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Nitrate and ammonium ions contents in field minibioreactors with Antarctic freshwater autotrophs

Pavel Coufalík[1], Peter Váczi[2], Ondřej Zvěřina[1], Kateřina Trnková[2], Kateřina Skácelová[2], Miloš Barták[2], Josef Komárek[1]

[1]*Department of Chemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic*
[2]*Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic*

Abstract

The content of nitrate and ammonium ions in aquatic environment is an important factor in the development of microorganisms colonies especially in low-nutrient environment. In this study, field experiments using small-volume minibioreactors were performed directly on the James Ross Island in Antarctica in order to describe changes in nitrate and ammonium ions contents in the Antarctic environment The ion concentrations in minibioreactors with local freshwater autotrophs was monitored for increased eutrophication conditions. The content of nitrogen forms was determined in water samples taken from the minibioreactors regularly. Samples were taken to the laboratory of the Johann Gregor Mendel station where nitrogen content was evaluated using ion-selective electrodes. Furthermore, the freshwater autotrophs was subjected to basic taxonomic study. Closed system of the minibioreactors allowed the monitoring of nitrogen speciation changes which take place in the environment. These changes can be attributed to both the biological activity of microorganisms and external conditions. Increased eutrophication of water did not induce a rapid development of the freshwater autotrophs.

Key words: nitrate, ammonium, minibioreactor, microorganism, James Ross Island

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Introduction

Antarctica represents unique natural laboratory for the ecosystem study. An intense growth of communities of organisms can be observed in deglaciated parts of costal parts of the continent and nearby islands mainly those located in maritime Antarctica, such as *e.g.* South Shetlands. Recently, there is a new classification of Antarctica based on biodiversity that distinguish three zones in the Antarctic Peninsula and neighbouring islands (Terauds et al. 2012). Using the above classification of bioregions, the James Ross Island belongs to North-east Antarctic peninsula. In spite

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*Corresponding author: Pavel Coufalík <272713@mail.muni.cz>
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of numerous vegetation oases found at deglaciated parts of the James Ross Island (Láska et al. 2011) their formation and development is limited by physical and chemical characteristics. Climatic conditions as well as eutrophication of the environment (Hawes et Brazier 1991) and the content of mineral components (Nedbalová et al. 2013) are essential for growing of the Antarctic freshwater autotrophs. The interactions between biotic and abiotic components of the ecosystem are in Antarctica less complex than elsewhere (Bargagli et al. 1998). Within last two decades, numerous studies focused on nutrient aviability in Antarctic streams and lakes, their short- and long-term changes (*e.g.* Hawes et al. 2013). A variety of freshwater ecosystems has been studied, *e.g.* Davey (1993) and Moorhead et al. (1998) reported changes in nitrogen availability in maritime and dry valleys streams, respectively. The role of temperature and the rate of microbial community growth as dependent on nutrient availability were also investigated (Reay et al. 1999, Izaguierre et al. 2011).

Surface streams and lakes of the James Ross Island are supplied from melting snow and ice and are similar to other maritime regions of Antarctica. Development of microbial communities, rate of photosynthesis, respiration and biomass production varies according to ecophysiological conditions of individual localities (Hawes et Brazier 1991). Specifically for the James Ross Island, ecophysiological parameters of the Lachman Lakes have been intensively studied since 2006 (Váczi et Barták 2011, Váczi et al. 2011). Water temperature of the lakes during austral summer may reach the values of about 10°C (Nedbalová et al. 2013) which may cause an oxygen oversaturation (Váczi et Barták 2011). Actual amount of dissolved oxygen depends on chemical composition of the water and on physiological activity of photosynthesising microorganisms. During austral summer season, amount of nutrients varies in time in streams and lakes of James Ross Island, however amount of nitrogen is generally low (Coufalík, *unpublished results*). The extension of snow-free period increases an input of nutrients, mixing of lake water and thus primary production (Váczi et Barták 2011, Váczi et al. 2011). A fast growth of cyanobacterial mats with high diversity and number of morphotypes is reported for James Ross Island and the period of austral summer (Komárek et al. 2008).

