


Eutrophication as a driver of microbial community structure in lake sediments

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1 **Title: Eutrophication as a driver of microbial community structure in**
2 **lake sediments**

3 **Running title: Eutrophication shapes lake microbial communities**

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23 **Summary**

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

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48 **Introduction**

49 Despite only accounting for 2% of Earth's land area, lake sediments are globally important organic
50 carbon (OC) sinks (Dean and Gorham, 1998; Cole *et al.*, 2007; Mendonca *et al.*, 2017). Most of this OC
51 comes from autochthonous production by phytoplankton and aquatic macrophytes and from
52 allochthonous sources in surrounding watersheds (Meyers and Ishiwatari, 1993; Einsele *et al.*, 2001). A
53 major fraction of the OC deposited to lake sediments is mineralized by microorganisms, which gain
54 energy for growth and survival by degrading this OC to carbon dioxide (CO₂) and methane (CH₄)
55 through a network of reactions. The microbial production of CH₄ in sediments results in lakes being
56 important greenhouse gas sources in addition to OC sinks (Dean and Gorham, 1998; Einsele *et al.*, 2001;
57 Bastviken *et al.*, 2011).

58 Microbial OC degradation in sediments comprises aerobic and anaerobic processes. In all cases, the
59 hydrolysis of organic macromolecules (e.g., proteins, carbohydrates, lipids) into smaller molecules, such
60 as monomers, by extracellular enzymes is a crucial first step that enables OC uptake for energy
61 conservation. While many aerobic microorganisms completely oxidize monomers to CO₂, OC
62 dissimilation under anaerobic conditions is a multi-stage process undertaken by incomplete and terminal
63 oxidizers (Canfield *et al.*, 2005). Primary fermenters transform monomers into low molecular weight
64 compounds, such as H₂, CO₂, short-chain organic acids, and alcohols. Secondary fermenters then
65 catabolize short-chain organic acids and alcohols to H₂, acetate, and C1 compounds (Capone and Kiene,
66 1988; Schink, 1997). The terminal oxidation of fermentation products to CO₂ is performed by respiring
67 microorganisms, which use nitrate (NO₃⁻), manganese(IV), iron(III), sulfate (SO₄²⁻) or CO₂ as electron
68 acceptors (Canfield *et al.*, 2005; Drake *et al.*, 2006). These electron acceptors are utilized in order of
69 free energy yields, which is highest for O₂ and lowest for CO₂. This may result in a vertical zonation of
70 dominant respiration reactions from oxic to anoxic sediments, the latter consisting of distinct depth
71 intervals that are dominated by nitrate reduction (denitrification), Mn(IV) reduction, Fe(III) reduction,
72 sulfate reduction, and CO₂ reduction (methanogenesis) as sediment depth increases (Capone and Kiene,
73 1988).

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

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

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

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

106 **Results**

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

113 **Distributions of OM sources and respiration reactions**

114 Lipid biomarker data (Figure 1) indicate that aquatic OM sources, i.e. short-chain n-alkanes
115 (C15+C17+C19) and FAs (C14+C16+C18), and steroids from diatoms (brassicasterol+24-
116 methylenecholesterol) and dinoflagellates (dinosterol + dinostanol), decrease with sediment depth in all
117 lakes. Consistent with stimulation of aquatic primary production by eutrophication, Lake Greifen, Lake
118 Baldegg, Lake Zug, and Lake Zurich contain higher amounts of these biomarkers in surface sediments
119 than Lake Lucerne. Both lignin phenol and long-chain FA contents have strong depth-related
120 fluctuations (Figure 1, Supplementary Figure S1). Yet, consistent with lower microbial degradation rates
121 of OM from higher plants compared to aquatic OM, contributions of long-chain n-alkanes
122 $[(C27+C29+C31)/(C15+C17+C19)]$ and lignin phenols increase or show no clear changes with depth.

123 Distributions of respiration reactions have strong vertical overlaps but appear influenced by trophic state
124 (Table 1). Consistent with higher microbial activity fueled by higher OC input, the water column-derived
125 electron acceptors O₂, nitrate and sulfate are depleted at shallower depths in the eutrophic Lake Greifen,

126 Lake Baldegg, and Lake Zug compared to the mesotrophic Lake Zurich and oligotrophic Lake Lucerne.
 127 The only exception is the site from the hypoxic deep basin of Lake Zurich, where O₂ and nitrate are
 128 absent from bottom water. The respiration of the less reactive electron acceptors Mn(IV), Fe(III) and
 129 CO₂ extends into deeper layers. Mn reduction rates are mainly controlled by Mn supply, which is highest
 130 in Lake Zurich due to geochemical focusing (Schaller and Wehrli, 1996; Naeher *et al.*, 2013), whereas
 131 iron reduction and in particular methanogenesis rates increase with trophic state (Fiskal *et al.*, 2019).

