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# The use of plant-specific pyrolysis products as biomarkers in peat deposits

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ABSTRACT

Peatlands are archives of environmental change that can be driven by climate and human activity. Proxies for peatland vegetation composition provide records of (local) environmental conditions that can be linked to both autogenic and allogenic factors. Analytical pyrolysis offers a molecular fingerprint of peat, and thereby a suite of environmental proxies. Here we investigate analytical pyrolysis as a method for biomarker analysis. Pyrolysates of 48 peatland plant species were compared, comprising seventeen lichens, three *Sphagnum* species, four non-*Sphagnum* mosses, eleven graminoids (Cyperaceae, Juncaceae, Poaceae), five Ericaceae and six species from other families. This resulted in twenty-one potential biomarkers, including new markers for lichens (3-methoxy-5-methylphenol) and graminoids (ferulic acid methyl ester). The potential of the identified biomarkers to reconstruct vegetation composition is discussed according to their depth records in cores from six peatlands from boreal, temperate and tropical biomes. The occurrence of markers for *Sphagnum*, graminoids and lichens in all six studied peat deposits indicates that they persist in peat of thousands of years old, in different vegetation types and under different conditions. In order to facilitate the quantification of biomarkers from pyrolysates, typically expressed as proportion (%) of the total quantified pyrolysis products, an internal standard (5-α-androstane) was introduced. Depth records of the *Sphagnum* marker 4-isopropenylphenol from the upper 3 m of a *Sphagnum*-dominated peat, from samples analysed with and without internal standard showed a strong positive correlation ($r^2 = 0.72$, $P < 0.0005$, $n = 12$). This indicates that application of an internal standard is a reliable method to assess biomarker depth records, which enormously facilitates the use of analytical pyrolysis in biomarker research by avoiding quantification of a high number of products.



## 1. Introduction

Peatlands respond to changes in environmental conditions. Proxies for such changes are preserved in the peat and may provide records of past environmental change (Chambers et al., 2012). Several studies of plant macrofossils in ombrotrophic peat have shown good correlations between vegetation composition and local hydrology (Blackford, 2000; Castro et al., 2015). Because in highly decomposed peat the preservation of plant remains is usually poor, plant-specific recalcitrant compounds (biomarkers) have been used instead of macrofossils to reconstruct plant species composition. Identified peatland biomarkers are relatively scarce (Nichols, 2010) and are mainly restricted to free solvent-extractable lipids (Dehmer, 1995; Pancost et al., 2002).

A biomarker approach assumes that biomarker abundance accurately reflects the original surface vegetation at the time of peat deposition (Blackford, 2000). Peat decomposition and changes in vegetation type have been found to influence biomarkers that are not plant-specific, such as the distribution of *n*-alkanes and the

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composition of lignin phenols. Decomposition may interfere with the plant-specific distribution of such compounds and cause errors in the hydrological interpretation (Pancost et al., 2002; Huang et al., 2012; Andersson and Meijers, 2012; Jex et al., 2014). The influence of decomposition, vegetation type and intrinsic plant characteristics appears more straightforward for plant specific markers, because these – contrary to *n*-alkanes and lignin phenols – have a single source. Nevertheless, effects of decomposition on specific markers have rarely been studied (Sinninghe-Damste et al., 2002). Therefore, the question arises to which extent the variation of a marker depends on the contribution from that particular plant species to the peat. It has recently been shown that the abundance of the marker for sphagnum acid, 4-isopropenylphenol, in *Sphagnum*-dominated peatlands reflects decomposition rather than the contribution from *Sphagnum* to the surface vegetation (Schellekens et al., 2015a). This demonstrates the need to study botanical changes and the degree of decomposition simultaneously.

Pyrolysis gas chromatography mass spectrometry (pyrolysis-GC/MS) gives a detailed fingerprint of organic material at the molecular level and enables studying the composition of biomacromolecules. The use of analytical pyrolysis to gain insight into peat decomposition processes has been repeatedly demonstrated (Halma et al., 1984; van Smeerdijk and Boon, 1987; Durig et al., 1989; van der Heijden et al., 1997; Kuder et al., 1998; Huang et al., 1998; Gleixner and Kracht, 2001; González et al., 2003; Buurman et al., 2006). Well-established macromolecular markers to differentiate between mosses and vascular plants include lignin phenols from lignin (Tsutsuki et al., 1994; Williams et al., 1998; Bourdon et al., 2000) and 4-isopropenylphenol from sphagnum acid (van der Heijden et al., 1997; Schellekens et al., 2009, 2015a; McClymont et al., 2011; Abbott et al., 2013; Swain and Abbott, 2013).

In addition to lignin phenols and 4-isopropenylphenol, the application of analytical pyrolysis in peat biomarker research was explored for a *Sphagnum*-dominated (Schellekens et al., 2009) and a graminoid-dominated (Schellekens et al., 2011) peatland. The results suggested that in addition to pyrolysis products of lignin and sphagnum acid, a number of specific markers can be used. Although within each study the hydrological interpretation of depth records of these markers agreed well with that of data obtained from other methods, their application needs verification.

In the present study, pyrolysates from 48 plants from *Sphagnum*-dominated and graminoid-dominated peatlands were combined in order to establish new biomarkers. The presence and behaviour of potential markers was tested and the ecological interpretation of their source plants discussed for six peat deposits from different climatic regions. In order to simplify the quantification procedure, depth records of the marker for sphagnum acid obtained by the traditional quantification (relative abundance) and by addition of an internal standard (5-α-androstane; absolute abundance by normalisation for organic carbon content) were compared. Thus, the purpose of this study was to establish methodological improvements in peatland biomarker research by i) the introduction of an internal standard using analytical pyrolysis, ii) identification of new biomarkers from pyrolysates of peatland plants and iii) identify whether the interpretation of biomarker depth records is consistent in diverse peatlands.

## 2. Material and methods

### 2.1. Peatlands

The selection of peatlands was designed to optimise testing the applicability of the markers. On the one hand, highly diverse peatlands were selected including different vegetation types (*Sphagnum* and graminoid-dominated) from boreal, temperate and tropical biomes, to test the application of potential biomarkers under different conditions. On the other hand, the solidity of the interpretation of marker records requires support from other hydrological proxies; therefore, the selected peatlands were sampled at high resolution and well-studied by other methods. The studied peatlands comprise three *Sphagnum*-dominated peatlands, including Harberton (HRB; Tierra del Fuego, Argentina; Schellekens et al., 2009; Schellekens and Buurman, 2011), Königsmoor (KM; Germany; Biester et al., 2014) and Rödmossamyren (RMM; northern Sweden; Schellekens et al., 2015a), and three graminoid-dominated peatlands, including Penido Vello (PVO; Spain; Schellekens et al., 2011, 2012, 2015b; Pontevedra-Pombal et al., 2013), Pena da Cadela (PDC; Spain; Pontevedra-Pombal et al., 2013; Castro et al., 2015), and Pau de Fruta (PF; Brazil; Horák-Terra et al., 2014; Schellekens et al., 2014). For details on location, sampling and peat characteristics we refer to those studies. The main characteristics of the peatlands are given in Table 1. All peatlands were ombrotrophic in nature, except for PF (mesotrophic) and the deepest part of HRB (minerotrophic).

