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1 **Contribution of microbial photosynthesis to peatland carbon uptake along a**
2 **latitudinal gradient**

3

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28 **ABSTRACT**

29 1. Phototrophic microbes, also known as micro-algae, display a high abundance in many terrestrial
30 surface soils. They contribute to atmospheric carbon dioxide fluxes through their photosynthesis, and
31 thus regulate climate similar to plants. However, microbial photosynthesis remains overlooked in most
32 terrestrial ecosystems. Here, we hypothesize that phototrophic microbes significantly contribute to
33 peatland C uptake, unless environmental conditions limit their development and their photosynthetic
34 activity.

35 2. To test our hypothesis, we studied phototrophic microbial communities in five peatlands distributed
36 along a latitudinal gradient in Europe. By means of metabarcoding, microscopy and cytometry
37 analyses, as well as measures of photosynthesis, we investigated the diversity, absolute abundance
38 and photosynthetic rates of the phototrophic microbial communities.

39 3. We identified 351 photosynthetic prokaryotic and eukaryotic operational taxonomic units (OTU)
40 across the five peatlands. We found that water availability and plant composition were important
41 determinants of the composition and the structure of phototrophic microbial communities. Despite
42 environmental shifts in community structure and composition, we showed that microbial C-fixation
43 rates remained similar along the latitudinal gradient. Our results further revealed that phototrophic
44 microbes accounted for approximately 10% of peatland C uptake.

45 4. *Synthesis*. Our findings show that phototrophic microbes are extremely diverse and abundant in
46 peatlands. While species turnover with environmental conditions, microbial photosynthesis similarly
47 contributed to peatland C uptake at all latitudes. We estimate that phototrophic microbes take up
48 around 75 MT CO₂ per year in northern peatlands. This amount roughly equals the magnitude of
49 projected peatland C loss due to climate warming and highlight the importance of phototrophic
50 microbes for the peatland C cycle.

51

52 **KEYWORDS**

53 Algae, Carbon cycle, Metabarcoding, Microbial diversity, Peatland, Photosynthesis, Phototrophs,
54 Primary productivity.

55

56 **1 INTRODUCTION**

57 Microbes only represent one-sixth of the living biomass on Earth (93 PgC for fungi, protists, bacteria
58 and archaea; Bar-On et al., 2018) but they drive some of the most important carbon (C) fluxes at the
59 global scale (Caron et al., 2017; Fierer, 2017; Singh et al., 2010). For instance, oceanic phytoplankton
60 contributes to one third of the global photosynthetic CO₂ fixation with 50 PgC fixed each year, while
61 the remaining amount is consensually attributed to terrestrial plants (Ciais et al., 2013). The input of
62 organic matter to oceanic and terrestrial food webs is therefore attributed to the photosynthetic
63 activity of either oceanic phytoplankton or terrestrial plants, respectively (Krumins et al., 2013; Liang
64 et al., 2017; Worden et al., 2015). This consensus, however, ignores terrestrial photosynthetic
65 microorganisms also known as micro-algae (hereafter, phototrophic microbes), which are natural
66 components of the soil flora (Bates et al., 2013; Cano-Díaz et al., 2019; Oliverio et al., 2020), and can
67 reach several millions of individuals per gram of soil (Zancan et al., 2006).

68 Soil phototrophic microbes display myriads of species with a wide range of morphologies and
69 life styles (Caron et al., 2017; Delgado-Baquerizo et al., 2018; Oliverio et al., 2020). Prokaryotes and
70 eukaryotes that compose the soil phototrophic microbial communities can be either photo-autotrophs
71 (i.e., strict phototrophs using only mineral C as C source), photo-heterotrophs (i.e., using only organic
72 C; Geisen et al., 2018; Lynn et al., 2017), or mixotrophs (i.e., using both mineral and organic C; Mitra
73 et al., 2016). Recent studies suggest that phototrophic microbes could account for large amounts of
74 net primary production, and play a much larger role in terrestrial C dynamics than previously
75 acknowledged (Elbert et al., 2012). However, our understanding of their contribution to the C balance
76 and productivity of terrestrial ecosystems is largely restricted to drylands (Elbert et al., 2012; Maier et

77 al., 2018). Phototrophic microbial contribution to total C fixation remains largely unexplored in most
78 other ecosystems, especially in C-accreting systems such as peatlands.

79 Peatlands play an important role in the global C cycle as they are major C sinks and store a
80 large pool of soil C (500 – 1000 PgC; Bridgham et al., 2006; Nichols & Peteet, 2019; Yu, 2012). Peat
81 mosses (i.e., *Sphagnum* spp.) serve a variety of key functions in peatlands, notably primary production
82 and C sequestration (Turetsky, 2003; van Breemen, 1995). *Sphagnum* mosses also provide a habitat
83 for a large diversity of microbes living either inside or at the surface of *Sphagnum* leaves (Gilbert et al.,
84 1998). This association between *Sphagnum* mosses and microbes forms the bryosphere (Lindo &
85 Gonzalez, 2010). *Sphagnum*-associated phototrophic microbes include bacteria (e.g., Cyanobacteria,
86 Alphaproteobacteria), protists (e.g., Chlorophyta) and other mixotrophic micro-eukaryotes (e.g., many
87 Ochrophyta or endosymbiotic Lobosa) (Gilbert et al., 1998; Gilbert & Mitchell, 2006; Jassey et al., 2015;
88 Lara et al., 2011; Tian et al., 2019) and can be highly abundant. For instance, the biomass of
89 phototrophic microbes in surface peat (0.5 mg C.l⁻¹; Gilbert & Mitchell, 2006) can exceed the
90 phytoplankton biomass of some of the most productive oceanic locations (c.a. 0.08 mg C.l⁻¹;
91 Behrenfeld, 2014). Yet, despite such high abundance, only a handful studies focused on algal
92 productivity in peatlands, mostly in water-logged rich fens (Gilbert et al., 1998; Goldsborough &
93 Robinson, 1996; Wyatt et al., 2012). Phototrophic microbial productivity remains largely overlooked
94 in all other peatland types including *Sphagnum*-dominated bogs and poor fens, which constitute the
95 largest part of peatlands at a global scale (Hugelius et al., 2020). *Sphagnum*-dominated peatlands
96 occupy extensive areas of land at different latitudes and across a wide variety of climates (Halsey et
97 al., 2000; Robroek et al., 2017). Hence, improving our understanding of the spatial and environmental
98 patterns of phototrophic microbial contribution to bryosphere C uptake across environmental
99 gradients is likely to improve our understanding of biogeochemical cycles in peatlands.

100 Here, we explore the diversity of phototrophic microbes and their primary productivity in
101 different peatlands under varying climates and peatland conditions, using a latitudinal transect

102 covering a broad range of environmental conditions. Specifically, we tested the relationships between
103 phototrophic microbial community composition, structure, and photosynthetic rate in five European
104 *Sphagnum*-dominated peatlands distributed from southern France to northern Sweden. We
105 hypothesized (i) that phototrophic microbes make a significant contribution to the bryosphere C
106 uptake, and that (ii) this contribution depends on environmental conditions such as climate
107 (temperature, precipitation) and/or edaphic factors. More specifically, we expected (iii) that
108 environmental conditions strongly influence the taxonomic composition of phototrophic microbial
109 communities, affecting community structure with cascading effects on phototrophic abundance and C
110 fixation rates. We analysed phototrophic microbial community composition by means of
111 environmental 16S and 18S rDNA gene sequencing for prokaryotes and eukaryotes, respectively. We
112 further evaluated phototrophic microbial abundance through flux cytometry and microscopy, and we
113 assessed phototrophic microbial C fixation rates by measuring their photosynthetic efficiency and
114 photosynthetic pigments content.

115

116 **2 METHODS**

117 **2.1 Sites description and sampling**

118 We collected samples in five European peatlands distributed along a latitudinal gradient ranging from
119 42 °N (south of France) to 68°N (north of Sweden) in July 2018. From south to north (3000 km as the
120 crow flies), the five peatlands were Counozouls (Lapazeuil) in France (42°41'16 N, 2°14'18 E, 1350 m
121 a.s.l.), Kusowo (Kusowkie Bagno) in Poland (53°48'48 N, 16°35'12 E, 250 m a.s.l.), Männikjärve in
122 Estonia (58°52'30 N, 26°15'04 E, 78 m a.s.l.), Siikaneva in Finland (61°50'00 N, 24°11'32 E, 170 m a.s.l.)
123 and Abisko (Stordalen) in Sweden (68°20'54 N, 19°04'09 E, 350 m a.s.l.) (Fig. 1A). These sites were
124 characterised by flat topographies on a local scale and were associated with different peatland types.
125 Counozouls is a minerotrophic fen, Kusowo and Männikjärve are ombrotrophic bogs, Siikaneva is a
126 boreal oligotrophic fen, and Abisko a palsa mire. The vegetation at all five sites contained a sparse

127 vascular plant layer, and was dominated by bryophytes from the *Sphagnum* genus: *S. warnstorffii* in
128 Counozouls, *S. magellanicum* and *S. fallax* in Kusowo, *S. rubellum*, *S. magellanicum* and *S. fuscum* in
129 Männikjärve, *S. papillosum* in Siikaneva and *S. balticum* in Abisko. At each site, we selected five
130 homogeneous plots (50 x 50 cm). In each plot we sampled the apical part of *Sphagnum* mosses (0-3
131 cm from the capitulum) for analysis of microbial diversity, abundance and biomass as well as
132 photosynthetic rates. We further quantified bryosphere C fixation rates from the same location (see
133 below).

