcode sequencing libraries were prepared for the V3/V4 region of the bacterial 16S ribosomal RNA gene following Illumina's "16S Metagenomic Sequencing Library Preparation" manual without any modifications. Sequencing libraries to the OIST Sequencing Center for 2x300-bp sequencing on the Illumina MiSeq platform with v3 chemistry.

Metabarcode analyses

Sequencing reads were denoised using the Divisive Amplicon Denoising Algorithm (Callahan *et al.* 2016) with the DADA2 plug-in for Qiime2 (Bolyen *et al.* 2018). Taxonomy was assigned to representative Amplicon Sequence Variants (ASVs) using a Naive Bayes classifier trained on the SILVA 97% consensus taxonomy (version 132, (Quast *et al.* 2013) with the Qiime2 feature-classifier plug-in (Bokulich *et al.* 2018). The results were imported into the R statistical environment (R Core Team 2018) for further analysis with phyloseq (McMurdie and Holmes 2013). The ASV richness for each sample was estimated using the R package breakaway (Willis and Bunge 2015) and differences in estimated richness between sample types were tested for with the betta function (Willis, Bunge and Whitman 2017). In order to minimize compositional bias inherent in metabarcoding data as much as possible, we used the Aitchison distance between samples (Gloor *et al.* 2017) for principal coordinate analyses (PCoA). Lastly, we used the DESeq2 bioconductor package (Love, Huber and Anders 2014) to determine which ASVs were significantly differentially abundant (False Discovery Rate adjusted p-value < 0.05)

in water samples collected from field sites before, during, and after storms. We repeated these pairwise comparisons after filtering data from field water samples to remove ASVs that were also found in soil samples to determine if differentially abundant ASVs represented soil bacteria.

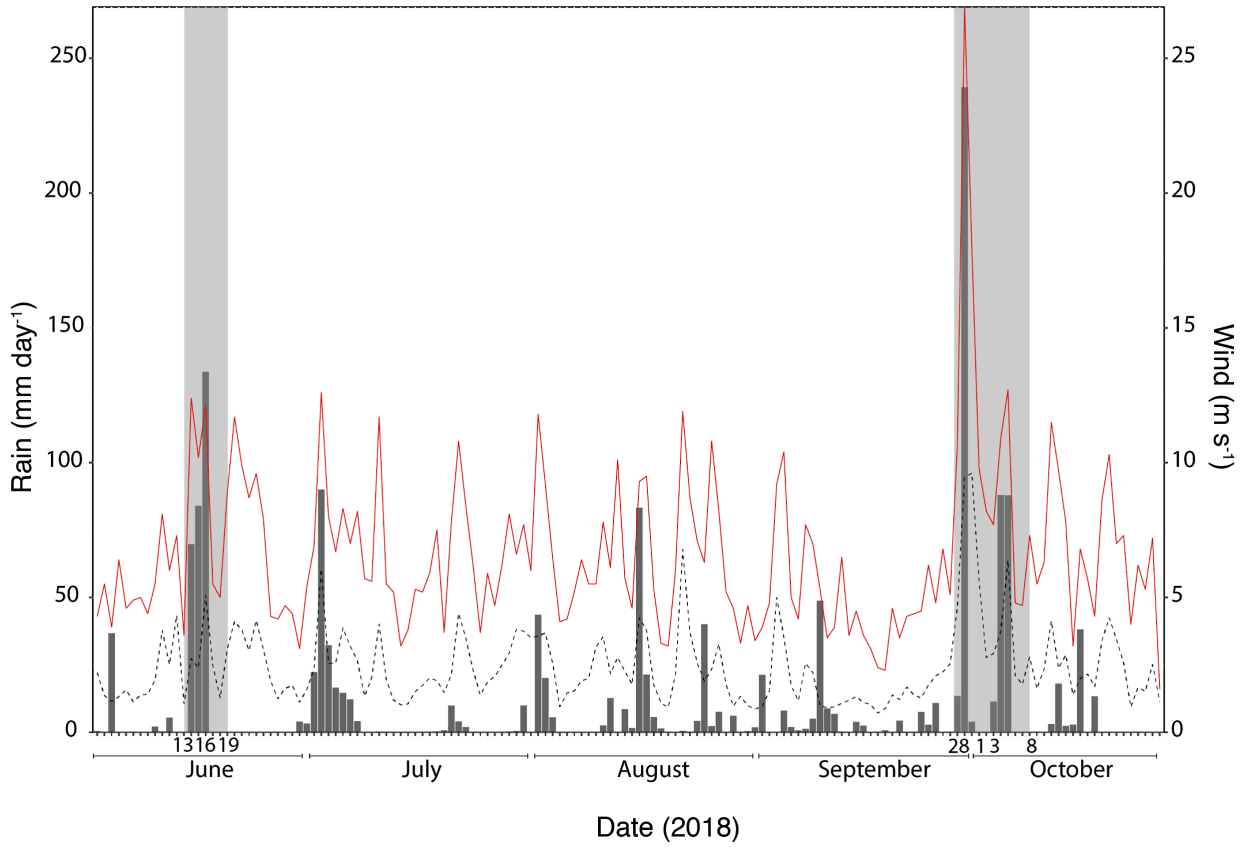

Figure 1. Precipitation (in mm day^{-1}) and wind speed (m s^{-1}) during the 2018 typhoon season in Okinawa, Japan. Data were collected with the meteorological station located at OIST Marine Science Station (26.510046 ˚N, 127.871721 ˚E) from June through October, corresponding to the duration of the typhoon season in Okinawa. Bars represent the daily amount of rain (in mm); dashed black and red lines indicate daily mean and maximum wind speeds (in m s^{-1}), respectively; shaded areas represent the two red soil pollution events monitored in this study: Tropical Storm Gaemi on June 16 and the super-typhoons Trami and Kong-Rey on September 29 and October 5. The dates when water samples were collected for chemical and DNA analyses are noted on the x-axis.

Results

Physicochemical responses to extreme storm events and red soil run-off

Temperature (˚C), DO (mg L^{-1}), salinity (‰) and turbidity (NTU) were measured *in situ* at field sites when water samples were collected. We observed a decrease in salinity (~15‰) and concurrent increase of turbidity (~10-fold) during the storm on June 16 compared to before the

storm on June 13, indicating a significant run-off event was caused by the storm (Figure 2). We did not observe significant changes in turbidity or salinity during the October event (Oct 1 and 3) despite the storms in October being larger and more intense than the storm in June (Figure 1).

The concentrations of dissolved nutrients, including NO_2, NO_3, NH_4, PO_4, SiO_2, and dFe, were measured in seawater samples collected in June and October (Figure 3) and throughout the October mesocosm experiment (Figure 4). Field values were generally higher in June than October, with the exception of dFe and SiO_2, which had similar values in both sampling months (Figure 3). A nonparametric Kruskall-Wallis test was applied to detect significant storm-induced differences in field nutrient concentrations (Supplemental Table 1). Results showed significant increases ($p < 0.05$) in NO_2 concentrations during and following storm events in June and October, whereas SiO_2 and PO_4 were only significantly elevated during and after the storm in June. Nutrient concentrations were generally more clearly elevated during the storm in June. While this trend was less obvious in October, moderate increases in nutrient concentrations were detected during and after the October event.

Red soil addition in the October mesocosm experiment caused a significant increase in SiO_2 concentration (Figure 4, Supplemental Table 2). Additionally, red soil addition caused PO_4 concentrations to increase above the LOD 4 hours following soil addition, whereas PO_4 was below the LOD in control mesocosms after t0 (Figure 4). Two-way ANOVA results (Supplemental Table 2) indicate that time had a greater effect on nutrient concentration ($p < 0.05$ for NO_2, SiO_2 and dFe) than red-soil treatment ($p < 0.05$ for SiO2 and dFe). The treatment by time interaction was only significant in the case of SiO_2 ($p < 0.05$), for which higher concentrations were found for soil treatment. These results suggest that red soil run-off contributes to the increase in SiO_2 observed in coastal waters during storms.

