Environmental DNA

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Using eDNA to Assess Freshwater Bacterial Diversity Along a Forest–Non-Forest Gradient in the Afrotropics

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ABSTRACT

Healthy ecosystems are critical for maintaining ecosystem services and water security; yet many freshwater ecosystems have been subject to environmental degradation. Impacts are often greatest in water-scarce and developing regions, including across much of Sub-Saharan Africa, where many people lack access to basic drinking water. However, environmental monitoring programmes to track ecosystem health are generally lacking across this region due to limited resources and funding. Recent advances in environmental DNA (eDNA) methods offer an increasingly cost-effective and information-rich solution. Here, we explore the potential of eDNA as a tool for ecological monitoring of freshwater ecosystems in Uganda, East Africa. We sampled eDNA to quantify the bacterial diversity of rivers, streams, and swamps across a gradient of human disturbance in and around Kibale National Park, using off-the-shelf sampling methods that require minimal pre-existing infrastructure. We found distinct bacterial communities between intact and degraded habitats, but the bacterial community in rivers converged when flowing through intact forest. We identified several taxa with differential abundances that might serve as potential bioindicators of degraded ecosystems, and showed that a machine learning tool trained on eDNA can accurately differentiate between intact and degraded habitats. Our proof-of-concept study demonstrates the potential of eDNA as a practical and cost-effective biomonitoring tool for freshwater ecosystems in resource-limited regions, including Sub-Saharan Africa. We also highlight the potential benefits of protected forest in modulating bacterial composition in freshwater ecosystems.

1 | Introduction

Freshwater ecosystems support a wide range of essential ecosystem services and enhance water security by providing natural freshwater storage, regulating flows, and through water purification and the replenishment of groundwater (Creed et al. 2017; Vári et al. 2022; Piczak et al. 2023). Degradation and damage to these ecosystems can have catastrophic social, economic, and ecological consequences, particularly in Sub-Saharan Africa where water resources are often scarce,

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vulnerable to external pressures, and lack effective planning and management (Sanon et al. 2020; Jones et al. 2023). More than half of the population in Sub-Saharan Africa lacks access to basic drinking water, and an estimated 160 million people live under conditions of water scarcity (United Nations 2021). Environmental degradation in this region has exacerbated the water crisis, exposing populations to water-borne diseases, threatening agricultural production and livestock health, and escalating conflicts, population displacement, and poverty (Almer et al. 2017; Matchawe et al. 2022). Here, we explore the use of environmental DNA (eDNA) in quantifying freshwater ecosystem health and evaluate the contribution of protected areas in restoring water quality in and around Kibale National Park, Uganda (Taberlet et al. 2012; Deiner et al. 2017; Pawlowski et al. 2018; Altermatt et al. 2025). Located in tropical East Africa, Uganda supports a rich animal diversity, including the critically endangered chimpanzee, and a human population of almost 50 million, of which only 15% have access to potable drinking water (Nayebare et al. 2013).

Deforestation has been recognized as a primary driver of environmental degradation in Sub-Saharan Africa with direct and indirect effects on water quality and freshwater ecosystems (Dudgeon et al. 2006; Strayer and Dudgeon 2010; Vörösmarty et al. 2010; Arthington et al. 2016; Reid et al. 2019). Direct effects of deforestation include increased sedimentation and water turbidity from soil erosion and raised water temperature due to reduced shading and greater irradiance, which can impact dissolved oxygen content and aquatic organisms (Kasangaki et al. 2008; Fugère et al. 2016, 2018; Wilkinson et al. 2018). Indirect effects of deforestation include a reduction in the ability to regulate nutrient cycling and filter pollution, allowing chemicals from agricultural and urban areas, such as fertilizers, pesticides, and heavy metals, to enter water bodies, leading to nutrient pollution, toxicity, and ecosystem disruption (Bashir et al. 2020; Shah et al. 2022). Understanding the impact of human activities is essential for protecting and restoring freshwater ecosystems but requires monitoring at appropriate temporal and spatial scales.

Over the past decades, significant effort has been invested in developing biological indicators and monitoring systems for assessing the ecological status of aquatic ecosystems. A wide range of aquatic organisms from macroinvertebrates to fish, plankton and some other microorganisms have been used in biomonitoring, with their diversity, physical structures, function, and genetics informing metrics of environmental quality (Sumudumali and Jayawardana 2021; Mezgebu 2022). The development of increasingly sophisticated biomonitoring systems has provided essential information for the management and conservation of freshwater ecosystems, underpinning national and international environmental legislation in the U.S., Canada, and Europe (e.g., the U.S. Clean Water Act, the Canadian Protection Act and the EU's Water Framework Directive), and guides environmental funding (e.g., almost 80% of the total EU environmental budget involves water-related expenditure that relies on accurate estimation of water quality, Friberg et al. 2011). However, equivalent advances are critically lagging in Sub-Saharan Africa, despite recent efforts in developing South African environmental monitoring systems, such as the South Africa Scoring System and citizen science-based biomonitoring programs (Dallas 1997; Kitaka et al. 2024).

Directly applying the knowledge obtained from other regions to Sub-Saharan Africa is challenging for several reasons. First, human impacts in Sub-Saharan Africa are different from those in more developed regions. For example, fertilizer use in agriculture is lower, and there is less industrial pollution but greater input of untreated sewage, especially in urban areas. Additionally, rapid deforestation in less urban areas due to demand for arable land has transformed the abiotic and biotic structure of the landscape (Fugère et al. 2018; Dimkpa et al. 2023; Ali and Gujiba 2024). Second, biomonitoring systems rely on deep knowledge of organismal responses to environmental change. However, research on deforestation in freshwater ecosystems in Sub-Saharan Africa is still limited, and we lack detailed information on much of the biological diversity within them (Fugère et al. 2016). Third, in many developed countries, there is financial and legislative support for biomonitoring that is not available or cost-effective in less economically developed countries such as many within Sub-Saharan Africa. Developing biomonitoring programs in Sub-Saharan Africa using traditional approaches would require extensive local studies of the freshwater ecosystem that would be both time-consuming and costly (Ochieng et al. 2019; Masese et al. 2021).

In recent decades, eDNA has been developed as a powerful biomonitoring tool and as an alternative to more traditional taxonomic approaches (Pawlowski et al. 2020; Blackman, Couton, et al. 2024), and it has recently been outlined how aquatic eDNA biomonitoring can be used to address global biodiversity targets, particularly in understudied regions (Altermatt et al. 2025). Traditional methods typically rely on extensive sampling of individual organisms and meticulous taxonomic identification based on morphological features (or using staining and microscopy for detecting protists), which requires significant effort and specialized knowledge (Ochieng et al. 2019). Sequencing eDNA fragments and comparing them to reference databases allows us to capture information on the whole biotic community from environmental samples (Taberlet et al. 2012; Deiner et al. 2017). Such eDNA approaches generate vast amounts of data compared to traditional methods, and thus provide a holistic assessment of the diversity and composition of local biological communities (Pawlowski et al. 2018). Common bioindicators for water quality are often multicellular eukaryotes or diatoms, whereas the use of microbes has historically been limited to a few microorganisms such as fecal indicator bacteria and water-borne pathogens (Sagova-Mareckova et al. 2021). The recent application of eDNA methods has revealed unexpected bacterial diversity in freshwater ecosystems and enhanced our understanding of how bacterial communities respond to environmental change. For example, nitrogen enrichment from fertilizers and wastewater treatment plant effluent can lead to significantly increased abundance of *Nitrospirae* and *Sphingobacteriales* and decreased abundance of *Actinobacteria* (Wan et al. 2017). These shifts in bacterial community composition (and function) are expected to occur quickly given the short generation time and high sensitivity to environmental perturbation, making bacterial communities potentially effective bioindicators for environmental monitoring (Sagova-Mareckova et al. 2021; Altermatt et al. 2025).