### 2.2. Plant samples

Because the tropical peatland (PF) has a relatively high biodiversity (>60 families; Horák-Terra, 2014) and studies on its botanical composition and ecology are scarce compared with boreal and temperate peatlands, plants from the tropical peatlands were not included here. The tropical peatlands are dominated by graminoids (Poaceae and Cyperaceae) and contain patches of trees called 'Capões' (Schellekens et al., 2014). Samples of lichens (17), mosses (7) and vascular plants (24) were collected from the peatlands HRB, PVO, PDC, RMM and KM. Samples were taken from fresh tissue of fully developed plants. The included tissue in terms of roots, leaves and stems is indicated in Table 2. The term graminoids is used here to indicate gramineous monocotyledons, and thus includes Poaceae, Cyperaceae and Juncaceae. The selection of plant species was based on their present abundance as well as their value as indicators of hydrologic conditions in the peatlands (Fraga et al., 2001, 2005; Romero-Pedreira et al., 2008; Markgraf, 1993; Baumann, 2009; Rydberg et al., 2010). The samples were washed, oven dried at 35 °C for 1 week, ground, and analysed with pyrolysis-GC/MS.

### 2.3. Pyrolysis-GC/MS

For the studies included here, different pyrolysis devices have been used, including a Micro-furnace (ESALQ – University of São Paulo, Brazil), a Curie-Point (Wageningen University, The Netherlands), and Pt Filament coil probe pyrolysers (Pyroprobe 5000, University of Santiago de Compostela, Spain; Pyroprobe 1000, Newcastle University, UK; Table 3). The pyrolysis temperature was set at 600 °C; except for the Filament pyrolysers (650 °C; due to a T uncertainty caused by the heat transfer from the wire to the quartz tube). Helium was used as carrier gas.

The Micro-furnace pyrolyser used a single shot PY-3030S pyrolyser coupled to a GCMS-QP2010 (Frontier Laboratories LTD.). The injection T of the GC (split 1:20) and the GC/MS interface were set at 320 °C. The GC oven was heated from 50 to 320 °C (held 10 min) at 15 °C $min^{-1}$. The GC instrument was equipped with a UltraAlloy-5 column (Frontier Laboratories LTD.), length 30 m, thickness 0.25 μm, diameter 0.25 mm. The MS was scanning in the range of *m*/*z* 45–600.

The Curie-Point pyrolyser was connected to a Carlo Erba GC8000 gas chromatograph. The pyrolysis products were separated in a fused silica column (Chrompack 25 m, 0.25 mm i.d.) coated with CP-Sil 51 b (film thickness 0.40 μm). The initial oven temperature

**Table 1**
Characteristics of the studied peatlands.

| Peatland[a] | Location | Coordinates | Height (m a.s.l.) | P (mm)[b] | T (°C)[c] | Depth (cm) | Age (cal ka BP) | $n$[d] | Vegetation type |
|---|---|---|---|---|---|---|---|---|---|
| Harberton (HRB) | Tierra del Fuego, Argentina | 54°53 S, 67°20 E | 20 | 600 | 5 | | | | |
| HRB1 | | | | | | 0–340 | 0–3.9 | 15 | *Sphagnum* |
| HRB2 | | | | | | 340–540 | 3.9–5.7 | 17 | *Sphagnum*/graminoids |
| HRB3 | | | | | | 540–850 | 5.7–13.3 | 31 | Graminoids/woody species |
| Rödmossamyran (RMM) | Northern Sweden | 63°47 N, 20°20 E | 40 | 650 | 2–3 | 0–255 | – | 53 | |
| Lawn | | | | | | | | | *Sphagnum* |
| Forest | | | | | | | | | *Sphagnum*/woody species |
| Königsmoor (KM) | Harz Mountains, Germany | 51°45 N, 10°34 E | 730 | 790 | 8 | 0–80 | – | 42 | *Sphagnum* |
| Penido Vello (PVO) | Galicia, Spain | 43°32′N, 7°30′W | 780 | 1600 | 8.8 | 0–300 | 0–8 | 101 | Graminoids |
| Pena da Cadela (PDC) | Galicia, Spain | 43°30′N, 7°33′W | 970 | 1800 | 7.5 | 0–185 | 0–5.3 | 34 | Graminoids |
| Pau de Fruta (PF) | Minas Gerais, Brazil | 18°15′S, 43°40′W | 1350 | 1500 | 18.7 | 0–398 | 0–9 | 44 | Graminoids/Capões[e] |

[a] References for the characteristics of the peatlands: HRB (Schellekens et al., 2009), RMM (Rydberg et al., 2010; Schellekens et al., 2015), KM (Biester et al., 2014), PVO (Pontevedra-Pombal et al., 2013), PDC (Pontevedra-Pombal et al., 2013; Castro et al., 2015), PF (Horák-Terra et al., 2014; Schellekens et al., 2014).
[b] Mean annual precipitation.
[c] Mean annual temperature.
[d] $n$ = number of samples analysed with analytical pyrolysis.
[e] The word 'Capões' does not refer to a specific botanical composition but indicates a group of trees within an open landscape.

was 40 °C and the heating rate was 7 °C $min^{-1}$. The final temperature, 320 °C, was maintained for 20 min. The GC column was connected to a Fisons MD800 mass spectrometer ($m/z$ 45–650, cycle time 1 s).

Pt filament coil probe pyrolysis (Chemical Data Systems, Oxford, USA) was performed at 650 °C for 2 s (heating rate 10 °C $ms^{-1}$). The GC oven was heated from 40 to 320 °C (held 10 min) at 7 °C $min^{-1}$. The pyroprobe interface was maintained at 320 °C. The Pyroprobe 5000 was coupled to a 6890 N GC and 5975B MSD GC/MS system from Agilent Technologies (Palo Alto, USA). The GC instrument was equipped with a (non-polar) HP-5MS 5% phenyl, 95% dimethylpolysiloxane column (length 30 m; i.d. 0.25 mm; film thickness 0.25 μm). The Pyroprobe 1000 was fitted with a platinum coil and a CDS 1500 valved interface, with the products passing into an HP5890 GC with an open split (40 mL/min), with a 60 m HP5-MS column (0.25 mm internal diameter, 0.25 μm film thickness; J&W Scientific, USA). Product detection was carried out using an HP5972 series mass selective detector in full scan mode ($m/z$ 50–700).

### 2.4. Identification and quantification

A marker is here defined as a pyrolysis product that was 1) exclusively found in one of the investigated species, genera or taxonomic group and 2) preserved in the peat. Partial chromatograms of specific fragment ions ($m/z$ values) were used to establish the absence of potential markers in other species. The 'abundance' of a marker in plant and/or peat pyrolysates is not a measure for its reliability as a marker; even pyrolysis products that are only visible in a partial chromatogram may be reliable markers. The abundance of markers varied between 0.01% and 3% of the total quantified peak area (TIC). The quantification of the peat samples was mostly based on the dominant peaks; inherent to the different composition of pyrolysates for different peatlands the choice of some peaks differed between the datasets. Contents of pyrolysis products reflect relative abundances that allow us to assess the variations along a core (Jacob et al., 2007). Although a product with a very low abundance (e.g. <1% of the total quantified pyrolysis products) can be considered as statistically independent, a large number of pyrolysis products has to be quantified to reach this independence. Pyrolysis-GC/MS does not allow a quantitative analysis due to differences in response factors of the MS for different molecules and a residue of unknown quantity and quality that remains after pyrolysis (Moldoveanu, 1998). The use of an internal standard provides an alternative to overcome the problems encountered with quantification of pyrolysis products.

