Redox-dependent niche differentiation provides evidence for multiple bacterial sources of glycerol tetraether lipids in lakes

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Terrestrial paleoclimate archives such as lake sediments are essential for our understanding of the continental climate system and for the modeling of future climate scenarios. However, quantitative proxies for the determination of paleotemperatures are sparse. The relative abundances of certain bacterial lipids, i.e., branched glycerol dialkylglycerol tetraethers (brGDGTs), respond to changes in environmental temperature, and thus have great potential for climate reconstruction. Their application to lake deposits, however, is hampered by the lack of fundamental knowledge on the ecology of brGDGT-producing microbes in lakes. Here, we show that brGDGTs are synthesized by multiple groups of bacteria thriving under contrasting redox regimes in a deep meromictic Swiss lake (Lake Lugano). This niche partitioning is evidenced by highly distinct brGDGT inventories in oxic vs. anoxic water masses, and corresponding vertical patterns in bacterial 16S rRNA gene abundances, implying that sedimentary brGDGT records are affected by temperature-independent changes in the community composition of their microbial producers. Furthermore, the stable carbon isotope composition (δ13C) of brGDGTs in Lake Lugano and 34 other (peri-)Alpine lakes attests to the widespread heterotrophic incorporation of 13C-depleted, methane-derived biomass at the redox transition zone of mesotrophic to eutrophic lake systems. The brGDGTs produced under such hypoxic/methanotrophic conditions reflect nontemperature-dependent changes in the community composition of their microbial producers. Moreover, the stable carbon isotope composition (δ13C) of brGDGTs in Lake Lugano and 34 other (peri-)Alpine lakes attests to the widespread heterotrophic incorporation of 13C-depleted, methane-derived biomass at the redox transition zone of mesotrophic to eutrophic lake systems. The brGDGTs produced under such hypoxic/methanotrophic conditions reflect temperature-independent changes in the community composition of their microbial producers. Furthermore, terrestrial archives, and linking continental paleoclimate records with oak sediments are based on the empirical correlation of the brGDGT distribution in contemporary soils with mean annual air temperature (MAT) and pH, which, in turn, gave rise to the hypothesis that brGDGT-producing microbes adjust the composition of their membranes in response to changing environmental conditions (4, 5). This mechanism is known as “membrane lipid homeostasis” (6), and is also pertinent to other molecular proxies such as archaeal tetraether lipids (7). Quantitative links between the brGDGT composition and latitudinal/altitudinal MAT gradients are evident in surface sediments of lakes (8, 9), enabling the reconstruction of past climate variability in lacustrine sedimentary archives, and linking continental paleoclimate records with those from the marine realm (e.g., ref. 10).

Significance

Reliable prediction of future climate conditions requires a thorough understanding of climate variability throughout Earth’s history. Microbial molecular fossils, such as bacterial membrane-spanning tetraether lipids [branched glycerol dialkyl glycerol tetraethers (brGDGTs)], have proven to be particularly useful for the assessment of past climatic conditions, because they occur ubiquitously in the environment and show compositional changes related to temperature. However, the identity and ecology of brGDGT-producing bacteria is largely unknown, and a mechanistic basis for brGDGT-based paleoclimate reconstruction is still lacking. Here, we present insights into the ecological parameters that affect brGDGT synthesis in lakes, demonstrating that eutrophic waters with oxygen-deprived bottom waters are the preferred sites for brGDGT-based reconstructions of continental climate.

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Despite the ubiquitous presence of brGDGTs in both terrestrial and freshwater environments, knowledge of the identity and ecophysiology of their source organisms is still severely limited. The glycerol stereochromy of brGDGTs in peat revealed that these compounds are produced by bacteria (11), which is remarkable in light of the fact that the vast majority of cultured bacterial strains exclusively contain ester- (instead of ether) bound membrane lipids. The divergent and still poorly characterized Acidobacteria represent an important component of microbial communities in soils and peat bogs (12, 13), and were suggested as potential biological sources because brGDGT abundance correlates with acidobacterial 16S rRNA gene copy numbers (11, 14). So far, only soil-inhabiting members of this phylum have been found to produce building blocks of brGDGTs, i.e., iso diabolic acid and derivatives, whereas full brGDGT structures are largely absent in the available acidobacterial isolates (15). Information on the occurrence and diversity of Acidobacteria in lakes, however, is extremely rare (e.g., refs. 16–18), and potential links between microbial ecology and aquatic brGDGT synthesis are uncertain.

