MICROBIAL AND EXTRACELLULAR POLYMERIC SUBSTANCE DYNAMICS IN ARID–ZONE TEMPORARY PAN ECOSYSTEMS

by

TAFARA FRANK BUTE

A thesis submitted in fulfilment of the requirements for the degree of Master of Science in the Department of Zoology and Entomology, Rhodes University

SUPERVISORS: PROF. RYAN J WASSERMAN AND DR TATENDA DALU

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https://orcid.org/0000-0002-0875-9949

ABSTRACT

Microbial communities of bacteria, viruses, algae, protozoans and fungi participate profoundly in aquatic systems, particularly in mediating processes such as primary production, decomposition, and biogeochemical cycles. In addition, microbiomes produce extracellular polymeric substances (EPS) which encompass a hydrated exopolymer mainly constituted of carbohydrates and proteins. The exopolymer aid proliferation and persistence of biofilms on their resident surfaces. There is however paucity of data on functional diversity of microbiomes in arid zone temporary wetlands with previous research having mainly focused on permanent systems in the northern hemisphere. In the face of ongoing climatic changes and anthropogenic threats to wetlands, it is imperative to assess the health status of aquatic systems in relation to microbial productivity dynamics. In this thesis, colorimetric methods and sequence–based metagenomics were conducted to quantify microbial EPS production and bacterial metagenome functions, respectively. This study was conducted in Khakhea–Bray region (North–West, South Africa) in June 2021 and January 2022 with a focus on evaluating microbial patterns of distribution between seasons (i.e., Dry and Wet) and varying depth i.e., deepest zones (Deep), intermediate depth (Mid) and shallowest regions (Edge). Additionally, potential relationships between EPS and either water content or organic matter content (OM content) were evaluated. In this study it was hypothesized that wet phases and deeper zones will have high EPS production and support more functions in comparison to shallowest regions and dry phases.

Carbohydrates and proteins were quantified using the Dubois method and modified Lowry procedure, respectively. Carbohydrates generally occurred in higher proportions than proteins, suggesting that EPS found in these systems was largely diatom produced. The wet phases (wet season and inundation periods) supported more EPS production compared to the dry phases. The results of principal components analysis (PCA) and Spearman's correlations suggested that EPS was highly correlated with sediment water content among other assessed variables. No significant associations were established between EPS and organic matter content. Spatial distribution of EPS demonstrated similar patterns between the deepest (Deep) and the intermediate depth zones (Mid) however the shallow regions (Edge) had significantly lower concentrations.

Bacterial characterization was established by amplification of the 16S rRNA gene using illumina–sequencing protocol. Enzyme functions associated with biogeochemical pathways were predicted in PICRUSt2 bioinformatics pipeline. A total of 15 042 Unique Amplicon Sequence Variants (ASVs) were observed to be affiliated to 51 bacterial phyla and 1 127 genera. All top genera had commonality in heat tolerance. Firmicutes, dominated at phyla level with 59 % (mean ± sd, 19 ± 13 %) relative abundance followed by Actinobacteria and Proteobacteria both at 34 % (18 ± 7 %) and (18 ± 6 %), respectively. Microbial diversity matrices highlighted significant differences in beta diversity more than alpha diversity. Bacterial microbiomes were more distinct between seasons compared to within season, suggesting that functions were seasonally driven. These findings were supported by highest rates of denitrification, carbohydrate degradation and EPS production by core microbiomes in the wet season as compared to low rates of nitrogen mineralisation, carbon fixation and nitrification in the dry season.

The present findings represent a first attempt in evaluating sequence–based metagenomics in semi–arid southern African temporary pan ecosystem. Both microbial EPS and bacterial functional potential were highly driven by water availability, with highest rates mainly associated with maximum inundation compared to dry states of pans. It can therefore be suggested that extended dry periods are threatening to microbially mediated processes in temporary wetlands, with implications to loss of biodiversity due to desiccation resulting in poor nutrient cycling.

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To all my friends and family, I express my deep appreciation in the positive energy you imparted in my academic journey. A special mention to my parents Mr. and Mrs. Bute, without you I wouldn't be who I'm today. Thank you for your support and everything you have done I will always be indebted to you and may God bless you abundantly. To my bother Donald Bute and my sisters Chengetai Bute and Tinovimbanashe Bute I love you guys, we are destined for greatness.

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DECLARATION

I, Tafara Frank Bute, hereby declare that this thesis entitled "Microbial and extracellular polymeric substance dynamics in arid–zone temporary pan ecosystems" is my original work carried out in the Department of Zoology and Entomology, Rhodes University under the supervision of Prof. Ryan J. Wasserman and Dr. Tatenda Dalu. All components of this thesis have not been submitted for any degree or examination at any other university or tertiary institution.

Signed: 14 December 2022

PREFACE

This work was presented at the following conferences (*first and presenting author only*):

1. **Tafara Frank Bute**, Ryan Wasserman, Adam Wyness, Tatenda Dalu. *Microbial dynamics in temporary pans of Khakhea-Bray Transboundary Aquifer quantified using metagenomics techniques*. 5th Southern African Development Committee (SADC) Groundwater 2022 Conference, from 16–18 November 2022, Windhoek, Namibia.

2. **Tafara Frank Bute**, Ryan Wasserman, Adam Wyness, Tatenda Dalu. *Microbial and Extracellular Polymeric Substances (EPS) dynamics in temporary pans of the Khakhea-Bray Transboundary Aquifer region.* Southern African Society for Aquatic Scientists (59th SASAqS) Conference, from 26–30 June 2022, Brandford Bloemfontein, South Africa.

3. **Tafara Frank Bute**, Ryan Wasserman, Adam Wyness, Tatenda Dalu. *Microbial dynamics in freshwater pans of Khakhea-Bray Transboundary Aquifer quantified using metagenomics techniques*. Southern African Society for Aquatic Scientists (58th SASAqS) Virtual Conference, from 2–4 November 2021, University of Free State (UFS), South Africa.

4. **Tafara Frank Bute**, Ryan Wasserman, Adam Wyness, Tatenda Dalu. *Microbial dynamics in freshwater pans of Khakhea-Bray Transboundary Aquifer quantified using metagenomics techniques*. 4th Southern African Development Committee (SADC) Groundwater 2021 Virtual Conference, from 10–12 November 2021.

DEDICATION

I dedicate this thesis to my family; Abgarl Materesa Bute, Norbert Bute, Donald Nyasha Bute, Charleene Chengetai Bute, and Tinovimbanashe Derleen Bute. I also dedicate this thesis to the field of microbial ecology in aquatic systems.

CHAPTER 1

GENERAL INTRODUCTION

Plate 1. Aerial view highlighting the mosaic of inundated and dry pans during the dry season, in the Khakhea–Bray temporary pan network in the North–West Province, South Africa. Photo credit: Dr. Adam J. Wyness, June 2021.

1.1 BACKGROUND

1.1.1 Wetlands

Globally, wetlands are considered valuable ecosystems (Bhowmik, 2019; Xu et al., 2019; Dymond et al., 2021; Adeeyo et al., 2022) and are formed as a result of temporal or permanent inundation on pieces of land. In these systems, precipitation is the main source of recharge and the water table is situated adjacent to the water surface (Brinson, 1993; Ellery et al., 2016). Given this, wetlands are highly controlled by the availability of water in supporting ecosystem functioning (Niering, 1985; Wasserman and Dalu, 2022).

Intermittently inundated wetlands commonly referred to as temporary wetlands (Figure 1.1), are characteristically small and of shallow depth (Calhoun et al., 2017). They are distinguished from permanent wetlands by their cyclical and alternating drying and rewetting phases (Olde Venterink et al., 2002). Hydroperiod defines the length of the inundation phase, which usually follows precipitation and is the major driver of community assemblages in these systems (Batzer and Boix, 2016). Although hosting similar communities, there are various types of temporary wetlands which include ponds, rock pools, pans (inland endorheic pans), playas, potholes, tarns and bays (Sharitz, 2003; Rodríguez–Rodríguez, 2007; Johnson and Poiani, 2016; Dunnink et al., 2016).

1.1.2 Characteristics of pans

In the Global South, pans are the most prominent geographical feature throughout much of the region (Goudie and Thomas, 1985; Riato et al., 2014). More often, pans are referred to as depressions that are flat–bottomed (Ollis et al., 2013) and mainly characterised by closed elevation contours, with gradual increase in depth (less than 3m) from the edges to the central zone (Cilliers and Bredenkamp, 2003). In shape pans appear circular to oval without any outlet. Although most frequently pans are situated near the water table, they are highly controlled by seasonal water recharge (De Klerk et al., 2016). In South Africa, most pans are seasonally dry and fill up during the rainy season. Furthermore, these habitats were observed to oscillate between freshwater and saline conditions as a response of change in physico-chemical characteristics of the pan soil driven by the progression and intensification of evaporation (Allan et al., 1995, Meintjies et al., 1994).

1.1.3 Ecological significance of pan ecosystems

Pans have important ecological contributions such as providing habitat for unique biodiversity (Necker et al., 2016) including migratory birds (Allan, 1987). In rural areas pans save as both a source of water and of nutritious plants for grazers. Additionally, permanently inundated pans are often used to supply water for domestic use and/or irrigation by humans in arid regions. As a type of wetland, pans also play important roles in biogeochemistry however the data is scarce for the Global South. Despite studies which have evaluated plant communities in endorheic pans in the region (Cilliers and Bredenkamp, 2003) and macroinvertebrate diversities (Nhiwatiwa et al., 2011; Nhiwatiwa et al., 2017) current achievements in EPS and 16S microbial communities in the study of pans is severely limited yet important in elucidating the contributions of microbes in these arid ecosystems.

The importance of microbial communities in freshwater ecology have been widely reported for permanent systems. With much research addressing issues pertaining to i) spatio-temporal diversity of microbial communities (Villarino et al., 2022), ii) importance of EPS in aquatic food webs (Hede and Khandeparker, 2020) and iii) role of microbes in global nutrient cycling (Arrigo, 2005, Zhang et al., 2020). This study therefore seeks to bridge the knowledge gap in microbial ecology between permanent systems and temporary systems (pans), with much more emphasis on EPS and nutrient cycling.

Figure 1.1. A representative view of (a) inundation state and (b) dry phase of a temporary pan in Khakhea–Bray Transboundary region in the North–West Province of South Africa, in period June 2021 and January 2022, respectively. Photo credit: Dr Tatenda Dalu

1.1.4 Wetland microbial ecology

Microbiomes in wetlands which include diatoms, bacteria and viruses are critically important in the ecosystem functioning and contribute significantly to high ecological productivity in these systems (Chandra et al., 2020; Olszewski et al., 2020). In the natural environment including wetlands, microbes aggregate to form biofilms so as to enhance chances of survival against external environmental harsh conditions (Chandki et al., 2011; Yin et al., 2019). More so, the ecological significance of diatoms emanates from their fundamental participation at the base of aquatic food chains as primary producers (Goldman, 1993) and extracellular polymeric substance production (Smith and Underwood, 1998) to biomonitoring (Dalu and Froneman, 2016). Other microbes such as bacteria, viruses (Jackson and Jackson, 2008) and fungi also mediate important biogeochemical processes (Grossart et al., 2020), in addition to EPS production with implications to dynamics in atmospheric chemistry. Biogeochemical cycles are critical for ecosystem sustainability (Prosser et al., 2007). More so, the efficiency of microbes is highly facilitated by the microbial loop (Okafor, 2011), which aggregates specialist microbiomes involved in channelling energy into higher trophic levels (Prog et al., 1983). The advances in molecular ecology such as polymerase chain reaction (PCR) and illumina sequencing (Jiang et al., 2013) in combination with bioinformatics tools (Schloss et al., 2009; Bolyen et al., 2019) have allowed profiling of functional microbiomes, without cultivation (Okafor, 2011).

1.1.5 Global contribution of temporary wetlands

Despite wetlands covering less than 10 % of the earth's surface, their ecosystem functions and services contribute more at the global level (Robinson, 1995; Zedler and Kercher, 2005).

Temporary wetlands are renowned biodiversity hotspots due to their higher species richness generated by dry and wet distinct phases which support unique taxa best adapted to these episodes (Keddy, 2010; Mlambo et al., 2011; De Carvalho and Caramujo, 2014). Furthermore, the participation of temporary wetlands in biogeochemical cycling and organic matter content decomposition brings balance to the recycling of matter necessary to support existence of life on earth (Gaunt et al., 1995; Reddy and DeLaune, 2008). Alternatively temporary wetlands provide socioeconomic value regionally and internationally which also include cultural services and flood control (Terer et al., 2004; Gichuki and Macharia, 2006; Musamba et al., 2017).

1.1.6 Current threats to temporary wetlands

Given their relative smaller size and shallow depth, temporary wetlands are often indistinguishable during dry phase hence very vulnerable to degradation (Boix et al., 2016). The greatest threat of temporary wetlands remain anthropogenic invasions (Dube et al., 2020) and climate changes (Nielsen et al., 2009; Johnson and Poiani, 2016). Humans destroy temporary wetlands in various ways which include urbanization (Horner, 2001; Mahmud et al., 2017) and excessive groundwater water abstraction for agriculture resulting in the lowering of water table (Li et al., 2021). Furthermore, temporary wetlands in semi–arid zones such as Khakhea–Bray (North–West Province, South Africa) are more susceptible to climatic changes due to an imbalance in the total amount of rainfall received and evapotranspiration (LaBaugh et al., 1996).

1.2 AIM AND OBJECTIVES

The aim of the study is to assess the dynamics of microbial communities and extracellular polymeric substances (EPS) production in the Khakhea–Bray semi–arid zone temporary pans using colorimetric methods and metagenomics techniques.

1.2.1 Objectives

The objectives of this thesis are to:

1. quantify and assess distributions of major EPS constituents (carbohydrates and proteins) present in sediment biofilms.

2. explore interrelationships between EPS and sediment characteristics of water content and organic matter content (OM content).

3. characterise bacterial communities in benthic sediments by targeting 16S rRNA gene using illumina high–throughput sequencing technology.

3. examine the abundance and diversity of bacteria across seasons and a depth gradient,

4. predict and compare bacterial metagenome functions central in mediating important biogeochemical pathways in temporary wetland ecosystems.

1.3 RESEARCH HYPOTHESES

The study hypothesised that;

i) there would be an increased yield of EPS during wet phases in comparison to dry phases as sediment water content directly impacts nutrient availability for EPS producing microorganisms.

ii) spatial variation of EPS, organic matter content and water content within each system would be limited.

iii) linear relationships would persist between EPS, organic matter content and water content due to interdependence of these factors in the ecosystem.

(iv) more heat tolerant taxa would dominate given the arid nature of Khakhea–Bray.

(v) core microbiomes in the wet season would have higher diversity than dry season, due to increased sediment water content which supports proliferation of hydrated biofilms.

(vi) predicted metagenome functions for enzymes involved in biogeochemical cycles would be seasonally correlated, with more in the deeper areas of inundated pans in wet season, as these zones have relatively more depth and dry out last in comparison to the outer areas.

1.4 THESIS OUTLINE

The thesis constitutes of four (4) chapters, which address microbial dynamics and their associated activities in the Khakhea–Bray temporary pan ecosystem region.

Chapter One is a general background of the study and an outline of the importance of temporary wetlands and the role microbiomes play in these systems. In addition, this chapter follows the aim and objectives, research questions, and description of the study site.

Chapter Two assesses microbial extracellular polymeric substance dynamics between dry and wet season and with varying depths, evaluating correlations with water content/organic matter content. This chapter provides detailed sections which include introduction, methods, results, and discussion on key findings.

Chapter Three demonstrates bacterial dynamics in temporary pans and evaluates distributions between seasons and with varying depths. This chapter further addresses participation of functional microbiomes in biogeochemical cycling and the relative dynamics associated. The following list of sections provide detailed information for this chapter i.e., introduction, methods, results, and discussion on main findings.

Chapter Four is General discussion that consolidates the findings of the two empirical data chapters and makes conclusions and recommendations around microbial ecology in temporary wetland ecosystems.

References Combined reference list for all chapters at the end ensured limited repetition.

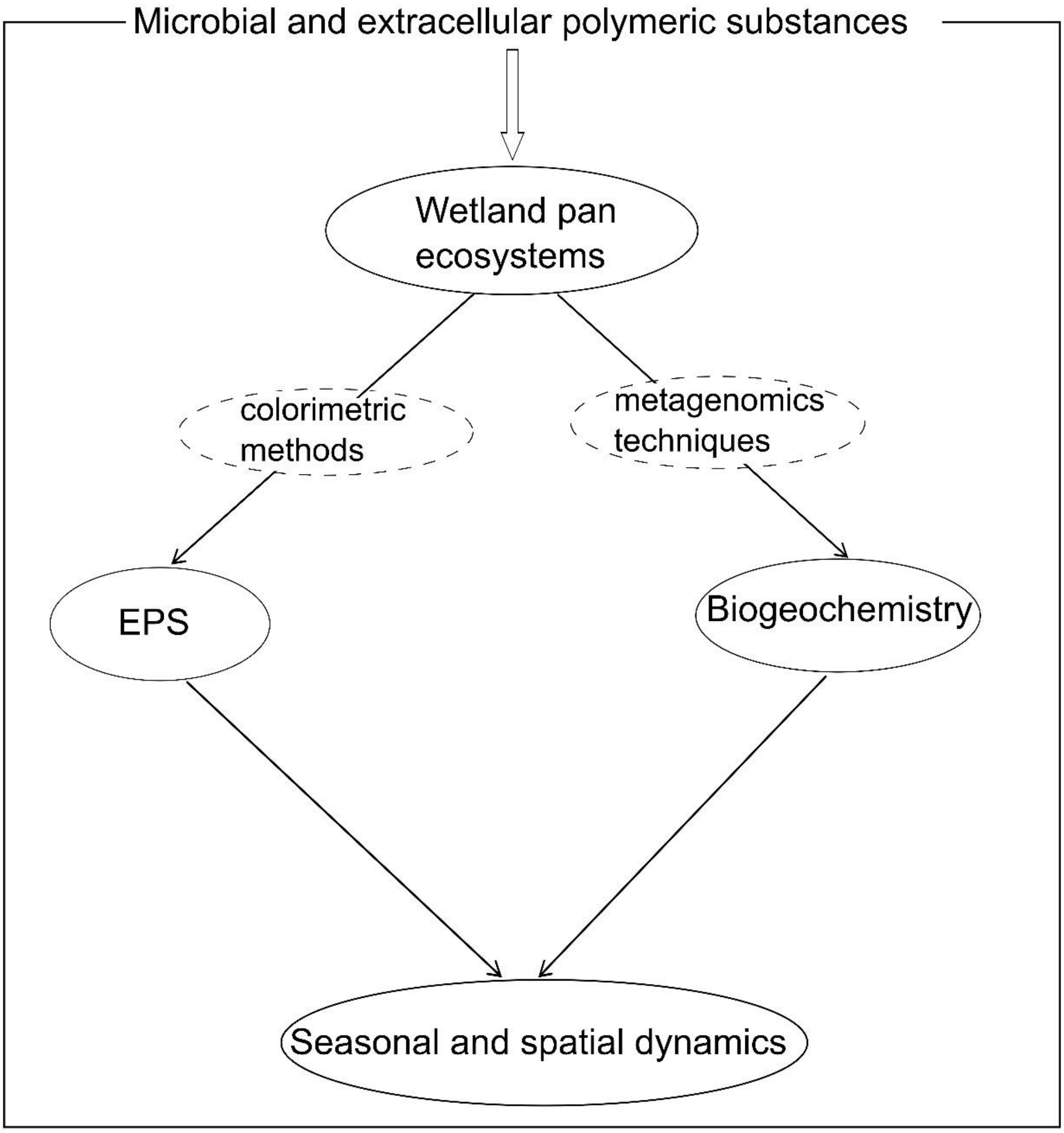

Figure 1.2. Logical framework showing how chapters in this thesis are connected.

