



Climate and Environmental Variables Drive Stream Biofilm Bacterial and Fungal Diversity on Tropical Mountainsides

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Abstract

High mountain freshwater systems are particularly sensitive to the impacts of global warming and relevant environmental changes. Microorganisms contribute substantially to biogeochemical processes, yet their distribution patterns and driving mechanism in alpine streams remain understudied. Here, we examined the bacterial and fungal community compositions in stream biofilm along the elevational gradient of 745–1874 m on Mt. Kilimanjaro and explored their alpha and beta diversity patterns and the underlying environmental drivers. We found that the species richness and evenness monotonically increased towards higher elevations for bacteria, while were non-significant for fungi. However, both bacterial and fungal communities showed consistent elevational distance-decay relationships, i.e., the dissimilarity of assemblage composition increased with greater elevational differences. Bacterial alpha diversity patterns were mainly affected by chemical variables such as total nitrogen and phosphorus, while fungi were affected by physical variables such as riparian shading and stream width. Notably, climatic variables such as mean annual temperature strongly affected the elevational succession of bacterial and fungal community compositions. Our study is the first exploration of microbial biodiversity and their underlying driving mechanisms for stream ecosystems in tropical alpine regions. Our findings provide insights on the response patterns of tropical aquatic microbial community composition and diversity under climate change.

Keywords Bacteria · Fungi · Community composition · Diversity · Elevational pattern · Streams

Introduction

Exploring elevational patterns of biodiversity has been one of the central research topics in ecology and biogeography [1, 2]. High mountain areas are home to aggregations of small-ranged species [3] and support approximately one third of terrestrial and aquatic biodiversity [4]. They are also natural laboratories to examine biodiversity patterns given its dramatically changes in biotic and abiotic environments within a relatively small spatial area [5]. Unlike the diversity and distribution of macroorganisms that have been studied for centuries, elevational patterns of microbial community have only been explored for less than two decades and thus still remain poorly understood [2, 6]. Compared to terrestrial environments [7, 8], aquatic microbes such as bacteria, fungi, and diatoms are still relatively understudied [2]. Therefore, the general diversity patterns and driving mechanisms remain to be explored especially for aquatic microorganisms on mountainsides.

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Microbes are an important component of ecosystem in response to global change [9], and their biodiversity usually differs across regional and global scales as driven by various environmental variables [10, 11]. Biodiversity consists of two complementary facets, i.e., alpha and beta diversity, with the former usually measured by species richness and species abundance distribution, and the latter by community composition variations [12, 13]. For alpha diversity, species richness measures the total number of species at local sites, whereas species abundance distribution, often examined as evenness, measures the similarity level of species abundances, being both linked to multiple ecosystem processes and functioning [13, 14]. Microbes may not always follow the decreasing or hump-shaped elevational patterns in diversity traditionally observed in plants and animals [7, 15], but may show unexpected monotonically increasing patterns [11]. For beta diversity, it is originally proposed as the variation in species richness among communities in a geographical region [16] with additional methods developed since then, such as to disentangle the similarity decay in species composition with spatial distance [17]. Beta diversity has been recognized as an important facet to reveal the biodiversity patterns, and the ecological processes in maintaining the biodiversity [14, 18].

Local and regional environmental variables typically shape species occurrence across various spatial and temporal scales and constrain the biodiversity and community composition in freshwater ecosystems [19]. Among local environmental variables, pH and nutrient concentrations are the main factors in shaping the alpha diversity of diatom and bacteria in streams [11] or limiting the spatial distribution of bacterioplankton in lakes [20]. For regional environmental variables, the mean temperature of the warmest season or the mean precipitation of the coldest season serving as climatic factors is the best predictor of microbial alpha diversity and composition in three mountainsides streams across Eurasia [21]. Considering multiple aspects of biodiversity and their environmental drivers simultaneously may be helpful to improve our comprehension of the general biodiversity patterns and underlying mechanisms.

Here, we explored the patterns of biofilm bacterial and fungal diversity and community composition in the streams along an elevational gradient of 745–1874 m on Mt. Kilimanjaro, Tanzania. Mt. Kilimanjaro is located in a seasonally dry tropical climate zone, where climatic conditions are dominated by the Indian Ocean monsoon and vary greatly with elevation [22, 23]. This mountain is one of the world's biodiversity hotspots and refuges for endangered species, and offers an outstanding example to investigate microbial biodiversity in African tropical habitats along elevational gradients due to its unique topographical conditions [24]. The mountain is affected by climate change and anthropogenic activities in the past decades, such as the pronounced

decrease in glaciers thickness, leaving only a small portion covered by ice cap [25–27]. The unique microbial communities may be gradually compromised and lost with the continued recede of the glaciers on top of Mt. Kilimanjaro [28]. There is thus an urgent need to systematically study microbial diversity and ecosystem functions across the elevational gradients. In 2020, we sampled the bacteria and fungi of stream biofilm on Mt. Kilimanjaro and focused on three key objectives: (i) Are there general elevational patterns in bacterial and fungal species richness and evenness in the stream biofilm on Mt. Kilimanjaro? (ii) How do the community compositions change along the elevational gradients for bacteria and fungi? (iii) What are the key drivers among environmental factors underlying biodiversity patterns for bacteria and fungi?

Materials and Methods

Study Area and Field Sampling

Mt. Kilimanjaro, the Africa's highest mountain, is located on the Kenya-Tanzania border, about 300 km south of the equator [28]. The nature diversity of this site is exceptional: from lowland savannah to upper icecap, a massive range of ecosystems is presented in its slopes [29]. Our study area (3° 10' 27"–3° 26' 30" S, 37° 12' 19"–37° 34' 40" E) is located in the south slope of Mt. Kilimanjaro with an elevational range of 745–1874 m. The whole forest zone above 1700 m has been part of Kilimanjaro National Park since 2005 [30]. The region is classified as a seasonal dry tropical climate zone, with two distinct rainy seasons: long rains from March to May and short rains around November [31]. The southern slope of Mt. Kilimanjaro can be distinguished into seven vegetation zones: natural savanna woodlands (700–1100 m), traditional home gardens (1100–1700 m), lower montane forests (1700–2100 m), montane zone that consists of a mixture of dense natural and disturbed *Ocotea* forests (2100–2800 m), upper montane zone that consists of natural and disturbed *Podocarpus* forest (2800–3200 m), sub-alpine zone that consists of natural *Erica trimera* forest and degraded bushland (3500–4000 m), alpine zone dominated by several species of *Helichrysum* dwarf shrubs, and grasses (above 4000 m) [23, 30].

In February and March 2020, we sampled stream water to determine environmental variables, and scraped stream biofilm from stone surface for microbial analyses at each sampling location. Forty locations were selected along elevations, and eventually a total of 40 water and biofilm samples were separately collected. Water samples and stream biofilm were acquired according to the methods reported in previous literature [32]. Briefly, each site was divided into five or ten transects depending on the width

of streams. Ten stones were randomly selected from the shallow/stream habitats along the transects, and biofilms were obtained by scarping the upside surfaces (~9 cm² per stone) of each stone with a sterile sponge. The biofilms were collected into a composite sample as the microbial sample from each site. Water and biofilm samples were immediately stored in mobile refrigerator at -20 °C on site and were kept at -20 °C in the laboratory until the analyses of chemical variables and microbial communities.