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Importantly, standardized eDNA protocols, from sampling to analysis, require only limited training compared to the decades of acquired experience necessary for ecosystem specialists and taxonomic experts, and therefore might be more easily transferable to less-studied ecosystems in developing regions (von der Heyden 2023). However, the lack of suitable infrastructure and the technical and logistical challenges of sampling eDNA have limited its application, especially in remote locations where clean laboratory facilities, fridges and freezers, or even electricity may be unavailable (Deplazes-Zemp et al. 2018; Perry et al. 2022; von der Heyden 2023; Schilling et al. 2024). Recent advances in eDNA technology, including cheaper sequencing of samples and the development of simple and transportable sampling equipment, have reduced these barriers and provided opportunities for developing eDNA-based biomonitoring systems in Sub-Saharan Africa (Stat et al. 2017; Bessey et al. 2021; Blackman et al. 2021).

Over the past few years, an increasing number of studies have explored eDNA methods to identify bacterial bioindicators of freshwater ecosystem health and revealed high sensitivity of bacterial diversity and composition to environmental degradation (Hermans et al. 2024). However, few studies have explored the freshwater bacterial diversity in Sub-Saharan Africa (but see Farrell et al. 2019). This limits efficient biomonitoring in the region where deforestation poses a primary threat to freshwater ecosystems (Shapiro et al. 2023; Masolele et al. 2024). Here, using easily available and off-the-shelf field sampling equipment, we explore the potential application of eDNA as a tool for monitoring freshwater ecosystem health in and around Kibale National Park, Uganda. We sample from freshwater ecosystems inside and outside Kibale National Park, encompassing a gradient of human disturbance, to examine whether bacterial eDNA can distinguish among them and to characterize the bacterial eDNA signature of intact, healthy ecosystems as a reference point for monitoring ecosystem status.

2 | Materials and Methods

2.1 | Study Area

Kibale National Park is a 795-km^2 mid-altitude (1100–1600 m) rainforest located in southwestern Uganda (0.436° N, 30.367° E, Figure 1a). Kibale is one of the last few large forest fragments remaining in Uganda and one of the few remaining mid-altitude rain forests in East Africa. The park is situated in the foothills of the Ruwenzori Mountains and is located within the Albertine Rift Valley, which forms part of the "Eastern Afromontane" biodiversity hotspot described by Conservation International (Mittermeier et al. 2011). In 1932, Kibale was designated as a forest reserve (with controlled logging) and in 1993, the reserve was reclassified as a National Park.

About 60% of the Kibale National Park consists of tall forest (canopy > 25 m high), with the remainder comprised of wetlands, grasslands, and regenerating forest (from cut exotic timber plantations, Figure 1a). Mean annual rainfall is 1655 mm (1970–2022, range 1205–2140 mm, measured at Makerere University Biological Field Station), with two distinct wet and dry seasons (March–May and September–November are the wetter months, while June–August and December–February are the drier months). Kibale National Park is drained by two major river systems (Dura River and Mpanga River), which eventually feed into the Lake George system, and flow both within and outside of the park boundaries. The park is surrounded by a human dominated matrix of intensive agriculture of food and cash crops (e.g., bananas, cassava), pastures for goats and cows (< 10 animals/ha), exotic trees planted for timber (pine and eucalyptus trees) and large tea plantations (Fugère et al. 2020). Kibale National Park and its surrounding areas exemplify the complex interface between a growing human population and biodiversity conservation. Previous work in the park and surrounding areas outside the park has demonstrated strong impacts of land use change on freshwater chemistry and documented a reduced alpha diversity and increased beta diversity of aquatic communities in agricultural land versus forests (Fugère et al. 2016, 2018).

2.2 | Sampling

We broadly classify the freshwater ecosystems in and around Kibale National Park into three habitat types: swamp, stream, and river. These habitat types are distributed both within the protected area (hereon referred to as 'in forest'), and in more degraded sites outside the park, adjacent to human settlements, agriculture, and forest (hereon referred to as 'out forest'). We sampled the two main rivers that drain the forest, the Mpanga and Dura, both of which flow outside and inside the park. The Dura River starts close to the park and is characterized by a large swamp at its origin, while the Mpanga River flows through extensive agricultural land before entering the forest and is much more likely to be impacted by anthropogenic pressure in upstream areas (Figure 1a). For purposes here, we collected samples at three locations on each river, allowing us to compare sites out forest, adjacent to forest, and in forest (Figure 1a). We additionally collected samples in two stream and two swamp systems to compare in forest versus out forest sites across habitat types (Bigodi Swamp and Rwembaita Swamp, and Emmanuel Adegenyira Farm Stream and Mikana Stream, as examples of out and in forest habitats, respectively; Figure 1b). In total, we collected samples across 10 locations encompassing different habitat types and a gradient of ecosystem intactness. Samples were collected in June 2023, a time of the year when the climate is typically cooler and drier.

At each site, we collected five water samples within a radius (swamps) or linear distance (rivers and streams) of approximately 1 km. This number of samples has been used to capture the composition and potential heterogeneity in aquatic communities within sites (Fugère et al. 2016). Up to 1000 mL of water was sampled from just below the water surface using the eDNA Citizen Scientist Sampler powered by a 12 V DC battery and using 5-μm eDNA self-preserving filters (Smith-Root, Vancouver, Washington, USA). The sampler is designed for easy and fast water sampling in the field, and the filters use a hydrophilic resin to absorb moisture from the membrane, effectively preserving captured eDNA without the need for cold storage or a preservation buffer (Thomas et al. 2019). In addition, at four randomly selected sites, we also took duplicate samples using disposable sterile syringes and Sterivex filters to contrast the performance of the self-preserving filters with typically more

