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## Peatland microhabitat heterogeneity drives phototrophic microbe distribution and photosynthetic activity

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1 **Peatland microhabitat heterogeneity drives phototrophic microbes**  
2 **distribution and photosynthetic activity.**

3

4 **Author list**

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22 **Running title:**

23 **Microbial photosynthesis in peatland**

24

25 **Keywords**

26 Algae, Carbon cycle, Metabarcoding, Microbial diversity, Peatland, Phototrophs, Primary productivity,

27 Soil carbon uptake.

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31 **Originality-Significance Statement:**

32 Phototrophic microbes are, together with heterotrophic microorganisms, a constitutive part of the  
33 microbiome in surface soils, yet their contribution to global and regional biogeochemical C cycling still  
34 remains virtually unknown in many ecosystems. Our work combines 23S metabarcoding and  
35 ecophysiological measurements to complete our current view on phototrophic microbes in peatlands.  
36 Our results show for the first time that phototrophic microbial communities are very diverse across  
37 peatland microhabitats, and more importantly, that phototrophic microbial C uptake is widespread  
38 although the highest in relatively dry microhabitats such as hummocks. These findings are of high  
39 importance because the role of phototrophic microbes in peatland C fixation remains overlooked and  
40 has until now only been estimated in the wettest microhabitats. Therefore, we show here that the  
41 role of phototrophic microbes in peatlands is underestimated, and that our current understanding of  
42 the peatland C dynamics lacks a significant piece of the puzzle.

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53 **Summary**

54 Phototrophic microbes are widespread in soils but their contribution to soil carbon (C) uptake remains  
55 underexplored in most terrestrial systems, including C-accreting systems such as peatlands. Here, by  
56 means of metabarcoding and ecophysiological measurements, we examined how microbial  
57 photosynthesis and its biotic (e.g., phototrophic community structure, biomass) and abiotic drivers  
58 (e.g., *Sphagnum* moisture, light intensity) vary across peatland microhabitats. Using a natural gradient  
59 of microhabitat conditions from pool to forest, we show that the structure of phototrophic microbial  
60 communities shifted from a dominance of eukaryotes in pools to prokaryotes in forests. We identified  
61 five groups of co-occurring phototrophic operational taxonomic units (OTUs) with specific  
62 environmental preferences across the gradient. Along with such structural changes, we found that  
63 microbial C uptake was the highest in the driest and shadiest microhabitats. This study renews and  
64 improves current views on phototrophic microbes in peatlands, as the contribution of microbial  
65 photosynthesis to peatland C uptake has essentially been studied in wet microhabitats.

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## 78 **Introduction**

79 The functioning of ecosystems across the Earth is inextricably linked with the existence of complex and  
80 diverse microbial communities (Falkowski *et al.*, 2008). Microbes are omnipresent in soils, where they  
81 show a broad functional diversity and drive key ecosystem processes such as carbon (C) cycling (e.g.  
82 Bardgett and van der Putten, 2014; Fierer, 2017). A better understanding of soil microbial communities  
83 across the globe is therefore crucial for modelling and predicting ecosystem functioning and global  
84 biogeochemistry (Bardgett and van der Putten, 2014; Fierer, 2017; Crowther *et al.*, 2019).

85         Until recently, most studies focused on the biogeographic patterns (e.g. Fierer and Jackson,  
86 2006; Martiny *et al.*, 2006; Chu *et al.*, 2020) and the functional role of heterotrophic microorganisms  
87 in soil biogeochemical cycles (e.g. Bates *et al.*, 2013; Tedersoo *et al.*, 2014; Karimi *et al.*, 2018),  
88 overlooking other life forms. Heterotrophic microbes indeed drive important ecosystem processes  
89 such as the recycling of essential elements (Gougoulas *et al.*, 2014; Wagg *et al.*, 2014). Aside from  
90 heterotrophs, phototrophic microbes are also omnipresent and widely distributed in soils (Cano-Díaz  
91 *et al.*, 2020; Oliverio *et al.*, 2020), and therefore potentially contribute to biogeochemical cycles too  
92 (Elbert *et al.*, 2012). They provide organic matter to soils through the fixation of atmospheric C by  
93 photosynthesis. Hence, they can balance the catabolic fluxes of C and actively take part in the global C  
94 cycle (Elbert *et al.* 2012). However, despite their potential global importance, soil phototrophic  
95 microbes remain essentially studied in systems with few or no plants, where they dominate the C  
96 fixation (e.g. drylands, glacial systems; Anesio *et al.*, 2009; Maier *et al.*, 2018; Chen *et al.*, 2020). They  
97 are thus largely overlooked in vegetated ecosystems, including the ones that play a key role in the  
98 global C cycle such as peatlands (Schimel *et al.*, 1994; Bardgett *et al.*, 2008; Yu *et al.*, 2011).

99         Phototrophic microbes are very abundant in northern peatlands (Hamard *et al.*, 2021) where  
100 the dominant plants, *Sphagnum* mosses, provide a suitable habitat for microbial communities (Gilbert  
101 and Mitchell, 2006; Bragina *et al.*, 2012), and moist conditions foster phototrophic growth (Starks *et*  
102 *al.*, 1981). Phototrophic microbes live either freely in pore water or in *Sphagnum* dead cells (Gilbert *et*  
103 *al.*, 1998b) and show a tremendous diversity of taxa and life styles (Gilbert and Mitchell, 2006a; Lara

104 *et al.*, 2011; Hamard *et al.*, 2021). Not all phototrophic microbes in peatlands are strict autotrophs.  
105 They rather form a continuum between autotrophy and heterotrophy, with the presence of diverse  
106 taxa including strict photoautotrophs (e.g. protists such as Chlorophyta), mixotrophs (i.e. organisms  
107 combining photoautotrophy and heterotrophy such as some Ciliophora, Lobosa, many  
108 Alphaproteobacteria or Cyanobacteria), or photoheterotrophs species (e.g. Chloroflexia) (Jassey *et al.*,  
109 2015; Nowicka and Kruk, 2016; Hamard *et al.*, 2021). Only a handful studies suggest that phototrophic  
110 microbes fix significant amounts of atmospheric C in northern peatlands (Goldsborough and Robinson,  
111 1996; Gilbert *et al.*, 1998b; Wyatt *et al.*, 2012; Jassey *et al.*, 2015; Hamard *et al.*, 2021). The most recent  
112 studies estimated the contribution of phototrophic microbes to approximately 10% of total peatland  
113 C uptake (Hamard *et al.*, 2021), highlighting the crucial importance of these organisms to the peatland  
114 C cycle. However, these estimations remain partial as they do not embrace the diversity of peatland  
115 microhabitats, which strongly limits our understanding of the importance of phototrophic microbes  
116 into peatland C dynamics.

