1   **Phytoplankton response to experimental thermocline deepening: a mesocosm experiment**

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12

13   **Abstract**

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15   A number of modelling results suggested thermocline shifts as a consequence of global climate

16   change in stratifying lakes. Abundance and composition of the phytoplankton assemblage is

17   strongly affected by the stratification patterns, therefore, change in the thermocline position

18   might have a substantial effect on this community or even on the whole lake ecosystem. In this

19   study, thermocline depths in large mesocosms installed in Lake Stechlin (Germany) were

20   deepened by 2 meters and phytoplankton changes were analysed by comparing changes to

21   untreated mesocosms. Higher amounts of SRP were registered in the hypolimnion of treatment

22   mesocosms than in the controls, and there were no differences in the epilimnion. Small but

23   significant changes were observed on the phytoplankton community composition related to the

24   effect of deepening the thermocline; however, it was weaker than the yearly successional

25   changes. The most remarkable differences were caused by *Planktothrix rubescens* and by

26   chlorophytes. *P. rubescens* became strongly dominant at the end of the experiment in the

27   mesocosms, and in the open lake as well. The results of the experiment cannot clearly support the

28   proliferation of cyanobacteria in general; however, the deepened thermocline can modify the

29   behaviour of some species, as was observed in case of *P. rubescens*.

30

31   **Introduction**

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33   Global climate change has a significant effect both on terrestrial and aquatic ecosystems and its

34   consequences will accelerate in the future (IPCC, 2007; IPCC, 2013). One of the most significant

35   effects of climate change on phytoplankton communities in stratifying lakes will be presumably

36   related to changes in stratification patterns. Because of the climate warming, some polymictic

37   lakes are expected to become dimictic, dimictic lakes may become warm monomictic and

38   numerous monomictic lakes may turn into oligomictic (Gerten and Adrian, 2002). Several key

39   variables, which are driving the phytoplankton community assembly depend on the stratification

40   processes (Winder and Sommer, 2012). The duration and intensity of thermal stratification

41   strongly affect the nutrient input from the hypolimnion to the upper layers (Behrenfeld et al.,

42   2006). Stratification in itself results in complex physical and chemical gradients, which increase

43   the heterogeneity of the water column, thus increase habitat heterogeneity (Selmeczy et al.,

44   2016). The turbulence is supressed in a stratified waterbody (Turner, 1979), which favours motile

45 (Gervais, 1997) or buoyant phytoplankton species (Huisman et al., 2004) and negatively affects

46 most planktonic diatoms with high sinking rates (Reynolds, 2006) and also some green algal

47 species (Huisman et al., 2004). Thus, it is expected that mainly because of the physical processes

48 altered by climate change diatoms and other non-motile species will be replaced by other groups,

49 which are able to cope with reduced mixing (Findlay et al., 2001). Though in a few other cases

50 diatoms dominated over other taxonomic groups (Winder et al., 2009; Medeiros et al., 2015) in

51 stratifying lakes, according to most of the scenarios increase of cyanobacteria is foreseen.

52   Cyanobacteria have several unique abilities to surpass other taxonomic groups in different

53 environments affected by climate change. The most important eco-physiological features, which

54 help them to adapt to the changing environment are: (i) the ability to grow at warmer

55 temperatures, (ii) the buoyancy regulation by gas vesicles, (iii) potential nitrogen-fixation with

56 heterocytes, (iv) high affinity for, and ability to store phosphorus, (v) potential of akinete

57 production, (vi) very good light harvesting in a wide range of wavelengths by chromatic

58 adaptation, (vii) good UV resistance (Ehling-Schulz and Scherer, 1999; Carey et al., 2012), (viii)

59 high level of ecophysiological plasticity (Üveges et al., 2011) and different antipredator

60 properties. Obviously, not all cyanobacteria species possess all these abilities because of the great

61 diversity of this taxonomic group, however these features could help a given species to become

62 the dominant member of the phytoplankton assemblages in different kinds of water bodies.

63 Increase of dominance of cyanobacteria as a consequence of climate change has been an overall

64 emerging issue in phytoplankton ecology (Salmaso et al., 2015; Sukenik et al., 2015).

65   The main goal of the joint experiment was to mimic a deepened thermocline during the

66 summer stratification in large size mesocosms and to follow the changes in the food web and

67 matter transport (Fuchs et al., 2017). This study tries to answer the following questions: (i) are

68 there any changes in the phytoplankton community because of the altered stratification, if yes, (ii)

69 can we confirm the proliferation of cyanobacteria, if not, (iii) what kind of species, taxonomic

70 groups or functional groups will get advantages from the changed environment?

71

72 **Materials and methods**

73

74 Lake Stechlin is a dimictic, meso-oligotrophic, hardwater lake in Northern Germany, which is

75 one of the best studied lakes in the region. The mean depth of the lake is 23.3 m, the maximum

76 depth is 69.5 m, and the surface are is 4.25 km^2 (Casper, 1985). The phytoplankton community of

77 Lake Stechlin has been studied since 1959, however, regular surveys are performed only from

78 1994 (Padisák et al. 2010). Seasonal patterns of the phytoplankton show a bimodal distribution.

79 The spring assemblages are dominated by species of Codon **B** (such as *Stephanodiscus*

80 *neoastraea* Håkansson & Hickel) and the most common species during summer belong to the **H1**

81 and **Lo** coda in the epilimnion. In most years, the metalimnion and/or upper hypolimnion is

82 dominated by picocyanobacteria species (codon **Z**), although in some years *Planktothrix*

83 *rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek population (codon **R**) were

84 present in the upper hypolimnion with significant biomass (Padisák et al. 2010; Selmeczy et al.,

85 2016).

86 An experimental setup consisting of 24 large-sized enclosures and a central reservoir was built in

87 the south basin of Lake Stechlin (Bauchrowitz, 2012). This facility is called "LakeLab"

88 (http://www.lake-lab.de). Diameters of the enclosures are around 9 m and their plastic walls are

89 anchored to the bottom of the lake. Since the bottom of the lake is not horizontal under the

90 LakeLab, the depths of the enclosures vary between 17 and 20 m. Twelve enclosures were

91 randomly selected for the experiments from Fields I-III (Fig. 1). Prior to the experiment, water

92 exchange was performed in all mesocosms to ensure the possible highest similarity. The

93   thermocline was deepened by 2 m experimentally in 6 of 12 mesocosms; the other six mesocosms

94   served as controls. The water exchange and the alteration of the stratification was performed by

95   submerged pumps (SUPS 4-12-5, SPECK Pumpen Verkaufsgesellschaft GmbH, Neunkirchen am

96   Sand, Germany) transporting nearly 6 $m^3 h^{-1}$ of water via aluminium release rings. The graphical

97   design of the system is found in Fuchs et al. (2017). During the experiment surface water was

98   pumped down to a given depth only during daytime to allow the natural cooling over nights.

99   During the first month, rhythm of pumping activity was 8 h per day, then until the end of the

100 experiment it was increased to 12 h per day. In the control mesocosms, aluminium rings were

101 placed in the depth of the lake's thermocline in order to affect equally all systems by pumping

102 activities. The depth of the release rings were moved 1 m down at 24 July, 25 July and 04

103 September to follow the natural thermocline depth in the lake.

104   Physical and chemical parameters (temperature, conductivity, pH, redox potential, oxygen

105 concentration, oxygen saturation and the photosynthetically available radiation - PAR) were

106 measured with YSI (Yellow Springs Instruments, OH, USA) and PAR sensors. These data were

107 recorded in half a meter increments from the surface (0.5 m) to the bottom in every hour.

