

# Stable Nitrogen Isotopes ( $\delta^{15}\text{N}$ ) in Podetias of Lichenized Fungi *Cladonia pocillum* from Different Altitudes of Habitats

Lev G. Biazrov

Institute of Ecology & Evolution, Russian Academy of Sciences, Moscow, Russia  
Email: [lev.biazrov@rambler.ru](mailto:lev.biazrov@rambler.ru)

Received 9 April 2014; revised 15 May 2014; accepted 21 June 2014

Copyright © 2014 by author and OALib.

This work is licensed under the Creative Commons Attribution International License (CC BY).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

---

## Abstract

The  $\delta^{15}\text{N}$  values of stable nitrogen isotopes were determined in samples of organic matter (OM) of podetias of lichen *Cladonia pocillum* (Ach.) Grognot, collected across 10 altitudinal levels within the range of 1550 - 2900 m.a.s.l. of both steppes and highland meadows of the Khangai Mountains (Mongolia). No correlation between the  $\delta^{15}\text{N}$  values of lichen OM and the altitude range was detected at the regional scale. However, there is a positive correlation  $\delta^{15}\text{N}$  values in OM *C. pocillum* with of the nitrogen content in the podetias.

## Keywords

Lichens, *Cladonia pocillum*, Stable Isotopes,  $\delta^{15}\text{N}$ , Nitrogen, Local Scale, Regional Scale, Altitude, Mountain Steppes, Highland Meadows, Khangai Mountains, Mongolia

---

## 1. Introduction

Study of the isotopic composition of nutrients (nitrogen, oxygen, sulfur, carbon, and others) and the fractionation, *i.e.* changes in the ratio of isotopes during the metabolic processes in the last decade is used extensively in studies of animal ecology, fungi, plants [1]-[6]. Nitrogen is one of the main elements of proteins, which are the predominant metabolites of living organisms. This element has two stable isotopes, one of which is  $^{14}\text{N}$  and it makes up the vast majority of nitrogen in the environment (99.636%), whereas the content of the second isotope ( $^{15}\text{N}$ ) is 0.364% [7]. The average ratio of the heavy isotope to the light isotope on the planet is equal to  $3.677 \times 10^{-3}$  [7]. However, some variations of this average ratio can be observed in nature depending on the material and the properties of environmental factors under which this material functions; these variations are monitored and used in ecological and some other investigations. If the nitrogen isotope ratio in the investigated material is less

than the value cited above, it is assumed that the substrate material is enriched in the  $^{14}\text{N}$  isotope, and conversely, the substrate material is considered to be enriched in the  $^{15}\text{N}$  isotope when the isotope ratio is above the global average value [7]. However, these values are so low that in practice the stable isotope ratios of substrates are determined relative to their ratio in the widely accepted standard (in the case of nitrogen, this is atmospheric nitrogen  $\text{N}_2$ ); this value is symbolized as  $\delta^{15}\text{N}$  and it is measured in ‰ [4]. The naturally occurring ratio of the stable isotopes of nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) in organic matter (OM) of individual organisms as well as populations and other organism communities is considered to reflect, on one hand, an integral index of the nitrogen cycle intensity in the investigated objects, and, on the other hand, this ratio is regarded as a marker of nitrogen sources and trophic relationships between organisms [3]-[5] [8].

The majority of studies regarding the detection of the  $^{15}\text{N}/^{14}\text{N}$  ratio in OM were carried out in higher plants [2] [3]. The amount of similar research articles where measurements of  $^{15}\text{N}/^{14}\text{N}$  ratios in OM of algae, cyanobacteria, and lichens were described is relatively low, even though these photoautotrophic organisms are widely represented in both land and water ecosystems. Lichens (lichenized fungi), for example, are quantitatively abundant in organism communities which occupy about 8% of the land surface [9]. They inhabit soil, tree bark, leaves, rocks, bones, and even man-made substrates. In any habitat, lichens are a source of food for many groups of animals [10] [11]; this has been confirmed by direct observations [12] as well as using measurements of the natural stable nitrogen isotope ratio in OM of lichens and animals [13] [14]. Apparently, depending on the species compositions of groups formed by lichens and the mass produced by them, lichenized fungi are somewhat involved in the nitrogen cycle of ecosystems [15].

Lichens are the symbiotic phenotype of nutritionally specialized fungi (mycobionts), ecologically obligate biotrophs which acquire fixed carbon and in some cases nitrogen from minute cells of a population of their alga or cyanobacterium symbionts (photobionts) [16].

The fungi which can form lichens are also called lichenized. They all belong to various taxonomic groups of the fungi kingdom. Their common feature is nutritional specialization; their name—lichens or lichenized fungi—falls into the same category of terms as, for example, mycorrhizal fungi or plant pathogenic fungi. The interaction of all lichen bionts results in the lichen body formation which is otherwise called thallus.