To test the consistency of pyrolysis results using an internal standard with the traditional quantification, the upper part of the *Sphagnum*-dominated HRB record was selected for analysis with an internal standard (5-α-androstane), and the depth record of 4-isopropenylphenol compared between both quantification approaches. For quantification, peat samples were weighed (ca. 2 mg) and a known amount (3 μL) of 5-α-androstane was added as internal standard prior to pyrolysis. Mass yield of 4-isopropenylphenol was determined based on the relative response of the total ion current to that of the internal standard, using a relative response factor of 1. Results were then normalised to mg $g^{-1}$ organic carbon (OC). Total OC values for each sample were obtained using a Leco CS230 carbon-sulphur analyser.

## 3. Results and discussion

### 3.1. Quantification of biomarkers from pyrolysates

The use of an internal standard to analyse biomarkers using analytical pyrolysis has been tested for the well-known marker for *Sphagnum*, 4-isopropenylphenol. For this purpose, samples from the upper 3 m of the *Sphagnum*-dominated HRB peat deposit were used. Depth records of 4-isopropenylphenol obtained with internal standard (mg 100 $g^{-1}$) and traditional quantification (% of the total quantified pyrolysis products) are given in Fig. 1. The correlation between both is significant ($r^2 = 0.72$, $P < 0.0005$, $n = 12$). First, this indicates that analytical pyrolysis, although usually considered to be semi-quantitative, reliably quantifies marker compounds when expressed as proportion of the total quantified peak area. Second, it means that using an internal standard enables analysis of well-established biomarkers without quantification of large numbers of compounds as is elaborated in Section 2.4.

### 3.2. Review of markers

The markers obtained from peatland plant pyrolysates and their characteristic fragment ions, molecular ions and source species are given in Table 4. The markers were checked in other analysed plants using partial chromatograms of their specific fragment ions. The results were consistent; the presence of markers in peatland plants is given in Table 2.

#### 3.2.1. Lichens

The phenolic compound 3-methoxy-5-methylphenol (compound 1, Table 2; Figs. 2 and 3) was present in six out of seventeen

**Table 2**
Specific compounds in pyrolysates of plant samples.[a]

| Species | Tissue[b] | Origin[c] | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lichens** | | | | | | | | | | | | | | | | | | | | |
| *Cetraria aculeata* (Schreber) Fr. | – | TF | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Cetraria islandica* (L.) Ach. | – | X | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Cladia aggregata* (Sw.) Nyl. | – | TF | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Cladonia arbuscula* (Wallr.) Flot. | – | X | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Cladonia cervicornis* (Ach.) Flot. | – | X | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Cladonia coccifera* (L.) Willd. | – | X | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Cladonia fimbriata* (L.) Fr. | – | TF | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Cladonia furcata* (Huds.) Schrad. | – | X | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Cladonia portentosa* (Dufour) Coem. | – | X | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Cladonia pyxidata* (L.) Hoffm. | – | TF | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Cladonia rangiferina* (L.) Weber ex F.H. Wigg. | – | TF | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Cladonia squamosa* (Scop.) Hoffm. | – | X | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Cladonia uncinalis* (L.) G.F. Weber | – | X | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Ochrolechia frigida* (Sw.) Lynge | – | TF | + | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Pseudocyphellaria freycinetii* (Delise) Malme | – | TF | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | + |
| *Psoroma hisutulum* Hyl. ex Grombie | – | TF | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Pycnothelia papillaria* (Ehrh.) L.N. Dufour | – | TF | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| ***Sphagnum* mosses** | | | | | | | | | | | | | | | | | | | | |
| *S. magellanicum* Brid. | – | TF, KM, RMM | – | – | + | + | + | – | – | – | – | +[d] | – | – | – | – | – | – | – | – |
| *S. capillifolium* (Ehrh.) Hedw. | – | X | – | – | + | + | + | – | – | – | – | +[d] | – | – | – | – | – | – | – | – |
| *S. centrale* C. Jens. | – | RMM | – | – | + | + | + | – | – | – | – | +[d] | – | – | – | – | – | – | – | – |
| **Non-*Sphagnum* mosses** | | | | | | | | | | | | | | | | | | | | |
| *Campylopus* sp. | – | X | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Hypnum cypressiforme* Hedw. | – | X | – | – | – | – | – | + | + | – | + | – | – | – | – | – | – | – | – | – |
| *Leucobryum glaucum* (Hedw.) Angstr. | – | X | – | – | – | – | – | + | + | + | + | – | – | – | – | – | – | – | – | – |
| *Racomitrium lanuginosum* (Hedw.) Brid. | – | X | – | – | – | – | – | + | – | + | – | – | – | – | – | – | – | – | – | – |
| **Graminoids** | | | | | | | | | | | | | | | | | | | | |
| Cyperaceae | | | | | | | | | | | | | | | | | | | | |
| *Carex demissa* Hornem. | c | X | – | – | – | – | – | – | – | – | – | + | + | – | – | – | – | + | + | – |
| *Carex binervis* Sm. | c | X | – | – | – | – | – | – | – | – | – | + | + | – | + | – | – | + | + | – |
| *Carex durieui* Steud. Ex Kunze | a, b | X | – | – | – | – | – | – | – | – | – | + | + | – | – | – | – | – | + | – |
| *Eriophorum angustifolium* Honckeny | a, b | X | – | – | – | – | – | – | – | – | – | + | + | – | – | – | – | – | + | – |
| *Eriophorum vaginatum* L. | a, b | KM, RMM | – | – | – | – | – | – | – | – | – | + | + | – | – | – | – | – | + | – |
| Juncaceae | | | | | | | | | | | | | | | | | | | | |
| *Juncus bulbosus* L. | c | X | – | – | – | – | – | – | – | – | – | + | + | – | – | + | – | + | + | – |
| *Juncus effuses* L. | c | X | – | – | – | – | – | – | – | – | – | + | + | – | – | – | – | + | + | – |
| *Juncus squarrosus* L. | c | X | – | – | – | – | – | – | – | – | – | + | + | – | – | – | – | + | + | – |
| *Marsippospermum grandiflorum* (L.f.) Hook. | a[e] | TDF | – | – | – | – | – | – | – | – | – | + | + | – | – | – | – | – | – | – |
| Poaceae | | | | | | | | | | | | | | | | | | | | |
| *Agrostis curtisii* Kerguélen | a, b | X | – | – | – | – | – | – | – | – | – | + | + | – | – | – | – | – | + | – |
| *Deschampsia flexuosa* (L.) Trin. | a, b | X | – | – | – | – | – | – | – | – | – | + | + | – | – | – | – | – | + | – |
| *Festuca rubra* L. | a, b | X | – | – | – | – | – | – | – | – | – | + | + | – | – | – | – | – | + | – |
| *Molinia caerulea* L. Moench | a, b | X | – | – | – | – | – | – | – | – | – | + | + | – | – | – | – | – | + | – |
| **Non-graminoid herbaceous** | | | | | | | | | | | | | | | | | | | | |
| Asteraceae | | | | | | | | | | | | | | | | | | | | |
| *Pilosella officinarum* F.W. Sch.&Sch. Bip. | c | X | – | – | – | – | – | – | – | – | – | + | – | – | – | – | – | + | + | – |
| Droseraceae | | | | | | | | | | | | | | | | | | | | |
| *Drosera rotundifolia* L. | c | X | – | – | – | – | – | – | – | – | – | + | – | – | – | – | – | – | – | – |
| **Ericoids** | | | | | | | | | | | | | | | | | | | | |
| Ericaceae | | | | | | | | | | | | | | | | | | | | |
| *Calluna vulgaris* (L.) Hull. | a, b | X, KM, RMM | – | – | – | – | – | – | – | – | – | + | – | – | – | – | – | + | + | – |
| *Empetrum rubrum* Vahl ex Willd. | a[e] | TF | – | – | – | – | – | – | – | – | – | + | – | – | – | – | – | + | | – |
| *Erica cinérea* L. | a[e] | X | – | – | – | – | – | – | – | – | – | + | – | – | – | – | + | + | + | – |
| *Erica mackaiana* Bab. | a, b | X | – | – | – | – | – | – | – | – | – | + | – | – | – | – | + | – | + | – |
| *Ledum palustre* L. | a[e] | RMM | – | – | – | – | – | – | – | – | – | + | – | – | – | – | – | – | – | + |
| Thymelaceae | | | | | | | | | | | | | | | | | | | | |
| *Thymelaea cordifolia* (Lam.) Endl. | a[e] | X | – | – | – | – | – | – | – | – | – | + | – | – | – | – | – | + | + | – |
| **Non-ericoid woody** | | | | | | | | | | | | | | | | | | | – | |
| Nothofagaceae | | | | | | | | | | | | | | | | | | | | |
| *Nothofagus antarctica* (Forster f.) Oersted | a[e] | TF | – | – | – | – | – | – | – | – | – | + | – | – | – | – | – | – | + | – |
| *Nothofagus pumilio* (Poeppig & Endl.) Krasser | a[e] | TF | – | – | – | – | – | – | – | – | – | + | – | – | – | – | – | – | + | – |
| Pinaceae | | | | | | | | | | | | | | | | | | | | |
| *Pinus sylvestris* L. | a[e] | RMM, KM | – | – | – | – | – | – | – | – | – | + | – | + | – | – | – | – | – | – |

