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Compound-specific carbon and hydrogen isotope analysis traces archaeal lipid signatures in cold seep marine systems

KATHERINE KELLER[1], SEBASTIAN KOPF[2], JAMIE MCFARLIN[3], ASHLEY MALONEY[2], JEEMIN H RHIM[4], DR. FELIX J. ELLING[5], KEMI ASHING-GIWA[6], ISABEL R BAKER[7], AMANDA CALHOUN[1] AND **ANN PEARSON**[1]

[1]Harvard University
[2]University of Colorado Boulder
[3]University of Wyoming
[4]University of California, Santa Barbara
[5]Christian-Albrechts-Universität zu Kiel
[6]Stanford University
[7]Johns Hopkins University

Compound-specific stable isotope analyses of archaeal lipid biomarkers, including isoprenoid glycerol dialkyl glycerol tetraether lipids (iGDGTs) and their integral biphytane (BP) moieties, provide powerful constraints on biogeochemical cycling in marine systems. The widely applied TEX_{86} sea surface temperature proxy estimates temperature from the degree of iGDGT cyclization and traditionally assumes a dominant contribution from planktonic, ammonia-oxidizing Nitrososphaerota (*syn.* Thaumarchaeota). However, contributions from additional archaeal sources complicate TEX_{86} application and interpretation, particularly in methane-influenced environments.

Previously, we reported compound-specific $\delta^{13}C$ values of iGDGTs as a means of discriminating archaeal lipid sources in sediments from Astoria Canyon, a methane-influenced cold seep along the Cascadia Margin [1]. These results demonstrated that the iGDGT pool is dominated by planktonic and benthic methane-cycling archaea, with methane influence varying substantially across three sites (approximately 2% to 45%). Here, we extend this work by investigating compound-specific $\delta^{2}H$ values of the BPs derived from the individually purified iGDGTs [2]. Despite their potential to resolve lipid sources and metabolic diversity, hydrogen isotopic compositions of archaeal lipids remain underexplored in sedimentary settings. Using a mixing model applied to biphytane $\delta^{2}H$ values from individual iGDGTs, we estimate the hydrogen isotopic signatures of the planktonic and benthic methane-cycling archaeal endmembers and resolve the specific biphytane structural combinations comprising each parent iGDGT.

Our results indicate that both archaeal groups produce highly fractionated lipids, with modeled $\delta^{2}H$ values of –308 ‰ for planktonic archaea and –222 ‰ for benthic methane-cycling archaea. When combined with the prior compound-specific $\delta^{13}C$ data, this dual-isotope approach refines endmember constraints for archaeal lipid source apportionment and links isotopic variability to underlying lipid structure. Together, these findings show how the sedimentary methane cycle can influence iGDGT distributions and proxy behavior. Integrating $\delta^{2}H$ and $\delta^{13}C$ measurements with structural information provides a more nuanced framework for disentangling archaeal lipid sources, assessing methane cycling, and improving interpretation of iGDGT-based proxies in methane-impacted marine environments.

[1] Keller KJ et al. (2025a) *Organic Geochemistry* 200:104882.

[2] Keller KJ et al. (2025b) *Geochimica et Cosmochimica Acta* 407:309-321.