108 Integrated water samples were taken on 25 June, 10 July, 23 July, 06 August, 20 August, and 11

109 September 2013 both from the epi- and hypolimnion using an integrated water sampler (IWS II,

110 Volume: 5L, Hydro-Bios, Kiel, Germany). Concentrations of TP, SRP, TN, NO_2^-, NO_3^-, NH_4^+

111 and SRSi were measured according to APHA (1998) in these samples. All inorganic N fractions

112 (NO_2^--N, NO_3^--N and NH_4^+-N) were added for estimating dissolved inorganic nitrogen (DIN),

113 and nutrient ratio (DIN/SRP). Samples for phytoplankton analyses were taken on 25 June, 23

114 July, 20 August 2013. These samples were preserved in Lugol's solution and were stored in a

115 dark at room temperature. Phytoplankton numbers were determined with the classical

116 methodology by Utermöhl (1958) and Lund et al. (1958). Altogether 400 settling units (cells,

117 filaments and colonies) were counted at minimum in each sample using an inverted microscope

118 (Zeiss Axiovert 100, Oberkochen, Germany). Volume of the cells was calculated by the most

119 similar geometric form according to Hillebrand et al. (1999), then biovolume was converted to

120 biomass using the 1 mm^3L^{-1} = 1 mgL^{-1} conversion factor. Opticount cell counting software

121 (Opticount, 2008) was used to estimate the biomass. Phytoplankton species were sorted into

122 different functional groups (FG) according the classification of Reynolds et al. (2002) and

123 Padisák et al. (2009).

124 Analysis of variance using distance matrices was used to test how depth (epilimnion or

125 hypolimnion), month of the sampling (June, July, August) and treatment (control, treatment)

126 influence the community composition using function ADONIS in R software (R Core Team,

127 2015). Bray-Curtis dissimilarity was used for the distance matrix. Indicator species analysis

128 according to Dufrene and Legendre (1997) was run to identify characteristic taxa of depths,

129 months and treatments. INDVAL function was used by the *labdsv* package (Roberts, 2012) in R

130 environment.

131

132 **Results**

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134 The alteration of the thermocline depth resulted in multiple changes in the stratification pattern:

135 the border between hypolimnion and metalimnion sank, and according to the temperature the

136 epilimnion had two parts. The temperature of upper part (from the surface until 4 meter) was

137 homogenous and the lower part had a small temperature decrease until metalimnion. The

138 temperature was rather smooth in the epilimnion of control mesocosms. The typical temperature

139 profile of the treatment and control mesocosms are shown in Fig. 2. Similar patterns were

140 observed in the control and treatment mesocosms related to SRP values: at the beginning, the

141   amounts of SRP fall near to the detection limit in both in the epilimnion and hypolimnion, later

142   SRP in the epilimnion remained low during the whole experiment, but it increased in the

143   hypolimnion after mid-July. In spite of the similar pattern, the medians of SRP were higher in the

144   hypolimnion of treatment mesocosms compared to the controls (Fig. 3), however there were no

145   significant differences between the treatment and control mesocosms according to t-test analyses.

146   The DIN values were close to 0.05 mgL^{-1} at beginning of the experiment in the hypolimnion and

147   epilimnion as well, in both types of mesocosms (Fig. 4). Later, it decreased in the epilimnion and

148   increased in the hypolimnion both in the treatment and control mesocosms. The maximum level

149   of DIN/SRP (92) was calculated in a hypolimnetic sample at the beginning of September and the

150   lowest value (4) was calculated at the end of the experiment in several epilimnetic samples. In

151   general, the DIN/SRP values decreased in the epilimnion and alternated in the hypolimnion.

152   Altogether 78 samples were analysed and 130 taxa were found in these samples. Species

153   can be categorised in 21 functional groups of which **F**, **H1**, **X2**, **X3**, and **Y** were the most

154   frequently occurring FGs present in more than 90 % of the samples and **R**, **H1** and **Y** were the

155   most dominant ones. The main representatives of the FG's are shown in Table 1.

156   Cryptophytes were the most dominant phytoplankton group in the epilimnetic samples at

157   the beginning of the experiment (Fig. 5 A, C). Later this group strongly decreased in the control

158   mesocosms until the end of the experiment. Chlorophytes showed maximum abundance during

159   July in the epilimnetic samples of the treatment mesocosm, but reached maximum level during

160   August in the control enclosures. The biomass of cyanobacteria showed increasing pattern during

161   the experiment, and reached the highest amount during August as well.

162   Maximum abundance of cryptophytes was registered during July in the hypolimnion of

163   both types of mesocosms (Fig. 5 B, D). Biomass of dinoflagellates showed increasing pattern in

164   the treatment and control mesocosms as well. Similar amount of Chlorophytes were present in the

165   two types of mesocosms in the hypolimnion. Furthermore, cyanobacteria was the most dominant

166   taxonomic group reaching 59% contribution to total biomass in the treatment mesocosms and

167   75% in the control enclosures. Species belonging to the chrysophytes were observed in negligible

168   amounts. Diatoms occurred rarely in our experiment, although higher amounts were registered in

169   the hypolimnetic samples but remaining below 12% of the total biomass.

170   The changes of the seven most dominant functional groups are shown on Figure 6. At the

171   beginning, **H1**, **X2** and **Y** coda dominated in the epilimnetic samples. The abundance of the latter

172   was higher in the epilimnion of control mesocosms, than in the treatment one (Fig. 6 A, C). At

173   the end of the experiment, decrease of the total biomass was observed in the control mesocosms,

174   but a slight increase was registered in the treatment enclosures. This difference was caused by

175   high amounts of *Planktothrix rubescens* (the only member of codon **R**), which started to increase

176   after July in the treatment enclosures. This species dominated in the hypolimnetic samples and

177   started to increase from the beginning of the experiment and became the most dominant by

178   August. That time, nearly 70% of the total biomass belonged to this species (functional group) in

179   the control mesocosms and reached 51% in the treatment enclosures in the hypolimnion.

180   However, in the epilimnia *P. rubescens* reached 8% (32.9±62.2) in the control mesocosms and

181   44% (342.3±381.6) in the treatment enclosures (Fig. 7). This difference was significant according

182   to Wilcoxon test: W=2, p=0.03175.

183   *Phacotus lenticularis* (Ehrenberg) Deising was the only member of X_{Ph} codon, and did not

184   belong to the frequently occurring functional groups. However, during July it became the

185   dominant member of the phytoplankton community in mesocosm T6; while remaining low in

186   other mesocosms (not shown) and nearly disappeared from T6 during August. Thus this codon

187   caused the highest uncertainty during the experiment.

188   ADONIS revealed that the depths (epi-, or hypolimnion), months and treatment

189   individually, further the interaction of months and depths affect significantly the phytoplankton

190   community composition of mesocosms (Table 2). INDVAL analyses were performed to

191   investigate, which functional groups indicate the different categories of months, depths and

192   treatment (Table 3). Six functional groups (**X2**, **L$_0$**, **X1**, **Y**, **F** and **H1)** were indicators of

193   epilimnetic samples and 3 coda (**R**, **X3** and **C)** were indicators of hypolimnetic samples (Fig. 8).

