Preferential degradation of polyphenols from *Sphagnum* – 4-isopropenylphenol as a proxy for past hydrological conditions in *Sphagnum*-dominated peat

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Highlights

- Depth records of C/N, markers for lignin, cellulose and sphagnum acid are compared for peat cores
- Their relation to bog hydrology is established in five peatlands from different climatic zones
- Under aerobic conditions polyphenols degrade faster than polysaccharides in *Sphagnum* peat
- 4-Isopropenylphenol reflects past hydrological conditions in *Sphagnum* peat
- Interpretation of vegetation and decomposition proxies in *Sphagnum* peat is site-dependent

Keywords

Sphagnum degradation; Rödmossamyran peatland; Pyrolysis-GC/MS; C/N ratio; Biomarker.

ABSTRACT

The net accumulation of remains of *Sphagnum* spp. is fundamental to the development of many peatlands. The effect of polyphenols from *Sphagnum* on decomposition processes is frequently cited but has barely been studied. The central area of the Rödmossamyran peatland (Sweden) is an open lawn that consists mostly of *Sphagnum* spp. with a very low contribution from vascular plants. In order to determine the effects of decay on sphagnum phenols, 53 samples of a 2.7 m deep core from this lawn were analysed with pyrolysis gas chromatography-mass spectrometry (pyrolysis-GC/MS) and compared with more traditional decomposition proxies such as C/N ratio, UV light transmission of alkaline peat extracts, and bulk density. Factor Analysis of 72

quantified pyrolysis products suggested that the variation in 4-isopropenylphenol was largely determined by aerobic decomposition instead of *Sphagnum* abundance. In order to evaluate the effects of aerobic decay in *Sphagnum* peat, down-core records from different climatic regions were compared using molecular markers for plant biopolymers and C/N ratio. These included markers for lignin from vascular plants ((di)methoxyphenols), polyphenols from *Sphagnum* spp. (4-isopropenylphenol), and cellulose (levoglucosan). Our results indicate that polyphenols from *Sphagnum* are preferentially degraded over polysaccharides; consequently the variability of the marker for sphagnum acid, 4-isopropenylphenol, was found indicative of decomposition instead of reflecting the abundance of *Sphagnum* remains. The fact that 4-isopropenylphenol is aerobically degraded in combination with its specificity for *Sphagnum* spp. makes it a consistent indicator of past hydrological conditions in *Sphagnum*-dominated peat. In contrast, the variability of C/N records in *Sphagnum*-dominated peat was influenced by both vegetation shifts and decomposition, and the dominant effect differed between the studied peatlands. Our results provide direction for modelling studies that try to predict possible feedback mechanisms between peatlands and future climate change, and indicate that the focus in *Sphagnum* decay studies should be on carbohydrates rather than on phenolic compounds.

1. Introduction

To understand the consequences of a changing climate, knowledge of past environmental change as well as the feedback mechanisms between natural ecosystems and climate is essential. Peatlands are important sources of information for such studies because they respond strongly to changes in environmental conditions, in particular to hydrology (Philben et al., 2013). First, peatlands are a major terrestrial carbon store (Yu, 2012), due to incomplete decomposition of

plant materials after death. Under a changing climate, the carbon that is stored by plant photosynthesis can be released back into the atmosphere as the greenhouse gasses carbon dioxide and methane (Freeman et al., 2001). Second, the relationship between hydrology, botanical composition and degree of peat decomposition in peatlands provides an important contribution in their functioning as a climate archive. A number of biological, physical and chemical proxies have been used to study past environmental and climate changes in peatlands (Chambers et al., 2012). However, many of the available proxies are influenced by multiple processes, which may limit or complicate the interpretation of their environmental implications. For example, recent geochemical studies indicate that the effects of decomposition should be considered when using source proxies, including for stable isotopes of plant biopolymers (DeBond et al., 2013), trace elements (Biester et al., 2012), biomarkers (Sinninghe-Damsté et al., 2002; Jex et al., 2014) and the distribution (Andersson et al., 2012) and isotopic composition (Huang et al., 2014) of *n*-alkanes.

The controls on peat decomposition are a key factor in our understanding of the relationship between peatlands and climate. Plant inputs and their subsequent decomposition leave a chemical fingerprint on the composition of the peat. Decomposition includes mineralisation to carbon dioxide and water, as well as transformations of (macro)molecules that include fragmentation and oxidation. Mass loss is a measure for mineralisation and can be studied by the quantification of gas fluxes *in situ*; however, this only provides information of on-going decomposition. The ratio of total organic carbon to total nitrogen (C/N) has been proposed as a proxy for estimating mass loss, with relatively low C/N values indicating drier conditions because carbon is lost during decomposition while nitrogen is preferentially preserved (Biester et al., 2014). Transformations of plant remains can be studied by the chemical composition of the

remaining material, i.e., the peat. For example, the degree of peat humification can be inferred from the colour intensity of humic substances measured using UV light transmission (%T) following an alkaline extraction (8% NaOH; Blackford and Chambers, 1993). As proxies for decomposition, C/N and %T will decline with increasing decomposition: C/N due to the preferential loss of carbon, and %T due to the increased content of soluble organic matter. However, C/N and %T do not always infer the same decomposition profile and an important role for the original vegetation inputs in controlling these proxies has been proposed (Yeloff and Mauquoy, 2006; Hansson et al., 2013). Based on preferential decomposition, the content and composition of biomacromolecules such as polysaccharides and lignin have also been used as proxies (Comont et al., 2006; Jia et al., 2008; Jex et al., 2014).

Our current knowledge on the three-way relationship between the botanical composition, decomposition and environmental factors in peatlands is limited (Abbott et al., 2013) making the interpretation of their proxy records intricate. To obtain a fundamental understanding of peatland organic matter dynamics, pyrolysis techniques are a powerful tool because they provide molecular information for complex unknown mixtures of organic matter (Nierop et al., 2005). This enables the study of both the plant sources of the organic matter and the superimposed decomposition processes. Pyrolysis results in cleavage of chemical bonds within macromolecular structures by adding heat to a sample, which in combination with gas chromatography/ mass spectrometry (GC/MS), makes it possible to directly analyse non-volatile complex mixtures of organic material. Pyrolysis-GC-MS provides detailed structural information, including the contribution of chemical groups as well as the composition within these groups (Huang et al., 1998). Pyrolysis-GC-MS has been successfully applied to study past peatland dynamics, providing the composition of lignin phenols and the abundance of plant vs. microbial sugars that

117 can be related to plant source and degradation state (Kuder et al., 1998; Huang et al., 1998), as
118 well as plant-specific pyrolysis products (Schellekens et al., 2011). In addition, knowledge of the
119 molecular structure of sphagnum phenols largely depends on pyrolysis techniques (van der
120 Heijden et al., 1997).

121 Although *Sphagnum*-dominated peatlands are frequently used for palaeoclimate
122 reconstructions (Chambers et al., 2012), the decay of *Sphagnum* itself is poorly understood.
123 *Sphagnum* litter is known to have a very slow decomposition rate (Clymo, 1965), which is
124 attributed to an abundance of phenolic macromolecules (van Breemen, 1995; van der Heijden et
125 al., 1997; Freeman, 2001) that contain sphagnum acid (*p*-hydroxy-β-[carboxymethyl]-cinnamic
126 acid) or to pectin-like polysaccharides (sphagnan; Painter, 1991; Hájek et al., 2011). Sphagnum
127 acid (Hesse and Rudolph, 1992) and sphagnan (Mitchel, 1996) are mainly found in the hyaline
128 cell walls of *Sphagnum* mosses in which it is covalently linked to other cell wall biopolymers
129 (Painter, 1991; van der Heijden, 1994). Sphagnum acid is specific for *Sphagnum* spp. (van der
130 Heijden et al., 1997), and has been used to trace the abundance of *Sphagnum* in peat records
131 using pyrolysis techniques (McClymont et al., 2011; Schellekens and Buurman, 2011). There is
132 recent evidence that the decay of sphagnum phenols is related to water table depth (Abbott et al.,
133 2013; Swain and Abbott, 2013) but how this compares to other plant biopolymers remains
134 unclear. In addition, plant-specific monosaccharide compositions agreed with the macrofossil
135 record in a *Sphagnum*-dominated peat core (Jia et al., 2008) suggesting a minor effect of
136 decomposition on sphagnum polysaccharides. Indeed, these structural polysaccharides from
137 *Sphagnum* were related to its resistance against degradation (Hájek et al., 2011).

138 Here we hypothesise that sphagnum acid is preferentially lost during aerobic surface decay
139 and that its variation with depth thereby provides a sensitive proxy for past mire surface wetness

in (homogenous) *Sphagnum* peat. In order to test this hypothesis, a *Sphagnum*-dominated peat core was studied in detail with analytical pyrolysis, C/N and %T. Pyrolysates will provide the molecular structure of bulk peat samples, while C/N and %T support pyrolysis data and are used to estimate mass loss and decomposition state, respectively. The Rödmossamyran mire (RMM; northern Sweden) was chosen for this purpose because its central lawn is covered almost exclusively by *Sphagnum* and has very wet conditions throughout the year (Rydberg et al., 2010).

To further examine the mechanisms of *Sphagnum* decay, depth records of C/N and pyrolysis parameters were applied to cores from RMM and four previously studied peatlands from other climatic regions. Large areas of high-latitude peatlands are dominated by *Sphagnum*, although with local differences in climate and ecology. Here we test whether local differences in botanical composition, both the contribution of different *Sphagnum* species and the type and contribution of vascular plants, bias the interpretation of peat chemistry. Pyrolytic parameters included products specific for sphagnum acid (4-isopropenylphenol; van der Heijden et al., 1997), lignin ((di)methoxyphenols) and cellulose (levoglucosan; Pouwels et al., 1989), thereby providing markers for the relevant plant biopolymers in *Sphagnum* peat. From these parameters, lignin provides evidence for vascular plants in peat dominated by *Sphagnum*, because *Sphagnum* contains no lignin and anaerobic degradation of lignin proceeds at a relatively slow rate (Benner et al., 1984). The relative abundance of cellulose and sphagnum acid can be related to preferential decay of those plant compounds. By assessing the signals recorded in peatlands from different regions (with different vegetation and hydrology), we test the impact of botanical differences on sphagnum phenol degradation and extend its application. Along with changes in the assemblages of testate amoebae (e.g. van Bellen et al., 2014), the C/N ratio is one of the few

proxies that can be used to infer surface wetness in homogeneous *Sphagnum* peat (Yeloff and Mauquoy, 2006). The C/N ratio was determined for all peatlands, providing a measure for mass loss during decomposition upon aerobic conditions. In addition, comparison of C/N records with molecular parameters in peatlands which have different contribution from *Sphagnum* allows an examination of the influence of source vegetation on its use as decomposition proxy.

2. Methods

2.1. Location and sampling

The RMM peatland is a nutrient-poor (oligotrophic) fen in northern Sweden that is approximately 7 ha in extent (Table 1). Based on the basal age of a nearby mire (Stor Åmyran, 2400 BP; Oldfield et al., 1997) and the rates of isostatic rebound in this region, RMM transformed into a mire between 2500 and 2800 years ago. Macrofossils characteristic of marsh vegetation (e.g., *Equisetum palustre*) were visible in the basal peat layers. For details on the location of the core see Rydberg et al. (2010). Much of the mire surface is covered by small, thinly spaced pines (*Pinus sylvestris* L.) with a field layer consisting of mosses and dwarf shrubs. In the central area of the southern half of the mire there is an open *Sphagnum* lawn of 0.25 ha that consists mostly of *Sphagnum*. There is no open water on the mire surface nor are there any surface inlets or outlets or signs of historical ditching in its close proximity. The vegetation of the *Sphagnum* lawn is dominated by *S. centrale* and *S. subsecundum* (with minor contributions from *S. palustre* and *S. magellanicum*) and some contributions from *Eriophorum vaginatum*. The dominant fen peat characterising most of RMM surrounding the *Sphagnum* lawn includes *S. centrale*, field-layer vegetation including *Calluna vulgaris* (L.) Hull and *Ledum palustre* L., and sparcely spaced small pine (*P. sylvestris*).

