

1 **Decreased snow cover stimulates under ice primary producers, but** 2 **impairs methanotrophic capacity**

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13

14 **Abstract (150 words)**

15

16 Climate change scenarios anticipate decrease of spring snow cover in boreal and subarctic
17 regions. Forest lakes are abundant in these regions and substantial contributors of methane
18 emissions. We performed an experiment on an anoxic frozen lake and observed that the
19 removal of snow increased light penetration through the ice into the water modifying the
20 microbial composition across depths. A shift in photosynthetic primary production was
21 reflected by the increase of chlorophyll a and b concentrations in the upper depths of the
22 water column, while Chlorobia, one of the key photosynthetic bacteria in anoxic lakes, shifted
23 to lower depths. Moreover, a decrease in abundance of methanotrophs, such as
24 Methylococcaceae, was noted concurrently to an increase in methane concentration in the
25 water column. These results indicate that decrease of snow cover impacts both primary
26 production and methane production/consumption, ultimately leading to increased methane
27 emissions after spring ice off.

28

29 **Key words:** Snow cover, autotrophy, methanotrophy, light climate, climate change

30 Introduction

31 Small forest lakes are a typical feature of the boreal and subarctic region ¹. These small water
 32 bodies with high organic loads are hotspots in the carbon cycle and one of the most
 33 prominent environmental sources of greenhouse gas emissions in these regions ^{2,3}. The
 34 microorganisms inhabiting such lakes are the main drivers of these biogeochemical
 35 processes ⁴. However, the community structure and functioning of microorganisms is bound
 36 to the environmental conditions and seasonality. One very important factor related to
 37 seasonality is light availability. Light is the driving force of primary production at the base of
 38 the food web. Algae provide substrates and oxygen for lake bacteria and are an important
 39 food source of zooplankton. In wintertime, ice cover inhibits oxygen transfer from the
 40 atmosphere into the water column while snow cover impedes light transfer, further curtailing
 41 photosynthesis beneath the ice ^{5,6}. Moreover, aerobic microorganisms consume residual
 42 oxygen in the water beneath the ice ⁷, leading to decreasing oxygen gradient and anoxia from
 43 the lake surface to the bottom. The resulting anoxic conditions facilitate anaerobic processes
 44 like methanogenesis and decrease methanotrophy ⁸. These conditions result in methane
 45 accumulation under ice and consequently high methane emissions during ice break-up in the
 46 spring ^{9,10}. Currently, climate change is altering seasonal patterns in the subarctic region ¹¹
 47 and also changing patterns of snow cover worldwide ¹². In the northern hemisphere these
 48 changes include sudden extreme conditions, as seen during the 2007/08 winter, when a
 49 sudden loss and reformation of snow cover occurred due to a warming event ¹³. Also long
 50 term trends have been observed, such as a decrease in spring snow cover ¹². This project
 51 tests the impact of reduced snow cover on a humic lake ecosystem with the emphasis on
 52 methane cycle and primary producers.

53 We hypothesized that (a) the decrease in snow cover increases light penetration and
 54 stimulates primary production. Furthermore, we hypothesized that more primary production
 55 would (b) increase the availability of oxygen having an impact on aerobic bacteria and (c)
 56 increase methanotrophic activity as an effect of improved oxygen availability. The project was
 57 conducted as a whole lake manipulation in Lake Lomtjärnen (63°20'56.9"N 14°27'28.3"E) a
 58 small boreal lake in central Sweden.

59

60 **Results and Discussions**

61 *Snow cover removal increased light penetration and its effect on chlorophyll*

62 Our sampling scheme included 6 depths (depth 1 was sampled at 0.65 cm and thereafter
63 approx. every 40 cm) and 6 time points. We sampled a vertical profile of the lake on three
64 occasions before the snow removal and three times after; with one day between each
65 sampling. Thus, the total duration of the experiment was two weeks. Snow depth on the
66 frozen lake was 18 to 21 cm through the experiment. The snow was removed from
67 approximately an area of 400 m² in the middle of the lake. During the experiment the ice
68 thickness was approximately 50 cm. Prior to the snow removal, the lake had a shallow
69 epilimnion, with a steep oxygen depletion layer from 0.6 to 0.7 m, where the oxygen
70 concentration was close to detection limit (Figure S1).

71 We observed light intensity increase in all depths of the lake after the snow removal (depth 1
72 from 177 to 3764 lx, depth 2 from 63 to 1378 lux, depth 3 from 39 to 826 lux, depth 4 from 20
73 to 480 lux, depth 5 from 9 to 299 lux and depth 6 from 0 to 112 lux). Light intensity was
74 significantly higher only in the first three layers (0.65 m to 1.35 m) (Figure 1). Moreover, the
75 chlorophyll a concentration increased significantly in those top three layers (0.65 m to 1.35
76 m), whereas chlorophyll b increased in layers 4 and 5 (1.85 m to 2.53 m). In comparison, the
77 concentration of bacteriochlorophyll d and e appeared to increase in depth 5 (Figure 1).
78 Based on these results we presume light had a direct effect on the phytoplankton of the water
79 column affecting their chlorophyll production and photosynthesis activity and subsequent
80 oxygen production. After the snow removal an increase in oxygen concentration in the top
81 layer under the ice was observed which rapidly decreased as time progressed (Figure S1).
82 However, the differences in oxygen concentration were not statistically significant. This might
83 indicate that heterotrophic organisms immediately utilized the produced oxygen, which is
84 supported by the fact that bacterial abundance doubled in the upper layer of the lake (Figure
85 1).

