MICROBIAL ECOLOGY AND NUTRIENT DYNAMICS OF THE RAJANG RIVER

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Abstract

The Rajang River is a peat-draining river in Sarawak, Malaysia of which 50% of its 5,500 km² peatlands have been exploited and converted into plantations. However, there has been no comprehensive research on the nutrient dynamics and the microbial diversity in this region. Hence, this thesis aims to study the nutrient dynamics and the microbial community structure (bacteria, phytoplankton and picoplankton) that occur spatially as well as seasonally and integrate these two components along ~300 km of the Rajang River to South China Sea continuum. Amplicon sequencing of 16S rRNA genes via Illumina Miseq was utilized for profiling microbial communities while CHEMTAX was utilized for the prediction of phytoplankton community structure coupled with physico-chemical conditions using a distance-based linear model (distLM). Nutrients analyses were executed via a SKALAR San^{plus} continuous flow analyser, and picoplankton abundance was determined via flow cytometry. The findings in this study can be classified under three recurring themes: (1) Changes according to spatial variation. DIP concentrations varied along the salinity gradient whereby DIP and DOP exhibited non-conservative behaviour, with the DIP subjected to 57.78% removal and DOP 44.07% addition towards the South China Sea. The microbial communities (bacteria and phytoplankton) showed distinct patterns linked to changes in salinity and other biogeochemical parameters. However, PICRUSt predictions showed minor variations. Alpha diversity indices for bacteria indicated that the diversity was higher upstream. (2) Changes according to seasonal variation. Both DIP and DOP may have supported phytoplankton biomass; Spearman correlations show possible switch in preference for DOP vs. DIP depending on concentrations due to seasonality. Different seasons showed changes in NO3N:DIP ratios which influenced the phytoplankton biomass. Furthermore, shifts in bacterial community composition and particle association indicate the influence of seasonality. Lastly, resource availability as a result of seasonal changes led to changes in the distribution of phytoplankton size classes; (3) Possible anthropogenic influences. The sources of P were likely anthropogenic in nature based on dSi:P ratios, whereby oil palm plantations increased the richness but decreased the diversity of microbial populations. The presence of CFB-group bacteria and Cryptophytes should be cause for concern as indicators for eutrophication. Overall, the findings of this study improve the understanding of nutrient dynamics, P budgets, and the microbial communities of the Rajang River-South China Sea continuum to inform public administration and support future research.

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Declaration

I, Edwin Sia Sien Aun, hereby declare that no portion of the work referred to in this thesis:

1. contains no material that has been accepted for the award of any other degree or diploma;

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(Edwin Sia Sien Aun)

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Abbreviations

⁰∕₀	Percentage
°C	Degree Celsius
μm	Micrometre
ACE	Australian Centre for Ecogenomics
ANOSIM	Analysis of similarities
ANOVA	Analysis of variance
С	Carbon
CH ₄	Methane
Chl a	Chlorophyll a
CO ₂	Carbon Dioxide
CTD	Conductivity Temperature and Depth
dbRDA	Distance-based Redundancy Analysis
DIN	Dissolved Inorganic Nitrogen
DIP	Dissolved Inorganic Phosphorus
DistLM	Distance-based linear model
DO $L^{-1} d^{-1}$	Litre of dissolved oxygen per day
DOC	Dissolved Organic Carbon
DON	Dissolved Organic Nitrogen
DOP	Dissolved Organic Phosphate
dSi	Dissolved Silicate
GDP	Gross Domestic Product
GIS	Geographic Information System
Gt	Giga-ton
HPLC	High performance liquid chromatography
HUMAnN2	HMP Unified Metabolic Analysis Network
km ²	Square kilometre
LOICZ	Land-Ocean Interactions in the Coastal Zone
m^2	Square metre
m ³	Cubic metre
$m^{3}s^{-1}$	Cubic metre per second
mbar	Millibar
$mg L^1$	Milligram per litre
mL	Millilitre
mm	Millimetre
mM	Millimolar

Ν	Nitrogen
$\mathrm{NH_4}^+$	Ammonium
nm	Nanometre
NMDS	Non-metric multidimensional scaling
NO ₂ ⁻	Nitrite
NO ₃ -	Nitrate
OTU	Operational Taxonomic Unit
Р	Phosphorus
Peuk	Pico-eukaryotes
Pg	Peta-gram
PICRUSt	Phylogenetic Investigation of Communities by
	Reconstruction of Unobserved States
POC	Particulate Organic Carbon
РР	Particulate Phosphate
ppm	Parts per million
Pro	Prochlorococcus
PSU	Practical Salinity Unit
rpm	Rotations per minute
Sal	Salinity
SE	Standard Error
Si	Silicate
SOP	Standard Operating Protocol
SPM	Suspended Particulate Matter
Syn	Synechococcus
t DIP mth ⁻¹	Tonnes of Dissolved Inorganic Phosphate per
	month
TDN	Total Dissolved Nitrogen
TDP	Total Dissolved Phosphate
v/v	Volume per volume

Publications

Edwin Sien Aun SIA, Zhuoyi ZHU, Jing ZHANG, Wee CHEAH, Gonzalo CARRASCO, Aazani MUJAHID, Moritz MUELLER 'Biogeographical Distribution of Microbial Communities Along the Rajang River-Sea Continuum' (in preparation for submission to Biogeosciences Special Issue: *Biogeochemical processes in highly dynamic peat-draining rivers and estuaries in Borneo*)

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Zhuoyi ZHU, Joanne OAKES, Bradley EYRE, Youyou HAO, Edwin Sien Aun SIA, Shan JIANG, Moritz MUELLER, Jing ZHANG, 'The non-conservative distribution pattern of organic matter composition in Rajang, a tropical river with peatland in its estuary', *Biogeosciences*, (manuscript submitted: bg-2019-157)

Ying WU, Kun ZHU, Jing ZHANG, Moritz MUELLER, Shan JIANG, Aazani MUJAHID and Edwin Sien Aun SIA, 'Distribution and degradation of terrestrial organic matter in the sediments of peatdraining rivers, Sarawak, Malaysian Borneo', *Biogeosciences*, (manuscript submitted: bg-2019-94)

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Oral Presentations

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Poster Presentations

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Chapter 1 Introduction

1.1 Thesis Scope and Objectives

This thesis aims to study the spatio-temporal microbial diversity and nutrient dynamics of the longest river in Malaysia, the Rajang River. The Rajang is studied in a river-to-sea continuum approach, with the lotic water column the focus of this thesis. Besides the changes from freshwater to marine water, the Rajang is also characterized by the existence of peat and mineral soils as well as relatively pristine and anthropogenic areas, offering an opportunity to assess impact of changes in land use on microbial diversity and nutrient dynamics as well.

While it cannot be denied that microbes in a broad definition influence the biogeochemistry in riverto-sea continuums, the physico-chemical conditions should be studied as well in order to assess their role in influencing the microbial diversity and/or biogeography. To address microbial community structure, bacterial, phytoplankton and picoplankton communities and their relationship with nutrients will be discussed. Hence, this thesis has two broad objectives, which are 1) to examine the spatial and seasonal nutrient dynamics of the river-to-sea continuum, and 2) understand the microbial community structure and its functional potential as a whole. This would:

- Address the need for understanding the composition of the microbial community within a dynamic environment (spatial heterogeneity and/or homogeneity, biogeographical differences, edaphic niches as well as seasonal fluctuations) and the changes that come with it
- Determine the fate and fluctuation of nutrients as part of the dynamic changes occuring along the river-to-sea continuum
- Highlight the need for understanding and integrating nutrient dynamics with the microbial community of river-to-sea continuums, particularly peat-related riverine continuums, in order to further elucidate their biogeochemical roles within such dynamic environments

1.2 Thesis outline

Chapter 1 provides the relevant context highlighting knowledge gaps related to the scope of the thesis. Chapter 2 provides an overview of the study site and the methods utilized in the proceeding chapters. In each of the following studies, the underlying hypotheses and rationale are provided in the introduction and end with a concluding remark and proposed future work.

Chapter 3 details the study of the dissolved phosphorus with associated nutrients in relation to phytoplankton biomass along the Rajang River-South China Sea continuum across seasonal changes (Objective 1). Understanding the nutrient dynamics aids in Chapters 4 and 5.

Chapter 4 discusses the biogeographical distribution of the bacterial communities and Chapter 5 the biogeographical distribution of phytoplankton along the Rajang River-South China Sea continuum (Objective 2).

The findings of these studies are then synthesized as a concluding summary in Chapter 6, which also addresses the existing knowledge gap and provides recommendations for future research directions.

1.3 Introduction

1.3.1 Brief Introduction to Microbial Ecology

Bacterial communities in natural environments are highly dynamic and extraordinarily diverse (Crump et al. 2009). According to Whitman et al. (1998), there are about 10³⁰ organisms present in the vast microbial world on Earth; they may only be observed through relatively small proportions at discrete points across time and space (Sloan et al. 2007). As put into perspective by Curtis and Sloan (2004), there are 10^9 times more bacteria existing on Earth as compared to the number of stars in the Universe. Furthermore, it was theorized for years (Everything is everywhere theory) that microorganisms are ubiquitous globally (Baas Becking 1934), but studies have begun to show restricted, distinctive microbial populations. Current knowledge now points to evidence that time and spatial environmental gradients cause shifts in natural communities of bacterioplankton (Fenchel 2003; Crump and Hobbie 2005). With the advent of high-throughput sequencing (Quince, Curtis and Sloan 2008) and its recent improvements (Faust et al. 2015), there has been a rise in longitudinal studies which are able to document the variation of microbiological communities from a vast range of environments. New frontier environments explored in recent times include deep subseafloor extremophiles (Morono et al. 2011; Nunoura et al. 2013), high altitude snow (Chuvochina et al. 2011) and even space on the International Space Station (Ichijo et al. 2013). While these are exciting times to explore new frontiers with the new advances in technology, however, there is still a lack of awareness of the importance of peatlands and of the microbial ecology of tropical peat-draining rivers.

1.3.1.1 Importance of Microorganisms

As microorganisms are the most abundant organisms in aquatic ecosystems, they play a major role in the biogeochemistry and ecosystem productivity of aquatic bodies (Labatte et al. 2016). According to Schlesinger and Bernhardt (2013), microorganisms and their metabolic activities predominantly govern ecosystem processes to a large extent. The global biogeochemical cycles of major Earth elements such as carbon, nitrogen, sulfur, phosphorus and a host of metals (Falkowski and Raven 2013) are driven by microbial communities (Battin et al. 2003; Cardinale et al. 2011; Alsterberg et al. 2017). For example, in a pelagic food web (**Fig. 1.1**), the major path of organic matter flux is dependent on the microbial loop within it (Azam 1998).



Fig. 1.1: The microbial loop (Azam 1998)

Biota in the streams rely on allochthonous particulate organic sources as critical energy supply which would subsequently influence the metabolic activity of the ecosystem as well as energy flow within the aquatic food web (Tank et al. 2010). Conrad (1996) showed that greenhouse gases such as CO_2 and CH₄ are released into the environment due to the decomposition of organic material via microbes, which thus play a critical role in the global carbon (C) cycle. Furthermore, nitrogen transformations by microorganisms are reliant on carbon supplies that are able to undergo oxidation (Bernhardt and Likens 2002). Within the N cycle, denitrification by microbial denitrifiers is one of the major known biological process that are involved in the removal of reactive N species (Castellano-Hinojosa et al. 2017). As put forth by Seitzinger (1994), denitrification rates are functionally coupled to the carbon cycle in large due to the dependence on organic carbon availability. Moreover, microbial populations play an important role in the biogeochemical cycling of P due to their role in organic phosphorus mineralization, which assists the release of bioavailable phosphate and supplies phosphorus to other oganisms (LeBrun et al. 2018). This is particularly important for cyanobacteria, which are responsible for fixing atmospheric nitrogen and utilize phosphorus (Andersson et al. 2015); nitrogen fixation in turn promotes primary productivity (Worden et al. 2004; Jardillier et al. 2010). Rieck et al. (2015) further stated that major environmental drivers for community structure and activities of aquatic organisms are the availability of nutrients, pH, salinity and temperature. Therefore, the community composition of aquatic organisms and its metabolic activities are shaped by the spatial and temporal gradients in environmental factors, which naturally shapes the biogeochemistry of the aquatic ecosystems as well (Rieck et al. 2015). Hence, it is essential to cross-examine the various components in zones of "coalescence" or mixing, which are: 1) mineral soil freshwater to peat freshwater coalescence 2) peat freshwater to peat brackish waters 3) peat-brackish waters to marine waters. Furthermore, in addition to that, serial discontinuities [WHY serial?] such as anthropogenic coalescence events should also be taken into consideration (Mansour et al. 2017). These are important in order to improve and enhance predictions regarding how communities would change over space and time as community composition is linked with ecosystem function. For instance, according to Reed and Martiny (2013), bacterial community composition in estuaries are intrinsically linked with its ecosystem functioning such as nitrification, enzymatic activities, CH₄ flux as well as CO₂ production.

1.3.1.2 Anthropogenic Threats to Microbial Biogeochemistry

Human activities are increasingly affecting global biogeochemical cycles of carbon and other nutrients (Griggs et al. 2013). However, the anthropogenic impacts on the function of aquatic microbial assemblages and ecology are often multi-faceted and largely undefined (Labatte et al. 2016). Furthermore, Evans and Wallenstein (2011) and Yavitt et al. (2011) stated that global warming, anthropogenic enrichments and alterations in water availability have received particular attention due to the effects on microbes. The disruption of microbial activities by environmental disturbances such as changes in direct physico-chemical properties and nutrient availability leads to the alteration of the rate of their processes (Schimel, Balser and Wallenstein 2007). Anthropogenic bursts of microbial carbon and nitrogen mineralization were attributed to positive priming effects that were due to the enhanced turnover of microbial biomass or induced cell lysis resulting in the increase of labile substrates (Schimel and Clein 1996; Herrmann and Witter 2002). Furthermore, there has been a lack of attention to the role of organic C in the regulation of P as mediated by the organophosphohydrolytic microbial enzymes (Anderson 2018). This is truer for riverine cycling of phosphate via microorganisms. Anderson (et al. 2018) demonstrated that biogeochemical changes of C and P link microbial communities to a complex network of interactions in organic-rich Arctic and mineral rich temperate soils. With this in mind, it is also essential to understand the interaction and activity between microorganisms and primary producers (Treseder et al. 2012). Thus, microbes can potentially accentuate or mitigate climate change (Strengborn et al. 2002; Wagner and Liebner 2009) due to the alteration of decomposition rates of carbon (Treseder et al. 2012).

1.3.1.3 Role of microorganisms in Peat-Draining Environments

There is a lack of current literature regarding microbial community structure in tropical, lotic environments such as South East Asia, particularly in peat-draining rivers. According to Heino (2011), interactions between local habitat conditions and regional-scale factors such as dendritic network energy transport from upstream to downstream, adjacent riparian ecosystem interactions drives the local aquatic communities. Such drivers are, in turn, influenced by the dynamicity of river discharge that is temporal in nature (Poff et al. 1997). While microorganisms are indeed essential in rivers, i.e.

they drive biogeochemical cycles (Zeglin 2015), interact with the abiotic component as well as macroorganisms, propel biogeochemical cycles, and are predominantly involved in decomposition and the degradation of pollutants. However, recent studies do not include peat-draining rivers in the consideration of river-to-sea continuum microbial composition. Hence, it is important to consider the "peat-draining" component while examining microbial community composition of the boundaries especially where "zones" of mixing occur. This then leads to the question: What happens when the microbial communities encounter such zones of mixing and how do the microbial communities adapt/evolve in terms of their composition as well as their functional role when met with physicochemical changes. This will be further explored in **Chapter 4**.

1.3.2 Tropical Peat Swamps

1.3.2.1 General Features of Peat



Fig. 1.2: Formation of Tropical Peat Swamps (UNDP 2006)

According to Shotyk (1988), tropical peat swamp forests are consistently waterlogged environments where organic matter consisting of organic carbon, nitrogen, phosphorus and sulphur derived from plant debris accumulates due it being predominantly anaerobic. As shown in **Fig 1.2** the accumulation of carbon in peat swamps is due to the slow degradation of plant biomass caused by its oligotrophic nature, low pH and high amount of tannins which inhibit microbial activity (Kanokratana et al. 2011). Such habitats play a major role in conserving biodiversity as well as in carbon emissions reduction by sequestering carbon (Harrison 2013).



Fig. 1.3: Structure of the acrotelm and catotelm (Thornton 2019)

An acrotelm, as defined by Ingram's definition (Holden and Burt 2003) is rich in peat-forming aerobic bacteria and other microorganisms, has a live matrix of growing plant material, has high hydraulic conductivity, fluctuating water content and variable water table; the base of the acrotelm is where the lowest water table depth is (**Fig 1.3**). On the other hand, the catotelm has fixed water content, has anaerobic microorganisms instead of peat-forming aerobic ones, and has limited hydraulic conductivity. Furthermore, as put forth by Holden and Burt (2003), most nutrient transfer and the production of runoff will occur at, close to, or within the peat surface, implied by the acrotelm-catotelm model.



Fig. 1.4: Global distribution of peat (%) (Pravettoni 2009)

The global peat carbon pool is estimated to be between 489 Gt to 622 Gt of carbon (C) (Page, Rieley and Banks 2011; Dargie et al. 2017). As put forth by Kiew et al. (2018), the fundamental characteristics that differentiate boreal peat and tropical peat ecosystems are the existence of waterlogged woody peat together with the occurence of tropical rainforests. The diminshed carbon dioxide (CO_2) under anoxic conditions caused by the high groundwater levels (GWL), as well as increased biomass productivity, contribute to the efficacy of the tropical peat swamp forests as efficient carbon (C) sinks. Furthermore, the tropical peat carbon pool comprises 17 - 19% of the estimated global peat carbon pool. However, within this one-fifth of the carbon pool, South East Asia constitute 88.6 Gt of carbon accumulation or 15 - 19% of the global carbon peat pool (Immirzi, Maltby and Clymo 1992; Page et al. 2011), indicating that South East Asia has the highest carbon densities globally, signifying its importance in global C cycles as well as highlighting the fundamental need to incorporate South East Asian tropical peat carbon in the assessment of global C stocks. As compared to tropical rainforests, peatlands cover less than half of the total area but contain 3.5 times more carbon (IGBP 1999). As shown in Fig. 1.5, the accumulation of peat in Sarawak within South East Asia cannot be ignored (further explained in Chapter 2.1.1), as these peatlands are subjected to multiple anthropogenic influences and land use changes.



Fig. 1.5: Location of Sarawak, Malaysia in Borneo (Inset). The map shows all peat deposits that are greater than 1m thick as well as the major peat forming regions. The drainage basin as well as the drainage from proximal hills are shown. (Staub, Among and Gastaldo 2000).

1.3.2.2 Anthropogenic influences and Land Use Change on Peatlands and Rivers



Fig. 1.6: Threats to peatlands (IUCN 2019)

Currently, literature points to anthropogenic activities (namely: draining or mining) which contribute to 10% global peatlands being converted from long-term sinks into sources by three main pathways (Joosten 2019, see **Fig. 1.6**). These three pathways are: 1) combustion of mined peat that leads to carbon monoxide and methane emissions, 2) leaching of dissolved carbon and 3) carbon dioxide emission from microbial oxidation of peat (IPCC 2014). Moreover, peatlands can be altered into sources from sinks by draining which degrades peatlands by releasing a significant amount of N₂O (Leifeld 2013). Due to the balance that exists between the peat, vegetation and hydrological aspects of tropical peat swamps, they are much more susceptible to the synergistic effects of multiple human disturbances (Posa et al. 2011). Among the major threats to tropical peat swamps are logging, land conversion (land-use change) and fires. The economic value of selected timber species in peat swamp forests, such as the ramin trees (*Gonystylus bancanus*), has led to intensive exploitation of these areas in South East Asia. According to Rashid and Ibrahim (1994), intensive extraction which led to greater damage of the residual forests was due to the mechanization of extraction methods. For example, illegal logging activities often include building canals to floating out logs; this has caused hydrological conditions of the tropical peat swamps to be altered. Such adulterations have caused the decrease in flora and fauna; the orang utan densities have decreased by 21 - 22% versus pristine peat swamp forests (Felton et al. 2003; Johnson et al. 2005). As the peat substances are extremely flammable when it is dry, tropical peat swamps are particularly vulnerable to destruction by fire (Langner, Miettinen and Siegert 2007; Langner and Siegert 2009). Langner et al. (2007) reported that 77% and 55% of forest fires in Borneo in 2002 and 2005, respectively were peat forests. As extreme droughts which are brought upon by strong El Niño events in South East Asia, human disturbances further exacerbate the vulnerability of peat swamps to burning (Page et al. 2009). Among the tree crops that were successfully grown on peat soils are the *Elaeis guineensis* (oil palm) and *Acacia* crassicarpa (pulp trees). Due to the physical and chemical properties of peat, agricultural activities on peat soils require large-scale land conversion via drainage, clearing of forests, application of fertilizers, increase pH with liming and enhanced microbial activities (Posa et al. 2011). The drainage of peat causes a chain-effect which first causes enhanced microbial oxidation that decomposes organic matter at a faster rate. This in turn causes subsidence, which means the peat surface is lowered (Wösten, Ismail and De Wijk 1997). Next, the greenhouse gases such as methane and carbon dioxide are released from the oxidation of organic matter which contributes significantly to global warming (van der Werf et al. 2009; Couwenberg, Dommain and Joosten. 2009).

According to Cobb et al. (2017), in the development and preservation of tropical peat, water is integral whereby the overall hydrological characteristics of peatland are regulated by rainfall and surface topography. In the Southeast Asian region, peatlands are one of the extensive land types which have developed mainly in coastal lowland plains in between large-scale rivers (peat portal 2016). According to Wetlands International (2010), of the 23% of peatlands in Malaysia, Sarawak, the eastern state of Malaysia consists of 17%. Out of the 17%, only 1.5% of the Sarawakian peatlands remains entirely pristine. The geophysiology, climatology and the socioeconomic activities of the Rajang River is further discussed in detail in **Chapter 2.1**. Apart from being a powerhouse in storing carbon, when compared with other peatland types globally, tropical peat swamp forests have the highest diversity of flora and fauna whereby a substantial precentage of birds and mammals were recorded as endangered, vulnerable or threatened under the IUCN Red list status (Posa et al. 2011).

1.3.3 Nutrient Budgets associated with Rivers to Sea continuum

Rivers were often thought to be just passive channels in the global and regional determination of carbon (C) and weathering products; later the importance of rivers in regulating the transfer of nutrients from land to coastal areas was recognised (Smith and Holibaugh 1993). According to Aufdenkampe et al. (2011), an integral part of characterizing the riverine biogeochemical function and land use change is understanding the components (coupling of landscape components, transport and reactivity). This is in line with the River Continuum concept (Vannote et al. 1980), whereby fluvial geomorphic processes largely govern the organic matter transport and storage, energy input, and use by the corresponding functional feeding groups. It is hence important to primarily understand the physical processes that govern a river in order to further understand and predict the patterns of community structures and their functions, metabolic strategies and growth patterns. Moreover, as put forth by Aufdenkampe et al. (2011), outgassing from inland surface waters, based on published estimates, ranged from 0.75 - 1.4 Pg C yr⁻¹ justifying its importance when compared with net estimates of C accumulation in both oceans and even on continents (both at 2.2 Pg C yr⁻¹).

According to Cotrim da Cunha et al. (2007), rivers are important drivers in altering the hydrography and consequently the biogeochemistry of oceans. In the past, global estimates of the impact of river-transported nutrients and carbon on the coastal and global ocean were faulty due to the lack of estimates of of river nutrients discharge on a global scale. One concern is that the coastal ocean biogeochemistry is affected by fluvial systems which input nutrients (Meybeck 1998). As put forth by Longhurst (2000), this in turn affects the seawater composition on a geological timescale. In a natural environment, leaching and eroding processes in the catchments leads to the delivery of nutrients that are being drained to the coastal oceans (Smith et al. 2003). The main components which are identified and studied (which will be further discussed in the thesis) are based on the dominant components which influence freshwater ecosystems (**Fig. 1.7**).



Fig. 1.7: Conceptual Model of the dominant components which influence freshwater ecosystems (Ecological Society of America 2003)

According to Seitzinger et al. (2010), the concentrations of N and P in the majority of rivers worldwide have at least doubled due to anthropogenic inputs. Moreover, as put forth by Baron et al. (2003), watersheds or catchments are closely correlated to the function and structure of freshwater ecosystems. The water body which passes through the landscapes links it to three dimensions: 1) from upstream to downstream, 2) tributaries which connect to floodplains as well as riparian wetlands, and 3) the hyporheic zones which link surface waters to groundwaters. Therefore, such systems are immensely determined by terrestrial-based activities, including anthropogenic activities, whereby land-generated materials across the landscape would ultimately arrive in water bodies (lakes, rivers and other freshwater ecosystems). Moreover, as put forth by Vinita et al. (2015), the net transport of nutrients from upstream of the estuarine (headwaters) to the ocean (land-ocean fluxes) is essential in the assessment of environmental impacts. This is therefore a critical step, as it would allow for the determination of the dynamics between nutrients and other immediate components - such as plankton dynamics (Sooria et al. 2015) as well as microbial community composition and function - that thrive from the nutrient fluxes.

TROPICAL



Fig. 1.8: Conceptual Model of Land-Ocean Interactions in the Coastal Zone (LOICZ) in Tropical Coastal Ecosystems (LOICZ 2019)

The coastal zone represents a long narrow interface between the land and ocean which is a naturally dynamic zone and is subject to increasing human use (LOICZ 2005). As shown in **Fig. 1.8**, the LOICZ conceptual model includes numerous factors that are involved within each section as it moves from land to ocean. For example, the increase in intensity and frequency of tropical storms as well as more irregular monsoonal rainfall results in pulsed run-off in which the communities within the coastal ecosystem would respond in the positive or negative feedback loop. Ergo, while the conceptual model does not equally represent all tropical coastal ecosystems worldwide, it is a representation of the coastal ecosystem; the spatial and temporal heterogeneity of such coastal zones are extensive (Vafeidis et al. 2004). This would in turn cause methodological complexities in developing global perspectives on the scale and roles of the coastal ecosystem in Earth System functioning. For instance, within Rajang coastal ecosystem, several other factors come into play, such as the presence of peat domes (refer to **Chapter 2.1.1**) where there are interactions between the peat riparian region and the lotic water column of the Rajang river.

1.3.3.1 Phosphorus



Fig. 1.9: The global phosphorus cycle (Ruttenberg 2003)

1.3.3.1.1 Phosphorus in Freshwater Ecosystems

As put forth by Ruttenberg (2003), the major conduits of phosphorus transfer into lakes and oceans are rivers and streams (**Fig 1.9**). As such, rivers and streams function as an ecosystem whereby the biogeochemical processes that occur during the aforementioned transport will modify the P form en route. That in turn influences the biological availability and chemical reactivity once it reaches the interface of the receiving water bodies (Reynolds and Davies, 2001; Withers & Jarvie 2008). According to Correll (1998), the availability of essential nutrients would become limited for the growth of plant and bacteria in an aquatic system. One of the important drivers for biological activity in flowing waters is the supply of phosphorus (P) in terms of its flux as well as concentration (Withers & Jarvie et al. 2008). The management of the aforementioned nutrient is hence crucial in order to avert the impacts of eutrophication that are correlated with the upsurge in agricultural activity as well as urbanisation. Furthermore, the occurrence and severity of eutrophication are dependent on a diverse set of factors other than P, such as nitrogen (N), silica (Si), carbon (C) and other physic-chemical parameters such as flow regime, water temperature etc. (Soballe and Kimmel, 1987; Dodds 2007).

1.3.3.1.2 Phosphorus in Coastal Zones

Valiela (2015) states that phosphorus can be found in marine environments as dissolved inorganic phosphorus (DIP), dissolved organic phosphorus (DOP) and particulate phosphorus (in this thesis mostly DIP and DOP will be discussed). The low phosphate concentrations in surface waters are typical scenarios due to the uptake by primary producers and bacteria. The residence time of DOP is limited to only a few hours (Van Wazer 1973) due to the rapid lysis process, whereas abiotic hydrolysis occurs at a rate that is $10^3 - 10^4$ times slower than microbial activity. Furthermore, the hydrolysis of esters of DOP as well as viral lysis will contribute to the formation of DIP. According to Smith et al. (2003), 70% of the total material loads (i.e. Dissolved Inorganic Phosphorus) to the coastal zones originate from regions with low to intermediate area-specific yields. However, parts of Europe, sub-tropical, island-dominated and particularly, tropical regions are medium to high yields. At a global scale, these areas are which are of medium to high yields come from either small or small-medium river influenced or ocean-dominated coasts. This indicates that at a global scale, a large proportion of the aforementioned rivers dominate coastal processes as compared to continental-scale influences of large rivers (Solomon et al. 2007).

1.3.3.2 Nitrogen

1.3.1.2.1 Nitrogen Cycling in Freshwater Ecosystems

While Carpenter (2008) and Schindler et al. (2008) state that the main nutrient limiting primary productivity in lakes is phosphorus (P), nitrogen is often co-limiting or limiting in many freshwater ecosystems (Elser et al. 2007, Lewis et al. 2011). However, there is a need to highlight that phosphorus management may lead to elevated dissolved inorganic nitrogen (DIN) via the decreased denitrification process (Finlay et al. 2013).



Fig. 1.10: Simplified Nitrogen cycle (Valiela 2015)

The nitrogen cycle has three phases which are sedimentary, aquatic and gaseous phases (**Fig 1.10**). In this thesis, the form of N that will be discussed is the Dissolved Inorganic Nitrogen (DIN) that is formed from Nitrate (NO₃), Nitrite (NO₂⁻) and Ammonium (NH₄⁺). In aerobic environments, the nitrogen form that is the most oxidized form, NO₃⁻ is being taken up by microorganisms such as bacteria, phytoplankton and plants. Usually, the concentration of NO₂⁻ is low in the water column. Heterotrophs decompose the large quantities of organic-N that are released from dead animals to form NH₄⁺. Other sources of NH₄⁺ included zooplankton excretion, which also releases Dissolved organic nitrate (DON). At high temperatures, NH₄⁺ is abundant in shallow productive regions due to the high rates of microbial degradation caused by the high temperatures. Due to the interaction of nitrogen with the gaseous phase, a unique pathway that N undergoes is its fixation (N₂) into organic-N forms from the atmosphere, which acts as a new source of nitrogen for the marine ecosystem. This process is inhibited by high NH₄⁺ concentrations.

