Authors' Replies to Reviewers

Dear editor,

We have very much appreciated the overall effort of both reviewers as well as their fair criticism and thorough reading of our manuscript. We have revised the text, accepted most reviewers' comments and added new figures as suggested. At the same time, two figures were moved to supplementary information. Below, we are responding stepwise to comments made the reviewers (our answers are given in italics). In the revised text we highlighted (by tracking changes in the reviewing mode) all the changes to our manuscript and kept major deletions to enable easy comparison with the original text.

Reviewer #1:

First, the microbial mats should be described more thoroughly. I don't see the microbial mats in Fig. 8. These micrographs (recorded under cross polars, which is not mentioned) only show the minerals, and it is impossible to see the claimed microbial mats – dark zones may simply represent pure epoxy resin. At least plane polarized photomicrographs of the same zones should be added to show, e.g. green-pigmented mats. It would be better to use fluorescence techniques (e.g. Gérard et al. ISMEJ 2013,: : :) to clearly shown the microorganisms.

Figure 8 shows thin sections of dry samples from the black zone visible on Figure 2a. That is why the biofilm is represented only by a very thin black layer.

We are sorry for this unclear situation and we modified the accordingly the figure legend as well as the text itself. The use of cross polars method was also added. Moreover the fluorescence technique was used to demonstrate the presence of living mats on the rock surface (added as new Fig. 6).

Also, I'm not convinced with the rock pictures that the dark surface of the rocks is dark because of the presence of cyanobacteria, since the basaltic rocks would also be darks: could the cyanobacteria be very thin and transparent? Or do they have very dark pigments? Microscopy pictures of the cyano and algae are lacking in the paper. *The black colour of the rock surface shown of Fig. 6 (as Fig. 5 in the new version) is definitely caused by the presence of cyanobacteria. The dominant species Calothrix elsteri contains high amount of the dark pigment scytonemin (data not shown here). The black colour of this species is demonstrated on the micrphoto added as new Fig. 4 (together with photo of the co-dominating filamentous alga as requested above).*

In Fig.7b, arrows should be used to better distinguish mucilage and filaments. *Arrows were added to the Fig. 7b to make the picture clearer.*

It is claimed that "The regular leather biofilm structure with distinct cyanobacterialmicroalgal composition and incorporated mineral grains is a modern analogue of some of the oldest well-described 10 Archean stromatolites (sensu Allwood et al., 2006)." – this is wrong, there is no similarity at all. Paleoarchean stromatolites do not show trapping and binding, they only show alternance of carbonaceous laminae and silica/carbonate laminae, which is not observed in your modern samples as you apparently only have a single mat layer with scarce carbonate precipitation. *This statement was deleted from the text.* Second, the finding of the carbonates spicules is very interesting and well documented and should be discussed in more depth. Many references are cited on carbonates precipitated from evaporation: do any of those resemble the spicules (I guess not, which could be a biogenicity argument)?

As far as we know, structures similar to the spicules observed in our study have not been documented, yet. We tried to reflect this in the title of the paper as well as in the discussion, where a sentence was added to stress more this biogenicity argument.

Moreover, a tubular hollow is shown in Fig. 8d in a carbonate spicule and claimed as a cyanobacterial filaments: abiotic carbonates may form hollow mineral tubes without requiring the presence of a filament of cells (Fan & Wang, Advanced Materials, 2005).

The possibility of abiogenic formation of these hollows was added to the discussion.

More importantly, the surface textures of the spicules, interpreted as "worn" etching figures can also be interpreted as primary structures: in Fig. 9 I see a new type of mesostructured carbonate crystal formed through highly oriented growth of micro/nanocrystals: see the references below showing and discussing in vitro and biotic growth of mesocrystalline structures. In general, mesostructured crystals strongly suggest growth in presence of organic matter, such as mucilage. *The possibility of primary origin of the surface textures of spicule presented on Fig. 9a-b was added to the text together with some of the suggested references.*

Minor comments:

"lake water was mostly formed by detached benthic species; no substantial phytoplankton developed in the lakes." – is that published elsewhere (please cite) or not (maybe show pictures of floating microorganisms)

The characteristics of phytoplankton in the lakes was not published. The low biomass in the open water was formed by detached filaments or tiny filament clumps, and no floating mats were observed. This part of the text was amended to describe better the situation.

What do you mean by "soft mineral"? "

By "soft mineral" we mean the mixture of dry or semi-dry cells, sheets and mucilage of cyanobacteria and algae biofilm infilled with crystals of feldspathoids (tectosilicate minerals) and mineral grains (semi-rounded quartz grains). In the text, we changed it to "soft mineral matter".

Studies based on field or laboratory experiments have shown that some cyanobacteria and algae are able to tolerate prolonged periods of desiccation." – please cite these studies

The references Tashyreva and Elster (2015) and Pichrtová et al. (2014) were added to demonstrate the ability of both cyanobateria and algae to tolerate dessication.

The segregation of Ca2+ and HCO 3 between ice and the residual solution depend on the freezing rate and hydrogen – oxygen isotope fractionation" – I don't understand this sentence. Do you mean that H/O isotope fractionations can distinguish biotic and freezing-related precipitations? The sentence above was deleted in the revised text. Based on our measurements, we cannot clearly decide if the winter abiotic calcite precipitation accompanies microbial lithogenic processes.

Reviewer #2:

General comments:

The present paper describes characteristics of unique organosediment structured from shallow Antarctic endorheic lakes. The paper is comprehensive and well written and I have only few comments.

The thermal regime section (3.2) is too descriptive and unnecessary, given that majority of the information can be gathered by looking at Figures 3, 4 and 5. Having three figures (Fig. 3-5) showing seasonal changes in air and water temperatures is also unnecessary as well. I would recommend using only one figure and moving the others into supplementary materials.

The section 3.2 was significantly shortened, and the former Figures 4 and 5 were moved to Supplementary materials as suggested.

The biological characteristics of the organosediment structures (section 3.3) could be better described and better presented and would benefit from including light microscopy photographs of the different cyanobacterial and algal species. *The microphotos of the dominant species were added as new Figure 4.*

The discussion should be tightened up and some sections could be either entirely omitted or moved to the Results, particularly the descriptions of the environmental properties (section 4.1.).

The section 4.1 was shortened. However, we believe that the discussion of environmental properties (chemistry and thermal regime) is crucial for the understanding of benthic community characteristics and functioning and also for the explanation of possible origins of the calcite structures.

The discussion would benefit from adding a section discussing the possible origins of the structures as well as clearly describing the process of their formation. *The section discussing possible origin of calcite spicules was amended and new aspects based on available literature were added to the text Unfortunately, process of formation of the spicules could not be assessed in detail based on available data.*

There are too many figures in the manuscript and some of them are not very informative and thus could be moved to supplementary materials. *The original figures 4 and 5 were moved to supplementary materials.*

Specific comments:

Page 13594, Line 26: Remove "on a range of spatial scales" *Removed.*

Page 13595, Line 3: Preclude the development of larger organisms *Corrected.*

Page 13595, Line 4: Organosedimentary structures instead of Organisedimentary The sentence is not entirely clear *Corrected.*

Page 13595, Line 14: related "to" rather than related "with" Sentence starting with Calcite is too complex and should be divided into several sentences *Preposition corrected and the sentence was divided.*

Page 13596, Line 6: Pleistocene and Holocene *Corrected.*

Page 13596, Line 16: Missing ")" after Nedbalova *Corrected.*

Page 13597, Line 12: Catchment areas would be more meaningful in km2 than in m2 *Catchment area units changed to km2.*

Page 13597, Line 15: How dramatically? A sentence was added to clarify the extent of water level fluctuations.