Physicochemical and Climatic Variables

The latitude, longitude, and elevation of each sampling point were recorded using a GPS device. Physical factors of streams, including water depth and width, velocity, shading (% canopy coverage), temperature, and stone size, were determined at each sample site as shown in previous literature [32]. Briefly, water electrical conductivity and pH were determined on site by using mobile pH meters (Sanxin, China). Nitrogen oxides (NO_x⁻-N), nitrite (NO₂⁻-N), ammonium (NH₄⁺-N), and phosphate (PO₄³⁻-P) were measured using a flow injection analyzer (Skalar SA1000). Total nitrogen (TN) and total phosphorus (TP) were measured using peroxodisulfate oxidation with the spectrophotometric method [33]. Dissolved organic matter (DOM) is a diverse and complex blend of labile and persistent ingredient that actively governs the biogeochemical cycles of ecosystems [34]. The availability of resources, especially nutrients and carbon, shapes the structure and function of microbial communities. UV-vis absorption spectroscopy is a widely used tool to effectively investigate the quantity and quality of light absorbing compounds in DOM (i.e., chromophoric DOM, cDOM) [35]. Therefore, the water samples were scanned in the wavelength ranging from 200 to 800 nm with a UV-visible spectrophotometer (UV2700, Shimadzu) to obtain the absorbance data for cDOM. Mili-Q water was used as the blank. The fluorescence excitation-emission matrices (EEMs) offer a plethora of information about various fluorophores. The EEMs fluorescence spectroscopy was generated with Mili-Q water as the background using a fluorescence spectrophotometer (Fluorolog-3, Horiba). Scans were conducted at excitation wavelengths in the range of 250–450 nm at 5 nm intervals and emission wavelengths in 250–580 nm at 1 nm intervals [36].

Climatic variables were extracted using the package “raster” V3.6-3 from “WordClim” website, which is a database of globally interpolated climate data at a high spatial resolution (approximately 1 km²) [37, 38]. These variables are derived from the monthly temperature and rainfall values, and represent annual trends, seasonality, and extreme or limiting environmental factors [39].

Bacteria and Fungi Community Analysis

Genomic DNA was extracted from stream biofilm using MoBio PowerSoil DNA Isolation Kit (MoBio, Carlsbad, USA). The 16S rRNA genes of bacteria were amplified using universal primers [515F, 5'-GTG YCA GCM GCC GCG GTA A-3' and 806R, 5'-GGA CTA CNV GGG TWT CTA AT-3'] targeting the V4 region [40]. For fungi, amplicon of ITS2-2 region was targeted using the primers [gITS7F, 5'-GTG ART CAT CGA RTC TTT G-3' and ITS4R, 5'-TCC TCC GCT TAT TGA TAT GC-3'] [14]. We obtained amplification products and normalized them at equal molar concentration, and then paired-end sequenced (2 × 250bp) on the Illumina NovaSeq 6000 sequencing platform. Before sequence analysis processes, quality control was performed to select high quality sequences for bioinformatic and statistical analyses. Briefly, any reads that are shorter than 200 bp were excluded, while those with over 40% unqualified bases were discarded. Sequencing data for each bacterial or fungal sample were included in an individual fastq file. The raw sequence data were obtained after removing the barcode sequence and divided it into paired-end read files. Raw sequence data were firstly removed potential primers from the obtained 16S rRNA and ITS2-2 using qiime2-cutadapt in QIIME2[41, 42], and then were denoised and quality control using DADA2 for obtaining high-quality amplicon sequence variants (ASVs). The taxonomic assignment of each ASV for both bacteria and fungi was performed using the q2-feature-classifier, by comparing to the databases of SILVA 138 and UNITE (99% threshold levels, release 10.05.2021) [43], respectively. The fragments that could not be mapped were defined as unknown sequences. ASV tables were then generated based on the mapped sequences. All above analyses were performed in QIIME2 software (version 2022.8) [44]. The mapped ASV results for each dataset in USEARCH tabular format were imported directly into the R software and merged into the integrated ASV tables for further analysis. Our study was currently focused on bacteria and fungi, and the ASVs designated as “mitochondria”, “chloroplasts”, “archaea”, and “eukaryotes” were removed from the bacterial communities before further analyses. The sequences were rarefied to 30,007 and 51,166 per sample for bacteria (30,007–73,717 sequences) and fungi (51,166–106,161 sequences), respectively, using the “rrarefy” function in R package “vegan” V 2.6-2.

Statistical Analyses

The stream chromophoric dissolved organic matter (cDOM) spectroscopic data of absorbance and fluorescence were analyzed using the R package “staRdom” V1.1.25. Briefly,

we used “staRdom” to perform parallel factor analysis (PARAFAC) of excitation-emission matrices (EEMs) [45], including “peak picking” of EEM. Fluorescence indices, absorbance indices and absorbance slope indices were calculated from EEMs and absorbance spectra [46]. The indices of cDOM included classical peaks based on manual peak picking: B (tyrosine-like), T (tryptophan-like), A (humic-like), M (marine humic-like), C (humic-like), which are proxies for tyrosine-like, tryptophan-like, UV humic-like, microbial-derived and visible humic-like DOM, respectively [47, 48]. Other indices were also included to reflect various aspects of DOM: Humification index (HIX) ranges from 0 to 1, and higher values mean greater degree of DOM humification [49]. Autochthonous productivity index (BIX) has values < 1 suggesting the presence of autochthonous and fresh DOM, and < 0.6 indicating low or zero autochthonous DOM production [50]. Fluorescence index (FI) is proxy of the DOM origin, with high values suggesting the prevalence of stream-produced DOM [51]. Absorbance coefficient at 254 nm (a_{254}) is used to indicate the concentration of cDOM [52]. Spectral slope ($S_{275-295}$) is a reliable proxy of and negatively correlated with cDOM average molecular weight (MW) [53]. More details of these indices could be found in the Supporting Information (Tables S2 - S4). A four-component model was validated via split-half test and random initialization analysis along with the residuals analysis in our study, with the components being identified according to the shapes and locations of the spectral peaks [46, 54, 55]. The components identified by PARAFAC were compared with previous studies based on their Ex/Em maxima online at the “OpenFluor” database [35, 56].

We firstly examined the elevational patterns of environmental variables through linear or quadratic model based on lower value of Akaike’s information criterion (AIC) [57]. We grouped the environmental variables into four categories: that is, climatic, physical, chemical, and cDOM. The variables for these categories were: for climatic, including mean annual temperature and precipitation; for physical, including water temperature, speed, stream width, water depth, shading, and stone size; for chemical, including pH, conductivity, TN, TP, NO_x^- -N, NO_2^- -N, NH_4^+ -N, and PO_4^{3-} -P; for cDOM, including B, T, A, M, C, HIX, BIX, FI, a_{254} , and $S_{275-295}$. Pearson correlations were used to determine the covariation among environmental variables, and the highly correlated variables ($|r| > 0.75$) were excluded to minimize collinearity in the subsequent analyses.

The metrics of species richness and evenness were calculated to characterize the two aspects of bacterial or fungal alpha diversity using the R package “vegan” V 2.6-2 [58]. Chao1 is a good measure for species richness, and Pielou’s evenness for distribution of relative abundance in a community [59]. Pielou’s evenness was calculated by

$J = H/\log(S)$, where H is the Shannon-Weaver diversity index and S is the number of species [60]. The relationship between elevation and Chao1 or evenness was explored with linear or quadratic model. The better fitting model was selected based on lower value of AIC. The elevational patterns of dominant phyla/genera were also explored using a similar approach. Nonmetric multidimensional scaling (nMDS) based on the pairwise Bray-Curtis dissimilarity was carried out using the “metaMDS” function to visualize the variation in the community composition of bacteria and fungi across elevations [61, 62]. The distance-decay relationships between Bray-Curtis dissimilarity and geographical distance were estimated for bacteria or fungi [63]. In addition, we calculated the Spearman correlation between environmental factors and the relative abundance of dominant phyla using “corr.test” function. The bacterial and fungal ASVs with the threshold of incidence $> 50\%$ were separately selected, and then Spearman correlations between these ASVs and the environmental variables were calculated using “corr.test” function.