[a] Numbers correspond to pyrolysis products provided in Table 4.
[b] a = aerial parts; b = roots; c = mixture of roots and aerial parts.
[c] KM = Königsmoor (Harz Mountains, Germany); RMM = Rödmossamyren (Sweden); TF = National Park Tierra del Fuego (Tierra del Fuego, Argentina); X = Xistral Mountains (Galicia, Spain).
[d] Contained low amounts of guaiacyl lignin, see Section 3.2.4.
[e] Roots were not analysed.

**Table 3**
Number of samples analysed on the pyrolysis devices used for analysis of the studied peat and plant material.[a]

| Material | Location | Pyrolysis device | | | |
|---|---|---|---|---|---|
| | | Micro-furnace | Curie-Point | Filament 5000 | Filament 1000 |
| Peat | HRB | – | 63 | – | 12 |
| | RMM | – | – | 53 | – |
| | KM | – | – | 42 | – |
| | PVO | – | 101 | – | – |
| | PDC | 34 | – | – | – |
| | PF | – | – | 42 | – |
| Plant | HRB | 9 | – | 14 | – |
| | RMM | – | – | 6 | – |
| | KM | – | – | 4 | – |
| | PVO | – | 20 | – | – |
| | PDC | 12 | – | – | – |

[a] For codes of peatlands and for plant species see Tables 1 and 2, respectively; for details on apparatus parameters see Section 2.3.

lichens (Table 2). Its presence did not relate to a specific family or location. 3-Methoxy-5-methylphenol was found in species collected from both Galicia and Tierra del Fuego, and included *Cetraria islandica*, *Cladonia arbuscula*, *Cladonia cervicornis*, *Cladonia furcata*, *Ochrolechia frigida* and *Pseudocyphellaria freycinetii*. A synonym used in perfumery for 3-methoxy-5-methylphenol is 'oak moss phenol', because it has been isolated from oak moss (*Evernia prunastri* (L.) Ach.) by ter Heide et al. (1975). This observation supports its use as a marker for lichens.

The identification of this marker is important because lichens are generally not recognised in the macrofossil record, and lichens occur only under relatively dry conditions in peatlands (see Section 3.3). Lipid distribution (Ficken et al., 1998) and monosaccharide composition (Jia et al., 2008) of lichens have been studied as potential markers in peatland ecosystems. Although the distribution of these compounds may be specific for the lichen species, (part of) the compounds are common in many organisms so that their distribution in mixtures of several plant species and tissues (i.e., peat) complicates the interpretation of their depth records. The depth record of 3-methoxy-5-methylphenol in the studied peatlands will be further discussed in Section 3.4.4.

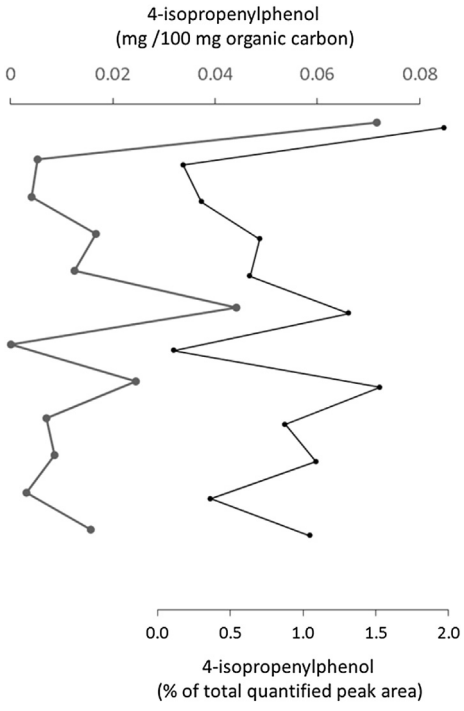



**Fig. 1.** Depth records of 4-isopropenylphenol obtained with internal standard and as % of the total quantified peak area.

In addition to 3-methoxy-5-methylphenol, other specific compounds were detected in the lichens, most of them being (poly) aromatic or benzofuran compounds, of which the major part contained (di)methoxygroups (SI_Table 1). These compounds probably originate from substituted phenolic esters (depsides, depsidones) and dibenzofurans that are abundant in lichens (Wachtmeister, 1958; Honda, 2006). Most chemical studies for lichens have been carried out on extracts, though the distribution of depsides necessarily involves a destruction (Edwards et al., 2003). Most of the compounds from SI_Table 1 were not found in the peat pyrolysates, except for an aromatic compound that originated from *O. frigida* (compound 2, Tables 2 and 4) and was found in the deepest part of the HRB peat record (Table 5). The abundance of compound 2 was much lower than that of the more general lichen marker 3-methoxy-5-methylphenol (<0.05% and <0.25% TIC, respectively).

### 3.2.2. *Sphagnum spp.*

The specificity of 4-isopropenylphenol (compound 3, Table 3; Figs. 2 and 3) as a marker for sphagnum acid has been tested thoroughly (van der Heijden et al., 1997; McClymont et al., 2011; Abbott et al., 2013) and is confirmed by its presence in the analysed *Sphagnum* species and its absence in all other plant pyrolysates (Table 2). 4-Isopropenylphenol was proposed by van der Heijden et al. (1997) as a marker for *Sphagnum* in peat cores. Recently it has been shown that the marker for sphagnum acid is very sensitive to water table height, and that its abundance in *Sphagnum*-dominated peat is determined by aerobic decay rather than the contribution from *Sphagnum* spp. to the surface vegetation (Schellekens et al., 2015a). In the graminoid-dominated peatlands, with a low contribution from *Sphagnum*, the depth record of 4-isopropenylphenol and other markers agreed well with the hydrological preference of the plants (see Section 3.3). This in combination with the fact that decomposition has much more progressed in these graminoid-dominated peatlands suggests that its depth record reflected the contribution from *Sphagnum* to the surface vegetation. The sensitivity to decay of sphagnum acid under aerobic conditions in *Sphagnum*-dominated peat in combination with its persistence in graminoid-dominated peat suggests that part of sphagnum acid is very resistant against degradation. The presence of four different thermochemolysis products of sphagnum acid and their different behaviour with depth in *Sphagnum* peat (Abbott et al., 2013) support a heterogeneous character of the biopolymers from which sphagnum acid originates.

