学号：D2018065

分类号 X5 密级 公开

U D C 502

河 海 大 學

博 士 学 位 论 文

Characterization and comparison of microbial communities in biofilms on different substrates in tropic lakes and hot springs of Rwanda.

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申请学位级别 工学博士 专业名称 环境科学与工程

论文提交日期 2022 年 04 月 11 日 论文答辩日期 2022 年 10 月 14 日

学位授予单位 河海大学 学位授予日期 年 月 日

答辩委员会主席：倪利晓 教授 论文评阅人: 宋海亮 教授 、王圣森 教授 、 谭啸 教授、 苗令占 教授

2022 年 10 月 中 国 南 京

分类号（中图法） X5 U D C 502　密级 公开

论文作者姓名 Benjamin MANIRAKIZA 学号 D2018065 单位 河海大学

论文中文题名:卢旺达热带湖泊和温泉中不同介质表面生物膜微生物群落结构特征及对比研究

论文中文副题名 无

论文英文题名 Characterization and comparison of microbial communities in biofilms on different substrates in tropic lakes and hot springs of Rwanda

论文英文副题名 无

论文语种： 英 语 论文摘要语种： 英，汉语 论文页数 228 论文字数 59127

论文主题词 真核微生物群落，温泉，生物膜，表层沉积物、组装过程

申请学位级别 工学博士 专业名称 环境科学与工程

研 究 方 向 水生态修复

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论文答辩日期 2022 年 10 月 14 日

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Dissertation submitted to

Hohai University

In fulfillment of the requirement

For the Degree of

Doctor of Philosophy in Environmental Science and Engineering

by

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(College of Environment)

Dissertation Supervisor: Professor Zhang Songhe

October 2022, Nanjing P.R China

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Preface

Microorganisms do not exist as planktonic cells but in the form of microbial aggregates on surfaces (e.g., macrophytes, sand/sediments, rocks, and animals) such as films, mats, mucus, and accumulations of biomass in the form of sludges or flocs in suspension. They are complex and comprise bacteria, protozoa, fungi, and other microorganisms enclosed in a self-produced hydrated extracellular polymer substance (EPS) matrix. The ecological functions of biofilms are closely linked to water purification, nutrient uptake and nutrient cycling, pollutant removal, trophic interactions, and microbial gene pool preservation in freshwater ecosystems. However, the microhabitats and their biofilms are susceptible to pollution and climate change, thus creating new challenges to aquatic health and microbial gene pool preservation.

This research was conducted to provide updated knowledge on the influence of microhabitat type and environmental variables on biodiversity, composition, assembly mechanisms, interactions, and ecological functions of biofilm-dwelling microbial communities in natural tropical lakes and hot springs. Written in the form of a thesis, this article encompasses the comprehensive and current research knowledge on the influence of microhabitat type and environmental variables on epiphytic, epipelic, and mat biofilms using high-throughput sequencing of the 16S and 18S rRNA genes. An attempt was made to ensure that the results and findings of this thesis were properly described in clear language that experts and non-experts could easily understand.

This research thoroughly investigated the influence of various microhabitats (aquatic macrophytes, surface sediments, and hot spring mats) and environmental variables on microbial communities in biofilms in shallow tropical lakes and hot springs of Rwanda. The main topics were provided in different chapters to give a clear data presentation and sufficient discussion of the results. Chapter 1 provides the theoretical background and study objectives; chapter 2 explores the microbial diversity and ecological function of epiphytic and surface sediment biofilm communities in a shallow tropical lake. Chapter 3 described the temporal dynamics of microbial structures, co-occurrence patterns, and assembly mechanisms in epiphytic and surface sediment biofilm communities in a shallow tropical lake, while chapter 4 investigated the microbial community diversity, composition, assembly processes, interactions, and ecological function in

hot spring mats. The findings were then summarized in the 5th chapter. Most of the results are provided in figures and tables embedded in the body of the thesis.

Novelty and identification of knowledge gaps

1. Microhabitat types drive the microbial composition in epiphytic biofilms: The main innovation of this study is that it proved that microhabitat types (submerged plants, floating plants, and surface sediments) were the dominant drivers of microbial community composition in biofilms. Prior studies focused on phytoplankton and epiphytic bacteria of submerged plants in constructed wetlands, rivers, and lakes. However, there is insufficient knowledge about the influence of floating macrophytes and surface sediments on microeukaryotic community structure in epiphytic and surface sediment biofilms in shallow tropical lakes.

2. Microbial assembly processes in periphytic biofilms: The microbial assembly processes have been researched widely in freshwater, groundwater, hot spring sediments, soils, marine environments, and wastewater treatment settings. However, little is known about the relative importance of stochastic and deterministic assembly processes in periphytic biofilms. Thus, this research constitutes the first attempt to investigate the relative importance of stochastic and deterministic assembly processes in epiphytic microeukaryotes in epiphytic biofilms, superficial sediments, and hot spring mats. This study indicates the null and neutral models revealed that stochastic processes dominated the microbial community assembly in epiphytic and surface sediment biofilms in shallow tropical lakes and moderate-temperature springs. However, in high-temperature springs, the microbial community was potentially shaped by temperature. Notwithstanding the dominance of stochastic processes in periphytic biofilms, the deterministic processes still represented a significant part of the assembly processes.

3. Ecological function of periphytic biofilms: Few studies explored the ecological function of bacteria in submerged plants, water system, seas, and oceans. However, bacterial metabolic function in epiphytic biofilm of aquatic and mats in tropical lakes and extreme environment have mostly been overlooked. This research constitutes the first attempt to investigate the ecological function of bacterial communities in floating plants, superficial sediments, and mats of shallow tropical lakes and hot springs. This study showed that microhabitat types significantly shaped the ecological role of biofilms in tropical lakes and hot springs. The findings of this study provide

new insights in the understanding of the influence of microhabitat/substrate types and environmental variables on biodiversity, composition assembly processes, interactions, and ecological functions of biofilm-dwelling microbial communities in tropical lacustrine and geothermal ecosystems, which holds good for the ecological restoration projects, water purification, and hot spring sustainability.

Abstract

Biofilms are complex and dynamic structures, mainly composed of viable cells (bacteria, archaea, algae, fungi, protozoa, and other metazoans) embedded in a self-produced hydrated extracellular polymer substance (EPS) matrix that binds them together and adhere to the solid surface or liquid-solid interface of various substrates. Based on the attachment surface, biofilms in aquatic ecosystems can be categorized as, for example, epipelon/ epipsammon (sediment/sand), mats (rock), or epiphytic (plant surface) biofilms. The submerged and floating macrophyte leaves provide unique niches and nutrients for microbial growth, forming plant-biofilm symbiotic systems that represent the basic components of aquatic ecosystems and play important roles in transforming pollutants and maintaining ecological balance. The ecological functions of biofilms are closely linked to nutrient chelating, (re) cycling, and the detoxification of environmental pollutants by biofilm-dwelling communities. Previous studies explored the planktonic microbial diversity and composition in the water column and rarely in epiphytic biofilms. However, the influence of environmental variables (e.g., seasons and water chemistry) and microhabitat types (e.g., submerged and floating macrophytes, surface sediments, and mats) on the microbial biodiversity, assemblages, interactions, and ecological functions are poorly understood, especially in the tropical Lakes and hot springs. In this study, epiphytic bacterial and eukaryotic biofilm communities in submerged and floating macrophytes and surface sediments were investigated in two tropical Lakes (Rumira and Cyohoha North), Rwanda in August and November 2019, and mat samples from two hot spring regions (Bugarama hot pool and Gisenyi hot springs); the water quality and environmental parameters were also determined, and the differences in microbial communities were compared under these environments. The main findings are as follows:

1) The exploration of microbial composition in Rumira Lake revealed that eight phyla, including Firmicutes, Proteobacteria, Cyanobacteria, Actinobacteria, Chloroflexi, Bacteriodetes, Verrumicrobia, and Myxomycota, dominated bacterial communities, while the microeukaryotic communities were dominated by Unclassified (uncl) SAR (Stramenopiles, Alveolata, Rhizaria), Rotifers, Ascomycota, Gastrotricha, Platyhelminthes, Chloroplastida, and Arthropoda. Interestingly, the eukaryotic OTUs (operational taxonomic units) number and Shannon indices were significantly higher in sediments and epiphytic biofilms on *Eichhornia crassipes* than *Ceratophyllum demersum* ($P < 0.05$), while no differences were observed in bacterial OTUs number and Shannon values among substrates. Redundancy analysis (RDA) shows that water temperature, pH, dissolved oxygen (DO), total nitrogen (TN), and electrical conductivity (EC) were the most important abiotic factors closely related to the microbial community on *C. demersum* and *E. crassipes*. Furthermore, co-occurrence networks analysis ($|r|>0.7$, $P < 0.05$) and functional prediction revealed more complex interactions among microbes on *C. demersum* than on *E. crassipes* and sediments, and those interactions include cross-feeding, parasitism, symbiosis, and predatism among organisms in biofilms.

2) The influence of *Ceratophyllum demersum*, *Eichhornia crassipes*, and surface sediments on the microbial structures, co-occurrence patterns, and community assembly mechanisms of epiphytic bacterial and eukaryotic biofilm communities in Lake Cyohoha North was investigated. Illumina sequencing method reveals that phylum Cyanobacteria, Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Chloroflexi dominated bacterial communities microeukaryotic communities were dominated by Rotifera, SAR (Stramenopiles, Alveolata, and Rhizaria), Platyhelminthes, Chloroplastida, Phragmoplastophyta, and Ascomycota. There were no significant differences in microbial alpha diversity indices (except OTU richness in microeukaryotes) among

substrates across seasons. Interestingly, the bacterial community dissimilarity was significantly different among substrates ($P < 0.05$). The null model analysis shows that stochastic processes dominated the microbial community assembly in epiphytic (except for bacteria on *E. crassipes*) and surface sediment biofilms. Notwithstanding the dominance of stochastic processes in microbial community assembly, the deterministic processes still represented a significant part of the assembly processes. Thus, the stochastic and deterministic processes drove microbial community assembly on aquatic plants and sediments. Moreover, co-occurrence network analysis revealed stable and complex food chains in surface sediments compared to other substrates.

3) The study on microbial assemblage and interaction has been conducted in hot springs Bugarama (40.2-47.3℃) and Gisenyi (58-71.4 ℃). The results indicate that bacterial community β-diversity in moderate temperature was strongly driven by stochastic processes, whereas in high-temperature springs, the variable selection in microeukaryotic and bacterial communities was potentially shaped by temperature. For example, some Amoebozoa (e.g., *Echinamoeba,* BOLA868, and *Telaepolella*) and SAR taxa have adapted to high-temperature in hot spring microbial mats. RDA revealed that microeukaryotic communities were strongly driven by temperature in high-temperature microbial mats. Furthermore, co-occurrence networks and functional prediction showed more stable interactions among microbes in high-temperature mats, including cross-feeding, symbiosis, parasitism, and predation. Even though microeukaryotic communities are often overlooked in hot spring mats and other extreme environments, our findings shed new insights into that microeukaryotic communities harbor complex interactions and adaptations to high-temperature in tropical hot spring microbial mats, which may provide a basis to understand better microbial communities inhabiting extreme environments.

4）Lastly, we compared of influence of environmental parameters on microbial interactions and predicted bacterial functions on microbial communities in epiphytic, epipelic, and mat biofilms in shallow lakes and hot springs. Bacterial and microeukaryotic communities on *C. demersum* and *E. crassipes* were positively associated with DO, pH, EC, TN, TP, and temperature in tropical lakes, whereas bacterial communities in *C. demersum* and mats were commonly positively related to temperature, TDS, pH, and EC. Co-occurrence networks of possible interactions among microbial genera in aquatic macrophytes, surface sediment and mat biofilms were different. However, there were similarities of top microbial phyla in networks among lakes and hot springs. Specifically, all microbial networks shared phylum Ascomycota and SAR super group, whereas GHS mat network displayed the higher microeukaryotic taxa and Cyanobacteria. Furthermore, this study suggests that all microbial networks share organic matter decomposition, predation and parasitism relationships, and primary production. The predicted metabolic functions (FAPROTAX) of the bacterial communities to all substrates were related to carbon and nitrogen cycling and xenobiotic degradation. Phototrophic functions were significantly dominant in epiphytic and mat bacterial communities, whereas methanogenesis dominated the sediment bacteria. The higher bacterial functional abundance of sediment and *C. demersum* bacteria was detected in the wet season, while high-temperature mats exhibited the high bacterial functional abundance

The findings provide new insights or cues to understand better the influence of microhabitat/substratum type and environmental variables on bacterial and microeukaryotic biodiversity, interactions, assemblages, and ecological functions in epiphytic, surface sediment, and mat biofilms from tropical lakes and hot springs. Furthermore, this study not only attempts to fill the knowledge gaps but also acts as a basis for future studies – with a particular emphasis on

including epiphytic, sediment, and mat biofilm to understand, maintain, and improve aquatic ecosystem health and integrity.

Keywords: Hot spring, Epiphytic biofilms, Surface sediments, Assembly processes, Lake Rumira, Lake Cyohoha.

摘要

生物膜是由活细胞（细菌，古菌，藻类，真菌，原生动物和其他后生动物）组成的复杂和动态的结构，这些微生物包裹在生产的细胞外聚合物物质（EPS）基质中，通常形成于各种固体介质-液体界面上。水生生态系统中，根据其附着的基质类型将生物膜分为不同类型，比如固着/表层生物膜（在沉积物/沙子表面）、生物甸（在岩石）或附生（植物表面）生物膜。沉水植物和漂浮植物叶片为微生物生长提供了独特的生态位和养分，形成了植物-生物膜共生系统，代表了水生生态系统的基本组成部分，在转化污染物和维持生态平衡方面也起着重要作用。在膜内微生物的作用下，生物膜的生态功能与环境内污染物的养分螯合和（再）循环和解毒密切相关。 以前的研究分析了水体内浮游微生物及及少量附着生物膜内微生物的多样性和组成。然而，关于环境因子（如季节和水体理化因子）和基质类型（如浮叶和沉水植物、表层沉积物和生物甸）对微生物的多样性、组装过程、互作机制和生态功能了解甚少，尤其在热带湖泊和温泉水体中。本研究，以不同环境内生物膜为对象，2019 年 8 月和 11 月分析了卢旺达内两座热带湖泊(Rumira and Cyohoha North)内沉水植物和浮叶植物及表层沉积物、以及两座温泉(Bugarama hot pool and Gisenyi hot springs)内底层生物甸生物膜内细菌和真核微生物的群落结构，水质及水环境参数；并对比了生物膜间的差异及对外界环境的响应。主要结果如下：

1) 在 Rumira 湖中，通过高通量测序共鉴定出 8 个细菌优势门，包括厚壁菌门、变形菌门、蓝藻门、放线菌、绿弯曲菌、拟杆菌、疣微菌和粘菌门；而微真核生物群落以未分类 SAR（Stramenopiles、Alveolata、Rhizaria）、轮虫、子囊菌、胃毛、扁形动物、叶绿体和节肢动物门为主。有趣的是，*Eicchornia crassipes* 的沉积物和附生生物膜中的真核 OTU（操作分类单位）数量和香农指数显着高于 *Ceratophyllum demersum*（$p < 0.05$），而底物之间的细菌 OTU 数量和香农值没有观察到差异。冗余分析 (RDA) 表明，水温、pH、溶解氧 (DO) 、总氮 (TN) 和电导率 (EC) 与 *C. demersum* 和 *E. crassipes* 微生物群落密切相关。 此外，共现网络分析 ($|r|>0.7, p<0.05$) 和功能预测揭示了 *C. demersum* 微生物之间的相互作用比 E. *crassipes* 和沉积物更复杂，这些相互作用包括生物膜中生物之间的交叉捕食、寄生和共生等关系。

2）对旱季和湿季 Cyohoha North 湖内 *Ceratophylum demersum* 和 *Eichhornia crassipes* 附着生物膜和表层沉积物内微生物群落结构、共生模式和群落组装机制的影响进行了研究，结果发现：不同介质内蓝细菌门、厚壁菌门、变形菌门、拟杆菌门、放线菌门和绿曲菌门以细菌群落为主，而微真核生物群落以轮虫门、SAR（Stramenopiles、Alveolata 和 Rhizaria）、扁形虫、叶绿体、Phragmoplastophyta 和子囊菌门为主。底物之间和季节之间的微生物 α 多样性指数（微真核生物中的 OTU 丰富度除外）没有显着差异。三种基质之间的细菌群落差异显着不同（$p<0.05$）。零模型分析表明，随机过程主导了附生植物（*E. crassipes* 上的细菌除外）和表面沉积物生物膜中的微生物群落组装，而确定性过程对群落组装过程也起重要作用。因此，随机和确定性过程在驱动水生植物和沉积物上的微生物群落组装时相结合。此外，与其他底物相比，共现网络分析揭示了表面沉积物中稳定而复杂的食物链。

3)调研了卢旺达两座温泉 Bugarama (40.2-47.3℃) 和 Gisenyi (58-71.4 ℃)内岩石上微生物甸型生物膜的组装和互作机制。结果表明，中等温度下的细菌群落 β 多样性受到随机过程的强烈驱动，而在高温泉水中，微真核生物和细菌群落的变量选择可能受温度影响。例如，一些变形虫（例如，*Echinamoeba*、*BOLA868* 和 *Telaepolella*）和 SAR 类群已经适应了温泉微生物垫中的高温。 RDA 揭示了微真核生物群落受高温微生物垫中温度的强烈驱动。此外，共现网络和功能预测表明高温垫中微生物之间的相互作用更加稳定，这些相互作用包括交叉喂养、共生、寄生和捕食。尽管微真核生物群落在温泉垫和其他极端环境中经常被忽视，但我们的研究结果揭示了微真核生物群落在热带温泉微生物垫中具有复杂的相互作用和对高温的适应能力，这可能为更好地了解栖息在极端环境中中微生物群落提供基础。

4）最后，我们比较了两座湖泊和两处温泉内环境参数对生物膜微生物群落内微生物相互作用和预测细菌功能的影响。发现 *C. demersum* 和 *E. crassipes* 上的细菌和微型真核微生物群落与热带湖泊中的 DO，pH，EC，TN，TP 和温度呈正相关，而 *C. demersum* 表面附着生物膜和温泉微生物甸型生物膜中的细菌群落通常与温度，TDS，pH 和 EC 呈正相关。共现性网络分析表明，水生植物表面、表层沉积物和微生物甸型生物膜中微生物属之间可能存在不同的相互作用机制。

综上所述，这些发现为更好地了解微生境/底层类型和环境变量对热带湖泊和温泉附生，表层沉积物和垫状生物膜中的细菌和真核微生物的生物多样性，相互作用，组装机制和生态功能提供了新的见解或线索。此外，这项研究不仅试图填补知识空白，而且还作为设计未来研究的基础 – 尤其包括附生植物，沉积物和垫子生物膜的了解，进而为维护和改善水生生态系统的健康和完整性服务。

关键词：温泉，附生生物膜，表面沉积物，组装过程， Rumira 湖，Cyohoha 湖。

Abbreviations

EPS: Extracellular Polymeric Substances

TN: Total nitrogen

HGT: Horizontal gene transfer

eDNA: Extracellular Deoxyribonucleic Acid

QS: Quorum sensing

AIPs: Autoinducer peptides

AHLs: N-acyl-homoserine lactones.

SAR: Stramenopiles, Alveolates, and Rhizaria

YNP: Yellowstone National Park

OTUs: Operational Taxonomic Units

PCR: Polymerase Chain Reaction

PICRUSt: Phylogenetic Investigation of Communities by Reconstruction of Unobserved States

RDA: Redundancy Analysis

CANOCO: Canonical Correspondence Analysis

NMDS: Non-metric Multidimensional Scaling

STAMP: Statistical Analysis of Metagenomic Profiles

PERMANOVA: Permutational Multivariate Analysis of Variance

WHO: World Health Organization

BCL: Biotechnology Complex Laboratory

PCoA: Principal Coordinate Analysis

βNTI: Beta Nearest Taxon Index

Table of contents

List of figures

List of Tables

Chapter 1. Introduction

1.1 Background

Tropical lakes worldwide are vital ecosystems, providing ecology, regional hydrology and irrigation, fishery, and water sources for the local population. Natural rivers or reservoirs account for more than 1/2 of all tropical lakes, and degradation of water quality in rivers will have direct negative effects on the majority of lakes in the tropics. Tropical lakes are more sensitive than temperate lakes to increases in nutrient supply and show higher proportionate changes in water quality and biotic communities in response to eutrophication[1]. They have been drastically altered due to increased population density, economic growth, and changes in land cover. To ensure the sustainable use of tropical lakes, its water quality must be protected. Lake surficial sediment is the upper-most layer of subaquatic sediment (usually 1 cm or less). Sediment is a critical component of lake ecosystems and can act as both a repository/sink of processes occurring in the pelagic environment and a source of pollution (heavy metals, nutrients, and pathogens) [2].

Hot springs provide thermal waters for health (therapeutic effects), recreation or tourism, and balneology and comprise the unique hot spots for thermophilic microbial communities (bacteria, archaea, virus, and eukaryotes)[3,4]. Bathing in hot springs relieves arthritis, muscular stress and strains, joint pains, neuralgia, myalgia, osteosis, cardiac diseases, bowel-associated diseases, and inflammatory skin diseases[5]. The chemical concentrations in thermal waters are associated with their therapeutic effects[6]. In addition, the bioproducts of photosynthetic microbes against oxidative stress comprise an additional value for the balneotherapy industry. Even though hot springs are devoid of pathogenic microbes, many patients suffering from various diseases bathe in them regularly. Previous works reported disease outbreaks from hot springs in Taiwan and Mexico[7]. Most serious outbreaks were caused by fungi (*Microsporidia* sp.), free-living amoeba (*Acanthamoeba* sp. and *Naegleria sp*.), and bacteria (*Listeria sp*., *Legionella sp*., and *Leptospira sp*.).

Macrophytes are macroscopic autotrophs growing as submerged, emergent, and floating forms in aquatic ecosystems. Aquatic macrophytes are known as ‘ecosystem engineers’ due to their ability to shape aquatic freshwater ecosystems' physical properties and functions, including hydraulic change by resisting water flow, aiding in sediment particle settlement, and influence

light availability by shading and maintaining clear water status[8]. They regulate water chemistry or improve water quality by absorbing nutrients, heavy metals, and other inorganic or organic contaminants from the water column, wastewater treatment wetlands, and sediments [9,10]. However, due to improper anthropogenic activities, aquatic macrophytes have declined significantly, and biodiversity in freshwater has decreased[11]. Thus, the deterioration of aquatic plants across the globe is a serious issue that needs attention. The aquatic macrophytes and biofilms represent the basic components of aquatic ecosystems. The submerged and floating macrophyte leaves are ideal substrates or hosts for microbial growth (e.g., biofilms), forming plant-biofilm platforms that display unique, complex, and interdependent biological interactions[12]. For example, strong competitive, mutualistic, and commensalistic relationships between epiphytic biofilm and macrophytes have resulted from interactions for resources (e.g., light and nutrients) and trophic and allelopathic dynamics.

Biofilms are complex aggregates and dynamic structures, mainly composed of viable cells (bacteria, archaea, algae, fungi, protozoa, and other metazoans) embedded in a self-produced hydrated extracellular polymer substance (EPS) matrix[13] and growing on a solid surface and exist in the form of mucus, mats, films, and flocs in suspension. Biofilms can develop at the interface of various phases (liquid-solid, liquid-gas, and solid-gas phases). Based on the attachment surface, biofilms in aquatic ecosystems can be categorized as, for example, epizoic (animal), epipelon/ epipsammon (sediment/sand), mats (rock/stone), or epiphytic (plant surface) biofilms. Epiphytic biofilms are key components in shallow freshwater ecosystems and play multiple roles, including maintaining ecosystem structure (community composition and diversity), primary production and respiration, nutrient uptake and cycling, decomposition, pollutant removal and microbial gene pool preservation[8]. Therefore, epiphytic biofilms significantly influence plant health, growth, and the biogeochemical cycling of lake elements. Contrary, climate warming may influence the relationship among macrophyte–periphyton–phytoplankton and change the producer community structure in shallow lakes, as the elevated temperature has been suggested to stimulate the dominance of phytoplankton[14].

Bacterial and microeukaryotic communities in surface sediment biofilm (epipelic) play significant roles in biogeochemical cycling (e.g., carbon, nitrogen, phosphorus, and sulfur), energy transport, and feeding relationships (predation, competition parasitism, symbiosis, commensalism

and amansalism)[15,16]. For example, microorganisms in surface sediments have renowned importance in the carbon cycle through methanogenesis (methanogens), organic matter decomposition, and sulfate reduction[17]. Previous studies have indicated that microbial communities in marine sediments were closely related to surface waters[18]. Compant et al. [19] reported a significant connection between water, aquatic macrophytes, and sediments, which may facilitate the sediment bacteria migration to the roots, then stem and leaf surfaces.

Recent works showed that environmental parameters, especially sediment characteristics (sediment water depth, chlorophyll content, median grain size, silt-clay percentage, organic matter content, and pheophorbide content), strongly shaped microbial community structure and symbiotic networks[20]. Contrary to their positive functions, sediment bacterial communities are prone to various natural and anthropogenic stressors, such as seasonal changes, rapid urbanization and land use changes[21,22]. Sun et al. [23] and Saxena et al. [21] indicated that the nitrogen, phosphorus, heavy metals, and organic pollutant from industrial effluent, domestic sewage, and agricultural runoff considerably shaped microbial community structure in sediments. For example, after a long period of precipitation and accumulation in the sediment, heavy metals can be released from sediments by resuspension and bioturbation, leading to secondary pollution[24]. Hence, exploring the key factors influencing microbial community compositions in lake sediments may provide insights into the biogeochemical functions in the lacustrine ecosystem[25].

Microbial mats are horizontally layered benthic microbial communities comprising milliards of microorganisms (bacteria, archaea, fungi, protists, viruses, and a few metazoans) enclosed in EPS, developing in the liquid-solid interface of various environments (e.g., hot springs and hypersaline waters) and exhibit a structure defined by gradients, which models microbial diversity, physiological activities, and their dynamics as a whole system[26]. Hot spring mats are the first natural ecosystems together with stromatolites capable of producing gases (CH_4, CO_2, and H_2) for biofuels[27], thermostable enzymes used in paper, detergent, leather and shoe processing[28], antimicrobial compounds (tetramic compound and ophiosetin), biosurfactants, and quorum sensing inhibitors[29]. Thus, microbial mats present enormous eco-friendly (renewable energy) and sustainable biotechnological applications in medicine, industries, bioremediation, and agriculture [26,28]. Furthermore, they are unquestionably a natural laboratory where microbial diversity, evolutionary processes, adaptation to extreme environments, and climate change can be explored.

Previous field studies have focused on the diversity, composition, and interaction of bacteria and algae in biofilms or planktonic cells on different substrates, including surface sediments[30], green alga[31], submerged macrophytes[32], floating macrophytes[33], and emergent plants[34]. Epiphytic biofilms on live macrophytes are different and unique in structure and function compared to the other periphytic biofilms in inert freshwater habitats[35]. Epiphytic biofilms are understudied compared to other periphytic biofilms in freshwater ecosystems. This is surprising as epiphytic biofilm-macrophyte-specific interactions interfere with important ecosystem processes and these interactions are highly complex. Microbial communities in epiphytic and surface sediments biofilms and mats play a vital role in the biogeochemical cycles of the major chemical elements in tropical lakes and hot springs. The microbial community diversity, composition, and co-occurrence networks might not be enough to disentangle microbial community impact on ecosystem functions. Few reports indicate submerged macrophytes' influence on bacterial metabolic functions[36,37]. However, little is known about the effect of various aquatic plant species, seasons, and hot springs on the ecological functions of epiphytic and surface sediment biofilms and mats. Therefore, it is of utmost importance to explore the effect of various microhabitats on the functional profile of periphytic bacterial biofilms.

Unquestionably, environmental variables can, directly and indirectly, affect microbial community diversity, composition, interaction, and function in different microhabitats (e.g., aquatic plants and sediments)[38]. For example, bacterial diversity and composition in epiphytic biofilms and sediments were influenced differently by seasons in marine environments [39,40]. Some studies suggest that epiphytic biofilm is less sensitive to environmental variables and more dependent on the interactions between macrophytes and biofilm[41]. Community assembly is a key topic in ecology[42]. This topic has been explored extensively in microbial ecology across various habitats, such as soils[43], freshwater[44], hot spring water and sediments[45], and membrane bioreactors (MBR)[46]. However, the microbial communities in aquatic ecosystems have largely been studied from a deterministic perspective, especially in terms of abiotic factors.

Additionally, only three studies explored the role of stochastic processes in epiphytic bacteria. For example, He at al. [47] reported that deterministic and stochastic processes synergistically influenced the assembly processes in epiphytic bacteria; however, the deterministic processes dominated only in the growing season (May). Contrary to this study, Shi et al. [48] indicated that

deterministic processes dominated the assembly processes in epiphytic bacteria. These authors attributed this observation to the colonization events, seasons, mature biofilms, and regularly circling water flows. Unfortunately, no study has explored the effect of aquatic macrophytes, surface sediments, and mats on microeukaryotic assembly mechanisms in tropical lakes and hot springs. Consequently, exploring microbial communities on various substrates across seasons are indispensable to clarify the assembly mechanisms and factors influencing them.

Taken together, aquatic macrophyte effects on epiphytic biofilm were mostly conducted in the constructed wetlands (CW). Furthermore, the influence of aquatic macrophytes, surface sediments, and hot springs on periphytic biofilms focused on bacterial communities, and most studies came from Asia (China), America (USA and Canada), Europe (Denmark and Germany), and Oceania (Australia). However, studies on the influences of the above microhabitats and seasonal shifts on microeukaryotic diversity, composition, assembly, interaction and ecological function remain largely unexplored and poorly understood. Moreover, there is a scarcity of periphytic research on the African continent.

To the best of my knowledge, this study is the first of its kind to provide a comprehensive exploration of the influence of microhabitat type (*C. demersum*, *E. crassipes*, surface sediments, and hot springs) and environmental variables (season and water chemistry) on the bacterial and microeukaryotic diversity, composition, assembly processes, interactions, and ecological function in epiphytic and surface sediment biofilm communities and mats.

1.1.1 Biofilms

It is only a few decades ago since microbes growing on or in agar broth, soil, wounds, plant leaves, rocks, sediments, and teeth, turned from a nuisance into a highly active field of research in which biofilms were recognized as the dominant microbial lifestyle on earth[49]. Biofilm life can be found everywhere, even in extreme environments (glaciers, hot vents, ultra-pure water, highly salty solutions, and nuclear power plants). Microorganisms do not exist as pure cultures of planktonic cells but in the form of microbial aggregates on surfaces such as plant leaves, rocks, sediments, soil, and animals. These aggregates may exist in the form of mucus, thick mats, films, and flocs in suspension[50,51]. Biofilms are microbial aggregates mainly comprised of living cells (bacteria, archaea, algae, fungi, protozoa, and other metazoans) encased in a self-produced

hydrated exopolymeric matrix[13] that binds them to the surface[52]. Extracellular polymeric substances (EPS) determine the characteristic features of the microbial aggregates as forms of microbial life[53].

1.1.2 EPS composition and functions

EPS are biopolymers produced by microorganisms where microbial cells are enclosed. Nucleic acids (DNA), polysaccharides, proteins, and lipids constitute most of the biofilm EPS structure. The biofilm matrix differs in various microbial taxa, and the extracellular layer contains polysaccharides (glycocalyx or mucous membrane) such as dextran, hyaluronic acid, cellulose, and others. Notably, this fraction is the most pronounced and accounted for about 40–95%. The concentration of other chemical components varies greatly. Furthermore, the proportion of proteins can be up to 60%, lipids up to 40%, and nucleic acids 1–20%. These compounds are hydrated since 80–H_2O occupies 90% of the biofilm volume. However, in some microbial species, such as *P. aeruginosa*, the exopolysaccharide matrix is predominantly anionic and consists of alginate.

EPS provides mechanical stability and adhesion to substrates and forms a 3D-polymer matrix that links and temporarily immobilizes microbial cells. Besides, the biofilm matrix serves as an exogenous digestive system by keeping extracellular enzymes around the cells, allowing them to digest colloidal, dissolved, and hard biopolymer[50]. Cells in the mucous matrix are not arranged randomly but in a certain way. For example, the structure of multicellular clusters is presented in mushroom-like and pillar-like formations, which makes it possible to delay and maintain the concentration of nutrients necessary for population growth. It also protects microbial cells from dehydration, humoral, and cellular resistance [54].

Moreover, the matrix comprised channels filled with water, cavities, and voids. Pores and channels penetrating the entire biofilm are essential in its structure. Bacteria communities in biofilms form many phenotypes with broad metabolic and replicative properties. Such a microbial community can tremendously resist stress factors[54]. **Table 1.1** provides further details about EPS composition and functions, indicating a wide spectrum of functions for the biofilm lifestyle.

Table 1. 1 EPS composition and functions

EPS components	Examples	Functions
Exopolysaccharides	Homopolysaccharides (sucrose-derived glycan and fructans, and cellulose Heteropolysaccharides (Alginate, Xanthan, Colanic acid, Uronic acids and adhesin)	In many bacteria, exopolysaccharides are indispensable for biofilm formation and provide mechanical stability to mature biofilms.
Extracellular proteins	Enzymes, surface proteins, amyloids, and proteinaceous appendages (pili, fimbriae, and flagella	Involved in adhesion to inanimate surfaces and host cells, degradation of biopolymers and promoting the detachment of bacteria from biofilms, and act as virulence factors.
Surfactants and lipids	Lipopolysaccharides, surfactin, viscosin, emulsan, and rhamnolipids	They may be useful for adherence or attachment, bioremediation of oil spills, initial microcolony formation, and biofilm dispersion [55,56].
Others	Extracellular DNA (eDNA), minerals, and H_20	eDNA is integral to the matrix and biofilm lifestyle[57]. H2O is the largest component of the matrix and buffers the biofilm cells against fluctuations in water potential.

1.1.3 Biofilm formation

Biofilm formation is a multifaceted event involving individual and microbial mixed species. The biofilm development in bacteria is a multi-step mechanical process that starts with the microbial attachment to the substrate with the help of intermolecular forces and hydrophobicity, followed by the production of the EPS, which facilitates the bacteria adhesion and movement to a substrate surface. The process ends with the detachment process of the biofilm.

This process includes three major steps: attachment, colonization and maturation, and active dispersal and propagation [58]. All steps of biofilm formation are presented in **Figure 1.1**.

Attachment: the planktonic cells attach to the surface (moisturized) through physical (e.g., van der Waals) and chemical (e.g., cohesion) forces. This stage is reversible, making it relevant for preventing biofilm growth.

Colonization and maturation: microbial attachment on substratum is irreversibly fortified via hydrophilic/hydrophobic interactions by fimbria, flagella, pili, lipopolysaccharides, exopolysaccharides, and collagen-binding adhesive proteins. Multilayered cells accumulate by proliferation, and microbes secrete EPS, which holds the entire colony together and provides strong adhesion. In addition, the nutrients amass, and cells begin to divide. The stable formation of a mature biofilm (3D-community) comprises channels to effectively distribute nutrients and signaling molecules within the biofilm[59].

Active dispersal (bacteria release) and propagation: as a result of division, microbial cells are periodically detached in clumps or separated, due to interactions with either intrinsic or extrinsic factors, with the disseminated cells forming a new colony in other locations [60].

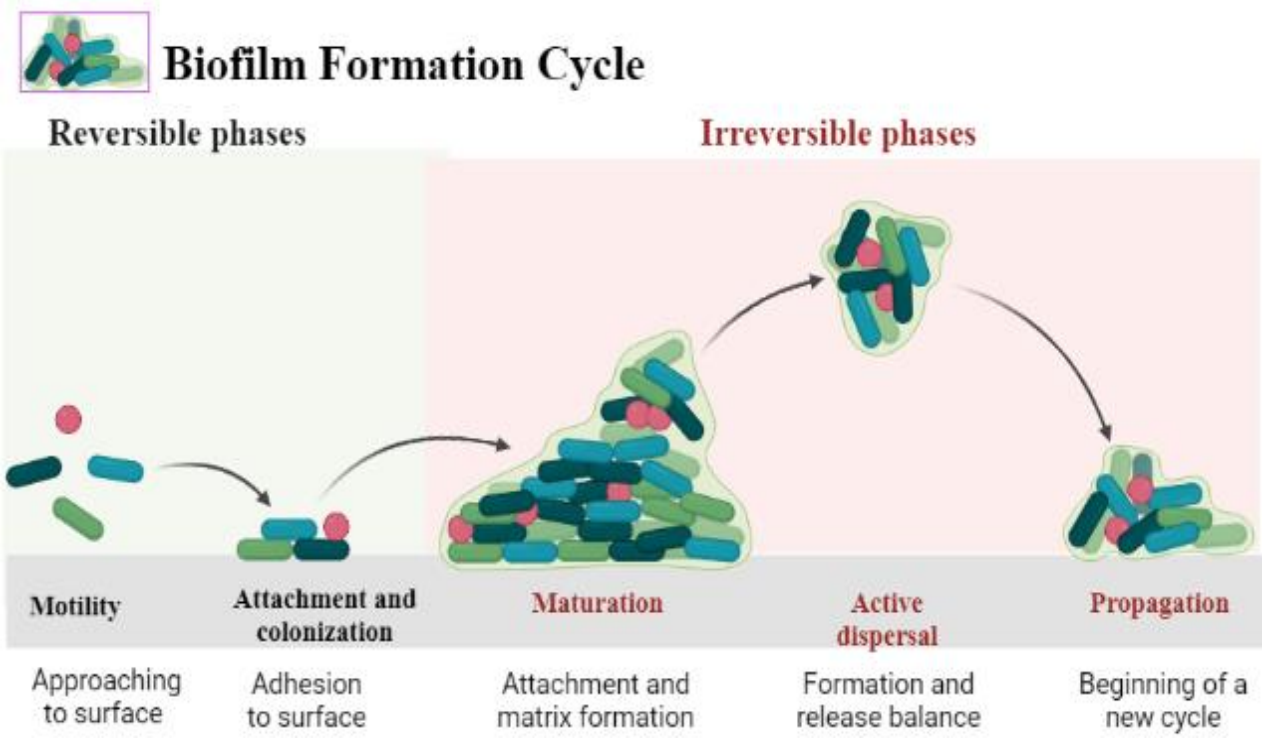


Figure 1. 1 Scheme of biofilm development (created with biorender.com)

1.1.4 Regulatory mechanisms (Quorum sensing)

Quorum sensing is a communication mechanism that links two microbial cells (bacteria) to coordinate their behavior through chemical interactions[61,62]. Auto-inducer (AI) molecules are produced inside the bacterial cell and processed internally or externally in the organism via specific transporters. QS has an important function in coordinating biofilm developmental mechanisms, regulating various genes in various bacterial species[63], maintaining microbial diversity, community parameters, and the performance of bacterial cells under stressful environmental conditions[64,65]. Moreover, QS systems affect ecosystem functioning, environmental restoration and cleaning processes[66]. It is a cell density-dependent phenomenon that is dynamically partaking in biofilm secretion and progression, extracellular polysaccharide secretion, biosurfactant production, horizontal gene transfer (HGT), cellular motility, and specific gene expressions [67]. Currently, QS signaling molecules in bacteria can be divided into three well-defined classes: oligopeptides or autoinducer peptides (AIPs), *N*-acyl-homoserine lactones (AHLs) and the LuxS/autoinducer-2 class[66,68].

AHLs are specifically secreted in Gram-negative (e.g., *Vibrio* sp. and *Pseudomonas* sp.) for cell-cell communication and cell population density-dependent regulatory system. These molecules can pass through the cell membranes[69]. Oligopeptides (AIP) are specific to Gram-positive bacteria (*Bacillus subtilis)*. These molecules are commonly produced inside the bacterial cell as pre-AIP, which enable the cell interaction mechanisms via specific transport proteins and are subsequently followed by phosphorylation and dephosphorylation reactions [70].

The LuxS/AI-2 molecule (known as the universal signaling molecule) is transported within the cell via specialized membrane transporters and is found in Gram-negative and Gram-positive bacteria[71,72]. Additionally, several other signaling molecules, such as diffusion signaling factors (DSF), *α*–hydroxyketones (AHKs), and *Pseudomonas* quinolone signals (PQS), have also been described [73–75].

1.1.5 Classification of biofilms

Biofilms can develop at the interface of various phases: liquid and solid (water - solid phase), liquid and gas (water-air), and solid and gas (building surface-atmosphere).

The conditions for the emergence of biofilms are surfaces for microbial colonization, water (humidity), biogenic elements, and microorganisms. These conditions are prevalent in the natural environment; biofilms are widely distributed in nature, medical settings, and technical systems [5]. There are practically no interfaces in the environment that are not inhabited by microorganisms (**Table 1.2**). Depending on the surface, the water content in biofilm is different. In the aquatic environment, biofilm can contain up to 98% water, while biofilm on rocks or in the atmosphere (dry air) contains significantly less water and is often a component of the "mud layer," which also includes a large amount of water. The biofilm matrix performs the fundamental function of a water retention system, ensuring microbial communities' survival in biofilm for decades under desiccation. Biofilms can be formed on biological and non-biological surfaces in almost every moist environment [76]. Based on the attachment surface, biofilms in aquatic ecosystems can be categorized as, for example, epizoic (animal), epipelon/ epipsammon (sediment/sand), or epiphytic (plant surface) biofilms. **Figure 1.2** (**A-G**) illustrates the aquatic macrophytes (epiphytic microbes on *C. demersum* and *E. crassipes*) in tropical shallow lakes Rumira and Cyohoha, Rwanda.

Table 1. 2 Biofilms on surfaces [77]

Interface	Examples of encountered biofilms
Solid Surface / Liquid	Biofilms can attach to underwater rocks and stones (epilithic biofilms), sediments, inner walls of water pipes and vessels, ship surface, medical prostheses, catheters, plant and animal tissues (algae, human, and animal epithelium), and the surface of tooth enamel.
Solid surface/air (often in contact with liquid)	Biological lawns in drip filters for air and wastewater treatment, soil, agar media, and lichens.
Liquid/air	Floating biofilm on the surface of water bodies and in reserve water tanks.
Liquid/ Liquid	Hydrocarbon-oxidizing biofilms at the oil/water interface.

1.1.5.1 Epiphytic biofilms

Aquatic macrophytes shape the physical properties of aquatic ecosystems by resisting their physical effects[78], regulating water chemistry, and maintaining clear water status[79]. Furthermore, the free/floating and submerged macrophytes are ideal substrates for microbial growth, forming a biofilm-macrophyte system that displays unique, complex, and interdependent biological interactions[12]. Epiphytic biofilms are complex matrix-dueling microbial communities on the plant surface, composed of bacteria, fungi, algae, protozoa, and other metazoans. Epiphyton influences plant health and the biogeochemical cycling of lake elements[47,80].

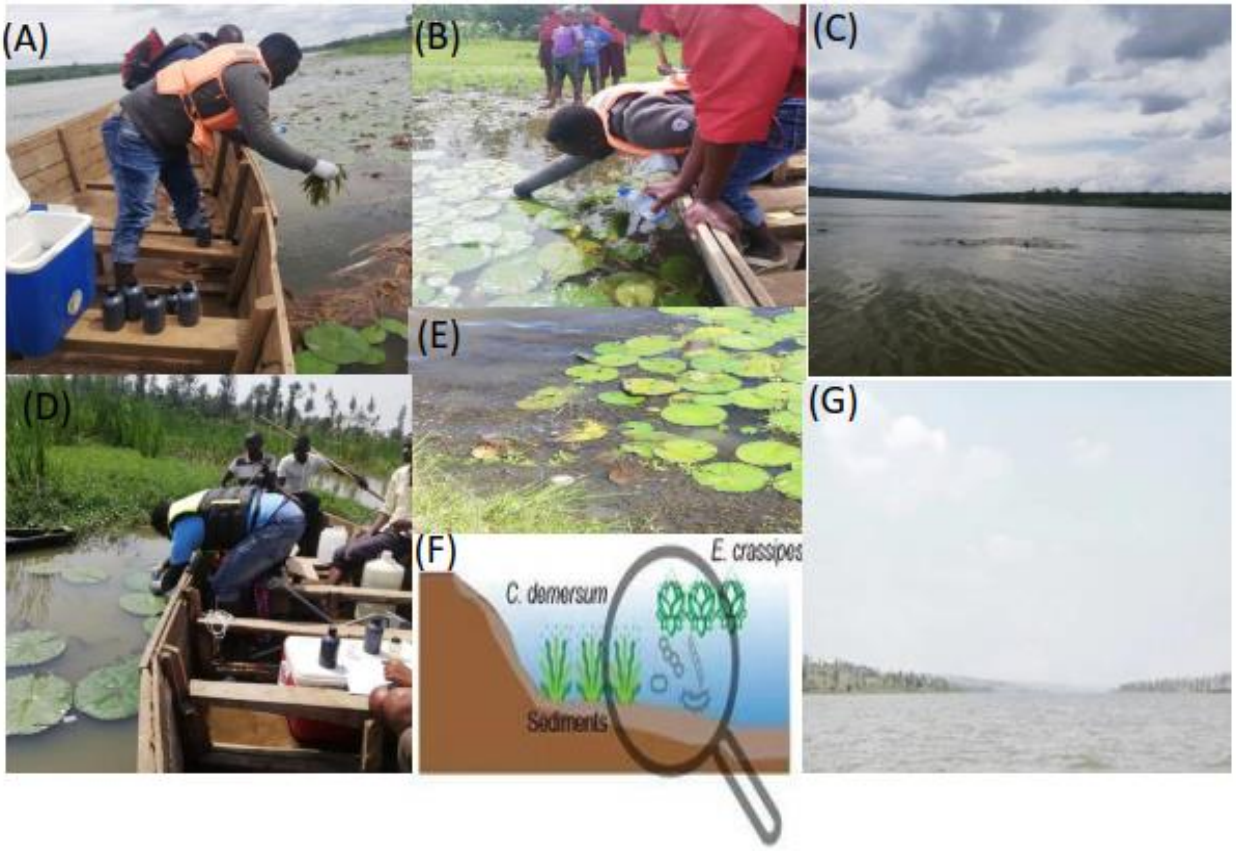


Figure 1. 2 (A-G) shows aquatic macrophytes (epiphytic microbes on *C. demersum* and *E. crassipes*) in tropical shallow lakes Rumira and Cyohoha, Rwanda

Microbial diversity and composition

Biofilm on aquatic macrophytes is different and unique in structure and function compared to the other periphytic biofilms on inert freshwater habitats (e.g., sand, stone/rock, and sediment)[35]. Previous reports indicated that bacterial dissimilarity was different among aquatic macrophytes[16,47,81–83]. Bojorge-García et al. [84] and Levi et al. [35] reported that epiphytic biofilm has a higher species diversity and distinctive species than epilithic and sediment biofilms. On the contrary, Liu et al.[82] and Liu et al. [85] revealed that most α-diversities were significantly higher in sediment than in epiphytic biofilm samples. In contrast, Manirakiza et al. [16] reported a lack of significant difference in all the α- diversity indices of the bacterial community in biofilm among microhabitats (*C. demersum*, *E. crassipes*, and sediments). Furthermore, the Shannon index of bacterial community was generally higher on artificial plants than on *Myriophyllum verticillatum* [82]. On the other hand, the alpha diversity (OTU richness, Shannon diversity, evenness, Chao1 phylogenetic diversity) indices for all the microeukaryotes were higher in sediments than those in epiphytic biofilms [16,82]. Moreover, previous studies emphasized that the epiphytic biofilm has

lower algal biomass and C: N: P ratios compared to epilithon in both lentic[86,87] and lotic ecosystems[88]. The difference in microbial diversity on different aquatic macrophytes may be ascribed to the host-specificity (allelopathy and physiology), plant species, and spatial heterogeneity. However, the significance of these differences of epiphytic biofilms on natural macrophytes relative to inert substrates in eutrophic freshwater ecosystems is still being debated.

Proteobacteria (α-, β- and γ-), Bacteroidetes, Cyanobacteria, Firmicutes, Actinobacteria, Planctomycetes, and Verrumicrobia have been reported worldwide as the top dominant bacterial phyla/classes in biofilms on aquatic macrophytes, such as *Vallisneria* spp.[50], *Ceratophyllum* spp. [51], *Potamogeton* spp. [66], *Nympha* spp.[33,83], and *Eichhornia* spp.[16,93,94] (**Table 1.3**). Furthermore, other taxonomic groups typically present in epiphytic biofilms include Chloroflexi, Acidobacteria, Gemmatimonadetes, Delta-Proteobacteria, and Deinococcus-Thermus.

On the other hand, microeukaryotes are ubiquitous and fundamental components of aquatic ecosystems [95]. Previous studies reported the eukaryotic community composition and dynamics in water samples [96,97]. However, few reports have studied the eukaryotic communities in epiphytic biofilms. The most prominent eukaryotic Kingdoms/phyla/classes/genera in biofilms on various aquatic macrophytes are typically Metazoa (Rotifers, Gastrotricha, Platyhelminthes, Annelida, and Arthropoda), Viridiplantae (Chloroplastida, Bacillariophyta, and Euglenophyta), Fungi (Ascomycota), and SAR group (Stramenopiles, Alveolata, and Rhizaria)[16,36,82,89–91,98–100]. Taken together, the relative abundance of dominant bacterial and microeukaryotic phyla varied in various aquatic macrphytes and seasons. These findings may be attributed to the plant-specific effects (allelopathy and physiology), environmental filtering, and succession that shaped the microbial dynamics at the phylum level; however, these viewpoints need further research.

Phylum Proteobacteria is metabolically diverse and comprises taxa involved in nitrification, N_2- fixation, methylotrophy, anoxygenic photosynthesis, and degradation of organic pollutants [101,102]. Betaproteobacteria and Bacteroidetes share the capability to decompose complex organic macromolecules[103]. A current study demonstrated that photosynthetic cyanobacteria sharing the same niche with diatoms formed thick biofilms during a nutrient influx event, and both participated in stream primary production [75]. Several genera in Firmicutes play a significant role in the microbial community structure and function in biofilm. For example, the functional role may be

associated with organic pollutants biodegradation (*Bacillus*)[104], plant growth promotion and antibiotic resistance control (*Paenibacillus*) [105], iron reduction (*Solibacillus*) [106], and fermentative hydrogen production (*Clostridium Sensi Stricto_1*) [107].

Fungi (e.g., phylum Ascomycota) are a prominent component of epiphytic biofilms next to bacteria due to their capability to degrade organic matter (nutrient cyclers) and cause diseases [108]. The fungal population comprises various organisms whose populations are greatly influenced during seasonal change [109], mainly growing season and senescence. Metazoans such as Rotifera, Gastrotricha, and some SAR taxa are important consumers or predators of picophytoplankton (algae and protozoans), bacteria, fungi (fungal zoospores), and particulate organic matter[96]. Additionally, they serve as prey for metazoans such as flatworms (Platyhelminthes)[110]. It is worthy of note that photosynthetic microbes (e.g., Chlorophyceae, photosynthetic taxa in SAR clade, and *Rhodobacter*) on aquatic macrophytes act as the primary producers (release oxygen through the photosynthetic process) and have a decisive influence on the entire aquatic ecosystem structural function and stability[111]. Algae, most commonly Bacillariophyta and Chlorophyta, provide substrates by exudates and lysis products and are a major carbon source for heterotrophic biofilm microbes [112–114]. **Table 1.3** summarizes aquatic plants' dominant bacterial and eukaryotic taxa at different taxonomic levels.

Table 1. 3 Dominant bacterial and eukaryotic kingdom/phylum/class/family/genera on aquatic macrophytes

Habitat	Aquatic macrophyte	Dominant bacterial and eukaryotic kingdom/phylum/class/family/genera	References
CW	*V. spiralis*	Proteobacteria, Bacteroidites, Cyanobacteria, Actinobacteria, Acidobacteria, Metazoa, Chloroplastida, Fungi, Stramenopiles, Rhizaria, Alveolata, and Amoebozoa	[98]
CW	*M. spicatum*	Proteobacteria, Bacteroidites, Firmicutes, Actinobacteria, Cyanobacteria, Annelida, Platyhelminthes, Arthropoda, Porifera, and Chlorophyta	[89]
CW	*C. demersum*	Cyanobacteria, α-proteobacteria, β-proteobacteria, Actinobacteria, Planctomycetes, Bacteroidetes, and γ-proteobacteria Metazoan (Arthropoda, Rotifera, Gastrotricha, Annelida, and Nematoda) and algae (Bacillariophyta, Chlorophyta, and Streptophyta)	[91]
CW	*Artificial plants, P.malaianus, V.natans, and H. verticillata*	Chlorophyta and Bacillariophyta	[115]
CW	*Cyperus giganteus, E.crassipes, Typha domingensis*	Protozoa, Rotifera, and *Vorticella* sp.	[93]

Habitat	**Aquatic macrophyte**	**Dominant bacterial and eukaryotic kingdom/phylum/class/family/genera**	**References**
Natural wetland	*M. verticillatum*, *Nymphoides peltatum and T. japonica*	Proteobacteria, Bacteroidites, Chloroflexi, Firmicutes, and Verrucomicrobia	[83]
Lake	*Potamogetonaceae*, *M.verticillatum, C. demersum and Ottelia acuminata var. acuminata*	γ-proteobacteria, Bacteroidites, and Firmicutes, Chlorophyta, Bacillariophyta, and Euglenophyta	[32]
Lake	*Nymphoides peltate*, *Trapa natans*	Proteobacteria, Bacteroidites, Chloroflexi, Firmicutes, Verrucomicrobia, and Acidobacteria	[33]
Lakes	*P. crispus*	α-proteobacteria, β-proteobacteria, γ-proteobacteria, Actinobacteria, Bacteroidites, Firmicutes, Armatimonadetes, and Planctomycetes	[92]
Lake	*Chara aspera*	*Cytophaga-Flabobacteria-Bacteroidites* and α-proteobacteria, β-proteobacteria, γ-proteobacteria, and Actinomycetes	[116]
Lake	*M. spicatum*, *M. sibericum*, and *M. alterniflorum*	Gastropods, oligochaetes, Amphipods, Chironomids , *Oxyethira*, and *Acentria ephemerella*	[117]

1.1.5.2 Sediment biofilms

The palaeoenvironmental records or history showed that lake surficial sediment samples potentially store much empirical data readily available for developing and testing ecological theory[118,119]. A lake surface sediment is the upper-most layer of subaquatic sediment comprising a multifaceted biogeochemical sample that characterizes a unified record of current lake microbial algae communities. The remains of algal communities formed an assemblage entombed several years ago by environmental change [118]. The time factor and cost-effectiveness of surface sediment samples compared with other diversity sampling approaches have stimulated a number of recent studies that used surface sediments to assess diversity patterns through environmental gradients and ecotones[120–122].

Microbial diversity and composition

Notwithstanding that 1g of surface sediment may contain milliards of microbial species, the main issue of using surface sediments is its temporal and spatial integration of variability, which may influence diversity. Previous works have documented the bacterial community structures in sediments[22,123]. Most α-diversity indices (Chao1, Ace, PD, evenness, OTU richness, and the Shannon index) of bacterial communities were significantly higher in sediments than those in epiphytic biofilm samples[82,83,85], while Manirakiza et al. [16] and Qiu et al. [90] reported a lack of significant difference in the bacterial α- diversity in epiphytic and sediment biofilms, planktonic microbes, and among sites in constructed wetlands and eutrophic shallow lakes. Interestingly, Shannon diversity and OTUs richness of microeukaryotes were higher in sediments than those in epiphytic biofilm samples from *C. demersum*[16]. The discrepancies in bacterial and eukaryotic diversity in surface sediments from lakes, rivers, costal water, and other water bodies may be attributed to the difference in sediment structure, environmental conditions (e.g., salinity, seasonality and depth), spatial variation, stochastic processes, and anthropogenic stressors (heavy metals, PAH, and nutrients), [22,96,124–129].

Based on composition, Proteobacteria (α-proteobacteria, β-proteobacteria, γ-proteobacteria, Deltaproteobacteria and Epsilonproteobacteria) Bacteriodetes, Firmicutes, Cyanobacteria, Chloroflexi (Anaerolineae), Actinobacteria, Acidobacteria, Nitrospirae, Verrumicrobia, Spirochaetes, and Methanomicrobia were the most dominant bacterial phyla in sediments worldwide[16,30,82,83,85,130–137].

On the other hand, microbial eukaryotes (nematodes, protists, fungi, etc., loosely referred to as meiofauna) are ubiquitous in sediments (lakes, seas, rivers and oceans) and likely play pivotal roles in maintaining ecosystem function. The most abundant eukaryotic Kingdoms/phyla in sediment biofilms are typically Rotifera (Brachionidae), SAR (Stramenopiles, Alveolates, and Rhizaria), Platyhelminthes, Bacillariophyta, Chlorophyta, Annelida, Nematoda, Arthropoda, Fungi (Ascomycota, Basidiomycota), Cercozoa, Dinoflagellates, Chrysophyceae, Streptophyta, and Ochrophyta, Mollusca, and Chlorophyta[15,16,82,138–142]. Taken together, the relative abundance of dominant bacterial and microeukaryotic phyla varied in surface sediments. These findings may be attributed to the difference in sediment type, environmental variables (temperature, pH, depth, season and nutrients), effluent pollution, and succession that shaped the microbial dynamics at the phylum level, consistent with previous reports [161–163], however, these viewpoints need further research.

Sediments are a repository of the events occurring in the pelagic environment and the processes occurring. The bacterial and microeukaryotic communities inhabiting the surface sediments play significant roles in biogeochemical cycling (carbon, phosphorus, nitrogen, and sulfur) and feeding relationships (predation, competition parasitism, symbiosis, commensalism and amansalism) [143–146]. For example, Phylum Proteobacteria plays a key role in degradation and metabolism in lake sediments[123,147]. Studies have shown that Chloroflexi prefers to live in a nutrient-rich environment [148], which is an indicator of nutrient enrichment sediment [149,150]. Methanomicrobia belongs to methanogens and has renowned importance in the carbon cycle through methanogens in anoxic marine sediment and sulfate reduction[17]. In addition, bacteria such as Actinobacteria and Bacteroidetes were the main microorganisms in river sediments and were usually important contributors to biogeochemical processes[151–153]. Bacillariophyta, Chlorophyceae, and Ascomycota are major components in microbial food webs, playing important roles in primary production and organic matter decomposition[108]. Metazoans are important consumers or predators of algae bacteria, fungal zoospores, and particulate organic matter[96].