The growth of algae and cyanobacteria in low-mineral environment of streams depends mainly on secondary enrichment of melting water of the snowfields. The development of algae and cyanobacteria is considerably limited by the content of available nitrogen and phosphorus (Elster et al. 2002) whose concentrations are retained at low levels due to an uptake of those nutrients by photosynthesising organisms. Dissolved nitrogen has a minimum in the water in late afternoon – nitrogen uptake is endogenous process which is stimulated by light (Hawes et Brazier 1991). Increased amount of nitrogen in closed system would enable objective monitoring of the influence of eutrophication on cultures development and vice versa. Therefore, we focused on the field study of changes in nitrate and ammonium ions in water with autotrophic organisms under conditions close to Antarctic environment. We hypothesized that, increased content of mineral nitrogen (approach of controlled N addition) would lead to altered rate of N uptake by freshwater alage and cyanobacteria.

Material and Methods

Locality of experimental set up

The James Ross Island (64° 10' S, 57° 45' W) located on the eastern side of the Antarctic Peninsula represents a transitional zone between maritime and continental Antarctica (Komárek et Elster 2008, Nedbalová et al. 2013). The experiments exploiting field-installed minibioreactors and nutrient enrichment were performed in the northern part of the James Ross Island (Ulu Peninsula) from January to February 2013. Two different habitats were selected for the study of the Antarctic freshwater autotrophs, the pools Interlagos at Lachman Lakes (Fig. 1) – *experiment 1* and Bohemian Stream – *experiment 2*. Collected biota from seepages near the J. G. Mendel station was also used in the *experiment 2* (Fig. 1).

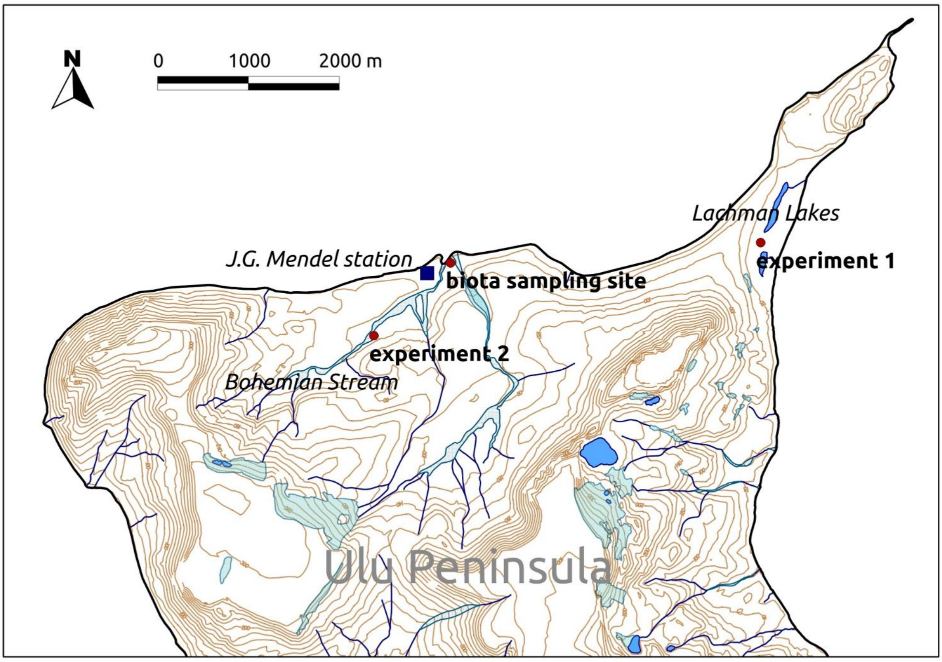

Fig. 1. Locations of the study (*modified from* CGS 2009).

