Exploring Northern Peatland Biogeochemistry as a Function of Climate Change

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A thesis submitted to Newcastle University in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Science, Agriculture and Engineering.

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Declaration

I hereby certify that the work presented in this thesis is my own original research, with the exception of the identification of lichen and graminoid markers through pyrolysis-GCMS in chapter 4, which was performed by Judith Schellekens. Climatic data and branched GDGT analysis from chapter 5 was provided by Arnaud Huguet within the framework of the French Agence Nationale de la Recherche project PEATWARM, however all interpretation in relation to my results is my own. Wherever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature, and acknowledgement of collaborative research and discussions. No part of this work has been submitted previously for a degree at this or any other university.

Jonathan Alexander Bradley

Abstract

Soil organic matter (SOM) is a major store of terrestrial carbon (C), and peat soils make up a large proportion of this terrestrial C sink. Northern peatlands themselves cover an area of around 4 x 10^6 km^2, and currently store around 547 Gt of organic carbon as waterlogged peat. Some of the major peat forming plants are the *Sphagnum* mosses, which are known to synthesize phenolic compounds such as *trans*-sphagnum acid as structural support components.

The use of molecular marker compounds to identify specific plant species or SOM inputs is well established, as is the use of molecular proxies to assess existing or past climatic conditions and/or SOM dynamics and turnover. Samples from a global range of peatlands were analysed using thermally assisted hydrolysis and methylation (THM) in the presence of ^{13}C-labelled tetramethylammonium hydroxide (^{13}C-TMAH) in order to confirm the presence of a suite of *Sphagnum* marker compounds. The corresponding *Sphagnum* yield parameter (σ), along with the parameter for total lignin-derived phenols (Λ) was assessed in relation to branched glycerol dialkyl glycerol tetraether (brGDGT) derived proxies under simulated climatic warming conditions in the Frasne Peatland, France. In addition to this, analytical pyrolysis gas chromatography mass spectrometry (Py-GCMS) was carried out on peat samples in order to obtain new plant-specific biomarkers, and the viability of these markers was assessed with ^{13}C-TMAH thermochemolysis.

The proposed *Sphagnum* marker compounds **I**, **IIa**, **IIb** and **III** were observed across all *Sphagnum* capitula sampled and all peat deposits with *Sphagnum* input, while being absent from all vascular plants or non-*Sphagnum* bryophytes studied. Ferulic acid methyl ester was proposed as a graminoid-specific marker after being found exclusively in graminoid pyrolysates, and 3-methoxy-5-methylphenol was proposed as a marker for lichens after being observed in six of the seventeen lichen species sampled. ^{13}C-TMAH thermochemolysis of these proposed markers confirmed a primarily monomethoxy source, confirming their viability as biomarkers.

The observed decrease in σ and Λ in surficial peats under simulated warming conditions suggests an effect due to drying and aeration of surficial peat layers as a result of increasing temperatures, which corresponds with brGDGT-based proxies such as the methylation index of branched tetraethers (MBT), which is seen as an indicator of air temperature.

List of Publications

1. Schellekens, J., Bradley, J.A., Kuyper, T.W., Fraga, I., Pontevedra-Pombal, X., Vidal-Torrado, P., Abbott, G.D., Buurman, P., 2015b. The use of plant-specific pyrolysis products as biomarkers in peat deposits. Quaternary Science Reviews 123, 254-264.

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Chapter 1 – Introduction

1.1 Soil organic carbon

The global carbon (C) cycle consists of three main pools: the atmospheric pool (760 Gt C), the oceanic pool (38000 Gt) and the terrestrial pool (8060 Gt). Carbon cycles continuously between these pools, with each behaving simultaneously as a source and a sink of carbon (Lal, 2004). The terrestrial pool comprises the geologic (5000 Pg C), pedologic (soil; 2500 Pg C) and biotic pools (560 Pg C). Of the pedologic section, soil organic carbon (SOC) makes up 1550 Gt, with the remaining 950 Gt being soil inorganic carbon (SIC; Lal, 2004).

Inputs of carbon to the pedologic pool are primarily from the atmospheric pool via the biotic pool in the form of CO_2 conversion to terrestrial biomass, followed by sequestration as soil organic matter (SOM) through biochemical and physical stabilisation processes, with a proportion released back into the atmospheric pool as CO_2 and CH_4 via respiration (Kay and Angers, 2000; Dawson and Smith, 2007; Lorenz et al., 2007).

The size of the soil organic carbon pool is determined by the difference between inputs from biota, primarily vegetation, and outputs via respiration. Soil can act as either a sink of carbon or a source, depending on the balance of inputs and outputs (Powlson et al., 2008; Malhi and Grace, 2000).

SOM accounts for around 60% of the pedologic carbon pool (Post et al., 2001; Lal, 2004). It originates from vegetation in the form of leaf, root and wood litter, root and microbial exudates, and dead soil fauna. This material undergoes decomposition as it is incorporated into the soil profile, where 60-80% of labile OM can undergo conversion to CO_2, mostly within the first year of residence in the soil profile (González-Pérez et al., 2004). The material not decomposed within the labile pool can enter the intermediate pool or the stable pool, with residence times of 10-100 and over 1000 years respectively (Trumbore, 1997; von Lützow et al., 2006; Lorenz et al., 2007). The long-term residence of SOM within the stable pool is dependent on the structural composition of the material, spatial accessibility, and presence of certain minerals and metal ions (Sollins et al., 1996; Six et al., 2002; von Lützow et al., 2006). Although SOM can remain within the stable fraction for thousands of years, some studies have shown that this recalcitrant material can be degraded rapidly under certain conditions, including elevated temperature (von Lützow et al., 2006; Karhu et al., 2010; Kleber et al., 2011).

The dependence of the stable fraction of SOM on conditions such as pH, soil moisture and temperature is particularly relevant for peatlands, where pH and moisture levels play an

important role in the accumulation of peat (Rydin and Jeglum, 2013). It is therefore important to assess the impact of changing climatic conditions on peatlands and the impact this may have on their status as a sink of organic carbon.

1.2. Peat formation and diagenesis

The Intergovernmental Panel on Climate Change (IPCC) estimates the global carbon stocks in soils to be between 1500 and 2400 Gt, plus a further 1700 Gt in permanently frozen soils (IPCC, 2014). Northern peatlands are a particuarly important store of organic carbon (OC), covering an area of around 4 x 10^6 km^2, and currently storing around 547 Gt of OC as waterlogged peat (Yu et al., 2010). Scientific studies of peatlands, encompassing ecology, hydrology and chemistry have taken place for over 100 years, with Weber (1902) performing one of the first recorded scientific studies on a raised bog in Lithuania. Although the effects of climatic change on peatlands remains uncertain, it is thought that increasing drought frequency at high latitudes (IPCC 2007) could potentially lead to large scale degradation of northern peatlands due to increased aeration and loss of anoxic conditions in surficial peat (Dorrepaal et al., 2009; Fenner & Freeman, 2011; Abbott et al., 2013). Throughout the Holocene, peatlands have acted as a CO_2 sink and a source of CH_4 emissions via decomposition of organic material (OM) in an anoxic environment (Baird et al., 2009). Due to the significance of CH_4 as a greenhouse gas, changes in the equilibrium of peatlands has potentially large implications for the global climate, with the release of 100 Gt C corresponding to a 0.2 °C increase over a decadal timescale (IPCC, 2014).

Peat can be defined as soil material that contains at least 30% organic material by dry mass (Joosten and Clarke, 2002), and is formed when inputs of OM, primarily in the form of plant litter, exceed rates of decomposition due to factors including wetness, temperature and pH (FAO, 2014). As primary productivity continues to exceed decomposition, excess organic matter is buried and is transferred from a relatively dry, oxygen rich environment at the surface, to an anoxic, water saturated environment, where decomposition processes decrease to an extremely slow rate (Reddy and DeLaune, 2008).

A peatland is an area of land covered by peat, generally to a depth of 30 cm or more (Rydin and Jeglum, 2013), however a variety of classification systems exist worldwide, based on water level and nutrient status (Finland; Cajnder, 1913), hydrological regime (Canada; Tarnocai and Stolbovoy, 2006), and vegetation classification (UK; Rydin et al., 2013).

Peatland types can be broadly distributed along a gradient based on hydrology and pH, with groundwater-fed, nutrient-rich minerotrophic fens at one end, and rain-fed, acidic ombrotrophic bogs at the other. In these examples, minerogenous and ombrogenous, respectively, would refer to the hydrological regime, with minerotrophic and ombrotrophic referring to the provision of nutrients (Rydin and Jeglum, 2013). Peatland hydrology governs fundamental processes such as ecology and decomposition of OM (Clymo and Hayward, 1982), and peatlands can be further split into three distinct layers based on their hydrological regime; the oxygen rich acrotelm, at the surface of the peat, which is unsaturated by the water table, and where more rapid decomposition of OM takes place; the mesotelm, where the water table fluctuates between its medium and maximum levels and OM is not permanently saturated, creating a wet, oxygen rich environment where rapid decomposition of OM can take place, and the permanently saturated catotelm, which is below the minimum water table level, and is an anoxic environment where decomposition rates are extremely slow (Rydin et al., 2013). The slow decomposition rates in the catotelm allow peat to accumulate, and this in turn causes the water table to rise as the catotelm grows in depth, due to the hydraulic conductivity of the peat material (Baird, 1997).

Peat can be formed from a variety of different vegetation types, depending on the hydrological and pH regime of the peatland (Rydin et al., 2013). *Sphagnum* mosses are one of the main peat forming plants found in temperate ombrotrophic bogs (Andrus et al., 1986; Gajewski et al., 2001). As well as contributing large amounts of OC in the form of litter, *Sphagnum* has also been considered an ecosystem engineer in that it creates waterlogged, acidic conditions which inhibit the growth of vascular plant species and slow the rate of litter decomposition (van Breemen, 1995). Alongside *Sphagnum*-derived inputs, lignin is another major component of peatland OM, and is present in peat deposits due to input from vascular plants such as *Eriophorum vaginatum* (cottongrass) and *Vaccinium microcarpum* (cranberry). Other inputs include phenolic compounds from non-*Sphagnum* bryophytes such as *Pleurozium schreberi* (red-stemmed feathermoss) and *Polytrichum commune* (common haircap moss; Freeman et al., 2012).

1.3. Sphagnum acid

Sphagnum and other bryophytes do not synthesize lignin (Weng and Chapple, 2010), with *Sphagnum* being known to synthesize other phenolics as structural support components, such as *trans*-sphagnum acid (Rasmussen et al., 1995; Fig 1.1). Sphagnum acid and other phenolic constituents of *Sphagnum* can also act as inhibitors of microbial decomposition of *Sphagnum*

tissue. Cellulose is locked within a polymeric network within the cell wall, with sphagnum acid providing structural support similar to lignin in vascular plants, albeit with lower structural rigidity (Verhoeven and Liefveld, 1997). This cell wall polymeric network, in combination with the cell wall lipid coating, and a further polymeric network of condensed tannins (van der Heijden, 1994) leads to slow decomposition of dead *Sphagnum* tissue (Verhoeven and Liefveld, 1997).

In addition to their structural role, *Sphagnum*-derived phenolics also act as enzyme inhibitors. The enzyme phenol oxidase is thought to be one of the few enzymes capable of degrading these phenolic components (Freeman et al., 2001), however its activity is inhibited in peatland environments due to oxygen constraints and the level of acidity created by *Sphagnum* (van Breemen, 1995), which in turn inhibits degradation of phenolics and other enzyme activity (Freeman et al., 2001).

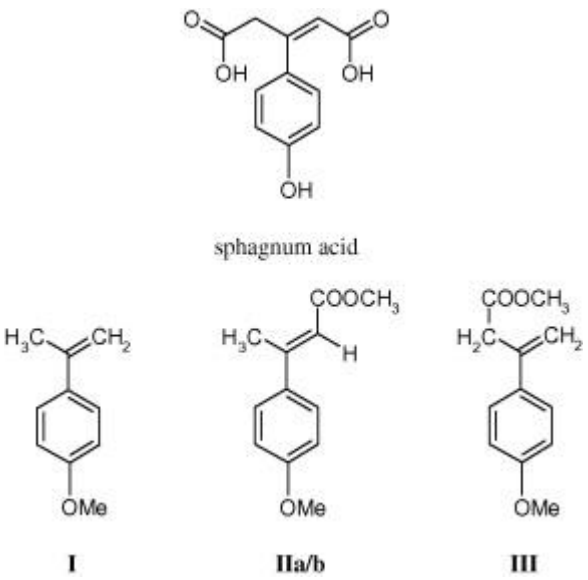

Fig. 1.1. The structure of *p*-hydroxy-*β*-[carboxymethyl]-cinnamic acid (sphagnum acid) and its thermochemolysis products (**I**) methylated 4-isopropenylphenol (IUPAC: 1-methoxy-4-(prop-1-en-2-yl)benzene), (**IIa**/**b**) methylated *cis*- and *trans*-3-(4′-hydroxyphen-1-yl)but-2-enoic acid (IUPAC: (*E*/*Z*)-methyl 3-(4-methoxyphenyl)but-2-enoate), and (**III**) methylated 3-(4′-hydroxyphen-1-yl)but-3-enoic acid (IUPAC: methyl 3-(4-methoxyphenyl)but-3-enoate) (van der Heijden et al., 1997; Abbott et al., 2013).

1.4. TMAH Thermochemolysis

Several methods have been used to attempt to characterise SOM inputs, particularly from vascular plants. Cupric oxide (CuO) oxidation has previously been used to characterise

vascular plant inputs to mineral soils (Hedges and Parker, 1976; Hedges and Mann 1979; Hedges and Ertel, 1982; Kögel, 1986), however the propyl side chain of the associated degradation products is not retained with this method (Hatcher et al., 1995; Filley et al., 2000).

Thermally assisted hydrolysis and methylation (THM) in the presence of tetramethylammonium hydroxide (TMAH) is an analytical method used to methylate phenols and phenolic acids in plant litter and soils, making them more amenable to GC analysis than the equivalent Pyrolysis-GCMS products (Filley et al., 1999). TMAH is used as a methylating agent in order to mitigate the issue of poor chromatographic separation of Py-GCMS products with polar functional groups (Challinor, 1989; Saiz-Jimenez, 1994; Klinberg et al., 2005). The reaction is described as a thermally assisted chemolysis reaction as opposed to a standard pyrolysis reaction, distinguishing the technique from Py-GCMS (de Leeuw and Largeau, 1993; Challinor, 2001).

The technique has been successfully used to characterise lignin-derived phenolic compounds in plant litter and soils (Mason et al., 2012), as well as *Sphagnum*-derived compounds in peat (Abbott et al., 2013). However, conventional TMAH thermochemolysis is unable to distinguish between methyl groups originally present, and those added during the THM process, meaning that tannin- and demethylated lignin-derived phenols can contribute to the intact lignin signal. In order to distinguish between original and added methyl groups, ^{13}C-labelled TMAH is used (Filley et al., 2006; Fig. 1.2).

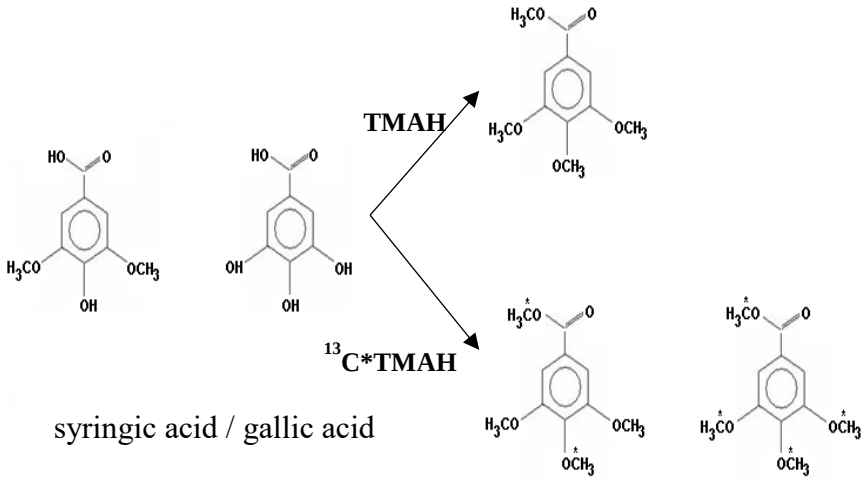

Fig. 1.2. ^{13}C-labelled/unlabelled TMAH thermochemolysis comparison.

1.5. Biomarkers and Proxies

1.5.1. Peat humification, testate amoebae and plant macrofossils

Ombrotrophic peat deposits can contain organic material thousands of years old (Rydin and Jeglum, 2013). They are also known to respond to changes in environmental conditions, and a record of these changes can be obtained from proxies preserved in the peat, such as plant macrofossils, degree of peat humification, and testate amoebae. Observation of plant macrofossils can be used to reconstruct past surface wetness conditions and water table levels by assessing vegetation composition, with, for example, hummock forming *Sphagnum* species and ericaceous shrubs proliferating in drier conditions. The degree of peat humification can give an insight into water table processes in the acrotelm, with greater humification evident during periods of drier conditions, allowing degree of humification to be used to infer environmental conditions during peat accumulation. Abundances of testate amoebae, a group of moisture sensitive ameboid protists, can also be used to reconstruct surface wetness levels (Chambers et al., 2012). Vegetation has been shown to correlate to peatland hydrology (Blackford et al., 2000), however given that use of plant macrofossils as an indicator of vegetation cover can be limited by the degree of peat humification, with extensive degradation of macrofossils making them visually unrecognisable, it can be necessary to use the chemical makeup of the plant, in the form of plant-specific molecular markers to reconstruct past vegetation composition (Schellekens et al., 2015a).

The existing range of peat biomarkers is relatively limited, and predominantly confined to the extractable lipid fraction (Dehmer, 1995; Pancost et al., 2002; Nichols, 2010). In order to accurately reconstruct past vegetation cover using a biomarker approach, it is important that the markers accurately reflect individual plant species or, more commonly, families or genera, such as vascular plants, *Sphagnum* mosses and other non-*Sphagnum* bryophytes. Non plant-specific biomarkers, such as distribution of *n*-alkanes, can be influenced by decomposition of the peat material, leading to potential inaccuracies in the interpretation of the bog hydrology due to varying inputs from different tissue types from an individual species (Pancost et al., 2002), selective preservation of higher molecular weight compounds (Huang et al., 2012), and alteration of other lipids during humification (Andersson and Meijers, 2012).

1.5.2. Py-GCMS and TMAH thermochemolysis-derived markers and proxies

The use of pyrolysis gas chromatography mass spectrometry (Py-GCMS) as an analytical tool for the study of organic material in peat at a molecular level is well established (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; Gonzalez et al., 2003). Established molecular markers from the non-extractable fraction of peat include lignin phenols as an indicator of vascular plant input (Tsutsuki et al., 1994; Williams et al., 1998; Bourdon et al., 2000), and the *Sphagnum*-specific 4-isopropenylphenol from sphagnum acid, which has been proposed as a *Sphagnum*-specific marker from Py-GCMS (Schellekens et al., 2009, 2015b; McClymont et al., 2011) and, in its methylated form, from thermally assisted hydrolysis and methylation in the presence of tetramethylammonium hydroxide (TMAH thermochemolysis; van der Heijden et al., 1997; Abbott et al., 2013; Swain and Abbott, 2013).

van der Heijden et al. (1997) identified six thermochemolysis products of sphagnum acid, including 4-isopropenylphenol, which, using flash pyrolysis without TMAH, has been proposed as a marker for *Sphagnum* inputs to bulk peat deposits (McClymont et al., 2011). Abbott et al. (2013) and Swain & Abbott (2013) quantified compounds **I** (methylated 4-isopropenylphenol (IUPAC: 1-methoxy-4-(prop-1-en-2-yl)benzene)), **IIa** and **IIb** (methylated cis- and trans-3-(4´-hydroxyphen-1-yl)but-2-enoic acid (IUPAC: (E/Z)-methyl 3-(4-methoxyphenyl)but-2-enoate)), and **III** (methylated 3-(4´-hydroxyphen-1-yl)but-3-enoic acid (IUPAC: methyl 3-(4-methoxyphenyl)but-3-enoate)) as the dominant TMAH thermochemolysis products of sphagnum acid, and proposed these as markers for *Sphagnum* input to peat deposits. These products were observed down eight peat cores from hummocks and hollows along four stages of the Ryggmossen peatland, Sweden. A *Sphagnum* yield parameter (σ) was proposed, using the sum of the individual amounts of the four *Sphagnum*-derived phenols, normalised to 100 mg of OC (Abbott et al., 2013).

$$\sigma \text{ (mg/100mg OC)} = [\mathbf{I}] + [\mathbf{IIa}] + [\mathbf{IIb}] + [\mathbf{III}]$$

Using the parameter for total amount of lignin-derived phenols (Λ), obtained from the sum of the individual amounts of guaiacyl, syringyl and cinnamyl moieties G4, G5, G6, G18, S4, S5, S6, and P18, normalised to 100 mg OC (Hedges and Parker, 1976; Hedges and Mann, 1979), a measure of the relative contribution of *Sphagnum*-derived phenols to vascular plant-derived phenols, known as the *Sphagnum* ratio (SR%) was developed (Abbott et al., 2013).

$$SR\% = [\sigma / (\sigma + \Lambda)] * 100$$

The efficacy of a plant- or taxon-specific biomarker is dependent on it being derived from a single source. It is therefore important to determine the origin of any newly identified peat biomarkers before they can be used as effective proxies. Thermally assisted hydrolysis and methylation in the presence of ^{13}C-labelled tetramethylammonium hydroxide (^{13}C-TMAH thermochemolysis) has been used to effectively elucidate the source of lignin-derived phenols in mineral soils (Filley et al., 2006; Mason et al., 2009), as well as *Sphagnum*-specific markers in peat deposits (Abbott et al., 2013).

1.6. Aims and Objectives of this thesis

The project aims to find and thoroughly assess molecular proxies for identifying and quantifying inputs of both *Sphagnum* and vascular plants into SOM so that the sensitivity of different plant inputs to climate-induced changes on subsurface processes can be assessed. The project was subdivided into sections with specific aims as follows:

1) Assessment of the specificity and viability of existing sphagnum acid markers using ^{13}C-labelled TMAH thermochemolysis on a suite of *Sphagnum* tissue samples and *Sphagnum* based peat samples from a range of geographic locations (chapter 3).

2) Identification of novel plant-specific biomarkers from existing peat deposits and assessment of their specificity and viability using ^{13}C-labelled TMAH thermochemolysis (chapter 4).

3) Study of peat deposits subjected to a simulated climatic warming experiment, with a focus on i) The effect of the warming experiment on developed peatland proxies and ii) the interaction and relationship between TMAH thermochemolysis-derived proxies and brGDGT based proxies (chapter 5).

Chapter 2 - Methods

2.1. Standard laboratory procedures

Laboratory grade solvents, dichloromethane (DCM) and methanol (MeOH) were supplied by Leading Solvent Supplies Ltd. (UK) and distilled on a 50 plate Oldershaw column to >99% purity. Glassware for general laboratory use was cleaned in a laboratory dishwasher (Miele 7783CD) with Lancerclean and Lancerinse (Lancer Ltd., UK) before being rinsed in 15 MΩ.cm deionised water and dried in an oven at 60 °C. Repeated rinses with DCM then took place before a final three washes in the solvent or other substance to be used. Glassware used in the synthesis of ^{13}C-labelled tetramethylammonium hydroxide (TMAH) was also cleaned in a solution of chromic acid prior to the standard cleaning procedure.

2.2. Synthesis of ^{13}C labelled tetramethylammonium hydroxide (^{13}C-TMAH)

^{13}C-labelled tetramethylammonium hydroxide (^{13}C-TMAH) was synthesized using a method adapted from Filley et al (1999) (Fig. 2.1). Following method development with unlabelled TMAH synthesis, the vacuum filtration step from Filley et al. (1999) was replaced with washing of the precipitated tetramethylammonium iodide (TMAI) crystals with cold methanol (MeOH) and subsequent drying by evaporation of excess solvent under nitrogen (N_2) flow. This increased yield significantly as losses from TMAI crystals remaining in the filter and filter paper were eliminated.

The synthesis of ^{13}C-labelled TMAH was achieved over two defined steps. In the first step, ammonium hydroxide is methylated with labelled methyl iodide to give ^{13}C tetramethylammonium iodide (TMAI). A pre-weighed conical flask with a mechanical stirrer was placed in an ice bath, and 2 g (14 mmol) ^{13}C methyl iodide was added with a glass syringe. 0.24 ml (3.6 mmol) ammonium hydroxide and 6 ml (115 mmol) acetonitrile were then added, before the addition of 4.8 ml (14 mmol) 3 M sodium hydroxide (NaOH), dropwise over a period of 5 minutes. The conical flask was subsequently removed from the ice bath, covered with aluminium foil, and left to stir gently for 72 hours at room temperature. After this, the flask was placed in a fridge for 24 hours to allow any remaining ^{13}C-TMAI crystals to precipitate.

To recover the ^{13}C-TMAI crystals, the supernatant was gradually syringed off to leave the crystals in the conical flask. Silica wool was added to the neck of the flask, and the crystals were washed with MeOH by adding 2 ml MeOH and leaving to evaporate under N_2 flow for 30 minutes. This step was repeated five times. Any remaining MeOH was then evaporated

under N_2 flow, the flask was re-weighed, then left overnight at room temperature and weighed again. The ^{13}C-TMAI crystals were then recovered from the conical flask.

The second step involved the conversion of the labelled TMAI to TMAH by a reaction with silver oxide (Ag_2O). Silver nitrate ($AgNO_3$) was measured out in a 2:1 molar ratio to the existing ^{13}C TMAI and dissolved in 20 ml degassed H_2O in a 3-necked round bottom flask (3RB), attached to another 3RB via a glass fritted tube, under constant N_2 flow. 10 ml concentrated NaOH solution was added dropwise to produce silver oxide (Ag_2O) precipitate until Ag_2O no longer precipitated then to excess, and the solution was then stirred for 40 minutes. Distilled H_2O was then added and removed 10 ml at a time with a glass syringe, and the pH tested with indicator paper until the effluent was neutral (pH 7). The ^{13}C-TMAI crystals were then dissolved in 22 ml hot (60-70 °C) distilled H_2O, and added dropwise to the 3RB, then left to stir for 3 hours. After this period, the apparatus was inverted, and the ^{13}C-TMAH solution passed through the glass frit between the two 3RB, filtering out the AgI precipitate. The ^{13}C-TMAH solution collected in the second 3RB, and was syringed into a Teflon centrifuge tube. This was then centrifuged at 4000 rpm for 2 minutes, repeated twice, to remove any remaining solid AgO. The remaining solution was then freeze dried using a Thermo Scientific Modulyo D 230 freeze dryer (Thermo Fisher Scientific, USA) to give white ^{13}C-TMAH crystals. These were then made up into a 25% w/w aqueous solution with distilled H_2O. Yield from the successful synthesis was 0.4073g TMAH, or 20.4% of theoretical yield. Attempts using the vacuum filtration method on the TMAI had resulted in yields as low as 5%. In total, five attempts were made at the synthetic process during the method development phase, followed by one unsuccessful run with ^{13}C-labelled components, before the successful synthesis was achieved, amounting to several months of laboratory work.

