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Streambed microbial diversity in an intermittent river system in the Andean Chocó: a genetic approach to assess the effects of seasonality

Tesis

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RESUMEN

Las comunidades microbianas en los sedimentos del fondo de los ríos pueden cumplir hasta el 96% del metabolismo de todo el ecosistema. En ríos intermitentes, mismos que experimentan condiciones de desconexión del caudal o sequía durante la época seca, los cambios en las comunidades microbianas pueden verse exacerbados en respuesta a la estacionalidad. Así mismo, los roles de los microorganismos en los ríos se vuelven más relevantes en condiciones extremas en que otros grupos no pueden estar presentes. En nuestro estudio, investigamos como cambian las comunidades de la Cuenca del Río Cube, Esmeraldas, en la estación seca y de lluvia. Nuestros principales hallazgos sugieren que la diversidad alfa es mayor en la época seca, dada por condiciones ambientales ideales para el crecimiento bacteriano y su funcionalidad. Así mismo, encontramos grupos clave en los ciclos de fósforo y nitrógeno, claves en el metabolismo de los ríos. Adicionalmente, evaluamos qué variables ambientales pueden estar afectando la diversidad alfa y encontramos que la temperatura y conductividad explican parte de la diversidad, junto a la altitud y la estacionalidad. Nuestros hallazgos tienen relevancia en vista del escenario de cambio climático que esperamos, con condiciones extremas en los que los microorganismos serán jugadores clave para mantener la funcionalidad de los ríos en esta zona.

Palabras clave: comunidades microbianas, estacionalidad, ríos intermitentes, metabolismo, 16s rRNA

ABSTRACT

Microbial communities in river sediment can account for up to 96% of the ecosystem's metabolism. In intermittent rivers, which experience flow disconnection or drought conditions during the dry season, changes in microbial communities can be exacerbated in response to seasonality. Furthermore, the roles of microorganisms in rivers become more relevant under extreme conditions where other groups may not be present. In our study, we investigated how the communities in the Cube River Basin in Esmeraldas change during the dry and rainy seasons. Our main findings suggest that alpha diversity is higher during the dry season due to ideal environmental conditions for bacterial growth and functionality. We also identified key groups involved in the phosphorus and nitrogen cycles, which are crucial for river metabolism. Moreover, we evaluated which environmental variables could be driving shifts in alpha diversity, and found that temperature and conductivity explain art of the variation in alfa diversity, along altitude and seasonality. Our findings are relevant considering the expected scenario of climate change, with extreme conditions in which microorganisms will play a key role in maintaining river functionality in this area.

Keywords: microbial communities, seasonality, intermittent rivers, metabolism, 16s rRNA

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1. INTRODUCTION

Streambed sediment microbial communities are key players in river's biogeochemical cycles and can be in charge of nearly 96% of stream's production [1]. In intermittent systems, characterized by rivers and streams where water ceases to flow for some part of the year [2], streambed dynamics are constantly modified due to the high spatiotemporal variability of streamflow [3].

Streamflow variability in intermittent systems can occur at a temporal scale, with modifications in the duration, timing, and intensity of the longitudinal, lateral, and vertical connectivity, and at the spatial scale where river fragments or pools distribute throughout the drainage area [4]. Drying phenomena occur around the world affecting all rivers and is considered that nearly 50% of the global river network is experiencing some level of intermittency [5].

Traditionally, intermittent rivers and ephemeral streams (IRES) have been observed in arid and semi-arid regions, but increasing evidence shows that intermittency can be found in almost all biomes (i.e., Mediterranean, Temperate, Alpine, Boreal, and Polar regions) [5–7]. Additionally, permanent rivers and streams are experiencing drying due to anthropogenic stressors such as over-allocation of water for human consumption [8]. Pressing effects from climate change in synergy with anthropogenic stressors are hypothesized to become the main source of change from permanent to intermittent river regimes for the next decades [9].

Across ecoregions, well-established and emerging intermittent systems are a source of cutting-edge research [10]. Australia, for instance, where 70% of rivers are considered intermittent [11], has appointed data generation as a priority for well-informed decisions on sustainable water management [12]. The South-West region of the United States and several territories of Europe have pushed research on intermittent systems to secure biodiversity and ecosystem services [13–16]. Despite evident advances, there is very limited information on intermittency in the Neotropical region (i.e., Cattinga desert in Brazil and Bolivian Altiplano) [17, 18].

In most biomes, seasonality is influenced by local environmental stressors like wind patterns, fluvial geomorphology, hydrogeology, and tectonic activity [19]. In the Neotropics, these factors might be subjacent to the meridional oscillations of the Inter-Tropical Convergence Zone (ITCZ) [20]. Therefore, precipitation (magnitude and frequency) could be critical to explain neotropical seasonality. Hence, exploring the response of fluvial systems to

seasonality in the Neotropics is of utmost relevance [21] because biodiversity and ecological functions in streams can unveil the resilience and resistance mechanisms of aquatic organisms when dry-wet seasonality prompts streamflow intermittency.

Seasonality is particularly relevant to streambed sediment dynamics (i.e., drying-rewetting regimes) as substrates configurate in mosaics according to streamflow variations [22], where microbial communities have shown to adjust and restructure in response to mobilization, desiccation, and anoxic conditions [23].

Microbial communities in the streambed become especially relevant as sediment-entrained cells´ activity drives biogeochemical processes at reach, watershed, and continental scales [24–26]. Pressing conditions of drying periods control streambed metabolic activity as a series of proximal (direct) and distal (indirect) drivers [27], that can lead to gain or loss of functionality or to significant cell death [28]. Therefore, microbial communities could be affected indirectly by distal drivers such as catchment geomorphology, land use, riparian vegetation cover, and seasonal precipitation [29], and directly by proximal drivers such as Nitrogen: Phosphorus ratio, water temperature, dissolved oxygen availability, sediment characteristics, and organic matter content [23].

In lotic ecosystems microbial diversity might unveil an entirely new contribution from global stream networks, as it has received less attention than marine and lentic ecosystems [30]. Particularly, in intermittent streams, microbial communities are considered to be the sole source of diversity to maintain ecosystem functions by metabolic activity, when other organisms like fish and macroinvertebrates might be inhibited to grow under such conditions [22].

In temperate regions, extensive research has been conducted to understand environmental factors affecting microbial communities in response to hydrology [31–33]. However, dominant controls for streambed microbial communities in the temperate regions might contrast with environmental conditions in the Neotropics.

The Andean Chocó, a Neotropical biome among the 25 global biodiversity hotspots, provides an “all-in-one” setting to start understanding the effect of seasonality on streambed dominant players.

The Andean Chocó ranges from Panamá to Ecuador (Figure 1a), it presents a unique species assemblage as the result of evolution through local and regional climatic, biogeographic, and environmental features. The Chocó separated from the Amazon (circa., 25 million years) by the uplift of the Andes. This geological effect generated strong divergent evolutionary processes, followed by the emergence of several endemic species [34]. In the Pacific lowlands of the Andean Chocó, the evaporation driving from the Ocean reaches the Colonche ridge creating a shadow effect over tropical and dry forests. This effect displaces inland precipitation creating clear seasonality in watersheds like the Cube River Basin (Figure 1c).

The Cube River is an intermittent fluvial system in the Ecuadorian Chocó, where some streams cease to flow during the dry period (Figure 1c). It exhibits drastic streamflow fluctuations changing from rivers completely inundating the riparian margins to dry rivers with disconnected stream channels. Like many humid and temperate regions, intermittency tends to concentrate in the headwaters of drying river networks, where steep slopes and small drainage areas trigger rapid delivery of water to the river channel, intensifying the influence of precipitation on the variations in streamflow [35]. In the Cube River system, streams from the upper basin experience evident desiccation in comparison to lower basin streams.

Given the importance of streambed microbes to global biogeochemical cycles and the rapidly increasing accessibility of molecular tools, understanding streambed microbial communities, metabolism, and functional diversity in the Neotropics is critical for managing aquatic ecosystems in biomes like the Chocó. Identifying drivers for microbial diversity in streambed sediments of intermittent systems in the Neotropics is of utmost importance to the global contribution of freshwater ecosystems [36].

In this context, in the streambed along the Cube River basin of the Ecuadorian Chocó, we propose to: 1) Evaluate the main stream environmental differences along the watershed in the wet and dry seasons, 2) describe streambed microbial alpha diversity using a molecular techniques to understand the main differences between the wet and dry seasons along the river watershed, and 3) identify potential drivers that contribute to explain microbial alpha diversity in intermittent systems along the watershed. We hypothesize that alpha diversity will be higher in the dry season and that seasonality could modulate proximal drivers, that would in turn explain the observed changes in microbial diversity the Cube River basin.

Our results will be the first to describe microbial diversity in streambed sediments from intermittent system in the Neotropics and will contribute to complying with the Chocó Bioregion biodiversity hotspot to understand the drivers of change to a highly threatened biome.

2. METHODS

2.1 Study area

We conducted our study in the Cube River Basin (Figure 1a) located in Northwestern Ecuador. The drainage area is 165.15 km^2 and comprises tropical-humid forests in the headwaters that become less humid towards the lowlands, along an altitudinal gradient from ~50-650 m (Figure 1d). The Northern part of the Cube River Basin is located inside the Mache-Chindul Ecological Reserve (REMACH) [37] and covering part of this territory, two private NGOs protect the last remnant of primary and secondary forests in the basin. However, despite its high levels of diversity and endemism, with crucial roles in ecosystem services provision, the rest of the basin experiments strong levels of anthropogenic pressures including pasture, agriculture, and timber extraction, with the later representing the stronger impact in the basin. Additional pressures due to mining and oil extraction have not affected this ecosystem, yet local activities driving domestic contamination might still represent a strong impact in the basin.

The main stem of the Cube River Basin receives most of its tributaries from the ecological reserve in the headwaters at ~650 m asl. (Figure 1d). Third and fourth order tributaries to the main stem are characterized by wide reaches (~37 m), while the channel width of the main stem at this elevation ranges from 10 to 27 m. with streambeds predominantly composed by cobbles and gravel, and low forest cover. Headwater streams, on the contrary, present narrower reaches (~1-15 m), abundant forest cover and bedrock as the predominant type of the streambed.

The Cube River Basin experiments 1500 mm of annual precipitation, with 80% of the total precipitation occurring in the first half of the year [38]. The basin exhibits drastic streamflow fluctuations changing from rivers completely inundating the riparian margins to dry rivers with disconnected stream channels. Therefore, streams from the headwaters experience evident desiccation in comparison to those in the lowlands.

2.2 Sampling design and field data collection

2.2.1 Sampling design

Sampling was designed to include a wide array of aquatic habitats (pools, riffles; bedrock, gravel) in the headwaters and the middle and lowland parts of the basin. To cover differences between sites in the upper and lower basin, a balanced design chose ten sites within the headwaters and middle section and ten sites from the middle section to the lowlands. With a total of twenty sample sites, distributed along the altitudinal gradient from 50 to 532 m, sampling was conducted in the wet (April-May) and the dry (Oct-Nov) seasons of 2021.