1.1.5.3 Microbial mats

Microbial mats are vertically stratified and self-sustaining microbial communities that develop in the liquid-solid interface of various substrates in a wide range of environments, including hot springs, hypersaline ponds, coastal intertidal zones, and oligotrophic environments[154]. These communities range from single specific biofilm to complex microbial mats comprised of a wide variety of microorganisms in the bacterial domain; however, archaea, viruses (bacteriophages), and eukaryotes (fungi, protists, and a few metazoans) are also involved in forming microbial mats, although less diverse and abundant in nature[155]. The microbes in the mats are embedded in the extracellular polymer substances (EPS), where they interact and exchange signals and nutrients to enable a greater flow of resources and energy for the community's survival [156]. The microbial diversity and composition in the microbial mat are strongly shaped by physicochemical parameters such as temperature, light, O2, pH, redox potential, pressure, salinity, and electron donor and acceptor compounds[157]. In addition, biotic interactions, dispersal limitations, and neutral stochastic resource-independent processes may shape microbial community structures [158,159].

Life at the extreme

The organisms can endure certain tolerance limits of environmental conditions and cannot survive below or above these limits. This is known as the "limits of life" [160]. However, special microorganisms, "Extremophiles," can successfully colonize extreme environments (hot springs, hypersaline lakes, acid mine drainages, ice caps, and deserts). **Table 1.4** shows the environmental ranges of the most studied extremophilic microorganisms. Thanks to the discovery of the extremophilic bacteria *Thermus aquaticus* by Thomas D. Brock [161] in Yellowstone hot springs, the extraction and use of heat-stable enzyme Taq polymerase in the polymerase chain reaction (PCR) has put vitality into the biotechnology industry and stimulated research improvements in molecular biology ever since[162]. Brock reported that he could not discover this enzyme without field studies, enlightening the significance of explorations such as the present thesis.

Table 1. 4 Extreme definitions and some examples of extremophilic microbes

	Extremophile	**Definition**	**Example**
pH	Acidophile	Low pH loving	*Ferroplasma* sp.
	Alkaliphile	High pH loving (pH > 9)	*Spirulina* sp.
Temperature	Hyperthermophile	Growth > 80 ^{o} C	*Pyrolobus fumarii* (113^{o}C)
	Thermophile	Growth 60-80 ^{o} C	*Synechococcus lividus*
	Psychrophile	<15^{o}C	*Psychrobacter* sp.
Oxygen	Anaerobe	Cannot tolerate O_2	*Methanococcus jannaschii*
	Microaerophile	Tolerates some O_2	*Clostridium* spp.
Salinity	Halophile	Salt-loving (2-5M NaCl)	*Halobacteriacea*
Pressure	Piezophile	Pressure loving	*Shewanella violaceae*
Radiation	Radioresistant	Infra-red Ionizing radiation tolerant	*Deinococcus radiodurans*

Modified from Stojanovic et al. [163]

Hot spring mats

The hot spring mats can develop in various thermal habitats such as hot springs, fumaroles, eruption vents, and steaming ground [164]. The hot springs augment the solubility gases (CO_2 and O_2) and stimulate microbial enzyme denaturation. Microbes have developed physiological adaptations to curb these environmental stresses, making them thrive in high temperatures using various ionic bonds and internal forces that stabilize all enzymes [165]. The environmental DNAs harbor crucial genetic information about the microbial communities inhabiting various thermal environments[166]. The previous reports exposed how physicochemical conditions and biological interactions have shaped these microbial communities within their particular environments[166]. The thermophilic microbial communities are physiologically diverse. They comprise primary producers and nutrient cyclers or primary consumers. The trophic relationships rely on electron acceptors (oxygen, nitrate, sulfur, iron, and sulfate) or the fermentation process[160]. Pace et al. [167] reported that the last common ancestor of all live forms could have been using H_2 for energy at high temperatures. The deeply branched bacterial taxa such as *Thermotoga* and *Aquifex* are also

thermophilic chemolithotrophs, proposing that adaptations of temperature-loving organisms (thermophiles and hyperthermophiles) are important traits in the early history of life on earth.

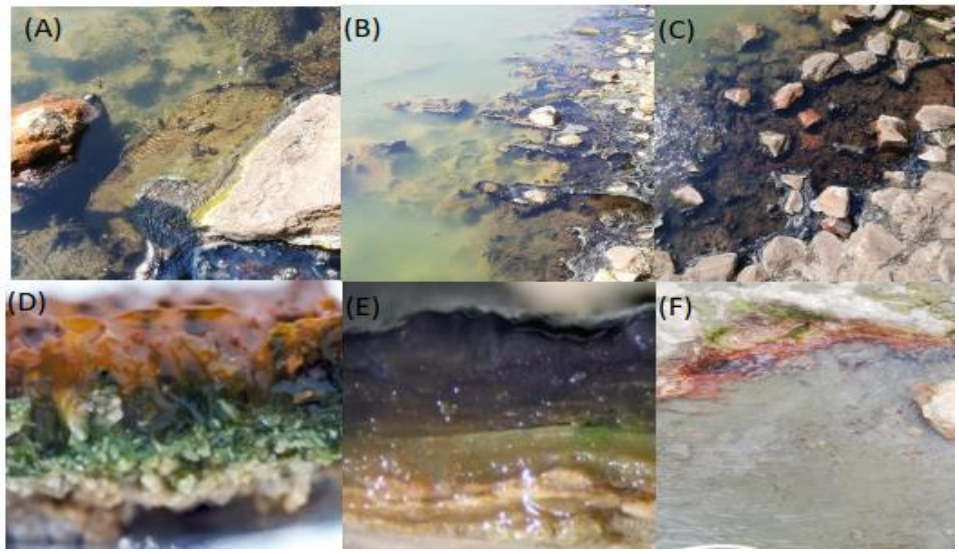


Figure 1. 3 Microbial mats in hot springs. Image (D and E) were adapted from Pieto-Barajas et al.[26]

Microbial diversity and composition

The microbial composition in the biomats from various extreme environments worldwide has been reported by several authors [26,168,169] (**Table 1.5**). However, studies on microeukaryotic communities in hot spring microbial mats are scarce, and little is known about their microbial diversity, assembly processes, and interactions. Hot springs assessed were located in the North-West part of Rwanda, Africa. Given the limited scientific data available in this part of the globe, the significance of the fourth chapter of the thesis was to explore the bacterial and microeukaryotic diversity, assembly processes, and interactions in different hot spring mats. Thanks to the advancements in the sequencing techniques that allow us to explore the microbial diversity, composition, assembly, interactions, and ecological function in hot spring mats. The knowledge of microbial diversity, interactions, and succession of these delicate ecosystems underpin the need for their protection. Furthermore, this biodiversity could be the basis for further biotechnological applications, symbolizing a key foundation for a developing region.

Table 1. 5 Dominant microbial diversity of various mat ecosystems worldwide.

Microbial mat type	Country/Continent	Dominant microbial diversity	References
Hypersaline mats	USA, Chile, Australia, and Spain	Cyanobacteria (Oscillatoriales, Nostocales, and Leptolyngbya), Chloroflexi (Anaerolineae and Chloroflexus), Bacteroidetes, Proteobacteria (α-proteobacteria, γ-proteobacteria, and δ-proteobacteria), Planctomycetes (Brocadiae), Spirochaetes, Verrucomicrobia, and Firmicutes Nematodes, arthropods, stramenopiles, alveolates, fungi, and chlorophytes	[170–176]
Coastal mats	Netherlands and USA	Proteobacteria (α-proteobacteria, γ-proteobacteria, and δ-proteobacteria), Bacteroidetes (Flavobacteriales and Sphingobacteriales), Cyanobacteria, Diatoms (*Navicula* sp., *Diploneis* sp., Amphora sp. and *Cylindrotheca*), algae (Chlorophyta and *Enteromorpha* sp.)	[177–180]
Microbial mats in oligotrophic environments	Mexico	Firmicute (Bacillus coahuilensis), Proteobacteria, Cyanobacteria, and Bacteroidetes	[181,182]
Psychrophile microbial mats	Antarctica and the Arctic shelter	Cyanobacteria (orders: Dichothrix, Nostocales-Tolypothrix, and Oscillatoriales-Tychonema), Actinobacteria, Proteobacteria, Bacteroidetes, Firmicutes and Deinococcus–Thermus, diatoms, algae, flagellates, ciliates, nematodes, rotifers and microinvertebrates	[183–186]

Microbial mat type	Country/Continent	Dominant microbial diversity	References
Hot springs microbial mats	Thailand, Patagonia, Tibet, Romania, USA and Mexico	Cyanobacteria (*Synechococcus*), Proteobacteria (*Pseudomonas* and *Aeromonas*), Chloroflexi, Bacteroidetes, Deinococcus–Thermus, Firmicutes (*Bacillus*, *Paenibacillus* and *Exiguobacterium*), and Actinobacteria (*Microbacterium*)	[168,187–193]
Acid microbial mats	USA	Actinobacteria, Firmicutes, δ-Proteobacteria, Nitrospira, Leptospirillum, Acidomicrobium, Hydrogenobaculum spp., Metallosphaera, Yellowstonensis,	[194–198]

1.1.6 Assembly mechanisms in biofilms

Microbial community assembly, the processes shaping the microbial community diversity, functions, succession, distribution, and biogeography, is a vital but poorly overlooked topic in aquatic microbial ecology[42], specifically in lacustrine and hot spring ecosystems. Deterministic and stochastic processes are the two categories of microbial community assembly[199]. The niche-based processes consider that microbial communities are shaped by the deterministic abiotic (environmental filtering) and biotic factors (species interactions such as cooperation, symbiosis, and predation) due to different habitat preferences and microbial fitness[200]. On the contrary, the neutral theory asserts that stochastic processes, such as birth, death, immigration, speciation, and limited dispersal, shape the microbial community structure[201,202] and assumes that microbes exhibit a stochastic balance between the loss and gain of taxa.

This topic has been investigated comprehensively in microbial ecology across different natural habitats such as freshwater lakes[203], sediments[204], streams[205], groundwater[202], soils[206], marine environments[207], engineering settings such as wastewater treatment plants[208] and membrane bioreactors (MBR)[46], and even some terrestrial extreme environments such as saline lakes[209] and hot spring water and sediments [45]. From an ecological perspective, both deterministic and stochastic processes play pivotal roles in regulating the assembly of ecological

communities[46]. However, their relative importance varies across different spatial and temporal scales, depending on the strength of environmental gradients and the sensitivity of the microbes to environmental changes. Microbial communities in aquatic ecosystems have largely been explored from a deterministic perspective, especially regarding abiotic factors. For example, physicochemical parameters (such as water flow velocity, temperature, pH, and heavy metals), host condition[210], interspecies interactions[211], and plant diversity[212] were important drivers for microbial diversity, community structures, and the biogeochemical processes in aquatic ecosystems. However, stochastic and deterministic (biotic) processes receive little attention in aquatic ecosystems, especially the impacts of stochastic and deterministic assembly processes on microbial community in epiphytic, surface sediment, and hot spring mat biofilms.

Few studies on epiphytic and hot spring bacteria community assembly have been investigated. For example, Shi et al. [48] revealed that the environmental filtering (deterministic processes) prevailed over stochastic processes in shaping epiphytic bacteria assembly on *Najas marina* and *Potamogeton lucens* in Honzhe lake (China), whereas He et al. [47] and Lear et al. [213] reported that deterministic and stochastic processes cooperatively influenced the bacterial composition and diversity in epiphyton on *Hydrilla verticillata* and *Vallisneria natans* in Lake Taihu and epilithon in the stream. Furthermore, He et al.[214] (2020a) indicated that stochastic processes primarily affected endosphere bacterial composition in *Phragmites australis*, whereas both deterministic and stochastic processes dictated bacterial assemblages of the rhizosphere, with the relative importance of stochastic versus deterministic processes depending on the season. Thus, the role of assembly processes for epiphytic communities is the topic of ongoing debate.

In Tengchong hot spring (China), He et al. [45] demonstrated that stochasticity was the major assembly mechanism in the water community, while determinism was the major assembly mechanism in the sediment community. Kuang et al. [204] reported that a peak in eutrophication enhanced bacteria deterministic processes in surface sediments. Noteworthy, Chen et al.[125] revealed that stochastic processes shape microeukaryotic community assembly in a subtropical Tingjiang river across wet and dry seasons. Compared with that of the bacterial community, studies on microeukaryotic community assembly on aquatic plants, surface sediments, and hot spring mats remain largely unexplored and poorly understood. Therefore, it is of utmost importance to clarify the relative contributions of the abovementioned processes in understanding the microbial

community (bacteria and microeukaryotes) assembly in aquatic macrophytes, surface sediments, and hot spring mats.

The neutral community model (NCM) and null model analysis are the main techniques used to infer ecological stochasticity[215]. A neutral community model is used to characterize the community assembly processes. Sloan et al.[215] stated that the probability of appearance of species in a sample at abundance above a detection threshold could be predicted by using the beta probability density function. The null modeling approach compares the observed phylogenetic turnover in OTUs between sites relative to a stochastic model and the impact of deterministic processes on community assembly. The modeling is based on phylogenetic and taxonomic β-diversity metrics, indicated by the β-nearest taxon index (βNTI) and Bray-Curtis-based Raup-Crick (RCBray) [199]. Notably, the β-nearest taxon index (βNTI) based on a null model provides a quantitative description of the assembly processes.

1.1.7 Microbial interactions and ecological functions in biofilms

Periphytic biofilm on aquatic macrophytes, surface sediments, and mats form a highly interactive unit with substrate provision, competition for nutrients, allelopathic interactions, and trophic interactions. Epiphytic biofilms are understudied compared to other periphytic biofilms in freshwater ecosystems. This is surprising as epiphytic biofilm-macrophyte-specific interactions interfere with important ecosystem processes and these interactions are highly complex. Therefore, we need to gain a better understanding of biofilm-macrophyte interactions. Lack of understanding of these interactions may underestimate the importance of macrophyte habitats in freshwater ecosystems due to ignorance of macrophytes' role as a substrate for microbial biofilm.

Topological properties, such as average degree, average path length, modularity class, and average clustering coefficient, are computed to describe the complex pattern of correlations among the microbial genera in the network[216]. Various approaches are adopted to choose network hubs or keystones. For example, nodes with degrees greater than 90% of the maximum degree are designated network hubs or keystones, which play a pivotal part in maintaining microbial community stability in biofilms[217]. Furthermore, the keystone taxa of the food web can be determined based on the closeness centrality index. The classification system based on determining the ecological outcome of each organism in a pairwise interaction[218] can be either positive, neutral

or negative. It can encompass all possible pairs of effects: mutualism (+/+) (interaction where both participants experience a positive outcome), competition (-/-) (interaction where both participants experience a negative outcome), and commensalism (+/0) (interaction between individuals of two species in which one species obtains food or other benefits from the other without either harming or benefiting the latter). Between these two extremes are combinations of positive, neutral and negative outcomes such as amensalism (0/0), in which the actor experiences no benefit or detriment and the recipient experiences a negative outcome. This framework has formed the basis for a broad corpus of ecology research[219].

Trophic interactions

More attention is being directed toward the myriad ways microbes interact and the crucial roles these interactions play in defining community function. Based on the feeding style, the food webs were categorized into three trophic ranks, namely, the producers (photosynthetic microeukaryotes and prokaryotes), consumers (metazoans), and decomposers (bacteria and fungi). It is worthy of note that photosynthetic microbes (e.g., Chlorophyceae, photosynthetic taxa in SAR clade, Cyanobacteria, and *Rhodobacter*) are important primary producers (release oxygen through the photosynthetic process) in aquatic macrophytes, surface sediments, and mats and have a decisive influence on the entire aquatic ecosystem structure-function and stability. For example, epiphytic biofilms have a crucial role in primary production and have the site of trophic interactions benefitting both macrophytes and the epiphytic biofilm. They initiate a crucial food web that includes lower trophic level invertebrates (e.g., micrograzers and meiofauna). In return, macrophytes provide substrate to epiphytic algae and bacteria by improving biofilm stoichiometry and bacterial growth[87].

Primary consumers; phylum Bacteroidetes and Proteobacteria, are aerobic bacteria involved in nutrient removal. Flavobacterium has an important role in polysaccharides degradation whereas. *Pseudomonas* is important for the assimilation and degradation of nutrients by nitrification and denitrification processes [220]. Other bacteria roles may be associated with PHA degradation (g_Sphingorhabdus)[221], Facultative and methane degradation (f_Methylophilaceae)[222], plastic degradation in the gut of nematodes (Exiguobacterium)[223], Benzene degradation (g_Methyloversatilis)[224], nitrogen fixation (g_Cellvibrio)[225]. Positive interactions, such as cooperation, cause bacteria to co-occur in similar niches, while negative interactions, such as

competition for space and resources, result in co-exclusion [226]. Fungi and bacteria are responsible for decomposing coarse particulate organic matter and detrital particulate and dissolved organic matter [227]. Nonetheless, there are often antagonistic interactions between bacteria and fungi fueled by competition for resources [228]. The interaction between algae and bacteria involves nutrient exchange, signal transfer, and gene transfer [229]. Furthermore, symbiotic algae-bacteria systems were key in removing and converting nutrients [230]. Metazoans such as Gastrotricha, Rotifers (o_Philodinida and o_Ploimida), and particular SAR taxa are important consumers of picophytoplankton (algae and protozoans), bacteria, fungi (fungal zoospores), and particulate organic matter [96]. Additionally, they serve as prey for large metazoans such as flatworms (Rhabdocoela) [110]. For example, secondary consumers such as *Echinamoeba, c_Tubulinea* in phylum Amoebozoa, and *o_Monhysterida* in Phylum Nematoda have been reported in hot springs and are considered as fierce predators [231,232]. Nematodes show high preference toward stalked and tubular diatoms, whereas both rotifers and ciliates show a preference for Cocconeis-type diatoms[233]. Annelida (o_Haplotaxida) play important role in decomposition and predation[98]. **Table 1.6** shows a summary of microbial taxa (Kingdom/phylum/genus) recorded in food webs of aquatic macrophytes, surface sediments, and hot springs.

Taken together, the negative and positive interactions in networks may be attributed to the microhabitats' distinctive lifestyles, food and energy sources, niches, allelopathy, and seasons [234].This shows that complex food webs with interrelationships can exist in microbial communities in epiphytic, surface sediment, and mat biofilms, such as feeding relationships, predation, parasitism, and synergism for biogeochemical cycling.

Previous studies explored microbial interactions in natural habitats such as soils [206], freshwater [32], rivers [37], and hot spring water[231]. However, microbial interactions in aquatic macrophytes, surface sediment, and mats remain largely unexplored. Few reports explored the microbial interaction in aquatic macrophytes, surface sediment, and mats[91,235,236]. However, most of these studies were conducted in china and focused mainly on a bacterial community under pollutant or nutrient loading in constructed wetlands, whereas little is known about the microeukaryotic interaction in the abovementioned substrates. Therefore, it is of utmost importance to investigate the microbial (bacteria and microeukaryotes) interaction in aquatic macrophytes, surface sediment, and mats in tropical lakes and hot springs.

The microbial community diversity, composition, and co-occurrence networks might not be enough to disentangle microbial community impact on ecosystem functions[36]. To this end, researchers have developed a variety of methods based on 16S rRNA high-throughput sequencing to predict bacterial community functions, including the FAPROTAX (Functional Annotation of Prokaryotic Taxa)[237], Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt)[238], Tax4Fun2[239], and Piphillin[240]. Among these, PICRUSt2 and FAPROTAX provide useful perspectives on community functions and can guide the separation of functional bacteria. Additionally, they are most flexible, accurate, and interoperable with the OTU-denoising algorithm and high correlation mean predictions[237,241]. FAPROTAX is a manually built database that uses current research on cultivated strains to link bacterial clades to recognized metabolic or other ecologically important activities[237].

PICRUSt and FAPROTAX have been used to predict metabolic functions in epiphytic bacteria associated with Myriophyllum spicatum, *Sargassum horneri, Hydrilla verticillata*, *Vallisneria natans*, and *Potamogeton maackianus* in Lake Baiyangdian (China), the Yellow Sea, and Lake Jinhu, respectively[242–244]. Furthermore, predicted functions were recently studied in sediment biofilms of aquaculture sites[245] and wetlands[246]. However, this topic remains unexplored, especially in aquatic plants (e.g., floating plants), surface sediments, and hot spring mats. Functions of the bacterial community are performed using KEGG function annotations to obtain information on OTUs at each functional level. Spearman's rank correlation is used to measure the correlation between the bacterial genus and KEGG orthology metabolic pathways in all samples[247]. In addition, STAMP analysis can be used to check the significant difference in predicted functions.

The top bacterial genera in epiphytic biofilms from different aquatic macrophytes were most related to the metabolism (nucleotide, amino acid, carbohydrate, lipid, energy, cofactors and vitamin) in Lake Rumira[16], methylotrophy, human pathogen-related functions, and denitrification-related functions in three rivers connected to Lake Taihu[37], carbon cycling (carbon fixation, methane metabolism, and photosynthesis), nitrogen metabolism, oxidative phosphorylation, and sulfur metabolism in Lake Jinhu[244], chemoheterophy, nitrate reduction, fermentation, aerobic chemoheterophy, and degradation of aromatic compounds in Lake Baiyangdian [242].

Table 1. 6 Summary of microbial taxa (Kingdom/phylum/genus) recorded in aquatic macrophytes, surface sediment, and hot spring food webs.

Interaction type	**Positive or negative association**	**Significance (+/-) of microbial taxa detected in epiphytic biofilms**	**Substrate**	**References**
Bacteria-Bacteria	Proteobacteria vs. Bacteroidites	β-Proteobacteria and Bacteroides work together to degrade (+) complex organic macromolecules	*V. natans* Hot springs	[89,103]
Bacteria-Algae	*Synechococcus* vs *Lutibacterium* and *Erythrobacter*	*Synechococcus* was positively (+) correlated with *Lutibacterium* and *Erythrobacter* They allow nutrient exchange, signal transfer, and gene transfer. Furthermore, symbiotic algae-bacteria systems played a key role in removing and converting nutrients	*Ceratophyllum demersum*	[91]
Bacteria-Fungi	Bacteria vs. Fungi	Bacteria and Fungi compete (-) for resources.	*M. verticillatum* *M. spicatum*	[82,89]
Fungi -Algae	*Aspergillus* vs *Cladochytrium* and Chlorophyta	*Aspergillus* and *Cladochytrium* were positively (+) related to genera in phylum Chlorophyta or Ochrophyta	*M. verticillatum*	[82]
Metazoans-bacteria, Fungi, Algae, Picoplancktons, Small protozoans	Gastrotricha and Rotifers vs algae, protozoa, bacteria (Firmicutes), and fungi	Gastrotricha and Rotifers predate (-) on algae, protozoan, bacteria, and fungi	*Ceratophyllum demersum* Sediments	[16]

Interaction type	Positive or negative association	Significance (+/-) of microbial taxa detected in epiphytic biofilms	Substrate	References
Others (Fungi-Fungi, Algae-algae, etc.)	Proteobacteria vs. Firmicutes	Bacteria compete (-) with their neighbors for space and resources.	*C. demersum*, *E. crassipes* and surface sediments	[16,98]
	Fungi vs. Fungi	Fungi inhibit (-) other fungi via toxin production and parasitism		
	Algae vs. algae	Algae inhibit (-) other Algae via toxin release		

1.2 Problem statement

Rumira and Cyohoha North are the largest tropical shallow lakes in Bugesera district, Eastern Province, Rwanda. They are part of the Akagera river system in the Nile basin at an altitude of 1328-1348 m above sea level[248] and an average depth range of 3-5 m. *Ceratophyllum demersum* and *Eichhornia crassipes* are the most common aquatic plants in these lakes. Some farming and agricultural activities are carried out at the lakeshore of the sampling sites. These tropical lakes are essential for the local population's ecology, regional hydrology, fishery, and water sources.

Tropical lakes worldwide are vital ecosystems, providing ecology, regional hydrology and irrigation, fishery, and water sources for the local population. However, they are more sensitive to the increase in nutrient supply and show higher proportionate changes in water quality and biotic communities in response to eutrophication. To ensure the sustainable use of tropical lakes, its water quality must be protected. Furthermore, sediment is a critical component of lake ecosystems and can act as both a repository/sink of processes occurring in the pelagic environment and a source of pollution (heavy metals, nutrients, and pathogens). Hot springs provide thermal waters for health (therapeutic effects), recreation or tourism, and balneology, and comprise the unique hot spots for thermophilic microbial communities (bacteria, archaea, viruses, and eukaryotes) [3,4].

Aquatic macrophytes are known as ‘ecosystem engineers due to their ability to shape aquatic freshwater ecosystems' physical properties and functions (e.g., regulate water chemistry). However, due to improper anthropogenic activities, they have declined significantly, and biodiversity in freshwater has decreased[11]. Thus, the deterioration of aquatic plants across the globe is a serious issue that needs attention. The submerged and floating macrophyte leaves are ideal substrates for microbial growth (e.g., biofilms), forming plant-biofilm platforms, which display unique, complex, and interdependent biological interactions[12]. Epiphytic biofilms are key components in shallow freshwater ecosystems and play multiple roles, including maintaining ecosystem structure (community composition and diversity), primary production and respiration, nutrient uptake and cycling, decomposition, pollutant removal and microbial gene pool preservation[8]. Compant et al.[19] (2010) reported a significant connection between water, aquatic macrophytes, and sediments, which may facilitate the sediment bacteria migration to the roots, stem, and leaf surfaces.

Therefore, epiphytic biofilms significantly influence plant health, growth, interactions, and the biogeochemical cycling of lake elements. However, global warming may influence the relationship among substrate–periphyton–phytoplankton and change the producer community structure in shallow lakes, as the elevated temperature has been suggested to stimulate the dominance of phytoplankton[14]. Thus, epiphytic biofilms can serve as bioindicators of climate change.

Bacterial and microeukaryotic communities in surface sediment biofilm (epipelic) play significant roles in biogeochemical cycling, energy transport, and feeding relationships[15,16]. Contrary to their positive functions, sediment bacterial communities are prone to a variety of natural and anthropogenic stressors, such as seasonal changes, rapid urbanization and land use changes [21,22]. Hence, exploring the key factors influencing microbial community compositions in lake sediments may provide insights into the biogeochemical functions in the lacustrine ecosystem[25].

Previous field studies have focused on the diversity, composition, and interaction of bacteria and algae in biofilms or planktonic cells on different substrates, including surface sediments[30], hot spring water and bulk sediment[45], saline mats[249], green alga[250], submerged macrophytes[32], and floating macrophytes[33]. Unquestionably, environmental variables can, directly and indirectly, affect microbial community diversity, composition, interaction, and function in different

microhabitats (e.g., aquatic plants and sediments)[38]. However, the effect of environmental variables on bacterial and microeukaryotic communities in epiphytic biofilms and mats is poorly understood. Compared with the bacterial community, studies on the influences of microhabitats and seasonal shifts on eukaryotic diversity, composition, and interaction in epiphytic and surface sediment biofilms and mats remain largely unexplored and poorly understood.

The microbial communities in aquatic ecosystems have largely been studied from a deterministic perspective, especially in terms of abiotic factors. Additionally, only three studies explored the role of stochastic and deterministic processes in aquatic epiphytic bacteria[47,48,244]. However, no study has explored the effect of aquatic macrophytes, surface sediments, and mats on microeukaryotic assembly mechanisms in tropical eutrophic lakes and hot springs. Furthermore, exploring microbial communities on various substrates across seasons is indispensable to clarifying the assembly mechanisms and factors influencing them.

Microbial communities in epiphytic and surface sediment biofilms and mats play a vital role in the biogeochemical cycles of the major chemical elements in tropical lakes and hot springs. Few reports explored the influence of microhabitats on periphytic bacterial functions[36,37]. Thus, little is known about the effect of aquatic plants, surface sediments, and hot spring mats on bacterial ecological functions. Therefore, it is of utmost importance to explore the effect of various microhabitats on the functional profile of periphytic bacterial biofilms.

Taken together, previous studies of epiphytic biofilm were mostly conducted under pollutant loading in constructed wetlands (CW) dominated by submerged macrophytes. Most of these studies focused on epiphytic bacterial communities. Additionally, most works on epiphytic biofilms and mats came from China, Denmark, and the USA, while the African continent still lags behind in this regard.

To the best of my knowledge, this study is the first of its kind to provide a comprehensive exploration of the influence of microhabitats (*C. demersum*, *E. crassipes*, surface sediments, and hot springs) and environmental variables (season and water chemistry) on the bacterial and microeukaryotic diversity, composition, assembly processes, interactions, and ecological function in epiphytic and surface sediment biofilms and mats.

1.3 Aim and objectives

The overarching aim of this study is to explore the influence of microhabitats (*C. demersum*, *E. crassipes*, surface sediments, and hot springs) and environmental variables on microbial diversity, composition, assembly processes, interactions, and ecological function in epiphytic and surface sediment biofilms and mats. The specific objectives designed to address the research aim are as follows:

1) To comprehensively explore the impact of microhabitat type (*C. demersum*, *E. crassipes*, surface sediments, and hot springs) and seasonal change on the bacterial and microeukaryotic community dynamics in periphytic biofilms.
2) To disentangle the assembly processes driving microbial communities in periphytic biofilms of shallow tropical lakes and hot springs.
3) To comprehensively explore the potential interactions and ecological functions of microbes in periphytic biofilms
4) To comprehensively evaluate the response of microbial communities in periphytic biofilms to environmental variables.
5) To assess the water quality of tropical lakes and hot springs.
6) To compare the microbial communities in different microhabitats on the ground of response to environmental variables, interactions, and ecological functions.

Table 1. 7 Research objectives and respective research questions

Thesis objective	Research questions
Specific	1) What is the influence of microhabitats/substrates and seasons on microbial diversity and composition in epiphytic and surface sediment biofilms? 2) What is the impact of the environmental variables on microbial communities (bacteria and microeukaryotes) in epiphytic biofilms? 3) What are the potential ecological functions of microbes in epiphytic and sediment biofilms? 4) What are the stochastic and deterministic assembly processes shaping the microbial communities in epiphytic and surface sediment biofilms? 5) What is the influence of the microhabitats (*C.demersum*, *E.crassipes*, and surface sediments) on the microbial community co-occurrence? 6) What is the influence of the temperature gradient on microbial diversity and composition in hot spring mats? 7) What are the stochastic and deterministic assembly processes driving the microbial communities in hot spring mats? 8) What is the impact of the environmental variables on microbial communities (bacteria and microeukaryotes) in hot spring mats

Thesis objective	Research questions
	9) What are the potential microbial interactions and ecological functions among microbes in hot spring mats?

1.4 Hypothesis

The hypothesis established and tested by this study includes the following:

- ✓ Microhabitat type and seasons can influence microbial diversity, composition, and ecological function in biofilms.
- ✓ Environmental variables may directly or indirectly influence the microbial structure and water quality in tropical lakes and hot springs.
- ✓ Seasonal and substrate changes may shape the assembly processes of biofilms.
- ✓ Substrate type may drive the dynamics of the biofilm ecosystem food webs.

1.5 Justification of the research

The macrophyte-biofilm platform and mat communities provide an environmentally friendly and cost-effective technology for biogeochemical cycling of elements, plant health, water and wastewater purification, and search for novel thermophilic enzymes and antibiotics with various biotechnological applications. The research on the biofilm-aquatic plant relationship will deepen our understanding of the costs and benefits of current management practices, such as employing hydrophyte-associated microbes to promote the development of hydrophytes, which will be essential for increasing the utility of hydrophyte microbiomes in the future and enhancing aquatic ecological restoration.

The plant species and environmental variables influence the microbial diversity and composition in epiphytic and sediments biofilms and mats. Furthermore, most studies were conducted on submerged macrophytes (*Vallisneria* and *Myriophyllum* species) and bulk sediments in greenhouses focusing on planktonic microbes and bacteria or algae biofilms. However, the effect of various substrates (*E. crassipes*, *C. demersum*, surface sediment, and hot spring) and environmental variables (seasons and water chemistry) on the bacterial and microeukaryotic diversity, interactions, and ecological functions in epiphytic and surface sediment biofilms and mats are unexplored and poorly understood.

The microbial communities in aquatic ecosystems have largely been studied from a deterministic perspective (abiotic factors). Few reports explored the role of stochastic and deterministic processes in epiphytic bacteria. However, no research has explored the effect of aquatic macrophytes, surface sediments, and mats on microeukaryotic assembly mechanisms in tropical eutrophic lakes and hot springs. Therefore, it is of utmost importance to seize the advantages that substrate-associated biofilms offer by exploring new insights into biodiversity, assembly mechanisms, interactions, and ecological roles of dwelling microbial communities, which are essential in understanding the trophic relationships, biogeochemical cycling, species conservation, and valorization of tropical lakes and geothermal springs. The updated information on the topics discussed in this research would be relevant to the research community and government agencies for informed policy-making on the lake and hot springs protection.

1.6 Thesis outline and research contents

This study explores the influence of various microhabitats/substrates (*C. demersum*, *E. crassipes*, surface sediments, and hot springs) and environmental variables on the microbial diversity, composition, assembly processes, interactions, and ecological function in epiphytic and surface sediment biofilms and hot spring mats of Rwanda. The main topics were divided into sections to give a clear data presentation and sufficient discussion of the results. Therefore, the thesis outline and research contents are as follow:

- Provide the theoretical background, rationale, and study objectives.
- The epiphytic bacterial and eukaryotic biofilm communities on submerged and floating macrophytes and surface sediments were explored in Lake Rumira, Rwanda, in August and November 2019 using high-throughput sequencing. The study aimed to investigate 1) whether microhabitats and seasonal shift affect microbial diversity and composition in epiphytic biofilms and surface sediments, 2) the response of epiphytic microbes to environmental factors, and 3) the potential ecological functions of microbes in epiphytic and sediment biofilms.
- The epiphytic bacterial and eukaryotic biofilm communities on submerged and floating macrophytes and surface sediments were investigated in Lake Cyohoha, Rwanda, in August (dry season) and November (wet season) 2019 by high-throughput sequencing of the 16S and 18S rRNA genes. The study aimed to investigate 1) whether *C. C. demersum*,

E. crassipes, surface sediments, and seasons influence the microbial community co-occurrence, and 2) the relative importance of stochastic and deterministic processes in shaping epiphytic and epipelic microbial assembly. The co-occurrence patterns and ecological processes were computed by phylogenetic molecular ecological network analysis and statistical methods based on the null and neutral models.

- Furthermore, the microbial assemblage and interactions in mats for two hot springs were evaluated by high-throughput sequencing. Field measurements and sample collections were conducted in Bugarama hot pool (40.2-47.3 ^{o}C) and Gisenyi hot springs (58-71.4 ^{o}C), Rwanda, in October 2019. The study aimed to investigate: 1) the microbial diversity and composition along a temperature gradient in microbial mats, 2) the effect of temperature gradient on assembly processes in microbial mats, 3) the response of microbial communities in mats to environmental parameters, and 4) the interactions and ecological roles among microbes in microbial mats.
- Compare and contrast the microbial community response to environmental variables, biotic interactions, and ecological functions in epiphytic and surface sediments biofilms and mats for tropical lakes and hot springs.
- Summarizes the findings and contributions of this thesis to scientific knowledge and provides a series of recommendations

1.7 Study framework

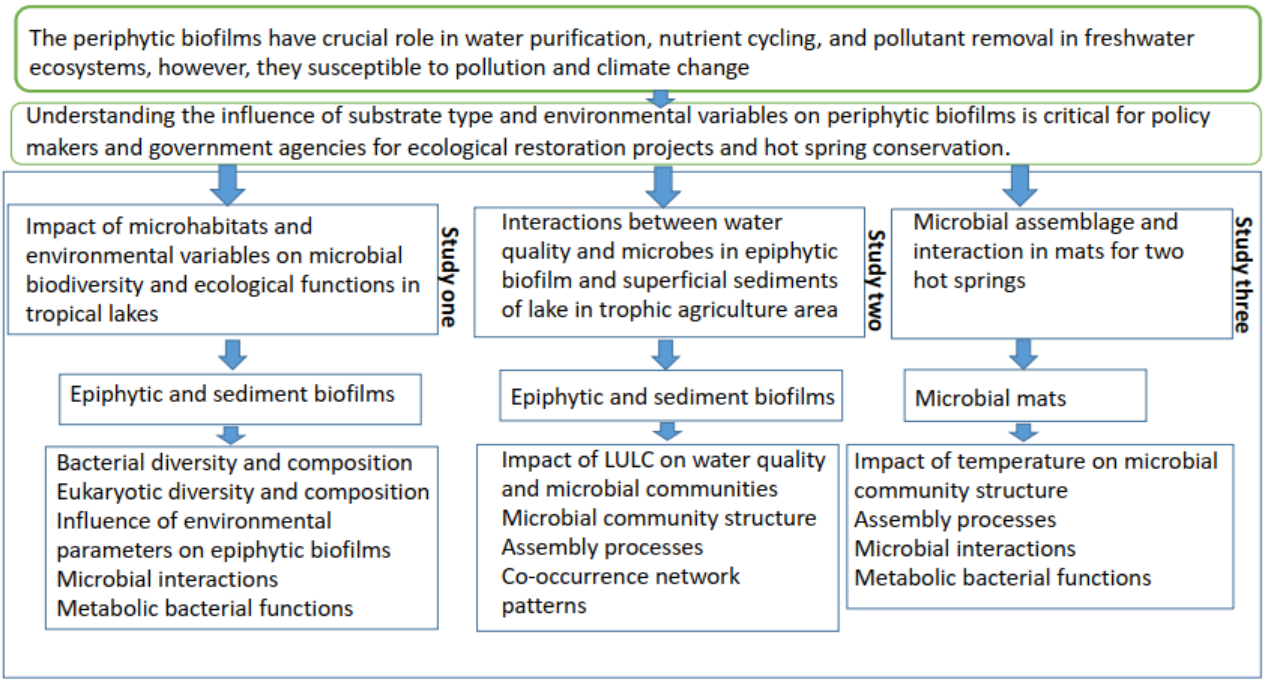


Figure 1.5. Technical route of study

Chapter 2. Exploring microbial diversity and ecological function of epiphytic and epipelic biofilm communities in a shallow tropical lake.

2.1 **Background**

Biofilms are complex and dynamic structures, mainly composed of viable cells (bacteria, archaea, algae, fungi, protozoa, and other metazoans) embedded in a self-produced hydrated extracellular polymer substance (EPS) matrix [13]. Based on the attachment surface, biofilms in aquatic ecosystems can be categorized as, for example, epipelon (sediment), epipsammon (sand), epilithon (rock) or epiphytic (plant surface) biofilms. The aquatic vegetation provides an ideal attachment or substrate for microbial growth, forming a plant-biofilm platform that represents the basic components of aquatic ecosystems and also plays important roles in transforming pollutants and maintaining ecological balance [251]. The ecological functions of biofilms are closely linked to the nutrient biogeochemical cycling and detoxification of environmental pollutants by biofilm-dwelling communities [252,253].

Previous field studies have focused on the diversity, composition, and interaction of planktonic forms or bacteria and algae biofilm on green alga [250], submerged macrophytes [32], floating macrophytes [33] and emergent plants [254], and surface sediments[82]. Unquestionably, physical, biochemical, and other environmental variables affect microbial community diversity, composition, interaction, and function in different microhabitats. Furthermore, it has been reported that bacterial diversity and composition in epiphytic biofilms and sediments are influenced differently by seasons in marine environments [39,40]. For instance, the growing season played a prominent role in driving the alpha and beta diversity of bacterial communities on submerged macrophytes [47]. Recent research on bacterial communities associated with sediments showed that alpha diversity was significantly higher in the dry season than in the wet season [39]. Therefore, there is a need for further research on bacterial diversity in two marine microhabitats during wet and dry seasons. In addition, compared with that of the bacterial community, studies on the influences of microhabitats and seasonal shifts on eukaryotic diversity, composition, and interaction in epiphytic biofilms and surface sediments remain largely unexplored and poorly

understood. It was revealed by Kettner et al. [95] found that bacterial communities are, to a large extent, controlled by the dynamics of eukaryotes, both as substrate source and as an interaction partner. Furthermore, epiphytic eukaryotes shape prokaryotic communities on eelgrass leaves by releasing chemical attractants [256], while the epiphytic ciliates on *E. crassipes* were highly diverse and depended on nutrient availability [263].

The microbial community diversity, composition, and co-occurrence networks might not be enough to disentangle microbial community impact on ecosystem functions[36]. Therefore, it is of utmost importance to explore the functional characteristics of microbial communities. To this end, researchers have developed a variety of methods based on 16S rRNA high-throughput sequencing to predict bacterial community functions, including the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) [238], Tax4Fun2 [239], and Piphillin [240]. PICRUSt2 is the most flexible, accurate, and interoperable among the OTU-denoising algorithm and has high correlation mean predictions [241]. Recently, it has been used to predict metabolic functions in epiphytic bacteria associated with *Myriophyllum spicatum* and *Sargasum horneri* in Lake Baiyangdian (China) and the Yellow Sea, respectively [242,243]. Furthermore, predicted functions were recently studied in sediment biofilms of aquaculture sites [245] and wetlands [246]. To the authors' best knowledge, no research has so far comprehensively investigated the diversity and ecological functions of biofilm-dwelling microbial communities in submerged plants, floating plants, and surface sediments in natural tropical lakes.

Therefore, to fill this gap, the present study aimed to investigate 1) whether microhabitats (substrate-specificity) and seasonal shift affect microbial diversity and composition in epiphytic biofilms and surface sediments, 2) microbial interactions in biofilms on *Ceratophyllum demersum* and *Eichhornia crassipes* in response to environmental factors, and 3) the potential ecological functions of microbes in epiphytic and sediment biofilms. In-depth research of the various factors influencing the diversity, composition, and ecological functioning of the microbial communities in epiphytic biofilms and surface sediments is crucial for understanding the interactions among biofilm-dwelling microorganisms in tropical lacustrine ecosystems, which holds good for the management of aquatic plants, water purification, and other important biogeochemical processes.

2.2 Materials and methods

2.2.1 Study area and sampling

Bugesera district hydrographical network is mainly marked by three rivers: Akagera, Akanyaru, and Nyabarongo[259]. In addition, it is marked by nine lakes, including Rumira, Mirayi, Kirimbi, Gaharwa, Rweru, Cyohoha North, Cyohoha South, Gashanga, and Kidogo. Seven lakes were formed due to the Akagera river overflow, except Lake Rweru and Cyohoho South. Lake Rumira is one of the largest tropical shallow lakes located in Bugesera district, Eastern Province, Rwanda (30°14'9.96"E, -2°11'6"S) **(Figure 2.1)**. It is part of the Akagera river system in the Nile river basin at an altitude of 1328 m above sea level [248] and an average depth range of 3-5m. In Rwanda, there is no more monitoring of water quality and little research indicated that major water pollutants are sediments and nutrients transported into watershed with soil topography (steep-slope)[260]. Soil degradation affects water quality and quantity, and this threat is linked to inadequate soil erosion control and unsustainable land-use practices[261]. High sediment loads reduce the size of river channels and the water-holding capacities of lakes. *C. demersum* and *E. crassipes* are among the most common submerged plant species in Lake Rumira. Some farming and agricultural activities are carried out at the lakeshore of the sampling sites. This tropical lake is essential for the local population's ecology, regional hydrology, fishery, and water sources.

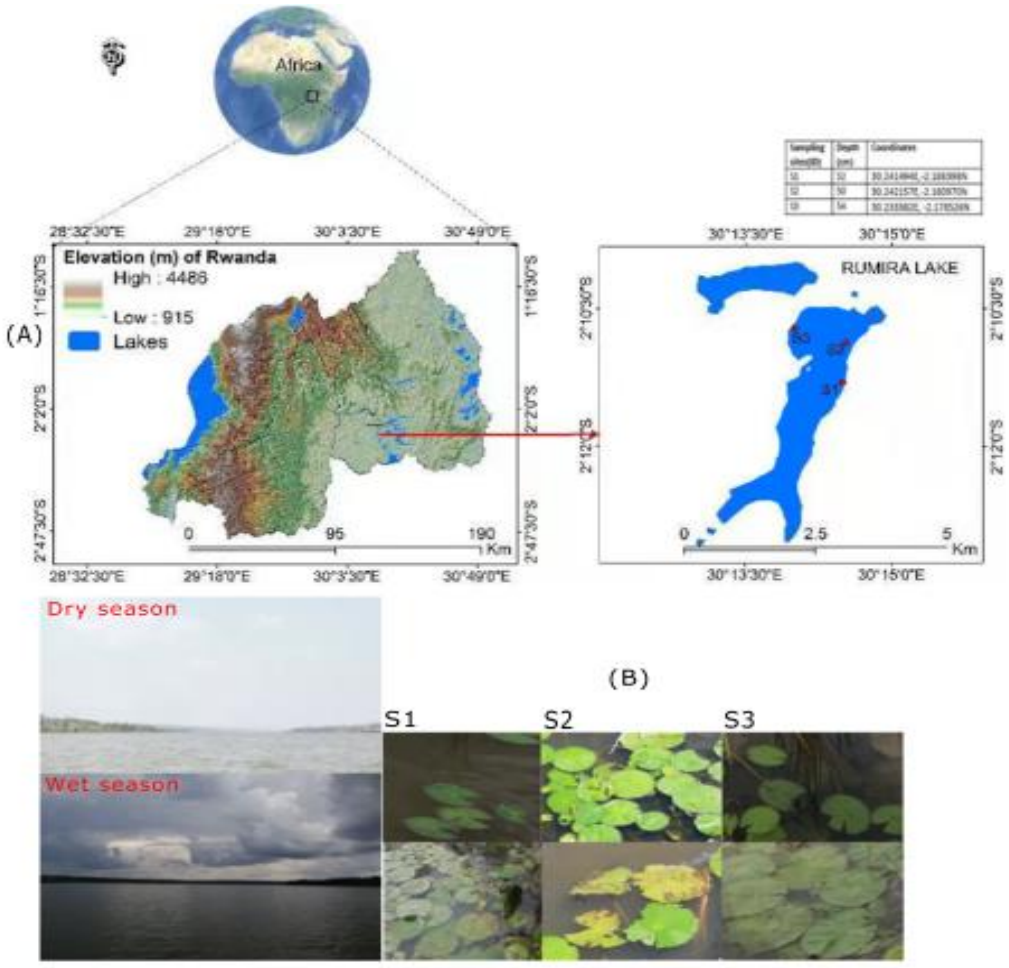


Figure 2. 1 Map of Rumira Lake (A: shows the map of Rumira Lake in Rwanda, B: shows the photos of aquatic plants in Rumira Lake).

2.2.2 Sampling and determination of environmental parameters

The aquatic macrophytes, surface sediments, and surface water samples were collected in Lake Rumira in the dry season (August) and wet season (November) in 2019. Dry season samples were taken on 10th August, and wet season samples were taken on 22nd November between 8:00 and 13:00 h. A total of 24 samples were collected from three sampling sites (S1, S2, and S3): 12 samples (*C. demersum*, *E. crassipes*, surface sediment, and surface water; termed C, E, S, and Sdry&Swet) were collected from S1(CD1, ED1, SD1, and S1dry), S2 (CD2, ED2, SD2, and S2dry), and S3 (CD3, ED3, SD3, and S3dry) in the dry season, while the remaining 12 samples were again sampled from S1 (CW1, EW1, SW1, and S1wet), S2 (CW2, EW2, SW2, and S2wet), and S3 (CW3, EW3, SW3, and S3wet) in the wet season (Fig.1). In the dry season, both *C. demersum* and *E. crassipes* were in their juvenile period with younger leaves; and in the wet season, both plants

began to senesce and decay [262]. The *E. crassipes* are rooted in sediments, while *C. demersum* does not root but can exist free-floating in the water column or near the sediment, depending on nutrient and light availability. At each site, three replicates of healthy aquatic macrophyte leaves (CD and ECr) were collected by hand with gloves or using a stainless hook, slightly washed with dH_2O, and then incubated immediately in a sterilized wild-mouth polyethylene bottle (500 ml) containing 350 ml 95% ethyl alcohol.

The surface water samples were collected below the overlying water at 0.5 m. About 1L of water around the cohabiting aquatic plants at each sampling spot was collected using an aseptic plastic bottle. Approximately 500 ml of water was used for chemical analyses.

Surface water samples collected were preserved and kept cool for less than 6 h before transporting them to the laboratory for proper analysis. A Peterson dredge was used to collect three replicates of surface sediment samples thoroughly mixed to create one sample. About 3g of superficial sediment (0-5cm) was collected and stored in a 10 mL sterile plastic tube containing 95% ethanol for at least 15 min. The V: V ratio of sediment to ethanol was > 3:7.

Physicochemical properties of the water samples, such as water temperature (WT), pH, electric conductivity (EC), total dissolved solids (TDS), oxidation-reduction potential (ORP), and dissolved oxygen (DO), were measured *in situ* using HQ30d portable multi-parameter digital analyzers (HACH, USA). Other environmental variables such as total nitrogen (TN), total phosphorus (TP), ammonia nitrogen (NH_4-N), and nitrate-nitrogen (NO_3-N) were measured in the laboratory following previously defined methods [263].

2.2.3 Treatment of epiphytic and surface sediment biofilm samples

In the laboratory, the epiphytic microorganisms on harvested samples were detached by taking approximately 50 g mixtures of ethanol (95%) and plant leaves of each species (*C. demersum* and *E. crassipes*) and then subjected to 1 hour of mechanical shaking (225 r/min) [81]. The suspensions from the same sample were combined and passed through a sieve with a mesh size of 0.8 mm to remove plant debris and small animals. This was then subsequently centrifuged at 8000 rpm for 10 min. Conversely, a mixture of sediments and ethanol was centrifuged at 8000 rpm for 5 minutes. Supernatants were discarded, and sediments of 2g per tube were stored. Epiphytic pellets and

sediment were mixed with 80% ethanol for a minimum of 1 min, centrifuged, and then stored carefully in clean 5 ml sterilized plastic tubes with a cap for shipping and DNA extraction in China.

2.2.4 DNA extraction and PCR amplification

DNA samples were extracted from 0.25 g of epiphytic and sediment biofilm using Fast DNA® SPIN extraction Kits (MP Biomedical, OH, USA) following the methods described in the manufacturer's protocol. DNA samples were checked by running on a 1% agarose gel electrophoresis and were quantified using a NanoDrop 2000 UV– Vis spectrophotometer (Thermo Scientific, USA) [264]. The bacterial V4-V5 hypervariable region of the 16S rRNA gene was amplified by a thermocycler PCR system (GeneAmp 9700, ABI, USA) using the primers 515F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), and eukaryotic V4 region of 18S rRNA using 3NDF (5'-GCGGTAATTCCAGCTCCAA-3') and V4-euk-R2 (5'-AATCCRAGAATTTCACCTCCAA-3').

2.2.5 Illumina MiSeq sequencing of 16S rRNA and 18S rRNA gene

Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, USA) following the standard protocols supplied by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

2.2.6 Bioinformatics

Raw reads (FASTQ files) were quality-filtered by Trimmomatic and merged by FLASH (https:// ccb.jhu.edu/software/FLASH/index.shtml) with the following criteria: The reads were truncated at any site receiving an average quality score < 20 over a 50 bp sliding window. OTUs were clustered with a 97% similarity cutoff using UPARSE (http://drive5.com/uparse/, version 7.1) with a novel 'greedy' algorithm that performs chimera filtering and OTU clustering simultaneously. The taxonomy of each 16S rRNA and 18S rRNA gene sequence was assigned by the RDP Classifier algorithm (http://rdp.cme.msu.edu/) and mapped against the Silva (SSU123) and (SSU138) database, respectively, with a confidence threshold of 70%.

2.2.7 Data analysis

Data analysis and visualization were performed on the free online platform of Majorbio Cloud Platform (www.majorbio.com) and in the R environment (https://www.r-project.org/). The 'Stats' and the 'spicy' R packages were used to perform the statistical comparisons. A one-way ANOVA was used to compare the environmental parameters among groups, and statistical significance was determined at $p < 0.05$. Pearson correlations analyses were done using IBM SPSS Statistics (https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-22, version 22.0). Alpha and beta diversity were computed using the 'vegan' package and were visualized using the 'ggplot2' graphic library in R. Permutational multivariate analysis of variance (PERMANOVA) test were performed to evaluate the difference in beta diversity among seasons and hosts using the Bray-Curtis index. The relative abundance of taxonomic compositions was visualized using the 'circlize' package. RDA (Redundancy analysis) was performed to describe the relationship between epiphytic biofilms and environmental parameters. Variation partitioning analysis (VPA) was used to determine key variables (microhabitat types versus physicochemical parameters), how much variation each explained, and the size of their shared effects.

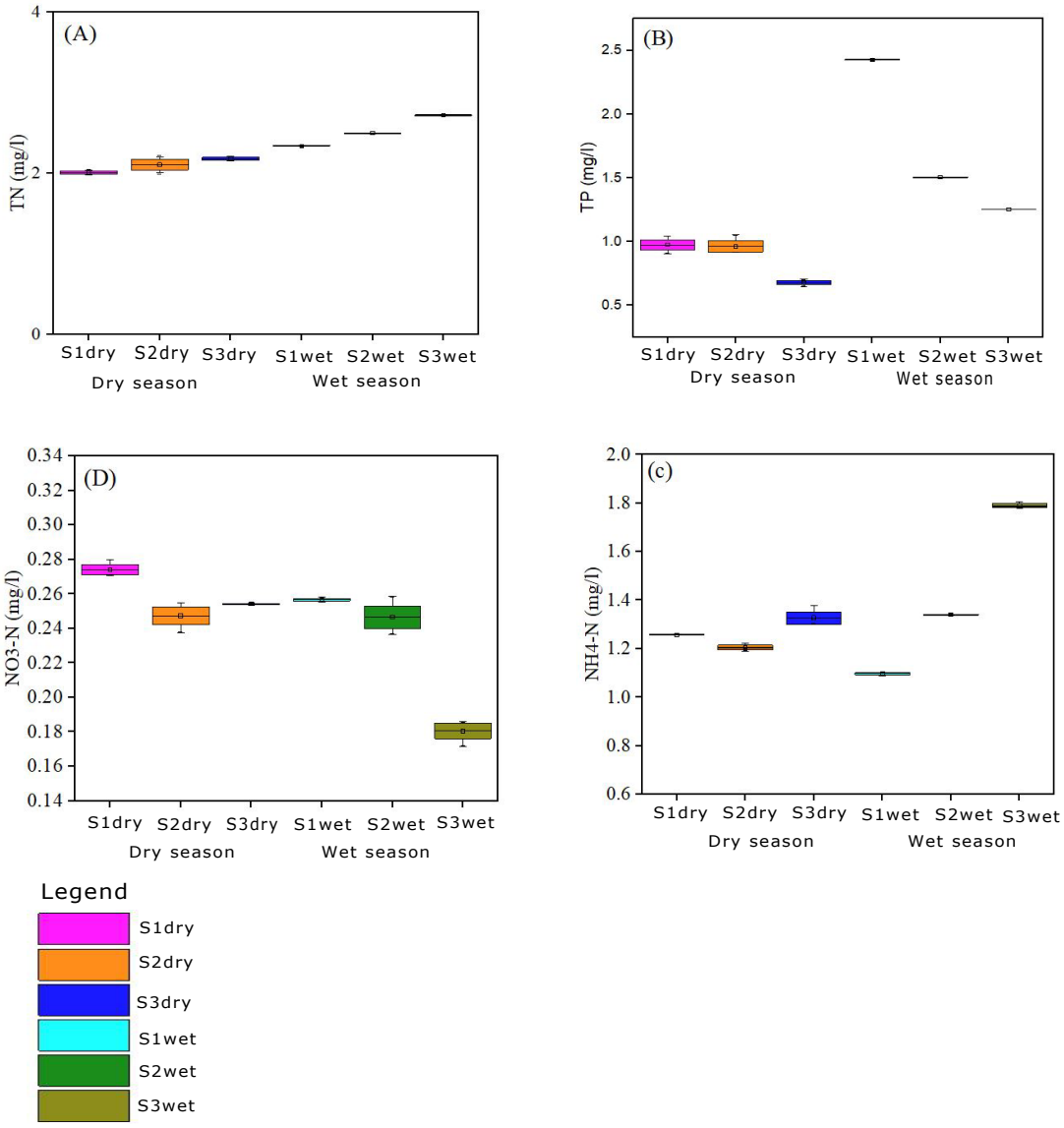
The Monte Carlo permutation test (permutations = 499) was used to assess the statistical significance of these relationships. Both RDA and VPA analyses were performed using CANOCO 5.0 software (https://www.wur.nl/en/show/Canoco-for-visualization-of-multivariate-data.htm, version 5.0). Network analysis was conducted based on Spearman's correlations calculated using the corr. test function of the 'psych' packages in R (https://www.r-project.org/, version 4) and visualized using Gephi (https://gephi.org/, version 0.9.2). Robust correlations were considered if Spearman's correlation coefficient (r) was > 0.7 or <−0.7 used for further analysis ($p < 0.05$) [265]. Based on OTU data, functional metabolic pathways of a bacterial community 16S rRNA gene sequences were predicted by PICRUSt [238]. PICRUSt2 (https://github. com/picrust/picrust2) was used to obtain information at different pathway levels (levels 1–3) compared with the clusters of orthologous groups (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases, and visualized in heatmap via R package (version 3.6.3). The above functional metabolic pathways were compared between the seasons and substrates using Statistical Analysis of Metagenomic Profiles (STAMP) (https://beikolab.cs.dal.ca/software/STAMP, version2.1.3) based on the Bonferroni-test ($p<0.05$). The sampling map was drawn using QGIS (QGIS Development Team, 2017, v2.18).