CHAPTER 2.

MICROBIAL EXTRACELLULAR POLYMERIC SUBSTANCES IN SEMI–ARID TEMPORARY PANS OF THE KHAKHEA–BRAY REGION, NORTH–WEST PROVINCE OF SOUTH AFRICA.

Plate 2. A representative view of the partial inundation in temporary pans of the Khakhea-Bray region in the North–West Province of South Africa. The Edge gradually experience increased drying resulting in water accumulating towards the centre and more deeper zones. Photo Credit: Dr. Chad Keates, June 2021.

ABSTRACT

Microbial extracellular polymeric substances in the form of carbohydrates and proteins in temporary wetland systems are often overlooked. This is despite their various ecological contributions which include inter-particle binding in benthic sediment stability, protection of biofilms and being a source of nutrients in the benthic–pelagic food webs. The study of extracellular polymeric substances (EPS) in aquatic ecology has been centralised in more permanent systems with limited information currently available for temporary ecosystems such as pans. With forecasted climate change that is associated with less volume and frequency of rain, it is critical to evaluate the potential impacts of such environmental perturbations to ecosystem functioning dynamics. The present study investigated seasonal and spatial variations in extracellular polymeric substances (EPS) production by benthic biofilms and evaluated correlations with sediment water content and organic matter content. To achieve these objectives, ten pans were sampled in the Khakhea–Bray region, North–West Province of South Africa in the dry season (June 2021) and wet season (January 2022). Sediment cores were extracted across varying depth starting from deepest zones (Deep), intermediate depth (Mid points) and shallowest regions (Edge). To determine EPS production colorimetric methods were employed where carbohydrates were measured using phenol–sulfuric acid assay and total protein (PRT) analysis was conducted using the modified Lowry procedure. Results demonstrated significant differences between the seasons and across varying depths. The wet phases had higher EPS production compared to dry phases. The deepest zones (Deep) could not be differentiated from intermediate depth (Mid) however the shallowest regions (Edge) significantly had lower concentrations. Carbohydrates were consistently higher in proportion compared to proteins, suggesting the nature of EPS found in these systems to be largely diatom produced. Extracellular polymeric substances were highly correlated with water content but reflected no association with organic matter content. The findings of this study suggest that EPS production seems to be very dependent on sediment moisture content as a function of water availability in pans. This preliminary investigation provides evidence of EPS dynamics in temporary pans, with implications on wetland ecosystem functioning and climate change in the region.

Key words: Extracellular polymeric substances (EPS), benthic biofilms, temporary wetlands, sediment water content, microbiomes.

2.1 INTRODUCTION

Temporary wetlands inclusive of pans and ponds, are characteristically distinguished by their small sizes, shallow depth and alternating hydroperiods (Downing et al., 2006; Calhoun et al., 2017). Benthic sediment is a crucial feature of shallow water aquatic environments, with implications for various biotic and abiotic factors that relate to ecosystem function (Bolam et al., 2002; Schratzberger and Ingels, 2018). For example, sediment size and type, have implications for water quality dynamics as sediment–water chemical exchange, with implications for biotic organisms (Bilotta and Brazier, 2008; Chapman, 2021; Arya et al., 2022). Similarly, sediment typically serves as habitat for plants, animals and microbes (Sanders, 1958; Boon et al., 1996; Janadeleh, et al., 2016;). One important and often overlooked aspect is the role biofilms play in wetland sediments and subsequently ecosystem ecology (Battin et al., 2016a). Sediments are typically characterised by the presence of biofilms, which support ecosystem functioning contributing to improved sediment quality (Carvajal–Arroyo et al., 2016). Biofilm communities are comprised of algae, bacteria, diatoms and fungi which also secrete extracellular polymeric substances (Schnurr and Allen, 2015; Pinto et al., 2020; Osemwegie et al., 2020). This chapter seeks to examine extracellular polymeric substances (EPS) production by benthic biofilms in temporary pans.

The biological composition of biofilms is complex and widely diverse, which include communities of algae, protozoa, fungi and bacteria (Jain et al., 2005; Guasch et al., 2016; Osemwegie et al., 2020). Photosynthetic biofilms are dominated by algae and cyanobacteria, with a considerable contribution of diatoms and green algae in total biomass of these biofilms (Schnurr and Allen, 2015). In contrast, bacterial biofilms are comprised of autotrophic and heterotrophic microbiomes which participate in various biogeochemical pathways including carbon, nitrogen, phosphate cycling (Leff et al., 2016) and organic matter decomposition through extracellular digestion. Biofilms also act as accumulation zones for nutrients (Lock et al., 1984) and through this, biofilm communities support uptake of materials (organic and inorganic) of lower bioavailability (Battin et al., 2003), which is beneficial in wetland food web dynamics. Additionally, recent advances in biofilm ecotoxicological studies highlights that, biofilms can be used as a tool to quantify pollutants in aquatic environments (Guasch et al., 2016). Water quality monitoring studies using biofilms as indicators so far have been reported for pesticide contamination and metal pollution in aquatic systems (Guasch et al., 2012).

The production of EPS by microbial communities is initially stimulated as an adaptation mechanism to the environment (Donot et al., 2012; Tuson and Weibel, 2013), whereby the microorganisms secrete high–molecular weight biopolymers (Underwood and Paterson, 2003; Roca et al., 2015). These polymers vary with regard to biological and physico-chemical properties (Decho, 2000; Liu and Fang, 2002). The accumulation of EPS around the microbiome community give rise to the so–called EPS biofilm–matrix (Tuson and Weibel, 2013). The EPS matrix is typically hydrated, and the moist environment promote community assemblages of microbial cells (Flemming et al., 2007). The microhabitat generated by EPS around a community of microbial cells is critical for the survival of the biofilm. Through these interactions, microorganisms maximise their survival rate (Huang et al., 1998) and strengthen symbiotic relationships between species in the EPS biofilm matrix (Møller et al., 1998).

The major constituents of EPS are carbohydrates and proteins, which are predominantly secreted by diatoms and bacteria, respectively (Underwood et al., 1995; Underwood and Paterson, 2003b; Gerbersdorf et al., 2009). Diatoms significantly support freshwater primary productivity and benthic food webs (Dalu et al., 2020) through mediating nutrient bioavailability for phytoplankton species by enzyme metabolism (Hassler et al., 2011).

Proteins in the EPS matrix represent a vital source of nutrition in the benthic–pelagic food webs as food to aquatic invertebrates and additionally provide structural support to carbohydrate complexes through facilitating adhesion among polysaccharide branches (Costerton et al.,1978; Pennisi, 2002). More so, EPS has been reported to contribute to stability of benthic sediments through inter–particle binding (Underwood et al., 1995; Yallop et al., 2000), which is beneficial in aquatic systems (Hauer et al., 2018) and contributes to minimised turbidity in the water column, with implications for planktonic and nektonic animals that use this zone. Microbial EPS production remains a key component in facilitating nutrient cycling and removal of pollutants in aquatic systems (Cunha et al., 2020). Globally, there has been increased advances in sediment microbial ecology (Chaudhary et al., 2013; Otte et al., 2018; Zhu et al., 2018). It is increasingly recognised that understanding EPS dynamics and their interrelationships with bacterial communities, is vital in elucidating biogeochemical processes of aquatic habitats, such as Carbon (C) (Arnosti, 2011), Sulphur (S) (Bhatt, 2021) and Iron (Fe) cycling (Otte et al., 2018). There is, however, a paucity of information regarding EPS biofilm–matrix in temporary wetland ecosystems, with knowledge on the sediment microbial EPS dynamics being particularly scant in these environments.

In support of the widely accepted functional role of EPS in permanent freshwater habitats (Decho, 2000; Underwood and Paterson, 2003; Wotton, 2011) extensive and comprehensive studies of EPS in permanent aquatic systems have taken place including marine (Bhaskar and Bhosle, 2006; Grassi et al., 2020), and recent advances in large rivers (Gerbersdorf et al., 2007; Gerbersdorf et al., 2008; Gerbersdorf et al., 2009). The aforementioned studies critically. evaluated the nature of EPS found in these systems and assessed, seasonal and spatial variability (horizontal and vertical) in the distributions of EPS in these environments. Furthermore, it's important to highlight the advanced chemical extraction techniques which have been so far employed to quantify EPS in these systems which include ethylenediamine tetra-acetic acid (EDTA) and cation exchange resin (CER).

However, a major gap in EPS studies exist in the role they play in temporary aquatic ecosystems. Many freshwater systems do not hold water permanently, cycling through variable inundation and dry phase periods (De Meester et al., 2005; Solimini et al., 2005). These systems are renowned for their productivity in the wet phase, with implications for organic matter accumulation. These dynamics would presumably also have considerable implications for the EPS biofilm–matrix, given the importance of the inter–relationships of soil water content and organic matter content in sediment ecology in general (Avnimelech et al., 2001). Sediment moisture content dynamics influences microbiome diversity through direct and indirect impacts on nutrient concentrations and oxygen availability (Drenovsky et al., 2004). Wetting phases are associated with low sediment oxygen saturation levels and favours proliferation of obligate and facultatively anaerobic bacteria, whereas dry phases generally reduce microbial action, and favours fungi sporulation (Sylvia et al., 2005). Natural organic matter defines a highly complex macromolecule, containing locked up energy, which is not directly accessible by consumers

(Sillanpää, 2015). Microbial mediation facilitates the release of energy through organic matter degradation (Leichtfried, 1996). Low organic matter availability can limit proliferation of microorganisms in sediments (Aldén et al, 2001; Sylvia et al., 2005). How these dynamics shape microbial EPS dynamics in temporary wetland ecosystems has yet to be fully explored.

To contribute to this knowledge gap, the present study evaluated EPS productivity patterns in temporary wetland sediments in an extreme environment characterised by prolonged and regular dry phase dynamics. Carbohydrates and proteins were measured from temporary pans across varying depths, contrasting sections that hold water for longer (deepest zones) with those that hold water for relatively shorter periods (shallowest regions).

The objectives of this study were to i) quantify and assess seasonal and spatial distributions of major EPS constituents (carbohydrates and proteins) present in sediment biofilms. ii). explore interrelationships between EPS and sediment characteristics of water content and organic matter content (OM content).It was hypothesised that there would be i) an increased yield of EPS in the wet phases in comparison to the dry phases as sediment water content directly impacts nutrient availability for EPS producing microorganisms ii) limited spatial variation of EPS, organic matter content and water content within each system would be limited, but that iii) linear relationships would persist between EPS, organic matter content and water content due to inter–dependence of these factors in the ecosystem.

2.2 MATERIALS AND METHODS

2.2.1 Study area

This study was conducted in the Khakhea–Bray Transboundary Aquifer (KBTA) region located in Tosca (25.880°S 23.959°E), the North–West Province of South–Africa, close to border of Botswana (Figure 2.1). Further detail on pan coordinates is provided for in Supplementary Table S1. Formerly known as the Pomfret–Vergelegen Transboundary aquifer system, the KBTA has an estimated area of over 28 000 km^2 in size with approximately 5300 km^2 of the area covered in a single dolomitic aquifer (Mpakairi et al., 2022). The major geographical features in the region consist of the non–perennial Molopo River and the presence of an extensive mosaic of small temporary pan wetlands. The building of three reservoir dams upstream of the Molopo river, has resulted in the river flowing less than five times since 1980 after heavy rains (Godfrey and Van Dyk, 2002). As a result, local villagers only rely entirely on groundwater and water from pans for domestic and agricultural irrigation.

The area receives an estimated rainfall ranging from 107–928 mm in summer, hence KTBA is classified as a semi–arid region (Godfrey and Van Dyk, 2002). More so, high evaporation rates dominate the area (2050–2250 mm per annum) hence there is always a deficit in water recharges (Turton et al., 2006; Altchenko and Villholth, 2013). Historically, the study area was spread over game reserves in large pieces of land with minimal irrigation. With migration, socio–economic pressures and agricultural activities water table and groundwater levels severely went down from 20 to 60 metres in a space of 10 years i.e., 1990–2000 (Turton et al., 2006).

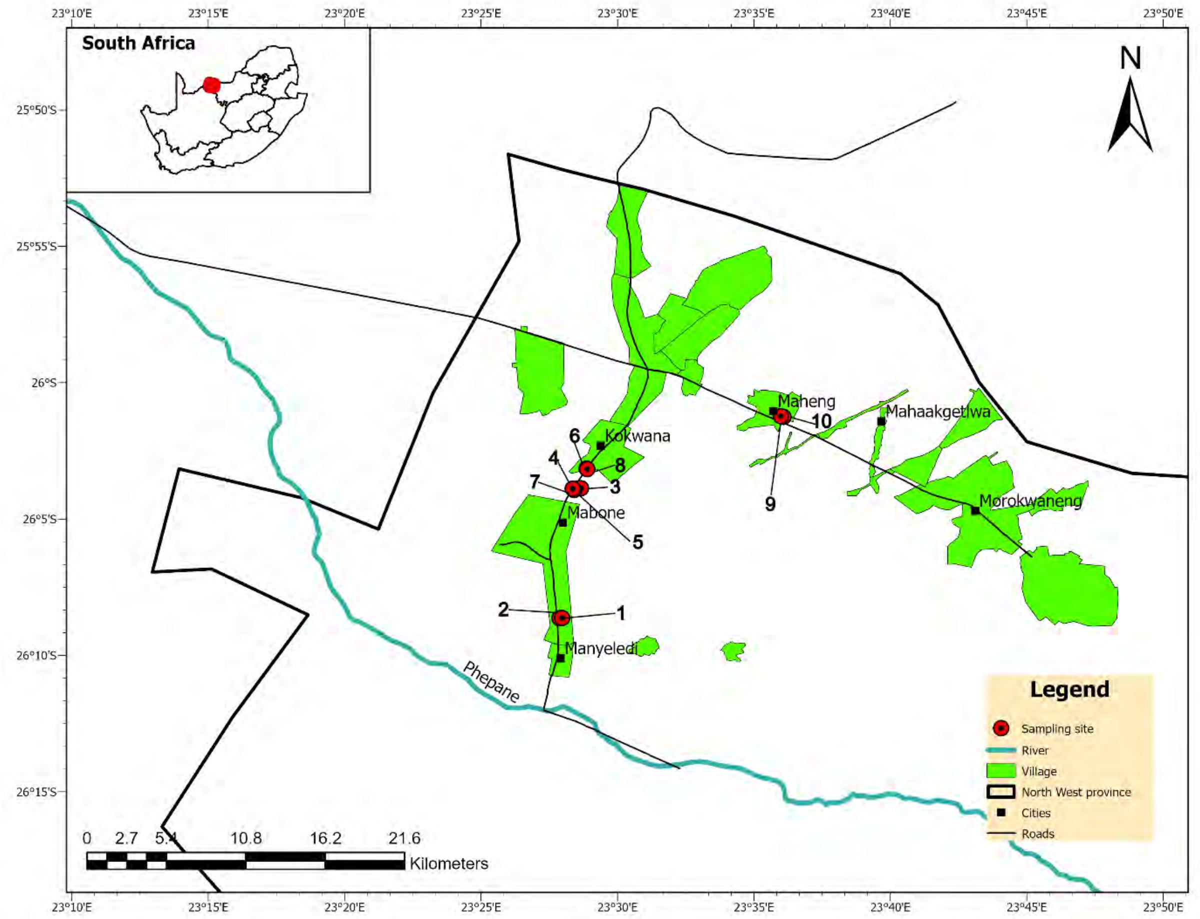

Figure 2.1. Map showing the Khakhea–Bray region in North–West Province, South Africa. Numerical numbers (1–10) highlight exact location of pans sampled in the sampling period, June 2021 and January 2022.

Sampling was conducted for two separate seasons dry season (June 2021) and wet season (January 2022). A total of ten pans of similar shape and size were randomly selected for sampling between the seasons. In the dry season, six (6) contained water which were identified as (pan 2, 3, 4, 5, 7, 10) and four (4) were dry (pan 1, 6, 8 and 9). All ten pans were inundated in the wet season. At each pan, a transect was generated from the deepest point in the depression, to the shallowest end of the depression. Sample points along each transect were set at deepest point (Deep), midway between the deepest and edge of depression (Mid), and at the edge of each depression (Edge) (Figure 2.2). At each sampling point within each pan, three sediment samples were collected, within 10 cm radius of each other. These samples were used for EPS, water content and organic matter content determination.

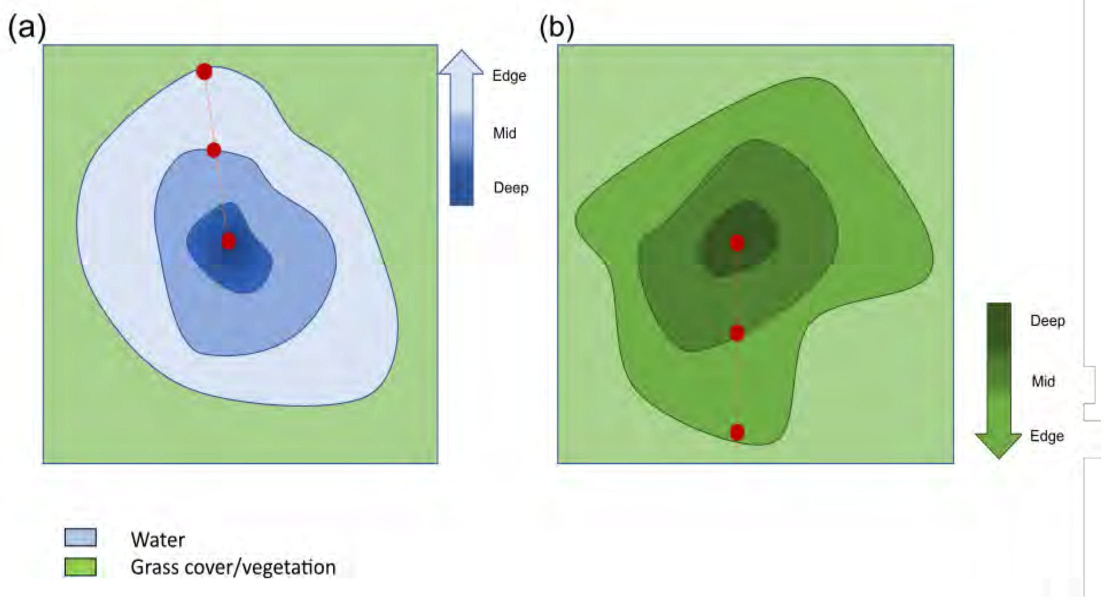

Figure 2.2. Schematic diagram representing aerial view of (a) wet and (b) dry pans, outlying the nature of the sampling protocol employed at each pan. Red dots represent points where sediment samples ($n = 3$ per point) were attained. Colour intensity is proportional to the amount of water and grass cover found in respective sampling zones.

2.2.2 Sediment collection

Clean latex gloves were used during all sampling and laboratory analysis steps in the study. Sediment was collected using a coring method by Wyness et al. (2019) whereby a modified 5 mL graduated plastic disposable syringe was employed as a core. In the laboratory, each 5 mL (13.5 mm diameter) syringe was modified by cutting off the base of the syringe (using a sterilised blade), just above the luer–slip tip, thereby creating a corer (Figure 2.3). Sampling collection using these corers involved vertical insertion up to 4 cm deep into benthic sediment. However, the study only utilised the top 3 cm for analyses. Upon removal, parafilm was then used to seal the end of the corer before being placed individually into 50 mL centrifuge tube.

Centrifuge tubes were then immediately placed into a cooler box with ice and transported to a freezer where they were frozen and stored at –22 °C within 12 hours of sampling.

Figure 2.3. Five mL cut syringe corer (a) with sediment and parafilm at the end (b) and falcon tubes containing sediment sample ready for freezing prior analysis.

