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Hydrologic History Regulates Microbial Biofilm Diversity and Ecosystem Function

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ABSTRACT

Aquatic biofilms are an understudied component of northern peatlands and are expected to play a more prominent role in ecosystem processes in areas where aquatic habitat is expanding. The goal of this study was to investigate how hydrologic history influences biofilm diversity and functional genes. This study was conducted in a long-term water table manipulation that simulates drought (lowered water table treatment) and flooding (raised water table treatment) conditions relative to a control treatment (no manipulation). We used a combination of metabarcoding and metagenomic approaches to (1) examine the diversity of eukaryotic algae, cyanobacteria, bacteria and fungi within the biofilm and (2) identify functional genes associated with alternating wet-dry transitional states. Historical flooding, but not drought, led to broad changes in composition and functional genes, especially those associated with carbon metabolism and nitrogen cycling. Differences were related to changes in relative abundance rather than the presence/absence of individual taxa or genes. Hydrologic history influenced community diversity by reducing interspecific competition or by alleviating resource limitation. These findings show that hydrologic history regulates species membership of the community (and thereby associated genes) but differences in water chemistry and interspecific interactions alter the relative abundance of species and their functional potential.

1 | Introduction

Aquatic microbial biofilms are assemblages of algae, bacteria and fungi attached to submerged surfaces that represent a metabolically active and structurally complex component of freshwater ecosystems. The communities of microorganisms that make up a microbial biofilm can be diverse and are a driving force in ecosystem metabolism and biogeochemical cycling (Battin et al. 2016; Kuehn 2016; Wyatt et al. 2019; Grossart et al. 2020; Hamard, Küttim, et al. 2021; Arias-Real et al. 2023; Wyatt et al. 2025). For example, the balance of carbon uptake and respiration in these communities is regulated by producers (eukaryotic algae and cyanobacteria) and decomposers (bacteria and fungi) found within the biofilm matrix. Biofilm diversity and community assemblage are dependent on environmental factors and niche availability, which can determine the distribution and the functional roles of the biofilm members (Besemer et al. 2009, 2012; Bengtsson et al. 2018; Seballos et al. 2020; Allen et al. 2021; Arias-Real et al. 2023).

Inundation frequency and duration (i.e., hydrologic regime) play a critical role in regulating aquatic community structure

(Arias-Real et al. 2024), particularly in wetland ecosystems (Kneitel 2014; Mazumder et al. 2017; Emsens et al. 2020; Lamit et al. 2021). Across large wetland expanses of the northern boreal landscape, climate-driven changes in precipitation patterns (both rain and snow) and hydrologic connectivity (following permafrost degradation) have increased the frequency and duration of inundation (Jorgenson et al. 2020; Douglas et al. 2020; Tank et al. 2020; Barel et al. 2021). For example, at the Alaska Peatland Experiment (APEX) site, where this study was conducted, 11 of the last 18 years have exhibited standing water above the peat surface for most of the growing season (Euskirchen et al. 2024). Although peatlands naturally exhibit both wet and dry transitional states, in the past, periods of flooding were considered temporary disturbances (Wyatt et al. 2012). However, it has become apparent that flooding is becoming a common feature of this landscape (Kane et al. 2021; Euskirchen et al. 2024) and similar trends have emerged from other long-term study sites (Varner et al. 2021). Notably, aquatic microbial biofilms are expected to play a more significant role in ecosystem processes (e.g., carbon cycling) in areas where aquatic habitat is expanding (Wyatt et al. 2025).

Species composition is important for determining the flux and transformation of carbon and nutrients in both terrestrial and aquatic environments (Strickland et al. 2013; Ernakovich et al. 2022; Jassey, Walcker, et al. 2022). Northern boreal peatlands are widely recognised for their role in carbon sequestration (Yu 2012) and carbon uptake and storage have been largely attributed to regulation by the soil microbial community (Zak and Kling 2006; Potter et al. 2017; Waldrop et al. 2023). However, research has shown that microbial interactions in aboveground communities can influence belowground carbon storage through changes in the form and availability of energy inputs into the microbial loop (Strickland et al. 2013; Schmidt et al. 2016; Schmitz and Leroux 2020; Jassey, Walcker, et al. 2022). For example, during periods of inundation, the primary mode of production shifts from mosses and vascular plants to aquatic biofilms that form on submerged surfaces (van Duinen et al. 2013; Vesterinen et al. 2016; Ferguson et al. 2021). However, current efforts to describe peatland microbial diversity have focused on soil and plant-root-associated microbes (archaea, bacteria, fungi) (Haynes et al. 2015; Potter et al. 2017; Emsens et al. 2020; Seward et al. 2020; Lamit et al. 2021; Rupp et al. 2021; Waldrop et al. 2023) and have mostly ignored the aboveground microbiome and thereby aquatic biofilms. Therefore, our understanding of microbial communities in peatlands and by extension their contribution to ecosystem function, is likely incomplete.

The goal of this study was to examine the diversity (i.e., who is there?) of both the producer and decomposer components of a peatland biofilm, including eukaryotic algae, cyanobacteria (also known as blue-green algae), bacteria and fungi, and identify functional genes (i.e., what can they do?) along a gradient of hydrologic history. Since alternating wet-dry transitional states are often coupled with changes in environmental conditions that influence microbial biofilm growth and metabolism (Wyatt et al. 2012, 2024; Kneitel 2014), we expected that examining the microbial biofilm among habitats with differing hydrologic history would reveal: (1) differences in the composition of producers and decomposers with variation in water table position, (2) the influence of environmental conditions shaping species diversity, particularly, flooding and flood-history and (3) the influence of hydrologic history on the relative abundance of functional genes.

2 | Materials and Methods

2.1 | Study Site

This study was conducted at the APEX site, which is located within the Tanana River floodplain, approximately 35 km southeast of Fairbanks, Alaska. The APEX site was established in 2005 and is part of the Bonanza Creek Long Term Ecological Research Program (Turetsky et al. 2008). The water table manipulation is located within a moderately rich fen and the vegetation and environmental characteristics have been described in detail elsewhere (Churchill et al. 2015; Euskirchen et al. 2024). Experimental water table treatments are 120 m^2 (each spaced approximately 25 m apart) and simulate drought (lowered treatment) and flooding (raised treatment) conditions expected for this region due to climate change relative to a control treatment (no manipulation) (Figure 1). Despite experimental manipulation, all three experimental treatments also experience natural flooding which can confound the water table manipulation (Wyatt et al. 2012; DeColibus et al. 2017; Kane et al. 2021; Euskirchen et al. 2024). This study was conducted during such an event in 2022, when all experimental sites were flooded at the time of sampling (11 June 2022; Figure S1). While biofilm research at the APEX study site has widely focused on temporal variation (Wyatt et al. 2012, 2024; Rober et al. 2013; DeColibus et al. 2017; Kane et al. 2021), the goal of this study was to describe the diversity and functional potential of the biofilm community using molecular tools. Having no prior knowledge of the microbial community warranted single time point sampling rather than time series analyses. The timing of sample collection was selected to maximise genetic material and was based on our prior research of microbial biomass at the site (Rober et al. 2013; DeColibus et al. 2017; Kane et al. 2021; Wyatt et al. 2024).

2.2 | Sampling Methods

Biofilm samples were collected within a 1 m^2 quadrat at six randomly selected locations (each spaced 5–10 m apart) within each of the three water table treatments (Figure 1; Wyatt et al. 2012; Rober et al. 2013; DeColibus et al. 2017; Kane et al. 2021; Wyatt et al. 2024). To capture microscale heterogeneity within quadrats, each sample was a composite of four subsamples that were collected from dead submerged *Carex utriculata* stems (each 10 cm in length) (Figure 1). Stems with attached microbial biofilm were placed in a sterile centrifuge tube and kept in a dark cooler on ice until returning to the lab where they were frozen at −80°C until analysis. Prior to analysis, the microbial biofilm was removed from each plant stem using sterile toothbrushes and the combined slurry was used for molecular analyses described below (Figure 1).

