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Summary

Lake sediments are globally important carbon sinks. Although the fate of organic carbon (OC) in lake sediments depends significantly on microorganisms, only few studies have investigated controls on lake sedimentary microbial communities. Here we investigate the impact of anthropogenic eutrophication, which affects redox chemistry and organic matter (OM) sources in sediments, on microbial communities across five lakes in central Switzerland. Lipid biomarkers and distributions of microbial respiration reactions indicate strong increases in aquatic OM contributions and microbial activity with increasing trophic state. Across all lakes, 16S rRNA genes analyses indicate similar depth-dependent zonations at the phylum- and class-level that follow vertical distributions of OM sources and respiration reactions. Yet, there are notable differences, such as higher abundances of nitrifying Bacteria and Archaea in oligotrophic lake. Furthermore, analyses at order-level and below suggest changes in OM sources due to eutrophication cause permanent changes in bacterial community structure. By contrast, archaeal communities are differentiated according to trophic state in recently deposited layers, but converge in older sediments deposited under different trophic regimes. Our study indicates an important role for trophic state in driving lacustrine sediment microbial communities and reveals fundamental differences in the temporal responses of sediment Bacteria and Archaea to eutrophication.

Introduction

Despite only accounting for 2% of Earth's land area, lake sediments are globally important organic carbon (OC) sinks (Dean and Gorham, 1998; Cole *et al.*, 2007; Mendonca *et al.*, 2017). Most of this OC comes from autochthonous production by phytoplankton and aquatic macrophytes and from allochthonous sources in surrounding watersheds (Meyers and Ishiwatari, 1993; Einsele *et al.*, 2001). A major fraction of the OC deposited to lake sediments is mineralized by microorganisms, which gain energy for growth and survival by degrading this OC to carbon dioxide (CO_2) and methane (CH_4) through a network of reactions. The microbial production of CH_4 in sediments results in lakes being important greenhouse gas sources in addition to OC sinks (Dean and Gorham, 1998; Einsele *et al.*, 2001; Bastviken *et al.*, 2011).

Microbial OC degradation in sediments comprises aerobic and anaerobic processes. In all cases, the hydrolysis of organic macromolecules (e.g., proteins, carbohydrates, lipids) into smaller molecules, such as monomers, by extracellular enzymes is a crucial first step that enables OC uptake for energy conservation. While many aerobic microorganisms completely oxidize monomers to CO_2, OC dissimilation under anaerobic conditions is a multi-stage process undertaken by incomplete and terminal oxidizers (Canfield *et al.*, 2005). Primary fermenters transform monomers into low molecular weight compounds, such as H_2, CO_2, short-chain organic acids, and alcohols. Secondary fermenters then catabolize short-chain organic acids and alcohols to H_2, acetate, and C1 compounds (Capone and Kiene, 1988; Schink, 1997). The terminal oxidation of fermentation products to CO_2 is performed by respiring microorganisms, which use nitrate (NO_3^-), manganese(IV), iron(III), sulfate (SO_4^{2-}) or CO_2 as electron acceptors (Canfield *et al.*, 2005; Drake *et al.*, 2006). These electron acceptors are utilized in order of free energy yields, which is highest for O_2 and lowest for CO_2. This may result in a vertical zonation of dominant respiration reactions from oxic to anoxic sediments, the latter consisting of distinct depth intervals that are dominated by nitrate reduction (denitrification), Mn(IV) reduction, Fe(III) reduction, sulfate reduction, and CO_2 reduction (methanogenesis) as sediment depth increases (Capone and Kiene, 1988).

In addition to electron acceptors, OM sources may drive microbial community structure. This is because different microorganisms specialize in the breakdown and fermentation of different polymers and monomers, and because sedimentary OM sources differ significantly in chemical constituents. For instance, terrestrial vascular plants are largely made up of degradation-resistant (hemi)cellulose and lignin, whereas aquatic phytoplankton mainly consists of more labile lipids, carbohydrates, and proteins (Meyers and Ishiwatari, 1993; Hedges *et al.*, 1997; Killops and Killops, 2005). Despite well-known differences in hydrolytic and fermentative capabilities among microorganisms, the relationships between OM sources and microbial community structure in sediments have only been documented in a few cases (West *et al.*, 2012; Fagervold *et al.*, 2014; Xiong *et al.*, 2015). In these studies, the dominant operational taxonomic units (OTUs) of β-*Proteobacteria* and *Bacteroidetes* were positively correlated with terrestrial and phytoplankton derived fatty acids (FAs), respectively, whereas δ-*Proteobacteria* decreased with elevated input of C4 plant-derived OC, and methanogen communities did not change in relation to algal OM contributions.

The impact of human activity on microbial community structure in lake sediment is also not well understood. Enhanced anthropogenic input of nitrogen and phosphorus (P) to lakes promotes water column primary production (eutrophication), which in turn increases deposition of algal OM to sediments (Bechtel and Schubert, 2009; Anderson *et al.*, 2014). Land-use changes (e.g., deforestation, agriculture, hydroelectric dam construction) furthermore impact the contribution of land-derived OM (Enters *et al.*, 2006; Dubois and Jacob, 2016). Analyses of sedimentary lipid biomarker (e.g., n-alkanes, FAs, steroids) and lignin records are an efficient tool for determining past changes in aquatic and terrestrial OM sources (Meyers and Ishiwatari, 1993; Volkman *et al.*, 1998; Dubois and Jacob, 2016), and can be used to trace the impact of eutrophication on aquatic primary production (Bechtel and Schubert, 2009; Naeher *et al.*, 2012). Sedimentary compositions of lignin phenols, on the other hand, enable the reconstruction of vegetation histories and soil erosion in surrounding lake catchments (Leopold *et al.*, 1982; Hu *et al.*, 1999).

Here we test the hypothesis that anthropogenic eutrophication, through its impact on dominant OM sources and respiration reactions, drives microbial community structure in lake sediment. Based on the oligotrophic Lake Lucerne, the mesotrophic Lake Zurich, and the eutrophic Lake Zug, Lake Greifen,

and Lake Baldegg, we analyze relationships between microbial community structure, determined based on bacterial and archaeal 16S rRNA gene sequences, and (1) trophic history over the past 180 years, (2) dominant OM sources, revealed by lipid biomarker and lignin analyses, and (3) distributions of respiration reactions.

Results

In this section we first present the distributions of OM biomarkers and respiration zones across the five lakes before documenting patterns in microbial abundance, diversity, and community structure. We conclude with an analysis of relationships between microbial community structure and OM sources, respiration reactions, and trophic history. For an overview of the trophic history of the five lakes we refer to the subsection "Sampling sites and sample collection" at the beginning of the Experimental Procedures.

Distributions of OM sources and respiration reactions

Lipid biomarker data (Figure 1) indicate that aquatic OM sources, i.e. short-chain n-alkanes (C15+C17+C19) and FAs (C14+C16+C18), and steroids from diatoms (brassicasterol+24-methylenecholesterol) and dinoflagellates (dinosterol + dinostanol), decrease with sediment depth in all lakes. Consistent with stimulation of aquatic primary production by eutrophication, Lake Greifen, Lake Baldegg, Lake Zug, and Lake Zurich contain higher amounts of these biomarkers in surface sediments than Lake Lucerne. Both lignin phenol and long-chain FA contents have strong depth-related fluctuations (Figure 1, Supplementary Figure S1). Yet, consistent with lower microbial degradation rates of OM from higher plants compared to aquatic OM, contributions of long-chain n-alkanes [(C27+C29+C31)/(C15+C17+C19)] and lignin phenols increase or show no clear changes with depth.

Distributions of respiration reactions have strong vertical overlaps but appear influenced by trophic state (Table 1). Consistent with higher microbial activity fueled by higher OC input, the water column-derived electron acceptors O_2, nitrate and sulfate are depleted at shallower depths in the eutrophic Lake Greifen,

Lake Baldegg, and Lake Zug compared to the mesotrophic Lake Zurich and oligotrophic Lake Lucerne. The only exception is the site from the hypoxic deep basin of Lake Zurich, where O_2 and nitrate are absent from bottom water. The respiration of the less reactive electron acceptors Mn(IV), Fe(III) and CO_2 extends into deeper layers. Mn reduction rates are mainly controlled by Mn supply, which is highest in Lake Zurich due to geochemical focusing (Schaller and Wehrli, 1996; Naeher *et al.*, 2013), whereas iron reduction and in particular methanogenesis rates increase with trophic state (Fiskal *et al.*, 2019).