2.2.3 Sample preparation

The sample was extruded from the corer for processing of the 3 cm of the core (containing the upper top sediment from the benthic zone). The sediment was homogenised in small, autoclaved petri dishes using a sterilised scalpel and then split into two sections for microbial and EPS analyses, respectively. The microbial subsample component is dealt with in next chapter (Chapter 3).

2.2.4 *Sediment water content*

The EPS subsample of sediment was measured into clean pre–weighed 2 mL Eppendorf tubes prior to freeze drying. The tubes were weighed before and after addition of sediment to allow calculation of water content (equation 1). Freeze drying was carried out for 48 hrs at –48 °C and 0.014 mBar (Labconco 4.5 plus freezone benchtop freeze dryer). Tubes were tightly covered with GF/C filter paper to avoid sediment escape as pressure draws out moisture during freeze drying process. The tubes were re–weighed for the determination of water content after freeze drying using equation 1.

$$Y = \left\lfloor \frac{(X1 - X2)}{X1} \right\rfloor \times 100$$

Equation 1

Where Y represents percentage (%) water content, $X1$ and $X2$ denotes the mass (g) of wet and dry sediment core, respectively.

2.2.5 *Organic matter content*

Sediment cores were analysed in triplicate, with three at each position along the transect (Deep, Mid, and Edge). Sediment from the freeze drier after determination of water content was weighed into clear glass vials. The glass vials were weighed pre– and post–addition of sediment to allow calculation of organic matter content using equation 2:

$$Y = \left\lfloor \frac{(X1 - X2)}{X1} \right\rfloor \times 100$$

Equation 2

Where Y represents percentage (%) organic matter content, $X1$ and $X2$ denotes the mass (g) of dried sediment core and furnaced core, respectively.

Vials were then furnaced at 505 °C for 5 h and re–weighed for establishment of OM content by loss on ignition. From the furnace samples were exposed to room temperature until they had cooled down, pending the final weight measurements.

2.2.6 *Extracellular polymeric substances*

Extracellular polymeric substances were quantified using the water extraction method on dry weight sediment (*DW)*. Carbohydrates and proteins were quantified using the Dubois and the modified Lowry procedure, respectively. Standard curves were established by running a combination of standards formed as part of various sets of samples for carbohydrate (Figure 2.4a) and protein analyses (Figure 2.4b). Three replicates of each i.e., glucose and bovine serum albumin (BSA) (Whitehead Scientific, South Africa) were used to come up with carbohydrates and proteins standard curves, respectively. Standards of 0, 25, 50, 75, 100, 200, 300, 400, 500 and 750 $\mu g\ mL^{-1}$ in deionised water (DI H_2O) were adopted for both analyses, following serial dilutions from a 1 $mg\ mL^{-1}$ stock solution. For each run, a corresponding standard curve equation was calculated before integration into equation 3 below.

$$Y = \left\lfloor \frac{(X1 - B)/M}{X2} \right\rfloor \times 1$$

Equation 3

Where Y represent total carbohydrates and protein contents in ($\mu g\ g^{-1}$). EPS is measured in glucose and BSA equivalents per gram of dry sediment and $X1$ represents Absorbance at 486 nm and 750 nm for measurement of carbohydrates and proteins, respectively. The constant of the equation was represented by B and M was the slope of an individual standard curve calculated for each set of samples. The denominator $X2$, represents the measured weight of sediment used in the initial stage of EPS extraction. The results were then multiplied by the volume of water used in extraction, which in this case was (1 mL).

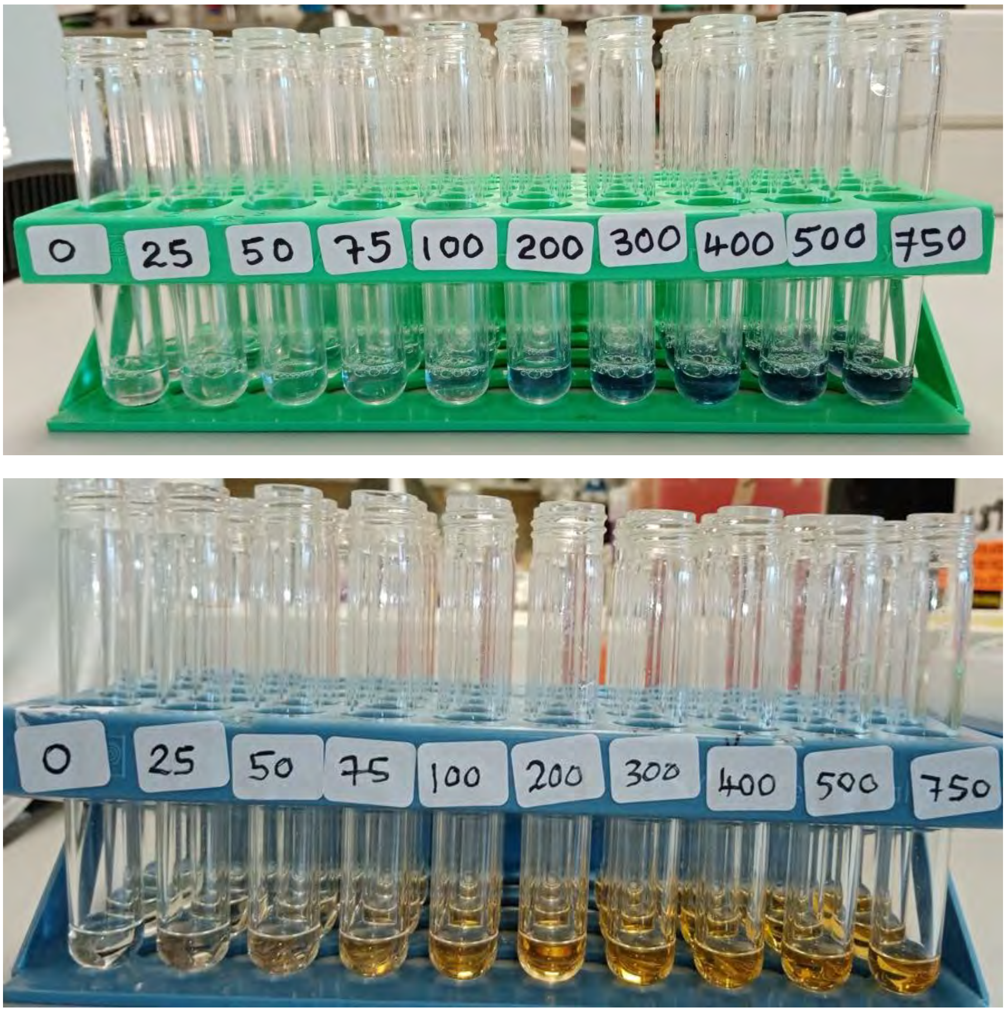

Figure 2.4. Optically clear glass test tubes with (a) completed reaction of total carbohydrate analysis of the standard curve, and (b) completed reaction of total protein .

2.2.7 Carbohydrates in sediment

Total carbohydrates were analysed using a method similar to Taylor and Paterson (1998). Into triplicate set of test tubes for each site, 107.1 µL of supernatant or glucose standard solution was pipetted. Then, subsequently the same volume 107.1 µL of 5 % weight/volume (w/v) phenol (Whitehead Scientific, South Africa) was transferred using a single channelled pipette. A final volume of 535.8 µL of concentrated sulphuric acid (Acechem, Johannesburg) was added and solution was homogenised by pipetting. The reaction test tubes for carbohydrates analysis were incubated for 40 minutes at 30 °C in a lab oven (ProLab Systems, South Africa) followed by spectrophotometry Absorbance readings at 486nm (Genesys 20, Thermo Scientific, UK). Total carbohydrate quantities are given as µg g^{-1} glucose equivalent.

2.2.8 Proteins in sediment

The modified Lowry procedure was adapted for use in the glass test tubes, whilst maintaining the concentrations and ratio of reagents according to Raunkjaer et al. (1994). The working reagent was constituted of 143 mM NaOH (Acechem, South Africa) 270 mM Na_2CO_3 (Acechem, South Africa) solution with 57 mM $CuSO_4$ (Acechem, South Africa) and 124mM $Na_2C_4H_4O_6$ (Acechem, South Africa) in ratio 100:1:1. Triplicate sets of test tubes for each site were pipetted 144 µL each of supernatant or BSA standard solution. The same volume 144 µL of SDS 2 % (Life technologies, USA) was added, followed by an incubation period of 15 minutes at room temperature to allow proteins to lineate. Subsequently, 403.5 µL of the working reagent and 58.5 µL of Folin and Ciocalteu's reagent (Whitehead Scientific, South Africa) (dilution was 5:6 with deionised water) were added, respectively and solution was homogenised by pipetting. The reaction test tubes for protein analysis were incubated for 50 minutes at 30 °C in a lab oven (ProLab Systems, South Africa) followed by spectrophotometry Absorbance readings at 750nm (Genesys 20, Thermo Scientific, UK). Total protein quantities are given as μg g^{-1} glucose equivalent.

2.2.9 Data analysis

Two–way ANOVAs were assessed in R (version 4.2.1) using R Studio to test for differences in EPS constituents, water content and organic matter content between seasons and across depth. In R, EPS and sediment characteristics data were transformed using $\log_{10}$ for (carbohydrates and proteins), organic matter content and water content values were arcsine square-root-transformed, to achieve parametric assumptions of normality and homogeneity of variance. Arcsine transformations have been widely used to improve interpretability of percentage data in ecological studies (Makee and Saour, 2001; Agrawal and Kotanen, 2003; Dobbs et al., 2022). Additionally, TukeyHSD test was also performed in R to evaluate depth specific variations suggested by ANOVAs.

The potential relationships between EPS, water content and organic matter content were assessed by Principal Components Analysis (PCA) in PRIMER version 6 (Clarke and Gorley, 2006) using Euclidean distance. Data were normalised and transformed using overall transformation Log(x+1) as a pre–treatment procedure. The significance of correlations in the PCA output were assessed using Pearson correlation coefficient at 5% significance level in R version 4.2.1.

2.3 RESULTS

2.3.1 Seasonal dynamics in extracellular polymeric substances production

Carbohydrates had higher concentrations between the seasons compared to proteins, (mean 73 $\mu g\ g^{-1}$) in the dry season and (mean 102 $\mu g\ g^{-1}$) in the wet season (Figure 2.5a). Proteins proportionally occurred in lower concentrations mean (26 $\mu g\ g^{-1}$) in the dry season and mean (65 $\mu g\ g^{-1}$) in the wet season. All EPS analyses were performed on freeze dried sediment (*DW).* There was a significant difference between the two seasons in EPS production of proteins (F =13.640, $p < 0.001$) and carbohydrates (F = 5.257, $p = 0.034$; Table 2.1). Carbohydrate concentration in sediments were consistently high across the season as compared to proteins (Figure 2.5a). In addition, ratio of carbohydrate to protein varied from 2.8 to 1.5 (Table 2.2), suggesting more proteins in the wet phases.

Percentage variation in sediment water content ranged from 7.6 – 17.8 % between dry and wet season, with a significant maximum value recorded at 22 % (Figure 2.5b) in the wet season (F = 19.21, $p < 0.001$). Organic matter content did not show significant variation across seasons (F = 0.512, $p > 0.088$), however differences were significantly across depth (F = 20.826, $p < 0.001$) with no interactions between site and season.

Table 2.1. Two–way ANOVA table results for EPS and sediment properties. Statistically significant differences ($p < 0.05$) are indicated in bold.

Variable	**Factor**	**F–statistic**	***p*–value**
Protein	Depth	5.799	**0.005**
	season	13.640	**<0.001**
Carbohydrates	depth	8.62	**<0.001**
	season	15.96	**<0.001**
Water content	depth	10.40	**<0.001**
	season	19.21	**<0.001**
OM content	depth	20.83	**<0.001**
	season	0.51	**0.477**

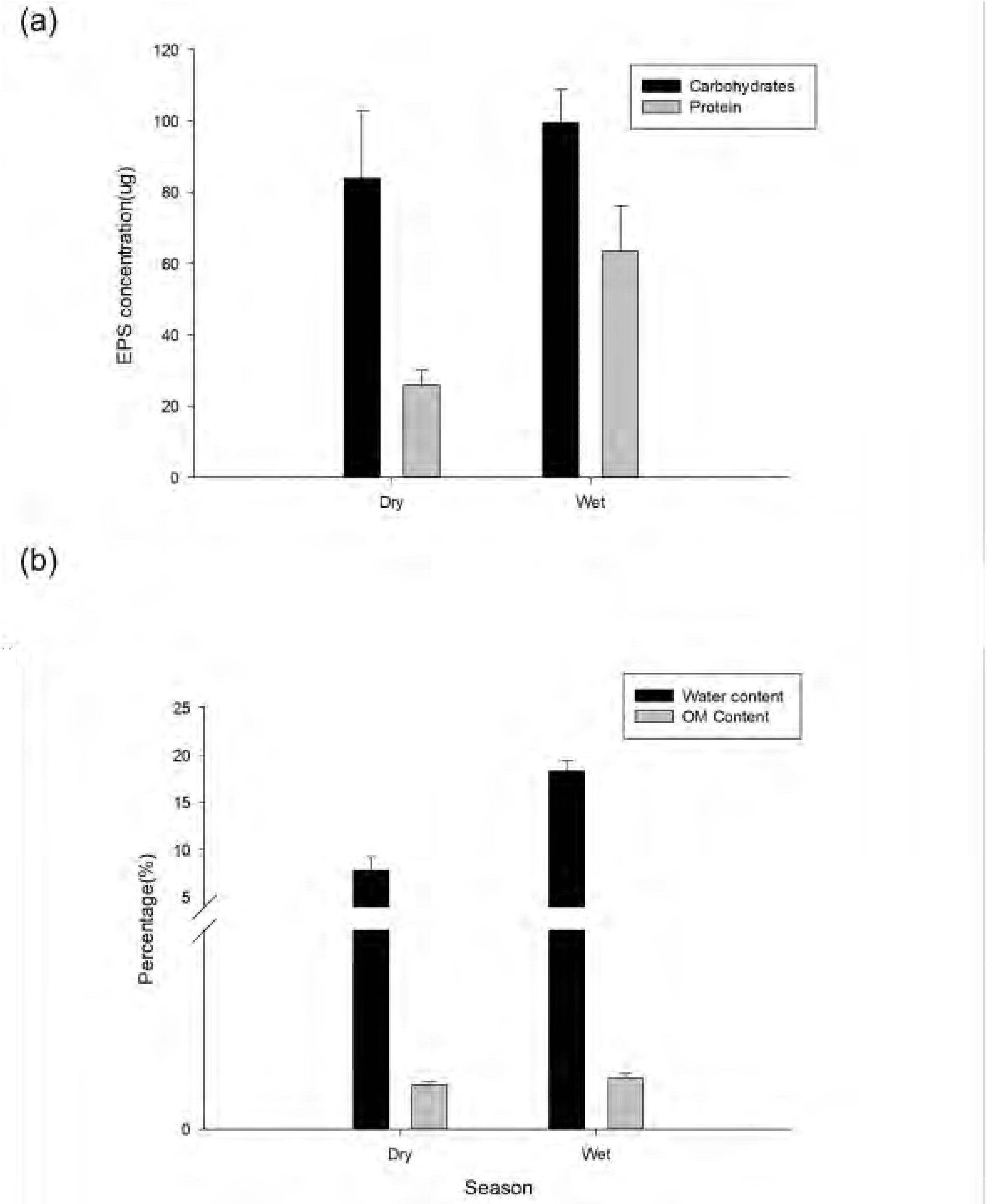

Figure 2.5. Seasonal variations in (a) Carbohydrate and protein concentrations measured in glucose equivalents and Bovine Serum Albumin (BSA) equivalents, per dry weight sediment g^{-1} ($\mu g\ g^{-1}$ *DW*), respectively, and (b) water content and organic matter content given as percentages (%), y–axis bar cut between 2 % and 4 %. Mean values, ± standard error, (n = 60). The study assessed 10 pans between the dry and wet season (June 2021 and January 2022) in Khakhea–Bray region.

2.3.2 Spatial variation in microbial extracellular polymeric substances dynamics

Total protein and carbohydrates concentrations followed a similar trend in respective proportions, with the highest quantities recovered from the Deep followed by Mid and Edge points (Figure 2.6a). The decrease in mean concentration of carbohydrates and proteins was consistent from the Deep to Edge, although C:P ratio remained the same for Mid and Edge samples (Table 2.2). The Deep points yielded the highest (mean) carbohydrates 102 $\mu g\ g^{-1}$ DW and proteins 68 $\mu g\ g^{-1}$ DW (Figure 2.6a). Proteins were significantly highest at the Deep than Edge points (Tukey HSD, $p = 0.006$). There were significant differences in mean carbohydrates concentration from the Deep compared to pan Edges (Tukey HSD, $p < 0.001$). The Deep and the Mid of pans could not be differentiated in both proteins (Tukey HSD, $p > 0.795$) and carbohydrates (Tukey HSD, $p > 0.396$) production. Organic matter content was relatively low ranging 0.2 –0.8 %. However, it had significant variations at each level from the Deep, Mid points, and Edge (ANOVA, $p < 0.\ 001$).

The Deep, Mid and Edge systematically followed a decreasing order in water and organic matter content percentage (Figure 2.7b). Sediments from Deep had the highest water retention capacity (mean 17.8 %), compared to Edge of pans (Tukey HSD, $p < 0.001$). There was a significant difference in water content between the Mid and Edge points (TukeyHSD, $p = 0.003$). Furthermore, Deep points were found to be matching the Mid points (TukeyHSD, $p > 0.667$). On the other hand, organic matter content varied significantly across depth (TukeyHSD, $p < 0.014$).

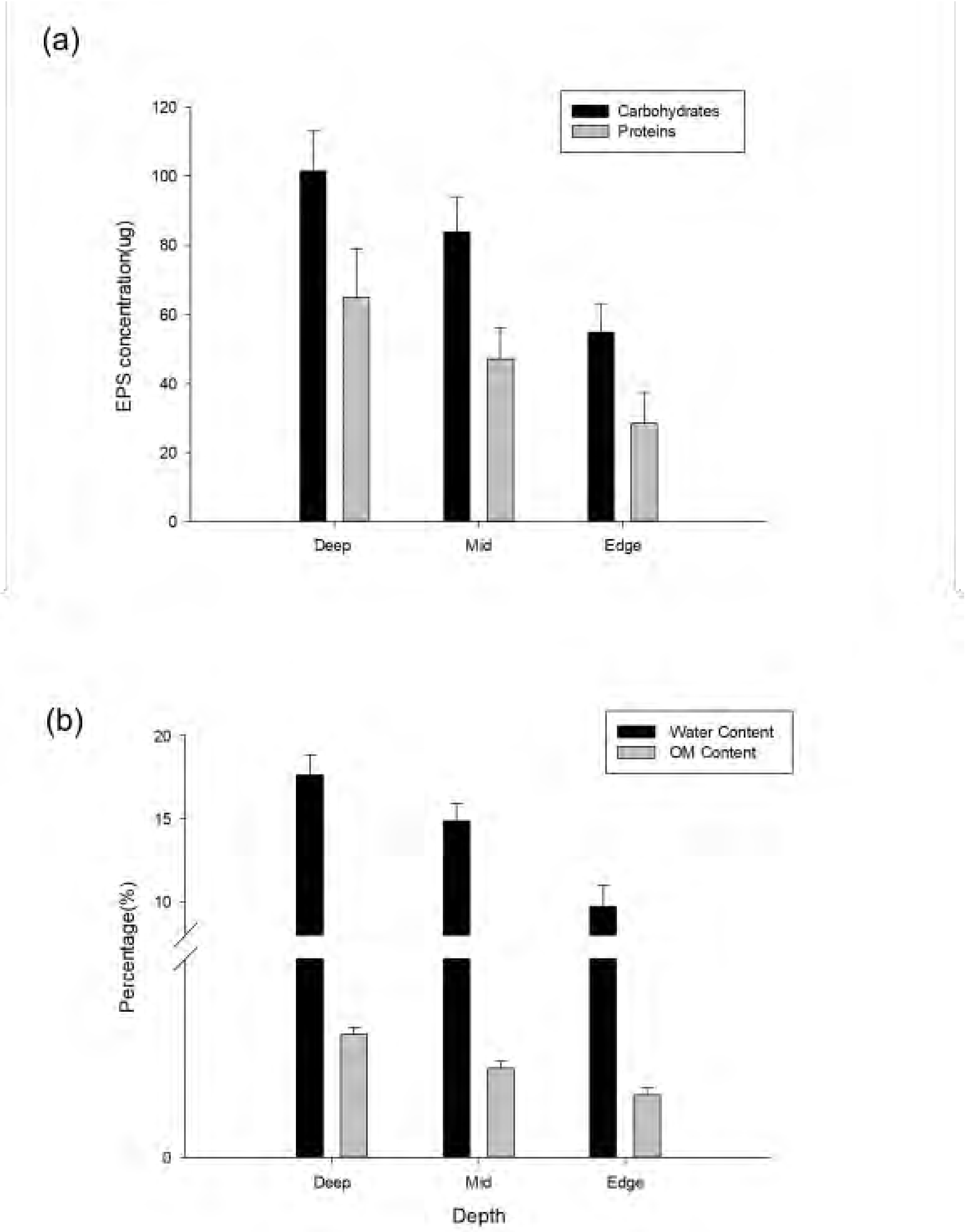

Figure 2.6. (a) Spatial variation of extracellular polymeric substances quantified from the Deep, Mid and Edge. Concentrations of carbohydrates and proteins are µg glucose and Bovine serum albumin (BSA) equivalents per dry weight sediment (µg g^{-1} *DW*), respectively. (b) Spatial variation in percentage water content and organic matter content across varying depth within the pan (Deep, Mid and Edge), y–axis bar cut between 1 and 8. Error bars represent standard error around the mean.