Environmental conditions were measured at the same six locations as biofilm collection. Water depth (cm) was measured with a metre stick. Water temperature (°C), pH, conductivity

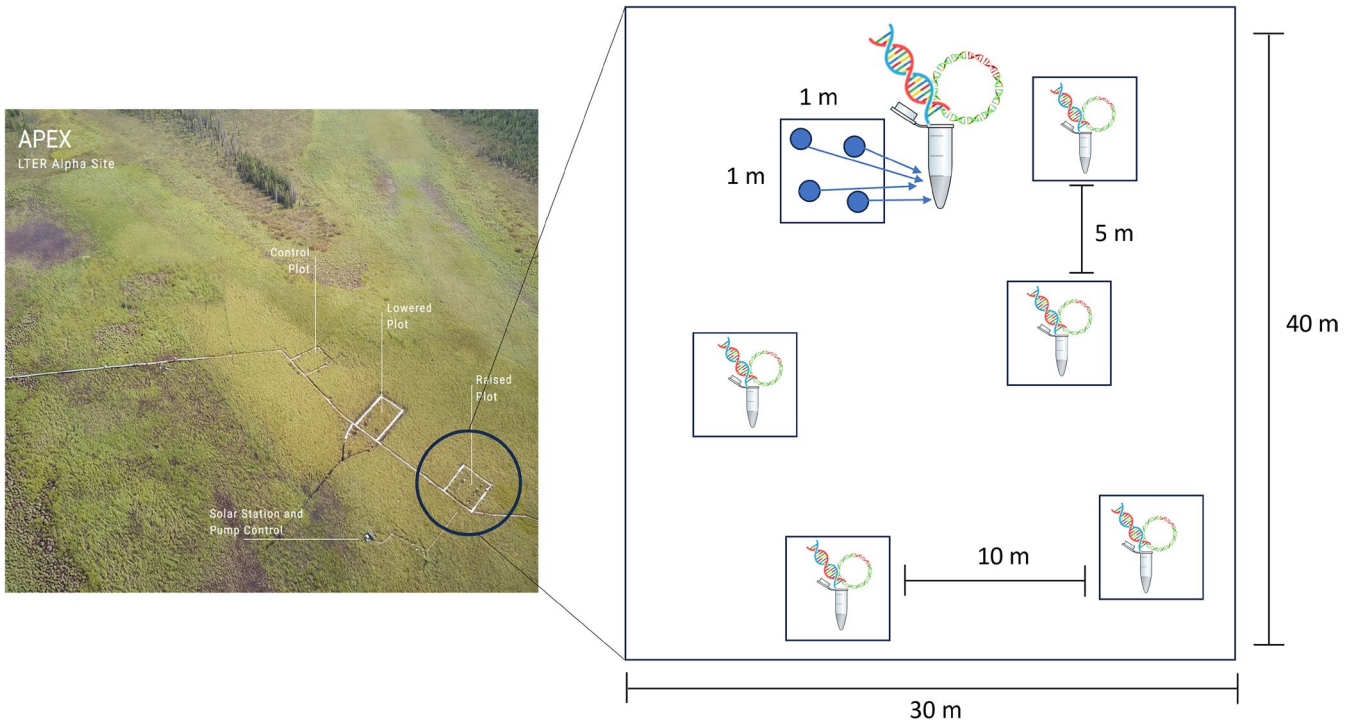

FIGURE 1 | Aerial photograph of Alaska Peatland Experiment (APEX) study site examining long-term changes in hydrology including drought (lowered plot), flooding (raised plot), relative to a control plot without manipulation. Experimental water table treatments are 120 m^2, spaced approximately 25 m apart and outlined by a raised boardwalk for site access. Within each plot, biofilm samples were collected within six randomly selected 1 m^2 quadrats (each spaced 5–10 m apart) within each of the three water table treatments. Microscale heterogeneity was captured by combining four subsamples collected from dead submerged *Carex utriculata* stems (each 10 cm in length) within each quadrat into a single composite sample.

($\mu S\, m^{-2}\, s^{-1}$) and dissolved oxygen (DO; $mg\, L^{-1}$) were measured using a Hydrolab sonde (Hach Company, Loveland, CO, USA). Samples for dissolved nutrient analysis (NO_2, NO_3, PO_4) and dissolved organic carbon (DOC) were collected with a syringe and filtered through a 0.45 μm filter (Millipore Corporation, Bedford, MA, USA) into 60-mL acid-washed amber polyethylene bottles. Dissolved nutrient samples were stored on ice in the field and frozen until analysis using ion chromatography (Dionex Corporation, Sunnyvale, CA, USA). Dissolved organic carbon was analysed using a Shimadzu TOC analyser (Shimadzu Scientific Instruments, Columbia, MD, USA). A subsample of filtered samples was analysed for ultraviolet absorption at 254 nm using an Agilent Cary 60 UV–VIS spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Specific UV absorbance at 254 nm ($SUVA_{254}$) was calculated by dividing ultraviolet absorption at 254 nm by DOC concentration.

2.3 | DNA Extraction

Biofilm DNA was extracted for both metabarcoding and metagenomic analyses. Following removal from plant stems, the detached microbial biofilm slurry was centrifuged to produce a pellet ($n = 6$ from each water table treatment). Biofilm pellets were transferred to 2 mL tissue disruption tubes and extracted using a DNeasy Plant Pro Kit following the manufacturer's protocol (Qiagen, Germantown, MD, USA). Extracted DNA was quantified with a NanoPhotometer N60 (Implen, Munich, Germany) and resultant DNA was normalised to a concentration of 20 $ng\, \mu L^{-1}$ before polymerase chain reaction (PCR) amplification.

2.4 | Library Preparation and High Throughput Sequencing

Metabarcoding was used to determine the taxonomic composition of the individual components of the biofilm (bacteria, cyanobacteria, eukaryotic algae and fungi). Metagenomic sequencing was used to identify differences in functional genes among water table treatments. Biofilm amplicon libraries were generated separately for bacteria (including cyanobacteria), fungi and eukaryotic algae by amplifying the bacterial 16S and algal 18S V4 regions and the fungal Internal Transcribed Spacer Region 2 (ITS2) using a two-step amplification process (Brown et al. 2018). To target the bacterial community, the V4 region of the ribosomal RNA (rRNA) was amplified using 515F and 806R primers (Apprill et al. 2015; Parada et al. 2016). The fungal ITS2 region of rRNA was amplified using the primer pair flTS7 and ITS4 (White et al. 1990; Ihrmark et al. 2012). The eukaryotic algal community was targeted by amplifying the V4 region of rRNA using the primers TAReuk454FWD1 and TAReukREV3 (Tanabe et al. 2016; Hamard, Céréghino, et al. 2021). The targeted 16S, 18S and ITS2 rRNA gene regions were amplified using PCR separately for each sample. Primary PCRs consisted of 50 μL reactions with 2.5 μL DNA template (50 ng), 25 μL 5× Phusion High-fidelity Buffer (mastermix), 5 μL of each forward and reverse primer and 12.5 μL molecular grade water. The PCR conditions were 98°C for 30 s, 25 cycles of 98°C for 10 s, 52.5°C for

30 s and 72°C for 40 s, followed by a final extension at 72°C for 10 min; all ramp rates were set to 1°C s^{-1}. The amplification was confirmed using gel electrophoresis (1.5% agarose w:v in tris-borate-EDTA [TBE]). Secondary PCRs were performed using forward and reverse primers that include the P5-i5-overlap and P7-i7-overlap, where P5 and P7 are the Illumina Adaptor sequences, i5 and i7 are 8 bp unique dual barcodes and the overlap consists of partial nexF and nexR sequences that act as the annealing sites for the secondary PCRs (Seballos et al. 2020). The secondary PCR reactions consisted of 5 μL of the primary PCR product, 25 μL 5× Phusion High-fidelity Buffer, 2.5 μL of each primer and 15 μL molecular grade water with the PCR conditions of 98°C for 30 s, 12 cycles of 98°C for 10 s, 52.5°C for 30 s and 72°C for 40 s, followed by a final extension at 72°C for 10 min. This produced the final amplicon constructs with a total of 32 cycles. Amplification of the secondary PCR was confirmed using gel electrophoresis (1.5% agarose w:v in TBE). The secondary PCR DNA product was cleaned using the Just-a-Plate 96 PCR Purification and Normalisation kit, following the manufacturer's protocol, to produce the final product of the original pure DNA. Libraries for metabarcoding ($n = 18$) were sequenced in one reaction of Illumina MiSeq (300PE) at the Kansas State University Integrated Genomics Facility (Manhattan, KS, USA). Quality control of the raw MiSeq reads was performed using the Galaxy web application (Blankenberg et al. 2010).

Metagenomic sequencing was used to survey gene content profiles to investigate potential functional aspects of the biofilm microbiome. Libraries were prepared by combining a 10 μL subsample of purified DNA from individual replicates ($n = 6$ per water table treatment) to produce a single composite sample for each water table treatment ($n = 3$ total metagenomic samples). DNA fragments were sequenced using the Illumina NovaSeq 6000 Sequencing System by Novogene (Novogene Corporation Inc.), producing paired ends 350 bp. Read ends were trimmed where quality values were < 38. Filtered reads were assembled using MegaHit (Table S1).