2.3 Results and discussion

2.3.1 Physicochemical parameters

Environmental parameters are essential for plants and microbes' growth in the water column. Temporal and spatial variations of physical-chemical variables of surface water in Rumira Lake are shown in **Figure 2.2 & 2.3**. The mean concentration of TDS, EC, NO_3-N, and pH were significantly higher ($p < 0.05$) in the dry season (85.4 mg/L, 188.7 μS/cm, 0.26 mg/L, and 8.4, respectively) compared to the wet season (80.3 mg/L, 164.2 μS/cm, 0.23 mg/L, and 7, respectively) (**Figure 2.3**). However, the mean concentration of TP and TN revealed a reverse trend. Furthermore, water temperature (27.7^{o}C) and DO (7.6 mg/L) values were also higher ($p > 0.05$) in the dry season compared to the wet season (26.3^{o}C and 2.8mg/L, respectively), whereas NH_4-N showed a reverse trend. Invariably, most of the surface water physicochemical parameters determined for the 3 sites of lake Rumira had the same spatial variation regularity with the exception of NO_3-N at site WW2 ($P > 0.05$) (**Figure 2.2**). The nutrients, such as TP, TN, NO_3-N, and NH_4-N, were noticeably beyond the maximum permissible limit of the World Health Organization (WHO) for surface water quality[266]. The low pH could aggravate the acidic state of the surface water, thus leading to a corrosive and bitter metallic taste of water[267]. More so, the persistence of low pH in the water environment could negatively affect aquatic plant nutrient availability and the fate of other contaminants [268]. The EC is the capacity of a solution to potentially conduct electric current, which implies the availability and the concentration of ionic species (salinity indicator) [269]. Thus, causing a disequilibrium of aquatic biota in water bodies and leading to depletion of DO concentration[270].

Excess nitrate and phosphate concentrations in Lake Rumira could instigate the eutrophication process[271], leading to depletion of DO in surface water, which could stimulate algae bloom and lead to suffocation and death of aquatic microbes[272]. Compared to lakes Poyang and Lake Dongting (China) and Awash River (Ethiopia), the results obtained for the nutrient values in Lake Rumira are higher[273,274] but comparatively lower than those obtained for the eutrophic Beseka Lake (Ethiopia) [275]**.** This condition of increased nutrient pollution in Lake Rumira can be ascribed to the inlet rivers, run-off, agricultural activities, siltation, and other land use types in the tropical region, as reported in a previous study[276].


(A)
TN (mg/l)
0
2
4
S1dry S2dry S3dry S1wet S2wet S3wet
Dry season
Wet season
(B)
TP (mg/l)
0.5
1.0
1.5
2.0
2.5
S1dry S2dry S3dry S1wet S2wet S3wet
Dry season
Wet season
(D)
NO3-N (mg/l)
0.14
0.16
0.18
0.20
0.22
0.24
0.26
0.28
0.30
0.32
0.34
S1dry S2dry S3dry S1wet S2wet S3wet
Dry season
Wet season
(c)
NH4-N (mg/l)
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
S1dry S2dry S3dry S1wet S2wet S3wet
Dry season
Wet season
Legend
S1dry
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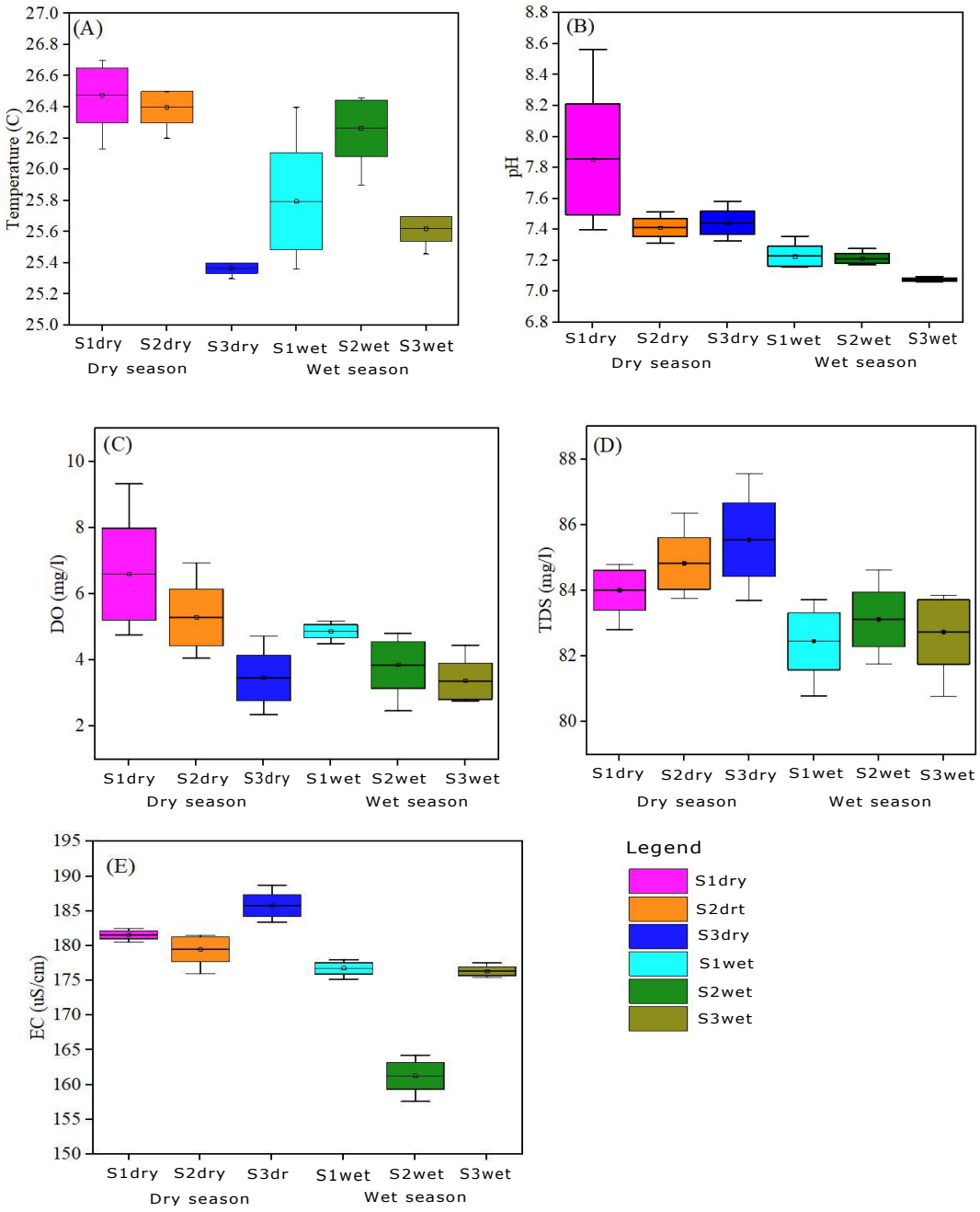


Figure 2. 2 Chemical parameters of the water in different sites of Lake Rumira. (A) Total nitrogen, (B) Total phosphorous, (C) Ammonia nitrogen, and (D) Nitrate nitrogen. While Physical parameters are (A) Temperature, (B) pH, (C) Dissolved oxygen, (D) Total dissolved solids, and (E) Electrical conductivity.

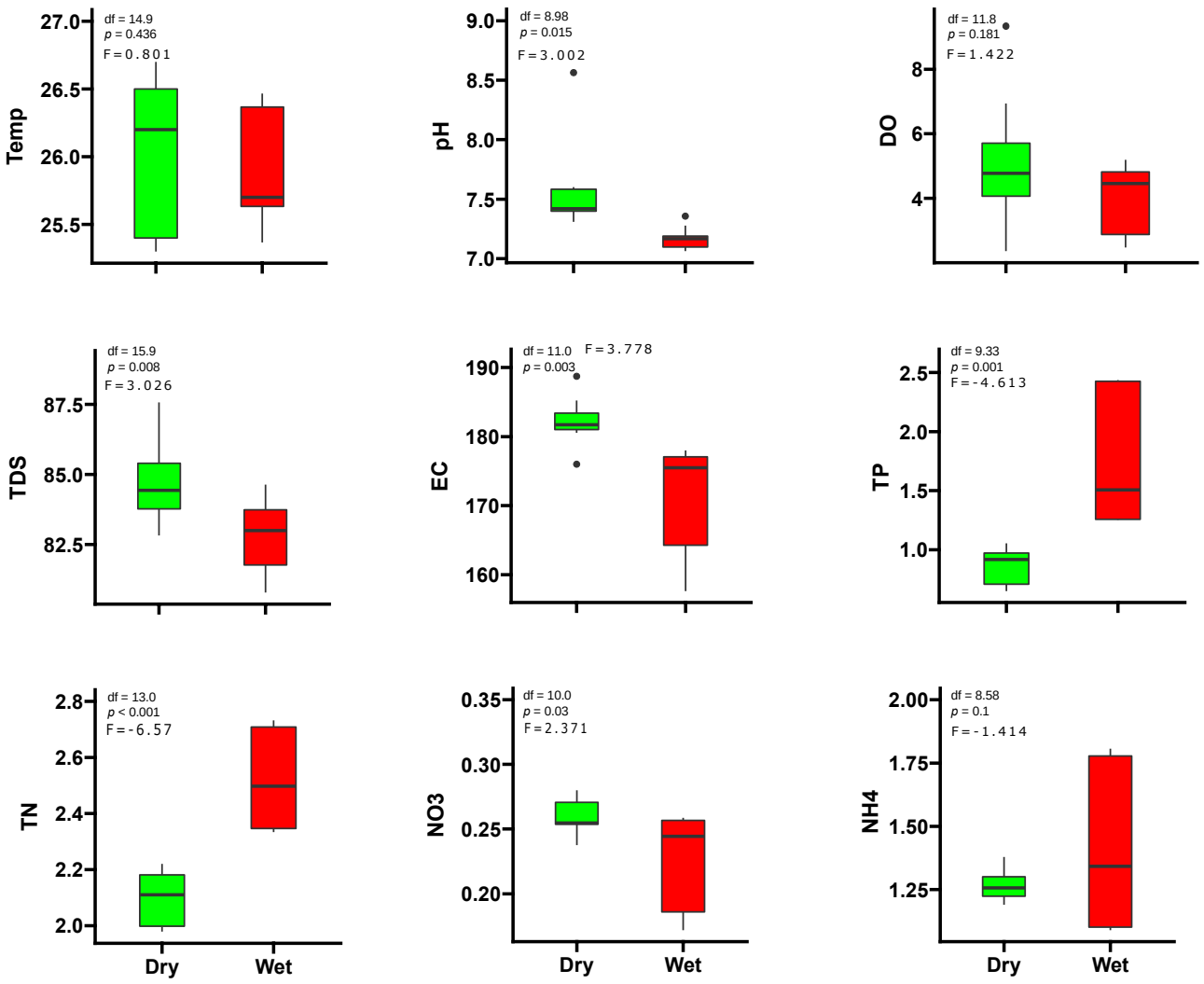


Figure 2. 3 Physicochemical parameters of the surface water in Lake Rumira across seasons (dry and wet).

2.3.2 Influence of microhabitats on bacterial community structure in epiphytic biofilm

A total of 1,133,117 16S rRNA clean reads were normalized to 1,120,342 in all samples. There was no significant difference ($P > 0.05$) in all the α-diversity indices (OTU richness, Shannon values, and evenness) (**Figure 2.4A**) of the bacterial community in biofilm among microhabitats (*C. demersum*, *E. crassipes*, and sediments) and seasons ($P > 0.05$), however, OTUs richness was generally higher on *C. demersum* when compared to *E.crassipes* and sediments (**Figure 2.4A**). Contrary to this study, a recent mesocosm study revealed a reverse trend [82], where most α-diversities were significantly higher in sediment than those in epiphytic biofilm samples. Notably, a higher abundance of OTUs in epiphytic biofilm (*C. demersum*) than in sediments was observed in this study (**Figure 2.4A**). This may be due to the connection between water, aquatic plants, and sediments, which in turn facilitated the sediment bacteria migration to the roots, then stem and leaf surfaces [19]. In addition, Liu et al. [85] proved that sediments rather than the water column were the seeding bank of a large number of epiphytic bacterial species, suggesting the enrichment of the epiphytic bacterial pool from sediments. The principal coordinate analysis (PCoA) for the bacterial community explained about 70 % of the total variance and revealed that the bacterial community dissimilarity was different among substrates ($R^2 = 0.41$, $P = 0.001$) but not among seasons ($R^2=0.03$, $p=0.53$) (**Figure 2.4B**)**.** The OTUs richness superiority in *C. demersum* over other microhabitats may be attributed to the host-specificity (allelopathy, physiology) or microhabitat type and spatial heterogeneity, and this is in tandem with previous reports [33,82,91].

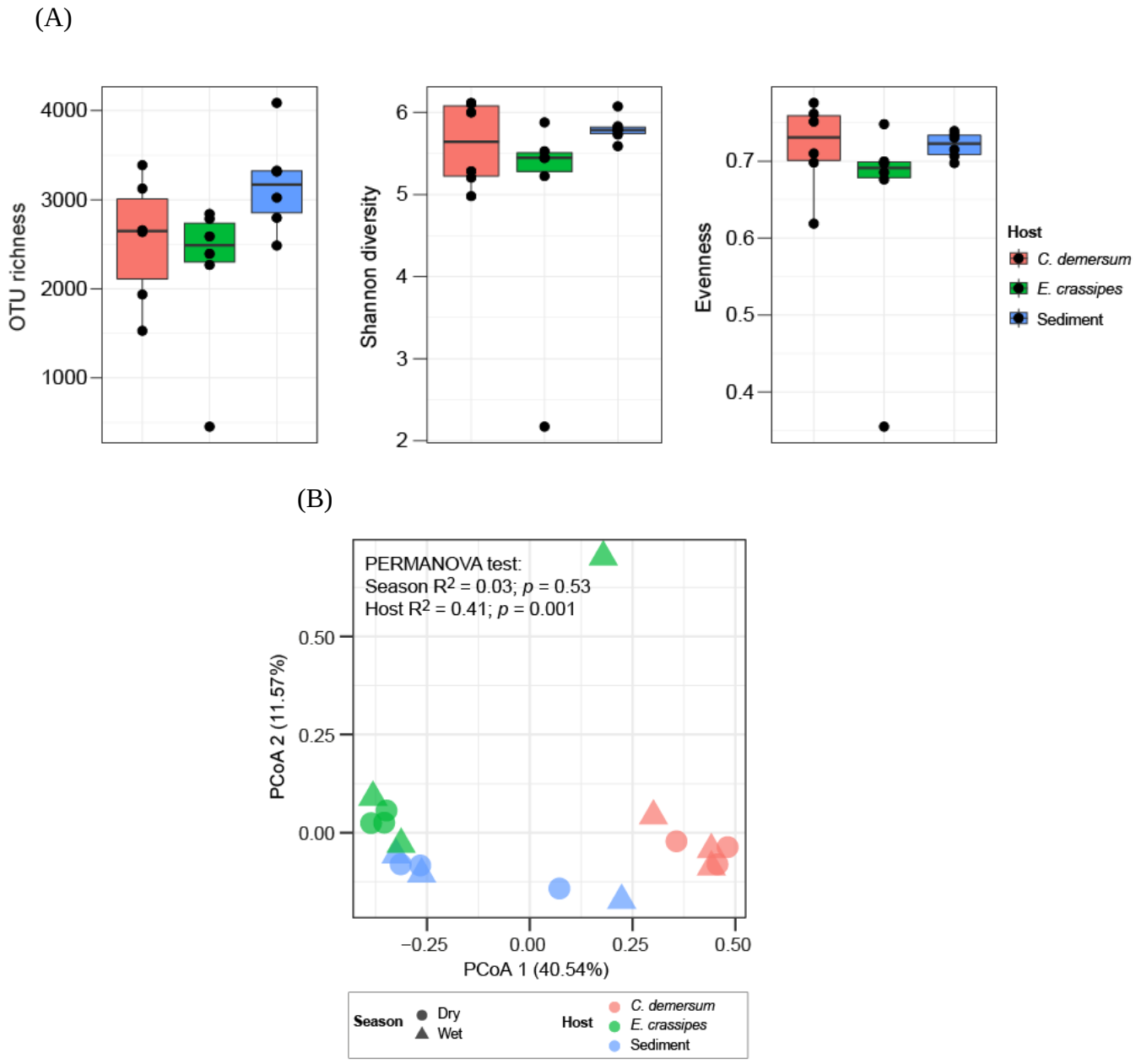


Figure 2. 4 Changes in the alpha (A) and beta (B) diversities of the bacterial community in the biofilms of floating macrophytes (*E. crassipes*), submerged macrophytes (*C. demersum*) and sediments in Lake Rumira (Rwanda) in the dry and wet seasons. OTU richness, Shannon index and evenness were not statistically different among microhabitat types

Approximately 64 and 143 OTUs common to all samples from *C. demersum*, *E. crassipes*, and sediments were identified in the dry and wet seasons, respectively. OTUs numbers were higher in wet than dry seasons **(**except for CD2 and SD1), and this value was more in *C. demersum* than in sediments and *E. crassipes* in two seasons (**Figure 2.5A-B**). This finding is similar to that of Xia et al.[32], which confirmed that the lower diversity found in the growing period is a consequence of the natural colonization process of the epiphytic biofilm. Meanwhile, the bacterial community in sediments was higher than in epiphytic biofilms in another study (Liu et al., 2020), which is contrary to our findings in this study. Significantly, the higher OTUs number in epiphytic biofilms (*C. demersum*) than in sediments is envisaged to be caused by sediments resuspension and/or sediment bacteria migration to the surface of stems and leaves [19].

A total of nine dominant bacterial phyla (relative abundance >1% in at least one sample) were detected in samples from *C. demersum*, *E. crassipes*, and sediments (**Figure 2.5C-D**). Compared to the wet season, higher Firmicutes abundance was detected in three sampling sources in the dry season, while higher Proteobacteria and Cyanobacteria were detected in *C. demersum* in the wet season. The most dominant bacterial phylum was Firmicutes in sediments and *C. demersum* and Proteobacteria in *E. crassipes*. Cyanobacteria were higher in *E. crassipes* and *C. demersum* than in sediments. Notably, the variation in the other six bacterial phyla across microhabitats, seasons, and sampling sites was insignificant. Nine bacterial phyla (except for Cyanobacteria) were found to be dominated in biofilms on many aquatic macrophytes such as *Vallisneria natans* and *Hydrilla verticillata* in Taihu Lake [81], *E. crassipes* in Brazilian wetlands [94], and *C. demersum* [91] and sediments [82].

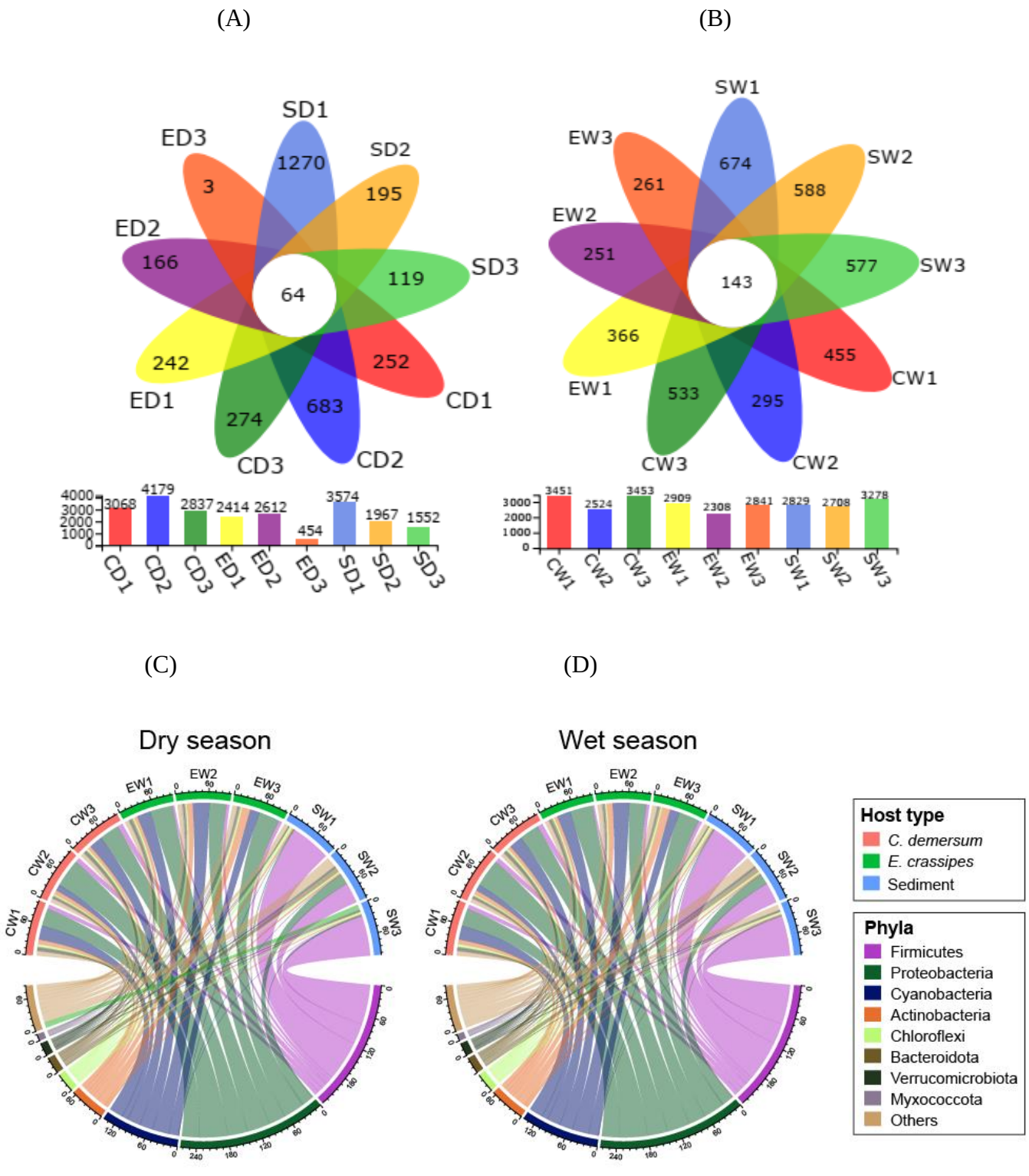


Figure 2. 5 Taxonomic composition of bacterial community OTUs (A) and Phylum (B) in the biofilm of floating macrophytes (*E. crassipes*), submerged macrophytes (*C. demersum*) and sediments in Lake Rumira (Rwanda) during the dry and wet seasons of the year 2019

Most dominant bacterial phyla were significantly different among *C. demersum*, *E. crassipes*, and sediments in the dry and wet seasons **(Figure 2.6).** The Firmicutes abundance was significantly higher in sediment ($P < 0.05$) than other microhabitats, whereas Actinobacteria, Cyanobacteria, and Chloroflexi phyla were significantly ($P < 0.05$, $p < 0.01$) higher in *C. demersum* than *E. crassipes*. Moreover, the relative abundance of Proteobacteria was significantly ($p < 0.01$) higher in *E. crassipes*. On the other hand, Verrucomicrobiota was of higher significant ($p < 0.05$) occurring phylum in the wet season than during the dry season (**Figure 2.7**). The distributions of dominant phyla (Cyanobacteria, Chloroflexi, and Actinobacteria, proteobacteria) were similar to those detected in sediments [133,277], submersed macrophytes (e.g. *C. demersum*, *Potamogetonaceae*, *Vallisneria natans*) [32,81,91], *E. crassipes* [278], and in wet season[279]. However, the relative abundance of dominant phyla varied in various microhabitats and seasons. These findings may be attributed to the plant-specific effects (allelopathy and physiology), environmental filtering (e.g., inorganic nutrients), and succession that shaped the microbial dynamics at the phylum level; however, these viewpoints need further research.

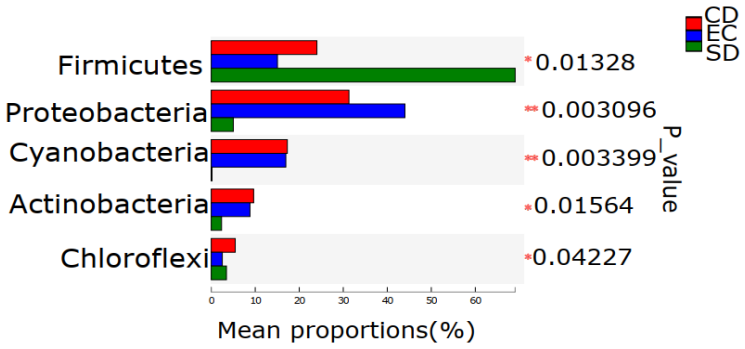


Figure 2. 6 Kruskal-Wallis test's sample comparison in bacterial phyla on *C. demersum* (CD), *E.crassipes* (EC), and sediments (SD). * signifies $p<0.05$, ** $p<0.01$.

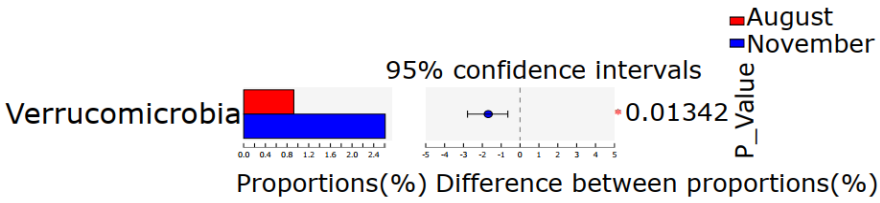


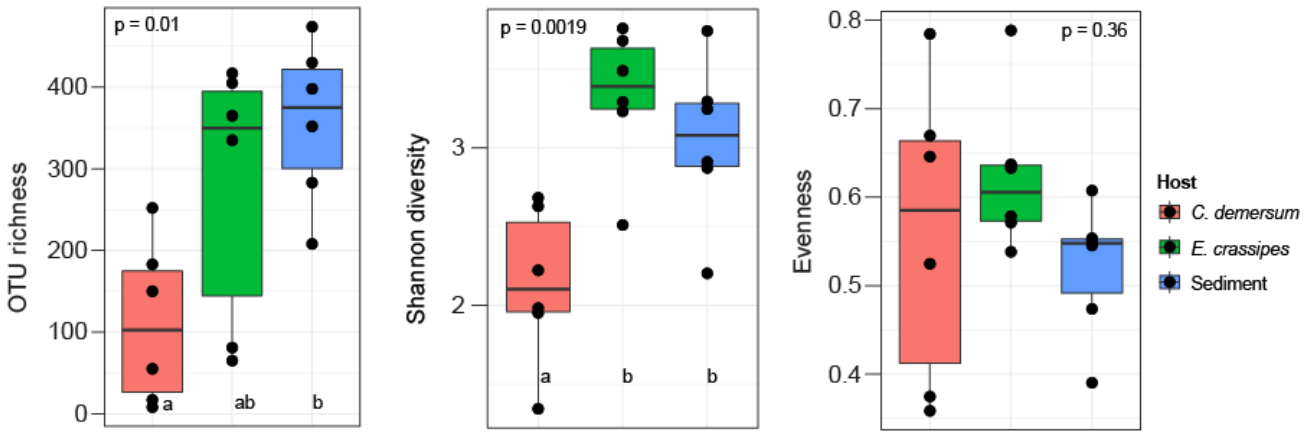
Figure 2. 7 Wilcoxon rank-sum test's sample comparison in bacterial phyla in dry and wet seasons* signifies $p<0.05$.

2.3.3 Influence of microhabitats on microeukaryotic community structure in epiphytic biofilms

The eukaryotic community is an essential component of aquatic ecosystems and occupies special ecological niches in epiphytic biofilms. In this study, the diversity of eukaryotes based on 18S rRNA in biofilms on *C. demersum*, *E. crassipes*, and sediments was analyzed. The alpha diversity (OTU richness, Shannon diversity, and evenness) indices for all the eukaryotic samples are provided in **Figure 2.8A**. In agreement with Liu et al.[82], Shannon diversity and OTUs richness of microeukaryotes were higher in sediments than those in epiphytic biofilm samples from *C. demersum (*$P < 0.05$*)*. The reason for the higher eukaryotic community diversity in surface sediments than epiphytic biofilms observed in this study is yet unknown, but we assume that it is likely due to the strong environmental gradients, spatial variation, stochastic processes, and microhabitat characteristics (leaf morphology) [92,96,125]. For example, the sediment communities experienced a higher degree of resource heterogeneity within the sediment matrix than the epiphytic communities. Furthermore, the 3D matrix of the sediment habitat provided the epipelon with more surface area for biofilm growth than the surfaces of the macrophyte leaves [35]. The generally lower eukaryotic diversity in the epiphyton relative to the surface sediments suggests that intense competition (e.g., inhibition) and species filtering dictated the diversity of the eukaryotic community on the aquatic macrophytes [112,280]; however, these viewpoints need further research.

The PCoA of the microeukaryotic community also had a significant amount of variation (74.5 %) of the total variance, and it revealed that the microeukaryotic community was statistically different ($R^2 = 0.26$, $P < 0.01$) among substrate types but not among seasons **(Figure 2.8)**. Similar to bacteria, the microeukaryotic community in sediments was more closely related to *E. crassipes* than to *C. demersum*. Only two and four OTUs were shared by all samples in dry and wet seasons, respectively (**Figure 2.8A-B**), and a low number of eukaryotic OTUs from *C. demersum* and sediments in the wet season was similar to the dry season one (except for the site SD3). Previous studies have revealed that abiotic factors such as pH [281], depth [138], and various nutrients [282] could influence the distributions of microeukaryotic communities in aquatic environments.

(A)



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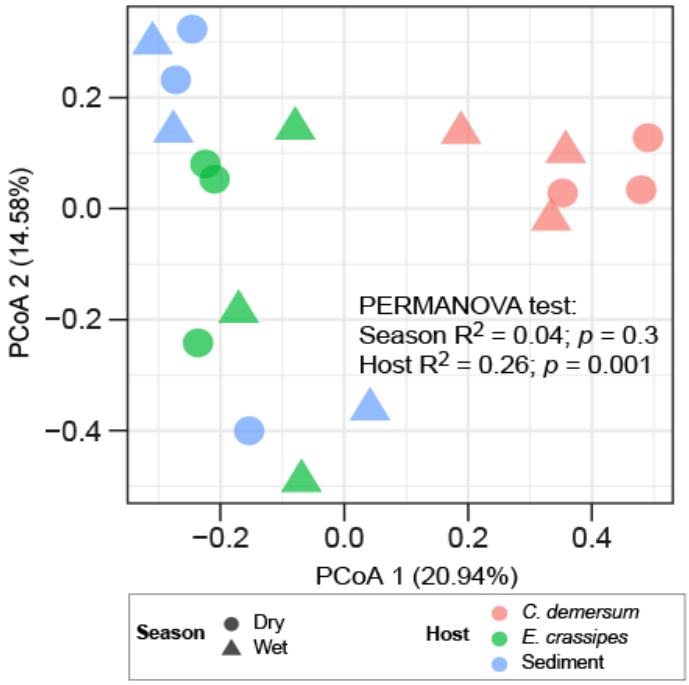


Figure 2. 8 Alpha (A) and beta (B) diversities of microeukaryotic communities in the biofilms of floating macrophytes (*E. crassipes*), submerged macrophytes (*C. demersum*) and sediments in Lake Rumira. Submerged macrophytes (*C. demersum*) exhibited lower microeukaryotic Shannon diversity than floating macrophytes and sediment biofilms.

According to 18S rRNA gene annotation, kingdom Metazoa, Chloroplastida, Fungi, Amoebozoa, Discoba, Cryptophyceae, and undefined clades were detected in all samples. In addition to that, not less than eight phyla with relative abundance >1% in at least one sample were detected in the microeukaryotic community of three microhabitats **(Figure 2.9C-D)**. In epiphytic biofilms, the Unclassified SAR, Rotifera, and Gastrotricha were the dominant kingdoms, while Ascomycota and Platyhelminthes dominated surface sediments. Unclassified SAR and Platyhelminthes were the most abundant kingdoms in the dry season (**Figure 2.9C**), while Rotifera and Gastrotricha dominated in the wet season (**Figure 2.9D**).

No distinctive difference in temperature between dry and wet seasons was recorded in this study. Meanwhile, in previous studies, Morley [283] and Ana et al. [284] revealed that warmer seasons/months and water temperature have a direct influence on Platyhelminthes occurrence, high-density variation, and population dynamics, which can be connected to the increase of the planarian's predation resulting from high food availability in warm months [285]. However, the influence of temperature on density is less clear; in a population of *Schmidtea mediterranea* [286] from Tunisia, Harrath et al. [287] proposed a decrease in the density of planarians in summer due to an increase in water temperature. Furthermore, Francavilla et al. [288] indicated no relationship between water temperature and the density of planarians (*G. capacivasa*). Therefore, the contrasting trend in the influence of water temperature on Platyhelminthes dynamics is probably due to the broad-spectrum of the thermal range of Platyhelminthes offering more tolerance to temperature variations. Alveolates in the SAR clade are abundant during the summer and known to encyst and revive when conditions become hospitable, suggesting a community turnover from the microbial seed bank in lake sediments [289,290].

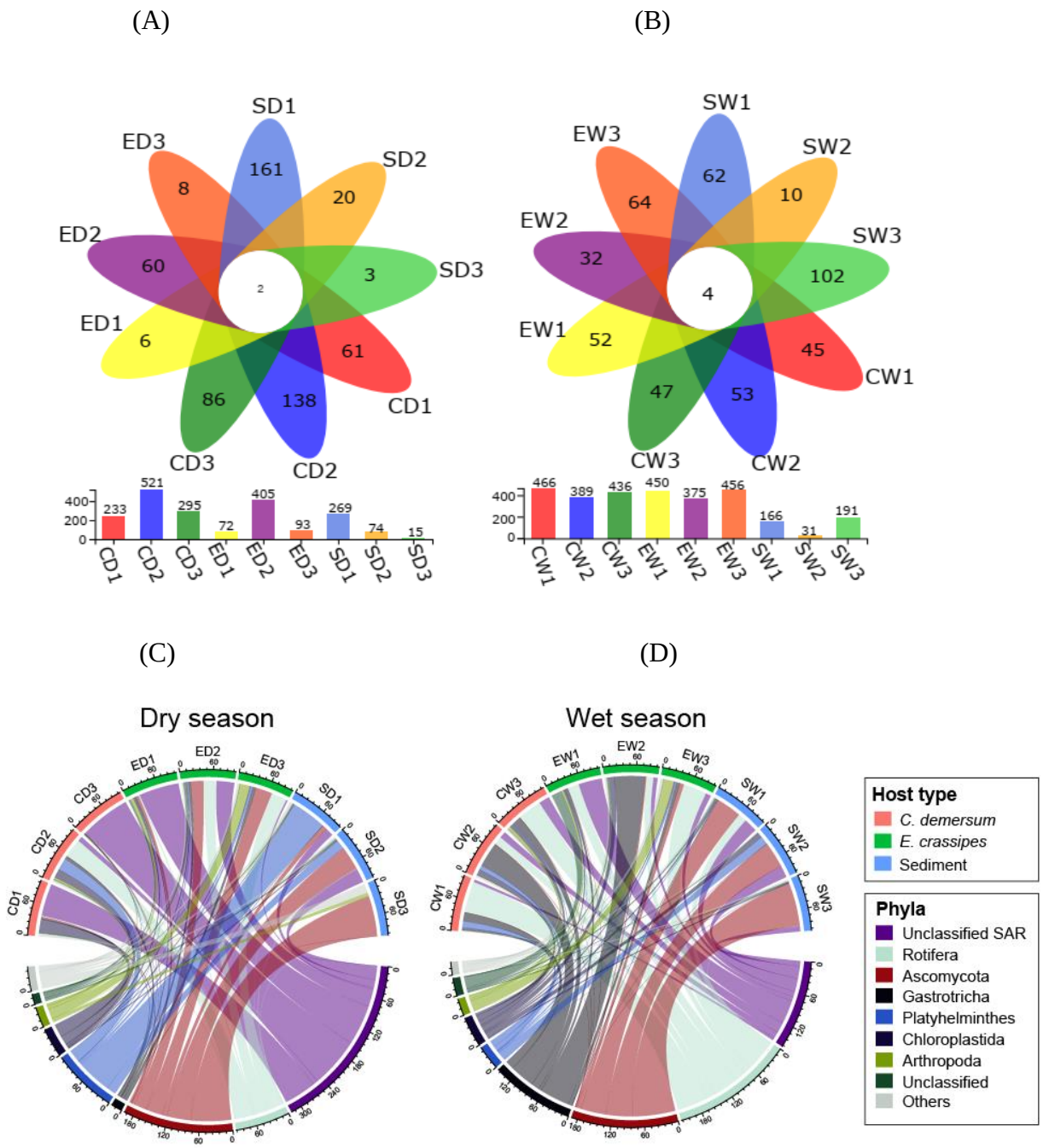


Figure 2. 9 Taxonomic composition of microeukaryotic community OTUs (A-B) and Phylum (C-D) in the biofilms of floating macrophytes (*E. crassipes*), submerged macrophytes (*C. demersum*) and surface sediments in Lake Rumira (Rwanda) during the dry and wet seasons of the year 2019.

The relative abundance of Rotifera, Gastrotricha, and Chloroplastida was significantly ($P < 0.05$, $P < 0.01$) **(Figure 2.10)** higher in *C. demersum* than in *E. crassipes* and sediments. However, the relative abundance of Ascomycota was significantly ($P < 0.01$) higher in sediments than in aquatic plants. It should be noted that seasonal shifts also had an insignificant effect on the dominant eukaryotic phyla. Rotifers and other metazoans, such as Gastrotricha, live in unfavorable or harsh environments and serve as bio-indicators in the water environment [291,292]. Additionally, members of Rotifers prey on bacteria, mold, algae, protozoa, and organic particles [293]. Therefore, an increase in the relative abundance of Rotifers and Gastrotricha indicate that they can adapt to high organic nutrients brought by effluents and runoff from agriculture activities (total phosphorus, ammonium, and total nitrogen)[294].

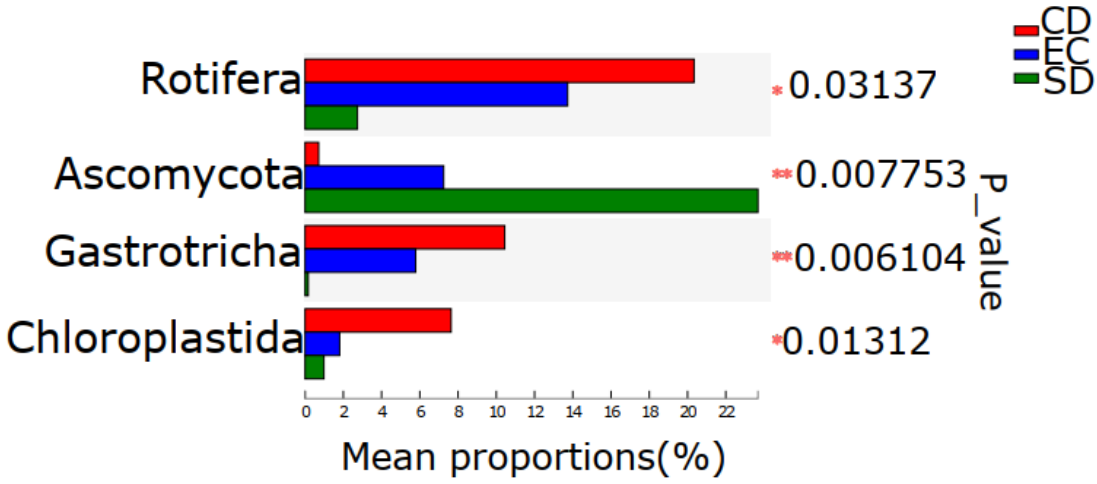


Figure 2. 10 Kruskal-Wallis test's sample comparison in eukaryotic phyla on *C. demersum* (CD), *E. crassipes* (EC), and sediments (SD). * signifies $p<0.05$, ** $p<0.01$.

2.3.4 Influence of environmental parameters on microbial community in epiphytic biofilms

A total of 12 bacterial and 9 dominant eukaryotic genera in biofilms on *C. demersum* and *E. crassipes* were significantly correlated with environmental parameters either positively or negatively (**Figure 2.12A and 2.12B**). RDA explored the relationship between microbial genera on two aquatic plants and environmental parameters (**Figure 2.12**). Furthermore, VPA was conducted to assess which variables (microhabitat types versus physicochemical parameters) were dominant in driving the distribution of microbial communities among all samples, and how much variance each explained alone or in combination. Microhabitat types and physicochemical variables together explained 68% of total variation (**Figure 2.11C,** $P < 0.5$). The conditional effect of microhabitat types was 37% and physicochemical variables account 21%.The shared effect of these two variables was 10%, while an unexplained variation was 32%.

The Pearson correlation revealed that DO and pH were significantly positively correlated (* $P < 0.05$, ** $P < 0.01$; $r > 0.7$) with *Methylomagnum* and *Amaricoccus* genera in biofilm on *C. demersum*, while *Methylomagnum* was significantly positively correlated with TN. *Amaricoccus* and family *Commamonadaceae* are negatively correlated (* $P < 0.05$, ** $P < 0.01$; $r > -0.7$) with temperature and EC on *E. crassipes* (**Appendix Table S1 & 3**). *Methylomagnum* is a methane-oxidizing bacterium (methanotroph), active in the presence of O_2[295] and also showed a nitrogen fixation capability in the rice rhizosphere[296]. Consistent with the previous studies where water hyacinth parts (surface or roots)[297] and duckweeds [298] provide habitat and stimulate the growth of various methanotrophs (*Methylomonas*, *Methylocaldum*, *Methylospira*) for CH_4 oxidation, this study suggests that *Methylomagnum* may be involved in methane oxidation from *E. crassipes* in the lake. The *Amaricoccus* grow well in neutral to alkaline pH in mangrove soil and subtropical coastal vegetation[299]. However, the negative correlation of *Amaricoccu*s with temperature and EC and f_(family) *Commamonadaceae* with EC on *E. crassipes* may be associated with direct exposure of floating leaf to sunlight (UV and desiccation) and plant physiology [251,300].

Bacterial genera (*Acinetobacter*, *Solibacillus*, *Romboutsa*, and *Lysinibacillus*) on *E. crassipes* significantly positively correlated with TP, NH_4-N, pH, and temperature, while *Bacillus*, Alicyclobacillus, f_ *JG30.KF.CM45*, *Solibacillus*, and *Paenibacillus* were significantly negatively correlated with TN. The positive correlation is associated with solubilization of phosphate using nitrogen compounds such as ammonium and nitrate [83,301], circumneutral and mesophilic

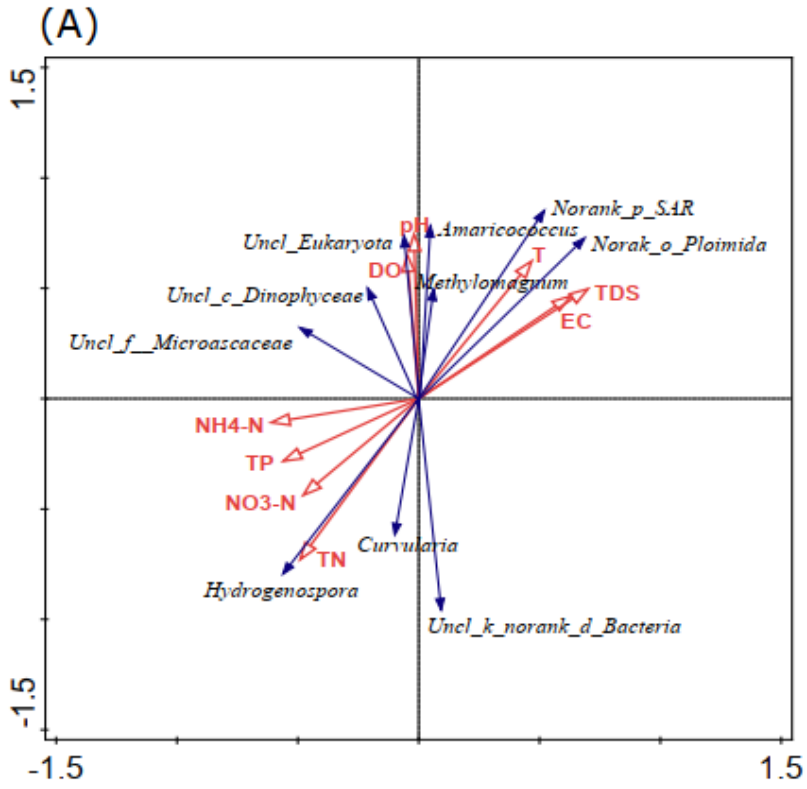
endophytes[302], cellulose degraders during floating plant decline period optimum temperature and pH [303] and entomological control and bioremediation capability in a broad spectrum of pH [304]. Eukaryotic community in o_ (order) Rhabdocoela and o_Ploimida on *C. demersum* showed a significant positive correlation with NO_3-N, temperature, TDS, pH, and DO, while order Adinetida and Asperigillaceae on *E. crassipes* revealed a significant positive correlation with TN and pH (**Appendix Table S2 & 4**).

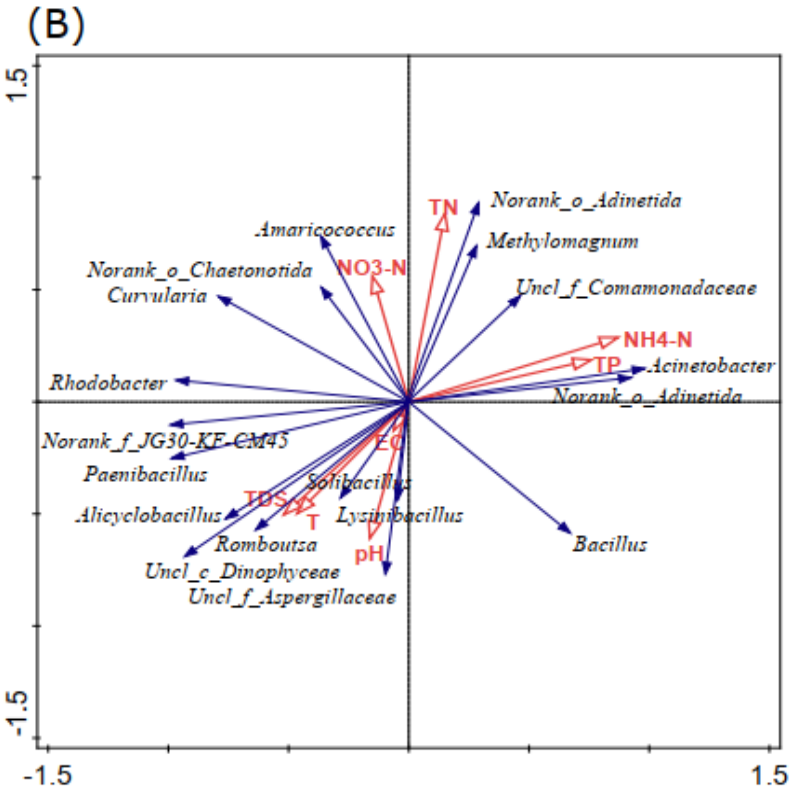
However, a number of taxa in eukaryotic phyla such as Rotifera, Fungi, Algae, and Gastrotricha on *C. demersum* and *E.crassipes* were significantly negatively correlated with TN, NH_4-N, EC, temperature, and TDS (**Appendix Table S2 & 4).** Weihrauch et al. [305] investigated mechanistic studies of ammonia excretion of nephridia and epidermal tissues in freshwater Schmidtea Mediterranean, suggesting the positive link between Platyhelminthes (o_Rhabdocoela) with nitrate in this study. Ploimida (Rotifers) feed on organic detritus in a wide range of temperatures, and Adinetida lives in a hostile environment, including a nitrogen-rich (TN) environment[294].

The negative correlation of Ploimida with TN and NH_4-N on *C. demersum* and that of Adinetida with temperature and TDS on *E. crassipes* may be due to the toxicity from high concentrations of TN and NH_4-N in the water column [306,307]. Meanwhile, Adinetida can be inhibited by elevated temperature (UV radiation) and total dissolved solids from exudates on the floating leaves. Also, Asperigellaceae in Ascomycota phylum can produce organic compounds (antibiotics and weak acids) and perform submerged fermentation at 20-30 °C and pH (5-7) [308,309], indicating that Asperigillaceae can produce its end-products in weak to neutral pH as supported by the findings this study.

Although Rotifers and fungi thrive well in organic matter-rich environments, it is envisaged in this study that the Lake may harbor diverse genera of Rotifers (Ploimida and Adinetida) and fungi (Curvularia and Microascarceae), responding differently to aquatic plant metabolism and nutrient loads (salinity and nitrogen compounds). Dinoflagellate species density has been related to low nutrient availability [310]. However, recent reports of Kutlu et al.[311] and Accoroni et al. [312] showed that Dinoflagellates (Dinophyceae) were negatively and positively correlated with nutrients such as NO_2-N, NO_3-N, TN, and TP, suggesting TN toxicity in the Dinophyceae family; however, this viewpoint needs further research.

Altogether, the microbial community on *C. demersum* was more positively correlated with pH, DO, and temperature, while microbes on *E.crassipes* were more negatively correlated with TN and EC, suggesting that the oxygen release and submersed lifestyle by submerged macrophytes. Suitable temperature and pH in the water column also allow epiphytic biofilms to thrive well on *C. demersum*. In addition, submerged macrophytes provide a larger accessible surface area for epiphytic microbes than floating plants, which present a hostile environment on the upper surface leaf.





(C)

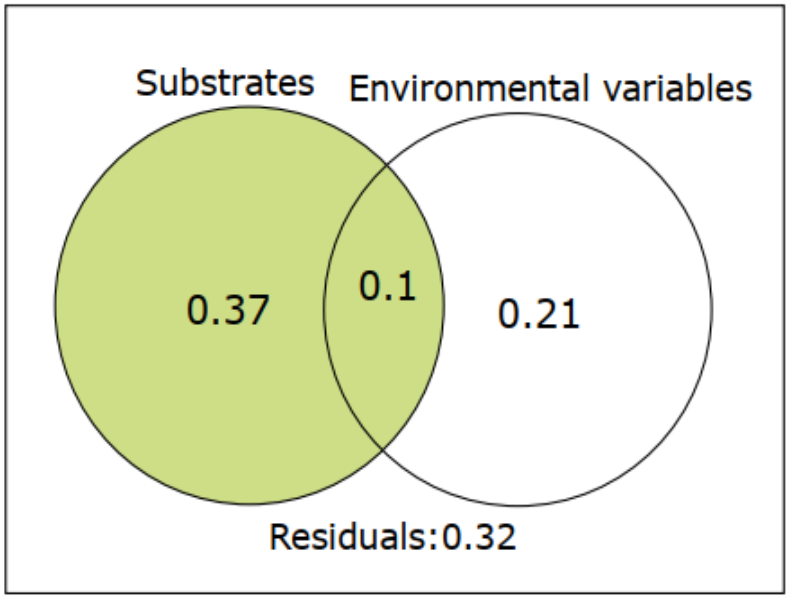


Figure 2. 11 Redundancy analysis (RDA) ordination showing associations between microbial communities in epiphytic biofilms to environmental parameters (A-B) and Variation Partitioning of epiphytic biofilms by physicochemical variables and microhabitat types (C).

2.3.5 Microbial interactions and ecological functions in epiphytic and epipelic biofilms

Co-occurrence networks were employed to investigate the possible interactions among the top 25 dominant microbial genera in each microhabitat (*C. demersum*, *E. crassipes*, and surface sediments) **(Figure 2.13A-C)**. A total of 197 edges by 46 nodes (genera), 142 edges by 45 nodes, and 68 edges by 39 nodes were identified in *C. demersum*, *E. crassipes*, and surface sediments, respectively. Topological properties, such as average degree, average path length, modularity class, and average clustering coefficient, were calculated to describe the complex pattern of correlations among the microbial genera in the network [216]. The computation of the average degree, average path length, modularity class, and average clustering coefficient were, respectively, 8.56, 3.15, 5.28, and 0.55 in *C. demersum*; 6.31, 3.18, 2.22, and 0.68 in *E. crassipes*; and 3.25, 5.77, 1.1 and 0.51 in surface sediments samples. In addition, the calculated modularity values were 5.28, 2.22, and 1.1 in *C. demersum*, *E. crassipes*, and sediments, respectively. The modularity values were both > 0.4, which indicated that the networks have evident modular structures[313]. These results suggested that interactions are more complex in *C. demersum* than *E. crassipes* and surface sediments. The nodes (genera) with degrees greater than 90% of the maximum degree are designated network hubs or keystones, which play a pivotal part in maintaining microbial community stability in biofilms [217].

Depending on the growth form of macrophytes, submerged plants grow below the water surface, which allows higher light penetration, and provides complex and large surface area for epiphyte development [314,315]. Based on the feeding style, the food webs were categorized into three trophic ranks namely, the producers (photosynthetic microeukaryotes and prokaryotes), consumers (metazoans), and decomposers (bacteria and fungi). Significantly, the microbial food web in *C. demersum* comprised feeding relationships among 9 producers (Pinnularia, Symbiodinium, Ulnaria, Dinophyceae, Heterocapsa, SAR_k_norank, Glenodinium, Chlorophycea, and Rhodobacter), 10 consumers (Ploimida, Adinetida, Flocullarceae, Bdelloidea, Philodinida, Platyhelminthes, Nematozoa, Eugregarinorida, Arthropoda, and Gastrotricha), and 23 decomposers (*Exiguobacterium*, *Acinetobacter*, *uncl_f_T34*, *Methylococcaceae*, *Bacillus*, *Solibacillus*, *Fictibacillus*, *Paenibacillus*, *Hungateiclostridium*, *clostridium Sensi Stricto_1*, *norank_f_JG30.KF.CM45*, *Amaricoccus*, *uncl_f_Commamonadaceae*, *Hydrogenospora*,

Romboutsa, *Crenothrix*, *Methylomagnum*, *Alicyclobacillus*, *Lysinibacillus*, *Aspergillaceae*, *uncl_f_Microascaceae*, *Curvularia*, and *Onygenales*). Remarkably, dominant microbial taxa in trophic levels of this food web were also dominant in *E. crassipes* and surface sediments.

The keystone taxa of the food web were determined based on the closeness centrality index. Genera of Firmicutes (*Exiguobacterium*), Proteobacteria (*Acinetobacter*, family T34, and *Methylococcaceae*), Fungi (*Aspergillaceae*), and algae (*Symbiodinium*) were the keystone taxa in *E. crassipes* (**Figure 2.13A**). On the other hand, genera of Firmicutes (*Bacillu*s, *Solibacillus*, *Fictibacillus*, *Paenibacillus*, *Hungateiclostridium*, and *Clostridium Sensi Stricto*_1), Arthropoda (Arachnida), Platyhelminthes (Rhabdocoela), and Rotifera (Ploimida) were special keystone taxa on *C. demersum* (**Figure 2.13B**). In sediment samples, genera in SAR clade, Rotifers (Bdelloidea and Philodinida), Firmicutes (*Exiguobacterium*), and algae (Dinophyceae) were the keystone taxa (**Figure 2.13C**). The dominance of the keystone bacterial and eukaryotic genera in Firmicutes, Proteobacteria, Fungi, and Algae in *E. crassipes* showed syntrophic, symbiotic, and parasitic interactions. The bacterial and eukaryotic keystone genera in *E. crassipes* are important for plastic degradation in the gut of nematodes (*Exiguobacterium*) [223], methane oxidation (Methylococcaceae) [316], bioremediation of xenobiotics and denitrification process (*Acinetobacter*) [317,318], photosynthesis and endosymbiosis in tropical metazoans (*Symbiodinium*) [319], and parasitism and organic matter plant decomposition (*Aspergillaceae*) [320].

In *C. demersum*, the dominance of keystone genera in Firmicutes and metazoans indicated that organic matter decomposition and predation were the main feeding relationship in this Food web. For example, the functional role may be associated with organic pollutants biodegradation (*Bacillus*) [104], plant growth promotion and antibiotic resistance control (*Paenibacillus*) [105], acetate assimilation and iron reduction (*Solibacillus*) [106] and fermentative hydrogen production (*Clostridium Sensi Stricto*_1) [107]. Metazoans such as Gastrotricha and Rotifers are important consumers of picophytoplankton (algae and protozoans), bacteria, fungi (fungal zoospores), and particulate organic matter [96]. Additionally, they serve as prey for metazoans such as flatworms [110]. It is worthy of note that photosynthetic microbes (e.g., Chlorophyceae, photosynthetic taxa in SAR clade, and *Rhodobacter*) on submerged macrophytes act as the primary producers (release oxygen through the photosynthetic process) and have a decisive influence on the entire aquatic ecosystem structure-function and stability [321,322]. Furthermore, the dominant keystone taxa in

sediments revealed a predation relationship, organic matter decomposition, and photosynthesis or toxin production. Sediments receive carbon from plant die-off with carbohydrates degradation and fermentation of plant-derived carbon [94]. The negative and positive network interactions may be attributed to the microhabitats' distinctive lifestyles, food and energy sources, niches, allelopathy, and seasons [234]. This study showed the interrelationship between the microbial community in biofilms, such as feeding relationships, predation, parasitism, and synergism for biogeochemical cycling.

Functions of the bacterial community in epiphytic biofilms and sediments were performed using KEGG function annotations to obtain information on OTUs at each functional level. Spearman's rank correlation ($|r| > 0.7$ and $P < 0.05$) was used to measure the correlation between the bacterial genus and KEGG orthology metabolic pathways in all samples[247]. The predicted metabolic functions (Pathway level 2) (**Figure 2.14**) of the top 17 bacterial genera were most related to the metabolism (nucleotide metabolism, amino acid metabolism, carbohydrate metabolism, lipid metabolism, energy metabolism, and cofactors and vitamin metabolism). In addition, results from STAMP analysis showed that all predicted functions (especially metabolism) were statistically different ($P > 0.05$) between surface sediments and epiphytic biofilms (**Figure 2.15A**), with seasonal shift inclusive (**Figure 2.15B**).