132 **Bacterial and archaeal abundance trends**

133 16S rRNA gene abundances show similar trends within and between lakes and are higher in Bacteria
 134 than in Archaea (Figure 2, Supplementary Figure S2). Despite having higher microbial activity, gene
 135 abundances are not higher in the eutrophic compared to the meso- and oligotrophic lakes. Bacterial gene
 136 abundances decline approximately tenfold over the depth interval studied (surface: ~10⁹ copies cm⁻³;
 137 bottom: ~10⁸ copies cm⁻³) (Figure 2A). Archaeal gene abundances generally increase from the sediment
 138 surface (~10⁷ copies cm⁻³) to ~15-20 cm (>10⁸ copies cm⁻³), and decrease slightly or remain stable below
 139 (Figure 2B). Bacteria-to-Archaea gene ratios (BARs) decrease from surface sediments downward
 140 (Figure 2C). BAR values in surface sediments are lower in eutrophic lakes (Lake Greifen: 15.6±3.0;
 141 Lake Baldegg: 24.0±5.4; Lake Zug: 8.7±3.7) than in Lake Zurich (34.2±19.1) or Lake Lucerne
 142 (41.7±18.0), but are uniformly <10 in sediments below 20 cm (Figure 2C).

143 **General sequencing results and microbial richness and diversity trends**

144 Seven to nine samples, which included one bottom water (BW) sample and covered the entire cored
 145 sediment interval, were sequenced from each station. The total of 3,114,837 16S rRNA gene sequence
 146 reads for 112 samples (average: 27,800 reads per sample; all samples had >12,000 reads) were clustered
 147 into 8,976 bacterial and 840 archaeal 97%-similarity Zero-radius operational taxonomic units (ZOTUs)
 148 and fall into 62 bacterial and 13 archaeal phyla. The observed Richness and Shannon diversity are clearly
 149 higher in Bacteria than in Archaea (Supplementary Figure S3). Bacterial Observed Richness and
 150 Shannon Diversity Indices are highly similar between and within lakes, and change little with sediment
 151 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

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

185 ZOTU-level variations in bacterial and archaeal communities

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

199 The observed trophic state-related trends are consistent at multiple phylogenetic levels. For Bacteria,
200 trophic state-related trends are already clear at the order-level, and increase only marginally at the
201 family-level or for ZOTU-cutoffs that mimic the class-level (80%), order-level (87%), family-level
202 (92%), genus-level (95%), or species-level (97%) (Supplementary Figure S7A; also see “Sequencing
203 data processing” in “Experimental Procedures). While the vast majority of Archaea could not be
204 phylogenetically classified below the class rank, ZOTU-cutoffs that mimic the same phylogenetic levels
205 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

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

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

249 Discussion

250 To our knowledge, this is the first study to investigate the relationship between trophic history and
 251 bacterial and archaeal community structure, and one of only a few studies that have investigated
 252 relationships between respiration reactions or OM source gradients and overall microbial community
 253 structure in lake sediment (Xiong *et al.*, 2015; Rissanen *et al.*, 2017). [By comparison, several studies](#)
 254 [have documented the effects of trophic state on bacterial community structure focusing on the N-cycle](#)
 255 (Small *et al.*, 2016; Palacin-Lizarbe *et al.*, 2019; Zeng *et al.*, 2019; Li *et al.*, 2020). Bacteria dominate
 256 all samples, with BARs being highest in oxic surface sediment of oligotrophic and mesotrophic lakes
 257 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% \pm 0.68%).

284 At the order-level and below, trophic state-related trends in bacterial and archaeal communities become
285 much more evident (Figure 4, Supplementary Figure S7). While bacterial communities remain separated
286 by trophic state with increasing sediment depth and age, archaeal communities converge in deeper and
287 older sediment layers. The drivers behind these general differences in bacterial and archaeal community
288 trajectories over time are unclear. A potential explanation is that trophic state permanently alters the
289 chemical composition of insoluble organic macromolecules and that major groups of Bacteria are
290 primary degraders of these macromolecules. This explanation is consistent with the fact that bacterial
291 communities in pre-eutrophication era sediment of currently eutrophic lakes in numerous cases cluster
292 with bacterial communities of oligotrophic Lake Lucerne and not with bacterial communities from the
293 same lakes that were deposited after the onset of eutrophication. By contrast, dominant Archaea in deep,
294 permanently anoxic layers might rely on energy sources that are ubiquitous independent of trophic state,
295 e.g. fermentation intermediates, OM functional groups, terrestrial OM, and/or electron acceptors such
296 as CO₂ or Fe(III).