Page 13604, Line 17: FSD instead of FDS *Corrected*

Page 13608, Line 24: Please add reference for the trapping of the mineral grains *The reference Riding (2011) was added.*

1	Unusual biogenic calcite structures in two shallow lakes,
2	James Ross Island, Antarctica
3	
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18	Abstract
19	The floors of two shallow endorheic lakes, located on volcanic surfaces on James Ross Island,
20	are covered with calcareous organosedimentary structures. Their biological and chemical
21	composition, lake water characteristics, and seasonal variability of the thermal regime are
22	introduced. The lakes are frozen down to the bottom eight-nine months per year and their

water chemistry is characterized by low conductivity and neutral to slightly alkaline pH. The
 photosynthetic microbial mat is composed of filamentous cyanobacteria and microalgae that

are considered to be Antarctic endemic species. The mucilaginous black biofilm is covered by green spots formed by a green microalga and the macroscopic structures are packed together

27 with fine material. Thin sections consist of rock substrate, soft biofilm, calcite spicules and

28 mineral grains originating from different sources. The morphology of the spicules is typical of

29 calcium carbonate monocrystals having a layered structure and worn surfacespecific surface

30 <u>texture</u>, which reflect growth and degradation processes. The spicules chemical composition 31 and structure correspond to pure calcite. Lakes age, altitude, morphometry, geomorphological 32 and hydrological stability, including low sedimentation rates, together with thermal regime 33 predispose the existence of this community. We hypothesize that the precipitation of calcite is 34 connected with the photosynthetic activity of the green microalgae that were not recorded in 35 any other lake in the region. This study has shown that the unique community producing 36 biogenic calcite spicules is quite different to any yet described.

37

38 1 Introduction

The floors of most Antarctic lakes are covered with photosynthetic microbial mats (Vincent 39 and Laybourn-Parry, 2008). However, the degree of disturbance plays a key role in the 40 41 development of microbial mats. When growing in low-disturbance habitats, interactions between benthic microbial communities and their environments can produce complex 42 emergent structures on a range of spatial scales. Such structures are best developed in extreme 43 environments, including benthic communities of deep, perennially ice-covered Antarctic 44 lakes, where physical and chemical conditions, and/or geographical isolation preclude the 45 development of larger organisms that could otherwise disrupt organised microbial structures 46 (Wharton, 1994; Andersen et al., 2011). Many organoisedimentary structures that emerge in 47 these conditions are laminated and accrete through episodic trapping of sediments or grains 48 and precipitation of minerals within a growing biogenic matrix (e.g. Arp et al., 2001; Reid et 49 50 al., 2003). In perennially ice-covered lakes, the seasonality of growth imposed by the summer-winter light-dark conditions can induce annual growth laminations (Hawes et al., 51 2001), reinforced by calcite precipitation during growth and sediment diagenesis (Wharton et 52 al., 1982; Wharton, 1994; Sutherland and Hawes, 2009). Calcite precipitation is not, however, 53 a prerequisite for laminated, stromatolite-like communities (Walter, 1976; Schieber, 1999; 54 Yamamoto et al., 2009). A diversity of micro- to nanostructured CaCO₃ associated with 55 extracellular polymeric substances and prokaryotes was described from the sediments of an 56 57 East Antarctic lake (Lepot et al, 2014). There is also a growing experimental evidence that some carbonate precipitates are only produced in the presence of organic matter (Cölfen and 58 Antonietti, 1998; Pedley et al., 2000). 59

Precipitation of calcite by expulsion (segregation) is also a common process in the nature related <u>towith</u> the freezing of common low ionic strength Ca^{2+} - HCO_3^- waters. Calcite precipitation related to water freezing was observed and described also from various polar-

alpine settings, e.g. from lake bottoms of Dry Valleys in Antarctica (Nakai et al., 1975) or; as 63 a results of aufeis (icing, naled) formation in Northern Canada (Clark and Lauriol, 1997). 64 eCrystalline precipitates that form subglacially on bedrock, were reported from numerous 65 locations (Ng and Hallet, 2002), for example f; as fine-grained calcite powders were observed 66 in subglacial deposits and in aufeis formations, Svalbard (Wadham et al., 2000) or ;-in basal 67 ice and subglacial clastic deposits of continental glaciers of Switzerland (Fairchild et al., 68 1993) <u>case</u> calcite pendants occurreding beneath coarse clasts in well-drained sediments on 5 69 Svalbard (Courty et al., 1994) and; as calcite coatings were found in cavities in cold-climate 70 Pleistocene deposits of Western Transbaikalia, Russia, and in modern surface deposits at 71 Seaymour Island, Antarctica (Vogt and Corte, 1996).; eryogenic calcite powder from modern 72 cave environment (Clark and Lauriol, 1992). 73

James Ross Island belongs to a transitory zone between the maritime and continental 74 Antarctic regions (Øvstedal and Lewis Smith, 2001). Air temperature records indicate 75 progressive warming trends from 1.5 °C to 3.0 °C over the Antarctic Peninsula during the past 76 77 50 years (Turner et al., 2014). More than 80% of the island surface is covered with ice (Rabassa et al., 1982). Only the northernmost part of the island, the Ulu Peninsula, is 78 79 significantly deglaciated and represents one of the largest ice-free areas in the northern part of the Antarctic Peninsula. The origin of the lakes on James Ross Island is related to the last 80 glaciations of the Antarctic Peninsula ice sheet and retreat of the James Ross Island ice cap 81 during the late Pleistocene -and-the Holocene (Nývlt et al., 2011; Nedbalová et al., 2013). 82 Interactions between volcanic landforms and glacial geomorphology during previous glacial-83 84 interglacial cycles, the Holocene paraglacial and periglacial processes and relative sea level change have resulted in the complex present-day landscape of James Ross Island (Davies et 85 al., 2013). All of these processes have influenced the development of the lakes which are 86 found on the Ulu Peninsula at altitudes from <20 m above sea level (a.s.l.) near the coast to e-87 400 m a.s.l. in the mountain areas (Nedbalová et al., 2013). 88

During two Czech research expeditions (2008 and 2009) to James Ross Island, lake ecosystems of the Ulu Peninsula were studied in respect to their origin, morphometry, physical, chemical and biological characteristics (Nedbalová et al., 2013), together with detailed cyanobacterial and microalgal diversity descriptions (Komárek and Elster, 2008; Komárek et al., 2011; Kopalová et al., 2013; Škaloud et al., 2013; Komárek et al., 2015). As part of this study, we encountered 1 to 5 millimetres scale calcareous organosedimentary structures on the floor of two endorheic lakes, 1 and 2, which are quite different to any 96 microbially mediated structures yet described from modern environments. These shallow

97 lakes on higher-lying levelled surfaces originated after the deglaciation of volcanic mesas
98 which became ice-free some 6.5–8 ka ago (Johnson et al., 2011) and are considered among

one of the oldest in the region. However, a later appearance of these lakes is also possible, as

100 we have no exact dates from their sediments (Nedbalová et al., 2013).