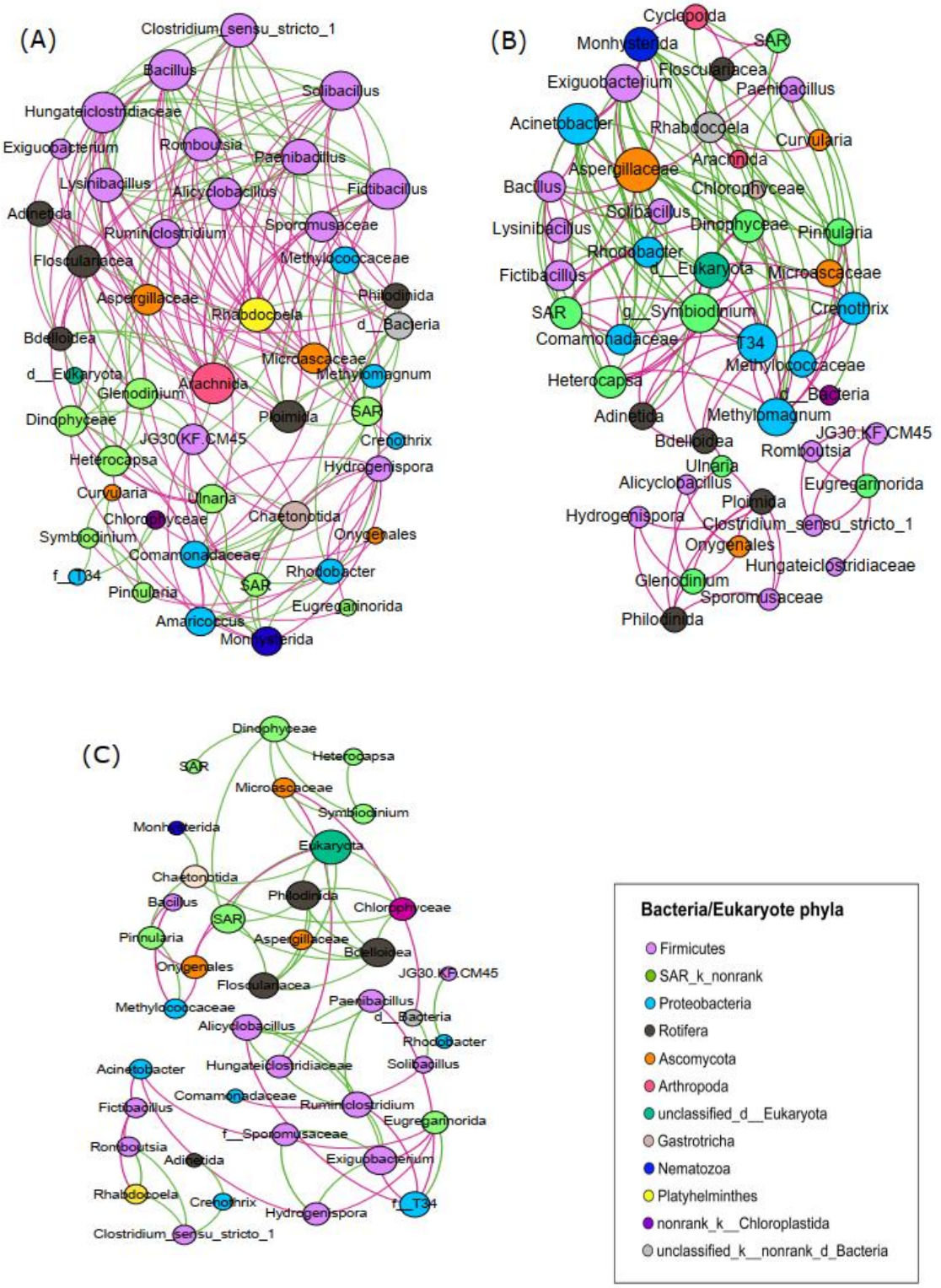


Figure 2. 12 Network analysis showing interactions among bacterial and eukaryotic communities in the top 50 genera in *C. demersum* (A), *E. crassipes* (B), and sediments (C). Each connection shows a strong Spearman correlation ($|r| > 0.7$ and $P < 0.05$); the blue and red lines represent, respectively, significantly strong positive and negative relationships

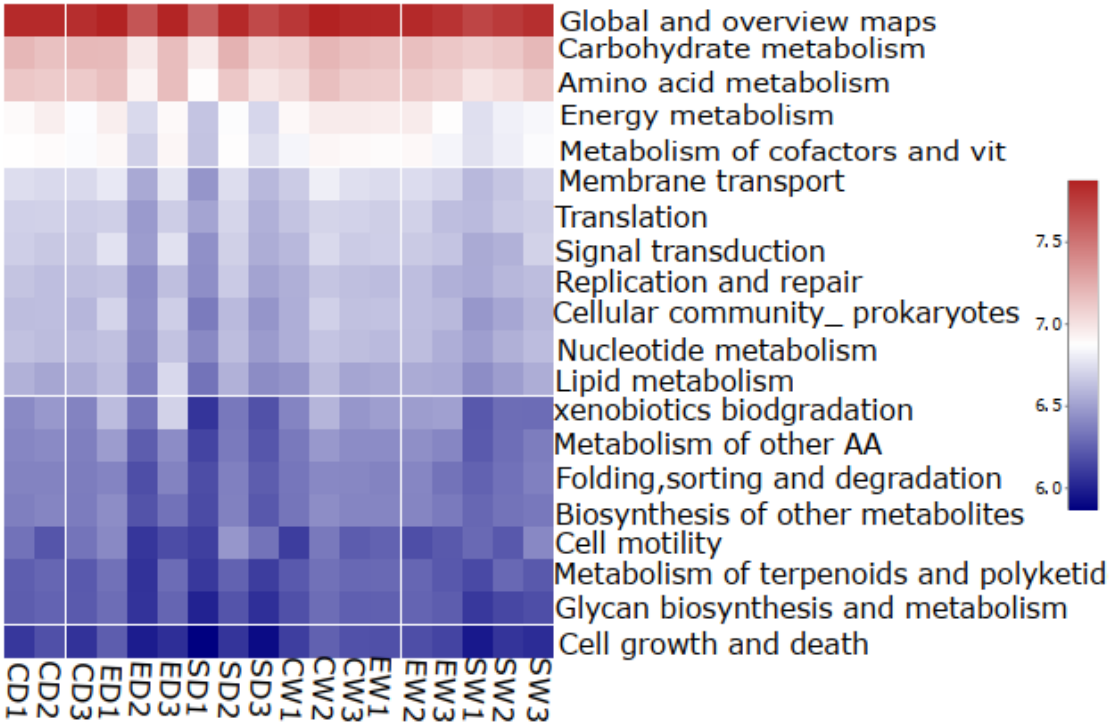
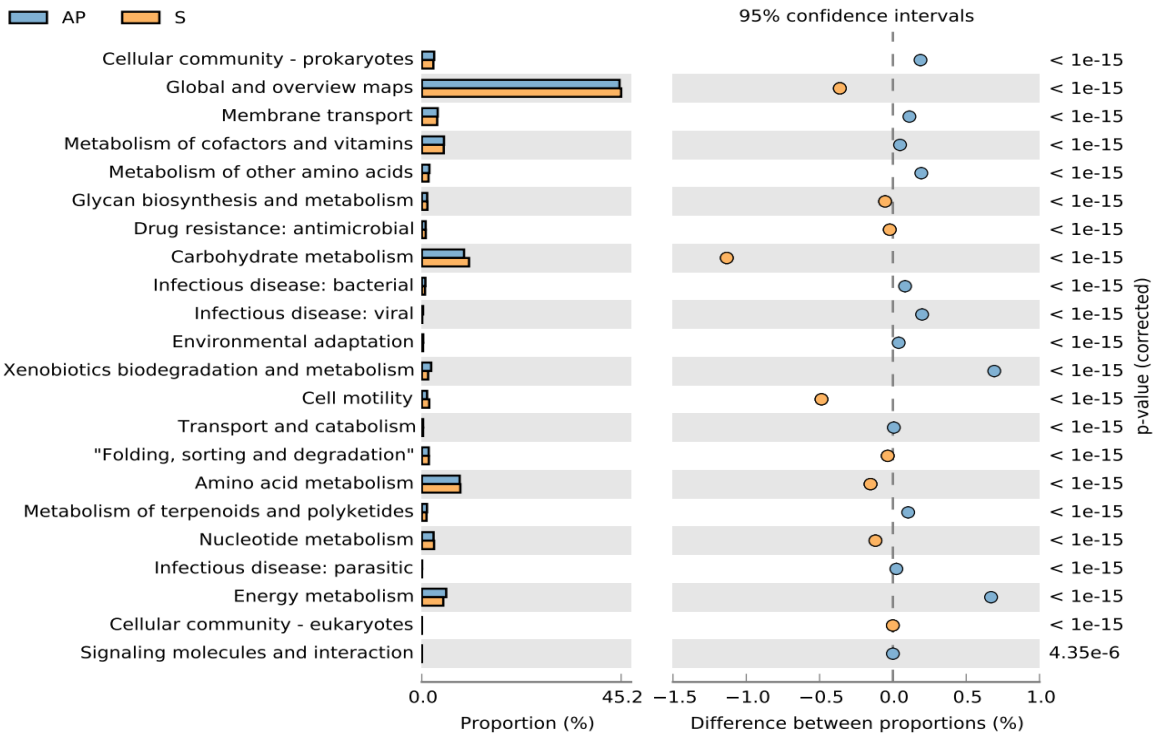


Figure 2. 13 Predicted metabolic functions of the top 20 bacteria genera by PICRUSt

(A)



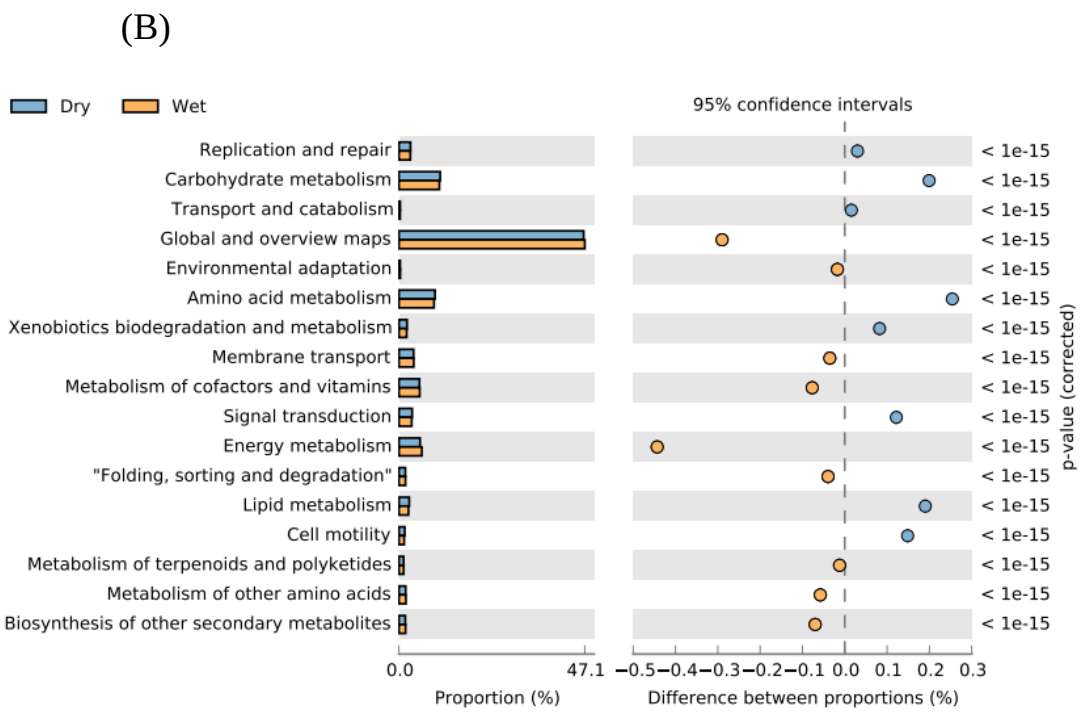


Figure 2. 14 STAMP analysis in predicted metabolic functions on aquatic plants (AP) and surface sediment (S) (A), and seasons (dry & wet) (B) by PICRUSt.

2.4 Summary

This study comprehensively explored the microbial diversity and ecological function of epiphytic and surface sediment biofilm communities during dry and wet seasons in a shallow tropical lake (Rumira). The main conclusions are as follows:

1) The eukaryotic OTUs number and Shannon indices were significantly higher in sediments and epiphytic biofilms on *Eichhornia crassipes* than *Ceratophyllum demersum*, while no differences were observed in bacterial OTUs number and Shannon values among substrates.
2) Phyla Actinobacteria, Cyanobacteria, Chloroflexi, Rotifera, Chloroplastida, and Gastrotricha were more dominant in epiphytic biofilms on *C. demersum* than on *E. crassipes* and surface sediments, while phyla Firmicutes, Ascomycota, and Proteobacteria on surface sediments and *E. crassipes* were significantly higher than on *C. demersum*.
3) RDA results indicate that the diversity and structure of the microbial community in epiphytic biofilms on *C. demersum* and *E. crassipes* were mainly related to pH, DO, temperature, TN, and EC.

4) Analysis of the co-occurrence network reveals the presence of more complex interactions between bacteria and eukaryotes on *C. demersum* than on *E. crassipes* and surface sediments. Ecological roles through network interactions and functional predictive analysis included degradation of organic pollutants, hydrolysis of polysaccharides, nutrient cycling, and energy production, which could have positive effects on lake ecosystems and the sustainability of local activities. These results shed light on the microbial diversity and ecological role of epiphytic biofilm and surface sediment communities in shallow tropical lake ecosystems.

Chapter 3. Interactions between water quality and microbes in epiphytic biofilm and superficial sediments of lake in trophic agriculture area

3.1 Background

Microorganisms do not live as pure cultures of discrete single-living cells (phytoplanktons) but instead amass at interfaces to form aggregates in the form of biofilms, biomats, sludge or flocs [50]. In most biofilms, the microorganisms account for less than 10% of the dry mass, while the matrix can account for > 90%. Biofilms are complex matrix-embedded communities consisting of bacteria, archaea, algae, fungi, protists, and other metazoans. These microbial communities play an essential role in primary production, pollutant removal, maintaining ecosystem structure (diversity and composition), trophic interactions, nutrient uptake and cycling, and microbial gene pool conservation in aquatic environments[8].

Depending on the substratum type, aquatic biofilms can be classified as epizoic (animal), epipelon/epipsammon (sediment), or epiphytic (aquatic vegetation) biofilms. However, they can colonize artificial substrates.Aquatic macrophytes (submersed and floating plants) - biofilm platforms are fundamental components of aquatic ecosystems and have a synergic effect of transforming pollutants and maintaining ecological balance[251]. Submersed and floating macrophytes are widely scattered in shallow water systems and provide unique substratum or attachments and food for microbial growth[251], while epiphytic biofilms impact aquatic plant health through protection from pathogenic microbes and invertebrates. Investigating epiphytic microbial communities and their variations may provide a valuable understanding of the plant-microbe interactions associated with plant health and bio-purification in aquatic environments [323,324]. Microbial biodiversity comprises the number of taxa, abundance, and ecological interactions between various microbial phylotypes.

Prior reports have addressed the key effects of physicochemical water parameters[116], macrophyte species [92,325], geographical location [326], and seasons [327] on the community variations of epiphytic bacteria and algae on aquatic macrophytes (freshwater or marine). Despite providing information on the influential factors of the epiphytic bacterial communities, we know little about their relative importance. Moreover, limited research tools have resulted in little

knowledge about microbial community interactions. Currently, network analysis techniques are used to analyze co-occurrence patterns among microbes in biofilm communities with symbiotic relationships and complex interactions[328–331]. In addition, the network analysis allows for the statistical identification of keystone species in the networks, which play an indispensable role in maintaining microbial structure and function and network complexity and stability in planktonic and benthic bacterial communities[332].

It is becoming more widely accepted that both deterministic (e.g., filtering by physicochemical water parameters or plant physiology) and stochastic (e.g., ecological drift and dispersal) processes play pivotal roles in regulating the assembly of ecological communities[199,333]. But it is still up for debate which dominates the other. This topic has been explored extensively in microbial ecology across various habitats, including soils [206], freshwater [44], sediments[45], groundwater[202], membrane bioreactors[46] and wastewater treatment plants[208], and some extreme environments[209]. Additionally, the bacterial communities in epiphytic biofilms have largely been studied from a deterministic perspective, especially in terms of abiotic factors. Investigating microbial community co-occurrence patterns and community assembly mechanisms (stochastic and biotic deterministic processes) is imperative for comprehending biological ecology[334].

Unfortunately, the community assembly processes and co-occurrence patterns of microbial communities (bacteria and microeukaryotes) in epiphytic and surface sediment biofilms in shallow lake are poorly understood. For instance, there is a lack of research considering the assessment of the relative importance of deterministic and stochastic processes in microbial communities (bacteria and microeukaryotes) on various aquatic macrophytes (submerged, floating and emergent) and surface sediments. Recently, He et al. [47] and D. He et al. [214] have studied the assembly processes (deterministic and stochastic processes) in epiphytic bacterial communities in submerged macrophytes and emergent plant rhizosphere and bulk sediments.

To the authors' best knowledge, no research has so far comprehensively explored the deterministic and stochastic processes of bacterial and microeukaryotic communities in submerged plants, floating plants, and surface sediments in natural tropical lakes.

Therefore, to fill this gap, the present study aimed to investigate 1) whether microhabitats (*C. Ceratophyllum demersum* and *Eichhornia crassipes* and surface sediments) and seasonal shift

affect microbial community co-occurrence, 2) the relative importance of stochastic and deterministic processes in shaping epiphytic and epipelic microbial community assembly.

In this study, we explored the microbial diversity and composition in epiphytic and surface sediment biofilms in dry and wet seasons in Cyohoha South Lake by high-throughput sequencing of the 16S and 18S rRNA genes. Furthermore, the co-occurrence patterns and ecological processes were computed by phylogenetic molecular ecological network analysis and statistical methods based on the null model. Our results would be valuable for gaining insights into assembly processes and co-occurrence of microbes in epiphytic and sediment biofilms in tropical lacustrine ecosystems.

3.2 Materials and methods

3.2.1 Study area and sampling

Lake Cyohoha North is a shallow lake located in the Eastern Province of Rwanda, East Africa (02.23009°S, 030.09976°E) (**Figure 3.1**). It is at a surface elevation of 1348 m[248], 27 km long, 5 to 2 km wide, and an average depth of 5 m [335]. *C. demersum* and *E. crassipes are* among the most common submerged plant species in Lake Cyohoha North, and some farming and agricultural activities are carried out at the lakeshore of the sampling sites. This shallow lake is essential for the ecosystem, navigation, fishery, tourism, and water sources for the nearby residents. The Cyohoha North catchment extends to an area of 508 km^2. Series of swamps up to 9 km separate the lake and the river Akanyaru, which is a tributary of the Akagera River, the biggest among 23 rivers that drain into Lake Victoria [336].

The main types of ecosystems found around Lake Cyohoha North are agricultural landscapes, build-up, forestland, grassland, waterbodies, and wetlands. Due to the agricultural expansion and inappropriate agricultural activities, sediments transport (e.g., fertilizers and pesticides), settlement, and persistent drought (1999/2000), the lake water quality is deteriorated (eutrophication) and dry up. Lake Cyohoha north degradation can be spotted on the LULC (land use and land cover) maps of the catchment throughout 2002, 2010, and 2018 (**Figure 3.2**) [337]. It is clear that different LULC changes occurred enhanced the reduction in size of Lake Cyohoha. However, despite these efforts made by the Rwandan government and other institutions to restore the lake Cyohoha north, the water quantity and quality remained under ambiguity due to continued

excessive growth of aquatic weeds (*E. crassipes*) and buffer zone deterioration by local farmers (**Figure 3.3A-B**). The abovementioned factors contributed to the reduction of Lake Cyohoha north water quantity and accelerated the water quality depletion that resulted in the eutrophication of the lake (**Figure 3.4A**). Most of the buffer zone of Lake Cyohoha north has been encroached and cultivated by local farmers (**Figure 3.4B-C**), and they cut papyruses (*Cyperus papyrus*) used to control erosion and sustain water filtration. Consequently, the erosion goes directly into the lake and negatively affects the water quality, lake evaporation, water level, buffer zone, fish production, and biodiversity.

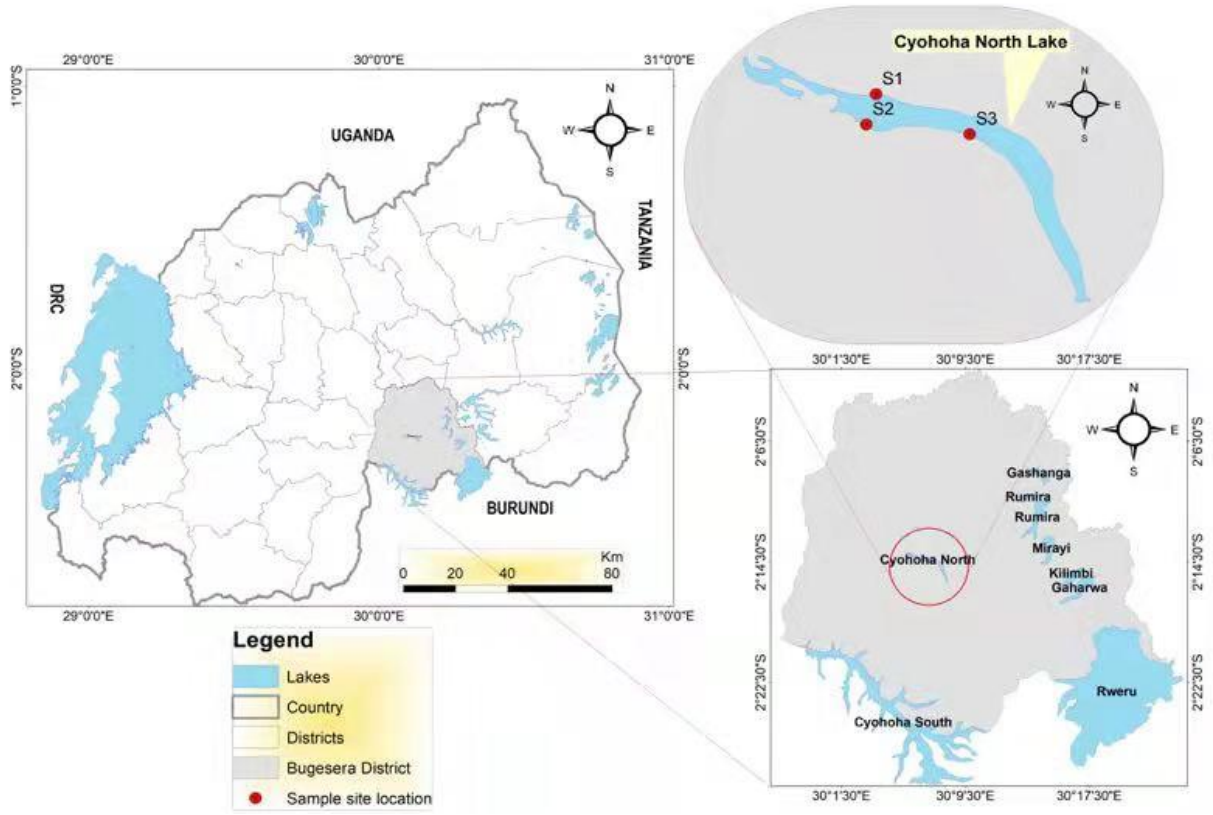


Figure 3. 1 Map of Cyohoha North Lake

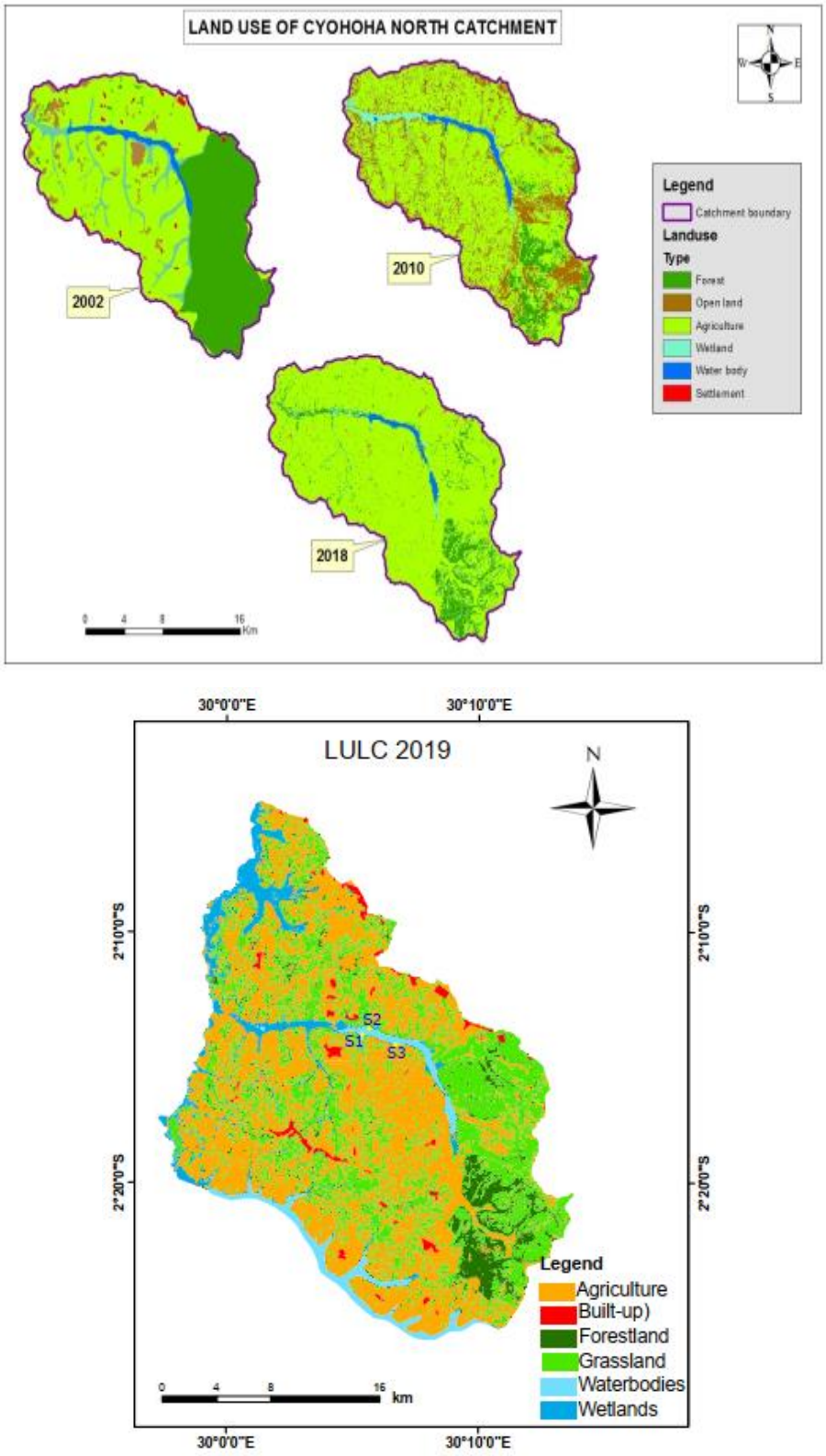


Figure 3. 2 Cyohoha North Catchment LULC maps for years 2002, 2010, & 2018 [337] and 2019 (this study.

Figure 3. 3 Inappropriate agricultural activities around the Lake Cyohoha North [337].

Figure 3. 4 Water eutrophication (A) and encroachment (B-C) in the Lake Cyohoha North buffer zone[337].

The aquatic macrophytes, surface sediments, and surface water samples were collected in Lake Cyohoha North in the dry season (August) and wet season (November) in 2019. Dry season samples were taken on 15th August, and wet season samples were taken on 20th November, between 8:00 and 12:00 h. A total of 24 samples were collected from three sampling sites (S1, S2, and S3): 12 samples (*C. demersum*, *E. crassipes*, surface sediment, and surface water; termed CP, EC, S, and Sdry&Swet) were collected from S1 (CPD1, ECD1, SD1, and S1dry), S2 (CPD2, ECD2, SD2,

and S2dry), and S3 (CPD3, ECD3, SD3, and S3dry) in the dry season, while the remaining 12 samples were again sampled from S1 (CPW1, ECW1, SW1, and S1wet), S2 (CPW2, ECW2, SW2, and S2wet), and S3 (CPW3, ECW3, SW3, and S3wet) in the wet season (**Figure 3.1**). In the dry season, both *C. demersum* and *E. crassipes* were in their juvenile period with younger leaves; and in the wet season, both plants began to senesce and decay[262]. At each site, three replicates of healthy aquatic macrophyte leaves (CPD and EC) were collected by hand with gloves or using a stainless hook, slightly washed with dH_2O, and then incubated immediately in a sterilized wild-mouth polyethylene bottle (500 ml) containing 350 ml 95% ethyl alcohol.

The surface water samples were collected below the overlying water at a depth of 0.5 m, and about 1L of water around the vicinity of the cohabiting aquatic plants at each sampling spot was also collected using an aseptic plastic bottle. Approximately 500 ml of water was used for chemical analyses. Surface water samples collected were preserved and kept cool for less than 6 h before transporting them to the laboratory for proper analysis. A Peterson dredge was used to collect three replicates of surface sediment samples thoroughly mixed to create one sample. About 3g of superficial sediment (0-5cm) was collected and stored in a 10 mL sterile plastic tube containing 95% ethanol for at least 15 min. The V: V ratio of sediment to ethanol was > 3:7.

Physicochemical properties of the water samples, such as water temperature, pH, EC, TDS, ORP, and DO, were measured *in situ* using HQ30d portable multi-parameter digital analyzers (HACH, USA). Other environmental variables such as TN, TP, NH_4-N, and NO_3-N were measured in the laboratory following previously defined methods [263].

3.2.2 Treatment of epiphytic and surface sediment biofilm samples

In the laboratory, the epiphytic microorganisms on harvested samples were detached by taking approximately 50 g mixtures of ethanol (95%) and plant leaves of each species (*C. demersum* and *E. crassipes*) and then subjected to 1 hour of mechanical shaking (225 r/min) [81]. The suspensions from the same sample were combined and passed through a sieve with a mesh size of 0.8 mm to remove plant debris and small animals. This was then subsequently centrifuged at 8000 rpm for 10 min. Conversely, a mixture of sediments and ethanol was centrifuged at 8000 rpm for 5 minutes. Supernatants were discarded, and sediments of 2g per tube were stored. Epiphytic pellets and sediment samples were both mixed with 80% ethanol for a minimum of 1 min, centrifuged, and then stored carefully in clean 5 ml sterilized plastic tubes with a cap for shipping and DNA extraction in China. Furthermore, DNA extraction and PCR amplification, Illumina MiSeq sequencing of rRNA genes, and Bioinformatics were performed following the standard protocols supplied by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). For more details, see **section 2.2.4-2.2.6**.

3.2.3 Data analysis

Data analysis and visualization were performed on Majorbio Cloud platform (www.majorbio.com) and in R (R Core Team, 2015). The ‘Stats’ and the ‘spicy’ R packages were used to perform the statistical comparisons. A one-way ANOVA was used to compare the environmental parameters among groups, and statistical significance was determined at $P < 0.05$. The alpha diversity indices (Shannon, Richness and Evenness) were computed using the vegan package 2.5 (https://CRAN.R- project.org/package=vegan) and visualized with ggplot2 package 3.2.0. The PCoA beta diversity analysis was used to explore the similarity between microbial community structures at the OTU level adopting the Bray-Curtis distance algorithm and visualized via the ‘plot2D’ package in R. Permutational multivariate analysis of variance (PERMANOVA) test was performed to evaluate the difference in beta diversity among seasons and hosts, using the Bray-Curtis index. The diversity (ß-metrics) between microbial communities in epiphytic and surface sediment biofilms was computed by Beta Nearest Taxon Index (βNTI) using the "ape" and "Picante" functions of R (https://www.r-project.org/, v3.5.3) in accordance with Stegen et al. [199]. The value of $|\beta NTI| > 2$ represented the deterministic process ($\beta NTI > +2$, variable selection; $\beta NTI < -2$, homogeneous selection), while $|\beta NTI| < 2$ was regarded as the stochastic process. The relative abundance of taxonomic compositions

was visualized using the 'ggplot2' Package. Furthermore, to explore the potential importance of stochastic mechanisms on community assembly, we employed a neutral community model (NCM) to predict the relationship between OTU detection frequency and their relative abundance. The model used here is a version of the neutral theory[201], and all computations were performed in R (https://www.r-project.org/, version 3.2.3). Co-occurrence networks were constructed from all samples. Then, we divided all samples into three groups (CP, EC, and surface sediments) according to substrate type. Network topological parameters such as modularity, average degree, average weight degree, average path length, and average clustering coefficient were calculated using the igraph package in R (https://www.r-project.org/, v4.0.4).

Spearman rank correlations based on the relative abundance (Phylum level) were calculated using the corr. test function of the 'psych' packages in R (version 4) and visualized using Gephi (https://gephi.org/,version0.9.2) and Cytoscape (https://manual.cytoscape.org/en/stable/, v3.8.3). Robust correlations were considered if Spearman's correlation coefficient (r) was > 0.7 or <-0.7 used for further analysis ($p < 0.05$)[265]. This map was classified into six classes, namely, agriculture, forest, grassland, built-up, wetland, and water bodies, and the percentage contribution of each land-use type were obtained. To account for the direct and indirect effects of LULC, physico-chemical variables, and water quality index (WQI), the partial least squares path modelling (PLS-PM) was run using functions available in the plspm package. The sampling map was drawn using QGIS (QGIS Development Team, 2017, v2.18).

3.3 Results and discussion

3.3.1 Physicochemical parameters and land use analysis

Seasonal variations of physical-chemical parameters of surface water in Cyohoha North Lake are shown in **Figure 3.5**. The mean concentration of TP, TN, NO_3-N, and NH_3-N were significantly higher ($P < 0.05$) in the wet season (2.59 mg/L, 3.74 mg/L, 0.54 mg/L, and 2.56, respectively) compared to the dry season (1.29 mg/L, 2.76 mg/L, 0.39 mg/L, and 1.91, respectively) (**Appendix Table S5**). However, the mean concentration of EC and TDS were significantly higher ($P < 0.05$) in the dry season (**Figure 3.5**), consistent with the study of Zhang et al.[338]. There was an increase in water depth across all sampling sites during the wet season compared to dry season. This study suggests that water depth fluctuation during the rainy season may be ascribed to the influence of surface runoff from precipitation (rain) of October than September according to the monthly weather data from Climate data (https://en.climate-data.org/).

Notably, there was a variation of surface water physicochemical parameters across sites. The EC implies the availability and the concentration of salts[269], thus, disturbing the microorganisms in surface water and leading to depletion of DO concentration[270], consistent with Manirakiza et al.[16]. The high concentration of nitrogen (NO_3-N, NH_3-N, and TN) and phosphorus compounds (TP) in Lake Cyohoha North could promote the eutrophication process[271], leading to depletion of DO in lake water, the condition which could fuel algae bloom and suffocation of aquatic microorganisms[272].

Previous studies reported a significant temporal and spatial variation in physical and chemical parameters, such as EC, CODcr, NH_4-N, NO_3−N, TN, and TP [16,274,339]. The nutrients, such as TP, TN, NO_3-N, and NH_4-N, were beyond the maximum permissible limit of the Chinese Environmental Quality Standards for Surface Water [340,341]. Compared to lakes Rumira (Rwanda), Taihu and Poyang (China), and Awash River (Ethiopia), the results obtained for the nutrient values in Lake Cyohoha are generally higher [16,273,274,342], however, the NO3-N was lower than that obtained for the eutrophic Beseka Lake (Ethiopia) [275].This study suggests that nutrient pollution in Lake Cyohoha can be attributed to the increase in runoff, chemical pollutants, siltation, and river inflows during the rainy season, which play a vital role in modifying the water parameters in the region [276,343].

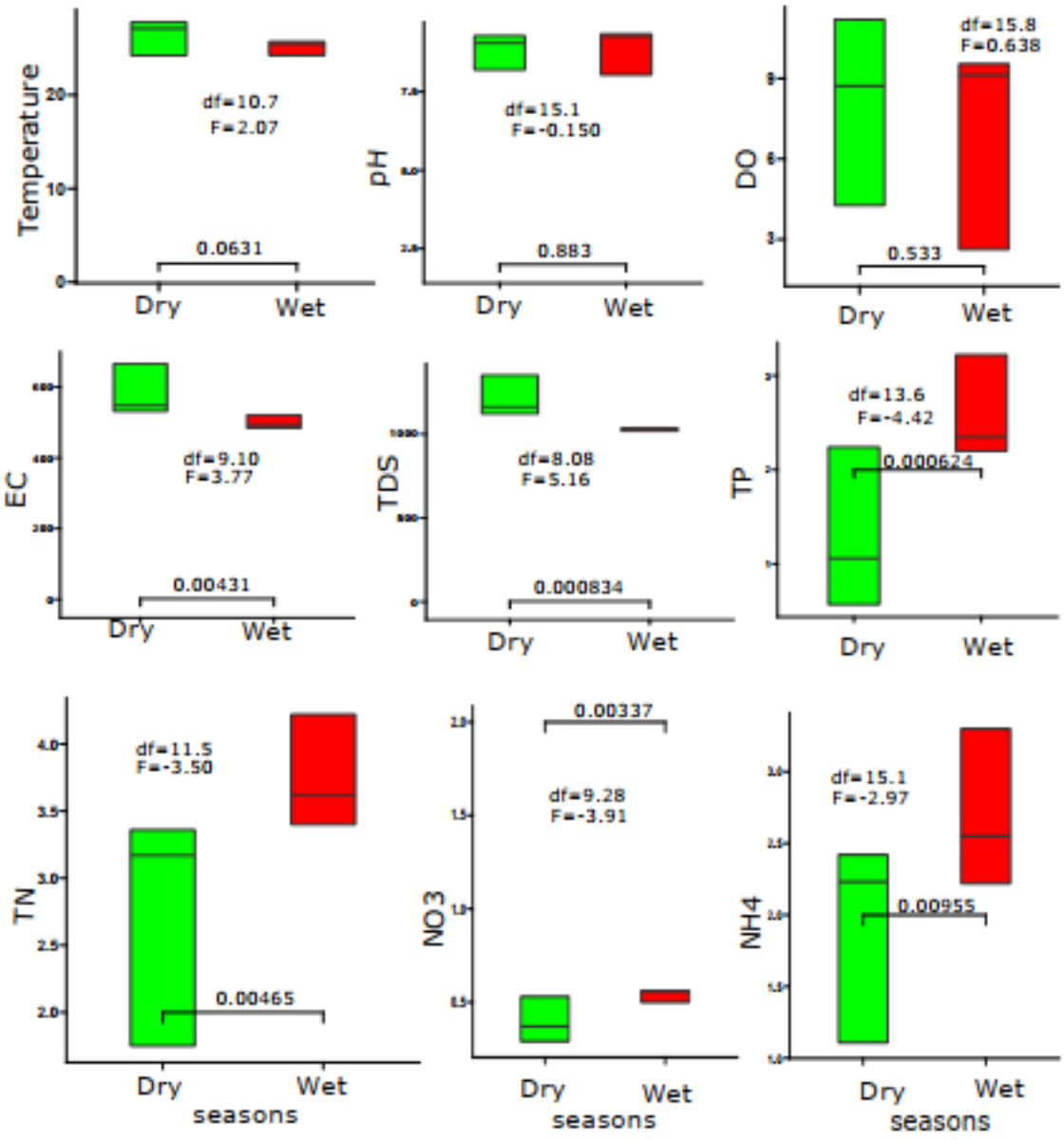


Figure 3. 5 Physicochemical parameters of the surface water in Lake Cyohoha across seasons (dry and wet).

On the other hand, the study explores the direct and indirect effects of land use variables on physicochemical parameters (Temperature, pH, DO, EC, TDS, TP, TN, NO3-N, and NH3-N), microbial structure (bacteria and microeukaryotes), and water quality index (WQI). By percentages, agriculture (52.78%), grassland (31.18%), forestland (6.16%), wetland (5.11%), waterbodies (3.32%), and built-up (1.54%) were identified around the Lake Cyohoha North (**Figure 3.2**). Of all the LULC classes, we noted that agriculture covers the highest proportion (52.78%), which explains the availability of different pollutants (fertilizers, pesticides, antibiotics,

sediments, etc.) in the lake from agricultural sources. The PLS-PM (**Figure 3.6**) showed a higher direct positive correlation (R^2= 0.9301 vs R^2= 0.6914) between LULC and physicochemical variables in the rainy than dry seasons, however, weak positive correlation (R^2= 0.28 vs R^2= 0.06) with water quality index in both seasons. Furthermore, there was a higher positive correlations between physicochemical variables and water quality index in rainy season (R^2= 0.599) than dry season (R^2= 0.1055) (**Figure 3.6A-B**), with a great impact of agriculture on the water quality parameters. This study suggests that the lake was seasonally affected by anthropogenic activities, consistent with previous reports[344,345].

Although land use types positively influenced the physicochemical variables during the wet season (**Fig. 6B**) than the dry season, the later showed little positive influence on microbial communities and water quality index, respectively. In addition, the water quality index showed a negative direct influence on microbial communities in both seasons. Prior studies revealed positive correlation between environmental variables and microbial community structure [346,347]. Isabwe et al. [345] demonstrated the effect of land use types on phytoplankton and bacterioplankton, however, the effect of land use types and water quality index on periphytic biofilms remains unclear. Similar to this study, Marmen et al. [346] and Catherine et al. [348] reported a partial or weak explanatory power of land-use types and water chemical properties on water microbiome. Therefore, the impact of land use types and WQI on the epiphytic and surface sediment biofilms should be of high priority for future research.

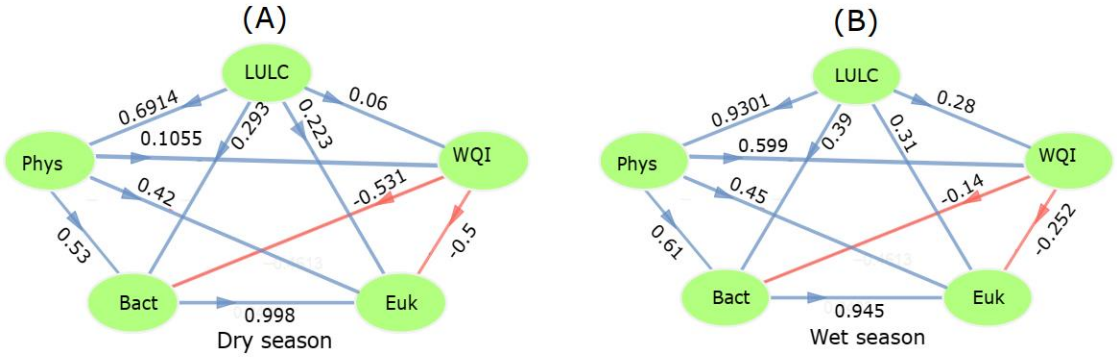


Figure 3. 6 PLS-PM model showing direct and indirect effects of land-use types on physico-chemical parameters, water quality index, and microbial community accross seasons (A-B) for the lake Cyohoha North. Blue and red lines represent positive and negative effects, respectively.

3.3.2 Seasonal influence on bacterial community structure in epiphytic and epipelic biofilms

A total of 871,378 16S rRNA clean reads were normalized to 534,684 in all samples. There was no significant difference ($P > 0.05$) in all the- diversity indices (Shannon values, OTU richness, and evenness) (**Figure 3.7A)** of the bacterial community in biofilms across two seasons (dry and wet) and among microhabitats (*C. demersum*, *E. crassipes*, and sediments). Consistent with this study, a recent mesocosm study reported a lack of significant difference ($P > 0.05$) in all α-diversity indices of epiphytic microbes on artificial plants, *Myriophyllum verticillatum* and surface sediments. Interestingly, Shannon index, OTU richness, and evenness were higher in sediments than those of *C. demersum* and *E. crassipes* (**Figure 3.7A**). Contrary to this study, our current report indicated a higher OTUs richness in epiphytic biofilm (*C. demersum*) than in surface sediments [349]. Furthermore, the above-mentioned α- diversity values were higher in the dry season (August) compared to the wet season (November). R. He et al. [214] reported that bacterial diversities of the bulk sediment and *P. australis* rhizosphere in summer were significantly higher than those in winter[214]. Contrarily, D. He et al. [47] and Shi et al. [48] detected a lower alpha diversity on epiphytic biofilms in summer-autumn (May-November) than winter season. The difference in alpha diversity from various studies may be attributed to the difference in seasons, substrate type, anthropogenic pollution, and water chemistry.

The principal coordinate analysis (PCoA) for the bacterial communities explained about 47.58 % of the total variance and revealed that the bacterial community dissimilarity was significantly different among substrates ($R^2 = 0.28$, $P = 0.002$), but not across seasons ($R^2 = 0.038$, $p = 0.621$) (**Figure 3.7B**). Notably, the PCoA and hierarchical clustering plots showed patterns in the distribution of bacterial species (OTUs) among substrates and across seasons, except for some sites (ECD1, CPW1). The significant difference in the bacterial community among substrates may be ascribed to the microhabitat type and spatial heterogeneity, which is in tandem with previous reports [16,82].

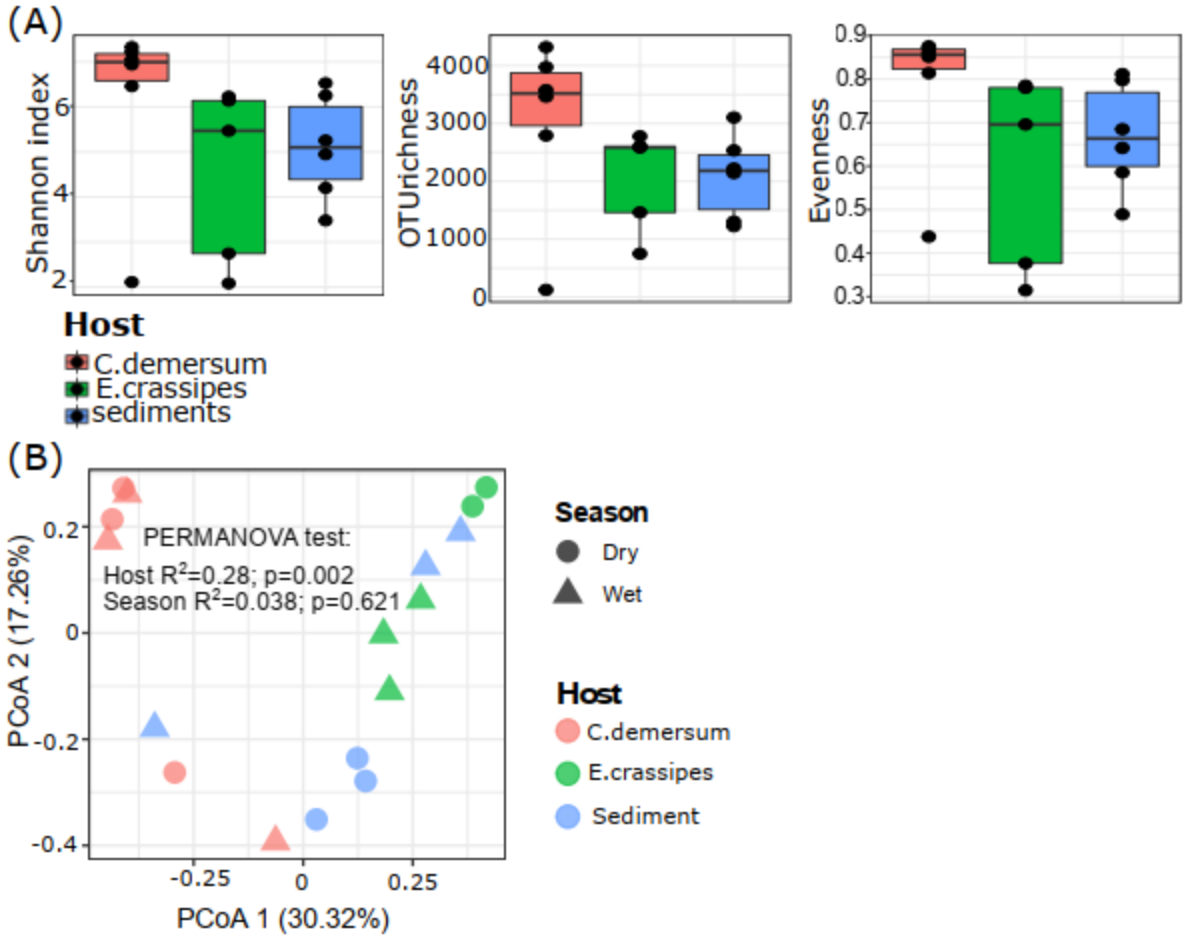


Figure 3. 7 Changes in the alpha (A) and beta (B) diversities of the bacterial community in the biofilms of floating macrophytes (*E. crassipes*), submerged macrophytes (*C. demersum*) and sediments in Cyohoha North Lake (Rwanda) in the dry and wet seasons. OTU richness, Shannon index and evenness were not statistically different among microhabitat types.

A total of nine dominant bacterial phyla (relative abundance >1% in at least one sample) were also investigated in samples from *C. demersum*, *E. crassipes*, and sediments (**Figure 3.8A**). Cyanobacteria was the dominant phylum of *C. demersum* and *E. crassipes* and Firmicutes in surface sediments. The top phyla were Cyanobacteria (27.87 %), Firmicutes (23.41%), Proteobacteria (19.71%), Bacteroidetes (6.98%), and others (8.36%) in *C. demersum*, whereas Cyanobacteria (41.55 %), Firmicutes (34.82%), Proteobacteria (11.41%), Actinobacteria (2.51%), and others (3.64%) were subsequently present in *E. crassipes*. Furthermore, Firmicutes, Chloroflexi, Actinobacteria, Bacteroidetes, and others accounted for 20.12%, 15.28%, 9.27 %, 8.17%, and 27.77 % in surface sediments, respectively. The above bacterial phyla were found to

be dominated in biofilms on various aquatic plants, including *C. demersum*, *Vallisneria natans*, and *Hydrilla verticillata* in Rumira and Taihu lakes[16,81], floating plants (*E. crassipes*) in Brazil wetlands and Rumira lake (Rwanda)[16,94], and surface sediments [16,82].

Compared to the dry season, higher Cyanobacteria abundance was detected in *C. demersum* and *E. crassipes* in the wet season, while higher Proteobacteria and lower Firmicutes were detected in *E. crassipes* and surface sediments. Interestingly, Chloroflexi, Bacteroidetes, and Proteobacteria declined in the wet season on *C. demersum*, while surface sediments revealed a reverse trend. The most dominant bacterial phyla were significantly different among *C. demersum*, *E. crassipes*, and sediments in the dry and wet seasons.

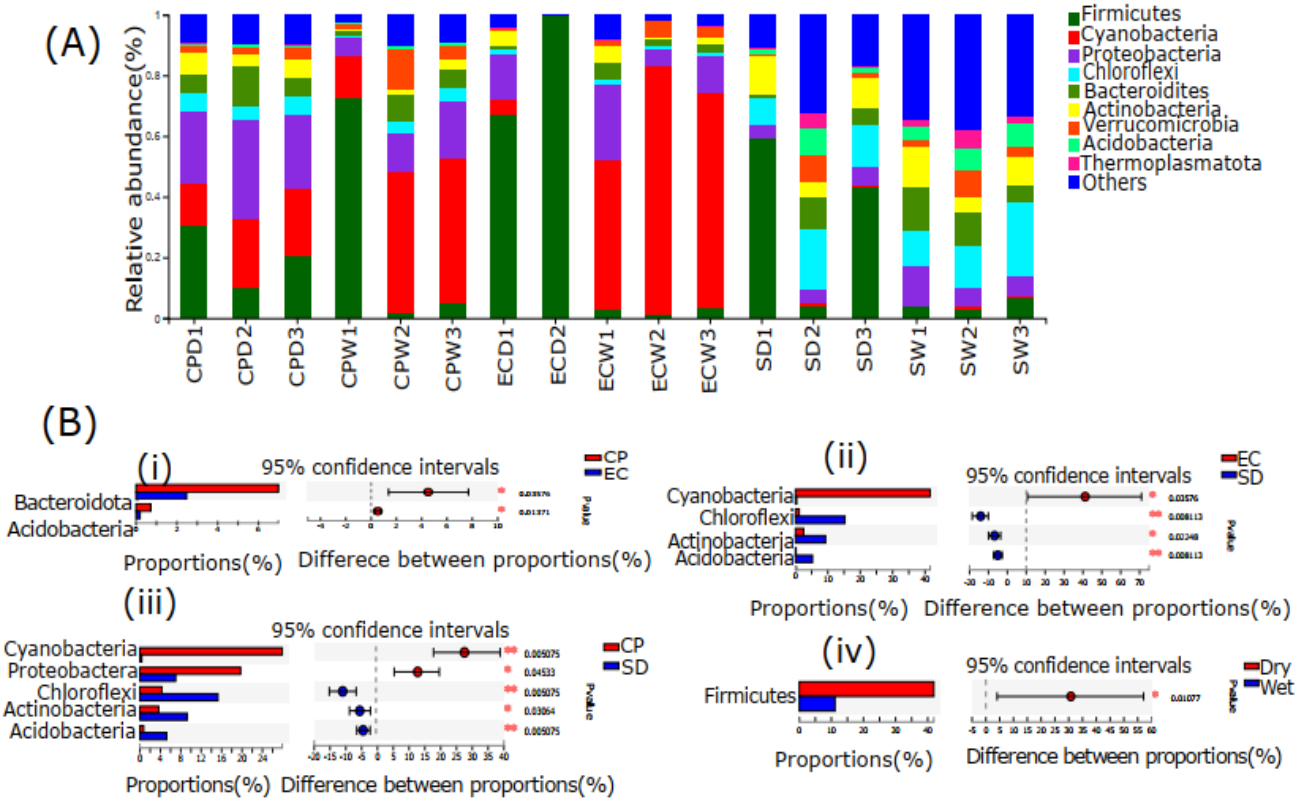


Figure 3. 8. The relative bacterial abundance at the phylum level (A) in the epiphytic (*E. crassipes* and *C. demersum*) and surface sediment biofilms for lake Cyohoha North (Rwanda) during the dry and wet seasons. Fischer's sample comparison in bacterial phyla (B) on *C. demersum* (CD) (i&iii), *E. crassipes* (EC) (i-ii), sediments (SD) (ii-iii), and seasons (dry and wet) (iv).* signifies $P < 0.05$, ** $P < 0.01$.

For instance, Chloroflexi and Cyanobacteria relative abundances were significantly higher in surface sediment and *E. crassipes* ($P < 0.05$, $P < 0.01$), respectively. Furthermore, phylum Bacteroidetes, Cyanobacteria, and Proteobacteria were significantly ($P < 0.05$, $P < 0.01$) higher in *C. demersum* than in *E. crassipes* and surface sediments (**Figure 3.8B (i-iii)**). On the other hand, Firmicutes was of higher significant ($P < 0.05$) occurring phylum in the dry season than during the wet season (**Figure 3.8B (iv)**). The distributions of dominant bacterial phyla (Chloroflexi, Cyanobacteria, Proteobacteria, and Firmicutes) were similar to those detected in sediments [16,277], submerged plants (e.g. *C. demersum*, *Vallisneria natans*) [16,32,81], and *E. crassipes* [16,278]. Nonetheless, the relative abundance of dominant bacterial phyla varied among microhabitats and seasons. These results may be ascribed to the host-specificity, and environmental filtering (water chemistry), that drove the microbial dynamics at the phylum level, however, these viewpoints need further research.

3.3.3 Seasonal influence on microeukaryotic community structure in epiphytic and epipelic biofilms

A total of 921,010 18S rRNA clean reads were normalized and rarified to 557,216 in all samples. In this study, the diversity of microeukaryotes based on 18S rRNA in epiphytic biofilms (*C. demersum* and *E. crassipes*) and surface sediments was inspected. The OTU richness, Shannon and evenness indices for all the eukaryotic samples are provided in **Figure 3.9A-C**. Inversely to Liu et al.[82], Shannon index of microeukaryotes was higher in *C. demersum* than those in sediments and *E. crassipes* ($p > 0.01$). However, there was a significant difference ($p = .0289$) in OTU richness in surface sediments compared to *E. crassipes*, consistent with our recent study [16].

The PCoA of the microeukaryotic community showed a variation (47.73 %) of the total variance and it revealed that the microeukaryotic community was not statistically different among substrates ($R^2 = 0.173$, $p = 0.177$) and across seasons ($R^2 = 0.067$, $P = 0.404$) (**Fig. 3.9D**). Conversely to bacterial communities, the microeukaryotic communities on *C. demersum* were more closely related to sediments than to *E. crassipes*. Previous studies reported that abiotic factors such as depth [138] and various nutrients [282] could influence the distributions of microeukaryotic communities in aquatic environments. This study suggests that substrate type, inorganic nutrients, and close proximity of *C. demersum* leaves to surface sediments compared to *E. crassipes* may be

essential factors for microeukaryotic community discrepancy; nevertheless, these standpoints need further exploration. Notwithstanding the lack of statistical difference among substrates and across seasons, the PCoA and hierarchical clustering plots showed patterns in the distribution of microeukaryotic species (OTUs) among substrates and across seasons, except for some sites (ECW1 and ECD1).