Lachman Lakes and associated pools belong to the category of coastal intermittent lakes (Komarek et Elster 2008) which are influenced by marine aerosols and melting water from snowfields. The lakes are shallow, with relatively high temperatures of water and well developed and biologically active freshwater autotrophs. The second location was Bohemian Stream – few kilometers long creek with many inflows. It is a typical surface watercourse of the James Ross Island with variable stream bed which is supplied from snowfields; the water temperature is close to zero. Its water surface varies significantly throughout the day depending on temperature conditions.

Minibioreactors

Field minibioreactor was designed for the study of the behaviour of freshwater aquatic organisms under controlled conditions (*see* Fig. 2). It involved polypropylene cylindrical container with the capacity of 1.9 L. The transparent minibioreactor was closed with transparent cap with silicone seal; 15 cm high, 13.3 cm inner diameter. The reactor had controlled inlet and exhaust of the air. The air was supplied through silicone tubing from air pump located in a vicinity of the reactor. The pump was powered from accumulators direct in the terrain. The air was introduced to minibioreactor bottom, the outlet was closed with a frit. A filter was placed on the outlet of gases from the reactor. Thus, the minibioreactor represents closed system allowing an exchange of gases. In addition, a service aperture was in the cap for the introduction of temperature sensor or sampling. Water temperature in the reactor was recorded using datalogger. It was used in total six minibioreactors, four temperature sensors with a datalogger, datalogger Minikin for the measurement of temperature of lake water, two air pumps, batteries for power, silicone hoses and filters for air filtration.

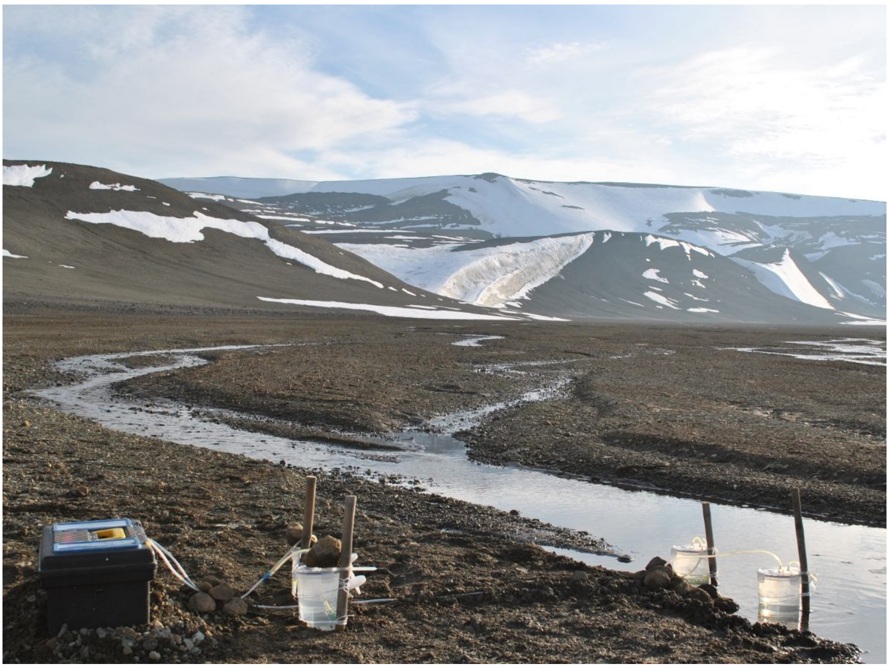

Fig. 2. Minibioreactors placed in the Bohemian stream and on the surface of sedimentary deposits (Photo: Pavel Coufalík).