The aim of this paper is to describe in detail the chemical and biological composition of the organosedimentary structures (stromatolites sensu Allwood et al., 2006) together with the limnological characteristics of the two lakes. A hypothesis concerning the formation of calcite spicules is also presented. The results of this study can serve as a baseline for understanding microbial behaviors in forming these organosedimentary structures, which will provide insight into the interpretation of fossil forms from early Earth.

107

108 2 Materials and methods

109 2.1 Study site

Endorheic lake 1 (63°54'11.7" S, 57°46'49.9" W, altitude 65 m a.s.l.) and endorheic lake 2 110 (63°53'54.6" S, 57°46'33.8" W, altitude 40 m a.s.l.) are shallow lakes located near Andreassen 111 112 Point on the E coast of the deglaciated Ulu Peninsula, in the northern part of James Ross Island, NE Antarctic Peninsula (Figure 1). They are shallow with maximum depth of 1.1 and 113 114 0.9 m, and mean depth of 0.5 and 0.3 m. Their catchment areas are 0,340,515 and 0,3698,586 km^2 , lake area 4220 and 2970 m² and water volume 2183 and 1037 m³, respectively 115 (Nedbalová et al., 2013). Melt water from the surrounding snowfields feed the lakes for a few 116 weeks during the austral summer. The water level in both lakes fluctuated dramatically. Water 117 is mainly lost through evaporation from the ice free water surface. During this period, intense 118 119 evaporation in both lakes is coupled with macroscopic changes in the littoral belt. The extent of water level fluctuation was documented for lake 1-(Figure 2). 120

Climate conditions of the Ulu Peninsula are characterized by mean annual air temperatures around -7 °C and mean summer temperatures above 0 °C for up to four months (Láska et al., 2011a). The mean global solar radiation is around 250 W m⁻² in summer (December-February), with large day-to-day variation affected by extended cyclonic activity in the circumpolar trough and orographic effects over the Antarctic Peninsula (Láska et al., 2011b). The bedrock is composed of two main geological units, namely Cretaceous back-arc basin sediments and mostly subglacial Neogene to Quaternary volcanic rocks (Olivero et al., 1986).

128 The terrestrial vegetation is limited to non-vascular plants and composed predominantly of

129 lichen and bryophyte tundra. A large number of lakes can be found in this area, formed by

130 glacial erosion and deposition, followed by glacier retreat during the Holocene (Nedbalová et

131 al., 2013).

132 2.2 Sampling procedures

Lake 1 was sampled on 22 February 2008. In 2009, lake 1 was sampled on 5 January, lake 2 133 134 on 12 January. Air temperature at 2 m above ground was measured by an automatic weather station (AWS) located nearby (Figure 1). Incident global solar radiation was monitored with a 135 136 LI-200 pyranometer (LI-COR, USA) at Mendel Station, located 11 km northwest of the study 137 site (Figure 1). The LI-200 spectral response curve covers wavelengths from 400 to 1100 nm with absolute error typically of $\pm 3\%$ under natural daylight conditions. Global radiation was 138 measured at 10s time interval and stored as 30-min average values, while air temperature was 139 recorded at 1 hour intervals from 1 February 2009 to 30 November 2010. In lake 1, water 140 141 temperature was monitored from 10 February 2009 to 30 November 2010 at 1 hour intervals using a platinum resistance thermometer with Minikin T data logger (EMS Brno, Czech 142 143 Republic) installed on the lake bottom.

Conductivity, pH, temperature and dissolved oxygen were measured in situ with a portable 144 meter (YSI 600) at the time the lakes were ice free. Water samples were collected from the 145 146 surface layer, immediately filtered through a 200-µm polyamide sieve to remove zooplankton and coarse particles. Chlorophyll-a was extracted from particles retained on Whatman GF/F 147 glass microfiber filters according to Pechar (1987). After centrifugation, chlorophyll-a was 148 measured by a Turner TD-700 fluorometer equipped with a non-acidification optical kit. The 149 150 remaining water was kept frozen until analyzed at the Institute of Hydrobiology (Czech 151 Republic). The chemical analytical methods are given in Nedbalová et al. (2013). The stones covered by photoautotrophic mats - biofilm collected in the field were transported to the 152 153 Czech Republic in a frozen and/or dry state, documented with stereomicroscope (Bresser, HG 424018) and imaging fluorometer (FluorCam, PSI) and used for a) phytobenthos community 154 description and isolation of dominant species, b) fix for thin section analyses, c) scanning 155 electron and optical microscopy, and d) determination of the structure and chemical 156 157 composition of calcium carbonate spicules.

158 2.3 Thin section analyses

Thin section analyses were made to observe both rock substrate and inorganic particles within biofilms. Dry microbial mat were saturated with epoxy resins in vacuum, subsequently cut perpendicularly and saturated again with epoxy resin. The sample was cemented to a glass slide after grinding and polishing, and a thin section was prepared by final sectioning, grinding and polishing to a desired thickness of 50–55 μ m. Thin sections of rocks were studied in transmitted (PPL) and polarized (XPL) light (Olympus BX-51M) and documented in transmitted light of a Nikon SMZ-645 optical microscope using NIS-Elements software.

166 **2.4 Biofilm scanning electron and optical microscopy**

The morphology of photoautotrophic mats and calcareous spicules was studied using standard methods of scanning electron microscopy (SEM) using back-scattered electrons (BSE) (Jeol JSM-6380, Faculty of Science, Charles University) and optical microscopy (Nikon SMZ-645 using NIS-Elements software). Calcareous spicules were collected directly from the surface of biofilms. Samples studied in SEM were completely dried for 5 months at room temperature, then mounted on stubs with carbon paste and coated with gold prior to photomicrographing.

173 2.5 Structure and chemical composition of calcium carbonate spicules – EDS 174 and EBSD analyses

The chemical composition of the analyzed spicules was measured by using the Link ISIS 300 175 system with 10 mm² Si-Li EDS detector on a CamScan 3200 scanning electron microscope 176 (Czech Geological Survey, Prague). Analyses were performed using an accelerating voltage 177 178 of 15 kV, 2 nA beam current, 1 µm beam size and ZAF correction procedures. Natural carbonate standards (calcite, magnesite, rhodochrosite, siderite and smithsonite) were used for 179 180 standardization. Subsequent structural identification was confirmed by electron backscattered diffraction (EBSD). Identification data and crystallographic orientation measurements were 181 performed on the same scanning electron microscope using an Oxford Instruments Nordlys S 182 EBSD detector. The thick sections used for EBSD applications were prepared by the process 183 of chemo-mechanical polishing using colloidal silica suspension. The acquired EBSD patterns 184 were indexed within Channe 15 EBSD software (Schmidt and Olensen, 1989) applying calcite 185 and aragonite crystallographic models (Effenberger et al., 1981; Caspi et al., 2005). 186 Orientation contrast images were collected from a 4-diodes forescatter electron detector 187

188 (FSD) integrated into the Nordlys S camera. EBSD pattern acquisition was carried out at 20

189 kV acceleration voltage, 3 nA beam current, 33 mm working distance and 70° sample tilt.