To identify the locus of brGDGT biosynthesis within the water column, and to constrain the ecology of their microbial producers in lakes, we investigated the distribution and stable carbon isotope composition of their alkyl moieties in the water column of Lake Lugano (Switzerland), as well as in surface sediments of 34 other lakes in the European Alps. In Lake Lugano, we further compared the vertical distribution and abundance of bacterial 16S rRNA genes with those of individual brGDGT isomers, and assessed the potential for aquatic brGDGT synthesis by lipid stable isotope probing. We provide conclusive evidence for lacustrine brGDGT production by spatially segregated microbial communities thriving under both oxic and hypoxic/anoxic conditions, and the export of deeper water-derived brGDGTs to the sediments. These insights into the ecology of tetraether lipid-producing bacteria are important for unraveling the biological source(s) of these ubiquitous bacterial lipids, and will allow for a more causative understanding of brGDGT paleo records in lake deposits.

Results and Discussion

Multiple Microbial BrGDGT Sources in Lake Lugano. The North Basin of Lake Lugano is 288 m deep, mesotrophic, and meromictic, with a permanent oxygencline at ~100 m and a seasonal thermocline at 10 m to 20 m water depth (Fig. 2 A). The brGDGT pool in suspended particulate matter (SPM) was dominated by noncyclic hexamethylated brGDGTs comprising three structural isomers (i.e., IIIa, III′a, and III′′a; Fig. 1), which accounted for 46 to 94% of all brGDGTs in the water column (data for the less abundant brGDGTs are shown in SI Appendix, Figs. S2 and S3). The organic carbon-normalized total concentration of brGDGTs was low in near-surface waters (~4 ng g−1 at 10 m depth), but increased to ~100 ng g−1 just below the thermocline (Fig. 2 B). Similarly, the fraction of intact polar brGDGTs that still retain the labile headgroup moieties present in living cells increased with depth from ~30% at the base of the thermocline to >70% (Fig. 2 B; see SI Appendix for further information). This is consistent with pronounced brGDGT synthesis in the deeper (anoxic) waters of the lake, which has previously been observed in other (seasonally) stratified lake systems (19–21).

We found profound changes in the relative contribution of brGDGTs IIIa, III′a, and III′′a throughout the water column (Fig. 2 C). While brGDGT IIIa showed a continuous increase with depth across the redox transition zone (RTZ), III′a was most abundant within oxygenated waters ~20 m above the oxic–anoxic interface, and rapidly decreased below. In stark contrast to all other brGDGTs, the III′′a isomer, which was recently identified in anoxic sediments of another Alpine lake (22), occurred exclusively at depths below 90 m. Moreover, it was detected neither in catchment soils nor in riverbed sediments collected from the lake’s watershed (SI Appendix, Fig. S5 and Table S1), suggesting that it is exclusively produced by anaerobic bacteria within the deeper waters (cf. ref. 22).

The spatially segregated production of these structurally similar brGDGTs in oxic and hypoxic/anoxic water masses is also reflected in their sinking fluxes, which we assessed by analyzing settling particles collected in sediment traps from three different depths throughout an annual cycle (Fig. 2 C). Fluxes of IIIa increased ~10-fold between the upper (20 m) and middle (85 m) trap but did not further increase below, indicating that it is predominantly biosynthesized in the lower part of the oxygenated water column. In contrast, III′a was only present in settling particles below the RTZ (176 m), further attesting to its exclusive production within oxygen-deprived waters. IIIa, on the other hand, showed increasing fluxes across the RTZ, suggesting that it is produced by both aerobic and anaerobic, or facultative anaerobic, bacteria.

Distinct sources of these specific brGDGTs within Lake Lugano are also indicated by the stable carbon isotope signature of their alkyl moieties. In contrast to the bulk of brGDGT-derived alkanes that showed significantly decreasing δ13C values with depth (i.e., up to 10‰; Fig. 2 D), the C isotope signature of alkane α that is exclusively contained in brGDGT III′a (compare Fig. 1) remained unchanged across the RTZ, suggesting that it is only produced in the oxygenated part of the lake, from where it is exported to the sediment. To further explore the potential of brGDGT biosynthesis under oxic conditions, we amended near-surface SPM from Lake Lugano (10 m depth) with 13C-enriched organic substrates (see SI Appendix for details). Indeed, we observed pronounced production of the brGDGT III′a and other C6-methylated isomers, providing evidence for an aerobic metabolism of their producers, whereas IIIa, III′a′, and all other C5-methylated brGDGTs were not detectable at the end of the experiment (SI Appendix, Fig. S6).

