Univerzita Karlova v Praze

Přírodovědecká fakulta

Studijní program: Biologie

Studijní obor: BBI

Tereza Jaroměřská

Stoichiometric patterns in cold oligotrophic aquatic systems

Ekologická stechiometrie chladných oligotrofních sladkovodních ekosystémů

Bakalářská práce

Školitel: Mgr. Jakub Žárský, Ph.D.

Praha, 2016

Prohlášení:

Prohlašuji, že jsem tuto bakalářskou práci vypracovala samostatně a že jsem řádně ocitovala všechny použité zdroje a literaturu. Dále pak prohlašuji, že práce nebyla použita na zisk jiného nebo obdobného druhu vysokoškolské klasifikace.

V Praze dne 25. 7. 2016

Podpis

Poděkování

Ráda bych poděkovala všem, kteří se na mé práci velkou i menší měrou podíleli. Je to především školitel Jakub Žárský, jehož věcné připomínky a poznámky vedly k ucelení informací i celého textu. Dále jsou to David Novotný a Tyler Kohler, kteří se postarali o správnost gramatiky, syntax a formát textu. A v neposlední řadě celému svému okolí a celé mé rodině, která je mou stálou oporou a podporou.

Abstract

Ecological stoichiometry is a conceptual framework which helps us to describe an ecosystem through its elemental composition, fluxes of matter and balance of energy. In glacier habitats, ecological patterns are different than in the terrestrial environment, and the research has been done mostly in the last decade. The result is that stoichiometric data only exists in small amounts, and are influenced by searching area and preference of the researcher. Stoichiometry of glacial hydrological systems has patterns which are specific for these habitats, and the knowledge about invertebrate stoichiometry living in those waters is still in its early days.

Abstrakt

Ekologická stechiometrie je způsob popisu a interpretace, zabývající se hlavně ekosystémem z pohledu jeho prvkového složení. Zároveň se také zabývá energetickými rovnováhami a toky prvků mezi jednotlivými komponenty. Stechiometrie ledovců je v tomto ohledu velmi specifická. Hydrologický systém na ledovcích má mnoho znaků, které nejsou srovnatelné s terestrickými systémy. To se odráží na stechiometrii živočichů, kteří musí být adaptováni na místní extrémní podmínky a jejichž výzkum je teprve v počátcích.

Summary

List of abbreviations

ATP – adenosine triphosphate
DNA – deoxyribonucleic acid
C:N:P – carbon:nitrogen:phosphorus ratio
DIN – dissolved inorganic nitrogen
DOC – dissolved organic carbon
DON – dissolved organic nitrogen
ER – endoplasmic reticulum
Fo-Me – forest and meadow
IC – inorganic carbon
IN – inorganic nitrogen
IP – inorganic phosphorus
LEA – late abundant embryogenesis
Me-Ro – meadow and rock
OC – organic carbon
OP – organic phosphorus
ON – organic nitrogen
PC – particulate carbon
POC – particulate organic carbon
PP – particulate phosphorus
rRNA – ribosomal ribonucleic acid
RNA – ribonucleic acid
RUBISCO – Ribulose-1, 5-bisphosphate carboxylase/oxygenase
SRP – soluble reactive phosphorus
TC – total carbon
TN – total nitrogen
TON – total organic nitrogen
TP – total phosphorus
UV – ultra violet
ER – Endoplasmic Reticulum

1 Introduction

The gap in our knowledge about the stoichiometry of biota inside cryoconite (accumulated sediment on the glacier surface) is huge (Zawierucha et al. 2015). However, glaciers are a place with increasing interest in recent times. These ecosystems are amazing not only due to their changing structure, but also due to their history and age. In this work, we focus mainly on ice caps and ice sheets. Ice caps are glaciers mainly in Svalbard, Iceland and Canada. Ice sheets are glaciers found in Antarctica and Greenland. The main goal of this work is to collect knowledge about glacial aquatic systems and their biota in a stoichiometric view. Ecological stoichiometry also helps us to take glacial aquatic systems apart and see the processes in more detail. Knowledge of stoichiometry also enables investigators to view different ecological pathways, trophic strategies, and relationships between organisms and their environment (Sterner and Elser 2002). This knowledge makes a good base for future planned work, which will focus on elemental composition and stoichiometry of Tardigrada and Rotifera.

In this work are some terms and definitions which need to be described. First, I would like to explain how we understand the term nutrient: Nutrients are everything which builds an organism excluding water and carbon. Secondly, I would like to explain the term imbalanced ratio. This is a ratio not in direct consensus with Redfield ratio (Redfield 1958; Robert W. Sterner and James J. Elser 2002), which is an established ratio of molar C, N and P with a value of 106:16:1, Imbalanced ratio can be changed with various factors of the environment. And at last, I would like to explain the term hyporheic zone. This nomenclature has its origin in limnology, where it is an ecotone between surface water and ground water. The hyporheic zone is a place with a high abundance of microorganisms. There is also a strong gradient of flushing and nutrient transformation (Kalff 2002). In this work I use the term hyporheic for liquid water seeping through the porous layer of temperate ice which undergoes melting. This area can be a great supply of nutrients and minerals for the glacial environment and also for cryoconite by weathering of mineral particles and flushing out of nutrients from melting ice.

2 Introduction to Ecological Stoichiometry

In general, ecological stoichiometry is a conceptual framework which looks at the organisms and their ecosystem through their elementary chemical composition. At the same time, stoichiometry obeys physiological and biochemical constraints of biological function of organisms. Ecological stoichiometry also operates with a balanced system following the law of conservation of mass (Sterner and Elser 2002). It also works with the chemistry of surrounding environment, which influences chemical reactions in the bodies of organisms (Vrede et al. 2004). This view gives us tools to predict ecological processes and interactions from the level of the elemental composition.

There are two main views on the use of metabolites among organisms:

1. Through biochemical reactions: elements and their compounds are reacting between each other

2. Through organization of metabolisms depending on mass and energy flow: deals with the supply of nutrients, costs of living (feeding, breathing, competition etc.) and environmental conditions.

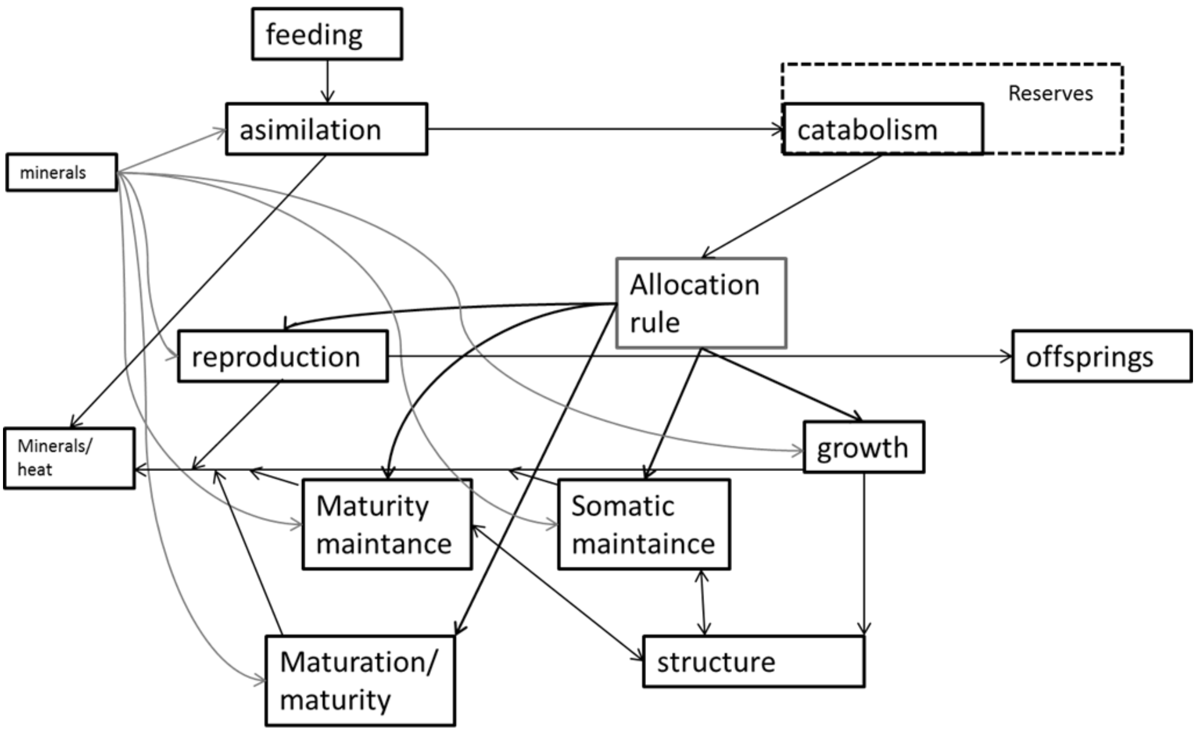

Fig. 1. Main biochemical pathways in organisms according to (Sousa, Domingos, and Kooijman 2008).

In this table, basic biochemical pathways, which form the dynamic energy budget, are shown. These pathways are located inside organisms and provide basic life mechanisms. For a better explanation, by reserves are meant body compartments, which have an important role in active metabolism of organisms. Inside them is converted energy from food, which is not directly used for growth or other processes in the body of an organism. Reserves are formed by lipids, carbohydrates, proteins and nucleic acids. In the body, there is not a strict division between elements found in reserves and elements found in structures (Vrede et al. 2004).

2.1 The major players in typical nutrient limitations

From the view of different ecological stoichiometric patterns, it seems to be obvious that carbon, nitrogen and phosphorus are presumably the main macro elements, which help us understand interactions inside glacial aquatic systems.

Cryoconite, compared to low altitude and latitude freshwater systems, has lower pH and nitrogen content. These factors, which influence cryoconite, form an absolutely different environment for the survival of microorganisms than factors on the glacier surface or inside proglacial soils. In table 1, main characteristics and comparison of glacier habitats are shown. The table is taken from the research work of Stibal et al. (2006) in which bulk C:N:P of the supraglacial sediment was also measured. These ratios are very useful for us because they show composition of the sediment in glacial aquatic habitats. TC:TN:TP inside them was: for cryoconite 5:0.4:1, for moraine 5:1:1 and for kame (deeper sediment) 21:3:1. For the analysis of C:N:P, carbonates were removed from samples. Organic nitrogen was converted to elemental nitrogen and carbon was converted to carbon dioxide. Phosphorus was extracted and measured separately. For the interpretation of the ratio, it is important to note that total phosphorus has a large proportion of P that is biologically unavailable and covalently bound (Stibal, Šabacká, and Kaštovská 2006).

		Supraglacial kames	*Cryoconite*	*Medial moraine*
pH	Mean	7.62	6.03	8.29
	SD	0.69	0.82	0.27
	Tukey	a	b	a
Coarse fraction content (% dw)	Mean	89.56	10.04	65.30
	SD	6.74	8.88	11.12
	Tukey	a	b	c
Water content (% w)	Mean	8.74	50.01	9.45
	SD	4.95	14.44	3.88
	Tukey	a	b	c
Organic matter content (% dw)	Mean	0.92	8.36	0.73
	SD	0.71	5.04	0.32
	Tukey	a	b	a
Total organic carbon (% dw)	Mean	0.713	2.803	0.700
	SD	0.348	0.858	0.178
	Tukey	a	b	a
Total nitrogen (% dw)	Mean	0.096	0.240	0.143
	SD	0.059	0.091	0.033
	Tukey	a	b	a
Total phosphorus (% dw)	Mean	0.033	0.664	0.137
	SD	0.009	0.625	0.128
	Tukey	a	b	a

Tab. 1. Main characteristics of a glacier ecosystem (Marek Stibal, Šabacká, and Kaštovská 2006)

Enzymes are one source of information about nutrient limitation in the system (for us glacial aquatic ecosystems). These proteins are employed in the acquisition of carbon, nitrogen and phosphorus (Burpee et al. 2016). These enzymes are e.g. β-1, 4-glucosidase, which degrades cellulose by hydrolyzing of cellobiose to glucose, β-N-acetylglucosaminidase, which degrades chitin, Leucine aminopeptidase, which hydrolyses leucine and hydrophobic amino acids, and Phosphatase, which degrades phosphomonoesters and phosphodiesters (Sinsabaugh et al. 2008).

These enzymes are closely related to changes in mean temperature and subsequent increases or decreases of organism activity. Also, they can vary with soil pH (Sinsabaugh et al. 2008). For example, nitrogen and phosphorus can be somewhere more available in colder climate and somewhere else not (Morales-Baquero et al. 2006). It is necessary to tell that melting ice buffers the temperature of water around the melting point, so the activity of enzymes on glaciers can vary with more factors than only with activities of microorganisms or with pH.

In nutrient limited environments, relationships between organisms and nutrient sources are important. But also important are relationships of organisms within a community and metacommunity. As an example of these relationship we can use the research done by Morales-Baquero (2006). This study observed high mountain lakes and their changes dependent on temperature and nutrient supply. When there were changes in nutrient content, there were also changes in structure and trophic interactions between organisms.