134

135 **2.2 Vegetation, climatic and edaphic parameters**

136 The vegetation cover (Supplementary Table S1) was assessed in each site and in each plot by taking
137 four pictures of each plot as explained in Sytiuk et al. (2020). The quality and quantity of *Sphagnum*
138 water-extractable organic matter (Supplementary Table S2) was assessed in each site according to
139 Jassey et al. (2018). Several physico-chemical properties were analysed including pH, dissolved organic
140 carbon and total nitrogen (measured by combustion on a Shimadzu TOC-L), ion concentration
141 (measured on Dionex Ics-5000+ and Dionex DX-120) and dissolved organic matter quality (measured
142 by spectrometry and fluorescence following Hansen et al. (2016). All values were corrected by a blank
143 consisting of demineralized water. *Sphagnum* shoots were weighted fresh just after sampling. Then
144 they were oven-dried (60°C for 48 hours) and weighted after extraction to calculate *Sphagnum* water
145 content and to express the variables per g of *Sphagnum* dry weight (g DW). *Sphagnum* water content
146 was expressed in g of water per g of *Sphagnum* DW. Finally, long-term bioclimatic variables such as
147 mean annual temperature, annual precipitation and temperature seasonality were retrieved from
148 WorldClim 2.0 (Supplementary Table S3).

149

150

151

152

153 **2.3 Diversity and community structure of peatland phototrophic microbes**

154 2.3.1 Microbial DNA extraction

155 Three shoots of *Sphagnum* (0-3 cm) were sampled with sterile equipment from each plot, cut into
156 small pieces and placed into sterile 5 mL tubes containing 3 mL of Lifeguard (Qiagen). For each sample,
157 DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen) and following the manufacturer's
158 instructions. A negative extraction (i.e., without *Sphagnum*) was performed to control for possible
159 contaminations during extraction. For mechanical lysis of the cells, we included two runs of bead
160 beating on a FastPrep FP120 Instrument (MP Biomedicals) for 40 s at speed 5.0 m.s⁻¹. DNA was eluted
161 in 70 µl of final solution and DNA concentration in each extract was quantified using a Nanodrop ND-
162 1000 spectrophotometer. Extracts were stored at -80°C before proceeding with DNA amplification.

163

164 2.3.2 Microbial DNA sequencing

165 To investigate prokaryote communities, we amplified a part of the 16S rDNA gene, using the primers
166 PCR1_515F and PCR1_928R (Wang & Qian, 2009). Eukaryote communities were investigated by
167 amplifying a part of the 18S rDNA gene with the primers TAREuk454FWD1 and TAREukREV3 (Tanabe
168 et al., 2016). Both prokaryotic and eukaryotic pairs of primers were tagged with Illumina adapter
169 sequences. PCRs were conducted in a total volume of 50 µl containing 13 µl of Mastermix AmpliTaq
170 Gold (ThermoFisher), 1µl of each primer from the chosen pair (0.4 µM final concentration), 35 ng of
171 DNA (up to 10 µl) and supplemented to 50 µl with DNA-free water. The primers' sequences and the
172 PCR programs used are presented in Supplementary Table S4. All PCR products were checked on an
173 agarose gel and samples were then frozen at -80°C until sequencing was performed by the GeT-PlaGe
174 platform (Genotoul, Toulouse, France) with Illumina MiSeq technology and using the V3 chemistry
175 (2x250 bp).

176 Paired-end fastq sequences (851 910 prokaryotic reads and 891 422 eukaryotic reads for 25
177 samples) were analysed using the FROGS pipeline (Find Rapidly Operational Taxonomic Units Galaxy
178 Solution) on the Galaxy platform (Escudié et al., 2018). Paired-end reads were merged using Vsearch

179 (10% of mismatch; Rognes et al., 2016). Sequences were filtered based on their length and primer
180 mismatches were removed, leading to a total of 770 602 prokaryotic sequences and 813 098 eukaryotic
181 sequences for all samples. Sequences were then de-replicated and clustered into operational
182 taxonomic units (OTUs) using the Swarm clustering method with an aggregation distance of 3 (Mahé
183 et al., 2014). Chimera were identified and removed using Vsearch. Filters were then applied to remove
184 singletons. In total 677 947 sequences clustered in 2 063 OTUs were kept for prokaryotes, and 718 684
185 sequences clustered in 2 050 OTUs were kept for eukaryotes. OTUs were assigned at different
186 taxonomic levels using Blast. We used the Silva 138 database (Quast et al., 2013) for prokaryotic
187 assignation and the PR² v4.12.0 database (Guillou et al., 2013) for eukaryotic assignation.

188

189 2.3.3 Identification of phototrophic OTUs

190 Further analyses were done using the Phyloseq R package (McMurdie & Holmes, 2013). Prokaryotic
191 and micro-eukaryotic sequences were retrieved by removing chloroplast, mitochondria and plant
192 associated sequences. The 25 samples were further rarefied to a total of 50 400 prokaryotic sequences
193 clustered into 1 172 operational taxonomy units (OTUs), and 21 672 micro-eukaryotic sequences
194 clustered in 622 OTUs. Microbial phototrophic OTUs were identified at the genus level, and we
195 outlined the structure of phototrophic microbial communities from the relative abundance of
196 phototrophic OTUs. We considered that an OTU was phototrophic if its affiliated genus was referred
197 as photoautotrophic, photoheterotrophic or mixotrophic in the literature (e.g. (Jia et al., 2015;
198 Kulichevskaya et al., 2014; Li et al., 1999; Okamura et al., 2009; Yurkov et al., 1993)). For instance, we
199 included endosymbiotic mixotrophs (Mitra et al., 2016) among phototrophic OTUs such as the testate
200 amoeba *Archerella flavum* and *Hyalosphenia papilio*. Further, OTUs of Chrysophyceae were specifically
201 aligned with the GenBank database using BLAST v. 2.9.0+ as implemented on the NCBI website in order
202 to obtain a precise functional assignation. Indeed, Chrysophyceae have swapped trophic modes
203 several times during their evolutionary history (Graupner et al., 2018). The overwhelming majority of
204 Chrysophyceae OTUs were phototrophic or mixotrophic.

205 **2.4 Absolute abundance of phototrophic microbes**

206 To determine the absolute abundance of phototrophs in every site, we sampled five shoots of
207 *Sphagnum* (0-3 cm) in each plot, fixed them in 20 ml of glutaraldehyde (2% final concentration), and
208 extracted microorganisms from *Sphagnum* tissues following the method of Jassey et al. (2011). Then,
209 we quantified the absolute abundance of phototrophic microbes from microbial extracts by means of
210 either flow cytometry or microscope analyses according to microbial body size.

211 Phototrophic microbes with a body length smaller than 40 μm were enumerated using flow
212 cytometry. To do so, we filtrated 300 μl aliquots of microbial extract with 40 μm Nitex[®] filters, and
213 placed them in a 96-well microplate. Then, aliquots were run unstained in a Guava[®] easyCyte[™] 11HT
214 cytometer at a flow of 0.59 $\mu\text{l}\cdot\text{s}^{-1}$. A maximum of 5000 events were recorded in each sample. Forward
215 and side light scatter (size indication) as well as green fluorescence (presence or absence of
216 chlorophyll) were used to discriminate photosynthetic microorganisms from non-photosynthetic
217 microorganisms (bacteria) (Olson et al., 1989).

218 Phototrophic microbes with a body size larger than 40 μm as well as all Cyanobacteria were
219 identified and counted directly using an inverted microscope. In our samples, Cyanobacteria formed
220 colonies and/or long filaments over 40 μm that were not counted by flow cytometry. We double
221 checked that the subsamples filtrated at 40 μm did not contain Cyanobacteria under the microscope.
222 Under the microscope, we identified and enumerated phototrophic microbe until species level when
223 possible. We differentiated between strictly phototrophic microbes (microalgae, Cyanobacteria), and
224 mixotrophic microbes (endosymbiotic microbes). Mixotrophic species included testate amoebae (e.g.
225 *Archerella flavum*, *Amphitrema wrightianum*, *Heleopera sphagni* and *Hyalosphenia papilio*) and ciliates
226 (e.g. *Paramecium bursaria* and *Platyophrya sphagni*).

227 Flow cytometry and inverted microscope abundance data were expressed as the number of
228 individuals per gram of *Sphagnum* dry weight (DW). We further converted abundance data to C
229 biomass (μg C per gram of *Sphagnum* DW) by measuring the biovolume of each species using
230 geometrical shapes under microscope (Gilbert et al., 1998; Mitchell et al., 2003). Biovolumes were

231 then converted to C biomass using conversion factors from the literature (Borsheim & Bratbak, 1987;
232 Gilbert et al., 1998; Weisse et al., 1990).

233

234 **2.5 Microbial photosynthetic rates**

235 Chlorophyll *a* (Chl *a*) concentrations and the quantum yield of photosystem II (Φ_{PSII}) were measured in
236 each plot. Chl *a* concentrations provide an estimate of the amount of photosynthetic machinery, while
237 Φ_{PSII} gives the fraction of the absorbed quanta that are used for photosynthetic electron transport and
238 thus provides a measure of photosynthetic efficiency (Wilken et al., 2013). For these analyses, five
239 shoots of *Sphagnum* (0-3 cm) were sampled in each plot and immersed in 20 mL demineralized water.
240 Samples were kept at 4°C in the dark and microorganisms were extracted immediately upon return to
241 the laboratory. Samples were shaken at 150 rpm for 1.5 h and then squeezed to extract
242 microorganisms. The remaining solution was filtered at 100 μm with Nitex® filters to remove any
243 *Sphagnum* residue, while the remaining *Sphagnum* material was dried at 80°C for 48 h and weighted.
244 Each microbial extract was filtered on a GF/F Whatman® filter (0.7 μm) to recover the microbial
245 community.