Table 2.2. Spatial and temporal variations in carbohydrate: protein ratio

Category	C:P ratio
Wet season	2.8
Dry season	1.5
Deep	1.5
Mid	2.0
Edge	2.0

2.3.3 Temporal and spatial correlations in extracellular polymeric substances, water content and organic matter content

Principal Component Analysis (PCA) was employed to evaluate the relationship between EPS (carbohydrates and proteins) and sediment characteristics across depth and between seasons. In total, component 1 and 2 of PCA for *Season* (Figure 2.7a) explained 84 % in total variation with eigen values 2.26 and 1.10, respectively. The total variation explained by the PCA for *State* (Figure 2.7b) also accounted 84 % with eigen values 2.24 and 1.08. Similarly, PCA for *Depth* (Figure 2.8) component 1 and 2 explained 82 % of total variability in EPS dynamics across the Deep, Mid and Edge with eigen values 2.47 and 0.82, respectively. The dry pans represented by 1, 6, 8 and 9 in the two biplots (Figure 2.6) were consistently distanced from other pans suggesting variable degree in EPS production between wet and dry phases. Pans identified as 2, 10, 11, 13, 17 (Figure 2.6) were characterised with very low organic matter content however were better related to carbohydrates production. On the other hand, relative positioning of pans 5, 14, 20 suggested high association with proteins whilst pans 12 and 16 were characterised by more water content.

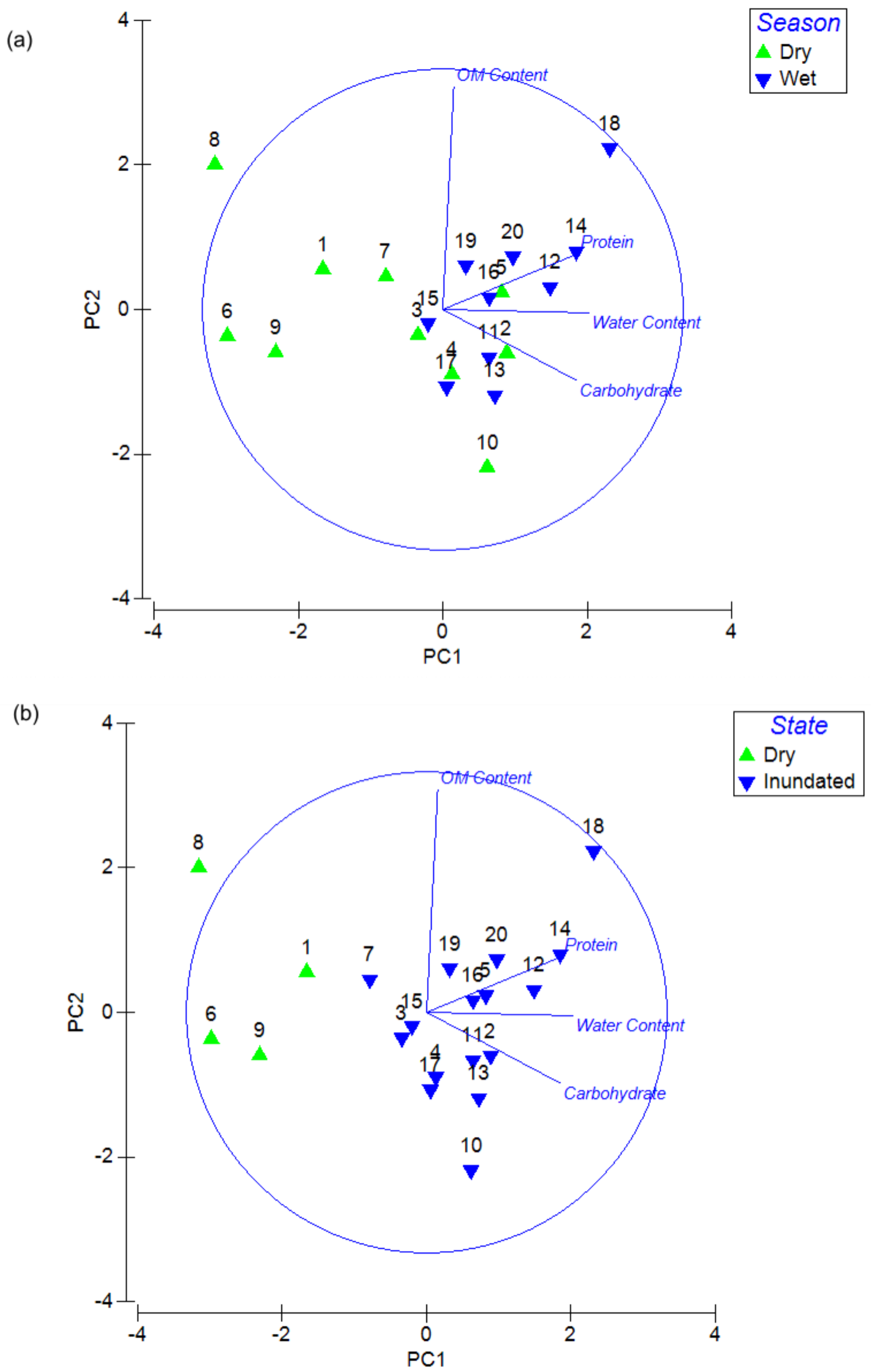

Figure 2.7. Principal Components Analysis (PCA) biplots showing (a) (PCA for *Season*) and (b) spatial variations (PCA for *State*) in extracellular polymeric substances, water content and organic matter content in Khakhea–Bray temporary pans (June 2021 and January 2022). The ordination plot explained 84% of variation with symbols colour coded as defined by key. Numbers on top of symbols represent pans.

2.3.4 Extracellular polymeric substances relationship with dry/wet season and inundation phases

Wet season was characterised by EPS and water content as compared to dry season, based on grouping by similarity observations (Figure 2.6a). Water content correlated independently with proteins and carbohydrates as displayed by smaller shared angles between the vectors and these observations were statistically significant ($p < 0.001$; Table 2.3). Organic matter content had relatively the longest vector and its relationship with other vectors was almost at 90–degree angle, resulting in non–significant correlations between organic matter content and EPS i.e., carbohydrates ($p = 0.221$) and proteins ($p = 0.18$; Table 2.3).

Table 2.3. Pearson's correlations between extracellular polymeric substances and sediment characteristics between dry and wet seasons. Statistically significant differences ($p < 0.05$) presented in bold.

Relationships	***r*–value**	***p*–value**
Proteins– carbohydrates	0.72	<0.001
Proteins–water content	0.75	<0.001
Carbohydrates–water content	0.74	<0.001
Organic content–carbohydrates	0.29	**0.221**
Organic content–proteins	0.37	**0.181**

2.3.5 Extracellular polymeric substances interaction with sediment water content and organic matter content across varying depth

Similar grouping, and clustering in the biplot (Figure 2.8) showed that varying depth influenced distributions in EPS concentrations production, organic matter content production and sediment water content dynamics. The Deep and Mid were characterised by EPS and water content more than the Edge based on relative positioning of these vectors in the biplot. Among EPS constituents, proteins had the longest vector and demonstrated correlations with water content as suggested by the tight angle formed between these variables (Figure 2.8). Pearson correlation confirmed these findings to be significant at the Deep ($r^2 = 0.70$ $p < 0.001$; Table 2.4) compared to Mid and Edge. Component 2 of PCA for *Depth* also differentiated organic matter content from other variables at an angle approximately 90–degrees, resulting in no significant correlations with carbohydrates and proteins (Table 2.4). Edges were similarly characterised by an almost negative correlation with organic matter content, as shown by opposite clustering (Figure 2.7).

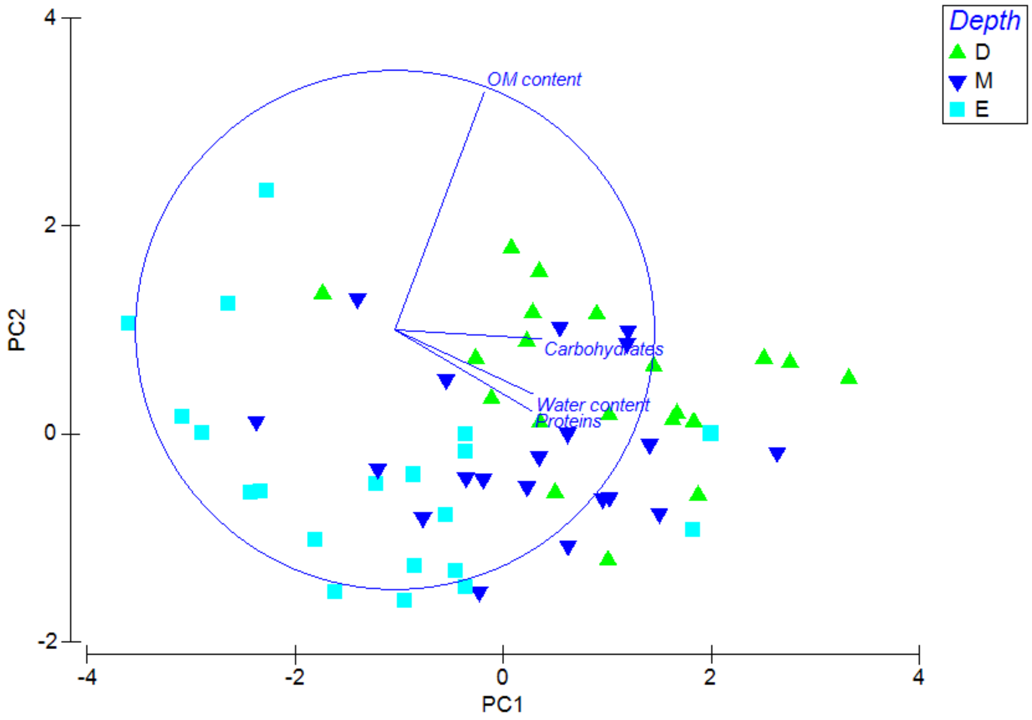

Figure 2.8. Principal Components Analysis (PCA) for *Depth* biplot showing spatial variations in EPS and sediment characteristics in Khakhea–Bray pans between June 2021 and January 2022. The ordination plot explained 82 % of variation with symbols colour coded as defined by key. Letter D– Deep, M– Mid and E– Edge.

Table 2.4. Pearson's correlations between EPS and sediment characteristics within different spatial levels and across seasons. Statistically significant differences ($p < 0.05$) are indicated as **bold.**

Depth	**relationships**	***r*–value**	***p*–value**
Deep	proteins–carbohydrates	0.66	**0.001**
	proteins–water content	0.70	**0.001**
	carbohydrates–water content	0.38	0.097
	OM content–carbohydrates	0.20	0.375
	OM content–proteins	0.16	0.351
Mid	proteins–carbohydrates	0.65	**0.002**
	proteins–water content	0.48	**0.034**
	carbohydrates–water content	0.54	**0.013**
	OM content–carbohydrates	0.33	0.154
	OM content—protein	0.22	0.381
Edge	proteins–carbohydrates	0.81	**<0.001**
	carbohydrates–water content	0.74	**<0.001**
	proteins–water content	0.56	**0.009**
	OM content–carbohydrates	0.18	0.391
	OM content—proteins	0.14	0.446

2.4 DISCUSSION

Globally, temporary wetlands are renowned for high levels of productivity during hydroperiods (Arnott and Vanni 1993; Waterkeyn et al., 2008; Florencio et al., 2009). This study is the first of its kind to be conducted in arid–zone freshwater temporary pan systems in the global south. The present results confirm increased production of EPS by benthic biofilms in the wet season compared to dry season and this support our first hypothesis. Furthermore, the deepest zones (Deep) could not be differentiated from intermediate depth (Mid), however the shallowest regions (Edge) significantly had lower EPS concentrations, resulting in rejection of our second hypothesis. Extracellular polymeric substances highly correlated with water content however demonstrated no association with organic matter content, which did not substantiate our third hypothesis. These findings contribute to the body of knowledge in microbial ecology particularly extracellular polymeric substance production in temporary pan ecosystems

Carbohydrates as the major constituents in EPS produced by diatoms and algae as compared to bacteria (Underwood, 2010), were consistently higher than proteins between the dry and wet seasons. The present study employed water extraction method for EPS quantification and carbohydrates concentrations retrieved were comparable to more permanent systems (Friend et al., 2003) In addition, an increased yield of EPS in the wet season highlighted changes in EPS concentrations over time, similar to findings by (Gerbersdorf et al., 2009a). Furthermore, the changes in carbohydrate: protein (C:P) ratio between the two seasons suggested distinct nature of EPS found in temporary pans at different times of the season with implications to ecosystem functioning.

Although organic matter content did not differ or show correlations between seasons, its variation was depth specific, from highest in the deepest zones and lowest in the shallowest regions. These findings agree with Shuman (2003) who reported similar variations in cores extracted from, the deepest points and edges in shallow lakes. The observed spatial variation in organic matter content in this study could be a function of differences in sediment composition, influenced by different rates of productivity and decomposition (Reddy and Patrick, 1975) in the assessed zones. It is therefore possible for the deepest sections to have higher organic matter content, as these zones hold water for the longest and with time all aquatic life migrates to the center and dies off. Similarly, more vegetation grows in these saturated zones and cumulative death of aquatic biota and plants results in organic matter accumulation (Wantzen et al., 2008).

The contribution of erratic rainfall patterns and high evaporation rates in Khakhea–Bray region (Altchenko and Villholth, 2013), likely affect EPS productivity dynamics in the pan ecosystem. Indeed, all shallowest regions (Edge) had less influence in supporting EPS production despite time of the season. This implies that even when pans receive water in wet season, their total EPS production from the Edge do not match that of the Deep. High evaporation rates results in early desiccation of the Edge , with implications of low diversity and biomass in key EPS producing microorganisms, such as diatoms (Souffreau et al., 2019) and bacteria (Baldwin and Mitchell, 2000). In this way, some pans have been limited to production of more EPS at the Deep where water accumulates for the longest time supporting proliferation of EPS producing species.

The linear correlations between EPS producing microbiomes which include diatoms, green algae, fungi, bacteria and wet season have been previously investigated, (Boggs et al., 2008; Ezra and Nwankwo, 2001; Cantrell and Duval–Perez 2012; Pineda–Mora et al., 2020). This suggestively explain higher correlations found between EPS and water content in this study. Flemming et al. (2007) emphasized the importance of moist environments in promoting microorganism's community assemblages. It can therefore be suggested that inundation supported more hydrated biofilms thereby ensuring the maximum survival rate of EPS producing microbiomes. At the Deep zones, correlations of EPS and water content favoured more proteins production than carbohydrates suggesting the significant contribution of bacterial biofilms to total EPS biomass in these zones (Flemming and Wingender, 2001).

It has been reported that, EPS play an important role in shaping benthic microbial productivity and participate in sediment stabilization in permanent freshwater ecosystems (Underwood et al., 1995; Dalu et al., 2020). However, whether carbohydrates and proteins recovered from this study directly provided nutrition to benthic macroinvertebrates (Decho and Lopez, 1993; Cyr and Morton, 2006) or were involved in remediation of pollutants (Liu and Fang, 2003) is a question for further investigation.

In general this study contributes to the better understanding of ecosystem functioning in temporary wetlands, which are constantly under threat of deterioration due to climatic changes and human interferences (Bouahim et al., 2014; Dube et al., 2020). In addition this study is informative within the context of ongoing global climatic shifts (Canziani and Parry 2007; Tuytens et al., 2014), and ongoing predictions of permanent systems becoming more temporary due to extended warming and drying periods. Hence, understanding temporary wetlands dynamics is potentially more relevant in elucidating the function of aquatic systems at large in the face of climate change.

2.5 CONCLUSIONS

Extracellular polymeric substances production seems to be very dependent on water availability, contributing to the spatial and seasonal variability observed in Khakhea–Bray temporary pans. With forecasted climate change, irregular hydroperiods, less volume and frequency of rain, it is potentially threatening to wetlands with implications on ecosystem functioning dynamics. Whilst this study did not establish a significant correlation between organic matter content, and EPS/water content, it did partially substantiate depth specific variations in the distributions of organic matter content in semi–arid temporary pans. Taken together, this research extends our knowledge of EPS productivity dynamics in temporary wetlands. Furthermore, some EPS patterns and trends observed in this study partially corroborate findings from permanent systems, indicative of the potential in using temporary wetlands as models to understand the global climate changes. However, more follow up and comprehensive studies are recommended to fully understand and explore these dimensions in temporary wetland ecology. Suggestively these include, evaluating the links between EPS dynamics and secondary productivity biomass or exploring EPS function in heavy metal polluted wetlands.

CHAPTER 3.

BACTERIAL DIVERSITY AND ECOSYSTEM FUNCTION DYNAMICS IN TEMPORARY PAN SEDIMENT QUANTIFIED USING METAGENOMICS

PLATE 3. A representative view of sediment collection in the field using a 5 mL syringe corer tightened with parafilm at the tip. Photo Credit: Dr. Chad Keates.

ABSTRACT

Temporary wetland ecosystems have the potential to offer unique insight into how shifting climates, and associated predicted drying of permanent water bodies, may alter aquatic microbial dynamics and associated ecosystem functioning. Here, bacterial diversity and ecosystem functioning dynamics were explored in a series of small temporary pan ecosystems in an arid region characterized by limited permanent water bodies. The objective of the study was to characterize bacterial communities and predict associated biogeochemical pathways. To achieve this, the study employed sequence–based metagenomics approach on sediment cores collected across different depths within 20 pans in the dry season (June 2021) and wet season (January 2022). The study targeted the 16S rRNA gene to profile functional bacterial communities and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (Picrust2) pipeline to infer targeted metabolic pathways. The main bacteria phyla were dominated by Firmicutes, at maximum relative abundance 59 % (mean ± sd, 19 ± 13 %). Actinobacteria and Proteobacteria both at 34 % (18 ± 7 %) and (18 ± 6 %) respectively. Thermophilic genera were found to be highly affiliated across top 20 genera, with highest relative abundance found in *Bacillus at* 54 % (13 ± 12 %) followed by *Rubrobacter* 16 % (7 ± 4 %). Shannon–Wiener index did not demonstrate alpha diversity differences within seasons and across deepest zones, intermediate depth and shallowest regions. Seasonal and spatial beta diversities were however significantly different. The predicted functions were observed to be significantly seasonally driven by bacterial core microbiomes in the wet season contributing to the highest rates of denitrification, carbohydrate degradation and exopolymers production (EPS). In the dry season, a unique set of enzymes catalysed low rates of EPS degradation, nitrogen mineralisation, carbon fixation and nitrification. Site specific variations observed highest activity of dissimilatory sulphate reduction (DSR) by nitrogen fixing bacteria in the deepest zones of dry pans. Our investigation provides insights into how climatic shifts associated with drought conditions may impact functional microbial communities in aquatic systems. However, more comprehensive future studies in Southern Africa are recommended to substantiate the present findings.

