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Microbial community and extracellular polymeric substance dynamics in arid–zone temporary pan ecosystems

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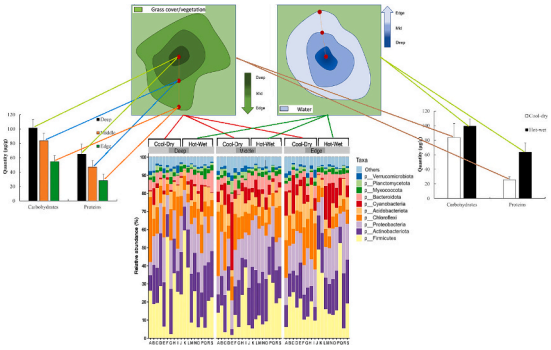
HIGHLIGHTS

- Carbohydrate concentrations exceeded protein, i.e., 2.8:1 (Wet Season) and 1.6:1 (Dry Season) suggesting EPS produced was largely diatom.
- EPS quantities in the hot–wet season was significantly higher than cool–dry season.
- Firmicutes, in particular characteristically thermo–tolerant *Bacillus* had the highest abundance accounting for 54%.
- Whilst the hot–wet season had high levels of denitrification and EPS production, the cool–dry season experienced low levels of nitrification.
- Extended dry periods are threatening to microbially mediated processes in arid-zone temporary pans.

GRAPHICAL ABSTRACT



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ABSTRACT

Microbial extracellular polymeric substances (EPS) are an important component in sediment ecology. However, most research is highly skewed towards the northern hemisphere and in more permanent systems. This paper investigates EPS (i.e., carbohydrates and proteins) dynamics in arid Austral zone temporary pans sediments. Colorimetric methods and sequence–based metagenomics techniques were employed in a series of small temporary pan ecosystems characterised by alternating wet and dry hydroperiods. Microbial community patterns of distribution were evaluated between seasons (hot–wet and cool–dry) and across depths (and inferred inundation period) based on estimated elevation. Carbohydrates generally occurred in relatively higher proportions than proteins; the carbohydrate:protein ratio was 2.8:1 and 1.6:1 for the dry and wet season respectively, suggesting that EPS found in these systems was largely diatom produced. The wet– hydroperiods (Carbohydrate mean 102 μg g^{-1}; Protein mean 65 μg g^{-1}) supported more EPS production as compared to the dry– hydroperiods (Carbohydrate mean 73 μg g^{-1}; Protein mean 26 μg g^{-1}). A total of 15,042 Unique Amplicon Sequence Variants (ASVs) were allocated to 51 bacterial phyla and 1127 genera. The most abundant genera had commonality in

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high temperature tolerance, with Firmicutes, Actinobacteria and Proteobacteria in high abundances. Microbial communities were more distinct between seasons compared to within seasons which further suggested that the observed metagenome functions could be seasonally driven. This study's findings implied that there were high levels of denitrification by mostly nitric oxide reductase and nitrite reductase enzymes. EPS production was high in the hot–wet season as compared to relatively lower rates of nitrification in the cool–dry season by ammonia monooxygenases. Both EPS quantities and metagenome functions were highly associated with availability of water, with high rates being mainly associated with wet– hydroperiods compared to dry– hydroperiods. These data suggest that extended dry periods threaten microbially mediated processes in temporary wetlands, with implications to loss of biodiversity by desiccation.

1. Introduction

A unifying character of all temporary pans is their cyclical nature, which involves alternating drying and inundation phases (Mitsch et al., 2009; Wasserman and Dalu, 2022). These features make them unique among aquatic ecosystems and facilitate the persistence of specialist aquatic fauna and flora, in addition to the generalist biota that inhabit these ecosystems (Bird et al., 2013). Microbes are no exception, with alternating drying and inundation phases supporting the coexistence of transient microbial species which are adapted to survive distinctively between the hydroperiods (Bouahim et al., 2014). However, there is a general scarcity of information on microbial community dynamics in the small temporary wetland literature globally. Intermittently inundated wetlands commonly referred to as temporary wetlands, 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). These systems, which includes the temporary pans of the Khakhea–Bray region, 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 (Meng et al., 2022; Rousk and Bengtson, 2014) and also secrete extracellular polymeric substances (EPS) (Pinto et al., 2020; Osemwegie et al., 2020). The major constituents of EPS are carbohydrates and proteins, which are predominantly secreted by diatoms and bacteria, respectively (Underwood and Paterson, 2003). The production of EPS by microbes is initially stimulated as an adaptation mechanism to the environment (Donot et al., 2012; Tuson and Weibel, 2013), whereby microorganisms secrete high–molecular weight biopolymers (Underwood and Paterson, 2003).

In the EPS matrix, carbohydrates support the extracellular storage of photosynthetically fixed carbon and provide structural support for sediments in the benthic zone through its alpha 1.6 glycosidic linkages (Gavande et al., 2023). Hence, biologically cohesive EPS contributes to stability of benthic sediments through inter–particle binding (Malarkey et al., 2015; Yallop et al., 2000). This necessitates the consideration of sediment organic cohesion levels which are influenced by various factors including variability in particle size and percentage of clay content (Grabowski et al., 2011). Proteins represent a vital nutritional food source in the benthic–pelagic food webs to aquatic invertebrates (Pennisi, 2002). Microbial EPS production remains a key component in facilitating nutrient cycling and removal of pollutants in aquatic systems (Kuang et al., 2023; Shao et al., 2023). Globally, there have been increased advances in sediment microbial ecology (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 cycling (Wu et al., 2024).

Freshwater wetlands are important habitats for the global carbon cycle, being capable of the sequestration and release of carbon (Moyo, 2022). Pan wetland systems maintain the carbon balance by mediating conversion of organic matter into dissolved organic carbon (Lam et al., 2007), a process highly mediated by various functional guilds of heterotrophic anerobic and aerobic bacteria, well adapted in these environments (Pester et al., 2012). However, there has been growing concern of unresolved challenges in understanding the interactions between bacterial communities and ecosystem functioning (Thukral et al., 2023; Widder et al., 2016). More specifically, the impact of episodic drying and rewetting phases of sediments in arid regions on microbial communities' diversity and function remain unclear.

