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研究課題名（和文）Study of plankton community structure by environmental DNA metabarcoding under red soil pollution

研究課題名（英文）Study of plankton community structure by environmental DNA metabarcoding under red soil pollution

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　1,000,000円

研究成果の概要（和文）：気候変動シナリオではより頻繁で激しい台風が予測され、沿岸生態系に影響を及ぼす土壌流出量を増大させるだろう。細菌は生態系へ多大な影響をもたらすが、台風への応答はあまり知られていない。この研究では、沖縄での台風時の赤土汚染における、細菌群集のダイナミックスと物理化学的特性の変化を調査及び評価した。台風は土壌流出の大規模で迅速な流入及び主要栄養素の増加を引き起こし、サンゴ及び人の病原体を含む、海洋及び陸生由来の細菌の相対的存在量の変化が発見された。台風が影響する期間は短いが、それは亜熱帯沿岸生態系での台風への応答がその時々の環境に大きく依存することを示す。

研究成果の学術的意義や社会的意義
Red Soil Pollution contributed to the coral reefs degradation in Okinawa, and hence an important impact in fisheries and tourism. This project provided insights into prokaryote responses to RSP and their potential to be used as bioindicators to be used as an easy and efficient environmental tool.

研究成果の概要（英文）：Climate change scenarios predict more frequent and intense tropical storms which will increase the amount of terrestrial run-off affecting coastal ecosystems. Bacteria are key contributors to the ecosystem, but little is known about how they respond to storm events. In this project, we combined field and mesocosm observations to assessed bacterial community dynamics and changes in physicochemical properties during early- and late-season tropical typhoons under Red Soil Pollution in Okinawa. Storms caused large and fast influxes of soil runoff and caused increases of macronutrients. We detected shifts in relative abundances of marine and terrestrially derived bacteria, including putative coral and human pathogens, during storm events. The storm effects were short-lived but the early- and late-season storms caused different physicochemical and bacterial community changes, demonstrating the context-dependency of extreme storm responses in a subtropical coastal ecosystem

研究分野：environmental science

キーワード：ecotoxicology　marine microbiology　red soil pollution　coastal pollution

１．研究開始当初の背景

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. In addition to influencing salinity and turbidity, flood plumes often include elevated concentrations of bacteria, nutrients (i.e. C, N, P) and other chemicals, such as herbicides or heavy metals which can act synergistically to negatively affect coastal ecosystems. Especially in tropical and subtropical regions experiencing severe seasonal storms, large volumes of terrestrial run-off entering coastal waters can degrade coastal ecosystems, including coral reefs, through sedimentation or disease. Such run-off events can also cause harm more indirectly, through eutrophication, hypoxia and decreased water quality. As global climate change is expected to enhance the frequency and intensity of extreme storm events,it is increasingly important to better understand how such storms impact coastal ecosystem functioning.

The western North Pacific (where Okinawa is located) there is an average of 27 named storms per year, being the most active region in the world for tropical cyclones. Landfalling typhoons, which most affect coastal ecosystems, have intensified in the region; the proportion of category 4 and 5 typhoons striking land more than doubled in the last four decades. Current climate models predict continued intensification of landfalling typhoons affecting mainland China, Taiwan, Korea and Japan, indicating these regions will suffer even more storm-caused losses of life, property, and coastal habitat. Okinawa Island is an ideal natural laboratory for studying storm effects on coastal ecosystems (Fig. 1). Okinawa's coral reefs have experienced significant declines in recent decades, due in part to increased storm induced run-off and sedimentation, which is exacerbated by agricultural practices and large coastal development projects. 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 colour during the frequent tropical cyclones. These events are locally referred to as **Red Soil Pollution**.

While the biological consequences of storm-induced run-off have been investigated for corals and fish species in Okinawa, less is known about how tropical cyclones and associated run-off affects coastal microbial communities and especially bacteria. Microbial communities contribute to marine ecosystems through primary production and by recycling dissolved organic carbon and nutrients through the microbial loop, but can also draw down dissolved oxygen or cause opportunistic infections in marine organisms. Therefore, changes in microbial community compositions in response to storms could precipitate large-scale ecosystem effects. Microbial responses can occur extremely quickly; Gammaproteobacteria, Flavobacteria and many Alphaproteobacteria 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 1 day to about a week. Rapid microbial response times to changing environmental conditions make microbes valuable early warning bioindicators 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 intensities. Therefore, the dynamics of microbial communities under red soil pollution in nearshore seawater is under researched.

２．研究の目的

In this study, we characterize nearshore bacterial community dynamics in response to tropical cyclones affecting Okinawa Island and isolate the effects of sediment input through controlled mesocosm experiments. 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 evaluated physicochemical properties and bacterial community compositions in seawater samples collected before, during/between, and after storms in June and October and in samples taken from mesocosms with and without red soil amendment. The specific aims for this study were to (i) assess how bacterial community composition and physicochemical parameters respond in time to tropical cyclones and sediment input; (ii) evaluate the speed of the responses and recovery; and (iii) identify potential ecosystem consequences due to extreme storms and sediment input.

