MarineGEO Network Project

The Good, the Bad, & the Ugly: Carbon storage, eutrophication effects, and emergent health risks in the global marine sediment microbiome

Coastal marine environments provide ecosystem services that are essential to human well-being, including water and nutrient cycling, fisheries, coastal protection and tourism. These services are underpinned by microorganisms that comprise the greatest biomass in the oceans and ultimately regulate their local environment through biogeochemical cycling and primary productivity. However, relatively few studies have investigated the interface between coastal microbiomes and their impact on ecosystem functioning. Here, we propose a collaborative project using the **global MarineGEO Network** to answer critical questions pertaining to the effect of eutrophication on microbiome composition and resulting impacts on decomposition and carbon storage. With the marriage of next gen microbial sequencing with simple decomposition (tea bag) assays **we aim to test the hypothesis that eutrophication 1) increases the rate of decomposition and thus, reduces carbon storage and 2) the microbial biodiversity and decomposition rate vary as a function of annual mean temperature (latitude).** This proposed work has the potential to highlight the importance of water quality in climate change mitigation and will supply partner sites with detailed information on their sediment microbiome including the prevalence of pathogens and virulence factors.

Introduction

"**The Good" - Oceans & Carbon Cycling -** The ocean regulates Earth's climate. It is a sink for carbon dioxide (CO_2), storing 50 times more carbon than the atmosphere. As such, we depend on the ocean to modulate climate and to facilitate the sequestration of greenhouse gasses. Yet, coastal oceans and the services they provide are under threat from global climate change, eutrophication, overharvesting and habitat destruction[1,2] which affects the very organisms which drive those functions, particularly microbes. Indeed, anthropogenic activities are leading to environmental changes affecting marine biodiversity across a gamut of species including critical foundational groups such as corals, seagrass, and oysters[3–5]. Common to these ecosystems is a cryptic "microbial jungle" within marine sediments that has a critical role in biogeochemical cycling and underpins ecosystem productivity. Marine bacteria are particularly important in ocean sediments through the action of decomposition, carbon storage[6] and as a reservoir of pollutants and pathogens[7].

As an ecosystem, marine sediments are <u>ubiquitous</u> and provide essential ecosystem services. Therefore, understanding the response of microbial communities to global and local disturbances is essential to properly assessing the functioning and stability of the coastal oceans[8].

"The Bad" - Eutrophication - Human impacts on water quality affect the diversity and function of microbial communities and influence the degradation of organic matter in

marine sediments[13]. Indeed, organic matter deposited on the seafloor is processed by microorganisms and rapidly oxidized into CO_2. In characteristically oligotrophic systems, such as tropical seas, nutrient limitation (nitrogen, phosphorus, trace elements, etc.) can suppress decomposition. In oligotrophic systems, microbial abundance and decomposition activity is nutrient-limited owing to a lack of resources for growth and metabolism, respectively. In such cases, marine sediments can more effectively sequester organic matter. Conversely, eutrophication, the increase of inorganic nitrogen and phosphorus concentrations, enhances the decomposition of labile organic matter and inhibits the breakdown of recalcitrant organic matter[14]. In eutrophic systems, microbial species compete for abundant resources and quickly metabolize labile organic matter. The 'leftovers', a pool of recalcitrant organic matter, requires relatively more energy to process, meaning that this pool is effectively stable within sediments while the labile fraction is rapidly respired. Thus, as more nutrients are added to the system, more labile organic matter is decomposed, while the recalcitrant components remain relatively stable. This process ultimately transforms marine carbon sinks into carbon sources that contribute to ocean acidification and increasing atmospheric CO_2 via outgassing.

