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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