The use of molecular chemistry (pyrolysis-GC/MS) in the environmental interpretation of peat

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Thesis

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Summary

The molecular composition of organic matter in peatlands reflects local conditions and stores information about botanical composition (plant source) as well as the degree of and conditions during decomposition. A reliable hydrological (and hence palaeoclimatological) interpretation of source and decomposition proxies in peatlands requires the understanding of the interactions between decomposition and botanical composition and the reaction of both to changes in the water table. Only few studies combined vegetation and decomposition characteristics to investigate peat OM dynamics and reconstruct environmental changes, and these included only a limited number of samples. In order to study the three-way relationship between botanical composition, decomposition and environmental conditions, pyrolysis-GC/MS of high-resolution sampled peat cores was used on two contrasting ombrotrophic peatlands. The two sites comprise a *Sphagnum*-dominated peatland in Tierra del Fuego (Argentina) and a graminoid-dominated peatland in Galicia (Spain). The purpose of this study was to examine the use of analytical pyrolysis in the environmental interpretation of peat profiles and to gain an improved understanding of peat OM dynamics and thereby develop parameters that reliably reflect past environmental conditions.

The molecular composition of the *Sphagnum*-dominated Harberton peatland was first explored for a selection of samples. Several peat fractions have been analysed, including NaOH-extract, NaOH-insoluble residue and bulk sample. A large number of pyrolysis products was identified and quantified for all peat samples; additionally total C, N and ash content were measured. Molecular parameters were identified by a systematic methodological approach. Peatland plants have been analysed in search for specific markers. Factor analysis was used to reduce the number of pyrolysis products for quantification and to assess differences in peat chemistry and relate these to environmental factors. This resulted in a number of molecular parameters that reflect botanical changes, (an)aerobic decomposition and fire incidence.

The results of the different peat fractions and plant analysis were applied to a large number of bulk samples (67), and used to reconstruct the 12,000 years of vegetation history of the Harberton core, which was then interpreted in terms of past hydrological conditions. The C:N ratio showed a perfect agreement with the botanical composition according to pyrolytic plant markers. It appeared that the *n*-alkane distribution showed remarkable changes with respect to shifts in the abundance of *Sphagnum*. Consequently, the detailed vegetation reconstruction was used to discuss the application of the *n*-alkane distribution in ombrotrophic peat. The simultaneous effects of botanical shifts (source material) and decomposition may cause conflicting hydrological interpretations for non-specific plant markers such as *n*-alkanes. The results indicated a considerable effect of aerobic decomposition on the distribution of *n*-alkanes. Although pyrolysis is not the regular method to establish the *n*-alkane distribution, comparison of *n*-alkanes in the NaOH-extract and residue peat samples supported the findings. The results emphasise the importance of combining vegetation and decomposition characteristics.

For the graminoid-dominated Penido Vello peatland, all 101 bulk peat samples of the 3 m thick peat deposit have been analysed. In addition to the search for specific compounds of peatland plants, the same pyrolysis products were quantified for plant pyrolysates and peat. Depth records of plant markers agreed well with their preferential habitat, with the degree of decomposition and with the transition from minerotrophic to ombrotrophic peat. Factor analysis was used to reconstruct past hydrology. The results showed the importance of high-resolution sampling through the higher correlations between molecular parameters in the part sampled each 2 cm compared to 5 cm. Furthermore, good correlations of pyrolysis results were found with mineral content, solid-state ^{13}C CPMAS NMR data and total N.

The hydrological reconstruction based on depth records of plant markers and factor scores obtained by factor analysis of all quantified pyrolysis products, and the low contribution of *Sphagnum* to the graminoid-dominated Penido Vello peat allowed studying the effects of source and decay on the lignin composition. A large number of lignin pyrolysis products were quantified for peatland plants and for the three different peat fractions for a selection of 15 samples. Lignin composition of woody and graminoid plant species were compared as well as the lignin composition in different peat fractions, and their relation to source or decay was established.

To determine whether the lignin parameters, derived from analysis of plants and peat fractions, can be used to reconstruct peat environment, they were applied to all 51 bulk peat samples of the upper meter of the Penido Vello core. The results show a strong effect of vegetation type and anaerobicity on generally-used lignin decomposition proxies, which indicates that such proxies are not reliable without information on the context.

To examine the interaction between vegetation type and decomposition processes, the results of *Sphagnum*-dominated peat and graminoid-dominated peat were combined. In addition to the well-known pyrolytic marker from *Sphagnum* (4-isopropenylphenol), depth records of pyrolysis products specific for lichens and graminoids functioned well in both peatlands and are considered reliable markers for application in other peatlands. A number of other identified markers only functioned well within one of both peatlands. The different functioning of these markers in the two peatlands emphasises the importance of plant analysis prior to the use of pyrolytic biomarkers with low specificity, but also shows that supposedly non-specific pyrolysis products can be specific within a certain peatland ecosystem. The use of different peat fractions (NaOH-extractable and non-extractable peat) provided information on the degree of decomposition and allowed comparison with studies on peat humic acids and humin, and the generally applied colorimetric method to determine peat humification. Furthermore, the comparison of pyrolysates of extracts and residues for a selection of peat samples, in combination with factor analysis, allowed separation of the effects of source, and several stages of aerobic and anaerobic decay. Degradation of lignin-cellulose (vascular plants) and polyphenol-cellulose (*Sphagnum*) is discussed using the abundance of their markers (lignin moieties, levoglucosan and 4-isopropenylphenol) in pyrolysates of the different peat fractions, and gives insight into degradation mechanisms. The results indicated that, in *Sphagnum* litter, polyphenols are more easily degraded than polysaccharides. Comparison of pyrolysis results and C:N ratio showed that differences in litter quality between *Sphagnum* and vascular plants is a major factor that determines the variance of C:N with depth. Large part of the variation in C:N in *Sphagnum*-

dominated peat is caused by decomposition rather than small increases of vascular plants upon drier conditions, while in graminoid-dominated peat this is not true and several processes may disturb the effect of mass loss on the C:N ratio. The C:N ratio can thus be a consistent decomposition proxy in *Sphagnum*-dominated peat but not in graminoid-dominated peat. Pyrolysis-GC/MS in combination with the applied research design provided detailed chemical information that gave insight in both vegetation and decomposition characteristics. This methodology thus has a high potential for the reconstruction of past environmental conditions.

Samenvatting

De moleculaire samenstelling van veen weerspiegelt lokale omstandigheden en bevat informatie over de soortensamenstelling van de vegetatie als ook over de mate van en condities tijdens afbraak. Een betrouwbare hydrologische (en daarmee paleo-klimatologische) interpretatie van plant- en afbraakindicatoren in veen vereist het begrip van de wederzijdse interacties tussen afbraak, botanische samenstelling, en de reactie van beide op veranderingen in het waterpeil. Er zijn maar weinig studies die vegetatie en afbraakeigenschappen combineren in onderzoek naar de dynamiek van organische stof en omgevingsveranderingen in venen, en deze studies gebruiken maar een beperkt aantal monsters. Om de driehoeksrelatie tussen botanische samenstelling, afbraak en veranderingen in omgevingsfactoren te bestuderen is pyrolyse-GC/MS toegepast op met hoge resolutie bemonsterde boorkernen van twee uiteenlopende ombrotrofe venen. Een daarvan is gedomineerd door veenmos (*Sphagnum*) en gelegen in Harberton, Vuurland (Tierra del Fuego, Argentinië), de ander is een door grassen en zegge-achtigen (graminoiden) gedomineerd veen uit Penido Vello, Galicië (Spanje). Het doel van deze studie is om het gebruik van analytische pyrolyse voor de omgevingsinterpretatie van veenprofielen verder te onderzoeken en een verbeterd begrip te verkrijgen van veendynamiek, en daarmee het ontwikkelen van parameters voor de reconstructie van omgevingscondities.

Ten eerste is een aantal monsters van het door *Sphagnum* gedomineerde profiel geselecteerd om de moleculaire samenstelling van het veen te verkennen. Hierbij zijn verschillende veenfracties geanalyseerd, waaronder NaOH-extracten, de niet extraheerbare residuen, en onbehandelde totaalmonsters ('bulk' monsters). Een groot aantal pyrolyseproducten is geïdentificeerd en gekwantificeerd voor deze veenmonsters; daarnaast is het koolstof- (C) en stikstofgehalte (N) en de hoeveelheid anorganisch materiaal bepaald. Moleculaire parameters zijn geïdentificeerd door middel van een systematische methodische benadering. Om plant-specifieke pyrolyseproducten (markers) te zoeken zijn veenplanten geanalyseerd. Factoranalyse is gebruikt om het aantal te kwantificeren pyrolyseproducten te verminderen, en om de chemische verschillen tussen monsters te beoordelen en in verband te brengen met omgevingsfactoren in het verleden. De resultaten hiervan hebben geleid tot een aantal moleculaire parameters welke veranderingen in botanische samenstelling, (an)aerobe afbraak en brand aangeven.

De resultaten van analyse van veenplanten en de verschillende veenfracties zijn toegepast op een groot aantal totaalmonsters (67), en gebruikt om de vegetatiegeschiedenis van de 12,000 jaar oude Harberton-kern te reconstrueren, welke vervolgens hydrologisch geïnterpreteerd is. De C:N-verhouding bleek in perfecte overeenstemming met de botanische samenstelling volgens de moleculaire plantenmarkers. De *n*-alkaan verdeling liet opmerkelijke veranderingen zien in relatie tot verschuivingen in de bijdrage van *Sphagnum*. Als gevolg daarvan is een gedetailleerde vegetatiereconstructie gebruikt om het gebruik van *n*-alkaan verdelingen in ombrotroof veen te bediscussiëren. De gelijktijdig optredende effecten van botanische veranderingen (uitgangsmateriaal) en afbraak kunnen tot tegenstrijdige hydrologische interpretaties leiden voor niet-specifieke plantmarkers zoals *n*-alkanen. De resultaten wijzen op een aanzienlijk effect van aerobe afbraak op de concentratie van *n*-alkanen. Hoewel pyrolyse niet de meest voor de hand

liggende methode is om *n*-alkaan verdelingen te bestuderen, werden de resultaten bevestigd door vergelijking van de *n*-alkaan verdeling in NaOH-extract en residu van veenmonsters. De resultaten benadrukken het belang om vegetatie en afbraakkarakteristieken te combineren.

Voor het door graminoiden gedomineerde Penido Vello veen zijn alle 101 totaalmonsters geanalyseerd voor de 3 m dikke boorkern. Naast het zoeken naar plant-specifieke pyrolyseproducten is voor veenplanten dezelfde set pyrolyseproducten als voor het veen gekwantificeerd. Diepteprofielen van plant-specifieke pyrolyseproducten kwamen goed overeen met zowel de preferentiële habitat, de mate van afbraak, alsook met de overgang van minerotroof (grondwater-gedomineerd) naar ombrotroof (regenwater-gedomineerd) veen. Factoranalyse is gebruikt om de hydrologie te reconstrueren. Uit de resultaten bleek het belang van bemonstering met hoge resolutie door de betere correlaties tussen moleculaire parameters in het elke 2 cm bemonsterde gedeelte van de boorkern vergeleken met het elke 5 cm bemonsterde gedeelte. Daarnaast zijn goede correlaties gevonden tussen pyrolyseresultaten en de anorganische fractie, solid-state ^{13}C CPMAS NMR gegevens, en totaal stikstofgehalte.

De hydrologische reconstructie gebaseerd op plant-specifieke markers en factorscores verkregen met factoranalyse toegepast op alle gekwantificeerde pyrolyseproducten, en de lage bijdrage van *Sphagnum* aan het door graminoiden gedomineerde Penido Vello veen, zorgde ervoor dat de invloed van herkomst en afbraak op de ligninesamenstelling goed bestudeerd kon worden. Een groot aantal pyrolyseproducten van lignine is gekwantificeerd voor de veenplanten en alle drie organische stoffracties voor een selectie van 15 veenmonsters. De ligninesamenstelling van hout- en kruidachtige plantensoorten zijn vergeleken alsook de ligninesamenstelling van de verschillende veenfracties, en de relatie tot botanische herkomst en de effecten van afbraak is vastgesteld.

Om te bepalen of de lignineparameters gebruikt kunnen worden voor de reconstructie van veenmilieu, zijn de met behulp van plantenanalyse en veenfracties verkregen parameters toegepast op alle 51 totaalmonsters van de bovenste meter van de Penido Vello boorkern. De resultaten geven aan dat vegetatietype en anaerobiciteit een sterke invloed hebben op algemeen toegepaste indicatoren voor lignineafbraak, en dit wijst er op dat zulke indicatoren niet betrouwbaar zijn zonder informatie over de context.

Om de interactie tussen vegetatietype en afbraakprocessen verder te onderzoeken zijn de resultaten van het *Sphagnum*-gedomineerde en graminoid-gedomineerde veen gecombineerd. Naast de welbekende pyrolytische marker voor *Sphagnum* (4-isopropenylphenol), gaven diepteprofielen van pyrolyseproducten specifiek voor korstmossen en graminoiden aan dat het betrouwbare markers zijn voor toepassing in andere venen. Een aantal andere geïdentificeerde markers was alleen bruikbaar binnen een van beide venen. Het verschil in bruikbaarheid van deze markers in beide venen benadrukt het belang van analyse van veenplanten voordat een niet-specifiek pyrolyseproduct gebruikt kan worden als marker, maar geeft ook aan dat niet-specifiek veronderstelde pyrolyseproducten specifiek kunnen zijn binnen een bepaald veenecosysteem. Het gebruik van de verschillende veenfracties (NaOH extraheerbaar en niet-extraheerbaar veen) gaf informatie over de mate van afbraak en is vergeleken met studies van verschillende humusfacties en humuszuren van veen, en met de algemeen toegepaste methode om veenafbraak vast te stellen (door het bepalen van de kleurverzadigingsgraad van veenextracten).

Daarnaast gaf de vergelijking van pyrolyseproducten van extracten en residuen (voor een selectie van monsters) in combinatie met factoranalyse, de verschillen aan tussen botanische herkomst en de effecten van verschillende stadia van aerobe en anaerobe afbraak. De afbraak van het lignine-cellulose-complex (vaatplanten) en polyfenol-cellulose-complex (*Sphagnum*) is besproken door gebruik te maken van de concentratie van hun markers (ligninefragmenten, levoglucosan en 4-isopropenylphenol) in pyrolyseproducten van de verschillende veenfracties. Dit leverde inzicht op in de afbraakmechanismen in veen. De resultaten laten zien dat in *Sphagnum* polyfenolen makkelijker afbreken dan polysachariden. Uit de vergelijking van pyrolyseresultaten en de C:N-verhouding bleek dat verschillen in strooiselkwaliteit tussen *Sphagnum* en vaatplanten een belangrijke factor is voor de variatie in de C:N-verhouding met de diepte. Een groot deel van de variatie in C:N-verhouding in *Sphagnum*-gedomineerd veen is veroorzaakt door afbraak in plaats van door kleine toename van vaatplanten bij drogere omstandigheden, terwijl dit niet geldt voor veen gedomineerd door graminoiden waar verschillende processen dit effect van gewichtsverlies op de C:N-verhouding kunnen verstoren. De C:N-verhouding is dus een degelijke indicator voor afbraak in *Sphagnum*veen maar niet in graminoid veen. Pyrolyse-GC/MS in combinatie met de toegepaste onderzoeksopzet gaf gedetailleerde chemische informatie welke inzicht gaf in zowel vegetatie als afbraakkarakteristieken. Deze methodologie heeft dus een hoge potentie voor de reconstructie van omgevingscondities in het verleden.

Chapter 1

Introduction

1.1. Peatlands and climate variability

The influence of human activity on climate has strongly increased in recent years. In order to understand the effects of anthropogenic C emissions and their consequences for climate change, knowledge of past climate is es sential as well as the responses of natural ecosystems to a changing climate. Palaeoclimate research provides valuable insights into natural climate variability, and such understanding is important for modelling future climate variability (IPPC, 2007).

Peatlands play an important role in global climate change. First, peatlands store large amounts of organic C (Gorham, 1991; Yu et al., 2010) fixed from the atmosphere by photosynthesis, which can be released back into the atmosphere by microbial degradation as the greenhouse gases carbon dioxide and methane. Peatlands can thus amplify or reduce the consequences of climate change. Second, the well-preserved stratigraphy of peat deposits in combination with dating techniques can be used to reconstruct past environmental conditions (Blackford, 2000; Chambers and Charman, 2004). Peatlands are formed when the rate of production of organic matter (OM) is larger than its decomposition, causing the accumulation of plant remains. Peat is generally growing upwards with time, which leads to the formation of organic soils that may reach several meters in thickness and may span periods of thousands of years. The accumulation of peat OM is thought to be mainly dependent on the slow decomposition rate. In peatlands, the height of the water table is the main factor that determines the degree of decomposition (Clymo, 1984). In addition, plant species composition on peat bogs is highly dependent on hydrological conditions (McMullen et al., 2004; Kleinebecker, 2007). Thus, both vegetation composition and the degree of decomposition of its remains are mainly determined by depth and fluctuation of the water table.

In ombrotrophic bogs, all moisture and nutrients come from precipitation, making them particularly suitable to study variations in climate (Barber, 1993). Palaeoclimate reconstructions from peat deposits include several techniques, recently reviewed by Chambers et al. (2012). Proxies related to atmospheric deposition reflect regional conditions and include pollen (Mighall et al., 2006), charcoal (Whitlock et al., 2007; Markgraf and Huber, 2010) and inorganic geochemical proxies (Shotyk, 1996). However, such regional proxies may be influenced by local conditions or autogenic processes that complicate their interpretation. For example, the ecological interpretation of *Empetrum* and *Nothofagus* pollen is ambiguous unless it can be related to local conditions (Markgraf and Huber, 2010) because it has different environmental preferences locally (the peatland) compared to regionally (the surrounding mineral soils). Another example is that peat OM decomposition can influence trace element records (Biester et al., 2012). In addition to proxies that reflect the height of the water table, such as non-pollen palynomorphs (van Geel, 2001) and testate amoebae (Charman, 1997; Woodland et al., 1998), the composition of peat OM itself exclusively reflects local conditions.

Several studies of plant macrofossils in ombrotrophic peat have shown good correlations between past plant species distribution and local hydrology (Blackford, 2000; Barber et al., 2003). Because in highly decomposed peat the preservation of plant remains is poor, plant-specific recalcitrant compounds (biomarkers) have been used to reconstruct plant species composition (Pancost et al., 2002; Xie et al., 2004). Several methods have been applied to study the degree of decomposition of peat OM. Von Post (1922) based the degree of peat decomposition on the colour of peat pore water and of the remaining solid material. Blackford and Chambers (1993) introduced an alkaline extraction procedure combined with UV absorption measurements to determine differences in the degree of peat humification based on colour intensity of the leachate. Furthermore, elemental ratios have been used to reflect changes in decomposition, including C:N (Kuhry and Vitt, 1996) and H:C and O:C ratios (Lu et al., 2000). Stable isotopes of (specific plant parts of) peat OM have also been used to reflect decomposition (Dupont and Brenningkmeijer, 1984; Pendall et al., 2001). However, also other factors, such as botanical changes, fire incidence and selective anaerobic decomposition, can influence humification (Yeloff and Mauquoy, 2006), C:N ratio (Hornibrook et al., 2000) or stable isotopes (Boutton and Yamasaki, 1996) of peat OM.

There is a large deficiency in studies that combine vegetation and decomposition characteristics to investigate peat OM dynamics, and most of these include only a limited number of samples (Zheng et al., 2007). This combination of information on vegetation and decomposition is essential for a solid palaeohydrological interpretation of a peat deposit, because the distribution of plant species and decomposition of their remains in peat are closely associated (Blackford and Chambers, 1993; Yeloff and Mauquoy, 2006), and the height of the water table affects both simultaneously. This level of complexity requires a combination of decomposition and vegetation characteristics and a detailed methodological approach, which can be provided by pyrolysis-GC/MS analysis of high-resolution sampled peat deposits.

1.2. Peat OM chemistry

Peat OM is primarily composed of plant remains (including mosses). Because these plant remains represent a mixture of species and tissues that differ in chemical composition, the chemical composition of peat OM at a given depth depends on the botanical composition of the peatland at the time of deposition. After deposition, this mixture of plant species and plant tissues is subject to decomposition. The degree of decomposition is controlled by 1) the intrinsic characteristics of the plant remains, 2) the abiotic conditions (pH, water table, temperature, nutrients), and 3) the decomposer community (Berg and McClaugherty, 2007). Both the botanical composition and the factors that influence decay in peatlands are predominantly controlled by the depth and fluctuation of the water table, i.e. oxygen availability. Hydrology is thus the main factor that determines differences in chemistry within a given peatland ecosystem. In order to reconstruct hydrology from peat OM chemistry the complex interactions between botany, hydrology and decomposition need to be studied and understood.

1.2.1. Botanical composition – Hydrology

Plant species on ombrotrophic peatlands are adapted to wet, acidic and nutrient-poor conditions. Within such an environment, species have preferential habitat conditions under which they perform better. Some species are restricted to specific conditions (location, hydrology, nutrients), whereas others appear everywhere but with differences in abundance. Because this relationship between plant and environment at present was the same in the past, the botanical composition on peatlands reflects its environmental history (Fraga et al., 2008). Ombrotrophic peatlands only receive water from precipitation and thus precipitation predominantly controls the water table level and thereby the composition of plant species. Knowledge of plant ecology is important to understand the influence of botanical composition on the ecosystem and to interpret peat OM chemistry. In peatlands, mosses and vascular plants occur together, but they have different growth strategies, differ in interactions regarding light and nutrients, and determine micro-topography (Malmer et al., 1994). Thus, plants also influence local conditions and thereby indirectly accompanying plants, but environmental conditions are the major factors that control the botanical composition on ombrotrophic peatlands (Kleinebecker et al., 2007; McMullen et al., 2004).

1.2.2. Botanical composition – Decay

Although fungi, bacteria and insect material contribute, plant remains comprise the majority of peat. Major differences in chemical composition are found between plant types that contribute to peatlands: mosses, lichens, herbaceous species, and woody species. The chemical composition may also differ between closely related species of the same growth form and between plant parts (e.g. roots, leaves, wood). Knowledge of these chemical differences is important for botanical reconstruction and for the interpretation of decomposition effects. Therefore it is important to investigate the chemical composition of plant material. Plants are composed of easily degradable and more resistant ('recalcitrant') tissues (Webster and Benfield, 1986). The composition and architecture of plant macromolecules are therefore important factors that control decomposition (Overbeck, 1975; Berg and McClaugherty, 2007; Cornwell et al., 2008). Several classes of chemical compounds have intrinsic properties related to decay. Major groups of chemical compounds in plants include cellulose, hemicellulose, lignin, tannin, proteins, cutin, suberin, and waxes. In litter (i.e., the above and belowground remains of plants), the quantitatively most common compounds are structural macromolecules of the plant cell wall. Depending on species and on its position in the plant, cell walls differ in content and composition of chemical compounds. The composition and degradation characteristics of the different chemical groups will be discussed below.

Cellulose and lignin are the major plant cell wall components. Cellulose is the most abundant natural polymer and is the major structural component of plants for both mosses and vascular plants. Cellulose is composed of β-(1-4)-linked glucan chains. Because of their linear structure, glucan chains can easily form intermolecular and intramolecular hydrogen bonds causing the strength of the cellulose fibre (Updegraff, 1969; Gardner and Blackwell, 1974). The cellulose microfibrils are bound via hydrogen bonds to each other by hemicelluloses that in turn are associated to lignin. Hemicelluloses are polymers of sugars other than β-(1-4)-linked glucose, and form more branched structures. The monosaccharide composition of the chains is composed of mannans,

galactans, arabinans and xylans, the composition of which may strongly differ between plant species (Kirk, 1973; Cowie and Hedge, 1984). Carbohydrates of graminoids have a higher contribution of hemicellulose compared to *Sphagnum* mosses (Laggoun Défarge et al., 2007) and in hemicellulose of graminoids xylose is the major sugar (Wicks et al., 1991; Bourdon et al., 2000; Peng and Wu, 2010; Christensen et al., 2010), while in *Sphagnum* rhamnose is relatively abundant (Popper and Fry, 2003; Ballance et al., 2007). Lichen polysaccharides are mainly composed of glucans and galactomannans (Olafsdottir and Ingólfsdottir, 2001).

Lignin is exclusively plant-derived and is an important chemical component of the cell wall of vascular plants; mosses do not synthesise lignin (Weng and Chapple, 2010). Lignin is composed of *p*-hydroxyphenyl, guaiacyl and syringyl units that are bound together by ether and C-C bonds, forming a complex polymer that lacks the regular repetitive units found in other polymers such as cellulose. The heterogeneity exhibited by lignin is due to variations in building blocks, degree of cross-linking, and functional groups, which vary considerably among plant species and plant tissue (Sarkanen and Ludwig, 1971; Hedges and Mann, 1979; Buranov and Mazza, 2008). Lignified plant cell walls are formed by successive deposition of cellulose, hemicellulose and lignin and form a composite in which these components are physically and chemically connected (Lu and Ralph, 2010). This association is called lignin-carbohydrate complex (LCC) or lignocellulose. The cell wall contains lignified and non-lignified polysaccharides; the amount of lignin differs between plant species and between plant parts, and thus also the amount of polysaccharides that are associated with lignin. In addition to lignin, non-woody tissue may contain a high content of free and bound phenolics (Hedges and Mann, 1979) that can form bridges between lignin and polysaccharides. In wood of dicotyledonous species, the lignin is more directly associated with polysaccharides through the formation of benzyl-ether and other alkali-stable bonds (Watanabe et al., 1989). The composition and architecture of lignocellulose in the plant cell wall is an important factor that controls decomposition (Blanchette, 1995). Polysaccharides that are associated with lignin are more resistant to decay (Chesson, 1997) than free polysaccharides. Hemicellulose is easier degraded than cellulose. Free and bound phenolic acids are more easily decomposed than macromolecular lignin. Of the different lignin building blocks syringyl lignin is more easily degraded than guaiacyl lignin. Because the composition and architecture of lignin differs between plant species, it is expected that decomposition of lignin in peat OM is highly influenced by the dominant source vegetation.

Plant proteins include storage and structural proteins, and are relatively easy to decompose. Proteins are large polypeptides composed of amino acids and account for most of the N present in organisms. Bacteria may contain high amounts of murein, a peptidoglycan (Killops and Killops, 2005). Chitin is a polysaccharide with a structure similar to that cellulose, but composed of N-acetylglucosamine units (Rudall and Kenchington, 1973). Chitin in peat may originate from fungi and the exoskeletons of insects, in which it is the main constituent. Organic N in peatlands has a relatively large contribution from microbes.

Lipids include a large range of compounds such as fats, waxes, and terpenoids that occur as free molecules or as macromolecules in both microbes and plants. Waxes mainly function as protective coatings and include alkanes, fatty acids, alcohols, ketones, aldehydes, and esters, of which the chain length can be specific for the organism. Cutin and suberin are high-molecular-weight natural polyesters that occur in plants. Cutin is characteristic of the cuticular membrane, an extracellular layer covering the aerial parts of plants where no secondary thickening occurs (e.g. leaves, non-woody stems). Suberin is an intracellular component in the walls of stems of woody plants and in roots where it forms protective barriers. Cutin and suberin are composed of aliphatic molecules and phenolic acids; cutin is composed of C_{16} and C_{18} aliphatic monomers while C_{20}-C_{30} aliphatic monomers dominate in suberin (Kolattukudy, 1980; Tegelaar et al., 1989; Kögel-Knabner, 2002). In addition to cutin and suberin, non-hydrolysable aliphatic biopolymers, labeled cutan and suberan, were detected in the cuticle and bark of some plants; cutan and suberan mainly consist of *n*-alkane and *n*-alkene molecules with chain lengths ranging from C_5 to C_{35} (Nip et al., 1986; Tegelaar et al., 1989; McKinney et al., 1996).

Plants may contain phenolic (macro)molecules other than lignin. Tannins are natural polyphenols. They are especially abundant in the bark of dicotyledons, and of minor importance in mosses, grasses and fungi (Haslam, 1981; Kögel-Knabner, 2002). Lichens are also known for their specific and highly variable phenolic chemistry (Wachtmeister, 1958). *Sphagnum* litter is known to have a very slow decomposition rate (Clymo, 1965; Dickinson and Maggs, 1974; Coulson and Butterfield, 1978; Johnson and Damman, 1991; van der Heijden, 1990, 1991) which is attributed to an abundant phenolic macromolecule (Painter, 1991; van der Heijden, 1994; van der Heijden et al., 1997) containing sphagnum acid (*p*-hydroxy-β-[carboxymethyl]-cinnamic acid). Sphagnum acid is specific for *Sphagnum* spp. (Tutschek et al., 1973, 1978; Rudolph and Samland, 1985; van der Heijden et al., 1994; Rasmussen et al., 1995; Verhoeven and Liefveld, 1997; McClymont et al., 2011; Abbott et al., 2013); the major part of sphagnum acid is present in the cell wall (Wilschke et al., 1989; Hesse and Rudolph, 1992) and is probably ether-linked to other cell wall biopolymers (van der Heijden, 1994; Swain and Abbott, 2013).

Thus content, composition and architecture of chemical compounds differ between species and influence decomposition. If these differences are known and their intrinsic properties related to decomposition understood, plant-specific chemistry can be used to trace past abundance of a species or to determine the degree of decomposition in peat.

1.2.3. Decay – Hydrology

Plant remains are enzymatically decomposed by microorganisms to carbon dioxide and water, and under anaerobic conditions to carbon dioxide, water and methane. The microbial community in peatlands is highly variable and changes with depth in composition and size; OM is decomposed by several interacting groups of microbes with complementary enzymatic activities that are influenced by different environmental conditions (Andersen et al., 2013). Although temperature, acidity and nutrients influence the microbial community, these factors are more or less constant at a given site, and the availability of oxygen (and other electron acceptors) and litter quality are major factors that influence the microbial community.

Thus, decomposition in peatlands is highly dependent on the availability of oxygen. In the upper, aerated and partly living, peat layer (acrotelm) substantial litter mass loss occurs in a relatively short time interval. The degree of decomposition of a given peat layer is therefore generally used in water table reconstructions. However, decay of the partly decomposed OM continues, both in the lower permanently waterlogged anaerobic layer (catotelm), although at a substantially slower rate (Clymo, 1984; Kuhry and Vitt, 1996), and during secondary aerobic decomposition that may occur upon lowering of the water table (Tipping, 1995). These differences in oxygen availability have a large influence on the composition and activity of the microbial community (Andersen et al., 2013), and thereby the degree and rate of decomposition and the composition and transformations of OM. An example is the degradation of lignocellulose. Cellulose can be decomposed by a variety of fungi and bacteria under both aerobic and anaerobic conditions; oxygen availability determines which microbes dominate. Cellulose-degrading organisms produce a series of enzymes with different specificities (Béguin and Aubert, 1994). Decomposition of lignin is considered an aerobic process catalysed by unspecific enzymes such as peroxidases and laccases (Williams and Yavitt, 2003; Sinsabaugh, 2010). Anaerobic degradation of lignin proceeds at a much lower rate and is only partial, although smaller fragments of lignin (monomers, dimers) may be completely broken down (Young and Frazer, 1987). Under anaerobic conditions holocellulose is preferentially degraded over lignin and hemicellulose more rapidly than cellulose (Hedges et al., 1985; Benner et al., 1987).

In addition to the effects of the chemical groups and their architecture, decomposition is also influenced by elemental concentrations in plants (Aerts et al., 1999; Turetsky, 2003), and decomposition also directly influences the botanical composition by the availability of inorganic nutrients (Scheffer, 1998). N plays an important role in peatlands, as both peat OM production and initial decomposition are N-limited (Weedon et al., 2013). The abundance of C and N and the C:N ratio in peat, is determined by input material and by the degree of decay. The C:N ratio decreases with decomposition but also strongly differs between *Sphagnum* spp. and vascular plants (Hornibrook et al., 2000). In peat, N may originate from plant remains, microbes, N-fixing organisms, atmospheric deposition and manure. Increased nutrient levels, in particular N, P and S that are the main limiting nutrients for microbial growth, stimulate the degradation of cellulose, hemicellulose and soluble carbohydrates (Berg and McClaugherty, 2007).

1.2.4. Methods used for the molecular characterisation of peat OM

The molecular composition of peat OM provides information on local past environmental conditions, including botanical composition, degree of decomposition and fire history. Chemical characterisation of OM comprises analysis of free molecules (e.g. lipids) or of moieties that originate from macromolecules (e.g. lignin). Free lipids can be directly analysed by gas chromatography/mass spectrometry (GC/MS; Eglinton and Hamilton, 1967) after extraction of the lipid fraction. In an anaerobic environment, lipids are relatively resistant against decomposition and thus their abundance or distribution in peat provides information on past abundance of peatland plants (Dehmer, 1993; Pancost et al., 2002; Xie et al., 2004) or microbes (Zheng et al., 2007; Peterse et al., 2011) that can then be related to climate.

Analysis of macromolecular markers in peat deposits includes analysis of lignin (Williams et al., 1998; Tsutsuki et al., 1994; Bourdon et al., 2000), polysaccharides (Boon et al., 1986; Moers et al., 1989; Macko et al., 1991; Comont et al., 2006; Jia et al., 2008; Delarue et al., 2011), sphagnum acid (van der Heijden et al., 1997; Abbott et al., 2013; Swain and Abbott, 2013), tannin (Mason et al., 2009), suberin (van Smeerdijk and Boon, 1987) and polyaromatics (Pontevedra-Pombal et al., 2012). The content, composition and character of moieties that derive from macromolecules provide information on the botanical composition and/or post-depositional processes (decomposition and burning). To obtain information on the (macro)molecular composition of heterogeneous mixtures, several techniques have been developed including non-destructive methods (^{13}C cross polarisation magic angle spinning nuclear magnetic resonance spectroscopy (CPMAS NMR) and Fourier transform infrared (FTIR) spectra), chemolytic methods (CuO oxidation, acid hydrolysis), thermochemolysis and pyrolysis-GC-MS (Kögel-Knabner, 2000). Although secondary reactions are avoided using non-destructive methods, ^{13}C CPMAS NMR or FTIR analysis provides information on the atom environment only, and for complex mixtures of OM this can only be translated to general molecular information (e.g. polysaccharides). Chemolytic methods provide more detailed molecular information, but are concentrated on a certain fraction of the OM (e.g. lignin or polysaccharides). The used methods may, in addition to plant chemistry and post-depositional processes, also influence the results of (macromolecular) analysis, such as is reported for lignin (Lu and Ralph, 2010).

Several authors have shown that plant community (vegetation type) or litter chemistry (plant species) highly influence decomposition characteristics in peatlands (Scheffer, 1998). These studies demonstrated the need to study botanical changes and the degree of decomposition simultaneously, which can be obtained with pyrolysis-GC/MS. Although the interpretation of pyrolysates may be complex due to rearrangements during the pyrolysis process, pyrolysis-GC/MS gives a detailed fingerprint of organic material at the molecular level. The use of pyrolysis products as markers of peatland plants and to gain insight into peat decomposition processes has been repeatedly demonstrated (Halma et al., 1984; van Smeerdijk and Boon, 1987; Durig et al., 1989, 1991; van der Heijden, 1994; Kuder et al., 1998; Huang et al., 1998; Gleixner and Kracht, 2001; González et al., 2003; Buurman et al., 2006; McClymont et al., 2011). Thus the characterisation of peat by pyrolysis-GC/MS allows conclusions to be drawn about both past vegetation composition and the degree of decomposition. Some studies have used the method successfully to relate the chemical signature of peat OM to palaeoclimate. However, at the start of this thesis, in 2010, no studies existed that used high-resolution sampled peat cores (Table 1.1).

1.3. Study area

Two contrasting ombrotrophic peatlands were selected to study peat OM dynamics and past environmental changes. The two sites comprise a *Sphagnum*-dominated peatland in Tierra del Fuego, and a graminoid-dominated peatland in Galicia, respectively. Both peatlands had been relatively well-studied, cover large part of the Holocene and have received a minimum of industrial and agricultural influences (Godoy et al., 2001).

Table 1.1 Pyrolysis-GC/MS studies (until 2010) that studied peat chemistry to investigate past environmental conditions

Reference	Depth/Age	Number of samples
Boon et al., 1986	111–150 cm	2 samples (10 samples Py-MS)
Moers et al., 1990	0–254 cm	6 samples
Calvert et al., 1991	0-10 m	8 samples
van der Heijden et al., 1997	0–70,000 years	4 samples
Kuder and Kruge, 1998	0–111 cm	12 samples
Kuder et al., 1998	0–600 cm	4 samples
Bourdon et al., 2000	0–99 cm	10 samples
González et al., 2003	0–70 cm	2 samples
McClymont et al., 2005	?	?
Buurman et al., 2006	0–250 cm	24 samples

The Harberton bog is located along the Beagle Channel, Puerto Harberton, Tierra del Fuego, Argentina (54°53'S, 67°20'W; 20 m a.s.l.). The seasonal temperature amplitude is small, winter temperature remains above freezing and mean annual temperature is 7 °C, while mean annual precipitation is 600 mm (Markgraf and Hüber, 2010). After the retreat of the glacier that occupied the Beagle Channel during the late glacial maximum, peatlands started to develop. The age at the basis of the bog is 16 ka (Pendall et al., 2001). At present the bog is dominated by *Sphagnum magellanicum* and it is surrounded by a *Nothofagus* forest, of which *Nothofagus antarctica* also grows locally in the peatland (Markgraf and Hüber, 2010). In addition to pollen (Heusser, 1989; Markgraf, 1993) the bog was analysed for macrofossils and charcoal (Markgraf, 1993), and stable isotopes (White et al., 1994; Pendall et al., 2001). A large vegetation shift from peat dominated by graminoids and woody species to *Sphagnum*-dominated (>6 m) peat was recorded by macrofossil analysis (Markgraf, 1993). No traces of European plants were found, indicating the relatively undisturbed local environment along the eastern Beagle Channel (Markgraf and Hüber, 2010).

The Penido Vello peatland is located at a remote site in the Xistral Mountains, Galicia, NW Spain (43°31'19''N, 7°30'45''W; 700 m a.s.l.) The main growth of peatlands in this area started at 1100 years BP, the basal age of the Penido Vello bog is 8000 years BP. The Penido Vello peatland is located 20 km south of the coast, on a summit, a flat surface resulting from the weathering and erosion of granite. The mean annual temperature in the Xistral Mountains ranges from 7.5 to 10 °C, and annual precipitation from 1400 to 1800 mm (Martínez-Cortizas et al., 1997, 2002). The bog is dominated by graminoids, both Poaceae and Cyperaceae, with contributions of ericoids and mosses, including a number of endemic species (Fraga et al., 2001). *Sphagnum* is almost completely absent.

1.4. Methodology

1.4.1. Pyrolysis-GC/MS

Pyrolysis is defined as a chemical degradation reaction that is caused by thermal energy alone. Pyrolysis makes it possible to directly analyse non-volatile macromolecular materials (e.g. coal, wood, peat) by adding heat to a sample, and thereby extends the applicability of GC/MS for material characterisation. Pyrolysis is carried out in an inert atmosphere (heating of organic

material in the presence of oxygen will only result in carbon dioxide and water), which results in cleavage of chemical bonds within the macromolecular structure, producing a number of pyrolysis products (chemical moieties) that are reproducible using the same conditions. However, due to recombination during the pyrolysis process the fragments may be structurally somewhat different from the source compound; especially polar molecules are easily changed during pyrolysis. The optimum pyrolysis conditions (temperature, pyrolysis time, etc.) depend on the sample material. Several types of pyrolysis currently exist, including Curie-Point, Micro-furnace and Filament pyrolysis, each with its own advantages and disadvantages. For most of the peat and plant analysis used here, Curie-Point pyrolysis had been used. A common detection device to obtain analytical data after pyrolysis is gas chromatography (GC) mass spectrometry (MS). The mixture of volatile pyrolysis products are separated by the GC during their travel through the column according to their chemical differences and a temperature programme of the oven. After separation by the GC, the pyrolysis products enter the MS where each molecule may break apart into ionised fragments that can be detected according to their mass-to-charge values (*m*/*z*). Software subsequently translates the data into a Total Ion Chromatogram or pyrogram of which each of the peaks can be identified by comparison with libraries and literature according to their mass spectra and retention time (Moldoveanu, 1998). A schematic picture of a typical pyrolysis-GC/MS devise and its output is given in Figure 1.1.

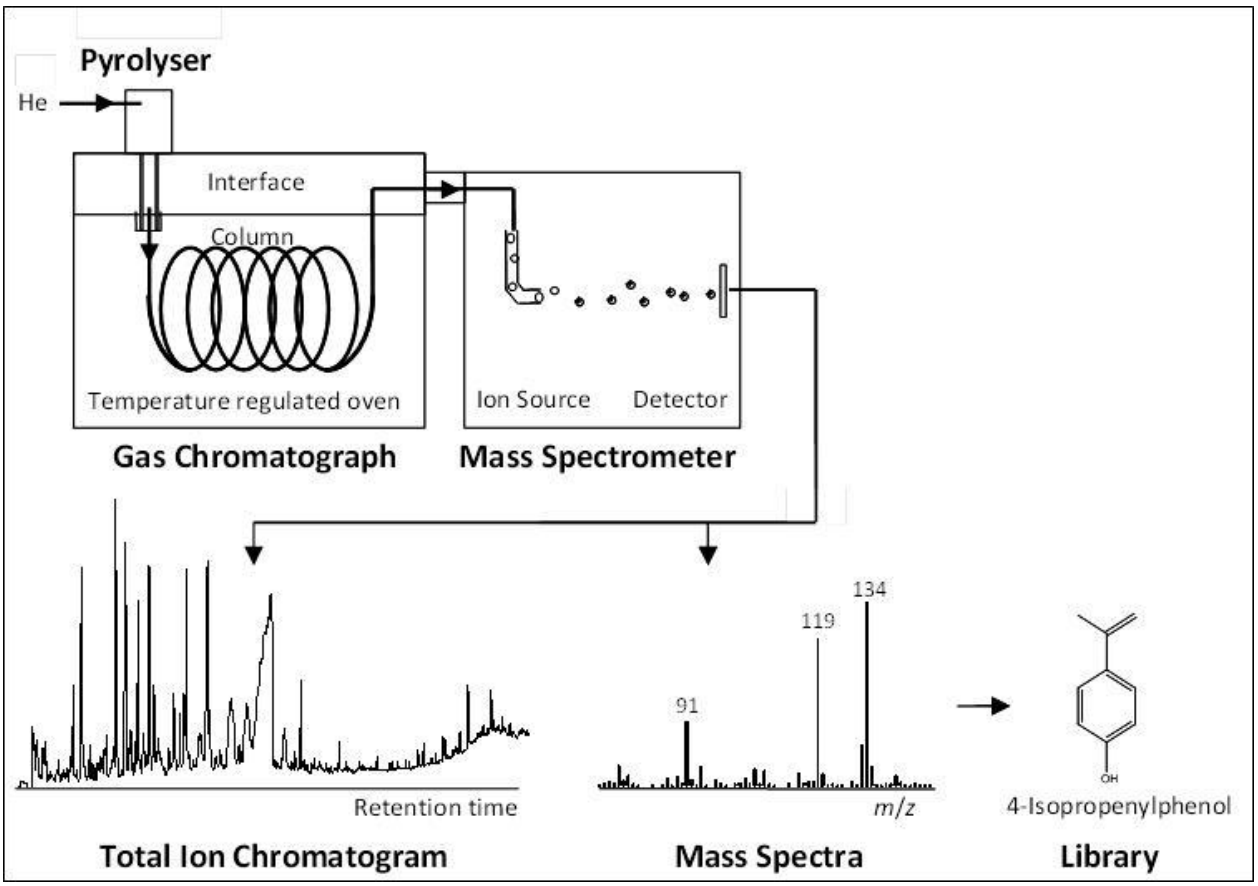

Fig. 1.1. Schematic overview of a pyrolysis-GC/MS measuring system (modified after Frontier Laboratories Ltd.)

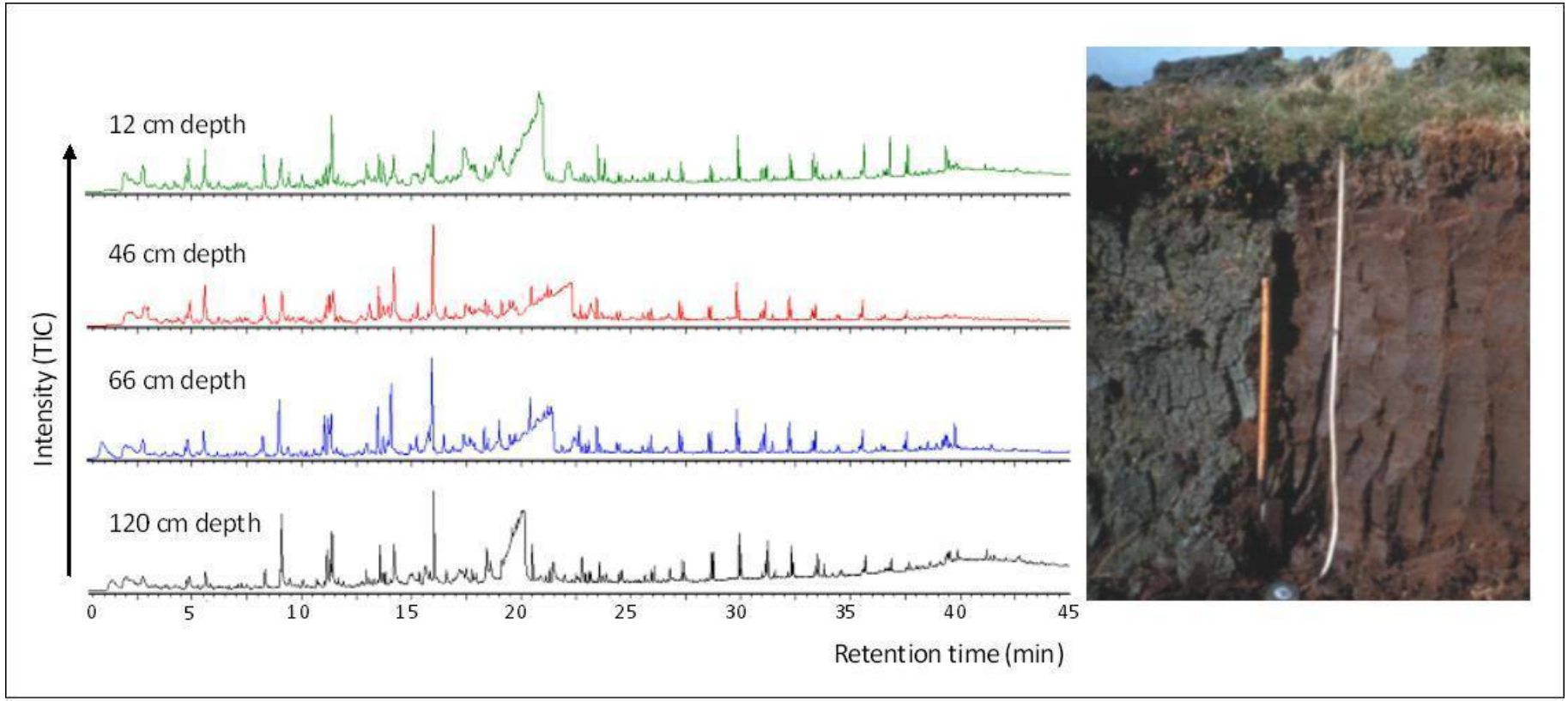

Fig. 1.2. Depth profile of the Penido Vello peatland with pyrograms obtained from four samples at different depths (photograph: courtesy of Xabier Pontevedra Pombal).

1.4.2. Interpretation of peat pyrolysates

A macromolecule can be identified by its combination of pyrolysis products. However, the interpretation of peat pyrolysates, representing a mixture of plant material at various stages of decomposition and possible admixture of microbial or charred material, is complex for several reasons. First, without pre-treatment pyrolysates of natural OM may include free molecules (evaporation products) as well as fragments from macromolecules (bound molecules). A single pyrolysis product may originate from both sources, for example 4-vinylguaiacol is a pyrolysis product of ferulic acid (Boon et al., 1982; van der Hage et al., 1993) but it can also originate from macromolecular lignin (Saíz-Jimenez and de Leeuw, 1984). Generally, low-molecular-weight pyrolysis products (e.g. toluene), can originate from many sources (e.g. toluene may originate from proteins, lignin, sugars, or charcoal). The interpretation of such pyrolysis products is thus highly dependent on the complete picture. Second, in addition to the influence of pyrolysis conditions, GC column and MS response factors (Section 1.4.1), the presence of mineral material may influence the abundance and types of pyrolysis products (Alcañiz et al., 1989; Schitzer et al., 1994; Miltner and Zech 1997). In ombrotrophic peat this effect is not relevant. Third, pyrolysis of peat OM typically results in hundreds of pyrolysis products that may only show small changes in their relative abundance with depth (Fig. 1.2).

1.4.3. Methodological approach

Apart from the difficulty in the interpretation of pyrolysates (Section 1.4.2), the processes that affect the composition of peat OM are extremely complex (Section 1.2). Therefore the following approach was used for the interpretation of pyrolysates of both peat cores:

- *Vegetation type*. In order to examine the influence of dominant vegetation on peat molecular chemistry, two contrasting ombrotrophic peatlands were selected, including a *Sphagnum*-dominated and a graminoid-dominated peatland. The different vegetation types of these two peatlands may allow us to study the interactions between vegetation composition and decomposition characteristics.
- *Plant analysis*. In addition to peat samples, peatland plants were selected in order to search for potential markers of peatland plants and to establish plant chemical characteristics. The selection of plants for analysis was based on their present abundance in both peatlands, as well as their value as indicators of hydrological conditions in the bog.
- *High-resolution sampling*. For an accurate reconstruction it is important to avoid averaging out of long time periods, and thus the sampling intensity should take into account the peat accumulation rate. Sampling heights of 2, 3 or 5 cm were chosen, taking into account peat stratigraphy. In addition, for a correct interpretation of peat records the sampling resolution is important. Therefore all 101 samples from the Penido Vello peat core (3 m) were analysed with pyrolysis-GC/MS, while for Harberton (9 m) 67 samples were analysed with pyrolysis-GC/MS and all 268 samples were analysed for ash content and total C and total N.

- *Fractionation of peat OM*. Peat OM is composed of plant material at various stages of decomposition. In order to separate the influence of vegetation and decomposition on the chemical composition of peat, different peat fractions were compared. The NaOH-extractable OM gives information of more decomposed material while the NaOH-resistant OM (the residues after extraction) more reflect intact plant material (Buurman et al., 2006). Therefore, pyrolysates of extract, residue and bulk samples were compared for a selection of samples for both cores (24 samples for Penido Vello, 13 samples for Harberton).
- *Factor analysis*. The high number of samples and pyrolysis products and the complexity of peat OM dynamics make the interpretation of pyrolysates of peat profiles difficult. Therefore, Factor Analysis has been used in order to separate the effects between source and post-depositional processes and to extract the most significant differences.
- *Comparison with conventional methods*. To support the interpretation of pyrolysis results, the studied peat samples were additionally analysed for ash content, total C and total N (both cores), and ^{13}C CPMAS NMR (Penido Vello). ^{14}C ages were determined for a number of samples of both cores to enable comparison with other studies.

1.5. Objectives and outline of the thesis

The purpose of this study is to examine the use of analytical pyrolysis in the environmental interpretation of peat records and to gain an improved understanding of the three-way interactions between botanical composition, decomposition and environmental factors such as depth of the water table. The thesis is composed of five individual papers divided into two parts according to vegetation type: dominance of *Sphagnum* (Chapters 2 and 3) and dominance of graminoids (Chapters 4-6).

In the first part, the *Sphagnum*-dominated Harberton peatland has been studied. **Chapter 2** is a survey of the general chemistry of the Harberton peat core, with a focus on the identification of molecular parameters. This has been done by a systematic methodological approach. Peatland plants have been analysed in search for specific markers. Several peat fractions have been analysed for a selection of samples (13), including NaOH-extract, NaOH-insoluble residue and bulk sample. A large number of pyrolysis products (177) was identified and quantified, additionally total C and N and ash content were measured. Factor analysis was used to assess differences in peat chemistry and to relate these to environmental factors. This resulted in a number of molecular parameters that reflect botanical changes, (an)aerobic decomposition and fire incidence.

In **Chapter 3** the results of the different peat fractions and plant analysis (Chapter 2) were applied to a large number of bulk samples (67). These were used to reconstruct the 12.000 years of vegetation history of the Harberton core, which was then interpreted in terms of past hydrological conditions. The marker of sphagnum acid (4-isopropenylphenol) has been applied to reconstruct past abundance of *Sphagnum*, as proposed by van der Heijden et al. (1997). The C:N ratio showed a perfect agreement with the botanical composition according to pyrolytic markers. It appeared that the *n*-alkane distribution showed remarkable changes with respect to shifts in the abundance of

Sphagnum. Consequently, the detailed vegetation reconstruction was used to discuss the application of the *n*-alkane distribution in ombrotrophic peat. The difference in contribution of alkanes to the total OM between *Sphagnum* and woody plants, as suggested by Pancost et al. (2002), was confirmed; and the results additionally indicated a considerable effect of aerobic decomposition on the distribution of alkanes, which are generally used to indicate botanical changes in peat deposits. Although pyrolysis is not the regular method to establish the *n*-alkane distribution, comparison of *n*-alkanes in the NaOH-extract and residue peat samples supported the findings. Chapter 3 stresses the importance of combining vegetation and decomposition characteristics. Although the effect of decomposition on alkane distributions was already published for soils (Buggle et al., 2010, Zech et al., 2011), I showed that this is also evident in peatlands. The effects of decomposition on the *n*-alkane distribution in peatlands were supported by GC/MS studies of alkanes in peatlands (Andersson et al., 2011).

The second part discusses the graminoid-dominated Penido Vello peatland. **Chapter 4** provides a detailed record of pyrolytic markers of peatland plants, and the effects of decomposition on such markers are discussed. All 101 bulk peat samples of the 3 m thick peat deposit had been analysed. In addition to the search for specific compounds in pyrolysates of plants, plant pyrolysates were quantified for the same pyrolysis products as for the peat. Depth records of plant markers agreed well with their preferential habitat, with the degree of decomposition and with nutrient content. The hydrological interpretation agreed well with other European studies. The results showed the importance of high-resolution sampling by the better correlations between molecular parameters in the part sampled each 2 cm compared to 5 cm. Furthermore, good correlations of pyrolysis results were found with mineral content, ^{13}C CPMAS NMR data and total N.

Lignin parameters are frequently used to indicate botanical source or degree of decomposition. Because of the low contribution of (*Sphagnum*) moss to the Penido Vello peat, the effects of source and decay on the lignin composition were examined for the high-resolution sampled upper meter (51 samples of 2 cm in height; **Chapter 5**). A large number of lignin pyrolysis products were quantified for peatland plants and for the three different peat fractions for a selection of samples previously analysed by Buurman et al. (2006). Lignin composition of woody and graminoid plant species were compared as well as the lignin composition in different peat fractions, and their relation to source or decay was established.

To determine if the lignin parameters, derived from analysis of plants and peat fractions, can be used to reconstruct peat environment, they were applied to all 51 bulk peat samples of the upper meter of the Penido Vello core (**Chapter 6**).

To further examine the interaction between vegetation type and decomposition processes, the results of *Sphagnum*-dominated peat (part I) and graminoid-dominated peat (part II) are combined in **Chapter 7** (General Discussion). In addition, the main subjects of the applied methodology are elaborated and compared with more classical methods, and ideas for further research are defined.

Chapter 2

Selecting parameters for the environmental interpretation of peat molecular chemistry – a pyrolysis-GC/MS study

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Abstract

A number of samples from a deep peat bog in Tierra del Fuego were analyzed using pyrolysis-gas chromatography/ mass spectrometry (Py-GC/MS) in order to extract parameters that might be used to interpret the peat chemistry in terms of vegetation change, anaerobic and aerobic decomposition, and fire incidence. The choice of parameters was based on factor analysis of 177 pyrolysis products, quantified for 13 samples, separated into extract and residue, as well as the total samples. Factor analysis of extracts, residues and total samples yielded similar classifications in terms of vegetation and decomposition. Pyrolysis products and ratios that most clearly differentiated samples were used to interpret the depth profile. Although interpretation was not always straightforward, indications of parameters to describe vegetation shifts, aerobic and anaerobic decomposition, and fire largely coincided. These parameters will be used in a forthcoming study for a more complete interpretation of the peat profile.

