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Characterisation of humic acids in boreal mires depending on a peat type

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The main question addressed in this study was the ongoing discussion about the humification of organic matter in different environments. We aimed at demonstrating the relation between organic matter transformation in boreal fens and raised bogs and related humic acid properties, depending on the precursor biota and environmental conditions. To highlight the differences between humic acids in different peat types, the main methods applied were based on the elemental and spectroscopic analysis and the comparison of the data obtained. The main findings pointed to differences in the concentration of aromatic compounds in humic acids, depending on peat type, i.e., the aromaticity of humic acids in fen peat was comparatively higher than that of humic acids in raised bog peat, which appeared to be related to fen peat formation from lignin-rich higher vegetation that provided aromatic macromolecules. While raised bog peat-forming bryophytes did contain molecules rich in phenols, the aliphatic compounds prevailed in the composition of humic acids. We found that fen peat humic acids were also more affected by carbohydrate degradation over time, leading to apparent functional differences between boreal fen and raised bog peat humic acids. The humification process itself seems to be initialized similarly in boreal fens and raised bogs, while comparatively higher aromaticity of fen peat humic acids appears to be related to high lignin content, which joins the humification process at a later stage of peat formation due to slow decomposition rate and thus it per se does not indicate a humification processes being different from raised bog peat. Albeit lignin involvement changes the microbial pathways to degrade the organic material in fens at a later stage, and in raised bogs due to uniform lower vegetation cover, the microbial pathways do not change significantly over time.

Introduction

The boreal biogeographical region within the European Union covers the Baltic States, Finland and most of Sweden. The present topography of the boreal region was formed due to the movement of the last glacier and its melting waters that formed the main relief macroforms, such as uplands and lowlands (Sundseth 2009). During the last ice age, the whole region was covered in ice, but approximately 10 000 years ago, when the ice retreated, on the bedrock carved by the ice first mires were formed which gradually became a major part of the modern boreal environment. The most extensive formation of mires in the boreal biogeographical

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region took place in the Boreal period approximately between 8 800 and 7 500 years ago, when the climate in northern Europe became warmer and more continental, allowing mire vegetation to spread and decompose.

Peat may be considered as a slowly renewable energy source, and it is considered a solid fossil fuel rather than a biomass fuel (Seyboth *et al.* 2012). The decomposition and diagenesis of peat-forming plant organic matter over millennia has resulted in the accumulation of carbon (C), making mires one of the major reservoirs for C worldwide, therefore peat humic substances (HSs) are playing a vital role in the global C cycle. It is estimated that mires store approximately one third of the soil organic C on a global scale (Falkowski *et al.* 2000). Studies on the character of HSs within peat of different origins and environmental settings are essential for understanding the biogeochemical cycling of C (Frimmel and Christman 1987).

The formation of peat types is due to varied environmental and climatic settings in mires. In the boreal biogeographical region, whilst mires are distributed throughout the whole region, the area they cover and the diversity of vegetation in them are determined by the geomorphology of the site and local sedimentary cover in the adjacent area and beneath mires, as well as by atmospheric, hydrological and hydrogeological factors. For instance, in Latvia, approximately 35% of mires have formed as fens due to basin fill-in in the relief depressions, while the remaining 75% of mires have formed as raised bogs due to ground paludification on sedimentary rocks with low water permeability. The natural conditions and the biological diversity in Latvian mires essentially are determined by the geographical location of the country near the Baltic Sea, fragmented geological conditions and structured terrain. The climate in Latvia generally is affected by the Atlantic air masses and the Baltic Sea, which altogether cause high atmospheric humidity and moderate temperature regime, which supports mire development. In addition, in January, the range of temperature varies from –2.6°C to –6.6°C, but in July, the temperature varies from +16.8°C to +17.6°C, while the annual precipitation in Latvia is between 500–800 mm. Meanwhile, the vegetation period is observed when the daily average temperature exceeds +5°C, which begins in mid-April and lasts for approximately 180–200 days (Kalnina 1995).

Boreal fens are peat-forming mires with major nutrient-rich groundwater and surface water input. High availability of mineral nutrients results in the growth of higher vegetation, rich in lignin and cellulose. In addition, the high nutrient content in fen peat is directly related to decomposition processes in fens, that is — high nutrient content enhances peat decomposition rate. When the organic matter is broken down to humic substances and CO_2, a simultaneous release of nutrients is taking place, thus creating a fertile substrate for the development of a new generation of higher vegetation (Bodker *et al.* 2015). Accordingly, fen peat can be described as a complex of disintegrated, moderately-to-highly decomposed higher plant remains with an additional mineral matter content. Varied overall environmental settings in fens, depending on the location, lead to differences in fen peat composition, such as the botanical composition, fibre content and the decomposition degree, as well as in HS content and properties (Bragazza *et al.* 2007). In fact, the fibre content and the decomposition degree of fen peat are essential not only for HS content, but also for the quality and properties of HSs. Boreal fen vegetation, like tropical bog vegetation, consists of rather diverse higher vascular plants (Keddy 2002, Ptereka *et al.* 2017). Those commonly include different types of grasses, sedges, reeds, shrubs and trees that is the basis of hemic-to-sapric peat. However, due to obvious differences in the climatic and environmental conditions, there are major differences in plant species between tropical bogs and boreal fens. For example, in the early stage of fen formation and development in Latvia, common reed (*Phragmites australis*) and sedge species (*Carex dioica, C. nigra, C. panicea*, etc.) dominated in the vegetation cover and their remains are now accumulated in thick continuous fen peat layers in mire profiles. The majority of fens in Latvia were formed due to overgrowing of lakes, lagoons, rivers and their meanders, thus they compromise gyttja (sapropel) sediments at the bottom of peat layer. Fen peat formation and accumulation in the boreal environment took place in close-to-neutral pH conditions and is significantly affected by the local groundwater composition. Therefore, fen peat HS properties are expected to be different from those in raised bog peat without groundwater impact, because when peat accumulates above the mineral groundwater, it becomes increasingly isolated from this nutrient source (Klavins *et al.* 2009; Silamikele *et al.* 2010b; Silamikele et al. 2011). The impact of groundwater on the overlying peat layers is generally reflected in respect to elemental accumulation and correlates with the elemental concentration in peat, especially with the elements of natural origin, such as Fe and Mn. Peat layers that are in the contact with groundwater have different composition than those without groundwater impact, thus there is a difference in these parameters between boreal fens and raised bogs. At the same time, given the natural range of ecological and hydrological conditions across different types of mires, it is not clear if the exact pattern will always be consistent (Macrae *et al.* 2012). The concentration of Fe^{3+} and Mn^{4+} in peat is important when it comes to the decomposition of lignin in fens as these metals can be involved in the microbial pathways to decompose lignin. As shown by Krumins (2016), Fe concentration (mg kg^{-1}) in top peat layers (above groundwater level) of studied fens were somewhat different from the rest of peat profiles. For instance, in the first 0.20 m of the Viku mire (Latvia) peat profile, the Fe concentration was from 2600–4700 mg kg^{-1}, while below the groundwater level it was approximately from 400–500 mg kg^{-1} in average. In addition, differences could be noticed also in the Mn concentration, from 23–60 mg kg^{-1} above the groundwater level and from 2–20 mg kg^{-1} below the groundwater level (Krumins 2016).

Raised bogs in the boreal region are peat-forming mires with the main contribution of moisture from atmospheric precipitation. The low overall availability of nutrients leads to the growth of lower vegetation (Schaffhauser *et al.* 2016). However, atmospheric precipitation has a complex chemical composition that varies geographically and seasonally, which consequentially may change nutrient availability in raised bogs and allow the introduction of new plant species. At the same time, considering the raised structure of a bog, essential mineral nutrients cannot flow up onto it and thus their content is scarce and therefore bog vegetation has to develop with nutrient shortage. Raised bog peat may be described as a complex of disintegrated, weakly-to-moderately decomposed lower plant remains with a minor mineral matter content. Raised bog vegetation commonly consists of a variety of bryophytes. For the major part, those are different *Sphagnum* species that form fibric-to-hemic peat. At the beginning of raised bog formation in Latvia due to ground paludification around 10 000 years ago, the first common plant species were bog-myrtle (*Myrica gale*), swamp sawgrass (*Cladium mariscus*), water caltrop (*Trapa natans*), swamp willow (*Salix myrtilloides)* and bog orchid (*Hammarbya paludosa*). The further development of raised bogs in Latvia was associated with a rapid increase of various *Sphagnum* species. The species in the vegetation were primarily determined by the local microclimate (Kalnina 1998). The formation of raised bogs in the boreal environment was causally linked to the atmospheric conditions during the peat accumulation process, and it took place in acidic and anaerobic environments (Zaccone *et al.* 2018a). The main factors that were promoting mire formation in Latvia, especially due to ground paludification and formation of raised bogs, were local geological processes, geomorphology and sedimentary cover. Due to these factors, the most paludified areas in Latvia are now located in the continental lowlands where the relief is relatively flat and covered with till, glaciolimnic clay and silt with low water permeability.

The information provided by peat parameters is of high importance for analysing the development of boreal environment, and the impact of climate change and anthropogenic activities on the environment, as mires contain records of global and local changes (Zaccone *et al.* 2007b). Peat types in boreal mires differ from each other by composition and properties. In general, peat is a dark-coloured and mostly organic material, which is mainly formed in water-saturated and anaerobic mire environment. However if peat decomposition was restrained, its colour is light, from white to brownish grey. In the boreal region, there are profound differences between raised bog and fen peat. Fen peat contains average to highly decomposed lignin-rich plant remains, but the raised bog peat commonly consists of lignin-lacking bryophytes with a low to average decomposition degree. The main component of peat is HSs, which is the product of peat-forming plant decomposition. The HSs consist of three major groups that can be distinguished depending on their solubility in the environment. These groups are humin, which is insoluble; fulvic acid (FA), which is soluble at all pH conditions; and it is humic acid (HA), which is soluble at pH below 2, the latter being analysed in this paper. The HSs belong to a general category of naturally occurring, heterogeneous, organic substances of high molecular weight and refractory to degradation (Stevenson 1994). Recent discussions about HSs stress the importance of defining an operational approach for their isolation with alkaline extractants from organic sediments and other sources. The most often used extractants are sodium hydroxide (NaOH) and potassium hydroxide (KOH) (Olk *et al.* 2019). It is estimated that HSs can form as much as 60–85% of the organic matter in peat. However, the extraction yield can also be much lower, depending on the source material (e.g., *Carex* vs. *Sphagnum* peat) (Garcia *et al.* 1993, Zaccone *et al.* 2007b, Yu *et al.* 2012, Zaccone *et al.* 2018a). Presently, studies on organic matter humification processes become increasingly important and are heatedly debated (Tfaily *et al.* 2014, Hodgkins *et al.* 2018). There are heated discussions regarding HSs and their concept, such as studies by Kleber & Lehmann (2019) and others, where HSs are considered as an artifact of organic matter. Indeed, there is growing evidence expressing doubts on the soil organic matter concept as there is an issue to apply this concept to all environments where refractory part of the natural organic matter is formed. However, it is especially important to consider that HSs nowadays are industrially produced in thousands of tons, at first for agricultural applications using peat as the source material. Thus, HSs properties remains a crucial topic for studies. A study of the HS properties, especially the characteristics of HAs extracted from different organic sources, allows to identify source-specific properties and differentiate not only the HAs of varied origins but also variations in their properties, providing a valuable technological aid for many industrial sectors, including for the development of environmentally friendly technologies and applications.

However, regardless of their origin, peat HAs form as the final product of microbial pathways *via* degradation and transformation of organic matter and synthetic oxidation of phenolic compounds (Zaccone *et al.* 2018b). Notably, not only the source of peat, but also the decomposition conditions (e.g., alternative than O_2 electron acceptors) are important for the content and structure of peat HAs. The industrial importance of peat HAs is related to their properties and capacity to interact with other substances. Peat HAs are able to interact with biopolymers, organic and inorganic compounds of low molecular weight, such as metallic elements, with which they form chelate complexes. Peat HAs are used in agriculture, environmental remediation, medicine and many other areas (Tipping *et al.* 2003). Among the key useful properties of peat HAs are carboxyl and hydroxyl groups in the composition, the redox and association-dissociation potentials and high ionised nutrient holding capacity that allow HAs to work as mediums for nutrient transportation from soil to plant (Nardi *et al.* 2002). Although the composition of HAs at present is not definitely determined, it directly bears on the ways of their commercial use. Therefore, the evaluation of peat HAs of different origins and peat types is of major importance not only for extension of the scope of use but also for more efficient utilisation of local natural resources.