In every location, the length of the sampled reach was determined based on the stream width. For stream widths of less than 3 m, the sampled reach corresponded to 50 m length. Widths between 3 and 10 m had a sampling stream reach that ranged between 50 and 100 m. Finally, for widths over 10 m, the maximum stream reach was 150 m of length.

2.2.2 Environmental variables

Six environmental variables (temperature, pH, conductivity, dissolved oxygen, salinity, suspended solids) were measured *in situ* using a YSI multiparameter (ProDSS®). To do this, the sampled reach was divided into five transects of similar length and measures were collected in the middle of each transect at 50% of stream depth. Discharge or flow was measured using the stage method and a Doppler velocimeter OTT (Hydromet®). At the stream reach, the riparian vegetation cover, slope, and shade were calculated in the wet and dry seasons.

Additionally, for laboratory analyses of nutrients and other elements (e.g., SO_4, NO_3, PO_4, SS, TOC Ba, Ca, K, Zn, Na, Mg, Mn), water samples were collected in 500 ml Nalgene containers previously rinsed with 10% HCl. For metal analysis (e.g., Hg, Pb, Ca, Cd, Fe) 60 ml of filtered (0.45 µm) water was collected and preserved with 0.3% HSO_4. Samples were stored at -4 ^{o}C and transported to the lab. All chemical analyses were conducted at CoreLab, Universidad San Francisco de Quito.

To characterize the sediment grain size for the streambed of every location, sediment samples for the wet and dry seasons were pooled (n=20), dried at 65°C for 72 hours and screened for different grain sizes. The screening was conducted by sieving the samples in a tower of standardized sieves with the mesh size increasing from the bottom to the top (0.125mm, 0.25mm, 0.5mm, 1mm, 2mm, 4mm). After computation of the granulometric curve, the d60 coefficient was calculated as the 60% finer size of each sample.

2.2.3 Streambed sediment collection

To describe the microbial communities from the streambed, we collected sediment samples in every stream in the wet and dry seasons (n = 20), using a metal shovel previously sterilized with 96% etOH and a burner flame. For standardization, we collected different numbers of subsamples in each site depending on the length of the sampling reach. For a stream reach of 50 m length, we collected5 subsamples. In sampling stream reaches that ranged between 50 and 100 m, we collected10 subsamples. Finally, for a stream reach that was 150 m in length, the number of subsamples was 15. We selected subsamples in order to cover as many habitats present in the reach length. When possible, we collected all subsamples at streambed depths ranging from 0 to 20 cm, aiming to capture the diversity from the superficial and hyporheic zone. We recoveredtreambed sediment subsamples in a thin container and then pooled and mixed to leave for 10 min for decantation. After the samples were ready, we used a No. 10 stain-steel sieve to filter grains of 2 mm. From pooled samples, we recovered three replicates of 50 mL of sediment and stored them in falcon tubes at approximately 4 ºC during transport to the lab. Samples were stored at -20 ºC until they were analyzed [10].

2.3 Microbial DNA extraction and sequencing

To describe the microbial communities of the streambed sediments of the Cube River basin, we used one replicate (50 mL) of streambed sediment sample for DNA extraction. We extracted DNA from 0.25 g of soil using the *DNeasy PowerSoil Pro DNA Isolation Kit* (MoBio®, USA*)* according to manufacturer's instructions, with a slight modification. After adding solution CD1, wehomogenized the samples by vortex and let overnight in agitation at 60 C. The protocol was resumed from the bead-beating step. For the final DNA elution, we

used30 µl of solution C6 was used. We measured DNA quality and quantity using a Nanodrop spectrophotometer.

We purified the DNA using AmpureXP magnetic beads at a concentration of 1.8X. We measured DNA concentration in a total of 15 samples from the dry and wet seasons , followed by metagenomic sequencing of 16s rRNA marker gene (Bakt_341F: CCTACGGGNGGCWGCAG Bakt_805R: GACTACHVGGGTATCTAATCC). Sequencing was performed in an Illumina MiSeq PE300 platform (Illumina®, USA) at Macrogen, Korea.

2.4 Data analysis

2.4.1 Environmental variables

To understand the environmental setting and potential differences within the Cube River Basin, we performed aa Principal Components Analysis (PCA) with 17 environmental variables (Table S1) that were measured in the wet and dry seasons. Sites with no data or under the detection limit in water chemistry were excluded from the analysis. Based on this analysis, 10 environmental variables were selected, five ecohydrological variables: discharge, temperature, pH, conductivity, and the d60 grain size, and five chemical variables: phosphate, nitrate, ammonia, total organic carbon, and suspended solids. We compared environmenta variables between seasons using a Wilcoxon ranking-sum test at a 0.05 significance level, after testing for all statistical assumptions. We performed all these analysis in R environment, version 4.2.

2.4.2 Sequence analysis

We imported to Qiime2 software [39] paired-end 16s rRNA sequence reads from 12 paired samples (Wet and Dry season): 1, 2, 4, 6, 8, 9, 14, 15, 16, 17, 18 and 19; and 6 unpaired samples: 3, 12 and 13 (Wet Season); 7, 11 and 20 (Dry season). To do this, we used the Fastq Manifest (Phred 33) method for paired-end sequences with quality information. We performed sequence quality control using DADA2 from the q2-dada2 plug-in, which included quality filtering, chimera checking, and paired-end read joining. Forward and reverse reads

were equally truncated at 290 bp based on Q scores. We clustered sequence reads de novo into amplicon sequence variants (ASVs) at 100% sequence similarity, using DADA2 vsearch. We did axonomic classification of ASVs using qiime2-feature-classifier classify-sklearn with the naïve Bayes pre-trained Silva 138 database. We removed ASVs with unclassified domains or that were taxonomically assigned as chloroplast and mitochondria. Finally, we rarefied samples to the lowest number of reads, using using the function "rarefy_even_depth" from the "phyloseq" package in R version 4.2 [40]. 1111 ASVs were removed as they were not present in any sample after random subsampling.

We used ASVs beforeOTUs as they have shown increased resolution that allow higher accuracy in taxonomic identification and quantification [41]. As ASVs are generated using one universal grouping algorithm (100% sequence similarity), they have better consistency and reproducibility [42].

2.4.3. Microbial community diversity

We calculated microbial community alpha diversity (observed richness, Chao1, and Simpson´s reciprocal index) at the Amplicon Sequence Variant level using the function "estimate_richness" from the phyloseq package (Reference here). We calculated Pielou's evenness manually, as the Shannon´s Index divided by the logarithm of the observed ASV richness. To test for differences in alpha diversity between the dry and wet seasons we used a Wilcoxon Ranked-Sum test, prior verification of data statistical assumptions.

We DESeq2 used to calculate the relative abundance of ASVs collapsed to Class level, following the method adapted from Lee 2019. We groupedlasses with relative abundances lower than 0.05 as "Others", resulting in 11 dominant Classes. To test for differences in the relative abundances of the dominant classes between seasons we used Wilcoxon Ranked-Sum test, prior verification of data assumptions. Plots were built in R version 4.2 (package: ggplot2). (R Core Team).

2.4.4. The effect of seasonality on microbial community diversity

To analyze the effect of seasonality on microbial community diversity we used a mixed effects model [44] (Equation 1). First, we built a full model with environmental variables that presented significant differences between seasons (Eq. 1). Seasons were assigned as the fixed effect and altitude as the random effect to consider the variability that this parameter could introduce to the response variable. Using the AIC value we compared the full model to several models with all the possible combinations of variables. The selected model (Eq. 1) was tested to asses the contribution of each variable. A scatter plot of microbial diversity was used to represent the effect of significant variables from the model considering the regional effect of seasonality and the intrinsic effect of altitude on each sampling site. Analyses were carried out in the R environment, version 4.2. (R Core Team)

$$Chao1 \sim Temp + Cond + pH + TOC + (1|Altitude) + (1|Seasons) + \varepsilon \quad \text{(Eq. 1)}$$

3. RESULTS

3.1 Seasonal effects and environmental conditions

The PCA biplot shows that altitude exerts a strong effect on all site distribution on the *X-axis*, as it is strongly correlated with Dimension 1 (53.8%). The PCA shows how stream sites in the dry season separate from stream sites in the wet season and within each season's sites separate between headwaters (sites from 1 to 13) and lowlands (sites from 14 to 20). In the Y-axis several variables like nitrate and phosphate, as well as pH and conductivity, are associated with sites in the dry season. Discharge (Q), water velocity (V), channel width (Width) and suspended solids (SS), explained the distribution of sites in the wet season (Figure 2a). Environmental variables compared between seasons exhibited that discharge, temperature, suspended solids, and phosphate where higher in the wet season than the dry season. Only discharge and temperature showed statistical differences (Figure 3, Table S2). Environmental variables like pH, conductivity, and total organic carbon were significantly higher in the wet season compared to the dry season. Nutrients like nitrate, ammonia, were also higher in the wet season than the dry season but differences were not significant. Grain size characterized by d60 showed no differences between seasons (Figure 3, Table S2).

3.2 Microbial alpha diversity in the Cube River basin

We obtained 7088 ASVs in the complete set of 16s rRNA sequence reads, where 26% of the total was shared between seasons. The dry season had a higher count of unique ASVs than the wet season with 2401 and 1986, respectively (Figure 4).

Taxonomic diversity in the Cube river basin comprised 145 different classes corresponding to 43 phyla. ASVs for both seasons together were mainly distributed among Vicinamibacteria (16,70%), Bacteroidia (6,74%), Gammaproteobacteria (5,92%) Actinobacteria (4,82%), Alphaproteobacteria (4,75%) and Thermoleophilia (4,38%) (Figure 5). Remarkably, Vicinamibacteria (Acidobacteriota) surpassed with a ~3-fold increase in abundance the second and third most abundant classes (Figure 5; Table 1). Other dominant bacterial classes present in both seasons included: Acidobacteriae (3.90%), Anaerolineae (3.88%), Planctomycetes (3.15%), Polyangia (2.62%), Acidimicrobiia (2.12%), Bacilli (2.01%), and Holophagae (1.95%). Among the dominant bacterial groups (relative abundance >5%) we also found class-level taxa that could not be classified within the Acidobacteriota phylum, therefore we kept the taxonomic assignation as subgroup 5 (1.82%) and 22 (2%). Non-dominant classes (129) add up to 32.38% and correspond to 33 different phyla. Among the classes with minor abundance we also grouped 7 classes of archaea (Nanoarchaeia, Thermoplasmata, Nitrososphaeria, Methanosarcinia, Ordinarchaeia and the Deep Sea Euryarchaeotic Group (DSEG)), classified within 6 different phyla.