The other two pyrolysis products that appeared specific for *Sphagnum* (*p*-hydroxybiphenyl and 4-methyl-2-phenylphenol, compounds 4 and 5, Table 2) are not considered suitable markers

**Table 4**
Characteristics of marker compounds, separated for (A) compounds that are potential generally valid biomarkers and (B) compounds that were specific but rapidly degraded or compounds that are less specific (common pyrolysis products of soil OM), though applicable as marker in one of the peatlands.

| | Compound | Fragment ions[a] | $M^{+}$[b] | Source plant(s) |
|---|---|---|---|---|
| 1 | 3-Methoxy-5-methylphenol | 138, 107, 109, 108, 95, 77 | 138 | Lichens[c] |
| 2 | Dimethoxy aromatic compound | 152, 151, 165, 236, 121, 91, 77, 194 | 236 | *O. frigida* |
| 3 | 4-Isopropenylphenol | 134, 119, 91 | 134 | *Sphagnum* spp. |
| 4 | *p*-Hydroxybiphenyl | 170, 115, 141 | 170 | *Sphagnum* spp. |
| 5 | 4-Methyl-2-phenylphenol | 184, 183, 185 | 184 | *Sphagnum* spp. |
| 6 | 4-(2-Phenylethenyl)phenol | 196, 195, 97, 165, 152, 181 | 196 | *R. lanuginosum*, *L. glaucum*, *H. cupressiforme* |
| 7 | Dihydroxy polyaromatic compound | 241, 256, 257, 242, 120, 213, 225 | 256 | *L. glaucum*, *H. cupressiforme* |
| 8 | Benzofuran, 2,3-dihydro-2-methyl-4-phenyl | 210, 195, 209, 165 | 210 | *R. lanuginosum*, *L. glaucum* |
| 9 | 3-Ring polyaromatic (N) compound | 209, 224, 153, 181, 210, 104, 76 | 224 | *L. glaucum*, *H. cupressiforme* |
| 10 | Guaiacyl and syringyl lignin phenols[d] | – | – | Vascular plants |
| 11 | Ferulic acid methyl ester | 208, 177, 145, 117, 89, 77 | 208 | Graminoids |
| 12 | Diterpene derivatives[e] | – | – | *Pinus* spp. |
| 13 | $C_1$-Phenanthrene | 192, 191, 189, 193, 188 | 192 | *C. binervis* |
| 14 | 4-Hydroxybenzene acetonitrile | 133, 78, 106, 132, 77, 105, 104, 90 | 133 | *J. bulbosus* |
| 15 | Triterpenoid product | 218, 203, 189 | – | *E. mackaina*, *E. cinerea* |
| 16 | Benzoic acid | 105, 122, 77, 51 | 122 | *E. rubrum* |
| 17 | $C_3H_3$ guaiacols | 162, 147, 91, 119, 130, 102, 89 | 162 | *N. antartica*, *N. pumilio* |
| 18 | Sesquiterpenes | 105, 119, 91, 133, 161, 204, 189 | 204 (222) | – |

[a] Decreasing intensity.
[b] $M^{+}$ molecular weight.
[c] See Table 2 for its presence in the analysed lichens.
[d] For typical fragment ions of lignin pyrolysis products we refer to Schellekens et al. (2015b).
[e] For typical fragment ions of pine pyrolysis products we refer to Hautevelle et al. (2006) and Schellekens et al. (2013).

because they rapidly decreased with depth suggesting that these compounds are lost during the first stage of decay (not shown).

### 3.2.3. Non-*Sphagnum* mosses

Some polyaromatic compounds were found specific for non-*Sphagnum* mosses, including 4-(2-phenylethenyl)phenol, a dihydroxypolyaromatic compound, 2,3-dihydro-2-methyl-4-phenylbenzofuran, and a polyaromatic N-containing compound (compounds 6–9, Tables 2 and 4). All of them were detected in the HRB peat samples, while in most other peatlands only the dihydroxypolyaromatic compound (compound 7) was detected.

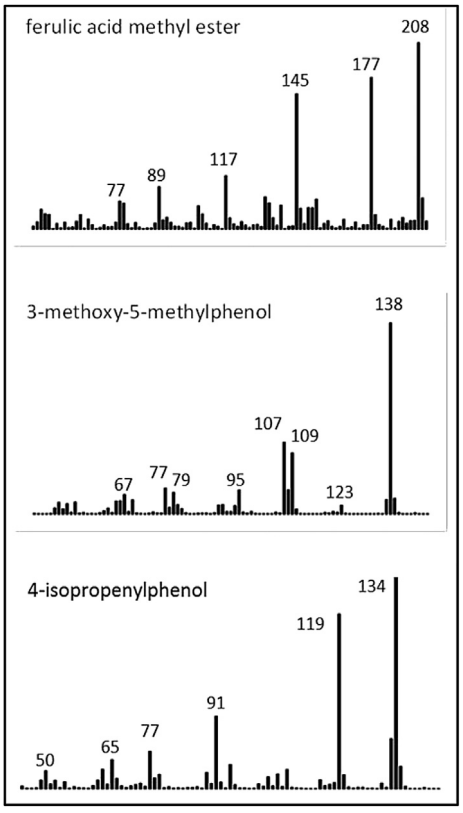



**Fig. 2.** Mass spectra of ferulic acid methyl ester, 3-methoxy-5-methylphenol, and 4-isopropenylphenol.

It must be mentioned that *n*-methyl ketones were present in all mosses, of which the $C_{19}$ was dominant in most of them, and even a dominant peak in *H. cupressiforme* (not shown). *H. cupressiforme* also had the *n*-$C_{19}$ alkyldione (fragment ions *m*/*z* 85 and 100). Because *n*-methyl ketones have been supposed as biomarkers being oxidation products of *n*-alkanes (Jansen and Nierop, 2009), it is important to mention that most samples from both Galician peatlands (PVO, PDC) also showed a dominance of the $C_{19}$ *n*-methyl ketone, suggesting a moss source instead of an *n*-alkane oxidation product in these peatlands.

### 3.2.4. Vascular plants

Lignin phenols are well-known and important markers for vascular plants. Syringyl lignin moieties were only detected in vascular plants, not in mosses and lichens. Very low amounts of guaiacyl moieties detected in *Sphagnum* pyrolysates (Table 2) do not originate from *Sphagnum* itself, but are probably derived from vascular plants and migrated into *Sphagnum* capitula with dissolved organic matter (Abbott et al., 2013). The lignin phenol composition in pyrolysates of plant species from the PVO and PDC peatlands was earlier discussed in detail by Schellekens et al. (2012).