190

191 **3 Results**

192 **3.1 General description of the lakes and water chemistry**

Pictures and detailed bathymetric parameters of both lakes together with marked lines of
water level and the maximum extent of the photosynthetic microbial mat littoral belt in lake 1
are presented in Figure 2.

196 The physico-chemical characteristics of the lake water for both lakes are given in Table 1. The sampling of lake 1 (pH 7.4–7.9, saturation of oxygen 98.9 %) was performed during 197 cloudy days. Oxygen supersaturation (128%) together with a relatively high pH (8.6) was 198 observed in lake 2 during a sunny day. Conductivity was below 100 µS cm⁻¹ in both lakes. 199 The concentrations of dissolved inorganic nitrogen forms were low, whereas the 200 concentration of dissolved reactive phosphorus (SRP) was 19.3 μ g L⁻¹ in lake 2. Relatively 201 high concentrations of dissolved organic carbon, particulate nutrients and chlorophyll-a were 202 also characteristic for lake 2 (Table 1). The Low autotrophic biomass in lake open water was 203 mostly formed by detached benthic species; no substantial phytoplankton neither floating 204 mats developed occurred in the lakes. The comparison of the two sampling dates available for 205 206 lake 1 suggested high fluctuations of dissolved nutrient concentrations.

207 3.2 Thermal regime

208 Figure 3a shows the annual variation of daily mean water temperature in lake 1 and of daily mean air temperature measured 2 m above ground in the Solorina Valley (locations of air 209 temperature, global radiation and water temperature sensors are marked in Figures 1 and 2). 210 211 Lake 1 is-was frozen to the bottom from the end of March to the end of October or beginning of November. Air temperatures were frequently lower than water temperatures. Minimum 212 daily mean winter temperatures on the bottom of the lake were about -12 °C and -10 °C for 213 2009 and 2010, respectively. Minimum daily mean air temperatures in the same period were 214 between -32 °C and -25 °C. Positive summer daily mean water temperatures on the bottom of 215 the lake were between 5 °C to 9 °C from the beginning of November to the end of March. Air 216 temperatures were frequently lower than water temperatures. Mean monthly water 217

temperatures in the lake ranged from -10.4 °C (August 2009) to 5.8 °C (February 2010), while monthly mean air temperatures were between -18.7 °C to 0.7 °C. The differences were greater at the beginning of the winter season (June–July), due to a rapid drop of air temperature.

Diurnal water and air temperature amplitudes in lake 1 and the Solorina Valley are shown in Figure 3b. The highest night-day air temperature fluctuations (up to 28 °C) were recorded during the winter months, while the lowest, in contrast, occurred in summer. ConverselyIn contrast, the highest night-day amplitudes of lake water temperature on the bottom of the lake were recorded from November to February, with typical values between 2 °C and 4 °C (Figure 3b).

The course of global solar radiation (Figure 3c) is was smooth, with the maximum daily mean of 385 W m⁻² during clear sky conditions around the summer solstice. Global radiation reacheds the bottom of both lakes during the ice free period.

231 The relative frequency of hourly values of lake 1 water and air temperature at the bottom of lake 1 and air temperature measured 2-m above ground in the Solorina Valley in the period 232 233 from 10 February 2009 to 30 November 2010-is shown in Figure 4S1. Water temperature fluctuation is-was narrow, ranging from -15-16 to 8 °C. This plot also documents that tThe 234 bottom of the lake is wasdry frozen, with temperatures from 15°C to most frequently 2 to 235 <u>-6° for most of the year, C and t (Figure 4a).</u> The growing season, with liquid-water at 236 temperatures from 2 to 4-8 °C, covereds only two-three months (Figure S1a).- In contrast to 237 lake water thermal regime, aWith respect to air temperature fluctuations (Figure 4b), the 238 seasonal thermal regime in the Solorina Valley iswere much wider (typically from -38 °C to 8 239 °C) (Figure S1b). in comparison with the thermal regime at the bottom of lake 1, where was 240 from 16 °C up to 8 °C. 241

242

The temperature at the lake bottom was permanently belowoecurrence of days with water temperature higher than -4 °C in lake 1 and monthly mean global solar radiation in the period from February 2009 to November 2010 is shown in Figure 5. Owing to the variability of the weather conditions, lake water at the bottom is completely frozen only during the coldest onetwo-three months per year (Fig. S2) (July August); water temperature is higher than -4 °C in the rest of the year. The water temperature is-_above 0 °C (liquid phase) was recorded from November to April (139 days in average). The A number of days with temperature between 0 and -4 °C remains the same as for liquid water occurrence with small changes in the start and
end dates towards to the transition period (February-June and September-November,
respectively). In such thermal conditions, the benthic littoral community can be metabolically
active.

254 **3.3 Littoral phytobenthos – biofilm community description**

The littoral benthic community in lakes 1 and 2 are dominated by the heterocytous 255 cyanobacterium Calothrix elsteri Komárek 2011 (Figure 4a), which forms a flat black biofilm 256 257 on the upper surface of bottom stones (Figure 56), followed by Hassallia andreassenni Komárek 2011 and Hassallia antarctica Komárek 2011. Hassallia andreassenni is associated 258 259 with calcium precipitation, as described later. Hassallia antarctica was found in stone crevices, being only loosely attached to the substrate. Littoral benthic mats - biofilms on 260 stones (Figure 6) are co-dominated on the surface of the blackish cyanobacterial biofilm by 261 the green filamentous and richly-branched alga Hazenia broadyi Škaloud et Komárek 2013 262 (Ulotrichales, Chlorophyceae) (Figure 4b). Hazenia broadyi grew in macroscopic colonies 263 producing green spots (Figure 56, d). Later in the summer season, the green spots connected 264 micro fortified mucilaginous lines (Figure 56c,d). Figure 56a shows the community in early 265 266 spring whereas Figure 56b,d originated from later summer when the littoral benthic community was already well developed with a dense coverage of Hazenia broadyi green 267 spots. More detailed pictures (Figure 56e,f) documented the structure of the black leather like 268 biofilm with mucilaginous marble on its surface covered by green spots. When the biofilm 269 gets dry, the net of precipitated micro fortified mucilage mixed with soft mineral matter 270 271 particles and crystals of calcium carbonate is visible (Figure 65g,h).

Scanning electron micrographs document the structure of the biofilm (Figure 7). Figure 7a
shows a lateral view (cross section) of a biofilm with cyanobacterial filaments (*Calothrix elsteri* and *Hassallia andreassenni*). A biofilm upper view (Figure 7b,d) shows the structure
of the cyanobacterial-microalgae community producing the mucilaginous micro fortified net
of filaments with spots on its surface.