2.5 | Amplicon Sequencing Analysis

Sequence data were processed with the programme mothur (Schloss et al. 2009), generally following methods described by Kozich et al. (2013). The forward and reverse reads were contiged and files were merged into single fasta file for bacterial (including cyanobacteria), fungal and eukaryotic algae where technical replicates were merged. Primers were trimmed using cutadapt and remaining sequences were aligned with SILVA and filtered to exclude non-16S or 18S V4 regions or any misaligned reads (fungal ITS sequences cannot be globally aligned). Sequences were screened for chimeric reads using VSEARCH (Huse et al. 2010; Rognes et al. 2016) and all identified putative chimeras were culled. Sequences were taxonomically classified using a naïve Bayesian classifier against the ribosomal database project training set (Wang et al. 2007). Any non-target lineages for bacterial sequences were culled and cyanobacterial target lineages were retained among bacterial sequences for future separation. Sequences were clustered into operational taxonomic units (OTUs) using OptiClust at 3% dissimilarity (Westcott and Schloss 2017). All OTUs that had fewer than 10 global sequences were considered potentially spurious and removed (Brown et al. 2015). At this point, cyanobacteria OTUs were separated from bacteria OTUs and classified independently due to their ecological classification as blue–green algae. The algae are not a formal taxonomic group of organisms but are instead a polyphyletic group with representatives in several kingdoms that are aquatic, photosynthetic (possessing chlorophyll *a*) and have simple vegetative structures without a vascular system (Graham et al. 2008). Algae, including cyanobacteria, can be unicellular, colonial, filamentous or pseudo-filamentous (i.e., uni- or multicellular). Nevertheless, since cyanobacteria are prokaryotic, they were also kept separate from eukaryotic algae OTUs. Bacterial OTUs were assigned taxon affinities based on the most representative sequence of the OTU (centroid) and the fungal OTUs were classified to the level of Species Hypothesis using UNITE (Nilsson et al. 2018). Eukaryotic algae and cyanobacterial OTU's that did not have 100% support for phylum level identities were manually confirmed using MOLE-BLAST (NCBI) against GenBank (nr/nt) while excluding environmental sequences.

2.6 | Distribution of Functional Genes

To determine how functional genes were distributed across water table treatments, we annotated metagenome reads that included all components of the biofilm through comparison to the Kyoto Encyclopaedia of Genes and Genomes (KEGG) database (Kanehisa et al. 2017). Gene count matrices were generated that included the number of times each KEGG Orthology was observed among treatments. Custom functional profiles were computed based on the proportions of genes associated with metabolic functions of particular ecological relevance in our study system (e.g., photoautotrophy, methanotrophy, nitrogen fixation) (Louca et al. 2016). We also generated functional gene profiles using FAPROTAX, a bioinformatics tool designed to predict the relative abundances of various functional groups in the absence of transcriptomics. FAPROTAX is based on taxon abundances derived from 16S sequences (i.e., prokaryotes only) (Louca et al. 2016). FAPROTAX-based functional profiles considered similar metabolic functions of ecological relevance as the metagenome-based whole biofilm functional profiles, but only considered functions that could be attributed to prokaryotes (bacteria and cyanobacteria).

2.7 | Statistical Analysis

We calculated relative OTU richness, diversity (both Shannon–Wiener and Simpson's Index of diversity) and Shannon's Evenness separately for bacteria, cyanobacteria, fungi and eukaryotic algae using mothur. Following a Shapiro–Wilks test for normality, multivariate general linear models (GLM) were used to evaluate differences in richness, diversity and evenness among water table treatments as well as the effect of water table treatment on physical and chemical conditions. When differences were significant, Tukey's test for post hoc comparison of means was used to discriminate among treatments. All tests for normality and GLMs were performed using SPSS 20 (IMB Statistics, Chicago, IL, USA).

Non-metric multidimensional scaling (nMDS) of Bray–Curtis dissimilarity values were used to visualise individual components of the microbial biofilm among water table treatments using the ggtreatment package in R. To test if individual components of the

TABLE 1 | Mean and standard deviation of physical and chemical parameters among water table treatments.

	Control		Lowered		Raised	
Environmental factor	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Water depth (cm)	50.3[a]	1.90	53.8[a]	1.90	48.3[a]	1.90
pH	5.83[a]	0.21	6.04[a]	0.09	6.16 [a]	0.37
Temperature (°C)	15.0[a]	1.29	13.4[b]	0.50	12.9[b]	0.13
Dissolved oxygen (DO; $mg\,L^{-l}$)	2.83[a]	0.50	2.50[a]	0.13	4.00[b]	0.90
Conductivity ($\mu S\,m^{-2}\,s^{-1}$)	41.9[a]	0.51	44.7 [b]	1.00	41.3[a]	0.86
Dissolved organic carbon (DOC; $mg\,L^{-l}$)	50.2[a]	3.69	52.3[a]	0.79	50.7[a]	0.73
SUVA ($L\,mg\,C^{-1}\,m^{-1}$)	2.99[a]	0.20	3.01[a]	0.16	3.14[a]	0.10
Total dissolved nitrogen (TDN; $mg\,L^{-l}$)	0.65[a]	0.06	0.71[a]	0.10	0.68[a]	0.10
Phosphate (PO_4; $mg\,L^{-l}$)	0.45[a]	0.60	0.54[b]	0.02	0.52[b]	0.01
Nitrite (NO_2; $mg\,L^{-l}$)	0.84[a]	0.08	1.08[b]	0.11	0.91[a]	0.05
Nitrate (NO_3; $mg\,L^{-l}$)	0.22[a]	0.03	0.27[b]	0.01	0.29[b]	0.02

Note: Different letter superscripts indicate significant differences among treatments ($p < 0.05$).

biofilm differed among water table treatments, we used 999 permutations of pairwise comparisons using a permutational multivariate analysis of variance (PerMANOVA) (Anderson 2001) with Bonferroni's post hoc test using the 'ecodist' (Goslee and Urban 2017), 'vegan' (Oksanen et al. 2022) and 'pairwiseAdonis' (Martinez 2019) packages in R. Only the OTU sequences found at >1% relative abundance were used to visualise the dominant taxa for each biofilm community among treatments. We note that water table treatment was not replicated and thus, the effects of the water table treatment were confounded with location. While caution is warranted due to the lack of true replicates, we believe that differences in our data are most parsimoniously interpreted as differences in historical water table manipulation. To determine which environmental variables were associated with community composition, we performed a correlation analysis using PAST4.03 by correlating environmental variables with nMDS axes (Kendall τ correlations). Only environmental parameters that were significantly correlated with at least one nMDS axis were plotted.

Patterns of co-occurrence among cyanobacteria, fungi, algae and bacteria within each water table treatment were determined with a Gaussian copula-based network analysis using the ecoCopula package (Popovic et al. 2019) following methods described by Niku et al. (2019). Since OTU tables were generated separately for each component of the biofilm, OTU matrices were transformed into a presence/absence matrix where 1 = OTU presence in a sample and 0 = OTU absence, so that all biofilm components could be compared in a single matrix. Only taxa present at greater than 5% relative abundance were used to generate the presence/absence matrix. Correlation matrices were visualised separately for each water table treatment. Gaussian Copula Graphical Models (GCGMs) were used to visualise co-occurrences (Popovic et al. 2019). GCGMs use a Bayesian approach for graphical model determination that can accommodate binary (presence/absence) variables and incorporate conditional dependence relationships to describe how pairs of species are related after controlling for all the other species in the dataset.

Metagenomic reads were not replicated (all six replicates from each water table treatment were consolidated into a single sample prior to analysis; $n = 1$ per treatment). This pooling facilitated the visualisation of the relative abundances of KEGG genes among water table treatments; however, it precluded any statistical analysis of observed differences. Since FAPROTAX-based taxonomic profiles were replicated ($n = 6$ per treatment), both visualisation and statistical analyses were performed using PerMANOVA.

3 | Results

3.1 | Physical and Chemical Conditions

At the time of sampling, water depth, pH, DOC, SUVA and TDN were not significantly different among water table treatments ($p \geq 0.12$; Table 1). Water temperature was 1.8°C higher in the control treatment compared to lowered or raised treatments ($p \leq 0.005$), which were not different from each other ($p = 0.06$). The DO concentration was nearly 2-fold higher in the raised treatment compared to the control or lowered treatments ($p = 0.04$), which were not different from each other ($p = 0.09$). Conductivity was elevated by $3.08\,\mu S\,m^{-2}\,s^{-1}$ in the lowered treatment compared to the control or raised treatments ($p < 0.001$), which were not different from each other ($p = 0.3$). The concentration of dissolved inorganic nutrients (NO_3, NO_2, PO_4) were elevated in the lowered and raised treatments compared to the control ($p < 0.02$), but were not significantly different from each other ($p = 0.6$).