Four plant species from RMM were selected for chemical analysis, these included *S. centrale*, *S. magellanicum*, *E. vaginatum* and *C. vulgaris*. The 270-cm thick profile from RMM consists of a Wardenaar (1987) surface core (0–66 cm) and an overlapping series of four Russian peat cores (length 1 m; diameter 7.5 cm; Rydberg et al., 2010). The Wardenaar core was taken back to the laboratory intact and stored frozen, whereas the Russian peat cores were cut in the field into 10-cm sections. In the laboratory all field-sectioned samples were weighed and then stored frozen at -18 °C until processing. In a freezer room (-18 °C) the Wardenaar core was cut in half length-wise, the outermost 1 cm removed on a band saw with a stainless steel blade, hand planed into even dimensions, and then cut into 2-cm-thick slices. The samples were freeze-dried, weighed for calculating dry bulk density and ground in an agate ball mill before further analysis.

Peat cores from *Sphagnum*-dominated peatlands from other climatic zones included Königsmoor (KM; Harz Mountains, Germany; Biester et al., 2012, 2014), and Butterburn Flow (BBF; UK; McClymont et al., 2008, 2011), while the Harberton core (HRB; Tierra del Fuego, Argentina; Schellekens et al., 2009, Schellekens and Buurman, 2011) is composed of three vegetation zones, the upper two being dominated by *Sphagnum* (HRB1, HRB2) and the deepest one dominated by graminoids and woody species (HRB3). A graminoid-dominated peatland that has contributions from *Sphagnum* was also studied, Penido Vello (PVO; northern Spain; Schellekens et al., 2011, 2012, 2015). The main characteristics of these peatlands are given in Table 1.

The *Sphagnum*-dominated peatlands (RMM, KM, BBF and HRB) differed slightly in *Sphagnum* and vascular plant species (Table 1), though all peatlands shared the same ecology showing an increase of vascular plant species upon drier conditions (McClymont et al., 2008; Rydberg et al., 2010; Schellekens et al., 2009; Biester et al., 2014). PVO is dominated by

graminoids including Poaceae, Juncaceae and Cyperaceae and has a low contribution from several *Sphagnum* species (for details on the vegetation composition of this peatland see Schellekens et al., 2011). KM and PVO were sampled at a resolution of 2 cm; for HRB the upper 174 cm were sampled according to the morphology/stratigraphy, the deeper part of the core was sampled in sections of 3 cm, except at the bottom of each sub-core (5 cm); BFF was sampled at 1 cm intervals, of which a selection of 56 samples were analysed, because carbon and nitrogen content and analytical pyrolysis were not always determined on samples from the same depths, only the overlapping samples (32) were used to determine correlation coefficients. Depth, age and number of samples for each core are given in Table 1.

2.2. Bulk density, ash content and %T (RMM)

All samples were weighed for calculating dry bulk density. The ash content of the peat samples was determined following re-drying of the sample at 105 °C and then heating the samples at 450 °C for 4 h. %T was measured following an alkaline extraction based on the methods of Blackford and Chambers (1993). In brief, 0.020 g of peat were digested in 10 mL 8% NaOH (95 °C, 1 h), diluted with an equal volume of deionised water, filtered (Whatman no.1), diluted again with an equal volume of deionised water and light transmission (%T) measured in triplicate using a 1 cm quartz cuvette in a Hitachi U-1100 spectrophotometer at a wavelength of 540 nm. Replicate analyses, both within run and between days, were within ±3%.

2.3. Carbon and nitrogen

Elemental compositions were determined as proportion (%) of the dry weight of peat analysed. The carbon and nitrogen contents of the RMM peat and plant samples were determined using a

232 PerkinElmer 2400 series analyser operating in CHN mode only. Replicate samples, included
233 approximately every 10^{th} sample, showed a precision within ±3% for carbon and nitrogen. C/N
234 ratios are reported here based on a mass basis.

235 Concentrations of carbon and nitrogen for KM peat and plant samples were determined by gas
236 chromatography and thermal conductivity detection after thermal combustion in an elemental
237 analyser (Euro EA3000, Eurovector). Reproducibility of duplicates was always better than ~8%
238 RSD (Biester et al., 2014). For BBF, freeze-dried subsamples of *c*. 3–4 cm^3 volume were
239 homogenised and ground to pass through a 0.5 mm sieve. Aliquots were analysed in duplicate
240 using a Carlo-Erba EA1108 elemental analyser (McClymont et al., 2011). For HRB, peat
241 samples were analysed by complete combustion in an auto-analyser, Fisons CHNS-O EA-1108
242 for carbon, and LECO CHNS-932 for nitrogen (Schellekens and Buurman, 2011). For PVO, peat
243 samples were analysed by complete combustion in a Leco CHN-1000 auto-analyser (Pontevedra-
244 Pombal et al., 2004).

245

246 *2.4. Pyrolysis-GC/MS (RMM)*

247 Platinum filament coil probe pyrolysis (temperature 650 ºC) was performed with a Pyroprobe
248 5000 pyrolyser (CDS, Oxford, USA) coupled to a 6890N gas chromatograph and 5975B mass
249 selective detector (MSD) system from Agilent Technologies (PaloAlto, USA). Samples were
250 embedded in quartz tubes using glass wool. The pyrolysis interface and GC inlet (split ratio 1:20)
251 were set at 325 ºC. The GC instrument was equipped with a (non-polar) HP-5MS 5% phenyl,
252 95% dimethyl-polysiloxane column (30 m x; 0.25 mm i.d.; film thickness 0.25 µm). Helium was
253 the carrier gas at constant flow of 1 mL/min. The oven temperature program was 50 to 325 ºC
254 (held 10 min) at 15 ºC/min. The GC/MS transfer line was held at 270 ºC, the ion source (electron

impact mode, 70eV) at 230 ºC the quadrupole ion filter and the detector at 150 ºC scanning a range between *m*/*z* 50 and 500. Compounds were identified using the NIST '05 library.

Seventy-two pyrolysis products were quantified for 53 peat samples, including all prominent peaks. In addition, the presence of some compounds was established by partial chromatograms of their specific fragment ions, these included *n*-alkanes and *n*-methyl ketones, a series of lignin phenols, and biomarkers that have been previously identified from pyrolysates of peatland plants (Schellekens et al., 2009, 2011). The searched biomarkers included ferulic acid methyl ester (graminoids) and 3-methoxy-5-methylphenol (lichens); in addition fragment ions of diterpenes (*Pinus* spp.) and sesquiterpenes were also searched for. From those plant-specific pyrolysis products, only ferulic acid methyl ester was detected in the RMM peat samples, but it was very low in some samples (<0.02% TIC) and absent in others. Quantification was based on the peak area of characteristic fragment ions (*m*/*z*) for each pyrolysis product (SI_Table 1). For each sample, the sum of the quantified peak areas was set at 100% and amounts were calculated relative to this. According to probable origin and similarity, the pyrolysis products were grouped as follows: polysaccharides, aliphatic compounds (*n*-alkanes, *n*-alkenes, *n*-methyl ketones, *n*-fatty acids, other aliphatic compounds), lignin moieties (syringyl, guaiacyl and *p*-hydroxyphenyl), phenols (including polyphenols), aromatics, polyaromatic hydrocarbons, and nitrogen containing compounds. To compare the abundance of phenols and polysaccharide pyrolysis products between living *Sphagnum* and *Sphagnum* peat, the same 72 products quantified for the peat also were quantified for pyrolysates of living *Sphagnum*.

For details on analytical conditions and quantification for pyrolysis of previous studies we refer to Biester et al. (2014) for KM, Schellekens and Buurman (2011) for HRB, McClymont et

al. (2011) for BBF and Schellekens et al. (2011) for PVO; whether the 72 quantified RMM pyrolysis products were included in the quantification of those cores is indicated in SI_Table 1.

2.5. Data analysis and statistics

The abundance of pyrolysis products depends on several factors that, apart from the composition of the peat, differ between laboratories. These are, for example: the selection of pyrolysis products for quantification, because they are expressed as the proportion (%) of the total quantified products; the choice of *m*/*z* values for quantification; MS response factors; and pyrolysis as well as GC conditions. Therefore, comparison of numerical values between different peat pyrolysis studies is difficult, but the general trends will allow us to assess the variations along a core (Jacob et al., 2007). Although a product with a low abundance (e.g. <1% of the total quantified pyrolysis products) can be considered as statistically independent, a large number of pyrolysis products have to be quantified to reach this independence. An alternative to the quantification of large data sets is the use of ratios; frequently applied examples from lignin geochemistry include syringyl to guaiacyl and acid to aldehyde ratios (Jex et al., 2014). In relation to analytical pyrolysis, the advantage of using ratios is that quantification is simple and data sets can be compared. A disadvantage is that its variation is determined by changes in both numerator and denominator; because this can be influenced by several factors, information may be lost. Therefore, we chose to use depth records of (groups of) pyrolysis products expressed as proportion of the total quantified pyrolysis products (% TIC), as well as ratios. Ratios included that of 4-isopropenylphenol to lignin pyrolysis products (I%; McClymont et al., 2011) and (di)methoxyphenols to levoglucosan. The parameters are given in Table 2.

300 *2.5.1. Factor analysis (RMM)*

301 The main processes that influence the molecular composition of peat organic matter interact with
302 environmental factors and comprise changes in input material (shifts in the composition of the
303 plant cover) and decay processes. In order to identify and separate the effects of these factors on
304 peat chemistry, factor analysis was applied. Factor analysis was carried out using Statistica
305 software, version 6 (StatSoft, Tulsa).

306 Factor analysis extracts trends from complex data sets by searching for linear correlations
307 between variables, reducing this variation to a number of factors. Factor analysis was performed
308 on the 53 RMM peat samples using all 72 quantified pyrolysis products as variables (SI_Table
309 1). The identification of the underlying processes was based on the loadings of pyrolysis
310 products on each statistical factor; scores reflect the weight of each statistical factor to a given
311 sample and allows comparing the molecular chemistry with other characteristics (C/N, %T, ash
312 content and bulk density). Thus, while the variation in individual pyrolysis products can be
313 influenced by several aspects (decomposition, input, wildfire, etc.), the scores of an extracted
314 factor take into account the shared variation in all quantified pyrolysis products thereby
315 reflecting the effect of a single process on the peat chemistry.

316 The total variance explained by a factor is not indicative of its value as proxy, because it
317 depends on the number and combination of variables that show a similar distribution; a variance
318 with a higher level of explanation thus reflects major changes in peat chemistry (provided that
319 the pyrolysates are reflecting this). Although the largest set of correlated variables, i.e. the major
320 process affecting peat chemistry, is allocated into the first factor this does not mean that variables
321 with low loadings on this factor cannot have value as a proxy indicator. For example, if the first
322 factor reflects preferential degradation of polysaccharides over lignin, polysaccharide and lignin

pyrolysis products will have high and opposite loadings on this factor; but minor differences within the lignin phenol group can be enlarged when the ratio has been included as a variable. Thus, minor differences in loading or low loadings of pyrolysis products still can be noteworthy, but interpretation must be carefully reasoned.