This paper offers original data on the properties of fen peat HAs and compares them to raised bog peat HAs that have been studied in high detail in boreal mires both locally and worldwide (Stevenson 1994, Tipping *et al.* 2003, Zaccone *et al.* 2007a, Kuske *et al.* 2010, Dudare and Klavins 2012, Tfaily *et al.* 2014, Schaffhauser *et al.* 2016). The study was aimed to establish the difference between peat HAs formed of lignin-lacking vegetation and those formed of lignin-rich higher vegetation and advocated the use of spectroscopic methods in the determination of properties of HAs of various origins. The aim of the present study was to contribute to the discussion regarding the relevance of soil organic matter concept, demonstrating the relation between organic matter transformation and HS properties, gradual transformation processes depending on the precursor biota, environmental conditions and other aspects. The study was mainly focused on the differences in the structure and properties of HAs, depending on the peat type, to determine possible differences in different peat types in the boreal mires.

Material and methods

Study Site

In this study, we analysed fen peat and HAs from fen peat. Raised bog peat was analysed by Kuske *et al.* (2010), whereas raised bog peat HAs were extracted by Dudare and Klavins (2012). A total of 12 fen and 12 raised bog peat samples and related HAs were analysed. The HAs were isolated from common types of boreal fen and raised bog peat in mires dominated by the *Sphagnum* and *Carex* species. Two boreal fens that were chosen for the study was Viku mire (56°30′58′′N, 22°54′32′′E) and Opulu mire (56°25′24′′N, 28°10′22′′E). Two raised bogs used for data comparison were Dzelves bog (57°13′’58′′N, 22°54′’32′′E) and Eipurs bog (57°14′53′′N, 24°37′00′′E). Peat sampling for HA isolation was performed at different depths in mire profiles, representing different fen and raised bog peat layers. Fen peat sampling in raised bogs were excluded due to significant changes in the environmental conditions that led to change in the type of mire, which can have significant impact on peat layers.

All the chemicals used in this study were of analytical grade, and all statistical correlations ($n = 22$) had a significance level of $p < 0.05$. Peat sampling for HA isolation was done in three spatial subunits at each site during a single visit. To reduce false variability of experimental results, absence or presence of data, the laboratory analysis was done on three sample replicates using the standardised methods listed below.

Peat botanical composition and decomposition degree

Peat decomposition degree was determined by the centrifugation method according to the GOST 10650-72 (1974) standard using Sigma-Aldrich Hettich universal 320 centrifuge (USA) and expressed as a percentage of the total peat sample mass.

The botanical composition of peat was determined with a Carl-Zeiss binocular microscope (Germany) at 56/140x magnification according to the GOST 28245-89 (1990) standard. The principle of this analysis was to identify the percentage of specific plant residues in a defined area, where the total of all recognised plant remains constituted 100% (Malterer *et al.* 1992, Krumins *et al.* 2012).

Loss-on-ignition analysis

Peat decomposition degree was determined by the centrifugation method according to the GOST 10650-72 (1974) standard using Sigma-Aldrich Hettich universal 320 centrifuge (USA) and expressed as a percentage of the total peat sample mass.

Radiocarbon dating

The ^{14}C dating was done using the conventional liquid scintillation counting technique with the liquid scintillation counter LKB (Wallack, Australia) following the standard AAA (acid-alkali-acid) procedure. The peat samples were treated with 0.5 M HCl at 50°C for 2 h (acid) and with 0.1 M NaOH at 50°C for 2 h (alkali) (Bronic *et al.* 2009). The absolute age was calculated using the Clam ver. 1.0.2 add-on for R ver. 2.11.0 software (Reimer *et al.* 2009, Blaauw 2010).

Humic acid extraction procedure

The HAs were extracted from 12 fen and 12 raised bog peat samples, following the procedure suggested by the International Humic Substance Society (Swift 1996). Under the

N_2 atmosphere, 0.1 M KOH was added to a ground, oven-dried (air convection drying oven, Nabertherm, Germany) peat sample until the final extractant-to-peat ratio of 10:1. The suspension was extracted with intermittent shaking for 4 h and allowed to settle overnight, then centrifuged (a Sigma-Aldrich Hettich universal 320 centrifuge, USA) and filtrated. The solution was acidified with 6 M HCl with constant stirring to pH = 1.0 and allowed to settle for 12 h and centrifuged and filtered again to separate the precipitated HA, which was then re-dissolved with 0.1 M KOH under N2 atmosphere. KCl powder was added to the solution to attain the concentration of 0.3 M [K+]. The obtained solution was centrifuged and filtered to remove any suspended solids left in the liquid. The HA was then re-precipitated from the filtrate by adding 6 M HCl, with constant stirring to pH = 1.0. The acidified solution could settle for 12 h, then centrifuged and filtrated. Even though the repeated extraction can help to increase the yield of HAs; however, a single extraction was preferred. This was chosen, because during repeated extraction the hydrolytic processes can prevail which can then increase the yield of FAs, which is not desirable.

To remove ash, the HA precipitate was suspended in 0.1 M HCl solution, shaken overnight at room temperature and then centrifuged. The treatment of HAs with 0.1 M HCl is well known approach for ash removal and the efficiency can be tested using the ashing method at 900°C. Finally, the HA precipitate was dialysed against distilled water and freeze-dried. The yield of HAs was measured by the gravimetric method. The concentration of HAs was calculated as the difference between a filter paper with precipitated HAs and an empty filter paper and expressed as grams of HA per g of dry and ash free peat (Dick *et al.* 2002).

Elemental analysis

The total C, H, N and S concentrations in HA samples were determined in triplicate on freeze-dried samples, using dry combustion with an elemental analyser Fison Model EA-1108 (Carlo Erba Instruments, Italy). The analyser was calibrated using BBOT [2,5-Bis-(5-tert-butylbenzoxazol-2-yl)-thiophen] and the phenanthrene-enriched standard (Fison/Carlo Erba Italia). To limit potential errors in the elemental data, the removal of hygroscopic water-adhering HA molecules was performed overnight at 105°C prior to analysis. The O concentration was calculated by the difference (O% = 100 – (C + H + N + S)%. The values obtained were corrected for ash and moisture content. The result of the elemental analysis was used to calculate the elemental ratios (i.e., N/C, O/C and H/C atomic ratios), the degree of oxidation (ω) and the index of hydrogen deficiency (φ) (Eqs. 1 and 2) (Fong and Mohamed 2007):

$$\omega = (2O + 3N) / (H/C) \quad (1)$$

$$\varphi = ((2C + 2) - H) / 2 \quad (2)$$

The concentration of total organic C (TOC) in peat HAs was calculated as the difference between total C and inorganic C, both of which were determined using an elemental analyser Shimadzu (Japan) TOC Analyser TOC-VCSN with urea analytical standard. The calibration of data was done with a solid sample. Precautions taken to avoid interferences included combustion at high temperature in an O-rich atmosphere. The TOC analyser was calibrated in the range of 0.5–100 mg L^{-1} for organic and inorganic C.

Fourier-transform infrared spectroscopy

The Fourier-transform infrared (FTIR) spectra were recorded in the transmittance mode using a Perkin Elmer (USA) Spectrum BX FT-IR spectrophotometer. Potassium bromide (KBr) pellets (in triplicate) were obtained by pressing under vacuum 30 mg of a homogenised mixture of infrared-grade KBr and HA (ratio 10:1). Spectra were recorded in the wavelength range of 4000–450 cm^{-1} with a 4 cm^{-1} resolution. The results of FTIR spectroscopy were used to calculate the A1610/A2920 and A3400/A1610 IR light absorption ratios. The FTIR spectroscopy provided noteworthy information about the reactivity and structural arrangement of functional groups in peat HAs.

Ultraviolet-visible light spectroscopy

The ultraviolet-visible light (UV/Vis) spectroscopy was conducted in triplicate on solutions of 10 mg L^{-1} of dried and ash free HA at a pH of 7.0. Solutions were prepared by dissolving 10 mg of HA in 10 mL of 0.05 M NaOH. The supernatants were stirred under the N_2 atmosphere for 30 min, then 10 mL of 1 M HCl were added. The pH of solutions was adjusted to 7.0 by addition of 0.1 NaOH and bringing the total volume of the solution to 100 mL with deionised distilled water. The supernatants were diluted 1:10 with 1 M KCl at pH 7.0. The UV/Vis spectra were recorded on a Thermo spectronic Helios γ UV (ThermoFisher, USA) spectrophotometer in 1 cm quartz cuvettes. Finally, the E_{465}/E_{665}, E_{280}/E_{472} and E_{280}/E_{664} absorbance ratios were calculated based on the UV/Vis data obtained (Chen *et al.* 1977, Uyguner and Bekbolet 2005, Albrecht *et al.* 2011).

Fluorescence spectroscopy

The fluorescence spectroscopy was conducted on samples that were prepared for the UV/Vis spectroscopy. The fluorescence spectra of peat HAs were recorded using a Perkin Elmer (USA) LS 55 fluorescence spectrometer. The emission (Em) spectra were recorded with a scanning speed of 500 nm min^{-1} in the wavelength range of 380–650 nm, with the fixed excitation (Ex) wavelength of 350 nm. The ratio of fluorescence intensity at 460 nm and 510 nm (I_{460}/I_{510}) was considered as the humification index.

Excitation-emission matrix fluorescence spectroscopy

The excitation-emission matrix (EEM) fluorescence spectroscopy was conducted in triplicate (in 1 cm-path-length cuvettes) on solutions prepared for the UV/Vis spectroscopy. The fluorescence EEMs were measured using a Horiba (Japan) Aqualog total luminescence spectrometer (Ex: 250–600 nm at 5 nm increments and a 5 nm bandwidth; Em: 250–600 nm at 5 nm increments and a 5 nm bandwidth). The laboratory reagent blank was made of distilled water. The fluorescence signals were normalised to the lamp reference intensity, with spectral corrections applied by the instrument software. The EEMs obtained were a three-dimensional scan showing a contour plot of Ex and Em wavelengths and fluorescence intensity. The EEMs provided a molecular fingerprint for organic samples and reflected fluorescent components therein. The EEMs were combined with parallel factor (PARAFAC) analysis, which was a multivariate software method considered as a standard algorithm to process EEM data (Bro 1997). The Em loadings of fluorescence data that follow PARAFAC models were estimates of the real Em spectra of real fluorophores (Bro 1997). The PARAFAC analysis was performed using the MATLAB R2014a ver. 5.3.0.532 software with a drEEM toolbox used for the correction for inner filter effects. The fluorescence intensity was converted to Raman units, and the blanks were subtracted (Lawaetz and Stedmon 2009, Kothawala *et al.* 2013, Murphy *et al.* 2013, Krumins 2018). In the PARAFAC analysis, EEMs were decomposed into independent components that can be linked to different functional compounds (Murphy *et al.* 2013). A four-component model was generated for peat HAs and validated by the residual examination and split-half analysis.

^{13}C nuclear magnetic resonance spectroscopy

The solid-state cross-polarisation magic angle spinning ^{13}C nuclear magnetic resonance (NMR) spectra were obtained using a Bruker Avance (Germany) Wide-bore 600 MHz Solid-state NMR spectrometer, equipped with a 4 mm magic-angle spinning (MAS) double-resonance probe and having a contact time of 2 ms and a recycle delay of 2 s. The spectra were baseline-corrected and calibrated relative to Adamantane (left peak) at 38.48 ppm. Solid-state ^{1}H-^{13}C CP/MAS NMR spectra were recorded on an 800 MHz Bruker Avance III HD spectrometer equipped with a 3.2 mm H/X MAS probe. The MAS frequency was set to 10 kHz, and the temperature was regulated to 298 K. Cross-polarisation (CP) from ^{1}H to ^{13}C was performed using a linear ramp from 98.0–78.4 kHz on ^{1}H, while the ^{13}C amplitude was held constant at 88.3 kHz. The CP contact time was 0.75 ms. Spinal64 decoupling was applied during acquisition with a ^{1}H radiofrequency (RF) field amplitude of 94.3 kHz and a pulse length of 5.1 µs. The acquisition time was 10 ms. The recycle delay was 2 s. The chemical shifts were referenced externally relative to the adamantane CH_2 group (at 38.48 ppm relative to TMS) (Morcombe and Zilm 2003).