297 Our CAP analyses indicate that both OM sources and dominant respiration reactions are strongly
298 correlated with bacterial and archaeal community structure and explain much of the observed bacterial
299 and archaeal community variation across the five lakes (Supplementary Figure S8; Table 2). Hereby the
300 increased sedimentary input of more easily degradable aquatic OM in eutrophic lakes appears to be a
301 very important driver behind the observed patterns in microbial community structure related to trophic
302 state. We furthermore show that dominant microbial groups differ systematically in their distributions
303 relative to OM sources and respiration reactions, suggesting diverse ecophysiological niches among
304 these organisms (Figure 6). Hereby the distributions of *oxic*, *suboxic*, and *anoxic group* taxa, and the
305 correlations of these taxa with OM sources, are largely consistent with published metabolic functions of
306 their closest relatives (for a detailed analysis, we refer to the section “Potential drivers of microbial
307 community structure” in the Supplementary Materials). Pure culture and genomic data on
308 microorganisms from the same taxonomic groups as *oxic group* (mainly α -, β -, and γ -*Proteobacteria*,
309 *Bacteroidetes*, *Acidobacteria*, *Verrucomicrobia*, *Planctomycetes*) indicate that closest known relatives
310 mainly consist of aerobic and facultatively anaerobic carbohydrate-, amino acid-, and fatty acid-
311 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

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

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

366 **Conclusions**

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

381 **Experimental Procedures**

382 **Sampling sites and sample collection**

383 We studied sediment cores from the eutrophic Lake Greifen, Lake Baldegg, and Lake Zug, the
384 mesotrophic Lake Zurich, and the oligotrophic Lake Lucerne in central Switzerland ([Supplementary](#)
385 [Figure S11, Table S5](#)). The four eutrophic and mesotrophic lakes became highly eutrophic during the
386 late 19th or early 20th century (Lake Greifen: ~1920; Lake Baldegg: ~1890; Lake Zug: ~1930; Lake
387 Zurich: ~1890) due to high anthropogenic P inputs from sewage, detergents, and agricultural fertilizers,
388 whereas Lake Lucerne only had a slight increase in P input and remained oligotrophic. Starting in the
389 1970s, wastewater treatment plants and P bans on detergents, along with artificial mixing and aeration
390 in Lake Baldegg (1982/3) and Lake Greifen (2009), led to strong P concentration decreases. Since then
391 Lake Zurich has become mesotrophic. Yet, Lake Greifen, Lake Baldegg, and Lake Zug remain eutrophic
392 due to P remobilization from sediments. Lake Greifen still experiences seasonal hypoxia in bottom water,

393 whereas the deep basin of Lake Zurich remains hypoxic due to the low frequency of deep mixing events.
394 For more details on the trophic histories of these lakes, see Fiskal *et al.* (2019). Within each lake, we
395 sampled the top ~40 cm of sediment with a 150-mm diameter gravity corer (UWITEC, AT) at three
396 stations (shallow, medium and deep, ~20 samples per station), which extended from the shallow
397 sublittoral to the profundal zone (for sample depths and ages see [Supplementary Table S6](#)). Samples for
398 DNA analyses were taken by sterile cut-off syringes, immediately frozen in liquid N₂, and subsequently
399 stored at -80°C. Biomarker samples were taken using metal spatulas, stored on ice during sampling, and
400 then frozen at -20°C.

401 **Lipid biomarkers, lignin and chlorophyll *a* analyses**

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

410 **DNA extraction**

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

415 **Quantification and sequencing of 16S rRNA genes**

416 16S rRNA genes were quantified SYBR Green I Master on a LightCycler 480 II (Roche Molecular
417 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

445 studies. Because most ZOTUs could not be assigned to the genus rank for Bacteria and order rank for
446 Archaea, we explored additional ZOTU sequence similarity cutoffs to investigate microbial community
447 differences at different taxonomic levels (phylum-level: 75% sequence similarity; class: 80%; order:
448 87%; family: 92%; genus: 95% (Lever and Teske, 2015). All ZOTU sequences are publicly available
449 under Genbank accession number KDOW00000000. Raw sequences have been deposited in Sequence
450 Read Archive with accession number SAMN13038023 under the project PRJNA577818.