2.5.2. Comparison of proxy records

In order to discuss the influence of botanical source on decay parameters, an ANOVA test was applied to check for significant differences of the parameters between vegetation types. For each peatland, the selected proxies (Table 2) were compared using their depth records and correlation coefficients. Because of local differences in environmental conditions, botany and ecology and their complex relationships with peat chemistry, we rely on the major trend. For a detailed interpretation of the chemistry of KM (Biester et al., 2014), HRB (Schellekens and Buurman, 2011), BBF (McClymont et al., 2011) and PVO (Schellekens et al., 2011) we refer to previous studies.

3. Results

3.1. Plant analysis

C/N values for the dominant plant species from RMM and KM are given in Table 3. RMM plants showed higher C/N ratios compared to the same species from KM, while within both peatlands C/N values decreased from *Sphagnum* to *C. vulgaris* with lowest values for *E. vaginatum*. For analytical pyrolysis of *E. vaginatum*, *S. centrale*, *S. magellanicum* and *C. vulgaris*, 4-isopropenylphenol was specific for *Sphagnum* spp. (Ph5; van der Heijden et al., 1997) and additionally two biphenyl compounds were detected solely in *Sphagnum* (Ph6, Ph7,

see SI_Table 1; Biester et al., 2014). Furthermore, all peat pyrograms showed a double peak for *m*/*z* 137+152 (G3 and Ph4, SI_Table 1). This mass spectrum is generally identified as 4-ethylguaiacol (G3), the structure of which does not allow isomers. The second peak (longer retention time) was detected in pyrolysates of fresh *Sphagnum* but was absent in pyrolysates of the other plant species, which indicates that it is related to *Sphagnum*. The marker for graminoids (ferulic acid methyl ester; Schellekens et al., 2012) was found in the *Eriophorum* plant sample, but its abundance in the RMM peat samples was near the limit of detection (Section 2.4). A minor amount of guaiacyl lignin was present in pyrolysates of living *Sphagnum*; this was also found in pyrolysates of living *Sphagnum* from other sites (McClymont et al., 2011; Schellekens et al., 2009). The fact that the abundance of guaiacyl moieties was clearly higher in the *Sphagnum* samples collected from the dominant pine-covered area of RMM compared with the open area of the *Sphagnum* lawn supports the explanation of this phenomenon by Abbott et al. (2013), who suggested that the lignin phenols dissolved in peat water have been mobilised into the hyaline cells of the *Sphagnum* capitula where they are physically entrapped or bound.

3.2. Peat analysis

The ash content at RMM varied between 0 and 1.9% indicating the ombrotrophic nature of the lawn peat, except for the two deepest samples with 9.5 and 26.6% ash (not shown) that were deposited during the isostatic isolation and early formation of the mire. The chemical composition of these two samples differed extremely from the other samples; they were therefore excluded from the depth records. A detailed list of all quantified pyrolysis products from the RMM peat samples and their mean abundance is given in SI_Table 1.

368 The first three factors from factor analysis applied to the pyrolysates from RMM explained
369 67.5% of the total variance. The loadings of the first two factors are plotted against each other in
370 Fig. 1. Factor 1 (F1) explained 43.9% of the variance. Factor 2 (F2) explained 12.8% of the
371 variance. The scores of F1 and F2 are plotted against depth and compared with depth records of
372 C/N, %T, bulk density and ash content (Fig. 2).

373 Mean values of the parameters provide a broad indication of peat chemistry and allow
374 comparison between peatlands (Table 2). The results of the ANOVA test indicate highly
375 significant (P<0.01) differences between bogs for all parameters (Table 2). In general terms,
376 RMM chemical nature is closer to that of HRB1 (both *Sphagnum*-dominated peat), characterised
377 by the highest C/N ratios, I% values, 4-isopropenylphenol, total polysaccharides and
378 levoglucosan content, and the lowest lignin content and lignin/levoglucosan ratios; while HRB3
379 and PVO (graminoid-dominated peat) represent the opposite nature (lowest C/N ratios, I%, 4-
380 isopropenylphenol, total polysaccharides and levoglucosan, and highest lignin and
381 lignin/levoglucosan ratio). KM and HRB2 showed similar, somewhat intermediate values for the
382 parameters, although much closer to those of RMM-HRB1 than to PVO-HRB3 (as indicated by a
383 cluster analysis on standardised average values of the parameters in Table 2; data not shown).

384 The relationship of each pyrolysis parameter with C/N is indicated by a correlation, and
385 provides a measure for the influence of source material and decomposition (SI_Table 2). Depth
386 records of C/N and pyrolysis parameters are shown in Fig. 3 for each peatland. The extent to
387 which molecular parameters varied together down-core in each bog is in addition indicated by a
388 correlation (SI_Table 2) and showed considerable differences between the vegetation types. In
389 the *Sphagnum*-dominated peatlands 4-isopropenylphenol and the summed (di)methoxyphenols
390 showed no correlation (RMM) or a rather weak negative correlation for KM (r^2=0.32; n=42;

391 P<0.0001) HRB2 (r^2=0.50; n=18; P<0.001) and HRB1 (r^2=0.34; n=15; P<0.02), though when
392 the upper three samples of HRB1 were excluded the correlation increased to highly significant
393 values (r^2=0.74; n=12; P<0.0005). The correlation between (di)methoxyphenols and
394 polysaccharides was only evident in the graminoid-dominated PVO peat core (r^2=0.59; n=80;
395 P=0.000000), while the correlation between 4-isopropenylphenol and polysaccharides was
396 absent in RMM, HRB1 and HRB3, and only weak in HRB2, KM and PVO (r^2<0.36; SI_Table
397 2).

398 To provide an indication for the relative decomposition rates of polyphenols and
399 polysaccharides, the change of their pyrolysis products from living *Sphagnum* to peat and from
400 surface peat to deeper peat is indicated in Figure 4 for the *Sphagnum*-dominated peatlands.
401 Because of differences in the quantification between the peatlands only phenol, 4-vinylphenol,
402 and levoglucosan (being dominant products + present in the quantification from all peatlands)
403 and 4-isopropenylphenol (specific for *Sphagnum* + present in all quantifications) were indicated
404 individually; the change in abundance of other phenolic/(poly)aromatic and polysaccharide
405 products are indicated by their sum. The polyphenol-derived pyrolysis products together
406 accounted for a decrease from surface to deeper peat from 26.2 to 9.3% and from 24.9 to 15.2%
407 of TIC in HRB and KM, respectively; while in RMM a decrease from 53.4 to 18.1% of TIC was
408 found from living *Sphagnum* to peat. Polysaccharides on the other hand clearly increased from
409 living *Sphagnum* to peat (from 41.4 to 76.8% TIC, from 63.9 to 74.2% TIC and from 48.3 to
410 59.6% of TIC for RMM, HRB and KM, respectively).

411

412 **4. Discussion**

413 *4.1. C/N in living plants*

Between plant species there is a similar pattern at RMM and KM where C/N decreased from *Sphagnum* to *C. vulgaris* to *E. vaginatum*, which is in agreement with the generally higher C/N values in *Sphagnum* compared with vascular plants (Hornibrook et al., 2000; Kleinebecker et al., 2007). The C/N values of the same plant species clearly differ between the sites (Table 3). The difference between sites is most prominent for *Sphagnum* and can be explained by differences in nitrogen deposition, because living *Sphagnum* assimilates nitrogen from the atmosphere (Heijmans et al. 2002) and central Germany receives much higher nitrogen deposition than northern Sweden (Akselsson et al., 2010).

4.2. The Rödmossamyran peat record

The absence of markers for lichens (3-methoxy-5-methylphenol) and pine and the very low abundance of the marker for graminoids (ferulic acid methyl ester) in the peat samples (Section 2.4) indicate a complete dominance by *Sphagnum* and prevailing wet conditions, which is in agreement with the present-day situation at the centre of the mire (Rydberg et al., 2010). This notwithstanding, the peat has a substantial contribution from (di)methoxyphenols (Table 2), indicating a contribution from vascular plants. The high syringyl/guaiacyl ratio (mean value 0.39; S.D.=0.09; n=53; SI_Fig. 1) excludes pine as the dominant source of this lignin (Hedges and Mann, 1979). In addition to ferulic acid methyl ester, parameters indicative of graminoids (including *Eriophorum*) are high ratios of 4-vinylguaiacol to the total guaiacyl compounds and 4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one to the summed polysaccharide pyrolysis products (Schellekens et al., 2015). They are positively correlated (r^2=0.41; P=0.000; n=53; SI_Fig. 2a) suggesting that a substantial part of these compounds has an *Eriophorum* source in the RMM peat, which is also consistent with the present-day composition. However, no correlation

between either compound (4-vinylguaiacol and 4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one) and (di)methoxyphenols was found (SI_Fig. 2bc). Catechol, on the other hand, showed high positive correlation with the (di)methoxyphenol content (r^2=0.62; *n*=52; *P*=0.000, uppermost sample excluded; SI_Fig. 2d). Catechol is a pyrolysis product of tannin (Nierop et al., 2005), which has a relatively high abundance in bark or berries (Kögel-Knabner, 2002), pointing towards *C. vulgaris* and/or *L. palustre* in the RMM peat. Although catechol was also significantly present in pyrolysates of *Sphagnum*, the similarity of depth records of catechol and (di)methoxyphenols in the peat (except for the uppermost sample) suggests that the variation in lignin is related to changes in the abundance of *C. vulgaris*, and indicates that the precursor of catechol from *Sphagnum* (polyphenols) is rapidly degraded compared with its precursor from *C. vulgaris* (lignin and/or tannin).

4.2.1. Identification of environmental processes from peat pyrolysates using factor analysis

Most (di)methoxyphenols showed high negative loadings on F1. The fact that *Sphagnum* contains no lignin indicates that F1 reflects mainly the contribution from vascular plants to the peat organic matter (high negative loadings). This is confirmed by the positive loading of the markers of *Sphagnum* (Ph5–Ph7); their lower loading on F1 (up to 0.4) is explained by the dominance of *Sphagnum*, because a minor increase of vascular plants will not significantly change the abundance of *Sphagnum* markers if the peat matrix is predominantly composed of *Sphagnum* litter.

For peat with a relatively high contribution from vascular plant material (negative loadings on F1) F2 seems to reflect aerobic degradation, compounds with positive loadings indicating a higher degree of decomposition. This interpretation is based on: (i) the separation of aliphatic

460 (positive) and lignin (negative) pyrolysis products (Fig. 1), because lignin is preferentially
461 degraded over aliphatic macromolecules (Klotzbücher et al., 2011); (ii) a high negative loading
462 of 4-vinylphenol (H1), 4-vinylguaiacol (G4) and 4-vinylsyringol (S4), pyrolysis products that
463 largely originate from non-lignin phenolics (Boon et al., 1982), which are abundant in non-
464 woody tissue (Hedges and Mann, 1979) and easier to decompose than macromolecular lignin;
465 and (iii) (di)methoxyphenols with a C_3 alkyl side chain (G6, S6), which are indicative of intact
466 lignin (van der Hage et al., 1993) and showed more negative loadings compared with the other G
467 and S moieties.