Key words: Bacteria, sediments, temporary pans, biogeochemical cycles, metagenomics, microbial communities.

3.1 INTRODUCTION

A unifying character of all temporary pools is their cyclical nature, which involves alternating drying and inundation phases (Boven et al., 2008; Mitsch and Gooselink, 2009). These features make them unique among aquatic ecosystems and facilitates persistence of specialist aquatic fauna and flora, in addition to the generalist biota that exploit these ecosystems as habitat (Bird et al., 2018). Microbes seem to be of no exception, with alternating drying and inundation phases supporting the coexistence of transient bacterial species which are adapted to survive distinctively between the cycles (Bouahim et al., 2014). However, there is generally a paucity of information on microbial dynamics in the small temporary wetland literature globally. These systems have the potential to offer unique insight into how shifting climates, and associated predicted drying of permanent water bodies, may alter aquatic microbial dynamics and associated ecosystem functioning.

It has been widely established that freshwater sediments harbor diverse microbial communities which mediate important biogeochemical pathways (Rousk and Bengtson, 2014; Meng et al., 2022). The functional and taxonomic composition of bacterial abundance in sediments has been reported to be relatively high in comparison to the water column (Ren et al., 2019; Zhao et al., 2022). These microbiomes occupy a crucial link in aquatic food webs, through facilitating nutrients provision to higher trophic levels (Findlay, 2010). This is achieved via the combined processes of biodegradation and mineralization of complex compounds into simpler forms readily available for consumption by aquatic organisms (Brusseau, 2019). Two of the most important biogeochemical processes in aquatic systems include carbon cycling (Moomaw et al., 2018) and nitrogen cycling (Huygens et al., 2015). Freshwater wetlands have been reported to participate in storage and release of carbon hence contributing significantly to global carbon cycle (Moyo, 2022).

Aquatic ecosystems maintain the carbon balance by mediating conversion of organic matter into dissolved organic carbon (DOC) (Lam et al., 2007), a process highly mediated by various functional guilds of heterotrophic anerobic and aerobic bacteria, well adapted in these environments (Zhou et al., 2012; Pester et al., 2012). The nitrogen cycle, however, hinges on the ability of nitrogenase enzymes to perform nitrogen fixation by converting nitrogen to ammonium (NH_4^+) (Bentzon–tilia et al., 2015; Einsle and Rees, 2020). Furthermore, oxidative nitrification of ammonia give rise to nitrite (NO_2^-) through the action of *Nitrosomonas* an Ammonia Oxidizing Bacteria (AOB). On the other hand, Nitrite Oxidizing Bacteria (NOB) such as *Nitrospira* facilitates further conversion of nitrite to nitrate (Prosser, 1990). Denitrification is a stepwise conversion of nitrites and nitrates to gaseous forms of Nitrous oxide (N_2O) and dinitrogen gas (Seitzinger, 2018), hence this pathway is responsible for the nitrogen loss in aquatic systems. The genus *Nitrospira* was reported as one of taxa central in denitrification pathway (Hayatsu et al., 2010).

Contrary to denitrification, a competing pathway in the nitrogen cycle with reduced emission of nitrous oxide (N_2O) and Nitrogen as (N_2) gas is the Dissimilatory Nitrate Reduction to Ammonium pathway (DNRA) which converts nitrates (NO_3–) and nitrites (NO_2–) to ammonium (NH_4^+) (Pandey et al., 2020). This process is mediated by heterotrophic anaerobic fermenting bacteria (Silver et al., 2001) and chemolithoautotrophic organisms (Giblin et al., 2013). More specifically, through the action of nitrate/nitrate reductase genes such as the nrfA functional gene (Smith et al., 2007). The nrfA gene was observed to occur in Bacteroides, Proteobacteria and Firmicutes (Mohan et al., 2004) hence their ability to participate as DNRA organisms. The recycling of fixed nitrogen through DNRA allows retention of bioavailable nitrogen in aquatic systems (Kamp et al., 2015) enriching the ecosystem with soluble ammonium vital for primary productivity. The functional ability of ecosystems to cycle nitrogen is important for sustaining their productive capacity, at equilibrium nitrogen concentrations.

Metagenomics have revolutionised the field of molecular ecology, through allowing direct acquisition of genetic material from diverse environments including soil and water (Handelsman, 2004), with subsequent generation of microbial data which help in understanding diversity patterns and functional roles of microbes in aquatic environments (Huson et al., 2009). Metagenomics employs techniques to sequence genetic material such as the Next–Generation Sequencing (NGS) which use the high–throughput approach in generating thousands of DNA reads per sample at a lower cost as compared to its predecessor the Sanger sequencing method (Baudhuin et al., 2015; Reuter et al., 2015). As such, the molecular characterization of all bacteria found in the environment is now possible by targeting genes conserved between diverse taxa such as the 16S rRNA gene (Janda and Abbott 2007; Pearman et al., 2022). The 16S rRNA gene is characterized by highly conservative partitions interspersed by the hypervariable regions (V1–V9), which represent various diversities in different bacterial taxa (Chakravorty et al., 2007). Recent developments in computational tools have also allowed the addressing of ecologically relevant questions using information gained from the sequencing of the 16S rRNA gene (Schloss et al., 2009; Bolyen et al., 2019). The result is an ability to quantify microbial communities in a classic community ecology framework. Given that the functional roles of microbial groups are well characterized, this approach offers greater insights into how shifts in community dynamics can have implications for ecosystem functioning dynamics. This study employs a sequence–based metagenomic approach to address the knowledge gap on microbiomes and ecosystem functioning dynamics of temporary wetland ecosystems. This study aims to characterize bacterial communities buy targeting 16S rRNA gene and high throughput sequencing, examine the abundance and diversity of bacteria across seasons and varying depth, and predict and compare the functional roles of enzymes central in mediating important biogeochemical cycles in Khakhea–Bray pan ecosystem. It was hypothesized that (i) more heat tolerant taxa would dominate given the arid nature of Khakhea–Bray, (ii) core microbiomes in the wet season would have higher diversity than dry season, due to increased sediment water content which supports proliferation of hydrated biofilms, and (iii) predicted functions for enzymes would be seasonally correlated, with more in the deeper zones and wet phases and, as these zones have relatively more depth and dry out last in comparison to the shallowest (Edge).

3.2 MATERIALS AND METHODS

3.2.1 DNA extraction

Sediment for microbial analyses was collected and processed as described in sections 2.2.1 – 2.1.3 of Chapter 2. Using <250 mg subsamples of sediment, the extraction of genomic DNA for metagenome sequencing was conducted using E.Z.N.A® Soil DNA Kit (Omega Biotek, Norcross, USA), following the manufactures protocol for DNA Extraction and Purification. The samples were pooled from twenty (20) pans following two (2) sampling events in which sediment corers were used to collect sediments at three zones along the pan (Deep, Mid and Edge) in replicates of three. Roughly 220 mg of sediment was accurately weighed directly into the omega biotech disrupter tubes using the tare function on an electronic balance. A negative control of autoclaved deionised water was used as a quality check in the extraction processes. The quantity and quality of DNA extracted from benthic sediments was assessed by fluorometric analysis using a nanodrop spectrophotometer (ThermoFisher Scientific, South Africa) with 2 µL of DNA sample pipetted onto the sensor. The DNA concentrations were retrieved in µg/mL whilst A260/280 and A260/230 ratios evaluated quality of the genetic material. The readings were captured prior the generation of polymerase chain reaction (PCR) reaction mixtures, and the DNA material was stored in the freezer at –22 °C.

3.2.2 DNA quantification

Agarose gel was prepared by accurately weighing 1 g of agarose stock, (Whitehead Scientific, South Africa) and mixed with 1x TE buffer Tris base (Anatech, South Africa) in the ratio 1 g:100 mL to make 1 % Agarose. The mixture was microwaved on medium power, with intermittent swirling until the agarose powder was completely dissolved, making sure the mixture did not overboil, as this could affect the final gel percentage concentration. The gel was cooled at room temperature, to about 50 °C, following addition of 2.5 μL Ethidium Bromide (ETBr) lab stock per 100 mL (1 % Agarose gel); ETBr allows DNA staining and transluminescence under UV light. Furthermore, 4 μL of DNA sample was constituted with 1.6 μL of 40 % sucrose loading dye (40 % w/v sucrose and 0.20 % bromophenol blue (w/v). The DNA was loaded by carefully pipetting into wells of a 12 cm × 14 cm, (1 % agarose gel tray). Gel electrophoresis was run at room temperature for 35 minutes at 90 V. The electrophoresis images were captured using Bio–Rad ChemiDoc ^{TM} XRS + Bio–Rad Gel Doc XR Imaging System under UV light.

3.2.3 Amplification of the bacterial gene (16S rRNA) using polymerase chain reaction

Prior to the PCR amplification, samples with higher DNA concentration were proportionally diluted with deionised water to fit the range 20–30 ng/μL by using equation 1 below. The 16S rRNA gene was amplified using 20 μL Master mix containing 4 μL Buffer (Whitehead Scientific, South Africa), 1 μL forward primer, 1 μL reverse primer, 0.2 μL taq enzyme (Whitehead Scientific, South Africa), and 1.5 μL of 30 ng/μL of template DNA, 12.3 μL deionised water. The PCR targeted V3 –V4 region of 16S RNA bacteria, which is a highly variable region in the Kingdom bacteria but has highly conserved regions either side to ensure primer specificity. The primers used were Forward Primer f351

5' CCTACGGGNGGCWGCAG

and Reverse Primer r765

5'GACTACHVGGGTATCTAATCC (Klindworth et al., 2013).

The initial PCR amplification of samples was run on (ABI Veriti 96 well thermal cycler Applied Biosystems, MA, USA), at the Zoology and Entomology Molecular Laboratory (Grahamstown, South Africa) with the following conditions: initial denaturation 95 °C for 3 minutes; 95 °C for 30 seconds 25 cycles, 55 °C for 30 seconds 25 cycles, 72 °C for 30 seconds 25 cycles, 72 °C for 5 minutes, hold at 4 °C. Annealing was done for 30 seconds at 55 °C, elongation for 1 minute at 72 °C.

$$C1V1 = C2V2$$

Where $C1$ = primary concentration, $V1$ = primary volume, $C2$ = final concentration, $V2$ = final volume

3.2.4 Polymerase chain reaction product cleaning and indexing

The success of the DNA extraction process was assessed by running gel electrophoresis for visualization of DNA bands. The PCR product was cleaned using PCR Clean–Up System (Promega, ThermoFisher Scientific, South Africa), which remove enzymes, nucleotides and all other components that might interfere with the downstream processes. Prior to clean up, all three replicates per each site were pooled into one well resulting in a total of 60 samples from 180. This increased the volume of product to 60 μL per well, and after cleaning up the cleaned product was eluted in the same volume of 60 μL. The amplicon products of clean up purification process (50 μL) were attached to adapters and assigned dual indices following Nextera XT Index Kit protocol (Illumina® Inc., USA). This process allowed samples to be unified with overhang adapters.

Forward overhang: 5’TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[locus specific sequence]

Reverse overhang: 5’GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[locus specific sequence].

Indexing enabled unique identification of samples after high throughput–sequencing, allowing demultiplexing and identification of the correct origins of each sample in the study. The sequencing of the metagenome library was conducted by Aquatic Genomics Research Platform (AGRP) at the South African Institute of Aquatic Biodiversity (Grahamstown, South Africa) using (2 × 300 bp) paired–end sequencing on an illumina MiSeq high–throughput sequencing machine.

3.2.5 Processing data using Quantitative Insights Into Microbial Ecology (QIIME2)

The sequencing data generated by the Illumina MiSeq platform, was obtained in fastq.gz file format which were demultiplexed reads, for all 60 samples. The data was imported into QIIME2 environment QIIME 2 version 2022.2 (Bolyen et al., 2019), as CasavaOneEightSingleLanePerSampleDirFmt. QIIME2 is an online free software which uses data provenance and plug–ins to analyse amplicon sequences (Hall and Beiko, 2018). Denoising and quality filtering of DNA reads was done using DADA2 pipeline to remove chimeras. The quality drop in the forward and reverse reads were removed by —p–trim–left–f 17, —p–trim–left–r 21 respectively to remove non–biotic primer regions. The sequence reads were truncated at —p–trunc–len–f 300, —p–trunc–len–r 232, removing poor quality regions after analysing the quality score plots, but leaving long enough fragment to allow for the minimum of 50 bp overlap needed to join paired end sequences as per illumina 16S metagenome protocol. A feature table/Amplicon Sequence Variance (ASVs) table (Supplementary Table S2) was retrieved after denoising with DADA2 for downstream analyses.

3.2.6 Taxonomy assignment and core metrics

Taxa were assigned to the feature table at 99 % similarly index using the silva database v132 (Quast et al., 2013) trained using a naïve bayes classifier for v3–v4, trained with scikit–learn 0.24.1. Taxonomy–based filtering was employed for both the feature table and representative sequence reads to remove Archaea, Chloroplasts and Mitochondria and Eukaryotes. After denoising, a total of 15040 sequences were obtained from 60 samples. Samples were rarefied (McMurdie and Holmes, 2014) to even sampling depth of 6 533 reads per each sample (Supplementary Figure S1), which retained 365 848 (51.6 %) features in 56 (94.9 %) samples. The QIIME2 phylogeny and mafft–fasttree for alignment plugins were used to run core–metrics–phylogenetics. Four samples that had fewer sequences than 6 533 were dropped from the core–metrics–phylogenetic analyses. To establish the most dominant taxa, core microbiota was analysed using core–features plugin in QIIME2 across all seasons and sites at minimum prevalence of 85 %, similar to (Wyness et al., 2022). This value was selected based on frequency threshold for common core microbiomes often found between 30 % and 95 % (Huse et al., 2012; Ainsworth et al., 2015).

3.2.7 Alpha and beta diversity

Alpha and beta diversity measures were conducted on rarefied data at an even sequencing depth of 6533 (Figure S2). The QIIME2 artifacts were integrated into R using the phyloseq package (McMurdie and Holmes, 2013). Alpha diversity metrices employed for this study include the Observed (OTUs) and Shannon–Weiner Index (community richness), with statistical significance assessed using the non–parametric Kruskal–Wallis (all groups) test in QIIME2. Differences in Beta diversity between treatments was evaluated by running a PERMANOVA in QIIME2. Weighted unifrac and unweighted unifrac distance metrices were used to plot Ordination plots in R Studio.

3.2.8 Metagenome prediction of functional bacterial communities

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) (Douglas et al., 2020) was performed in QIIME2 to predict metagenome functions and the following outputs; i) EC (enzymes), ii) KO (orthologs) and iii) predicted pathway abundances. The pipeline implicitly uses the MetaCyc database to infer MetaCyc pathways (Caspi et al., 2016). The Kyoto Encyclopedia of Genes and Genomes (KEGG) data base was used to infer molecular functions retrieved as functional orthologs (KO) from PICRUSt2 (Kanehisa and Subramaniam, 2002). The exploration of biological functions using PICRUSt2 and pathway databases, allows a deeper understanding into enzymatic pathways discussed in section (3.1 Introduction) of this study. The selection of ECs used in this study was motivated by Wyness et al., (2021) micro communities investigation. A heatmap of Z–scores were generated in R studio (Figure 3.10) using relative abundances of enzymes to allow the interpretation of predicted metabolic pathways across seasons and sites.

3.3 RESULTS

3.3.1 Absolute and relative abundance of bacterial community composition

A total of 15 042 Unique Amplicon Sequence Variances (ASVs) were discovered across all samples from 691 934 16S rRNA sequence reads. These unique features contributed to a total of 51 bacterial phyla found to be present in Khakhea–Bray pan ecosystem network. Of these, 7 phyla were observed to be dominant (Figure 3.1). Firmicutes had high relative occurrence, at maximum 59 % (mean ± sd, 19 ± 13 %). Actinobacteria and Proteobacteria both had maximum prevalence at 34% with (18 ± 7 %) and (18 ± 6 %), respectively. Cyanobacteria occurred to maximum of 38 % and Acidobacteria 15 %, however with similar mean ranges (8 ± 7 %) and (7 ± 3 %), respectively. The total distribution of less prominent phyla (<4 %) was represented by the top blue (Figure 3.1).

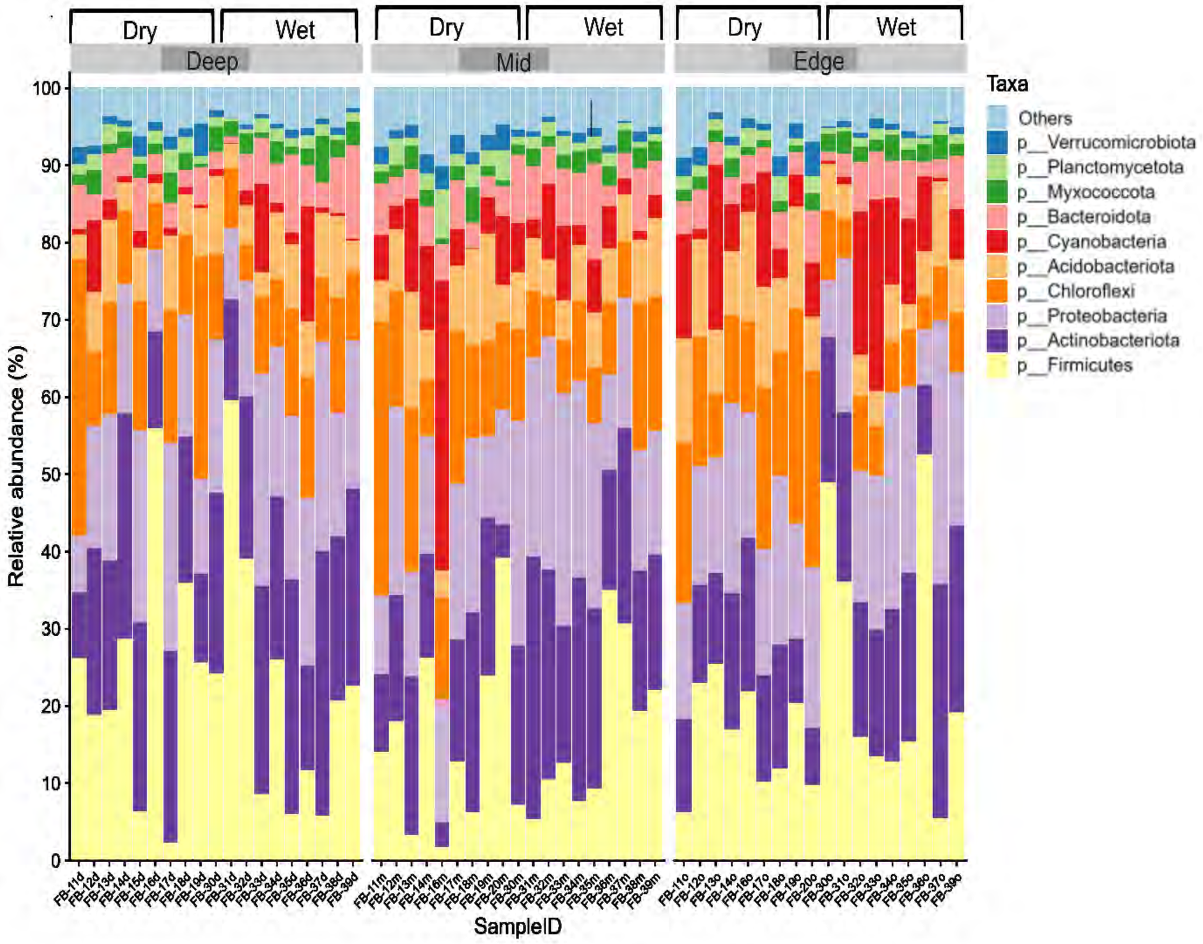

Figure 3.1. Stacked bar plot highlighting spatial distribution of phyla occurring in relative abundances (>4 %) in the Khakhea–Bray pan ecosystems in June 2021 and January 2022. Deep, Mid, and Edge represent the sites sampled across varying depth and Dry/Wet represent the season.

