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SEASONAL VARIATION IN BRYOPHYTES COVER IN THE CALCAREOUS MIRE BELIANSKE LUKY, SLOVAKIA

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Abstract

While making investigations of glacial relic bryophytes in Belianske lúky mire, we recorded seasonal change in cover of selected bryophytes. It was decided to perform repeated monitoring between 2008-2010, in three replicates each year. In particular, the main observation was the decreasing cover of *Campylium stellatum* and *Drepanocladus cossonii* in the course of the year. In order to explain the seasonal change, we have sampled physico-chemical features of the examined sites: pH, redox potential (ORP), conductivity, total dissolved solids (TDS), salinity, dissolved oxygen, chemical oxygen demand (COD), $CaCO_3$, NO_3^-, SO_4^{2-}, NH_4^+ and Cl^-.

Results show that within investigated sites the distribution of *Campylium stellatum* and *Drepanocladus cossonii* was mostly limited by decreasing concentration of NH_4^+ and increasing concentration of NO_3^- in the course of the year. The phylogenetic diversity of the bacterial community in the water sample from Belianske luky meadows was investigated using a PCR of the 16S rRNA gene. After screening by Terminal Restriction Fragment Length Polymorphism (T-RFLP) method, we made *in silico* analysis, confirming the presence of nitrifying bacteria.The seasonal variation in bryophytes cover is induced by the synergic influence of decomposition of organic substances in soils, by nitrifying bacteria activity and by unstable the water table.

Key words: Bryophytes cover, Seasonal change, Minerotrophic fen, Nitrifying bacteria, Water table, Slovakia.

Introduction

Belianske lúky meadows are the largest unspoilt Slovakian minerotrophic fen. It covers an area of about 90 ha and at the altitude of 670–695 m a. s. l. The mire lies between the settlements Lendak, Slovenská Ves, Rakúsy and the town of Spišská Belá in the Poprad basin. This site of Europaean interest harbours many glacial relic species, not only vascular plants (*Carex diandra, C. dioica, C. limosa, Pedicularis sceptrum-carolinum*), but also bryophytes (*Calliergon trifarium, Meesia triquetra, Catoscopium nigritum* and others). The mire is more than 10000 years old dating from the Early Holocene.

The most important feature within the mire are the pools (bog hollows). The water table is unstable and the pools are most full after snow thaw in the springtime or during heavy rainfall whereas during the summer the lakes are often dried up. Grootjans *et al*. (2005) evaluated the possibilities for restoration of the calcareous spring mire Belianske lúky meadows that have been damaged by man made due to changes in the hydrology. In terms of evaluating the ecology very important are micro-organisms - still unidentified coating the bottom of the lakes. The first record of the presence of bacteria on the Belianske lúky meadows came from Dražil *et al*. (2008), who observed purple anaerobic bacteria at the bottom of the pools.

While making bryological investigation in the sites, we have recorded seasonal change in the cover of some bryophytes in the pools. We thought it is necessary to explain the reasons for these variations, which is why we decided to fill this gap in our knowledge. The following questions are addressed in this paper:

1. How is bryophyte species composition in the pools of Belianske lúky meadows related to physico-chemical factors, i.e., conductivity, pH, redox potential, chemical oxygen demand, dissolved oxygen, chlorides, ammonia, sulphates, nitrates and calcium carbonate.
2. How is the vegetation of the pools endangered by long-term changes, mainly lowering of the water table.

Material and Methods

Sampling: The investigation have been carried out in three permanent replicates (sites) harbouring glacial moss relic species, established in 2008. Quadrats of the dimension of 1 x 1 m, divided into 100 squares of 1 dm^2 has been used for sampling. Total bryophyte cover growing inside the squares has been estimated in all the 100 squares twice a year (early spring and late autumn) in the period 2008-2010. The geographical coordinates are recorded in the system WGS 84, device Garmin eTrex Vista.

Nomenclature: The nomenclature of bryophytes follows Kubinská and Janovicová (1998) and Marhold (1998) for vascular plants. That of bacteria follows Wang *et al*. (2007).