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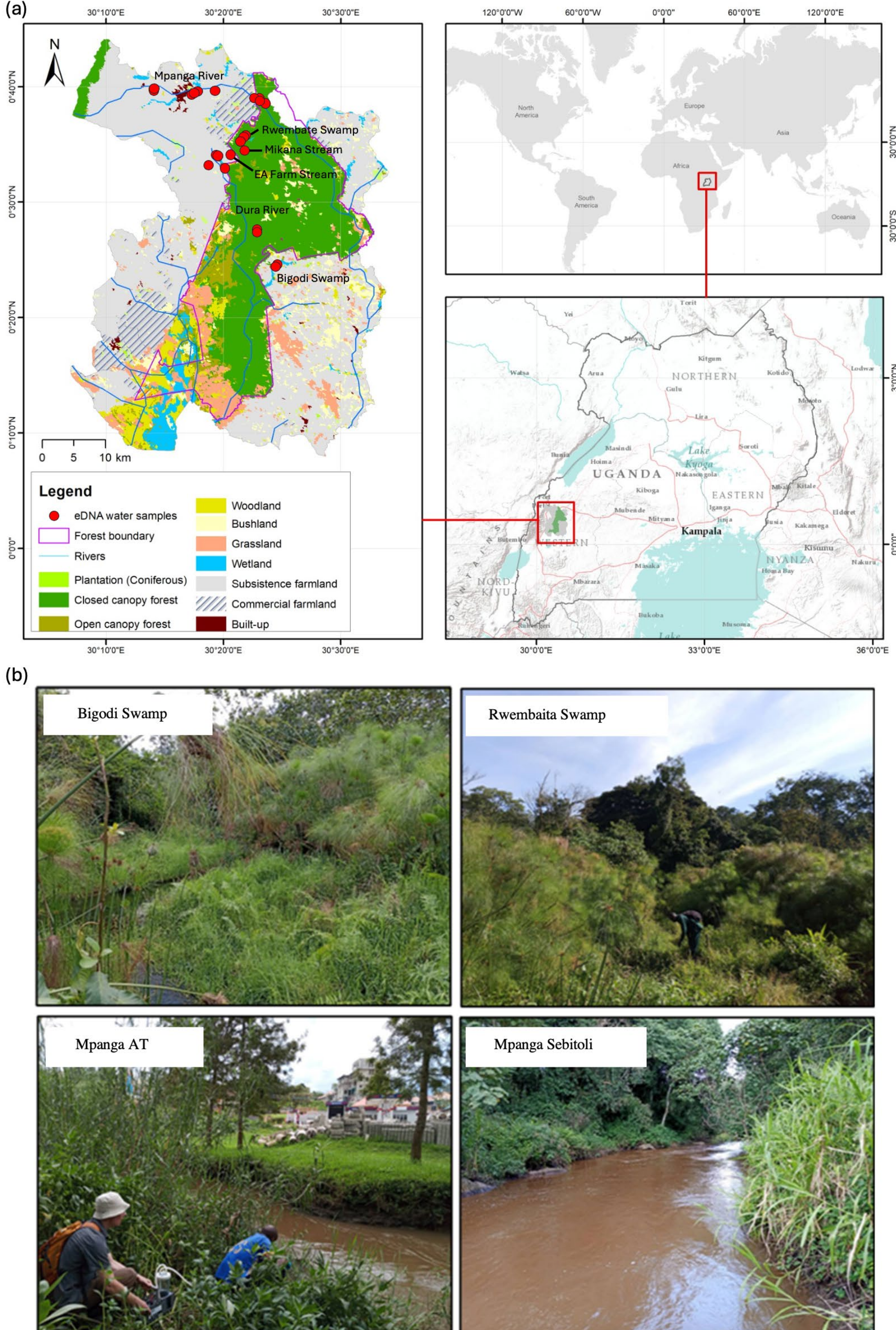

FIGURE 1 | Map of Kibale National Park with study sites annotated (a) and photographs illustrating some sampling sites (b).

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widely used sampling methods (Pawlowski et al. 2020). Because of high turbidity, filter clogging reduced the total volume of water sampled, especially for the Sterivex filters; the median volume for the eDNA citizen scientist sampler was 500 mL, and the median volume for the Sterivex filters was 200–300 mL. Finally, whilst in the field, we filtered four control samples using commercially available UV-sterilized water purchased at a local general store (Rwenzori, Rwenzori Bottling Company, Uganda). After sampling, filters were sealed in Ziploc bags with silica gel (Allison et al. 2021) and stored at ambient temperature for 1–2 weeks prior to transport to Makerere University, Kampala, for longer-term storage at −20°C. Samples were later exported to the University of Zurich, Switzerland, for DNA extraction and sequencing.

2.3 | Molecular Analyses

DNA extraction, as well as the first PCR steps, were performed in a dedicated lab with constant air overpressure and the exclusion of PCR products to limit the risk of cross-contamination (Deiner et al. 2015). DNA extractions were performed using the DNeasy PowerWater Sterivex Kit following the manufacturer's protocol (Qiagen, Germany). While DNA was extracted from Sterivex filters following the manufacturer's instructions without the bead-beating step, the self-preserving filters were removed from their casing and cut into several pieces with sterilized equipment. Half of this filter was used for DNA extraction; the other half was stored at −20°C as a backup in case of problems with extractions. The filter pieces were placed in a tube with 900 μL of lysis buffer ST1B and vortexed at minimum speed for 10 min. The rest of the protocol followed that for the Sterivex filters. For both filter types, the extracted DNA was eluted in a 100 μL elution buffer and stored at −20°C. We used blank filters to create four extraction controls that were treated with the same protocol as the actual samples.

The DNA library was created by amplifying the V3-V4 region of 16S rDNA using primers 341F and 785R from (Mühling et al. 2008). We performed a 2-step PCR approach. First, extracted DNA for each sample was amplified in three separate PCR replicates, each assigned a unique 8-bp tag (identifying the replicate). We performed the reaction in a volume of 30 μL comprising 15 μL of Qiagen Multiplex MasterMix, 0.9 μL of both forward and reverse primers (with an original concentration of 10 μM), 3 μL of DNA, and 10.2 μL of molecular grade water. We performed the PCR cycling in an Eppendorf Mastercycler X50s, starting with a denaturation step at 95°C for 15 min, followed by 31 cycles of 95°C for 45 s, 55°C for 60 s, and 72°C for 60 s. The final extension step lasted 10 min at 72°C. Amplification success was checked using a QiAxcel Screening Cartridge, and the three PCR replicates were pooled using the intensity of the QiAxcel band as a guide. We then performed a bead cleaning step using the Agencourt AMPure XP beads from Beckman Coulter with a ratio of 0.8 and following the manufacturer's protocol.

In order to bind the Nextera index adapters to our amplified fragments, we performed a second PCR step in a total volume of 30 μL, consisting of 15 μL of KAPA HiFi HotStart ReadyMix, 3 μL of cleaned PCR product from the first PCR step, 3 μL of each forward and reverse Nextera primers, and 6 μL of molecular grade water. PCR cycling started with a denaturation step at 95°C for 3 min, followed by 10 cycles of 98°C for 30 s, 55°C for 30 s, and 72°C for 30 s. The final extension step lasted 5 min at 72°C. The indexed amplicons were again cleaned with Agencourt AMPure XP beads at a ratio of 0.8. Each PCR step includes one negative control.