Mantel test with Spearman’s rank correlation [64] was deployed to determine the impacts of environmental variables on the bacterial and fungal community. We also calculated the Spearman’s rank correlation between environmental variables and microbial biodiversity by considering species richness and the community composition represented by nMDS1, that is, the first axis of the nonmetric multidimensional scaling (nMDS). Random Forest modeling was then used to predict the importance of the climatic and physicochemical factors on the species richness, evenness, and community composition of bacteria and fungi. Model cross-validation for random forests was performed using 75% of the dataset for training, with the remainder validated at each resampling iteration. We trained the model using 500 trees and sampled 2 predictors per node for splitting selected to minimize the root-mean-squares (measures of variance, RMSE) [65]. The R packages included “vegan” V2.6-4, “raster” V3.6-23 [66], “FactoMineR” V2.7 [67], “caret” V6.0-93 [68], and “randomForest” V4.7-1.1 [69]. Finally, the variation of microbial biodiversity was partitioned into pure effects of the four environmental categories (that is, climatic, physical, chemical, and cDOM), and their joint effects using variation partitioning analysis [70]. We used forward selection model to identify a best explained subset of environmental variables to avoid overloading the model, by using “ordiR2step” function in R package “vegan” V 2.6-2 [71]. Variation partitioning was conducted using the “varpart” function in the R package “vegan” V 2.6-2. All statistical analyses were performed in R version 4.2.1 unless otherwise indicated.

Results

Climate and Environmental Variables along Elevation Gradients

There were considerably variations of the measured environmental variables across the 40 sampling sites, such as temperature, stream morphology, and nutrient conditions (Table S1). For instance, water temperature ranged from 16.50 to 28.20 °C ($SD = 2.83$), conductivity from 16 to 251 $mS\ m^{-1}$ ($SD = 47.68$), total nitrogen from 0.23 to 1.68 $mg\ L^{-1}$ ($SD = 0.44$), and total phosphorus from 0.03 to 0.15 ($SD = 0.03$). There were generally significant ($P < 0.05$) elevational patterns for more than half of the climate and environmental variables (Figs. 1, S1). For instance, phosphate and nitrite showed a significant decreasing pattern towards higher elevations, whereas total nitrogen and nitrogen oxides first increased slightly then decreased towards higher elevations (Figs. 1, S1).

For cDOM, we found that humic substances (A, C), tyrosine (B) and absorption coefficient (a_{254}) showed significantly decreasing elevational patterns (Figs. 1, S1). Across the 40 sampling sites, HIX value ranged from 0.09

to 0.45, and only five samples had BIX values larger than 1, which indicates that most of DOM was input from surrounding terrestrial lands and showed a lower degree of humification. This is because HIX values ranged from 0 to 1 with increasing degree of humification [49], and high BIX values (>1) suggest the presence of autochthonous and fresh DOM [72] (Table S2).

The Structure and Composition of Bacteria and Fungi

The bacterial and fungal groups dominant at different taxonomic levels, i.e., phylum and genus, demonstrated multiple elevational patterns which illustrated the strong influence of elevation on community (Figs. 2, S2). For bacteria, the most dominant phylum was *Proteobacteria*, which accounted for 56.9% of the bacterial community, followed by *Cyanobacteria* (25.61%) and *Deinococcota* (4.16%). The relative abundance of *Proteobacteria* varied from 28.15 to 66.67% in the bacterial communities across the 40 sampling sites, but showed a nonsignificant elevational pattern ($P > 0.05$). Four dominant phyla showed significant elevational trends ($P < 0.01$, Fig. S2), such as a decreasing trend for *Deinococcota*, while U-shaped trends

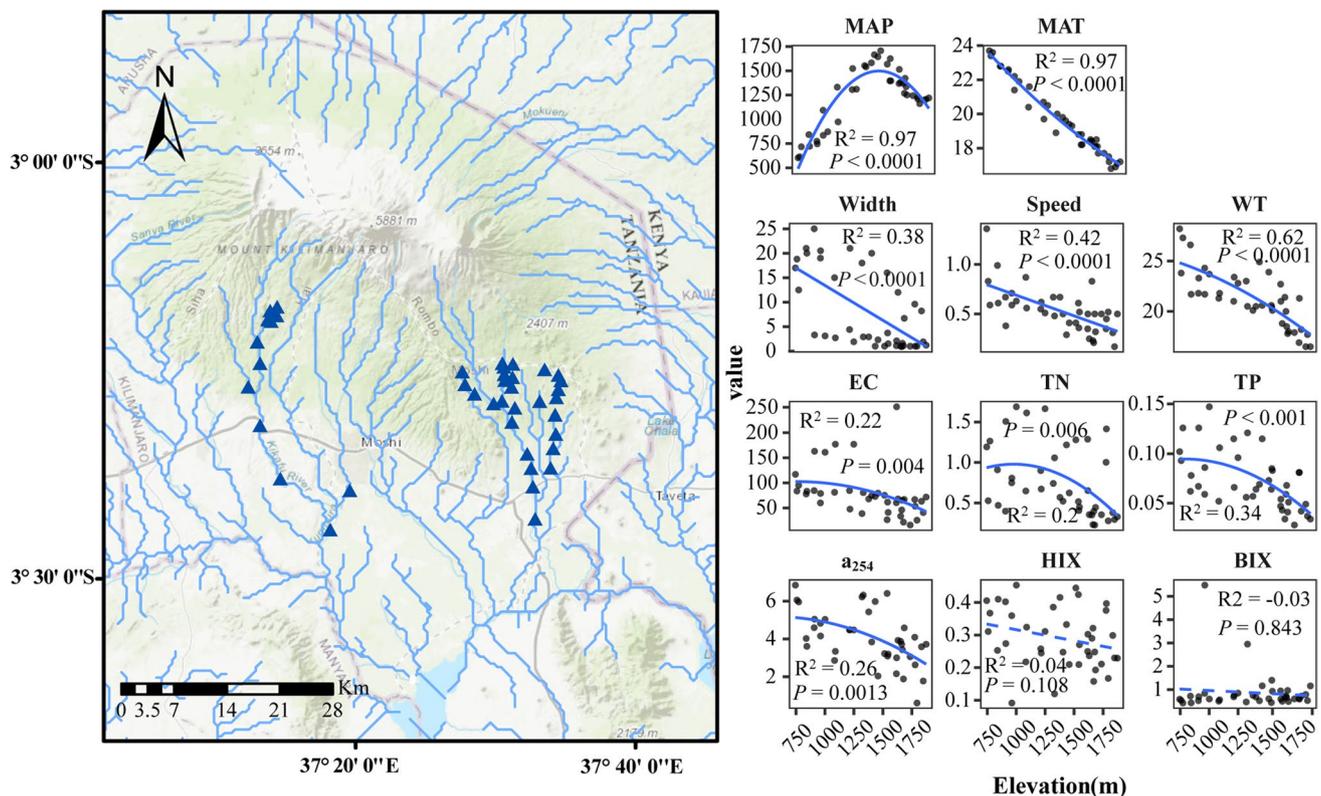


Fig. 1 Map of stream sampling sites on the Mt. Kilimanjaro, Africa, and the elevational trends of partly main climatic and local variables. The lines were fitted with linear or quadratic models. The model was

chosen based on the lower value of Akaike's information criterion. The significant ($P < 0.05$) models are shown as solid lines, and non-significant trends ($P > 0.05$) are shown as dotted lines