Instrumental analysis: Based on environmental data collected in 2008-2009 (conductivity and pH), we were unable to explain the seasonal change in cover of some bryophyte species. In 2010, we therefore decided sampling of physico-chemical features of the examined sites: pH, ORP (redox potential), conductivity, TDS (total dissolved solids), salinity, dissolved oxygen, COD (chemical oxygen demand), $CaCO_3$, NO_3^-, SO_4^{2-}, NH_4^+ and Cl. Conductivity, TDS and salinity have been measured using device YSI EC 300 (USA), pH using aYSI pH 100 (USA) device. Determination of dissolved oxygen is based on fluorescence detection using device YSI DO 200 (USA). Determination of chlorides, sulphates, amonia and nitrates is based on colorimetry using direct-reading photometer YSI 9500 (USA). Redox potential (ORP) using YSI ORP 15 (USA) device. Volumetric methods have been used for determination of the following features: Manganometry for chemical oxygen demand (COD) determination and chelatometry for $CaCO_3$ determination.

Terminal restriction fragment length polymorphism (T-RFLP) analysis: The phylogenetic diversity of the bacterial community in the water sample from Belianske luky meadows was investigated using a PCR of the 16S rRNA gene. After screening by Terminal Restriction Fragment Length Polymorphism (Table 7) method, made by in silico analysis which confirmed the presence of nitrifying bacteria.

DNA was extracted by the Power Water DNA Isolation Kit (MoBio Laboratories, Inc.) according to the manufacturer's protocol. DNA amplification was carried out in 50 µl reaction mixture containing FailSafe™ PCR PreMix Selection Kit (Epicentre Biotechnologies); 0.10 µM of both primers and 25 ng of DNA extracted from water. Bacterial universal primers for the 16S rRNA gene: 8F (AGAGTTTGATCCTGGCTCAG, FAM labelled) and 926R (CCGTCAATTCCTTTRAGTTT) were used. PCR was performed using the program: 3 min at 95°C, 35 cycles of 30 s at 94°C, 30 s at 47°C, 1 min at 72°C and final polymerization 10 min at 72°C in GeneAmp PCR System 9700 (Applied Biosystems). Triplicate reactions were pooled, and PCR products were purified by 3 M sodium acetate (pH 5.2) and ethanol precipitation. After purification, PCR products were digested separately with CfoI, MspI and RsaI (Roche) restriction enzymes. Digestion mixture (total volume 20 µl) containing 10 U of restriction enzyme, 2 µl of 10 × buffer, and 10 µl of purified PCR mix was incubated for 3 h at 37°C. After digestion samples were purified by ethanol precipitation. Terminal Restriction Fragments (T-RFs) were separated by electrophoresis using an ABI Prism 3100 Avant apparatus (Applied Biosystems) with LIZ 1200 internal standard. Electrophoretograms were analyzed by GeneMapper 3.5 (Applied Biosystems). Only fragments between 60 bp and 924 bp were used for evaluation. Using the program MiCA 3 (Microbial Community Analysis III, Shyu *et al*., 2007) experimentally obtained sizes of T-RFs were compared with *in silico* sizes of T-RFs taken from a database of 16S rDNA sequences.

Statistics: CANOCO 4.5 for Windows package (Ter Braak & Šmilauer, 2002) was used for statistical analysis. We used the unimodal methods - CCA. The statistical significance of the explanatory (environmental) variables in canonical methods were determined by Monte Carlo permutation tests. Explanatory variables were tested separately (partial tests).

Results and Discussion

The observed regular changes in bryophytes cover values of the all examined sites are shown in Tables 1, 3, 5. We have recorded decreasing values of *Drepanocladus cossonii* and *Campylium stellatum* coverage in the year's cycle in every site in the period 2008–2010. The composition of the vegetation is mainly determined by the chemistry of the ecosystem.

The most important disolved gas is oxygen, a requirement for life in waters. Positive correlation between dissolved oxygen and oxydo-reduction potential is expected and easy to explain. The value of redox potential gives information on oxidative or reducing conditions in the waters. Positive value indicates the oxidative situation in the waters and negative values indicate reducing status. In natural waters, oxidative situations are associated with dissolved oxygen, thus positive correlation is expected. However, potential changes of redox state and pH may remobilize the metals bound to carbonates (Hnaťuková, 2011).

Nitrogen is an important components of plant nutrition and limits plant growth in many wetlands (Gürsoy *et al*., 2013). A significant source of nitrogen to ecosystems are agricultural activities, e. g. poultry plants, animal husbandry (Kondratyev & Trumbull, 2012). Sources of nitrogen having origin in agricultural activities, are in the case of Belianske lúky excluded. In addition to anthropogenic sources, an important source of chemical substances into the ecosystem are from precipitation. Whilst rainfall is a source of sulphates or nitrates, these also arise from the decomposition of organic substances. Chloride concentration in waters mainly derived from atmospheric precipitation.