Bacterial and archaeal abundance trends

16S rRNA gene abundances show similar trends within and between lakes and are higher in Bacteria than in Archaea (Figure 2, Supplementary Figure S2). Despite having higher microbial activity, gene abundances are not higher in the eutrophic compared to the meso- and oligotrophic lakes. Bacterial gene abundances decline approximately tenfold over the depth interval studied (surface: ~10^9 copies cm^{-3}; bottom: ~10^8 copies cm^{-3}) (Figure 2A). Archaeal gene abundances generally increase from the sediment surface (~10^7 copies cm^{-3}) to ~15-20 cm (>10^8 copies cm^{-3}), and decrease slightly or remain stable below (Figure 2B). Bacteria-to-Archaea gene ratios (BARs) decrease from surface sediments downward (Figure 2C). BAR values in surface sediments are lower in eutrophic lakes (Lake Greifen: 15.6±3.0; Lake Baldegg: 24.0±5.4; Lake Zug: 8.7±3.7) than in Lake Zurich (34.2±19.1) or Lake Lucerne (41.7±18.0), but are uniformly <10 in sediments below 20 cm (Figure 2C).

General sequencing results and microbial richness and diversity trends

Seven to nine samples, which included one bottom water (BW) sample and covered the entire cored sediment interval, were sequenced from each station. The total of 3,114,837 16S rRNA gene sequence reads for 112 samples (average: 27,800 reads per sample; all samples had >12,000 reads) were clustered into 8,976 bacterial and 840 archaeal 97%-similarity Zero-radius operational taxonomic units (ZOTUs) and fall into 62 bacterial and 13 archaeal phyla. The observed Richness and Shannon diversity are clearly higher in Bacteria than in Archaea (Supplementary Figure S3). Bacterial Observed Richness and Shannon Diversity Indices are highly similar between and within lakes, and change little with sediment depth (Observed: ~ 2,000 ZOTUs; Shannon: ~6 (5-7)). In all lakes, archaeal Observed Richness and

152 Shannon Diversity Indices increase in the top 0-5 cm, and stabilize below (Observed: ~200; Shannon:
153 ~4 (3-5)).

154 Phylum- and class-level variations in microbial communities

155 Relative abundances of major bacterial and archaeal groups indicate high similarities at the phylum- and
156 class-level groups across lakes (Figure 3). Among Bacteria, there is a decline in β- and γ-*Proteobacteria*,
157 *Bacteroidetes*, *Verrucomicrobia*, *Cyanobacteria* and *Acidobacteria*, but an increase in δ-*Proteobacteria*,
158 *Chloroflexi*, *Firmicutes*, *Planctomycetes*, *Actinobacteria*, *Armatimonadetes*, *Acetothermia* and
159 *Aminicenantes* with sediment depth at nearly all stations. Among Archaea, *Pacearchaeota* and
160 *Woesearchaeota* generally decrease, while *Thermoplasmata*, *Altiarchaeales*, *Diapherotrites* and
161 *Lokiarchaeota* overall increase with sediment depth. Co-occurrence patterns at the phylum- and class-
162 level (Supplementary Figure S4) show that the above-mentioned Bacteria and Archaea form two large,
163 separated surface and subsurface sedimentary clusters. Other, smaller clusters are formed by Marine
164 Group I (MGI), *Nitrospirae* and *Gemmatimonadetes*, and by the bathyarchaeotal MCG-6 and Group C3
165 subgroups. The clustering that was observed at the phylum- and class-level is similar, but less clear,
166 when dominant orders are incorporated into the analysis (Supplementary Figure S5).

167 Despite the similarities, there are also clear differences in community profiles across lakes. For Bacteria,
168 Lake Lucerne and shallow and medium stations of Lake Zurich have higher relative abundances of
169 *Nitrospirae* and lower relative abundances of *Bacteroidetes* compared to the other locations (Figure 3A).
170 SIMPER analyses show that relative abundances of the orders *Nitrospirales* (*Nitrospirae*), Sva0485,
171 *Syntrophobacterales*, 43F-1404R, NB1-j (all δ-*Proteobacteria*), vadinHA17 (*Bacteroidetes*), and
172 *Xanthomonadales* (γ-*Proteobacteria*) differ significantly between the three eutrophic lakes and
173 oligotrophic Lake Lucerne (>1%, $p<0.05$, Supplementary Table S1). The same groups except
174 *Syntrophobacterales* and vadinHA17 also contribute significantly to the differences between Lake
175 Zurich and Lake Lucerne. By contrast, no major orders differ significantly in relative abundances
176 between Lake Zurich and the three eutrophic lakes. For Archaea, the eutrophic lakes have elevated
177 fractions of MCG-6 and Soil Crenarchaeotic Group (SCG) and lower fractions of MGI compared to
178 oligotrophic Lake Lucerne and deep sediment layers of Lake Greifen and Lake Zug that were deposited

prior to the eutrophication era (Figure 3B). SIMPER analyses indicate differences between the three eutrophic lakes and oligotrophic Lake Lucerne to be mostly driven by MGI (14.6%, $p<0.001$) and *Thermoplasmata* (9.9%, $p<0.01$), and differences between Lake Zurich and Lake Lucerne to be largely controlled by MGI (14.0%, $p<0.001$) and *Woesarchaeota* (6.6%, $p<0.001$; Supplementary Table S1). Differences between Lake Zurich and the eutrophic lakes are significantly influenced by *Woesearchaeota* (6.6%, $p<0.01$).

ZOTU-level variations in bacterial and archaeal communities

Principal coordinate analysis (PCoA) plots at the 97% ZOTU-level show that microbial communities have similar depth-related trends within each lake (Supplementary Figure S6). When all lakes are analyzed together, additional, trophic state-related trends emerge (Figure 4A). Within similar depth horizons, bacterial communities strongly overlap between the three eutrophic lakes and the deep station in mesotrophic Lake Zurich. Yet, the shallower stations from Lake Zurich and all stations from oligotrophic Lake Lucerne are clearly separated, and cluster separately from each other. Within the meso- and eutrophic lakes, deep samples from all three stations in Lake Zug, shallow and deep stations in Lake Greifen, and the shallow station in Lake Zurich are exceptions. Bacterial communities from these deep samples, which were deposited before the onset of eutrophication (Figure 3), cluster with those in deep layers from oligotrophic Lake Lucerne (Figure 4A). Archaeal communities are also clearly structured in relation to trophic state, albeit mainly in the top 10 cm (Figure 4B). With increasing sediment depth, archaeal communities from all lakes become increasingly similar, suggesting a convergence in community structure that is independent of the trophic state at the time of deposition.

The observed trophic state-related trends are consistent at multiple phylogenetic levels. For Bacteria, trophic state-related trends are already clear at the order-level, and increase only marginally at the family-level or for ZOTU-cutoffs that mimic the class-level (80%), order-level (87%), family-level (92%), genus-level (95%)), or species-level (97%) (Supplementary Figure S7A; also see "Sequencing data processing" in "Experimental Procedures). While the vast majority of Archaea could not be phylogenetically classified below the class rank, ZOTU-cutoffs that mimic the same phylogenetic levels as for Bacteria also show a clear separation of archaeal communities at the order-level and below

206 (Supplementary Figure S7B). Statistical analyses based on Adonis and ANOSIM confirm the observed
207 structuring of bacterial and archaeal communities in relation to trophic state (Supplementary Table S2).

208 Microbial community structuring according to trophic state is also illustrated by shared numbers of 97%
209 ZOTUs. On average, 55% of the bacterial ZOTUs and 41% of the archaeal ZOTUs are shared between
210 Lake Greifen, Lake Baldegg, Lake Zug and Lake Zurich, while the value is only 31% and 28%,
211 respectively between these lakes and Lake Lucerne (Figure 5A). Geographic distance or connectivity
212 by rivers do not explain these patterns. Shared ZOTU percentages show no relationship with geographic
213 distance (Figure 5B), and, though all lakes belong to the Rhine River watershed, none are serially
214 connected to each other by the same tributaries. The only lakes whose outflows merge into the same
215 tributary (Reuss River), Lake Lucerne and Lake Zug, have low similarities in microbial community
216 structure.