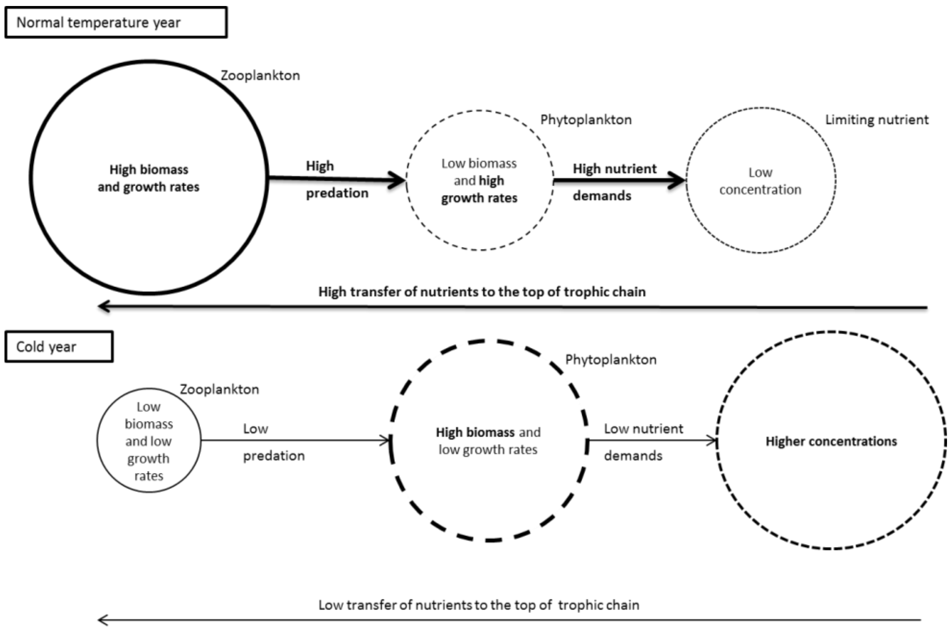

Fig. 3. Trophic interactions in the high mountain lakes according to (Morales-Baquero et al. 2006)

Molecular analyses carried out on samples of glacial communities from the Arctic and Antarctic have shown that in organisms, which live in polar glacial environments, are groups of coding genes for enzymes important in carbon and nitrogen cycling. Some of these cycles are photosynthesis, nitrification and denitrification. Main groups involved in these processes are Cyanobacteria, Chloroplastida, Alpha-, Beta- and Gammaproteobacteria, Rhizobiales (Cameron, Hodson, and Osborn 2012) and Metazoa.

2.1.1 Carbon

Carbon, as a source of energy in cryoconite, can be found inside the system in two main forms – organic carbon (OC) and inorganic carbon (IC) (Allison, Bollen, and Moodie 1965; Sherrod et al. 2002). Organic carbon can be distinguished as particulate organic carbon (POC) and dissolved organic carbon (DOC). The soluble form of inorganic carbon is HCO_3^- (carbonate ion) and the particulate forms are carbonate minerals. Inorganic carbon is mostly converted to organic forms by microorganisms (Tranter et al. 2004). Input of OC into cryoconite can be divided into autochthonous and allochthonous. Autochthonous carbon is the carbon which is contained in the biomass of organisms and is incorporated in the system. Allochthonous carbon is a carbon from the surrounding environment. And there is also legacy carbon, which is the carbon released from melted ice or cryoconite sediment (Tranter et al. 2004). Carbon from streams is mainly in the form of POC (particulate organic carbon). Organic carbon can vary between cryoconite holes and reflects very close relationships between abiotic and biotic components. Also, it can be contained in the excretion of organisms and in decomposed matter made by microbes (Tranter et al. 2004; Anesio et al. 2010; Takacs, Priscu, and McKnight 2001).

In measuring of OC, it is necessary to take into consideration the parameters of each cryoconite and surrounding environment. For example, when a cryoconite hole is closed, bacterial activity is lower than when is opened. Also, sources of carbon can differ, and getting correct results is very difficult (Anesio et al. 2010). For a better explanation of carbon cycles, the analysis of carbon isotopes is used. Through this analysis, it is possible to measure changes in carbon depending on the different environmental conditions in time. When there is low allochthonous inputs, ^{13}C is reduced due to the reduced growth of the phototrophic organisms, limitation of light, or increased concentrations of CO_2. Biological membranes of organisms vary in the ability to transmit CO_2, which also affects the composition of compounds in the environment and measuring (Lawson et al. 2004).

According to the supply from the surrounding environment and climatic patterns of the study site, it is possible to form main dynamic pathways.

When there is lower content of N and organic matter in the environment, there is also lower C turnover because of energy and nitrogen limitation of decomposers. There is a possibility to mobilize nitrogen from storage in the soil. Nevertheless, when sources of N in biomass are immobilized, productivity can be lower and communities become limited (Moorhead and Reynolds 1991).

Carbon can be, similarly to nitrogen, deposited into the soil. This can happen when the elemental ratio becomes imbalanced and trophic interactions inside ecosystem change.

Imbalanced ratios can be related to the input of fresh water (melted ice, rainfall) and NPP (net primary production). When the supply of nutrients and water is low, there is also low NPP. This causes a low abundance of decomposers and thus it is possible that soil carbon will be accumulated into the soil more than it would be with enough decomposition (Cao and Woodward 1998).

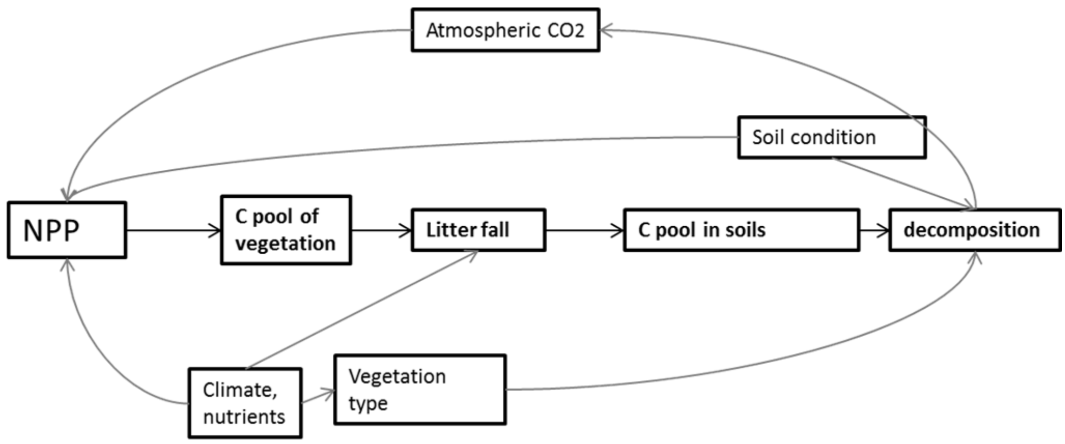

Fig. 4. Model of the carbon exchange in terrestrial habitats according to (Cao and Woodward 1998).

Important players in carbon cycling inside the ecosystem are photosynthesis and respiration. Due to low temperatures and high radiation, photosynthesis works more efficiently than respiration and more carbon can be deposited (Markager, Vincent, and Tang 1999). With the input of DOC, UV radiation incident can be lowered by transforming the high energy radiation in the decomposition process of DOC on the water surface and it can allow the growth of UV sensitive taxa (Weidman et al. 2014).

In the lack of light, compounds of dissolved organic carbon are decomposed by bacteria and used for respiration. When the light is sufficient, DOC is produced by phytoplankton. This process depends on the input of the light radiation (Takacs, Priscu, and McKnight 2001). But in cryoconite, only a small amount of OC is formed by photosynthesis (Telling et al. 2012). The ratio between photosynthesis and respiration can vary between holes (Tranter et al. 2004).

Productivity is also influenced by DOC, which can fluctuate with temperature (Weidman et al. 2014). Attesting to the fact, this was also found by Sinsabaugh (2008), in the work aimed on stoichiometry of enzymatic activity. In this paper, he claimed that C:N acquisition is related to the mean annual temperature and C:P lower in relation to mean annual temperature and mean annual precipitation (Sinsabaugh et al. 2008). Warming often causes an increase of DOC and N and a decrease of P. It may be due to a change in nutrient content and different demands on organisms. Therefore it depends on habitat and environmental conditions (Weidman et al. 2014). This hypothesis can be supported by considering that Arctic lakes with low input of DOC become rather N-limited than P-limited (Burpee et al. 2016).

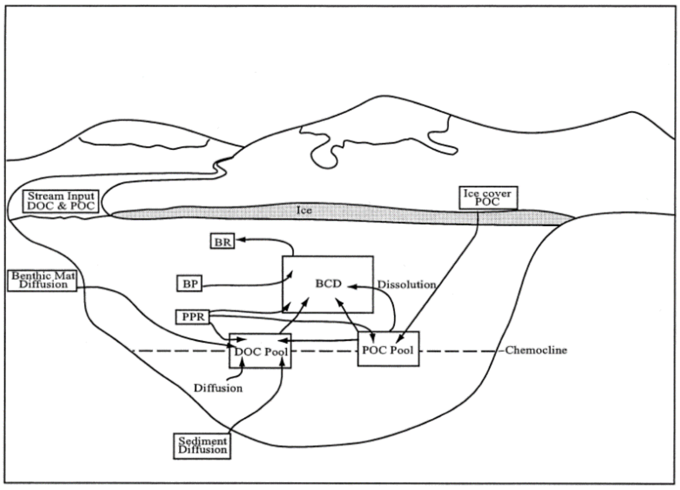

Fig. 5. Summer DOC supply and bacterial carbon demand of Taylor Valley Lakes (Takacs, Priscu, and McKnight 2001)

Based on the knowledge gained from this I infer, that when there is a low energy input from DOC, then there can´t be a big amount of fast growing organisms, and therefore there is a low consumption of P and high consumption of N.

Because subglacial runoff has much higher nutrient values than cryoconite or supraglacial streams, flushed carbon from these places is also a source of nutrients for subglacial and proglacial ecosystems. Some glaciers are bigger reservoirs of carbon then others and the accumulation of organic matter in the cryoconite can take tenths of years. (Stibal, Tranter, Benning, et al. 2008).

	pH[A]	DIC[A] (mg l^{-1})	DOC[A] (mg l^{-1})	DOC (%TDC)	DIN[A] (µg l^{-1})	DON[A] (µg l^{-1})	DON (%TDN)	DIC:DIN	DOC:DON
Supraglacial channel	5.1 ± 0.27a	1.3 ± 0.52a	1.8 ± 1.2a	58 ± 15a	48 ± 17a	130 ± 87a	68 ± 20a	34 ± 20a	22 ± 15a
Cryoconite holes	5.4 ± 0.19b	1.3 ± 0.93a	2.1 ± 1.6a	59 ± 19a	49 ± 22a	130 ± 95a	68 ± 17a	34 ± 23a	26 ± 27a
Glacier runoff	6.9 ± 0.33c	4.1 ± 0.57b	3.4 ± 3.5a	38 ± 18b	55 ± 15a	180 ± 130a	72 ± 13a	85 ± 36b	30 ± 31a

Tab. 2. Main stoichiometric parameters of carbon and nitrogen in the glacial hydrological systems in Werenskioldbreen (Marek Stibal, Tranter, Telling, et al. 2008)

2.1.2 Nitrogen

One of the most important nitrogen inputs into the glacial environment is nitrogen deposition. Nitrogen can be added to the system by nitrogen fixation and dry or wet deposition, mostly in nitrate form (NO_3^-). Dry deposition is less frequent. Wet deposition is influenced by weather: precipitation (snow, liquid water) and wind (Björkman et al. 2013; Smith, Smith, and Thomas 1998). Deposition of nitrogen is very important in the water environment, where nitrogen cycling depends on the microbial activity and where processing cycles are very fast (Gooseff et al. 2004). The three main processes in nitrogen cycle are nitrogen fixation, assimilation and denitrification (Townsend, Begon, and Harper 2010).

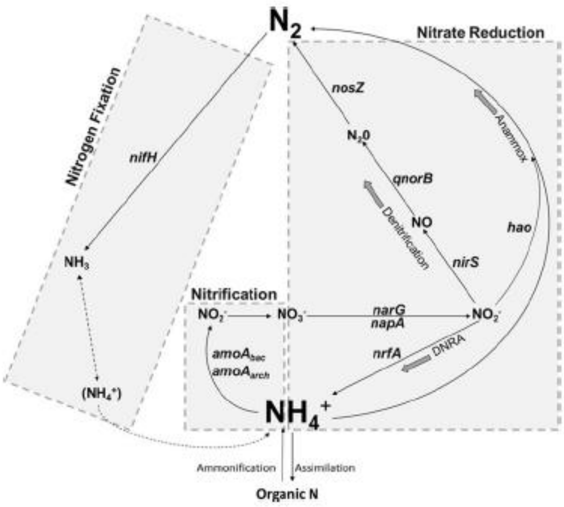

Fig. 6. Biochemical pathways of a nitrogen (Cameron, Hodson, and Osborn 2012)

Nitrogen fixation is a process, in which atmospheric nitrogen is converted to reactive forms, due to an enzyme called nitrogenase. This enzyme consists from two metalloproteins. For the reaction, it is necessary to reduce nitrogen molecules to ammonia. In this reaction, ATP is consumed and O_2 must be absent (Knowles 1982; Rees et al. 2005; Berendt et al. 2012).

Denitrification is less frequent than assimilation in aerobic conditions. Assimilation is the incorporation of N to the body of an organism. Bacteria incorporate NO_3^- and thereby lower the concentration of nitrate in the water. Conversely in denitrification, bacteria reduce nitrite to the molecular form of N_2 and return N back to the atmosphere. This process is anaerobic (Gooseff et al. 2004; Smith, Smith, and Thomas 1998).

Denitrification has two pathways. One of them is the reduction of $NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$ and the second is the reduction of $NO_3^- \rightarrow NH_4^+$ (Knowles 1982). Ammonium is toxic by removing oxo-glutarate from Krebs cycle, so it must be removed from the body of organisms (Matouš 2010). In aquatic systems, ammonium is excreted directly to the water.

Because of the variable abundance of organisms during the year, ratios of elements may vary (Stibal, Tranter, Telling, et al. 2008). There is also an effect on nutrient fluxes by the changes of weather conditions during the season. Moreover, main biochemical pathways of nutrients such as P, N and C change with the warming of a climate (Kohler et al. 2016). During summer season N:P lowers due to a bigger abundance and activity of microorganisms and also due to the decreasing snow pack, which is one of the major supply of nitrate in a glacier. Nitrogen depletion over season causes their limitation in the end of the season. OC:TN (11-12) and TN:OP (2,7-8,9) are similar to the global microbial biomass ratios (Stibal, Tranter, Telling, et al. 2008; Dibb et al. 2002).