Depending on the type of photobiont, lichens demonstrate different ways of nitrogen production. According to this characteristic, they can be correspondingly divided into at least two different groups [15]. The first group consists of chlorolichens containing two bionts (about 85% of the total number of all known lichen species), the primary photobiont of which is usually represented by green algae, mainly by representatives of *Trebouxia* species. As other eukaryotic organisms, they are not capable of the direct assimilation of atmospheric molecular nitrogen. Their nitrogen supply is fully determined by the inflow of inorganic (nitrate, ammonia  $\text{NH}_3$ , and ammonium ions) as well as organic nitrogen compounds (amino acids, ergosterol, chitin, and others) on the surface of lichen thalli in the form of both solid and liquid depositions. This explains low nitrogen concentration in thalli of chlorolichens; this value is on average  $10\ \mu\text{g/g}$  of dry mass [17].

The second group is represented by cyanolichens consisting of two bionts (about 10% of the total number of all known lichen species), which contain cyanobacterium, often a representative of *Nostoc* species, as the primary photobiont. Similar to independently living cyanobacteria, these photobionts are able to fix atmospheric  $\text{N}_2$ , thus providing an additional nitrogen intake in lichen thalli. Additionally, cephaloidal lichens containing three bionts (3% - 4% of all existing species of lichens) can also be classified as lichens corresponding to the same group; these lichens contain green algae of *Coccomyxa*, *Trentepohlia*, and *Dictyochloropsis* species as the primary photobiont, whereas their third biont is represented by nitrogen-fixing bacteria that are located in specific formations called cephalodia. Generally the lichens of this group are characterized by high concentrations of thallus nitrogen; this value is on average 34 and  $24\ \mu\text{g/g}$  of dry mass in the case of lichens containing two and three bionts, respectively [17]. It should also be noted that, in the case of lichens containing cyanobacterium symbionts, the fixation of molecular nitrogen is just an additional source of this element intake, whereas the inflow of compounds containing nitrogen on the thallus surface in the form of solid and liquid depositions remains the main source of nitrogen supply [17].

The  $\delta^{15}\text{N}$  values of various lichens were determined to vary from  $-21.5\text{‰}$  [18] to  $+18\text{‰}$  [19]; the highest values were found in thalli collected from rocks near bird colonies of Antarctica. The highest values of the total  $\text{NO}_3^-$ ,  $\text{NO}_4^+$  nitrogen content in lichen thalli were also detected in the same regions.

Lichens belong to the group of poikilohydric organisms; this means that they contain no structures responsible for the water cycle regulation of their thalli with the environment [20]. In dry conditions, they become cryptic,

as there are no metabolic processes in their thalli. Chlorolichens are capable of photosynthesis in the case of a high percentage of water vapor in the air, that is, in the early morning after sunrise and later in the evening just before sunset. Cyanolichens, on the other hand, require their thalli to be saturated with liquid water to perform photosynthesis [21].

It should be noted that there are many lichen species which occupy spacious natural habitats. For example, more than a quarter of 400 species living in Antarctica dwell also in the Northern Hemisphere [22]. Moreover, similar species can reside in different organism communities. This fact together with their slow growth and belonging to poikilohydric organisms makes lichens a very convenient object for studying of the dependence of various nitrogen metabolism processes at a local, regional, and global scale; mainly, this is related to the fact that OM of lichens integrates the influence of different ecological factors in their specific microhabitat over a long period of time.

One of the environmental factors which influence the whole complex of features of organism habitats is the altitude of a region since this characteristic affects dramatically the air temperature and the temperature of a substrate, the amount of atmospheric precipitation, the air pressure, the solar spectrum, and other parameters. There are many lichen species which dwell within a wide range of altitudes [23], so that the use of such species for measurements of the  $^{15}\text{N}/^{14}\text{N}$  ratio in OM allows one to obtain useful data about variations in metabolic processes depending on environmental conditions on the species level. The aim of this work was to reveal the dependence of the naturally occurring stable nitrogen isotope ratio in lichen OM on its habitat altitude at both local and regional scales.

## 2. Materials and Methods

The  $^{14}\text{N}/^{15}\text{N}$  ratio was measured in specimens of the lichen *Cladonia pocillums* (Ach.) Grognot from the herbarium of lichens kept at the Laboratory for Radioecological Monitoring of Nuclear Power Plant Regions and Bioindication, Severtsov Institute of Ecology and Evolution Russian Academy of Sciences. The lichens were collected by this author on the Khangai Mountains, Mongolia, in the course of research performed by the Joint Soviet-Mongolian Biological Expedition (table). The collection material has been repeatedly used to measure the ratios of stable isotopes in lichen thalli [24]-[26].

The Mountains are located in the central part of western Mongolia. The western and eastern edges of the Khangai are located near 92°E and 106°E, respectively; the southern edge is situated slightly to the south of 46°N, whereas the northern edge is located north of 50°N. Overall, the Khangai is considered to be a typical medium-high mountain country containing a combination of mountain ridges within a height range of 2000 - 3500 m together with intermountain valleys of different width, the altitude of which is more than 1000 m above sea level [27]. The maximum elevation of the main mountain ridge of the Khangai, which is a part of the global water divide, is located in the Otgon Khaikhan Nuru mountain range, the highest peak of which is called Otgon-tenger (3905 m.a.s.l.) and a deep layer of snow has been preserved there in summer for many decades.