In South Africa, pans are characteristically closed drainage basins (De Klerk et al., 2016). In these systems, organic matter accumulates in the sediment from various sources such as macroinvertebrates feeding, natural accumulation of terrestrial input, defecation, and death of aquatic life. In particular, the Khakhea–Bray region is predominantly dolomitic, with weathering supplying the greater part of the coarse sediments' characteristic of the region. Suspended particulate matter (SPM) is the major driver of downward flux for sediment leading to the formation of natural sediment flocs (Lawrence et al., 2022). Whilst, EPS acts as a traditional flocculant in promoting flocculation (Deng et al., 2023), increased OM content has been reported to correlate with floc size (Mietta et al., 2009).

Metagenomics have revolutionised the field of microbial ecology, through allowing the direct acquisition of genetic material from diverse environments including soil and water (Handelsman, 2004), with subsequent generation of molecular data which help in understanding diversity patterns and functional roles of microbes in aquatic environments (Huson et al., 2009). As such, the molecular characterization of bacteria found in the environment is now possible by targeting genes conserved between diverse taxa such as the 16S rRNA gene (Pearman et al., 2022). 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 (Bolyen et al., 2019; Schloss et al., 2009). The result is an ability to quantify microbial communities in a classic community ecology framework. The functional roles of microbial groups are of ever–increasing interest, and a focus of much research. Both classic approaches (e.g., shotgun metagenomics), and more recent metagenomic predictions such as BugBase (Ward et al., 2017), PICRUSt2 (Douglas et al., 2020) and Tax4Fun2 (Wemheuer et al., 2020) offer greater insights into how shifts in community dynamics might influence ecosystem functioning. Although PICRUSt2 infers microbial functions and does not rely on RNA sequencing data, its predictions are supported by large and reputable databases such as MetaCyc (Caspi et al., 2016) and the Kyoto Encyclopedia of Genes and Genomes (KEGG). This study employs a sequence–based metagenomic function prediction approach to address the knowledge gap on microbial communities and ecosystem functioning dynamics in temporary wetland ecosystems.

Pan wetland systems remain threatened habitats, especially in arid areas. With limited knowledge on the impacts of increased temperatures and irregular rainfall patterns on microbes in arid pan ecosystems because of climate change, this study aimed to i) quantify and assess seasonal and spatial distributions of major EPS constituents

(carbohydrates and proteins) present in sediment biofilms ii) examine seasonal and spatial microbial diversity patterns and iii) predict and compare microbiome functions central in biogeochemical pathways for temporary wetland ecosystems. It was hypothesised that there would be i) an increased yield of EPS in the wet phases (wet hydroperiod and hot–wet season) in comparison to the dry phases (dry hydroperiod and cool–dry) as sediment water content directly impacts nutrient availability for EPS producing microorganisms (ii) hot–wet season would have higher microbial richness and diversity than the cool–dry season, due to increased sediment water content which supports proliferation of hydrated biofilms, and (iii) microbial functions related to isolated bacterial communities would be higher in the deeper zones (inundated for longer) and wet phases, as these zones have dry out later than the shallowest edges and will therefore retain a higher water content for longer.

The present paper aims to address the following questions: What are the major microbial taxa found inhabiting arid pan wetland ecosystem? Are the seasonal and spatial distributions of EPS (carbohydrate and protein) in temporary pan systems comparable to more permanent systems such as rivers and oceans? What are the characteristic pathways and specific enzymes likely driving temporary pan wetland biogeochemistry?

2. Materials and methods

2.1. Study area

This study was conducted in the Khakhea–Bray Transboundary Aquifer (KBTA) region (25.880°S, 23.959°E) in the North–West Province of South–Africa (Table S1). 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 which lies on outcropping dolomite on South African side of the border. The KBTA lies on top of dolomitic aquifer which extends an area of above 5000 km^2. Rainfall is the major source of recharge especially in the summer (November–March) and ranges from 100 to 920 mm annually with rates of evaporation ranging as high as 2000–2250 mm per year (Godfrey and van Dyk, 2002).

Sampling of pans was performed from the 23rd – 27th of June 2021 (cool–dry season) and 9th – 14th of January 2022 (hot–wet season). A total of ten pans of similar size and shape were randomly sampled from the South African side of the transboundary region. Majority of pans had proximity to the main roads suggesting possible exposure to human usage. In cool–dry season, six pans contained water (W1–W6), and four pans were found dry (D1–D4) (Table S1).

2.2. Sediment collection

Sediment was collected using a modified 20 mL graduated plastic disposable syringe as a corer (Lubarsky, 2011). At each pan, a transect was generated from the deepest point in the depression to the edge of the depression. Sample points along each transect were set at deepest point (deep), middle of the deepest and edge of pan (middle), and at the edge of each pan (edge) based on estimated elevation. At each sampling point within each pan, triplicate sediment cores were collected within 10 cm radius of each other by vertical insertion up to 4 cm deep into the ground. In the field, the samples were stored in a cooler box with ice prior placement into a chest freezer. The upper 3 cm of each replicate core was homogenized and subsetted for DNA extraction and analyses of EPS, water and organic matter content determination.

2.3. DNA extraction and quality control

Using a sterilised scalpel knife, a 220 mg subsample of each homogenized sediment core was used for the extraction of genomic DNA using the E.Z.N.A® Soil DNA Kit (Omega Biotek, Norcross, USA) following the manufacturers protocol. The blade was flame sterilised between successive cuts of sediment cores belonging to different sites. All DNA extraction protocols were carried out in a laminar flow hood. The v3–v4 region of the bacterial 16S rRNA gene was amplified following the "Illumina 16S Metagenomic Sequencing Library Preparation guide". The quality and quantity of DNA was determined using nanodrop 1000 spectrophotometer. The sequencing of the prepared metagenome library was conducted by Aquatic Genomics Research Platform (AGRP) at the South African Institute of Aquatic Biodiversity (Grahamstown, South Africa) using 300 bp paired–end sequencing on an Illumina MiSeq using v3 chemistry. Three replicates were sequenced for each environmental sample. Sequence data were deposited and are publicly available in the NCBI Sequence Read Archive (SRA) under the BioProject ID PRJNA1083343.

All data curation steps of raw reads were carried out within Quantitative Insights Into Microbial Ecology (QIIME2) v2021.8 (Bolyen et al., 2019) which include filtering, trimming, dereplication, merging of paired end reads, denoising and removal of chimeras using DADA2 (Callahan et al., 2016). Taxonomy was assigned to the Amplicon Sequence Variance (ASVs) feature table at 99 % similarly index using the SILVA database v132 (Quast et al., 2013) and a naïve bayes classifier. Taxa based filtering was used to remove sequences assigned to archaea, chloroplasts, mitochondria and eukaryotes.