The clear vertical segregation of the isomers III′a′ and III′′a, as well as their distinct stable carbon isotope signatures, strongly suggests that they are produced by distinct (groups of) microorganisms with different redox requirements. To elaborate links between brGDGT synthesis and microbial niche differentiation, we investigated vertical trends in the bacterial population by 16S rRNA gene sequencing. The data indicated an increase in the number of brGDGT producers across the RTZ accompanied by a marked shift in the bacterial community, with only 11% of the operational taxonomic units (OTUs) being shared between oxic (10 m to 80 m) and anoxic (90 m to 275 m) water masses. Furthermore, we compared OTU-specific DNA concentrations with the concentration
of each individual brGDGT in the water column (see SI Appendix for details). A substantial fraction of the bacterial community (i.e., 30% of the OTUs) showed a strong correlation (r > 0.75) with the abundance of at least one of the major brGDGTs, whereas this correlation was much weaker when all brGDGTs were considered as a whole (r ≤ 0.5; SI Appendix, Fig. S7). Moreover, the bacterial clades that correlated with individual brGDGTs were often taxonomically distinct from one another (Fig. 3). In particular, the OTUs correlating well with brGDGT IIIa had taxonomic affiliations distinct from those corresponding to brGDGTs IIIa, in support of brGDGT synthesis by multiple (groups of) bacteria that reside in different ecological niches (e.g., oxic vs. hypoxic/anoxic).

Acidobacteria are a plausible biological source of brGDGTs, due to their membrane lipid composition (15). In Lake Lugano, 88% of all acidobacterial OTUs correlated well (r > 0.75) with one or more of the major brGDGTs, and the diversity between the acidobacterial groups associated with different brGDGTs varied strongly (Fig. 3). This attests to a marked niche partitioning among members of the Acidobacteria that seems to resemble the differential vertical trends of carbon isotope ratios measured at different depths (see SI Appendix, Figs. S8 and S9). However, the vertical distribution of brGDGTs also corresponded to OTUs belonging to many other bacterial clades (Fig. 3 and SI Appendix, Figs. S8 and S9), suggesting that the trait of brGDGT synthesis extends beyond the phylum of Acidobacteria. Because ecological niches within the water column are often shared by diverse groups of microbes due to syntrophic relationships (23) or similar metabolic lifestyles (17), we cannot draw definite conclusions on the identity of brGDGT producers in Lake Lugano.

Carbon Metabolism of brGDGT-Producing Bacteria in Lake Lugano.

In Lake Lugano’s water column, the 13C content of brGDGTs (assessed by their alkyl moieties) provides insight into the potential C substrates utilized by their bacterial producers. Coinciding with the marked shift in the brGDGT distribution, the 13C content of most brGDGTs (i.e., the 13C values of the alkyl moieties a, b, and c) decreased from ~33 to ~42‰ across the RTZ (Fig. 2D). This may, in part, reflect the vertical 13C gradient of the C substrate utilized by brGDGT producers, and/or may be due to a distinct biosynthetic 13C fractionation expressed by different (groups of) source organisms. To elucidate this further, we measured 13C in the inorganic and organic C pools that may serve as substrates for brGDGT production within the water column. In fact, bulk suspended particulate organic matter (POM) showed vertical 13C trends similar to the brGDGTs (i.e., a decline across the RTZ; Fig. 2D), whereas 13C values of dissolved inorganic carbon (DIC) did not change substantially with depth (SI Appendix, Fig. S9). We therefore conclude that brGDGT-producing bacteria in Lake Lugano are likely involved in the heterotrophic breakdown and assimilation of particulate organic C in the water column, which is in agreement with the known metabolic capabilities of acidobacterial isolates (24), and analogous to what has previously been suggested for peat bogs and soils (25, 26). The more negative 13C values encountered at the RTZ, in turn, are predominantly due to the presence of methanotrophic bacteria that oxidize and incorporate 13C-depleted methane (~60‰) at and below the anoxic interface (27). We thus argue that at least some of the brGDGT producers thriving within the RTZ are part of a methane-based microbial food web (22, 28), in which they assimilate organic matter that is derived either directly from
methanotrophic bacteria and/or from other organisms that have previously incorporated methanotroph-derived carbon (29–31).