2.1. Introduction

Because of its low temperature, strong oceanic influence and limited seasonality, Tierra del Fuego, at the southern tip of South America, is sensitive to variation in climatic conditions (Thukanen, 1992). This makes it a good location for investigating climate change. It harbours some well-preserved peat bogs. In peat, the organic material is sequentially deposited for thousands of years. Because of the gradual upward growth, the peat composition reflects the changes in vegetation and hydrology over the time of its development. In combination with dating techniques, the climate can be reconstructed over a considerable period of time. Techniques for palaeoclimate reconstruction from peat deposits include pollen analysis (e.g. Mancini et al., 2005), plant macrofossil analysis (e.g. Barber et al., 2003), humification indices (e.g. Roos-Barraclough et al., 2004), stable isotopic records (Pancost et al., 2000), testate amoebae (e.g. Charman, 1997), ^{14}C dating (Mauquoy et al., 2002a), charcoal abundance (Whitlock et al., 2007) and beetles (Heusser et al., 1996). In Tierra del Fuego, most climate reconstructions are based on pollen data (e.g. Markgraf et al., 2002). White et al. (1994) used the $^{13}C/^{12}C$ ratio of cellulose from mosses and sedges to reconstruct atmospheric CO_2 concentration, and thereby temperature. Markgraf (1993) reconstructed climate with macrofossil analysis, charcoal abundance and pollen analysis. Pendall et al., (2001) reconstructed temperature and relative humidity with stable hydrogen isotope analysis of mosses.

Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) of peat deposits is used to characterise the chemical composition of the organic matter (OM) and thereby gain insight in to the decomposition process (e.g. Huang et al., 1998; Gleixner and Kracht, 2001; González et al., 2003). It gives a fingerprint of OM at the molecular level and therefore information about past vegetation, aerobic and anaerobic decomposition, and addition of microbial products. Some studies have used the method successfully to relate the chemical signature of peat to palaeoclimate (Boon et al., 1986; Kuder and Kruge, 1998; Buurman et al., 2006).

The objective of this study was to find chemical parameters that reflect shifts in decomposition and vegetation in a peat profile and relate these to past environmental circumstances. Peat OM includes many different organic compounds, ranging from plant residues to microbial biomass. All this OM is subject to chemical, physical, or biological transformation (Dai et al., 2002) and its composition changes as a result of differences in ''palatability''. The general trend is anaerobic decay, which causes a loss of polysaccharides and an increase in non-oxygenated aliphatics and aromatics with depth (Pontevedra-Pombal et al., 2001). Deviations from this general trend are caused by, e.g. changes in vegetation composition (different input chemistry), periods of drought (different vegetation + prevalent aerobic decay) and fires (charring of OM). Decomposition causes loss of plant structure and formation of an amorphous, soluble organic fraction. Thus, peat consists of an extractable, decomposed fraction and a non-extractable fraction. This paper focusses on defining appropriate parameters for relating organic chemistry to changes in vegetation and decomposition, using information from extractable, non-extractable and total samples. The study is based on relatively few samples, but the chemical parameters selected will be used subsequently to analyse the profile using a large set of samples.

2.2. Methods

2.2.1. Samples

2.2.1.1. Peat samples

Samples were obtained from a peat core (895 cm) from an interdrumlin raised bog located at Puerto Harberton (54°53'S–67°20'W; 20 m a.s.l.), Tierra del Fuego (South Argentina). The core was taken at a distance of 1 m from the site in Heusser (1989) and 20 m from that in Pendall et al. (2001); for the location see Markgraf (1993). The peat, dating back to 14 ka, is primarily composed of *Sphagnum magellanicum* in the upper 600 cm, and different genera and species of brown mosses below 850 cm. In addition to pollen analysis (Heusser, 1989; Markgraf, 1993; Pendall et al., 2001) the core was analyzed for charcoal abundance and macrofossils (Markgraf, 1993; White et al., 1994). Cores were taken with a 5 x 50 cm Russian (Macaulay) peat sampler. The upper 174 cm were sampled according to the morphology/stratigraphy. The deeper part of the profile was sampled in sections of 3 cm, except at the end of each sub-core where the samples were 5 cm thick. The volcanic ash layers were sampled individually. The resulting 264 samples were air dried, ground, homogenised and divided into sub-samples for the various analyses.

Based on depth and ash content (which might indicate differences between ombrotrophic and minerotrophic peat), 13 samples were selected (Fig. 2.1). They were expected to contain the most diverse chemical information stored in the core. Three fractions of the selected samples were analyzed: bulk samples, extractable OM and non-extractable OM (residue). Extracts are supposed to reflect more decomposed material and microbial products, while residues more closely reflect relatively intact plant material (Buurman et al., 2006).

2.2.1.2. Vegetation samples

Samples of the dominant vegetation species found in the Harberton peat core (Markgraf, 1993) were collected from actual growing vegetation on Tierra del Fuego peat. The samples were oven dried at 35 °C for 1 week, ground and analyzed with Py-GC/MS. Plant species and analyzed parts are given in Table 2.1.

2.2.2. Extraction

An aliquot (0.5 g) of each peat sample was extracted with NaOH (0.1 M, 20 ml), shaken for 24 h under N_2 and centrifuged (1 h) at 4000g. The extract was decanted and the extraction repeated a second time. The extracts were combined, acidified to pH 1 with HCl, shaken for 24 h, dialyzed against distilled water (cut off 1000 D) and freeze-dried. The residues were acidified, dialyzed against distilled water and freeze-dried. Total samples were ground for pyrolysis without further pre-treatment.

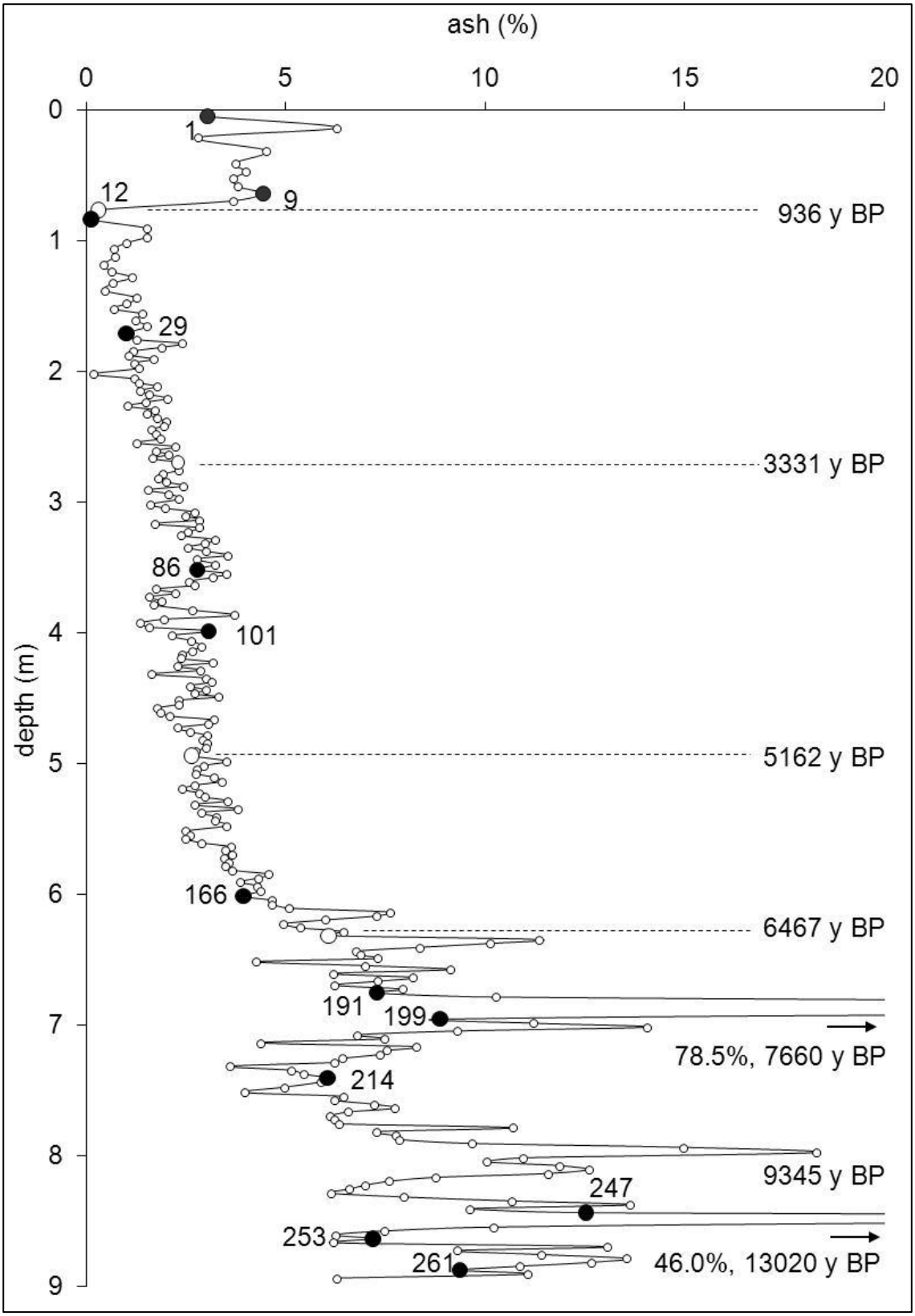

Fig. 2.1. Ash content of the (selected) samples. The high values at 685 cm, 795 cm, and 845 cm correspond to tephra layers. ^{14}C dating of tephra layers: 685 and 795 cm (Stern, 2008), 845 cm (Franzén, 2006).

2.2.3. Pyrolysis-GC/MS

2.2.3.1. Peat samples

The three fractions: bulk sample, extract and residue were pyrolyzed using a Curie-Point pyrolyser (Curie temperature 600 °C) connected to a Carlo Erba GC8000 gas chromatograph. The pyrolysis products were separated on a fused silica column (Chrompack 25 m, 0.25 mm i.d.) coated with CP-Sil 51 b (film thickness 0.40 µm), with He as carrier gas. The initial oven temperature was 40 °C and the heating rate 7 °C min^{-1}. The final temperature, 320 °C, was maintained for 20 min. The GC column was connected to a Fisons MD800 mass spectrometer (*m/z* 45–650, cycle time 1 s).

Of the 39 samples, 12 pyrograms were interpreted in detail, resulting in 380 different pyrolysis products. Based on frequency and abundance the number was reduced to 177. These were quantified for all 39 pyrograms using the two main fragment ions for each pyrolysis product (Appendix 2). All quantifications were checked manually.

2.2.3.2. Vegetation samples

Pt filament coil probe Py-GC/MS was performed with a Pyroprobe 5000 (Chemical Data Systems, Oxford, USA) coupled to a 6890 N GC and 5975B MSD GC/MS system from Agilent Technologies (Palo Alto, USA). The samples were pyrolyzed at 650 °C for 2 s (heating rate 10 °C ms^{-1}). The interface and GC inlet (in splitless mode) were set at 325 and 320 °C, respectively. The GC oven was heated from 40 to 320 °C (held 10 min) at 7 °C min^{-1}. 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), with He as carrier gas.

Samples S1, S2 and S3 were analyzed in duplicate (R^2 = 0.89, 0.94 and 0.93, respectively) and mean values of pyrolysis products were used. All peaks were identified and quantified, except for the sterols, whose identification from Py-CC/MS is doubtful. This resulted in 187 different pyrolysis products, of which 21 were detected in the peat samples and exclusively found in one of the investigated species. However, most of these 21 potential vegetation markers were not used because they also have a common or microbial origin.

2.2.3.3. Quantification

The total ion current (TIC) of all quantified pyrolysis products was set at 100% and relative amounts were calculated with respect to this to give the relative abundance of each pyrolysis product. This quantification is essentially different from wt%, because (i) MS response factors of pyrolysis products vary and (ii) molecular masses of the pyrolysis products are widely different. Changes in relative abundance of pyrolysis products can be interpreted in the same way as data quantified otherwise.

The pyrolysis products were grouped according to probable origin and chemical similarity into a number of source groups: polysaccharides, aliphatic biopolymers, methyl ketones, lignins, phenols, (other) aromatics, polyaromatics, N compounds, lipids (steroids) and triterpenoids. The quantified data sets for extracts, residues and bulk samples were analyzed with factor analysis using Statistica® Version 6 (Statsoft Inc., Tulsa).

Table 2.1. Abundances of quantified groups (%) of 187 pyrolysis products of vegetation samples[a].
[a]Ps polysaccharides; Al aliphatics; *n*-C:o alkanes; *n*-C:1 alkenes; Lg lignins; G, S and C are guaiacyl, syringyl and cinnamyl as % of the lingin group; Ph phenols; Ar aromatics; PA polyaromatics; Lp lipids; MK methylketones; FA fatty acids; N N-compounds; ? unidentified. [b]Leaves/stem. [c]Cortex. [d]Xylem.

	Vegetation	Ps	Al	*n*-	*n*-	other	Lg	G	S	C	Ph	Ar	PA	Mk	FA	N	?
S1	*Sphagnum*	62.6	3.4	0.6	0.1	2.6	6.5	2.0	0.0	98.0	24.0	1.6	0.0	0.1	0.2	0.0	1.6
S2	*Empetrum rubrum*	19.6	41.3	27.5	2.0	11.8	7.9	55.2	17.6	27.2	8.9	9.8	0.1	0.1	5.4	0.3	6.5
S3	*Pseudocyphellaria*	66.2	3.7	1.8	0.1	1.7	0.0				2.9	12.2	0.0	0.0	10.3	0.8	3.8
S5	*Marsippospermum*	53.4	2.4	1.9	0.1	0.4	38.2	65.0	14.8	20.2	1.7	1.4	0.1	0.0	0.3	0.0	2.5
S6	*Pseudocyphellaria*	71.5	3.2	1.3	0.3	1.6	0.2				1.1	20.5	0.1	0.2	1.9	0.0	1.3
S7	*Nothofagus*	33.8	7.5	0.8	1.2	5.5	44.6	72.5	26.9	0.5	6.2	3.6	0.1	0.0	2.5	0.3	1.6
S8	*Nothofagus*	39.3	0.3	0.1	0.0	0.2	50.8	23.7	76.3	0.0	5.2	2.1	0.0	0.0	0.4	0.0	1.9
S9	Lichen 1	92.6	.04	0.1	0.0	0.3	0.0				1.9	0.1	0.0	0.0	0.2	0.0	4.9
S10	Lichen 2	93.1	1.7	0.0	0.0	1.7	0.0				1.6	0.7	0.0	0.0	0.4	0.0	2.6
S11	*Nothofagus pumilio*	28.1	26.8	16.5	3.4	6.9	16.1	66.3	12.1	21.5	10.7	10.8	0.1	0.3	4.8	1.1	1.2

Table 2.2. *n*-Alkane distribution of vegetation samples (as % of alkanes).

	Vegetation	C_{15}	C_{17}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{27}	C_{29}	C_{31}
S1	*Sphagnum magellanicum* [b]	0.0	2.5	0.0	8.7	6.1	33.9	5.2	30.1	6.0	7.5	0.0
S2	*Empetrum rubrum* [b]	0.6	0.4	0.0	0.5	0.5	0.7	0.6	1.0	7.1	56.1	29.4
S3	*Pseudocyphellaria freycinettii*	18.9	15.8	0.0	3.2	3.9	3.3	1.6	2.6	5.2	26.6	17.3
S5	*Marsippospermum* sp. [b]	4.8	3.5	0.0	1.8	1.7	3.9	1.9	2.2	5.1	32.8	38.2
S6	*Pseudocyphellaria*	6.0	4.6	0.0	5.8	5.7	5.4	2.4	6.8	10.1	32.8	17.2
S7	*Nothofagus antarctica* [c]	8.3	6.6	31.0	17.9	30.4	0.0	5.9	0.0	0.0	0.0	0.0
S8	*Nothofagus antarctica* [d]	0.0	0.0	0.0	10.1	8.9	12.2	5.5	0.0	5.5	29.3	28.5
S9	Lichen 1	0.0	0.0	0.0	0.0	0.0	8.1	7.8	11.8	37.8	26.8	7.6
S10	Lichen 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S11	*Nothofagus pumilio* [b]	1.5	1.2	0.0	2.2	2.7	16.1	2.7	6.3	11.8	46.0	8.8

2.3. Results and discussion

2.3.1. Vegetation samples

Table 2.1 shows the general chemical composition of the vegetation samples. The lichens (S3, S6, S9 and S10) predominantly consist of polysaccharides, *Sphagnum* (S1) has high phenolic content, and *Empetrum* (S2) a high aliphatic contribution. Large differences in lignin content are found between the woody species *Empetrum* (7.9%) and *Nothofagus antarctica* (45–50%). The xylem of *N. antarctica* (S8) predominantly consists of syringols, with high contributions from C_3 syringols, 4-vinylsyringol and syringol (8%, 9% and 10% of TIC, respectively), while cinnamyl compounds are typically absent. *Marsippospermum* showed a large peak of 4-vinylguaiacol (20% TIC) and a relatively low percentage of C_3 guaiacols.

The distribution of *n*-alkanes (Table 2.2) is in agreement with most literature data. The dominance of the C_{23} and C_{25} alkanes for *S. magellanicum* is in agreement with Baas et al. (2000) and Corrigan et al. (1973). The other plants show a dominance of C_{29} or C_{31} alkane or both. High contributions of C_{29} and C_{31} occur in *Empetrum* where the aliphatics comprise 41.3% of the total OM, dominated by the alkanes (see Tables 2.1 and 2.2); also, pristene (Al2) had a high contribution (1.2% compared to >0.1% TIC in the other samples, not specified in Table 2.1). The cortex of *N. antarctica* (S7) shows a dominance of even alkanes and a relatively high contribution from alkenes. These aliphatics may be derived from bacteria associated with the bark of *Nothofagus* (Brassell et al., 1978). This sample (S7) was the only vegetation sample in which C_{18}, C_{20} and C_{22} alcohols were detected. The lichen S3 shows a dominance of C_{31} and C_{15} alkanes.

Typical markers of the various species (Section 2.2.3.2) were benzoic acid in *Empetrum rubrum* (7.0% TIC), a benzene compound with ions at *m/z* 107 + 138 in the *Pseudocyphellaria* lichens S3 (8.3% TIC) and S6 (17.0% TIC), and 4-propyneguaiacol and 4-alleneguaiacol in both bark and sapwood of *N. antartica* wood (0.5, 0.5% TIC) (Appendix 2 and Table 2.1). No markers were found for *Marsippospermum* (S5) and lichens S9 and S10.

Although benzoic acid might be a degradation product of lignin and is found as a pyrolysis product in peat fulvic acids and podzol soils (Martín et al., 1994), and 4-propyneguaiacol was also found in eucalyptus (Ibarra et al., 2007) and willow wood (Ralph and Hatfield, 1991), with respect to the present samples they probably indicate the presence of *Empetrum* and *N. antarctica*, respectively.

2.3.2. Peat samples

All pyrolysis products and their mean abundance in the three fractions are listed in Appendix 2. The information sought can be obtained by quantifying large numbers of pyrolysis products, such as the 177 in this study. This, however, is time consuming and inefficient, because some pyrolysis products contain more information than others. Factor analysis was applied to the results of each fraction, first to interpret the meaning of the plot of samples and products in factor space and thereafter to select the pyrolysis products containing the required information. Extracts are expected to give more information on decomposition (and addition of microbial products), while residues are supposed to

reflect more the undecomposed vegetation. Both are used to interpret the chemistry of the total samples.

2.3.2.1. Residues

In factor analysis of the residue samples, four factors explain 73.3% of the variation in all pyrolysis products; Factors 1 and 2 together explain 54.2%. The loadings of the pyrolysis products in F1–F2 space are shown in Fig. 2.2. Products with loadings <0.5 are not discussed in detail because they barely contribute to distinction among samples.

Lignin compounds group together with short and mid-chain alkanes and alkenes and have a positive loading on Factor 1. The main lignin compound that plots outside this group is 4-vinylphenol (Lg3), with a negative loading on Factor 1. It is probably a marker for *Sphagnum* moss as it was found in high abundance in *S. magellanicum* (see cinnamyl content in Table 2.1; the 2% guaiacyl is probably a result of contamination in the *Sphagnum* vegetation), and plots very close to 4-isopropenylphenol (Ph8), which is a typical *Sphagnum* marker (Stankiewicz et al., 1997).

The long chain alkanes ($C_{28–31}$) and pristene (Al2) plot at the bottom of the diagram. This may indicate *Empetrum*, which has a high contribution of these aliphatic compounds and low lignin content (see Section 2.3.1 and Table 2.1). Other pyrolysis products which plot at the bottom are, among others, long chain methyl ketones, levoglucosan (Ps29), sterols and a lignin dimer. These are indicative of fresh plant material (e.g. Buurman et al., 2007). The *Empetrum* marker (Ar14) plots within the lignin group and is probably more stable than the long chain alkanes.

The top centre of the diagram, opposite the markers of fresh material, may represent the strongest decay, with a relative accumulation of aromatics and phenols.

There is a decrease in chain length for alkanes, alkenes, methyl ketones, alcohols and fatty acids (FAs) from the bottom toward the top, indicated by the arrow in Fig. 2.2. This was also found for a Spanish peat and is ascribed to degradation (Buurman et al., 2006). Factor 2 divides the lignin group into guaiacols (positive loading) and syringols (negative loading). Within both groups, the compounds with short side-chains have loadings that are more positive on Factor 2. This reduction in (side)chain length also shows that Factor 2 indicates a (aerobic) decay process, from fresh material at the bottom to decayed material at the top (Kuder and Kruge, 1998; Buurman et al., 2006).

Most polysaccharides have a negative loading on Factor 1 and plot in the upper left quadrant with the moss marker 4-isopropenylphenol. This suggests that they may represent (partially decayed) cellulose from moss material; 5-hydroxymethyl-2-furancarboxaldehyde (Ps22), 1,4-dideoxy-D-glycero-hex-1-enopyranose-3-ulose (Ps24) and levomannosan (Ps28) plot in the lower left quadrant, suggesting an origin in relatively fresh moss material.

The aromatics benzene, toluene, dimethylbenzene, ethylbenzene and styrene (Ar1–Ar5) plot within the polysaccharide cluster at the upper left of the diagram. The fact that these aromatics plot with the moss markers may indicate that they result from decay of phenolic compounds in *Sphagnum* moss (compare the phenolic content of *Sphagnum* in Table 2.1 with that of the *Sphagnum*-dominated upper samples in Table 2.4).

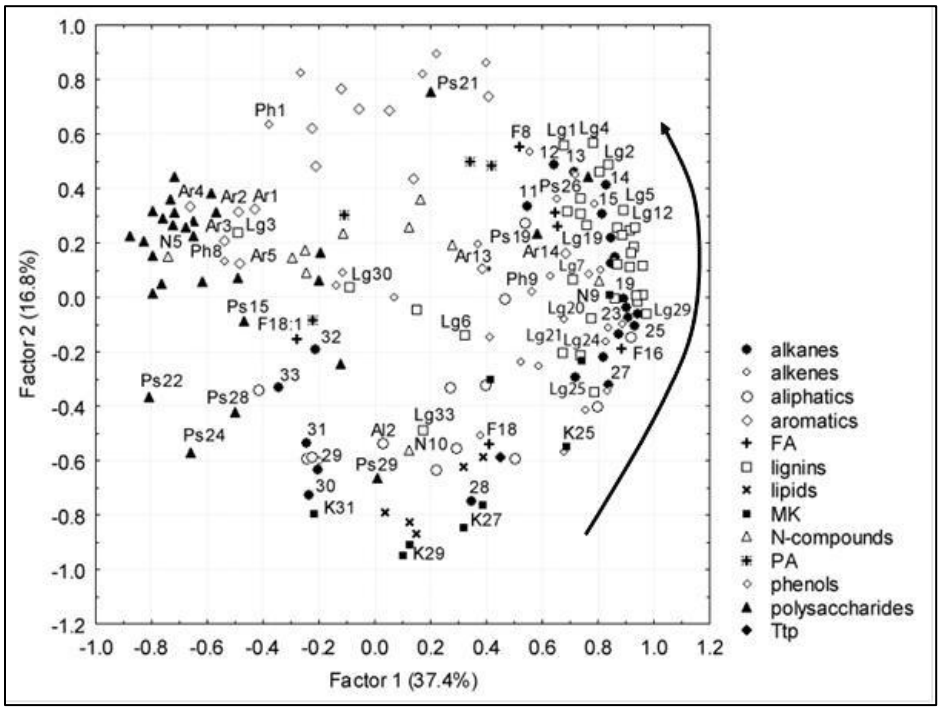

Fig. 2.2. Factor loadings for residue samples in F1–F2 space. Arrow denotes aerobic degradation in woody material.

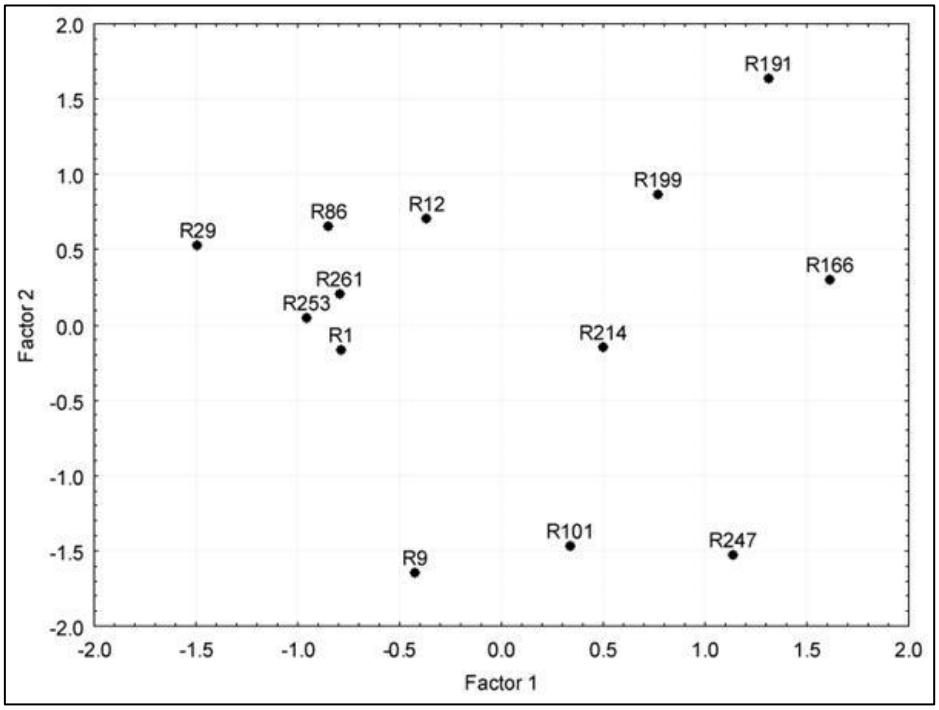

Fig. 2.3. Factor scores for residue samples in F1–F2 space.

Most N compounds do not differentiate among the residue samples and plot in the centre of the diagram, except for diketodipyrrole (N9) and 3-pyridinamine (N5), which plot with the lignin group and the polysaccharide group, respectively, suggesting that the first is indicative of woody plants (e.g. Buurman et al., 2007) and the second of moss vegetation.

The distribution of the loadings indicates that Factor 1 differentiates between woody (positive loading) and moss vegetation (negative loading). Within both vegetation types, negative loadings on Factor 2 indicate relatively fresh plant-derived material, while positive loadings indicate rather strong (aerobic) decomposition, with stronger decomposition resulting in accumulation of aromatics and phenols. The lower right quadrant, with relatively intact and oxygenated syringyl moieties, probably indicates anaerobic degradation (relative accumulation) of woody material.

The location of the samples in F1–F2 space (Fig. 2.3) indicates that samples R101, R166, R191, R199, R214 and R247 are derived from more woody vegetation and samples R1, R9, R12, R29, R86, R253 and R263 originate from a dominant moss vegetation. This is in accord with macrofossil analysis of the same peat bog by Markgraf (1993). The location of samples R9 and R101 indicates a relatively high contribution from *Empetrum* to a moss and wood dominated peat, respectively.

Within the woody vegetation there is a distinct decomposition trend (see arrow in Fig. 2.2) from relatively undecomposed at bottom centre to strongly decomposed at top right. If the top right quadrant represents aerobic decay, the effect is most strongly represented in sample R191, while anaerobic decay (preservation) is strongest for sample R247. Within the moss-dominated samples, a higher score on Factor 2 probably indicates stronger decomposition. However, the differences between the moss-dominated samples on Factor 2 are small, possibly because the high phenol content of *Sphagnum* inhibits decay (e.g. Freeman et al., 2001). Additionally, aerobic circumstances (which facilitate decomposition) induce replacement of *Sphagnum* by other vegetation.

2.3.2.2. Extracts

In factor analysis of the extracts, four factors explain 69.6% of the variation, while Factors 1 and 2 together explain 46.5%. The distribution of the pyrolysis products is shown in Fig. 2.4. In contrast to the residues, Factor 1 now separates lignin compounds (negative loading) and alkanes/alkenes (positive loading). Mid-chain alkanes and alkenes (C_{14}–C_{25}) plot in the lower right quadrant negative on Factor 2, with the C_{19}–C_{25} alkanes clearly separated from their alkene counterparts. Long chain alkanes and alkenes have low loadings on Factor 2, while the (microbial) C_{12} alkane and alkene plot closer to the centre. Close to the long chain alkanes other markers of fresh material plot, as indicated in the previous section. Most lignin compounds group in the lower left quadrant. Guaiacol (Lg1) is separated from the group and plots positively on Factor 2. Most syringols are concentrated at the bottom part of the lignin group. Lignins with a C_3 side-chain plot more on the right hand side of the group while oxygenated lignin compounds plot more towards the bottom left.

The top part of the diagram contains low molecular weight (MW) polysaccharides (Ps1, Ps2, Ps3, Ps6 and Ps8), N compounds N1–N4 and aromatics Ar1–Ar4 and Ar6. This combination is indicative of a microbial origin (Buurman et al., 2007). Between the microbial pyrolysis products in the upper part of

the diagram and the fresh components on the right hand side, is a scattered group of some (plant-derived?) polysaccharides, lipids (Lp2 and Lp3), C8 FA, one aromatic (Ar8) and catechol (Ph6).

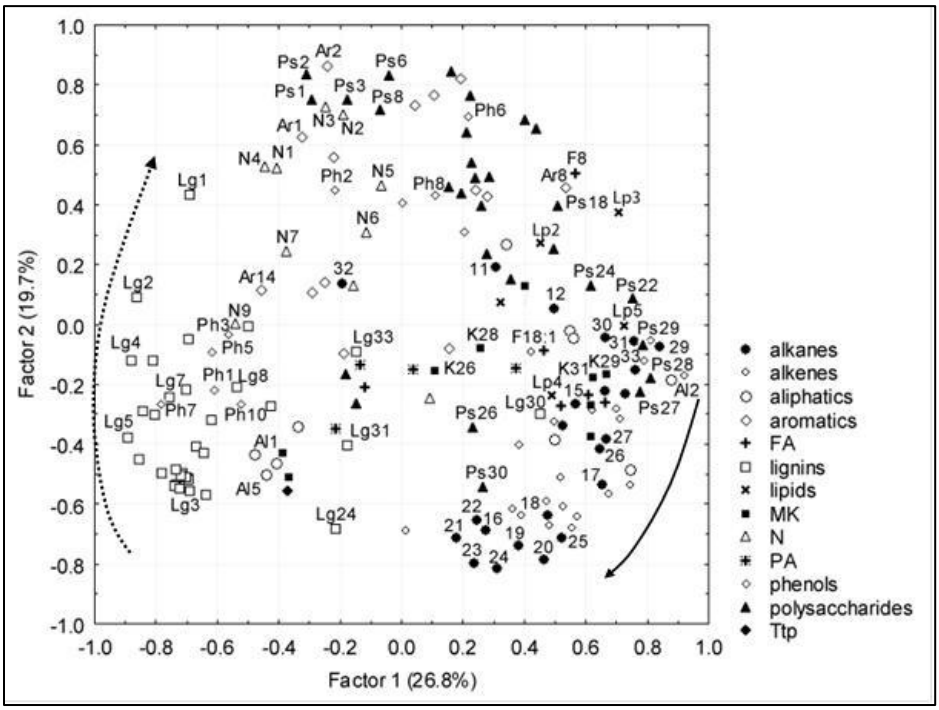

Fig. 2.4. Factor loadings for extract samples in F1–F2 space. The dtted arrow indicates aerobic degradation of lignin: the drawn arrow denotes anaerobic (residual) accumulation of alkanes.

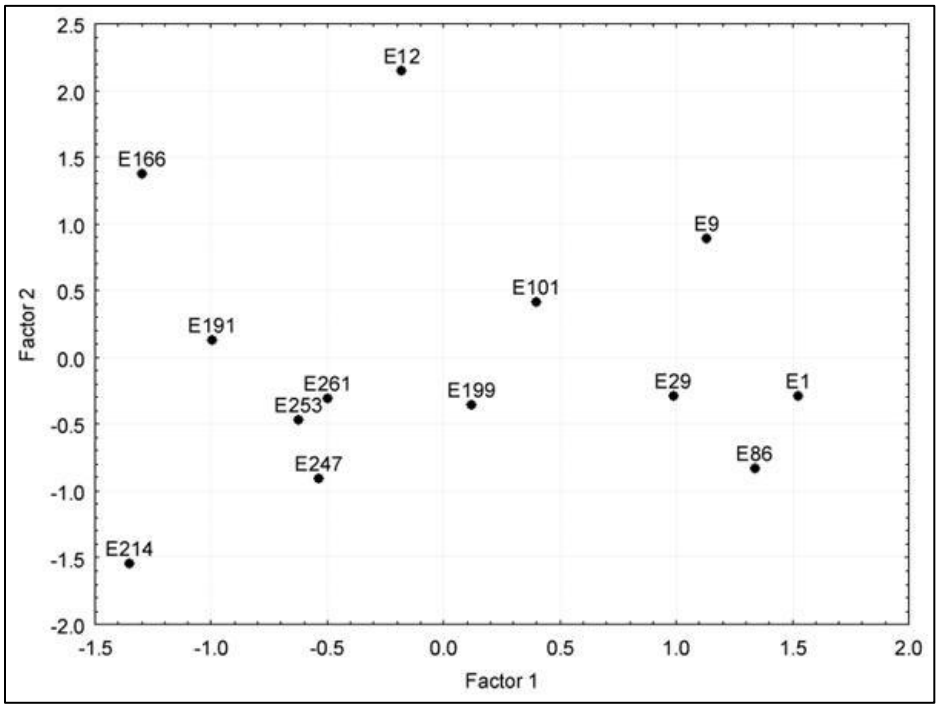

Fig. 2.5. Factor scores for extract samples in F1–F2 space.

The projection of the various pyrolysis products in F1–F2 space suggests the following interpretation. In the extracts 4-vinylphenol (Lg3) plots within the lignin cluster opposite to the *Sphagnum* marker 4-isopropenylphenol, while factor analysis of the residues showed that Lg3 is indicative of *Sphagnum*. This discrepancy may be explained by the differences in composition of both fractions. The residues predominantly consist of litter and clearly show a *Sphagnum* origin. The location of Lg3 in factor loadings of the extracts and the fact that extracts contain more decomposed plant material suggests that this compound in the extracts predominantly originates from demethoxylation of guaiacols or syringols, and thus from woody vegetation. The moss marker Ph8 plots positively on Factor 2 and may therefore be residually accumulated upon decay. The separation between lignin and alkane/alkene compounds suggests that the left hand part of the diagram represents (degraded) woody material, while the right hand part, by way of an absence of lignin, represents non-woody material. The fact that both Factors have nearly equal contributions to the explained variance shows that vegetation (F1) is of less importance in the extracts compared with residue and total samples. Since aerobic and anaerobic circumstances influence both vegetation composition and decay process, the separation of lignins and alkanes in the extracts becomes more clear.

There is a decay trend for both the woody and the non-woody material. For the woody material (negative loading on Factor 1), the aerobic decay points upwards, with Lg1 as the ultimate lignin pyrolysis product. For both the woody and the non-woody fraction, aerobic decay shifts the chemical composition towards the top of the diagram, which denotes accumulation of microbial products. For both woody and non-woody material, shifts towards the bottom of the diagram denote anaerobic decay. For the non-woody material, this leads to a relative accumulation of mid-chain alkanes (and alkenes). For the woody material, it is characterised by a preservation of (oxygenated) C_3 chains on lignin moieties.

The fully drawn arrow in Fig. 2.4 therefore indicates anaerobic (relative) accumulation of alkanes and alkenes in non-woody material, while the dotted arrow indicates aerobic decay of woody material. Anaerobic decomposition does not appear to cause accumulation of microbial matter, while the arrow for aerobic degradation points in the direction of the microbial compounds. The separation of mid-chain alkanes and alkenes in factor space suggests that, once the source material (e.g. suberan) is broken up into smaller units and the ether bonds have been destroyed, pyrolysis produces fewer alkene moieties.

The scores of the extract samples in F1–F2 space (Fig. 2.5) show that samples E1 and E29 plot at the right hand side of the diagram, where fresh, non-woody material is dominant. Sample E86 shows a slight residual accumulation of aliphatics, while E9 has some accumulation of microbial material. Samples E12 and E166 plot in the upper left quadrant where the microbial products are dominant, aliphatics are depleted and lignins are strongly degraded. Samples E101, E199 and E261 do not show specific degradation or vegetation characteristics. The samples in the lower left quadrant are all rich in lignin compounds, with strongest (anaerobic) preservation in sample E214.

2.3.2.3. Total samples

In the analysis of the total samples, four factors explain 77.9% of the variation in all pyrolysis products, while Factors 1 and 2 together explain 60.3%. The loadings in F1–F2 space are shown in Fig. 2.6. The polysaccharides separate into three groups. The benzofurans (Ps21, Ps26 and Ps30) have negative loadings on Factor 1. The levosugars Ps27-29, indicative of fresh plant material, plot in the lower right quadrant, while the group indicative of microbial material, including all furans, plots in the upper right quadrant. Of the aliphatics, pristene (Al2) and the C_{29} and C_{31} alkanes plot at bottom-centre. The mid-chain and short chain alkanes, alkenes and methyl ketones largely plot in the lower left quadrant. Exceptions are the short chain C_{11} and especially C_{12} members, with much higher loadings on Factor 2, supporting the non-plant origin of the latter. The location of the C_{15} alkane, close to the long chain alkanes, is probably a result of the high contribution of this alkane to lichen S3 (Section 2.3.2). Lignin compounds plot in the upper left quadrant, with guaiacol (Lg1) and 4-ethylguaiacol (Lg4) at the top of the distribution. Low loadings on Factor 2 are found for lignin compounds with partially oxidised side-chain (acetyl and formyl guaiacols and syringols). Although most syringol compounds plot towards the left side of the lignin cluster, it does not appear possible to separate various lignin types. The potential moss marker Lg3 plots inside the lignin cluster negative on Factor 1, but the real *Sphagnum* marker Ph8 plots at the far right. This indicates that, for total samples, 4-vinylphenol is not valid as a *Sphagnum* marker because, as for the extracts, the cinnamyl moieties of the (decayed) woody vegetation play a major role. Lignin compounds with C_3 side chain plot in the middle of the lignin group with the C_3 syringols (Lg23, 26, 27) in the left hand part and the C_3 guaiacols (Lg8, 11, 13) more towards the centre of the diagram. As suggested above, this may indicate that anaerobic degradation of lignin leads to oxygenated side-chains (shift towards the F1 axis), while aerobic degradation leads to complete side-chain removal, ending with guaiacol (Lg1). This is compatible with the behaviour of alkanes/alkenes, because residually accumulated mid-chain alkanes/alkenes plot close to the oxygenated lignin compounds. Phenols and aromatics plot at the top of the diagram, with the phenols more towards the lignins. N compounds N1–N4 plot close to toluene (Ar2) suggesting that these products again represent (microbial) proteins. Some N compounds plot with the lignin cluster, suggesting that these are pyrolysis products of (woody) plant proteins (N9, see Section 2.3.2.1) or are of microbial origin associated with woody vegetation (N6, N7, N8 and N10).

Combining these interpretations, plots of total samples in F1–F2 space can be read as follows: the bottom right quadrant represents relatively fresh plant-derived material; the lower left quadrant represents relative accumulation of recalcitrant plant-derived material because of anaerobic degradation. As found by Pontevedra-Pombal et al. (2001), anaerobic residual accumulation of alkanes/ alkenes is more pronounced than that of lignin. The upper left quadrant represents aerobic degradation of lignin, while the top and the upper right quadrant represent accumulation of microbial biomass and strong degradation (depletion) of the plant-derived compounds.

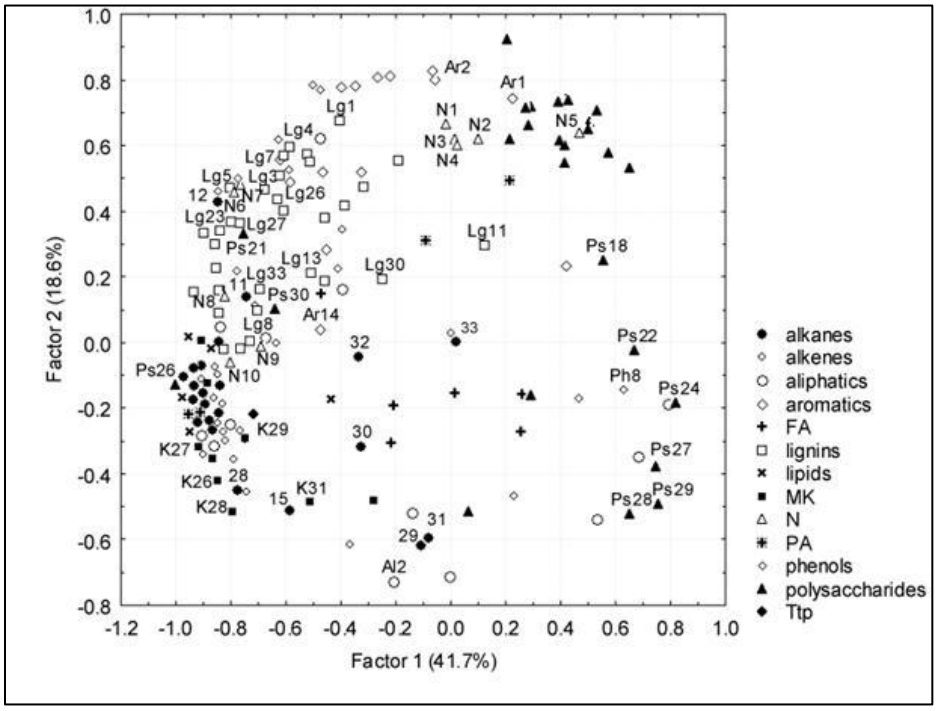

Fig. 2.6. Factor loadings for total samples in F1–F2 space.

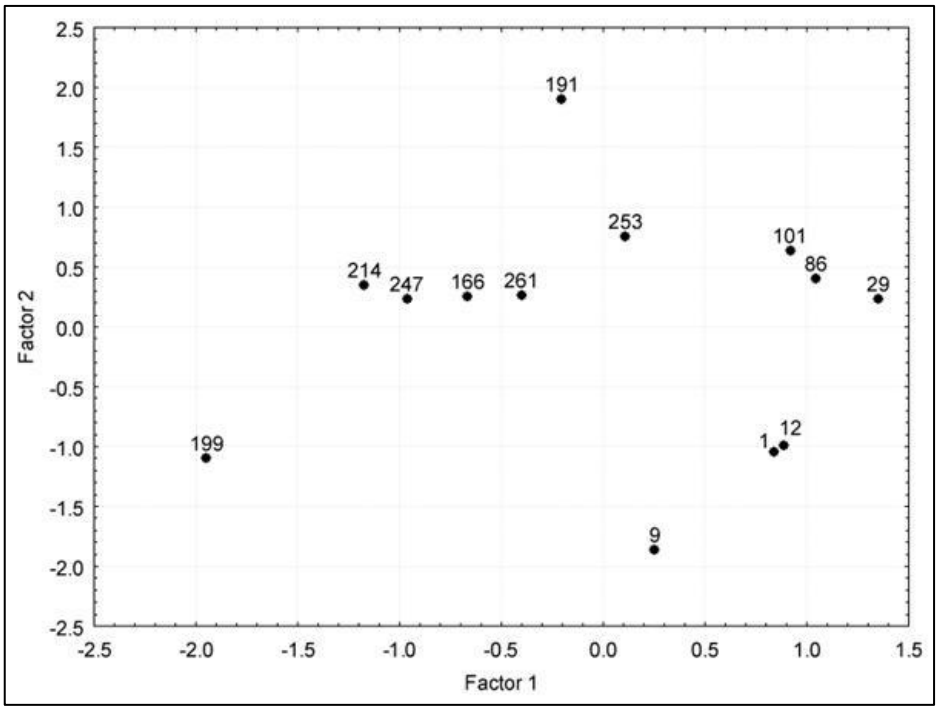

Fig. 2.7. Factor scores for total samples in F1–F2 space.

The distribution of total samples in factor space (Fig. 2.7) can therefore be interpreted as follows: The deepest samples (253 and 261) plot in the centre of the diagram and do not allow any distinction of vegetation or degradation state. The top samples (1–12) plot in the lower right quadrant and represent relatively fresh material. Samples 29, 86 and 101 plot in the upper right quadrant and are dominated by moss markers with relatively low amounts of especially aliphatics, but also of lignin. Sample 191 appears to combine the strongest aerobic degradation of aliphatics and lignin with the largest accumulation of (microbial) proteinaceous material. Samples 214, 247 and 166 have lost fresh cellulose, but degradation of aliphatics and lignin is moderate. Finally, sample 199 is a woody sample that has the greatest residual accumulation of plant-derived alkanes and alkenes, together with the lowest contents of fresh plant material and microbial products.

Table 2.3. Interpretation for different fractions from factor analysis[a].

Sample	Residue	Total	Extract
001	–	Fresh	Fresh
	Non-woody	Non-woody	Non-woody
009	Fresh	Fresh	Microbial
	Mixed	Mixed	Non-woody
012	Degraded	Fresh	Microbial
	Non-woody	Non-woody	Mixed
029	–	–	Fresh
	Non-woody	Non-woody	Non-woody
086	Degraded	Degraded	Residual
	Non-woody	Non-woody	Non-woody
101	Fresh	Microbial	Microbial
	Mixed	Non-woody	Mixed
166	–	–	Microbial
	Woody	Woody	Woody
191	Microbial	Microbial	–
	Woody	Mixed	Woody
199	Degraded	Residual	Residual
	Woody	Woody	Mixed
214	–	–	Residual
	Woody	Woody	Woody
247	Residual	–	Residual
	Woody	Woody	Woody
253	–	–	?
	Non-woody	Mixed	Woody
261	–	–	?
	Non-woody	Mixed	Woody

[a] For all samples first line indicates decomposition state and second line vegetation.

2.3.2.4. All fractions

The interpretations for extracts, residues and total samples are summarised in Table 2.3. The interpretations for the three fractions largely coincide. Some samples show contradictory interpretations for the different fractions. Samples 9 and 12 are classified as microbial from the extracts while they indicate fresh material for the total samples. Sample 101 shows a dominance of microbial material in the extract and total sample while this sample is classified as fresh in the residue. Samples 253 and 261 differ in interpretation of vegetation, with a different classification for each fraction. Discrepancies may result from relative abundances of the various fractions. In a fresh peat sample, the total sample and the residue may reflect the same characteristics, while the extractable fraction may be dominated by microbial matter on the surface of vegetation remains, thereby giving a completely different picture. This is further elaborated in the Conclusions below.

2.3.3. Selection of parameters

Because not all pyrolysis products give valuable information and 177 are too many for quantification of large numbers of samples, the number of pyrolysis products was reduced. The factor analyses indicate which pyrolysis products contain the information that distinguishes samples. The number of pyrolysis products was reduced by deleting those with one or more of the following characteristics: (i) products with loadings between 0.5 and -0.5 in all data sets, (ii) products with relative intensity <0.5% TIC, (iii) products that show little variation with depth and (iv) products that plot together in factor analysis of all fractions and behave similarly with depth, those with the lowest intensity being omitted. The remaining 86 pyrolysis products are printed in bold in Appendix 2. The elimination of half the original pyrolysis products did not change the previous interpretation of the (total) samples as reflected in Table 2.3, neither did it significantly change the general depth trends of groups. The number of products was not reduced to a minimum because the totals for the groups (polysaccharides, N compounds, lignins, etc.) also contain valuable information on vegetation, decomposition, or effects of burning. Relative abundances of products were recalculated for the reduced data set.

The reduced data set was used to derive a number of parameters that reflect vegetation, decomposition and burning history, and which are used to interpret the chemistry of the total samples. Because it is still unknown which parameters best reflect the sought after characteristics, a number of parameters are tested for each feature.

2.3.3.1. Vegetation parameters

Table 2.5 identifies the parameters used for characterization of the original vegetation. The total lignin content (parameter V1) reflects the relative contribution of woody and non-woody vegetation. The total polysaccharide content (V2) may reflect the same characteristic. The syringyl/guaiacyl ratio (V3) indicates the relative contributions of different plant species or parts.

Table 2.4. Abundances of quantified groups (%) of 86 pyrolysis products in total samples[a].

Sample	Depth (cm)	Ps	Al	Lg	Ph	Ar	PA	Lp	Mk	FA	N
001	5	65.8	18.5	3.5	7.2	1.0	0.2	0.2	1.8	1.3	0.4
009	64	63.5	20.1	4.5	5.9	0.4	0.2	0.5	4.1	0.4	0.4
012	83	76.3	5.6	7.3	8.8	0.1	0.1	0.0	1.2	0.1	0.5
029	171	77.2	5.4	4.4	10.6	0.9	0.0	0.2	0.9	0.2	0.2
086	352	76.1	4.2	7.0	8.2	2.4	0.2	0.2	0.6	0.5	0.6
101	399	71.4	4.8	9.5	9.8	2.5	0.2	0.1	0.4	0.5	0.8
166	602	36.6	14.8	24.3	11.5	5.3	0.1	0.8	4.3	0.5	1.8
191	676	38.5	12.3	21.3	16.5	6.0	0.2	0.3	1.7	0.5	2.8
199	696	26.0	30.3	18.6	11.2	2.2	0.5	0.5	7.7	0.8	2.2
214	741	32.2	19.6	22.6	14.4	3.2	0.4	0.7	5.1	0.6	1.3
247	844	34.7	19.3	20.4	15.4	1.7	0.4	0.6	5.7	0.4	1.5
253	864	51.4	15.7	16.4	10.5	1.5	0.2	0.2	2.4	0.1	1.5
261	889	48.1	15.9	15.1	10.4	2.7	0.3	0.7	5.1	1.0	0.8

[a] Ps, polysaccharides; Al, aliphatics (including alkanes alkenes and other aliphatics); Lg, lignins; Ph, phenols; Ar, aromatics; PA, polyaromatics; Lp, lipids; MK, methylketones; FA, fatty acids; N, N-compounds.

Four parameters potentially measure the abundance of *Sphagnum*. In the total samples, the conflicting interpretation of 4-vinylphenol in extracts and residues can be avoided when it is used relative to 4-vinylguaiacol + 4-vinylsyringol (parameter V4) as woody vegetation contains large amounts of the latter. Also 4-isopropenylphenol is a marker of *Sphagnum* (Stankiewicz et al., 1997) and ratio V5 indicates the relative abundance of mosses vs. higher plants. Rhamnose (Ps18) and 5-hydroxymethyl-2-furancarboxaldehyde (Ps22) plot together with 4-isopropenylphenol and 4-vinylphenol in factor analysis of the residues. Both Comont et al. (2006) and Popper and Fry (2003) found rhamnose and mannose as the dominant monosaccharides in *Sphagnum* species and other bryophytes. Parameters V6a and V6b may therefore also indicate the abundance of *Sphagnum*.

Benzoic acid was exclusively found in *Empetrum* (Section 2.3.2) and V7 may reflect (partially decomposed) *Empetrum*. Fresh *Empetrum* had a relatively high abundance of pristene, a degradation product of chlorophyll (V8).

Nothofagus is characterised by 4-propyneguiacol and 4-alleneguaiacol (parameter V9). The abundance of the marker of *Pseudocyphellaria* (Ar13) was too low in the peat samples (see Appendix 2), so it is therefore not used as a vegetation parameter. The ratio of 4-vinylguaiacol to the summed guaiacols with a C_3 side-chain may be indicative of *Juncus* vegetation (V10) (Section 2.3.2). The C_{23} alkane is dominant in *Sphagnum magellanicum*, while all other species show a dominant C_{29} or C_{31} alkane (V11a,b) (Table 2.2).

Table 2.5. Parameters used to characterise the peat vegetation.

V1	Sum of abundances of all lignin compounds (% TIC)
V2	Sum of abundances of polysaccharides (% TIC)
V3	Sum syringyl/Sum guaiacyl
V4	4-Vinylphenol/(4-Vinylguaiacol + 4-vinylsyringol)
V5	4-Isopropenylphenol (Ph8)/Guaiacols+syringols)
V6a	Dianhydrorhamnose (Ps18) (% TIC)
V6b	5-Hydroxymethyl-2-furancarboxaldehyde (Ps22) (% TIC)
V7	Benzoic acid (% TIC)
V8	Pristene (% TIC)
V9	Lg15 + Lg16 (% TIC)
V10	4-Vinylguaiacol (Lg5)/Sum C_3 guaiacols
V11a	C_{23} alkane/sum alkanes
V11b	C_{29}+C_{31} alkanes/sum alkanes

2.3.3.2. Decomposition parameters

Table 2.6 lists parameters that can be used to judge the decomposition (of plant-derived compounds). The total amount of polysaccharide compounds (V2, in addition to characterising vegetation, may be a useful parameter for judging anaerobic decomposition. With proteins, polysaccharides are the most easily degradable plant components, especially under anaerobic conditions, and low temperature and pH. Factor analysis (Section 2.3.2) indicated that residual accumulation of alkanes/alkenes upon anaerobic decomposition is not accompanied by significant accumulation of microbial sugars, while aerobic degradation is. If other parameters indicate aerobic decomposition (and addition of microbial sugars), V2 is not valid as a decomposition parameter.

Levoglucosan is the most common polysaccharide pyrolysis product and is derived from relatively fresh cellulose (Poirier et al., 2005). High values for parameter D1 therefore indicate relatively fresh (ligno-cellulose) material. A high abundance of acetic acid (parameter D9a), a low MW polysaccharide pyrolysis product, reflects strong decomposition of the polysaccharide fraction (Buurman et al., 2006). High amounts of acetic acid usually coincide with those of 2-furaldehyde, which, in some systems, is a pyrolysis product of microbial sugars and therefore also denotes decomposition (parameter D9b).

Although lignin does not show residual accumulation under anaerobic conditions (Pontevedra-Pombal et al., 2001), it is not easily decomposed under anaerobic conditions (Tsutsuki et al., 1994), cold temperatures (Dai et al., 2002) and low pH (van Bergen et al., 1998). Aerobic degradation of peat causes shortening of alkyl side-chains of lignins (Kuder and Kruge, 1998) and decreases the ratios of guaiacyl and syringyl units with intact side-chains to, respectively, guaiacol or syringol (D4ab). Parameter D2 reflects the relative degradation of the syringol and guaiacol C_3 side-chain. In aerobic systems (soils) side-chain degradation in syringols is more rapid than in guaiacols (e.g. van der Heijden, 1994). Anaerobic degradation of lignin increases oxygenation of the C_3 side-chain (Zaccone et al., 2008) and parameters D7a and D7b reflect this aspect.

Sterols are important plant constituents that are well-preserved under anaerobic conditions in peat (e.g. Kondo and Tsutsuki, 2001). A high abundance might therefore indicate anaerobic conditions

and less decomposed peat (parameters D3a and D3b). If the sterols belong to specific plant species, this may interfere with their interpretation as preservation parameters.