1.3.3.2.2 Nitrogen in Coastal Zones

According to Nixon (1986), one of the most highly fertilized ecosystems (where N is concerned) are coastal zones. Hence, there is a primary concern about the loading of nitrogen to coastal watersheds due to primary productivity being limited by the nitrogen supply (Caraco et al., 1987; Howarth 1988; Valiela 2015). Thus, it appears that anthropogenic transformation of coastal ecosystems (eutrophication of coastal waters), elicited via nitrogen loading, is ubiquitous (National Research Council 1994). The usage of fertilizers, contamination of the atmosphere and the disposing of waste waters (Lee & Olsen 1985) are all major sources of nitrogen loading in coastal watersheds; such

sources are subsequently carried to waters via river discharge, groundwater flow, deposition in the atmosphere and sewage discharge.

1.3.3.3 Silicon Cycle

According to Wollast and Mackenzie (2005), Silicon comprises 27% by weight of the elements in the Earth's crust and is the second most abundant element after oxygen. Silicon is crucial in the formation of skeletal structures of numerous aquatic planktons such as radiolarians, diatoms, silicoflagellates and sponges. Hence, in primary productivity processes silica is an important nutrient in aquatic systems, whereby the cycling of silicon is regulated between physiochemical and biological processes. Canfield, Kristensen & Thandrup (2005) state that the weathering of rock-forming silicate minerals is the main source of dissolved silica in aquatic environments by acidic dissolution. The rate of weathering is dependent on the complex interaction between the temperature, topography, vegetation apart from the precipitation, runoff as well as its lithology (Drever 1994). It is interesting to note however, that a considerable portion of riverine load of dissolved silica (about $0.6 \times 10^{12} \text{ mol yr}^{-1}$) is removed by biological uptake in estuaries and does not reach the ocean (this is further explored in Chapter 5). On the other hand, a considerable amount of Si flux into the ocean is via the transport by rivers of amorphous silica from terrestrial environment (Conley 1997). While various studies show that there is a strong relationship between dissolved Si depletion and nutrient loading (high discharges of nitrogen and phosphorus) and damming of rivers in coastal marine areas (Conley, Schelske and Stoermer 1993), the condition of the Rajang river is different as the damming causes the river to be enriched in dSi.
1.3.4 Primary Productivity

1.3.4.1 Role of phytoplankton in global carbon cycle

According to Bracher et al. (2017), the phytoplankton community composition/structure is paramount to many essential biogeochemical processes, especially nutrient uptake and cycling, gas exchange with the atmosphere, energy transfer through the marine food web, as well as deep-ocean carbon transport. One such biogeochemical cycle is the global carbon cycle via the biological carbon pump as put forth by IPCC (2013), in which marine phytoplankton contributes to about 50% of the global primary production (Field et al. 1998).

1.3.4.2 Role of nutrient dynamics in phytoplankton production

Fishery productions in marine ecosystems are supported by phytoplankton production via a bottom-up effect (Kimmerer 2002; Connolly, Schlacher and Gaston 2009; Kostecki et al. 2010). This is supported by the fact that the distributions of phytoplankton biomass influence the global distribution of fishery resources (Caddy & Bakun 1994). The phytoplankton primary production in turn, is supported by nutrient limitation and the availability of light, whereby the fluctuations in the aforementioned factors would regulate seasonal and annual fluctuations in the primary productivity of phytoplankton (Cloern & Jassby 2008). This in turn would then regulate secondary production and further influence the survival of larval fish and annual fish stock recruitment. However, as put forth by Thomas et al. (2004) and Smith et al. (2012), in relation to temperate zone rivers and estuaries, the characteristics and processing of nutrients of tropical rivers and estuaries are a lot less studied.

1.3.4.3 Primary Productivity of Freshwater Ecosystems

While it is shown that freshwater flow and upwelling are the two major processes that supply nutrients in estuarine and coastal systems that do not undergo strong tidal mixing (Caddy & Bakun 1994), the input of each nutrient source to the production of phytoplankton in open coastal systems influenced by oceanic water is still less well studied. Watanabe et al. (2017) showed that open coastal systems are dominantly driven by entrainment of oceanic nutrients and are altered by both freshwater inflow and coastal conditions that are dissimilar from semi-enclosed bays. This is due to the fact that nutrient composition and concentrations as well as the volume of the aforementioned two parameters could differ seasonally (Watanabe et al. 2017).

1.3.4.4 Importance of Tropical Estuaries for Primary Productivity

A feature of tropical estuaries is that they are among the most biogeochemically active zones which are much more susceptible to anthropogenic nutrient loading than are higher latitude estuaries (Yule et al. 2010; Smith et al. 2012). Hence, this is a crucial region as the primary productivity within

estuaries and coastal areas accounts for about 30% of the total global net oceanic productivity (Gattuso et al. 1998; Dürr et al. 2011).

1.3.4.5 Nutrient dynamics and influence on Primary Productivity

According to Harrison (2000), the species composition of marine primary producers is significantly influenced by the alterations in Si inputs to marine ecosystems, particularly the balance of production between non-siliceous phytoplankton and diatoms. Furthermore, it was hypothesized that there was a higher contribution of diatoms to total oceanic phytoplanktonic biomass during the Last Glacial Maximum (79% as compared to 54% today) due to the increased aeolian (wind) inputs of Si (Tréguer & Pondaven 2000). Also, while total algal growth is essentially governed by the availability of P and N respectively, the relative abundance and availability of Si in relation to P and N, i.e. the Si:N and Si:P ratios, are able to influence the phytoplankton community composition (Conley, Schelske and Stoermer 1993), hence proving Si to be a key element to sustain diatom growth. Paasche (1980) also states that the depletion of oceanic and coastal DSi concentrations to near limitation is due to the production of new diatoms (<5µM DSi). Moreover, diatoms (apart from phytoliths and sponges) make up important amorphous silicon pools in wetland ecosystems (Struyf et al. 2009; Clarke 2003) and at the interface of aquatic and terrestrial continuums. Even though most of the DSi is sequestered by diatoms (97% of the ASi that is settling) back into DSi before settling to the sediments of ocean floors, oceanic diatom production would eventually decline in the long term without constant replenishing of the remainder (3%) from terrestrial ecosystems. This would in turn affect the production of oceans as well as burial of carbon (Struyf et al. 2009). Thus, diatoms play an important role in primary productivity in terms of the eutrophication of coastal zones and oceanic C-sink.

Additionally, one of the main causes for concern for regional-scale alteration and threat to the biosphere (in terms of rivers) is human interference (land-use change) (Sala et al. 2000) and in particular the building of dams. From an ecological standpoint, river corridors are decentralized or fragmented by dams. Such conversion of stream-flow dynamics and fluvial processes poses serious threats to the native river biodiversity (Poff et al. 2007). Based on the study done by Poff et al (2007), river dynamics in the United States were shown to homogenize due to the construction of dams; they contended that the concentration of dams in the country would create conditions that would support non-indigenous, urban species at the expense of the local biodiversity. Hence, while dams cause widespread, regional homogenization of the regional dynamics, as the Rajang river is heterogeneous in its riparian ecosystem, it would be interesting to note if such homogenization is indeed the major force of driving nutrient dynamics and the subsequent microbial community, or if the riparian run-off would be the major driving force.

Documentation of large-scale alterations in nutrient fluxes are less studied than those demonstrated in other tropical aquatic ecosystems within similar latitudes (i.e. Amazon, African continent). Of the studies shown, increased nutrient loads (especially N) by riverine transport have contributed to the increased input of nutrients to numerous major coastal zones (Meybeck 1982; Turner and Rabalais 1991). Furthermore, such increases in loads (riverine suspended sediment) caused by watershed/riparian development lead to extensive consequences on sensitive marine ecosystems including seagrass meadows (Restrepo et al. 2006), coral reefs (Fabricius 2005; Bartley et al. 2014) and wetlands (White et al. 2019), even though wetlands were shown to act as a filter/barrier. Furthermore, inputs of P have increased globally as rock phosphates are used in detergent additives, animal supplements and most importantly fertilizers for agricultural crops (Gomes et al. 2018). Globally, while the estuarine processes were not taken into consideration, it was estimated that 80% of Dissolved Inorganic Phosphate (DIP) reaches the open ocean (Seitzinger et al. 2010; Sharples et al. 2017). According to Jickells et al. (2017), a weak tropical Coriolis force allows river plumes to move directly across the shelf as compared to temperate and polar regions, thus causing tropical and subtropical rivers to be most important for the delivery of nutrients to the open ocean (Sharples et al 2017). Hence, a major source of nutrients for open oceans and seas comes from low-latitude rivers, particularly in the Amazon as well as rivers in South East Asia. This further highlights the importance of deducing the nutrient outputs of the Rajang River and the biogeochemical processes that come with it.

Chapter 2

General Methodologies

General Methodologies

Each of the following chapters includes methods that are specific to each chapter. The following methodologies encompass methods which are common to all chapters as outlined below.

2.1 Study Area

2.1.1 Geophysiology and climatology of the Rajang River

This study was conducted along ~300km of the Rajang River in Sarawak, Malaysia, which is located on the north-western region of the Borneo Island. This region has an equatorial climate characterized by constant temperatures, high extensive rainfall and high humidity (Wang et al. 2005, Wang et al. 2009). Based on the statistics provided by the Malaysian Department of Statistics (2019), the level of urbanization within the Sarawak state was at 53.8% of which the estimated total population in Sarawak for the year of 2018 was 2.79 Million with a GDP of RM 113.982 billion in 2017. It was reported that 60% of the total wood revenue in 1973 were from wood products originating from peat swamp forests in Sarawak, amounting to RM 150 million (36.7 million USD) at the time (FTU 2002).



Fig. 2.1: Location of Rajang River within Sarawak, Malaysia (Inset). The four tributaries of the Rajang River are as labelled (clockwise: Rajang, Paloh, Lassa and Igan Tributary).

According to MacKinnon et al. (1996), the Rajang River originates near the border of Indonesia and Malaysia where the Iran mountain range is situated, where elevations can reach 1800 m (Milliman

and Farnsworth, 2011). From Staub and Gastaldo (2003), the main trunk of the Rajang river drains an approximately 52,100 km² area (Lehner, Verdin and Jardis 2006; DID 2017). It follows a comparatively straight path through the Central Borneo Massif (separation of planet's crust by faults or flexures without changing its internal structure). The Rajang River flows around 530km from East to West; the river starts bifurcating close to the town of Sibu in a rectilinear north-south position, and finally discharges into the South China Sea (Milliman and Farnsworth 2011). This bifurcation then results in 5 main tributaries from northeast to southwest: Igan, Lassa, Paloh, Rajang and Belawai (not shown). The shoreline experiences tides and seasonally strong waves ranging from 3 - 6 m, with intensity increasing from the east to the west. In addition, maximum saltwater incursion occurs during the dry season (Staub and Esterle, 1994), by which monsoonal climate causes the fluctuation in saltwater influence. According to Müller-Dum et al. (2019), saltwater intrusion occurs until a few kilometres downstream of the town of Sibu, whereas tidal influence extends further inland up to 120 km to the town of Kanowit (Staub and Gastaldo 2000). The aforementioned influence is restrained by diurnal or semi-diurnal tides ranging from meso- to macro-tidal (about 2 to 6 metres). The Rajang delta can be segmented into three regions, namely the distal region in which Nipa is the most pronounced species, the tidal flats in which marine to brackish water-fed mangroves are colonized by Rhizophora, Avicennia and Sonneratia, and lastly the river channels which are flanked by riparian vegetation (Gastaldo 2010).

The Rajang river drainage basin area is approximately 50,000 km² (Staub, Among & Gastaldo 2000). According to Nachtergaele et al. (2009), 11% of the catchment size corresponds to peatlands which extend over the aforementioned area. Furthermore, only 1.5% of Sarawak's 17% of peatlands of the entire state remains entirely pristine (Wetlands International 2010). The upper reaches of the Rajang river drain mineral soils until the town of Sibu (Müller-Dum et al. 2019), from which multiple distributary channels branch out to drain peat towards the South China Sea. Apart from that, the proximal hills region also releases discharge and sediment (Gastaldo 2000) whereby its delta plain covers approximately 6500 km². The Rajang delta system consists of an alluvial valley, an associated coastal plain and a delta plain (Staub and Esterle 1994). According to Gastaldo (2010) the Rajang river delta is a coastal plain system mainly accumulated with thick peat; it can be greater than 1 m thick; it is low-ash and low-sulphur acquired over the past 7 – 7.5 ka (Staub and Esterle 1994; Staub and Gastaldo 2003).



Fig. 2.2: Licensed sago and oil palm plantations that was digitized from the NREB map and determined based on Landsat data (Wetlands International 2015)

According to Wetlands International (2015), the land surrounding the study sites is characterised by a range of anthropogenic activities, ranging from oil palm and sago plantations to human settlements and transportation and sand dredging activities (see **Fig. 2.2**). In terms of human settlements, apart from the main settlements (Sibu, Kanowit and Kapit), a large number of traditional buildings, called longhouses, are inhabited by the local communities along the river and the tributaries (Ling et al. 2017). Industrial oil palm plantations (Gaveau et al. 2016) and sago plantations (Wetlands International 2015) were mostly converted from these peatlands; Miettinen et al (2016) claim that the plantation industry accounts for more than 50% of the peatlands (11% of the total catchment size) in the Rajang watershed. Furthermore, timber processing, logging and fisheries are the main socioeconomic activities for the local residents (Abdul Salam & Gopinath 2006, Miettinen et al. 2016).

2.1.2 Classification of Seasons

Two monsoonal periods occur within this region, whereby the southwestern monsoon which occurs from May until September is normally associated with relatively drier weather whereas the northeastern monsoon which is normally associated with enhanced rainfall and subsequently frequent flooding occurs from December to February. Nonetheless, rainfall is high throughout the year despite the monsoon which is associated with the drier season (Sa'adi et al. 2017). The discharge rates for the Rajang river drainage basin vary from $1000 - 6000 \text{ m}^3 \text{s}^{-1}$ for each month (data obtained from 30 years of rainfall data) whereby the average is around $3600 \text{ m}^3 \text{s}^{-1}$.



Fig 2.3: Monthly Mean Precipitation (mm) from Jan 2016 to Sep 2017. Months of the field campaigns are highlighted red (Aug 2016), blue (Mar 2017) and dark blue (Sep 2017)

Monthly precipitation for the period in between the cruises (August 2016 to September 2017) were obtained from the Tropical Rainfall Measuring Mission website (NASA 2019) in order to gauge the seasonality (wet or dry). As the rainfall data do not correlate with the monsoonal periods, the seasons into which the sampling cruises were classified were based on the mean rainfall that occurred for each month. The August 2016 cruise (colored red) is classified as the dry season based on the lower mean

rainfall value as compared to the other two (March 2017 and September 2017), which are both classified as the wet season.

2.2 Sample Collection Strategy

Two cruises were undertaken in August 2016 over a span of seven days and in March 2017 over four days on board a live-aboard fishing boat, and in September 2017 over four days on a speedboat. All samples were collected on board and filtered and preserved directly.



Fig 2.4: Fishing boat used for August 2016 and March 2017 cruises

The cruise in August 2016 represented the highest sampling frequency in order to obtain complete coverage of representative regions with marine and freshwater end-members in mind, while the cruises in March and September 2017 were carried out on a lower frequency and were aimed to obtain seasonal representatives but with similar spatial coverage and end-members. Based on the monthly mean precipitation, the first cruise (August 2016) was sampled during the dry season, while the March 2017 and September 2017 cruises were carried out during the wet season. The specific sampling sites for each chapter will be shown in each individual chapter (**Chapters 3, 4 and 5**). The samples were collected within the upper 1 m (as a representative of surface waters) using a throw-away bucket. The bucket was thoroughly rinsed with sample waters at the start of each station.

2.3 Filtration of Samples

Filtration of the samples was carried out on-board immediately after collecting the sample waters with the throw-away bucket using a portable filtration set connected to a vacuum pump (Fig. 2.5). About 250 - 1000 mL of waters were sampled depending on the turbidity of the waters. The vacuum pump was set at around 100 mbar. For nutrient samples, refer to Chapter 3.3. For bacteria samples, refer to Chapter 4.3.1.



Fig. 2.5: Portable Filtration Set

2.4 Classification of Samples

The sampling area can be divided into four categories according to salinity and/or source types: 1) marine, 2) brackish peat, 3) freshwater peat, and 4) freshwater mineral soil. The classification was based on salinity and type of soil (peat or mineral soils) based on earlier descriptions of Müller-Dum et al. (2019) and salinity data obtained from the cruises. The classification of land-use is based on descriptions by Wetlands International (2015), Gaveau et al. (2016), Miettinen et al (2016) and Ling et al. (2017) to assess the possible anthropogenic influences. The classification of land use can be separated into several categories, namely: 1) coastal zone, 2) coastal zone with plantation influence, 3) oil palm plantation, 4) human settlements, 5) secondary forests.

2.5 Physico-chemical Measurements

A multi-parameter probe that was pre-rinsed with Milli-Q[®] and sample waters was used at the start of every station to obtain the physico-chemical parameters, i.e. salinity, temperature, DO and pH of the surface waters utilizing an Aquared[®]. The Global Positioning System (GPS) coordinates were obtained with a GPS in-built in the YSI CastAway CTD[©]. The salinity data obtained from the CTD was also compared with the multi-parameter probe.

2.6 Sample Collection Preparation

Sampling bottles for nutrients were all soaked in 4% HCl for 3 days and thoroughly rinsed with Milli- $Q^{\text{(B)}}$ water for 6 times prior to being air-dried in a laminar flow. For the storage of filters and also picoplankton samples, 2 mL and 4 mL cryo-preservation tubes were sterilized at 121°C for 15 mins. 0.7 GF/F filters for Chl *a* (refer to **Chl** *a* **determination**) and pigments analyses (refer to **Chapter 5.3**) were also individually sterilized at 121°C for 15 mins. Also, 3.0 µm, 0.4 µm (for nutrients) and 0.2 µm pore size polycarbonate (GF/C) filters (Cyclopore, Whatman, Germany) were already presterilized.

2.7 Nutrients Analyses

The concentrations of nutrients were determined in the laboratory utilizing a Skalar SAN^{plus} auto analyser (see Fig. 2.6) based on the procedures of Grasshoff et al. (1999). The nutrients measured included: Nitrate (NO₃⁻), Nitrite (NO₂⁻), Ammonium (NH₄⁺), Dissolved Inorganic Phosphate (DIP), Dissolved Silicate (dSi), Total Dissolved Nitrogen (TDN) and Total Dissolved Phosphate (TDP). The sum of NO₃⁻, NO₂⁻ and NH₄⁺ was classified as dissolved inorganic nitrogen (DIN). The analytical precision for all nutrient components measured was <5%. The samples were analyzed in batches based on the salinities.



Fig 2.6: Skalar San^{plus} auto analyzer (Left). Automated sampler for the Skalar San^{plus} (Right)

2.7.1 Principle and Preparation of Reagents and Standards

For each of the nutrient components, reagents and standards were prepared before the analyses. The components and principle for each of the components are as stated below according to standard references provided by EPA (1983), American Public Health Association (1989) and International Organisation for Standardisation (ISO-5667-3 2018). For samples of known salinities, it should be noted that the instrument wash water was replaced with ultra-pure sodium chloride solution to match the salinities of the samples. The samples were fed into the system with the auto-sampler and reacted with the reagents stated below for each of the nutrient component.

2.7.1.1 Phosphate

The determination of phosphate was based on the formation of an antimony-phospho-molybdate complex formed from the reaction between a solution of diluted phosphate, potassium antimony tartrate and ammonium molybdate in an acidic medium (Boltz and Mellon 1948; Walinga et al. 1989). The complex formed was measured at 880 nm due to the complete reduction of the intensely blue

coloured complex by ascorbic acid. The reagents required for the determination of phosphate were as follows: (1) ammonium molybdate solution prepared with 230 mg of potassium antimony (K(SbO)C₄H₄O₆· $\frac{1}{2}$ H₂O) added to ±800 mL MilliQ[®] water in a 1 L Erlenmeyer flask. Next, 69.4 mL of 97% sulphuric acid (H₂SO₄) was slowly added to the solution and allowed to cool with constant swirling. Then, 6 g of ammonium molybdate ((NH₄)₆Mo₇O₂₄·4H₂O) were added to the mix and topped up with MilliQ[®] water to 1 L. The solution was then topped up with 2 mL of FFD6 (an anionic surfactant). (2) ascorbic acid solution was prepared by dissolving 11 g of ascorbic acid (C₆H₈O₆) in ± 800 mL MilliQ[®] water. Then, 60 mL of acetone was added to the solution of 100 ppm standard for phosphate was prepared by dissolving 0.4394 g of potassium dihydrogen o-phosphate (KH₂PO₄) in ±800 mL of MilliQ[®] water in a 1 L Erlenmeyer flask. The flask was then filled up to the 1 L mark with MilliQ[®] water and mixed well.

2.7.1.2 Nitrate and Nitrite

Nitrate and nitrite were determined based on the reduction of cadmium. To reduce the nitrate to nitrite, the samples were passed through a column with granulated copper-cadmium. The determination of the originally present nitrite and reduced nitrate was based on the diazotization with sulfanilamide. A highly coloured azo dye was formed when the reaction was completed by the coupling with α -napthylethylenediamine dihydrochloride which was then measured at 540 nm. The preparation of the buffer solution required for the determination of both components included: (1) a buffer solution which was prepared by dissolving 50 g of ammonium chloride (NH₄Cl) in 800 mL of MilliQ[©] water in an Erlenmeyer flask and adjusted to pH 8.2 with an ammonium hydroxide (25%) solution. The flask was then filled to 1 L and 3 mL of Brij 35 (30%, a non-ionic surfactant) were added to the solution and mixed well; (2) a colour reagent was prepared by adding 150 mL of Phosphoric acid (H₃PO₄, 85%) in \pm 700 mL of MilliQ[©] water in an Erlenmeyer flask. Then, 10 g of sulfanilamide $(C_6H_8N_2O_2S)$ and 0.5 g of α -napthylethylenediamine dihydrochloride (C10H7NHCH2CH2NH2·2HCl) were dissolved into the solution. The solution was then topped up with MilliQ[©] water to the 1 L mark and mixed well. For the preparation of 100 ppm N to use as standards in order to construct the calibration curve, 0.6068 g of sodium nitrate (NaNO₃) was added into \pm 800 mL of MilliQ[©] water in an Erlenmeyer flask. The solution was well mixed after the addition of MilliQ[©] water up to the 1 L mark.

The determination of nitrate and nitrite requires another step which is the activation of the cadmium column. The activation of the aforementioned column required: (1) the dilution of 400 mL of 32% hydrochloric acid (HCl) in 600 mL of MilliQ[©] water to obtain 4 M HCl; (2) dissolving 20 g of cupric sulphate (CuSO₄·5H₂O) in \pm 800 mL of MilliQ[©] water, subsequently topped up to the 1 L mark in an

Erlenmeyer flask; (3) \pm 4.5 g of 0.3–1.0 mm sieved cadmium granules. About 30 mL of 4M HCl solution was mixed with cadmium granules and stirred for approximately 1 minute. The solution was decanted and washed with MilliQ[©] water until it was acid free and then topped up with \pm 50 mL of cupric sulphate solution and stirred for 5 minutes. The dirt was washed out with additional MilliQ[©] water. The cadmium granules were then dried with filter paper. The column was filled with the cadmium granules with the aid of a funnel. Care was ensured to pack the column with the granules. A small piece of polyethylene tube was added with a sharpened inlet into the column in order to avoid the granules from spilling. The column was filled with the buffer solution prepared in step 1 with the aid of a syringe. The column was then placed into the system.



2.7.1.2.1 Digestion of Dissolved Organic Nitrate and Dissolved Organic Phoshphate

Fig. 2.7: Graphical representation of DON and DOP digestion (illustration from Jin, unpublished)

The concentrations of the dissolved organic phosphorus (DOP) and dissolved organic nitrogen (DON) were calculated by subtraction of DIP from TDP and DIN from TDN respectively. A digestion method (see Fig. 2.7) to determine total dissolved phosphorus (TDP) and total dissolved nitrogen (TDN) was designed according to methods of Ebina et al. (1983) using the alkaline persulfate oxidation method with the aforementioned auto analyzer. The materials prepared for the oxidizing agent were: 1.5 g of low-N sodium hydroxide (NaOH) added to 90 mL of MilliQ[©] water in a 125 mL bottle. Then, 5 g of low-N potassium persulfate (K₂S₂O₈) and 3 g of boric acid (H₃BO₃) were added into the solution and topped up to 100 mL (~100 g by weight). A series of working standards were prepared for nitrogen and phosphate utilizing potassium nitrate (KNO₃) and potassium dihydrogen phosphate (KH₂PO₄), respectively prior to analyses of samples. In order to ensure total digestion,

digestion check standards were carried out with glutamic acid and glycerophosphate for nitrogen and phosphorus digestion checks, respectively. For the digestion of the samples, 20 mL of the sample was added with 2 mL of the oxidizing reagent that was prepared. For blanks, the samples were replaced with 20 mL of MilliQ[®] water. The samples were digested utilizing an autoclave machine at 120°C at 1 atm for 30 mins and allowed to cool. The determination of the total dissolved phosphate and nitrogen procedure is similar to the protocol for phosphate as well as nitrate and nitrite.

2.7.1.3 Ammonia

The determination of ammonia was based on the modification of the Berthelot's reaction (Krom 1980; Searle 1984) whereby phenol reacted with chlorinated ammonia (monochloramine). After the oxidative coupling and oxidation, it results in the formation of a green coloured complex. Then, the reaction was catalysed by nitroprusside, whereby chlorine donation occurs when reacted with sodium hypochlorite. The absorption of the formed complex was measured at 630 nm. The reagents required for the analysis of ammonia were: (1) a buffer solution prepared by dissolving 33 g of potassium sodium tartrate (KNaC₄H₄O₆·4H₂O) in \pm 800 mL of MilliQ[©] water and added with 24 g of sodium citrate (C₆H₅O₇Na₃·H₂O) in an Erlenmeyer flask until it was dissolved. The pH of the solution was then adjusted with 0.5 M sulphuric acid to pH = 5.0. Then, the solution was topped up to the 1 L mark with MilliQ[©] water and 0.5 mL of Brij 35 (35%) added; (2) phenol solution was prepared by dissolving 83 g of phenol in ± 80 mL MilliQ[©] water in a 1 L Erlenmeyer flask and 40 g of sodium hydroxide (NaOH) slowly added to the mixture. The solution was then mixed after the addition of MilliO[©] water to the 1 L mark; (3) sodium hypochlorite solution was prepared by the addition of 200 mL of 13% active chlorine sodium hypochlorite in ± 700 mL of MilliQ[©] water in a 1 L Erlenmeyer flask. Next, the solution was topped up to the 1 L mark with MilliQ[©] water; (4) sodium nitroprusside solution was prepared by the dissolution of 0.5 g of sodium nitroprusside (Na₂[Fe(CN)₅NO]·2H₂O) in about 800 mL of MilliQ[©] water and subsequently topped up to the 1 L mark in an Erlenmeyer flask; (5) The air scrubber solution was prepared by careful dilution of 139 mL of 97% H₂SO₄ sulphuric acid in ± 800 mL of MilliQ[©] water and topped up to the 1 L mark in an Erlenmeyer flask. The preparation of 100 ppm N for the determination of ammonia required dissolving 0.3819 g of ammonium chloride (NH₄Cl) in ± 800 mL of MilliQ[©] water, mixed well after filling to the 1 L mark in an Erlenmeyer flask.

2.7.1.4 Silicate

The principal behind the determination of silicate is based on the formation of molybdosilisic acid from the acidification of the sample that was mixed with an ammonium molybdate solution (Smits and Milne 1981; Babulak 1973). The acid was reduced to a blue dye with ascorbic acid and was added with oxalic acid to avoid the interference of phosphate. The blue dye was measured at 810 nm. The reagents required for the reactions were: (1) the preparation of sulphuric acid solution by diluting 10

mL of 97% sulphuric acid (H₂SO₄) in \pm 800 mL of MilliQ[©] water and subsequently filled to 1 L in a 1 L Erlenmeyer flask; (2) 20 g of ammonium molybdate ((NH₄)₆Mo₇O₂₄·4H₂O) was added to 800 mL of MilliQ[©] water and topped up to 1 L in an Erlenmeyer flask; (3) oxalic acid solution was prepared by adding 44 g of oxalic acid (C₂H₂O₄·2H₂O) in \pm 800 mL of MilliQ[©] water and mixed after subsequent addition of MilliQ[©] water to the 1 L mark in an Erlenmeyer flask; (4) ascorbic acid solution was prepared with 800 mL of MilliQ[©] water and the addition of 40 g of ascorbic acid (C₆H₈O₆) and filled to the 1 L mark in an Erlenmeyer flask. The standard stock solution of 100 ppm Si was prepared by the dissolution of 1.0119 g of sodium metasilicate Na₂SiO₃·9H₂O in 1 L of MilliQ[©] water in an Erlenmeyer flask.

2.7.2 Calibration Curve

The calibration curves for each of the components are shown in Table 2.1 below:

Parameter	Slope	a	\mathbf{R}^2
Si	8.34359	-30.87108	0.99987
Nitrate/Nitrite	45.75879	-3.09998	0.99969
Ammonium	6.7604	-1.66455	0.99819
Phosphate	10.01598	-31.71001	0.9992

Table 2.1: Calibration curve of each parameter analysed

2.8 Pyrosequencing, DNA Processing and Quality Control Pipeline

In traditional culture-dependent techniques for the identification of bacteria in environmental samples, the discrepancy between the actual number of culturable bacteria and direct microscopic count is one of its many limitations (Amann, Ludwig and Schleifer 1995). In order to improve both basic science understanding and informed progress towards the monitoring of water quality, it is essential to study whole microbial communities instead of selected proxies; the latter are often biased and incomplete representations of the microbial diversity in the genomic databases and literature of microbial ecology (Van Rossum 2017). Furthermore, the actual bacterial community structure cannot be accurately reflected due to the selectivity of the growth media which is limited to certain bacteria (Theron and Cloete 2000). According to Dahllöf, Baillie, H and Kjelleberg (2000), molecular methods allow high resolution and rapid description of microbial communities and provide new insights for microbial diversity as compared to traditional, culture-dependent approaches. Based on the review by Douterelo et al. (2014), high-throughput sequencing techniques such as the Illumina or Roche 454 pyrosequencing methods are less expensive and faster than the traditional Sanger sequencing, as they can combine multiple samples in a single run and are often utilized for the analysis of microbial

diversity and structures of environmental samples. Hence, the Illumina Miseq sequencing platform was utilized, as the variability of the highly diverse microbial communities in both marine and freshwater ecosystems is unknown (Fortunato et al. 2012).

2.8.1 Amplicon Sequencing

The 3.0 μ m and 0.2 μ m filters were sent to the Australian Centre for Ecogenomics, Brisbane in order to undergo amplicon sequencing (16s ribosomal Ribonucleic Acid, rRNA) using the Illumina Miseq (Caporaso et al. 2012) platform. It should be noted that the platform utilized runs on the forward (F1) read only. As shown in the workflow below (**Fig. 2.8**), the raw .fastq files were first processed with fastqc (Babraham Bioinformatics).