In oxygenated sediments, oxygen reduction is the dominant organic matter decomposition pathway. However, in hypoxic sediments organic carbon oxidation often uses sulfate as an electron-donor mediated by sulfate-reducing microbes (SRMs). SRMs are a major contributor to carbon storage and release[15]. Neither the quantification of SRM community composition[16] nor decomposition rates[17] in marine sediments have been extensively studied together despite both components being vitally linked to organic carbon oxidation[18] and driving carbon storage in the ocean[19]. This is especially relevant to eutrophic coastal sediments, which remain understudied and therefore a critical gap for carbon inventory accounting. In recent years, several studies have quantified carbon stocks and their vulnerability to climate change. While some have traced the corresponding impact on CO_2 emissions to the atmosphere[20], information is fragmented, especially for the marine realm. Moreover, a significant gap remains in understanding the human welfare impacts of eutrophication from a climate change perspective. Therefore, quantitative assessment of the biological–social–economic risks of eutrophication-driven CO_2 emissions warrants attention.

"**& The Ugly" - Pollutants, Pathogens, AMR -** The abundance and diversity of microorganisms are correlated with human impacts and their genetic markers can be used as an indicator for pollution, pathogen loads, and antimicrobial resistance genes (AMR)[9–11]. However, this field of research is still new and more studies are required to provide a comprehensive genetic profiling of those communities, especially within anaerobic habitats which remain poorly understood. Our proposed work will be able to extract information about human impacts on the marine microbiome, specifically the identity of putative human pathogens and the prevalence of virulence factors such as AMR. These metrics serve as an indicator of human influence on the ocean microbiome, and subsequent feedbacks of these influences on human and marine organisms health.

To fill this gap, as part of MarineGeo-Hong Kong, we have (1) measured organic matter decomposition rates and carbon storage capacities using the tea-bag assay, (2) estimated CO_2 efflux rates, and (3) correlated the role and composition of SRMs in coastal marine sediments along a pollution gradient between the north-eastern coast of Hong Kong and mainland China [21] (Figure 1). We showed that the carbon storage capacity resembled that of bogs and mangroves in the least eutrophic site. However, this was true only when nitrogen pollution was low. With nutrient pollution, decomposition rates were higher, in concert with greater abundance of SRMs. **This study has provided the first estimate of the carbon cost of eutrophication in marine sediments which provides a critical link between global climate change and localized water quality management.** We have estimated that if global coastal marine sediments were as eutrophic as urbanized sites in Hong Kong, the resulting carbon cost would exceed US$ 11 billion. These estimates become more meaningful when compared with the cost of losing blue carbon sinks (US$ 9 billion[20]). This implies that pristine coastal marine sediments perform carbon storage services that are comparable to coral reefs, mangroves and seagrasses.

(A)

