The Evaluation of Stable Isotopic Ratios δ^{13}C and δ^{15}N in Humic Acids along a Fen Peat Profile

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Abstract—**Mires are known as consistent environmental archives, but humic acids are the fraction of peat that is most recalcitrant and refractory to organic matter degradation, thus data on environmental changes during mire development can be recorded into them. This work was focused on the studies of stable isotopic ratios delta carbon-13 and delta nitrogen-15, and their distribution in humic acids within fen peat layers of different depths and peat composition. The variations in delta carbon-13 reflect isotopic variations in peat-forming plants over time and can be considered as a function of photosynthetic pathway that is being used to fix carbon dioxide. At the same time, variations in delta nitrogen-15 show nitrogen fixation in peat-forming plants and can be traced along with peat decomposition degree and depth. Properties of humic acids were studied in 2018 at 3 fens located in Latvia and Southern Finland, and comparatively they show properties. The method used for the determination of stable isotopes was the isotope-ratio mass spectrometry that was performed in the Faculty of Chemistry, University of Latvia. Results on delta carbon-13 indicate signal of C3 peat-forming plants, while signal of C4 peat-forming plants is not evident, which can be explained by non-efficient carbon dioxide fixation in fen vegetation. Results on delta nitrogen-15 show variations in nitrogen fixation in fen vegetation. Data show nitrogen fixation in terrestrial plants, however significant shifts in absolute isotope values indicate dependence on variations in peat decomposition degree and botanical composition. Data suggest that differences in peat botanical composition, decomposition degree and site dependent characteristics reflect in differences in delta carbon-13 and delta nitrogen-15 variations.**

Keywords— environmental changes, fen peat, humic acids, stable isotopes.

INTRODUCTION

The formation of raised bogs is directly linked to the atmospheric conditions during peat accumulation, while the development of fens is also significantly affected by terrestrial conditions, notably - groundwater composition. Therefore, mires are known as consistent environmental archives and peat profiles can be used to reconstruct past environmental conditions in both minor and major scale [1].

Humic acids are the fraction of peat that is most recalcitrant and refractory to organic matter degradation over time, thus data on environmental changes during mire development can be obtained from their analysis [1]. Fen peat humic acids form due to microbial decomposition of peat-forming plants in fens and, alongside fulvic acids and humin, are the major part of peat organic material.

The vegetation of fen ecosystems has developed in nutrient-rich conditions and thus fen peat consists of disintegrated and decomposed remains of higher vegetation, containing both C_3 and C_4 plants [2]. The majority of plants in fen vegetation are C_3 plants (most grasses, reeds, sedges etc.) and have no special features to combat photorespiration that initially reduces the efficiency of photosynthesis. The photorespiration takes place during plant metabolism when the enzyme of ribulose-1,5-bisphosphate carboxylate or oxygenase oxygenates ribulose 1,5-bisphosphate and in the process reduces photosynthetic output up to 25 %. At the same time, the C_3 carbon fixation is the most common of metabolic pathways for carbon fixation in photosynthesis [3], [4]. The minority of plants are C_4 plants (several reed and sedge species) and they differentiate from C_3 plants with the ability to minimize the photorespiration by separating the initial CO_2 fixation and the Calvin cycle. In the C_4 plants CO_2 typically is drawn out of malate (salt of malic acid, which is produced by plants), but not directly from the air. The Calvin cycle is the set of light-independent chemical reactions that take place in plant chloroplasts during photosynthesis after the energy has been captured from sunlight [4].

Therefore, it can be concluded that fen peat-forming vegetation is an assemblage of C_3 and C_4 plant communities and their signal can be found in fen peat humic acids in the form of stable isotope signatures.

Stable isotope variations in humic acids depend on the isotopic compositions of reactants, the pathways and kinetic modes of reaction dynamics, and physicochemical conditions [1]. The isotopic signature is unique to the

origin and history of the substance and thus each organic material has its own specific signature. To establish an isotopic signature for fen peat humic acids, the ratios of stable isotopes ^{13}C and ^{12}C, and ^{15}N and ^{14}N can be measured. The isotopic abundances of carbon and nitrogen were fixed when the planet was formed and, on a global scale, has not change ever since [5]. At the same time, the variation of isotopic composition of fen peat humic acids was introduced during various biological, chemical and physical processes within mire ecosystem and thus allow to monitor changes in the environmental and ecological settings due to peat-forming plant production. These variations in the natural abundance of stable isotopes usually are expressed using delta (δ) notion. Accordingly, - $^{13}C/^{12}C$ is expressed as δ¹³C but $^{15}N/^{14}N$ is expressed as δ¹⁵N. The delta values are commonly reported in parts per thousand (‰). One of the most common techniques for measurements of the relative abundance of stable isotopes in organic materials, including humic acids, is the isotope-ratio mass spectrometry [5].

