様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　５ 年　 ６ 月 １５ 日現在

機関番号：３８００５
研究種目：若手研究
研究期間：2020～2022
課題番号：２０Ｋ１９９８６

研究課題名（和文）Biomonitoring of Red Soil Pollution by DNA-based methods in Okinawa

研究課題名（英文）Biomonitoring of Red Soil Pollution by DNA-based methods in Okinawa

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交付決定額（研究期間全体）：（直接経費）　　2,600,000円

研究成果の概要（和文）：環境への圧力に極めて素早く反応することで、微生物の集合体は沿岸の健康状態の生物指標として重要な役割を果たすことができます。
ただし、台風に対する微生物の短期的な反応は不明瞭です。このプロジェクトでは、DNAベースの方法と物理化学的な観察を組み合わせて、異なる土地利用によって影響を受ける沿岸域での原核生物コミュニティの季節変動と赤土汚染（RSP）に対する転写反応をより良く理解するために使用されます。このプロジェクトで得られる結果は、RSPに対する原核生物の反応および沖縄のサンゴ礁生態系のモニタリングにおけるDNAベースの方法の適用可能性についての示唆を提供します。

研究成果の学術的意義や社会的意義
We believe that these results demonstrate that urbanization is strongly impacting physicochemical and nutrient conditions in Okinawa’s nearshore ecosystems.
This study highlights the importance of vegetation to limit run-off to coastal systems, as well as wastewater management.

研究成果の概要（英文）：By responding extremely quickly to environmental pressures, microbial assemblages can play an essential role as bioindicators of coastal health.
However, short-term microbial responses to typhoons are poorly characterized. In this project, DNA based methods in combination with physicochemical observations will be used to understand better the seasonal variability of prokaryote communities and transcriptional response to Red Soil Pollution (RSP), contributed to the coral reefs degradation, in nearshore areas affected by different land uses. The results obtained in this project will provide insights into prokaryote responses to RSP as well as the applicability of DNA based methods in coral reef ecosystem monitoring in Okinawa.

研究分野：Marine environmental Science

キーワード：Biomonitoring　coastal pollution　coastal microbiology　DNA metabarcoding

１．研究開始当初の背景

Coral reefs are among the most productive and diverse ecosystems on earth. They provide essential goods and services to society, such as fisheries, tourism, and natural products with biomedical applications. In Japan alone, the overall benefits of coral reefs have been estimated as equivalent to 1665 million USD/year. Unfortunately, over the last 30 years, half of the total reefs on earth have disappeared, and it is expected that more than 90 percent of the world's coral reefs will die by 2100 due to impacts from climate change, such as the rise of temperatures, acidification or terrestrial run-off. Regarding the latter, various types of land uses in the watershed (e.g. urban, industrial, agricultural, rural) will determine the physico-chemical (e.g. type of pollutants or nutrients) and biological composition of terrestrial run-off plumes reaching coastal ecosystems –especially under extreme rain events (e.g. tropical storms or typhoons)– largely affecting the extent of the impact.

In the Western North Pacific, extreme storm events are common and expected to be more frequent and intense in the future as a result of climate change. Okinawa Island is an ideal natural laboratory for studying storm effects on coastal ecosystems since storm-induced run-off and sedimentation have, contributed to the significant declines in coral reefs. The fine-particle, laterite soils with high iron concentrations found in Okinawa are easily suspended and turn coastal waters a deep, cloudy red colour –triggered by intensive agriculture and large coastal development projects– during the frequent tropical cyclones. These events are locally referred to as Red Soil Pollution (RSP).

In a recent study, we characterized nearshore physico-chemical properties and microbial community dynamics during two of the major tropical cyclones occurring in Okinawa in 2018. We observed that the microbial community composition was highly influenced by terrestrially derived bacteria, including potential coral and human pathogenic taxons, but that the speed and signal of the response was highly context-dependent: in the late season typhoon more terrestrial bacteria were found before the storm and less terrestrial bacteria was found right after the storm compared to early season storm. However, the interpretation of these results is still challenging due to the lack of well-established baselines for various physico-chemical and biological parameters.