468 From the compounds with positive loadings on F1 (peat with a very low contribution from
469 vascular plants), 4-isopropenylphenol (Ph5) showed the highest negative loadings on F2, while
470 most polysaccharide pyrolysis products showed moderate to high positive loadings on F2.
471 Because F2 is identified as reflecting aerobic decomposition, this means that the phenolic
472 macromolecule is preferentially degraded over polysaccharides in *Sphagnum* litter, which is not
473 in agreement with the general assumption that *Sphagnum* decay is hindered by its polyphenolic
474 network (van Breemen, 1995). Abbott et al. (2013) indeed found that the abundance of markers
475 of sphagnum acid correlated with water table fluctuations, indicating aerobic degradation of
476 sphagnum acid. Thus, it is concluded that negative values on F1 reflect the contribution from
477 vascular plant species (especially the woody species, Section 4.2), while F2 reflects aerobic
478 degradation with positive values indicating a higher degree of decomposition.

479

480 *4.2.2. Chemical interpretation for RMM*

481 C/N and %T are positively correlated (r^2=0.57; P<0.01; n=53; SI_Fig. 2e), and both showed a
482 similar trend as F1 (Fig. 3; r^2=0.44; n=53; P=0.001 and 0.52; n=53; P<0.01, respectively;

483 SI_Fig. 2fg). Bulk density, on the other hand, showed similarities with F2 (r^2=0.36; *n*=50;
484 *P*<0.01 without the deepest three samples; SI_Fig. 2h). This supports identification of F2
485 because bulk density has been linked to decomposition in peatlands (Charman et al., 2013). This
486 suggests that in RMM the variation in C/N and %T is related mainly to the contribution from
487 vascular plants and not solely to aerobic degradation. However, although the chemical
488 transformations caused by vascular plant input and aerobic decay can be separated using factor
489 analysis, both are related to the height of the water table. An increase in the relative importance
490 of vascular plants is thus accompanied by increasing degradation of *Sphagnum* litter in the
491 acrotelm. The opposite is not necessarily true; that is, in nearly homogeneous *Sphagnum* peat
492 such as the RMM peatland, degradation of the polyphenolic network, from which sphagnum acid
493 originates, continues under aerobic conditions independently of a small simultaneous increase of
494 vascular plants. C/N values and transmission data on the other hand are undoubtedly affected by
495 aerobic decomposition, but a small increase of vascular plants may strongly influence the
496 variance of both the C/N ratio (Table 3) and %T (Yeloff and Mauqouy, 2006), thereby enlarging
497 the decomposition effect during low water table conditions. 4-Isopropenylphenol, being
498 indisputably of *Sphagnum* origin, may therefore be a good indicator of aerobic decomposition
499 (water table height) in *Sphagnum*-dominated peat. In order to test whether these results can be
500 extrapolated, depth records of a number of molecular parameters as well as C/N were compared
501 among a number of well-documented *Sphagnum*-dominated peatlands.
502
503 *4.3. Comparison of vegetation and decomposition proxies with other peat records*
504 *4.3.1. Polyphenols*

505 Several observations confirm rapid degradation of sphagnum polyphenols under aerobic
506 conditions as proposed in Sections 4.2.1 and 4.2.2. First, considering the extreme dominance of
507 *Sphagnum* in the RMM peat core, it is very unlikely that the variation in 4-isopropenylphenol
508 (from 0.21 to 2.85% TIC; Fig. 3a) reflects changes in the contribution from *Sphagnum* moss to
509 the peat. This is also evident from the much higher variation in 4-isopropenylphenol in
510 *Sphagnum*-dominated peat from Harberton (HRB1 and HRB2) compared with that of
511 polysaccharides and lignin (Fig. 3d).

512 Second, the abundance of 4-isopropenylphenol rapidly declined from fresh *Sphagnum* to peat
513 organic matter from 2.7 to 0.9% TIC for RMM (Fig. 3a), or from the surface sample to the
514 deeper peat samples from 0.7 to 0.1% TIC for KM (Fig. 3b), from 0.6 to 0.2 for I% in BBF (Fig.
515 3c) and from 2 to 0.1% TIC for HRB (Fig. 3d). Other phenolic and (poly)aromatic pyrolysis
516 products also showed a decline from living *Sphagnum* material to peat in RMM (except for Ph6;
517 SI_Table 1). A large decrease of most phenolic and some aromatic pyrolysis products was found
518 in the upper part of the *Sphagnum* peatlands (Fig. 4). Although most of these pyrolysis products
519 are not specific for *Sphagnum*, their identity, abundance and behaviour suggests sphagnum
520 polyphenols as their main source. However, in peat, a contribution from other sources is
521 apparent, demonstrated by the difference in intensity of the decrease in phenolic compounds,
522 being largest in RMM (living *Sphagnum* to peat), and decreasing from HRB1 to KM (surface to
523 deeper peat; Fig. 4). This is assigned to the contribution from vascular plants that was higher in
524 KM (Table 2). When the contribution from vascular plants increases, the proportion of phenolic
525 and aromatic pyrolysis products that originates from other sources than *Sphagnum* is increasing
526 and therefore obscuring the decay of sphagnum phenols. Thus, although the decrease of phenolic

and aromatic products supports decay of sphagnum polyphenols, only 4-isopropenylphenol, being specific for *Sphagnum*, can be assigned to *Sphagnum* decay in peat.

4.3.2. Lignin and polysaccharides

Contrary to phenolic pyrolysis products, polysaccharides showed an increase from living *Sphagnum* to peat (RMM) and from surface peat to deeper peat (HRB1, KM) in *Sphagnum*-dominated peat (Figs. 3 and 4). For the peat records discussed here, the abundance of (di)methoxyphenols reflects changes in the water table, with a higher abundance indicating relatively drier conditions. However, the underlying mechanism differs between graminoid and *Sphagnum* peat. We suggest that in *Sphagnum*-dominated peat (RMM, HRB1, HRB2, KM, BBF) higher (di)methoxyphenols contents indicate a higher contribution from vascular plants, which increase under drier conditions. Conversely, in the peatlands that were dominated by graminoids (PVO and HRB3), a high abundance of (di)methoxyphenols reflected the preferential decay of polysaccharides over lignin (r^2=0.59 for PVO; SI_Table 2; Schellekens et al., 2011, 2012, 2015). The absence of a correlation between lignin and polysaccharide pyrolysis products in the deeper part of HRB3 is due to the increasing mineral content and several volcanic ash layers (Schellekens et al., 2009), and accordingly a considerably higher degradation state in this section. In highly decomposed peat, the abundance of lignin reflects relatively fresh material, because after decomposition of plant-derived polysaccharides lignin is preferentially lost over highly resistant aliphatic polymers (Schellekens et al., 2014).

The record of the summed polysaccharide pyrolysis products showed a decreasing depth trend in PVO and HRB (Fig. 3de), which indicates that polysaccharides are also degraded during long-term anaerobic decay, while lignin is not mineralised anaerobically (Jex et al., 2014). The

550 decrease of polysaccharides with depth seems to be related to the contribution from vascular
551 plants (Table 1), and, apart from the generally lower abundance of polysaccharides in the
552 different vegetation zones of HRB, the depth trend within these zones is absent in HRB1
553 (*Sphagnum*-dominated), minor in HRB2 (*Sphagnum*+graminoids) and clear in HRB3
554 (graminoids+woody plants) and the PVO peatland (graminoids). The peat dominated by
555 *Sphagnum* did not show this decrease of polysaccharides with depth (RMM, HRB1, KM, Fig.
556 4a-d), thus supporting the resistance to decay of *Sphagnum*-derived polysaccharides (Hájek et
557 al., 2011).
558 From factor analysis of the RMM peat pyrolysates (Section 4.2.2) it appeared that 4-
559 isopropenylphenol reflects aerobic surface decomposition while (di)methoxyphenols indicate
560 vascular plant input. The correlation between both varied between the peatlands and was rather
561 weak (Section 3.2; SI_Table 2). This indicates that water-table drop downs do not always occur
562 together with an increase of vascular plants in *Sphagnum* peat. The variable and weak correlation
563 of 4-isopropenylphenol with (di)methoxyphenols may have various causes, such as differences
564 between the peatlands in nitrogen deposition, fluctuations of the water table, plant ecology,
565 nutrient status, and human disturbance. Though from Fig. 3 it is evident that a major increase in
566 vascular plants, indicated by the (di)methoxyphenol record, occurred together with a decrease of
567 4-isopropenylphenol while the reverse is not always true. Major shifts occurred between 35–45
568 cm (RMM) and 40–50 cm (KM), and at 182, 245 (HRB1) and 410 cm (HRB2), and also the
569 variation of both proxies at these depths was much larger for 4-isopropenylphenol than for
570 (di)methoxyphenols. Thus suggesting that decomposition of sphagnum phenols is a more
571 sensitive proxy for past water table.
572

573 *4.3.3. C/N ratio*

574 The C/N ratio clearly differed between the peat records and generally showed increasing values
575 with increasing contribution from *Sphagnum* (Table 2). Some exceptions are found. First, the
576 low C/N ratio in the upper 40 cm of KM and the upper 1 m of BBF is probably not related to the
577 contribution from vascular plants but instead to the high nitrogen deposition in those areas,
578 indicating an overruling influence of nitrogen deposition (Section 4.1). Second, in graminoid-
579 dominated peat, the contribution from *Sphagnum* does not play a role because its abundance is
580 low. The differences between peatlands (Table 2) clearly demonstrate the prevailing influence of
581 vegetation type (i.e. source) on the C/N ratio, caused by the lower nitrogen content in *Sphagnum*
582 than in vascular plants. Therefore, the question arises to what extent botanical shifts within a
583 single vegetation type contribute to the variation in C/N, or whether most of this variation is
584 related to mass loss during decomposition.

585 Within each of the peat records, the variation in C/N is differently correlated to pyrolysis
586 parameters. In all *Sphagnum*-dominated peatlands the correlation between C/N and
587 (di)methoxyphenols (vascular plants) was weaker than that with 4-isopropenylphenol or
588 polysaccharides (SI_Table 2), which suggests that polyphenols from *Sphagnum* are more easily
589 degraded than both lignin (dimethoxyphenols) and cellulose (polysaccharide products) in
590 *Sphagnum*-dominated peat. In *Sphagnum*-dominated cores with a low contribution from vascular
591 plants (RMM and HRB1, indicated by the (di)methoxyphenol content; Table 2), 4-
592 isopropenylphenol showed a positive correlation with C/N (r^2=0.60 and 0.75 for RMM and
593 HRB1 respectively; SI_Table 2). In *Sphagnum*-dominated cores with a higher contribution from
594 vascular plants such a correlation was absent (HRB2) or weaker (KM; r^2=0.49). The better
595 correlation of 4-isopropenylphenol with C/N in HRB1 and RMM supports that a large part of the

596 variation in C/N in these peatlands is caused by decomposition rather than small increases of
597 vascular plants upon drier conditions, which is in agreement with the interpretation of 4-
598 isopropenylphenol in the factor analysis of RMM pyrolysates; Section 4.2.1).
599
600 The decrease in polyphenol and increase in polysaccharide pyrolysis products from living
601 *Sphagnum* to peat (Sections 4.3.1 and 4.3.2) provide clear evidence for the preferential decay of
602 sphagnum phenols over sphagnum polysaccharides, which should largely explain the mass loss
603 as suggested by the decrease in C/N from 80 to 45 in HRB1 (Fig. 3d), from 60 to 40 in BBF (Fig.
604 3c), and from 35 to 20 in KM (Fig. 3b) in these samples. This is in agreement with the mass loss
605 of up to 30% during the first stage of decay found for *Sphagnum* litter (Asada et al., 2005).
606 The variation in C/N within graminoid-dominated peat (PVO and HRB3) differed from that in
607 *Sphagnum*-dominated peat. The C/N ratio in both graminoid-dominated peatlands showed a
608 much lower variation and no correlation with 4-isopropenylphenol (SI_Table 2), which is
609 explained by the low contribution from *Sphagnum* to these peats. Because cellulose is
610 preferentially lost over lignin in such peat, a negative correlation of polysaccharides with C/N
611 should be expected. However, such a correlation was only weak in HRB3 (r^2=0.43), and even
612 slightly negative in PVO (r^2=0.37; SI_Table 2). Furthermore, the C/N ratio is determined mainly
613 by nitrogen in all peatlands, while in the upper 80 cm of PVO carbon also contributed to its
614 variance (Schellekens, 2013). It is not exactly clear which factors cause these differences and
615 several processes may influence these discrepancies, including nitrogen mining (Lindahl et al.,
616 2007; Craine et al., 2007), nitrogen deposition (Heijmans et al., 2002) and the preferential
617 decomposition of carbon-poor compounds such as polysaccharides (e.g. glucose: $C_6H_{12}O_6$) over
618 compounds richer in carbon such as lignin (e.g. C_3-guaiacol: $C_{10}H_{12}O_2$). The higher contribution

from carbon in phenolics (e.g. sphagnum acid: $C_{11}H_{10}O_5$) compared with polysaccharides and the preferential degradation of phenolics over polysaccharides may also contribute to the strong correlation between 4-isopropenylphenol and C/N in *Sphagnum*-dominated peat.