117 Northern peatlands exhibit a mosaic of various microhabitats, characterised by different  
118 vegetation communities (Kvillner and Sonesson, 1980). Microhabitat changes are underpinned by  
119 different environmental factors shaping plant assemblages, such as microtopography, distance to bog-  
120 pools, water table depth or nutrient availability (Rydin *et al.*, 2013). For instance, peatlands  
121 microhabitats range from wet to dry as the distance to bog-pools and to water table increases, with  
122 consequences on *Sphagnum* and vascular plant assemblages. Denser *Sphagnum* species are found far  
123 from the water table, while vascular plants shift from graminoids to shrubs and eventually to tree  
124 species as distance from the water table increases (Kvillner and Sonesson, 1980; Gunnarsson, 2005).  
125 These changes in the vegetation cover generate additional environmental gradients, for instance light  
126 gradients as the light intensity reaching the ground surface decreases as the vascular plant layer  
127 increases.

128 We also know that peatland microbial communities are sensitive to plant diversity and  
129 community composition (Jassey *et al.*, 2014; Robroek *et al.*, 2015). This suggests a turnover of microbial

130 phototrophic species across microhabitats. As microbial photosynthetic rates in peatlands strongly  
131 depend on the phototrophic community structure (Hamard *et al.*, 2021), these structural changes may  
132 alter phototrophic C fixation rates among peatland microhabitats. Therefore, studying phototrophic  
133 communities and their C uptake along different peatland microhabitats would bring relevant  
134 information on the role of phototrophic microbes in peatland C dynamics.

135         In this study, we explored the community structure and the photosynthetic activity of  
136 phototrophic microbes along a gradient of microhabitats in a boreal peatland, the Männikjärve  
137 complex (Estonia). This gradient began from bog-pools, and included the following six microhabitats  
138 that came in succession as the distance to bog-pools increased: pools, *Sphagnum*-pools (*Sphagnum*  
139 growing in the shallow edge of the pools), lawns, hummocks, wooded-hummocks and forested-  
140 ditches. Specifically, we studied the relationships between the structure, the abundance and the C  
141 fixation rate of phototrophic microbial communities along three transects covering the six  
142 microhabitats (6 microhabitats x 3 transects = 18 plots in total; Supplementary Fig. S1). In each plot,  
143 we monitored the phototrophic microbial community composition using 23S rDNA gene sequencing  
144 (Sherwood and Presting, 2007), their absolute abundance and photosynthetic properties  
145 (photosynthetic efficiency, photosynthetic pigments content and C fixation rates). We hypothesized (i)  
146 that microbial photosynthetic activity varies between microhabitats, and (ii) that this variation is the  
147 consequence of species turnover along with micro-environmental conditions of the microhabitats. In  
148 particular, we expected to find higher photosynthetic rates in microhabitats close to the water table,  
149 where water and light availability are not limited and allow favourable conditions for phototrophic  
150 growth.

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## 156 Results

### 157 Description of the microhabitats

158 The depth to the water table increased from pools (open water) to wooded-hummocks (> 50cm), and  
159 was only 4 cm in the forested-ditches (Table 1). While all microhabitats (except pools) were dominated  
160 by *Sphagnum* mosses, the nature of the vascular plant layer shifted from a dominance of graminoids  
161 in lawns, to shrubs and trees in wooded-hummocks and forested-ditches. These variations of the  
162 vascular plant layer induced a light gradient at the surface of microhabitats, with only 50% of  
163 photosynthetically active radiations reaching the ground in forested-ditches, versus 90% in lawns and  
164 100% in pools ( $F_{(4,11)} = 7.02, P = 0.013$ ). Further, *Sphagnum* species differed in each microhabitat except  
165 in hummocks and wooded-hummocks where *S. fuscum* dominated. Physical characteristics of the  
166 *Sphagnum* mat varied along with *Sphagnum* species, with, in particular, a higher *Sphagnum* water  
167 content in the less dense *Sphagnum* species present in *Sphagnum*-pools and forested-ditches  
168 ( $F_{(4,11)} = 10.62, P = 0.004$ ).

169

170 Preferred position Table 1

171

### 172 Community structure and environmental preferences of phototrophic microbes

173 Phototroph-specific 23S metabarcoding analyses revealed the presence of 129 phototrophic  
174 operational taxonomic units (OTUs), gathering a total of 1 116 phototrophic microbial sequences in  
175 the 18 plots. Among the 129 OTUs, 68 were associated to phototrophic micro-eukaryotes, 60 to  
176 phototrophic prokaryotes, and one unassigned. We identified nine phyla of phototrophic microbes,  
177 out of which seven were eukaryotic and two were prokaryotic (Supplementary Fig. S2). On average,  
178 phototrophic microbial communities were dominated by one eukaryotic phylum, Chlorophyta (relative  
179 abundance of 34% on average), and two prokaryotic phyla, Cyanobacteria (33%) and Proteobacteria  
180 (15%). Trebouxiophyceae, Cyanobacteria and Alphaproteobacteria were the main representative class  
181 of these respective phyla on average (Supplementary Fig. S2; Supplementary Table S1).



182 We found important discrepancies in the composition and the structure of phototrophic  
183 microbial communities along the microhabitats. First, the OTU richness (Chao1 index) of the  
184 phototrophic community was altered along the microhabitats ( $F_{(5, 12)} = 3.81$ ,  $P = 0.034$ ), with a richness  
185 significantly lower in the forested-ditches (5 OTUs on average) compared to the other microhabitats  
186 (28 OTUs on average) (Fig. 1A). Second, the OTU diversity (Shannon index) showed a similar pattern  
187 with a lower diversity found in the forested-ditches compared to other microhabitats ( $F_{(5, 12)} = 6.92$ ,  
188  $P = 0.005$ ; Fig. 1A). Third, in each microhabitat, 45% of OTUs on average were specific of the site  
189 (Supplementary Fig. S3). This proportion was the highest in the pools where 80% of the OTUs were  
190 microhabitat specific ( $F_{(5, 12)} = 5.43$ ,  $P = 0.011$ ). On one hand, wooded-hummocks, hummocks and  
191 lawns, and on the other hand, pools and *Sphagnum*-pools were the microhabitats that shared the  
192 highest number of OTUs in common (Supplementary Fig. S3).

193

194 Preferred position Figure 1

195

196 A permutational multivariate analysis of variance revealed that the community structure of  
197 phototrophic microbes strongly depended on the microhabitats ( $R^2 = 0.63$ ,  $P < 0.001$ ). At the domain  
198 level, we found a shift in the phototrophic community structure between the different microhabitats  
199 ( $F_{(5,12)} = 27.72$ ,  $P < 0.001$ ; Fig. 1B). Whilst micro-eukaryotes strongly dominated the phototrophic  
200 community in pools (95.2%), the relative abundance of phototrophic prokaryotes gradually increased  
201 along the microhabitat gradient, and prokaryotes strongly dominated in forested-ditches (98.4%). In  
202 parallel with these changes, the number of micro-eukaryotic OTUs strongly decreased, from 32 OTUs  
203 in pools to three OTUs in forested-ditches. Furthermore, we identified five clusters of highly co-  
204 occurring phototrophic OTUs (Fig. 2A). They evidenced a clear transition from the pools to the  
205 forested-ditches ( $R^2 = 0.53$ ,  $P < 0.001$ ; Fig. 2B). Cluster 1 and 2 were specific of pools and *Sphagnum*-  
206 pools, being almost absent in the other microhabitats. Cluster 1 dominated the pools (65% of the  
207 phototrophic relative abundance), while cluster 2 dominated the *Sphagnum*-pools microhabitats

208 (73%). Cluster 3 showed a low abundant on both extremes of the transects (3% in pools and forested-  
209 ditches), but increased toward the middle of the transect to dominate in lawns (87%). Cluster 5  
210 gradually increased from the pools (0%) to the forested-ditches (97% while Cluster 4 was present from  
211 *Sphagnum*-pools to wooded-hummocks and dominated in hummocks (85%).