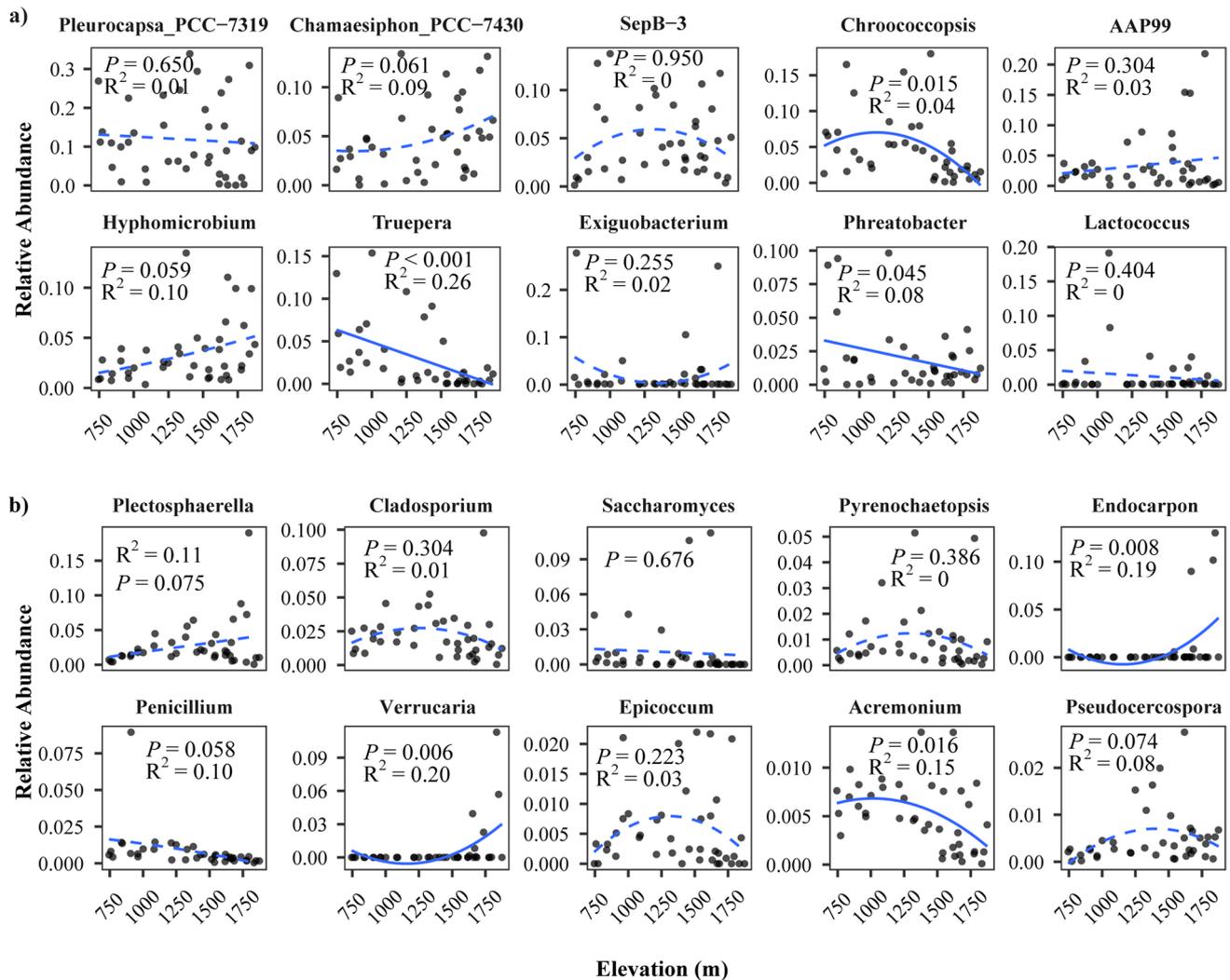


Fig. 2 The elevational trends of the relative abundance of the top 10 genera for bacteria (a) and fungi (b). The lines were fitted with linear or quadratic models. The model was chosen based on the lower value

of Akaike's information criterion. The significant ($P < 0.05$) models are shown as solid lines, and nonsignificant trends ($P > 0.05$) are shown as dotted lines.

for *Acidobacteriota*, *Planctomycetota*, and *Actinobacteriota*. Although *Proteobacteria* was the most dominant bacterial phylum, the genera belonging to the *Cyanobacteria* occupied the top four positions in the dominant genera. The genera *Chroococcopsis* showed a significantly hump-shaped elevational pattern ($P < 0.05$), while *Pleurocapsa_PCC-7319*, *Chamaesiphon_PCC-7430*, and *SpeB-3* were nonsignificant ($P > 0.05$, Fig. 2). For fungi, the most dominant phylum was *Ascomycota* (34.86%), followed by *Basidiomycota* (4.02%). The proportion of *Ascomycota* fluctuated between 14.49% and 66.01% in fungal communities across 40 sampling sites, with a nonsignificant elevational pattern (Fig. S2). Except for *Rozellomycota* and *Mortierellomycota*, the other dominant fungal phyla showed non-significant elevational trends. The most dominant genera for fungi all belong to the members

of *Ascomycota* (Fig. 2). The drivers for the dominant phyla of bacteria and fungi differed greatly, such as *Ascomycota* primarily driven by water depth, whereas *Cyanobacteria* by total phosphorus (Fig. S4).

At the ASV level, the Spearman correlations between the relative abundance of microbial ASVs with incidence $> 50\%$ and the environmental variables were mainly positive, which was particularly true for fungi with over 62.30% ASV showing positive correlations (Figs. S5, S6). Further analysis revealed that negative correlations were prevalent between elevation and the dominant ASVs for bacteria and fungi, e.g., ASVs belonging to the genera *Chroococcopsis* and *Truepera* in the bacterial community, and *Penicillium* and *Pyrenochaetopsis* in the fungal community, whereas positive correlations prevailing with other environmental factors (Fig. S7).

Bacteria and Fungi Diversity Patterns

The elevational trends of species richness and evenness showed different elevational trends between bacteria and fungi (Fig. 3). The bacteria had significant, monotonically increasing elevational patterns in both species richness and evenness ($R^2 = 0.16$, $P < 0.01$; $R^2 = 0.09$, $P < 0.05$), while non-significant elevational patterns for fungi. There were significant correlations between species richness and evenness for both bacteria and fungi (Fig. S7).

The bacterial or fungal communities were more similar at lower elevations than those at higher elevations indicated by Bray-Curtis dissimilarity (Fig. 4). Specifically, both microbial groups showed more pronounced separation toward higher elevations in nMDS plots, implying higher microbial community dissimilarity. The community compositional dissimilarity significantly increased with larger elevational distance for bacteria and fungi with Mantel's r of 0.35 and 0.23, respectively ($P = 0.0001$ and 0.0004), indicating there were predictable turnover patterns of microbial assemblage

composition along the elevational gradients (that is, elevational distance-decay relationship).