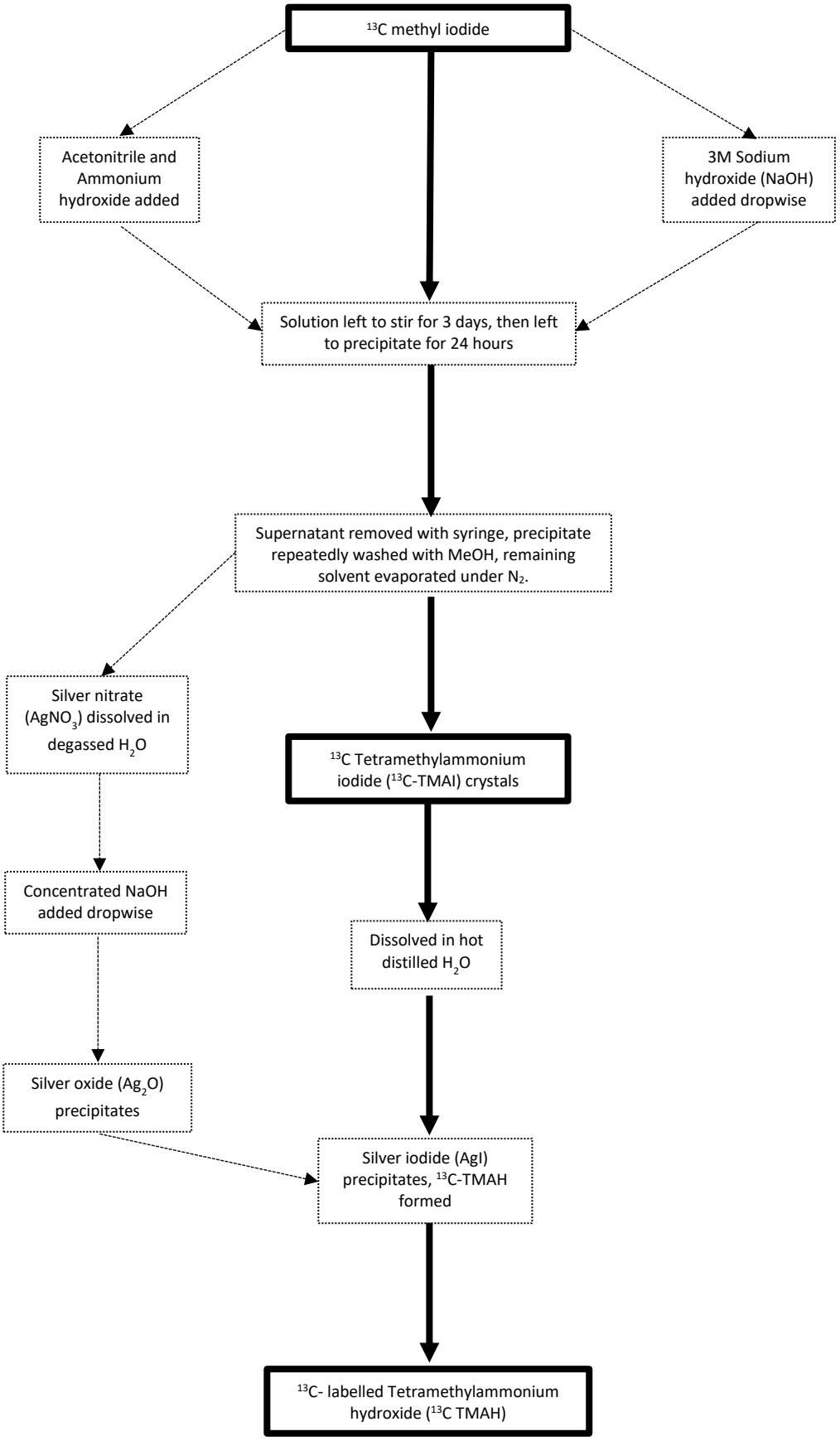

Fig. 2.1. Flow chart of ^{13}C-TMAH synthesis

The synthesized ^{13}C-TMAH crystallizes as tetramethylammonium hydroxide pentahydrate ($(CH_3)_4N(OH) \cdot 5H_2O$). This was analysed with proton and ^{13}C NMR and found to be clean, with the ^{13}C showing coupling to nitrogen (Fig. 2.2) and the proton NMR spectrum exhibiting two peaks, due to the methyl groups and water, suggesting that the synthesized TMAH did indeed crystallise as TMAH pentahydrate (Fig. 2.3). Proton NMR shows successful labelling of the compound with ^{13}C. The methyl protons appear as a doublet of quartets with a shift of 3.0 ppm (1JCH = 144.5 Hz, 3JCH = 3.3 Hz). The split into a large doublet (144.5 Hz) indicates a large one bond ^{13}C-H coupling, which confirms that the carbons are labelled. The further splitting into quartets is caused by the 3 bond ^{13}C-H coupling from the adjacent carbons (Corinne Wills, personal communication).

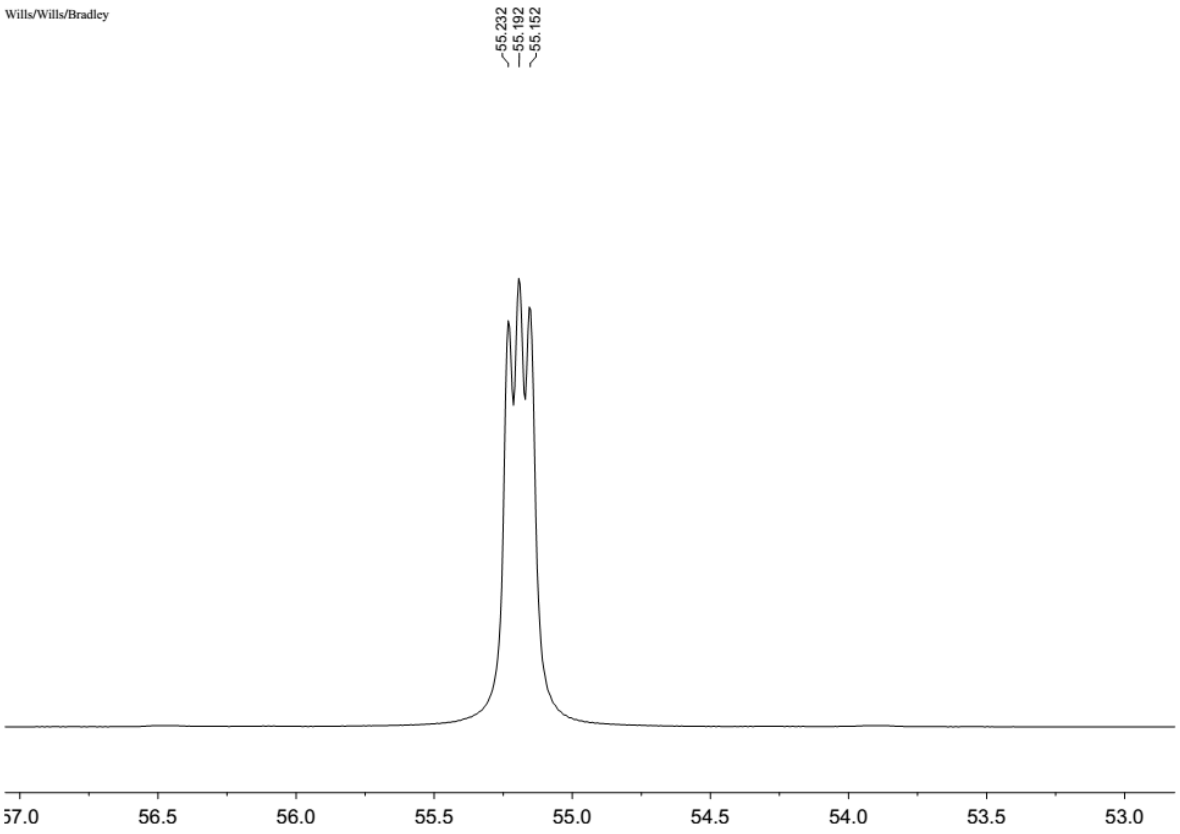

Fig. 2.2. ^{13}C NMR spectrum for synthesized ^{13}C-TMAH.

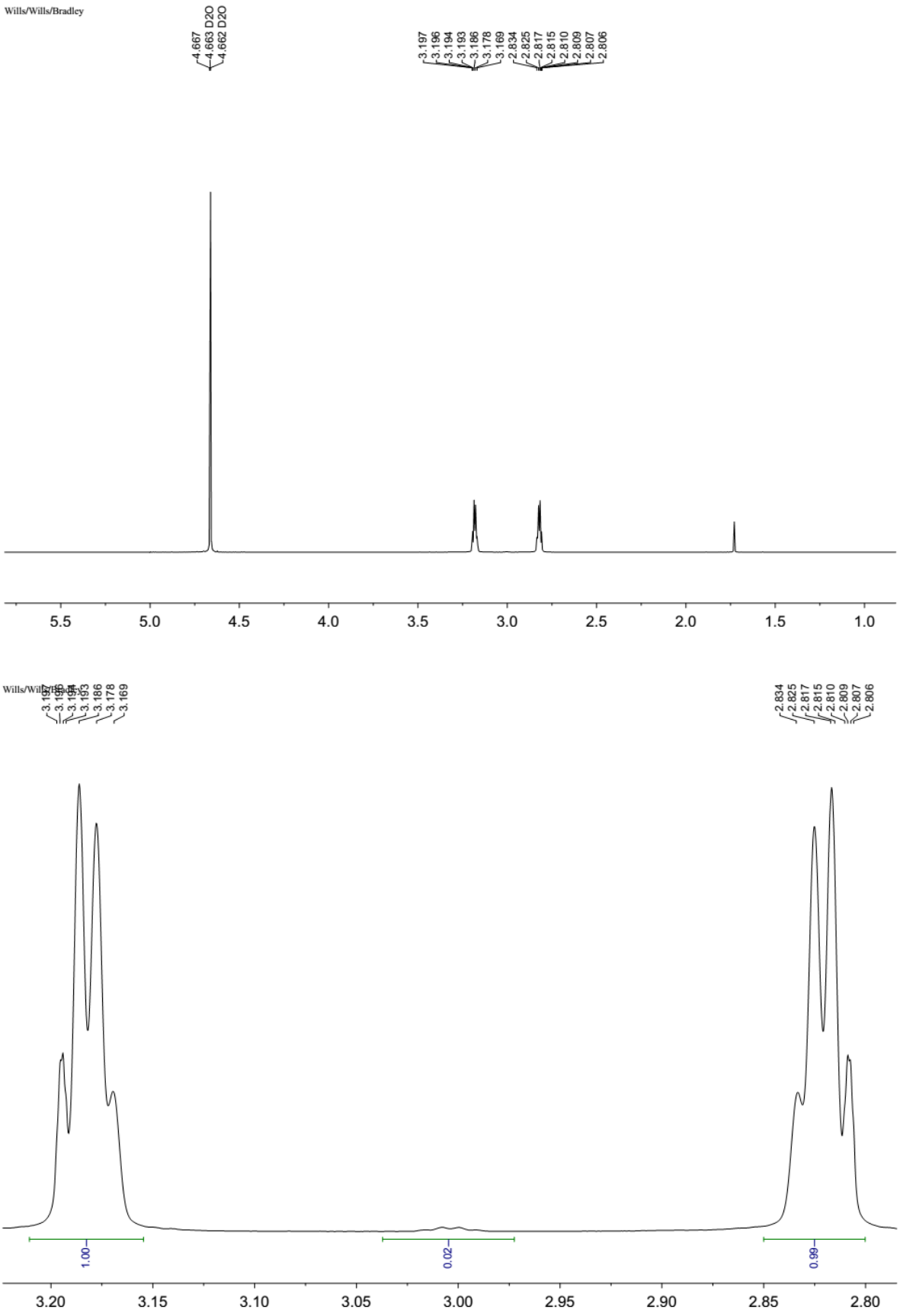

Fig. 2.3. Proton NMR for synthesized ^{13}C-TMAH

	% C	% H	% N
Result	30.66	11.82	7.81
Expected	26.5	12.8	7.7

Table. 2.1. Elemental analysis of synthesized ^{13}C-TMAH pentahydrate $(CH_3)_4N(OH) \bullet 5H_2O$)

Despite the NMR spectra indicating a clean product, the elemental analysis showed results slightly outside the normal acceptable limit for purity (expected results for TMAH pentahydrate are 26.51% C, 12.79% H, 7.73% N) (Table 2.1). This suggests that the product is not entirely pure, and may contain a small amount of contaminant. In order to test this, the product was run with an authentic standard of syringic acid (Fig. 2.4) alongside certified TMAH purchased from Sigma Aldrich, using the method of on-line thermally assisted hydrolysis and methylation (THM) in the presence of TMAH (TMAH thermochemolysis) (Fig. 2.5, 2.6).

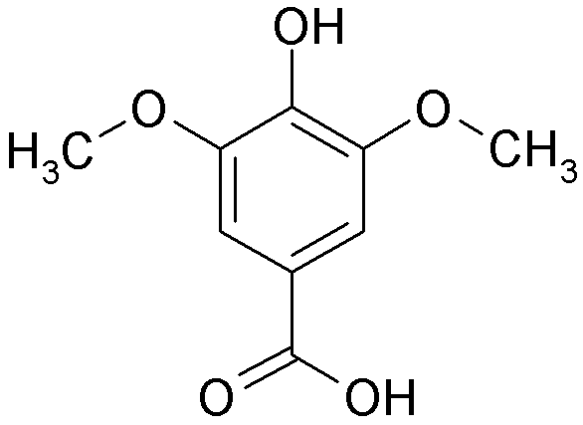

Fig. 2.4. Chemical structure of syringic acid.

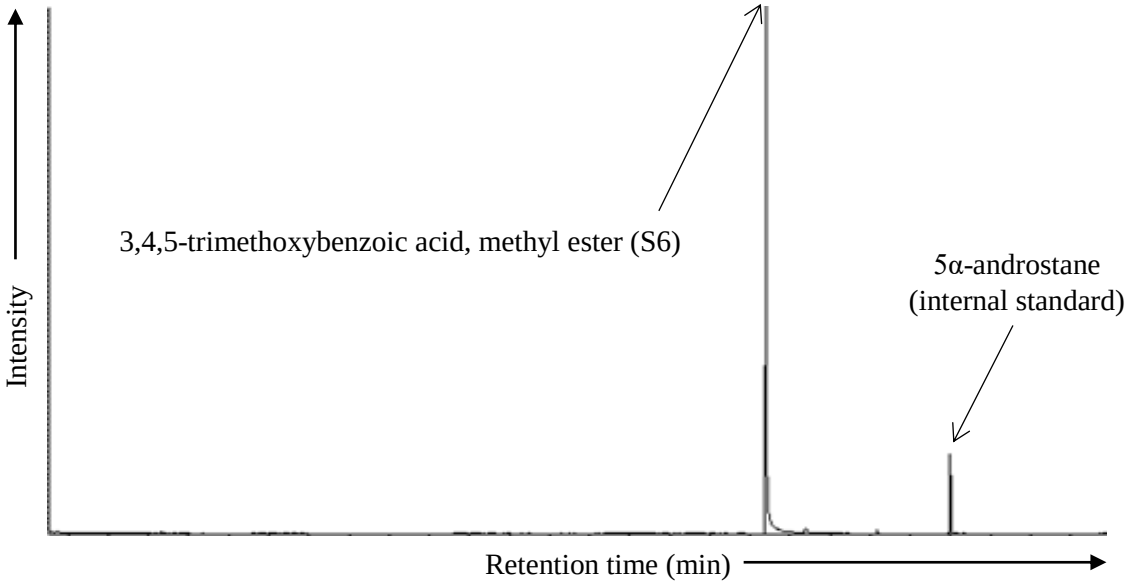

Fig. 2.5a. Partial chromatogram for the total ion current (TIC) of the TMAH thermochemolysis products from 10 µl Syringic acid in DCM (1 mg/ml) + 5 µl standard + 10 µl TMAH (Sigma Aldrich).

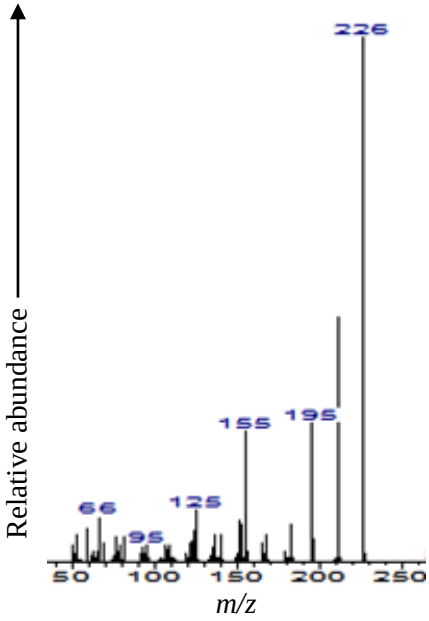

Fig. 2.5b. Mass spectrum for 3,4,5-trimethoxybenzoic acid, methyl ester (S6).

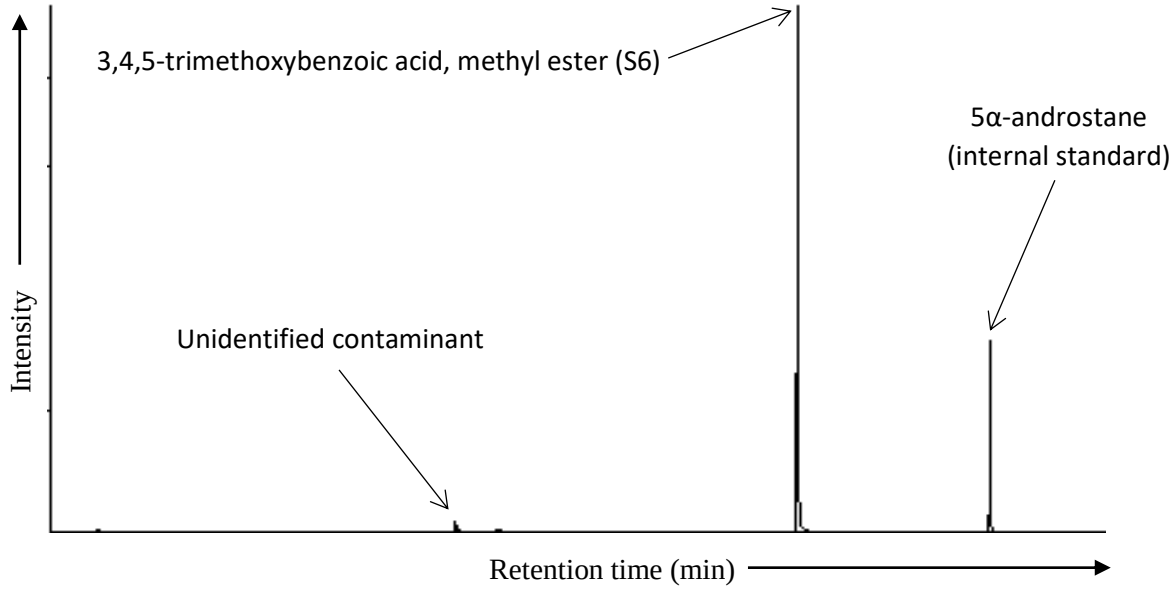

Fig. 2.6a. Partial chromatogram for the TIC of the TMAH thermochemolysis products from 10 µl Syringic acid in DCM (1 mg/ml) + 5 µl standard + 5 µl ^{13}C-TMAH.

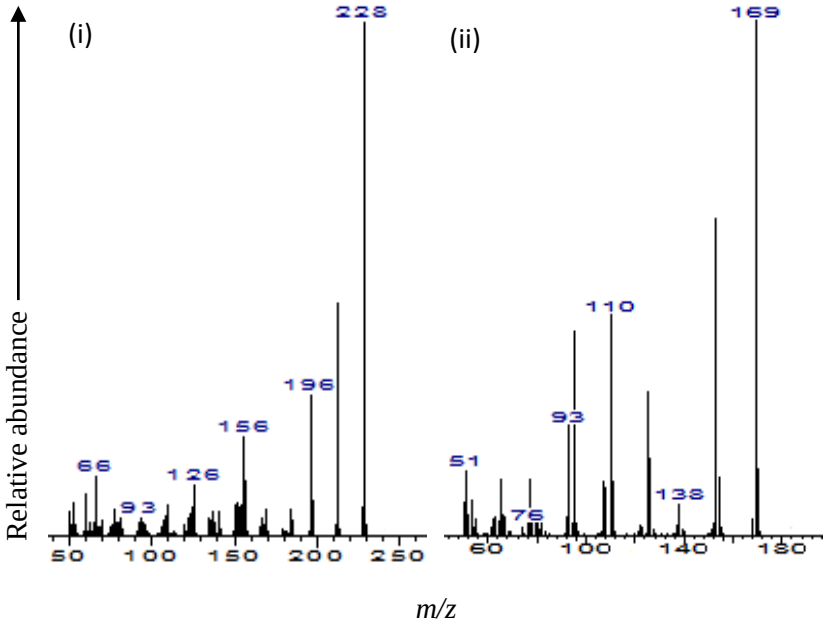

Fig. 2.6b. Mass spectrum for thermochemolysis products observed from ^{13}C-TMAH thermochemolysis of Syringic acid: (i) ^{13}C-labelled 3,4,5-trimethoxybenzoic acid, methyl ester (S6), (ii) unidentified contaminant.

A small amount of an unidentified contaminant with molecular ion 169 was observed at 48 minutes on the TIC of syringic acid with ^{13}C-labelled TMAH (Fig. 2.6a, 2.6b). However, due

to the very small amount present and the fact that the elution time and mass spectrum do not interfere with any observed lignin, *Sphagnum*, carbohydrate or lipid thermochemolysis products, the ^{13}C-TMAH can be considered acceptable for use, as indicated by Fig. 2.6b (i), which shows the increase in mass of the molecular ion of S6 by two units relative to Fig. 2.5b, indicating that both added methyl groups are labelled with ^{13}C. It is also possible that the observed purity was affected by the product taking on H_2O in storage, as the contaminant appears to make up less than 5% of the sample.

2.3. Thermally assisted hydrolysis and methylation in the presence of ^{13}C-labelled and unlabelled tetramethylammonium hydroxide (TMAH)

On-line thermally assisted hydrolysis and methylation (THM) in the presence of ^{13}C-labelled and unlabelled tetramethylammonium hydroxide (TMAH) (TMAH thermochemolysis) was performed using a platinum filament coil probe pyrolysis unit (Chemical Data Systems, Oxford, USA). The Pyroprobe 5000 was coupled to a 6890 N GC and 5975B MSD GC/MS system from Agilent Technologies (Palo Alto, USA).

Approximately 1 mg of powdered extracted peat or vegetation sample was weighed into a quartz pyrolysis tube (CDS Analytical, Oxford PA, USA) plugged with pre-extracted silica wool. An internal standard, 5α-androstane, and an aqueous solution of unlabelled or ^{13}C-labelled TMAH (25% w/w) was added to the sample immediately prior to THM.

Pyrolysis was performed at 610 °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 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 5000 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). Compound identification was based on the NIST98 mass spectral library, on ion fragmentation patterns and following the conventions described in other studies (van der Heijden, 1994; Clifford et al., 1995; Hatcher et al., 1995; Filley et al., 2000; Mason et al., 2009; Abbott et al., 2013). GC program and THM method remained constant for all analyses unless stated otherwise.

2.3.1. Aromatic hydroxyl content

The original aromatic hydroxyl content of phenolic THM products was calculated by determining the number of ^{13}C-labelled methyl groups added during the ^{13}C-TMAH thermochemolysis process. During ^{13}C-TMAH thermochemolysis, hydroxyl functional groups are methylated by ^{13}C labelled methyl groups from the ^{13}C-TMAH. Therefore, the number of labelled methyl groups added is equivalent to the original amount of aromatic hydroxyl groups. Given that ^{13}C and ^{12}C have a difference in mass of one unit, the number of added methyl groups can be calculated by the difference in the mass of the molecular ion between thermochemolysis products obtained with unlabelled and ^{13}C-labelled TMAH.

Aromatic hydroxyl content was determined using methods set out in previous work (Filley et al., 1999; Filley et al., 2006; Mason et al., 2009), using baseline ion fragment ratios obtained from unlabelled TMAH thermochemolysis. Original % 3-methoxyl, 4-hydroxyl content of TMAH thermochemolysis products with 3,4-dimethoxy substitution (guaiacyl) and original relative amount of 1, 2, or 3 hydroxyls in products with 3,4,5-trimethoxy substitution (syringyl) was calculated using a series of equations (from Filley et al., 2006; Mason et al., 2009; appendix 1):

% Hydroxyl(G6, G7, G8, G14, G15, G18)

$$= 100 \text{ x } \left[\frac{(M_{L+1} - (M_{L+1})_{calc})}{M_L + M_{L+1} - (M_{L+1})_{calc}}\right]$$

% Hydroxl(G4)

$$= 100 \text{ x } \left[\frac{(M_{L+2})_{calc}}{(M_L)_{calc} + (M_{L+2})_{calc}}\right]$$

% 1 Hydroxyl(S6, S7, S8, S14, S15)

$$= 100 \text{ x } \left[\frac{(M_L)}{M_L + [M_{L+1} - (M_{L+1})_{calc}] + [M_{L+2} - (M_{L+2})_{calc}]}\right]$$

% 2 Hydroxyls(S6, S7, S8, S14, S15)

% 3 Hydroxyl

$$= 100 \text{ x } \left[\frac{M_{L+1} - (M_{L+1})_{calc}}{M_L + [M_{L+1} - (M_{L+1})_{calc}] + [M_{L+2} - (M_{L+2})_{calc}]}\right]$$

$$= 100 \text{ x } \left[\frac{M_{L+2} - (M_{L+2})_{calc}}{M_L + [M_{L+1} - (M_{L+1})_{calc}] + [M_{L+2} - (M_{L+2})_{calc}]}\right]$$

% 1 Hydroxyl(S4)

$$= 100 \text{ x } \left[\frac{(M_{L-1})}{(M_{L-1}) + [(M_{L+1})_{calc}] + [M_{L+2} - (M_{L+2})_{calc}]}\right]$$

% 2 Hydroxyls(S4)

$$= 100 \text{ x } \left[\frac{(M_{L+1})_{calc}}{(M_{L-1}) + [(M_{L+1})_{calc}] + [(M_{L+2})_{calc}]}\right]$$

% 3 Hydroxyls(S4)

$$= 100 \text{ x } \left[\frac{(M_{L+2})_{calc}}{(M_{L-1}) + [(M_{L+1})_{calc}] + [(M_{L+2})_{calc}]}\right]$$

(M_L) refers to the abundance of the molecular ion of the labelled fragment that contains the aryl methoxyl groups where methylation has taken place during TMAH thermochemolysis. (M_{L+1}) is the abundance of the corresponding fragment that has a mass of 1 unit greater than (M_L). Given that position 4 on the benzene ring in syringyl and guaiacyl structures is the principle site of the β-O-4 bond in lignin structure, there is no original methoxyl group present at this position, therefore it can be assumed that the methoxyl carbon atom at this point on the thermochemolysis product is always ^{13}C labelled.

The term N_L represents the number of C atoms in a ^{13}C-labelled methylated lignin monomer that contribute to (M_{L+1}) and (M_{UL+1}), or every ^{12}C atom in a ^{13}C-labelled product, whereas N_{UL} represents the contributing C atoms in an unlabelled product, or the total amount of C atoms in the fragment. (M_{UL+1}) is used to represent and account for natural presence of ^{13}C in organic material.

$(M_{L+1})_{calc}$ is the calculated intensity of (M_{L+1}), and is determined by multiplication of M_L by the ratio $(M_{UL+1})/M_{UL}$ derived from the equivalent unlabelled TMAH thermochemolysis product, followed by normalisation by multiplication with the ratio N_L/N_{UL}. In the case of products with 3,4,5-trimethoxy substitution, $(M_{L+2})_{calc}$ represents the calculated intensity of (M_{L+2}), the molecular ion of the labelled fragment with +2 mass units. The value of $(M_{L+2})_{calc}$ is determined by the multiplication of the difference between (M_{L+1}) and $(M_{L+1})_{calc}$ by the ratio $(M_{UL+1})/(M_{UL})$, then normalised by multiplication with the ratio $N_L/N_{UL.}$

The aldehyde group present on the benzene ring of G4 and S4 can also be methylated by TMAH thermochemolysis. In this case, the values of $(M_{L+1})_{calc}$ and $(M_{L+2})_{calc}$ can be obtained by subtracting the normalised contributions of (M_{L+3}) relative to (M_{L+2}) and (M_{L+2}) relative to (M_{L+1}) from the abundance of (M_{L+1}).

The equations relating to structures with dihydroxy substitution were applied to sphagnum acid-derived thermochemolysis products I, IIa, IIb, III, however all calculated % 2 hydroxyl group (sphagnum acid-derived) values were 100%, suggesting no alternative precursors to I, IIa, IIb or III are present in peat.

2.3.2. Determination of mass yields and proxies

Mass yields of individual compounds were calculated based on the relative responses of their total ion currents to the internal standard (5α-androstane), with an assumed relative response factor of 1. Yields derived from unlabelled TMAH thermochemolysis were adjusted using the calculated percentage aromatic hydroxyl content, in order to distinguish between intact lignin-derived compounds, and those derived from degraded lignin or non-lignin sources such as tannins (Filley et al., 2006).