212

213 Preferred position Figure 2

214

215 Each cluster grouped OTUs belonging to various taxa, meaning that co-occurring OTUs formed  
216 phylogenetically complex communities (Supplementary Fig. S4; Supplementary Table S2). Unifrac  
217 distance showed that the cluster 1 had the highest phylogenetic distance from the other clusters  
218 (Unifrac distance of 0.81 with the other clusters on average), while the clusters 4 and 5 were the most  
219 similar (distance of 0.52 between them; Supplementary Table S3). In terms of relative abundance,  
220 Chlorophyta and Proteobacteria were well-represented in the five clusters, contributing on average to  
221 nearly 66% of the cluster sequences. Chlorophyta especially dominated in the cluster 3 (78%), while  
222 Proteobacteria dominated in the cluster 1 and 5 (35% and 56%, respectively). Cyanobacteria,  
223 Streptophyta and Ochrophyta constituted respectively a large part of the cluster 1, 2 and 4 (28%, 28%  
224 and 42%, respectively; Supplementary Table S2). In line with this uneven distribution of phyla, only few  
225 OTUs dominated the different clusters (Fig. 2A). Two OTUs belonging to *Nostocaceae* (Cyanobacteria)  
226 dominated the clusters 4 and 5 (28% and 22% respectively), while an OTU belonging to *Mesotaenium*  
227 (Streptophyta) accounted for 21% of the cluster 2. One OTU belonging to *Chlorella* dominated the  
228 cluster 3 with 30% of the sequences.

229 Environmental parameters affected the relative abundance of the clusters 3, 4 and 5 (Fig. 3).

230 The cluster 3 was negatively related to *Sphagnum* water content ( $F_{(1,16)} = 4.55$ ,  $P = 0.050$ ), while the  
231 cluster 5 was positively related to shadow ( $F_{(1,16)} = 43.78$ ,  $P < 0.001$ ). The cluster 4 was negatively  
232 related to *Sphagnum* water content ( $F_{(1,16)} = 8.03$ ,  $P = 0.014$ ) and positively related to water table depth  
233 ( $F_{(1,16)} = 15.46$ ,  $P = 0.001$ ).

234

235 Preferred position Figure 3

236

### 237 **Phototrophic metabolic rates and C fixation**

238 We quantified the chlorophyll *a* (chl<sub>a</sub>) content and the maximum electron transport rate (ETR) per unit  
239 of phototrophic biomass (μgC) to estimate the metabolic and photosynthetic rates of phototrophic  
240 microbes. The chl<sub>a</sub> content per biomass of phototrophic microbes significantly differed between the  
241 microhabitats ( $F_{(5,12)} = 4.00$ ,  $P = 0.034$ ; Fig. 4A), and was the lowest in pools and lawns ( $8.8 \pm 6.1$  and  
242  $7.7 \pm 2.9$  ng chl<sub>a</sub> per μgC, respectively), while it was nearly five and four times higher in hummocks and  
243 wooded-hummocks ( $44.1 \pm 30.6$  and  $28.8 \pm 10.4$  ng chl<sub>a</sub> per μgC, respectively). The maximum ETR (Em)  
244 was reached for low light intensities, with an average PAR of  $85 \pm 24$  μmol.m<sup>-2</sup>.s<sup>-1</sup> (Supplementary Fig.  
245 S5). The maximum ETR followed the same pattern than the chl<sub>a</sub> content, being the lowest in pools and  
246 lawns ( $67.3 \pm 35.5$  and  $1316 \pm 376$  fmol e<sup>-</sup>.s<sup>-1</sup>.μgC<sup>-1</sup>, respectively) and the highest in hummocks and  
247 wooded-hummocks ( $6290 \pm 3194$  and  $7060 \pm 2666$  fmol e<sup>-</sup>.s<sup>-1</sup>.μgC<sup>-1</sup>, respectively) ( $F_{(5,12)} = 24.93$ ,  
248  $P < 0.001$ ; Fig. 4B).

249

250 Preferred position Figure 4

251

252 We then quantified the community-weighted mean biomass per cell (μgC.cell<sup>-1</sup>) and the total  
253 biomass of phototrophic microbes per cubic centimetre of *Sphagnum* (μgC.cm<sup>-3</sup>), allowing an  
254 estimation of microbial photosynthetic and C fixation rates at the microhabitat level. We observed  
255 variations in the phototrophic biomass between microhabitats. The phototrophic biomass per  
256 phototrophic cell was on average 2.5 times lower in emerged *Sphagnum* than in pools ( $F_{(5,12)} = 9.59$ ,  
257  $P = 0.002$ ; Supplementary Fig. S6), while the total biomass was the highest in lawns, hummocks and  
258 wooded-hummocks ( $1.67 \pm 0.47$  μgC.cm<sup>-3</sup> on average) and the lowest in forested-ditches ( $0.26 \pm 0.11$   
259 μgC.cm<sup>-3</sup>) ( $F_{(5,12)} = 3.31$ ,  $P = 0.041$ ; Fig. 4C). Concerning C fixation rates, phototrophic microbes fixed

260  $174 \pm 41 \text{ ngCO}_2\text{.h}^{-1}\text{.cm}^{-3}$  on average along the microhabitat gradient. However large discrepancies could  
261 be observed between microhabitats ( $F_{(5,12)} = 29.66$ ,  $P < 0.001$ ; Fig. 4d). Microbial C fixation was the  
262 highest in hummocks and wooded-hummocks ( $350 \pm 93 \text{ ngCO}_2\text{.h}^{-1}\text{.cm}^{-3}$  on average), intermediate in  
263 *Sphagnum*-pools and lawns ( $161 \pm 64 \text{ ngCO}_2\text{.h}^{-1}\text{.cm}^{-3}$ ), and the lowest in pools and forested-ditches ( $11$   
264  $\pm 7 \text{ ngCO}_2\text{.h}^{-1}\text{.cm}^{-3}$ ).