We quantified DNA in each sample using a selective fluorescence dye QubitTM BR DNA Assay Kit, read on a Spark Multimode Microplate Reader, and we pooled all samples at equimolar concentration. We cleaned the library pool one last time using the AMPure XP beads at a ratio of 0.8. We then assessed the quality of the library on an Agilent 4200 TapeStation System and quantified its concentration using the QubitTM (1.0) fluorometer following the manufacturer's protocol for the dsDNA HS Assay. Paired-end sequencing was performed on an Illumina NovaSeq platform at the Functional Genomic Center (FGCZ) from the University of Zürich, using a Reagent Kit v.1.5 (500 cycles) on a SP flow cell.

2.4 | Bioinformatics

We demultiplexed PCR replicates based on their internal tags and removed primer sequences using cutadapt v-2.8 (Martin 2011), allowing one mismatch on tag sequences and six on primers. We then used the DADA2 package in R (package version 1.24.0, R version 4.2) to identify the amplicon sequence variants (ASV) present in each sample (Callahan et al. 2016). Amplicon sequences were trimmed at the 200 bp and 220 bp positions of forward and reverse sequences, respectively. Reads with expected errors higher than 2% of the total nucleotide sites (i.e., four nucleotides) were discarded. Error structures were modeled using the *learnErrors* function in DADA2 with a modified error estimation function, in which we altered the loess function arguments (setting parameter span to 0.95, degree to 1 and using log-transformed totals as weights) and enforced monotonicity. This modification has better performance in predicting the error structure for our sequencing data, which uses binned error rates as standard for Novaseq sequencing. Amplicon sequences were then dereplicated and ASVs were inferred using DADA2 default parameters. Forward and reverse reads with an overlap of > 12 nucleotides were merged to construct a sample-by-sequence observation matrix. Taxonomy was assigned to each ASV by comparison to the SILVA ribosomal RNA gene database (v.138.1; Quast et al. 2013).

The total number of sequences per sample ranged from a few hundred to tens of thousands. To account for this large variation in sequencing depth, we rarefied the total number of sequences to 1000 per sample using the R package 'vegan' (v.2.6.2) and excluded sequencing files with lower sequencing depth. We found a few ASVs in our PCR negative controls but not in our field sampling negative controls, indicating some potential external contamination during molecular processing. These possible contaminant ASVs were removed based on the PCR blank controls. We then pooled PCR replicates to obtain the averaged abundance of ASVs in the 50 water samples (i.e., 10 sites * 5 water samples per site). Two samples were removed due to the low sequencing depth, resulting in 2703 ASVs across 48 samples. Most ASVs (> 90%) were identified to order or higher taxonomic level,

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67% were identified at the family level, and 49% and 4% were identified at the genus and species level, respectively. All raw sequences are deposited at European Nucleotide Archive with the study accession number 'ERP172274'.

2.5 | Bacterial Diversity and Composition

We first calculated the Shannon diversity of bacterial communities using the *diversity* function in the 'vegan' package and compared diversity estimates between site pairs using Student's *t*-test. To explore the relationship between bacterial diversity and the rivers length in degraded and preserved areas, we modeled the bacterial diversity in each sample as a function of distance from park edge for the Dura and Mpanga rivers separately, using a linear regression.

We visualized the variation in bacterial community composition between in forest and out forest sites for rivers, streams and swamps, separately, using non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarity. Statistical significance was assessed by permutational multivariate analysis of variance (PERMANOVA) for each habitat separately and for all data combined. In the latter case, the two rivers, stream and swamp were considered as four-level blocks to incorporate the nested data structure. To test whether bacterial communities in the two rivers (Mpanga and Dura) showed evidence for convergence in forest, we calculated the Sorensen pair-wise dissimilarity between rivers using the 'betapart' package, and decomposed it into turnover and nestedness components (Baselga and Orme 2012). Large values of turnover indicate the replacement of some species by others between communities, while large values of nestedness indicate that species in one sample are a subset of the other's, emphasizing the loss of certain species. In total, we compared four out-forest sites (two sites along the Mpanga paired with two sites along the Dura) and two in-forest sites (Mpanga Sebitoli versus Dura Mid). We used Tukey's HSD to test for bacterial community dissimilarity among groups.

2.6 | Indicator Species of Ecosystem Health

We conducted differential abundance analysis to identify bacterial indicators of intact (in forest) versus degraded (out forest) sites. Specifically, we applied Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) on bacterial community data of the two rivers, streams, and swamps, separately. ANCOM-BC uses bias-corrected microbial compositions to identify bacterial ASVs of differential abundance between sites (Lin and Peddada 2020). The ANCOM-BC analysis was implemented using the *ancombc* function in the ANCOMBC package (v.1.6) following default settings. Last, we tested whether habitat quality (in forests vs. out forest) could be predicted by eDNA using a machine learning algorithm. We trained binary classifiers using a random forest algorithm based on the total bacterial community data. ASVs that only occurred in one sample were removed. We split the data into training and validation datasets, with 70% of the samples used to train the classifier and the remaining 30% used for validation. The analysis was implemented in the 'caret' package (v.6.0), with the number of randomly drawn candidate variables ('mtry' parameter) tuned based on the largest ROC from a 3-fold cross-validation, and with other parameters set as default. We ran the analysis for all data combined and separately for the combined river systems; we did not train separate models for streams and swamps due to their limited number of sampling sites.

3 | Results

The bacterial communities in freshwater habitats in and around Kibale National Park were dominated by the phyla *Actinobacteriota* and *Proteobacteria*, which account for 40% and 38% of the relative abundance, respectively (Figure 2). In rivers and streams, the relative abundance of *Proteobacteria* increased while that of *Actinobacteriota* decreased from out forest to in forest sites. However, we did not observe a matching shift in bacterial composition for swamps (Figure 2). At the family (and order) level, the most abundant bacterial taxa were Microbacteriaceae (Micrococcales), Sphingomonadaceae (Sphingomonadales) and Rhodobacteraceae (Rhodobacterales), accounting for 23%, 16%, and 10% of total bacterial abundance, respectively. The abundance of these taxa also differed between out forest and in forest sites for rivers and streams. For example, Sphingomonadaceae and Rhodobacteraceae were relatively more abundant in forest while Microbacteriaceae was relatively more abundant out forest; however, once again this shift in relative abundance among taxa was not apparent in swamps (Figure S1). The Sterivex filters captured fewer bacterial taxa than the eDNA citizen scientist sampler, possibly due to the smaller volume of water filtered (Figures S2 and S3).

3.1 | Bacterial Diversity and Community Composition

Along the Dura River, Shannon diversity was higher in forest, while along the Mpanga River, Shannon diversity was higher out of forest (mean ± SD of Shannon diversity in vs. out forest are 4.13 ± 0.32 vs. 3.10 ± 0.85 in Dura River, and 3.29 ± 0.29 vs. 4.05 ± 0.58 in Mpanga River, Figure 3a,b). We did not find a significant difference in bacterial diversity between the two streams or between the two swamps (mean Shannon diversity: 3.78 ± 0.93 and 3.36 ± 0.42 for streams and swamps, respectively; Figure 3c,d). When measuring distance to forest edge, we found the lowest bacterial diversity closest to the edge for both river systems, with increased bacterial diversity towards the upstream of Mpanga River and the downstream of Dura River (Figure 4).