The location of the Khangai Mountains in the center of the powerful Asian anticyclone results in the extreme continental climate of the region. The difference in average temperatures between January and July reaches 32°C - 43°C and the change in temperature from day to night is as significant. According to the system of solar climate zones, the Khangai is situated in the dry steppe zone between the 200 and 350 mm isohyets, -20°C and -25°C January isotherms, and 16°C and 18°C July isotherms [28]. Another extremely important factor is that the Khangai is located in the permafrost zone. However, all these common parameters are transformed quite significantly under the influence of the orography, which thus determines the specific climatic features of different parts of the Mountains as well as the mixed character of both top soil and plant cover [23] [28]. Here, against the background of zonal changes from a semidesert to a steppe and even a forest, various forms of vertical climate zone aspects can be observed. Taking into account both vertical and horizontal zoning, the whole territory can be divided into six regions: Northern, Western, Central, Northeastern, Eastern, and Southern Khangai [29]. Overall, grasslands such as steppes and meadows prevail on the territory under study, in which lichens play a specific and in some cases quite significant role [30] [31].

Lichen *C. pocillum* represented a life form of epigeic plagio-orthotropic squamous-fruticose lichens with awl-like podetia [32]. It should also be noted that the investigated species belongs to chlorolichens, so that its photosynthetic biont is represented by green algae in the thallus. In this case, this was a representative of the *Trebouxia* species. Correspondingly, this lichen is not able to  $\text{N}_2$  fixation. Thallus consists of a horizontal, formed into squamules of 5 - 10 mm diameter, tightly pressed against the substrate and vertical formed podetia 1

- 3 cm in height with cup-like correct shape. Representatives of this species in Mongolia are found mainly on the ground, among moss on the rocks in the steppe, meadow, forest communities of low-, medium- and high-altitude belts of Khangai, Hovsgol, Khentei, Mongolian Dauria, Hovd, Mongolian Altai, Gobi-Altai botanic-geographic provinces [33] [34]. The presence of this species they recorded on all continents of Earth from the islands of the Arctic to Antarctica, as in the plains and in the mountains. To perform the measurements, thalli collected on ground within the range of 1550 - 2900 m.a.s.l. in highland and midland meadows and steppes of the Central, Eastern and North-Eastern Khangai were selected from the herbarium collection (Table 1). The altitude during the collection process was determined using an aviation altimeter with an accuracy of 10 m. The distance between edge points of the material collection was about 300 km along the west-east direction.

To determine the natural ratio of the  $^{14}\text{N}$  and  $^{15}\text{N}$  stable isotopes, four intact thalli were separated from each lichen sample stored after the expedition in paper bags in dry conditions, that is, in the cryptic state. The thalli were thoroughly washed with deionized water to remove any pulverulent particles of some other samples from their surface that were stored in the same herbarium bag. The thalli were then dried at  $40^\circ\text{C}$  for 24 h. To perform the isotope analysis, a sample (1 - 2 mg) was separated from each individual thallus using a pair of metal forceps. It was apical parts of cup-like podetia.

The prepared dry samples were weighed using a Mettler Toledo MX5 scales with an accuracy of  $1\ \mu\text{g}$  and then encapsulated in tin foil. The  $^{15}\text{N}$  and  $^{14}\text{N}$  stable isotope ratio as well as the nitrogen content were measured in 40 samples prepared as described above and representing 10 different altitudinal levels. The measurements were carried out at the Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, using a set of equipment consisting of a Thermo Flash EA 1112 element analyzer and a Thermo-Finnigan Delta V Plus isotope mass spectrometer (Germany). In detail, a sample encapsulated in tin foil is first delivered to a reactor preliminarily heated to  $1020^\circ\text{C}$  and then combusted in the presence of oxygen. The combustion products are catalytically oxidized to  $\text{CO}_2$  and  $\text{N}_2$ , then separated on a chromatographic column in a helium flow and further delivered to the mass spectrometer. At the next stage, ionized nitrogen molecules of different mass (28/29/30) are separated in the mass spectrometer in the presence of a strong magnetic field. The current intensity of molecules of different mass is estimated using Faraday detectors; this procedure therefore allows one to determine the nitrogen isotopic composition of the investigated sample.

**Table 1.** The locations of sampling sites of *Cladonia pocillum* thalli on different altitudes in the communities of the Khangai Mountains and average ( $M \pm SE$ ,  $n = 4$ ) of  $\delta^{15}\text{N}$ , ‰ and N, % values in organic matter of podetia, in brackets is coefficient of variation.

