



Genus richness of microalgae and Cyanobacteria in biological soil crusts from Svalbard and Livingston Island: morphological versus molecular approaches

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Abstract

Biological soil crusts (BSCs) are key components of polar ecosystems. These complex communities are important for terrestrial polar habitats as they include major primary producers that fix nitrogen, prevent soil erosion and can be regarded as indicators for climate change. To study the genus richness of microalgae and Cyanobacteria in BSCs, two different methodologies were employed and the outcomes were compared: morphological identification using light microscopy and the annotation of ribosomal sequences taken from metatranscriptomes. The analyzed samples were collected from Ny-Ålesund, Svalbard, Norway, and the Juan Carlos I Antarctic Base, Livingston Island, Antarctica. This study focused on the following taxonomic groups: Klebsormidiophyceae, Chlorophyceae, Trebouxiophyceae, Xanthophyceae and Cyanobacteria. In total, combining both approaches, 143 and 103 genera were identified in the Arctic and Antarctic samples, respectively. Furthermore, both techniques concordantly determined 15 taxa in the Arctic and 7 taxa in the Antarctic BSC. In general, the molecular analysis indicated a higher microalgal and cyanobacterial genus richness (about 11 times higher) than the morphological approach. In terms of eukaryotic algae, the two sampling sites displayed comparable genus counts while the cyanobacterial genus richness was much higher in the BSC from Ny-Ålesund. For the first time, the presence of the genera *Chloroidium*, *Ankistrodesmus* and *Dunaliella* in polar regions was determined by the metatranscriptomic analysis. Overall, these findings illustrate that only the combination of morphological and molecular techniques, in contrast to one single approach, reveals higher genus richness for complex communities such as polar BSCs.

Keywords Biological soil crust · Eukaryotic algae · Cyanobacteria · Morphological identification · Metatranscriptomics

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Introduction

The Arctic region and Antarctica are extreme environments defined by low temperatures year-round, low water availability, and large seasonal fluctuations with long periods of darkness during winter versus continuous irradiance in summer (Thomas et al. 2008a). Thus, plant cover is sparse and biodiversity is generally low, e.g. only two autochthonous flowering plants occur in Antarctica and these are mainly found in a few suitable areas along the coast (Thomas et al. 2008b; Pointing et al. 2015). These ecosystems are instead dominated by biological soil crusts (BSCs), which are the main terrestrial primary producers and also important ecosystem engineers (Evans and Johansen 1999; Yoshitake et al. 2010; Pointing and Belnap 2012; Williams et al. 2017). BSCs are communities of eukaryotic algae, Cyanobacteria, lichens, bryophytes, as well as heterotrophic fungi and bacteria

which colonize the surface and top millimeters of the soil (Belnap et al. 2001a; Belnap 2006; Büdel et al. 2016). Typically occurring phyla of eukaryotic microalgae are Streptophyta (Klebsormidiophyceae, Zygnematiophyceae), Chlorophyta (Chlorophyceae, Trebouxiophyceae, Ulvophyceae) and Ochrophyta (Bacillariophyceae, Eustigmatophyceae, Xanthophyceae) (Büdel et al. 2016). Certain crust organisms, such as Cyanobacteria and eukaryotic algae, form a matrix which aggregates the living cells and soil particles (Belnap 2006; Breen and Lévesque 2008). Hence, BSCs provide protection against soil erosion and cryoturbation (Evans and Johansen 1999). Moreover, BSCs enhance water and nutrient retention and the crust-associated Cyanobacteria fix nitrogen which in turn creates improved conditions (fertilization) for seed germination and plant growth (Belnap et al. 2001b; Breen and Lévesque 2008). This accumulation of biomass forms the nutritional basis for higher trophic levels such as the Svalbard reindeer (*Rangifer tarandus platyrhynchus*; Elster et al. 1999; Cooper and Wookey 2001). In general, BSCs are essential components of sparse ecosystems such as the polar deserts. These communities are valuable biological indicators for abiotic factors, ecological health and climate change (Belnap et al. 2001b; Pushkareva et al. 2016). Due to global warming which particularly affects the Arctic and the Antarctic Peninsula, an invasion by alien species is likely, which in turn might alter the BSC composition (Frenot et al. 2005; Chown et al. 2012; Pushkareva et al. 2016; Lee et al. 2017). Hence, studying individual BSC organisms, as well as the whole community, is important to fully address biodiversity, which is a prerequisite for monitoring changes, as well as for predictions of future climate change (Pushkareva et al. 2016).

The identification of autotrophic microorganisms in BSCs, such as eukaryotic algae and Cyanobacteria, is traditionally performed using light microscopy to examine BSCs in situ, or through established cultures of selected crust organisms (Skinner 1932; Bischoff and Bold 1963; Waterbury and Stanier 1978; Büdel et al. 2009). The taxa of interest are typically identified using morphological traits such as color, cell size and shape, or motility (Prescott 1964; Lind and Brook 1980; Cox 1996; John et al. 2002). The utilization of light microscopy for identification is rather quick and inexpensive compared to molecular techniques (Misawa 1999), but requires expert knowledge for many taxa as morphological features are often difficult to recognize and distinguish (Manoylov 2014). In addition, morphological features are not always stable as they can change in response to environmental factors (Albrecht et al. 2017). As an alternative, a number of molecular markers, so-called *barcodes*, can be used if unialgal cultures are available (Vieira et al. 2016). Typical barcode sequences are the 16S/18S rRNA gene and the internal transcribed spacer (ITS) rDNA, the RuBisCO large subunit (*rbcL*), the plastid elongation factor *tufA*, the

cytochrome oxidase I (COX I), as well as internal simple sequence repeats (ISSR) of microsatellite regions (Buchheim and Chapman 1991; Wilmotte 1994; Doyle et al. 1997; An et al. 1999; Evans et al. 2007; Shen 2008; Sherwood et al. 2008; Hall et al. 2010). A huge pitfall is that the majority of microorganisms present in environmental samples, such as BSCs, are unculturable (Ward et al. 1990; Schloss and Handelsman 2005; Shi et al. 2009; Massana et al. 2014). To overcome the limitation of cultivation, biodiversity assessments can be performed by metabarcoding (Taberlet et al. 2012; Yoon et al. 2016; Elferink et al. 2017). For this purpose, total DNA is extracted from an environmental sample (eDNA) and used as a template to generate an amplicon mixture from a barcoding gene (Taberlet et al. 2012). Subsequently, the generated PCR products are sequenced with a high-throughput sequencing (HTS) technique, such as Roche 454 or Illumina (Margulies et al. 2005; Bentley et al. 2008; Taberlet et al. 2012). The identification of taxa is carried out by annotating the obtained sequence reads against an adequate database and sequence counts provide information about taxonomic abundance in the sample (Pawlowski et al. 2011; Taberlet et al. 2012). However, metabarcoding also exhibits various pitfalls such as the introduction of sequence errors during PCR, the design of suitable metabarcoding primers, covering all taxa of interest, and, again, the need for appropriate reference databases (Taberlet et al. 2012). Fortunately, PCR-dependent bias and the dependency on single barcodes can be avoided by exploiting shotgun metagenomics and metatranscriptomics (Urich et al. 2008, 2014). Similar to metabarcoding, total nucleic acids are isolated from an environmental sample but the amplification step is omitted (Urich et al. 2008, 2014). Instead, total DNA or cDNA is directly applied to HTS and the resulting sequences are assembled for any desired gene or transcript of interest, e.g. the small ribosomal subunit RNA (SSU; Geisen et al. 2015). This powerful approach enables a more reliable taxonomic identification compared to metabarcoding but relies on the availability of correctly determined sequence data (Urich et al. 2008; Klimke et al. 2011). Additionally, the isolation of high-quality nucleic acids from soil samples, suitable for HTS, can be difficult due to the presence of humic substances and exocellular RNase activity (Greaves and Wilson 1970; Rippin et al. 2016).