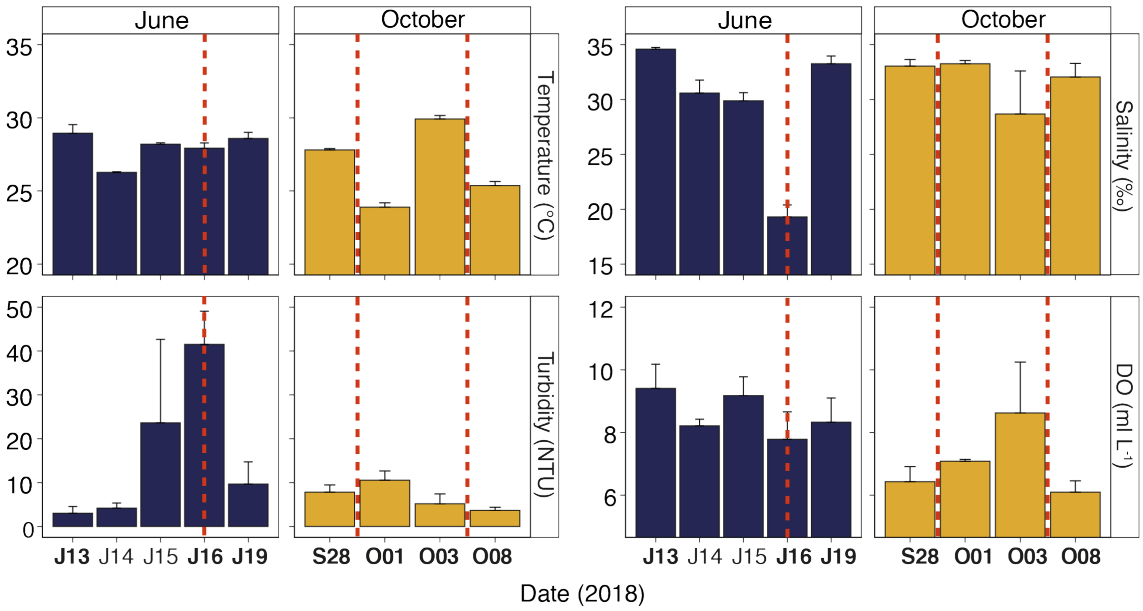

Figure 2. Temporal variation of temperature (°C), turbidity (NTU), salinity (‰) and dissolved oxygen (DO; mg L^{-1}) before, during, and after storm events in June and October, 2018. Bars represent mean concentrations of dissolved temperature (°C), turbidity (NTU), salinity (‰) and DO (mg L^{-1}) for four sampling sites along the central west coast of Okinawa, Japan. Sampling dates when samples were also processed for metabarcode analyses are indicated in bold on the x-axis. Red dashed vertical lines represent the timing of storms and corresponding red soil pollution events. All measurements were taken with a CTD profiler.

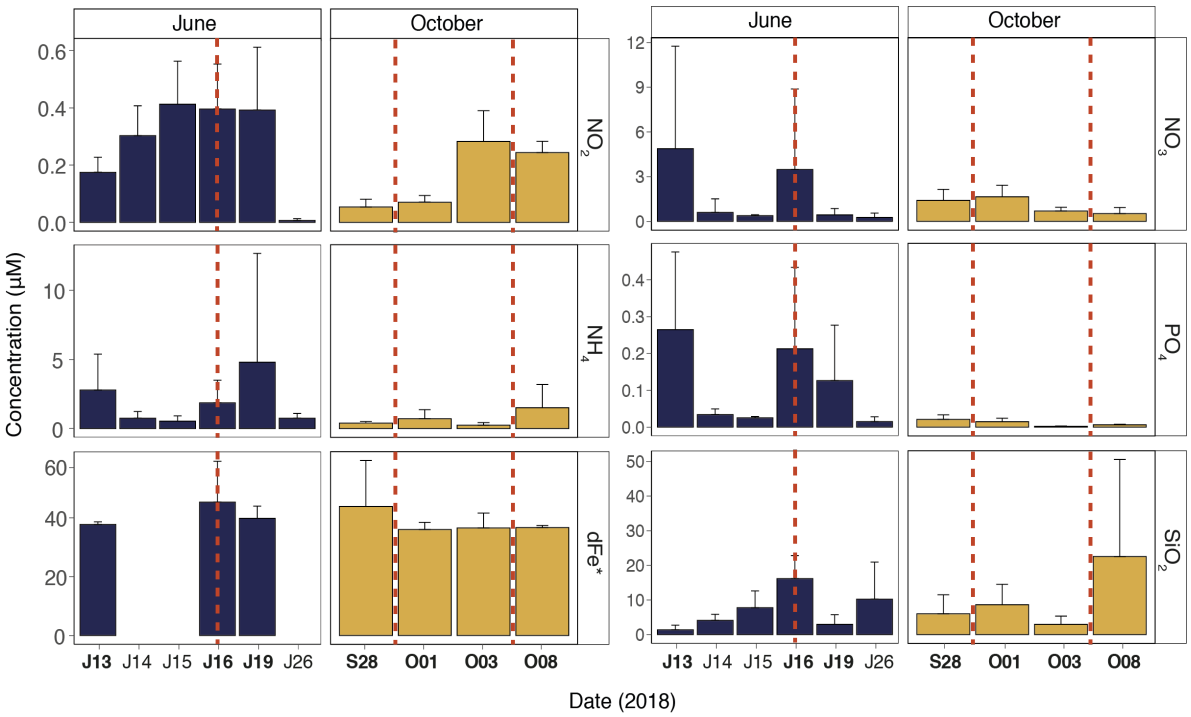

Figure 3. Temporal variation of micro- and macro-nutrient concentrations before, during, and after storm events in June and October, 2018. Bars represent mean concentrations of dissolved NO_2, NO_3, NH_4, PO_4, SiO_2, and dFe (* dFe concentration is in nM) for four sampling sites along the central west coast of Okinawa, Japan. Error bars represent one standard deviation. Red dashed vertical lines represent the timing of major storms and associated red soil pollution events. Sampling dates when samples were also processed for metabarcode analyses are indicated in bold on the x-axis. Nutrient concentrations were determined on a QuAAtro39 Continuous Segmented Flow Analyzer and dFe concentration was determined by ICP-MS after Mg(OH)2 co-precipitation using the isotope dilution method (Wu and Boyle 1998).