Index	Altitude, m.a.s.l.	Location of site, community	$\delta^{15}\text{N}$ , ‰	N, %
I	2900	Central Khangai, southern slope of Khuh-Nur lake basin, $47^\circ32'\text{N}$ , $98^\circ30'\text{E}$ . Kobresia meadow [ <i>Kobresia sibirica</i> (Turcz.ex Ledeb.) Boeck.] with various herbs.	$-2.0 \pm 0.6$ (59%)	$0.7 \pm 0.1$ (16%)
II	2800	Central Khangai, southern slope of Khuh-Nur lake, $47^\circ32'\text{N}$ , $98^\circ30'\text{E}$ . Sedge-kobresia meadow.	$-2.5 \pm 0.3$ (27%)	$0.6 \pm 0.1$ (17%)
III	2500	Eastern Khangai, flat top of Ikh-Khairkhan Mountain, $47^\circ07'\text{N}$ , $101^\circ59'\text{E}$ . Kobresia meadow with slide rocks.	$-3.8 \pm 0.9$ (49%)	$0.7 \pm 0.1$ (30%)
IV	2390	Eastern Khangai, flat top of peak, $47^\circ17'\text{N}$ , $101^\circ50'\text{E}$ . Kobresia meadow.	$-4.4 \pm 0.7$ (30%)	$0.8 \pm 0.1$ (18%)
V	2080	North-eastern Khangai, the flat top of Burgut ridge, $48^\circ10'\text{N}$ , $103^\circ15'\text{E}$ . Kobresia meadow with various herbs and <i>Betula fruticosa</i> .	$-3.0 \pm 0.2$ (14%)	$0.8 \pm 0.2$ (38%)
VI	2000	Central Khangai, sands valley, $48^\circ35'\text{N}$ , $96^\circ37'\text{E}$ .	$-3.9 \pm 0.4$ (22%)	$0.8 \pm 0.1$ (18%)
VII	1900	Eastern Khangai, eastern slope of river basin, $47^\circ15'\text{N}$ , $102^\circ00'\text{E}$ . Petrophyte steppe.	$-4.4 \pm 0.6$ (29%)	$0.6 \pm 0.1$ (20%)
VIII	1800	North-eastern Khangai, the top of peak, $49^\circ20'\text{N}$ , $104^\circ05'\text{E}$ . Petrophyte steppe.	$-3.2 \pm 1.2$ (76%)	$0.8 \pm 0.1$ (14%)
IX	1700	Eastern Khangai, the flat top of ridge. $47^\circ26'\text{N}$ , $102^\circ01'\text{E}$ . Petrophyte steppe.	$-1.4 \pm 0.7$ (99%)	$1.4 \pm 0.3$ (36%)
X	1550	North-eastern Khangai, southern slope of ridge, $49^\circ10'\text{N}$ , $103^\circ46'\text{E}$ . Stipa steppe.	$-1.8 \pm 0.7$ (81%)	$0.9 \pm 0.1$ (14%)
Average for all samples (40)			$-3.0 \pm 0.3$ (53%)	$0.8 \pm 0.1$ (37%)

The isotopic composition ( $\delta^{15}\text{N}$ , ‰) was expressed in parts per thousand relative to a standard according to the following equation:

$$\delta^{15}\text{N}\text{‰} = \left[ \left( \frac{R_{lich} - R_{st}}{R_{st}} \right) \right] \times 10^3,$$

where  $R_{lich}$  is the  $^{15}\text{N}/^{14}\text{N}$  ratio experimentally determined in the lichen sample, and  $R_{st}$  is the same ratio in the standard, *i.e.*, in atmospheric nitrogen:  $R_{st} = 0.0036765$  [2].

To calibrate the equipment used in this study, glutamic acid with the annotated  $\delta^{15}\text{N}$  value (IAEA reference materials USGS-40, USGS-41) was utilized; acetanilide served as the laboratory standard. The analytical error of the  $\delta^{15}\text{N}$  value detection did not exceed  $\pm 0.3\text{‰}$ .

Experimentally determined values of  $\delta^{15}\text{N}$ , total N (%), and elevation (a.s.l.) at lichen collection sites were processed statistically at a  $p = 0.05$  significance level using corresponding applications in the Microsoft Excel 2003 program package.

### 3. Results and Discussion

The results of measurements of the stable nitrogen isotope ratio ( $^{15}\text{N}/^{14}\text{N}$ ) in OM of *C. pocillum* expressed in average  $\delta^{15}\text{N}$  values as well as the nitrogen content (%) in thalli of lichens collected at different altitudes are presented in **Table 1**.

The average  $\delta^{15}\text{N}$  values in OM of *C. pocillum* lichens collected across 10 different altitudinal levels vary from  $-4.4\text{‰}$  to  $-1.4\text{‰}$ , whereas the nitrogen content in the same samples varies within 0.6% - 1.4% (**Table 1**). Specific  $\delta^{15}\text{N}$  values in all 40 investigated samples cover a wider range; that is, they vary from  $-6.7\text{‰}$  to  $+0.1\text{‰}$  with average value for all 40 samples  $-3.0\text{‰} \pm 0.3\text{‰}$ , whereas the nitrogen percentage values in this case were determined to be within the range of 0.5 - 1.9.

The coefficient variation of  $\delta^{15}\text{N}$  values for all samples is 53%, and for each level of this altitude ranges is from 14% to 99% (**Table 1**). The highest values of the last parameter marked samples of the habitats in the range of altitudes 1550 - 1800 m (76% - 99%), while the lowest are at altitudes of 2000 and 2080 m, 22% and 14%, respectively. The coefficient of variation of N% values in all samples is 37%, and for each level of this altitude ranges is from 14% to 38%. The highest value of the last parameter marked for habitat at altitudes of 2080 and 1700 m (38% and 36%), while the lowest—at altitudes of 1800 and 1550 m (14%).