Alpha and beta diversity measures were conducted within QIIME2 on rarefied data at a sequencing depth of 6533, where accumulation curves of samples had plateaued (Fig. S1). Weighted and unweighted Unifrac distance matrices were integrated into R using the phyloseq package (McMurdie and Holmes, 2013). Microbial community function was predicted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2; Douglas et al., 2020), performed using the QIIME2 plugin. The PICRUSt2 output used here consisted of predicted enzyme commission (E.C) numbers, with a subset selected on their relevance to microbial production in intertidal sediments and biofilms by (Wyness et al., 2021) using the inferred MetaCyc pathways (Caspi et al., 2016).

2.4. Determination of sediment water content and organic matter content

A subsample of approximately 2.5 g of each homogenized sediment core was measured into clean pre–weighed 2 mL centrifuge tubes prior to freeze drying for 48 h at −48 °C at 0.014 mBar (Labconco 4.5 plus freezone benchtop freeze dryer). Tubes were tightly covered with GF/C filter paper 1.2 μm (Whatman® glass microfibre filters) 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. A 1 g subsample of the freeze–dried sediment was then weighed into pre–weighed clear glass vials for determination of organic matter content (OM content). The glass vials were furnaced at 505 °C for 5 h and re–weighed for establishment of OM content by loss on ignition.

2.5. Sediment carbohydrate content

Extracellular polymeric substances were extracted by measuring 1 g of freeze–dried sediment into 1 mL deionised water and incubating at room temperature for 15 min. All reactions were performed in triplicate glass test tubes. Carbohydrates were analysed using a method similar to Taylor and Paterson (1998). In a glass test tube 107.1 μL of extracted EPS supernatant was pipetted, followed by equal volume of 5 % (*w/v*) phenol (Whitehead Scientific, South Africa), and then 535.8 μL of concentrated sulphuric acid (Acechem, Johannesburg), and the solution homogenized by pipetting. The tubes were then incubated for 40 mins at 30 °C before the absorbance at 486 nm was determined using a spectrophotometer (Genesys 20, Thermo Scientific, UK). Carbohydrate quantities are presented as μg g^{-1} glucose equivalent. Further details of analyses are provided in (File S1).

2.6. Sediment protein content

Protein content was determined using the modified Lowry procedure (Lowry et al., 1951). A 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 124 mM $Na_2C_4H_4O_6$ (Acechem, South Africa) in a 100:1:1 ratio. In a glass test tube, 144 μL each supernatant of extracted EPS was added, followed by 144 μL of SDS 2 % (Life technologies, USA) and incubated for 15 mins 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) (diluted 5:6 with deionised water) were added, and the solution homogenized by pipetting. Tubes were then incubated for 50 mins at 30 °C before the absorbance at 750 nm determined using a spectrophotometer (Genesys 20, Thermo Scientific, UK). Protein quantities are given as μg g^{-1} bovine serum albumin equivalent. Further details of analyses are provided in (File S1).

2.7. Data analyses

To achieve parametric assumptions of normality and homogeneity of variance, EPS data were log_{10} transformed, and OM content and water content data were arcsine square–root transformed (Agrawal and Kotanen, 2003; Dobbs et al., 2022). Two–way Analysis of Variance (ANOVAs) were performed in R version 4.2.1 (R Core Team, 2022) using R Studio to test for differences in EPS constituents, water content and OM content between treatments. Additionally, Tukey's HSD test was also performed to evaluate depth specific variations suggested by ANOVAs. Statistical significance between treatments was assessed using the non–parametric Kruskal–Wallis within QIIME2 and beta diversity was evaluated by PERMANOVA in QIIME2. The generalized linear model (GLM) was fitted in R using the inverse Gaussian distribution for Shannon Weiner–diversity index as the data did not conform to normality. However, the Gaussian distribution was employed for Chao1 and ACE indices with site and season as predictors. Jaccard and Bray Curtis distance matrices were created in QIIME2 to evaluate microbial beta diversity patterns.

3. Results

To assess EPS concentrations, colorimetric methods of carbohydrate and protein assays (Dubois assay and modified Lowry procedure respectively) were employed. Furthermore, sequence–based metagenomics was employed to characterise microbial communities and predict their functions using bioinformatics pipeline QIIME2 (Bolyen et al., 2019) and PICRUSt2 (Douglas et al., 2020).

3.1. Temporal dynamics in extracellular polymeric substances and sediment

Carbohydrate content ranged from 23 μg g^{-1}–145 μg g^{-1} and existed in high concentrations for both seasons, in the cool–dry season (mean 73 μg g^{-1}) and in the hot–wet season (mean 102 μg g^{-1}) (Table S2). Protein content ranged from 2 μg g^{-1}–84 μg g^{-1} proportionally occurred in low concentrations mean (26 μg g^{-1}) in the cool–dry season and mean (65 μg g^{-1}) in the hot–wet season. There was a significant difference between the two seasons in the production of carbohydrates (ANOVA: $F_{1,18} = 5.25$, $P = 0.034$) and proteins (ANOVA: $F_{1,18} = 13.640$, $P < 0.001$) (Fig. 1A; Table S3). Carbohydrate and protein shared similar strong correlations with sediment water content (Supplementary Table S6). The variation in mean sediment water content ranged from 7 to 18 % between cool–dry and hot–wet seasons, with higher water content being recorded in the wet season (ANOVA: $F_{1,18} = 19.21$, $P < 0.001$) (Fig. 1B). Organic matter content did not vary significantly across the seasons