The present study focused on a methodological comparison of morphological and molecular approaches to analyze the microalgal and cyanobacterial genus richness of Arctic and Antarctic BSCs. Studying polar BSCs improves the knowledge on these microecosystems, their biodiversity and ecological structure. In this study, the identification of various groups of eukaryotic algae (Klebsormidiophyceae, Chlorophyceae, Trebouxiophyceae, Xanthophyceae) and Cyanobacteria in one Arctic and one Antarctic BSC sample was carried out. Eukaryotic and prokaryotic algae were

identified using light microscopy in combination with suitable taxonomic keys. Furthermore, active species in the crust were targeted by applying a metatranscriptomic approach and algal as well as cyanobacterial taxa were identified using fully assembled SSUs. The final results were combined and compared to assess the comprehensiveness of both techniques regarding the genus richness.

Materials and methods

Sampling

BSC samples were collected during expeditions to the Arctic and Antarctica in August 2014 and February 2015, respectively. The Arctic BSC (NÅ; cf. Rippin et al. 2016; Borchhardt et al. 2017; Williams et al. 2017, Jung et al. (in preparation)) was sampled in close vicinity to Ny-Ålesund, Svalbard, Norway (78°55'26.33"N 11°55'23.84"E; Fig. 1a). The research station Ny-Ålesund is located at Kongsfjorden and its climate is classified as polar tundra (ET) according to the Köppen-Geiger system (Peel et al. 2007; Vogel et al. 2012). The temperature is low year-round with an annual average of $-4.5\text{ }^{\circ}\text{C}$, the highest monthly temperature is in July with $5.8\text{ }^{\circ}\text{C}$ and the lowest in March with $-12\text{ }^{\circ}\text{C}$ (all temperatures are averaged from August 1993 to July 2011; Maturilli et al. 2013). The mean annual precipitation is 433 mm (Førland et al. 2011). Williams et al. (2017) found 90% of the area to be covered by BSCs. The Antarctic BSC (Gr1; cf. Williams et al. 2017) was collected close by the Spanish Juan Carlos I Antarctic Base at Livingston Island, South Shetland Islands (62°39'55.44"S 60°23'42.76"W; Fig. 1b). Similar to Svalbard, the South Shetland Islands can be regarded as polar tundra (ET) according to the Köppen-Geiger system (Pereira et al. 2006; Michel et al. 2014). The temperature at the Juan Carlos I Antarctic Base averages $-1\text{ }^{\circ}\text{C}$ annually, with the highest mean temperature ($2.1\text{ }^{\circ}\text{C}$) in austral summer and the lowest mean temperature ($-4.6\text{ }^{\circ}\text{C}$) in winter (all temperatures are averaged from December 2001 to April 2003 and January 2007 to February 2011; Bañón et al. 2013). The site receives on average 444.5 mm of precipitation annually (Bañón et al. 2013). The BSC coverage is approximately 43% with skeletal soils being predominant (Williams et al. 2017). BSC samples for metatranscriptomic analysis (1 g) were preserved using the LifeGuard™ Soil Preservation Solution (MO BIO Laboratories, Carlsbad, CA, USA).

Cultivation and microscopy

Enrichment cultures of eukaryotic algae were established by transferring the sampled BSCs (NÅ, Gr1) onto solid 1.5% Difco™ Agar (Becton Dickonson, Heidelberg,

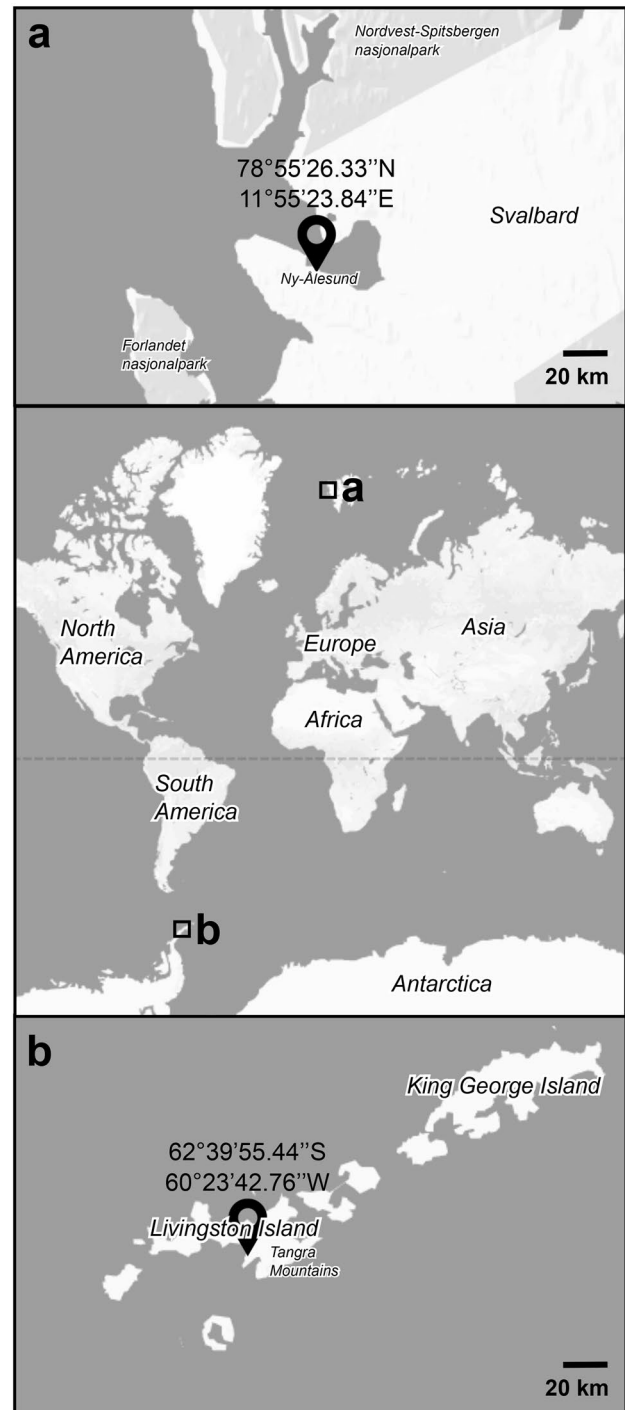


Fig. 1 Overview map of sampling sites in the Arctic and Antarctica. **a** Svalbard in the Arctic. Samples were taken at Ny-Ålesund (NÅ). **b** Livingston Island which is part of the South Shetland Islands. Isolates were collected in close vicinity of the Juan Carlos I Antarctic Base (Gr1). Maps were created using snazzymaps.com