There were compositional differences in class proportions between seasons (Figure 6), however, the dominant bacterial classes were conserved for both seasons (Table 1). Vicinamibacteria was the most prevalent class across all samples in both seasons, with higher relative abundance in the wet season (Wet = 19.93%; Dry = 13.47%). Bacteroidia was the second most prevalent class, followed by Gammaproteobacteria. Both Bacteroidia and Gammaproteobacteria were also present in both seasons, yet their abundance changed in different directions. Bacteroidia represented 6.82% of the microbial community in the wet season and 6.66% in the dry seasons. Gammaproteobacteria, on the other hand, increased from 5.40% in the wet season to 6.43% in the dry season. (Table 1, Figure 6)

We found significant differences in the relative abundance of Alphaproteobacteria and Dehalococcoidia (p=0.05). Alphaproteobacteria covered a higher proportion of the community in the dry season, while Dehalococcoidia had stronger representation in the wet season. No significant differences were found in any other dominant group.

3.3 Seasonal effect on microbial alpha diversity

Diversity analyses between seasons (ASV-level) revealed that alpha diversity was significantly higher in the dry season compared to the wet season (**Chao1 Index:** W = 157; $p = 0.023$) (Figure 7a). Observed richness of microbial communities was significantly higher in the dry season compared to the wet season (**ObsRich:** W = 152*;* $p = 0.042$) (Figure 7c). The Inverse Simpson diversity index showed no statistical differences for a higher diversity found in the dry season compared to the wet season (Figure 7b). The microbial community evenness showed no differences between seasons (Figure 7d).

3.4 Environmental variables driving microbial community diversity

We were able to explain 64% of the variation in microbial diversity caused by conductivity and temperature using the mixed effects model, considering seasons as the fixed effect and altitude as the random effect (AIC = 672.1) (Equation 2). The full model showed less contribution to explain microbial diversity (AIC = 688.5), as pH and total organic carbon had no significant effects on the response variable (Table 2). The mixed effects model showed conductivity had a significant positive effect on diversity as Chao1 increases with conductivity, that is higher in the dry season compared to the wet season (Figure 8a). At the same time, the temperature increases towards the wet season had a negative effect on microbial diversity, as Chao1 decreases with increasing temperature towards the wet season (Figure 8b).

$$Chao1 \sim Temp + Conductivity + (1|Seasons) + (1|Altitude) + \varepsilon \quad \text{(Eq. 2)}$$

The effect of altitude as a random effect allowed to separate the intrinsic conditions that each site had due to its location in the watershed. An error on the model allows to explain part of

the diversity that can not be attributed to environmental variables analyzed. Microbial diversity increases from the wet to the dry season when conductivity increase and temperature decrease (Figure 8).

4. DISCUSSION

4.1 Microbial community composition and its response to seasonality

4.1.1 Taxonomic diversity and seasonal prevalence

The microbial community of the Cube river basin is comprised by 145 classes and 43 phyla among bacteria (138 classes; 37 phyla) and archaea (7 classes; 6 phyla). These counts are within the range reported by other studies in the neotropical regions, going from 19 to 73 phyla and 167 to 200 classes of bacteria [45–47]. Contrastingly, in the temperate region, near 26 bacterial classes [48–50] and 14 to 17 bacterial phyla [51, 52] were found in streambed sediment microbial communities across three different biomes. This suggests higher bacterial taxonomic richness in the neotropics than in the temperate zone, and agrees with previous research showing that tropical and neotropical ecosystems harbor higher biodiversity than any other region [53, 54].

Streambed sediments of the Cube river were dominated by classes corresponding to the Acidobacteriota, Bacteroidota, Actinobacteriota and Proteobacteria phyla, which are known to dominate streambed microbial communitie [22, 49, 55]. Vicinamibacteria, from the Acidobacteriota lineage, was by far the most prevalent class across sites and seasons (Fig. 5). The Vicinamibacteria class comprises aerobic, gram-negative bacteria that are able to adapt to various pH ranges [56]. Members of this class are known to carry enzymes that confer inorganic phosphorus solubilization capacity, therefore play a crucial role in soil phosphorus cycle processes, as this element is considered a limiting nutrient for primary productivity in streams and rivers [57]. However, it is interesting to point that this role might be diminished during the dry season, as Vicinamibacteria abundance decrease in response to drying. Other studies have suggested a significant decrease in abundance of Vicinamibacteria in soil in response to drought, which might also indicate drying sensitivity in this group [58, 59]. These

findings could be explained by the absence of a peptidoglycan layer in the cell wall of this bacteria and the incapacity to sporulate.

Other dominant bacterial classes were members of the Proteobacteria phylum (Gammaproteobacteria and Alphaproteobacteria). These groups have demonstrated to play important roles in nitrogen cycling where Alphaproteobacteria is a key player in atmospheric nitrogen fixation [60]. The significant increase of Alphaproteobacteria observed in the dry season (Fig. 6) maximizes the importance of their role in the face of drying conditions that limit the presence and activity of other major taxa such as fish and macroinvertebrates.

Dehalococcoidia was the only class that showed significantly highprevalence during the wet season (Table 1), which we hypothesize that may be partly due to their thermophilic traits [61]. All the lineages within the Dehalococcoidia class are anaerobic and can only obtain their energy from the rupture of carbon-chlorine bonds [62]. Most Dehalococcoidia are strict hydrogenotrophic as they require hydrogen as electron donor for their metabolism [63]. These features make this class specially interesting as dechlorination can reduce the presence of recalcitrant compounds by transforming them into organic compounds that can be taken up by other microbes [62]. Additionally, their ability to incorporate and reduce hydrogen, allows H+ to be available for other bacteria and engage in syntrophic relations [64]. Overall, Dehalococcoidia could play crucial roles in carbon cycling and their presence in these streams represents a novel finding. In our study, the presence of this taxa might indicate the presence of recalcitrant compounds in the streambed if the Cube River basin, encouraging the need to evaluate the occurrence of this compounds and their possible source.

Although bacterial taxa were highly represented in our riverine microbial communities, other domains as archaea were also present, which agrees with other studies discussing their roles in the ecological processes of freshwater ecosystems [22, 65]. The archaeal community in our study was dominated by Nanoarchaeia (Nanoarchaeota) and Nitrososphaeria (Crenarcheota), which are known to easily cope with extreme conditions such as high temperatures and acidic environments [66, 67]. Regarding ecological roles of this groups in stream sediments we can infer from studies in lakes and other environments that they might have dominant roles in ammonia oxidation in environments like streambed sediments [68, 69]. This is relevant to our

study because, despite their low abundance, archaea might be relevant players in streambed microbial metabolism under a climate change scenario, where extreme environmental conditions are expected.

4.1.2 Microbial diversity modulated by seasonal intermittency

Results from this study reveal that microbial diversity in the Cube river basin is strongly affected by seasonality, increasing in the dry season as hypothesized (Fig. 8). Shifts in bacterial diversity from the wet to the dry season suggest that environmental conditions favor for more bacterial taxa [70]. This finding contrasts with other studies assessing microbial diversity across climatic seasons in intermittent river systems in the temperate region, where richness and diversity are higher in the wet season [31, 71]. To our knowledge, the effect of seasonality in microbial alpha-diversity of intermittent of temporary streams has not been examined in the neotropics.

Our data suggest that seasonal differences in alpha-diversity must be driven by environmental differences in the Cube River basin between seasons. It has been reported that temporal or seasonal variations in microbial diversity can be associated with multiple environmental drivers, with the direction of the relation direction depending on the type of ecosystem [51, 72, 73]. Some environmental variables that have been addressed as drivers of microbial diversity shifts in rivers in the temperate zone include temperature, organic matter availability, dissolved oxygen, and nutrient concentrations [55].

4.2 Drivers of microbial diversity in an intermittency scenario

Based on PCA results and significant differences found in environmental variables between seasons, we have addressed Temperature, conductivity, pH, discharge, suspended solids and TOC as potential drivers of seasonal patterns in alpha diversity. Looking to understand if and how they could explain ASV richness (Chao1) in response to seasonality, we found that only temperature and conductivity were actual predictors of richness, in opposite directions.

As conductivity is considered a proxy of salinity, its increase in the dry season the concentration of salts rise partly due to the decrease in water level [74, 75]. This is consistent

with previous assessments of stream environmental conditions in the face of seasonality, where conductivity also shows to increase in low flow conditions. Regarding the relation of conductivity with microbial alpha-diversity, contrasting findings were reported by [51, 76], where diversity increased with the decrease of conductivity levels. . In the streambed microbial community that we describe from samples of the Cube river basin, we consider the potential presence of taxa from biofilms stablished in sediment layers. Because, despite being counterintuitive, natural beds of sediment can provide excellent substratum for biofilm growth. Therefore, we suggest that the positive relationship of conductivity with microbial diversity of the Cube river might be explained due to the effects of electrical conductivity on biofilm formation. Conductivity can increase the electrostatic exchange between microbial cells and surfaces, and promote the transport of ions and nutrients, facilitating biofilm formation and cell attachment [77, 78]. Moreover, biofilm formation and growth can, in turn, affect the electrical conductivity of the media as its matrix can trap ions, leading to an increase in conductivity [77]. This increase in conductivity can provide a beneficial environment for the presence of other microorganisms, further promoting biofilm formation [78]. In our study area, the dry season is characterized by low-flow, where microbes use different strategies to adapt, including biofilm formation, also favored by permanent streambed moisture. On the contrary, in temperate ecosystems, the dry season is characterized by total streambed drying, which might explain the absence of favoring conditions for biofilm formation and therefore the lack of positive relations between conductivity and microbial diversity.

Temperature has been reported to covary positively with microbial activity on the temperate zone, particularly below 20°C [72, 79]. It has been observed that when temperature surpasses this threshold, its effect on activity diminishes, probably due to a change in microbial composition towards taxa adapted to warmer conditions [73, 80]. In our study, the pattern is opposite, higher temperatures negatively affect microbial richness. We hypothesize that microbial taxa might respond negatively to temperature increases, as temperature rises above a certain level start selecting against non-thermotolerant taxa. The latter is partially consistent with the studies reporting a threshold in the positive effect of temperature on microbial communities [72, 73]. Therefore, a concerning implication of the negative effect of the temperature increase in microbial diversity relies on predictions that climate change will

affect the neotropics by an increase in water temperature. Temperature rising will narrow the niche for non-thermo tolerant groups and because bacterial communities are key to maintain ecosystem functions in rivers and streams, a fall in stream metabolism could occur. Nevertheless, functional redundancy would have to be assessed in order to understand whether decreases in bacterial alpha-diversity accurately represent loss of functional groups or if ecological roles are being fulfilled by various different taxa and therefore draw conclusions regarding metabolic integrity of streams in the Cube basin.

5. CONCLUSIONS

We evaluated how seasonality could affect microbial communities through seasonal shifts in the environmental settings of an intermittent river system in the Chocó. For this purpose, firstly we described taxonomic diversity in this system for the first time, finding a community dominated by key players in the cycles of nitrogen, carbon and phosphorus, besides some novel taxa as the bacterial class Dehalococcoidia and members of the archaea domain. It is also key to point that some taxa with roles in nutrient processing like nitrogen cycling are more prevalent in the dry season, which increases their functional relevance under low-flow conditions.