265

### 266 **Drivers of microbial photosynthesis**

267 We built a path analysis (structural equation model, SEM) that explained 85% of the total variation of  
268 microbial C fixation at the microhabitat level (Fig. 5). First, the SEM showed that microbial C fixation  
269 was directly mediated by the total biomass (path = 0.62) and the photosynthetic rates of phototrophs  
270 per unit of biomass (path = 0.75). Second, the SEM highlighted the importance of micro-environmental  
271 conditions in determining microbial C fixation through cascading effects: environmental parameters  
272 affected phototrophic community structure that in turn affected both total biomass and  
273 photosynthetic rates of phototrophic microbes. More precisely, we found that shadow was a good  
274 predictor of the relative abundance of the cluster 5 (path = 0.91), while *Sphagnum* density was a good  
275 predictor of the cluster 4 (path = 0.79). Clusters 4 and 5 altered the diversity of the phototrophic  
276 microbial community (path = -1.06 and path = -0.92, respectively), which in turn influenced the  
277 photosynthetic rates of phototrophic microbes (path = 0.95) and their total biomass (path = 0.56). In  
278 particular, additional linear models confirmed that the total biomass of phototrophs was positively  
279 correlated with their diversity (Shannon index;  $F_{(1,16)} = 5.18$ ,  $P = 0.037$ ,  $r = 0.44$ ). Additional linear  
280 models further showed correlations between the relative abundance of specific OTUs and  
281 phototrophic biomass and photosynthetic rates. For instance, we found a positive correlation between  
282 a cyanobacteria from the *Nostoc* genus, dominant in the cluster 4, and the photosynthetic rates of  
283 phototrophs ( $F_{(1,16)} = 6.94$ ,  $P = 0.018$ ,  $r = 0.51$ ). We also found a negative correlation between the  
284 dominant OTU of the cluster 5, another cyanobacteria from the *Nostoc* genus, and the total biomass  
285 of phototrophic microbes ( $F_{(1,16)} = 9.21$ ,  $P = 0.008$ ,  $r = -0.57$ ).

286

287 Preferred position Figure 5

288

289

290

## 291 **Discussion**

292 Recent studies have suggested that phototrophic microbes play an important role in the C cycle of  
293 northern peatlands, fixing significant amounts of atmospheric C through photosynthesis (Wyatt *et al.*,  
294 2012; Hamard *et al.*, 2021). However, the role of microhabitat diversity in supporting C fixation by  
295 photosynthetic microbes remained unexplored. Here, by means of metabarcoding and eco-  
296 physiological analyses, we show that the community structure of phototrophic microbes change  
297 drastically among peatland microhabitats, with consequences on microbial C fixation rates. In  
298 particular and contrary to our hypothesis, we reveal that the most productive areas were neither the  
299 moistest nor the open microhabitats, but instead the relatively dry and closed microhabitats found on  
300 hummocks and wooded-hummocks.

301

## 302 **Phototrophic microbial diversity and community structure**

303 The use of the 23S marker for metabarcoding analysis, which allows for a simultaneous study of both  
304 eukaryotic and prokaryotic phototrophic microbes, showed that the structure of phototrophic  
305 microbial communities was highly variable among peatland microhabitats. While several studies  
306 revealed a high spatial heterogeneity of microbial communities in different peatlands microhabitats  
307 (Oloo *et al.*, 2016; Küttim *et al.*, 2017; Heger *et al.*, 2018; Chroňáková *et al.*, 2019), we showed here  
308 that such heterogeneity also exists for phototrophic microbes. In total we identified 129 phototrophic  
309 OTUs distributed in nine phyla. This richness is lower than the one found in peatland lawns along a  
310 3000 km latitudinal gradient (351 OTUs and 11 phyla with 16S/18S metabarcoding; Hamard *et al.*,  
311 2021) or than the one found in a peatland along a gradient of drainage (794 OTUs with 18S

312 metabarcoding; Heger *et al.*, 2018). The 23S metabarcoding analyses should perform better than the  
313 18S or 16S analyses to reveal the presence of phototrophic OTUs (Marcelino and Verbruggen, 2016).  
314 We found, using additional metabarcoding analyses, that 16S/18S gave comparable perceptions of the  
315 phototrophic community structure in our samples than 23S (Supplementary Fig. S7). Therefore, the  
316 low number of phototrophic OTUs in our study may rather come from a low sequencing depth.  
317 Rarefaction curves revealed nevertheless a near saturation at the microhabitat level (Supplementary  
318 Fig. S8), which indicates that a majority of the diversity of the six microhabitats was captured. Although  
319 our 23S metabarcoding analyses did not give an exact picture of the phototrophic community, it  
320 remains an interesting proxy to study phototrophic community changes along peatland microhabitats.

321 Our results showed that phototrophic microbial communities experienced strong structural  
322 variations along the few dozen meters forming the microhabitat gradients. In particular, a key finding  
323 of this study was the drastic shift in the phototrophic prokaryote to eukaryote ratio, which strongly  
324 increased from the pools toward the forested-ditches. Such variations emphasized the importance of  
325 micro-environmental conditions in the structure of phototrophic microbial communities, and pointed  
326 out that phototrophic prokaryotes and eukaryotes responded in antagonistic ways to changes in  
327 environmental conditions. More particularly, we identified five clusters of co-occurring OTUs, forming  
328 phylogenetically complex communities and grouping both eukaryotic and prokaryotic OTUs.  
329 Environmental conditions, mainly water and light availability, influenced the proportions of the  
330 clusters in the phototrophic community, and led to the prokaryotic to eukaryotic shift. This result is in  
331 line with another study, where a higher sensitivity of prokaryotes (Cyanobacteria) to high solar and UV  
332 radiations led to a partitioning between phototrophic prokaryotes and eukaryotes in a lake (Winder,  
333 2009).

334 Besides water and light availability, other environmental factors that we did not take into  
335 account likely intervened in the structural variations of phototrophic communities along the  
336 microhabitat gradient. For instance, differences in the nutrient status in the different microhabitats  
337 could drive species turnovers as calcium content affects phototrophic eukaryotes (Heger *et al.*, 2018)

338 and/or nitrogen supply increases the abundance of Cyanobacteria compared to microalgae in  
339 peatlands (Gilbert *et al.*, 1998a). As the vegetation changes across the gradient, it is also likely that pH  
340 changes between pools and forested-ditches, which could feedback on phototrophic microbial  
341 communities (Heger *et al.*, 2018). Furthermore, the abundance of predators among microhabitats  
342 could also be an important factor to take into account. Indeed, many algivorous protozoa and  
343 metazoan are sensitive to water table depth (Lamentowicz and Mitchell, 2005; Jassey *et al.*, 2011).  
344 Predator shifts along microhabitats could induce different top-down controls on phototrophic  
345 communities and favour some phototrophic taxa, as shown in marine systems where high predation  
346 rates induce a selection toward smaller phototrophic cells and favour prokaryotic abundance  
347 (Tamigneaux *et al.*, 1995). Finally, the plant community composition may also influence phototrophic  
348 communities (Heger *et al.*, 2018; Hamard *et al.*, 2021). In temperate forests, needle litter favour  
349 Chlorophyta while leaf debris favour Cyanobacteria (Maltsev *et al.*, 2017; Maltsev and Maltseva, 2018).  
350 In our study we observed similar patterns with more Chlorophyta in the pine-covered wooded-  
351 hummocks, and more Cyanobacteria in the forested-ditches, where birch and deciduous trees were  
352 also present. However, this pattern could also be related to water regime and nutrient status, as  
353 vegetation changes are underpinned by shifts in nutrients, water and light availability as well (Song *et*  
354 *al.*, 2019).