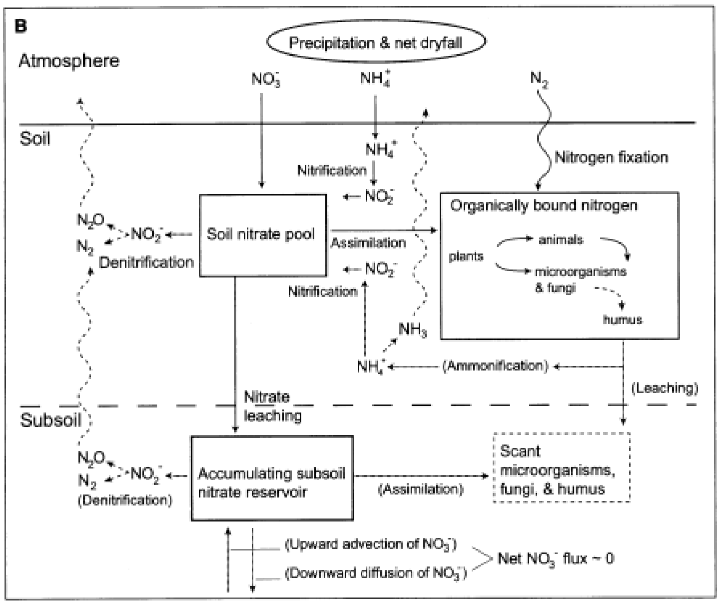

Fig. 8. Model of the pathways of nitrogen in the ecosystem (Michelle A. Walvoord et al. 2003)

Nitrogen can be found in greater amounts in areas with ancient glaciation, when it was accumulated during a dry period of glaciation. Nitrogen acquisition from organic matter is very complex and there is also a significant effect on the exchange of water between the supraglacial and the subglacial, which influences the nutrient dynamics. Dissolved organic nitrogen (DON) is very unstable in ecosystems (Gooseff et al. 2004; Tranter et al. 2004; Sinsabaugh et al. 2008).

2.1.3 Phosphorus

Phosphorus fluxes in ecosystems are necessary for the growth and proliferation of microorganisms and bigger eukaryotes. Bacterial communities have relatively high C:P and N:P. However, they are considered phosphorus sinks and their turnover can be high at the same time (Karl 2000). In study published by Clarke (2008), it was found that phosphorus contained in phospholipids is necessary for mating females of marine invertebrates.

Concentrations of phosphorus in croconite debris is much higher than in surrounding rocks and also higher than in the supraglacial stream. Although the majority is OP (57 %) in cryoconite, the other half is represented by IP (Stibal, Tranter, Telling, et al. 2008). Some unavailable phosphorus can be used by microbes due to the active enzyme called phosphatase. This enzyme helps microorganisms to use limited sources of phosphorus across the glacier environment (Stibal et al. 2009). Organic phosphorus has mostly allochthonous inputs from wind (plants, small animals, debris) or is released from the rocks by leaching. Organic phosphorus is very rapidly re-absorbed to the material (Stibal, Tranter, Telling, et al. 2008). Amazing information was presented by Hobbie et al. (1999), who examined lakes in a permafrost landscape. They found, that phosphorus added to the water was bound to the iron in the sediment. Thus, bound phosphorus was not available in the water and measured concentrations did not increase for a long time. Significant increases came after six years from the addition of phosphorus to the water. This increase caused that algae depleted the iron oxide layer for the production of iron sulfide. And this caused a big flux of phosphorus from the sediment (Hobbie et al. 1999).

Organic phosphorus is available in the melt season but also during the autumn. In this period, organic matter can be transported because it is in a frozen form. In the summer period, is impossible to transport wet debris by the wind, because is wet, but there is a lot of moraine material which is dry and easy to transport by a strong wind. Phosphorus can be also transported when the pH of water or in the soil is in such values, that it is released from rocks or when the temperature of water increases (Stibal, Tranter, Telling, et al. 2008; Hobbie et al. 1999). Value of pH varies over the glacial hydrological system. In habitats where water circulates slowly, there is a higher pH (cryoconite) because of a higher rate of photosynthesis. In supraglacial streams, pH is close to the pH of distilled water, where CO_2 is in the balance with atmospheric CO_2 (Stibal, Tranter, Telling, et al. 2008).

	SRP (μg l^{-1})	DOP (μg l^{-1})	DOP (%TDP)	DIC:SRP ($\times10^3$)	DIN:SRP	DOC:DOP	DON:DOP
Supraglacial channel	0.91 ± 0.80a	5.9 ± 1.0a	87 ± 12a	6.4 ± 4.7a	204 ± 160a	890 ± 640a	59 ± 51a
Cryoconite holes	0.75 ± 0.47a	6.1 ± 0.9a	89 ± 6.8a	6.7 ± 9.3a	202 ± 130a	990 ± 780a	58 ± 39a
Glacier runoff	0.80 ± 0.83a	5.7 ± 1.1a	88 ± 13a	24 ± 12b	240 ± 150a	2400 ± 4200a	97 ± 130a

Tab. 3. Main stoichiometric parameters of phosphorus in the glacial hydrological system of Werenskioldbreen (Marek Stibal, Tranter, Telling, et al. 2008)

In cases of more intense soil weathering, P availability declines relative to carbon (Sinsabaugh et al. 2008).

Phosphorus also affects Metazoa in oligotrophic waters. Research was done on small Crustaceans, mostly Daphnia and Copepoda, where impacts of phosphorus had influences on PC:PP and PN:PP ratios. These ratios varied in different habitats (Weidman et al. 2014).

2.2 Biological chemistry: Linking elements to functions

Life is based on chemical processes and chemistry, thus providing the tools and constraints for accomplishing biological functions. Biological chemistry is a substantial component of recent biology.

I would like to present the most important section for this work, which is the elementary composition of cells focused on carbon, nitrogen and phosphorus (Sterner and Elser 2002). There are similar patterns in the elementary composition of terrestrial and aquatic organisms (Elser et al. 2003). However, they may vary among different taxa (Clarke 2008).

Main sources of C, N and P are various biomolecules including nucleic acids, proteins, chitin, adenylates (ATP), phospholipids, triglycerides and carbohydrates. These compounds make the vast majority of dry mass of organisms (Elser et al. 1996).

This stoichiometric diagram shows major relationships between the content of P and N in important body compounds. There is also shown that dynamic structures have high content of P. Higher content of P in those struc-

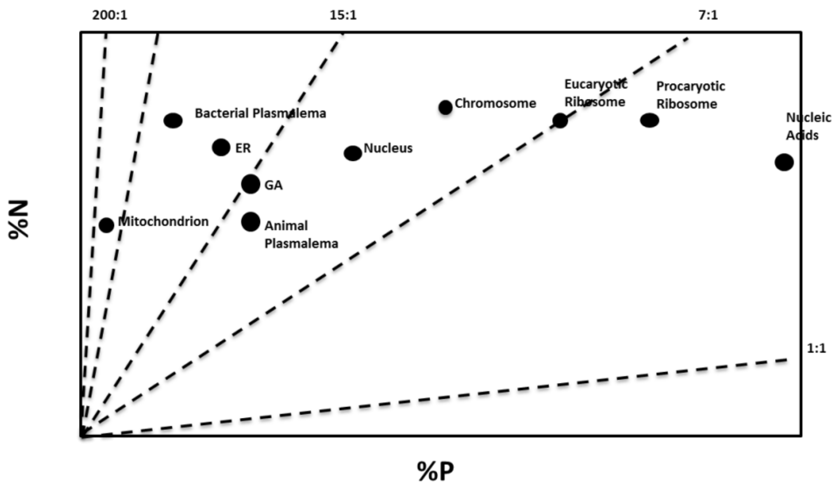

Fig. 9. N:P stoichiometry of main body compounds according to (Sterner and Elser 2002). Cell wall has 95 % of C.

tures can signify that there is a high growth rate. Content of P varies between Vertebrata and invertebrates, but here we focus only on invertebrates.

2.3 Stoichiometry of an organismal body

Stoichiometry of organisms is not possible to summarize into one group. It is necessary to analyze the size of organisms, quantity and composition of body mass, life strategy, growth rate and many other aspects. There are many methods how to calculate elemental content and estimate nutrient content in the bodies of organisms. But despite the often fluctuating results, the most significant differences in body composition are very important (Boros and Mozsár 2015).

At first, I would like to mention stoichiometric influence of RNA to an organism and then continue to other cell structures.

2.3.1 RNA and nucleus

RNA is one of the essential macromolecules, which organisms have in their bodies. RNA is often located inside the nucleus, which is an important organelle for animal growth, but also high amount is in the Endoplasmic Reticulum. Elementary composition of the nucleus can be different between organisms with different growth rates and cell types, although form of genetic information (DNA, RNA) is almost the same (Alberts et al. 1995).

Despite the fact that the nucleus is composed from a major part of nucleic acids, which are P-rich, its stoichiometry has high nitrogen ratio (N:P = 11:1) (Bowen 1979). It is probably due to the presence of N-rich bases of nucleotides and proteins.

Content of RNA can vary with many factors which influence organisms: their body structure, life strategy, habitat and many others. The most visible factor in stoichiometry is growth. During growth, content of RNA in the body of unicellular organisms can increase to about 45 % of dry weight. This means that content of phosphorus will increase and also stoichiometry of whole organisms will move towards higher proportion of phosphorus (Sterner and Elser 2002).

The fact that there is a higher content of RNA in organisms from the same species shows that stoichiometry can also vary with the availability of food. This availability influences reserves in bodies of organisms. Higher food intake increases RNA and then protein synthesis, which starts the growth of organisms (Vrede et al. 2004).

The content of RNA is the highest in embryos, juveniles and adults are almost constant. This is caused by higher growth rate in embryo stage, when ribosomal RNA is necessary for protein synthesis, which is situated on the ribosomes (Vrede et al. 2004).

2.3.2 Ribosomes

Ribosomes are components of the cell with a key role in proteosynthesis and metabolism of phosphorus. They consist of two main structures: RNA and ribosomal proteins. Their two subunits interact with tRNA and mRNA and thus operate proteosynthesis (Daines, Clark, and Lenton 2014).

Ribosomes are cellular compounds, which have high P content (Daines, Clark, and Lenton 2014). Ribosomes of prokarya are more P-rich than those of Eukarya. Nevertheless, ribosomes mostly do not comprise more than 50 % of the P content in cells among different taxa (Vertebrates are not included in this calculation) (Elser et al. 2003).

2.3.3 Proteins

Organisms have high N:P ratio because proteins are a big reservoir of nitrogen. Amino acids have more or less primary or secondary amino groups, so the content of nitrogen can vary among different species. In prokaryotes, proteosynthesis is situated in the cytoplasm of the cell. In eukaryotes, there is a strict division between replication and transcription, which take place inside the nucleus on one hand and protesynthesis, which is inside the cytoplasm on the other hand. Proteins have an essential function on metabolism and are divided into two major groups: structural proteins and enzymes. One of the aspects of stoichiometry of the enzymes is, that enzymes included in metabolisms with majority of one element (carbon, sulfur, etc.) are low in the content of this element. Other enzymes, like RUBISCO, can influence whole stoichiometry of an organism due to their metabolic pathways. In RUBISCO it is photosynthesis, which increases the demand for N (Sterner and Elser 2002).

2.3.4 Membrane organelles

A cell membrane delimitates the outer surface of the cell. The membrane is a bilayer of phospholipids consisting of a hydrophobic strain of fatty acids and a hydrophilic head, which is a polar structure with choline, phosphate and glycerol (Alberts et al. 1995). Membranes are full of other components including membrane proteins, sterols and signal molecules (Alberts et al. 1995). Proteins represent 25–62 % of membrane mass, lipids 25–56 % and carbohydrates 10 % (Da Silva and Williams 2001). Stoichiometry of membranes in bacteria is C:N:P = 165:35:1 and in an animal cell it is C:N:P = 100:15:1 (Sterner and Elser 2002).

Golgi complex and Endoplasmic Reticulum are both high in N:P ratio (Becker et al. 2003). Endoplasmic reticulum is one of the places where ribosomes are located. We distinguish the Rough Endoplasmic Reticulum and the Smooth Endoplasmic Reticulum. ER has synthetic function. Golgi apparatus has mostly secretory function (Elser et al. 1996; Alberts et al. 1995).

Crucial role in the oxidative metabolism is played by mitochondria. They are necessary for cell energetics because of respiration (Merzendorfer 2003; Alberts et al. 1995). In mitochondria is a high concentration of Ca^{2+}. Metabolic pathways of mitochondria are mainly focused on enzymes and energetic macromolecules (Rossi and Lehninger 1964; Ramakrishna et al. 2001). Because mitochondrial activities lead to an increase of ATP, we can expect increase of P content in organisms with a great need of breathing. Also organisms with high mitochondrial content have increased N content due to their increase of enzymatic activity (Rossi and Lehninger 1964; Ramakrishna et al. 2001). Mitochondria have very similar N:P stoichiometry as chloroplasts (N:P = 80) (Sterner and Elser 2002)

Body surfaces of polar invertebrates often form resistant structures like cuticles. These structures protect these animals from the surrounding extreme conditions and help them to survive.

In invertebrates, chitin structures are big reservoirs of carbon (Merzendorfer 2003). Despite this, chitin also includes acetyl amine groups, and therefore N is also present (Roer, Abehsera, and Sagi 2015).

Extracellular materials with high permeability can help invertebrates to dehydrate themselves and decrease the melting point of their body fluids close to the temperature of the surrounding environment (Holmstrup, Bayley, and Ramløv 2002). Another important macromolecule which help invertebrates (especially from polar marine habitats) to survive are triacylglycerols as storage pools. These lipids increase the content of C, N and P. Phosphorus is mostly contained in phospholipids (Clarke 2008).

There are many factors that influence C:N:P ratios. Among them, there is genetic information, living conditions, availability of nutrient rich food, foraging strategies, energetic constraints and life history.