246 We measured Φ_{PSII} of the microbial community with a Phyto-PAM (Walz, Effeltrich, Germany) after
247 exposing filters to low light for 30 min (PAR, 32 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$). We chose a PAR of 32 μmol
248 $\text{photons.m}^{-2}.\text{s}^{-1}$ following light response curve analyses previously ran on test samples (Supplementary
249 Fig. S1). We further quantified the microbial photosynthetic rates in each plot by calculating the
250 photosynthetic electron transport rate (ETR) as described in Wilken et al. (2013), and following
251 Falkowski & Raven (2013):

$$252 \quad (1) \text{ ETR} = 0.5 \times I \times \frac{\text{Chla}}{\text{cell}} \times \Phi_{\text{PSII}} \times a^*$$

253 where ETR is the photosynthetic rate, expressed per cell as a measure of the community-weighted
254 mean photosynthetic rate. It was calculated at a light intensity (*I*) of 32 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$. The calculation of
255 ETR relies on the importance of the microbial photosynthetic machinery (cellular Chl*a* content, $\frac{\text{Chla}}{\text{cell}}$)

256 and on its photosynthetic efficiency (effective quantum yield of photosystem II, Φ_{PSII} , measured at a
257 PAR of $32 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Here, a^* was the spectrally averaged chlorophyll-specific absorption cross
258 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 ETR
259 was expressed in $\text{mol e}^-\cdot\text{cell}^{-1}\cdot\text{s}^{-1}$.

260 To obtain Chl *a* concentrations, the filters used for microbial photosynthetic efficiency and
261 containing microbial communities were frozen at -80°C and freeze-dried in the dark. Adapting the
262 methodology from Capdeville et al. (2019), we soaked lyophilized filters in 1.5 ml of methanol buffered
263 with 2 %v of ammonium acetate ($1 \text{ mol}\cdot\text{l}^{-1}$). Samples were sonicated for 2 min in an ice bath, then
264 cooled at -20°C in the dark for 15 min and centrifuged for 5 min at 10 000 rpm. The supernatant was
265 recovered (solution 1) while 1 ml of buffered methanol was added to the pellet (solution 2). The
266 process was repeated for solution 2 and its supernatant was merged with solution 1. Then, 1.5 ml of
267 this composite solution was filtered at $0.2 \mu\text{m}$ with a syringe filter and frozen at -80°C before analysis
268 on an HPLC (Barlow et al., 1997). We assessed the concentration of several pigments including Chl *a*,
269 pheophytin *a* and pheophorbide *a*. As pheophytin *a* and pheophorbide *a* are alteration products of Chl
270 *a* (Jeffrey et al., 1997), we summed the abundance of these three pigments to have an estimate of
271 overall Chl *a* in the samples (hereafter named Chl *a*). Chl *a* was expressed in mg per g of *Sphagnum*
272 DW and divided by phototroph abundance to have an estimate of cellular Chl *a* content (expressed in
273 $\text{mg}\cdot\text{cell}^{-1}$).

274

275 **2.6 Microbial C fixation rate and contribution to bryosphere C fixation rate**

276 Phototrophic microbial C fixation rates (C_{fixed}) were calculated in each plot from ETR:

$$277 \quad (2) \quad C_{\text{fixed}} = 0.25 \times \text{ETR} \times \text{cell} \times \text{Area}_{\text{DW}}$$

278 where we assumed a maximum fixation of 0.25 mol CO_2 per mol of electron (Wilken et al., 2013), and
279 took into account phototrophic microbial abundance (*cell*) and specific surface of *Sphagnum* (Area_{DW})

280 measured in each plot as the area per gram of *Sphagnum* DW (0-3 cm). Carbon fixation rates were
281 expressed in mg of CO₂ fixed per hour and per m² of *Sphagnum*.

282 We measured the bryosphere photosynthetic capacity (A_{MAX} , maximum net CO₂ assimilation
283 rate) in each plot from three *Sphagnum* shoots as described in Jassey & Signarbieux (2019). The A_{MAX}
284 was measured with an open-path infrared gas analyser (IRGA) system connected to a 2.5 cm² PLC-5
285 chamber (TARGAS-1; PP-Systems) under optimum conditions for light (i.e., 600 μmol of photons m⁻² .s⁻¹
286 ¹, which was previously determined in the field). Bryosphere C fixation was expressed in mg CO₂ h⁻¹ .m⁻²
287 ², allowing comparisons with microbial C fixation rates. To estimate bryosphere C fixation per Chl *a*, we
288 also estimated the Chl *a* content of *Sphagnum* in each plot. We extracted Chl *a* from two lyophilized
289 *Sphagnum* shoots and followed the same extraction method as for phototrophic microbes.

290

291 **2.7 Numerical analyses**

292 Meta-barcoding data were used to explore the diversity and taxonomy of phototrophic microbes, and
293 their community composition. Biostatistics were conducted separately on prokaryotic and eukaryotic
294 microbial communities. Alpha-diversity, non-metric multidimensional scaling (NMDS), and beta-
295 diversity metrics based on Bray-Curtis dissimilarity were used to estimate diversity and species
296 turnover between sites. Environmental drivers of phototrophic microbial species turnover were tested
297 using generalized dissimilarity modelling (GDM). The environmental matrix included a selection of
298 variables such as plant community composition (Supplementary Table S1), *Sphagnum* physico-
299 chemical parameters (Supplementary Table S2), and climatic variables (Supplementary Table S3). We
300 selected the most representative and least collinear environmental variables using the package
301 ClustOfVar (12) with an ascendant hierarchical clustering of all available environmental variables. To
302 maximise the stability of partition, nine clusters of variables were retained. In each cluster, the most
303 representative variables were kept for GDM (Supplementary Fig. S2). Variable significance in GDM was
304 determined using matrix permutations and comparing models with each variable permuted and un-
305 permuted. At each step, the least important variables were dropped until all non-significant predictors

306 were removed. The GDM was then fit using only significant environmental predictors, and model
307 significance was checked.

308 All data were tested for normality and transformed if necessary. We tested variation of
309 phototrophic microbial abundance, chlorophyll content, electron transport rate, and C fixation rates
310 between sites (explicative qualitative variable) using analysis of variance (ANOVA). Further, we used
311 linear mixed effects models to test the effects of environmental variables (mean annual temperature,
312 water table depth, annual precipitation; fixed effects) on phototrophic microbial abundance, while
313 accounting for measurements repeated in the same sites (random effect).

314 The variability of phototrophic C fixation can be determined by numerous interconnected eco-
315 physiological mechanisms that operate simultaneously at different individual to community scales
316 (Padfield et al., 2018; Yvon-Durocher et al., 2015). One approach to studying such complex interactions
317 is Structural Equation Modelling (SEM; Grace et al. 2014). Following current knowledge on
318 phototrophic eco-physiology (Huete-Ortega et al., 2012; Kalchev et al., 1996; Padfield et al., 2018), we
319 developed an *a priori* model of hypothesized relationships (Supplementary Fig. S3) within a path
320 diagram allowing a causal interpretation of microbial C fixation rates in relation to microbial
321 community structure (bacteria and protists), to allometric features (abundance, community-weighted
322 mean body size) and metabolic parameters (Chl *a*, photosynthetic rate) (Supplementary Table S5). In
323 this diagram, each path is a component of the model, and represent a linear model between the
324 explanatory and the explained variable. All components of the model were united using the `psem`
325 function from the `piecewiseSEM` R package (Lefcheck, 2016). The goodness-of-fit of our *a priori* SEM
326 model was evaluated using Fisher's statistic and Akaike Information Criterion (AIC). Based on the
327 outputs of the *a priori* model and by step-wise exclusion and selection of variables, we found the model
328 with the lowest AIC value. The main drivers of microbial C fixation were determined from this final
329 model. We used the first axes of phototrophic prokaryotic and eukaryotic NMDS as indicators of
330 microbial community structure, and electron transport rate (ETR) as indicator of photosynthetic
331 activity. To test to what extent environmental variables affected microbial C fixation and the

332 phototrophic mechanisms underpinning it, we ran our SEM model a second time with microbial
333 variables corrected for the co-varying environmental variables (residual data). To correct microbial
334 variables for environmental conditions, we used the residuals of linear models computed between
335 every microbial variable and the environmental variables identified in the GDM (i.e., precipitation,
336 *Sphagnum* water content, plant community composition and dissolved organic carbon).

337 All statistical analyses were performed using R version 3.6.2 (R Core Team, 2019).

338

339

340 **3 RESULTS**

341 **3.1 Diversity and community composition of phototrophic microbes**

342 The relative abundance of phototrophic microbes varied strongly across sites (Fig. 1A; Supplementary
343 Fig. S4). Phototrophic bacteria represented on average 12% of the prokaryotic microbial community,
344 and had their largest relative abundance in COUNOZOULS (34% of prokaryotic gene copies) compared to
345 the other sites (<13 %; $F_{(4,12)} = 6.21$, $P = 0.006$). Phototrophic protists constituted a large part of the
346 micro-eukaryotic community, with on average 30% of micro-eukaryotes gene copies. The relative
347 abundance of phototrophic protists ranged from 53% in MÄNNIKJÄRVE to 17% in SIKANEVA ($F_{(4,12)} = 9.53$,
348 $P = 0.001$).

349 Overall, we observed 74 OTUs of phototrophic bacteria and 277 OTUs of phototrophic protists.
350 For phototrophic bacteria, COUNOZOULS had by far the highest OTU richness with on average 30 species
351 per sample ($F_{(4,12)} = 28.0$, $P < 0.001$; Fig. 2B), whilst SIKANEVA had the lowest richness (10 species per
352 sample on average). Despite significant variation in OTU richness, we found that phototrophic bacteria
353 diversity (Shannon's entropy) was stable across sites ($F_{(4,12)} = 0.9$, $P = 0.48$, Fig. 2B). For phototrophic
354 protists, we found the highest OTU richness in MÄNNIKJÄRVE (52 species) ($F_{(4,12)} = 3.2$, $P = 0.05$; Fig. 2B)
355 and the lowest in ABISKO (31 species). Phototrophic protist diversity followed the latitudinal gradient
356 with a slight but significant decrease from COUNOZOULS to ABISKO ($F_{(4,12)} = 4.6$, $P = 0.02$; Fig. 2B).

