RGC Ref. No.:
UGC/FDS16/M02/21
(please insert ref. above)

RESEARCH GRANTS COUNCIL
COMPETITIVE RESEARCH FUNDING SCHEMES FOR THE LOCAL SELF-FINANCING DEGREE SECTOR

FACULTY DEVELOPMENT SCHEME (FDS)

Completion Report

(for completed projects only)

Submission Deadlines:

1. *Auditor's report with unspent balance, if any: within **six** months of the approved project completion date.*
2. *Completion report: within **12** months of the approved project completion date.*

Part A: The Project and Investigator(s)

1. Project Title

Community Structure and Metaproteomics of Microorganism Assemblages Collected from Pneumatohpores of *Avicennia marina* in Mangrove Ecosystem

2. Investigator(s) and Academic Department(s) / Unit(s) Involved

Research Team	**Name / Post**	**Unit / Department / Institution**
Principal Investigator	LEE Wang-fat Professor	School of Science and Technology, Hong Kong Metropolitan University
Co-Investigator(s)	CHEN Luzhen Professor	College of The Environment and Ecology, Xiamen University
Others		

3. Project Duration

	Original	**Revised**	**Date of RGC / Institution Approval** *(must be quoted)*
Project Start Date	01/01/2022	NA	NA
Project Completion Date	31/12/2023	30/06/2024	14 August 2023
Duration *(in month)*	24	30	14 August 2023
Deadline for Submission of Completion Report	31/12/2024	30/06/2025	14 August 2023

4.4 Please attach photo(s) of acknowledgement of RGC-funded facilities / equipment.

N/A

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. ***Compare*** the biodiversity, species composition and abundance of dominant microbial groups (microalgae and bacteria) collected from pneumatophores of *Avicennia marina* in cleaned and polluted sites under different seasons of a year
2. ***Determine*** correlations between the microbial community structure and parameters of pneumatophores (different distances away from the main trunk, number of lenticels, vertical segments), and environmental variables
3. ***Elucidate*** effects of pneumatophores associated (natural) and dissociated (excised pneumatophores) to the parent plant on the microbial community structure
4. ***Investigate*** the recolonization of microalgae and their interactions with bacteria on excised pneumatophores under the condition with and without the access of free-living microalgal cells from tidal water and sediment
5. ***Explore*** the protein expression profiles and metabolic activities of the microalgae-bacteria-pneumatophore communities using metaproteomic approach
6. ***Reveal*** possible functional roles and interaction mechanism between the pneumatophores and their associated microalgal and bacterial assemblages

5.2 Revised objectives

Date of approval from the RGC: N/A

Reasons for the change: N/A

5.3 Realisation of the objectives
(Maximum 1 page; please state how and to what extent the project objectives have been achieved; give reasons for under-achievements and outline attempts to overcome problems, if any)

All objectives were successfully completed without any significant issues. The realization of the objectives is summarized below.

Objective #1: We conducted a literature review to gather updated information and refine our experimental protocols. We then conducted field sampling at six mangrove sites (Tai

O, Mai Po, Futian, Sam Mun Tsai, Ting Kok, and Tai Tan) to analyze microbial community structure using metagenomic sequencing. Spatial patterns revealed that northwest mangroves (Mai Po and Futian) had the most diverse microbial communities, followed by Tai O and northeast sites (Sam Mun Tsai, Ting Kok, and Tai Tan). *Pseudomonadota* was the dominant microbial phylum across all sites, with *Euryarchaeota* prevalent in northwest mangroves and *Cyanobacteriota* dominating in the other four sites. Northeast pneumatophores near Mirs Bay harbored higher microalgal abundance, with *Synechococcus*, *Tolypothrix*, and diatoms being prominent. Seasonal analysis at Mai Po and Ting Kok showed greater microbial diversity during the wet season at Mai Po, while no significant seasonal differences were observed at Ting Kok. Overall, site-specific factors had a stronger influence on microbial community structure than seasonal variation.

Objective #2: We investigated how microbial community structure on pneumatophores responds to various physicochemical parameters and pneumatophore characteristics. Our analysis revealed significant correlations between microbial communities and all measured environmental variables. Among these, the strongest correlations were observed with chromium and zinc concentrations in tidal water, as well as ammonia levels in the topsoil. Among pneumatophore characteristics, only diameter significantly influenced microbial communities, while length and density had no measurable effect. Tidal position had a stronger impact on microbial composition than vertical positions of pneumatophore, though vertical effects were more pronounced in the higher tide zone. Microalgae dominated the lower tide zone across all vertical positions, while drought-tolerant bacteria were enriched in the higher tide zone.