86 *Microbial community changes*

87 Against our expectation, snow removal did not have a significant effect on the total community
88 composition (PERMANOVA: $p_{DNA} = 0.594$, $p_{RNA} = 0.636$). However, when the depths were
89

considered individually, the composition of microbial communities before and after snow removal were significantly different in depth 1 (0.65 m) and in depth 4 (1.85 m) (PERMANOVA $p_{\text{depth1}} = 0.007$ and $p_{\text{depth4}} = 0.046$). Depth was an important driver of community composition (PERMANOVA, $p < 0.001$), as oxygen concentration and redox potential are key factors structuring bacterial communities^{14,15}. The major microbial groups in the first depth were Betaproteobacteria (26% before and 32% after snow removal in relative abundance of DNA-based bacterial community composition, 30% before and 46% after snow removal in RNA-based community) and Gammaproteobacteria (23% before and 7% after snow removal in DNA, 37% before and 18% after snow removal in RNA) (Figure 2). Most of the betaproteobacterial OTUs were classified as Comamonadaceae, while most of the gammaproteobacterial were classified as Methylococcaceae. Parcubacteria also contributed to the community change post snow removal in depth 1 (5% before and 10% after snow removal in DNA, not present in RNA). The major microbial groups in the fourth depth were Chlorobia (33% before and 17% after snow removal in DNA, 40% before and 28% after snow removal in RNA), Chloroflexia (12% before and 10% after snow removal in DNA, 13% both before and after snow removal in RNA) and Anaerolineae (3% before and 4% after snow removal in both DNA and RNA) (Figure 2). Changes in both DNA and RNA were consequent in most of the groups in which we observed the biggest alterations post treatment. Overall, the 11 most abundant OTUs contained each at least 1% of the sequences. Together these 11 OTUs accounted for 32% of the sequences. The most abundant OTU belonged to the Chlorobiaceae family, while the second and third most abundant OTUs belonged to the Methylococcaceae family.

Because we expected the oxygen conditions to improve the fastest in the top layer of the water column, we looked into the most abundant families in the first depth. We observed an increased relative abundance of the family Comamonadaceae (15% to 19% in DNA, 18% to 30% in RNA) and Flavobacteriaceae (10% to 14% in DNA, 5% to 6% in RNA) (Figure 3). Comamonadaceae and Flavobacteriaceae are both aerobic heterotrophs¹⁶⁻¹⁸. Both of these organisms that increased in relative abundance after snow removal could take advantage of the increased availability of oxygen and organic compounds originating from increased

119 activity of the primary producers, while keeping the oxygen concentration seemingly
120 unchanged.

121 Chlorobia are photoautotrophic bacteria that are common in boreal lakes such as our study
122 lake ¹⁹⁻²¹. Because of the extraordinarily large numbers of bacteriochlorophylls in their
123 antenna complexes, some of these green sulfur bacteria are able to grow at extremely low
124 light irradiances ($1-10 \text{ nmol photons m}^{-2} \text{ s}^{-1}$) under which no other types of chlorophototrophs
125 can grow ^{22,23}. It is possible that the decrease in the abundance of Chlorobia in depth 4 after
126 snow removal was due to light intensity increase, making the conditions more favorable for
127 organisms using chlorophyll a and b (Figure 1). Moreover, an increase of Chlorobi both in
128 DNA and RNA relative abundances was observed in depth 5 and 6 of the lake (Figure 3). This
129 together with the increase in bacteriochlorophyll d and e in depth 5 could mean that
130 decreased snow cover switches the taxonomical composition of the primary producers of the
131 lake pushing the optimal conditions for Chlorobia to lower depths of the lake. This shift in
132 primary producers might have implications for the carbon balance of the lake, following the
133 possible shifts in efficiency of carbon dioxide uptake and microbial interactions.

134

135 *Methane after snow removal*

136 As expected methane concentration increased with depth, but opposite to our hypothesis
137 methane concentration was significantly higher after the snow removal (two-way ANOVA
138 $p_{\text{depth}} < 0.001$, $p_{\text{treatment}} = 0.020$) (Figure 1). Most of the gammaproteobacterial organisms
139 observed in the water column belonged to the family Methylococcaceae, which consists solely
140 of methane consuming bacteria. Methylococcaceae decreased in relative abundance in DNA
141 after the treatment on all of the depths except for depth 4 (Figure 3). We also observed a
142 decreasing trend in phosphate concentration in depths 1, 3, 4 and 5 after the treatment. In a
143 previous study, low phosphate concentration has been linked to impaired methanotrophic
144 activity ²⁴. Considering that many methanotrophs are slow growing ²⁵ and the increased algal
145 activity in the lake after snow removal, as suggested by the increased chlorophyll
146 concentrations, it is possible that the methanotrophs were unable to compete for phosphorus
147 with the phytoplankton. Alternatively, the increased primary production could have contributed
148 more substrate to methanogenesis in surface sediments, increasing overall the concentration

149 of methane in the water column¹⁰. Decreased methanotrophy and increased methanogenesis
150 would explain the increased methane concentrations after the snow removal. In any case,
151 increased methane concentrations in the water column translate into increased methane
152 emissions to the atmosphere once the ice melts in the spring.

153

154 *Conclusions*

155 Our results show that the impact of decreased snow cover to the lake microbiome and also to
156 total lake metabolism is a complex interaction between the biotic and abiotic lake
157 characteristics. While our first hypothesis (a), the increase of light and stimulation of primary
158 production following the snow removal proved to be correct, (b) the increase of oxygen and
159 (c) increase of methanotrophic activity appeared to be false. However, it should be noted that
160 the duration of our experiment was rather short and it might be possible that on a longer time
161 scale the oxygen conditions might improve. Nevertheless, we conclude that the final outcome
162 of the decreased snow cover can be expected to change the total lake metabolism and
163 potentially increase methane emission after the ice break.