357 We identified four phyla of phototrophic bacteria, distributed in four classes and eight orders
358 (Supplementary Fig. S5). Proteobacteria and Cyanobacteria dominated phototrophic bacteria across
359 sites. Proteobacteria ($F_{(4,12)} = 113.24$, $P < 0.001$) were relatively more abundant in Kusowo,
360 Männikjärve and Siikaneva (>80 %), while Cyanobacteria ($F_{(4,12)} = 10.4$, $P < 0.001$) relatively dominated
361 in Counozouls (60.8 %) and Abisko (68.5 %) (Fig. 2A). Gemmatimonadetes and Chloroflexi were only
362 present in Counozouls (Fig. 2A). Proteobacteria were primarily represented by the orders
363 Acetobacterales and Rhizobiales, whereas Cyanobacteria were mostly represented by Nostocales
364 (Supplementary Fig. S5). We further identified seven phyla of phototrophic protists, distributed in 13
365 classes and 21 orders (Supplementary Fig. S5). Chlorophyta (75.1 % of the average phototrophic protist
366 relative abundance), Ochrophyta (15.3 %) and Streptophyta (7.4 %) were the three most relatively
367 abundant phyla (Fig. 2A, Supplementary Fig. S5). At the class level, Chlorophyta were mainly
368 represented by Trebouxiophyceae and Chlorophyceae; Ochrophyta by Chrysophyceae; and
369 Streptophyta by Zygnemophyceae and Klebsormidiophyceae. The relative abundance of the different
370 classes varied significantly along the latitudinal gradient (Fig. 2A, Supplementary Fig. S5).

371 Both phototrophic bacteria and protist assemblages clustered at the site level (NMDS with
372 Bray-Curtis dissimilarity; Fig. 1B and Fig. 1C). Pairwise comparisons of beta-diversity based on Bray-
373 Curtis dissimilarity showed substantial species turnover between sites (Supplementary Table S6). More
374 than half of all phototrophic microbial OTUs identified were indeed found in only one site
375 (Supplementary Fig. S6) and only 13 OTUs (6 affiliated to bacteria and 7 to protists) were found in all
376 sites. Furthermore, we found that both phototrophic bacteria and protist turnover (i.e., pairwise
377 community compositional dissimilarity) increased with environmental distance among sites (i.e.,
378 pairwise environmental dissimilarity; Fig. 3A, 3C). Phototrophic microbial communities of both
379 domains were increasingly dissimilar as shrubs replaced herbaceous plants in the vegetation (Fig. 3B,
380 3D). Phototrophic bacteria turnover was also driven by an increase in precipitation and dissolved
381 organic carbon (Fig. 3B), while the turnover of phototrophic protists was strongly driven by changes in
382 *Sphagnum* water content (Fig. 3D).

383

384 **3.2 Absolute abundance, biomass and size structure of phototrophs**

385 The absolute abundance of phototrophic microbes strongly decreased along the latitudinal gradient
386 with a drop from 194.0×10^6 ind.g⁻¹ DW in COUNZOULS to 45.3×10^6 ind.g⁻¹ DW in ABISKO ($F_{(4,16)} = 25.1$,
387 $P < 0.001$; Fig. 4A). This pattern was mostly driven by phototrophic microbes with a body size $< 40 \mu\text{m}$
388 that constituted the majority of microbial phototrophs (98.5 %, Supplementary Fig. S7). Large
389 phototrophic microbes and large mixotrophs (body size $> 40 \mu\text{m}$) were less abundant and showed
390 opposite patterns with an increase of their absolute abundance toward high latitudes ($F_{(4,16)} = 11.8$, P
391 < 0.001 and $F_{(4,16)} = 20.3$, $P < 0.001$ respectively, Supplementary Fig. S7, Fig. 4B). Consequently, the
392 community-weighted mean body size per cell increased toward high latitudes ($F_{(4,16)} = 20.3$, $P < 0.001$),
393 with phototrophic microbes being on average 28 times larger in Abisko than in COUNZOULS (Fig. 4C).
394 On the whole, the total biomass of the phototrophic community tended to increase toward high
395 latitudes, from $1049 \mu\text{gC.g}^{-1}\text{DW}$ in COUNZOULS to $1634 \mu\text{gC.g}^{-1}\text{DW}$ in ABISKO ($F_{(4,16)} = 46.3$, $P < 0.001$;
396 Fig. 4D). Within the phototrophic community, Cyanobacteria had a higher absolute abundance in
397 COUNZOULS and in ABISKO ($1.04 \times 10^6 \pm 0.01$ ind.g⁻¹ DW) than in the other sites ($0.20 \times 10^6 \pm 0.04$ ind.g⁻¹
398 DW) ($F_{(4,16)} = 20.3$, $P < 0.001$; Supplementary Fig. S8). However, considering biomasses, the ratio
399 Cyanobacteria to phototrophic microbes was higher in COUNZOULS and KUSOWO (0.61 ± 0.17) compared
400 to the other sites (0.13 ± 0.03) ($F_{(4,16)} = 16.9$, $P < 0.001$; Supplementary Fig. S8).

401 Phototrophic microbial abundance correlated positively with annual precipitation ($r = 0.75$,
402 $F_{(1,23)} = 29.4$, $P < 0.001$), and to a lesser extent with plant composition ($r = 0.55$, $F_{(1,23)} = 10.29$, $P = 0.004$),
403 phototrophic microbes being more abundant when shrubs were absent. The abundance of large
404 phototrophic microbes was negatively correlated with the water table depth ($r = -0.80$, $F_{(1,23)} = 15.05$,
405 $P < 0.001$), meaning that large phototrophic microbes were more abundant when water was close to
406 the surface. The abundance of large mixotrophs correlated negatively with both annual precipitation
407 ($r = -0.87$, $F_{(1,23)} = 73.83$, $P < 0.001$) and mean annual temperature ($r = -0.83$, $F_{(1,23)} = 51.10$, $P < 0.001$).

408

409

410 **3.3 Photosynthetic rates and C fixation of phototrophic microbial communities**

411 The chlorophyll content of phototrophic microbes increased along the gradient (+400 % between
412 COUNOZOULS and ABISKO; $F_{(4,16)} = 5.78$, $P = 0.005$; Fig. 4E). Similar patterns were found for photosynthetic
413 rates (+380 % ; $F_{(4,16)} = 3.7$, $P = 0.02$; Fig. 4F). On average, phototrophic microbes fixed 8.8 (3.8 – 16.2)
414 mg CO₂.h⁻¹.m⁻² over the gradient. Phototrophic microbial C fixation varied between sites, from 16.2 mg
415 CO₂.h⁻¹.m⁻² in MÄNNIKJÄRVE to 3.8 mg CO₂.h⁻¹.m⁻² in KUSOWO, but differences among sites were not
416 significant ($F_{(4,12)} = 1.10$, $P = 0.39$; Fig. 5A).

417 Our SEM model showed that microbial C fixation was directly mediated by the direct influence
418 of phototrophic abundance (path = 0.42) and metabolism (photosynthetic rate, path = 0.70) (Fig. 6).
419 Indirectly, microbial C fixation was mediated by the community structure of phototrophic bacteria and
420 protists, which influenced phototrophic allometry. In particular, community structure influenced the
421 microbial abundance (path = 1.09 and path = -0.43 for bacteria and protist community structure,
422 respectively) and the community-weighted mean body size (path = -0.46 and path = 0.70 for bacteria
423 and protist community structure, respectively), which had cascading effects on phototrophs
424 metabolism (chlorophyll content per individual, and finally photosynthetic rate). A comparison with
425 the SEM model corrected from environmental variables did not reveal strong alterations of the
426 pathways (Supplementary Fig. S9). This indicated that the phototrophic mechanisms identified in our
427 SEM model were independent from environmental changes.

428 In comparison with microbial C fixation, bryosphere C fixation showed a different latitudinal
429 pattern with higher C uptake in the three northern sites compared to the two southern sites
430 ($F_{(4,12)} = 6.70$, $P = 0.002$; Fig. 5A). The bryosphere fixed on average 111.5 mg CO₂.h⁻¹.m⁻². The microbial
431 C fixation normalised by Chl *a* showed that phototrophic microbes fixed nearly four times more C than
432 the bryosphere for a given amount of Chl *a* ($F_{(1,48)} = 79.15$, $P < 0.001$; Fig. 5B). We further estimated
433 that phototrophic microbes contributed by 9.4% (1.1% - 37.5%) to the bryosphere C fixation across all

434 sites (Fig. 5C). This contribution ranged on average from 4.1% in Siikaneva to 12.4% in Abisko (Fig. 5C),
435 but differences among sites were not significant ($F_{(4,12)} = 1.44$, $P = 0.27$).

436

437

438 **4 DISCUSSION**

439 Microbial communities play an important role in maintaining multiple ecosystem functions that are
440 important for C cycling. Whilst methanogens, fungi and other bacteria are known for their key role in
441 C mineralization and respiration (e.g., (Galand et al., 2005; Myers et al., 2012; Thormann, 2006)), we
442 show that, across a wide range of climatic conditions, phototrophic microbial communities are
443 important for peatland C dynamics through their contribution to C uptake. Our study revealed that
444 phototrophic microbes were phylogenetically diverse and highly abundant in peatlands throughout
445 the latitudinal gradient. The structure and abundance of phototrophic microbial communities varied
446 along the latitudinal gradient, driven by shifts in biotic and abiotic environmental factors (e.g., climate,
447 plant composition). Despite microbial species turnover, our findings demonstrated a constant
448 microbial CO₂ fixation along the gradient. Our results therefore provide the first *in situ* evidence for
449 the role of phototrophic microbes in peatland C uptake at a continental scale.