Values of the correlation coefficient between all altitudes used for the sample collection and the  $\delta^{15}\text{N}$  values and the nitrogen percentage in lichen OM are very low (**Table 2**); this confirms the absence of any functional dependence of the stable nitrogen isotope ratio on these parameters at a regional scale where lichen thalli were collected. But it should be noted once again that the Khangai Mountains territory is characterized by spatial heterogeneity and as mentioned above the region-averaged climate characteristics are transformed significantly by the orography, which determines the specific climatic characteristics of different parts of the Mountains and, in the end, the mixed character of both topsoil and plant cover as well as the role of lichens in ecosystems.

The *C. pocillum* samples were collected in different parts of the Khangai, such as the Central, Eastern, and North-Eastern districts of region, as well as in various types of grasslands, including highland meadows together with lowland and midland petrophyte steppes.

One series of samples was collected in the Central district of the Khangai. If these samples were analyzed separately from the rest of the collected material, we would find that an increase in altitude of habitats, where the *C. pocillum* samples were collected, correlates with the heavy  $^{15}\text{N}$  isotope increase in lichen OM (the correlation coefficient is 1.0). The correlation coefficient between the nitrogen content in thalli and the altitude was calculated to be  $-0.5$  (**Table 2**).

Another series of samples represents the Eastern and North-Eastern districts of the Khangai; in these cases, the correlation coefficient between the  $\delta^{15}\text{N}$  values in lichen OM and the sampling point altitude is significantly negative (**Table 2**). The ratio of stable isotopes in lichen thalli in this case is most probably related to the content of nitrogen (the correlation coefficient is 0.8 - 0.9).

Among all the collected samples, there is also a group of specimens collected in highland meadows and in steppes (**Table 1**). The correlation coefficients between the  $\delta^{15}\text{N}$  values in OM of lichen and the altitude of steppe habitats of lichen were also found to be significantly negative, whereas same parameter for meadow habitats of lichen is positively (**Table 2**). Apparently, the  $\delta^{15}\text{N}$  value in OM of *C. pocillum* lichen depends somewhat on the nitrogen content in thalli, and the latter value in most cases shows dependence on the lichen habitat altitude (**Table 2**).

**Table 2.** Correlation coefficients between  $\delta^{15}\text{N}$  (‰) values or the nitrogen content (N, %) in OM of *C. pocillum* lichen and an altitude ( $H$ , m) of habitats at a regional and a local scale.

Object	Parameter	H	$\delta^{15}\text{N}$
Whole territory	$\delta^{15}\text{N}$	-0.1	#
	N	-0.4	0.6
Steppes, all districts	$\delta^{15}\text{N}$	-0.8	#
	N	-0.5	0.8
Highland meadows, all districts	$\delta^{15}\text{N}$	0.6	#
	N	-0.7	-0.3
Eastern Khangai	$\delta^{15}\text{N}$	-0.6	#
	N	-0.5	0.9
Central Khangai	$\delta^{15}\text{N}$	1.0	#
	N	-0.5	-0.4
North-Eastern Khangai	$\delta^{15}\text{N}$	-0.8	#
	N	-0.3	0.8

The data on the values of  $\delta^{15}\text{N}$  in OM *C. pocillum* from other regions are absent in available to me publications. I found no information about this one, and for species of the genus *Cladonia* similar life form. In the species of this genus belonging to the life form of orthotropic fruticose branched lichens (*C. portentosa*, *C. stellaris* and other) averages  $\delta^{15}\text{N}$  values are in the range of  $-8.0\text{‰}$  to  $+2.5\text{‰}$  [35].

Nevertheless, the majority of similar studies of  $\delta^{15}\text{N}$  values in OM of vascular plants show that an increase in an altitude where samples for the measurement of the nitrogen stable isotope ratio were collected correlates with a decrease in  $\delta^{15}\text{N}$  values in plant OM. This dependence was observed for representatives of a few vascular epiphyte species (for example, *Bromeliaceae*, pteridophytes and others) of mountain forests of Mexico [36], plants of highland meadows of Austria [37] [38], and different vascular plants of Ethiopia collected in the altitude range of 930 - 4050 m [39].

However, there are also less unambiguous data available in the case of the distribution of  $\delta^{15}\text{N}$  values in OM of vascular plants. For example, plants for the measurement of  $\delta^{15}\text{N}$  values were collected at 18 different sampling points in mountains near Beijing (China) in the range of altitudes varying from 400 to 2300 m [40]. The average  $\delta^{15}\text{N}$  values in OM of a few plant species collected at the same altitudinal level demonstrated their parabolic dependence on the sampling point altitude; that is, the average  $\delta^{15}\text{N}$  values decreased with an altitude increase from 400 to 1350 m and then they increased up to the altitude of 2300 m. However, the graphs presented in this article, which illustrate this correlation for each species separately, confirm that some species do not demonstrate the above described dependence; some species are characterized by a simultaneous increase in  $\delta^{15}\text{N}$  values with altitude, whereas others demonstrate an inverse correlation [41]. In the above mentioned article about the studies carried out in the mountains of Mexico [36], the investigation of  $\delta^{15}\text{N}$  values in OM of representatives of 98 vascular epiphyte plant species showed that there is no correlation between  $\delta^{15}\text{N}$  values in OM of all plants collected at a certain altitudinal level (six different levels within the altitude range of 720 - 2370 m were extensively studied) and the sampling point altitude (the correlation coefficient is equal to 0.15).