Fig. 2.8: Workflow of processing of raw data (ACE 2019)

Using the Trimmomatic software (Bolger, Lohse and Usadel 2014), the primer sequence was removed by trimming the first 20 bases of all fastq files. Then, the primer-trimmed fastq files were then quality trimmed with the criteria of an average base quality of >15 with a sliding window of 4 bases.

2.8.2 Downstream Processing of High Quality Raw Sequences

While the amplicon clustering and taxonomy assignment was carried out by ACE, in this study the clustering and taxonomy assignment was carried out with mothur v1.39.5 (Schloss et al. 2009) using the supercomputer (OzSTAR) provided at Swinburne University of Technology, Melbourne, Australia, which is an open-source bioinformatics pipeline that is widely used for bioinformatics analyses. The standard operating procedures are according to Kozich et al. (2013). The standard operating procedure is as shown in **Fig. 2.9**.



Fig. 2.9: General overview of the MiSeq SOP using mothur (image from Chunlab Inc.^(C) 2019)

Prior to analyses, the FASTA file had the barcodes removed and the paired-end sequences merged; it was filtered for quality and length (based on the ACE steps). The EzTaxon (v.1.5) (Kim et al. 2012) database was utilized over the conventional SILVA database as the EzTaxon database contains uncultured phylotypes which were suitable for the unknown samples obtained from the Rajang River-South China Sea continuum. The minimum length of base pairs was set at 400, and maximum homopolymer value was set at 8. The values below 400 base pairs were discarded from subsequent analyses. The *pre.cluster* command was utilized to remove sequences as a result of pyrosequencing errors. Chimeric sequences were then removed via the *chimera.vsearch* command. The *classify.seqs* command was utilized with a cutoff of 80 to assign taxonomy to the sequences. The remove lineage option was utilized to only include taxa from bacteria. The uncorrected pairwise distances were calculated with the *dist.seqs* option with cutoff = 0.15 and lastly the *cluster* command with a cutoff = 0.03 was utilized to assign OTUs to the sequences. Then, to obtain the consensus taxonomy for the OTU, whereby a taxonomic table was generated, the *classify.otu* command was used. Then, the split.abund option was utilized in order to remove singleton OTUs (alpha diversity analyses exclude the removal of singleton OTUs). A .biom file was generated utilizing the make.shared command and make.biom command. MEGAN community edition (Huson et al. 2016) was utilized for graphical representation of the microbial (bacteria) community analyses.

2.9 Chl a and pigments determination

As a proxy for phytoplankton biomass, chlorophyll a (Chl a) was utilized. The extraction of Chl a is as provided by Martin et al. (2018). The methods for pigments extraction is as provided by Zhu et al. (2009) and Biswas et al. (2015). The filters were first grounded and extracted utilizing methanol and extracted with an ultrasonicator (VCX644, Sonics and Materials, USA) in an ice bath. Then, 0.45 µm PTFE membrane was utilized to filter supernatant of the extracts after centrifugation at 3000 rpm. Prior to HPLC analyses, the extracts were stored at -40 °C. Milli-Q water was added immediately before the injection at a water:extract ratio of 1:5 v/v to the samples. This is to prevent distortion of analyses due to the earlier eluting peaks (Zapata and Garrido 1991). Then the HPLC with gradient elution (Zapata et al. 2000) was utilized to analyze 100 μ L (extract + water) aliquots. The HPLC system used was the Agilent 1100 series that contains an auto-sampler, diode array detector (DAD), fluorescence detector (FLD), on-line vacuum degasser, quaternary pump and a column fitted with a thermostat. The samples were stored after being scanned at 300 to 750 nm. Then, at 440 nm with a 20nm bandwith, 4 nm slit peak and >0.1 peak width (2s), the pigments were quantified. At 665 nm (DAD) and FLD (ex.:440nm, em.: 650 nm), the sample chlorophylls were also quantified. After being passed through the corresponding guard column, the pigments were separated using a C8 column (ZORBAX Eclipse XDB-C8, 4.6×150 nM) at 25°C. Based on the comparison of the spectra and retention time, the pigments were quantified with authentic standards. Chl a standards were purchased from Sigma-Aldrich. The remaining pigment standards were bought from DHI company.

2.9.1 Phytoplankton community Determination

For the prediction of phytoplankton community using the pigments concentration via CHEMTAX, refer to **Chapter 5.2**.

2.10 Picoplankton Abundance Determination and Biomass Calculations

The picoplankton samples were sent to Institute of Ocean and Earth Sciences, University of Malaya for enumeration. The method for picoplankton enumeration is as described by Marie et al. (2000) A Flow cytometer (Partec CyFlow Space, Partec, Germany) was used to determine the cell abundance (cells mL⁻¹) of *Prochlorococcus, Synechococcus*, and pico-eukaryotes based on their auto-fluorescence of the chlorophyll (FL3 channel), phycoerythrin (FL2 channel) and side scattering characteristics. The samples were first thawed to 37 °C and diluted with pre-filtered by 0.2 pore size syringe filtered seawater or freshwater depending on the salinity of the samples.

The absolute cell concentration is as follows:

$$Cpop = T * Npop / R * (\frac{Vtotal}{Vsample})$$

whereby:

 C_{pop} = concentration of population (cells μL^{-1}),

 $N_{pop} =$ Number of cells obtained

T = Time of Acquisition (minutes)

R =Sample flow rate ($\mu L \min^{-1}$)

 $V = volume of sample (\mu L)$

 V_{total} = Volume of sample (including fixatives, beads etc).

The cell abundance was calculated and converted into biomass (ug C L⁻¹) based on literature values (Buitenhuis et al. 2012). For *Prochlorococcus* (*Pro*), each cell mL⁻¹ = 60 fg C mL⁻¹, *Synechococcus* (*Syn*) = 154 fg C mL⁻¹ and Pico-eukaryotes (*Peuk*) = 1319 fg C mL⁻¹. The calculated biomass was utilized for correlation with selected nutrient parameters. The equation for the calculation of the picoplankton biomass is as follows:

Biomass ($\mu g L^{-1}$)

= ((Abundance (cells mL^{-1}) × Literature Biomass cell⁻¹(fg C mL^{-1}) ÷ 1.0 × 10⁹) × 1000

2.11 Statistical analyses and Modeling

Averages of measured parameters were reported as \pm standard error (SE) unless stated otherwise. For statistical correlations, SPSS (IBM SPSS Statistics 22) was utilized for calculations of Independent sampling *t*-test (between seasons), one-way ANOVA (between source types) and Spearman's ranking (Bivariate correlation). Graphs were produced using Prism 6 (GraphPad Software, Inc). By utilizing PRIMER 7 (Clarke and Gorley 2015), the biomass (pigments concentration or picoplankton) or abundance data (OTU abundance table for bacteria) were first standardised and transformed (Square root), i.e. Hellinger Transformed. Bray-Curtis dissimilarities were calculated utilizing the Hellinger Transformed OTU/biomass matrix whereby ordination visualization, non-metric multidimensional

scaling (NMDS), and similarity analyses (ANOSIM) were then executed. The physico-chemical parameters were then standardized and a Euclidean distance matrix was calculated from the physico-chemical parameters that were standardized. Multi-collinearity between variables was tested utilizing the 'Draftsman Plot' function in Primer 7 as well as normalizing transformations of the environmental variables were carried out prior to execution of DistLM analyses. Using the partition of community variation, distance-based linear models (DistLM) were used to determine the extent to which the community structure can be explained by environmental variables (Legendre and Anderson 1999). Hellinger Transformed abundance tables were used as the response variable for the variation partition analysis, whereby the best fit selection procedure was selected with AIC (Akaike information criterion) as the selection criterion (Kim et al. 2012). The significance of each correlation was tested with 999 permutations, and the distance-based redundancy analyses (dbRDA) was plotted.

Chapter 3

Behavior of Dissolved Phosphorus with the associated nutrients in relation to phytoplankton biomass of the Rajang River-South China Sea continuum

Behavior of Dissolved Phosphorus with the associated nutrients in relation to phytoplankton biomass of the Rajang River-South China Sea continuum

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3.1 Abstract

The Rajang river is a tropical peat-draining river which passes through peat-domes in the estuary and has mass discharge of organic matter into the South China Sea. This study aims to understand the nutrient biogeochemistry of the Rajang River, especially modifications implicated by anthropogenic activities. Hence, the main aim is to determine the spatial and seasonal variations in dissolved phosphorus budgets and the associated nutrients and their influence on the phytoplankton biomass. Three sampling campaigns in August 2016, March 2017 and September 2017 were undertaken along \sim 300 km of the Rajang river to study both spatial and seasonal distribution of nutrients and their fate in the coastal region. The analyses for nutrients encompass both inorganic (i.e. Nitrate, NO₃, Nitrite, NO₂, Ammonium, NH₄⁺, Phosphate, PO₄⁻ (DIP) and Silicate, dSi) and organic (Dissolved organic nitrate, DON and Dissolved organic phosphate, DOP) fractions. It was found that DIP concentration was not seasonally influenced but was spatially different along the salinity gradient whereas DOP was both seasonally and spatially different. Both DIP and DOP exhibited non-conservative behaviour in the mixing. DIP was subjected to 57.78% removal whereas DOP was subjected to 44.07% addition along the salinity gradient towards the South China Sea. The bulk of the dissolved phosphate is from DOP (73.84%), but both DIP and DOP may have contributed to the phytoplankton biomass. Spearman's correlations show that there was a switch in preference for DOP as compared to DIP depending on the concentrations of DIP or DOP due to seasonality. The main limitation in the Rajang River was assumed to be DIP based on the Redfield ratio. During the dry season, the NO₃-N:DIP ratios were lower, which were ideal conditions for phytoplankton proliferation while in the wet season, the increased NO₃-N:DIP ratios led to lower phytoplankton biomass. Overall, the Rajang River exports 0.12 t DIP mth⁻¹ into the South China Sea, which is relatively low compared to other major peat-draining rivers in the world. At the current pace of deforestation and the projected intensification of rainfall in the region, this finding provides an important baseline of the inventory of DIP into the South China Sea.

Keywords: Dissolved inorganic phosphate, dissolved organic phosphate, Rajang River, South China Sea, phosphate limitation

3.2 Introduction

The view of rivers as passive transporters have been recently been deemed null by studies (Richey et al 2002; Tranvik et al. 2009). Aufdenkampe et al. (2011) and Marwick et al. (2015) state that rivers are now well acknowledged as key players in regional and global carbon budgets, with the majority of the terrestrial input fraction being processed along the transit towards the coastal zone.

As the major pathway for nutrients dispersal from the continents to the oceans is through riverine transport (Liang & Xian 2018), the N and P riverine loading to the estuarine ecosystems have increased on a global scale due to nutrient enrichment (Nixon 1995). Nonetheless, eutrophication occurs due to enhanced nutrient levels vary from one aquatic environment to another (Di & Cameron 2002). While tropical aquatic environments support an extensive amount of biodiversity, there are few or no studies of nutrient mass balances of tropical regions (Liljeström, Kummu and Varis 2012). Furthermore, Yule et al (2010) and Smith et al. (2012) stated that tropical estuaries are the most biogeochemically active zones and are much more vulnerable towards anthropogenic nutrient loading as compared to estuaries at higher latitudes. Rapid economic development as a result of population growth has resulted in the extensive modification of tropical South East Asian rivers and degradation of catchments (Jennerjahn et al. 2008; Yule et al. 2010). This is even more true for peat draining rivers, yet there are limited studies of nutrient transport and in particular the dynamics of phosphate (P) in such environments.

The Rajang River is subjected to human developments which may alter the quantity and quality of nutrients and carbon (Rixen et al. 2016). Development may influence nutrient dynamics and subsequent alterations towards primary productivity and microbiological function (Henson et al. 2018). Primary productivity and biomass accumulation in coastal and freshwater ecosystems are driven by seasonally high NO₃⁻ concentrations (Sieracki, Verity and Stoecker. 1993; Kristiansen, Farbrot, Naustvoll 2001). However, as the Rajang river is tidal, has fluvially-driven inputs of terrestrial mineral soils in the upper altitudes, and drains peat domes in the lower altitudes (towards the coastal regions), it is imperative to understand the anthropogenic variability in nutrient dynamics in the landscape to better understand how such systems may respond to disturbance.

A macronutrient that is essential but often limiting in freshwater systems is phosphorus (Elser et al. 2007); under specific conditions it can also limit the primary productivity of terrestrial and coastal ecosystems (Sylvan et al. 2006, Street, Mielke 2018; Woodin 2018,). In the second half of the 20th century, anthropogenic activities have caused the global riverine phosphorus and nitrogen inputs to increase by three times (Jennerjahn et al. 2004). On a global scale, it was estimated that the riverine DIP loading for the world's largest rivers, which include 37% of the earth's watershed area and half of the earth's population, is 2.6 Tg yr⁻¹ (Turner et al. 2002). This value will undoubtedly increase due

to the increasing anthropogenic pressures. Runoff and leaching from animal production and agricultural fields (Van Drecht et al. 2009) would lead to changes in primary productivity, ecosystem functioning, hypoxic events, harmful algal blooms, damaged water quality as well as increased greenhouse gas emissions (Schindler 1974; Deemer et al. 2016; Macdonald et al. 2016; Ho & Michalak 2017). It is essential to understand the influence of peat on the riverine phosphate loading into the South China Sea due to the knowledge gaps about tropical peat-draining rivers, particularly the Rajang River. As the South China Sea supports one third of the global marine biodiversity (Ooi, Samah and Braesicke 2013), the contribution of the Rajang River towards the South China Sea in terms of primary productivity cannot be ignored.

The carbon pools in tropical peatlands are globally significant, with the current estimates ranging from 40 to 90 Gt of C (Yu et al. 2010; Page, Rieley and Banks 2011; Warren et al. 2016). The disturbance of peatlands due to anthropogenic activities such as deforestation and conversion of peatlands for agricultural activities poses a threat to the environment. This is because disturbed peat soil changes from carbon sink into carbon source, contributing to the greenhouse gases in the atmosphere (Hooijer et al. 2010, Hirano et al. 2012). Among recent studies of lateral transport of CO₂ of tropical peat-draining rivers (Müller et al 2015, Wit et al. 2015), the river of Maludam National Park seems to have a moderate amount of outgassing of CO₂ as compared to other peat-draining rivers globally. While the Rajang River is considered a medium-sized river based on its discharge (Sa'adi et al. 2017), 11% of its catchment area is part of the 15-19% of the global carbon peat pool in South East Asia (Page, Rieley and Banks 2011).

Therefore, the aim of this study is to 1) better understand the spatial and temporal distribution of nutrients, with particular focus on dissolved inorganic phosphate (DIP) and dissolved organic phosphate (DOP) in the Rajang river with consideration of the diverse inputs and influences, and 2) determine its influence on the phytoplankton biomass.

3.3 Methodology

3.3.1 Study Area

The sites of samples that were collected for nutrient analyses are shown in **Fig. 3.1**. The red triangles represent the samples collected from the dry season whereas the blue circles represent the samples collected for the wet season.



Fig. 3.1: Location of the Rajang River in Sarawak, Malaysia (Inset). Close up map of the Rajang basin and the stations sampled along the Rajang river and its tributaries (Red triangle: dry season, blue circle: wet season). The bold cross indicates the location of Sibu.

3.3.2 Sampling

For the two sampling campaigns, all samples were collected within the upper 1 m (surface) using 1 L HDPE sampling bottles that were pre-washed with 4% hydrochloric acid (HCl) via a pole-sampler to reduce contamination from the surface of the boat and engine coolant waters (Zhang et al., 2015). All samples to be analysed for nutrients were filtered through 0.4 μ m pore-size polycarbonate membrane filters (Whatman) into 100 mL bottles that were pre-rinsed with the filtrate. About 100 mL of the filtrate was collected in pre-acid washed polyethylene bottles. These samples were then killed with 10 μ L of concentrated mercury chloride, HgCl₂ and kept in a cool, dark room before chemical analyses. For chlorophyll *a*, the samples (250 – 1000 mL) were filtered through 0.7 μ m pore-size GF/F filters (Whatman) and carefully wrapped in aluminium foil before being immediately stored at -20 °C.

3.3.3 Nutrients Analyses

The concentrations for nutrients were determined in the laboratory utilizing a Skalar SAN^{plus} auto analyser (Liu et al. 2010). Total dissolved phosphorus (TDP) and total dissolved nitrogen (TDN) followed the methods of Grasshoff et al. (1999) using the aforementioned auto analyzer. The components of nutrients that were measured include: Nitrate (NO₃⁻), Nitrite (NO₂⁻), Ammonium (NH₄⁺), Dissolved Inorganic Phosphate (DIP), Dissolved Silicate (dSi), Total Dissolved Nitrogen (TDN) and Total Dissolved Phosphate (TDP). The sum of NO₃⁻, NO₂⁻ and NH₄⁺ were classified as dissolved inorganic nitrogen (DIN), whereas the concentrations of the dissolved organic phosphorus (DOP) and dissolved organic nitrogen (DON) were calculated by subtraction of DIP from TDP and DIN from TDN respectively. The analytical precision for all nutrients components measured was <5%. In order to analyse correlation between humic acids and DIP or DOP, dissolved organic carbon concentrations (DOC) were used as a proxy as part of the hydrophobic fraction of dissolved organic matter are generally derived from humic substances (Findlay et al. 2003). Lastly, for DOC concentrations the results were obtained from Martin et al. (2018), whereas SPM, PN and POC values were reported by Müller-Dum et al. (2019).

In this study particulate P was excluded from the total determination of P loading. While DIP is more biologically available as compared to particulate P (PP), Harrison et al. (2019) suggested that Particulate P is usually the dominant form of P that is being exported to the coastal areas. Thus, the bioavailability of particulate P should be further studied and modelled to better understand the significance of P loading model outputs. However, as suggested by Jordan et al. (2008), most of the biologically available DIP in estuaries is converted from fluvial PP which is enhanced by increasing salinities. Consequently, the DIP in estuaries could serve as a proxy for the PP that originated from headwaters and its importance can still be reflected in the concentration of biologically available DIP.

3.3.4 Chlorophyll a determination

Refer to Chapter 2.9

3.3.5 Data analyses

The spatial distribution of the physico-chemical parameters was plotted in Surfer 13 and all graphs were plotted utilizing Prism 6 (GraphPad Software, Inc). Averages of measured parameters were reported as \pm Standard Error (SE) unless stated otherwise. For statistical correlations, SPSS (IBM SPSS Statistics 22) was utilized for calculations of Independent sampling *t*-test (between seasons), one-way ANOVA (between source types) and Spearman's ranking (Bivariate correlation, for nutrients correlation).

3.3.6 Export calculations

For calculations of the discharge of the entire Rajang river, precipitation values for the entire Rajang river catchment were obtained from the Tropical Rainfall Measuring Mission (TRMM) website (NASA 2019). The precipitation values were converted into m³ from mm and multiplied by the conversion factor to obtain the discharge s⁻¹ and further multiplied with 60% (0.6) (Whitmore 1984) to obtain the discharge values after taking into consideration the surface run-off values. Furthermore, the value for the entire catchment area was derived from the values provided in Müller-Dum et al. (2019).

 $Discharge = Mean \ precipitation \times area \ of \ basin \ \times \ conversion \ factor \ to \ s^{-1} \ imes \ surface \ runoff \ percentage$

River loads for DIP and Si were calculated for the entire Rajang river with the assumption that the total loading from the headwaters at the Upper Rajang river (input) would equal the output (into the South China Sea). The average concentrations (μ mol L⁻¹) of the selected nutrient parameters were calculated based on the nutrient concentrations of the samples obtained at salinity $\equiv 0$ (Liang & Xian 2018). The average concentrations were then used for the estimation of river loads utilizing the equation provided in Müller-Dum et al. (2019) with slight modifications provided by the conversion factor from (International Council for the Exploration of Seas 2019).

The nutrient loads of Phosphate Phosphorus (PO₄-P) were obtained from DIP and were calculated based on the conversion factors (International Council for the Exploration of Seas 2019) whereby:

1 µg PO4
$$L^{-1} = 1 \div MW PO_4$$
 µg $L^{-1} = 0.010529$ µmol $L^{-1} = C$
 $C = \text{conversion factor for DIP}$
 $f = \text{conversion factor from s}^{-1} - \text{mth}^{-1}$
 $g = \text{conversion factor from g to t}$
 $d = \text{discharge (m}^3 \text{ s}^{-1})$

Hence, the equation for yield is:

 $t DIP mth^{-1} = Conc. of Average DIP \times C \times Discharge \times f \div g$

3.4 RESULTS

3.4.1 Physico-chemical parameters and nutrient concentrations

Table 3.1: Average values of measured parameter along the four source types (geographical distribution) of the Rajang river (mean \pm SE)

		Source Type (Mean ± SE)				
Parameters	Season	Marine	Brackish Peat	Freshwater Peat	Mineral Soil	
Temperature (°C)	Dry	31.10 ± 0.41 (n=3)	30.40 ± 0.21 (n=13)	30.00 ± 0.18 (n=4)	26.00 ± 0.17 (n=9)	
	Wet	30.25 ± 0.15 (n=2)	28.84 ± 0.31 (n=8)	27.76 ± 0.25 (n=5)	26.60* (n=1)	
Salinity (PSU)	Dry	31.50 ± 0.32 (n=3)	$15.40 \pm (n=13)$	$0.28 \pm (n=4)$	0.00*(n=9)	
	Wet	30.01 ± 0.01 (n=2)	14.52 ± 2.46 (n=8)	0.00 (n=5)	0.00* (n=1)	
Dissolved oxygen, DO (mg L ⁻¹)	Dry	$4.03 \pm 0.08 \text{ (n=3)}$	3.51 ± 0.16 (n=13)	3.68 ± 0.19 (n=4)	4.33 ± 0.13 (n=9)	
	Wet	$6.52 \pm 0.02(n=2)$	$6.01 \pm 0.27 \ (n=8)$	5.88 ± 0.36 (n=5)	5.96*(n=1)	
DIP(µM)	Dry	$0.17 \pm 0.05 \text{ (n=3)}$	0.11 ± 0.04 (n=13)	0.04 ± 0.01 (n=4)	$0.04 \pm 0.01(n=8)$	
	Wet	0.13* (n=1)	0.10 ± 0.01 (n=8)	$0.08 \pm 0.03 \ (n=5)$	0.06 * (n=1)	
DOP(µM)	Dry	$0.25 \pm 0.01 \text{ (n=3)}$	$0.25 \pm 0.01 \ (n=13)$	0.22 ± 0.02 (n=4)	0.20 ± 0.01 (n=9)	
	Wet	0.33 ± 0.04 (n=2)	$0.19 \pm 0.03(n=8)$	$0.10 \pm 0.02 \ (n=5)$	0.09 * (n=1)	
TDP(µM)	Dry	$0.42 \pm 0.04 \text{ (n=3)}$	$0.36 \pm 0.02 \ (n=13)$	0.25 ± 0.02 (n=4)	0.23 ± 0.01 (n=9)	
	Wet	0.42* (n=1)	$0.29 \pm 0.03 \ (n=8)$	$0.18 \pm 0.02 \ (n=5)$	0.16 * (n=1)	
Dissolved Inorganic	Dry	$11.36 \pm 1.69 \text{ (n=3)}$	21.86 ± 1.59 (n=13)	13.33 ± 1.14 (n=4)	$10.90 \pm 1.76 (n=9)$	
Nitrogen, DIN (µM)	Wet	$10.57 \pm 0.46 \ (n=2)$	$13.41 \pm 0.93 \ (n=8)$	$13.44 \pm 1.95 \ (n=5)$	10.34 * (n=1)	
dSi (μM)	Dry	4.63 ± 0.32 (n=3)	$80.50 \pm 12.96 \ (n=13)$	152.00 ± 3.13 (n=4)	$143.00 \pm 3.21(n=9)$	
	Wet	10.77 ± 4.78 (n=2)	$76.50 \pm 12.01 \ (n=8)$	$146.94 \pm 2.98 \ (n=5)$	157.00* (n=1)	
Suspended Particulate	Dry	49.30 ± 20.51 (n=3)	$86.10 \pm 11.06 \ (n=13)$	56.00 ± 12.76 (n=4)	$74.00 \pm 14.85 (n=9)$	
Matter, SPM (mg L ⁻¹)	Wet	55.47 ± 8.32 (n=2)	52.46 ± 6.27 (n=8)	$264.09 \pm 58.58 \ (n=5)$	226.73* (n=1)	
Dissolved Organic Carbon	Dry	$0.1\overline{833 \pm 0.0189}$ (n=3)	$0.2\overline{678} \pm 0.0151$ (n=13)	0.2355 ± 0.0119 (n=4)	0.2281 ± 0.0214 (n=9)	
(DOC) (mM)	Wet	0.0896 ± 0.0066 (n=2)	0.1452 ± 0.0066 (n=8)	0.1839 ± 0.0094 (n=5)	0.1253* (n=1)	

*Indicate only one (1) sample was available for calculations

The physico-chemical parameters of temperature (°C), salinity (PSU), dissolved oxygen, DO (mg L^1) and suspended particulate matter, SPM (mg L^{-1}) of dry and wet seasons were plotted along the Rajang River-South China Sea continuum (**Fig. 3.2**).



Fig. 3.2: Distribution of temperature (°C), salinity (PSU), dissolved oxygen, DO (mg L^1) and suspended particulate matter, SPM (mg L^{-1}) in the dry and wet season along the Rajang River-South China Sea continuum

Based on **Table 3.1**, the temperature in the dry season was 29.92 ± 0.20 °C whereas for the wet season the temperature was 28.54 ± 0.30 °C. For both seasons, the variation of temperature between the cruises was limited (**Fig. 3.2**). The full range of salinities from freshwater to marine water were sampled in both cruises, ranging from 0 to 33 PSU. In the dry season, dissolved oxygen ranged between 2.7 mg L⁻¹ to 4.9 mg L⁻¹ whereas in the wet season, the range was from 4.5 - 7.58 mg L⁻¹. The mean values for dissolved oxygen during the wet season were 6.03 ± 0.17 mg L⁻¹ as compared to the dry season with only 3.84 ± 0.11 mg L⁻¹. The SPM concentrations of both the dry and wet seasons decreased from headwaters (freshwater mineral soil) towards the coastal region (marine), with a range of 25.01 - 161.27 mg L⁻¹ in the dry season and 36.06 - 494.46 mg L⁻¹ in the wet season.

The nutrient concentrations of dissolved inorganic nitrate, DIN (μ M), dissolved organic carbon, DOC (mM) and dissolved silicate, dSi (μ M) are plotted in **Fig. 3.3**.



Fig. 3.3: Concentration of DIN (μ M), DOC (μ M) and dSi (μ M) in both dry and wet seasons along the Rajang River-South China Sea continuum

The range of DIN in both dry and wet seasons is from 7.1 to 28.7 μ M. However, the measured DIN concentrations for the dry season varied, with the highest mean occurring in the brackish peat 21.86 ± 1.59 μ M as compared to marine, freshwater peat and freshwater mineral soils (11.36 ± 1.69 μ M, 13.33 ± 1.14 μ M and 10.90 ± 1.76 μ M, respectively). In terms of DOC, the concentrations ranged from 0.08 to 0.40 μ M (Martin et al., 2018). For dSi, the range in the dry and wet season was from 4 –

179.1. The dSi concentration in the wet season had an average of 147.72 \pm 32.79 μM as compared to the dry season with an average 106.67 \pm 11.06 $\mu M.$

The concentrations of dissolved inorganic phosphate, DIP (μ M), dissolved organic phosphate, DOP (μ M) and total dissolved phosphate, TDP (μ M) are plotted in **Fig. 3.4**.



Fig. 3.4: The distribution of DIP (μ M), DOP (μ M) and TDP (μ M) concentrations in the dry and wet season along the Rajang River-South China Sea continuum


Fig. 3.5: (A) Distribution of DIP along salinity gradient in the dry and wet season and theoretical conservative line calculated based on integration. (B) Distribution of DOP along salinity gradient in the dry and wet season and theoretical conservative line. (C) Composition (%) of Phosphates in the Rajang River. (D) DIP composition based on different classifications/anthropogenic source (E) Dissolved inorganic phosphorus, DIP (μ M) and dissolved silicate, dSi (μ M) against salinity (PSU) (F) Dissolved inorganic phosphorus, DIP (μ M) and suspended particulate matter, SPM (g L-1) against Salinity (PSU) of surface waters along the Rajang river

From Fig. 3.4, the range of DIP is $0.0 - 0.27 \,\mu$ M. The overall range of DOP for both seasons is from 0.04 to 0.11μ M. Combining the two parameters (DIP and DOP), the concentrations of TDP ranged from $0.23 - 0.42 \mu$ M during the dry season and $0.16 - 0.42 \mu$ M during the wet season. Collectively, the range of TDP is from $0.13 - 0.53 \ \mu\text{M}$ across both seasons. The concentrations of DIP and DOP were also plotted along the integrated conservative mixing line against salinity (Fig. 3.5(A and B)). In terms of the DIP concentrations, both dry and wet season consistently increased from headwaters towards the coastal region with the mean concentrations of each source type ranging from 0.03 - 0.17 μ M whereas the wet season had mean concentrations of 0.06 – 0.13 μ M. On the other hand, DOP concentrations during the dry season were relatively stable with a mean concentration of 0.23 ± 0.01 uM. In contrast, the mean concentrations during the wet season increased from headwaters towards the coastal region $(0.09 - 0.33 \,\mu\text{M})$. In the dry season DIP is 26.16% and DOP 73.84% of the total TDP pool (Fig. 3.5(C)). In the wet season the DIP is 34.70% and DOP represents 65.30% of the total TDP. The average concentrations for DIP when they are classified under different land use are 0.11 ± 0.02 (coastal zone), 0.117 ± 0.019 (coastal zone with plantation influence), 0.087 ± 0.012 (oil palm plantation), 0.085 ± 0.027 (human settlement) and 0.032 ± 0.031 (secondary forest) (Fig. 3.5(D)). Based on Fig. 3.5(E) and Table 2, dSi and DIP were negatively correlated into both dry and wet seasons (-0.819 and -0.550, respectively). Lastly, there were no significant correlations between DIP and SPM in both dry and wet seasons. However, when plotted against salinity, it can be seen that the SPM decreases and DIP increases along the salinity gradient (Fig. 3.5(F)).