Regarding seasonality, we found that microbial diversity increased in the dry season, mainly driven by shifts in temperature and conductivity. Under the predictions that climate change will affect freshwater ecosystems by an increase in water temperature and extended and more intense drought periods, our study reports relevant findings regarding microbial community responses to seasonal changes in the face of stream intermittency.

Based on our current findings, we aim to continue investigating the microbial communities of this intermittent freshwater system to directly address its functional diversity and provide key information on the effect of seasonality in stream metabolic integrity, in the face of climate change.

6. REFERENCES

1. Pusch M, Fiebig DM, Brettar I, Eisenmann H, Ellis B, Kaplan L, et al. The role of micro-organisms in the ecological connectivity of running waters. *Freshwater Biology* 1998; **40**.
2. Datry T, Bonada N, Boulton AJ (eds). Intermittent rivers and ephemeral streams: ecology and management. 2017. Academic Press, an imprint of Elsevier, London ; San Diego, CA.
3. Lautz L, Fanelli R. Seasonal Biogeochemical Hotspots in the Streambed around Restoration Structures. *Biogeochemistry* 2008; **91**: 85–104.
4. Costigan KH, Kennard MJ, Leigh C, Sauquet E, Datry T, Boulton AJ. Flow Regimes in Intermittent Rivers and Ephemeral Streams. *Intermittent Rivers and Ephemeral Streams*. 2017. Elsevier, pp 51–78.
5. Fovet O, Belemtougri A, Boithias L, Braud I, Charlier J, Cottet M, et al. Intermittent rivers and ephemeral streams: Perspectives for critical zone science and research on socio-ecosystems. *WIREs Water* 2021; **8**.
6. Larned ST, Datry T, Arscott DB, Tockner K. Emerging concepts in temporary-river ecology. *Freshwater Biology* 2010; **55**: 717–738.
7. Vander Vorste R, Sarremejane R, Datry T. Intermittent Rivers and Ephemeral Streams: A Unique Biome With Important Contributions to Biodiversity and Ecosystem Services. 2019.
8. Steward AL, von Schiller D, Tockner K, Marshall JC, Bunn SE. When the river runs dry: human and ecological values of dry riverbeds. *Frontiers in Ecology and the Environment* 2012; **10**: 202–209.

9. Magand, Claire, Alves, Maria Helena, Calleja, Eman, Datry, Thibault, Dörflinger, Gerald, England, Judy, et al. Intermittent rivers and ephemeral streams: what water managers need to know. 2020. Zenodo.

10. Datry T, Allen D, Argelich R, Barquin J, Bonada N, Boulton A, et al. Securing Biodiversity, Functional Integrity, and Ecosystem Services in Drying River Networks (DRYvER). *RIO* 2021; **7**: e77750.

11. Sheldon F, Bunn SE, Hughes JM, Arthington AH, Balcombe SR, Fellows CS. Ecological roles and threats to aquatic refugia in arid landscapes: dryland river waterholes. *Mar Freshwater Res* 2010; **61**: 885.

12. Olden MS S Godsey, T Datry, R Hale, SC Zipper, K Costigan, CA Krabbenhoft, WK Dodds, M Zimmer, DC Allen, M Bogan, KE Kaiser, RM Burrows, JC Hammond, M Busch, S Kampf, MC Mims, A Burgin, JD. Science Gets Up to Speed on Dry Rivers. *Eos*. http://eos.org/opinions/science-gets-up-to-speed-on-dry-rivers. Accessed 1 Dec 2022.

13. Stevens ANP. Factors Affecting Global Climate. *ature Education Knowledge* . 2011. , **10**

14. Datry T, Corti R, Foulquier A, Von Schiller D, Tockner K. One for All, All for One: A Global River Research Network. *Eos* 2016; **97**.

15. Sauquet E, Shanafield M, Hammond JC, Sefton C, Leigh C, Datry T. Classification and trends in intermittent river flow regimes in Australia, northwestern Europe and USA: A global perspective. *Journal of Hydrology* 2021; **597**: 126170.

16. Steinman BA, Stansell ND, Mann ME, Cooke CA, Abbott MB, Vuille M, et al. Interhemispheric antiphasing of neotropical precipitation during the past millennium. *Proceedings of the National Academy of Sciences* 2022; **119**: e2120015119.

17. Datry T, Moya N, Zubieta J, Oberdorff T. Determinants of local and regional communities in intermittent and perennial headwaters of the Bolivian Amazon. *Freshwater Biology* 2016; **61**: 1335–1349.

18. Abrantes YG, Medeiros LS de, Bennemann ABA, Bento D de M, Teixeira FK, Rezende CF, et al. Geographic distribution and conservation of seasonal killifishes (Cyprinodontiformes, Rivulidae) from the Mid-Northeastern Caatinga ecoregion, northeastern Brazil. *NBC* 2020; **15**: 301–315.

19. Boulton AJ, Rolls RJ, Jaeger KL, Datry T. Hydrological Connectivity in Intermittent Rivers and Ephemeral Streams. *Intermittent Rivers and Ephemeral Streams*. 2017. Elsevier, pp 79–108.

20. Arias PA, Garreaud R, Poveda G, Espinoza JC, Molina-Carpio J, Masiokas M, et al. Hydroclimate of the Andes Part II: Hydroclimate Variability and Sub-Continental Patterns. *Frontiers in Earth Science* 2021; **8**.

21. Molinero J, Barrado M, Guijarro M, Ortiz M, Castaño OC, Rey-Baltar DZ. The Teaone River: a snapshot of a tropical river from the coastal region of Ecuador. *Limnetica* 2019; **38**: 587–605.

22. Romaní AM, Chauvet E, Febria C, Mora-Gómez J, Risse-Buhl U, Timoner X, et al. Chapter 4.1 - The Biota of Intermittent Rivers and Ephemeral Streams: Prokaryotes, Fungi, and Protozoans. In: Datry T, Bonada N, Boulton A (eds). *Intermittent Rivers and Ephemeral Streams*. 2017. Academic Press, pp 161–188.

23. Sala M, Güde H. Seasonal dynamics of pelagic and benthic (littoral and profundal) bacterial abundances and activities in a deep prealpine lake (L. Constance). *Archiv für Hydrobiologie* 2006; **167**: 351–369.

24. Battin TJ. Hydrodynamics is a major determinant of streambed biofilm activity: From the sediment to the reach scale. *Limnol Oceanogr* 2000; **45**: 1308–1319.

25. Mulholland PJ, Helton AM, Poole GC, Hall RO, Hamilton SK, Peterson BJ, et al. Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature* 2008; **452**: 202–205.

26. Rosemond AD, Benstead JP, Bumpers PM, Gulis V, Kominoski JS, Manning DWP, et al. Freshwater ecology. Experimental nutrient additions accelerate terrestrial carbon loss from stream ecosystems. *Science* 2015; **347**: 1142–1145.

27. Arce MI, von Schiller D, Bengtsson MM, Hinze C, Jung H, Alves RJE, et al. Drying and Rainfall Shape the Structure and Functioning of Nitrifying Microbial Communities in Riverbed Sediments. *Front Microbiol* 2018; **9**: 2794.

28. Timoner X, Acuña V, Von Schiller D, Sabater S. Functional responses of stream biofilms to flow cessation, desiccation and rewetting: Flow intermittency effects on stream biofilms. *Freshwater Biology* 2012; **57**: 1565–1578.

29. von Schiller D, Datry T, Corti R, Foulquier A, Tockner K, Marcé R, et al. Sediment Respiration Pulses in Intermittent Rivers and Ephemeral Streams. *Global Biogeochemical Cycles* 2019; **33**: 1251–1263.

30. Nakayama T. Development of an advanced eco-hydrologic and biogeochemical coupling model aimed at clarifying the missing role of inland water in the global biogeochemical cycle. *Journal of Geophysical Research: Biogeosciences* 2017; **122**: 966–988.

31. Febria CM, Beddoes P, Fulthorpe RR, Williams DD. Bacterial community dynamics in the hyporheic zone of an intermittent stream. *ISME J* 2012; **6**: 1078–1088.

32. Arias-Real R, Muñoz I, Gutierrez-Cánovas C, Granados V, Lopez-Laseras P, Menéndez M. Subsurface zones in intermittent streams are hotspots of microbial decomposition during the non-flow period. *Science of The Total Environment* 2020; **703**: 135485.

33. Gionchetta G, Artigas J, Arias-Real R, Oliva F, Romaní A. Multi-model assessment of hydrological and environmental impacts on streambed microbes in Mediterranean catchments. *Environmental Microbiology* 2020; **22**.

34. Fagua JC, Ramsey RD. Geospatial modeling of land cover change in the Chocó-Darien global ecoregion of South America; One of most biodiverse and rainy areas in the world. *PLOS ONE* 2019; **14**: e0211324.

35. Messager ML, Lehner B, Cockburn C, Lamouroux N, Pella H, Snelder T, et al. Global prevalence of non-perennial rivers and streams. *Nature* 2021; **594**: 391–397.

36. Zeglin LH. Stream microbial diversity in response to environmental changes: review and synthesis of existing research. *Front Microbiol* 2015; **6**: 454.

37. Flora y Fauna. http://cube.gob.ec/index.php/ct-menu-item-11/ct-menu-item-29. Accessed 29 May 2023.

38. Mosquera G, Rosero-López D, Escobar-Camacho D, Daza J, Datry T, Encalada A. Delineation of water flow paths in a tropical intermittent stream system under changing land use: Isotopic and geochemical assessment. 2023. GFZ German Research Centre for Geosciences.

39. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 2019; **37**: 852–857.

40. McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* 2013; **8**: e61217.

41. García-García N, Tamames J, Linz AM, Pedrós-Alió C, Puente-Sánchez F. Microdiversity ensures the maintenance of functional microbial communities under changing environmental conditions. *ISME J* 2019; **13**: 2969–2983.

42. Joos L, Beirinckx S, Haegeman A, Debode J, Vandecasteele B, Baeyen S, et al. Daring to be differential: metabarcoding analysis of soil and plant-related microbial communities using amplicon sequence variants and operational taxonomical units. *BMC Genomics* 2020; **21**: 733.

43. Lee MD. Happy Belly Bioinformatics: an open-source resource dedicated to helping biologists utilize bioinformatics. *Journal of Open Source Education* 2019; **4**: 53.

44. Bates D, Mächler M, Bolker B, Walker S. Package Lme4: Linear Mixed-Effects Models Using Eigen and S4. *R package version* . 2014.

45. Wang Y, Sheng H-F, He Y, Wu J-Y, Jiang Y-X, Tam NF-Y, et al. Comparison of the Levels of Bacterial Diversity in Freshwater, Intertidal Wetland, and Marine Sediments by Using Millions of Illumina Tags. *Appl Environ Microbiol* 2012; **78**: 8264–8271.

46. Liao H, Yen JY, Guan Y, Ke D, Liu C. Differential responses of stream water and bed sediment microbial communities to watershed degradation. *Environment International* 2020; **134**: 105198.

47. Chavarria KA, Saltonstall K, Vinda J, Batista J, Lindmark M, Stallard RF, et al. Land use influences stream bacterial communities in lowland tropical watersheds. *Sci Rep* 2021; **11**: 21752.