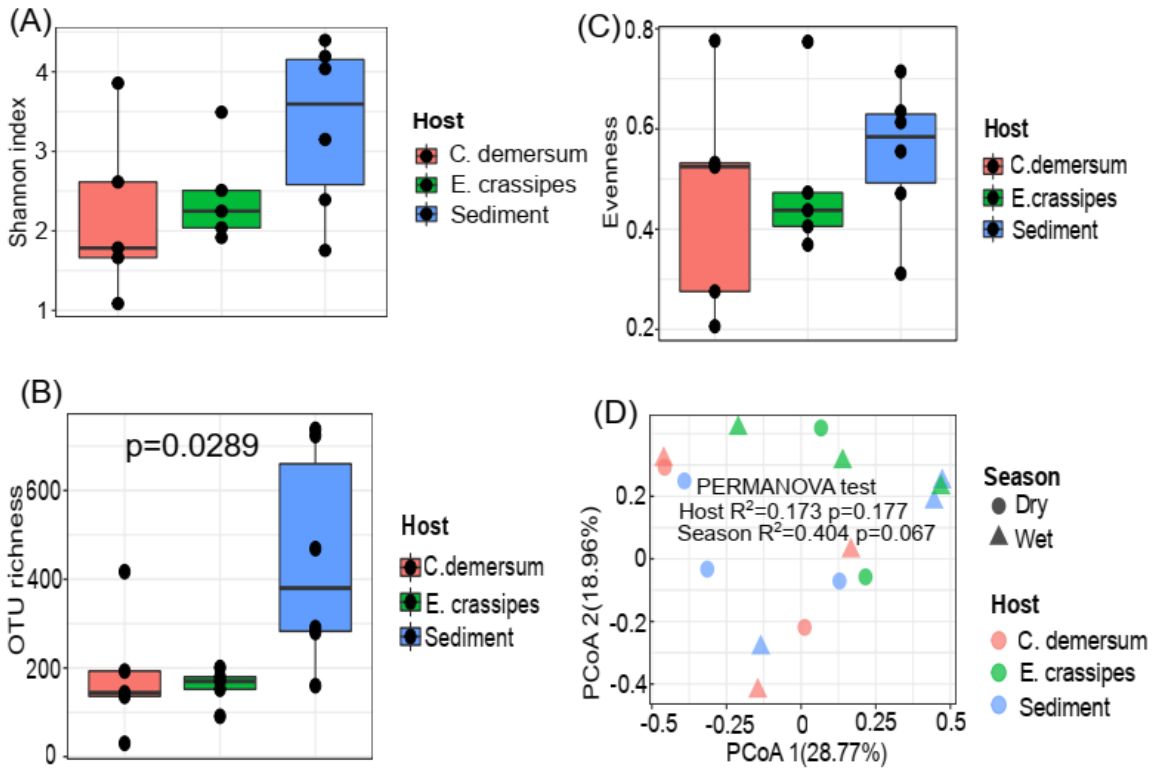


Figure 3. 9 Alpha (A) and beta (B) diversities of microeukaryotic communities in the biofilms of floating macrophytes (*E. crassipes*), submerged macrophytes (*C. demersum*) and sediments in Cyohoha North Lake (Rwanda) in the dry and wet seasons. OTU richness, Shannon index and evenness were not statistically different (except the OTUs richness) among microhabitat types.

According to 18S rRNA gene annotation, phylum Rotifera, SAR (Stramenopiles, Alveolata and Rhizaria), Platyhelminthes, Chloroplastida, Phragmoplastophyta, and Ascomycota dominated in all samples (**Figure 3.10A**), in agreement with our recent study [16]. Furthermore, Phragmoplastophyta and Ascomycota were unique to *E. crassipes* and surface sediments in both seasons, suggesting organic decomposition in *E. crassipes* and surface sediments. Remarkably, Rotifera was the most abundant phylum of *C. demersum* and *E. crassipes*. Based on the season, Rotifera was the sole phylum that peaked during the wet season on *C. demersum* and *E. crassipes*,

whereas SAR increased in surface sediments in the same season. Nevertheless, other dominant microeukaryotic phyla declined in the wet season. Rotifers and other metazoans live in unhospitable environments, such with high organic nutrients and serve as bioindicators in the water environment [291,292]. Additionally, Rotifers graze on bacteria, mold, algae, protozoa, and organic particles [293], suggesting predation in epiphytic biofilms. Sediment can act as a sink of microeukaryotes and may re-enter the water by physical processes (resuspension and diffusion). For instance, the increase in supergroups such as SAR taxa and Opisthokonta have been reported in river sediments during the wet season, attributed to the influents. A possible reason for this event can be that the relatively high precipitation/runoff during the wet season may feed extra SAR taxa in the sediments via dispersal. However, the relatively higher proportions of the SAR taxa from the sediments might also be attributed to the physical disturbance during the sampling activities.

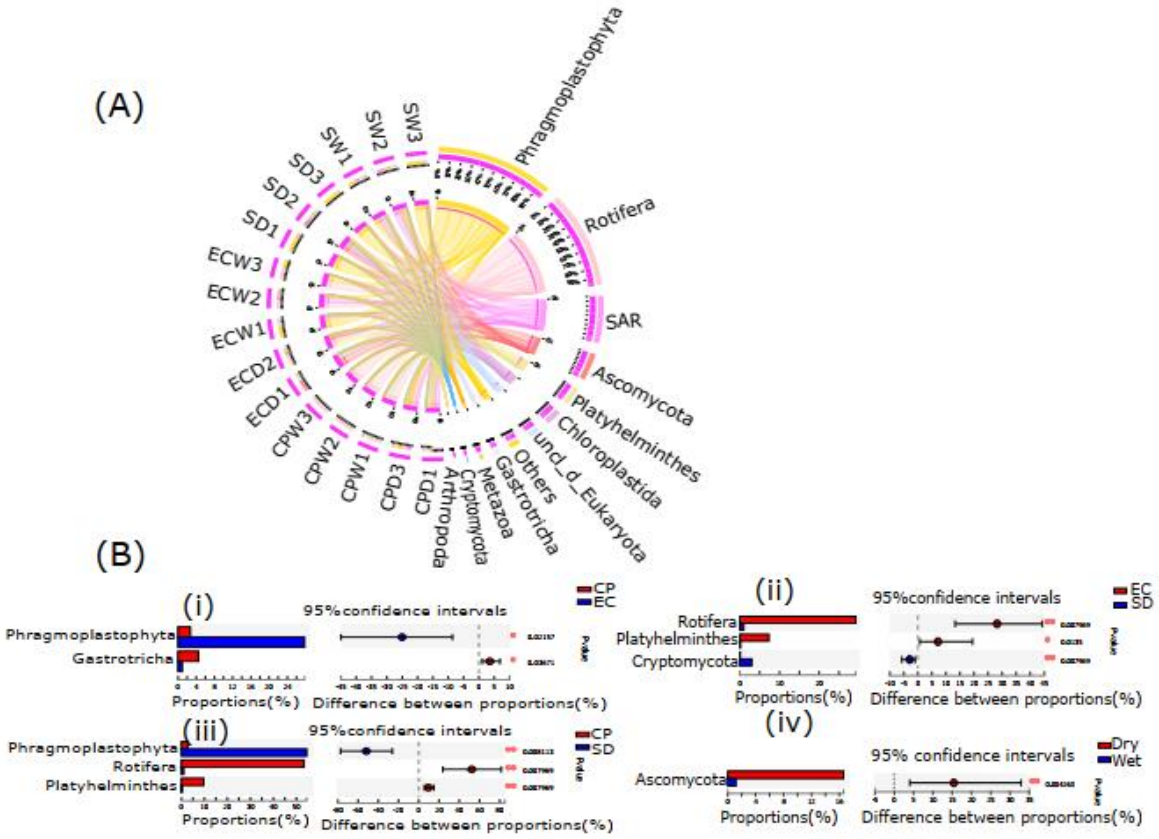


Figure 3. 10 The relative abundance of microeukaryotic at phyla (A) in the epiphytic (*E. crassipes* and *C. demersum*) and surface sediment biofilms for lake Cyohoha North (Rwanda) during the dry and wet seasons. Fischer's sample comparison at phylum (B) on *C. demersum* (CD) (i&iii), *E. crassipes* (EC) (i-ii), sediments (SD) (ii-iii), and seasons (dry and wet) (iv).* signifies $P < 0.05$, ** $P < 0.01$.

The relative abundance of Gastrotricha, Rotifera, and k_Metazoa was significantly ($P < 0.05$, $P < 0.01$) (**Figure 3.10B (i-iii)**) higher in *C. demersum* than in *E. crassipes* and sediments. Furthermore, phylum Platyhelminthes was unique to *C. demersum* and *E. crassipes* and significantly higher ($P < 0.05$, $P < 0.01$) than surface sediments. Ascomycota was of higher significant ($P < 0.01$) occurring phylum in the dry season than during the wet season (**Figure 3.6B (iv)**). These findings imply the dominance of predation among microbes on submersed and floating macrophytes, whereas fungal decomposition dominated in summer.

3.3.4 Ecological processes in biofilms

3.3.4.1 Null model of community assembly

The null model-based analyses were employed to explore the relative contributions of deterministic and stochastic processes to bacteria and microeukaryotes in epiphytic biofilms and surface sediments (**Figure 3.11**). From an ecological perspective, both deterministic and stochastic processes govern microbial community assembly [350]. The neutral theory asserts that stochastic processes (e.g., immigration, random colonization through dispersal, speciation/extinction, random birth/death, and ecological drift [351,352] shape the microbial community structure, and assumes that microbes exhibit a stochastic balance between the loss and gain of taxa[215,353]. However, the deterministic processes shape the microbial communities via environmental filtering (e.g., pH and temperature) and biological interactions (e.g., competition and predation).

The bacterial community of *C. demersum* was dominated by stochastic processes, specifically ecological drift (67%), whereas environment selection accounted for 33% (**Figure 3.11A**). Notwithstanding the dominance of ecological drift (55.5%) in the bacterial community in surface sediments, the environment selection also represented a bigger part of the assembly processes (deterministic process) with 44.4%. Interestingly, ecological drift (50%) and environment selection (50%) contributed equally to the bacterial assembly processes of *E. crassipes.* On the other hand, the microeukaryotic communities in *E. crassipes* and surface sediment were dominated by ecological drift (100%) (**Figure 3.11B)**. Although the dominance of ecological drift (60%) in the microeukaryotic community on *C. demersum*, environment selection still played a significant part in the assembly processes with 40%. Taken together, the high proportion of stochastic processes (ecological drift: $RC_{bray} > +0.95$) in the bacterial (except for *E. crassipes*) and

microeukaryotic communities in epiphytic and sediments biofilms implies that stochastic processes exert a greater influence on these microbial communities. Notwithstanding the dominance of stochastic processes (ecological drift) detected in bacteria and microeukaryotes, the deterministic processes (environment selection: $-2 < \beta NTI < 2$) still represented a significant part of the assembly processes. Furthermore, the stochastic and deterministic processes were coupled in shaping bacterial community assembly on *E. crassipes*.

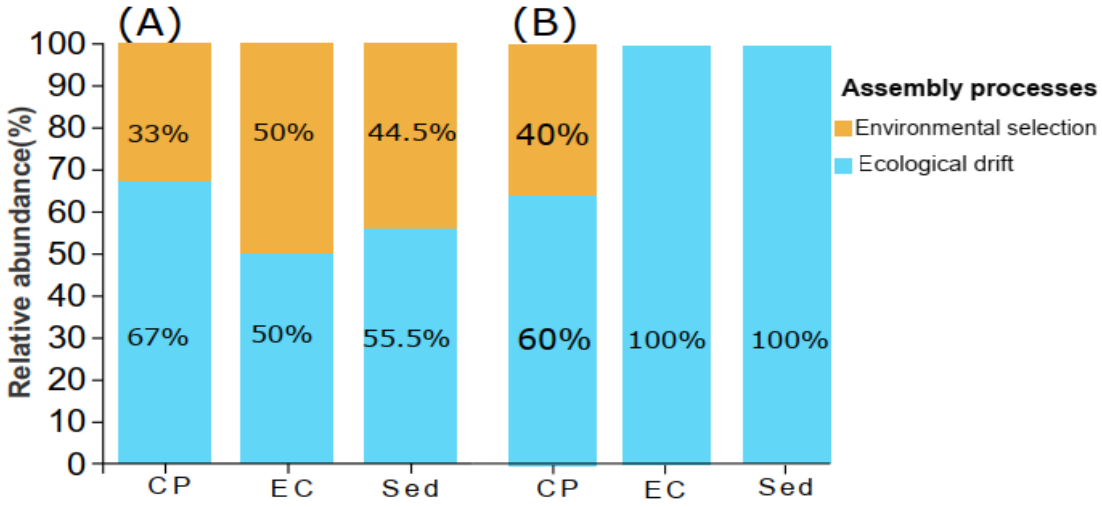


Figure 3. 11 The relative contributions of ecological processes to bacterial (A) and microeukaryotic (B) community assembly in epiphytic and sediment biofilms using the null model.

In long shallow lakes like Cyohoha North, water flows from Inlet Rivers, circling through the whole lake; anthropogenic pollution, boat movements, and wind or waves may allow a high dispersal rate of microbes and result in lower spatial heterogeneity for epiphytic microbes. Thus, a good opportunity existed for random events to affect epiphytic microbes in the context of temporal and spatial shifts. Furthermore, the high stochasticity may also lie in the high possibility of new recruitment to the local community (more colonization events) from the regional species pool, especially in aquatic habitats where the dispersal limitation of water bacteria was low. Moreover, we detected dense and mature epiphytic biofilms on *C. demersum* and *E. crassipes* leaf surfaces in the plant growing period (November), which might cause reduced selection strength; thus, more microbial species (bacteria and microeukaryotes) could successfully colonize leaf surfaces under high dispersal rates, consistent with recent study [47]. The mature epiphytic biofilms produce complex exopolymeric substances (EPS), where robust interactions, such as horizontal

gene transfer, occurs among bacterial members [354]. The EPS can sustain, to some extent, its own architecture and lessen influences coming from inside the plant[323,355]. Thus, the selection from the plant might be undermined and allow more neutral processes to occur.

Concurrently, a recent report showed that deterministic processes played an important part in shaping assembly processes in epiphytic bacteria[48]. Furthermore, D. He et al. [47] reported that deterministic and stochastic processes cooperatively influenced the assembly processes in epiphytic bacteria; however, the deterministic processes dominated only in the growing season (May). Contrary to this study, Shi et al. [48] indicated that deterministic processes dominated the assembly processes in epiphytic bacteria. Our study suggests that the equal influence of deterministic and stochastic processes in epiphytic bacteria on *E. crassipes* may be due to its location (air-water interface), which is prone to air disposal, high temperature (sunlight), and UV radiation in the case of stochastic processes, whereas the lower part of the plant may be influenced by water chemistry in case of determinism.

3.3.4.2 Neutral model of community assembly

To complement the null model, we adopted the neutral community model (NCM) to quantify the importance of neutral processes, which are difficult to detect directly but can have a large influence on microbial communities (i.e., dispersal and ecological drift)[215]. The NCM has been successfully applied to a wide range of ecological phenomena[356]. Points above the prediction represent taxa that are found more frequently than expected, suggesting that they have higher migration ability and can disperse to more locations, whereas points below the prediction represent taxa found less frequently than expected, suggesting their lower dispersal ability in the Lake.

The neutral community model estimated a large portion of the variation in the frequency of occurrence of microeukaryotic communities (R^2= 0.679, 0.526, and 0.757 in wet, dry, and both seasons, respectively) (**Fig. 3. 12B (i-iii)**), suggesting an important role for neutral or stochastic process in shaping the microeukaryotic community assembly. In addition, bacterial communities showed a moderate fit to the neutral model, whose variances R^2 were 0.63, 0.628, and 0.55 in wet, dry, and both seasons, respectively (**Fig. 3.12A(i-iii)**). These results suggest that stochastic processes were very important in shaping the microeukaryotic and bacterial communities in both seasons.

Furthermore, Nm-values were higher for bacterial and microeukaryotic taxa in the wet season (Nm= 18,949 and Nm = 23,570) than in the dry season (Nm = 16,134 and Nm = 13,093), indicating that species dispersal of microbial communities in biofilms was higher in the wet than dry seasons. The microbes are dispersed more easily due to their smaller size, and they cannot counteract the water flow's unidirectional movement. Precipitation always peaks in the wet season and can cause high water levels and river flow. The river flow facilitates higher habitat homogeneity and river connectivity in the wet season than in the dry season [355], and the microorganisms can easily disperse with water flow to remote areas (Lake) in the wet season and successfully colonize the different substrates.

Previous works also revealed similar results to our findings. For example, Roguet et al. [203] investigated the bacterial community in 49 lakes in the Paris area, France, and showed that bacterial community structure was mainly driven by stochastic processes (R^2 = 0.76 explained by NCM). In addition, Chen et al.[125] revealed a high stochastic process of microeukaryotic communities in the wet season (R^2 =0.899) from the Tingjiang River. It should be noted that there might be a difference in microhabitat type and environmental factors, which may affect the microbial assembly process in these studies. These reports were conducted on planktonic microbes in temperate lakes and rivers, which may be different from periphytic biofilms (epiphytic and epipelic biofilms) in tropical lakes. However, further study on the influence of the substrate type and seasons for epiphytic and epipelic biofilms is needed in the future. Although the NCM had a good fit to microbial data, it did not explain 100% of the microbial community variation. Thus, it is difficult to infer the absence of the deterministic processes such as environmental variables and species interactions[357,358].

In summary, the null and neutral models revealed that stochastic processes exert a greater influence on the bacterial (except for *E. crassipes*) and microeukaryotic communities in epiphytic and sediments biofilms in both seasons. This study suggests inlet rivers circling (water flow) through the whole lake and river connectivity in the wet season facilitates habitat homogeneity and microbial dispersal to remote areas (e.g., shallow tropical Lakes). Furthermore, dense and mature epiphytic biofilms in the plant growing period (November) might cause reduced selection strength; thus, more microbial taxa could successfully colonize leaf surfaces under high dispersal rates. Although the dominance of stochastic processes in microbial communities across seasons, the deterministic processes still represented a significant part of the assembly processes. In different shallow lakes, the

bacterial and microeukaryotic communities in epiphytic and surface sediment biofilm communities may be inconsistent and related to lake nutrient status, temperature, seasons, and substrate type. Consequently, exploring microbial communities on various substrates, lakes, and seasons is indispensable to clarifying the assembly mechanisms and factors influencing them.

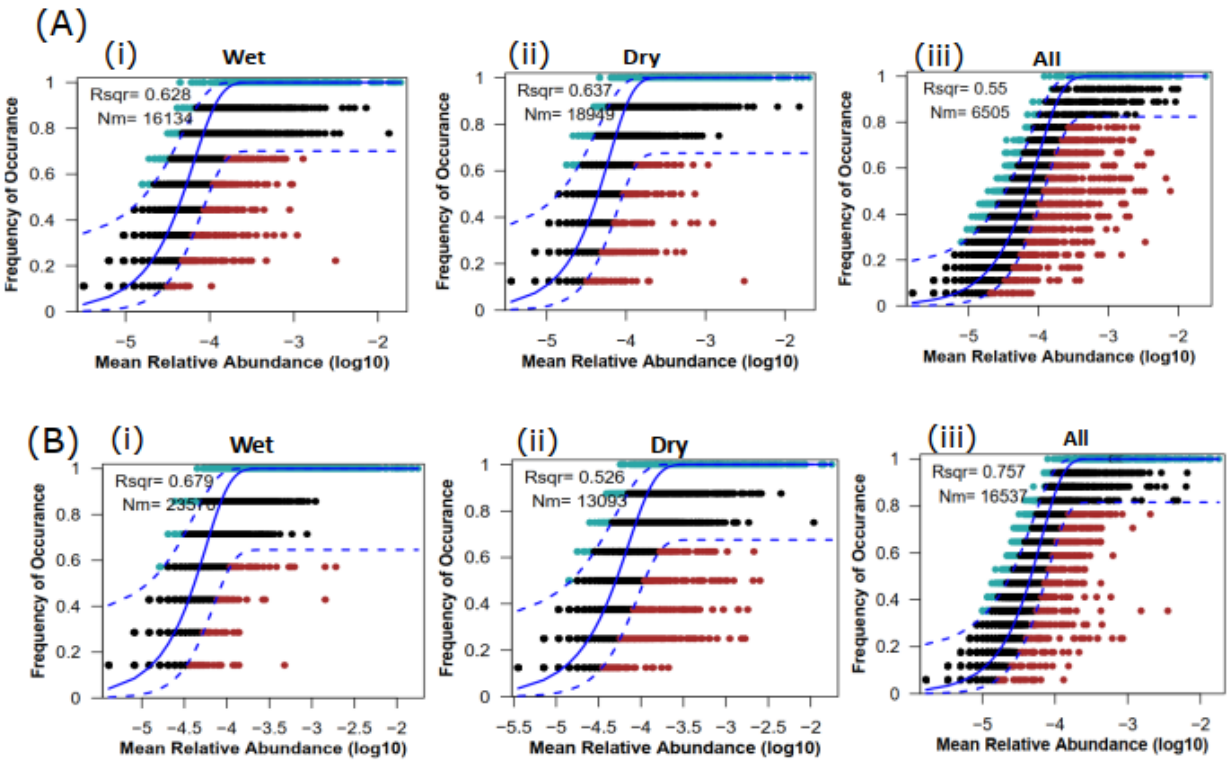


Figure 3. 12 Fit the neutral community model (NCM) of community assembly. The predicted occurrence frequencies for wet, dry, and all represent bacterially (A) and microeukaryotic(B) communities in epiphytic and surface sediment biofilms during wet (i), dry(ii), and both seasons (iii), respectively. The solid blue lines indicate the best fit to the NCM as in Sloan et al.[215], and the dashed blue lines represent 95% confidence intervals around the model prediction. OTUs that occur more or less frequently than predicted by the NCM are shown in different colors. Nm indicates the metacommunity size times immigration, and R^2 indicates the fit to this model.

3.3.5 Co-occurrence patterns of epiphytic and epipelic microbial communities

The network diagrams were employed to explore the possible interactions among bacterial and microeukaryotic communities in *C. demersum*, *E. crassipes*, and surface sediments (**Figure 3.13 A-C**). A total of 2865 edges by 261 nodes (OTU), 3744 edges by 261 nodes, and 3271 edges by 261 nodes were identified in *C. demersum*, *E. crassipes*, and surface sediments, respectively. Topological properties, such as average degree, average path length, modularity class, and average clustering coefficient, were calculated to describe the complex pattern of correlations among the microbial OTUs in the network [216]. The computation of the average degree, average path length, modularity class, and average clustering coefficient was, respectively, 21.95, 2.85, 0.68, and 0.52 in *C. demersum*; 28.69, 2.72, 0.65, and 0.68 in *E. crassipes*; and 25.06, 3.14, 7.96 and 0.52 in surface sediments. **Table 3.1** below summarizes the network topological characteristics for the different microhabitats.

Table 3. 1 Network topological characteristics for the different microhabitats

Parameters	*C. demersum*	*E. crassipes*	Sediment
Nodes	261	261	261
Edges	2865	3744	3271
Average degree	21.95	28.69	25.06
Average clustering coefficient	0.52	0.68	0.52
Average path length	2.85	2.72	3.147
Modularity index	0.68	0.65	7.96

Average degree describes how well a node is connected with its neighbors, while modularity describes the system's resistance to the environment [359]. Besides, the modularity values were > 0.4 in both epiphytic biofilms and surface sediments, demonstrating that the networks have higher module classes[313]. This study suggests that interactions are more complex in surface sediments compared to *C. demersum* and *E. crassipes* based on modularity indices, consistent with previous studies [82]. The higher average degree and shorter path length of the *E. crassipes* network unveiled the possibility that microbial interaction was more intensive[360]. In this study, the average degree and modularity values were 21.95 and 0.68 in *C. demersum*, 28.69 and 0.65 in *E. crassipes*, and 25.06 and 7.96 in surface sediments. A lower average degree and higher modularity in surface

sediments and *C. demersum* than in *E. crassipes* showed that microbial interactions were more intensive in *E. crassipes*, and microbial communities were more vulnerable in *E. crassipes* than surface sediments and *C. demersum*[360].

A module is defined as a group of OTUs that are linked more tightly together. The division of the network into modules may shed light on the different groups of nodes performing different functions [361]. Here, *C. demersum* and surface sediment networks were parsed into 5 major modules, of which modules I and II accounted for 25.29 and 23.37% of the *C. demersum* network, respectively (**Figure. 3.13A**), and modules I and II accounted 60% of the surface sediment network, respectively (**Figure 3.13C**). Notably, the *E. crassipes* network was parsed into 9 modules (**Figure. 3.13B**), of which modules I, II and III accounted for 78.7 % of the whole network.

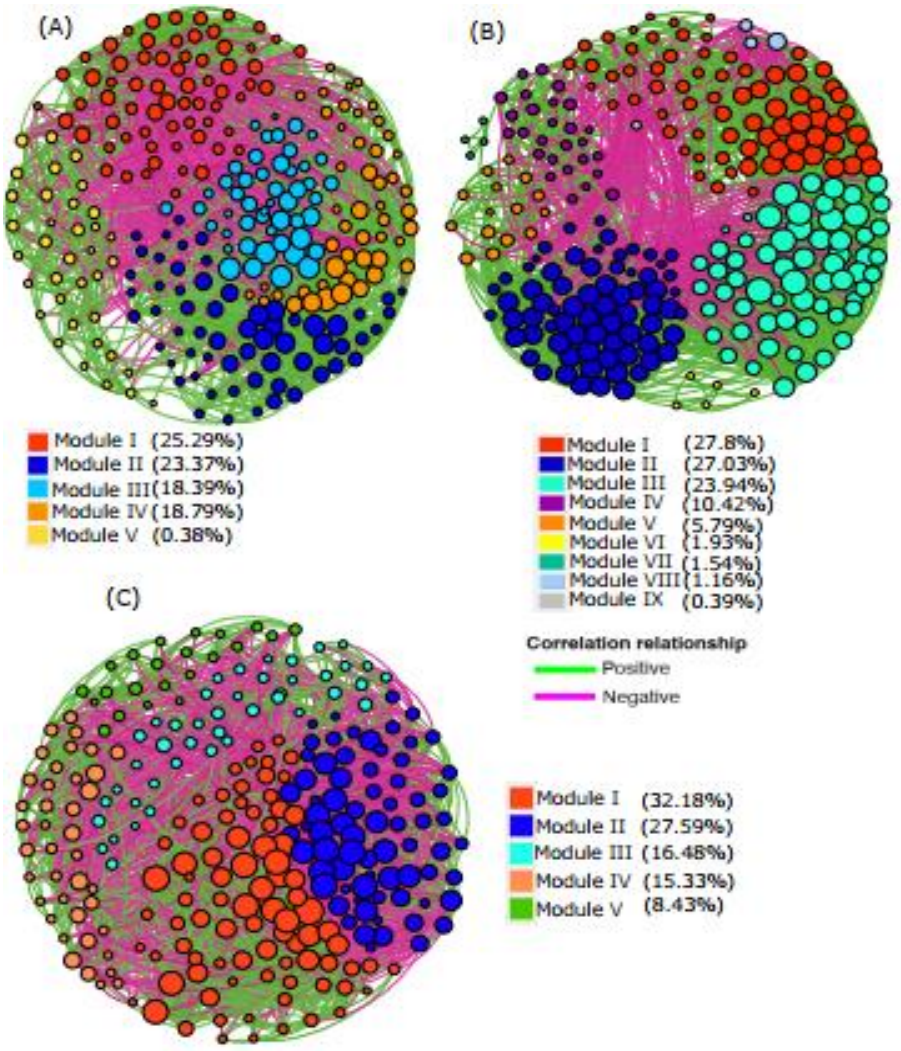


Figure 3. 13 Co-occurrence networks of the bacterial and microeukaryotic communities in *C. demersum* (A), *E. crassipes* (B), and sediments (C) based on pairwise Spearman's correlations between OTUs. The nodes were colored according to different modularity classes, and the size of each node is based on betweenness centrality scores. A connection stands for a strong (Spearman $r > +0.7$ or $r < -0.7$) and significant (P-value < 0.05) correlation. The green and pink lines (edges) represent, respectively, significantly strong positive and negative relationships.

Figure 3.14 shows the identification of putative keystone types according to within-module connectivity (Zi) and among-module connectivity (Pi) of OTUs in all microhabitats. The modularity analysis revealed that most OTUs in the three networks were ultra-peripheral and peripheral nodes and accounted for more than 80% of the total nodes (**Table 3.2**). Notably, the number of ultra-peripheral nodes, peripheral nodes, non-hub connectors, non-hub kinless nodes, and provincial nodes varied in different microhabitats. Based on the topological roles analysis of each OTU, we found that the surface sediment network comprised a much stronger clustered topology (modularity). The Zi-Pi plot displayed that the Proteobacteria phylum was the sole provincial hub (1 OTU) in the sediment network **(Figure 3.14C)**, while *C. demersum* and *E. crassipes* networks shared a unique provincial hub (1 OTU) belonging to the Firmicutes phylum (**Figure 3.14A-B**).

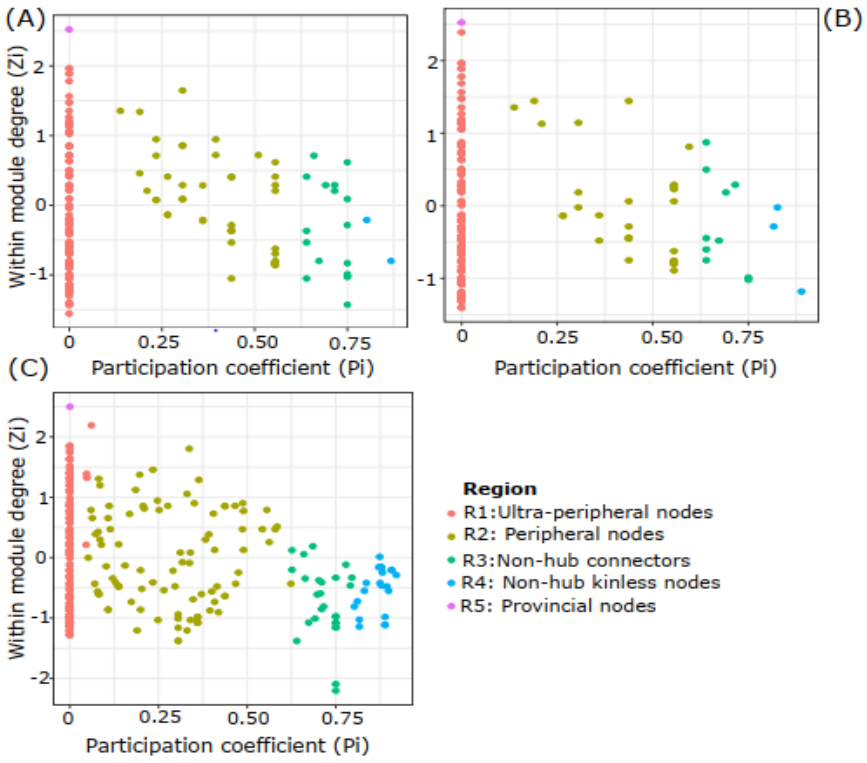


Figure 3. 14 Zi-Pi plot showing the distribution of bacterial and microeukaryotic OTUs (A-C) in *C. demersum* (A), *E. crassipes* (B), and sediments based on their topological roles. Each symbol represents an OTU. The topological role of each OTU was determined according to the scatter plot of within-module connectivity (Zi) and among-module connectivity (Pi).

Table 3. 2 Scatter plots of within-module connectivity (Zi) and among-module connectivity (Pi).

Region/Cluster	*C. demersum*	*E.crassipes*	Sediment	Zi	Pi
R1	64%	66%	53%	Zi< 2.5	Pi<0.05
R2	18%	17%	34%	Zi< 2.5	0.05<Pi<0.625
R3	7%	5%	7%	Zi< 2.5	0.625<Pi<0.8
R4	1%	1%	5%	Zi< 2.5	Pi>0.8
R5	0.4%	0.4%	0.25	Zi>2.5	Pi<0.3

The links showing positive relationships occurred at 83% and 90.9% in *C. demersum* and *E. crassipes*, respectively. This indicates ecological mutualistic relationships or cooperation in epiphytic biofilms, while a much higher proportion of negative correlations among edges was found in the sediments network (47.23%), highlighting the effect of competition in sediment biofilm. The nodes (OTUs) in the three networks were specified to sixteen microbial phyla (bacteria and microeukaryotes). Among these, seven bacterial phyla (Proteobacteria, Firmicutes, Actinobacteria, Chloroflexi, Bacteroidetes, Verrucomicrobia, and Cyanobacteria) were widely distributed, accounting for nearly 75% of nodes (**Table 3.3**).

Table 3. 8 Bacterial/Microeukaryotic Phylum proportions (%) affiliated to the dominant OTUs in epiphytic and sediment networks.

Bacterial/Microeukaryotic Phylum	*C. demersum*	*E. crassipes*	Sediment
Proteobacteria	23.92%	25.1%	25.59
Firmicutes	19.61%	19.12%	19.29
Actinobacteria	14.51%	13.94%	14.57
Chloroflexi	9.02%	8.76%	8.27
Bacteroidetes	4.71%	4.78%	5.12
Verrucomicrobia	4.31%	3.98%	3.94
Cyanobacteria	3.92%	3.59%	3.94
Gastrotricha	1.18%	1.2%	0.79
SAR	1.18%	1.59%	1.57
Rotifera	1.18%	1.2%	1.18
Acidobacteria	1.57%	1.99%	1.97
Spirochaetota	1.57%	1.59%	1.57
Planctomycetota	1.57%	1.59%	1.57
Platyhelminthes	0.78%	-	0.79
Chloroplastida	0.39%	-	0.39
Phragmoplastophyta	0.78%	0.8%	0.79
Ascomycota	-	0.4%	-
Others	9.8%	10.37%	8.66

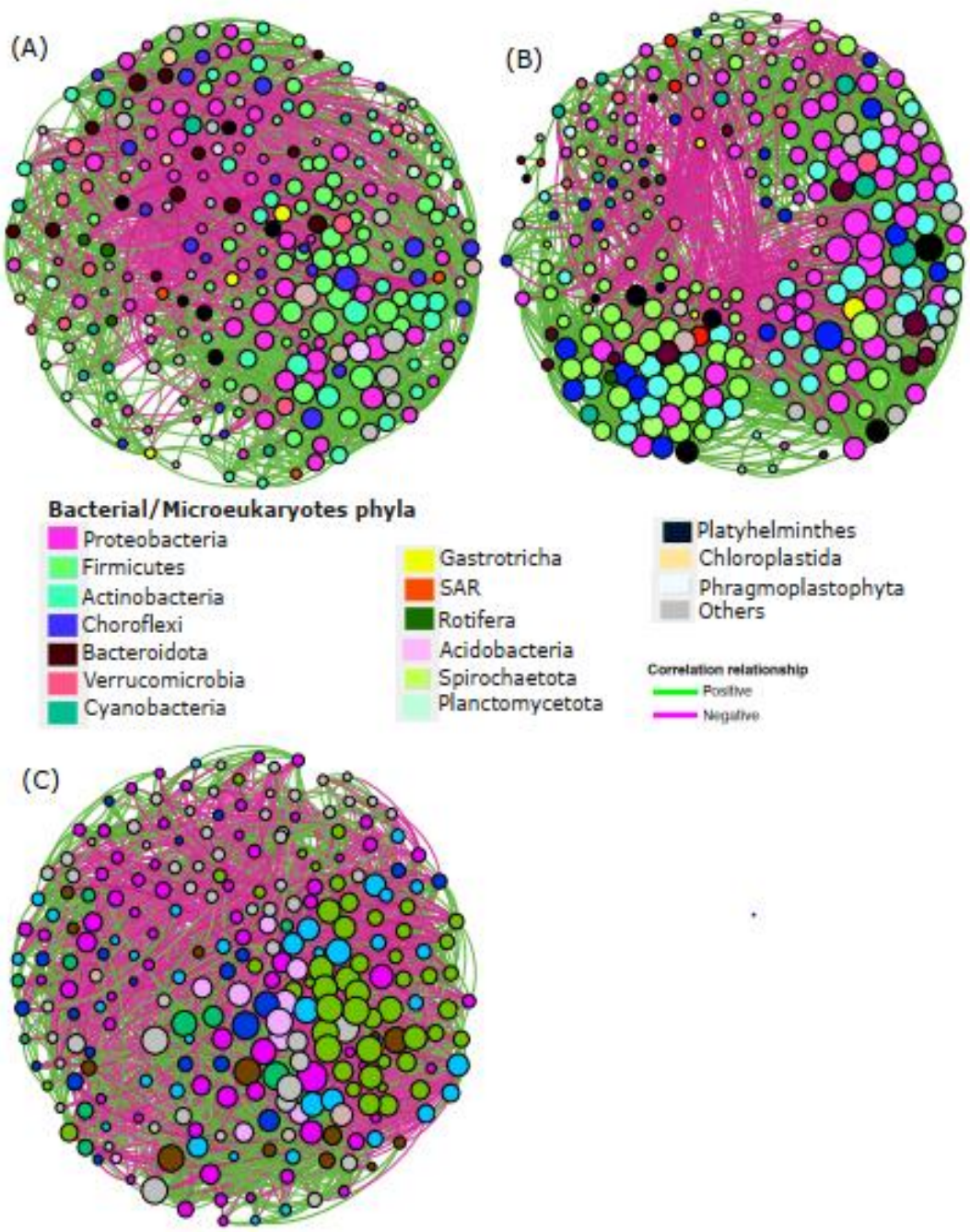


Figure 3. 15 Co-occurrence networks of the bacterial and microeukaryotic communities in *C. demersum* (A), *E. crassipes* (B), and sediments (C) based on pairwise Spearman's correlations between OTUs. The nodes were colored according to different microbial phyla, and the size of each node is based on betweenness centrality scores. A connection stands for a strong (Spearman $r > +0.7$ or $r < -0.7$) and significant (P-value < 0.05) correlation. The green and pink lines (edges) represent significantly strong positive and negative relationships.

Proteobacteria (23.92-25.59%) was the most dominant phylum in all microhabitat networks (**Figure 3.15**). Although microeukaryotic phyla exhibited a low proportion (0.4-1.6%) compared to bacteria, they perform complex interactions with bacteria [16,89]. The most represented were SAR, Rotifera, Gastrotricha, Platyhelminthes, and Chloroplastida. Our recent report showed that complex microbial interactions (predation, symbiosis and competition) existed in epiphytic biofilms and surface sediments [16]. In this study, the proportion of positive and negative correlations between bacteria and eukaryotes infer that predation and symbiosis relationships may occur among biofilm microbes. Interestingly, predators (Rotifera, Gastrotricha and SAR) dominated the microeukaryotic phyla in three networks (**Figure 3.15A-C**), suggesting that they were the top consumers (predation) in microbial food webs. Rotifers, Gastrotricha, and some SAR taxa are important consumers of small phytoplanktons and bacteria, and their association shows predation in the food web[96].

Banerjee et al. [362] reported that the keystone species could be defined in the system by betweenness centrality. The top five species (OTUs) could be confirmed as keystone taxa on the ground of the betweenness centrality score[363], which is fateful for sustaining the stability of communities[364]. In *C. demersum*, these keystone species (OTUs) belong to Verrucomicrobia, Chloroflexi, Proteobacteria, Bacteroidetes, and Firmicutes phyla (**Figure 3.15A**). In *E. crassipes*, the top five keystone species (OTUs) belong to Actinobacteria, Firmicutes, Proteobacteria, Rotifera, and Ascomycota (**Figure 3.15B**), whereas in surface sediments, those keystone taxa belong to Proteobacteria, Verrucomicrobia, Firmicutes, Actinobacteria, and Chloroflexi (**Figure 3.15C**). Proteobacteria and Bacteroides work synergistically to decompose complex organic matter[103]. The fungi and metazoans are known as organic matter decomposers and predators in aquatic systems [32,227]. The dominance of keystone genera in phylum Rotifera and Ascomycota in *E. crassipes* implied that predation and organic matter decomposition were the important feeding relationship in this food web. This study suggests that fungi decompose organic matter from decayed *E. crassipes*.

Several primary consumers (bacteria and fungi) played a significant role in the microbial community structure and function in freshwater lakes' epiphytic and sediment biofilms. For example, the functional role may be associated with carbon cycling/anoxygenic phototrophy (p_(phylum)Chloroflexi) [150], lignocellulose decomposition and production of secondary

metabolites (p_Actinobacteria) [365,366], nitrogen fixation and diverse carbon metabolism (p_Verrucomicrobia) [367], methane oxidation (p_Firmicutes) [316], and fermentation and organic matter decomposition (p_Ascomycota) [227,320]. Although our results revealed general complex interactions (predation, symbiosis, commensalism and competitive relationships) among microbes in all microhabitats, the surface sediments food web was more complex and stable. The difference in food web structure and complexity in aquatic plants and surface sediments may be attributed to the substrate type, plant physiology (secondary metabolites) and morphology [368,369], distinctive microbial lifestyles, water chemistry, and seasons[234].

3.4. Summary

In this study, we broadly explored the effect of aquatic macrophytes (*C. demersum* and *E. crassipes*) and surface sediments on microbial community structure, assembly processes, and co-occurrence patterns during dry and wet seasons in a shallow tropical lake (Cyohoha). The main conclusions are as follows:

1) We found significant differences in the bacterial community dissimilarity and microeukaryotic OTU richness among microhabitats.
2) Stochastic processes dominated the microbial community assembly in epiphytic (except for bacteria on *E. crassipes*) and surface sediment biofilms. Notwithstanding the dominance of stochastic processes in microbial community assembly, the deterministic processes still represented a significant part of the assembly processes
3) The stability and complexity of networks differed among substrates, significantly higher in surface sediments than in *C. demersum* and *E. crassipes*.
4) Phylum Proteobacteria, Firmicutes, Actinobacteria, Chloroflexi, Bacteroidetes, Verrucomicrobia, Cyanobacteria, SAR, Rotifera, Gastrotricha, Platyhelminthes, and Chloroplastida were the key role players in the microhabitat networks.

Chapter 4. Microbial assemblage and interaction in mats for two hot springs

4.1 Background

Microbial communities exhibit diverse metabolic capabilities in microbial mats and play central roles in biogeochemical cycles and food-webs. Microbial mats are complex and self-sustaining biofilms embedded vertically in extracellular polymeric substances (EPS) as a result of physical gradients [178]. They form at the liquid-solid interface of various habitats, including hot springs and hypersaline waters [26,154]. Microbial mats comprise a wide variety of microorganisms, mainly bacteria and archaea. However, eukaryotes (e.g., Fungi, Microalgae, Protists, and rarely Metazoans) and viruses are the integral parts of the microbial mats, although less diverse and abundant in nature [26,179,184].

The microbial communities in EPS communicate by quorum sensing and exchanging nutrients to facilitate a greater flux of resources and energy for survival [156,370]. Dynamic physiochemical gradients in microbial mats greatly affect biological diversity, modified mainly by the biological processes of the dwelling bacteria. Due to these biological processes and physical gradients, microorganisms with specific preferences choose the optimal microenvironments and ecological niches [154,170]. Thermophiles are micro/organisms that can reach optimal growth at a temperature between 50 and 80 °C [371]. This group is generally subdivided into 3 subgroups: (i) hyperthermophiles (≥ 80 °C), (ii) extreme thermophiles (70-80°C), and (iii) moderate thermophiles (45-70°C). Furthermore, eukaryotes are restricted to growing in a narrow temperature range due to the persistent paradigm that eukaryotic organisms are not well adapted to survive in harsh conditions, notwithstanding increasing evidence that they are present and active in these extreme habitats [231]. One recent report suggests that several protistan taxa may survive in hot springs at 70 °C[371]. To thrive in these extreme environments, they maintain the cytoplasmic pH neutral via altered internal physiology, and there may be substantial gene transfer from bacteria and archaea [372,373]. Sharp et al.[374] indicated that temperature was the controlling factor of microbial diversity in many hot springs across Canada and New Zealand. At a broad range of geochemical gradients, Power et al. [375] concluded that pH was the main factor determining diversity in the temperature range of 20-70ºC, but the temperature impact was more significant above 70°C.

A series of biological interactions occurring in the mats must be considered since they are interconnected with crucial processes such as photosynthesis, nitrogen fixation, denitrification, metal reduction, sulfate reduction, and methanogenesis [376,377]. Previous studies focused on bacterial and archaeal diversity and composition in thermophilic microbial mats worldwide, and their distribution is directly correlated with geothermal zones [378,379]. Nevertheless, most studies were conducted in the USA Yellowstone National Park (YNP) [380]. Recently, microeukaryotic communities were reported to actively grow in the hydrothermal and hot springs' hot water, where they play potential ecosystem functions [231,370]. Furthermore, a couple of discrete reports addressed microeukaryotic diversity and composition in saline and hot spring mats [249,381]. However, most of these reports explored one group of eukaryotes and failed to comprehensively investigate the microeukaryotic diversity, assembly processes, interactions, and ecological role in hot spring mats.

Geothermal hot springs are distributed at multiple locations across the continents and the inhabiting microbial communities received much attention in Oceania, South America, North America, an Asia [375,382–384]. The African Rift Valley is composed of milliards of alkaline geothermal hot springs, formed by upwelling geo-thermally heated waters belowground and due to volcanic activity that started about 30 million years [385]. Because temperature is considered as one of the main factors controlling the diversity and distribution of microbial mat communities, exploring hot spring microbial mats could improve our understanding of microbial community changes in extreme environments.

To fill this gap, the present study aimed to investigate: 1) microbial diversity and composition along a temperature gradient in microbial mats, 2) the response of microbial communities in mats to environmental parameters, and 3) the interactions and ecological roles among microbes in microbial mats. To achieve these aims, we collected samples from geothermal hot springs in Bugarama and Gisenyi districts located in South and North-Western Province, Rwanda, known for their large fluctuation in temperature. In-depth research on microbial mats may shade new light in understanding the biodiversity, interactions, and ecological roles among mat-dwelling microorganisms in tropical geothermal springs, which holds good for trophic relationships and biogeochemical cycling.

4.2 Materials and methods

4.2.1 Study area

Field measurements and sample collections were conducted in 2019 from Bugarama hot pool (BHP) (2º34’57” S, 29º00’59” E) and Gisenyi hot springs (GHS) (1°44’ 23” S, 29°16’26” E), distanced with 243.5 km in the Western Province of Rwanda **(Figure 4.1)**. The sampling locations are geothermal areas associated with the western branch of the East-Africa Rift Valley composed of deep lacustrine basins and structural heights characterized by active volcanism and aquifer lithology. BHP emerges from groundwater reservoirs across limestone query and exhibits lower temperatures. However, the higher temperature GHS is located at the north shore of Lake Kivu, where they emerge from Cenozoic to recent volcanic rocks, such as basalt, mica schist, sandstone, and travertine. These rocks are a volcanic, highly alkaline extension of the Virunga volcanic area [386].

4.2.2 Field measurements and sample collection

A total of 20 samples (10 microbial mats and 10 water samples) were collected in BHP (BG1-5) and GHS (GS1-5). Triplicates of microbial mat samples were collected beneath the water surface and preserved and treated following previous reports [16,378]. HQ30d portable multi-parameter digital analyzers (HACH, USA) were used to measure *in situ* the physico-chemical parameters of the water samples, including water temperature, pH, EC, TDS, and ORP. Other chemical parameters such as TN, TP, NH_4-N, and NO_3-N were quantified at the Biotechnology Complex Laboratory (BCL) of the University of Rwanda (UR) laboratory previously described methods [263].

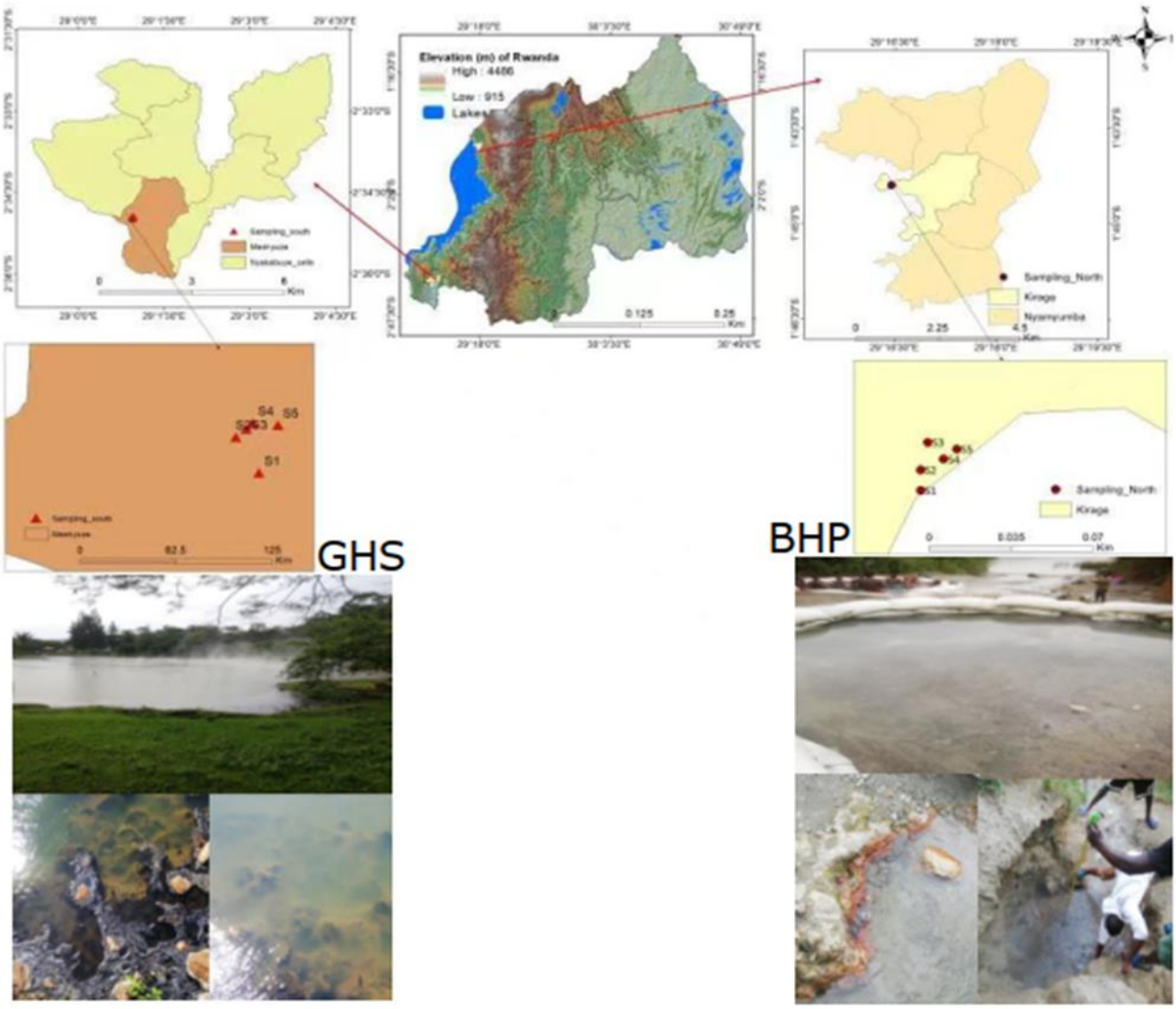


Figure 4. 1 Map of the Bugarama hot pool (BHP) and Gisenyi hot springs (GHS), Rwanda. The geothermal fields from which samples were collected are presented in red.

4.2.3 DNA extraction and PCR amplification

Samples were vigorously homogenized, and metagenomic DNAs were extracted from 0.25 g of microbial mats using Fast DNA® SPIN extraction Kits (MP Biomedical, Ohio, USA) as described in the manufacturer's protocol. DNA quality was checked on 1% agarose gel electrophoresis and quantified using a NanoDrop™ 2000 UV– Vis spectrophotometer (Thermo Scientific, USA) [264]. Three aliquots of DNA samples from the triplicates were combined for PCR amplification (GeneAmp 9700, ABI, USA). For Illumina high-throughput sequencing, the bacterial V4-V5 hypervariable region of the 16S rRNA gene was amplified by thermocycler PCR

(Polymerase Chain Reaction) system (GeneAmp 9700, ABI, USA) using the primers 515F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), and eukaryotic V4 region of 18S rRNA using 3NDF (5'-GCGGTAATTCCAGCTCCAA-3') and V4-euk-R2 (5'-AATCCRAGAATTTCACCTCCAA-3').

4.2.4 Illumina MiSeq sequencing of 16S rRNA and 18S rRNA gene

The purified amplicons were pooled in equimolar fractions, and paired-end sequences were run on the MiSeq PE300/NovaSeq PE250 platforms (Illumina, San Diego, CA, USA) according to the standard protocols provided by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

4.2.5 Bioinformatics

FASTQ files (raw reads) were quality filtered by Trimmomatic before merging by FLASH (https:// ccb.jhu.edu/software/FLASH/index.shtml) with the following criteria: Reads were truncated at sites receiving an average quality score of < 20 within a 50 bp sliding window. Simultaneously, UPARSE[387], the taxonomy of each 16S rRNA and 18S rRNA gene sequence was assigned and mapped to the Silva (SSU123) and (SSU138) databases, respectively, with a 70% confidence threshold.

4.2.6 Functional predictions

FAPROTAX is a database that maps prokaryotic clades (e.g., genera or species) to established metabolic or other ecologically relevant functions.

4.2.7 Data analysis

Data analysis and visualization were performed on Majorbio Cloud platforms (www.majorbio.com), OmicShare (https://www.omicshare.com/tools), and in R (R Core Team, 2015). The 'Stats' and the 'spicy' R packages were used to perform the statistical comparisons. One-way ANOVA using the software SPSS (https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-22, version 22.0) was used to compare the groups. Alpha diversity was computed using the vegan package 2.5 (https://CRAN.R- project.org/package=vegan) and was visualized using ggplot2 package 3.2.0. The NMDs beta diversity analysis was used to explore the similarity between microbial community structures at the OTU level using the Bray-Curtis distance algorithm [388]. The diversity (*ß*-metrics) between microbial communities along a temperature gradient was computed by Beta Nearest Taxon Index (βNTI) using the "ape" and "Picante" functions of R (https://www.r-project.org/, v3.5.3) in accordance with Stegen et al. [199]. This phylogenic signal was used to assess the relative importance of the assembly processes generating the observed patterns. For this, value of $|\beta NTI| > 2$ represented the deterministic process ($\beta NTI > +2$, variable selection; $\beta NTI < -2$, homogeneous selection), while $|\beta NTI| < 2$ was regarded as the stochastic processes. The relative abundance of taxonomic compositions was visualized using the 'circlize' package and Omicshare online platform. With the help of CANOCO (https://www.wur.nl/en/show/Canoco-for-visualization-of-multivariate-data.htm, version 5.0, v5.0) [389], we performed a Redundancy Analysis (RDA) to describe the relationship between mat biofilms and environmental parameters. The network analysis was performed using Spearman correlations calculated via the corr. test function of the 'psych' packages in R (https://www.r-project.org/, V4.1.2) and visualized via Gephi (https://gephi.org/, v0.9.2). Correlations were considered robust if Spearman's correlation coefficients (r) was (> 0.7 or <-0.7; $P < 0.05$)[265]. The ecological relevant functions of bacteria were predicted by FAPROTAX to obtain information at different pathway levels (levels 1–3) and visualized in a heatmap via the Omicshare platform. The predicted functions were compared between hot springs using STAMP (Statistical Analysis of Metagenomic Profiles version 2.1.3, https://beikolab.cs.dal.ca/software/STAMP) based on the Bonferroni-test ($P < 0.05$). The sampling map was drawn using QGIS (v2.18, QGIS Development Team, 2017).

4.3 Results and discussion

4.3.1 Physicochemical parameters

Environmental variables are important for microbial growth in the hot springs. Eleven physicochemical parameters of the water column were measured at the 10 sampling sites in the Bugarama and Gisenyi hot springs (**Figure 4.2**). In general, water temperature (40–71.4 °C), pH (6.48–7.1), DO (ND-5.2mg/L), TDS (912.3-1183.3mg/L), EC (2.5-4.85μS/cm), ORP (-2.63-32.4mV), TP (0.82-2.3mg/L), TN (5-6.2mg/L), NO_3-N (1-1.5 mg/L), NH_4-N (2.3-3.4mg/L), and elevation (1174-1470m) varied across two geothermal springs. In the present study, there was a significant difference ($P < 0.01$, $P < 0.05$) in temperature, pH, ORP, TP, TN, and NO_3-N across the two hot springs (**Figure 4.2 and Table 4.1**), in line with previous studies[45,390,391]. Furthermore, temperature, pH, TDS, EC and elevation values were higher in GHS than in BHP, while ORP, TP, and DO revealed a reverse trend. Temperature and pH are key drivers of microbial community structure in the hot springs[374,375]. The high temperatures could significantly negatively affect the DO concentration in hot springs, and the negative ORP represents a potential reducing condition. Additionally, the high TP in BHP may leach from sediments/soil and fallen leaves (seen in BHP). Previous work indicated that GHS contains higher ratios of cations (Na^+/$K^+$and SiO_2) and anions (Cl^-, HCO_3^-, and SO_4^{2-}), while BHP is rich in bicarbonate (HCO_3^-), Mg^{2+}, Ca^{2+}, and Li^+, consistent with previous studies [392,393]. These observed differences or similarities in the physicochemical parameters may be due to the divergences in the geological substrate, geothermal activity, and geographic distance between the two hot springs.

