

THE TROPICAL PEATLAND ARCHAEAL LIPIDOME – INFLUENCE OF VEGETATION AND REDOX ON DIVERSITY

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The nature, variability, and diversity of environmental microbiomes and lipidomes are vital to understanding soil health, biogeochemical processes and reconstructing past climates. Such research on peatlands – especially tropical peatlands – is limited, despite their importance to the global carbon cycle through the sequestration of organic matter (OM) and production of methane. Here, we explore the distribution of archaea and their isoprenoidal glycerol dialkyl glycerol tetraether lipids (isoGDGTs) across a range of wetlands, in order to ascertain the controls on their distribution. We focus specifically on vegetation and OM composition to explore the relationships between archaeal ecology and carbon cycling in tropical contexts.

Through international collaboration, we created a database of core archaeal and bacteria lipid distributions of hundreds of peats from globally widespread sites (the TGRES Peat Database, Naafs et al., 2017). This formed the basis for peat-specific temperature and pH proxies based on the distribution of bacterial branched GDGTs as initially pioneered for mineral soils. However, clear environmental controls and patterns in the distribution of archaeal lipids are ambiguous (Naafs et al., 2018). For example, isoGDGT-5 is restricted to high temperature and low pH settings, but other isoGDGT and overly methylated isoprenoidal GDGT (Me-GDGTs) ring indices are poorly correlated with temperature and pH (Blewett et al., 2020). This suggests that in comparison to previously established GDGT-based environmental proxies the archaeal GDGTs of peatlands derive from an ecologically diverse group of organisms that confound simple environmental comparisons. Given the increased recognition of archaeal metabolic diversity, including a range of heterotrophic, methanotrophic and methanogen ecologies, it seems likely that changes in vegetation, peat OM composition and water level depth will impose significant controls on the archaeal community – and that of the lipids they produce.

To explore this further, we examined downcore and vegetation controls on archaeal assemblages at San San Pond Sak (SSPS), a wetland area in the Republic of Panama. To ascertain the impact of vegetation and therefore organic matter composition on archaeal assemblages, we collected a transect of cores that represent a variety of tropical peatland ecosystems – mangrove, mixed tropical forest, stunted forest with sawgrass (sedge) and ombrotrophic sawgrass bog. All sites experienced the same mean annual temperature ($\sim 26^{\circ}$ C) and rainfall ($\sim 3000 \text{ mm}$) and pH ($\sim 3 \text{ to } 4$) was largely invariant (except for an elevated near-seawater pH for the mangrove). We compare biomarker (core lipids and IPLs) and 16S rRNA gene derived community profiles from these sites with data from previously published tropical swamps (Everglades and Indonesia) and temperate bogs (Blewett et al., 2020).

Some parameters exhibit clear differences between temperate/boreal and tropical sites, including brGDGGT/isoGDGT ratios, but others do not despite our expectations. By analogy



with other environments, we expect isoGDGT ring number to increase with temperature and decrease with pH, but we observe only weak correlations. For example, ring indices vary dramatically among sites and with depth in the SSPS, where temperature is invariant. The index is highest in the mangrove setting (despite highest pH) and the mesotrophic mixed tropical forest site, apparently reflecting a contribution from Nitrososphaeria and anaerobic oxidisers of methane (AOM). However, it also decreases with depth in the other three sites (as well as at the Everglades and Tor Royal), which could reflect the dominance of Bathyarchaeia in the catotelm at these sites. These data highlight that downcore as well as intra-site community changes impose a significant control on the archaeal lipid assemblage.

Tropical peats also contain abundant BDGTs (butanetriol dialkyl glycerol tetraethers) produced by the methanogenic archaeon *Methanomassiliicoccus luminyensis;* however, BDGTs are also abundant in some temperate and even boreal peats, especially sedge-dominated peatland. We have previously speculated that the low δ^{13} C values of BDGTs in freshwater wetlands reflects their utilization of methyl substrates (Blewett et al., 2022). If so, we anticipate greater proportions of BDGTs in peats comprised of organic substrates rich in oxidized methyl moieties (lignin, pectin), and these are abundant in all of the SSPS cores. Thus, BDGT abundances appear to be dictated by ecology rather than temperature, with tropical peats typically characterized by woody plants and elevated BDGTs. BDGT proportions also increase with depth at all sites in the SPSS and globally, also consistent with a methanogen source.

These examples are representative of features in the wider core lipid distribution (and preliminary analyses of IPL diversity): 1) archaeal lipids are widespread through the peat column with assemblages changing and becoming more diverse with depth, largely due to greater proportions of isoGDGT isomers and BDGTs; 2) although depth is the dominant discriminant of archaeal lipid assemblages there are strong differences between tropical and temperate peats, and this likely reflects vegetation and OM composition as well as temperature; 3) differences among vegetation types in the SSPS are significant but subordinate to downcore differences. Comparison with OM composition and 16S rRNA gene derived community profiles suggests that archaeal heterotrophs/fermenters likely contribute significantly to the GDGT pool, especially in tropical settings where high temperatures give rise to rapid rates of OM degradation and a large heterotrophic microbial population. Our 16S rRNA analyses suggest that the metabolically versatile Bathyarchaeia are very important contributors to the peat microbiome especially in the tropics and below the water table. Enhanced heterotrophy at tropical temperatures undoubtedly fuels high rates of archaeal methanogenesis, but it is becoming increasingly clear that methanogens are not the sole (or even dominant) source of archaeal lipids in these settings. (We acknowledge the Smithsonian Tropical Research Institute for support as well as Eric Brown for guidance and insight into the SSPS peatland.)

References

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