48. Bucci JP, Szempruch AJ, Caldwell JM, Ellis JC, Levine JF. Seasonal Changes in Microbial Community Structure in Freshwater Stream Sediment in a North Carolina River Basin. *Diversity* 2014; **6**: 18–32.

49. Gweon HS, Bowes MJ, Moorhouse HL, Oliver AE, Bailey MJ, Acreman MC, et al. Contrasting community assembly processes structure lotic bacteria metacommunities along the river continuum. *Environmental Microbiology* 2021; **23**: 484–498.

50. Schreckinger J, Mutz M, Mendoza-Lera C, Frossard A. Attributes of Drying Define the Structure and Functioning of Microbial Communities in Temperate Riverbed Sediment. *Frontiers in Microbiology* 2021; **12**.

51. Zeglin LH, Dahm CN, Barrett JE, Gooseff MN, Fitpatrick SK, Takacs-Vesbach CD. Bacterial community structure along moisture gradients in the parafluvial sediments of two ephemeral desert streams. *Microb Ecol* 2011; **61**: 543–556.

52. Beattie RE, Bandla A, Swarup S, Hristova KR. Freshwater Sediment Microbial Communities Are Not Resilient to Disturbance From Agricultural Land Runoff. *Frontiers in Microbiology* 2020; **11**.

53. Gardner TA, Barlow J, Chazdon R, Ewers RM, Harvey CA, Peres CA, et al. Prospects for tropical forest biodiversity in a human-modified world. *Ecology Letters* 2009; **12**: 561–582.

54. Kivlin SN, Hawkes CV. Temporal and Spatial Variation of Soil Bacteria Richness, Composition, and Function in a Neotropical Rainforest. *PLOS ONE* 2016; **11**: e0159131.

55. Zeglin LH. Stream microbial diversity in response to environmental changes: review and synthesis of existing research. *Front Microbiol* 2015; **6**: 454.

56. Ottoni J, Grignet R, Barros M, Prado Fernandes S, Panatta A, Lacerda Júnior G, et al. DNA Metabarcoding from Microbial Communities Recovered from Stream and Its Potential for Bioremediation Processes. *Current Microbiology* 2022; **79**.

57. Wu X, Peng J, Liu P, Bei Q, Rensing C, Li Y, et al. Metagenomic insights into nitrogen and phosphorus cycling at the soil aggregate scale driven by organic material amendments. *Science of The Total Environment* 2021; **785**: 147329.

58. Hartmann M, Brunner I, Hagedorn F, Bardgett RD, Stierli B, Herzog C, et al. A decade of irrigation transforms the soil microbiome of a semi-arid pine forest. *Mol Ecol* 2017; **26**: 1190–1206.

59. Huber KJ, Vieira S, Sikorski J, Wüst PK, Fösel BU, Gröngröft A, et al. Differential Response of Acidobacteria to Water Content, Soil Type, and Land Use During an Extended Drought in African Savannah Soils. *Front Microbiol* 2022; **13**: 750456.

60. Hou L, Zhou Q, Wu Q, Gu Q, Sun M, Zhang J. Spatiotemporal changes in bacterial community and microbial activity in a full-scale drinking water treatment plant. *Science of The Total Environment* 2018; **625**: 449–459.

61. Palmer M, Covington JK, Zhou E-M, Thomas SC, Habib N, Seymour CO, et al. Thermophilic Dehalococcoidia with unusual traits shed light on an unexpected past. *ISME J* 2023; 1–15.

62. Yang Y, Sanford R, Yan J, Chen G, Cápiro NL, Li X, et al. Roles of Organohalide-Respiring Dehalococcoidia in Carbon Cycling. *mSystems* 2020; **5**: e00757-19.

63. Taş N, Van Eekert MHA, De Vos WM, Smidt H. The little bacteria that can – diversity, genomics and ecophysiology of 'Dehalococcoides' spp. in contaminated environments. *Microb Biotechnol* 2010; **3**: 389–402.

64. Caldwell SL, Laidler JR, Brewer EA, Eberly JO, Sandborgh SC, Colwell FS. Anaerobic Oxidation of Methane: Mechanisms, Bioenergetics, and the Ecology of Associated Microorganisms. *Environ Sci Technol* 2008; **42**: 6791–6799.

65. Gionchetta G. At the edge of aquatic systems: intermittent streambed microbial communities' responses to hydrological alterations. *TDX (Tesis Doctorals en Xarxa)* . 2019. Ph.D. Thesis, Universitat de Girona.

66. Kemnitz D, Chin K-J, Bodelier P, Conrad R. Community analysis of methanogenic archaea within a riparian flooding gradient. *Environ Microbiol* 2004; **6**: 449–461.

67. Rastogi G, Sani RK, Peyton BM, Moberly JG, Ginn TR. Molecular studies on the microbial diversity associated with mining-impacted Coeur d'Alene River sediments. *Microb Ecol* 2009; **58**: 129–139.

68. Buriánková I, Brablcová L, Mach V, Dvořák P, Chaudhary PP, Rulík M. Identification of Methanogenic archaea in the Hyporheic Sediment of Sitka Stream. *PLOS ONE* 2013; **8**: e80804.

69. Compte-Port S, Subirats J, Fillol M, Sànchez-Melsió A, Marcé R, Rivas-Ruiz P, et al. Abundance and Co-Distribution of Widespread Marine Archaeal Lineages in Surface Sediments of Freshwater Water Bodies across the Iberian Peninsula. *Microb Ecol* 2017; **74**: 776–787.

70. Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, et al. Patterns and Processes of Microbial Community Assembly. *Microbiol Mol Biol Rev* 2013; **77**: 342–356.

71. Gionchetta G, Romaní AM, Oliva F, Artigas J. Distinct responses from bacterial, archaeal and fungal streambed communities to severe hydrological disturbances. *Sci Rep* 2019; **9**: 13506.

72. Sutton SD, Findlay RH. Sedimentary microbial community dynamics in a regulated stream: East Fork of the Little Miami River, Ohio. *Environmental Microbiology* 2003; **5**: 256–266.

73. Hullar MAJ, Kaplan LA, Stahl DA. Recurring Seasonal Dynamics of Microbial Communities in Stream Habitats. *Applied and Environmental Microbiology* 2006; **72**: 713–722.

74. Smith REW, Pearson RG. The macro-invertebrate communities of temporary pools in an intermittent stream in tropical Queensland. *Hydrobiologia* 1987; **150**: 45–61.

75. Boulton AJ, Lake PS. The ecology of two intermittent streams in Victoria, Australia. I. Multivariate analyses of physicochemical features. *Freshwater Biology* 1990; **24**: 123–141.

76. Febria CM, Hosen JD, Crump BC, Palmer MA, Williams DD. Microbial responses to changes in flow status in temporary headwater streams: a cross-system comparison. *Frontiers in Microbiology* 2015; **6**.

77. Flemming H-C, Wingender J. The biofilm matrix. *Nat Rev Microbiol* 2010; **8**: 623–633.

78. Malvankar NS, Lau J, Nevin KP, Franks AE, Tuominen MT, Lovley DR. Electrical Conductivity in a Mixed-Species Biofilm. *Appl Environ Microbiol* 2012; **78**: 5967–5971.

79. Kaplan LA, Bott TL. Diel fluctuations in bacterial activity on streambed substrata during vernal algal blooms: Effects of temperature, water chemistry, and habitat: Diel patterns in streams. *Limnol Oceanogr* 1989; **34**: 718–733.

80. Shiah F-K, Ducklow HW. Multiscale variability in bacterioplankton abundance, production, and specific growth rate in a temperate salt-marsh tidal creek. *Limnol Oceanogr* 1995; **40**: 55–66.

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8. TABLES

Table 1. Mean relative abundance of dominant taxa and seasonal differences

	Mean relative abundance (%)			Difference between seasons
Class	**Wet**	**Dry**	**All**	**p-value**
Other*	29.52%	35.25%	32.38%	0.02
Vicinamibacteria	19.93%	13.47%	16.70%	0.08
Bacteroidia	6.82%	6.66%	6.74%	0.96
Gammaproteobacteria	5.40%	6.43%	5.92%	0.12
Actinobacteria	4.97%	4.67%	4.82%	0.65
Alphaproteobacteria*	3.96%	5.54%	4.75%	0.05
Thermoleophilia	4.72%	4.04%	4.38%	0.46
Acidobacteriae	4.71%	3.08%	3.90%	0.49
Anaerolineae	3.34%	4.42%	3.88%	0.16
Planctomycetes	2.75%	3.54%	3.15%	0.16
Polyangia	2.23%	3.00%	2.62%	0.16
Acidimicrobiia	2.51%	1.74%	2.12%	0.17
Bacilli	1.74%	2.29%	2.01%	0.43
Acidobacteriota subgroup 22	2.08%	1.91%	2.00%	1
Holophagae	1.89%	2.02%	1.95%	0.33
Acidobacteriota subgroup 5	2.16%	1.48%	1.82%	0.32
Dehalococcoidia*	1.28%	0.47%	0.88%	0.05

Mean relative abundance of dominant taxa in the Cube river basin (>5%) expressed as percentage for the wet and dry season and individually and both seasons combined.

Table 2. Summary statistics of the linear mixed model

	Estimate	**SE**	**p-value**
Season	0.43463	0.49682	0.3907
Temperature	-12.09014	538351	0.0346*
Conductivity	0.90310	0.33998	0.0141*
TOC	-5.465	0.38958	0.8897

Summary statistics of the linear mixed model of microbial community diversity (Chao1) in response to season, temperature conductivity and total organic carbon (TOC). Significance levels are set below 0.05

9. FIGURES

Figure 1.

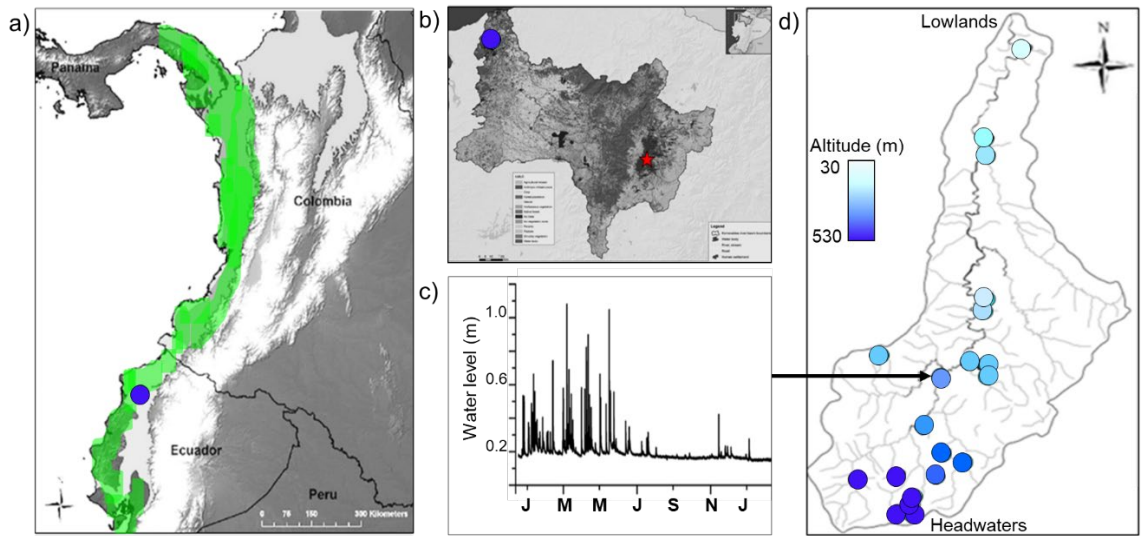

Figure 1. a) The Andean Chocó Bioregion extending from Darien in Panamá to Southern Ecuador (green shade) and the study area (blue circle), b) the Esmeraldas River Basin of which the Cube River basin is tributary, c) water level variation from sampling location number 11 in 202, denoting the seasonality in this area evidencing the wet (January-May) and dry periods (June - December), d) The Cube River Basin and 20 sampling reaches distributed along an altitudinal gradient (blue shaded circles).