217 Relationships between microbial community structure, dominant respiration 218 reactions, and OM sources

219 We investigated the importance of organic biomarkers and respiration reactions in driving overall
220 microbial community structure (97% ZOTU-level) based on a constrained analysis of principal
221 coordinates (CAP; Supplementary Figure S8) and Adonis statistical test (Table 2). Measured OM
222 biomarkers explain a significant part of the variation in bacterial (R^2=0.61) and archaeal (R^2=0.56)
223 community structure (Table 2), in particular in eutrophic lakes (Supplementary Figure S8A). While
224 aquatic biomarkers and terrestrial long-chain FAs show highly significant correlations, lignin phenols
225 are not or less significantly correlated with bacterial or archaeal community structure. Furthermore, in
226 all lakes microbial respiration educts (O_2, nitrate, sulfate) and end products (Mn^{2+}, Fe^{2+}, CH_4) show clear
227 trends in relation to and explain significant fractions of bacterial (R^2=0.28) and archaeal (R^2=0.21)
228 community variation (Table 2; Supplementary Figure S8B).

229 To investigate the importance of OM sources and respiration reactions in driving the relative abundances
230 of individual groups of microorganisms across the five lakes, we performed a heatmap analysis (Figure
231 6). BARs show a clear respiration zone-related pattern, being highest in oxic sediment and lowest in

methanogenic sediment. BARs can furthermore be explained with quantities of labile aquatic OM, i.e. short-chain n-alkanes (R^2=0.27), diatom sterols (R^2=0.37) and short-chain FAs (R^2=0.47). Among Bacteria, there are stronger correlations of *oxic group* organisms with chlorophyll *a*, most aquatic biomarkers, and several long-chain alkanes and FAs. These correlations include *Holophagales* (*Acidobacteria*), *Cyanobacteria*, *Chlorobi*, *Sphingobacteriales* (*Bacteroidetes*), most *Verrucomicrobia*, *Planctomycetales*, *Phycisphaeraceae* (all *Planctomycetes*), *Xanthomonadaceae*, *Legionellales*, *Cellvibrionales*, *Crenotrichaceae* (all γ-*Proteobacteria*), *Burkholderiales*, *Rhodocyclales* (all β-*Proteobacteria*), and *Rhodobacterales* (α-*Proteobacteria*). While no significant correlations between percentages of *suboxic group* and OM sources are observed, several *anoxic group* Bacteria, i.e. the dominant orders *Syntrophobacterales* (δ-*Proteobacteria*), SJA-15, GIF9 (both *Chloroflexi*), *Gaiellales* (*Actinobacteria*) and *Firmicutes* show strong correlations with terrestrial OM.

Patterns are similar for Archaea, i.e. *oxic group* shows stronger correlations with aquatic biomarkers and *anoxic group* with terrestrial OM sources. *Oxic group* organisms consist of MGI and SCG (both *Thaumarchaeota*), and *suboxic group* of *Pace*- and *Woesearchaeota*. Within *anoxic group*, lignin phenols are significantly correlated with Marine Benthic Group D (MBG-D) (*Thermosplasmata*), *Micrarcheia* (*Diapherotrites*), *Beta Subgroup* (*Lokiarchaeota*) and *Aenigmarchaeota*, but not with any of the major bathyarchaeotal subgroups.

Discussion

To our knowledge, this is the first study to investigate the relationship between trophic history and bacterial and archaeal community structure, and one of only a few studies that have investigated relationships between respiration reactions or OM source gradients and overall microbial community structure in lake sediment (Xiong *et al.*, 2015; Rissanen *et al.*, 2017). By comparison, several studies have documented the effects of trophic state on bacterial community structure focusing on the N-cycle (Small *et al.*, 2016; Palacin-Lizarbe *et al.*, 2019; Zeng *et al.*, 2019; Li *et al.*, 2020). Bacteria dominate all samples, with BARs being highest in oxic surface sediment of oligotrophic and mesotrophic lakes and decreasing with sediment depth (Figure 2). Similar to other lakes (Ye *et al.*, 2009; Wurzbacher *et*

258 *al.*, 2017), phylogenetic richness and diversity of Bacteria exceed those of Archaea (Figure 3;
259 Supplementary Figure S3). Yet, Archaea show more pronounced changes at the phylum- and class-level
260 in relation to lake trophic state than Bacteria (Figure 3, Supplementary Table S1). In the following
261 paragraphs we highlight the most important trends in microbial community structure, and how these are
262 likely related to eutrophication history, as well as sedimentary redox conditions and OM sources.

263 Microbial communities at the phylum- and class-level show similar sediment depth-related trends across
264 all lakes (Figure 3). Independent of trophic state, microbial communities in surface sediments are
265 dominated by groups that are often abundant in lacustrine surface sediments, i.e. α-, β-, and γ-
266 *Proteobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Planctomycetes*, *Acidobacteria*, and MGI Archaea
267 (Kadnikov *et al.*, 2012; Ruuskanen *et al.*, 2018). Phyla that are common in anoxic (subsurface)
268 sediments, i.e. *Chloroflexi*, δ-*Proteobacteria, Acetothermia, Aminicenantes*, *Bathyarchaeota*,
269 *Lokiarchaeota* and *Altiarchaeales* (Borrel *et al.*, 2012; Vuillemin *et al.*, 2018), increase in the top 5-10
270 cm and dominate below. Unlike a previous study (Wu *et al.*, 2019), we observe no trends in community
271 profiles in relation to water depth. A notable feature of all lakes is, however, the dominance of surface
272 sedimentary archaeal communities by the sister phyla *Pace-* and *Woesearchaeota*. Both phyla are
273 dominant water column Archaea in oligotrophic, high-altitude lakes (Ortiz-Alvarez and Casamayor,
274 2016), and *Woesearchaeota* have been reported to dominate Archaea in oligotrophic lake sediment
275 (Ruuskanen *et al.*, 2018). Yet, the dominant contribution of *Pacearchaeota* in lake sediment, and of
276 *Woesearchaeota* in eutrophic lake sediment is new. Interestingly, the percentages of *Pace-* and
277 *Woesearchaeota* have negative linear correlations with those of MBG-D (*Thermoplasmata*)
278 (R^2=0.28/0.23; both *p*-values<0.001), suggesting different redox preferences and/or competition for
279 similar resources between these dominant archaeal groups. While there are strong overlaps in phyla and
280 classes between bottom water (BW) and sediments, certain groups, e.g. MCG-6, Group C3, MCG-5 (all
281 *Bathyarchaeota*) and MBG-D (*Thermoplasmata*), are restricted to sediments. Moreover, *Atribacteria*,
282 which often dominate anoxic marine and lacustrine subsurface sediments (Orcutt et al., 2011; Vuillemin
283 *et al.*, 2016), only account for a small fraction of bacterial reads (0.21%±0.68%).

At the order-level and below, trophic state-related trends in bacterial and archaeal communities become much more evident (Figure 4, Supplementary Figure S7). While bacterial communities remain separated by trophic state with increasing sediment depth and age, archaeal communities converge in deeper and older sediment layers. The drivers behind these general differences in bacterial and archaeal community trajectories over time are unclear. A potential explanation is that trophic state permanently alters the chemical composition of insoluble organic macromolecules and that major groups of Bacteria are primary degraders of these macromolecules. This explanation is consistent with the fact that bacterial communities in pre-eutrophication era sediment of currently eutrophic lakes in numerous cases cluster with bacterial communities of oligotrophic Lake Lucerne and not with bacterial communities from the same lakes that were deposited after the onset of eutrophication. By contrast, dominant Archaea in deep, permanently anoxic layers might rely on energy sources that are ubiquitous independent of trophic state, e.g. fermentation intermediates, OM functional groups, terrestrial OM, and/or electron acceptors such as CO_2 or Fe(III).