Stable isotope-ratio mass spectrometry

The analysis by stable isotope-ratio mass spectrometry (IRMS) were carried out in triplicates with a Nu Horizon isotope-ratio mass spectrometry instrument (United Kingdom), using certified reference materials USGS-40 and USGS-41. The δ^{13}C values were expressed relative to Vienna Pee Dee Belemnite, the δ^{15}N values — relative to the air (atmospheric N_2). For internal quality control, L-Glutamic acid was used as a laboratory reference material after every ten samples. For the IRMS measurements, ~1000 ± 50 µg samples were weighed into tin capsules (5 × 9 mm, Euro Vector), carefully pressed into a pellet (< 4 mm) and introduced into an autosampler. For the determination of the δ^{13}C and δ^{15}N values, IRMS was used to measure the ratio of ions that correspond to CO_2 and N_2 gasses. Peat HA samples were subject to combustion in the O_2 atmosphere. The molecules of generated CO_2 and N_2 gasses were ionised, and ions were separated and detected in the mass spectrometer (Carter and Barwick 2011).

Results

Peat characterisation

Peat characterisation in this study was based on the assessment of peat botanical composition, decomposition degree and mineral matter content, all of which were of major significance for the HA content. Both fens and bogs chosen for the peat sampling, separation of HSs and isolation of HAs were dominated by living, peat-forming plants common to boreal mires. Viku fen was covered with woody and herbaceous plants (i.e., pine, birch, *Carex* species). The vegetation in Opulu fen was dominated by the *Carex* species. The sapric peat layers in both fens covered a dense layer of gyttja (sapropel) sediments, which indicated fen formation due to a waterbody overgrowing. Both raised bogs had a profound *Sphagnum* dominance in the current vegetation. According to the mire profile composition, it appears that Dzelves bog, containing fibric-to-sapric peat, was formed on sand deposits due to ground paludification, while Eipurs bog (fibric-to-sapric peat) originated as a fen ecosystem and had transformed into a raised bog over time (Kuske *et al.* 2010, Dudare and Klavins 2012).

The complete peat profile (2.50 m) of the Viku fen consisted of seven peat types (Fig. 1): wood, grass, sedge, wood-*sphagnum*, wood-grass, wood-sedge and sedge-*hypnum* peat, respectively. The fen peat profile (2.00 m) of Opulu fen consisted of wood-grass fen peat only. The most representative peat-forming plant species in the vegetation of both fens were birch, tufted sedge (*Carex elata*), woolly fruit sedge (*Carex lasiocarpa*), common reed (*Phragmites australis*) and broad-leaved cotton grass (*Eriophorum latifolium*), which are all common species to boreal fens. The complete peat profile (3.45 m) of Dzelves bog consisted of *Sphagnum*, wood-*Sphagnum*, wood-cotton grass and cotton-grass bog peat types (Fig. 1), while the Eipurs peat profile (4.70 m) contained layers of raised bog peat (0.00–3.40 m), transitional mire peat (3.40–3.45 m) and fen peat (3.45–4.70 m) (Kuske *et al.* 2010, Dudare and Klavins 2012). The most representative peat-forming plant species in the raised bog vegetation were pine trees, tussock cotton grass (*Eriophorum vaginatum*), the rusty bog mosses (*Sphagnum fuscum*) and the Magellanic bog mosses (*Sphagnum magellanicum*).

The decomposition degree of peat in both fens varied from 27–44% and followed similar pattern with highest decomposed peat being at the upper part of mire profiles. The decomposition degree of peat in raised bogs was comparatively similar, in Eipurs bog it was from 10–50%, while in Dzelves bog from 10–58%

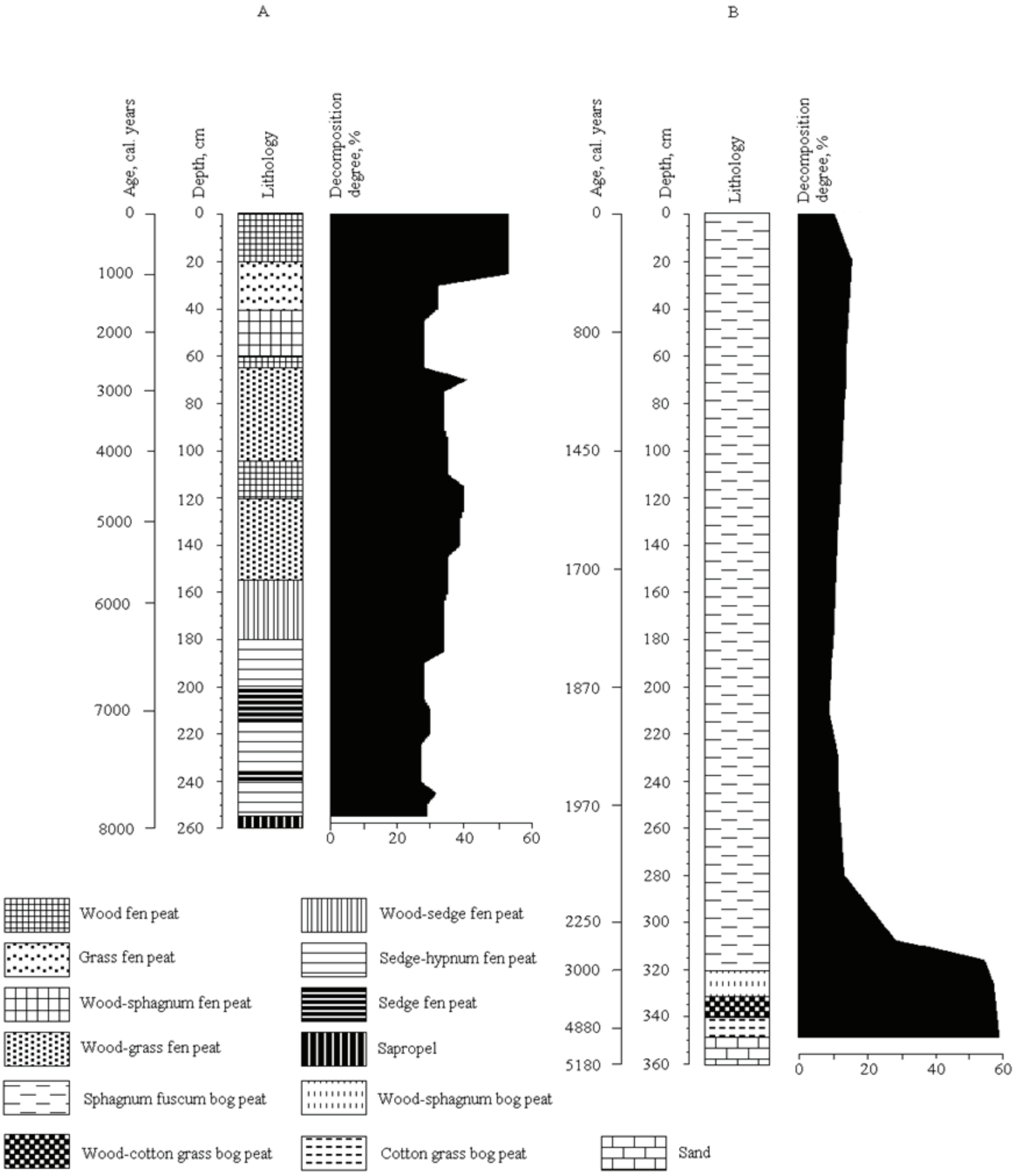

Fig. 1. The vertical cross-sections of the studied peat profiles: (a) Viku fen profile and (b) Dzelves bog profile. Representation of the peat subtype and peat decomposition degree changes due to changes in the environmental settings.

and the more decomposed peat was at the bottom part of both peat profiles. Differences between fen and raised bog peat were also noticeable in organic (Table 1) and inorganic matter content, which determined varied HS concentration and HA yield among different peat types. Especially notable differences were observed in the inorganic matter content. The mineral matter content in the peat samples from Viku fen varied from 2.4–30.1%, in the peat profile of Opulu fen — from 9.5–12.9%, while it did not exceed 5% in both raised bogs — from 1.4–4.1% in Eipurs bog and from 0.5–0.6% in Dzelves bog, respectively (Kuske *et al.* 2010, Dudare and

Klavins 2012). Highest mineral matter content in all mires were observed in peat from upper mire layers.

Humic acids in peat

Peat samples for HA extraction were selected depending on the differences in the botanical composition at different depth ranges and ages (Table 2). However, the results indicated no significant correlation between a peat type and the amount of HA in peat (r = 0.54 in fen peat and r = 0.31 in raised bog peat). At the same time, there was a connection between the peat age and HA content in fen peat (r = 0.79), while age seemed not to affect the amount of HAs in raised bog peat (r = –0.24). The organic matter content has shown a much higher impact on the HA content in peat. In fen peat, there was a strong correlation between peat-forming organic matter and HA content (r = 0.84); to a lesser extent, this could also be noticed in raised bog peat (r = 0.51). Yet, the results have shown no significant relation between the peat decomposition degree and the concentration of HAs. The gravimetric results indicated that the analysed fen peat contained from 0.02–0.70 g g^{-1} of ash free HA (Table 2), which was up to 80% of the total peat mass. The amount of HA in raised bog peat was around 0.50 g g^{-1} (Table 2) and did not exceed 50% of the peat mass.

Table 1. The organic matter (OM) content (%) in studied peat samples with increasing depth range: (**a**) Viku fen peat; (**b**) Opulu fen peat; (**c**) Dzelves raised bog peat; and (**d**) Eipurs raised bog peat.

Fen peat	Peat type	OM	Bog peat	Peat type	OM
A 0.00–0.20 m	Wood	69.9	C 0.00–0.10 m	*Sphagnum fuscum*	99.5
A 0.40–0.60 m	Wood-*Sphagnum*	93.3	C 0.20–0.30 m	*Sphagnum fuscum*	99.5
A 0.65–1.05 m	Wood-grass	95.7	C 0.40–0.60 m	*Sphagnum fuscum*	99.4
A 1.20–1.55 m	Wood-grass	97.0	C 0.70–0.80 m	*Sphagnum fuscum*	99.4
A 2.15–2.35 m	Sedge-*hypnum*	97.6	C 0.80–0.90 m	*Sphagnum fuscum*	99.4
A 2.40–2.55 m	Sedge-*hypnum*	97.4	C 0.90–1.00 m	*Sphagnum fuscum*	98.9
B 0.00–0.15 m	Wood-grass	87.1	D 0.00–0.25 m	*Sphagnum fuscum*	98.6
B 0.40–0.65 m	Wood-grass	89.9	D 0.50–0.70 m	*Sphagnum fuscum*	98.2
B 0.80–1.10 m	Wood-grass	89.1	D 1.20–1.35 m	Cotton grass-*Sphagnum*	97.9
B 1.10–1.50 m	Wood-grass	90.5	D 1.70–1.90 m	Cotton grass-*Sphagnum*	98.1
B 1.50–1.80 m	Wood-grass	90.3	D 2.20–2.30 m	*Sphagnum fuscum*	96.2
B 1.80–2.00 m	Wood-grass	90.2	D 2.40–2.50 m	Cotton grass-*Sphagnum*	95.9

Table 2. The humic acid (HA) content (g g^{-1} of dry and ash free) in the selected peat samples and their corresponding age (cal. years): (**a**) Viku HAs; (**b**) Opulu HAs; (**c**) Dzelves HAs; and (**d**) Eipurs HAs.