*3.2.4.1. Graminoids.* Ferulic acid methyl ester was found in pyrolysates of all analysed graminoids and not in other plants. Its marker status for graminoids is supported by analytical pyrolysis of 32 Mediterranean plant species in which ferulic acid methyl ester was detected in both roots and aerial parts of eight out of nine graminoids, while it was absent from all 23 other analysed plant species, including a non-graminoid monocotyledon (Schellekens et al., 2013). Graminoids are known to have different lignin-carbohydrate complexes compared to other plant species. Ferulic acid dehydrodimers from grass cell walls that cross-link polysaccharides (Ralph et al., 1994) are proposed as the source of this pyrolysis product. The reliability of ferulic acid methyl ester as a graminoid marker is well established (Table 2); but its abundance in plant (0.04–0.14% TIC) and peat pyrolysates (<0.07% TIC) was

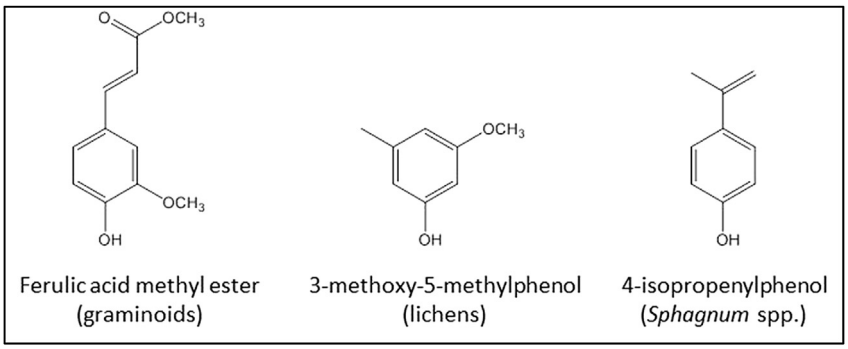



**Fig. 3.** Structure formula for the marker of lichens, graminoids and *Sphagnum*.

**Table 5**
Presence of the markers in the studied peatlands, for codes of peatlands and marker compounds see Tables 1 and 4, respectively.

| Peatland | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HRB1 | + | − | + | + | + | + | + | + | + | + | − | − | − | − | +[a] | + | + | − |
| HRB2 | + | − | + | + | + | + | + | + | + | + | + | − | + | − | +[a] | + | + | + |
| HRB3 | + | + | + | − | − | + | + | + | + | + | + | + | + | − | +[a] | + | + | + |
| RMM | + | − | + | + | + | − | − | − | − | + | + | − | − | − | − | − | − | − |
| KM | + | − | + | + | + | − | − | − | − | + | + | − | +[b] | − | +[a] | − | + | + |
| PVO | + | − | + | − | − | − | + | − | − | + | + | − | + | + | + | + | + | − |
| PDC | + | − | + | − | − | − | + | + | − | + | + | − | + | − | + | − | + | + |
| PF | + | − | + | − | − | − | + | − | − | + | + | − | + | − | − | − | + | + |

[a] Similar but not the same compound.
[b] Very low abundance.

low, and sometimes near the detection limit (e.g., in RMM, Section 3.4.3). The mass spectrum and structure formula of ferulic acid methyl ester is given in Figs. 2 and 3.

*3.2.4.2. Pinus sylvestris.* Diterpenes were common in pyrolysates of *P. sylvestris* (Thomas, 1970; Otto et al., 2007). For the peatlands, diterpenoids were only detected in HRB3, which is consistent with the lack of pine trees growing on the peatlands at present.

*3.2.4.3. Other vascular plants. Carex binervis.* $C_1$-phenanthrene (compound 13, Table 4) is considered to have a pyrogenic origin, and may indicate resinous materials (del Rio et al., 1992). Among the analysed plant species it was only detected in *C. binervis*, and its depth record in the PVO peat showed good agreement with its preferential habitat and with other markers (Schellekens et al., 2011). Polycyclic antimicrobial compounds have been found in the roots of *Carex* species (Kawabata et al., 1995), and these compounds can be a source of phenanthrenes.

*Juncus bulbosus.* 4-Hydroxybenzene acetonitrile (4-hydroxybenzyl cyanide) was not detected in other analysed species (Table 2) and occurred exclusively in pyrolysates from PVO peat samples (Table 5). It has been reported from the fungus *Aspergillus fumigatus* (Packter and Collins, 1974). It has also been reported from a marine sponge (Goclik et al., 1999), although the authors suggested that the substance could have been produced by a fungal symbiont. However, *Aspergillus* is not a symbiotic fungus, and members of the genus *Juncus* do not form beneficial root symbioses. The source of the product therefore remains unclear.

*Erica.* The mass spectrum of the marker for the *Erica* species analysed, a triterpenoid product with fragment ions *m*/*z* 218, 203 and 189, showed the best fit with alpha- or beta-amyrin, but the molecular ion of alpha- or beta-amyrin (426) did not correspond with that of the marker for *Erica mackaiana* and *E. cinerea* (408). A high abundance of such triterpenoids was also reported with GC/MS in Ericaceae by Pancost et al. (2002). Although its depth record showed good agreement with other markers from the same peatland (PVO), identification of triterpenoids with pyrolysis is problematic because many have the same basic skeleton, and edge groups are removed upon pyrolysis.

*Empetrum rubrum.* Benzoic acid was a major peak in pyrolysates of *E. rubrum* (7% TIC; Schellekens et al., 2009) and was not detected in other analysed HRB peatland plant species, though also occurred in pyrolysates from other species from other peatlands. The depth record of benzoic acid in HRB showed a clear trend (Schellekens and Buurman, 2011) that was in good agreement with the pollen record of *E. rubrum* from a core of the same peatland (Markgraf and Huber, 2010).

*Nothofagus.* The two isomers identified as $C_3H_3$-guaiacols were solely detected in wood of *Nothofagus antarctica* in the HRB plant selection and its depth record showed a clear trend that was in agreement with other markers and with the peatland ecology (Schellekens and Buurman, 2011).

*Ledum palustre.* A number of sesquiterpenes was detected in pyrolysates of *L. palustre*. These probably originate from the sesquiterpenoid ledol (Butkiene et al., 2008). Sesquiterpenes were detected in some of the peatlands (Table 5), though their source is too widespread to allocate them reliably to a specific species; a sesquiterpene was also found in one of the lichens (Table 2).

*Drosera rotundifolia.* A number of specific products were detected in pyrolysates from *D. rotundifolia*, many of them were polyaromatic compounds that contained nitrogen and/or oxygen functional groups (SI_Table 2). The fact that none of them were detected in the peat may be related to a low contribution from *D. rotundifolia* to the surface vegetation.

Most markers discussed in this Section probably only function well within a specific peatland ecosystem, because 1) the marker was a rather common pyrolysis product, being specific only within pyrolysates of the plant set of a single peatland (*Erica*, *E. rubrum*,

*Nothofagus* and *L. palustre*) or 2) its origin from the plant is not well established (*C. binervis* and *J. bulbosus*). The potential of pyrolysis products specific for *D. rotundifolia* could not be discussed due to their absence in the peat pyrolysates. The differences between the peatlands emphasises the importance of plant analysis and ecological knowledge (for the ecology of peatland plants see for example Rydin and Jeglum (2013)) prior to the use of pyrolytic biomarkers with low specificity, but also shows that supposedly non-specific pyrolysis products may be suitable markers within a certain peatland ecosystem.