Our CAP analyses indicate that both OM sources and dominant respiration reactions are strongly correlated with bacterial and archaeal community structure and explain much of the observed bacterial and archaeal community variation across the five lakes (Supplementary Figure S8; Table 2). Hereby the increased sedimentary input of more easily degradable aquatic OM in eutrophic lakes appears to be a very important driver behind the observed patterns in microbial community structure related to trophic state. We furthermore show that dominant microbial groups differ systematically in their distributions relative to OM sources and respiration reactions, suggesting diverse ecophysiological niches among these organisms (Figure 6). Hereby the distributions of *oxic*, *suboxic*, and *anoxic group* taxa, and the correlations of these taxa with OM sources, are largely consistent with published metabolic functions of their closest relatives (for a detailed analysis, we refer to the section "Potential drivers of microbial community structure" in the Supplementary Materials). Pure culture and genomic data on microorganisms from the same taxonomic groups as oxic group (mainly α-, β-, and γ-*Proteobacteria*, *Bacteroidetes*, *Acidobacteria, Verrucomicrobia, Planctomycetes*) indicate that closest known relatives mainly consist of aerobic and facultatively anaerobic carbohydrate-, amino acid-, and fatty acid-oxidizing bacteria (Rouf and Stokes, 1964; Coates *et al.*, 1999; Stein, 2001; Yoon *et al.*, 2008; Guo *et*

312 *al.*, 2014; Pujalte, 2014; Sun *et al.*, 2016), and aerobic nitrifying archaea (MGI and SCG) (Spang *et al.*,
313 2012; Kitzinger *et al.*, 2019). Closest relatives of *suboxic group* Bacteria include microaerophilic
314 nitrifiers, and microorganisms capable of anaerobic growth by denitrification, sulfate or metal reduction,
315 or fermentation (Balk *et al.*, 2008; Podosokorskaya *et al.*, 2013; Daims, 2014; Suzuki *et al.*, 2019),
316 whereas genomic data on *suboxic group* Archaea, i.e. *Pace*- and *Woesearchaeota*, suggest capacity for
317 fermentative and/or syntrophic metabolism (Castelle *et al.*, 2015; Liu *et al.*, 2018). Matching their
318 distributions, genomic and pure culture data indicate most *anoxic group* lineages to be obligate
319 anaerobes. Though members of *anoxic group* have highest percentages in Fe(III)-reducing and
320 methanogenic sediment, only a few groups, that are too rare to be included in Figure 6, can be linked to
321 Fe(III) reduction (*Clostridium* (*Firmicutes*): 0.14%±0.04% (Lovley, 1987)) or methanogenesis
322 (*Methanofastidiosa* (*Euryarchaeota*): 0.07%±0.26% (Nobu *et al.*, 2016); *note: the dominant*
323 *methanogenic class, Methanobacteria, are an outlier and have an oxic group distribution*). Instead
324 published data suggest the majority of *anoxic group* Bacteria and Archaea to be anaerobes that are
325 involved in the fermentation of carbohydrates and proteins, and degradation of aromatic compounds,
326 including lignin (Lloyd *et al.*, 2013; Kuever, 2014; McIlroy *et al.*, 2017; Yu *et al.*, 2018).

327 In addition to being driven by changes in OM sources and respiration reactions, the observed changes
328 in microbial community structure with depth could also be caused by selective survival. Accordingly,
329 taxa that were already present at the time of sediment deposition but are better equipped to persist under
330 the low energy conditions associated with burial than other taxa would become dominant with increasing
331 sediment depth (Lever *et al.*, 2015b; Petro *et al.*, 2017; Starnawski *et al.*, 2017; Rissanen *et al.*, 2019).
332 Strong decreases in microbial diversity, that are consistent with survival of a small number of low-
333 energy resilient taxa, have been documented for the top 30 cm of Lake Stechlin (Wurzbacher *et al.*,
334 2017). Yet, we observe no clear changes in microbial diversity over the top 40 cm of sediment across
335 the lakes we investigated (Supplementary Figure S3). Furthermore, similar to a recent study on marine
336 sediment (Chen *et al.*, 2017), we observe significant (2-4fold) increases in average archaeal 16S rRNA
337 gene copy numbers per volume or mass of wet sediment within the top 20 cm of sediment across all
338 lakes, suggesting net growth of archaeal populations (Figure 2; Table 3). These increases are unlikely
339 to be the outcome of past, eutrophication-related changes in archaeal deposition rates or archaeal growth

rates within surface sediments, since they are most pronounced in oligotrophic Lake Lucerne and mesotrophic Lake Zurich, which were least affected by eutrophication. Year-to-year fluctuations in deposition or growth rates are also unlikely drivers, given that the observed increases in archaeal gene copies represent trends over many decades. We explored the possibility of microbial growth further by calculating depth-related changes in abundances of major "subsurface" groups per volume or mass of wet sediment (Table 3; Supplementary Table S3A; Supplementary Figures S9A-B and S10A-B). While the abundances of *δ-Proteobacteria* and *Chloroflexi* do not change significantly with depth, consistent with stable population size and selective survival, significant increases are observed in bacterial *Acetothermia*, *Aminicenantes*, and the *Chloroflexi* class *Dehalococcoidia* across the entire data set and most individual lakes. Moreover, dominant subsurface archaeal groups, i.e. *Thermoplasmata* (mainly MBG-D), *Altiarchaeales*, *Lokiarchaeota*, *Diapherotrites*, and bathyarchaeotal MCG-6 and C3, increase significantly in abundance within the top 20 cm across the entire data set and in most individual lakes. This interpretation changes, however, if gene copy numbers are analyzed in relation to the mass of dry sediment. Under this scenario, total archaeal gene copy numbers do not change significantly with depth, and gene copy numbers of *Deltaproteobacteria* and *Chloroflexi* even decrease significantly with depth (Supplementary Table S3B; Supplementary Figures S9C and S10C). Yet, even under this scenario we observe significant increases in *Dehalococcoidia*, *Acetothermia*, *Thermoplasmata*, and Group C3 with depth, suggesting net growth of certain microbial groups with sediment depth.

The observed quantitative increases or stable gene copy numbers in total Archaea and specific subsurface bacterial and archaeal groups are also evidence against relic DNA (Torti *et al.*, 2015; Carini *et al.*, 2017) being the driver behind the observed vertical community shifts. Relic DNA from microorganisms, that were deposited from overlying water or thrived in surface sediments, would be expected to decrease, not increase, in content with sediment depth (Torti *et al.*, 2015). Furthermore, recent studies on marine sediments suggest that the preservation potential for microbial relic DNA is low, and that instead almost all microbial relic DNA comes from recently deceased sediment microorganisms (Ramirez *et al.*, 2018; Torti *et al.*, 2018).

Conclusions

Our study produces novel insights into the impact of anthropogenic eutrophication on microbial communities in sediments. We show that, independent of geographic distance or connectivity by rivers, eutrophic lakes have more similar microbiomes than lakes that differ in trophic state. Differences in bacterial and archaeal communities in relation to trophic state increase considerably from the phylum- to the order-level and less so from the order- to lower taxonomic levels, suggesting that microbial community filtering by trophic state is strongest at the order-level. Hereby trophic state-related changes in distributions and quantities of OM sources appear to be the main drivers behind the permanent changes in bacterial community structure. By contrast, archaeal communities are differentiated according to trophic state in recently deposited layers, but converge in older sediments deposited under different trophic regimes. Given that total Archaea, as well as several typical "subsurface" bacterial and archaeal taxa, increase significantly in population size with depth in the top 20 cm, there appears to be significant subsurface growth among microbial subpopulations. Thus, rather than consisting solely of persisting cells or survivors from surface sediments, shallow subsurface sediments in lakes appear to be favorable or even preferred habitats for certain groups of microorganisms.