Fen peat HAs	Age	Content	Bog peat HAs	Age	Content
A 0.00–0.20 m	0–800	0.02	C 0.00–0.10 m	0–150	0.50
A 0.40–0.60 m	1 600–2 450	0.64	C 0.20–0.30 m	200–450	0.48
A 0.65–1.05 m	2 600–4 100	0.66	C 0.40–0.60 m	700–1 000	0.49
A 1.20–1.55 m	4 550–5 750	0.70	C 0.70–0.80 m	1 200–1 350	0.47
A 2.15–2.35 m	7 100–7 500	0.70	C 0.80–0.90 m	1 350–1 380	0.45
A 2.40–2.55 m	7 600–7 900	0.68	C 0.90–1.00 m	1 380–1 450	0.46
B 0.00–0.15 m	0–700	0.10	D 0.00–0.25 m	0–150	0.46
B 0.40–0.65 m	1 725–2 000	0.11	D 0.50–0.70 m	175–300	0.39
B 0.80–1.10 m	3 100–4 300	0.34	D 1.20–1.35 m	680–850	0.49
B 1.10–1.50 m	4 300–5 500	0.49	D 1.70–1.90 m	1 680–2 000	0.50
B 1.50–1.80 m	5 500–6 500	0.45	D 2.20–2.30 m	2 600–2 800	0.42
B 1.80–2.00 m	6 500–7 000	0.47	D 2.40–2.50 m	2 900–3 000	0.45

Peat humic acid elemental composition

The C content in fen peat HAs ranged from 49–53% (Table 3) of the total elemental content; in raised bog peat HAs — from 42–54% (Kuske *et al.* 2010, Dudare and Klavins 2012). The N content in fen peat HAs ranged from 1.6–4.9%, and in raised bog peat HAs from 1.8–2.8% (Table 3), showing a slight difference from fen peat HAs (Kuske *et al.* 2010, Dudare and Klavins 2012). The H content in the studied fen peat HAs ranged from 4.2–5.3% (Table 3), in raised bog peat HAs - from 4.1–5.4% (Kuske *et al.* 2010, Dudare and Klavins 2012). The O in fen peat HAs constituted around 39–43%, in raised bog HAs — 37.5–49.8%, again showing a minor difference from fen peat HAs (Kuske *et al.* 2010, Dudare and Klavins 2012). The S content in bog peat HAs (around 1.0%) was higher than in fen peat HAs (around 0.3%) (Kuske *et al.* 2010, Dudare and Klavins 2012). The HAs from Viku fen peat contained more N and C than those in Opulu fen peat. Opulu fen peat HAs contained more S than those of Viku fen peat. HAs from Dzelves bog contained comparatively more N and O than HAs from Eipurs bog. At the same time, the HAs from the Eipurs bog contained more C, H and S in their composition.

In this study, the concentration of C in raised bog peat HAs tended to increase with depth, while O decreased. In fen HAs, the concentration of C tended to decrease with depth, while O increased. In raised bog peat HAs, the C content appeared to be affected by the peat depth range, age and organic matter content. At the same time, the C content in fen peat HAs was more affected by the peat decomposition degree. Overall trends of C and O concentrations as the function of time were rather different between fen and raised bog peat HAs, this was due to diversity in peat humification conditions. Respectively, in raised bogs with less intensive organic matter humification C tended to increase in concentration, but in fens with intensive organic matter humification the

Table 3. The elemental composition of selected humic acid (HA) samples from peat with increasing depth range, expressed as a percentage (%) of the total composition and related atomic elemental ratios: (**a**) Viku HAs; (**b**) Opulu HAs; (**c**) Dzelves HAs; and (**d**) Eipurs HAs.

Sample	N	C	H	S	O	N/C	O/C	H/C
A 0.00–0.20 m	1.9	53.1	5.1	0.3	39.5	0.03	0.56	1.52
A 0.40–0.60 m	3.0	49.2	4.2	0.3	43.3	0.05	0.66	1.35
A 0.65–1.05 m	4.9	52.2	5.1	0.3	37.5	0.08	0.54	1.55
A 1.20–1.55 m	3.8	51.1	5.3	0.3	39.5	0.06	0.58	1.65
A 2.15–2.35 m	2.8	50.6	4.9	0.3	41.4	0.05	0.61	1.54
A 2.40–2.55 m	2.9	49.8	4.6	0.3	42.6	0.05	0.64	1.47
B 0.00–0.15 m	2.9	49.8	4.8	0.3	42.2	0.05	0.64	1.53
B 0.40–0.65 m	1.9	53.3	5.0	0.3	39.6	0.03	0.56	1.49
B 0.80–1.10 m	2.3	53.2	4.8	0.3	39.4	0.04	0.59	1.49
B 1.10–1.50 m	2.9	49.7	4.7	0.3	42.3	0.05	0.64	1.50
B 1.50–1.80 m	3.4	49.3	4.8	0.5	42.0	0.06	0.63	1.59
B 1.80–2.00 m	3.5	49.5	5.0	0.8	41.2	0.06	0.62	1.60
C 0.00–0.10 m	2.3	42.7	4.2	0.9	49.8	0.05	0.88	1.56
C 0.20–0.30 m	2.8	51.9	4.9	0.9	39.4	0.05	0.57	1.50
C 0.40–0.60 m	2.2	53.4	4.9	0.8	38.6	0.04	0.54	1.46
C 0.70–0.80 m	2.0	48.4	4.1	0.6	44.9	0.04	0.70	1.34
C 0.80–0.90 m	2.1	48.3	4.0	0.6	45.0	0.04	0.69	1.33
C 0.90–1.00 m	2.1	48.4	4.1	0.6	44.8	0.04	0.69	1.33
D 0.00–0.25 m	2.4	52.3	5.4	1.5	38.3	0.04	0.55	1.64
D 0.50–0.70 m	2.5	53.5	5.4	1.1	37.5	0.04	0.53	1.60
D 1.20–1.35 m	2.1	48.8	4.4	0.9	43.8	0.04	0.67	1.43
D 1.70–1.90 m	1.8	54.4	4.8	1.4	37.7	0.03	0.52	1.40
D 2.20–2.30 m	1.8	53.8	4.9	0.8	38.7	0.03	0.54	1.45
D 2.40–2.50 m	2.0	54.7	5.0	0.8	37.5	0.03	0.51	1.45

concentration of C was decreasing. In raised bog peat HAs, the N content had a positive relation with the organic matter content and negatively correlated with peat depth, age and peat decomposition degree. In fen peat, however, the N content has shown no negative correlations, and its strongest connection was with the organic matter content. The H content in fen peat HAs appeared to be affected by the peat decomposition degree. The concentration of O in raised bog peat HAs was correlated with the organic matter content, while in fen peat HAs, it was more affected by the peat decomposition degree.

The minor differences in the elemental composition of fen and raised bog peat HAs resulted in differences in the N/C, O/C and H/C atomic ratios (Table 3). In raised bog peat HAs, the N/C ratio was strongly affected by peat depth, age and organic matter content, while in fen peat HAs, the N/C ratio could be correlated with the organic matter only. The O/C ratio in the studied HA samples was generally affected by sample depth, age and organic matter content. However, data have shown no statistically significant correlations, except for the decomposition degree of fen peat. The H/C ratio in raised bog peat HAs was affected by the depth range, age and decomposition degree, while in fen peat HAs, this connection was not clear. The O/C ratios in fen and raised bog peat HAs (from 0.54–0.66 and from 0.52–0.88, respectively) appeared to be as expected from peat HAs. At the same time, the H/C ratios were rather high (from 1.35–1.65 and from 1.34–1.64, respectively), which was not quite common to peat. However, the H/C ratios were like those that other authors have found in peat HAs in other Latvian mires and reflected slight hydrogenation with depth throughout the fen peat HA samples (Klavins and Sire 2010, Silamikele *et al.* 2010b).

Statistically significant correlations ($p < 0.05$, $n = 22$) among HA elemental composition and peat parameters (depth, age, organic matter content and decomposition degree) were few, although it did not prove that such relation does not exist. For instance, in this study it was found that there is a relation between organic matter content in fen peat and N concentration ($r = 0.66$) and N/C atomic ratio of fen peat HAs ($r = 0.61$); this relation was also found between organic matter content in raised bog peat and relevant parameters: $r = 0.56$ with N and $r = 0.81$ with N/C, respectively. The organic matter content in raised bog peat also correlated with C concentration ($r = –0.53$) and O concentration ($r = 0.46$) in raised bog peat HAs, while in fen peat such relation was not found. In addition, results also indicated relation between depth range and C ($r = 0.54$) and N ($r = –0.76$) concentration, and N/C ($r = –0.89$) and H/C ($r = –0.52$) atomic ratio in raised bog peat HAs. As the depth increases, so does the age. Accordingly, correlation between age and relevant parameters in raised bog peat HAs was also found, with C concentration ($r = 0.50$), N concentration ($r = –0.76$), N/C ($r = –0.84$) and H/C ($r = –0.63$)

Table 4. The organic matter (OM) content in studied peat samples with increasing depth range: (**a**) Viku fen peat; (**b**) Opulu fen peat; (**c**) Dzelves raised bog peat; and (**d**) Eipurs raised bog peat.

Fen peat HAs	ϕ	ω	Bog peat HAs	ϕ	ω
A 0.00–0.20 m	51.5	84.6	C 0.00–0.10 m	41.6	106.4
A 0.40–0.60 m	48.1	95.5	C 0.20–0.30 m	50.4	87.1
A 0.65–1.05 m	50.6	89.6	C 0.40–0.60 m	51.9	83.7
A 1.20–1.55 m	49.4	90.3	C 0.70–0.80 m	47.3	95.7
A 2.15–2.35 m	49.1	91.1	C 0.80–0.90 m	47.5	95.8
A 2.40–2.55 m	48.5	93.8	C 0.90–1.00 m	47.8	96.0
B 0.00–0.15 m	48.4	93.0	D 0.00–0.25 m	50.6	83.7
B 0.40–0.65 m	51.8	84.8	D 0.50–0.70 m	51.8	82.4
B 0.80–1.10 m	49.7	90.3	D 1.20–1.35 m	47.6	93.8
B 1.10–1.50 m	48.3	93.2	D 1.70–1.90 m	53.0	80.7
B 1.50–1.80 m	48.2	93.0	D 2.20–2.30 m	52.3	82.7
B 1.80–2.00 m	48.0	92.8	D 2.40–2.50 m	53.2	80.9

atomic ratio, respectively. Finally, the data have shown relation between peat decomposition degree and N concentration (r = –0.51) and H/C atomic ratio (*r* = –0.46) in raised bog peat HAs, while in fen peat HAs, peat decomposition degree has shown a relation between C concentration (*r* = 0.63), H concentration (*r* = 0.55), O concentration (*r* = –0.60) and O/C (*r* = –0.63) atomic ratio.

As the data on elemental composition suggested, there were minor differences in the degree of oxidation (ω) of the HAs, depending on the type of peat. The ω value in the studied fen peat HA samples was 91% on average, in raised bog peat HAs — around 88% (Table 4). The HAs from Opulu fen were more oxidised than those from Viku fen, while HAs from Dzelves bog were more oxidised than those from Eipurs bog. At the same time, the index of hydrogen deficiency (φ) was similar in HAs of both types of peat and was around 50% (Table 4). The ω value in raised bog peat HAs was affected by the depth range (*r* = –0.51), peat age (*r* = –0.46) and organic matter content (*r* = 0.51), whereas in fen peat HAs, there was also a strong correlation with the decomposition degree (*r* = –0.64). The φ value in raised bog peat HAs also have shown a connection with the depth range (*r* = 0.56), age (*r* = 0.54) and the amount of organic matter (*r* = –0.53). Meanwhile in the fen peat HAs a similarly strong correlation was found with the decomposition degree (*r* = 0.61).

Table 5. Total organic carbon (TOC), mg L^{-1} of selected humic acid (HA) samples from peat with increasing depth range: (**a**) Viku HAs; (**b**) Opulu HAs; (**c**) Dzelves HAs; and (**d**) Eipurs HAs.

Fen peat HAs	TOC	Bog peat HAs	TOC
A 0.00–0.20 m	37.4	C 0.00–0.10 m	20.5
A 0.40–0.60 m	14.4	C 0.20–0.30 m	48.2
A 0.65–1.05 m	33.6	C 0.40–0.60 m	16.9
A 1.20–1.55 m	29.4	C 0.70–0.80 m	27.9
A 2.15–2.35 m	30.3	C 0.80–0.90 m	29.8
A 2.40–2.55 m	28.5	C 0.90–1.00 m	29.2
B 0.00–0.15 m	120.8	D 0.00–0.25 m	31.3
B 0.40–0.65 m	152.6	D 0.50–0.70 m	36.7
B 0.80–1.10 m	137.8	D 1.20–1.35 m	43.3
B 1.10–1.50 m	124.1	D 1.70–1.90 m	43.8
B 1.50–1.80 m	117.4	D 2.20–2.30 m	62.8

The organic C in different soil types allows modelling of the speciation, mobility and bioavailability of trace elements in soils, the reactive organic C concentration being a major controlling parameter, as well as in assessments of C cycling between soil and water. The amount of the TOC in HAs (Table 5) varied, depending on the peat type and site-specific characteristics, such as peat composition and decomposition degrees, ash content and depth range. At the same time, this concentration was consistent with the concentration of elemental C. However, a pronounced correlation between the TOC and various peat parameters at a statistically significant level could only be found for raised bog HAs (depth *r* = 0.90, age *r* = 0.87, organic matter *r* = –0.96, decomposition degree *r* = 0.64). In HAs from the Viku fen, TOC was between 14.4 mg L^{-1} and 37.4 mg L^{-1}, and there was no specific distribution pattern. This could be explained by the diverse botanical compositions of peat. The amount of TOC in HAs from Opulu fen peat ranged between 97.4 and 152.6 mg L^{-1}, increasing from top to bottom peat layers. At the same time, the TOC content in HAs from the Opulu fen was not substantially different from that in HAs from the Viku fen. In HAs from the Dzelves raised bog peat, the TOC was from 29.8–48.2 mg L^{-1}, while the HAs from the Eipurs bog peat showed 31.3–57.6 mg L^{-1}.

