Microbial composition and photosynthesis in Antarctic snow algae communities: Integrating metabarcoding and pulse amplitude modulation fluorometry

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ABSTRACT

Antarctic snow microbial communities are complex biomes formed by different groups of microorganisms that include algae, bacteria, fungi, and archaea. During spring-summer season, abundant microalgae generate red, orange, pink, green and even yellow patches of snow. The presence of microalgae in snow ecosystems is pivotal for carbon fixation and has the potential to decrease snow albedo. Up to now, the relationship between microorganism diversity and functionality in these ecosystems in Maritime Antarctica is not well understood. In the present study, the microbial composition of different types of colored snow was determined by metabarcoding at Fildes Peninsula, King George Island (Maritime Antarctica). Additionally, light use characteristics were assessed to gain insights into the photosynthetic functionality of different algal groups causing snow blooms. Results from this study indicated that green algae of the Trebouxiophyceae and Chlorophyceae classes dominated the eukaryotes. A high abundance of Stramenopiles was also detected. Besides these findings, Principal Component Analysis was employed, revealing taxonomic distance among sampling sites. Bacteroidetes and Proteobacteria were found to be the most abundant groups in the bacterial communities with an absence of taxonomic distance among sampling sites. However, the presence of Flavobacteria and γ-Proteobacteria varied among samples. Photosynthetic parameters determined for dominating algae within the blooms were dependent on the sites and snow colors. All in all, these results show that snow algae blooms at Fildes Peninsula represent a mixture of algal species with different light requirements.

1. Introduction

Snow algae are extremophilic microorganisms that thrive in the snowfields, glaciers of polar regions, and the snow of mid-latitude mountains. During the melting season, extensive snow algal blooms occur in polar and alpine regions due to the increase in photosynthetically active radiation (PAR), nutrients, and liquid water. Snow algae can adjust their predominant pigmentation from chlorophylls to carotenoids, causing a green or reddish coloration. The accumulation of secondary carotenoids is part of the changes in the algal life cycle and a mechanism of protection from high irradiation [1,2]. Along with algal species, these microbial communities also include bacteria, archaea, and fungi [3–5].

The diversity of microbial communities forming blooms in the snow has been evaluated extensively in the Arctic [3,4,6–10], and to a lesser extent, Antarctica [10,11] and mid-latitude mountains [5,10]. A usual approach to assessing algal snow blooms is by metabarcoding hypervariable regions of the 18S rRNA gene, which provides insights into the rough diversity of eukaryotes [4–6,8]. According to several studies, green algae from the Chlamydomonadales order stands out as the principal snow colonizers [4,5,9,10]. The presence of green algae in snow ecosystems is crucial for carbon fixation, and the survival of heterotrophic bacteria [3,12].

Photosynthesis of snow algae has been measured in monospecific blooms of Chlamydomonas nivalis [2], Chloromonas polyptera [13], Chloromonas brevispina [14], Chlamydomonas nivalis [1], and Chlainomonas sp. [15,16]. However, a broader survey of algae-causing colored snow is necessary for determining their functionality in lesser studied areas like Antarctica. This survey is especially pertinent considering the evidence of endemism in some snow algae populations [10,13,17], and their potential to reduce albedo [9,18].

West Antarctic Peninsula (also known as Maritime Antarctica) is a...
region where the snowfields are in close interaction with the marine realm. In these coastal areas, snow algae can play important subsidiary roles, through the melting runoff, food web, and degradation products in the biogeochemical cycles of the whole coastal system [19–21]. Coastal zones of Fildes Peninsula at the south of King George Island (South Shetland Islands, Maritime Antarctica) are covered by seasonal and semi-permanent snow. These areas are exposed to different environmental conditions such as a high nutrient input due to proximity to animal colonies [22] or the presence of light-absorbing impurities (e.g. soot, dust, black carbon) [18]. In contrast, snowfields associated with glaciers show a lower concentration of impurities compared to snow from areas influenced by bird colonies or higher human impact. During summer-season, the presence of snow algae blooms results in the occurrence of green, red, pink and/or orange snow [23], reflecting a diverse cryoflora.