5. Conclusions

Phenolic compounds (sphagnum acid) as well as pectin-like polysaccharides (sphagnan) have been identified as causal agents for the inhibition of *Sphagnum* decay. Our results indicate preferential degradation of phenolics over polysaccharides. This suggests that inhibition of degradation is not caused by sphagnum acid (van Breemen, 1995; van der Heijden et al., 1997; Freeman et al., 2001), which supports the claim that polysaccharides such as sphagnan provide a degree of recalcitrance to the *Sphagnum* cell walls (Hájek et al., 2011). The preferential degradation of phenolics over polysaccharides in *Sphagnum* litter explains the higher polysaccharide content found in *Sphagnum* peat compared with *Sphagnum* plants (Moers et al., 1989; Comont et al., 2006; Schellekens et al., 2009).

The changes in C/N values with depth are highly influenced by plant species (*Sphagnum* vs. vascular plants), decomposition and atmospheric deposition. Therefore, molecular proxies are essential. The fact that 4-isopropenylphenol is relatively rapidly degraded aerobically in combination with its specificity for *Sphagnum* makes it a reliable proxy-indicator of past hydrologic conditions in *Sphagnum* peat, because effects of vegetation changes that may occur simultaneously cannot influence its abundance.

The different understanding of chemical parameters in the studied *Sphagnum*-dominated peatlands highlights the complexity of natural systems and the difficulty to extrapolate chemical

641 parameters, it further emphasises the importance of ecological knowledge and botanical changes
642 in the environmental interpretation of peat decomposition proxies.
643
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649
650 **References**
651 Abbott G. D., Swain E. Y., Muhammad A. B., Allton K., Belyea L. R., Laing C. G. and Cowie G. L. (2013) Effect
652 of water-table fluctuations on the degradation of *Sphagnum* phenols in surficial peats. *Geochim. Cosmochim.*
653 *Acta* **106**, 177–191.
654 Akselsson C., Belyazid S., Hellsten S., Klarqvist M., Pihl-Karlsson G., Karlsson P. –E. and Lundlin L. (2010)
655 Assessing the risk of N leaching from forest soils across a steep N deposition gradient in Sweden. *Environ.*
656 *Pollut*. **158**, 3588–3595.
657 Andersson R. A., Kuhry P., Meyers P., Zebühr Y., Crill P. and Mörth M. (2011) Impacts of paleohydrological
658 changes on *n*-alkane biomarker compositions of a Holocene peat sequence in the eastern European Russian
659 Arctic. *Org. Geochem*. **42**, 1065–1075.
660 Asada T., Warner B. and Aravena R. (2005) Effects of the early stage of decomposition on change in carbon and
661 nitrogen isotopes in *Sphagnum* litter. *J. Plant Interact*. **1**, 229–237.
662 Benner R., MacCubbin A. E. and Hodson R. E. (1984) Anaerobic biodegradation of the lignin and polysaccharide
663 components of lignocellulose and synthetic lignin by sediment microflora. *Appl. Environ. Microb*. **47**, 998–1004.
664 Biester H., Hermanns Y. M. and Martínez-Cortizas A. (2012) The influence of organic matter decay on the
665 distribution of major and trace elements in ombrotrophic mires - a case study from the Harz Mountains.
666 *Geochim. Cosmochim. Acta* **84**, 126–136.

667 Biester H., Knorr K. H., Schellekens J., Basler A. and Hermanns Y. (2014) Comparison of different methods to
668 determine the degree of peat decomposition in ombrotrophic mires. *Biogeosciences* **11**, 2691–2707.
669 Blackford J. J. and Chambers F. M. (1993) Determining the degree of peat decomposition for peat-based
670 palaeoclimatic studies. *Int. Peat J.* **5**, 7–24.
671 Boon J. J., Wetzel R. G. and Godshalk G. L. (1982) Pyrolysis mass spectrometry of some *Scirpus* species and their
672 decomposition products. *Limnol. Oceanogr.* **27**, 839–848.
673 Chambers F. M., Booth R. K., de Vleeschouwer F., Lamentowicz M., le Roux G., Mauquoy D. M., Nichols J. E. and
674 van Geel B. (2012) Development and refinement of proxy-climate indicators from peats. *Quatern. Int.* **268**, 21–
675 33.
676 Charman D. J., Beilman D. W., Blaauw M., Booth R. K., Brewer S., Chambers F. M., Christen J. A., Gallego-Sala
677 A., Harrison S. P., Hughes P. D. M., Jackson S. T., Korhola A., Mauquoy D., Mitchell F. J. G., Prentice I. C.,
678 van der Linden M., de Vleeschouwer F., Yu Z. C., Alm J., Bauer I. E., Corish Y. M. C., Garneau M., Hohl V.,
679 Huang Y., Karofeld E., le Roux G., Loisel J., Moschen R., Nichols J. E., Nieminen T. M., MacDonald G. M.,
680 Phadtare N. R., Rausch N., Sillasoo Ü., Swindles G. T., Tuittila E. –S., Ukonmaanaho L., Väliranta M., van
681 Bellen S., van Geel B., Vitt D. H. and Zhao Y. (2013) Climate-related changes in peatland carbon accumulation
682 during the last millennium. *Biogeosciences* **10**, 929–944.
683 Clymo R. S. (1965) Experiments on breakdown of *Sphagnum* in two bogs. *J. Ecol.* **53**, 747–758.
684 Comont L., Laggoun-Défarge F. and Disnar J. R. (2006) Evolution of organic matter indicators in response to major
685 environmental changes: The case of a formerly cut-over peat bog (Le Russey, Jura Mountains, France). *Org.*
686 *Geochem.* **37**, 1736–1751.
687 Craine J. M., Morrow C. and Fierer N. (2007) Microbial nitrogen limitation increases decomposition. *Ecology* **88**,
688 2105–2113.
689 DeBond N., Fogel M. L., Morril P. L., Benner R., Bowden R. and Ziegler S. (2013) Variable δD values among
690 major biochemicals in plants: Implications for environmental studies. *Geochim. Cosmochim. Acta* **111**, 117–127.
691 Freeman C., Ostle N. and Kang H. (2001) An enzymic 'latch' on a global carbon store. *Nature* **409**, 149–149.
692 Hájek T., Ballance S., Limpens J., Zijlstra M. and Verhoeven J. T. A. (2011) Cell-wall polysaccharides play an
693 important role in decay resistance of *Sphagnum* and actively depressed decomposition in vitro. *Biogeochemistry*
694 **103**, 45–57.

695 Hansson S. V., Rydberg J., Kylander M., Gallagher K. and Bindler R. (2013) Evaluating paleoproxies for peat
696 decomposition and their relationship to peat geochemistry. *Holocene* **23**, 1666–1671.
697 Hedges J. I. and Mann D. C. (1979) The characterization of plant tissues by their lignin oxidation products.
698 *Geochim. Cosmochim. Acta* **43**, 1803–1807.
699 Heijmans M. M. P. D., Klees H., de Visser W. and Berendse F. (2002) Effects of increased nitrogen deposition on
700 the distribution of ^{15}N-labeled nitrogen between *Sphagnum* and vascular plants. *Ecosystems* **5**, 500–508.
701 Hesse K. F. and Rudolph H. (1992) Crystal structure of sphagnum acid $C_{11}H_{10}O_5$. *Z. Kristallogr*. **200**, 133–139.
702 Hornibrook E. R. C., Longstaffe F. J., Fyfe W. S. and Bloom Y. (2000) Carbon-isotope ratios and carbon, nitrogen
703 and sulfur abundances in flora and soil organic matter from a temperate-zone bog and marsh. *Geochem. J.* **34**,
704 237–245.
705 Huang W., Stankiewicz B. A., Eglinton G., Snape C. E., Evans B., Latter P. M. and Ineson P. (1998) Monitoring
706 biomacromolecular degradation of *Calluna vulgaris* in a 23 year field experiment using solid state ^{13}C-NMR and
707 pyrolysis-GC/MS. *Soil Biol. Biochem.* **30**, 1517–1528.
708 Huang X., Xue J., Meyers P. A., Gong L., Wang X., Liu Q., Qin Y. and Wang H. (2014) Hydrologic influence on
709 the δ^{13}C variation in long chain *n*-alkanes in the Dajiuhu peatland, central China. *Org. Geochem.* **69** 114–119.
710 Jacob J., Disnar J. R., Boussafir M., Albuquerque A. L. S., Sifeddine A. and Turcq B. (2007) Contrasted
711 distributions of triterpene derivatives in the sediments of Lake Caçó reflect paleoenvironmental changes during
712 the last 20,000 yrs in NE Brazil. *Org. Geochem.* **38** 180–197.
713 Jex C. N., Pate G. H., Blyth A. J., Spencer R. G. M., Hernes P. J., Khan S. J. and Baker A. (2014) Lignin
714 biogeochemistry: from modern processes to Quaternary archives. *Quaternary Sci. Rev.* **87**, 46–59.
715 Jia G., Dungait J. A. J., Bingham E., Valiranta M., Korhola A. and Evershed R. P. (2008) Neutral monosaccharides
716 as biomarker proxies for bog-forming plants for application to palaeovegetation reconstruction in ombrotrophic
717 peat deposits. *Org. Geochem.* **39**, 1790–1799.
718 Kleinebecker T., Hölzel N. and Vogel A. (2007) Gradients of continentality and moisture in South Patagonian
719 ombrotrophic peatland vegetation. *Folia Geobot.* **42**, 363–382.
720 Klotzbücher T., Kaiser, K., Guggenberger G., Gatzek C. and Kalbitz K. (2011) A new conceptual model for the fate
721 of lignin in decomposing plant litter. *Ecology* **92**, 1052–1062.