Spectral characterisation of peat humic acids

As expected, the IR of isolated fen and raised bog (Kuske *et al.* 2010, Dudare and Klavins 2012) peat HA fractions reflected typical peat-derived HA absorption bands (Fig. 2), with some minor differences in their relative intensity and between peat types in the wavelength range between 4000 and 450 cm^{-1} (Aerts *et al.* 1999, Saito and Seckler 2014). Although the collected FTIR spectra had only few broad bands, it was decided to not calculate the second derivative spectra, to prevent possible derivative spectra distortion due to the use of a wide smoothing window. The IR spectra of peat HAs contained characteristic absorption bands at 3500–3300 cm^{-1} (OH-stretching vibrations, hydrogen-bonded

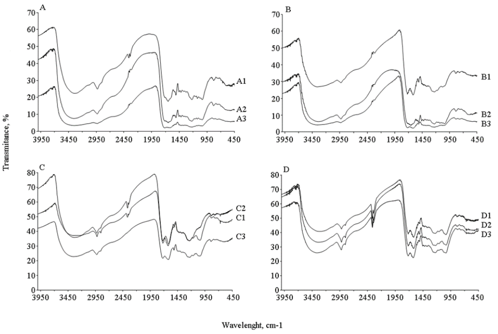

Fig. 2. The infrared spectra (FT-IR) of selected humic acid (HA) samples: (**a**) Viku HAs (A1: 0.00–0.20 m, A2: 1.20–1.55 m, A3: 2.40–2.55 m); (**b**) Opulu HAs (B1: 0.00–0.15 m, B2: 1.10–1.50 m, B3: 1.80–2.00 m); (**c**) Dzelves HAs (C1: 0.00–0.10 m, C2: 0.40–0.60 m, C3: 0.70–0.80 m); and (**d**) Eipurs HAs (D1: 0.00–0.25 m, D2: 1.70–1.90 m, D3: 2.40–2.50 m).

OH groups), at 2920, 2850 and 700–900 cm^{-1} (stretching vibrations of -CH_3 and -CH_2 in side chains), at 1725–1700 cm^{-1} (stretching vibrations of C=O groups in ketones, aldehydes, carboxylic acids and their functional derivatives), at 1625–1610 cm^{-1} (in-plane vibrations of conjugated C=C bands and C=O bands in an aromatic skeleton and quinones), at 1510–1500 cm^{-1} (uncondensed aromatic compounds bound to N and O atoms), at 1250–1225 cm^{-1} (C-O-stretching and O-H-bending vibrations), and at 1030–1150 cm^{-1} (alcohol and carbohydrate OH-stretching vibrations). Notably, the conjugated C=C and C=O bands in an aromatic skeleton and quinones could not be differentiated from conjugated olefinic C=C and enolic C=O bands by the IR spectroscopy alone.

The calculation of light absorbance ratios was applied for quantification of the relative concentration of functional groups in HAs and determination of the intensity of light absorption bands in the IR region. The data have shown evident differences between HAs of raised bog and fen peats (Table 6). The IR light absorption A_{1610}/A_{2920} (absorption at 1610 and 2920 cm^{-1}) and A_{3400}/A_{1610} (absorption at 3400 and 1610 cm^{-1}) ratios generally reflect optical densities for aromatic, aliphatic and OH groups in HAs (Zaccone *et al.* 2007a, Vialykh *et al.* 2014). The results indicated visible differences between fen and raised bog peat HA samples. These differences apparently were linked to peat type, depth range, age and peat decomposition degree. The A_{1610}/A_{2920} ratio (between aromatic and aliphatic groups) in fen peat HAs ranged widely between 0.41 and 0.78 (higher aromaticity was in recently formed HAs), with major dependence on the organic matter content in peat (r = 0.91) and depth range (r = –0.78). The A_{1610}/A_{2920} ratio in raised bog HAs was more diverse and ranged between 0.80 and 1.18. Notably, there was a difference between HAs from Dzelves (1.13–1.18) and Eipurs (0.80–0.91) bogs, and the values were linked to the sample depth (r = –0.84), age (r = –0.86) and decomposition degree (r = –0.64). The IR light absorption ratios in HAs at 1610 cm^{-1} and 2920 cm^{-1}

Table 6. The infrared light absorption ratios at 1 610, 2 920 and 3 400 cm-1 of selected humic acid (HA) samples from peat with increasing depth range: (**a**) Viku HAs; (**b**) Opulu HAs; (**c**) Dzelves HAs; (**d**) Eipurs HAs.

Fen peat HAs	A_{1610}/A_{2920}	A_{3400}/A_{1610}	Bog peat HAs	A_{1610}/A_{2920}	A_{3400}/A_{1610}
A 0.00–0.20 m	0.78	1.19	C 0.00–0.10 m	1.13	1.03
A 0.40–0.60 m	0.70	1.23	C 0.20–0.30 m	1.15	1.01
A 0.65–1.05 m	0.64	1.27	C 0.40–0.60 m	1.18	0.99
A 1.20–1.55 m	0.56	1.32	C 0.70–0.80 m	1.17	0.95
A 2.15–2.35 m	0.52	1.41	C 0.80–0.90 m	1.16	0.93
A 2.40–2.55 m	0.49	1.53	C 0.90–1.00 m	1.16	0.94
B 0.00–0.15 m	0.75	1.23	D 0.00–0.25 m	0.91	1.07
B 0.40–0.65 m	0.57	2.12	D 0.50–0.70 m	0.92	1.14
B 0.80–1.10 m	0.52	2.33	D 1.20–1.35 m	0.88	1.13
B 1.10–1.50 m	0.41	2.73	D 1.70–1.90 m	0.88	1.15
B 1.50–1.80 m	0.41	1.99	D 2.20–2.30 m	0.79	1.15
B 1.80–2.00 m	0.42	1.84	D 2.40–2.50 m	0.80	1.16

have shown that in HAs of both fens and in Eipurs HAs, the aliphatic contribution relatively to olefinic/aromatic one was increasing with increasing age and depth. At the same time in Dzelves HAs, the aliphatic contribution was slightly decreasing. Results suggest that with age, the relative contribution of aliphatic carbon in the structure of HAs is increasing.

The range of the A_{3400}/A_{1610} ratio (between OH and aromatic groups) in fen peat HAs was between 1.19 and 2.73 (higher reactivity in the HAs that have formed earlier). However, the values were not linked to other parameters at a significant level. The range of the A_{3400}/A_{1610} ratio in raised bog peat HAs was between 0.99 and 1.16; i.e., comparatively lower than for fen peat HAs. This could be explained by lower aromaticity. The A_{3400}/A_{1610} ratio in raised bog peat HAs was related to peat depth range ($r = 0.72$), organic matter content ($r = –0.85$) and peat decomposition degree ($r = 0.64$). This ratio was increasing in HAs of both fens and Eipurs bog and indicated that the relative contribution of the olefinic and aromatic C=C double bonds, and C=O bonds were increasing relative to OH-bonds with increasing age of peat.

Whilst with the aid of UV/VIS spectroscopy it is not possible to explicitly identify the structure of HAs as aromatic, it provided significant information on compounds with conjugated double bonds. The UV/Vis spectra of fen and raised bog peat HAs reflected typical peat-derived light absorption, and as expected, the intensity decreased consistently from $\lambda = 200$ nm to $\lambda = 665$ nm. An inconspicuous curvature was noticeable around $\lambda = 270–280$ nm, which indicated the overlapping absorbance of chromophores that are present in the humic core (Mathur and Schnitzer 1978). Light absorbance at $\lambda = 270–280$ nm reflected the organic material at the beginning of its transformation, i.e. lignin and quinone moieties. The chromophores that gave light absorption signals in the UV region (less than $\lambda = 380$ nm) were phenolic arenes, benzoic acids, aniline derivatives, polyenes and polycyclic aromatic hydrocarbons with two or more rings (Uyguner and Bekbolet 2005). At the same time, light absorbance in the visible region (more than $\lambda = 380$ nm) was related to extended conjugation in aliphatic or polyaromatic structures and in quinoid structures, and to the presence of metal complexes and donor-acceptor complexes. This could be attributed to the condensation of aromatic rings and organic molecules of high molecular weight (Uyguner and Bekbolet 2005). Light absorbance above $\lambda = 600$ nm reflected a strongly humified organic material. Although fen peat was more aromatic and raised bog peat – more aliphatic, the difference in peat HAs in this context is not very pronounced, as increase in aromaticity appears to be related to lignin decomposition which occurs at later stage of humification.

The degree of organic matter humification could be determined based on light absorbance ratios. These ratios at different wavelengths

Table 7. Humification indicators in the humic acid (HA) samples from peat with increasing depth ranges: (**a**) Viku HAs; (**b**) Opulu HAs; (**c**) Dzelves HAs; and (**d**) Eipurs HAs.

Fen peat HAs	E_{280}/E_{472}	E_{465}/E_{665}	E_{280}/E_{664}	Bog peat HAs	E_{280}/E_{472}	E_{465}/E_{665}	E_{280}/E_{664}
A 0.00–0.20 m	2.70	5.40	14.40	C 0.00–0.10 m	10.00	10.00	10.01
A 0.40–0.60 m	1.84	6.54	15.11	C 0.20–0.30 m	8.53	8.02	7.51
A 0.65–1.05 m	1.30	7.20	15.80	C 0.40–0.60 m	6.21	4.04	3.82
A 1.20–1.55 m	1.63	7.31	14.39	C 0.70–0.80 m	9.77	7.03	6.53
A 2.15–2.35 m	1.87	7.49	12.74	C 0.80–0.90 m	9.75	7.04	6.54
A 2.40–2.55 m	2.10	7.50	12.10	C 0.90–1.00 m	9.76	7.04	6.54
B 0.00–0.15 m	1.80	8.30	20.20	D 0.00–0.25 m	10.05	5.01	4.75
B 0.40–0.65 m	3.12	9.12	25.66	D 0.50–0.70 m	9.67	5.86	5.57
B 0.80–1.10 m	3.25	9.41	28.78	D 1.20–1.35 m	7.62	6.86	6.43
B 1.10–1.50 m	4.70	9.50	31.90	D 1.70–1.90 m	7.03	6.58	5.57
B 1.50–1.80 m	1.85	10.04	32.11	D 2.20–2.30 m	7.38	6.36	6.00
B 1.80–2.00 m	1.60	11.50	34.10	D 2.40–2.50 m	7.62	6.77	6.44

could be calculated from the UV-Vis spectra, thereby obtaining information on the aromaticity or aliphaticity of HAs. The E_{280}/E_{472} ratio (Table 7) in fen peat HAs ranged between 1.30 and 4.70, representing the presence of lignin and other weakly humified materials in the composition of HAs. There was a slight difference in this ratio between HAs from the Viku and Opulu fens, which appeared to be caused by the differences in the peat botanical composition. The E_{280}/E_{472} ratio in raised bog peat HAs was comparatively much higher, ranging between 6.21 and 10.05, although the variability in this case was not as high as in fen peat HAs. The E_{280}/E_{664} ratio (Table 7) also served as an indication of a large amount of lignin in fen peat HAs. This ratio ranged between 12.10 and 34.1, also showing a large share of weakly humified compounds in the structure of fen peat HAs. The E_{280}/E_{664} ratio in raised bog peat HAs ranged between 3.80 and 10.00. The E_{280}/E_{472} and E_{280}/E_{664} ratio values did not seem to follow any specific pattern; nevertheless, there were apparent differences between fen and raised bog peat HAs: in raised bog peat HAs, a stronger correlation between the E_{280}/E_{664} ratio and elemental composition could be observed (C $r = -0.77$, H $r = -0.60$, O $r = 0.77$). The E_{465}/E_{665} ratio in isolated fen peat HAs (Table 7) ranged between 5.4 and 11.5, showing the presence of non-humified organic material with plant proteins and carbohydrates, low quantities of aliphatic structures and high content of condensed aromatic structures. Thus, this parameter appeared to be linked to the peat decomposition degree ($r = -0.65$). Moreover, it was consistent with the ratios between aromatic and aliphatic compounds and between the -OH and aromatic groups found in fen peat HAs. The highest ratio values indicated weakly humified organic material that contains proteins and carbohydrates, while the lowest ratio values were typically associated with a higher quantity of aromatic components and higher condensation of HA structure. The E_{465}/E_{665} ratio in raised bog peat HAs was quite similar (between 4.0 and 10.0). Minor and site-dependent differences in the humification indicators for HAs of different peat types could be noticed. The UV/Vis absorption typically was the strongest through the UV wavelengths to the blue light wavelengths, therefore E_{280}/E_{472} appeared to be the most important ratio amongst three. At the same time, all three presented ratios of raised bog peat HAs had at first decreasing trend, which then changed to increasing trend, this appeared to be due to olefinic/aromatic conjugated C=C bonds first to be reduced and then increased.