The aim of this study was to gain insights into the microbial diversity and composition of algal blooms occurring in different sites of the Fildes Peninsula. Also, it determines the light use characteristics of the main algal groups in Antarctic snow. To achieve these objectives, we employed a next-generation sequencing technique (metabarcoding) to describe the composition of bacteria and eukaryotes. Finally, we determined the photosynthetic characteristics of snow algae using pulse amplitude modulation fluorometry.

Green and red snow algal blooms can differ in their dominant phyla, pigments, carbohydrates, fatty acids, protein, and metabolites profiles [4,11]. Besides these variations, the bacterial composition of algal blooms may also present geographical separation, as reported in an Arctic snow survey [9]. Thus, we hypothesize that different snow microbial assemblages have different photo-physiological characteristics. We predict that the composition of the microbial community should also vary among sampling sites and snow colors.

2. Materials and methods

2.1. Field sites and sampling

Colored snow fields were sampled at Ardley Island (62°12′34″ S; 58°56′40″ W), Punta Duran (62°11′52″ S; 58°56′35″ W), and Collins glacier (62°10′4″ S; 58°51′17″ W) in Fildes Peninsula, King George Island, South Shetlands, Antarctica, during January 2017 (Fig. 1).

To collect the snow samples, the surface in contact with the air was first scratched using a sterile spoon and discarded. Three samples of red, orange, and green snow from each site were then collected using sterile gloves and a 50 mL centrifuge tube (Falcon). Snow samples were immediately transported to the laboratory of Base Julio Escudero with glaciers show a lower concentration of impurities compared to snow from areas influenced by bird colonies or higher human impact. During summer-season, the presence of snow algae blooms results in the occurrence of green, red, pink and/or orange snow [23], reflecting a diverse cryoflora.

The photosynthetic characteristics comparison was assessed by the Photosynth ETR E FPSII II= × × PowerSoil® DNA Isolation kit 11 (MoBio Laboratories, USA). Extraction was performed following the instructions from the manufacturer, with the exception that the DNA was eluted with 25 μL of Milli-Q water instead of Solution C1. DNA quantification was performed using the Kit Qubit®dsDNA HS Assay Kit and a Qubit 3.0 fluorometer. Samples were normalized to a DNA concentration of 5 ng/μL. Because the extracted DNA had a low concentration, samples of the same color collected in the same site were pooled. Therefore, at each sampling site, one pooled sample of each color was analyzed.

2.3. Amplicon sequencing

Next-generation sequencing libraries and bioinformatic analyses were performed in the AUSTRAL-omics core research facility at the Universidad Austral de Chile (Valdivia, Chile). For bacteria analysis, the hypervariable regions V3–V4 of the 16S rRNA gene were amplified using the specific primers 341F 5′CCTACGGGNGGCWGGCAG3′ and 805R 5′CTACCHVGGGTATCTAAATCC3′. Eukaryotes, the hypervariable region V4-V5 of the 18S rRNA gene were amplified using the primers 528F 5′GGCTAATTCACGGGCTCAAA3′ and 706R 5′AATCTCRAAGATTCACCTCT3′. Additionally, sequences were complemented with the design described in Fadrosh et al. 2014 containing a linker sequence optimized for sequencing on the Illumina, an index sequence and a heterogeneity spacer. The Illumina protocol 16S Metagenomic Sequencing Library was followed for the library construction.

Amplicon sequencing was performed using 250-bp paired-end sequencing on an Illumina MiSeq sequencer (Illumina, San Diego, CA). Raw sequences were filtered according to their quality (q-value > 30), using PRINSEQ software. Finally, paired-end reads were assembled using PANDASeq and sequences were analyzed using QIIME v1.9.1 software.