The reaction factor was calculated for both DIP and DOP with both seasons combined to obtain the average reaction factor. The reaction factors are given in **Table 3.2**:

Table 3.2: The calculated reaction factor and percentage addition or removal of DIP and DC	P along
the salinity gradient towards the South China Sea	

	DIP	DOP
Reaction Factor	0.58	2.27
Percentage Addition or Removal (%)	57.78% Removal	44.07% Addition

3.4.2 Nutrient Ratios across the Rajang River-South China Sea continuum

	Source Type (Mean ± SE)							
Nutrients Ratios	Season	Marine	Brackish Peat	Freshwater Peat	Mineral Soil			
	Drv	73.61 ± 12.55	203.36 ± 24.69	404.50 ± 62.45	$438.00\pm$			
ΠΙΝ·ΠΙΡ		(n=3)	(n=13)	(n=4)	83.11 (n=8)			
	Wat	77.73*	152.78 ± 19.01	265.60 ± 97.69	161.91*(n-1)			
	wei	(n=1)	(n=8)	(n=5)	101.81 (II-1)			
	D	17.74 ± 1.15	114.63 ± 16.35	209.19 ± 31.74	$229.39\pm$			
NO ₃ -N:DIP	Dry	(n=3)	(n=13)	(n=4)	40.63 (n=8)			
	Wet	29.93*	69.85 ± 11.78	199.49 ± 104.28	112.97*(n-1)			
		(n=1)	(n=8)	(n=5)	$112.87^{\circ}(n-1)$			
	Dura	31.86 ± 8.23	883.04 ± 206.16	4793.68 ± 923.36	$6615.26\pm$			
S: DID	Dry	(n=3)	(n=13)	(n=4)	1429.10 (n=8)			
SI:DIF	Wat	119.57*	897.00 ± 182.63	$4001.02 \pm$	2459*(n-1)			
	wet	(n=1)	(n=8)	2183.14 (n=5)	2438* (n=1)			
Si:DIN	D	0.42 ± 0.04	3.90 ± 0.81	11.71 ± 0.85	16.47 ± 1.71			
	Dry	(n=3)	(n=13)	(n=4)	10.47 ± 1.71			
	Wat	1.04 ± 0.50	5.40 ± 0.69	12.10 ± 2.12	15.19*			
	wei	(n=2)	(n=8)	(n=5)	(n=1)			

Table 3.3: Nutrient ratios of the selected parameters along four source types (mean \pm SE)

* Indicates only one sample

The DIN:DIP ratios were high throughout the Rajang River (**Table 3.3**), which can be correlated with the low DIP concentrations. The same trend can be seen for the other two nutrient ratios (Si:DIP and Si:DIN). In a study carried out by Liang & Xian (2018), the two components that were utilized were the NO₃-N:DIP as these two were the main components that were utilized or incorporated by phytoplankton for growth. Hence, for discussion in this study, the NO₃-N:DIP were utilized for discussions.

Davamatava	Dr	У	Wet		
rarameters	DIP	DOP	DIP	DOP	
DIP	N/A	0.237	N/A	0.416	
DOP	0.237	N/A	0.416	N/A	
DIN	0.476**	0.005	0.447	-0.282	
DON	-0.520**	-0.226	-0.631*	-0.427	
TDN	-0.081	-0.148	0.111	-0.466	
DOC	0.192	0.123	-0.563	-0.688**	
dSi	-0.819**	-0.328	-0.550*	-0.844**	
SPM	0.21	0.004	-0.014	-0.557*	
Sal	0.839**	0.453*	0.450	0.880**	
DO	-0.537**	-0.121	-0.207	0.413	

Table 3.4: Spearman's rank correlation of various parameters against DIP and DOP in the dry and wet seasons. Bolded values indicate statistical significance

** means significant at the 0.01 level (two tailed)

* means significant at the 0.05 level (two tailed)

Based on Table **3.4**, the parameters which were highly positively or negatively correlated with DIP in the dry seasons were DON, Silicate, Salinity and DO (-0.520, -0.819, 0.839 and -0.537, respectively) whereas for DOP in the dry season, none of the parameters were highly correlated. On the other hand, in the wet season, the parameters that were highly correlated with DIP were DON and Silicate (-0.631 and -0.550 respectively) whereas for DOP, the parameters that were highly correlated were DOC, dSi SPM and Salinity (-0.688, -0.557, -0.844 and 0.880 respectively).

3.4.3 Factors influencing phytoplankton biomass



Fig. 3.6: (A) Dissolved organic phosphate, DOP (μ M) and dissolved organic carbon, DOC in both wet and dry season (μ M) against salinity (PSU) (B) Concentration of CHLa (mg L⁻¹) and dSi (mM) in dry and wet season against salinity (PSU) (C) CHLa (mg L⁻¹) concentrations in the dry and wet and suspended particulate matter , SPM (g L⁻¹) against salinity

Wet
Chlorophyll a
0.189
0.691*
0.770*
0.815**
-0.223
-0.713**
-0.733*
-0.499
0.545

Table 3.5: Spearman's Rank correlation of Chl a in dry vs wet with selected parameters. Bolded values indicate statistical significance

* means significant at the 0.01 level (two tailed)

* means significant at the 0.05 level (two tailed

DOP was further plotted against DOC (**Fig.3.6(A**)) against the salinity gradient in which there is an observed trend whereby there is an increase in DOP with the decrease in DOC concentrations along the salinity gradient. **Fig. 3.6(B and C**) show that Chl *a* increased significantly with salinity only in the dry season, while SPM decreased drastically in the wet season and silicate decreased against the salinity gradient for both seasons. From **Table 3.5**, Chl *a* correlated positively with DIP and TDP in the dry season (0.562 and 0.631, respectively) and with DOP, TDP, Salinity in the wet season (0.692, 0.770 and 0.815, respectively). Chl *a* correlated negatively with dSi in both seasons (dry: -0.796, wet: -0.713) and with SPM in the wet season (-0.733).

3.4.4 Average Discharge of the Rajang River

Table 3.6: Average precipitation calculations over the month of the sampling campaigns (dry and wet) and the calculations for discharge

Season	Average precipitation (mm)	Average precipitation (m)	Days per month	Area of Basin (m ²)	Total Precipitation (m ³)	Precipitation (m ³ s ⁻¹)	Discharge after consideration of surface runoff) (m ³ s ⁻¹)
Dry	209.79	0.20979	21	5.20E+10	1.09E+10	4073.74	2444.24
Wet	338.68	0.33868	31	5.20E+10	1.76E+10	6576.49	3945.89

Discharge during the dry season (2444.24 m³ s⁻¹), as shown in **Table 3.6**, was below the annual average of 3355 m³ s⁻¹ as described by Müller-Dum et al. (2019) (or 3780.57 m³ s⁻¹ based on own calculation) whereas for the wet season, the discharge rate (3945.89 m³ s⁻¹) was below the annual estimated average of 4197.39 m³ s⁻¹ (own calculation).

3.4.5 P yield calculations and comparisons with other global peat-draining rivers



Fig. 3.7: The yield of DIP and the DIP:dSi ratio in selected blackwater rivers along increasing discharge (t DIP mth⁻¹). The dotted line represents the DIP:Si soil reference for the Rajang River (Funakawa et al. 1996)

River	Country	Catchment Size (km ²)	Discharge (m3 s ⁻¹)	Classification	DIP (µmol L ⁻¹)	DOP (µmol L ⁻¹)	dSi (µmol L ⁻¹)	DIN (µmol L ⁻¹)	Reference
Pearl River	China	453,700	10,464	Peat	0.43 - 1.44	0.58	138.3	112.6	Li et al. (2017)
Rajang	Malaysia	52,009	3600	Peat (11% of total)	0.002 - 0.26	0.14 – 0.32	4.01 – 179.00	7.10 - 28.68	This study
Amazon (Morth)	Brazil	6,300,000	180,000	Peat	0.7	-	144	-	Demaster & Pope (1996)
Dumai, Sumatra (Black water)	Indonesia	7,500	16	Peat	0.017 - 0.033	-	0.7	1.0	Alkhatib, Jennerjahn & Samiaji (2007)
Siak, Sumatra (Polluted Black water)	Indonesia	10,500	99 - 684	Peat (21.9)	0.2 - 36.7	-	1.6 - 89.1	7.9 - 67.9	Baum, Rixen and Samiaji. (2007)

Table 3.7: Comparison of nutrient concentrations of major global rivers or other peat-draining rivers vs. Rajang river (µmol L⁻¹)

Among the tropical/subtropical blackwater rivers compared (**Table 3.7, Fig. 3.7**), the highest yield was the Amazon River (31.02 t DIP mth⁻¹), followed by the Pearl River (2.65 t DIP mth⁻¹). Next, the Siak River had DIP yields of 1.78 t DIP mth⁻¹. The Rajang River and the Dumai River have yields of 0.12 t DIP mth⁻¹ and 0.00001 t DIP mth⁻¹, respectively.

3.5 Discussion

3.5.1 DIP sources and behavior

The concentrations of DIP increased from the headwaters (from mineral soils) to the coastal region along with salinity (F(3, 40)= 12.009, $\rho = 0.000$ (**Fig. 3.4** and **Table 1**). However, the difference in DIP concentrations between the dry and the wet season was not found to be significant (t(42)=-0.514, $\rho = 0.610$). The increase in DIP towards the coastal region can be explained by probable desorption of DIP from particles (Froelich et al. 1985, Fox 1990) as well as from estuarine and marine sediments (Caraco 1990, Pagnotta 1989), both caused by increasing salinities (Zhang & Huang 2011)

Non-conservative behaviour was observed in the dry season (Fig. 3.5(A)), indicating a constant removal of DIP towards the coastal region (average of 57.87% removal across both seasons, Table 3.2). This was similar to DIP behaviour shown in the Changjiang estuary (Kwon et al. 2018) which showed possible PO₄²⁻ removal within the estuary due to biological removal or buffering actions of suspensions and sediments of the estuary, the phosphate buffering mechanism. Furthermore, studies in Europe and North America (Lebo & Sharp 1992; Nixon et al. 1996; Sanders et al. 1997) also show large scale removal of DIP by suspended particles in estuaries. In the wet season, DIP showed nonconservative behavior as well. The varying DIP concentrations might indicate probable point sources of DIP. In another study by Ling et al. (2017) on the Rajang river, it was reported that the total phosphorus and SRP (DIP) was higher in the stations located at the upper part of river. However, this study was carried out only during the wet season and in tributaries different from this study. Hence, the values obtained could likely originate from point sources. Another possible explanation for the increase in DIP is due to the resuspension of sediments as shown by the higher SPM levels (Fig. 3.2) near the coastal region. Oenema and Roest (1998) stated that the bioavailability of P transported from land is only a fraction whereby its movement is dependent on the transport and mobilisation of soil particles (Jarvie et al. 1998; Stanley and Doyle 2002). Furthermore, as put forth by Stumm and Morgan (1996), 10% of naturally weathered phosphorus is only available to the marine biota in the form of orthophosphate (i.e. DIP). As shown in Fig. 3.5(D), it is likely that the concentration of dissolved inorganic phosphate originated from probable leaching from anthropogenic activities (from oil palm plantations) as well as desorption from sediments under increasing salinity (coastal zone). It is interesting to note that in a study by Funakawa et al. (1996) on peat soils in Sarawak, the concentrations of N and P were fairly high in the soil solution, even in those classified as oligotrophic peat, except for the concentrations of P adjacent to the centre of the peat dome. However, depletion of phosphate was observed during the rainy season at a sago plantation farm grown on deep peat which was associated with the clear-cutting of forests and the successive disruption in nutrient cycling. Thus, it can be inferred that the higher average DIP values in the wet season (Fig. 3.5 (C)) in this study were a result of probable run-off from the disturbed peat.

3.5.2 DOP sources and behaviour

With relation to the TDP (Fig. 3.5(C)), the DOP represents a significant percentage compared to the DIP pool. Even though there is mounting evidence that phytoplankton and/or zooplankton and even microbial populations are able to hydrolyze a considerable amount of DOP in natural waters (Chrost et al. 1986), many studies exclude DOP and it is hence infrequently measured. It is, however, of importance to consider DOP when assessing nutrient budgets and nutrient limitations (Monbet, McKelvie and Worsfold et al. 2009). It was shown that DOP (referred to as Filtrate Hydrolysable Phosphate) formed 85% of the Total Filterable Pool (Ellwood and Whitton, 2007) with DOP originating from the drainage of peat and underlying limestones. Both dry and wet seasons showed addition of DOP (44.07% addition, see Table 3.2) towards the coastal region (Fig. 3.5(B)). Based on the independent t-test, DOP differed slightly between dry and wet seasons (t(22.218)=1.777, $\rho = 0.09$) but was significantly different between source types (F(3,41)=3.927, $\rho = 0.015$). Furthermore, DOP concentrations were negatively correlated with DOC (-0.688, as shown in Table 3.4 and Fig. 3.6(A)) in the wet season which was in line with a study by Whitton and Neal (2011) who showed that DOC concentrations were low when the DOP pools were at its highest. Besides probable sources such as sewage effluents or agricultural soils, Whitton and Neal (2011) also showed that DOP pools in downstream sites might have originated upstream but have yet to be utilized by organisms or be hydrolysed by soluble phosphatases in the water. In the wet season, the concentrations of DOP exceeded that of the dry season (Fig. 3.6(A)), likely due to the higher run-off induced by higher precipitation during the sampling campaign. According to Nissenbaum (1979), it was estimated that 20-50% of the organic phosphorus reservoir in sediments are bound by humic acids. As a large proportion of peat is made up of humic substances (Klavins & Purmalis 2013), the draining of peat would then lead to the probable release of high amounts of DOP. However, the highest correlation of humic substances (DOC) was with DOP during the wet season (-0.688, see Table 3.4). A similar pattern was observed for DOC run-off from the peatlands (Martin et al. 2018) which was accelerated by higher precipitation as indicated in the steeper DOC gradient in the wet season in Fig. 3.6(A). suggesting probable higher DOP run-off as compared to DOC. This was in line with a prediction model (Harrison, Caraco & Seitzinger 2005) in which DOC:DOP ratios tend to be lower in regions with intensive agricultural activities.

3.5.3 Nutrient ratios and fate in the estuarine and coastal region

Generally, the ratios for NO₃N:DIP are extremely high (Table 3.3), indicating that the river is naturally low in phosphate, which could possibly be limiting nutrient in the Rajang river. According to Justić et al. (1995), P limitation could potentially occur when N:P is greater than 22. Based on the NO_3N :DIP ratios in the dry season, the ratio of 17.74 (1.15) is less than the aforementioned possible P limitation (when N:P>22) as suggested by Justić et al. (1995). Hence, the dry season is in favour of the Redfield's ratio of 16:1, indicating optimal conditions for phytoplankton growth as compared to the wet season. Si limitation occurs when Si:DIN is greater than 1 and Si:P is less than 10. In the Rajang River, the Si:P ratios were higher than the Redfield ratio across both seasons and source type. All Si:N ratios were greater than 1 across both seasons and source type except for the dry season (0.42 \pm 0.04, **Table 3.3**). Cloern (2001) and Kemp et al. (2009) highlighted that estuaries that are highly turbid, strongly mixed and that exchange high amounts of organic inputs from livestock production or watershed with agricultural activities will not exhibit a relationship between primary productivity and nitrogen. However, in this study, the NO₃N:DIP ratios differed between the dry and wet seasons, especially within the brackish peat region (Table 3.3). The NO₃N:DIP ratios were higher in the dry season as compared to the wet season. This could be due to the increased DIN concentrations in the dry season due to the decomposition of dissolved organic nitrogen as demonstrated by Jiang et al (2019). Furthermore, as shown in Fig. 3.2, the lower SPM levels in the brackish peat during the dry season led to the enhancement of light, which favours the growth of phytoplankton, which can be reflected in the increased Chl a concentrations (Fig. 3.6(B) and Fig. 3.6(C)). The uptake of DIP by phytoplankton may have led to the drawdown of DIP (Li et al. 2017). In estuarine zones, silicate is usually conservative whereby it is influenced mainly by the flux from dry to wet season (Zhang 1996). The highly negative correlation of Chl a with silicate (-0.796) and the positive correlation with DIP (0.562) in the dry season may explain the net removal of Silicate within the estuarine to coastal region by phytoplankton, i.e. diatoms, and the increase in Chl a is enhanced by the increased presence of DIP. Conversely, the intensity of ammonification and nitrification in the Rajang River was reduced during the wet season, which led to lower DIN concentrations as compared to the dry season (Jiang et al. 2019), thus reflecting the generally lower NO₃N:DIP ratios which were closer to but still not at the optimal Redfield ratio. Furthermore, Chl a was not correlated with DIP in the wet season (Table 3.5) as reflected in the higher NO3N:DIP ratios (Table 3.3) in the brackish peat region. This was identical to the scenario in the Chesapeake Bay where phytoplankton bloom was delayed due to higher rapid flushing in the wet season (Malone et al. 1988). When river flow was higher, the downstream mass transport of biomass was relatively more important versus production utilizing DIP as a source of biomass. In addition to that, during periods of high discharge (i.e. wet season), seaward advective transport driven by freshwater inflow prevents biomass accumulation due to its flow being faster than phytoplankton growth rate (Cloern et al. 2014). This can be further supported by the fact that there was almost a two-fold increase in SPM (**Fig 3.2**) during the wet season, which could have constrained phytoplankton production due to light attenuation and altered spectral quality sediments (Wetsteyn & Kromkamp 1994). Furthermore, during the wet season, the ratios for NO₃N:DIP were much lower than in the dry season (**Table 3.3**), with the exception of the marine region, which was possibly caused by higher run-off of phosphates or nitrogen from anthropogenic activities such as oil palm and sago plantation (**Fig. 3.5(D**)). As put forth by Tarmizi &Tayeb (2006), oil palm plantations require more phosphate rock fertilizer in the mixing of the Nitrogen (N):Phosphate (P):Potassium (K) ratios in order to compensate for the phosphates that are immobilized by the soils, implying that there is an abundance of phosphates within the agricultural soils. This would support the notion that greater runoff from higher precipitation during the wet season would lead to higher leaching of phosphates into the Rajang river. While Thevenot et al. (2010) illustrated that tropical soils are naturally poor in N and P compounds, intensive land-use changes such as deforestation will increase recalcitrant compounds which are readily decomposed. Furthermore, drained peatlands export more phosphorus than mineral soils after clear-cutting of peat forests, as peat has lower phosphate adsorption capacity (Cuttle 1983; Nieminen and Jarva 1996).

Numerous studies have shown the importance of DOP as a source of phosphorus (Bentzen, Taylor & Millard 1992; Boyer et al. 2006) in aquatic environments to support algal metabolism and growth when the other bioavailable P pools drop below critical threshold concentrations with regards to other requisite nutrients (Lin, Litaker and Sunda 2015). It is more advantageous for phytoplankton to utilize DIP as it can be directly taken up and assimilated, whereas DOP requires more energy (Falkowski & Raven 2013) as it requires phosphatases catalysing the hydrolysis of phosphate monoesters found within DOP compounds. Consequently, this would result in the liberation of inorganic phosphate as well as organic matter (Labry, Delmas and Herbland 2005). Thus, as the Rajang River has a greater pool of DOP as compared to DIP (Fig.3.5(C)), there is a probable switch in preference for DOP as compared to DIP depending on the concentrations of DIP or DOP. From Table 3.5, the change of Chl a being positively correlated with DIP to a correlation with DOP reflects a switch in the roles of DIP and DOP as the preferred phosphate sources for the phytoplankton biomass (refer to Chapter 5.3, Table 5.10 and 5.11). As described by Lin et al. (2016), the operational measurement of DOP is defined as the difference between TDP and DIP, thus polyphosphate esters and inorganic polyphosphate as well as two other DIP species, which are phosphite (PO₃³⁻) and phosphine (PH₃), are included operationally in the determination of DOP. This is reflected in the prediction of functional genes (see Chapter 4.4.5) which indicate the presence of phosphonate and phosphinate metabolism even though in low abundance.

3.5.4 Nutrient loads & Comparisons with worldwide systems: other peat and non-peat draining rivers

It should be noted that this paper discusses the estimation of P loads based on the freshwater inputs, which excludes addition and removal (fluxes) from the calculations. As reported by Statham (2012), while freshwater inputs in estuarine environments will frequently be exceeded by tidally driven fluxes of seawater, nutrients in river waters will typically have greater concentrations as compared to the adjoining seawater. While the estimated figures in t P mth⁻¹ (**Fig. 3.7**) are an underestimation due to the exclusion of particulate phosphates and sedimentary phosphates, they are still useful for estimation purposes.

Globally, the export of P from Rajang is comparatively minor when compared to other major rivers. When compared with other peat draining rivers in Southeast Asia, the Rajang river exports 1,178 times more t DIP mth⁻¹ than the Dumai river, which is a pristine peat-draining river, whereas the Rajang was 15 times lower than the Siak river (highly polluted blackwater river). When compared to the Amazon, the export of the Rajang river was 267 times lower. Considering another major anthropogenically influenced river draining into the South China Sea, the Pearl River (third largest river in China; Strokal et al. 2015), the Rajang exports about 23 times less.

Regarding the dSi:DIP ratios in the Rajang, while DIP yields were variable, their sources are likely anthropogenic in nature as dSi originates from natural chemical and physical weathering, which are relatively stable compared to riverine N and P loads (Beusen et al. 2009). In the Siak River, the DIP:dSi ratios were the highest; however, the yield of the Siak was lower than the Pearl or the Amazon River. The yields of the Siak River was lower than the Pearl River as a result of less discharge. However, the DIP concentrations by of the Siak River increased by 470% due to the domestic wastewater discharges. A similar pattern was observed in the Dumai River. While the DIP yields of the Amazon as well as the Pearl River were higher than that of the Rajang River, the DIP:dSi ratios were similar, indicating that the DIP yield in the Rajang River was likely anthropogenic in nature. The vast difference in DIP yields in the Pearl River was due to agriculture and industrial activities as well as sewage (Vitousek et al. 2009; Qu and Kroeze 2010; Maimaitiming et al. 2013). On the other hand, the DIP yield in the Amazon was the highest but was attributed to the high discharge which was about 18 times higher than the Pearl River (Table 5). Even though the addition as well as removal rate of both DIP and DOP is known, the P accumulation rate, which is largely dependent on several factors such as the sedimentation rate or bottom-water oxygen content, is largely unknown. By referencing the soil P:Si ratios (obtained from Funakawa et al. 1996) in a peat swamp forest along the Rajang River, it can be inferred that the Rajang River may be subjected to high burial and sedimentation of P, as reflected by the low DIP:dSi in the water column compared to the soil.

Since these estimations are only based on DIP exports, the actual P load of the Rajang River and its contribution to the adjacent South China Sea and global P loads should be determined to better inform government authorities for proper management of the Rajang river basin. As proposed by Jiang et al. (2019), the mild DIN input likely supports primary productivity within the region. Likewise, the P loads similarly contribute towards sustaining primary productivity and subsequently the fisheries industry (Ikhwanuddin et al. 2011).

3.6 Conclusion

This study represents an in-depth look into the nutrient dynamics of the Rajang river and its tributaries. The DIP concentrations in the Rajang River increased along the salinity gradient and were variable with source types but were not significantly different between seasons. Seasonality slightly exhibited for DOP but was significantly different between source types. Both DIP and DOP exhibited non-conservative behaviour, with DIP subjected to 57.78% removal whereas DOP was subjected to 44.07% addition along the salinity gradient towards the South China Sea. In the Rajang River, the bulk of the dissolved phosphate is from DOP (73.84%), in which both DIP and DOP may have contributed to the phytoplankton biomass. Spearman's correlations show that there was a switch in preference for DOP as compared to DIP depending on the concentrations of DIP or DOP due to seasonality. The complexity of DOP formation, supply and degradation is due to variation in origins such as river supplies, algal excretion, cell lysis etc. as well as variation in the degradation process of DOP (both enzymatic and chemical). Much of this requires further examination. During the dry season, the NO₃N:DIP ratios were lower, which were ideal conditions for phytoplankton proliferation, while in the wet season, the increased NO₃N:DIP ratios led to lower phytoplankton biomass. In terms of export loads of P, while the Rajang River exports more DIP compared to Dumai (a pristine peat draining river), it is much less compared to the Pearl and the Amazon rivers. In order to further understand the dynamics of phosphorus on the Rajang River and the coastal region, long term observations with higher frequency should be carried out. While the loading of P is not as extensive as other major rivers, including those that discharge into the South China Sea, with further understanding of the addition and removal rates of the P components as well as the sedimentation rates, more can be known about the contributions of P export from the Rajang River into the South China Sea, which is essential as a reference to improve regional as well as global P budget estimations.

Chapter 4 Biogeographical distribution of Microbial Communities along the Rajang River-South China Sea Continuum

Biogeographical distribution of Microbial Communities along the Rajang River-South China Sea Continuum

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4.1 Abstract

The Rajang River is the main drainage system for central Sarawak in Malaysian Borneo, eventually feeding into the southern South China Sea. Due to rapid development, there is mass discharge of organic matter into the Rajang River caused by logging and sand-mining operations. Its many tributaries also pass through peat domes where peat-rich material is fed into the system. Microbial communities found within peat-rich systems are important biogeochemical cyclers in terms of methane and carbon dioxide sequestration. To address the critical lack of knowledge about microbial communities in tropical (peat-draining) rivers, this study of the Rajang River aimed to (1) investigate the microbial community structure, diversity and probable function across wet and dry seasons, and (2) determine the underlying factors that may influence the spatial and seasonal distribution of the prokaryotic communities and the nutrient dynamics. This was carried out utilizing 16S rRNA gene amplicon sequencing via Illumina MiSeq in size-fractionated samples (0.2 and 3.0 \Box m GF/C filter membranes) covering different biogeographical features/sources from headwaters to coastal waters. Amplicon sequencing of 16S rRNA genes via Illumina MiSeq in size-fractionated samples (0.2 and 3.0 µm GF/C filter membranes) was utilized for the identification of free-living and particleassociated bacterial communities. The microbial communities found along the Rajang river exhibited taxa common to rivers (i.e. the predominance of β -Proteobacteria), while estuarine and marine regions exhibited taxa that were common to the aforementioned regions as well (i.e. predominance of α - and γ -*Proteobacteria*). This is in agreement with studies from other rivers which observed similar changes along the salinity gradients. In terms of particulate versus free-living bacteria, nonmetric

multi-dimensional scaling (NMDS) results showed similarly distributed microbial communities with varying separation between seasons. Distinct patterns were observed as a result of the changes in salinity along with variation of other biogeochemical parameters. Alpha diversity indices indicated that microbial communities were higher in diversity upstream compared to the marine and estuarine regions, whereas anthropogenic perturbations led to increased richness but less diversity. Despite the observed changes in bacterial community composition and diversity that occur along the Rajang River-to-sea continuum, the PICRUSt predictions showed minor variations. The present study represents the first seasonal assessment targeted at establishing a foundational understanding of the microbial communities of the Rajang River-South China Sea continuum. The results provide essential context for future studies such as further analyses of the ecosystem health in response to anthropogenic land-use practices and probable development of biomarkers to improve the monitoring of water quality in this region.

Keywords: particle-associated microbes, free-living microbes, 16S rRNA, River-sea continuum

4.2 Introduction

Biogeochemical transformations are primarily governed by microbial communities (Konopka 2009), and it is crucial to understand their dynamics in order to predict biosphere modulations in response to a changing climate. Despite the importance of freshwater to society and despite hosting the highest microbial diversity (Besemer et al. 2013), microbial community composition and diversity in freshwater habitats, especially in lotic environments, are much less studied compared to marine and soil communities (Kan 2018).

Lotic environments are the interface between soil and aquatic environments. Until not long ago, rivers were thought to be passive channels in the global and regional determination of carbon (C) and weathering products; then it became clear that rivers regulate, for example, the transfer of nutrients from land to coastal areas (Smith and Holibaugh 1993). Several studies have shown that bacteria are key players in nutrient processing in freshwater systems (Cotner and Biddanda 2002; Findlay 2010; Madsen 2011). Zhang et al. (2018a) stated that the organic matter composition and resistance to degradation are strongly modified by bacteria. Recent studies in the Rajang river have demonstrated that bacteria strongly influence the soil humic substances (Zhu et al., 2019) as indicated by high concentrations of D-form amino acids (Dittmar, Fitznar, and Kattner 2001). Moreover, it was demonstrated by Jiang et al. (2019) that Dissolved Organic Nitrogen was reduced to NH_4^+ via mineralization and ammonification, again highlighting the biogeochemical activity and the importance of microbes in the Rajang River. However, until now there has been no study on their diversity - a gap that this study aims to fill.

Next-generation sequencing technologies have enabled a better understanding of the rare or unculturable biosphere which traditional culture methods would not have been able to elucidate (Cao et al. 2017; Pough and Singh 2016). Only few studies assessing bacterial community composition have been undertaken in lotic/riverine environments (Zwart et al. 2002; Fortunato et al. 2012; Ladau et al. 2013), with even fewer focusing on the diversity of surface-attached biofilms in lotic environments, particularly in comparison to biofilm studies in benthic habitats (Zeglin 2015). Furthermore, bacterial assemblages on suspended particles were shown to differ from free-living bacterioplankton in a number of studies in which the ratios between both fractions were often influenced by the quality of suspended particulate matter (Bidle and Fletcher 1995; Crump, Armbrust and Baross 1999; Doxaran et al. 2012). Even fewer studies attempted to map bacterial community composition in a river-to-sea continuum across multiple seasons and habitats (Fortunato et al. 2012), and it was only recently reported that the most abundant riverine bacterioplankton resemble lake bacteria and can be regarded as 'typical' freshwater bacteria (Zwart et al. 2002; Lozupone and Knight 2007). Metagenomics studies substantiated the dominance of *Proteobacteria* and *Actinobacteria*, but *Bacteroidetes, Cyanobacteria, and Verrucomicrobia* were also found to be abundant in rivers

(Cottrell et al. 2005; Lemke et al. 2008; Newton et al. 2011; Staley et al. 2013; Kolmakova et al. 2014; Read et al. 2015). Crump and Hobbie (2005) and Fortunato et al. (2013) studied the freshwatermarine gradients of rivers, and Kanokratana et al. (2011), Mishra et al. (2014), Yule et al. (2016) and Too et al. (2018) studied tropical peatlands. But to the author's knowledge, this is the first study which links both freshwater-marine gradients as well as tropical peatlands as a cohesive system (i.e. tropical peat-draining river to coastal ecosystem). Due to the high diversity and fast generation time, the first responders to environmental changes (both natural and anthropogenic events such as storms, upwelling and pollutants) are microbial communities (Hunt and Ward 2015). Liao et al. (2019) show that extensive agricultural land-use in the inter-tidal region of a watershed resulted in the prevalence of bacteria pathogen-like sequences. Bruland et al. (2008) stated that the assemblages of microbes also vary temporally as a function of oceanographic conditions, river discharge, tidal phase and season. Thus, as the Rajang River experiences two monsoonal seasons (Sa'adi et al. 2017) and is subject to anthropogenic disturbances (Gaveau et al. 2016; Miettinen et al. 2016), it is thus fundamental to consider both seasonal and anthropogenic influences on the microbial communities of the Rajang River.

