The University of South Bohemia in České Budějovice
Faculty of Science

Origin of resistance against desiccation and cryoinjuries in soil crust microalgae of High Arctic

Bachelor thesis

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Annotation

Microalgae of polar soil crusts are of significant ecological importance by regulating numerous vital processes in the extreme Arctic climate. To protect themselves from these harsh conditions they developed various adaption mechanisms. Hardening and nutrient starvation during the late summer months are expected to positively impact the resistance of microalgae against cryoinjuries and desiccation. The first aim of this study was to assess the vitality of microalgae in biological soil crusts within the High Arctic (Central Svalbard) during the summer using multiple approaches. The second objective was to perform an evaluation of the biodiversity and a viability growth test of microalgae within the cryptogamic crusts, providing an overview of the species composition and vitality.

Declaration

I declare that I am the author of this qualification thesis and that in writing it I have used the sources and literature displayed in the list of used sources only.

České Budějovice, 17.08.2023

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Elisa Frank

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Abstract

The Arctic regions are one of the Earth's climatic extremes, exerting selective influence on the life of numerous organisms. One of the most successfully adapted organisms in this prevailing climate are soil crust microalgae. Different strategies enable them to survive the cold and dry conditions occurring in the High Arctic. Even though these survival mechanisms have been extensively described by now, their origin remains unknown. Thus, late-summer hardening and nutrient starvation are expected to be positively influencing the resistance of microalgae against cryoinjuries and desiccation. The aim of this bachelor thesis is to assess the vitality of soil crust microalgae in the High Arctic during the summer. Additionally, their distribution, biodiversity and growth along an elevation gradient was investigated. Samples were collected from three different study sites in three different altitudes. Their cell conditions were initially assessed using a multiparameter staining protocol followed by growth tests appraised through chlorophyll fluorescence imaging. Contrary to the expectations, the fluorescence staining of soil crust microalgae showed to be ineffective in the distinction of living and dead cells. This can be attributed to unexpected correlations between the sediment, cells and utilized stains. However, the comparison of growth and vitality parameters of the fluorescence imagining overall indicates a growth in soil crust microalgae with a higher vitality in samples collected from the study sites located in higher elevation (Breinosa) than in lower elevation (Bjørndalen). Furthermore, the delay of low values in the maximum quantum yield (F_V/F_M) at the beginning of the experiment suggest an early preparation for winter.

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List of Abbreviations

APC	allophycocyanin
ATP	adenosine triphosphate
BSC	biological soil crust
CTC	5-Cyano-2,3-Ditolyl Tetrazolium Chloride
DAPI	4′,6-diamidin-2-fenylindol
DNA	deoxyribonucleic acid
EPS	extracellular polymeric substances/ exopolysaccharides
F_0	minimum fluorescence in the dark
F_M	maximum quantum yield after the saturation pulse in the dark-adapted state
F_V	maximum variable chlorophyll fluorescence yield in the dark-adapted state
Φ_{Po}, F_V/F_M	maximum quantum yield
PAA	total photosynthetically active area
PAR	photosynthetically active radiation
PC	phycocyanin
PE	phycoerythrin
PSII	photosystem II
RNA	ribonucleic acid
rRNA	ribosomal RNA
UVR	ultraviolet radiation

1 Introduction

1.1 The High Arctic

Climate change has become a global problem in recent decades and has a wide-ranging impact on the entire planet. However, few places on Earth are experiencing this impact as strongly as the polar regions (Huntington et al., 2005; Olsen et al., 2017). Located at the northernmost and southernmost part of the Earth, the Arctic and Antarctic regions are undergoing rapid and profound transformations due to rising temperatures and shifting climate patterns, majorly influenced by enhanced greenhouse gas emissions (Hassol & Corell, 2006; Huntington et al., 2005; Overland et al., 2017; Walsh et al., 2011). In fact, with an air temperature rise of up to 4°C in the winter, the Arctic regions are found to have the highest increase in temperature per year, resulting in the continuous melting of ice and glaciers as well as an upward trend in precipitation (Huntington et al., 2005). This not only affects the ecosystems and ecology of the Arctic, but also strongly impacts the climate systems and sea levels on a global scale (Hobbie et al., 2017). Therefore, the changes in the Arctic and Antarctic can be seen as a measure of global warming and the investigation of their causes and consequences takes on great importance (Anisimov et al., 2007; Hassol & Corell, 2006).

The geographical definition of the Arctic is referenced by the Arctic Circle (66° 33'N), which is dependent on the solar radiation. This region experiences a minimum energy of one day without daylight during winter and the phenomenon of the midnight sun during summer, with deviations due to the irregular topography of the Earth being neglected (Huntington et al., 2005; Nilsson & Huntington, 2002).

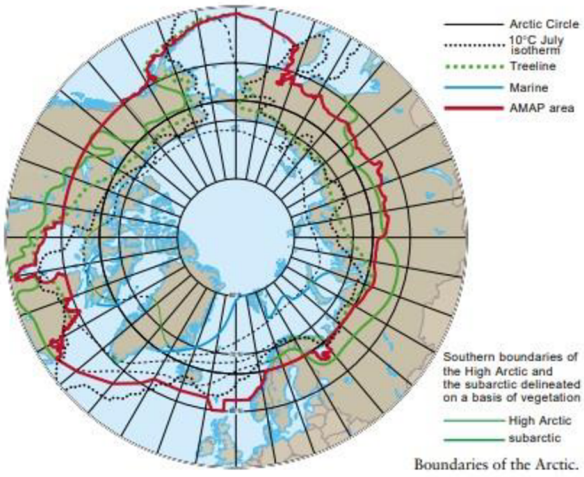

Figure 1: Graphic depiction of the circumpolar North (Nilsson & Huntington, 2002)

1.1.1 The impact of the Arctic climate on the global climate system

The Arctic climate changes seasonally and regionally with a perennial temperature below 10°C (Arctic Centre: University of Lapland, n.d.; Armstrong et al., 2023; McBean et al., 2005). As the Arctic is connected to the other regions of the world by rivers, the ocean and atmosphere, it contributes significantly to the overall global climate system (McBean et al., 2005).

Albedo effect

The cryosphere in the Arctic region is an important contributor in terms of climate change as it has a positive feedback on the Earth's albedo (Baede et al., 2001; McBean et al., 2005). Landmasses covered in ice and snow, along with the sea ice cover, reflect incoming solar radiation, thereby partially insulating the Arctic Ocean during the colder months (Baede et al., 2001). The warming of the Arctic inevitably leads to a decline in the cryosphere with significant changes in the Earth's energy balance (Albritton et al., 2001; Hinzman et al., 2005). Longer melting seasons during spring and summer as well as the thinning of multiyear sea ice decrease the Arctic albedo and consequently increase the absorption of solar radiation on the surface (Wadhams, 2020). As a result, the climate gets warmer which facilitates an even more pronounced shrinkage of the cryosphere zones and a parallel elevation of the water levels (Albritton et al., 2001; McBean et al., 2005; Wadhams, 2020).

Heat flux around the globe

The heat flow around the Earth is primarily interconnected by two factors: the ocean and the atmosphere (Turner & Marshall, 2011). The Arctic Ocean is linked to the rest of the global seas via the thermohaline circulation and therefore has a strong impact on the global heat distribution. At northern latitudes the mixing of upper, warmer water layers with deeper, colder water layers is regulated by the water's salt content, which impacts the density of the water. Decreased salinity, often caused by the inflow of melted terrestrial ice and snow due to higher air temperatures, prevents the surface water from sinking, thereby slowing the deep-ocean conveyor belt down (Albritton et al., 2001; Loeng et al., 2005; Olsen et al., 2017; Turner & Marshall, 2011; Wadhams, 2020). Additionally, rivers flowing through warmer land areas, increase the oceanic water temperature. This accelerates the decline of sea ice, which, in turn decreases the Earth's albedo (Albritton et al., 2001; Farmer et al., 2021; Loeng et al., 2005; Turner & Marshall, 2011; Wadhams, 2020).

In contrast to the marine Arctic, the atmosphere carries heat as warm air in the northeast direction and as cool air in the western direction (Turner & Marshall, 2011). They are both interlinked through the constant exchange of heat, gases and water vapor. Increasing air temperatures can easily disrupt this equilibrium leading to the retreat of the snow line and a positive feedback due to the evaporated water. This is accompanied by more frequent heatwaves, droughts, and heavy rainfall. The interconversion of heat facilitates the warming of the ocean, resulting in the melting of the sea-ice cover and a subsequent rise in the sea levels. Consequently, the Earth's albedo is further increased (AMAP, 2017; Carmack et al., 2015; Wadhams, 2020).

Carbon cycle

The Arctic carbon cycle represents an ongoing interplay of carbon and its derivatives in the atmosphere, land and marine environment. It is significantly influenced by anthropogenic greenhouse emissions caused by fossil fuel burning, deforestation and agriculture. The amount of marine carbon, mostly in the form of CO_2, is dependent on several factors including the partial pressure difference (pCO_2) for the gas exchange at the air-sea interface and the sea-ice cover. These physical factors are accompanied by the photoautotrophic incorporation of atmospheric CO_2 into the Arctic Sea (Christensen et al., 2017; Loeng et al., 2005; Prentice et al., 2001). The global temperature rise as well as the melting of sea ice lead to a higher uptake of CO_2 causing an acidification of the water with severe consequences for the aquatic animals (AMAP, 2017; Parmentier et al., 2013; Prentice et al., 2001).

The largest sink for soil carbon has been found to be stored in the Arctic as prevailing cold temperatures, wet conditions and permafrost create an environment that promotes low decomposition rates (Christensen et al., 2017; Hugelius et al., 2014; Parmentier et al., 2017; Tarnocai et al., 2009). Miner et al. (2022) estimated that the amount of carbon stored in the Arctic permafrost amounts to 1,700 billion metric tons. However, over the last few decades, a notable increase in the melting of permafrost has been observed. Consequently, long preserved soil organic matter is made accessible for microbial decomposition which causes the outgassing of greenhouse gases, such as CO_2 and CH_4. This in turn leads to a positive CO_2 feedback and thereby accelerates climate change (Christensen et al., 2017; Parmentier et al., 2017; Wickland et al., 2006).

1.2 Climate change and the greening of the Arctic

Over the past decade, the anthropogenic influence emerged as an increasingly significant factor contributing to climatic changes in the High Arctic. On land, this becomes evident through the melting of snow, permafrost, and glaciers (Overland et al., 2017). However, the subsequent succession of the newly deglaciated areas entails several difficulties (Bockheim & Ugolini, 1990; Dinh, 2019; Szymański et al., 2015). As a result, only well adapted organisms can inhabit the newly available surface, including biological soil crust microalgae (Agnelli et al., 2021). Their ability to protect themselves from cryoinjuries and desiccation enables them to thrive even under the harsh climatic conditions prevailing in the Arctic. Furthermore, they are predicted to facilitate the "greening of the Arctic" as their growth is a driving factor for the subsequent proliferation of shrub tundra in the northern latitudes (Myers-Smith et al., 2015; Pointing et al., 2015). Moreover, the increase of the CO_2-concentration in the atmosphere and rising air temperatures as well as unthawed soil surface promote the growth of primary producers (Belshe et al., 2013; IPCC, 2001). As shown in laboratory experiments can elevated CO_2-levels directly increase the rate of photosynthesis resulting in better biomass production and carbon fixation. Consequently, this rise acts as additional carbon storage in the northern latitudes (Belshe et al., 2013; Prentice et al., 2001).

1.3 Biological soil crusts (BSC)

Biological soil crusts are diverse communities consisting of microalgae, cyanobacteria, microfungi, lichens and bacteria that form a thin crust at the soil surface (<1 cm) by aggregating surrounding soil particles (Belnap et al., 2003; Gold & Bliss, 1995). In Arctic deserts, the reduced crust cover is predominantly composed of cyanobacteria and eukaryotic microalgae (Elster et al., 1999; Williams et al., 2017).