Experiment and measurement

Field minibioreactors were used in the following arrangement of experiments. The *experiment 1* was conducted in the Interlagos from Jan. 16th to Feb. 11th 2013. Two minibioreactors were placed in shallow water of Interlagos pool in order to allow submersion of the minibioreactor bottom (about 5 cm of reactors height) into water of the pools. Thermal regime of the minibioreactors was semi-controlled in this way so that water temperature in the minibioreactor followed the fluctuations in pool temperature. A datalogger Minikin (Environmental Monitoring Systems, Brno, Czech Republic) was used to measure the water temperature in the vicinity of the minibioreactors. The volume of 1 liter of water from Interlagos pond was dosed into both minibioreactors. Nitrogen was added into the water as NH_4NO_3 in 715 µM concentration. It represented 10 mg of nitrogen in the form of ammonium cation and 10 mg of nitrogen in the form of nitrate anion.

The *experiment 2* was performed in the Bohemian Stream (Fig. 2) from Jan. 17th to Feb. 8th, 2013. Altogether, four minibioreactors were used. Two were located on stream bank and two in stream bed. (The water surface surrounding the minibioreactors fluctuated during the day according to temperatures and sunshine.) Thermal sensor was inserted into each minibioreactor. Water temperature was recorded by a datalogger from Jan. 18th to Feb. 8th, 2013. The volume of 1 liter of water from the Bohemian Stream was dosed into all minibioreactors and eutrophicated using NH_4NO_3 about the same concentration as in the *experiment 1*. In addition, microbiological mat collected from seepages near the station (Long-term reasearch plot, Fig. 1) was added within two minibioreactors (one on stream bank and one in stream bed) – about 2.5 ml of living matter (Table 1).

Experiment 1	**Location**	**Remark**	**Dates of samples collecting**
Bioreactor A	Interlago		16.1., 20.1., 23.1., 28.1., 4.2., 11.2.
Bioreactor B	Interlago		16.1., 20.1., 23.1., 28.1., 4.2., 11.2.
Experiment 2			
Bioreactor 1	Stream bank		17.1., 19.1., 22.1., 27.1., 29.1., 4.2., 8.2.
Bioreactor 2	Stream bed		17.1., 19.1., 22.1., 27.1., 29.1., 4.2., 8.2.
Bioreactor 3	Stream bank	Added biota	17.1., 19.1., 22.1., 27.1., 29.1., 4.2., 8.2.
Bioreactor 4	Stream bed	Added biota	17.1., 19.1., 22.1., 27.1., 29.1., 4.2., 8.2.

Table 1. Experimental design and samples collecting.

In both experiments, the air flow into reactors was regulated to approximately 1 liter per hour. The aeration also served for mixing of water and reduced probability of the freezing of reactors considerably. Water sampling was carried out according to external climatic conditions, *i.e.* in irregular intervals. Individual sample was about 40 ml of water during each sampling in case of the *experiment 1*, and 20 ml of water for the *experiment 2*. Water samples in PE vials were immediately transported to the laboratory of the station. The determination of ammonium and nitrate ions concentrations was carried out by potentiometry using ion-selective electrodes (Theta 90). Finally, remaining volumes of water in minibioreactors were filtered through glass fiber filters with pore size of 1 µm (Whatman, GF/B) after the experiments ending. A half of each filter was dried out to determine dry weight of the microbiota formed in the minibioreactor and the other half was placed in 4% formaldehyde for subsequent taxonomical study by means of optical microscopy (Olympus BX41, Japan). Several samples were taken from each sample and dominating genera/ species were determined using morphological approach.

Results and Discussion

Environmental conditions

Temperature effect on the activity of autotrophic organisms in location of the *experiment 1* is described in the paper of Váczi et Barták (2011). Water temperature course of the Interlagos was measured nearby parallel minibioreactors of the *experiment 1*, which were placed on the bottom, close to the bank and presented in Fig. 3. Maximum temperatures correspond with peaks of solar radiation (data not shown). Minima are found in early morning hours due to a short-term dark period of a day. The Interlagos did not freeze within duration of the experiment, thus autotrophic organisms were maintained biologically active during the whole period of the experiment. Water temperature was found above 10°C frequently (*see* Fig. 3). Only on 6 days, water temperature was found close to zero.