277 3.4 Inorganic compounds of biofilms

Thin sections, showing both <u>dry</u> biofilms and rock substrate (Figure 8), provided information on various inorganic compounds associated with the soft tissue of the cyanobacterial – microalgal community. These inorganic compounds are represented by (1) allochthonous

- 281 mineral grains that are overgrown and incorporated by biofilms and (2) calcareous spicules of
- different sizes ranging from 0.5 mm to 1 cm that are precipitated within the cyanobacterial-
- 283 microalgal community.

The rock substrate of biofilms is formed by subangular to subrounded pebbles to boulders of basaltic rock, which is dark-grey in colour, compact and usually with a microcrystalline porhyric texture. The rock is not homogenous, but contains numerous ball-like empty voids, which are often partly filled with feldspathoids (Figure 8a). Crystals of plagioclase (feldspar group) and augite (pyroxene group) are easily recognizable in thin sections (Figures 8a–c).

- Biofilms are often partly covered with various mineral grains and rock fragments, but all
 specimens studied also contain these particles incorporated directly within soft cyanobacterial
 microalgal filaments (Figure 8a–c).
- Mineral grains embedded within biofilms close to the basaltic rock surface are mainly angular to subangular crystal fragments of plagioclase and augite (Figures 8b,c), i.e. the main mineral components of the basaltic rock substrate described above. In the upper part of biofilms, however, partly or fully incorporated grains of quartz occur, being typically rounded or partly rounded (Figures 8a,b). One of the thin sections shows a calcareous spicule in situ and mineral grains within the biofilm (Figures 8c,d).
- The structure and morphology of calcareous spicules was studied on SEM (Figure 9). The spicules (see also Methods) show an intensively worn surface (Figure 9a), partial or intense recrystallization (Figures 9a,b) and dissolution (Figure 9b). Crystal facets on the surface and cleavage (crystallographic structural planes) in the interior of the spicules (Figures 9a,b) are typical characteristics of calcium carbonate monocrystals.
- A non-recrystallized superficial layer of microcrystalline calcite (e.g., Figure 9b) shows the structure of parallel needle-like calcite microcrystals (Figures 9d–f). Partial corrosion and dissolution of spicules show distinct layering of these needle-like microcrystals (Figure 9d). The layered structure of even partly recrystallized spicules is confirmed in the ring-like structures with a cyanobacterial filament in the centre (Figure 10).
- The chemical composition of the studied calcareous spicules determined by F<u>SDDS</u> corresponds to pure CaCO₃. Following chemical composition, calcite and aragonite structural models were applied for the EBSD study focused on structural identification of the crystals forming the spicule. Structural identification of the studied specimen especially prepared for the EBSD study confirmed the absolute agreement between the recorded EBSD patterns and

modelled patterns for calcite. The presence of aragonite was not confirmed. FSD images acquired for chemical and orientation contrasts (Figure 10) show a layered structure especially visible in orientation contrast. This feature reflects continual growing processes on layers with very similar crystallographic orientation. Absolute angular differences between individual layers are below 0.8°.

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319 4 Discussion

320 4.1 Environmental properties

The Ulu Peninsula is a region of high limnological diversity that is related to differences in lake age, bedrock and altitude. The endorheic-lakes under study are characterized by a low content of major ions due to their volcanic bedrock and lower marine influence. In comparison with other lakes of this area, the two lakes show no specific lake water chemistry characteristics with moderate SRP and nitrate concentrations frequently below the detection limit (Nedbalová et al., 2013). High pH together with oxygen supersaturation recorded in lake 2 could be associated with high photosynthetic activity of the mats at the time of sampling.

Because water in either liquid or solid form has a large heat storage capacity, it acts as an 328 important buffer to temperature change. Local climatic conditions of shallow freshwater lakes 329 is the principal external factor controlling their ecological functionality. Lake 1 is frozen to 330 the bottom with minimum daily mean temperatures between -12 °C and -10 °C 331 332 approximately eight-nine months per year. For most of the year, however, the temperature of 333 the littoral and lake bottom is only from -2 to -4 °C. In such conditions, a thin layer of water probably covers the surface of the littoral benthic community that (the community can be 334 335 metabolically active at temperatures of about 4 °C) (Davey et al., 1992). The growing season, with liquid water at temperatures between 2 to 4 °C, covers only two-three months. 336

In regards to heat balance, the studied shallow lakes are pond (wetlands) environments which freeze solid during the winter. This inevitability is a strong habitat-defining characteristic, which places considerable stress on resident organisms (Hawes et al., 1992; Elster, 2002). In summer, they must withstand drying in large parts of the littoral zone due to a considerable drop in water level. In freezing and desiccation resistance studies of freshwater phytobenthos in shallow Antarctic lakes, several ecological measurements have recorded seasonal, diurnal, and year round temperature fluctuations and changes in water state transitions (e.g., Davey,

1989; Hawes et al., 1992, Hawes et al., 1999). In localities with steady moisture and nutrient 344 supplies, the abundance and species diversity of algae is relatively high. However, as the 345 severity and instability of living conditions increases (mainly due to changes in mechanical 346 disturbances, desiccation-rehydration and subsequent changes in salinity), algal abundance 347 and species diversity decreases (Elster and Benson, 2004). The speed at which water state can 348 349 change between liquid, ice, and complete dryness, is one of the most important ecological and physiological factors of these lakes. Studies based on field or laboratory experiments have 350 351 shown that some cyanobacteria and algae are able to tolerate prolonged periods of desiccation (Pichrtová et al., 2014; Tashyreva and Elster, 2015). It is also obvious that there are 352 strain/species specific differences in the overwintering strategies, and also between 353 strains/species inhabiting different habitats (Davey, 1989; Hawes et al., 1992; Jacob et al., 354 1992; Šabacká and Elster, 2006; Elster et al., 2008). The ice and snow which cover the 355 endorheie lakes for about eight-nine months per year serve as a natural incubator which 356 357 moderate potential mechanical disturbances and stabilise the thermal regimes of the lakes.

358 4.2 Biodiversity

Patterns of endemism and alien establishment in Antarctica are very different across taxa and habitat types (terrestrial, freshwater or marine) (Barnes et al. 2006). Environmental conditions, as well as dispersal abilities, are important in limiting alien establishment (Barnes et al., 2006). Antarctic microbial (cyanobacteria, algae) diversity is still poorly known, although recent molecular and ecophysiological evidence support a high level of endemism and speciation/taxon distinctness (Taton et al., 2003; Rybalka et al., 2009; de Wever et al., 2009; Komárek et al., 2011; Strunecký et al., 2012; Škaloud et al., 2013).