Lotic environments are the interface between soil and aquatic environments as terrestrial environments seed microbes into the adjacent water column (Crump et al. 2012). Thus, it is essential to understand the dynamics and structure of microbial communities in them to assess their contribution towards biogeochemical fluxes such as carbon and nitrogen (Battin et al. 2008; Raymond et al. 2013), as well as phosphate cycling (Hall et al. 2013). In addition, the fluxes as well as transformations of organic matter as well as nutrients in aquatic systems are environmentally driven by parameters such as temperature or the availability of nutrients in these ecosystems (Welti et al. 2017). In turn, various gradients (i.e. physical, chemical, hydrological or even biological) contribute to the changes in the microbial diversity and distribution living within the lotic environments (Zeglin et al. 2015).

Given the rapid development in Sarawak and the hypothesized importance of microbes in several biogeochemical processes in the Rajang river (Martin et al. 2018; Müller-Dum et al. 2019; Shan et al. 2019; Zhu et al. 2019), it is imperative to study the microbial communities to enable future predictions and management responses. The Rajang river offers the opportunity to study the microbial diversity along a river to sea continuum and at the same time assess influence of natural conditions such as seasons (dry *vs.* wet), different soil types (peat *vs.* mineral soil), as well as anthropogenic disturbances such as plantations. Linear models are used to examine the relationship between the microbial community structure and their environment. This study of the Rajang River aimed to (1) investigate the microbial community structure, diversity and probable function across wet and dry

seasons, and (2) determine the underlying factors that may influence the spatial and seasonal distribution of the prokaryotic communities and the nutrient dynamics.

4.3 Methodology



4.3.1 Study area and sampling strategy

Fig. 4.1: Location of Rajang River within Sarawak, Malaysia (inset). (A) shows the stations sampled during three (3) different cruises; August 2016 (red triangles), March 2017 (blue circles) and September 2017 (cyan diamonds). (B) GIS data from 2010 (Sarawak Geoportal, 2018) indicating various forest types. Red colour represents non-forest areas (2010), yellow represents non-forest areas (2013), light green represents primary forests, teal represents secondary forests whereas dark green represents potential peat swamp forests.

A total of 59 water samples were collected along salinity gradients during three (3) cruises (**Fig. 4.1(A**)), covering both wet and dry seasons as well as different source types (i.e. mineral or peat soils).

Source types sampled were grouped as follows: 1) marine, 2) brackish peat, 3) freshwater peat and 4) mineral soils. From Sibu towards Kapit (upriver), the riparian zone is mineral soil whereas from Sibu downwards to the coast it consists of peat which was then further divided into freshwater (salinity 0 to ~ 1 PSU) and brackish (salinity 2- 28 PSU) (as described in **Fig. 4.1(B)**). The cruise in August 2016 represented the highest sampling frequency in order to obtain complete coverage of representative regions, while the cruises in March and September 2017 were aimed to obtain seasonal representatives for each region. About 250 – 500 mL of water were filtered through 3.0 μ m pore size polycarbonate filters GF/C (Cyclopore, Whatman, Germany) via vacuum filtration. This was referred to as the 'Particulate-attached' fraction. The filtrate from the 3.0 μ m portion was collected in a sterile glass bottle and subsequently filtered through 0.2 μ m pore size polycarbonate (GF/C) filters (Cyclopore, Whatman, Germany). The smaller fraction was referred to as 'free-living' fraction. All filters (117 in total as 1 3.0 μ m filter was contaminated and discarded during the filtration process) were immediately stored at -20 °C and sent to the Australian Centre for Ecogenomics (ACE), Brisbane for processing utilizing Illumina platform (Caporaso et al. 2012).

4.3.2 Pyrosequencing and Bioinformatics Analyses

Initial processes were carried out by the Australian Centre for Ecogenomics utilizing the ACE mitag pipeline (ACE 2016). In short, fastq files generated from the Illumina platform were processed with fastqc, primer sequences trimmed with Trimmomatic, and poor quality sequences removed using a sliding window of 4 bases with an average base quality of more than 15. Subsequent processing steps were then performed utilizing the mothur pipeline. Sequences were aligned against the SILVA alignment (Quast et al. 2013, Yilmaz et al. 2014), 'pre.cluster' command executed for denoising, and chimeric sequences removed using the 'chimera.vsearch' function. Chimera-free 16s rRNA bacterial gene sequences were taxonomically assigned against the EzTaxon database (Kim et al. 2012) using the Naïve Bayesian classifier with a threshold of 80%. The quality-filtered sequences were then clustered into operational taxonomic units (OTUs) at 97% similarity cutoff with singleton OTUs being omitted. In order to reduce bias caused by variations in sample size, high-quality reads were randomly subsampled to 923 reads per sample. The alpha diversity was calculated using the phyloseq package R (v.3.5.3). For the analyses of functional genes, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt, Langille et al. 2013) was utilized. The metagenomics prediction table produced from PICRUSt was utilized to produce pathway abundance profiles using HUMAnN2 (Franzosa et al. 2018). It should be noted that the reconstructed functional genes were based on the GreenGenes database and not the EzTaxon database used for the phylogeny.

4.3.3 Physico-chemical Data and Geochemical Analyses

Monthly precipitation for the period in between the cruises (August 2016 to September 2017) was obtained from the Tropical Rainfall Measuring Mission website (NASA 2019) in order to gauge the seasonality (wet or dry; **see Fig. 2.3**, **Chapter 2.1.2**). The analyses for nutrients encompassing both inorganic (i.e. Nitrate, NO₃⁻, Nitrite, NO₂⁻, Ammonium, NH₄⁺, Phosphate, PO₄⁻ and Silicate, SiO₄⁴⁻) and organic (dissolved organic nitrate, DON, and dissolved organic phosphate, DOP) fractions were photometrically determined utilizing a SKALAR San^{plus} continuous flow analyser in the State Key Laboratory for Estuarine and Coastal Research (SKLEC), Shanghai (details described in **Chapter 2.7**). NH₄⁺ and PO₄³⁻ were determined manually following Grasshoff et al. (1999), while Total Dissolved Nitrogen, TDN, and Total Dissolved Phosphate, TDP, were determined indirectly by obtaining the values for NO₃⁻ and PO₄³⁻ via oxidation with boracid-acid-persulfate solution (Ebina et al. 1983).

4.3.4 Statistical Analyses and distLM model

Refer to Chapter 2.10 for statistical analyses and distLM model.

Ordination visualization, non-metric multidimensional scaling (NMDS), and similarity analyses (ANOSIM) were executed using PRIMER 7 (Clarke and Gorley 2015) to determine if for example the various terrestrial source types or different land uses determine the structural differences of the bacterial community. By partitioning the community variation, distance-based linear models (DistLM) were used to determine the extent to which the bacterial community structure can be explained by environmental variables (Legendre and Anderson 1999). Normalizing transformations of the environmental variables were carried out prior to execution of DistLM analyses. Hellinger Transformed OTU abundance table was used as the response variable for the variation partition analysis. The authors would like to note that the distLM models are based on only the August 2016 and March 2017 cruise as there was a lack of physico-chemical data from the September 2017 cruise due to malfunctioning equipment. However, it is sufficient to draw linkages between the major drivers of microbial communities between seasons as March 2017 and September 2017 were considered wet seasons based on the average precipitation (see Fig. 2.3). Multi-collinearity between variables was tested utilizing the 'Draftsman Plot' function in Primer 7 (Clarke and Gorley 2006; Supplementary Fig. 1).

4.4 Results

4.4.1 Clustering of Samples according to ANOSIM Global Test Scores

Parameters tested, 999 permutations, random sampling	ANOSIM Global Test, R	P value
Cruise (Wet/Dry season)	0.439	0.001
Source Type	0.422	0.001
Land use	0.182	0.001
Particle Association	0.037	0.001
Source Type, Land use	0.415	0.001
Cruise, Source Type, Particle Association,	0.708	0.001
Cruise, Source Type, Land use	0.737	0.001

Table 4.1: ANOSIM Global Test scores based on various parameters

74,690 high quality bacterial sequences were obtained from a total of 117 samples, with 200 to 2,615 sequence reads per sample. The sequences were clustered into 2,087 OTUs at the 97% confidence interval. Instead of displaying bacterial diversity by station, bacterial communities were grouped together according to the R scores obtained from the ANOSIM Global test, with the parameters 'cruise', 'source type' and 'land use' showing the highest scores (ANOSIM Global R = 0.737, P < 0.001, **Table 4.1**).

4.4.2 Shifts in bacterial community structure

The NMDS graph (2D stress score: 0.18, **Fig. 4.2**), supported ANOSIM results by clustering samples according to (i) source type and land use as well as (ii) cruises.



Fig. 4.2: Non-metric Multi-dimensional Scaling (NMDS) graph of samples according to cruise, source type as well as land use.

The X axis (MDS1 scores) clearly reflects changes in terms of salinity (river-sea continuum) while the Y axis (MDS2 scores) emulates the different cruises. It is apparent that there were seasonal variations as shown from the lighter shade points, representing the August 2016 samples, compared to those with darker shades representing both March 2017 and September 2017 samples (**Fig. 4.2**). There are apparent overlaps of samples from mineral soil and brackish peat origin. It can also be observed that there is a gradual shift of samples from mineral soils and freshwater peat towards brackish and then marine samples, with evident transitioning between samples.



Fig. 4.3: Non-metric Multi-dimensional Scaling (NMDS) diagram of seasonal (August 2016, March 2017 and September 2017) and particle association (particle-attached or free-living)

Seasonality was observed within the three cruises irrespective of the particle association (**Fig. 4.3**). The August 2016 cruise was found to cluster with the September 2017 whereas the March 2017 cruise clustered separately from the other two cruises. However, it can be seen that there is greater partitioning of free-living and particle-associated samples in the March 2017 samples.

4.4.3 Bacterial Distribution according to source type and cruise

To further investigate whether the four different source types support distinct bacterial communities, the relative abundance was mapped into a percentage plot (Fig. 4.4).



Fig. 4.4: Relative abundance (%) of dominant bacterial (at phylum level, top 10) along the various source types (Marine, Brackish Peat, Freshwater Peat, Mineral Soils) across 3 cruises/seasons

Fig 4.4 shows that the phylum Deinococcus-Thermus was abundant in freshwater peat and in mineral soils, albeit to a lesser extent compared to freshwater peat. Taking seasonality into consideration, the relative abundance (%) of Deinococcus-Thermus drastically decreased in September 2017. On the contrary, the abundance of Cyanobacteria was greater within marine as well as brackish peat for the cruises of March 2017 and September 2017 but not for August 2016. For the August 2016 cruise, Cvanobacteria were found throughout all source types albeit at lower counts compared to the other cruises. Similar changes in bacterial communities were observed during different cruises but at different sections of the river. For the marine and brackish peat portions, the cruises of March 2017 and September 2017 were more similar to each other than to the August 2016 cruise, with the anomaly of the *Bacteroidetes* phylum. On the other hand, for the freshwater peat and mineral soils, the cruises of August 2016 and March 2017 had greater resemblance. Furthermore, there was a distinct split in terms of the bacterial community composition for the four source types across all sampling cruises, i.e. marine and brackish peat had similar composition and freshwater peat and mineral soils had similar composition. In terms of a river-sea continuum, the most apparent changes in the community composition were observed during March 2017, which presented an almost step-wise change in bacterial community composition.



The diversity of *Proteobacteria* was examined in more detail as it was the predominant phyla regardless of source type (Fig. 4.5).

Fig. 4.5: Relative abundance (%) of dominant classes of *Proteobacteria* along the various source types (Marine, Brackish Peat, Freshwater Peat, Mineral soils) across 3 sampling cruises

In the marine region, the abundance of α -*Proteobacteria* was higher than β -*Proteobacteria*. However, γ -*Proteobacteria* were found to be the predominant class in the marine and brackish peat regions in the March 2017 samples as well as the marine region for the September 2017 samples. It was also shown that the β -*Proteobacteria* was the predominant class of *Proteobacteria* in the August 2016 as well as September 2017 samples. However, in the March 2017 samples the proportion of γ -*Proteobacteria* was greater than that of α -*Proteobacteria* within the freshwater peat and the mineral soils region.

The separation of groups was also shown down to the genus level as shown in the heatmap (Fig 4.6) whereby the marine and brackish peat groups are distinct from the freshwater peat and mineral soil, with the exception of the groups that fall in the marine and brackish peat for September 2017.



Fig. 4.6: Heatmap of the bacterial community composition (OTU reads, genus level). The relative abundance of each taxon is indicated by the intensity of the colour ranging from black (indicative of 0) to white (500) with the green scale as the values in between.

The heatmap also showed different distribution pattern for i) the sampling cruise as well as ii) the different source types. *Salinimicrobium* for example was present in August 2016 in the marine and brackish peat samples but absent from freshwater peat and mineral soils. Similar patterns were observed for *Erythrobacter*, *Sphingomonas*, *Psychrobacter*, and *Bacillus*. On the other hand, *Deinococcus*, *Exiguobacterium*, and *Masilia* were the major genera present in freshwater peat and mineral soil.

4.4.4 Alpha Diversity Indices



Fig. 4.7: The calculated α -diversity indices (Observed, Chao1, Shannon, Simpson and Inverse Simpson) of the four different source type along the salinity gradient.

Based on the Observed indices (**Fig. 4.7**), mineral soils generally had the highest counts of unique OTUs. However, during the September 2017 cruise, the freshwater region had the highest values. Based on the Chao1 indices, there was a significant effect of the source type on the observed richness (p<0.001), with increasing values from marine to mineral soils. In the March 2017 and September 2017 cruise, the Chao1 indices were found to have greater variability as compared to the August 2016 cruise. For the September 17 cruise, the values for Chao1 across the brackish peat, freshwater peat as well as mineral soils were all observed to have increased values of Chao1. According to the Shannon indices, the diversity of the microbial communities were significantly different along the different source types (p<0.001). In the dry season the Shannon indices were found to be higher than that found in March 17 and September 2017 samples, except for the Brackish peat September 2017 samples. In terms of the Simpson diversity indices, the August 2016 season was found to have the higher values as compared to the March 2017 and September 2017 season.



Fig. 4.8: The calculated α -diversity indices (Observed, Chao1, Shannon, Simpson and Inverse Simpson) of the Land Use types (Coastal Zone, Coastal Zone with Plantation (OP) influence) Coastal Zone with Plantation (Sago and Oil Palm influence), Human Settlement, Oil Palm and Sago mixed Plantation, Oil Palm Plantation and Secondary Forest)

Based on the effects of land use on the diversity indices (**Fig. 4.8**), the sites which are surrounded by human settlements had higher observed indices (regardless of the cruise), with the exception of the Shannon indices in August 2016. Samples surrounded by secondary forest had the second-highest values, with samples from August 2016 repeatedly higher than the other two cruises. There were significant differences (p<0.001) between samples from the coastal region with generally lower indices compared to upstream samples.

4.4.5 Functional Profile of Bacterial Communities



Fig. 4.9: The relative abundance of predicted functional profiles in the four source types across two seasons based on KEGG Pathways

Based on the KEGG pathways (Fig. 4.9), the functional profiles of the microbial communities were predicted for the August 2016 and March 2017 samples. The metabolic pathways that were selected were based on the active pathways that were exhibited, including the metabolism of Nitrogen, Carbohydrate, Methane and Sulfur metabolism. The main functions found were oxidative phosphorylation (20.09%), carbon fixation pathways in prokaryotes (19.00%) and methane metabolism (18.36%), respectively. These were followed by nitrogen metabolism (11.50%), carbon fixation in photosynthetic organisms (7.67%), and inorganic ion transport and metabolism (5.68%). The remaining functional groups were photosynthesis (4.92%), sulphur metabolism (4.31%), inositol phosphate metabolism (2.96%), phosphotransferase system (PTS, 2.34%), carbohydrate metabolism (1.83%), phosphonate and phosphinate metabolism (1.11%) and lastly mineral absorption (0.23%). From Fig. 4.9, it can be seen that the functional gene profiles that were derived from the metagenomic profile were very similar. This was similar to a study by Fortunato & Crump (2015) who observed that the average similarities of the functional gene profiles were 82% from river to ocean. In terms of gene abundances, the March 2017 samples (wet season) were found to have higher gene abundances with the highest counts in brackish peat followed by marine samples. However, marine samples in August 2016 displayed slightly higher gene counts compared to the brackish peat.
4.4.6 Distance-based Linear Model of bacterial communities and environmental parameters

Category	Variable	Pseudo-F	<i>P-</i> value	Proportion
explained by each predictor	r variable using two c	cruises (August 20	16 and March 201	7)

Table 4.2: Proportion of combined community variation based on marginal DistLM test that is

Category	Variable	Pseudo-F	<i>P</i> -value	explained (%)
Dhusiaa ahamiaal	Salinity	9.6128	0.001	13.42
Physico-chemical	Dissolved oxygen	6.6151	0.001	9.64
parameters	SPM	4.3486	0.001	6.55
	DIP	4.2218	0.001	10.57
Biogeochemical	Silicate	9.269	0.001	9.27
parameters	DOP	5.4246	0.001	8.04
	DON	4.2218	0.001	6.37

Marginal DistLM was performed in order to gauge the extent of physicochemical parameters or environmental variables accounting for a compelling proportion of variation in the bacterial communities. Salinity was the single best predictor variable explaining bacterial community variation (15.27%), followed by Dissolved Inorganic Phosphate at 10.57%. The remaining physico-chemical parameters were dissolved oxygen (9.64%) and Suspended Particulate Matter (6.55%) whereas for the biogeochemical parameters, Silicate (9.27%), Dissolved Organic Phosphate (8.04%), Dissolved Organic Nitrogen (6.37%), Dissolved Organic Carbon (5.27%) and lastly Dissolved Inorganic Nitrogen (4.29%) made up the remaining variables (all variables P = 0.001, except for DIN, P=0.002).

Significant vectors of environmental variables (R^2 >0.3892, P <0.001) were calculated based on a linear model (DistLM) and plotted against the bacterial community composition as shown in **Fig 4.10**.



Fig. 4.10: Distance-based Redundancy Analysis (dbRDA) plot based on a linear model (DistLM) and plotted against the bacterial community composition.

From **Fig. 4.10**, the distLM model clustered samples from the August 2016 cruise away from the samples of the March 2017 cruise (as seen from the plot points with lighter shades as August 2016 and darker shades as March 2017). Samples originating from the brackish peat as well as marine region (August 2016) irrespective of land use were shown to cluster more strongly towards salinity (as shown from the longer vector from salinity) as well as DIN and DOP, followed by DIP. On the other hand, the brackish peat and marine samples from the March 2017 were found to cluster in between DIP and DO. In addition, the samples from August 2016 for freshwater peat and mineral soil -irrespective of land use- clustered towards silicate and DON whereas for March 2017, the samples were shown to cluster towards the SPM vector. Lastly, it was found that samples which are of peat origin were also adjacent to the DOC vector.

4.5 Discussion

This study presents seasonal and spatial distribution of particulate-attached and free-living bacteria in the longest river in Malaysia in an attempt to map the bacterial community composition of the water column across several habitats with relation to the riparian zones and anthropogenic activities in a river-to-sea continuum. Our dataset allows comparison of the microbial community across two dimensions: 1) spatial biogeography from headwaters to the coastal zone, and 2) through time (seasonally). The rich supporting dataset also allows us to assess underlying nutrient dynamics influencing the microbial communities.

4.5.1 General bacterial community composition

The core microbial communities along the Rajang River-South China Sea continuum consist of *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Deinococcus-Thermus* and *Cyanobacteria* in varying abundances (**Fig. 4.4**), indicating high variation within the system. Staley et al (2015) proposed that variability in microbial communities are due less to the presence/absence, but likely due to shifts in relative abundance of OTUs. As shown in **Fig. 4.4**, the bulk bacterial taxa were restricted to a relatively small number of assemblages. However, due to the heterogeneity of the Rajang River, substantial shifts in OTU diversity were shown. While exhibiting successional changes in community composition downstream, there were abrupt shifts in terms of richness, diversity and bacterial distribution, which was structured according to macro-scale source types.

4.5.2 Diversity and shifts in bacterial communities along the Rajang river-South China Sea continuum

The predominance of the *Proteobacteria* phylum, especially within the brackish peat region (**Fig. 4.4**) was similar to a recent study on the Pearl River Delta (Chen et al. 2019). In another study by Doherty et al. (2017) on the mainstem of the Amazon River (a blackwater influenced river, similar to the Rajang River), *Actinobacteria* were much more abundant (25.8%) compared to the Rajang River (11.95%). However, the second-most abundant taxon were the *Proteobacteria* (β -*Proteobacteria*) which peaked during seasons of high discharge. The same pattern of peaking during high discharge can be observed in the Rajang River with considerably higher relative abundance in the wet season (**Fig. 4.4**). This could be a result of the intense rainfall that led to the large input of freshwater (Silveira et al. 2011), and ultimately resulting in a "trickling" over microbial pattern from the freshwater to the brackish region. The predominance of β -*Proteobacteria* in the freshwater region and the predominance of α - and γ -*Proteobacteria* (**Fig. 4.5**) in the estuarine region is typical as the main group in seawaters (Nogales et al. 2011) and similar to findings by Silveira et al. (2011) on the bacterioplankton community along the Parnaioca River continuum towards the Atlantic Ocean. Hence, this shows that salinity exhibited a strong influence on the abundances of *Proteobacteria* and *Firmicutes*.

Among the proteobacterial classes, γ -Proteobacteria was the most dominant, followed by α -*Proteobacteria*. The high abundance of γ -*Proteobacteria* is in line with Fuchsman et al. (2012) which states that the group is commonly regarded as particle-associated bacteria. When compared across the river-to-sea continuum, the low abundance of β-Proteobacteria is in contrast to other literature (Ghai et al. 2012; Brown et al. 2015), as the majority of freshwater systems have β -Proteobacteria as the most dominant taxa. This was a because the determination of the Proteobacteria phylum on the Rajang takes into account the estuarine as well as the marine regions. The phylum Proteobacteria was dominant in all the samples, indicating its role in nitrogen cycling (Yang et al. 2013). The presence of Proteobacteria is complementary to the Cyanobacteria blooms which occur as evidently shown in Fig. 4.4. Furthermore, the higher presence of Chloroflexi (Ward et al. 2018) and Cyanobacteria (Guida et al. 2017) within the marine and brackish peat regions - as reflected by the higher gene counts (carbon fixation pathways in prokaryotes) in those regions as compared to the freshwater peat and mineral soil (Fig. 4.9) - indicated their probable role in carbon fixation. Furthermore, the presence of the genus Sphingomonas indicated the presence of purple-sulfur bacteria which were able to utilize carbon dioxide (carbon fixation pathways in prokaryotes) and oxidation of hydrogen sulfide (sulphur metabolism, Fig. 4.9) (Pfennig 1975). The higher abundance of Firmicutes in the brackish region was reflective of the overall production as opposed to selective growth of the particular source type, as Firmicutes were found throughout all four source types. The highest presence of Deinococcus-Thermus (Fig. 4.4) was found in freshwater peat environments, indicating its preference for the aforementioned environment. This is interesting to note as most studies on bacterial community composition show that the phylum *Deinococcus-Thermus* occurs in a higher abundance in extreme environments such as hot springs (Zhang et al. 2018b) or in environments that are analogous for Mars (2019). In contrast, *Deinococcus-Thermus* was found in low percentages in extreme environments such as Antarctic marine environments (1%, Giudice & Azzaro 2019), or hypersaline soils (1.5%, Vera-Gargallo et al. 2019). Considering the major genera, there is a fundamental shift in bacterial community composition along the continuum (Fig 4.4, Fig. 4.7). Together with the bacterial richness and diversity indices, there was a distinct difference between the dry season (August 2016) and both wet seasons, with September 2017 having higher observed indices, while March 2017 had lower or variable observed indices. This difference in the two wet season samples could be due to the different stages of phytoplankton bloom as mentioned earlier; September 2017 was during an algal bloom while March 2017 was after an algal bloom event. This was reflected in the Simpson index as well as the indices for September 2017 being lower than those of the August 2016 or March 2017 samples. Similarly, Zhou et al. (2018) demonstrated that the Simpson Indices for bacteria increased after the onset of an algal bloom (Brackish peat, September 2017) whereas the Shannon indices were at the lowest (Brackish peat, March 2017), assuming that the region in which phytoplankton blooms occur is the brackish peat region. Overall, there was greater diversity (based on Shannon Indices) in the dry season (August 2016) than the wet seasons (March and September 2017), whereas there were greater OTUs in the wet season (Observed index). The decrease in richness and evenness was similar to a study conducted by Savio et al. (2015) in which the bacterial evenness and richness declined downriver, which is in line with the River Continuum Concept (Vannote et al. 1980). The presence of peat did not affect the alpha-diversity indices; this is reflected in the shift in taxa occurring from freshwater (which includes freshwater peat) towards the saline region (which includes brackish peat). Dominant phyla such as *Proteobacteria*, typically found in Malaysian peat swamps (Kanokratana et al. 2011; Tripathi et al. 2016; Too et al. 2018), are found throughout the Rajang river, whereas *Acidobacteria* is not a major phylum in the Rajang river.

4.5.3 Factors determining bacterial community composition

While there is difficulty in assessing microbial communities in lotic environments due to the heterogeneity of the physicochemical parameters that lotic environments are subjected to (Zeglin 2015), the major drivers of microbial communities should still be assessed. While only two cruises (August 2016 and March 2017) were used due to the lack of physico-chemical data for the September 2017 cruise, it is sufficient to draw linkages between the major drivers of microbial communities between seasons (see Fig. 2.3, Chapter 2.1.2). As shown in Fig. 4.2, it can be observed that there is a continual shift in microbial communities, suggesting mixing of the communities from the headwaters to the coast (Fortunato et al 2012), which has also been observed along the Upper Mississippi River (Staley et al. 2015) and along the Danube River (Savio et al. 2015). Based on the linear model (Fig. **4.10**), salinity is an important factor in driving the shift in microbial communities (**Table 4.2**), akin to findings by Herlemann et al. (2011) along a 200 km salinity gradient in the Baltic Sea. The dispersal of taxa of microbial communities from fresh to marine waters faces a strong barrier due to salinity (Fortunato and Crump, 2015), likely explaining the reduced relative abundances of Chloroflexi upstream and in turn the reduced *Deinococcus-Thermus* downstream (Fig. 4.4). Such dispersals are further influenced by transitional waters such as estuaries and plumes where the microbial communities are exposed to rapidly changing physico-chemical conditions such as salinity gradients, nutrients, temperature as well as sporadic anthropogenic inputs (Crump et al. 2004). While the distribution of the core microbial communities are indicative of the river-sea continuum, it is noteworthy that several phyla were distinctly associated with specific source types. The distinct shift in bacterial taxa for example from freshwater to brackish waters (and lack thereof between freshwater peat and brackish peat; Fig. 4.4) indicates that peat did not have a significant effect on the distribution of bacterial taxa. This is further supported by the fact that DOC (as a proxy for organic matter of peat origin) only accounts for 5.27% of the community variation (Table 4.2). A study on blackwater rivers in the Orinoco Basin, Venezuela (Castillo et al. 2004) showed that increased DOC resulted in higher bacterial production. However, the change in bacterial production is not a reflection of its influence on the community composition. This was supported by a simple respiration experiment conducted in August 2016 (Supplementary **Table 1**), whereby the respiration rate $(0.44 \pm 0.16 \text{ g DO } \text{L}^{-1} \text{ d}^{-1})$ was higher than that of the primary production rate $(0.39 \pm 0.08 \text{ g DO } \text{L}^{-1} \text{ d}^{-1})$.

According to Peter et al. (2011) and Wilhelm et al. (2015) salinity, DIP (biogeochemical parameter) and Dissolved Oxygen (physical parameter) had major impacts on the distribution of species. This is neatly supported by the distribution of samples on the distLM fitted dbRDA graph (Fig. 4.10). The affinity for each of the samples correlates to the physical environment, e.g. the samples which group along the salinity vector were those which correlate with the marine as well as brackish peat regions. Samples influenced by dissolved oxygen (Fig. 4.10) are from the estuarine region which showed an almost anoxic zone (refer to Fig. 3.2). The low availability of oxygen is mirrored in higher counts; samples belonging to the brackish peat category showed highest counts regardless of phyla as well as season; Supplementary Fig. 2. However, higher counts (particularly Chloroflexi and Cyanobacteria) do not reflect higher primary production within this zone. While zones of coastal estuaries are usually deemed to have higher primary productivity, it can be inferred that the depletion in oxygen and higher pCO₂ emissions (Mueller-Dum et al. 2019) within the brackish peat region of the August 2016 campaign was a result of high bacterial productivity. This can be further supported by the high suspended particulate matter (SPM) as a proxy for turbidity of the brackish peat (Fig 3.2) which may have resulted in the reduced primary productivity, which in turn can explain the lower dissolved oxygen values. As mentioned earlier, the respiration rate $(0.44 \pm 016 \text{ g DO L}^{-1} \text{ d}^{-1})$ was higher than that of the primary production rate $(0.39 \pm 0.08 \text{ DO L}^{-1} \text{ d}^{-1})$. This was similar to a study in the Scheldt river, where the higher bacterial production occurred in the turbidity maxima together with the depletion of oxygen (Goosen, Rijswijk & Brockmann 1995). However, the relative abundance of bacterial OTUs were higher in the estuary as well as marine region, reflecting that while the microbial communities are structured by salinity, their abundance is more a reflection of the nutrients available, especially in estuaries which exhibit circulation patterns which can result in localised nutrient-rich conditions (They et al. 2019). This was supported by the higher relative abundance of oxidative phosphorylation genes as well as nitrogen metabolism within the brackish peat, and by Jiang et al. (2019), who demonstrated through incubation studies that N transformations in the Rajang estuary mixing zone was higher than in the Rajang River and coastal region.

While the development of unique community structures is strongly influenced by spatial factors, an influence of seasonality could also be observed with samples from March 2017 being distinctly different from the other two cruises (August 2016 and September 2017; **Fig. 4.3**). Seasonal variability was also observed between the source types, particle association and down to the genus level (**Fig 4.2**, **Fig 4.3** and **Fig. 4.6**)). Based on the precipitation as an indicator of the seasonality, a probable "transitioning" phase was observed in the dry season (August 2016) with the microbial communities being more alike with the March 2017 samples (**Fig. 4.4**) than with the September 2017 ones. Within

the phylum rank (Fig. 4.4), the presence of *Cyanobacteria* during the March and September 2017 cruises indicates the influence of seasonality. However, while March 2017 and September 2017 were both considered to be wet seasons based on the precipitation, in terms of the relative abundance there are considerable differences between the two cruises. The greater abundance of *Bacteroidetes* in March 2017 may be indicative of the community composition adjusting following an algal bloom (Pinhassi et al. 2004). In the September 2017 season, it is probable that the time sampled was still during an algal bloom, as indicated by the higher abundance of *Cyanobacteria*. Moreover, the shifts in community composition from August 2016 to March 2017 and from March 2017 to September 2017 are indicative of the influence of seasonality. While March 2017 and September 2017 were similar in terms of seasons, September 2017 had higher precipitation during that month, which led to higher run-off from the riparian region as compared with the March 2017 wet season. This could have led to the increase in cyanobacteria, which was also reflected increase of picoplankton size class during the wet season (**Chapter 5.4, Fig 5.3**). Furthermore, in comparison, August 2016 and March 2017 were similar in terms of the proportion of the relative abundance of the community composition (**Fig. 4.4**).