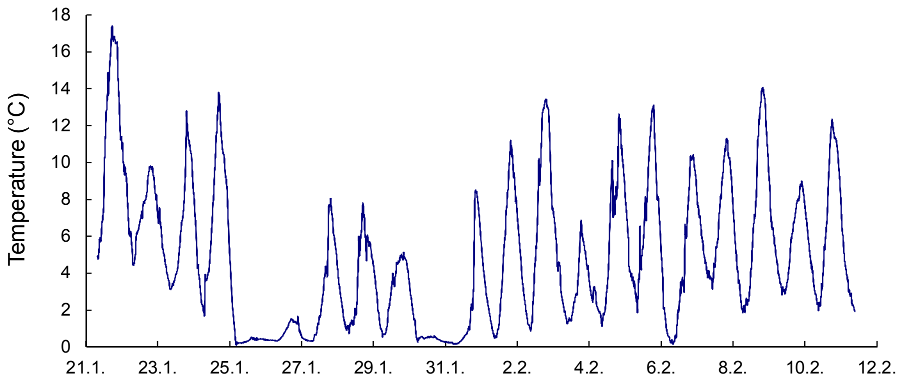

Fig. 3. Course of temperature in Interlagos lake.

Water temperature in the minibioreactors included into the *experiment 2* are shown on the Fig. 4 and Fig. 5. The minibioreactors placed on the bank (Fig. 4) evinced higher fluctuations of the temperature than in case of the minibioreactors placed directly in stream bed (Fig. 5) The minibioreactors placed on the bank did not have so good agreement of measured temperatures as reactors in stream bed. Partial freezing of all minibioreactors was observed up to one half of the volume. Thus, a part of the volume remained liquid despite the freezing of temperature sensor.

Species of the freshwater autotrophs

Since the 90-ies, taxonomy of cyanobacteria and other microorganisms in lakes, streams or seepages of the James Ross Island has been intensively studied (Hawes et Brazier 1991, Komárek et Elster 2008, Komárek et al. 2008, Komárek et al. 2012, Kopalová et al. 2012). Cyanobacterial communities found in lakes of the James Ross Island are considered connecting link between the maritime and continental Antarctic lakes (Komárek et Elster 2008). The lakes are often characterized by a dominance of few taxa (Komárek et al. 2008).

The freshwater autotrophs in the minibioreactors of the *experiment 1* were represented primarily by green alga *Zygnema* sp. and diatoms. Then *Phormidium* sp. and *Calotrix* sp. were found in minimal numbers. The biomass of biota in the minibioreactors at experiment ending corresponded to approximately 15 mg of dry weight per 1 L of water.

The biota in the minibioreactors 1 and 2 of the *experiment 2* (water from the Bohemian Stream) consisted mainly of diatoms (60%). The the minibioreactor 1 contained about 30% of *Phormidium* sp., the minibioreactor 2 about 35% of *Chroococcus* sp. The biomass of biota in both reactors corresponded to about 2 mg / 1 L. The minibioreactor 3 and 4 contained added biota from seepages. The main share (80%) was represented by *Zygnema* sp., *Phormidium* sp. reached approximately 15%. Furthermore, the presence of diatoms and *Oscillatoria* sp. was confirmed in these minibioreactors as well. Dry biomass of biota in both reactors was about 50 mg / 1 L.