1.4 Photosynthetic microorganisms

Microalgae encompass a wide range of taxonomic groups ranging from prokaryotic cyanobacteria to eukaryotic microalgae (Guiry, 2012; Sánchez-Bayo et al., 2020). These small and simply built microorganisms are characterized by their ability to perform oxygenic photosynthesis due to the presence of photosynthetic pigments (e.g. chlorophylls, carotenoids, phycobiliproteins) (Begum et al., 2016; Vítová, 2020). Furthermore, they exhibit a remarkable morphological diversity which not only depends on the species themselves but also on their respective life stages and adaptions (Andersen, 2013). In this thesis, the term "microalgae" refers to both algae and cyanobacteria.

1.4.1 Cyanobacteria

Cyanobacteria are one of the oldest, most primitive photosynthetic organisms and have made a significant contribution to the evolution of the Earth by oxygenating the atmosphere (Hogg, 2005). Although cyanobacteria are commonly termed as blue-green algae, they are morphologically and phylogenetically more closely related to bacteria (Oren & Ventura, 2017). This confusion can be attributed to certain species which possess photosynthetic pigments such as C-phycocyanin and C-phycoerythrin. These pigments, along with xanthophylls, chlorophyll a and β-carotenes, are located in the thylakoids which run parallel to the organismic cell membrane and enable these prokaryotes to perform oxygenic photosynthesis similar to plants or eukaryotic algae (Hogg, 2005; Trivedi et al., 2010).

The taxonomical evaluation of cyanobacteria proves to be difficult as several different approaches for the classification exist. According to bacteriologists are cyanobacteria classified into 5 different subgroups (Waterbury, 2006):

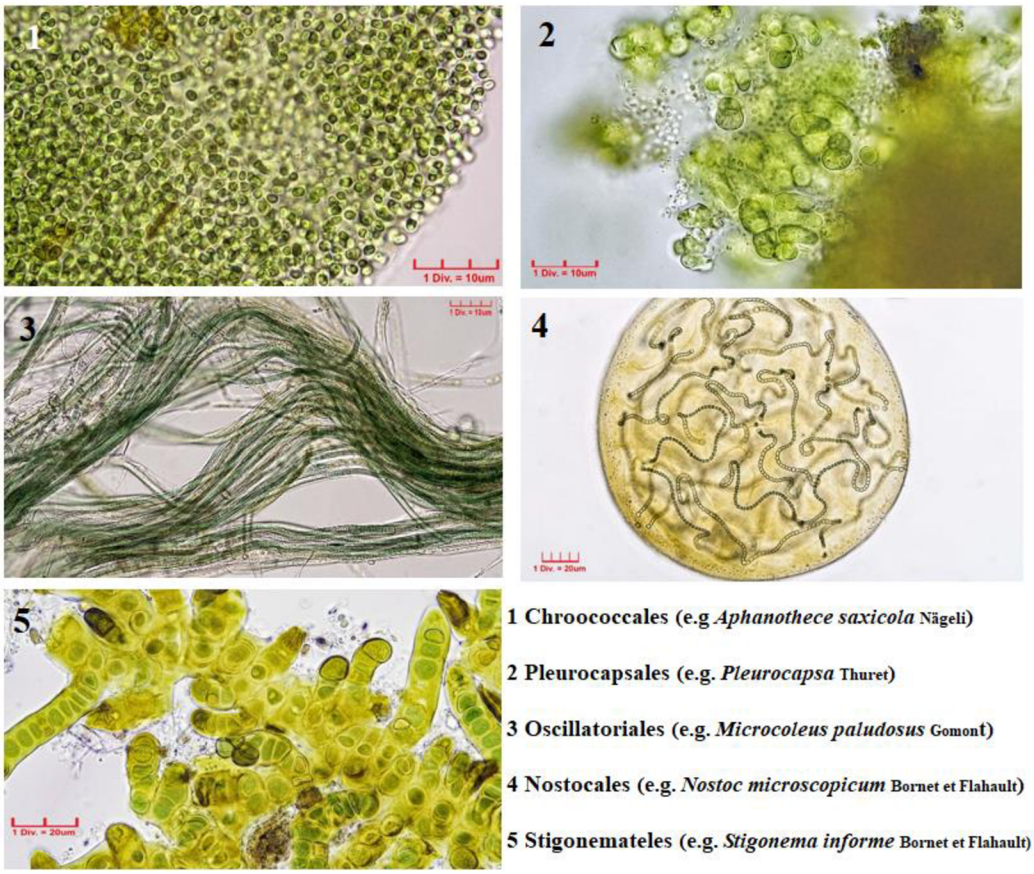

Figure 2: Comprehensive Overview of dominant cyanobacterial orders and representatives (Gerald Oemig, n.d.)

Based on a polyphasic approach, a novel system of classification was introduced by Komárek et al. (2014), dividing the cyanobacteria phylum into eight main groups (Oscillatoriales, Gloeobacterales, Synechococcales, Chroococcales, Spirulinales, Pleurocapsales, Nostocales and Chroococcidiopsidales).

1.4.2 Eukaryotic microalgae

Eukaryotic microalgae differ from prokaryotic cyanobacteria in the possession of membrane bound organelles such as nucleus, mitochondria or chloroplasts. The present chloroplast acts as their main photosynthetic organelle (Liberton et al., 2011). They exhibit a diverse array of morphological forms ranging from amoeboid over plamelloid to filamentous or coccoid (Andersen, 2013). Some species are even characterized by the presence of one or more flagella (Fleurence, 2021). There exist several different approaches for the classification of the eukaryotic algal phylum. Traditionally, the positioning of the pigments in the organism is analysed. All members of this phylum are classified by the possession of the photosynthetic pigment chlorophyll a like higher plants and cyanobacteria. This leads to a taxonomic division into three subgroups (Fleurence, 2021). The presence of additional pigments determines the further classification of an individual into a certain algal lineage (Morançais et al., 2018).

Table 1: Possible taxonomic classification of algae based on their photosynthetic pigment composition

Phylum	Branch	Common Pigment	Signature pigments
Chlorophyta (green algae)	Chlorophycophyta	chlorophyll a	chlorophyll b
Chromophyta (golden-brown algae)	Pheophycophyta	chlorophyll a	- chlorophyll c, e - excess carotenoids (β-carotenes, diatoxanthin, fucoxanthin, etc.)
	Chrysophycophyta		
	Pyrrophycophyta		
Rhodophyta (red algae)	Rhodophycophyta	chlorophyll a	- chlorophyll d - PE - PC - APC

Note: Adapted from Microalgae: from future food to cellular factory, by J.Fleurence, 2021, p. 3f. Copyright 2021 by ISTE Ltd

Furthermore, also a phylogenetic approach with morphological verification can be used for establishing the taxonomy of eukaryotic microalgae. However, the comparison of species by means of ribosomal RNA or DNA analysis results in a different classification scheme which shows the evolutionary relationship among different classes of algae more prominently (Fleurence, 2021).

1.5 Ecological role of microalgae (cyanobacteria and eukaryotic algae) in BSC

Both eukaryotic algae and cyanobacteria are important contributors to the ecological system of biological soil crusts by fulfilling different functional roles.

1.5.1 Soil stabilization

The primary function is the stabilization of soil surfaces which subsequently results in a reduction of soil erosions (St. Clair & Johansen, 1993). One contributing factor is the formation of soil aggregates. These aggregates are formed from sticky exopolysaccharides (EPS), produced by filamentous cyanobacteria and green algae, and act as binding agents entrapping individual soil and clay sediments (Evans & Johansen, 1999). Additionally, the presence of eukaryotic microalgae that are attached to both, the primary crust forming organisms and surrounding loose soil particles, enhances the process (Büdel et al., 2016). Rooting structures of mosses, liverworts and lichens further stabilize the crust by filling empty soil pores, thus preventing soil detachment (Belnap, 2003a; Belnap & Eldridge, 2003; Büdel et al., 2016; Evans et al., 2003).

1.5.2 Hydrology

Enhanced aggregation and pore formation can considerably impact arid land hydrology. Whether the impact is positive or negative remains unclear (Evans & Johansen, 1999). However, it is certain that the water infiltration capacity strongly depends on the size of the particles included in the biological soil crust and the diversity of the contributors involved in the crust formation process (Brotherson & Rusforth, 1983; Warren, 2003).

1.5.3 Influence on the nutrient cycle

Besides retaining moisture, biological soil crusts are important primary producers that influence the nutrient content within the soil. The use of photosynthesis by microalgae facilitates the fixation of carbon and contributes significantly to the carbon pool of the polar ecosystems. The carbon formed can be partially restored into the surrounding soil thereby enriching the terrestrial hummus reservoir (Austin et al., 2004; Lange, 2003). However, the overall carbon contribution varies with

the prevailing climate conditions as their active metabolism and the rate of photosynthesis are linearly linked to the availability of water, the temperature and the light intensity (Austin et al., 2004; Evans & Johansen, 1999; Lange, 2003).

Another important requirement for surviving the changing conditions in the polar regions is the presence of nitrogen. N_2-fixating cyanobacteria or cyanolichens embedded in the complex system of BSC can reduce atmospheric nitrogen into ammonia thereby increasing the nitrogen content in the cells for biosynthesis (Belnap, 2003b; Evans & Lange, 2003). The reaction follows equation (1) (Evans & Johansen, 1999):

$$N_2 + 8\,H^+ + 8e^- + 16\,ATP \rightarrow 2NH_3 + 16\,ADP + 16P_i \quad (1)$$

However, this process can only take place in an anaerobic environment as the catalysing enzyme nitrogenase is inactivated by the presence of oxygen (Evans & Johansen, 1999). Depending on the tolerance of these organism to oxygen, they either fixate nitrogen in permanently oxygen-free environments or have developed special cells that create an anaerobic space for the fixation reaction (heterocysts) (Gallon, 1981). Those heterocysts shield the nitrogen fixing enzyme by thickened cell walls and a degrading photosystem II (PSII) (Belnap, 2003b).

1.5.4 Impacts of BSC on vegetation

Given the influence on the nutrient content and water retention in the soil, microalgae are also significantly impacting the colonization potential of vascular plants in cold deserts. The rough surfaces of the formed soil crusts make good augmentation sites for seed germination and the enhanced soil stability increases the longevity of trapped seeds (Zhang et al., 2016). Additionally, elevated levels of carbon and nitrogen lead to better-fertilized soils that further affect growth and germination (Belnap et al., 2003; Harper & Pendleton, 1993). Between certain species even the symbiotic formation of arbuscular mycorrhizae has been observed (Harper & Pendleton, 1993). The resulting enhanced vegetation cover once again exerts a positive influence on the sequestration of atmospheric CO_2 which acts as a feedback reaction in global warming.

1.6 Biodiversity of Microalgae in the High Arctic

The biodiversity of microalgae of the BSC in the Arctic regions is strongly dependent on the prevailing climatic conditions. Cyanobacteria of BSC exhibit a predominantly filamentous morphology with Chroococcales, Pseudanavaenales, Oscillatoriales and Nostocales being the most prominent orders (Pushkareva et al., 2016). Moreover, taxonomic differences can be observed depending on the latitude and the microvariations in the polar environment (Rippin et al., 2018). Aquatic habitats characterized by nitrogen-demanding soil show a high abundance of *Nostoc* ssp. and several species of the order Oscillatoriales (Lan et al., 2012; Pushkareva & Elster, 2013) whilst *Rivularia minutula, Nostoc commune* and *Tolypothrix tenuis* appear to prevail in or near rivers and lakes (Belnap & Eldridge, 2003; Sheath et al., 1996).