The result of the fluorescence emission spectroscopy indicated no major differences between fen and raised bog peat HAs taken from top peat layers (Fig. 3). In general, the data have shown two major signals: the first at $\lambda = 450$–455 nm and the second at $\lambda = 480$–490 nm, with the latter signal being more intense in fen peat HAs. The signal at $\lambda = 450$–455 nm could be attributed to quinone structures in HAs, while the signal at $\lambda = 480$–490 nm was related to aromatic and polyaromatic structures that are more common in fen peat HAs than in raised bog peat HAs, as supported by the FT-IR and UV-Vis analysis data.

The humification degree of HAs could be traced in the fluorescence emission spectra using the ratio between light absorption at 460 nm and 510 nm (I_{460}/I_{510}). The data have shown that this parameter, although variable, was generally higher in raised bog peat HAs (Eipurs HAs: 1.36, Dzelves HAs: 2.48) compared to fen peat HAs (Viku HAs: 1.08, Opulu HAs: 1.15) and in peat taken from mire top layers, and it was sensitive to the O content in HAs. The EEM information obtained from the 3D fluorescence analysis of fen and raised bog peat HAs reflected the presence of complex systems with several fluorescent components. The fluorescence peaks with high relative intensity at short Em wavelengths could be associated with the presence of simple structural components of wide molecular heterogeneity and small molecular weight, a small degree of aromatic condensation, low degree of humification and low quantity of conjugated fluorophores (Enev *et al.* 2014). At the same time, the fluorescence peaks at long Em wavelengths indicated the presence of more complex structural components with higher degree of aromatic condensation with more conjugated fluorophores (Enev *et al.* 2014). Regarding Ex wavelengths, fen and raised bog peat HAs exhibited similar characteristic fluorescence peaks around $\lambda = 450$–500 nm, which can be attributed to aromatic fluorophores

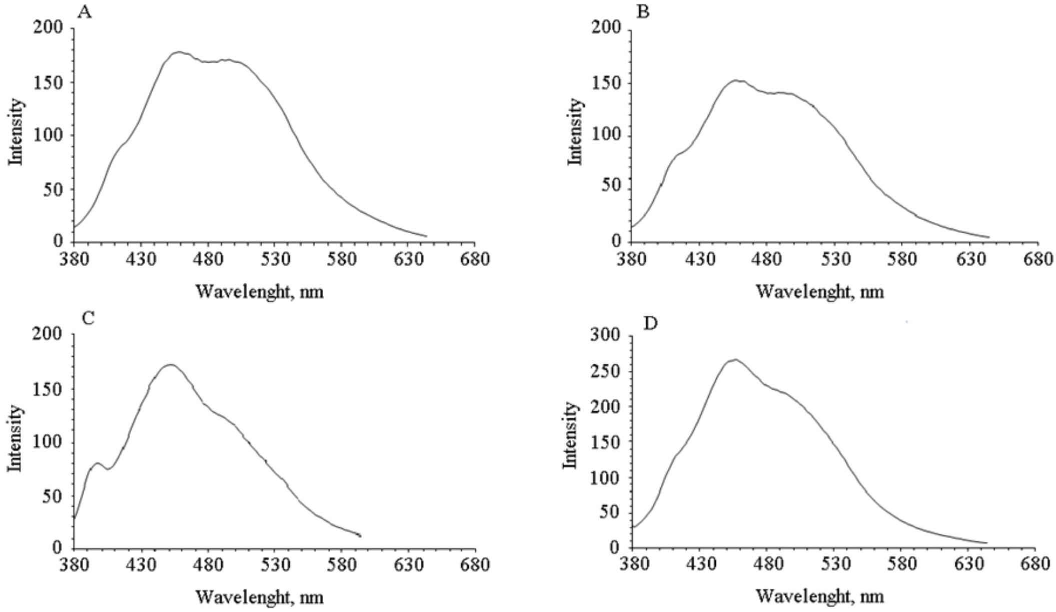

Fig. 3. The fluorescence emission spectra of selected humic acids (HAs) from peat at top layers of mires (excitation: 350 nm): (**a**) Viku HAs from peat at 0.00–0.20 m; (**b**) Opulu HAs from peat at 0.00–0.15 m; (**c**) Dzelves HAs from peat at 0.00–0.10 m; and (**d**) Eipurs HAs from peat at 0.00–0.25 m.

with electron-donating functional groups (Enev *et al.* 2014). However, in raised bog peat HAs, the fluorescence at this wavelength interval less expressed, pointing to lower aromaticity. Moreover, the overall fluorescence intensity of raised bog peat HAs was lower when compared to fen peat HAs; aside from that, both types of HAs had similar EEMs (Fig. 4), which were consistent with the data obtained from FT-IR and UV/Vis spectroscopic analysis.

Stable carbon and nitrogen isotope ratios in peat humic acids

The δ^{13}C isotopic ratio in fen peat HAs (Table 8) reflected CO_2 fixation in peat-forming plants, and its values were like ratios typically found in soil and peat HAs, such as reported by Zaccone *et al.* (2011), Krumins *et al.* (2019), Nissenbaum and Schallinger (1974), Katsumi *et al.* (2015). In HAs from Viku fen, the δ^{13}C values were between –28.2 and –27.4‰, reflecting a dominant contribution of C3 plant species and a possible change in dominant peat-forming plant species over time. At the same time, these values also have shown the depletion of ^{13}C, which might point on the presence of lignin-poor plant species, while the slight enrichment could have been caused by the production of fresh peat layers from the higher plant decomposition. The distribution of δ^{13}C in HAs from Opulu fen followed similar pattern, although the absolute δ^{13}C values were different and indicated slight enrichment: they varied between –27.5 and –23.8‰. Low δ^{13}C values correlated with a high decomposition degree. The δ^{13}C isotopic ratio in raised bog peat HAs was equally similar and ranged between –28.2 and –23.4‰, with no major differences between HAs from Dzelves and Eipurs bogs. The distribution of δ^{13}C varied with HA parameters and appeared to depend on the origin of the organic material. A noticeable enrichment could be observed with the complexation of a botanical composition, while a relatively simple botanical composition has shown stable isotopic distribution – such as in fen peat HAs, where a strong correlation between δ^{13}C and total organic carbon (r = –0.68), peat decomposition degree (r = 0.61) and peat type (r = –0.56) could be observed. Generally, δ^{13}C shown the CO_2 fixation in C3 plants: the ratio was higher in freshly formed peat layers.

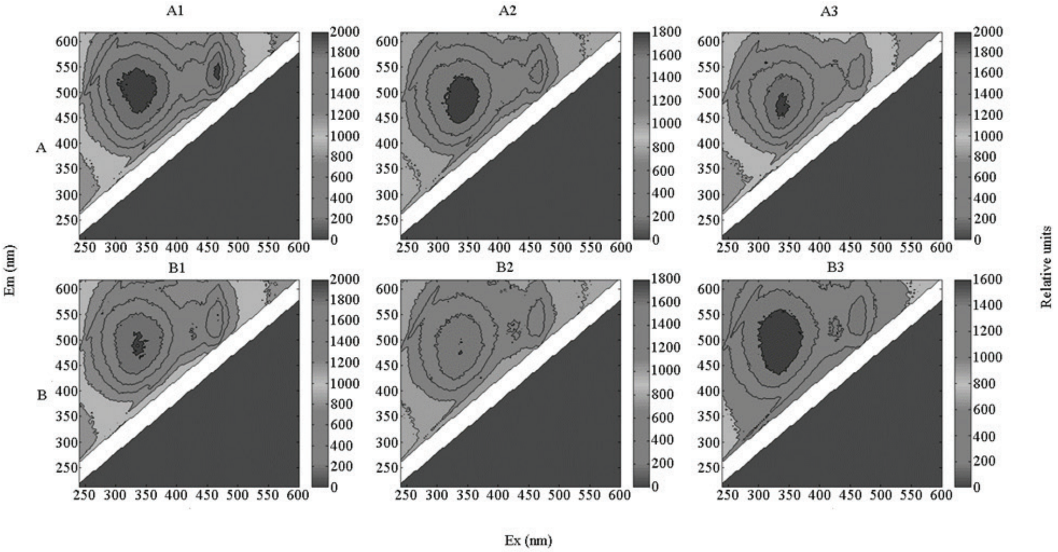

Fig. 4. The excitation-emission matrices (EEMs) of the studied humic acid (HA) samples: (**a**) Viku HAs (A1: 0.00–0.20 m, A2: 1.20–1.55 m, A3: 2.40–2.55 m) and (**b**) Dzelves HAs (B1: 0.00–0.10 m, B2: 0.40–0.50 m, B3: 0.70–0.80 m).

The δ^{15}N isotopic ratio in fen and raised bog peat HAs reflected the N_2 fixation in peat-forming plants, N input and peat mineralisation. In HAs from Viku fen, the δ^{15}N values were indicating enrichment and varied between 1.0 and 2.4‰ (Table 8). Considerably high values might be indicative of the main peat-forming species and preferential loss of ^{14}N through fractionation during biological transformation. Possible causes for the enrichment were N stress, the fractionation of stable N isotopes during e.g. denitrification and mixing of N from internal and external sources. In HAs from Opulu fen, the δ^{15}N values were indicating both enrichment and depletion and varied between –0.4 and 0.9‰, showing peat mineralisation and both input and depletion of N. The N depletion could be identified in HAs from the bottom layer of the Opulu fen peat profile, while the top layer has shown enrichment that might have been caused by anthropogenic activities, such as agriculture, in the local area. The higher δ^{15}N values correlated

Table 8. The δ^{13}C ratio and the δ^{15}N ratio (‰) in the humic acid (HA) samples from peat with increasing depth range: (**a**) Viku HAs; (**b**) Opulu HAs; (**c**) Dzelves HAs; and (**d**) Eipurs HAs.

Fen peat HAs	δ^{13}C	δ^{15}N	Bog peat HAs	δ^{13}C	δ^{15}N
A 0.00–0.20 m	–26.77	0.94	C 0.00–0.10 m	–27.83	3.49
A 0.40–0.60 m	–27.16	0.01	C 0.20–0.30 m	–28.69	–1.33
A 0.65–1.05 m	–27.83	0.11	C 0.40–0.60 m	–26.83	0.95
A 1.20–1.55 m	–27.67	0.07	C 0.70–0.80 m	–27.92	2.27
A 2.15–2.35 m	–27.48	–0.05	C 0.80–0.90 m	–27.55	2.78
A 2.40–2.55 m	–27.39	–0.37	C 0.90–1.00 m	–27.74	2.68
B 0.00–0.15 m	–27.92	1.87	D 0.00–0.25 m	–27.02	–1.24
B 0.40–0.65 m	–27.93	2.41	D 0.50–0.70 m	–27.78	3.19
B 0.80–1.10 m	–27.83	2.16	D 1.20–1.35 m	–23.49	–0.32
B 1.10–1.50 m	–28.16	1.66	D 1.70–1.90 m	–25.72	0.65
B 1.50–1.80 m	–28.03	1.08	D 2.20–2.30 m	–25.48	0.78
B 1.80–2.00 m	–27.74	1.05	D 2.40–2.50 m	–26.01	0.95