Lambda (Λ) is calculated as the sum of the amounts of the eight dominant lignin-derived phenols (G4+G5+G6+S4+S5+S6+G18+P18) normalised to 100 mg OC (Hedges et al., 1982; Hedges and Mann, 1979; Kögel, 1986), and represents total lignin-derived inputs to SOM. The acid/aldehyde ratio (Ad/Al) records the state of oxidation for either guaiacyl lignin

components (G6/G4) or syringyl lignin components (S6/S4) and was determined as the sum of 3,4-dimethoxybenzoic acid methyl ester (G6) divided by 3,4-dimethoxybenzaldehyde (G4), or the sum of 3,4,5-trimethoxybenzoic acid methyl ester (S6) divided by 3,4,5-trimethoxybenzaldehyde (S4) (Filley et al., 2006).

Sigma (σ) is calculated as the sum of the amounts of four sphagnum acid-derived phenols (I+IIa+IIb+III) normalised to 100 mg OC (Abbott et al. 2013) and can be taken to represent *Sphagnum*-derived inputs to SOM.

2.4. Temperature calibration of pyroprobe

The pyroprobe used for TMAH thermochemolysis was calibrated according to Bashir (1999) to determine the exact temperature of pyrolysis within the quartz pyrolysis tubes. The pyroprobe was calibrated using inorganic salts of known melting temperatures (lead chloride, lithium chloride, potassium iodide, calcium chloride). The salts were placed in quartz tubes, mounted in the platinum coil of the pyroprobe as per the determined method, and inserted in to a brass heating block, which was maintained at a temperature of 340 °C. The helium carrier gas flow rate was maintained at 2-10 mL/min. A glass window allowed the melting of salts to be observed under the microscope. The salts were pyrolysed at defined increasing temperature intervals and the observed melting point was recorded as displayed on the pyroprobe control panel. The average melting temperature of the three replicates of each salt was plotted against the actual known melting points, and an equation to calculate the necessary input temperature was derived from this (Fig. 2.7).

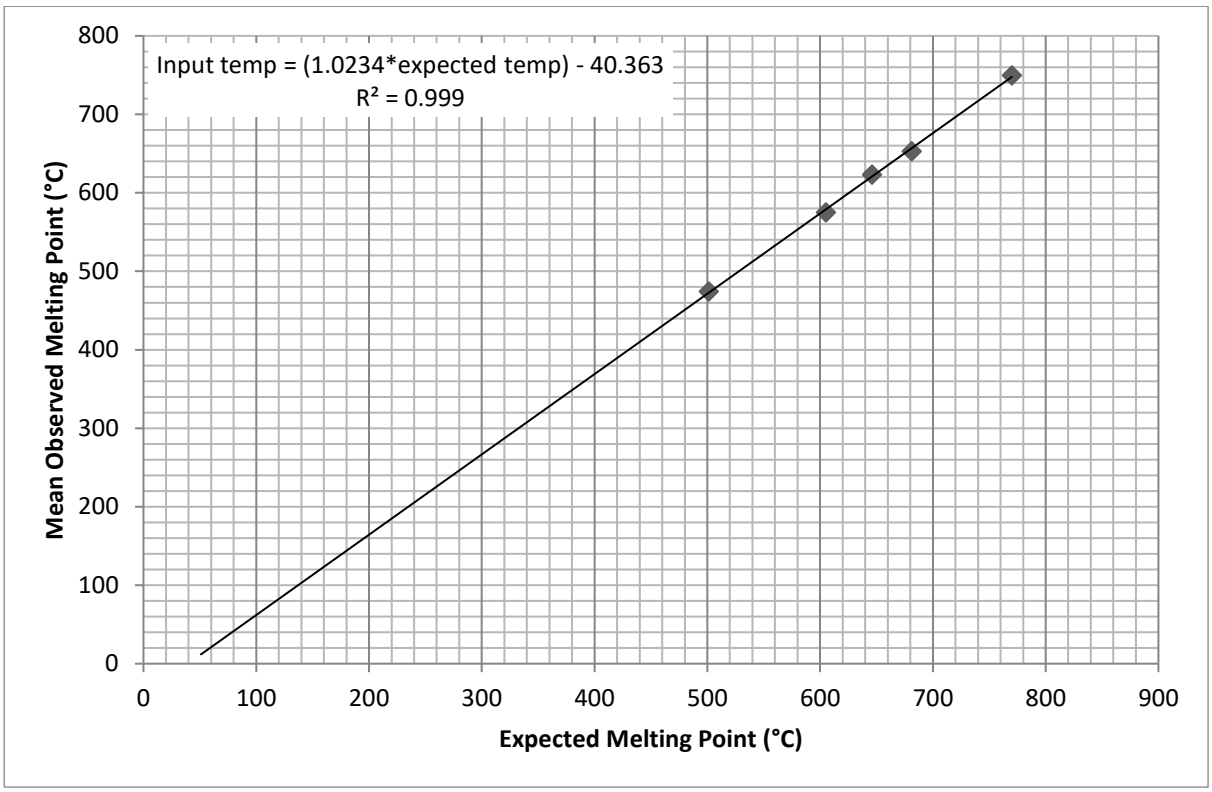

Fig. 2.7. Temperature calibration of pyroprobe.

Calibration of the pyroprobe determined that in order to achieve the desired pyrolysis temperature of 610 °C, a temperature of 584 °C should be input on the pyrolysis unit control panel.

2.5. Determination of total organic carbon (TOC)

Approximately 0.1 g of sample was weighed into a porous crucible, and hot (60-70 °C) 4 mol/L hydrochloric acid (HCl) was added dropwise to remove carbonates. Acid was then drained from the crucible, and the crucible rinsed with distilled H_2O to remove residual acid. The crucibles were then dried overnight at 65 °C, and the organic carbon content of the sample determined using a Leco CS230 Carbon - Sulphur analyser (Leco Corporation, USA).

2.6. Description of sampling sites and sample preparation

2.6.1. Ryggmossen peatland

2.6.1.1. Sampling area

Peatland vegetation samples and peat cores were collected from the Ryggmossen peatland, around 25km northwest of Uppsala, in the boreonemoral zone of central Sweden (60° 3′ N, 17° 20′ E; 60m above sea level; Rydin, 1993; Bragazza et al., 2003; Abbott et al., 2013).

Average precipitation in the area is 544 mm yr^{-1}, and mean annual air temperature (MAAT) is 5.6 °C. The raised bog has remained relatively undisturbed by human activities such as the construction of drainage ditches, and is a roughly circular domed shape with a diameter of around 800m (Fig. 2.8).

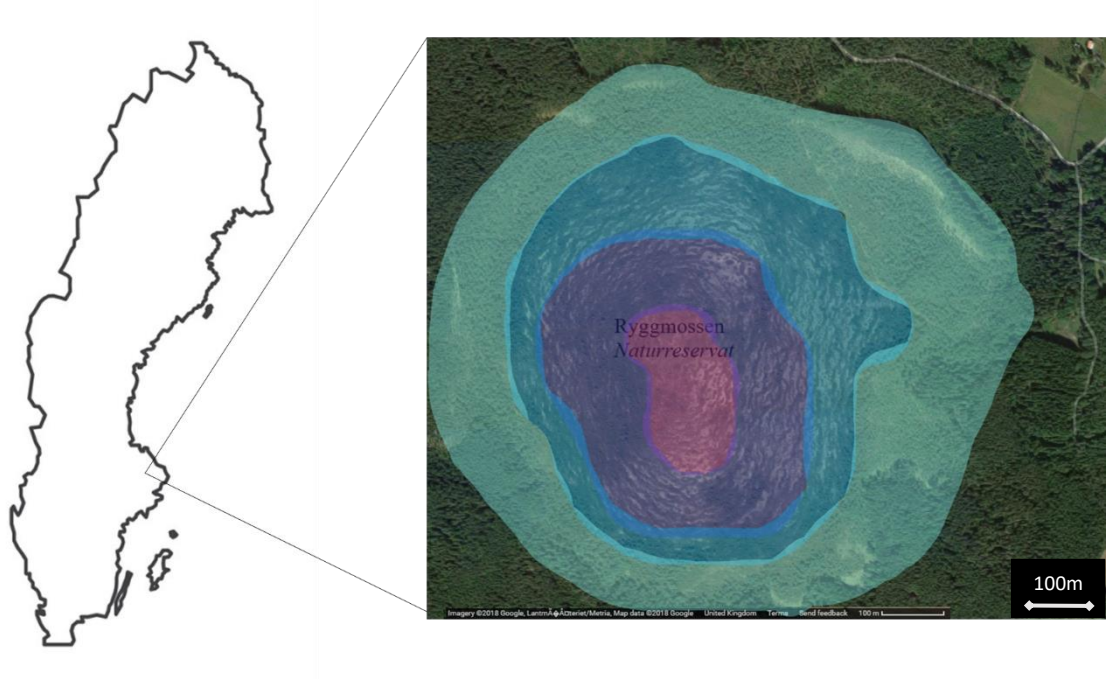

Fig. 2.8. Location and topographic position of Ryggmossen peatland. Coloured overlay corresponds to macrotopographic gradient stages in table 2.2.

The site has a well-developed hummock-lawn-hollow microtopography, with hummocks forming concentric circles along defined contours (Rydin, 1993). A macrotopographic gradient can be observed from the centre of the bog to the peatland-forest ecotone, showing increases in pH and mineral cations, as well as distinct vegetation succession and changes in water table regimes (Table. 2.2; Bragazza et al., 2003). Ground-layer vegetation surveys performed at the coring sites illustrate the differences in vegetation composition at each stage (Table 2.3). Samples were collected as part of a collaboration between Dr Geoffrey Abbott, Dr Eleanor Swain, Dr Aminu Muhammad (Newcastle University), Dr Lisa Belyea, Dr Kathryn Allton, Dr Chris Laing (Queen Mary University, London), and Dr Greg Cowie (Edinburgh University). Peatland vegetation species identification was performed by Prof. Håkan Rydin and Dr Gustaf Granath (Uppsala University, Sweden).

Stage		Description
BP	Bog Plateau	Raised ombrogenous bog dominated by dry *Sphagnum fuscum* hummocks and wet *Sphagnum balticum* hollows.
BM	Bog Margin	Well-drained ombrogenous bog with sparse *Pinus sylvestris* tree cover and ericaceous shrub understory (*Calluna vulgaris*). *Sphagnum fuscum* hummocks and *Sphagnum balticum* lawns.
FL	Fen Lagg	Geogenous peatland with a range of peatland vegetation including graminoids (*Carex lasiocarpa, Phragmites australis*) and peat-forming mosses (*Sphagnum majus, Sphagnum fallax*). *Sphagnum fallax* hollows interspersed with *Sphagnum fuscum* hummocks.
SF	Swamp Forest	Seasonally flooded mixed woodland (*Pinus sylvestris, Betula pubescens*) with an understory of shrubs (*Salix aurita, Juniperus communis*), non-*Sphagnum* bryophyte (*Polytrichum commune*) hummocks and wet *Sphagnum angustifolium* hollows.

Table 2.2. Description of Ryggmossen macrotopographic gradient stages. Colours correspond to overlay on Fig. 2.8.

Species	**%**	**Species**	**%**
BP Hummock		BP Hollow	
S. fuscum	57.5	*S. balticum*	71
Calluna vulgaris	30	*Eriophorum vaginatum*	25
Rubus chamaemorus	5	*Andromeda polifolia*	3
Vaccinium microcarpum	5	*Vaccinium microcarpum*	1
Andromeda polifolia	2		
Empetrum nigrum	1		
BM Hummock		BM Hollow	
S. fuscum	67	*S. angustifolium*	65
Calluna vulgaris	25	*Rubus chamaemorus*	26
Empetrum nigrum	2	*Calluna vulgaris*	20
Vaccinium microcarpum	3		
S. magellanicum	3		
FL Hummock		FL Hollow	
S. fuscum	47	*S. fallax*	88
Empetrum nigrum	30	*Andromeda polifolia*	10
Polytrichum strictum	15	*Vaccinium microcarpum*	1
Andromeda polifolia	3	*Eriophorum vaginatum*	1
Calluna vulgaris	2		
Drosera rotundifolia	1		
Vaccinium microcarpum	1		
Polytrichum commune	1		
SF Hummock		SF Hollow	
Polytrichum commune	91	*S. angustifolium*	95
S. angustifolium	6	*Vaccinium uliginosum*	4
Vaccinium uliginosum	3	*Carex* sp.	1

Table 2.3. Ground-layer vegetation percentage of area covered at each coring site. BM and SF *Pinus sylvestris* canopy not recorded (Abbott et al., 2013).

2.6.1.2. Sample collection and preparation

Peat cores were collected from a hummock and a hollow at each stage along the macrotopographic gradient (BP, BM, FL, SF). A core with a diameter of 20 cm and a length of 30-50 cm was collected by cutting the peat around a PVC pipe and inserting the pipe into the peat, repeating this process to as great a depth as possible. The intact pipe was then dug out, sealed, and transported in an upright position. Cores were initially stored at 4 °C, then frozen at -18 °C, before being removed from the pipe and cut into sections of 2 cm thickness using a water lubricated bandsaw. Samples were then taken from these sections using a power drill with a holesaw bit (2.9 cm diameter).

Living material from a variety of vegetation species was collected from a field monitoring site in April 2008. Material from eight vegetation species including *Sphagnum* mosses, non-*Sphagnum* bryophytes and vascular plants (Table 2.4), as well as surficial peat sections from a BP hummock and hollow were selected for analysis. Samples were freeze dried using a Thermo Scientific Modulyo D 230 freeze dryer (Thermo Fisher Scientific, USA), and milled using a SPEX CertiPrep 6750 freezer mill (SPEX SamplePrep, USA), before being further ground into a fine powder using an agate mortar and pestle, and stored in glass vials. Samples were initially ultrasonically extracted in a 50 mL Teflon centrifuge tube using a mixture of DCM and MeOH (1:1, v/v) at room temperature for 2 h. The tube was then centrifuged at 12,000 rpm for 15 minutes in a Sorvall RC5B Plus centrifuge (Thermo Fisher Scientific, USA) and the supernatant was transferred into a second centrifuge tube. Following addition of another 20 mL of the DCM/MeOH solvent to the residue in the first tube, the above procedure was repeated. Following transfer of the supernatant liquid to the other centrifuge tube, the residue was dried at 60 ˚C overnight before being analysed in triplicate with unlabelled TMAH and once with ^{13}C-TMAH.

Vegetation type	Species
Sphagnum mosses	*Sphagnum capillifolium* *Sphagnum fuscum* *Sphagnum angustifolium*
Non-*Sphagnum* bryophytes	*Eriophorum vaginatum* *Polytrichum commune* *Pleurozium schreberi*
Vascular plants	*Rhododendron tomentosum* *Vaccinium microcarpum*

Table 2.4. Selected Ryggmossen vegetation species.

2.6.2. Frasne peatland

2.6.2.1. Sampling Area

Peat cores were collected from the Frasne peatland (46° 49′ N, 6° 10′ E, 800 m above sea level; Delarue et al., 2011; Huguet et al., 2013), a *Sphagnum*-dominated mire located in the Jura Mountains in Eastern France (Fig. 2.9). The site has been classified as a Region Natural Reserve for over 20 years, and is protected under the EU Habitat Directive, and is therefore subject to little human interference and remains in a relatively undisturbed condition. MAAT is 6.2 °C, with a winter average of -1.4 °C and a summer average of 14.6 °C. Annual precipitation varies from 1300-1500 mm yr^{-1}.

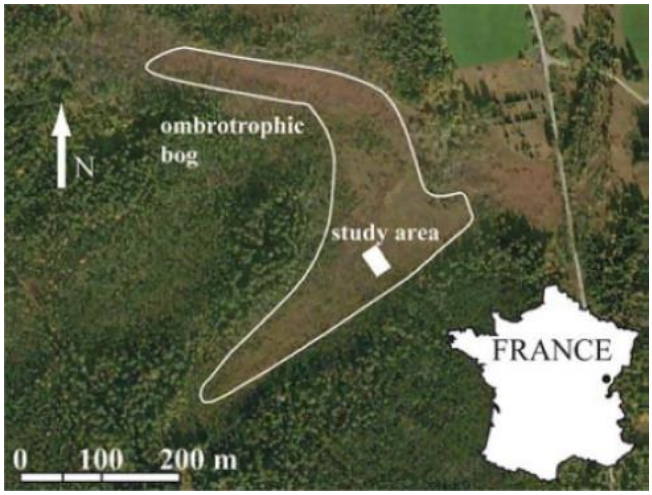

Fig. 2.9. Location and topographic position of Frasne peatland (Huguet et al., 2013).

The study site can be divided into two distinct adjacent sections based on a trophic gradient inferred from vegetation composition and hydrology: the ‘wet’ fen site and the ‘dry’ bog site (Table 2.5; Delarue et al., 2011). The mean water table level, measured between 2009 and 2010, was at 14 cm depth at the fen site, and 16.5 cm depth at the bog site.

Stage	Description
'Wet' Fen	*Sphagnum*-dominated nutrient poor fen with a semi-homogenous lawn-hollow microtopography. *Sphagnum fallax* dominant, some vascular plants present in very low abundances (*Eriophorum vaginatum, Vaccinum oxycoccus, Andromeda polifolia*).
'Dry' Bog	*Sphagnum*-dominated bog with hummock-lawn-hollow microtopography. Hummocks dominated by *S. magellanicum*, with *V. oxycoccus*, *E. vaginatum* and *Calluna vulgaris* also present. Hollows and lawns characterised by *S. fallax*, *Carex rostrate* and *A. polifolia.*

Table 2.5. Descriptions of Frasne trophic and wetness gradient stages.

2.6.2.2. Experimental Design

An experimental in-situ warming experiment was installed at the site in May 2008, undertaken within the framework of the French Agence Nationale de la Recherche project PEATWARM. Six plots were selected at each site and three were randomly allocated the warming treatment, with three remaining as controls. Warming plots were then fitted with polycarbonate open topped chambers (OTCs) in order to induce passive warming. The OTCs are of trapezoidal shape and 50 cm high, 1.7 m across at the top, and 2.4 m wide at the base (Fig. 2.10). Surficial peat (7 cm depth) temperature and air (10 cm above vegetation) temperature were automatically monitored every 30 minutes using thermocouple probes and a Campbell CR-1000 data logger (Campbell Scientific, UK), starting from November 2008 and July 2009, respectively.

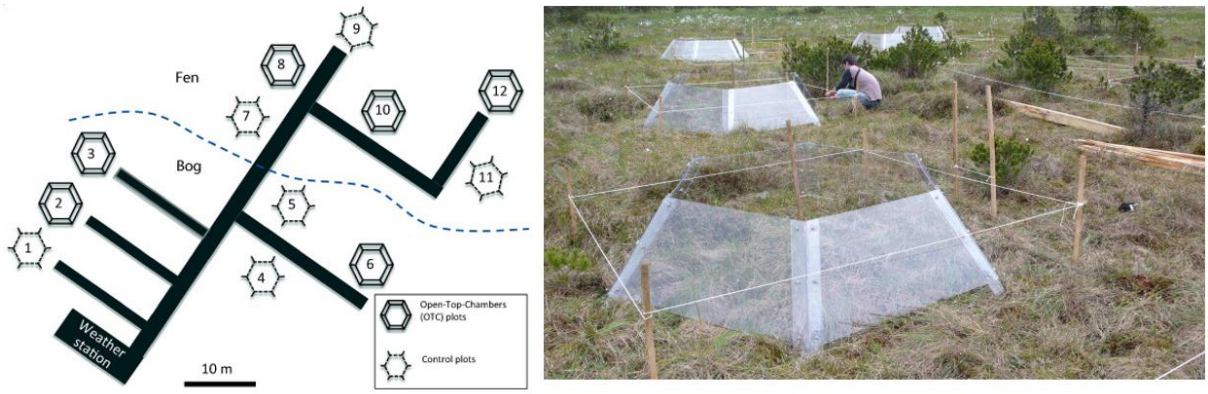

Fig. 2.10. Sampling plot locations and OTC setup at Frasne peatland (Huguet et al., 2013).

2.6.2.3. Sample collection and preparation

Peat cores were collected in June 2010, after 26 months of OTC treatment, and again in June 2013. After collection of cores, the living *Sphagnum* material comprising the first 5 cm of the core (0-5 cm depth) was discarded. Cores were then frozen at -18 °C and cut into 3 sections: 5-7 cm, 7-12 cm and 12-17 cm. These sections were then freeze dried, powdered with an agate mortar and pestle and homogenised. Samples were extracted for lipid analysis using a modified Bligh and Dyer technique (Huguet et al., 2013), and the residues analysed in triplicate with unlabelled TMAH and once with ^{13}C-TMAH.

2.6.3. Rödmossamyran peatland

2.6.3.1. Sampling Area

The Rödmossamyran peatland is an oligotrophic fen located near Umeå in northern Sweden (63° 47′ N, 20° 20′ E; 40 m above sea level; Rydberg et al. 2010; Fig. 2.11). The site vegetation cover consists primarily of thinly spaced pine trees (*Pinus sylvestris*) over a field layer of shrubs and mosses. The central southern part of the site comprises an open lawn of around 0.25 ha, predominantly covered by various *Sphagnum* species (Rydberg et al., 2010; Schellekens et al., 2015b; Fig. 2.11). MAAT for the Umeå region is 3.5 °C, with around 650 mm total annual precipitation.

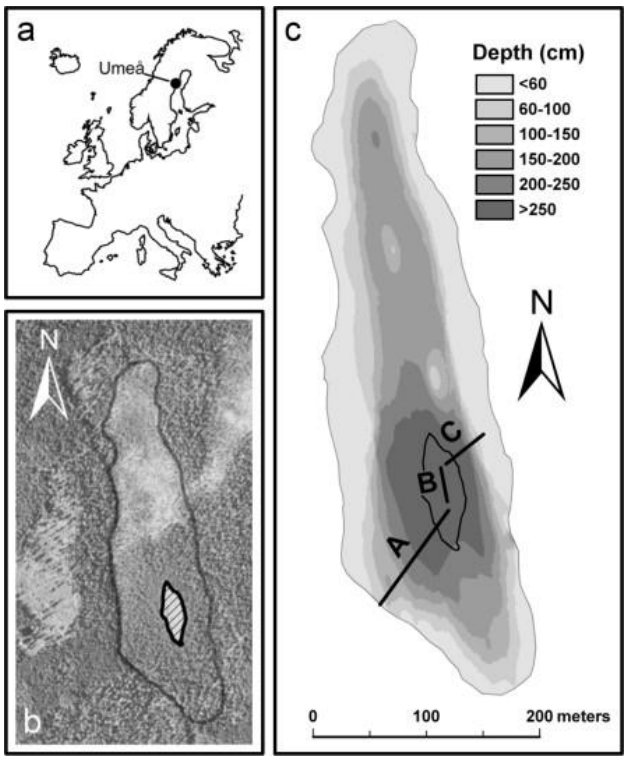

Fig. 2.11. a) Location of Rödmossamyran peatland in northern Sweden. b) Aerial photograph showing area of pine-covered fen (outer line) and open *Sphagnum* area (hatched area). c) Map showing transects where peat (A) and vegetation (A-C) samples were collected. From Rydberg et al. (2010).

2.6.3.2. Sample collection and preparation

One *Sphagnum* species (*S. magellanicum*), one grass (*E. vaginatum*) and one surficial peat sample from Rödmossamyran were selected for analysis. Peat cores were collected in autumn 2009 along a southwest transect (transect A, Fig. 2.11c). Cores were taken using a combination of a Wardenaar corer (Wardenaar, 1987) for the upper ~75 cm, followed by an overlapping (by ~20 cm) series of Russian peat cores 0.75-1 m in length. The cores were cut into 10 cm sections in the field and transported in polyethylene bags.

Vegetation samples were collected in September 2008 along a transect within the open *Sphagnum*-dominated area (transect B, Fig. 2.11c). Samples from several individual plants of the desired species within a 1 m^2 plot were combined to give one composite sample. Samples were taken by cutting the vegetation onto a clean weighing boat using stainless steel scissors, and transferring into a polyethylene bag. Samples were stored at -18 °C at Umeå University prior to transport and processing (Rydberg et al., 2010). Samples were processed and extracted using the methods outlined in section 2.6.1.2., before being analysed in triplicate with unlabelled TMAH and once with ^{13}C-TMAH.

2.6.4. Harberton peatland

2.6.4.1. Sampling area

The Harberton peatland (HRB) is a raised bog located at Puerto Harberton (54° 53′ S, 67° 20′ W; 20 m a.s.l.), Tierra del Fuego, Argentina. *Sphagnum magellanicum* is the dominant peat-forming plant, and is the main component of the upper 600 cm of peat (Schellekens et al., 2009).

2.6.4.2. Sample collection and preparation

Peat cores were taken with a 5 x 50 cm Russian (Macaulay) peat sampler. Samples were air dried, ground, homogenized and divided into sub-samples for analysis. Samples of the dominant individual plant species were taken from the bog surface, oven dried at 35 °C for 1 week, then ground and stored for analysis.

2.6.5. Pena da Cadela

2.6.5.1. Sampling area

Tremoal da Pena da Cadela (PDC; 43° 30′1 2″ N, 7° 30′ 00″ W) is an ombrotrophic peatland located in the Xistral Mountains, Spain. The bog microtopography consists of shallow hummocks and hollows (<10 cm in height). Mean annual air temperature is 7.5 °C, with a mean annual precipitation of 1800 mm. Vegetation cover is dominated by sedges (*Carex durieui, Carex panicea, Eleocharis multicaulis*), grasses (*Agrostis curtisii, Agrostis hesperica, Molinia caerulea, Deschampsia flexuosa*) and heathers (*Erica mackaiana*; Pontevedra-Pombal et al., 2013).

2.6.5.2. Sample collection and preparation

Peat cores were obtained in October 1998 by cutting directly into sections of peat newly exposed by ditches. 25 x 25 cm sections were cut to a depth of 185 cm and placed in plastic bags and aluminium foil for transport. These large sections were then sliced into 2 cm thick sections, dried at 30 °C, milled to a powder using an agate mortar and pestle, then homogenised (Pontevedra-Pombal et al., 2013).

Chapter 3 – Identification and determination of *Sphagnum*-specific biomarkers in northern peatlands using TMAH thermochemolysis

3.1. Introduction

Thermally assisted hydrolysis and methylation in the presence of tetramethylammonium hydroxide (TMAH), or TMAH thermochemolysis, has been widely used as a method to characterise lignin-derived phenolic compounds in plant litter and soils (Mason et al., 2012), as well as *Sphagnum*-derived compounds in peat (Abbott et al., 2013). van der Heijden et al. (1997) identified six thermochemolysis products of sphagnum acid, including 4-isopropenylphenol, which, using flash pyrolysis without TMAH, has been proposed as a marker for *Sphagnum* inputs to bulk peat deposits (McClymont et al., 2011). Abbott et al. (2013) and Swain & Abbott (2013) quantified compounds **I** (methylated 4-isopropenylphenol (IUPAC: 1-methoxy-4-(prop-1-en-2-yl)benzene)), **IIa** and **IIb** (methylated cis- and trans-3-(4´-hydroxyphen-1-yl)but-2-enoic acid (IUPAC: (E/Z)-methyl 3-(4-methoxyphenyl)but-2-enoate)), and **III** (methylated 3-(4´-hydroxyphen-1-yl)but-3-enoic acid (IUPAC: methyl 3-(4-methoxyphenyl)but-3-enoate)) as the dominant TMAH thermochemolysis products of sphagnum acid, and proposed these as markers for *Sphagnum* input to peat deposits. These products were observed down eight peat cores from hummocks and hollows along four stages of the Ryggmossen peatland, Sweden. A *Sphagnum* yield parameter (σ) was proposed, using the sum of the individual amounts of the four *Sphagnum*-derived phenols, normalised to 100 mg of OC (Abbott et al., 2013).

$$\sigma \text{ (mg/100mg OC)} = [\mathbf{I}] + [\mathbf{IIa}] + [\mathbf{IIb}] + [\mathbf{III}]$$

Using the parameter for total amount of lignin-derived phenols (Λ), obtained from the sum of the individual amounts of guaiacyl, syringyl and cinnamyl moieties G4, G5, G6, G18, S4, S5, S6, and P18, normalised to 100 mg OC (Hedges and Parker, 1976; Hedges and Mann, 1979), a measure of the relative contribution of *Sphagnum*-derived phenols to vascular plant-derived phenols, known as the *Sphagnum* ratio (SR%) was developed (Abbott et al., 2013).

$$SR\% = [\sigma / (\sigma + \Lambda)] * 100$$

It is important to note that, due to the potential for unlabelled TMAH thermochemolysis to produce an apparent lignin signal from non-intact lignin sources such as tannins and demethylated lignin (Filley et al., 2006), Λ in this case is taken as an indicator of total vascular plant-derived phenol input.