Site 1 (N 49°12,876′; E 20°23,614′)

The site is dominated by *Menyanthes trifolia* and *Carex lepidocarpa*. An important species in Site 1 is *Meesia triquetra*. In Central Europe, *Meesia triquetra* is a rare species found in fens and considered a glacial relic species. In Slovakia, its occurence is concentrated in the Northern part of the country, mainly in the Orava region, extending to the Tatra region.

Site 2 (N 49°12,875′; E 20°23,619′)

The site is dominated by *Triglochin palustre*. An important bryophyte is *Catoscopium nigritum*. This is a bipolar species, has a large circumpolar occurrence in the North Hemisphere with its commonest occurrence at high latitudes. In its most southerly range it is restricted mainly to the mountains and the Westcarpathians' localities (Slovakia) represent the southernmost limit of the species distribution. Other than the mountains, the species has been found rarely in the rich fens between altitudes of 680-940 m a.s.l. (Dítě *et al*., 2011).

Table 1. Seasonal changes of moss layer in 2008-2010, Site 1.

Site 1	2008 (cm^2)			2009 (cm^2)			2010 (cm^2)		
	Apr.	Oct.	Diff.	Apr.	Oct.	Diff.	Apr.	Oct.	Diff.
Meesia triquetra	27	21	-6	20	8	-12	4	7	3
Campylium stellatum	1001	935	-59	960	755	-255	1485	685	-800
Drepanocladus cossonii	3576	2745	-831	2937	2107	-830	3246	2263	-983
Aneura pinguis	14	79	65	38	30	-8	21	38	17
Bryum pseudotriquetrum	10	56	46	29	25	-4	2	17	15
Calliergon trifarium	1	4	3	1	4	3	2	4	2
Fissidens adianthoides	0	2	2	0	1	1	1	0	-1
Philonotis tomentella	0	0	0	0	1	1	6	10	4
Sum	4634	3854	-780	3985	2881	-1104	4767	3024	-1743

Table 2. Physico-chemical features of the Site 1 during vegetation period 2010.

	Apr	May	June	July	Aug	Sept	Oct
pH	7.52	7.52	7.69	7.60	7.65	7.40	7.36
ORP mV	231	235	235	240	221	195	159
Conductivity µS	358.8	435.6	397.6	356.9	433.0	492.1	328.2
TDS $g.L^{-1}$	0.302	0.301	0.314	0.271	0.296	0.350	0.353
Salinity $ng.L^{-1}$	0.2	0.2	0.2	0.2	0.2	0.3	0.3
Dissolved O_2 $mg.L^{-1}$	9.22	4.66	5.08	6.24	7.57	6.20	6.91
COD $mg.L^{-1}$	7.900	6.600	10.767	10.100	11.633	7.800	4.500
$CaCO_3$ $mg.L^{-1}$	210	255	310	210	210	265	245
NO_3^- mg.. L^{-1}	0.253	0.478	1.103	1.586	1.452	1.723	2.405
NH_4^+ mg. L^{-1}	0.40	0.10	0.12	0.09	0.09	0.04	0.08
Cl^- mg. L^{-1}	1.1	0.9	1.3	1.6	0.4	0.5	0.7
SO_4^{2-} mg. L^{-1}	5	6	10	7	1	4	4

Table 3. Seasonal changes of moss layer in 2008 - 2010, Site 2.

Site 2	2008 (cm^2)			2009 (cm^2)			2010 (cm^2)		
	Apr	Oct	Diff.	Apr	Oct	Diff.	Apr	Oct	Diff.
Catoscopium nigritum	167	234	67	268	121	-147	397	250	-147
Campylium stellatum	238	148	-90	134	129	-5	294	142	-152
Drepanocladus cossonii	860	708	-152	685	582	-103	853	758	-95
Aneura pinguis	1	9	8	7	8	1	8	14	6
Philonotis tomentella	15	18	3	29	57	28	73	234	161
Meesia triquetra	1	0	-1	0	1	1	1	0	-1
Bryum pseudotriquetrum	3	5	2	16	25	9	3	32	29
Calliergon trifarium	0	0	0	0	5	5	1	17	16
Aulacomnium palustre	0	0	0	0	0	0	1	0	-1
Sum	1285	1122	-163	1139	928	-211	1631	1447	-184

Table 4. Physico-chemical features of the Site 2 during growing season of 2010.