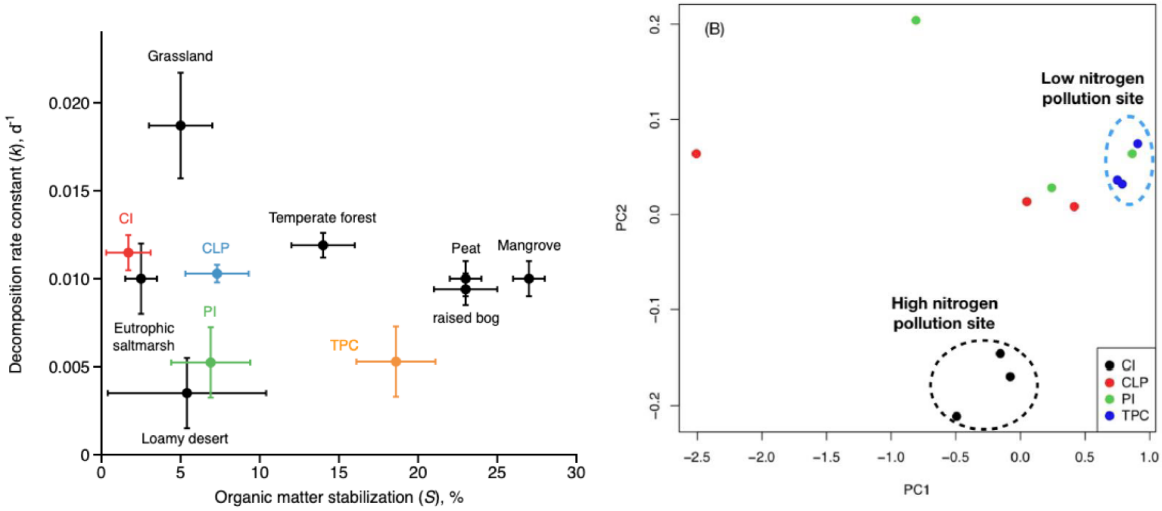

Fig. 1: (A) Comparison of Hong Kong's marine sediments to other ecosystems and geographies in their decomposition rate and stabilization, (B) Principal Component Analysis depicting SRM community structure in each sample. Four Sites in Hong Kong along a nutrient pollution gradient: Center Island, CI; Che Lei Pai, CLP; Port Island, PI; Tung Ping Chau, TPC).

Another related study[12] conducted under the umbrella of MarineGeo-Hong Kong evaluated the microbial community composition, the functional characteristics of the microbial community, and the prevalence of pathogenic bacteria in marine sediments along a punctuated pollution gradient (<10km). A shotgun metagenomic approach provided a comprehensive understanding of taxonomic, functional and antimicrobial resistance profiles. We highlighted that the marine sediment microbiome composition and function are influenced by human activity and pollution discharges. These data set the stage for testing exciting hypotheses pertaining to the role of marine biodiversity to mitigate the spread of pathogens and virulence factors (dilution hypothesis).

Methods

Smithsonian MarineGEO commits to provide: coordination in the form of participant recruitment, planning fieldwork with each participant, standardized protocols and templates, metagenomics analysis, data management, data synthesis and lead writing of the publications.

Each partner commits to: conducting the required fieldwork, processing the samples collected, submitting the data in standard format to MarineGEO, and contributing to data interpretation and manuscript preparation.

In this project, we propose to conduct a comprehensive global study to evaluate the effect of latitude, temperature and relative anthropogenic impacts on ubiquitous marine sediment ecosystems. Based on a simple methodology using the core research decomposition assay, experimental fertilization treatments, and sediment sampling, this project aims to characterize; 1) the sediment microbiome and its function and 2) the recalcitrant organic carbon decomposition within that environment under the influence of nutrient fertilization relative to a control across a network of global partner sites. As such, this proposal represents a first of its kind study to examine the impact of temperature (latitude) and nutrients on microbial communities and their ability to sequester carbon. Moreover, this project will allow us to identify existing and emergent human and environmental health risks through analysis of pathogen abundance and virulence factors. To do so, we will (1) coordinate a global survey among all the participants of the MarineGEO Network as well as potential future partners, and (2) provide materials and coordinate samplings at all sites and conduct a centralized analysis and reporting of the data.

(1) Global survey
Using Google sheet we will distribute a call for metadata from potential partner sites. The survey will generate a database of all observatories, partners and emerging partner sites with emphasis on their related characteristics (*e.g.* ecosystem type coral reef, seagrass, oysters; anthropogenic impacts; temperature and water quality records, etc.). This survey will be of paramount importance for this study but also for MarineGEO providing a database of participating sites and their characteristics for future projects and activities.

(2) Sampling and data analysis
Each participant will receive a package of materials for the tea bag assay and sediment sampling. The tea bag assay was developed[22] to provide a standardized, cost-effective and simple plant-litter assay for decomposition rates. It is now widely used across diverse ecosystems, including mangroves, grasslands, deserts and forests. Moreover, the previous results using this methodology correlate well with the calculation of terrestrial carbon sequestration potential, suggesting that reliable estimates of carbon storage potential for other habitats can be derived.