Relatively high abundances for toluene and pyridine (parameters D8a and D8b) indicate more decomposed samples. In such decomposed samples, these pyrolysis products represent the microbial fraction. Anaerobic decay is reflected in an increase in mid-chain (C_{16}–C_{26}) alkanes (parameter D6) as shown by factor loadings of the extracts (Section 2.3.2.2). Methyl ketones can result from microbial oxidation of *n*-alkanes (e.g. Xie et al., 2003; Jansen and Nierop, 2009). The ratio of (long chain) odd methyl ketones to long chain odd alkanes may therefore also reflect anaerobic decay (parameter D5); the C_{29} and C_{31} alkanes are not included as the plant origin of these compounds may dominate the abundance.

Table 2.6. Parameters used to judge decomposition.

D1	Levoglucosan (Ps29)/Sum polysaccharides
D2	Sum C_3 syringyl/C_3 guaiacyl
D3a	Lp2 (% TIC)
D3b	Lp3 (% TIC)
D4a	Sum C_3 guaiacols/Guaiacol
D4b	Sum C_3 syringols/Syringol
D5	Sum MK (C_{25}, C_{27})/Sum alkanes (C_{25}, C_{27})
D6	Sum mid-chain alkanes (C_{16}-C_{26})
D7a	4-formyl- + 4-acetyl-guaiacol/Guaiacol
D7b	4-formyl- + 4-acetyl-syringol/Syringol
D8a	Toluene (% TIC)
D8b	Pyridine (% TIC)
D9a	Acetic acid (Ps2) (% TIC)
D9b	2-Furaldehyde (Ps8) (% TIC)

2.3.3.3. Fire parameters

The fire parameters are listed in Table 2.7. Large contributions from naphthalene (F3), benzonitrile (F2) and benzene (F4) are an indication of the presence of charcoal, while benzofurans (F1) are also considered to represent the influence of burning (e.g. Kaal et al., 2008). High values of the fire parameters, together with a strong decrease in polysaccharide (V2) content, might indicate peat fires. Another indication is a decrease in lignin compounds via the loss of methoxy groups during fire (González-Pérez et al., 2004). Since a phenol/lignin ratio in the profile is predominantly influenced by vegetation differences (see parameter V4) the summed lignin compounds (V1) best reflect the loss of lignin methoxy groups.

Table 2.7. Parameters used to indicate fire history.

F1a	Dibenzofuran (% TIC)
F1b	Methylbenzofuran (% TIC)
F2	Benzonitrile (% TIC)
F3	Naphthalene (% TIC)
F4	Benzene (% TIC)

With depth profiles of the above parameters and the relative abundance of groups (Table 2.4) the profile can be described in terms of vegetation, aerobic and anaerobic decomposition, and fire history, and differences between these variables can be correlated with differences in past climate. This will be done in detail with a large set of (total) samples.

2.4. Conclusions

Analysis of different fractions of peat samples confirms that vegetation characteristics are best reflected in residues, while decomposition characteristics are best reflected in the extracts, as mentioned by Buurman et al. (2006).

Analysis of the residues provides vegetation-specific information stored in the peat profile (confirmed by modern vegetation analysis), which can be used to distinguish among the dominant vegetation in depth profiles of the total peat samples (vegetation parameters). Analysis of the extracts allows distinction between aerobic and anaerobic decomposition, which is not clear from total samples or residues. Thus, analysis of extracts and residues gives additional information about vegetation and decomposition, respectively, which is less clear from analysis of total samples alone.

Parameters based on loadings in factor space of pyrolytic products reduce the data requirements. The chosen parameters enable distinction between dominant vegetation, aerobic and anaerobic decomposition, and fire incidence. There is some redundancy between parameters, but the small number of samples does not allow us yet to interpret the meaning or choose the best.

In factor analysis of residues, alkanes and lignins plot together, which correctly illustrates the link between these compounds in fresh wood. Further, aerobic decomposition trends are clearly reflected in the distribution of alkanes, alkenes, lignins, methyl ketones and FAs in factor space. The different susceptibility to aerobic decay of syringols and guaiacols separates these groups in factor space. The fact that aerobic decomposition is clearly linked to a woody vegetation supports the interpretation of residue chemistry in terms of vegetation. In factor analysis of extracts, the separation of aliphatics and lignin compounds indicates different effects of anaerobic and aerobic decay and a loss of vegetation information.

At the end of Section 2.3.2.4, we discussed the discrepancy between classifications based on factor analysis of extracts, residues and total samples. Analysis of different fractions of peat samples with Py-GC/MS allows better interpretation of past environmental circumstances than that of total samples alone. However, despite the clear differentiation and additional information in (factor) analysis of extracts and residues, interpretation of the samples with both fractions can be erroneous due to differences in relative amounts of fractions. Therefore, total samples seem to best reflect the information sought. In the analysis of total samples, depth profiles of the chosen parameters based on the different fractions may provide, in some cases, information not visible in factor analysis.

The chemical signal is strongly influenced by the original vegetation, as Factor 1 separates vegetation in all fractions. Decomposition and fire parameters probably should be interpreted differently for moss or wood dominated vegetation.

Appendix 2. Quantified pyrolysis products. Bold type indicates selected pyrolysis products. M, molecular weight; *m*/*z*, typical mass; RT, retention time; % TIC, ave. % total ion current (13 samples) for residue, total, and extract samples.

Code	Name	M	*m*/z	RT(min)	% TIC Res	% TIC Tot	% TIC Ex
11	C_{11}	156	57+71	12.762	0.11	0.90	0.43
12	C_{12}	170	57+71	15.000	0.16	0.08	0.50
13	C_{13}	184	57+71	17.155	0.12	0.10	0.54
14	C_{14}	198	57+71	19.157	0.13	0.14	0.59
15	C_{15}	212	57+71	21.060	0.15	0.15	0.70
16	C_{16}	226	57+71	22.840	0.24	0.22	0.45
17	C_{17}	240	57+71	24.527	0.18	0.16	0.51
18	C_{18}	254	57+71	26.128	0.16	0.16	0.34
19	C_{19}	268	57+71	27.648	0.17	0.16	0.36
20	C_{20}	282	57+71	29.093	0.20	0.18	0.34
21	C_{21}	296	57+71	30.464	0.23	0.21	0.39
22	C_{22}	310	57+71	31.786	0.28	0.23	0.39
23	C_{23}	324	57+71	33.041	0.33	0.32	0.39
24	C_{24}	338	57+71	34.246	0.24	0.28	0.29
25	C_{25}	352	57+71	35.402	0.48	0.52	0.44
26	C_{26}	366	57+71	36.506	0.16	0.20	0.14
27	C_{27}	380	57+71	37.572	0.41	0.43	0.29
28	C_{28}	394	57+71	38.605	0.15	0.17	0.14
29	C_{29}	408	57+71	39.608	1.59	1.77	1.05
30	C_{30}	422	57+71	40.548	0.13	0.23	0.15
31	C_{31}	436	57+71	41.595	1.36	1.70	0.83
32	C_{32}	450	57+71	42.706	0.05	0.14	0.02
33	C_{33}	464	57+71	44.015	0.27	0.39	0.09
11:1	$C_{11:1}$	154	55+69	12.514	0.20	0.18	0.37
12:1	$C_{12:1}$	168	55+69	14.776	0.41	0.26	0.47
13:1	$C_{13:1}$	182	55+69	16.937	0.16	0.21	0.34
14:1	$C_{14:1}$	196	55+69	18.964	0.14	0.14	0.27
15:1	$C_{15:1}$	210	55+69	20.872	0.14	0.14	0.27
16:1	$C_{16:1}$	224	55+69	22.674	0.35	0.33	0.32
17:1	$C_{17:1}$	238	55+69	24.375	0.15	0.17	0.22
18:1	$C_{18:1}$	252	55+69	25.985	0.23	0.24	0.23
19:1	$C_{19:1}$	266	55+69	27.518	0.12	0.13	0.16
20:1	$C_{20:1}$	280	55+69	28.974	0.21	0.20	0.19
21:1	$C_{21:1}$	294	55+69	30.362	0.12	0.13	0.16
22:1	$C_{22:1}$	308	55+69	31.686	0.29	0.26	0.22
23:1	$C_{23:1}$	322	55+69	32.953	0.13	0.16	0.13

Appendix 2. (*continued*).

Code	Name	M	*m*/z	RT(min)	% TIC Res	% TIC Tot	% TIC Ex
24:1	$C_{24:1}$	336	55+69	34.163	0.20	0.23	0.16
25:1	$C_{25:1}$	350	55+69	35.313	0.17	0.10	0.08
26:1	$C_{26:1}$	364	55+69	36.438	0.14	0.18	0.13
27:1	$C_{27:1}$	378	55+69	37.511	0.19	0.26	0.14
28:1	$C_{28:1}$	392	55+69	38.546	0.12	0.16	0.11
30:1	$C_{30:1}$	420	55+69	40.481	0.06	0.11	0.08
Al1	branched alkane		57+69	13.905	1.07	1.00	0.35
Al2	prist-1-ene	266	56+57	25.045	1.56	1.31	1.60
Al3	C_{16} alcohol	242	55+57	27.227	0.22	0.26	0.14
Al4	branched alkene 1		55+69	29.694	0.01	0.02	0.00
Al5	C_{18} alcohol	270	55+57	30.152	0.20	0.20	0.12
Al7	C_{20} alcohol	298	55+57	32.818	0.18	0.21	0.13
Al8	branched alkene 2	280	83	33.461	0.16	0.21	0.21
Al9	4,8,12,16-tetramethylheptadecan-4-olide	324	99	33.621	0.04	0.02	0.04
Al10	alkanal 1		57+82	34.489	0.08	0.01	0.00
Al11	C_{22} alcohol	326	55+57	35.259	0.22	0.25	0.13
Al13	alkanal 2		57+82	40.912	0.12	0.02	0.01
Al14	alkanal 3		57+82	43.262	0.17	0.02	0.00
Al15	alcohol		55+57	44.139	0.16	0.21	0.01
Ar1	benzene	78	77+78	3.350	0.31	0.20	0.49
Ar2	toluene	92	91+92	4.971	0.74	0.94	2.53
Ar3	ethylbenzene	106	91+106	6.965	0.08	0.12	0.42
Ar4	1,2/1,4-dimethylbenzene	106	91+106	7.139	0.22	0.24	0.92
Ar5	styrene	104	78+104	7.571	0.15	0.16	0.52
Ar6	xylene	106	91+106	7.679	0.17	0.10	0.46
Ar7	propylbenzene	120	91+120	9.134	0.04	0.04	0.41
Ar8	ethyl-methylbenzene/trimethylbenzene	120	105+120	10.119	0.09	0.09	0.67
Ar9	benzeneacetaldehyde	120	91+92	10.845	0.11	0.18	0.33
Ar10	indene		115+116	11.278	0.20	0.17	0.48
Ar11	acetophenone	120	77+105	11.488	0.18	0.13	0.51
Ar12	butyl benzene	134	91+134	11.607	0.05	0.05	0.21
Ar13	benzene compound		107+138	14.349	0.02	0.01	0.02
Ar14	benzoic acid	122	105+122	14.600	0.37	0.57	0.32
F8	fatty acid (C_8)	144	60+73	14.180	0.24	0.13	0.25
F14	fatty acid (C_{14})	228	60+73	25.314	0.11	0.09	0.19
F15	fatty acid (C_{15})	242	60+73	26.890	0.05	0.03	0.03
F16	fatty acid (C_{16})	256	60+73	28.374	0.39	0.33	0.89
F18:1	fatty acid ($C_{18:1}$)	240	55+69	30.889	0.03	0.26	0.37

Appendix 2. (*continued*).

Code	Name	M	*m*/z	RT(min)	% TIC Res	% TIC Tot	% TIC Ex
F18	fatty acid (C_{18})	284	60+73	31.177	0.08	0.08	0.26
Lg1	guaiacol	124	109+124	12.108	1.45	0.89	1.96
Lg2	4-methylguaiacol	138	123+138	14.531	1.73	1.01	0.85
Lg3	4-vinylphenol	120	91+120	15.060	1.53	2.10	2.59
Lg4	4-ethylguaiacol	152	137+152	16.431	0.71	0.44	1.00
Lg5	4-vinylguaiacol	150	135+150	17.135	3.31	2.83	2.98
Lg6	4-(2-propenyl)phenol	134	133+134	17.675	0.24	0.17	0.14
Lg7	syringol	154	139+154	17.714	0.27	0.24	0.66
Lg8	eugenol, 4-(1-propenyl)guaiacol	164	77+164	18.031	0.31	0.18	0.08
Lg9	4-propylguaiacol	166	137+166	18.293	0.12	0.05	0.05
Lg10	vanillin, 4-formylguaiacol	152	151+152	18.654	0.77	0.65	0.49
Lg11	*cis*-4(2-propenyl)guaiacol	164	149+164	19.064	0.18	0.14	0.05
Lg12	4-methylsyringol	168	153+168	19.657	0.32	0.26	0.39
Lg13	*trans*-4-(2-Propenyl)guaiacol	196	77+164	19.861	0.92	0.52	0.23
Lg14	4-Acetylguaiacol	166	151+166	20.385	0.56	0.58	0.80
Lg15	4-propyneguaiacol		147+162	20.527	0.12	0.14	0.09
Lg16	4-alleneguaiacol		147+162	20.660	0.09	0.09	0.07
Lg17	vanillic acid methyl ester	182	151+182	20.986	0.03	0.04	0.04
Lg18	guaiacylacetone, (4-propan-2-one)guaiacol	180	137+180	21.162	0.28	0.18	0.23
Lg19	4-ethylsyringol	182	167+182	21.175	0.09	0.07	0.29
Lg20	4-vinylsyringol	180	165+180	21.863	0.52	0.52	0.73
Lg21	vanillic acid, 4-hydroxy-3methoxy benzoic acid	168	153+168	21.973	0.10	0.22	0.16
Lg22	4-propandione guaiacol	180	151+180	22.188	0.16	0.17	0.18
Lg23	4-(prop-1-enyl)syringol	194	91+194	22.530	0.12	0.08	0.07
Lg24	4-formyl syringol	182	181+182	23.374	0.19	0.19	0.24
Lg25	4-propynesyringol		131+192	24.004	0.05	0.10	0.08
Lg26	*cis*-4-(prop-2-enyl)syringol	194	91+194	23.400	0.09	0.11	0.07
Lg27	*trans*-4-(prop-2-enyl)syringol	194	91+194	24.233	0.41	0.31	0.24
Lg28	4-acetylsyringol	196	181+196	24.659	0.19	0.25	0.40
Lg29	4-(propan-2-one)syringol	210	167+210	25.198	0.12	0.12	0.27
Lg30	phenol, 4(2-phenylethenyl)-	196	195+196	25.601	0.12	0.07	0.20
Lg31	syringic acid	198	183+198	26.121	0.00	0.01	0.03
Lg32	4-(propan-3-one)syringol	210	181+210	26.147	0.05	0.05	0.11
Lg33	alpha-beta-diguaiacylacylethene	272	272+273	37.178	0.03	0.05	0.00
Lp1	sterol		147+191	40.789	0.04	0.13	0.02
Lp2	urs-20-en-16-one (hopanoid)	424	149+191	41.328	0.05	0.12	0.02
Lp3	gamma -tocopherol		151+416	41.351	0.16	0.17	0.13
Lp4	cholesta-4,6-dien-3-ol compound		135+143	42.321	0.05	0.12	0.04

Appendix 2. (*continued*).

Code	Name	M	*m*/z	RT(min)	% TIC Res	% TIC Tot	% TIC Ex
Lp5	stigmastan-3-ol compound		147+396	42.631	0.10	0.18	0.12
K1	methyl ketone (2-one)		58+59	26.683	0.08	0.08	0.06
K23	C_{23} methyl ketone		58+59	35.441	0.09	0.11	0.08
K24	C_{24} methyl ketone	352	58+59	36.588	0.03	0.03	0.03
K25	C_{25} methyl ketone	366	58+59	37.655	0.41	0.49	0.31
K26	C_{26} methyl ketone	380	58+59	38.704	0.13	0.16	0.09
K27	C_{27} methyl ketone	394	58+59	39.711	0.99	1.44	0.67
K28	C_{28} methyl ketone	408	58+59	40.708	0.06	0.08	0.03
K29	C_{29} methyl ketone	422	58+59	41.786	0.43	0.66	0.29
K31	C_{31} methyl ketone	450	58+59	44.376	0.19	0.28	0.10
N1	pyridine	79	52+79	4.462	0.10	0.15	0.39
N2	C_1-pyrrole 1	81	80+81	6.163	0.03	0.02	0.23
N3	C_1-pyrrole 2	81	80+81	6.416	0.02	0.01	0.21
N4	3-methyl-pyridine	93	66+93	6.676	0.01	0.03	0.19
N5	3-pyridinamine?	94	66+94	7.017	0.05	0.04	0.02
N6	benzonitrile	103	76+103	9.334	0.01	0.03	0.12
N7	benzeneacetonitril	117	116+117	12.849	0.05	0.10	0.31
N8	indole	117	90+117	16.619	0.12	0.23	0.83
N9	diketodipyrrole	186	93+186	24.477	0.20	0.32	0.62
N10	9-octadecanamide		59+72	38.315	0.04	0.11	0.05
PA1	naphthalene	128	128	14.560	0.09	0.13	0.38
PA2	naphthalene compound		141+142	17.352	0.08	0.09	0.42
PA3	polyaromatic		227+242	24.887	0.06	0.04	0.17
PA4	1H-indene-1-one, 2,3-dihydro-3-methoxy-2-(2-	216	199+214	26.777	0.05	0.03	0.04
Ph1	phenol	94	66+94	9.647	4.37	4.36	9.63
Ph2	2-methylphenol	108	107+108	11.361	0.72	0.52	1.31
Ph3	3/4-methylphenol	108	107+108	11.849	2.92	2.83	6.40
Ph4	2,4-dimethylphenol	122	107+122	13.548	0.41	0.36	0.92
Ph5	2,6-dimethylphenol	122	107+122	13.941	1.08	0.95	2.18
Ph6	catechol, dihydroxybenzene	110	64+110	14.763	1.41	2.06	2.55
Ph7	1,2-benzenediol, 3-methoxy	140	125+140	16.054	0.18	0.19	0.42
Ph8	4-isopropenylphenol	134	91+119	16.870	0.55	0.36	0.13
Ph9	1,2-benzenediol, 4-methyl	124	78+124	16.721	0.39	0.31	0.45
Ph10	1,2-benzenediol, 4-ethyl	138	123+138	18.636	0.44	0.67	0.66
Ph11	1,2-benzenedicarboxylic acid, 4 hydroxy	182	181+182	23.584	0.36	0.32	0.49
Ps1	2-methylfuran	82	53+82	2.729	0.50	0.46	0.71
Ps2	acetic acid	60	60	2.777	0.51	0.37	0.68
Ps3	2,5-dimethylfuran	96	95+96	3.930	0.13	0.09	0.15

Appendix 2. (*continued*).

Code	Name	M	*m*/z	RT(min)	% TIC Res	% TIC Tot	% TIC Ex
Ps4	2-cyclopenten-1-one	82	53+82	4.381	0.13	0.10	0.02
Ps5	(2*H*)-furan-3-one	84	54+84	5.119	1.24	1.57	0.62
Ps6	3-furaldehyde	96	95+96	5.493	0.23	0.21	0.09
Ps7	2,4-pentadienal	82	53+82	5.870	0.81	0.43	0.43
Ps8	2-furaldehyde	96	95+96	5.924	2.75	3.47	1.68
Ps9	2-(hydroxymethyl)furan	98	55+98	6.642	0.27	0.20	0.13
Ps10	(5*H*)-furan-2-one	84	55+84	7.304	0.50	0.28	0.21
Ps11	2-cyclopenten-1-one, 2-methyl	96	67+96	7.589	0.25	0.12	0.24
Ps12	2-acetylfuran	110	95+110	7.721	0.24	0.24	0.22
Ps13	2,3-dihydro-5-methylfuran-2-one	98	55+98	8.041	1.13	0.70	0.41
Ps14	5-methyl-2-furaldehyde	110	109+110	8.904	2.16	2.23	1.65
Ps15	4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one	114	58+114	9.698	2.50	1.88	1.22
Ps16	3-hydroxy-2-methyl-2-cyclopenten-1-one	112	55+112	10.133	0.50	0.36	0.26
Ps17	2-hydroxy-3-methyl-2-cyclopenten-1-one	112	55+112	10.529	0.57	0.27	0.25
Ps18	dianhydrorhamnose	128	113+128	10.793	0.90	0.60	0.47
Ps19	levoglucosenone	126	68+98	12.215	0.60	0.92	0.58
Ps20	3-hydroxy-2-methyl-(4*H*)-pyran-4-one	126	71+126	12.546	0.29	0.37	0.27
Ps21	2/7-methyl benzofuran	132	131+132	12.752	0.18	0.17	0.50
Ps22	5-hydroxymethyl-2-furancarboxaldehyde	126	97+126	14.945	0.82	0.75	0.23
Ps23	1,4-anhydroxylofuranose (C_5-sugar)		57+73	15.872	1.51	1.18	0.50
Ps24	1,4-dideoxy-D-glycero-hex-1-enopyranose-3-	144	87+144	16.718	0.93	0.88	0.26
Ps26	4/7 methyl benzofuran	132	131+132	18.025	0.34	0.30	0.33
Ps27	levogalactosan		60+73	18.455	3.86	3.24	2.50
Ps28	levomannosan	162	60+73	19.741	2.45	3.21	2.21
Ps29	levoglucosan	162	60+73	21.055	21.05	24.68	6.83
Ps30	dibenzofuran	168	139+168	21.440	0.16	0.22	0.38
Ttp	triterpene, squalene	410	69+81	38.893	1.13	0.66	1.21

Codes: Al, aliphatics; Ar, aromatics; F, fatty acids; Lg, lignins; Lp, lipids; K, methylketones; N, N-compounds; PA, polyaromtics; Ph, phenols; Ps, polysaccharides; Ttp, triterpenoids.

Chapter 3

n-Alkane distributions as palaeoclimatic proxies in ombrotrophic peat: the role of decomposition and dominant vegetation

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Abstract

n-Alkane distributions are frequently used as palaeoclimate proxies in ombrotrophic peat deposits. Although *n*-alkane distributions differ strongly between plant species, *n*-alkanes are not species-specific molecules. For a proper interpretation, it is important to understand the different abundances of *n*-alkanes in various plant species as well as the changes that occur when plant litter is transformed to peat. In particular because molecular markers are especially valuable in highly decomposed peat where plant remains are no longer recognizable, it is important to understand the effects of decomposition on *n*-alkane distributions. The organic matter (OM) of a high-resolution sampled, 9 m thick, ombrotrophic peat deposit from Tierra del Fuego was analysed with pyrolysis-gas chromatography/mass spectrometry (pyrolysis-GC/MS). The same samples were analysed for carbon (C) and nitrogen (N) content. Depth profiles of C:N ratio, the summed lignin and summed polysaccharide pyrolysis products, and markers specific for *Sphagnum* spp., *Empetrum rubrum* and *Nothofagus antarctica*, enabled a reconstruction of changes in vegetation composition to be made. This reconstruction was used to examine the validity of the *n*-C_{23} alkane to indicate *Sphagnum* and the summed long chain *n*-alkanes (C_{29} and C_{31}) to reflect leaf input of the woody species *E. rubrum* and *N. antarctica*. Our results show that even in *Sphagnum*-dominated peat, the *n*-alkane distribution is not determined by *Sphagnum* but by leaf input of *E. rubrum* and *N. antarctica*. However, good correlations between the *n*-C_{23} alkane and the *Sphagnum* marker 4-isopropenylphenol, and between the summed *n*-C_{29} and *n*-C_{31} alkanes and the marker of *N. antarctica* support that their relative change with depth can be used to indicate the abundance of these species in *Sphagnum*-dominated peat. In peat with relatively low contributions of *Sphagnum*, both *n*-alkane proxies (C_{23} and C_{29}+C_{31}) reflect the degree of decomposition. We evaluated the influence of *Sphagnum* dominance, decomposition, and pyrolysis on the *n*-alkane distributions in peat OM.

3.1. Introduction

The well-preserved stratigraphy of peat deposits can be used to reconstruct past environmental conditions (Blackford, 2000). In ombrotrophic bogs all moisture input comes from precipitation, making them particularly sensitive to variations in climate (e.g. Barber, 1993). The OM of ombrotrophic bogs stores information of local hydrology by both the composition and the degree of decay of its plant remains. Vegetation composition on peat bogs is largely determined by hydrological conditions (e.g. McMullen et al., 2004), while the degree of decay primarily depends on the supply of oxygen. Thus, both vegetation composition and the degree of decomposition of its remains are determined by depth and fluctuation of the water table.

Several studies of plant macrofossils in ombrotrophic peat have shown good correlations between past vegetation composition and local hydrology (e.g. Barber et al., 2003). Because macrofossils are often insufficiently preserved in peat deposits, recent studies have used molecular markers for a reconstruction of past vegetation. The use of biomarkers to trace the occurrence of a given plant species demands a marker to be both species-specific and recalcitrant to decomposition. Triterpenoids, wax esters (Pancost et al., 2002) and 5-*n*-alkyl-resorcinols (Xie et al., 2004) were found to meet these requirements. In addition to these specific compounds, characteristic *n*-alkane distributions are used as molecular proxies to trace plant species. *n*-Alkane distributions in peat are commonly obtained by gas chromatography/mass spectrometry (GC/MS) of the extracted lipid fraction, and compared with *n*-alkane distributions of the plant species that contribute to the peat OM (e.g. Nichols et al., 2006). However, the contribution of different plants and plant tissues to the peat OM (Pancost et al., 2002) and the effects of decomposition (Lehtonen and Ketola, 1993) influence the *n*-alkane distribution, and thereby complicate its interpretation.

n-Alkane distributions can also be obtained with pyrolysis-GC/MS. Pyrolysis-GC/MS gives a fingerprint of the molecular composition of the total OM, but its interpretation may be complex due to chemical rearrangements during the pyrolysis process. In addition to potential lipid biomarkers, it provides other markers, for example methoxyphenols from lignin (vascular plants) or the *Sphagnum* marker 4-isopropenylphenol (Stankiewicz et al., 1997; van der Heijden et al., 1997). Furthermore, pyrolysis-GC/MS gives information on the degree of decomposition (e.g. Kuder et al., 1998), which is important because the distribution of plant species and decomposition of their remains in peat are closely associated (Yeloff and Mauquoy, 2006).

The Harberton peat deposit (Tierra del Fuego, Argentina) represents more than 13,000 years of peat accumulation and includes a large vegetation shift from peat dominated by *Juncus* and woody species to *Sphagnum*-dominated peat (Schellekens et al., 2009 and references therein). Because studies on *n*-alkane distributions are generally related to *Sphagnum* peat, this peat record provides a good stratigraphic context for testing the validity of *n*-alkane proxies to reflect vegetation changes. Furthermore, molecular markers for *N. antarctica*, *E. rubrum* (Schellekens et al., 2009) and *Sphagnum* spp. (Stankiewicz et al., 1997; van der Heijden et al., 1997), which have been dominant plant species at Harberton according to macrofossil analysis, have been applied to the peat record. Because these plant species show large differences in their *n*-alkane distribution (Schellekens et al., 2009), changes with

depth of their markers enables interpretation of *n*-alkane proxies between and within both vegetation zones (*Sphagnum* and *Juncus*-dominated peat). The objective of this study is to evaluate the influence of dominant vegetation and decomposition on the abundance and distribution of *n*-alkanes in peat OM. In addition, it offers an opportunity to validate some of the previously selected vegetation parameters by Schellekens et al. (2009).

3.2. Methods

3.2.1. Samples

Samples were obtained from a peat core (895 cm) from a raised bog located at Puerto Harberton (Tierra del Fuego, Argentina). The core was taken at a distance of 1 m from Heusser's (1989) and 20 m from that of Pendall et al. (2001). For details of the location see Markgraf (1993). The peat is primarily composed of *Sphagnum magellanicum* in the upper 600 cm, of brown mosses below 850 cm, and of *Juncus* sp. in between. In addition to pollen analysis (Heusser, 1989; Markgraf, 1993; Markgraf and Huber, 2010; Pendall et al., 2001) the core was analysed for charcoal abundance and macrofossils (Markgraf, 1993; Markgraf and Huber, 2010; White et al., 1994). Cores were taken with a 5×50 cm Russian (Macaulay) peat sampler. The upper 174 cm were sampled according to themorphology/stratigraphy. The deeper part of the core was sampled in sections of 3 cm, except at the bottom of each sub-core where the samples were 5 cm thick. The core contained three volcanic ash layers, which were sampled individually. The resulting 264 samples were air-dried, ground, homogenised and divided into sub-samples for the various analyses. Selection of samples for pyrolysis-GC/MS was based on depth, ash content, and the abundance of (wood) macro-remains, resulting in a total of 67 samples. In addition, *n*-alkane distributions of NaOH-extractable and non-extractable OM fractions of 13 of these samples previously analysed by Schellekens et al. (2009) were compared. The NaOH-extractable OM is supposed to reflect more decomposed material, and the non-extractable residue to more closely reflect relatively intact plant material (Buurman et al., 2006). Furthermore, *n*-alkane distributions of the pyrolysates of the dominant plant species at Harberton (see Sections 3.3 and 3.4.1) were compared. Bulk samples of these species were previously analysed with pyrolysis-GC/MS by Schellekens et al. (2009).

3.2.2. Extraction

An aliquot (0.5 g) of 13 peat samples was extracted with NaOH (0.1 M, 20 ml), shaken for 24 h under N_2 and centrifuged (1 h) at 4000 g. The extract was decanted and the extraction repeated a second time. The extracts were combined, acidified to pH 1 with HCl, shaken for 24 h, dialysed against distilled water (cut off 1000 D) and freeze-dried. The residues were acidified, dialysed against distilled water and freeze-dried.

3.2.3. Elementary analysis

All 264 samples were finely powdered (<50 µm) in an automatic agate mortar pre-cleaned with diluted HCl and MilliQ water, dried, and stored in the dark before further analysis. They were analysed for C and N by complete combustion in an auto-analyzer, Fisons CHNS-O EA-1108 for C, and LECO CHNS-932 for N.

3.2.4. Pyrolysis-GC/MS

The samples were pyrolysed using a Curie-Point pyrolyser (Curie temperature 600 °C) connected to a Carlo Erba GC8000 gas chromatograph. The pyrolysis products were separated on a fused silica column (Chrompack 25 m, 0.25 mm i.d.) coated with CP-Sil 51 b (film thickness 0.40 µm),with He as carrier gas. The initial oven temperature was 40 °C and the heating rate 7 °C min^{-1}. The final temperature, 320 °C, was maintained for 20 min. The GC column was connected to a Fisons MD800 mass spectrometer (*m/z* 45–650, cycle time 1 s).

Based on factor analysis of 177 pyrolysis products quantified for 13 samples of different fractions of the same peat core by Schellekens et al. (2009), 86 pyrolysis products were selected which contain information on vegetation composition and decomposition. A number of *n*-alkanes and *n*-alkenes were added, so that all chain lengths between C_{17} and C_{33} were included, which raised the number of pyrolysis products to 98. These 98 pyrolysis products were quantified for all 67 pyrograms using the peak area of the two main fragment ions for each pyrolysis product (for a list of quantified pyrolysis products and their mean abundance in the peat OM we refer to Schellekens et al. (2009)). All quantifications were checked manually. For each sample, the sum of the peak areas (total ion current, TIC) was set at 100% and relative amounts were calculated with respect to this sum. The resulting quantification gives the relative abundance of each pyrolysis product. The pyrolysis products were grouped, according to probable origin and chemical similarity, into a number of source groups: polysaccharides, aliphatic biopolymers, methyl ketones, lignins, phenols, aromatics, polyaromatics, N-compounds, steroids and triterpenoids.

3.3. Review of vegetation parameters

The term 'molecular marker' is used here for specific pyrolysis products of *Sphagnum* spp., *N. antarctica* and *E. rubrum*. It must be mentioned that the markers for *N. antarctica* and *E. rubrum* may only be valid within the current study; notwithstanding the good correlations with other vegetation characteristics, these markers have not been validated as species-specific pyrolysis products such as the marker for *Sphagnum*, and thus the use of the term '(molecular) marker' is provisional.

In a previous paper (Schellekens et al., 2009) parameters based on (ratios of) abundances of pyrolysis products were selected to reflect vegetation, decomposition and fire history for the Harberton peat. This selection was based on factor analysis of extractable and non-extractable fractions of 13 peat samples and on analysis of modern plant species growing on this bog. A number of the selected vegetation parameters and their interpretations are given in Table 3.1. Specific markers for *Sphagnum* spp., *E. rubrum* and *N. antarctica* were 4-isopropenylphenol, benzoic acid and the sum of

two guaiacol derivatives with specific masses 147+162, respectively. Although these guaiacol derivatives have a C_3H_3 side-chain, their previous identification as 4-propyneguaiacol and 4-alleneguaiacol (Schellekens et al., 2009) was not correct. The sum of both is here referred to as C_3H_3-guaiacols. In total lignin content, used to differentiate between mosses (*Sphagnum*) and vascular plant species (*Juncus* and woody species), the phenolic lignin compounds were excluded because these compounds have a high contribution from *Sphagnum* mosses. High total polysaccharides may indicate moss vegetation and/or a low degree of decomposition, as polysaccharides are easily decomposed (e.g. Benner et al., 1984; Schellekens et al., 2011). The relative abundance of the *n*-C_{23} alkane was found to reflect *Sphagnum* while the summed *n*-C_{29} and *n*-C_{31} alkanes indicated leaf input from *E. rubrum* and *N. antarctica* (Schellekens et al., 2009). The *n*-alkane distribution of the Harberton plant species analysed with pyrolysis-GC/MS showed a dominance of the C_{23} in *S. magellanicum* while other species showed a dominance of one or both of the C_{29} and C_{31}. The contribution of both long chain *n*-alkanes to the total OM was extremely high in the leaves of *E. rubrum* and *Nothofagus pumilio* (Table 3.2; no leaves of *N. antarctica* were available for analysis, because *N. pumilio* and *N. antarctica* are both deciduous *Nothofagus* species and *n*-alkanes predominantly originate from cuticular waxes, it is assumed that *n*-alkane distribution and abundance present in leaves of *N. pumilio* are similar to those of *N. antarctica*). These values agree with *n*-alkane distributions of plant species that contribute to *Sphagnum* peat as analysed by GC/MS (Baas et al., 2000; Ficken et al., 1998; Nott et al., 2000; Ortíz et al., 2010; Vonk and Gustafsson, 2009), and with the generally much higher abundance of *n*-alkanes in leaves compared to roots (Jansen et al., 2006; Pancost et al., 2002). The good agreement between *n*-alkane distributions analysed with GC/MS and pyrolysis-GC/MS supports that the summed *n*-C_{29} and *n*-C_{31} mainly reflect leaf input of *E. rubrum* and *N. antarctica*, while the *n*-C_{23} alkane may indicate *Sphagnum*. The translation of molecular biomarkers obtained with pyrolysis-GC/MS, via peat OM, into former vegetation composition was recently discussed by Schellekens et al. (2011) for a *Carex*-

Table 3.1. Parameters used to reconstruct vegetation composition (see Section 3.3). Interpretations refer to high values. Zones refer to the vegetation zones as mentioned in Section 3.4.1 and Figs. 3.1 and 3.3. The water demand of the species increases in the following order: *Nothofagus*, *Empetrum*, *Juncus*, *Sphagnum* (see Section 3.4.1); % TIC = percentage of total ion current.

Parameter	High values denote	Hydrological interpretation	
		zone 1	zone 2
4-isopropenylphenol (% TIC)	*Sphagnum* spp.	wet	wet
benzoic acid (% TIC)	*Empetrum nigrum*	dry/wet	dry
C_3H_3-guaiacols (% TIC)	*Nothofagus antarctica*	dry	dry
sum of lignins (% TIC) [a]	woody plant species or *Juncus*	dry	dry
sum of polysaccharides (% TIC)	moss or 'fresh' OM	wet	wet
n-C_{23} (% *n*-alkanes)	*Sphagnum* spp. or aerobic decomposition	dry	wet (dry)
n-C_{29} + *n*-C_{31} (% *n*-alkanes)	fresh leaves of *E. nigrum* and *N. antartica*	wet	dry

[a] Except phenolic lignin compounds

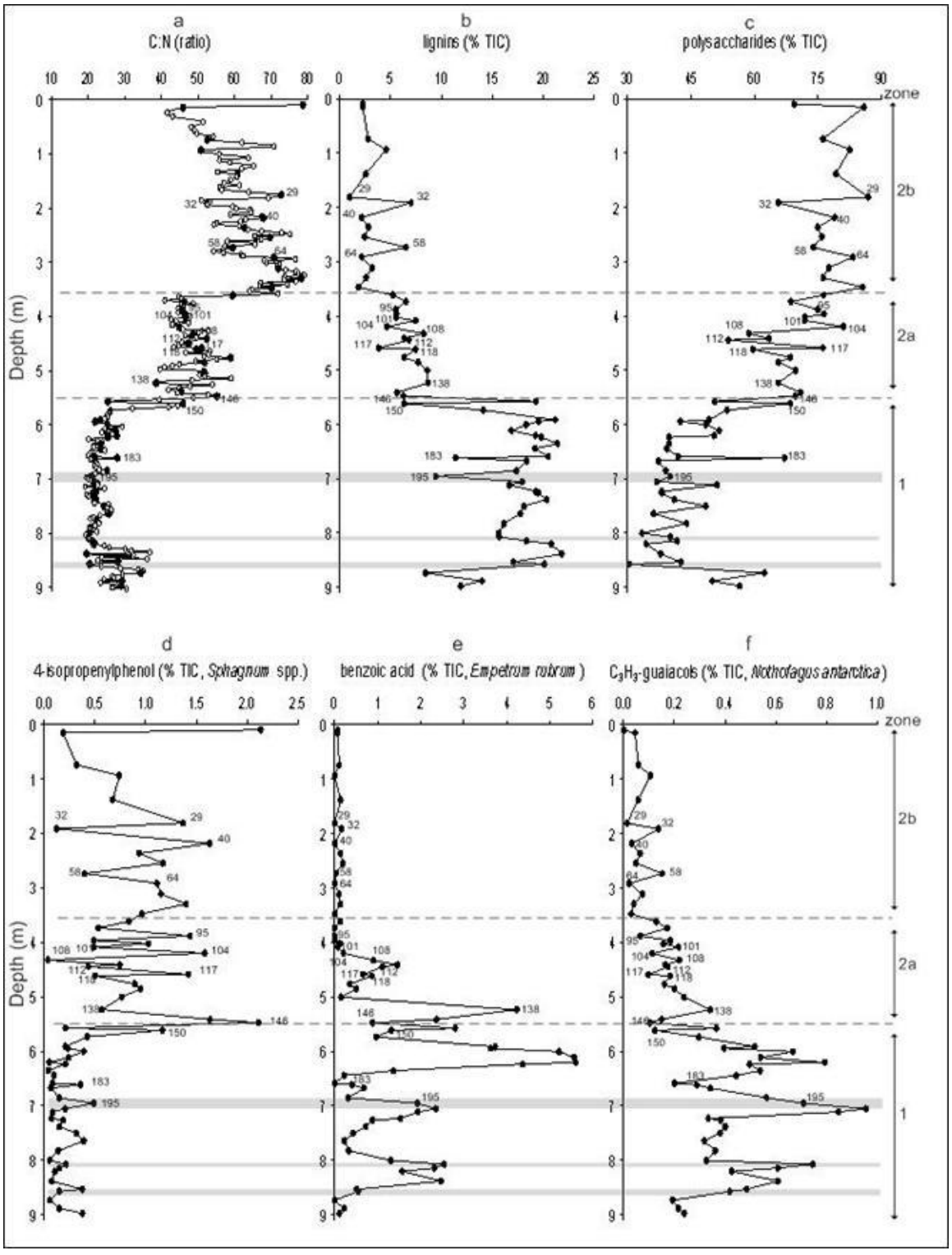

Fig. 3.1. Depth profiles of vegetation parameters, (a) C:N ratio, (b) summed lignin pyrolysis products, (c) summed polysaccharide pyrolysis products, (d) 4-isopropenylphenol, (e) benzoic acid and (f) summed C_3H_3-guaiacols. Dotted lines separate vegetation zones (codes on right), shaded bars are tephra layers. The (chemical) meaning of the parameters is given in Table 3.1.

dominated bog in Northwest Spain. Their reconstruction of peat hydrology showed excellent agreement with other European climate studies.

In the present study, the vegetation parameters of Table 3.1 are applied to a larger number of samples of the same peat record. The C:N ratio is used to evaluate the interpretation of pyrolysis products, as this ratio is high in *Sphagnum* and low in vascular plants (e.g. Hornibrook et al., 2000). With the above parameters the peat record can be described in terms of changes in vegetation composition (Section 3.4.1), which will be used as a context to interpret the distribution of *n*-alkanes with depth (Section 3.4.2).

3.4. Results and Discussion

3.4.1. Main vegetation shifts

According to macrofossil analysis of the peat deposit, the dominant species were *S. magellanicum*, *Juncus* sp., *E. rubrum* and *N. antarctica* (Markgraf and Huber, 2010; White et al., 1994). The tolerance to permanently wet conditions decreases from *Sphagnum* through *Juncus* to *E. rubrum*, and *N. antarctica* (Kleinebecker et al., 2007; Roig and Roig, 2004).Molecular markers were found for most of these species, except for *Juncus* (see Section 3.3). Depth profiles of the vegetation parameters (Table 3.1) and C:N ratio, are shown in Fig. 3.1. Based on the simultaneous, opposite shifts in lignin content (Fig. 3.1b) and the *Sphagnum* marker 4-isopropenylphenol (Fig. 3.1d), the peat record can be divided into two main vegetation zones, from 8.9 to 5.5 m (zone 1) and from 5.4 to 0 m depth (zone 2). This shift is also evident in the lower C:N ratio, higher values for markers of *E. rubrum* and *N. antarctica* and generally lower polysaccharide contents in zone 1, and suggests a change towards generally wetter conditions from 5.5 m upwards. The relatively high lignin content in zone 1 is explained by dominance of *Juncus* and more woody vegetation. Zone 2, with low lignin contents and high but fluctuating values for the *Sphagnum* marker, shows clearly lower C:N ratios and higher lignin contents in its lower part (zone 2a) compared to the upper part (zone 2b). This may originate from a combination of *Juncus* and *Sphagnum* in zone 2a and dominance of *Sphagnum* in zone 2b. This interpretation is largely in agreement with previous findings for the same bog (Heusser, 1989; Markgraf, 1993; Markgraf and Huber, 2010; Pendall et al., 2001; White et al., 1994). Although *Sphagnum* also contributes to zone 1, in the following the main vegetation zones are referred to as *Sphagnum*-dominated (zone 2) and *Juncus*-dominated (zone 1) peat. In addition to these main vegetation zones, the various vegetation parameters can be used to indicate changes in hydrological conditions within each zone. In zone 2, where *Sphagnum* dominates, there is a good positive correlation between total lignin content and the marker of *N. antarctica* (R^2=0.86, Fig. 3.2a). Minima for lignin content (Fig. 3.1b) correspond to maxima for the marker of *Sphagnum* (Fig. 3.1d) and vice versa, which indicates relatively dry conditions in samples 32, 58, 101, 108–112, 118 and 138, and relatively wet ones in samples 29, 40, 104, 117 and 146.

In zone 1, where *Juncus*, *E. rubrum* and *N. antarctica* dominated, interpretation of total lignin content in terms of vegetation is more difficult. The OM in this part of the peat record has no significant contribution of lignin-less *Sphagnum* and no marker was found for *Juncus*. Markers of *E.*

rubrum and *N. antarctica* (Fig. 3.1e,f) show a large variation and a similar behaviour, while the total lignin content (Fig. 3.1b) has high values and varies less. Consequently, high lignin contents in samples with a low contribution of the markers of woody species must originate from *Juncus*. Samples 183 and 150 show very low lignin contents together with high polysaccharide contents and higher values for the *Sphagnum* marker 4-isopropenylphenol, which points to short periods with a strong increase of *Sphagnum*, and thus wet conditions (Section 3.3; Table 3.1). In sample 195, the very low lignin content is not accompanied by high polysaccharide content. As this sample represents a tephra layer, its interpretation is more complex and will not be discussed here.

Thus, in the *Sphagnum*-dominated peat of zone 2 good correlations between vegetation parameters enable identification of relatively dry periods, while in the *Juncus*-dominated peat (zone 1), identification of relatively wet/dry periods according to vegetation markers is more complex due to the absence of a marker for *Juncus*. However, a high abundance of polysaccharides indicates relatively fresh material (Section 3.3) and thus can serve to identify relatively wet periods in the *Juncus*-dominated peat.

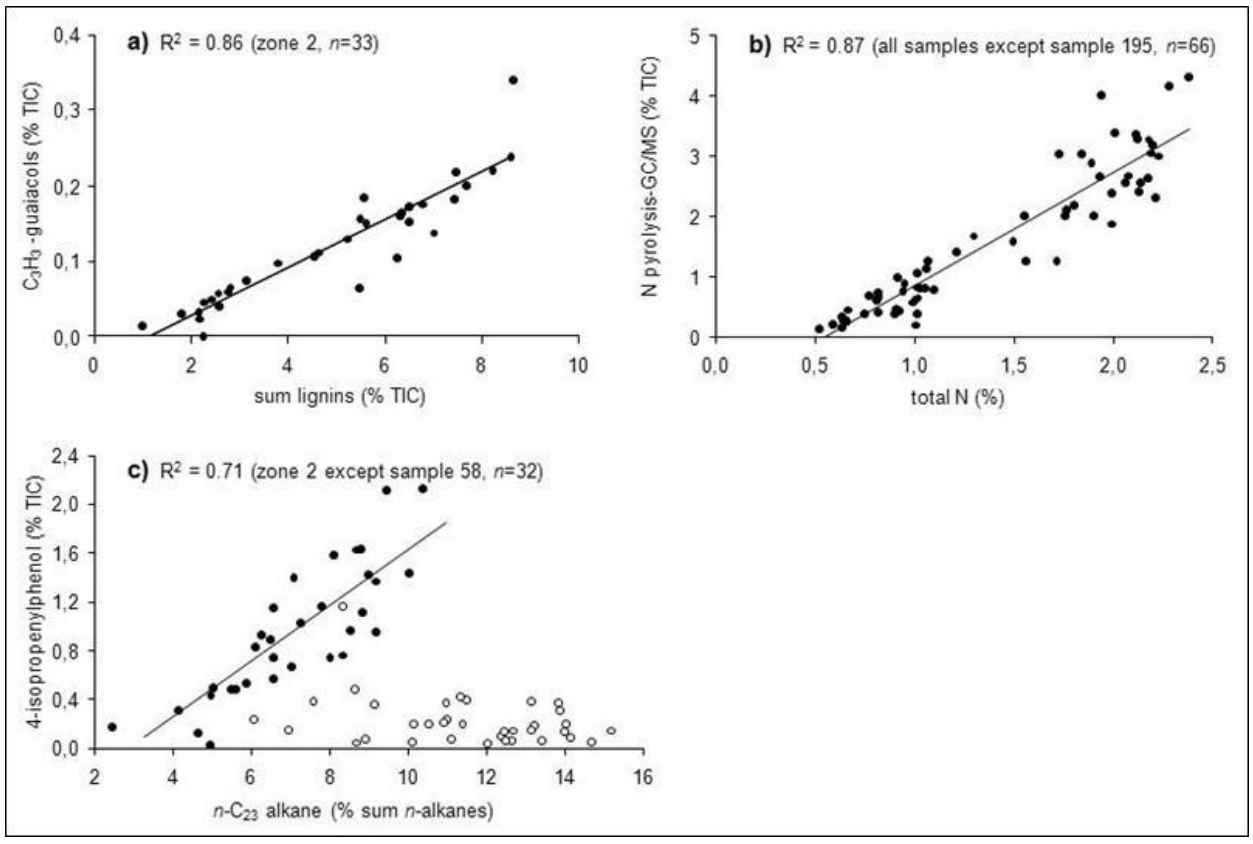

Fig. 3.2. Correlations between (a) summed lignins and summed C_3H_3-guaiacols, (b) total N and summed pyrolytic N-compounds, and (c) *n*-C_{23} alkane and 4-isopropenylphenol (marker of *Sphagnum* spp.), filled points originate from zone 2 and open points from zone 1. The (chemical) meaning of the parameters is given in Table 3.1.

The good agreement between pyrolytic vegetation parameters and vegetation composition according to previous studies indicates that pyrolysis products allow a vegetation reconstruction to be made. The validity of the pyrolysis results is further supported by good correlations between groups of pyrolysis products (high% TIC) and the various vegetation markers (low% TIC, Schellekens et al., 2011) and between groups of pyrolysis products and elemental analysis. The sum of N-containing pyrolysis products and the N content show a good correlation (R^2=0.87, *n*=66 tephra sample 195 omitted, Fig. 3.2b). Similarly, the sum of lignin pyrolysis products is well correlated with the C:N ratio (R^2=0.83 *n*=67, not shown). The latter correlation is probably caused by 1) the lower N content in *Sphagnum* compared to vascular plant species, and 2) the higher amount of microbial products that accompany aerobic decomposition associated with better drainage and woody plants.

3.4.2. n-Alkane distributions in the Harberton peat record

The summed *n*-C_{29} and *n*-C_{31} alkanes mainly reflect leaf input of *E. rubrum* and *N. antarctica*, while the *n*-C_{23} alkane may indicate *Sphagnum* (Section 3.3). Depth profiles of the summed *n*-alkanes, the *n*-C_{23} and the summed *n*-C_{29} and *n*-C_{31} alkanes in the peat are shown in Fig. 3.3. The opposite pattern of the *n*-C_{23} and that of the summed *n*-C_{29} and *n*-C_{31} may reflect the mutual exclusion of *Sphagnum* and *N. antarctica*/*E. rubrum* in the past peat vegetation (see Section 3.4.1).

Two observations, however, indicate that the interpretation is less straightforward. The first observation is, that C_{29} and C_{31} and not C_{23} are the dominant *n*-alkanes in the *Sphagnum*-dominated peat (zone 2, except sample 58), while in the peat dominated by *Juncus* and woody species (zone 1), the dominance varies strongly (not shown). Dominance of long chain *n*-alkanes in *Sphagnum* peat was also found by GC/MS analysis (Farrimond and Flanagan, 1996; Ficken et al., 1998; Pancost et al., 2002; Zheng et al., 2007), in most samples by Nott et al. (2000), Xie et al. (2004) and Zhou et al. (2010), but was less prominent in peat samples from northern Spain (Ortíz et al., 2010).

The seeming discrepancy can be understood when we consider the *n*-alkane abundance in the various plant species. The contribution of *n*-alkanes to the total quantified OM was only 0.6% in pyrolysates of *Sphagnum*, while it was extremely high in the leaves of *E. rubrum* (27.5%) and *N. pumilio* (16.5%; Table 3.2). This largely coincides with GC/MS analyses of the few studies that gave, besides the *n*-alkanedistributions, also the weight percentage of the *n*-alkanes in the total OM. Baas et al. (2000) found an *n*-alkane content of 0.3% in *S. magellanicum*. Pancost et al. (2002) found that the abundance of individual lipids in all tissue types of Ericaceous species was an order of magnitude higher than in *Sphagnum*. Ficken et al. (1998) found a five times higher concentration of *n*-alkanes in *Empetrum nigrum* compared to *Sphagnum* species. Since such Ericaceous shrubs grow on the dryer spots in *Sphagnum*-dominated peat, they may considerably increase the contribution of long chain *n*-alkanes at such spots (Pancost et al., 2002). The continuous leaf input of woody species together with strongly limited decomposition in a *Sphagnum* environment explains the dominance of long chain *n*-alkanes in the *Sphagnum*-dominated upper part of the peat record (zone 2). This is confirmed by the higher abundance of the summed *n*-alkanes for the samples that show maxima for the long chain *n*-

alkanes (Fig. 3.3a,c), and by maxima for the long chain *n*-alkanes in the woody samples (samples 32, 108, 112, 118; Figs. 3.1b and 3.3c) except for sample 58.

The second observation is that the *Sphagnum* marker 4-isopropenylphenol and the *Sphagnum*-indicating *n*-C_{23} alkane show a good correlation in zone 2, but not in zone 1 (Fig. 3.2c). Furthermore, vegetation shift from more woody material to *Sphagnum* dominance (Fig. 3.3b,c), but opposite to expectation, the *n*-C_{23} increases towards the *Juncus*-dominated peat. In zone 1, the relatively high abundance of the *n*-C_{23} alkane and the absence of a correlation with 4-isopropenylphenol, indicate that other sources than *Sphagnum* define the abundance of the *n*-C_{23} in this vegetation zone. The absence of a consistent dominance of the long chain *n*-alkanes may be due to aerobic decomposition, since this causes chain length reduction in peat OM (Buurman et al., 2006; Schellekens et al., 2009). Assuming that there is no preferential decay for *n*-alkanes with a specific chain length, chain length reduction will cause a relative increase of *n*-alkanes with mid-chain length (provided that longer chain lengths were initially dominant; Table 3.2).

The *n*-C_{23} alkane shows a general increase with depth in the woody peat of zone 1 parallel to a decrease of the summed *n*-C_{29} and *n*-C_{31} alkanes (Fig. 3.3b,c), which indicates that chain length reduction also occurs during diagenesis under anaerobic conditions in the *Juncus*-dominated peat. The absence of depth trends in zone 2 is possibly caused by continuous water saturation and/or protection against degradation by the high polyphenolic content in *Sphagnum* (e.g. Freeman et al., 2001).

In addition to the effects of decomposition, alkyl side-chains that are included in better protected plant structures may contribute to the *n*-alkane distribution as these may be released with pyrolysis. Therefore, the origin of *n*-alkanes in pyrolysates of peat OM will be discussed in detail prior to further interpretation of the *n*-alkane proxies in the Harberton peat record (Section 3.4.3).

Table 3.2. Analysed plant species and their abundance of *n*-alkanes and *n*-alkenes as percentage of the total ion current (% TIC), the contribution of long chain alkanes to the *n*-alkane content and carbon preference index values for *n*-alkanes and *n*-alkenes with mid-chain lengths (CPI_{21-25}). CPI values according to Wiesenberg et al. (2009).

	Species	Tissue	*n*-Alkanes			*n*-Alkenes	
			sum (% TIC)	*n*-C_{29} + *n*-C_{31} (% alkanes)	CPI_{21-25} [a]	sum (% TIC)	CPI_{21-25} [a]
Mosses	*Sphagnum magellanicum*	Leaves/stem	0.6	7.2	5.1	0.1	0.7
Juncaceae	*Marsipossperum* sp.	Leaves/stem	1.9	54.3	1.5	0.1	
Woody shrubs	*Empetrum rubrum*	Leaves/stem	27.5	81.8	1.5	2.0	0.5
	Nothofagus antartica	Cortex	0.8	2.9	0.4	1.2	1.6
	N. antartica	Xylem	0.1	57.8	1.6	0.0	1.2
	Nothofagus pumilio	Leaves/stem	16.5	50.6	3.7	3.4	0.7

[a] CPI_{21-25}=0.5 * [((*n*-C_{21}+*n*-C_{23}+*n*-C_{25})/(*n*-C_{20}+*n*-C_{22}+*n*-C_{24}))+((*n*-C_{21}+*n*-C_{23}+*n*-C_{25})/(*n*-C_{22}+*n*-C_{24}+*n*-C_{26}))].

3.4.3. Origin of aliphatic hydrocarbons in pyrolysates of peat and plant OM

n-Alkanes obtained with GC/MS after a lipid extraction are 'free *n*-alkanes' and thus occur as such in the peat OM. In leaf waxes of higher plants they mainly occur in the C_{23}–C_{33} range with a strong odd carbon number predominance and maximizing at C_{27}, C_{29} or C_{31} but lack alkene homologues (Eglinton and Hamilton, 1967). The *n*-alkanes in pyrolysates of the peat, however, might also be generated by thermal cracking of alkyl chains of macromolecular OM (Lichtfouse et al., 1998 and references therein). *n*-Alkanes formed upon thermal cleavage of alkyl chains are observed along with the corresponding nalkenes (Derenne et al., 1991), do not show odd/even predominance, and are characterised by a regular decrease in intensity with increasing carbon number (Dignac et al., 2006). The two possible origins of *n*-alkanes in pyrolysates can thus be distinguished through their different distributions.