	Apr	May	June	July	Aug	Sept	Oct
pH	7.37	7.38	7.27	7.56	7.37	7.40	7.24
ORP mV	157	197	238	249	226	195	171
Conductivity µS	382.3	488.1	473.0	222.4	421.3	454.6	304.4
TDS $g.L^{-1}$	0.331	0.331	0.351	0.162	0.284	0.332	0.324
Salinity $ng.L^{-1}$	0.2	0.3	0.3	0.1	0.2	0.2	0.2
Dissolved O_2 $mg.L^{-1}$	8.40	4.92	4.56	5.74	5.27	4.25	5.67
COD $mg.L^{-1}$	16.800	11.900	9.700	9.400	7.567	6.167	4.517
$CaCO_3$ $mg.L^{-1}$	250	275	330	245	230	260	230
NO_3^- mg. L^{-1}	0.615	0.944	0.642	0.585	1.108	1.104	1.258
NH_4^+ mg. L^{-1}	0.80	0.12	0.13	0.21	0.12	0.06	0.10
Cl^- mg. L^{-1}	1.5	0.8	0.6	0.6	0.2	0.8	0.9
SO_4^{2-} mg. L^{-1}	8	7	9	3	1	4	7

Site 3 (N 49°12,848′;E 20°23,485′)

The site is dominated by *Carex lepidocarpa* and *C. limosa.* An important bryophyte in Site 3 is *Calliergon trifarium*. The species is rare in Slovakia and confined to marshy meadows, i. e. to the most vulnerable habitats, which were in the past strongly influenced by underground water management.

Ammonia concentration were shown to have rapidly decreased in the time period April – May in all the sites, presumably by oxidation of ammonia due to bacterial activity. After May the ammonia concentrations remain relatively stable at low concentrations (usually 0.04-0.12 mg.1000 ml^{-1}, Fig. 1a, 1b, 1c, Tables 2, 4, 6) for the rest of the year. Similarly, this pattern was seen in respect to nitrogen. Despite the consumption of nitrogen during growth of vegetation, its concentrations are seen to increase during the year. Nitrate production by nitrifying bacteria or decomposition of organic substances in soils is most important in the nitrate consumption by the vegetation.

The highest $CaCO_3$ concentrations have usually been recorded in the Spring months (May and June) and in September also though these events do not always correlate with pH level (Table 1), because the acidity is influenced by many other factors such as ammonia, nitrate, sulphate and other ions concentrations.

Based on physico-chemical features (Tables 2, 4, 6), pH is usually highly negatively correlated with TDS (r= *-0,7406; *-0,8228; -0,2154) and with salinity (r= *-0,8569; *-0,6548; -0,5076). TDS and conductivity are usually highly positively correlated (r= 0,1812; *0,7784; *0,9080) and similarly conductivity and salinity are positively correlated (r= 0,1210; *0,8583; *0,8056). Oxygen is signifincantly positivelly correlated with ammonium (r= *0,6954; *0,9472; *0,7925). Asterisk indicates significance. Correlations between TDS-conductivity and salinity-conductivity are easy to explain where the greater the dissolved solids, the higher the conductivity. The ammonia-oxygen correlation is due to the oxidation of ammonia as an aerobic process. The pH has a low positive correlation with ammonium and highly negatively correlates with nitrates (r= -0,5636; -0,6573; -0,6794). These correlations are understandable with respect to acido-basic characteristics.

Seasonal variation in bryophytes physiology and cover has been the subject of interest of many bryologists. Dynamic changes in bryophytes cover is probably influenced by increasing nitrate concentration and desiccation of pools.

Table 5. Seasonal changes of moss layer in 2008 - 2010, Site 3.

Site 3	2008 (cm^2)			2009 (cm^2)			2010 (cm^2)		
	Apr	Oct	Diff.	Apr	Oct	Diff.	Apr	Oct	Diff.
Calliergon trifarium	78	135	57	276	37	-239	68	52	-16
Drepanocladus cossonii	439	310	-129	347	87	-260	299	93	-206
Campylium stellatum	1246	235	-1011	1974	136	-1838	403	252	-151
Aneura pinguis	0	0	0	5	1	-4	3	0	-3
Sum	1763	680	-1083	2602	261	-2341	773	397	-376

Table 6. Physico-chemical features of Site 3, during the growing season in 2010.