Figure 2.

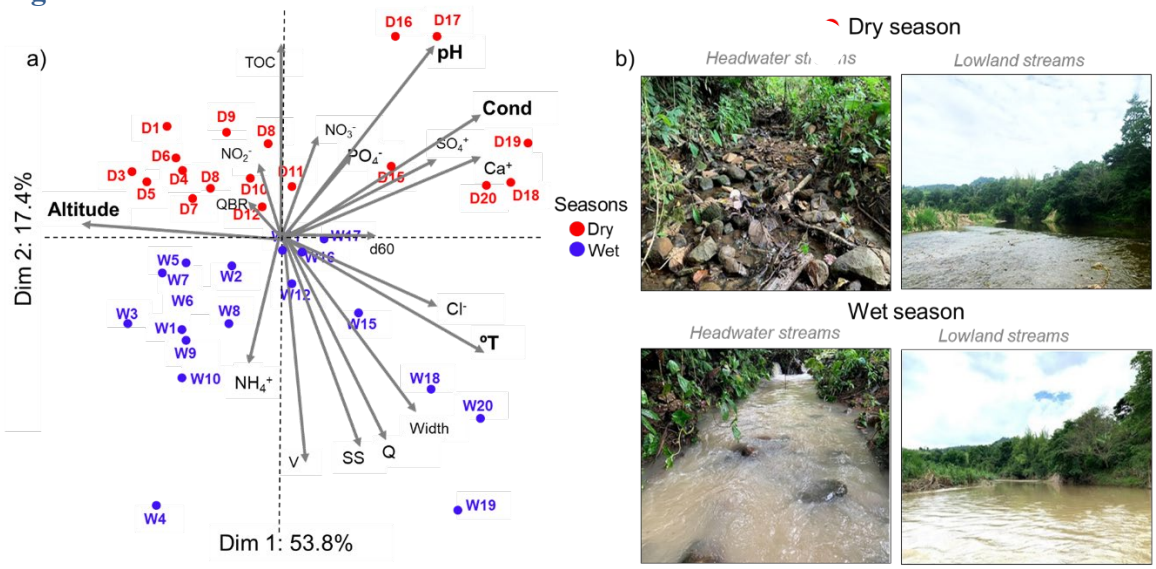

Figure 2. a) Principal Components Analysis of environmental variables of streams (*n = 20*) showing the distribution of sampling locations between the dry (red numbers-coded locations) and wet seasons (blue numbers-coded locations), principal variables responsible of data ordination are Discharge (Q), pH, Conductivity (Cond), Total Organic Carbon (TOC), and Temperature (ºT), arranged along the *X-axis* = 53.8% and the *Y-axis* = 17.4%; b) Stream reaches from the headwaters (left panels) and lowlands (right panels) in the dry (top panels) and wet (bottom panels) seasons.

Figure 3.

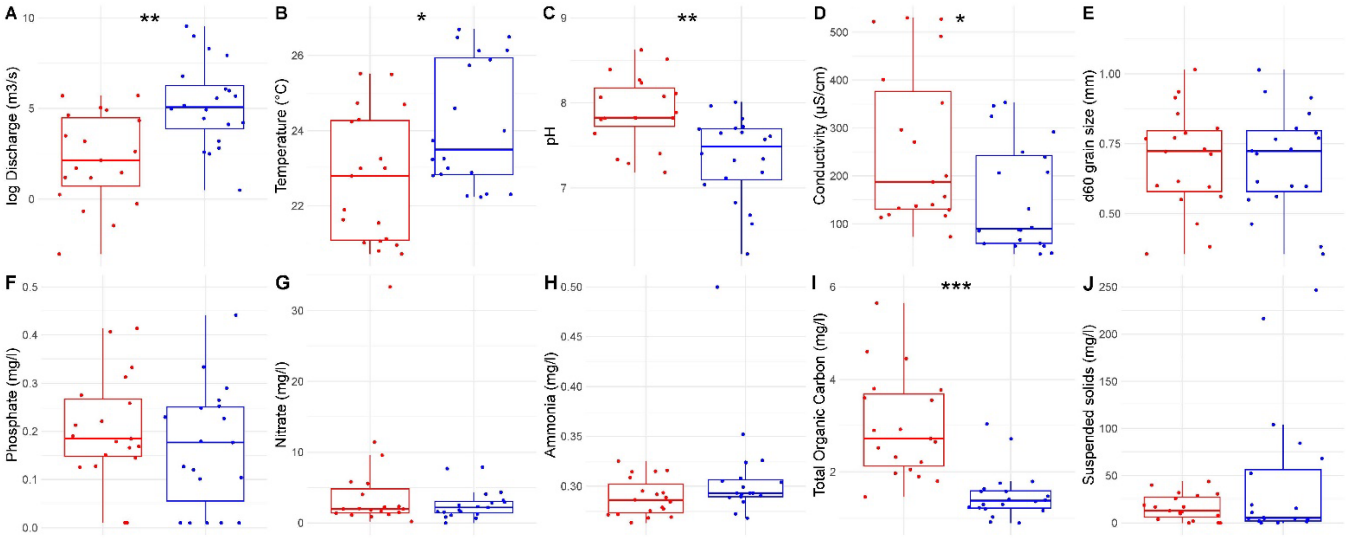

Figure 3. Boxplots for environmental variables collected at all sampling reaches (*n = 20*) in the Cube River Basin, grouped by wet (red) and dry (blue) seasons for Discharge (A), Temperature (B), Ph (C), Conductivity (D), d60 grain size (E), Phosphate (F), Nitrate (G), Ammonia (H), Total Organic Carbon (I), and Suspended Solids (J). The horizontal lines represent the first, second (median), and third quartiles. Wilcoxon analyses show *0.05, **0.005, and ***0.0005 significance levels.

Figure 4.

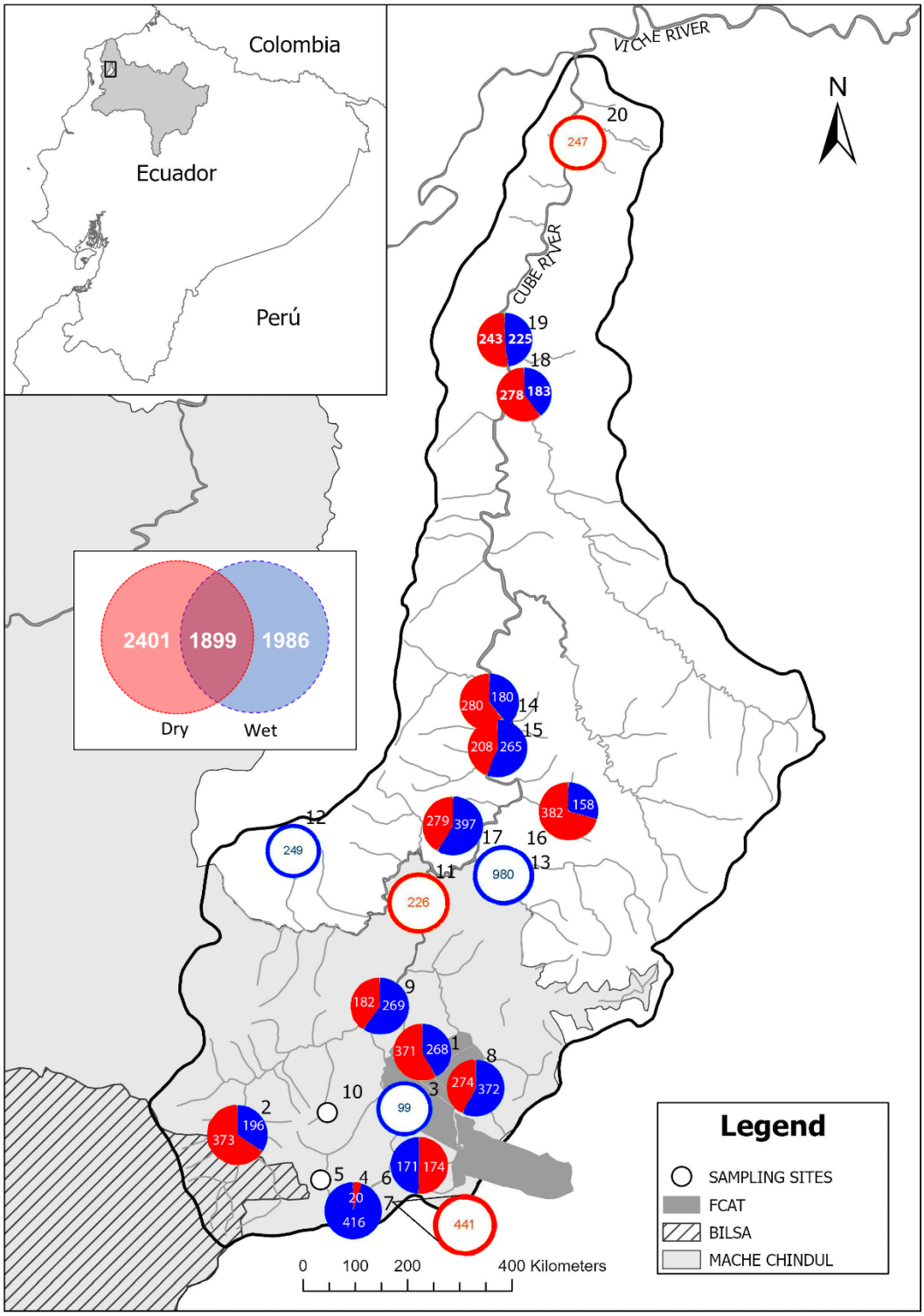

Figure 4. Map of the Cube River basin area, showing ASV abundance across seasons for each sampling location; seasons are color coded as red and blue for dry and wet, respectively. White circles correspond to locations where data was available only for one of the seasons; border colors represent the season. Venn diagram displays the number of ASVs (ASV richness) for each season as well as the number of shared ASVs (30%).

Figure 5.