Cluster identity was 97%, and a minimum of 5 reads was defined to generate an OTU. Removal of OTUs chimeras was performed using the identify_chimeric_seq.py Python script and BLAST as a method of search. For taxonomic annotation of OTUs, the assign_taxonomy.py script, and uclust method were used against the SILVA database v138. Additionally, a further taxonomic assignation of eukaryotic most abundant OTUs (relative abundance > 5%) was determined using BLAST (megablast) against nucleotide collection nr/nt database (Genbank database). Query cover, identity, and E-value were considered for determining the best hits for every OTU. The same was done with bacterial abundant sequences (relative abundance > 5%) that were assigned as cyanobacteria: chloroplast against the SILVA database.

Finally, the alpha diversity analysis was carried out with the alpha_rarefaction.py script using shannon and chao1 as methods (Fig. A1).

2.4. OTUs analysis

After filtering steps, 16S rRNA (Bacteria) and 18S rRNA (Eukarya) sequences generated 1,151,211 and 1,183,680 reads, respectively. Finally, chimeric OTUs were detected and eliminated originating a final count of 10,355 OTUs for Bacteria, and 2,484 OTUs for Eukarya, defined at a 97% similarity (Table A1).

2.5. PAM fluorescence measurements

The photosynthetic characteristics comparison was assessed by electron transport rate (ETR) based on photosynthesis-irradiance curves (PI curves). For this, PSII early photochemical reactions of algae in melted snow were measured using the PHYTO-PAM-II chlorophyll fluorometer (Heinz Walz GmbH, Germany) according to principles described in Jakob et al. 2005 [28]:

\[ ETR = \Phi_{PSII} \times E \times F_I \]

where \( \Phi_{PSII} \) is the effective PSII-quantum yield, \( E \) is the incident actinic
irradiance (16–441 μmol m$^{-2}$ s$^{-1}$) increasing at intervals of 20 s, and \( F_{II} \) is the fraction of chlorophyll associated with photosystem II. The unified factor of 0.5 for \( F_{II} \) was used, assuming that two quanta are required for the transport of one electron in the two photosystems. A modified nonlinear function of Platt et al. 1981 was fitted to obtain \( ETR_{\text{max}} \) (the maximal ETR), \( \alpha \) (the initial slope of the PI curve as an indicator of photosynthetic efficiency) and \( E_k \) (the saturating irradiance of photosynthesis) [29]. Photosynthetic parameters were calculated using the software KaleidaGraph version 4.1 (Synergy Software, Reading, PA, USA).

Based on in vivo chlorophyll a content, the PHYTO-PAM-II can differentiate green algae from cyanobacteria, algae containing phycoerythrin (PE), and algae containing chlorophyll c (Chl-c). The method is based on the excitation of the sample with five measuring light wavelengths (440, 480, 540, 590 and 625 nm), which consider the absorbance spectra of the antenna pigment arrangement for each functional algal group [28].

2.6. Statistical analysis

Comparison of ecological diversity indices among sampling sites and snow colors was performed using 11,743 and 88,522 reads for bacteria and eukaryotes, respectively. Because the sample of orange snow at Ardley Island used for 16S metabarcoding did not reach this minimum number of readings, it was discarded and not included in the analysis.

Richness, diversity, and photosynthetic parameters were evaluated among sites and snow colors using the R package \texttt{stats}, and the Welch Two Sample t-test [30] or two-way ANOVA. Principal component analysis (PCA) was performed in the R environment (v 3.5.1) using the \texttt{prcomp} function (library \texttt{stats}). Figures were generated using the R library \texttt{factoextra}, and the \texttt{fviz_pca_var} and \texttt{fviz_pca_biplot} functions.

Heatmap was constructed using Heatplus package in the R environment, and square root transformed data of relative abundance of most abundant OTUs. Hierarchical Clustering was generated using the default functions \texttt{dist} and \texttt{hclust} (\texttt{stats} v3.6.1 package).

Finally, light curves were graphed, and photosynthetic parameters were calculated using the software KaleidaGraph version 4.1 (Synergy Software, Reading, PA, USA).