The data on the altitudinal distribution of  $\delta^{15}\text{N}$  values in OM of bryophytic organisms depending on the habitat altitude confirm that the nitrogen stable isotope ratio in their tissues simultaneously increases with altitude. The same trend was observed in the southwestern part of China where  $\delta^{15}\text{N}$  values in both young and old tissues of *Haplocladium microphyllum* moss increase with an increase in the sampling point altitude (the samples were collected at 990 and 3276 m above sea level) [42]. It should be noted, however, that the lowest sampling point was located on the territory of a city with a large population, that is, the region was enriched in nitrogen-containing compounds. In Austria, the studies of  $\delta^{15}\text{N}$  values in tissues of a few moss species, which were collected from 220 sampling points that were located pretty much evenly on the territory of the country (2,5 sampling points per 1000 km<sup>2</sup>), demonstrated the highly significant positive correlation between  $\delta^{15}\text{N}$  values in moss tissues and the sampling point altitude [43]. To summarize, mosses, which, as well as lichens, belong to thallophyte and poikilohydric organisms, demonstrate a different dependence of  $\delta^{15}\text{N}$  values on the habitat altitude

compared to the *C. pocillum* lichen in the Khangai Mountains.

#### 4. Conclusion

Today interpretation of the results is difficult because of the lack of such data on  $\delta^{15}\text{N}$  values in OM for representatives of *C. pocillum* from other regions. Undoubtedly, more research needs to be performed to fully explain the data. At the moment, unfortunately, the obtained values cannot be related to any specific data on the air temperature, the amount of atmospheric precipitation, or any other environmental parameters of sampling point locations since there are only poor data on climatic characteristics available for the territory of Mongolia. The revealed dependences allow us to speculate that the results obtained on a local scale are not always in agreement with those that characterize the region overall. The data on  $\delta^{15}\text{N}$  values in OM of the average sample from several species of community at a certain altitudinal level are undoubtedly of great importance for the establishment of variation on altitude; however, the most important information is represented by the data on  $\delta^{15}\text{N}$  values of concrete species that dwell at all altitudinal levels.

#### Acknowledgements

The author thanks A.V. Tiunov and K.B. Gongal'skii for measuring the  $\delta^{15}\text{N}$  values and the nitrogen content in lichen samples.

#### References

- [1] Galimov, E.M. (1981) Priroda Biologicheskogo Fraktsionirovaniya Izotopov (The Nature of Biological Fractionation of Isotopes). Nauka, Moscow.
- [2] Robinson, D. (2001)  $\delta^{15}\text{N}$  as an Integrator of the Nitrogen Cycle. *Trends in Ecology & Evolution*, **16**, 153-162. [http://dx.doi.org/10.1016/S0169-5347\(00\)02098-X](http://dx.doi.org/10.1016/S0169-5347(00)02098-X)
- [3] Dawson, T.E., Mambelli, S., Plamboeck, A.H., Temper, P.H. and Tu., K.P. (2002) Stable Isotopes in Plant Ecology. *Annual Review of Ecology, Evolution, and Systematics*, **33**, 507-559. <http://dx.doi.org/10.1146/annurev.ecolsys.33.020602.095451>
- [4] Tiunov, A.V. (2007) Stable Isotopes of Carbon and Nitrogen in Soil Ecological Studies. *Biology Bulletin*, **34**, 395-407. <http://dx.doi.org/10.1134/S1062359007040127>
- [5] Makarov, M.I. (2009) The Nitrogen Isotopic Composition in Soils and Plants: Its Use in Environmental Studies (Review). *European Journal of Soil Science*, **42**, 1335-1348. <http://dx.doi.org/10.1134/S1064229309120035>
- [6] Hoefs, J. (2009) Stable Isotope Geochemistry. 6th Edition, Springer-Verlag, Berlin Heidelberg.
- [7] Aelion, C.M., Hohener, P., Hunkeler, D. and Aravena, R. (2010) Environmental Isotopes in Biodegradation and Bioremediation. CRC Press, Boca Raton.
- [8] Fry, B. (2006) Stable Isotope Ecology. Springer Science + Business Media, LLC, Berlin.
- [9] Lange, O. (1992) Pflanzenleben unter Stress: Flechten als Pioniere der Vegetation an Extremstandorten der Erde. Rostera Universitatis Wirceburgensis.
- [10] Biazrov, L.G., Medvedev, L.N. and Chernova, N.M. (1971) Lichen Consortias in Deciduous Fir Forests of Moscow Suburbs. In: *Biogeotsenologicheskie Issledovaniya v Shirokolistvenno-Elovykh Lesakh (Biogeocenological Studies in Deciduous Fir Forests)*, Nauka, Moscow, 252-270.
- [11] Biazrov, L.G. (1995) Microarthropods and Decomposition Rate of Dead Epiphytic Lichen *Hypogymnia physodes*. *Acta Zoologica Fennica*, **196**, 45-47.
- [12] Baur, B. and Baur, A. (1997) *Xanthoria parietina* as a Food Resource and Shelter for the Land Snail *Balea perversa*. *Lichenologist*, **29**, 99-102. <http://dx.doi.org/10.1017/S0024282997000145>
- [13] Schneider, K., Migge, S., Norton, R.A., Scheu, S., Langel, R., Reineking, A. and Maraun, M. (2004) Trophic Niche Differentiation in Soil Microarthropods (Oribatida, Acari): Evidence from Stable Isotope Ratios ( $^{15}\text{N}/^{14}\text{N}$ ). *Soil Biology & Biochemistry*, **36**, 1769-1774. <http://dx.doi.org/10.1016/j.soilbio.2004.04.033>
- [14] Erdmann, G., Otte, V., Langel, R., Scheu, S. and Maraun, M. (2007) The Trophic Structure of Bark-Living Oribatid Mite Communities Analysed with Stable Isotopes ( $^{15}\text{N}$ ,  $^{13}\text{C}$ ) Indicates Strong Niche Differentiation. *Experimental & Applied Acarology*, **41**, 1-10. <http://dx.doi.org/10.1007/s10493-007-9060-7>
- [15] Nash, T.H. (2008) Lichen Biology. 2nd Edition, Cambridge University Press, Cambridge. <http://dx.doi.org/10.1017/CBO9780511790478>