Lignin thermochemolysis products are also present in peat deposits due to input from vascular plants such as *Eriophorum vaginatum* (cottongrass) and *Vaccinium microcarpum* (cranberry), as well as other phenolic compounds from non-*Sphagnum* bryophytes such as *Pleurozium*

schreberi (red-stemmed feathermoss) and *Polytrichum commune* (common haircap moss; Freeman et al., 2012). In order to distinguish between existing intact lignin, degraded lignin, and non-lignin products such as tannins, it is necessary to use ^{13}C-labelled TMAH thermochemolysis Filley et al., 1999). As well as being used to analyse lignin phenols in litter and SOM (Filley et al., 2000; Filley et al., 2002; Filley et al., 2006; Mason et al., 2012), this technique can be applied to *Sphagnum* and peat samples in order to identify any other possible sources of, or precursors to, compounds **I**, **IIa**, **IIb** and **III**.

It is important to confirm the presence of the proposed *Sphagnum* markers in peat deposits and *Sphagnum* tissue, as well as their absence from vascular plant and non-*Sphagnum* bryophyte tissue from a variety of geographical locations in order to evaluate their viability as biomarkers and potential use as a proxy for the state of degradation of existing peat deposits or as a possible paleoclimate proxy. It is currently unclear whether relative quantities of the sphagnum acid THM products differ between either individual *Sphagnum* species, or between different geographic locations.

3.2. Materials and Methods

3.2.1. TMAH thermochemolysis

^{13}C-labelled and unlabelled TMAH thermochemolysis was performed using a platinum filament coil probe pyrolysis unit (Chemical Data Systems, Oxford, USA). The Pyroprobe 5000 was coupled to a 6890 N GC and 5975B MSD GC/MS system from Agilent Technologies (Palo Alto, USA).

Approximately 1 mg of powdered extracted peat or vegetation sample was weighed into a quartz pyrolysis tube (CDS Analytical, Oxford PA, USA) plugged with pre-extracted silica wool. An internal standard, 5α-androstane, and an aqueous solution of unlabelled or ^{13}C-labelled TMAH (25% w/w) was added to the sample immediately prior to THM.

Pyrolysis was performed at 610 °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 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 5000 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). Compound identification was based on the NIST98 mass spectral library, on ion fragmentation patterns and following the conventions described in other studies (van der Heijden, 1994; Clifford et al., 1995; Hatcher et al., 1995; Filley et al., 2000; Mason et al., 2009; Abbott et al., 2013).

3.2.2. Sampling

Samples were collected from a range of locations, namely the Ryggmossen peatland (section 2.2.1) and the Rödmossamyran peatland, both in Sweden, the Frasne peatland, France (section 2.6.2; Fig. 3.2), and locations across North America (sample set provided by Prof Merritt Turetsky, University of Guelph and Prof. Jonathan Shaw, Duke University). Samples were run in triplicate with a single additional run using ^{13}C-TMAH per sample on selected samples.

Fig. 3.2. European map showing sampling locations Frasne peatland (FR), Ryggmossen (RYG), Rödmossamyran (RMM).

3.3. Results and Discussion

3.3.1. TMAH thermochemolysis of Sphagnum capitula and surficial peats

The compounds **I**, **IIa**, **IIb** and **III** were observed across all *Sphagnum* capitula sampled (fig. 3.3), as well as in all peat deposits with *Sphagnum* input (fig. 3.4; fig. 3.5). Average σ yield was 0.497mg/100 mg OC for the *Sphagnum* capitula and extremely similar at 0.494 mg/100 mg OC for the surficial peats, with an overall average σ yield of 0.496 mg per 100 mg OC. The sphagnum acid products were not observed in any of the vascular plants or non-*Sphagnum* bryophytes studied (fig. 3.6). Lignin-derived phenols G4, G6, S4, S6, P18 and G18 were observed in varying abundances in all samples (Fig. 3.7). It is unclear why lignin-derived phenols were observed in non-*Sphagnum* bryophyte samples, as these species are known not to synthesize lignin (Weng and Chapple, 2010).

The presence of lignin-derived phenols in *Sphagnum* tissue is due to the hyaline cells of the *Sphagnum* mosses – large, porous cells occurring throughout the structure of the plant. These cells account for the large water holding capacity of *Sphagnum* by allowing free entry of surrounding water from the bog environment into the cell body (Hayward and Clymo, 1982). Lignin phenols form one of the more stable fractions of dissolved organic matter (DOM; Kalbitz and Kaiser, 2008), and are present in a peatland environment from the degradation of peat forming vascular plants. These lignin phenols suspended in the groundwater can therefore be taken into the living *Sphagnum* tissue via the hyaline cells.

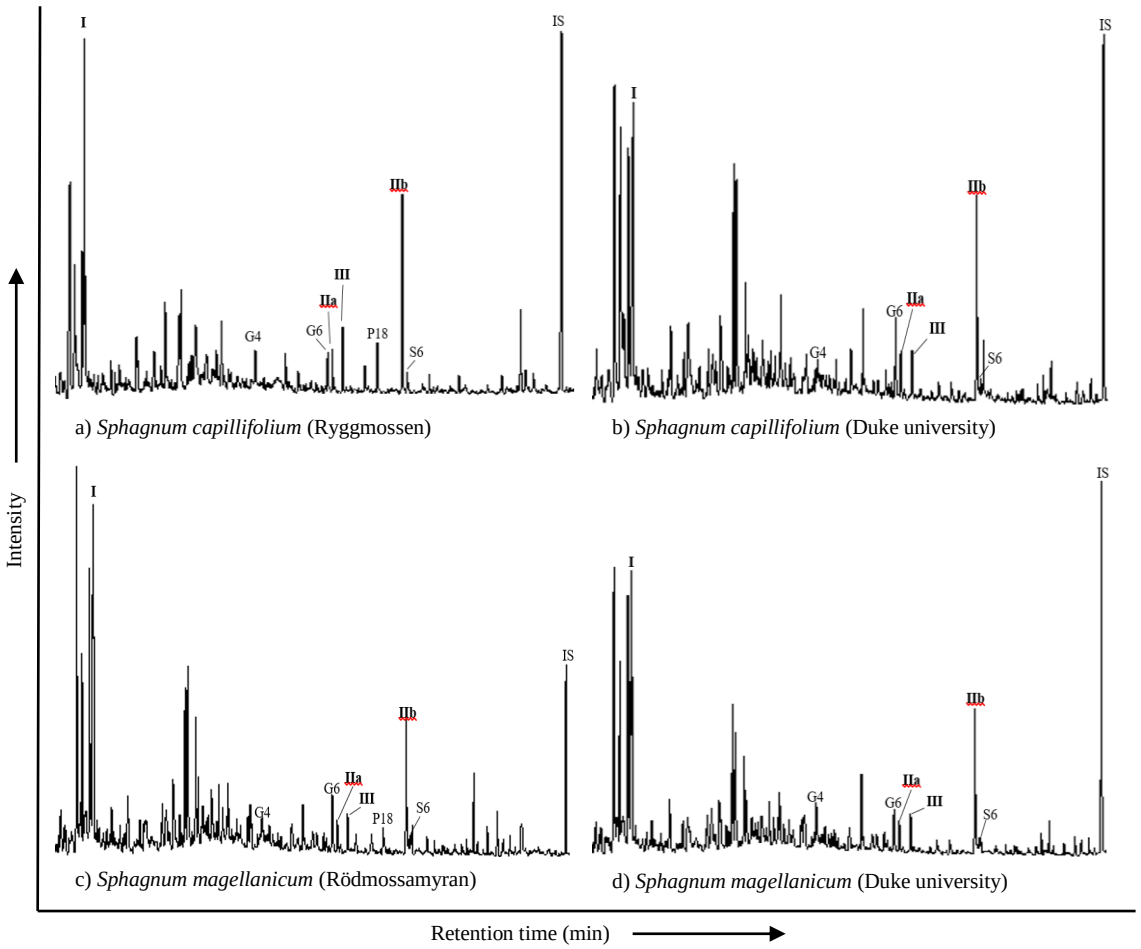

Fig. 3.3. Partial chromatogram for the total ion current (TIC) of the thermochemolysis products from the capitula of *Sphagnum* species a) *Sphagnum capillifolium* (Ryggmossen peatland) b) *Sphagnum capillifolium* (North America) c) *Sphagnum magellanicum* (Rödmossamyran peatland) and d) *Sphagnum magellanicum* (North America).

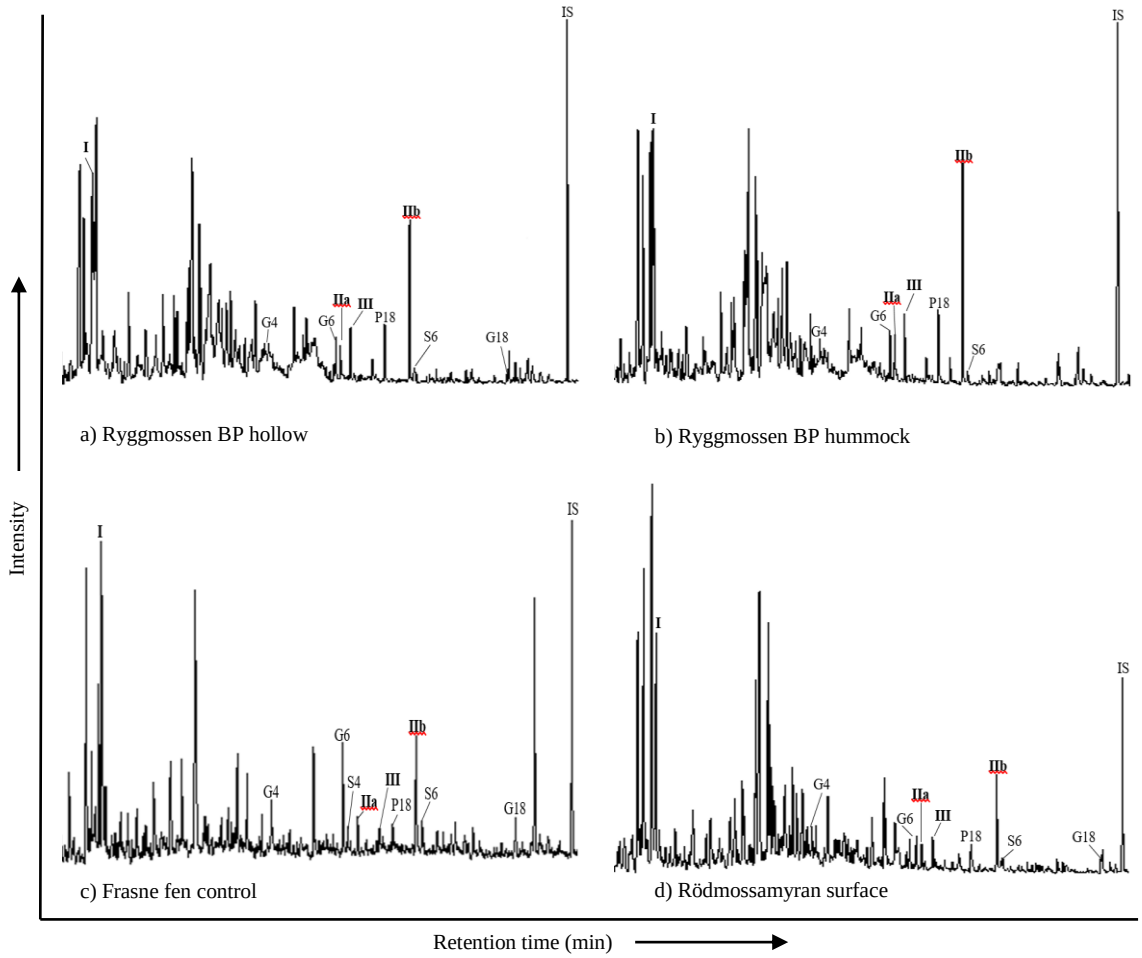

Fig. 3.4. Partial chromatogram for the total ion current (TIC) of the thermochemolysis products from surficial peat samples from a) Ryggmossen peatland, bog plateau (BP) stage hollow b) Ryggmossen peatland, bog plateau (BP) stage hummock c) Frasne peatland 'wet' fen site and d) Rödmossamyran peatland.

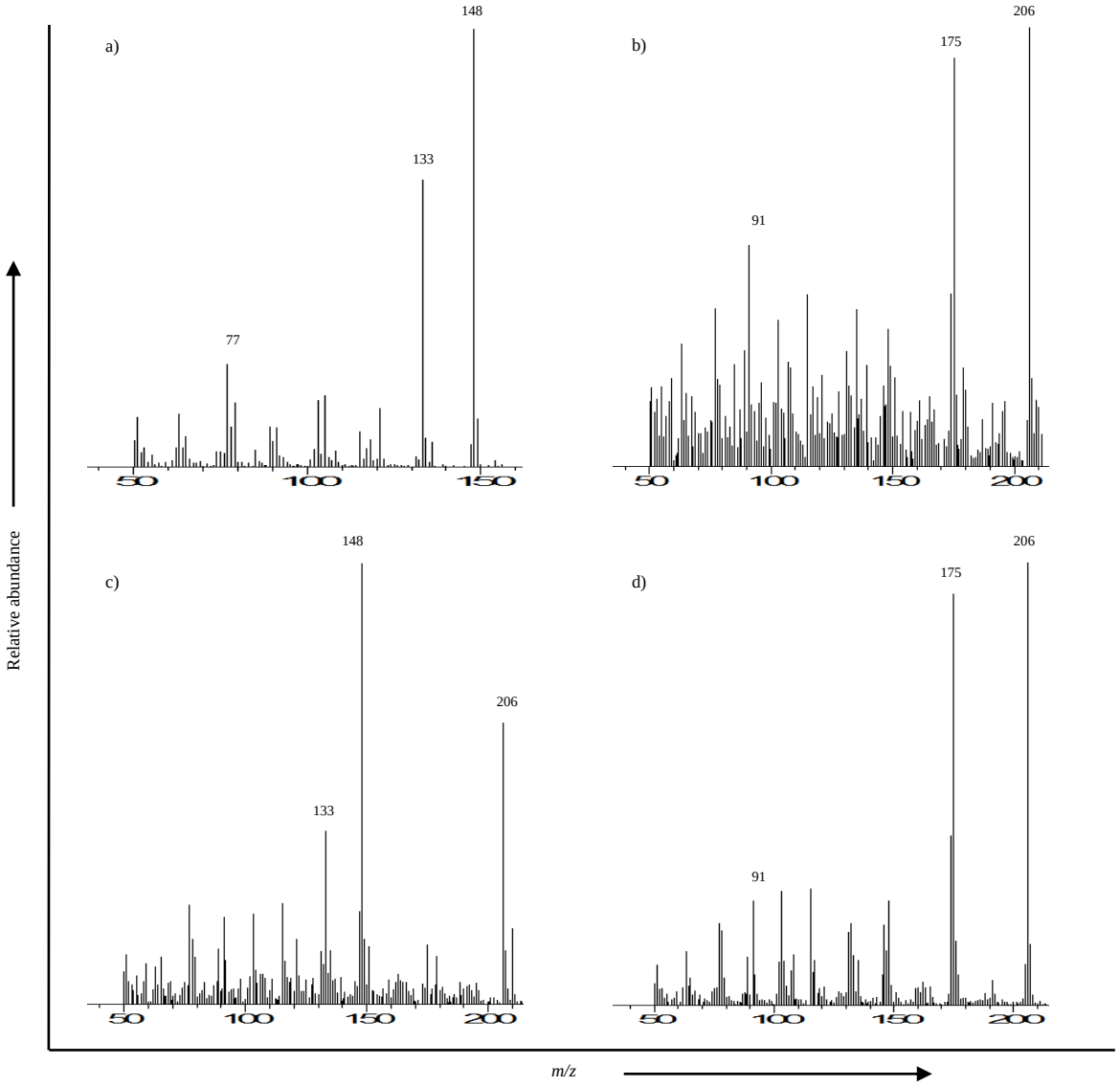

Fig. 3.5. The mass spectra of the sphagnum acid products formed during TMAH thermochemolysis: a) component **I** methylated 4-isopropenylphenol, b) component **IIa** methylated *cis*-3-(4′-hydroxyphen-1-yl)but-2-enoic acid, c) component **III** methylated 3-(4′-hydroxyphen-1-yl)but-3-enoic acid and d) component **IIb** *trans*-3-(4′-hydroxyphen-1-yl)but-2-enoic acid.

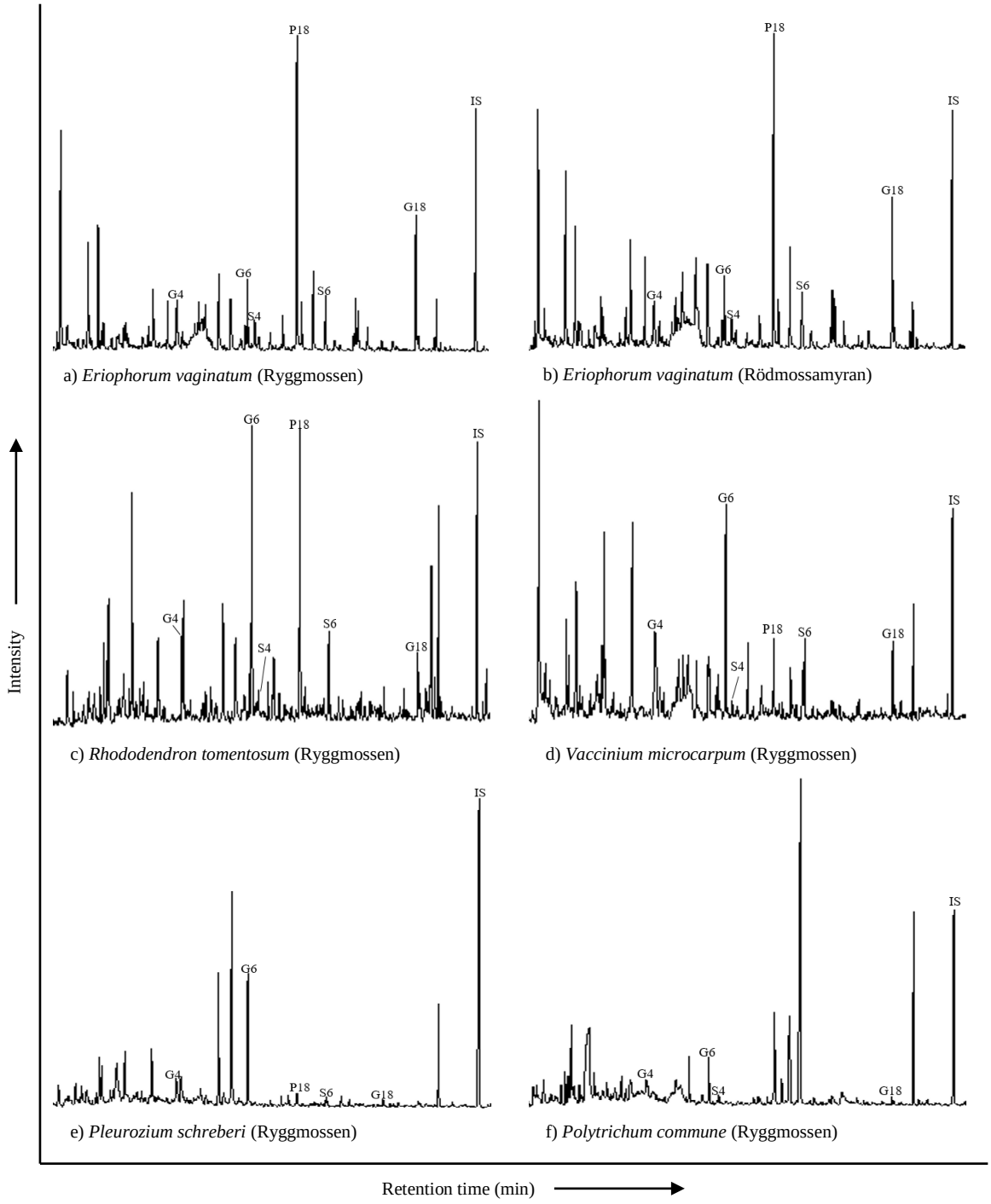

Fig. 3.6. Partial chromatogram for the total ion current (TIC) of the thermochemolysis products from vascular plant species a) *Eriophorum vaginatum* (Ryggmossen peatland) b) *Eriophorum vaginatum* (Rödmossamyran peatland) c) *Rhododendron tomentosum* (Ryggmossen peatland) d) *Vaccinium microcarpum* (Ryggmossen peatland) and non-*Sphagnum* bryophyte species e) *Pleurozium schreberi* (Ryggmossen peatland) and f) *Polytrichum commune* (Ryggmossen peatland).

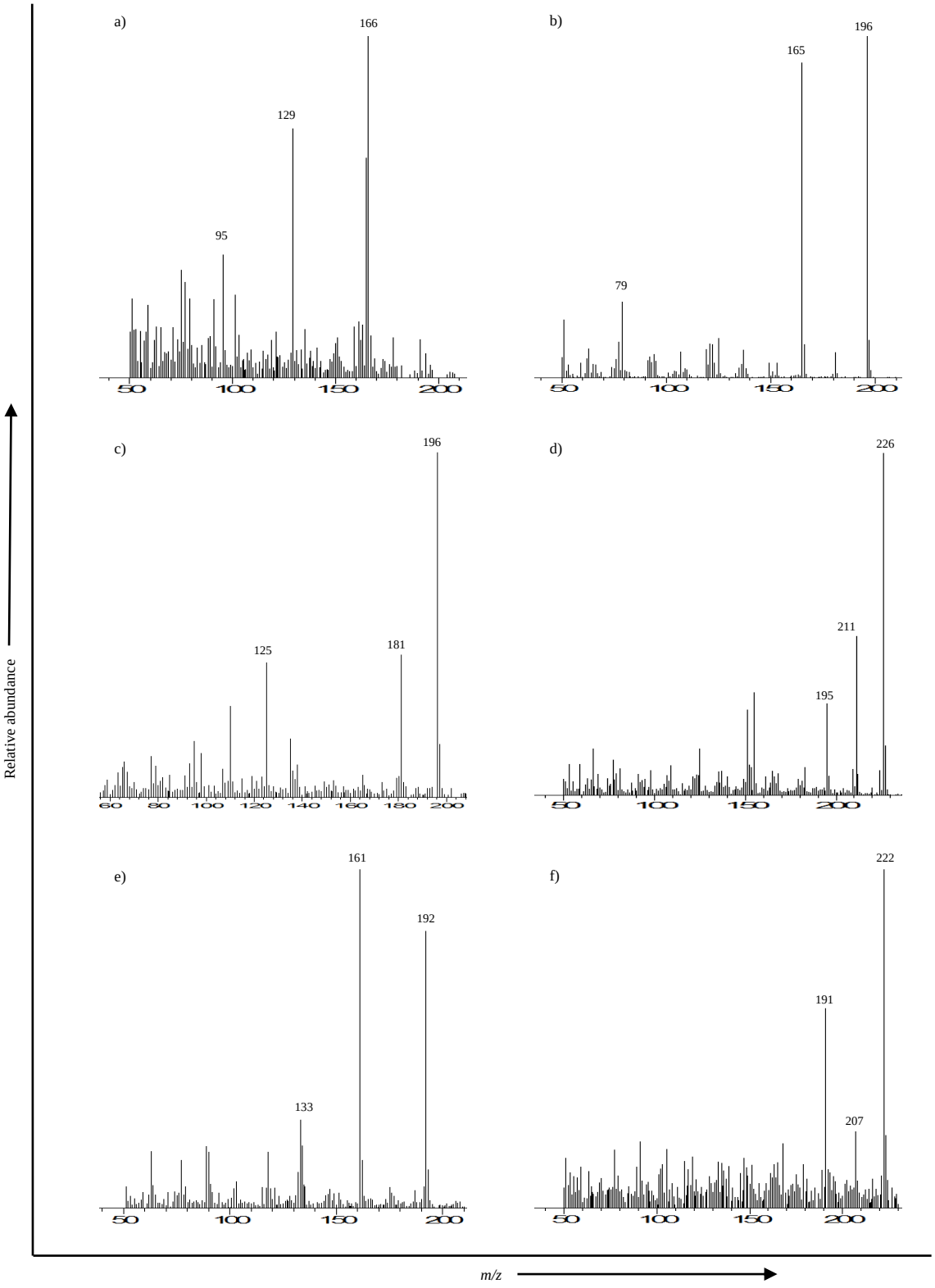

Fig. 3.7. The mass spectra of the lignin-derived TMAH thermochemolysis products: a) G4 (3,4-dimethoxybenzaldehyde), b) G6 (3,4-dimethoxybenzoic acid, methyl ester), c) S4 (3,4,5-trimethoxybenzaldehyde), d) S6 (3,4,5-trimethoxybenzoic acid, methyl ester), e) P18 (*trans*-3-(4-methoxyphenyl)-3-propenoic acid, methyl ester) and f) G18 (*trans*-3-(3,4-methoxyphenyl)-3-propenoic acid, methyl ester).

Despite almost identical overall σ yields in the *Sphagnum* capitula and surficial peats, individual abundances of the four sphagnum acid-derived compounds **I, IIa, IIb** and **III** differed between the two (table 3.1), albeit with a relatively large degree of uncertainty (fig. 3.8). This indicates that there is a possibility that different sphagnum acid components are degraded at different rates upon entering bulk peat deposits.

Sample	I	IIa	IIb	III	**σ**
S. capillifolium Ryggmossen	0.24	0.03	0.17	0.05	**0.48**
S. fuscum Ryggmossen	0.16	0.06	0.26	0.07	**0.55**
S. angustifolium Ryggmossen	0.21	0.02	0.16	0.04	**0.44**
S. capillifolium Duke University	0.10	0.03	0.11	0.03	**0.26**
S. magellanicum Duke University	0.33	0.05	0.23	0.05	**0.66**
S. magellanicum Rödmossamyran	0.34	0.04	0.17	0.05	**0.60**
Surface Peat BP hollow Ryggmossen	0.30	0.02	0.20	0.05	**0.57**
Surface Peat BP hummock Ryggmossen	0.28	0.02	0.20	0.03	**0.53**
Surface Peat Rödmossamyran	0.25	0.05	0.17	0.05	**0.51**
Surface Peat Frasne Bog	0.36	0.03	0.20	0.03	**0.62**
Surface Peat Frasne Fen	0.15	0.03	0.01	0.03	**0.23**

Table 3.1. Abundances of individual sphagnum acid-derived TMAH thermochemolysis products and σ parameter for *Sphagnum* capitula samples and surficial peats expressed in mg 100 mg^{-1} OC.