3. Results

3.1. Diversity and composition of eukaryotes in snow microbial communities

Alpha diversity indices (Table A2) of snow samples did not present any significant difference among sampling sites or snow color (two-way ANOVA, \( p \)-value > .05).

The composition determined by metabarcoding revealed that eukaryotic microorganisms were represented by six major groups (Fig. 2A). The most abundant group was \textit{Chloroplastida}, with an average relative abundance of 47%. \textit{Stramenopiles} were present in all sites and snow samples with a mean relative abundance of 17% but exhibited higher relative abundances (> 50%) in red and orange snow samples from Ardley Island. Besides this finding, the \textit{Rhizaria}, \textit{Alveolata}, \textit{Nucleomycota}, and \textit{Holoza} were found to be the least abundant groups with an average relative abundance lower than 10%. Finally, 21% of eukaryotic taxa were unassigned.

PCA analysis of eukaryotic taxa indicated that the two principal components represent the 90.3% of the total variability among samples, with the \textit{Chloroplastida}, \textit{Stramenopiles}, and Unassigned taxa groups contributing to the most variability (Fig. 3A). Although the data set is limited, it was possible to outline some taxonomic separation among sampling sites. \textit{Chloroplastida} algae, for example, were found to be
abundant at Collins glacier, while plenty of Stramenopiles algae and Unassigned taxa were found at Ardley Island and Punta Duran, respectively.

A deeper taxonomic assignation of prevailing OTUs (relative abundance > 5%) showed that snow algae blooms at Fildes Peninsula might be either monospecific-like the orange snow from Ardley Island (Fig. 4, Table A3) - or consists of two or more algal groups. Among the Chlorophyta classification, *Chloromonas* spp. were present in four of the nine samples analyzed (Fig. 4) with a relative abundance of 37 to 58% of the entire population. Microalgae of the genus *Chlorella* and *Sanguina* spp. were present in two snow samples with abundances ranging between 10 and 14% and 6–12%, respectively.

Interestingly, at Ardley Island algae of the genus *Hydrurus* (*Chrysophyceae*) were abundant in red and orange snow samples (relative abundances of 66% and 98%, respectively). *Chromulina* spp., another alga of the class *Chrysophyceae*, was present in green snow of Punta Duran exhibiting a relative abundance of 37 to 58% of the entire population. Microalgae of the genus *Chlorella* and *Sanguina* spp. were present in two snow samples with abundances ranging between 10 and 14% and 6–12%, respectively.

Interestingly, at Ardley Island algae of the genus *Hydrurus* (*Chrysophyceae*) were abundant in red and orange snow samples (relative abundances of 66% and 98%, respectively). *Chromulina* spp., another alga of the class *Chrysophyceae*, was present in green snow of Punta Duran exhibiting a relative abundance of 6%. Finally, some taxa of the phylum *Chlorophyta*, found in orange and red snow samples from Collins glacier, and green and red snow samples from Ardley Island, could not be assigned deeply and thus were described as Uncultured alga in the GenBank database.

3.2. Bacterial composition of Antarctic snow

With regards to bacterial composition (Fig. 2B), *Flavobacteria*, *Sphingobacteria*, and *γ-Proteobacteria* being the most abundant bacterial groups each, reached an average relative abundance of 15% from the total population. Considering the contribution of *α-Proteobacteria* (4%)
and β-Proteobacteria (13%), Proteobacteria was the most abundant phylum with an average relative abundance of 32%.

Cyanobacteria OTUs were present in all samples and were more abundant in red snow from Punta Duran (17%) and Collins Glacier (32%). However, a deeper taxonomic assignation against SILVA database identified them as chloroplast. Further BLAST analysis results of abundant OTUs (relative abundance > 1%) identified as cyanobacteria by metabarcoding are presented in Table A4.