Objectives #3 & 4: Manipulative field experiments assessed the role of host connection and microbial recolonization on pneumatophores were conducted. Three setups were used: (1) excision (pneumatophores disconnected from the host), (2) sterilization (microorganisms removed but host connection maintained), and (3) combined (excised and sterilized). Microbial communities connected to the host consistently exhibited higher alpha diversity, particularly in recolonized communities. Further analysis revealed that both excision and sterilization influenced microbial community structure, but excision had a far greater impact. Notably, the diversity and abundance of microalgae were significantly reduced on excised pneumatophores compared to non-excised ones. These findings highlight the host plant's critical role in shaping microbial communities and suggest a mutualistic relationship between pneumatophores and microalgae.

Objectives #5& 6: Omics analyses explored molecular interactions between A. marina pneumatophores and epiphytic microbes were performed. Proteomics data matched against the NCBI-curated genome of A. marina and sample-specific metagenomes revealed striking similarities in gene ontology (GO) terms between host and microbial communities, suggesting cooperative interactions. Specifically, proteins associated with ATP and DNA binding may play roles in genetic information processing and energy metabolism, indicating functional overlap between the host and its epiphytes. Comparing excised and non-excised pneumatophores showed that severing host connection led to significant downregulation of functional genes, indicating a loss of microbial capabilities. These results underscore the importance of host-microbe interactions in maintaining functional diversity.

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
*1.**Compare*** the biodiversity, species composition and abundance of dominant microbial groups (microalgae and bacteria) collected from pneumatophores of *Avicennia marina* in cleaned and polluted sites under different seasons of a year	✓	100%
*2.**Determine*** correlations between the microbial community structure and parameters of pneumatophores (different distances away from the main trunk, number of lenticels, vertical segments), and environmental variables	✓	100%
*3.**Elucidate*** effects of pneumatophores associated (natural) and dissociated (excised pneumatophores) to the parent plant on the microbial community structure	✓	100%
*4.**Investigate*** the recolonization of microalgae and their interactions with bacteria on excised pneumatophores under the condition with and without the access of free-living microalgal cells from tidal water and sediment	✓	100%
*5.**Explore*** the protein expression profiles and metabolic activities of the microalgae-bacteria-pneumatophore communities using metaproteomic approach	✓	100%
*6.**Reveal*** possible functional roles and interaction mechanism between the pneumatophores and their associated microalgal and bacterial assemblages	✓	100%

6. Research Outcome

6.1 Major findings and research outcome

(Maximum 1 page; please make reference to Part C where necessary)

Characterization of Microbial Community Structure on Pneumatophores: The community structure of epiphytic microbial species on pneumatophores was found to be influenced by the physicochemical properties of their microhabitats and the characteristics of the pneumatophores. Significant differences in the epiphytic microbial structures colonizing on the surface of pneumatophores and their adhered soil were observed. It indicated that microhabitats provided by these two substrates are varied. Our data showed that bacteria is the dominating microorganism (over 96% of identified taxa) across all pneumatophore samples we have tested, with Proteobacteria being the most prevalent bacterial phylum. The community structure would be affected by several key environmental factors, including pneumatophore water content, soil salinity, and NH_4^+-N concentration. Increased soil salinity enhanced species richness and diversity, favoring the growth of Nitzschia, while soil water content and NH_4^+-N concentration were particularly influential for microalgal communities on adhered soil. We also noticed that vertical position along the pneumatophores is one of the major factors in determining the microbial community structure. These findings demonstrate the importance of environmental factors and microhabitat characteristics in shaping microbial diversity and structure colonizing on the pneumatophores.

The Effect of the Mangrove Plant Host on Microbial Community Structure on Pneumatophores: Our manipulative field investigations involving “excision” and “sterilization” treatments on pneumatophores demonstrated significant effects on the colonization of the microorganisms, with much higher effects were observed from the excision treatment. The abundant microbial species on the excised pneumatophores were found to be very different from that of the non-excised one. The result indicated that the mangrove plant (the host) was found to facilitate a rich and diverse microbial community on the pneumatophores, and it also suggests a possible mutualistic relationship between the host and its associated epiphytic microbes. The host effect on alpha diversity was more pronounced in recolonized microbial communities, because larger differences in microbial diversity and richness between excised and non-excised pneumatophores under sterilized conditions were observed. These findings suggest that pneumatophores not only serve as physical substrate but also create favorable environments to the colonizing microbes through various biological processes. In return, colonizing microbes may contribute to the stability and functionality of pneumatophores.