### 3.3. Ecological and hydrological understanding of marker records

Among the plant species selected as reference for reconstruction of the history of peatland vegetation in relation to mire hydrology, there is a cluster of cosmopolitan and widespread species and a cluster of species restricted to relatively small geographical areas or even endemic (SI_Table 3), which enables us to analyse the validity of the markers at different geographical scales.

Lichens are good indicators of soil dryness because lichens do not need soil water for their growth, given that they are able to utilise only dew, fog or water vapour as hydrological resources. Moreover, lichens can withstand dry environmental conditions by deactivating their metabolism and being biologically active only for short time periods (Lange et al., 1982, 1986; Kappen, 1988; Green et al., 2011). The analysed lichen species are mainly terricolous, living over peat or on bryophytes, so that most of them are tolerant to water table fluctuations, although all of these species are unable to live submerged and show a strong preference for the drier places of the peatland.

*Sphagnum* is a genus with around 200 species, some of which are typical of peatlands where each species usually occupies a habitat range determined by the depth of the water table (Daniels and Eddy, 1990; Clymo, 1997). Since 4-isopropenylphenol exclusively indicates the presence of *Sphagnum* spp. in the peat, without species differentiation, this marker is difficult to interpret in peatlands that are not dominated by *Sphagnum*. However, according to Blackford (2000), the presence of *Sphagnum*, relative to the abundance of plants indicative for drier conditions, such as Ericaceae, can be used as an indicator of relative surface wetness.

Similar to *Sphagnum* spp., graminoids in peatlands comprise a high number of species from the families Juncaceae, Poaceae and Cyperaceae, which may have different ecological niches especially in graminoid-dominated peatlands; its marker should therefore be interpreted with caution.

*J. bulbosus*, *C. binervis* and *Eriophorum angustifolium* correspond to wet and damp phases in the mire development, while *Deschampsia flexuosa*, *E. cinerea*, *E. mackaiana* and *P. sylvestris* are indicative of the dry phases. Some species can tolerate various environmental conditions, even opposite to their usual ecological niches. Examples are *E. mackaiana* and *P. sylvestris*, both indicators of dryness, but tolerant to wet conditions, albeit with an anomalous and poor growth. Such morphological characteristics cannot be detected in their corresponding marker records. So, in order to determine the hydrological changes throughout the history of the peatland, it is more important to take into account the relative frequencies of all markers (or other plant proxies), along the peat core, than the existence of one particular marker of wetness or dryness indicator value.

### 3.4. Effect of decomposition and vegetation type on marker abundance

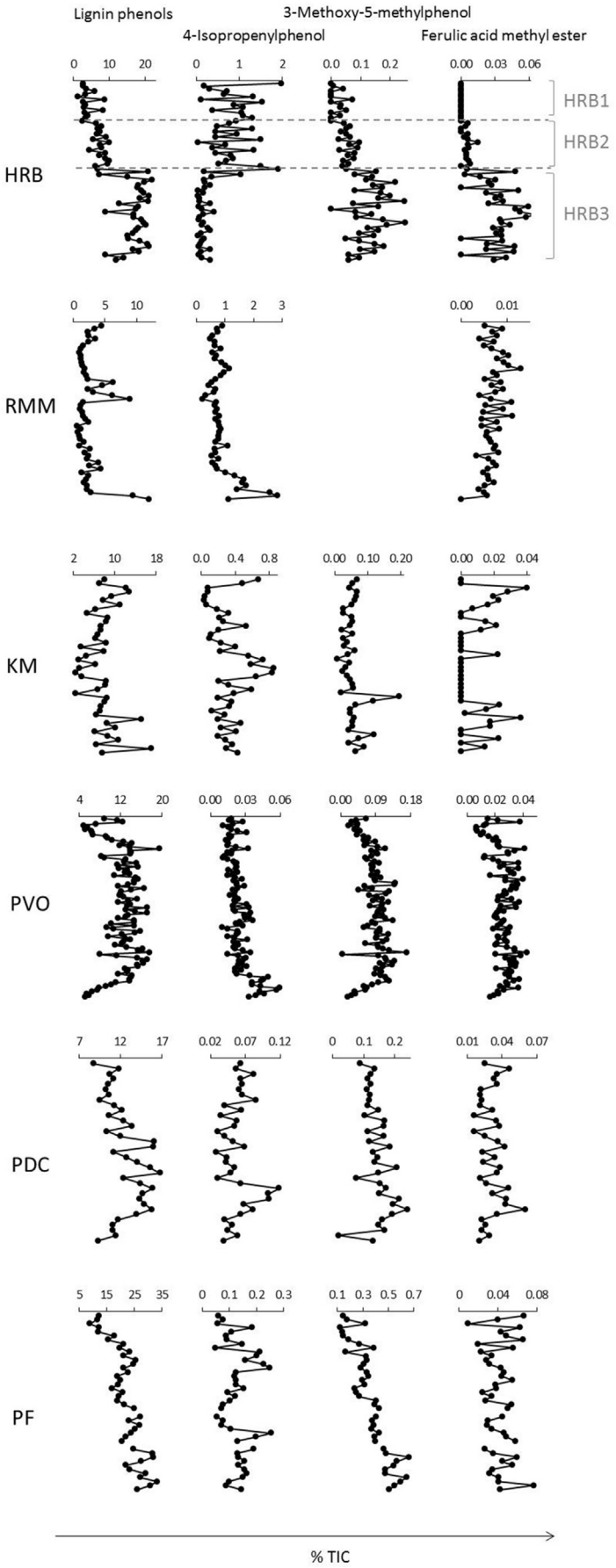
In the investigated peatlands, depth records of the plant markers generally showed good agreement with the preferential hydrological habitat of the source species. The pyrolysis results agreed with other paleo-environmental proxies established for the same peatlands, including both local and regional proxies. For HRB, depth records of the markers agreed well with macrofossil analysis, pollen, ash content, C/N ratio, charcoal abundance and moisture levels obtained with the deuterium/hydrogen isotope ratios (Heusser, 1989; Markgraf, 1993; White et al., 1994; Pendall et al., 2001; Schellekens et al., 2009; Markgraf and Huber, 2010; Schellekens and Buurman, 2011); for PVO, the marker records agreed well with pollen and non-pollen palynomorphs, ash content, C/N ratio, and Holocene climate shifts in the same area according to other studies (Martínez-Cortizas et al., 1999; Muñoz-Sobrino et al., 2005; Mighall et al., 2006; Schellekens et al., 2011; Castro et al., 2015); the marker records from PF agreed well with those obtained from $\delta^{13}C$ and $\delta^{15}N$ isotopes, C/N ratio, ash content, inorganic geochemistry and pollen data (Horák-Terra et al., 2014; Schellekens et al., 2014); the marker records from KM were compared with several decomposition proxies, Fourier transform infrared spectra absorption intensities, Rock Eval oxygen and hydrogen indices, $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures and UV-absorption (UV-ABS) of NaOH peat extracts (Biester et al., 2014); and marker records from RMM were compared with C/N ratio and UV-absorption (UV-ABS) of NaOH peat extracts (Schellekens et al., 2015a). The good agreement between the results presented here and those obtained with other methods implies that the effect of decomposition on molecular markers does not necessarily prohibit their use for most of them. Nevertheless, the effects of decomposition on the variation of a marker may be masked by a preferential wet habitat of the species, wet conditions causing both an increase of the species and less decomposition. The effects of vegetation type and decomposition on the variation of a marker will be discussed for the markers that were relevant in all peatlands, and include lignin (vascular plants), 4-isopropenylphenol (*Sphagnum*), ferulic acid methyl ester (graminoids), and 3-methoxy-5-methylphenol (lichens; Tables 2 and 5). Depth records of those markers are given in Fig. 4 for all peatlands. The hydrological interpretation of the marker records for the different peatlands is summarised in Table 6.