At class level, Bacilli and Clostridia represented the phylum Firmicutes (Figure 3.2a). Similarly, Proteobacteria was represented by the Alpha– and Gammaproteobacteria. At genus level a sum of 1 129 genera were observed to be the subsidiaries of 51 bacterial phyla. Evidently, most top genera found in this study had commonality as thermophiles (Figure 3.2b), with *Bacillus* having the highest relative abundance at 54 % (13 ± 12 %).

Bacterial communities in the wet season were generally low in ASV richness (Figure 3.3a) compared dry the season (ASVs, wet median – 523, dry median – 569), however these differences were not significant (Kruskal–Wallis; observed features, H = 3.28, *p* = 0.07). Similarly, Shannon's diversity could not be differentiated between the two seasons (ASVs, wet median – 5.69, dry median – 5.71), hence there were no significant differences in total microbe richness between seasons (Kruskal–Wallis; Shannon's Diversity: H = 0.11 *p* = 0.74).

The number of ASVs observed in deep points were relatively low as compared to the mid and edge points (Figure 3.3b, ASVs; deep median – 514, mid median – 569, edge median – 569). However, these differences were not significant when all three depth levels were assessed (Kruskal–Wallis; observed features, H = 5.71, *p* = 0.06). Shannon's diversity suggested a uniform distribution of microbial communities within all samples examined across the depth gradient (ASVs, Deep median– 5.69, Mid median– 5.74 and Edge median– 5.67, respectively), and the differences were not significant (Kruskal–Wallis, H = 4.98, *p* = 0.08).

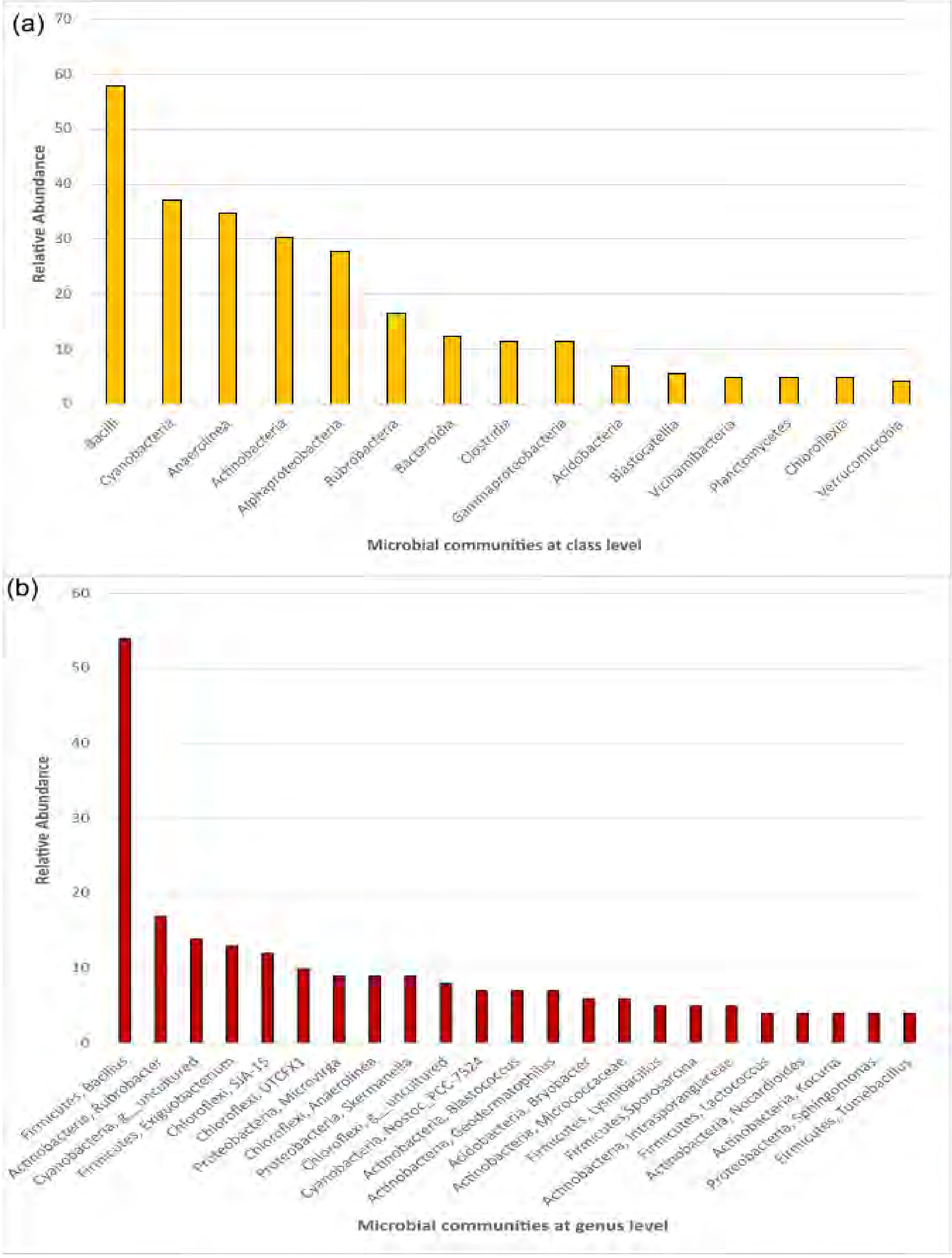

Figure 3.2. Taxa bar plots highlighting the distribution of bacterial communities in Khakhea–Bray pan ecosystem network at (a) class and (b) genus level for taxa with relative abundances >4 %. The total sum of less prominent bacterial classes and genera accumulated to 75 and 419, respectively.

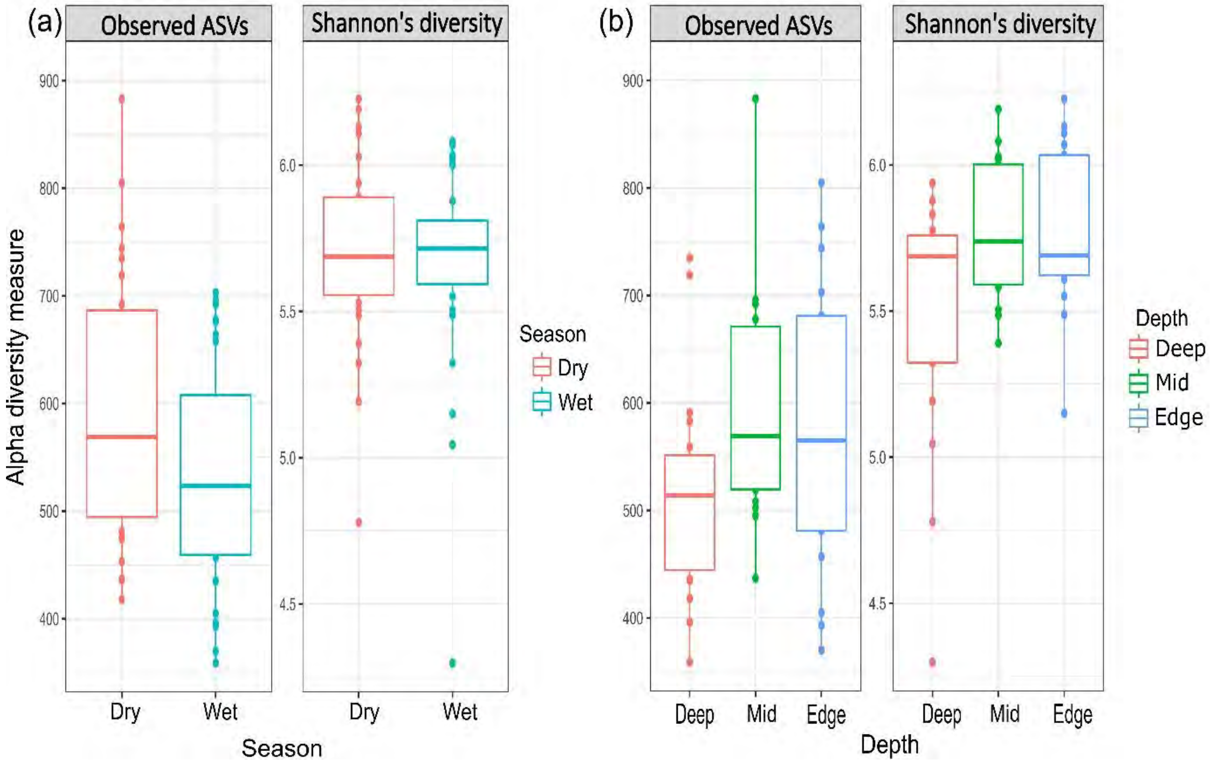

Figure 3.3. Alpha diversity box plots indices showing variations in observed features and Shannon's diversity of bacterial communities in in the Khakhea–Bray temporary pan ecosystem in June 2021 and January 2022 (a) between seasons and (b) across depth.

3.3.2 Beta diversity

The PCoA biplot suggest existence of distinct types of bacterial communities between dry and wet seasons, as demonstrated by the clusters (Figure 3.4a). While majority of differences were explained by Axis 1 in both graphs, bacterial communities within the dry season phase seem to be more dispersed apart as compared to the wet phase (Figure 3.4b). Weighted unifrac, which represents community composition and phylogeny, explained about 68 % variation in microbial community compositions on axis 1. The compact clustering in the PCoA suggests a high degree in phylogenetic relationships in structure of bacterial communities within the wet season compared to dry season (Figure 3.4b). The seasonal differences were confirmed to be significant, based on weighted unifrac (PERMANOVA, Pseudo–F = 5.742, p = 0.001) and unweighted unifrac distances (PERMANOVA, Pseudo–F = 2.561, p = 0.001). Unweighted unifrac represent a classic diversity matrix which only explores the absence or presence of bacterial taxa whereas weighted unifrac further explores abundance of microbes in establishing community differences (Chang, Luan and Sun, 2011). In addition, the variations were persistent across varying depth, weighted unifrac (PERMANOVA, Pseudo–F = 1.884, p = 0.013), unweighted unifrac (PERMANOVA, Pseudo–F = 1.556, p = 0.001). Jaccard and Bray Curtis distance matrices also showed similar responses and were significant across varying depth and seasons ($p < 0.05$).

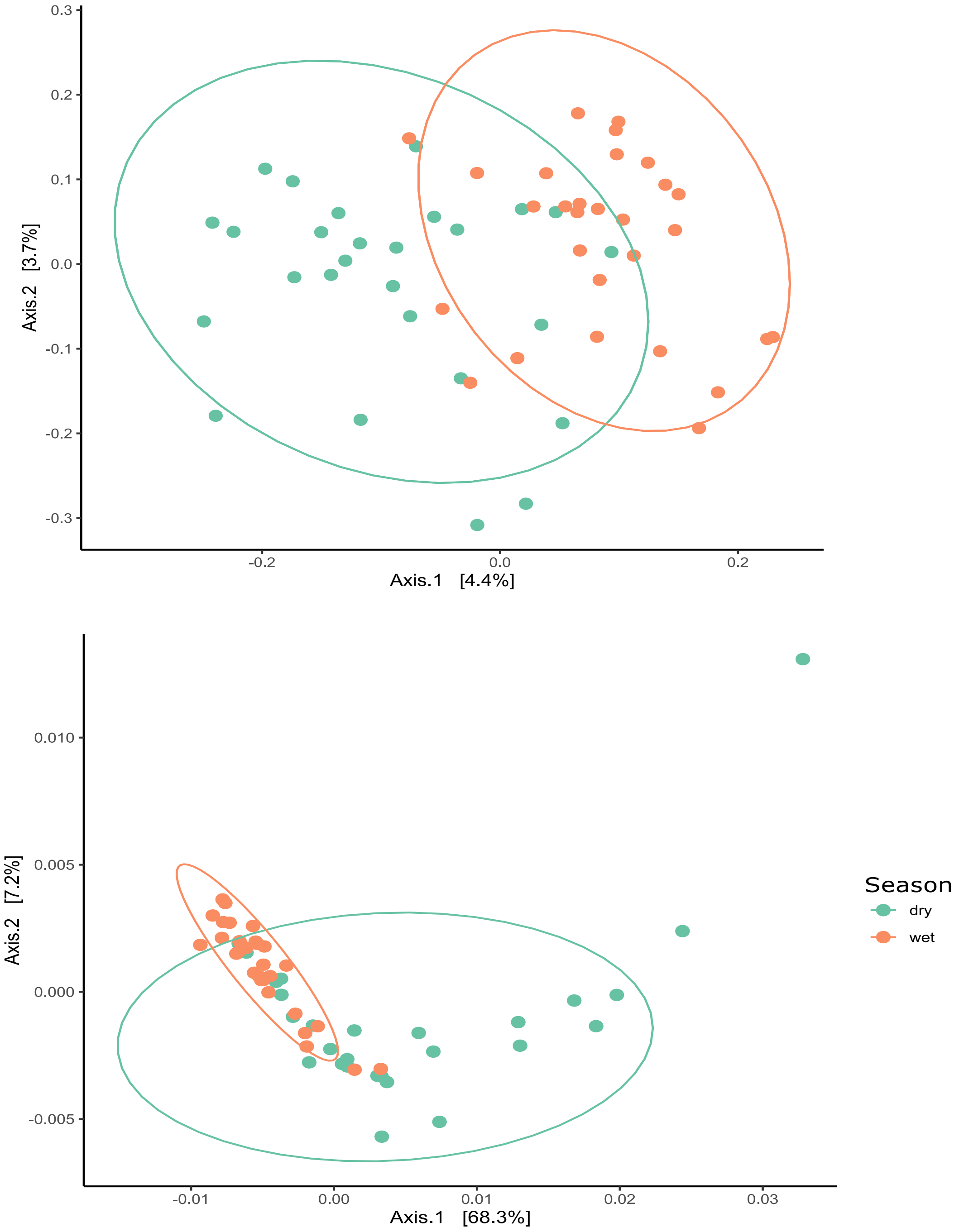

Figure 3.4. Diagrammatic illustration of PCoA, on Beta diversity phylogenetics across the seasons, ellipses delineate clustering of (a) unweighted unifrac and (b) weighted unifrac distance matrices, highlighting differences in bacterial community structure between the dry and wet phases.

Nonmetric Multidimension Scaling (NMDS) highlighted notable differences in the type of microbial communities which exist between the dry state of pans and wet state of pans within the dry season and this observation somehow explains the dispersion observed in PCoA (Figure 3.4). There was a large variation in the distribution of microbial communities within dry season and shallowest regions (Figure 3.5) likely a function of differences in sediment water retention capacity. The 'Deep, Mid and Edge' highlighted some overlaps in microbial communities (Figure 3.5b) supporting the existence of a continuum in the distribution of microbiomes across a pan. More specialised bacteria were however found to be limited within respective zones different from one another. Ecologically adaptable and flexible microbial communities dominated the intersections (Figure 3.5b). The above observations define microbial community structure which make up the Khakhea–Bray temporary pan ecosystem.

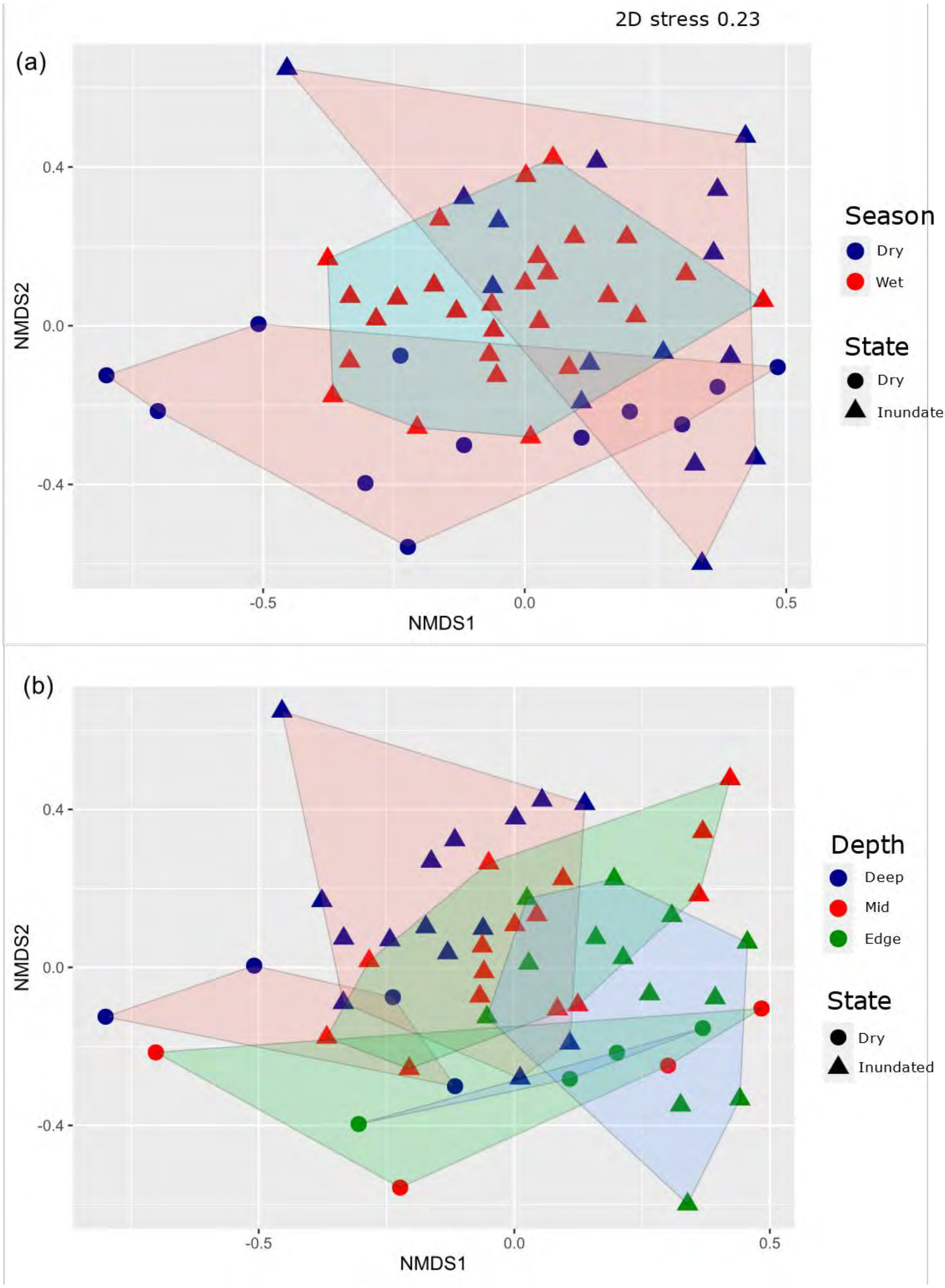

Figure 3.5. Non–metric Multidimensional scaling based on Bray–Curtis distance matrix for rarefied ASVs showing effect of the state of pans on the distribution of bacterial microbiomes in Khakhea–Bray pan ecosystem (a) between season and (b) across depth. The ellipses are delineating microbial communities belonging to the same category as defined by the key.