Experimental Procedures

Sampling sites and sample collection

We studied sediment cores from the eutrophic Lake Greifen, Lake Baldegg, and Lake Zug, the mesotrophic Lake Zurich, and the oligotrophic Lake Lucerne in central Switzerland (Supplementary Figure S11, Table S5). The four eutrophic and mesotrophic lakes became highly eutrophic during the late 19th or early 20th century (Lake Greifen: ~1920; Lake Baldegg: ~1890; Lake Zug: ~1930; Lake Zurich: ~1890) due to high anthropogenic P inputs from sewage, detergents, and agricultural fertilizers, whereas Lake Lucerne only had a slight increase in P input and remained oligotrophic. Starting in the 1970s, wastewater treatment plants and P bans on detergents, along with artificial mixing and aeration in Lake Baldegg (1982/3) and Lake Greifen (2009), led to strong P concentration decreases. Since then Lake Zurich has become mesotrophic. Yet, Lake Greifen, Lake Baldegg, and Lake Zug remain eutrophic due to P remobilization from sediments. Lake Greifen still experiences seasonal hypoxia in bottom water,

whereas the deep basin of Lake Zurich remains hypoxic due to the low frequency of deep mixing events. For more details on the trophic histories of these lakes, see Fiskal *et al.* (2019). Within each lake, we sampled the top ~40 cm of sediment with a 150-mm diameter gravity corer (UWITEC, AT) at three stations (shallow, medium and deep, ~20 samples per station), which extended from the shallow sublittoral to the profundal zone (for sample depths and ages see Supplementary Table S6). Samples for DNA analyses were taken by sterile cut-off syringes, immediately frozen in liquid N_2, and subsequently stored at -80°C. Biomarker samples were taken using metal spatulas, stored on ice during sampling, and then frozen at -20°C.

Lipid biomarkers, lignin and chlorophyll *a* analyses

FAs and neutral lipids (n-alkanes, sterols, stanols) were extracted with methanol and dichloromethane, followed by potassium hydroxide saponification and derivatization with N,O-bis(trimethylsilyl) trifluoroacetamide for neutral lipids and boron trifluoride/methanol for FAs (modified from Naeher *et al.* (2012)). Lignin-derived phenols were characterized after alkaline cupric oxide oxidation, followed by acidification, ethyl acetate extraction and derivatization (Goni and Montgomery, 2000). All quantifications were done by gas chromatography with flame ionisation detection (Shimadzu, Kyoto, Japan). For further details see Methods section in Supplementary Materials. Chlorophyll *a* was extracted using acetone and quantified spectrophotometrically (Lever and Valiela, 2005).

DNA extraction

DNA was extracted according to Lever *et al.* (2015a). Samples from Lake Zug, Lake Zurich, and Lake Lucerne were extracted with lysis protocol II while those from Lake Greifen and Lake Baldegg underwent an additional humic acid removal step (lysis protocol III). For further details see Methods section in Supplementary Materials.

Quantification and sequencing of 16S rRNA genes

16S rRNA genes were quantified SYBR Green I Master on a LightCycler 480 II (Roche Molecular Systems, Inc.) (Lever *et al.*, 2015a). PCR inhibition was not detectable. The primer pairs for Bacteria

418 and Archaea were Bac908F_mod (5´-AAC TCA AAK GAA TTG ACG GG-3´) (Lever *et al.*, 2015a) /
419 Bac1075R (5´-CAC GAG CTG ACG ACA RCC-3´) (Ohkuma and Kudo, 1998) and Arch915F_mod
420 (5´-AAT TGG CGG GGG AGC AC-3´) (Cadillo-Quiroz *et al.*, 2006) / Arch1059R (5´-GCC ATG CAC
421 CWC CTC T-3´) (Yu *et al.*, 2005), respectively.

422 Seven to nine samples, which included one BW and covered the entire cored sediment interval and its
423 changes in OM contributions and respiration zones, were chosen for amplicon sequencing from each
424 station (further details see Supplementary Table S6). A fragment with ~283 bp that included the V4
425 hypervariable regions of bacterial and archaeal 16S rRNA genes was amplified by the universal primers
426 Univ519F (5´-CAG CMG CCG CGG TAA-3´) / Univ802R (5´-TAC NVG GGT ATC TAA TCC-3´)
427 (Claesson *et al.*, 2009; Wang and Qian, 2009). Library preparation started with a booster PCR, consisting
428 of the threshold cycle number of the bacterial qPCR plus three additional cycles, to obtain similar
429 amplicon concentrations across all samples, and was followed by tailed-primer (10 cycles) and index
430 PCRs (8 cycles). Sequencing (600 cycles) was done by paired-end sequencing using a MiSeq Personal
431 Sequencer (Illumina Inc., San Diego, California, USA). For further details, see Methods section in
432 Supplementary Materials.

433 Sequencing data processing

434 Raw sequence reads were first quality-checked by FastQC (Andrews, 2010). Read ends were trimmed
435 using seqtk (https://github.com/lh3/seqtk), and merged into amplicons by flash (Magoc and Salzberg,
436 2011) (max mismatches density, 0.15; max and min length, 500 and 150). Primer sites were trimmed by
437 usearch (in-silico PCR) (Martin, 2011). Quality filtering was done by prinseq (Schmieder and Edwards,
438 2011) (GC range, 30-70; Min Q mean, 20). ZOTUs were generated using the USEARCH unoise3()
439 algorithm with a 97% identity, which includes the removal of chimeric sequences (Edgar, 2016). A total
440 of 8,976 bacterial and 840 archaeal ZOTUs falling into 62 bacterial and 13 archaeal phyla were detected.
441 ZOTU tables were generated by USEARCH otutab() and phylogenetically assigned using USEARCH
442 utax() based on the SILVA SSU Ref NR release 128 database for bacterial 16S rRNA genes. Archaeal
443 16S rRNA genes were assigned in ARB (www.arb-home.de) using neighbor-joining phylogenetic trees
444 that were based on a manually optimized SILVA database with 16S gene sequences from whole-genome

studies. Because most ZOTUs could not be assigned to the genus rank for Bacteria and order rank for Archaea, we explored additional ZOTU sequence similarity cutoffs to investigate microbial community differences at different taxonomic levels (phylum-level: 75% sequence similarity; class: 80%; order: 87%; family: 92%; genus: 95% (Lever and Teske, 2015). All ZOTU sequences are publicly available under Genbank accession number KDOW00000000. Raw sequences have been deposited in Sequence Read Archive with accession number SAMN13038023 under the project PRJNA577818.

Statistical analyses

Analyses were done in R v3.4.0 (http://R-project.org) using R Studio v1.1.442 (http://rstudio.com). ZOTU files, mapfiles with biogeochemical data, tree files, and sequence files were merged into a "phyloseq" class with import_qiime() from phyloseq package (McMurdie and Holmes, 2013). Calculations were done on the read percentage (relative abundance) of each ZOTU or other phylogenetic group per sample. Variations in microbial communities were analyzed by PCoA based on Bray-Curtis dissimilarities (Beals, 1984). Significant differences in microbial communities between lakes were calculated by anosim() (ANOSIM) and adonis() (Permutational multivariate analysis of variance (PERMANOVA); Permutations: 1000), both based on Bray-Curtis dissimilarities using vegan (Clarke, 1993)). A SIMPER analysis was applied to identify orders that contribute the most to differences in microbial compositions between lakes (Clarke, 1993). Constrained analysis of principal coordinates (CAP, also called distance-based RDA) was done by capscale() using Bray-Curtis distance (Anderson and Willis, 2003). The contributions of environmental variables to microbial community structures were calculated by Adonis() (permutational multivariate analysis of variance with Bray-Curtis dissimilarities). Shared ZOTUs (Bacteria (top 800); Archaea (top 200)) between lakes were obtained using venn() from the gplots package. A correlation matrix between microbial community composition and biogeochemical parameters was produced based on Spearman coefficients and calculated and plotted using cor() and cor.mtest() from the Hmisc and Corrplot packages. To identify co-occurrence patterns of dominant microbial groups, a co-occurrence network was constructed based on Spearman correlation coefficients (ρ<0.5, p<0.01) (Junker and Schreiber, 2008). To remove false-positives, we adjusted P-

values with a multiple testing correction using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995).

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Conflict of Interest

The authors declare no conflict of interest.

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Tables

Table 1. Depth distributions (cmblf) of respiration reactions (average±standard deviation (SD)) across the five lakes (modified from Fiskal *et al.* (2019)). The deepest samples from the bottom of cores were typically from a depth interval of 36-40 cm.