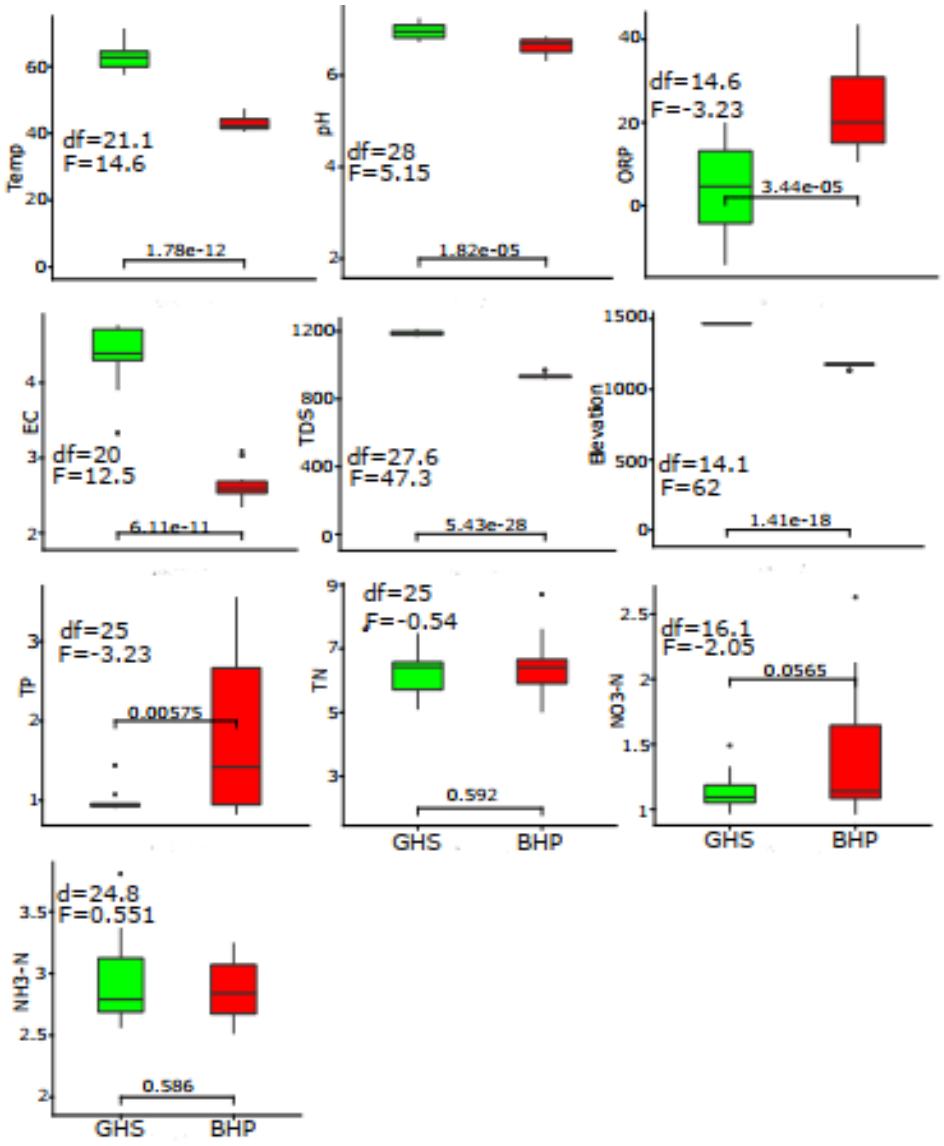


Figure 4. 2 Physicochemical Parameters of Bugarama (BHP) and Gisenyi (GHS) hot springs

Table 4. 1 Physicochemical parameter of Bugarama and Gisenyi hot spring

Parameters	Temperature	pH	DO(mg/L)	TDS (mg/L)	EC(µS/cm)	ORP (mv)	TP(mg/L)	TN(mg/L)	NO_3-N(mg/L)	NH_4-N(mg/L)
BG1	47.33±0.05	6.48±0.01	4.2±0.08	929.33±4.58	2.64±0.06	32.36±0.13	0.82±0.00	5.34±0.06	1.11±0.01	3.14±0.05
BG2	42.63±0.28	6.66±0.02	2.75±0.04	912.33±3.14	2.58±0.03	20.63±0.49	2.35±0.06	6.22±0.18	1.58±0.07	2.75±0.05
BG3	41.63±0.28	6.74±0.02	4.35±0.03	937.33±2.25	2.53±0.01	15.4±0.47	0.94±0.00	5.85±0.06	1.30±0.06	2.40±0.05
BG4	40.2±0.17	6.96±0.02	5.20±0.08	917.33±1.86	2.47±0.02	8±0.32	1.06±0.02	5.03±0.01	1.13±0.00	2.82±0.01
BG5	44.43±0.44	6.60±0.03	2.67±0.03	922.33±2.25	2.60±0.02	24.76±0.18	1.60±0.01	6.10±0.04	1.10±0.00	3.24±0.03
GS1	62.8±0.22	6.93±0.04	ND	1183.33±13.27	4.47±0.12	3.03±0.40	0.90±0.00	6.02±0.13	1.20±0.12	2.90±0.06
GS2	64.8±0.84	7.06±0.00	ND	1165.67±22.06	4.81±0.39	-2.63±0.22	0.91±0.01	5.9±0.46	1.02±0.06	2.50±0.05
GS3	71.4±0.27	6.8±0.08	ND	1157±8.53	4.85±0.18	14.8±0.23	0.90±0.00	5.47±1	1±0.00	2.30±1.03
GS4	58±1.05	7.12±33.4	ND	1161.33±4.50	4.5±0.06	0.36±0.13	0.94±0.01	5.22±0.05	1.14±0.01	2.74±0.09
GS5	60±0.62	6.78±0.06	ND	1166.66±7.71	4.32±0.06	12.26±0.57	0.96±0.00	5.45±0.04	1.00±0.00	3.41±0.02

4.3.2 The stochastic processes and temperature drive the microbial biodiversity (β-) in mats

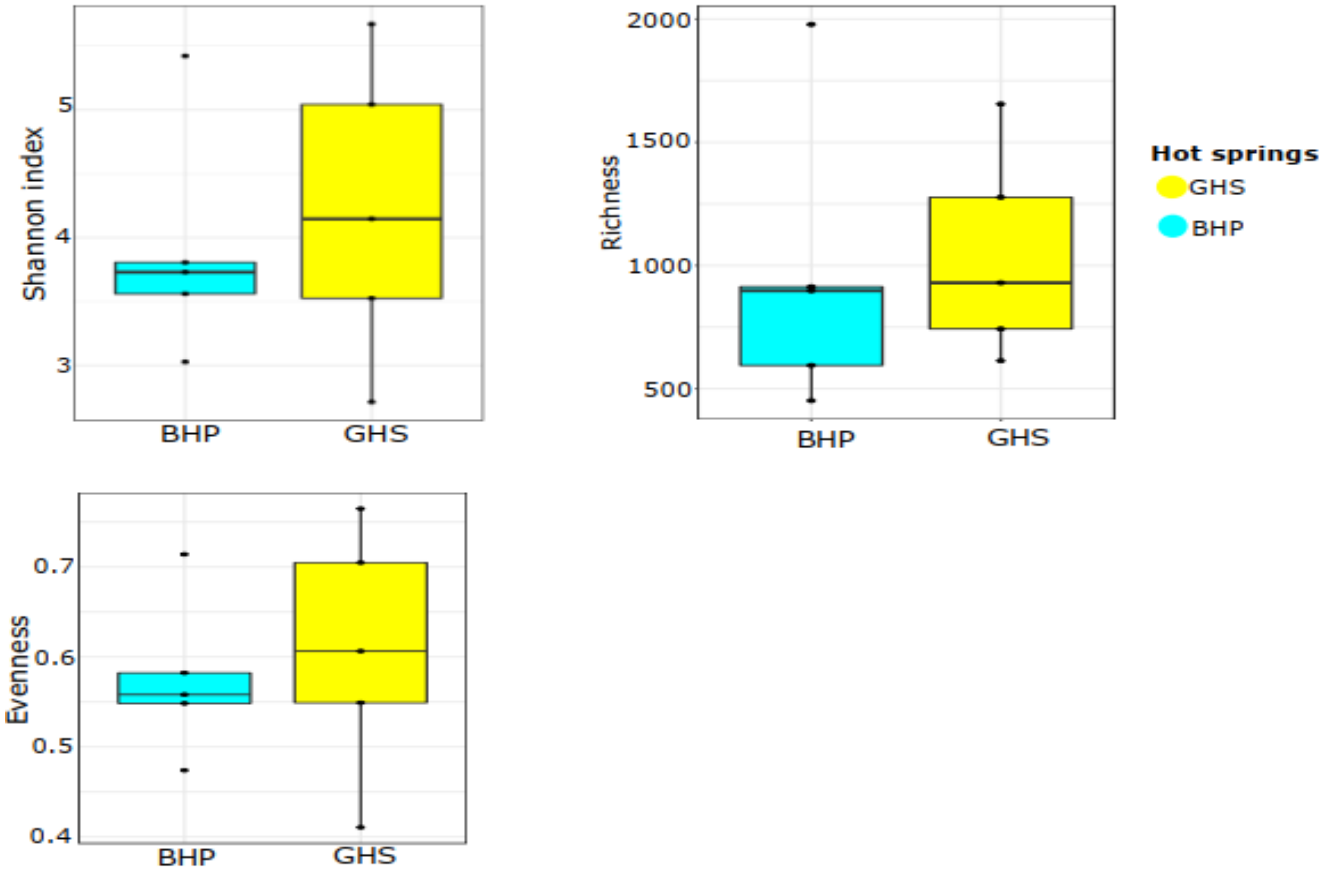
A total of 585780 (16S rRNA) and 506208 (18S rRNA) clean reads were normalized in all samples. The Shannon, OTUs richness and Pielou's evenness values for all samples were provided in **Table 4.2**. There was no significant difference ($P > 0.05$) in microbial (bacteria and microeukaryotes) α-diversity (OTU richness, Shannon index, and Pielou's evenness) along temperature gradients between BHP and GHS (**Figure 4.3A-B**), consistent with previous reports [10, 14]. In addition, GHS showed higher microbial α-diversity values (except Shannon index for eukaryotes) than BHP. These findings may be due to the differences in temperature and pH **(Figure 4.2 and Table 4.1)** or shaped by other unmeasured water parameters such as heavy metals and organic compounds (e.g., COD, BOD) in hot springs. Notably, the low-temperature range (< 60ºC) favored the microeukaryotes (e.g., protists), and they thrive well in optimum pH compared to bacteria, congruent with previous studies [231,394]. Thus, this study envisaged that this might be due to the more limited temperature range considered in this work (40-72 °C), niche preference, and the diversity of hot spring mats that may be more closely associated with extreme temperatures. However, a substantial viewpoint of this finding in microeukaryotic mats needs further research.

Non-metric multidimensional scaling (NMDS) plot of β-diversity showed two groups, i.e., the BHP group (Group I) and the GHS group (Group II) (**Figure 4.4A-B**). NMDS showed a significant difference ($R^2 = 0.25$, $P = 0.003$) in the bacterial communities of two hot springs, suggesting their different distribution based on temperature gradients. These findings indicate that temperature is critical in bacterial community distribution in microbial mats.

Table 4. 2 Microbial alpha diversity indices

Alpha index	Bacteria	Microeukryotes
Shannon index	0.65	0.51
Richness	0.82	0.52
Pielou-evenness	0.67	0.5

(A)



(B)

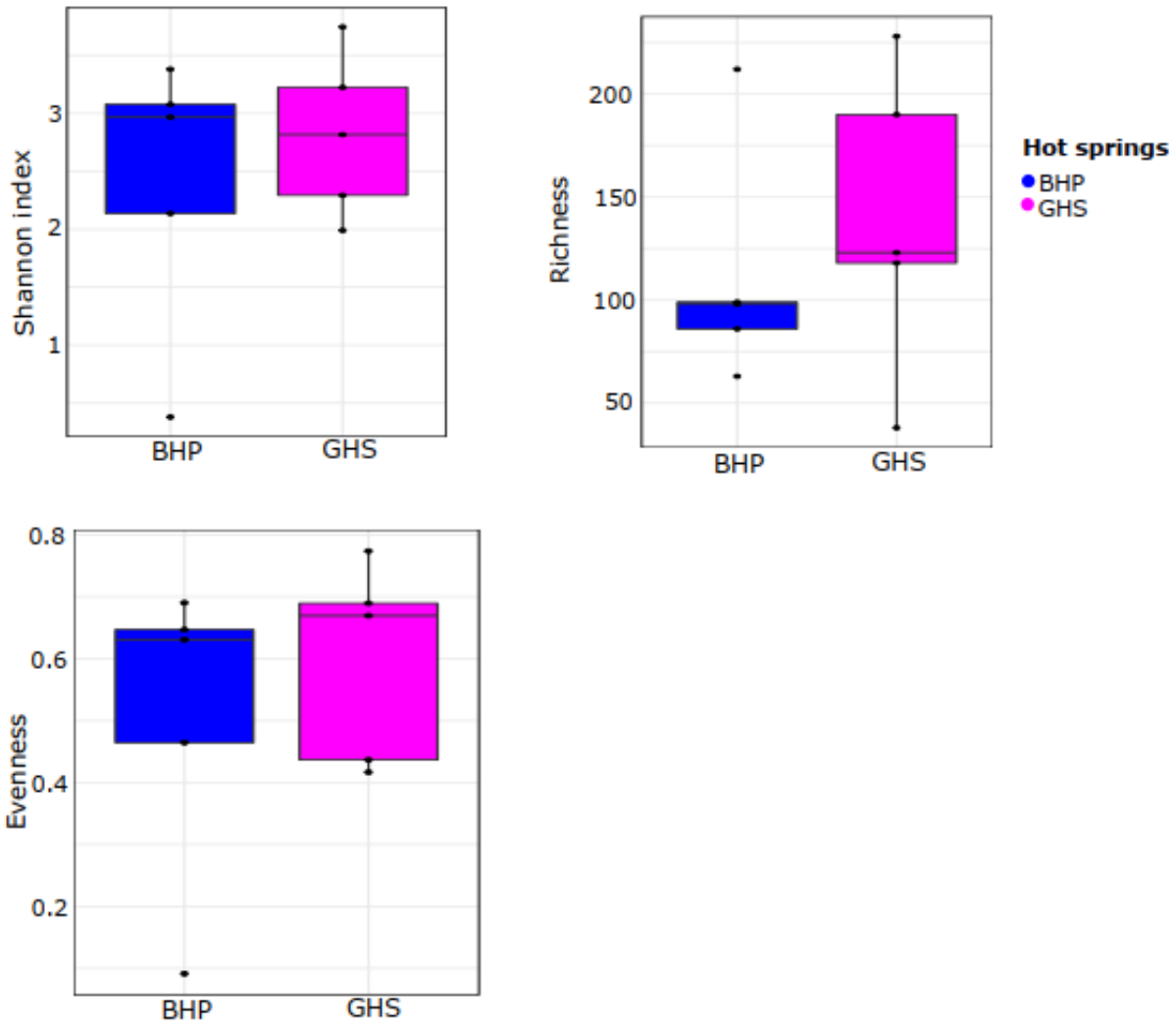


Figure 4. 3 Bacterial (A) and microeukaryotic (B) alpha diversity indices along temperature gradients in two hot springs

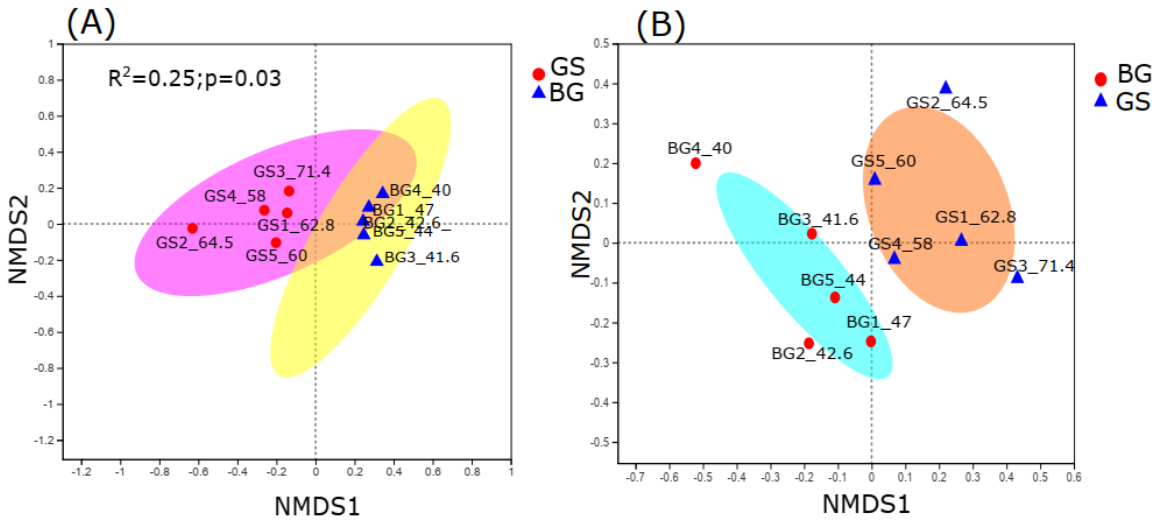


Figure 4. 4 NMDS plot of beta diversity (via Bray–Curtis dissimilarities) in bacterial (A) and microeukaryotic (B) communities in hot spring mats.

The null model-based analyses were employed to explore further the relative contributions of deterministic and stochastic processes to microbial β-diversity in the mat biofilms. From an ecological perspective, both deterministic and stochastic processes govern microbial community assembly [350]. To comprehend the mechanisms of community assembly that underpin microbial diversity is a crucial topic in ecology [42].This topic has been researched widely in microbial ecology across various habitats such as freshwater[44], groundwater[202], hot spring sediments[45], soils[206], marine environments[207], and water and wastewater treatment settings[46]. However, little is known about the relative importance of stochastic and deterministic assembly processes in microbial hot spring mats.

The bacterial community in BHP and GHS mats was completely structured by stochastic processes, including ecological drift (80%) and homogenizing dispersal (20%) (**Figure 4.5A**). In contrast, the microeukaryotic community was dominated by variable selection (40%), followed by homogenizing dispersal (30%) and ecological drift (30%). The high proportion of stochastic processes in the bacterial community implies that stochastic processes exert a greater influence on this community. In addition, stochastic and deterministic processes were coupled in shaping microeukaryotic diversity; however, the stochastic processes further dominated microeukaryotes of the hot spring mats **(Figure 4.5A)**. The potential relationship between βNTI (β-Nearest Taxon Index) and temperature gradient was examined to infer deterministic and stochastic assembly

processes (Fig. 2B). The pairwise comparisons of βNTI for bacterial communities were strongly driven by stochastic processes and negatively correlated with temperature in BHP (**Figure 4.5B (i)**), whereas microeukaryotes revealed no correlation with temperature (**Figure 4.5B (ii)**). However, the pairwise comparisons of βNTI for microeukaryotic and bacterial communities were driven by deterministic processes (selective pressure) at high temperatures (GHS) (**Figure 4.5B (iii & iv)**). This indicates that bacterial communities in moderate temperature were driven by stochastic processes, whereas at high temperature springs (GHS), the variable selection in microeukaryotic and bacterial communities was potentially induced by temperature.

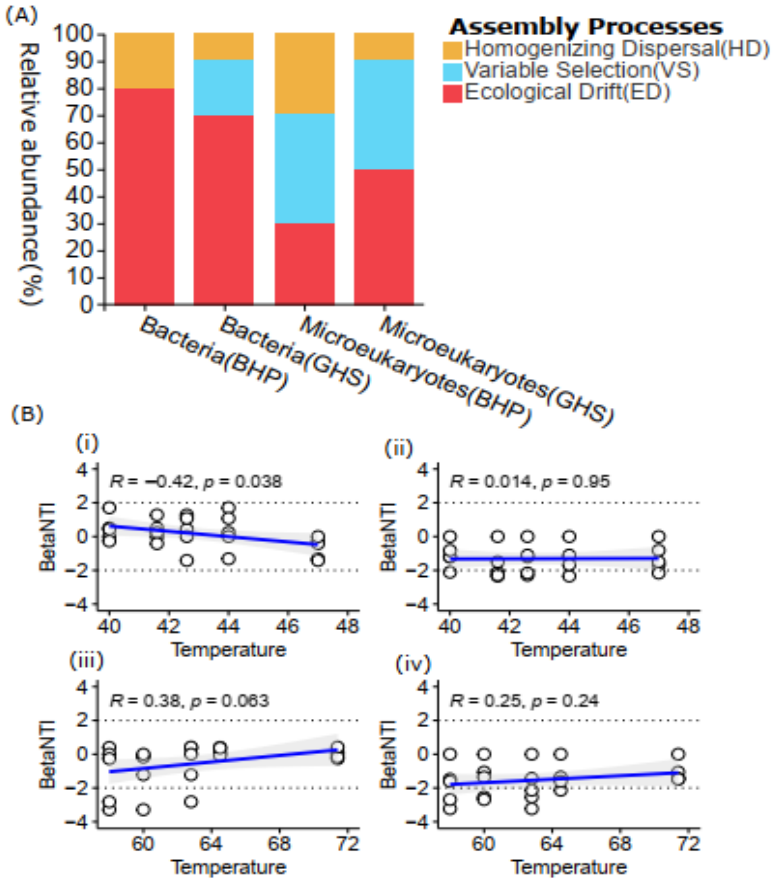


Figure 4. 5 Relative influences of deterministic and stochastic processes on hot spring microbial mat assembly (A). The fraction of ecological processes (deterministic: homogeneous and variable selection; stochastic: homogenizing dispersal and ecological drift governing the community assemblies in hot spring mats. (B) Relationships between βNTI and temperature gradients in microbial mats (bacteria and microeukaryotes). Linear regression models (shown as blue lines) and associated correlation coefficients and P values are provided on each panel. Horizontal dashed lines indicate the βNTI significance thresholds of +2 and −2. The dots below – 2 indicate the presence of variable selection induced by temperature.

4.3.3 Phylum Chloroflexi dominate bacterial communities in hot spring mats

A total of 17 and 11 bacterial phyla (relative abundance >1% in at least one sample) dominated the samples from BHP and GHS, respectively (**Figure 4.6A-B**). Chloroflexi, Proteobacteria, Cyanobacteria, and Bacteroidota were the most abundant phyla in all samples. These bacterial phyla were commonly reported in hot springs/mats worldwide [395–397]. Notwithstanding, the bacterial communities shared these four dominant phyla but differed in overall phyla composition. Phylum Chloroflexi (18.6-74.1%) and Proteobacteria (1.7-30%) dominated in BHP, whereas Cyanobacteria (5.3-59.4%) revealed a reverse trend in GHS (**Figure 4.6A-B**). The differences in the bacterial composition recorded in this study may be attributed to spring temperature variations that are important in shaping microbial communities in an extreme environment [166].

Furthermore, phylum Desulfobacterota, Patescibacteria, Campylobacteria, WOR-1, and Armatimonadota phyla were exclusively abundant in BHP. Most of these phyla are novel lineages and minor groups in hot springs/mats [398,399] and were reported as groundwater dwellers [400] and sediment microbes [401]**.** This study suggests that these bacterial phyla may thrive well in Bugarama hot pool with temperature below 50^{o}C and may originate from groundwater systems, sediments/soil, and fallen leaves (seen in BHP) via dispersal mechanism.

The relative abundance of phylum Planctomycetes was significantly higher ($P < 0.05$) in GHS than in BHP (**Figure 4.6C**). Planctomycetota comprises thermophilic taxa growing in hot spring mats [164], which can play a critical role in the global carbon cycle and nitrogen cycle [36], suggesting their high-temperature preference, organic matter decomposition, anaerobic ammonium oxidation in GHS hot spring mats. Besides, Cyanobacteria, Acidobacteriae, Verrucomicrobiae, and Bacilli were the most abundant bacterial classes in all samples. It should be noted that the dominant bacterial classes revealed no significant difference ($P > 0.05$) (**Figure 4.7A)**; however, there was a statistical difference among rare classes such as Phycisphaerae, Planctomycetes, Deinococci, Blastocatellia, Sumerlaeia, Gemmatimonadetes, Rhodothermia, and Chloroflexia (*$P < 0.05$, ** $P < 0.01$) in GHS mats (**Figure 4.7 B**).

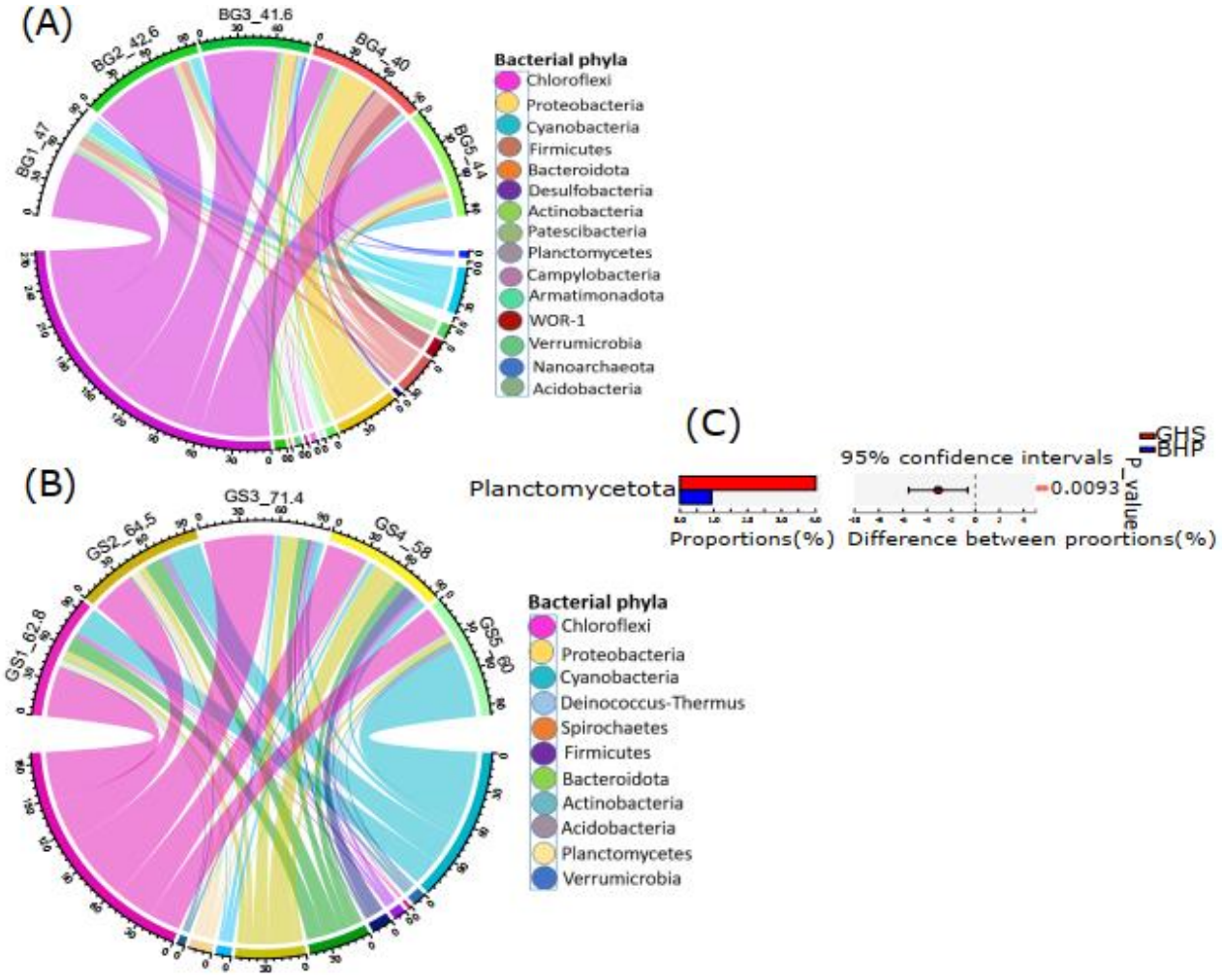


Figure 4. 6 The relative abundance of bacterial phyla (A-B) in all samples. *** signifies $P < 0.01$ by Welch's sample comparison (C).

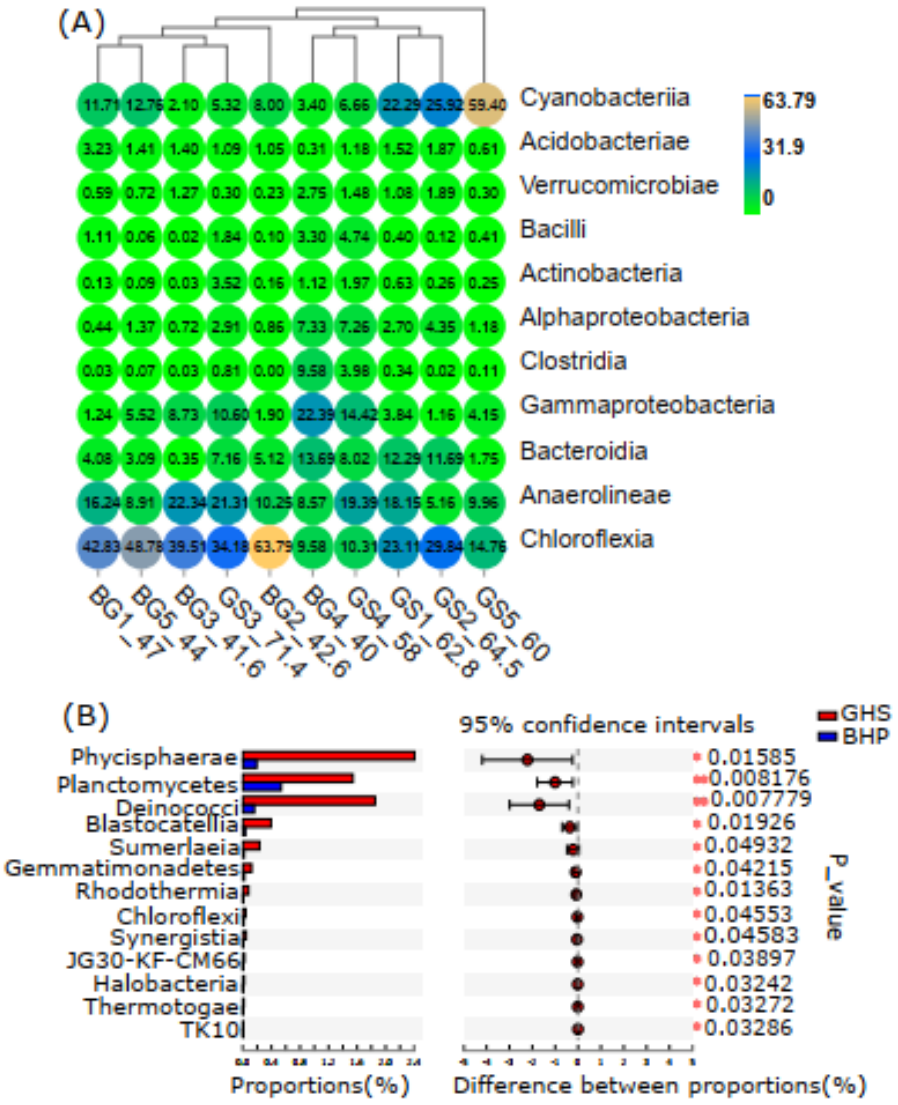


Figure 4. 7 Relative abundance of bacterial classes (A) and sample comparison analysis (class level) based on student's t-test (B).* indicates $P < 0.05$, ** $P < 0.01$.

4.3.4 Temperature governed the microeukaryotic composition in mats

Among the 14 dominant microeukaryotic phyla (relative abundance >1% in at least one sample) (**Figure 4.8A-B**), the phylum Amoebozoa, Arthropoda, Ascomycota, Phragmoplastophyta, and SAR (Stramenopiles, Alveolata, and Rhizaria) were the highly abundant phyla in all hot spring mats. Correspondingly, microeukaryotic taxa in Amoebozoa, Fungi, Microalgae, and Metazoa kingdoms have been reported in hot springs/mats worldwide [26,231,402,403]; however, they differed in composition. Compared to GHS, higher relative abundance of Viridiplantae or Phragmoplastophyta (0.8-33%) and Cryptomycota (0-16.9%) were detected in BHP, while Amoebozoa (14.1-45.2%), Ascomycota (10.3-37.2%), and SAR (4.6-17.3%) dominated in GHS mats (**Figure 4.8A-B**). Some fungal (Cryptomycota) and microalgal phyla were detected in moderate-temperature hot springs [169,399], whereas taxa in Amoebozoa and Ascomycota selected high-temperature springs [231,404], demonstrating microeukaryotic community temperature spectrum preference. Notably, Arthropoda and Blastocladiomycota were exclusively detected in BHP, whereas Platyhelminthes occurred merely in GHS. The relative abundance of Amoebozoa and SAR was of higher significance ($p < 0.05$) occurring phyla in GHS (**Figure 4.8C**), similar to a recent report [231].

Moreover, *Amoebozoa*, *Mucoromycota*, *Tubulinea*, *Echinamoeba*, and *Podocopida* were the most abundant genera in all samples (**Figure 4.9A**). Notably, *Boeremia* and *Cryptosporidium* abundances were significantly higher in GHS ($P < 0.05$, $P < 0.01$) than in BHP (**Figure 4.9B**), suggesting that some microeukaryotic genera have adapted to the high-temperature spring mats.

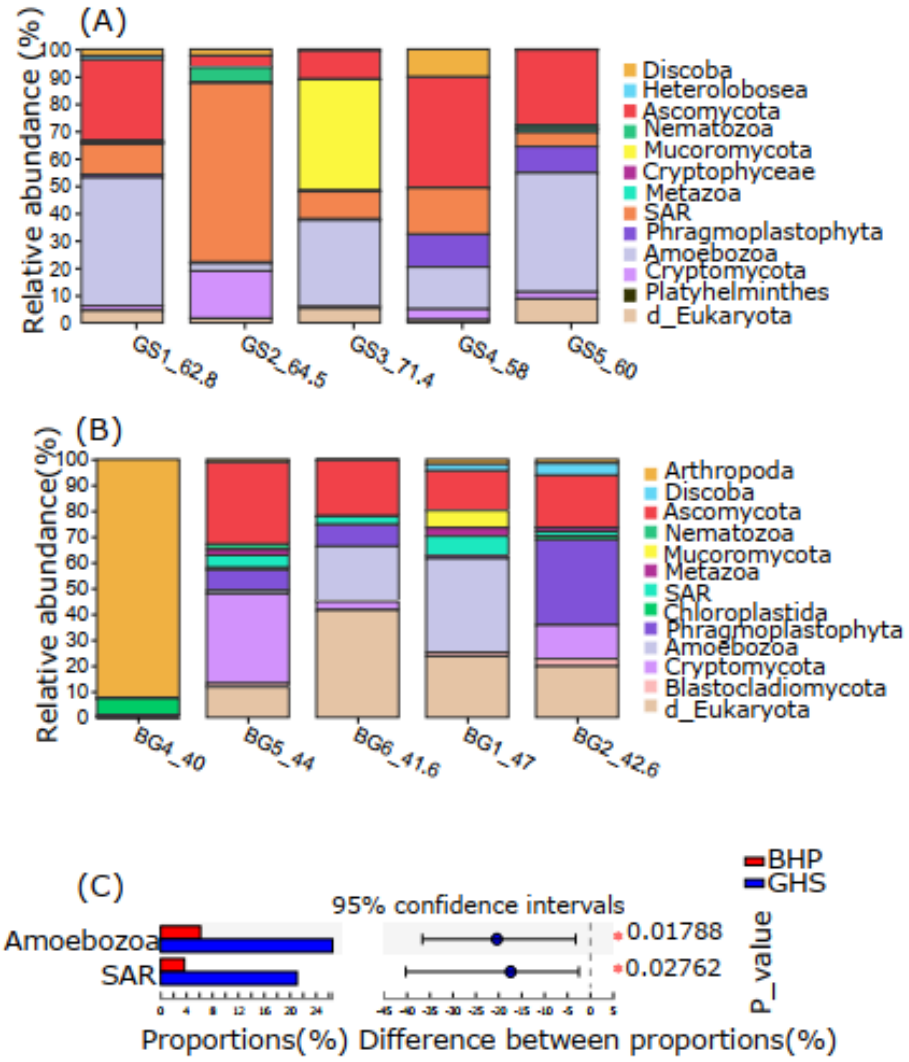


Figure 4. 8 The relative abundance of microeukaryotic phyla (A-B) in all samples. * $P < 0.05$ by Wilcoxon rank-sum (C) sample comparison.

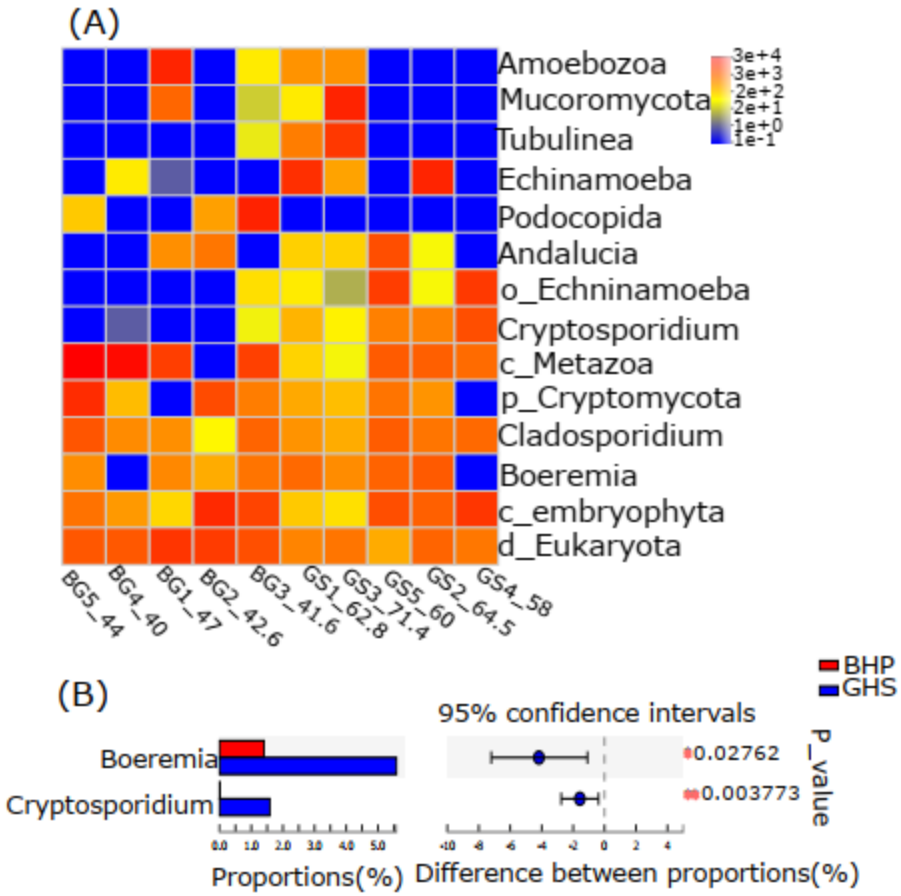


Figure 4. 9 The relative abundance of microeukaryotic genera in GHS (A) and BHP (B) samples and statistical difference at genus level based on Wilcoxon rank-sum test (C).* indicates $P < 0.05$ and ** $P < 0.01$.

4.3.5 Response of microbial communities to environmental parameters in the mats

RDA analysis was used to explore the relationship between microbes and environmental parameters (**Figure 4.10 A-D**). EC, pH, and NO_3^- were significantly positively correlated (* $P < 0.05$, ** $P < 0.01$; $r > 0.7$) with *Planktotrichoides_SR001*, *Chthoniobacterales*, *Lentisphaerae* (f_Lenti-02), *novel Ca.o_OPB41*, and *f_A4b* in BHP mats, whereas c(class)*_Anaerolineae* was significantly negatively correlated (*$P < 0.05$; $r >- 0.7$) with TP **(Figure 4.10A and Appendix Table S6)**. Previous reports in extreme environments have shown that bacterial taxa in Cyanobacteria (*Planktotrichoides_SR001*), Verrucomicrobiota (*Chthoniobacterales*), and Actinobacteria (*OPB41*) thrive well in saline environments (as indicated by EC)[333,405]**,** and this finding is factual because salinity and electrical conductivity are strongly connected. Taxa

Anaerolinae (*family A4b*) and *Lentisphaerae* were positively related to nitrate reduction and pH range of 7-9, respectively [406,407].

In GHS mats **(Figure 4.10 B and Appendix Table S7)**, bacterial genera (*Thermus*, *Hydrogenophilus*, *Chloroflexus*, *f_A4b*, *f__Rhodobacteraceae*, *Pseudanabaena_NgrPSln22*, *Leptococcus_JA-3-3Ab*, and *Trichodesmium_IMS101*) were significantly positively correlated (* $P < 0.05$, ** $P < 0.01$; $r > 0.7$) with Temperature, ORP, EC, TDS, TN, NO_3^-, and NH_3-N. Bacterial genera in phylum Cyanobacteria and Chloroflexi rely on nitrogen metabolism [408,409] and thrive in hypersaline mats[410]. The *Thermus* genus is an oxygenic thermophilic bacterium relying on oxygen solubility [411], while *Hydrogenophilus* is a H_2-oxidizing thermophile that thrives well in the hot spring mats[412].

However, some nitrogen-cycle related bacterial genera (*Tepidimonas*, *Chloroflexi bacterium OLB13*, *o_Xanthomonadales*, *f_Rhodobacteraceae*, *f_Comamonadaceae*, and *f_ML635J-40_aquatic_group*) were significantly negatively correlated (* $P < 0.05$, ** $P < 0.01$; $r > -0.7$) with TN and NH_3-N, respectively. *Tepidimonas* is an aerobic thermophilic bacterium (50-55 ^{o}C) capable of oxidizing $S_2O_3^{2-}$ and $S_4O_6^{2-}$ to SO_4^{2-} in the presence of a digestible carbon source and has a clear preference for sulfur compounds instead of TN[413]. Recently, Yin et al. [414] reported that Chloroflexi taxa OLB13 may help microbial species harboring the nirK/nirS gene to complete the Anammox process and partial denitrification in the organotrophic reactor; however, the same genera may carry a crucial functional gene (*nrf* or *nir*) important in partial dissimilatory nitrate/nitrite reduction to ammonium process[414]. Therefore, this study proposes that the Chloroflexi bacterium OLB13 preferred DNRA over the Anammox process. However, this viewpoint needs further research due to the research gap in hot spring mats.

As shown in **Figure 4.10 C and Appendix Table S8**, the eukaryotic genera *Kurtzmaniella-Candida_clade*, *Candida-Lodderomyces_clade*, *f_Plectosphaerellaceae*, *Clavispora-Candida_clade*, *f_Chaetomiaceae*, *Knufia*, *c_(class)Trebouxiophyceae*, and *c_Novel_Clade_Gran-5* were significantly positively correlated (* $P < 0.05$; $r > 0.7$) with NO_3^- in BHP mats. As a ubiquitous component of microbial mats, Fungi were found to survive in anoxic environments by respiring nitrate [415]. Furthermore, diatoms in the SAR clade could directly use nitrate as a nitrogen source and thrive well in the nitrate-enriched medium [416]. In contrast, the

Cercozoa group dominated the microeukaryotic community composition and was often accompanied by diatom blooms [417]. This study demonstrates that fungi, diatoms, and Cercozoans may grow in nitrate-enriched hot springs, where they may exchange nutrients. *Vermamoeba*, *o_Podocopida*, p_*Ascomycota*, and p_*Cryptomycota* were also significantly positively correlated with EC, pH, and TN, congruent with previous reports where the *Hartmanella*, *Podocopida*, Ascomycota, and yeasts thrived well in a thermal saline bath, neutral-alkaline pH, and nitrogen-rich environments, respectively [418–420]

Interestingly, the temperature was significantly positively correlated ($p<0.05$) with *Cryptomonas*, *BOLA868*, *o_Rhabditida*, *Cryptosporidium*, *o_Rhabdocoela*, *f_Cordycipitaceae* in GHS mats **(Figure 4.10 D and Appendix Table S9)**. These findings are consistent with previous studies on thermal springs[232,421,422]. This study implies that microeukaryotes can grow in extreme environments (e.g., high temperature) due to their broad-adaptive anatomical (cysts) and physiological characteristics.

Curvularia, *Arthrinium*, *f_Chaetomiaceae*, *f_Plectosphaerellaceae*, and *Poteriospumella* were significantly positively correlated with pH. Fungal clade and SAR group were positively related to the pH of the hot springs/mats in previous reports [169,404].Further, *Vampyrellidae* and *Cryptosporidium* were significantly positively correlated with TDS, TN, and ORP, possibly due to their capabilities to grow in a broad range of salinity (TDS) in the sea and desert hot springs [423,424], and they are known to be closely related with nitrogen mineralization [425]. The cytosolic milieu of the *Cryptosporidium* may facilitate spontaneous oxidation due to its higher O_2 concentration and lower ORP compared to mitochondria[426]**.** This study suggests that inorganic nutrients such as TN, TDS, and ORP in GHS mats/hot water were suitable for *Vampyrellida* and *Cryptosporidium* survival. Taken together, bacterial communities in both hot spring mats (BHP&GHS) shared a significant positive correlation with NO_3-N and EC, whereas microeukaryotic communities were significantly positively correlated with pH and TN. These data suggest that pH, salinity, and nitrogen compounds were the driving factors for microbial communities in the mats

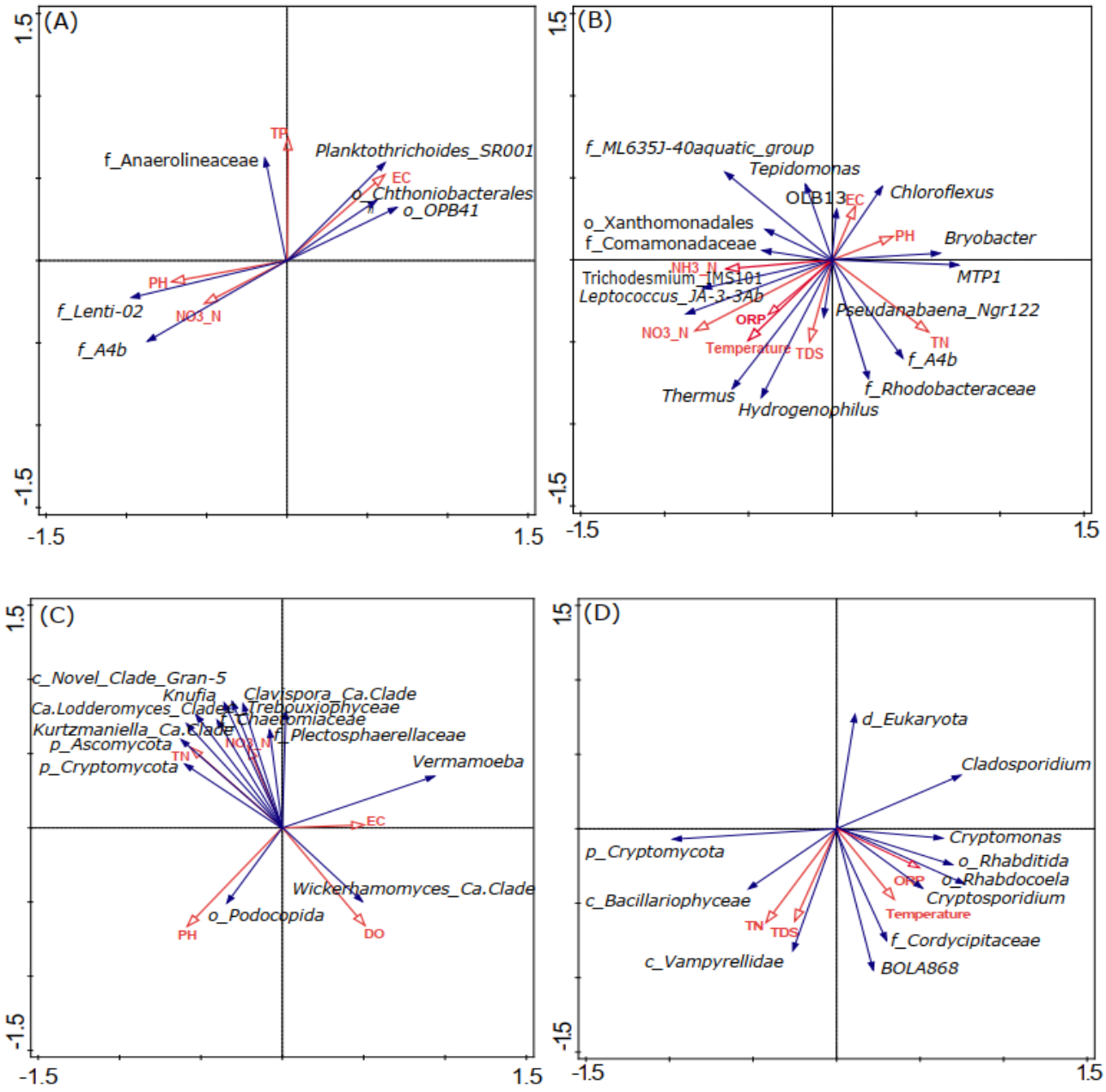


Figure 4. 10 Redundancy analysis (RDA) of dominant bacterial (A-B) and eukaryotic (C-D) genera to environmental parameters in hot spring microbial mats.

4.3.6 High temperature revealed complex and stable interactions in the mats

According to co-occurrence networks (**Figure 4.11),** 59 nodes (genera with relative abundance > 1%) with 130 edges and 75 nodes with 235 edges were generated in BHP and GHS, respectively. To describe the complex pattern of correlations among the microbial genera in the network[216], topological properties were calculated, including average degree, average path length, and modularity index. The computation of the average degree, average path length, and modularity were, respectively, 4.09, 4.56, and 1.47 in BHP and 5.68, 3.32, and 1.86 in GHS mat samples. The higher average degree and shorter path length in the GHS mats' network unveiled the possibility that microbial interactions were more intensive [360]. Based on betweenness centrality [362], the top 5 genera can also be regarded as keystone taxa [363], which are fateful for maintaining stable communities [217,427].

Based on the feeding lifestyle, the food-web was categorized into three trophic levels, i.e., the primary producers (Algae, Cyanobacteria), primary consumers (nutrient cyclers and decomposers), and secondary consumers (predators, pathogens, and parasites). The dominant keystone taxa of the food-webs, based on the betweenness centrality index, include; *c_Trebouxiophyceae* (primary producer), *f_Hydrogenophilaceae,o_Chthoniobacteriales*, and *Clavispora.Candida_clade* (primary consumers), and *Andalucia* (secondary consumer) in BHP (**Figure 4.11A**), whereas *Leptolyngbya_RV74L*, *o_Oxyphotobacteria_Incertae_Sedis* (primary producers), *Hydrogenophilus*, *Saccharomyces* (primary consumers), and *Cryptosporidium* (secondary consumer) were keystone taxa in GHS (**Figure 4.11B**). The feeding relationships between microbial communities are critical in understanding the ecosystem function; for example, *c_Trebouxiophyceae*, *Leptolyngbya_RV74L*, and *o_Oxyphotobacteria_Incertae_Sedis* are photosynthetic microbes and keystone taxa in all mat samples, therefore have a decisive influence on the structure, function, and stability of the entire aquatic system[322].

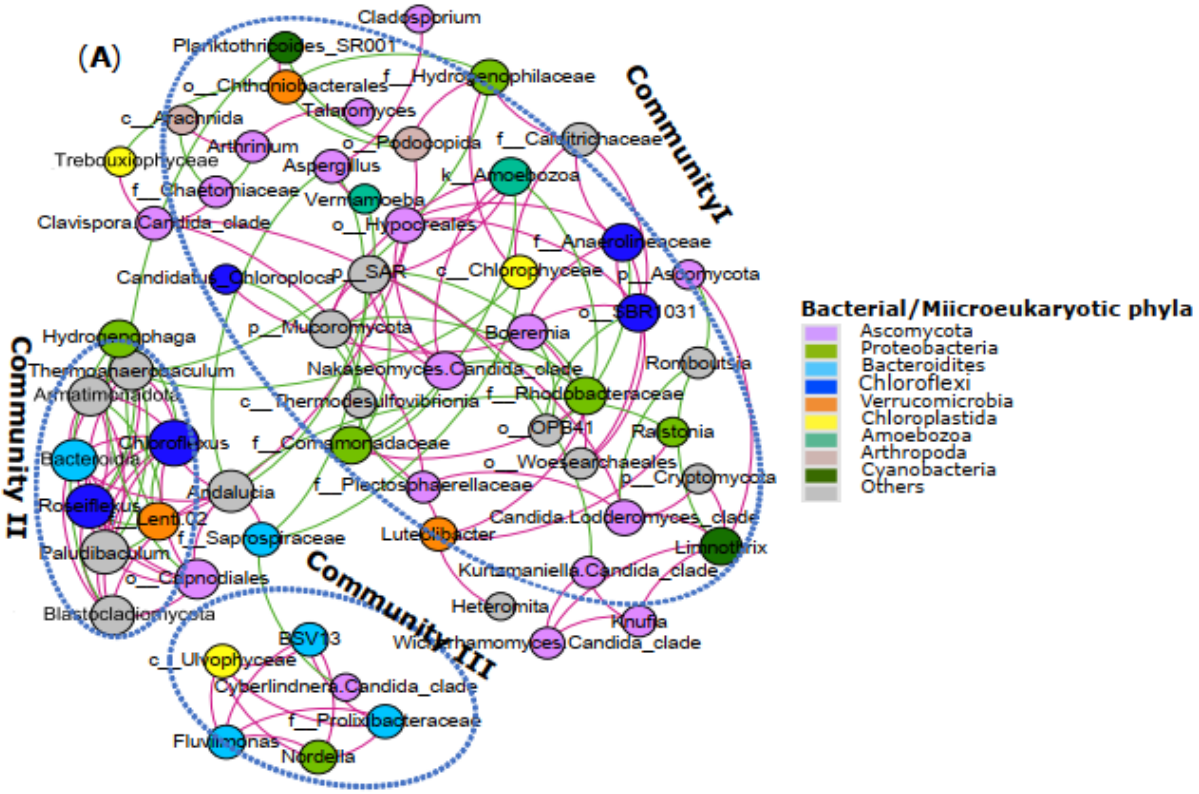
Notably, three dominant communities were observed in BHP and GHS mats (**Figure 4.11A and B**). Genera in the community I of BHP comprised 4 primary producers, 32 primary consumers, and 5 secondary consumers, including keystone taxa *c_Trebouxiophyceae* and *f_Hydrogenophilaceae*, *o_Chthoniobacteriales*, *Clavispora.Candida_clade*. As for GHS, there were 12 primary producers, 24 primary consumers, and 5 secondary consumers in community I;

and the keystone taxa were *Leptolyngbya_RV74L* (primary production), *Hydrogenophilus* (nutrient cycling), and *Cryptosporidium* (predation). There were more primary consumers but lower primary producer numbers in Community I of BHP than GHS, suggesting taxa ecological preferences and environmental filtering. For instance, *Hydrogenophilaceae*was positively related to *o_Podocopida*, *Chlorophyta*, and *f_Calditrichaceae*, but negatively related to Chthoniobacteriales and *Thermoanaerobaculum*from BHP samples. Nevertheless, *Hydrogenophilus* was positively related to *Cordycipitaceae* and *Cryptosporidium* while negatively related to *Ca. Chlorothrix, f__Plectosphaerellaceae, Chaetomiaceae, Curvularia*, *Andalucia*, and *Poteriospumella* in GHS.

The interaction of algae and bacteria was shown in a recent study, including nutrient exchange, signal transfer, and gene transfer [229]. Additionally, symbiotic algae-bacteria systems were key in removing and converting nutrients [230]. Fungi and bacteria are primarily responsible for decomposing coarse particulate organic matter and detrital particulate and dissolved organic matter [227]. Nonetheless, there are often antagonistic interactions between bacteria and fungi fueled by competition for resources [228]. Sasha et al. [428] and Dézerald et al.[429] reported that detritivores (Arthropod such as; *o_Podocopida* and *c_Arachnida*) and environmental bacteria interactions may stimulate antimicrobial components of aquatic bacterial communities and contribute to nutrient cycling and detritus processing, both devour and provide nutrients (feces) to bacterial communities. Furthermore, oocysts may end up in surface- and groundwater during the rainy season [78], and *Cryptosporidium perfringens* spores can resist temperatures higher than 70°C [430], suggesting commensalism between *Hydrogenophilus* and *Cryptosporidium*, where both can cooperate within the same niche in high-temperature GHS. Metazoans such as o_Rhabdocoela, o_Rhabditida, and SAR taxa are prime consumers of picophytoplankton, bacteria, and fungi (fungal zoospores), indicating predation in the food-webs [96]. Positive interactions, such as cooperation, cause bacteria to co-occur in similar niches, while negative interactions, such as competition for space and resources, result in co-exclusion [226].

Community II-III of BHP comprised feeding relationships among 1 primary producer (c_Ulvophyceae), 16 primary consumers, and 1 secondary consumer (*Andalucia*), whereas Community II-III of GHS comprised feeding relationships among 5 primary producers, 22 primary consumers, and 6 secondary consumers. The keystone taxa of these communities (II-III) were

Andalucia, *o_Oxyphotobacteria_Incertae_Sedis*, and *Saccharomyces*. These findings support more stable and complex communities in GHS with abundant taxa comprising interkingdom interrelationships. Several primary consumers played a substantial role in the microbial community structure and function in mat biofilm. For example, the functional role may be associated with H_2-production (*Hydrogenophilaceae*)[412], methane oxidation (*Methyloacidophylaceae*)[431], phototrophy and phenanthrene and nitrogen transformation (*f_Rhodobacteraceae*, *f_Commammonadaceae*)[432,433], fermentative growth on pyruvate/ proteinaceous substrates and Fe (III) and Mn (IV) reduction (*Thermoanaerobaculum*) [434], decomposition (*p_Cryptomycota*, *f_Saprospiraceae*)[435]. In addition, secondary consumers such as *Echinamoeba*, *c_Tubulinea* in phylum Amoebozoa, and *o_Monhysterida* in Phylum Nematoda can be fierce predators in hot springs[231,232]. The negative and positive interactions observed in this study may be due to the differences in food and energy sources, niche preferences or coexistence, and environmental modification [436,437]. In summary, this study showed the interrelationship between the microbial communities in mats, such as feeding relationships, predation, parasitism, and synergism for biogeochemical cycling.