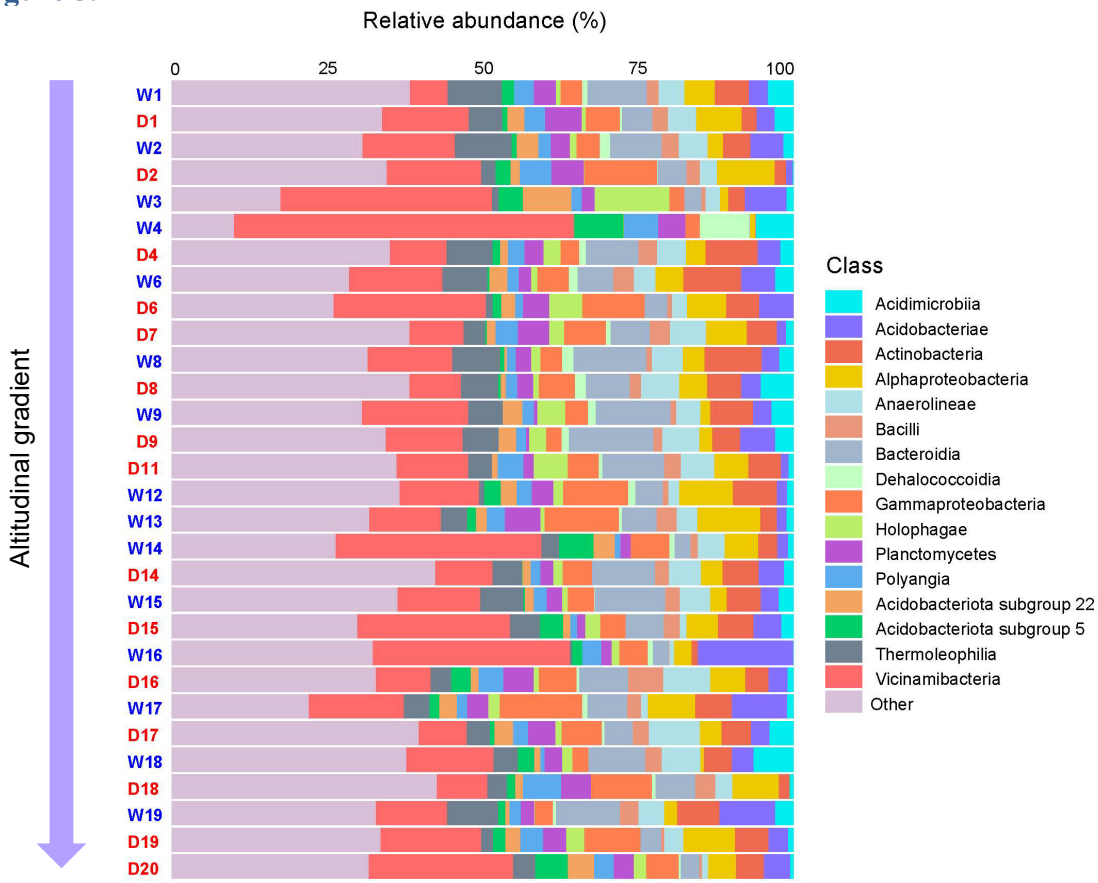

Figure 5. Relative abundance (%) of microbial communities at the Class level, with >5% of presence in all samples, described by the community structure reported for the dry and wet seasons for locations distributed along the altitudinal gradient of the Cube River Basin.

Figure 6.

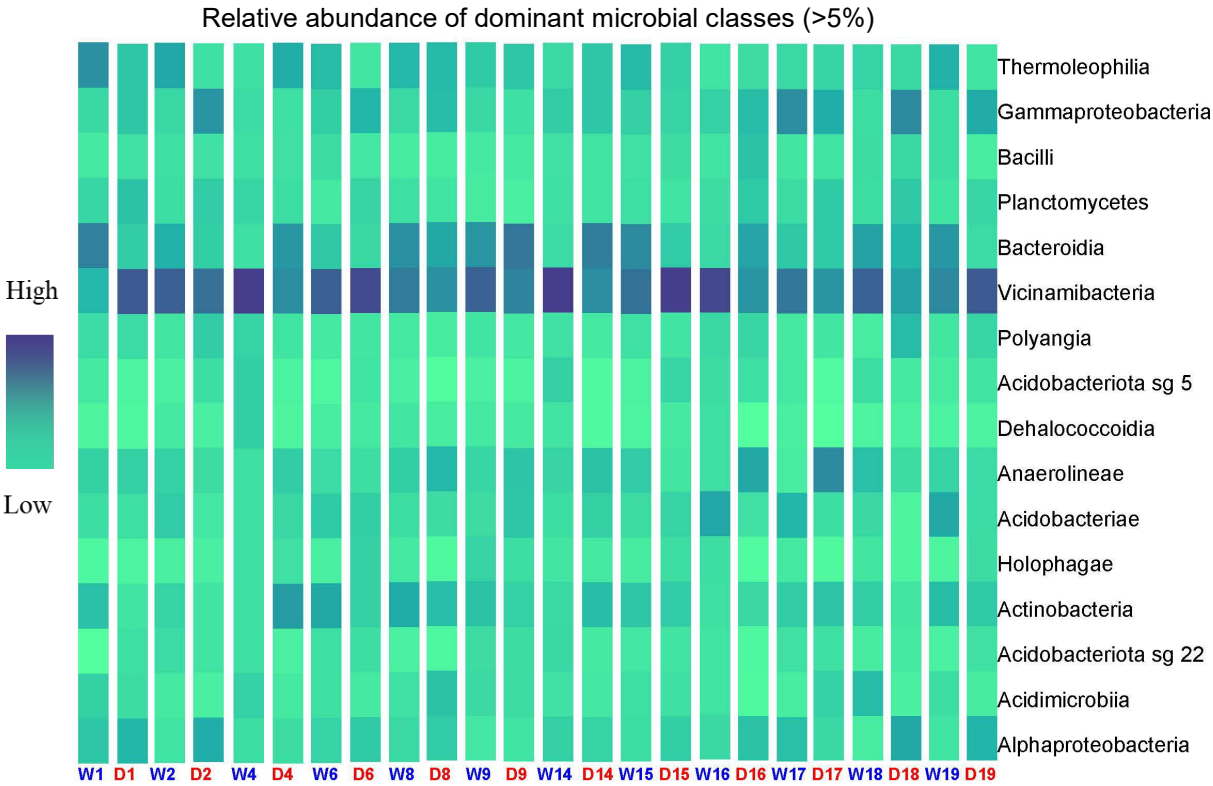

Figure 6. Heatmap of microbial communities' relative abundance (%) at the Class level, for paired locations ordinated according to the wet and dry seasons, colored abundance changes according to changes in the abundance of each Class.

Figure 7.

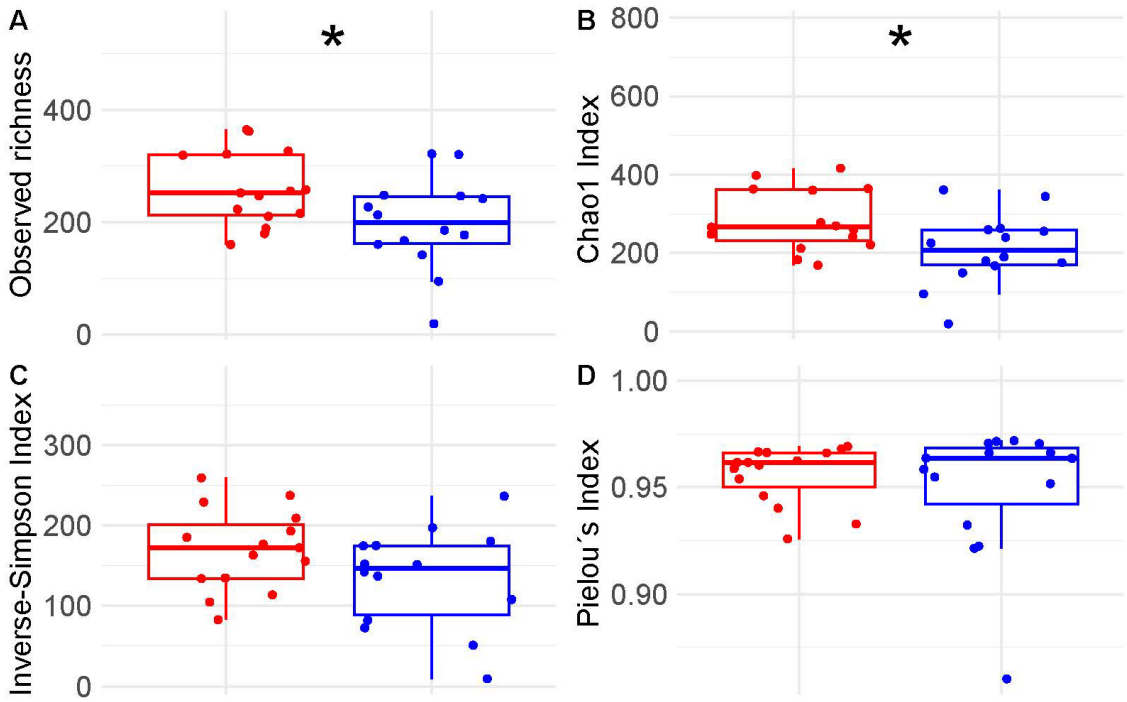

Figure 7. Boxplots of microbial community alpha diversity from amplicon sequence variants (ASVs) data calculated for the dry (red) and wet (blue) seasons, (A) Chao1 index from the dry season is significantly higher ($p < 0.05$) than diversity in the wet season; B) Observed richness show that ASVs number in the dry season was significantly higher ($p < 0.05$) than ASVs from the wet season; (C) Inverse Simpson diversity index that accounted for proportional abundance show no differences between seasons; D) Pielou Index show that microbial communities are even for both seasons. The horizontal lines represent the first, second (median), and third quartiles.

Figure 8.