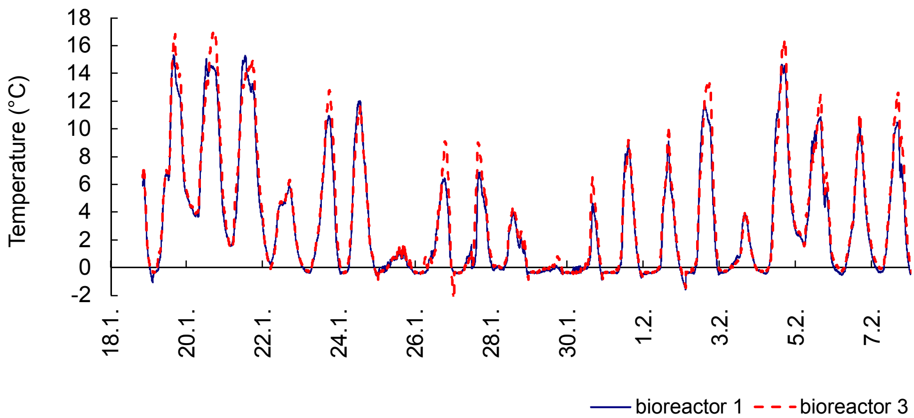

Fig. 4. Course of temperature in the minibioreactors 1 and 3 (Bohemian Stream).

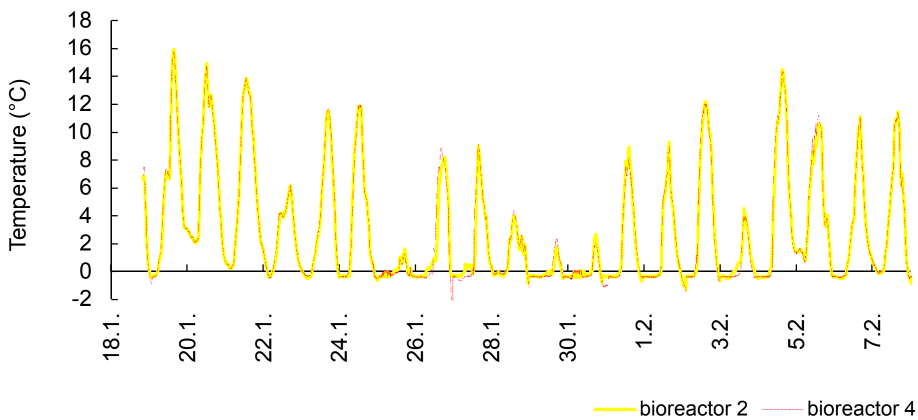

Fig. 5. Course of temperature in the minibioreactors 2 and 4 (Bohemian Stream).

Contents of nitrate and ammonium ions

Generally, the content of available nitrogen in water is limiting for the development of aquatic communities. In the performed experiments, additional nitrogen was supplied to increase water eutrophication and promote growth of fresh water autotrophs. Nitrogen can exist in inorganic and organic forms and its speciation depends primarily on redox conditions. Nitrate ion dominates in surface water under oxidizing conditions, ammonium ion under reducing conditions (Hanrahan et Chan 2005). In this research, the ratio of these ions and their total content in the minibioreactors were observed.

The change of ion contents and the sum of nitrate and ammonium nitrogen are presented on the Fig. 6. for parallel minibioreactors used in the *experiment 1*. Original content of ions in water from the Interlagos was 38 µM NO_3^- and 209 µM NH_4^+. Ion contents after the eutrophication (nitrogen addition) were 753 µM NO_3^- and 924 µM NH_4^+. These values are also shown in Fig. 6 as initial. Since freezing did not occur during experimental time, the uptake of N ions were not affected by sudden changes in the quantity of living biomass of autotrophs.

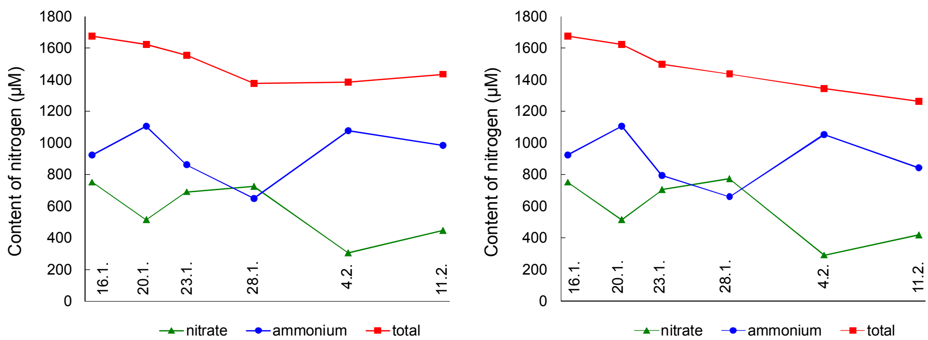

Fig. 6. Contents of nitrate and ammonium ions in paralel minibioreactors of the *experiment 1*.