**Fig. 3.** Diversity of OTUs that show strong empirical correlation ($r > 0.75$) with at least one of the six major brGDGTs in Lake Lugano (30% of all 1,581 OTUs analyzed). OTU-specific DNA concentrations were estimated based on bacterial 16S rRNA gene abundances and total DNA concentrations (see SI Appendix for details), and the vertical concentration profiles of OTUs and individual brGDGTs were then compared by bivariate correlation analysis (SI Appendix, Fig. S7; https://github.com/yukiweber/phylo.lipids). The phylogenetic affiliation of the highly correlated OTUs ($r > 0.75$) is shown for each brGDGT on the phylum level (large pie charts) and is further differentiated for the Acidobacteria (class level), smaller pie charts, red). The diversity of the (acido) bacteria associated with each brGDGT is markedly distinct, suggesting differential brGDGT synthesis by multiple (acido) bacterial taxa. Key: a, Acidobacteria; b, Actinobacteria; c, Alphaproteobacteria; d, Armamonadetes; e, Bacteroidetes; f, Betaproteobacteria; g, Chloroflexi; h, Deltaproteobacteria; i, Firmicutes; j, Gammaproteobacteria; k, Planctomycetes; l, Spirochaetes; m, TM6-Dependentiae; n, Verrucomicrobia; unk., unknown phylum; SD3 to SD21, subdivisions of the Acidobacteria. Phyla contributing less than 4% to the total number of OTUs in each group are subsampled as “other.” The number of OTUs in each group (n) is shown for each pie chart.

**Fig. 4.** Stable C isotope composition of brGDGT-derived alkanes (a to f) and TOC in lake surface sediments from the European Alps. (A) Alkane e that exclusively originates from brGDGT IIIa′ (alkane e) had substantially higher δ13C values (≥24‰) than those originating from brGDGTs IIIa and IIIa” (alkanes d and f; Fig. 4B). This isotopic offset further seems to be associated with lakes that are more eutrophic and have relatively lower δ13Cdaf values. We therefore assume that the production of brGDGT IIIa′ in these lakes is also spatially separated from that of the bulk of the other major brGDGTs, similar to what we show for Lake Lugano (compare Fig. 2D). Moreover, in >50% of the lake sediments, the most common alkyl moieties of brGDGTs (i.e., alkane a, b, and c) were depleted in 13C relative to total organic carbon (TOC) (i.e., ≥5‰ lower δ13C values) (Fig. 4B), again indicating a substantial contribution of brGDGTs produced in methanotrophic water and/or sediment layers, as we propose for Lake Lugano (see also SI Appendix, Fig. S5). Remarkably, these low δ13C(alkane) values are consistently associated with an elevated trophic state of the lake (Fig. 4C), which, in turn, gives rise to an enhanced export production, as well as anoxia (Fig. 4D) and methane accumulation in bottom waters (32). Eutrophication and redox stratification thus likely promote the growth of brGDGT-producing microbes at the RZTW where methanotrophy may play an important role in microbial food webs. As indicated by the δ13C data, these deeper water-derived brGDGTs substantially contribute to the sedimentary brGDGT pool. In Lake Lugano, the production of brGDGT IIIa′ is restricted to hypoxic/anoxic water (compare Fig. 2C). Corroboratively, we detected this compound preferentially in sediments of mesotrophic to eutrophic lakes (9 out of 11 cases; SI Appendix, Table S1), where biological oxygen consumption is likely to cause anoxia within the bottom water and/or the top layer of the surface sediment. The presence of brGDGT IIIa′ may thus be used as a redox indicator and, indirectly, as a proxy for the trophic state. Taken together, redox-dependent differentiation of brGDGT-producing bacterial communities, as shown here for Lake Lugano, seems to be prevalent in a substantial fraction of lake systems. Compositional shifts in sedimentary brGDGT records may thus not only reflect the ability of bacteria to adapt the chemical properties of their membrane in response to temperature and pH (5, 6), but may also be strongly dependent on changes in the trophic state (33) and the microbial community composition.