355 Another key result of this study is that phototrophic communities in pools were more dissimilar  
356 compared to the phototrophic communities from *Sphagnum* mosses. Several taxa such as Rhodophyta  
357 or Cryptophyta and a high proportion of OTUs were specific to the pools, while different phototrophic  
358 taxa were found in *Sphagnum* habitats. We acknowledge that we sampled only the surface water of  
359 the pools, which might lead to an underestimation of the phototrophic richness. However, bog-pools  
360 are particular systems with very dark water that limit phototrophic growth deep in the water column  
361 (Lew *et al.*, 2016; Umanskaya *et al.*, 2020). The difference between pools and *Sphagnum* phototrophic  
362 communities nevertheless reveals that a sampling of free or pore water is not sufficient to capture the  
363 full composition of the phototrophic community in *Sphagnum*, most probably because many microbes

364 live in close association with mosses (Gilbert *et al.*, 1998b; Bragina *et al.*, 2012). Our results further  
365 suggest that *Sphagnum* mosses are important in structuring phototrophic microbial communities, even  
366 if the *Sphagnum* species differ. Such close association could derive from similar physical properties  
367 provided by *Sphagnum* mosses (e.g. hyaline dead cells), but also from specific chemical interactions  
368 between *Sphagnum* mosses and microbes. Indeed, recent findings evidenced the strong linkages  
369 between *Sphagnum* chemicals and phototrophic microbes in peatlands (Sytiuk *et al.*, 2021).

370         Despite alterations in the phototrophic community structure along the microhabitats, OTU  
371 richness and diversity remained similar in most of the microhabitats except in the forested-ditches  
372 (lower richness and diversity). Especially, we found a strong decrease in the number of micro-  
373 eukaryotic OTUs in the forested-ditches (three OTUS while there were 32 in the pools), which suggests  
374 that only few phototrophic micro-eukaryotic species were able to live under the environmental  
375 conditions present in the forested-ditches. On the contrary, the number of prokaryotic OTUs stayed  
376 the same. This could be link with the fact that Cyanobacteria can establish symbiosis with other plants  
377 (Black and Osborne, 2004) or have a high plasticity in their photosynthetic machinery (Bengtsson *et*  
378 *al.*, 2018), which could help them to overcome the light limitation of forested-ditches microhabitats.  
379 As microbial richness and diversity drive soil functions (e.g., Nannipieri *et al.*, 2003; Coleman and  
380 Whitman, 2005; Delgado-Baquerizo *et al.*, 2017), this result suggested that phototrophic functions  
381 were probably not affected along the microhabitat gradient at the exception of forested-ditches, due  
382 to functional redundancy (Louca *et al.*, 2018). However, the measurements of microbial  
383 photosynthetic rates among microhabitats rebutted such hypothesis.

384

### 385 **Spatial changes in microbial C fixation rates**

386 We observed higher C fixation rates in hummocks and wooded-hummocks. By comparison, C fixation  
387 rates were much higher than in the other microhabitats, being for instance nearly four time higher  
388 than in lawns and 17 times higher than in forested-ditches. While previous studies showed the  
389 importance of phototrophic microbes in *Sphagnum* lawns, where they contributed to 10% of C uptake



390 (Wyatt *et al.*, 2012; Hamard *et al.*, 2021), we show here that phototrophic microbes had even a higher  
391 primary productivity in hummocks and wooded-hummocks. This striking result is important because it  
392 shows that phototrophic communities are more productive in microhabitat distant from the water  
393 table and under dense plant cover darkening the incident light. In fact, we found that phototrophic  
394 microbes were well adapted to these environments, reaching their maximal C fixation rates for low  
395 light intensities (under  $115 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ ).

396 Our structural equation model (SEM) revealed the drivers of microbial C fixation rates. In  
397 particular, our SEM showed that the community structure drove microbial C fixation through a dual  
398 effect. First, the community structure affected the total biomass of phototrophs, which can be seen as  
399 the photosynthetic workforce. Second, the community structure affected the averaged metabolic  
400 rates of phototrophs, which can be seen as the photosynthetic potential. The combination of both  
401 photosynthetic workforce and potential determined the microbial C fixation rates.

402 Overall, the total biomass of phototrophic microbes positively correlated with phototrophic  
403 diversity, denoting resource limited environments (Bastida *et al.*, 2021). The total biomass of  
404 phototrophic microbes was further intimately correlated with specific OTUs. Only few studies explored  
405 the role of specific phototrophic species in driving phototrophic biomass, yet it has been showed that  
406 colonial species could increase the total phototrophic biomass in oceanic environments (Finkel *et al.*,  
407 2010). In our study, we found a negative correlation between one *Nostoc* cyanobacteria and the total  
408 phototrophic biomass. This was somehow unexpected because Cyanobacteria species from the *Nostoc*  
409 genus often form colonies in peatlands (e.g., Elster *et al.*, 2012), and are able to fix nitrogen, a nutrient  
410 known to promote algal production (Piorreck *et al.*, 1984). However, some cyanobacteria, and more  
411 particularly, *Nostoc* species can also produce toxic and allelopathic compounds (Nowruzi *et al.*, 2012),  
412 thus potentially limiting the development of other phototrophs and competitors, as shown in  
413 freshwater ecosystems (e.g., Carmichael, 1997; Leflaive and Ten-Hage, 2007).

414 The metabolic rates of phototrophic microbes depended on phototrophic community  
415 structure. While metabolic rates negatively correlated with the relative abundance of cluster 3 and

416 positively correlated with cluster 4, we did not find significant correlations with the relative abundance  
417 of specific phototrophic taxa. This shows that metabolic rates are not driven by a little number of taxa  
418 or OTUs but rather by an association of multiple OTUs. In particular, as fluorescence measurements of  
419 photosynthetic efficiency often underestimate cyanobacterial photosynthetic rates (Schuurmans *et*  
420 *al.*, 2015), we expected to find a negative correlation between metabolic rates and the proportion of  
421 cyanobacteria in the phototrophic community. We however did not find such bias in our study  
422 (Supplementary Fig. S9). Instead, we found higher photosynthetic rates in hummocks and wooded-  
423 hummocks, where phototrophic cells were the smallest; a results in line with a general observation  
424 showing that metabolic rates per biomass unit are negatively correlated with body size (Finkel *et al.*,  
425 2010).