Germany) containing Bold's Basal Medium modified according to Starr and Jeffrey (1993). The cultures were kept at $15\text{ }^{\circ}\text{C}$, $30\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$ under a 16:8-h

light–dark regime. A detailed outline on the procedure is given in Borchhardt et al. (2017b). The identification of genera or species was carried out by light microscopy (BX51, Olympus, Tokyo, Japan) with oil-immersion and a 1000-fold magnification. Relevant identification keys are listed in Borchhardt et al. (2017b). A complete list of the identified Arctic algae (NĀ) was published by Borchhardt et al. (2017a) and is used for comparison in the present study.

Cyanobacterial genera, present in the Arctic (NĀ) and Antarctic (Gr1) BSC were directly determined using light microscopy with oil immersion and a 630-fold magnification using identification keys (Geitler 1932; Komárek and Anagnostidis 1998, 2005). Cyanobacteria were additionally pre-cultivated on BG-11 medium at 15–17 °C, 20–50 μm photons $\text{m}^{-2} \text{s}^{-1}$ under a 14:10-h light–dark cycle and identified using suitable taxonomic keys as described by Williams et al. (2016). The data of Jung et al. (in preparation), which examined the cyanobacterial diversity of both sites, were compared to the molecular dataset.

RNA isolation and sequencing

The Arctic sample (NĀ) was processed according to Rippin et al. (2016) using the CTAB protocol, DNase I (Thermo Fisher Scientific, Waltham, MA, USA) and the RNeasy MinElute Cleanup Kit (Qiagen, Hilden, Germany). Due to low RNA yields, a total of six biological replicates were extracted and combined to obtain three pooled replicates. RNA from three biological replicates, collected at Livingston Island (Gr1), was isolated using the Spectrum™ Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO, USA), treated with DNase I (Thermo Fisher Scientific, Waltham, MA, USA) and purified using the RNeasy MinElute Cleanup Kit (Qiagen, Hilden, Germany) as described by Rippin et al. (2016). Single samples yielded sufficient amounts of RNA.

All RNA samples were further processed by Eurofins Genomics (Ebersberg, Germany). The processing included quality control utilizing the BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) and library preparation for both triplicates. Eukaryotic mRNA was enriched using oligo-(dT) beads, fragmented and, subsequently, cDNA was synthesized using random hexamers. Finally, Illumina compatible adapters were ligated. The libraries of the individual samples NĀ and Gr1 were applied to an Illumina HiSeq 2500, all triplicates multiplexed on one lane, using 125 bp paired-end and single-end mode, respectively. For sample NĀ, the HiSeq Control Software 2.2.58, RTA 1.18.64 and bcl2fastq-1.8.4 were used while the detected signals from sample Gr1 were processed by operating the HiSeq Control Software 2.2.38, RTA 1.18.61 and bcl2fastq-1.8.4 (Illumina, San Diego, CA, USA).

Bioinformatics

The obtained raw reads (SRA bioproject PRJNA421095) were quality trimmed using Trimmomatic 0.35 (Bolger et al. 2014) and rRNA gene reads were filtered out using SortMeRNA 2.1 (Kopylova et al. 2012) and the SILVA SSU NR Ref 119. All rRNA SSU reads were assembled using EMIRGE 0.61.0 (Miller et al. 2011) combined with the SILVA SSU NR Ref 128. The script `emirge_amplicon.py` for single-end reads with the flag `phred33` was run. Only successfully assembled SSUs were considered. The assigned genera for Klebsormidiophyceae, Chlorophyceae, Trebouxiophyceae, Xanthophyceae and Cyanobacteria were extracted and compiled with an in-house *R*-script (Version 3.3.2).

Results

The genus richness of phototrophic organisms in BSCs, isolated from Svalbard (NĀ) and Livingston Island (Gr1), was assessed using morphological features and annotated environmental rRNA SSU sequences. We obtained 18,041,893, 28,446,946 and 116,112,134 forward raw reads for sample NĀ and 62,235,763, 70,163,318 and 48,605,923 for Gr1. After quality trimming and rRNA SSU filtering we ended up with 8936, 7818 and 38,320 sequences for NĀ and 706,549, 1081,090 and 8646 for Gr1. This study focused on genera belonging to the Klebsormidiophyceae, Chlorophyceae, Trebouxiophyceae, Xanthophyceae or Cyanobacteria. All the taxa that were identified are included in the supplementary information (Online Resource 1). Additional taxonomic groups, for example Fungi, that were identified in the molecular analysis were not further investigated in this study.

The Arctic BSC

The Arctic sample contained 26 genera of eukaryotic algae and Cyanobacteria identified by light microscopy while 132 were assigned by metatranscriptomic analysis. The average number of genera in the metatranscriptomic triplicates was 71.7 ± 36.1 (SD). In total, 15 genera were determined by both methodologies, compared to 117 and 11 genera which were solely found by molecular and morphological identification, respectively (Fig. 2a). The utilized 18S reference database contained sequences for 5 of the 11 genera identified by morphology. Figures 3a and 4a display all genera identified with 1, 44, 25, 6 and 67 taxa belonging to Klebsormidiophyceae, Chlorophyceae, Trebouxiophyceae, Xanthophyceae and Cyanobacteria, respectively. The genera *Coccomyxa*, *Elliptochloris*, *Stichococcus* (Trebouxiophyceae), *Leptolyngbya*, *Microcoleus*, *Nostoc* and *Phormidium* (Cyanobacteria) were found in the culture isolates and in all three metatranscriptomes while *Klebsormidium*