3.2 | Biofilm Community Diversity and Evenness

Measures of biofilm OTU richness, Shannon diversity, Simpson's diversity and evenness varied among water table treatments ($p \leq 0.04$; Figure 2A–D). Species richness was greater in the lowered treatment compared to the raised

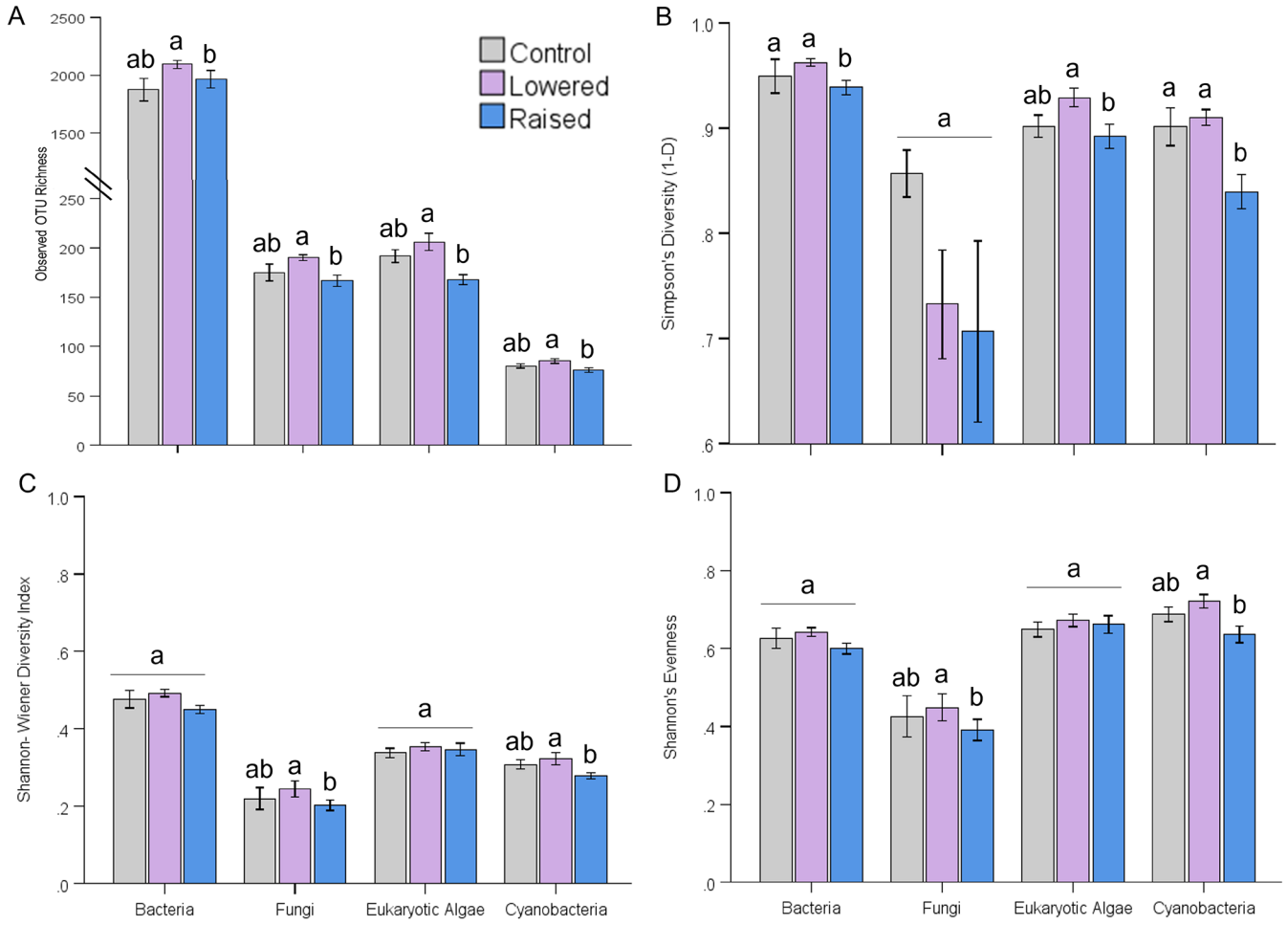

FIGURE 2 | Richness and diversity metrics for individual components of the biofilm among water table treatments. Bars represent mean ± 1 SD of (A) relative observed operational taxonomic unit (OTU) richness, (B) Simpson's index of diversity, (C) Shannon–Wiener diversity index and (D) Shannon's evenness. Bars with the same letter are not significantly different among treatments ($\alpha = 0.05$). Diagonal lines on *y*-axis of (A) indicate a change in scale.

treatment ($p = 0.002$), but species richness in both water table treatments were similar to the control treatment ($p > 0.06$). Simpson and Shannon diversity metrics revealed that differences in biofilm community composition were driven by the most common species (Simpson's diversity) of bacteria, eukaryotic algae and cyanobacteria ($p \leq 0.01$; Figure 2B), but the least common species (Shannon's diversity) of fungi ($p < 0.02$; Figure 2C). Bacterial and algal species were similarly distributed among water table treatments (i.e., there were no differences in the evenness) ($p > 0.2$), but a less even distribution of fungal and cyanobacterial species indicated that a few species dominated the community in the raised treatment compared to the lowered treatment ($p < 0.03$), although neither were different from the control ($p > 0.2$; Figure 2D).

3.3 | Biofilm Composition Among Water Table Treatments

We observed clear separation in bacteria, cyanobacteria, fungi and eukaryotic algae between the control and raised water table treatments but biofilm composition in the lowered treatment overlapped with both the control and raised treatments (Figure 3A–D). There was greater dissimilarity in bacteria (Figure 3A) and cyanobacteria (Figure 3D) community composition among all three treatments than fungi (Figure 3B) or eukaryotic algae (Figure 3C). Patterns in fungi (Figure 3C) and eukaryotic algae (Figure 3D) showed overlap between the control and lowered treatments, but little overlap with the raised treatment.

Bacteria that correlated with water table treatment were the phyla *Acidobacteria*, *Proteobacteria (subclass α-proteobacteria)*, *Bacteroidetes, Plantymmocycetes* and *Sphingobacteria* ($p \leq 0.02$; Figure 3A). Fungi that correlated with water table treatments were the orders *Botrysophaeriales* and *Capnodiales* ($p < 0.03$; Figure 3B). Algae that correlated with water table treatments were the green algae *Stigeoclonium* and *Oedogonium* ($p = 0.01$; Figure 3C) and cyanobacteria *Nostoc* and *Sphaerospermopsis* ($p < 0.01$; Figure 3D).

Environmental parameters that were predictors of variation in community structure among water table treatments included DO, conductivity and dissolved nutrients (PO_4, NO_2 and NO_3) (Figure 3A–D). Differences in DO and NO_3 among water table treatments were positively correlated with variation in the