426

#### 427 **Consequences for peatland functioning**

428 These differences in the amount of microbial C fixation between microhabitats might profoundly affect  
429 their C dynamics. Phototrophic microbes release a great part (40%) of the C they fix as labile exudates  
430 and hence promote dissolved organic C and heterotrophic respiration in peatlands (Wyatt and  
431 Turetsky, 2015). It is therefore highly probable that the balance between algal and plant C input  
432 determine the chemical composition of organic matter and eventually its fate in the ecosystem, as it  
433 is the case in several soil systems (Kögel-Knabner, 2002). In hummocks and wooded-hummocks,  
434 phototrophic microbes are more productive, but at the opposite, *Sphagnum* species living in these  
435 microhabitats are less productive than the one living in lawns (Gunnarsson, 2005; Küttim *et al.*, 2020).  
436 This indicates that phototrophic microbes contribute more to C uptake in hummocks and wooded-  
437 hummocks than in other microhabitats, and subsequently affect the C dynamics of these  
438 microhabitats. Further research would be needed to explore this hypothesis and to quantify the net  
439 effects of microbial C fixation on peatland C fluxes. Indeed, the fate of phototrophic-fixed C is still  
440 virtually unknown, and species turnover among microhabitats may influence this dynamic. Further,  
441 the role of phototrophic microbes in peatland is certainly not limited to the contribution to C fixation.

442 Phototrophic microbes could affect peatland functioning in many other ways, for instance by fixing  
443 nitrogen (Granhall and Selander, 1973), or by structuring global microbial communities, either as food  
444 for predators (Seppey et al. 2017 SBB), through allelochemical interactions (Sytiuk et al. 2021), or  
445 through competition as recently suggested (Hsu and Moeller, 2021).

446

447

#### 448 **Conclusion**

449 Microhabitats are important drivers of the structure of heterotrophic microbial communities in  
450 peatlands with consequences on the peatland C dynamics (Robroek *et al.*, 2015; Barreto and Lindo,  
451 2018). Microhabitats are also primordial in the structure of phototrophic communities, as structural  
452 shifts are associated with important alterations of microbial primary activity. Microbial C fixation  
453 indeed experience huge variations along short microhabitats gradients, with an increase of up to 17  
454 times over few decametres. As the structure of peatland phototrophic communities changes  
455 throughout the year (Reczuga *et al.*, 2020) it is probable that the microbial C fixation could change in  
456 the different microhabitats according to the sampling season. Despite this limitation, our study shows  
457 that peatlands are complex ecosystems where phototrophic microbes might play a significant role. Our  
458 findings further highlight that the current understanding of peatland C dynamics is lacking a significant  
459 piece. A better understanding of the role of phototrophic microbes in peatlands, and to the whole  
460 diversity of peatlands microhabitats is urgently needed to improve our predictions of the peatland C  
461 balance under future global changes.

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## 468 **Methods**

### 469 **Study site and sampling**

470 The Männikjärve ombrotrophic bog is situated in central Estonia (58°52'30 N, 26°15'04 E, 78 m a.s.l.)  
471 and is representative of northern (or boreal) peatland types (Masing, 1984). It has a well-developed  
472 microhabitat complex, structured along gradients extending from pools to wooded-hummocks. The  
473 margins of the Männikjärve bog are colonized by a wooded bog and a forest (Masing, 1982; Lode *et*  
474 *al.*, 2017). In early October 2019, we selected three different transects in the bog that were related  
475 with three different pools (Supplementary Fig. S1). Getting some distance from the pools, we set plot  
476 locations that were representative of five different microhabitats: pools (Po), *Sphagnum*-pools (Sp,  
477 corresponding to *Sphagnum* growing in the shallow edge of the pools), lawns (La), hummocks (Hu) and  
478 wooded-hummocks (Wh). On top of this, we set three other plot locations in the ditches of the margin  
479 forest (forested-ditches, Fd; Supplementary Fig. S1). The plots had an area of 20 cm per 20 cm. Except  
480 for the pools (water), we made an inventory of the vegetation of the different microhabitats. They  
481 were all dominated by *Sphagnum* species, that formed a continuous *Sphagnum* carpet. In each plot,  
482 we measured the light intensity at the ground surface. Measurements were done in comparable  
483 conditions and at the same time. We also collected *Sphagnum* in PVC-tubes (diameter 8.5 cm; height  
484 6 cm), and weighted it fresh and dry in order to determine the *Sphagnum* mat density and the  
485 *Sphagnum* water content (pools excepted). Further, we sampled surface water in the pools and apical  
486 part of *Sphagnum* mosses (0-3 cm from the capitulum) in other plots for analysis of microbial diversity,  
487 abundance and biomass as well as photosynthetic rate.

488

### 489 **DNA extraction and metabarcoding**

490 We sampled three capitula of *Sphagnum* with sterile equipment from each plot, cut them into small  
491 pieces and placed them into sterile tubes containing 3 ml of Lifeguard (Qiagen). For pools, we filtered  
492 5 ml of pool surface water on 0.25 µm filters using sterile equipment, and we placed the filters into 3  
493 ml of Lifeguard. For each sample, DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen) and

494 following the manufacturer's instructions. The DNA was eluted in 70 µl of final solution and its  
495 concentration in each extract was quantified using a Nanodrop ND-1000 spectrophotometer. Extracts  
496 were stored at -80°C before proceeding to DNA amplification.

497 To investigate photosynthetic microorganisms, we used the 23S rDNA metabarcoding marker,  
498 allowing the replication of a DNA fragment of 365 base pair (Presting, 2006). The pair of primers was  
499 tagged with Illumina adapter sequences. PCRs were conducted in a total volume of 50 µl containing 13  
500 µl of Mastermix AmpliTaq Gold (Thermofisher), 1µl of each primer (0.4 µM final concentration), 35 ng  
501 of DNA (up to 10 µl) and completed to 50 µl with DNA-free water. The primers' sequences and the PCR  
502 programs used are presented in Supplementary Table S4. The sequencing was performed by the GeT-  
503 PlaGe platform (Genotoul, Toulouse, France) with Illumina MiSeq technology and using the V3  
504 chemistry (2x250 bp).

505 Paired-end fastq sequences were analysed using the FROGS pipeline (Find Rapidly Operational  
506 Taxonomic Units Galaxy Solution) on the Galaxy platform (Escudié *et al.*, 2018). Paired-end reads were  
507 merged using VSEARCH (10% of mismatch; (Rognes *et al.*, 2016)). Sequences were filtered based on  
508 their length and primer mismatches were removed. Sequences were then dereplicated and clustered  
509 into OTUs using the SWARM clustering method with an aggregation distance of three (Mahé *et al.*,  
510 2014). Chimera were identified and removed using VSEARCH. Filters were then applied to remove  
511 singletons. OTUs were assigned at different taxonomic levels using Blast. We used the Silva 138  
512 database (Quast *et al.*, 2013) for the 23S assignation. After these steps, the 18 samples contained  
513 418 618 curated reads in total, belonging to 589 phototrophic OTUs associated to plants and  
514 phototrophic microbes.