194   Interestingly, INDVAL did not find any indicator codon for treatment or control mesocosms.

195   NMDS analyses (Fig. 9) were carried out to visualize differences between samples

196   belonging to different months, treatment and depths suggested by ADONIS analyses. Epilimnetic

197   and hypolimnetic samples are separated according mainly to the vertical axis, other samples from

198   different months differentiated based mainly on the horizontal axis.

199

200   **Discussion**

201

202   The heat-balance of lakes is determined basically by meteorological forcing at the air-water

203   interface, therefore, it is possible to raise general predictions for changes of the thermal

204   characteristics of lakes in relation to climate change. However, other features such as

205   morphometry, residence time of water, optical properties and landscape setting can have a major

206   effect on the thermal characteristic of lakes (Arvola et al., 2010). For this reason, lakes respond

207   individually to the effect of climate change concerning changes of stratification pattern and very

208   likely to numerous other hydrological and chemical changes as well. For example, both shrinking

209   and deepening of thermoclines were predicted and observed in a number of studies. Model

210   predictions for four Finish lakes suggested thermocline deepening in three cases and a shallower

211   thermocline in one case (Elo et al., 1998). In North-temperate lakes in Wisconsin (USA),

analyses of 10-year thermal records predicted changes in thermocline depth ranging from 3.5 m deeper to 4.0 m shallower compared to the average depth (DeStasio et al., 1996). Thermocline deepening was observed during long term studies (1970-1990) in Canadian boreal lakes by Schindler et al. (1996) and it was explained by three reasons: (i) increasing wind velocities, (ii) increasing effects of wind, because of decreasing number of trees in the area, however, the most important reason was (iii) the rising temperature in sub-thermocline water layers caused by increasing transparency of epilimnetic water. Fee et al. (1996) got similar outcome and the authors emphasised that the increasing transparency is an important factor especially in case of small lakes (<500 ha). The increase in Secchi-depths was the consequence of decreasing DOC level because of the less precipitation runoff caused by longer periods of droughts.

The general findings of numerous studies is that warmer air temperatures result in warmer surface water temperature and this layer of warmer and lighter water weakens wind induced mixing, thus shallower and warmer epilimnia are predicted in the future (Robertson and Ragotzkie, 1990; King et al., 1997; Vincent, 2009; De Senerpont Domis et al., 2013). Coats et al. (2006) analysed the thermal structure of Lake Tahoe (USA) from 1970 to 2002 and observed a strong decrease of depth of the October thermocline, but the reasons remained unrevealed. Straile et al. (2003) and Livingstone (2003) analysed long dataset from deep European lakes (Lake Constance and Lake Zürich) and they found increasing epilimnetic temperatures like in most of the studied lakes around the globe, although these two studies did not observe clear changes related to thermocline depth.

Thus it is possible to draw an important lesson from these examples, namely that we must be cautious with general statements related to lake responses to climate change, because even quite similar lakes can react at different ways to changing climatic conditions.

235   Either the thermocline depth of Lake Stechlin will decrease or increase in the future, it is

236   likely to result in changes in overall phytoplankton biomass and taxonomic composition, because

237   mixing depth is a key factor determining light availability and sedimentation losses (Reynolds,

238   1984; Visser et al., 1996).

239   In our experiment, the thermocline of treated mesocosms was deepened by 2 meters

240   compared to the control mesocosms and small, but strongly significant (P<0.001) differences

241   were observed between the phytoplankton community composition of the treated and control

242   enclosures. The observed differences confirm the prominent importance of position of the mixing

243   depth. The most obvious difference between the control and treatment mesocosms is the high

244   abundance of *Planktothrix rubescens* in the epilimnion of treatment- and in the hypolimnion of

245   control enclosures. Moreover, the amounts of chlorophytes were higher in the epilimnion of

246   control mesocosms during the last month, as a consequence of the considerable abundance of **F**

247   and **MP** coda.

248   Similar, but more spectacular results were found by Cantin et al. (2011) in an experiment of

249   thermocline deepening, however at a whole basin scale not in mesocosms. The authors could

250   demonstrate an important shift in the structure of the phytoplankton community towards

251   dominance of chlorophytes in the epilimnion in response to thermocline deepening. In our

252   experiment we did not observe this phenomenon, although green algae belonging to codon **F**,

253   such as *Oocystis lacustris* Chodat, frequently occurred in epilimnetic samples, and reached higher

254   biomass in the control mesocosms.

255   Ptacnik et al. (2003) analysed the phytoplankton community changes in a gradient of

256   mixing depths in a mesocosm experiment. High biomass of diatoms was observed in this

257   experiment even at low mixing depth and it was explained by the fast growth rates of diatoms

258   under sufficient supply of available silica. Our experiment cannot support the increase of

259   diatoms, because this group never exceeded a 12% contribution to the total biomass, though in

260   more than 80% of the samples the SRSi concentration was higher than 0.1 mgL^{-1} but none of

261   them was higher than 0.5 mgL^{-1}. According to Reynolds (2006) silica concentration below 0.5

262   mgL^{-1} begins to interfere the growth of diatoms, but the growth-limiting threshold is 0.1 mgL^{-1} or

263   less in most lacustrine environments. Sommer (1988) reviewed a number of experimental and

264   field observations and concluded that the limitation is strongly species-specific and ranges

265   between 0.9 μM Si (~0.023 mgL^{-1}) and 20 μM Si (~0.5 mgL^{-1}). Others indicate 0.5 mgL^{-1} as

266   limitation threshold for *Asterionella formosa* Hassall, (Lund, 1950; Vaccari et al., 2006), which

267   was the most dominant diatom species during our experiment.

268   At beginning of the experiment cryptophytes were the dominant taxonomic group in all the

269   epilimnetic samples in the treatment and control mesocosms as well, which can be explained by

270   the effect of pumping activity which can be considered as a kind of disturbance. This group can

271   be prominent in post-stratification community or can peaks after disturbances such as

272   precipitation periods or wind activity (Reynolds and Reynolds, 1985; Bicudo et al., 2009). During

273   July cryptophytes increased in the hypolimnetic samples and decreased in the epilimnion. The

274   epilimnetic decline could be related to the increasing zooplankton grazing and may the deepened

275   thermocline favoured to cryptophytes to increase their biomass in the hypolimnion. Cryptophytes

276   can have competitive advantage there, because in case of deeper thermocline the nutrient-rich

277   hypolimnion has lower light levels, which they can utilize with special pigments such as carotene

278   or phycoerythrin (Gervais, 1997) or they can compensate with mixotrophic strategy (Cantin et al.,

279   2011). However, the reason of the cryptophytes increase was most probably their disturbance

280   tolerance.

281   *Planktothrix rubescens* is an important member of the phytoplankton community of Lake

282   Stechlin for a long time (Krieger, 1927). The abundance of this species is commonly low,

283 however, if the circumstances are appropriate it can become the dominant taxon in the lake, such

284 as in 1998 (Padisák et al., 2003) or in the year of this study (2013) (Selmeczy et al., 2016).

285 During these periods, this species can be classified as an "ecosystem engineer", because it can

286 strongly modify the annual phytoplankton succession of the lake (Padisák et al., 2010).