The floors of the studied lakes are covered with photosynthetic microbial mats composed of 366 367 previously described species of heterocytous cyanobacteria, mostly Calothrix elsteri Komárek 368 2011 followed by Hassallia andreassenni Komárek 2011 and Hassallia antarctica Komárek 2011 (Komárek et al., 2011). They are co-dominated by a newly described species of green 369 370 filamentous and richly branched algae Hazenia broadyi Škaloud et Komárek 2013 (Ulotrichales, Chlorophyceae) (Škaloud et al., 2013). All the previously mentioned recently 371 described species have special taxonomic positions together with special ecology and are 372 considered at present as Antarctic endemic species. 373

The black leather like biofilm with mucilaginous marble on its surface is covered by green spots. These macroscopic structures form mats a few mm thick consisting of the above 376 mentioned species packed in mucilage glued together with fine material. The regular leather

biofilm structure with distinct cyanobacterial-microalgal composition and incorporated 377 mineral grains is a modern analogue of some of the oldest well described Archean 378 stromatolites (sensu Allwood et al., 2006)to our knowledge unique. During the limnological 379 380 survey of the whole Ulu Peninsula (Nedbalová et al., 2013), this specific biofilm structure was observed only in these two endorheic lakes, although lakes with very similar morphometric 381 and chemical characteristics are found in the area. The mat structure is thus apparently tightly 382 linked to the species composition (Andersen et al., 2011). 383

The low abundance of benthic diatoms in the lakes is unusual, but not unprecedented as there 384 are other areas in Antarctica where diatoms are scarce or absent (Broady, 1996, Wagner et al., 385 2004). The reason underlying the absence of diatoms is not immediately obvious, because 386 diatoms are quite a common and frequently dominant component of microbial communities in 387 most freshwater habitats of the Ulu Peninsula, James Ross Island (Kopalová et al., 2013). 388 Local geographical separation of lakes 1 and 2 together with founder effect may have 389 390 precluded successful colonization by the subset of diatoms that are common in the surrounding freshwater habitats. Although it has long been held that diatoms are dispersed 391 392 widely, some recent reports document very small scale microbial distributions and endemism 393 (Kopalová et al., 2012; Kopalová et al., 2013).

4.3 Inorganic compounds of biofilms 394

395 Based on the character of the rock substrate and lake sediments it is suggested, that one of the main prerequisites for existence of this cyanobacterial-microalgal community producing 396 unusual biogenic calcite structures is; (1) flat and stable substrate in both lakes and (2) low 397 398 sedimentation rate.

The substrate for biofilms is composed of boulders and pebbles of the stony littoral zone, 399 petrographically corresponding to compact and massive basaltoids (Smellie et al., 2008; 400 401 Svojtka et al., 2009). Rounded or sub-rounded quartz grains that are incorporated ("trapped") within biofilms cannot originate from basaltic volcanic rocks forming the bottom of both 402 lakes and substrate of the studied biofilms. This is evidenced by the petrographic character of 403 the basaltoids, which do not contain any quartz. The presence of abraded quartz grains in lake 404 405 1 and 2 can be easily explained by wind transport (e.g., Shao, 2008).

The specific cyanobacterial-microalgal community described above can prosper in the two 406 shallow endorheic lakes, because of low sedimentation rates resulting from minor water input. 407

408 Low sedimentary input is the main necessary ecological parameter which facilitates the existence of this special microbial community. The community is, however, well adapted to 409 seasonally elevated sedimentation rates coming from frequent and intense winds. During wind 410 storms, the wind is carrying a relatively large amount of small mineral grains and rock 411 412 microfragments (intense eolic erosion; e.g., Shao (2008) and references therein). These grains 413 and particles are usually derived from erosion of the rocks either in the very close vicinity of the locality (weathering of basaltic rocks), but mainly come from remote locations where 414 415 especially Upper Cretaceous marine sedimentary sequences are outcropping (Smellie et al., 2008; Svojtka et al., 2009). Even elevated amounts of mineral grains transported into the lake 416 by wind do not stop the growth of cyanobacterial-microalgae biofilms, due to their ability of 417 incorporating and "trapping" mineral grains within the living tissue (Riding, 2011). 418

This study has shown that inorganic substances precipitated by microbial lithogenetic 419 420 processes are exclusively represented by calcite spicules. Precipitation of carbonate outside of 421 microorganisms during photosynthesis as a mechanism of carbonate construction was 422 described for many filamentous cyanobacterial species (Schneider and Le Campion-Alsumard, 1999). However, the biogenic calcite structures in both lakes are quite different to 423 424 any microbially mediated structures yet described from modern environments (Kremer et al., 2008; Couradeau et al., 2011) and also to structures formed by abiotic precipitation (e.g., Vogt 425 and Corte, 1996). Although there are many lakes with thick mats and similar chemical 426 characteristics on the Ulu Peninsula, the calcite spicules were found exclusively in the two 427 428 endorheic lakes. We believe that their formation is linked to the specific photoautotrophic 429 mats present in the lakes. From Figures 6g,h it is clearly visible that the calcareous organosedimentary structures keep contours of viable photosynthetic microbial mat after 430 431 desiccation or calcite spicules precipitation. More specifically, the co-dominance of a green 432 microalga is unique since mats in Antarctic lakes are most frequently formed by filamentous cyanobacteria (Vincent and Laybourn-Parry 2008). Therefore, we hypothesise that the more 433 rapid photosynthesis rate of *Hazenia* in comparison with cyanobacteria may induce conditions 434 435 necessary for carbonate precipitation in the lakes (Schneider and Le Campion-Alsumard, 1999; Vincent, 2000). However, some role of abiotic precipitation of calcite is also possible. 436 The segregation of Ca²⁺ and HCO₃⁻⁻ between ice and the residual solution depend on the 437 freezing rate and hydrogen oxygen isotope fractionation (O'Neil, 1968; Žák et al. 2004). 438 From our measurements observations we cannot clearly decide if the winter abiotic calcite 439 precipitation accompany microbial lithogenetic processes. 440

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Although we interpret the tubular hollow observed in the centre of some spicules as the result
of the presence of cyanobacterial filament during the process of crystallization, such
structures may form also as the result of abiotic precipitation of calcite –(Vogt and Corte,
1996; Fan and Wang, 2005).

It is striking that some calcite spicules <u>probably</u> exhibit recrystallization, forming spicules
with the structure of calcite monocrystals. <u>However, these spicules could be also interpreted</u>
as primary structures: mesostructured carbonate crystals formed through highly oriented
growth of micro/nanocrystals and characterized by a specific surface texture (Fig. 9a-b).
There is already evidence that some biominerals including calcite are mesocrystals (Cölfen
and Antonietti, 2005) and the importance of extracellular polymeric substances for the
formation of some types of carbonate precipfitates was documented (Pedley et al., 2009).

Determining the structure and material of precipitated inorganic substances brought another relevant question: "Do calcite spicules have fossilisation potential"? Microcrystalline calcite forming the recrystallized spicule is a typical material of calcite shells of fossil invertebrates (e.g., Vodrážka, 2009). Although calcite fossils may be partly or completely dissolved during diagenetical processes in the fossil record (e.g., Schneider et al., 2011; Švábenická et al., 2012), their preservation potential is relatively high. Therefore, we expect to find fossil and/or sub-fossil calcite spicules from the Quaternary lake sediments of the studied area.

459

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719 Table 1. Physico-chemical characteristics and chlorophyll-a concentrations in lake water.