Drivers of Bacterial and Fungal Biodiversity

The Spearman correlations between fungal and bacterial diversity indices and environmental variables were mainly negative, with only four positive correlations (Fig. S8). For instance, the Chao1 for bacteria negatively correlated with Comp.1 ($\rho = 0.47$, $P < 0.01$) a_{254} ($\rho = -0.46$, $P < 0.01$), and $\text{PO}_4^{3-}\text{-P}$ ($\rho = -0.45$, $P < 0.01$). The bacterial and fungal assemblage compositions represented by nMDS1 were strongly correlated with mean annual temperature with ρ values of -0.86 and -0.90 , respectively ($P < 0.001$). It was worth noting that bacterial and fungal nMDS1 had positive Spearman correlation with mean annual precipitation with ρ values of 0.42 and 0.40 ($P < 0.05$).

Compared to fungi, the community of bacteria generally exhibited stronger correlations with various environmental variables with ρ values ranging from 0.15 to 0.41 in

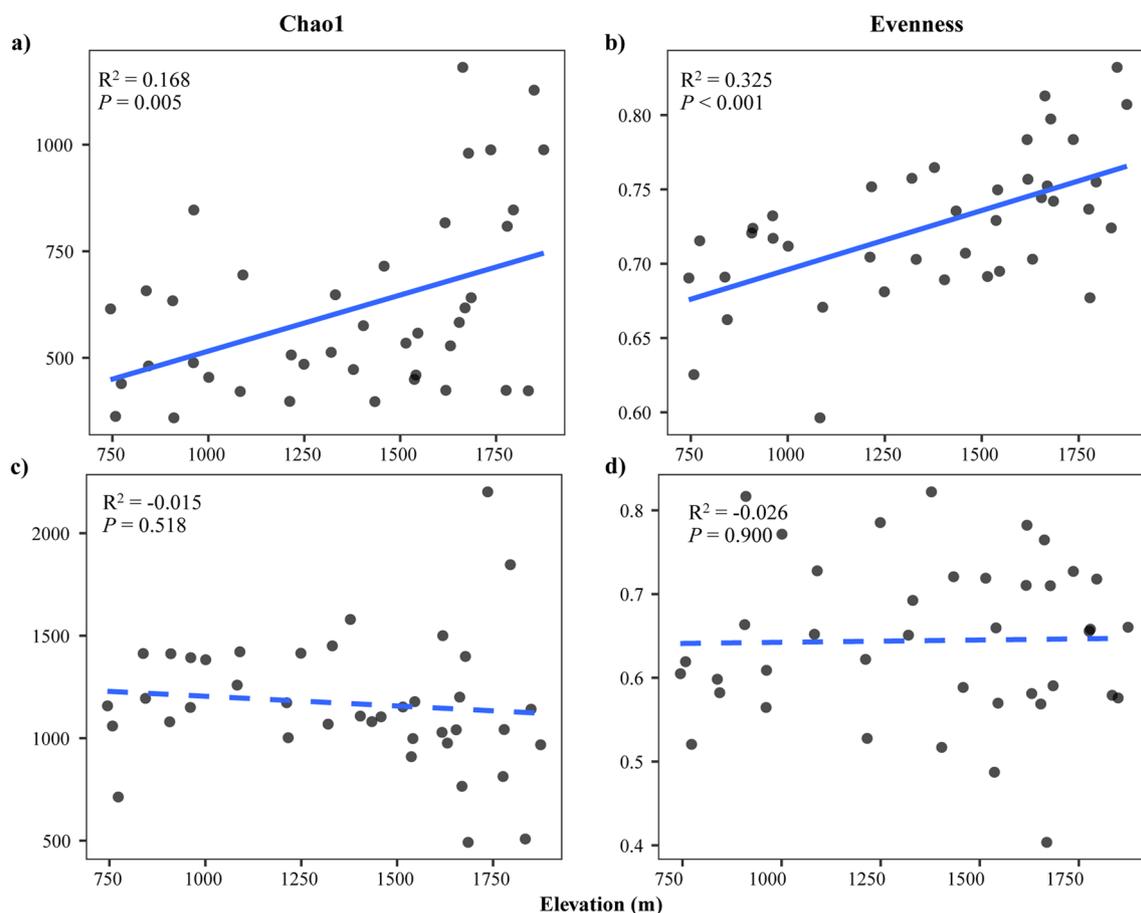


Fig. 3 Elevational alpha diversity patterns for bacteria (a–b) and fungi (c–d). For each group, we considered two indicators: Chao1 (a, c) and Evenness (b, d). The relationships between elevation and alpha

diversity were modeled by linear models. The significant ($P < 0.05$) models are shown as solid lines, and nonsignificant trends ($P > 0.05$) are shown as dotted lines

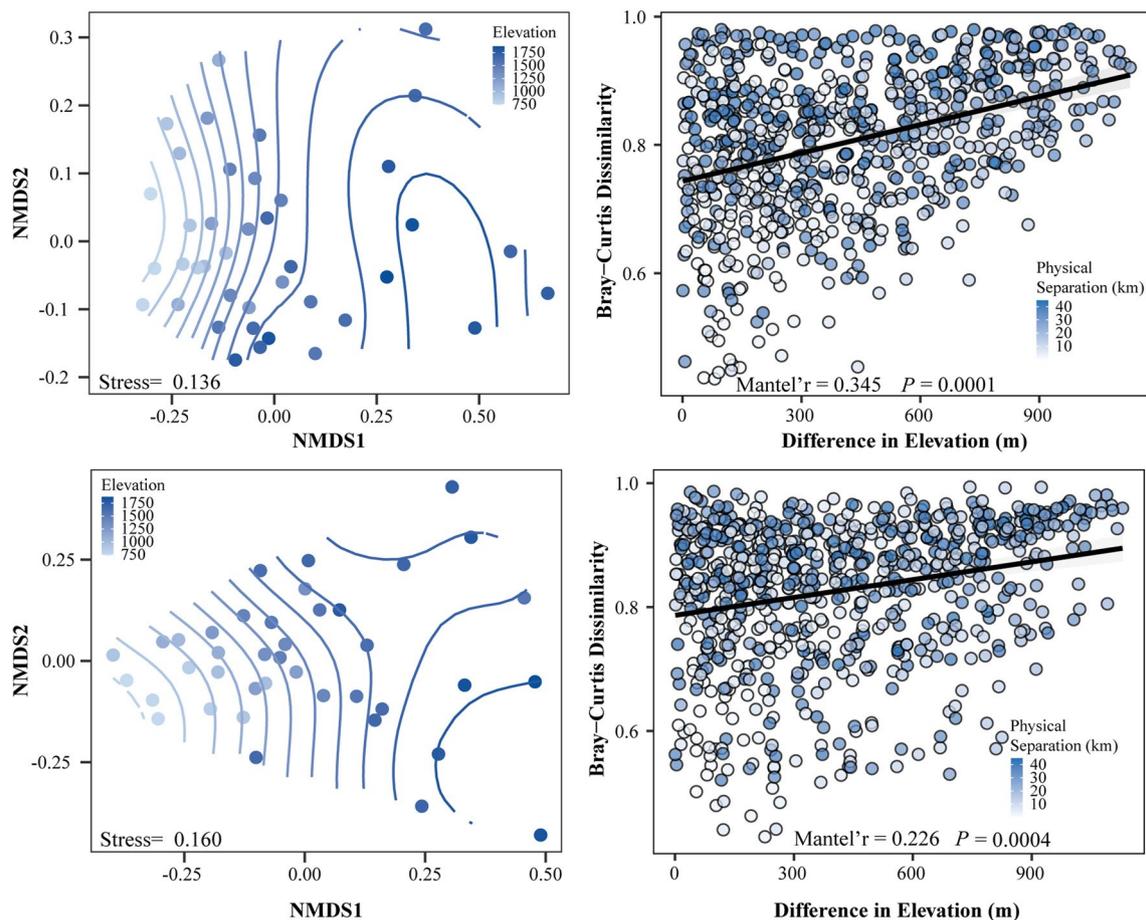


Fig. 4 The variation of community composition along the elevational gradient. The nMDS of bacterial (a) and fungal (c) communities and the elevations of the sample points were distinguished by color. The relationships between community dissimilarity and elevational change for bacteria (b) and fungi (d). The regression slope of

the linear relationship based on the Gauss generalized linear model is expressed by a solid line. According to the Mantel test (9999 permutations, $P < 0.05$), the relationships of bacteria and fungi and elevation were statistically significant