The observed *n*-alkane distribution in the pyrolysates of *E. rubrum*, with a clearly higher abundance of the longer chain lengths (Fig. 3.4a) and an odd over even predominance for the mid-chain alkanes, illustrated by the carbon preference index value ($CPI_{21–25}$=1.5 Table 3.2; CPI>1 indicates odd over even predominance), suggests that the *n*-alkanes predominantly originate from plant waxes. However, the mid-chain *n*-alkanes, notwithstanding their low abundance, are accompanied by their *n*-alkene homologues that show an even over odd predominance ($CPI_{21–25}$=0.5, Table 3.2), which is compatible with a contribution from highly aliphatic non-hydrolysable biomacromolecules such as cutans and suberans from cuticular and suberised plant tissues (Tegelaar et al., 1995) and suberan-like structures in roots (Nierop, 1998).

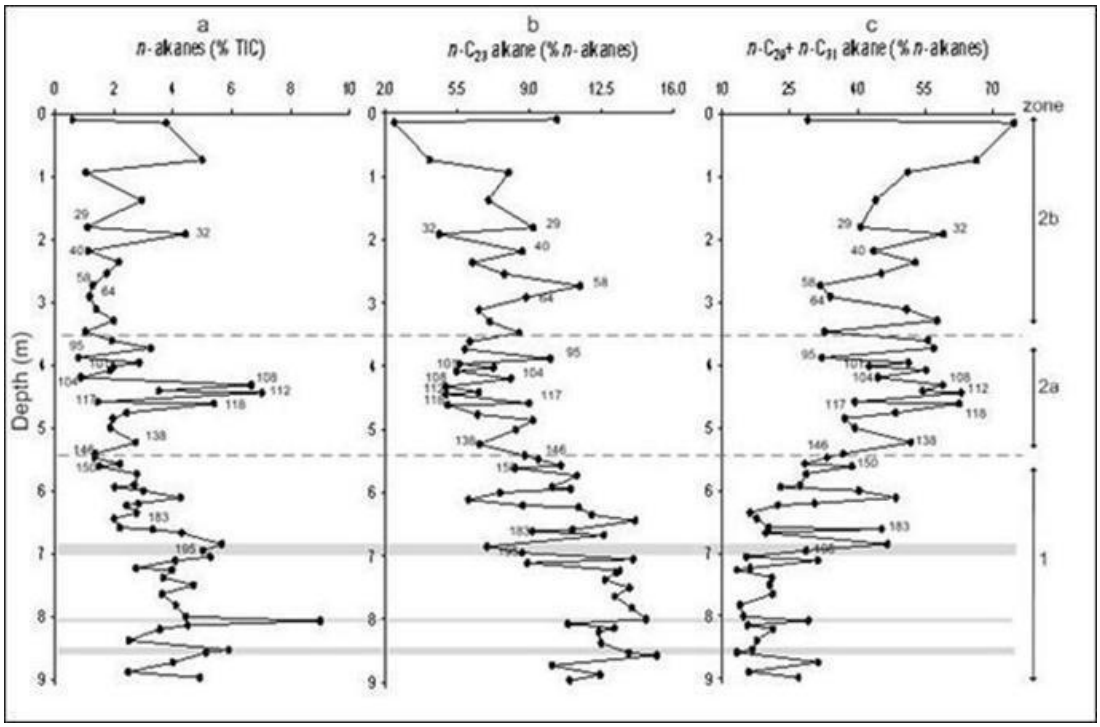

Fig. 3.3. Depth profiles of (a) summed *n*-alkanes, (b) the *n*-C23 alkane and (c) the summed long chain *n*-alkanes (C_{29}, C_{31}).

Examples of the *n*-alkane and *n*-alkene distribution for *Sphagnum* and *Juncus*-dominated peat are given in Fig. 3.4b,c. To obtain more insight in the origin of mid-chain *n*-alkanes (C_{21}–C_{26}) in the pyrolysates of peat OM, their distributions in pyrolysates of NaOH-extracted peat (extract) and the residue after extraction (residue) were compared. The residues are expected to include more refractory, macromolecular constituents than the extracted material. Thus, *n*-alkanes that originate from thermal cracking of alkyl chains (recognised by the presence of alkene homologues and the absence of an odd/even predominance) are expected to be less abundant in the pyrolysates of the extracts. The summed mid-chain *n*-alkanes divided by their alkene homologues gives a measure for the contribution of *n*-alkenes (Table 3.3a). Indeed, the extracts clearly have a lower contribution of *n*-alkenes, which point towards a contribution of *n*-alkanes that originate from thermal cracking of alkyl chains during the pyrolysis process.

Table 3.3. (**a**) Average values of parameters used to indicate the contribution of thermal cracking during the pyrolysis process and of decomposition on the mid-chain *n*-alkane distribution in different fractions of peat OM (calculated with percentages of the total quantified OM, see Section 3.2.4); (**b**) factors which influence the above mentioned parameters in pyrolysates of peat OM, and the peat fraction that is predominantly represented by this factor (see Section 3.4.3). Zones refer to the vegetation zones as mentioned in Section 3.4.1. $CPI_{21–25}$=carbon preference index values for *n*-alkanes and *n*-alkenes with mid-chain lengths, CPI values according to Wiesenberg et al. (2009). S.D.=standard deviation.

a	*n*-Alkanes$_{21-26}$ / *n*-alkenes$_{21-26}$				CPI_{21-25} *n*-alkanes [a]			
	Extracts	S.D.	Residues	S.D.	Extracts	S.D.	Residues	S.D.
Zone 2, *n*=6	2.1	0.4	1.5	0.3	1.3	0.1	1.4	0.1
Zone 1, *n*=7	2.8	0.3	1.9	0.5	1.4	0.2	1.5	0.1

b	*n*-Alkanes$_{21-26}$ / *n*-alkenes$_{21-26}$	CPI_{21-25} *n*-alkanes [a]	CPI_{21-25} *n*-alkenes [a]	Dominant fraction
Plant leaf waxes	Alkanes >> alkenes	>>1	-	Residues >> extracts
Thermal cracking of macromolecular structures	Alkanes < alkenes	1	<<1	Residues >> extracts
Decomposition	-	1	-	Residues << extracts

[a] $CPI_{21–25}$=0.5 * [((n-C_{21}+n-C_{23}+n-C_{25})/(n-C_{20}+n-C_{22}+n-C_{24}))+((n-C_{21}+n-C_{23}+n-C_{25})/(n-C_{22}+n-C_{24}+n-C_{26}))].

Chain shortening of long chain *n*-alkanes derived from plant waxes can be a third source of *n*-alkanes, causing an enhanced accumulation of even carbon numbered homologues (Eglinton and Hamilton, 1967; Meyers and Ishiwatari, 1993; Zegouagh et al., 1998). Chain length reduction of *n*-alkanes upon aerobic decomposition was also reported for peat (Buurman et al., 2006; Schellekens et al., 2009). Comparison of *n*-alkane distributions within the different peat fractions gives an indication of the influence of decomposition (chain length reduction). The residues contain more intact plant material, while the extracts reflect more decomposed material (Buurman et al., 2006). The residues should therefore more closely reflect the pattern typical of plants (odd over even predominance) than the extracted fraction. The mid-chain *n*-alkanes of both fractions exhibit clear odd over even predominance ($CPI_{21–25}$ between 1.3 and 1.5 Table 3.3a), which reflects their original source. Because extracts contain more decomposed material they have a higher contribution of even numbered mid-

chain *n*-alkanes compared to the residues (Table 3.3a). In the extracted fraction, the contribution of even numbered mid-chain *n*-alkanes that originate from macromolecular structures counteracts the effect of decomposition. Chain length reduction due to decomposition is therefore much stronger than indicated by the small differences in CPI values between the different peat fractions. The above-discussed factors that influence the *n*-alkane distribution in pyrolysates of peat OM and the peat fraction that predominantly represents this factor are summarised in Table 3.3b.

Two other mechanisms that may influence the *n*-alkane distribution in the pyrolysates of peat OM must be mentioned. First, burning of the peat surface may lead to a significant increase in (even numbered) mid-chain *n*-alkanes (Almendros et al., 1988; Wiesenberg et al., 2009). Because this also affects the *n*-alkane distributions obtained with GC/MS, it is not discussed here. Second, the presence of inorganic material may influence the *n*-alkane distribution in pyrolysates (e.g. Nierop and van Bergen, 2002). This should not be significant for the Harberton peat, because mineral contents are below 10% and for most samples below 5% (with exception of the tephra layers; Schellekens et al., 2009). Furthermore, no correlations were found between ash content and *n*-alkane or *n*-alkene distributions. Finally, organic matter derived of microorganisms may contribute to the *n*-alkane distribution. In the present case, however, this source is considered negligible because accumulation of microbial matter was not evident in the factor analysis (Schellekens et al., 2009).

Thus, the long chain *n*-alkanes (C_{29} and C_{31}) originate from plant waxes. The *n*-C_{23} alkane, on the other hand, which occurs as a free lipid in *Sphagnum*, has contributions from thermal cracking of alkyl chains of macromolecular OM and from decomposition of *n*-alkanes with longer chain lengths. Because *n*-alkane distributions and abundances are very similar between pyrolysis-GC/MS and GC/MS in both plant (Section 3.3) and *Sphagnum*-dominated peat (Section 3.4.2) the contribution of thermal cracking should be of minor importance. The contribution of decomposition to the abundance of the *n*-C_{23} alkane, however, may be substantial as indicated by comparison of the different peat fractions (Table 3.3).

The good correlation between the *n*-C_{23} and the summed *n*-C_{29} and *n*-C_{31} alkanes and markers of *Sphagnum* and woody vegetation, respectively, in the *Sphagnum*-dominated peat (zone 2), indicates that in *Sphagnum*-dominated peat *n*-alkanes predominantly originate from free *n*-alkanes from plant waxes, and the relative change of the *n*-C_{23} and the summed *n*-C_{29} and *n*-C_{31} alkanes predominantly reflect the abundance of *Sphagnum* and woody vegetation, respectively.

Contrarily, in the *Juncus*-dominated peat the absence of a correlation between *n*-alkane parameters and vegetation markers indicates that the relative change of the *n*-C_{23} and the summed *n*-C_{29} and *n*-C_{31} alkanes do not reflect past vegetation composition. It was shown that aerobic decomposition may significantly contribute to the abundance of mid-chain *n*-alkanes, and thus to the *n*-C_{23}, in peat OM. Although *Juncus*-dominated peat may mainly consist of roots and thus root-derived, pyrolytic mid-chain *n*-alkanes can be high, the contribution of decomposition is substantially higher according to Table 3.3. This explains the previously mentioned observations for zone 1 (Section 3.4.2), and suggests that in the *Juncus*-dominated peat, the *n*-alkane distribution reflects aerobic decomposition rather than plant species contribution, with a high abundance of long chain

alkanes indicating relatively fresh OM and that of C_{23} (and other mid-chain alkanes) indicating more decomposed OM. This is supported by positive correlations between long chain *n*-alkanes and polysaccharides (R^2=0.55 zone 1 *n*=34) and between polysaccharides and C:N ratio (R^2=0.60 zone 1 *n*=34, not shown), as high polysaccharide contents indicate fresh material (Section 3.3) and lower C:N ratios indicate greater decomposition (e.g. Kuhry and Vitt, 1996).

Our results indicate that the interpretation of *n*-alkane distributions in peat OM highly depends on the dominant vegetation composition and bog hydrology. In *Juncus*-dominated peat with low contributions of *Sphagnum*, a relatively high abundance of long chain *n*-alkanes indicates relatively wet conditions (preservation), while it indicates relatively dry ones in *Sphagnum*-dominated peat (increased input from shrub vegetation). Using the above considerations, a final hydrological interpretation of the parameters for the various vegetation zones is given in Table 3.1.

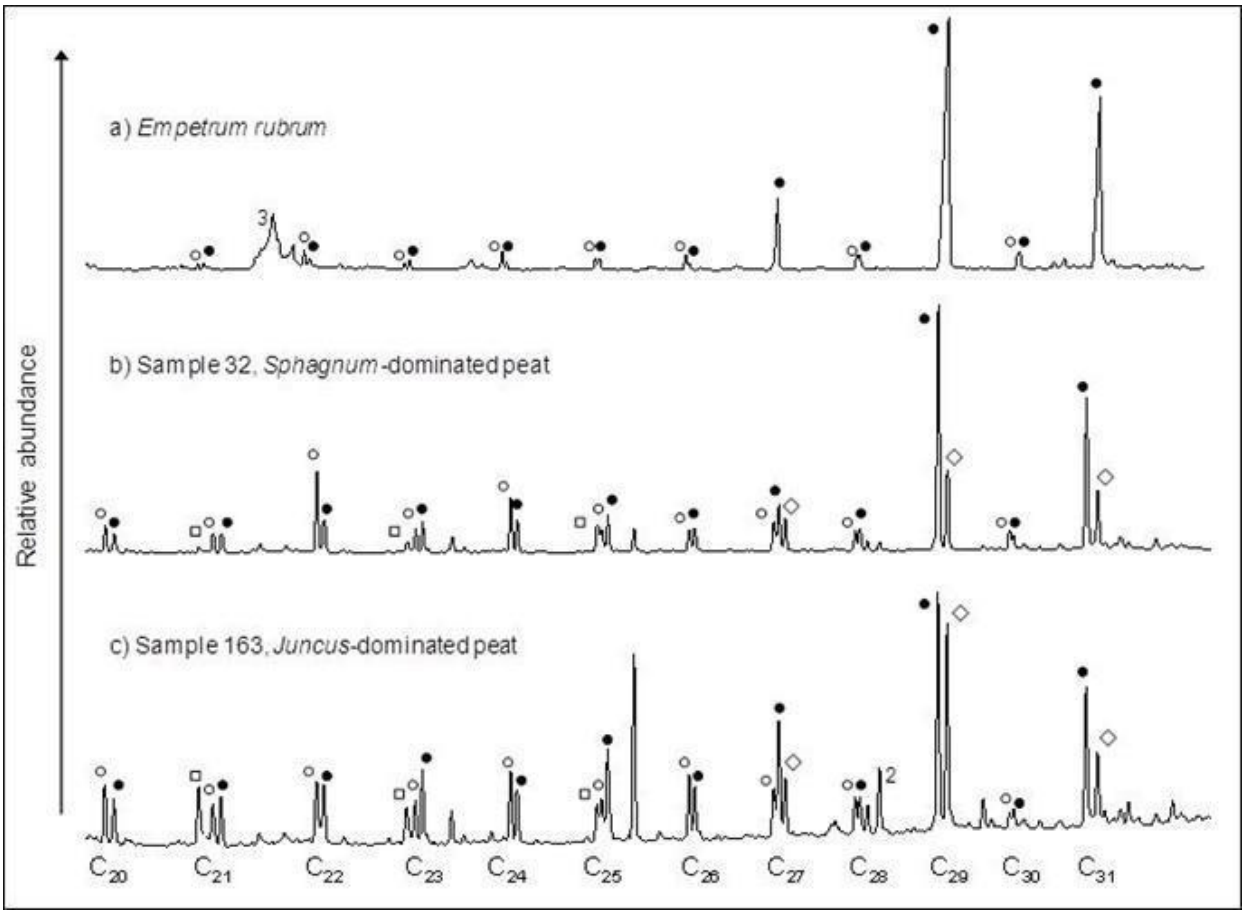

Fig. 3.4. Chromatograms for *m/z* 57+71+55+69 indicative for *n*-alkanes and *n*-alkenes of *Empetrum rubrum* (branch with leaves) and peat samples. Indicated carbon numbers refer to chain lengths of *n*-alkanes and *n*-alkenes. ●=alkanes, ○=alkenes, □=alcohols, ◊=methyl ketones. 1 Phthalate, 2 Squalene, 3Unsaturated C_{18} fatty acid.

3.4.4. Comparison with previous studies

From Sections 3.3, 3.4.2 and 3.4.3 it follows that, in *Sphagnum*-dominated peat, the *n*-alkane distribution provided by pyrolysis-GC/MS is consistent with that of GC/MS analysis (for the equipment and material used in this study). The GC/MS results from literature can therefore be compared with the pyrolysis-GC/MS results of the present study.

The varying abundances of *n*-alkanes between plant species and the different effects of decomposition in *Sphagnum* and *Juncus*-dominated peat may explain the discrepancies found in studies where the *n*-alkane distributions in (*Sphagnum*-dominated) peat deposits did not or not completely match the macrofossil analysis (Ficken et al., 1998; Nott et al., 2000), pollen analysis (Farrimond and Flanagan, 1996), or testate amoeba (Nichols et al., 2006). It may also explain why surface peat chemistry does not always reflect the *n*-alkane distribution of the present moss vegetation (Huang et al., 2010), and indicate possible contribution of other plants or the influence of early decomposition. Because the contribution of *Sphagnum* to the peat *n*-alkane fraction is very low, and shrubs with much higher *n*-alkane contents are continuously present on the dryer spots in *Sphagnum*-dominated peat (Kleinebecker et al., 2007), sparse vascular plant species may contribute more to the abundance of *n*-alkanes in the peat OM than *Sphagnum* itself (Pancost et al., 2002). Therefore, the use of *n*-alkane distributions to differentiate between different *Sphagnum* species (McClymont et al., 2008; Nott et al., 2000) needs to be taken with caution. In the Butterburn Flow peat, analysed for macrofossils and *n*-alkane distributions (McClymont et al., 2008), the simultaneous shift of long chain *n*-alkanes (towards higher values) at the depth were *Sphagnum imbricatum* is replaced by *S. magellanicum* peat can also be explained by an increase of Ericaceous shrubs under changed hydrological conditions, supported by a simultaneous shift in dominant *Sphagnum* species with an increase of Ericaceous species found in *Sphagnum*-dominated peat by Breeuwer et al. (2009).

A *dominance* of specific *n*-alkanes cannot be used to reconstruct vegetation changes. Long chain *n*-alkanes, derived from woody plant species, dominate in *Sphagnum* peat, while the *Sphagnum*-indicating *n*-C_{23} increases in non-*Sphagnum* peat as a result of aerobic decomposition. This may also explain the dominance of the *n*-C_{23} in one-third of the peat samples analysed by GC/MS reported by Ortíz et al. (2010), as *Sphagnum* is not dominant in northern Spain (see Section 3.4.2). Nonetheless, the relative change of *n*-alkane distributions with depth can be used to reconstruct *Sphagnum* (C_{23}) and woody species (C_{29}+C_{31}) in *Sphagnum*-dominated peat. However, relatively long or intense dry periods within a *Sphagnum* environment could lead to opposite interpretations, such as in sample 58 where the high values of the *n*-C_{23} do not indicate a high abundance of *Sphagnum*, and thus a relatively wet period, but strong aerobic decomposition and thus a relatively dry period. Similarly, in *Juncus*-dominated peat (zone 1), a short period of *Sphagnum* dominance shows similar high values for the *n*-C_{23} alkane as in the *Sphagnum*-dominated samples of zone 2 (e.g. compare sample 150 of zone 1 with samples 29 and 40 of zone 2, Fig. 3.3b), but it appears as a minimum within zone 1, where the *n*-C_{23} has high contributions from decomposition in underlying and overlying layers. Thus, the use of the *n*-C_{23} alkane to indicate *Sphagnum* only functions in continuously *Sphagnum*-dominated peat (see Table 3.1).

The positive correlation between long chain *n*-alkanes and humification found by Lehtonen and Ketola (1993) may therefore not be a direct relation, but might rather be explained by simultaneous increase of woody shrubs (e.g. Ericaceae) and decomposition upon better drainage. The higher contribution of the *n*-C_{29} and *n*-C_{31} alkanes to decomposed peat is therefore likely caused by a (minor) vegetation change and not by decomposition of *Sphagnum* itself. The increase in long chain *n*-alkanes with decomposition was highest in *Sphagnum*-dominated peat, compared to *Carex*, *Bryales* and *Carex-Bryales* peat (Lehtonen and Ketola, 1993), which supports our interpretations.

3.5. Conclusions

Pyrolysis-GC/MS is a solid method for investigating OM composition in peat bogs. The selected vegetation parameters are thus reliable for a hydrologic reconstruction of the Harberton peat, which will be done in a forthcoming study.

Interpretation of *n*-alkane distributions should take into account the effects of decomposition and vegetation type (dominance of *Sphagnum*) in ombrotrophic peat records. Peat decomposition clearly interferes with the interpretation of its initial (plant) chemistry, which may lead to contradicting hydrological interpretations. Although, models to correct *n*-alkane patterns for decomposition effects may function well in terrestrial sediments (Buggle et al., 2010; Zech et al., 2009) our results demonstrate that a combination of markers or other proxies is necessary for a correct interpretation of *n*-alkane distributions in peat. Therefore, both high-resolution sampling and comparison with other (vegetation) parameters are recommended.

Chapter 4

Holocene vegetation and hydrologic changes inferred from molecular vegetation markers in peat, Penido Vello (Galicia, Spain)

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Abstract

Peat molecular chemistry reflects a combination of plant input and decomposition. Both vegetation community and the degree of decomposition of plant remains are highly dependent on depth and fluctuation of the water table and thus peat organic matter (OM) chemistry reflects past hydrological conditions. Changes in hydrology according to the OM composition (by pyrolysis-gas chromatography/mass spectrometry, pyrolysis-GC/MS) in a high-resolution sampled monolith of an 8000 years old peat deposit are presented. Analysis of 18 modern vegetation species resulted in molecular markers for *Erica* spp., *Deschampsia flexuosa*, *Juncus bulbosus* and *Carex binervis*, in addition to more general markers which enabled differentiation between woody, grass and moss vegetation. Factor analysis of 106 pyrolysis products quantified for all peat samples enabled identification of mineral (Factor 1) and hydrological (Factor 2) conditions of the bog. Depth profiles of vegetation markers showed good agreement with those of the scores of both factors and enabled the identification of 14 relatively wet periods, dating to 1430–1865 AD, 930–1045 AD, 640 AD, 270–385 AD, 190–215 AD, 135 AD, 45 BC–15 AD, 260–140 BC, 640–440 BC, 1055–960 BC, 1505–1260 BC, 2300 BC, 4190–2945 BC and 5700–5205 BC, which show excellent agreement with other palaeoclimatic studies in Europe. The results emphasize the importance of high-resolution sampling, in combination with the use of multiple vegetation markers and other peat OM characteristics for a proper interpretation of a peat record.

4.1. Introduction

Peat bogs store information on past environmental conditions because of their sequentially upward growth, and have been used as such by many authors using both biological (e.g. Barber et al., 2003) and geochemical proxies (e.g. Shotyk, 1996). The Penido Vello bog, located in the Xistral mountains (Galicia, Spain), has been analysed for geochemical properties in previous studies. The geochemical characteristics indicate an ombrotrophic nature for the majority of the peat deposit (Martínez-Cortizas et al., 1997), and several elemental and isotopic characteristics have been interpreted in terms of past climatic events and human influence (Kylander et al., 2005; Martínez-Cortizas et al., 1997, 1999, 2002; Rauch et al., 2010). Together, these studies show good agreement between the signals preserved in the bog and both past climatic changes and historical events. A pollen record of the first 2 m of the same bog was made by Muñoz-Sobrino et al. (2005) and includes local species of the genus *Erica*, *Calluna* and *Sphagnum*, and the families Cyperaceae and Gramineae (Poaceae). However, their chronology was not based on dating of the sampled core, but on that obtained from the core studied by Martínez-Cortizas et al. (1997, 1999, 2002), without any common property that enabled a stratigraphic correlation to be made.

While pollen and non-pollen palynomorphs in the peat (partially) originate from the wider surroundings, the peat OM itself reflects local environmental conditions. The vegetation community is very sensitive to changes in hydrology, and (aerobic or anaerobic) decomposition of plant remains is also determined by the depth and fluctuation of the water table. Information on past changes in site hydrology is thus stored in the peat OM.

The OM of the Penido Vello peat core used in the present study was analysed by ^{13}C CPMAS NMR (Pontevedra-Pombal, 2002), and revealed a decrease in polysaccharides and an increase in aliphatics with depth, which was interpreted as the overall effect of long-term anaerobic decomposition. Deviations from this trend might be due to both changes in vegetation and to aerobic decomposition. To find such influences, Buurman et al. (2006) analysed a small number of samples by pyrolysis-GC/MS. This method provides more detailed insight on structures at the molecular (rather than at the atomic) level, and allows conclusions to be drawn about vegetation changes and decomposition. These authors were able to identify some major vegetation shifts and decomposition effects related to dry periods. However, the low number of samples analysed did not allow a detailed reconstruction of vegetation or hydrological changes to be made.

Because vegetation responds to environmental factors such as hydrology, temperature, pollution, grazing pressure, fires, and mineral admixtures, its changes in composition over time can be used to reconstruct environmental changes. In this study this is done using pyrolysis-GC/MS analysis of a high-resolution sampled core (see Section 4.3.2.1) in combination with analysis of modern vegetation species. Our purpose is to reconstruct changes in vegetation composition and interpret them in terms of hydrological conditions.

4.2. Materials and methods

4.2.1. Samples

4.2.1.1. Vegetation samples

The plant cover of Penido Vello was sampled in March 2009. From the 32 species recorded, 18 were selected for chemical analysis (Table 4.1). The selection was based on their present abundance on Galician ombrotrophic mires as well as their value as indicators of hydrologic conditions in the bog (Fraga et al., 2001, 2005; Romero-Pedreira et al., 2008). The samples were washed, oven dried at 35 °C for 1 week, ground, and analysed with pyrolysis-GC/MS. Recorded plant species and analysed plant parts are given in Table 4.2.

4.2.1.2. Peat samples

The Penido Vello peat bog is located in Galicia (NW Spain). Location and peat are described in detail by Martínez-Cortizas et al. (1997, 2002). The sampling was done in two parts. The upper 245 cm, the depth of the water table at the time of sampling, was sampled in 1996 as peat monoliths in a recently opened ditch by cutting sections of 25×25×50 cm. The deeper section was sampled with a Wardenaar corer (10×10 cm), in 2001, during a lowering of the water table in a parallel fresh cut, 25 cm distant from the bottom surface of the previous sampling point, thereby adding another 47 cm to the studied sample column. The upper meter of the peat monoliths and the second core were sectioned into slices 2 cm thick, while between 100 and 245 cm the monoliths were sectioned into slices of 5 cm in thickness, resulting in 101 samples. Wood macro-remains were removed. All samples were dried at 35 °C for one week, and then ground for pyrolysis without further pre-treatment.

4.2.2. Pyrolysis-GC/MS

The samples were pyrolysed using a Curie-Point pyrolyser (Curie temperature 600 °C) connected to a Carlo Erba GC8000 gas chromatograph. The pyrolysis products were separated in a fused silica column (Chrompack 25 m, 0.25 mm i.d.) coated with CP-Sil 51 b (film thickness 0.40 μm), with He as carrier gas. The initial oven temperature was 40 °C and the heating rate was 7 °C min^{-1}. The final temperature, 320 °C, was maintained for 20 min. The GC column was connected to a Fisons MD800 mass spectrometer (*m/z* 45–650, cycle time 1 s).

4.2.3. Quantification

Of the 18 analysed plant species all peaks were identified and quantified. This resulted in 354 pyrolysis products, nine of which appeared to be valuable as markers for one species or for a group of species. A marker is here defined as a pyrolysis product which is 1) exclusively found in one of the investigated species and 2) well preserved in the peat.

Of the 101 peat samples, six of the most distinct pyrograms were interpreted in detail, resulting in a total of 298 different pyrolysis products. Based on frequency, abundance, and potential as vegetation markers, 106 pyrolysis products were quantified for all 101 pyrograms using the area

Table 4.1. Ecological and morphological features of the analysed plant species in mires from the Xistral Mountains.[a]

Species	Abundance			Habit	Preferential habitat	Roots	Gregariousness
	O	M-wet	M-dry				
Carex durieui Steud. ex Kunze	4	2	3	Caespitose sedge, 20-40 cm	Damp	Thin and short	Forming turfs
Agrostis curtisii Kerguélen	4	1	3	Densely caespitose grass,10-40 cm, with many very fine basal leaves	Dry	Thin and short	Forming turfs
Molinea caerulea L. Moench. subsp. *caerulea*	4	3	3	Caespitose grass, 20-80 cm	Wet, damp and fluctuating water table	Tough and superficial	Often forming large tussocks
Erica mackaiana Bab.	4	1	4	Variable. In wet places it is a thin and stunted dwarf shrub, 12-20 cm high. In well drained places it is a vigorous bushy shrub, 50-100 cm	Damp and dry	The root system springing from underground horizontal woody stems is composed of short, fine and fibrous roots, upper 15 cm	Very gregarious in dry places
Calluna vulgaris (L.) Hull.	4	1	3	Very polymorphic shrub, 20-100 cm	Damp and dry	Very branched, the ultimate branches fine and fibrous. Rooting depth is determined by the water table, in damp places most roots are concentrated in the upper 15 cm of the soil, in dry places the deepest roots can reach 90 cm	Forming large patches in dry places
Erica cinerea L.	3	-	3	Dwarf shrub, 20-60 cm, usually semi prostrate	Dry, often linked to anthropogenic activity	Stout, woody, up to 1 cm diameter, from woody rootstock and with many fine branches rootlets. upper 15 cm	Forming patches of various sizes
Deschampsia flexuosa (L.) Trin.	3	1	-	Caespitose grass, 20-100 cm	Dry	Short, thin, often with slender stolons	Often forming turfs
Festuca rubra L.	3	1	-	Creeping grass, 20-100 cm	Dry	Short,, thin with long slender rhizomes	Forming tufted patches
Sphagnum spp.	3	5	-	Several different moss species, small to robust	Wet and damp	-	Forming patches, loose to dense turfs and hummocks
Eriophorum angustifolium Honckeny	3	5	-	Perennial herb, 20-60 cm	Wet and shallow water	Short and fine, from long rhizomes. Generally in the top 15 cm of peat	Very gregarious, generally forming large patches
Lichens	2	1	2	Epiphyte	Dry	-	
Carex binervis Sm.	2	2	-	Caespitose sedge, 30-50 cm	Wet	Thin and short, from short creeping rhizomes	Forming small patches
Juncus squarrosus L.	2	2	-	Caespitose rush, 10-30 cm	Damp	Thin and short, from branched rhizomes	Isolated plants to small patches
Campylopus spp.	1	-	-	Small to robust	Damp	-	Forming dense, usually glossy turfs
Thymelaea coridifolia (Lam.) Endl.	1	-	-	Dwarf shrub with spreading branches, 10-40 cm	Dry	Underground branched woody stems and large taproot with thin lateral roots	Isolated plants or very small patches
Pilosella officinarum F.W. Sch. & Sch. Bip.	1	-	-	Perennial herb with a basal rosette of leaves and scapes 5-20 cm	Pastured places	Tough and superficial roots from oblique or horizontal rhizomes	Isolated plants or very small patches
Juncus bulbosus L.	1	4	-	Caespitose perennial rush, 3-15 cm	Wet, often submerged	Short, fine and superficial	Forming patches
Carex demissa Hornem.	1	2	-	Caespitose sedge, 10-25 cm	Damp	Thin and short, from short rhizomes	Forming small patches
Juncus effusus L.	1	2	-	Densely caespitose rush, 50-150 cm	Wet and damp	Thin and short, from stout rhizomes. Usually in the top 15 cm	Forming patches

[a] O = ombrotrophic peatlands, M = minerotrophic peatlands, 1 = very scarce, 2 = scarce, 3 = common, 4 = abundant, 5 = very abundant.

of two main fragment ions (see Appendix 4). For comparison, the plant samples were also quantified for these 106 pyrolysis products. Although the chemical composition of fresh plants differs from that of the peat, it may contribute to the interpretation of the transformation from vegetation to peat OM. For each sample, the sum of the quantified peak areas was set at 100% and relative proportions were calculated with respect to this sum. The resulting quantification gives the relative abundance of each pyrolysis product, expressed as percentage of the Total Ion Current (% TIC).

The pyrolysis products were grouped, according to probable origin and chemical similarity, into a number of source groups: polysaccharides, aliphatic biopolymers, methyl ketones, fatty acids, lignins, phenols, aromatics, poly-aromatics, nitrogen compounds, steroids and triterpenoids (see Appendix 4).

Factor analysis was undertaken using Statistica® Version 6 (Statsoft Inc., Tulsa), and hierarchical cluster analysis using SPSS v15.

4.2.4. Radiocarbon dating

Radiocarbon analysis was performed earlier on 12 selected samples of the Penido Vello peat monolith, at Beta Analytic Inc. (Miami, USA) and the Centre for Isotope Research at the University of Groningen (Groningen, The Netherlands) (Kylander et al., 2005; Martínez-Cortizas et al., 1997). The bulk radiocarbon and calibrated ages at different depths are given in Table 4.3. Here we use the ages estimated from the age/depth model obtained by Rauch et al. (2010) which is constructed by fitting a cubic smoothing spline. The growth rates are approximately 1 cm/20 years in ombrotrophic peat and 1 cm/65 years in minerotrophic peat. In the following account, all ages are given in calibrated years.

4.3. Results

4.3.1. Vegetation markers

Vegetation markers and their abundances in the analysed plant species are summarised in Table 4.2. The typical marker for *Sphagnum* is 4-isopropenylphenol (Ph5) (Schellekens et al., 2009; Stankiewicz et al., 1997), while a sugar compound with dominant masses 101+116 (Ps12) may characterise mosses in general, as it was found in high amounts in both *Campylopus* sp. and *Sphagnum capillifolium* (Ehrh.) Hedw. 3-Methoxy-5-methylphenol with dominant masses 107+138 (Ph4), that is characteristic for several lichens from Tierra del Fuego (*Pseudocyphellaria freycinetii* (Delise) Malme and *Ochrolechia frigida* (Sw.) Lynge; Schellekens et al., 2009), was detected in all peat samples. Although no lichens were analysed in this study and neither of the mentioned lichen species is found in Galicia, it is assumed that this compound also reflects other lichen species.

Of the vascular plants, *Juncus bulbosus* L. is characterised by 4-hydroxybenzeneacetonitrile (N3), *Carex binervis* Sm. by a phenanthrene compound (PA2), and *Deschampsia flexuosa* (L.) Trin. by a sterol with dominant masses 191+231 (S1).

Table 4.2. Relative abundance of the vegetation markers and the summarised polysaccharides and lignins in plant samples (% TIC).[a]

	Mosses		Juncaceae			Cyperaceae			Poaceae				Ericaceae				Other	
(Groups of) pyrolysis products	*Sphagnum capillifolium*[1]	*Campylopus* sp.[1]	*Juncus effusus*[1,2]	*Juncus bulbosus*[1,2,3]	*Juncus squarrosus*[1,2]	*Carex durieui*[1,2]	*Carex demissa*[1,2]	*Carex binervis*[1,2]	*Eriophorum angustifolium*[1]	*Festuca rubra*[1,2]	*Agrostis curtisii*[1,2]	*Deschampsia flexuosa*[1,3]	*Molinea caerulea*[1,2,3]	*Calluna vulgaris*[1,2]	*Erica cinerea*[1,3]	*Erica mackaiana*[1,2,3]	*Thymelaea coridifolia*[1,3]	*Pilosella officinarum*[1,2]
4-Isopropenylphenol (Ph5)	**2.07**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sugar compound (Ps12)	**2.28**	**2.73**	0.07	0.00	0.08	0.00	0.00	0.00	0.10	0.05	0.05	0.00	0.05	0.16	0.13	0.20	0.10	0.07
4-Hydroxybenzene acetonitrile (N3)	0.00	0.00	0.00	**0.90**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
x-Methyl-phenanthrene (PA2)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.13**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-(2-Propenoic acid methyl ester)guaiacol (Lg29)	0.00	0.00	**0.07**	**0.04**	**0.04**	**0.06**	**0.05**	**0.04**	0.00	**0.10**	**0.14**	0.00	**0.08**	0.00	0.00	0.00	0.00	0.00
Squalene (Ttp)	0.20	0.05	0.07	0.29	0.03	0.08	0.02	0.04	**14.7**	0.44	0.20	0.08	0.72	0.13	0.11	0.51	0.08	0.16
Sterol (S1)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.04**	0.00	0.00	0.00	0.00	0.00	0.00
Sterol (S3)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**1.55**	**0.75**	0.02	0.06
Aliphatic compound (Al2)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.13**	**1.69**	**1.36**	**0.24**	0.00
Aromatics	1.62	2.13	3.08	4.34	4.40	1.98	4.02	4.03	1.89	1.62	1.44	1.21	1.64	2.84	5.38	3.26	3.22	**9.53**
Nitrogen compounds	0.23	0.85	1.24	1.56	1.72	0.42	1.62	1.22	1.27	1.67	1.20	1.20	1.17	0.57	0.77	1.37	1.17	**4.36**
Lignins	2.8*	0.7*	40.4	28.7	39.1	33.8	35.1	41.1	27.8	28.5	36.6	32.9	41.4	24.6	16.1	22.4	25.3	15.6
Polysaccharides	67.9	82.2	39.1	37.8	32.2	35.4	30.9	30.0	34.7	46.1	42.6	34.0	34.9	38.9	37.9	41.5	37.9	38.7

[a]TIC = Total Ion Current; values in bold indicate that the compound may be used as a marker for this species (see Section 4.3.1)

[1]Leaves/stem

[2]roots

3flowers

[*]The values of lignin for both mosses are due to 4-vinylphenol, this compound is included in the lignin group as it is known to be abundant in grass lignins. For mean relative abundancesof vegetation markers in the peat OM see Appendix 4.

Some pyrolysis products were not specific for one species but may represent a (group of) species. The triterpenoid squalene (Ttp) was present with low abundances in all plant species (<0.7% TIC) but showed a large peak in *Eriophorum angustifolium* Honckeny (14.8% TIC). A sterol with masses 189+218 (S3) was only found in *Erica mackaiana* Bab. and *Erica cinerea* L. Compared to *E. mackaiana*, *E. cinerea* makes only a minor contribution to the vegetation composition on peatlands in the Xistral mountains (see Section 4.3.2.1), in the following it is therefore assumed that marker S3 reflects the abundance of *E. mackaiana*. An aliphatic compound with specific masses 67+81 (Al2) was only detected in the woody species (*E. mackaiana*, *E. cinerea*, *Calluna vulgaris* (L.) Hull. and *Thymelaea coridifolia* (Lam.) Endl.). Although 4-(2-propenoic acid methyl ester)guaiacol (Lg29) was present in all Juncaceae, Poaceae and Cyperaceae (except for *Deschampsia flexuosa* and *E. angustifolium*) it may mainly reflect *Carex durieui* Steud. ex Kunze and the grasses (see Section 4.3.2.2). Although nitrogen compounds and aromatics are common pyrolysis products of plant and microbial matter *Pilosella officinarum* F.W. Sch.&Sch. Bip. showed especially high contents of these products.

Table 4.3. Bulk radiocarbon and calibrated ages taken from Kylander et al. (2005).[a]

Depth (cm)	Radiocarbon age (^{14}C year BP)	Calibrated age (calendar year)	Laboratory no.
15	180±50	1725-1815 AD	B-099389
25	610±40	1290-1400 AD	GrN-22288
45	1050±60	970-1025 AD	B-099390
75	1470±60	555-650 AD	B-099391
100	1860±40	115-220 AD	GrN-22289
180	2810±60	1015-890 BC	B-099393
215	3440±60	1775-1670 BC	B-099394
245	4070±50	2700-2570 BC	GrN-22290
253	4540±70	3370-3100 BC	B-145569
263	5320±60	4170-4050 BC	B-145570
277	6200±70	5180-5050 BC	B-145571
287	6600±80	5560-5480 BC	B-145572

[a] Laboratory no.: B=Analysed at Beta Analytic Inc. (Miami, USA); GrN=analysed at the Centre for Isotope Research at the University of Groningen (Groningen, The Netherlands).

A cluster analysis was applied to the vegetation samples, using the 106 pyrolysis products that were used for characterization of the peat OM (see Section 4.2.3). Fig. 4.1 shows that the plant species are clustered into three main groups in which plant species have a similar chemical composition, these are 1) mosses, 2) dicotyledons, and 3) monocotyledons. The second group can be divided into a woody sub-group and a sub-group containing only *Pilosella officinarum*. The last group can be divided into four sub-groups, of which *Eriophorum angustifolium* separates at a comparatively high level in the hierarchy, while the other three sub-groups are closely related. *Molinea caerulea* L. Moench. and *Juncus squarrosus* L. separate from the other monocotyledons; the species with preference for relatively dry conditions (*Festuca rubra* L., *Agrostis curtisii* Kerguélen, *Carex durieui*, and *Deschampsia flexuosa*) form a sub-group with a very similar chemical composition, and the species

which perform better under minerotrophic conditions (*Juncus bulbosus*, *Carex demissa* Hornem., and *Carex binervis*) also group together (except for *Juncus effusus* L. which groups together with the species which perform better under dry conditions).

4.3.2. Interpretation of vegetation markers

In order to interpret the vegetation parameters correctly, the assumptions and methodology will be discussed in detail.

4.3.2.1. Interpretation from peat OM to vegetation composition

In studies of living vegetation, the abundance of a species is usually expressed as percentage of plant cover. However, the contribution of a plant species to peat OM may, besides its above-ground abundance, also depend on other characteristics. For example the plant cover (per m^2) of one *Calluna vulgaris* plant is equivalent to that of hundreds of grass plants, whereas the biomass of 1 m^2 covered with grasses is much higher. The composition of plant remains in peat may therefore differ significantly from the composition of the living vegetation as described in vegetation studies. However, the relative change in the contribution of a species to the peat OM with time may not be affected by this discrepancy.

Due to selective decay, the contribution of a species to the peat OM may not correspond to proportions existing in the original vegetation (Mauquoy and Yeloff, 2008) but compared to macrofossil analysis, the abundance of molecular vegetation markers is expected to be less influenced by decomposition (see also Section 4.3.2.2). Furthermore, roots may be better preserved because they are less exposed to aerobic conditions compared to above-ground material. Differences in rooting depth between plant species may thus also influence the contribution of a species to the peat OM.

Roots may penetrate into peat layers accumulated decades to centuries earlier. Peat growth rate and sample thickness define how many years of peat accumulation are contained within one peat sample. The Penido Vello core was sampled every 2 cm for the upper 1 m and between 247 and 287 cm, and every 5 cm from 100 to 245 cm. This means that changes in vegetation composition are averaged over 40, 130 or 100 years, respectively (see Sections 4.2.1.2 and 4.2.4). Thus, the sampling method, the peat growth rate, and the rooting depth and preferential habitat of actual growing species, determine the influence of contemporary and later vegetation to the composition of a given sample.

Table 4.1 shows the morphological characteristics and ecological behaviour of the analysed Penido Vello plant species and the contribution they make to the current plant cover. Table 4.1 indicates that the vegetation composition is dominated by *Carex durieui* and the grasses *Agrostis curtisii* and *Molinea caerulea* and to a lesser extent *Deschampsia flexuosa* and *Festuca rubra*. The ericaceous species *Erica mackaiana* and *Calluna vulgaris* also show a high contribution to the plant cover. *Sphagnum* spp. and *Eriophorum angustifolium* also contribute significantly, while *Campylopus* spp., *Pilosella officinarum* and *Thymelaea coridifolia* have a low contribution. The other species in

Table 4.1 make only a minor contribution or only contribute significantly to the minerotrophic part (*Carex demissa*, *Carex binervis* and *Juncus* spp.).

If a similar vegetation composition is assumed for the past, it can be expected that root influences are restricted to the upper 5 cm of the peat, except for *Eriophorum angustifolium* and the ericaceous species (Table 4.1). The roots of *E. angustifolium* are not hindered by anoxic conditions (Boggie et al., 1958), while those of ericaceous species are limited to varying degrees by the depth of the water table.

Thus, in the ombrotrophic part, the peat samples reflect an average of 40 (upper 1 m) to 100 years (100–245 cm) of peat accumulation – a mixture of root and above-ground material of the standing vegetation. In addition, it may have received a contribution of root material of vegetation growing during the subsequent 100 years, although this influence decreases with time. Notwithstanding these mixing effects, it is assumed that the relative abundance of litter of a specific plant species will reflect its change in abundance with time.

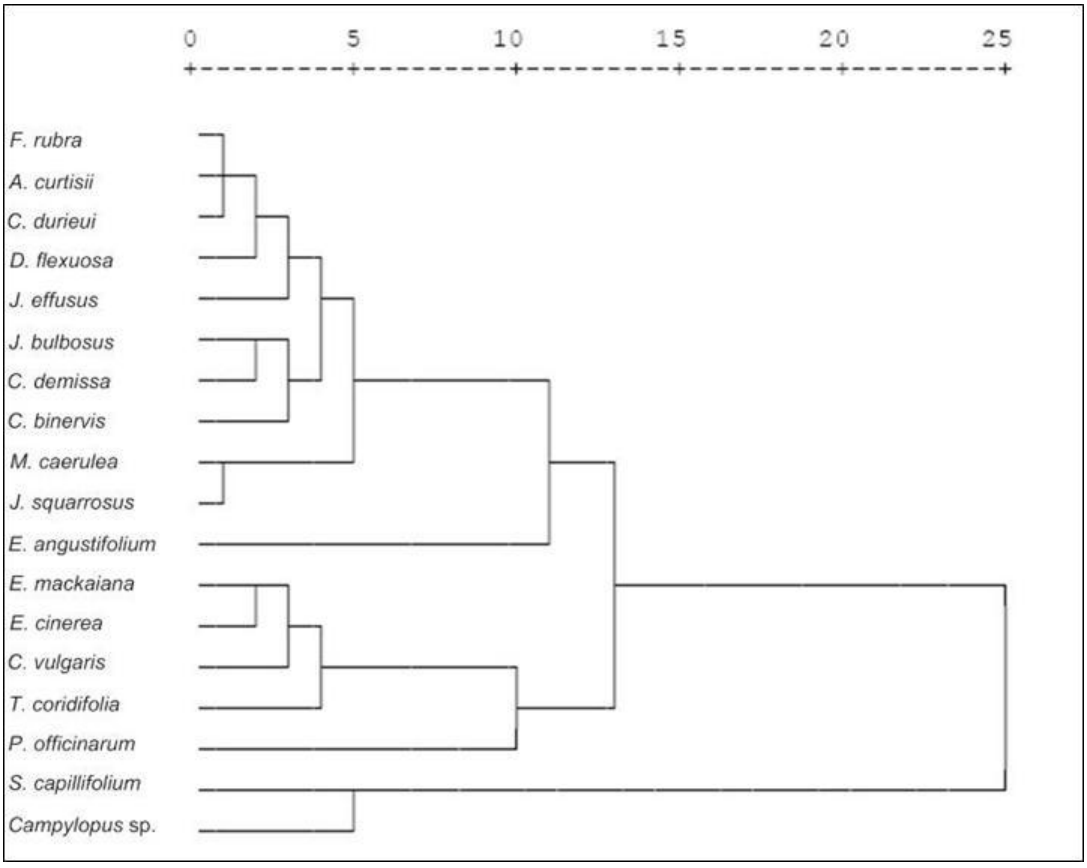

Fig. 4.1. Dendrogram based on the molecular composition of the analysed vegetation species. The hierarchical cluster analysis is performed on the standardised data (Z-scores) using the squared Euclidian distance as a measure of proximity; x-values are rescaled statistical distance units.

4.3.2.2. Interpretation of pyrolysis products as vegetation markers

Species-specific pyrolysis products can be used as markers to reconstruct past vegetation, but several aspects may influence the interpretation of such markers: 1) pyrolysis products are relative abundances and thus depend on the abundances of other quantified compounds; 2) the marker does not reflect the absolute contribution of a species to the total vegetation composition, only the relative change of the species to itself over time is indicated; 3) the abundance of a vegetation-specific pyrolysis product in a peat deposit may be influenced by (an)aerobic decay and fire incidence; and 4) because we analysed whole plants (see Table 4.2), we cannot separate the effect of roots or surface litter.

Although quantified pyrolysis products are expressed as relative abundances, a product with a very low abundance (e.g. <1% of the total quantified pyrolysis products) can be considered as statistically independent – a proposal which can be supported by good correlations with other markers or indicators.

Correlations between vegetation markers (0.1–5% TIC) and groups of pyrolysis products (20–80% TIC) may provide an estimate of the contribution of a species to the peat OM (see Section 4.3.2.2).

A decrease with depth of specific pyrolysis products may indicate that they are prone to anaerobic decay. Some markers may not be exclusively plant derived, but have contributions due to decay or fire. Such influences can be ruled out if markers of species growing under wet conditions show a negative correlation with those of species growing under dry conditions. It is therefore important to check the behaviour of markers against each other.

Table 4.4. Ages of the identified wet periods and comparison with other studies. All ages in calibrated years.

Wet period	Samples	Depth (cm)	This study	Barber et al., 2003 and references therein	Langdon et al., 2003	Desprat et al., 2003	Chambers et al., 2007	van Geel and Renssen, 1998
B12	12-3	24-6	1430–1865 AD	1150–1550 AD	1700–1800 AD	1400–1860 AD		
B11	24-21	48-42	930–1045 AD	950–1000 AD				
B10	32	64	640 AD	550 AD				
B9	44-40	88-80	270–385 AD	150–350 AD				
B8	47-46	94-92	190–215 AD	200–250 AD				
B7	49	98	135 AD					
B6	52-51	110-105	45 BC- 15 AD					
B5	56-54	130-120	260–140 BC					
B4	62-59	160-145	640–440 BC	800–400 BC	700 BC		850 BC	700 BC
B3	67-66	190-185	1055–960 BC	1800–1070 BC	1450 BC			
B2	71-69	205-195	1505–1260 BC					
B1	76	230	2300 BC	2570–1970 BC				
A2	90-79	267-245	4190–2945 BC	4250–3250 BC				
A1	100-95	287-281	5700–5205 BC	5850–5550 BC				

The presence or absence of the marker in the roots of species with large rooting depth (Ericaceae and *Eriophorum angustifolium*) defines their influence on the underlying peat. Under extremely dry conditions the roots of ericaceous species extend to large depths and may contribute to layers where the plant was originally not present. Roots of *E. angustifolium*, which tolerate wet anaerobic conditions, may contribute to peat OM originating from earlier dryer periods. From Table 4.2 it follows that the markers of *E. angustifolium*, both *Erica* species (*Erica mackaiana* and *Erica cinerea*) and of woody species (Al2) are undoubtedly present in the above-ground material, while presence in the roots is not guaranteed.

4.3.3. Peat record

4.3.3.1. General characteristics of the peat record

Because vegetation markers comprise only a small part of the total peat OM (0.01–2% TIC, Fig. 4.2) it is necessary to create a general context in which the vegetation markers can be interpreted. Such a context can be provided by general characteristics such as the mineral content (Martínez-Cortizas et al., 1997), polysaccharides, lignins, and values of Factors 1 and 2 obtained from a factor analysis applied to all 106 quantified pyrolysis products (see Section 4.2.3).

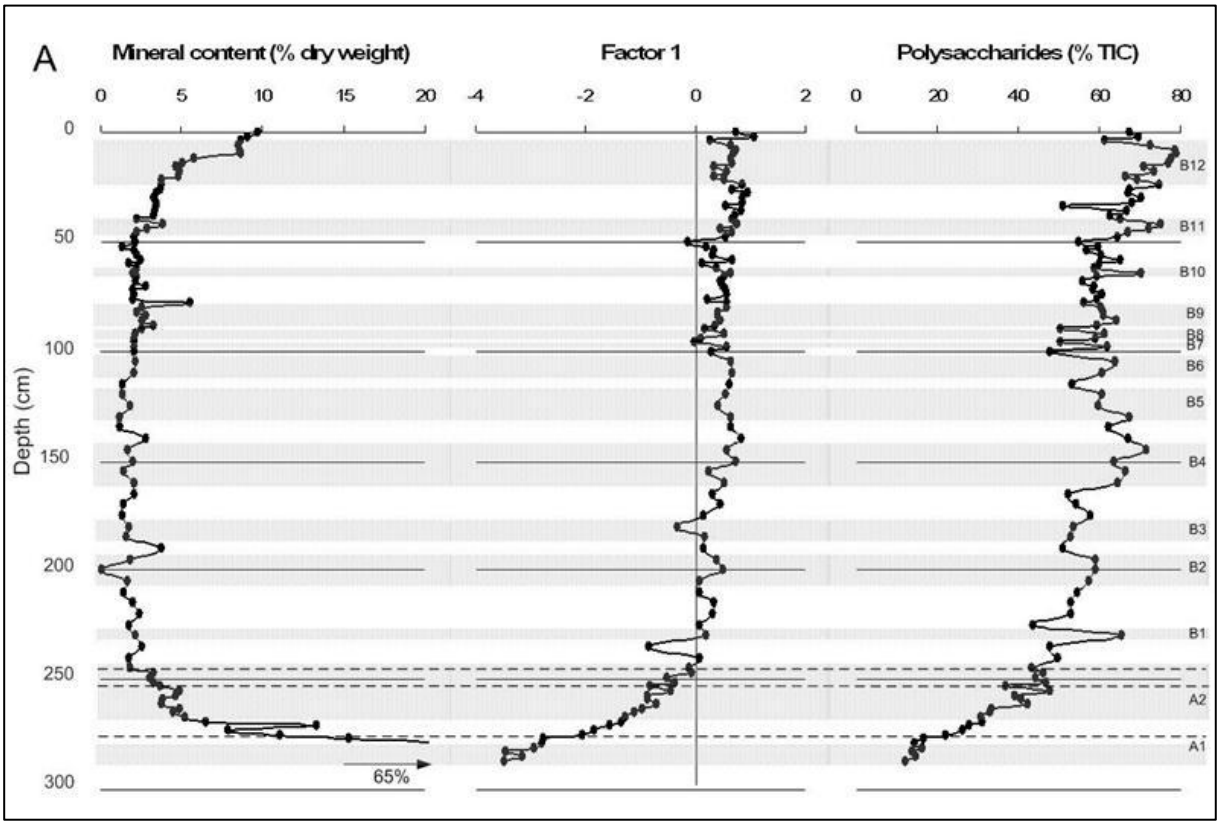

Fig. 4.2. Depth profiles of mineral fraction, factor scores of Factors 1 and 2, summarised polysaccharides and lignins (A-B), and vegetation markers (C-E). For details of vegetation markers see Table 4.2 and Appendix 4. The broken lines indicate the changes from mineral soil to minerotrophic peat to ombrotrophic peat. The shaded areas indicate relatively wet periods.