287 *Planktothrix rubescens* is typical a deep-chlorophyll maximum (DCM) forming cyanobacterium

288 in the metalimnion or in the upper hypolimnion in deep lakes (Micheletti et al., 1998; Camacho,

289 2006), thus the depth of the thermocline is very likely crucial for this species. The euphotic depth

290 of Lake Stechlin extends to the upper 20-25 meter and the thermocline develops around 8 meter

291 below the surface during the stratified period, thus there is a more or less 10-15 meter thick water

292 layer available for DCM formation. If the thermocline will lower because of the climate change

293 by two or even more meters, still there is "enough space" for DCM forming phytoplankton

294 species. Thus we can conclude that the thermocline deepening is not likely to affect the

295 development of DCM by *Planktothrix rubescens*. However, other species such as

296 *Aphanizomenon flosaquae* Ralfs ex Bornet & Flahault, or more frequently *Cyanobium* sp. can

297 form DCM in Lake Stechlin as well. According to our experience these species are forming DCM

298 in the middle of the thermocline (Padisák, 2003; Selmeczy et al., 2016) mainly spatially separated

299 from the population of *Planktothrix rubescens*. However, if the thermocline depth will increase,

300 the amount of available light will decrease, which basically influences the community of DCM

301 forming species. Consequently, *P. rubescens* may outcompete the above mentioned species, if the

302 thermocline depth increases until a certain point, because *P. rubescens*, can utilize low light

303 levels much more effectively than either *Aphanizomenon flosaquae* or *Cyanobium* sp.

304 Additionally, *Planktothrix rubescens* can produce cyanotoxins in Lake Stechlin (Dadheech et al.,

305 2014), which justifies the necessity of regular monitoring of the species. In spite of the typical

306 characteristic of this species, according to our experiment *P. rubescens* can be a good competitor

307   in the epilimnion as well, because during August it became the dominant species in the

308   epilimnion of treatment mesocosms. This phenomenon is interesting, because it was rarely

309   described (e.g. Anneville et al., 2014) that higher or at least comparable biomass of *P. rubescens*

310   occurred in the epilimnion, as in the meta,- or upper hypolimnion in case of deep lakes. A

311   possible explanation is that the lower part of epilimnion offered good conditions for *P. rubescens*.

312   This zone had a slightly lower temperature compared to the same depths of the control

313   mesocosms, however, because of the lack of samples from different depth increments, it is not

314   possible to confirm this hypotheses.

315   IndVal analyses did not detect any functional group which would be specific to the

316   phytoplankton assemblage of either the treatment or the control mesocosms, however statistically

317   significant differences were registered between them. Thus, the differences between the two

318   communities related to numerous smaller or bigger differences in the proportion of different

319   functional groups, instead of emerging one or few groups, which are present just in one type of

320   mesocosms. However, on species level, significant difference was found related to the biomass of

321   *P. rubescens*, but only in the epilimnetic samples during August. This was the most remarkable

322   difference, which was registered during end of the experiment and this can be explained by the

323   high resilience of the community. Additionally, according to IndVal analyses the temporal

324   changes (different months) and different depths have a stronger effect on the community, than the

325   altered thermocline.

326   As a conclusion, the artificial deepening of the thermocline led to small, but significant

327   changes in the epilimnetic phytoplankton community: higher level of biomass of *Planktothix*

328   *rubescens* (codon **R**) and lower amounts of coda **F** and **MP** were registered after the treatment.

329   Additionally, *P. rubescens* was the dominant member of the phytoplankton community in the

330   treatment mesocosms, however it is not a clear confirmation about the proliferation of

331　cyanobacteria related to a deepened thermocline, because in 2013, when the study was

332　conducted, *P. rubescens* were present with high biomass during the whole vegetation period,

333　similarly to 1998 (Padisák et al., 2010), therefore presumably the summer phytoplankton

334　assemblage was considerably affected by *P. rubescens*.

335

336　**Acknowledgement**

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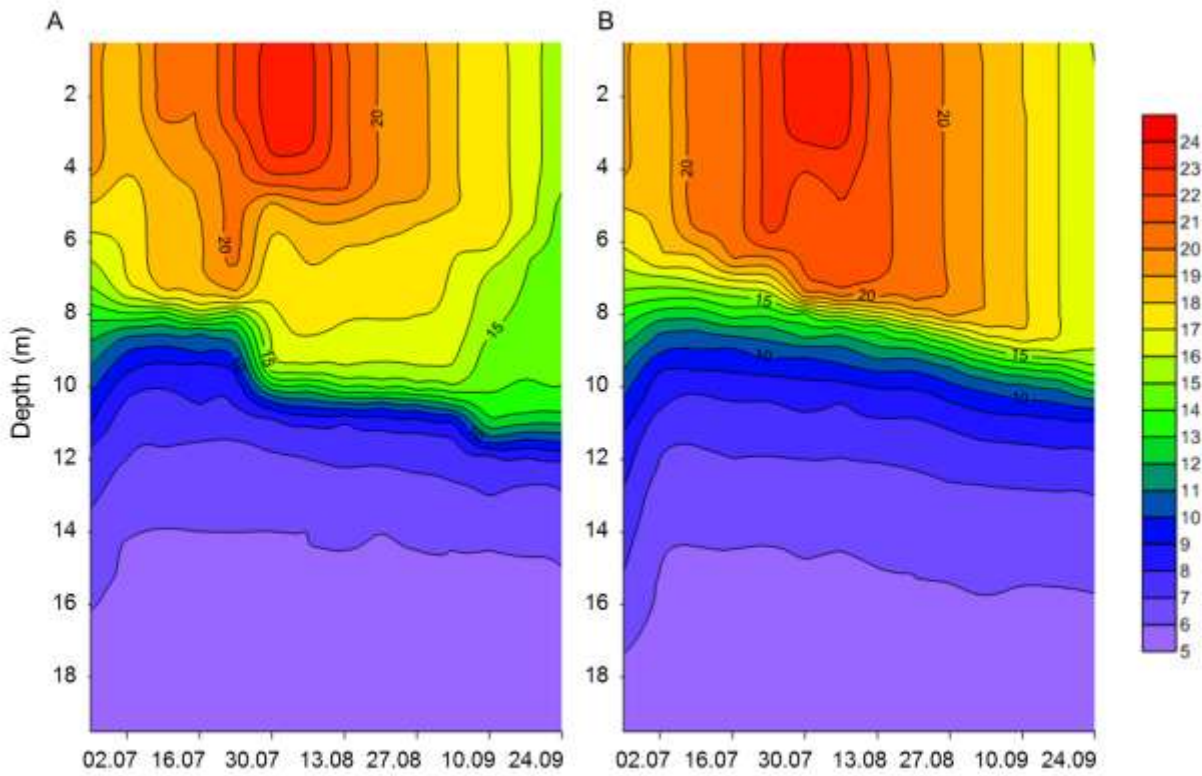
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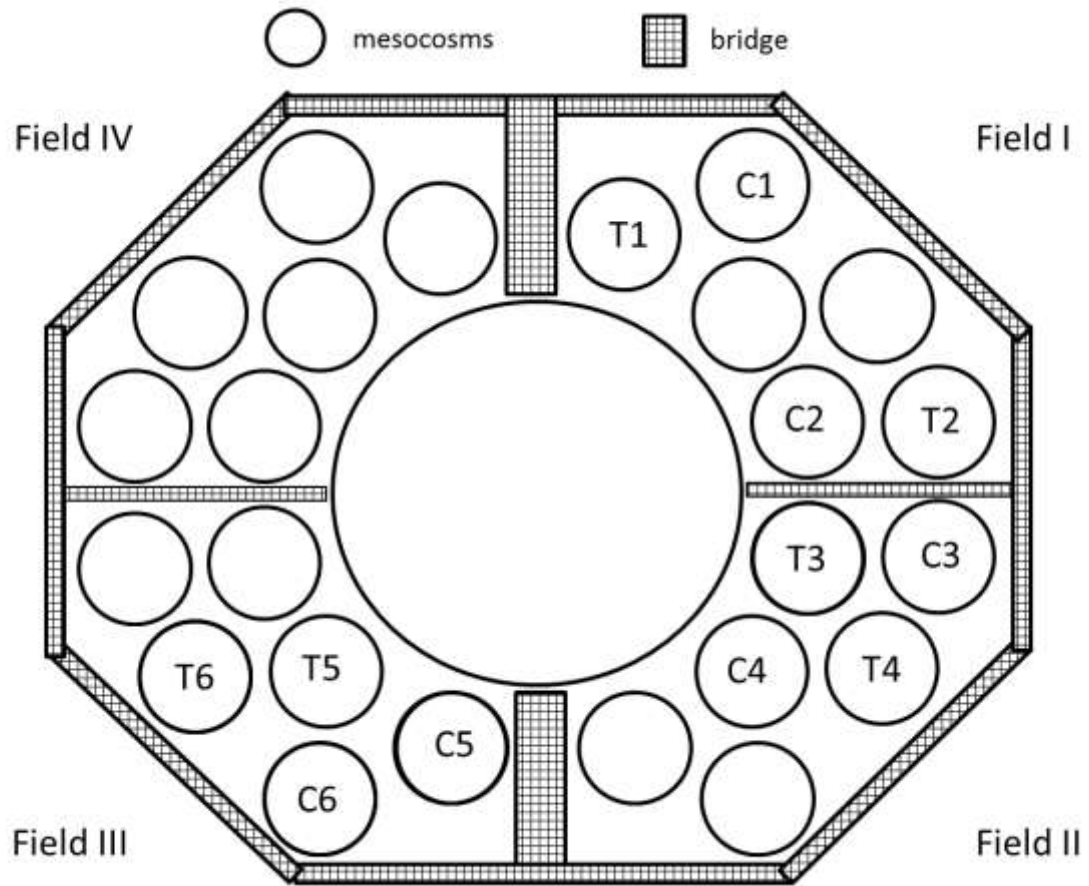
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504 Fig. 1 Lake Lab platform with the experimental design, C1, C2, C3, C4, C5, C6 indicate control

