

## Production of branched tetraether lipids in soil enrichment cultures

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Branched glycerol dialkyl glycerol tetraethers (bGDGTs) are unique ether-linked cell membrane lipids detected in diverse environments. *Acidobacteria* are considered to produce them in acidic soils and peat bogs. However, the biological source of bGDGTs in alkaline soils and the mechanism of their response to environmental change are still obscure. We designed a 1-year incubation experiment using different media under varying temperature conditions (30, 45, 69 and 85 °C) in order to understand the mechanisms underlying the change in bGDGTs in the non-acidic soil environment. The best results were obtained at 45 °C, which showed 10fold increase in abundance of both PL-bGDGTs (polar lipid-derived bGDGTs, “living”) and CL-bGDGTs (core lipid bGDGTs, “fossil”) relative to the original concentration after 8 month incubation, indicative of biosynthesis of the bGDGTs during the incubation. The MBT/CBT-derived temperature was close to the incubation temperature (45 °C) at the end of experiment, suggesting that MBT/CBT proxy correctly reflected the growth temperature of bGDGT producing organisms under the laboratory conditions. High throughput sequencing of the enrichment cultures showed the absence of *Acidobacteria*, suggesting that bGDGTs were produced by other bacteria growing in the enrichment.