4.5.4 Possible pathogenic bacteria and/or anthropogenic influence and land-use change

According to Reza et al. (2018) the taxa Flavobacterium is a potential fish pathogen which is commonly found in freshwater habitats (Lee and Eom 2016) as well as coastal pelagic zones (Eilers et al. 2001). In the Rajang river, it is the sixth most abundant class (Supplementary Fig. 2). This is cause for concern as it was found to be high in the coastal regions as well as brackish regions where fisheries and fishing activities are concentrated. Furthermore, the Cytophaga-Flavobacterium-Bacteroidetes group, or rather known as the CFB group, is commonly associated with humans (Weller et al. 2000), reflecting anthropogenic influences on the samples, especially within the brackish areas which have several human settlements and plantations. Lee-Cruz et al. (2013) demonstrated that conversions from tropical forests to oil palm plantations are much more severe than logged forests in terms of bacterial community composition; logged forests were shown to exhibit some resilience and resistance (to a certain extent). There has been little to no literature regarding the changes in microbial community composition as a result of land-use changes that occur within this region, particularly throughout the catchment area of the Rajang River. However, the results obtained from this study evidently suggest that the run-off from anthropogenic activities alters the microbial community composition. Anthropogenic disturbances, in particular settlements and logging (secondary forest), led to higher diversity indices (Fig. 4.9). On the contrary, sites surrounded by oil palm plantations displayed the lowest diversity indices, supporting results by Mishra et al. (2014), who found similar results in peatlands. In a study by Fernandes et al. (2014), anthropogenically-influenced mangroves had 2x higher amounts of *y-Proteobacteria* compared to pristine mangroves. This was similar to the March 2017 cruise along the Rajang river, whereby *y*-Proteobacteria was the predominant class in the marine and brackish peat region along with the significant increase in Bacteroidetes as mentioned,

which can be associated to anthropogenic activities. On the other hand, during the dry season, the diversity of the "less-disturbed" region was higher than the disturbed regions. However, it should be noted that the coastal zone generally has the lowest richness and diversity amongst the regions regardless of the presence or absence of anthropogenic activities. Hence, the extent of salinity intrusion may also result in the loss of diversity and richness of the microbial communities in the Rajang river (Shen, Langenheder & Jürgens, 2018).

4.6 Conclusion

This study represents the first assessment of the microbial communities of the Rajang River, the longest river in Malaysia, expanding our knowledge of microbial ecology in tropical regions. The predominant taxa are Proteobacteria (50.29%), followed by Firmicutes (22.35%) and Actinobacteria (11.95%). The microbial communities were found to change according to the source type, whereby distinct patterns were observed as a result of the changes in salinity along with variation of other biogeochemical parameters. Alpha diversity indices indicate that the microbial diversity was higher upstream as compared to the marine and estuarine regions, whereas anthropogenic perturbations led to increased richness but less diversity in the less pristine environments. Even though there were observed changes in bacterial community composition and diversity that occur along the Rajang River to sea continuum, the PICRUSt predictions showed minor variations. Areas surrounded by oil palm plantations showed the lowest diversity; other signs of anthropogenic impacts included the presence of CFB-groups as well as probable algal blooms. In order to further gauge and substantiate the functional and metabolic capacity of the microbial communities within each specific source type, metaproteomics as well as metabolomics should be carried out along with mixing experiments. These could show the response of the microbial communities towards anthropogenic perturbations as well as the role of microbial communities in degrading peat-related run-off from the surrounding riparian regions.

Chapter 5

Phytoplankton and picoplankton dynamics along the Rajang river-South China Sea continuum

Phytoplankton and picoplankton dynamics along the Rajang river-South China Sea continuum

5.1 Abstract

In the recent years, there has been an increase in the number of studies on phytoplankton in the South China Sea. As the Rajang River is a unique tropical peat-draining river which discharges a large amount of organic matter into the South China Sea, little is known about the relative contribution of the Rajang River towards the phytoplankton and picoplankton assemblages and vice versa. Furthermore, there are limited studies which are conducted along blackwater dominated rivers and estuaries in general with regards to phytoplankton and picoplankton. Therefore, the aim of this study is to assess phytoplankton community via CHEMTAX (program for the analysis of pigment markers) and elucidate its dynamics with environmental parameters. Also, the distribution of picoplankton will be investigated in order to determine the seasonal and spatial distribution and dominance of either phytoplankton or picoplankton. CHEMTAX was utilized to predict the phytoplankton community from the obtained pigment concentrations, and picoplankton abundance was obtained via flow cytometry. The profiles of phytoplankton pigments revealed distinct contributions of phytoplankton communities towards the total Chl a, which change according to source type. Distributions of the phytoplankton were commonly found based on the salinity profiles, i.e. diatoms were found in the salinity influenced regions whereas the chlorophytes were common in freshwater ecosystems. Resource availability was a major factor in the phytoplankton and picoplankton communities of the Rajang River. In the wet season, the upstream Rajang River has lower nutrient concentrations due to higher flow, which led to the dominance of picoplankton upstream; larger sized phytoplanktons dominated the coastal regions due to higher abundance of nutrients. In conclusion, the present study represents a first assessment of the spatial and seasonal distribution of both freshwater and marine phytoplankton assemblages, and of picoplankton abundance along the Rajang River-South China Sea continuum. The results contribute towards improved understanding of the phytoplankton and picoplankton communities of this region, which can provide insights towards the ecophysiological health of the region. They should also assist long-term monitoring of harmful algal blooms, especially with indicator phytoplankton species that respond to anthropogenic activities.

5.2 Introduction

Phytoplankton play an important role in the marine ecosystems and biogeochemical cycles (Le Quéré et al. 2005; Doney et al. 2009; Weber & Deutsch 2010) such as the carbon cycle (Zhu et al. 2009). In addition, planktonic algae play an essential role in the functioning of large rivers as major producers of organic carbon and food source for planktonic consumers (Wehr & Descy 1998). The diversity of phytoplankton, such as their shape, size and pigmentation, strongly influences biogeochemical processes such as the efficiency of phytosynthesis, trophic interactions (Legendre and Lefevre 1989; Jennings et al. 2002) and global carbon fluxes originating from the euphotic zone (Michaels and Silver 1988; Buesseler 1998).

Paerl et al. (2003) claim that phytoplankton groups are seasonally influenced, whereby drought conditions -which cause the reduction of freshwater discharge- result in longer water residence time and reduced nutrient concentrations, which favour slower growing phytoplankton taxa such as cyanobacteria and dinoflagellates. Other phytoplankton groups are able to grow under reduced saline conditions and competitively utilize nutrients; they subsequently grow rapidly during increased river flow rates caused for example by higher precipitation. Hence, the elucidation of spatiotemporal nutrient availability is essential for the basic understanding of the riverine and coastal/estuarine ecosystem structure and overall productivity (Downing et al. 1997, Paerl et al. 2004). It has been established that regions of high nutrients are usually found in estuaries and are mostly characterized by the dominance of large celled-diatoms in the phytoplankton biomass (Sarthou et al. 2005). Thus, fucoxanthin, the diagnostic pigments related to diatoms, are provisionally higher in these areas (Wysocki et al. 2006). Cyanobacteria are commonly found in many aquatic systems, including tropical and temperate lakes, rivers and estuaries (Whitton and Potts 2000). Picoplankton are groups of phytoplankton with sizes less than 2 or 3 µm in diameter (Sieburth et al. 1978; Takashi & Hori 1984; Vaulot et al. 2008). According to Alvain et al (2005), the phytoplankton biomass that dominates under oligotrophic conditions, such as in subtropical gyres, is picoplankton due to their advantage of a high surface-to-volume ratio which makes them the best at competing for low nutrient concentrations (Raven 1998). The abundance of eukaryotes is often inversely associated with that of prokaryotes, whereby the prokaryotes are generally favoured in physically active mixed layers (Bouman et al. 2011). Due to the increasing warming of oceans, picoplankton (with specific reference to picoeukaryotes) were found in increasing fractions of the total chlorophyll concentrations (Li et al. 2009; Moran et al. 2010).

The estuarine and coastal ecosystem dynamics are strongly driven by hydrological forcing as a result of intra- and inter-annual climatic variability (Goldenberg et al. 2001; Cloern 2001; Paerl et al. 2006). Such hydrological forcing would then aid in triggering biogeochemical as well as trophic responses which would in turn change the functional properties of the ecosystem.

Several studies on the ecology of phytoplankton communities in river-to-sea continua have been carried out, for example along the Neuse River estuary (Arhonditsis et al. 2007) and along the subtropical Wanquan River in China. Studies on the phytoplankton ecology along blackwater influenced tropical rivers are, however, very limited. One exception is a study conducted on a blackwater dominated estuary in the Gulf of Mexico (Quinland & Phlips 2007). Even though riverine phytoplankton communities consist of a diverse assemblage of benthic macrophytic, smaller epilithic, epiphytes and sediment–dwelling forms other than the suspended algae (Reynolds and Descy 1996), this chapter will focus only on the phytoplankton and picoplankton distribution in the surface waters or the epipelagic zone of the Rajang river-South China Sea continuum.

As the Rajang river is characterized as being phosphate limited (Sia et al., in preparation), the question arises if this nutrient limitation results in a greater proportion of picoplankton compared to phytoplankton, or does the excess in nitrogen limit the abundance of both picoplankton and phytoplankton? In this study, phytoplankton communities were elucidated using pigment data, and their seasonal dynamics and correlation with environmental parameters were assessed. Respiration experiments were conducted in order to determine the primary productivity of the region. Lastly, the distribution of picoplankton was investigated as well in order to determine the seasonal and spatial distribution and dominance of either phytoplankton or picoplankton.

5.3 Methodology

5.3.1 Study Site & Sample Collection

Two cruises were undertaken in August 2016 and March 2017 on a live-aboard fishing boat; all samples were collected on board and filtered and preserved immediately. The sites where the phytoplankton samples were obtained are shown in **Fig 5.1**. The samples of each picoplankton type are shown in **Fig. 5.5**, **Fig. 5.6** and **Fig. 5.7**.



Fig. 5.1: Location of Rajang River within Sarawak, Malaysia (inset). The map shows the stations sampled during two different cruises; August 2016 (dry season; red diamonds), March 2017 (wet season; blue squares)

The samples were collected within the upper 1 m using a throw-away bucket. The bucket was thoroughly rinsed with sample waters at the start of each station. A multi-parameter probe was used at every station to obtain the physico-chemical parameters such as salinity, temperature, turbidity and pH of the surface waters. The interpretation of the season is based on the monthly averages of the precipitation (Refer to **Chapter 2.1.2**, **Fig. 2.3**), whereby August 2016 represents the dry season, and March 2017 represents the wet season.

The abbreviations, names and formulae for the pigments and that are pertinent to this study are found in the table (**Table 5.1**) below:

Abbreviation	Name or Formulae
ALLO	Alloxanthin
BUT	19'Butanoyloxyfucoxanthin
Chl a	Chlorophyll <i>a</i>
Chl b	Chlorophyll <i>b</i>
dv-Chl a	Divinyl chlorophyll a
dv-Chl b	Divinyl chlorophyll b
TChl a	CHLa + dvCHLa
	CHLb + dvCHLb*
TChl b	*In this study the TChl <i>b</i> could not be determined due to the lack of data for dv-
	CHLb. Hence it is assumed here that Tchl b = Chl b
DIAD	Diadinoxanthin
DIAT	Diatoxanthin
FUCO	Fucoxanthin
HEX	19'Hexanoyloxyfucoxanthin
LUT	Lutein
NEO	Neoxanthin
PER	Peridinin
PRA	Prasinoxanthin
VIO	Violaxanthin
ZEA	Zeaxanthin

Table 5.1: Overview of the Abbreviations, Name and formulae of the pigments studied

5.3.2 Chemical Analyses of Phytoplankton Pigments

The extraction of the phytoplankton pigments was carried out according to methods provided by Zapata et al. (2000) (Refer to **Chapter 2.9**).

5.3.3 Phytoplankton community structure estimates via CHEMTAX

While conventional light microscopy is the main tool for the enumeration and the identification of phytoplankton, it has its limitations, such as being unable to differentiate small-sized phytoplankton groups. This is pertinent in regions where small flagellates are the dominant species (Peterson et al. 1988; Rodriguez, Varela and Zapata. 2002). As some pigments are characteristic of specific phytoplankton groups (Schlüter et al. 2000; Ediger et al. 2006), the phytoplankton pigments can be utilized as diagnostic markers in the classification of phytoplankton assemblages. According to Mackey et al. (1996), the CHEMTAX program is one of the most robust methods for the analysis of pigment markers. A steep descent algorithm is employed with a factor analysis in order to identify the best fit data based on initial estimates of the most applicable pigment ratio(s) for each class of phytoplankton. While CHEMTAX has mostly been used for oceanic environments (Mackey et al. 1998; Rodriguez Rodriguez, Varela and Zapata 2002; Muylaert et al. 2006), it has also been used for

estuarine regions (Zhu et al. 2009; Seoane et al. 2011; Parab et al. 2013) freshwater regions (Simmons and Simmons 2012) and neotropical lakes, lagoons and swamps (Guisande et al. 2008). Hence, in this study, the initial pigment ratios selected were based on pigment ratios that are analogous to the physico-chemical conditions of the Rajang River.

By utilizing the CHEMTAX program (Mackey et al. 1996), the estimation of phytoplankton group abundances (in terms of contributions to Chl a) were based on the HPLC phytoplankton pigment measurements. Mackey et al. (1997) stated that a pre-study of the region is necessary (based on microscopic taxanomic observations) in order to obtain the representative of the main species within the class that are representative based on the pigment ratios. However, as there is little to no literature on the phytoplankton ratios within this region, the pigment ratios had to be derived based on studies by Cartaxana et al (2009) (lakes), Gameiro et al. (2007) (estuaries) and Zhu et al. (2015) on the Wanquan River (a tropical/subtropical river in Hainan, China which extends out to the northern South China Sea). The ratios from these studies were chosen as the environment closely resembled the conditions of the Rajang River for the estuarine regions. The pigment ratios for the samples obtained from the freshwater region were adapted from Guisande et al. (2008) which was based on multiple freshwater regions. Hence, pigment ratios utilized for this study were divided into two categories: (1) when salinity = 0 and (2) when salinity > 0. In the selection of pigments to be utilized in the CHEMTAX program, pigments such as DIAT, which is formed by the rapid degradation of DIAD, and pigments that are present in all algal classes were excluded as the aforementioned pigments are unable to provide suitable information regarding the phytoplankton composition (Mackey et al. 1997; Zhu et al. 2015). The composition of the diagnostic pigments included cryptophytes, haptophytes and prasinophytes for the estuarine (salinity > 0) region. Prochlorophytes were excluded as the determination of prochlorophytes requires the pigments to be normalized to divinyl chlorophyll a which was unavailable in this study. Furthermore, for the riverine region (salinity = 0), prochlorophytes, haptophytes and prasinophytes were excluded due to the lack of diagnostic pigments that correspond to the taxa involved. However, while the wet season lacked the pigments needed to run the CHEMTAX program (PER, FUCO, NEO, VIO and ALLO), the aforementioned pigments remained in the pigments ratio matrix in order to properly run the program. It should also be noted that terrestrial plant detritus would affect the prediction of the CHEMTAX results as it contains the pigments LUT as well as Chl b, which is often present in rivers and estuarines (Zhu et al. 2015). This could lead to the overestimation of chlorophytes (Lionard et al. 2008). The run configuration of CHEMTAX is shown in Table 5.2 below.

Run Configuration	Value
Verbosity	2 (Low)
Iteration Limit	100
Epsilon Limit	0.0001
Initial Step Size	10
Step Ratio	1.3
Cutoff Step	1000
Elements Varied	5
Subiterations	1
Weighting	3
Weight Bound	30 (Bounded Relative)
Ratio Limits	500

Table 5.2: CHEMTAX Configuration

5.3.4 Size Structure of Algal Populations

The relative biomass proportions that were correlated to micro-phytoplankton $(20 - 200 \ \mu m)$, nanophytoplankton $(2-20\mu m)$ and picophytoplankton $(<2 \ \mu m)$ in natural populations were estimated based on those pigment concentrations which were shown to be significant in terms of taxonomic determination and which were able to be associated to a size class (Vidussi et al. 2001). Each size class and the associated biomass proportions were computed as (Bricaud et al. 2004; Uitz et al. 2006):

% picophytoplankton = 100 * (0.86[Zea] + 1.01[TChl b]/DP (1))

% nano = 100 * (0.6[ALLO] + 0.35 [BUT – FUCO] + 1.27 [HEX-FUCO])/DP (2)

% micro = 100 * (1.41[FUCO] + 1.41 [PER])/DP (3)

Whereby the sum of the seven diagnostic pigment concentrations (DP):

$$DP = 0.6[ALLO] + 0.35[BUT - FUCO] + 1.41[FUCO] + 1.27[HEX-Fuco] + 1.41[PER] + 1.01[TChlb] + 0.86[ZEA]$$

Claustre (1994) and Uitz et al. (2006, 2008) specified that the assumptions of pigment/size relationships may occasionally lead to errors, as some diagnostic pigments are common among several phytoplankton groups which may cover a broad range of sizes. For example zeaxanthin, which is normally used for the classification of *Cyanobacteria*, is also present in *Trichodesmium* (Kheireddine et al. 2017). However, Uitz et al. (2006) and Ras, Claustre and Uitz (2008) justified that the aforementioned approach is still important and relevant in order to elucidate the trends of the dominant phytoplankton communities and the regional and seasonal scales of size structures. Tchl b was assumed to be equal to Chl **b** in order to estimate the size structure of the algal populations

5.3.5 Picoplankton biomass calculations

Refer to Chapter 2.9

5.3.6 Data analysis and statistics

Refer to Chapter 2.10

5.4 Results

5.4.1 Determination of Phytoplankton Community Structure Estimates

Based on the output pigment ratios (**Table 5.6** and **Table 5.8**), the input remains generally similar or within the range (Mackey et al .1996), which exemplifies the robustness of the fitting. However, as CHEMTAX makes a prediction of the available phytoplankton community based on literature, it has to be interpreted with caution. As put forth by Zhu et al (2009) and Higgins and Mackey (2000), tight limits cannot be set as pigment ratios from the literature were based on laboratory cultures, whereas the pigments obtained in this study were subject to variable conditions as opposed to laboratory conditions. Hence, tight limits on the pigment ratios were not set, and the fluctuations in the pigment ratios were accepted.

Table 5.3: Phytoplankton pigment concentrations (μ g L⁻¹) of the Rajang River-South China Sea continuum in the Dry Season. ALLO = Alloxanthin; BUT=19'Butanoyloxyfucoxanthin; Chl *a* = Chlorophyll *a*; Chl *b* = Chlorophyll *b*; dv-Chl *a* = Divinyl chlorophyll *a*; DIAD = Diadinoxanthin; FUCO = Fucoxanthin; HEX = 19'Hexanoyloxyfucoxanthin; LUT = Lutein; NEO = Neoxanthin; PER = Peridinin; PRA= Prasinoxanthin; VIO = Violaxanthin; ZEA = Zeaxanthin

Season		Dry													
							Pigme	nts (mg L ⁻¹)						
Stations	ALLO	BUT	Chl a	ChI b	dv-Chl a	DIAD	DIAT	FUCO	НЕХ	LUT	NEO	PER	PRA	VIO	ZEA
AUG16ST2	2.533	0	180.51	7.767	0	7.041	1.043	78.203	1.830	0.526	0.839	5.131	0.978	0.650	11.068
AUG16ST3	1.762	0	65.37	3.970	0	2.347	0.532	19.853	0	1.206	0.775	1.775	0	0	3.089
AUG16RAJST5	1.982	0	62.84	10.787	0	1.327	0	2.072	0	4.713	1.775	0.321	0	0.960	2.445
AUG16RAJST6	2.312	0	67.06	8.630	0	2.449	0	5.352	0	4.385	2.259	2.138	0	1.033	2.831
AUG16RAJST7	0.947	0	42.18	5.178	0	1.429	0	6.560	0	3.179	1.468	0	0	0.599	1.930
AUG16RAJST8	1.982	0	47.24	6.472	0	1.531	0.448	6.905	0.717	3.398	1.517	1.155	0	0.681	2.188
AUG16RAJST10	0.727	0	21.93	3.107	0	1.123	0	4.316	0	3.288	0.871	1.304	0	0	1.544
AUG16RAJST15	2.092	0	37.96	4.746	0	0.980	0	3.798	0	2.850	1.614	0.428	0	0.867	1.930
AUG16RAJST16	3.523	0	61.58	8.198	0	1.531	0	2.762	0	3.508	2.098	0.513	0	1.136	2.059
AUG16RAJST28	1.090	0	32.47	4.746	0	0.633	0	2.072	0	2.302	0.920	0	0	0.743	2.703
AUG16RAJST30	0.716	0	24.04	1.553	0	1.429	0	5.524	0	0.537	0	1.261	0	0	1.145
AUG16RAJST31	5.836	0	87.30	4.746	0	4.388	1.147	24.514	0	0.976	0.468	6.200	0	1.136	2.059
AUG16RAJST32	4.514	0	132.01	5.178	0	6.735	1.032	40.051	0	0.274	0	7.269	0	0.712	3.475
AUG16RAJST33	7.818	0	128.21	5.609	0	5.613	3.232	40.051	1.525	0.866	0.694	2.010	1.793	0.661	21.621
Mean ± SE	2.702 ± 0.554	0	70.763 ± 12.412	5.763 ± 0.645	0	$\begin{array}{c} 2.754 \pm \\ 0.594 \end{array}$	$\begin{array}{c} 0.531 \pm \\ 0.240 \end{array}$	$\begin{array}{c} 17.288 \pm \\ 5.899 \end{array}$	$\begin{array}{c} 0.291 \pm \\ 0.166 \end{array}$	$\begin{array}{c} 2.286 \pm \\ 0.408 \end{array}$	$\begin{array}{c} 1.093 \pm \\ 0.191 \end{array}$	$\begin{array}{c} 2.107 \pm \\ 0.631 \end{array}$	$\begin{array}{c} 0.198 \pm \\ 0.141 \end{array}$	$\begin{array}{c} 0.656 \pm \\ 0.106 \end{array}$	4.292 ± 1.481

Table 5.4: Phytoplankton pigment concentrations (μ g L⁻¹) of the Rajang River-South China Sea continuum in the Wet Season. ALLO = Alloxanthin; BUT=19'Butanoyloxyfucoxanthin; Chl *a* = Chlorophyll *a*; Chl *b* = Chlorophyll *b*; dv-Chl *a* = Divinyl chlorophyll *a*; DIAD = Diadinoxanthin; FUCO = Fucoxanthin; HEX = 19'Hexanoyloxyfucoxanthin; LUT = Lutein; NEO = Neoxanthin; PER = Peridinin; PRA= Prasinoxanthin; VIO = Violaxanthin; ZEA = Zeaxanthin

Season								We	t						
								Pigments	(mg L ⁻¹)						
Stations	ALLO	BUT	Chl a	Chl b	dv-Chl a	DIAD	DIAT	FUCO	HEX	LUT	NEO	PER	PRA	VIO	ZEA
MAR17RAJST1	0	0	0.022	0.004	0	0	0.008	0	0	0.004	0	0	0	0	0.002
MAR17RAJST3	0	0	0.034	0.009	0	0	0.008	0	0	0.008	0	0	0	0	0.003
MAR17RAJST5	0	0	0.022	0.005	0	0	0.007	0	0	0.005	0	0	0	0	0.002
MAR17RAJST6	0	0	0.049	0.015	0	0	0.011	0	0	0.015	0	0	0	0	0.007
MAR17RAJST7	0.011	0	0.076	0.004	0	0	0.008	0.008	0	0.002	0	0	0	0	0.003
MAR17RAJST8	0.012	0	0.232	0.012	0	0.017	0.008	0.123	0	0.003	0	0.015	0	0	0.014
MAR17RAJST9	0.014	0	0.264	0.013	0	0.012	0.009	0.078	0	0.001	0.002	0.015	0	0.003	0.010
MAR17RAJST10	0.006	0	0.210	0.010	0	0.010	0.009	0.071	0	0.002	0	0.004	0	0	0.008
MAR17RAJST11	0.043	0	0.622	0.063	0	0.035	0.010	0.191	0.003	0.003	0.010	0.053	0.013	0.007	0.030
MAR17RAJST12	0.013	0	0.608	0.021	0	0.040	0.009	0.429	0.005	0.003	0.003	0.022	0.004	0.004	0.022
MAR17RAJST13	0.030	0	0.800	0.100	0	0.030	0.009	0.400	0.010	0	0.010	0.020	0.030	0.010	0.100
MAR17RAJST14	0.014	0	0.662	0.074	0	0.013	0.007	0.177	0.016	0	0.012	0.009	0.026	0.005	0.213
MAR17RAJST15	0.007	0	0.165	0.009	0	0.007	0.007	0.087	0	0.002	0	0	0	0	0.007
MAR17RAJST16	0	0	0.090	0.006	0	0.003	0.008	0.020	0.006	0	0	0	0	0	0.030
Mean ± SE	$\begin{array}{c} 0.011 \\ \pm \\ 0.003 \end{array}$	0	$\begin{array}{c} 0.275 \pm \\ 0.074 \end{array}$	$\begin{array}{c} 0.025 \pm \\ 0.008 \end{array}$	0	$0.012 \\ \pm \\ 0.004$	$0.008 \\ \pm \\ 0.001$	0.113 ± 0.038	$\begin{array}{c} 0.003 \pm \\ 0.001 \end{array}$	$0.003 \\ \pm \\ 0.001$	0.003 ± 0.001	$0.011 \\ \pm \\ 0.004$	0.005 ± 0.003	$\begin{array}{c} 0.002 \pm \\ 0.001 \end{array}$	$\begin{array}{c} 0.032 \pm \\ 0.015 \end{array}$

Table 5.5: CHEMTAX calculated input pigment ratios for the estuarine region (Salinity>0; all pigment ratios are normalized to Chl *a*). PER = Peridinin; BUT=19'Butanoyloxyfucoxanthin; FUCO = Fucoxanthin; HEX = 19'Hexanoyloxyfucoxanthin; NEO = Neoxanthin; PRA= Prasinoxanthin; VIO = Violaxanthin; ALLO = Alloxanthin; LUT = Lutein; ZEA = Zeaxanthin; Chl b = Chlorophyll b

Ratios	Taxa	PER	BUT	FUCO	HEX	NEO	PRA	VIO	ALLO	LUT	ZEA	Chl b
	Chlorophytes	0	0	0	0	0.043	0	0.032	0	0.16	0.001	0.277
	Chrysophytes	0	0.933	0.625	0	0	0	0	0	0	0	0
	Cryptophytes	0	0	0	0	0	0	0	0.405	0	0	0
	Cyanobacteria	0	0	0	0	0	0	0	0	0	0.984	0
Input	Diatoms	0	0	0.779	0	0	0	0	0	0	0	0
	Dinoflagellates	0.533	0	0	0	0	0	0	0	0	0	0
	Euglenophytes	0	0	0	0	0.069	0	0.007	0	0	0.056	0.219
_	Haptophytes	0	0	0.233	0.462	0	0	0	0	0	0	0
	Prasinophytes	0	0	0	0	0.082	0.497	0	0	0.032	0.157	0.568

Table 5.6: CHEMTAX calculated output pigment ratios for the estuarine region (Salinity>0; all pigment ratios are normalized to Chl a). PER = Peridinin;BUT=19'Butanoyloxyfucoxanthin; FUCO = Fucoxanthin; HEX = 19'Hexanoyloxyfucoxanthin; NEO = Neoxanthin; PRA= Prasinoxanthin; VIO =Violaxanthin; ALLO = Alloxanthin; LUT = Lutein; ZEA = Zeaxanthin; Chl b = Chlorophyll b

Ratios	Taxa	PER	BUT	FUCO	HEX	NEO	PRA	VIO	ALLO	LUT	ZEA	Chl b
	Chlorophytes	0	N/A	0	0	0.023	0	0.019	0	0.116	0.001	0.183
	Chrysophytes	0	N/A	0.308	0	0	0	0	0	0	0	0
	Cryptophytes	0	N/A	0	0	0	0	0	0.288	0	0	0
Output	Cyanobacteria	0	N/A	0	0	0	0	0	0	0	0.496	0
(Dry)	Diatoms	0	N/A	0.438	0	0	0	0	0	0	0	0
(Dry)	Dinoflagellates	0.348	N/A	0	0	0	0	0	0	0	0	0
	Euglenophytes	0	N/A	0	0	0.009	0	0.006	0	0	0.045	0.135
	Haptophytes	0	N/A	0.137	0.273	0	0	0	0	0	0	0
	Prasinophytes	0	N/A	0	0	0.035	0.213	0	0	0.014	0.067	0.243
	Chlorophytes	0	N/A	0	0	0.005	0	0.019	0	0.109	0.001	0.188
	Chrysophytes	0	N/A	0.258	0	0	0	0	0	0	0	0
	Cryptophytes	0	N/A	0	0	0	0	0	0.288	0	0	0
Outnut	Cyanobacteria	0	N/A	0	0	0	0	0	0	0	0.496	0
(Wot)	Diatoms	0	N/A	0.585	0	0	0	0	0	0	0	0
(Wet)	Dinoflagellates	0.348	N/A	0	0	0	0	0	0	0	0	0
	Euglenophytes	0	N/A	0	0	0.016	0	0.008	0	0	0.043	0.166
	Haptophytes	0	N/A	0.137	0.273	0	0	0	0	0	0	0
	Prasinophytes	0	N/A	0	0.0351	0.213	0	0	0.014	0.067	0.243	0.243

The output of the calculated pigment ratios are shown in **Table 5.6**. The bolded values indicate changes in original values that were input in the to CHEMTAX. The pigment 19'Hexanoyloxyfucoxanthin in the final output matrix was not included due to the pigment being absent in both seasons.