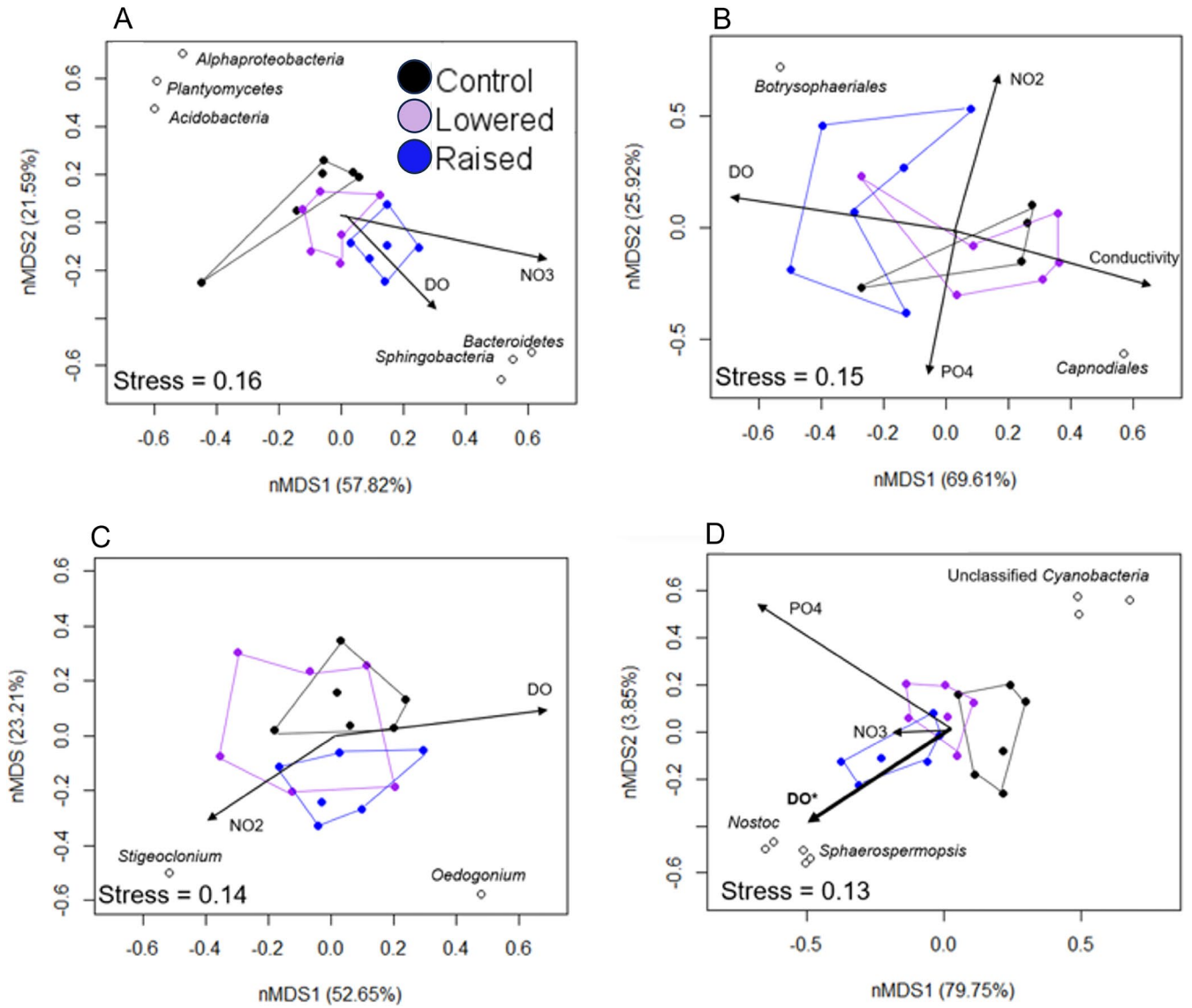

FIGURE 3 | Non-metric multidimensional scaling (nMDS) ordinations based on Bray–Curtis dissimilarity values demonstrating the differences and procrustean relationships among individual components of the biofilm (A; bacteria, B; fungi, C; eukaryotic algae and D; cyanobacteria) among control, lowered and raised water table treatments. Significant correlations between individual species and water table treatments are denoted by black open circles with taxonomic names. Arrows indicate the strength and direction of the correlation of environmental variables with the ordination axes. Bold arrows with an asterisk represent a correlation with both axes. Axes percentages explain captured variation. Stress values were used to determine how well each community was represented in two-dimensional space where values between 0.1 and 0.2 were considered acceptable.

bacterial community ($p = 0.005$; Figure 3A). Variation in fungal community structure among treatments was positively correlated with differences in NO_2 and conductivity ($p < 0.005$) and negatively correlated with differences in PO_4 and DO ($p = 0.009$; Figure 3B). Variation in algal community composition among treatments was negatively correlated with NO_2 and positively correlated with DO ($p < 0.03$; Figure 3C). Differences in DO, PO_4 and NO_3 among water table treatments were negatively correlated with variation in the cyanobacteria community ($p < 0.04$; Figure 3D).

Bacterial OTUs were determined to the phyla- and class- levels, while fungi, eukaryotic algae and cyanobacteria were identified to the genus level (Figure 4A–D). Taxonomic composition was

TABLE 2 | Permutational analysis of variance (PerMANOVA) results evaluating differences in the community composition of individual components of the biofilm among water table treatments.

Microbial community	df	SS	R^2	F	p
Bacteria	2	0.42	0.47	5.86	0.001
Fungi	2	1.29	0.34	3.72	0.001
Eukaryotic algae	2	0.72	0.40	4.82	0.001
Cyanobacteria	2	0.66	0.49	5.10	0.002

Note: Owing to their role as producers, cyanobacteria were analysed separately from the rest of the bacteria. F-value by permutation; R^2 for Bray–Curtis distance matrices values were calculated using 999 permutations. Significant differences are indicated by $p < 0.05$.

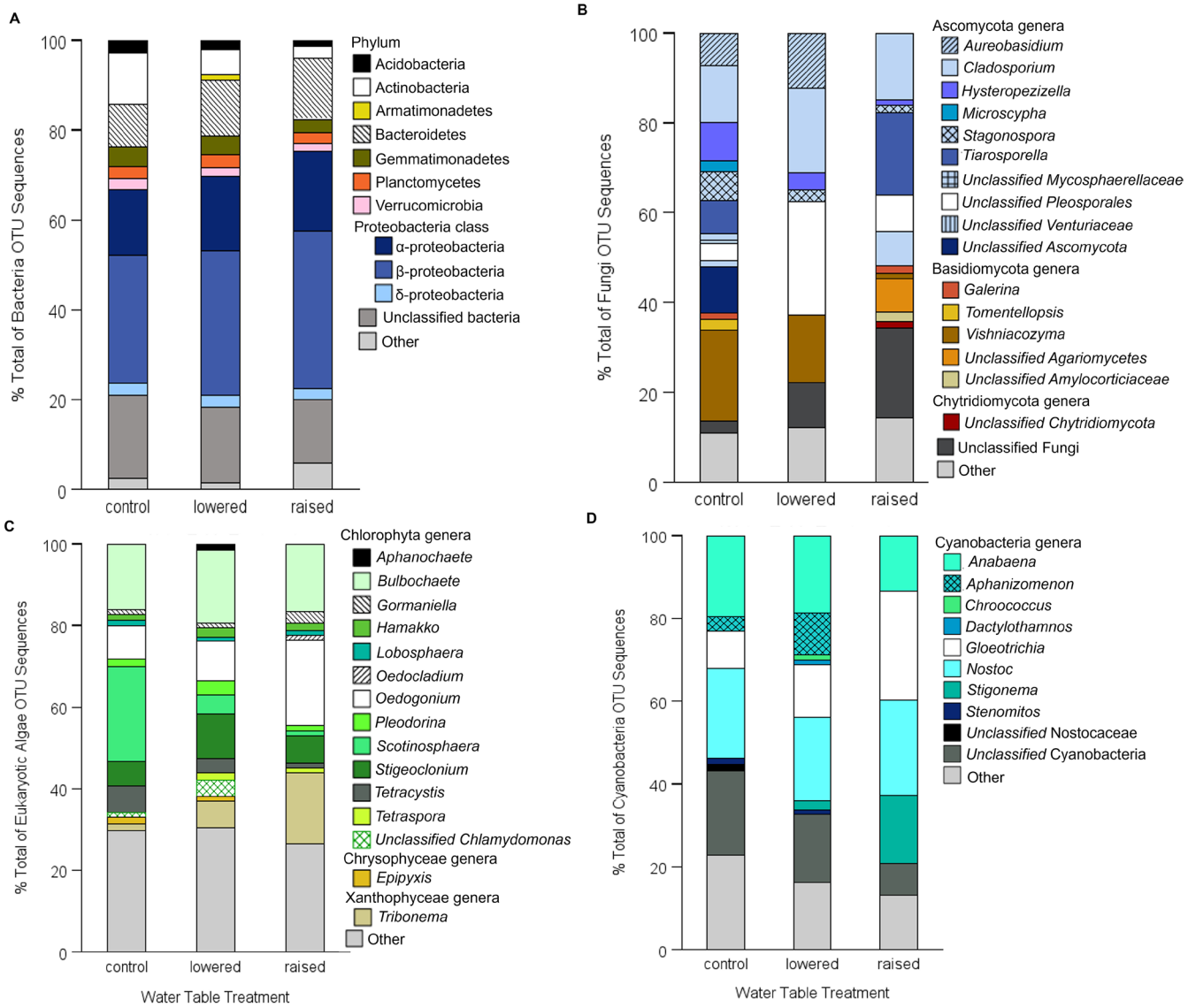

FIGURE 4 | Percent of total operational taxonomic unit (OTU) sequences of (A) bacterial phyla (and *Proteobacterial* classes), (B) fungal genera, (C) eukaryotic algae genera and (D) cyanobacteria genera present at > 1% relative abundance among control, lowered and raised water table treatments.

similar across treatments, but relative abundances differed among water table treatments (Table 2). Approximately 95% of bacteria were from seven common phyla and the phylum *Proteobacteria* (classes *α*-, *β*- and *δ*) made up the greatest proportion (46%–55%) of the bacterial community (Figure 4A). The fungal community was largely comprised of members from the phyla *Ascomycota* (53%–65%) and *Basidiomycota* (12%–48%; Figure 4B). The division *Chlorophyta* (i.e., green algae) made up 56%–65% of the algal community across treatments, but the proportion of representative genera varied among treatments (Figure 4C). The eukaryotic algae had the greatest proportion (20%–30%) of taxa in the 'other' category compared to the other members of the biofilm, likely owing to presently limited libraries for identification of algae (Pawlowski et al. 2012; Del Campo et al. 2014; West et al. 2018). Among the eukaryotic algae that have been previously identified as part of the peatland biofilm community that were not quantified here were the Bacillariophyceae (diatoms) (Rober et al. 2014; Hamilton et al. 2023; Araujo et al. 2024). The cyanobacteria community was largely comprised of genera capable of nitrogen-fixation (Figure 4D).