Bacterial community composition varied between in forest and out forest sites in both rivers; for example, bacterial communities in Mpanga Sebitoli and Dura Mid sites differ in composition from those in river sites outside forest (Figure 5a,b). Additionally, the bacteria community shows different composition between the EA Farm Stream and Mikana Stream (Figure 5d). However, there was no significant variation in bacterial composition between the two swamps (PERMANOVA, $p = 0.07$, Figure 5c, Table S1). When calculating the beta dissimilarity between the rivers, we found that bacterial composition was highly divergent out forest, but converged in forest (Figure 6a, Table S2). By examining the turnover and nestedness components, we show that the convergence in bacterial composition in forest is largely

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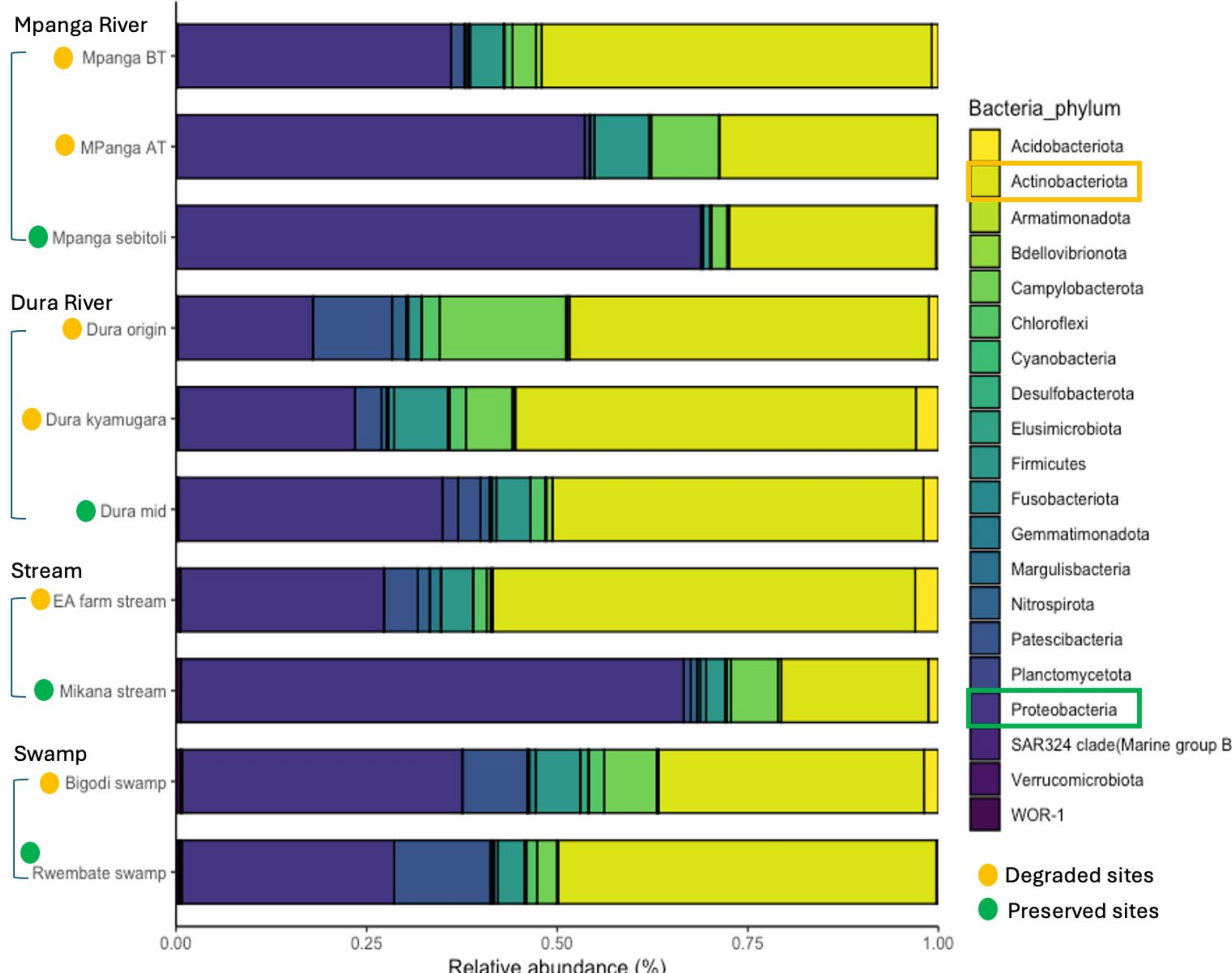

FIGURE 2 | Phylum level bacterial community composition across 10 study sites. The two most abundant bacterial phyla (*Actinobacteriota* and *Proteobacteria*) are indicated.

driven by reduced species turnover despite a small increase in nestedness, that is, the two rivers harbor distinct bacterial taxa out forest, but are more likely to share the same bacterial taxa in forest (Figure 6b,c).

3.2 | Indicator Species of Ecosystem Health

We identified bacterial ASVs that differed in relative abundance between in- forest and out-forest sites in both river systems, including 61 ASVs in the Mpanga River and 101 ASVs in the Dura River (Figure S4). For example, an ASV in the genus *Pseudorhodobacter* (ASV_54) had a relative abundance of 7.4% in the forest site Mpanga Sebitoli, but was totally absent in out-forest sites Mpanga BT and Mpanga AT. In contrast, an ASV identified as a potential pathogen, *Arcobacter cryaerophilus* (ASV_63), accounted for 4% of the relative abundance in Mpanga AT, a site close to human settlement, but was absent from the Mpanga Sebitoli site (Figure S4a). Additionally, we identified 32 ASVs that significantly differed in relative abundance between the two swamps and 94 ASVs that differed in relative abundance between the two streams ($p < 0.05$). ASVs that showed higher abundance in the forest stream than in the out-forest stream were commonly associated with *Rhodobacter*, *Novosphingobium*, and *Pseudarcobacter* (Figure S4c), while ASVs with enriched abundance in forest swamps were primarily Microbacteriaceae (e.g., ASV_46, ASV_27, and ASV_72, Figure S4d). As the abundance of these ASVs differs greatly between degraded and preserved areas, they may serve as potential indicator taxa of habitat integrity.

We showed that in forest and out forest sites could be distinguished with high accuracy on the basis of the whole bacterial community using Random Forest. The models built based on the whole dataset (combing rivers, streams and swamps) achieved a high accuracy with AUC of 0.8, with the model trained on river system data only slightly more predictive (AUC = 0.9; Figure S5).

4 | Discussion

The degradation of freshwater ecosystems has led to a severe water crisis in water-scarce and developing regions across much of Sub-Saharan Africa (Sanon et al. 2020; Jones et al. 2023).