Finally, PCA analysis of bacterial taxa indicated that the two principal components explain 77.5% of total variability among samples and that Flavobacteriia and γ-Proteobacteria are the taxa with the highest contribution (Fig. 3B). No taxonomic distance among sampling sites was observed in bacterial communities, except for γ-Proteobacteria abundant in red/orange snow from Ardley Island.

3.3. Photosynthetic characteristics

Photosynthetic parameters of snow algae were determined using multiwavelength-excitation pulse amplitude modulation (PAM) fluorometry. Values of ETR$_{\text{max}}$, α, and E$_k$ of algal groups identified by metabarcoding are shown in Table 1.

Green algae exhibited ETR$_{\text{max}}$ values between 17.6 ± 7.7 and 55.8 ± 7.1 (μmol photons$^{-} m^{-2} s^{-1}$), α values between 0.10 ± 0.01 and 0.28 ± 0.01 (μmol photons$^{-} m^{-2} s^{-1}$) (μmol m$^{-2} s^{-1}$)$^{-1}$, and E$_k$ values between 126.4 ± 67.5 and 311.4 ± 61.5 (μmol m$^{-2} s^{-1}$).

Photosynthetic parameters of green algae showed differences among snow colors (green, orange, and red) and sampling sites (Table A5). Considering color as a factor within sampling sites, at Collins glacier, quantum efficiency (α) was significantly higher in red snow compared with orange snow. The same was observed at Ardley Island, where α was significantly higher in red snow compared with green snow. Instead, at Punta Duran, the parameters ETR$_{\text{max}}$ and E$_k$ were higher in red snow compared with green snow.

Photosynthetic parameters of red-snow algae varied significantly among sampling sites. The highest value of α was recorded at Ardley Island, and the lower value at Collins glacier. Additionally, the parameters E$_k$ and ETR$_{\text{max}}$ were higher at Punta Duran compared with Ardley Island and Collins glacier. In algae from green snow, differences in ETR$_{\text{max}}$ and E$_k$ were detected between Ardley Island and Punta Duran, being higher at Ardley.

Finally, Chl-c algae exhibited an average value of 57.5 ± 9.8 for ETR$_{\text{max}}$, 0.27 ± 0.00 for α, and 214.2 ± 39.9 for E$_k$.

4. Discussion

The diversity and composition of Antarctic snow microbial communities are influenced by the geographical characteristics (isolation of the Antarctic continent), long-range atmospheric transport, ocean currents, birds, and human vectors [31]. The present study assessed the diversity of snow algae and bacteria at Fildes Peninsula (King George Island, Maritime Antarctica) a region with important bird, and anthropogenic influence [32–35].
4.1. Snow algae diversity and composition at Fildes Peninsula

Previous studies performed at different sites in the Arctic region suggested that the structure of snow algae is conserved [9]. However, our results revealed that there is a taxonomic distance between eukaryotic organisms found in the relatively close sampling sites of the Maritime Antarctica. Another factor related to algal composition is the color of its bloom. Davey et al. 2019 reported that this feature differs between bloom types (green and red) [11], but in our case, the color of the bloom seems to affect the community structure to a lesser extent than the geographic separation (Fig. 3). Finally, our results show that regardless of the composition of the eukaryotic community, algal diversity (Chao 1 and Shannon) indices did not show differences among sampling sites or among blooms with a different color.

Within the Chloroplastida clade we were able to detect algae of the genus *Sanguina*, cosmopolitan microorganisms common in snowfields worldwide [36]. In addition to this, we also identified both *Chloromonas* and *Chlorella* algae in concordance with previous studies that detected these microorganisms along the Antarctic Peninsula and continental Antarctica [11,13,37], highlighting their wide distribution. Finally, uncultured algae (*Chlorophyta*) identified earlier in red snow from Antarctica and the Arctic [10] were detected in samples from Collins glacier and Ardley Island.