- [16] Honegger, R. (2009) Lichen-Forming Fungi and Their Photobionts. *The Mycota*, **5**, 307-333.
- [17] Palmqvist, K., Dahlman, L., Valladares, F., Tehler, A., Sancho, L.G. and Mattsson, J.E. (2002) CO<sub>2</sub> Exchange and Thallus Nitrogen across 75 Contrasting Lichen Associations from Different Climate Zones. *Oecologia*, **133**, 295-306. <http://dx.doi.org/10.1007/s00442-002-1019-0>
- [18] Fogel, M.L., Wooller, M.J., Cheeseman, J., Smallwood, B.J., Roberts, Q., Romero, I. and Meyers, M.J. (2008) Unusually Negative Nitrogen Isotopic Compositions ( $\delta^{15}\text{N}$ ) of Mangroves and Lichens in an Oligotrophic, Microbially-Influenced Ecosystem. *Biogeosciences*, **5**, 1693-1704. <http://dx.doi.org/10.5194/bg-5-1693-2008>
- [19] Huiskes, A.H.L., Boschker, H.T.S., Lud, D. and Moerdijk-Poortvliet, T.C.W. (2006) Stable Isotope Ratios as a Tool for Assessing Changes in Carbon and Nutrient Sources in Antarctic Terrestrial Ecosystems. *Plant Ecology*, **182**, 79-86.
- [20] Kappen, L. and Valladares, F. (2007) Opportunistic Growth and Desiccation Tolerance: The Ecological Success of Poikilohydrous Autotrophs. In: *Functional Plant Ecology*, 7-65.
- [21] Lange, O.L., Green, T.G.A. and Ziegler, H. (1988) Water Status Related Photosynthesis and Carbon Isotope Discrimination in Species of the Lichen Genus *Pseudocyphellaria* with Green or Blue-Green Photobionts and in Photosymbiodemes. *Oecologia*, **75**, 494-501. <http://dx.doi.org/10.1007/BF00776410>
- [22] Øvstedal, D.O. and Smith, R.I.L. (2001) Lichens of Antarctica and South Georgia: A Guide for Their Identification and Ecology. Cambridge University Press, Cambridge.
- [23] Biazrov, L.G., Ganbold, E., Gubanov, I.A. and Ulziikhutag, N. (1989) Flora Khangaya [The Khangai Flora]. Nauka, Leningrad.
- [24] Máguas, C. and Brugnoli, E. (1996) Spatial Variation in Carbon Isotope Discrimination across the Thalli of Several Lichen Species. *Plant, Cell & Environment*, **19**, 437-446. <http://dx.doi.org/10.1111/j.1365-3040.1996.tb00335.x>
- [25] Cuna, S., Balas, G. and Hauer, E. (2007) Effects of Natural Environmental Factors on  $\delta^{13}\text{C}$  of Lichens. *Isotopes in Environmental and Health Studies*, **43**, 95-104. <http://dx.doi.org/10.1080/10256010701362401>
- [26] Biazrov, L.G., Gongalsky, K.B., Pelgunova, L.A. and Tiunov, A.V. (2010) Izotopnyi sostav ugleroda ( $\delta^{13}\text{C}$ ) tallomov lishainikov v lesakh vblizi Chernobyl'skoi AES [Carbon Stable Isotope Composition ( $\delta^{13}\text{C}$ ) of Lichen Thalli in the Forests in the Vicinity of the Chernobyl Atomic Power Station]. *Radiacionnaya Biologiya. Radioekologiya*, **50**, 98-105.
- [27] (1982) Geomorfologiya Mongol'skoi Narodnoi Respubliki (Geomorphology of People's Republic of Mongolia). Nauka, Moscow.
- [28] Beresneva, I.A. (2006) Klimaty Aridnoi Zony Azii [Climatic Conditions of the Arid Zone of Asia]. Nauka, Moscow.
- [29] Karamysheva, Z.V. and Banzragch, D. (1977) Some Botanical-Geographical Characteristics of the Khangai Related to Its Zoning. In: *Rastitel'nyi i Zhivotnyi Mir Mongolii (Flora and Fauna of Mongolia)*, Nauka, Leningrad, 7-26.
- [30] Biazrov, L.G. (1974) Lishainikovye Sinusii v Listvennichnike Raznotravnom (Lichen Synusiae in Herb Larch Forests). *Botanicheskii Zhurnal*, **59**, 1425-1438.
- [31] Biazrov, L.G. (1980) O Raspredelenii Fitomassy Lishainikov v Kedrovo-Listvennichnom Soobshchestve (Kangai, MNR) [The Distribution of Lichen Biomass in the Cedar-Larch Community (Khangai, MPR)]. Bjuulleten' Moskovskogo Obscestva Ispytatelej Pirody. *Otdel Biologiceskij*, **85**, 117-123.
- [32] Golubkova, N.S. and Biazrov, L.G. (1989) Life Forms of Lichen and Lichensynusiae. *Botanicheskii Zhurnal*, **74**, 794-805.
- [33] Golubkova, N.S. (1981) Konspekt Flory Lishainikov Mongol'skoi Narodnoi Respubliki (Synopsis Lichen Flora of the Mongolian People's Republic). Nauka, Leningrad.
- [34] Biazrov, L.G. (2013) Checklist of the Mongolian Lichens. Version 8. [http://www.sevin.ru/laboratories\\_eng/biazrov\\_mong.html](http://www.sevin.ru/laboratories_eng/biazrov_mong.html)
- [35] Ellis, C.J., Crittenden, P.D., Scrimgeour, C.M. and Ashcroft, C. (2003) The Natural Abundance of  $^{15}\text{N}$  in Mat-Forming Lichens. *Oecologia*, **136**, 115-123. <http://dx.doi.org/10.1007/s00442-003-1201-z>
- [36] Hietz, P., Wanek, W. and Popp, M. (1999) Stable Isotopic Composition of Carbon and Nitrogen and Nitrogen Content in Vascular Epiphytes along an Altitudinal Transect. *Plant, Cell & Environment*, **22**, 1435-1443. <http://dx.doi.org/10.1046/j.1365-3040.1999.00502.x>
- [37] Huber, E., Wanek, W., Gottfried, M., Pauli, H., Schweiger, P., Arndt, S.K., Reiter, K. and Richter, A. (2007) Shift in Soil-Plant Nitrogen Dynamics of an Alpine-Nival Ecotone. *Plant Soil*, **301**, 65-76.
- [38] Männel, T.T., Auerswald, K. and Schnyder, H.T. (2007) Altitudinal Gradients of Grassland Carbon and Nitrogen Isotope Composition Are Recorded in the Hair of Grazers. *Global Ecology and Biogeography*, **16**, 583-592.
- [39] Liu, X.H., Zhao, L.J., Gasaw, M., Gao, D.Y., Qin, D.H. and Ren, J.W. (2007) Foliar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  Values of C<sub>3</sub> Plants in the Ethiopia Rift Valley and Their Environmental Controls. *Chinese Science Bulletin*, **52**, 1265-1273. <http://dx.doi.org/10.1007/s11434-007-0165-5>