164

165 **Methods**

166 The experiment was conducted in March 2016 on Lake Lomtjärnan (63°20'56.9"N
167 14°27'28.3"E), a small lake located in Krokum (Sweden). The lake is located on a mire
168 surrounded by a coniferous forest and it is ice covered during the winter months,
169 approximately from November to April. The experiment consisted of two parts; monitoring of
170 the lake while there was a snow cover over the whole ice and observing the impact snow
171 cover removal after the snow was removed from an area of approximately 400 m² in the
172 middle of the lake. Snow removal was performed on day 6 manually using snow shovels. For
173 each sampling a hole was drilled through the ice on a different location to avoid interference
174 of unnatural ice formation as the holes were always filled and covered with snow to limit
175 oxygen and light penetration to the water column.

176

177 Light intensity was measured with 18 HOBO loggers with the capacity to measure light
178 intensity and temperature (HOBO Pendant® Temperature/Light 64K Data Logger, Onset,

179 USA). The loggers were placed under ice from surface to the bottom every 0.1 to 0.75 m on
180 the first day of the experiment and kept there throughout the experiment. Laterally, the
181 sensors were approximately 50 cm from the hole in the ice. The light values are presented as
182 daily averages for the time between sunrise and sunset (approximately 10 AM to 3 PM).

183

184 On each sampling occasion samples were taken for the measurement of chemical
185 parameters (Chlorophylls, Nitrite, Nitrate, Phosphate, Sulfate, Ammonia, Fluoride, Chloride,
186 O₂, CH₄, CO₂), and DNA- and RNA-based community analyses. For DNA and RNA the
187 samples were taken with Sterivex-filters (Millipore, Billerica, MA, USA). For RNA the filtering
188 was limited to 15 minutes, while for DNA the filtering was continued until the filter was
189 clogging. Samples were taken from six different depths and on days 1, 3 and 5 (before the
190 snow removal) and on days 7, 9 and 12 (after the snow removal).

191

192 Chlorophyll pigments were extracted on ice in 2ml 90% acetone using an ultrasonic bath and
193 left at -20°C overnight. After that, the samples were centrifuged (3000x 10min at 4°C) and
194 filtrated (0.45µm syringe filters). The extracts were analyzed using HPLC on an Agilent 1100
195 Series HPLC system (Agilent Technologies, Waldbronn, Germany) fitted with three RP-18e
196 100x4.6mm Chromolith Performance columns (Merck, Darmstadt, Germany) connected in
197 series. The flow rate was 1.4ml/min and the gradient program followed as described ²⁶. The
198 column temperature was 25°C and the injection volume was 100µl (70µl sample + 30µl 0.5M
199 ammonium acetate). The absorbance was measured with a diode array detector between
200 300-800nm (resolution 2nm and slit with 4nm). Chlorophyll a and b were identified and
201 quantified using standard solutions (DHI Laboratory Products, Hoersholm, Denmark) and the
202 bacteriochlorophylls by using previously published chromatograms, spectra and extinction
203 coefficients ²⁷⁻³¹. Methane concentration was analysed as described ³², except that room air
204 was used instead of nitrogen for the headspace. The methane concentration was analysed
205 also from room air and subtracted from the final gas concentrations.

206

207 DNA and RNA was co-extracted using phenol-chloroform method ³³ as modified in ²¹. RNA
208 was then transcribed into cDNA as previously described ³⁴ using RevertAid H Minus First

209 Strand cDNA synthesis kit (Thermo Scientific). After this, both, the RNA and DNA samples
210 were amplified for bacterial 16S rRNA genes using primers 341r and 805f³⁵. PCR protocol
211 was done as previously³⁶. The samples were then pooled in equimolar amounts and
212 sequenced with Illumina MiSeq at Science for Life Laboratories (Uppsala, Sweden). The
213 resulting 2.5 million sequences were processed using Mothur³⁷ as described³⁸, except that
214 OTU clustering was done using abundance-based greedy clustering. Raw sequences have
215 been submitted to ENA under the accession number ERS2597919 - ERS2597988.

216
217 The effect of snow removal and water depth was tested by two-way analyses of variance
218 (ANOVA) with corresponding Tukey's post-hoc tests for the environmental parameters and by
219 two-way permutational ANOVA (PERMANOVA) with 9999 permutations for the community
220 composition data. The normal distribution of the residuals of the ANOVA models was tested
221 using Shapiro-Wilk normality test and where needed, data was log transformed. All statistical
222 analyses were done in R version 3.4.3³⁹. Packages phyloseq, vegan and ggplot2 were used
223 ⁴⁰⁻⁴².

224

225 **Author Contributions**

226 SLG and SP originated the research. SLG, AS, MS, SP participated in the sampling
227 campaign. SP, AS and CB conducted the laboratory work including DNA, RNA, nutrient, and
228 chlorophyll analyses. SLG, AS, SP performed the data analysis. All authors contributed to
229 discussions of data and manuscript review.

230

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239 **Conflict of interest Statement:** The author declares no conflict of interest.