The variations in δ¹³C values typically reflect isotopic variations in plants over time, the signature of an inorganic source, organic carbon burial and the type of vegetation, and can be considered as a function of photosynthetic pathway that is being used to fix CO_2. The δ¹³C value most commonly is affected by primary production and organic carbon burial [6]. Plants typically take up lighter ^{12}C isotope and depending on metabolic pathways have the δ¹³C value around -25 ‰. An increase in this value usually indicates decrease in the production of vegetation. The δ¹³C value in fen peat humic acids can be described as a function of the amount of organic carbon burial. After the burial more ^{12}C isotope is locked out of the cycle in peat. The C_3 and C_4 plants have different δ¹³C signatures [7]. In C_3 plants the δ¹³C is in the range between -33 and -24 ‰, while in C_4 this parameter is ranging between -16 and -10 ‰ [8].

The δ¹⁵N values of a variety of nitrogen pools in vegetation depend on its source nitrogen in the fractionation of ^{15}N and ^{14}N during nitrogen assimilation, and on nitrogen transport within and nitrogen loss from plants. Typically, the δ¹⁵N values reflect the interaction between nitrogen sources and metabolic δ¹⁵N fractionation [9]. The δ¹⁵N values for non-N_2-fixing plants are usually greater than for N_2-fixing plants and therefore differences in this parameter provide nitrogen transfer information between plants [10]. Positive δ¹⁵N values indicate ^{15}N enrichment, whilst negative values show ^{15}N isotope depletion in plants. However, there is an uncertainty about the meaning of particular δ¹⁵N values due to nitrogen pool mixing and fractionation [11]. Regarding fen peat humic acids, δ¹⁵N can be used to detect the assimilation of NH_3 by peat-forming plants [11]. Nitrogen is lost from peat-forming plant by efflux reaction from roots and by volatilization from plant leaves [11]. Part of nitrogen is also transformed during assimilation, amino acid synthesis and protein turnover [11].

This work was focused on studies of stable isotope ratios δ¹³C and δ¹⁵N, and their distribution in humic acids

within fen peat layers of different botanical composition, depth, decomposition degree and research site to detect differences in humic acids of peat of different origin.

MATERIALS AND METHODS

Properties of fen peat humic acids were studied in 2018 at 3 fens located in Latvia and Southern Finland. Fen peat samples were collected in two Latvian mires – Opulu mire (56°25'24"N and 28°10'22"E) and Viku mire (56°30'58"N and 22°54'32"E), with an additional fen peat sample from Southern Finland (Table 1).

TABLE I. Basic properties of sampled fen peat

Mire	Parameters		
	Depth range, m	Type	Decomposition degree
Finnish peat	0.50 – 1.25	Grass-sedge	29
Finnish peat	1.25 – 1.50	Grass-sedge	29
Finnish peat	1.50 – 1.75	Grass-sedge	40
Finnish peat	1.75 – 2.00	Grass-sedge	40
Opulu mire	0.00 – 0.50	Wood-grass	44
Opulu mire	0.50 – 1.00	Wood-grass	44
Opulu mire	1.00 – 1.50	Wood-grass	44
Opulu mire	1.50 – 2.00	Wood-grass	44
Viku mire	0.00 – 0.10	Wood	41
Viku mire	0.10 – 0.20	Wood	41
Viku mire	0.80 – 0.90	Grass	40
Viku mire	0.90 – 1.00	Sedge	27

Peat botanical composition and decomposition degree were determined microscopically according to the methodology suggested by Lishtvan and Korol (1975) [12].

Fen peat was treated with 0.1 M KOH extracting solution at room temperature and shaken for 24 h and filtered. After this procedure the alkaline extract was acidified with 50 % H_2SO_4 to pH 1.5 and allowed to precipitate for 48 h in cold storage at 4°C. Humic acids were precipitated from acidified solution and washed until neutral pH.

The method that was used for the determination of stable isotopes in humic acids was the stable isotope-ratio mass spectrometry (SIRMS) that was performed at the faculty of chemistry, University of Latvia. The analyses were carried out with Nu Horizon SIRMS using certified reference materials USGS-40 and USGS-41 (L-Glutamic acid). The δ¹³C values were expressed relative to VPDB (Vienna Pee Dee Belemnite) and the δ¹⁵N values relative to AIR. For internal quality control L-Glutamic acid (analytical grade, Sigma-Aldrich) as laboratory reference material was used after each ten samples. For the SIRMS measurements ~1000 ± 50 µg of samples were

weighed in to tin capsules (5 x 9 mm, EuroVector) and carefully pressed in to a ball (<4 mm) and inserted in to autosampler. For the determination of $\delta^{13}C$ and $\delta^{15}N$ values, the isotope-ratio mass spectrometry measured the ratio of ions that correspond to CO_2 and N_2 gasses [5]. Fen peat humic acid samples underwent combustion in O_2 atmosphere. The molecules of evolved gasses CO_2 and N_2 were ionised, but ions were separated and detected in the mass spectrometer.