3.3.3 Core microbiota

The core microbiome analysis composition was performed for bacterial taxa that appeared in more than 85 % of all samples at genus level (Figure 3.6a, b). A total of 6 species (10 % core) were exclusively found in the dry season, and included *Blastocatella*, *Pedosphaeraceae*, *uncultured bacterium (Choloroflexi)*, *Anaerolinea* and *Bryobacter.* In contrast, the wet season had a considerable number of species exclusively adapted to the wetting period (44 % core microbiomes). A total of 25 species (46 % core) were found to be persistent though out the wet and dry season, suggesting a more robust adaption of these taxa to environmental changes.

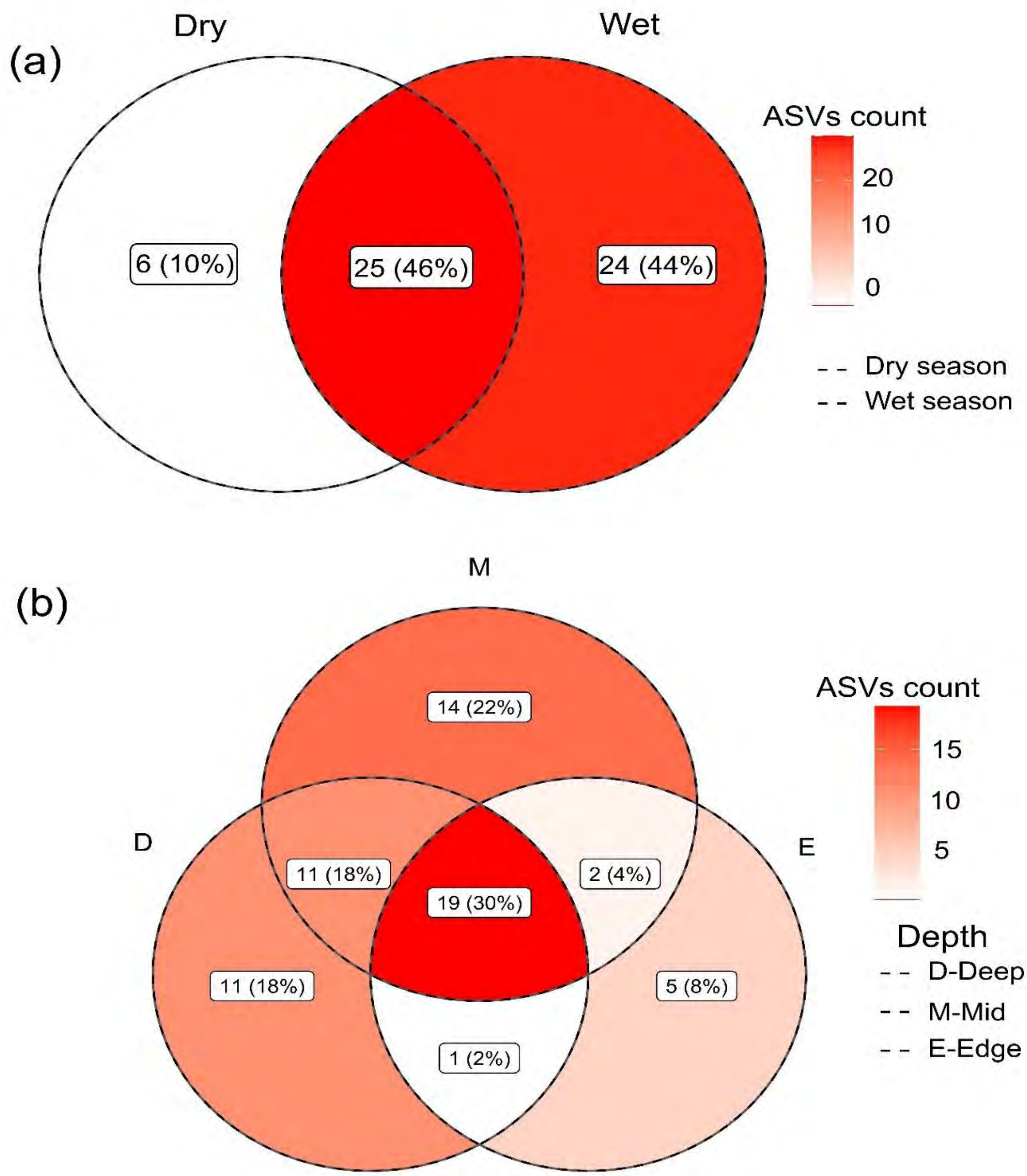

Figure 3.6. (a)Venn diagram showing distribution of core microbiota between Dry and Wet season and (b) depth gradient at >85 % prevalence in all samples for a genus to be considered a core feature.

A total of 19 genera (30 % core microbiomes) were observed to be dominating across depth from the deepest zones (Deep), intermediate depth (Mid), and shallowest regions (Edge). Generally, the Edge contributed much lesser core microbiomes relative to the mid and the Deep points (Figure 3.6b). The Mid points had the highest number species 14 (22 % core) exclusively, which might suggest that it’s a transition zone likely experiencing a cocktail of Deep and Edge functional microbiomes. Two taxa were shared between the Mid and Edges i.e genus *Skermanella* and unknown family which belongs to *Cyanobacteria*.

The patterns of distribution in the predicted metagenome functions suggested by Principal coordinate analysis PCoA (Figure 3.7) showed not only that season distinguished temporary pans in bacterial functions but also clearly demonstrated that wet season supports more functions compared to the dry season. Although some functions seem to overlap (Figure 3.7) others were distinct and found only at the bottom and top of the biplot highlighting the narrow range of operation in some bacterial functions specific to season. The functions responsible for driving important biogeochemical cycles in Khakhea–Bray ecosystem were interpreted using Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway identifier, denoted by Enzyme Commission number (E.C) (Bairoch, 2000). The predicted metagenome functions for enzymes in bacterial microbiomes were significantly different across dry and wet season (PERMANOVA,Pseudo–F = 5.136, $p = 0.004$) with Bray Curtis distance and (PERMANOVA, Pseudo–F = 3.186, $p = 0.001$) by Jaccard. The 1st axis explained majority of variation with 52 %, whilst the second axis explained the remaining 20 %. The above current patterns agree with beta diversity PCoA for ASVs using weighted and unweighted unifrac distance matrix, therefore validating the PICRUSt2 predictions.

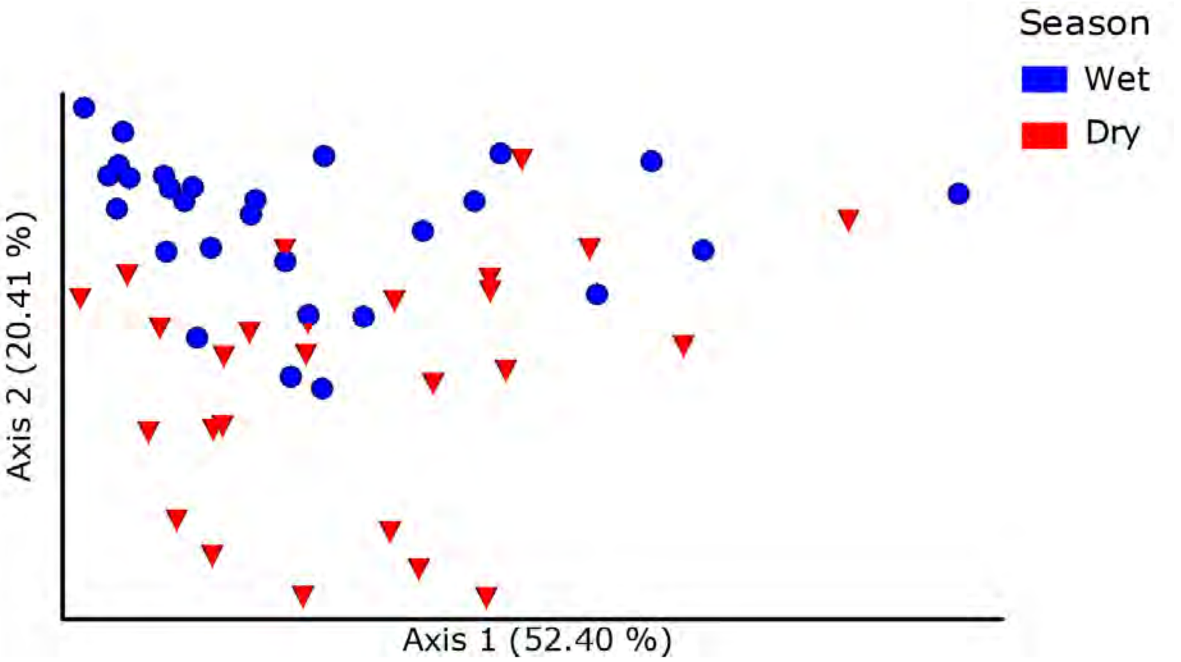

Figure 3.7. Principal Coordinate Analysis (PCoA) plot showing seasonal distribution of predicted enzyme metagenome functions as based on Jaccard distance matrix.

3.3.4 Predicted functional potential of bacterial microbiomes using PICRUSt2

The predicted metabolic pathways for functional bacterial microbiomes adapted from (Wyness et al., 2021) as novel, were generated as relative abundances and Enzyme commission numbers (E.C) (Table 3.1) and interpreted as Z–scores (Figure 3.8) to draw conclusions as motivated by Clark–Carter, (2005). Enzyme commission numbers (E.C) used in this section were previously discussed in section 3.3.3 paragraph 3 of this chapter. In the wet season, inundated pans were exclusively dominated by the following functions from the deepest to the shallowest i) denitrification by nitrite reductase (E.C:1.7.1.15) and nitrous oxide reductase (E.C: 1.7.2.4) ii) carbon degradation (E.C:1.11.1.1.9 and iii) eps production (E.C:1.1.1.132). In relatively less proportions there was assimilatory sulphate reduction (E.C:1.8.4.8) and urea hydrolysis (E.C:3.5.1.5). Furthermore, site specific variations were observed in other enzyme abundances in summer wet pans, with high Z–scores of close to 3 namely the deepest zones, eps degradation (E.C:3.2.1.21) and nitrogen mineralisation (E.C:1.4.1.2). Intermediate depth, eps degradation (E.C:3.2.1.8), denitrification (E.C:1.7.2.4) and DNRA (E.C:1.7.7.2). Shallowest regions, eps production (E.C:24.1.12) and denitrification (E.C:1.7.2.5). These functions and their associated microbiomes are therefore suggested to be the main drivers of significant seasonal differences (Figures 3.4 and 3.9).

Table 3.1. Ortholog Functions and Enzyme Commission numbers (E.C) for predicted gene functions expressed as Absolute abundances of total sums across all samples.

Ortholog Pathway	**E.C Number**	**Gene name**	**Relative Abundance**
Carbon fixation	E.C:6.3.4.3	Formate–tetrahydrofolate ligase (FTSH)	28760
Carbon fixation	E.C:4.1.1.39	Ribulose–bisphosphate carboxylase	8334
Carbon degradation	EC:1.11.1.9	Glutathione peroxidase (btuE)	31366
Urea hydrolysis	E.C:3.5.1.5	Urease (Urea A,B,C)	38232
Nitrogen fixation	E.C:1.18.6.1	Nitrogenase reductase (NifD/NifH)	10677
Nitrogen mineralization	E.C:1.4.1.1	Alanine dehydrogenase (AlaDH)	60622
Nitrogen mineralization	E.C:1.4.1.2	Glutamate dehydrogenase (gdh)	27833
Nitrogen mineralization	E.C:1.4.1.3	Glutamate dehydrogenase [NAD(P)+	23600
Nitrification	E.C:1.14.99.39	Ammonia monooxygenase (amoA)	496
Nitrification	E.C:1.7.2.6	Hydroxylamine dehydrogenase (hao)	337
Denitrification	E.C:1.7.1.15	Nitrite reductase (NADH) nirB/nirD	37973
Denitrification	E.C:1.7.2.4	Nitrous–oxide reductase (nosZ)	3525
Denitrification	E.C:1.7.2.5	Nitric oxide reductase (cytochrome c)	3134
DNRA	E.C:1.7.7.2	Nitrite reductase nrfA	3440
DNRA	E.C:1.7.7.1	Nitrite reductase nrfA	9529
ASR	E.C:1.8.4.8	Phosphoadenylyl–sulfate reductase	36535
DNRA	E.C:1.8.99.5	Dissimilatory sulfite reductase (dsrA/B)	917
EPS production	E.C:2.4.1.12	Cellulose synthase (bcsA/B)	8444
EPS production	E.C:1.1.1.132	GDP–mannose 6–dehydrogenase	1957
EPS degradation	E.C:3.2.1.21	Beta–glucosidase	87513
EPS degradation	E.C:3.2.1.4	Cellulase	51079
EPS degradation	E.C:3.2.1.8	Endo–1,4–beta–xylanase	6672

**ASR–Assimilatory Sulphate Reduction, *DNRA–Dissimilatory nitrate reduction to ammonium*

The dry season was defined by a different set of enzymes suggesting a shift in the bacterial communities in response to season. However, functions in the dry season had relatively less abundance (<2 Z–Scores). The deepest to the shallowest zones within dry season pans supported EPS degradation (E.C:3.2.14), nitrogen mineralisation (E.C:1.4.1.3), carbon fixation (E.C:6.3.4.3), and nitrification (E.C:1.14.99.39; E.C:1.7.2.6) (Figure 3.8). The above observations also corroborate significant seasonal differences in bacterial community structure (Figures 3.4 and 3.7), highlighting less functions in the dry season compared to wet season. However, there were site specific variations which demonstrated very high activity of dissimilatory sulphate reductase (E.C:1.8.99.5) and nitrogen fixation (E.C:1.18.6.1), shown by the unique pair of dark red colored boxes close to the bottom (Figure 3.8).

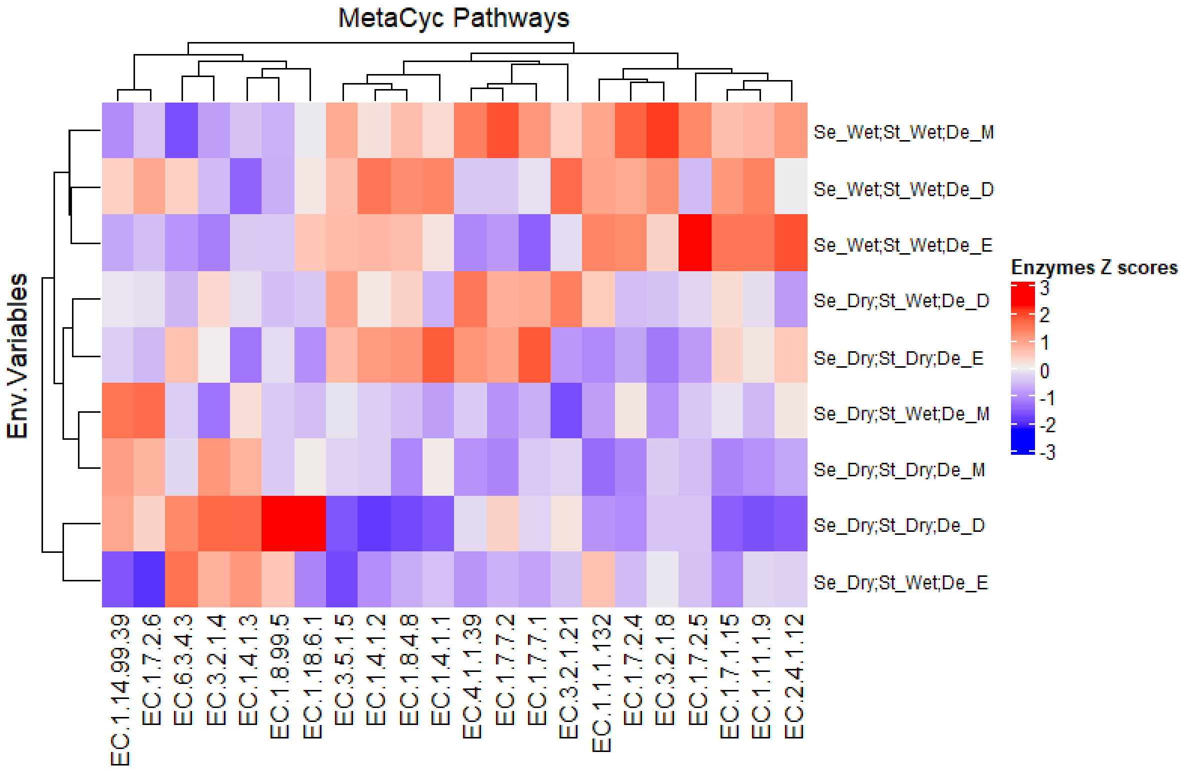

Figure 3.8. A heatmap plot of relative abundances Z–scores, highlighting functional clustering of predicted metagenomes as represented by Enzyme Commission (E:C) numbers using PICRUSt2. Colour intensity represent Z–scores of enzymes ranging from the lowest blue to the highest red. The metagenome functions associated with E:C numbers were predicted using MetaCyc database. Se_ represent season, St_ state, De_ depth where D = Deep, M = Mid and E = Edge.

3.4 DISCUSSION

This study is among the first to explore microbial diversity and ecosystem functioning dynamics in arid–zone temporary pan ecosystems using a metagenomics approach. As hypothesized, heat tolerant taxa dominated given the arid nature of Khakhea–Bray. All top 20 genera in their relative abundances shared thermophilic tendencies. Additionally, beta diversity between communities was higher in the wet season and varied across depth with more microbiomes found in the deeper zones compared to the shallower regions. This confirmed our second hypothesis which stated that core microbiomes in the wet season would have higher diversity than dry season. The associated functions for enzymes predicted were greater in the wet season and across depth, partially confirming our third hypothesis which stated that predicted functional metabolic roles for enzymes would be seasonally correlated, with more in the deeper areas of inundated pans in wet season. These findings contribute to the existing knowledge gap in microbial functional ecology, particularly arid–zone temporary pans.

The dominance of Firmicutes, Actinobacteria and Chloroflexi largely represented by *Bacillus, Rubrobacter and Anaerolineae* respectively, confirmed our first hypothesis which predicted the dominance of heat tolerant taxa. The thermophilic adaptation allowed each group to become prolific in the ecosystem due to their ability to tolerate the increased heat associated with Khakhea–Bray (Sekiguchi et al., 2003; Albuquerque et al., 2014). The high prevalence of *Bacillus* might have been necessitated by its ability to produce heat resistant endospores (Nicholson et al., 2000; Checinska et al., 2015).

Bacillus species occupy prominent roles in nitrogen cycling, which include ammonification (Hui et al., 2019), nitrification (Rout et al., 2017), and denitrification (Verbaendert et al., 2011; Bachar et al., 2012). In addition, *Rubrobacter* can mineralize sulphate compounds (Vikram et al., 2016) and participate in the global carbon cycle through organic matter decomposition (Araujo et al., 2020). However, *Anaerolinea* is involved in sediment aggregate formation and EPS secretion, critical for nitrogen removal in aquatic systems by anammox (Zhao et al., 2018; Zhao et al., 2019).

The observed non–significant differences in Shannon–Wiener indices representing within community diversity in dry and wet season, suggested an even community richness and overall greater diversity, which corroborate (Silveira et al., 2020). Spatial variations across depth gradient in aquatic systems has been reported to shape alpha and beta diversity distributions of microbial communities (Gong et al., 2015). However, alpha diversity differences did not demonstrate any distinct pattern across the deepest zones, intermediate depth and shallowest regions. Taken together, this is encouraging for rapid assessments as it suggests the sampling of targeted seasons and/or sites across depth to get a representative picture of microbial consortia present at that time.