*Stramenopiles* algae were detected in orange and red snow samples collected from Ardley Island. It has been reported that algae of the *Hydrurus* genus grow in snowpack influenced by strong meltwater streams forming blooms with a yellowish-brownish coloration due to the presence of xanthophylls [38,39]. In the case of red snow samples, the presence of *Chlorophyta* algae probably masked the coloration caused by the *Hydrurus* algae. However, in the case of the orange snow, the relative abundance of *Hydrurus* genus was close to 96%, which contrasts with the dominance of *Chloromonas polyptera* species reported by Remias et al. in snow that exhibited this coloration in Maritime Antarctica [13]. A possible explanation for this observation is that the low pigmentation of snow samples from Ardley Island (Fig. A1) made it difficult to distinguish between light orange and yellow coloration.

A large proportion of unassigned taxa was detected at Punta Duran, especially in green and orange snow samples. In addition, at this site, snow samples presented a higher abundance of *Bacillariophyceae* (relative abundance ~1%) compared to the other sampling sites (Fig. A1). Also, plenty of ciliates (*Alveolata*) were detected in orange snow samples from Punta Duran. A high relative abundance of these microorganisms was previously reported by Davey et al. in green and red snow samples from Ryder Bay, Antarctic Peninsula [11].

Finally, an important consideration about our results is that the sequencing of the hypervariable regions V4-V5 of the 18S RNA gene, and an OTU clustering at 97% of similarity, may hide several species of green algae in one OTU. This is because some algal species could share up to 100% identity of this marker [40]. Therefore, we cannot discard a higher variability of green algae at species level, which was not described in this study. To improve the resolution of amplicon sequencing, a multi-marker approach (e.g. 18S + ITS2), and the use of methods like metaproteomic approaches in water samples from coastal areas of East Antarctica, it has been established that *Proteobacteria* can degrade labile compounds produced by *Flavobacteria* from algal-derived polymers [51]. Moreover, studies carried out in algal strains cultured in a laboratory suggest that these bacteria can use the available carbon sources produced by snow algae and in turn, promoting their growth [5]. Thus, both bacterial groups take part in the degradation of complex molecules in snow algae blooms.

The presence and abundance of *Cyanobacteria* in our samples remains unclear due to that OTU assignation classified them as chloroplasts. The potential absence of *Cyanobacteria* in our snow samples, contrasts with previous studies that documented the presence of *Cyanobacteria* in snow from the Arctic region, alpine regions, and Antarctica [54–60].

Finally, despite that snow bacterial diversity has been recorded as a feature dependent on geographic separation [9,61], at Fildes Peninsula bacterial communities did not present taxonomic separation among sampling sites.

4.2. PHYTO-PAM measurements

In principle, the Phyto-PAM fluorometer can determine the chlorophyll content and differentiate upon four algal groups in mixture samples. However, although green algae, cyanobacteria, Chl-c algae, and PE algae were detected through PAM fluorometry (Fig. A2), only green and Chl-c algae were confirmed by metabarcoding analysis. However, PAM fluorometry underestimated the presence of *Chrysophyta* algae (Chl-c algae) in the red snow from Ardley Island.

In the case of PE algae, they were absent at metabarcoding analysis and were detected only in sample FDGS by PAM fluorometry. Although PAM fluorometry indicated the presence of cyanobacteria in all samples, metabarcoding did not support this finding. It has been described in previous studies, that this group presents a high variability in PS II/PS I-ratios [62]. Thus, the discordance in our results might be explained because of the limitation of fluorometry in determining the chlorophyll content of cyanobacteria.

Reliable fluorescence-based Chl estimation using multichannel PAM fluorometry is possible only with the suitable calibration of the Chl/F-ratios of the major algal groups [27]. In the case of snow algae, the use of mono-specific blooms as standards might be an option to perform a suitable calibration and analyze blooms formed by mixtures of algal groups. This approach was used previously in phytoplankton blooms, improving the determination of chlorophyll content [27].