Results and Discussion

Properties of studied fen peat comparatively show similar botanical composition between study sites, while peat decomposition degree is rather varied (Table 1). Finnish fen peat consist of grass and sedge remains with the decomposition degree between 29 and 40 %. At the same time, Opulu mire peat contain remains of wood and grass with the decomposition degree of 44 %, but Viku mire peat contains wood, grass and sedge remains with the decomposition degree between 27 and 41 %. In the case of Finnish peat, samples seem to have higher decomposition degree with an increase of depth range, while Viku mire peat shows the opposite. The variations of $\delta^{13}C$ in studied Finnish (Fig. 1), Opulu mire (Fig. 2) and Viku mire (Fig. 3) humic acids reflect isotopic variations in peat-forming plants over time and can be considered as a function of photosynthetic pathway that is being used to fix CO_2. Although overall range of $\delta^{13}C$ values amongst study sites is rather similar, the distribution of stable carbon isotope in humic acids varies with peat parameters.

The $\delta^{13}C$ value in Finnish fen peat humic acids is between -27.5 and -27.2 ‰ and represent CO_2 fixation in C_3 plants and humic acids from top peat layers seem to have more stable carbon isotope than bottom peat layers, that can be explained by the production of current vegetation in this mire and lower peat decomposition degree.
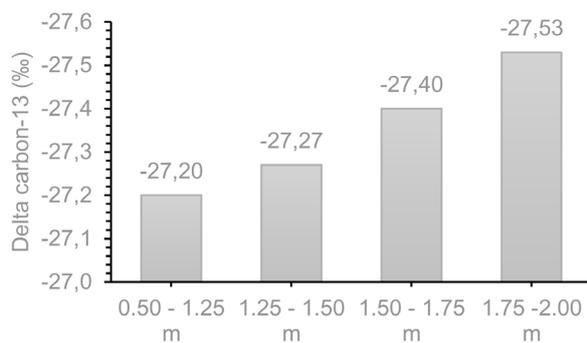

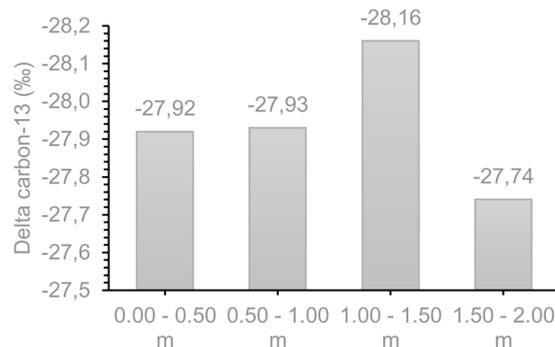**Fig. 1.** Delta carbon-13 values of Finnish fen peat humic acids.

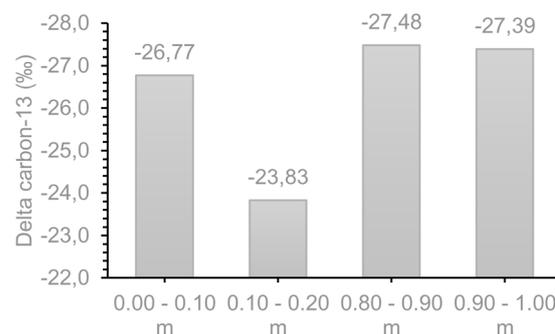**Fig. 2.** Delta carbon-13 values of Opulu fen peat humic acids.

Fig. 3. Delta carbon-13 values of Viki fen peat humic acids.

The value of $\delta^{13}C$ in Opulu mire humic acids vary between -28.2 and -27.4 ‰, which overall is very similar to Finnish fen peat humic acids, and the distribution of $\delta^{13}C$ seems to follow the pattern that can be traced in Finnish fen peat humic acids, with the exception of bottom peat layer, that can be explained with differences in the origin of both mires. The value of $\delta^{13}C$ in Viki mire fen peat humic acids is between -27.5 and -23.8 ‰ and thus it is rather different from carbon isotopes in Finnish and Opulu mire humic acids. Moreover, the $\delta^{13}C$ distribution pattern in Viki mire humic acids due to complex botanical composition and rather varied peat decomposition degree does not follow any particular pattern.