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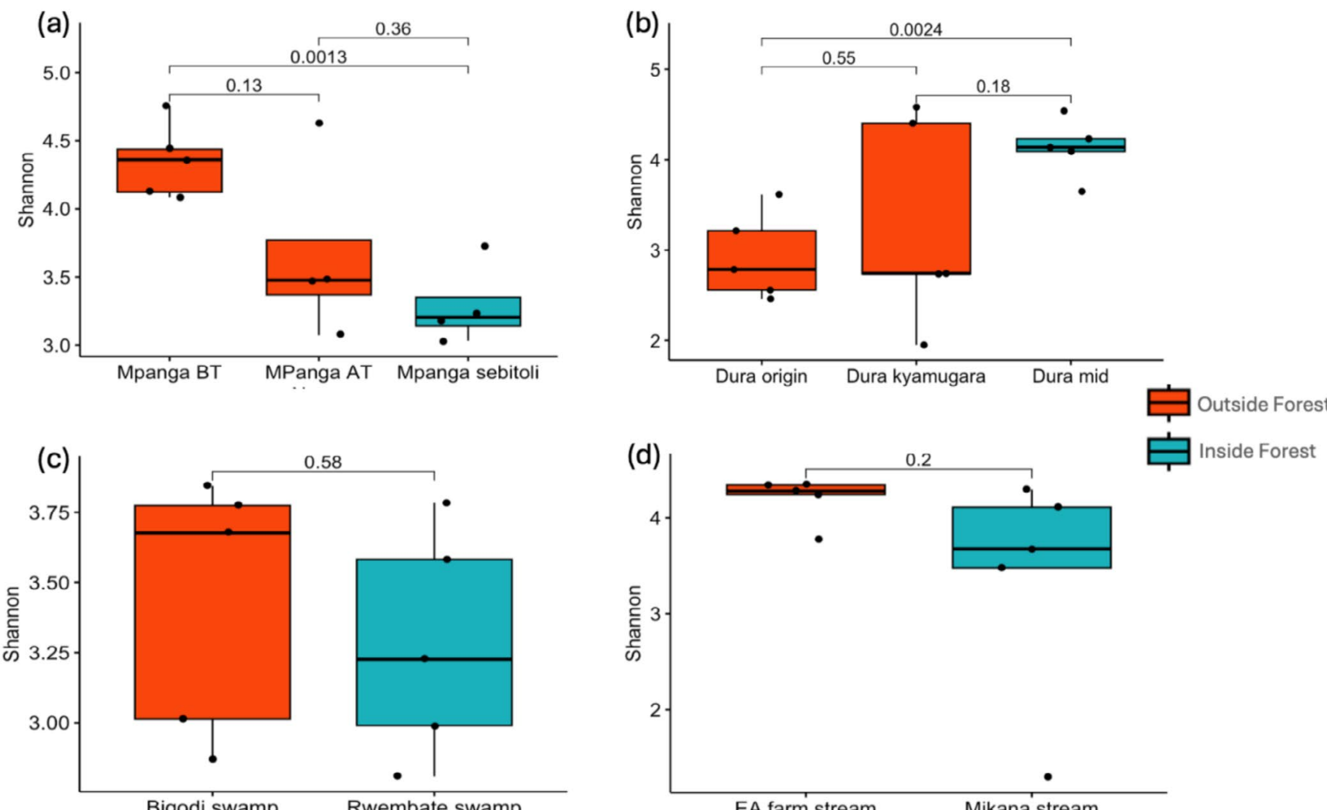

FIGURE 3 | Variation in bacterial Shannon diversity between sampling sites in the Mpanga River (a), the Dura River (b), swamps (c) and streams (d). In-forest sites include Mpanga Sebitoli, Dura Mid, Mikana Stream and Rwembaita Swamp (blue) while other sites are outside the forest (red). *p* values from pairwise *t*-tests.

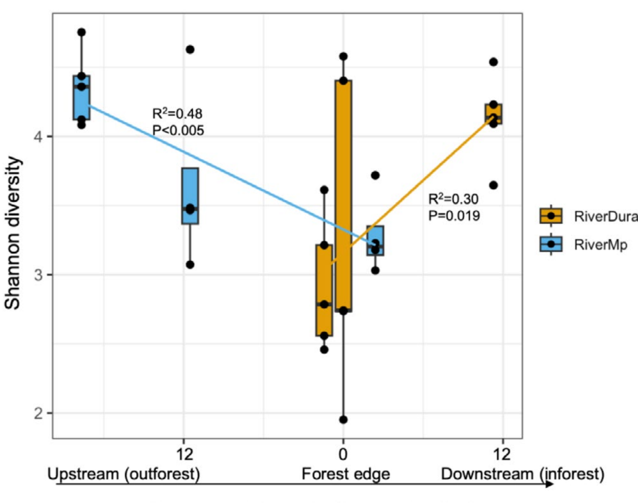

FIGURE 4 | The relationship between river length from the forest edge and the Shannon diversity in the river system. Shannon diversity was fitted as a function of river length from the forest edge for Dura River (RiverDura) and Mpanga River (RiverMp), separately. The R^2 and *p* value of the linear regression are shown.

Investment in research, monitoring, and conservation is crucial to addressing this challenge, but funding and infrastructure are often lacking (Masese et al. 2023). Here, leveraging recent advances in eDNA methods, we highlight how this developing field can help bridge this gap. Using standard and easily available sampling equipment and in collaboration with local researchers, we illustrate the potential of eDNA as a tool for interactional collaborative biomonitoring of freshwater ecosystems in Sub-Saharan Africa.

Sampling freshwater habitats in and around Kibale National Park, Uganda, we found evidence suggesting that bacterial diversity in river and stream systems is highly sensitive to habitat degradation. Anthropogenic stressors—agriculture and human settlement—appeared to select for distinct bacterial communities compared to preserved habitats; however, river systems flowing through the protected forest ecosystem converged in their bacterial community composition and were enriched in their bacterial Shannon diversity. In contrast to river and stream habitats, we did not find a clear differentiation in bacterial diversity or composition between forested and degraded swamp habitats. Using the Random Forest machine learning tool and differentially abundant bacterial taxa, we were able to differentiate between intact versus degraded ecosystems with high accuracy, corroborating approaches that have hitherto only been applied to temperate freshwater ecosystems (Keck et al. 2023). In this proof-of-concept study, we thus demonstrate the potential of eDNA as a tool for monitoring and classifying ecosystem health, and suggest that protected forest habitat may provide a valuable ecosystem service in restoring freshwater systems via reconditioning their bacterial community composition.