The species diversity of eukaryotic algae within Arctic BSC strongly depends on the chemical and physical properties of the soil. Thus, green coccoid algae (Chlorophyta) appear as the dominant genera (Pushkareva et al., 2016; Pushkareva & Elster, 2013). Furthermore, studies conducted by Kaštovská et al. (2005) showed that algae of the species *Klebsomordium* and *Chlorella* occur frequently. These findings were confirmed by the evaluation of the algal biodiversity of Ny- Ålesund in Svalbard (Kim et al., 2008). Additionally, species of the class Xanthohyceae showed a high abundance in the polar climate including *Botrydiopsis, Tribonema, Xanthonema*, and *Heterococcus* (Büdel et al., 2016). Their presence in the higher latitudes was additionally corroborated by Borchhardt et al. (2017). Several species of the class Trebouxiophyceae, including *Myrmecia* and *Stichococcus,* were found in the European and Canadian Arctic (Pushkareva et al., 2016). These green algae form symbiotic relationships with or on lichens in biological soil crusts. The specific function of those chlorolichen associations, however, depends on the type of the photobiont partner (Büdel et al., 2016; Rosentreter et al., 2016).

In general, it remains unclear whether certain species of terrestrial cyanobacteria and eukaryotic algae are confined solely to this geographical high latitude region or if they have simply adapted to the prevailing climatic conditions (Pushkareva et al., 2016).

1.7 General adaption/acclimatisation mechanisms to the High Arctic climate

Due to the highly demanding climatic conditions in the polar regions, microalgae have developed different adaption mechanisms to cope with occurring stress factors, including strong fluctuation in temperature, limited water as well as nutrient availability, varying salinity and significant changes in the ultraviolet radiation (UVR) with the seasons. Currently, two major strategies for surviving the polar climate have been identified: Stress avoidance and Stress tolerance (Kvíderová et al., 2019; Pushkareva et al., 2016). The former includes survival mechanisms that primarily ensure the absence of stress within the organisms. On the contrary, serves stress tolerance the purpose of coping with already present stress. Whether organisms only pursue one strategy or different combinations depends on the duration and intensity of the prevailing stressor (Kvíderová et al., 2019; Schulze et al., 2005).

1.7.1 Stress avoidance

Active migration

Certain microalgae react and adapt to changing environmental factors by actively moving towards more favourable environmental conditions (Elster & Benson, 2004). Eukaryotic algae most commonly employ a flagellum for motility while cyanobacteria of the order Oscillatoriales prefer straight-lined gliding (Elster & Benson, 2004).

Habitat selection

Some species can select their habitat according to optimal environmental conditions. To avoid excessive solar radiation, some cyanobacteria inhabit subsurface microenvironments with the depth in the soil being an important factor in ensuring sufficient absorption of photosynthetically active radiation (PAR) whilst avoiding UVR (Ehling-Schulz & Scherer, 1999; Whitton & Potts, 2002).

Complex life cycles

Seasonal temperature changes affect the metabolic processes within microalgae and therefore restrict their growth (Vincent, 2007). Consequently, polar algae developed specialized life stages that enable them to survive harsh climatic conditions. Some microalgae have adapted the concept of dormancy. During unfavourable conditions they fall into an anabolic state and return their metabolic activity to a normal rate once the conditions have returned to being propitious for growth (Tashyreva & Elster, 2012). This process is accompanied by several changes in the

organismic physiology and the development of protective mechanisms (Elster, 1999; Tashyreva & Elster, 2012).

Modifications in the structural morphology

Frequent changes in water availability are common in the Arctic. Therefore, some microalgae produce a sticky layer out of EPS to prevent desiccation. These mucopolysaccharides strengthen the cell wall, aid in the cellular retention of water and serve as protection against damage associated with the fluctuations in cell size (Caiola et al., 1993, 1996; Elster & Benson, 2004; Hu et al., 2012). Upon soil moistening, the trichomes of the cyanobacteria slide out of their sheaths leaving them behind empty. This contributes to the stabilization of the soil even in the absence of the organisms. Decreasing water content, however, initiates new sheath production (Pushkareva et al., 2016). Furthermore, these biopolymers operate as an accumulation matrix for UV-absorbing pigments protecting against excessive solar radiation (Caiola et al., 1996). In addition, some cyanobacteria, including *Nostoc* spp., form akinetes which provide protection against freezing and desiccation due to a thick cellular wall (Flechtner, 2007).

Symbiotic and mutualistic relationships

In order to minimize external stress factors microalgae have entered into symbioses with other organisms living in the Arctic (Pushkareva et al., 2016). The most common symbiotic relationship occurring in this climate are lichens with fungi as mycobionts and green algae or cyanobacteria as photobionts (Bjerke, 2011; Inoue et al., 2011). Living in this mutualistic relationship protects them from desiccation and cryoinjuries (Pushkareva et al., 2016). Furthermore, cyanobacteria and mosses form another important life-association in the polar regions. Their relationship is primarily epiphytic, serving the purpose of nutrient exchange and desiccation protection (Rousk, 2022; Zielke et al., 2005).

1.7.2 Stress tolerance

Alternations in the composition of fatty acids

The maintenance of the membrane fluidity is of great importance for preserving cellular equilibrium (Lyon & Mock, 2014). One strategy observed in microalgae is the desaturation of fatty acids (Vincent, 2007). In the presence of fatty acid desaturases, the number of double bonds is increased leading to the production of polyunsaturated fatty acids (PUFAs) in the membrane (Los & Murata, 2004; Lyon & Mock, 2014; Russell, 2008). These lipids maintain their fluidity

even at cold temperatures and therefore support the acclimatization of microalgae to the Arctic conditions (Vincent, 2007). The degree of unsaturation of membrane lipids is influenced by the salinity status, level of dryness, light exposure and the nutrient availability in the habitat (Morgan-Kiss et al., 2006).

Osmotic solutes and anti-freeze proteins

To resist the climate conditions in the polar regions some microalgae take advantage of compatible solutes including polyols (glycerol), sugars (mannose), amino acids (proline) and their derivatives (DMSP) (Oren, 2007). These solutes decrease osmotic stress in the events of high salinity and freeze-up by stabilizing proteins, DNA and cell membranes (Raymond-Bouchard & Whyte, 2017). Furthermore, some microalgae employ anti-freeze proteins to prevent cryoinjuries, as they bind to the ice crystals and change their structure, thus inhibiting growth (Davies et al., 2002).

Removal of reactive oxygen species and DNA repair mechanisms

Excessive exposure to high UVR or PAR can have severe consequences on Arctic microalgae as it leads to the damage of the genetic material and photoinhibition (Jungblut & Vincent, 2017; Stal, 2007). Additionally, low temperatures and other stressors facilitate the production of reactive oxygen species which can lead to different cell aging mechanisms (Adelman et al., 1988; Benson et al., 2007). To minimize the oxidative damage, antioxidant enzymes are activated (Lyon & Mock, 2014). Furthermore, several well-adapted species of cyanobacteria employ different methods for the reparation of their DNA upon damage (Ehling-Schulz & Scherer, 1999; Jungblut & Vincent, 2017; Potts, 1999).

Photoprotective screening and quenching pigments

Another mechanism for the protection against high-intensity solar radiation is the production of photoprotective-screening pigments such as Scytonemin. This is one of the most frequently occurring pigments in the EPS of cyanobacteria and eukaryotic microalgae and protects the soil crust embedded organisms by shielding > 90% of the incoming UV-A radiation (Vincent, 2007). However, UV-B screening pigments including mycosporine-like amino acids seem to have a higher abundance in the Arctic regions (Quesada et al., 1999).

1.8 Hardening of polar microorganisms

Microalgae of biological soil crusts growing under suboptimal conditions, especially in environments with low nitrogen levels, are found to exhibit a greater resistance against the prevailing challenges associated with the Arctic winter, than under conditions more propitious for growth. The process of hardening and starvation causes physiological reactions within the affected microalgae which are accompanied by morphological and ultrastructural modifications. Since the vast majority of adaptation/acclimation mechanisms are connected to metabolic rearrangements, also their (meta)genomic and (meta)transcriptomic profiles are altered (Billi & Caiola, 1996; Pichrtová et al., 2020; Pichrtová al., 2014; Tashyreva & Elster, 2015).

1.8.1 Acclimation to freezing/ melting and desiccation/rewetting injuries by nutrient starvation

In the spring, polar soils are supplied with a surge of meltwater from snow and ice (with high content of mineral nutrients) which increases the microalgal growth. This period is usually followed by longer periods of drought during summer when water and mineral nutrients are scarer. Furthermore, microalgae of biological soil crust are subject to seasonal extremes such as hypo- and hypersalinity, desiccation and inundation, diurnal and seasonal temperature fluctuations, frequent freeze-thaw cycles and, ultimately, deep-freeze temperatures down to -34°C in winter (Láska et al., 2012; Schmidt et al., 2009). The warming of the Arctic with more frequent winter thaws and decreases in snow cover, depth, and its cover duration also influences their survival (Bokhorst et al., 2015). However, they can handle these long periods of desiccation and strong fluctuations in temperature by using very different survival strategies. The selection is dependent on their origin and phylogeny, which is why the extent of their cryo- and desiccation resistance differs among individual strains and/or species (Alpert, 2005; Elster & Benson, 2004; Potts, 1999).

During winter the terrestrial microalgal communities are completely desiccated and frozen until liquid water is available again in spring or during winter melts (Láska et al., 2012). Reasons that influence the survival of these conditions are presumed to be the stable autumn temperatures and numerous day-to-day freeze-thaw cycles during summer as they promote the cold/desiccation hardiness of the BSC microalgae before the onset of lower winter temperatures (Davey & Rothery, 1992; Elster & Komárek, 2003; Hawes, 1990; Hawes et al., 1992; Pichrtová et al., 2014; Pichrtová et al., 2014; Šabacká & Elster, 2006; Tashyreva & Elster, 2015). Nutrient starvation is connected

to hardening (Siegele & Kolter, 1992; Votyakova et al., 1994), as the lack of mineral nutrients increases resistance to different stress factors. The associated changes, including the development of vegetative cells adapted to prolonged freezing – with thickened cell walls, structurally reduced chloroplasts, accumulated storage materials, and diminished physiological activity (pre-akinetes) (Sheath et al., 1996), might account for the increased tolerance of starved cells against different stress factors (Tashyreva and Elster 2015), including freezing and desiccation (Billi & Caiola, 1996).

2 Aims

Polar regions are characterized by highly demanding climatic conditions with short, cold, and dry summers followed by long, icy and dark winters. BSC microalgae are capable of handling these seasonal extremes, including long periods of desiccation and strong fluctuations in temperature, by using very different survival strategies. With the help of a combined methodological approach (field observation, manipulation studies, laboratory experiments and analyses), this Czech-German collaborative research project intends to prove that hardening including nutrient starvation are the main ecophysiological mechanisms promoting microalgal cryo- and desiccation resistance. In the framework of this bachelor thesis, the following objectives were examined:

i. Preliminary determination of the differences in the microalgal abundance and biodiversity with changes in the altitudes
ii. Assessment of the vitality of the BSC community of the High Arctic during the summer months via:
    - Evaluation of the cell condition by fluorescence staining
    - Observation of algal growth during a laboratory cultivation period by monitoring of the photosynthetically active area using variable chlorophyll fluorescence imaging and growth rate evaluation by light microscopy (tentative data)

3 Materials and Methods

3.1 Study site description

The field study was conducted in three localities with BSC near Longyearbyen, Central Svalbard, at different elevations. The climate in this Arctic Archipelago is characterized by very short, cold and dry summers (June -August). In the year 2022 the average temperature ranged from 5.6 to 6.5°C. The mean precipitation amounted to lowest at 4.5mm in July up to 36.6 mm in August (Norwegian Centre for Climate Services, n.d.). The mountainous landscape is covered by glaciers and polar deserts with U-shaped valleys interspersed by seasonally present rivers and freshwater wetlands (lakes). Two of the three study sites are located at plateaus of the mountain Breinosa (2: 78°09'20.3"N 16°01'52.7"E; 3: 78°08'47.4"N 16°02'21.5"E) at 409 m and 519 m above sea level. The last site is located on the western part of the coast in Bjørndalen (1: 78°13'11.5"N 15°19'54.9"E, 47 m a.s.l.).