722 Kögel-Knabner I. (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil
723 organic matter. *Soil Biol. Biochem.* **34**, 139–162.
724 Kuder T., Kruge M. A., Shearer J. C. and Miller S. L. (1998) Environmental and botanical controls on peatification -
725 a comparative study of two New Zealand restiad bogs using Py-GC/MS, petrography and fungal analysis. *Int. J.*
726 *Coal Geol.* **37**, 3–27.
727 Lindahl B. D., Ihrmark K., Boberg J., Trumbore S. E., Högberg P., Stenlid J. and Finlay R. D. (2007) Spatial
728 separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytol.* **173**, 611–620.
729 Yeloff D. and Mauquoy D. (2006) The influence of vegetation composition on peat humification: implications for
730 palaeoclimatic studies. *Boreas* **35**, 662–673.
731 McClymont E. L., Mauquoy D., Yeloff D., Broekens P., van Geel B., Charman D. J., Pancost R. D., Chambers F.
732 M. and Evershed R. P. (2008) The disappearance of *Sphagnum imbraticum* from Butterburn Flow, UK.
733 *Holocene* **18**, 991–1002.
734 McClymont E. L., Bingham E. M., Nott C. J., Chambers F. M., Pancost R. D. and Evershed R. P. (2011) Pyrolysis
735 GC-MS as a rapid screening tool for determination of peat-forming plant composition in cores from
736 ombrotrophic peat. *Org. Geochem.* **42**, 1420–1435.
737 Mitchel J. (1996) Role of polysaccharides in the preservation of bodies in peat. *Carbohyd. Polym.* **29**, 193.
738 Moers M. E. C., Boon J. J., de Leeuw J. W., Baas M. and Schenk P. A. (1989) Carbohydrate speciation and py-MS
739 mapping of peat samples from a tropical open marsh environment. *Geochim. Cosmochim. Acta* **53**, 2011–2021.
740 Nierop K. G. J., van Bergen P. F., Buurman P. and van Lagen, B. (2005) NaOH and $Na_4P_2O_7$ extractable organic
741 matter in two allophanic volcanic ash soils of the Azores Islands – a pyrolysis GC/MS study. *Geoderma* **127**,
742 36–51.
743 Oldfield F., Crooks P. R. J., Harkness D. D. and Petterson G. (1997) AMS radiocarbon dating of organic fractions
744 from varved lake sediments: An empirical test of reliability. *J. Paleolimnol.* **18**, 87–91.
745 Painter T. J. (1991) Lindow man, Tollund man and other peat-bog bodies: the preservative and antimicrobial action
746 of sphagnan, a reactive glycuronologlycan with tanning and sequestering properties. *Carbohyd. Polym.* **15**, 123–
747 142.
748 Philben M., Kaiser K. and Benner R. (2013) Does oxygen exposure time control the extent of organic matter
749 decomposition in peatlands? Journal of Geophysical Research: *Biogeosciences* **119**, 897–909.

Pontevedra-Pombal X., Martínez-Cortizas A. and Buurman P. (2004) Las turberas de montaña de Galicia como sumideros de carbono. *Edafologia* **11**, 295–307.

Pouwels A. D., Eijkel G. B. and Boon J. J. (1989) Curie-point pyrolysis high-resolution gas chromatography–mass spectrometry of microcrystalline cellulose. *J. Anal. Appl. Pyrol.* **14**, 237–280.

Rydberg J., Karlsson J., Nyman, R., Wanhatalo, I., Näthe K. and Bindler R. (2010) Importance of vegetation type for mercury sequestration in the northern Swedish mire, Rödmossamyran. *Geochim. Cosmochim. Acta* **74**, 7116–7126.

Schellekens J. (2013) The use of molecular chemistry (pyrolysis-GC/MS) in the environmental interpretation of peat. Ph.D. thesis, Wageningen University.

Schellekens J. and Buurman P. (2011) *n*-Alkane distributions as palaeoclimatic proxies in ombrotrophic peat: the role of decomposition and dominant vegetation. *Geoderma* **164**, 112–121.

Schellekens J., Buurman P. and Pontevedra-Pombal X. (2009) Selecting parameters for the environmental interpretation of peat molecular chemistry - A pyrolysis-GC/MS study. *Org. Geochem.* **40**, 678–691.

Schellekens J., Buurman P., Fraga I. and Martínez-Cortizas A. (2011) Holocene hydrologic changes inferred from molecular vegetation markers in peat, Penido Vello (Galicia, Spain). *Palaeogeogr. Palaeocl.* **299**, 56–69.

Schellekens J., Buurman P. and Kuyper T. W. (2012) Source and transformations of lignin in *Carex*-dominated peat. *Soil Biol. Biochem.* **53**, 32–42.

Schellekens J., Horák-Terra I., Buurman, P. Christófaro-Silva, A. and Vidal-Torrado' P. (2014) Holocene vegetation and fire dynamics in central-eastern Brazil: Molecular records from the Pau de Fruta peatland. *Org. Geochem.* **77**, 32–42.

Schellekens J., Buurman P., Kuyper T. W., Abbott G. D., Pontevedra-Pombal X. and Martínez-Cortizas A. (2015) Influence of source vegetation and redox conditions on lignin-based decomposition proxies in graminoid-dominated ombrotrophic peat (Penido Vello, NW Spain). *Geoderma* **237–238**, 270–282.

Sinninghe-Damsté J., Rijpstra W. I. and Reichart G. (2002) The influence of oxic degradation on the sedimentary biomarker record II. Evidence from Arabian Sea sediments. *Geochim. Cosmochim. Acta* **66**, 2737–2754.

Swain E. Y. and Abbott G. D. (2013) The effect of redox conditions on sphagnum acid thermochemolysis product distributions in a northern peatland. *J. Anal. Appl. Pyrol.* **103**, 2–7.

van Bellen S., Mauquoy D., Payne R. J., Roland T. P., Daley T. J., Hughes P. D. M., Loader N. J., Street-Perrott F. A., Rice E. M. and Pancotto V. A. (2014) Testate amoebae as a proxy for reconstructing Holocene water table dynamics in southern Patagonian peat bogs. *J. Quaternary Sci.* **29**, 463–474.

van Breemen N. (1995) How *Sphagnum* bogs down other plants. *TREE* **10**, 270–275.

van der Hage E. R. E., Mulder M. M. and Boon J. J. (1993) Structural characterization of lignin polymers by temperature-resolved in-source pyrolysis mass spectrometry and Curie-point pyrolysis-gas chromatography/mass spectrometry. *J. Anal. Appl. Pyrol.* **25**, 149–183.

van der Heijden E., Boon J. J., Rasmussen S. and Rudolph H. (1997) *Sphagnum* acid and its decarboxylation product isopropenylphenol as biomarkers for fossilised *Sphagnum* in peats. *Anc. Biomol.* **1**, 93–107.

van Smeerdijk D. G. and Boon J. J. (1987) Characterisation of subfossil *Sphagnum* leaves, rootlets of Ericaceae and their peat by pyrolysis-high-resolution gas chromatography-mass spectrometry. *J. Anal. Appl. Pyrol.* **11**, 377–402.

Yu, Z. C. (2012) Northern peatland carbon stocks and dynamics: a review. *Biogeosciences* **9**, 4070–4085.

Figure Captions

Fig. 1. Factor loadings of F1–F2 projection obtained with factor analysis of pyrolysates from the Rödmossamyran peat samples.*

*Al Aliphatic hydrocarbon (including *n*-alkanes, *n*-alkenes, *n*-methyl ketones, *n*-fatty acids); G methoxyphenol; S dimethoxyphenol; Ph phenolic compound; Ps polysaccharide; PA polyaromatic hydrocarbon; Ar aromatic; N nitrogen containing compound.

Fig. 2. Depth records of C/N, %T, bulk density, ash content and factor scores of F1 and F2 for RMM. *

*Note that y-axis is not on scale, each sample reflects a mixture of 2 cm in the 0–65 cm interval and of 10 cm in the 65–255 cm interval.

Fig. 3. Depth records of C/N and proxy-indicators for selective degradation of polyphenols, cellulose and lignin for several ombrotrophic peatlands.*

*Note that depth is not on scale, see Section 2.1 for sample heights; pyrolysis and C/N were not determined on the same sub-samples for BBF; the upper sample of the pyrolysis parameters for RMM reflects values of living *Sphagnum* (open point).

Fig. 4. Proportion (% TIC) of polyphenol-derived and polysaccharide pyrolysis products in living *Sphagnum* and *Sphagnum*-dominated peat. *

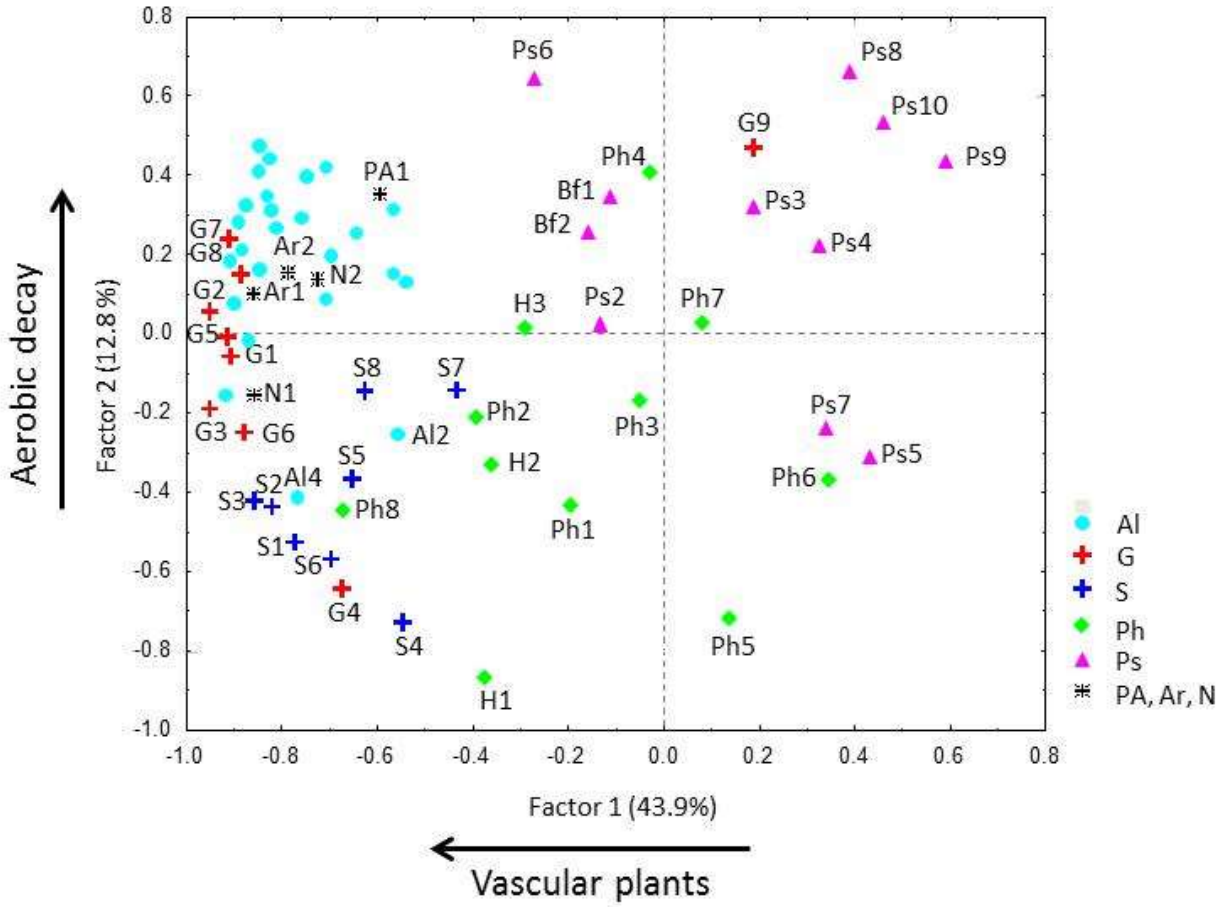
*Ph1 phenol; H1 4-vinylphenol; Ph5 4-isopropenylphenol; ∑Ph sum of polyphenol-derived pyrolysis products; ∑Ps sum polysaccharide pyrolysis products. ∑Ph included 4-isopropenylphenol, phenol, 4-ethylphenol, 4-vinylphenol in all peatlands, and additionally: C_1-hydroxybiphenyl, phenolic compound (Ph4), and toluene in RMM and KM; styrene in KM; 4-methylphenol, 4-acetylphenol and 4-(prop-1-enyl)phenol in RMM and HRB; 4-formylphenol in HRB; and C_1-naphthalene and styrene in RMM. ∑Ps included all polysaccharide pyrolysis

products from SI_Table 1 for RMM; for KM: compounds Ps5, Ps6, Ps9 and Ps10 (SI_Table 1), a sugar compound with *m*/*z* 72+128 and 1,4:3,6-dianhydro-α-D-glucose; for HRB: Ps5, Ps7, Ps8, Ps9, Ps10 (SI_Table 1),acetic acid, 2-furaldehyde, 2-hydroxy-3-methyl-2-cyclopenten-1-one, 1,4-anhydroxylofuranose, levogalactosan and levomannosan.

