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# Watershed-scale patterns of microbial diversity in the Congo River ecosystem

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Watersheds serve as vital links between terrestrial and marine ecosystems. Studies in primarily temperate regions show that along this flowpath, river microbial communities develop to both transform terrestrial organic matter and mediate autochthonous biogeochemical processes. Yet, we know relatively little about this hydrology-biogeochemistry-microbial axis in tropical watersheds, especially in Africa. In this study, we describe patterns of microbial diversity and function, as well as links between microbes, hydrology, and biogeochemistry in the Congo River watershed.

The Congo is the 2<sup>nd</sup>-largest watershed on earth and drains a significant portion of the African continent. Synoptic samples were collected at 27 sites in the Republic of Congo, and monthly sampling occurred at two sites in Brazzaville for one year. Biogeochemical and physical parameters were collected either stream-side or via remote sensing. Microbial beta-diversity was assessed with 16S rRNA gene amplicon sequencing, and calculated as weighted unifrac distances among samples. Principal coordinates analysis explained 59.7% of variation in communities and showed that microbial community similarity increased with increasing stream order. While headwater microbial communities varied widely, a common planktonic community appeared to develop across the watershed. Mantel tests using Spearman correlation indicated that microbial community structure correlated with both geographic measurements (upland watershed area  $r=0.3569$ ,  $p=0.002$ , and dendritic distance  $r=0.345$ ,  $p=0.003$ ), and water chemistry (dissolved organic matter slope ratio  $r=0.3851$ ,  $p=0.005$ ; temperature  $r=0.3826$ ,  $p=0.001$ ; pH  $r=0.246$ ,  $p=0.036$ ; NO<sub>x</sub>  $r=0.201$ ,  $p=0.044$ ). Thus, DOM character, temperature, and spatial gradients may in part structure river microbial communities in the tropics. Ongoing analyses are focusing on additional DOM and landscape metrics, 'omics functional modeling, and seasonal patterns at end-member sites on the Congo.

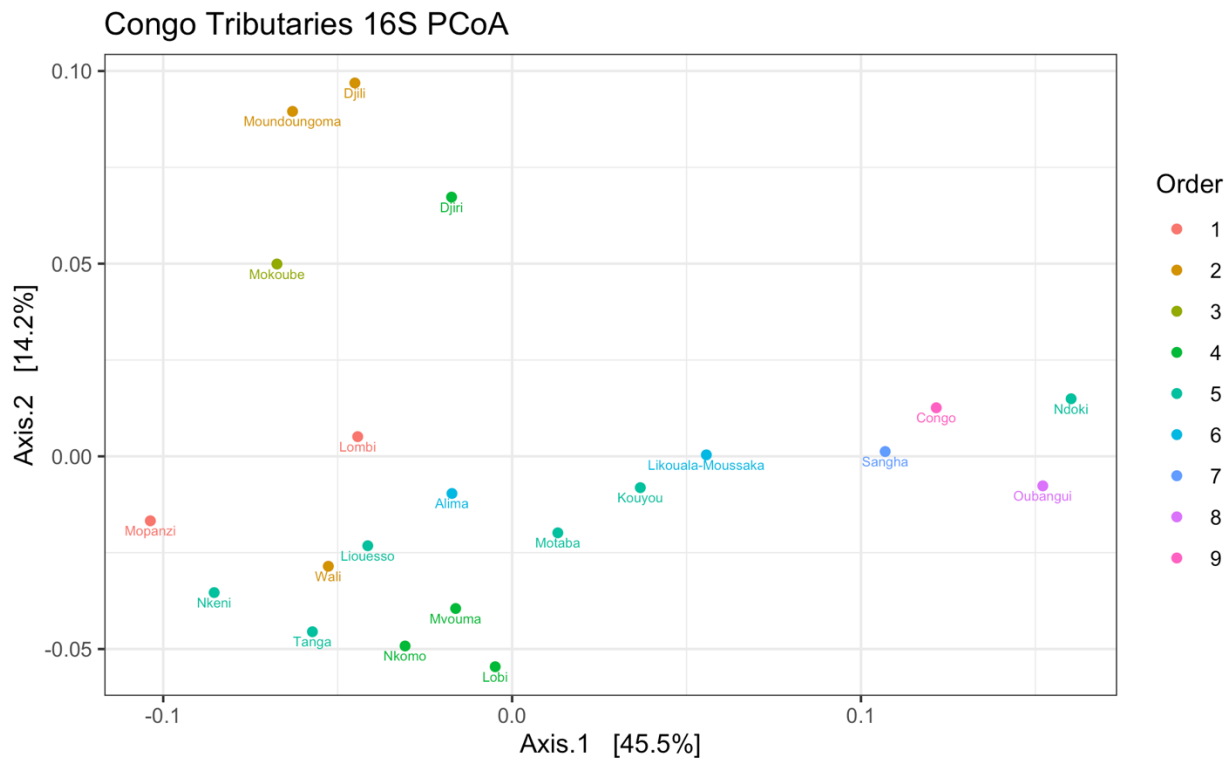


Figure. Principal coordinates analysis plot of 16S community structure from synoptic samples in the Congo River watershed. Weighted unfrac distances was computed for each sample, and colors indicate Strahler stream order.

Table. Correlation between 6S community structure and selected environmental variables using Spearman Mantel tests. Only statistically significant variables are shown ( $p < 0.05$ )

	Mantel $r$	p-value
Temperature	0.3826	0.001
Dendritic distance	0.345	0.003
Upland km <sup>2</sup>	0.3569	0.002
pH	0.246	0.036
Slope-ratio	0.3851	0.005
NOx	0.201	0.044