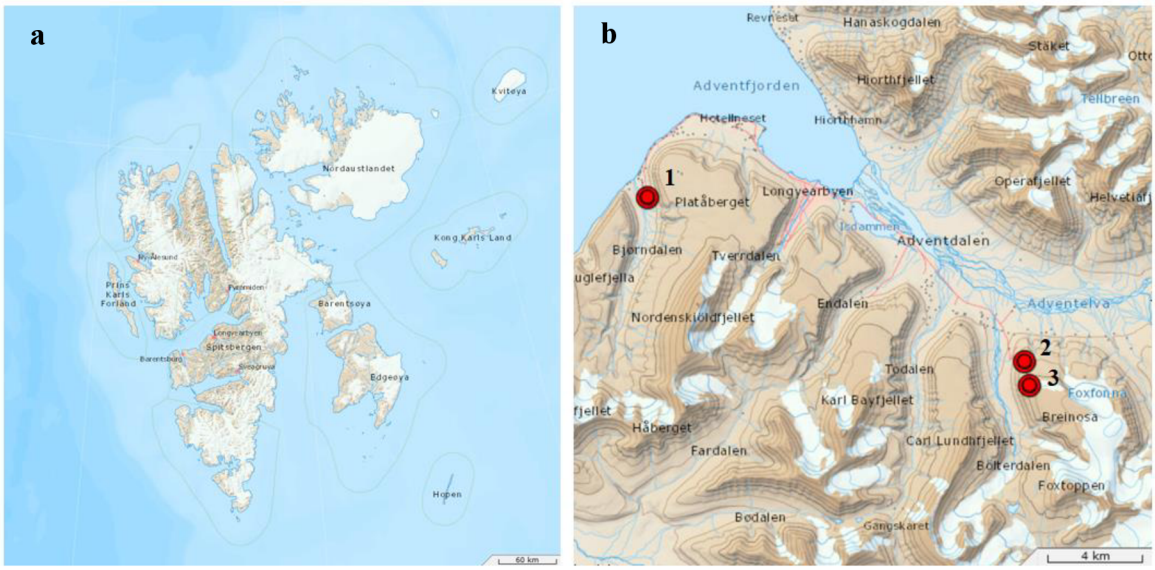

Figure 3: Map of Svalbard (**a**) and map of Central Svalbard showing the distribution of the sampling sites (**b**): Bjørndalen (**1**), Breinosa 1 (**2**), Breinosa 2 (**3**) (Norwegian Polar Institute, n.d.)

At all sites, the bedrock consisted of sandstone with siltstone and shale intercalations, covered in a unique vegetation. Bjørndalen (1) is surrounded by a wet meadow with an abundant growth of different species of mosses and grasses such as the polar willow (*Salix polaris*), cotton grass (*Eriophorum* sp.) and *Silene acaulis*. Site two in Breinosa (1) is a sparsely vegetated area on moist substrate with small patches of mosses and lichens between detritus.

Common plants found in this area include *Polytrichum* sp., *Luzula confuse*, *Cerastium arcticum* and *Saxifraga cespitosa*. Breinosa 2 was mainly covered in biological soil crusts and various types of rocks with little to no vegetation.

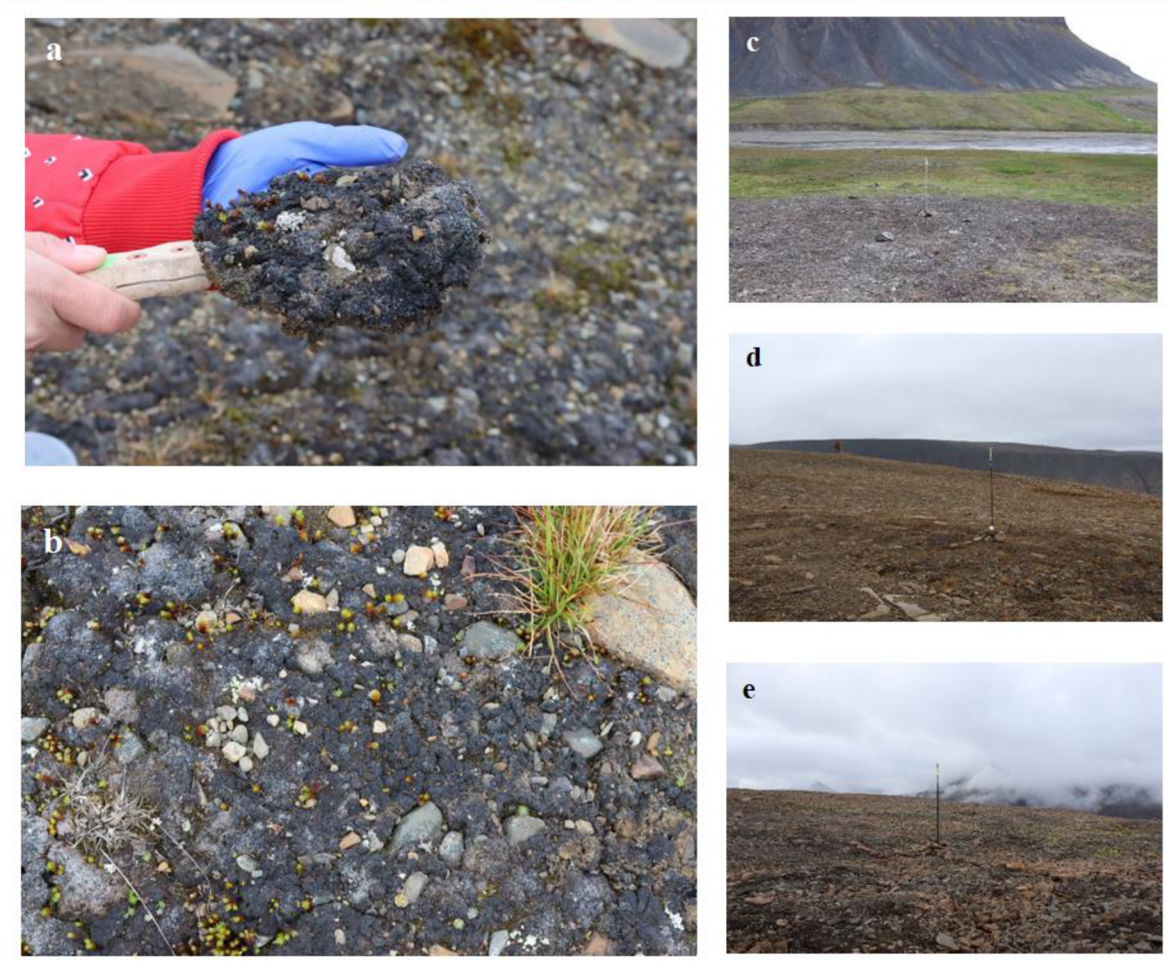

Figure 4: Exemplary photographs of BSC in Breinosa at sampling site 2 (**a,b**) and pictures of the vegetation at different experimental sites in August 2022: Bjørndalen (**c**), Breinosa 1 (**d**), Breinosa 2 (**e**)

3.2 Experimental setup

The vitality assessment of microalgae was carried out as part of a biological soil crust field manipulation experiment (Fig. 5). Nutrients and water as well as simulated weather events are supposed to show the positive or negative effects of starvation and hardening on the resistance of microalgae towards cryoinjuries and desiccation in comparison to untreated controls. In summer, water and nutrient experiments are conducted, while in winter, melting and weather manipulation experiments take place. Each experimental site was set up with bowls as well as petri dishes in a beforehand specified area (site 1: 53.76 m^2; site 2: 21.11 m^2; site 3: 63.50 m^2).

The bowls and the petri dishes were assigned and labelled according to their different treatments: control, additional watering (summer), nutrient supply (summer), freeze-thaw cycle simulations (winter) and rain-on-snow event simulations (winter).

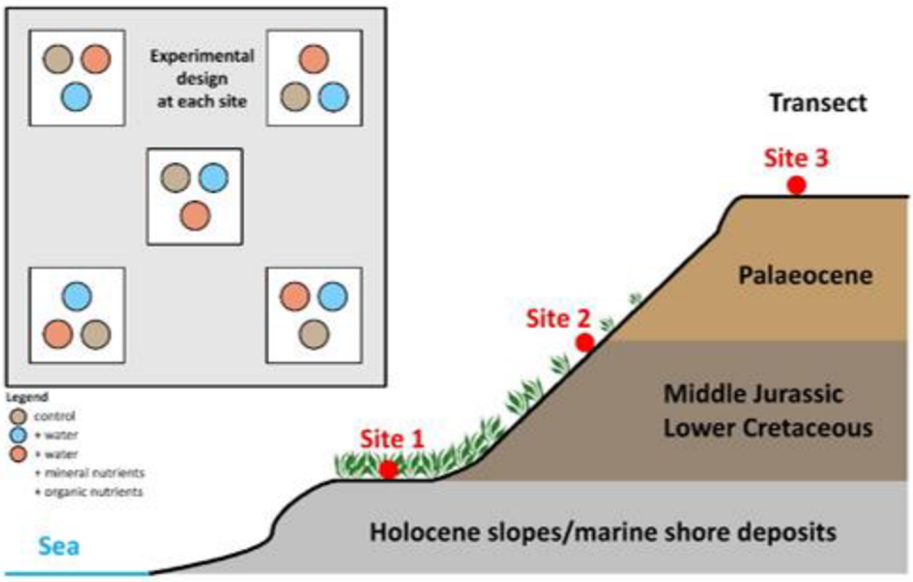

Figure 5: Experimental design at each sampling site (Kvíderová, 2022)

3.3 Experimental Sampling

The sampling for the vitality assessment was performed in August 2022. Therefore, six samples of each study side were collected. The biocrusts were discernible as brown to black areas without vegetative cover. Each sample was cut out by pressing the bottom of a petri dish (diameter: 7cm, depth: 1.5cm) onto the crust and removing it carefully with a scraper. The samples were transported back into the station in a cooler. Afterwards the upper most millimetres of the crust cover from each study site were transferred into a collected sample tube with the help of tweezers and a spatula. Both samples were diluted with distilled water. Excess soil and sediment were removed using a sieve. Vital staining was performed at the Josef Svoboda Czech Arctic Research Station in Svalbard.

Furthermore, additional soil crust samples were collected for the growth tests in the beginning of September. To this end, the uppermost layer was extracted with a spoon from different spots at each sampling site and transferred into a 15ml centrifugation tube. The collected biomass was stored at a temperature of -20°C and protected from light during the transportation to the Centre for Phycology of the Institute of Botany CAS in Třeboň, where further analysis was performed.

3.4 Viability evaluation

Vitality evaluation via fluorescence staining was based on the multiparameter staining protocol originally developed by Tashyreva et al (2013). Nonetheless, due to complications with the execution of algal field samples, growth tests with fluorescence imaging were employed as an alternative method.

3.4.1 Multiparameter staining protocol

The vital state of the microalgae within the crust was tested by evaluating the presence/shape of their nucleoids, the membrane state and respiration rate. Therefore, three different fluorescence dyes were used: DAPI (4′,6-diamidino-2-phenylindole, Molecular Probes, Eugene, OR, USA), SYTOX® Green (S7020, Molecular Probes, Eugene, OR, USA) and CTC (5-cyano-2,3-ditolyl tetrazolium chloride, Polysciences Europe GmbH, Eppelheim, Germany). DAPI is a blue-fluorescent stain that is visible in both living and dead cells. The excitation and emission peaks range around wavelengths of 350 and 470 nm. SYTOX® Green nucleic acid stain is able to penetrate disintegrated cell membranes. By binding to DNA within the nucleus, the stains produce a green-fluorescent signal with an excitation/emission peak of 504/523 nm, leading to the detection of dead cells. The rate of respiration is assigned by using CTC stain. Upon active respiration the tetrazolium salt gets reduced to CTC formazan. When exited with light in the range of 430-480nm, these crystals become visible as red fluorescent dots within the cell (emission: 630 nm).