Table 5.7: CHEMTAX input pigment ratios for the freshwater region (Salinity=0). PER = Peridinin; FUCO = Fucoxanthin; NEO = Neoxanthin; VIO =Violaxanthin; ALLO = Alloxanthin; LUT = Lutein; ZEA = Zeaxanthin; Chl b = Chlorophyll b

Ratios	Taxa	PER	FUCO	NEO	VIO	ALLO	LUT	ZEA	CHLb
	Chlorophytes	0	0	0.006	0.031	0	0.124	0.015	0.166
	Chrysophytes	0	0.261	0	0.116	0	0	0.021	0
	Cryptophytes	0	0	0	0	0.295	0	0	0
Input	Cyanobacteria	0	0	0	0	0	0	0.32	0
	Diatoms	0	1.057	0	0	0	0	0	0
	Dinoflagellates	0.636	0	0	0	0	0	0	0
	Euglenophytes	0	0	0	0	0	0	0	0.241

Ratios	Taxa	PER	FUCO	NEO	VIO	ALLO	LUT	ZEA	Chl b
	Chlorophytes	0	0	0.011	0.026	0	0.105	0.013	0.141
	Chrysophytes	0	0.187	0	0.083	0	0	0.015	0
	Cryptophytes	0	0	0	0	0.228	0	0	0
Output	Cyanobacteria	0	0	0	0	0	0	0.242	0
(Dry)	Diatoms	0	0.514	0	0	0	0	0	0
	Dinoflagellates	0.389	0	0	0	0	0	0	0
	Euglenophytes	0	0	0	0	0	0	0	0.194
	Chlorophytes	0	0	0.001	0.004	0	0.323	0.017	0.283
	Chrysophytes	0	0.187	0	0.083	0	0	0.015	0
Qutmut	Cryptophytes	0	0	0	0	0.228	0	0	0
(Wet)	Cyanobacteria	0	0	0	0	0	0	0.310	0
(wet)	Diatoms	0	0.514	0	0	0	0	0	0
	Dinoflagellates	0.389	0	0	0	0	0	0	0
	Euglenophytes	0	0	0	0	0	0	0	0.065

Table 5.8: CHEMTAX output pigment ratios for the freshwater region (Salinity=0). PER = Peridinin; FUCO = Fucoxanthin; NEO = Neoxanthin; VIO =Violaxanthin; ALLO = Alloxanthin; LUT = Lutein; ZEA = Zeaxanthin; Chl b = Chlorophyll b

The relative phytoplankton contribution to total Chl a was calculated and plotted in a graphical representation as shown in Fig. 5.2.



Fig. 5.2: Relative phytoplankton contribution to total Chl a as per calculated via CHEMTAX in both dry and wet seasons according to source type (%)

Reading Fig. 5.2, the diatoms had the highest percentage in the marine and the brackish peat regions in the dry season (37.85%). The second highest phylum in the marine region during the dry season were the Chrysophytes (16.15%), followed by Euglenophytes (15.29%). The remaining phyla were Cyanobacteria (10.47%), Cryptophytes (9.24%), Dinoflagellates (4.13%), Chlorophytes (2.56%), Haptophytes (2.38%), and Prasinophytes (1.93%). For the brackish peat in the dry season, the highest contributor were the Chrysophytes (34.09%), followed by Euglenophytes (19.88%) and Diatoms (16.40%). The remaining phyla were Cryptophytes (9.72%), Dinoflagellates (9.64%), Chlorophytes (7.69%), Cyanobacteria (2.54%), Prasinophytes (0.04%) and Haptophytes (negligible percentage). On the other hand, the chlorophytes were the dominant taxa in the freshwater peat in the dry season were Cryptophytes (14.66%) followed by Euglenophytes (13.19%), Cyanobacteria (12.62%) and Diatoms (5.38%). The remaining taxa were Dinoflagellates (0.95%) and Chrysophtes (0.60%). The highest contributor in the freshwater peat in the dry season were the Chlorophytes (13.29%). followed by Euglenophytes (0.60%). The highest contributor in the freshwater peat in the dry season were the Chlorophytes (14.66%) followed by Euglenophytes (13.19%), Cyanobacteria (2.52%), followed by Euglenophytes (13.19%), Cyanobacteria (12.62%) and Diatoms (5.38%). The remaining taxa were Dinoflagellates (0.95%) and Chrysophtes (0.60%). The highest contributor in the freshwater peat in the dry season were the Chlorophytes (52.59%), followed by

Cryptophytes (14.66%) and Euglenophytes (13.19%). The remaining phyla were Cyanobacteria (12.62%), Diatoms (5.38%), Dinoflagellates (0.95%) and lastly Chrysophytes (0.60%). For mineral soils in the the dry season, the highest taxa were also Chlorophytes (52.54%), followed by Diatoms (12.77%), Cyanobacteria (11.71%), Cryptophytes (10.29%), Euglenophytes (8.51%) and Dinoflagellates (4.18%), respectively.

On the other hand, in the wet season, the highest contributors in the marine region were the Euglenophytes (30.83%), Diatoms (20.90%), followed closely by Cyanobacteria (20.70%). The remaining phyla were Haptophytes (8.52%), Chrysophytes (7.63%), Cryptophytes (4.41%), Prasinophytes (3.60%), Dinoflagellates (2.23%) and lastly, Chlorophytes (1.17%), respectively. For brackish peat in the wet season, the highest contributor belonged to the Chrysophytes (26.40%), followed by Diatoms (20.89%), and Euglenophytes (17.47%). The remaining phyla belonged to the Cryptophytes (13.51%), Cyanobacteria (6.74%), Dinoflagellates (6.51%), Chlorophytes (5.89%), Prasinophytes (1.62%) and lastly, Haptophytes (0.98%). For the freshwater peat in the wet season, the highest contributor belonged to the Euglenophytes (35.48%), followed by Cyanobacteria (33.71%) and Chlorophytes (30.80%). The remaining phyla occured in trace amounts (Chrysophytes, Cryptophytes and Dinoflagellates). Lastly for the mineral soils in the wet season, the biggest contributor also belonged to the Euglenophytes (42.21%), followed by Chlorophytes (30.03%) and Cyanobacteria (27.76%).

5.4.2 Size Structure of Algal Class (Determination of phytoplankton size class)

The relative proportion of phytoplankton size class was calculated as shown in **Supplementary Table 3 and 4 respectively.**

The average values for each source type was calculated and plotted on a bar graph as shown in **Fig. 5.3**



Fig. 5.3: Graphical representation of the average relative proportion of phytoplankton size class (%) in both dry and wet seasons according to source type

In the dry season, the highest proportion of phytoplankton in the marine region were microphytoplankton (75.23%), followed by picoplankton (19.71%) and nanoplankton (5.06%). For the brackish peat in the dry season, the largest contributor were microphytoplankton as well (80.77%), followed by picoplankton (15.17%) and 4.06% nanophytoplankton. For the freshwater peat region, the picoplankton dominated in the wet season (61.92%) followed by microphytoplankton (29.41%) with the nanoplankton having the smallest contribution (4.61%), respectively. Lastly for the mineral soils in the dry season, the biggest contributor were microphytoplankton at 54.26%, followed by picoplankton (41.13%), and lastly nanoplankton (4.61%).

For the wet season, the highest contributor in the marine region were also microphytoplankton (57.39%), followed by picoplankton (35.08%) and nanoplankton (7.52%). For the brackish peat in the wet season, microphytoplankton dominated the region (74.82%), followed by picoplankton (18.48%) and nanoplankton (6.70%). It is unclear why during the wet season, both freshwater peat and mineral soils were completely dominated by the picoplankton (100.0%).

5.4.3 Environmental Drivers of Phytoplankton Community Composition

Among the environmental parameters selected, distLM clustered the samples according to source types for the regions with salinity influence whereas for the freshwater regions, the samples were clustered according to season as shown in **Fig. 5.4**. The environmental parameters were fitted in a best fit distLM model in **Fig. 5.4** ($R^2 > 0.839$, P>0.001).



Fig. 5.4: Significant vectors of environmental variables were calculated based on a linear model (Dist LM) and plotted against phytoplankton pigments composition

Samples in the marine and brackish peat regardless of season were found to cluster in between DOP and Salinity and DIP. For the mineral soils and freshwater peat, the samples were found to cluster separately as compared to the marine and brackish peat. For the dry season, freshwater peat and mineral soils clustered towards DOC and DON; in the wet season, the freshwater peat and mineral soil clustered along the SPM vector.

 Table 5.9: Proportion of combined community variation based on marginal DistLM test that is

 explained by each predictor variable for phytoplankton

Category	Variable	Pseudo-F	<i>P</i> -value	Proportion explained (%)
Dhusiaa ahamiaal	Salinity	11.073	0.002	38.08
parameters	SPM	10.04	0.002	35.81
	DO	1.5988	0.201	8.16
	Silicate	12.653	0.001	41.28
	DOP	11.044	0.001	38.03
Biogeochemical	DIP	7.3363	0.004	28.96
parameters	DON	1.7933	0.161	9.06
	DOC	1.6631	0.176	8.46
	DIN	1.1137	0.305	5.83

In **Table 5.9**, the environmental variables or physico-chemical parameters that were able to account for most of the variation in the phytoplankton community are arranged according to the highest proportion. Silicate was the single best predictor variable that explained the variation in phytoplankton community (41.28%), followed by Salinity (38.08%) and DOP (38.03%). This was then followed by SPM (35.81%) and DIP (28.96%). The remaining variables (DO, DON, DOC and DIN) all had *P*-values of more than 0.05, and were excluded from the interpretations in this study.

Table 5.10: Spearman's Correlation of Phytoplankton Community in the Dry season (From Predicted Percentage via CHEMTAX) with selected physico

 chemical parameters

					Dry				
				Phyt	oplankton	Туре			
Parameters	Chlorophytes	Chrysophytes	Cryptophytes	Cyanobacteria	Diatoms	Dinoflagellates	Euglenophytes	Haptophytes	Prasinophytes
DIP	-0.762**	0.836**	-0.352	-0.467	0.537*	0.326	0.520	0.819**	0.702**
DOP	-0.699*	0.694**	0.207	-0.630*	0.420	0.655*	0.367	0.608*	0.248
TDP	-0.716**	0.852**	-0.066	-0.521	0.636*	0.584*	0.349	0.810**	0.566*
DIN	-0.538**	0.743**	-0.446	-0.499	0.200	0.446	0.387	0.428	0.284
DON	0.429	-0.760**	0.17	0.258	-0.258	-0.044	-0.151	-0.432	-0.494
DOC	0.073	0.023	-0.086	-0.266	-0.196	0.415	0.112	-0.192	-0.235
Sal	-0.820**	0.788**	-0.447	-0.326	0.641*	0.474	0.394	0.862**	0.697**
Silicate	0.909**	-0.696**	0.328	0.603*	-0.51*	-0.596*	-0.688**	-0.838**	-0.677**
SPM	-0.330	0.571*	-0.599*	-0.560*	0.077	0.495	0.412	0.197	0.175

** means significant at the 0.01 level (two tailed)

* means significant at the 0.05 level (two tailed)

Table 5.11: Spearman's Correlation of Phytoplankton Community in the Wet season (From Predicted Percentage via CHEMTAX) with selected physico

 chemical parameters

	Wet								
	Phytoplankton Type								
Parameters	Chlorophytes	Chrysophytes	Cryptophytes	Cyanobacteria	Diatoms	Dinoflagellates	Euglenophytes	Haptophytes	Prasinophytes
DIP	-0.459	0.537	0.253	-0.371	0.355	0.167	-0.145	0.221	0.091
DOP	-0.821**	0.100	0.179	-0.207	0.593*	0.167	-0.189	0.857**	0.513
TDP	-0.909**	0.112	0.353	-0.424	0.682*	0.434	-0.052	0.760**	0.646*
DIN	0.154	0.591*	0.53	-0.231	-0.181	0.254	-0.11	-0.317	-0.266
DON	0.539*	0.133	-0.051	0.037	-0.357	-0.282	0.086	-0.472	-0.616*
DOC	0.679**	0.209	0.202	-0.191	-0.284	-0.130	-0.145	-0.810**	-0.448
Sal	-0.947**	0.277	0.272	-0.433	0.745**	0.342	-0.184	0.781**	0.534
Silicate	0.896**	-0.304	-0.26	0.374	-0.763*	-0.306	0.17	-0.717**	-0.482
SPM	0.682*	-0.468	-0.615	0.564	-0.667**	-0.447	0.400	-0.337	-0.451

**means significant at the 0.01 level (two tailed)

*means significant at the 0.05 level (two tailed)

The correlation between selected parameters and the phytoplankton type were examined and are presented in Table 5.10 and Table 5.11 for dry and wet seasons, respectively. Chrysophytes, Haptophytes, Diatoms and Prasinophytes were positively correlated with DIP in the dry season (0.836, 0.819, 0.537 and 0.702, respectively), whereas Chlorophytes were negatively correlated (-0.762). However, in the wet season, none of the phytoplankton were correlated to DIP. For DOP, Chrysophytes, Dinoflagellates and Haptophytes were positively correlated with DOP (0.694, 0.655, and 0.810 respectively) but negatively correlated with Chlorophytes (-0.699) in the dry season. For the wet season, DOP was positively correlated with Diatoms and Haptophytes (0.593 and 0.857, respectively) and was negatively correlated with Chlorophytes (-0.821). For TDP, it was found to be positively correlated with Chrysophytes, Diatoms, Dinoflagellates, Haptophytes and Prasinophytes (0.852, 0.636, 0.584, 0.810 and 0.566, respectively) in the dry season whereas in the wet season it was positively correlated with Diatoms, Haptophytes and Prasinophytes (0.682, 0.760 and 0.646, respectively) and negatively correlated with only Chlorophytes (-0.947). In terms of DIN, in the dry season Chrysophytes was the only parameter that was positively correlated (0.743), and Chlorophytes the only one negatively correlated (-0.538). In the wet season, DIN was positively correlated with Chrysophytes (0.591) only. On the other hand, in the dry season DON was only negatively correlated with Chyrosphytes (-0.760), whereas in the wet season it was positively correlated with Chlorophytes (0.539) and negatively correlated with Prasinophytes (-0.616). For DOC, there was no correlation in the dry season, but it was positively correlated with Chlorophytes (0.679) and negatively correlated with Haptophytes (-0.810) in the wet season. In terms of salinity, it was found to be positively correlated with Chrysophytes (0.788), Diatoms (0.641), Haptophytes (0.862), and Prasinophytes (0.697), and negatively correlated with only Chlorophytes (-0.820) in the dry season. In the wet season, salinity was shown to be positively correlated with Diatoms and Haptophytes (0.745 and 0.781, respectively). In the dry season silicate was positively correlated with Chlorophytes and Cyanobacteria (0.909 and 0.603, respectively) and negatively correlated with Chrysophytes, Dinoflagellates, Euglenophytes, Haptophytes and Prasinophytes (-0.696, -0.596, -0.688, -0.838 and -0.677, respectively). In the wet season, silicate was found to be positively correlated to only Chlorophytes (0.896) and negatively correlated to two phytoplankton types, namely Diatoms and Haptophytes (-0.763 and -0.717, respectively). Lastly, for SPM, it was found to be positively correlated only with Chrysophytes (0.571) and negatively correlated with Cryptophytes and Cyanobacteria (-0.599 and -0.560) in the dry season, and positively correlated with Chlorophytes (0.682) and negatively correlated with Diatoms (-0.677) in the wet season.



5.4.4 Spatial and Seasonal abundance of Picoplankton

Fig. 5.5: Spatial distribution of *Prochlorococcus* (cells mL⁻¹) in dry and wet season



Fig. 5.6: Spatial distribution of *Synechococcus* (cells mL⁻¹) in dry and wet season



Fig. 5.7: Spatial distribution of Pico-eukaryotes (cells mL⁻¹) in dry and wet season

Fig. 5.5, Fig. 5.6 and Fig 5.7 illustrate the spatial and seasonal distribution of *Prochlorcoccus*, *Synechococcus* and Pico-eukaryotes. For *Prochlorococcus*, the abundance ranged from 10 - 106200 cells mL¹. For *Syn*, the range was from 1390 - 118200 cells mL⁻¹ whereas Pico-eukaryotes ranged from 5 - 1370 cells mL⁻¹.

5.4.5 Environmental Drivers of Picoplankton Composition

The environmental variables that contributed the most to variation in the picoplankton community biomass are shown in **Fig. 5.8** (R^2 >0.5316, P>0.001).



Fig. 5.8: Significant vectors of environmental variables based on calculated linear model (DistLM) and plotted against picoplankton biomass

Samples from the marine region and brackish peat were clustered towards Sal, DO, DOP, DIP and DIN, regardless of the season. For freshwater peat and mineral soils, the pattern of clustering was similar, albeit at greater variability, with samples clustered around Silicate, DON, SPM and DOC.
Table 5.12: Proportion of combined community variation based on marginal DistLM test that is

 explained by each predictor variable for picoplankton

Category	Variable	Pseudo-F	<i>P</i> -value	Proportion explained (%)	
Dhygiaa ahamiaal	Salinity	26.149	0.001	43.47	
parameters	SPM	4.177	0.027	10.94	
	DO	1.9128	0.171	5.32	
Biogeochemical parameters	Silicate	25.997	0.001	43.33	
	DIP	15.887	0.002	31.85	
	DON	5.873	0.008	14.72	
	DOP	3.230	0.063	8.67	
	DOC	2.718	0.112	7.40	
	DIN	0.259	0.693	0.75	

From **Table 5.12**, the highest variable predictors were salinity (43.47%), silicate (43.33%) and DIP (31.85%). These were followed by DON (14.72%) and SPM (10.94%). The remaining variables (DO, DOC and DIN) all had *P*-values greater than 0.05 and were not taken into account.

Season	Dry			Wet			
	Picoplankton type			Picoplankton type			
Parameters	Pro	Syn	Peuk	Pro	Syn	Peuk	
DIP	0.761**	0.149	0.818**	0.495	0.221	0.357	
DOP	0.397*	0.119	0.250	0.811**	0.550*	0.620*	
TDP	0.710**	0.182	0.634**	0.805**	0.372	0.691**	
DIN	0.346	0.017	0.300	-0.131	-0.462	-0.086	
DON	-0.642**	-0.265	-0.581**	-0.374	-0.347	-0.375	
TDN	-0.353	-0.028	-0.331	-0.292	-0.543*	-0.26	
DOC	0.046	-0.61	-0.54	-0.482	-0.182	-0.449	
Silicate	-0.854**	-0.402*	-0.833**	-0.923**	-0.424	-0.768**	
Sal	0.905**	0.278	0.894**	0.914**	0.409	0.779	
DO	-0.242	0.114	-0.275	0.193	0.159	0.116	
SPM	-0.076	-0.074	-0.05	-0.729**	-0.165	-0.777**	

Table 5.13: Spearman's correlation of picoplankton type in Dry vs Wet with selected physico

 chemical parameters

** means significant at the 0.01 level (two tailed)

* means significant at the 0.05 level (two tailed)

A correlation between picoplankton type and selected physico-chemical parameters (Table 5.13) it shows that in the dry season DOP did not correlate with any picoplankton type, but in the wet season was positively correlated to all picoplankton types (Prochlorococcus, Synechococcus, Pico-eukaryotes at 0.811, 0.550 and 0.620, respectively). DIP was positively correlated with Prochlorococcus and Pico-eukaryotes (0.761 and 0.818, respectively), but not with any picoplankton type. For Silicate, Prochlorococcus and Pico-eukaryotes were negatively correlated (-0.854 and -0.833, respectively) in the dry season. In the wet season, Silicate was negatively correlated to both Prochlorococcus and Pico-eukaryotes (-0.923 and -0.768, respectively). For DON, only Prochlorococcus and Picoeukaryotes in the dry season were negatively correlated (-0.642 and -0.581, respectively). There was no picoplankton type that was correlated with DIN. For SPM, only Prochlorococcus and Picoeukaryotes were negatively correlated in the wet season (-0.729 and -0.777, respectively). In the dry season, Prochlorococcus and Pico-eukaryotes were positively correlated to salinity (0.905 and 0.894, respectively). In the wet season, only Prochlorococcus was positively correlated to salinity (0.914). Furthermore, there were no correlation of the DO and any picoplankton type. None of the picoplankton were correlated to DOC in either season, but Synechococcus was negatively correlated with TDN in the wet season (-0.543). For TDP, Prochlorococcus and Pico-eukaryotes were positively correlated (0.710 and 0.634, respectively) in the dry season. In the wet season, Prochlorococcus and Pico-eukaryotes were found to be positively correlated with TDP (0.805 and 0.691, respectively).

5.5 Discussion

5.5.1 Spatial and Temporal Distribution of phytoplankton community composition

5.5.1.1 Phytoplankton Groups

Based on the phytoplankton community composition (Fig 5.2), regions influenced by salinity were generally higher in diversity. The interface between freshwater and marine waters has diverse nutrient conditions due to steep salinity gradients and water availability, which leads to diverse phytoplankton communities within the coastal region (Gaiser et al. 2005). The presence of diatoms within the coastal region was typical of regions with high turbulence and a high supply of nutrients (Bode et al. 2017). This was corroborated by a microscopic study conducted along the coasts of South China Sea where diatoms, dinoflagellates and 2 species of blue green alga were found (Boonyapiwat 1998). The trend of increasing diatom presence with increasing salinity is similar to the study done by Zhu et al. (2015). Furthermore, the presence of diatoms in the freshwater region indicates that there are species which are able to tolerate low salinities (Zhang, Gradinger and Spindler 1999). The dominance of chlorophytes within the freshwater region was also expected, similar to studies by Zhu et al. (2015) on the Wanquan River and Duan and Bianchi (2006) along the Mississippi River and the Pearl River whereby the decrease in abundance was observed along the salinity gradients]. Galvão et al. (2008) suggested that large river inputs into estuaries would lead to high numbers of Cyanophyceae during the wet season; however, in this study it seems that the difference in biogeochemical factors led to decreased Cyanobacteria. Furthermore, the dominance of Euglenophytes during the wet seasons is consistent with sampling during higher riverine inputs which induce bloom-forming Euglenophyte species, such as the *Eutreptiella eupharyngea*, which often bloom in many countries (Yoo et al. 2018). However, the predominance of Cryptophytes within the brackish region during both seasons should be cause for concern. Warming and eutrophication due to nitrogen and phosphate led to increased presence of Cryptophytes (Brito et al. 2014; Šupraha et al. 2014; Sin and Jeong 2015).

5.5.1.2 Picoplankton Groups

For the distribution near the headwaters, the region near Kapit was sampled only in the dry season, which explains the lack of picoplankton data in that region in the wet season. *Prochlorococcus* were abundant throughout the Rajang River (**Fig. 5.5**), however, this should be carefully interpreted as almost undetectable amounts of dv-CHLa can imply the absence of *Prochlorococcus* (Chai et al. 2011). However, as shown by **Fig. 5.5**, *Prochlorococcus* was the most abundant class within the brackish and estuarine regions, which is normally associated with high productivity and nutrient concentrations. The existence of different strains with freshwater origin might be the reason why *Prochlorococcus* was also found in bays, estuaries and riverine regions (Vaulot et al. 1990; Shimada et al, 1995; Shang et al. 2007; Mitbavkar et al. 2009). The abundance of *Synechococcus* throughout the Rajang River, especially in the dry season (**Fig 5.6**), can be explained by differences in species as

well. Xia et al. (2015) studied estuarine and coastal waters of Hong Kong and observed that phycoerythrin-rich *Synechococcus* were ubiquitous throughout the region as they are able to accommodate large variations in salinity. In another study by Xia et al. (2017) along the Pearl River estuary, different *Synechococcus* species displayed specific preference for either marine or freshwater regions. This was reflected in this study in the relative phytoplankton contribution (**Fig. 5.2**); the relative abundance of freshwater cyanobacteria contrasts with the cyanobacteria associated with the marine region. Lastly, in terms of Pico-eukaryotes, the distribution pattern regardless of season was typical of Pico-eukaryotes distributions globally. Pico-eukaryotes are comparably more abundant in coastal waters as compared to oceanic waters (Pan et al. 2005), a pattern that was also observed in our data (**Fig 5.7**).

5.5.2 Environmental Drivers of Phytoplankton Community Composition, Picoplankton distribution and Size Distribution

Phytoplankton diversity and size are known to be affected by environmental factors and biogeochemistry of the surrounding waters (Finkel et al. 2007, Finkel et al. 2009). Silicate and salinity (**Fig. 4** and **Table 5.9**) appear to be the two factors that correlate the most with the shifts in phytoplankton taxa along the Rajang River-South China Sea continuum, but there are other factors. The distribution patterns are a reflection of the resource availability which results in different functional groups such as nitrogen fixers or silica specialists (Quinlan and Phlips 2007). The distribution of diatoms was negatively correlated with silicate (-0.51, dry; -0.763), SPM (-0.677 in the wet season). Its positive correlation with salinity in both dry and wet seasons (0.651; 0.745, respectively; see **Table 5.10** and **Table 5.11**) indicate its predominance in marine and brackish peat, as shown in **Fig. 5.2**. It can be inferred that the reduction in silicate concentration (refer to Chapter 3 **Table 3.1** and **Fig 3.3**) is correlated to the increase in Diatoms biomass (Biswas et al. 2015), as silicate was not the limiting factor for the production of Diatoms, with ratios higher than the optimum Redfield ratio of ~15 (McLaughlin et al. 2019).

Similarly, salinity was found to be the second highest driver of the phytoplankton community composition (**Fig 5.4** and **Table 5.9**), which correlates with the observed selective dominance of phytoplankton species (Bode et al. 2017; also see **Figure 5.2**). However, this study is in contrast to an "ecotone" model (Bolton 1983, Attrill and Rundle 2002), which exhibited an abrupt transition zone, indicating the heterogeneity of the estuarine region.

The classification of the regions suggests that the presence of DOC (proxy for humic acids derived from peat) might play a role in the abrupt shifts in the phytoplankton community as opposed to clustering based on salinity alone. However, based on the distLM model, the clustering of the phytoplankton community showed that DOC was not the main driver. This can be attributed to next highest environmental variables, DOP and SPM. As discussed in Chapter 3 and also shown within the

distLM model (**Fig 5.4**), higher DOP pools compared to DIP pools in the Rajang River led to the utilization of DOP over DIP. Fu et al. (2013) suggested that *Thalassiosira pseudonana*, a diatom, can utilize dissolved phosphine, and it was also found that the dinoflagellate *Symbiodinium kawagutii* contains a phosphite transporter (Lin et al. 2015a). While we did not identify dinoflagellates and diatoms down to the genus level, these studies still highlight the possible utilization of DOP pools by phytoplankton.

In terms of environmental drivers of picoplankton, the variables were similar to phytoplankton, in particular salinity, silicate and phosphate (Fig. 5.8 and Table 5.12). As put forth by Margalef (1978), Huete-Ortega et al. (2010) and Reul et al. (2006), waters which have high nutrient concentrations and are turbulent, are preferentially dominated by larger phytoplanktons. Waters which have low nutrient concentrations and are stratified are generally preferred by small phytoplanktons (Chisholmm 1992; Kiørboe 1993 and Marañon 2009). Based on the phytoplankton signature pigments, the microphytoplankton dominated the regions that were salinity influenced (marine and brackish peat region) in both dry and wet season (Fig. 5.3). This was exhibited in the dominance of diatoms as well as Chrysophytes (Fig. 5.2). Picoplankton dominated the freshwater region during the wet season (Fig. 5.3). It has been shown that when river flow is high, the downstream mass transport of biomass is relatively more important versus production utilizing DIP as a source of biomass (Malone et al. 1998). Thus, it can be implied that the higher flow during the wet season (as discussed in Chapter 3) led to the dominance of the picoplankton size class due to the lower nutrient concentrations in the upper reaches of the Rajang River as shown by increasing nutrient ratios (see Chapter 3, Table 3.2) (Thingstad, 1997; Roy et al. 2006). Cvanobacteria dominated the freshwater regions of the Rajang River regardless of peat or mineral soils. As shown by Zhang et al. (2013), the abundance of picoplankton was also negatively correlated with inorganic nutrients, hence further supporting the notion that the distribution of inorganic nutrients led to the aforementioned distribution of the phytoplankton size classes.

5.6 Conclusion

The present study represents a first assessment of the spatial and seasonal distribution of both freshwater and marine phytoplankton assemblages and picoplankton abundance. The profiles of phytoplankton pigments revealed distinct contribution of phytoplankton communities towards the total Chl a via CHEMTAX which changes according to source type. The main contributor towards the phytoplankton biomass in the marine and brackish regions in the dry season was diatoms (37.85%) in the marine region and chrysophytes (34.09%), respectively. On the other hand, in the wet season, the main phyla shifted to be Euglenophytes (30.84%) in the marine region whereas Chrysophytes remained the main contributor in the brackish peat (30.83\$). For the freshwater peat and mineral soils in the dry season, the main contributor was Chlorophytes (52.59% and 52.52%, respectively) which is commonly found in freshwater habitats. In the wet season, the highest contributor to both freshwater peat and mineral soil is the Euglenophytes (35.48% and 42.21%, respectively). Distributions of the picoplankton Synechococcus and Peuk were typical of global aquatic environments except for the occurrence of Pro within the freshwater region which may be attributed to Prochlorococcus-like picoplankton instead. The main drivers of the phytoplankton and picoplankton community of the Rajang River was dependent of the resource availability whereby silicate and salinity were the main drivers for phytoplankton, in which DOP and SPM also influences the phytoplankton communities while salinity, silicate and DIP influences the picoplankton community. This influenced the relative proportion of the phytoplankton size class whereby there was an increase in picoplankton abundance in the wet season as compared to the dry season. The lower nutrient concentrations upstream of the Rajang River were a result of higher flow in the wet season. This then led to the dominance of picoplankton upstream while larger sized phytoplanktons dominated the coastal regions due to higher abundance of nutrients. Hence, in order to further substantiate the phytoplankton and picoplankton communities (especially with regards to freshwater *Prochlorococcus*-like picoplankton) of the Rajang River-South China Sea continuum, further identification utilizing microscopy should be employed. The study of the photophysiological states of the phytoplankton assemblages in response to sediment turbation and anthropogenic activities, especially with increasing presence of cryptophytes within the brackish region should be cause for concern as an indicator warming and eutrophication due to nitrogen and phosphate. Lastly, mixotrophy studies should be further studied studies should also be taken into consideration together with studies of zooplankton in order to increase the understanding of the microbial loop within this region.