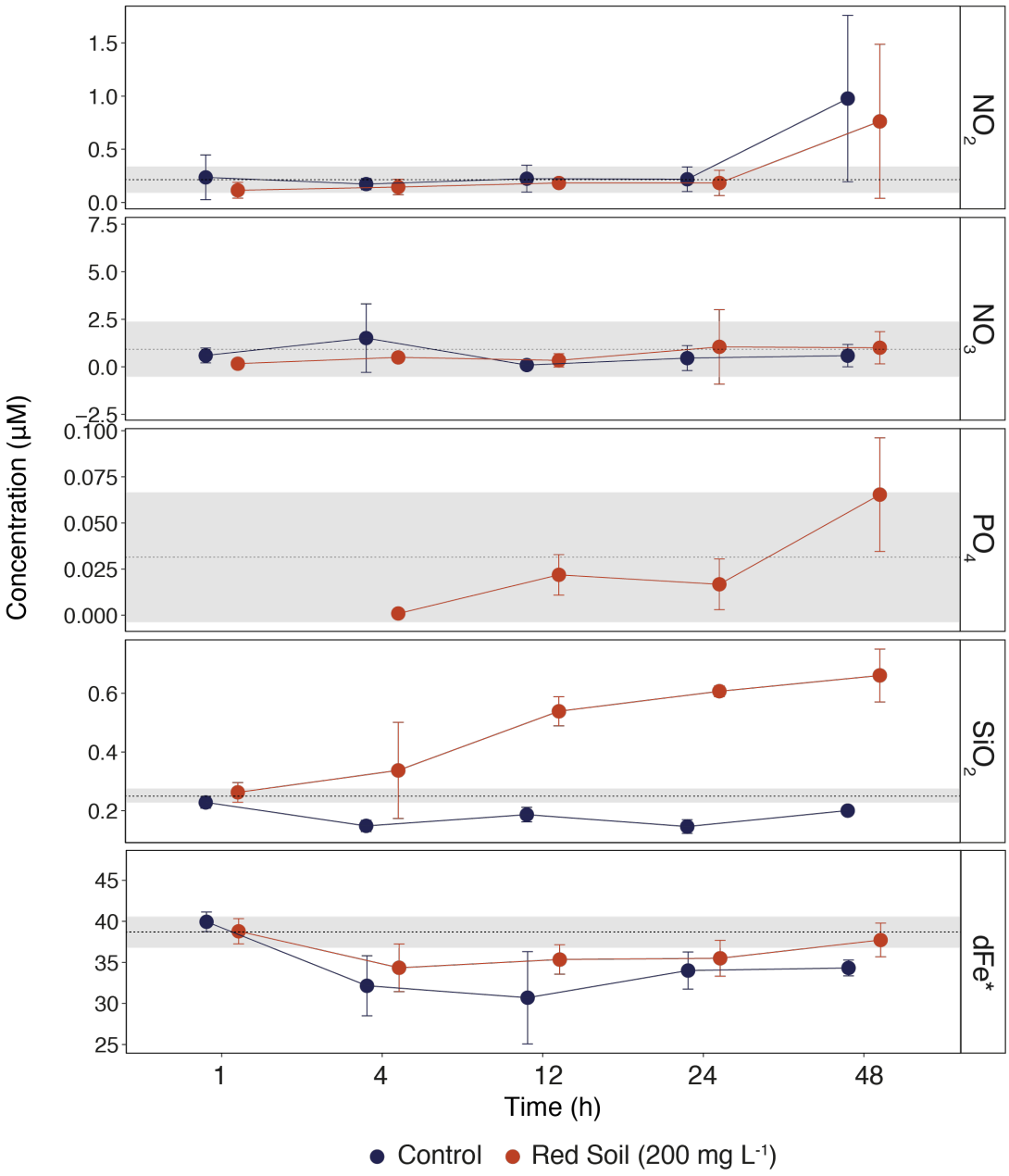

Figure 4. Temporal variation of micro- and macro-nutrient concentrations in a mesocosm experiment following red soil addition. Points represent mean concentrations of dissolved NO_2, NO_3, PO_4, SiO_2 and dFe (* dFe concentration is in nM) in control (blue points) and red soil treated (red points) mesocosms. Samples for nutrient analysis were taken at t0 and 1, 4, 12, 24, and 28 h after red soil (200 mg/L) was added to treatment mesocosms. Error bars on points denote ± one standard deviation. The dashed black horizontal line is the mean value for all mesocosms at t0 and the shaded region represents ± one standard deviation. Nutrient concentrations were determined on a QuAAtro39 Continuous Segmented Flow Analyzer and dFe concentration was determined by ICP-MS after $Mg(OH)_2$ co-precipitation using the isotope dilution method (Wu and Boyle 1998).

Bacterial community responses to storm events and red soil input

Overall, 18.4 million sequencing reads were generated in this study, with 76,217–219,584 sequencing reads per sample (mean = 137,585). Sequencing data are available from the NCBI Sequencing Read Archive under the accession PRJNA564579. Following denoising, 11.8 million sequences remained with 11,061–153,646 sequences per sample (mean = 88,033). A total of 36,007 ASVs were identified in our dataset, with 64–2,886 observed ASVs per sample (mean = 642).

Shifts in the relative abundance of bacterial phyla during the June storm event were clearly observable, with phyla that were predominant in the red soil samples becoming more abundant in water samples collected during the storm (Figure 5). The samples collected between the storms in October also showed an increased relative abundance of some bacterial phyla present in the soil samples, but some phyla present in soil were already present in samples collected on September 28th, before the storms (Figure 5). Likewise, ASV richness estimates for the samples collected in June were significantly higher for samples collected during the storm (Figure 6). In October, the estimated richness in soil samples was lower than in June soil samples and the richness in samples collected between rain events was not significantly higher than before or after events (Figure 6). Principal coordinate analysis (PCoA) of the Aitchison distance between samples further indicate a pulse of terrestrial bacteria into the coastal environment during storm events; in both June and October, the samples collected before and after the pollution event cluster together, while the samples collected during the rain event in June and between the rain events in October cluster separately on the primary axis (Figure 7). However, the differences in community by sample collection date were not significantly different when PERMANOVA analyses were performed with the adonis function in the vegan R package (Oksanen *et al.* 2019). The greatest number of significantly differentially abundant ASVs were between June 13 and June 16, with the vast majority being more abundant during the storm. When ASVs exclusively present in seawater samples were considered, the number of differentially abundant ASVs decreased substantially (Table 1).

Red soil addition to mesocosms in June caused an increased abundance of bacterial phyla that were also found in soil samples, although the effect was smaller than in field samples (Figure 5). In October, however, soil addition did not alter the prokaryotic communities in mesocosms (Figure 5, Supplemental Figure 7). Overall, bottle incubation seemed to have a larger effect on the bacterial community than red soil addition (Figure 5, Supplemental Figure 7), despite mesocosm conditions (temperature, salinity, DO) remaining similar to ambient conditions (Supplemental Figures 5–6). Similar to the field observations, richness estimates for June mesocosms increased following red soil addition, whereas soil addition did not increase richness in October mesocosms (Supplemental Figure 8).