Based on the survey responses collected, we will call on all locations to participate in the coordinated sampling. Within each location, one site will be targeted for experimentation and sampling. Targeted sites should be at 0.5m to 3m depth, with mud sand (fine sediment) and be unimpacted (e.g. remote area, MPA). The experimental components will include 1) deployment of organic matter (tea leaves) with different composition, and 2) fertilization of sediments using time-release fertilizers (Figure 2).

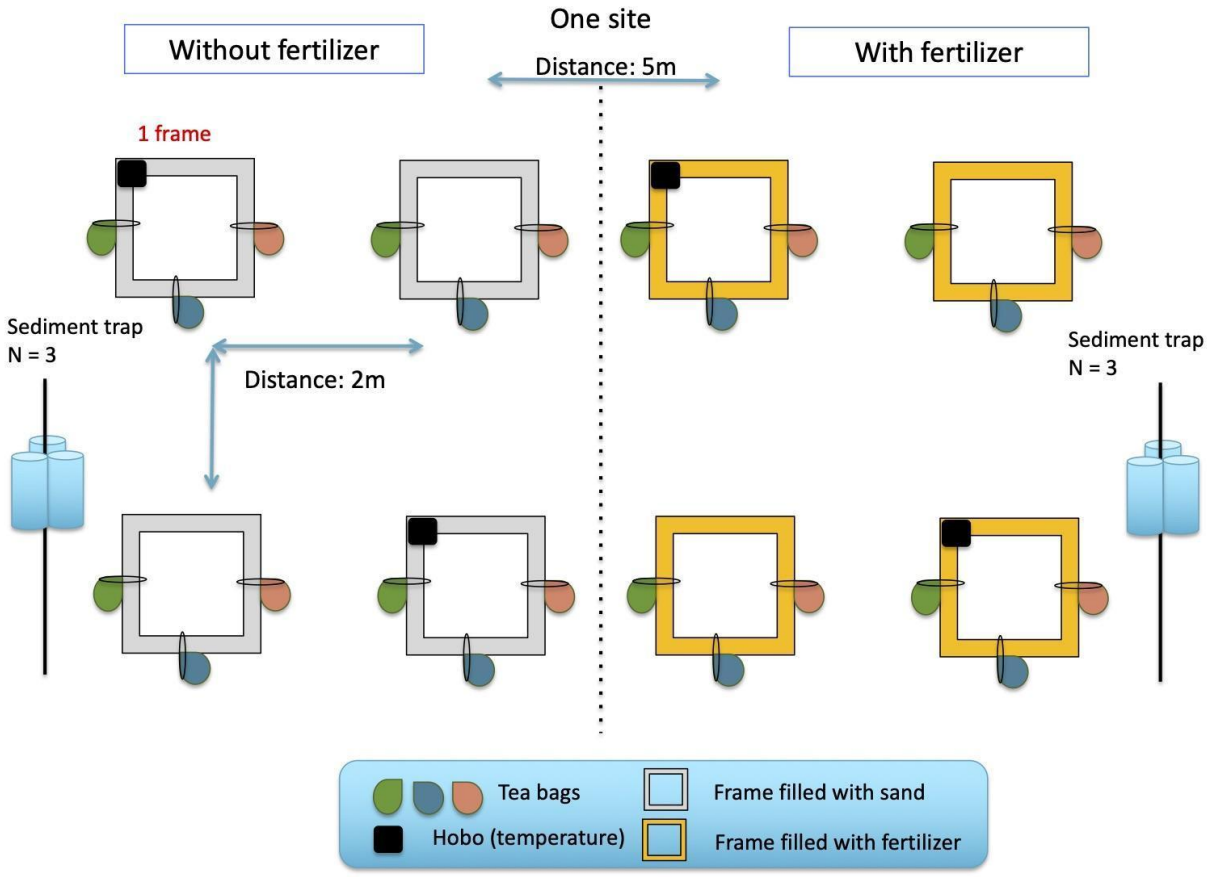

Fig. 2: Experiment layout: frames with or without fertilizer.