3.4 | Biofilm Co-Occurrence Patterns

Patterns of microbial biofilm co-occurrence varied among water table treatments (Figure 5). Deviations from expected co-occurrence were more prevalent in the control water table treatment, as shown by a greater number of both positive (blue) and negative (red) associations than either the lowered or raised treatment. Notable strong negative associations in the control treatment were between the cyanobacteria *Nostoc flagelliforme* and the fungus *Hysteropezizella*, as well as the green algae *Scotinosphaera* (Figure 5A). Among the notable positive associations in the control treatment were between the fungus *Hysteropezizella* and bacteria Intrasporangiaceae and an unidentified Actinobacteria. However, the same *Hysteropezizella* was strongly negatively associated with the green algae *Bulbochaete* in the lowered water table treatment, with none of the other positive or negative co-occurrences observed as in the control (Figure 5B). Many of the co-occurrences in the raised water table treatment were weak (pink or light blue) or neutral (white) (Figure 5C).

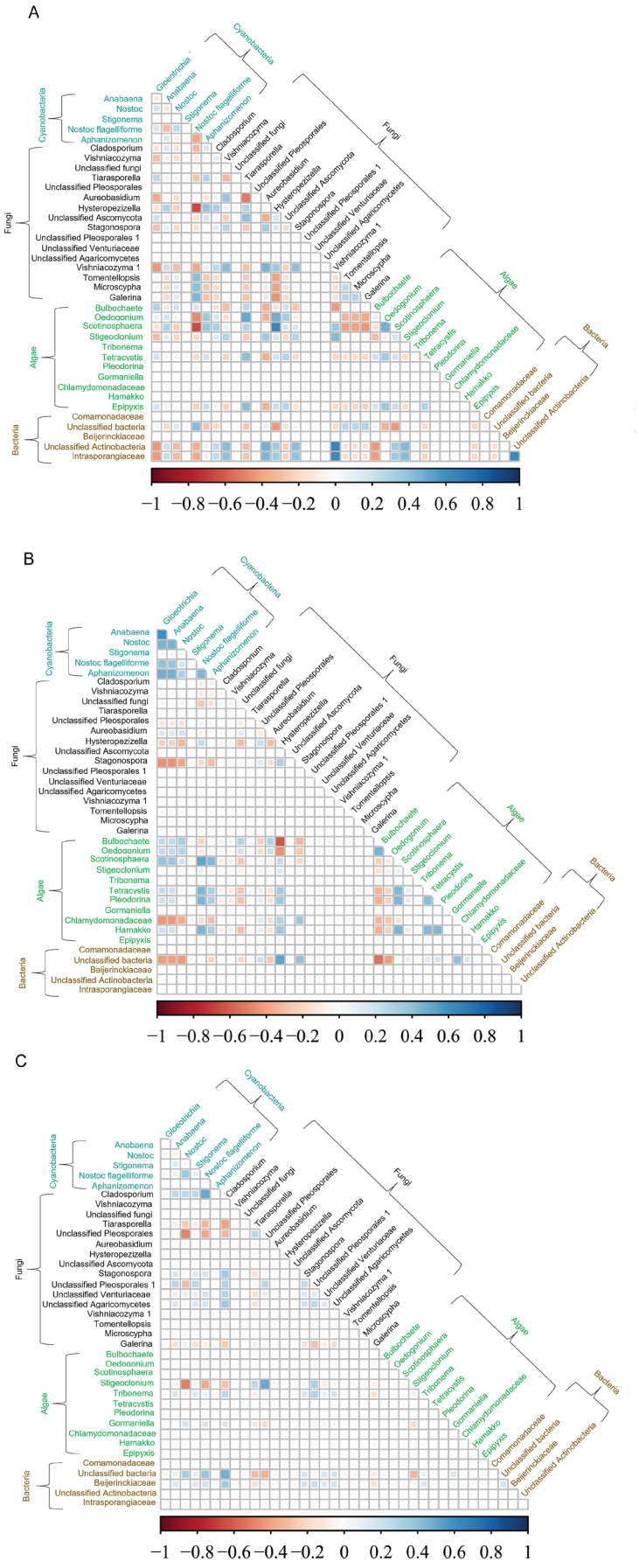

FIGURE 5 | Legend on next page.

FIGURE 5 | Correlation matrices based on latent factor loadings from Gaussian Copula Graphical Models (GCGMs) to determine complex conditional dependencies among cyanobacteria, fungi, algae and bacteria within each water table treatment. Correlations are positive (blue) if taxa co-occurred more often than expected by their prevalence, negative (red) if they co-occurred less often and neutral (white) if they co-occurred at the rate expected by their prevalence. (A) Control, (B) lowered and (C) raised.

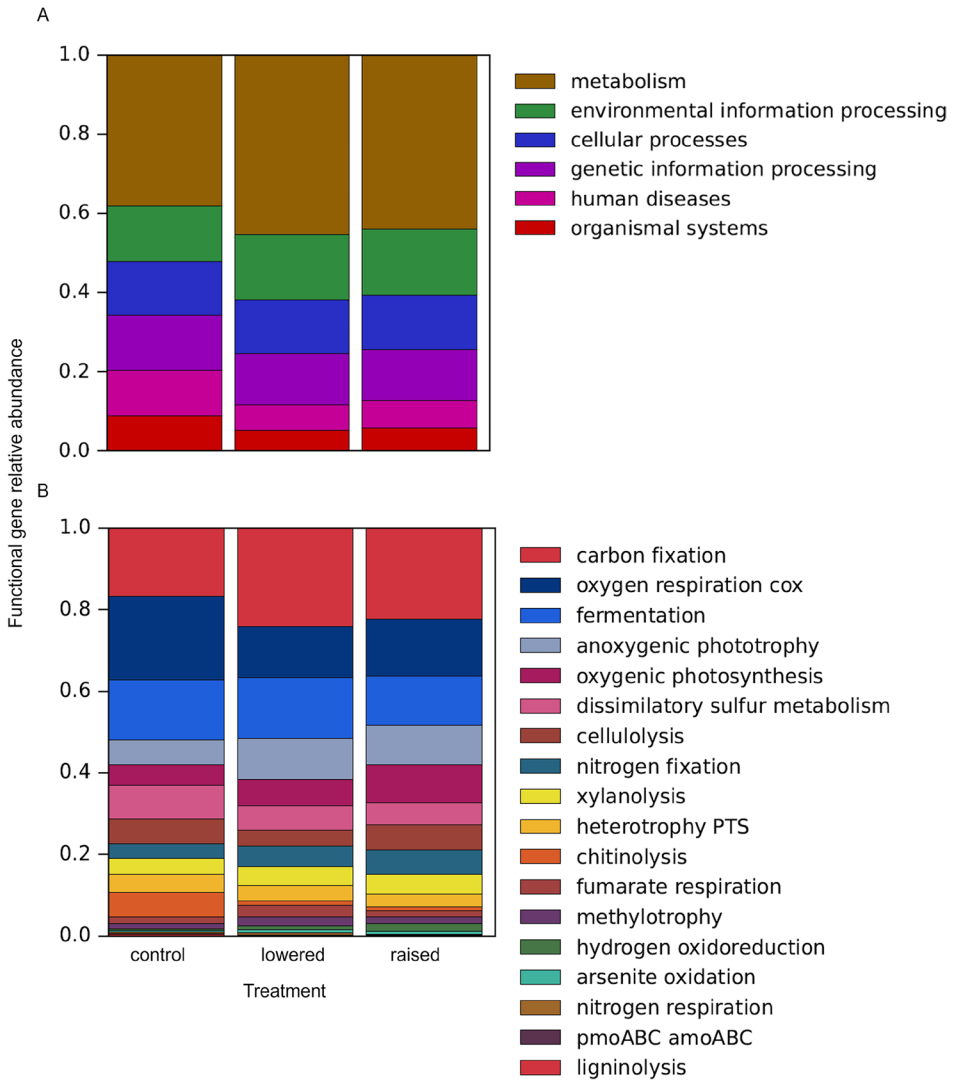

FIGURE 6 | Relative abundances of KEGG-annotated genes associated with (A) KEGG level A and (B) metabolic functions of ecological importance. Data include bacterial (including cyanobacteria) and eukaryotic (microalgae and fungi) functional genes. Bar sizes correspond to estimated gene proportions based on the number of reads averaged across all genes in each gene group.