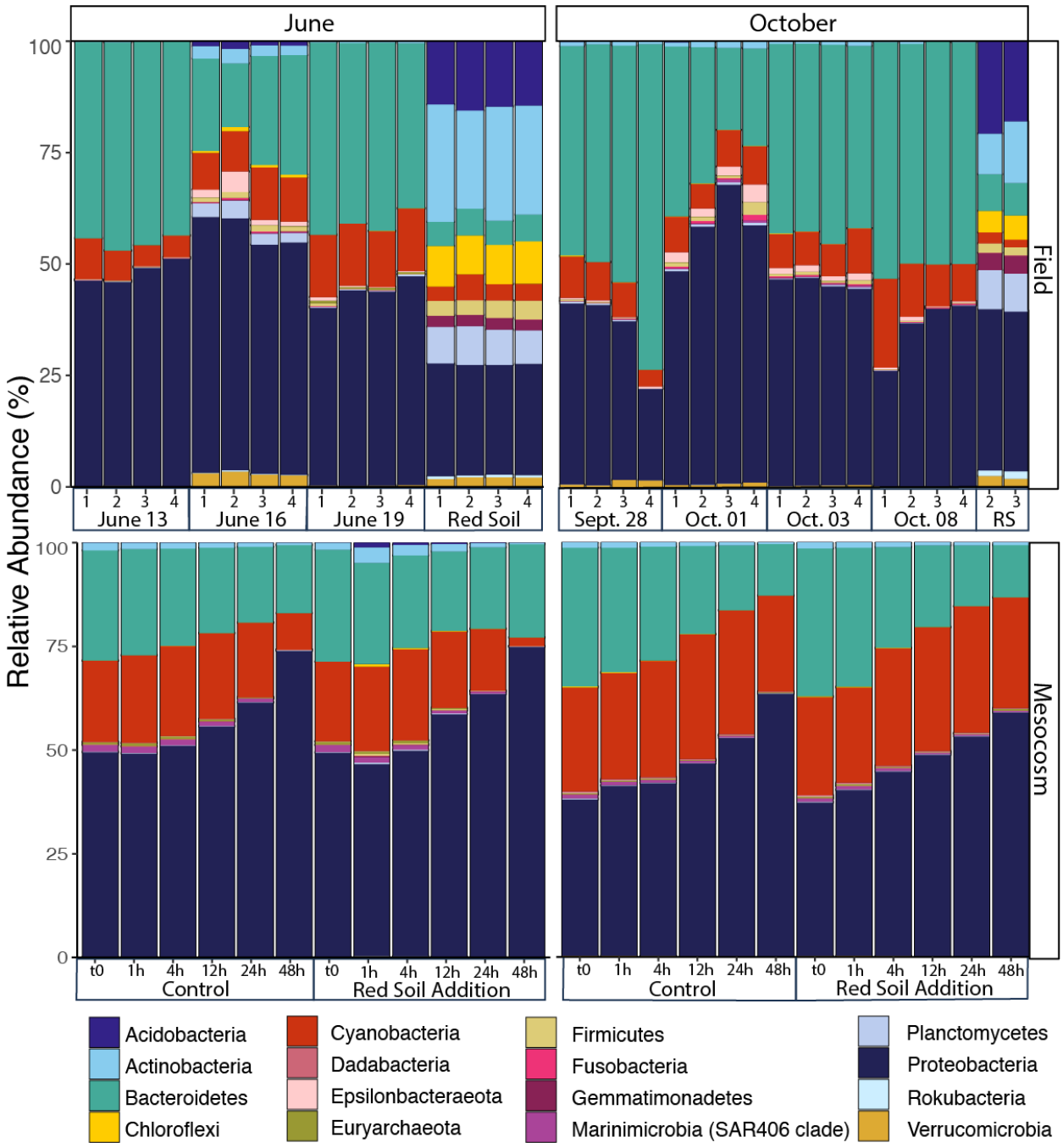

Figure 5. Relative abundance of prokaryotic phyla in field and mesocosm samples collected in June and October, 2018. Each stacked bar represents the relative contribution of major bacterial phyla to the total community at one sampling location, in one red soil sample, or at one time point in mesocosms (mesocosm bars represent aggregate data from 4–5 replicates depending on month and treatment). Sampling stations 1–4 were sampled before, during, and after red soil pollution events in June and October 2018. In June, 6/13 was before the event, 6/16 was during the event, and the 6/19 was after the event. In October, 9/28 was before the event, 10/01 and 10/03 were between events, and 10/08 was after events. Red soil (RS) samples are labeled with replicate number and were collected close to the onset date of storms

in June and October. Red Soil (200 mg L^{-1}) was added to treatment mesocosms immediately following the t0 sampling.

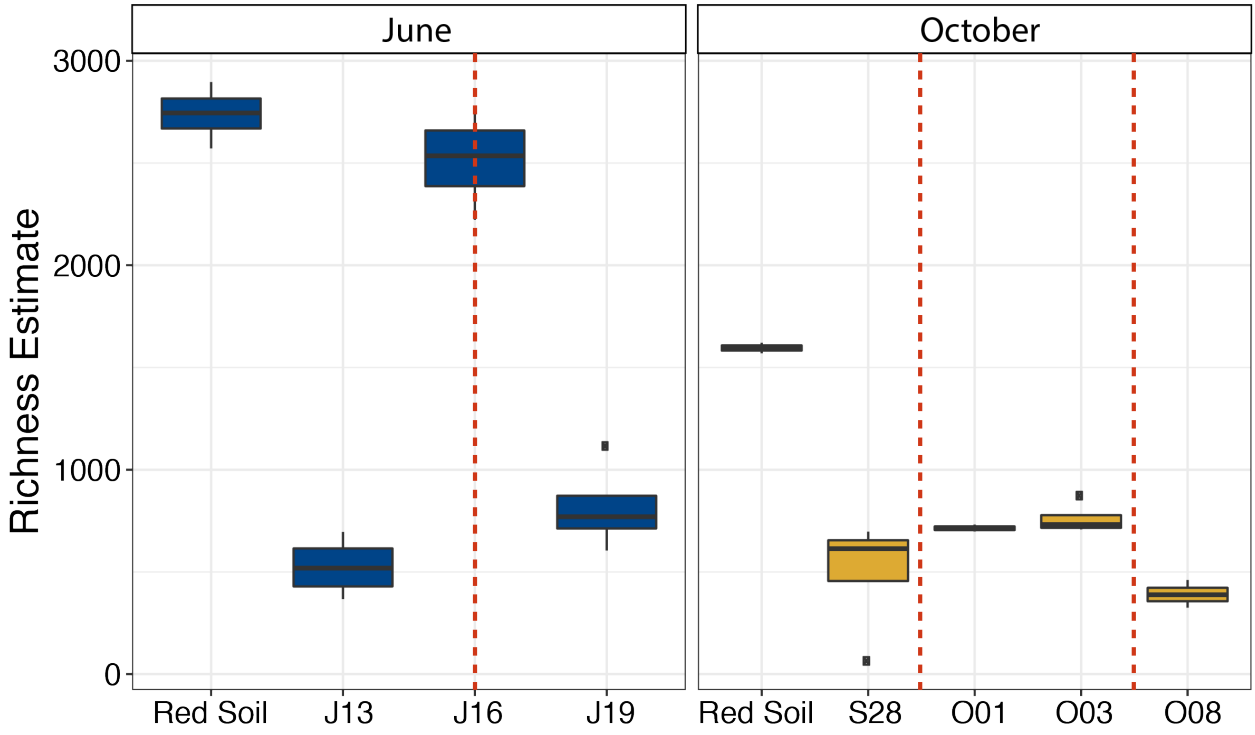

Figure 6. Richness estimates for bacterial communities in red soil samples and surface water samples collected before, during, and after storm events in June and October 2018. Red dashed vertical lines represent the timing of major storms and associated red soil pollution events. In June, the red soil samples had significantly higher richness than the water samples collected before and after the event, but not samples collected during the event. In October, red soil samples had significantly higher richness than all water samples and richness was not significantly different in water samples collected before, during, or after the storms. Differences in richness were considered significant when $p < 0.05$.