451 **Statistical analyses**

452 Analyses were done in R v3.4.0 (<http://R-project.org>) using R Studio v1.1.442 (<http://rstudio.com>).
453 ZOTU files, mapfiles with biogeochemical data, tree files, and sequence files were merged into a
454 “phyloseq” class with `import_qiime()` from phyloseq package (McMurdie and Holmes, 2013).
455 Calculations were done on the read percentage (relative abundance) of each ZOTU or other phylogenetic
456 group per sample. Variations in microbial communities were analyzed by PCoA based on Bray-Curtis
457 dissimilarities (Beals, 1984). Significant differences in microbial communities between lakes were
458 calculated by `anosim()` (ANOSIM) and `adonis()` (Permutational multivariate analysis of variance
459 (PERMANOVA); Permutations: 1000), both based on Bray-Curtis dissimilarities using `vegan` (Clarke,
460 1993)). A SIMPER analysis was applied to identify orders that contribute the most to differences in
461 microbial compositions between lakes (Clarke, 1993). Constrained analysis of principal coordinates
462 (CAP, also called distance-based RDA) was done by `capscale()` using Bray-Curtis distance (Anderson
463 and Willis, 2003). The contributions of environmental variables to microbial community structures were
464 calculated by `Adonis()` (permutational multivariate analysis of variance with Bray-Curtis dissimilarities).
465 Shared ZOTUs (Bacteria (top 800); Archaea (top 200)) between lakes were obtained using `venn()` from
466 the `gplots` package. A correlation matrix between microbial community composition and
467 biogeochemical parameters was produced based on Spearman coefficients and calculated and plotted
468 using `cor()` and `cor.mtest()` from the `Hmisc` and `Corrplot` packages. To identify co-occurrence patterns
469 of dominant microbial groups, a co-occurrence network was constructed based on Spearman correlation
470 coefficients ($p < 0.5$, $p < 0.01$) (Junker and Schreiber, 2008). To remove false-positives, we adjusted P-

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

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480 **Conflict of Interest**

481 The authors declare no conflict of interest.

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715 freshwater lakes. *Ecol Indic* **106**(105491):1-13.

716 **Tables**

717 **Table 1. Depth distributions (cmlbf) of respiration reactions (average±standard deviation (SD))**
 718 **across the five lakes (modified from Fiskal *et al.* (2019)).** The deepest samples from the bottom of
 719 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

720

721 **Table 2. Relative contributions of environmental variables to driving microbial community**
 722 **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	**	

723 **Note:** Steroids A: Diatom: Brassicasterol+24-methylenecholesterol; Steroids B: Dinoflagellates: Dinosterol+Dinostanol.

724 * P < 0.05; ** P < 0.01; *** P < 0.001.

725 **Table 3. Spearman correlations of bacterial and archaeal 16S rRNA gene abundances based on**
 726 **qPCR, as well as calculated abundances of dominant subsurface bacteria and archaeal groups vs.**
 727 **sediment depth (0-20 cm).** We show correlations for the entire data set (All lakes) and for all data from
 728 each individual lake. All significant correlations are positive, except those of bacterial 16S rRNA gene
 729 copy numbers vs. depth, which are negative. Abundances of dominant subsurface bacterial and archaeal
 730 groups were calculated by multiplying their fractions of total bacterial and archaeal 16S gene reads with
 731 total bacterial and archaeal 16S gene copy numbers cm⁻³ sediment, respectively. *Dehalococcoidia* are
 732 shown in addition to total *Chloroflexi* due to the frequent dominance of subsurface *Chloroflexi* by this
 733 class (Orcutt *et al.*, 2011; Teske *et al.*, 2014). MCG-6 and Group C3 are the dominant class-level
 734 subgroups of *Bathyarchaeota* in our study. Depth related trends across all lakes and stations are shown
 735 in [Supplementary Figure S9](#) and [Figure S10](#). Correlation analyses for additional sediment depth intervals
 736 (0-10, 0-15, 0-30, and 0-40 cm) are displayed in [Supplementary Table S3](#). Correlations based on
 737 bacterial and archaeal 16S gene copy numbers g⁻¹ wet and g⁻¹ dry sediment are shown in [Supplementary](#)
 738 [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	***	0.00		0.28	***	0.06		0.55	***	0.25	***
<i>Deltaproteobacteria</i>	0.03		0.10		0.02		0.13		0.03		0.001	
<i>Chloroflexi</i>	0.001		0.03		0.17		0.002		0.005		0.003	
<i>Dehalococcoidia</i>	0.29	***	0.40	**	0.74	***	0.37	**	0.26	*	0.57	***
<i>Acetothermia</i>	0.27	***	0.44	**	0.77	***	0.23		0.19		0.20	
<i>Aminicenantes</i>	0.23	***	0.47	*	0.79	***	0.49	**	0.28	*	0.23	*
<i>Thermoplasmata</i>	0.44	***	0.50	**	0.66	***	0.79	***	0.59	***	0.79	***
<i>Altiarchaeales</i>	0.18	***	0.06		0.68	***	0.01		0.47	**	0.32	*
<i>Lokiarchaeota</i>	0.15	***	0.01		0.002		0.57	***	0.33	*	0.44	**
<i>Diapherotrites</i>	0.12	**	0.03		0.08		0.15		0.25	*	0.34	*
MCG-6	0.16	***	0.00		0.39	*	0.16		0.35	*	0.53	***
Group C3	0.22	***	0.04		0.48	**	0.15		0.57	***	0.76	***