450

451 **4.1 Phototrophy is a dominant microbial process in surface peat**

452 On average, we found 7.5×10^7 microbial phototrophic cells per gram of dry moss in the top 3 cm of
453 the *Sphagnum* carpet. This number is in line with previous studies, where phototrophic microbial
454 abundance typically ranged between 10^6 and 10^8 cells per gram of dry moss (Basińska et al., 2020;
455 Jassey et al., 2015). We found that phototrophic microbes represented an approximate biomass of 1.0
456 mg C per gram of dry moss. This is, by comparison, higher than the heterotrophic bacterial biomass
457 (approximately 0.5 mg C per g DW), or the biomass of heterotrophic protists (approximately 0.15 mg
458 C per g DW) found in peatlands at the same depth (Jassey et al., 2011, 2015; Mitchell et al., 2003).
459 Phototrophic microbes constitute therefore the most important component of microbial C biomass in

460 the apical part of the *Sphagnum* carpet, which suggests that phototrophy is a dominant microbial
461 process in the upper peatland layer.

462 In terms of community composition, we found that, on average, 12% of prokaryotic sequences
463 and 30% of micro-eukaryotic sequences belonged to phototrophic microbial lineages. These relative
464 proportions were unexpectedly high, especially compared to previous meta-barcoding studies where
465 phototrophic microbes represented less than 1% of prokaryotes (Bragina et al., 2012, 2014) and only
466 10% of micro-eukaryotes (Geisen et al., 2015; Heger et al., 2018). These high relative abundances of
467 phototrophic microbes most probably result from our experimental design. We indeed sampled the
468 top 3 cm of the *Sphagnum* carpet, where light conditions are favourable for phototrophic microbial
469 abundance and activity (Jassey et al., 2013; Reczuga et al., 2020; Robroek et al., 2009).

470 Our results further revealed that both communities of phototrophic bacteria and protists were
471 relatively more abundant in peatlands than in other terrestrial ecosystems (Cano-Díaz et al., 2019;
472 Oliverio et al., 2020). This alters our perception of the global distribution of soil phototrophic microbes,
473 as until now, drylands were considered as the main phototrophic microbial hotspots (Bates et al., 2013;
474 Oliverio et al., 2020). These findings therefore highlight the potential importance of peatland
475 phototrophic microbes for the global C cycle.

476

477 **4.2 Peatland phototrophic communities are diverse and environment-specific**

478 We identified 351 phototrophic microbial OTUs over the gradient, belonging to diverse prokaryotic
479 and eukaryotic taxonomic clades. While phototrophic communities were relatively dominated by
480 Chlorophyta (micro-eukaryote) and Alphaproteobacteria (prokaryotes), we nevertheless identified
481 seven phyla of phototrophic micro-eukaryote, and four out of the seven existing phyla of phototrophic
482 prokaryotes (Zeng et al., 2014). In link with this high taxonomic diversity, we identified a wide range of
483 photosynthetic pathways. Within micro-eukaryotes, we found strict phototrophic organisms (e.g.,
484 phototrophic Ochrophyta, Chlorophyta) and mixotrophic organisms (e.g., endosymbiotic Ciliophora,
485 Lobosa). Within prokaryotes, the OTUs identified formed a continuum between photoautotrophy and

486 photoheterotrophy (Kulichevskaya et al., 2014; Yurkov et al., 1993), with uncertainty about the levels
487 of autotrophy. For instance, Cyanobacteria can acquire organic C and downregulate their
488 photosynthesis when in symbiosis with other plants (Black & Osborne, 2004), while some phototrophic
489 Proteobacteria can grow both photoautotrophically and photoheterotrophically (Tang et al., 2011).
490 Even though the occurrence of Cyanobacteria and phototrophic Proteobacteria is generally accepted
491 in peatlands, they remain poorly explored and this calls for a deeper consideration of their role in
492 peatland C cycling.

493 Beyond the high taxonomic diversity, our results revealed that phototrophic microbial
494 communities were site-specific due to a high species turnover across sites. Species turnover was largely
495 driven by water availability (precipitation and *Sphagnum* water content), plant cover and dissolved
496 organic C. This indicates that complex interactions among climate, *Sphagnum* properties and
497 vegetation determine the community structure of phototrophic microbes in peatlands. For instance,
498 by reducing light availability at the *Sphagnum* surface, vascular plant cover could structure
499 phototrophic microbial communities as shown in other terrestrial systems such as grasslands or alpine
500 deserts (Davies et al., 2013; Řeháková et al., 2017). Moreover, this close link between phototrophic
501 microbial and plant communities suggests that plants affect phototrophic microbes through the
502 nutrients and numerous allelochemical compounds they release in their surrounding environment, by
503 favouring or inhibiting specific taxa (Asao & Madigan, 2010; Hamard et al., 2019; Řeháková et al., 2017;
504 Stoler & Relyea, 2011; Sytiuk et al., 2021).

505

506 **4.3 The microbial C fixation depends on the community structure of phototrophic microbes**

507 Our structural equation modelling revealed the links by which species turnover affected microbial C
508 fixation rates. We identified two indirect pathways: first, microbial community structure influenced C
509 fixation rates through an alteration of microbial abundance; and second, through an alteration of
510 community-weighted mean body size and individual-level photosynthetic rates. Both pathways were
511 antagonists: when the phototrophic community structure shifted and induced an increase of the

512 microbial abundance, it also led to a decrease of the community-weighted mean body size and a
513 decrease of the individual-level photosynthetic rates. This compensation resulted in constant C fixation
514 rates across the gradient. This result is in line with previous studies where isometric laws between
515 body size, metabolic rates and abundance of phytoplankton have been found (Cermeño et al., 2006;
516 Huete-Ortega et al., 2012). Altogether, these findings suggest that the total energy processed by
517 phototrophic microbes in peatlands for C fixation was constant across our large latitudinal gradient.

518 The influence of microbial community structure on C fixation rates could be related to specific
519 mechanisms on the two identified pathways. For instance, the presence of certain species, such as
520 colonial phototrophic microbes, could increase the abundance of phototrophic microbes (Finkel et al.,
521 2010). Some colonial phototrophic species (e.g. *Anabaena* sp.) can indeed form long filaments
522 composed by numerous cells (Gilbert & Mitchell, 2006). These filaments are hardly eaten by grazers
523 (DeColibus et al., 2017), thus favouring phototrophic abundance. In addition, the community-weighted
524 mean body size, and hence, the size of the photosynthetic apparatus at the individual level (Finkel et
525 al., 2010; Kalchev et al., 1996), depends on the phototrophic species present in the community.
526 Therefore, communities composed of larger phototrophic microbes had higher individual-level
527 photosynthetic rates, as was the case in the northern sites, where large mixotrophic species were
528 present. This result suggests that mixotrophic species are key organisms by their size, driving microbial
529 photosynthesis rates and C uptake in peatlands. This is in line with previous studies on the contribution
530 of mixotrophic testate amoebae to bryosphere C fixation (Jassey et al., 2015). It also echoes
531 observations in oceanic environments where mixotrophic plankton plays a paramount role in
532 ecosystem primary productivity (Ward & Follows, 2016; Worden et al., 2015).

533

534 **4.4 Implications of microbial C fixation for peatland C dynamics**

535 Phototrophic microbes fixed on average 8.8 (3.8 – 16.2) mg CO₂ h⁻¹ m⁻² across sites, which represented
536 on average 9% (4% - 12%) of the total bryosphere C fixation. This contribution might seem relatively
537 high considering the low C biomass of phototrophs in *Sphagnum* (1 mg C.g⁻¹ DW). Yet, the

538 photosynthetic rate per chlorophyll content of phototrophs is four to ten times higher than for
539 *Sphagnum*. It explains why phototrophic microbes contribute significantly to the bryosphere C fixation
540 despite their small size and biomass. It further indicates that the small fraction of C found in
541 phototrophic biomass does not reflect the amount of C they fix. This result is in line with a previous
542 study suggesting that Cyanobacteria and green algae can display a 50-fold higher efficiency in CO₂
543 fixation compared to plants due to their faster growth rate (Rossi et al., 2015).