Samples were collected from surface of lakes. ND – not determined, ANC – acid
 neutralization capacity, PN – particulate nitrogen, DP – dissolved phosphorus, PP –

particulate phosphorus, SRP – dissolved reactive phosphorus, DOC – dissolved organic
 carbon, PC – particulate carbon, * – laboratory values.

			iooratory var	
Lake		Green 1		Green 2
Date		22.2.2008	5.1.2009	12.1.2009
Temperature	°C	3.5	ND	12.3
O_2	mg L^{-1}	13.1	ND	13.7
O ₂ saturation	%	98.7	ND	128.0
pН		7.9	7.4*	8.6
Conductivity	$\mathbf{v}\mathbf{S}$ \mathbf{cm}^{-1}	<i></i>	48*	07
(25 °C)	$\mu S \text{ cm}^{-1}$	54	48.	97
ANC	mmol L^{-1}	236	246	455
Na ⁺	mg L^{-1}	4.7	5.9	12.5
K^+	${ m mg}~{ m L}^{-1}$	0.24	0.29	0.60
Ca ²⁺	${ m mg}~{ m L}^{-1}$	2.12	1.26	2.32
Mg^{2+}	${ m mg}~{ m L}^{-1}$	1.24	0.77	1.65
$\mathrm{SO_4}^{2-}$	${ m mg}~{ m L}^{-1}$	1.74	1.33	2.60
Cl ⁻	${ m mg}~{ m L}^{-1}$	5.3	5.1	10.6
NO ₃ -N	$\mu g \; L^{-1}$	<5	11	<5
NO ₂ -N	$\mu g \ L^{-1}$	0.6	0.2	0.1
NH ₄ -N	$\mu g \ L^{-1}$	6	<5	<5
PN	$\mu g \; L^{-1}$	20	50	73
DP	$\mu g \ L^{-1}$	7.8	20.2	30.4
РР	$\mu g \; L^{-1}$	4.6	5.9	11.7
SRP	$\mu g L^{-1}$	4.0	11.6	19.3
DOC	mg L^{-1}	1.25	1.13	2.17
PC	mg L^{-1}	0.13	0.41	1.33
Si	mg L^{-1}	1.45	0.87	2.85
chl-a	$\mu g \; L^{-1}$	0.9	ND	6.0

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725

726	Figure captions
727	Figure 1. Location of lake 1 and 2 and air temperature measurements (ASW) in the Solorina
728	Valley.
729	
730	Figure 2. Bathymetric parameters of lake 1 (a) and 2 (b) together with marked lines of water
731	level and maximum extent of the photosynthetic microbial mat littoral belt in lake 1.
732	
733	Figure 3. a – annual variation of daily mean water temperature in lake 1 (L1water) and annual
734	variation of daily mean air temperature 2-m above ground-in the Solorina Valley (SV air), b-
735	diurnal temperature amplitudes in lake 1 (L1 water) and diurnal air temperature amplitudes in
736	the Solorina Valley (SV air), respectively. c - daily mean global radiation at Mendel Station.
737	All parameters measured from February 2009 to November 2010.
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739	Figure 4. Dominant species in the photoautotrophic mats.
740	<u>a – Calothrix elsteri,</u>
741	<u>b – Hazenia broadyi</u>
742	
743	Figure 65 . Photoautotrophic mats in lakes 1 and 2.
744	a,b - rapid development of the mat in January 2009 (lake 1). The two photos show the mat at
745	a one week interval, note the growth of gelatinous clusters of densely agglomerated filaments
746	of the green alga Hazenia broadyi,
747	c,d – fully developed mats with mosaic-like structures on the surfaces of stones in the littoral
748	zone of lake 2,
749	e,f – detail,
750	g-drying of the mat in the littoral zone leaves a characteristic structure on the surface of
751	stones,
752	h – calcium carbonate spicule in situ (arrowed)
753	

754 Figure 6. Photoautotrophic mat covering a stone visualized using imaging fluorometry.

755 <u>a – upper view,</u>

756 <u>b – lateral view</u>

- 757
- Figure 7. SEM macrographs showing the structure of the dried mat in the lakes.
- 759 a transversal section of the mat with visible cyanobacterial filaments,
- b surface structure of the mat, the position of cyanobacterial filaments incorporated within
 mucilaginous matrix is indicated by arrows,
- c general view of the surface structure of the mat with the net formed by mucilage (compare with Fig. 7d),
- 764 d detail of the same mucilaginous structure
- 765
- Figure 8. Perpendicular thin sections of rock substrate <u>coveredovergrown</u> by <u>dry</u> biofilms
 (recorded under cross polars). Note that biofilms are partly detached from the surface of the
 rock due to complete drying of the sample.
- a conspicuous U-shaped empty void (arrowed "2") near the surface of basaltic rock partly
 infilled with crystals of feldspathoids (tectosilicate minerals, arrowed "1"); empty void is
 bridged by biofilm (arrowed "3") with partly incorporated mineral clasts, represented by semirounded quartz grains (arrowed "4"),
- b rather thick biofilm with numerous incorporated mineral grains. Note that close to the
 rock substrate the angular grains of plagioclase (feldspar group) and augite (pyroxene group)
 dominate, being derived from basaltoids, whereas close to the surface rounded grains of
 quartz occur (arrowed),
- c in situ calcium carbonate spicule penetrating biofilm and surrounded by incorporated
 grains of feldspars (two arrows on the left) and pyroxene (arrow on the right),
- d close up of the same calcium carbonate spicule with a cyanobacterial filament in its centre
- 780

Figure 9. SEM macrographs showing the morphology of partly recrystallized calcium carbonate spicules. Spicules were washed away from the living tissue and collected directly from the surface of biofilms, although residence time on the bottom cannot be determined.

a – calcareous spicule showing <u>specific surface texture intensively ("</u>worn surface<u>"</u>) and
 complete recrystallization of the spicule interior. The spicule shows crystal facets on the
 surface and cleavage (crystallographic structural planes) in the interior (arrowed) – i.e. typical
 characteristics of calcium carbonate monocrystal,

b – detail of previous image; two parallel systems of deep furrows on the surface are
crystallographic structural planes of calcite monocrystal; remnants of a superficial layer of
microcrystalline calcite are, however, preserved in places on the surface of the crystal
(arrowed),

c-f – poorly recrystallized spicule, formed mainly by microcrystalline calcite,

793 c - lateral view of the spicule,

- d detail of the surface showing corrosion of needle-like calcite microcrystals with distinct
 layering,
- re parallel needle-like calcite microcrystals on the surface of the central part of the spicule,

f - tops of parallel needle-like calcite microcrystals on the surface of the terminal part of the spicule; the view is perpendicular with respect to the previous macrograph

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Figure 10. FSD image of a transversely sectioned, partly recrystallized calcite spicule
acquired in (a) chemical contrast, (b) orientation contrast