Mantel test ($P < 0.05$, Fig. 5). Specifically, the bacterial communities were more correlated with several chemical and climatic variables such as conductivity and mean annual temperature (Mantel's $r = 0.41$ and 0.35 , $P = 0.0001$), while the fungal communities were with the physicochemical variables, i.e., water depth (Mantel's $r = 0.27$, $P = 0.0001$) and conductivity (Mantel's $r = 0.31$, $P = 0.002$). In addition, bacterial and fungal communities were both significantly related to cDOM, such as a_{254} (Bacteria: Mantel's $r = 0.28$, $P = 0.0001$; Fungi: Mantel's $r = 0.16$, $P = 0.026$) and FI (Bacteria: Mantel's $r = 0.17$, $P = 0.022$; Fungi: Mantel's $r = 0.26$, $P = 0.001$).

Bacterial and fungal alpha diversity were mainly driven by chemical and physical variables, respectively, while community compositions by climatic factors, according to random forest analysis (Fig. 6). Regarding bacteria, total nitrogen was the most important factors governing species richness and evenness (Fig. 6a, b). Further research revealed

a significantly negative correlation between total nitrogen and both bacterial species richness and evenness (Fig. S7). Regarding fungi, shading was the best driver for species richness, and stream width for evenness, with shading showing a positive significant correlation with both indices (Figs. 6d, e; S7). Different from the diversity indices, the microbial community compositions were mainly driven by climatic variables with mean annual temperature being the strongest driver for both bacterial and fungal community compositions (Fig. 6c, f).

The alpha diversity were explained by different categories of explanatory variables between bacteria and fungi, while variations in community composition were mainly explained by the climatic variables, as revealed by the variation partitioning analysis (Fig. 6). Regarding alpha diversity, the physical (9.3%) and climatic variables (5.2%), and the joint effect of the them (8.7%) accounted for most of the variation in bacterial species richness, and the climatic variables

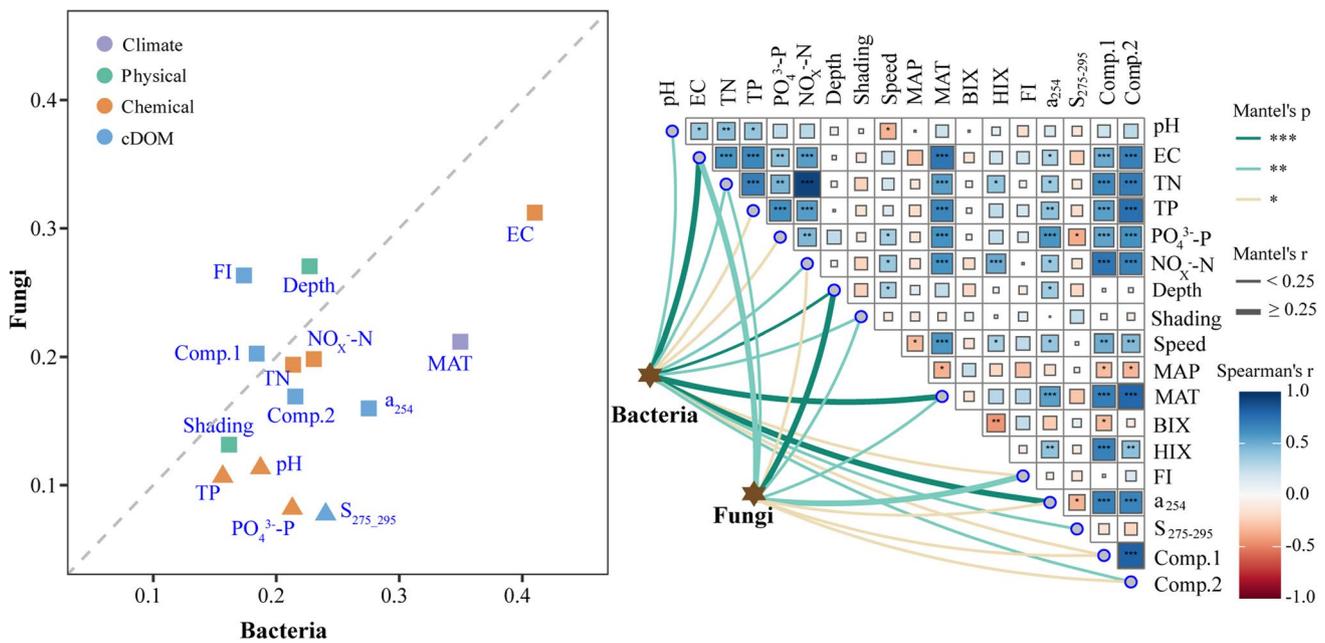


Fig. 5 Relationship between environmental factors and bacterial and fungal community. The color gradient is used to represent Spearman's correlation coefficient of pairwise comparisons of environmental variables. Spearman's correlation was used to analyze the correlation of bacterial and fungal community with each environmental factor. EC, electrical conductivity; TN, total nitrogen; TP, total phosphorus;

PO₄³⁻-P, orthophosphate; NO_x⁻-N, nitrogen oxides; Depth, water depth; Shading, riparian shading; Speed, stream flow speed; MAP, mean annual precipitation; and MAT, mean annual temperature. BIX, autochthonous productivity index; HIX, humification index; FI, fluorescence index; a₂₅₄, absorbance at 254 nm; S₂₇₅₋₂₉₅, spectral slope; Comp.1, microbial humic-like; Comp.2, Dark-incubation peak

(13.4%) explained most in bacterial evenness (Fig. 6g, h). For fungi, physical variables explained most of the variation in species richness (27.0%) and evenness (27.6%) (Fig. 6j, k). Regarding community composition, the climatic variables explained the largest variation in both bacteria and fungi with 23.2% and 34.6%, respectively (Fig. 6i, l).

Discussion

Despite the increasing number of studies on the distribution, characteristics, and diversity of microorganisms along elevational gradients, studies on freshwater ecosystems are still scarce, but are an important part of understanding the global microbial geographic distribution patterns. Here, by examining the elevational biodiversity patterns and drivers of stream bacteria and fungi on the southern slope of Mt. Kilimanjaro, we had three main findings: (1) The species richness and evenness of bacteria increased significantly towards higher elevations, whereas fungi showed non-significant trends. (2) The dissimilarity of assemblage composition increased significantly with greater elevational distances for both bacteria and fungi. (3) Bacterial and fungal alpha diversity were predominantly driven by chemical and physical factors, respectively, whereas community compositions by climatic factors.