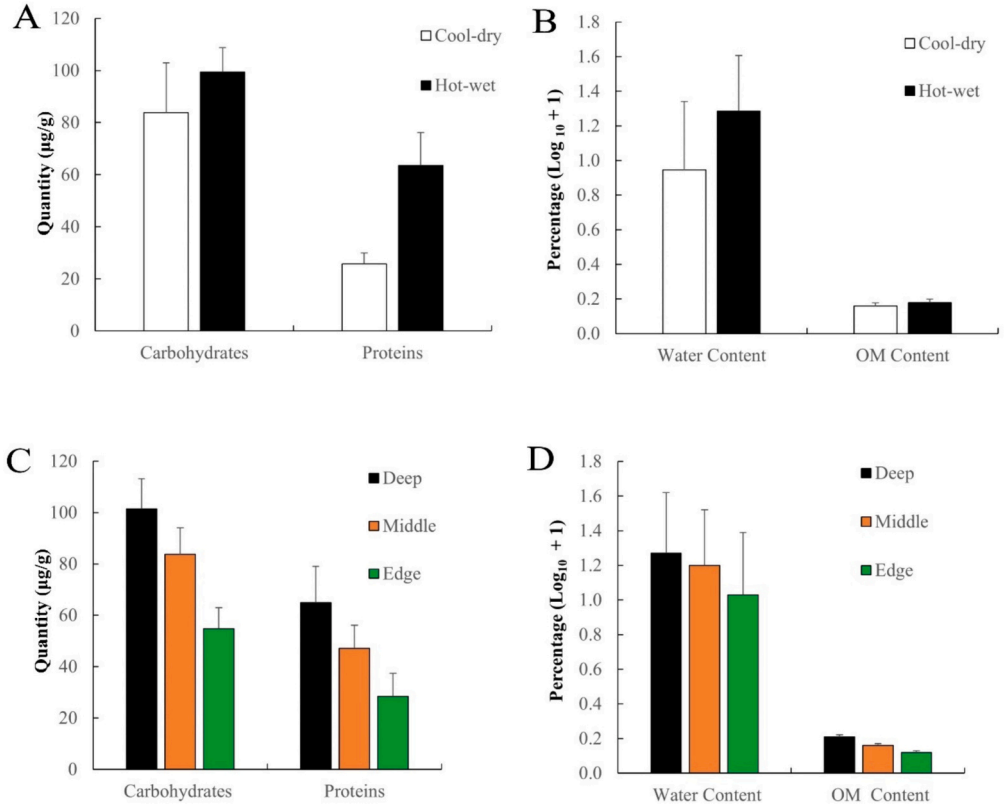

Fig. 1. Mean temporal variation (A, B), and spatial variation (C, D) in extracellular polymeric substance content (A, C); water content and organic matter (OM) content (B, D) expressed as $log_{10} + 1$ transformed data. Carbohydrates and proteins were measured by spectrophotometry (File S2) and are expressed in glucose and Bovine Serum Albumin (BSA) equivalents, per dry weight sediment (μg g^{-1} *DWt*). Error bars represent standard error around the mean.

(ANOVA: $F_{1,18} = 0.512$, $P = 0.088$) (Table S4).

3.2. Spatial variation in microbial extracellular polymeric substances and sediment properties

Carbohydrates and proteins followed a similar trend to each other, where high quantities of both were recovered from the deep and decreased towards the edge (Fig. 1C). The carbohydrate quantities found were significantly different across sites (ANOVA: $F_{2,57} = 8.62$, $P < 0.001$) with the deep and middle having higher quantities compared to the edges (Tukey HSD, $p < 0.001$) (Fig. 1C). However, no differences in carbohydrates were found between the deep and the middle (Tukey HSD: 0.396). Protein content differed significantly between sites (ANOVA: $F_{2,57} = 5.80$, $P = 0.005$), where the outer sites had significantly less protein content than the deep (Tukey HSD: $p = 0.006$) and middle had more than the edge (Tukey HSD: $p = 0.034$), however there were no differences in protein content between the middle and deep ($p = 0.795$) (Fig. 1C).

With reference to sediment properties, the deep, middle and edge systematically followed a decreasing order starting from the deep to the edge in relation to both water content and organic matter content. The variation in water content was significant across sites (ANOVA: $F_{2.57} = 10.40$, $P < 0.001$) where lower percentages existed at the edge compared to the deep (Tukey HSD: $p < 0.001$) and middle (Tukey HSD: $p = 0.003$) (Table S5), with no significant differences between the deep and middle (Tukey HSD: $p = 0.667$).

Organic matter (OM) content ranged from 0.2 to 0.8 %, with significant differences between sites (ANOVA: $F_{2,57} = 20.83$, $P < 0.001$). The outer had significantly less OM content compared to the deep (Tukey HSD: $p < 0.001$) and middle (Tukey HSD: $p = 0.014$). The deep had more OM content than the middle (Tukey HSD: $p = 0.002$) (Fig. 1D).

3.3. Bacterial community structure

A total of 15,042 unique Amplicon Sequence Variances (ASVs) were observed across all samples from 692,162 rRNA sequence reads. Of these, the most dominant bacterial phyla included, Firmicutes (39 %), Actinobacteria (24 %) and Proteobacteria (24 %) (Fig. 2). At genus level, thermophilic bacteria were found to be highly abundant within top 20 (Fig. S5) and having high relative abundances of *Bacillus* at 54 % and *Rubrobacter* at 16 %.

3.4. Microbial diversity patterns

3.4.1. Alpha diversity

There were no significant differences in alpha diversity between the cool–dry season and hot–wet season (Fig. 3), Shannon–Weiner: t(52) = 1.04, $p = 0.30$ and Chao1: t(54) = −0.68, $p = 0.50$. However, ACE diversity index suggested slightly more richness in the hot–wet season compared to dry season, t(52) = −2.16, $p = 0.04$. There were site specific differences (Fig. 3) with the middle of pans having more diversity and richness than the deep zones (Shannon–Weiner: t(52) = −2.27, $p = 0.03$ and ACE: t(52) = 2.07, p = 0.04).