	Lake Greifen	**Lake Baldegg**	**Lake Zug**	**Lake Zurich**	**Lake Lucerne**
Trophic state	eutrophic	eutrophic	eutrophic	mesotrophic	oligotrophic
Aerobic	0.0 - 0.17±0.03	0.0 - 0.08±0.02	0.0 - 0.23±0.03	0.0 - 0.22±0.08	0.0 - 0.73±0.25
Nitrate reduction	0.0 - 2.5±1.0	0.0 - 2.8±1.2	0.0 - 7.7±3.1	0.0 - 3.3±1.4	0.0 - 9.0±2.0
Sulfate reduction	0.0 - 5.8±2.0	0.0 - 6.2±3.3	0.0 - 11.7±3.1	0.0 - 10.3±1.2	0.0 - 11.0±2.0
Mn(IV) reduction	0.3±0.3 - 8.2±4.9	0.3±0.3 - 14±7	0.5±0.0 - 5.0±0.0	0.3±0.3 - 24±15	0.7±0.8 - bottom
Fe(III) reduction	0.5±0.0 - bottom	0.0 - bottom	0.5±0.0 - bottom	0.5±0.0 - bottom	0.8±0.6 - bottom
Methanogenesis	0.0 - bottom	0.0 - bottom	0.0 - bottom	2.6±2.4 - bottom	3.0±1.7 - bottom

Table 2. Relative contributions of environmental variables to driving microbial community structures across the five lakes based on Adonis (Permutational multivariate analysis of variance).

			Bacteria		**Archaea**	
			R^2	**P**	**R^2**	**P**
OM sources		Total OM	0.61	***	0.56	**
		TOC	0.12	***	0.07	**
		Chlorophyll a	0.08	*	0.08	**
	Aquatic	C15+C17+C19	0.13	***	0.10	***
		C23+C25	0.12	***	0.10	***
		C14+C16+C18	0.11	***	0.10	***
		(a+i)C15+C16:1	0.12	***	0.11	***
		Steroids A	0.15	***	0.12	***
		Steroids B	0.12	***	0.09	**
	Terrestrial	C27+C29+C31	0.14	***	0.09	**
		C24+C26+C28	0.12	***	0.08	**
		Vanillyl	0.05		0.06	*
		Syringyl	0.05		0.07	*
		Cinnamyl	0.05		0.05	
Respiration reaction		Total respiration	0.28	***	0.21	***
		Oxygen	0.05	***	0.05	***
		Nitrate	0.06	***	0.05	***
		Sulfate	0.10	***	0.08	***
		Manganese(II)	0.04	***	0.02	*
		Iron(II)	0.08	***	0.05	**
		Methane	0.12	***	0.08	**

Note: Steroids A: Diatom: Brassicasterol+24-methylenecholesterol; Steroids B: Dinoflagellates: Dinosterol+Dinostanol.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3. Spearman correlations of bacterial and archaeal 16S rRNA gene abundances based on qPCR, as well as calculated abundances of dominant subsurface bacteria and archaeal groups vs. sediment depth (0-20 cm). We show correlations for the entire data set (All lakes) and for all data from each individual lake. All significant correlations are positive, except those of bacterial 16S rRNA gene copy numbers vs. depth, which are negative. Abundances of dominant subsurface bacterial and archaeal groups were calculated by multiplying their fractions of total bacterial and archaeal 16S gene reads with total bacterial and archaeal 16S gene copy numbers cm^{-3} sediment, respectively. *Dehalococcoidia* are shown in addition to total *Chloroflexi* due to the frequent dominance of subsurface *Chloroflexi* by this class (Orcutt *et al.*, 2011; Teske *et al.*, 2014). MCG-6 and Group C3 are the dominant class-level subgroups of *Bathyarchaeota* in our study. Depth related trends across all lakes and stations are shown in Supplementary Figure S9 and Figure S10. Correlation analyses for additional sediment depth intervals (0-10, 0-15, 0-30, and 0-40 cm) are displayed in Supplementary Table S3. Correlations based on bacterial and archaeal 16S gene copy numbers g^{-1} wet and g^{-1} dry sediment are shown in Supplementary Table S4.

	All lakes		Lake Greifen		Lake Baldegg		Lake Zug		Lake Zurich		Lake Lucerne	
	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P
Bacterial 16S genes	0.16	***	0.25	***	0.08		0.56	***	0.16	**	0.37	***
Archaeal 16S genes	0.17	***	0.007		0.28	***	0.06		0.55	***	0.25	***
Deltaproteobacteria	0.03		0.10		0.02		0.13		0.03		0.001	
Chloroflexi	0.001		0.03		0.17		0.002		0.005		0.003	
Dehalococcoidia	0.29	***	0.40	**	0.74	***	0.37	**	0.26	*	0.57	***
Acetothermia	0.27	***	0.44	**	0.77	***	0.23		0.19		0.20	
Aminicenantes	0.23	***	0.47	*	0.79	***	0.49	**	0.28	*	0.23	*
Thermoplasmata	0.44	***	0.50	**	0.66	***	0.79	***	0.59	***	0.79	***
Altiarchaeales	0.18	***	0.06		0.68	***	0.01		0.47	**	0.32	*
Lokiarchaeota	0.15	***	0.01		0.002		0.57	***	0.33	*	0.44	**
Diapherotrites	0.12	**	0.03		0.08		0.15		0.25	*	0.34	*
MCG-6	0.16	***	0.007		0.39	*	0.16		0.35	*	0.53	***
Group C3	0.22	***	0.04		0.48	**	0.15		0.57	***	0.76	***

Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure legends

Figure 1. Depth profiles of (A) n-alkanes, (B) fatty acids, (C) steroids, and (D) lignin phenols. The onset of eutrophication is indicated by the black dot-dashed lines (Lake Greifen: ~1920; Lake Baldegg: ~1890; Lake Zug: ~1930; Lake Zurich: ~1890). The beginning of artificial mixing and aeration to prevent water column anoxia in Lake Baldegg (1982/3) and Lake Greifen (2009) is marked with red solid lines. The transition from eutrophic to mesotrophic in Zurich took place around 1980 (green dashed line). P concentrations in Lake Lucerne increased slightly, without a change in trophic state, from 1960-1990 (samples between dotted black lines). Abbreviations in (D): V = vanillyl phenols (incl. vanillin, acetovanillone and vanillic acid); S = syringyl phenols (incl. syringealdehyde, acetosyringone and syringic acid); C = cinnamyl phenols (incl. p-coumaric acid and ferulic acid.)

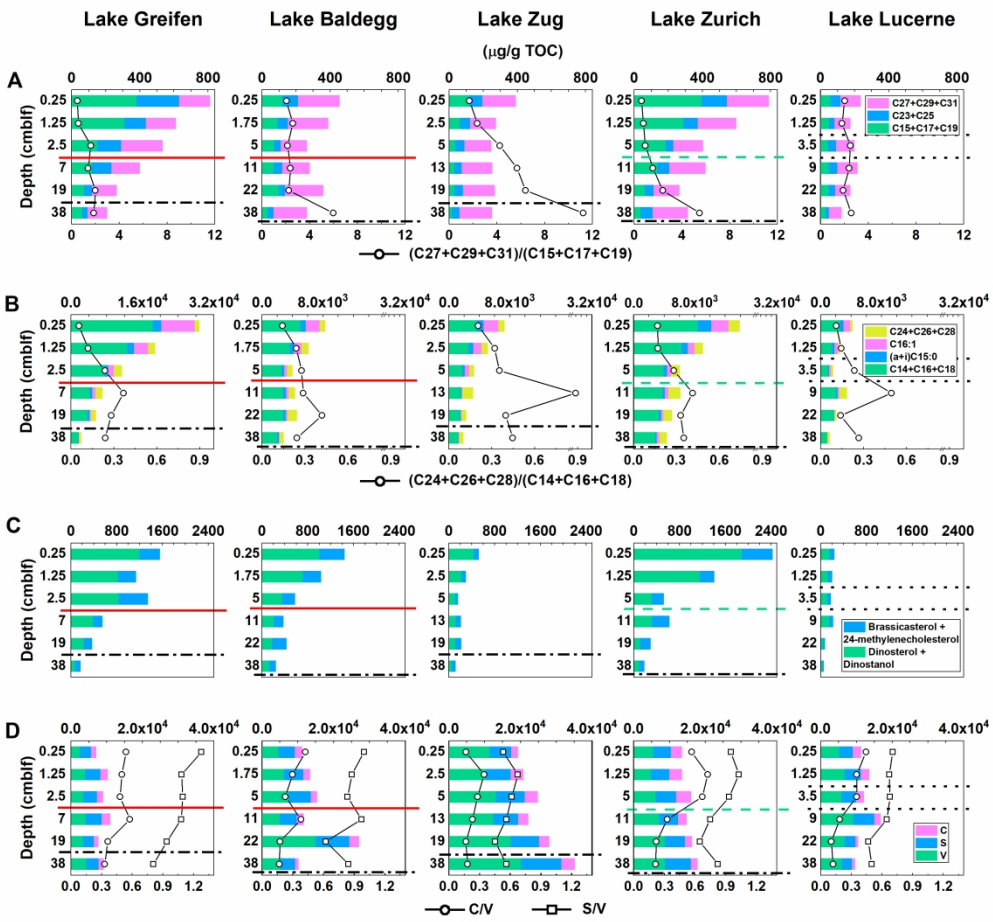
Figure 2. Copy numbers (average±standard deviation (SD)) of (A) bacterial and (B) archaeal 16S rRNA genes, and (C) Bacteria to Archaea ratios (BARs) across the five lakes ordered from most eutrophic (Lake Greifen) to oligotrophic (Lake Lucerne).