3.5 | Variation in Biofilm Functional Genes Among Treatments

Community-level functional profiles based on the proportions of KEGG-annotated genes (Figure 6) revealed differences in relative abundances of functional groups among water table treatments. Across all three water table treatments ≥ 50% of considered and identified genes were associated with metabolism (e.g., carbon and nitrogen uptake, transformation and degradation) and environmental information processing (Figure 6A). Both the lowered and raised treatments had a greater proportion of genes (~10%) associated with metabolism and relative abundances were more similar to each other than to the control (Figure 6A,B). Of the metabolic genes, ≥ 60% were associated with carbon metabolism (Figure 6B). Together, carbon fixation and respiration made up ~40% of genes across all three treatments, but both the lowered and raised treatments had a greater proportion of carbon fixation genes (20%–25%) whereas the control had a higher proportion of respiration genes (25%). Fermentation represented between 10% and 15% of genes across treatments. The proportion of genes associated with phototrophy (i.e., photosynthesis, photoheterotrophy, anoxygenic phototrophy) was higher in the lowered and raised treatments compared to the control, but in the raised treatment the relative abundance of oxygenic photosynthesis genes was nearly double that of the lowered treatment. The remaining 25%–30% of the genetic profile was largely similar among treatments and comprised of genes associated with nitrogen-fixation, sulphur reduction,

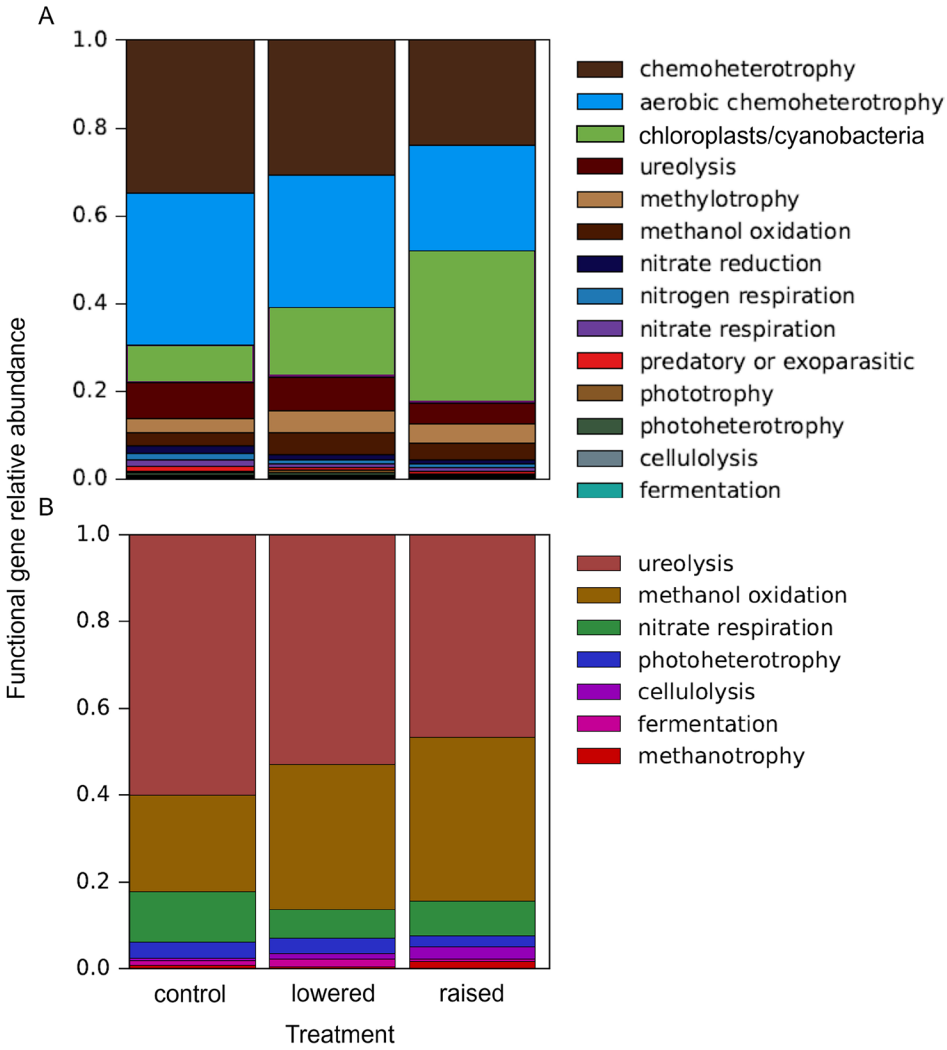

FIGURE 7 | Estimated proportions (relative abundances) of (A) FAPROTAX-inferred metabolic functions and (B) metabolic functions of ecological importance. Data include bacterial members of the biofilm only. Bar sizes correspond to OTU proportions, which are based on the sum of reads mapped to each OTU in each sample.

polysaccharide degradation (e.g., chitonolysis, xylanolysis) and carbon uptake (e.g., heterotrophic phosphotransferase).

FAPROTAX estimated functional profiles revealed differences in relative abundances of inferred metabolic functions driven by prokaryotes among water table treatments (PerMANOVA $F_{108,216} = 29.1$, $p < 0.001$; Figure 7). Estimated metabolic functions attributed to just the bacterial component of the biofilm (including cyanobacteria), were dominated by genes associated with energy acquisition (Figure 7A). Genes for forms of chemoheterotrophy made up 45%–70% of the inferred metabolic functions among water table treatments. However, the relative abundances of genes associated with heterotrophic metabolism pathways were surpassed by photosynthesis (i.e., cyanobacteria) in the raised treatment where chloroplasts made up ~40% of the gene profile compared to ~10% in the control treatment and 20% in the lowered treatment (Figure 7A). Inferred metabolic pathways associated with methylotrophy, methanol oxidation and nitrogen metabolism (ureoloysis, nitrate reduction, nitrate/nitrite respiration) were prevalent across all treatments but made up a greater proportion in the control and lowered treatments compared to the raised treatment (Figure 7A). Of the genes of particular ecological relevance, those associated with nitrogen and carbon metabolism made up 82%–85% of the metabolic profile among treatments (Figure 7B). Genes for nitrogen metabolism (ureolysis and nitrate respiration) made up a greater proportion of the profile in the control treatment compared to the lowered or raised treatments. Conversely, genes for carbon metabolism (methanol oxidation) were two to threefold more prevalent in the lowered and raised treatments compared to the control. The remaining genes for metabolic pathways made up $\leq 10\%$ across treatments and were all related to carbon uptake (photoheterotrophy) or degradation (cellulolysis, fermentation, methanotrophy) (Figure 7B).

4 | Discussion

We examined species diversity within an aquatic microbial biofilm and identified differences in community structure and function associated with alternating wet-dry transitional states in a boreal peatland. Biofilm community structure and genes associated with metabolic functions differed among water table treatments, even though water depth was not different among

treatments at the time of sampling. We observed clear separation in all components of the biofilm (bacteria, fungi, eukaryotic algae and cyanobacteria) between the raised treatment and the control, but minimal separation with the lowered treatment. Differences in community composition and metabolic functions were manifested mostly by changes in relative abundance rather than the presence/absence of individual taxa or genes, which appears to be a trend broadly consistent across studies and ecosystems (Rober et al. 2014; Bengtsson et al. 2018; Emsens et al. 2020; Seward et al. 2020; Veach and Zeglin 2020; Waldrop et al. 2023; Soufi et al. 2024). The presence of similar biofilm taxa and functional potential among treatments together with differences in relative abundance indicates that hydrologic history (long timescale) regulates species membership (and thereby associated genes), but environmental conditions and interspecific interactions within the biofilm (short timescale) alters the relative abundance of species and the potential for metabolic functions. These findings are consistent with research showing that peatland microbial community assembly is structured by a combination of short-term high-disturbance and longer timescale low-intensity disturbance events (Ernakovich et al. 2022). In contrast to ecosystems where water-driven disturbance events cause non-reversible shifts in community structure (i.e., regime shift) (Beisner et al. 2003), the reoccurring nature of hydrologic variation in wetlands appears to select for a microbial community able to tolerate alternating transitional states (Arias-Real et al. 2024).

Despite similar water table position at the time of sampling, measures of species richness and diversity of all components of the biofilm were higher in the lowered treatment compared to the raised treatment. The depth of water-drawdown (30 cm) prior to the natural flooding event may have shaped the higher level of taxonomic richness observed in the lowered treatment. All three water table treatments experienced natural drought at the end of the 2021 growing season (August) and reflooded the following spring (May 2022; Figure S1) (Wyatt et al. 2024). However, owing to the experimental manipulation, water table drawdown was approximately 30 cm below the surface in the lowered treatment prior to rewetting, whereas the water table in the raised treatment remained at or near the surface of the peat (Wyatt et al. 2024). Not only do brief periods of drought disrupt competitive interactions within the microbial biofilm (Philippot et al. 2021), but previous research has demonstrated that alternating dry-wet transitional events facilitate the transport of limiting resources such as organic carbon (Kane et al. 2010; Wyatt et al. 2024) and nutrients (Wyatt et al. 2012; DeColibus et al. 2017) into the water-column that influence microbial biofilm development during flooding (Rober et al. 2023). Taken together, these findings suggest that the frequency and severity of drought influenced aquatic microbial community diversity either by reducing interspecific competition or by alleviating resource limitation. These findings are consistent with studies documenting subtle shifts in microbial diversity in response to altered hydrology in peatlands that have ecosystem-level consequences (Peltoniemi et al. 2015; Potter et al. 2017; Emsens et al. 2020; Varner et al. 2021; Le Geay et al. 2024).