4.3. Photosynthetic parameters

PI-curves of snow algae present at Fildes Peninsula revealed moderate light requirements for photosynthesis of green algae and *Chrysophyta* algae, based on their saturating irradiances values (Eₖ). These values match the maximum Photosynthetically Active Radiation (PAR) measured in the snow column at King George Island in the late summer (February). At this region, the average value of PAR was 435 ± 261.92 (μmol photons m⁻² s⁻¹) at the surface of the snow, and 113 ± 77.92 (μmol photons m⁻² s⁻¹) at 10 cm depth [63]. Due to that our samples were taken removing the first layer of snow (2–3 cm), it is possible to assume that algal species were exposed to mid-light conditions considering these field values as reference. Moreover, at Fildes Peninsula global radiation is low because of the low solar elevation and considerable cloudiness [23]. However, during a sunny day, PAR might reach values as high as 1276 μmol photon m⁻² s⁻¹ at the surface [63]. Therefore, it is possible to affirm that light requirements of the algal mixtures present in our snow samples, were consistent in their relationship to the environmental irradiance of Fildes Peninsula. In addition, the variability of PAR levels at Fildes Peninsula, suggests that the snow algae species at this locality should be quite flexible to cope
with variable light conditions.

The average values of ETR$_{\text{max}}$ (36 ± 15.9 μmol photons$^{-2}$ s$^{-1}$), α (0.18 ± 0.06 (μmol photons$^{-2}$ s$^{-1}$)(μmol photons$^{-2}$ s$^{-1}$)$^{-1}$), and E$_{0}$ (208.4 ± (μmol m$^{-2}$ s$^{-1}$) of green algae at Filides Peninsula, were between the values reported for Chloromonas nivalis (ETR$_{\text{max}}$ = 23.4; α = 0.222; E$_{0}$ = 106 μmol photons m$^{-2}$ s$^{-1}$) [2], and Chloromonas brevispina (ETR$_{\text{max}}$ = 73.4; α = 0.29; E$_{0}$ = 250 μmol photons m$^{-2}$ s$^{-1}$) [14]. Both of these algal species were described previously, like algae adapted to low-mid irradiance conditions [2,14]. Our results show that the photosynthesis of snow algae (green algae) described in this study, is less efficient at low light compared with the Chloromonas species cited above.

All in all, photosynthetic characteristics of snow algae communities varied significantly among sampling sites. Due to that the three studied sites differed in closeness to animal colonies, water content, and light transmittance in the snow [18], it is possible to argue that light requirements of snow algae might have been influenced by particular abiotic factors of the sampling sites. Moreover, within sampling sites, differences among types of blooms (green, orange, red) were also detected. These results might be related to the variable composition of snow algae communities within the sampling site. Thus, different microbial assemblages were characterized by different photo-physiological performances.

5. Conclusions

In this study, we conclude that snow algae at King George Island, Maritime Antarctica were dominated by a mixture of green algae including Trebouxiapectyceae and Chlorophyceae.

However, despite the dominance of green algae, Stramenopiles of the Hydrurus genus formed almost monospecific blooms in orange/yellow snow from Ardley Island. Additionally, they were found to be growing together with the green algae in the red snow at this site. Thus, a taxonomic distance among sampling sites was detected.

In contrast to the algae community, the bacterial community structure remained conserved among sampling sites. Therefore, we concluded that the distribution of bacteria was not affected by geographical separation at the spatial scale measured in this study.

The use of multi-channel PAM fluorometry offers the possibility of monitoring different algal groups in snow samples. However, this technique may result in an underestimation of the chlorophyll content by mixtures of algae.

Photosynthetic parameters of snow algae at Filides Peninsula were consistent with the prevailing light availability. Moderate light requirements of green algae and Hydrurus algae were determined at this region characterized by variable light conditions due to the cloudiness, and sometimes clear sunny days.

Although photosynthesis varied among sampling sites and snow colors, no clear patterns were found. All in all, physiological adaptation reflected in different light requirements suggested that there is a functional difference between different microbial communities.

Declaration of competing interest

Authors declare no conflict of interest.

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