#### 3.4.1. Lignin phenols (vascular plants)

The interpretation of the lignin phenol depth record depends on the vegetation type. In *Sphagnum*-dominated peat it reflects vascular plant input (RMM, HRB, KM), which increases under drier conditions (Schellekens et al., 2015a). In non-*Sphagnum* peat the abundance of lignin phenols reflects the degree of decomposition, though its hydrological interpretation depends on the degradation state of the peat. In moderately decomposed peat, high values indicate relatively decomposed organic matter (dry conditions) caused by preferential decomposition of carbohydrates over lignin (PVO, PDC; Schellekens et al., 2015b). In highly decomposed peat, the abundance of lignin reflects less decomposed material (wet conditions), because after decomposition of plant-derived polysaccharides lignin is preferentially lost over highly resistant aliphatic biopolymers (Schellekens et al., 2014). In systems with alternating vegetation types, lignin phenols in highly decomposed peat may also reflect vascular vs. aquatic plant input (Tsutsuki et al., 1994; Bourdon et al., 2000; Kaal et al., 2014).

#### 3.4.2. 4-Isopropenylphenol (Sphagnum spp.)

The 4-isopropenylphenol record is predominantly determined by aerobic degradation in *Sphagnum*-dominated peat instead of the contribution of *Sphagnum* to the surface vegetation (Schellekens et al., 2015a). The presence of 4-isopropenylphenol in peat samples of up to 13.000 years old in combination with an absence of




depth trends indicates that once in the permanently anaerobic layer no further degradation occurs. The abundance of the marker for sphagnum acid in *Sphagnum*-dominated peat (0–3% TIC) was generally an order of magnitude higher compared to graminoid-dominated peat (0–0.3% TIC; Fig. 4). In all *Sphagnum*-dominated peatlands, 4-isopropenylphenol showed a negative correlation with (di)methoxyphenols, reflecting that under aerobic conditions sphagnum acid is easily decomposed while vascular plants increase (Schellekens et al., 2015a). Depth records of 4-isopropenylphenol in the non-*Sphagnum* peatlands (PVO, PDC, PF) appear to reflect the abundance of *Sphagnum*, which suggests that part of sphagnum acid is very resistant against degradation. This is most clear from the PF deposit, where 4-isopropenylphenol showed large variation and no correlation with groups of pyrolysis products (aliphatics, carbohydrates, lignin), suggesting that decomposition did not control its variation. In PVO, 4-isopropenylphenol consistently showed high values during dry periods and low values during wet periods in the ombrotrophic part; this was interpreted by the occurrence of a species of *Sphagnum* that prefers relatively dry conditions such as *S. compactum* (Schellekens et al., 2011).

### 3.4.3. Ferulic acid methyl ester (graminoids)

Ferulic acid methyl ester is specific for graminoids (Table 2). Depth records of ferulic acid methyl ester in *Sphagnum*-dominated peat (HRB, RMM, KM) showed an opposite trend to that of 4-isopropenylphenol, suggesting that it reliably reflects the abundance of graminoids. This is most clear from HRB, where it showed a large shift from generally high values in the graminoid-dominated peat, much lower values in the *Sphagnum* peat with considerable contribution from graminoids, and was not detected in the upper *Sphagnum*-dominated peat. In RMM its abundance was near the detection limit which did not allow a reliable interpretation (<0.01% TIC).

In the non-*Sphagnum* peat deposits, ferulic acid methyl ester was positively correlated to (di)methoxyphenols in PVO and PDC ($r^2$ 0.46 and 0.44, respectively). This can be related to the dominance and diversity of graminoids in these profiles, causing that the abundance of this marker is affected by several processes: 1) the contribution of a large set of graminoid species and their different ecology, 2) the effects of decomposition and 3) a different contribution of the marker to pyrolysates from the graminoid species. In PF there was no correlation of ferulic acid methyl ester with (di) methoxyphenols, which can be related to the fact that in PF also wood contributed significantly to the lignin content. The interpretation of the marker for graminoids is not clear for non-*Sphagnum* peatlands. The depth record of ferulic acid methyl ester may indicate large vegetation changes, but not minor shifts within a certain vegetation type.

### 3.4.4. 3-Methoxy-5-methylphenol (lichens)

Depth records of the marker for lichens, 3-methoxy-5-methylphenol, showed good agreement with other markers in HRB, PVO and PF and support its use as marker for lichens in peatlands (Schellekens et al., 2009, 2014; Schellekens and Buurman, 2011). Its absence in RMM is probably caused by the very wet conditions throughout the year at this site (Rydberg et al., 2010) inhibiting growth of lichens.

**Fig. 4.** Depth records of markers for lignin (methoxyphenols), *Sphagnum* spp. (4-isopropenylphenol), lichens (3-methoxy-5-methylphenol) and graminoids (ferulic acid methyl ester) for the different peatlands.

**Table 6**
Hydrological interpretation and corresponding process for markers those were present in all peatlands.[a]

| Peatland | 4-Isopropenylphenol | 3-Methoxy-5-Methylphenol | Lignin phenols | Ferulic acid methyl ester |
|---|---|---|---|---|
| *Sphagnum* dominated | | | | |
| HRB | **Wet** (less aerobic decomposition) | **Dry** | **Dry** (increase in vascular plants) | **Dry** (contribution from graminoids) |
| RMM | **Wet** (less aerobic decomposition) | – | **Dry** (increase in vascular plants) | **Dry** (contribution from graminoids) |
| KM | **Wet** (less aerobic decomposition) | **Dry** | **Dry** (increase in vascular plants) | **Dry** (contribution from graminoids) |
| Graminoid dominated | | | | |
| PVO | **Dry** (contribution from *Sphagnum*) | **Dry** | **Dry** (preferential loss of carbohydrates over lignin) | Not clear |
| PDC | | | **Dry** (preferential loss of carbohydrates over lignin) | Not clear |
| PF | **Wet** (contribution from *Sphagnum*) | **Dry** | **Wet** (preferential loss of lignin over aliphatic biopolymers)[b] | Not clear |

[a] Interpretation is based on depth records of markers in the different peatlands (Fig. 4) and comparison with other markers and proxies obtained from other methods in the corresponding publications; interpretation refers to high values.
[b] The majority of plant polysaccharides already being lost.

## 4. Conclusions

Plant specific pyrolysis products (biomarkers) were detected in all six peatlands, making pyrolysis-GC/MS a powerful method to reconstruct past vegetation composition from peatlands. Markers for lichens, graminoids and *Sphagnum* were present in all peatlands. The absence of depth trends indicates that none of the markers were subject to long-term anaerobic decomposition.

The similarity between depth records of the *Sphagnum* marker (4-isopropenylphenol) using an internal standard and the traditional quantification indicates that markers can be obtained without time-consuming quantification of pyrolysates, thereby enormously simplifying the use of pyrolysis in biomarker research.

The interpretation of marker records depended on species dominance and environmental conditions; this emphasises the importance to interpret geochemical records in the context of the investigated ecosystem and comparison with other proxies.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.quascirev.2015.06.028.

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