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

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742

743 **Figure legends**

744 **Figure 1. Depth profiles of (A) n-alkanes, (B) fatty acids, (C) steroids, and (D) lignin phenols.** The
 745 onset of eutrophication is indicated by the black dot-dashed lines (Lake Greifen: ~1920; Lake Baldegg:
 746 ~1890; Lake Zug: ~1930; Lake Zurich: ~1890). The beginning of artificial mixing and aeration to
 747 prevent water column anoxia in Lake Baldegg (1982/3) and Lake Greifen (2009) is marked with red
 748 solid lines. The transition from eutrophic to mesotrophic in Zurich took place around 1980 (green dashed
 749 line). P concentrations in Lake Lucerne increased slightly, without a change in trophic state, from 1960-
 750 1990 (samples between dotted black lines). Abbreviations in (D): V = vanillyl phenols (incl. vanillin,
 751 acetovanillone and vanillic acid); S = syringyl phenols (incl. syringaldehyde, acetosyringone and
 752 syringic acid); C = cinnamyl phenols (incl. p-coumaric acid and ferulic acid.)

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

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

763 **Figure 4. PCoA plots of bacterial (A) and archaeal (B) communities on a 97% ZOTU level based**
 764 **on Bray-Curtis dissimilarity.** The numbers next to the shapes indicate the sediment depths, e.g. 0-0.5
 765 denotes 0-0.5 cm sediment depth. **Arrows within plots highlight depth- and trophic state-related trends**
 766 **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.