Variations in $\delta^{15}N$ in studied Finnish (Fig. 4), Opulu mire (Fig. 5) and Viku mire (Fig. 6) humic acids show nitrogen fixation in peat-forming plants and seem to be site dependent as data show significant differences amongst study sites, depth range, peat botanical composition and decomposition degree. Moreover, due to differences in the environment amongst study sites there can be noticed both enrichment and depletion of ^{15}N isotope that are of different rates depending on botanical composition and properties of particular peat, - most likely due to the ratio between remains of N_2-fixing and non-N_2-fixing plants and nitrogen transformation during assimilation, amino acid synthesis and protein turnover.
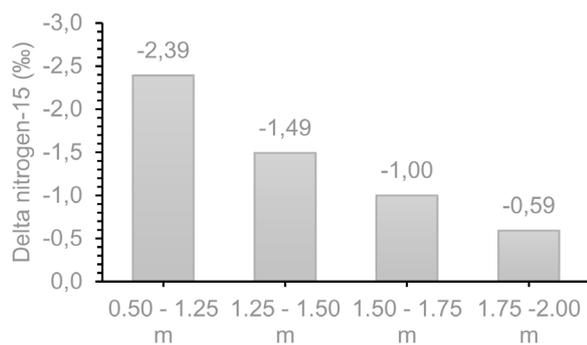
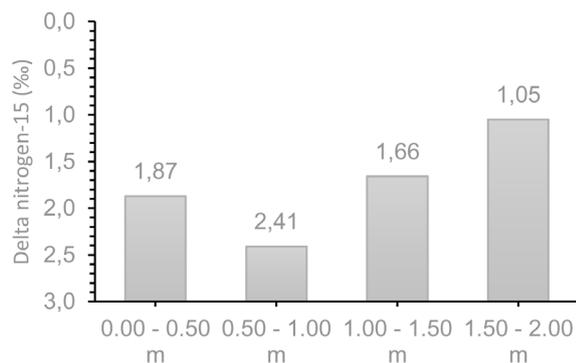**Fig. 4.** Delta nitrogen-15 values of Finnish fen peat humic acids.

Fig. 5. Delta nitrogen-15 values of Opulu fen peat humic acids.

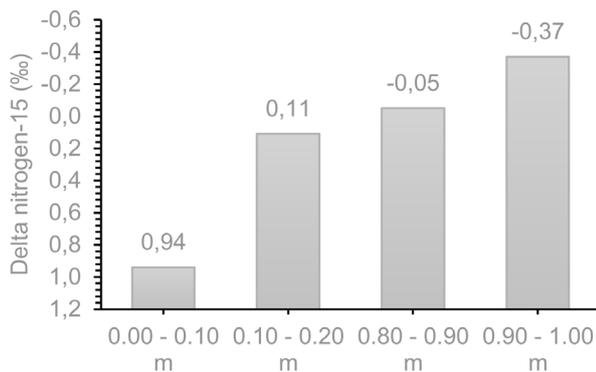**Fig. 6.** Delta nitrogen-15 values of Viki fen peat humic acids.

The $\delta^{15}N$ value in Finnish fen peat humic acids varies between -2.4 and -0.6 ‰ that describes stable ^{15}N isotope depletion, the most active nitrogen depletion is characteristic to the top part of peat profile and seems to be reversely proportional to ^{13}C isotope in Finnish peat humic acids. The $\delta^{15}N$ value in Opulu mire fen peat humic acids vary between 1.0 and 2.4 ‰ and indicates the ^{15}N enrichment throughout full peat profile that might be caused by nitrogen stress and the fractionation of stable nitrogen isotopes and/or by mixing of nitrogen from internal and external sources (anthropogenic activity). The $\delta^{15}N$ value in Viki mire fen peat humic acids is between -0.4 and 0.9 ‰ and shows on both enrichment and depletion of ^{15}N. The depletion can be identified in the bottom part of the peat profile, while the top part shows an enrichment that might be caused by the anthropogenic activities (agricul-

ture) in the local area. Data on $\delta^{15}N$ distribution in Opulu and Viku mire humic acids show no relation with $\delta^{13}C$ distribution in these humic acids.

CONCLUSIONS

Results suggest that differences in peat botanical composition, decomposition degree and site dependent organic matter transformation into humic acids also reflect in differences of $\delta^{13}C$ and $\delta^{15}N$ distribution. Although data show typical nitrogen fixation in terrestrial plants, however significant shifts in absolute isotope values amongst study sites can be explained with variations in the botanical composition of fen peat material and human activities in particular areas.

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