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387

388 **Figure Legends**

389 **Figure 1** Chemical and physical parameters of the lake before (grey) and after snow removal
390 (white). Depth 1 = 0.65 m, 2 = 1.0 m, 3 = 1.35 m, 4 = 1.85 m, 5 = 2.4 m, 6 = 2.7 m

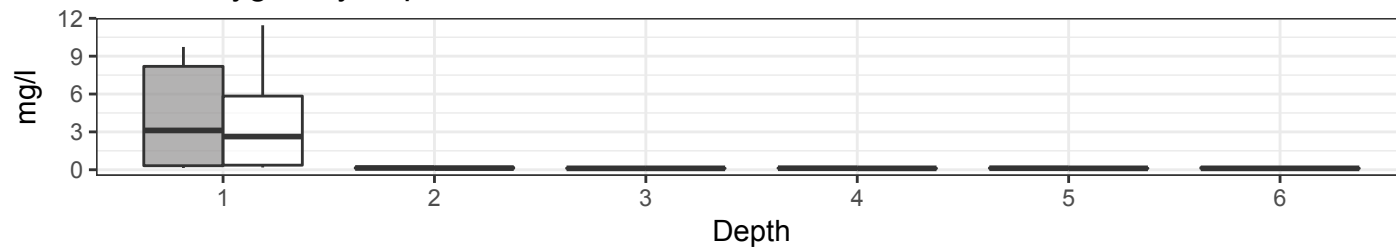
391 **Figure 2** Relative abundance of microbial communities of each of the samples. Color coded
392 according to taxonomical class. In the x axis 1 = 100%. Numbers in upper boxes represent
393 the depth number (1 = 0.65 m, 2 = 1.0 m, 3 = 1.35 m, 4 = 1.85 m, 5 = 2.4 m, 6 = 2.7 m). Bars
394 inside each box are ordered according to time being the first bar the time 1 and the last bar
395 time 6. First bar in depth 6 is missing as on the first day of sampling we could not obtain a
396 sample for depth 6.

397 **Figure 3** Relative abundance of some specific taxonomical groups. Samples taken before the
398 snow removal are in grey and after snow removal in white. In the x axis 1 = 100%. Numbers
399 in depth number; 1 = 0.65 m, 2 = 1.0 m, 3 = 1.35 m, 4 = 1.85 m, 5 = 2.4 m, 6 = 2.7 m.

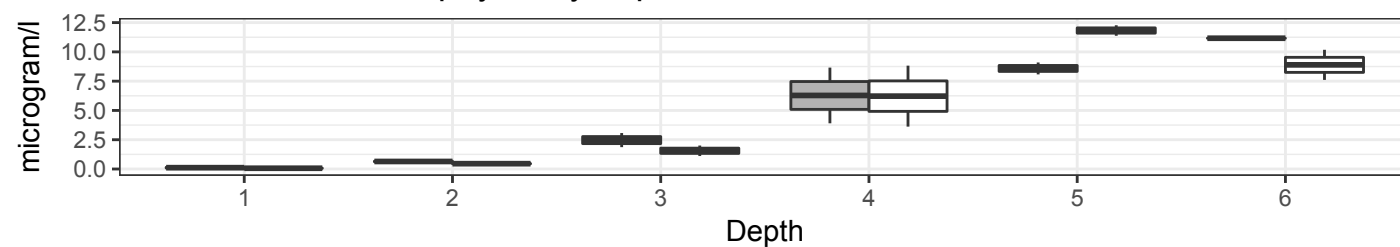
400

401 **Supplementary Figure S1.** Oxygen concentration in the water column across the experiment
402 in the sampling depths.
403