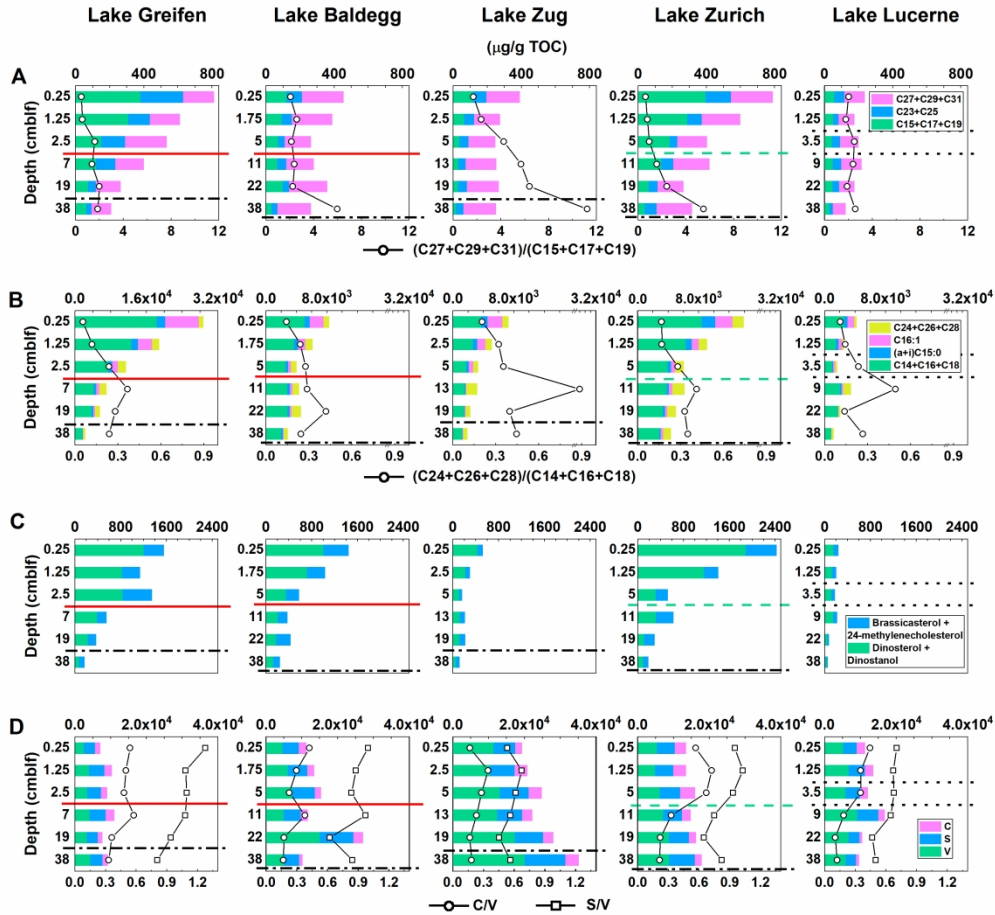


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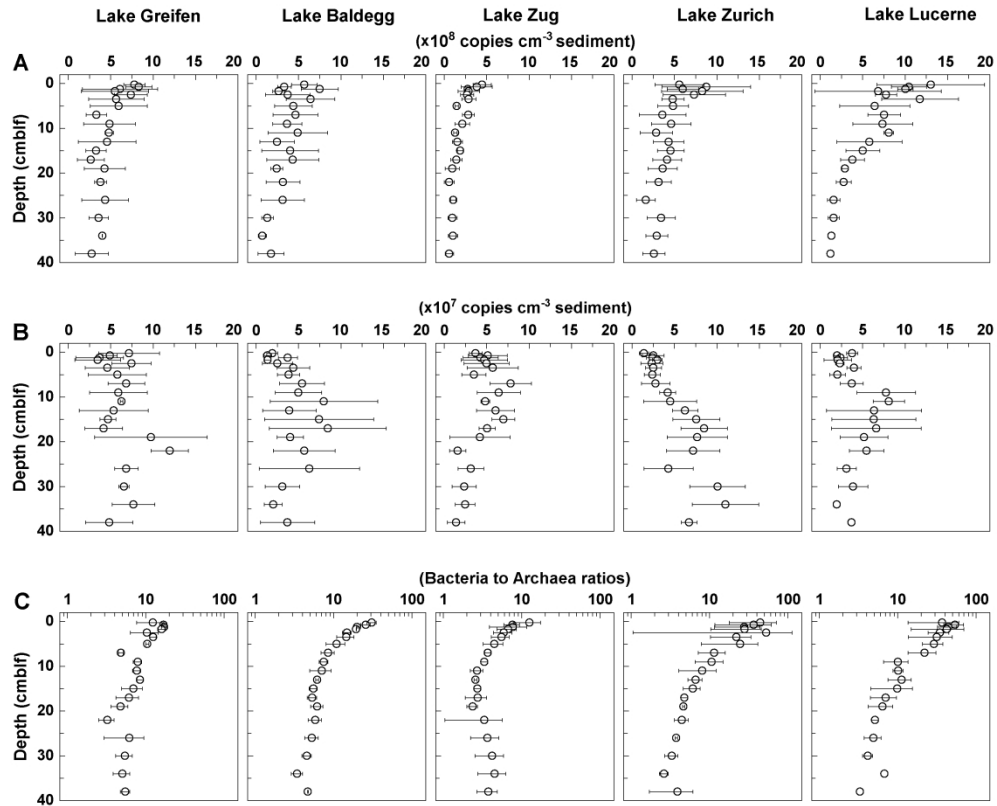