	Apr	May	June	July	Aug	Sept	Oct
pH	7.77	7.63	7.63	7.55	7.46	7.19	7.28
ORP mV	174	226	255	219	206	207	193
Conductivity µS	357.1	456.3	487.0	195.8	456.5	467.0	369.9
TDS $g.L^{-1}$	0.305	0.342	0.355	0.146	0.315	0.349	0.346
Salinity $ng.L^{-1}$	0.2	0.2	0.3	0.1	0.3	0.3	0.3
Dissolved O_2 $mg.L^{-1}$	10.13	5.00	4.84	6.15	4.73	5.54	7.46
COD $mg.L^{-1}$	6.800	7.233	11.517	9.400	7.700	7.783	2.333
$CaCO_3$ $mg.L^{-1}$	235	260	290	300	275	300	245
NO_3^- mg.. L^{-1}	0.253	0.461	0.346	0.363	0.629	0.670	0.904
NH_4^+ mg. L^{-1}	0.61	0.25	0.10	0.09	0.06	0.12	0.09
Cl^- mg. L^{-1}	1.3	0.4	0.5	1.5	0.3	0.5	0.8
SO_4^{2-} mg. L^{-1}	5	5	10	4	7	11	5

Increasing nitrate concentration: Nitrogen is generally considered one of the major limiting nutrients in relation to plant growth. The biological process responsible for reduction of molecular nitrogen into ammonia is referred to as nitrogen fixation (Franche *et al*., 2009). In addition to the fixation of nitrogen, there are another possible sources of nitrates – decomposition of organic substances in soils, activity of nitrifying bacteria or atmospheric deposition.

In all the examined sites we have recorded a remarkable correlation - increasing nitrate concentration in the course of the years and a decrease in cover of *Drepanocladus cossonii* and *Campylium stellatum*. Other authors confirmed a negative correlation between bryophytes cover and nitrogen and drew attention to the increasing nitrogen deposition (nitrate, nitrogen dioxide, nitric acid and ammonium) in Europe. Atmospheric nitrogen deposition has increased throughout Europe during the last two decades from 2–6 kg to 15–60 kg N

ha^{-1} $year^{-1}$ (Pitcairn *et al*., 1995). The highest concentrations (>16 $g.kg^{-1}$ in moss tissues) were found in parts of Belgium, France, Germany, Slovakia, Slovenia and Bulgaria (Harmens *et al*., 2011). Paulissen *et al*. (2004) found, that brown mosses subjected to high nitrogen deposition have declined markedly. High nitrogen inputs pose a serious threat to the brown moss flora of rich fens. Nordin *et al*. (2006) recorded negative correlation between moss N concentration and abundance. Similar results were obtained by Gordon *et al*. (2001) in High Arctic Heath. Swedish experiments using ammonium nitrate showed that abundance of some species like *Hylocomium splendens* and *Pleurozium schreberi* declined strongly (Dirkse & Martakis, 1992). Mitchell *et a*l. (2004) studied the relationship between growth and tissue nitrogen concentration of epiphytic bryophytes and found that growth declined following an increase in atmospheric N deposition. Hallingbäck (1992) compared the occurence of 10 epiphytic moss species in southern Sweden to pre-1950. The author found that the majority of examined species are now restricted to regions with comparatively low air pollution and harmful impact is attributed mainly to sulphur and nitrogen oxides.

Despite the fact that atmospheric nitrogen deposition has increased in general, we have not seen any seasonal changes in depositions neither during our own investigation (Šoltés & Ciriaková, 2011) nor in published sources. Seasonal changes in the deposition of nitrogen are considered unlikely.

The impact of chemical effects of nitrates on mosses has been the subject of research of many researchers. Saxena (2006) found seasonal pattern of nitrate reductase activity in moss samples collected from Kainchi, India. The nitrogen metabolism is significantly affected by cadmium. Higher nitrate reductase activity were recorded during the monsoon after winter, whereas asharp decline was observed during the summer. Increasing nitrogen concentrations influence nitrogen cycling: nitrate – nitrite – ammonium - incorporation of ammonium into amino acids (Morgan *et al*., 1992). Increased nitrate applications induce decreasing nitrate reductase activity (Woodin & Lee, 1987). The cause of the seasonal changes in the bryophytes cover may also be competition relationships (Ilomets *et al*., 2010).

The most plausible reason for seasonal changes in nitrate concentration are nitrifying bacterial activity and this is indicated by Chemical Oxygen Demand (COD). COD test indicates organic water pollution, including biological contamination. In all the sites the COD is culminating in high summer, when maximal biological activities are assumed, decreasing gradually after this period (Tables 2, 4, 6).