Regional monitoring programs in areas with well-distinguished land uses, including comprehensive microbiology data, are essential for a better interpretation of ecological consequences of extreme storm events.

２．研究の目的

Coastal bio-monitoring approaches -based on plankton, benthic macrofauna observations- are often imprecise, time-consuming and appropriate only for mid- and long-term responses. Microbes, on the other hand, display rapid and sensitive response to changing environmental conditions, making them valuable early-warning bioindicators of anthropogenic impacts– such as the presence of fecal bacteria derived from wastewater overflow–. However, bacteria communities have not been considered for bio-monitoring purposes until very recently with the development of genetic methods. By yielding faster, easier and more reliable taxonomic identification from monitoring, genetic sequencing-based methods (including DNA metabarcoding, metatranscriptomics) provide a better assessment of the environmental status of marine waters. DNA metabarcoding –large scale taxonomic identification of environmental samples via analysis of few orthologous DNA regions named as barcodes– has emerged as a powerful tool to assess and detect spatiotemporal changes in aquatic community structure, enabling the correlation with specific pollution sources due to different land uses. Additionally, meta-transcriptomics provides valuable insights about microbial community interactions and their metabolic pathways as essential components of ecosystem function. Therefore, this project aims to apply genetic approaches in order to study seasonal variability of prokaryote communities and RSP effects on the microbial genetic response in nearshore environments affected by different land uses. In particular, the present project proposal aims to address 3 main general scientific questions (SQ):

SQ1- What is the seasonal variability of prokaryotes and how does this correlates with site specific land uses and hydrological regimes?

SQ2- What are the prokaryote gene expression patterns under RSP events?

SQ3- What is the environmental risk at each of the areas affected by different land use?

３．研究の方法

To investigate prokaryote community structure and genetic responses to RSP, high throughput sequencing methods -DNA metabarcoding- was used. Environmental water samples were collected in 4 nearshore areas with well-distinguished land uses: Ginowan and Nago as urban and Tancha and Ogimi as rural. These areas were selected because, in contrast to the mixed land use pattern found in most of the Okinawa territory, the selected watershed were occupied by at least 70% of urban territory (to be determined as urban) and 70% of rural territory (to be determined as rural). Okinawa land mapping was already carried

out by Kenneth Lynn Dudley a colleague from the Environmental Science and Information section at OIST.

Water samples for chemical (macronutrient analysis) and biological characterization were collected on a regular basis each 2 weeks for 1 year at these four sampling sites starting the 1st of May of 2020. Additionally, a data logger was deployed in the water to collect physicochemical data (e.g. temperature, salinity, turbidity, dissolved oxygen, etc). In all cases, samples will be collected in triplicates (n=3/site). All the analysis will be performed at OIST (DNA Sequencing Section and at Instrumental Analysis

４．研究成果

We have performed all the genetic and chemical analysis and we are now working on the manuscript. One of the main observations is that both genetic as well as chemical composition showed big differences between rural and urban sites all around the year.

Both urban sites showed higher concentrations of total nitrogen and phosphate and these differences were constant all the year. Higher concentrations were found when water was collected right at the output of the watershed main stream while concentrations were found much diluted when water was collected few meters away from the main stream output.

As for microbes, several groups of bacteria derived from living organisms feaces were found with higher prevalence in urban sites. Some of these includes the order Clostridiales and Campilobacterales.

We believe that these results demonstrate that urbanization is strongly impacting physicochemical and nutrient conditions in Okinawa's nearshore ecosystems.

Bacterial communities at urban sites are consistently disturbed compared to more rural sites—year-round effect with limited correlation to environmental variables.

In summary this study highlights the importance of vegetation to limit run-off to coastal systems, as well as wastewater management.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Margaret Mars Brisbin, Angela Ares, Kenneth Dudley, Satoshi Mitarai
２．発表標題 Impacts of urbanization on near-shore bacterioplankton dynamics along Okinawa Island, Japan
３．学会等名 ASLO Aquatic Sciences Meeting 2023（国際学会）
４．発表年 2023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