On the other hand, beta diversity results suggested distinct types of microbial communities between the two seasons and across depth. Seasonal dynamics had a bigger influence in shaping bacterial communities. Higher microbial diversity in the wet season by core microbiomes of Alphaproteobacteria, Acidobacteriota and Actinobacteriota, were highly associated with elevated rates of biogeochemical cycling in comparison to the to dry season. The cyclical variations in sediment moisture content driven by seasonal effects can therefore be suggested to influence microbial diversities, with possible modification in metabolic functions, as demonstrated by highest rates in sulphate reduction by nitrogen fixing bacteria in extremely dry pans.

The majority of predicted functional metabolic roles for enzymes were greater in the wet season and across depth of inundated pans, without any obvious bias towards the deepest zones. These findings highly suggest the importance of hydroperiods associated with maximum pond filling with implications to overall pond productivity from deepest zones to shallowest regions. However, denitrification (E.C:1.7.2.5), EPS production, and EPS and carbohydrate degradation were relatively higher on Edge and Mid points respectively, compared to the Deep. Whilst Gemmatimonadetes, Nitrospira, Rubrobacter, *Bacillus* core microbiomes are implicated in the denitrification process mentioned above, Proteobacteria and Bacteroides dominant at the Mid points, have been reported to be key carbohydrate degraders (Cottrell and Kirchman, 2000; Edwards et al., 2010). The microbial degradation of polysaccharides in aquatic systems constitutes an important initial step in the carbon cycling process (Biddanda and Benner, 1997). Furthermore, Nichols et al. (2005) demonstrated that Gammaproteobacteria and Bacteroidetes can synthesize EPS. Phylum Actinobacteria, Acidobacteria, and Planctomycetes recovered in this study have been also been linked to EPS production (Costa et al., 2020). In a nutshell, Mid points demonstrated much more significance in holding taxa abundance, suggesting potential target zones for rapid assessments.

A different set of enzymes dominated the dry season, which mediated processes including nitrification and nutrient mineralization at however relatively lower levels. This complete switch in microbiome function confirmed the cyclical nature of temporary systems which have been reported to support distinct microbial consortium in respective times (de Nijs et al., 2019; Fromin et al., 2010). Depth specific variations in extremely dry phases such as highest rates of DNRA by nitrogen fixation at the central zone (usually deep region during inundation phase) suggest the impact of low rainfall and high evaporation rates on pan ecosystem. These sites are likely hypersaline and subsequent evaporation of water from the sediment creates an ideal environment for the proliferation of nitrogen fixing sulphate–reducing bacteria (Brandt et al., 2001; Roychoudhury et al., 2013). Dissimilatory sulphate reduction plays a key role in global sulfur and carbon cycle (Crowe et al., 2014), with an estimated over 50 % contribution to organic carbon mineralization in aquatic sediments (Jørgensen, 1982; Mußmann et al., 2005).

3.5 CONCLUSIONS

This study provides metagenomic evidence for the participation of temporary pans in important global biogeochemical cycling through microbial mediation by bacterial microbiomes. The results observed here demonstrate positive relationship between wet season, hydroperiods and level of inundation within pans with implications to proliferation of functional microbiomes. This investigation highlighted the importance of pan filling as a determinant of sediment moisture content which influences microbiome function across varying depth. Arid climates are often exposed to drying periods; therefore, it is considerable for extremophiles/heat tolerant taxa to be the backbone of major biogeochemical cycles during these extremely dry and hot periods. Hence making it a unique feature of Khakhea–Bray pan ecosystem network. The deepest zones are more buffered hence in this system they did not house diverse microbes compared to the higher diversity that was found in the transition zones, especially the Mid, with limited species occurring in the Edge. Taken together, the response of microbiomes and differences in their associated functions outlined by this study can help us predict response of these systems in face of climatic changes characterized with extended drought periods. These findings enhance our understanding of microbial ecology in temporary wetlands and make several contributions to small temporary wetland literature globally despite its relatively small scale.

CHAPTER 4.

GENERAL DISCUSSION

PLATE 4. View of inundated pan sampled for aquatic biodiversity including benthic microbiomes. From left to right Prof. Ryan J. Wasserman, Chipo Mungenge, Tafara Frank Bute, Dr Tatenda Dalu and Mr Farai Dondofema. Photo Credit: Kudzai Shawn Mpakairi, January 2022.

4.1 Overview

Molecular ecology has been underrepresented in studies of temporary ecosystems despite tremendous insights this field has offered over the last decade in global aquatic ecology (Kennedy et al., 2010; Ustick et al., 2021).The two data chapters of this thesis, chapters 2 and 3 provide a detailed metagenomic investigation of microbial functions in temporary pans of the Khakhea–Bray region, quantified as EPS concentrations and 16S rRNA sequenced bacterial communities. The influence of seasonal and spatial variation is also explored in these two chapters. One of the leading findings of this research, is that the variation in sediment water content influenced bacterial community structure and biogeochemical pathways as previously reported in other studies (Schimel, 2018; Ouyang and Li, 2020; De Vicente, 2021). In this study both EPS and functional bacterial microbiomes decreased with a declining sediment moisture content i.e., from the deepest zones to the shallowest regions (Edge). It has been confirmed that, low sediment moisture content slows down microbially mediated biogeochemical rates (Ochoa–Hueso et al., 2018) leading to increased selection pressure among microbial communities through competition for limited resources (Barnard et al., 2013). This thesis contributes significantly to literature on microbial productivity in temporary pans which have been a neglected topic in Global South wetland systems.

4.2 Extracellular polymeric substances

This study in temporary pans stands out as the first metagenomic study in the Global South, despite other biodiversity assessment studies of temporary ponds in the region (Bird et al., 2013; Mlambo et al., 2011; Dube et al., 2020). In Chapter 2 the trends observed in EPS composition of more carbohydrates than proteins have been reported in more permanent systems in other parts of the world (Hirst et al., 2003). The widely accepted explanation to this, implicates diatoms as high polysaccharide producers in the EPS–matrix. (Myklestad, 1974; Smith and Underwood, 2000; Urbani et al., 2005). However, the major challenge in comparing EPS studies stems from the various methods used to extract complex extracellular components. This study in Chapter 2 used water extraction method for retrieval of total EPS and the results were comparable to studies elsewhere in the world (Friend et al., 2003; Paterson et al., 2000). In this study EPS production and its degradation by bacteria were confirmed by metagenome prediction in Chapter 3, which supports global (Behera et al., 2020; Ahmad et al., 2021; Nguyen et al., 2022) and regional (Abia et al., 2018; Lukhele et al., 2019; Alegbeleye et al., 2021) advances in aquatic microbial ecology.

4.3 Microbiomes and their functional diversity

One of the main objectives in Chapter 3 was to characterize bacterial diversity and associated functional potential of microbiomes in nutrient cycling. An important discovery highlighted by this thesis is the dominance of heat tolerant taxa in Khakhea–Bray arid zone temporary pans (Chapter 3). This revelation brings to light how microorganisms survive extreme conditions such as hot arid zones, which are structured by irregular and unpredictable variations in hydrological regimes and physico–chemical conditions (Walton, 2017). The various tactics of adaptation by other fauna in temporary ponds of extreme environments are well documented (Hibshman et al., 2020) and the main strategies include dormancy and dispersal (Lahr, 1997). Chapter 3 expands our knowledge in the adaptation of microbes in arid environments, which include formation of spores and carrying of heat tolerant genes as in the case of *Bacillus.* We conclude that such characteristics allows specific microbiomes to be prolific and drive ecosystem functioning in temporary pan wetlands.

In Chapter 3 the genomic capacity for denitrification, carbon degradation and EPS production was observed to be high in the wet season and from deep zones to shallowest regions. As mentioned in the first paragraph of this chapter, the major seasonal differences in bacterial functions were driven by precipitation and the level of inundation in temporary pans. It is known, small aquatic systems in semi–arid habitats are characterised by seasonal hydroperiod fluctuations (Msiteli–Shumba et al., 2018) which could be a function of interacting processes, including precipitation, evapotranspiration and surface or groundwater flows (Tamea et al., 2010). This study demonstrated high microbial productivity when temporary pans were at full capacity during the wet hydroperiod phase compared to dry hydroperiod state and/or partial inundation.

Spatial variations across depth gradient in aquatic systems has been reported to shape community richness of microbial communities (Gong et al., 2015) and influence distribution of microbially mediated biogeochemical pathways (Tran et al., 2021). This thesis findings (Chapters 2 and 3) demonstrated that pan Edge was significantly low in microbial productivity compared to the Deep zones. Such highlighted similar patterns of change across depth have been confirmed in other related studies elsewhere in the northern hemisphere (Burford et al., 1998; Bryant et al., 2012; Signori et al., 2014; Lu et al., 2020). The differences in microbial functions between the Deep and Edge was high of any combination between designated points. Therefore, in the event of rapid assessments or follow up studies, sampling effort should be limited to the Deep and Edge. In addition, to saving time this also allows cost effective monitoring of these systems. From a Global South standpoint this investigation provides a foundation for further exploratory studies to evaluate dynamics in microbial profiles in arid zone small aquatic ecosystems and associated changes over time. This is highly motivated by the current trends in global warming and associated predicted climatic changes likely impacting aquatic ecosystems (O'Reilly et al., 2003; Prakash, 2021; Zhao et al., 2022).

4.4 Global climatic changes and future prospects

The global temperature rises in the last century has been reported to have gone up by approx. ~0.5 ºC (Houghton et al., 2001; Houghton, 2005) and models have predicted increasing aridity of inland water bodies. The influence and magnitude of these changes in microbially mediated biogeochemical pathways remains unclear, with a risk in increased potent pathogens in response to global warming as predicted in more permanent systems (Abirami et al., 2021). However, microorganisms are good bioindicators of ecosystem reaction to drought induced stress (Peguero et al., 2021). The findings of this study (Chapter 3) demonstrated prevalence of drought tolerant species such as *Bacillus*, *Anaerolinea* and *Microviga*. The assessment of microbial community responses to drought provide an understanding of the reaction of temporary systems to climate change perturbations and support the establishment of new global system models targeted to predict climatic changes (Schimel, 2018).

This study outlined the importance of microbial mediation in EPS production and important biogeochemical processes in temporary wetlands. The data generated by the current investigation has valuable insights into microbial functions within arid zone temporary pans in the Global South. There is however a pressing need to extend the study in other provinces across the region, which are highly characterised by various types of temporary wetlands (Day et al., 2010). Future research should also investigate bound EPS using chemical extraction techniques such as cation exchange resin (CER) and ethylenediamine tetra-acetic acid (EDTA)

(Jahn and Nielsen, 1995; Wong et al., 2022). As these have demonstrated better yield and high recovery rate of EPS from biofilms (Hirst et al., 2003; Cyr and Morton, 2006; Gerbersdorf et al., 2009). Furthermore microbial assessments in aquatic systems can be more comprehensive by evaluating vertical profiles in assessing sediment EPS dynamics (Gerbersdorf et al., 2008). Although this study managed to evaluate bacterial EPS degradation by metagenome prediction, there is need to comprehensively quantify other EPS degradation pathways from wetlands habitats such as solubilisation and consumption.

4.5 Limitations of the study

The current study was unable to sample more than 2 periods due to financial and time constraints. Temporary wetland ecosystems are highly dynamic over their hydroperiod cycles (Wasserman et al. 2015) and future studies can assess how microbes respond at finer temporal resolutions, identifying succession patterns vital for thorough understanding of pan community dynamics.

As with any study, some caution must be exercised when interpreting these findings. Variation in DNA extraction techniques can influence both quantity and quality of recovered genetic material (Lombard et al., 2011). Despite high accuracy in short reads generated by NGS platforms, comparative studies have argued that long–read sequencing techniques produce high taxonomic characterization of species (Pearman et al., 2020). Furthermore, some difficulties persist in contrasting bacteria against other members belonging to same family which might share high degree of similarity in their 16S rRNA hypervariable regions (Jovel et al., 2016). Notwithstanding these challenges, the NGS remains the best method so far in sequencing technologies with high diagnostic accuracy, shorter turnaround time and identification of unculturable bacteria (Rizal et al., 2020). More so, the continuous development of bioinformatics pipelines such as DADA2 used in this study ensures maximum correction of sequencing errors hence improving the taxonomic resolution (Callahan et al., 2016).

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Plate 5.; Emerging plastic pollution of temporary pans in the Khakhea-Bray, North–West Province of South Africa; a potential threat to aquatic biodiversity in this region. Photo credit: Dr Chad Keates.

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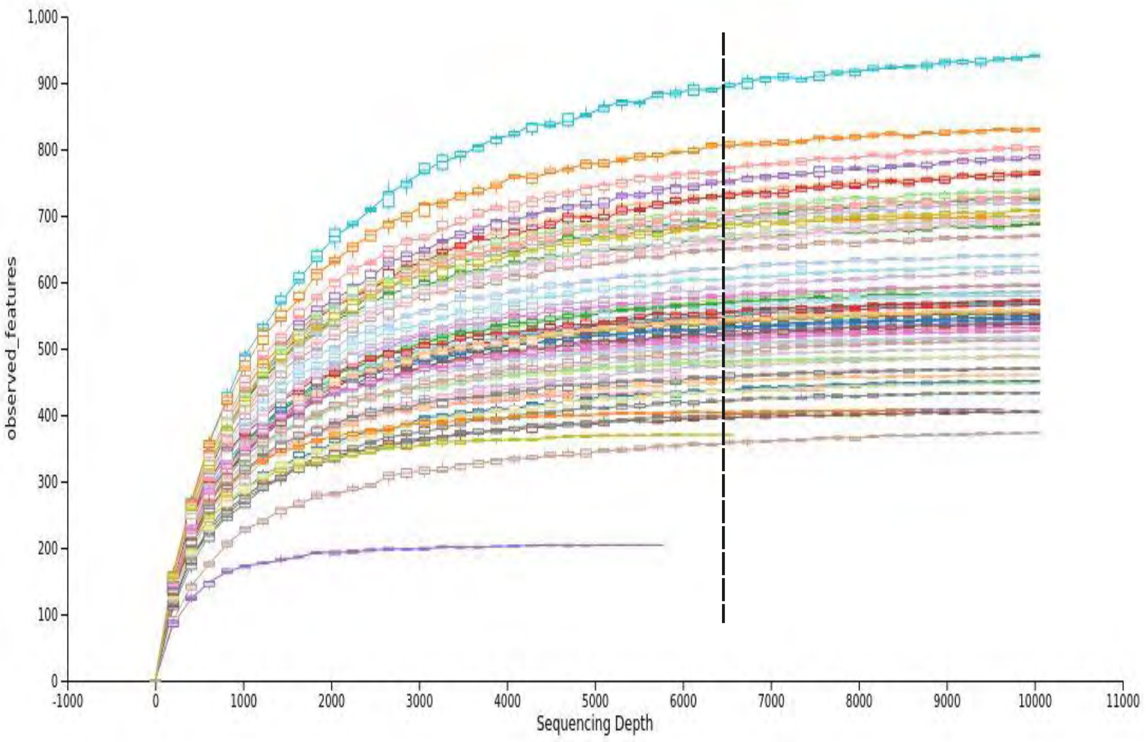
SUPPLEMENTARY MATERIAL



Supplementary Figure S1. A rarefaction curve plot showing the various sequencing depths of observed features in all samples. The sample DNA reads were rarefied to a maximum of 10 000 reads at 50–iterations. The even sampling depth selected for core–metrics phylogenetics was 6 533 reads denoted by the diagonal dashed line.

Supplementary Table S1. Geographical pan location in the Khakhea-Bray Transboundary Aquifer region (June 2021 and January 2022).

Pan	**Dry Season State**	**Wet Season State**	**Latitude**	**Longitude**
1	dry	wet	23.4645	-26.1439
2	wet	wet	23.4661	-26.1438
3	wet	wet	23.4754	-26.0646
4	wet	wet	23.4777	-26.0645
5	wet	wet	23.4735	-26.0656
6	dry	wet	23.4726	-26.0648
7	wet	wet	23.4811	-26.0525
8	dry	wet	23.4815	-26.0528
9	dry	wet	23.6014	-26.0209
10	wet	wet	23.5998	-26.0205

SUPPLEMENTARY TABLE

Supplementary Table S2. A representative Amplicon Sequence Variants (ASVs) table of twenty pans retrieved from microbiome analysis using DADA2 in QIIME2. Taxa were identified to species level were possible, p_ represent phylum, f_ family, g_ genus and s_ species.

Pan	Depth	State	Season	p__Bacteroidota; s__*uncultured_bacterium*	p__Verrucomicrobiota; f__Pedosphaeraceae	p__Proteobacteria; g__*Rhizobium*	p__Spirochaetota; g__*Spirochaeta*	p__Bacteroidota; g__*uncultured*
1	Deep	Dry	Dry	0	0	12	0	0
1	Mid	Dry	Dry	0	0	9	0	0
1	Edge	Dry	Dry	0	0	0	2	0
2	Deep	Wet	Dry	0	0	0	0	0
2	Mid	Wet	Dry	0	0	0	0	0
2	Edge	Wet	Dry	0	0	0	0	0
3	Deep	Dry	Dry	0	0	0	0	0
3	Mid	Dry	Dry	0	0	0	0	0
3	Edge	Dry	Dry	4	0	0	0	0
4	Deep	Wet	Dry	0	0	0	0	0
4	Mid	Wet	Dry	0	0	15	0	0
4	Edge	Wet	Dry	0	0	0	0	0
5	Deep	Wet	Dry	0	0	128	0	0
6	Deep	Wet	Dry	0	0	0	0	0

6	Mid	Wet	Dry	0	0	0	0	0
6	Edge	Wet	Dry	0	0	0	0	0
7	Deep	Dry	Dry	0	0	0	0	0
7	Mid	Dry	Dry	0	0	0	0	0
7	Edge	Dry	Dry	0	0	0	0	0
8	Deep	Wet	Dry	0	0	0	0	0
8	Mid	Wet	Dry	0	0	0	0	0
8	Edge	Wet	Dry	0	0	0	0	0
9	Deep	Dry	Dry	0	0	0	0	0
9	Mid	Dry	Dry	0	10	0	0	0
9	Edge	Dry	Dry	0	0	0	0	0
10	Mid	Wet	Dry	0	0	14	0	0
10	Edge	Wet	Dry	0	0	0	0	2
11	Deep	Wet	Wet	0	0	0	0	0
11	Mid	Wet	Wet	0	0	0	0	0
11	Edge	Wet	Wet	0	0	0	0	0
12	Deep	Wet	Wet	0	0	0	0	0
12	Mid	Wet	Wet	0	0	0	0	0
12	Edge	Wet	Wet	0	0	0	0	0
13	Deep	Wet	Wet	0	0	0	0	0
13	Mid	Wet	Wet	0	0	0	0	0

13	Edge	Wet	Wet	0	0	0	0	0
14	Deep	Wet	Wet	0	0	0	0	0
14	Mid	Wet	Wet	0	0	0	0	0
14	Edge	Wet	Wet	0	0	0	0	0
15	Deep	Wet	Wet	0	0	0	0	0
15	Mid	Wet	Wet	0	0	0	0	0
15	Edge	Wet	Wet	0	0	0	0	0
16	Deep	Wet	Wet	0	0	0	0	0
16	Mid	Wet	Wet	0	0	0	0	0
16	Edge	Wet	Wet	0	0	0	0	0
17	Deep	Wet	Wet	0	0	0	0	0
17	Mid	Wet	Wet	0	0	0	0	0
17	Edge	Wet	Wet	0	0	0	0	0
18	Deep	Wet	Wet	0	0	0	0	0
18	Mid	Wet	Wet	0	0	0	0	0
18	Edge	Wet	Wet	0	0	0	0	0
19	Deep	Wet	Wet	0	0	0	0	0
19	Mid	Wet	Wet	0	0	0	0	0
20	Deep	Wet	Wet	0	0	0	0	0
20	Mid	Wet	Wet	0	0	0	0	0
20	Edge	Wet	Wet	0	0	0	0	0