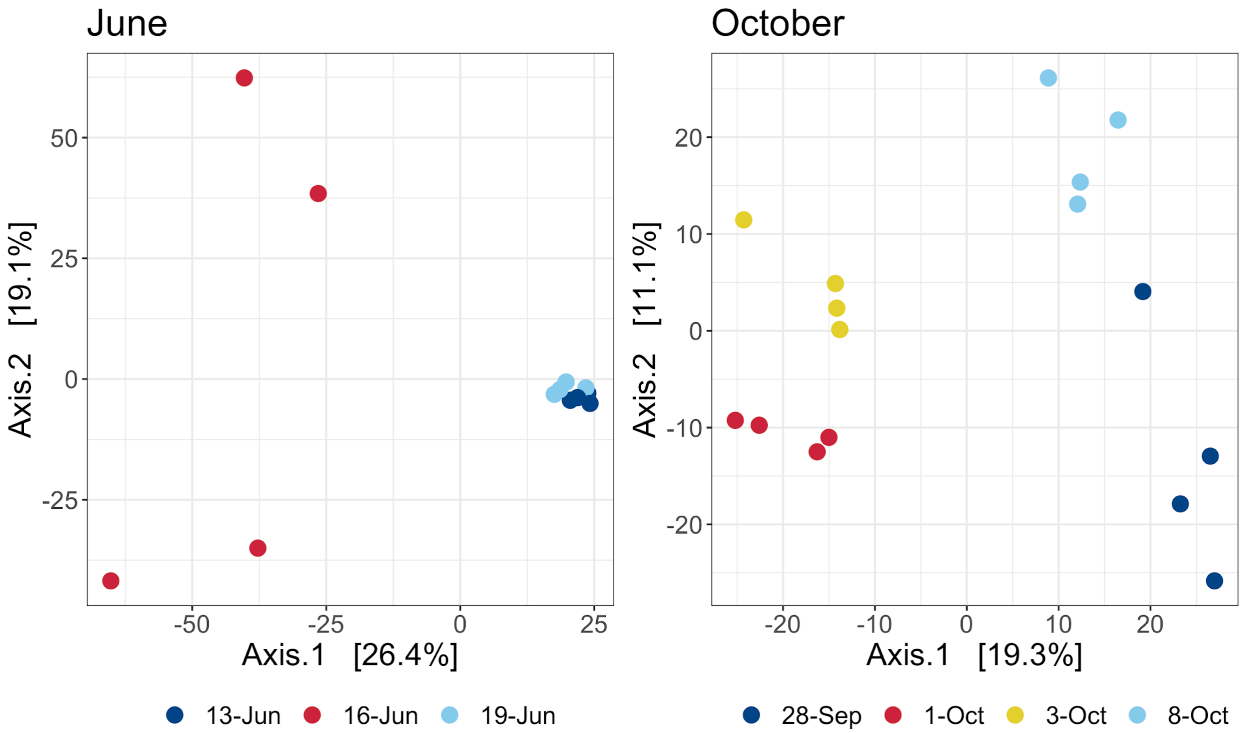

Figure 7. Principal coordinates analysis (PCoA) of Aitchison distances between prokaryotic community composition before, during, and after storm events in June and October, 2018. When June and October data are plotted together, samples cluster most strongly by sampling month. June 13 was before the June storm, 6/16 was during, and 6/19 was after. For the October event, 9/28 was before the storms, 10/01 and 10/03 were between, and 10/08 was after. Samples collected during/between storm events cluster separately from samples collected before and after for both the June and October events.

Table 1. Amplicon Sequence Variants (ASVs) significantly differentially abundant (FDR-adjusted *p*-value < 0.05) in pairwise tests between field samples collected before, during/between and after the run-off events in June and October. Pairwise tests were performed on full datasets ("Full") for seawater samples and on seawater datasets where all ASVs also present in soil samples were removed ("Filtered"). Samples collected during/between tropical cyclones are indicated in bold and red font.

			Abundance		
		Pairwise tests	+	-	All
June	Full	J13/ **J16**	317	23	360
		J16/ J19	22	144	133
		J13/J19	11	10	23
	Filtered	J13/ **J16**	41	10	51
		J16/ J19	6	13	19
		J13/ J19	5	2	9
October	Full	S28/ **O01** + **O03**	76	9	85
		O01 + **O03**/ O08	13	33	46
		S28/ O08	39	9	48
	Filtered	S28/ **O01** + **O03**	70	0	70
		O01 + **O03**/ O08	7	32	39
		S28/ O08	25	1	26

Discussion

As the severity and frequency of extreme storm events increases with global climate change, it is increasingly important to understand how these events impact ecological functioning in marine ecosystems (Wetz and Paerl 2008; Du *et al.* 2019). However, characterizing the effects of extreme storm events, such as typhoons, on coastal ecosystems is a complicated task, due both to forecast unpredictability, which makes sampling before and after events challenging, and the dangerous conditions that accompany storms (Chen *et al.* 2018). This study reports on the coastal microbial community dynamics and relevant environmental parameters in two short time-series encompassing major storms during the 2018 typhoon season in Okinawa, Japan. In addition, concurrent, controlled mesocosm experiments were performed to supplement field observations and isolate impacts of red soil pollution that accompanies storms in Okinawa. Predictably, storms caused large influxes of freshwater and terrestrial sediment into the coastal marine environment (Figure 2, Supplemental Figure 1), which included soil-derived bacteria and bacteria from other terrestrial sources (Figure 5). Remarkably though, the effects of the storms were extremely short-lived, and microbial community compositions quickly returned to baseline conditions. While field samples were collected three days following storm events, mesocosm

experiments showed that the microbial community began recovering only four hours after soil addition and was fully recovered after just 24 hours. Despite remaining in the water column for a short time following run-off events, terrestrially derived bacteria can still be detrimental to coastal ecosystems and dangerous to human health. Bacterial phyla that became more abundant following storms can cause diseases in corals (e.g. *Vibrio spp.*, *Pseudoalteromonas sp.*, Rhodobacteraceae bacteria) and humans (e.g. Campylobacter, Fusobacteria, Enterobacter), thus necessitating a better understanding of the sources and sinks for these bacteria in the coastal environment.

Storms cause rapid, context-dependent changes in environmental conditions and microbial community composition

During the storm event in June, we measured a drastic drop in salinity and an increase in the turbidity of coastal surface waters (Figure 2), which is similar to previous reports following major storm events (De Carlo *et al.* 2007; Zhou *et al.* 2012; Chen *et al.* 2018). The influx of freshwater and sediment was accompanied by moderate increases in concentrations of NO_2, NO_3, NH_4, PO_4, SiO_2 and dFe (Figure 3), which is consistent with terrestrial run-off from agricultural areas with high Fe soil, as is found in Okinawa (Arakaki *et al.* 2005). Likewise, there was an increase in bacterial diversity (Figure 6) and a clear influx of soil-derived bacteria in coastal waters during the June storm event, including bacteria belonging to the phyla: Acidobacteria, Actinobacteria, Chloroflexi, Firmicutes, Planctomycetes, Rokubacteria and Verrucomicrobia (Figure 5, (Freitas *et al.* 2012; Witt, Wild and Uthicke 2012; Balmonte *et al.* 2016; Lin *et al.* 2019). Three days following the storm, on June 19, the soil-derived bacteria were almost undetectable (Figure 5) and only 23 ASVs were significantly differentially abundant between June 13 and June 19 compared to 360 ASVs different between June 13 and June 16 (Table 1), demonstrating the incredible speed at which the microbial community returned to baseline. The ephemeral presence of soil bacteria was also apparent in the June mesocosm results. One hour after red soil was added to treatment mesocosms, Acidobacteria, Chloroflexi, Firmicutes, and Planctomycetes were detected, but their relative abundance decreased in samples taken four and twelve hours later, and they were absent in samples taken 24 hours later.

Our results indicate that the October storm event affected coastal physicochemistry and microbial community composition differently than the June event. Although differences may be due to disparate timing of sampling, it is likely they were also influenced by differences in rain intensity and the context in which the two events occurred. Typhoon Trami, in October, caused twice as much precipitation as Gaemi in June (Figure 1). Additionally, Gaemi was the first major storm of the 2018 typhoon season (June–October) and occurred following a relatively prolonged dry period. By October, several tropical storms and typhoons had already affected Okinawa (Figure 1) and caused severe run-off events; the 2018 Pacific typhoon season had higher than average storm frequency and included 29 tropical storms, 13 typhoons, and 7 super typhoons, although not all made landfall with Okinawa (Japan Meteorological Agency, https://www.jma.go.jp/jma/indexe.html). The intervening events could have stripped topsoil and depleted soil nutrients and microbiomes, so that October storm run-off carried less nutrients, organic material, and terrestrial bacteria into coastal water. Antecedent soil moisture will further

affect dissolved nutrient content in run-off, especially in clay-based soils like Okinawa red soil (Perrone and Madramootoo 1998). Rain intensity can also differentially affect nutrient loading; when flushing rate is high, less phosphate is desorbed from sediments and there is a dilution effect for both dissolved phosphorus and nitrogen in run-off (Blanco *et al.* 2010). These factors are evident in our results; for example, we did not measure a significant increase in NO_2, NO_3, NH_4, PO_4, or dFe from September 28 to October 1, despite several of these nutrients being significantly elevated during the June storm (Figure 3). Likewise, microbial diversity did not significantly increase from September 28 to October 1 or 3 (Figure 6) and water samples collected on October 1 and 3 contained less soil-derived bacterial taxa compared to those collected during the storm on June 16; Acidobacteria, Chloroflexi, and Rokubacteria were not observed in October 1 samples but were present in samples collected during the June storm event (Figure 5).