３．研究の方法

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 (Fig. 1). Samples were collected before, during and three days after the tropical storm Gaemi (16th June 2018) and the super typhoon Trami (October 1).

Seawater sampling for DNA and physicochemical analysis: Surface seawater was collected for DNA metabarcoding and chemical analysis of nutrient concentrations (including nitrate (NO3−), nitrite (NO2−), ammonium (NH4+), phosphate (PO43−) and Silica (SiO2) and dissolved iron (dFe)). Physicochemical properties – dissolved oxygen (DO), salinity, temperature, and turbidity – were measured with a CTD probe (RINKO, JFE Advantech, Japan) at each site.

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. Nutrient concentrations were determined on a QuAAtro39 Continuous Segmented Flow Analyser (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 Centre.

DNA extraction and metabarcode sequencing: DNA was extracted from frozen PTFE filters following the manufacturer protocol for the DNeasy PowerWater 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 were transferred to the OIST Sequencing Center for 2 × 300-bp sequencing on the Illumina MiSeq platform with v3 chemistry. Overall, 18.4 million sequencing reads were generated in this study, with 76 217–219 584 sequencing reads per sample (mean =

137 585).

Metabarcode analyses: Sequencing reads were denoized using the Divisive Amplicon Denoising Algorithm with the DADA2 plug-in for QIIME 2. The results were imported into the R statistical environment (R Core Team 2018) for further analysis with the Bioconductor phyloseq package. Permutational analyses of variance (PERMANOVA) on Aitchison distances were performed with the adonis function (999 permutations) in the R package vegan to test whether shifts in community composition were statistically significant. Lastly, we used the DESeq2 bioconductor package to determine which ASVs (amplicon sequence variants) were significantly differentially abundant (false discovery rate adjusted P-value <0.05) in water samples collected from field sites before, during, and after storms.

４．研究成果

Despite challenges associated with sampling marine ecosystems during tropical storms and typhoons, this study describes the timing and nature of storm effects on coastal bacterial communities in Okinawa, Japan. Surprisingly, we found that storm effects were transient, but highly context-dependent. We also found a number of bacteria accessions potentially pathogenic to corals or fish, so the effect of bacteria carried with the red soil runoff should be carefully studied with more detail in future assessments.

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. 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. Therefore, we suggest that the short-term study of typhoon events follow an adaptive sampling strategy, which involves the definition of well-established baselines for various physicochemical and biological parameters. The continuation of this line of work is seminal to achieve the Sustainable Development Goals (SDGs) on marine life.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　1件／うち国際共著　2件／うちオープンアクセス　1件）

１．著者名 Angela Ares, Margaret Mars Brisbin, Kirk N. Sato, Juan P. Martin, Yoshiteru Iinuma, Satoshi Mitarai	４．巻 1
２．論文標題 Extreme storm-induced run-off causes rapid, context-dependent shifts in nearshore subtropical bacterial communities	５．発行年 2019年
３．雑誌名 Biorxiv	６．最初と最後の頁 1-24
掲載論文のDOI（デジタルオブジェクト識別子） 10.1101/801886	査読の有無 無
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Angela Ares, Margaret Mars Brisbin, Kirk N. Sato, Juan P. Martin, Yoshiteru Iinuma, Satoshi Mitarai	４．巻 22 (11)
２．論文標題 Extreme storms cause rapid but short‐lived shifts in nearshore subtropical bacterial communities	５．発行年 2020年
３．雑誌名 Environmental Microbiology	６．最初と最後の頁 4571-4588
掲載論文のDOI（デジタルオブジェクト識別子） 10.1111/1462-2920.15178	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計3件（うち招待講演　1件／うち国際学会　1件）

１．発表者名 Ares A.
２．発表標題 Plankton community dynamics under red soil pollution in nearshore waters.
３．学会等名 The 4th Okinawa Marine Science Workshop Program, December 6th 2019.（招待講演）
４．発表年 2019年

１．発表者名 Ares A, Brisbin MM, Sato KN, Martin JP, Iinuma Y, Mitarai S.
２．発表標題 Prokaryote community and physicochemical shifts due to storm induced terrestrial runoff in subtropical coastal ecosystems.
３．学会等名 Ocean Science Meeting. 16-21 February 2020. San Diego, California, USA.（国際学会）
４．発表年 2020年

１．発表者名 Angela Ares
２．発表標題 Red Soil Pollution within coral reef ecosystems
３．学会等名 ASLO Aquatic Sciences Meeting
４．発表年 2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関