The sum of contents of observed N ions decreased slightly during the experiment which indicated the assimilation of mineral nitrogen together with the development of the microbial community in the minibioreactors. The ratio of nitrate to ammonium ions evinced a complementarity of speciation changes during the entire period. The scheme of transformation of nitrogen forms which are mediated by various species of microorganisms is shown in Fig. 7. The decrease of N ion contents in the minibioreactors and changes in nitrate/ammonium ratio could be attributed to the processes such as nitrification, assimilatory nitrate reduction and ammonification (Hanrahan et Chan 2005). Subsequently, total amount of nitrogen involved in these transformations remains approximately the same.

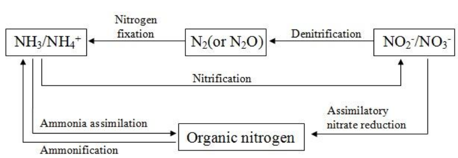

Fig. 7. The scheme of biological transformations of nitrogen species (*modified from* Hanrahan et Chan 2005).

Contrastingly to *experiment 1*, nitrogen content changes had different time courses in the *experiment 2* minibioreactors. The changes of ion contents and their sum in four bioreactors of the *experiment 2* is presented in Fig. 8. Initial contents of ions in water from the Bohemian Stream were 26 µM NO_3^- and 193 µM NH_4^+. Ion contents increased to 741 µM NO_3^- and 908 µM NH_4^+ after adding the NH_4NO_3 (initial values on the Fig. 8). Following development produced unexpected results. The development of ion contents in all minibioreactors was similar despite different placement and biota contained (Table 1). (Nitrogen content in minibioreactors placed in stream bed was slightly higher then in minibioreactors on the bank.) Partial freezing occurred several times in all minibioreactors throughout the experiment (Fig. 4 and Fig. 5). Therefore, cell destruction of autotrophs followed by a decomposition can not be excluded. Such events may lead to a release of nitrogen into water.