515 Further analyses were conducted with the Phyloseq R package (McMurdie and Holmes, 2013).  
516 In particular, we removed plant-associated sequences and kept only microbial phototrophic OTUs. For  
517 the 18 samples, we obtained 26 981 microbial sequences belonging to 262 phototrophic microbial  
518 OTUs. To alleviate sequencing artefact, we then performed a rarefaction for each sample, and obtained  
519 1 116 microbial sequences (62 per sample) belonging to 129 phototrophic microbial OTUs (Supp Fig.

520 S8). Finally, we checked the profiles of the rarefaction curves. At the sample level, not every sample  
521 reached the saturation, but when considering the six microhabitats, rarefaction curves revealed a near  
522 saturation (Supplementary Fig. S8). This indicated that the majority of the diversity of the six  
523 microhabitats was captured.

524

#### 525 **Microbial abundance**

526 We sampled three shoots of *Sphagnum* (0-3 cm) in each plot and 5 ml of surface water in pools, and  
527 fixed them in 10 ml of glutaraldehyde (2% final concentration). Microorganisms were extracted by  
528 adapting the method of Jassey *et al.* (2011). We vigorously shook the samples for 2 min on a vortex,  
529 then we passed them through a 100  $\mu\text{m}$  mesh. The retained moss was pressed with a spatula to extract  
530 the maximum of filtrates. The remaining fraction was dried at 80°C for 48 h, weighted and converted  
531 into cubic centimetre using the *Sphagnum* density calculated previously. Phototrophic microbes were  
532 enumerated by flow cytometry with a Guava® easyCyte™ 11HT cytometer. Aliquots of 300  $\mu\text{l}$  were  
533 placed in a 96-well microplate and run unstained in the flow cytometer at a flow of 0.59  $\mu\text{l}\cdot\text{s}^{-1}$ . A total  
534 of 5000 events were recorded in each sample. Forward and side light scatter (size indication) as well  
535 as green fluorescence (presence or absence of chlorophyll) were used to discriminate photosynthetic  
536 microorganisms from non-photosynthetic microorganisms. Abundance data were expressed as the  
537 number of individuals per cubic centimetre and were then converted in biomass ( $\mu\text{g C}$ ) per cubic  
538 centimetre using the community-weighted mean biomass. To measure the community-weighted mean  
539 biomass of phototrophic microbes, 3 ml of each sample were settled for 2 h in a plankton chamber  
540 and analysed at x 400 magnification by inverted microscopy. Along several transects of the plankton  
541 chamber, we counted and measured the geometrical shapes of every phototrophic microbe  
542 encountered. Geometrical shapes were then converted to biovolume and C biomass using conversion  
543 factors from the literature (Borsheim and Bratbak, 1987; Weisse *et al.*, 1990; Gilbert *et al.*, 1998b). We  
544 counted a minimum of five transects or 500 phototrophic microbes per sample to estimate the  
545 community-weighted mean biomass.

546

547 **Microbial photosynthesis efficiency, chlorophyll *a* and electron transport rate**

548 For microbial photosynthesis measurements, five shoots of *Sphagnum* (0-3 cm) were sampled in each  
549 plot and immersed in 20 mL demineralized water. Microbial communities were extracted and filtered  
550 on a GF/F Whatman® filter, while the remaining *Sphagnum* material was dried at 80°C for 48 h,  
551 weighted and converted into cubic centimetre (following Hamard *et al.*, 2021). In pools, we filtered  
552 10 ml of pool surface water on GF/F Whatman® filters. Filters containing the microbial communities  
553 were dark adapted for 30 min after what they were exposed to increasing light in order to measure  
554 the light curve of the quantum yield of photosystem II ( $\Phi_{PSII}$ ). Measurement were conducted with a  
555 Phyto-PAM (Walz, Effeltrich, Germany). The chlorophyll *a* content of phototrophic microbes was  
556 assessed following Hamard *et al.*, 2021. The filters used for microbial photosynthetic efficiency and  
557 containing microbial communities were frozen at -80°C and freeze-dried in the dark. Pigments were  
558 extracted from the freeze-dried filters, then quantified on an HPLC (Barlow *et al.*, 1997). We assessed  
559 the concentration of several pigments including chlorophyll *a*, pheophytin *a* and pheophorbide *a*. As  
560 pheophytin *a* and pheophorbide *a* are alteration products of chlorophyll *a* (Jeffrey *et al.*, 1997), we  
561 summed the abundance of these three pigments to have an estimate of overall chlorophyll *a* in the  
562 samples (hereafter named chlorophyll *a*). Chlorophyll *a* (Chla) was expressed in ng per cubic  
563 centimetre. It was then divided by the total biomass of phototrophs to have an estimate of the  
564 chlorophyll *a* content per unit of biomass (expressed in ng.µg C<sup>-1</sup>).

565 We quantified the microbial photosynthetic activity in each plot by calculating the  
566 photosynthetic electron transport rate per biomass (ETR) as described in Wilken *et al.* (2013), and  
567 following Falkowski and Raven (2013):

568 (1)  $ETR^{(I)} = 0.5 \times I \times \frac{Chla}{biomass} \times \Phi_{PSII}^{(I)} \times a^*$

569 where ETR was calculated at increasing light intensity (*I*) from 0 to 400 µmol.m<sup>-2</sup>.s<sup>-1</sup> to provide a light  
570 curve. ETR at light intensity *I* ( $ETR^{(I)}$ ) depends on the importance of the microbial photosynthetic  
571 machinery (Chla per biomass,  $\frac{Chla}{biomass}$ ) and on its photosynthetic efficiency at light intensity *I* (quantum

572 yield of photosystem II,  $\Phi_{\text{PSII}}^{(0)}$ ). Here,  $a^*$  was the spectrally averaged chlorophyll-specific absorption  
573 cross section, for which we took a value of  $26.86 \text{ m}^2 \cdot \text{g Chla}^{-1}$  as estimated in (Wilken *et al.*, 2013). The  
574 ETR was expressed in  $\text{mol e}^- \cdot \mu\text{g C}^{-1} \cdot \text{s}^{-1}$ .

575 For each plot, we fitted the light curves of ETR and we calculated the photosynthetic-irradiance  
576 parameters using the fitPGH function from the Phytotools R package (Silsbe and Malkin, 2015). We  
577 extracted the maximal ETR ( $E_m$ ) from each light curve, as well as the light intensity at which  $E_m$  was  
578 reached ( $P_m$ ) and the light intensity at which ETR decreased to  $E_m/2$  as a measure of photoinhibition.