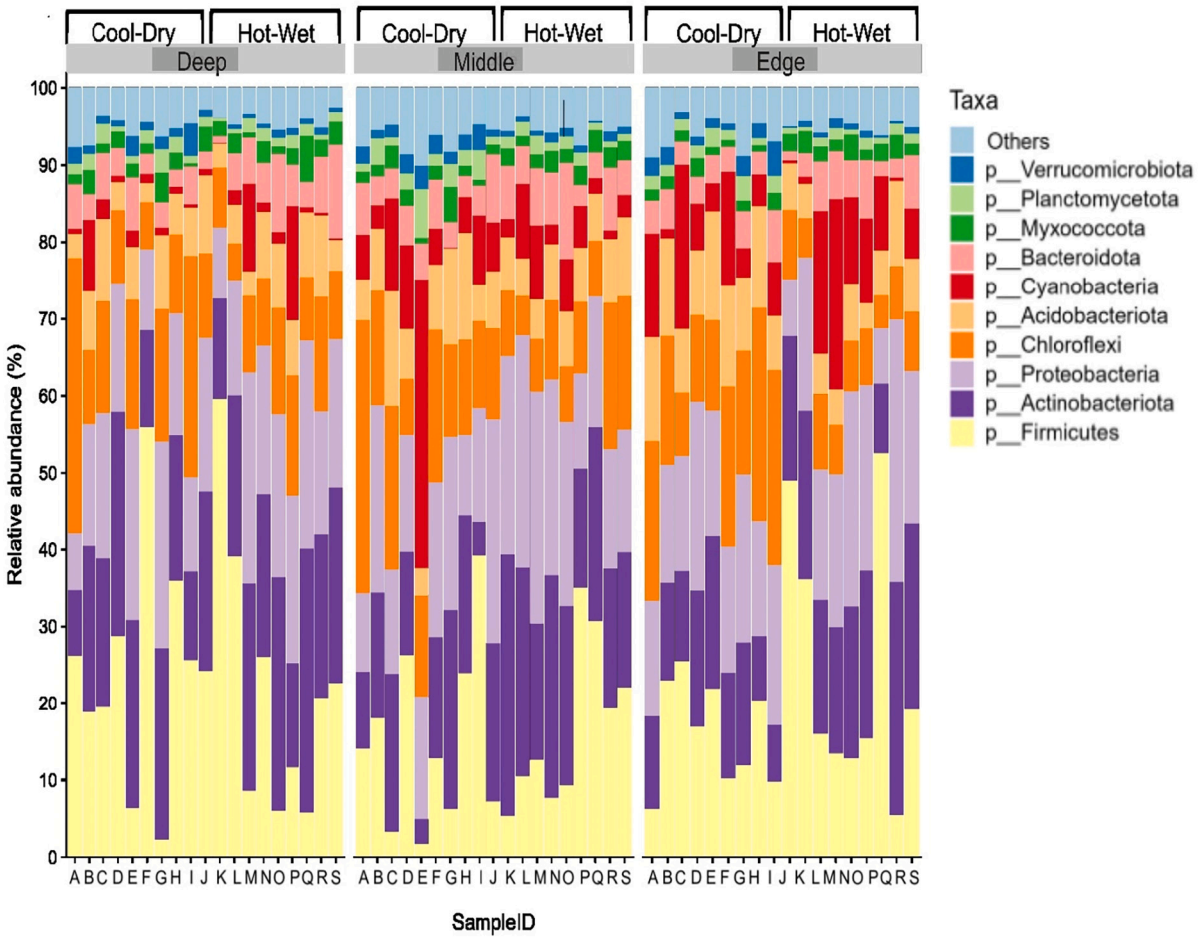

Fig. 2. Taxa bar plots highlighting the distribution of microbial community composition at phylum level for taxa with relative abundances >4 % found in the Khakhea–Bray region pan ecosystems in cool–dry season (June 2021) and hot–wet season (January 2022). Deep, middle, and edge represent the sites sampled within each pan.