Our study design was highly simplified, sampling a limited number of sites; we were nonetheless able to extract information

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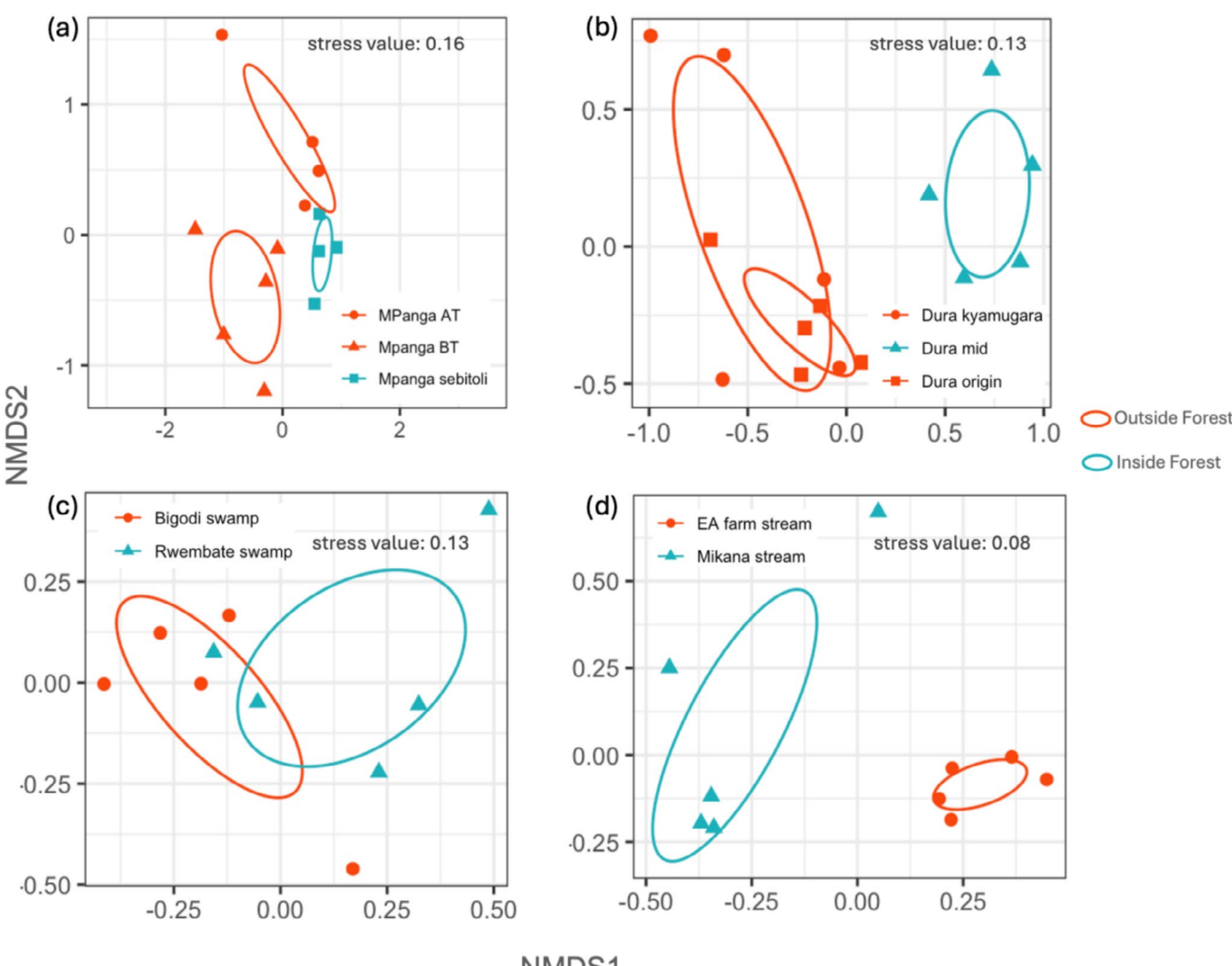

FIGURE 5 | Non-metric multidimensional scaling plot to visualize the variation in bacterial community composition between sampling sites in the Mpanga River (a), Dura River (b), swamps (c) and streams (d).

on the taxonomy and abundance of thousands of taxa, orders of magnitude more data than can be obtained from traditional ecological sampling (Fugère et al. 2016). While such benefits of eDNA are increasingly well-recognized (Stat et al. 2017; Bessey et al. 2021; Blackman et al. 2021), to date, the collection and analysis of eDNA samples have been limited across much of the global south, including sub-Saharan Africa, where budgets are constrained and infrastructure, research, and technical capacity in eDNA research have been limited (Masese et al. 2023). With advances in eDNA technology and through international research collaboration, many of these barriers are lowered. We suggest eDNA now provides an increasingly cost-effective and practical tool for studying, monitoring, and conserving freshwater ecosystems in research-resource limited regions (von der Heyden 2023; Lopes-Lima et al. 2024).

Sampling eDNA in remote locations with minimal infrastructure presents multiple challenges. The preprocessing, preservation and transportation of samples can all influence the quality of eDNA samples, particularly where it is not possible to establish a reliable 'cold-chain' of refrigeration (Deiner et al. 2015). In addition, like much of central Africa, a large part of the forest in Kibale National Park is not accessible by road, and is thus hard to access. We evaluated currently available off-the-shelf products for sample collection and preservation that required minimal infrastructure and training. By collecting samples using the highly portable eDNA Citizen Scientist Sampler and self-preserving filters, we were able to capture the majority of bacterial diversity despite some filter clogging (Figure S3). Performance (number of ASVs) was also generally better than an alternative widely used method (Sterivex filters with Longmire's buffer), likely due to the greater sampling volume (Thomas et al. 2019). In addition, we showed that locally bought UV sterilized drinking water provided reliable negative controls for field sampling with no obvious contamination detected. The self-preserving eDNA filter packs effectively preserved samples in field conditions, with longer-term storage on-site packed within commercial silica cat litter (Thomas et al. 2019). Overall, our study provided technical validation on applying eDNA methods for biomonitoring in Sub-Saharan Africa, where the application of such methods has been less common compared to more developed regions (von der Heyden 2023; Lopes-Lima et al. 2024).

We highlighted the convergent bacterial community composition in rivers flowing through preserved areas, indicating a robust microbial community in protected areas. This is consistent

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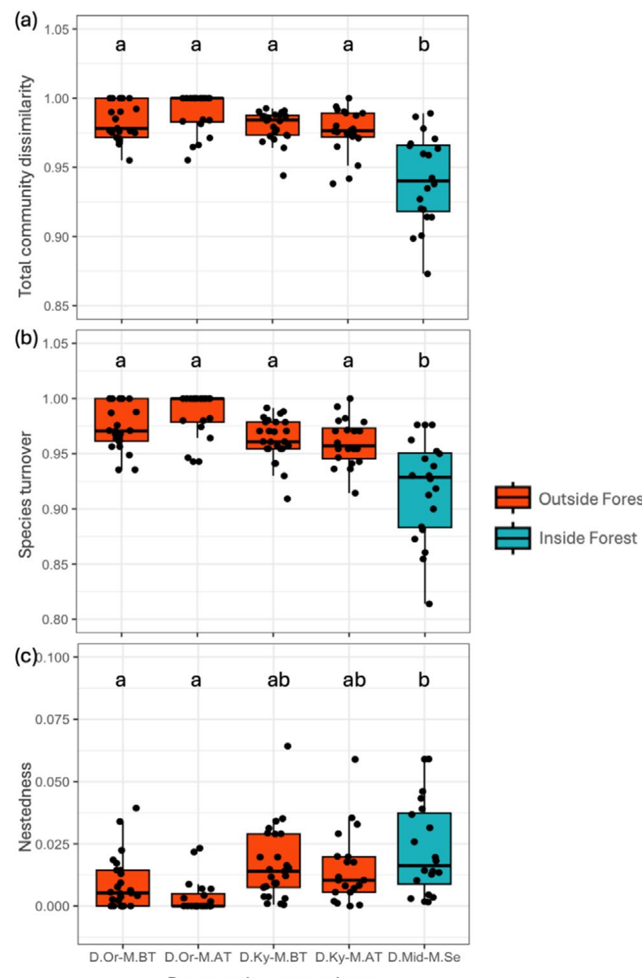