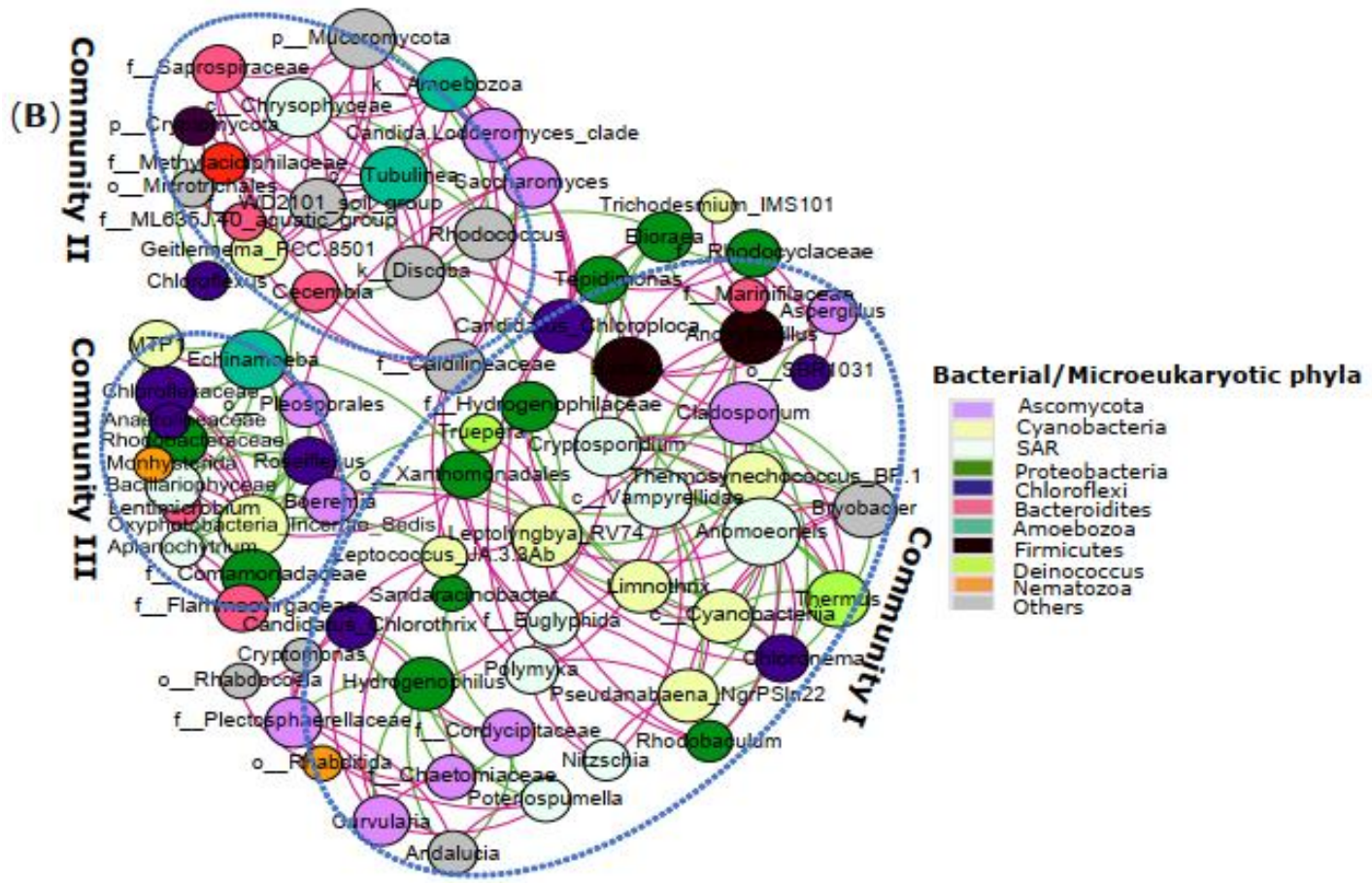


Figure 4. 11 Network analysis showing interactions among bacterial and microeukaryotic communities in BHP (A) and GHS (B). Each connection shows a strong Spearman correlation ($|r| > 0.7$ and $P < 0.05$); the pink and green lines represent, respectively, significantly strong positive and negative relationships.

4.3.7 Predicted bacterial functions in geothermal spring mats

Functional Annotation of Prokaryotic Taxa (FAPROTAX) was used to analyze the functions of biogeochemical cycles of bacteria, particularly the circulatory functions of carbon, sulfur, hydrogen, and nitrogen. These results reflect that the top 17 bacterial genera were most related to phototrophy, photoautotrophy, photo/chemo-heterotrophy, hydrocarbon degradation, methylotrophy, fermentation, dark oxidation and respiration of sulfur compounds, dark hydrogen oxidation, nitrogen cycling (reduction and ureolysis), and parasitism/pathogenicity (**Figure 4.12A**). Statistical Analysis of Metagenomic Profiles (STAMP) analysis showed a highly significant primary production ($P < 0.05$) in GHS than BHP, whereas other functions were

significantly higher (P < 0.05) in BHP (**Figure 4.12B)**, suggesting that temperatures may have a critical influence on predicted microbial functions in hot spring mats.

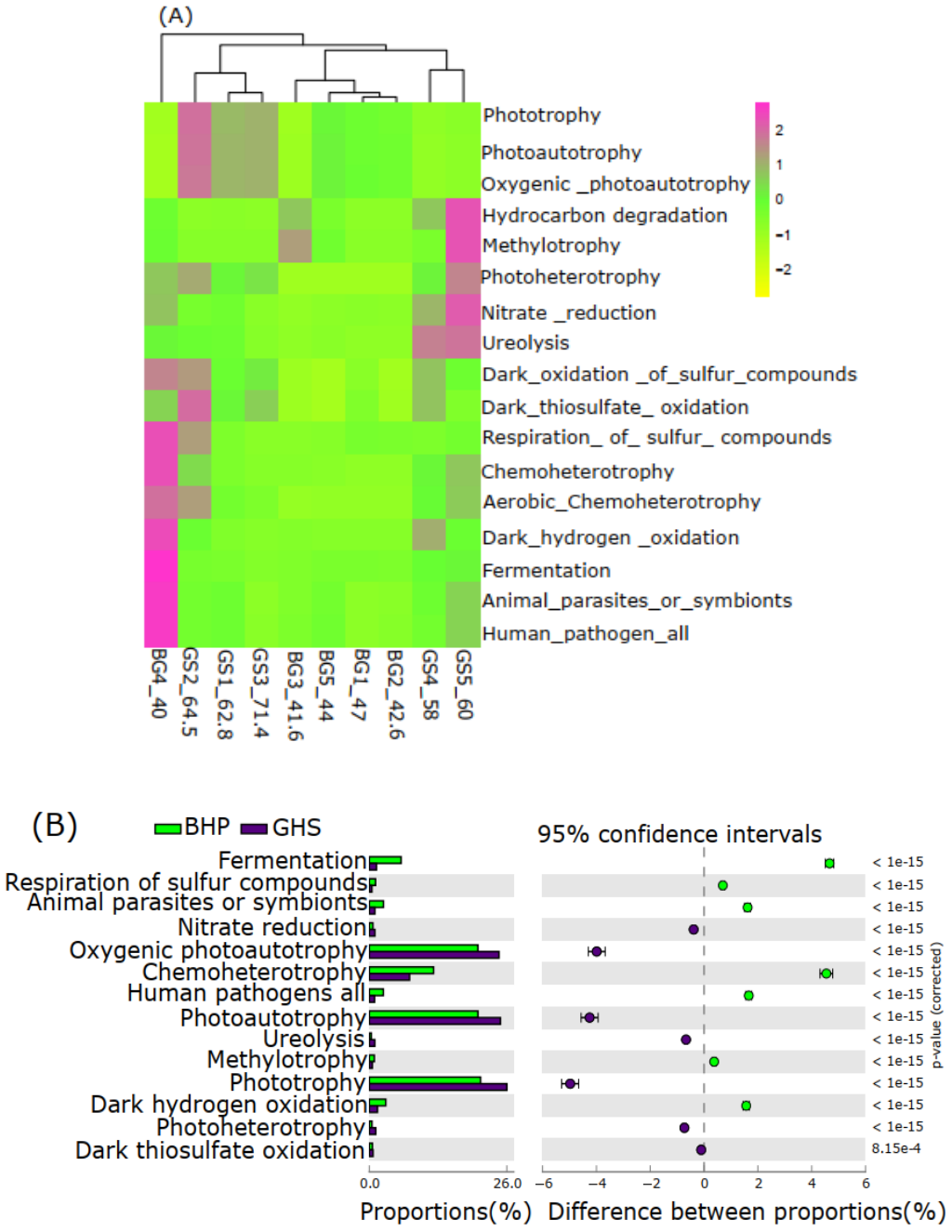


Figure 4. 12 Heatmap of bacterial relative abundance of metabolic pathways (level 2) using FAPROTAX annotation (A). Fisher's exact test on the 14 dominant metabolic pathways (B) between BHP and GHS mat samples (P < 0.05).

4.4 Summary

In this study, we extensively investigated the microbial assemblage and interaction in mats for two hot springs. The main conclusions are as follows.

1) According to our findings, some Amoebozoa (e.g., *Echinamoeba*, *BOLA868*, and *Telaepolella*) and SAR taxa have adapted to high-temperature in hot spring microbial mats. This knowledge could probe further any specific physiological adaptations that enable survival in harsh environments.
2) Bacterial community β-diversity in moderate temperature springs was strongly driven by stochastic processes
3) The variable selection in microeukaryotic and bacterial community β-diversity was potentially shaped by temperature.
4) RDA revealed that microeukaryotic communities were strongly driven by temperature in high-temperature microbial mats.
5) The exploration of co-occurrence networks demonstrated that microbial interactions in high-temperature mats were more stable and sophisticated. Although mostly ignored in hot spring mats, microeukaryotes are diverse and form complex interactions in extreme environments.
6) Statistical Analysis by STAMP analysis showed a highly significant primary production in GHS than BHP, while the remaining predicted functions were significantly higher in BHP.

Chapter 5. Comparison of microbial communities in periphytic: response to environmental variables, diversity, food-web structure, and ecological function.

5.1 Background

Chapters 2, 3, and 4 comprehensively investigated the influence of substrate type and environmental factors on biodiversity, composition, assembly mechanisms, interactions, and ecological functions of biofilm-dwelling microbial communities in natural tropical lakes and hot springs. Illumina sequencing and robust statistical tools were adopted to explore the data. To further strengthen the findings of this research, the microbial biofilm communities in *C. demersum*, *E.crassipes*, surface sediments, and mats were compared on the ground of response to environmental variables, biotic interactions, and predicted functions. In this section, we hypothesize that the microbial communities in epiphytic and sediment biofilms and mats differ in response to environmental variables, biotic interactions, and ecological functions.

5.2 Data processing and analysis

For better comparison, the data from Illumina sequencing in chapters 2, 3, and 4 were pooled together; the CDR, ECR, and SDR represent the lake Rumira sample group, CDC, ECC, and SDC represent the lake Cyohoha sample group, while the BHP and GHS represent the hot spring mats group. RDA was adopted to compare the relationship between epiphytic biofilms and mats to environmental parameters using the 'vegan' package and visualized by 'ggplot2' in R. Furthermore, co-occurrence analysis among microbial communities from different substrates was compared, and Spearman's correlations were computed using the corr.test function of the'psych' packages in R (https://www.r-project.org/, version 4.1) and visualized using Gephi (https://gephi.org/, version 0.9.2). Robust correlations were considered if Spearman's correlation coefficient (r) was > 0.7 or < -0.7 used for further analysis ($p < 0.05$) [265]. Based on OTU data, functional metabolic pathways of the bacterial community 16S rRNA gene sequences were predicted by FAPROTAX[237]. The above functional metabolic pathways were compared between bacterial biofilm communities on different substrates using STAMP (https://beikolab.cs.dal.ca/software/STAMP, version 2.1.3) based on fisher's exact test ($p < 0.05$).

5.3 Result and discussion

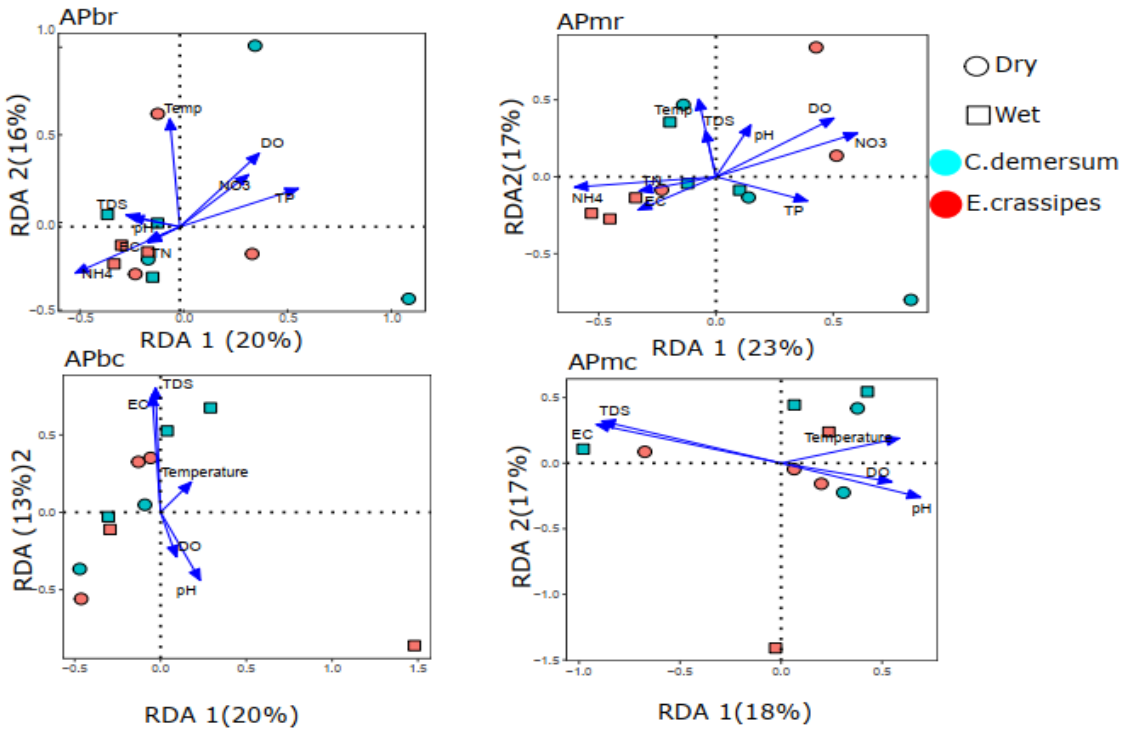
5.3.1 Response of microbial communities to environmental parameters in periphytic biofilms.

RDA results revealed the significant environmental variables closely related to bacterial and microeukaryotic communities in lakes Rumira and Cyohoha North, as well as in hot spring mats ($P < 0.05$, $P < 0.01$) (**Figure 5.1 A-H**). Bacterial and microeukaryotic communities on *C. demersum* and *E. crassipes* were mostly positively associated with DO, pH, TP, and TN in Lake Rumira (**Figure 5. A-B**) and EC, pH, and DO in Lake Cyohoha North (**Figure 5.1 C-D**). Specifically, microbial communities on *E. crassipes* were positively associated with nitrogenous compounds (TN, NH_4-N, and NO_3-N) in Lake Rumira and DO and pH in Lake Cyohoha North. The bacterial community in *C. demersum* and mats (**Figure 5.E-H**) was commonly positively related to temperature, TDS, EC, and pH. The *C. demersum* and *E.crassipes* epiphyton share the influence of pH and dissolve oxygen (DO) in both lakes. These physical parameters are important driving factors in aquatic macrophyte-biofilm systems [32]. For example, aquatic macrophytes can uptake dissolved CO_2 and release O_2 through a photosynthetic process and increase the pH value in the water column [438,439]. A CH_4-oxidizing bacterium *Methylomagnum* in phylum Firmicutes is active in the presence of O_2[295] and has demonstrated a nitrogen fixation capability in the rice rhizosphere[296]. Floating plants (e.g., hyacinth and duckweeds) can stimulate methanotrophic bacterial growth [297,298]. Furthermore, *Amaricoccus* grows well in neutral to alkaline pH in subtropical coastal vegetation [299], and *Asperigellaceae* in Ascomycota phylum can perform submerged fermentation at 20–30 °C and pH (5–7)[305]. These results suggest the key role of DO and pH in sustaining epiphyton metabolism and growth on aquatic macrophytes from shallow Lakes

Inorganic nutrients (TN, NO_3-N, NH_4-N, and TP) were more crucial for *E. crassipes* in Lake Rumira than in Lake Cyohoha. Notably, *E. crassipes* thrive well in a nitrogen-rich environment, consistent with the current report[83]. Therefore, this study hints at the nutrient pollution state of Lake Rumira. Temperature and pH are significant factors influencing microbial diversity and composition in hot spring mats and *C. demersum* epiphyton[32,440]. Additionally, ionic compounds (e.g., TDS and EC) from *C. demersum* leaf exudates and heavy metals (e.g., TDS and EC) from hot water can promote the microbial communities and polysaccharides formation in surface

biofilm EPS, resulting in higher total bacterial counts[441]. Therefore, higher TDS and EC may promote epiphytic biofilm and mat formation and growth.

The positive correlation of microbial communities of *E. crassipes* to NH_4-N, pH, and temperature in both lakes may be due to the solubilization of phosphate using nitrogenous compounds such as ammonium and nitrate[84], cellulose-degrading microbes during the floating plant decline period under optimum pH and temperature[303], and bacterial spore resistance to elevated temperature or UV radiation (sunlight) on *E. crassipes*. Moreover, the positive relation of epiphytic microeukaryotes on *E. crassipes* to EC and DO may infer that microeukaryotes thrive well in ionic compounds (e.g., TDS) from floating leaf exudates and dissolved oxygen from the air-water interface. Rotifers and fungi thrive well in organic matter-rich environments (nitrogen compounds, EC, and TDS)[294], suggesting that microeukaryotes may grow on submerged and floating macrophytes in eutrophic shallow lakes. The difference in response of microbial communities to environmental variables may be due to the influence of the substrate type and lifestyle, physicochemical variables of each Lake, seasons, anthropogenic pollution, and unmeasured variables.



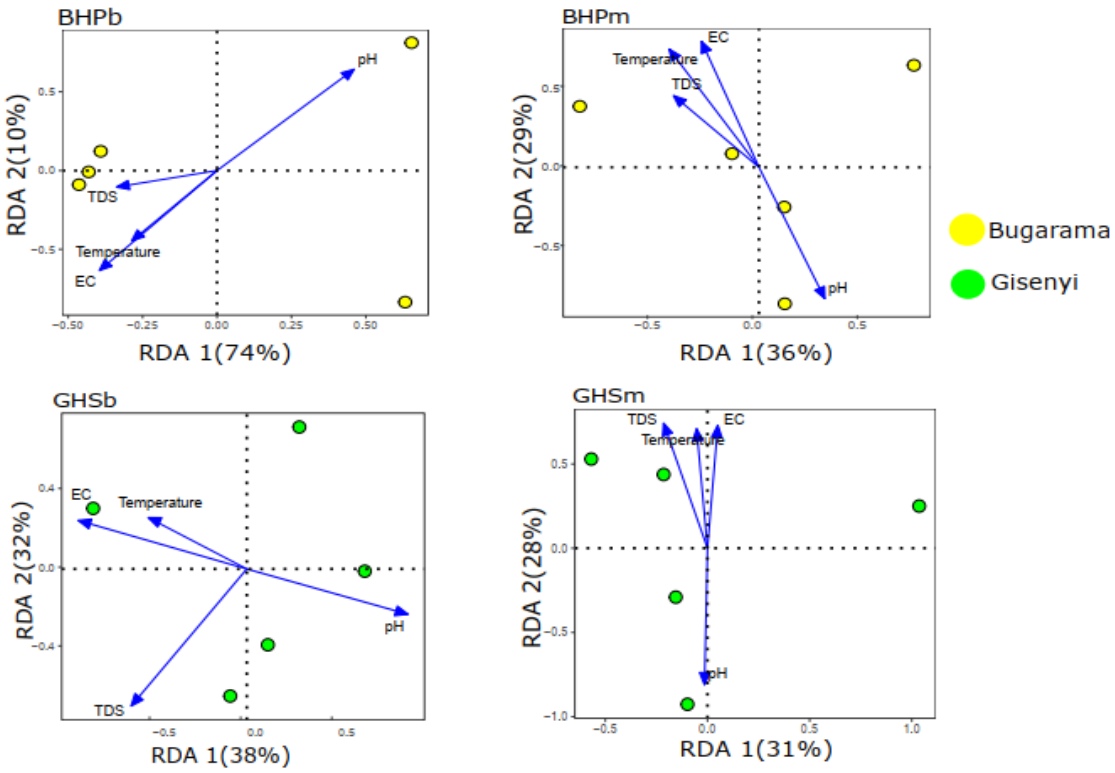


Figure 5. 1 Redundancy analysis (RDA) of dominant bacterial (A, C, E and G) and eukaryotic (B, D, F, and H) genera to environmental parameters in biofilms on aquatic plants (*C. demersum* and *E. crassipes*) and hot spring mats. **APbr**: Aquatic plant bacteria in Rumira Lake, **APmr**: Aquatic plant microeukaryotes in Rumira Lake, **APbc**: Aquatic plant bacteria in Cyohoha Lake, **APmc**: Aquatic plant microeukaryotes in Cyohoha Lake, **BHPb**: Bugarama hot pool bacteria, **BHPm**: Bugarama hot pool microeukaryotes, **GHSb**: Gisenyi hot spring bacteria, and **GHSm**: Gisenyi hot spring microeukaryotes.

5.3.2 Microbial diversity and interactions in periphytic biofilms

There was no significant difference in all the α-diversity indices of the bacterial community in biofilm among microhabitats ($P > 0.05$, $P > 0.01$) in lakes (Rumira and Cyohoha) and hot springs. However, OTU richness and Shannon index of the microeukaryotic community in biofilm among microhabitats and seasons revealed a significant difference ($P < 0.05$, $P < 0.01$) in two lakes. The PCoA showed that bacterial and microeukaryotic community dissimilarity was statistically different among substrates ($P < 0.05$, $P < 0.01$) in two lakes, while NMDS analysis showed only a significant difference ($P < 0.05$) in the bacterial community from hot spring mats.

For a better understanding of the microbial interactions in biofilms, co-occurrence network analysis (phylum level) among the top 50 dominant microbial genera (bacteria and microeukaryotes) on each substrate *(C. demersum*, *E. crassipes*, surface sediments, and mats) in shallow lakes and hot springs, was adopted based on robust and significant correlations (**Figure 5.1A (i&ii)-D (i&ii)**). Furthermore, we compare the epiphyton, epipelon, and mats in different lakes and hot springs. The key topological parameters of microbial networks of periphytic biofilms colonizing various substrates are listed in **Tables 5.1 &2**.

The microbial interactions on *C. demersum* in lakes Rumira (CDR) and Cyohoha (CDC) revealed similar dominant (top four modules and >5%) module proportions, with 91.3% and 89.79%, respectively (**Fig. 5A (i)**). However, networks of microbes on CDR have high modularity values (5.28) than those on CDC (2.1), suggesting that epiphyton of CDR had high modular structures[313]. The modularity reflects the system's resistance to the environment[359]. The edge/node rate of CDR (4.28) was slightly higher than those of CDC (4.08), reflecting the complexity of the microbial interactions[100]. For microbial networks, the nodes of biofilms on *C. demersum* for two lakes shared phyla Firmicutes, SAR, Proteobacteria, Rotifera, Chloroplastida, Ascomycota, and Gastrotricha (**Fig. 5A (ii)**). Notably, phyla Arthropoda, Platyhelminthes, and Nematoda were unique to CDR, whereas Cyanobacteria, Bacteroidetes, Verrucomicrobia, and Ochrophyta were exclusive to CDR, suggesting the dominance of epiphytic microeukaryotes on *C. demersun* in Lake Rumira. Interestingly, Firmicutes, SAR, and Proteobacteria were CDR's top three microbial phyla, while Firmicutes, Rotifera, and Chloroplastida dominated in CDC.

The microbial interactions on *E.crassipes* (97.87%) in Lake Rumira showed a high module proportion than those on *E.crassipes* (78.8%) in Lake Cyohoha (**Fig. 5B (iii)**). However, networks of ECC microbes have higher modularity values (4.7) than those on ECR (2.2), suggesting that the epiphyton of ECC had high modular structures. Furthermore, ECC's edge/node rate (3.88) was slightly higher than those of ECR (3.15). For microbial networks, the nodes of biofilms on *E.crassipes* for two lakes shared phyla Firmicutes, Proteobacteria, Rotifera, Ascomycota, and Chloroplastida (**Fig. 5B (iv)**). Remarkably, phyla SAR, Arthropoda, Platyhelminthes, and Nematoda were exclusive to ECR, whereas Cyanobacteria, Bacteroidetes, Gastrotricha, and Verrucomicrobia were unique to ECC, suggesting the dominance of epiphytic microeukaryotes on *E.crassipes* in Lake Rumira. Similar to *C. demersum*, Firmicutes, SAR, and Proteobacteria were

the top three microbial phyla in ECR, while Firmicutes, Rotifera, and Chloroplastida dominated in ECC.

Interactions among microbes in surface sediments of Lake Cyohoha (84.9 %) exhibited high module proportions than those of Lake Rumira (64.1%) (**Fig. 5C (v)**). Interestingly, microbial networks in both SDR and ECC exhibited similar modularity values and greater than 0.5 (**Table 5.2**), indicating high modular structure. Furthermore, the edge/node rate of SDC (2.92) was higher than those of SDR (1.74). For microbial networks, the nodes of biofilms in surface sediments for two lakes shared phyla Firmicutes, SAR, Proteobacteria, Rotifera, Ascomycota, and Gastrotricha (**Fig. 5C (vi)**). Notably, the phylum Nematoda was unique to SDR, whereas Cyanobacteria and Ochrophyta were unique to SDC. Similar to aquatic plants, Firmicutes, SAR, and Proteobacteria were the top three microbial phyla in SDR, while Firmicutes, Rotifera, and Chloroplastida dominated in SDC.

The microbial interactions in mats (75.93%) of Gisenyi hot springs showed a high module proportion than those on Bugarama hot pool (69.48%) (**Fig. 5D (vii)**). Furthermore, microbial networks in GHS mats has superior modularity values (1.86) than those on BHP (1.47), suggesting that microbial mats of GHS had high modular structures. Additionally, the edge/node rate of GHS mats (4.73) was higher than those of BHP (2.6). For microbial networks, the nodes of biofilms in mats for two hot springs shared phyla Ascomycota, Proteobacteria, Bacteroidetes, Chloroflexi, Amoebozoa, and Cyanobacteria (**Fig. 5C (viii)**). Noteworthy, phyla Verrucomicrobia, Chloroplastida, Arthropoda, and Acidobacteria, were unique to BHP mats, whereas SAR, Firmicutes, Deinococcus, and Nematozoa were exclusive to GHS mats. Ascomycota, Proteobacteria, and Bacteroidetes were the top three microbial phyla in BHP mats, whereas Ascomycota, Cyanobacteria, and SAR dominated GHS mats.

The edge/node rate in various substrates was in sequences: GHS (4.73) > CDR (4.28) > CDC (4.08) > ECC (3.88) > ECR (3.15) > SDC (2.92) > BHP (2.6) > SDR (1.74) (**Table 5.1 &2**). Based on modularity, substrates were in sequences: CDR (5.28) < ECC (4.7) < ECR (2.22) < CDC (2.1) < GHS (1.86) < BHP (1.47) < SDR (1.1) < SDC (1.08). Based on average degree and modularity class, the microbial interactions on *C. demersum* and *E. crassipes* in Lakes Rumira and Cyohoha showed the highest network complexity. The aquatic plant leaf exudates can release polyphenols and cyclic sulfur compounds (allelopathy), which may provide favorable or unfavorable chemical

environments shaping the epiphytic microbial diversity, composition, and food webs[8,81]. In return, epiphytic microbes enhance biogeochemical cycling and provide CO_2 to the macrophytes. The mechanisms underlying the close relationship between epiphytic microbes and their aquatic However, based on edge/node rate and positive relationship, GHS showed higher values compared to other substrates, suggesting that temperature and pH influenced the network complexity, consistent with previous studies[363]. In microbial food webs, positive and negative interactions (edges) were reflected as mutualistic and antagonistic associations between microbial taxa[442]. The co-occurrence networks analysis revealed more complex interactions among microbes in all substrates, and those interactions include cross-feeding, parasitism, symbiosis, and predatism among organisms in biofilms.

These findings illustrated that epiphytic biofilms on various aquatic macrophytes (*C. demersum and E. crassipes*) from different lakes might differ in microbial network complexity, keystone taxa, trophic interaction, and top microbial phyla. However, epiphyton and epipelon from the same shallow lake may share dominant microbial phyla. Interestingly, surface sediments from two lakes revealed similar microbial network complexity. Moreover, microbial interactions in high-temperature mats (GHS) were more stable and complex than those of BHP.

Taken together, all microbial networks in periphytic biofilms were complex and stable, with *C. demersum* networks .having a higher modular structure than other substrates.The difference in microbial diversity and network complexity in food webs may be ascribed to the influence of substrate type (e.g., plant allelopathy and physiology, leaf age, sediment structure, and rocks), water chemistry, land use types, and seasons in lakes and hot springs.

Table 5. 1 Key topological parameters of microbial networks in periphytic biofilms colonizing various substrates.

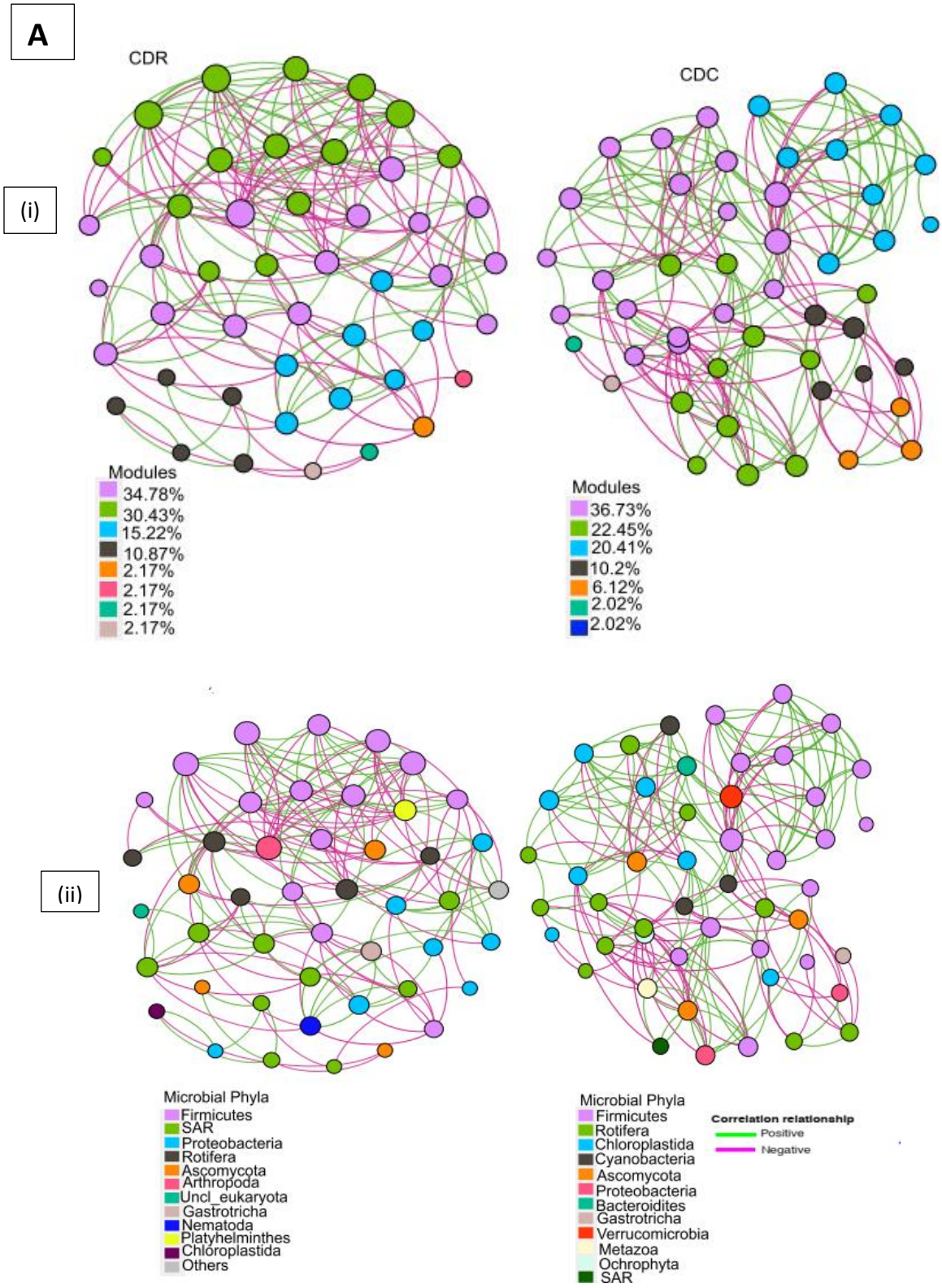
Groups	Nodes	Edges	AD	AWD	MD	ACC	APL
CDR	46	197	8.56	0.52	5.28	0.55	3.15
ECR	45	142	6.31	1.32	2.22	0.68	3.18
SDR	39	68	3.48	1.2	1.1	0.51	5.77
CDC	49	200	7.39	1.55	2.1	0.68	3.02
ECC	50	194	6.72	0.08	4.7	0.7	2.78
SDC	50	146	5.12	1.95	1.08	0.62	3.58
BHP	50	130	4.09	1.37	1.47	0.57	4.56
GHS	49	232	5.68	1.32	1.86	0.62	3.32

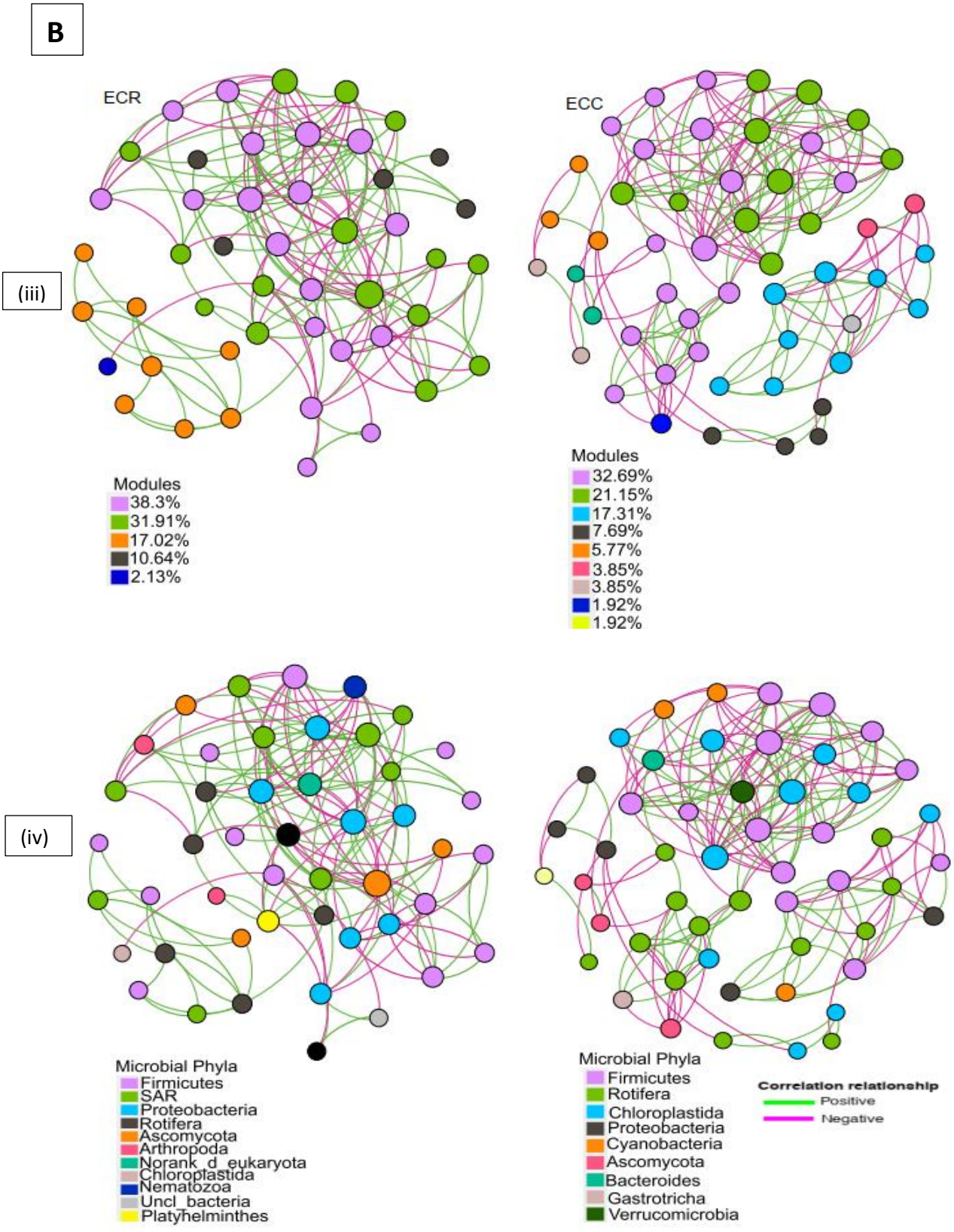
Note: AD, Average degree; AWD, Average weight degree; MD, Modularity; ACC, Average Clustering Coefficient; APL, Average Path length.

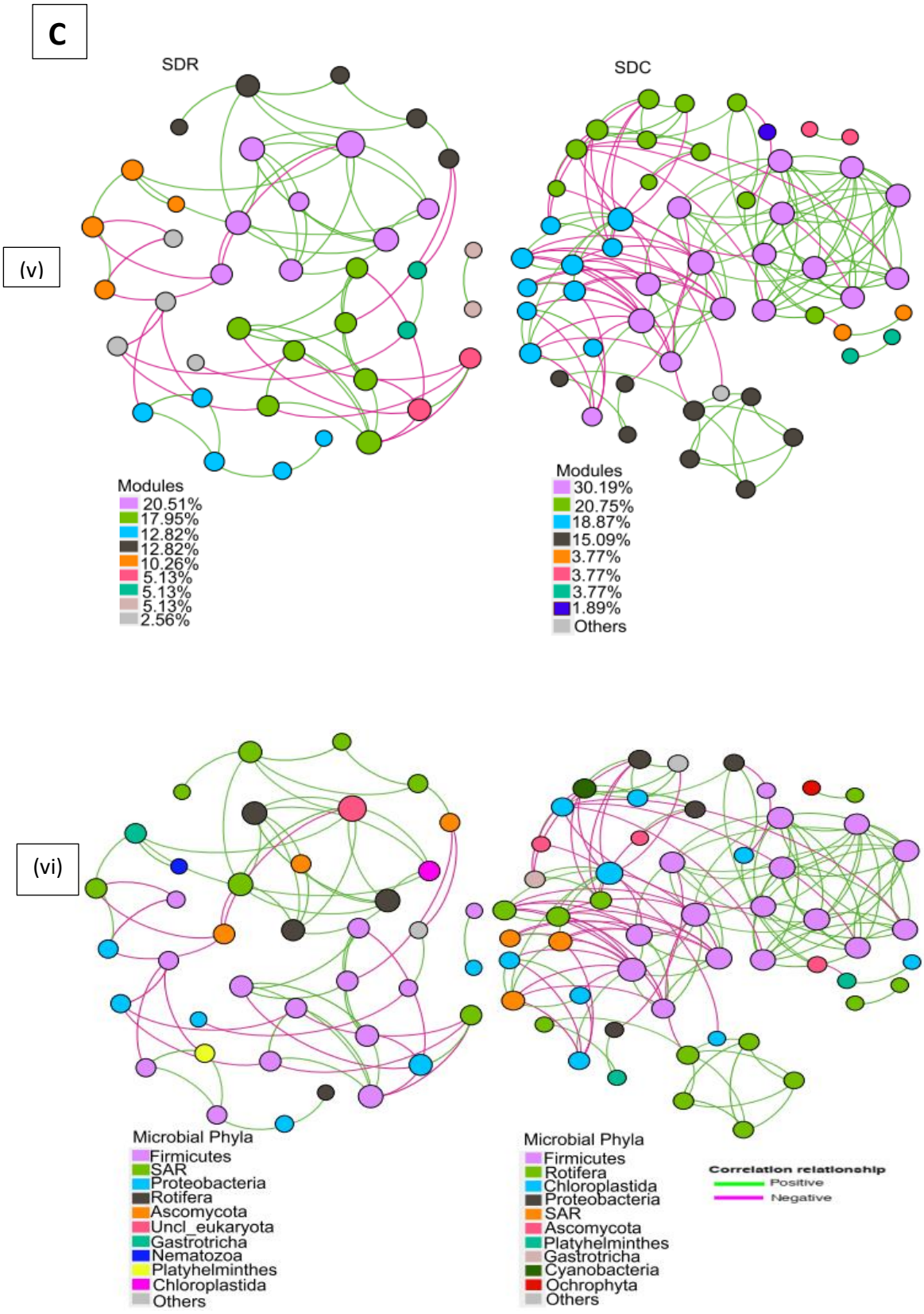
Table 5. 2 Keystone taxa and interaction relationships (edges) of microbial networks in periphytic biofilms colonizing various substrates.

Substrate	Keystone taxa	Positive relation	Negative relation
CDR	Bacillus, Solibacillus, Fictibacillus, Paenibacillus, Hungateiclostridium, Clostridium Sensi Stricto_1, Arachnida, Rhabdocoela, and Ploimida	105	92
ECR	Acinetobacter, family T34, Methylococcaceae, Aspergillaceae, and Symbiodinium.	88	42
SDR	SAR, Bdelloidea, Philodinida, Exiguobacterium, and Dinophyceae.	47	21
CDC	Methylacidiphilaceae, Philodinida, Tetraselmis, Pleosporales, Ploimida, Aspergillus, and Chaetonotida	121	79

ECC	Flosculariacea, Rhodobacter, Microscillaceae, Vorticella, and Chlorophyceae.	106	88
SDC	Paenibacillus, Cyanobium_PCC.6307, Methylacidiphilaceae, Romboutsia, Clostridium_sensu_stricto_1, Chlorophyceae, and Flosculariacea	102	44
BHP	Trebouxiophyceae, Hydrogenophilaceae, Chthoniobacteriales, Clavispora.Candida_clade, and Andalucia.	88	42
GHS	Leptolyngbya_RV74L, Oxyphotobacteria_Incertae_Sedis, Hydrogenophilus, Saccharomyces, and Cryptosporidium	147	88


A
(i)
CDR
CDC
Modules
34.78%
30.43%
15.22%
10.87%
2.17%
2.17%
2.17%
2.17%
Modules
36.73%
22.45%
20.41%
10.2%
6.12%
2.02%
2.02%
(ii)
Microbial Phyla
Firmicutes
SAR
Proteobacteria
Rotifera
Ascomycota
Arthropoda
Uncl_eukaryota
Gastrotricha
Nematoda
Platyhelminthes
Chloroplastida
Others
Microbial Phyla
Firmicutes
Rotifera
Chloroplastida
Cyanobacteria
Ascomycota
Proteobacteria
Bacteroidites
Gastrotricha
Verrucomicrobia
Metazoa
Ochrophyta
SAR
Correlation relationship
Positive
Negative


B
(iii)
ECR
Modules
38.3%
31.91%
17.02%
10.64%
2.13%
ECC
Modules
32.69%
21.15%
17.31%
7.69%
5.77%
3.85%
3.85%
1.92%
1.92%
(iv)
Microbial Phyla
Firmicutes
SAR
Proteobacteria
Rotifera
Ascomycota
Arthropoda
Norank_d_eukaryota
Chloroplastida
Nematozoa
Uncl_bacteria
Platyhelminthes
Microbial Phyla
Firmicutes
Rotifera
Chloroplastida
Proteobacteria
Cyanobacteria
Ascomycota
Bacteroides
Gastrotricha
Verrucomicrobia
Correlation relationship
Positive
Negative


C
SDR
SDC
(v)
Modules
20.51%
17.95%
12.82%
12.82%
10.26%
5.13%
5.13%
5.13%
2.56%
Modules
30.19%
20.75%
18.87%
15.09%
3.77%
3.77%
3.77%
1.89%
Others
(vi)
Microbial Phyla
Firmicutes
SAR
Proteobacteria
Rotifera
Ascomycota
Uncl_eukaryota
Gastrotricha
Nematozoa
Platyhelminthes
Chloroplastida
Others
Microbial Phyla
Firmicutes
Rotifera
Chloroplastida
Proteobacteria
SAR
Ascomycota
Platyhelminthes
Gastrotricha
Cyanobacteria
Ochrophyta
Others
Correlation relationship
Positive
Negative

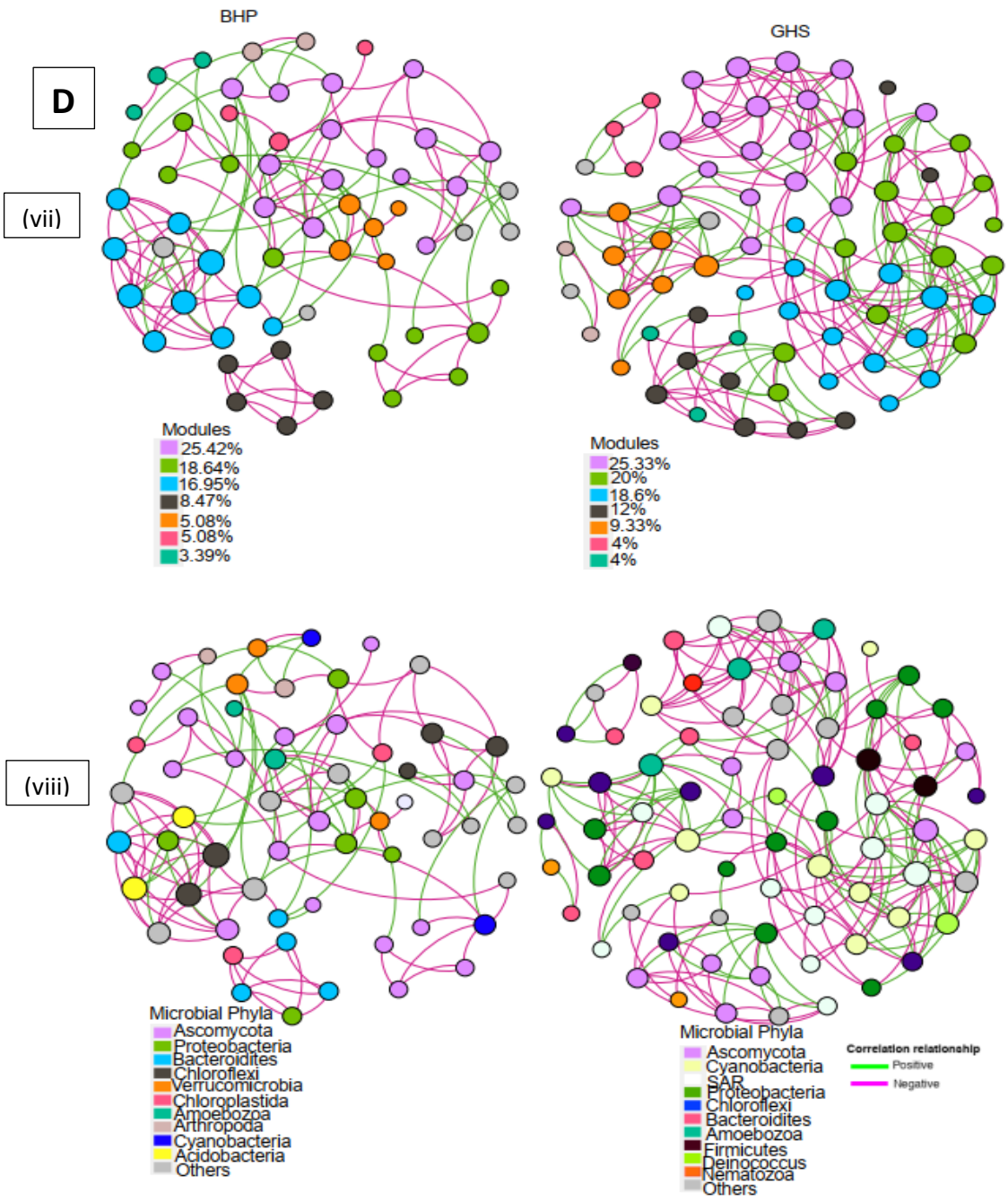


Figure 5. 2 Comparison of food web interactions among bacterial and eukaryotic communities in *C. demersum* (A), *E. crassipes* (B), sediments (C), and mats in tropical lakes and hot springs (D). Nodes were colored according to modular classes (i, iii, v, and vii) and different microbial phyla (ii, iv, vi, and viii), respectively. Each connection shows a strong Spearman correlation ($|r| > 0.7$ and $P < 0.05$). CDR: *C. demersum* in Rumira Lake, CDC: *C. demersum* in Cyohoha Lake, ECR: *E. crassipes* in Rumira Lake, ECC: *E. crassipes* in Cyohoha Lake, SDR: Sediment in Rumira Lake, SDR: Sediment in Cyohoha Lake, GHS: Gisenyi hot springs, and BHP: Bugarama hot pool.

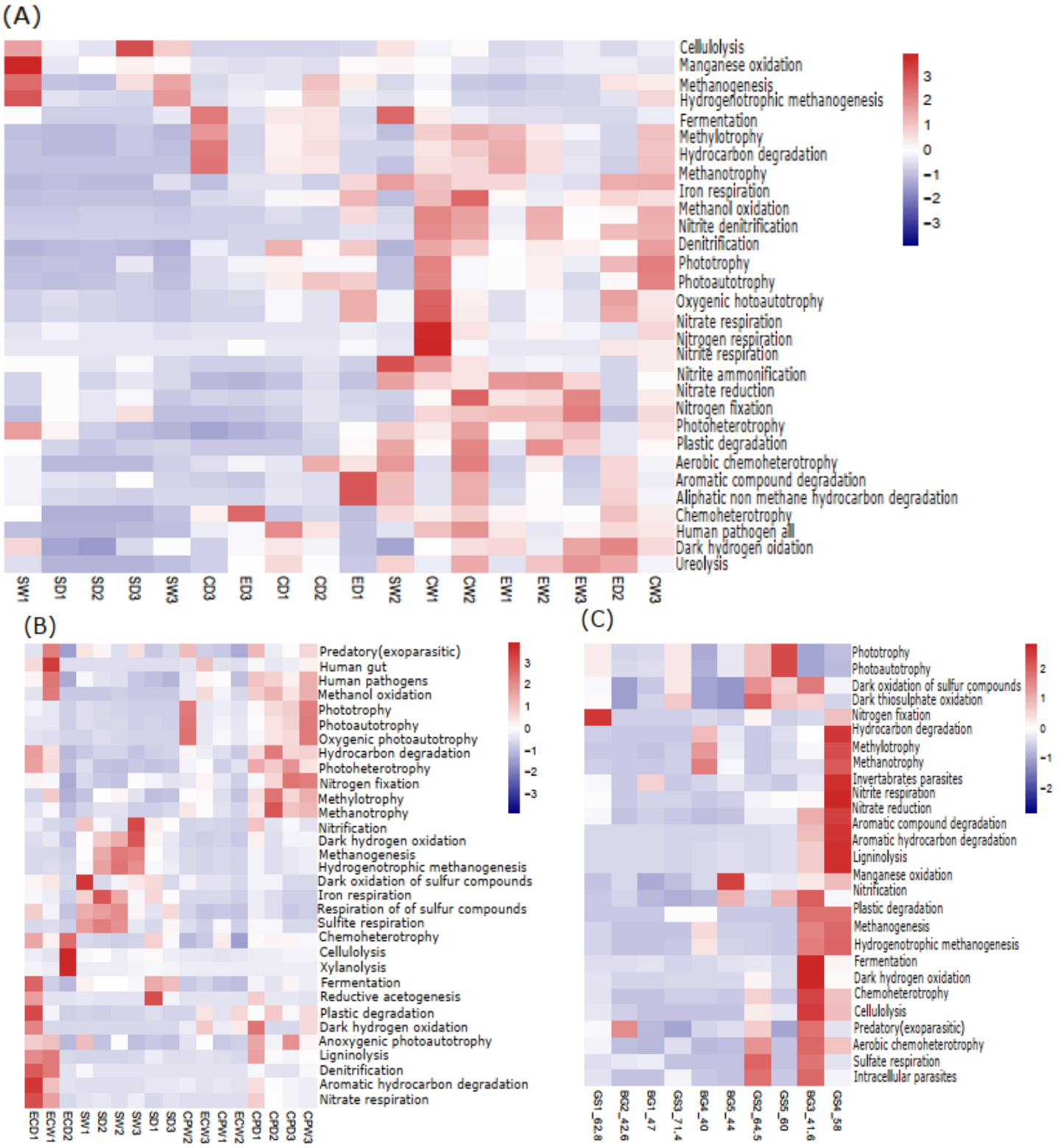
5.3.3 Predicted bacterial functions in periphytic biofilms

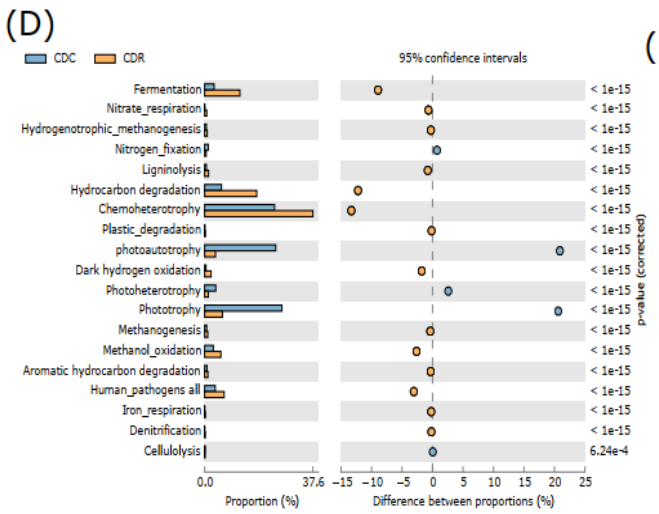
Recently, several gene functional annotation tools related to "predictive metagenomics", including Tax4Fun, PICRUSt and FAPROTAX, have been developed and successfully used to predict the functional traits of environmental microbial communities based on the linkage of microbial phylogeny and function using 16S rRNA gene data and a reference genome database[443,444].The potential functions of the bacterial communities in the samples were predicted using FAPROTAX; the results are illustrated in **Figure 5.3 A-F.** The top five metabolic pathways of the bacterial community to all substrates (*C.demersum*, *E.crassipes,* surface sediments, and hot spring mats) were assigned to fermentation, hydrogenotrophic methanogenesis, nitrogen fixation, hydrocarbon degradation, and ligninolysis. Specifically, fermentation, hydrogenotrophic methanogenesis, nitrogen fixation, hydrocarbon degradation, and ligninolysis functions were common to hot spring mats, surface sediments, and *C.demersum*. Interestingly, photoautotrophy and chemoheterotrophy were unique to hot spring mats, nitrate respiration distinctive to *C.demersum* and *E.crassipes,* and the higher bacterial functional abundance of sediment and epiphytic bacteria were detected in the wet season (except for *E. crassipes* bacteria in Lake Cyohoha). Notably, this study detected a higher abundance of bacterial functional groups in GHS mats than in the BHP.

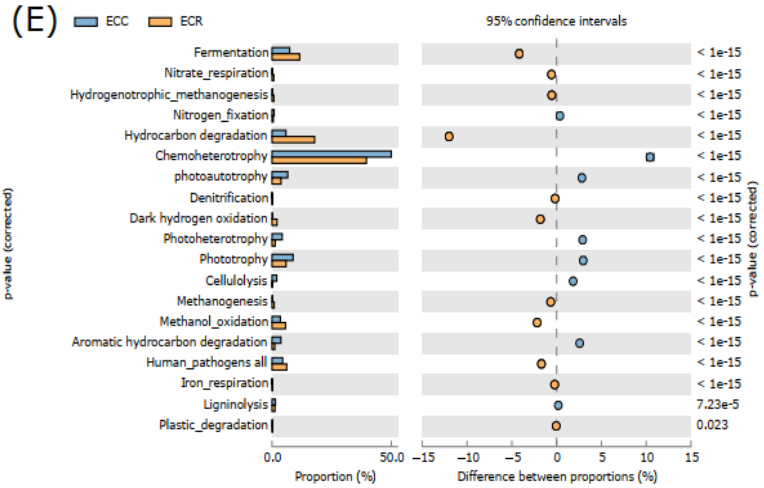
By adopting the fisher exact test and Shapiro-Wilk normality test in STAMP bioinformatics software, the dominant metabolic functions among bacterial communities in various substrates were compared. Phototrophic function dominated the hot spring mat bacterial communities. In addition, fermentation, chemoheterotrophy, methanogenesis, and hydrocarbon degradation were the dominant metabolic functions in surface sediments, whereas fermentation, hydrocarbon degradation, photoautotrophy, and chemoheterotrophy dominated the *C.demersum* and *E.crassipes*. Fermentation involves energy metabolism, which is regarded as the entirety of an organism's chemical processes and entails reactions generating ATP from nutrients. Previous studies reported complex polysaccharides (cellulose, hemicellulose, and lignin) degrading bacteria in decaying aquatic plants and sediments[216,445]. In addition, methanogenesis is an important terminal metabolic process during organic matter degradation in sediments[446,447]. These findings may be due to the stronger carbon cycle than other nutrient cycles in aquatic plants and surface sediments.

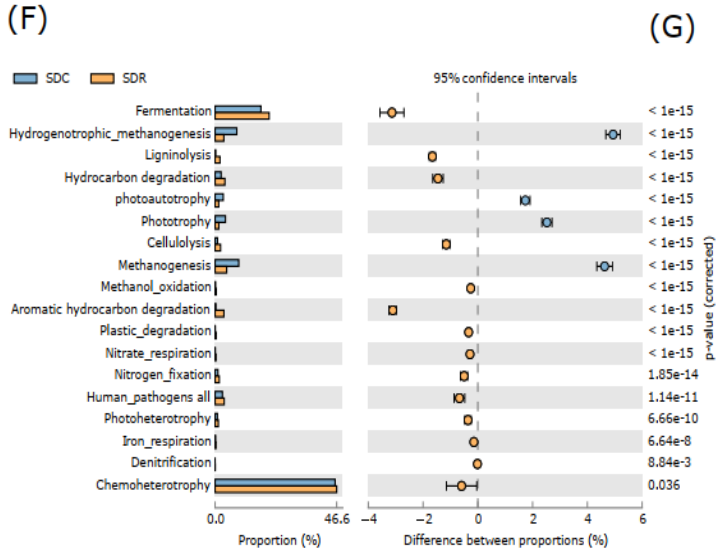
The higher chemoheterotrophic functions have been reported in epiphytic and sediment bacterial communities[36,448]. Heterotrophic bacteria are often decomposers and responsible for in situ bioremediation of polycyclic aromatic hydrocarbons (PAHs) and degradation of organic matter in aquatic plants and sediments[449,450]. Duraivadivel et al. [451] demonstrated that the bacterial community assisted the *E. crassipes* in reducing the toxic effect of heavy metals, antibiotics, and other xenobiotics in the Yamuna River. Furthermore, biofilms attached to *Potamogeton crispus* and *Wolffia australiana* showed nitrogen-rich cycle species and harbor-related functional genes[36,452].