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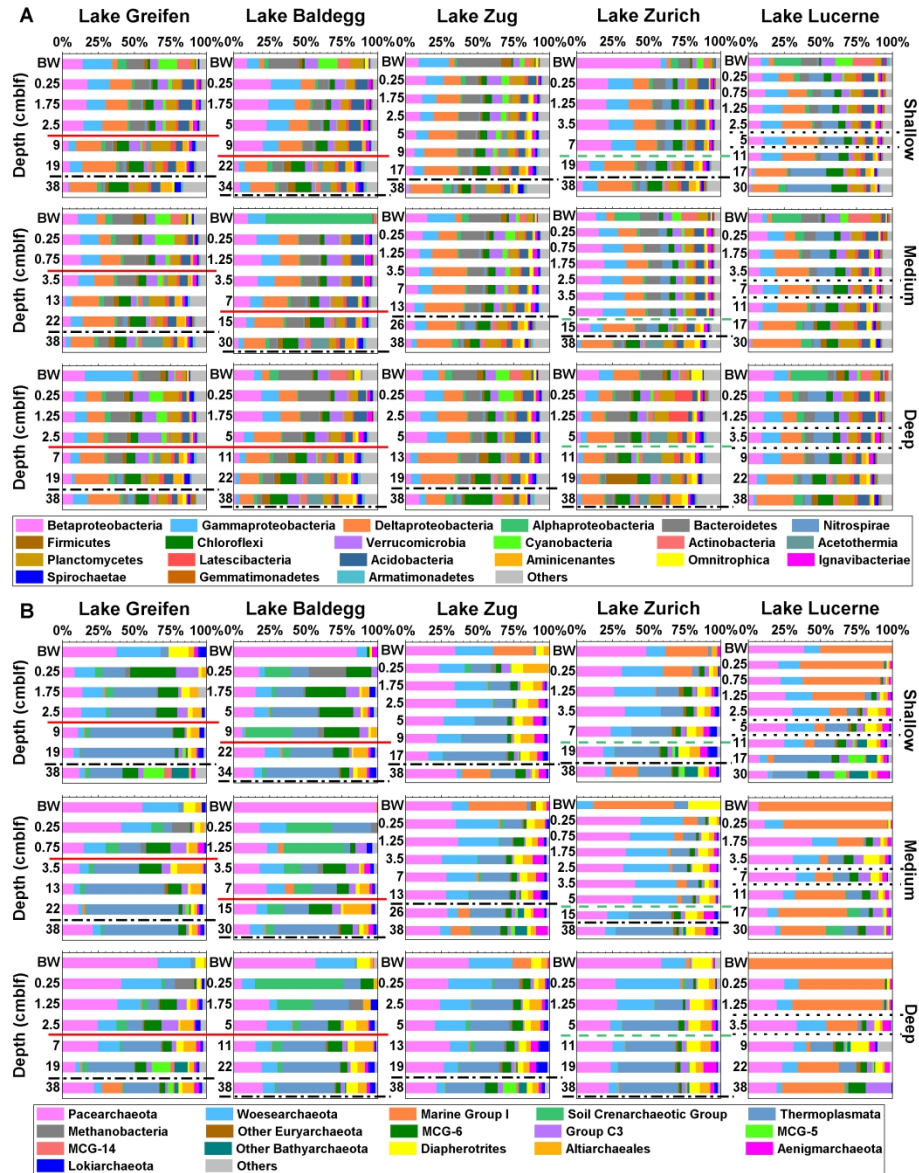


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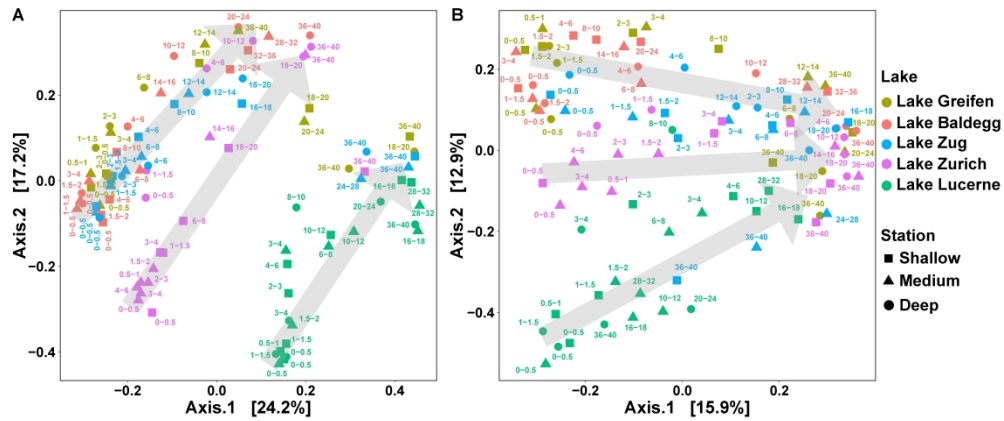