FIGURE 6 | The overall bacterial dissimilarity (a) and its turnover (b) and nestedness (c) components between Dura and Mpanga rivers. These dissimilarity indices were calculated for 4 pairs of out-forest sites (2 sites in Mpanga versus 2 sites in Dura, red) and a pair of in-forest sites (Mpanga Sebitoli versus Dura mid, blue), which were compared between groups using Tukey's HSD test. Letter labels indicate the significant difference across groups at $p < 0.05$. D.Or, Dura orgin; D.Ky, Dura Kyamugara; D.Mid, Dura Mid; M.BT, Mpanga BT; M.Se, Mpanga Sebitoli.

with previous findings that land use changes destabilize microbial biodiversity in freshwater ecosystems, further influencing microbial function and metabolic efficiency (Tolkkinen et al. 2020; Dang et al. 2021). A number of studies have examined the impact of land use change on water microbial communities, revealing correlations between anthropogenic pressure and microbial attributes such as richness, community composition, function, and interaction network structure (Kraemer et al. 2020; Mansfeldt et al. 2020; Martin et al. 2020). Our study further suggested that the converged bacterial community in protected habitats provides a potential standard for ecosystem health that may be used to quantify the impact of land use changes (Ladin et al. 2021; Keck et al. 2023).

We identified ASVs that differed in relative abundance between in forest and out forest samples. These ASVs were disproportionately present in either protected or degraded habitats, and thus they may serve as bioindicators of ecosystem intactness in the wetlands around Kibale (Blattner et al. 2021). The identified potential bioindicator ASVs fell into several distinct taxonomic groups, including Rhodobacteriaceae, Sphingomonadaceae, and Microbacteriaceae, consistent with previous findings indicating that the relative abundance of these bacterial groups is sensitive to environmental stressors such as nitrogen concentrations and wastewater treatment plant effluents (Sagova-Mareckova et al. 2021). Notably, we found the harmful bacterium *Arcobacter cryaerophilus* was present in high abundance (7%) in degraded habitat surrounded by human settlement, but was absent in forest habitat. Another ASV, from the genus *Sulfuricurvum* (ASV_97), was only found in an out-forest site on Dura River, and is indicative of a low oxygen environment with high concentration of sulfur compounds (Kodama and Watanabe 2004). In contrast, there were a number of bacteria only present in forest and absent from degraded environments, including *Pseudorhodobacter*, a bacterial genus often found in clean or chlorinated waters (Li et al. 2016). The association between the abundance of these bacterial taxa and habitat quality allows us to develop both taxon-specific and diversity-based indexes of water quality and ecosystem health, enhancing biomonitoring efforts (Pawlowski et al. 2018; Takahashi et al. 2023). However, we highlight that rivers, streams, and swamps may have different bacterial indicator taxa, and therefore developing habitat-specific eDNA bacterial indicators is necessary for accurate biomonitoring. In addition, we found that swamps exhibit less change in bacterial communities between forested and degraded environments, presumably because the unique biotic environment of swamp habitat imposes a strong filter on bacterial taxa that overwhelms external inputs (Brasil et al. 2021).

In the two river systems, we observed an unexpected gradient in bacterial diversity, with lowest diversity at the forest edge. The decreasing diversity of the Mpanga River may reflect the accumulated effect of anthropogenic impacts as it flows through increasingly degraded and transformed habitat, with environmental stress acting as a filter on bacterial diversity. However, the increasing bacterial diversity of the Dura River as it flows through the protected forests of Kibale National Park suggests that this forest habitat might help diversify aquatic microorganisms. Indeed, forests can restore water microbial diversity through many processes, such as recovering saturated hydraulic conductivity and flow paths, and seeding microbes from soil, plants, and animals (Barten and Ernst 2004; Ellison et al. 2017; Shah et al. 2022). While we emphasize that the pattern we observed is drawn from a few samples across just two separate river systems, it is consistent with recent work showing increased bacterial diversity in a freshwater forest ecosystem after the removal of disturbance (cattle), indicating the regenerative potential of intact forest (Chavarria et al. 2021).

Our study, while exploratory, adds importantly to our understanding of the microbial diversity of freshwater ecosystems in Sub-Saharan Africa. We identify key indicator taxa that allow us to differentiate between intact and degraded habitats and show how microbial community composition may be used to indicate ecosystem health. However, due to the limited sample size, this finding should be considered preliminary and further work is needed. More intensive sampling over larger spatial and temporal gradients is required to identify reliable bioindicators that are sensitive to environmental perturbation across multiple ecosystem types and to establish a robust biomonitoring system

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in the region (Goldberg et al. 2016; Altermatt et al. 2023). As a first step, within the Kibale system, we suggest expanded sampling along the Mpanga and Dura rivers both inside and outside Kibale National Park. Additionally, there is a need to sample across seasons, which can vary dramatically in precipitation regimes, to examine the stability and variability of microbial communities through time (Djurhuus et al. 2020). Data on water physical properties (pH, total nitrogen, temperature, conductivity, turbidity, etc.) and biotic conditions (e.g., vegetation diversity and density) should also be collected alongside eDNA samples to better connect microbial community composition to biotic and abiotic environments (Zhang et al. 2023; Blackman, Carraro, et al. 2024). Finally, it would be straightforward to include additional primers (e.g., those targeting ITS and 18S rRNA) in eDNA amplification to generate data on other taxonomic groups, including fungi, protists, and diatoms, which may also have potential as important bioindicators (Takahashi et al. 2023).

This work is a product of collaboration among researchers in North America, Europe, and Africa. Importantly, the study supported local graduate students (also co-authors on this manuscript), who required only minimal training prior to field sample collection, quickly building local capacity. Sample sequencing was conducted in Europe, bioinformatics analysis in Canada, with all data returned to researchers in Uganda. Through sharing knowledge, protocols, laboratories, and computing platforms, we believe it would be possible to easily scale up, sampling more intensively at local and regional scales, and across seasons, and such a model could be transferable to other Sub-Saharan African countries (Altermatt et al. 2025).

Author Contributions

T.J.D., F.A., and L.J.C. contributed to the design of the study. V.M., I.K., M.C., S.O., and N.A. contributed to data acquisition. Z.W. led the analysis with support from T.J.D. Z.W., T.J.D., L.J.C., and F.A. contributed to the interpretation of the results. Z.W. drafted the initial manuscript, and all authors contributed to the editing of the manuscript text.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All raw sequences and the associated meta data have been deposited at European Nucleotide Archive with the study accession number "ERP172274".

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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