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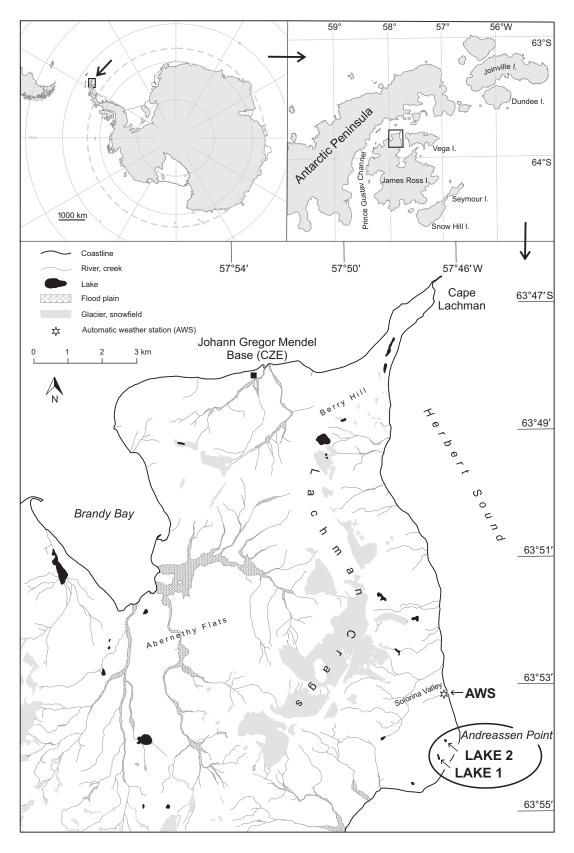
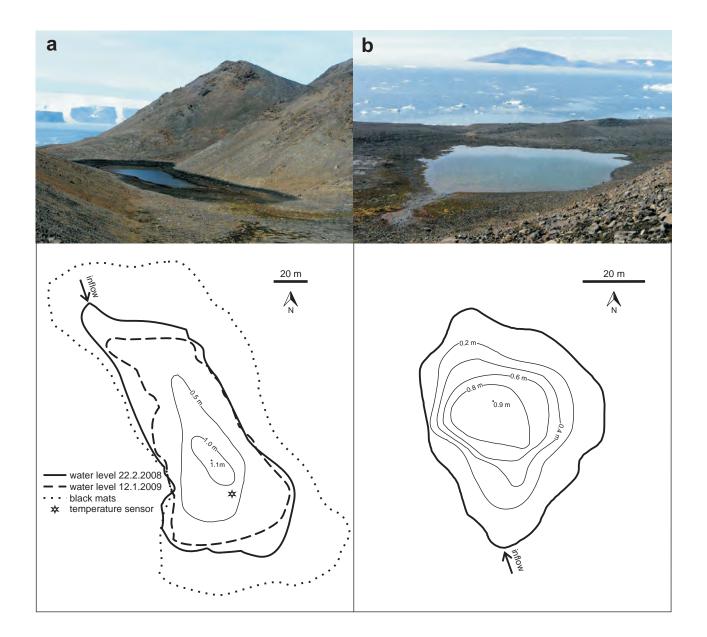


Fig. 1



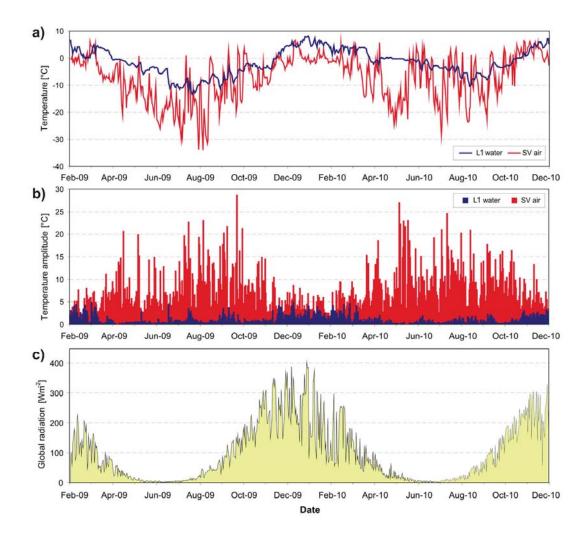
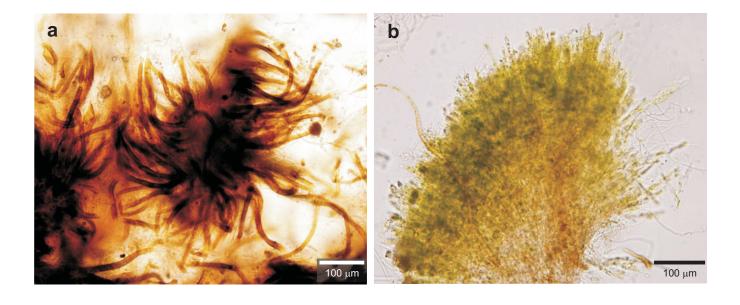


Fig. 3



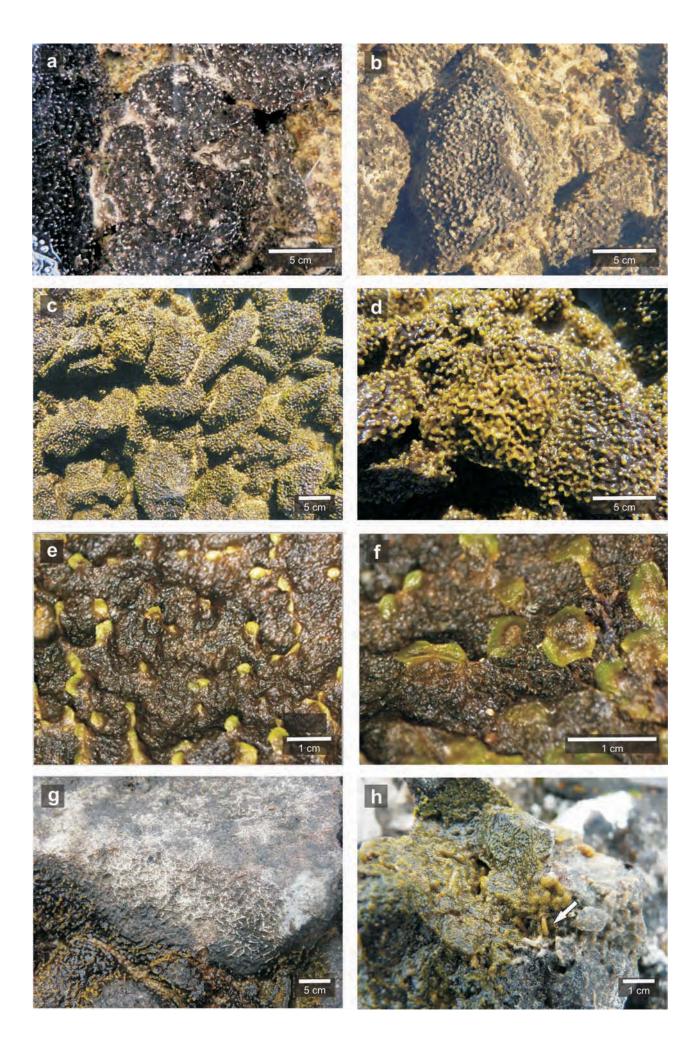


Fig. 5