Possible Functional Roles of the Epiphytic Microbes: This study carried out omics analysis to determine the possible functional roles of epiphytic microbes and their interactions with pneumatophores. Our results showed that metabolic pathways between microbes colonizing on the pneumatophore and those in surrounding sediments were different significantly. Genes related to sulfur metabolism and dissimilatory nitrate reduction were more abundant in pneumatophore matrices. These results indicate the essential role of epiphytic microbes in the contribution to nutrient cycling within the mangrove ecosystems. We observed increase in gene expressions with functions associated with cell motility, environmental information processing, and metabolic processes linked to osmoprotection and halotolerance. Such enrichment of genes suggests that microbial in pneumatophore matrices are well-equipped to respond to environmental stress and dynamic conditions that happened to pneumatophores. These findings provide valuable insights into the ecological significance of epiphytic microbes and their specialized adaptations to pneumatophores. This study forms the basis for future research into their functional roles in mangrove ecosystems.

The above findings led to the generation of 10 publications, including 4 SCI journal papers (with 2 published and 2 under review) and 6 conference posters / abstracts.

6.2 Potential for further development of the research and the proposed course of action *(Maximum half a page)*

Building on the results obtained in this study, two possible research directions could be further developed.

Molecular interactions between mangrove plants and their microbial communities, and their potential impact on restoration of mangroves: the mutual benefits between microbes and mangroves could be studied in greater detail to uncover specific mechanisms of nutrient and metabolites exchange. Questions such as how microbes provide various nutrients to the plant and how the plant provide essential metabolites or creating favorable conditions for maintaining the life and growth of the microbes remained to be resolved.

Development of microbial biomarkers: The identification of microbial biomarkers could be used to assess the status of mangrove restoration by monitoring the recolonization and activity of beneficial microbes on pneumatophores. These biomarkers could serve as early indications, helping researchers and mangrove professionals to detect ecosystem imbalances before they become severe. This approach would deepen the ecological insights gained from this research while offering practical tools for ecosystem management and restoration.

7. Layman's Summary

(Describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

This research investigates the microbes living on pneumatophores (a specialized root structures of mangrove trees that stick out from ground soil). We examined how the abundance and composition of these microbes change under various environmental conditions and the characteristics of the pneumatophores. This study highlights that pneumatophores create unique habitats for microbes, distinct from the surrounding soil, and that the mangrove tree actively influences the formation and diversity of these microbial communities. Additionally, we uncover how these microbes adapt to harsh conditions and their possible functional roles, such as nutrient cycling and sulfur metabolism. This study provides a solid foundation for future research and offers potential applications in the management, conservation, and restoration of mangrove ecosystem.

Part C: Research Output

8. Peer-Reviewed Journal Publication(s) Arising Directly From This Research Project

(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				**Author(s)** *(denote the corresponding author with an asterisk*)*	**Title and Journal / Book** *(with the volume, pages and other necessary publishing details specified)*	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
Year of Publication	**Year of Acceptance** *(For paper accepted but not yet published)*	**Under Review**	**Under Preparation** *(optional)*						
2024				Varsha Bohra, Nora Fung-Yee Tam, Luzhen Chen, Kaze King-Yip Lai, Winnie Lam, Steven Jing-Liang Xu, Hai-Chao Zhou, Tao Lang, Chak-Lam Lee, **Fred Wang-Fat Lee***	**Untangling structural and functional diversity of prokaryotic microbial assemblage on mangrove pneumatophores** **Journal of Marine Science and Engineering, 12(5) 802**	**No**	**Yes (Annex 1)**	**Yes**	**Yes**
2025				Varsha Bohra, , Kaze King-Yip Lai, Kit-Ling Lam, Nora Fung-Yee Tam, Steven Jing-Liang Xu, **Fred Wang-Fat Lee***	**Metagenomic surveillance reveals different structure and function of microbial community associated with mangrove pneumatophores and their surrounding matrices** **Marine Pollution Bulletin 213, 117614**	**No**	**Yes (Annex 2)**	**Yes**	**Yes**
		Yes		Kaze King-Yip Lai, Fenglan Li, Nora Fung-Yee Tam,	**Substrate and position variations of microalgal community on pneumatophores of three**	**No**	**Yes (Manuscript is attached in Annex 3)**	**Yes**	**Yes (after published)**