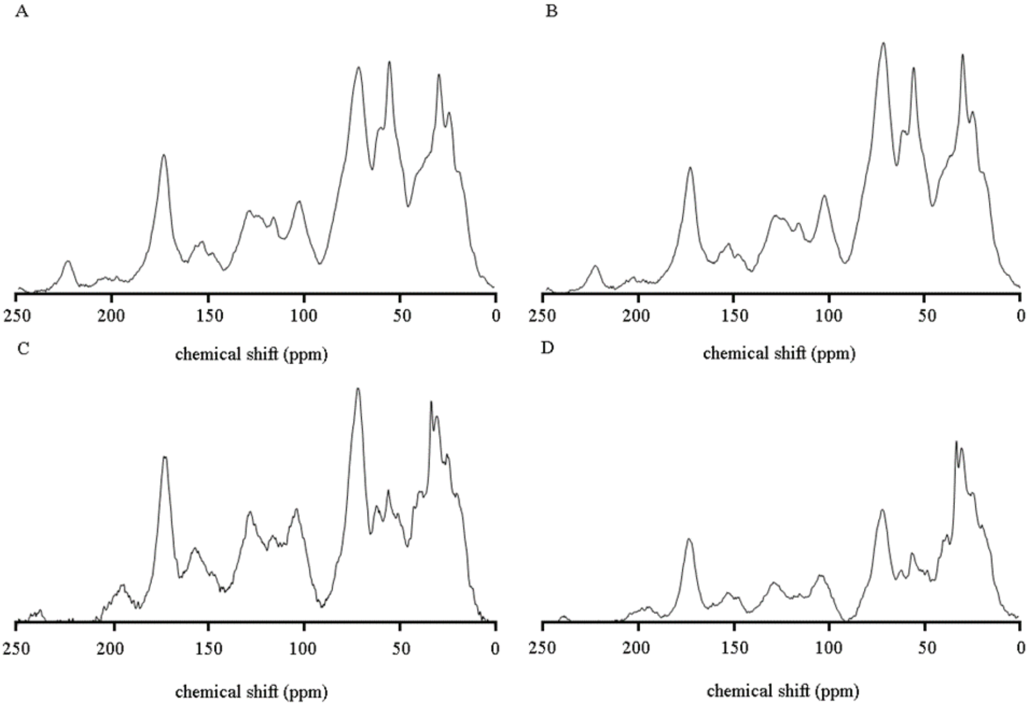

Fig. 5. The ^{13}C NMR spectra of selected humic acids (HAs) from peat at top layers of mires: (**a**) Viku HAs from peat at 0.00–0.20 m; (**b**) Opulu HAs from peat at 0.00–0.15 m; (**c**) Dzelves HAs from peat at 0.00–0.10 m; and (**d**) Eipurs HAs from peat at 0.00–0.25 m.

with a higher decomposition degree. In HAs from raised bog peat, the δ^{15}N values were somewhat similar, although there were site-dependent differences that possibly point to varied anthropogenic impacts on different sites. At the same time, overall δ^{15}N isotopic ratio values in HAs followed the same intervals as found in other peat HAs, e.g., as in the HAs studied by Zaccone *et al.* (2011) or Katsumi *et al.* (2015).

Peat humic acid ^{13}C nuclear magnetic resonance spectra

The ^{13}C NMR for the purposes of this study was performed in solid state as this in contrast to dissolved-state allowed to obtain results with higher resolution. The choice was based on the evidence that in dissolved-state the media contains comparatively lower concentration of HAs than solid state media. The content of aromatics/conjugated aliphatics in raised bog peat-forming plants, such as *Sphagnum magellanicum*, is known to be comparatively much lower than in fen peat-forming higher vegetation. At the same time, the ^{13}C NMR spectra of fen peat HAs and raised bog peat HAs had no major differences, and the chemical shifting of C manifested similarly, apart from intensity and differences between the integration areas for unsubstituted aromatic C and aromatic C substituted by O (Fig. 5). However, spinning side bands present in the spectra must be considered in the interpretation of the data.

The chemical shifting at 0–50 ppm reflected aliphatic carbon (C-C). While the chemical shifting at this interval in peat HAs shown no apparent pattern relative to the depth range of peat used for HA extraction, the relative shifting intensity in fen peat HAs was higher than in raised bog peat HAs. Shifting at 50–60 ppm represented methoxyl carbon (C-O). The values of fen peat HAs in this interval were similar in all depth ranges, but their relative shifting intensity was higher when compared to raised bog peat HAs. Although the signal produced by methoxyl carbon could coincide with that of N compounds in HAs, the results show no such correlation. Shifting at 60–90 ppm pointed to a simple carbon bond (C-O) and shifting in peat HAs to increase with an increase in the depth of a peat sample, while the relative intensity was higher in fen peat HAs compared to raised bog peat HAs. The O-alkyl signal possible carbohydrate degradation and dehydration in fen peat HAs (Fig. 5), and this is consistent with fen peat HA FTIR spectra. Shifting at 90–110 ppm has shown a simple carbon bond to two heteroatoms (C=C), and it also appeared to increase with the depth of a sample, with the relative intensity in fen peat HAs being higher. At around 105 ppm, there was primarily the anomeric C (C1) of carbohydrates. Shifting at 110–140 ppm indicated conjugated unsaturated carbon (-C-C-C-C- aromatic, but possibly also aliphatic), and the shifting increased with the depth of a sample, the relative intensity was highest in fen peat HAs. The conjugated olefinic C=C bands are shifting through the region between 110–140 ppm. Roughly around 115 ppm, there was a signal of unsubstituted aryl C, but around 130 ppm: C-substituted aryl C, while at around 150 ppm, there was indication of O-substituted aryl C. Shifting at 160–190 ppm suggested the presence of carbonyl, ester and amide (C=O), and the intensity of this shifting decreased with the sample depth, while showed no significant relation with N compounds in the studied HAs. Differences between the integration areas for unsubstituted aromatic C and aromatic C substituted by O suggested the presence of lignin products in fen peat HAs. The ratio between both areas in fen peat HAs was 1.73, while in raised bog peat HAs it was 0.76. Overall, the contribution of aliphatic C to the structure of HAs appears to be dominant in both raised and fen peat HAs.

Discussion

Mires in Northern Europe have been classified as large landscape units with common features in vegetation, hydrology, peat stratigraphy and general patterns of site conditions, such as minerotrophic (fen) and ombrotrophic (raised bog) conditions (Pakarinen 1995). Peat used for HA extraction was sampled in mires that represent typical environmental conditions in boreal mires, those being two fens and two raised bogs (Aerts *et al.* 1999). Viku fen is in the western geobotanical district of Latvia, while Opulu fen is situated in the north-eastern geobotanical district. Both raised bogs are located in the coastal geobotanical district. Differences amongst these geobotanical districts are reflected in contrast geological development, vegetation and soil features, which is resulting in different mire types, their distribution and vegetation cover. Therefore each of mires selected for this study serve as unique archive containing data on regional differences (Kabucis 1995). The dominant vegetation in the mires considered for peat sampling represents the most common plant species in mires of Northern Europe, depending on their type (Table 9), while

Table 9. The dominant peat-forming plant species (in Latin) in the botanical composition of the studied peat samples

Species in the raised bog vegetation	Species in the fen vegetation
Andromeda polifolia L.	*Carex elata* L.
Calluna vulgaris L.	*Carex lasiocarpa* L.
Drosera rotundifolia L.	*Carex diandra* L.
Eriophorum vaginatum L.	*Carex nigra* L.
Ledum palustre L.	*Menyanthes trifoliata* L.
Oxycoccus palustris L.	*Phragmites australis* L.
Rhynchospora alba L.	*Eriophorum latifolium* L.
Scheuchzeria palustris L.	*Drepanocladus revolvens* L.
Sphagnum magellanicum L.	*Scorpidium scorpioides* L.
Sphagnum fuscum L.	*Campylium stellatum* L.
Sphagnum rubellum L.	*Fissidens adianthoides* L.
Sphagnum angustifolium L.	*Calliergonella cuspidate* L.

also showing profound differences between fens and raised bogs of the boreal environment.

Due to differences in the formation and development conditions, including formation in different geobotanical regions, raised bogs are with thicker peat layers than fens that were considered in this study. Additionally, these mires are also of different age. Although the study was conducted in Latvia, which is only a small part of the boreal environment, we believe that the results obtained reflect fundamental trends in the formation of peat natural organic matter in the boreal region as a whole, since all mires considered for peat sampling were formed at the end of the last glacial period under similar climatic conditions (Behre and van der Plitcht 1992, Kuske *et al.* 2010). The age and thickness of the peat profiles indicate changes in peat accumulation rates over time (Fig. 1). The raised bog peat accumulation in Dzelves and Eipurs bogs apparently has been more intensive than fen peat formation in Viku and Opulu fens. Higher plant remains are generally more decomposed than lower plant (bryophyte) remains, and this difference is exemplified in Dzelves and Eipurs bog peat profiles, the first of which contains only raised bog peat, while the latter contains fen, transitional and raised bog peat types (Kuske *et al.* 2010). However, the environmental conditions of particular mire also have significant impact on the further development of the site. The decomposition of higher plant remains leads to formation of sapric peat layers in fens, while bryophyte decomposition ends with accumulation of fibric peat.

Due to differences in the environmental conditions, there are profound differences in the ratio between organic and inorganic matter content amongst peat types, which understandably also appears in HA outcome, which can be extracted from peat. Fens have formed in depressions between hills and hillocks from whose slopes mineral matter entered mire and accumulated within peat layers. In addition, sensitivity to changes in environmental settings are reflected in varied amounts of inorganic matter in Viku and Opulu fen. Simultaneously, raised bogs formed as ecosystems isolated from surroundings with little impact from mineral sources. Viku fen is surrounded by the quaternary deposits — gQ_3*ltv*, with additional areas of glQ_3*ltv* and gfQ_3*ltv*, all of which are mineral sources and source of high mineral matter content in fen peat. The gQ_3*ltv* are glaciogenic deposits, which are generally composed of till and form hills and hillocks in the surrounding areas. The glQ_3*ltv* are glaciolacustrine deposits, which contain sand, silt and clay. Generally, glaciolacustrine deposits cover glaciogenic deposits and from surrounding hill and hillock slopes are delivered in the fen ecosystem. The gfQ_3*ltv* are glaciofluvial deposits and contain sand, gravel and pebble. The surrounding areas of Opulu fen contain similar deposits to those of Viku fen, whilst there are also additional aQ_4 to the north of the fen, which are alluvial sediments and contain sand, gravel, pebble, and silt. Eipurs and Dzelves bog are located in close proximity and surroundings contain gQ_3*ltv*, gfQ3*ltv* and aQ_4 sediments, although the coastal area is relatively flat and there is no major influence of mineral sources on raised bog development (https://www.lvmgeo.lv/en/maps).

The interpretation of data on HAs in this study is based on the results of numerous studies dedicated to development of HS structural models. Examples of such studies are e.g., Schulten (1995), Saiz-Jimenez (1996) and De Paolis and Kukkonen (1997), who published on the presence of considerable lignin concentration in humic material and low thermodynamical stability of conjugated aliphatic chains in comparison to aromatic chains. The concept of organic matter humification is one of the key concepts in carbon biogeochemical flow and all the processes related to living organic matter transformation; however, due to the complexity of these processes, discussions are still open for interpretation. Comparatively, lignin-rich vegetation in fens is more sensitive to organic matter degradation than lignin-lacking vegetation in raised bogs. Moreover, the humification in fens takes more time in aerobic/hypoxic environment than in raised bogs and this results in more profound organic matter mineralization.

The amount of HAs that can be extracted greatly differ between fen and raised bog peat and the exact amount is sensitive to peat type, age and inorganic matter content present in peat mass. The results suggest that fen peat of a diverse botanical composition, low amount of detritus and the mineral matter content of less than 10% typically has the highest HA yield, as proven by the strong correlation between the amount of organic matter and the HA content. Apparently, there is a strong correlation between peat-forming organic matter (woody and herbaceous plant remains) in fen peat and HA content, while in raised bog peat this correlation is not a very pronounced indicator. Therefore, the amount of HAs in raised bog peat is more consistent than in fen peat HAs. The amount of HAs in fen peat is sensitive to peat botanical composition, while in raised bog peat it is rather stable due to homogeneous continuous peat layers. Notably, the amount of HAs that can be extracted from peat depends not only on the peat type and related properties but also on the extraction procedure. In this study, the extraction was done under same conditions using KOH that allows to extract comparatively more HAs than NaOH (Saito and Seckler 2014). It is unambiguous that analysis of samples that are of similar age can be highly informative and unique. Alas, analysed fen and raised bog peat HAs were of peat of systematically different age, thus differences are not per se related to peat type and could be related to time-depended environmental factors. However, while raised bogs are mostly environmentally isolated ecosystems and can be directly compared to one another, fens are variably affected by the surrounding environment both in time and space-scale making the comparison between two sites rather difficult as data will reflect manifestations of local influences, which can be highly varied. Moreover, comparison using same age frame would require differentiation between peat types, but as suggested by the results, the deeper parts of Eipurs bog contain transitional and fen peat, which accordingly cannot be used for comparison between fen and raised bog peat HAs.