Different composition of tea bags will be weighed and buried (3 replicates per frame) in mesh bags at a depth of ~8 cm. Slow-release fertilizer (Osmocote) will be added in half of the frames. The idea behind using slow-release fertilizer is to investigate the effect of nutrient pollution on the microbial activity in the marine sediments. Osmocote is comprised of a semipermeable membrane surrounding water-soluble inorganic Nitrate and Phosphate.

Upon retrieval, tea bags will be oven-dried at 60°C for at least 72 hours and re-weighed after removing the mesh bag. Decomposition rate (k, d-1) and organic matter stabilization (S, %) will be calculated using the following equations:

$$k = -\text{Ln}\ [(\text{final mass} - \text{initial mass})/\text{duration of deployment}]$$
$$S = 1 - [(\text{final mass} - \text{initial mass})/\text{initial mass of tea}] \times 100$$

During the coordinated tea bag retrieval, seawater and sediments will be collected. If information on the seawater nutrients can not be provided by the participant, 3 replicate seawater samples (50 mL) will be collected to be analyzed by flow injection analysis (FIA). The pore water will be collected using Rhizon sampler as well as 50 g of sediment from the top 8 cm. The sediment samples will be further divided into aliquots for grain size, meiofauna, metabarcoding and metagenomic analysis. The sediment samples will be stored at − 80 °C for DNA extraction, metagenomic shotgun sequencing and metabarcoding analysis (18S and COI).

Based on our previous study[12,21], we will investigate the taxonomic profile and community composition of the microbiome as well as their function. Special attention will

be given to the identification of biogeochemical cycles and potential pathogens. Antibiotic gene resistance will be annotated to determine the correlation between anthropogenic stressors and human health risk factors. The composition and abundance of SRMs that have functional capabilities for major anaerobic cycling and sulfur metabolisms will be characterized. The abundance of sulfite reductase (*dsrA* and *dsrB* genes[23]) encoded by the sulfate reducing microbes will be investigated.

With the use of simple protocols and materials, and a focus on a pan-global habitat (sediments), one of the strengths of this project is its accessibility across locations allowing all interested partners to be involved. As a value-add, this project will help us to 'on-ramp' more partner sites into the MarineGEO program and foster new collaborations. Moreover, the use of next-generation sequencing in tandem with functional response measurements will lead us to high impact research outputs. Similar work based out of our group alone has been published in *Microbiome* and a forthcoming manuscript was reviewed in *Science Advances.* We expect the outputs of a global-scale study to be of even higher impact.

Timeline and Products

June - July 2020: Interested parties indicate their participation. Review of site selection and methods.
August - September 2020: Production and shipping of supply packages to participants.
October - November 2020: Planning and preparation of the fieldwork by the participants in the south hemisphere.
November 2020 - February 2021: Tea bags and slow-release fertilizer study conducted by the participants in the south hemisphere during 3 the warmest months of the year.
March - May 2021: processing of the sediment and seawater samples. Planning and preparation of the fieldwork by the participants in the north hemisphere.
June - August 2021: Data analysis. Tea bags and slow-release fertilizer study conducted by the participants in the north hemisphere during 3 the warmest months of the year.
September - November 2022: processing of the sediment and seawater samples.
December 2022 - April 2023: write and submit manuscripts.

Prior to initiation of the project, we will distribute and discuss an agreement detailing guidelines for authorship, ethics, and responsibilities of each party associated with the project. The project will be led by Dr. Isis Guibert, Postdoctoral Fellow. Isis will also lead coordination, analysis, and writing of the manuscripts. All participants who were instrumental in conducting the proposed work (including idea generation, providing required data, and assisting in manuscript preparation and submission) will be co-authors on publications, and we anticipate that Isis will be primary author on these manuscripts. We are happy to work with individuals or groups to pursue additional questions related to the data, which will be made freely available to all partners after curation.

How to Join

Interested participants should complete the google sheet and contact Dr. Isis Guibert, at iguibert@hku.hk. Questions, comments, or suggestions are also welcome.

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