The list of identified bacteria in sampled pools is presented in Table 8, restriction enzymes used are listed in Table 7. The genus *Nitrosomonas* is represented by the species *N. eurotropha* and *N. europeae* (Table 8). *Nitrosomonas* sp. and *Spirillum volutans* are ammonia-oxidizing bacteria, which oxidize ammonia to nitrites. *Nitrospira* sp., *Nitrobacter winogradskyi*, *Magnetobacterium bavaricum* and *Thermodesulfovibrio* sp. are nitrite oxidizing bacteria, oxidize nitrites to nitrates.

The ordination of nitrifying bacteria is positively correlated with dissolved oxygen and with redox potential (ORP), relevant species are seen on the right of the Fig. 2. Denitrifying bacteria reducing nitrates are seen on the left of the Fig. 2.

The first axis shows a 55,8% relationship between the bacteria composition and the physico-chemical features (Eigenvalue 0.277), this axis highly positively correlates with ORP (r=0.9686), chlorides (r=0.9409), dissolved oxygen (r=0.9252) and to a lesser extent with conductivity (r=0.9023) and pH (r=0.8877). Ammonia highly negatively correlates with this axis (r=-0.9996).

The second axis shows a 44.2% relationship of species-environment relation (Eigenvalue 0.220), these axes are highly positively correlated with sulphates (r=0.9278) and to a lesser extent with salinity (r=0.8092). This axis negatively correlates with COD (r=-0.9015).

Conductivity and pH levels are positively correlated (r = 0.9995), also pH levels and dissolved oxygen (r = 0.9960), whereas pH is negatively correlated with ammonia (r=-0.8995). Reduction potential (ORP) is positively correlated with dissolved oxygen (r = 0.9905) and negatively correlated with ammonia (r=-0.9748). Disolved oxygen is positively correlated also vith TDS (r=0.9705), while salinity is negatively correlated with COD (r=-0.9838). While using the ordination analyses, no environmental variable showed significancy (p>0.05).

Decomposition is a complicated process dependent upon many conditions. During the processes of decomposition, the nitrogen in proteins is transformed to ammonium (NH_4^+) by bacterial activity. This process is called ammonification. Other kinds of bacteria change ammonia to nitrite, and others can change nitrite to nitrate (nitrification). Cold temperatures and low nutrient supply can account for the low decomposition rates (Sand-Jensen *et al*., 1999).

A significant negative correlation between soil temperature and moisture content was observed while investigating the intensity of decomposition (Bahuguna *et al*., 2012). Tahvanainen *et al*. (2003) investigated seasonal variation of water chemistry in three boreal fens where they found that concentrations of total nitrogen had very little seasonal variation. The level of nitrogen mineralization is affected not only by ecological conditions, but also by phytocoenological ones. Nitrogen mineralization was significantly faster in bogs with a *Sphagnum* cover than in phanerogam-dominated fens (Verhoeven *et al*., 1990). In our case on the Belianske lúky meadows the *Sphagnum* cover is not present.

Mineral-rich wetlands not only have higher pH and base cation levels than mineral-poor wetlands, but are also generally considered to be more nutrient rich, because litter decay and nutrient turnover are faster. The increase in net N-mineralization in mineral-poor fens was mainly due to ammonium (Kooijman & Hedenäs, 2009).

Nitrates are being consumed by vegetation and in anoerobic conditions denitrifying bacteria reduce nitrates to molecular nitrogen or rarely to ammonia (Sorensen, 1978; Hussain *et al*., 1994; Griffiths & Cole, 1990). Nitrite ammonification takes place before the beginning of the growing season, so that the stocks of ammonia are recovered. Higher supply of ammonia may also be the result of the decomposition of organic material.

Table 7. Detected numbers of individual terminal restriction fragments (T-RFs) in samples.

Restriction enzyme	No. of T-RFs		
	Site 1	Site 2	Site 3
CfoI	24	30	48
MspI	22	28	83
RsaI	8	18	17

Table 8. Identified bacteria *(in silico)* in investigated sites [No. of T-RFs/%].