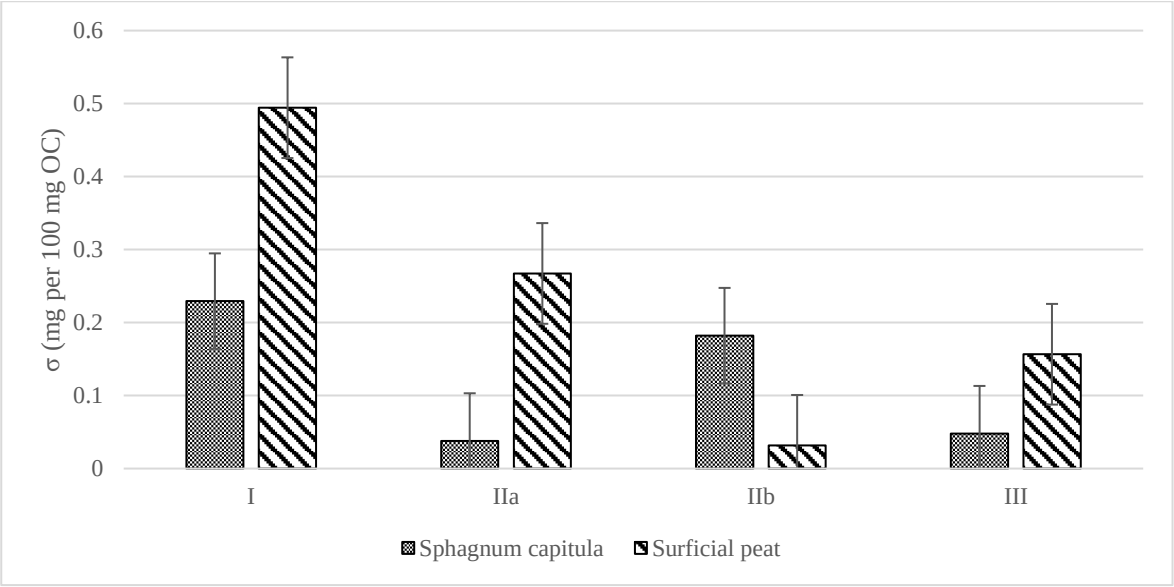

Fig. 3.8. Abundance (expressed in mg 100 mg^{-1} OC) of the four individual sphagnum acid-derived TMAH thermochemolysis products **I, IIa, IIb** and **III** in *Sphagnum* capitula and surficial peat samples. Error bars represent the standard error of the mean across all samples.

Comparing *Sphagnum capillifolium* to *Sphagnum magellanicum* (fig. 3.9) suggests that concentrations of **I, IIa, IIb** and **III** may be greater in *S. magellanicum*, however a regional comparison between *Sphagnum* species from Sweden and North America displays no significant differences in **I, IIa, IIb** or **III** (fig. 3.10). However, these comparisons are based on very small sample sizes, so it is not possible to draw truly representative conclusions from this.

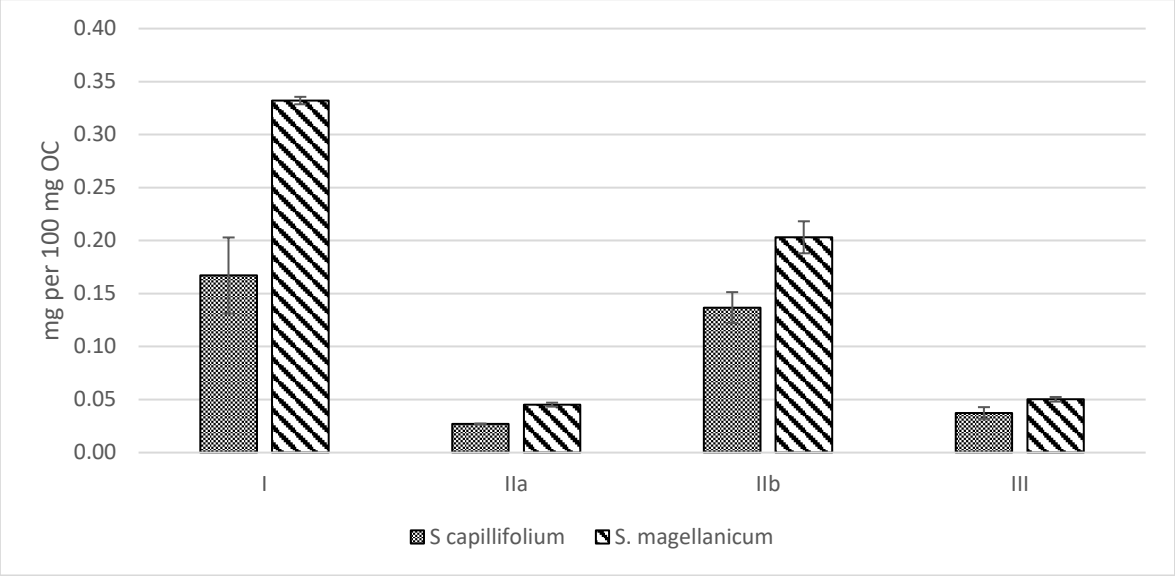

Fig. 3.9. Abundance (expressed in mg 100 mg^{-1} OC) of the four individual sphagnum acid-derived TMAH thermochemolysis products **I, IIa, IIb** and **III** in *Sphagnum capillifolium* and *Sphagnum magellanicum* samples. Error bars represent the standard error of the mean across all samples.

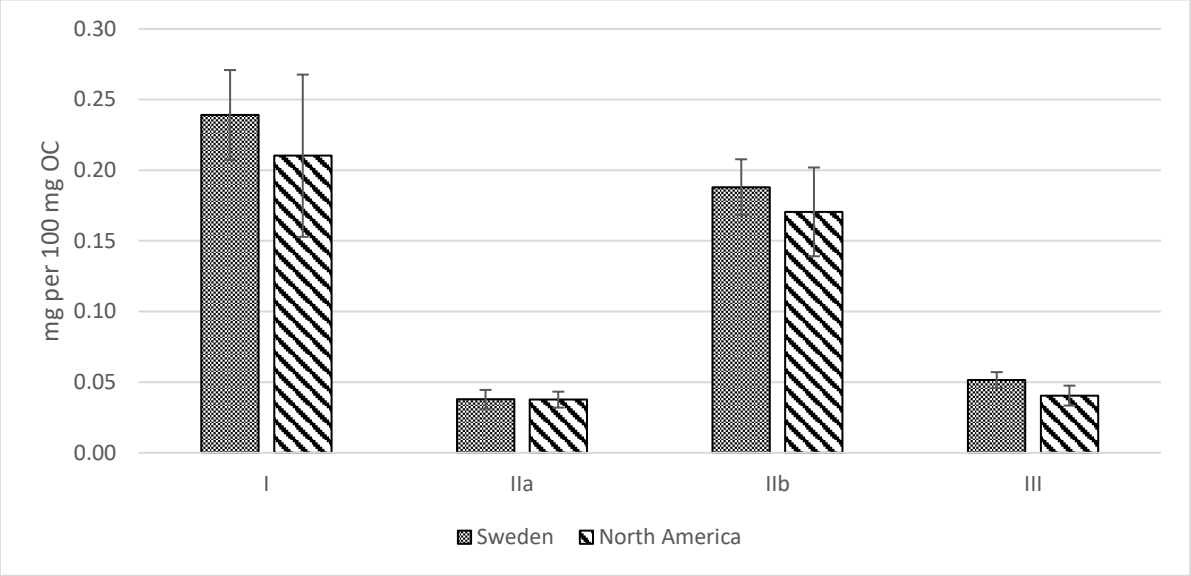

Fig. 3.10. Abundance (expressed in mg 100 mg^{-1} OC) of the four individual sphagnum acid-derived TMAH thermochemolysis products **I, IIa, IIb** and **III** in *Sphagnum* samples from Sweden and North America. Error bars represent the standard error of the mean across all samples.

3.3.2. ^{13}C-TMAH thermochemolysis of sphagnum acid products

It is possible to adapt the ^{13}C enrichment equations used to determine the aromatic hydroxyl content of G6 (Filley et al., 2006) to apply it to the sphagnum acid thermochemolysis products **I**, **IIa**, **IIb** and **III**. **IIa, IIb** and **III** show an increase of 2 mass units in the molecular ion when subjected to ^{13}C-TMAH thermochemolysis (Fig. 3.11), indicating that two methyl

groups are added during the process. If no alternative precursors are present, the aromatic hydroxyl content should be 100%. As the equation was designed to show the original % 3-methoxy, 4-hydroxy content in G6, it has been altered to subtract this figure from 100, instead giving the % 3,4-dihydroxy content:

% Dihydroxyl (**I**, **IIa**, **IIb**, **III**)

$$= 100 - [100 \text{ x } \left[\frac{(M_{L+1} - (M_{L+1})_{calc})}{M_L + M_{L+1} - (M_{L+1})_{calc}}\right]]$$

where

$$(M_{L+1})_{calc} = M_L \text{ x } \left(\frac{M_{UL+1}}{M_{UL}}\right) \text{ x } \left(\frac{N_L}{N_{UL}}\right)$$

The sphagnum acid products present in all samples analysed with ^{13}C-TMAH were found to have an aromatic hydroxyl content of around 100% (table. 3.2). This suggests that the observed peaks for **I**, **IIa**, **IIb** and **III** have no alternative sources or precursors other than these sphagnum acid thermochemolysis products. Tests carried out with an authentic standard of syringic acid ($C_9H_{10}O_5$) showed no ^{12}C-^{13}C exchange during the THM process, with an observed mass increase of 2 indicating methylation only at the initial hydroxyl sites (Fig. 3.12).

Location	Description	**I**	**IIa**	**IIb**	**III**	Average
Ryggmossen	*Sphagnum capillifolium*	99.6	99.3	100	100	99.9
Ryggmossen	*Sphagnum fuscum*	99.9	97.5	100	100	100
Ryggmossen	*Sphagnum angustifolium*	99.8	98.2	100	99.3	98
Rödmossamyran	*Sphagnum magellanicum*	99.9	99.4	100	99.1	99.8
North America	*Sphagnum magellanicum*	99.8	99.6	100	100	99.9
Frasne (Bog)	Surficial peat	99.8	98.9	100	100	99.9
Frasne (Fen)	Surficial peat	99.4	99.3	100	99.4	99.9

Table 3.2. Aromatic dihydroxy content (%) of *Sphagnum* capitula and surficial peat samples analysed with ^{13}C-TMAH thermochemolysis.

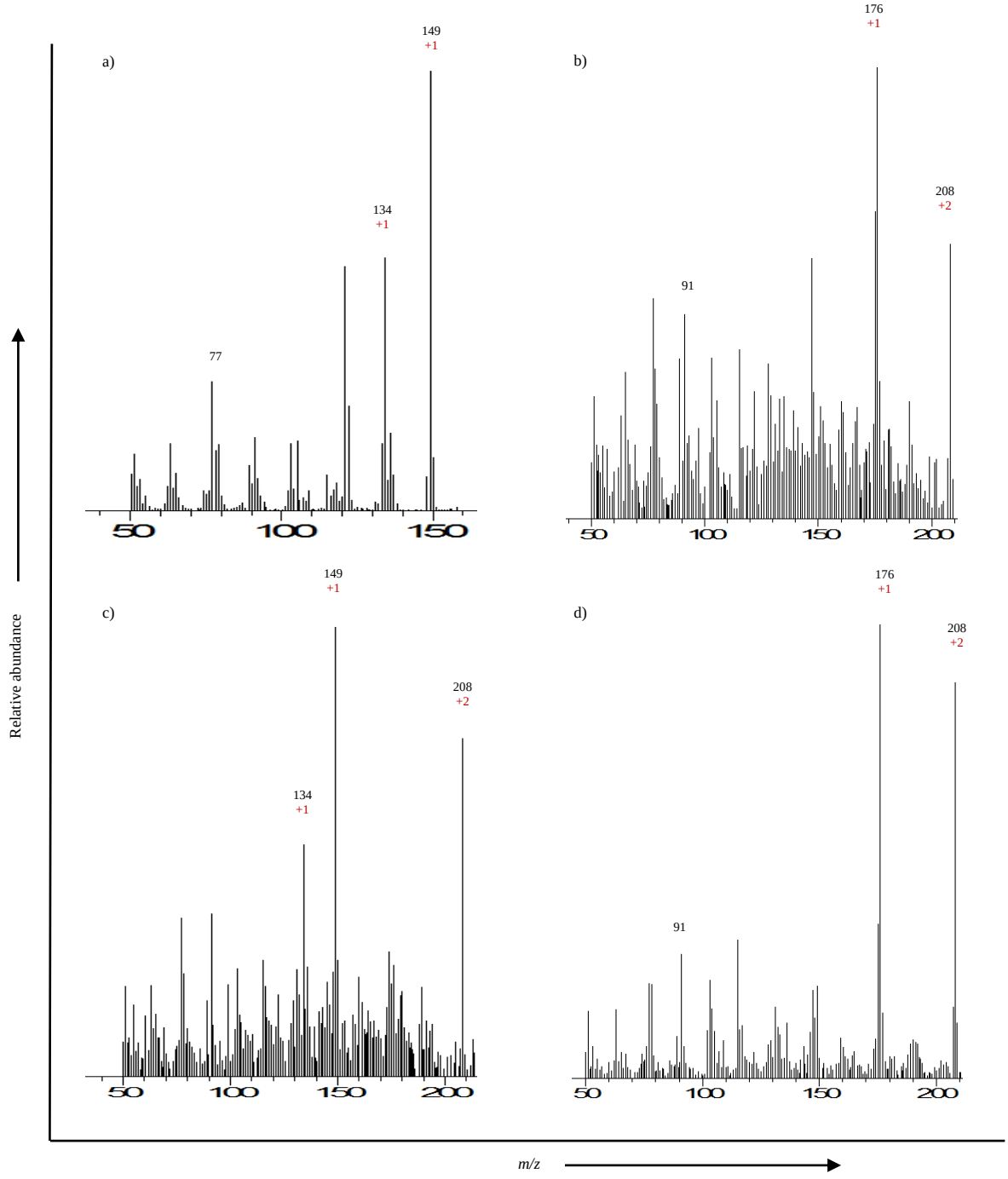

Fig. 3.11. The mass spectra of the sphagnum acid products formed during ^{13}C-labelled TMAH thermochemolysis: a) component **I** methylated 4-isopropenylphenol, b) component **IIa** methylated *cis*-3-(4′-hydroxyphen-1-yl)but-2-enoic acid, c) component **III** methylated 3-(4′-hydroxyphen-1-yl)but-3-enoic acid and d) component **IIb** *trans*-3-(4′-hydroxyphen-1-yl)but-2-enoic acid. Numbers in red reflect the change in M_L from the unlabelled mass spectra.

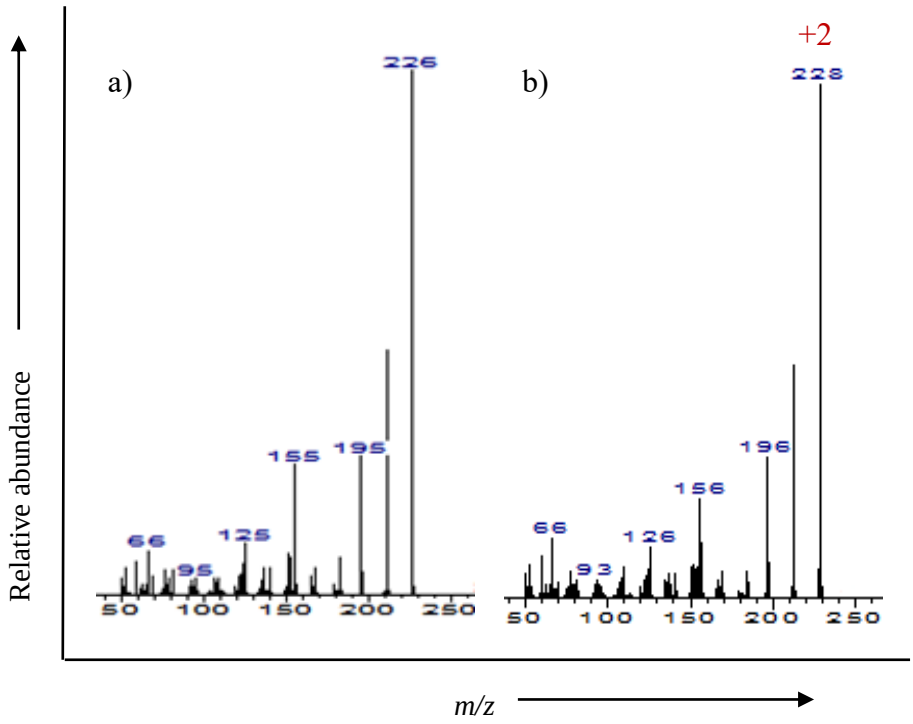

Fig. 3.12. The mass spectra of the thermochemolysis products of an authentic standard of syringic acid ($C_9H_{10}O_5$) formed during a) unlabelled TMAH thermochemolysis and b) ^{13}C-labelled TMAH thermochemolysis. Numbers in red reflect the change in M_L from the unlabelled mass spectra.

3.4. Conclusions

Analysis of a suite of *Sphagnum* moss samples from a variety of Northern hemisphere locations confirmed the specificity of the proposed marker compounds **I**, **IIa**, **IIb** and **III** to *Sphagnum* species. These TMAH thermochemolysis products were also found to have no alternative sources or precursors via ^{13}C-TMAH thermochemolysis, confirming their viability as biomarkers. Relative abundances of compounds **I, IIa, IIb** and **III** were found to differ between *Sphagnum* tissue and surficial peat deposits, suggesting that the different components of sphagnum acid degrade at different rates upon entry to the bulk peat deposit.

Chapter 4 - The use of plant-specific pyrolysis products as biomarkers in peat deposits

4.1. Introduction

This work was published in Quaternary Science Reviews (Schellekens et al., 2015; appendix 2).

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 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 (Py-GCMS) 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; Gonzalez 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 a range of 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 peat deposits from different climatic regions.

Although the use of plant-specific biomarkers can provide a detailed record of vegetation composition, quantification using abundances relative to the dominant peaks in the TIC, as has been used in previous analytical pyrolysis studies (Schellekens et al., 2015b), whilst providing a compositional guide to how amounts of certain compounds may be changing relative to one another, cannot be considered a quantitative guide, and will henceforth be referred to as a 'compositional assessment'. The addition of an internal standard allows for quantification with abundances of specific compounds relative to the known absolute abundance of the internal standard after normalisation for organic carbon content. An internal standard creates internally consistent conditions, meaning compound abundances can be reliably compared across different locations. This allows for more accurate determination of plant-specific marker abundances in peat deposits than the compositional assessment method. In order to achieve truly quantitative absolute abundances, it is necessary to calculate relative response factors for each compound based on calibrations using an authentic standard of the compound in question (Filley et al., 2006). However, it is often difficult to acquire authentic standards of every relevant compound in a given study, so a relative response factor of 1 is assumed to provide a semi-quantitative assessment (Mason et al., 2012).

The efficacy of a plant- or taxon-specific biomarker is dependent on it being derived from a single source. It is therefore important to determine the origin of any newly identified peat

biomarkers before they can be used as effective proxies. Thermally assisted hydrolysis and methylation in the presence of ^{13}C-labelled tetramethylammonium hydroxide (^{13}C-TMAH thermochemolysis) has been used to effectively elucidate the source of lignin-derived phenols in mineral soils (Filley et al., 2006; Mason et al., 2009), as well as *Sphagnum*-specific markers in peat deposits (Abbott et al., 2013). In this chapter, the origin of several proposed novel peatland plant-specific biomarkers identified using conventional Py-GCMS will be investigated using ^{13}C-TMAH thermochemolysis, and the compositional assessment analytical pyrolysis method will be compared to quantification using an internal standard.

4.2. Materials and Methods

4.2.1. Sampling

Samples were collected from a diverse range of peatlands, including the *Sphagnum*-dominated 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., 2015b; section 2.6.3.), and graminoid dominated peatlands Penido Vello (PVO; Spain; Schellekens et al., 2011, 2012, 2015c; 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; Horak-Terra et al., 2014; Schellekens et al., 2014; Fig. 4.1). The main characteristics of these peatlands can be found in table 4.1.

Samples from HRB were analysed with internal standard 5α-androstane, and lichen and graminoid samples from PDC were analysed with ^{13}C-TMAH thermochemolysis.

4.2.2. Pyrolysis-GCMS

Py-GCMS was performed using several methods as outlined in Schellekens et al. (2015a). Samples from HRB were run on a pulsed-mode open pyrolysis system, specifically a CDS 1000 pyroprobe unit (CDS Analytical, USA) fitted with a platinum coil and a CDS 1500 valved interface. Insoluble residues of peat and vegetation were weighed (ca. 2 mg) into clean quartz pyrolysis tubes plugged with extracted silica wool. For quantification, a known amount (3 µl) of internal standard (5α-androstane) was added prior to pyrolysis. Pyrolysis-GCMS was carried out at 650 ºC for 2 s (10 °C.ms^{-1} temperature ramp). The pyroprobe interface was maintained at 320 ºC with the products passing into an HP5890 gas chromatograph (GC) with an open split (40 mL/min), with a 60 m HP5-MS column (0.25 mm internal diameter, 0.25 lm

film thickness; J&W Scientific, USA). Helium was used as carrier gas at a flow rate of 1 mL/min. The GC oven was heated from 40 to 320 ºC (held 10 min) at 7 ºC.min^{-1}. Product detection was carried out using an HP5972 series mass selective detector in full scan mode (*m/z* 50–700) with compound identification based on the NIST98 mass spectral library and ion fragmentation patterns. 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} OC. Total OC values for each sample were obtained using a Leco CS230 carbon-sulphur analyser.

4.2.3. Identification and quantification

For the purposes of this study, a marker is defined as a product that is both preserved in the bulk peat material, and found exclusively in a single species, genera or taxonomic group (Schellekens et al., 2015a). Aside from the samples from HRB analysed with an internal standard, quantification of markers was based on the relative abundances of dominant peaks in the total ion current (TIC). In order to compare this quantification method to the use of an internal standard, samples from the upper 3 m of the HRB core were selected for analysis with internal standard 5α-androstane, and the depth record of 4-isopropenylphenol was compared between the two quantification methods.

4.2.4. ^{13}C-TMAH thermochemolysis

^{13}C-TMAH thermochemolysis was performed on lichen (*Pseudocyphellaria freycinetii*) and graminoid (*Molinia caerulea*) samples (Fig. 4.2), as well as a single peat sample from PDC, and aromatic hydroxyl content determined using methods outlined in section 2.3.

Peatland	Location	Coordinates	Height (m a.s.l.)	P (mm)[a]	T (°C)[b]	Depth (cm)	Age (cal ka BP)	*n*[c]	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	43º32'N, 7º30'W	780	1600	8.8	0–300	0–8	101	Graminoids
Pena da Cadela (PDC)	Galicia, Spain	43º30'N, 7º33'W	970	1800	7.5	0-185	0-5.3	34	Graminoids
Pau de Fruta (PF)	Minas Gerais, Brazil	18º15'S, 43º40'W	1350	1500	18.7	0–398	0–9	44	Graminoids/Capões[d]

[a] mean anual precipitation

[b] mean anual temperature

[c] *n* = number of samples analysed with analytical pyrolysis

[d] the word 'Capões' does not refer to a specific botanical composition but indicates a group of trees within an open landscape. This characteristic vegetation (Capões) is typical for the peatlands in the area (Schellekens et al., 2014).

Table 4.1. Characteristics of the studied peatlands. From Schellekens et al., 2015a

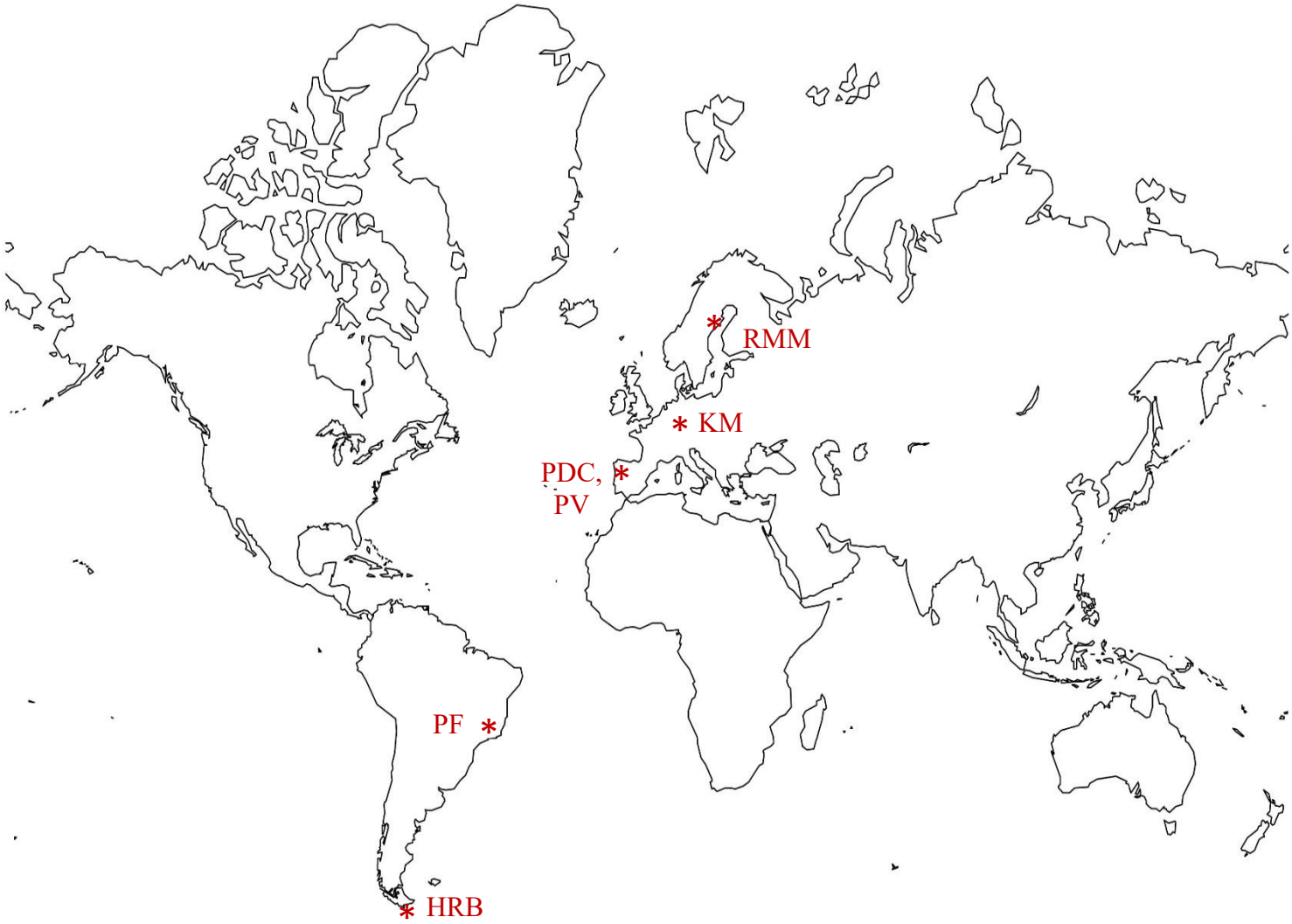

Fig. 4.1. Global map showing location of sampling sites in red.