				Luzhen Chen, Steven Jing-Liang Xu, Hai-Chao Zhou, Kit-Ling Lam, Emily Sze-Wan Wong, **Fred Wang-Fat Lee***	**mangrove species** **Submitted to Pedosphere**				
		Yes		Varsha Bohra, Nora Fung-Yee Tam, Luzhen Chen, Kaze King-Yip Lai, Steven Jing-Liang Xu, Hai-Chao Zhou, , Emily Sze-Wan Wong, Ping-Lung Chan, **Fred Wang-Fat Lee***	**Microbial life on pneumatophores: Insights into its adaptation and functional roles in mangrove ecosystems through metagenomics** **Submitted to Environmental Research**	**No**	**Yes (Manuscript is attached in Annex 4)**	**Yes**	**Yes (after published)**

9. Recognized International Conference(s) In Which Paper(s) Related To This Research Project Was / Were Delivered

(Please attach a copy of each conference abstract)

Month / Year / Place	**Title**	**Conference Name**	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
April/ 2023 Hong Kong, China	Microbial communities in mangrove ecosystem differs by intertidal location and microhabitat of pneumatophores	International Conference on Conservation and Sustainable Development of Coastal Wetland	No	Yes (Annex 5)	Yes	Yes
April/ 2023 Hong Kong, China	Establishment of Sample Preparation Workflow for Metataxonomic Analysis of Epiphytic Bacteria on Pneumatophores	International Conference on Conservation and Sustainable Development of Coastal Wetland	No	Yes (Annex 6)	Yes	Yes

November/ 2023 Hiroshima, Japan	Microalgal species isolation and the establishment of monoclonal culture from samples collected from mangrove water and pneumatophores	20th International conference on harmful algae 2023 Hiroshima Japan HAB science and Human well-being	No	Yes (Annex 7)	Yes	Yes
November/ 2023 Hiroshima, Japan	Functions of epiphytic microbiota on pneumatophores of Avicennia marina and possible host-microbe interactions through preliminary proteomic analysis	20th International conference on harmful algae 2023 Hiroshima Japan HAB science and Human well-being	No	Yes (Annex 8)	Yes	Yes
January/2024 Hong Kong, China	Metagenomics surveillance reveals different structure and function of prokaryotic microbial community associated with mangrove pneumatophores	10th International conference on marine pollution and ecotoxicology	No	Yes (Annex 9)	Yes	Yes
January/2024 Hong Kong, China	Exploring factors shaping the microalgal community structure on pneumatophores	10th International conference on marine pollution and ecotoxicology	No	Yes (Annex 10)	Yes	Yes

10. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning

(Please elaborate)

As a case study for undergraduates:

Relevant techniques and results of the study, such as metagenomic and proteomic analysis of the roots and microbiome, were adopted as case study to share with 120-150 year 3 students in the course named “Biochemical & DNA Technologies”.

STEM workshop:

We organized a STEM workshop for secondary school students and teachers. It was a half-day workshop (13:00 – 18:00) organized on 6 May 2024. Around 9 students and teachers attended the workshop. The participants were trained with both theory and practical skills in mangrove research, such as the determination of the abundance and distribution of mangrove plant species and associated sediment organisms [See Annex 11]

11. Student(s) Trained

(Please attach a copy of the title page of the thesis)

Name	Degree Registered for	Date of Registration	Date of Thesis Submission / Graduation
	Ph.D	4 September 2023	31 August 2026^

^ The student is expected to submit the PhD thesis on or before 31 August 2026. The title of the project is "Identification and Characterization of Epiphytic Microorganism from Pneumatophore of *Avicennia Marina*".

12. Other Impact

(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, teaching enhancement, etc.)

This project has strengthened the collaboration of our research team with two institutions in the Greater Bay Area, including Greater Bay Area Coastal Mangrove Wetland Research and Development Centre, Guangdong Neilingding Futian National Nature Reserve and the College of Life Sciences and Oceanography, Shenzhen University.

13. Statistics on Research Outputs

	Peer-reviewed Journal Publications	Conference Papers	Scholarly Books, Monographs and Chapters	Patents Awarded	Other Research Outputs (please specify)	
					Type	No.
No. of outputs arising directly from this research project	4 [2 published + 2 under review]	6	N/A	N/A	STEM training workshop Training of PhD student	1 1

14. Public Access Of Completion Report

(Please specify the information, if any, that cannot be provided for public access and give the reasons.)

Information that Cannot Be Provided for Public Access	Reasons
N/A	N/A