Organisms	Site 1			Site 2			Site 3		
	CfoI	MspI	RsaI	CfoI	MspI	RsaI	CfoI	MspI	RsaI
Nitrifying bacteria									
Nitrosomonas spp.		1/4.55		1/3.33				2/2.41	
Nitrospira sp.			1/12.50		2/7.14	3/16.68		4/4.81	1/5.88
Nitrobacter winogradskyi	1/4.17						1/2.08	1/1.20	
Magnetobacterium bavaricum								1/1.20	
Thermodesulfovibrio sp.			1/12.50			1/5.56			
Spirillum volutans				1/3.33					
Denitrifying bacteria									
Diaphorobacter nitroreducens	1/4.17			1/3.33				1/1.20	
Denitrovibrio acetiphilus				1/3.33					
Achromobacter denitrificans								1/1.20	
Alicycliphilus denitrificans								1/1.20	
Geobacillus thermodenitrificans					1/3.57			1/1.20	
Hyphomicrobium denitrificans						1/5.56	1/2.08		
Jonesia denitrificans								1/1.20	
Kingella denitrificans								1/1.20	
Unspecified denitrifying bacteria		1/4.55		1/3.33				2/2.41	

Recorded denitrifying bacteria are listed in Table 8. The largest number of denitrifying bacteria was recored in site 3, where we have found the lowest nitrate concentrations (Tables 2, 4, 6), in average of 0.52 mg L^{-1}, while in the other sites it was 0.89-1.29 52 mg L^{-1}. On this site have been recorded the highest concentration of sulphates (6.71 mg L^{-1}on average, Tables 2, 4, 6) and calcium carbonate (272.14 mg L^{-1}on average, Tables 2, 4, 6).

Pools desiccation: Desiccation or flooding could be important factors impeding the establishment of some species in the rich fens (Granath, 2012; Ilomets *et al*., 2010). Alteration of the water table may cause the retreat of sensitive species. The hydrophysical parameters and water flow in soils is significantly affected by vegetation cover (Lichner *et al*., 2013). Mälson & Rydin (2006) pointed to the high sensitivity of some rich fen bryophyte species that used to decrease rapidly after drainage, among them included *Drepanocladus cossonii* and *Campylium stellatum.* Thus we also showed that species show high sensitivity in relation to seasonal changes. Even small alterations of the water level (5 cm) resulted in differences in biomass growth of *Drepanocladus cossonii* and *Campylium stellatum* (Mälson & Rydin, 2006). Ilomets *et al*. (2010) came to different conclusions with respect to fluctuation of the water table. They found, that the cover of rich fen species decreased sharply as the seasonal fluctuation of water level rose to 25 cm. In our study of Belianske lúky meadows the depth of pools is about 25 cm in the spring months and in the hot summers the level drops to 10-15 cm and occasionaly full desiccation occurs.

The ground water level affects the nitrogen cycling as well as other physiological processes. Asada *et al*. (2002) found, that the deeper the groundwater table is, the more the nitrogen is depleted. The level of groundwater is subject to seasonal variations and mosses thrive better in moist, humid and wet environments. Aceto *et al*. (2003) and Ekpo *et al*. (2012) found, that levels of accumulated metal in the moss samples were higher during wet season than in dry season.

Understandably, the distribution of bryophytes is affected by other factors, such as permafrost (Turetsky *et al*., 2010), pH and conductivity (Wojtuń *et al*., 2013; Wu *et al*., 2001). In site 3, we've seen a smooth decrease of pH during the year whereas, in the other sites the regular seasonal changes of pH has not been detected.

Threat: All three glacial relic moss species *(Meesia triquetra, Catoscopium nigritum, Calliergon trifarium)* are endangered in the investigated area. In the past, the pools drying up was a rare event, but in recent times this has became a common phenomenon. An unstable water table is the most adverse factor for the vegetation of Belianske lúky meadows, but especially for glacial relic moss species. *Calliergon trifarium* is particularly sensitive to an unstable water table. Another potential threat to the glacial relic species is the degradation of the site by drainage of surrounding landscape and by the changes in the water regime related to the management of forests above the site.

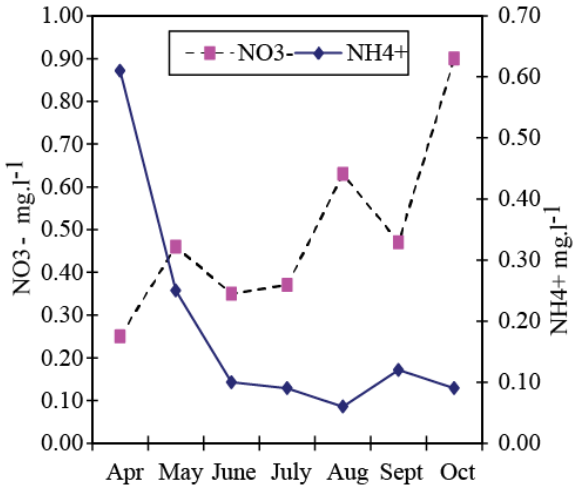

Fig. 1a. Nitrate and ammonia concentrations in the course of the year 2010 (Site 1).