3.4.1.1 Staining of laboratory samples

As the protocol was originally designed for cyanobacteria of the species *Phormidium* (Oscillatoriales) (Tashyreva et al., 2013), the procedure was adapted for microalgae with the help of algal mixtures in March 2022. Three different strains of eukaryotic microalgae and two cyanobacterial strains (*Klebsormidium* sp., *unidentified coccoid green alga, Monoraphidium* sp., *Phormidesmis* sp., *Phormidium uncinatum*) were included in the selection, since these genera are common in Arctic BSC. The staining procedure was started by preparing inactivated reference samples. To this end, the cell suspensions were treated with formaldehyde or subjected to boiling. Afterwards, 100 µl of the prepared samples were stained with 2, 10 and 20 µl of SYTOX® Green and incubated for 15 and 30 minutes, respectively. While staining, the samples were kept cool and covered in aluminium foil to protect them from light. Once the incubation time was reached, the

samples were washed three times with BG11 medium to remove excess dye. This exact procedure was repeated for 2, 10, and 20µl of CTC stain with a processing time of 60 and 150 minutes.

DAPI staining was performed with a sample volume of 100 µl and 3.33 µl of dye for two different incubation periods (15 and 30 minutes). All the samples were shortly centrifuged and subsequently evaluated under the microscope at different magnifications. For this purpose, an Olympus BX-51 with a 100-W ultrahigh-pressure mercury arc lamp (Olympus Corporation, Tokyo, Japan). The microscope was equipped with different filters for the detection of the fluorescence stains. DAPI observations were conducted by employing a U-FUN filter cube with band-pass excitation filter of 360–370 nm and a long-pass cutoff filter at 420 nm. The fluorescence of SYTOX® Green was detected using a U-FBWA cube with excitation filters in the range of 460–495 nm and emission filters ranging from 510 to 550 nm. For the visualization of CTC-formazan crystals, a U-FRFP cube equipped with filters for excitation/emission ranging between 535–555 nm and 570–625 nm was utilized.

3.4.1.2 Staining of field samples

The evaluation of microalgal abundance via light microscopy preceded the staining of the field samples. With the help of the laboratory assessment, the minimum staining concentration was then adjusted to the field conditions. Upon the arrival at the lab, test mixtures were prepared, consisting of 200 µl aliquots of the diluted samples from both Site 1 and Site 2 to ensure high cell density.

DAPI

The cell suspensions were stained at a DAPI dye concentration of 5 µg/ml under cold and dark conditions for 15 and 30 minutes, respectively. Excess staining material was removed by washing the samples three times with filtered water.

SYTOX® Green

To investigate the optimum staining concentration of SYTOX® Green for the field samples, two inactivated controls were prepared prior to staining. To this end, cell suspensions were subjected to boiling (10 min at +90°C) and treated with ethanol (70%) for 50 minutes. All sample solutions were then stained at final concentrations of 5, 10 and 20 µM of SYTOX® Green and incubated for 15 minutes protected from light. Samples were rinsed three times with filtered water and soaked for 15 minutes between each step.

Furthermore, homogenized suspensions of soil crusts and sediment were stained with a SYTOX® Green dye at a final concentration of 5, 10 and 20 µM for 15 minutes. Excess stain was removed by washing with a 30-minute soaking period during the third cycle. Inactivated controls were prepared according to the procedure mentioned above, with an extension of the ethanol incubation time to 5 hours. For the removal of excess soil and sediment, ultrasonic waves at a frequency of 45 kHz (using a VWR ultrasonic cleaner) were applied for 3 minutes at 24-27°C for one of the ethanol-treated samples.

To check for bias, two additional samples were prepared at a concentration of 20 µM of SYTOX® Green stain with an incubation time of 15 minutes: one was treated with ethanol pre-sonication and one was treated with ethanol post-sonication. Both samples were compared to a living reference. Additionally, measurements were conducted using 4% neutral buffered formalin inactivated controls (60 minutes) at the same staining concentrations.

Effects of sonication on the vitality of the sample cells were assessed by conducting a control experiment with four different probes (Living, Living sonicated, EtOH, EtOH sonicated). Inactivated references were prepared by treating the samples with 70% ethanol for 60 minutes; one of the living and one of the dead samples were further subjected to sonication for 3 minutes (24-27°C). The presence of cells was verified with the help of their autofluorescent activity. The optimal sonication time was investigated by treating two living samples with ultrasonic waves for 3 and 5 minutes, respectively. A living non-sonicated reference was used for comparison. All samples were stained at a SYTOX® Green concentration of 20 µM for 30 minutes.

CTC

In order to adjust the optimum staining concentration of CTC for the field samples, 100 µl of ethanol and formaldehyde treated samples were stained with 2, 10 and 20 µl of CTC stain for 60 minutes to reach a final concentration of 1, 5 and 10 mM. Afterwards, the excess dye was removed.

Following the staining process, all samples were immediately examined, and the number of stained cells was noted. For this purpose, sample solutions were transferred onto a slide and microscopically evaluated with an Olympus BX-53 microscope with a 100-W ultra-high pressure mercury arc lamp (Olympus Corporation, Tokyo, Japan) equipped with the same filters used for the assessment of the vitality in the laboratory strains.

Additionally, the cell quantity of the soil crusts from the field was compared by evaluating BSC samples taken from the backyard of the research station for their autofluorescent activity. Dilutions (400 µl, 800µl) of the mixed samples aided as test sample.

3.4.1.3 Image acquisition and processing

The microphotographs were taken by the Olympus DP-74 camera (Třeboň) / DP-73 (Svalbard) (both Olympus, Japan) attached to the microscope and processed by the cellSens Entry 3.1 software (Třeboň; Olympus, Japan) and QuickPhoto 2.3 software (Svalbard; ProMicra, Czech Republic).

3.4.2 Growth tests and diversity evaluation

The polar crusts were analysed for their vitality in the Centre for Phycology of the Institute of Botany CAS in Třeboň. 0.1g, 0.2g and 0.4g of soil crust from each study site were weight in. The soil crust sample was spread and cultured on agar plates in petri dishes with BG11 medium (Stanier et al., 1979). At the start of the cultivation 0.5 ml of liquid BG11 medium were added to ensure optimum conditions after the transport. This procedure was repeated three times for each weight and sample of each study site. All petri dishes were stored at a temperature of 5-7°C and illuminated continuously (irradiation: 30 µmol m^{-2} s^{-1}). In a cultivation period of seven days, chosen parts of the soil crusts were evaluated for their microalgal growth via light microscopy using an OLYMPUS BX-51 microscope (Olympus Corporation, Tokyo, Japan).

To visualize the growth of the individual colonies, pictures of the regions of interest were taken on each day measurements using an Olympus DP74 digital camera (Olympus, Japan) equipped with 20 × and 40 × magnification lenses. It is important to mention that the microscopic evaluation was exclusively performed with the petri dishes containing 0.1 g of soil crusts as the other samples had too much soil abundance. After the completion of the measurements, the morphotypes of the cyanobacteria and algae cultivated on the agar plates, were assessed with the help of the produced microphotographs. The photosynthetic microorganisms were identified according to the following manuals: Heering (1914), Geitler (1932), Batko (1975), Růžička (1977, 1981), Ettl (1978), Rieth (1980), Komárek & Fott (1983), Kadłubowska (1984), Mrozińska (1985), Krammer & Lange-Bertalot (1986, 1991a, 1991b), Lange-Bertalot (1993), Ettl & Gärtner (2014).

3.4.3 Fluorescence imaging

The photosynthetic activity of photosynthetic microorganisms in all petri dishes was evaluated via a variable chlorophyll *a* fluorescence approach using a closed FluorCam 700MF fluorescence imaging camera (Photon Systems Instruments, Brno, Czech Republic). Images were captured on a weekly basis, twice each measuring day (morning and afternoon). During the first week, the activity was assessed three times. The analysis was completed after four consecutive weeks as the growth rate exceeded detectable values. The acquired data were processed by the FluorCam 7.0 software (Photon Systems Instruments, Brno, Czech Republic).

After 15-minutes of dark adaptation, the pre-defined protocol for F_V/F_M was applied. The dark period to determine the minimum fluorescence (F_0) lasted 2 s during which red measurement pulses with the intensity of <1 µmol m^{-2} s^{-1} were applied. This was followed by a strong white saturating pulse with an intensity of 1100 µmol m^{-2} s^{-1} and a duration of 960 ms to determine the maximum chlorophyll fluorescence.

The maximum quantum yield (Φ_{Po}, F_V/F_M) of PSII was estimated according to equation (Roháček et al., 2008),

$$\Phi_{Po} = \frac{F_V}{F_M} = 1 - \frac{F_0}{F_M} = \frac{F_M - F_0}{F_M} \quad (2)$$

where F_0 is defined as the minimum fluorescence in the dark and the F_M corresponds to the maximum quantum yield after the saturation pulse in the dark-adapted state.

In addition to Φ_{Po}, the total photosynthetically active area (PAA) per petri dish was evaluated as a proxy of growth.

3.5 Statistical analysis

Fluorescence data were analysed in Statistica 10.0 software (StatSoft, USA). Variations in the PAA, F_0 and maximum quantum yield (F_V/F_M) at different sampling sites for different inoculation weights were evaluated using a one-way ANOVA at three different significance levels ($p < 0.05$; $p < 0.01$; $p < 0.001$). Additionally, a one-way ANOVA was performed to examine the statistically significant effects of the inoculation dose on the growth and vitality parameters.

4 Results

4.1 Biodiversity and abundance

The preliminary estimation of the biodiversity and abundance of microalgae at different study sites was based on morphological identification by light microscopy. In total 128 algal and cyanobacterial morphotypes were identified within nine cultivations from three sampling sites (see Appendix). All three sites differed significantly in their composition. In most cases, the biological crusts were dominated by unidentified coccoid green algae in initial stages of algal group's growth.

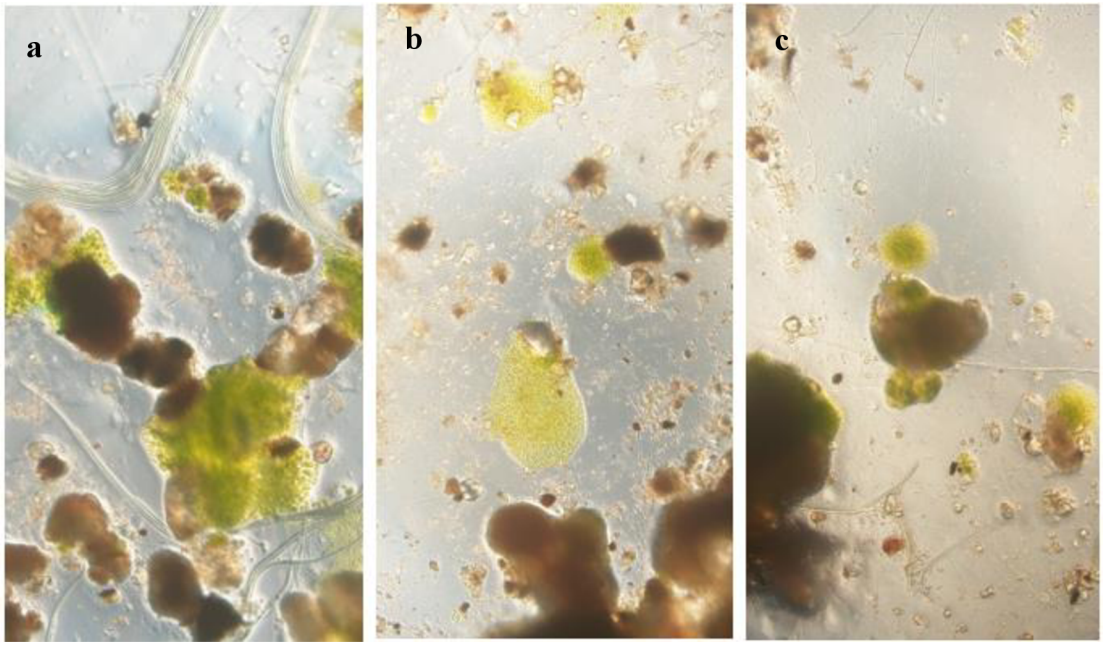

Figure 6: Example of microalgal diversity revealed under the light microscope in Bjørndalen (**a**), Breinosa 1 (**b**), Breinosa 2 (**c**) cultivated on agar plates

However, it is apparent from figure 7 that cyanobacteria are the predominant taxonomical group at study site 1 (Bjørndalen). Furthermore, a clear trend in the relative abundance becomes evident when comparing the two study sites in Berniosa with more frequently observed species from the class *Trebouxiophycae* and *Bacillariophyceae* than in Bjørndalen. Additionally, figure 7 emphasizes the absence of species from the filamentous group of *Klebsormidium* sp. in study site 2. Moreover, at least one filamentous microalga, including *Microcoleus* sp. (*cyanobacteria-Oscillatorialles*), *Klebsormidium* sp. and *Nostoc* sp. (*cyanobacteria-Nostocales*), was detected in each study site.