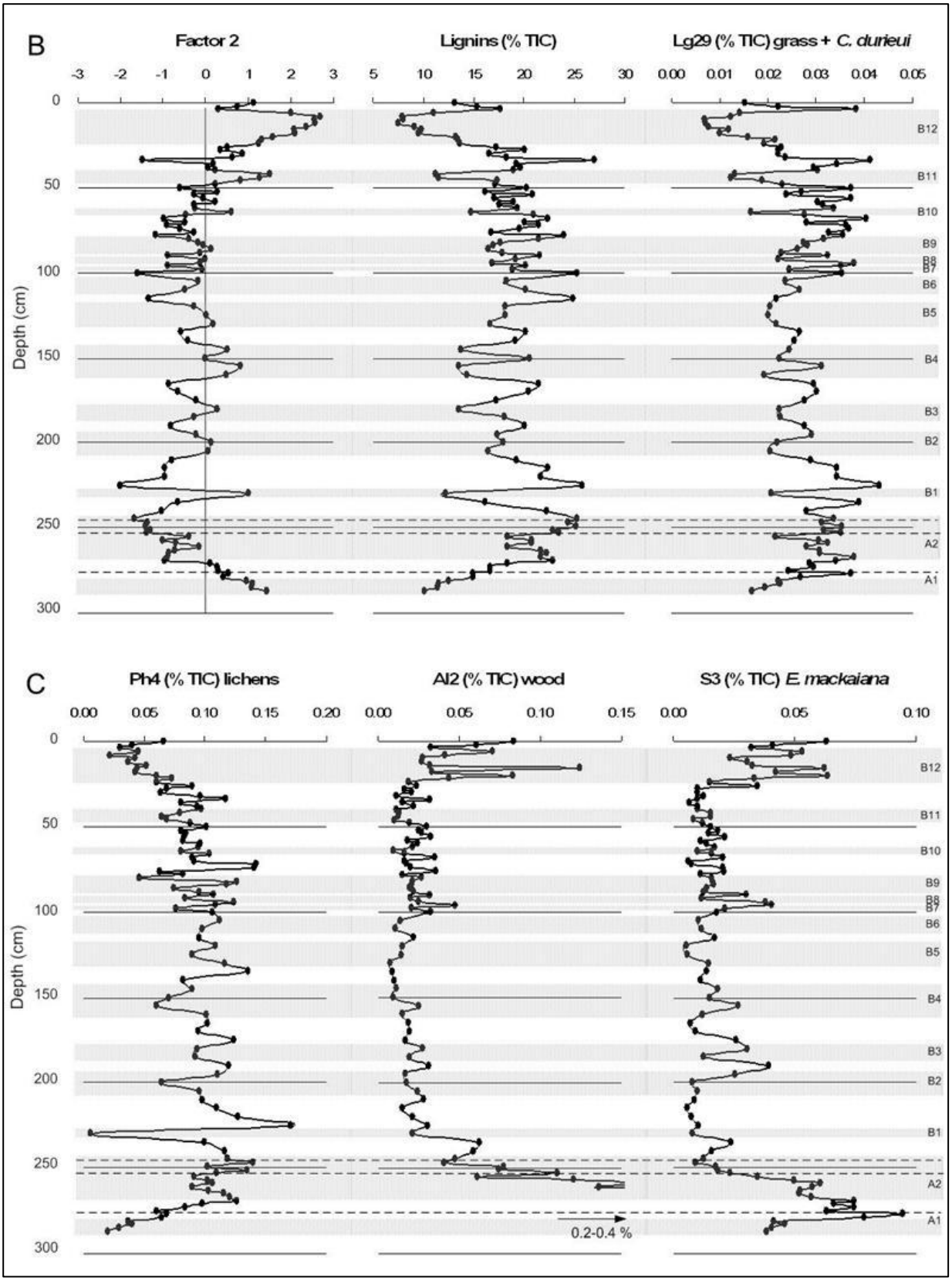

Fig. 4.2. (*continued*).

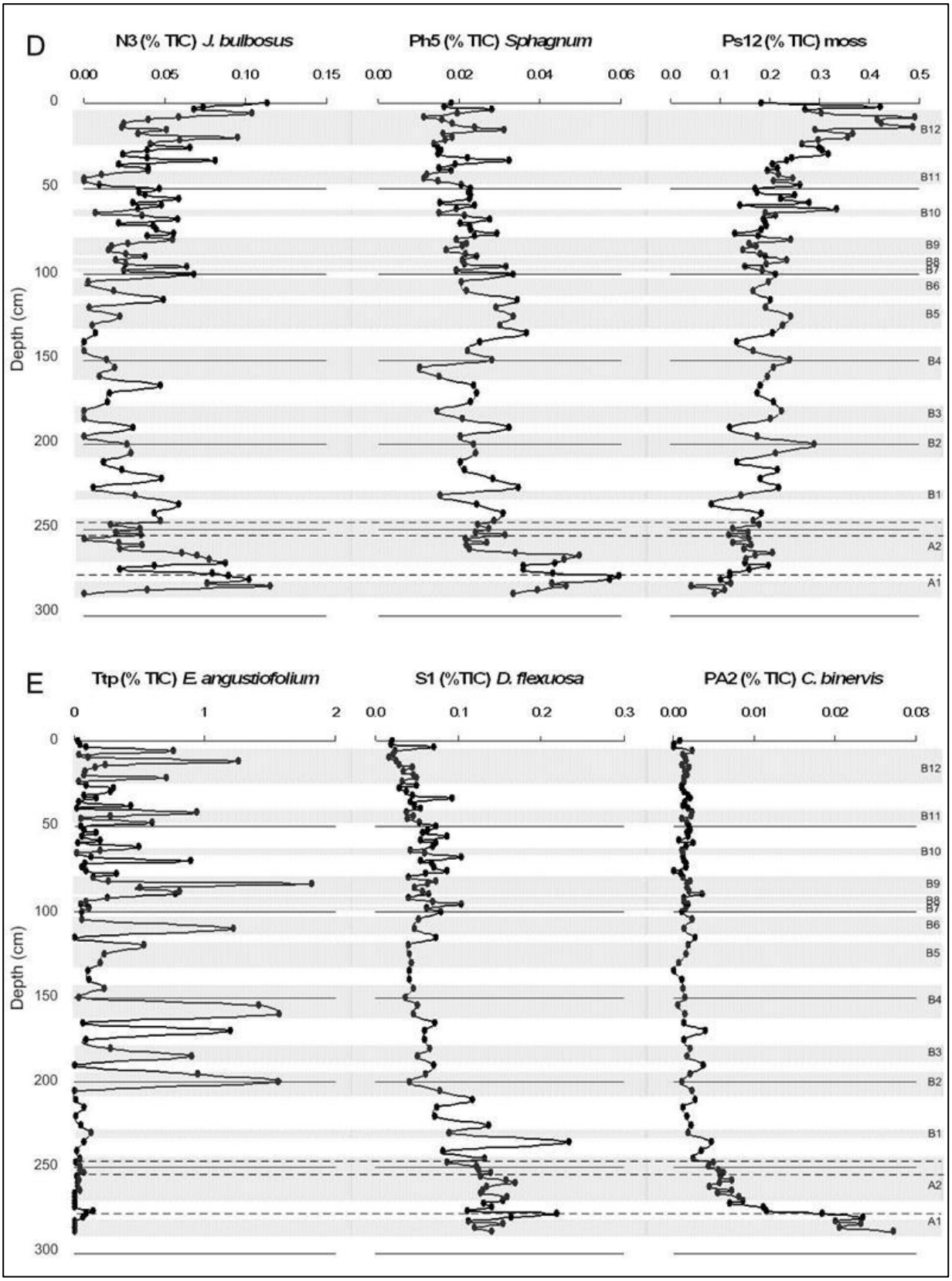

Fig. 4.2. (*continued*).

The mineral content (Fig. 4.2A) is related to vegetation composition and to peat decomposition. The deepest part of the core, from 287 to 281 cm, where mineral contents exceed 40%, represents an OM-rich mineral soil. In this part, plants had been rooted in the mineral soil and the incipient minerotrophic peat deposit was presumably still subjected to strong seasonal variations in groundwater level. From 281 to 268 cm, the mineral content decreases from 40% to 6%, indicating a build up of the peat deposit (Kylander et al., 2005). In such a situation, decomposition of plant remains is not yet restricted by a permanently anoxic environment or scarcity of nutrients. From 268 cm upwards the mineral content decreases more slowly until it reaches stable low values at 245 cm; this part probably reflects the transition from minerotrophic to ombrotrophic peat. Only in the uppermost part of the peat, from a depth of 40 cm upwards, does mineral content increase again, eventually reaching 10% in the upper 10 cm. This is probably due to the deposition of windblown material, resulting both from recent human disturbance of the vegetation and from increased soil erosion in the area (Martínez-Cortizas et al., 2005).

Polysaccharides and lignins comprise the major part of the OM (Fig. 4.2AB) and may reflect major differences in vegetation (mosses versus vascular plants, respectively). In addition, large quantities of polysaccharides indicate relatively little decay of OM and thus periods of relatively high water table. Both polysaccharides and lignins are strongly depleted below 250 cm, indicating aerobic decay in the earliest stages of peat formation (Fig. 4.2AB).

Extracted Factors 1 and 2 (F1 and F2) together explained a large part of the total variance (64.9%) of the molecular composition of the OM. Both polysaccharides and lignins have positive loadings on F1, while they are separated by F2 (Fig. 4.3). The scores on F1 (45.2% explained variance, Fig. 4.2A) are close to zero from the surface of the core to 263 cm, from where they decrease strongly downwards. The synchronous decrease of mineral content with the increase of F1 from the bottom towards 240 cm suggests that F1 reflects the change from minerotrophic to ombrotrophic peat. This is supported by the relatively low fluctuations of F1 scores in the upper (ombrotrophic) part of the core, and by the change from negative to positive values at the depth where mineral content becomes stable and low. This interpretation is in agreement with the ombrotrophic nature of the upper 250 cm of the same core (2945 BC until present) as found by Martínez-Cortizas et al. (1997), and with the general process of the formation of this kind of bogs. The scores of F1 are highly correlated with the summarised polysaccharides (R^2=0.86), suggesting that the preservation of polysaccharides is favoured by the absence of minerals and by an anoxic environment. Although F1 explains the major part of the variance, the usefulness of F1 is limited because it distinguishes only between minerotrophic and ombrotrophic conditions.

Scores of F2 (19.7% explained variance) show considerable variation throughout the core. Values are strongly (negatively) correlated with the summarised lignin pyrolysis products (R^2=0.89) and show a positive correlation with the polysaccharides (R^2=0.82, in the upper 245 cm). This may indicate that F2 reflects differences in 1) vegetation composition (mosses versus vascular plants, as mosses do not contain lignin), or 2) oxygen availability, as decomposition causes a relative increase in lignins and a decrease in polysaccharides (e.g. Benner et al., 1984). As both scenarios above are similarly

related to bog hydrology, low scores on F2 indicate relatively low water tables (vascular plants, associated with increased aerobic decomposition). In contrast high scores of F2 may indicate periods of high water table (moss vegetation, associated with reduced decomposition). This hydrologic interpretation is supported by the good negative correlation of the lichen marker (Ph4) with Factor 2 (R^2=0.61), as dry conditions are also the preferential habitat for lichens. Thus, though explaining less of the total variance than F1, F2 gives much better insight into conditions that prevailed once ombrotrophy was established.

The validity of the pyrolysis results is supported by good correlations between groups of pyrolysis products and data obtained from the same samples by other methods. Thus, O-alkyl analysed with ^{13}C CPMAS NMR (Pontevedra-Pombal, 2002) correlates well with total polysaccharides (Fig. 4.4). Similarly, nitrogen content correlates well with total nitrogen compounds determined by pyrolysis-GC/MS (Fig. 4.4). There is also a clearly-synchronous increase/decrease of mineral content and Factor 1 (Fig. 4.2A).

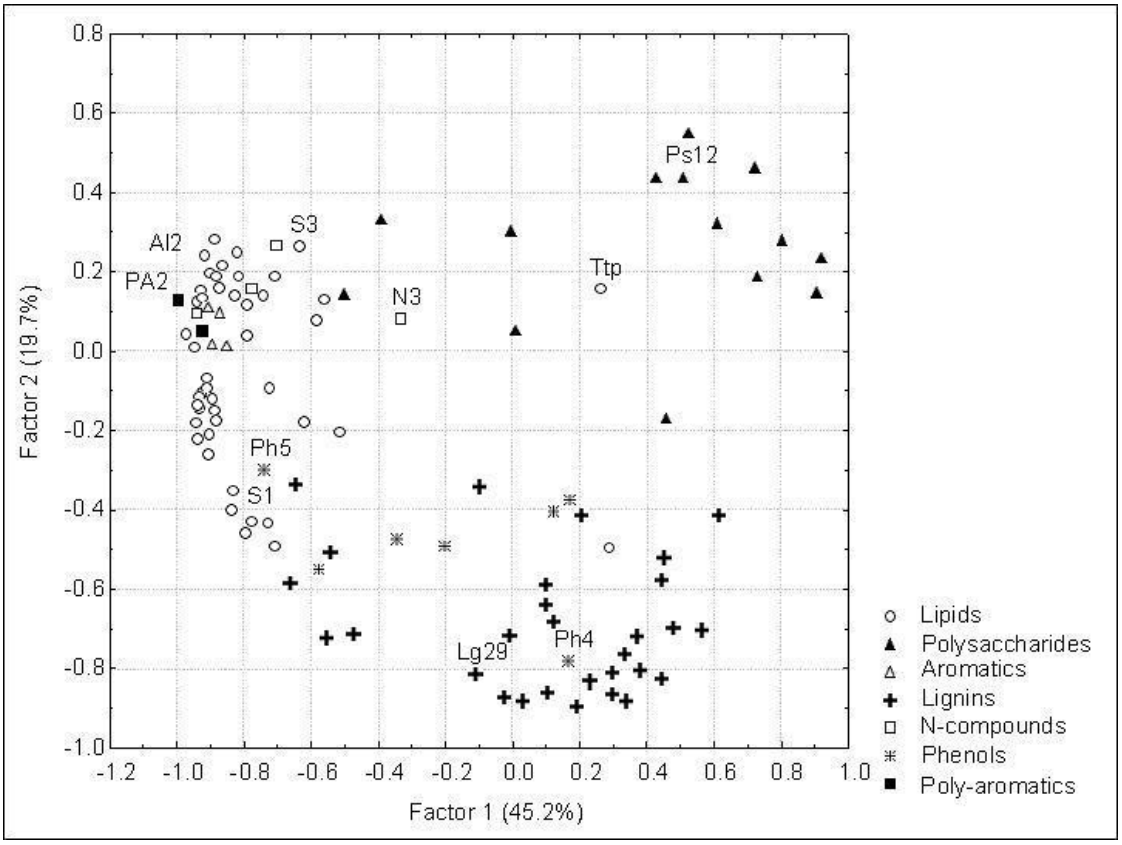

Fig. 4.3. Factor loadings for all 101 samples in F1–F2 space. Lipids include aliphatics, methyl ketones, fatty acids, steroids and triterpenoids; the labeled compounds are the vegetation markers (see Table 4.2), for labels and groups of components see Appendix 4.

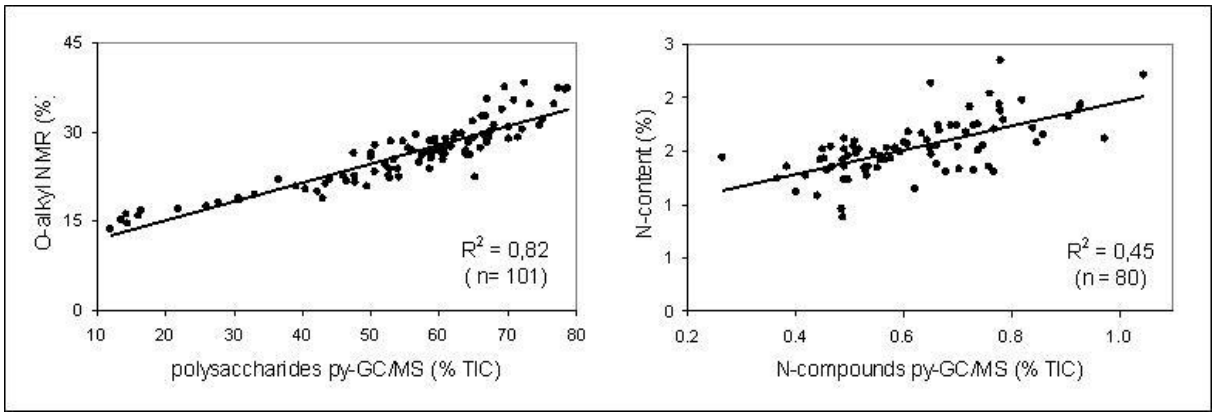

Fig. 4.4. Correlations between quantified pyrolysis results and other properties of the Penido Vello peat.

4.3.3.2. Reconstruction of the dominant vegetation

For the ombrotrophic peat, correlations between species markers and groups of pyrolysis products provide an indication of the contribution to the peat OM made by a specific species. The markers of *Erica mackaiana* and the woody species (S3 and Al2, respectively) show no correlation with lignin content (R^2=0.05). This indicates a generally marginal contribution of the woody species to the peat OM, and suggests that Poaceae, Cyperaceae and Juncaceae are the main lignin sources. Marker Lg29 was detected in all analysed Poaceae, Cyperaceae and Juncaceae species, except for *Eriophorum angustifolium* and *Deschampsia flexuosa* (Table 4.2). Juncaceae, *Carex demissa* and *Carex binervis*, make only a minor contribution to the present vegetation community of the ombrotrophic peatlands of the Xistral mountains (Table 4.1). For only two species that contained marker Lg29 (*Juncus bulbosus* and *C. binervis*) species-specific markers were found. The absence of a correlation between Lg29 and these markers (N3, PA2; R^2=0.02 and 0.04, respectively) indicates that *J. bulbosus* and *C. binervis* were not main contributors to the vegetation of the past. Therefore Lg29 must originate from *Carex durieui* and the grasses *Agrostis curtisii*, *Festuca rubra* and *Molinea caerulea*, which are also dominant species at present (Table 4.1). These species have a very similar chemical composition (Fig. 4.1), so a distinction cannot be made. Because *C. durieui*, *A. curtisii* and *F. rubra* are more abundant under relatively dry conditions (Fraga et al., 2005) and there is a good (negative) correlation between marker Lg29 and Factor 2 which, reflects bog hydrology (R^2=0.68, Section 4.3.3.1), it is assumed that marker Lg29 predominantly reflects *C. durieui*, *A. curtisii* and *F. rubra*, but other species may contribute at some depths.

The relatively high contribution of squalene (Ttp) to the peat OM (up to 2% TIC) in combination with its strong fluctuations in the core suggests a significant contribution of *Eriophorum angustifolium* to the vegetation at some depths (Fig. 4.2E).

The *Sphagnum* marker 4-isopropenylphenol shows a very low abundance in all peat layers (<0.06% TIC), compared to ~2% TIC in *Sphagnum*-dominated peat (Schellekens et al., 2009). Together with the fact that 4-isopropenylphenol shows no correlation with the more general moss markers

Ps12 (Section 4.3.1.1) and dianhydrorhamnose (Schellekens et al., 2009, not shown), this excludes a significant contribution of *Sphagnum* spp. to the peat OM.

Thus according to its chemical signature, the peat OM of the ombrotrophic section was dominated by *Carex durieui*, *Agrostis curtisii*, *Festuca rubra*, and *Molinea caerulea*, with a significant contribution of *Eriophorum angustifolium* at some depths, and a low contribution of *Sphagnum* spp., *Deschampsia flexuosa*, *Carex demissa*, *Carex binervis* and *Juncus* spp. This is in good agreement with the present day vegetation community (Table 4.1), except for the contribution of ericaceous species, which is much higher according to its cover in present vegetation than according to the peat OM composition. This aspect was discussed in Section 4.3.2.1.

The fact that the lignin content is largely determined by grasses and *Carex durieui*, may explain some discrepancies in the interpretation of the Penido Vello peat OM by Buurman et al. (2006), who assumed that *Calluna vulgaris* contributed significantly to the peat OM. The conclusion that a higher lignin content reflects a dryer bog surface is still valid, although it appears that the lignins may be derived from grasses and *C. durieui* rather than woody vegetation, while the increased relative abundance of lignin may be due to selective decomposition of polysaccharides.

The dominant vegetation shift upon changing hydrological conditions in the ombrotrophic part is thus expected to be between *Carex durieui*, *Agrostis curtisii*, and *Festuca rubra* (dry) and *Eriophorum angustifolium* (wet). Notwithstanding the low contribution of woody species and lichens to the vegetation community, a relative increase of those species is expected to reflect drier conditions, while an increase in mosses reflects wetter conditions. Based on this interpretation, changes in bog hydrology over time are discussed later.

4.3.3.3. Chronological reconstruction of major vegetation shifts and bog hydrology

Depth profiles of the vegetation markers are given in Fig. 4.2B–E (see Table 4.2 and Section 4.3.1). F2 scores, which reflect changes in bog hydrology (Sections 4.3.2.2 and 4.3.3.2), allow differentiation between relatively dry and wet periods. However, because F1 and F2 scores show a good (negative) correlation in the minerotrophic section (zone A, R^2=0.87 n=22), the use of F2 as indicator of hydrological conditions is limited to the ombrotrophic part. Thus, wet periods are indicated by high F2 scores in the ombrotrophic peat, and by the vegetation markers in the minerotrophic peat (Table 4.2). The wet periods are shaded in Fig. 4.2; ages and depths are given in Table 4.4.

The change from minerotrophic to ombrotrophic conditions according to the mineral content (269 cm, see Section 4.3.2.2) is not completely synchronous with the change of the vegetation community according to the vegetation markers. Markers of *Carex binervis* (Fig. 4.2E), *Juncus bulbosus* and *Sphagnum* (Fig. 4.2D) remain high until 263 cm and then decrease sharply. The marker of *Erica mackaiana* (Fig. 4.2C) has high values and is strongly correlated with marker Lg29 (Fig. 4.2B) up to 255 cm, from where the value for *E. mackaiana* decreases sharply and starts to deviate from marker Lg29. This delay in the change of vegetation community at the onset of the transition to ombrotrophic conditions (indicated by the decrease in mineral content), which is largest for *E. mackaiana*, is probably related to the rooting depth of the plants (Section 4.3.2.1 and Table 4.1).

According to the vegetation composition the section of 287–277 cm depth corresponds to mineral soil, followed by minerotrophic peat (275–255 cm), then a transition zone towards ombrotrophic peat (253–247 cm), and ombrotrophic peat from 245 cm to the surface. These zones are indicated by the dashed lines in Fig. 4.2.

The bottom of the mineral part reflects a relatively wet period (A1, 287–281 cm, 5700–5205 BC) according to high values for the markers of *Carex binervis* (Fig. 4.2E), *Sphagnum* and *Juncus bulbosus* (Fig. 4.2D), and low values for lichens and *Erica mackaiana* (Fig. 4.2C). This is in agreement with the beginning of peat formation, and coincides with an increase in wetness found in many (older) European bogs (e.g. Barber et al., 2003). Markers for *E. mackaiana* (Fig. 4.2C), *Deschampsia flexuosa* (Fig. 4.2E) and lichens (Fig. 4.2C) increase sharply from 279 to 277 cm (5045–4895 BC), which points towards dryer conditions. This is followed by another relatively wet period from 267 to 245 cm (A2, 4190–2945 BC), from where the peat starts to be truly ombrotrophic.

A distinct increase in wetness occurs at 230 cm (B1, 2300 BC). This is in agreement with a major shift to wet conditions between 1970 and 2570 BC found in many European palaeoclimatic studies (Table 4.4), and coincides with the definitive decrease in the marker for *Erica mackaiana* which remains low from here to the uppermost part of the core.

The next clear vegetation change occurs between 205 and 200 cm (B2, 1505–1380 BC), where the marker for *Deschampsia flexuosa* (Fig. 4.2E) decreases significantly. This decrease is directly followed by the appearance of the marker for *Eriophorum angustifolium* (Fig. 4.2E). This is in good agreement with the results of a bog at 5 km distance from Penido Vello, where a non-pollen palynomorph associated with *E. angustifolium* first appeared at ~1700 BC (Mighall et al., 2006; see Section 4.3.3.4). This ecological change probably indicates a significant increase in precipitation. After a short dry interruption, this wet period continues until 960 BC (B3). This coincides with wet shifts found in other palaeoclimatic studies, except that the short dry period between B2 and B3 has not been identified elsewhere (Table 4.4).

The wet periods between 930–1045 AD (48–42 cm, B11) and 640–440 BC (160–145 cm, B4) are also well documented in many studies (Table 4.4).

The dryer period, between B11 and B12 (1085–1385 AD, 40–26 cm) coincides with the Medieval Warm Period (MWP) (e.g. Desprat et al., 2003; Graumlich, 1993), while the wet period between 1430 and 1865 AD (24–6 cm, B12) coincides extremely well with the Little Ice Age (LIA). The LIA is characterised by relatively low temperatures and recognised in many palaeoclimatic studies. Although the literature largely agrees about the end of the LIA at about 1850 AD, coinciding with the start of the industrial period, the beginning of the LIA shows more variation (e.g. Desprat et al., 2003). According to the Penido Vello vegetation shifts, the LIA started at 1430 AD (24 cm), which is in good agreement with the results found in alpine peat (Chaline, 1985), tree ring data USA (Graumlich, 1993), pollen analysis from a Galician sediment (Desprat et al., 2003) and geochemical proxies of the same bog (Martínez-Cortizas et al., 1999). The Maunder minimum (e.g. Alcoforado et al., 2000) is also recognised at 1715–1670 AD (12–16 cm).

Except for some short periods (B5–B7), other wet periods in the ombrotrophic part (B8–B10) of the present study site coincide with those found in other palaeoclimatic studies (see Table 4.4).

4.4. Discussion

The correlation with palaeoclimatic studies lends support to our interpretation that high contents of markers for mosses (Fig. 4.2D), *Eriophorum angustifolium* (Fig. 4.2E), and of polysaccharides (Fig. 4.2A), together with low values of markers for *Carex durieui*, *Agrostis curtisii* and *Festuca rubra* (Fig. 4.2B), *Deschampsia flexuosa* (Fig. 4.2E) and lichens (Fig. 4.2C) reflect wet conditions; while the opposite is true for dry periods. However, the abundance of some species does not conform to expectations.

In the ombrotrophic peat the markers of *Sphagnum* and *Juncus bulbosus* consistently show relatively high values in dry and low values in wet periods. For *Sphagnum* this points to dominance of a species which performs better under relatively dry conditions, such as *Sphagnum compactum* (Fraga et al., 2005). The few peaks for the *Sphagnum* marker in wet periods B4, B5, and B12 are probably caused by an increase in other *Sphagnum* species. For *J. bulbosus*, the marker confirms its low abundances (Section 3.3.2) as this species needs standing water to attain high abundance. Markers for woody tissues and *Erica mackaiana* (Fig. 4.2C) do not show a clear relation with hydrology; peaks are found in both relatively wet and dry periods. This is most probably due to the morphology and ecology of the species (Table 4.1, Section 4.3.2). Under wet conditions individual plants remain small and roots grow horizontally, while under dry conditions individual plants may reach large sizes and roots penetrate to larger depth. This is confirmed by the peaks for markers of both species in wet period B12 in the upper part of the core. The peat was drained during road and wind farm construction (50 and 10 years ago, respectively; Fraga et al., 2008). This gave ericaceous species the opportunity to root to greater depths. Similarly, the two peaks of the *Eriophorum angustifolium* marker in dry periods (between B3–B4 and B9–B10) can be explained by the larger rooting depth of this species, and thus as originating from the next wet period. A clear increase in nitrogen content in the upper 54 cm (816 AD to present) may be related to an increase of *Pilosella officinarum* (not shown). This period coincides with the beginning of cattle grazing in the area to which *P. officinarum* is related (Mateo, 2007).

The relatively high abundance of markers of *Juncus bulbosus*, *Carex binervis* and *Sphagnum* in the minerotrophic peat (see Section 4.3.3.3) is in good agreement with present day vegetation community (Table 4.1). The absence of *Eriophorum angustifolium* in the minerotrophic peat may be due to competition with e.g. *J. bulbosus* as both species form mono-specific batches. Alternatively, highly fluctuating water tables may have favoured *J. bulbosus* as this species grows in the shallow margins and in the shallow pools of standing water while *E. angustifolium* can grow in water of considerable depth. The high abundance of the marker of *Erica mackaiana* in the minerotrophic peat (zone A) and its decrease towards the ombrotrophic peat (zone B) does not agree with the lack of ericaceous species in the present vegetation community on wet minerotrophic peatlands (Fraga et al., 2001). For the minerotrophic part of this core this suggests large fluctuations of the water table, with

alternating dry and wet conditions. This is in good agreement with strong lignin degradation in this part (see Section 4.3.3.1) and explains the high abundance of markers of both wet (*Sphagnum* spp., *C. binervis*, *J. bulbosus*) and dry (*E. mackaiana*, woody tissue) indicator species. In Section 4.3.2.2 the generally low contribution of ericaceous species to the peat OM, compared to the actual vegetation cover in the ombrotrophic part, was ascribed to the morphology of the species. However, the large decrease of the *Erica* marker towards the ombrotrophic part suggests that the contribution of *Erica* was indeed lower than indicated by present abundance of *Erica* on minerotrophic and ombrotrophic peatlands.

The interpretation of the various markers did not cause conflicts and showed good agreement with ecological conditions; major vegetation shifts were supported by the total (quantified) OM with factor analysis. The hydrological shifts show a chronology comparable to that described in other (European) palaeoclimatic studies on peat bogs (Table 4.4) and thus support the interpretation of the pyrolysis results. Palaeoclimatic interpretations based on records from bogs in the same area are valuable for a more detailed comparison because local and regional influences are expected to be the same.

The pollen record of the first 2 m made by Muñoz-Sobrino et al. (2005) shows a similar trend for *Erica* spp. to that shown in the present study. The maximum for marker Lg29 in the MWP (between B10 and B11 Fig. 4.2B) coincides with a clear maximum in Gramineae (Poaceae) pollen indicated by Muñoz-Sobrino.However, in the deeper part there is no correlation between marker Lg29 and the pollen of Gramineae (Poaceae) or Cyperaceae. *Sphagnum* spores and Cyperaceae pollen values obtained by Muñoz-Sobrino show a completely different picture compared to data obtained from the present study. These discrepancies are probably caused by the fact that pollen do not differentiate between regional and local inputs. Species defined as 'local' may also be growing on nearby mineral soil, while the abundance of such species may have an opposite interpretation on peat or mineral soil. For example, the abundance of *Erica arborea* may increase on mineral soil in the valley under increasing precipitation while the abundance of *Erica* species in the peat decreases under increasing wet conditions.

The abundance of non-pollen palynomorph Type 18, which is identified as the spore of a fungus that parasitises *Eriophorum vaginatum* (van Geel, 2001), has been used by Mighall et al. (2006) to indicate relatively wet periods in two other bogs in the Xistral mountains, Pena de Cadela (PDC) and Borralleiras da Cal Grande (BLL) located 5 km south and north, respectively, of Penido Vello, assuming that Type 18 is also present in *Eriophorum angustifolium* (the only species of *Eriophorum* found in the area). High values of Type 18 in these bogs largely agree with the wet periods indicated by the vegetation changes in Penido Vello. The appearance of *E. angustifolium* (according to the abundance of Type 18) in BLL coincides with wet period B1, while its appearance in the PDC core (which represents a shorter time span) coincides with B2. Wet periods B3 and B4 are also in good agreement with the abundance of Type 18 in both bogs. B5–B9 coincide well with one of the bogs, B10 shows no similarities, and B11 only shows slightly higher values in BLL. For wet period B12 (LIA) only the beginning coincides with high values of Type 18. The fact that high values of the non-pollen

palynomorph coincide with wet periods indicated by the peat OM, while the reverse fit is not always clear (some wet periods indicated by the OM are not confirmed by the non-pollen palynomorph), is probably caused by competition between *E. angustifolium* and other species which expand under wet conditions (e.g. *Juncus* spp. and mosses). For example, the start of wet period B12 coincides with high values of Type 18 which thereafter decreases. OM analysis shows that the whole period has a high contribution of mosses. Furthermore, a parasite associated with a particular species may not always be present on that species and its abundance may be influenced by wetness or other environmental conditions.

Mighall et al. (2006) developed a humidity index based on the thermal lability of atmospheric mercury in the ombrotrophic part of the Penido Vello peat (Martínez-Cortizas et al., 1999). It appears that, except for wet periods B4 and B6, this humidity index is not in agreement with the wet periods found by pyrolysis-GC/MS. Further research is required to explain this discrepancy.

4.5. Conclusions

Molecular chemistry by pyrolysis-GC/MS allows a valuable insight to be obtained into the reconstruction of hydrology and species composition of peat. Factors extracted by multivariate analysis of all quantified pyrolysis products give a useful picture of mineral content and hydrology. Because factor analysis addresses all variables, the extracted factors are perhaps more reliable indicators than the abundance of individual vegetation markers. The use of individual vegetation markers as indicators for hydrology is less reliable than the use of a combination of markers.

Depth profiles of vegetation markers and decomposition characteristics indicate a change from minerotrophic to ombrotrophic peat between 2945 and 2715 BC. Due to the rooting depth of plants, this effect is delayed compared to geochemical characteristics. In the minerotrophic peat, decomposition causes strong decay of both polysaccharides and lignins. In the ombrotrophic peat decomposition is less severe and polysaccharides are preferentially degraded.

Strong decomposition in the minerotrophic peat indicates highly fluctuating water tables. Therefore, vegetation markers more precisely show changes in hydrology, as vegetation reacts directly to changes in hydrology whereas decomposition may occur at a later stage.

In the ombrotrophic peat, OM composition indicates an almost homogeneous composition of the vegetation during the last 5000 years, with dominance of *Carex durieui* and grasses, as well as significant contributions from *Eriophorum angustifolium* and mosses at some periods, together with low contributions of *Juncus* spp., *Carex binervis* and *Carex demissa*.

Because in the ombrotrophic peat, the dominant species (*Carex durieui* and the grasses) have a very similar chemical composition and no specific markers could be identified with pyrolysis, major shifts within these species upon changes in hydrology are better shown by chemical changes due to decomposition processes. Thus a relative decrease in polysaccharides and increase of lignins indicates relatively dry conditions.

The part of the peat core sampled at higher resolution (2 cm) shows better correlations between the vegetation markers and other parameters, compared to the part sampled with lower resolution (5 cm). This suggests that root influence is shallow and supports high-resolution sampling as a useful approach for reconstructing past environmental conditions.

The fact that Factor 2 matches with the abundance of markers for both wet and dry species-indicators, suggests that the effect of roots and the transition from vegetation to peat OM is generally marginal. Consequently, the present study recommends high-resolution sampling, coupled with a combination of markers and support from other peat OM characteristics (e.g. the abundance of polysaccharides to indicate decomposition).

Appendix 4. Quantified pyrolysis products. M =molecular weight; m/z =typical mass; RT =retention time; % TIC = ave.% Total Ion Current; S.D.=tandard deviation (101 samples). Vegetation markers are printed in bold.

Code	Name	M	*m/z*	RT (min)	Ave. TIC (%)	S.D.
C10	$C_{10:0}$	142	57+71	9.703	0.14	0.09
C11	$C_{11:0}$	156	57+71	11.907	0.21	0.11
C12	$C_{12:0}$	170	57+71	14.019	0.23	0.14
C13	$C_{13:0}$	184	57+71	16.038	0.26	0.15
C14	$C_{14:0}$	198	57+71	17.938	0.30	0.17
C15	$C_{15:0}$	212	57+71	19.739	0.32	0.23
C16	$C_{16:0}$	226	57+71	21.444	0.38	0.23
C17	$C_{17:0}$	240	57+71	23.044	0.47	0.33
C18	$C_{18:0}$	254	57+71	24.566	0.41	0.25
C19	$C_{19:0}$	268	57+71	26.032	0.58	0.30
C20	$C_{20:0}$	282	57+71	27.403	0.57	0.28
C21	$C_{21:0}$	296	57+71	28.735	0.81	0.38
C22	$C_{22:0}$	310	57+71	29.993	0.81	0.40
C23	$C_{23:0}$	324	57+71	31.212	1.08	0.57
C24	$C_{24:0}$	338	57+71	32.373	0.57	0.34
C25	$C_{25:0}$	352	57+71	33.481	0.72	0.46
C26	$C_{26:0}$	366	57+71	34.553	0.32	0.33
C27	$C_{27:0}$	380	57+71	35.590	0.39	0.53
C28	$C_{28:0}$	394	57+71	36.590	0.20	0.29
C29	$C_{29:0}$	408	57+71	37.541	0.43	0.65
C31	$C_{31:0}$	436	57+71	39.375	0.94	1.34
C33	$C_{33:0}$	464	57+71	41.159	0.75	1.08
Al1	Prist-1-ene	266	56+57	23.546	0.68	0.45
Al2	Aliphatic compound		67+81	25.603	0.08	0.14
Al3	C_{18} alcohol	270	55+57	28.432	0.14	0.23
Al4	C_{20} alcohol	298	55+57	30.957	0.25	0.20
Al5	Branched alkene	280	83	31.531	0.29	0.17
Al6	C_{22} alcohol	326	55+57	33.314	0.30	0.27
Al7	C_{24} alcohol	354	55+57	35.427	0.13	0.12
Ps1	Acetic acid	60	60	2.792	1.69	0.64
Ps2	2-Furaldehyde	96	95+96	5.593	3.68	1.33
Ps3	5-Methyl-2-furaldehyde	110	109+110	8.300	1.44	0.58
Ps4	4-Hydroxy-5,6-dihydro-(2*H*)-pyran-2-one	114	58+114	9.011	1.51	0.64
Ps5	Dianhydrorhamnose	128	113+128	10.093	0.21	0.08
Ps6	Levoglucosenone	126	68+98	11.392	2.26	0.95
Ps7	1,4:3,6-Dianhydro-alpha-D-glucose	144	57+69	13.735	0.90	0.38
Ps8	5-Hydroxymethyl-2-furancarboxaldehyde	126	97+126	14.073	0.42	0.27
Ps9	1,4-Dideoxy-D-glycero-hex-1-enopyranose-3-ulose	144	87+144	15.683	1.33	0.52
Ps10	Levogalactosan	162	60+73	17.356	3.12	1.36
Ps11	Levomannosan	162	60+73	18.659	2.70	1.20
Ps12	Sugar compound		101+116	19,151	0.68	0.45
Ps13	Levoglucosan	162	60+73	20.359	32.91	12.14
Ps14	1,6-Anhydro-beta-D-glucofuranose	162	73+85	21.671	2.15	0.94
K19	C_{19} methyl ketone	282	58+59	28.616	1.16	1.46
K21	C_{21} methyl ketone	310	58+59	31.155	0.34	0.33
K23	C_{23} methyl ketone	338	58+59	33.474	0.25	0.10

Appendix 4A (*continued*).

Code	Name	M	*m/z*	RT (min)	Ave. TIC (%)	S.D.
K24	C_{24} methyl ketone	352	58+59	34.586	0.12	0.06
K25	C_{25} methyl ketone	366	58+59	35.640	0.68	0.25
K26	C_{26} methyl ketone	380	58+59	36.658	0.15	0.09
K27	C_{27} methyl ketone	394	58+59	37.641	0.66	0.35
K28	C_{28} methyl ketone	408	58+59	38.575	0.08	0.08
K29	C_{29} methyl ketone	422	58+59	39.492	0.36	0.31
K31	C_{31} methyl ketone	450	58+59	41.325	0.22	0.35
K33	C_{33} methyl ketone	478	58+59	43.644	0.14	0.25
Ar1	Benzene	78	77+78	3.324	0.61	0.48
Ar2	Toluene	92	91+92	4.733	1.52	1.01
Ar3	Styrene	104	78+104	7.080	0.24	0.21
Ar4	Benzoic acid	122	105+122	14.000	0.13	0.08
Lg1	Guiacol	124	109+124	11.301	1.30	0.38
Lg2	4-Methylguaiacol	138	123+138	13.539	1.19	0.40
Lg3	4-Vinylphenol	120	91+120	14.163	3.62	1.12
Lg4	4-Ethylguaiacol	152	137+152	15.330	0.48	0.16
Lg5	4-Vinylguaiacol	150	135+150	16.002	3.63	0.96
Lg6	Syringol	154	139+154	16.548	0.42	0.14
Lg7	4-(1-Propenyl)guaiacol, Eugenol	164	77+164	16.830	0.07	0.02
Lg8	5-Methyl-3-methoxy catechol	154	139+154	16.970	0.16	0.06
Lg9	4-Formylguaicol, Vanillin	152	151+152	17.445	0.67	0.17
Lg10	4-(2-Propenyl)guaiacol, *cis*	164	77+164	17.799	0.05	0.01
Lg11	4-Methylsyringol	168	153+168	18.393	0.54	0.22
Lg12	4-(2-Propenyl)guaiacol, *trans*	164	77+164	18.569	0.27	0.09
Lg13	4-Acetylguaiacol	166	151+166	19.057	1.00	0.30
Lg14	4-Propan-2-one-guaiacol	180	137+180	19.809	0.20	0.05
Lg15	4-Ethylsyringol	182	167+182	19.827	0.13	0.05
Lg16	Guaiacol derivative (C_3H_3 side chain)	162	147+162	19.927	0.10	0.04
Lg17	Guaiacol derivative (C_3H_3 side chain)	162	147+162	20.060	0.07	0.02
Lg18	4-Vinylsyringol	180	165+180	20.483	0.89	0.32
Lg19	Vanillic Acid	168	153+168	20.747	0.70	0.35
Lg20	4-(Prop-2-enyl)syringol	194	91+194	21.113	0.09	0.04
Lg21	4-(Prop-1-enyl)syringol, *cis*	194	91+194	21.932	0.09	0.03
Lg22	4-Formylsyringol	182	181+182	21.932	0.19	0.07
Lg23	4-(Prop-1-enyl)syringol, *trans*	194	91+194	22.731	0.52	0.24
Lg24	4-Hydroxy-1,2-benzenedicarboxilic acid	182	181+182	24.480	0.12	0.05
Lg25	Syringol derivative (C_3H_3 side chain)	192	131+192	22.471	0.16	0.07
Lg26	Syringol derivative (C_3H_3 side chain)	192	131+192	22.575	0.08	0.03
Lg27	4-Acetylsyringol	196	181+196	23.131	0.50	0.14
Lg28	4-(Propan-2-one)syringol	210	167+210	23.649	0.38	0.13
Lg29	4-(2-Propenoic acid methyl ester)guaiacol	208	177+208	23.863	0.03	0.01
Lg30	4-(Propan-3-one)syringol	210	181+210	24.531	0.16	0.07
N1	Pyridine	79	52+79	4.226	0.62	0.37
N2	Benzonitrile	103	76+103	8.750	0.02	0.02
N3	4-Hydroxy-benzeneacetonitrile	133	78+133	17.580	0.04	0.03
N4	Diketodipyrrole	186	93+186	22.870	0.12	0.08
Ph1	Phenol	94	66+94	9.065	4.31	1.97
Ph2	Catechol	110	64+110	14.001	1.29	0.68

Appendix 4A (*continued*).

Code	Name	M	*m/z*	RT (min)	Ave. TIC (%)	S.D.
Ph3	4-Methyl-1,2-benzenediol	124	78+124	14.753	0.21	0.15
Ph4	3-methoxy-5-methylphenol	138	107+138	15.259	0.09	0.03
Ph5	4-Isopropenylphenol	134	119+134	16.270	0.02	0.01
Ph6	4-Hydroxy benzoic acid	138	123+138	17.550	0.12	0.08
Ph7	4-Hydroxybenzoic acid methyl ester	152	121+152	18.345	0.02	0.02
PA1	Naphthalene	128	128	12.674	0.07	0.08
PA2	x-Methyl-phenanthrene	192	191+192	25.237	0.00	0.01
FA16	C_{16} fatty acid	256	60+73	26.753	0.34	0.29
Ttp	Squalene	410	69+81	36.808	0.26	0.40
S1	Sterol 1		191+231	38.078	0.08	0.04
S2	Gamma-tocopherol	416	151+416	39.009	0.14	0.08
S3	Sterol 2		189+218	39.432	0.03	0.02

Chapter 5

Source and transformation of lignin in *Carex*-dominated peat

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Abstract

We identified the effects of vegetation changes, and aerobic and anaerobic decay on the lignin composition in the Penido Vello peat record (Galicia, Spain). The ombrotrophic part of this peat record was dominated by graminoids and has significant contributions of ericoids at some depths. The organic matter (OM) of different peat fractions (bulk, NaOH-extractable fraction, and non-extractable residues) of 15 samples from the upper meter was analysed with pyrolysis-gas chromatography/mass spectrometry (pyrolysis-GC/MS). In addition, the dominant plant species were analysed, including *Carex durieui*, *Agrostis curtisii*, *Molinia caerulea*, *Deschampsia flexuosa*, *Festuca rubra*, *Eriophorum angustifolium*, *Erica mackaiana* and *Calluna vulgaris*, and their lignin composition compared to that of the peat OM. The high abundance of guaiacol and 4-formylguaiacol in fresh plant tissue compared to peat OM suggests that in addition to *p*-coumaric and ferulic acid (which are abundant in graminoids), other non-lignin phenolic monomers are contributed by graminoid species. For the non-lignin phenolics, graminoids differed from ericoids in the high abundance of ferulic acid (4-vinylguaiacol), while *p*-coumaric acid (4-vinylphenol) showed high and similar abundances in ericoids and graminoids. This result suggests that ratios between *p*-hydroxyphenyl (or *p*-coumaric acid) and other lignin moieties in (pyrolysates of) peat cannot be used as source indicator. Comparison of plant and peat fractions using factor analysis allowed a distinction between the effects of source (plant identity) and decay on the lignin composition of the Penido Vello peat, and different stages of decomposition were identified. Preferential decay of guaiacyl over syringyl moieties was found for the first stage of decay. This preferential decay is probably related to the large abundance of guaiacyl moieties in easily degradable non-lignin phenolics. Preferential decay of syringyl moieties occurred during subsequent aerobic decay.

5.1. Introduction

In ombrotrophic peatlands, fluctuations in the water table are determined by variation in precipitation only. Bog hydrology strongly influences the botanical composition and decomposition process. The molecular composition of OM in peatlands, soils and sediments supplies information on the botanical sources (plant identity) and on the extent of decomposition during litter decay. Therefore, the molecular composition of OM is often used as a proxy for past environmental conditions (e.g. McClymont et al., 2011), or to obtain information on the rate of carbon sequestration that is a crucial element in the global carbon cycle (Clymo et al., 1998).

Lignin is a significant component of OM in anaerobic ecosystems such as peatlands, as decomposition of lignin primarily depends on the supply of oxygen (Williams and Yavitt, 2003). The knowledge of lignin transformations in soils and sediments, recently reviewed by Thevenot et al. (2010), is mainly based on aerobic systems and uses the abundance of syringyl, guaiacyl and *p*-hydroxyphenyl moieties, oxygen functionality and chain length reduction of alkyl side-chains, and demethoxylation. In anaerobic systems, however, the interpretation of these characteristics may be different. In addition, the lignin composition varies strongly between plant species, plant parts and elements of plant cells, and its resistance to decay may show similar differences (Machinet et al., 2011). Because changes in hydrology drive changes in both plant species composition and degree of decomposition, the interpretation of chemical changes in peat in terms of decomposition is complex (Yeloff and Mauquoy, 2006).

Many methods have been developed to study the lignin composition in plant and soil OM (Lu and Ralph, 2010). Characterisation of lignin is difficult because it is not possible to isolate lignin in its intact state. Distinguishing non-lignin phenolics also found in the cell wall from macromolecular lignin can be difficult. In soil OM studies, the lignin composition is usually analysed with the CuO oxidation method (Thevenot et al., 2010), but also pyrolysis-gas chromatography/mass spectrometry (pyrolysis-GC/MS) and tetramethylammonium hydroxide thermochemolysis (Filley et al., 2006; Nierop, 2001) are frequently applied. Although pyrolysis-GC/MS is a destructive method and rearrangements may occur during the pyrolysis process, most lignin fragments produced during pyrolysis retain the substitution patterns of the lignin macromolecule (Martín et al., 1979; Ralph and Hatfield, 1991; Stout et al., 1988). In addition to the lignin composition, pyrolysis-GC/MS gives detailed information on the overall molecular composition, which benefits the interpretation. The method has been successfully applied to characterise peat deposits and provides valid information on both vegetation and decomposition characteristics (e.g. Kuder and Kruge, 1998; Schellekens et al., 2009).

The effects of decomposition on monocotyledon tissues have been studied by few authors (Kuder et al., 1998). The ombrotrophic part of the Penido Vello peat record was dominated by *Carex durieui* and grasses (*Agrostis curtisii*, *Molinia caerulea*, *Deschampsia flexuosa* and *Festuca rubra*) with significant contributions of *Erica mackaiana* and *Calluna vulgaris*, *Eriophorum angustifolium* and mosses at some depths. *M. caerulea*, *E. angustifolium* and mosses indicate wetter conditions, the other plant species drier conditions (Fraga et al., 2005). The molecular composition of the high-resolution sampled ombrotrophic Penido Vello bog (Galicia, Spain) has been previously studied with

pyrolysis-GC/MS (Schellekens et al., 2011). Combined interpretation of vegetation markers, groups of pyrolysis products and present-day vegetation composition enabled a reconstruction of bog hydrology. Wet and dry periods identified by molecular chemistry agreed well with other European studies. Thus, the Penido Vello bog, with its known changes in vegetation composition and hydrology, is particularly suited to study the effects of decomposition on the lignin composition in peat.

The purpose of this study is to identify and separate the effects of source and decomposition (aerobic and anaerobic) on the lignin composition of the Penido Vello peat record. Because polysaccharides and lignin-like phenolic monomers are associated with lignin (see Section 5.4.1), the effects of source and decomposition on polysaccharides and phenolic monomers were also studied.

5.2. Material and methods

5.2.1. Location and sampling

The Penido Vello peat is an ombrotrophic bog located in the Xistral mountains (Galicia, NW Spain). Location and bog are described in detail by Martínez-Cortizas et al. (1997, 2002). The 3 m thick peat record dates back to 6000 years BC. For the purpose of this study only the high-resolution sampled (slices of 2 cm thick) upper meter of the peat record was used, as it showed better correlations between vegetation markers than the deeper part, which was sectioned into 5 cm thick slices (Schellekens et al., 2011). The upper meter (51 samples) represents around 2000 years of peat accumulation.

5.2.2. Samples

Bulk samples of plant species and peat were previously analysed with pyrolysis-GC/MS (Schellekens et al., 2011). In addition, pyrolysates of NaOH-extractable and non-extractable OM of a selection of 15 samples, previously analysed by Buurman et al. (2006) were used. The selection of these samples was based on differences in quantified ^{13}C NMR data. The extractable fraction more reflects decomposed OM and the non-extractable residue more closely resembles intact plant material (Buurman et al., 2006; Schellekens et al., 2009; Schellekens and Buurman, 2011; Zaccone et al., 2008). Comparison of pyrolysates of fresh plant tissues and the extractable and non-extractable peat fraction allows the distinction between effects of source and those of decomposition on the lignin fraction.

5.2.3. Extraction

An aliquot (0.5 g) of each peat sample was extracted with NaOH (0.1 M, 20 mL), shaken for 24 h under N_2 and centrifuged (1 h) at 4000 g. The extract was decanted and the extraction repeated a second time. The extracts were combined, acidified to pH 1 with HCl, shaken for 24 h, dialysed against distilled water (cut off 10,000 D) and freeze-dried. The residues were acidified, dialysed against distilled water and freeze-dried.

5.2.4. Pyrolysis-GC/MS

The samples were pyrolysed using a Horizon Curie-Point pyrolyser (Curie temperature 600 °C) connected to a Carlo Erba GC8000 gas chromatograph. The pyrolysis products were separated on a non-polar fused silica column (Chrompack 25 m, 0.25 mm i.d.) coated with CP-Sil 51 b (film thickness 0.40 mm), with He as carrier gas. The initial oven temperature was 40 °C and the heating rate 7 °C min^{-1}. The final temperature, 320 °C, was maintained for 15 min. The GC column was connected to a Fisons MD800 mass spectrometer (*m/z* 45–650, cycle time 1 s).

5.2.5. Quantification

In addition to the 106 pyrolysis products quantified for the bulk samples (Schellekens et al., 2011), a number of less common lignin pyrolysis products were quantified. This resulted in a total of 120 quantified pyrolysis products. These 120 pyrolysis products were also quantified for the peat extracts and residues and for the plant samples. Quantifications were based on the peak area of two major fragment ions of each pyrolysis product (Appendix 5). All quantifications were checked manually. For each sample, the sum of the peak areas was set at 100% and relative proportions were calculated with respect to this sum. The resulting quantification gives the abundance of each pyrolysis product, expressed as percentage of the total ion current (TIC).

The pyrolysis products were grouped, according to probable origin and chemical similarity, into a number of source groups: polysaccharides, aliphatic biopolymers, methylketones, lignins, phenols, catechols, (other) aromatics, polyaromatics, nitrogen compounds, fatty acids, steroids and triterpenoids. Factor analysis was applied using Statistica® Version 6 (Statsoft Inc, Tulsa).

5.3. Results

The abundance of groups of pyrolysis products for plant and peat OM are given in Table 5.1. The abundance of lignin-derived pyrolysis products as percentage of the total quantified pyrolysis products are given in Table 5.2. Based on analysis of 18 plant species markers were found for graminoids, ericoids, and woody species (including ericoids but not exclusively ericoids; Schellekens et al., 2011).

5.3.1. The composition of lignin-derived pyrolysis products in plant species

To interpret the chemical composition of the lignin-carbohydrate complex (LCC), the ratio of lignin to polysaccharides is used in addition to groups of pyrolysis products (Table 5.1). In graminoids the abundance of lignin pyrolysis products was higher than that of polysaccharide pyrolysis products (lignin/polysaccharide ratios between 1.1 and 1.8) except for *F. rubra* with a slightly higher contribution of polysaccharides (0.9),while in the ericoids the abundance of polysaccharides exceeded that of lignins (ratios of 0.7 and 0.8).

The contribution of pyrolysis products derived from the various lignin-building blocks (Table 5.1) showed (i) large and unexpected differences between graminoids that perform better under

relatively dry and wet conditions, and (ii) considerable content of *p*-hydroxyphenyl pyrolysis products in ericoids.

Table 5.1. Percentages and ratios of groups of quantified pyrolysis products in plant and peat OM. Mean values (printed in bold) are given for graminoids that perform better under dry conditions (*A. curtisii*, *F. rubra*, *C. durieui*, *D. flexuosa*), graminoids that perform better under relatively wet conditions (*M. caerulea*, *E. angustifolium*) and ericoids (*E. mackaiana*, *C. vulgaris*), the peat fractions are mean values of 15 samples for extracts and residues and of 51 samples for the bulk OM. % TIC = percentage of the total quantified pyrolysis products. Pyrolysis products that contribute to each group are given in the Appendix 5, lignin moieties are listed in Table 5.2.

		GRAMINOIDS								ERICOIDS			PEAT		
Name	unit	*A. curtisii* [1,2]	*F. rubra* [1,2]	*C. durieui* [1,2]	*D. flexuosa* [1,3]	**mean (dry)**	*M. caerulea* [1,2,3]	*E. angustifolium* [1]	**mean (wet)**	*E. mackaiana* [1,2,3]	*C. vulgaris* [1,2]	**mean (ericoids)**	**residues**	**bulk**	**extracts**
Polysaccharides	% TIC	40.1	43.0	33.2	32.0	**37.1**	31.0	32.9	**32.0**	41.5	37.1	**39.3**	**73.0**	**61.8**	**31.0**
Lignins	% TIC	47.8	40.0	47.5	47.7	**45.8**	54.8	34.9	**44.8**	29.5	30.4	**29.9**	**9.8**	**17.4**	**33.3**
Phenols	% TIC	4.0	3.3	4.4	3.8	**3.9**	**5.0**	4.4	**4.7**	6.9	6.1	**6.5**	**1.9**	**5.4**	**14.9**
Catechols	% TIC	2.7	2.1	2.7	1.7	**2.3**	3.3	2.5	**2.9**	8.9	17.0	**13.0**	**0.9**	**1.8**	**4.8**
Aromatics	% TIC	1.8	2.8	2.4	2.4	**2.3**	2.2	2.7	**2.4**	4.2	3.5	**3.9**	**1.0**	**1.8**	**4.1**
Other	% TIC	3.6	8.8	9.9	12.4	**8.7**	3.8	22.6	**13.2**	9.1	5.9	**7.5**	**13.4**	**11.7**	**11.9**
p-Hydroxyphenyl (H)	% lignin	17.8	18.4	16.4	9.6	**15.6**	36.1	33.2	**34.6**	27.5	14.1	**20.8**	**15.6**	**21.9**	**19.8**
Guaiacyl (G)	% lignin	71.3	71.8	71.9	80.2	**73.8**	51.7	52.5	**52.1**	57.4	55.0	**56.2**	**66.9**	**57.1**	**56.2**
Syringyl (S)	% lignin	10.9	9.8	11.7	10.2	**10.6**	12.2	14.3	**13.3**	15.1	30.9	**23.0**	**17.5**	**21.0**	**24.0**
Lignins/Polysaccharides	-	1.19	0.93	1.43	1.49	**1.26**	1.76	1.06	**1.41**	0.71	0.82	**0.76**	**0.13**	**0.28**	**1.07**
H/G+S	-	0.22	0.23	0.20	0.11	**0.19**	0.56	0.50	**0.53**	0.38	0.16	**0.27**	**0.18**	**0.28**	**0.25**
S/G	-	0.15	0.14	016	0.13	**0.14**	0.24	0.26	**0.25**	0.26	0.56	**0.41**	**0.26**	**0.37**	**0.43**

[1]leaves/stem
[2]roots,
[3]flowers.