- [40] Liu, X.Z., Wang, G.A., Li, J.Z. and Wang, Q. (2010) Nitrogen Isotope Composition Characteristics of Modern Plants and Their Variations along an Altitudinal Gradient in Dongling Mountain in Beijing. *Science in China Series D: Earth Sciences*, **53**, 128-140. <http://dx.doi.org/10.1007/s11430-009-0175-z>
- [41] Skrzypek, G., Jezierski, P. and Szykiewicz, A. (2010) Preservation of Primary Stable Isotope Signatures of Peat-Forming Plants during Early Decomposition—Observation Along an Altitudinal Transect. *Chemical Geology*, **273**, 238-249. <http://dx.doi.org/10.1016/j.chemgeo.2010.02.025>
- [42] Liu, X.Y., Xiao, H.Y., Liu, C.Q. and Li, Y.Y. (2008) Stable Carbon and Nitrogen Isotopes of the Moss *Haplocladium microphyllum* in an Urban and a Background Area (SW China): The Role of Environmental Conditions and Atmospheric Nitrogen Deposition. *Atmospheric Environment*, **42**, 5413-5423. <http://dx.doi.org/10.1016/j.atmosenv.2008.02.038>
- [43] Zechmeister, H.G., Richter, A., Smidt, S., Hohenwallner, D., Roder, L., Maringer, S. and Wanek, W. (2008) Total Nitrogen Content and  $\delta^{15}\text{N}$  Signatures in Moss Tissue: Indicative Value for Nitrogen Deposition Patterns and Source Allocation on a Nationwide Scale. *Environmental Science & Technology*, **42**, 8661-8667. <http://dx.doi.org/10.1021/es801865d>