The controlled mesocosm experiments offer insight for interpreting differences in results from field observations in June and October. Despite collecting soil from the same place in June and October, the soil microbiome in October had approximately half the alpha diversity as in June (Figure 6) and soil addition to October mesocosms did not introduce soil bacteria or increase bacterial diversity as it had in June mesocosms (Figure 5, Supplemental Figure 8). These results suggest that soils in Okinawa could have lower bacterial loading towards the end of the typhoon season. Furthermore, soil addition in October mesocosms did not cause nitrogen (NO_2 or NO_3) or dFe to increase over baseline measurements, but did cause increases in SiO_2 and PO_4 (Figure 4). However, the increase in SiO_2 and PO_4 were gradual, demonstrating that time is required to release these compounds from red soil (De Carlo *et al.* 2007; Blanco *et al.* 2010; Chen *et al.* 2018). Soils in Okinawa could, therefore, have lower nitrogen content at the end of the typhoon season and intense rains may deliver less nutrients due to rapid flushing.

The influences of June and October storms on coastal microbial communities vary in magnitude, but the shift in microbial communities in both instances is extremely rapid. Additionally, shifts in community compositions were almost completely driven by influxes of terrestrially-derived bacteria rather than changes in relative abundances of marine bacteria (Figure 5). It is unsurprising that terrestrially-derived bacteria cannot persist in the marine environment (Korajkic *et al.* 2019), however, we anticipated that organic carbon and inorganic nutrients accompanying terrestrial run-off would differentially benefit various groups of marine microbes (Zhang et al. 2009; Chen et al. 2018; Zhang et al. 2009). There are several possible explanations for the minimal changes in marine bacterial community composition that we observed: (1) the coastal microbial community was not limited by compounds present in run-off, (2) nutrients in run-off were not biologically available, or (3) terrestrial run-off was quickly transported offshore. Nutrient concentrations that were elevated during the June storm (NO_2, NO_3, PO_4 and dFe) decreased after the storm passed, but not immediately and not as quickly as the microbial communities recovered (Figure 3). Since nutrients were not immediately drawn down, it is possible that the nearshore coastal microbial community was not nutrient limited in our study area. The limited bacterial response to soil addition in mesocosms further suggests that soil has negligible direct effects on marine microbial communities. However, run-off including soil could elicit different microbial responses than soil on its own. Furthermore, it

remains that we did not perform bacterial cell counts or otherwise measure bacterial biomass or metabolic activity, thus leaving the possibility that run-off evenly increased microbial biomass or differentially affected microbial physiology.

Potential ecosystem consequences due to terrestrial run-off from extreme storms

Despite being short-lived, the changes we observed in microbial community composition and environmental parameters during storms can nevertheless be detrimental to both the coastal ecosystem and human health. While many soil-derived bacteria are benign, many are potentially pathogenic to corals and other marine organisms (Sutherland, Porter and Torres 2004; Haapkylä *et al.* 2011; Pollock *et al.* 2014; Sheridan *et al.* 2014). Terrestrial run-off has been implicated in coral diseases, such as White Pox Disease and Red Band Disease, in many tropical and subtropical locations, including Madagascar (Haapkylä *et al.* 2011; Pollock *et al.* 2014; Sheridan *et al.* 2014), the Caribbean (Frias-Lopez *et al.* 2002; Patterson *et al.* 2002), and Australia's Great Barrier Reef (Pollock *et al.* 2014). Indeed, we found several strains of *Vibrio spp.*, *Pseudoalteromonas sp.*, Rhodobacteraceae bacteria specifically associated with coral disease (Sussman et al. 2008; Sheridan et al. 2014) significantly enriched in the samples we collected during or following storm events (Supplemental Table 3). Considering the additional stress caused by turbidity and sedimentation during run-off events alongside potentially decreased pH and DO due to enhanced heterotrophic bacterial respiration (Weber *et al.* 2012; Altieri *et al.* 2017), corals and other organisms may be especially susceptible to pathogen infection during and after storm events.

Heavy rains and floods have long been implicated in transporting human pathogens (e.g. fecal coliform bacteria) to the marine environment (Pandey *et al.* 2014; De Jesus Crespo *et al.* 2019). While we found bacterial taxa that are potentially dangerous to humans—including Campylobacter, Fusobacteria, and Enterobacter (Bennett and Eley 1993; Sharma, Sachdeva and Virdi 2003; Davin-Regli, Lavigne and Pagès 2019)—significantly enriched during and following storms, these bacteria were not present in our soil samples. Likewise, these taxa were not found in mesocosms following soil addition in June or October. Thus, these results demonstrate the larger effect of run-off on the coastal ecosystem than simply transporting soil into the water. Human pathogens may derive from live-stock, storm drains, or overwhelmed waste treatment plants during storms (Weiskel, Howes and Heufelder 1996; Jamieson *et al.* 2004). Interestingly, these taxa were already present in water samples collected on September 28—although they were more abundant on October 1 and 3—further indicating a cumulative effect of the typhoon season on the coastal ecosystem and emphasizing the context-dependency of storm effects. Ultimately, swimmers and other recreational users need to be aware that pathogenic bacteria are likely present in Okinawa coastal waters following large rain events.

Conclusions & Future Directions

Despite challenges associated with sampling marine ecosystems during tropical storms and typhoons, this study describes the timing and nature of storm effects on coastal microbial communities in Okinawa, Japan. We found that storm effects were transient, but highly context-dependent. The transient nature of storm effects should not be viewed as diminishing their potential impact on reef or human health. Terrestrially-derived bacteria entering coastal waters during storms may cause disease in marine organisms and humans. In this study, we coupled controlled mesocosm studies with field observations in an effort to disentangle the effects of extreme wind and waves and enhanced currents from the effects of soil run-off during storms. While the mesocosm results were useful, future studies would benefit from more realistic run-off simulation than the soil additions we employed. Additionally, while we did not observe strong shifts in the relative abundance of different marine microbes following storm events, marine microbial metabolism may have been significantly altered. Future studies may capture such responses by measuring bacterial respiration rates or by performing metatranscriptomics.

It is important to note that environmental effects of extreme storms will vary in terms of intensity, spatial extent and duration in different ecosystems and need to be evaluated locally (Paerl *et al.* 2006; Zhang *et al.* 2009; Herbeck *et al.* 2011). Storm effects were transient in the open, tidally-flushed Okinawa coast, but more prolonged storm effects have been observed in other coastal systems, particularly semi-enclosed areas, such as bays and estuaries, where terrestrial sediment loads can have residence times from weeks to years (Zhang et al. 2009; Herbeck et al. 2011; Paerl et al. 2001). Therefore, we suggest that the short-term study of typhoon events follow an adaptive sampling strategy (Wetz and Paerl 2008), which involves the definition of well-established baselines for various physicochemical and biological parameters. Regional monitoring programs, including a comprehensive understanding of background conditions, are essential for a better interpretation of ecological consequences of extreme storm events.