3 Glaciers and their history

As it is written in Oparin´s The Origin of Life (Oparin 1938), life has to start with the evolution of matter. In that time our planet was cooled down and hot waters connected together elements such as: carbon, nitrogen, oxygen and hydrogen and main compounds such as H_2O, NH_3, CO_2 and CH_4 were formed (Langmuir and Broecker 2012).

This work is focused on glacial environments, so the aim of this chapter is to give a general summary of glaciation events in the history of Earth, their consequences and general description of a glacier.

Probably the first large glaciation in the history of the Earth were a series of events called Snowball Earth. This events could be in the Protezoic period, in the time of the Neoprotezoic, which started 2.5 billion years before present (Hoffman et al. 1998). The first living organisms would have their origin in this period too. The first fossils of Metazoans were found in marine sediments and were dated to this time (Lipps and Signor 2013).

In the past 730 000 years, we know of about 10 major glaciations, therefore there was much more time with below average temperatures (Emiliani 1978).

For our study, Quaternary glacial events are the most important. In the Quaternary, consisting of Pleistocene and Holocene, glacial and interglacial episodes were changing (Holocene starts circa 10 000 radiocarbon years before presents). In the Pleistocene, which is also known as the Ice Age and lasted from 1.8 million years before present to 10 000 years before present (Lyle 2009), glaciers in Antarctica and Greenland were formed (Aber 1988).

Main characteristic of these stages is apparent from the definition of The American Commission from 1961: “A *Glaciation* is a climatic episode during which extensive glacier developed, attained a maximum extent, and receded. A *Stadial is* a climatic episode, representing a subdivision of a glaciation, during which a secondary advance of glaciers took place. An *Interstadial* is a climatic episode within a glaciation during which a secondary recession or standstill of glaciers took place. An *Interglacial* is an episode during which the climate was incompatible with the wide extent of glaciers that characterize a glaciation.” (Gibbard and van Kolfschoten 2004).

Around two thirds of Earth´s freshwater is stored in the ice. The majority of ice mass is stored in Polar Regions. Only a small amount of this fresh water is stored in alpine glaciers and surface waters (Sattler et al. 2007; Thomas et al. 2008).

An interface between the glacial ice and the bedrock is the glacial bed. The type of rock has strong effect on the glacial hydrology, ice dynamics and biogeochemistry of the glacial catchment. The main mass of the ice sheet is divided into two parts: the active ice lobe and the stagnant ice lobe. On the bottom of the active ice lobe, there is an end moraine forming ice margins and proglacial lakes. In the stagnant lobe, which is on the base of the glacier, sinking streams, debris band and also debris-rich ice are formed (Lyle 2009). Debris is easily transmitted with melting ice and it can vary with solar radiation during the day. Ablation is much greater on the peripheral zone of the glacier than on the ice surface, so dust material works differently on snow and on ice.

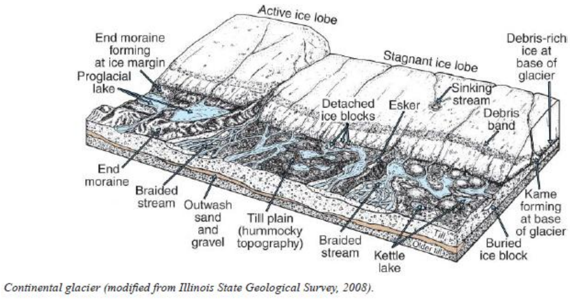

Fig. 10. Morphology of a glacier (Lyle 2009)

4 Glacial ecosystem analogues in the oligotrophic alpine lakes and ponds

Lakes can be classified from different points of views depending on the subject of investigation. In Kopáček et al. (2000) was used division to two groups. The first are forest lakes in the mountains, which are divided into two groups:

1. Forest – more than 80 % of catchment area is forested
2. Fo-Me – where meadows cover 30–70 % of catchment area.

The second type of mountain lakes are Alpine lakes (in Tatra Mountains situated above 1800 m). They can be divided into three groups:

1. Meadows – more than 70 % of catchment area is covered by meadows
2. Me-Ro – meadows and rocks cover 30–70 % of catchment area
3. Rocks – rocks (bare or with lichens) cover more than 70 % of catchment area.

This kind of division was used due to the nutrient connection of mountain lakes to surrounding vegetation or rock bed. I would like to give some parameters of alpine lakes with the use of this classification, because this research focused on nutrient cycling has similar needs as we do in our foreseen work on stoichiometry of glacial invertebrates. I have also chosen this research work because lakes in Tatra Mountains usually have glacial origin and environment of their occurrence often has some analogy with polar habitats. It's important to emphasize that a cryoconite holes are not stratified and are not limited by oxygen (with a special exception of cryoconite in continental Antarctica), and so the limnological patterns shown below have no direct use on glaciers. But they can be used as basics for understanding the processes in aquatic ecosystems.

Glacial lakes in the mountains are usually dimictic, which means that they are mixing twice a year (summer and winter). These lakes are often close to Rock-rock lakes without vegetation around their area. Sometimes they can be close to Ro-Me lakes, but vegetation in Polar Regions is very simple. Over the year, lakes are mostly stirred in epilimnion by the wind. In these lakes, we can also observe other water movements such are seiches, gravity waves, Kelvin waves or other laminar or turbulent movements. In Polar Regions, there are also epishelf lakes, which are connected to the ocean. Then there are halophilic lakes, which are permanently stratified due to their strong chemical and physical gradients. And finally permafrost lakes, which have temperature of epilimnion above 0 °C and hypolimnion with high concentrations of dissolved H_2S with temperature above 6 °C (Thomas et al. 2008; Kalff 2002). Here we are mainly interested in lakes lacking any extreme chemical or hydrogeological features for the comparison to glacial conditions.

Between upper layers of the water column, there are differences in the content of nutrients and abundance of zooplankton and phytoplankton. Hypolimnion is a hypoxic or even an anoxic base layer which is changed only during spring and autumn mixing. There are very sharp differences between the epilimnion, thermocline and hypolimnion. In the winter season lakes freeze, so the upper layer has temperatures below 0 °C. In the summer, upper layer of epilimnion can have about 13 °C (Luoto and Nevalainen 2013; Hartman, Štědronský, and Přikryl 1998; Kalff 2002; Brönmark and Hansson 2005; Thomas et al. 2008). The warming of the upper layer of the water column provides a rapid change in the nutrient content and also a change in dissolved nutrients. Here we could talk about ionic pulse, which is a rapid supply of nutrients from the surface of melting ice crystals formed from solutions, where the solutes were concentrated due to preferential freezing. This happens during early melt, when the release is about 80 % of solutes. Although lakes are different than cryoconite, we can find a connection between them in use of this process. Lakes are stirred during the year (if they are not amictic) and there are possibilities for nutrient supply from the upper layer to the lower layer and vice versa. In cryoconite on the ice sheet, this can also happen by the ionic pulse, even though they are not stratified and the nutrient supply is limited. The mechanism is different in both systems, but these consequences represent useful analogues in the interface between them.

Concentrations of nutrients in alpine lakes (incl. TON- Total Organic Nitrogen, TP-Total Phosphorus) is lower than in the lowlands, except TIC (Total inorganic carbon), which is mostly higher because of higher plankton abundance (Caputo, Alfonso, and Givovich 2013; Kopáček et al. 2000). There are also different concentrations of nutrients which are caused by different distribution in water column by water movements. In polar lakes, external input of C,N and P by excretion of birds from colonies around often play a substantial role in their trophic status. After that they are not oligotrophic any more (Thomas et al. 2008).

Cryoconite holes, compared to oligotrophic mountain lakes, are not stratified, but we can draw some basic analogies between them. For example the limitation of phosphorus or water movements, which are affecting holes with a bigger size. In these huge holes we need to know the parameters of the place where hole is situated: glacier ridge or glacier indentation; geomorphology of surrounding mountains because of the wind circulation and geological characteristics of glacier subsoil. But there are also big differences. A few of them are: liquid water content over the winter season, different contributions of sources and mechanisms of nutrient input, the duration of the summer season and the variety of animals living in the water column, not only in the sediment.

It is also necessary to mention some patterns of alpine ponds, because they are very close (mostly by their structure) to the cryoconite holes. Ponds are usually formed by rainfall or thawing snow in rock- depressions. They are not stratified and can dry during summer (Hartman, Štědronský, and Přikryl 1998). Ponds are variable environments and habitats for various types of species (Dodson 1975).

5 Glacier ecosystem, adjacent and downstream systems

A glacier ecosystem is a large-scale system with several distinctive components and a number of pathways linking the components on different spatiotemporal scales. Atmosphere, ocean, fauna, flora, organic or inorganic debris windblown and release from adjacent biotopes form very complex interactions which are, compared to other habitats, very unique.

Debris, as a one of the sources of nutrients, is easily transmitted with melting ice and it can vary with solar radiation during the day. Despite this, ablation is much greater on the peripheral parts of the glacier than on the ice surface, so the dust material works differently on the snow and on the ice (Adhikary et al. 2000).

Glaciers typically differ in fluxes and also sources of sediment. It is because of different types of adjacent areas, their geology and geomorphology and also with different size and geometry of the glacier. Sediment can be melted out of the ice and brought to the glacier together with avalanche from surrounding massifs, by wind flow or by melted water (Fountain et al. 2004). Wind supply of nutrients can be sometimes called aeolian input. This input can vary between glaciers and it is important for many processes (McKnight et al. 1999; Edwards et al. 2014).

In the glacier, there are also differences in the chemical parameters of waters and their temperature and pH, therefore the environment is very thermally stable. Supraglacial water is cold (0,1 °C) and low in pH, while glacier runoff is warmer than the supraglacial water (3 – 5 °C), higher in pH, and inorganic carbon content (Stibal, Tranter, Telling, et al. 2008).

One of the factors on the glacial surface forming an ideal place to endure winter in polar habitats is the presence of liquid water in the summer season. Liquid water has low albedo and a big heat capacity, so we can find a great diversity of organisms compared to the surrounding glacier there. Inside a cryoconite holes, viruses, bacteria, algae and Metazoa are accumulated (Porazinska et al. 2004). These microorganisms help to create cryoconite granules, so they are the factor conducting the variability between cryoconite holes on glaciers (Yoshimura 1993). This variability is reflected in complex stoichiometry and ecology of each glacier.

5.1 Proglacial soils as a source of nutrients

Proglacial soils are important sources of C, ON and IN (Beilke and Bockheim 2013). Nutrients and minerals in soils can be provided by wind and their concentrations are higher in low elevations. Relatively high are especially concentrations of SO_4^{2-} and NO_3^- (Berry Lyons et al. 2003).

Due to the age of the soils, C:N ratios differ across the glaciers. After deglaciation, concentrations of nutrients in soils decrease to a very low level. But after that, development of the soil also causes formation of the retention capacity and nutrients can again increase. In spite of that, OC can lower with elevation and climate. In drifts of ice core, which are low in inorganic nitrogen, there is usually high C content due to the presence of coal within the soil (Barrett et al. 2007; Beilke and Bockheim 2013).

Content of P in the soil usually differs among different soil types depending on the geological context, development of vegetation cover and also climatic factors as precipitation rate. In sand, the content of P is low and in the clay and silt, the P content is higher. It is because of a better ability of clay to bound P. Also, there are much more microorganisms in the clay. IP is found dominantly in Ca-bound fractions due to minimal weathering of P-bearing minerals such as apatite or basalt. There is a negative correlation between the P of biomass and soil pH due to leaching (Blecker et al. 2006).

Soils form a very extreme habitat with imbalanced stoichiometry, which is very often dependent on biotic controls (Barrett et al. 2007). Cryoconite sediment layer thickness influences the ratio of respiration and photosynthesis. More sediment indicates more OC, which can be used by organisms for these processes. However, higher sediment causes minor supply of the light (Telling et al. 2012). In habitats with small abundance of nutrient depositors, nitrogen can be accumulated within the soil. This accumulation occurs, because nitrogen is not used for assimilation and also it is not denitrificated (Michelle A. Walvoord et al. 2003). At last, geochemical C:N stoichiometry affects soil invertebrates (Barrett et al. 2007) and taking this into consideration we discuss our future research plans.

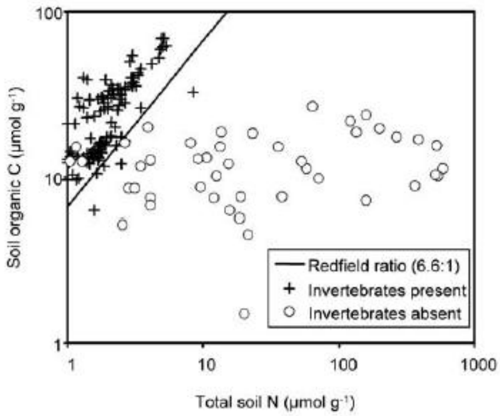

Fig. 11. Communities of invertebrates depended on content of the soil organic C and N (Barrett et al. 2007)

5.2 Cryoconite

On the melted glacier surface, there are usually spots of dark sediment which have thawed into the surface and filled with meltwater. These wells are called cryoconite holes. Inside them, there is a great diversity of organisms, including bacteria, cyanobacteria, viruses, algae and invertebrates (Porazinska et al. 2004; Takeuchi et al. 2005a).

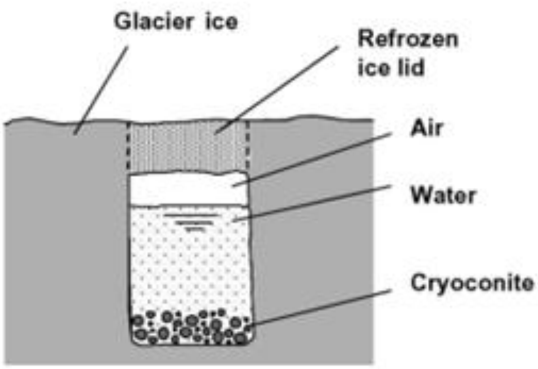

Fig. 12. Structure of a cryoconite hole (Porazinska et al. 2004)

These holes are formed by melting ice due to their low albedo and high absorption of radiation. Melting is up to 3 times higher than in the surrounding surface. This high absorption is caused by dark organic components in cryoconite granules (Takeuchi, Kohshima, and Seko 2001).