Microbial Community Structure in the Streams

For bacterial groups, we found that the most dominant phylum in the streams biofilms was *Proteobacteria*, with the prevalence of class *Alphaproteobacteria* (Figs. S2, S3), while *Betaproteobacteria* was not detected in our stream samples. Generally, *Alpha*-, *Beta*-, and *Gammaproteobacteria*, as members of *Proteobacteria*, are typically the main classes in the freshwater ecosystems [73, 74]. Our unusual findings are partly consistent with previous study in the Cloghoge River, County Wicklow, Ireland, where *Alphaproteobacteria* was the key component of freshwater biofilms, while *Betaproteobacteria* also occurred [75]. In addition, the relative abundance of *Cyanobacteria*, as a dominant phylum second to *Proteobacteria*, was significantly correlated with total phosphorus and PO₄³⁻. This may be attributed to the large phosphorus accumulation capacity of *Cyanobacteria* at low phosphorus concentrations [76].

For fungi groups, *Ascomycota* had the broadest distribution in the stream ecosystems (Figs. S2, S3). This finding is consistent with Hulun lake that the *Ascomycota* was the most dominant phyla in the lake water and sediment [77]. *Ascomycota* is probably a potentially critical structuring element of stream biofilms that facilitates the decomposition of submerged organic matter [78, 79], hence typically plays an important role in aquatic ecosystems. Previous studies

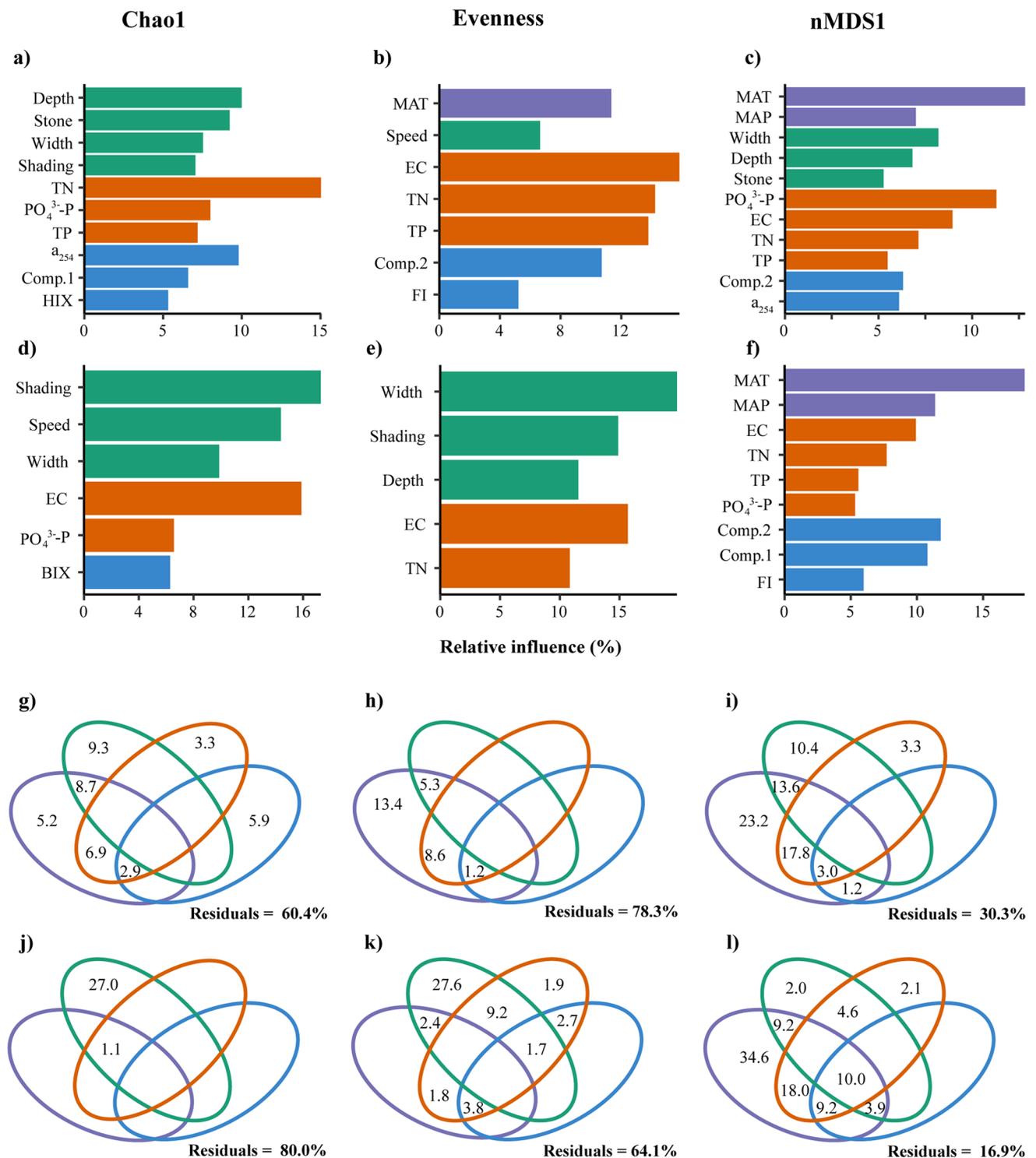


Fig. 6 Relative contribution of climate, physical, chemical, and cDOM variables in shaping the Chao1 diversity, Evenness diversity, and assemblage composition of bacteria and fungi. The independent effects of the selected variables on the biological indices for bacteria (a)–(c) and fungi (d)–(f) examined by random forest. The variances in Chao1 diversity, Evenness diversity, and assemblage composition for bacteria (g)–(i) and fungi (j)–(l) associated with the climatic and

local non-climatic variables were obtained using variation partitioning. The values in the diagrams refer to the coefficient of determination. Negative variance components were not displayed. The assemblage composition was estimated using first axis coordinates from nonmetric multidimensional scaling (nMDS) based on Bray-Curtis dissimilarity matrices. Purple, green, yellow, and blue colors represent climatic, physical, chemical, and cDOM factors, respectively.

in soil have demonstrated that the *Ascomycota* are mostly saprophytes, which breakdown difficult-to-degrade organic matter and play an important role in nutrient cycling [80]. The relative abundance of *Ascomycota* was significantly negatively correlated with water depth ($P < 0.001$, Fig. S4), which could be attributed to the fact that *Ascomycota* are aerobic fungi [81], while the oxygen availability gradually may decrease with depth. It should be noted that our findings are different from a global survey of terrestrial ecosystems showing the dominance of *Basidiomycota* [82].

The most abundant ASVs in the subset of ASVs with > 50% incidence in bacterial communities in stream biofilm were mainly belong to the genus *Pleurocapsa_PCC-7319*, followed by the genus *Chroococcopsis*. Significant correlations ($P < 0.001$) were observed between NH_4^+ and two prominent ASVs belonging to the genus *Pleurocapsa_PCC-7319*, which could be the potential nitrogen-fixing bacteria that fix atmospheric nitrogen gas to ammonia serving nitrogen in ecosystem [83]. Furthermore, four of the ten ASVs with the highest relative abundance in the subset of ASVs with >50% incidence in fungal communities were belonged to the genus *Plectosphaerella* and correlated negatively with PO_4^{3-} , while several ASVs from the genera *Cladosporium*, *Pyrenochaetopsis*, and *Penicillium* showed positive correlations with PO_4^{3-} . This phenomenon indicates that some taxa of fungi were sensitive to phosphorus conditions, which is likely relevant to human activities with phosphorus inputs. This highlights the importance of the further research on functional fungal genes such as those governing the phosphorus cycle.