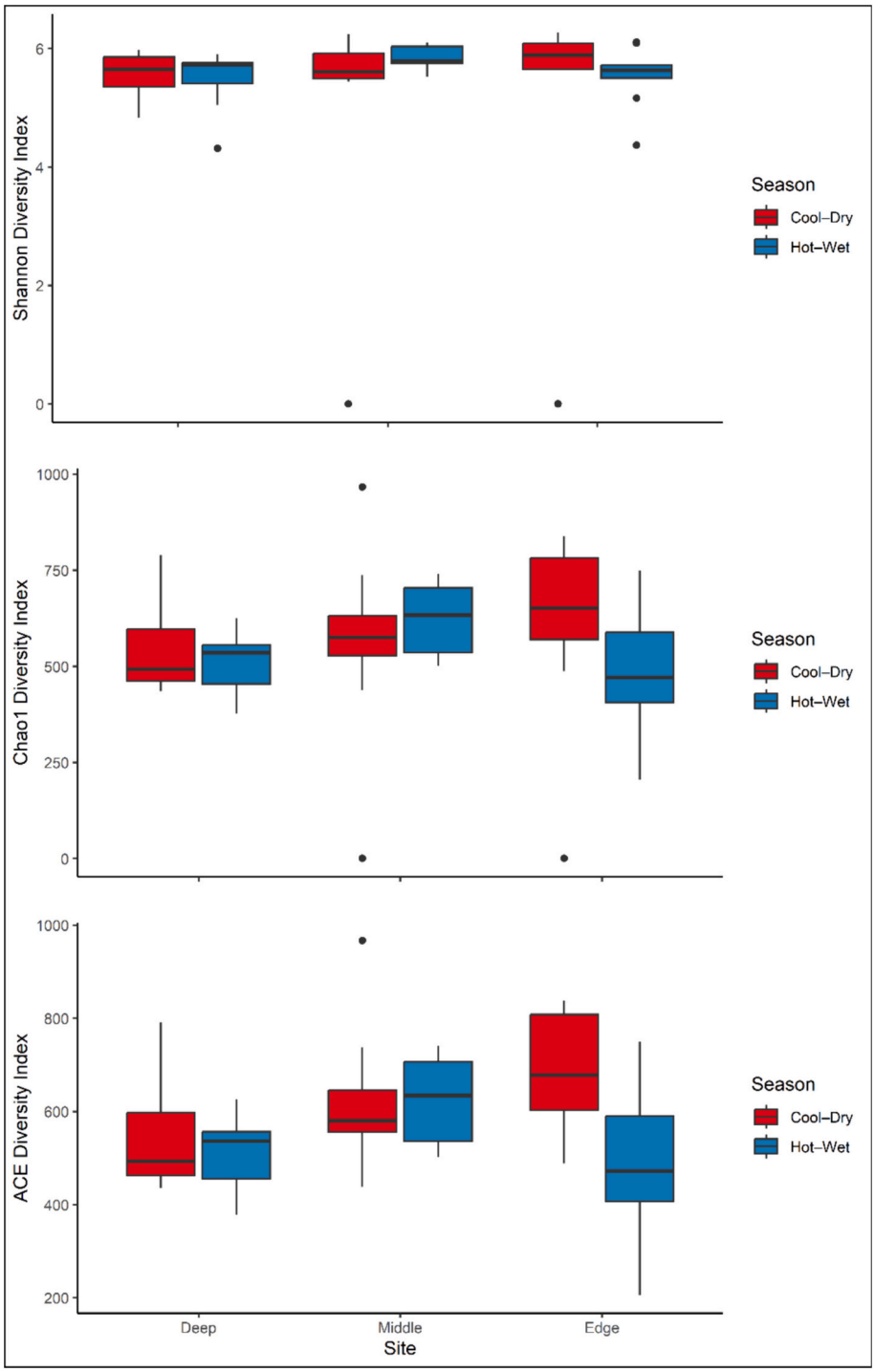

Fig. 3. Alpha diversity box plots indices showing variations in Shannon diversity index, Chao1 diversity index and ACE diversity index between seasons and across sites in the Khakhea–Bray region temporary pan ecosystem in cool–dry season (June 2021) and hot–wet season (January 2022). Supplementary File S2 provide detail on the analyses.

3.4.2. *Beta diversity*

The seasonal differences in microbial communities were significant based on weighted unifrac (PERMANOVA, 999 permutations, Pseudo–F = 5.742, $p = 0.001$) and unweighted unifrac distances (PERMANOVA, 999 permutations, Pseudo–F = 2.561, p = 0.001) (Fig. S3). 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 whilst incorporating phylogenetic distance. In addition, the differences were observed across depth (Fig. S4), weighted unifrac (PERMANOVA, 999 permutations, Pseudo–F = 1.884, $p = 0.013$), unweighted unifrac (PERMANOVA, 999 permutations, Pseudo–F = 1.556, p = 0.001). Jaccard and Bray Curtis distance matrices also demonstrated that microbiomes differed significantly across depth and seasons ($p < 0.05$).

3.5. *The microbiome functions*

During the wet season, inundated pans were exclusively dominated

by the following functions ranging from the deep to the shallow edges: (i) denitrification by nitrite reductase (E.C:1.7.1.15), 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). Processes known to be important in wetland biogeochemistry, but found in relatively lower abundances during this study, were 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 among inundated pans in the hot–wet season, with high abundance at the deep zones for EPS degradation (E.C:3.2.1.21) and nitrogen mineralization (E.C:1.4.1.2). For the middle regions EPS degradation (E.C:3.2.1.8), denitrification (E.C:1.7.2.4) and DNRA (E.C:1.7.7.2) were in high abundance. The EPS production (E.C:24.1.12) and denitrification (E.C:1.7.2.5) were high in the shallow regions.

The dry season (dry hydroperiods and cool–dry season) was defined by a different set of enzymes which suggested a shift in the microbial community composition responding to seasonality changes. However, there were fewer microbial functions in high abundance during the dry season than in the wet season. The deep zones to the shallow regions of pans in the dry season supported EPS degradation (E.C:3.2.14), nitrogen mineralization (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) (Fig. 4). 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) (Fig. 4).

4. Discussion

Despite temporary freshwater wetland pans being renowned for high levels of productivity during wet–hydroperiods (Arnott and Vanni, 1993; Waterkeyn et al., 2008), little information is available on microbial and EPS productivity dynamics in these systems. This study compliments the on–going research around EPS sediment ecology; the results presented here show an increased production of EPS by benthic biofilms in the hot–wet season compared to cool–dry season, supporting the first hypothesis. An increased yield of EPS in the wet phases (combination of wet hydroperiod and hot–wet season) in comparison to the dry phases (dry hydroperiod and cool–dry season) as a result of presumably low sediment water content reducing the activity of EPS producing microbes.