**Implications for Lacustrine Paleothermometry.** Observed correlations between brGDGT distributions in recent lake sediments and MAT gave rise to transfer functions that allow for the quantitative reconstruction of paleoclimatic conditions from lacustrine sediment archives (e.g., ref. 9). For instance, the Methylation Index of brGDGTs (MBT’SMP, reflecting the relative amount of C5-methyl branches; ref. 4) has been successfully applied to East African lake sediments, where it showed a strong correlation with MAT ($r = 0.96$; ref. 34). However, this correlation was poor for the surface sediments of the lakes investigated here ($r = 0.33, n = 36$). Several factors may affect the fidelity of brGDGT-based temperature proxies in lake sediments. One major concern is that teregerous brGDGTs may contribute to the sediment through soil organic matter inputs. The brGDGTs from soils have a composition
that is markedly distinct from those produced within lakes of the same climate regime (8), and, as a consequence, their admixture to the aquatically produced brGDGT pool can substantially bias temperature estimates derived from lake deposits. Another factor relates to the vertical temperature distribution within the water column. Besides a possible input of soil-derived brGDGTs, the water depth from where the in situ-produced brGDGTs are predominantly transferred to the sediments will ultimately determine the “temperature imprint” of brGDGT in the surface sediment. In Lake Lugano, the largest part of the brGDGT sinking flux can be attributed to production in subthermocline (i.e., hypolimnetic) water (compare Fig. 2C), and thus the pool of brGDGTs finally reaching the sediment reflects isothermal deeper water, rather than seasonal surface water conditions (refs. 19 and 35 and SI Appendix, Fig. S10). On the other hand, based in lakes with low water production, the sedimentary brGDGTs may more dominantly derive from surface water, shifting the proxy record toward higher temperatures (i.e., increased MBT index values). The relative contributions of soil-, (near) surface water-, and hypolimnion-derived proxy signals to the sediments may consequently vary largely among different study sites, depending on hydrochemical parameters (e.g., trophic state/redox conditions), morphological characteristics (i.e., maximal depth), and the depositional environment (i.e., soil organic matter input), which is likely to confound the quantitative relationships between atmospheric MAT and the sedimentary brGDGT record. Knowledge of the predominant brGDGT sources, therefore, is of prime importance for the use of brGDGTs in paleoenvironmental reconstructions.

Our data from Lake Lugano show that the brGDGTs produced at the RTZ—likely by utilization of methane-derived C compounds—are much more depleted in 13C than those synthesized in both theoxic part of the water column (≥35%; compare Fig. 2D) and soil (≤25 to ≤31% (25, 26); see also SI Appendix, Table S1). Such 13C-depleted sedimentary brGDGT pools in eutrophic lakes may thus be indicative of proxy signals that are exclusively reflective of variations in water temperature, and may hence be largely unaffected by above-mentioned differential brGDGT inputs from surface water and/or soils. To further investigate the temperature response of sediments with a deeper water-dominated brGDGT source, we reassessed the MAT−brGDGT correlation in our dataset for different δ13C domains. We found that, in mesotrophic to eutrophic lakes with low δ13C(a,b,c) values (−45 to −36‰), the correlation between MBT <S1> and related proxy indices; SI Appendix, Fig. S11</S1> and MAT was indeed significant (r = 0.67, n = 15, P < 0.01), and much stronger than in oligo- to mesotrophic lakes, where the δ13C(a,b,c) was higher (r = 0.16, n = 20, P = 0.5). This correlation was again substantially improved when only shallow lakes with a maximal depth of ≤40 m were considered (r = 0.87, n = 10, P < 0.01; Fig. 5B), in which the RTZ may overlap with the thermocline, and the low 13C brGDGTs thus probably reflect thermocline water temperatures. In contrast, MBT <S1>proxy values were more or less constantly low among the deeper lakes (>40 m) (Fig. 5D), which we attribute to the decoupling of atmospheric air and hypolimnetic water temperatures in lakes of the cool-temperate climate zone (i.e., MAT ≤ 11 °C; ref. 36). In warmer climates, on the other hand, the temperature of hypolimnetic water is, in fact, linearly related to that of the atmosphere (37, 38), and hypolimnion-derived brGDGTs from deeper lakes will therefore be more responsive to interannual MAT variations than in the Alpine lakes investigated here.

We thus conclude that deeper water communities of brGDGT-producing bacteria thriving at the RTZ “reset” the sedimentary brGDGT “thermometer” to (near)-bottom water temperature, thereby mitigating the interfering effects of surface water- and/or cathemal soil-derived brGDGT signatures. Depending on climate zone and water depth, such settings, which can be identified by the C isotopic imprint of brGDGTs inherited from biogenic methane, are expected to yield reliable records of climate variability, and are preferable sites for the application of brGDGT-based paleotemperature proxies in lakes.
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