5.3.2. Comparison of lignin-derived pyrolysis products in plant and peat OM

Factor analysis was applied to quantified lignin-derived pyrolysis products in plant species and peat fractions (mean values of extracts, residues and bulk samples). To this end, quantities of lignin pyrolysis products were expressed as a percentage of the summed lignin pyrolysis products (not of TIC). Loadings and scores of factors 1 and 2 (F1, F2) and factors 3 and 4 (F3, F4) are shown in Fig. 5.1. Loadings for the first four factors explain the major part of the variance (85.9%).

The clearly separated clusters of scores on the F1-F2 projection (Fig. 5.1a) show that graminoids, ericoids and peat fractions differ in composition of lignin-derived pyrolysis products. The projection of the peat fractions, with the residues closest to the plant species, confirms that the residues reflect more intact plant material, while the extracts reflect decomposed material. The factor loadings (Fig. 5.1b) indicate which lignin pyrolysis products are associated with graminoids, ericoids and peat OM. The variance of 4-vinylguaiacol (Lg12) is mainly determined by graminoids. Ericoids are characterised by a high abundance of C_3-guaiacols (Lg13, Lg14, Lg16, Lg17), C_3-syringols (Lg31, Lg33), 4-

formylsyringol (Lg34) and 4-(propan-3-ol) syringol (Lg43). While peat OM is associated with a relatively high abundance of lignin pyrolysis products with oxygenated alkyl side-chains (Fig. 5.1a and b). These are 4-formylphenol (Lg5), 4-hydroxybenzoic, vanillic and syringic acid methyl ester (Lg7, Lg21, Lg40), 4-hydroxybenzoic, vanillic and syringic acid (Lg8, Lg24, Lg42), 4-acetylphenol, 4-acetylguaiacol and 4-acetylsyringol (Lg6, Lg18, Lg38), lignin pyrolysis products with an oxygenated C_3 alkyl side-chain (Lg23, Lg39, Lg41), and syringyl pyrolysis products with a C_3H_3 side-chain (Lg35, Lg36).

In the F3–F4 projection (Fig. 5.1c), the graminoids are separated into two groups: those that perform better under relatively dry conditions have positive scores on F3, while those that prefer wet conditions have negative scores. The ericoids have low scores on F3. The factor loadings (Fig. 5.1d) show that F3 separates guaiacyl (positive loadings) from syringyl and *p*-hydroxyphenyl lignin pyrolysis products (negative loadings).

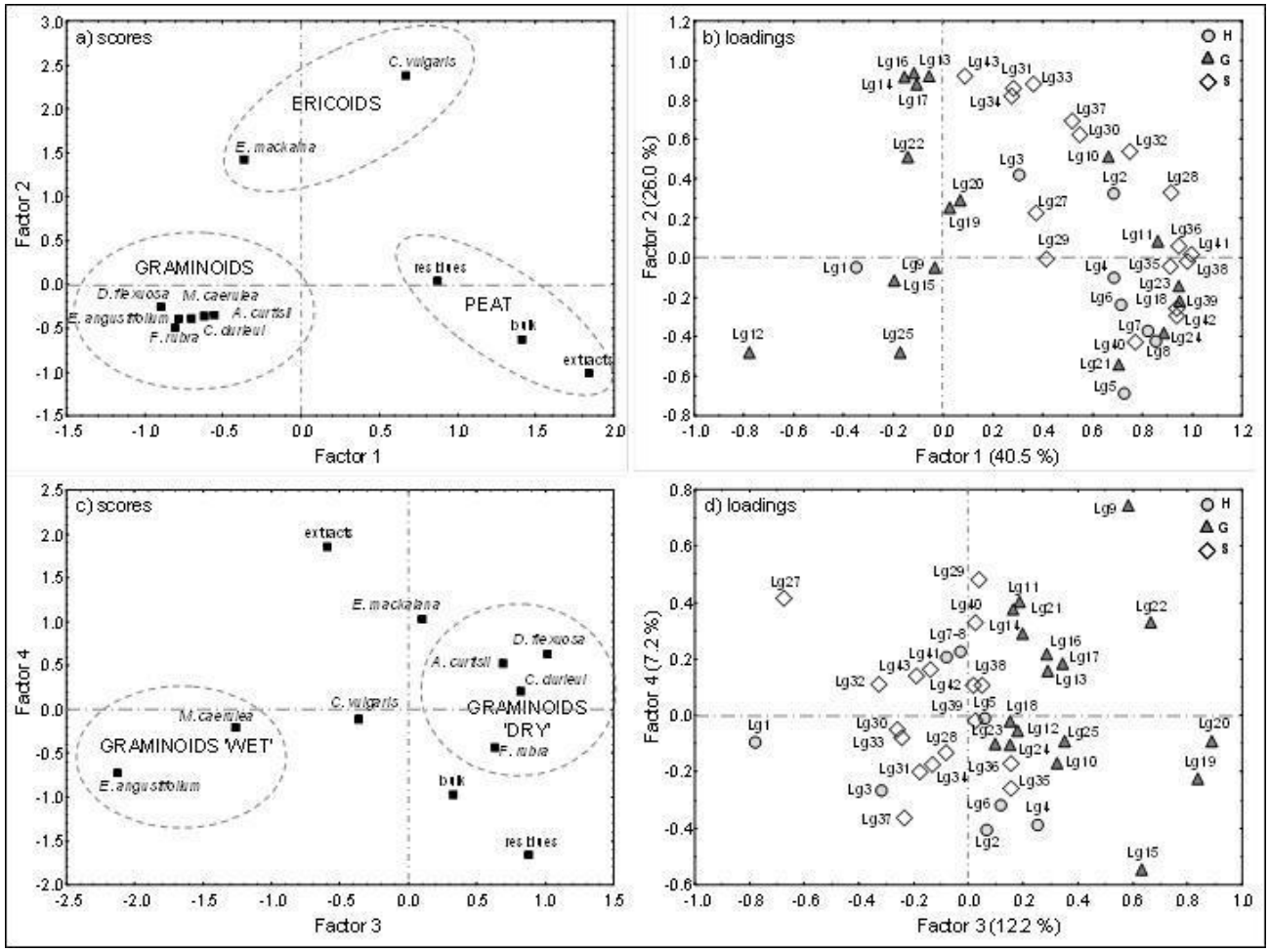

Fig. 5.1. F1–F2 projection of factor scores (a) and loadings (b), and F3–F4 projection of scores (c) and loadings (d) for lignin pyrolysis products of plant species and mean values of the peat fractions. H = *p*-hydroxyphenyl pyrolysis products; G = guaiacyl pyrolysis products; S = syringyl pyrolysis products. For codes see Table 5.2.

Table 5.2. Lignin pyrolysis products expressed as percentage of the total quantified pyrolysis products (see Appendix 5) in plant and peat OM. Mean values (printed in bold) are given for graminoids that perform better under dry conditions (*A. curtisii*, *F. rubra*, *C. durieui*, *D. flexuosa*), graminoids that perform better under relatively wet conditions (*M. caerulea*, *E. angustifolium*) and ericoids (*E. mackaiana*, *C. vulgaris*), the peat fractions are mean values of 15 samples for extracts and residues and of 51 samples for the bulk OM.

		GRAMINOIDS							ERICOIDS			PEAT		
code	Name	*A. curtisii*	*F. rubra*	*C. durieui*	*D. flexuosa*	**mean (dry)**	*M. caerulea*	*E. angustifolium*	**mean (wet)**	*E. mackaiana*	*C. vulgaris*	**mean (wood)**	**residues**	**bulk**	**extracts**
Lg1	4-vinylphenol	8.2	7.0	7.3	4.4	**6.7**	19.3	11.4	**15.3**	7.9	4.0	**6.0**	**1.2**	**3.1**	**5.1**
Lg2	4-(2-propenyl)phenol	0.0	0.0	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.1	**0.0**	**0.0**	**0.0**	**0.0**
Lg3	4-(1-propenyl)phenol, *cis*	0.1	0.1	0.0	0.0	**0.0**	0.1	0.1	**0.1**	0.1	0.1	**0.1**	**0.0**	**0.0**	**0.0**
Lg4	4-(1-propenyl)phenol, *trans*	0.1	0.1	0.2	0.1	**0.1**	0.2	0.1	**0.1**	0.1	0.1	**0.1**	**0.1**	**0.1**	**0.2**
Lg5	4-formylphenol	0.1	0.1	0.2	0.1	**0.1**	0.2	0.1	**0.1**	0.0	0.0	**0.0**	**0.1**	**0.1**	**0.3**
Lg6	4-acetylphenol	0.0	0.0	0.1	0.0	**0.0**	0.1	0.0	**0.0**	0.0	0.0	**0.0**	**0.0**	**0.2**	**0.1**
Lg7	4-hydroxybenzoic acid methyl ester	0.0	0.0	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	**0.0**	**0.0**	**0.1**
Lg8	4-hydroxybenzoic acid	0.0	0.0	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	**0.0**	**0.3**	**0.7**
Lg9	guaiacol	4.5	3.8	5.1	6.1	**4.9**	3.9	1.9	**2.9**	3.3	2.9	**3.1**	**0.8**	**1.2**	**3.9**
Lg10	4-methylguaiacol	2.2	1.2	1.4	1.2	**1.5**	1.1	0.6	**0.8**	2.3	2.3	**2.3**	**0.9**	**1.2**	**1.7**
Lg11	4-ethylguaiacol	1.2	0.7	0.8	0.9	**0.9**	0.9	0.4	**0.7**	0.7	0.8	**0.7**	**0.2**	**0.5**	**1.2**
Lg12	4-vinylguaiacol	18.4	17.5	20.4	23.3	**19.9**	17.6	13.5	**15.6**	4.7	4.7	**4.7**	**2.3**	**3.5**	**5.7**
Lg13	4-(prop-2-enyl)guaiacol	0.4	0.3	0.3	0.4	**0.3**	0.3	0.1	**0.2**	0.5	0.5	**0.5**	**0.1**	**0.1**	**0.1**
Lg14	4-propylguaiacol	0.2	0.1	0.1	0.2	**0.1**	0.1	0.0	**0.1**	0.3	0.2	**0.2**	**0.0**	**0.0**	**0.0**
Lg15	4-formylguaiacol	2.5	1.9	1.7	2.1	**2.0**	1.5	1.2	**1.4**	0.8	1.1	**1.0**	**0.5**	**0.7**	**0.8**
Lg16	4-(prop-1-enyl)guaiacol, *cis*	0.3	0.2	0.3	0.3	**0.3**	0.2	0.0	**0.1**	0.4	0.4	**0.4**	**0.1**	**0.0**	**0.1**
Lg17	4-(prop-1-enyl)guaiacol, *trans*	1.7	1.1	1.4	1.7	**1.5**	1.1	0.3	**0.7**	2.1	2.1	**2.1**	**0.3**	**0.3**	**0.3**
Lg18	4-acetylguaiacol	0.7	0.4	0.5	0.6	**0.6**	0.3	0.1	**0.2**	0.4	0.6	**0.5**	**0.5**	**0.9**	**1.9**
Lg19	guaiacol derivative	0.2	0.2	0.3	0.2	**0.2**	0.2	0.0	**0.1**	0.1	0.2	**0.1**	**0.1**	**0.1**	**0.1**
Lg20	guaiacol derivative	0.1	0.1	0.2	0.2	**0.2**	0.1	0.0	**0.1**	0.1	0.1	**0.1**	**0.0**	**0.1**	**0.1**
Lg21	Vanillic acid methyl ester	0.1	0.1	0.1	0.0	**0.1**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	**0.0**	**0.1**	**0.2**
Lg22	4-(propan-2-one)guaiacol	0.8	0.7	0.9	1.0	**0.9**	0.6	0.1	**0.4**	0.9	0.5	**0.7**	**0.2**	**0.2**	**0.4**
Lg23	4-(propan-1-one)guaiacol	0.0	0.1	0.1	0.0	**0.0**	0.0	0.0	**0.0**	0.2	0.1	**0.2**	**0.1**	**0.3**	**0.5**
Lg24	Vanillic acid	0.5	0.3	0.3	0.0	**0.3**	0.2	0.0	**0.1**	0.1	0.1	**0.1**	**0.4**	**0.6**	**1.4**
Lg25	ferulic acid methyl ester	0.2	0.1	0.1	0.0	**0.1**	0.1	0.0	**0.1**	0.0	0.0	**0.0**	**0.0**	**0.0**	**0.0**
Lg27	syringol	1.0	0.8	1.2	1.0	**1.0**	1.8	1.2	**1.5**	0.8	1.3	**1.0**	**0.2**	**0.4**	**1.5**
Lg28	4-methylsyringol	0.4	0.3	0.3	0.4	**0.3**	0.4	0.4	**0.4**	0.4	0.9	**0.6**	**0.2**	**0.5**	**0.9**
Lg29	4-ethylsyringol	0.8	0.1	0.1	0.1	**0.3**	0.1	0.1	**0.1**	0.1	0.3	**0.2**	**0.0**	**0.1**	**0.5**
Lg30	4-vinylsyringol	0.9	0.9	1.6	1.4	**1.2**	1.5	1.3	**1.4**	1.0	2.1	**1.5**	**0.3**	**0.7**	**1.4**
Lg31	4-(prop-2-enyl)syringol	0.1	0.1	0.2	0.2	**0.1**	0.2	0.2	**0.2**	0.2	0.4	**0.3**	**0.1**	**0.1**	**0.1**
Lg32	4-propylsyringol	0.0	0.0	0.0	0.0	**0.0**	0.1	0.0	**0.0**	0.0	0.1	**0.1**	**0.0**	**0.0**	**0.1**
Lg33	4-(prop-1-enyl)syringol, *cis*	0.1	0.1	0.1	0.1	**0.1**	0.2	0.2	**0.2**	0.2	0.3	**0.3**	**0.0**	**0.1**	**0.1**
Lg34	4-formylsyringol	0.4	0.3	0.3	0.2	**0.3**	0.4	0.3	**0.4**	0.3	1.0	**0.7**	**0.1**	**0.2**	**0.2**
Lg35	syringol derivative	0.1	0.1	0.1	0.1	**0.1**	0.1	0.1	**0.1**	0.0	0.1	**0.1**	**0.0**	**0.1**	**0.1**
Lg36	syringol derivative	0.0	0.0	0.1	0.1	**0.0**	0.0	0.0	**0.0**	0.0	0.1	**0.1**	**0.0**	**0.1**	**0.1**
Lg37	4-(prop-1-enyl)syringol, *trans*	0.6	0.6	0.7	0.5	**0.6**	1.0	0.8	**0.9**	0.6	1.4	**1.0**	**0.3**	**0.4**	**0.7**
Lg38	4-acetylsyringol	0.3	0.3	0.3	0.4	**0.3**	0.4	0.1	**0.3**	0.2	0.6	**0.4**	**0.1**	**0.5**	**1.0**
Lg39	4-(propan-2-one)syringol	0.3	0.3	0.4	0.4	**0.3**	0.4	0.2	**0.3**	0.1	0.3	**0.2**	**0.1**	**0.3**	**0.7**
Lg40	syringic acid methyl ester	0.0	0.0	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	**0.0**	**0.0**	**0.1**
Lg41	4-(propan-3-one)syringol	0.1	0.1	0.1	0.0	**0.0**	0.1	0.1	**0.1**	0.1	0.2	**0.1**	**0.1**	**0.1**	**0.4**
Lg42	syringic acid	0.1	0.0	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.1	**0.0**	**0.0**	**0.1**	**0.2**
Lg43	4-(propan-3-ol)syringol	0.0	0.0	0.0	0.0	**0.0**	0.1	0.0	**0.1**	0.2	0.3	**0.3**	**0.0**	**0.0**	**0.0**

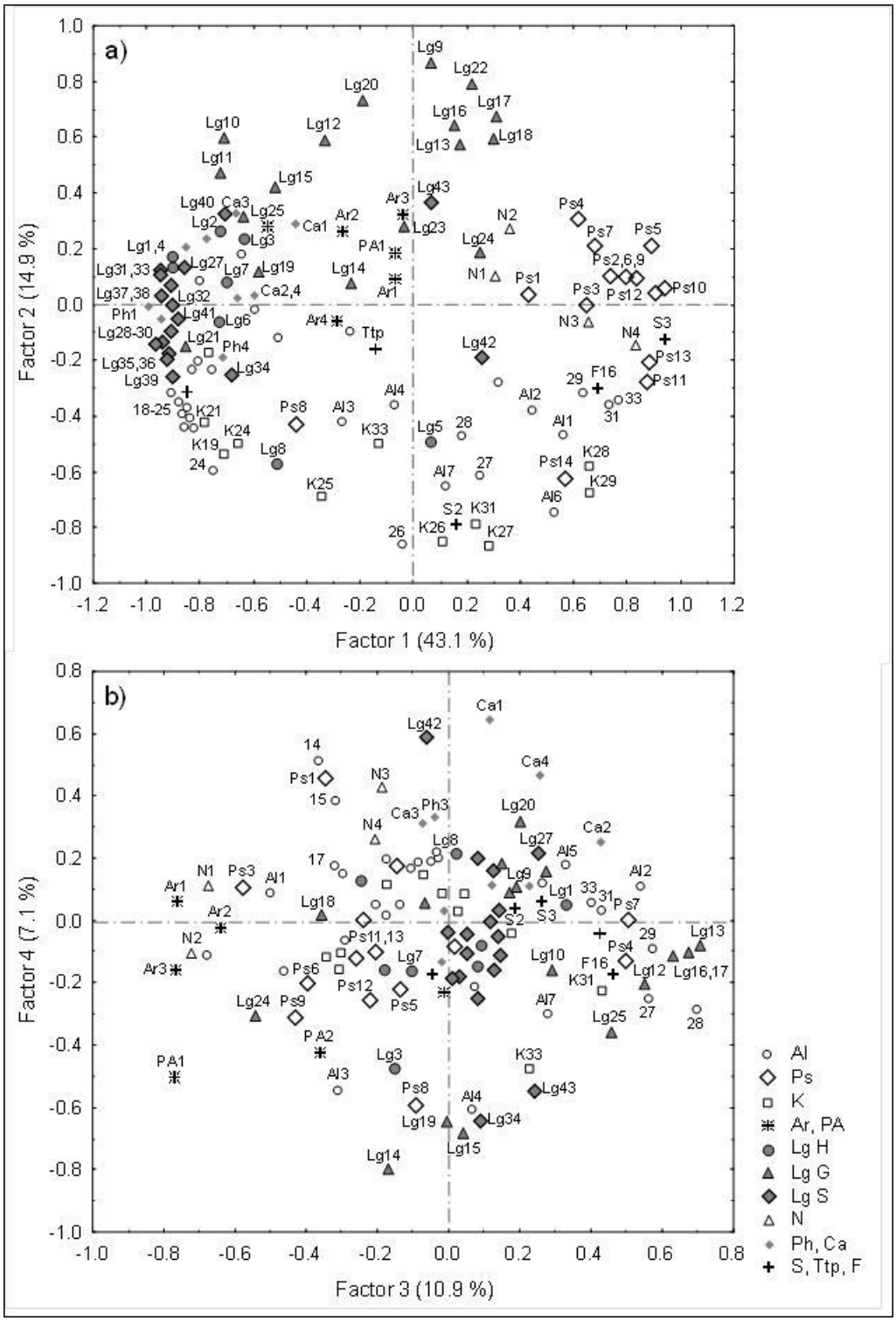

Fig. 5.2. Factor loadings of extracts (a) F1–F2 and (b) F3–F4. Al = aliphatics; Ps = polysaccharides; K = methylketones; Ar = aromatics; PA = polyaromatics; Lg H = *p*-hydroxyphenyl lignin; Lg G = guaiacyl lignin; Lg S = syringyl lignin; N = nitrogen compounds; Ph = phenols; Ca = catechols; S = sterols; Ttp = triterpenoids; F = fatty acids. For codes see Appendix 5.

5.3.3. Differences in molecular composition between peat fractions

To further identify the effects of botanical source and decomposition on the lignin composition in peat OM, pyrolysates of the different peat fractions are compared. Factor analysis was applied to all 120 quantified pyrolysis products for extracts and residues (Appendix 5). Factor loadings for the first four factors explain the major part of the variance for extracts (76.0%, Fig. 5.2) and residues (78.6%, Fig. 5.3).

Loadings in F1–F2 projection indicate major differences in the distribution of lignin pyrolysis products between extracts (Fig. 5.2a) and residues (Fig. 5.3a). In the extracts, a clear separation appears between guaiacyl and syringyl pyrolysis products, while in the residues, guaiacyl and syringyl pyrolysis products are not separated. This differential pattern suggests that decomposition (reflected by the extracts) rather than vegetation composition (reflected by the residues) affects the guaiacyl-to-syringyl ratio in the peat OM.

5.4. Discussion

5.4.1. Structure and composition of lignin-carbohydrate complex

There is a notable difference in the composition of the LCC between the two dominant lignin sources in the Penido Vello peat record, namely ericoids (woody species) and graminoids (herbaceous species). In addition to lignin, graminoids possess a high content of free and bound phenolics (Lewis and Yamamoto, 1990). *p*-Coumaric and ferulic acids are ester- and/or ether-linked to cell wall polymers. Ferulic acid is ether-linked to lignin and ester-linked to hemicellulose and may form bridges between them, while *p*-coumaric acid is mainly ester-linked to both lignin and hemicellulose (Kondo et al., 1989; Sun et al., 2011). In addition, many xylans of grasses contain dimers of ester-linked *p*-coumaric and ferulic acid that cross-link cell wall polysaccharides (Ishii, 1997; Lam et al., 1992). The structure of the LCC of graminoids is shown in Fig. 5.4. In wood of dicotyledonous species (but not necessarily in leaves), lignin is more directly associated with polysaccharides through the formation of benzyl-ether and other alkali-stable bonds (Watanabe et al., 1989).

The LCC can be recognised upon pyrolysis. The main pyrolysis product of cellulose is levoglucosan (Pouwels et al., 1989). Xylose, which has a high abundance in hemicelluloses of grasses (Smith and Harris, 1999; Wende and Fry, 1996) and sedges (Bourdon et al., 2000), results in 4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one upon pyrolysis (Pouwels et al., 1987). Typical pyrolysis products of *p*-coumaric and ferulic acid are 4-vinylphenol and 4-vinylguaiacol, respectively (Boon et al., 1982; van der Hage et al., 1993). The *β*-O-4 bonds, which form the primary link between lignin monomers, are broken upon pyrolysis and, in unaltered lignin, result in lignin moieties with a C_3 alkyl side-chain (van der Hage et al., 1993).

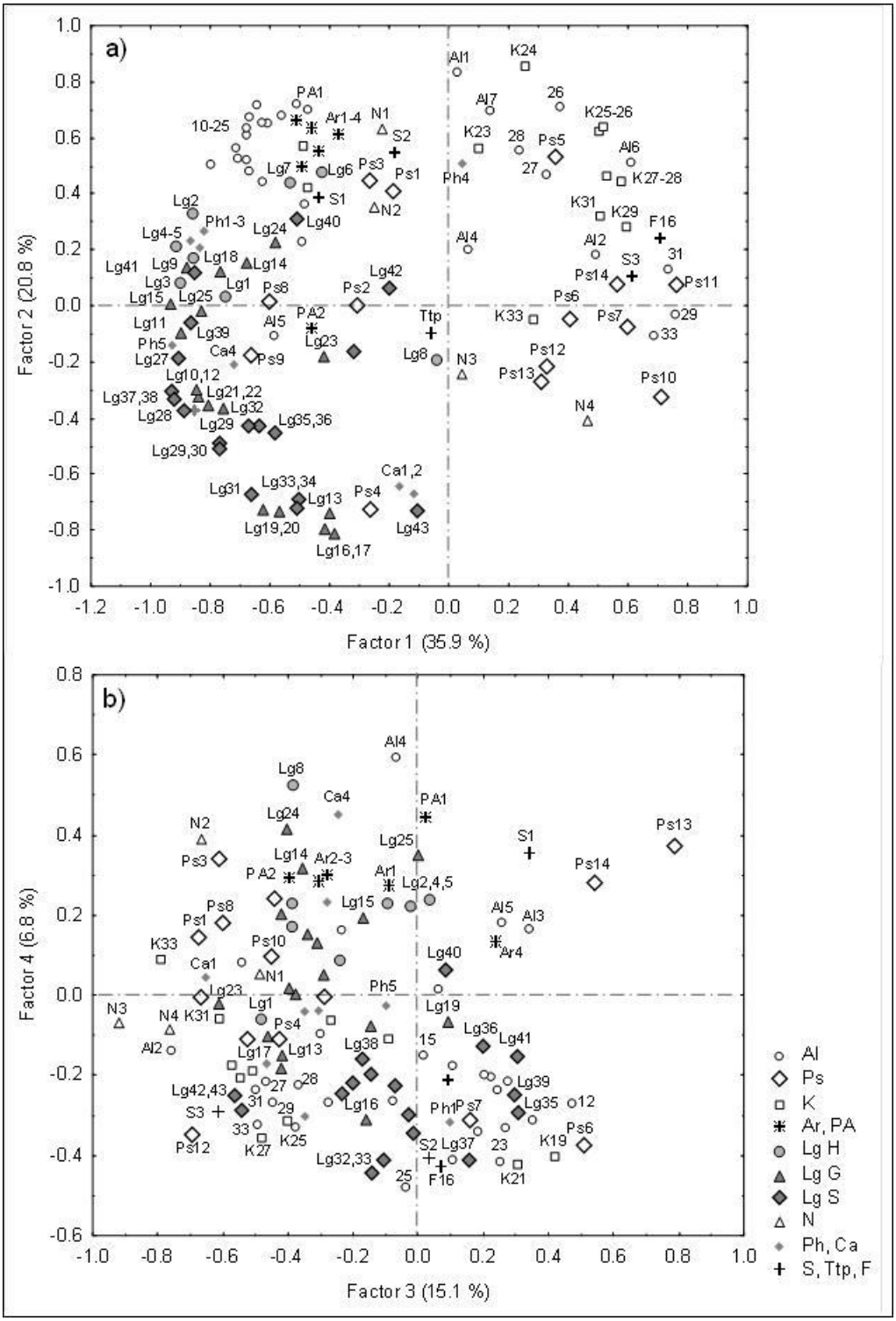

Fig. 5.3. Factor loadings of residues (a) F1–F2 and (b) F3–F4. Al = aliphatics; Ps = polysaccharides; K = methylketones; Ar = aromatics; PA = polyaromatics; Lg H = *p*-hydroxyphenyl lignin; Lg G = guaiacyl lignin; Lg S = syringyl lignin; N = nitrogen compounds; Ph = phenols; Ca = catechols; S = sterols; Ttp = triterpenoids; F = fatty acids. For codes see Appendix 5.

5.4.2. The lignin composition of dominant plant species

The relatively high content of lignin-derived pyrolysis products in graminoids (Table 5.1) is not conform expectations, as graminoids have lower lignin content than woody species such as ericoids. This discrepancy is caused by the high abundance of non-lignin phenolics (*p*-coumaric and ferulic acid) in graminoids, which can contribute to more than 50% to guaiacyl and *p*-hydroxyphenyl moieties (Martínez et al., 2001; Lu and Ralph, 2010), and pyrolysis-GC/MS does not allow differentiation between moieties derived from macromolecular lignin and non-lignin phenolics.

The differences in guaiacyl content between graminoids that perform better under relatively dry or wet conditions are mainly caused by the contribution of 4-vinylguaiacol and 4-vinylphenol (Table 5.2). This does not reflect the contribution of guaiacyl and *p*-hydroxyphenyl moieties to the lignin macromolecule but the abundance of ferulic and *p*-coumaric acid. The difference between the two graminoid groups will be further discussed in Section 5.4.3.

Notwithstanding the woody character of ericoids, *C. vulgaris* and *E. mackaiana* show a relatively high abundance of *p*-hydroxyphenyl pyrolysis products (Table 5.1), dominated by 4-vinylphenol (Table 5.2). 4-Vinylphenol was also found a dominant pyrolysis product in *C. vulgaris* shoots by Huang et al. (1998) and only in non-woody tissue of *C. vulgaris* by Nierop et al. (2001). Other phenolic plant biomacromolecules such as tannin and suberin may contribute to *p*-hydroxyphenyl moieties in ericoids. Tannin is abundant in ericoids (e.g. Monschein et al., 2010). Suberin consists of aliphatic and phenolic components including *p*-coumaric and ferulic acid (Hatfield, 2009). However, these macromolecules generally produce other phenolic compounds (e.g. catechol and phenol, Table 5.1) in much higher amounts than those included in the *p*-hydroxyphenyl group (Table 5.2). Knowledge of lignin of woody plants is generally restricted to woody tissue (e.g. Donaldson, 2001), while research on lignin in non-woody tissue has been concentrated on graminoids (Buranov and Mazza, 2008). In addition to monocotyledons, non-woody tissue from woody species may also contain *p*-coumaric and ferulic acids (Sarkanen and Ludwig, 1971; Hedges and Mann, 1979). In soil OM, lignin from woody species originates from a mixture of tissues (wood, leaves, roots, etc.) that have different lignin compositions, and thus knowledge of the paper industry – which deals with wood exclusively – cannot directly be translated to soil OM studies. Thus, the high values of *p*-hydroxyphenyl pyrolysis products in ericoids (Table 5.1) originate from their leaves, and ratios between *p*-hydroxyphenyl (or *p*-coumaric acid) and other lignin moieties in (pyrolysates of) peat cannot be used as source indicator, not even when it is assumed that decomposition does not affect such ratios. The lignin classification softwood-hardwood-grasses is thus inadequate (Boerjan et al., 2003; Lu and Ralph, 2010), which is supported by the results presented above.

5.4.3. Influence of source on lignin moieties in peat OM

The major differences in the composition of lignin pyrolysis products between plant families and peat OM are explained by F1–F2 (Fig. 5.1a and b). Most lignin pyrolysis products that were relatively enriched upon degradation showed oxygenation of alkyl side-chains while lignin moieties with a C_3 alkyl side-chain are abundant in ericoids (Section 5.3.2). This is conform the known differences

between fresh plant material and peat OM, as degradation increases oxygenation of lignin alkyl side-chains (Kögel-Knabner, 2002). All graminoids were characterised by a high abundance of ferulic acid (reflected by 4-vinylguaiacol Fig. 5.1a and b; Table 5.2). The fact that both 4-vinylphenol (Lg1) and 4-vinylguaiacol (Lg12) are clearly separated from the peat samples by F1 indicates that *p*-coumaric and ferulic acid are rapidly degraded after the plant dies (van Bergen et al., 1997; Karunen and Kalviainen, 1988; Kuder and Kruge, 1998; Nierop, 2001).

The high negative loadings of 4-vinylphenol (Lg1) on F3 shows that *p*-coumaric acid has a much higher abundance in the graminoids that perform better under wet conditions (Fig. 5.1c and d; Table 5.2). This may be related to the high syringyl content of these species (Fig. 5.1), as *p*-coumaric acid is mainly acetylated on the terminal carbon of syringyl alkyl side-chains (Shimada et al., 1971; Ralph et al., 1994; Grabber et al., 1996). The separation on F3 between graminoids that perform better under relatively dry or wet conditions by (Fig. 5.1d), was also found when a cluster analysis was applied to all quantified pyrolysis products of plant species (not limited to lignin pyrolysis products alone) by Schellekens et al. (2011), and may suggest environmental control.

In Fig. 5.1c and d, F4 mainly affects the peat fractions and separates residues (negative scores) from extracts (positive scores). F4 is strongly determined by the abundance of guaiacol (Lg9, positive loadings) and 4-formylguaiacol (Lg15, negative loadings). Together with their negative loadings on F1 (Fig. 5.1b) this indicates that guaiacol and 4-formylguaiacol, in addition to a lignin origin, partly originate from non-lignin phenolic monomers that are abundant in the LCC of grasses (Kuder et al., 1998). This suggests an origin from free phenolic acids for guaiacol, as these are easily extractable, whereas the phenolic compound from which 4-formylguaiacol originates is probably bound to cell wall polymers of the graminoids that perform better under relatively dry conditions. F4 also explains a large part of the variance of lignin pyrolysis products with a C_3 alkyl side-chain (Lg2–Lg4, Lg37), which is in agreement with their origin from the unaltered lignin macromolecule that is relatively abundant in the residues.

5.4.4. Transformations of lignin in peat OM

5.4.4.1. Extracts

In Fig. 5.2a, all polysaccharides except 5-hydroxymethyl-2-furancarboxaldehyde (Ps8) have positive loadings on F1, with the highest loadings for the levosugars (Ps10, Ps11, Ps13). Pyrolysis products with high negative loadings on F1 include most syringyl and *p*-hydroxyphenyl moieties, phenols, and mid-chain *n*-alkanes ($C_{18–25}$). In peat, polysaccharides are preferentially decayed (Benner et al., 1984; van der Heijden et al., 1990; Schellekens et al., 2011), F1 therefore reflects aerobic decay (bog hydrology), with highest positive scores for unaltered material. An increase in aerobic decay (more negative on F1) causes chain length reduction for *n*-alkanes (Eglinton and Hamilton, 1967; Schellekens and Buurman, 2011) and shortening of alkyl side-chains from lignin moieties (Thevenot et al., 2010). The location of guaiacol (Lg9) is not in agreement with the interpretation of guaiacyl alkyl chain length reduction, and its position confirms its partial origin from free phenolic acids (see Section 5.4.3) as these are rapidly degraded (Kuder et al., 1998). The fact that mainly guaiacyl pyrolysis products were affected is in

agreement with the findings of Kuder and Kruge (1998) for a southern hemisphere peat dominated by monocotyledons (restiads).

All guaiacyl pyrolysis products (except Lg21) have positive loadings on F2, while pyrolysis products with strong negative loadings on F2 are mainly methylketones and other aliphatics. The high negative loadings of aliphatics on F2 may indicate residual accumulation of these compounds upon diagenesis (long-term anaerobic decay), as an increase of aliphatics with depth was found with ^{13}C CPMAS NMR for the same peat record (Pontevedra-Pombal, 2002). This suggests that negative scores on F2 indicate (long-term) anaerobic decay.

Thevenot et al. (2010) reported preferential degradation of syringyl over guaiacyl lignin moieties, except for the first stage of decay. This first stage of decay is likely reflected in the extracts where the loadings indicate that guaiacyl moieties are preferentially degraded under both aerobic (F1) and anaerobic conditions (F2). An explanation would be the preferential decay of non-lignin phenolics in graminoids (dominated by guaiacyl and phenolic monomers). Kuder et al. (1998) also found guaiacyl more transformed during decay than syringyl lignin in peat, which supports this hypothesis. Pyrolysis products with positive values on F3 (Fig. 5.2b) are associated with relatively unaltered OM, C_3-guaiacols (Kuder and Kruge, 1998) and long chain *n*-alkanes (Eglinton and Hamilton, 1967).

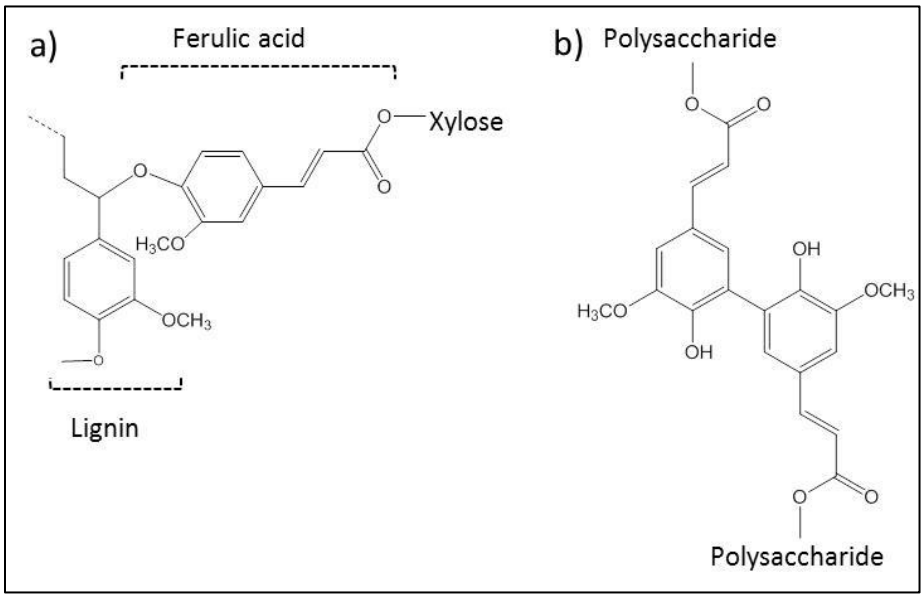

Fig. 5.4. (a) Ferulic acid cross-link between lignin and hemicellulose in graminoids, after Buranov and Mazza (2008), (b) Diferulate cross-link between polysaccharides after Ishii (1997).

Pyrolysis products with strong negative loadings on F3 -aromatics, naphthalene (PA1), pyridine (N1) and benzonitrile (N2)- are associated with strong aerobic decay or burning (Schellekens et al., 2009 and references therein). Therefore F3 may reflect aerobic decomposition, with negative loadings indicating more decomposed material. Of the lignin pyrolysis products, vanillic acid (Lg24) is located within this group, suggesting that it is a product of aerobic decay, which is in agreement with other lignin degradation studies (e.g. van Bergen et al., 1997). Pyrolysis products associated with the lignin-ferulic acid-xylose part of the LCC in graminoid material (the xylose marker (Ps4), markers of *p*-coumaric and ferulic acid (Lg1 and Lg12), and markers of intact lignin (Lg13, Lg16, Lg17)) show high positive values on F3, while the marker of cellulose (Ps13) is not affected by aerobic decay in the extracts. Markers of graminoids (Lg25), ericoids (S3) and wood (Al2) also show positive loadings on F3. Thus F1 reflects aerobic decay of easily degradable non-lignified polysaccharides and guaiacyl moieties (probably from non-lignin phenolics of graminoids); whereas F3 reflects aerobic decay of ericoid material and of a lignin-ferulic acid-xylose part of the LCC of graminoid material. This separation of ferulates in an easily degradable and a more resistant part is in agreement with Ralph et al. (1995) who found that part of the wall-bound ferulates in ryegrass could not be released by any chemical solubilisation method.

In Fig. 5.2b, some lignin pyrolysis products have high negative loadings on F4. These are 4-propylguaiacol (Lg14), 4-formylguaiacol (Lg15) and 4-formylsyringol (Lg34), 4-(propan-3-ol)syringol (Lg43) and guaiacol derivative (Lg19). Other pyrolysis products with high negative loadings on F4 are the alcohols and 5-hydroxymethyl-2-furancarboxaldehyde (Ps8). Syringic acid (Lg42) shows positive loadings on F4 together with catechols (Ca1, Ca4) and acetic acid (Ps1). The loadings of these compounds do not allow a straightforward interpretation of F4.

5.4.4.2. Residues

Contrary to the extractable fraction, the residues contain relatively large fragments and therefore more reflect plant characteristics. Pyrolysis products with positive loadings on F1 include compounds indicative of mosses (Ps5, Ps12, Ph4) and intact cellulose (Ps10, Ps11, Ps13) as well as markers of ericoids (S3) and wood (Al2, positive loadings; Fig. 5.3a). The corresponding samples originate from the Little Ice Age (Schellekens et al., 2011). The relatively high contribution of both mosses and ericoids is caused by (i) the prevailing wet conditions during this period followed by recent drainage during road and wind farm construction (Fraga et al., 2008) that may have caused roots of ericoid plants to enter to greater depths (Schellekens et al., 2011), and/or (ii) a better preservation of ericoid plant remains during wet periods (Buurman et al., 2006).

The distribution of pyrolysis products of residues on F2 resembles that in the extracts, and F2 in the residues is therefore also interpreted as (long-term) anaerobic decay (increasing towards positive loadings). Pyrolysis products with high positive loadings on F1 show little variation on F2, which suggests that remains of mosses and ericoids are less altered under anaerobic conditions. This is in agreement with an excellent preservation of polysaccharides of wood of ericoids under anaerobic conditions in peat (van der Heijden and Boon, 1994). Contrarily, anaerobic decay in the residues affects

lignin (negative on F1). Most pyrolysis products with high negative loadings on F2 are indicative of intact lignin (C_3-guaiacols (Lg13, Lg16, Lg17) and C_3-syringols (Lg31, Lg33), 4-(propan-3-ol)syringol (Lg43) and 4-formylsyringol (Lg34)). Furthermore, the xylose marker 4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one (Ps4) clusters with the C_3-guaiacols and may thus indicate intact LCC from graminoids (Fig. 5.4). The markers of ferulic (4-vinylguaiacol, Lg12) and *p*-coumaric acid (4-vinylphenol, Lg1) are not within this cluster but predominantly determined by F1. This confirms that a major part of ferulic and *p*-coumaric acid were degraded at first (Sections 5.4.3 and 5.4.4.1) and thus removed from the LCC in the residues, and indicates that in the residues degraded macromolecular lignin contributes more to the abundance of 4-vinylguaiacol and 4-vinylphenol. It further supports the interpretation of F1 as reflecting different botanical contributions. *p*-Hydroxyphenyl pyrolysis products cluster together, with positive loadings on F2, which points towards demethoxylation under anaerobic conditions. This is in agreement with other decomposition studies in peat (Kuder and Kruge, 1998; Zaccone et al., 2008).

In the F3–F4 projection (Fig. 5.3b), the residues show low loadings for most lignin pyrolysis products. However, a clear separation of syringyl and guaiacyl moieties appears. Levoglucosan (Ps13) shows high positive loading on F3; while pyrolysis products indicative of aerobic decay such as vanillic acid (Lg24) and pyridine (N1) have negative loadings on F3. Therefore, negative scores on F3 may reflect aerobic decay of remaining material. The preferential decay of syringyl (more positive on F3) over guaiacyl pyrolysis products (more negative on F3) then also corresponds to the general knowledge on lignin decay (Thevenot et al., 2010).

5.4.4.3. The influence of extraction on the molecular composition of peat OM

In order to correctly interpret the effects of decomposition on the LCC it is important to understand the side-effects of extraction.

5.4.4.3.1. Lignin and cellulose. Unaltered lignin and cellulose polymers are not extractable with the mild alkaline extractant used here. However, a major difference in the abundance of lignin and polysaccharide pyrolysis products is found between the peat fractions: residues have very high values for polysaccharides, while extracts have higher lignin, phenol and aromatic contents. Except for the topmost sample, the relatively high abundance of lignin pyrolysis products in the extracted fraction was not found for the C_3-guaiacols (Lg13, Lg16, Lg17). The high polysaccharide content in the residues is primarily due to levoglucosan, a pyrolysis product of cellulose (between 48 and 60% TIC in residues, compared to 6–20% in extracts). This indicates that the major part of cellulose (that is still present in the peat OM) is intact and remains in the residues, while a relatively large part of lignin is fragmented and rendered extractable.

5.4.4.3.2. Xylose. Most hemicelluloses are soluble in alkaline solutions so that they will be partly extracted by traditional methods (Kögel-Knabner, 2002). The xylose marker Ps4 showed high values in the topmost sample in both extract (7% TIC) and residue (3.5% TIC), while the deeper samples all showed low values and no difference between both peat fractions (1.5% TIC mean). This indicates that a large part of Ps4 is rapidly degraded after the plant dies, and that this rapidly degradable part is affected by extraction.

5.4.4.3.3. Non-lignin phenolic acids. Alkaline extractions can break the ester bonds of phenolic acids, while ether bonds remain intact (Ishii, 1997; Terrón et al., 1993; Hartley and Morrison, 1991; Kondo et al., 1989). Microbial degradation also more easily disrupts ester bonds of ferulic and *p*-coumaric acids than ether bonds (Kondo et al., 1991; Akin et al., 1996). The ratio of 4-vinylguaiacol to 4-vinylphenol is clearly higher in the residues than in the extracts (1.9 versus 1.1), except in the topmost sample where both extract and residue have a high value. This may indicate that ferulic acid (ether-linked to lignin) remains in the residues and *p*-coumaric acid (ester-linked to lignin) is more easily extracted. The high values in both fractions of the topmost sample (not shown) indicate that extraction did not break ester bonds, and that the difference between extracts and residues is caused by decomposition or vegetation characterist

From comparison of abundances of LCC-derived pyrolysis products between (i) residues and extracts and (ii) the topmost sample and deeper samples, it is concluded that the differences in OM composition between the fractions mainly reflect the effect of decomposition.

5.4.4.4. Synthesis

During the first stage of decay non-lignin phenolics from grasses, such as ferulic and *p*-coumaric acids (Section 5.4.3) and non-lignified polysaccharides (Section 5.4.4.1) are rapidly degraded. This stage is generally aerobic. It showed alkyl side-chain reduction for guaiacyl pyrolysis products, while syringyl pyrolysis products were not affected (Section 5.4.4.1). During subsequent anaerobic decomposition lignin moieties showed demethoxylation, oxygenation of alkyl side-chains and alkyl side-chain reduction. Cellulose is barely degraded or transformed under anaerobic conditions (Sections 5.4.4.1 and 5.4.4.2). Persistent aerobic decay further degrades the LCC of which the 'lignin phenolic acid-xylose' part is reflected in the extracts (Section 5.4.4.1) while the cellulose part is reflected in the residues (Section 5.4.4.2) and reflects the originally lignified cellulose. This interpretation is confirmed by the high abundance of lignin pyrolysis products in the extracts and of levoglucosan (cellulose) in the residues (Section 5.4.4.4).

Interpretation of the results obtained from pyrolysates of plant material and the different peat fractions should be tested on the high-resolution sampled peat record (bulk samples); this will be done in a forthcoming study.

Appendix 5. Quantified pyrolysis products. Grouped according to origin and thereafter retention time. *m/z* typical mass; M molecular weight; RT/RTG retention time relative to guaiacol; Ave. % TIC = mean values in the bulk samples relative to the sum of all quantified pyrolysis products (*n*=51); S.D.= standard deviation. Al: aliphatic; Ps: polysaccharide; K: methylketone; Lg: lignin; N: N-compound; Ph: phenol; Ca: catechol; S: sterol; Ttp: triterpene; FA: fatty acid; PA: polyaromatic. *The marker of graminoids, 4-(2-propenoic acid methyl ester)guaiacol (Schellekens et al., 2011) is here named with its synonym ferulic acid methyl ester.

code	name	*m/z*	M	RT/RT G	ave. % TIC	S.D.
10	*n*-C10 alkane	57+71	142	0.858	0.09	0.03
11	*n*-C11 alkane	57+71	156	1.054	0.15	0.04
12	*n*-C12 alkane	57+71	170	1.268	0.16	0.06
13	*n*-C13 alkane	57+71	184	1.419	0.18	0.07
14	*n*-C14 alkane	57+71	198	1.587	0.21	0.08
15	*n*-C15 alkane	57+71	212	1.746	0.18	0.07
16	*n*-C16 alkane	57+71	226	1.896	0.23	0.09
17	*n*-C17 alkane	57+71	240	2.038	0.26	0.11
18	*n*-C18 alkane	57+71	254	2.173	0.26	0.10
19	*n*-C19 alkane	57+71	268	2.301	0.39	0.17
20	*n*-C20 alkane	57+71	282	2.423	0.40	0.16
21	*n*-C21 alkane	57+71	296	2.540	0.58	0.28
22	*n*-C22 alkane	57+71	310	2.653	0.56	0.23
23	*n*-C23 alkane	57+71	324	2.759	0.70	0.36
24	*n*-C24 alkane	57+71	338	2.862	0.36	0.15
25	*n*-C25 alkane	57+71	352	2.961	0.47	0.18
26	*n*-C26 alkane	57+71	366	3.055	0.19	0.07
27	*n*-C27 alkane	57+71	380	3.147	0.21	0.07
28	*n*-C28 alkane	57+71	394	3.235	0.10	0.04
29	*n*-C29 alkane	57+71	408	3.321	0.22	0.14
31	*n*-C31 alkane	57+71	436	3.481	0.51	0.45
33	*n*-C33 alkane	57+71	464	3.640	0.39	0.43
Al1	prist-1-ene	56+57	266	2.082	0.44	0.12
Al2	aliphatic compound	67+81		2.277	0.03	0.02
Al3	*n*-C_{18} alcohol	55+57	270	2.512	0.06	0.02
Al4	*n*-C_{20} alcohol	55+57	298	2.738	0.15	0.04
Al5	branched alkene	83	280	2.788	0.31	0.15
Al6	*n*-C_{22} alcohol	55+57	326	2.946	0.20	0.09
Al7	*n*-C_{24} alcohol	55+57	354	3.138	0.10	0.06
Ps1	acetic acid	60	60	0.264	1.71	0.55
Ps2	2-furaldehyde	95+96	96	0.496	4.06	1.32
Ps3	5-methyl-2-furaldehyde	109+110	110	0.736	1.38	0.44
Ps4	4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one	58+114	114	0.807	1.78	0.48
Ps5	dianhydrorhamnose	113+128	128	0.877	0.24	0.09
Ps6	levoglucosenone	68+98	126	1.016	2.35	1.12
Ps7	1,4:3,6-dianhydro-alpha-D-glucose	57+69	144	1.239	1.08	0.34
Ps8	5-hydroxymethyl-2-furancarboxaldehyde	97+126	126	1.265	0.52	0.24
Ps9	1,4-dideoxy-D-glycero-hex-1-enopyranose-3-ulose	87+144	144	1.431	1.57	0.23
Ps10	levogalactosan	60+73	162	1.608	3.76	1.23
Ps11	levomannosan	60+73	162	1.739	2.95	1.23
Ps12	sugar compound	101+116		1.743	0.79	0.50
Ps13	levoglucosan	60+73	162	1.966	37.22	5.34

Appendix 5. (*continued*).

code	Name	*m/z*	M	RT/RT G	ave. % TIC	S.D.
Ps14	1,6-anhydro-beta-D-glucofuranose	73+85	162	2.062	2.51	0.56
K19	*n*-C_{19} methylketone	58+59	282	2.533	0.17	0.10
K21	*n*-C_{21} methylketone	58+59	310	2.818	0.11	0.05
K23	*n*-C_{23} methylketone	58+59	338	2.963	0.20	0.06
K24	*n*-C_{24} methylketone	58+59	352	3.058	0.09	0.02
K25	*n*-C_{25} methylketone	58+59	366	3.153	0.58	0.18
K26	*n*-C_{26} methylketone	58+59	380	3.241	0.11	0.04
K27	*n*-C_{27} methylketone	58+59	394	3.329	0.54	0.24
K28	*n*-C_{28} methylketone	58+59	408	3.412	0.05	0.03
K29	*n*-C_{29} methylketone	58+59	422	3.493	0.27	0.20
K31	*n*-C_{31} methylketone	58+59	450	3.655	0.11	0.11
K33	*n*-C_{33} methylketone	58+59	478	3.863	0.07	0.12
Ar1	benzene	77+78	78	0.293	0.40	0.09
Ar2	toluene	91+92	92	0.419	1.18	0.28
Ar3	styrene	78+104	104	0.626	0.17	0.04
Ar4	benzoic acid	105+122	122	1.191	0.10	0.04
Lg1	4-vinylphenol	91+120	120	1.256	3.08	0.99
Lg2	4-(prop-2-enyl)phenol	133+134	134	1.316	0.02	0.01
Lg3	4-(prop-1-enyl)phenol, *cis*	133+134	134	1.381	0.02	0.01
Lg4	4-(prop-1-enyl)phenol, *trans*	133+134	134	1.469	0.09	0.02
Lg5	4-formylphenol	121+122	122	1.527	0.15	0.10
Lg6	4-acetylphenol	121+136	136	1.650	0.18	0.08
Lg7	4-hydroxybenzoic acid methyl ester	121+152	152	1.662	0.01	0.01
Lg8	4-hydroxybenzoic acid	121+138	138	1.858	0.29	0.19
Lg9	guaiacol	109+124	124	1.000	1.22	0.34
Lg10	4-methylguaiacol	123+138	138	1.201	1.19	0.32
Lg11	4-ethylguaiacol	137+152	152	1.356	0.46	0.19
Lg12	4-vinylguaiacol	135+150	150	1.418	3.52	0.98
Lg13	4-(prop-1-enyl)guaiacol	77+164	164	1.491	0.07	0.02
Lg14	4-propylguaiacol	137+166	166	1.510	0.04	0.01
Lg15	4-formylguaiacol	151+152	152	1.549	0.67	0.16
Lg16	4-(prop-2-enyl)guaiacol, *cis*	77+164	164	1.576	0.05	0.01
Lg17	4-(prop-2-enyl)guaiacol, *trans*	77+164	164	1.644	0.28	0.09
Lg18	4-acetylguaiacol	151+166	166	1.692	0.95	0.20
Lg19	guaiacol derivative (C_3H_3)	147+162		1.693	0.09	0.02
Lg20	guaiacol derivative (C_3H_3)	147+162		1.702	0.06	0.01
Lg21	vanillic acid methyl ester	151+182	182	1.740	0.06	0.02
Lg22	4-(propan-2-one)guaiacol	137+180	180	1.758	0.20	0.04
Lg23	4-(propan-1-one)guaiacol	151+180	180	1.841	0.29	0.07
Lg24	vanillic acid	153+168	168	1.873	0.64	0.21
Lg25	ferulic acid methyl ester*	177+208	208	2.194	0.02	0.01
Lg27	syringol	139+154	154	1.468	0.39	0.14
Lg28	4-methylsyringol	153+168	168	1.630	0.49	0.20
Lg29	4-ethylsyringol	167+182	182	1.755	0.11	0.05
Lg30	4-vinylsyringol	165+180	180	1.813	0.74	0.30
Lg31	4-(prop-1-enyl)syringol	91+194	194	1.869	0.08	0.03
Lg32	4-propylsyringol	167+196	196	1.882	0.02	0.01
Lg33	4-(prop-2-enyl)syringol, *cis*	91+194	194	1.941	0.07	0.03

Appendix 5. (*continued*).

code	Name	m/z	M	RT/RT G	ave. % TIC	S.D.
Lg34	4-formylsyringol	181+182	182	1.948	0.17	0.07
Lg35	syringyl derivative (C_3H_3)	131+192		1.989	0.12	0.05
Lg36	syringyl derivative (C_3H_3)	131+192		1.999	0.06	0.03
Lg37	4-(prop-2-enyl)syringol, *trans*	91+194	194	2.012	0.43	0.19
Lg38	4-acetylsyringol	181+196	196	2.053	0.48	0.14
Lg39	4-(propan-2-one)syringol	167+210	210	2.099	0.33	0.14
Lg40	syringic acid methyl ester	181+212	212	2.100	0.01	0.00
Lg41	4-(propan-3-one)syringol	181+210	210	2.175	0.13	0.06
Lg42	syringic acid	183+198	198	2.218	0.11	0.06
Lg43	4-(propan-3-ol)syringol	168+212	212	2.249	0.01	0.00
N1	pyridine	52+79	79	0.383	0.50	0.10
N2	benzonitrile	76+103	103	0.772	0.01	0.00
N3	4-hydroxy-benzeneacetonitrile	78+133	133	1.554	0.04	0.02
N4	diketodipyrrole	93+186	186	2.024	0.11	0.05
Ph1	phenol	66+94	94	0.805	3.17	1.53
Ph2	4-methylphenol	107+108	108	0.989	1.84	0.60
Ph3	4-ethylphenol	107+122	122	1.159	0.36	0.16
Ph4	4-isopropenylphenol	119+134	134	1.404	0.02	0.01
Ph5	3-methoxy-5-methylphenol	107+138	138	1.431	0.08	0.03
Ca1	catechol	64+110	110	1.253	1.33	0.66
Ca2	4-methylcatechol	78+124	124	1.347	0.23	0.18
Ca3	5-methyl 3-methoxycatechol	139+154	154	1.509	0.15	0.06
Ca4	4-ethylcatechol	123+138	138	1.564	0.11	0.07
S1	sterol 1	191+231		3.384	0.05	0.02
S2	gamma-tocopherol	151+416	416	3.449	0.11	0.07
S3	sterol 2	189+218		3.533	0.02	0.01
Ttp	squalene	69+81	410	3.256	0.27	0.35
F16	C_{16} fatty acid	60+73	256	2.370	0.22	0.12
PA1	naphthalene	128	128	1.196	0.04	0.01
PA2	x-methylphenanthrene	191+192	192	2.321	0.00	0.00

Chapter 6

Influence of source vegetation and anaerobicity on lignin-based decomposition proxies in *Carex*-dominated ombrotrophic peat

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Abstract

Most knowledge about the degradation of lignocellulose in natural environments is based on woody tissue and aerobic systems; however, the contribution of graminoids to organic matter (OM) in anaerobic systems such as peatlands is high. In order to reconstruct past environmental conditions, a better understanding of decomposition of graminoid tissue in natural anaerobic ecosystems is needed. Samples (51) from the upper 1 m of the graminoid-dominated Penido Vello peatland (Galicia, NW Spain) were analysed with pyrolysis-gas chromatography-mass spectrometry (pyrolysis-GC/MS) and ^{13}C CPMAS NMR. C and N content were also determined. Molecular proxies were selected to reflect the state of decay of lignocellulose from peat pyrolysates, including macromolecular lignin, non-lignin phenolic acids, hemicellulose, and non-lignified and lignified cellulose. Factor analysis applied to 120 quantified pyrolysis products allowed identification of the different factors that influence the composition of peat OM. Botanical changes and several degradation stages including (an)aerobic decay directly after peat deposition, long term anaerobic decay and secondary aerobic decay all affected the values of the lignin-based decomposition proxies. Depth records of some lignin decomposition proxies were not in agreement with the literature. Guaiacyl (G) and syringyl (S) moieties with a C_3 alkyl side chain showed no correlation with bog hydrology at the time of peat deposition; G and S moieties with acetyl side chains were related to both relatively dry (secondary aerobic decay) and wet (first stage of decay) conditions; vanillic acid and syringic acid were related partly to ericoids (indicating dry conditions) and partly to free phenolic acids (less depleted under wet conditions); furthermore, preferential decay of G over S moieties was found during the first stage of decay (both aerobic and anaerobic) and long term anaerobic decay. These contradictions can be explained by the dominance of non-woody lignin sources (graminoids) and the prominence of anaerobic decay in peatlands. Our findings indicate that the effect of anaerobicity and source vegetation should be considered when using lignin proxies to deduce aerobic decay in peat records.