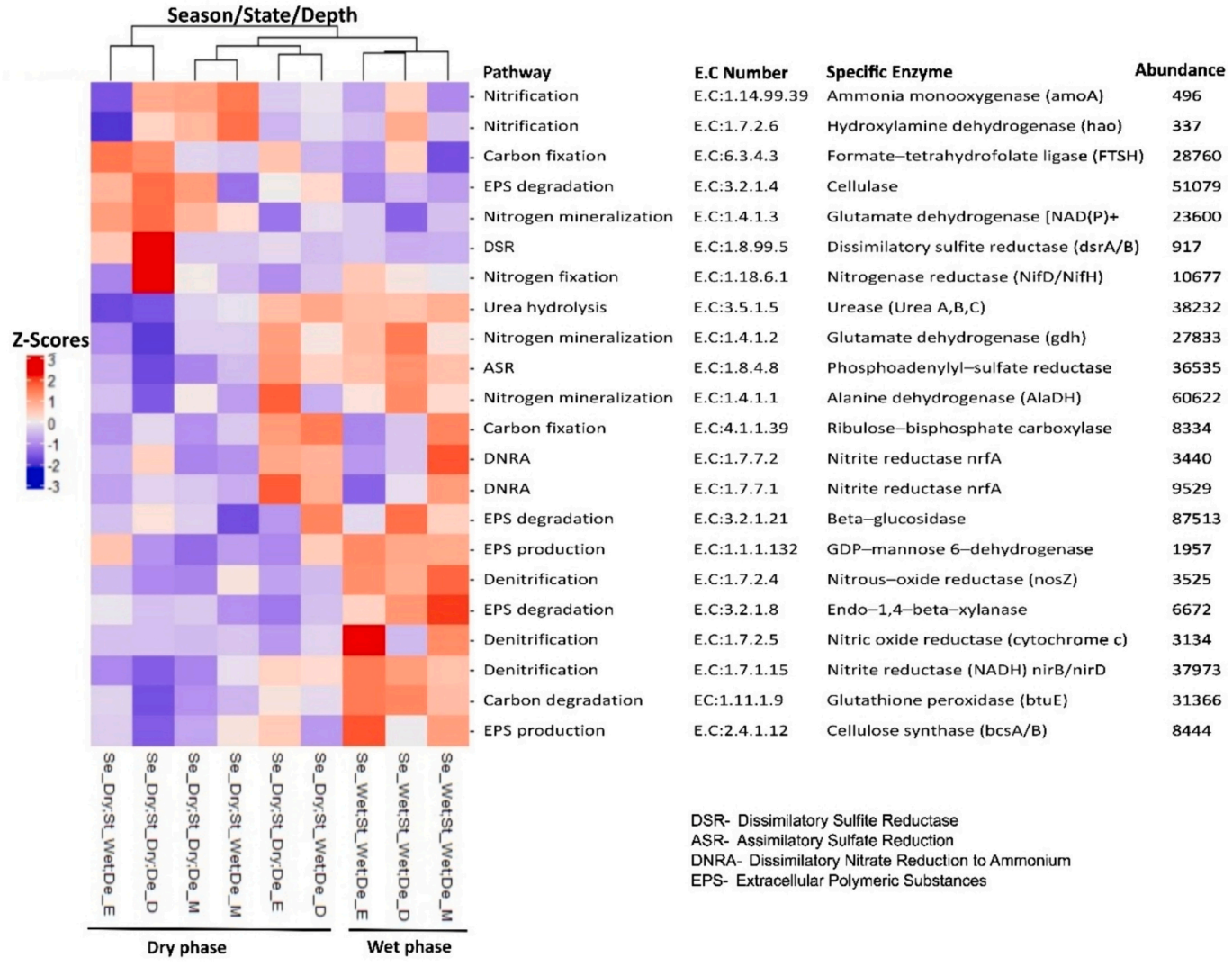

Fig. 4. A heatmap plot of relative abundances Z–scores, highlighting functional clustering of predicted metagenomes as represented by Enzyme Commission (E.C) numbers using PICRUSt2. Colour intensities represent Z–scores of enzymes ranging from the lowest blue to the highest red. The metagenome functions associated with E.C numbers were predicted using MetaCyc database. Dry phase represents (cool–dry season and dry hydroperiods) whereas wet phase represents (hot–wet season and wet–hydroperiods).

4.1. Extracellular polymeric substances seasonal and spatial variation

Extracellular polymeric substances are biosynthetic polymers, secreted by microbes (Flemming et al., 2007). The adhesion of microbial cells to surfaces activates transcription of EPS encoding genes (Dunne, 2002). Carbohydrates are the major diatom–produced constituents in EPS (Daly et al., 2023), whereas bacteria produce a higher protein content. Carbohydrates were consistently higher in proportion than proteins across seasons i.e., ratio of 2.8:1(dry–season) and 1.6:1 (wet–season). 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. Furthermore, these results confirmed changes that EPS go through over time as a function of microbial communities response to drying and rewetting phases as well as varying water levels in pans. Carbohydrates concentrations retrieved in this study by using water extraction method were comparable to more permanent systems (70–300 μg g^{-1} DW) (Friend et al., 2003). In addition, the increased yield of EPS in the hot–wet season correlated with sediment water content as also reported for permanent systems (Gerbersdorf et al., 2009). However, there may have been other factors drivers behind the increased EPS concentrations in the wet–season, such as chlorophyll–a concentration and light intensity (Loustau et al., 2021).

The variation of OM content with depth reported in this study, as previously observed (Du et al., 2022) could be a function of differences in sediment composition, influenced by different rates of microbial communities productivity and decomposition activities (Reddy and Patrick, 1975). Deep regions are likely to have high OM content as a result of these regions holding water for longer, therefore, with time, aquatic life migrates to the center and dies off (Bird et al., 2013). This migration is driven off by changes in water level dynamics and the amount of water present in a pan at a given point is highly dependent on factors such evaporation rates and human abstraction which include domestic and agricultural. Similarly, more vegetation grows in these saturated zones and their cumulative death will also result in OM accumulation (Wantzen et al., 2008).

The erratic rainfall patterns and high evaporation rates in Khakhea–Bray region, 107–928 mm and 2050–2250 mm per annum, respectively (Altchenko and Villholth, 2013), would likely affect the EPS productivity dynamics in the pan ecosystems. Previous research has indicated that, water content supports strong positive correlations with EPS (Gerbersdorf et al., 2009). Indeed, all shallow regions (edge) had the lowest quantities of EPS despite time of the season. This implies that even during wet–hydroperiods and high–water levels, EPS production at the edge do not match the deep in semi–arid temporary pans. High evaporation rates results in early desiccation of the edge, with subsequent low diversity and biomass in key EPS producing microorganisms, such as diatoms (Souffreau et al., 2010) and bacteria (Baldwin and Mitchell, 2000). In this way, the majority of pans have been limited in their ability to produce more EPS at the deep regions where water accumulates for longer.

Laboratory experiments have shown that microbial EPS of sufficient density can enhance stability of intertidal sediments (Paterson et al., 1990). The role of organic cohesion in relation to purely cohesive and naturally mixed sediment bedforms have been extensively studied (Parsons et al., 2016; Schindler et al., 2015). Although untested in this study, an increase in EPS is very likely to increase erosion thresholds of pan sediment. Therefore when there is high rainfall and water flow, such as during flood events, sediments and nutrients are not lost from the system. However as demonstrated in this study, if the pan dries–up, EPS quality and quantities diminishes. Therefore it is postulated that with more complete and frequent drying out due to the effects of climate change, erosion of sediments in the area may worsen, excacerbated by the intensity of intermittent flooding events.

4.2. Microbial communities' diversity and their functional profiles

Overall examination of the alpha diversity patterns suggested that neither diversity nor richness differed significantly between either seasons, despite ACE suggesting slightly more richness in the hot–wet season. Therefore, the second hypothesis was rejected, which stated that microbiomes in the wet season would have higher diversity. However, community composition differed significantly between seasons and depths, as outlined by beta–diversity patterns confirming previous findings in similar studies (He et al., 2023; Lu et al., 2021). More permanent systems were influenced less by dry–wet seasonal changes of the lake despite the sampling site (Liu et al., 2023), suggesting that microbial communities in temporal systems might be more sensitive to water level fluctuations than permanent systems.

Microbial functions were highly elevated for the hot/wet season compared to the cool/dry season, and higher at the deep than the middle and edge, partially confirming our third hypothesis which predicted that metagenome functions where to seasonally correlate, with higher functions at the deeper areas which are inundated for longer. These findings are however contradictory from (Ren et al., 2019), who asserts more functions in the dry season from permanent systems associated with water level fluctuations.