579

#### 580 **Microbial C fixation rate and contribution to bryosphere C uptake.**

581 Phototrophic microbial C fixation rates ( $C_{\text{fixed}}$ ) were calculated in each plot based on the maximum ETR  
582 ( $E_m$ ):

$$583 \quad (2) \quad C_{\text{fixed}} = 0.25 \times E_m \times \text{biomass}$$

584 where we assumed a maximum fixation of  $0.25 \text{ mol CO}_2$  per mol of electron (Wilken *et al.*, 2013). We  
585 took into account the total biomass of phototrophic microbes per cubic centimetre (biomass) and  
586 therefore expressed the microbial C fixation rates in  $\text{mol CO}_2 \cdot \text{s}^{-1} \cdot \text{cm}^{-3}$ . We then converted the microbial  
587 C fixation rates in  $\text{g CO}_2 \cdot \text{s}^{-1} \cdot \text{cm}^{-3}$ .

588

#### 589 **Numerical analyses**

590 Metabarcoding data were used to explore the relative abundance and the structure of phototrophic  
591 microbial communities. Alpha-diversity measures (OTU richness and Shannon's entropy) were used to  
592 estimate diversity in each plot and for each metabarcoding marker. We used analysis of variance  
593 (ANOVA) to test the variations of diversity indexes along microhabitats. We further used a  
594 permutational multivariate analysis of variance (PERMANOVA) to test for structural differences  
595 between the phototrophic communities of the different microhabitats.

596 To explore if some associations of phototrophic microbes were specific of some microhabitats,  
597 we built co-occurrence networks based on the 23S analyses and following (Borcard *et al.*, 2018). First,



598 we built a presence-absence matrix of the OTUs in the different plots. Then we computed a matrix  
599 composed of the a component of the Jaccard's  $S_7$  coefficient, which is a measure of co-occurrence  
600 among species (Borcard *et al.*, 2018). We assessed the probability of each "a component" by means of  
601 99999 permutations and obtained a "a" distance matrix. We then calculated an adjacency matrix from  
602 the "a" distance matrix in which we only kept significant co-occurrences. To know if co-occurrences  
603 were significant, we computed a matrix of the Spearman's rho rank correlation and assessed their P-  
604 values using the rcorr function from the Hmisc R package (Harrell and Dupont, 2008). Co-occurrences  
605 of the adjacency matrix were conserved if they were associated to a P-value lower than 0.05. Then we  
606 built the co-occurrence network. We looked for structures (or clusters) in the network, that is to say  
607 we looked for OTUs associations that were densely connected, using the cluster\_optimal function from  
608 the igraph R package (Csardi, 2008). Finally, we looked at the repartition of the different clusters in the  
609 different microhabitats. We calculated the Unifrac dissimilarity between the communities of the  
610 different clusters.

611 We tested variations of phototrophic microbial abundance, chlorophyll content, electron  
612 transport rate and C fixation rates between sites using analysis of variance (ANOVA).

613 We used structural equation modelling (SEM; Grace *et al.* 2014) to study the complex  
614 interactions determining the phototrophic C fixation. Following current knowledge on phototrophic  
615 eco-physiology (Kalchev *et al.*, 1996; Huete-Ortega *et al.*, 2012; Padfield *et al.*, 2018), we developed an  
616 *a priori* model of hypothesized relationships (Supplementary Fig. S10) within a path diagram allowing  
617 a causal interpretation of microbial C fixation rates in relation to microbial community structure  
618 (relative abundance of the five clusters), to total phototrophic biomass, to metabolic parameters  
619 (photosynthetic activity per unit of biomass) and to environmental parameters (Supplementary Table  
620 S5). In this diagram, each path is a component of the model, and represent a linear model between  
621 the explanatory and the explained variable. All components of the model were united using the psem  
622 function from the piecewiseSEM R package (Lefcheck, 2016). The goodness-of-fit of our *a priori* SEM  
623 model was evaluated using Akaike Information Criterion (AIC) and Fisher's statistic. Based on the

624 outputs of the *a priori* model and by step-wise exclusion/selection of variables, we found the model  
625 minimizing the AIC. The main drivers of microbial C fixation were determined from this final model.  
626 All statistical analyses were performed using R version 3.6.2 (R Core Team, 2019).

627

#### 628 **Data availability**

629 All data needed to evaluate the conclusions in the paper are present in the paper and/or the  
630 Supplementary Materials. Additional data and R codes related to this paper will be publicly available  
631 from Figshare (10.6084/m9.figshare.c.5190902). Amplicon data will be available upon publication on  
632 NCBI Sequence Archive (SRA) under project number: PRJNA727992.

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634

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896 **Table and Figure legends**

897 **Table 1:** Environmental variables measured in the six different microhabitats.

898 **Figure 1:** Biodiversity of the phototrophic communities found in the different microhabitats. OTU  
899 richness (Chao1 diversity; dark grey) and diversity (Shannon index; light grey) are presented in the A  
900 panel. The proportion of phototrophic prokaryotes and phototrophic micro-eukaryotes in the  
901 microbial phototrophic community is presented in the B panel.  
902

903 **Figure 2:** Clusters of co-occurring OTUs. In the network (A) one dot represent one OTU, and its  
904 diameter is proportional to its relative abundance averaged in the six microhabitats. The four dominant  
905 OTUs are *a: Nostoc sp* (15% of total sequences); *b: Cyndrospermum stagnale* (10%); *c: Chlorella*  
906 *variabilis* (9%) and *d: Mesotaenium caldariorum* (7%). The bar plot (B) shows the relative abundance  
907 of the different clusters within the phototrophic microbial communities of the six microhabitats.

908

909 **Figure 3:** Environmental parameters affecting the repartition of the different clusters and their relative  
910 abundance in the phototrophic community. The correlation coefficient ( $r$ ) is indicated for significant  
911 correlations ( $P < 0.05$ ) between environmental parameters and relative abundance of the clusters.  
912 \* $0.05 > P > 0.01$ ; \*\*  $0.01 > P > 0.001$ ; \*\*\* $P < 0.001$ .

913

914 **Figure 4:** Allometric features of phototrophic microbes in the six microhabitats. A: Chlorophyll *a*  
915 content per unit of phototrophic biomass phototrophic microbes. B: Maximum electron transport rate  
916 per unit of phototrophic biomass. C: Total biomass of phototrophic microbes expressed as  $\mu\text{g C per}$   
917  $\text{cm}^3$  of *Sphagnum* (or  $\mu\text{g C per cm}^3$  of water in pools). Error bars present standard error. Significant  
918 differences ( $P < 0.05$ ) are indicated by different letters above bars.

919

920 **Figure 5:** Structural equation modelling of the correlations between environmental parameters,  
921 structure of phototrophic microbial communities (relative abundance of the clusters and Shannon  
922 index), their total biomass per  $\text{cm}^3$  and metabolic features, and microbial C fixation per  $\text{cm}^3$ . Numbers  
923 in the boxes indicate the percentage of variance explained by the model (adjusted R-squared), while  
924 numbers along the arrows indicate the weight of the path relationship (\*  $0.01 < P < 0.05$ ; \*\*  
925  $0.001 < P < 0.01$ ; \*\*\*  $P < 0.001$ ). Grey arrows indicate non-significant relationships ( $0.05 < P < 0.10$ ).  
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