544 Regarding the proportion of C fixed by phototrophic microbes, we argue it is very likely that
545 phototrophic microbes play an important– and until now overlooked– role in peatland C cycling.
546 Assuming that our data are representative for northern peatlands, we roughly estimated that
547 phototrophic microbes would fix a global amount of *ca.* 75 MT C per year in northern peatlands
548 (Supplementary Methods, Supplementary Table S7). By comparison, this amount is about the same
549 magnitude as C loss from northern peatlands in response to climate warming (Dorrepaal et al., 2009).
550 We note that our estimates of microbial contribution to annual peatland C uptake are based on several
551 assumptions that might lead to uncertainty. For example, seasonal climatic variation in microbial
552 phototrophic activity could influence the values that we present here. In particular, water availability
553 and *Sphagnum* water content vary over the year, and thus shape the community structure and C
554 fixation rates of phototrophic microbes accordingly. Yet, phototrophic microbial communities respond
555 fast to water availability changes in peatlands (less than two weeks, Reczuga et al., 2018). We thus
556 believe that our estimations are representative of the summer conditions at the time we sampled.
557 Moreover, phototrophic microbial abundance have been shown to peak in spring and autumn
558 (Reczuga et al., 2020), when their abundance can double (Rober et al., 2014). This suggests that the
559 annual C fixation by microbial phototrophs might therefore be higher than expected. We also
560 acknowledge that our fluorescence-based measurements of microbial photosynthesis could introduce
561 some bias in our estimates. In particular, the high proportion of Cyanobacteria in some sites could lead
562 to an underestimation of photosynthetic rates, as chlorophyll fluorescence measurements are often
563 underestimated in Cyanobacteria due to their prokaryotic nature (Ogawa et al., 2017; Schuurmans et

564 al., 2015). We however did not observed this effect in our samples (Supplementary Fig. S10). Similarly,
565 measurements of chlorophyll fluorescence do not take into account the photosynthesis performed by
566 the bacteriochlorophyll of phototrophic prokaryotes. However, microbial C fixation rates estimated
567 using fluorescence were of the same magnitude as C fixation rates quantified using a gas analyser
568 (Supplementary Fig. S11). Finally, we caution that the estimate of peatland surface used for upscaling
569 (Leifeld & Menichetti, 2018) is not restricted to *Sphagnum*-dominated peatlands and includes rich fens.
570 However, *Sphagnum*-dominated peatlands (ombrotrophic bogs and poor fens) represent a high
571 proportion of total peatlands, accounting for instance for more than 65% in North America (Hugelius
572 et al., 2020). Further, phototrophic microbial C fixation in rich fens has been found of either similar or
573 higher magnitude than our estimates (Wyatt et al., 2012). Despite these limitations, our findings clearly
574 underline the importance of phototrophic microbes for peatland C cycle through the large amount of
575 C they fix.

576

577 **4.5 Conclusions and perspectives**

578 The current consensus on C pathways in terrestrial ecosystems is that plants are the only or at least
579 the main route for soil C uptake (Krumins et al., 2013; Liang et al., 2017). Our study questions this
580 assumption and shows that phototrophic microbial photosynthesis could represent about 10% of
581 peatland primary productivity, demonstrating that phototrophic microbes cannot be ignored in
582 peatland C dynamics. Their role remains nevertheless obscure in terms of C sequestration, as the fate
583 of microbial-fixed C in peatlands is currently unknown. The only study to date suggests that microbial
584 fixed C would not promote peatland C accumulation. This study shows that phototrophic microbes in
585 a fen ecosystem release 20 to 40% of the C they fix as exudates (Wyatt & Turetsky, 2015), contributing
586 to DOC concentrations and promoting heterotrophic respiration (Wyatt et al., 2014; Wyatt & Turetsky,
587 2015). The fate of the remaining C fixed (60 to 80%) is however unknown. If less labile than exudates,
588 this C could contribute to peat formation. We show here that our current understanding of peatland
589 C dynamics is missing a large piece of the jigsaw puzzle, highlighting the urgent need to further explore

590 phototrophic microbes. Including microbial C uptake in biogeochemical peatland C models would
591 certainly improve predictions of the peatland C balance under future global changes. Particular
592 attention should be given to seasonal variation across phototrophic microbial communities, and to the
593 fate of the C they fix. The incorporation of these aspects in future ecological models to refine our
594 understanding of peatland C biogeochemical cycles is a worthy challenge.

595

596

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610

611 **CONFLICT OF INTEREST**

612 The authors declare that the research was conducted in the absence of any commercial or financial
613 relationships that could be construed as a potential conflict of interest.

614

615 **AUTHOR CONTRIBUTION**

616 VEJJ conceived the ideas and designed methodology with the help of SH. VEJJ choose the sites with
617 the help of ML, MK, EST, GIR and ED. VEJJ and SH collected the data with the help of MK. SH and VEJJ
618 proceeded to laboratory work with the help of MB, AS, JL and EL. SH analysed the data with the help
619 of VEJJ, EL, MB and AS. SH and VEJJ led the writing of the manuscript with the help of RC and PK. All
620 authors contributed critically to the drafts and gave final approval for publication.

621

622 **DATA AVAILABILITY**

623 Data are available from Figshare (10.6084/m9.figshare.c.5190902).

624

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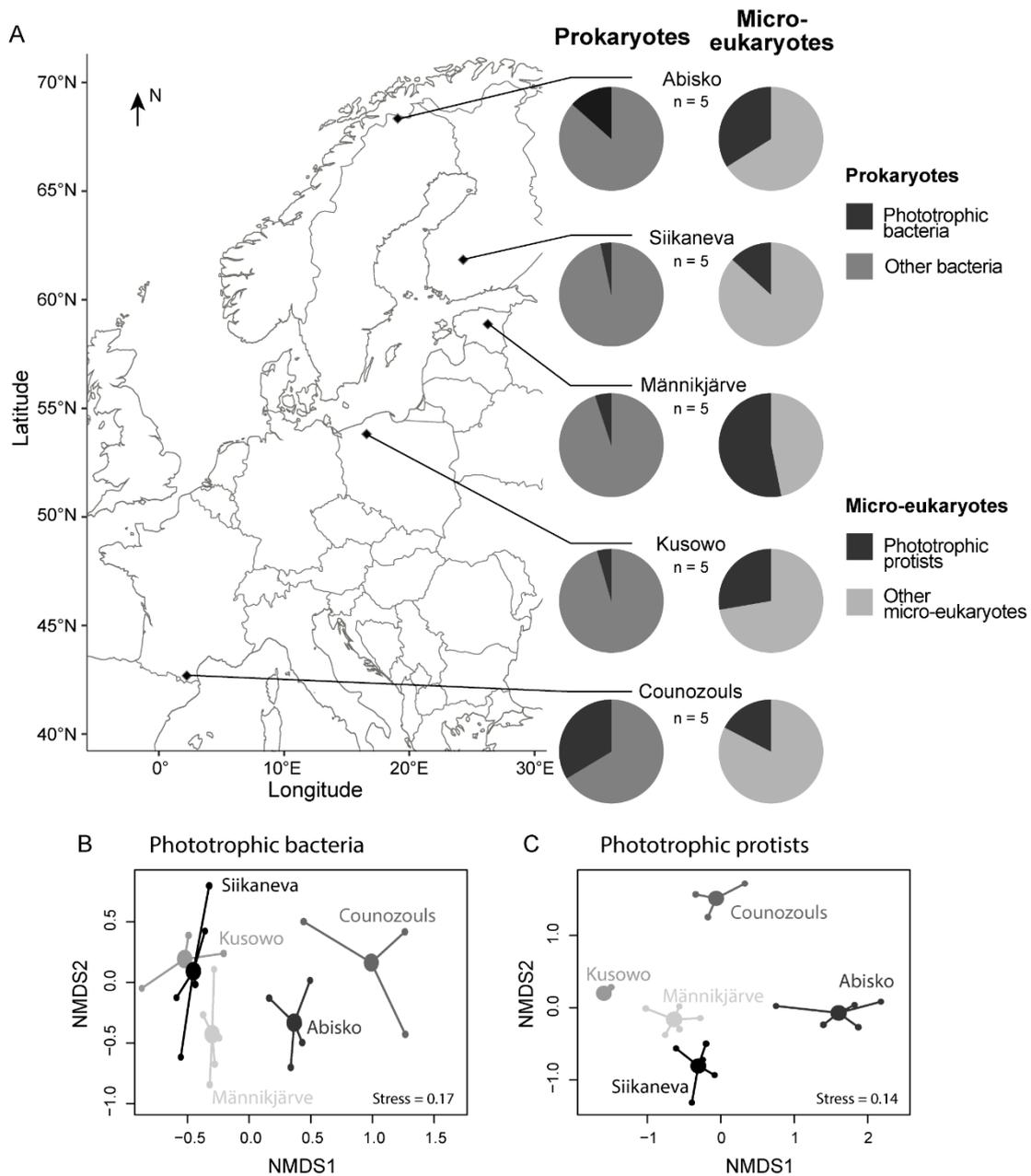
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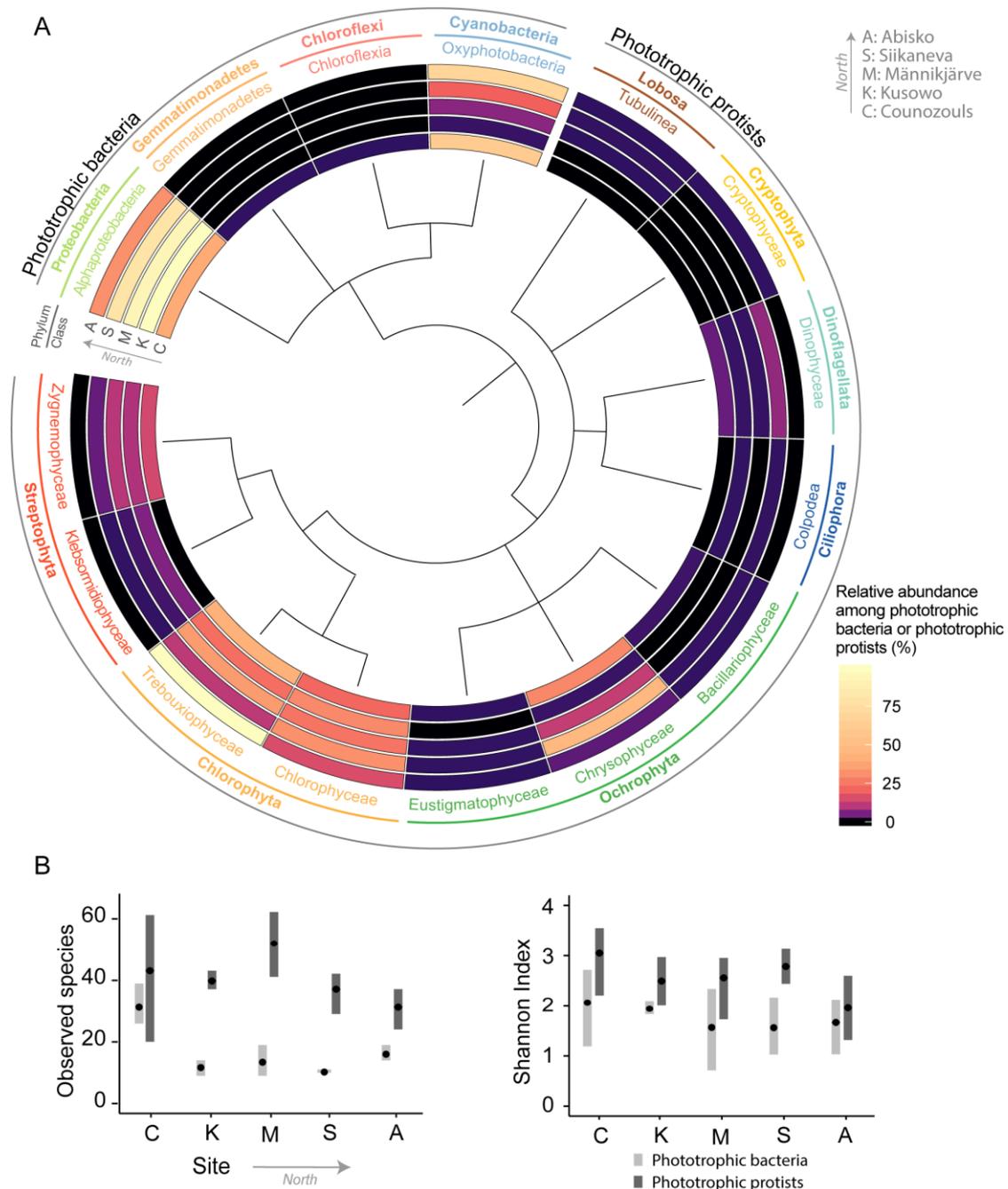
965 **FIGURES**