The result of elemental analysis suggest that HAs isolated from fen and raised bog peat do not differ significantly. However, the results may be seen as obstructed by different age distribution of fen and raised bog peat HAs. Simultaneously, differences in the relation between elemental composition and other properties of peat are evident. In raised bog peat HAs, C concentration appears to increase with the depth and age, while N concentration is decreasing. Simultaneously, C concentration comparatively is lower, but N content is higher in HAs isolated from raised bog with higher organic matter content. In fen peat HAs, only the positive corelation between organic matter and N concentration can be found. Fen peat HAs contain comparatively more N, but less O than raised bog peat HAs. Any differences in the concentration of C and H apparently are more site-dependent and not per se consistent with the type of peat. However, the results suggest that with advancing humification the concentrations of C and that of O changes. Nevertheless, the organic matter in peat and its decomposition degree appears to be of high importance for the quantitative elemental composition. Generally, the increase in peat humification is followed by a decrease in the N/C, H/C and O/C atomic ratios, which appear in response to the enrichment of total N relative to organic C, aromatic and aliphatic compounds (Stevenson 1994, Zaccone *et al.* 2018a).

The IR spectra of fen and raised bog peat HAs have shown no apparent differences in sample composition in terms of chemical groups present, apart from the IR light absorption intensity by molecules present in HAs. A difference in IR light absorption intensity between fen and raised bog peat HAs can be found at 1720 cm^{-1}, which characterizes carboxylic groups and typically is associated with aliphatic structures at 2920 cm^{-1}. Respectively, in fen peat HAs, the IR radiation absorption band for carboxylic groups is comparatively less pronounced. At the same time, there is no typical lignin pattern of lignocellulosic material in the analysed fen peat HAs (e.g., like in fen peat HAs described in the classic paper by Farmer and Morrison (1960). The absence of lignin pattern in studied fen peat HAs suggests a considerable carbohydrate degradation over time and HA dehydration, in addition, this is also consistent with organic matter degradation with increasing depth and age. Notably, while fen peat HAs and Eipurs bog HAs have shown similar traits in regard to IR light absorption and IR ratios, Dzelves bog HAs are considerably different. However, it should be considered that fen peat HAs and partly also Eipurs bog peat HAs are formed from peat with major contribution of higher vegetation to the composition, whereas Dzelves bog peat HAs are formed of peat with bryophyte dominance. The ratio between 1610 cm^{-1} and 3400 cm^{-1} (OH-stretching vibrations, hydrogen-bonded OH groups) is increasing with increasing depth in studied fen peat HAs and in Eipurs bog HA samples. This finding suggests that the relative contribution of the olefinic and aromatic C=C double bonds and C=O bonds is increasing in relation to OH-bonds with increasing depth and age of HAs. The ratio between 1610 cm^{-1} and 2920 cm^{-1} (in plane vibrations of conjugated olefinic and aromatic C=C bands and C=O bands, stretching vibrations of aliphatic $-CH_3$ and $-CH_2$ in the side chains) indicate that in fen peat HAs and in Eipurs bog HAs, the aliphatic contribution in relation to olefinic/aromatic contribution is increasing with increasing depth and age of HAs. In Dzelves bog HAs, the aliphatic contribution is slightly decreasing with depth and age of HAs. However, conjugated C=C and C=O bands in the aromatic skeleton of HAs and quinones cannot be differentiated from conjugated olefinic C=C and enolic C=O bands by FTIR data alone. Still, the obtained results suggest that the relative contribution of aliphatic carbon is increasing with depth and age of fen peat HAs. The UV/Vis spectroscopy can provide data on compounds with conjugated double bonds, confirm and supplement data obtained from FTIR analysis. However, it must be noted that with UV/Vis spectroscopy alone, it is not possible explicitly identify the structure of HAs as aromatic. The UV/Vis spectra of fen peat HAs have shown the presence of lignin, which can be explained by its high resistance to degradation and slow decomposition rate. Consequently, lignin can temporarily accumulate in fen peat, undergo biological and chemical transformation and become incorporated into fen peat HAs. For this reason, lignin compounds are amongst the main precursors of aromatic carbon in fen peat HAs (Adani *et al.* 2006). At the same time, less recalcitrant to degradation aliphatic compounds are humified and accumulated within peat mass before lignin. Therefore, aliphaticity and not aromaticity is a likely feature of both fen and raised bog peat HAs. In fen environment, peat humification adopted lignin as one of the constituents at later stage of peat formation and it is likely that the emergence of lignin did not have any profound influence on the humification itself, besides new microbial pathways to decompose the organic material. Nonetheless, the concept of the humification is one of key concepts for carbon biogeochemical flows and the processes related to transformation of living organic matter, however considering the huge complexity of these processes, especially amongst different environments, discussions are still open. A plot of the A_{1610}/A_{2920} IR absorption ratio and the E_{465}/E_{665} UV-Vis absorption ratio demonstrated a negative linear correlation ($r = -0.51$) and supported the conclusion that lower E_{465}/E_{665} ratio values are associated with more aromatic components in peat HA composition. However, high degradation degree of fen peat HAs obstructed the data validation. The main molecular components of fen and raised bog peat HAs that contribute to fluorescence most probably are chromone derivatives with the Ex-wavelength at $\lambda = 320–350$ nm and the Em wavelength at $\lambda = 400–500$ nm and flavones or isoflavones (phenolic compounds) with the Ex-wavelength at $\lambda = 310–365$ nm and the Em wavelength at $\lambda = 415–475$ nm (Enev *et al.* 2014). These phenolics are derived from lignin and other decomposed plant materials and are incorporated into peat HAs during their formation (Kleinert and Barth 2008). Therefore, their signal is more pronounced in fen peat HAs (Fig. 4).

Stable isotopes of C and N have been found useful in botanical studies, as they are a major aid to reconstruct the historical environment, to evaluate the origin and transformation of the organic material, to measure the anthropogenic interaction with the environment and in many other aspects (Fiorentino *et al.* 2015). Peat is commonly formed from assemblages of C3 and C4 plant communities with characteristic isotopic signatures, which can be detected in HAs. Changes in stable isotopic signatures reflect changes in the environmental conditions that can be both natural and human-caused. To identify changes in the historical environment, it is common to characterise stable isotopic ratios, such as, $\delta^{13}C$ and $\delta^{15}N$. The ratio $\delta^{13}C$ is a function of the photosynthetic pathways used by plants to fix CO_2 from the atmosphere. Thus, variations in this ratio indicate changes in vegetation over time. Moreover, peat-forming plants have unique δ^{13}C signature, which is not significantly altered during peat decomposition or HA formation. At the same time, our results suggest that in HAs of highly decomposed fen peat, the δ^{13}C ratio is comparatively higher than in HAs from weakly decomposed peat. Additionally, in HAs taken from top peat layers in fens, the value of δ^{13}C typically is higher than in HAs taken from deeper peat layers, which can be explained by formation of HAs in comparatively recent past and peat in top layers being with higher decomposition degree than in the rest of peat profile. Our data show that relation between δ^{13}C and decomposition is relatively high ($r = 0.60$). Peat is most commonly formed of plants with C3 photosynthesis, in which the δ^{13}C is around –27‰. Several C4 species common to peat material have δ^{13}C signature around –15‰. Noteworthy, C4 plant communities are very rare in the boreal region, because of their poor photosynthetic performance in the climatic conditions that are characteristic to this region. Still, individual parts of boreal ecosystems, for instance, within fens may still provide necessary conditions for C4 plant development (O'Leary 1988, Kubien and Sage 2003, Zhao *et al.* 2010). The ratio δ^{15}N represents N-fixation in plants over time. The ratio can also be used to analyse N-transformation during assimilation, amino acid synthesis and protein turnover, and N loss pathways such as loss of gaseous N products which fractionate heavily against ^{15}N (Bai *et al.* 2012). Our results suggest that δ^{15}N is linked to depth and age of peat where HAs are extracted from and it also depends on the organic matter content in peat and N concentration of HAs. At the same time, also differences in local anthropogenic and environmental impacts can be traced. Thus, different forms of N that are common to peat HAs may be interesting aspect for discussion in future studies, such as heterocyclic N, which can show possible impact of a historical wildfire, which has altered HA properties.

The data obtained from ^{13}C NMR point on the major contribution of aliphatic carbons to the structure of peat HAs, this finding is also consistent with information obtained from FTIR analysis. However, we believe that it is likely that conjugated double bonds in fen peat HAs are aromatic, as ^{13}C NMR regions where conjugated double bonds can be found (e.g. 116 ppm and 137 ppm) are also in the intervals where signals of aromatics, such as substituted and unsubstituted aromatics are located — from 110–144 ppm, respectively. This is also supported by Hasegawa *et al.* (1978) and Davis *et al.* (1999).

Conclusions

We conclude that HA content in peat is affected by the location of mire and the environmental settings. In fen peat, HA content appears to be related to peat decomposition degree, lignin content, elemental H and N content, and the amount of additional inorganic matter, which can greatly reduce or increase the organic matter content in the total peat mass. In raised bog peat, HA concentration also seems to be related to peat decomposition degree, whilst there is no apparent relation to N and H content or location of mires. HA content in peat HAs apparently is affected by the age and depth range of peat samples, these parameters also affect aromaticity and aliphaticity of HAs. In this study, fen peat samples were systematically older than raised peat bog samples, it per se caused potential differences which were not necessarily related to peat type, but a detailed comparison of HAs of same age would be required for a conclusion that peat type is a dependency. Still, a general conclusion is that fen and raised bog peats have significant differences in the concentration of HAs and this is because lignin-rich vegetation in fens is more sensitive to degradation than lignin-poor vegetation in raised bogs. Despite apparent differences in fen and raised bog peat HAs, the humification process itself is still a subject for discussions. We suggest that the pathways in microorganisms that are responsible for the decomposition and degradation of natural organic matter and the synthetic oxidation of phenolic compounds, such as lignin, are similar in all environments, and changes apparently occur at later stage due to subsequent conditions of organic matter transformation depending on the environment wherein the natural organic matter accumulates.

While trends of C and O concentrations as the function of time are different in fen HAs and in raised bogs HAs. the elemental ratios in HAs of both kinds are rather similar, indicating similar formation conditions irrespective of the environment where they have been forming. However, due to the environmental transformations over time, the elemental composition may be altered. With advancing humification the concentration of the C and O changes accordingly. Trends of C and O concentrations as the function of time appear to be rather different between fen and raised bog peat HAs due to diversity in peat humification conditions. Respectively, in raised bogs with less intensive organic matter humification C tend to increase in concentration, but in fens with intensive organic matter humification the concentration of C is decreasing.

The overall aromaticity of fen peat HAs is higher than that of raised bog peat HAs, due to peat formation from lignin-rich vegetation that provides aromatic macromolecules. This is supported by the spectroscopic analysis data. Some differences can be observed in the ^{13}C NMR spectra of HAs, depending on their type. That is, fen peat HAs contain higher concentration of aromatic C, and the ratio between unsubstituted aromatic C and aromatic C substituted by O is higher than that in raised bog peat HAs, pointing to the presence of lignin material in fen HAs. The O-alkyl signals, which can be observed in the fen peat HA ^{13}C NMR spectra, indicate possible degradation over time and dehydration of carbohydrates that are attached to the molecular structure of HAs. This finding is consistent with the lignin pattern in the fen peat HA FT-IR spectra. At the same time, peat-forming bryophytes also contain molecules rich in phenolics, although aliphatic compounds prevail here. Perhaps, lignin content in fen peat HAs should be observed separately from peat humification process as lignin is resistant to degradation, thus it gets involved in the HA composition when other compounds are humified and some portion of fen HAs already formed without any involvement of lignin.

The subsequent conditions of organic matter transformation in fens vs. raised bogs appear to be more important for the formation of HA properties than the source material itself. The results suggest that HAs formed due to polymerisation of water-soluble phenolic precursors in the heterophasic biocatalysis process due to a catalytic effect of immobilised phenol oxidases (Mala *et al.* 2013). Parameters like the depth range, age, organic matter content and decomposition degree show strong correlations with HA properties and composition, while the peat type is one of the major factors defining the amount of HAs, which consequently is sensitive to changes in the source material. The raised bog and fen peats from which the studied HAs were isolated have formed in considerably different environmental conditions and geobotanical regions and under varied impacts from adjacent areas. We suggest that future comparative analyses of these types of peat should use samples of a similar age to factor in global environmental impacts on their formation. Such analysis would also require a wider selection of bogs and fens, not just the four selected for this study.

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