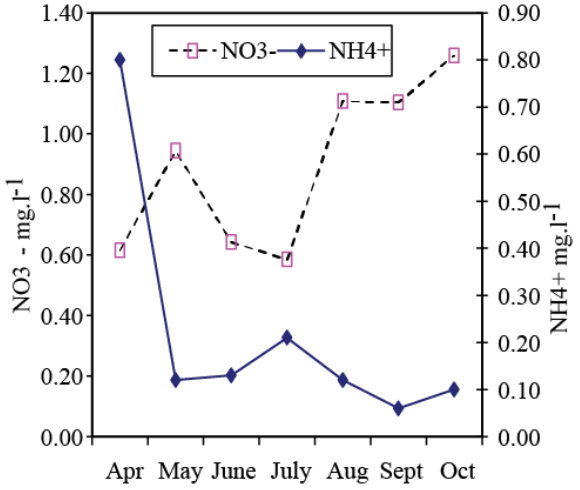

Fig. 1b. Nitrate and ammonia concentrations in the course of the year 2010 (Site 2).

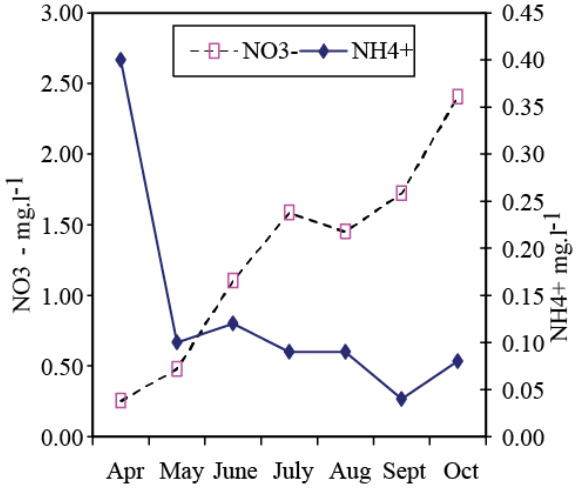

Fig. 1c. Nitrate and ammonia concentrations in the course of the year 2010 (Site 3).

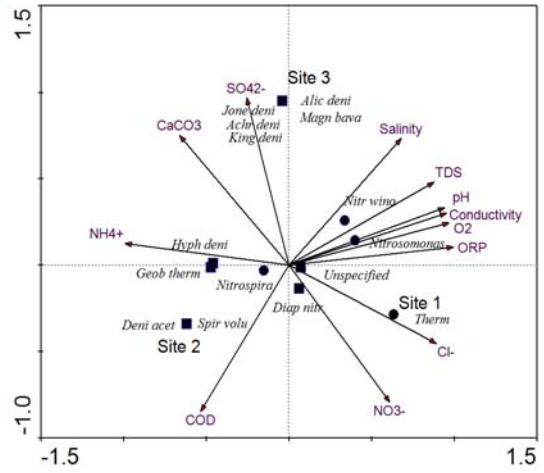

Fig. 2. Canonical correspondence analysis (CCA), triplot, ordination diagram of identified bacteria, physico-chemical features and sampling sites. Full squares – denitrifying bacteria (*Achr deni - Achromobacter denitrificans, Alic deni - Alicycliphilus denitrificans, Diap nitr - Diaphorobacter nitroreducens, Deni acet - Denitrovibrio acetiphilus, Geob term - Geobacillus thermodenitrificans, Hyph deni - Hyphomicrobium denitrificans, Jone deni - Jonesia denitrificans, King deni - Kingella denitrificans*), full circles – nitrifying bacteria (*Nitr vino - Nitrobacter winogradskyi, Magn bava - Magnetobacterium bavaricum,* symbol in the figure is overlapped by square*, Spir volu - Spirillum volutans*, symbol in the figure is overlapped by square), *Therm – Thermodesulfovibrio* sp.

Conclusion

The results of this study support the hypothesis that recent changes in the composition of the *Drepanocladus cossonii* and *Campylium stellatum* layers of the calcareous spring mire Belianske lúky meadows are caused by raised nitrogen levels in the course of the year. This is induced by synergic influence of decomposition of organic substances in soils and pools drying up. By comparing the obtained T-RFs with *in silico* T-RFs the presence was confirmed of nitrifying bacteria that change ammonia through nitrification to nitrite and subsequently to nitrate.

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