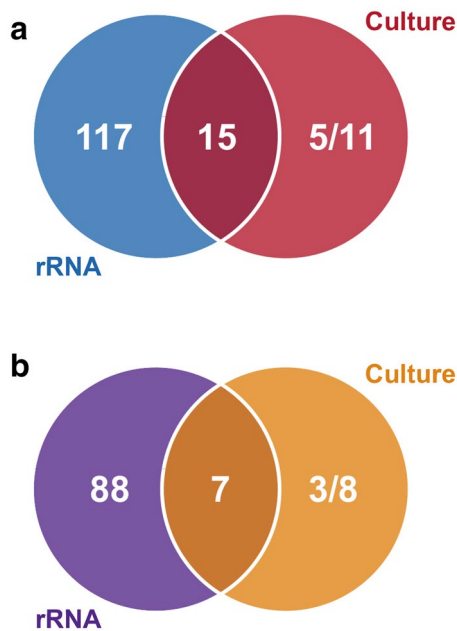


Fig. 2 Comparing the molecular (blue, violet) and morphological (red, orange) approach in terms of identified algal genera, both eukaryotic and prokaryotic. The numbers divided by a slash represent the taxa present in the reference database (left) and all genera identified (right). **a** NA; **b** GrI

(Klebsormidiophyceae), *Chlorococcum*, *Coelastrella*, *Desmodesmus*, *Lobochlamys* (Chlorophyceae), *Botrydiopsis*, *Heterococcus* (Xanthophyceae) and *Stigonema* (Cyanobacteria) were identified in at least one of the three replicates and by light microscopy. All three metatranscriptomes per site included rRNA SSU sequences of the following genera, which could not be cultivated: *Chloromonas*, *Dictyococcus*, *Oedogonium*, *Scenedesmus* (Chlorophyceae), *Chlorella*, *Chloroidium*, *Dictyochloropsis* (Trebouxiophyceae), *Anabaena*, *Aphanizonemon*, *Arthronema*, *Calothrix*, *Chroococciopsis*, *Crinalium*, *Cyanothece*, *Cylindrospermum*, *Gloeobacter*, *Nodularia*, *Tolypothrix* and *Trichormus* (Cyanobacteria). The morphology-based method identified the eukaryotic genera *Gloeocystis**, *Macrochloris**, *Neocystis**, *Tetracystis* (Chlorophyceae), *Desmococcus**, *Koliella*, *Lobosphaera* (Trebouxiophyceae), *Pleurochloris**, *Pleurogaster**, *Tribonema* and *Xanthonema* (Xanthophyceae), of which six were not represented by 18S rRNA gene sequences in the reference database (asterisk).

The Antarctic BSC

A total of 15 genera, present in the Antarctic BSC, were determined based on morphology while molecular identification yielded 95 taxa. The genera mean value of the three molecular replicates was 51.3 ± 5.7 (SD). Both techniques found seven genera while 8 and 88 genera were solely

determined by morphology or sequence homology search, respectively (Fig. 2b). For three genera, identified from culture isolates, rRNA reference sequences were present in the database. Regarding the different taxonomic groups, several genera were determined: one genus of Klebsormidiophyceae, 53 genera of Chlorophyceae, 28 genera of Trebouxiophyceae, six genera of Xanthophyceae and 16 genera of Cyanobacteria (Figs. 3b and 4b). The genera *Coccomyxa* (Trebouxiophyceae), *Leptolyngbya*, *Nostoc* and *Phormidium* (Cyanobacteria) could be determined morphologically and were identified in all replicates used for the molecular approach. In addition, the genera *Elliptochloris*, *Prasiola* (Trebouxiophyceae), *Heterococcus* (Xanthophyceae) were determined by morphology and present in at least one out of three metatranscriptomic replicates. All three replicates prepared for molecular identification included the genera *Ankistrodesmus*, *Bracteacoccus*, *Chlamydomonas*, *Chloromonas*, *Dunaliella*, *Hemiflagellochloris*, *Monoraphidium*, *Oedogonium*, *Scenedesmus* (Chlorophyceae), *Botryococcus*, *Chlorella*, *Chloroidium*, *Heterochlorella*, *Nannochloris*, *Stichococcus* (Trebouxiophyceae), *Botrydiopsis* (Xanthophyceae) and *Chamaesiphon* (Cyanobacteria); however, cultivation failed for these organisms. In contrast, cultures were established for the genera *Chlorococcum*, *Heterotetracystis*, *Mychonastes*, *Neocystis** (Chlorophyceae), *Desmococcus**, *Muriella**, *Myrmecia** (Trebouxiophyceae) and *Pleurochloris** (Xanthophyceae) which are missing in the rRNA dataset. Five of these genera were not represented by 18S sequences in the used database (asterisk).