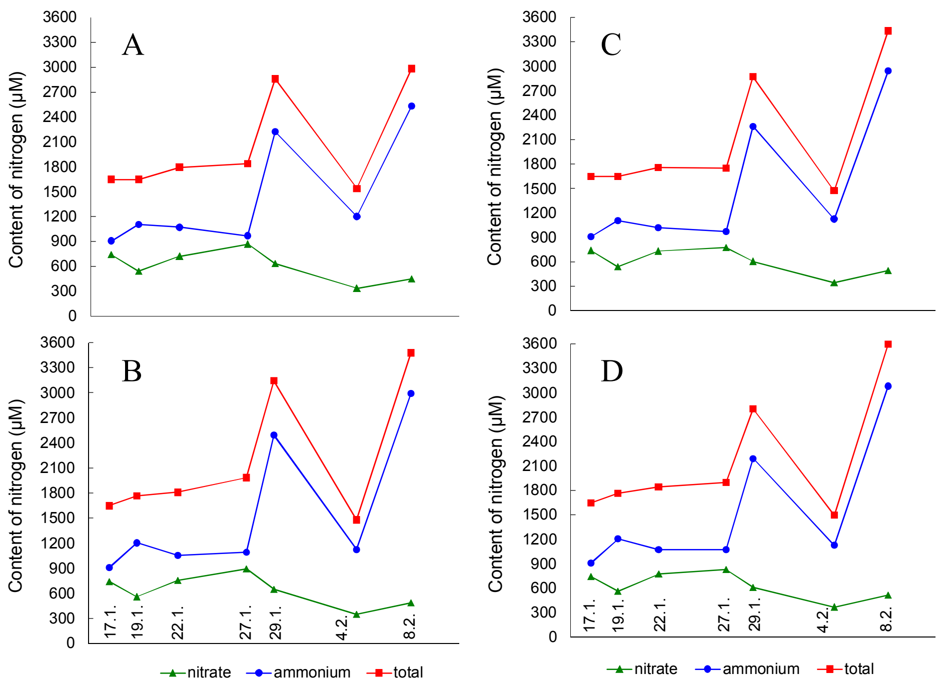

Fig. 8. Contents of nitrate and ammonium ions in minibioreactors 1 (A), 2 (B), 3 (C) and 4 (D) of the *experiment 2.*

After initial equilibration of N ion concentrations, an oscillation of NH_4^+ content, *i.e.* apparent increase followed by a drop, occured. Sudden variations in the content of dissolved nitrogen are also the result of biological processes. The increase may be attributed to freeze-induced release of nitrogen from destroyed autotrophs cells. Following fluctuation may be an interaction between a decrease of nitrogen content and an increase due to the presence of heterocystous cyanobacteria in seepage zones, notably *Nostoc* and *Nodularia* (Hawes et Brazier 1991). However, the presence of these species in the minibioreactors was not confirmed by microscopic study. The influence of external conditions could be considered as major factor. Increased content of ammonium ions could be attributed to a decay of organic matter, its decrease to a period of biomass growth. The rate of physiological processes of the Antarctic freshwater autotrophs is probably sufficient for an immediate utilization of bioavailable nutrients (Butler 1999). This phenomenon is also obvious in the case of measurement of ammonium ions in lake water of the James Ross Island (Coufalík, *unpublished results*). Recently deglaciated lakes may have an increased content of NH_4^+ with a decrease due to restored activity of algae and cyanobacteria communities. Thus, the development of contents of ammonium ions in the minibioreactors of the *experiment 2* could also be resulting from stagnation and activity of microorganisms.

Conclusion

The study reported here dealt with the time courses of nitrate and ammonium concentrations in field-installed minibioreactors with the Antarctic autotrophs. Addition of nitrogen into the water taken from the field led to the eutrophication of water in the minibioreactors. Generally, addition of mineral nitrogen increased biomass production of algae and cyanobacteria, particularly in low-mineral environment.

The experiments with increased water eutrophication in the presence of biota allowed to understand the main aspects of changes in concentrations of both ions in stagnant waters of the James Ross Island. A slow decline of nitrogen content (both forms) caused by nitrogen assimilation and primary production of the biomass can be expected for thermally stable conditions (unfrozen lakes). The concentrations of both N ions are dependent on speciation changes which are perpetually happening in water of Antarctic lakes. Based on these experiments, the influence of microbiological constituents, algae and cyanobacteria in particular, may suggest slightly preferential nitrate ion uptake. The effect of external conditions (water temperature) on nitrate/ammonium ratio can not be evaluated from our data.

Nevertheless, microbial communities under extreme Antarctic conditions are often exposed to sudden fluctuations of external conditions which is reflected in their physiological activity. Rapid freezing may lead to cell disruption of freshwater autotrophs. Close-to-zero temperature may also partly inhibit photosynthetic processes and thus rate of carbon and nitrogen assimilation. Therefore, periodic freezing and melting of small-area water pools may cause a stagnation in biological activity of autotrophic communities. On the other hand a release of ammonium ions from disrupted cells into aquatic environment may alter nitrogen concentration and availability for other organisms. The experiments we performed suggest that a significant increase in N availability do not cause a rapid increase in biomass of aquatic organisms forming the community in the minibioreactors. Follow-up studies carried out both in laboratory-based bioreactors (*see* e.g. Balarinová et al. 2013) and in the field are necessary to evaluate the potential of Antarctic autotrophs to utilize external N in field-installed bioreactors.

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