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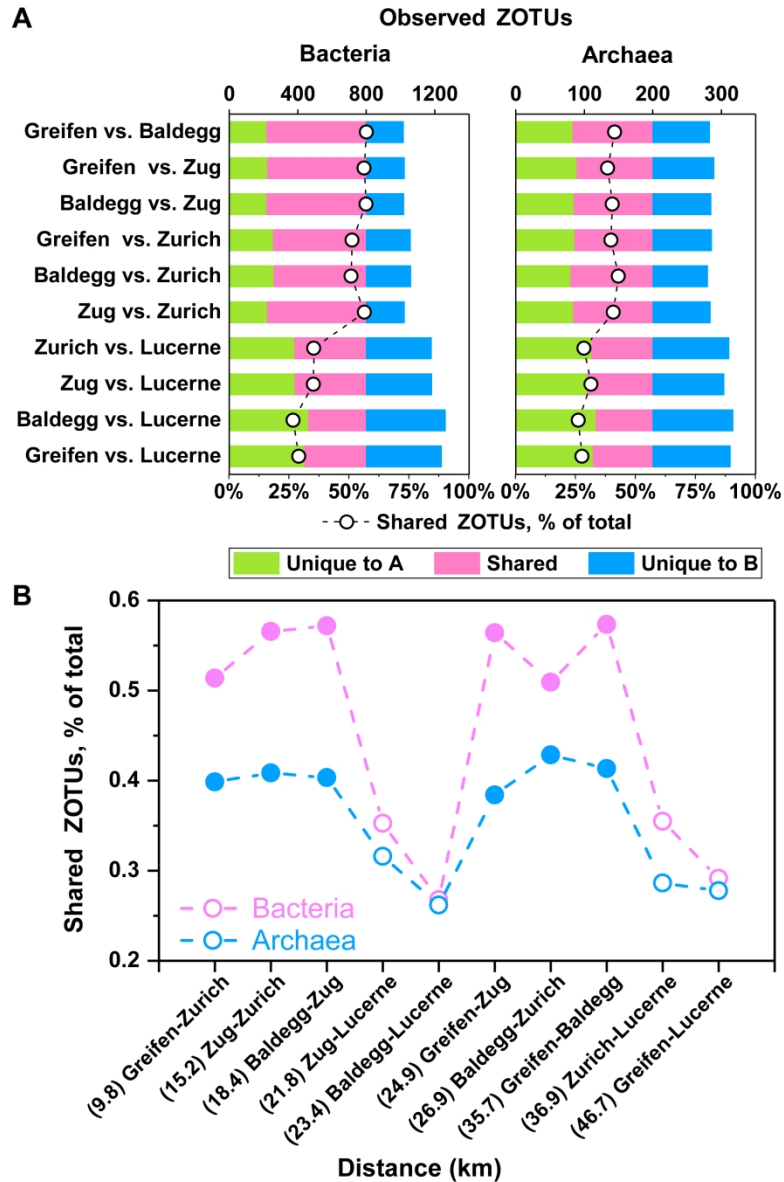


Figure 5. (A) Observed and shared ZOTUs of Bacteria and Archaea between different lakes. (B) Shared ZOTUs of Bacteria and Archaea in different lakes in relation to average geographic distance between sites. Unique to A: ZOTUs only belong to left lake; Unique to B: ZOTUs only belong to right lake. Dashed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

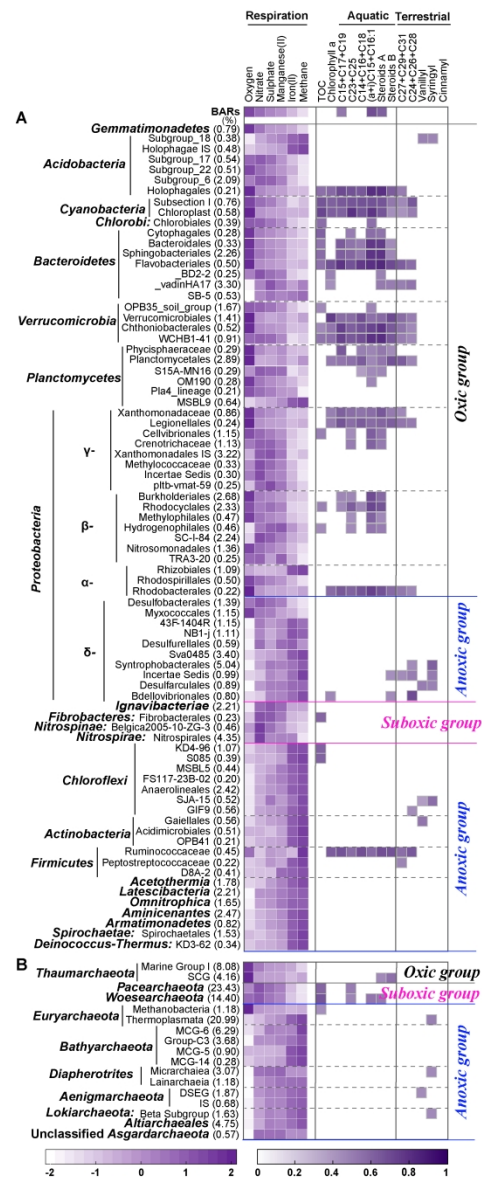


Figure 6. Heatmap showing relationships between dominant bacterial and archaeal groups and respiration reactions as well as aquatic and terrestrial OM sources. Average relative abundances of dominant microbial groups in each respiration zone were normalized based on their Z-scores. High Z-scores (to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

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