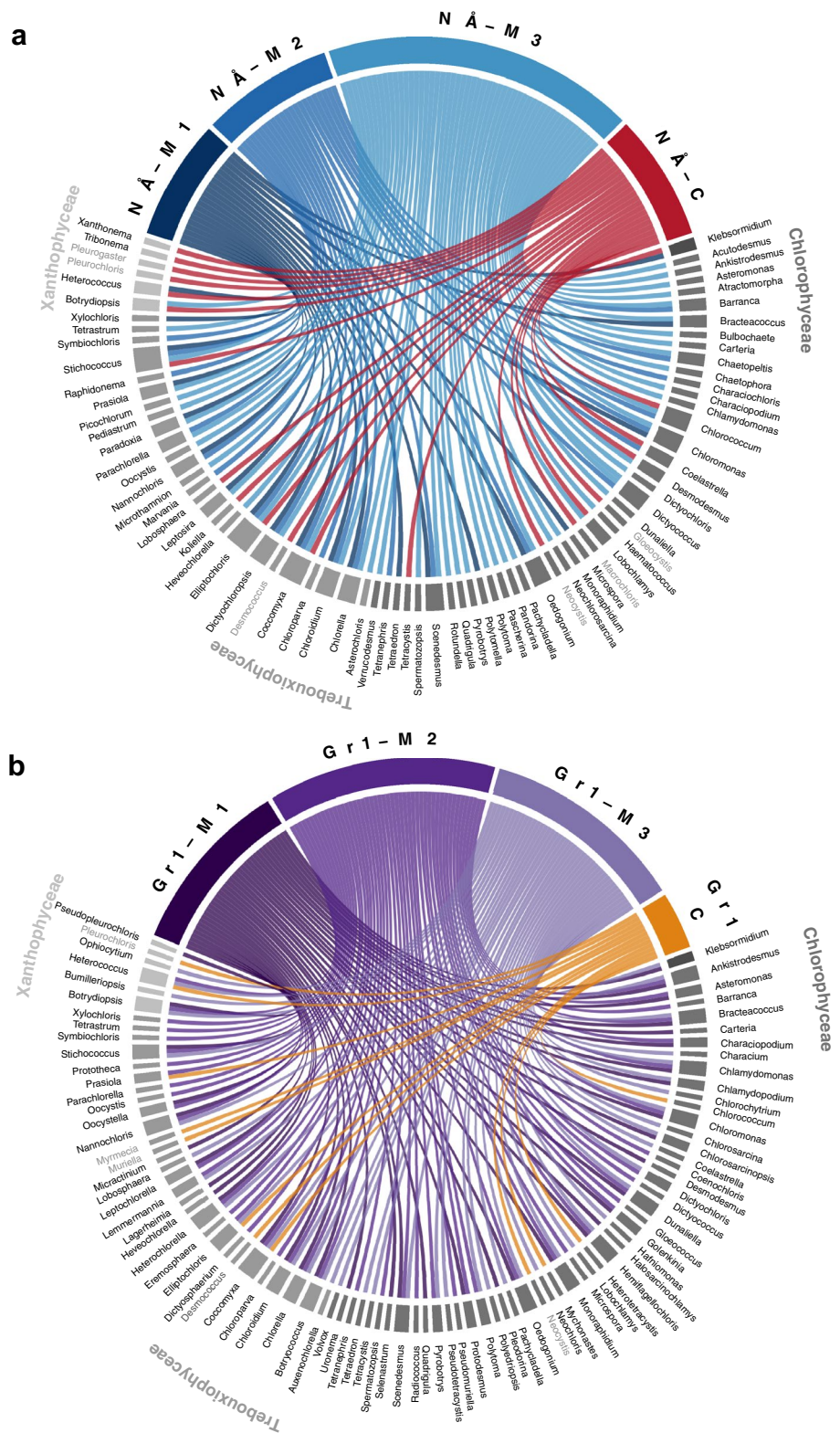
Site comparison

By comparing the Arctic and Antarctic crust samples we found 63 genera that were shared between both locations (Fig. 5). However, the BSC collected from Svalbard, included 80 microalgae and Cyanobacteria which were not shared with the Antarctic sample, although the sample from Livingston Island contained 40 additional taxa. The genus *Klebsormidium* occurred at both sites. The biggest portion of chlorophycean (28) and trebouxiophycean (16) genera was found in both samples. Regarding Xanthophyceae, both BSCs contained 6 genera of which 3 were shared between sites. The analyses of the Arctic and Antarctic samples revealed 67 and 16 cyanobacterial genera to be present, respectively. An overlap of 15 cyanobacterial genera was detected.

Discussion

Overall, the molecular survey of the BSC samples revealed a higher degree of genera richness for Chlorophyceae, Trebouxiophyceae and Cyanobacteria compared

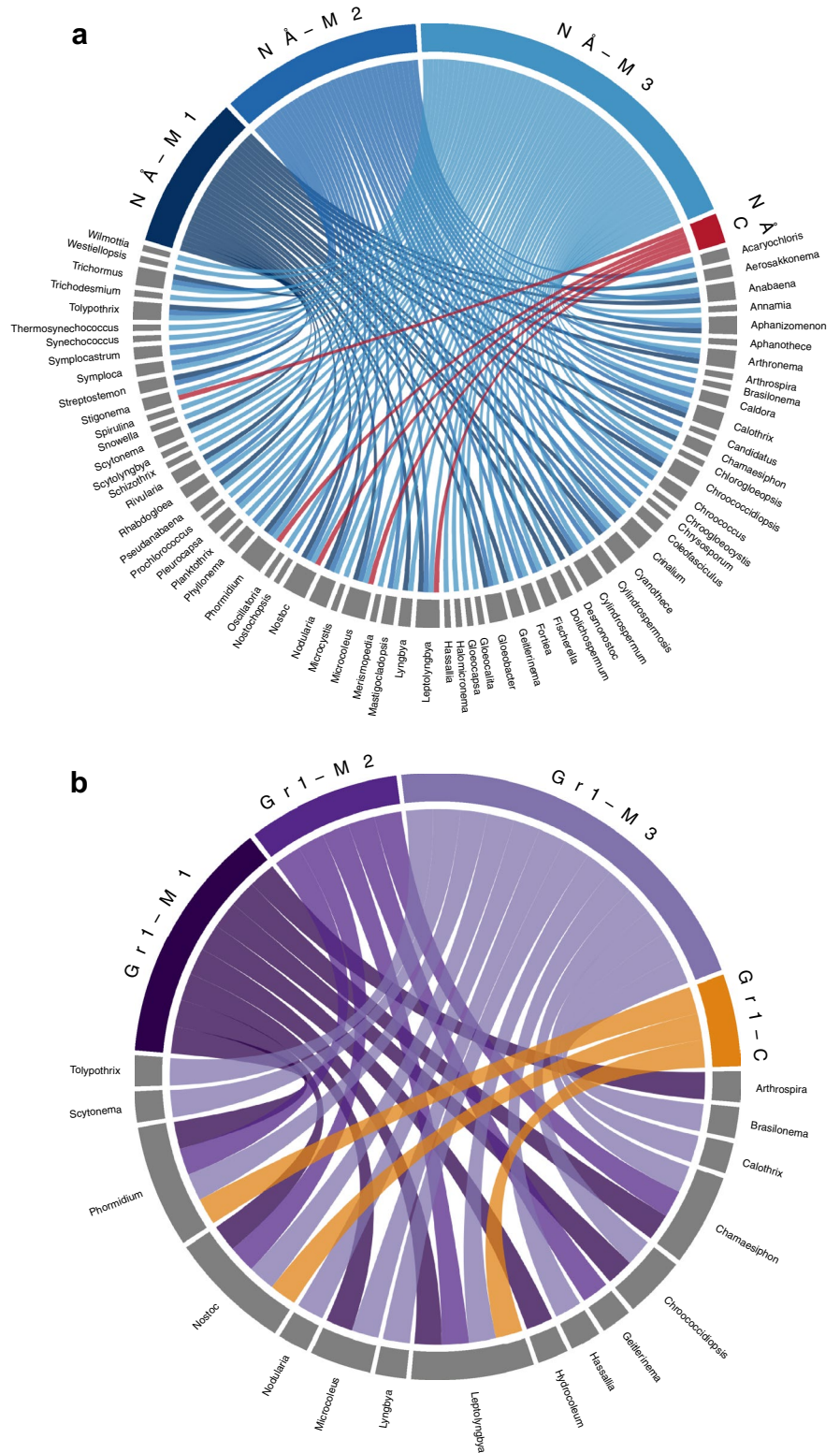
Fig. 3 All genera identified belonging to either Klebsormidiophyceae (*Klebsormidium*), Chlorophyceae, Trebouxiophyceae or Xanthophyceae. The metatranscriptome (blue and violet shades) is separated into three replicates while the culture isolates are represented by a single category (red, orange). Chords connect the methodologies with the identified genera. Genera displayed in gray were absent from the database used for the metatranscriptomic analyses. **a** NÁ; **b** Gr1



to the culture and morphology-based methodology. However, the rRNA SSU identification did not recover all genera determined by light microscopy. Regarding

Cyanobacteria, the Arctic crust sample exhibited a much higher variety compared to the sample collected at Livingston Island.