Chapter 6 General Summary

6.1 General Summary

This study is the first large-scale study covering almost 300 km of the Rajang River-South China Sea continuum that examines nutrient dynamics, budgets (with a focus on dissolved phosphorus) and the microbial communities (bacterial, phytoplankton and picoplankton). The research presented in this thesis is also one of very few studies which encompass both the nutrient dynamics and the microbial community composition of the "peat-draining" portion of tropical rivers. Overall, the findings based on observations across all three studies (nutrients, bacterial diversity and the phytoplankton assemblages and picoplankton distribution) can be classified under three recurring themes:

- 1) The spatial variation was apparent for all three components. DIP and DOP concentrations varied along the salinity gradient; they both exhibited non-conservative behaviour, with the DIP subjected to 57.78% removal and DOP subjected to 44.07% addition towards the South China Sea. Furthermore, the microbial communities changed according to the source type; distinct patterns were observed as a result of the changes in salinity along with variation of other biogeochemical parameters. The microbial communities found along the Rajang river exhibited taxa common to rivers (i.e. the predominance of β -Proteobacteria) while estuarine and marine regions exhibited taxa that were common to those regions as well (i.e. predominance of α - and γ -Proteobacteria). These results are in agreement with other salinity gradient profiled rivers and are substantiated by results obtained from linear models of various biogeochemical parameters. Alpha diversity indices indicated that the microbial diversity was higher upstream as compared to the marine and estuarine regions. However, despite the observed changes in bacterial community composition and diversity that occurred along the Rajang River to South China Sea continuum, the PICRUSt predictions showed minor variations. Lastly, the profiles of phytoplankton pigments revealed a distinct contribution of phytoplankton communities according to source type. The distributions of the picoplankton Synechococcus and Pico-eukaryotes were typical of global aquatic environments except for the occurrence of *Prochlorococcus* within the freshwater region, which may be attributed to Prochlorococcus-like picoplankton.
- 2) Changes according to seasonal variation. In the Rajang River, the bulk of the dissolved phosphate was in the form of DOP (73.84%), and both DIP and DOP may have supported phytoplankton biomass. Spearman correlations show that there was a possible seasonal switch in preference for DOP as compared to DIP depending on their relative concentrations. During the dry season the NO₃N:DIP ratios were lower, which created ideal conditions for phytoplankton proliferation, while in the wet season the increased NO₃N:DIP ratios led to lower phytoplankton biomass. The shifts in community composition from August 2016 to March 2017 and from March 2017 to September 2017 are indicative of the influence of seasonality. In terms of particulate versus free-living bacteria, nonmetric multi-dimensional scaling (NMDS) results showed similarly distributed

microbial communities with varying separation between seasons. The main drivers of the phytoplankton and picoplankton community of the Rajang River were resource availability; silicate and salinity were the main drivers for phytoplankton, with contributions from DOP and SPM. Salinity, silicate and DIP influence the picoplankton communities. The changes in phytoplankton size were also indicative of resource availability, as large sized phytoplankton were more abundant as compared to picoplankton when there was an availability of nutrients. While it was shown that spatial variation drives the phytoplankton and picoplankton population, it was observed that the seasonal variation led to greater changes in both the phytoplankton and the picoplankton community composition.

3) Possible Anthropogenic Influences. While the loading of P is not as extensive as in other major rivers, based on dSi:P ratios the source of P is likely anthropogenic in nature. Furthermore, anthropogenic perturbations, particularly oil palm plantations, led to increased richness but less diversity in the less pristine environments. Areas surrounded by oil palm plantations showed the lowest diversity; other signs of anthropogenic impacts included the presence of bacteria from the CFB-group and probable algal blooms. Especially the presence of *Cryptophytes* within the brackish region should be cause for concern as it is an indicator for eutrophication.

In conclusion, the findings of this study bridged the knowledge gap about spatial and seasonal variation of nutrient dynamics, budgets (with a focus on dissolved phosphorus) and microbial (bacterial, phytoplankton and picoplankton) communities of the Rajang River-South China Sea continuum. The insights and additional information gained can thus serve as a platform for future research.

6.2 Future outlook and recommendations

With the knowledge at hand, there are many opportunities to expand the knowledge and work together with the various stakeholders involved with the Rajang River and the South China Sea in order to preserve and provide sustainable solutions for future generations that are dependent on this region. Firstly, it is recommended that there should be an increase in the deployment of remote-sensing apparatus that can measure the hydrological and the physico-chemical properties of the Rajang River. This can aid in the long-term monitoring processes and assess any perturbations due to anthropogenic activities. In order to define and substantiate the sources of P (natural phosphorus has only one stable isotope - 31P), stable isotope studies utilizing the phosphate oxygen isotope (O'Neil et al. 2003) can be carried out as well. This can be conducted simultaneously with sediment records in order to obtain the paleo-ecology and the paleoclimate of the region prior to anthropogenic perturbation. Secondly, by understanding the bacterial and phytoplankton diversity and picoplankton abundance, further studies should be conducted, such as:

- Controlled experiments utilizing microfluidics to create microbial habitat structures (Aleklett et al. 2017) to further gauge potential changes in the microbial communities as a result of anthropogenic perturbations
- Incubation experiments that are coupled with metaproteomics that are based on the PICRUSt predictions of the functional potential of the bacterial communities
- Metabolomics which involve the direct relationship between the anthropogenic activities and the metabolites that are produced via microbial activity i.e. the metabolites that are produced by bacteria within the sediments of the riparian zone surrounding oil palm plantations
- Furthermore, potential biomarkers that are specific to the potential pathogenic bacteria can be developed from the metagenomic data obtained in order to rapidly assess the health of the ecosystem
- Also, the interface between the water column and sediments should be assessed for all three aspects especially the sedimentation of P, benthic algae and bacterial communities in order to fully understand the processes that occur along the Rajang River-South China Sea continuum.
- Lastly, mixotrophy studies should be further studied studies should also be taken into consideration together with studies of zooplankton in order to increase the understanding of the microbial loop within this region

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Appendix

Incubation Experiment

Position: Belawai of Rajang River Estuary													
Sampling time	e: 2016-	-08-28 at 06:00)										
Original samp	Original sample was collected from surface with bucket												
Water Depth: 6.8 m													
Transparency	Transparency: 60 cm												
Samples were	passed	through a 300	um size me	esh and mix	ed in a tank								
Volume of ind	cubation	n bottle: 1.5 L											
Temp (°C)pHTem (DO)SalinityDO (mg/l)DO (%)DO (mbr)													
27.7	7.99	25.8	33.8	4.25	63.2	129.8							

A respiration incubation was carried out for 9 hours in both dark and light bottles.

Supplementary Table 1: Incubation Experiment

Initial (mg L ⁻¹)	Bottle	Final Dark (mg L ⁻¹)	Respiration (g DO L ⁻¹ D ⁻¹)	Final Light (mg L ⁻¹)	Net Primary Productivity (g DO L ⁻¹ D ⁻¹)	
	1	4.18	0.19	4.6	0.51	
4.25	2	4.11	0.37	4.41	0.23	
	3	3.97	0.75	4.54	0.42	
		Average± SE	$(g DO L^{-1} D^{-1})$	Average± SE (g DO L ⁻¹ D ⁻¹)		
		0.44	± 016	0.39±0.08		

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	Sal	Temp	Do	pН	Nitrite	Amonium	Silicate	Nitrate	DIP	TDN	TDP	DON	DOP	DIN	SPM	POC

Supplementary Fig. 1: Draftsman Plot of normalised physico-chemical variables



Supplementary Fig. 2: Absolute values (counts) of the phyla present within all cruises

Based on the information above, the taxonomic data were classified based on the source type (i.e. mineral soil, freshwater peat) in which the stations fall under. According to Fig. 5, the bacterial phylum that was the most abundant across all samples was *Proteobacteria* (50.29%), followed by *Firmicutes* (22.35%) and *Actinobacteria* (11.95%). The remaining phyla belonged to *Bacteroidetes* (9.46%), *Deinococcus-Thermus* (2.69%), *Cyanobacteria* (1.61%), *Planctomycetes* (0.84%), *Chloroflexi* (0.34%), *Chlamydiae* (0.14%) and *Verrucomicrobia* (0.11%) respectively. Without taking into consideration the seasonality, spatial variation in the bacterial phyla based on the aforementioned sampling location is evidently apparent which were characteristic for each source types. The combined groups showed that the percentage of the *Proteobacteria* increased from marine (40.78% of total within marine samples), brackish peat (48.96%), freshwater peat (51.86%) to mineral soils (57.59%) while the percentage of *Firmicutes* decreased from marine (24.14%), brackish peat (25.31%), freshwater peat (19.34%), to mineral soils (16.26%). Furthermore, it can be seen that the phylum *Deinococcus-Thermus* generally has a higher relative abundance in freshwater peat (9.28%) and mineral soils (5.07%) as compared to marine (0.16%) and brackish peat (0.55%)

Supplementary Table 2: Metadata for Samples

Cruise	Source Type	Station	Long	Lat	Classification	DIP(µM)	DOP(µM)	TDP(µM)	Ammonium (µM)	Nitrate (µM)	Nitrite (µM)	DIN(µM)	DON(µM)	(MJ)N(JIM)	dSi (μM)	DO (mg/L)	Salinity (PSU)	SPM (mg/L)	DOC (µM)	Temp (°C)
Aug- 16	Marine	S1	110.9555	2.0203	Coastal Zone	0.09	0.27	0.36	6.78	1.54	0.04	8.37	1.56	9.92	4.02	4.14	32.10	25.02	0.15	31.47
Aug- 16	Marine	S2	111.3091	2.1157	Oil Palm Plantation	0.26	0.24	0.50	8.18	5.15	0.90	14.23	2.38	16.60	4.77	3.87	31.20	90.02	0.18	31.48
Aug- 16	Marine	S33	111.2047	2.4683	Coastal Zone with Plantation (OP) influence	0.17	0.23	0.40	7.14	2.59	1.76	11.49	4.14	15.64	5.08	4.08	31.10	32.70	0.22	30.26
Aug- 16	Brackish Peat	S3	111.5525	2.1663	Oil Palm Plantation	0.18	0.26	0.44	5.88	14.83	3.35	24.06	1.47	25.53	21.50	2.73	27.70	94.95	0.35	31.21
Aug- 16	Brackish Peat	S4	111.6524	2.2425	Oil Palm Plantation	0.07	0.27	0.35	6.05	14.45	3.08	23.58	4.44	28.02	145.89	3.01	4.80	67.66	0.36	30.29
Aug- 16	Brackish Peat	S20	111.6982	2.8249	Oil Palm and Sago mixed Plantation	0.06	0.31	0.37	6.85	7.21	0.17	14.23	1.81	16.04	149.68	4.28	5.60	57.51	0.26	30.64
Aug- 16	Brackish Peat	S21	111.6398	2.8641	Coastal Zone with Plantation (Sago and OP influence)	0.11	0.23	0.34	7.47	8.24	0.18	15.88	3.98	15.08	134.77	4.40	10.10	45.43	0.29	30.27
Aug- 16	Brackish Peat	S22	111.6	2.8646	Coastal Zone with Plantation (Sago and OP influence)	0.07	0.25	0.32	10.27	4.78	0.17	15.22	1.82	17.04	57.06	4.57	21.20	74.28	0.19	28.1
Aug- 16	Brackish Peat	S24	111.3976	2.5947	Oil Palm Plantation	0.13	0.24	0.37	7.76	15.85	0.26	23.87	6.21	30.08	69.60	3.41	19.10	116.10	0.22	30.78
Aug- 16	Brackish Peat	S25	111.4267	2.5005	Oil Palm Plantation	0.10	0.23	0.33	8.50	18.77	0.85	28.12	7.41	35.53	103.09	3.64	11.70	158.35	0.30	30.6
Aug- 16	Brackish Peat	S26	111.3856	2.4996	Oil Palm Plantation	0.12	0.22	0.34	7.25	17.00	0.37	24.62	3.21	27.83	82.98	3.28	15.90	161.27	0.26	30.58

Aug- 16	Brackish Peat	S27	111.4044	2.441	Oil Palm Plantation	0.13	0.19	0.32	9.63	14.55	2.71	26.89	1.90	28.79	69.52	2.98	19.40	53.70	0.21	31.01
Aug- 16	Brackish Peat	S29	111.5036	2.35	Oil Palm Plantation	0.08	0.18	0.27	9.07	16.40	3.21	28.68	3.19	21.68	107.86	3.20	4.30	42.27	0.21	30.01
Aug- 16	Brackish Peat	S30	111.4276	2.3738	Oil Palm Plantation	0.12	N/A	N/A	9.98	15.10	3.00	28.08	N/A	N/A	73.72	3.47	12.80	108.44	0.32	30.33
Aug- 16	Brackish Peat	S31	111.3644	2.3465	Oil Palm Plantation	0.15	0.32	0.47	7.44	7.84	1.73	17.00	6.52	23.52	22.85	2.95	19.90	81.17	0.28	30.42
Aug- 16	Brackish Peat	S32	111.3388	2.372	Oil Palm Plantation	0.14	0.27	0.41	7.39	5.78	0.72	13.88	2.71	16.59	7.88	3.69	27.90	58.12	0.23	30.55
Aug- 16	Freshwater Peat	S5	111.7545	2.2496	Oil Palm Plantation	0.03	0.17	0.20	3.86	6.62	0.12	10.59	13.86	24.45	145.04	4.10	0.00	84.05	0.26	30.24
Aug- 16	Freshwater Peat	S15	111.8464	2.4581	Oil Palm Plantation	0.02	0.23	0.25	6.17	6.18	0.14	12.49	4.28	16.77	156.36	3.53	0.00	N/A	0.23	29.39
Aug- 16	Freshwater Peat	S16	111.8075	2.6046	Oil Palm Plantation	0.04	0.27	0.31	7.94	6.33	0.16	14.43	1.93	16.36	152.27	3.87	0.00	34.41	0.25	29.84
Aug- 16	Freshwater Peat	S28	111.5036	2.35	Oil Palm Plantation	0.05	0.20	0.24	6.76	8.24	0.83	15.83	3.08	18.91	159.52	3.21	1.10	48.98	0.21	29.97
Aug- 16	Freshwater Mineral Soil	S6	111.9425	2.1805	Oil Palm Plantation	0.03	0.20	0.23	4.19	7.19	0.14	11.52	13.36	24.88	148.57	4.29	0.00	143.18	0.25	29.2
Aug- 16	Freshwater Mineral Soil	S7	112.2255	2.0743	Secondary Forest	0.04	0.17	0.21	3.50	5.95	0.14	9.59	5.47	15.06	154.39	4.83	0.00	31.78	0.20	28.85
Aug- 16	Freshwater Mineral Soil	S8	112.4888	2.0266	Secondary Forest	0.00	0.21	0.21	3.64	6.19	0.13	9.97	19.94	29.91	153.02	4.34	0.00	39.05	0.20	29.27
Aug- 16	Freshwater Mineral Soil	S9	112.6956	2.0097	Secondary Forest	0.03	0.15	0.18	17.34	6.92	0.13	24.40	2.41	26.81	151.22	4.36	0.00	52.63	0.18	27.83
Aug- 16	Freshwater Mineral	S10	112.8969	2.0176	Human Settlement	0.01	0.22	0.23	3.16	4.73	0.10	7.99	11.95	19.95	153.36	4.42	0.00	61.44	0.39	28.61

	Soil																			
Aug- 16	Freshwater Mineral Soil	S11	112.6965	2.0083	Secondary Forest	0.09	0.15	0.24	2.71	4.23	0.15	7.10	8.73	15.83	154.34	4.41	0.00	53.50	0.24	28.65
Aug- 16	Freshwater Mineral Soil	S12	112.4879	2.0272	Secondary Forest	0.02	0.20	0.22	5.82	5.33	0.10	11.25	19.84	31.09	158.42	4.25	0.00	65.64	0.20	28.01
Aug- 16	Freshwater Mineral Soil	S13	112.228	2.0755	Secondary Forest	0.01	0.28	0.30	2.82	4.79	1.19	8.80	8.79	17.59	179.00	4.61	0.00	N/A	0.20	28.24
Aug- 16	Freshwater Mineral Soil	S14	111.9413	2.1832	Oil Palm Plantation	0.05	0.19	0.24	2.89	4.49	0.12	7.49	19.56	27.06	167.57	3.43	0.00	144.63	0.19	28.82
Cruise	Source Type	Station	Long	Lat	Classification	(भर्म)तात	DOP(µM)	TDP(µM)	Ammonium (JuM)	Nitrate (µM)	Nitrite (µM)	(Wr ^f)NIC	(W ^{rl})NOQ	(Wil)NGL	dSi (μM)	(mg/L) DO	Salinity (PSU)	SPM (mg/L)	DOC (µM)	Temp (°C)
Mar- 17	Marine	S13	111.1311	2.4792	Coastal Zone	0.13	0.29	0.42	6.12	3.89	0.09	10.10	1.04	11.14	15.54	6.54	30.10	63.79	0.10	30.1
Mar- 17	Marine	S16	110.8344	1.8972	Coastal Zone	N/A	0.36	N/A	10.22	0.72	0.09	11.03	5.65	16.68	6.00	6.50	29.91	47.15	0.08	30.4
Mar- 17	Brackish Peat	S7	111.3648	2.3496	Oil Palm Plantation	0.08	0.13	0.20	7.53	7.51	0.53	15.57	5.51	21.08	126.00	5.53	5.41	66.72	0.16	27.5
Mar-	Brackish																			
17	Peat	S8	111.3555	2.3546	Oil Palm Plantation	0.07	0.12	0.19	6.71	7.05	1.24	15.01	6.81	21.82	90.09	6.13	9.99	36.06	0.16	28
Mar- 17	Peat Brackish Peat	\$8 \$9	111.3555 111.3304	2.3546 2.4033	Oil Palm Plantation Coastal Zone with Plantation influence	0.07	0.12	0.19	6.71	7.05	1.24	15.01 15.26	6.81 7.63	21.82	90.09 94.26	6.13 5.80	9.99 10.66	36.06 N/A	0.16 N/A	28 29.5
Mar- 17 Mar- 17	Peat Brackish Peat Brackish Peat	S8 S9 S10	111.3555 111.3304 111.3312	2.3546 2.4033 2.4062	Oil Palm Plantation Coastal Zone with Plantation influence Coastal Zone with Plantation influence	0.07	0.12 0.14 0.12	0.19 0.22 0.21	6.71 6.79 6.24	7.05 7.15 7.00	1.24 1.32 1.31	15.01 15.26 14.55	6.81 7.63 4.22	21.82 22.89 18.77	90.09 94.26 93.11	6.13 5.80 5.12	9.99 10.66 11.98	36.06 N/A N/A	0.16 N/A N/A	28 29.5 29.2

Mar- 17	Brackish Peat	S12	111.2442	2.4576	Coastal Zone with Plantation influence	0.18	0.26	0.44	4.61	6.27	1.81	12.69	2.90	15.59	56.48	5.95	20.99	76.06	0.14	28.9
Mar- 17	Brackish Peat	S14	111.4088	2.1407	Human Settlement	0.08	0.32	0.40	6.72	0.75	0.03	7.50	0.52	8.03	11.09	6.50	27.63	N/A	0.09	29.5
Mar- 17	Brackish Peat	S15	111.2537	2.1381	Human Settlement	0.12	0.23	0.34	2.86	6.88	4.25	13.99	6.03	20.03	63.47	5.46	14.48	42.22	0.17	28.13
Mar- 17	Freshwater Peat	S1	111.5531	2.1662	Oil Palm Plantation	0.17	0.04	0.21	9.83	8.09	0.00	17.92	3.26	21.18	142.22	4.55	0.00	244.76	N/A	27.7
Mar- 17	Freshwater Peat	S2	111.7569	2.2505	Human Settlement	0.13	0.13	0.26	9.71	8.33	0.00	18.04	2.93	20.97	143.91	6.67	0.00	494.46	N/A	28.3
Mar- 17	Freshwater Peat	S4	111.8212	2.4712	Oil Palm Plantation	0.05	0.09	0.14	4.09	6.95	0.17	11.22	6.77	17.98	147.07	6.28	0.00	196.61	0.18	28.2
Mar- 17	Freshwater Peat	S5	111.8454	2.4588	Oil Palm Plantation	0.06	0.12	0.17	3.99	7.69	0.22	11.89	7.14	19.04	143.11	6.09	0.00	179.49	0.20	26.9
Mar- 17	Freshwater Peat	S6	111.6528	2.3565	Oil Palm Plantation	0.01	0.12	0.13	0.46	7.68	0.01	8.14	4.96	13.11	158.42	5.82	0.00	205.12	0.17	27.7
Mar- 17	Freshwater Mineral Soils	S3	111.9154	2.2048	Oil Palm Plantation	0.06	0.09	0.16	2.97	7.21	0.16	10.34	8.43	18.77	157.00	5.96	0.00	226.73	0.13	26.6
L	50115																			
Cruise	Source Type	Station	Long	Lat	Classification	DIP(µM)	DOP(µM)	TDP(µM)	Ammonium (µM)	Nitrate (µM)	Nitrite (µM)	DIN(µM)	DON(µM)	TDN(µM)	dSi (µM)	OQ	Salinity (PSU)	SPM (mg/L)	DOC (µM)	Temp (°C)
Sep- 17	Soils Soils Mineral Soils	Station IS	112.5490	2.0124	U U Sassification O Human Settlement	N/A	(With) dod N/A	(MII)900	Ammonium (µM)	V/N Nitrate (µM)	V/N Nitrite (µM)	(Wr)NIQ N/A	(MI)NOO XA	(Wil)NGL	(Wit) isp N/A	0 7.25	o Salinity (PSU)	Z/M (mg/L)	DOC (µM)	(°C) Temp (°C)
Sep- 17 Sep- 17	ed, L Soils Mineral Soils Mineral Soils	Station S1	112.5490	ten 2.0124 2.0987	Human Settlement	(Wil)AIIO N/A	(WT) N/A N/A	(MI)AUT	Ammonium N/A N/A	V/V Nitrate (µM)	N/A N/A	(WI)NIQ N/A	(Wit)NOQ N/A	(Wt)NGL N/A	(WT) isp N/A	0 7.25 6.71	0 0.01	(T)/A (J/BU) (J/A (J/A)/A (J/A	DOC (hiM)	() 26.5 27.8
Sep- 17 Sep- 17 Sep- 17	ed, L end Soils Mineral Soils Freshwater Peat	Station S1 S3	112.5490 112.1628 111.5065	2.0124 2.0987 2.1394	Human Settlement Human Settlement Human Settlement	(Wtl)aid N/A N/A	(Wit)JOOQ N/A N/A	(WI)AQL N/A N/A	W/A N/A N/A	N/A N/A	N/A N/A	(WT)NIQ N/A N/A	(WH)NOQ N/A N/A	(Wt)NGL N/A N/A	(W ^{II}) isp N/A N/A	2 7.25 6.71 5.47	0 1.1	(T/BM) WdS N/A N/A	DOC (htm) N/A N/A	26.5 27.8 27.6

| Sep-
17 | Freshwater
Peat | S5 | 111.7303 | 2.8069 | Coastal Zone with
Plantation (OP)
influence | N/A | 5.4 | 0.01 | N/A | N/A | 30.18 |
|------------|--------------------|------------|----------|--------|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-------|-----|-----|-------|
| Sep-
17 | Brackish
Peat | S6 | 111.6260 | 2.8531 | Coastal Zone with
Plantation (OP)
influence | N/A | 5.76 | 17.86 | N/A | N/A | 29.93 |
| Sep-
17 | Brackish
Peat | S7 | 111.4114 | 2.3618 | Oil Palm Plantation | N/A | 4.85 | 5.12 | N/A | N/A | 27.9 |
| Sep-
17 | Brackish
Peat | S 8 | 111.3117 | 2.4195 | Oil Palm Plantation | N/A | 5.31 | 14.59 | N/A | N/A | 28.05 |

Source Type	Station	%pico	%nano	% micro
Marine	AUG16RAJST33	26.90	7.35	65.75
Marine	AUG16RAJST2	12.52	2.77	84.71
Brackish Peat	AUG16RAJST32	10.58	3.49	85.93
Brackish Peat	AUG16RAJST3	17.44	2.77	79.79
Brackish Peat	AUG16RAJST31	12.30	6.56	81.14
Brackish Peat	AUG16RAJST30	20.35	3.42	76.23
Freshwater Peat	AUG16RAJST5	74.02	6.77	19.21
Freshwater Peat	AUG16RAJST28	66.57	6.12	27.32
Freshwater Peat	AUG16RAJST16	59.89	12.60	27.52
Freshwater Peat	AUG16RAJST15	47.22	9.18	43.59
Mineral Soils	AUG16RAJST6	48.27	6.01	45.72
Mineral Soils	AUG16RAJST7	41.24	3.40	55.36
Mineral Soils	AUG16RAJST8	38.47	5.43	51.93
Mineral Soils	AUG16RAJST10	34.82	3.40	61.78

Supplementary Table 3: Relative proportion of phytoplankton size class (%) in the Dry season

Supplementary Table 4: Relative proportion of phytoplankton size class (%) in the Wet season

Source Type	Station	%pico	%nano	% micro
Marine	MAR17RAJST16	47.07	11.26	41.67
Marine	MAR17RAJST13	23.09	3.79	73.12
Brackish Peat	MAR17RAJST14	46.97	5.18	47.86
Brackish Peat	MAR17RAJST12	5.76	2.00	92.24
Brackish Peat	MAR17RAJST15	10.65	3.11	86.24
Brackish Peat	MAR17RAJST11	19.17	6.42	74.41
Brackish Peat	MAR17RAJST10	13.03	2.84	84.13
Brackish Peat	MAR17RAJST9	13.64	5.28	81.08
Brackish Peat	MAR17RAJST8	10.70	3.29	86.01
Brackish Peat	MAR17RAJST7	27.88	25.47	46.65
Freshwater Peat	MAR17RAJST1	100.00	0.00	0.00
Freshwater Peat	MAR17RAJST5	100.00	0.00	0.00
Freshwater Peat	MAR17RAJST6	100.00	0.00	0.00
Mineral Soil	MAR17RAJST3	100.00	0.00	0.00

Supplementary Data 1: mothur code carried out with OzSTAR (HPC, Swinburne Melbourne)

#!/bin/bash #SBATCH --nodes=1 #SBATCH --ntasks-per-node=1 #SBATCH --cpus-per-task=12 #SBATCH --time=5:00:00 #SBATCH --mem=64g #SBATCH -- job-name=MiSeq Rajang #SBATCH --output=slurm misegrajbac 13062018.out module load vsearch/2.8.0 module load mothur/1.39.5-python-2.7.14 ##mothur "#make.contigs(file=./bacteria.files, processors=4)" ##mothur "#summary.seqs(fasta=./bacteria.trim.contigs.fasta)" ##mothur "#unique.seqs(fasta=./bacteria.trim.contigs.fasta)" ##mothur "#summary.seqs(fasta=./bacteria.trim.contigs.unique.fasta, name=./bacteria.trim.contigs.names)" ##mothur "#align.seqs(fasta=./bacteria.trim.contigs.unique.fasta, reference=./silva.nr v132.align, processors=32)" ##mothur "#summary.seqs(fasta=./bacteria.trim.contigs.unique.align, name=./bacteria.trim.contigs.names)" ##mothur "#screen.seqs(fasta=./bacteria.trim.contigs.unique.align, name=./bacteria.trim.contigs.names, group=./bacteria.contigs.groups, optimize=start-end, minlength=400, maxambig=0, maxhomop=8, processors=32)" ##mothur "#summary.seqs(fasta=./bacteria.trim.contigs.unique.good.align, name=./bacteria.trim.contigs.good.names)" ##mothur "#filter.seqs(fasta=./bacteria.trim.contigs.unique.good.align, trump=., vertical=T, processors=32)" ##mothur "#summary.seqs(fasta=./bacteria.trim.contigs.unique.good.filter.fasta, name=./bacteria.trim.contigs.good.names, processors=32)" ##mothur "#unique.seqs(fasta=./bacteria.trim.contigs.unique.good.filter.fasta, name=./bacteria.trim.contigs.good.names)" ##mothur "#pre.cluster(fasta=./bacteria.trim.contigs.unique.good.filter.unique.fasta, name=./bacteria.trim.contigs.unique.good.filter.names, group=./bacteria.contigs.good.groups, diffs=2)" ##mothur "#summary.seqs(fasta=./bacteria.trim.contigs.unique.good.filter.unique.precluster.fasta, name=./bacteria.trim.contigs.unique.good.filter.unique.precluster.names)" ##mothur "#chimera.vsearch(fasta=./bacteria.trim.contigs.unique.good.filter.unique.precluster.fasta, name=./bacteria.trim.contigs.unique.good.filter.unique.precluster.names, group=./bacteria.contigs.good.groups, dereplicate=t, processors=32)" ##mothur"#remove.seqs(accnos=./bacteria.trim.contigs.unique.good.filter.unique.precluster.denovo.v search.accnos, fasta=./bacteria.trim.contigs.unique.good.filter.unique.precluster.fasta, name=./bacteria.trim.contigs.unique.good.filter.unique.precluster.names, group=./bacteria.contigs.good.groups)" ##mothur "#classify.seqs(fasta=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.fasta, name=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.names, template=./eztaxon full.align, taxonomy=./eztaxon id taxonomy.tax, cutoff=80, processors=36)" ##mothur"#remove.lineage(fasta=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.fast a, name=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.names, group=./bacteria.contigs.good.pick.groups, taxonomy=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.eztaxon id taxonomy.wan g.taxonomy, taxon=Eukaryota-Mitochondria-Eukarya-Chloroplast-Archaea-unknown)" ##mothur"#cluster.split(fasta=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.fas ta, name=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.names,

taxonomy=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.eztaxon_id_taxonomy.wan g.pick.taxonomy, splitmethod=classify, taxlevel=4, cutoff=0.03, processors=32)"

##mothur "#dist.seqs(fasta=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.fasta, cutoff=0.15, processors=32)"

##mothur "#cluster(column=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.dist, name=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.names, cutoff=0.03, processors=32)"

##mothur"#classify.otu(taxonomy=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.ezt axon id taxonomy.wang.pick.taxonomy,

list=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.opti_mcc.list,

name=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.names,

group=./bacteria.contigs.good.pick.pick.groups)"

##mothur"#split.abund(fasta=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.fast a, cutoff=1, list=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.opti_mcc.list, group=./bacteria.contigs.good.pick.pick.groups,

name=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.names, label=0.03)" ##mothur"#make.shared(list=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.opti

_mcc.0.03.abund.list, group=./bacteria.contigs.good.pick.pick.0.03.abund.groups, label=0.03)" ##mothur "#count.groups(group=./bacteria.contigs.good.pick.pick.0.03.abund.groups)"

##mothur"#sub.sample(list=./bacteria.trim.contigs.good.pick.pick.0.03.abund.groups size=115)"
##mothur"#make.shared(list=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.opti
mcc.0.03.abund.0.03.subsample.list,

group=./bacteria.contigs.good.pick.pick.0.03.abund.subsample.groups, label=0.03)" ##mothur "#summary.single(groupmode=t,

shared=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.opti_mcc.0.03.abund.0.0 3.subsample.shared, calc=nseqs-sobs-coverage-shannon-npshannon-simpson-invsimpson-chao-ace)" ##mothur"#summary.tax(taxonomy=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.e ztaxon id taxonomy.wang.pick.taxonomy,

name=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.names,

group=./bacteria.contigs.good.pick.pick.groups, relabund=T)"

##mothur"#make.biom(shared=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.o pti_mcc.0.03.abund.shared,

constaxonomy=./bacteria.trim.contigs.unique.good.filter.unique.precluster.pick.pick.opti_mcc.0.03.co ns.taxonomy)"

##skipped command to remove the chimeras sequences