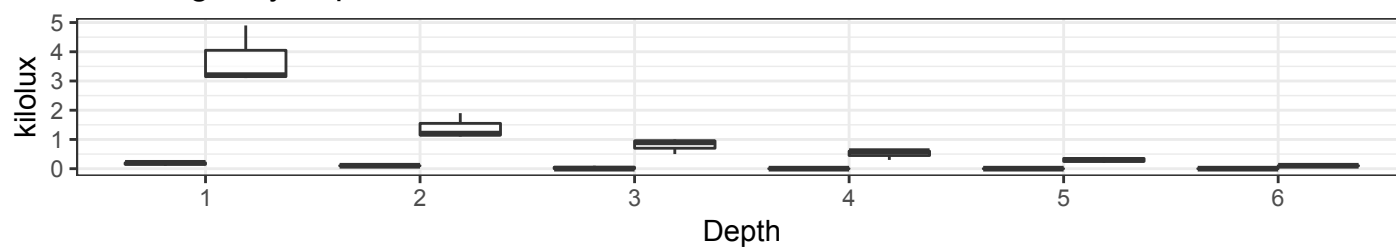
Mean oxygen by depth



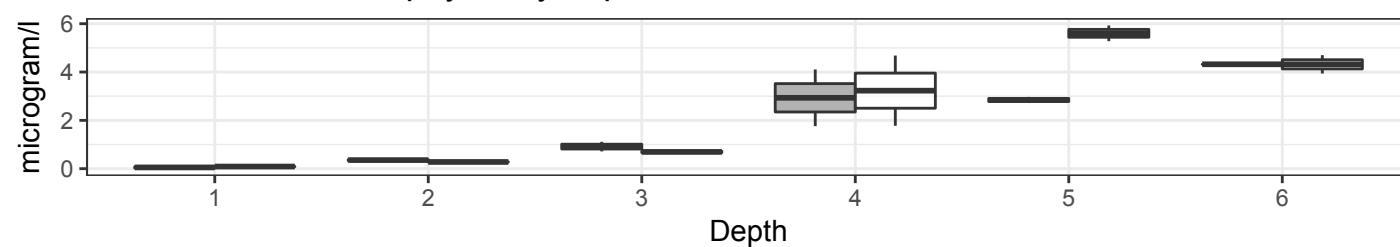
Mean bacteriochlorophyll d by depth



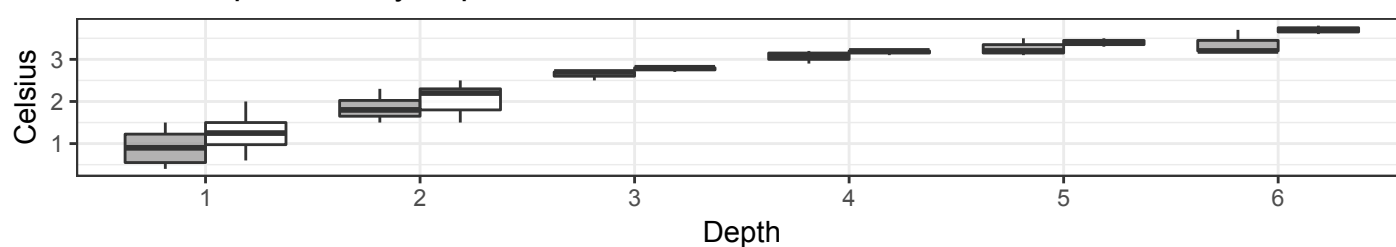
Mean light by depth



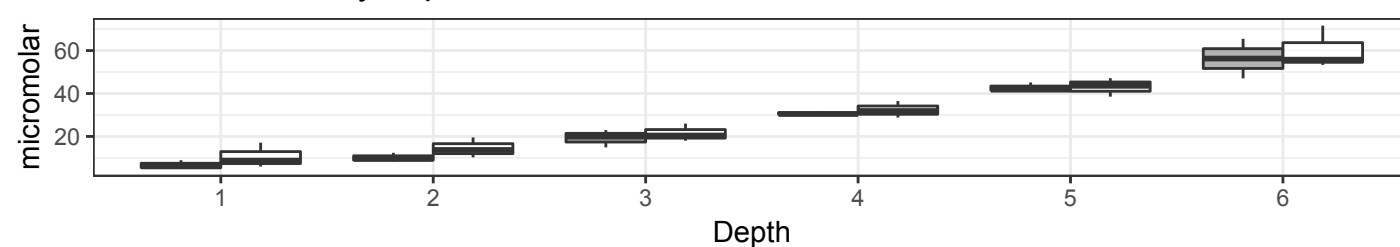
Mean bacteriochlorophyll e by depth



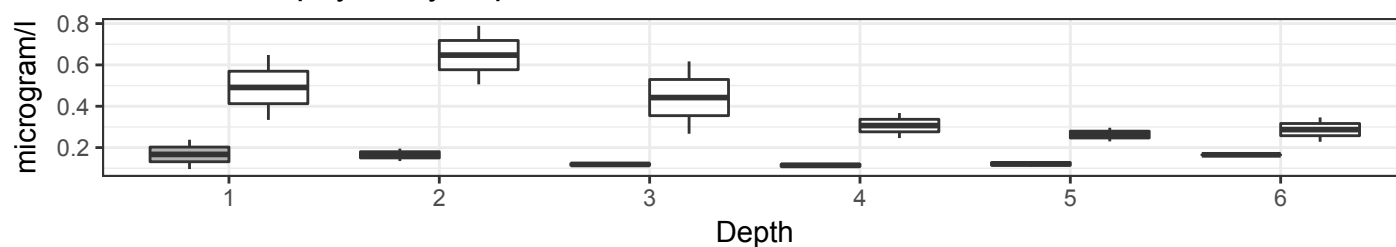
Mean temperature by depth