Acknowledgments

We thank Akinori Murata and Marine Le Gal for sample collection, Kazumi Inoha, Koichi Toda, Kosuke Mori and Okinawa Marine Science Support Section, OIST for experiment design and technical support and Koichi Kinjo from the Okinawa Prefectural Institute of Health and Environment for his advice. We also thank OIST sequencing center for DNA sequence support and Okinawa Prefecture Fisheries and Ocean Technology Center for nutrient analysis assistance. MMB was supported by a Japan Society for the Promotion of Science (JSPS) DC1 graduate student fellowship. This work was supported by the JSPS KAKENHI program [Early-Career Project No. 18K18203] and the OIST Marine Biophysics Unit.

Reference list

Altieri AH, Harrison SB, Seemann J *et al.* Tropical dead zones and mass mortalities on coral reefs. *Proc Natl Acad Sci U S A* 2017;**114**:3660–5.

Anderson TH, Taylor GT. Nutrient pulses, plankton blooms, and seasonal hypoxia in western Long Island Sound. *Estuaries* 2001;**24**:228–43.

Arakaki T, Fujimura H, Hamdun AM *et al.* Simultaneous Measurement of Hydrogen Peroxide and Fe Species (Fe(II) and Fe(tot)) in Okinawa Island Seawater: Impacts of Red Soil Pollution. *J Oceanogr* 2005;**61**:561–8.

Azam F, Fenchel T, Field JG *et al.* The Ecological Role of Water-Column Microbes in the Sea. *Marine Ecology Progress Series* 1983;**10**:257–63.

Balmonte JP, Arnosti C, Underwood S *et al.* Riverine Bacterial Communities Reveal Environmental Disturbance Signatures within the Betaproteobacteria and Verrucomicrobia. *Front Microbiol* 2016;**7**:1441.

Bennett KW, Eley A. Fusobacteria: new taxonomy and related diseases. *J Med Microbiol* 1993;**39**:246–54.

Blanco AC, Nadaoka K, Yamamoto T. Planktonic and benthic microalgal community composition as indicators of terrestrial influence on a fringing reef in Ishigaki Island, Southwest Japan. *Mar Environ Res* 2008;**66**:520–35.

Blanco AC, Nadaoka K, Yamamoto T *et al.* Dynamic evolution of nutrient discharge under stormflow and baseflow conditions in a coastal agricultural watershed in Ishigaki Island, Okinawa, Japan. *Hydrol Process* 2010;**24**:2601–16.

Bokulich NA, Kaehler BD, Rideout JR *et al.* Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* 2018;**6**:90.

Bolyen E, Rideout JR, Dillon MR *et al. QIIME 2: Reproducible, Interactive, Scalable, and Extensible Microbiome Data Science*. PeerJ Preprints, 2018.

Brodie JE, Kroon FJ, Schaffelke B *et al.* Terrestrial pollutant runoff to the Great Barrier Reef: An update of issues, priorities and management responses. *Mar Pollut Bull* 2012;**65**:81–100.

Callahan BJ, McMurdie PJ, Rosen MJ *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;**13**:581–3.

Chen N, Krom MD, Wu Y *et al.* Storm induced estuarine turbidity maxima and controls on nutrient fluxes across river-estuary-coast continuum. *Sci Total Environ* 2018;**628-629**:1108–20.

Chen N, Wu J, Hong H. Effect of storm events on riverine nitrogen dynamics in a subtropical watershed, southeastern China. *Sci Total Environ* 2012;**431**:357–65.

Davin-Regli A, Lavigne J-P, Pagès J-M. Enterobacter spp.: Update on Taxonomy, Clinical Aspects, and Emerging Antimicrobial Resistance. *Clin Microbiol Rev* 2019;**32**, DOI: 10.1128/CMR.00002-19.

De Carlo EH, Hoover DJ, Young CW *et al.* Impact of storm runoff from tropical watersheds on coastal water quality and productivity. *Appl Geochem* 2007;**22**:1777–97.

De Jesus Crespo R, Wu J, Myer M *et al.* Flood protection ecosystem services in the coast of Puerto Rico: Associations between extreme weather, flood hazard mitigation and gastrointestinal illness. *Sci Total Environ* 2019;**676**:343–55.

Du J, Park K, Dellapenna TM *et al.* Dramatic hydrodynamic and sedimentary responses in

Galveston Bay and adjacent inner shelf to Hurricane Harvey. *Sci Total Environ* 2019;**653**:554–64.

Fabricius KE. Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Mar Pollut Bull* 2005;**50**:125–46.

Freitas S, Hatosy S, Fuhrman JA *et al.* Global distribution and diversity of marine Verrucomicrobia. *ISME J* 2012;**6**:1499–505.

Frias-Lopez J, Zerkle AL, Bonheyo GT *et al.* Partitioning of bacterial communities between seawater and healthy, black band diseased, and dead coral surfaces. *Appl Environ Microbiol* 2002;**68**:2214–28.

Fuhrman JA, Cram JA, Needham DM. Marine microbial community dynamics and their ecological interpretation. *Nat Rev Microbiol* 2015;**13**:133–46.

Gao Y, Zhu B, Yu G *et al.* Coupled effects of biogeochemical and hydrological processes on C, N, and P export during extreme rainfall events in a purple soil watershed in southwestern China. *J Hydrol* 2014;**511**:692–702.

Gloor GB, Macklaim JM, Pawlowsky-Glahn V *et al.* Microbiome Datasets Are Compositional: And This Is Not Optional. *Front Microbiol* 2017;**8**, DOI: 10.3389/fmicb.2017.02224.

Groisman PY, Knight RW, Easterling DR *et al.* Trends in Intense Precipitation in the Climate Record. *J Clim* 2005;**18**:1326–50.

Haapkylä J, Unsworth RKF, Flavell M *et al.* Seasonal Rainfall and Runoff Promote Coral Disease on an Inshore Reef. *PLoS One* 2011;**6**:e16893.

Harii S, Hongo C, Ishihara M *et al.* Impacts of multiple disturbances on coral communities at Ishigaki Island, Okinawa, Japan, during a 15 year survey. *Mar Ecol Prog Ser* 2014;**509**:171–80.

Hennessy KJ, Gregory JM, Mitchell JFB. Changes in daily precipitation under enhanced greenhouse conditions. *Clim Dyn* 1997;**13**:667–80.

Herbeck LS, Unger D, Krumme U *et al.* Typhoon-induced precipitation impact on nutrient and suspended matter dynamics of a tropical estuary affected by human activities in Hainan, China. *Estuar Coast Shelf Sci* 2011;**93**:375–88.

Hongo C, Yamano H. Species-Specific Responses of Corals to Bleaching Events on Anthropogenically Turbid Reefs on Okinawa Island, Japan, over a 15-year Period (1995–2009). *PLoS One* 2013;**8**:e60952.

Inoue M, Ishikawa D, Miyaji T *et al.* Evaluation of Mn and Fe in coral skeletons (Porites spp.) as proxies for sediment loading and reconstruction of 50 yrs of land use on Ishigaki Island, Japan. *Coral Reefs* 2014;**33**:363–73.

Jamieson R, Gordon R, Joy D *et al.* Assessing microbial pollution of rural surface waters: A review of current watershed scale modeling approaches. *Agric Water Manage* 2004;**70**:1–17.

Korajkic A, Wanjugi P, Brooks L *et al.* Persistence and Decay of Fecal Microbiota in Aquatic

Habitats. *Microbiology and Molecular Biology Reviews* 2019;**83**, DOI: 10.1128/mmbr.00005-19.

Lewis SE, Schaffelke B, Shaw M *et al.* Assessing the additive risks of PSII herbicide exposure to the Great Barrier Reef. *Mar Pollut Bull* 2012;**65**:280–91.

Lin Y-T, Lin Y-F, Tsai IJ *et al.* Structure and Diversity of Soil Bacterial Communities in Offshore Islands. *Sci Rep* 2019;**9**:4689.

Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;**15**:550.

Masucci GD, Reimer JD. Expanding walls and shrinking beaches: loss of natural coastline in Okinawa Island, Japan. *PeerJ* 2019;**7**:e7520.

McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 2013;**8**:e61217.

Mei W, Xie S-P. Intensification of landfalling typhoons over the northwest Pacific since the late 1970s. *Nat Geosci* 2016;**9**:753.

Mistri M, Pitacco V, Granata T *et al.* When the levee breaks: Effects of flood on offshore water contamination and benthic community in the Mediterranean (Ionian Sea). *Mar Pollut Bull* 2019;**140**:588–96.

O'Connor JJ, Lecchini D, Beck HJ *et al.* Sediment pollution impacts sensory ability and performance of settling coral-reef fish. *Oecologia* 2016;**180**:11–21.

Oksanen J, Guillaume Blanchet F, Friendly M *et al.* vegan: Community Ecology Package. R package version 2.5-4. 2019.

Omija T. Terrestrial inflow of soils and nutrients. *Coral Reefs of Japan* 2004;**47**:64–8.

Omori M. Degradation and restoration of coral reefs: Experience in Okinawa, Japan. *Mar Biol Res* 2011;**7**:3–12.

Paerl HW, Bales JD, Ausley LW *et al.* Ecosystem impacts of three sequential hurricanes (Dennis, Floyd, and Irene) on the United States' largest lagoonal estuary, Pamlico Sound, NC. *Proc Natl Acad Sci U S A* 2001;**98**:5655–60.

Paerl HW, Crosswell JR, Van Dam B *et al.* Two decades of tropical cyclone impacts on North Carolina's estuarine carbon, nutrient and phytoplankton dynamics: implications for biogeochemical cycling and water quality in a stormier world. *Biogeochemistry* 2018;**141**:307–32.

Paerl HW, Valdes LM, Joyner AR *et al.* Ecological response to hurricane events in the Pamlico Sound system, North Carolina, and implications for assessment and management in a regime of increased frequency. *Estuaries Coasts* 2006;**29**:1033–45.

Pandey PK, Kass PH, Soupir ML *et al.* Contamination of water resources by pathogenic bacteria. *AMB Express* 2014;**4**:51.

Patterson KL, Porter JW, Ritchie KB *et al.* The etiology of white pox, a lethal disease of the

Caribbean elkhorn coral, Acropora palmata. *Proc Natl Acad Sci U S A* 2002;**99**:8725–30.

Pearman JK, Afandi F, Hong P *et al.* Plankton community assessment in anthropogenic-impacted oligotrophic coastal regions. *Environ Sci Pollut Res Int* 2018;**25**:31017–30.

Perrone J, Madramootoo CA. Improved curve number selection for runoff prediction. *Can J Civ Eng* 1998;**25**:728–34.

Peters EC. Diseases of Coral Reef Organisms. In: Birkeland C (ed.). *Coral Reefs in the Anthropocene*. Dordrecht: Springer Netherlands, 2015, 147–78.

Philipp E, Fabricius K. Photophysiological stress in scleractinian corals in response to short-term sedimentation. *J Exp Mar Bio Ecol* 2003;**287**:57–78.

Pollock FJ, Lamb JB, Field SN *et al.* Sediment and turbidity associated with offshore dredging increase coral disease prevalence on nearby reefs. *PLoS One* 2014;**9**:e102498.

Quast C, Pruesse E, Yilmaz P *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;**41**:D590–6.

Riegl B, Branch GM. Effects of sediment on the energy budgets of four scleractinian (Bourne 1900) and five alcyonacean (Lamouroux 1816) corals. *J Exp Mar Bio Ecol* 1995;**186**:259–75.

Sharma S, Sachdeva P, Virdi JS. Emerging water-borne pathogens. *Appl Microbiol Biotechnol* 2003;**61**:424–8.

Sheridan C, Baele JM, Kushmaro A *et al.* Terrestrial runoff influences white syndrome prevalence in SW Madagascar. *Mar Environ Res* 2014;**101**:44–51.

Shinn EA, Smith GW, Prospero JM *et al.* African dust and the demise of Caribbean Coral Reefs. *Geophys Res Lett* 2000;**27**:3029–32.

Solo-Gabriele HM, Wolfert MA, Desmarais TR *et al.* Sources of Escherichia coli in a coastal subtropical environment. *Appl Environ Microbiol* 2000;**66**:230–7.

Sutherland KP, Porter JW, Torres C. Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. *Mar Ecol Prog Ser* 2004;**266**:273–302.

Sutherland KP, Shaban S, Joyner JL *et al.* Human pathogen shown to cause disease in the threatened eklhorn coral Acropora palmata. *PLoS One* 2011;**6**:e23468.

Voss JD, Richardson LL. Coral diseases near Lee Stocking Island, Bahamas: patterns and potential drivers. *Dis Aquat Organ* 2006;**69**:33–40.

Wang B, Yang Y, Ding Q-H *et al.* Climate control of the global tropical storm days (1965-2008): CLIMATE CONTROL OF TROPICAL STORM DAYS. *Geophys Res Lett* 2010;**37**, DOI: 10.1029/2010GL042487.

Weber M, de Beer D, Lott C *et al.* Mechanisms of damage to corals exposed to sedimentation. *Proc Natl Acad Sci U S A* 2012;**109**:E1558–67.

Weiskel PK, Howes BL, Heufelder GR. Coliform Contamination of a Coastal Embayment:

Sources and Transport Pathways. *Environ Sci Technol* 1996;**30**:1872–81.

Wetz MS, Paerl HW. Estuarine Phytoplankton Responses to Hurricanes and Tropical Storms with Different Characteristics (Trajectory, Rainfall, Winds). *Estuaries Coasts* 2008;**31**:419–29.

Willis A, Bunge J. Estimating diversity via frequency ratios. *Biometrics* 2015;**71**:1042–9.

Willis A, Bunge J, Whitman T. Improved detection of changes in species richness in high diversity microbial communities. *J R Stat Soc C* 2017;**66**:963–77.

Witt V, Wild C, Uthicke S. Terrestrial runoff controls the bacterial community composition of biofilms along a water quality gradient in the Great Barrier Reef. *Appl Environ Microbiol* 2012;**78**:7786–91.

Wooldridge SA. Water quality and coral bleaching thresholds: formalising the linkage for the inshore reefs of the Great Barrier Reef, Australia. *Mar Pollut Bull* 2009;**58**:745–51.

Wu J, Boyle EA. Determination of iron in seawater by high-resolution isotope dilution inductively coupled plasma mass spectrometry after Mg(OH)2 coprecipitation. *Anal Chim Acta* 1998;**367**:183–91.

Yamano H, Watanabe T. Coupling Remote Sensing and Coral Annual Band Data to Investigate the History of Catchment Land Use and Coral Reef Status. In: Kayanne H (ed.). *Coral Reef Science: Strategy for Ecosystem Symbiosis and Coexistence with Humans under Multiple Stresses*. Tokyo: Springer Japan, 2016, 47–53.

Yamazaki A, Watanabe T, Tsunogai U *et al.* The coral δ15N record of terrestrial nitrate loading varies with river catchment land use. *Coral Reefs* 2015, DOI: 10.1007/s00338-014-1235-1.

Zhang J-Z, Kelble CR, Fischer CJ *et al.* Hurricane Katrina induced nutrient runoff from an agricultural area to coastal waters in Biscayne Bay, Florida. *Estuar Coast Shelf Sci* 2009;**84**:209–18.

Zhou W, Yin K, Harrison PJ *et al.* The influence of late summer typhoons and high river discharge on water quality in Hong Kong waters. *Estuar Coast Shelf Sci* 2012;**111**:35–47.