Figure 3. Bar charts of relative abundances of dominant phyla and classes of (A) Bacteria and (B) Archaea (BW: Bottom Water). The onset of eutrophication in Lake Greifen, Lake Baldegg, Lake Zug, and Lake Zurich is indicated by black dot-dashed lines. The beginning of artificial mixing and aeration in Lake Baldegg and Lake Greifen is marked with red solid lines. The transition from eutrophic to mesotrophic in Lake Zurich is marked by the green dashed line. The slight temporary increase in P concentrations in Lake Lucerne falls between the dotted black lines). MCG-6, Group C3, MCG-5, and MCG-14 are the dominant subgroups of *Bathyarchaeota*.

Figure 4. PCoA plots of bacterial (A) and archaeal (B) communities on a 97% ZOTU level based on Bray-Curtis dissimilarity. The numbers next to the shapes indicate the sediment depths, e.g. 0-0.5 denotes 0-0.5 cm sediment depth. Arrows within plots highlight depth- and trophic state-related trends in microbial community structure across the five lakes.

767 **Figure 5. (A) Observed and shared ZOTUs of Bacteria and Archaea between different lakes. (B)**
768 **Shared ZOTUs of Bacteria and Archaea in different lakes in relation to average geographic**
769 **distance between sites**. Unique to A: ZOTUs only belong to left lake; Unique to B: ZOTUs only belong
770 to right lake. Dashed lines with white circular symbols indicate percentages of shared ZOTUs between
771 each lake.

772 **Figure 6. Heatmap showing relationships between dominant bacterial and archaeal groups and**
773 **respiration reactions as well as aquatic and terrestrial OM sources.** Average relative abundances of
774 dominant microbial groups in each respiration zone were normalized based on their Z-scores. High Z-
775 scores (to +2) indicate higher average relative abundances within a specific respiration zone compared
776 to the complete data set. Low values (to -2) indicate lower average relative abundances in a specific
777 respiration zone compared to the complete data set. Z-scores were then used to vertically order dominant
778 microbial groups according to the respiration zones in which they have their highest average relative
779 abundances. The values in brackets are the relative percentages of groups throughout the whole sediment.
780 Phyla and proteobacterial classes that have their highest average percentages (and thus Z-scores) in oxic
781 sediment are termed 'oxic group'. *'Suboxic group'* phyla have their highest average percentages (and Z-
782 scores) in zones with nitrate-, sulfate- and/or Mn-reduction. *'Anoxic group'* phyla have their highest
783 average percentages (and Z-scores) in sediments with Fe(III)-reduction and methanogenesis.
784 Relationships between group percentages and OM sources were determined based on Spearman
785 correlations. Only significantly positive correlations ($P < 0.05$) are shown. *P*-values were adjusted by
786 the Benjamini-Hochberg method. Steroids A: Diatom: Brassicasterol+24-methylenecholesterol;
787 Steroids B: Dinoflagellates: Dinosterol+Dinostanol. (A): Bacteria; (B): Archaea.



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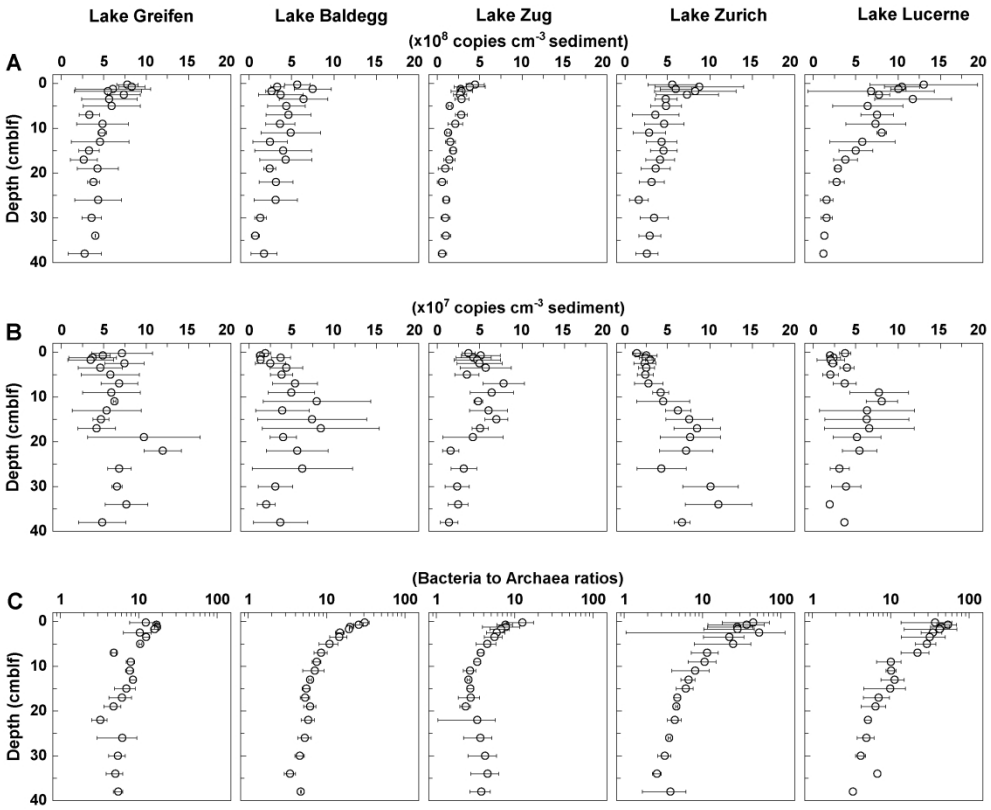