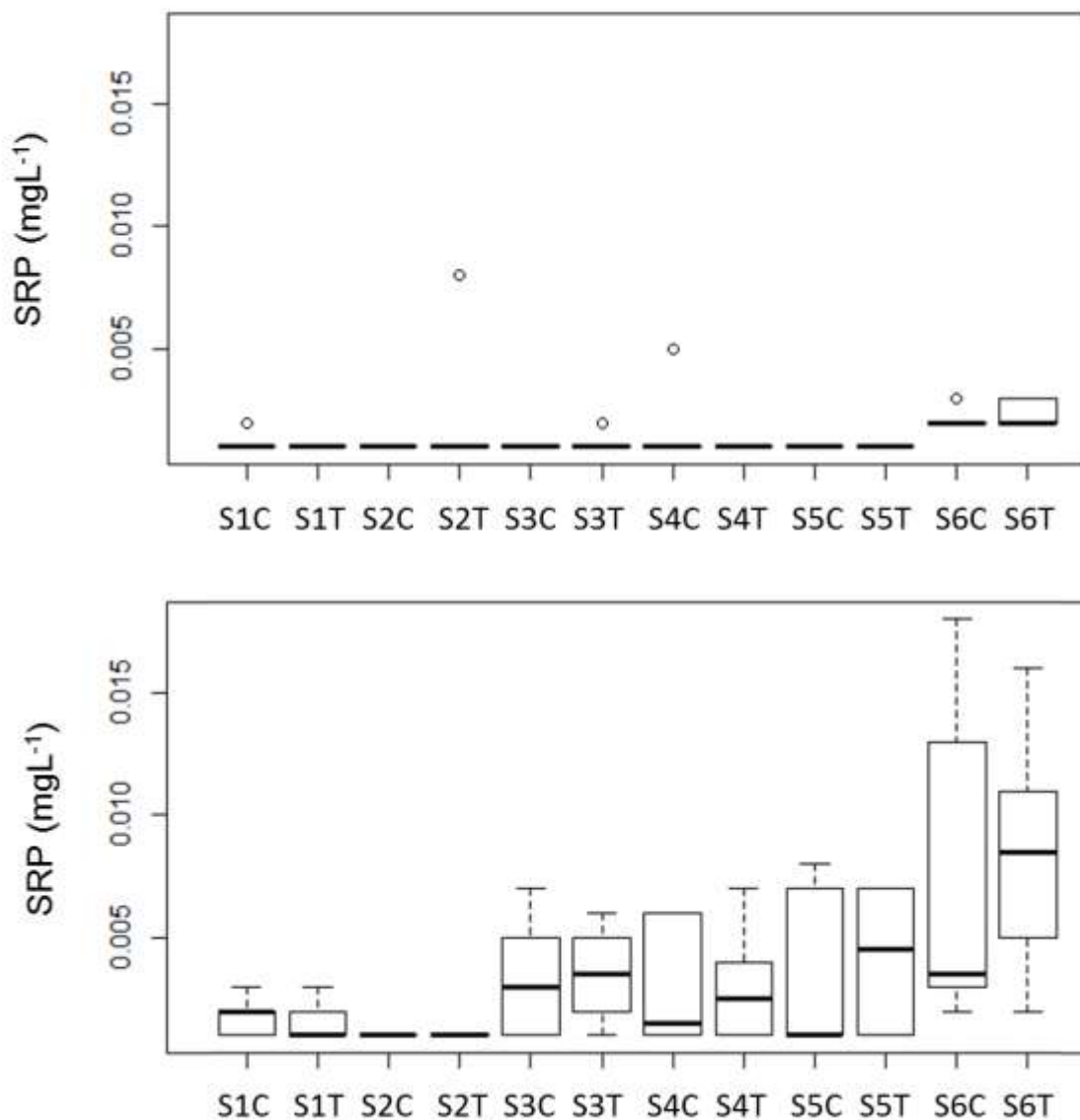505 mesocosms and T1, T2, T3, T4, T5, T6 indicate treated mesocosms

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Fig. 2 Typical temperature profiles of treatment (A) and control (B) mesocosms established in