The diameter of cryoconite holes varies from tens of centimeters to millimeters and there is a linear relationship between hole area and mass of the cryoconite sediment (Cook et al. 2010). Their depth can exceed 60 cm and does show low variability between different holes at the same locality (Gerdel and Drouet 1960). Temperature among a cryoconite holes is very similar, it can only vary very little with the hole size (Säwström et al. 2002). Differences between holes among different elevations were not found (Porazinska et al. 2004).

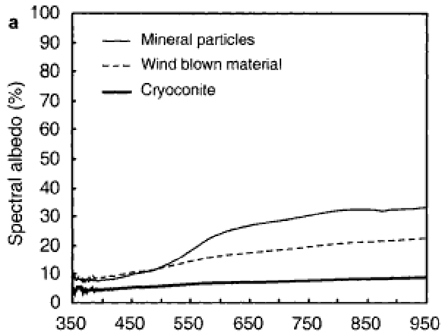

Fig. 13. Spectral albedo to wavelenght in the structures on the glacier surface (Takeuchi, Kohshima, and Seko 2001)

Organic dust found in a cryoconite can be stored in ice and gets to the surface by ablation of the glacier. Dust is also transported from surrounding places to the glacier by wind and by water flux (Gerdel and Drouet 1960). Water flux depends on the hydrological connection of the hole with surrounding streams. In some of them, water can change in a few minutes because of melting ice and snow, and some need hours (Marek Stibal, Tranter, Telling, et al. 2008). This dust is necessary for forming of cryoconite granules and also for nutrient input into the holes on the glacier.

Cryoconite holes are often situated in flat areas of the ablation zones on the glaciers, where the erosive effect of water flux does not cause major losses of sediment. It is questionable how much are particles in cryoconite (cryoconite granules) formed by dust from adjacent areas or by dust melted out of the glacier. I think that both sources are important, but can vary with the size of the glacier and distance from the margin.

The process of water flux through a cryoconite hole can influence communities of organisms and their living strategies. When there is a rapid turnover of water inside the cryoconite, the primary strategy is heterotrophy. It is because of the bigger input of nutrients and organic matter from surroundings. If there is a small flow through, then the dominant strategy is autotrophy (Telling et al. 2012). The water flow will probably differ on the flat sheet of the glacier and the glacier margin.

In polar habitats we can also find extremely large cryoconite holes, which are hydrologically connected to the glacier by drainages and glacier streams. These big holes became very large due to their low solar elevation angle (which is in maximally 36°). This angle causes warming of water by high input of radiation (Fountain et al. 2004). These holes would be amazing for discovering limnologic patterns and their influence on polar-glacier-surface-water biota, but they are very rare on glaciers. These holes are not at the forefront of our interests and thus are mentioned only briefly.

5.3 Stoichiometry of the glacier system

Stoichiometry of polar ecosystems depends on all its components. These components are soils, water (all phases of matter), organisms and atmosphere (Barrett et al. 2007). Some of these components received more attention than others and we would like to focus on a less known component, which is the metabolism of invertebrates.

A demonstration of the interconnections between glacier ecosystem and surrounding environment is in the figure below. There are shown the average molar N:P and C:N:P ratios of the components and their interweaving in a polar ecosystem.

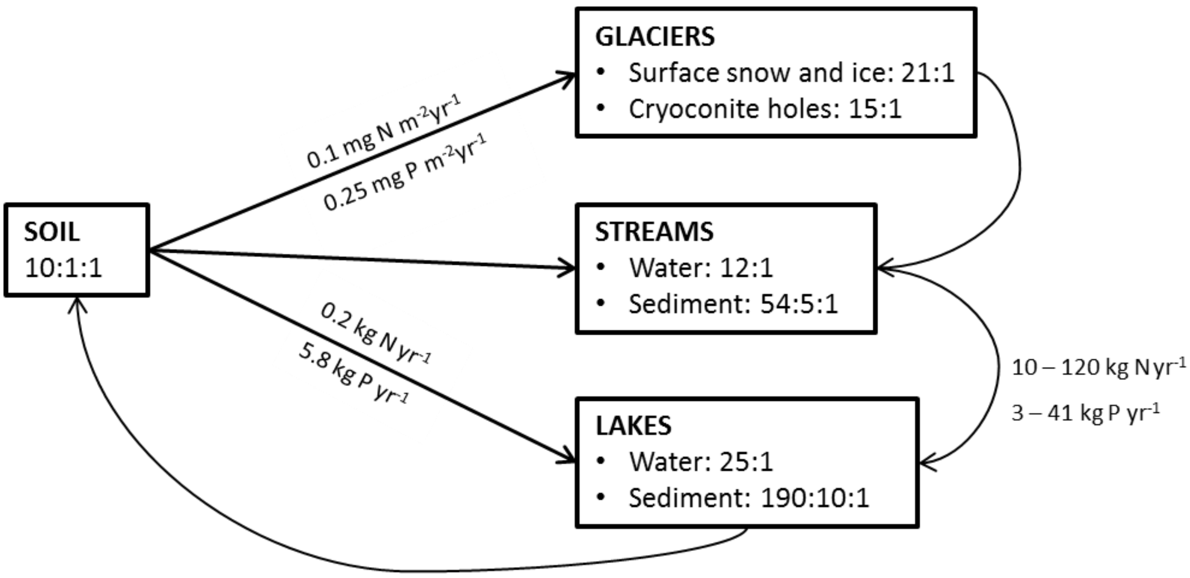

Fig. 14. Main stoichiometric pathways in the polar system according to (Barrett et al. 2007)

6 Aquatic habitats and biota found on glaciers

After getting a general view into functions of glacier systems and their non-glacier analogues, we can combine this acquired knowledge to generalize or stress differences between them.

Ablation zones provide melted snow and ice as a source of the water for cryoconite, lakes and streams (Berry Lyons et al. 2003). Due to the dissolution of the salts within the glacier ice, it tends to influence pH and ion concentrations (Tranter et al. 2004). For example, hydrolysis of $CaCO_3$ in dust and photosynthesis increase water pH and therefore may influence saturation of oxygen in water (Tranter et al. 2004).

The hyporheic zone in streams found in polar habitats represents a source of minerals and nutrients as well (McKnight et al. 1999). It is because the flowing water under the surface brings these compounds to the subglacial stream, lakes and water habitats within the glacier An important process, which takes place between the surface flowing layer and hyporheic zone, is a release and transfer of nutrients by weathering (Runkel, McKnight, and Andrews 1998). Weathering and nutrient fluxes depend on temperature, which influences solubility of ions and microbial activity (Gooseff et al. 2002).

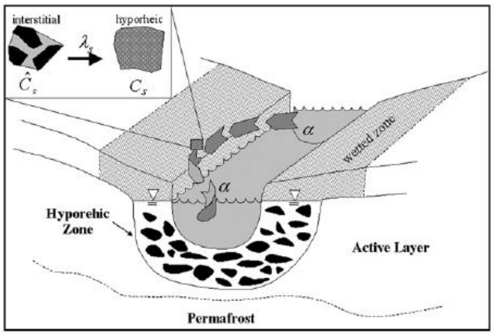

Fig. 15. Process of weathering in the hyporheic zone (Gooseff et al. 2002)

To give connection to the previous chapter, soils have an influence on the stoichiometry of glacial hydrologic systems too. This influence can differ with the size and location of the glacier. It is caused by the input of material from the neighborhood but also by leaching of soil particles from the ice. As was mentioned before, hydrological connections with soils and surrounding environment also affect nutrient fluxes and nutrient content among different sites (Bridgham, Updegraff, and Pastor 1998; Barrett et al. 2007). An example can be the nutrient contents in lakes within permafrost landscape where is soil pH and chemical solutions different than in for example, in lakes with apatite adjacent areas (Burpee et al. 2016).

Life depends on water, but some organisms are more sensitive to water availability than others. Warming is connected with melting and floods, while it releases an amount of particles which are the source of minerals or nutrient. There is also an amount of suspended solids, which, on the contrary, can lower the availability especially of reactive phosphorus. Particulate nitrogen and particulate carbon (in photic zones) to Chlorophyll-a ratios decreases after floods. In thermocline, DIN increases (Foreman, Wolf, and Priscu 2004). DIN: SRP ratios in glacial water systems are near to the Redfield ratio (Foreman, Wolf, and Priscu 2004). This study was focused on Antarctic dry valley lakes.

6.1 Organisms in stoichiometric view

As it was previously mentioned, stoichiometry of organisms is very diverse and interactions between them form a complex system, which is far from fully understood (Sterner and Elser 2002). This means that if we want to study stoichiometry of organisms, we need to have a very wide view. It is not possible to study only one or two interactions without taking account of other processes in a system.

What typically introduces large intraspecific variability in stoichiometry is that organisms have often more ontogenetic stages. They start with an egg and develop their body through embryo to an adult, who can produce other individuals. If organisms have enough nutrients, the growth is in the line with their ontogenetic plan (Sousa, Domingos, and Kooijman 2008).

The second aspect in an organism's stoichiometry is that imbalances between organisms in the ecosystem can disrupt the turnover of a nutrient. It happens very often between a producer and a consumer in nutrient poor systems, where nutrient turnover in consumers can influence, for example, P-limitation of the epilithon. Epilithon had in this case a lower C:P ratio compared to the consumer. Furthermore, more P and higher foraging pressure on consumers can lower C:N (Bowman, Chambers, and Schindler 2005).

Another example is from a process between the consumer and its food. When phytoplankton increase growth by increasing N, it also needs to keep balance between N:P. If consumers are large animals, phytoplankton is P-limited. When consumers are small animals, phytoplankton is N-limited. N:P requirements in zooplankton are lower compared to algae due to the higher ratio of N recycling, but it can also vary among different ecosystems (James J. Elser et al. 1988).

Here in this example, we understand large animals as organisms which invested more to their body size and body structures, so they have higher content of nitrogen and carbon and also need more phosphorus for building proteins (rRNA); they often follow K-strategy. On the other hand, we understand small animals as organisms, which are often R-strategists and have a large number of individuals. They need phosphorus for the body building machinery, but they also recycle more nitrogen. This is probably the reason why phytoplankton, grazed by these small animals, is N limited.

Interactions between nutrient limitation and populations of organisms are quite complex and may vary across different habitats (Elser et al. 1988).

Although terrestrial and freshwater habitats seem to be different, there are some aspects we can observe in both (Elser et al. 2000). From the figure behind, it is evident, that if there is a high content of P in the freshwater habitat, there is also a stronger gross growth efficiency compared to terrestrial environment, where the growth declines with lower content of N. That suggests a limitation of these systems by P in freshwater and N in terrestrial. Yet it must be noted that there were only two studied species and parameters in glaciers can differ in many aspects. C:P and N:P ratios are different, N:P ratios are almost similar in both.

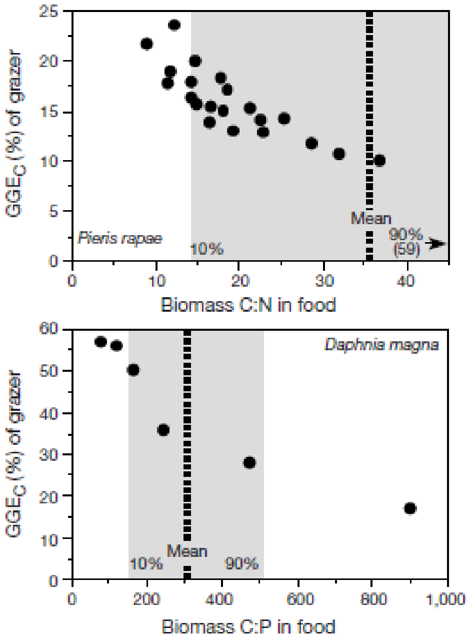

Fig. 16. Gross growth efficiency compared to a biomass C, N and P (James J. Elser et al. 2000)

6.2 Cryoconite granules, bacteria and algae in cryoconite

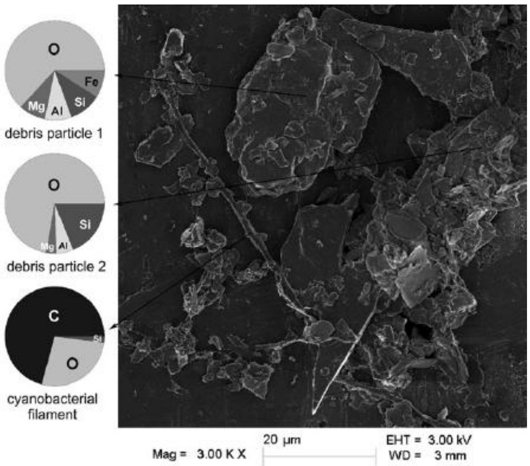

Fig. 17. Cryoconite granules under the scanning electron microscope (Marek Stibal, Tranter, Telling, et al. 2008)

Because cryoconite granules are not only inorganic material, they form a place where microorganisms can survive or live (Edwards et al. 2014; Langford et al. 2010). These organisms (bacteria, algae) play an important role in nutrient cycling (Cameron, Hodson and Osborn 2012) . Components of inorganic materials in a cryoconite can vary between glaciers. Variations are caused by different metabolic pathways and different structures of the communities of organisms inside holes (Edwards et al. 2011). Granules are formed primarily from dust found on the glacier surface and then they are associated with mineral particles, organic matter, bacteria and algae (Takeuchi et al. 2005b; Takeuchi et al. 2005a).

Although cryoconite granules are crucial for retention of nutrients in cryoconite holes, as it was mentioned before, supply of nutrients from the hyporheic zone and aeolian input are also very important.