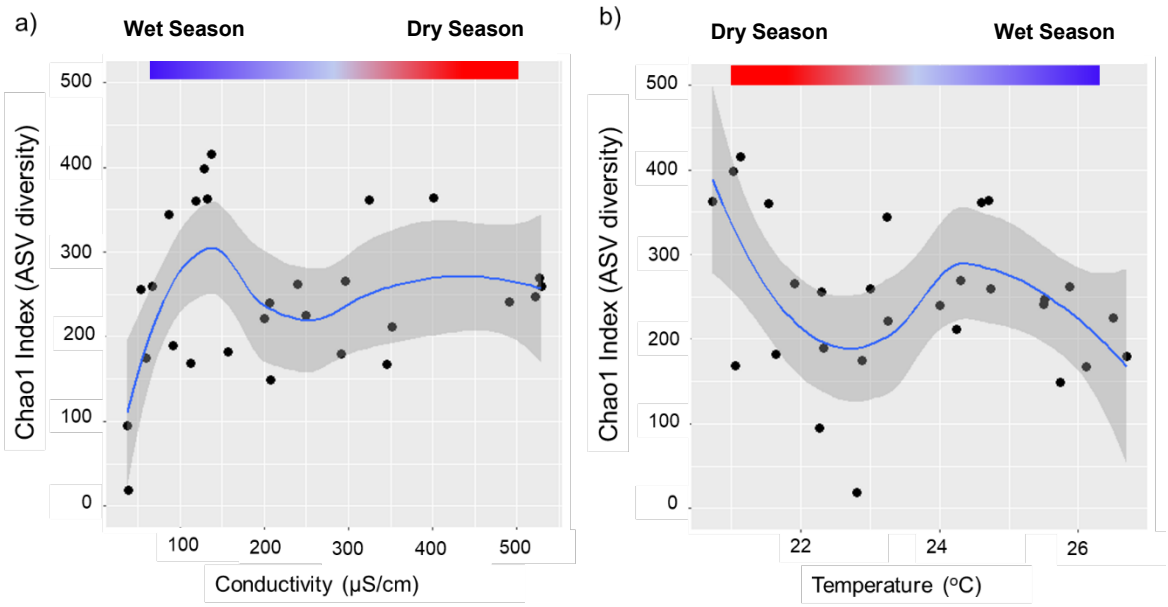

Figure 8. Linear mixed model of microbial community diversity (Chao1 Index at amplicon sequence variance ASVs level) according to temperature and conductivity considering seasons (dry and wet) as fixed effects and altitude (~500 m) as the random effect, A) the model shows that microbial diversity decreases with increasing temperature during the wet season, and b) microbial diversity increases with conductivity that is higher in the dry season compared to the wet season.

Appendices

Supplementary Information

Table S1. Variables describing the environmental settings of the Cube basin

Site	Id	Latitude	Longitude	Season	Alt	Slope	Temp	pH	Cond	DO	Discharge	SS	TOC	Cloruro	NO2	NO3	PO4	SO4	NH4	D60
Cub01W	W1	0,37805	-79,6646	Wet	507	6	22,3	6,68	53,6	8,422	12,1	52,5	1,296304	3,946	0,32	3,198	0,01	4,124	0,284	0,9367261
Cub02W	W2	0,36527	-79,70166	Wet	342	1,1	22,32	7,608	92,32	8,652	85,5	19	1,234329	6,518	0,199	1,281	0,12	10,392	0,29	1,0145218
Cub03W	W3	0,36888	-79,6688	Wet	472	10,8	22,26	6,578	37,12	7,888	1,63	68,5	0,916358	2,348	0,167	4,083	0,01	2,38	0,299	0,3817696
Cub04W	W4	0,351	-79,6688	Wet	518	3,4	22,8	6,224	39,026	8,476	61,45	84,5	1,756475	2,863	0,161	7,896	0,01	1,632	0,5	0,5990075
Cub05W	W5	0,35192	-79,683	Wet	512	2,22	22,84	7,404	53,74	8,384	16,626	1,5	2,714173	2,691	0,193	0,852	0,101	4,233	0,289	
Cub06W	W6	0,35124	-79,68103	Wet	464	10,2	22,88	7,184	59,46	8,49	24,45	4,5	0,895528	4,074	0,183	0,659	0,01	5,471	0,292	0,7235088
Cub07W	W7	0,353273	-79,67809	Wet	531	5	22,24	7,342	58,74	8,478	67,7	15,5	1,79366	2,793	0,165	1,094	0,104	5,701	0,268	0,731186
Cub08W	W8	0,37555	-79,6575	Wet	332	2,4	23,24	7,646	87,02	8,724	144,23	5,5	1,296649	5,094	0,171	2,856	0,127	8,283	0,304	0,787745
Cub09W	W9	0,38926	-79,6735	Wet	351	3,2	23	6,828	66,6	8,698	260,77	2	1,476034	2,397	0,164	1,601	0,01	3,18	0,326	0,7705724
Cub10W	W10	0,36833	-79,68583	Wet	526	1,2	23,26	7,04	85,42	8,566	2781,85	4	1,391678	1,803	0,16	2,309	0,177	6,002	0,293	0,8051638
Cub11W	W11	0,41222	-79,6675	Wet	198	2,4	23,74	7,964	131,44	8,684	884,84	2	1,225204	2,449	0,185	1,544	0,179	15,557	0,272	0,6148973
Cub12W	W12	0,423	-79,693	Wet	376	0,4	24	7,116	206,26	7,578	137,76	0	1,02516	7,265	0,318	4,336	0,441	16,172	0,293	0,5503037
Cub13W	W13	0,414	-79,649	Wet	215	0,5	26,14	7,326	87,86	7,938	396,32	1,5	3,035587							0,462906
Cub14W	W14	0,44527	-79,65138	Wet	133	1,8	26,12	7,72	346,12	8,048	13,28	5	1,1625399	13,121	0,219	7,695	0,265	64,372	0,352	0,7132969
Cub15W	W15	0,44388	-79,65138	Wet	135	1	25,88	7,572	239,6	8,112	4075,72	5	1,581049	4,385	0,349	1,593	0,227	31,669	0,305	0,8576487
Cub16W	W16	0,425002	-79, 622595	Wet	207	2,6	25,74	8,01	207,94	8,572	293,72	0	1,634417	3,529	0,145	<LD	0,23	34,582	0,324	0,7669521
Cub17W	W17	0,419	-79,65	Wet	208	0,6	24,6	7,702	324,34	8,07	173,97	11	1,406484	5,344	0,166	1,823	0,334	81,426	0,289	0,5966156
Cub18W	W18	0,514	-79, 6490	Wet	86	0,6	26,7	7,694	291,8	8,234	436,62	216,5	1,203513	3,533	0,175	2,193	0,29	59,852	0,308	0,9148447
Cub19W	W19	0,521389	-79,6495	Wet	78	0,6	26,5	7,654	249,7	8,162	13920,81	246,5	1,363445	10,062	0,169	2,53	0,249	51,964	0,293	0,5611116
Cub20W	W20	0,56277	-79,63388	Wet	52	1,1	26,48	7,814	353,36	8,088	8089,06	104	1,580877	6,233	0,164	2,95	0,252	60,883	0,29	0,3558251
CuB01D	D1	0,37805	-79,6646	Dry	507	6	20,72	7,334	132,5	6,306	0,51	11	3,55	1,531	0,176	1,209	0,19	16,517	0,286	0,9367261
CuB02D	D2	0,36527	-79,70166	Dry	342	1,1	21,54	7,824	119,4	8,866	5,5	0	4,6	1,871	0,191	2,332	0,179	13,341	0,295	1,0145218
CuB03D	D3	0,36888	-79,6688	Dry	472	10,8	20,96	7,184	73,08	4,288	0,048	32	3,77	1,47	0,158	1,122	0,169	7,528	0,289	0,3817696
CuB04D	D4	0,351	-79,6688	Dry	518	3,4	21,02	7,806	129,12	6,674	0,77	31	1,97	1,46	0,378	5,576	0,221	6,242	0,325	0,5990075
CuB05D	D5	0,35192	-79,683	Dry	512	2,22	20,8	7,288	116,82	8,696	3,21	2	2,05	1,16	0,194	1,654	0,145	9,093	0,277	
CuB06D	D6	0,35124	-79,68103	Dry	464	10,2	21,06	7,888	113,3	9,35	0,23	12	1,8	1,141	0,287	1,981	0,126	15,03	0,269	0,7235088
CuB07D	D7	0,353273	-79,67809	Dry	531	5	21,125	7,64	137,3	7,658	4,27	40	1,9	2,367	0,163	1,841	0,166	15,605	0,293	0,731186
CuB08D	D8	0,37555	-79,6575	Dry	332	2,4	21,9	8,236	296,14	8,978	1,28	10	2,21	2,598	0,183	1,981	0,151	47,568	0,263	0,787745
CuB09D	D9	0,38926	-79,6735	Dry	351	3,2	21,64	7,82	156,98	9,064	101,75	26	5,65	2,655	0,303	9,58	0,258	16,529	0,316	0,7705724
CuB10D	D10	0,36833	-79,68583	Dry	526	1,2	23	8,112	187,3	9,896	76,67	0	3,8	4,629	0,183	0,898	0,275	25,32	0,275	0,8051638
CuB11D	D11	0,41222	-79,6675	Dry	198	2,4	23,26	8,076	200,18	8,984	135,18	4	4,45	3,305	0,175	1,394	0,185	23,511	0,279	0,6148973
CuB12D	D12	0,423	-79,693	Dry	376	0,4	22,8	7,404	270,76	5,428	3,28	44	1,46	2,356	0,162	2,249	0,313	28,435	0,292	0,5503037
CuB13D	D13	0,414	-79,649	Dry	215	0,5	23	7,822	139,9	8,104	13,82	8	2,92	1,612	0,236	4,04	0,333	4,672	0,284	0,462906
CuB14D	D14	0,44527	-79,65138	Dry	133	1,8														0,7132969
CuB15D	D15	0,44388	-79,65138	Dry	135	1	24,24	8,066	352,26	9,834	154,46	13	2,9	5,334	0,211	5,825	0,213	60,57	0,315	0,8576487
CuB16D	D16	0,425002	-79, 622595	Dry	207	2,6	24,7	8,514	401,06	8,634	24,27	17	3,6	1,995	0,328	33,353	0,414	78,478	0,314	0,7669521
CuB17D	D17	0,419	-79,65	Dry	208	0,6	24,3	7,828	527,2	8,016	8,49	0	2,72	6,995	0,172	11,426	0,407	147,77	0,272	0,5966156
CuB18D	D18	0,514	-79, 6490	Dry	86	0,6	24,74	8,624	529,6	9,268	32,86	29	2,65	8,805	0,155	1,499	0,01	104,361	0,268	0,9148447
CuB19D	D19	0,521389	-79,6495	Dry	78	0,6	25,5	8,392	491,26	10,392	297,85	17	2,52	6,834	0,152	0,215	0,128	95,621	0,271	0,5611116
CuB20D	D20	0,56277	-79,63388	Dry	52	1,1	25,52	8,27	521,8	10,182	302,85	19	2,32	7,811	0,189	2,043	0,01	100,366	0,309	0,3558251

Environmental characteristics of the Cube river basin shown as physico-chemical and eco-hydrological variables, and water chemical (nutrients) composition.

Table S2. Environmental variables tested for differences between seasons

Environmental variables	Dry	Wet	W	p-value
Discharge*	61.44	1593.92	76	0.0009858
Temperature*	22.73	24.15	105	0.01755
pH*	7.90	7.36	310	0.0007851
Conductivity*	257.68	153.57	275	0.01636
D60 grain size	0.69	0.69	180.5	1
Phosphate	0.20	0.17	213.5	0.3412
Nitrate	4.75	2.81	193	0.7261
Ammonia	0.29	0.31	123.5	0.09869
Total Organic Carbon*	2.99	1.50	353	0.000421
Suspended Solids	16.58	42.45	195.5	0.8881

Seasonal mean values of environmental variables tested for differences between seasons; W statistic and p-values are shown for the difference between the wet and dry season.