The sediment microbiome drives several metabolic processes in the freshwater habitats, including elemental cycling (Underwood et al., 2022) and decomposition of organic matter (Raza et al., 2023). The high abundances of Firmicutes, Actinobacteria and Chloroflexi largely represented by *Bacillus, Rubrobacter* and *Anaerolineae* respectively, contradicts Parihar et al. (2022) who found dominance of Proteobacteria. This study confirmed dominance of heat and arid resilient microbial taxa and these revelations corroborate (Kaestli et al., 2019) in central Australian arid–land temporary water bodies. The thermophilic adaptation of the organism mentioned above will allow each group to become prolific in the ecosystem as they can tolerate the increased temperatures associated with Khakhea–Bray region in the North–West province, reported to range from 22 to 36 °C in hot summer seasons and often coupled with a series of heat waves in the region (Mbokodo et al., 2022). Additionally, this may demonstrate some resilience in the microbial community function of temporary pans to the increasing global temperatures (Harris et al., 2023).

The high prevalence of *Bacillus* may be a result of its ability to produce heat resistant endospores (Checinska et al., 2015; Nicholson et al., 2000). Furthermore, *Bacillus* species occupy prominent roles in nitrogen cycling, which include ammonification (Hui et al., 2019), nitrification and denitrification (Bachar et al., 2012; Verbaendert et al., 2011), possibly contributing to increased organic inputs addition into the pans. The genus *Rubrobacter* can mineralize sulphate compounds (Vikram et al., 2016) and participates in the global carbon cycle through organic matter decomposition (Araujo et al., 2020). However, *Anaerolinea* is involved in sediment aggregate formation and EPS secretion, and is critical for nitrogen removal in aquatic systems by anammox (Zhao et al., 2019). All the other species which were observed to occur less prominently contribute to a healthy status of KBTA pan ecosystem as part of the food web. The contribution of low abundance species found in this study is in fact very recognisable, as these communities are increasingly gaining attention in material and energy recycling (Jousset et al., 2017).

The differences observed in beta–diversity demonstrates that distinct types of microbial communities exist across seasons and among depths. Seasonal dynamics had a large influence in shaping bacterial communities. High microbial diversity in the hot–wet season supported more biogeochemical functions of high abundance relative to the to cool–dry season. The cyclical variations in sediment water content driven by seasonal changes might have influenced microbial community composition, with modification in metagenomic functions, such as high rates of sulphate reduction by sulphate–reducing bacteria in extremely dry phases. These findings contradict Weingarten et al. (2020) who argues

that bacterial composition did not differ between inundated and desiccated states of a salt–pan in the Mississippi Gulf Coast, USA.

Most predicted metagenome functions were in greater relative abundance during the hot/wet season and differed within inundated pans which exhibited different levels of pond filling. These findings strongly suggest the importance of both wet–hydroperiods and maximum pond filling. Denitrification, EPS production and its degradation were relatively higher on edge and middle regions, compared to the deep. Whilst Gemmatimonadetes, Nitrospira, Rubrobacter and *Bacillus* are implicated in the denitrification process mentioned above, Proteobacteria and Bacteroides found to dominate the middle (Fig. S2) 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). In summary, the middle demonstrated higher retention of taxa abundance as compare to the edge and the deep (Fig. S2), suggesting potential target areas for rapid assessments in the event of continuous monitoring of these systems.

A different set of enzymes dominated the cool–dry season, which mediated processes including nitrification and nutrient mineralization, albeit at relatively lower functional abundances than the wet season. This change in microbial community function demonstrates the cyclical nature of temporary systems which have been reported to support distinct microbial communities in respective seasonal times (De Nijs et al., 2019; Fromin et al., 2010). Functional depth specific variations in extremely dry phases such as high rates of DNRA by nitrogen fixation at the deep demonstrate the impact of low rainfall and high evaporation rates on pan ecosystems. These sites are likely hypersaline in nature and the high evaporation rates of water from the sediment in these zones 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, with an estimated over 50 % contribution to organic carbon mineralization in aquatic sediments (Mußmann et al., 2005; Crowe et al., 2014).

Biofilms are often characterised by mixed communities of multiple species (Davey and O'toole, 2000). Functional communites interact synergistically (Madsen et al., 2016), Although many of the patterns and mechanisms of such interactions remain cyrptic. This study speculates that seasonal dynamics drive certain synergistic interactions in the denitrification process, as it has been established that denitrifying bacteria reduce nitrate and also can provide nitrite for anammox bacteria (Wang et al., 2022). Furthermore, Wang et al. (2024) support that, synergism between DNRA microorganisms and anammox bacteria contributes to the complete denitrification process.

5. Conclusion

Overall, this study highlighted that microbial and EPS productivity dynamics are highly dependent on water availability, which contributed to the spatial and seasonal variations observed in the Khakhea–Bray temporary pan ecosystem. This study provided evidence that, colloidal EPS in semi–arid pan sediments ranged from 23 to 145 μg g^{-1} for carbohydrates and 2–84 μg g^{-1} for protein. With forecasted climate change signifying irregular hydroperiods, and less volume and frequency of rain, these effects will gradually degrade EPS concentrations. This investigation also substantiated depth–specific variations in the distributions of organic matter content in semi–arid temporary pans (ranging 0.2–0.8 %), with high content observed to occur at the deepest areas of pans. Furthermore, the present study provided metagenomic evidence for the important role of temporary pans in global biogeochemical cycling. These functions, which include denitrification, demonstrated high association with the rainy season and wet–hydroperiods.

The differences in functional microbial communities outlined by this study produced evidence of generally thermo–tolerant bacteria inhabiting pan ecosystems. 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 due to the ongoing temperature rises which might alter bacterial biomass. To achieve complete microbial characterization of arid ecosystems, future work aims to explore fungi, protozoa and archaea communities. More so, employing advanced techniques such as fourth–generation sequencing (Ari and Arikan, 2016) to explore viral genomics, specifically quantifying the impact of macrophages on bacterial communities, and investigating both short and long time-scale responses of microbial communities to inundation using RNA sequencing.

CRediT authorship contribution statement

Tafara F. Bute: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Adam Wyness:** Writing – review & editing, Supervision, Software, Resources, Methodology, Conceptualization. **Ryan J. Wasserman:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Farai Dondofema:** Project administration. **Chad Keates:** Writing – review & editing, Validation. **Tatenda Dalu:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare no known competing financial interest or personal inter–relationships that could have an influence on the work hereby conducted for this paper.

Data availability

Sequence data were deposited and are publicly available in the NCBI Sequence Read Archive (SRA) under the BioProject ID PRJNA1083343.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.173059.

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