Due to the structure of cryoconite granules, it is possible to observe something, what we could call the nutrients´ micro zones. These micro zones could be places were nutrients are accumulated. Micro zones are full of algae and bacteria and are easily observed by electron microscopy. Due to high content of chlorophyll in algae, micro zones are also evident under fluorescence (Takeuchi, Kohshima, and Seko 2001). The higher presence of chlorophyll is also a good marker of the bigger abundance of organisms in the environment (Schunemann and Werner 2005).

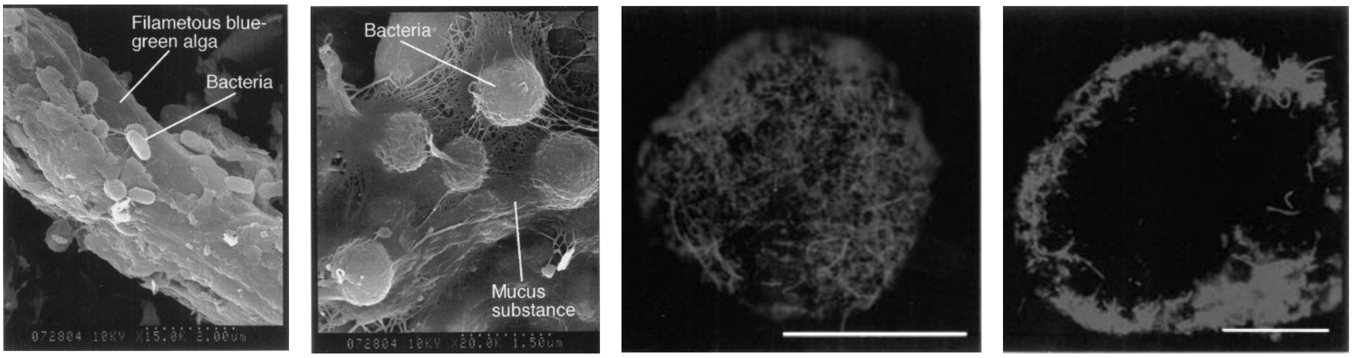

Fig. 18. Structure and autoflurescence of a algea in cryoconite granules (Takeuchi, Kohshima, and Seko 2001)

We need to expect that these surface micro zones are not only places where organisms can live. Inside cryoconite granules, nutrients are also accumulated and there is a big possibility of presence of life forms adapted to life in anoxic environment.

Among bacteria found in cryoconite, there are *Gammaproteobacteria*, *Firmicutes*, *Cyanobacteria* and *Proteobacteria* (Edwards et al. 2014). Abundance of Cyanobacteria was observed more than abundance of the microalgae. The main cyanobacterial species were *Phormidium, Nostoc* and *Leptolyngbya.* Species from groups *Chlorophyceae and Tribophyceae* were observed less often (Stibal, Šabacká, and Kaštovská 2006).

Nostocales and *Leptolyngbya* are also extremely adaptable, although they are not glacial specialists (Stibal, Šabacká, and Kaštovská 2006). On the ice, abundances are contrary to those in cryoconite.

6.3 Metazoa

Years of observations show that cryoconite is a place with diversity of metazoa. There are organisms as small invertebrates, insect or *Nematoda* (Zawierucha et al. 2015; Convey 2011).

Inside the polar habitats, palaeorefugia are created. In these places are favorable conditions for survival and they are therefore searched by very diverse species of animals (Pugh and Convey 2008; Christner, Kvitko II, and Reeve 2003). Also there is a big amount of endemic species, whose development was supported by isolation, different life cycles, nutrient limitation, different competitive pressures and several extinctions during glacial maximums. Their stabilization in Antarctica takes place from the Last Glacial Maximum (Convey and Stevens 2007).

Due to the isolation and extreme weather conditions, organisms have number of speciation. Also there were developed many adaptations including for example freezing protection (Vincent 2000). This protection forms antifreeze proteins, changes membrane proteins and influences the synthesis of carbohydrates, which can help organisms survive in low temperatures (Yeh and Feeney 1996; Alberts et al. 1995).

If we want to find information about polar water invertebrates, we need to be careful about their living habitat. It is because in marine habitats invertebrates are not limited by phosphorus like are invertebrates found on the glacier. Therefore, I expect that marine invertebrates will probably have higher content of P in their bodies (more RNA).

One of the aspects important for understanding stoichiometry of invertebrates is that biota of cryoconite is able to process N and P, so the contents of these nutrients and N:P ratio can vary between cryoconite and surface of the glacier. N:P ratio also increases with higher tropic level. It means that organisms low in the trophic interactions are often fast growing individuals with high P content. If we follow the trophic cascade, we can observe that organisms are often bigger, have longer life and hence also higher N content (Clarke 2008; Barrett et al. 2007). The other specific aspect in cryoconite is a great supply of oxygen. This aspect also changes stoichiometry, because mitochondrial activity in the presence of oxygen increases and thus increases also ATP and content of P in the body of an organism.

Due to the sensitivity to climate, cooling influences the abundance of primary producers (Doran et al. 2002). During the warming air temperature, glacial melt and ablation zone extend but temperature of the glacier is stable. This necessitated that growth rate and phosphorus content need to be calculated differently than in the temperate habitats. In the temperate habitats are fluctuations during the day and therefore ablation and process of ablation zone works different. Not only primary producers, but also animals show changes. These who live in low temperatures have low growth rate, which is combined with high RNA and P content (study was done on six species of Antarctic marine bentos) (Clarke 2008). But in this claim, there is not a complete consensus with Sterner and Elser, who mention cases where arctic species have higher body P and high growth rate due to maximizing use of the short reproductive season (Sterner and Elser 2002). This controversial information, which differs between studies, is one of the things which we would like to clarify with finding the exact stoichiometry of Metazoa inside a cryoconite holes.

6.3.1 Tardigrada

Tardigrada are small invertebrates mainly known for their living habitat. They can live in the moss, but also in very extreme conditions (Smrž 2015). The ability of survival in such extreme conditions is in Tardigrada permitted by cryptobiosis. This survival strategy is called anhydrobiosis. The ability to complete drying (which is necessary for successful anhydrobiosis) varies between different species and depends on environmental conditions (Guidetti et al. 2011). The mechanism of anhydrobiosis is unique due to the loss of intracellular and extracellular water and the change of body composition. In Tardigrada, it is mostly due to proteins, which belong to LEA family (late abundant embryogenesis). These proteins help to develop tolerance to water stress inside their bodies (Schokraie et al. 2010). The process of drying is physiologically very similar to the process of freezing.

Another survival strategy of Tardigrada is their protection of offspring as long as possible. The newborn individuals live in exuviae of mother and are protected from surrounding environment. Therefore, not all individuals of these taxa have the same survivability. It is because of their body size and energy stocks. These parameters can vary between species (Bertolani et al. 2004). In spite of this, distribution of adaptations of many species is limited (Pilato et al. 2006).

Tardigrada mostly consume algae, so their feeding apparatus must be resistant to mechanical stress. The resistance is caused by the content of calcium carbonate and chitin. Content of minerals in bodies of Tardigrada can vary between species (Guidetti et al. 2015). Tardigrada consume mostly moss and they are capable of acquiring carotenoids to their body structures. Carotenoids are used as a protection against the UV light (Bonifacio et al. 2012).

Vegetative season in the polar habitat is short, so a cryoconite holes are not covered by snow or ice only for 2 or 3 months. Short season usually forces sexual strategies such as the parthenogenetic reproduction (Altiero et al. 2015).

Oviposition (laying eggs) of Tardigrada can vary in time with the temperature of surrounding environment. Their development also depends on surrounding environment and conditions in living habitat (Tsujimoto, Suzuki, and Imura 2015).

6.3.2 Rotifera

In polar aqueous habitats, Rotifera are a relatively numerous taxon (Tang et al. 2011). Their variability is high among different places, which is probably due to their ability to inhabit various places with different types of soil. These places are polar deserts, tundra, nunataks and many others (Kaya, De Smet, and Fontaneto 2010). Predominant components of their food are bacteria (Pace and Orcutt 1981).

Rotifera are divided into two main groups. The first group is formed by Bdelloidea Rotifera with only sexual reproduction. The second is formed by Monogonea Rotifera with cyclical parthenogenesis. Within these groups, most of the major phylogenetic branches have different sexual strategies (King et al. 2005). These Metazoa have mechanisms to interrupt diapause and start to breed, when there are favorable conditions. They produce mictic or amictic females. Mictic females have haploid eggs and amictic females have diploid eggs. Strategy with the diploid eggs is called parthenogenesis (King and Serra 1998).

Rotifera have two main strategies to survive: the possibility of starvation (by consuming their own yolk granules and lipid inclusions) and anhydrobiosis. During these two processes, rotifera are able to change the number of organelles in the cells of their bodies including mitochondria or size of the ER (Marotta et al. 2012). They are able to take energy from degradation of mitochondria and this mechanism leads us to predict the changes in their stoichiometry, too.

Rotifera can enter anhydrobiosis more than once. They do not synthetize threhalose (protective disaccharide) and more anhydrobiosises do not cost more energy (Ricci and Caprioli 2005; Teramoto, Sachinvala, and Shibata 2008). The Rotifera body is mainly composed of proteins and carbohydrates. Lipid content is low (Barrett et al. 2007).

7 Results

The aim of this work was to make a general view into the stoichiometric patterns in a glacier ecosystem and find data about stoichiometry of Tardigrada and Rotifera. The main differences between these two groups of animals are in their body composition and biochemical changes during starvation or anhydrobiosis (Wang et al. 2014; Marotta et al. 2012). Also their growth rate compared to the content of RNA changes (Hessen et al. 2007; Kostopoulou, Miliou, and Verriopoulos 2015), but in this case we found data only for Rotifera. The changes in the content of RNA in Rotifera are probably connected to the change of their total body stoichiometry during anhydrobiosis. For the creating of the figure we use data from the paper made by Hessen (2007) and the original figure is from the book Ecological Stoichiometry, which is written by Sterner and Elser (2002). The data from the paper were [6.4;0.6], [7;0.7], [7.6;0.4]. Then we used the logarithm and charged it into the graph from the Sterner and Elser.

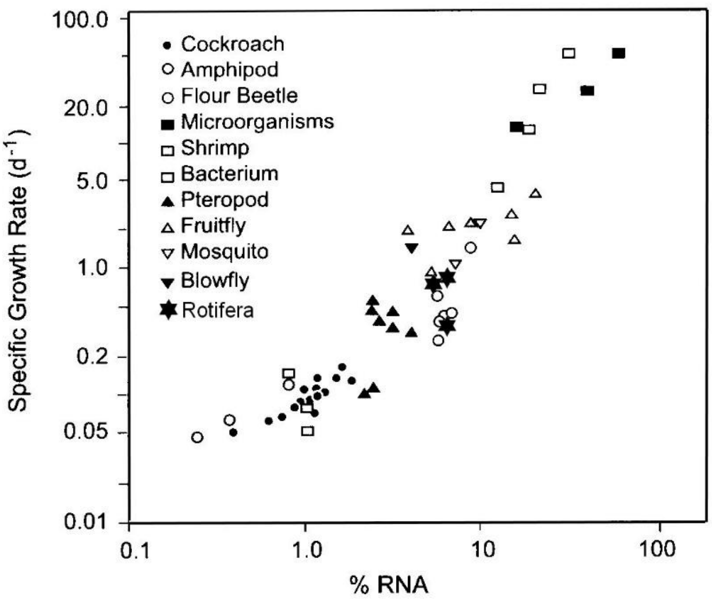

Fig. 20. Location of Rotifera in the animal kingdom according to (Robert W. Sterner and James J. Elser 2002; Hessen et al. 2007)

We consider that Rotifera has balanced RNA content compared to specific growth rate and are very close to Flour Beetles and Pteropoda. The important fact about Tardigrada is, that their structure of tRNA and genome size differs between species (Jørgensen et al. 2010; Dandekar et al. 2012).

8 Conclusions

This thesis should be used as a summary of the stoichiometric patterns inside glacier aquatic systems and adjacent areas. I chose the way through the stoichiometry and patterns of aquatic glacier systems in the whole amount with the use of papers from Svalbard, Greenland, Canada, Arctic, Antarctic and other mountain lakes and glaciers. It is necessary to emphasize that this information is useful only in some cases. The environment across these locations differs and also patterns, which influence local biota, can vary. The papers from Antarctica are mainly from Victoria Land and adjacent areas (Transantarctic Mountains etc.) and research work was done in summer seasons with average temperatures around -20 °C. Studied sites in papers from Northern hemisphere have much more places of interest and also wider seasonal spectrum (summer average temperatures are around 10 °C). I collected knowledge from both sites of the Earth, but it is possible that patterns, mainly mean annual temperature, inside them are different and it is a big question how much is possible to put them into one cap. Another aspect which is necessary to emphasize are nutrient sources in the glacier ecosystem. Very important is allochthonous inputs of carbon from adjacent areas, because carbon is the main element involved in energy metabolism of organisms. On the other hand, it is nitrogen, which has its main source through rain or snow falling into the glacier surface. Phosphorus is probably a limiting nutrient, because it can be only released from solid material, which can be melted out of the glacier or brought from adjacent areas. But all sources differ across different glaciers. Some adjacent areas of the glacier are formed by apatite and debris from them is rich in phosphorus. Some glaciers are near the sea and guano from bird colonies is an important supply of nitrogen and phosphorus. Another glaciers can be in valley with strong wind currents which bring to them debris with nutrients and some not. These sources form the main environment for the life of organisms and their knowledge helps us to understand the main pathways of the system interactions.

Glacier invertebrates are a group with a great diversity and many adaptations. There are similarities with terrestrial species as well as substantial differences due to the presence of extreme conditions. For the preparation of the foreseen research, it was necessary to mention the biochemical and molecular aspect of their body composition. As one of the most important information I consider the change in elemental composition of Rotifera during starvation and anhydrobiosis. They are able to perform the mitophagy (degradation of mitochondria) and it leads us to predict the decrease in phosphorus and nitrogen content. This fact is very important for future analyses, because stoichiometry of Rotifera fluctuates and it will be necessary to take it into consideration. However, their specific growth rate and content of RNA is not specific in the animal kingdom. Also there is a possibility that Tardigrada will have a similar mechanism of changing their intracellular composition, even though the process to get into anhydrobiosis is different.