A more detailed analysis of the species composition in the studied BSC is going to be provided by strain isolation and microalgal biovolume estimation, following a metabarcoding approach as part of this ongoing project.

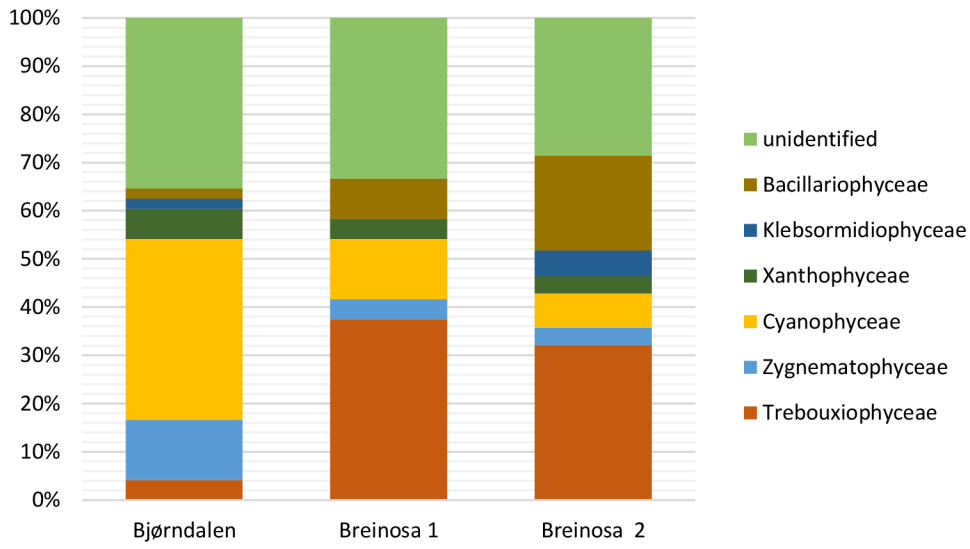

Figure 7: Relative comparison of the taxonomic diversity in biological soil crusts of the High Arctic at three different study sites

4.2 Fluorescent staining

Fluorescence staining, based on a multiparameter fluorescent staining protocol, aimed to assess the viability of microalgae in cryptogamic crusts of the High Arctic. Before applying the staining procedure to field samples, optimal concentrations of fluorescence dyes (DAPI, SYTOX® Green and CTC) were determined using laboratory strains. The results of these experiments are summarized in Table 2.

Table 2: The final concentrations of fluorescence dye for the laboratory assessment of vitality

Stain	concentration (per 100 µl of sample solution)	staining volume / µl	incubation time / min
DAPI	5 µg/ml	3.33	15
SYTOX® Green	5 µM	10	15
CTC	1 mM	2	150

In general, the staining of field samples appeared to be highly inconsistent. During the microscopic evaluation a number of issues were identified. Both DAPI and SYTOX® Green stained samples revealed a noticeably low cell abundance with sometimes less than one cell per evaluated sample drop. Overall, no significant difference was observed between the living cells and inactive controls. Bright green, fluorescent signals were also visible in most living cell samples stained with SYTOX® Green. During the second SYTOX® Green-staining attempt an overall higher cellular density was observed. Moreover, the microscopic evaluation showed fewer SYTOX®-Green positive cells in the living samples than previous attempts. However, in the inactivated controls the majority remained unstained with the lowest staining observed at a dye concentration of 5 µM. In addition, sonication led to no improvement in the stainability of ethanol treated samples as algal cells remained embedded in the cluster of moss and sediment. Living cells stained at a concentration of 20 µM showed little to no fluorescence signal with different sonication times. When changing the inactivation method from ethanol to formaldehyde no significant difference between the samples was found. None of the formalin treated samples at different concentrations showed a green-fluorescent signal although their presence was verified by their autofluorescent activity.

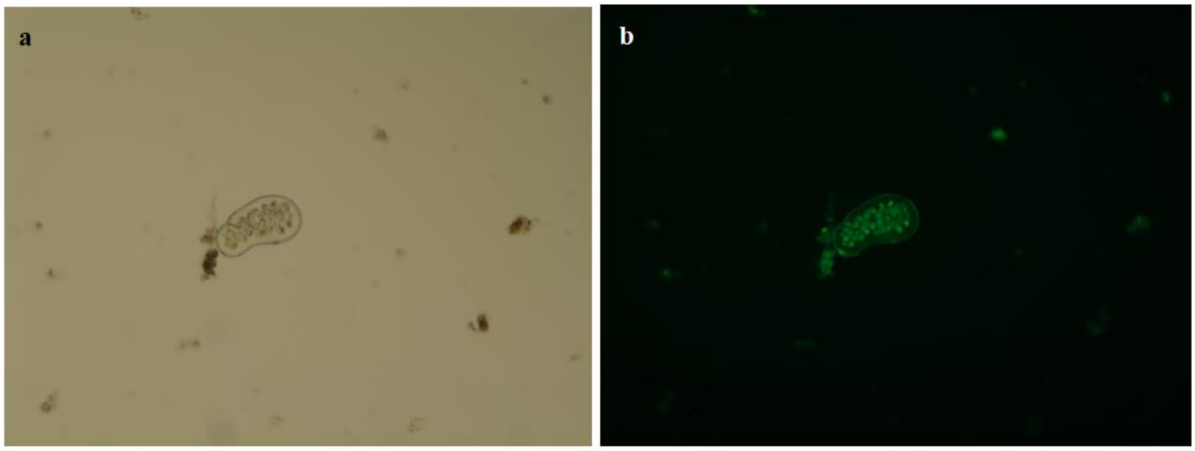

Figure 8: Microphotographs of *Nostoc sp*. under light microscope (**a**) and stained with SYTOX® Green (**b**)

Furthermore, changes in incubation times exhibited no relevant effects on the obtainment of DAPI-positive cells. Comparison of the outdoor sample with the dilutions from the mixed samples further raises the conjecture of a sparse cell population in the soil crusts. It is further apparent that the cell count in the backyard sample (400 µl dilution) is significantly higher than in both mixed samples with 10 cells per 20 µl of cell suspension.

Moreover, the sonication attempt showed in both sonicated samples little to no autofluorescence activity in contrast to untreated living and inactivated controls. However, samples stained with CTC at a concentration of 1 mM exhibited bright red fluorescent signals in approximately half of the cells contained in the living samples. Ethanol and formaldehyde inactivated controls yielded similar results. Additionally, in all stained samples, a substantial number of cells were observed to be surrounded or enveloped by a mucilaginous layer. This was particularly observed in cells of *Nostoc* ssp.

4.3 Analysis of the photosynthetic activity

4.3.1 Growth evaluation

Two parameters, the photosynthetically active area (PAA) and minimum fluorescence (F_0), derived from fluorescence imaging were considered for growth tracking, as they serve as chlorophyll *a,* and hence, biomass proxies. When PAA had been used for growth tracking, all samples expressed prolonged lag phase lasting at least 7 days, followed by a steep increase in PAA (Fig. 9, Table 3). Although after 24 days of cultivation the stationary phase was not reached, the differences between the sites became obvious and statistically significant in all inoculation doses. The lowest biomass content was found in Site 1 samples, higher and comparable biomass was observed in Sites 2 and 3 samples (Fig. 9, Table 3). Overall, site 2 showed the most profound increase in PAA.

The F_0 values were stable during the cultivation, and the observed increase and/or decrease were only minor (Fig. 10). The differences between the samples were rare, and no trends during cultivations were observed. However, samples obtained from study site 1, on average, reached the highest F_0 values (Table 4).

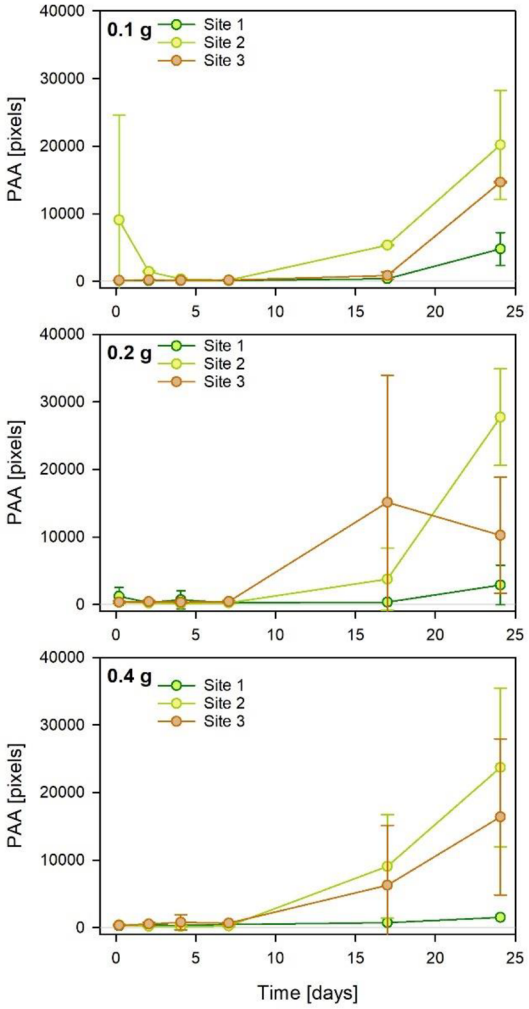

Figure 9: The growth of the photosynthetic microorganisms tracked by photosynthetically active area (PAA; mean ± standard deviation, n = 3) measurement at different inoculation doses

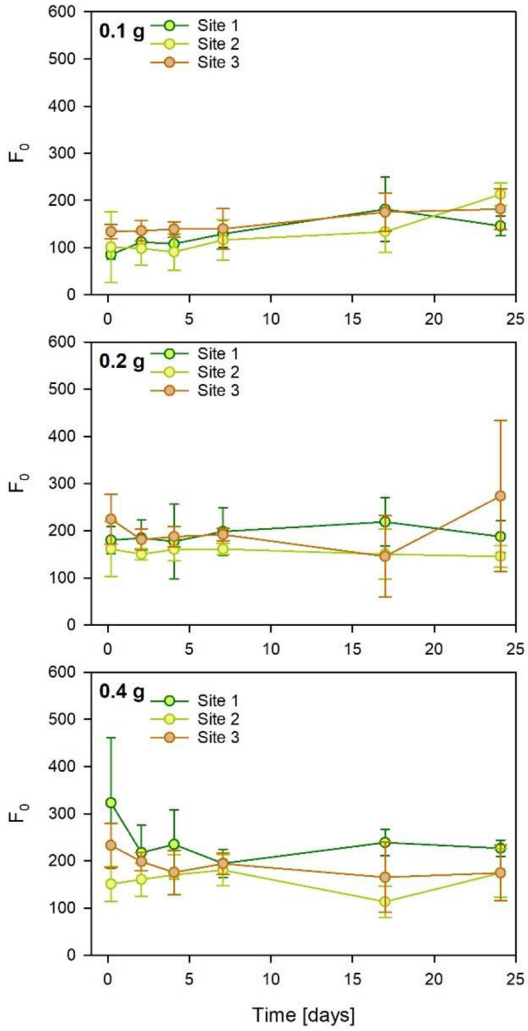

Figure 10: The growth of the photosynthetic microorganisms tracked by minimum fluorescence (F_0; mean ± standard deviation, n = 3) measurement at different inoculation doses