966 **Fig. 1:** Geographic locations of the five European peatlands and structure of microbial prokaryotic and
 967 eukaryotic communities **(A)**. NMDS of the communities of phototrophic bacteria **(B)** and phototrophic
 968 protists based on phototrophic OTUs **(C)**.



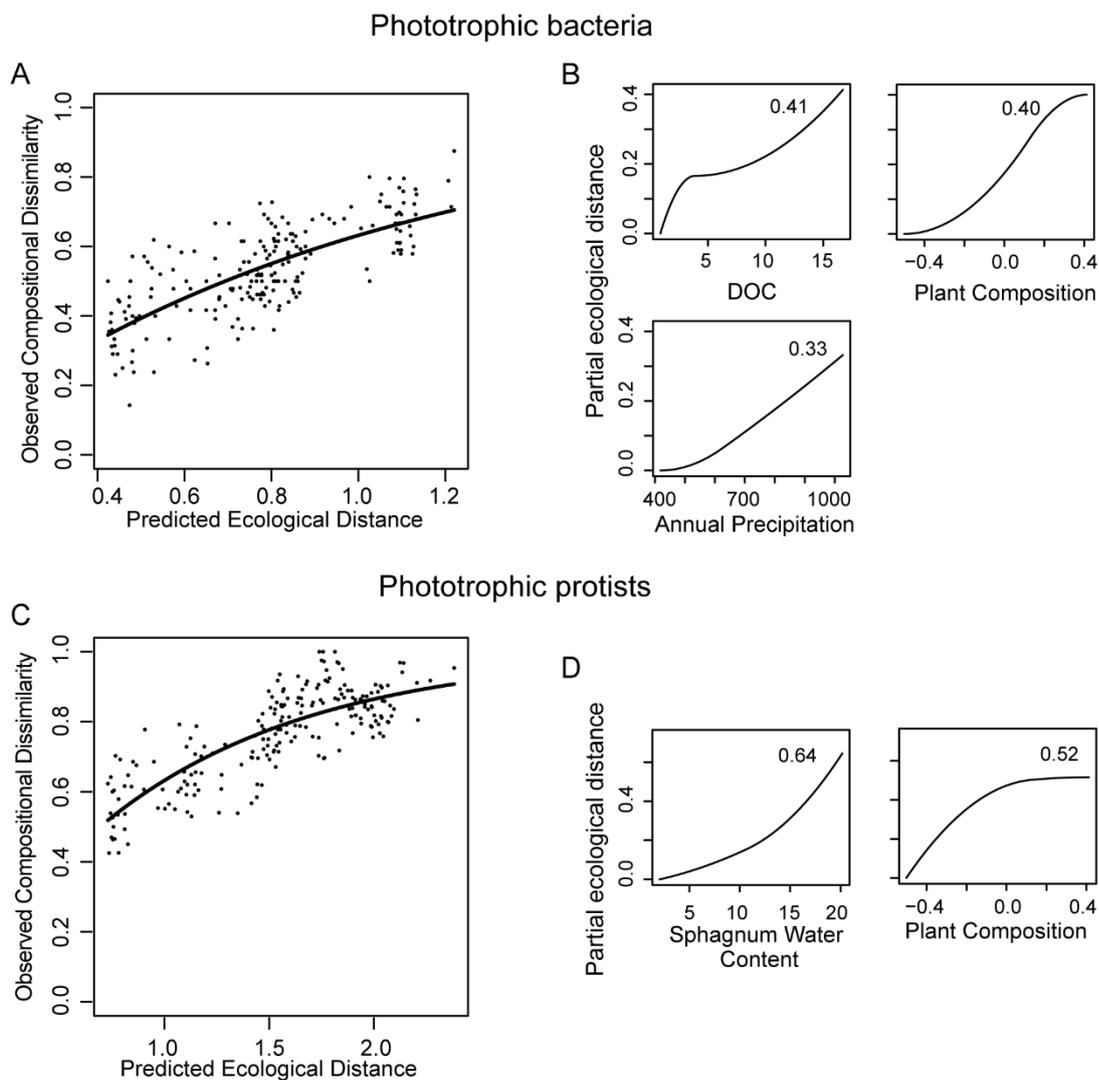
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970 **Fig. 2:** Phylogenetic tree, relative abundance (A), and diversity (B) of the phototrophic OTUs identified
 971 in the five peatland sites along the latitudinal gradient. Phototrophic OTUs include endosymbiotic
 972 mixotrophs (e.g., *Hyalosphenia papilio*, Lobosa). Relative abundance of phototrophic OTUs is
 973 calculated as a percentage of total phototrophic bacteria or total phototrophic protists. Alpha diversity
 974 metrics include observed richness (OTU richness) and Shannon diversity index for both phototrophic
 975 bacteria (light grey) and protists (dark grey). Bars range between the minimum and maximum values
 976 (n = 5 replicates), while points represent the mean value in each site.



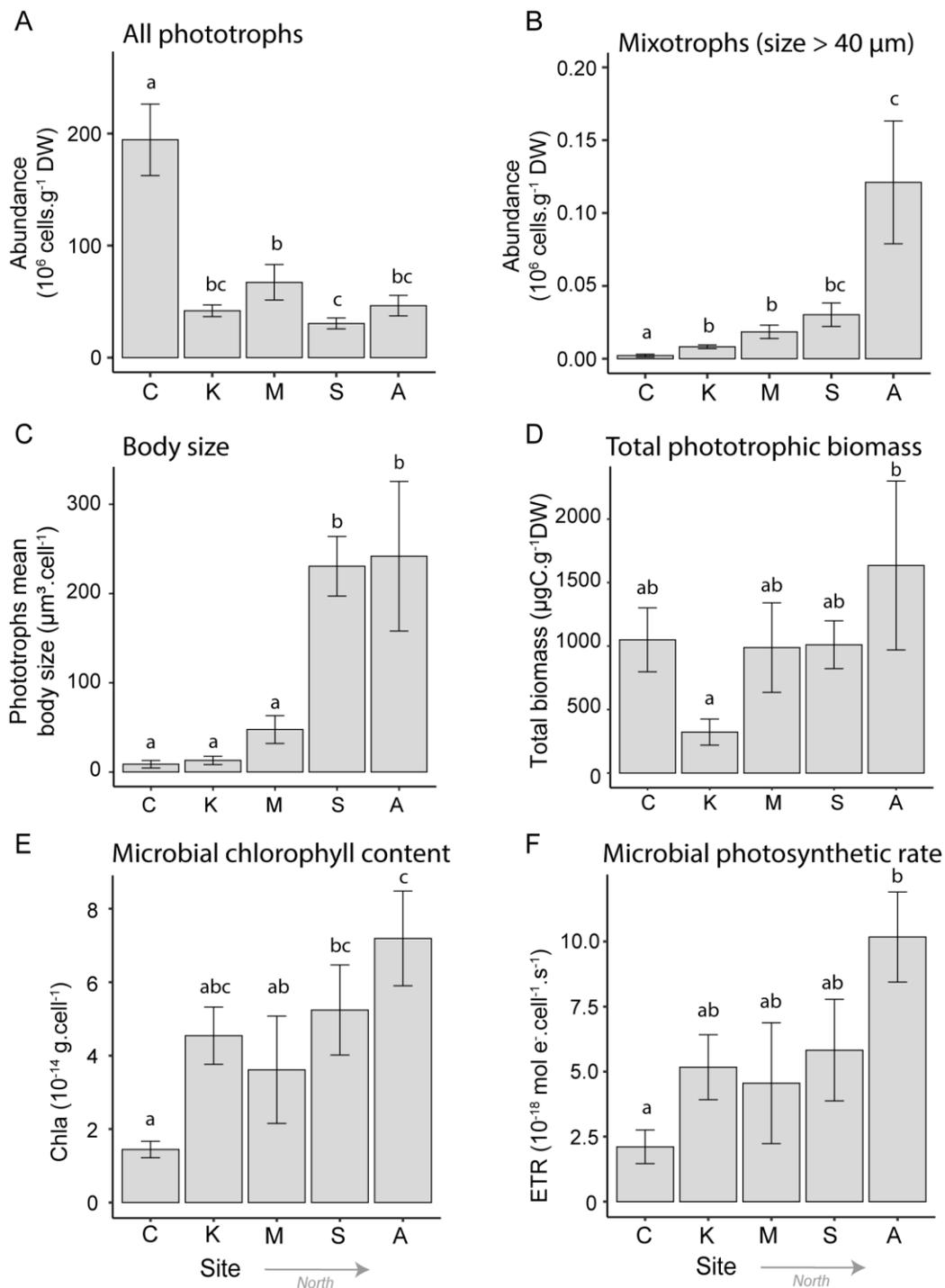
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978 **Fig. 3:** Generalised dissimilarity modelling and taxonomic turnover along environmental gradients.
 979 Relationship between compositional dissimilarity between site pairs (species turnover or beta-
 980 diversity) and their predicted environmental dissimilarity, for phototrophic bacteria communities **(A)**,
 981 and for phototrophic protists communities **(C)**. Partial regression fits (Model-fitted-I-splines) for factors
 982 significantly associated with phototrophic bacteria species turnover **(B)**, and phototrophic protist
 983 species turnover **(D)**. Factors identified with a significant effect on species turnover were Plant
 984 composition, *Sphagnum* Water Content, Dissolved Organic Carbon in *Sphagnum*-extractable water
 985 (DOC) and Annual precipitation. The maximum height (inset number) reached by the I-spline curve
 986 indicates the relative importance of that variable in explaining beta-diversity, keeping all other factors
 987 constant.