Focusing on stoichiometry of different groups of Tardigrada and Rotifera living on the glacial surface should enable us to delimitate mechanisms which can influence the dominance of different groups in different glaciers and can have a broad impact on nutrient acquisition and nutrient processing in the glacier ecosystem.

References

Aber J S 1988. “The Glaciation of Kansas: Heritage of the Great Plains.” v. XXI 4: 36.

Adhikary S, M Nakawo, K Seko, and B Shakya. 2000. “Dust Influence on the Melting Process of Glacier Ice: Experimental Results from Lirung Glacier, Nepal Himalayas.” *IAHS Publication*, 43–52.

Alberts B, D Bray, J Lewis, M R, K Roberts, J D Watson, and AV Grimstone. 1995. “Molecular Biology of the Cell (3rd Edn).” *Trends in Biochemical Sciences* 20 (5): 210–210.

Allison L E, W B Bollen, and C D Moodie. 1965. “Total Carbon.” *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, no. methodsofsoilanb: 1346–66.

Altiero T, I Giovannini, R Guidetti, and L Rebecchi. 2015. “Life History Traits and Reproductive Mode of the Tardigrade Acutuncus Antarcticus under Laboratory Conditions: Strategies to Colonize the Antarctic Environment.” *Hydrobiologia* 761 (1): 277–91.

Anesio A M, B Sattler, Ch Foreman, J Telling, A Hodson, M Tranter, and R Psenner. 2010. “Carbon Fluxes through Bacterial Communities on Glacier Surfaces.” *Annals of Glaciology* 51 (56): 32–40.

Barrett J E, R A Virginia, W B Lyons, D M McKnight, J C Priscu, P T Doran, A G Fountain, D H Wall, and D L Moorhead. 2007. “Biogeochemical Stoichiometry of Antarctic Dry Valley Ecosystems.” *Journal of Geophysical Research* 112 (G1).

Becker W M, L J Kleinsmith, J Hardin, and J Raasch. 2003. *The World of the Cell*. Vol. 6. Benjamin Cummings San Francisco.

Beilke A J, and J G Bockheim. 2013. “Carbon and Nitrogen Trends in Soil Chronosequences of the Transantarctic Mountains.” *Geoderma* 197–198 (April): 117–25.

Berendt S, J Lehner, Y V Zhang, T M Rasse, K Forchhammer, and I Maldener. 2012. “Cell Wall Amidase AmiC1 Is Required for Cellular Communication and Heterocyst Development in the Cyanobacterium Anabaena PCC 7120 but Not for Filament Integrity.” *Journal of Bacteriology* 194 (19): 5218–27.

Berry Lyons W, K A Welch, A G Fountain, G L Dana, B H Vaughn, and D M McKnight. 2003. “Surface Glaciochemistry of Taylor Valley, Southern Victoria Land, Antarctica and Its Relationship to Stream Chemistry.” *Hydrological Processes* 17 (1): 115–30.

Bertolani R, R Guidetti, I K Jonsson, T Altiero, D Boschini, and L Rebecchi. 2004. “Experiences with Dormancy in Tardigrades.” *Journal of Limnology* 63 (1s): 16–25.

Björkman M P, R Kühnel, D G Partridge, T J Roberts, W As, M Mazzola, A Viola, A Hodson, J Ström, and E Isaksson. 2013. “Nitrate Dry Deposition in Svalbard.” *Tellus B* 65 (0).

Blecker S W, J A Ippolito, J E Barrett, D H Wall, R A Virginia, and K L Norvell. 2006. “Phosphorus Fractions in Soils of Taylor Valley, Antarctica.” *Soil Science Society of America Journal* 70 (3): 806.

Bonifacio A, R Guidetti, T Altiero, V Sergo, and L Rebecchi. 2012. “Nature, Source and Function of Pigments in Tardigrades: In Vivo Raman Imaging of Carotenoids in Echiniscus Blumi.” Edited by Jochen Zeil. *PLoS ONE* 7 (11): e50162.

Boros G, and A Mozsár. 2015. “Comparison of Different Methods Used for Phosphorus Determination in Aquatic Organisms.” *Hydrobiologia* 758 (1): 235–42.

Bowen H J M. 1979. *Environmental Chemistry of the Elements.* Academic Press.

Bowman M F, P A Chambers, and D W Schindler. 2005. “Changes in Stoichiometric Constraints on Epilithon and Benthic Macroinvertebrates in Response to Slight Nutrient Enrichment of Mountain Rivers.” *Freshwater Biology* 50 (11): 1836–52.

Bridgham S D, K Updegraff, and J Pastor. 1998. “Carbon, Nitrogen, and Phosphorus Mineralization in Northern Wetlands.” *Ecology* 79 (5): 1545–61.

Brönmark Ch, and L A Hansson. 2005. *The Biology of Lakes and Ponds*. Oxford University Press.

Burpee B, J E Saros, R M Northington, and K S Simon. 2016. “Microbial Nutrient Limitation in Arctic Lakes in a Permafrost Landscape of Southwest Greenland.” *Biogeosciences* 13 (2): 365–74.

Cameron K A, A J Hodson, and A M Osborn. 2012. “Carbon and Nitrogen Biogeochemical Cycling Potentials of Supraglacial Cryoconite Communities.” *Polar Biology* 35 (9): 1375–93.

Cao M, and F I Woodward. 1998. “Net Primary and Ecosystem Production and Carbon Stocks of Terrestrial Ecosystems and Their Responses to Climate Change.” *Global Change Biology* 4 (2): 185–98.

Caputo L, G Alfonso, and A Givovich. 2013. “Limnological Features of Laguna Teno (35° S, Chile): A High Altitude Lake Impacted by Volcanic Activity.” *Fundamental and Applied Limnology / Archiv Für Hydrobiologie* 183 (4): 323–35.

Christner B C, B H Kvitko II, and J N Reeve. 2003. “Molecular Identification of Bacteria and Eukarya Inhabiting an Antarctic Cryoconite Hole.” *Extremophiles* 7 (3): 177–183.

Clarke A. 2008. “Ecological Stoichiometry in Six Species of Antarctic Marine Benthos.” *Marine Ecology Progress Series*.

Convey P. 2011. “Antarctic Terrestrial Biodiversity in a Changing World.” *Polar Biology* 34 (11): 1629–41.

Cook J, A Hodson, J Telling, A Anesio, T Irvine-Fynn, and C Bellas. 2010. “The Mass–area Relationship within Cryoconite Holes and Its Implications for Primary Production.” *Annals of Glaciology* 51 (56): 106–110.

Da Silva J, J R Frausto, and R J P Williams. 2001. *The Biological Chemistry of the Elements: The Inorganic Chemistry of Life*. Oxford University Press.

Daines S J, J R Clark, and T M Lenton. 2014. “Multiple Environmental Controls on Phytoplankton Growth Strategies Determine Adaptive Responses of the N: P Ratio.” *Ecology Letters* 17 (4): 414–25.

Dandekar T, F Foerster, D Beisser, M A Grohme, Ch Liang, B Mali, A M Siegl, et al. 2012. “Transcriptome Analysis in Tardigrade Species Reveals Specific Molecular Pathways for Stress Adaptations.” *Bioinformatics and Biology Insights*, April, 69.

Dibb J E, M Arsenault, M C Peterson, and R E Honrath. 2002. “Fast Nitrogen Oxide Photochemistry in Summit, Greenland Snow.” *Atmospheric Environment* 36 (15): 2501–11.

Dodson S I. 1975. “Predation Rates of Zooplankton in Arctic Ponds.” *Limnology and Oceanography* 20 (3): 426–433.

Edwards A, A M Anesio, S M Rassner, B Sattler, B Hubbard, W T Perkins, M Young, and G W Griffith. 2011. “Possible Interactions between Bacterial Diversity, Microbial Activity and Supraglacial Hydrology of Cryoconite Holes in Svalbard.” *The ISME Journal* 5 (1): 150–160.

Edwards A, L A J Mur, S E Girdwood, A M Anesio, M Stibal, S M E Rassner, K Hell, et al. 2014. “Coupled Cryoconite Ecosystem Structure-Function Relationships Are Revealed by Comparing Bacterial Communities in Alpine and Arctic Glaciers.” *FEMS Microbiology Ecology* 89 (2): 222–37.

Elser J J, K Acharya, M Kyle, J Cotner, W Makino, T Markow, T Watts, et al. 2003. “Growth Rate-Stoichiometry Couplings in Diverse Biota.” *Ecology Letters* 6 (10): 936–43.

Elser J J, D R Dobberfuhl, N A MacKay, and J H Schampel. 1996. “Organism Size, Life History, and N: P Stoichiometry toward a Unified View of Cellular and Ecosystem Processes.” *BioScience* 46 (9): 674–684.

Elser J J, M M Elser, N A MacKay, and S R Carpenter. 1988. “Zooplankton-Mediated Transitions between N-and P-Limited Algal Growth.” *Á Limnol. Oceanography* 33.

Elser J J, W F Fagan, R F Denno, D R Dobberfuhl, A Folarin, A Huberty, S Interlandi, et al. 2000. “Nutritional Constraints in Terrestrial and Freshwater Food Webs.” *Nature* 408 (6812): 578–80.

Emiliani C. 1978. “The Cause of the Ice Ages.” *Earth and Planetary Science Letters* 37 (3): 349–54.

Foreman Ch M, C F Wolf, and J C Priscu. 2004. “Impact of Episodic Warming Events.” *Aquatic Geochemistry* 10 (3–4): 239–268.

Fountain A G, M Tranter, T H Nylen, K J Lewis, and D R Mueller. 2004. “Evolution of Cryoconite Holes and Their Contribution to Meltwater Runoff from Glaciers in the McMurdo Dry Valleys, Antarctica.” *Journal of Glaciology* 50 (168): 35–45.

Gerdel R W, and F Drouet. 1960. “The Cryoconite of the Thule Area, Greenland.” *Transactions of the American Microscopical Society* 79 (3): 256–72.

Gibbard P, and T van Kolfschoten. 2004. *The Pleistocene and Holocene Epochs*. na.

Gooseff M N, D M McKnight, W Berry Lyons, and A E Blum. 2002. “Weathering Reactions and Hyporheic Exchange Controls on Stream Water Chemistry in a Glacial Meltwater Stream in the McMurdo Dry Valleys: Dry Valley Stream Hyporheic Zone Weathering.” *Water Resources Research* 38 (12): 15-1-15–17.

Gooseff M N, D M McKnight, R L Runkel, and J H Duff. 2004. “Denitrification and Hydrologic Transient Storage in a Glacial Meltwater Stream, McMurdo Dry Valleys, Antarctica.” *Limnology and Oceanography* 49 (5): 1884–1895.

Guidetti R, T Altiero, R Bertolani, P Grazioso, and L Rebecchi. 2011. “Survival of Freezing by Hydrated Tardigrades Inhabiting Terrestrial and Freshwater Habitats.” *Zoology* 114 (2): 123–28.

Guidetti R, A Bonifacio, T Altiero, R Bertolani, and L Rebecchi. 2015. “Distribution of Calcium and Chitin in the Tardigrade Feeding Apparatus in Relation to Its Function and Morphology.” *Integrative and Comparative Biology* 55 (2): 241–52.

Hartman P, E Štědronský, and I Přikryl. 1998. *Hydrobiologie*. Informatorium.

Hessen D O, T C Jensen, M Kyle, and J J Elser. 2007. “RNA Responses to N- and P-Limitation; Reciprocal Regulation of Stoichiometry and Growth Rate in Brachionus.” *Functional Ecology* 21 (5): 956–62.

Hobbie J E, B J Peterson, N Bettez, L Deegan, W J O’Brien, G W Kling, G W Kipphut, W B Bowden, and A E Hershey. 1999. “Impact of Global Change on the Biogeochemistry and Ecology of an Arctic Freshwater System.” *Polar Research* 18 (2): 207–214.

Hoffman P F, A J Kaufman, G P Halverson, and D P Schrag. 1998. “A Neoproterozoic Snowball Earth.” *Science* 281 (5381): 1342–46.

Holmstrup M, M Bayley, and H Ramløv. 2002. “Supercool or Dehydrate? An Experimental Analysis of Overwintering Strategies in Small Permeable Arctic Invertebrates.” *Proceedings of the National Academy of Sciences* 99 (8): 5716–5720.

Jørgensen A, S Faurby, J G Hansen, N Møbjerg, and R M Kristensen. 2010. “Molecular Phylogeny of Arthrotardigrada (Tardigrada).” *Molecular Phylogenetics and Evolution* 54 (3): 1006–15.

Kalff J. 2002. *Limnology: Inland Water Ecosystems*. Vol. 592. Prentice Hall New Jersey.

Karl D M. 2000. “Aquatic Ecology: Phosphorus, the Staff of Life.” *Nature* 406 (6791): 31–33.

Kaya M, W H De Smet, and D Fontaneto. 2010. “Survey of Moss-Dwelling Bdelloid Rotifers from Middle Arctic Spitsbergen (Svalbard).” *Polar Biology* 33 (6): 833–42.

King Ch E, C Ricci, J Schonfeld, and M Serra. 2005. “Evolutionary Dynamics of ‘the’ Bdelloid and Monogonont Rotifer Life-History Patterns.” *Hydrobiologia* 546 (1): 55–70.

King Ch E, and M Serra. 1998. “Seasonal Variation as a Determinant of Population Structure in Rotifers Reproducing by Cyclical Parthenogenesis.” *Hydrobiologia* 387: 361–372.

Knowles R. 1982. “Denitrification.” *Microbiological Reviews* 46 (1): 43.

Kohler T J, D J Van Horn, J P Darling, C D Takacs-Vesbach, and D M McKnight. 2016. “Nutrient Treatments Alter Microbial Mat Colonization in Two Glacial Meltwater Streams from the McMurdo Dry Valleys, Antarctica.” Edited by Dirk Wagner. *FEMS Microbiology Ecology* 92 (4): fiw049.