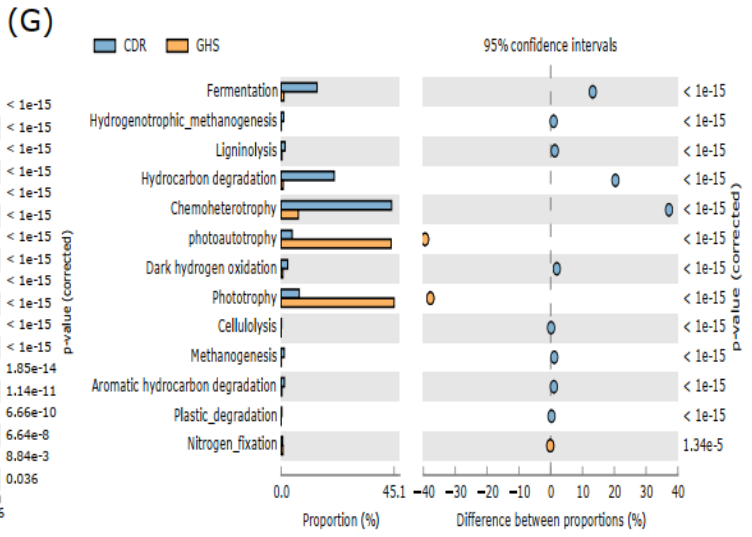
Photosynthesis (photoautotrophy) is the most important metabolic activity of aquatic plants and hot spring mats and has a significant impact on the structure and function of the entire aquatic ecosystem[453,454]. For example, in light-exposed hot springs, microbial mats are dominated by phototrophic bacteria at temperatures between 42–74°C[187]. The aquatic plant-biofilm platform plays multiple roles in aquatic ecosystems, including primary production (oxygen release) and respiration, trophic interactions, microbial gene pool conservation, and biogeochemical cycles[8]. Taken together, the differences in metabolic functions across various substrates might be related to the differences in microhabitats, plant physiology (secondary metabolites, allelopathy, immune system, and age) and morphology, and water chemistry.


(A)
Cellulolysis
Manganese oxidation
Methanogenesis
Hydrogenotrophic methanogenesis
Fermentation
Methylotrophy
Hydrocarbon degradation
Methanotrophy
Iron respiration
Methanol oxidation
Nitrite denitrification
Denitrification
Phototrophy
Photoautotrophy
Oxygenic hotoautotrophy
Nitrate respiration
Nitrogen respiration
Nitrite respiration
Nitrite ammonification
Nitrate reduction
Nitrogen fixation
Photoheterotrophy
Plastic degradation
Aerobic chemoheterotrophy
Aromatic compound degradation
Aliphatic non methane hydrocarbon degradation
Chemoheterotrophy
Human pathogen all
Dark hydrogen oidation
Ureolysis
3
2
1
0
−1
−2
−3
SW1 SD1 SD2 SD3 SW3 CD3 ED3 CD1 CD2 ED1 SW2 CW1 CW2 EW1 EW2 EW3 ED2 CW3
(B)
Predatory(exoparasitic)
Human gut
Human pathogens
Methanol oxidation
Phototrophy
Photoautotrophy
Oxygenic photoautotrophy
Hydrocarbon degradation
Photoheterotrophy
Nitrogen fixation
Methylotrophy
Methanotrophy
Nitrification
Dark hydrogen oxidation
Methanogenesis
Hydrogenotrophic methanogenesis
Dark oxidation of sulfur compounds
Iron respiration
Respiration of of sulfur compounds
Sulfite respiration
Chemoheterotrophy
Cellulolysis
Xylanolysis
Fermentation
Reductive acetogenesis
Plastic degradation
Dark hydrogen oxidation
Anoxygenic photoautotrophy
Ligninolysis
Denitrification
Aromatic hydrocarbon degradation
Nitrate respiration
3
2
1
0
−1
−2
−3
ECD1 ECW1 ECD2 SW1 SD2 SW2 SW3 SD1 SD3 CPW2 ECW3 CPW1 ECW2 CPD1 CPD2 CPD3 CPW3
(C)
Phototrophy
Photoautotrophy
Dark oxidation of sulfur compounds
Dark thiosulphate oxidation
Nitrogen fixation
Hydrocarbon degradation
Methylotrophy
Methanotrophy
Invertabrates parasites
Nitrite respiration
Nitrate reduction
Aromatic compound degradation
Aromatic hydrocarbon degradation
Ligninolysis
Manganese oxidation
Nitrification
Plastic degradation
Methanogenesis
Hydrogenotrophic methanogenesis
Fermentation
Dark hydrogen oxidation
Chemoheterotrophy
Cellulolysis
Predatory(exoparasitic)
Aerobic chemoheterotrophy
Sulfate respiration
Intracellular parasites
2
1
0
−1
−2
GS1_62.8 BG2_42.6 BG1_47 GS3_71.4 BG4_40 BG5_44 GS2_64.5 GS5_60 BG3_41.6 GS4_58


(D)
CDC
CDR
95% confidence intervals
Fermentation
Nitrate_respiration
Hydrogenotrophic_methanogenesis
Nitrogen_fixation
Ligninolysis
Hydrocarbon degradation
Chemoheterotrophy
Plastic_degradation
photoautotrophy
Dark hydrogen oxidation
Photoheterotrophy
Phototrophy
Methanogenesis
Methanol_oxidation
Aromatic hydrocarbon degradation
Human_pathogens all
Iron_respiration
Denitrification
Cellulolysis
< 1e-15
6.24e-4
p-value (corrected)
0.0
37.6
Proportion (%)
−15 −10 −5 0 5 10 15 20 25
Difference between proportions (%)


(E)
ECC
ECR
95% confidence intervals
Fermentation
Nitrate_respiration
Hydrogenotrophic_methanogenesis
Nitrogen_fixation
Hydrocarbon degradation
Chemoheterotrophy
photoautotrophy
Denitrification
Dark hydrogen oxidation
Photoheterotrophy
Phototrophy
Cellulolysis
Methanogenesis
Methanol_oxidation
Aromatic hydrocarbon degradation
Human_pathogens all
Iron_respiration
Ligninolysis
Plastic_degradation
< 1e-15
7.23e-5
0.023
p-value (corrected)
0.0
50.0
Proportion (%)
−15 −10 −5 0 5 10 15
Difference between proportions (%)


(F)
SDC
SDR
95% confidence intervals
Fermentation
Hydrogenotrophic_methanogenesis
Ligninolysis
Hydrocarbon degradation
photoautotrophy
Phototrophy
Cellulolysis
Methanogenesis
Methanol_oxidation
Aromatic hydrocarbon degradation
Plastic_degradation
Nitrate_respiration
Nitrogen_fixation
Human_pathogens all
Photoheterotrophy
Iron_respiration
Denitrification
Chemoheterotrophy
< 1e-15
1.85e-14
1.14e-11
6.66e-10
6.64e-8
8.84e-3
0.036
p-value (corrected)
0.0
46.6
Proportion (%)
−4 −2 0 2 4 6
Difference between proportions (%)


(G)
CDR
GHS
95% confidence intervals
Fermentation
Hydrogenotrophic_methanogenesis
Ligninolysis
Hydrocarbon degradation
Chemoheterotrophy
photoautotrophy
Dark hydrogen oxidation
Phototrophy
Cellulolysis
Methanogenesis
Aromatic hydrocarbon degradation
Plastic_degradation
Nitrogen_fixation
< 1e-15
1.34e-5
p-value (corrected)
0.0
45.1
Proportion (%)
−40 −30 −20 −10 0 10 20 30 40
Difference between proportions (%)

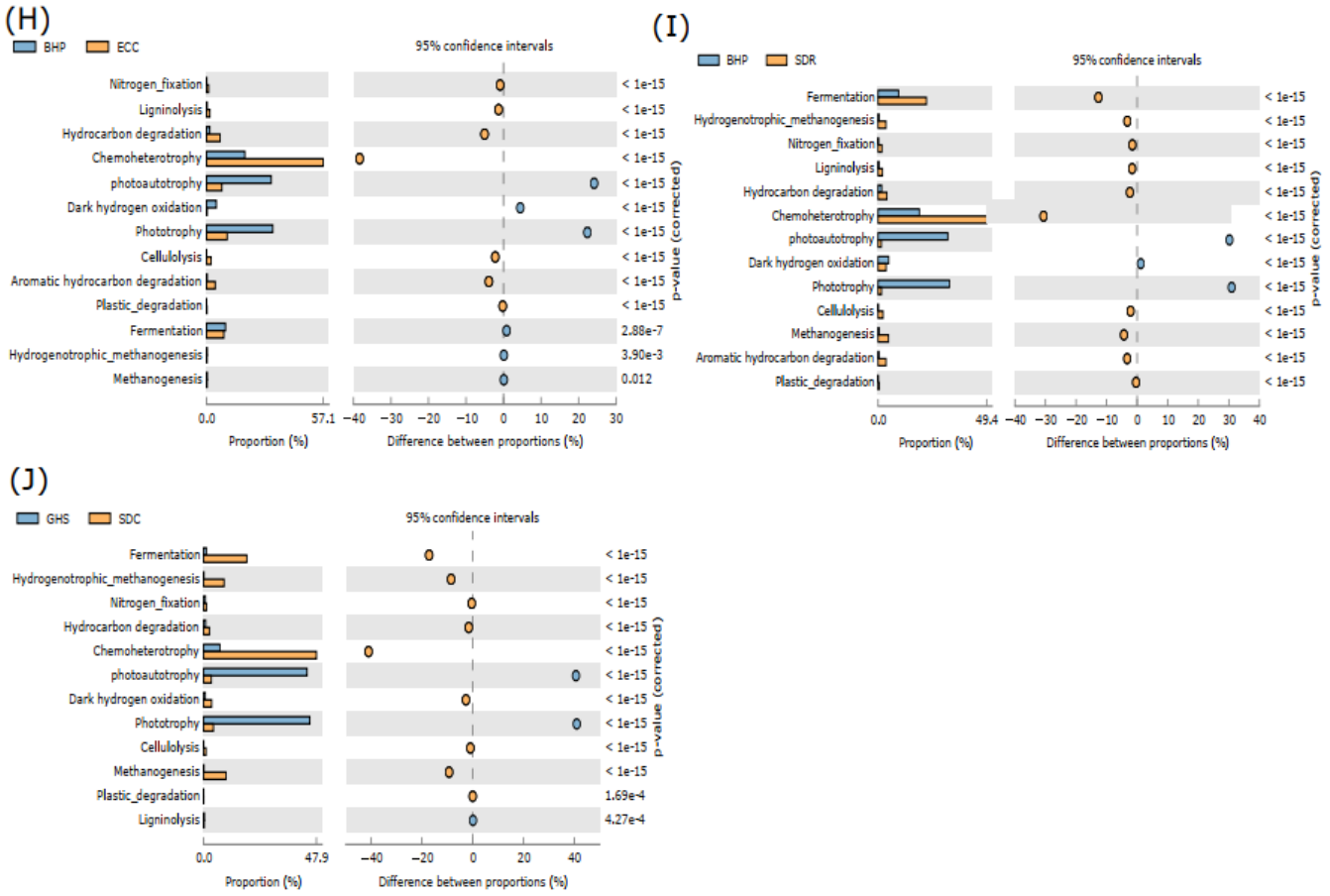


Figure 5. 3 Heatmap of percentage relative abundance of dominant metabolic pathways across different microhabitats (*C.demesum*, *E. crassipes*, surface sediment, and mats) using FAPROTAX annotation (**A-C**). Differences in metabolic functions between bacterial communities in epiphytic, epipelic and mat biofilms were computed using STAMP analysis (**D-J**). Fisher's exact test ($P < 0.05$, $P < 0.01$) followed by Bonferroni corrections were adopted. CDC, *C. demersum* in Lake Cyohoha; CDR, *C. demersum* in Lake Rumira; ECC, *E.crassipes* in Lake Cyohoha; ECR, *E.crassipes* in Lake Rumira; SDC, sediment in Lake Cyohoha; SDR, sediment in Lake Rumira; BHP, Bugarama hot spring mats; GHS, Gisenyi hot spring mats.

5.4 Summary

This study comprehensively compares the microbial biofilm communities in *C. demersum*, *E.crassipes*, surface sediments, and mats based on response to environmental variables, biotic interactions, and predicted functions. The main conclusions are as follows:

1) Bacterial and microeukaryotic communities on *C. demersum* and *E. crassipes* were positively associated with DO, pH, EC, TN, TP, and temperature in tropical lakes, whereas bacterial communities in *C. demersum* and mats were commonly positively related to temperature, TDS, pH, and EC.
2) Co-occurrence networks of possible interactions among microbial genera in aquatic macrophytes, surface sediment and mat biofilms were different. However, there were similarities of top microbial phyla in networks among lakes and hot springs. Specifically, all microbial networks shared phylum Ascomycota and SAR supergroup, whereas the GHS mat network displayed the higher microeukaryotic taxa and Cyanobacteria. Furthermore, this study suggests that all microbial networks share organic matter decomposition, predation and parasitism relationships, and primary production.
3) The predicted metabolic functions (FAPROTAX) of the bacterial communities to all substrates were related to carbon and nitrogen cycling and xenobiotic degradation. Phototrophic functions were significantly dominant in epiphytic and mat bacterial communities, whereas methanogenesis dominated the sediment bacteria. Lastly, the higher bacterial functional abundance of sediment and *C. demersum* bacteria was detected in the wet season, while high-temperature mats exhibited the high bacterial functional abundance.

Chapter 6. Conclusions and future perspectives

6.1 Conclusions

In this thesis, the influence of various microhabitats (aquatic macrophytes, surface sediments, and hot spring mats) on microbial communities in biofilms were thoroughly explored in shallow tropical lakes and hot springs of Rwanda. The first study suggested that microhabitat types and environmental conditions can have strong effects on the composition and diversity of bacterial and eukaryotic communities in biofilms and these effects may correlate with microbial interactions and ecological role in aquatic ecosystems. Phyla Actinobacteria, Cyanobacteria, Chloroflexi, Rotifera, Chloroplastida, and Gastrotricha were more dominant in epiphytic biofilms on *C. demersum* than on *E. crassipes* and surface sediments, while phyla Firmicutes, Ascomycota, and Proteobacteria on surface sediments and *E. crassipes* were significantly higher than on *C. demersum*. RDA results indicate that the diversity and structure of the microbial community in epiphytic biofilms on *C. demersum* and *E. crassipes* were mainly related to pH, DO, temperature, TN, and EC. The network analysis reveals the presence of more complex interactions between bacteria and eukaryotes on *C. demersum* than on *E. crassipes* and surface sediments. Ecological roles through network interactions and functional predictive analysis showed various biogeochemical cycling.

To further understand the interactions between water quality and microbes in epiphytic biofilm and superficial sediments of lake in trophic agriculture area. The effect of land use types on water quality and microbial community, characterization of microbial community, factors driving the microbial ecological analysis, and interaction between microbial communities were explored. This study found significant differences in bacterial dissimilarity and microeukaryotic OTU richness among microhabitats. The PLS-PM showed a higher direct positive correlation between land use types and physicochemical variables in the rainy than dry seasons. Furthermore, there was a higher positive correlation between physicochemical variables and water quality index in rainy season, with a great impact of agriculture on the water quality parameters. However, physicochemical variables showed a partial positive influence on microbial communities and water quality index, respectively. The stability and complexity of networks differed among substrates, being significantly higher in surface sediments than in *C. demersum* and *E. crassipes*. Proteobacteria,

Firmicutes, Actinobacteria, Chloroflexi, Bacteroidetes, Verrucomicrobia, Cyanobacteria, SAR, Rotifera, Gastrotricha, Platyhelminthes, and Chloroplastida were the key role players in the microhabitat networks. Stochastic processes dominated the microbial community assembly in epiphytic (except for bacteria on *E. crassipes*) and surface sediment biofilms. Notwithstanding the dominance of stochastic processes in microbial community assembly, the deterministic processes still represented a significant part of the assembly processes.

The study on the hot spring mats demonstrated that bacterial community β-diversity in moderate temperature pool was strongly driven by stochastic processes, whereas in high-temperature springs, the variable selection in microeukaryotic and bacterial communities was potentially shaped by temperature. For example, some Amoebozoa (e.g., *Echinamoeba*, *BOLA868*, and *Telaepolella*) and SAR taxa have adapted to high-temperature in hot spring microbial mats. In addition, the exploration of co-occurrence patterns and ecological roles demonstrated that microbial interaction in high-temperature mats were more stable and sophisticated, and the temperatures showed a critical influence on ecological functions. Although mostly ignored in hot spring mats, microeukaryotes are diverse and form complex interactions in extreme environments. In conclusion, this study provide new insights to understand better the influence of microhabitat types and environmental variables on microbial biodiversity, assemblages, interactions, and ecological functions from tropical lakes and hot springs.

Originality and contributions to science

This work systematically and comprehensively investigated the synergetic influence of microhabitat types and environmental variables on periphytic biofilm-dwelling microbial communities in shallow tropical lakes and hot springs. To the best of our knowledge, this study is the first of its kind to provide a comprehensive investigation of the biodiversity, assemblages, interactions, and ecological functions of bacterial and microeukaryotic communities in submerged and floating macrophytes, surface sediments, and mats. The following are the contributions of this study:

1. **Microhabitat types drive the microbial composition in epiphytic biofilms:** This study proved that microhabitat types (submerged plants, floating plants, and surface sediments) were the dominant drivers of microbial community composition in biofilms.

2. **Influence of land use types on water quality and microbial structure:** This study went further to investigate the potential impact of land use types and water quality on epiphytic biofilms in trophic agriculture area. The PLS-PM showed a higher direct positive correlation between land use types and physicochemical variables in the rainy than dry seasons. Furthermore, there was a higher positive correlation between physicochemical variables and water quality index in rainy season, with a great impact of agriculture on the water quality parameters. However, physicochemical variables showed a partial positive influence on microbial communities and water quality index, respectively.

3. **Microbial assembly processes in periphytic biofilms**: This research constitutes the first attempt to investigate the relative importance of stochastic and deterministic assembly processes of microeukaryotes in epiphytic biofilms, superficial sediments, and hot spring mats. The null and neutral models revealed that stochastic processes dominated the microbial community assembly in epiphytic biofilms and surface sediments of shallow tropical lakes and moderate-temperature springs. However, in high-temperature springs, the microbial community was potentially shaped by temperature. Notwithstanding the dominance of stochastic processes in periphytic biofilms, the deterministic processes still represented a significant part of the assembly processes.

4. **Microbial community in periphytic biofilms of African freshwater ecosystem:** Most studies on the exploration of microbial community in epiphytic biofilms, superficial sediments, and mats were mainly conducted in China, Denmark, Canada, Australia, and the USA, while the African continent still lags behind in this regards. To the best of our knowledge, this study is the first to explore the microbial community, in particular, microeukaryotes in epiphytic biofilms, superficial sediments, and mats in Africa.

5. **Microbial interaction in periphytic biofilms**: There was complex interactions among microbes in periphytic biofilms, and those interactions include cross feeding, symbiosis, parasitism, and predation. Particularly, this study revealed more complex and stable interactions among microbes on *C. demersum* than other substrates. This study went further to present an interesting evaluation of the interactions between bacteria and microeukaryotes in periphytic biofilms.

6. **Ecological function of periphytic biofilms**: This research constitutes the first attempt to investigate the ecological function of bacterial communities in floating plants, superficial

sediments, and mats of shallow tropical lakes and hot springs. This study showed that microhabitat types significantly shaped the ecological role of biofilms in tropical lakes and hot springs.

The findings of this study provide new insights in the understanding of the influence of microhabitat/substrate types and environmental variables on biodiversity, composition assembly processes, interactions, and ecological functions of biofilm-dwelling microbial communities in tropical lacustrine and geothermal ecosystems, which holds good for the ecological restoration projects, water purification, and hot spring sustainability.

6.2 Future perspectives

Based on the above conclusions, a collection of recommendations for future policy on aquatic macrophytes and hot springs conservation or management were provided. In addition, knowledge gaps and future research directions on epiphytic and surface sediment biofilms and hot spring mats should be addressed.

- **Aquatic health campaign.** The general public should be made aware of the significance of aquatic macrophytes and biofilms in water purification processes and the threat posed by emerging pollutants (e.g., pesticides, fertilizers, antibiotics, and microplastics) in aquatic ecosystems.
- **Strengthen surveillance:** Environmental monitoring studies should be conducted to know the status of freshwater water pollution. For example, various pollutants must be treated before being released into the wetland, Lakes, and rivers.

  Despite the current knowledge, there are several key areas where research is currently lacking. **Future studies should focus on**:

- Including epiphytic biofilms to understand, maintain, and improve freshwater ecosystem health and integrity.
- Global study of microbial biodiversity (e.g., bacteria, microeukaryotes, and archaea), assembly processes, and interactions in biofilms on different aquatic plant species and mats in different seasons. Moreover, rare and dominant microbial taxa in epiphytic and mat biofilms should be investigated.

- Microbial ecology research (e.g., aquatic biofilms) on the African continent must be strengthened to grasp the influence of biogeography on biofilm-dwelling microbial communities.
- Difference between Epiphytic bacteria, archaea, viruses and microeukaryotes in rivers, lakes, river-lake continuum, estuary, and Sea.
- Improved knowledge of the biofilm-macrophyte relationship can be used to enhance our understanding of the costs and benefits of current management practices, such as removing natural vegetation and re-oligotrophication.
- Advanced studies of biofilms on aquatic plants, surface sediments, and mats at the molecular level (metagenomics, transcriptomics, and metabolomics) using cut-edging technologies, such as neural networks, machine learning, big data, and artificial intelligence (AI).

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Acknowledgements

Special thanks to my supervisor Professor Songhe Zhang for his guidance towards the completion of this thesis.

Sincere thanks go to my team members especially Felix Gyawu Addo, Dr. Munyampundu Jean Pierre, Yu Ma, Salah Alden Alklaf, Guo shaozhuang, and Hezhou Chen, for their assistance in conducting the experiments. I would like to also show my appreciation to the international school and Chinese Scholarship Council for granting me CSC scholarship which funded my study.Special gratitude to my parents and friends for their moral support.

Benjamin Manirakiza

Appendix

Publication lists

1. Ohore, O.E., Zhang, S., Guo, S., **Manirakiza, B.**, Addo, F.G., Zhang, W., 2021b. The fate of tetracycline in vegetated mesocosmic wetlands and its impact on the water quality and epiphytic microbes. J. Hazard. Mater. 417, 126148. https://doi.org/10.1016/j.jhazmat.2021.126148

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ARTICLES UNDER REVIEW

Felix Gyawu Addo, Songhe Zhang, Benjamin Manirakiza, Yu Ma, Shudong Yuan, Salah Alden Alklaf, Shaozhang Guo, Godwin Abakari. Brown sugar addition enhanced nutrient removal rates, growth performance, and bacterial community in a rice straw biofloc shrimp culture system. Elsevier. First author (**I.F 5.013**)

Appendix Table S1. Pearson correlation coefficients between bacterial community on *C. demersum* and environmental parameters

	TP	**TN**	**NO_3-N**	**NH_4-N**	**T**	**pH**	**DO**	**TDS**	**EC**
Bacillus	-0.484	-0.322	-0.319	-0.61	0.306	-0.192	-0.249	0.569	0.434
Comamonadaceae	0.067	0.157	0.023	0.255	0.047	0.442	0.487	-0.235	-0.138
Clostridium_sensu_ stricto_1	-0.011	0.352	-0.049	-0.138	-0.231	-0.323	-0.192	-0.147	-0.193
Acinetobacter	-0.338	-0.513	-0.236	-0.319	0.388	0.024	-0.068	0.556	0.569
Exiguobacterium	-0.361	-0.454	-0.165	-0.349	0.366	-0.032	-0.149	0.545	0.553
Alicyclobacillus	-0.019	0.664	0.347	0.123	-0.286	-0.493	-0.35	-0.27	0.123
Lysinibacillus	-0.395	-0.528	-0.312	-0.405	0.409	-0.005	-0.079	0.61	0.595
Hydrogenispora	0.311	0.817*	0.545	0.174	-0.667	-0.791	-0.703	-0.558	-0.46
Fictibacillus	-0.342	-0.509	-0.234	-0.326	0.387	0.017	-0.076	0.558	0.568
uncl_f_Methylococcaceae	0.428	0.285	0.008	0.217	-0.423	-0.072	0.038	-0.476	-0.711
Solibacillus	-0.34	-0.514	-0.238	-0.321	0.389	0.024	-0.068	0.558	0.57
Romboutsia	-0.093	0.169	-0.215	-0.249	-0.11	-0.235	-0.112	0.003	-0.11
norank_f_JG30KFCM45	-0.016	0.281	-0.13	-0.153	-0.201	-0.303	-0.154	-0.101	-0.156
norank_f_T34	0.383	0.093	-0.319	0.504	-0.158	0.261	0.597	-0.343	-0.124
uncl_k_norank_d_Bacteria	0.518	0.733	0.467	0.412	-0.66	-0.368	-0.272	-0.747	-0.75
Amaricoccus	-0.391	-0.583	-0.449	-0.241	0.655	0.944**	0.81	0.416	0.219
Rhodobacter	-0.004	0.178	0.016	0.129	0.054	0.415	0.427	-0.203	-0.199
Crenothrix	0.663	0.421	-0.058	0.718	-0.536	-0.179	0.233	-0.638	-0.313
Methylomagnum	0.16	-0.223	-0.531	0.29	0.141	0.565	0.815*	-0.067	0.021
Paenibacillus	-0.386	-0.515	-0.262	-0.365	0.414	0.016	-0.071	0.593	0.609

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Appendix Table S2. Pearson correlation coefficients between eukaryotic community on *C. demersum* and environmental parameters

	TP	**TN**	**NO_3-N**	**NH_4-N**	**T**	**pH**	**DO**	**TDS**	**EC**
o_Flosculariacea	-0.421	0.002	0.457	-0.324	0.261	-0.008	-0.359	0.266	0.242
o_Chaetonotida	-0.567	-0.126	-0.389	-0.612	0.349	0.057	0.058	0.448	0.318
c_Dinophyceae	0.339	-0.261	-0.374	0.498	0.068	0.558	0.771	-0.161	-0.021
f_Aspergillaceae	-0.193	0.505	0.741	-0.094	-0.13	-0.43	-0.655	-0.102	0.066
o_Rhabdocoela	-0.028	0.519	0.826*	0.036	-0.24	-0.455	-0.692	-0.222	-0.085
Heterocapsa	0.313	-0.29	-0.346	0.436	0.089	0.602	0.754	-0.146	-0.099
f_Microascaceae	0.609	0.267	0.373	0.277	-0.599	-0.329	-0.397	-0.553	-0.906*
c_Chlorophyceae	-0.382	-0.254	-0.65	-0.307	0.392	0.368	0.539	0.357	0.37
Uncl_Eukaryota	-0.296	-0.538	-0.437	-0.159	0.578	0.920**	0.819*	0.33	0.139
o_Ploimida	-0.78	-0.853*	-0.544	-0.838*	0.829*	0.556	0.274	0.919**	0.518
c_Arachnida	-0.046	0.574	0.695	0.171	-0.172	-0.292	-0.399	-0.257	0.079
p_SAR	0.328	-0.282	-0.429	0.531	0.088	0.517	0.785	-0.119	0.126
Glenodinium	-0.393	-0.554	-0.194	-0.43	0.427	0.085	-0.101	0.59	0.458
Curvularia	-0.083	0.672	0.786	0.044	-0.238	-0.396	-0.564	-0.287	-0.08
o_Haplotaxida	-0.434	0.151	0.464	-0.305	0.183	-0.217	-0.474	0.242	0.371
o_Adinetida	0.055	-0.322	-0.265	0.086	0.236	0.728	0.673	-0.011	-0.253
o_Cyclopoida	-0.362	-0.51	-0.223	-0.335	0.404	0.033	-0.071	0.568	0.581

Appendix Table S3. Pearson correlation coefficients between bacterial community on *E.crassipes* and environmental parameters

	TP	TN	NO_3-N	NH_4-N	T	pH	DO	TDS	EC
Bacillus	-0.55	-0.838*	-0.478	-0.436	0.778	0.683	0.49	0.749	0.602
f__Comamonadaceae	0.689	0.657	0.441	0.434	-0.809	-0.567	-0.455	-0.779	-0.886*
Clostridium_sensu_stricto_1	-0.492	-0.672	-0.237	-0.363	0.692	0.692	0.42	0.593	0.421
Acinetobacter	0.821*	0.383	0.047	0.940**	-0.592	-0.211	0.212	-0.704	-0.248
Exiguobacterium	-0.239	-0.449	-0.071	-0.27	0.29	-0.003	-0.168	0.441	0.365
Alicyclobacillus	-0.556	-0.858*	-0.523	-0.428	0.803	0.737	0.559	0.757	0.616
Lysinibacillus	-0.513	-0.758	-0.513	-0.363	0.79	0.959**	0.777	0.607	0.395
Hydrogenispora	-0.452	-0.691	-0.382	-0.385	0.582	0.294	0.173	0.686	0.654
Fictibacillus	-0.541	-0.746	-0.383	-0.429	0.703	0.49	0.308	0.733	0.66
Uncl_f_Methylococcaceae	0.111	0.717	0.399	0.039	-0.478	-0.665	-0.569	-0.38	-0.242
Solibacillus	-0.54	-0.832*	-0.54	-0.386	0.822*	0.895*	0.717	0.691	0.52
Romboutsia	-0.584	-0.732	-0.412	-0.409	0.813*	0.880*	0.653	0.657	0.489
Norank_f_JG30-KF-CM45	-0.586	-0.849*	-0.523	-0.473	0.79	0.625	0.458	0.798	0.681
Norank_f_T34	-0.126	0.052	-0.353	-0.167	0.062	0.164	0.299	0.016	-0.083
Uncl_k_norank_d_Bacteria	0.233	0.475	0.205	-0.038	-0.504	-0.591	-0.522	-0.349	-0.508
Amaricoccus	0.637	0.771	0.791	0.396	-0.833*	-0.654	-0.714	-0.81	-0.943**
Rhodobacter	0.323	0.301	0.255	-0.046	-0.476	-0.365	-0.446	-0.378	-0.806
Crenothrix	0.163	-0.345	-0.258	0.353	0.225	0.656	0.717	-0.021	0.062
Methylomagnum	0.157	0.883*	0.744	0.172	-0.526	-0.617	-0.639	-0.533	-0.346
Paenibacillus	-0.561	-0.831*	-0.485	-0.45	0.768	0.617	0.44	0.77	0.651

Appendix Table S4. Pearson correlation coefficients between eukaryotic community on *E.crassipes* and environmental parameters

	TP	**TN**	**NO_3-N**	**NH_4-N**	**T**	**pH**	**DO**	**TDS**	**EC**
c__Embryophyta	0.252	0.16	0.226	0.332	-0.097	0.393	0.326	-0.373	-0.442
o__Floscullariacea	0.553	0.748	0.286	0.648	-0.666	-0.613	-0.238	-0.668	-0.16
o__Chaetonotida	0.713	0.598	0.621	0.424	-0.808	-0.562	-0.587	-0.773	-0.975**
c__Dinophyceae	-0.588	-0.865*	-0.539	-0.475	0.806	0.667	0.496	0.799	0.667
f__Aspergillaceae	-0.528	-0.799	-0.539	-0.395	0.807	0.957**	0.768	0.641	0.404
o__Rhabdocoela	0.096	0.141	-0.333	-0.022	-0.194	-0.259	0.009	-0.082	-0.054
Heterocapsa	-0.401	-0.594	-0.307	-0.373	0.474	0.124	0.018	0.626	0.607
f__Microascaceae	0.26	0.167	-0.386	0.299	-0.239	-0.236	0.192	-0.183	0.14
c__Chlorophyceae	-0.403	-0.204	-0.394	-0.462	0.363	0.482	0.412	0.272	-0.062
d__Eukaryota	-0.699	-0.691	-0.769	-0.783	0.704	0.512	0.432	0.783	0.424
o__Ploimida	0.16	0.622	0.197	0.103	-0.445	-0.561	-0.369	-0.366	-0.2
c__Arachnida	-0.34	-0.047	-0.416	-0.413	0.165	-0.058	0.059	0.279	0.213
p__SAR	0.054	0.43	0.028	-0.069	-0.296	-0.339	-0.209	-0.238	-0.274
Glenodinium	-0.414	-0.602	-0.32	-0.399	0.479	0.12	0.009	0.64	0.601
Curvularia	0.325	0.019	0.211	0.005	-0.282	0.023	-0.169	-0.296	-0.825*
o__Haplotaxida	-0.327	-0.027	-0.398	-0.388	0.179	0.081	0.171	0.219	0.1
o__Adinetida	0.714	0.957**	0.709	0.753	-0.874*	-0.752	-0.528	-0.900*	-0.483
o__Cyclopoida	-0.173	0.663	0.707	-0.06	-0.191	-0.403	-0.558	-0.218	-0.031

Appendix Table S5. Physicochemical parameters of Lake Cyohoha North

Sites	Temperature	Water depth(cm)	pH	DO(mg/L)	EC(μS/cm)	TDS(mg/L)	TP(mg/L)	TN (mg/L)	NO3-N (mg/L)	NH4-N(mg/L)
CWD1	24.2±0.89	66±0.02	8.20±0.22	4.27±0.06	665.66±0.14	1352±1.04	2.24±0.017	3.36±0.046	0.53±0.006	2.42±0.01
CWD2	27.1±0.13	85±0.03	9.05±0.19	11.20±0.08	531.66±0.91	1121.67±0.9	0.57±0.086	1.75±023	0.29±0.031	1.108±0.088
CWD3	27.8±0.51	50±0.35	9.28±0.16	8.72±0.05	548±0.72	1162.66±1.4	1.06±0.011	3.17±0.020	0.37±0.002	2.231±0.004
CWW1	24.23±0.72	74±0.45	8.04±0.045	2.63±0.077	521±0.57	1036.33±1.0	3.22±0.04	3.40±0.03	0.56±0.06	2.22±0.01
CWW2	25.46±0.818	96±0.45	9.34±0.040	9.54±0.076	484±1.05	1019±0.98	2.20±0.035	4.22±0.05	0.50±0.04	3.30±0.01
CWW3	25.7±1.15	59±0.05	9.27±0.76	9.13±0.0.85	493.33±0.82	1030.33±0.95	2.35±0.029	3.62±0.04	0.56±0.05	2.55±0.03

Appendix Table S6. Pearson correlation coefficients between bacterial communities in Bugarama hot pool (BHP) mats and environmental parameters

Bacterial genera		Temperature	pH	DO	TDS	EC	ORP	TP	TN	NO3-N	NH4-N
o__Woesearchaeales	Pearson Correlation	-.156	.067	.012	.327	-.361	-.115	-.680	.122	-.049	-.202
f__GW2011_GWC1_47_15	Pearson Correlation	-.357	.245	.110	.366	-.301	-.302	-.405	.230	.202	-.649
JdFR-76	Pearson Correlation	-.201	.059	-.536	-.022	-.263	-.073	-.126	.533	-.127	.236
o__RBG-13-54-9	Pearson Correlation	.534	-.537	-.220	-.080	.105	.541	-.151	-.029	.038	.507
Chloroflexus	Pearson Correlation	.206	-.320	-.639	-.484	-.015	.322	.338	.451	.612	.308
Luteolibacter	Pearson Correlation	-.590	.773	.529	-.392	-.414	-.738	-.309	-.722	-.209	-.017
c__Thermodesulfovibrionia	Pearson Correlation	.721	-.617	.265	.348	.371	.626	-.259	-.408	-.411	.389
Planktothricoides_SR001	Pearson Correlation	.806	-.743	.257	.695	.990**	.756	.658	-.007	-.415	-.077
o__Chthoniobacterales	Pearson Correlation	.763	-.697	.298	.695	.981**	.709	.670	-.023	-.379	-.151
JSC-12	Pearson Correlation	-.412	.284	.045	.291	-.346	-.342	-.369	.289	.291	-.662
o__SBR1031	Pearson Correlation	-.347	.266	.226	.360	-.343	-.322	-.532	.080	.192	-.649
Roseiflexus	Pearson Correlation	.434	-.487	-.470	-.240	-.018	.492	-.115	.170	.121	.623
Thermoanaerobaculum	Pearson Correlation	.565	-.503	.078	.098	.151	.508	-.311	-.296	-.168	.429
Romboutsia	Pearson Correlation	-.589	.773	.530	-.391	-.414	-.738	-.310	-.724	-.209	-.017
Fluviimonas	Pearson Correlation	-.589	.773	.530	-.391	-.414	-.738	-.309	-.723	-.209	-.018

Paludibaculum	Pearson Correlation	.525	-.522	-.186	-.001	.048	.524	-.321	-.077	-.122	.571
c__Anaerolineae	Pearson Correlation	-.061	-.124	-.358	.354	-.077	.077	-.094	.630	.093	-.252
f__Saprospiraceae	Pearson Correlation	.227	-.247	-.303	-.435	-.058	.255	.067	.043	.431	.332
f__A4b	Pearson Correlation	-.156	-.022	-.752	-.602	-.173	.013	.497	.692	.866*	.013
f__Prolixibacteraceae	Pearson Correlation	-.589	.773	.530	-.391	-.414	-.738	-.309	-.723	-.209	-.018
f__Lenti-02	Pearson Correlation	-.642	.817*	.561	-.351	-.455	-.789	-.364	-.714	-.184	-.105
f__Rhodobacteraceae	Pearson Correlation	-.598	.775	.474	-.421	-.420	-.738	-.282	-.683	-.222	.030
f__Hydrogenophilaceae	Pearson Correlation	-.505	.414	.162	.256	-.426	-.467	-.478	.134	.198	-.644
Ralstonia	Pearson Correlation	-.586	.770	.528	-.388	-.408	-.735	-.304	-.721	-.217	-.012
o__1-20	Pearson Correlation	-.481	.305	-.326	.052	-.432	-.350	-.237	.536	.258	-.344
norank_d__Bacteria	Pearson Correlation	.467	-.556	-.220	.465	.117	.527	-.356	.257	-.274	.250
p__Armatimonadota	Pearson Correlation	.502	-.439	.066	.076	.050	.444	-.441	-.321	-.243	.498
f__Calditrichaceae	Pearson Correlation	-.394	.305	.217	.360	-.316	-.361	-.434	.129	.221	-.717
Limnothrix	Pearson Correlation	-.141	-.040	-.802	-.595	-.114	.036	.595	.745	.801	.061
o__OPB41	Pearson Correlation	.652	-.568	.344	.637	.932**	.585	.677	-.081	-.416	-.162
o__Chthoniobacterales	Pearson Correlation	.741	-.674	.306	.695	.974**	.686	.671	-.025	-.387	-.162
BSV13	Pearson Correlation	-.589	.773	.530	-.391	-.414	-.738	-.309	-.723	-.209	-.018
c__Bacteroidia	Pearson Correlation	.260	-.334	-.602	-.519	.081	.351	.447	.362	.482	.426

o__SJA-15	Pearson Correlation	-.596	.523	.201	.109	-.568	-.574	-.594	.024	.300	-.633
o__Chthonomonadales	Pearson Correlation	.454	-.446	-.161	-.096	.000	.448	-.292	-.109	.009	.501
Candidatus_Chloroploca	Pearson Correlation	-.430	.192	-.745	-.335	-.540	-.231	-.104	.777	.596	-.066
Hydrogenophaga	Pearson Correlation	-.584	.769	.535	-.384	-.405	-.734	-.303	-.724	-.213	-.021
f__Comamonadaceae	Pearson Correlation	-.577	.714	.216	-.488	-.423	-.671	-.198	-.482	-.308	.252
Nordella	Pearson Correlation	-.589	.773	.530	-.391	-.414	-.738	-.309	-.723	-.209	-.018
f__Anaerolineaceae	Pearson Correlation	-.301	.278	.273	.222	-.497	-.321	-.845*	-.186	.005	-.307

Appendix Table S7. Pearson correlation coefficients between bacterial communities in Gisenyi hot spring (GHS) mats and environmental parameters

Bacterial genera		Temperature	pH	DO	TDS	EC	ORP	TP	TN	NO3-N	NH4-N
Leptococcus_JA-3-3Ab	Pearson Correlation	-.201	.475	.[b]	.131	-.088	-.206	-.217	-.099	.874*	-.123
GBChlB	Pearson Correlation	-.359	-.258	.[b]	-.115	.514	-.384	-.427	.236	-.166	-.405
Bryobacter	Pearson Correlation	-.818*	-.163	.[b]	.587	-.135	-.893*	-.200	.785	.245	.200
o__RBG-13-54-9	Pearson Correlation	.235	-.046	.[b]	-.260	-.292	.497	.732	-.448	-.433	.350
Chloroflexus	Pearson Correlation	.749	-.346	.[b]	-.589	.861*	.632	-.751	-.297	-.422	-.811
Trichodesmium_IMS101	Pearson Correlation	-.286	-.026	.[b]	.476	-.204	-.198	-.393	.328	.861*	.064
Elioraea	Pearson Correlation	-.652	-.154	.[b]	.114	.144	-.627	-.048	.363	-.112	-.028
Leptolyngbya_RV74	Pearson Correlation	-.574	-.198	.[b]	.800	-.678	-.565	.280	.753	.171	.729
o__Microtrichales	Pearson Correlation	-.563	-.158	.[b]	.797	-.703	-.555	.307	.726	.194	.743
o__SBR1031	Pearson Correlation	.292	.672	.[b]	-.239	.168	.117	-.277	-.450	.611	-.400
Roseiflexus	Pearson Correlation	-.247	-.303	.[b]	-.195	.292	-.090	-.033	.012	-.301	-.169
Truepera	Pearson Correlation	-.230	-.223	.[b]	-.230	.587	-.343	-.372	.177	-.412	-.445
SM1A02	Pearson Correlation	-.617	-.155	.[b]	.811	-.606	-.648	.153	.789	.266	.639
f__Coleofasciculaceae	Pearson Correlation	-.557	-.188	.[b]	.803	-.697	-.544	.293	.740	.185	.742
Pseudanabaena_NgrPSln22	Pearson Correlation	-.568	-.192	.[b]	.814*	-.698	-.554	.280	.752	.200	.741
o__Oxyphotobacteria_Incertae_Sedis	Pearson Correlation	-.531	-.194	.[b]	.791	-.682	-.525	.278	.733	.171	.727
o__Xanthomonadales	Pearson Correlation	-.496	.533	.[b]	.457	-.600	-.626	.444	.245	.405	.509
c__Anaerolineae	Pearson Correlation	.713	.529	.[b]	-.640	.476	.428	-.202	-.665	-.135	-.585
Geitlerinema_PCC-8501	Pearson Correlation	-.375	-.316	.[b]	.025	.472	-.372	-.594	.336	.083	-.405
Anoxybacillus	Pearson Correlation	.173	.979**	.[b]	-.462	.061	-.018	.275	-.706	.267	-.250
f__Saprospiraceae	Pearson Correlation	-.331	-.365	.[b]	.022	.494	-.325	-.633	.344	.063	-.422
norank_f__A4b	Pearson Correlation	-.667	-.327	.[b]	.939**	-.573	-.639	-.086	.932**	.473	.590
Hydrogenophilus	Pearson Correlation	.843*	-.208	.[b]	-.480	.583	.670	-.486	-.305	-.458	-.554
MTP1	Pearson Correlation	-.082	-.377	.[b]	-.232	.115	.147	.270	-.098	-.538	.044
Rhodobaculum	Pearson Correlation	-.583	-.186	.[b]	.783	-.664	-.580	.288	.745	.150	.719
f__Chloroflexaceae	Pearson Correlation	.088	-.223	.[b]	.357	-.220	-.040	-.054	.401	-.118	.263

f__Rhodobacteraceae	Pearson Correlation	-.367	-.048	.[b]	.650	-.471	-.483	.055	.623	.229	.473
f__Hydrogenophilaceae	Pearson Correlation	.145	.478	.[b]	-.217	-.357	.314	.622	-.593	.188	.238
Lentimicrobium	Pearson Correlation	.268	-.172	.[b]	.064	.274	.262	-.732	.061	.512	-.384
Rhodococcus	Pearson Correlation	.683	.590	.[b]	-.650	.460	.397	-.169	-.693	-.102	-.581
Sandaracinobacter	Pearson Correlation	-.149	.912*	.[b]	-.264	.182	-.432	-.031	-.355	.417	-.353
norank_d__Bacteria	Pearson Correlation	.036	-.363	.[b]	-.091	.716	-.106	-.951**	.279	.035	-.675
f__ML635J-40_aquatic_group	Pearson Correlation	-.569	.062	.[b]	.729	-.727	-.598	.399	.598	.265	.727
f__WD2101_soil_group	Pearson Correlation	-.407	-.298	.[b]	.014	.463	-.411	-.539	.335	.031	-.384
Limnothrix	Pearson Correlation	.218	-.160	.[b]	-.174	-.330	.512	.688	-.356	-.406	.397
Cecembia	Pearson Correlation	.009	.997**	.[b]	-.338	.002	-.206	.267	-.566	.345	-.189
f__Marinifilaceae	Pearson Correlation	-.169	-.197	.[b]	.378	-.066	-.047	-.487	.298	.712	-.037
Thermus	Pearson Correlation	.823*	-.203	.[b]	-.553	.155	.925**	.272	-.595	-.694	-.086
f__Caldilineaceae	Pearson Correlation	-.302	-.315	.[b]	.431	.037	-.212	-.620	.470	.650	-.096
Bellilinea	Pearson Correlation	-.036	.995**	.[b]	-.306	-.042	-.207	.284	-.566	.425	-.157
Tepidimonas	Pearson Correlation	.771	.548	.[b]	-.642	.318	.633	-.059	-.823*	.031	-.474
f__Rhodocyclaceae	Pearson Correlation	.182	.956**	.[b]	-.403	.112	-.039	.095	-.632	.397	-.324
c__Bacteroidia	Pearson Correlation	-.109	-.596	.[b]	.529	-.191	.099	-.414	.505	.444	.181
f__Methylacidiphilaceae	Pearson Correlation	-.363	-.036	.[b]	-.193	.559	-.465	-.422	.137	-.098	-.488
OLB13	Pearson Correlation	.636	-.308	.[b]	-.525	.875*	.442	-.786	-.176	-.437	-.812*
Thermosynechococcus_BP-1	Pearson Correlation	.280	-.216	.[b]	-.211	-.276	.566	.653	-.361	-.487	.360
Candidatus_Chloroploca	Pearson Correlation	-.696	-.443	.[b]	.565	-.045	-.591	-.414	.738	.394	.097
f__Flammeovirgaceae	Pearson Correlation	-.551	-.190	.[b]	.801	-.694	-.539	.289	.739	.181	.739
f__Chloroflexaceae	Pearson Correlation	-.555	-.240	.[b]	.805	-.627	-.558	.191	.786	.179	.680
Candidatus_Chlorothrix	Pearson Correlation	-.233	.928**	.[b]	-.307	.105	-.421	.161	-.443	.386	-.265
f__Comamonadaceae	Pearson Correlation	-.198	-.149	.[b]	.567	-.338	-.331	-.084	.588	.143	.351
c__Cyanobacteriia	Pearson Correlation	-.573	-.224	.[b]	.792	-.641	-.572	.243	.770	.149	.700
Nordella	Pearson Correlation	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]
Bacillus	Pearson Correlation	.048	.997**	.[b]	-.378	.013	-.149	.281	-.618	.333	-.203
Chloronema	Pearson Correlation	-.589	-.263	.[b]	.801	-.603	-.592	.183	.807	.150	.668

f__Anaerolineaceae	Pearson Correlation	.746	-.005	.[b]	-.398	.585	.559	-.691	-.315	.014	-.664
Chelatococcus	Pearson Correlation	-.013	1.000**	.[b]	-.339	-.014	-.206	.293	-.579	.360	-.174

Appendix Table 8. Pearson correlation coefficients between microeukaryotic communities in Bugarama hot pool (BHP) mats and environmental parameters

Microeukaryotic genera		**pH**	**DO**	**TDS**	**EC**	**ORP**	**TP**	**TN**	**NO3-N**	**NH4-N**
Kurtzmaniella-Candida_clade	Pearson Correlation	.052	-.661	-.618	-.169	-.058	.525	.616	.883*	-.062
Candida-Lodderomyces_clade	Pearson Correlation	.090	-.600	-.386	-.200	-.122	.439	.748	.966**	-.391
Aspergillus	Pearson Correlation	-.097	-.580	-.084	-.068	.107	.069	.465	-.302	.517
o__Podocopida	Pearson Correlation	.862*	.581	-.304	-.506	-.843*	-.421	-.679	-.134	-.218
Andalucia	Pearson Correlation	-.110	-.523	-.546	-.153	.104	.275	.384	.773	.085
p__Ascomycota	Pearson Correlation	-.242	-.633	-.125	.229	.221	.789	.861*	.716	-.300
o__Rhabditida	Pearson Correlation	-.085	-.625	-.197	-.103	.102	.074	.435	-.290	.625
f__Eugregarinorida	Pearson Correlation	.251	.182	.383	-.284	-.309	-.407	.175	.233	-.711
c__Chlorophyceae	Pearson Correlation	.297	.113	.335	-.339	-.352	-.428	.209	.175	-.626
Cladosporium	Pearson Correlation	-.293	-.250	.489	.124	.267	-.104	.432	-.331	.041
f__Plectosphaerellaceae	Pearson Correlation	.124	-.587	-.533	-.236	-.145	.433	.634	.969**	-.266
c__Thecofilosea	Pearson Correlation	-.377	.117	.074	-.001	.381	-.511	-.375	-.263	.476

Boeremia	Pearson Correlation	-.046	-.234	.128	-.403	-.004	-.591	.294	.229	-.103
c__Arachnida	Pearson Correlation	-.386	.078	.062	-.008	.392	-.511	-.352	-.284	.521
p__SAR	Pearson Correlation	-.355	.300	.450	.032	.328	-.610	-.281	-.203	.003
c__Trebouxiophyceae	Pearson Correlation	.261	-.284	-.130	-.356	-.315	.045	.559	.871*	-.687
Wickerhamomyces-Candida_clade	Pearson Correlation	.020	-.858*	-.667	-.199	-.020	.536	.749	.757	.156
p__Mucoromycota	Pearson Correlation	-.375	.119	.077	-.003	.380	-.515	-.375	-.261	.471
Talaromyces	Pearson Correlation	-.222	-.526	-.083	-.172	.229	-.289	.270	-.386	.734
c__Novel_Clade_Gran-5	Pearson Correlation	.059	-.582	-.586	-.154	-.067	.509	.559	.901*	-.130
o__Hypocreales	Pearson Correlation	-.320	.167	.167	-.072	.311	-.622	-.345	-.213	.313
Heteromita	Pearson Correlation	-.084	-.624	-.198	-.104	.101	.073	.434	-.290	.626
Clavispora-Candida_clade	Pearson Correlation	.129	-.542	-.491	-.235	-.154	.407	.617	.981**	-.328
Nakaseomyces-Candida_clade	Pearson Correlation	-.268	.194	.236	-.122	.248	-.680	-.299	-.162	.171
f__Chaetomiaceae	Pearson Correlation	-.006	-.446	-.297	-.017	-.021	.557	.627	.932**	-.451
c__Ulvophyceae	Pearson Correlation	.773	.530	-.391	-.414	-.738	-.309	-.723	-.209	-.018
Knufia	Pearson Correlation	.045	-.705	-.633	-.176	-.051	.533	.648	.867*	-.021

Vermamoeba	Pearson Correlation	-.637	.319	.719	.957**	.648	.656	-.018	-.409	-.193
Cyberlindnera-Candida_clade	Pearson Correlation	.037	.290	.631	.038	-.092	-.188	.173	.096	-.783
p__Cryptomycota	Pearson Correlation	-.026	-.951**	-.512	-.205	.028	.401	.825*	.391	.371
o__Hypocreales	Pearson Correlation	-.377	.117	.074	-.001	.381	-.511	-.375	-.263	.476
k__Amoebozoa	Pearson Correlation	-.376	.118	.076	-.003	.380	-.513	-.375	-.262	.473
Trichoderma	Pearson Correlation	.251	.182	.383	-.284	-.309	-.407	.175	.233	-.711
Arthrinium	Pearson Correlation	-.395	.016	.041	-.022	.403	-.512	-.312	-.319	.592
o__Polypodiales	Pearson Correlation	.190	.016	.339	-.334	-.246	-.464	.271	.165	-.517
p__Blastocladiomycota	Pearson Correlation	-.122	-.708	-.624	-.186	.120	.327	.525	.750	.225
o__Capnodiales	Pearson Correlation	-.117	-.720	-.591	-.237	.107	.248	.560	.779	.184
d(domain)__Eukaryota	Pearson Correlation	-.583	-.380	-.024	.215	.564	.208	.364	.474	.091

Appendix Table 9. Pearson correlation coefficients between microeukaryotic communities in Gisenyi hot spring (GHS) mats and environmental parameters

Microeukaryotic genera		Temperature	pH	DO	TDS	EC	ORP	TP	TN	NO3-N	NH4-N
Saccharomyces	Pearson Correlation	-.217	-.268	.[b]	.362	-.030	-.069	-.482	.322	.642	-.044
Candida-Lodderomyces_clade	Pearson Correlation	-.177	-.208	.[b]	.376	-.054	-.055	-.497	.306	.705	-.045
Aspergillus	Pearson Correlation	.454	.651	.[b]	-.415	.238	.320	-.205	-.634	.457	-.456
Andalucia	Pearson Correlation	-.187	.976**	.[b]	-.134	-.178	-.377	.344	-.390	.452	-.008
k__Discoba	Pearson Correlation	-.176	-.207	.[b]	.376	-.054	-.055	-.497	.305	.705	-.045
o__Rhabditida	Pearson Correlation	.842*	-.208	.[b]	-.479	.583	.668	-.488	-.304	-.457	-.555
Naegleria	Pearson Correlation	-.168	-.202	.[b]	.379	-.065	-.046	-.488	.300	.710	-.036
c__Tubulinea	Pearson Correlation	-.366	-.241	.[b]	-.121	.512	-.404	-.403	.236	-.195	-.397
c__Bacillariophyceae	Pearson Correlation	.786	-.280	.[b]	-.469	.672	.605	-.602	-.230	-.463	-.626
c__Chrysophyceae	Pearson Correlation	-.330	-.200	.[b]	-.188	.516	-.390	-.309	.178	-.321	-.385
Cladosporium	Pearson Correlation	.698	.589	.[b]	-.743	.260	.643	.197	-0.954	-.097	-.389
f__Plectosphaerellaceae	Pearson Correlation	-.075	.982**	.[b]	-.266	-.019	-.252	.196	-.515	.506	-.190
Boeremia	Pearson Correlation	-.088	.070	.[b]	.009	-.327	.198	.395	-.250	.248	.270
Aplanochytrium	Pearson Correlation	.702	-.264	.[b]	-.265	.400	.529	-.415	-.104	-.414	-.359
c__Tubulinea	Pearson Correlation	-.386	-.266	.[b]	-.067	.497	-.406	-.467	.275	-.094	-.398
Polymyxa	Pearson Correlation	-.178	-.206	.[b]	.391	-.073	-.055	-.487	.312	.713	-.027

c__Enoplea	Pearson Correlation	-.557	-.188	.[b]	.803	-.697	-.544	.293	.740	.185	.742
p__Mucoromycota	Pearson Correlation	-.326	-.195	.[b]	-.196	.515	-.388	-.297	.171	-.335	-.383
f__Euglyphida	Pearson Correlation	-.224	-.228	.[b]	.432	-.091	-.101	-.491	.364	.721	-.004
Anomoeoneis	Pearson Correlation	-.572	-.196	.[b]	.801	-.683	-.562	.284	.751	.173	.733
o__Pleosporales	Pearson Correlation	-.046	-.400	.[b]	.112	-.400	.318	.515	-.021	-.217	.487
Cryptomonas	Pearson Correlation	.842*	-.208	.[b]	-.479	.583	.668	-.488	-.304	-.457	-.555
c__Bacillariophyceae	Pearson Correlation	-.549	-.054	.[b]	.765	-.701	-.569	.323	.669	.237	.719
f__Chaetomiaceae	Pearson Correlation	-.187	.933**	.[b]	-.318	.138	-.384	.094	-.459	.430	-.312
Nitzschia	Pearson Correlation	-.560	-.191	.[b]	.810	-.700	-.546	.287	.746	.194	.743
Pottia	Pearson Correlation	.842*	-.207	.[b]	-.479	.584	.668	-.488	-.304	-.457	-.555
o__Echinamoebida	Pearson Correlation	.643	.564	.[b]	-.563	.397	.348	-.160	-.608	-.084	-.513
o__Monhysterida	Pearson Correlation	.136	-.259	.[b]	-.065	-.397	.445	.708	-.224	-.435	.491
Gregarina	Pearson Correlation	-.168	-.202	.[b]	.379	-.065	-.046	-.488	.300	.710	-.036
p__Cryptomycota	Pearson Correlation	-.613	-.033	.[b]	.802	-.789	-.592	.418	.665	.278	.801
k__Amoebozoa	Pearson Correlation	-.383	-.313	.[b]	.164	.333	-.329	-.623	.375	.330	-.317
Cryptosporidium	Pearson Correlation	.979**	-.040	.[b]	-.646	.496	.871*	-.229	-.602	-.486	-.499
BOLA868	Pearson Correlation	.857*	-.253	.[b]	-.466	.565	.707	-.482	-.298	-.462	-.532
Curvularia	Pearson Correlation	-.088	.995**	.[b]	-.287	-.017	-.281	.246	-.518	.431	-.177

Echinamoeba	Pearson Correlation	.041	-.335	.[b]	.147	-.298	.376	.180	-.033	.155	.294
Arthrinium	Pearson Correlation	-.031	.999**	.[b]	-.348	.001	-.226	.291	-.576	.346	-.185
o__Rhabdocoela	Pearson Correlation	.842*	-.208	.[b]	-.479	.583	.668	-.488	-.304	-.457	-.555
f__Cordycipitaceae	Pearson Correlation	.900*	-.290	.[b]	-.496	.531	.794	-.400	-.348	-.525	-.487
c__Vampyrellidae	Pearson Correlation	-.630	-.245	.[b]	.886*	-.697	-.599	.194	.832*	.295	.735
uncl_d__Eukaryota	Pearson Correlation	.201	-.578	.[b]	-.221	.119	.485	.135	-.126	-.522	.028
Poteriospumella	Pearson Correlation	-.031	1.000**	.[b]	-.315	-.034	-.223	.297	-.559	.375	-.156