6.1. Introduction

Ombrotrophic peatlands receive water from precipitation alone. Although autogenic processes may influence the water table (Malmer et al., 1994), the depth of the water table within a given peatland is to a large extent dependent on rainfall. Water table depth determines O_2 availability and thereby peat decomposition. The degree of decomposition is therefore often used as a proxy for climate (Blackford and Chambers, 1993), indicating the relative importance of drier vs. wetter periods, or to obtain information on the rate of C sequestration (Clymo et al., 1998).

Lignin is a major component of plant remains that decompose in peatlands, as decomposition of lignin is an aerobic process (Kirk and Farrel, 1987; Williams and Yavitt, 2003). Lignin content and composition are therefore of particular interest for reconstructing hydrological change in peat records. Lignin is a macromolecule composed of S, G and *p*-hydroxyphenyl (H) moieties that are irregularly bound to each other. Its composition differs between major plant groups. In addition, non-woody tissue possesses, besides lignin, a high content of free and bound phenolics that are ester- and/or ether-linked to lignin and polysaccharides in the cell wall (Hedges and Mann, 1979; Kondo et al., 1989; Lam et al., 1992; Sun et al., 2011). Apart from these source characteristics, the composition of lignin moieties can be influenced by demethoxylation (Tien and Kirk, 1983) and side chain oxidation and shortening (Kögel-Knabner, 2002) during decay. Thus, the composition of lignin in soils and sediments provides useful information about past environmental conditions (Bourdon et al., 2000; Tareq et al., 2006; Disnar et al., 2008).

Knowledge of lignin transformation in soils and sediments is based mainly on aerobic decay (Thevenot et al., 2010) and woody tissue (Donaldson, 2001), while research on non-woody tissue has been concentrated on forage quality in graminoids (Buranov and Mazza, 2008). Although anaerobic degradation of lignin proceeds at a slower rate (Opsahl and Benner, 1995) and lignocellulose can be completely degraded under anaerobic conditions (Benner et al., 1984), information about the pathways of anaerobic lignin degradation is scarce (Young and Frazer, 1987; van der Heijden and Boon, 1994). Because lignin composition varies between plant parts and elements of plant cells (Grabber et al., 2004), its resistance to decay may show a similar variation (van der Heijden et al., 1994; Williams and Yavitt, 2010; Machinet et al., 2011). Thus, in peatlands, with prevailing anaerobic conditions and significant contribution from graminoids, interpretation of lignin characteristics is intricate. Environmental interpretation of organic matter in peatlands is further complicated by the fact that changes in hydrology drive changes in both plant species composition and the nature and degree of decomposition (Yeloff and Mauquoy, 2006).

Pyrolysis-gas chromatography-mass spectrometry (pyrolysis-GC/MS) is applied frequently to the study of lignocellulosic material (Ralph and Hatfield, 1991; Meier et al., 1992). In addition to lignin characteristics, pyrolysis-GC/MS provides detailed information on the overall molecular composition, which benefits the interpretation of complex mixtures of OM such as in peatlands and soils.

A selection of samples from a core collected in the graminoid-dominated Penido Vello peatland had been analysed with pyrolysis-GC/MS to study the effects of source and decay on the lignin composition (Schellekens et al., 2012). Analysis of peatland plants and comparison of NaOH-extractable peat (reflecting decomposition) and the non-extractable residue (reflecting source vegetation;

Buurman et al., 2006) allowed separation of source effects (graminoid vs. ericoid material) and different stages of decay on the lignin composition. The effects were sometimes contrasting, for example G moieties were preferentially degraded during the first stage of decay, while S moieties were preferentially degraded during secondary aerobic decay. Based on these results a number of lignin parameters were defined and applied to the bulk samples of the same peat record, sampled at high resolution. To support the pyrolysis results, C:N and ^{13}C cross polarisation magic angle spinning nuclear magnetic resonance spectroscopy (CPMAS NMR) data were also used. The purpose was to (i) identify and separate the effects of botanical shifts and several decomposition stages (aerobic and anaerobic) on the lignin composition in bulk samples of the PVO peat core, and (ii) examine the use of lignin-based decomposition proxies as a tool for (palaeo)climatic interpretation.

6.2. Methods

6.2.1. Location and sampling

Penido Vello is an ombrotrophic mire in the Xistral Mountains (Galicia, NW Spain). Location, sampling and characteristics of the bog have been described in detail by Martínez-Cortizas et al. (1997, 2002). The 3 m dates back to 6000 yr BC. For this study, only the samples (2 cm) taken at high resolution from the upper 1 m were used, as this interval showed better correlation between vegetation markers than the deeper part, which had been sectioned into 5 cm slices (Schellekens et al., 2011). The upper 1 m (51 samples) represents ca. 2000 yr of peat accumulation. Samples were dried at 35 °C (1 week) and ground for pyrolysis without further treatment.

6.2.2. Total C and N

The samples were analysed for total C and total N, as described by Pontevedra-Pombal et al. (2004).

6.2.3. Solid state ^{13}C CPMAS NMR spectroscopy

The ^{13}C CPMAS NMR spectra were divided into chemical shift regions: 0-46 ppm, aliphatics; 46-60 ppm, methoxyl; 60-95 ppm, O-alkyl C; 95-110 ppm, acetyl; 110-140 ppm, aromatics; 140-160 ppm, phenolics; and 160-250 ppm, carboxyl (Pontevedra-Pombal et al., 2001; Kaal et al., 2007). For a detailed description of the methodology we refer to these studies.

6.2.4. Pyrolysis-GC/MS

A Curie-Point pyrolyser (Curie temperature 600 ºC) connected to a Carlo Erba GC8000 gas chromatograph was used. Pyrolysis products were separated on a non-polar fused silica column [Chrompack: 25 m, 0.25 mm i.d., CP-Sil 51 b (0.40 µm)], with He as carrier gas. The oven temperature programme was: 40 ºC to 320 ºC (held 15 min) at 7 ºC min^{-1}. The column was connected to a Fisons MD800 mass spectrometer (*m/z* 45–650, cycle time 1 s).

The core had been studied with pyrolysis-GC/MS and ^{13}C CPMAS NMR. The whole core was analysed with ^{13}C CPMAS NMR, mean values were presented for each stratigraphic layer (Pontevedra-Pombal et al., 2001) while depth records of the whole core were presented by Pontevedra-Pombal et al.

(2004). Different peat fractions were analysed for a selection of 25 samples from the whole record by Buurman et al. (2006; extract and residue), while Kaal et al. (2007) compared selected bulk samples with ^{13}C CPMAS NMR. Schellekens et al. (2011) analysed all 101 bulk samples of the whole record and quantified 106 pyrolysis products. Schellekens et al. (2012) analysed bulk, extractable and non-extractable fractions of 13 samples from the upper 1 m and quantified 120 pyrolysis products that included a number of less common lignin moieties. Quantification in the present study was identical to that of Schellekens et al. (2012; Appendix 5 Chapter 5) but was applied here to all 51 samples from the first 1 m. Quantification was based on the peak area of two major fragment ions from each pyrolysis product. All individual quantifications were checked manually. For each sample, the sum of the peak areas was set at 100 % and proportions were calculated relative to this.

According to probable origin and similarity, the pyrolysis products were grouped as follows: polysaccharides, aliphatic biopolymers, methylketones, lignins, phenols, catechols, (other) aromatics, polyaromatics, N-compounds, fatty acids, steroids and triterpenoids (Appendix 5 Chapter 5). Factor analysis was applied using Statistica® Version 6 (Statsoft Inc, Tulsa).

6.3. The Penido Vello peat record

The upper 1 m of the Penido Vello peatland was dominated by graminoids (*Carex durieui*, *Agrostis curtisii*, *Molinia caerulea* and *Festuca rubra*) with significant contributions from ericoids (*Erica mackaiana* and *Calluna vulgaris*), *Eriophorum angustifolium* and moss at some depths. Although *Sphagnum* was present in most samples its contribution to the moss was low. Major vegetation shifts upon changes in hydrology have been found between *C. durieui*, *A. curtisii*, *F. rubra*, *E. mackaiana* and *C. vulgaris* (dry) and *M. caerulea*, *E. angustifolium* and moss (wet). Assigned wet and dry periods agreed well with depth records of vegetation markers and with other European studies (Schellekens et al., 2011).

6.4. Results

Lignin-based decomposition parameters, extracted from the results of Schellekens et al. (2012), are given in Table 6.1. Structure formulae of lignin pyrolysis products used in the parameters are given in Fig. 6.1. Factor analysis was applied to all quantified pyrolysis products. In order to evaluate the selected lignin parameters they were also included in the factor analysis. The first four factors explained the major part of the variance (69.3%). Projections of the loadings of factors 1 and 2 (F1, F2) and factors 3 and 4 (F3, F4) are given in Fig. 6.2.

F1 (36.3% explained variance) separated mainly between lignin pyrolysis products and *n*-alkanes (C_{10-25}, positive loading) and polysaccharide pyrolysis products and long chain *n*-alkanes (C_{29-33}, negative loading; Fig. 6.2a). In peat, polysaccharides are preferentially degraded over lignin (Benner et al., 1984; van der Heijden et al., 1990; Disnar et al., 2008) and an increase in aerobic decay causes chain length reduction of *n*-alkanes (Eglinton and Hamilton, 1967; Schellekens and Buurman, 2011). F1 was therefore assigned as reflecting bog hydrology at the time of peat deposition and thus the first stage of decay.

The high positive loading of aliphatics on F2 (12.9% explained variance; Fig. 6.2a) indicated residual accumulation of these compounds upon diagenesis, as an increase in aliphatics was found for the whole (3 m) peat record with ^{13}C CPMAS NMR (Buurman et al, 2006) and pyrolysis-GC/MS (Schellekens et al., 2011). This indicates that F2 reflected long term anaerobic decay.

F3 (11.5% explained variance) showed high negative loadings for pyrolysis products associated with woody peatland plants: a sterol (S3) and an aliphatic product (Al2), that were obtained solely from ericoids and wood, respectively (Schellekens et al., 2011), and long chain *n*-alkanes (Schellekens and Buurman, 2011). Compounds with positive loadings on F3 were associated with lignocellulose of graminoids: 4-vinylguaiacol from ferulic acid (Lg12) and 4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one (Ps4; Section 6.5.4). Therefore, F3 separated ericoids (negative loadings) from graminoids (positive loadings, Fig. 6.2b).

F4 (8.6% explained variance) showed positive loadings for lignin pyrolysis products with a C_3 alkyl side chain that reflect unaltered lignin (van der Hage, 1993; Kuder and Kruge, 1998), while benzene (Ar1), toluene (Ar2), pyridine (N1) and polyaromatics (PA1, PA2) showed negative loadings on F4 and are associated with aerobic decay (Schellekens et al., 2009). F4 was therefore assigned as reflecting the second phase of aerobic decay that occurs due to events of lowering of the water table. Positive loadings on F4 indicated relatively undecomposed material (Fig. 6.2b) and thus high water table during development of the layer.

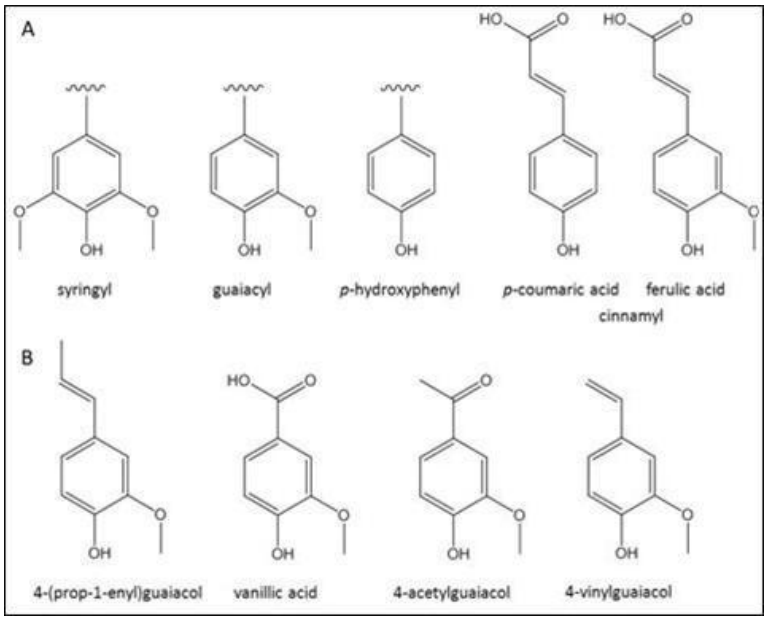

Fig. 6.1. Structure formulas of (a) lignin moieties and (b) pyrolysis products used for lignin parameters (presented for guaiacyl moieties).

Table 6.1a. Lignin-based parameters used (G, guaiacyl; S, syringyl; H, *p*-hydroxyphenyl; % TIC, amounts in terms of % of peak area compared to the total area of identified pyrolysis products.

	Parameter	
1a	Sum lignin	% TIC
1b	Sum polysaccharides	% TIC
2a	S/G	-
2b	C_3 G/C_3 S	-
2c	H/S+G[a]	-
3a	Vanillic acid	% G
3b	Syringic acid	% S
3c	4-Acetylguaiacol	% G
3d	4-Acetylsyringol	% S
4a	C_3 guaiacols	% G
4b	C_3 syringols	% S
5a	4-Vinylphenol	% H
5b	4-Vinylguaiacol	% Lignin
6	4-Hydroxy-5,6-dihydro-(2*H*)-pyran-2-one	% Polysaccharides
7	Levoglucosan	% Polysaccharides

[a]Without lignin moieties with a vinyl side-chain (Lg1, Lg12, Lg30).

Table 6.1b. Decay intervals.

	Decay stage	Water table	Decay type
1	Bog hydrology at plant death	Within acrotelm	Aerobic
		At surface	Anaerobic
2	Secondary aerobic decay	Within acrotelm	(repeating) Intervals of aerobicity
3	(long-term)Aanaerobic decay	Catotelm	Continuously anaerobic

Table 6.2. Correlation matrix (R^2) for ^{13}C CPMAS NMR data and factors (F1-F4) from factor analysis of pyrolysis products. [a]Underlined values denote to negative correlations; [b]values <0.1 not indicated.

	Pyrolysis-GC/MS			
^{13}C CPMAS NMR	F1	F2	F3	F4
Phenolic	0.78	-	-	-
Aromatic	0.86	-	-	-
Acetal	<u>0.66</u>	-	-	-
Methoxyl	0.23	-	-	-
Aliphatic	-	0.30	-	-
Carboxyl	0.21	-	-	-
O-alkyl	<u>0.82</u>	-	-	-

The explained variance for each individual variable accounted for by the factors was shown by the fractionation of communalities; the variance explained by the first four factors (eigenvalues >10) was given for the lignin parameters (Fig. 6.3). The fractionation of communalities for the remaining factors (F5–F16, eigenvalues between 1 and 8) and for the individual pyrolysis products, are not shown. Depth profiles of the lignin parameters are given in Fig. 6.4.

The factors extracted via factor analysis of the pyrolysates showed significant correlation with data obtained from ^{13}C CPMAS NMR (Fig. 6.5; Table 6.2). A negative correlation of O-alkyl C (R^2 0.82) and acetal C (R^2 0.66) was found with F1, while aromatic C (R^2 0.86) and phenolic C (R^2 0.78) were positively correlated with F1, reflecting preferential decay of polysaccharides over lignin.

C content (corrected for ash, R^2 0.75) and C:N (R^2 0.52) were positively correlated with F1, while N content was negatively correlated (corrected for ash, R^2 0.50; Fig. 6.5). These data indicate that higher C:N ratio values were found in more decomposed samples (Pontevedra-Pombal et al., 2004). The positive correlation of C with F1 presumably reflects the loss of polysaccharides during initial decay. High values of N in the upper part of the core were mainly responsible for the negative correlation of N and F1 (Fig. 6.5), reflecting the rapid recycling of N by plants in the aerobic layer.

6.5. Discussion

6.5.1. Abundance of lignin and polysaccharides

The summed lignin (parameter 1a) and summed polysaccharide (*parameter 1b*) pyrolysis products were for > 80% explained by F1 (Fig. 6.3), reflecting bog hydrology at the time of peat deposition (Table 6.1). High values of parameters 1a and 1b indicate relatively dry and wet periods, respectively (Fig. 6.2), and reflect the preferential decay of polysaccharides over lignin during the first stage of decay. The remaining variance in polysaccharides (parameter 1b) was explained by F2 and F3 (Fig. 6.3) and is discussed in Section 6.5.4. The remaining variance in lignin (parameter 1a) was explained by F2 (Fig. 6.3) and is predominantly related to G moieties (Fig. 6.2a; Section 6.5.2).

6.5.2. Preferential decay of lignin moieties

6.5.2.1. Syringyl to guaiacyl ratio

The analysis of different peat fractions indicated that differentiation between S and G moieties (parameter 2a) in the Penido Vello peat record was predominantly related to decomposition (Schellekens et al., 2012). To exclude a contribution from non-lignin phenolic acids (Section 6.5.4) to the abundance of lignin moieties, only the ratio of S to G units with a C_3 alkyl side chain was chosen (parameter 2b), as lignin moieties with a C_3 alkyl side chain reflect macromolecular lignin (Kuroda, 2000; van der Hage et al., 1993). Demethoxylation during pyrolysis was negligible (Harman-Ware et al., 2013) and can be excluded.

Both S/G ratios, parameters 2a and 2b, were explained for > 85% by the first four factors, of which the major part was related to F1 (Fig. 6.3). The projection of parameters 2a and 2b was negative on both factors F1 and F2 (Fig. 6.2a), and their depth profiles showed an increase until 80 cm from where they stabilised (Fig. 6.4). This pattern indicated preferential decay of G moieties, both during the first stage of decay (F1) and long term anaerobic decay (F2). For parameter 2a an additional part of the variance was explained by F4, reflecting the second phase of aerobic decay (Fig. 6.3). Parameter 2a de-trended for F1 and F2 (i.e. eliminating the effects of F1 and F2) showed a good positive correlation with F4 (R^2 0.63, not shown), indicating that, during secondary aerobic decay, S moieties were preferentially degraded over G moieties. The very low explained variance on F4 for parameter 2b (Fig. 6.3) indicates that preferential decay of S moieties is not valid for lignin C_3 alkyl side chains (see also Section 6.5.3).

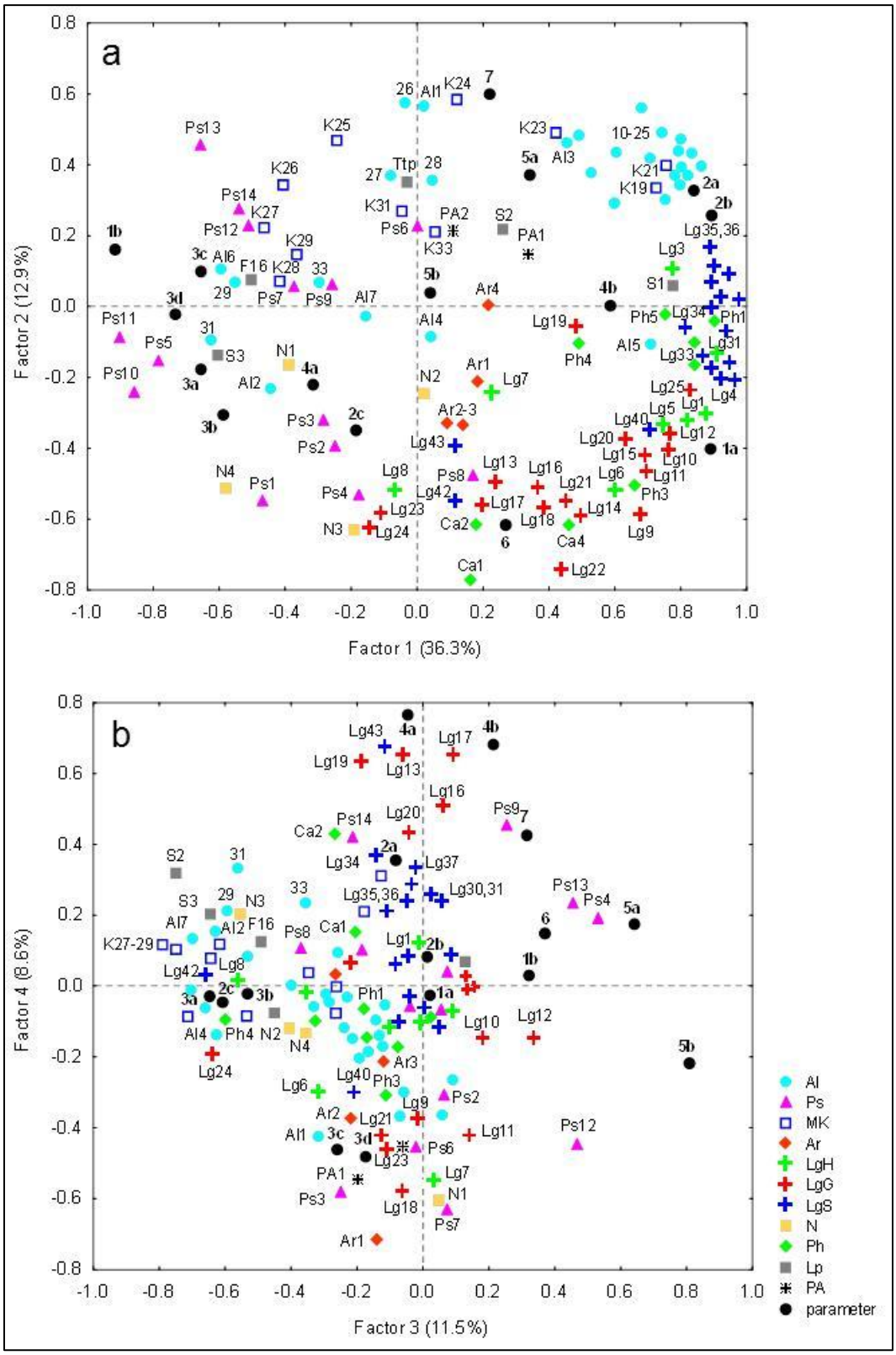

Fig. 6.2. Factor loadings of F1–F2 projection (a) and F3–F4 projection (b). Al, aliphatics; Ps, polysaccharides; K, methylketones; Ar, aromatics; LgH, *p*-hydroxyphenyl lignin; LgG, guaiacyl lignin; LgS, syringyl lignin; N, N-compounds; Ph, phenols; Ca, catechols; S, sterols; Ttp, triterpenoids; FA, fatty acids; PA, polyaromatics.

The preferential decay of G moieties was attributed by Schellekens et al. (2012) to that of G compounds originating from non-lignin phenolic acids and thus not from macromolecular lignin. An increase in S/G ratio under anaerobic conditions was also found for graminoid tissue by Opsahl and Benner (1995) and in graminoid-dominated peat by Kuder et al. (1998). Preferential decay of S lignin, related to its structure, is generally described from aerobic systems (Thevenot et al., 2010) except during the earliest stages of decay (Christman and Oglesby, 1971; Kögel, 1986). Because anaerobic conditions predominate in peat, this first stage of decay is prolonged, and the results thus correspond well with current knowledge. However, because C_3 alkyl side chains originate from the lignin macromolecule and parameters 2a and 2b showed similar behaviour in factor analysis, an additional explanation may be that of differential decay of plant tissues. Because structure and chemical composition of plant material may differ greatly within both plant parts and plant cells (Buranov and Mazza, 2008), it is likely that decay is connected to the anatomical structure of the plant, and thus to the accessibility of S or G lignin. This is in agreement with Grabber et al. (1997, 2004) whose results indicate that the degradability of grass cell walls was not affected by the composition of lignin moieties.

The graminoid species that dominate the Penido Vello peat have similar morphology, with thin leaves and a rigid stem (Schellekens et al., 2011), which suggests that the leaves are more easily degradable than the stems. According to macrofossil analysis of a similar peat record located at 2 km distance (Pena da Cadela), the peat is dominated by roots, rhizomes and internodes of graminoids. Remains of epidermis (which may originate from both leaves and stems) are also abundant but are much smaller in size and fragmented (I. Fraga, personal communication). The preferential decay of leaves vs. other plant organs in the peat may explain (part of) the abundance of G and S lignin during decay. This is supported by lignin studies of other graminoids. In bromegrass (Jung and Casler, 1990) and barley (Love et al., 1998), S lignin was found in higher abundance in the stem than the leaves and was more resistant to decay than G lignin, while the latter had a much higher abundance in the middle lamellae and was more easily decayed (Love et al., 1998). Furthermore, an increase in S/G with maturity in the stem of several graminoids (Grabber et al., 2004) was negatively correlated with enzymatic degradability in *Festuca arundinacea* (Chen et al., 2002). Thus in addition to preferential decay of G moieties from non-lignin phenolic acids, the observed decrease in G lignin under both aerobic and anaerobic conditions during the first stage of decay (Table 6.1b) is presumably related to plant anatomy. In woody tissue (e.g. from ericoids) the lignin composition and plant architecture is different and thus preferential decay of S moieties in peat in which ericoids determine the lignin content (van der Heijden and Boon, 1994; Huang et al., 1998) does not necessarily contradict to the preferential decay of G moieties in graminoid peat.

The source material (graminoids vs. ericoids) therefore exerts a strong influence over 'preferential decay' of G or S moieties in peat. However, F_3, which differentiated graminoids from ericoids, showed negligible explained variance for both S/G ratios (Fig. 6.3). Thus, for peat dominated by graminoids, parameters 2a and 2b would not be related to *shifts* between graminoids and ericoids, but would be almost completely determined by decay (F_1, F_2 and F_4).

6.5.2.2. p-Hydroxyphenyl moieties

Parameter 2c reflected the relative abundance of H moieties within the lignin macromolecule. Lignin moieties with a vinyl side chain (Lg1, Lg12 and Lg30) were excluded from this parameter because of their dual origin from both non-lignin phenolic acids and macromolecular lignin (Section 6.5.4).

The first four factors explained only 52% of parameter 2c (Fig. 6.3). F3, which distinguished graminoids from ericoids, accounted for the largest part of the explained variance. Thus, although demethoxylation of (G) lignin during the first stage of decay and long term anaerobic decay occurs in peat (Tsutuki, 1994; Schellekens et al., 2012), the influence of such decay (F1, F2) was lower than that of source vegetation. The negative loadings on F3 (Fig. 6.2b) indicated that H moieties had a relatively higher abundance in the peat samples with a higher contribution of ericoids. This higher abundance in ericoids contradicts the general interpretation of H lignin as being indicative for grass (Hedges and Mann, 1979; Thevenot et al., 2010), and is presumably related to the fact that H moieties may also originate from non-lignin phenolic polymers such as tannins (Wilson et al., 1985), that have a higher abundance in woody species (Kögel-Knabner, 2002).

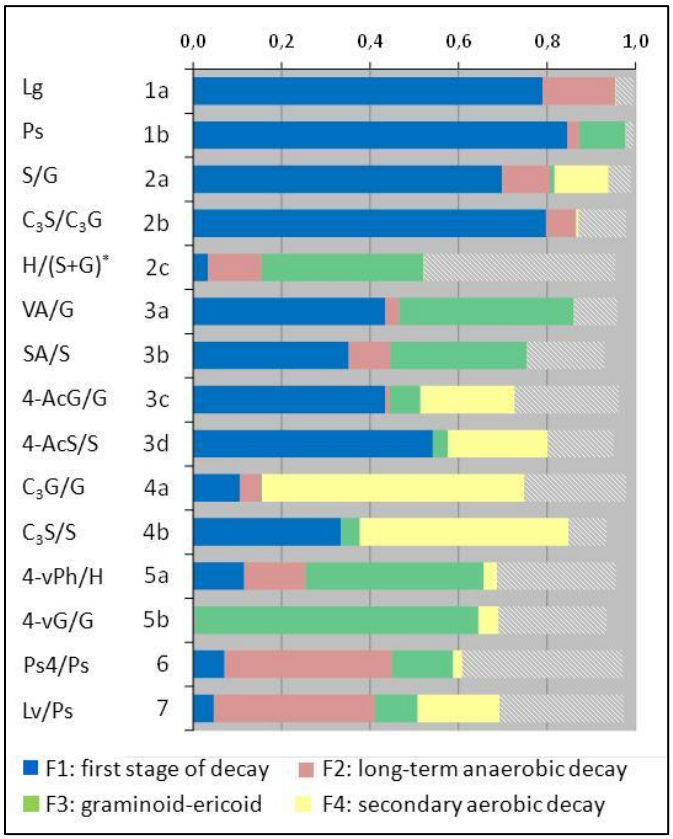

Fig. 6.3. Fractionation of communality for F1–F4 of the lignin decomposition parameters. The total length of the bar represents the communality for each parameter (i.e. the proportion of its variance explained by the first four factors), while each section of the bar corresponds to the proportion of variance accounted for by each specific factor.

6.5.3. Oxygenation and shortening of alkyl side chains

Side chain oxygenation highly increased from peatland plants to peat OM (Schellekens et al., 2012). A commonly used parameter for lignin degradation is the acid/aldehyde ratio (Ac/Ad) of G and S moieties after oxidation with CuO (Ertel and Hedges, 1985; Thevenot et al., 2010). With pyrolysis however, G and S moieties with a C_3 alkyl side chain, parameters 4a and 4b respectively, are generally interpreted as indicating intact lignin (Mulder et al., 1991; van der Heijden and Boon, 1994), and high values thus indicate relatively undecomposed material. For the present samples, the Ac/Ad ratio was completely dominated by the acid moieties (not shown). Therefore, vanillic acid and syringic acid were expressed as percentage of G, parameters 3a and 3b, respectively. In addition to the acids, 4-acetylguaiacol and 4-acetylsyringol were chosen to reflect oxygenation of lignin alkyl side chains (parameters 3c and 3d, respectively).

Although vanillic and syringic acid were expected to increase upon (secondary) aerobic decay in peat, parameters 3a and 3b had no loadings on F4 and a major part was related to undecomposed OM (F1) and ericoids (F3; Fig. 6.3). Low Ac/Ad values that did not correspond to other degradation parameters, were also found by Disnar et al. (2008) in a sedge-dominated peat. An explanation may be that the oxidation to acids is related to wood and thus in graminoid-dominated systems a high abundance is related to a change in vegetation composition. The knowledge that vanillic and syringic acid result from fungal oxidative cleavage of C_3 alkyl side chains (Saiz-Jimenez and de Leeuw, 1984; Hedges et al., 1988; Higuchi, 1990) is based on wood lignin. Parameter 3a showed a negative correlation with F1 only for the samples that had negative scores on F1 (R^2 0.64, n = 22; not shown). For these samples, vanillic acid may have originated from non-lignin phenolic monomers known to be abundant in vascular plants (Geissman and Crout, 1969) and were found abundantly in peat dominated by graminoids (Kuder et al., 1998; Schellekens et al., 2012). Furthermore, ester-bound vanillic acid is found in high amounts in seagrasses (Opsahl and Benner, 1993). The negative loadings on F1 are then explained by the rapid degradation of non-lignin phenolic monomers (Section 6.5.4).

Both acetyl ratios, parameters 3c and 3d, were positively correlated (R^2 0.86, not shown) and showed evidently higher values during wet period B12 (Fig. 6.4) that corresponds to the Little Ice Age. Parameters 3c and 3d showed similar communality for the factors and were negatively correlated with, explained mainly by, both F1 and F4 (Figs. 6.2, 6.3). Their similar behaviour indicates that parameters 3c and 3d increased under both relatively wet (negative on F1; Fig. 6.2a) and secondary dry conditions (negative on F4; Fig. 6.2b), which may explain the absence of spikes in their depth profiles (Fig. 6.4). The clearly higher values during the Little Ice Age can be explained by the duration of prevailing wet conditions. The initial increase in parameters 3c and 3d (upper 14 cm) suggest that the acetyl side chain is a result of anaerobic decay and cannot be interpreted as preservation due to the absence of aerobic decay. Thus, the formation of acetyl side chains occurs when the first step of aerobic decay is negligible and fresh material is subjected first to anaerobic conditions.

For the S moieties, the abundance of C_3 syringols (parameter 4b) was negatively correlated with parameter 3d (R^2 0.74, not shown), which suggests that C_3 syringols were transformed to 4-acetylsyringols during anoxic conditions at the first stage of decay. Anaerobic conditions during the first stage of decay (F1) and secondary aerobic decay (F4) explain the major part of the variance for parameter 4b (Fig. 6.3). Depletion of lignin moieties with a C_3 alkyl side chain also correlated with an increase in 4-acetylsyringol and 4-acetylguaiacol in peat investigated by Kuder and Kruge (1998). Although the effect of F4 dominated (Fig. 6.3), the abundance of C_3 syringols was obviously lower in the peat sections corresponding to the Little Ice Age (B12; Fig. 6.4). A low abundance of C_3 syringols during wet conditions is not in agreement with the literature (Kuroda, 2000; van der Hage et al., 1993), as high abundances of lignin with C_3 alkyl side chains are commonly interpreted as being from relatively intact lignin and thus indicating wet conditions.

Unlike the S moieties, for G moieties no correlation was found between C_3 and acetyl- alkyl side chains (R^2 0.01 for parameters 4a and 3c). C_3 guaiacols were mainly explained by F4 (Fig. 6.3) and showed particularly high values in the upper two samples (Fig. 6.4), indicating rapid decay of C_3 guaiacols. These rapidly degraded C_3 guaiacols probably originate from graminoids (Schellekens et al., 2012). Nevertheless, a clear maximum for parameter 3c occured during the Little Ice Age.

A possible explanation is that, during prevailing wet conditions, the undecomposed material, still rich in easily degradable polysaccharides, is anaerobically degraded by bacteria. During the first step in anaerobic cellulose degradation various simple O-containing compounds are released (Béguin and Aubert, 1994), which might be used for the oxygenation of lignin alkyl side chains. The good correlations of other lignin pyrolysis products with an oxygenated side chain with parameters 3c and 3d may be explained by the same mechanism. These include a correlation of syringic acid methylester (Lg38) with parameter 3d (R^2 0.84); vanillic acid methylester with parameter 3c (R^2 0.57); and 4-(propan-1-one)guaiacol with parameter 3c (R^2 0.55; not shown).

6.5.4. Non-lignin phenolic acids, hemicellulose and cellulose

Bourdon et al. (2000) used the ratio of cinnamic acids (i.e. ferulic acid and *p*-coumaric acid) to lignin as an indicator of Cyperaceae. Typical pyrolysis products of *p*-coumaric and ferulic acid are 4-vinylphenol and 4-vinylguaiacol, respectively (Boon et al., 1982; van der Hage et al., 1993). From analysis of peatland plants it appeared that 4-vinylguaiacol, from ferulic acid, was particularly associated with graminoids (parameter 5b), while 4-vinylphenol from p-coumaric acid (parameter 5a) showed a higher abundance in graminoids that perform better under wet conditions (Section 6.3). Also ericoids showed a relatively high abundance of 4-vinylphenol (Schellekens et al., 2012). However, 4-vinylguaiacol and 4-vinylphenol also originate from intact lignin (Harman et al., 2013) and chain length reduction of C_3 alkyl side chains during decay, which occurred under both aerobic and anaerobic conditions (Schellekens et al., 2012). Therefore the presence of phenolic acids cannot be evaluated properly via pyrolysis-GC/MS without methylation (del Rio et al., 2007), so the interpretation of 4-vinylguaiacol and 4-vinylphenol is problematic.

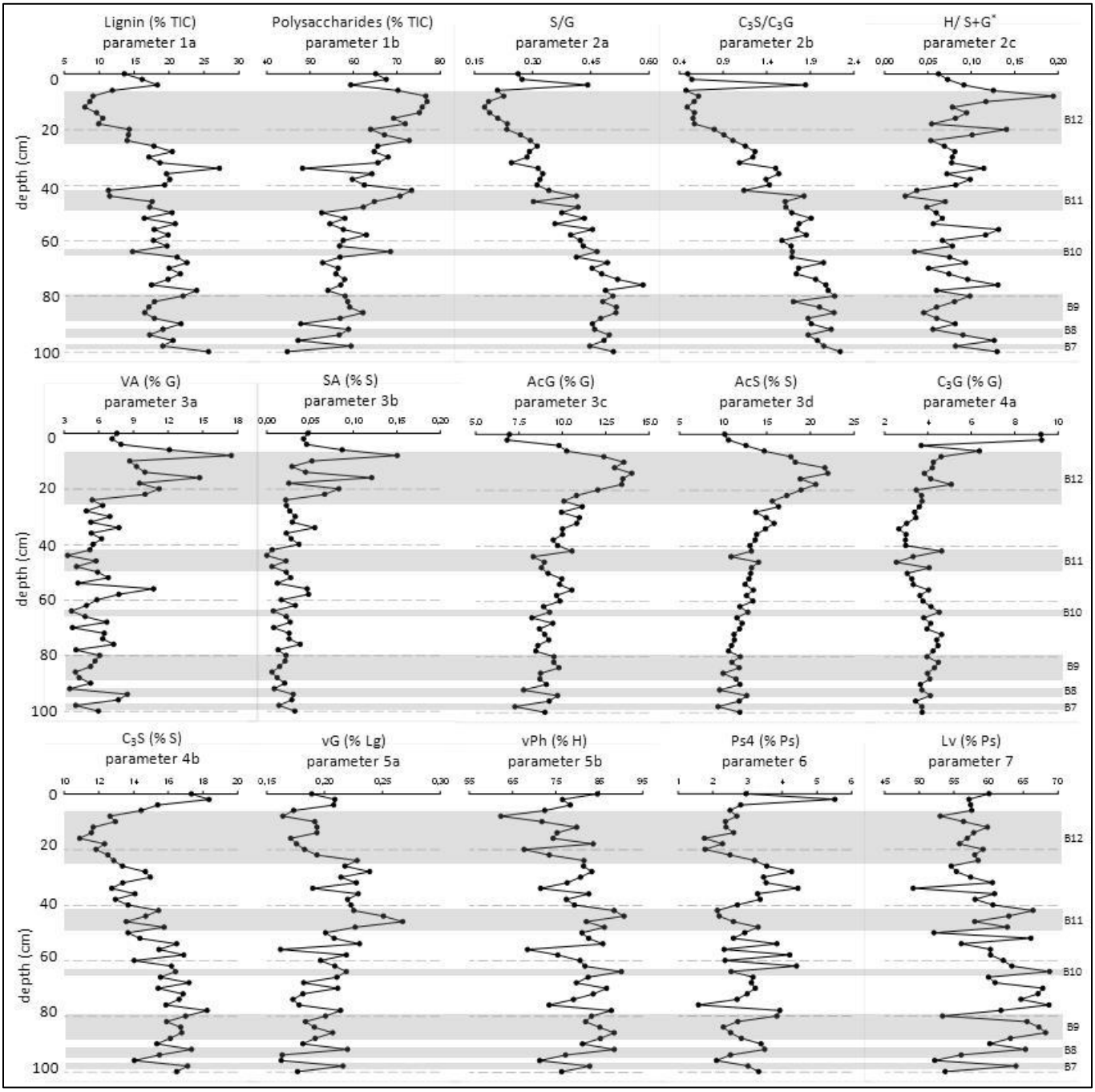

Fig. 6.4. Depth profiles of lignin parameters (see Table 6.1). The shaded areas indicate relatively wet periods, based on vegetation markers and the composition of peat pyrolysis products, after Schellekens et al. (2011).

Xylose, which is abundant in hemicelluloses of grass (Wende and Fry, 1996; Smith and Harris, 1999) and sedges (Bourdon et al., 2000), results in 4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one upon pyrolysis (parameter 6; Pouwels et al., 1987). The main pyrolysis product from cellulose is levoglucosan (Pouwels et al., 1989), of which non-lignified cellulose is reflected by parameter 1b and lignified cellulose by parameter 7.

The variance in parameter 5b is 64% explained by F3 (Fig. 6.3) and is related to graminoids (Fig. 6.2b), while the explained variance of parameter 5a is more distributed among other factors. Both ferulic acid and *p*-coumaric acid are rapidly degraded once the plant dies (Kuder and Kruger, 1998; Bourdon et al., 2000). A large part of both compounds is presumably already lost before it enters the peat. A smaller part of both is more resistant to decay (Tareq et al., 2006), which agrees with the maxima in parameters 5a and 5b during wet periods, except for a clear minimum during the Little Ice Age of parameter 5b (Fig. 6.4). These clearly lower values of 4-vinylguaiacol may indicate that ferulic acid was transformed to 4-acetylguaiacol under initial persistent wet conditions, as suggested in Section 6.5.3 for lignin C_3 alkyl side chains. Anaerobic degradation of ferulic acid can occur, with acetate being formed as an intermediate during metabolism (Healy et al., 1980), which supports this idea. Because the Penido Vello peat is dominated by graminoids and vegetation shifts upon lowering of the water table may be more prominent within graminoids than between ericoids and graminoids, the positive loadings of parameters 5a and 5b on F3 may also be related to graminoids that perform better under relatively wet conditions (*Eriophorum* and *Molinia*), as these showed particularly high values of non-lignin phenolic acids (Schellekens et al., 2012).

The most abundant polysaccharide pyrolysis product in the peat was levoglucosan (Appendix 5 Chapter 5). The loss of polysaccharides during the first stage of decay originates mainly from non-lignified cellulose, which is easier to access. Assuming that non-lignified cellulose is degraded as rapidly as other plant polysaccharides, levoglucosan expressed as proportion (%) of the total polysaccharide pyrolysis products reflects lignified cellulose (parameter 7). The increase with depth in parameter 7 (Fig. 6.4) showed the relative accumulation of lignified cellulose in an anaerobic environment (van der Heijden and Boon, 1994; Huang et al., 1998; Disnar et al., 2008; Fig. 6.3). The separation between parameters 6 and 7 on F2 (Fig. 6.2a), indicates that, during long term anaerobic decay, hemicellulose is preferentially degraded over lignified cellulose. The slightly positive loadings of parameter 7 on F1 also indicates that, during the first stage of decay, levoglucosan is less depleted than other polysaccharide pyrolysis products (Fig. 6.2a), indicating that a large part of the cellulose was lignified. The negative loadings of parameter 7 on F4 show that, during secondary aerobic decay, lignified cellulose is degraded.

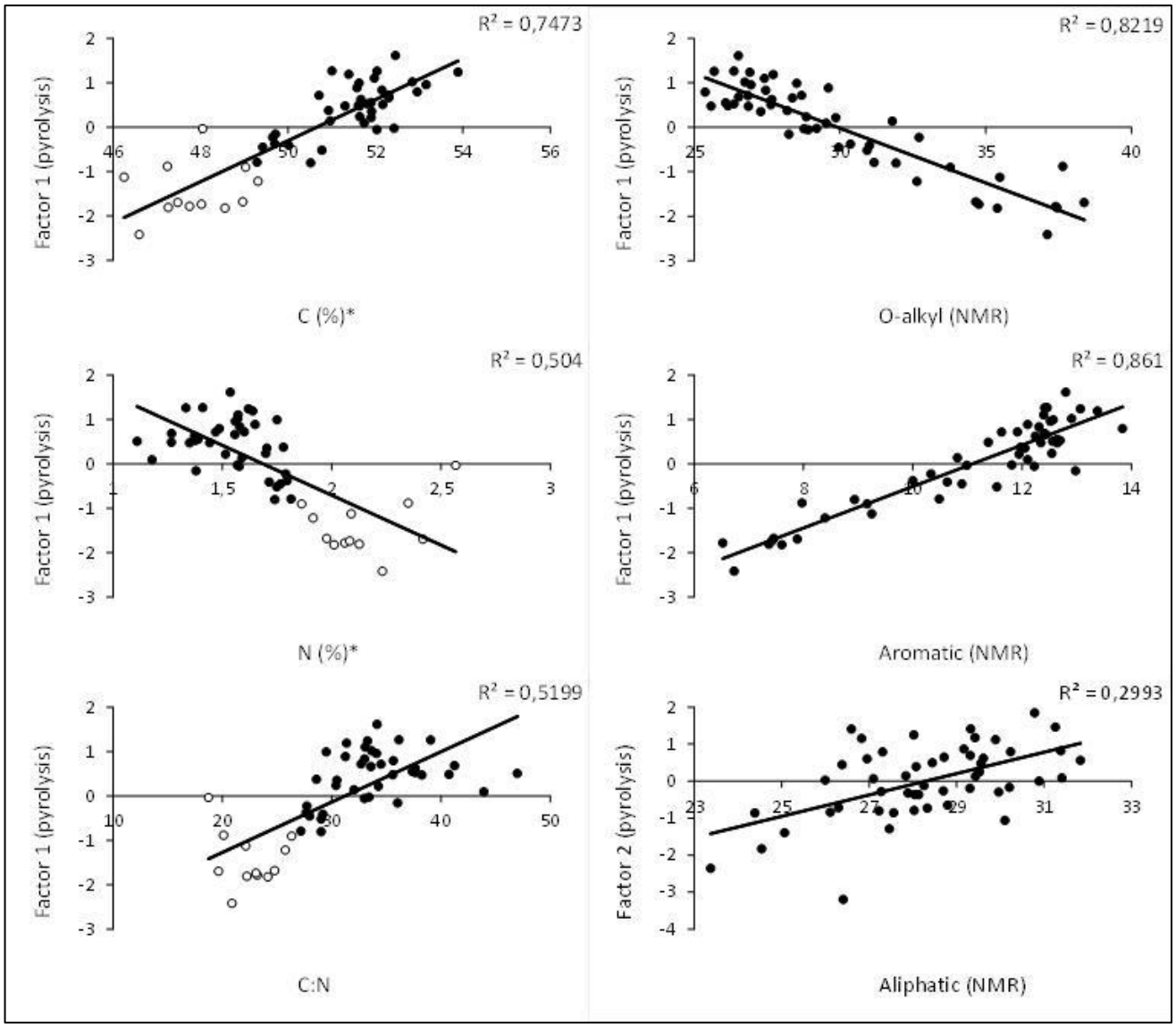

Fig. 6.5. Correlation between factors extracted from factor analysis of pyrolysates from peat OM with properties obtained with other methods. The open points reflect the uppermost samples; *corrected for ash content.

6.5.5. (Palaeo)climatic implications

The application of the parameters to reconstruct environmental conditions from peat records or to predict the effects of climate change related to the C stored in peatlands will be discussed for each parameter. Their usefulness as proxies for indicating bog hydrology is related to the first stage of decay. The contribution of the different decay stages and vegetation changes to the lignin parameters is indicated in Fig. 6.3.

6.5.5.1. Lignin and polysaccharide content (parameter 1)

Both polysaccharide and lignin content indicate bog hydrology at the moment of peat deposition, and reflect the preferential decay of polysaccharides over lignin. The lignin/polysaccharide ratio may thus be a reliable indicator of bog hydrology.

6.5.5.2. Syringyl, guaiacyl and p-hydroxyphenyl moieties (parameter 2)

The decrease in G lignin during the first stage of decay and long term anaerobic decay is presumably related to preferential decay of non-lignin phenolic acids and differential decay of plant parts that contain higher G content (leaves). During secondary aerobic decay the more resistant plant parts and macromolecular lignin are accessible and S is preferentially lost. Thus, in the Penido Vello core, the S/G ratio is completely determined by decomposition. However, interpretation is complex and highly dependent on prevailing decay phases.

Although parameter 2c had the lowest explained variance, the major part was related to source, namely ericoids. The contrast with the general assumption that a high abundance of H moieties in soils is related to graminoids (Thevenot et al., 2010), indicates that parameter 2c is not always a trustworthy indication of source material.

6.5.5.3. Alkyl side chain oxygenation (parameter 3)

Side chain oxygenation is generally related to fungal degradation and therefore used to indicate aerobic conditions in peat records. In the Penido Vello core, half of the abundance of vanillic and syringic acid was related to ericoids, and the other half to non-lignin phenolic acids from graminoids preserved under anoxic conditions during the first stage of decay (Fig. 6.3). Thus, a high abundance of vanillic and syringic acid may indicate (i) relatively wet conditions during the first stage of decay or (ii) an ericoid source, and thus indirectly relatively dry conditions. The absence of a relationship with secondary aerobic decay may be due to the fact that, under anoxic conditions, lignocellulose from graminoids is degraded more rapidly than that from wood, suggesting that wood is not yet strongly degraded.

Similarly acetyl side chains were related to both relatively dry (secondary aerobic decay) and wet (first stage of decay) conditions. The fact that the effect of anaerobic decay was more prominent in the depth records (Fig. 6.4) is related to the long duration of the Little Ice Age.

6.5.5.4. Alkyl side chain reduction (parameter 4)

A large part of the C_3 alkyl side chains is degraded during secondary aerobic decay. However, the interpretation of lignin moieties with a C_3 alkyl side chain to reflect intact lignin may lead to erroneous hydrological interpretation, as C_3 syringols in particular were depleted during wet periods. Neither of both parameters (4a and 4b) was correlated with bog hydrology at the time of peat deposition.

6.5.5.5. Free phenolic acids (parameter 5)

The cinnamyl/G ratio, generally used to differentiate between non-woody and woody tissue (Hedges and Mann, 1979), may be adequate for anaerobic systems with large changes in vegetation type (e.g. Tareq et al., 2006). However, no such correlation was found for the Penido Vello peat (not shown), probably because most G lignin pyrolysis products were as similarly rapidly degraded as 4-vinylguaiacol (from ferulic acid). However, the ratio 4-vinylguaiacol to *lignin* and the ratio of 4-vinylphenol (from *p*-coumaric acid) to H moieties are good indicators for graminoids, particularly those that perform better under relatively wet conditions (*Eriophorum* and *Molinia*, Section 6.5.4). The association of parameter 5a with graminoids is possibly caused by the fact that H moieties were related to ericoids (Section 6.5.5.2).

6.5.5.6. Hemicellulose and lignified cellulose (parameters 6 and 7)

During the first stage of decay hemicellulose and cellulose are degraded at equal rates, but relatively slowly compared with other polysaccharides. During long term anaerobic decay, hemicellulose is preferentially lost over cellulose; this was also found for *Calluna* tissue in peat and is presumably related to protection of cellulose by lignin (van der Heijden and Boon, 1994).

6.6. Conclusions

In the Penido Vello peat record the lignin composition is predominantly determined by decay, not by vegetation shift. However, the dominant vegetation source (graminoids) is the main factor that determines the consequences of decay on the composition of lignin.

Our results support the idea that lignin decay is a surface process, and that anatomical structure or accessibility control the decay of lignocellulose at different scales (van der Heijden and Boon, 1994; Grabber et al., 1997, 2004; Love et al., 1998; Talbot et al., 2012). Because plant groups differ in chemistry a general decay mechanism may not be valid for peat composed of unknown mixtures of different plant groups (ericoids-graminoids-mosses); most lignin knowledge comes from woody tissue and aerobic systems, so, some generally used lignin degradation parameters are not automatically valid for peatlands, where anaerobic conditions prevail and graminoids can be a dominant lignin source. Furthermore, because the lignin composition and the degree of cross-linking is highly variable (within graminoids, plant parts and plant tissue) and not understood, interpretation of the effects of decay in natural systems is complex. We recommend the use of a number of vegetation and decomposition proxies that provide similar information.

Chapter 7

General Discussion

Pyrolysis-GC/MS of peatland plants, comparison of different peat fractions, and analysis of bulk peat samples from cores sampled at high resolution in different vegetation types gave new insights into the three-way relationship between source vegetation, decomposition processes and environmental factors.

7.1. The use of specific markers for vegetation reconstructions

Identified peatland plant biomarkers are relatively scarce (Nichols, 2010) and are mainly restricted to free solvent-extractable lipids. They include 5-alkylresorcinols as markers of *Carex* (Avsejs et al., 2002), archaeol as marker of methanogens (Pancost et al., 2011), diterpenoids as markers of conifers (del Rio et al., 1992; Jansen et al., 2007) and triterpenoids. Triterpenoid markers include plant-derived pentacyclic triterpenoids and des-A-triterpenoids, bacterial-derived hopanoids, and steroids (Ourisson et al., 1979; Ekman and Ketola, 1981; Karunen et al., 1983; Ketola et al., 1987; del Rio et al., 1992; Dehmer, 1995; Pancost et al., 2002; Jansen et al., 2007; McClymont et al., 2008; Huang et al., 2008, 2012). Macromolecular markers are restricted to lignin to differentiate between mosses and vascular plants (Tsutsuki et al., 1994; Williams et al., 1998; Bourdon et al., 2000) and sphagnum acid (see below).

The molecular characterization of peatland plants with analytical pyrolysis provided a number of additional markers that allowed a vegetation reconstruction for *Sphagnum*-dominated (Chapters 2 and 3) and graminoid-dominated (Chapter 4) peatlands. The obtained markers for both peatlands are combined in Table 7.1; markers obtained from plant pyrolysates of Penido Vello were checked in those of plants from Harberton and *vice versa*. The results were consistent, and have further been tested and confirmed in pyrolysates obtained from plants from a very different ecosystem (Schellekens et al., 2013). The markers will be discussed separately.

The pyrolytic marker of sphagnum acid, **4-isopropenylphenol**, proposed by van der Heijden et al. (1997) as a marker of *Sphagnum* in peat cores, was identified in peat samples at several depths. Its occurrence along the profile confirms that sphagnum acid remains present in peat of thousands of years old (Chapter 2). Its depth record was in agreement with other studies and with other chemical parameters for the Harberton peat (Chapter 3). Its persistence has also been demonstrated by McClymont et al. (2011) for an English core. Quantification of 4-isopropenylphenol in the Penido Vello core showed that the contribution of *Sphagnum* to the past plant cover could also be reconstructed in graminoid-dominated peat with a much lower contribution of *Sphagnum* (Chapter 4). McClymont et al. (2011) used the ratio of 4-isopropenylphenol to lignin pyrolysis products to reflect the past abundance of *Sphagnum* relative to that of vascular plants. The advantage of using ratios is that

quantification is simple, but a disadvantage is that its variation is determined by changes in the numerator and / or denominator. Because the functional groups of sphagnum acid are lost during pyrolysis (resulting in 4-isopropenylphenol), TMAH thermochemolysis of *Sphagnum* peat may provide more information on the degradation state of *Sphagnum*. Recently, Abbott et al. (2013) and Swain and Abbott (2013) showed that sphagnum acid is subject to degradation under aerobic conditions, and the four TMAH thermochemolysis products of sphagnum acid have different characteristics with respect to decay for a number of surficial cores in a Swedish peat. More research is necessary to further explore the effects of degradation on sphagnum acid. The application of TMAH thermochemolysis is recommended as an additional methodology.