Kopáček J, E Stuchlík, V Straškrabová, P Pšenáková, and others. 2000. “Factors Governing Nutrient Status of Mountain Lakes in the Tatra Mountains.” *Freshwater Biology* 43 (3): 369–383.

Kostopoulou V, H Miliou, and G Verriopoulos. 2015. “Biochemical Composition and Fatty Acid Profile in a Strain of the Lineage ‘Nevada’, Belonging to the *Brachionus Plicatilis* (Rotifera) Complex, Fed Different Diets.” *Aquaculture Research* 46 (4): 813–24.

Langford H, A Hodson, S Banwart, and C Bøggild. 2010. “The Microstructure and Biogeochemistry of Arctic Cryoconite Granules.” *Annals of Glaciology* 51 (56): 87–94.

Langmuir Ch H, and W S Broecker. 2012. *How to Build a Habitable Planet: The Story of Earth from the Big Bang to Humankind*. Princeton University Press.

Lawson J, P T Doran, F Kenig, J C Priscu, and others. 2004. “Stable Carbon and Nitrogen Isotopic.” *Aquatic Geochemistry* 10 (3–4): 269–301.

Lipps J H, and P W Signor. 2013. *Origin and Early Evolution of the Metazoa*. Vol. 10. Springer Science & Business Media.

Luoto T, and L Nevalainen. 2013. “Climate-Driven Limnological Changes Determine Ecological Thresholds in an Alpine Lake.” *Aquatic Biology* 18 (1): 47–58.

Lyle S A. 2009. “Glaciers in Kansas.” *Kansas Geological Survey* 2009.
Markager S, W F Vincent, and E P Y Tang. 1999. “Carbon Fixation by Phytoplankton in High Arctic Lakes: Implications of Low Temperature for Photosynthesis.” *Limnology and Oceanography* 44 (3): 597–607.
Marotta R, A Uggetti, C Ricci, F Leasi, and G Melone. 2012. “Surviving Starvation: Changes Accompanying Starvation Tolerance in a Bdelloid Rotifer.” *Journal of Morphology* 273 (1): 1–7.
Matouš B. n.d. “Základy Lékařské Chemie a Biochemie. 1. Vyd. Praha: Galén, 2010, 540 S.” ISBN 978-807-2627-028.
McKnight D M, D K Niyogi, A S Alger, A Bomblies, P A Conovitz, and C M Tate. 1999. “Dry Valley Streams in Antarctica: Ecosystems Waiting for Water.” *Bioscience* 49 (12): 985–995.
Merzendorfer H 2003. “Chitin Metabolism in Insects: Structure, Function and Regulation of Chitin Synthases and Chitinases.” *Journal of Experimental Biology* 206 (24): 4393–4412.
Walvoord M A, M F Phillips, D A Stonestrom, D R Evans, P C Hartsough, B D Newman, and R G Strieg. 2003. “A Reservoir of Nitrate Beneath Desert Soils.” *Science* 302 (5647): 1018–1021.
Moorhead D L, and J F Reynolds. 1991. “A General Model of Litter Decomposition in the Northern Chihuahuan Desert.” *Ecological Modelling* 56 (January): 197–219.
Morales-Baquero R, C Presentacion, J Barea-Arco, C Perez-Martinez, and M Villar-Argaiz. 2006. “Climate-Driven Changes on Phytoplankton-Zooplankton Coupling and Nutrient Availability in High Mountain Lakes of Southern Europe.” *Freshwater Biology* 51 (6): 989–98.
Oparin A I. 1938. *The Origin of Life*. Courier Corporation.
Pace M L, and J D Orcutt. 1981. “The Relative Importance of Protozoans, Rotifers, and Crustaceans in a Freshwater Zooplankton Community.” *Limnology and Oceanography* 26 (5): 822–830.
Convey P, and M I Stevens. 2007. “Antarctic Biodiversity.” *Science* 317 (5846): 1877–79.
Doran P T, J C Priscu, L W Berry, J E Walsh, A G Fountain, D M McKnight, D Moorhead, et al. 2002. “Antarctic Climate Cooling and Terrestrial Ecosystem Response.” *Nature* 415 (6871): 514–517.
Pilato G, R Guidetti, L Rebecchi, O Lisi, J G Hansen, and R Bertolani. 2006. “Geonemy, Ecology, Reproductive Biology and Morphology of the Tardigrade Hypsibius Zetlandicus (Eutardigrada: Hypsibiidae) with Erection of Borealibius Gen. N.” *Polar Biology* 29 (7): 595–603.
Porazinska D L, A G Fountain, T H Nylen, M Tranter, R A Virginia, and D H Wall. 2004. “The Biodiversity and Biogeochemistry of Cryoconite Holes from McMurdo Dry Valley Glaciers, Antarctica.” *Arctic, Antarctic, and Alpine Research* 36 (1): 84–91.
Pugh P J A, and P Convey. 2008. “Surviving out in the Cold: Antarctic Endemic Invertebrates and Their Refugia.” *Journal of Biogeography* 35 (12): 2176–86.
Ramakrishna R, J S Edwards, A McCulloch, and B O Palsson. 2001. “Flux-Balance Analysis of Mitochondrial Energy Metabolism: Consequences of Systemic Stoichiometric Con-

straints." *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 280 (3): R695–704.

Redfield A C 1958. "The Biological Control of Chemical Factors in the Environment." *American Scientist* 46 (3): 230A–221.

Rees D C, F A Tezcan, Ch A Haynes, M Y Walton, S Andrade, O Einsle, and J B Howard. 2005. "Structural Basis of Biological Nitrogen Fixation." *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 363 (1829): 971–84.

Ricci C, and M Caprioli. 2005. "Anhydrobiosis in Bdelloid Species, Populations and Individuals." *Integrative and Comparative Biology* 45 (5): 759–763.

Sterner R W, and J J Elser. 2002. *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Woodstock: Princeton University Press.

Roer R, S Abehsera, and A Sagi. 2015. "Exoskeletons across the Pancrustacea: Comparative Morphology, Physiology, Biochemistry and Genetics." *Integrative and Comparative Biology* 55 (5): 771–91.

Rossi C, Stefano, and A L Lehninger. 1964. "Stoichiometry of Respiratory Stimulation, Accumulation of Ca++ and Phosphate, and Oxidative Phosphorylation in Rat Liver Mitochondria." *Journal of Biological Chemistry* 239 (11): 3971–3980.

Runkel R L, D M McKnight, and E D Andrews. 1998. "Analysis of Transient Storage Subject to Unsteady Flow: Diel Flow Variation in an Antarctic Stream." *Journal of the North American Benthological Society* 17 (2): 143–54.

Sattler B, A Anesio, I Antlinger, and H Lettner. 2007. "Microbial Communities of Cryoconites as Sensitive Indicators for Radioactive Impact on Arctic and Alpine Glaciers-Project Description." *Polarnet Technical Report,* 3.

Säwström Ch, P Mumford, W Marshall, A Hodson, and J Laybourn-Parry. 2002. "The Microbial Communities and Primary Productivity of Cryoconite Holes in an Arctic Glacier (Svalbard 79 N)." *Polar Biology* 25 (8): 591–596.

Schokraie E, A Hotz-Wagenblatt, U Warnken, B Mali, M Frohme, F Förster, T Dandekar, S Hengherr, R O Schill, and M Schnölzer. 2010. "Proteomic Analysis of Tardigrades: Towards a Better Understanding of Molecular Mechanisms by Anhydrobiotic Organisms." Edited by Anna Maria Delprato. *PLoS ONE* 5 (3): e9502.

Schunemann H, and I Werner. 2005. "Seasonal Variations in Distribution Patterns of Sympagic Meiofauna in Arctic Pack Ice." *Marine Biology* 146 (6): 1091–1102.

Sherrod L A, G Dunn, G A Peterson, and R L Kolberg. 2002. "Inorganic Carbon Analysis by Modified Pressure-Calcimeter Method." *Soil Science Society of America Journal* 66 (1): 299.

Sinsabaugh R L, Ch L Lauber, M N Weintraub, B Ahmed, S D Allison, Ch Crenshaw, A R Contosta, et al. 2008. "Stoichiometry of Soil Enzyme Activity at Global Scale." *Ecology Letters,* September.

Smith R L, T M Smith, and M S Thomas. 1998. *Elements of Ecology*.

Smrž J. 2015. *Základy Biologie, Ekologie a Systému Bezobratlých Živočichů*. Charles University in Prague, Karolinum Press.

Sousa T, T Domingos, and S A L M Kooijman. 2008. “From Empirical Patterns to Theory: A Formal Metabolic Theory of Life.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 363 (1502): 2453–64.

Stibal M, A M Anesio, C J D Blues, and M Tranter. 2009. “Phosphatase Activity and Organic Phosphorus Turnover on a High Arctic Glacier.” *Biogeosciences* 6 (5): 913–922.

Stibal M, M Šabacká, and K Kaštovská. 2006. “Microbial Communities on Glacier Surfaces in Svalbard: Impact of Physical and Chemical Properties on Abundance and Structure of Cyanobacteria and Algae.” *Microbial Ecology* 52 (4): 644–54.

Stibal M, M Tranter, L G Benning, and J Ehk. 2008. “Microbial Primary Production on an Arctic Glacier Is Insignificant in Comparison with Allochthonous Organic Carbon Input.” *Environmental Microbiology* 10 (8): 2172–78.

Stibal M, M Tranter, J Telling, and L G Benning. 2008. “Speciation, Phase Association and Potential Bioavailability of Phosphorus on a Svalbard Glacier.” *Biogeochemistry* 90 (1): 1–13.

Takacs C D, J C Priscu, and D M McKnight. 2001. “Bacterial Dissolved Organic Carbon Demand in McMurdo Dry Valley Lakes, Antarctica.” *Limnology and Oceanography* 46 (5): 1189–1194.

Takeuchi N, S Kohshima, and K Seko. 2001. “Structure, Formation, and Darkening Process of Albedo-Reducing Material (Cryoconite) on a Himalayan Glacier: A Granular Algal Mat Growing on the Glacier.” *Arctic, Antarctic, and Alpine Research*, 115–22.

Takeuchi N, Y Matsuda, A Sakai, and K Fujita. 2005a. “A Large Amount of Biogenic Surface Dust (Cryoconite) on a Glacier in the Qilian Mountains, China.” *Bull. Glaciol. Res* 22: 1–8.

Takeuchi N, Y Matsuda, A Sakai, and K Fujita. 2005b. “A Large Amount of Biogenic Surface Dust (Cryoconite) on a Glacier in the Qilian Mountains, China.” *Bull. Glaciol. Res* 22: 1–8.

Tang K, T Nielsen, P Munk, J Mortensen, E Møller, K Arendt, K Tönnesson, and T Juul-Pedersen. 2011. “Metazooplankton Community Structure, Feeding Rate Estimates, and Hydrography in a Meltwater-Influenced Greenlandic Fjord.” *Marine Ecology Progress Series* 434 (July): 77–90.

Telling J, A M Anesio, M Tranter, M Stibal, J Hawkings, T Irvine-Fynn, A Hodson, C Butler, M Yallop, and J Wadham. 2012. “Controls on the Autochthonous Production and Respiration of Organic Matter in Cryoconite Holes on High Arctic Glaciers: Carbon Production on Arctic Glaciers.” *Journal of Geophysical Research: Biogeosciences* 117 (G1): n/a-n/a.

Teramoto N, N D Sachinvala, and M Shibata. 2008. “Trehalose and Trehalose-Based Polymers for Environmentally Benign, Biocompatible and Bioactive Materials.” *Molecules* 13 (8): 1773–1816.

Thomas D N, G E Fogg, P Convey, Ch H Fritsen, J M Gili, R Gradinger, J Laybourn-Parry, K Reid, and D W H Walton. 2008. *The Biology of Polar Regions*. Oxford University Press.

Townsend C R, M Begon, and J L Harper. 2010. *Základy Ekologie*. Univerzita Palackého.

Tranter M, A G Fountain, Ch H Fritsen, W Berry Lyons, J C Priscu, P J Statham, and K A Welch. 2004. “Extreme Hydrochemical Conditions in Natural Microcosms Entombed within Antarctic Ice.” *Hydrological Processes* 18 (2): 379–87.

Tsujimoto M, A C Suzuki, and S Imura. 2015. “Life History of the Antarctic Tardigrade, Acutuncus Antarcticus, under a Constant Laboratory Environment.” *Polar Biology* 38 (10): 1575–81.

Vrede T, D R Dobberfuhl, S Kooijman, and J J Elser. 2004. “Fundamental Connections among Organism C: N: P Stoichiometry, Macromolecular Composition, and Growth.” *Ecology* 85 (5): 1217–1229.

Wang Ch, M A Grohme, B Mali, R O Schill, and M Frohme. 2014. “Towards Decrypting Cryptobiosis—Analyzing Anhydrobiosis in the Tardigrade Milnesium Tardigradum Using Transcriptome Sequencing.” Edited by Cynthia Gibas. *PLoS ONE* 9 (3): e92663.

Weidman P R, D W Schindler, P L Thompson, and R D Vinebrooke. 2014. “Interactive Effects of Higher Temperature and Dissolved Organic Carbon on Planktonic Communities in Fishless Mountain Lakes.” *Freshwater Biology* 59 (5): 889–904.

Yeh Y, and R E Feeney. 1996. “Antifreeze Proteins: Structures and Mechanisms of Function.” *Chemical Reviews* 96 (2): 601–18.

Yoshimura Y. 1993. “Biotic Acceleration of Glacier Melting in Yala Glacier Langtang Region, Nepal Himalaya.”

Zawierucha K, M Kolicka, N Takeuchi, and ł Kaczmarek. 2015. “What Animals Can Live in Cryoconite Holes? A Faunal Review: Cryoconite Holes Fauna.” *Journal of Zoology* 295 (3): 159–69.