Fig. 4 All genera identified belonging to Cyanobacteria. The metatranscriptome (blue and violet shades) is separated into three replicates while the culture isolates are represented by a single category (red, orange). Chords connect the methodologies with the genera identified. **a** NÁ; **b** Gr1



Assessment of microalgal and cyanobacterial genera richness

The genus *Klebsormidium* (Klebsormidiophyceae) is

typically found in BSC communities and has been previously found to colonize soils of both Livingston Island and Svalbard (Büdel 2001; Kastovská et al. 2005; Zidarova 2008; Borchhardt et al. 2017b). Our metatranscriptomic analyses

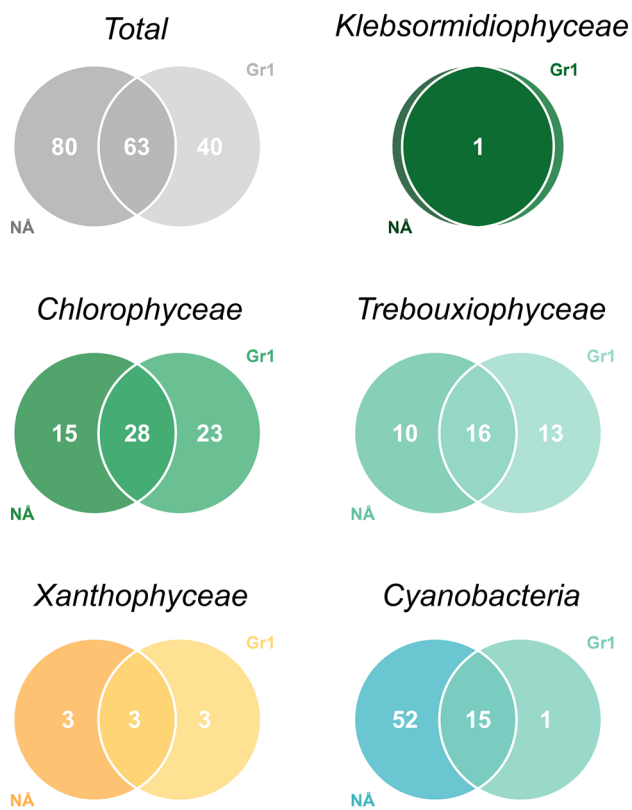


Fig. 5 Comparing the Arctic sample (NA) and the Antarctic BSC (Gr1) in terms of identified total genera (gray) and genera belonging to either Klebsormidiophyceae (dark green), Chlorophyceae (green), Trebouxiophyceae (light green), Xanthophyceae (yellow) or Cyanobacteria (turquoise)

confirmed the presence of this streptophytic taxon in the polar regions, although *Klebsormidium* was not identified by light microscopy in the sample collected from Livingston Island.

Several chlorophycean genera were identified in both the Arctic and the Antarctic samples by metatranscriptomic analysis: *Ankistrodesmus*, *Chlamydomonas*, *Chloromonas*, *Coelastrrella*, *Desmodesmus*, *Dictyococcus*, *Dunaliella*, *Lobochlamys*, *Monoraphidium*, *Oedogonium* and *Scenedesmus*. *Coelastrrella*, *Desmodesmus* and *Lobochlamys* were also found in Arctic culture isolates. All the aforementioned genera are known to occupy terrestrial habitats from temperate to cold climates (Büdel 2001; Borie and Ibraheem 2003; Lüring 2003; Tschakner et al. 2007; Matuła et al. 2007; Thorn and Lynch 2007; Uzunov et al. 2008; Zidarova 2008; de Wever et al. 2009; Wu et al. 2016; Büdel et al. 2016; Pushkareva et al. 2016; Schulz et al. 2016; Borchhardt et al. 2017b). For *Chlamydomonas*, *Chloromonas*, *Coelastrrella* and *Monoraphidium*, earlier records exist confirming their occurrence in both polar regions; however, *Dictyococcus*, *Oedogonium* and *Scenedesmus* have only been identified from Arctic BSCs (Matuła et al. 2007; Zidarova 2008;

Pushkareva et al. 2016; Borchhardt et al. 2017b). The genera *Chlamydomonas*, *Coelastrrella* and *Scenedesmus* were previously identified from Svalbard and *Monoraphidium* from Livingston Island (Matuła et al. 2007; Zidarova 2008; Pushkareva et al. 2016). Previous research describing the genera *Ankistrodesmus* and *Dunaliella* in polar habitats was not found; therefore, this is the first study which confirms their presence in Svalbard and Livingston Island BSCs. Species of *Chlorococcum* were found in culture isolates prepared from both the Arctic and Antarctic BSCs as well as in the metatranscriptomic dataset generated from the NA sample. Kastovská et al. (2005) also found *Chlorococcum* in close vicinity to Ny-Ålesund and Borchhardt et al. (2017b) identified this genus from samples collected from Ardley and King George Island, Antarctica. The genus *Tetracystis* was identified from both BSC communities. Based on morphology, *Tetracystis* was found in the Arctic sample and metatranscriptomics confirmed its presence in the BSC from Livingston Island. Evidence exists that suggests *Tetracystis* colonizes the soil of Arctic and Antarctica ecosystems (Broady 1986; Giełwanowska and Olech 2012; Patova et al. 2015; Pushkareva et al. 2016). The following eukaryotic algae were only identified in culture isolates: *Gloeocystis* and *Macrochloris* were present in isolates from the Arctic while *Heterotetracystis* and *Mychonastes* grew in the Antarctic enrichment cultures. The genus *Neocystis* was found in both samples. For almost five genera their presence in polar and alpine ecosystems has been reported previously (Matuła et al. 2007; Zidarova 2008; Lukešová et al. 2010; Andreyeva and Kurbatova 2014; Patova et al. 2015; Pushkareva et al. 2016; Borchhardt et al. 2017b).

Both the Arctic and Antarctic metatranscriptomic datasets and culture isolates contained the trebouxiophycean genus *Coccomyxa*. Previous studies also found that this genus is an inhabitant of Svalbard and Livingston Island ecosystems (Matuła et al. 2007; Zidarova 2008; Pushkareva et al. 2016). *Coccomyxa* has a ubiquitous distribution and exhibits an extraordinary versatility, it occurs in terrestrial mats, in associations with soil, Fungi and bryophytes, and is also planktonic in freshwater systems (Schmidle 1901; Vogel 1955; Peveling and Galun 1976; Büdel 2001; Darienko et al. 2015). An ecophysiological study on three *Coccomyxa* strains isolated from Antarctic BSC samples points to pronounced cold and drought tolerance (Pfaff et al. 2016). Rivasseau et al. (2016) even found a new species of *Coccomyxa*, *C. actinabiotis*, in the cooling pool of a nuclear power plant. Thus, it is not surprising that members of this genus can colonize the extreme habitats which prevail in the polar regions. The green alga *Elliptochloris* generally occupies terrestrial habitats and associates with Fungi to form lichens (Ettl and Gärtner 2014). Furthermore, this genus was found in the Canadian Arctic and King George Island, Antarctica (Pushkareva et al. 2016; Borchhardt et al. 2017b).