The phenolic compound specific for lichens, **3-methoxy-5-methylphenol**, was abundant in the analysed lichens (up to 17% of the total quantified peak area, TIC). Its depth record showed good agreement with other markers in both the Harberton (in preparation), and Penido Vello peat cores, and supported its use as marker for lichens in peatlands. Literature research of 3-methoxy-5-methylphenol revealed the synonym 'oak moss phenol', used in perfumery, as it has been isolated from oak moss (the lichen *Evernia prunastri*) by ter Heide et al. (1975). This supports its specificity and use as a marker for lichens and thus of dry conditions in peatlands. The identification of this marker is important as lichens are generally not recognised in the macrofossil record, and although their lipid distributions have been studied (Ficken et al., 1998), lipid distributions do not provide specific markers.

Benzoic acid was a major peak in pyrolysates of *Empetrum rubrum* (7% TIC) and was not detected in the other Harberton peatland plants. Although benzoic acid is a common pyrolysis product of soil organic matter, its depth record showed a clear trend that was in good agreement with the pollen record of *E. rubrum* from a core of the same peatland (Markgraf and Huber, 2010). Unlike its consistency in the Harberton peat core, it appeared to be less specific in other peats. Benzoic acid was identified in pyrolysates of half of the 18 analysed peatland plants from Penido Vello (between 0.20 and 0.45% TIC), including both ericoids and graminoids (Table 7.1). The two isomers identified as **C_3H_3-guaiacols** were solely detected in wood of *Nothofagus antarctica* in the Harberton plant selection and its depth record showed a clear trend that was in agreement with other markers and with the peatland ecology; these pyrolysis products were detected in all 18 plant pyrolysates from Penido Vello. Benzoic acid and C_3H_3-guaiacols can thus not be applied without analysis of the dominant plants in a given peatland.

The **aliphatic compound** with masses 67 and 81 was specific for woody plant species of the Penido Vello peatland. Verification of this compound in the peatland plants from Harberton confirmed its origin from wood as it was detected in *Nothofagus pumilio* and *N. antarctica*. Its presence in bark and absence in xylem of *N. antarctica* (not shown) suggests an origin from suberan (Tegelaar et al., 1995). However, it was not found in pyrolysates of *Empetrum rubrum* that also contained bark. Notwithstanding its specificity, its application to other peatlands is not recommended, as many aliphatics with similar masses are present in plant pyrograms (unpublished data), which complicates the detection of this specific one, especially when using another apparatus or different instrument

settings. Nevertheless, my research showed that even very common compounds may be specific markers within a given peatland and dataset.

Ferulic acid methyl ester was found in pyrolysates of all analysed graminoids and not in other plant species for both Harberton and Penido Vello 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 (except *Brachypodium retusum*), while it was absent from all 23 other analysed plant species (including a non-graminoid monocotyledon; Schellekens et al., 2013). A similar compound with a higher retention time (*m/z* 137, 165, 208) was also found in some graminoids and in some peat samples from Harberton. Graminoids are known to have different lignin-carbohydrate complexes compared to other plant species. Ferulic acid dehydrodimers from grass cell walls that cross-link polysaccharides (Ralph et al., 1994) are here proposed as the source of this pyrolysis product. The reliability of ferulic acid methyl ester as marker is well established; but its abundance in plant (0.04–0.14% TIC) and peat pyrolysates (<0.05% TIC Penido Vello (Chapter 4); <0.07% TIC in Harberton (not shown)) is low, which may complicate its use as marker. Nonetheless its depth record for both peats did not appear erroneous (Fig. 6.4.2b for Penido Vello; not shown for Harberton).

Although **C_1-phenanthrene** is considered to have a pyrolytic origin (either from fire residues or as a result of pyrolysis), and may indicate resinous materials (del Rio et al., 1992), for the selected peatland plants it was only detected in *Carex binervis* and its depth record in the peat showed good agreement with the preferential habitat of *C. binervis* and with other markers (Chapter 4). Some studies have found polycyclic antimicrobial compounds in the roots of *Carex* species (Kawabata et al., 1995), and these compounds can be a possible source of phenanthrenes.

4-Hydroxybenzene acetonitrile, used as marker for *Juncus bulbosus*, was not detected in other analysed plant species (Table 7.1). 4-Hydroxybenzene acetonitrile (4-hydroxybenzyl cyanide) 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, whether of plant or of fungal origin, remains unclear.

Although the depth record of the marker of *Deschampsia flexuosa*, **sterol 2** with *m/z* 191 and 231, gave a clear trend with a major shift to lower values that coincided with the other plant markers, its very low abundance in the plant pyrolysates and the fact that its origin is generally attributed to microbes (hopanoids), complicate its interpretation as marker of *D. flexuosa* (Chapter 4).

The mass spectrum of the marker of *Erica* (**sterol 4**) showed the best fit with alpha- or beta-amyrin according to the NIST library, but the molecular ion (426) did not agree with that of sterol 4 (408). A high abundance of such triterpenoids was also reported with GC/MS in Ericaceae by

Table 7.1. Presence of marker compounds in plants from the Harberton peatland, the Penido Vello peatland, and calcareous soils from Murcia (Spain)

Pyrolysis product (marker)	*m/z*	Harberton (*n* = 9; Chapter 2)[a]	Penido Vello (*n* = 18; Chapter 4)[a]	SE Spain (*n* = 32; Schellekens et al., 2013)[b]
4-Isopropenylphenol	119,134	*Sphagnum magellanicum*	*Sphagnum capillifolium*	-
Benzoic acid	105,122	*Empetrum rubrum*	*Thymelaea cordifolia*, *Calluna vulgaris*, *Erica cinerea*, *Juncus* spp., *Carex demissa*, *Carex binervis*, *Hieracium pilosella*	*Quercus coccifera*, *Pistacia lentiscus* (a), *Smilax aspera* (a)
C_3H_3 guaiacols	147,162	*Nothofagus antarctica, N. pumilio*	All 18 species (see Chapter 4)	8 species (including both herbaceous and woody species, and roots and aerial parts
3-Methoxy-5-methylphenol	107,109,137	Lichens	-	-
4-Hydroxybenzene acetonitrile	78,133		*Juncus bulbosus*	-
C_1-phenanthrene	191,192	-	*Carex binervis*	-
Ferulic acid methylester	177,208	*Marsippospermum* sp. (Juncaceae)	Juncaceae (*J. bulbosus*, *J. effusus*, *J. squarrosus*), Cyperaceae (*Eriophorum angustifolium*, *Carex durieui*, *C. demissa*, *C. binervis*), Poaceae (*Festuca rubra*, *Agrostis curtisii*, *Deschampsia flexuosa*, *Molinia caerulea*)	Poaceae (*Stipa tenacissima*, *Triticum* spp., *Hordeum vulgare*)
Sterol 2	191,231		*Deschampsia flexuosa*	
Sterol 4	189,218		*Erica cinerea*, *E. mackaiana*	Similar sterols in Fabaceae, *Rosmarinus officinalis*, *Cistus*
Sqaulene	69,81		*Eriophorum angustifolium*	10 random species
Aliphatic compound	67,81	Bark of *Nothofagus antarctica*, *N. pumilio*	Woody tissue of Ericacea, *Thymelaea cordifolia, Nothofagus*, *E. rubrum*)	*Pinus halepensis* (r), *Dorycnum pentaphyllum* (r)

[a]analysed with a Curie point pyrolyser, [b]analysed with a filament pyrolyser; r = roots, a = aerial parts

Pancost et al. (2002). The label 'sterol' was not correct, as it is a triterpenoid and not a steroid. Although their depth records showed good agreement with other markers, identification of triterpenoids with pyrolysis is problematic because many have the same basic skeleton, and edge groups are removed upon pyrolysis.

Although **Squalene** was found specific for *Eriophorum angustifolium*, it has not been detected in later plant analysis of a different sample of *E. angustifolium* analysed on a different apparatus (micro-furnace pyrolyser). Although it was a dominant pyrolysis product in some of the peat samples and its depth record showed good agreement with the other markers, it may also originate from contamination during sampling, and its attribution to *E. angustifolium* plants is not yet considered reliable.

Syringyl **lignin** moieties were only detected in vascular plants, not in mosses. Very low amounts of guaiacyl moieties were detected in *Sphagnum* (Table 2.1). However, these do probably not originate from *Sphagnum* itself, but are derived from vascular plants and migrated into *Sphagnum* capitula with dissolved organic matter (Abbott et al., 2013). The lignin depth record agreed well with that of the marker of sphagnum acid in the Harberton peat core, showing opposite trends. However, in the graminoid-dominated Penido Vello peat the abundance of lignin appeared to be correlated with decomposition, causing a preferential preservation of lignin over polysaccharides. Other studies have used depth records of lignin content in peatlands to reconstruct the contribution of mosses versus vascular plants (Boon et al., 1986; McClymont et al., 2011) or to indicate the degree of decomposition (Tsutsuki et al., 1994).

The specificity of 4-isopropenylphenol as a marker of sphagnum acid has been tested thoroughly (van der Heijden et al., 1997). In addition, markers that are specific for lichens and graminoids are considered reliable, while the other markers probably only function well within a given peatland ecosystem. The difference between the two peatlands emphasises the importance of plant analysis prior to the use of pyrolytic biomarkers with low specificity, but also shows that supposedly non-specific pyrolysis products could be specific within a certain peatland ecosystem (e.g. benzoic acid). In general, when using pyrolysis products as molecular markers it must be taken into account that the type of pyrolyser and pyrolysis conditions may influence the pyrolysis products (Moldoveanu, 1998). Furthermore, because bulk peat samples were used for analysis, markers may include both free molecules that did not change upon pyrolysis and (modified) chemical moieties from macromolecules. Their structural origin (free or bound) cannot be established by pyrolysis of bulk samples, unless literature has reported their original source and structure, as with 4-isopropenylphenol (pyrolysis product of sphagnum acid) and 3-methoxy-5-methylphenol (intact free molecule). The advantage of pyrolysis over other molecular marker techniques (GC/MS of solvent extractable lipids) to explore biomarkers of peatland plants is that no pre-treatment is necessary and no specific fractions need to be isolated (as is the case with solvent-extractable lipids (Nichols, 2010), and that with a single measurement a fingerprint of the complete sample is obtained. A disadvantage of pyrolysis is that the absolute quantification of a single marker is not possible, and

when using ratios with other compounds information may be lost, as changes of both numerator and denominator can be influenced by several factors. Contents of pyrolytic compounds reflect relative abundances that allow us to assess the variations along a core (Jacob et al., 2007). In both investigated peatlands, depth records of the plant markers (Figs. 6.3.1, 6.4.2) showed a good agreement with each other in relation to the preferential hydrological habitat of the species. The results of Harberton (Heusser, 1989; Markgraf, 1993; Markgraf and Huber, 2010; Pendall et al., 2001; White et al., 1994) and Penido Vello (Martínez-Cortizas et al., 1999; Muñoz-Sobrino et al. 2005; Mighall et al., 2006) also agreed with other studies on the same bog; and wet-dry cycles from other European bogs for Penido Vello (van Geel and Renssen, 1998; Desprat et al., 2003; Barber et al., 2003; Langdon et al., 2003; Chambers et al., 2007); a detailed palaeoenvironmental interpretation of the Harberton bog is in preparation. My findings and these other studies imply that the effect of decomposition on molecular markers does not prohibit their use. However, for less specific molecular markers such as lignin moieties or *n*-alkanes the effects of decay do interfere with interpretation as indicated by comparison of pyrolysates from different peat fractions and records of parameters sampled at high resolution (Chapters 3, 5 and 6). The 'abundance' of a marker in plant and/or peat pyrolysates is not a good measure for its reliability as a marker; even pyrolysis products that are only visible in a partial chromatogram may be reliable markers (e.g. 3-methoxy-5-methylphenol).

7.2. The use of different peat fractions

Bulk peat samples reflect a heterogeneous mixture of plant material at different stages of decomposition. Because the molecular composition of peat is affected by several factors, it can be informative to analyse different peat fractions. For example a solvent extraction removes free molecules, and pyrolysis of the remaining residue solely reflects bound molecules (Abbott et al., 2013; Swain and Abbott, 2013). This differentiation between free and bound molecules cannot be obtained by (single shot) pyrolysis. The objective of my study was to separate the effects of decomposition and botanical source on the molecular composition of bulk peat samples. Separation into NaOH-extractable (extract) and non-extractable (residue) peat according to Buurman et al. (2006) was expected to provide such information. Based on differences in size, the extract contains more decomposed material (smaller fragments) while the residue more closely reflects macromolecular structures and thus more intact plant material (Buurman et al., 2006). Most literature that reports molecular chemistry of different peat fractions is based on the conventional separation of soil organic matter into fulvic acid (FA), humic acid (HA) and humin, with varying purposes such as the understanding of coalification or humification (Hatcher et al., 1982; Given et al., 1984; Wilson et al., 1987; González-Vila et al., 1992; Klavins et al., 2009; Grasset et al., 2002; Guignard et al., 2005), metal sorption (Gondar et al., 2005; Groenenberg et al., 2010) or DOC properties (Kalbitz et al., 1999; Glatzel et al., 2003; Kalbitz and Geyer, 2002). The extract basically reflects the combined HA+ FA while the residue corresponds to humin. Comparison of peat HA, FA and humin for the environmental interpretation of peat records was explored by González et al. (2003). These authors did not find diagenetic differences, however, their conclusions were based on samples at only two different depths. Other authors compared bulk peat

samples with the HA fraction for a high-resolution sampled core, and the obtained differences were related to decomposition (Zaccone et al., 2007; Zaccone et al., 2008, 2011). The commonly applied method to determine the degree of decomposition in peat is based on the colour of alkaline peat extracts; the higher the degree of decomposition the more material is extracted and the darker the extract (Blackford and Chambers, 2003), supporting that the extract reflects decomposed material. Although the extraction for colorimetric determination of peat humification uses a stronger base (2M NaOH compared to 0.1–0.5 M for HAs), extraction time has been found more important than the molarity (Cook et al., 1998), and also the amount of HAs increased with decomposition in peat (Zaccone et al., 2008). Thus, plant-derived macromolecular constituents are better reflected in the residue while the extract contains more humified material. Nonetheless, the applied methodology reflects an operationally defined OM fraction; the size of fragments/molecules in the extract varies between 10 (dialysis membrane) and 3000 kD (0.45 µm), while the residue contains fragments >0.45 µm (Table 7.2). The 10 kD lower boundary does not reflect the minimum molecular weight of the fraction retained in the membrane. Because the sample is protonated, H-bridges restrict the removal of lower-molecular weight fragments. Both fractions present a continuum of sizes. For example Li et al. (2004) studied eight humic acid fractions between 1 and 300 kD, which all fall within the extracts here (Table 7.2); and Preston et al. (1989) studied six fractions between 75 and 2000 µm (all residues here). Both studies found that the degree of decomposition correlated with the size of the fraction, with the smaller fractions being more decomposed; the association between size and decomposition is further supported by other studies (Bracewell et al., 1980; Moers et al., 1990; Gonzalez et al., 2003).

The comparison of pyrolysates of extract and residue for a selection of peat samples was valuable for the interpretation of peat pyrolysates, which, in combination with factor analysis, allowed separating between the effects of source, and several stages of aerobic and anaerobic decay (Chapters 2,3,5). The results of the different fractions provided support for the interpretation of *n*-alkane (Chapter 3) and lignin (Chapter 5) parameters, and comparison of the vegetation types will be discussed below.

Table 7.2. Size ranges of peat fractions, (macro)molecules, and (parts of) plants and microbes.

	kD	µm		kD	µm
Dialysis membrane	10		*Sphagnum* cell wall		0.5
NaOH extract (HA+FA)	10<3000	<0.45	Cellulose macrofibril		0.5
Residue (humin)	>3000	>0.45	Cellulose microfibril		0.003–0.005
Colloid fraction		0.001–1	Hemicellulose	Up to 200	
FA	<30		Filamentous fungi		1–20
Plant cell		1–100	Bacteria		0.1–2
Sphagnum cell		60	Benzene		0.0006
S2 layer (secondary cell wall)		0.5–4			

7.2.1. Lignin : cellulose and sphagnum acid : cellulose ratios during degradation

The ratio of lignin pyrolysis products to levoglucosan (Eq. 2; Table 7.3a) is used to examine the degradation of the lignin-cellulose complex (levoglucosan being a marker of cellulose). In all three vegetation types the lignin : levoglucosan ratio clearly showed a higher value in the extracts compared to the residues, except for the upper sample of the *Sphagnum*-dominated peat (zone 1; Table 7.3a; Figure 7.1). This higher value agrees with a higher abundance of lignin in HAs compared to bulk peat as defined by CuO oxidation (Zaccone et al., 2008), and a slightly higher contribution of aromatic compounds in HA compared to the humin fraction for a 1000-year-old *Carex* peat sample (Guignard et al., 2005). The difference between extracts and residues increases from Harberton zone 2, to Harberton zone 1 to Penido Vello (Fig. 7.1). Within the Penido Vello peat the difference between extract and residue was smallest in the relatively undecomposed samples that correspond to the Little Ice Age (8–20 cm). Figure 5.1 (Chapter 5) clearly indicated that, of all peat fractions, the lignin composition of the residues best resembled that of plants. Concerning the difference in size between the fractions, this implies that lignin is fragmented during either degradation or extraction (alkali breaks ester bonds), as lignin is an insoluble macromolecule. A possible explanation could be that fragments are released from the lignin cellulose complex during degradation, or the lignin cellulose complex is degraded to such an extent that fragments are extractable with 0.1 M NaOH (Orem and Hatcher, 1987); the newly exposed cellulose of these fragments is then mineralised while the lignin is only partially degraded and transformed (Disnar et al., 2008). This explanation agrees with the fact that lignin can be fragmented anaerobically but not completely mineralised. The broken lignin-cellulose fragments, depleted in cellulose, are extractable due to their smaller size and / or higher oxidation state. The residual macromolecular lignin-cellulose structure remains in the non-extractable residue and is relatively enriched in cellulose as cellulose is not depolymerised by NaOH-extraction and protected by lignin against microbial attack. So, the more decomposed the peat, the higher the abundance of extractable lignin, as also found by Zaccone et al. (2008) for CuO oxidation products in bulk peat and HA. The proposed mechanism is supported by colorimetric humification studies (Blackford and Chambers, 1993), of which the principle is based on the increase of aromatic lignin fragments upon decomposition. Colorimetric determination of peat humification is a frequently applied method to determine the degree of decomposition but appears to be highly influenced by changes in the botanical composition (Yeloff and Mauquoy, 2006), (fresh) vascular plant species showing a much higher degree of 'humification' than mosses; this in combination with the above interpretation indicates that the colorimetric humification method in fact reflects the contribution of vascular plants (lignin) to peat, or the degradation of lignin (not of peat OM) in a botanical homogenous peat.

Table 7.3a. Parameters that reflect relative degradation of lignin and cellulose in peat OM

	Harberton zone 2 0-3 m; *n* = 6			Harberton zone 1b[a] 3-9 m; *n* = 5			Penido Vello 0-1 m; *n* = 15		
	Ex	Bulk	Res	Ex	Bulk	Res	Ex	Bulk	Res
Polysaccharides (% TIC)	38.6	71.8	56.2	18.4	33.6	44.9	31.0	60.6	73.0
Lignins (% TIC)	10.6	6.0	11.6	26.8	21.4	22.7	33.3	19.2	9.8
Lignin : Polysaccharides ratio (1)	0.27	0.08	0.21	1.57	0.62	0.51	1.22	0.32	0.14
Lignin : levoglucosan (2)	0.69	0.13	0.38	5.09	1.27	0.86	3.87	0.56	0.18

Table 7.3b. Ratio between extractable and non-extractable peat for parameters 1–2 from Table 7.3a.

	Harberton zone 2	Harberton zone 1[a]	Penido Vello
Eq.1 Extract / Eq.1 Residue	1.3	3.1	8.2
Eq.2 Extract / Eq.2 Residue	1.8	5.9	21.5

[a]zone 1a was not included in the average, as it was dominated by brown mosses.

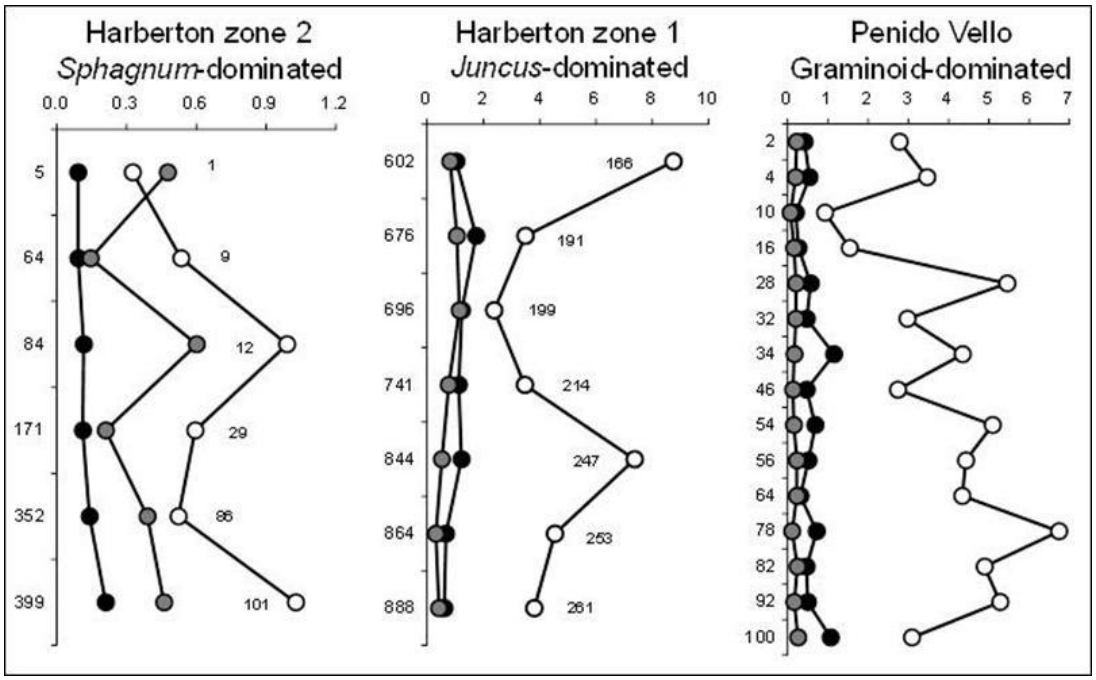

Figure 7.1. Lignin/levoglucosan ratios in extract (open), bulk (black) and residue (grey). Note the different scale of the axes. Sample numbers are indicated for Harberton.

The higher values of levoglucosan in bulk samples (and hence the lower ratios 1 and 2), compared to both extracts and residues, of peat dominated by *Sphagnum* (zone 2 Harberton, Table 7.3a, Figure 7.1) is remarkable, as bulk samples are expected to lie in between extracts and residues. This discrepancy could be related to the different structure of the polymeric network in the *Sphagnum* cell wall compared to lignin-cellulose in vascular plants. The main component that masks cellulose in *Sphagnum* is sphagnum acid (Tutschek et al., 1973; Wächter et al., 1986) which is thought to give *Sphagnum* its resistance to degradation (Rudolph and Samland, 1985), while other workers contribute this resistance

to structural polysaccharides in *Sphagnum* (Hájek et al., 2011). However, the marker of sphagnum acid, 4-isopropenylphenol, was clearly more abundant in the residues in zone 2 (Table 7.4). In peat with a lower contribution of *Sphagnum*, 4-isopropenylphenol showed similar (zone 1 Harberton) or lower values in the residues (Penido Vello) compared to the extracts. This difference in contribution of 4-isopropenylphenol to the fractions thus seems to be related to a different degree of decomposition in the vegetation types, and suggests that in peat with a lower contribution of *Sphagnum* (where decomposition has proceeded farther), sphagnum acid is degraded and rendered extractable (similar as is described above for lignin) while in *Sphagnum*-dominated peat (with a lower degree of decomposition) the polymeric network of sphagnum acid is less degraded and not extractable with 0.1 M NaOH. Specific polysaccharides, named sphagnan, have been identified in *Sphagnum* (Painter, 1991). In its native state, sphagnan occurs in the hyaline cell walls of *Sphagnum* mosses, where it is covalently linked to cellulose and an amyloid-like xyloglucomannan. Upon the death and decay of *Sphagnum*, however, it is slowly liberated in soluble form (Mitchel, 1996). More research on the architecture of polymeric phenols and sugars of *Sphagnum* and their degradation mechanisms is required to explain these differences.

Table 7.4 Abundance of markers in extract and residue for the different vegetation types

Pyrolysis product	Species	Harberton, zone 2	Harberton, zone 1	Penido Vello
4-Isopropenylphenol	*Sphagnum* spp.[a,b]	Ex << Res	Ex = Res	Ex >> Res
3-Methoxy-5-methylphenol	Lichens[a,b]	Ex = Res	Ex = Res	Ex >> Res
Ferulic acid methylester	Graminoids[a,b]	-	Ex > Res	Ex >> Res
Benzoic acid	*Empetrum rubrum*[a]	Ex = Res	Ex = Res	Ex >> Res
C_3H_3 guaiacols	*Nothofagus antarctica*[a]	Ex < Res	Ex = Res	Ex > Res
Squalene	*Eriophorum angustifolium*[b]	Ex = Res	Ex = Res	Ex = Res
Sterol 2	*Deschampsia flexuosa*[b]	-	-	Ex = Res
Sterol 4	*Erica*[b]	-	-	Ex < Res
Aliphatic	Woody tissue[b]	-	-	Ex < Res
4-Hydroxybenzene acetonitrile	*Juncus bulbosus*[b]	-	-	Ex >> Res
C_1-phenanthrene	*Carex binervis*[b]	-	-	Ex >> Res

[a]valid for Harberton; [b]valid for Penido Vello

7.2.2. Molecular markers

The better preservation of macromolecules in the residue compared to the extract does not necessarily mean that plant-specific molecules are better preserved in this fraction, since biomarkers comprise both 'free' and 'bound' molecules. Comparison of the abundance of the markers in both fractions did not yield consistent patterns in both peatlands (Table 7.4). The differences in abundance of markers between the fractions may be related to a different botanical source of the compound within each peatland (benzoic acid, C_3H_3 guaiacols), or reflect its origin from 'free' (3-methoxy-5-methylphenol) or bound (aliphatic compound) molecules (see Section 7.1). It could in addition be related to degradation state (4-isopropenylphenol).

Comparison of the different peat fractions supports the interpretation of the effects of decomposition on non-specific markers such as the *n*-alkane distribution (Chapter 3). This is further supported by the decrease in chain length of *n*-alkanes in the extract compared to the residue for the Penido Vello peat (Appendix 5 Chapter 5; Buurman et al., 2006).

7.3. OM dynamics in ombrotrophic peatlands

Two major problems with the interpretation of decomposition proxies in peatlands include the separation between the effects of source and decay, and between different decomposition stages (first and secondary decay) and decomposition types (aerobic and anaerobic). The degree of decomposition differed between the three vegetation types, with increasing contribution by *Sphagnum*, decomposition decreased. Because both the abundance of *Sphagnum* and the degree of decomposition are related to the water table, the question arises how these effects can be separated.

Vegetation type is a major factor that determines the interpretation of lignin decomposition parameters (Chapters 5 and 6). Furthermore, the simultaneous effects of botanical shifts (source material) and decomposition may cause conflicting hydrological interpretations for non-specific plant markers (e.g., *n*-C_{23} alkane, Chapter 3) or decomposition proxies (colorimetric determination of peat humification, Section 7.2). In addition to colorimetric humification (Mauquoy et al., 2002b; Langdon et al., 2003; Chambers et al., 2012), frequently used decomposition proxies in peat include the C:N ratio (Kuhry and Vitt, 1996; Borgmark and Schoning, 2006; Bindler, 2006; Martínez-Cortizas et al., 2007; Tillman et al., 2010; Biester et al., 2012), and the content or ratio of polysaccharides and lignin (Boon et al., 1986; Moers et al., 1989; Macko et al., 1991; Tsutsuki et al., 1994; Williams et al., 1998; Bourdon et al., 2000; Krull et al., 2004; Artz et al., 2006; Comont et al., 2006; Jia et al., 2008; Delarue et al., 2011; Broder et al., 2012). C:N ratio, and lignin or polysaccharide content, are similarly affected by the effects of both botanical changes and aerobic decomposition upon changes in hydrology. Wetter conditions cause a decrease of vascular plants and less decay, both resulting in a lower lignin content and higher C:N ratio compared to *Sphagnum* and more decomposed material, respectively. The hydrological interpretation is therefore probably not affected when the effects of source and decay cannot be separated. However, the underlying mechanisms that cause changes in C:N or lignin : carbohydrate ratios remain unclear, which can make the general application of these proxies problematical.

For all three vegetation types (*Sphagnum*-dominated, zone 2 Harberton; *Juncus*-dominated zone 1 Harberton; graminoid-dominated, Penido Vello) the polysaccharide and lignin content reflected changes in the water table, with a higher abundance of lignin reflecting relatively dry conditions. In Penido Vello, a high abundance of lignin reflected preferential decay of polysaccharides over lignin. In the *Sphagnum*-dominated peat of Harberton (zone 2) higher lignin contents indicated a higher contribution by vascular plants, which increase under drier conditions. In the *Juncus*-dominated peat of Harberton (zone 1), high values of polysaccharides were generally accompanied by low values of lignin, except for the deeper part with increasing mineral content. The absence of a correlation between lignin and polysaccharides in the deeper part of zone 1 may be due to a higher degree of decomposition, which causes a decrease of lignin (thus after decomposition of plant-derived

polysaccharides) while microbial material may contribute to the sugar content. It is known from some aerobic soils that microbial sugars contribute significantly to the overall carbohydrate content (Buurman et al., 2007). Thus in peatlands with a high degree of decomposition, such as tropical or minerotrophic peatlands, a high abundance of lignin may indicate relatively *wet* conditions, as in aerobic environments lignin is preferentially lost over highly resistant aliphatic polymers, and polysaccharides of microbial origin are newly produced. This hypothesis is in agreement with pyrolysis data of a core from a tropical peatland (Brazil, in preparation) where high lignin contents reflected relatively undecomposed OM. Thus, although for most high-latitude ombrotrophic peatlands the polysaccharide and lignin content probably reflect past surface wetness (water table), comparison with other proxies is recommended. In addition to aerobic decay, the presence of a depth trend indicates that polysaccharides are decomposed during long-term anaerobic decay, while lignin is not decomposed anaerobically.

Similarly to the lignin and polysaccharide content, the C:N ratio is affected by both botanical composition and by decomposition. High C:N values indicate relatively wet conditions because C is preferentially lost during aerobic decomposition (Malmer and Holm, 1984; Kuhry and Vitt, 1996). Botanical changes accompanying changes in hydrology also affect C:N ratios in peat (van Smeerdijk et al., 1989), as C:N ratios may highly differ between peatland plants. *Sphagnum* generally has higher C:N values compared to vascular plants (Hornibrook et al., 2000; Kleinebecker et al., 2007). In order to evaluate the influence of botanical composition on C:N, its behaviour in the different vegetation types was compared to pyrolysis results. Depth records of the C:N ratio are given in Figure 7.2 for both peatlands.

The C:N ratio clearly differed between the vegetation zones, and showed increasing values with increasing contribution of *Sphagnum* (or brown mosses, for zone 1a), with mean values of 63 and 47 in the *Sphagnum*-dominated peat (zones 2ab), of 28 in the bottom part of the core were brown mosses dominated (zone 1a) and of 23 in the *Juncus*-dominated peat (zone 1b); while in the graminoid-dominated peat of Penido Vello C:N varied between 18 and 47 with a mean value of 34. These differences between vegetation types clearly demonstrate the prevailing influence of source on the C:N ratio, caused by the lower N content in *Sphagnum* than vascular plants. However, within each vegetation type, the variation of C:N was differently correlated to pyrolysis results (Table 7.5).
In the *Sphagnum*-dominated peat (zone 2 Harberton), a positive correlation of C:N was found with the marker of sphagnum acid (R^2=0.75), while no correlation of C:N was found with the polysaccharide content and a weak correlation with lignin content (vascular plants) only when the upper three samples were excluded (Table 7.5). This combination suggests that 1) large part of the variation of C:N in *Sphagnum*-dominated peat is caused by decomposition rather than small increases of vascular plants upon drier conditions and 2) sphagnum acid is more easily degraded than both lignin and polysaccharides. This interpretation (both 1 and 2) is supported by the similar low lignin contents in the upper three samples of Harberton (indicating a low contribution of vascular plants), in combination with a high polysaccharide content and a large decrease in the marker of sphagnum acid from the upper sample (sample 0) compared to the other two (samples 1 and 9), and

by the much higher variation of the marker of sphagnum acid in *Sphagnum*-dominated peat (zone 2) compared to that of polysaccharides and lignin (Fig. 3.1a–d). A large decrease from sample 0 to sample 1 was also found for phenol, 4-methylphenol, 4-ethylphenol and 4-vinylphenol, together accounting for a decrease from 23 to 2% TIC. This decrease in phenols provides further support for the preferential decay of sphagnum phenols over polysaccharides, which should largely explain the mass loss as suggested by the decrease from 80 to 45 in C:N (Figs. 7.2 and 7.3) and is in agreement with the amount of mass loss during the first stage of decay found for *Sphagnum* litter (up to 30 %; Asada et al., 2005). The nature of the recalcitrance to decomposition of *Sphagnum* has been frequently discussed (van Breemen, 1995). Phenolic compounds (sphagnum acid) as well as pectin-like polysaccharides (sphagnan) have been identified as causal agents for low degradation. My study suggests that low degradation rate is not caused by sphagnum acid (van Breemen, 1995; van der Heijden et al., 1997), supporting the claim that polysaccharides such as sphagnan cause recalcitrance (Hájek et al., 2011). These authors noted that sphagnan did not serve as a C source for microbes but rather reduced decomposer activity, a property that would normally be associated with phenolics and lignin. The preferential degradation of phenolics over polysaccharides in *Sphagnum* litter may also explain the higher polysaccharide content found in *Sphagnum* peat compared to fresh plants (Moers et al., 1989; Comont et al., 2006; Tables 2.1 and 2.4).

The variation of C:N within graminoid-dominated peat differed from that in *Sphagnum*-dominated peat. The C:N ratio in both zone 1 of Harberton and Penido Vello showed a much lower variation and no correlation with the marker of *Sphagnum* (Table 7.5), which can be explained by the low contribution of *Sphagnum* to these peats (Table 7.4). Because polysaccharides are preferentially lost in these peats, a negative correlation with C:N should be expected. However, such a correlation was only weak in zone 1b of Harberton (R^2=0.43), and absent from Penido Vello where even a slightly negative correlation was found in the upper meter (Table 7.5). Furthermore, the C:N ratio is mainly determined by N in all vegetation types, while in the upper 80 cm of Penido Vello carbon also contributed to its variance (Fig. 7.3). It is not exactly clear which factors cause these differences and several processes may influence these discrepancies, including N mining (Lindahl et al., 2007), N deposition (Heijmans et al., 2002) and the preferential decomposition of C-poor compounds such as polysaccharides (e.g. glucose: $C_6H_{12}O_6$) over compounds richer in C (e.g. C_3-guaiacol: $C_{10}H_{12}O_2$). The higher contribution of C in phenolics (e.g. sphagnum acid: $C_{11}H_{10}O_5$) compared to polysaccharides and the preferential degradation of phenolics over polysaccharides may also contribute to the strong correlation beteen sphagnum acid and C:N in *Sphagnum*-dominated peat. The opposite correlation of C:N with polysaccharides in zone 1b of Harberton (positive) and the upper meter of Penido Vello (negative), both graminoid-dominated peat, can then be explained by the different degree of decomposition (i.e. polysaccharide content) between both. The upper 80 cm of Penido Vello reflects relatively young peat (<1900 years BP), while zone 1b of Harberton has ages between 5500 and 9500 years BP.

Another observation from the C:N depth records is that in Harberton the transition from moss to vascular plants (zone 1a to zone 1b) is gradual while the transition from vascular plants to *Sphagnum* (zone 1b to 2a and zone 2a to 2b) is extremely sharp. This sharp transition is probably related to the absence of roots in *Sphagnum*. The gradual transition then reflects a more mixed vegetation. The sharp transition is consistent with the claim of Scheffer et al. (2001) that litter quality (especially of *Sphagnum*) overrides habitat effects, thereby causing a disconnect between minerotrophic and ombrotrophic peat. The presence of roots of vascular plants (often ericoids) with mycorrhizal fungi may result in N mining (Craine et al., 2007), which could also contribute to explaining the increases in C:N ratio with depth in Penido Vello.

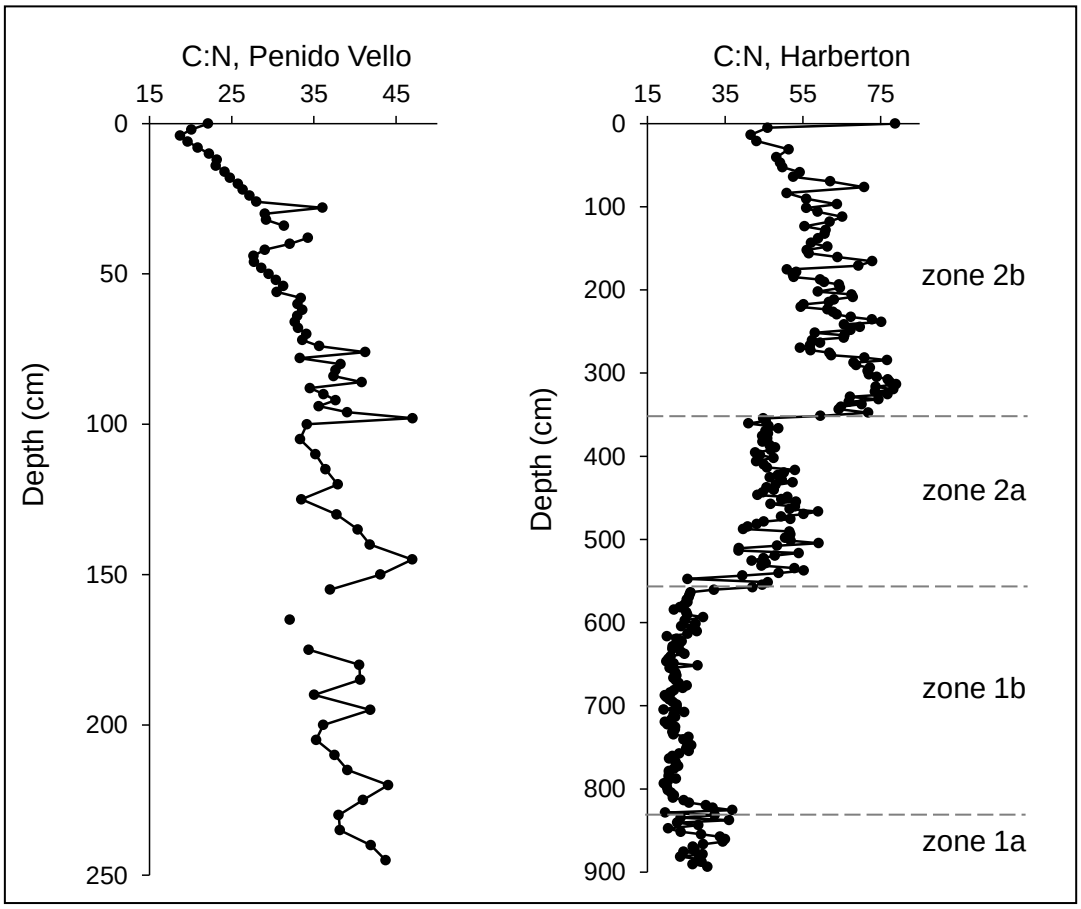

Fig. 7.2. Depth records of C:N ratio. Zone 1a = dominated by brown mosses, zone 2b is dominated by *Juncus* sp., zone 2 is dominated by *Sphagnum* of which 2a has a higher contribution of vascular plants.

Thus the difference in litter quality between *Sphagnum* and vascular plants is a major factor that determines the variance of C:N with depth. First, differences in C:N ratios between *Sphagnum* and vascular plants have a major impact on the C:N in peat. Second, within *Sphagnum*-dominated peat a decrease of the C:N ratio mainly reflects decomposition, during which C-rich compounds (phenolics) are preferentially degraded over C-poor compounds (sugars), while in graminoid-dominated peat this is not true and several processes may influence the effect of mass loss on the C:N ratio. The C:N ratio could thus be a consistent decomposition proxy in *Sphagnum*-dominated peat but not in graminoid-dominated peat. In *Sphagnum*-dominated peat also sphagnum acid (4-isopropenylphenol) reflects decomposition (Abbot et al., 2013). However, by using the C:N ratio in *Sphagnum*-dominated peat as a decomposition proxy, the effects of large botanical changes, minor increases of vascular plants, and secondary decay may disturb the hydrological interpretation (e.g. sample 183 in Fig. 3.1, which probably reflects a short period of *Sphagnum* dominance indicated by a sharp decrease in lignin content which coincides with a sharp increase of polysaccharides, while the markers of *Sphagnum* and C:N only show a minor peak). Lignin in *Sphagnum*-dominated peat is less degraded than in graminoid-dominated peat, and it is assumed that the small contribution of vascular plants completely determines the lignin content (not a preferential degradation of polysaccharides). The results support the reliability of the ratio of 4-isopropenylphenol to lignin (McClymont et al., 2011) to indicate bog hydrology, as sphagnum acid is easier degraded than lignin, and vascular plants (lignin) increase with drier conditions. But since both are affected by different processes, their separate depth records may provide more detailed information than the ratio.

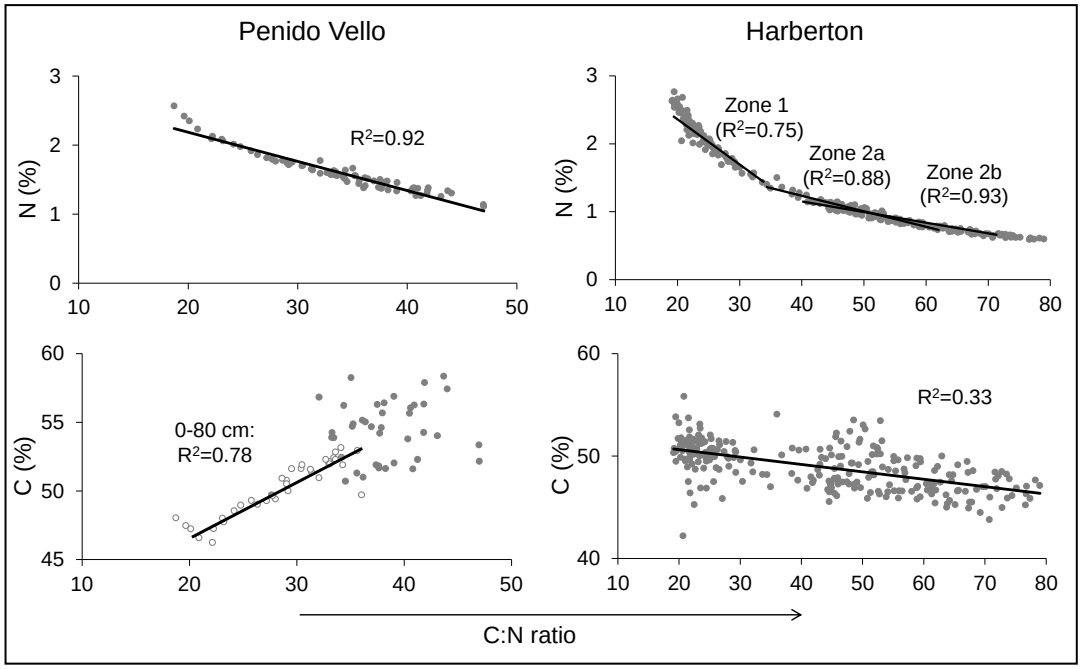

Fig. 7.3. Correlations (R^2) between C:N ratio and C and N content. Open points indicate samples from 0–80 cm.

Table 7.5. Correlation (R^2) of the C:N ratio with the summed polysaccharides, the summed lignin, and the marker of sphagnum acid (4-isopropenylphenol) in different vegetation types. Values <0.20 are not indicated; R^2 values refer to positive correlations unless printed in bold (negative).

	n	Polysaccharides	Lignin	4-isopropenylphenol
Harberton				
Sphagnum peat (zone 2b)	14	-	- (0.44[a])	0.75
Sphagnum-Juncus peat (zone 2a)	20	-	-	-
Juncus peat (zone 1b)[b]	27	0.44	-	-
Whole core (all zones)	67	0.77	**0.80**	0.58
Penido Vello (graminoid peat)				
0–100 cm	51	**0.33**	0.37	-
100–245 cm	28	-	-	-
All	79	**0.22**	-	-

[a]Without the upper three samples (0,1 and 9); [b]sample 150 excluded.

7.4. Conclusions and Outlook

The molecular composition of organic matter in peatlands reflects local conditions and stores information about botanical composition (plant source) as well as the degree of and conditions during decomposition. Both vegetation and decomposition proxies are frequently used to reconstruct past environmental conditions. A reliable hydrological (and hence palaeoclimatological) interpretation of such proxies in peatlands requires the understanding of the interactions between decomposition and botanical composition and their reaction to changes in the water table. The hypothesis that the use of molecular chemistry can improve our understanding of such interactions proved correct. Pyrolysis-GC/MS in combination with the applied research design provided detailed chemical information that gave insight in both vegetation and decomposition characteristics. This methodology thus has a high potential for the reconstruction of past environmental conditions.

Plant analysis

A biomarker approach assumes that plant characteristics reflect an accurate record of the original plant cover at the time of peat deposition (Blackford, 2000). Many biomarker researches in peatlands are concentrated on the plant-specific distribution of straight-chain aliphatic lipids such as *n*-alkanes (Farrimond and Flanagan, 1996; Ficken et al., 1998; Nott et al., 2000; Baas et al., 2000; Pancost et al., 2002; Nichols et al., 2006; McClymont et al., 2008; Vonk and Gustafsson, 2009; Ortíz et al., 2010; Huang et al., 2012; Andersson et al., 2011; Andersson and Meyers, 2012; López-Días et al., 2013; Ronkainen et al., 2013). However, these lipid distributions give a plant-specific combination of compounds and do not provide a specific compound (Jansen et al., 2007). If a molecule is not plant-specific within a given ecosystem, its use as a marker can be compromised, and the effects of decomposition and changes in vegetation type (Chapter 3) or the different contribution of *n*-alkanes to plant species (Pancost et al., 2002; Huang et al., 2012) may interfere

with the plant-specific distribution of such compounds and cause errors in the hydrological interpretation. A similar problem may occur when the lignin composition is used to reflect decomposition (Tsutsuki, 1994; Williams et al., 1998; Bourdon et al., 2000; Tareq et al., 2006; Disnar et al., 2008). Chapters 5 and 6 showed that lignin decomposition parameters are influenced by botanical source and anaerobic decomposition and part of them are not valid in graminoid-dominated peat.

The influence of decomposition, vegetation type and intrinsic plant characteristics is more straightforward for plant specific markers, as these - contrary to *n*-alkanes and lignin only have one source. Nevertheless, the simultaneous use of several markers is recommended as it allows validation by comparing their depth records and considering their preferential habitat. Cooperation with plant ecologists is therefore important, providing knowledge on plant morphology, growth characteristics and peatland ecology. Shifts in vegetation composition on peatlands is complex, may depend on other factors than hydrology (Malmer et al., 1994; Heijmans et al., 2002), and may differ between vegetation type and geographical location. The advantage of pyrolysis-GC/MS in plant analysis is that it gives information on both free and bound molecules, and that its application to peat OM provides a range of characteristics by which the markers can be validated. Depending on the results and the purposes it may be essential to use other techniques such as GC/MS after a solvent extraction for free molecules, as a contribution of bound molecules cannot easily be separated from the free target molecules (e.g. *n*-alkanes), and (^{13}C labeled) TMAH thermochemolysis for lignin and phenolics as polar groups are often lost during pyrolysis.

Fractionation of peat OM

Although one of the advantages of pyrolysis of peat is that no time consuming sample pre-treatments are needed, analysis of different peat fractions provided insight into decay processes and the effects of botanical shifts in both *Sphagnum* (Chapters 2, 3) and graminoid-dominated (Chapter 5) peat that would not have been obtained with analysis of bulk samples alone. Nevertheless, analysis of bulk peat is considered to be most reliable for the reconstruction of environmental conditions, as possible chemical transformations related to extraction are excluded.

Factor analysis

Factor analysis is a powerful method to explore large geochemical datasets, and can be used to reduce a high number of original variables (pyrolysis products) to a minimal number of new variables (the factors) based on the shared variance of the original variables in the samples. Factor analysis has been successfully used in combination with pyrolysis of peat OM (Moers et al., 1989; van der Heijden and Boon, 1994; Buurman et al., 2006). Factor analysis applied to a high number of pyrolysis products on a few samples allowed selection of the most relevant pyrolysis products and reduction of the number of pyrolysis products that needed to be quantified in a large number of samples (Chapter 2). The problem in interpreting decomposition proxies is that the unsaturated upper part of the peat, can be affected by secondary aerobic decomposition (Borgmark and Schoning, 2006). Furthermore, the influence of anaerobic decay during the first stages is generally neglected, but anaerobic decomposition of

fresh material also causes considerable mass loss with effects on the remaining OM (peat), that may be different from those of aerobic decay. Factor analysis was useful first to identify different processes (including source, first and second stage of decay, and aerobic and anaerobic decay), and thereafter to determine the influence of these different processes on individual pyrolysis products (Chapters 5 and 6). The vertical variation of the factor scores allowed comparison with other proxies (Chapter 4). Such information cannot be obtained by visible comparison of pyrograms.

Vegetation type

Peatland vegetation type has a major influence on botanical and decomposition proxies; examples include *n*-alkanes (Chapter 3), lignin composition (Chapters 5 and 6), C:N ratio and polysaccharide and lignin content (Section 7.3). The importance of vegetation type is supported by the fact that in Harberton, for most parameters, differences *within* vegetation types were smaller than *between* vegetation types. Major environmental changes (e.g. a change in vegetation type) may be invisible using such non-specific proxies.

The use of C:N ratio (Malmer and Holm, 1984; Kuhry and Vitt, 1996) and the colorimetric humification method (Blackford and Chambers, 1993) as decomposition proxies originated from studies in *Sphagnum*-dominated peatlands. It is remarkable that such operationally defined proxies are frequently applied to different peat types or peat-sediment systems (Turney et al., 2004, 2006; Wüst and Bustin, 2004; Martínez-Cortizas et al., 2007; Muller et al., 2008; Tipping et al., 2008; Ma et al., 2009; Wang et al., 2010; Swindles et al., 2012), assuming that they reflect a single characteristic that is similarly affected by environmental conditions. My data show that different processes act on C:N ratios in different peatlands; a warning that proxies cannot be used independent of context.

High-resolution sampling

Analytical pyrolysis of high-resolution sampled cores provided detailed information on the molecular composition of peat, which provides fundamental knowledge on peatland C dynamics that cannot be obtained from analysis of a low number of samples (relative to depth and age). The results showed the importance of analysis of peat cores sampled at high-resolution. In Penido Vello, better correlations were found between pyrolysis data for the upper meter sampled every 2 cm compared to the deeper part sampled every 5 cm. Although the Harberton peat core was sampled at high resolution and all samples were analysed for elemental composition and ash content, only a selection of samples has been analysed with pyrolysis. This approach is sufficient for the reconstruction of environmental conditions using well-defined chemical parameters, but in order to identify (the effects of) processes such as is done by comparing C:N ratio, 4-isopropenylphenol and polysaccharide and lignin content in the *Sphagnum*-dominated part (Section 7.3), a higher resolution is required (see Section Further research below).

Evaluation of pyrolysis-GC/MS in peat research

The purpose of this study was to examine the use of pyrolysis-GC/MS to reconstruct environmental conditions, including vegetation and decomposition proxies. Pyrolysis results showed good agreement with other proxies including elemental composition, solid-state ^{13}C NMR and ash content. The wet periods that my study identified agree with other environmental studies that are based on different methods. The use of pyrolysis in peat research has thus been validated, which indicates that the semi-quantification often referred to as a disadvantage of the method, is not disturbing the interpretation and the relative changes can be used to draw conclusions. Advantages of pyrolysis include the low amount of sample needed and that no time-consuming chemical pre-treatments are necessary. However, the main advantage is that pyrolysis provides a range of molecular characteristics, representing all chemical compound classes. Pyrolysis thus provides information on both the *abundance* of groups of compounds (lignin, polysaccharides, N-compounds, polyaromatics, etc.) and the *composition* within these groups.

The detailed information provided by pyrolysis-GC/MS gave insight into the molecular structure of peat OM that can be related to its source and degradation environment. This molecular information at a structural level cannot be obtained with non-destructive methods such as solid-state ^{13}C NMR and FTIR, or with operationally defined decomposition proxies (e.g. elemental ratios or the colour intensity of a leachate). Nevertheless, comparison with such proxies is useful given their common application in past studies. A disadvantage of pyrolysis is the complexity of data interpretation. However, considering that the data reflect the complexity of the studied system, the latter emphasises the importance of a fundamental understanding of peatland OM dynamics for a correct reconstruction of past environmental conditions.

Although the value of pyrolysis techniques in peat environmental research has been recognised by other authors (Boon et al., 1986; Moers et al., 1990; Calvert et al., 1991; van der Heijden et al., 1997; Huang et al., 1998; Kuder and Kruge, 1998, Kuder et al., 1998; Gleixner and Kracht, 2001; Bourdon et al., 2000; González et al., 2003; McClymont et al., 2005, 2011; Disnar et al., 2008; Abbott et al., 2013; Swain and Abbott, 2013), the number of scientific publications on peat using this method is surprisingly small compared to the importance of peatlands in climate research, both by its C storage (Charman et al., 2013) and by providing palaeoenvironmental records (Chambers et al., 2012), and the huge amount of climate-related peat research (thousands of publications). Most peat studies are, apart from biological proxies (macrofossils, pollen and non-pollen palynomorphs, testate amoebae, beetles), mainly concentrated on bulk density, elemental composition, colorimetric determination of humification, and greenhouse gas fluxes. Thus these studies measure the effects of decay and botanical composition with proxies that only contain very general information, while past environmental conditions are stored in detail by the molecular composition in the remaining material, peat.

Further research

A number of papers is in preparation in which pyrolysis results are compared with generally applied methods including both biological and decomposition proxies, in both peatlands in the same areas (Galicia and Tierra del Fuego) as well as in northern and tropical regions. This is expected to provide more detailed information on peatland OM dynamics.

The importance of peatlands in the global C cycle and the importance of *Sphagnum* in northern peatlands require a better understanding of the mechanisms underlying *Sphagnum* decay. Biological proxies are considered to be more precise in reflecting past changes because they reflect exactly the conditions during their time at the surface, while decomposition may also affect peat during later time periods of water table lowering (secondary decay). However, comparison of the C:N ratio and pyrolysis products of sphagnum acid, cellulose and lignin (Section 7.3) suggests that decomposition may be more sensitive to hydrological changes in *Sphagnum*-dominated peat. Differential degradation of sphagnum acid, cellulose and lignin may be a key for a very detailed water table reconstruction. More detailed and fundamental research is required to test this hypothesis.

Furthermore, the interaction with the inorganic phase, dissolved OM production, greenhouse gas fluxes and microbes require investigation. As the molecular composition of peat OM is the connecting factor between such studies, differences in peat molecular chemistry are expected to help explaining the results, together providing fundamental knowledge of peatland C dynamics and its relationship with environmental factors.

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