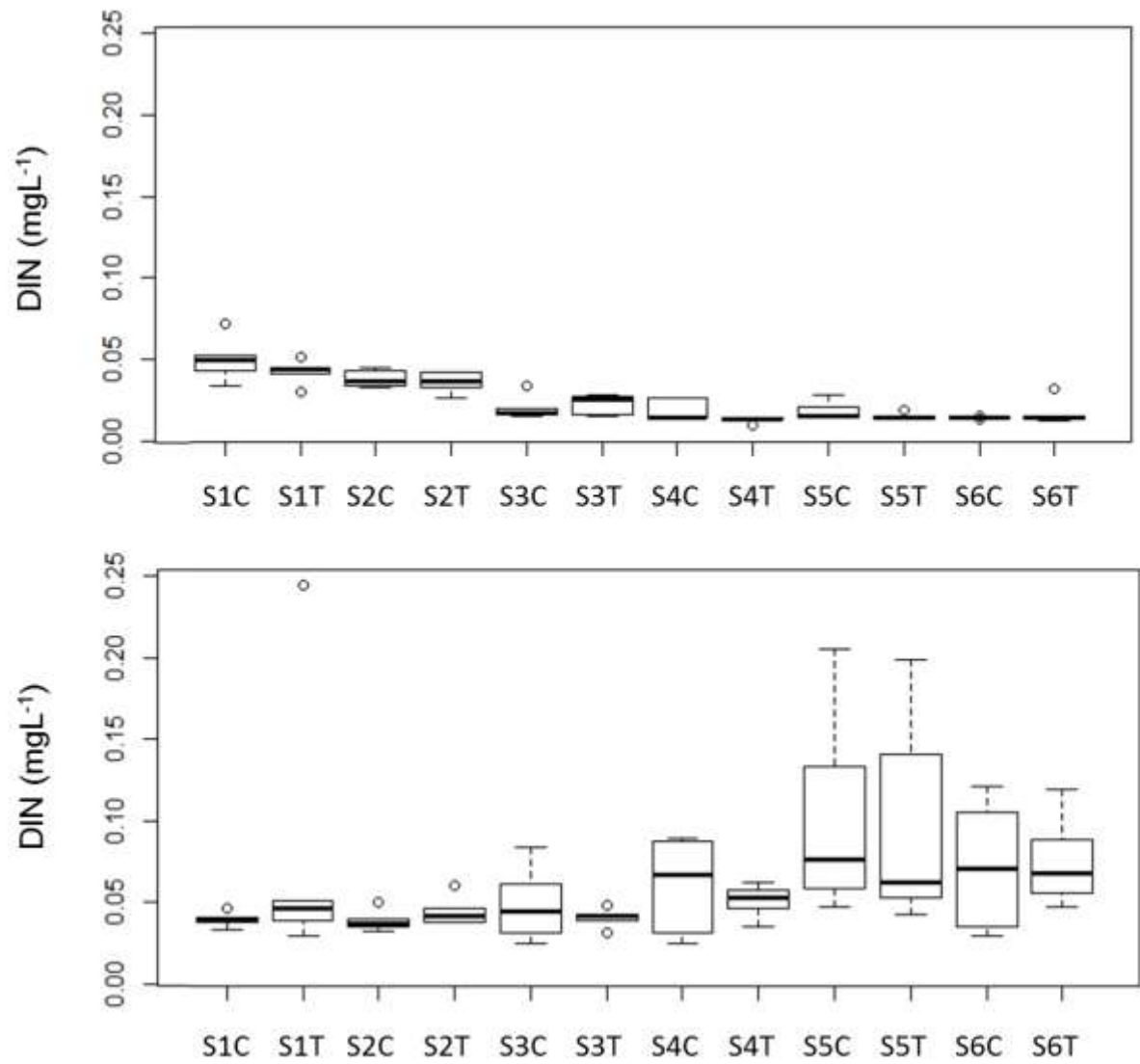Lake Stechlin between 25 June 2013 and 24 September 2013

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514 Fig. 3 Boxplots of SRP values during the experiment in the epilimnion (upper panel) and in the

515 hypolimnion (lower panel), S1: 29 May, S2: 25 June, S3: 10 July, S4: 23 July, S5: 06 August, S6:

516 20 August; C indicates control, T indicates treatment mesocosms
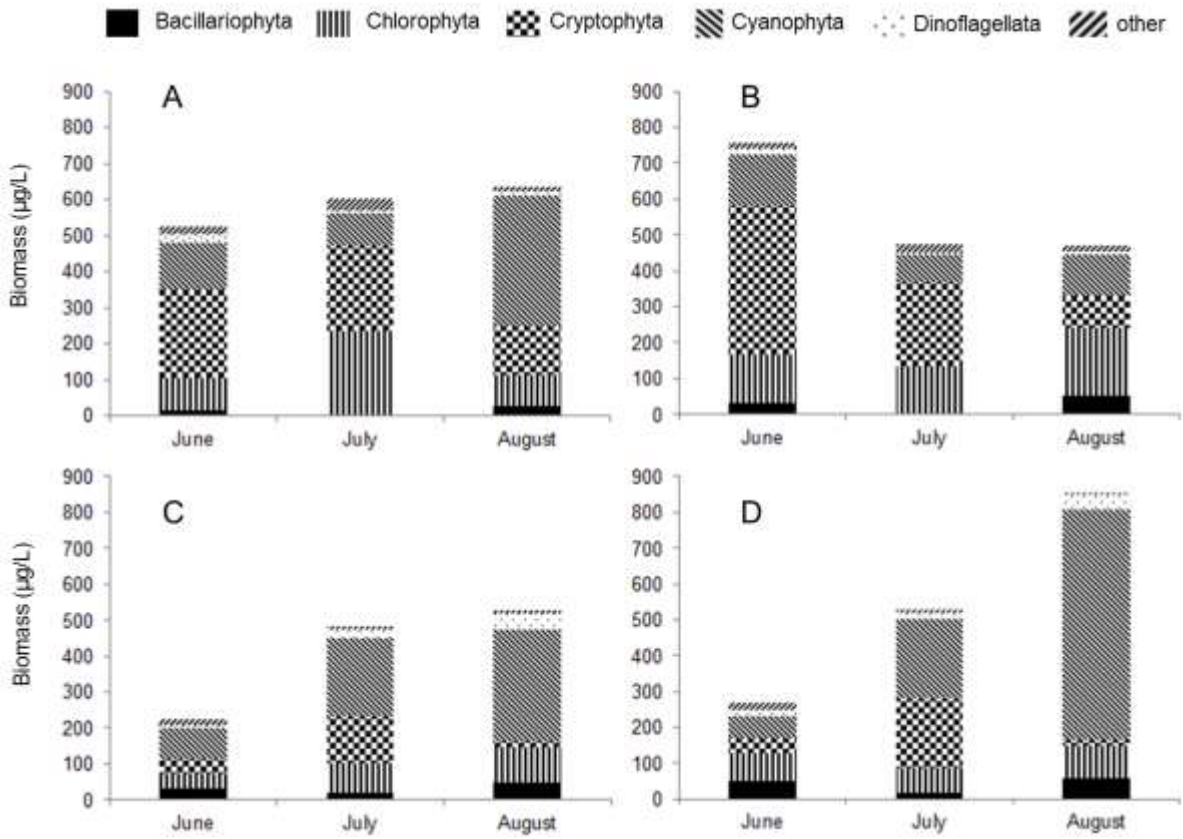
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520　Fig. 4 Boxplots of DIN values during the experiment in the epilimnion (upper panel) and in the

521　hypolimnion (lower panel), S1: 29 May, S2: 25 June, S3: 10 July, S4: 23 July, S5: 06 August, S6:

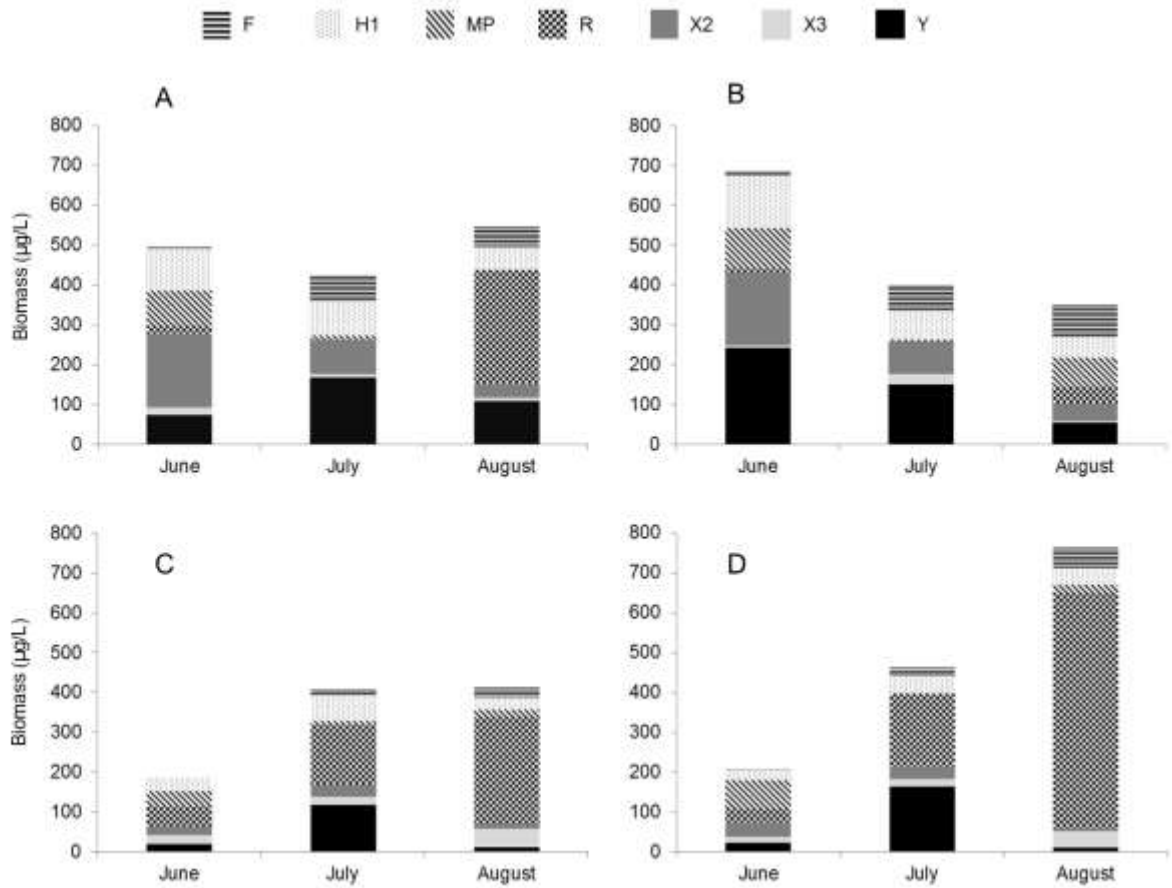522　20 August; C indicates control, T indicates treatment mesocosms

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Fig. 5 Average biomass of the different taxonomical groups during the experiment (A: Epilimnion of treatment mesocosms, B: Hypolimnion of treatment mesocosms, C: Epilimnion of control mesocosms, D: Hypolimnion of control mesocosms)
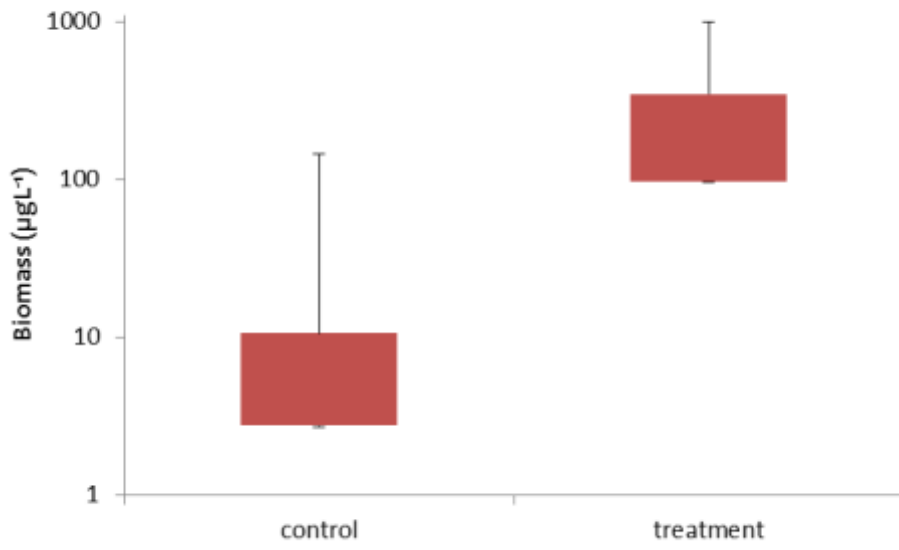
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Fig. 6 Average biomass of the most dominant functional groups during the experiment (A: Epilimnion of treatment mesocosms, B: Hypolimnion of treatment mesocosms, C: Epilimnion of control mesocosms, D: Hypolimnion of control mesocosms)