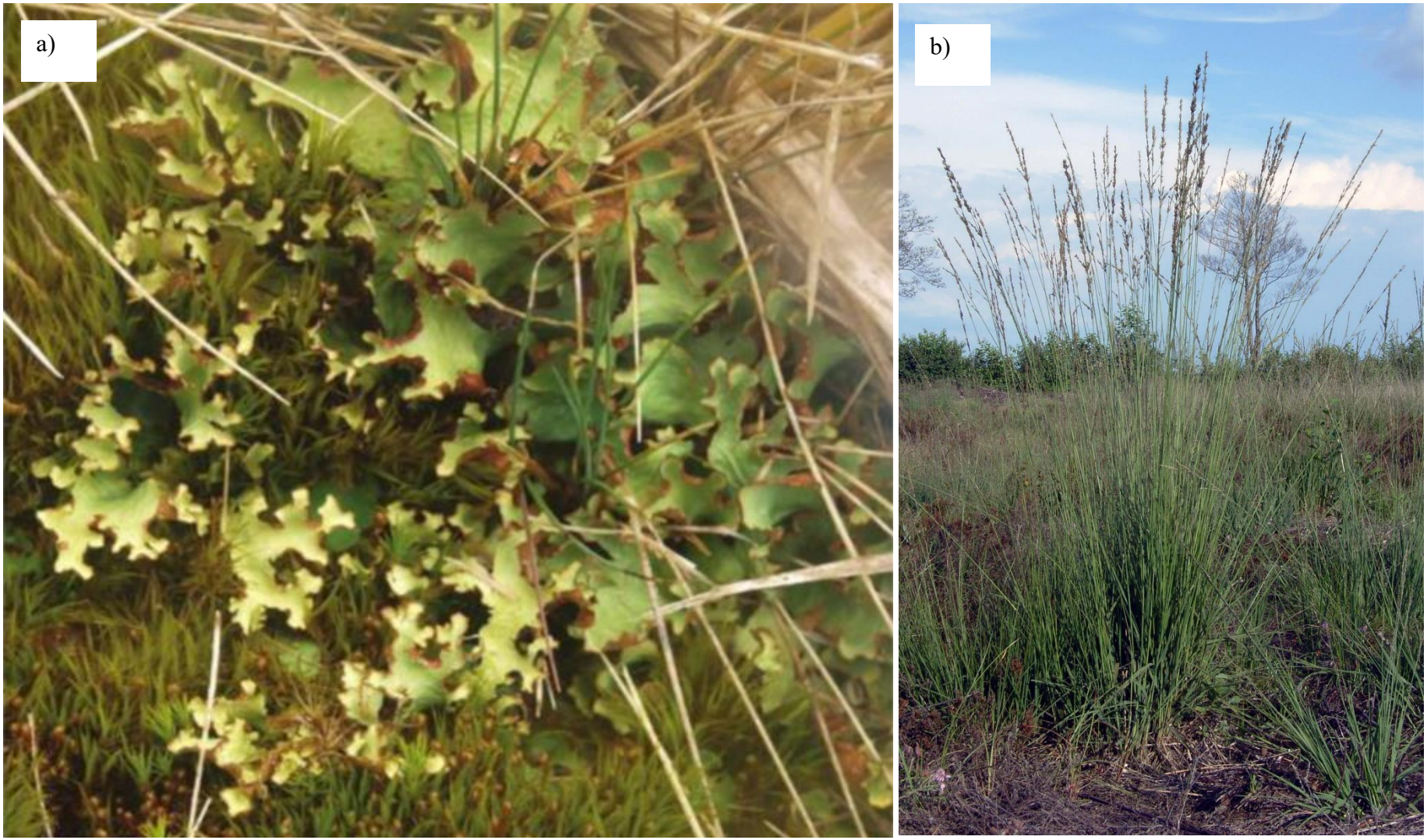

Fig. 4.2. a) *Pseudocyphellaria freycinetii* (Burton and Croxall, 2012) and b) *Molinia caerulea* (Wikipedia).

4.3. Results and Discussion

4.3.1. Quantification of biomarkers from pyrolysis

The use of an internal standard to analyse biomarkers using analytical pyrolysis has been tested for the *Sphagnum* biomarker 4-isopropenylphenol (Fig. 4.3, 4.4). Depth records of 4-isopropenylphenol from the upper 3m of the *Sphagnum*-dominated HRB obtained with internal standard (mg 100 g^{-1} OC) and compositional assessment (% of the total quantified pyrolysis products) were used for this comparison (Fig. 4.5). The correlation between both is significant ($r^2 = 0.72$, $P < 0.0005$, $n = 12$). This indicates that a compositional assessment from analytical pyrolysis, although usually not considered to be quantitative, can reliably quantify marker compounds when expressed as proportion of the total quantified peak area. Secondly, this shows that the use of an internal standard for analytical pyrolysis enables analysis of established biomarkers without the time consuming quantification of large numbers of compounds as is required in the compositional assessment method. It is important to note that although yields of phenolic compounds in these analyses are low, this is due to the volatility of phenolic compounds, many of which go to char from the high temperatures involved in Py-GCMS. As such, these markers will be present in a larger percentage of the biomass than is suggested from the phenolic yield.

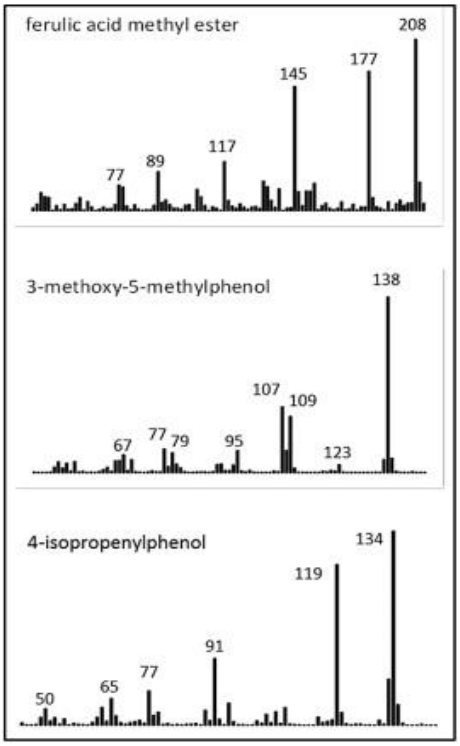

Fig. 4.3. Mass spectra of ferulic acid methyl ester, 3-methoxy-5-methylphenol, and 4-isopropenylphenol.

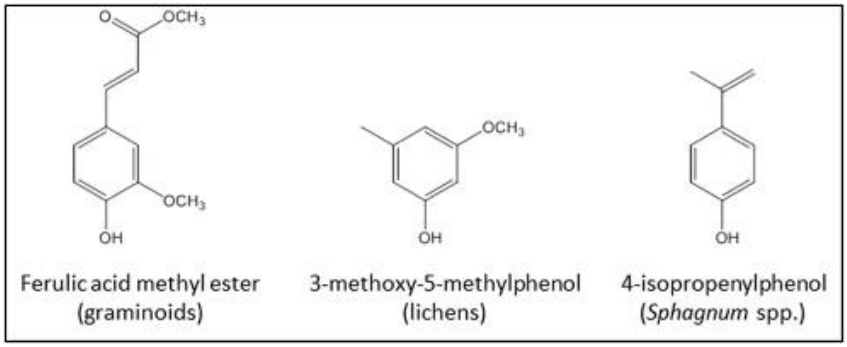

Fig. 4.4. Molecular structures of the identified marker compounds.

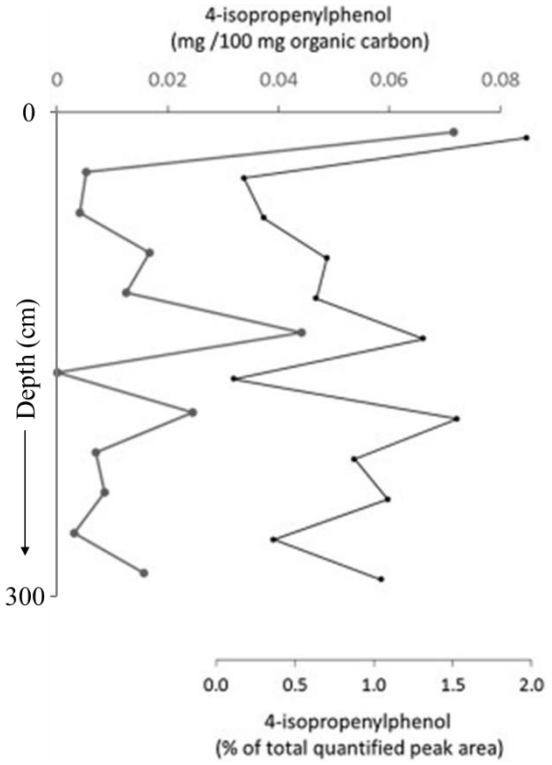

Fig. 4.5. Depth records of 4-isopropenylphenol obtained with internal standard 5α-androstane and as % of the total quantified peak area.

4.3.2. Identification of markers and analysis with ^{13}C-TMAH thermochemolysis

4.3.2.1. Lichen

The phenolic compound 3-methoxy-5-methylphenol (Fig. 4.3, 4.4) was proposed as a marker for lichens after being observed in six of the seventeen lichen species sampled (*Cetraria islandica, Cladonia arbuscula, Cladonia cervicornis, Cladonia furcata, Ochrolechia frigida, Pseudocyphellaria freycinetii*; Fig. 4.6). Presence of the marker was not specific to a single species or location, and was observed in samples taken from HRB, PDC and PVO (Fig. 4.7). Although 3-methoxy-5-methylphenol was not observed in all lichen species, it was absent from all other sampled taxa. It can therefore be considered a potential marker for lichens in that its presence indicates the presence of lichens, however its absence does not necessarily indicate their absence. After unlabelled TMAH thermochemolysis, the methylated form of 3-methoxy-5-methylphenol was present as 3,5-dimethoxytoluene in the *Pseudocypphelaria* sample (Fig. 4.8, 4.9). This suggests that the proposed marker did not undergo structural changes during pyrolysis without methylation. Correction for ^{13}C enrichment showed that 89% of the signal originated from a monomethoxy source, 3-methoxy-5-methylphenol. A small amount of a compound with *m/z* 91, 123, 152 was observed in the Py-GCMS TIC of the *Pseudocypphelaria* sample (Fig. 4.10, 4.11) and tentatively assigned as 3,5-dimethoxytoluene, suggesting that small amounts of 3,5-dimethoxytoluene are present in lichen pyrolysis products, therefore if using TMAH thermochemolysis as an analytical tool, it will be necessary to use ^{13}C-TMAH in order to distinguish between 3,5-dimethoxytoluene originally present in the lichen tissue, and 3-methoxy-5-methylphenol methylated during the THM process.

4.3.2.2. Graminoid

Ferulic acid methyl ester (Fig 4.3, 4.4) was found exclusively in graminoid pyrolysates, and has previously been observed in graminoid samples while being absent from non-graminoid species of a variety of Mediterranean plants (Schellekens et al., 2013). The methylated form of ferulic acid methyl ester, 3-(3,4-dimethoxyphenyl)-2-propenoic acid methyl ester, was observed in *Molinia caerulea* tissue following unlabelled TMAH thermochemolysis (Fig. 4.8, 4.9). ^{13}C-TMAH thermochemolysis revealed that 94% of this signal was derived from the monomethoxy source, ferulic acid methyl ester. 3-(3,4-dimethoxyphenyl)-2-propenoic acid methyl ester was also observed as one of the TMAH thermochemolysis products of a peat sample from PDC, with ^{13}C-TMAH thermochemolysis showing a 97% dihydroxy source, suggesting extensive demethylation of the marker compound may take place in bulk peat

deposits, possibly as part of a fungal degradation process, similar to demethylation of lignin-derived phenolics by white rot fungi (Filley et al., 2000).

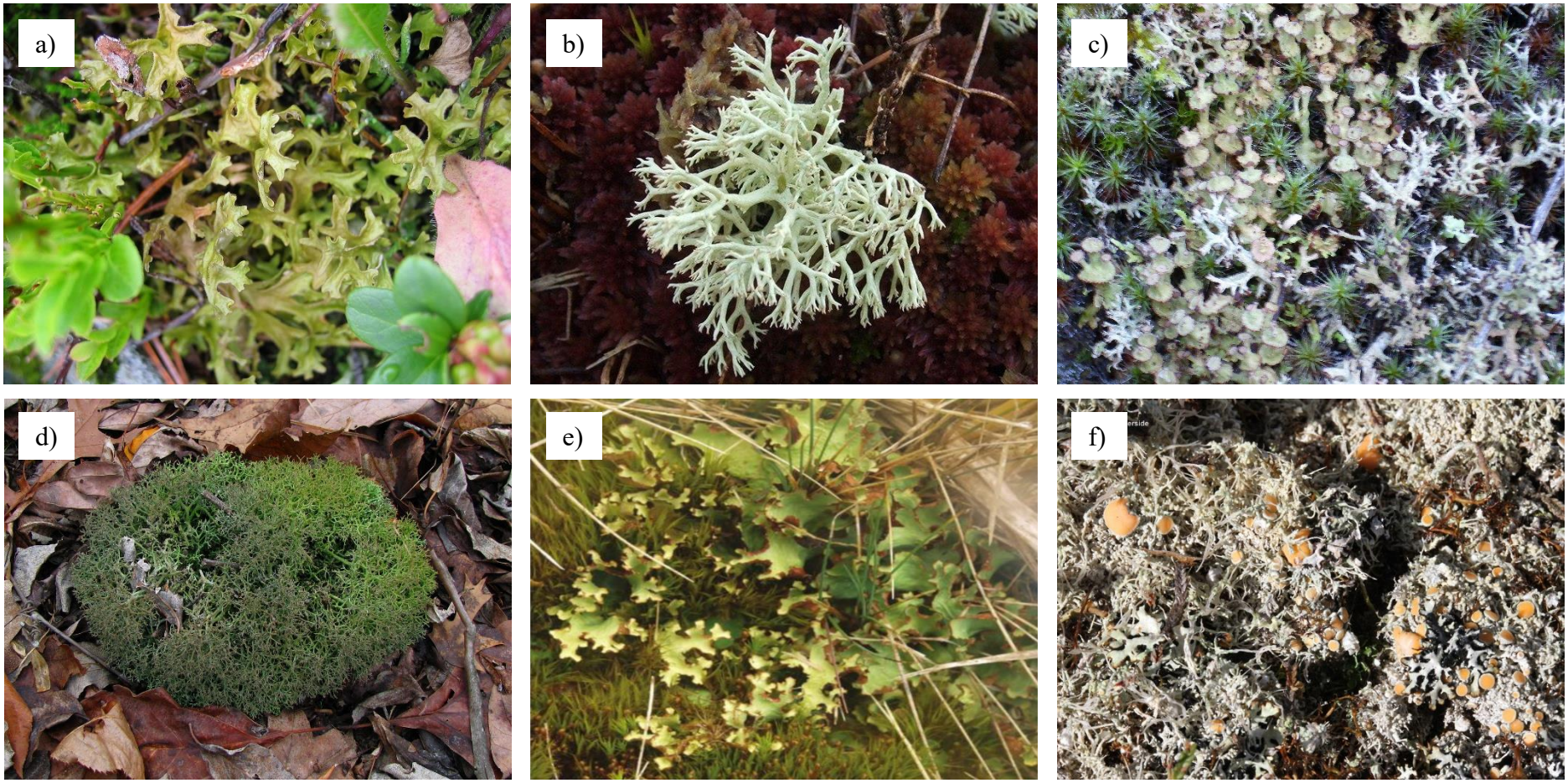

Fig. 4.6. a) Cetraria islandica, b) Cladonia arbuscula, c) Cladonia cervicornis, d) Cladonia furcata, e) Pseudocyphellaria freycinetii (Wikipedia) and f) Ochrolechia frigida (Images of British Lichens).

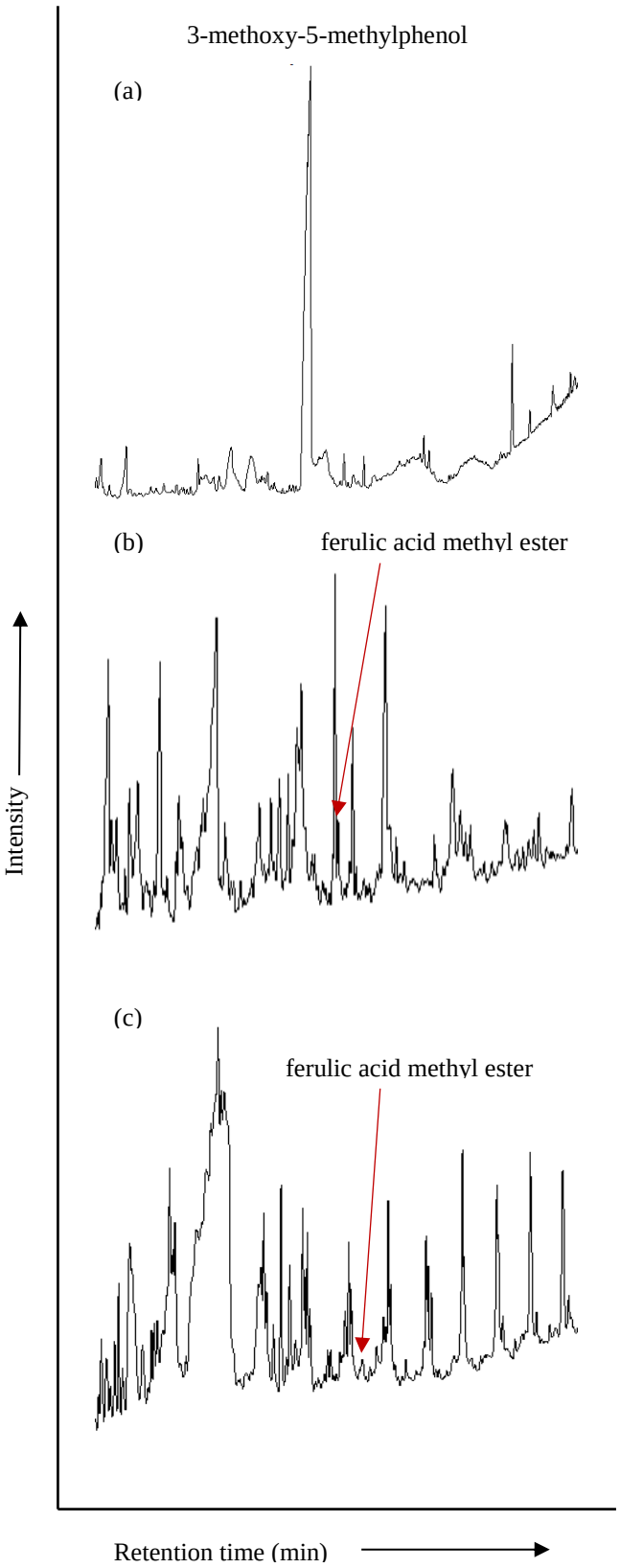

Fig. 4.7. Partial chromatogram for the total ion current (TIC) of the Pyrolysis-GCMS products from a) *Pseudocyphellaria freycinetii* (lichen) b) *Molinia caerulea* (graminoid) c) PDC surficial peat. Note absence of 3-methoxy-5-methylphenol from (b) and absence of ferulic acid methyl ester from (a).

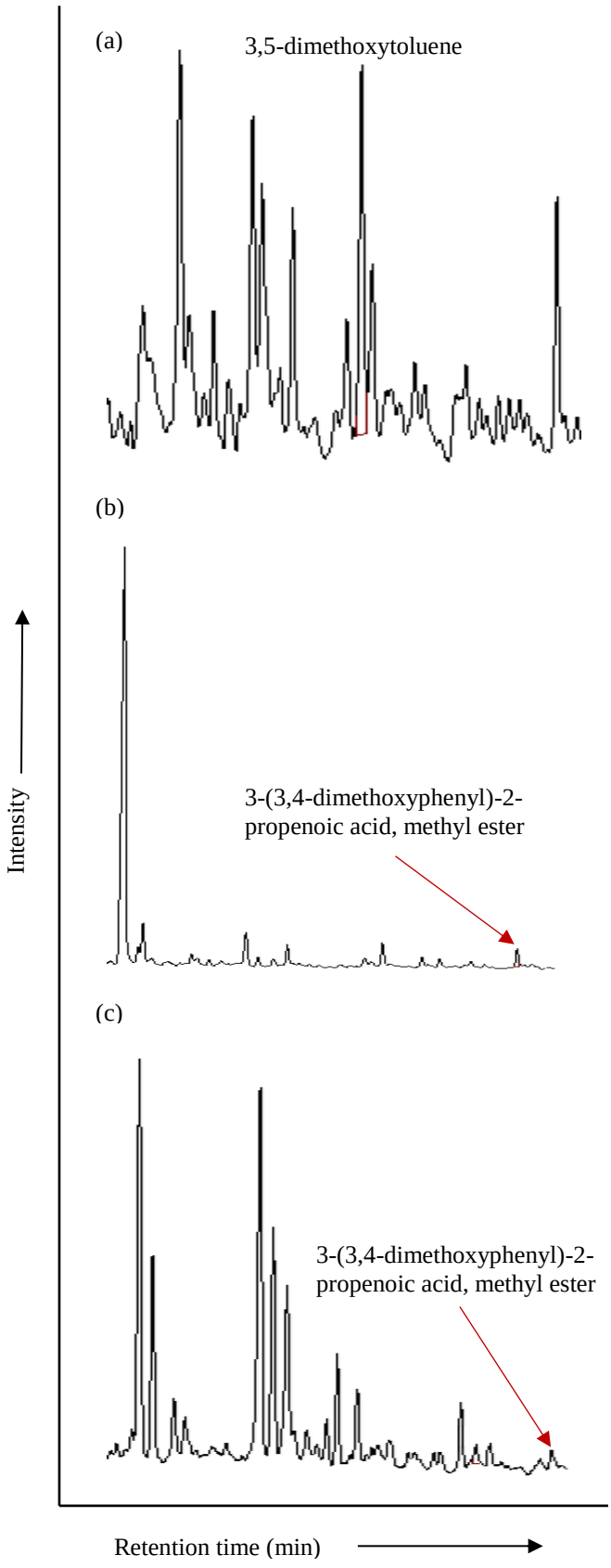

Fig. 4.8. Partial chromatogram for the total ion current (TIC) of the TMAH thermochemolysis products from a) *Pseudocyphellaria freycinetii* b) *Molinia caerulea* c) PDC surficial peat.

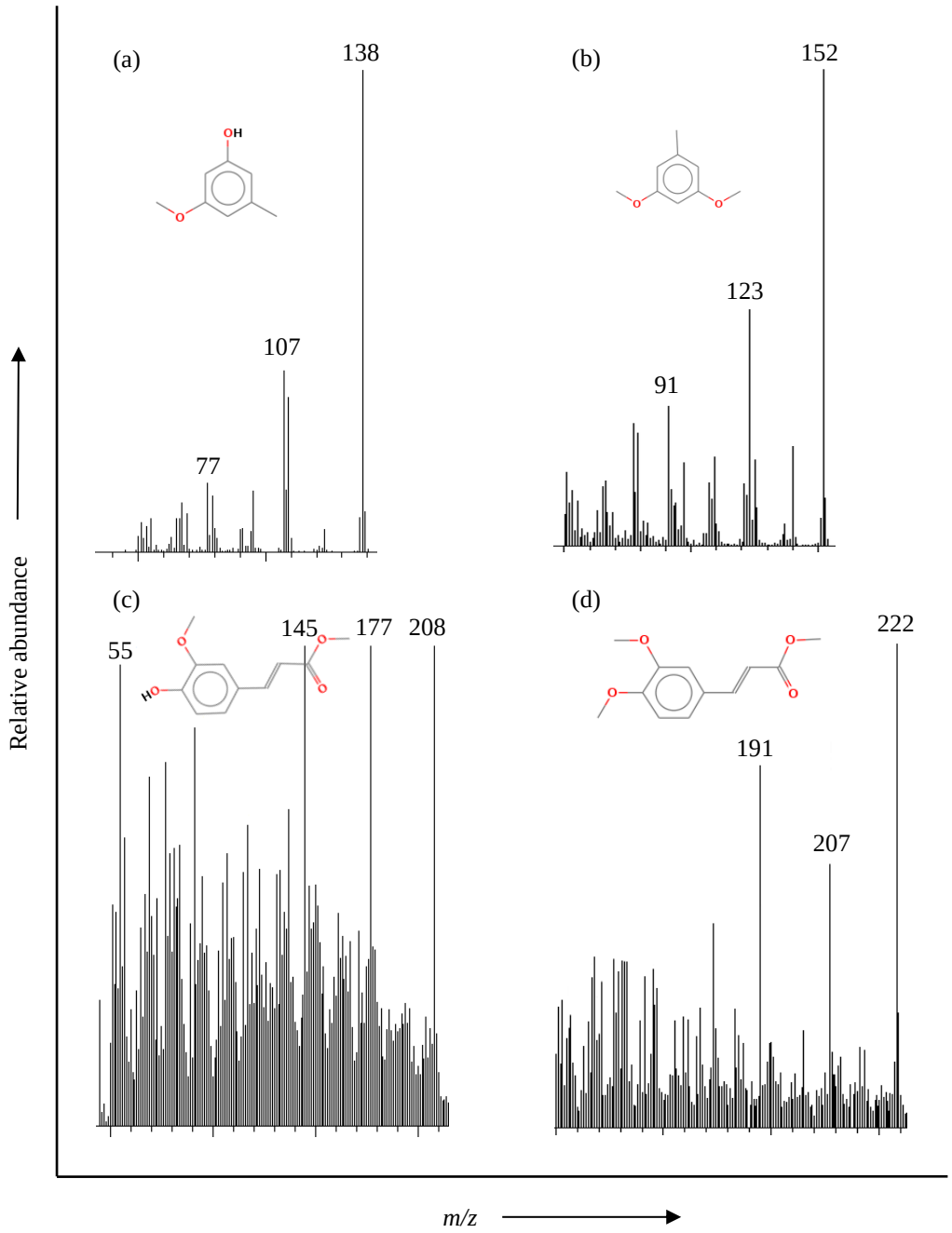

Fig. 4.9. The mass spectra of the Py-GCMS products (a, c) and TMAH thermochemolysis products (b, d): a) 3-methoxy-5-methylphenol, b) 3,5-dimethoxytoluene, c) ferulic acid methyl ester and d) 3-(3,4-dimethoxyphenyl)-2-propenoic acid, methyl ester.

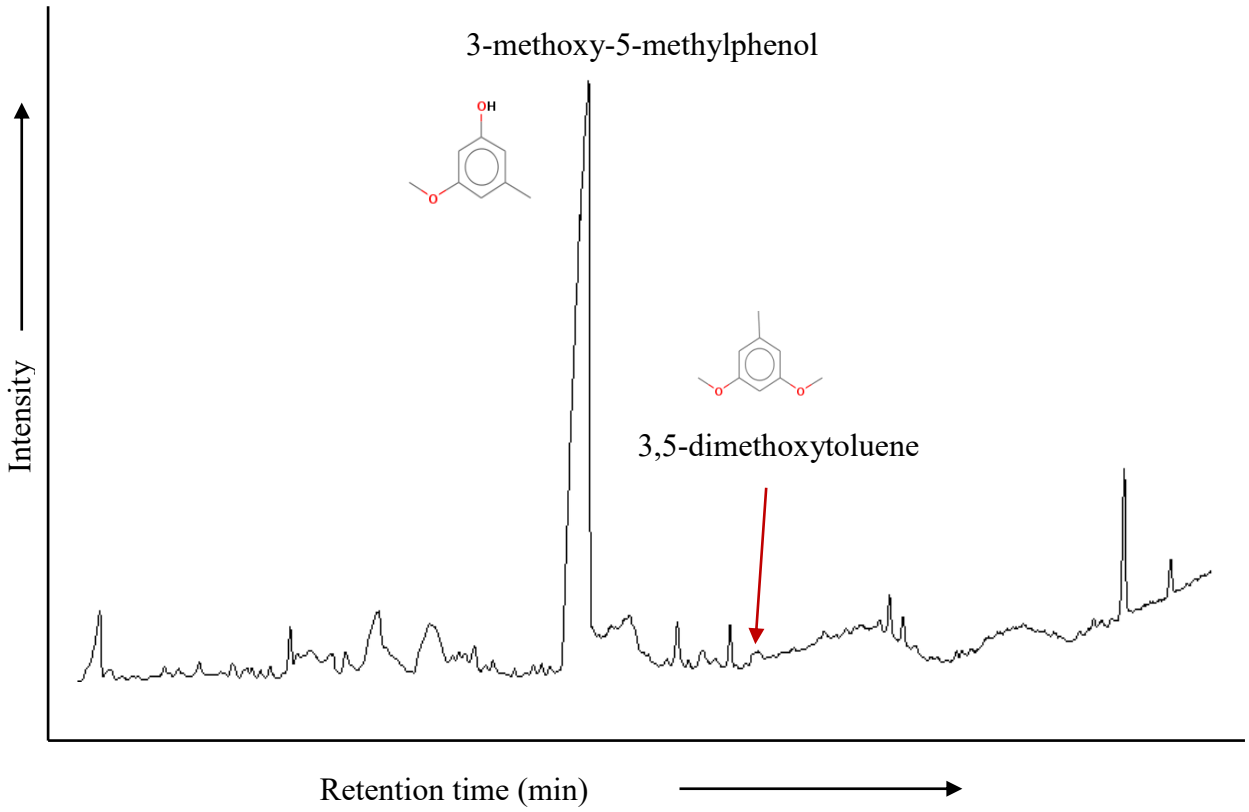

Fig. 4.10 Partial chromatogram for the total ion current (TIC) of the Pyrolysis-GCMS products from *Pseudocyphellaria freycinetii*.