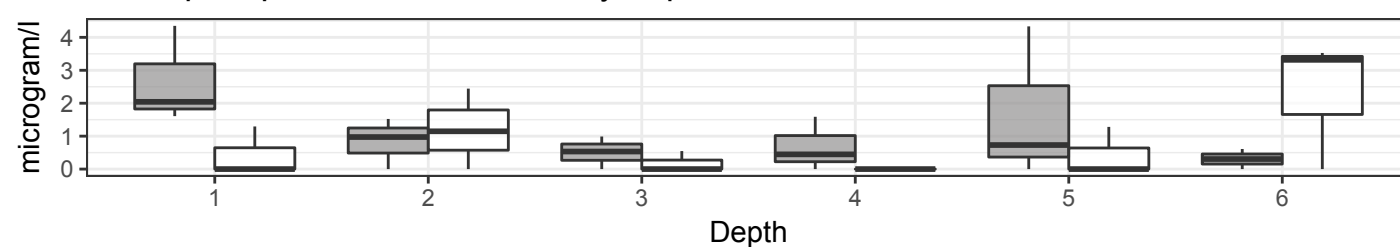
Mean methane by depth



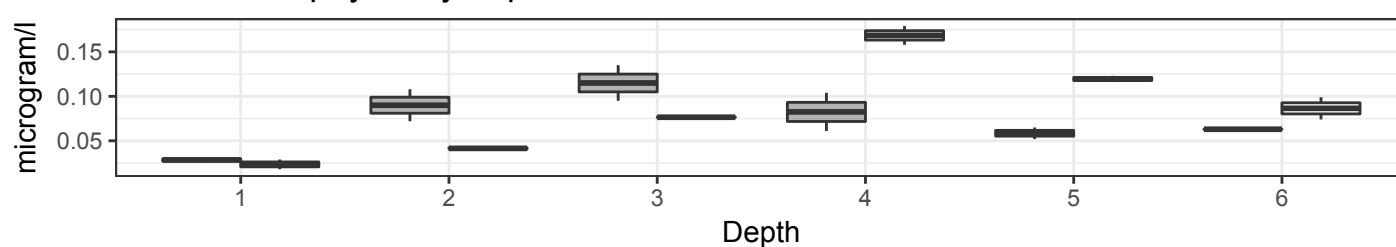
Mean chlorophyll a by depth



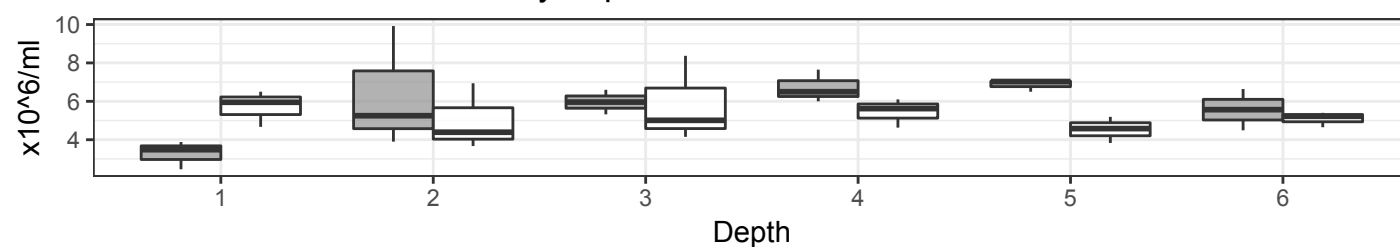
Mean phosphate concentration by depth



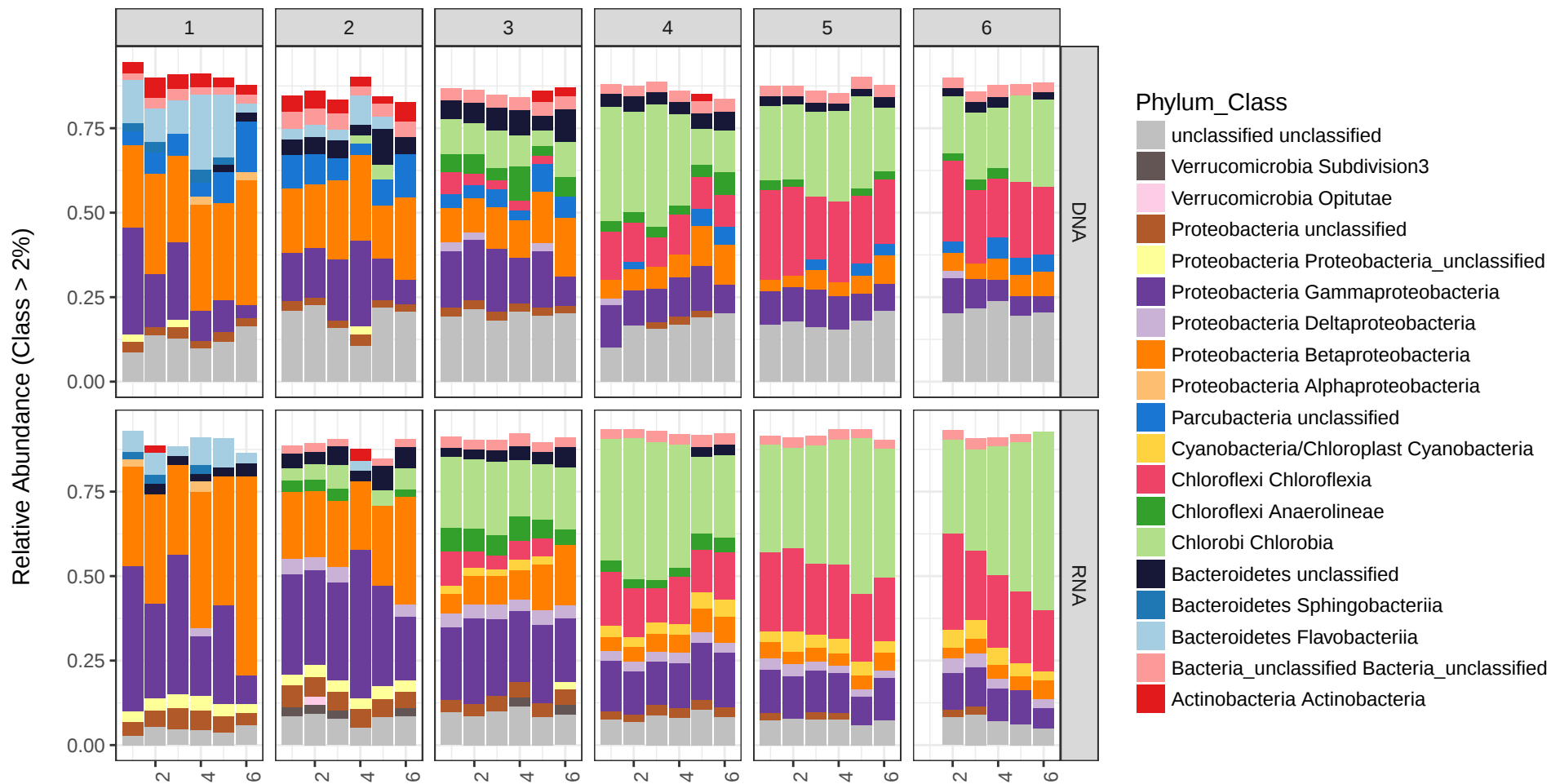
Mean chlorophyll b by depth



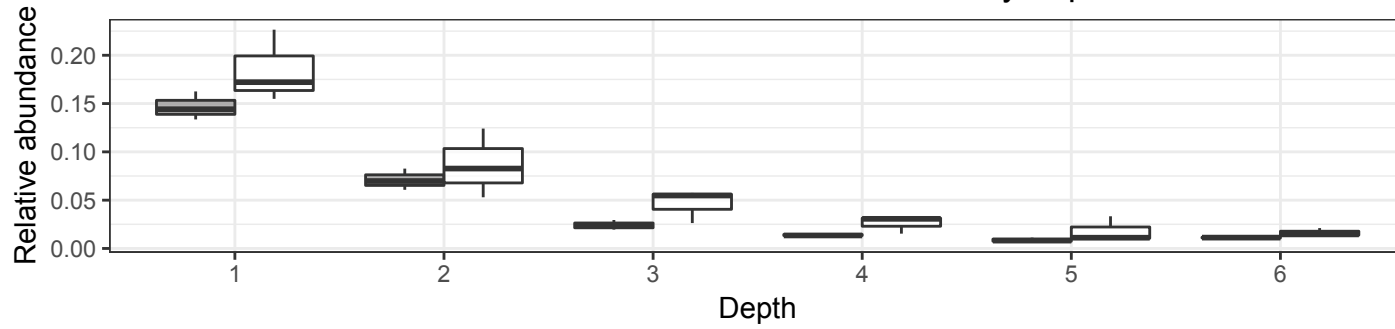