Algae (including cyanobacteria) are an integral component of natural microbial communities yet until recently most aquatic studies using molecular approaches to examine community structure have excluded eukaryotic algae (West et al. 2018; Linz et al. 2020; Hamard, Céréghino, et al. 2021; Krinos et al. 2024; Le Geay et al. 2024). This lack of information makes it difficult to compare community structure from this study with that of other peatlands or aquatic ecosystems, in general (Kwon et al. 2023; Borton et al. 2025). Nevertheless, our findings mostly confirm genus-level community composition identified using microscopy (Rober et al. 2013, 2014; Ferguson et al. 2021) and in moss-associated phototrophic communities (Hamard, Céréghino, et al. 2021; Jassey, Walcker, et al. 2022). Specifically, the dominance of filamentous green algae (Chlorophytes) and cyanobacteria from the Nostocales are consistent with the literature. Together, eukaryotic algae and cyanobacteria constituted fewer OTUs to the total microbiome compared to bacteria. However, previous research has shown that cyanobacteria and chlorophytes can display a 50-fold higher efficiency in carbon dioxide (CO_2) fixation compared to other autotrophs, including plants, owing to their faster growth rate (Rossi et al. 2015). This is interesting since we observed a greater relative abundance of genes associated with carbon fixation in both the lowered and raised treatments. While we cannot distinguish autotrophic (algae including cyanobacteria) from bacterial carbon fixation potential (e.g., α-proteobacteria), we attribute the increase in genes associated with carbon fixation to autotrophic members of the biofilm since cyanobacteria made up all the chloroplast-containing taxa in the FAPROTAX metabolic profile and bacteria species capable of phototrophy were poorly represented (Table S2). In a parallel study, we observed greater CO_2 uptake in the raised treatment whereas the control and lowered treatment were sources of CO_2 to the atmosphere (Wyatt et al. 2024). Interestingly, there were fewer algal OTUs in the raised treatment (where CO_2 uptake was higher) compared to the lowered treatment but nearly double the relative abundance of oxygenic photosynthesis and photosystem/photosynthesis genes (Figure S2). These findings add to a growing body of literature showing that despite their small size and biomass, photosynthetic microorganisms have the potential to contribute to peatland carbon uptake (Jassey, Hamard, et al. 2022; Kilner et al. 2024; Wyatt et al. 2024).

In contrast to microalgae, there has been considerable emphasis on bacterial taxonomic composition in peatlands, particularly, in response to permafrost thaw (Comte et al. 2016; Seward et al. 2020; Holland-Moritz et al. 2021; Fofana et al. 2022; Waldrop et al. 2023, 2025). As anticipated, bacteria made up the greatest proportion of OTUs recovered from the biofilm and were comprised of largely ubiquitous phyla (e.g., Proteobacteria, Bacteroidetes), many of which prefer wetter conditions (Lennon et al. 2012; Veach and Zeglin 2020) and have been linked to recalcitrant organic matter breakdown (Seward et al. 2020). A key difference between our findings and those from peatland soils is the lack of known methanogens and relatively few genes unambiguously associated with methanogenesis (Lamit et al. 2021; Fofana et al. 2022; Waldrop et al. 2023). This is not surprising given that methanogens are unlikely to survive in a biofilm matrix with microbial photoautotrophs (i.e., aerobic conditions inhibit methanogenesis), particularly, in the raised treatment where oxygenic phototrophy was greater. Instead, the bacterial metabolic profile was dominated by genes associated with chemoheterotrophy, specifically ureolysis (produces ammonia; NH_4^+) and methanol oxidation. Methanol is derived from the breakdown of plant tissue and its use or transformation by

bacteria often results in CO_2 or methane production (Kolb 2009; Zalman et al. 2018). The prevalence of genes associated with nitrogen and carbon intensive metabolic pathways at a time when all water table treatments were flooded may be an indicator of how bacterial communities will respond to the expansion of aquatic habitat and increases in vascular plants that release carbon-rich subsidies into the water column upon senescence (Rober et al. 2023). Taken together, these findings show that the bacterial community at the terrestrial-aquatic interface is unique compared to the belowground community and warrants further study.

To our knowledge, the aquatic fungal community has not previously been examined within the boreal peatland biofilm matrix. The dominant fungal phyla in this study were Ascomycota and Basidiomycota, which is consistent with root-associated fungi in peatlands (Peltoniemi et al. 2012; Asemaninejad et al. 2018; Lamit et al. 2021; Rupp et al. 2021) and aquatic ecosystems more broadly (Calabon et al. 2022; Grossart et al. 2022). However, at the genus level, the aquatic fungal community was unique (i.e., no overlapping genera) from root-associated fungi from the same site (Rupp et al. 2021). This is interesting because the plant exudates that serve as a source of nutrients and carbon in the rhizosphere (Rupp et al. 2021) are the same subsidies that promote aquatic biofilm development on the peat surface (Rober et al. 2023; Wyatt et al. 2024). These results suggest that differences in the availability of limiting resources between the surface biofilm matrix and belowground peat layers, even over distances of a few centimetres, may be reflected in competitive interactions within the respective microbial communities. Specifically, microbial interactions in the aboveground biofilm may influence belowground carbon storage and nutrient cycling through changes in the form and availability of energy inputs to the microbial loop (Perryman et al. 2022).

The overall findings of our study highlight linkages between hydrologic history, aquatic biofilm community composition and the relative abundance of functional genes. Despite flooding across all water table treatments at the time of sampling, we observed variation in microbial community structure and the relative abundance of functional genes linked to past water table manipulation. Interestingly, there was no single environmental parameter that explained variation in biofilm composition. Instead, hydrologic history created conditions in which environmental parameters had a unique relationship with individual components of the biofilm and their interactions with each other. By exploring the diversity of producers and decomposers, this study represents a comprehensive look at the community structure of a freshwater microbial biofilm thereby providing necessary baseline information for future studies. For example, our experimental hydrologic gradient provides an ideal opportunity for future research to examine temporal variation in the biofilm microbiome. A metagenomic time series could reveal the extent to which the aquatic microbiome is shaped by historical conditions versus short-term pulses (Rohwer et al. 2025) as well as how stable (resistant or resilient) the microbiome is to hydrologic change (Madrigal-Trejo et al. 2023). Further, by including contributions of microbial photoautotrophs, the findings from this study expand efforts to describe global peatland microbial diversity (Seward et al. 2020; Lamit et al. 2021; Kilner et al. 2024) and provide a comprehensive genetic potential profile (taxonomic and functional). Future research exploring microbiome transcripts could validate microbial activity or microbial process rates. These measurements would be especially relevant owing to the role that peatlands play in global carbon storage and the ability for microbes to mediate biogeochemical processes (Hamard, Küttim, et al. 2021; Wilson et al. 2021; Jassey, Walcker, et al. 2022; Kilner et al. 2024; Wyatt et al. 2024).

Author Contributions

Allison R. Rober: conceptualization, investigation, funding acquisition, writing – original draft, methodology, visualization, writing – review and editing, validation, project administration, formal analysis, data curation, supervision. **Leah C. Reese:** methodology, validation, visualization, writing – review and editing, formal analysis, investigation. **Shawn P. Brown:** funding acquisition, methodology, validation, visualization, writing – review and editing, formal analysis, investigation. **Katherine D. McMahon:** funding acquisition, writing – review and editing, formal analysis, investigation. **Stilianos Louca:** formal analysis, writing – review and editing, visualization, validation. **Jillian Cieslik:** methodology. **Evan S. Kane:** resources, writing – review and editing, project administration. **Merritt R. Turetsky:** writing – review and editing, resources, project administration. **Kevin H. Wyatt:** writing – review and editing, resources, project administration, conceptualization, investigation.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in National Center for Biotechnology Information (NCBI) at https://www.ncbi.nlm.nih.gov/, reference number PRJNA1237501.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Mean water-table position among control, lowered and raised water-table treatments 2005–2022. Positive values indicate water-table position above the peat surface. **Figure S2:** Relative abundances of a selection of functional genes among water table treatments. **Table S1:** Metagenome sequencing statistics. **Table S2:** Representative bacteria taxa within each of the FAPROTAX-inferred metabolic profiles.