Table 3: The differences in the PAA (mean ± standard deviation) at different experimental sites in time for different inoculation doses. The statistically significant differences (one-way ANOVA, n = 99) among the sites are marked by asterisks: * P < 0.05, ** P < 0.01, *** P < 0.001

CULTIVATION TIME (DAYS)	INOCULATION DOSE: 0.1 G			
	Site 1	**Site2**	**Site3**	
0.19	101 ± 113	9078 ± 1549	136 ± 12	
2.06	111 ± 48	1425 ± 86	192 ± 119	
4.06	108 ± 62	338 ± 143	143 ± 96	
7.06	99 ± 48	157 ± 95	173 ± 102	
16.99	387 ± 227	5351 ± 89	848 ± 543	
24.07	4781 ± 2431	20190 ± 8055	14663 ± 102	**
	INOCULATION DOSE: 0.2 G			
	Site 1	**Site2**	**Site3**	
0.19	1192 ± 1309	333 ± 232	352 ± 144	
2.06	299 ± 281	198 ± 56	417 ± 178	
4.06	696 ± 1338	114 ± 45	331 ± 161	
7.06	235 ± 125	238 ± 97	440 ± 147	*
16.99	342± 215	3754 ± 4600	15114 ± 18798	
24.07	2881 ± 2933	2777 ± 7146	10247 ± 8586	***
	INOCULATION DOSE: 0.4 G			
	Site 1	**Site2**	**Site3**	
0.19	257 ± 119	385 ± 154	329 ± 53	
2.06	344 ± 217	193 ± 73	524 ± 231	*
4.06	332 ± 172	171 ± 85	775 ± 1102	
7.06	455 ± 317	237 ± 103	656 ± 179	*
16.99	733 ± 196	9062 ± 7655	6263 ± 8876	
24.07	1506 ± 250	23686 ± 11733	16391 ± 11552	***

Table 4: The differences in the F_0 (mean ± standard deviation) at different experimental sites in time for different inoculation doses. The statistically significant differences (one-way ANOVA, n = 99) among the sites are marked by asterisks: * P < 0.05, ** P < 0.01, *** P < 0.001

CULTIVATION TIME (DAYS)	INOCULATION DOSE: 0.1 G			
	Site 1	**Site2**	**Site3**	
0.19	85 ± 9	101 ± 75	134 ± 15	
2.06	112 ± 19	98 ± 35	136 ± 22	
4.06	108 ± 19	91 ± 38	139 ± 16	*
7.06	129 ± 29	116± 43	140 ± 43	
16.99	181 ± 68	133 ± 43	175 ± 40	
24.07	146 ± 21	213 ± 24	182 ± 43	***
	INOCULATION DOSE: 0.2 G			
	Site 1	**Site2**	**Site3**	
0.19	180 ± 29	161 ± 58	224 ± 53	
2.06	184 ± 39	150 ± 12	181 ± 22	
4.06	177 ± 79	160 ± 23	187 ± 22	
7.06	198 ± 51	161 ± 12	192 ± 13	
16.99	219± 51	150 ± 53	145 ± 86	
24.07	187 ± 34	146 ± 23	273 ± 160	
	INOCULATION DOSE: 0.4 G			
	Site 1	**Site2**	**Site3**	
0.19	323 ± 138	151 ± 37	232 ± 47	
2.06	217 ± 58	160 ± 35	198 ± 19	
4.06	235 ± 73	170 ± 43	175 ± 47	
7.06	194 ± 29	180 ± 33	194 ± 23	
16.99	239 ± 28	113 ± 33	165 ± 74	**
24.07	226 ± 17	174 ± 52	174 ± 59	

4.3.2 Vitality assessment

The values of the maximum quantum yield (F_V/F_M) increased over time. The lowest value of 0.5 was found in the lightest inoculation dose at sampling site 1 (Fig. 11, Table 5). The differences among the sites varied at different inoculation doses and were most significant at 0.2 g per Petri dish. In general, the lowest values were found in samples from Site 1, the maximum ones were detected in Site 3 (Table 5).

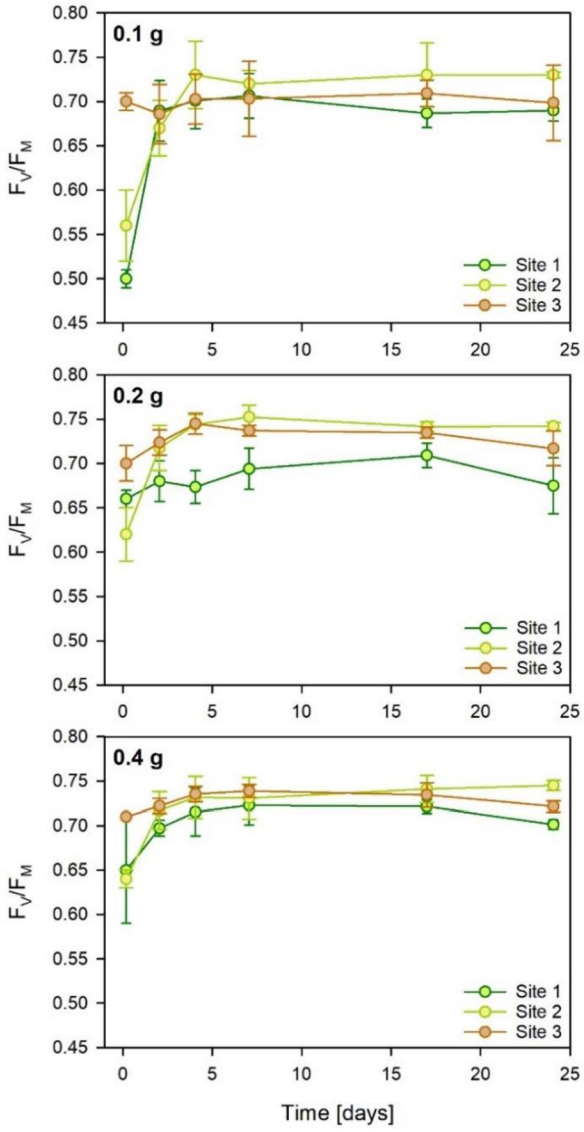

Figure 11: The change in vitality of photosynthetic microorganisms expressed as the maximum quantum yield (F_V/F_M; mean ± standard deviation, n = 3) in time at different inoculation doses

Table 5: The differences in the F_V/F_M (mean ± standard deviation) at different experimental sites in time for different inoculation densities. The statistically significant differences (one-way ANOVA, n = 99) among the sites are marked by asterisks: * P < 0.05, ** P < 0.01, *** P < 0.001

CULTIVATION TIME (DAYS)	INOCULATION DOSE: 0.1 G			
	Site 1	**Site2**	**Site3**	
	mn	mn	mn	
0.19	0.504 ± 0.007	0.557 ± 0.037	0.697 ± 0.014	**
2.06	0.690 ± 0.034	0.667 ± 0.031	0.686 ± 0.033	
4.06	0.701 ± 0.032	0.728 ± 0.038	0.703 ± 0.028	
7.06	0.706 ± 0.025	0.723 ± 0.015	0.703 ± 0.042	
16.99	0.687 ± 0.016	0.735 ± 0.036	0.709 ± 0.015	***
24.07	0.690 ± 0.012	0.734 ± 0.003	0.699 ± 0.042	***
	INOCULATION DOSE: 0.2 G			
	Site 1	**Site2**	**Site3**	
	mn	mn	mn	
0.19	0.656 ± 0.013	0.624 ± 0.026	0.705 ± 0.019	*
2.06	0.680 ± 0.023	0.718 ± 0.025	0.724 ± 0.014	***
4.06	0.673 ± 0.018	0.744 ± 0.011	0.745 ± 0.012	***
7.06	0.694 ± 0.023	0.752 ± 0.014	0.737 ± 0.006	***
16.99	0.709 ± 0.014	0.742 ± 0.006	0.735 ± 0.006	***
24.07	0.675 ± 0.032	0.742 ± 0.005	0.717 ± 0.019	***
	INOCULATION DOSE: 0.4 G			
	Site 1	**Site2**	**Site3**	
	mn	mn	mn	
0.19	0.647 ± 0.058	0.636 ± 0.013	0.710 ± 0.001	*
2.06	0.697 ± 0.009	0.717 ± 0.021	0.722 ± 0.008	*
4.06	0.715 ± 0.027	0.732 ± 0.024	0.735 ± 0.009	
7.06	0.723 ± 0.022	0.731 ± 0.023	0.739 ± 0.007	
16.99	0.721 ± 0.008	0.741 ± 0.015	0.734 ± 0.014	
24.07	0.701 ± 0.005	0.745 ± 0.006	0.721 ± 0.006	***

4.3.3 Effects of inoculation dose on studied growth and vitality parameters

The effects of the inoculation dose were site specific. In Site 1 samples, no differences were observed at the beginning of cultivation. The effects on PAA were found significant from 7th to 17th day of cultivation, while the differences in F_0 were detected even 2 days after the inoculation and remained detectable till the end of cultivation, with an exception on the 17th cultivation day. The differences in F_V/F_M were only occasional on days 4th and 17th (Table 6). No effects of inoculum dose on PAA were found in samples from Site 2. The effects of inoculum dose on F_0 were similar to the samples from Site 1. The differences in F_V/F_M were also only occasional on the first day, and on the 7th and 25th day of cultivation (Table 6). The statistically significant response of the growth and vitality parameters to inoculation dose in samples from the Site 3 was observed during the first 7 days of cultivation, with an exceptional PAA on the 4th day and F_V/F_M on the first day. In later stages of cultivation, only the differences in F_V/F_M till the end of the experiment were apparent (Table 6).

Table 6: The statistical significance (one-way ANOVA, n = 99) of the effects of inoculation dose on PAA, F_0 and F_V/F_M and their change during cultivation. Abbreviations: F – F-value, P – P-value. The statistically significant effects are marked in bold red

		SITE 1			SITE 2			SITE 3		
CULTIVATION TIME (DAYS)		PAA	F_0	F_V/F_M	PAA	F_0	F_V/F_M	PAA	F_0	F_V/F_M
0.19	F	1.129	3.058	0.072	0.949	0.892	**0.793**	**5.372**	**5.223**	0.680
	P	0.431	0.189	0.932	0.438	0.458	**0.024**	**0.046**	**0.049**	0.543
2.06	F	2.146	**9.971**	0.780	0.809	**7.889**	2.880	**5.217**	**14.21**	**5.910**
	P	0.151	**<0.001**	0.476	0.464	**<0.001**	0.087	**0.019**	**<0.001**	**0.013**
4.06	F	0.748	**6.043**	**3.930**	0.527	**9.009**	0.032	1.597	**4.208**	**9.210**
	P	0.491	**0.012**	**0.043**	0.601	**<0.001**	0.730	0.273	**0.037**	**0.003**
7.06	F	**4.916**	**6.296**	2.250	2.521	**5.437**	**4.620**	**16.51**	**6.605**	**3.830**
	P	**0.022**	**0.010**	0.140	0.114	**0.018**	**0.027**	**<0.001**	**0.009**	**0.045**
16.99	F	**6.079**	1.896	**9.760**	0.809	0.727	0.400	2.159	0.284	**8.440**
	P	**<0.001**	0.184	**0.002**	0.464	0.500	0.680	0.150	0.757	**0.003**
24.07	F	3.341	**15.57**	2.750	0.809	**8.054**	**9.600**	0.646	1.839	**5.500**
	P	0.063	**<0.001**	0.096	0.611	**0.004**	**0.002**	0.537	0.193	**0.016**

5 Discussion

Environmental stresses, such as freezing and desiccation, are important factors limiting the development of life in the High Arctic (Tashyreva & Elster, 2012). To survive the extreme fluctuations in the polar climate, microalgae in biological soil crusts developed a wide range of adaptive mechanisms (Borchhardt et al., 2017; Elster et al., 1999; Pichrtová et al., 2020; Williams et al., 2017). In the framework of this ongoing project, we hypothesized that their cryo- and desiccation resistance can be, inter alia, promoted by hardening and starvation. This study set out with the aim of investigating the viability and biodiversity of cyanobacteria and eukaryotic algae in biological soil crusts during the summer months to create a reference value for further studies.