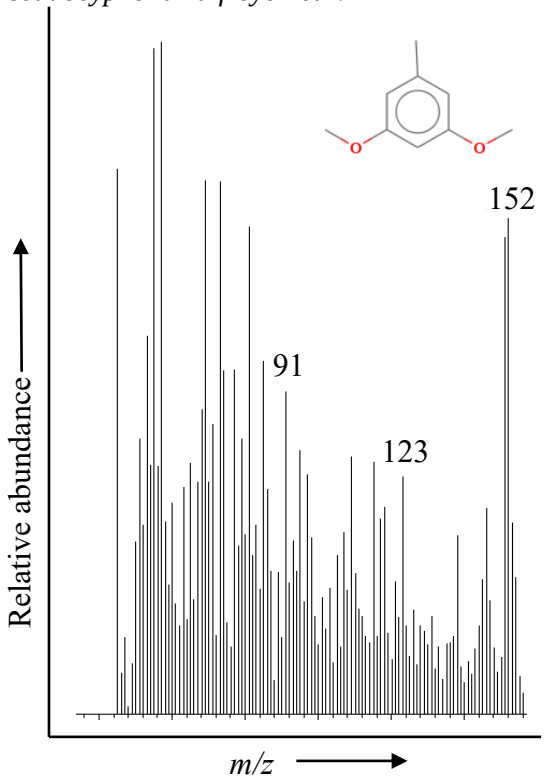

Fig. 4.11. The mass spectra of the tentatively assigned Py-GCMS product 3,5-dimethoxytoluene.

4.4. Conclusions

The significant correlation between the traditional Py-GCMS quantification method and the use of internal standard 5α-androstane suggests that the use of an internal standard for quantification is well suited to biomarker analysis using Py-GCMS, however in order for the method to be considered truly quantitative, it is necessary to calculate relative response factors for each individual compound. Tentative markers were suggested for lichens and graminoids, however the absence of the lichen marker from several of the sampled species indicates that the marker may only apply to certain lichen species. Both proposed markers were found to have a single source after ^{13}C-TMAH thermochemolysis, however the graminoid marker, ferulic acid methyl ester, was found to have a rapid turnover in bulk peat deposits, suggesting it may not be suitable for palaeoclimate studies.

Chapter 5 - Effects of experimental in situ microclimate warming on abundance and distribution of phenolic compounds in a *Sphagnum*-dominated peatland.

5.1. Introduction

Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are complex, high molecular weight lipids containing branched alkyl chains which occur ubiquitously in mineral soils and peats (Fig. 5.1; Sinninghe Damsté et al., 2000; Weijers et al., 2007a; Liu et al., 2010). brGDGT distribution in soil has been shown to depend primarily on air temperature and soil pH (Weijers et al., 2007a), making brGDGT records an effective palaeoenvironmental proxy through the application of two indices: i) the cyclisation ratio of branched tetraethers (CBT), the degree of cyclisation of the membrane lipids, which correlates with soil pH, and ii) the degree of methylation, expressed in the methylation index of branched tetraethers (MBT), which is dependent on mean annual air temperature (MAAT) and to a lesser extent on soil pH (Huguet et al., 2013). These indices have been successfully applied to the reconstruction of past air temperatures in a variety of different regions and environments through comparison with other proxies and instrumental records (Weijers et al., 2007b; Donders et al., 2009; Rueda et al., 2009; Peterse et al., 2011).

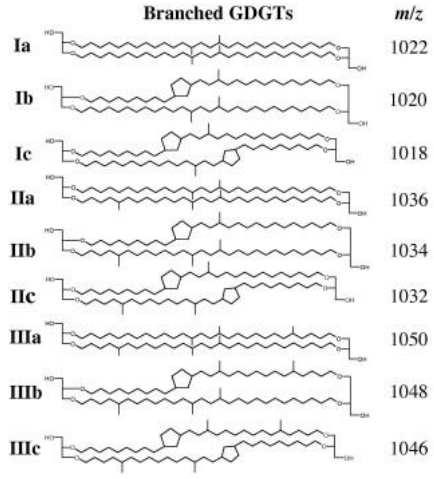

Fig. 5.1. Stuctures of branched glycerol dialkyl glycerol tetraether (GDGT) membrane lipids (Huguet et al., 2013).

Despite their widespread use as palaeoclimate proxies, few studies to date have been interested in the application of brGDGT-based proxies (MBT and CBT) to peatlands and their interaction with other environmental proxies such as abundance of *Sphagnum*-derived and lignin-derived (vascular plant) phenols, which can be used as a proxy for levels of oxidation of existing peat. Thermally assisted hydrolysis and methylation (THM) in the presence of tetramethylammonium hydroxide (TMAH) is an analytical method used to methylate phenols and phenolic acids in plant litter and soils, making them more amenable to GC analysis than

the equivalent Pyrolysis-GCMS products (Filley et al., 1999). The technique has been successfully used to characterise lignin-derived phenolic compounds in plant litter and soils (Mason et al., 2012), as well as *Sphagnum*-derived compounds in peat (Abbott et al., 2013). However, conventional TMAH thermochemolysis is unable to distinguish between methyl groups originally present, and those added during the THM process, meaning that tannin- and demethylated lignin-derived phenols can contribute to the intact lignin signal. In order to distinguish between original and added methyl groups, ^{13}C-labelled TMAH is used (Filley et al., 2006). Proxies developed from the use of TMAH thermochemolysis include the parameter for total lignin-derived phenols, Λ, obtained from the summed amounts of 8 dominant lignin-derived phenols (G4, G5, G6, S4, S5, S6, P18, and G18) normalised to 100 mg OC (Hedges and Parker, 1976; Hedges and Mann, 1979), and the corresponding parameter for total *Sphagnum*-derived phenols, σ, obtained from the summed amounts of sphagnum acid-derived phenols I, IIa, IIb and III, normalised to 100 mg OC (Abbott et al., 2013). The relative amounts of 'bound' sphagnum acid to 'bound' vascular plant phenols in peat moss and the surficial peat layers can be assessed from measuring the parameters σ and Λ using both ^{13}C-labelled and unlabelled TMAH thermochemolysis. This information can be used to gain a greater understanding of how different OM fractions are affected by changing climatic conditions, and study any interactions between developed proxies.

In this study, climatic change was simulated by an in situ warming experiment on a *Sphagnum*-dominated peatland (Jura Mountains, France) using a series of open top chambers (OTCs; Huguet et al., 2013). Temperature was artificially increased in half of the sampling plots, and was compared with control plots. Peat samples collected in 2010 and 2013 after 26 (t =1) and 62 (t =2) months of the warming experiment respectively, were extracted, then depolymerized using unlabelled and ^{13}C-labelled TMAH thermochemolysis. The results of this were compared to and discussed in the context of a previous experiment measuring brGDGT derived indices from the same experimental setup (Huguet et al., 2013).

5.2. Materials and Methods

5.2.1. TMAH thermochemolysis

^{13}C-labelled and unlabelled TMAH thermochemolysis was performed using a platinum filament coil probe pyrolysis unit (Chemical Data Systems, Oxford, USA). The Pyroprobe

5000 was coupled to a 6890 N GC and 5975B MSD GC/MS system from Agilent Technologies (Palo Alto, USA).

Approximately 1 mg of powdered extracted peat sample was weighed into a quartz pyrolysis tube (CDS Analytical, Oxford PA, USA) plugged with pre-extracted silica wool. An internal standard, 5α-androstane, and an aqueous solution of unlabelled or ^{13}C-labelled TMAH (25% w/w) was added to the sample immediately prior to THM.

Pyrolysis was performed at 610 °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 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 5000 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). Compound identification was based on the NIST98 mass spectral library, on ion fragmentation patterns and following the conventions described in other studies (van der Heijden, 1994; Clifford et al., 1995; Hatcher et al., 1995; Filley et al., 2000; Mason et al., 2009; Abbott et al., 2013).

5.2.2. Sampling

Peat cores were collected from the Frasne peatland (46° 49′ N, 6° 10′ E, 800 m above sea level; Delarue et al., 2011; Huguet et al., 2013), a *Sphagnum*-dominated mire located in the Jura Mountains in Eastern France. MAAT is 6.2 °C, with a winter average of -1.4 °C and a summer average of 14.6 °C. Annual precipitation varies from 1300-1500 mm yr^{-1}. For more detail, see section 2.6.2.

5.3. Results and Discussion

5.3.1. Effect of OTC treatment on air and soil temperature.

The OTC treatment was found to have a significant effect on both air and soil temperature. Mean annual maximal daytime temperature increased by around 2 °C between 2008 and 2010, with a 3 °C increase in spring and summer. This corresponded with an average air temperature increase of around 1 °C in summer. The OTC treatment also affected the peat moisture content, with the OTC plots being drier than the control (Huguet et al., 2013).

5.3.2. Effect of OTC treatment on brGDGT distribution.

A *t*-test showed that brGDGT distribution was significantly (P <0.05) affected by the OTC treatment, with higher MBT (degree of methylation) observed in OTC relative to control plots (Huguet et al., 2013).

5.3.3. Effect of OTC treatment on lignin- and Sphagnum-derived phenols.

5.3.3.1. Results after 26 months of warming experiment (t =1).

An increase in the parameters σ and Λ with depth was observed at the OTC site (Fig. 5.2). A decrease in σ and Λ was observed in surficial peats (5-7 cm depth) between control and OTC plots (Fig. 5.3).

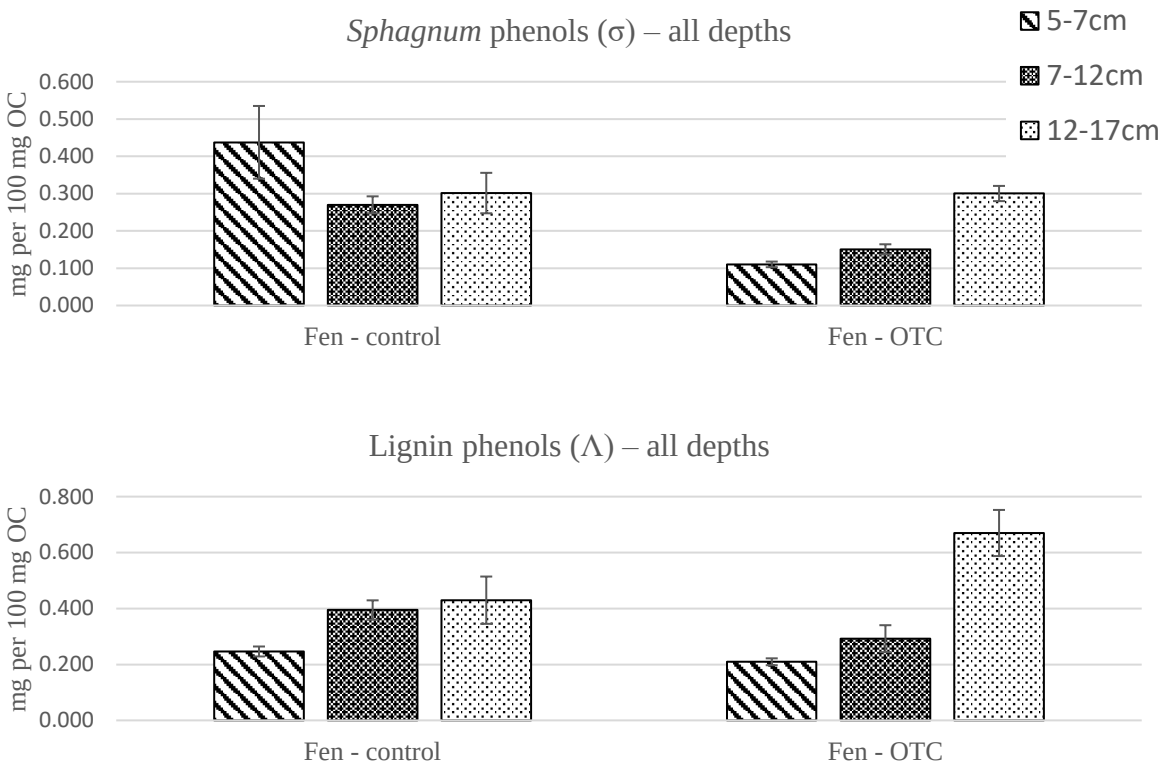

Fig. 5.2. Abundance of parameters σ and Λ expressed in mg 100 mg^{-1} OC for Fen control and OTC depth profiles. Error bars represent the standard error of the mean for three analytical replicates.

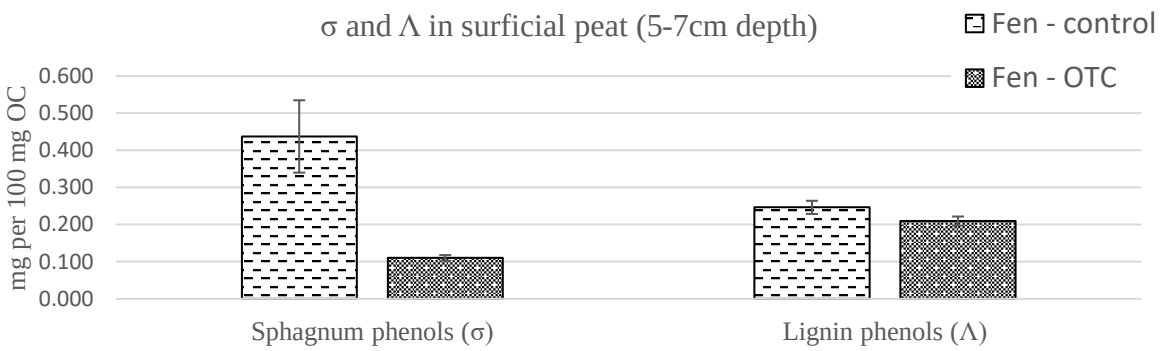

Fig. 5.3. Abundance of parameters σ and Λ expressed in mg 100 mg^{-1} OC for surficial peats (5-7cm depth) at Fen control and OTC sites. Error bars represent the standard error of the mean for three analytical replicates.

The observed decrease in σ and Λ in surficial peats suggests an accelerated oxidation due to drying and aeration of surficial peat layers as a result of increasing temperatures from the warming experiment. The increase in Λ and σ in deeper layers of peat at the OTC site suggests a possible preferential preservation of lignin phenols and sphagnum acid over other organic matter components such as carbohydrates. However, analysis of cellulose-derived carbohydrate components (Fabbri and Helleur, 1999; Fig. 5.4, 5.5), showed a similar trend to Λ and σ, in that cellulose-derived thermochemolysis products were observed to decrease in abundance at shallower depths from the control to the OTC site, with a defined increase with depth observed at the OTC site, suggesting a similar mechanism to that affecting lignin- and *Sphagnum*-derived products (fig. 5.6). It is possible that other carbohydrate-derived products, such as those derived from pectin or hemicellulose (Tanczos et al., 2003), may be being degraded at a greater rate than the products observed here, however data for these products was not available at the time of writing.

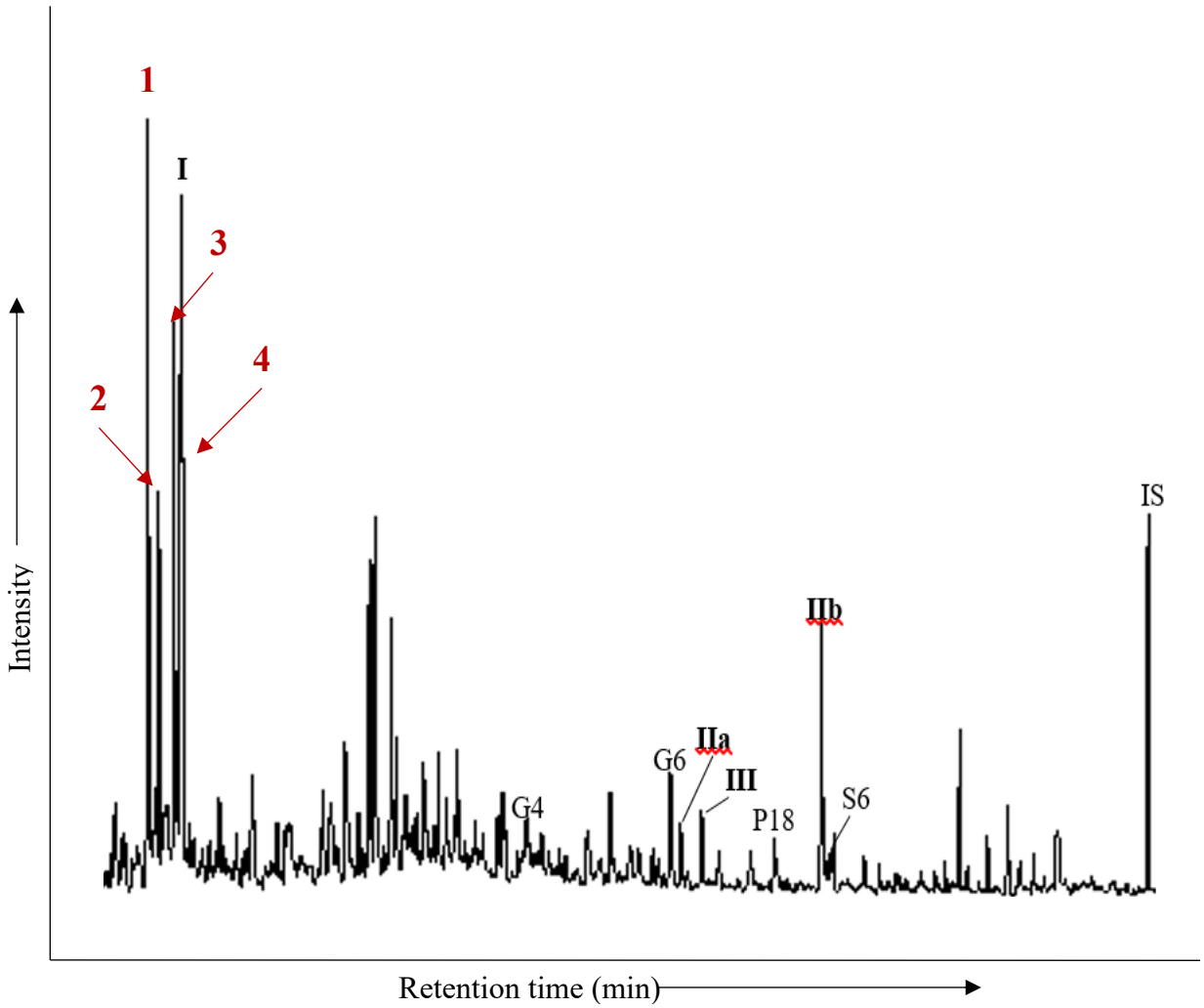

Fig. 5.4. Partial chromatogram for the total ion current (TIC) of the thermochemolysis products of cellulose (marked in red) as seen in a surficial peat sample.

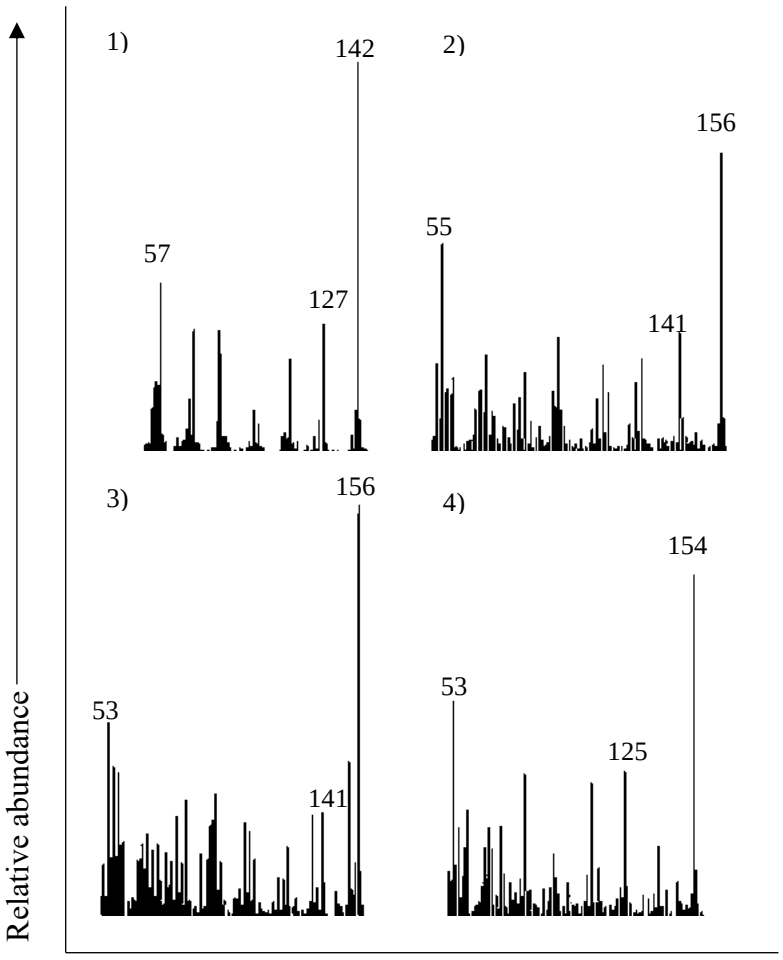

Fig. 5.5. Mass spectra of cellulose-derived TMAH thermochemolysis products 1, 2, 3 and 4.

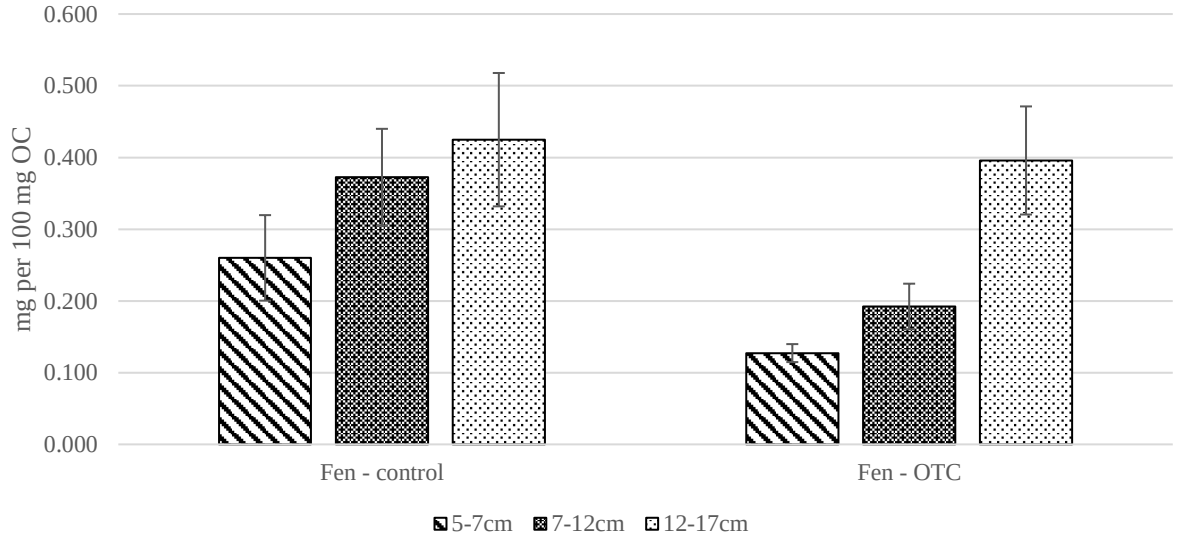

Fig. 5.6. Abundance of cellulose-derived TMAH thermochemolysis products expressed in mg 100 mg^{-1} OC for Fen control and OTC depth profiles. Error bars represent the standard error of the mean for three analytical replicates.

5.3.3.2. Results after 62 months of warming experiment (t =2).

An increase in σ was observed with increasing depth in the OTC plot at both t =1 and t =2. σ increased at both depth intervals between t =1 and t =2. No significant changes in σ abundance were observed at the control site with depth or time (Fig. 5.7). An increase in Λ was observed with increasing depth in the OTC plot at t =1 and t =2. Λ decreased in abundance significantly at both depth intervals between t =1 and t =2 at both the control and OTC plots (Fig. 5.8). SR% decreased with increasing depth at the OTC site at both t =1 and t =2. SR% almost doubled from t =1 to t =2 at both the control and OTC plot (Fig. 5.9). Ad/Al decreased with increasing depth at both t =1 and t =2 in the control plot, and increased with depth at t =2 in the OTC plot. Ad/Al decreased at both depth profiles in the control plot, but only in the 7-12 cm depth profile in the OTC plot (Fig. 5.10).

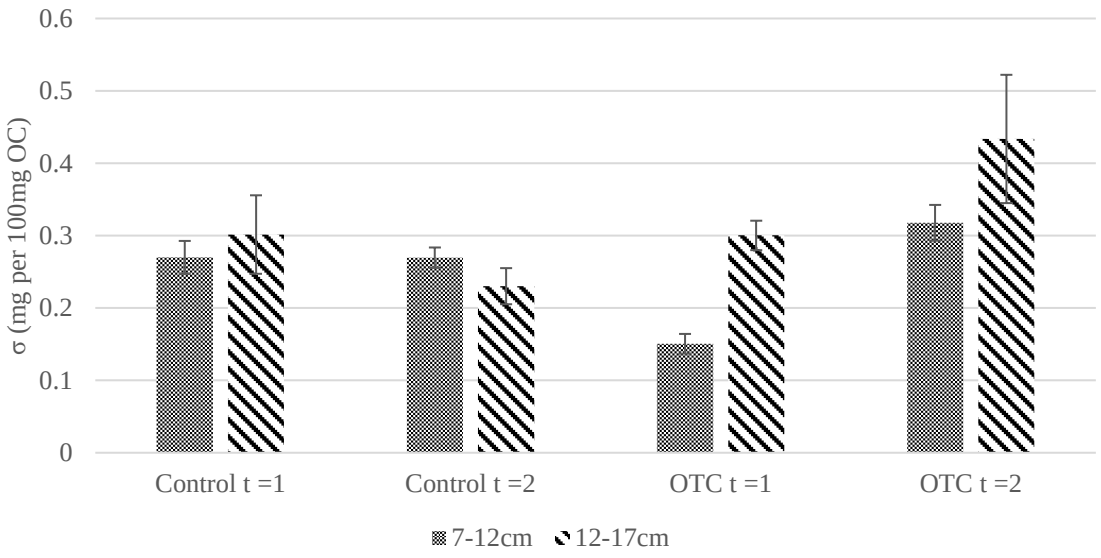

Fig. 5.7. Abundance of parameter σ expressed in mg 100 mg^{-1} OC for Fen control and OTC depth profiles. Error bars represent the standard error of the mean for three analytical replicates. 5-7cm section was unavailable for t =2 samples.

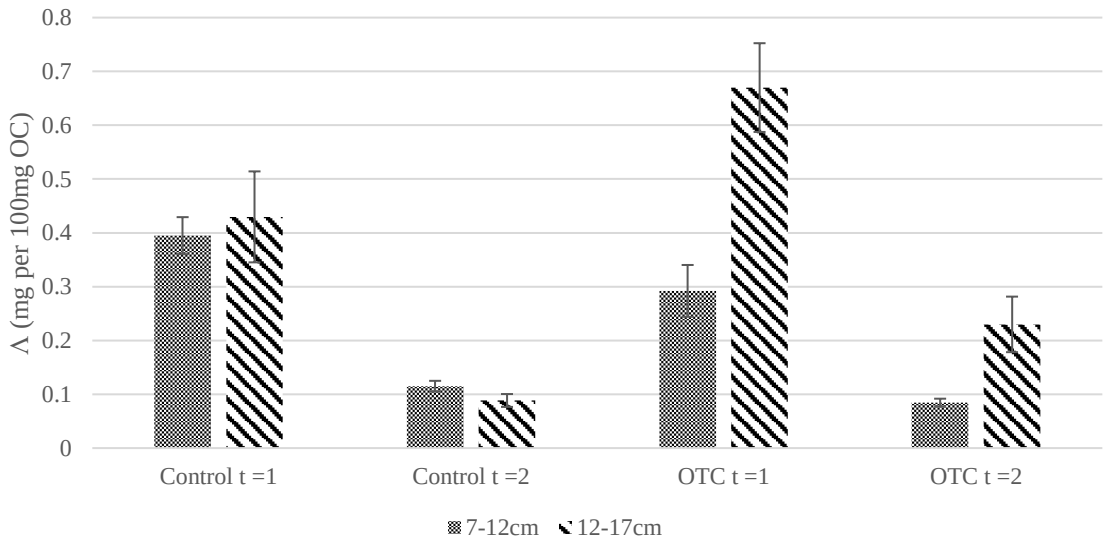

Fig. 5.8. Abundance of parameter Λ expressed in mg 100 mg^{-1} OC for Fen control and OTC depth profiles. Error bars represent the standard error of the mean for three analytical replicates. 5-7cm section was unavailable for t =2 samples.