Elevational Patterns of Microbial Diversity

Our results showed the contrasting elevational patterns in species richness or evenness of bacteria and fungi in the alpine stream ecosystems (Fig. 3). Regarding species richness, the monotonically increasing patterns towards higher elevations for bacteria are consistent with the previous observations for stream bacteria in the Pyrenees Mountain of Spain and the Haba Mountain of China [11], and contrary to biofilm microbial communities in the glacier-fed streams in Austria [84]. This increasing pattern does not follow the traditional ecological theory that the relationship between species richness and elevation generally shows a hump-shaped or decreasing pattern with increasing elevation for plants and animals in terrestrial ecosystems [85, 86]. The elevation-based increase in species richness observed may be linked to the sampling scale effect, as the extent of the geographical area or gradient of sampling has been confirmed to significantly affect the resulting species richness patterns [85, 87].

As for evenness, an index directly linked to ecosystem stability [88], there were also an increasing trend for

bacteria towards higher elevations (Fig. 3). This may be largely because high species evenness usually contributes to ecosystem stability, while the ecosystem at high altitudes is more vulnerable, and therefore they require high evenness for the maintenance of related functions [89]. Furthermore, we found that there was a significantly positive correlation between evenness and species richness for both bacteria and fungi (Fig. S7), which is opposite to the typically negative correlation for macroorganisms in stream ecosystems [90]. This could be explained by the lower extinctions of microorganisms, arising from the high dispersal capacity and large population, compared to macroorganisms [11, 91].

We found that the bacterial and fungal communities at the lower elevations exhibited greater similarity compared to those at higher elevations and showed a significant elevational distance-decay pattern (Fig. 4). This result suggested that elevation had crucial effects on structuring the composition of microbial communities, because elevation as a geographic factor has always been a complex and direct driving force for different climatic gradients, leading to the succession of habitats along gradients [85, 92]. The habitats at lower elevations are more benign that could allow many similar species to survive, while relatively harsher environments at higher elevations may be causing specialized species surviving in our study streams. For elevational distance-decay pattern, one possible explanation is that communities in close spatial proximity are likely to experience more similar environmental and climate condition and share more species through localized dispersal, resulting in more similar community structure than those located further apart in space [93].

Environment Effects on Microorganisms

The diversity patterns of bacterial community were primarily influenced by chemical factors, such as total nitrogen and phosphorus (Fig. 6), which are fundamental nutrient in aquatic ecosystems. For instance, the species richness and evenness of bacteria were both strongly driven by the total nitrogen, with a negative correlation observed between them (Figs. 6, S8). This may be related to the different strategies that the dominant groups in bacterial communities respond to environmental nutrient status. For example, *Gammaproteobacteria* prefer to high nutrient concentrations because they are typical R-strategists and are able to use the extra nutrients quickly (Shafi et al. 2021). In contrast, *Alphaproteobacteria* likely thrive in more oligotrophic environments as most of its species prefer to grow in oligotrophic environments [75]. At the ASV level, the ASVs sharing the same phylum also exhibited varying correlation to the environmental nutrient variables (Figs. S5, S6), further supporting that various dominant bacterial groups differently responded to environmental nutrient status. Furthermore, the

productive activities of microorganisms in the region may be limited by nitrogen levels, as the nutrient inputs East Africa are generally lower than in the densely populated subtropical and temperate zones of the Northern Hemisphere [94].

Bacterial evenness was also strongly driven by total phosphorus and showed negative correlations with total phosphorus, the concentrations of which typically correlate with human influence and are stronger at lower elevations [11]. The intensity of human activities, such as agricultural cultivation and domestic wastewater discharges, positively influences nitrogen and phosphorus contents in the aquatic environments, leading to changes in the diversity of microbial communities. On the slopes of Mt. Kilimanjaro, most of savanna woodlands have been converted to agriculture and thousands of hectares of forest were destroyed by slashing and burning, driven by human activity in search of resources [95]. Our sampling area gets closer to Kilimanjaro National Park as elevation increases; a gradual reduction in human impact may happen.

For fungi, both species richness and evenness were strongly driven by physical variables, such as riparian shading, with a significant positive correlation between both indices and riparian shading (Figs. 6, S8). Fungi are the primary decomposers of the leaf litter, therefore, more sensitive to variation in the litter environment [96]. Riparian shading could have important effects on stream organic inputs by directly affecting the amount of litter entering [97, 98], accordingly becoming a crucial driver of fungal species richness. The fungal species richness and evenness were also driven by other physical factors, such as flow speed and width, which characterized the hydrological changes (Fig. 6). This finding was consistent with previous researches that have demonstrated the significant influence of hydrological conditions in shaping bacterial diversity [99, 100]. This is because regional hydrological transport can directly influence the community, with microorganisms being actively or passively dispersing along hydrological flow paths, thereby reaching downstream habitats [101]. Stream width may also reflect human influence, as wider streams have larger catchments and more complex human impacts or nutrient conditions [17].

It should be noted that the community assemblages for bacteria and fungi were strongly driven by climatic factors (Figs. 6, S8). For instance, the mean annual temperature was the strongest driver for the dissimilarities of bacterial and fungal community compositions, which could be attributed to the effect of temperature on microbial enzyme activity and its role as a primary driver of biological metabolic processes [102, 103]. In addition, mean annual precipitation was also a predictor of fungal community composition in the streams, although its relative importance is relatively lower than that observed in other ecosystems such as soils [82]. Precipitation could arouse higher stream

flow, which causes increasing riparian soil input and sediment resuspension in rivers, eventually acting on microbial communities [17]. Generally, mean annual temperature and precipitation could directly regulate bacterial diversity and composition through speciation, competition, and dispersal of microbial communities, or indirectly through mediating other environmental factors [104].

Conclusion

In conclusion, we conducted a comprehensive study on the elevational patterns of stream bacteria and fungi in the Mt. Kilimanjaro region, and revealed the potential mechanisms underlying the observed patterns. We found that there were no consistent elevational patterns in species richness and evenness for the two microbial taxonomic groups bacteria and fungi, with the former showing a rarely monotonically increasing trend while the latter a nonsignificant trend. However, the beta diversity between the two taxonomic groups showed a consistent trend, i.e., significant elevational distance decay in community similarity. The inconsistency of species richness or evenness patterns between bacteria and fungi may be attributed to their different underlying drivers, with bacteria mainly by chemical factors such as total nitrogen and phosphorus, while fungi by physical factors such as riparian shading. For beta diversity, the consistent patterns may be related to the drivers of regional factors such as mean annual temperature and precipitation. Our study shows a new genetic data collection for better understanding the effects of multiple environmental stressors on stream microbes in this rarely studied region in Africa, and further provides novel insights on the response patterns of tropical aquatic microbial community composition and diversity under climate change.

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Author Contribution Y.Z.: data curation, formal analysis, writing—original draft, writing—review and editing. F.M.: data curation. B.O.: writing—review and editing. J.X.: data curation. L.Z.: resources. I.K.: resources. M.F.: resources. L.Z.: writing—review and editing. J.W.: conceptualization, formal analysis, writing—review and editing. All authors contributed to the article and approved the submitted version.

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Data Availability The raw sequencing data for bacterial and fungal communities have been submitted to the National Omics Data Encyclopedia (NODE: <https://www.biosino.org/node>) database under the accession number OEP004246.

Declarations

Competing of Interest The authors declare no competing interests.

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