SI_Fig. 1. Depth record of the syringyl/guaiacyl ratio in RMM.

SI_Fig. 2. Correlation between chemical parameters in the RMM core.*

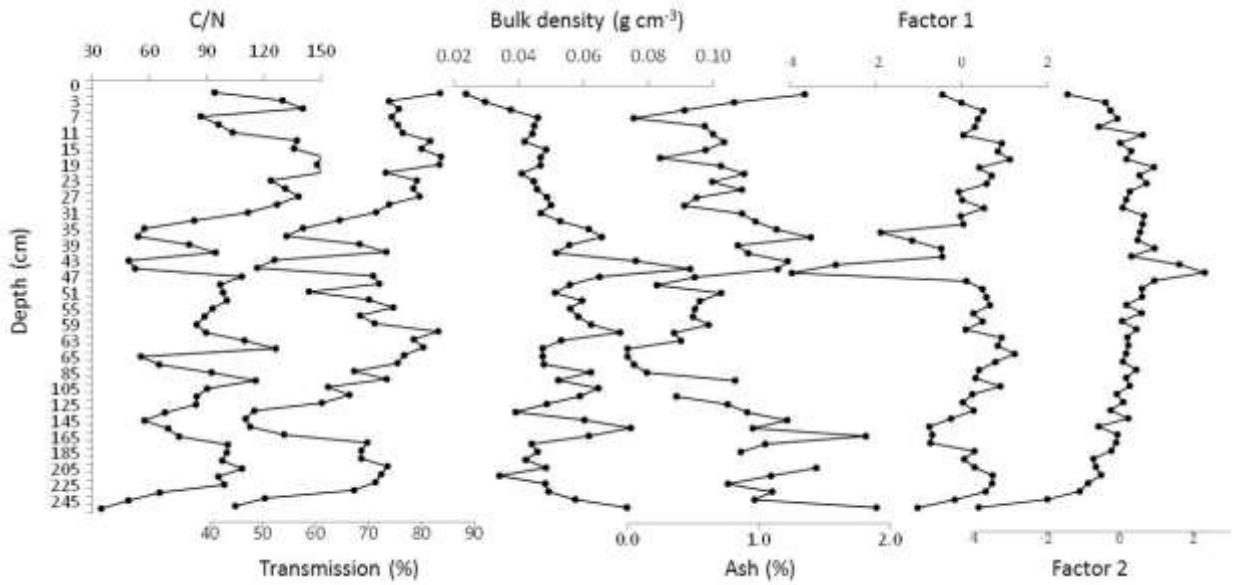
*Open symbols reflect samples that were excluded.



821

822 **Figure 1**

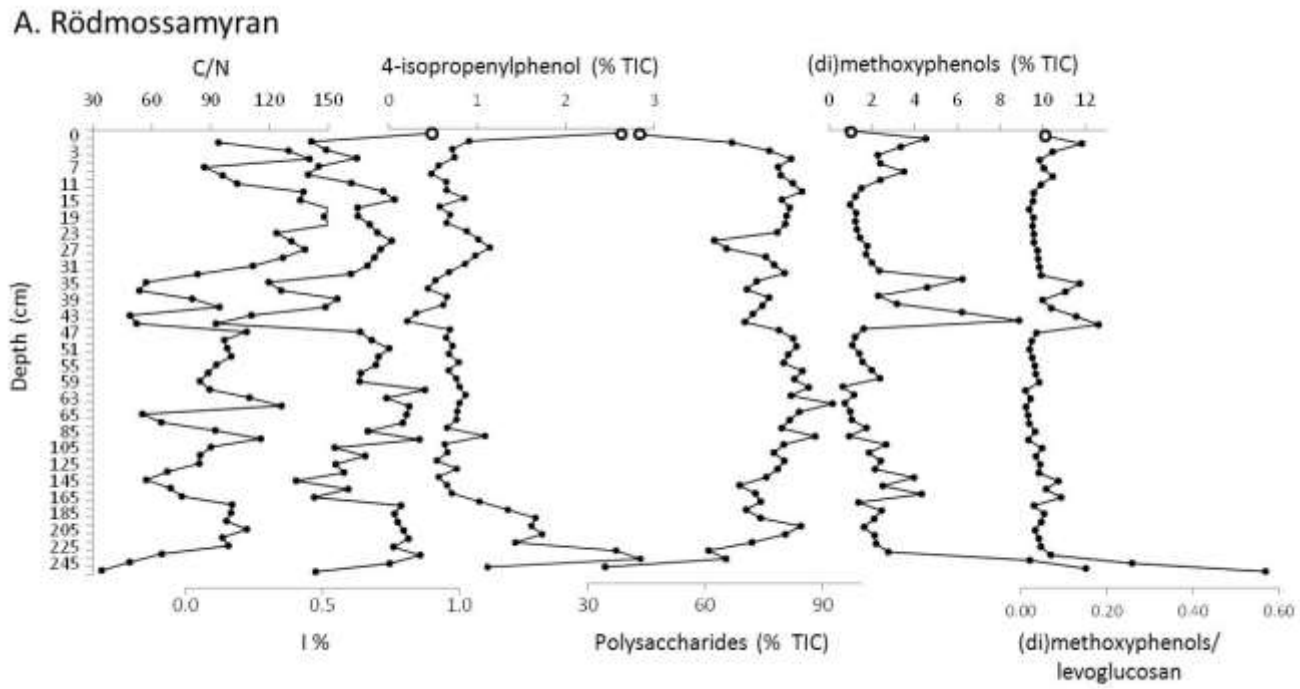
823



824

825 **Figure 2**

826


A. Rödmossamyran
C/N
4-isopropenylphenol (% TIC)
(di)methoxyphenols (% TIC)
Depth (cm)
I %
Polysaccharides (% TIC)
(di)methoxyphenols/
levoglucosan

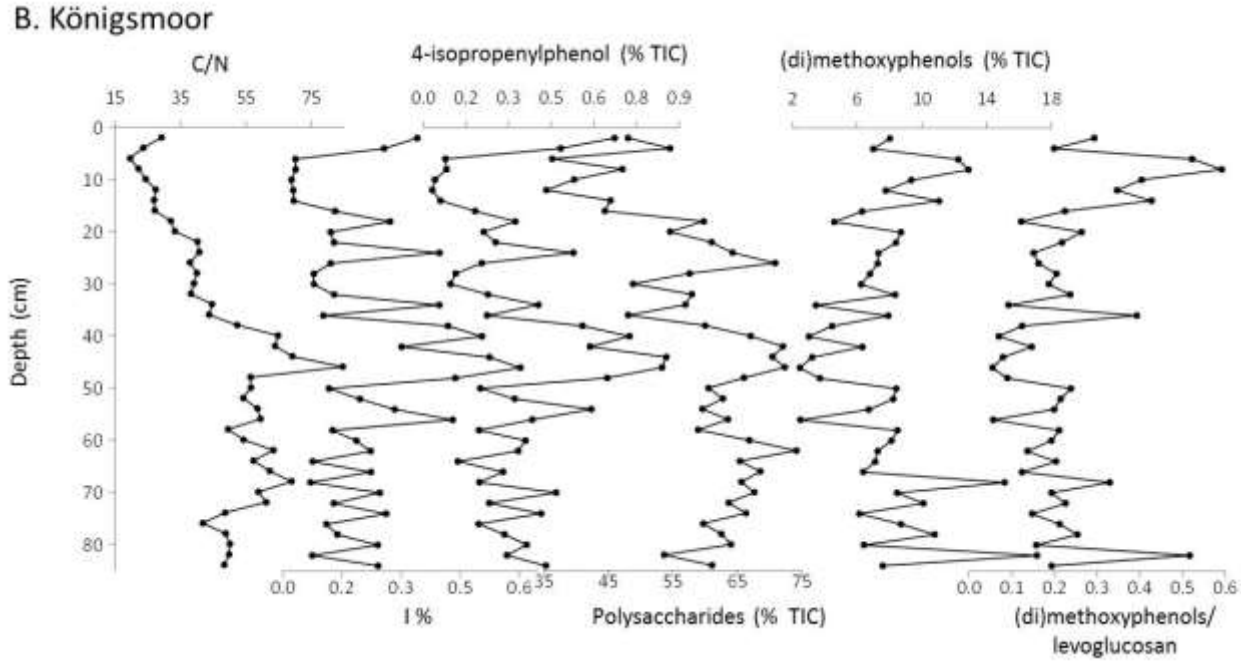
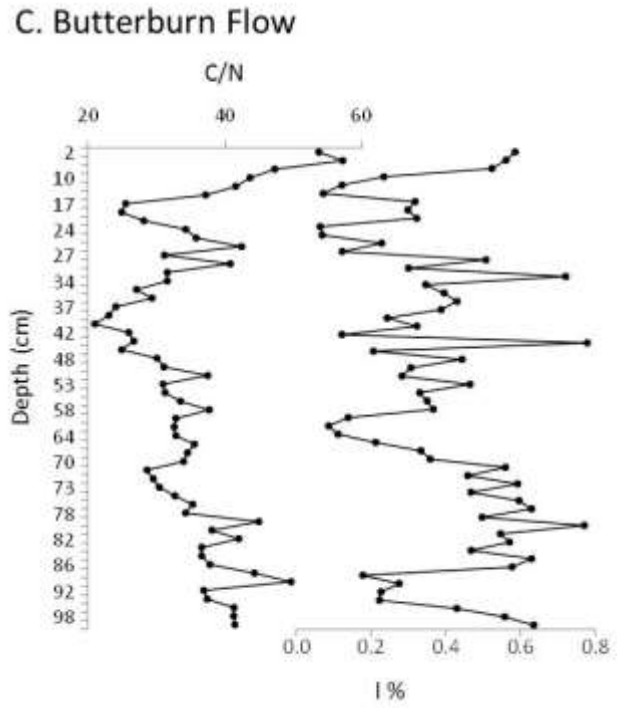

B. Königsmoor
C/N
4-isopropenylphenol (% TIC)
(di)methoxyphenols (% TIC)
Depth (cm)
I %
Polysaccharides (% TIC)
(di)methoxyphenols/
levoglucosan