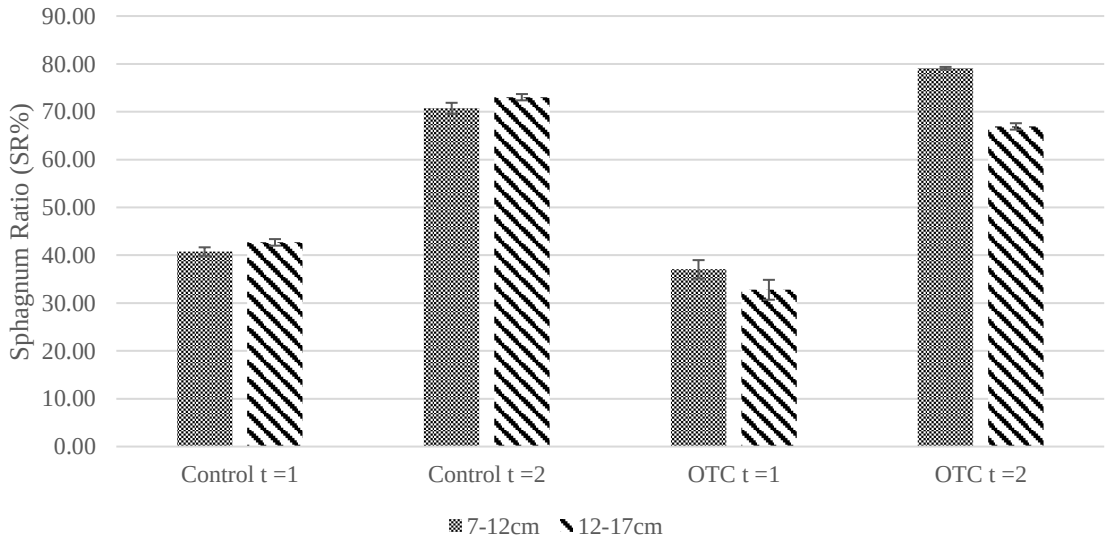

Fig. 5.9. Sphagnum ratio (SR%) expressed as percentage for Fen control and OTC depth profiles. Error bars represent the standard error of the mean for three analytical replicates. 5-7cm section was unavailable for t =2 samples.

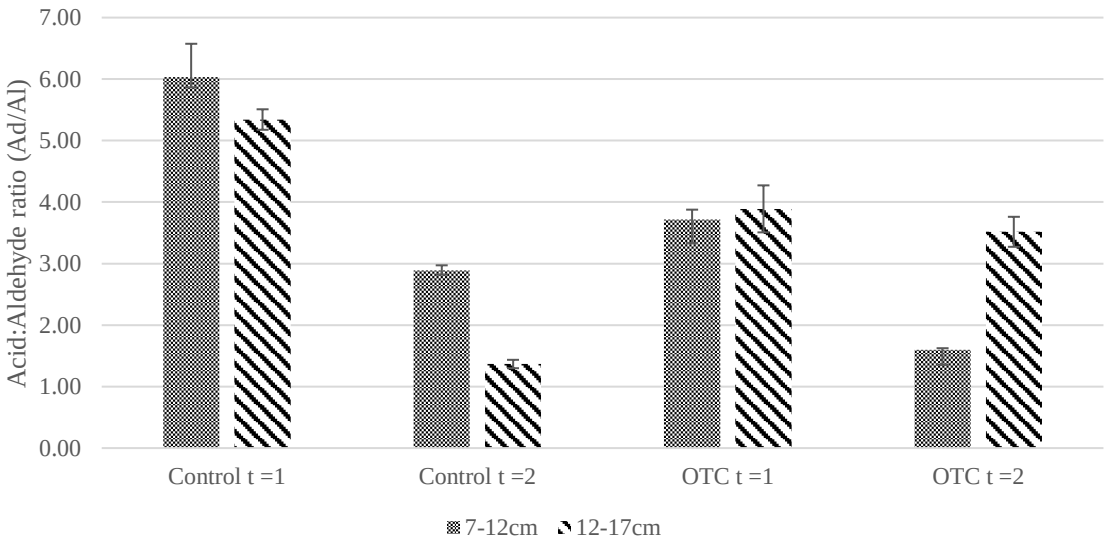

Fig. 5.10. Acid/Aldehyde ratio (Ad/Al) for Fen control and OTC depth profiles. Error bars represent the standard error of the mean for three analytical replicates. 5-7cm section was unavailable for t =2 samples.

As was observed at t =1, both σ and Λ were more abundant in deeper layers of peat at t =2, supporting the hypothesis that the drying and aeration effect of the OTCs is increasing degradation of OM components near the surface. The large increase in SR% indicated that a greater proportion of sphagnum acid-derived phenols appear to be preserved down the peat profile than lignin-derived phenols between t =1 and t =2 at both the control and OTC site. Therefore, as well as a possible preferential degradation of certain carbohydrates in deeper peat layers under warming conditions, as proposed from the results observed at t =1, it appears that lignin-derived phenolic products are also more readily degraded than sphagnum-acid derived components. This supports the proposal of Abbott et al (2013) that "bound" sphagnum acid remains stable in peat deposits not subject to drying and rewetting, as the aeration effect caused by the OTCs in this study does not extend to the deeper layers of peat. The proposed hypothesis that decreases in abundance of lignin- *Sphagnum*- and cellulose-derived products in surficial peat layers is due to a drying and aeration effect caused by increased air and soil temperature indicates that brGDGT-based proxies such as MBT can be used alongside lignin- and *Sphagnum*-based proxies in that elevated MBT, used as an indicator of air temperature, correlates with decreased abundances of Λ and σ in surficial peats. Carbohydrate abundance data for t=2 was not available at the time of writing.

5.4. Conclusions

Λ, σ, and cellulose-derived TMAH thermochemolysis products were all found to decrease in abundance in surficial peats when subjected to increased air temperatures as a result of the warming experiment. This is indicative of increased degradation of OM in the upper layers of peat as a result of increased drying and aeration of the acrotelm due to increased air and peat temperature. An increase in SR% from t =1 to t =2 suggests that sphagnum acid remains more stable than lignin-derived components in sections of peat that are not subject to a drying and rewetting cycle, indicating a longer residence time in older peat deposits. Due to the effect of air temperature on turnover of sphagnum acid- and lignin-derived components, the brGDGT-based proxy MBT can be used as a guide to the abundance of the parameters Λ and σ in peats.

Chapter 6 – Conclusions and Future Work

6.1. Conclusions

Analysis of a suite of *Sphagnum* moss samples from a variety of Northern hemisphere locations confirmed the specificity of the proposed marker compounds **I**, **IIa**, **IIb** and **III** to *Sphagnum* species. These TMAH thermochemolysis products were also found to have no alternative sources or precursors via ^{13}C-TMAH thermochemolysis, confirming their viability as biomarkers. Relative abundances of compounds **I, IIa, IIb** and **III** were found to differ between *Sphagnum* tissue and surficial peat deposits, suggesting that the different components of sphagnum acid degrade at different rates upon entry to the bulk peat deposit.

The significant correlation between the traditional Py-GCMS quantification method and the use of internal standard 5α-androstane suggests that the use of an internal standard for quantification is well suited to biomarker analysis using Py-GCMS, however in order for the method to be considered truly quantitative, it is necessary to calculate relative response factors for each individual compound. Tentative markers were suggested for lichens and graminoids, however the absence of the lichen marker from several of the sampled species indicates that the marker may only apply to certain lichen species. Both proposed markers were found to have a single source after ^{13}C-TMAH thermochemolysis, however the graminoid marker, ferulic acid methyl ester, was found to have a rapid turnover in bulk peat deposits, suggesting it may not be suitable for palaeoclimate studies.

Λ, σ, and cellulose-derived TMAH thermochemolysis products were all found to decrease in abundance in surficial peats when subjected to increased air temperatures as a result of the OTC warming experiment. This is indicative of increased degradation of OM in the upper layers of peat as a result of increased drying and aeration of the acrotelm due to increased air and peat temperature. An increase in SR% from t =1 to t =2 suggests that sphagnum acid remains more stable than lignin-derived components in sections of peat that are not subject to a drying and rewetting cycle, indicating a longer residence time in older peat deposits. Due to the effect of air temperature on turnover of sphagnum acid- and lignin-derived components, the brGDGT-based proxy MBT can be used as a guide to the abundance of the parameters Λ and σ in peats.

Overall, the thesis provides a review of a variety of ways to assess the impact of changing climatic conditions on peatlands, providing insight into the effects of changes in temperature and moisture levels on surficial peats, which may play a large role in the status of peatlands as

sinks or sources of carbon in years to come, and the impact this may have on future global climatic conditions.

6.2. Future Work

In order to truly assess any differences in relative abundances of sphagnum acid-derived markers between different *Sphagnum* species and different geographic locations, it will be necessary to analyse a much larger suite of samples, with a dedicated sampling methodology, for example analysing 100 *Sphagnum* tissue samples from at least 10 different species and 5 different locations, preferably multiple locations on each continent, subject to the presence of suitable peat deposits. Detailed analysis of such a large dataset would be likely to elucidate any true patterns in abundance of components **I, IIa, IIb** and **III**, and possibly give some insight into the mechanisms behind the apparent differing rates of degradation of the components in peat deposits.

In a similar vein, in order for 3-methoxy-5-methylphenol to be utilised as a lichen marker compound most effectively, it will be necessary to analyse a large suite of lichen samples from a variety of locations in order to determine which species 3-methoxy-5-methylphenol is found to be present in, and if there are any observable trends in ecosystem type, climatic conditions or ecological niche of the lichen species 3-methoxy-5-methylphenol is found in.

Access to a larger core and depth profile from the Frasne peatland would allow for a more detailed analysis of changes in *Sphagnum*- and lignin-derived parameters with depth, and combined with a detailed analysis of all available carbohydrate-derived thermochemolysis products, may help to clarify OM dynamics throughout the peat depth profile.

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Appendix

Appendix 1 – Aromatic hydroxyl content equations (from Filley et al., 2006; Mason et al., 2009)

% Hydroxyl(G6, G7, G8, G14, G15, G18)

$$= 100 \text{ x } \left[\frac{(M_{L+1} - (M_{L+1})_{calc})}{M_L + M_{L+1} - (M_{L+1})_{calc}}\right]$$

where

$$(M_{L+1})_{calc} = M_L \text{ x } \left(\frac{M_{UL+1}}{M_{UL}}\right) \text{ x } \left(\frac{N_L}{N_{UL}}\right)$$

% Hydroxl(G4)

$$= 100 \text{ x } \left[\frac{(M_{L+2})_{calc}}{(M_L)_{calc} + (M_{L+2})_{calc}}\right]$$

where

$$(M_L)_{calc} = \left(M_{L-1} \text{ x } \left(\frac{M_{UL}}{M_{UL-1}}\right) \text{ x } \left(\frac{N_L}{N_{UL}}\right)\right)$$

where

$$(M_{L+1})_{calc} = \left(M_L \text{ - } \left(M_{L-1} \text{ x} \frac{M_{UL+1}}{M_{UL}}\right) \text{ x } \left(\frac{N_L}{N_{UL}}\right)\right) \quad - \left(\left(M_{L+1} \text{ - } \left(M_{L+2} \text{ x} \frac{M_{UL+1}}{M_{UL}}\right) \text{ x } \left(\frac{M_{UL-1}}{M_{UL}}\right)\right) x \left(\frac{M_{UL-1}}{M_{UL}}\right)\right)\left(\frac{N_{L+1}}{N_{UL}}\right)\left(\frac{M_{UL+1}}{M_{UL}}\right)$$

where

$$(M_{L+2})_{calc} = M_{L+1} - \left(M_{L+2} \text{x} \frac{M_{UL-1}}{M_{UL}}\right) \text{x} \left(\frac{N_{L+1}}{N_{UL}}\right) - (M_{L+1})_{calc}$$

% 1 Hydroxyl(S6, S7, S8, S14, S15)

$$= 100 \text{ x} \left[\frac{(M_L)}{M_L + [M_{L+1} - (M_{L+1})_{calc}] + [M_{L+2} - (M_{L+2})_{calc}]}\right]$$

% 2 Hydroxyls(S6, S7, S8, S14, S15)

$$= 100 \text{ x} \left[\frac{M_{L+1} - (M_{L+1})_{calc}}{M_L + [M_{L+1} - (M_{L+1})_{calc}] + [M_{L+2} - (M_{L+2})_{calc}]}\right]$$

% 3 Hydroxyls(S6, S7, S8, S14, S15)

$$= 100 \text{ x} \left[\frac{M_{L+2} - (M_{L+2})_{calc}}{M_L + [M_{L+1} - (M_{L+1})_{calc}] + [M_{L+2} - (M_{L+2})_{calc}]}\right]$$

where

$$(M_{L+1})_{calc} = \left(M_L \text{ x} \left(\frac{M_{UL+1}}{M_{UL}}\right) \text{ x} \left(\frac{N_L}{N_{UL}}\right)\right)$$

where

$$(M_{L+2})_{calc} = \left([M_{L+1} - (M_{L+1})_{calc}] \text{ x} \left(\frac{M_{UL+1}}{M_{UL}}\right) \text{ x} \left(\frac{N_L}{N_{UL}}\right)\right)$$

% 1 Hydroxyl(S4)

$$= 100 \text{ x } \left[\frac{(M_{L-1})}{(M_{L-1}) + [(M_{L+1})_{calc}] + [M_{L+2} - (M_{L+2})_{calc}]}\right]$$

% 2 Hydroxyls(S4)

$$= 100 \text{ x } \left[\frac{(M_{L+1})_{calc}}{(M_{L-1}) + [(M_{L+1})_{calc}] + [(M_{L+2})_{calc}]}\right]$$

% 3 Hydroxyls(S4)

$$= 100 \text{ x } \left[\frac{(M_{L+2})_{calc}}{(M_{L-1}) + [(M_{L+1})_{calc}] + [(M_{L+2})_{calc}]}\right]$$

where

$$(M_{L+1})_{calc} = M_{L+1}$$

$$- \left[\left(M_L - M_{L-1} \text{ x } \left(\frac{M_{UL}}{M_{UL-1}}\right)\right) \text{ x } \left(\frac{M_{UL-1}}{M_{UL}}\right) \text{ x } \left(\frac{N_L}{N_{UL}}\right) + \left(M_{L+2} - M_{L+3} \text{ x } \frac{M_{UL+1}}{M_{UL}}\right) \text{ x } \left(\frac{M_{UL+1}}{M_{UL}}\right) x \left(\frac{N_{L+1}}{N_{UL}}\right)\right]$$

where

$$(M_{L+2})_{calc} = M_{L+2} - \left[M_{L+1} - \left(M_{L+2} - M_{L+3} \text{ x } \frac{M_{UL+1}}{M_{UL}}\right) \text{ x } \left(\frac{M_{UL+1}}{M_{UL}}\right) \text{ x } \left(\frac{N_{L+1}}{N_{UL}}\right) \text{ x } \left(\frac{M_{UL}}{M_{UL-1}}\right)\right.$$

Appendix 2 - Schellekens, J., Bradley, J.A., Kuyper, T.W., Fraga, I., Pontevedra-Pombal, X., Vidal-Torrado, P., Abbott, G.D., Buurman, P., 2015. The use of plant-specific pyrolysis products as biomarkers in peat deposits. Quaternary Science Reviews 123, 254-264.

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

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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-aandrostane) 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^2¼ 0.72, P< 0.0005, n¼ 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 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 e contrary to n-alkanes and lignin phenols e 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 (pyrolysisGC/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; Gonzalez 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-

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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-a-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 graminoiddominated 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; Horak-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; Horak-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 ‘Capoes~ ’ (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 ºC 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 e University of Sao~ 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 ºC; except for the Filament pyrolysers (650 ºC; 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 ºC. The GC oven was heated from 50 to 320 ºC (held 10 min) at 15 ºC min^{1}. The GC instrument was equipped with a UltraAlloy-5 column (Frontier Laboratories LTD.), length 30 m, thickness 0.25 mm, diameter 0.25 mm. The MS was scanning in the range of m/z 45e600.

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 mm). 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	5453 S, 6720 E	20	600	5				
HRB1						0e340	0e3.9	15	Sphagnum
HRB2						340e540	3.9e5.7	17	Sphagnum/graminoids
HRB3						540e850	5.7e13.3	31	Graminoids/woody species
Rodmossamyran (RMM)	Northern Sweden€	6347 N, 2020 E	40	650	2e3	0e255	e	53	
Lawn									Sphagnum
Forest									Sphagnum/woody species
Konigsmoor (KM)€	Harz Mountains, Germany	5145 N, 1034 E	730	790	8	0e80	e	42	Sphagnum
Penido Vello (PVO)	Galicia, Spain	43º32o0N, 7º30o0W	780	1600	8.8	0e300	0e8	101	Graminoids
Pena da Cadela (PDC)	Galicia, Spain	4330o0N, 733o0W	970	1800	7.5	0e185	0e5.3	34	Graminoids
Pau de Fruta (PF)	Minas Gerais, Brazil	18º15o0S, 43º40o0W	1350	1500	18.7	0e398	0e9	44	Graminoids/Capoes~[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 (Horak-Terra et al., 2014; Schellekens et al., 2014). [b] Mean annual precipitation.
[c] Mean annual temperature.
[d] n ¼ number of samples analysed with analytical pyrolysis. [e] The word 'Capoes~' does not refer to a specific botanical composition but indicates a group of trees within an open landscape.

was 40 ºC and the heating rate was 7 ºC min^{1}. The final temperature, 320 ºC, was maintained for 20 min. The GC column was connected to a Fisons MD800 mass spectrometer (m/z 45e650, cycle time 1 s).

Pt filament coil probe pyrolysis (Chemical Data Systems, Oxford, USA) was performed at 650 ºC for 2 s (heating rate 10 ºC ms^{1}). The GC oven was heated from 40 to 320 ºC (held 10 min) at 7 ºC min^{1}. The pyroprobe interface was maintained at 320 ºC. 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 mm). 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 mm film thickness; J&W Scientific, USA). Product detection was carried out using an HP5972 series mass selective detector in full scan mode (m/z 50e700).

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-a-androstane), and the depth record of 4isopropenylphenol compared between both quantification approaches. For quantification, peat samples were weighed (ca. 2 mg) and a known amount (3 mL) of 5-a-androstane was added as internal standard prior to pyrolysis. Mass yield of 4isopropenylphenol 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.

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

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}¼ 0.72, P < 0.0005, n ¼ 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 wellestablished 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

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

[a] Numbers correspond to pyrolysis products provided in Table 4.

b c aKM¼ aerial parts; b¼ Konigsmoor (Harz Mountains, Germany); RMM€ ¼ roots; c ¼ mixture of roots and aerial parts.¼ Rodmossamyren (Sweden); TF€ ¼

National Park Tierra del Fuego (Tierra del Fuego, Argentina); X ¼ 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	e	63	e	12
	RMM	e	e	53	e
	KM	e	e	42	e
	PVO	e	101	e	e
	PDC	34	e	e	e
	PF	e	e	42	e
Plant	HRB	9	e	14	e
	RMM	e	e	6	e
	KM	e	e	4	e
	PVO	e	20	e	e
	PDC	12	e	e	e

[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

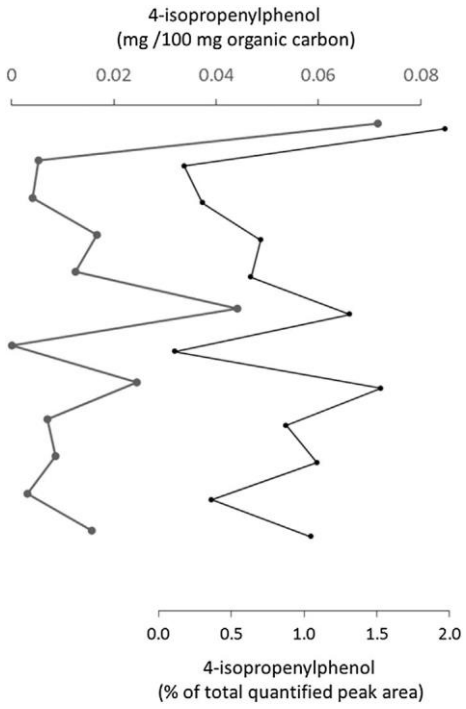

Fig. 1. Depth records of 4-isopropenylphenol obtained with internal standard and as % of the total quantified peak area.

record of 3-methoxy-5-methylphenol in the studied peatlands will be further discussed in Section 3.4.4.

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 3methoxy-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 4isopropenylphenol 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 a
less specific (common pyrolysis products of soil OM), though applicable as marker in one of the peatlands.

	Compound	Fragment ions[a]	Mb[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]	e	e	Vascular plants
11	Ferulic acid methyl ester	208, 177, 145, 117, 89, 77	208	Graminoids
12	Diterpene derivatives[e]	e	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	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)	e

[a] Decreasing intensity. [b] Mb 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 nonSphagnum mosses, including 4-(2-phenylethenyl)phenol, a dihydroxypolyaromatic compound, 2,3-dihydro-2-methyl-4phenylbenzofuran, and a polyaromatic N-containing compound

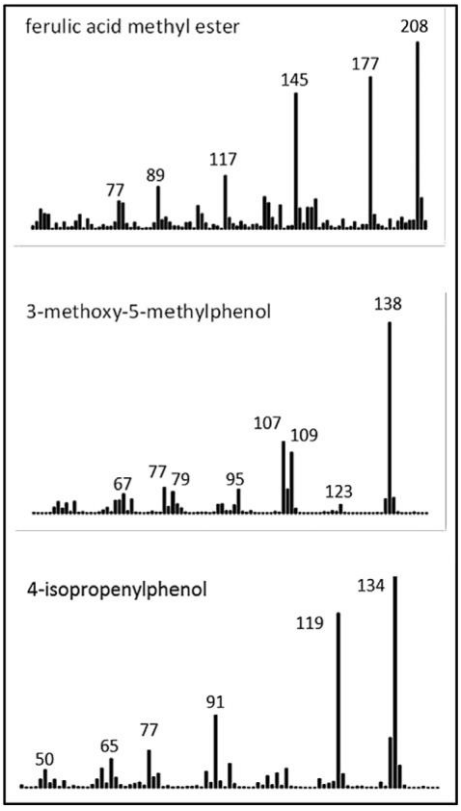

Fig. 2. Mass spectra of ferulic acid methyl ester, 3-methoxy-5-methylphenol, and 4isopropenylphenol.

(compounds 6e9, 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.

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 lignincarbohydrate 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 (0.04e0.14% TIC) and peat pyrolysates (<0.07% TIC) was

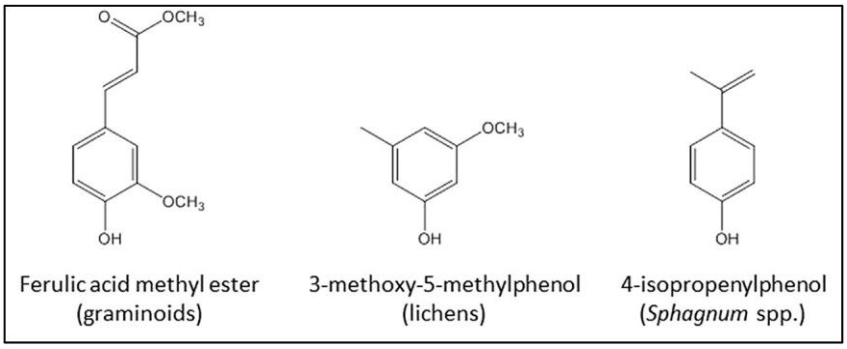

Fig. 3. Structure formula for the marker of lichens, graminoids and Sphagnum.

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	1
HRB1		e e		þ þ	þ þ	þ þ		þ þ	þ þ			e e		e e	þ[aa]	þ þ		e
HRB2		þ e		e þ	e þ	þ e		þ e	þ e			þ e		e e	þ[a] þ	þ e		þ
HRB3		e e		þ e	þ e	e e		e e	e e			e e		e þ	e	e þ		þ
RMM		e e		e e	e e	e e		þ e	e e			e e		e e	þ[a] þ	e e		e
KM	þ þ		þ þ				þ þ			þ þ	e þ		e þ		þ e		þ þ	þ
PVO	þ þ		þ þ				þ e			þ þ	þ þ		þ e				þ e	e
PDC	þ þ		þ þ				e þ			þ þ	þ þ		þ[b] þ				þ þ	þ
PF	þ þ		þ þ				þ þ			þ þ	þ þ		þ þ				þ þ	þ

[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 (4hydroxybenzyl 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; Munoz-~ 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 $d^{13}C$ and $d^{15}N$ isotopes, C/N ratio, ash content, inorganic geochemistry and pollen data (Horak-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, $d^{13}C$ and $d^{15}N$ isotopic signatures and UVabsorption (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-5methylphenol (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.

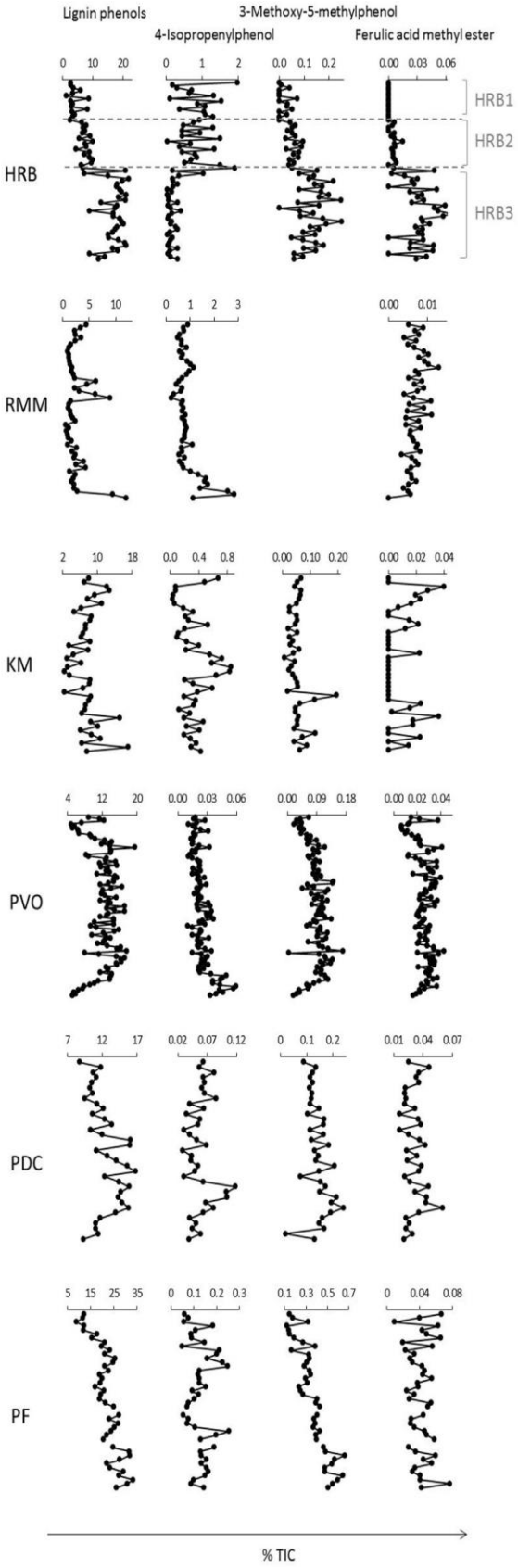

Fig. 4. Depth records of markers for lignin (methoxyphenols), Sphagnum spp. (4isopropenylphenol), lichens (3-methoxy-5-methylphenol) and graminoids (ferulic acid methyl ester) for the different peatlands.

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 (0e3% TIC) was generally an order of magnitude higher compared to graminoiddominated peat (0e0.3% 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 4isopropenylphenol, 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 graminoiddominated 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 nonSphagnum 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-5methylphenol, 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.

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

Peatland	4-Isopropenylphenol	3-Methoxy-5Methylphenol	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)	e	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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