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

Author list

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Originality-Significance Statement:

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

Summary

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

Introduction

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

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

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

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

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

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

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

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

Results

Description of the microhabitats

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

Preferred position Table 1

Community structure and environmental preferences of phototrophic microbes

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

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

Preferred position Figure 1

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

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

Preferred position Figure 2

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

Environmental parameters affected the relative abundance of the clusters 3, 4 and 5 (Fig. 3). The cluster 3 was negatively related to *Sphagnum* water content ($F_{(1,16)} = 4.55$, $P = 0.050$), while the cluster 5 was positively related to shadow ($F_{(1,16)} = 43.78$, $P < 0.001$). The cluster 4 was negatively related to *Sphagnum* water content ($F_{(1,16)} = 8.03$, $P = 0.014$) and positively related to water table depth ($F_{(1,16)} = 15.46$, $P = 0.001$).

Preferred position Figure 3

Phototrophic metabolic rates and C fixation

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

Preferred position Figure 4

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

174 $\pm$ 41 ngCO₂.h⁻¹.cm⁻³ on average along the microhabitat gradient. However large discrepancies could be observed between microhabitats ($F_{(5,12)} = 29.66$, $P < 0.001$; Fig. 4d). Microbial C fixation was the highest in hummocks and wooded-hummocks (350 $\pm$ 93 ngCO₂.h⁻¹.cm⁻³ on average), intermediate in *Sphagnum*-pools and lawns (161 $\pm$ 64 ngCO₂.h⁻¹.cm⁻³), and the lowest in pools and forested-ditches (11 $\pm$ 7 ngCO₂.h⁻¹.cm⁻³).

Drivers of microbial photosynthesis

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

Preferred position Figure 5

Discussion

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

Phototrophic microbial diversity and community structure

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

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

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

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

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

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

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

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

Spatial changes in microbial C fixation rates

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

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

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

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

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

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

Consequences for peatland functioning

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

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

Conclusion

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

Methods

Study site and sampling

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

DNA extraction and metabarcoding

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

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

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

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

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

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

Microbial abundance

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

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

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

yield of photosystem II, $\Phi_{PSII}^{(II)}$). Here, a^* was the spectrally averaged chlorophyll-specific absorption 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 ETR was expressed in $\text{mol e}^- \cdot \mu\text{g C}^{-1} \cdot \text{s}^{-1}$.

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

Microbial C fixation rate and contribution to bryosphere C uptake.

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

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

where we assumed a maximum fixation of 0.25 mol CO_2 per mol of electron (Wilken *et al.*, 2013). We took into account the total biomass of phototrophic microbes per cubic centimetre (biomass) and 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 C fixation rates in $\text{g CO}_2 \cdot \text{s}^{-1} \cdot \text{cm}^{-3}$.

Numerical analyses

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

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

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

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

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

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

Data availability

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

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Table and Figure legends

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

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

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

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

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

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