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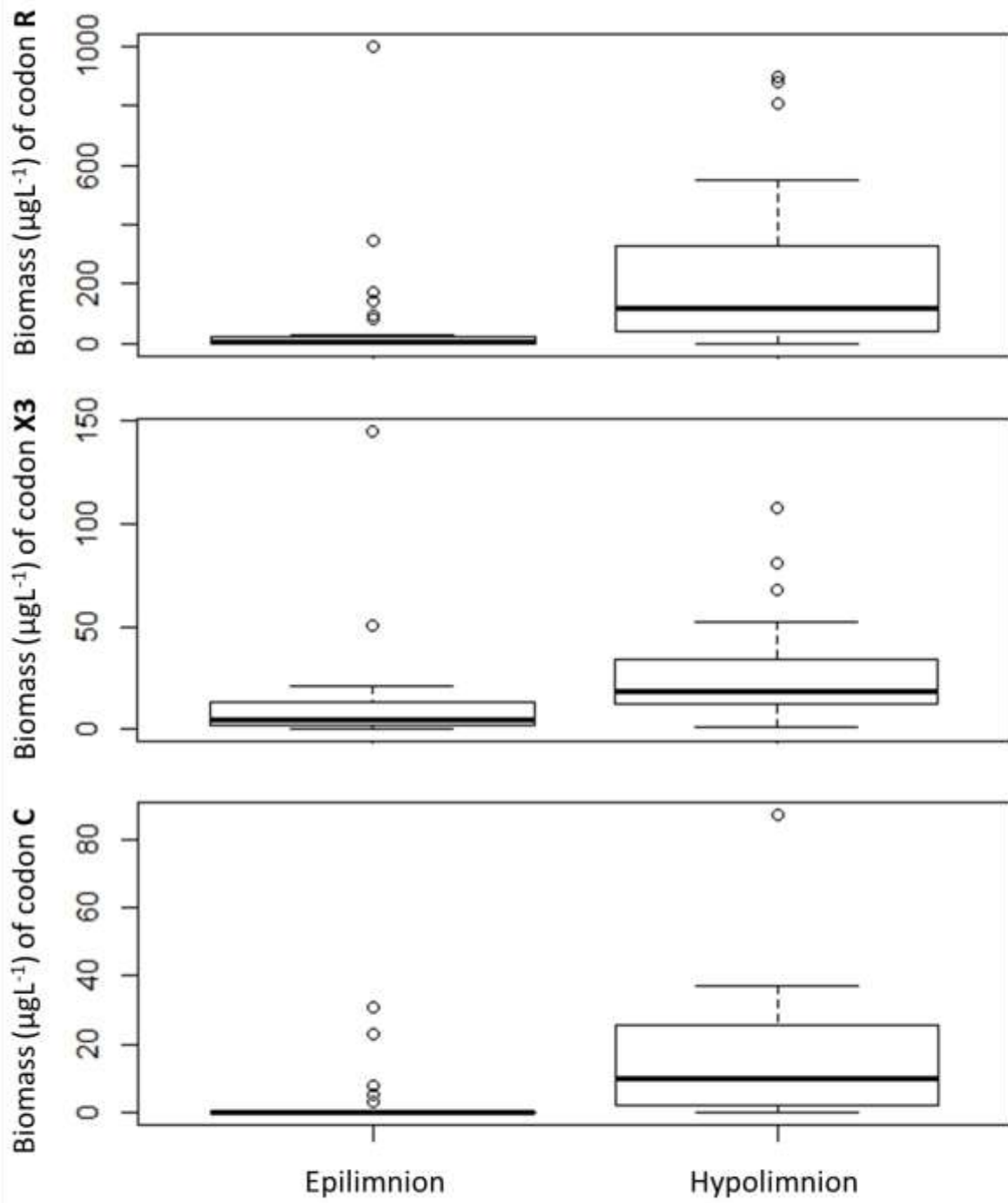538   Fig. 7 Boxplots of biomass (μgL^{-1}) of *Planktothrix rubescens* in epilimnion during August
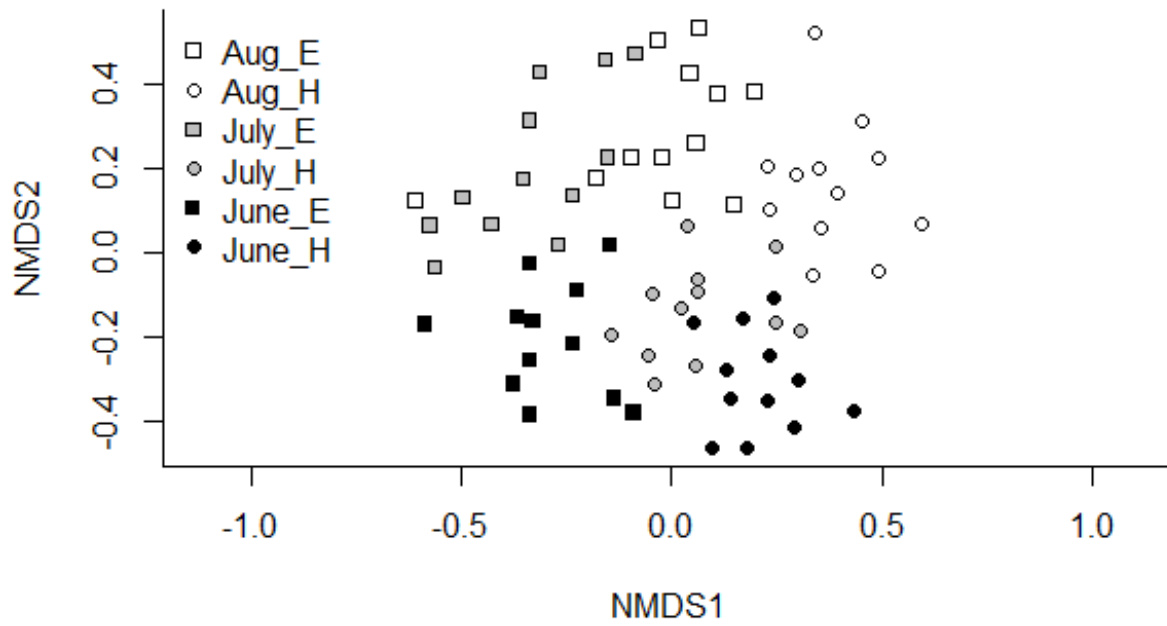
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542   Fig. 8 Boxplots of biomass (μgL^{-1}) of coda **C**, **X3** and **R** in epilimnetic and hypolimnetic samples

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546 Fig. 9 NMDS ordination diagram based on the functional group composition of the samples. E=

547 Epilimnetic samples, H=Hypolimnetic samples

548

549 **Table 1** Most important representatives of the main functional groups with their typical habitat

550 template according to Padisák et al. (2009)

Functional group	Typical habitat template	Dominant species
F	Clear, deeply mixed meso-eutrophic lakes	*Oocystis lacustris* Chodat *Coenochloris polycocca* (Korshikov) Hindák
H1	Eutrophic, both stratified and shallow lakes with low nitrogen content	*Aphanizomenon flosaquae* Ralfs ex Bornet & Flahault *Dolichospermum circinale* (Rabenhorst ex Bornet & Flahault) P. Wacklin, L. Hoffmann & J. Komárek *Dolichospermum solitarium* (Klebahn) Wacklin, L. Hoffmann & Komárek
X2	Shallow, meso-eutrophic environments	*Rhodomonas lacustris* Pascher & Ruttner *Rhodomonas lens* Pascher & Ruttner *Chrysocromulina parva* Lackey
X3	Shallow, well mixed oligotrophic environments	*Katablepharis ovalis* Skuja *Gymnodinium helveticum* Penard
Y	Almost all lentic ecosystems when grazing pressure is low	*Cryptomonas* sp.
R	In the metalimnion or upper hypolimnion of deep oligo-mesotrophic lakes	*Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek

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553 **Table 2** Summary of analyses of variance using distance matrices (ADONIS) testing the

554 individual and joint effects of month, treatment and depth on community composition.

555 Significant factors are highlighted in bold.

Factors	Df	SS	MS	F	R^2	P
Month	**2**	**3.8095**	**1.9047**	**14.0068**	**0.2307**	**0.001**
Treatment	**1**	**0.2225**	**0.2225**	**1.6361**	**0.0135**	**0.001**
Depth	**1**	**2.7321**	**2.7321**	**20.0906**	**0.1654**	**0.001**
Month: treatment	2	0.2306	0.1153	0.8478	0.0140	0.590
Month: depth	**2**	**0.8788**	**0.4394**	**3.2312**	**0.0532**	**0.002**
Treatment: depth	1	0.1351	0.1351	0.9931	0.0082	0.412
Month: treatment: depth	2	0.3467	0.1734	1.2748	0.0210	0.238
Residuals	60	8.1593	0.1360		0.4941	
Total	71	16.5146			1.0000	

556

557   **Table 3** Summary of IndVal analyses

558

Codon	Indication	Indicator value	P
X2	Epilimnion	0.81	0.001
L_0	Epilimnion	0.81	0.001
X1	Epilimnion	0.71	0.004
Y	Epilimnion	0.70	0.004
F	Epilimnion	0.67	0.041
H1	Epilimnion	0.63	0.039
R	Hypolimnion	0.76	0.001
X3	Hypolimnion	0.70	0.002
C	Hypolimnion	0.69	0.001
MP	June	0.62	0.001
X2	June	0.56	0.001
C	June	0.39	0.009
Y	July	0.52	0.001
X_{Ph}	July	0.52	0.007
A	August	0.76	0.001
R	August	0.72	0.001
J	August	0.62	0.009
P	August	0.56	0.001
L_0	August	0.52	0.020
F	August	0.52	0.031