Both methodologies that were used in this study confirm that *Elliptochloris* is present in Svalbard and Livingston Island BSCs. In contrast, the genus *Stichococcus* was found in both the Arctic and the Antarctic metatranscriptomic data and in isolates from Livingston Island but not from Svalbard. This alga has often been identified as a member of BSC communities (Kastovská et al. 2005). Different species of *Stichococcus* have been previously found to colonize the soils of both Livingston Island and Svalbard (Kastovská et al. 2005; Zidarova 2008). The green alga *Prasiola* was identified through both the metatranscriptomic and microscopic identification for Livingston Island terrestrial habitats. However, only the Arctic metatranscriptomes showed evidence for *Prasiola* species. These findings are supported by a previous survey on species richness which reported *Prasiola* from both Livingston Island and Svalbard (Matuła et al. 2007; Zidarova 2008). Based solely on the metatranscriptomic analysis, the genera *Chlorella*, *Chloroidium* and *Nannochloris* were identified in both the Arctic and Antarctic BSC samples. Different species of *Chlorella* have been previously isolated from Svalbard and Livingston Island (Matuła et al. 2007; Zidarova 2008; Pushkareva et al. 2016). Vishnivetskaya (2009) identified *Nannochloris* in Siberian permafrost using molecular techniques, which suggests that this alga can survive in extreme conditions. The genus *Chloroidium* has been previously identified in BSCs; however, it has not been reported from polar habitats so far (Schulz et al. 2016; Williams et al. 2016). Therefore, this study provides the first molecular record of *Chloroidium* in Arctic and Antarctic ecosystems. The presence of *Lobosphaera* in the Arctic and Antarctic BSC samples was supported by the morphological and metatranscriptomic identification. Additionally, Giełwanowska and Olech (2012) identified *Lobosphaera* as a photobiont of Antarctic lichens. Based on morphological identification, the genus *Desmococcus* was found in BSC samples from both sites, *Koliella* was identified in isolates from the Arctic, while *Muriella* and *Myrmecia* grew in the Antarctic enrichment cultures. These findings are in accordance with previous studies reporting the presence of these genera in polar and alpine habitats (Kawecka 1986; Takeuchi 2001; Zidarova 2008; Lukešová et al. 2010; Andreyeva and Kurbatova 2014; Czerwik-Marcinkowska et al. 2015; Patova et al. 2015; Pushkareva et al. 2016; Borchhardt et al. 2017b).

The xanthophyte *Heterococcus* was identified by both techniques from the Svalbard and Livingston Island samples. Pushkareva et al. (2016) and Borchhardt et al. (2017b) also identified this genus in BSCs collected in the Canadian Arctic and King George Island, Antarctica. The genus *Botrydiopsis* is also typically found in BSCs and was previously isolated from other Arctic and Antarctic locations (Broadly 1976; Büdel 2001; Pushkareva et al. 2016). Our metatranscriptomic analyses support the presence of this xanthophycean alga in the polar regions. However, it was

identified by light microscopy only in the sample collected from Livingston Island. On the other hand, the genera *Pleurogaster*, *Tribonema* and *Xanthonema* were only present in isolates from the Arctic, while *Pleurochloris* was identified in both samples by means of morphological methods. *Pleurochloris*, *Tribonema* and *Xanthonema* have been previously found in polar and alpine ecosystems (Matuła et al. 2007; Zidarova 2008; Patova et al. 2015; Pushkareva et al. 2016; Borchhardt et al. 2017b). However, the genus *Pleurogaster* had previously only been isolated from terrestrial habitats outside polar regions (Stoyneva 2000).

All metatranscriptomes and morphological analyses identified the cyanobacterial genera *Leptolyngbya*, *Nostoc* and *Phormidium* from Svalbard and Livingston Island samples, which was also reported by Pushkareva et al. (2016) and Zidarova (2008), respectively. All genera occur in various climates and in aquatic and terrestrial habitats (Rippka et al. 1979; Büdel 2001; Casamatta et al. 2005; Dojani et al. 2014). The cyanobacterium *Microcoleus* also has a ubiquitous distribution, is often associated with BSCs and has been found in both Arctic and Antarctic environments (Büdel 2001; Pushkareva et al. 2015). All metatranscriptomic datasets contained evidence of *Microcoleus*; however, it was only identified from Svalbard through the morphological investigation. The genus *Stigonema* was identified from Svalbard through both metatranscriptomic and morphological methods, which is in agreement with other reports (Matuła et al. 2007; Pushkareva et al. 2015). However, in contrast to our results *Stigonema* can also be found in Antarctic ecosystems (Büdel 2001). The cyanobacterial genera *Calothrix*, *Chamaesiphon*, *Chroococidiopsis*, *Nodularia* and *Tolypothrix*, typically found in soil biocoenoses (Büdel 2001; Wang et al. 2015), were identified in the Arctic and the Antarctic sample using metatranscriptomics. Earlier studies, assessing the species richness of aeroterrestrial algae from Svalbard, recorded all of these genera (Matuła et al. 2007; Pushkareva et al. 2015, 2016).

Interestingly, the Arctic crust community exhibited higher cyanobacterial genus richness than the BSC from Livingston Island, while the genus richness of eukaryotic algae was similar. Our morphological and molecular analyses found only 16 different genera of Cyanobacteria in the Antarctic sample compared to 67 in the Arctic. BSCs develop over time and undergo changes in species composition (Büdel et al. 2009). Early successional stages of BSCs are usually dominated by Cyanobacteria; however, bryophytes, lichens and/or liverworts may become the dominant groups in many ecosystems (Büdel et al. 2009). Thus, differences in cyanobacterial genus richness may be explained by the different developmental stages of the communities. Furthermore, Williams et al. (2017) reported a lower abundance of cyanobacterial crusts on Livingston Island than at Ny-Ålesund, Svalbard. Our observation might also be linked to the reduced

occurrence of Cyanobacteria in these soil communities which was previously reported by Colesie et al. (2014a, b).