Mean bacterial abundance by depth



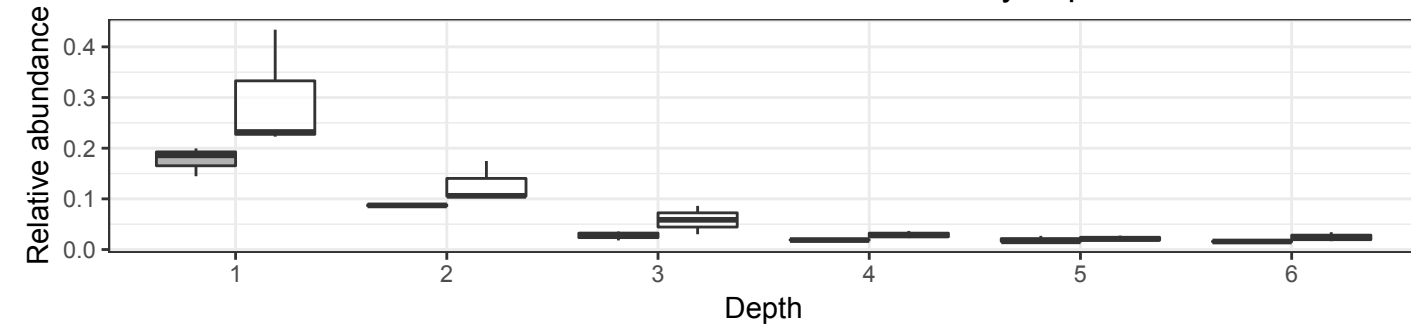
Class Composition of Lake Lomtjaernen Bacterial Communities Grouped by Depth



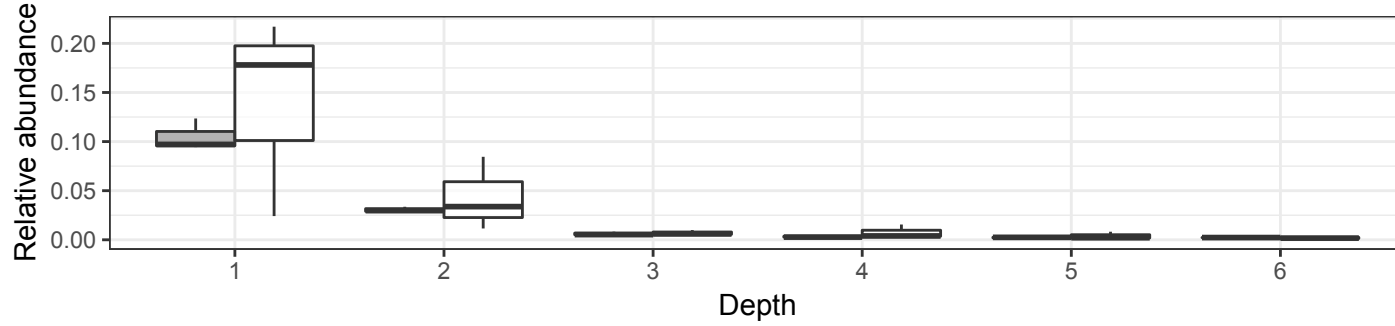
Mean relative abundance of Comamonadaceae in DNA by depth



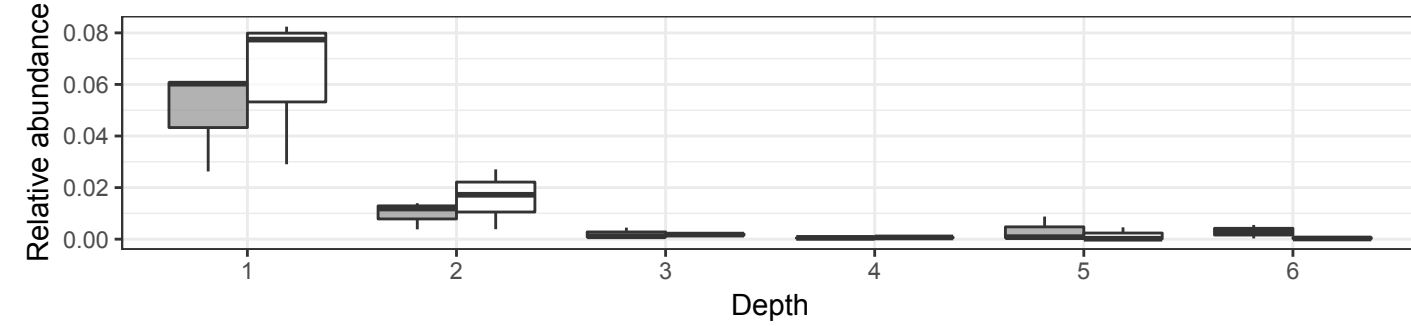
Mean relative abundance of Comamonadaceae in RNA by depth