Figure 3



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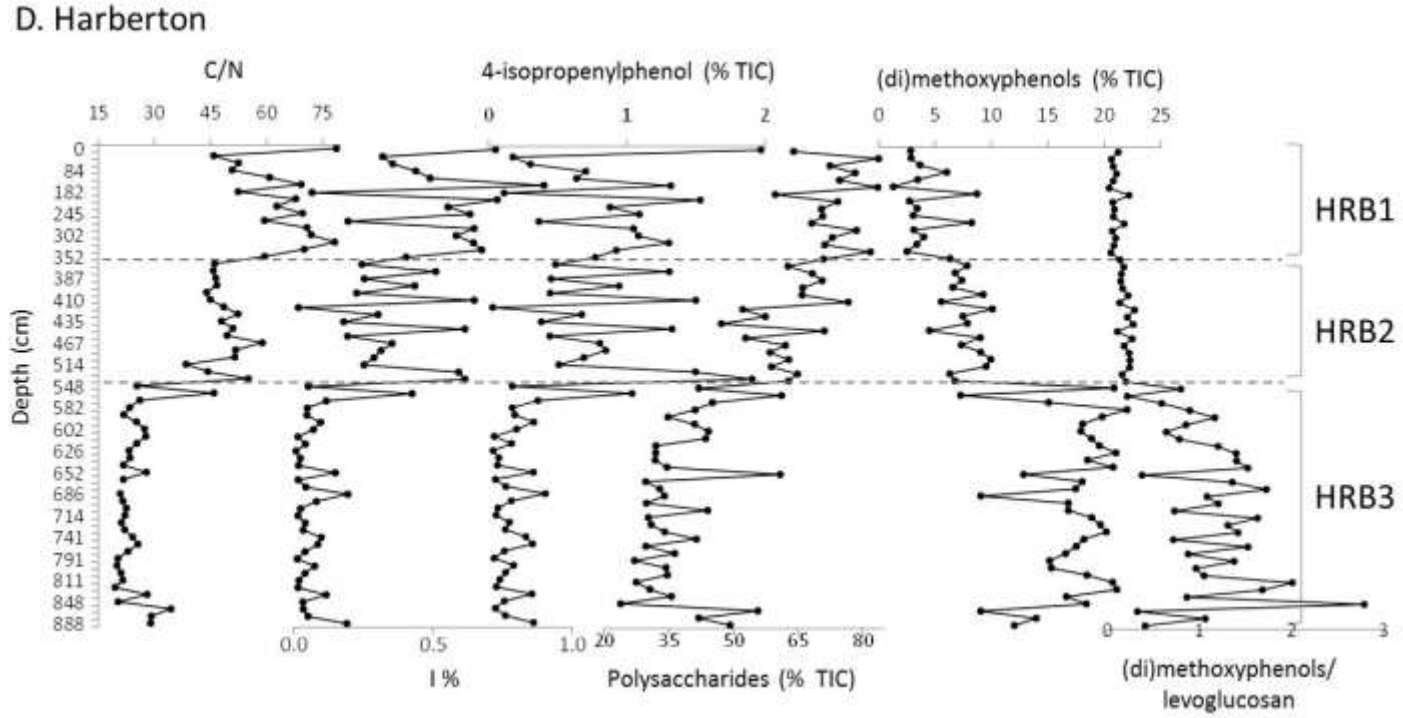


834

835 **Figure 3** ***Continued***

836

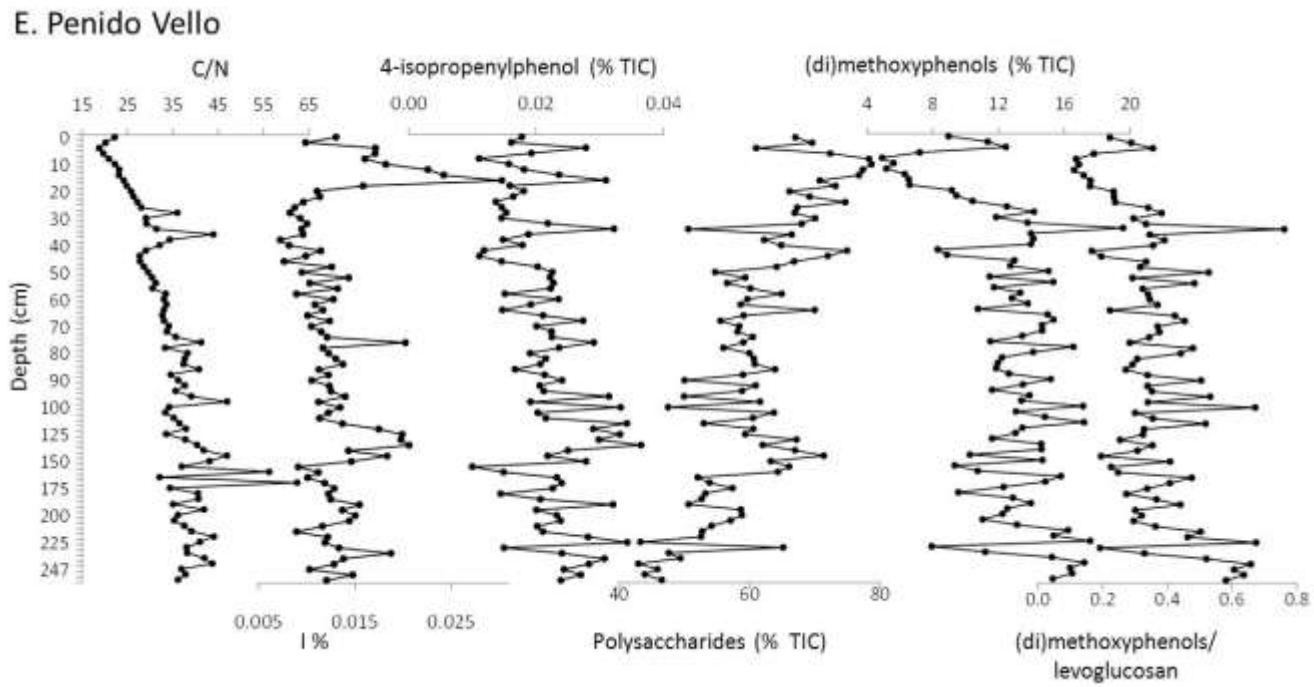
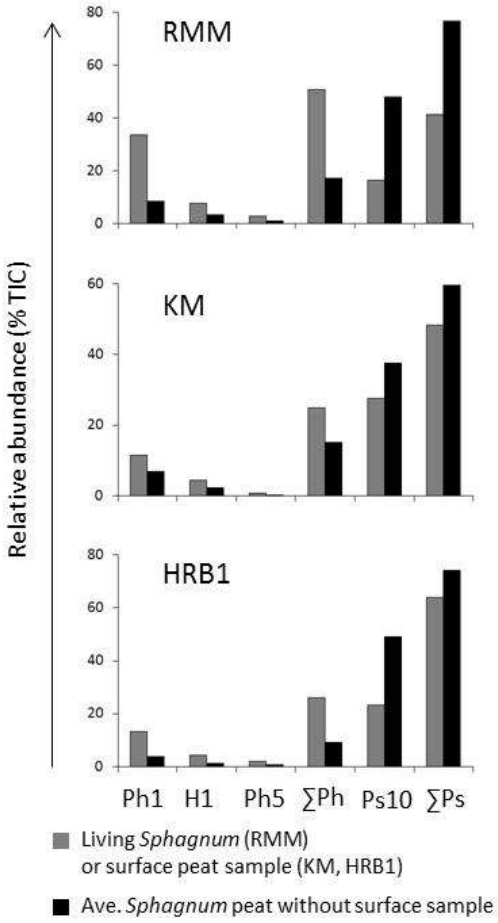


Figure 3 ***Continued***



840
841

842 **Figure 4**

843

Table 1

Characteristics of the studied peatlands

	Rödmossamyren (RMM)	Königsmoor (KM)	Butterburn Flow (BBF)	Harberton (HRB1)	(HRB2)	
Location	Northern Sweden	Harz Mountains Germany	England, UK	Tierra del Fuego Argentina		
Coordinates	63°47′N 20°20′E	51°45′N 10°34′E	55°05′N 02°30′W	54°53′S 67°20′E		
Height (m a.s.l.)	40	730	280	20		
Precipitation (mm)[a]	650	790	1280	600		
Temperature (°C)[a]	2–3	8	9.2	5		
Age (cal ka BP)	0–2.8[b]	-	850	0–3.9	3.9–5.7	
Depth (cm)	0–255	0–80	0–105	0–340	340–540	
Number of samples	53	42	56[c]	15	18	
Vegetation type	*Sphagnum*	*Sphagnum*	*Sphagnum*	*Sphagnum*	*Sphagnum*/ Graminoids	
Sphagnum species	*S. centrale* *S. subsecundum*	*S. magellanicum*	*S. magellanicum* *S. papillosum* *S. imbricatum*	*S. magellanicum*	*S. magellanicum*	
Vascular plants	*Eriophorum vaginatum*	*E. vaginatum* Ericoids	*E. vaginatum* Ericoids *Narthecium ossifragum* *Rynchospora alta*	*Empetrum rubrum* *Nothofagus Antarctica*	*E. rubrum* *N. antarctica* *Juncus* sp.	

[a] mean annual

[b] estimated (see Section 2.1)

[c] carbon and nitrogen were not always determined on the same samples as those used for pyrolysis

[d] sample at 552 cm excluded.

852 **Table 2**

853 Mean values of parameters that reflect degradation of lignin, sphagnum acid and cellulose in the
854 peatlands, and values for F, probability (*P*) and homogenous groups (letters to the right of the
855 numbers) of the ANOVA test.[a]

	unit	F	*P*	RMM	KM	BBF[b]	HRB1	HRB2	HRB3	PV
C/N	-	116.9	<0.01	96 e	47 c	35 b	64 d	49 c	24 a	
I %[c]	-	148.6	<0.01	0.63 e	0.21 b	0.38 c	0.53 d	0.36 c	0.06 a	0.
4-Isopropenylphenol	% TIC	69.2	<0.01	0.92 c	0.34 b		0.90 c	0.83 c	0.16 ab	0.
Polysaccharides	% TIC	97.9	<0.01	68.9 c	59.1 b		73.5 c	62.8 b	36.8 a	61
(di)Methoxyphenols[c]	% TIC	154.2	<0.01	2.7 a	7.6 b		3.9 a	7.6 b	17.4 d	19
Levoglucosan	% TIC	86.8	<0.01	47.6 c	37.3 b		47.5 c	38.8 b	17.7 a	38
(di)Methoxyphenols/Levoglucosan[d]	-	120.1	<0.01	0.07 a	0.22 bc		0.09 ab	0.20 abc	1.10 d	0.

856 [a] Bogs with the same letter showed no significant differences; for abbreviations of the peatlands
857 see Table 1.

858 [b] Due to the limited quantification in BBF, mean values were only determined for I%.

859 [c] 4-Isopropenylphenol / (4-isopropenylphenol + guaiacol + syringol).

860 [d] In order to homogenise the quantification, (di)methoxyphenols are limited to compounds G1–
861 G8 and S1–S8 (SI_Table 1) for all peat records.

862

863

Table 3

C/N values of living plant species collected at the peatlands.

Peatland	Species	*n*	C/N	C	S.D.	N	S.D.
RMM	*Sphagnum magellanicum*	3	96	44.2	0.04	0.5	0.03
	S. centrale	3	80	44.4	0.06	0.6	0.08
	Eriophorum vaginatum	2	45	47.3	0.12	1.1	0.29
	Calluna vulgaris	3	53	52.2	0.39	1.0	0.06
KM	*S. magellanicum*	3	48	44.0	0.08	0.92	0.03
	E. vaginatum	3	29	46.0	0.20	1.56	0.06
	C. vulgaris	3	35	49.0	0.20	1.40	0.04