Methodological differences and difficulties

The identification of microalgae through metatranscriptomics yielded a higher number of genera compared to the morphological determination of cultivated and directly observed organisms. This is an indication for the lack of culturability of most microorganisms associated with BSCs (Ward et al. 1990; Schloss and Handelsman 2005; Shi et al. 2009; Masana et al. 2014). The successful cultivation of microalgae is highly dependent on the applied growth conditions including temperature, irradiance and culture medium (Bold 1942; Hoham 1975; Singh and Singh 2015; Wu et al. 2016). For example, Hoham (1975) tested various cryophilic algae, isolated from alpine habitats in Washington, USA, and discovered that *Chloromonas pichincha* reached the highest growth rate at 1 °C while *Cylindrocystis brébissonii* grew fastest at 10 °C. Therefore, the genus *Chloromonas* might have been inhibited during our culturing procedure due to unsuitable temperatures. Regarding the growth medium, one single solution, that is suitable for all algae and Cyanobacteria, does not exist (Bold 1942; Lee et al. 2014). Even though the medium, used to establish the enrichment cultures, was optimized for a broad range of algae, certain strains may not exhibit growth due to individual nutrient requirements. Some species of the genus *Dunaliella*, for example, prefer higher salt concentrations compared to other algae (Oren 2005). The salt concentrations of Bold's Basal Medium, used to establish the enrichment cultures, could have been too low to enable the growth of *Dunaliella*. Similar reports exist for the cyanobacterium *Chroococcidiopsis*. Different strains of *Chroococcidiopsis*, isolated from different habitats, exhibited growth only if the medium was supplied with the appropriate amount of sea salt (Cumbers and Rothschild 2014). However, growth parameters and culture medium determine the culturability of microorganisms only to a certain extent. For many organisms, especially algae, the co-cultivation of certain helper organisms (other algae, bacteria, etc.) is essential for growth and development (Jones et al. 1973; Sanders et al. 2001; Vartoukian et al. 2010). Some Cyanobacteria, which form symbioses with eukaryotic algae, fail to grow in the absence of their partner (Thompson et al. 2012). It is likely that quorum sensing plays an important role for these biotic interactions as certain signal molecules from one organism influence growth and development of another (Poonguzhali et al. 2007; Williams 2007). Chi et al. (2017) studied the marine bacterium *Ponticoccus* sp. PD-2 and how it controls the growth of the dinoflagellates *Alexandrium tamarense*, *Prorocentrum donghaiense* and the haptophyte *Phaeocystis globosa*. Although this clear indication of microalgae interacting with other microbes exists,

hardly anything is known about the underlying mechanisms. BSCs are complex biocoenoses with many dependencies and co-dependencies which are not yet fully understood.

Another potential problem when comparing morphological and molecular methodologies is the false identification of taxa due to ambiguous morphological traits (Wu et al. 2001; Hodač et al. 2016). Some genera, such as *Chlorella*, are difficult to identify as their morphological characters are limited and changeable depending on environmental conditions (Bock et al. 2011; Hodač et al. 2016). This might be one possible explanation why the metatranscriptomic data confirmed the presence of *Chlorella* in the Arctic and the Antarctic sample but the morphology-based identification did not. However, it is more likely that the spatial heterogeneity of the sample replicates collected for molecular and light microscopic analysis caused some of the differences (Concostrina-Zubiri et al. 2013; Kim et al. 2015). A clear indication of heterogeneity is the differing results observed for the replicates of the same site in the molecular analysis. For example, the genera *Coelastrrella*, *Chlorococcum* and *Chloromonas* were found in one, two and three replicates of the Arctic metatranscriptome, respectively. Furthermore, the high standard deviation of the averaged genera number for the molecular dataset of the Arctic BSC suggests sample heterogeneity. Differences in taxonomic determination may also arise due to a lack of consensus in the algal taxonomy. Many species concepts are still being revised based on molecular analyses rendering an unambiguous morphological identification difficult (Stoyanov et al. 2014; Champenois et al. 2015; Kawasaki et al. 2015; Škaloud et al. 2016). The genus *Leptolyngbya*, for example, was revealed to be heterogeneous and only recently split up into 15 new genera (Komárek 2016).

Finally, we came across a number of genera which could be identified by light microscopy but not by the analysis of the rRNA SSU sequences. Regarding the genera *Gloeocystis*, *Macrochloris*, *Neocystis*, *Desmococcus*, *Muriella*, *Myrmecia*, *Pleurochloris* and *Pleurogaster*, the corresponding 18S sequences were missing from the database utilized in the course of identification. Incomplete and wrongly annotated references are a major problem when performing molecular analyses of any kind and impair the correct determination of genera in environmental samples (Klimke et al. 2011; Taberlet et al. 2012). The microbial dark matter, meaning the total of unculturable microorganisms, complicates this matter even more (Solden et al. 2016). However, by studying metagenomics, metatranscriptomics and single cell genomics researchers can overcome the limit of culturability and supply the databases with novel sequences (Solden et al. 2016). A number of algae, such as *Chlorococcum*, *Heterotetracystis*, *Mychonastes*, *Tetracystis*, *Koliella*, *Lobosphaera*, *Tribonema* and *Xanthonema*, were isolated from the BSC samples and were included in the reference database,

used for this study. However, they were not detected in the metatranscriptomes. One possible explanation is the spatial heterogeneity of the sample replicates (Concostrina-Zubiri et al. 2013; Kim et al. 2015). Furthermore, these organisms may have been inactive during sampling and were, therefore, undetectable during total RNA analysis (Geisen et al. 2015). Many algae are known to develop resting cells to survive unfavorable conditions such as drought (Holzinger and Karsten 2013; Li et al. 2015). Li et al. (2015) identified 35 different algae in resting stages, among them *Chlorococcum* which surrounds resting cells with a thin layer of mucus. A species of *Mychonastes*, *M. desiccatus*, may produce desiccation-resistant cysts if dried out (Margulis et al. 1988). Massalski et al. (1994) isolated *Lobosphaera* from King George Island, Antarctica, and studied its ultrastructure. The alga, colonizing volcanic rocks, was able to produce resting cysts with thickened cell walls to outlast detrimental conditions. Similar observations were made for *Tribonema bombycinum* which produced akinetes in response to starvation (Nagao et al. 1999). Our crust samples were collected in rather extreme habitats exhibiting low temperatures, high irradiance or complete darkness and low water availability throughout the year resulting in a hostile environment (Thomas et al. 2008a). Thus, the absence of certain genera in the molecular datasets might be attributed to the occurrence of resting stages, which likely occur under the conditions prevailing at Svalbard and Livingston Island.

Conclusion

BSCs represent an important part of polar ecosystems and serve as indicators for ecological health and climate change. Thus, these complex communities need to be closely examined to gain insights into not only taxonomy but also physiological plasticity and functionality. This study focused on comparing methodologies to analyze the genus richness of these interesting biocoenoses. Cultivating microorganisms, present in the BSC, combined with light microscopy offers individual analysis of certain organisms while metatranscriptomics enables investigation of the whole genera richness within the crust. The selection of one or the other technique should be based on the individual research focus as both come with certain benefits, as well as costs. The isolation of microorganisms and establishment of cultures enable detailed investigations and follow-up experiments on the isolated algae. However, unculturable genera will be neglected. In contrast, molecular surveys are always dependent on appropriate and complete reference datasets as it is difficult to correctly annotate taxa which are absent from, or misidentified in the database. Moreover, PCR biases and sequencing errors may falsify the results. In our opinion, the combination of both approaches will generate reliable results

of higher quality. Thus, we recommend conjoined studies to validate findings and maximize outcome.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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