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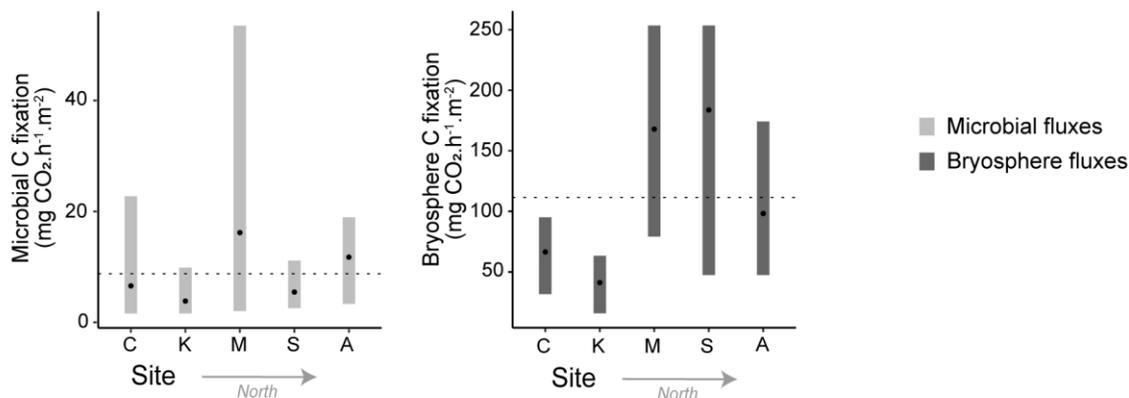
989 **Fig. 4:** Abundance of all phototrophs **(A)** and large mixotrophs (body size > 40 μm) **(B)** in the five
 990 peatlands. Community-weighted mean body size of phototrophic microbes **(C)** and total phototrophic
 991 biomass **(D)** in the five peatlands. Chlorophyll *a* cellular content of phototrophs in the five peatlands
 992 **(E)**. Photosynthetic rates (electron transport rate -ETR- per cell) of phototrophs in the five peatlands
 993 **(F)**. Error bars present standard error (n = 5 replicates). Significant differences ($P < 0.05$) are indicated
 994 by different letters above bars. C: Counouzouls; K: Kusowo; M: Männikjärve; S: Siikaneva; A: Abisko.



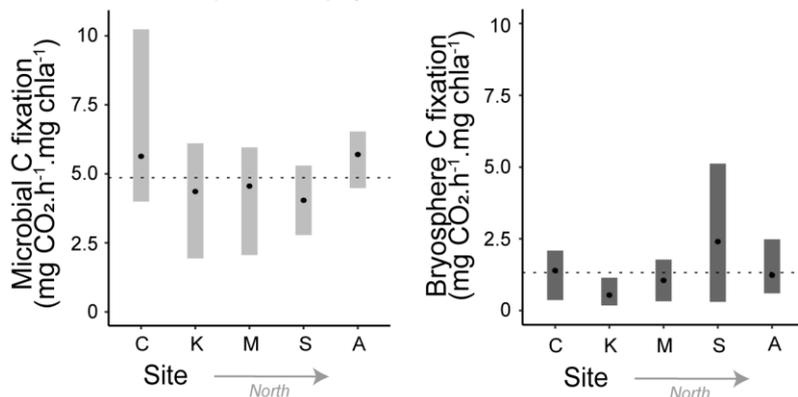
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996 **Fig. 5:** Microbial (light grey) and bryosphere (dark grey) C fixation rates, expressed per surface unit **(A)**
 997 or normalised by the chlorophyll *a* content **(B)**; and contribution of microorganisms to bryosphere C
 998 fixation **(C)** in the five peatlands. Bars range between minimum and maximum values (n = 5 replicates).
 999 Points represent the mean values at each site, while the dotted line in panel **(C)** represent the mean
 1000 values across sites. C: Counozouls; K: Kusowo; M: Männikjärve; S: Siikaneva; A: Abisko.

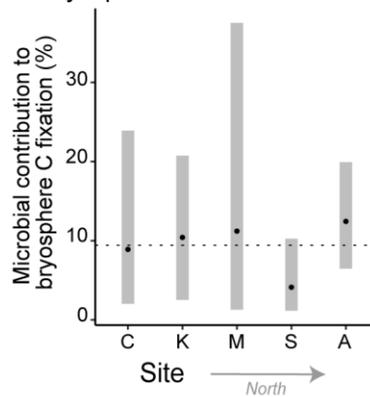
A Carbon fluxes per surface unit



B Carbon fluxes per chlorophyll content

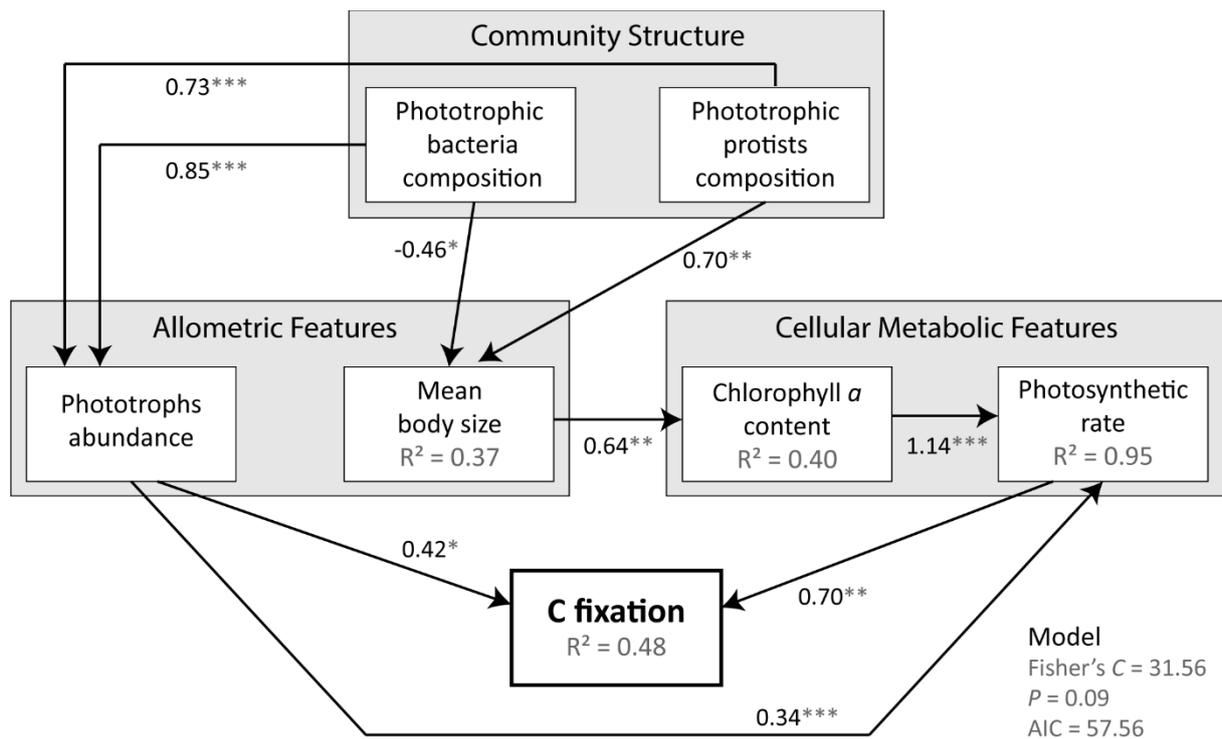


C Microbial contribution to bryosphere fluxes



1001

1002 **Fig. 6:** Structural equation model (SEM) of the correlations between the structure of phototrophic
 1003 microbial communities, their allometric and metabolic features and microbial C fixation. Numbers in
 1004 the boxes indicate the percentage of variance explained by the model (adjusted R-squared), while
 1005 numbers along the arrows indicate the weight of the path relationship (* $0.01 < P < 0.05$; **
 1006 $0.001 < P < 0.01$; *** $P < 0.001$).
 1007



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