5.1 Estimation of species diversity

Algal diversity in Arctic soil crusts is strongly influenced by the prevailing vegetation cover, geological type of substrate and microclimate (Elster et al., 1999). When comparing the results of the light microscopic evaluation with previous results of other studies, no notable difference could be observed (Borchhardt et al., 2017; Pushkareva et al., 2016; Pushkareva & Elster, 2013; Rippin et al., 2018). The dominant presence of green coccoid algae (Chlorophyta) at all three sites was in accordance with the findings of Pushkareva et al. (2013).The U-shaped valley of Bjørndalen at a lower elevation showed a significantly higher abundance of cyanobacteria compared to other sites. Moreover, species of the class Zygnematophyceae were observed in all sampling sites while Trebouxiophyceae were solely detected at higher latitudes. One possible explanation for this distribution may be the diverse characteristics of their microhabitats (Fierer, 2017; Rippin et al., 2018). Sampling sites at higher elevations are characterized by a substantially thicker and more persistent snow layer than the study site in Bjørndalen. Consequently, these areas experience a higher water supply with the onset of snowmelt in spring. The acidic and water-saturated soils in Bjørndalen furthermore support the presence of species like *Zygnema* sp. or *Spirogyra* sp. (Pushkareva et al., 2016). Contrary to the findings of Rippin et al. (2018), microalgae of the class Klebsormidiophyceae were observed in the study area of Breinosa 2. Furthermore, the altitudinal increase in the abundance of Bacillariophyceae could be attributed to the prevailing higher water content. However, it should be mentioned that this morphological approach utilized suffers from the limitation that microalgal cells and colonies can only be divided into the basic taxonomic classes because their initial stages exhibit an unclear morphology and ultrastructure.

5.2 Staining experiments

Prior studies have attained notable success with the employment of fluorescent staining for the assessment of algal vitality. Not only was its feasibility shown in the staining of laboratory strains and periphyton communities of cyanobacteria and diatoms but also in the filamentous alga *Tribonema* cf. *minus* originating from hydro-terrestrial habitats (Hejduková et al., 2020; Jimel et al., 2021; Tashyreva et al., 2013). However, the findings for the staining of BSC microalgae in the Arctic tundra exhibit significant differences to these previous research results. When examining the abundance of cells in the sample solutions, it becomes evident that the cell quantity was generally extremely low in all three study sites. This was further highlighted by the comparison of the site samples with the sample from the station backyard. A possible explanation for this result might be the diversity of the soil environment. The cell content of the soil exhibits continuous variety, ranging from high to low, depending on the piece of soil studied. Additionally, the sonication experiments did not detect any substantial evidence for an improvement in the stainability of the microalgal assemblages. The supposition that the process itself had harming effects on the viability of the samples cannot be excluded as algal disruption through the application of ultrasonic waves has been extensively documented in several studies (Hao et al., 2004; Yamamoto et al., 2015). Indirect effects such as cavitation bubbles caused by the surrounding water can additionally lead to the destruction of algal cells (Corrêa et al., 2020; Greenly & Tester, 2015; Miller, 1986). Kurokawa et al. (2016) further investigated that the disruption is highly dependent on the sonication frequency and varies with the type of algae. All this imposes challenges for the application of sonication for highly diverse biological soil crusts where numerous types of microalgae coexist.

Furthermore, inconsistent results in the staining with DAPI, SYTOX Green and CTC show that the viability assessment of microalgae embedded in biological soil crusts of the High Arctic by multifluorescent staining does not work. There are several possible explanations for this result. As the removal of excess soil and sediment from the microalgae proved to be difficult, a possible interference of the soil particles with the fluorescent stain cannot be ruled out. Assumptions suggest that the dye was either absorbed or chemically altered by the presence of soil particles. Gelatinous mucilage, as observed under the light microscope, may additionally implicate difficulties when staining different soil species as these coatings hinder fluorescent dyes to diffuse through the microalgal cell walls. This in turn would again be a possible explanation why the

staining did not work with some of the inactivated controls. The findings of the living sonicated samples additionally leave the question open as to whether they remain truly unstained because of their viability or simply due to the present protective layer. As mentioned in the literature review, the production of mucilage and thick cell walls is a common protection mechanism in nature against physiological stressors (Caiola et al., 1993; Elster & Benson, 2004; Hu et al., 2012). Their presence in the warmer season most probably aids in the retention of water against summerly day-to-day freezing and desiccation periods (Caiola et al., 1996; Davey & Rothery, 1992; Elster & Komárek, 2003; Kvíderová et al., 2019; Pereira et al., 2009; Potts, 1999; Tamaru et al., 2005). However, the occurrence of these reactions during the month of August might also be hypothesised as an earlier preparation for the upcoming winter months than previously assumed. Moreover, these staining results can be explained by the high diversity of cyanobacteria and eukaryotic algae occurring in Arctic soil crusts, as the determination of the optimal staining concentration and incubation time for each individual species is impossible. Nonetheless, even when considering all elucidating factors individually, a correlation between them cannot be excluded. Notwithstanding the lack of success of this method for soil crust microalgae, it is important to note that the vital staining of isolated and cultivated algal strains in laboratory conditions and for periphyton community (with low diversity), yields significant results (Hejduková et al., 2020; Tashyreva et al., 2013).

5.3 Evaluation of the photosynthetic activity

Overall, the increase in the photosynthetically active area (PAA) indicate the growth in biomass in all three locations. The 7-day lag phase in the beginning of the measurement could be an indicator for an early winter preparation, as it displays the time needed for the recovery of the already decreased metabolic and photosynthetic activity. However, clear differences in the growth rate were detected among the sampling sites. These variations can be possibly explained by the varying microenvironmental conditions. As this method allows to observe all present photosynthesizing organisms in the investigated soil crust samples, the contribution of unstudied organisms to the detected signal cannot be excluded. Thus, the in comparison steeper increase in the PAA (Fig. 9) at the study sites of Breinosa can be partially attributed to the presence of mosses or lichens. This which was verified by the augmented light microscopic observations of the moss *Paramecium* in sampling sites 1 and 2. Additionally, biological soil crusts of sampling site 1 in Bjørndalen are in constant nutrient competition with higher plants which could possibly influence

the inoculum or inoculation diversity. Furthermore, difficulties in the homogenization of samples could have caused the variability among the samples. The constant F_0 values shows that there was no change in the emission from the PSII antennae at all three sites during the observed period, further indicating that the chloroplasts are still intact (Krause & Weis, 1984). The slightly higher values at sampling site 1 can be linked to the prevailing dryer conditions, as environmental stressors increase the F_0 value (Krause & Weis, 1984). In contrary, the increased presence of cyanobacteria in Bjørndalen might also play a role in the slight differences, as their dark adaption can have a diminishing effect on F_0 value (Jallet et al., 2012; Schreiber et al., 1995; Stirbet et al., 2019). Furthermore, the discrepancy of increasing PAA data with consistent F_0 values might suggest that the growth of the colonies only takes place on the surface of the agar plate as described by Lukavský (1975). Moreover, the evaluation of the F_V/F_M ratios lead to the assumption that biological soil crusts at all experimental sites are vital. Analysis of the differences in the values showed a continuous increase in the ratio at all inoculation doses with overall value below 0.75. These results propose mild stress conditions at the beginning of the experiment with consecutive recovery as, according to Maxwell and Johnson (2000) as well as Björkman and Demming (1987), values below 0.83 are considered as an indicator for stress exposure in higher plants, however, in microalgae and cyanobacteria the maximum F_V/F_M values in optimum conditions could be lower due to the differences in the structure of the photosynthetic apparatus (Campbell et al., 1998). The decrease in the values due to prevailing stressors would imply a decrease in the photosynthetic activity (Maxwell & Johnson, 2000). This effect was most pronounced in samples from study site 1 (Bjørndalen). On the contrary, samples from study site 2 (Breinosa) seemed to exhibit enhanced recovery and the ratios in all three inoculation doses of sampling site 3 remained steadily constant over the observed period of time. There are several explanations for these findings. Samples from Bjørndalen exist in environments that are more nutrient and water scarce, which significantly impacts the viability. In contrast, the higher water supply during summer at the study sites in Breinosa, resulting from the melting of ice and snow at the beginning of spring, may potentially contribute to the resilience and recovery from the prevailing summer climate in the Arctic. However, it must be noted that these ratios might also be influenced by the presence of cyanobacteria in the biological soil crusts which generally exhibit lower F_V/F_M values at optimum conditions than higher plants or green algae (Stirbet et al., 2019). Furthermore, the assumption that sub-optimum conditions during the cultivation influenced the growth of the photosynthetic organisms cannot be excluded.

6 Conclusion

Although hardening and starving are presumed to play an important role in increasing the resistance of biological soil crust microalgae of the High Arctic against desiccation and cryoinjuries, the underlying morphological, ultrastructural and genomic alternations remain unexplored.

In this thesis, the viability of the soil crust microalgae during the summer months was assessed using two different approaches. Unfortunately, fluorescent staining proved to be ineffective in evaluating the vitality of microalgae in biological soil crusts due to the inconsistency of the results. Low cell content, soil-stain interactions as well as high species diversity could be possible causes for this problem. Mucilaginous coatings could have further prevented the diffusion of the stain into the microalgal cell. Nonetheless, this observation might be an indication for an early winter preparation.

Although the vital staining procedure was unsuccessful, the microalgal viability could be assessed by the evaluation of their photosynthetic activity. Thereby significant differences between the three experimental sites have been found. The analysis of the growth parameters PAA and F_0 as well as the maximum quantum yield (F_V/F_M) showed that samples from Bjørndalen are the least vital in comparison to biological soil crusts of Breinosa. Overall, site 2 appeared to be the most stress resistant exhibiting the fastest growth rate and highest recovery. These variations can be attributed to different microenvironmental conditions and a changing biodiversity with the study sites as the light microscopic evaluation showed notable variations in the species composition with changing altitude. However, further research is needed to precisely investigate the differences in the microalgal biodiversity at the study locations.

In general, the assessment of the microalgal viability in the summer was successful. Further research and the seasonal comparison of the vitality data together with an in situ and in vitro evaluation of the field manipulation experiments would provide valuable insights into the survival mechanisms of soil curst microalgae against the extreme climatic conditions prevailing in the High Arctic. Additionally, investigation and comparison of the microclimatic parameters in each sampling site are needed to establish a significant correlation between environmental factors and biodiversity.

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Appendix

Table 7: The list of found morphotypes and their occurrence at individual sites

Morphotype	*Bjørndalen*	*Breinosa 1*	*Breinosa 2*
Cyanobacteria	**18**	**3**	**4**
Leptolyngbya	4	0	1
Nostoc	3	3	2
Microcoleus	11	0	1
Bacillariophyceae	**1**	**2**	**11**
Trebouxiophyceae	**2**	**9**	**18**
Stichococcus	2	9	18
Xanthophyceae	**3**	**1**	**2**
Heterococcus	3	1	2
Klebsormidiophyceae	**1**	**0**	**3**
Klebsormidium	1	0	3
Zygnematophyceae	**6**	**1**	**2**
Zygnema	5	0	0
Desmids	1	1	2
unidentified green coccoid algae	**17**	**8**	**16**