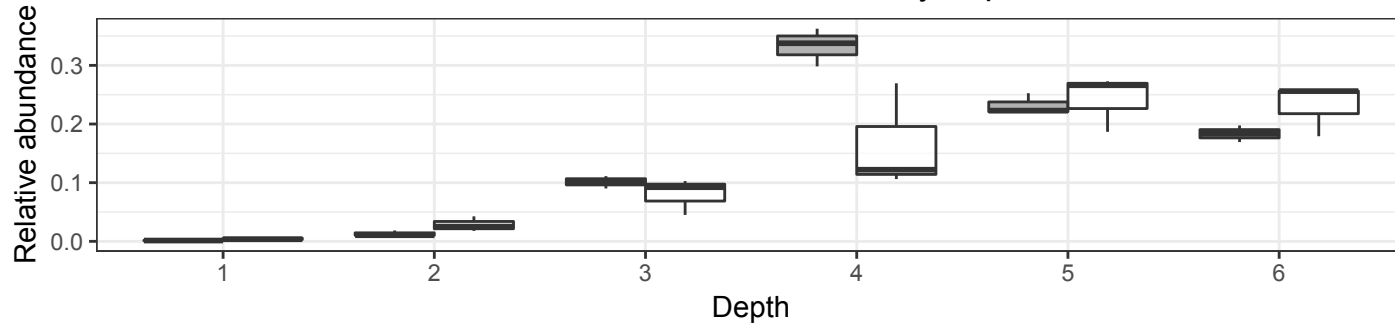
Mean relative abundance of Flavobacteriaceae in DNA by depth



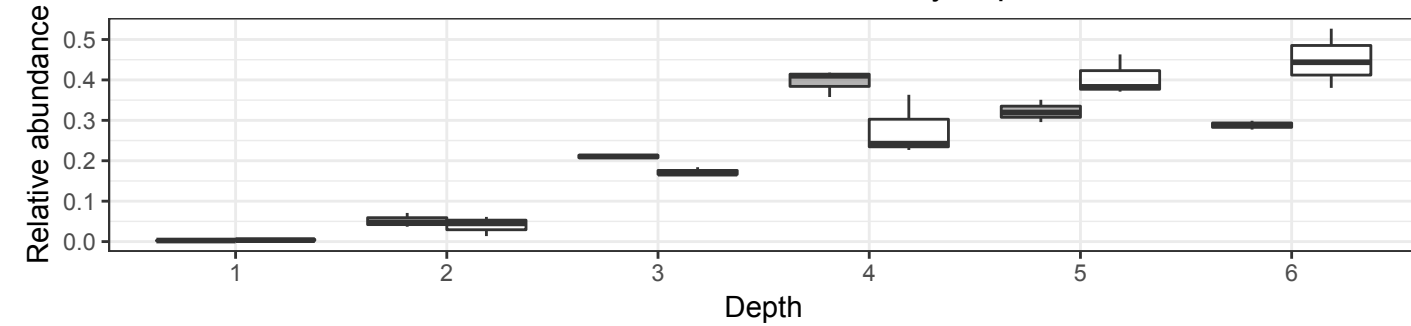
Mean relative abundance of Flavobacteriaceae in RNA by depth



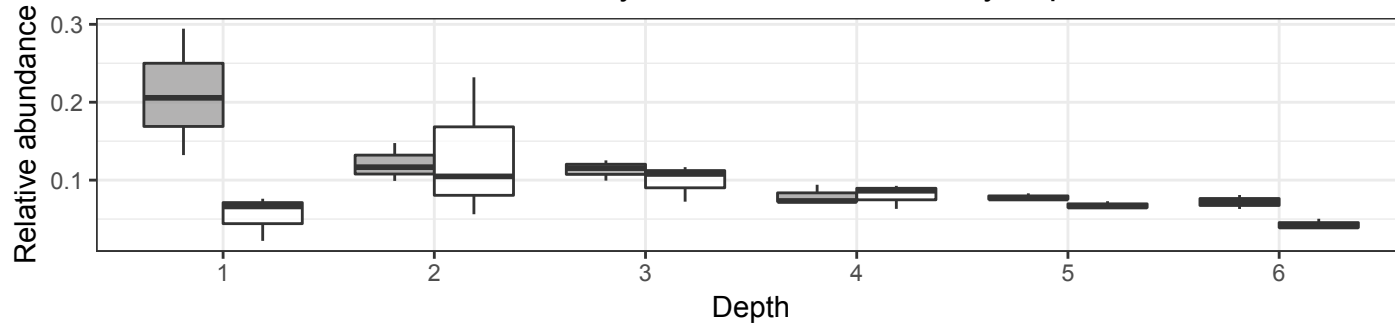
Mean relative abundance of Chlorobiaceae in DNA by depth



Mean relative abundance of Chlorobiaceae in RNA by depth



Mean relative abundance of Methylococcaceae in DNA by depth



Mean relative abundance of Methylococcaceae in RNA by depth