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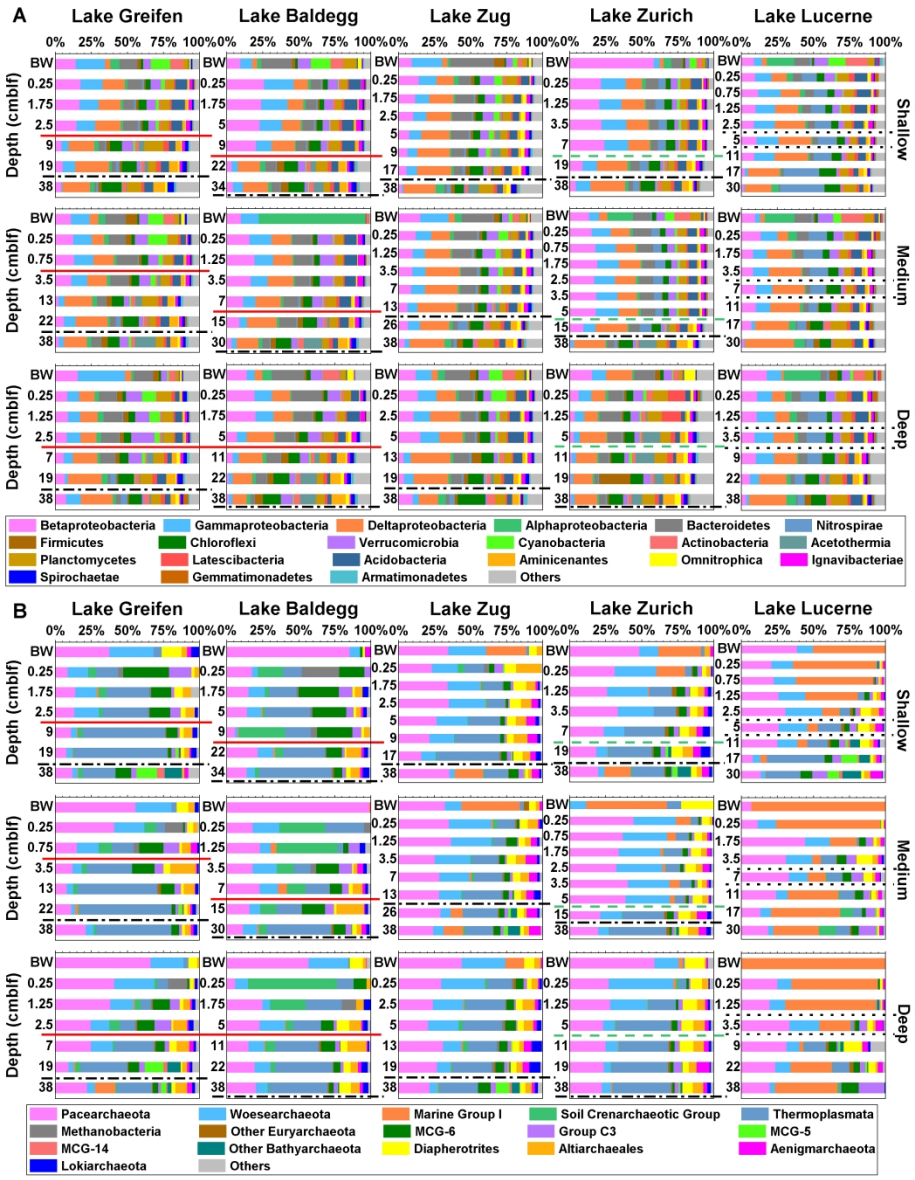

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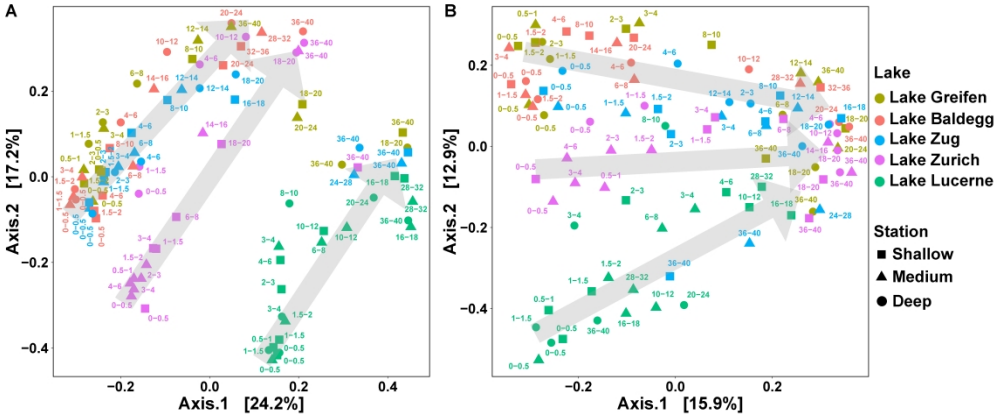

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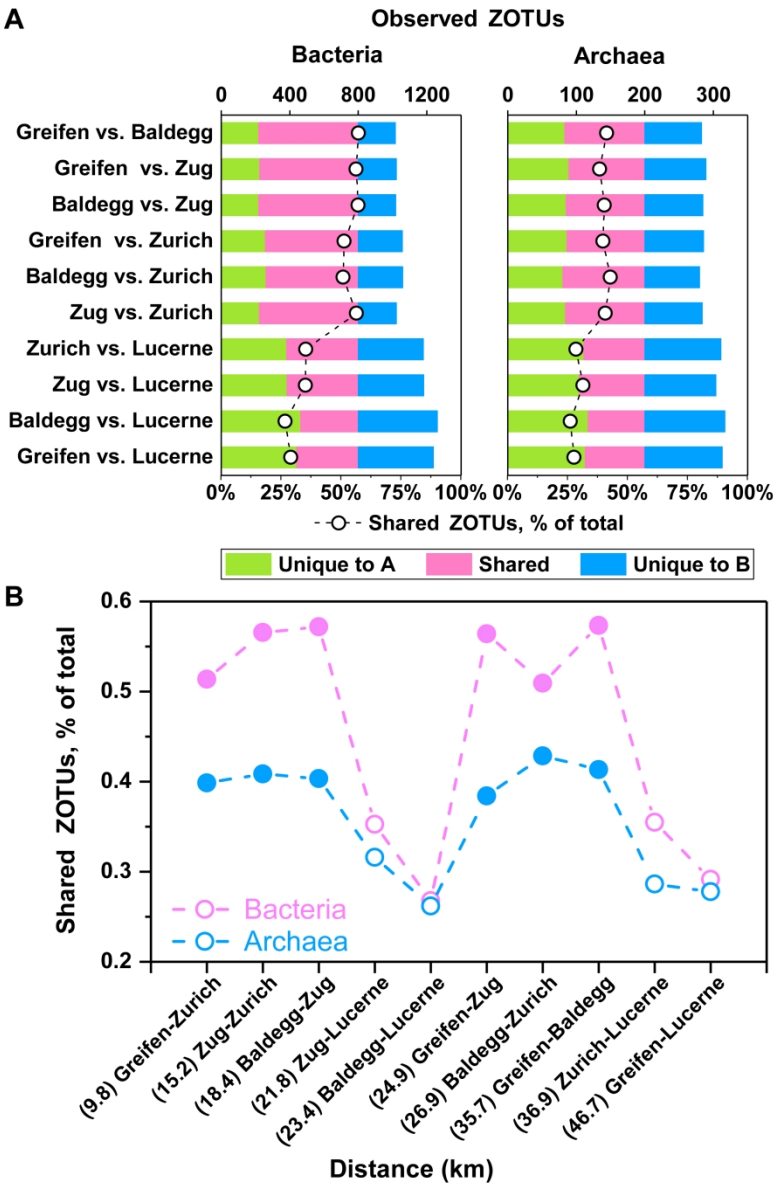

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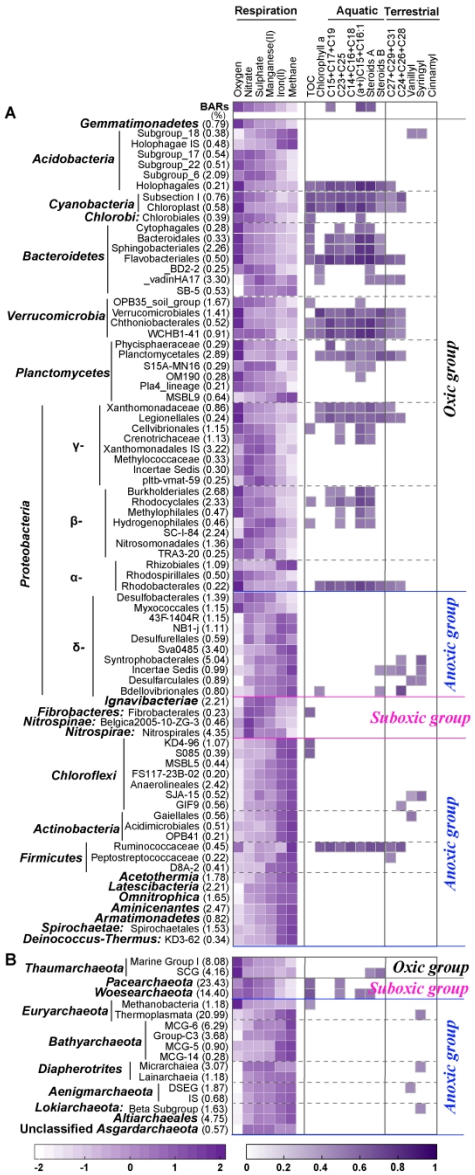

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