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Linking the molecular composition with peatbog status

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Gianluca Trifirò

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Abstract

Peatlands are organic matter rich soils that provide many ecosystem services from carbon storage to water filtration and flood management. Unfortunately, peatlands are fragile systems and as a result of human activities, such as afforestation as well as climate change, a large fraction of global peatlands are damaged. Restoration and protection of peatlands have, therefore, received world-wide attention and multimillion pound investment.

In order to understand if restoration methods are successful we need to investigate the peatland status, which in turn requires an understanding of the processes of peat decomposition and restoration at the molecular level. This work examined two peatlands, a raised bog located at the Red Moss of Balerno (Balerno, Edinburgh) and a blanket bog located in the Flow Country (Caithness and Sutherland) with different ecological status (near natural, drained/damaged, drain-blocked/restored). It was possible to link the molecular structure of those peatlands with their conditions using state-of-the-art spectroscopic techniques, namely Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR), Nuclear Magnetic Resonance (NMR) spectroscopy, Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) and Laser Desorption Ionization Mass Spectrometry (LDI-MS), and combine the results with traditional parameters, such as vegetation survey, hydrology and bulk peat characteristics. To identify key relationships from the complex data, a number of statistical methods and visualisation tools are required. The combination of both physical and chemical analysis will lead to an accurate evaluation of key indicators related to peatland status that are useful to predict the effects of restoration and contribute to successful restoration strategies.

A detailed molecular-level examination of both liquid and solid phases of one 50 cm peat core, divided into 13 different layers demonstrated the different capabilities of each spectroscopic or spectrometric technique for examining changes occurring with depth and relationships with hydrology or physicochemical factors. Specifically, FT-IR only showed functional group changes with depth irrespective of the position of the water table, while solid and liquid state NMR, detected a correlation between compound classes and water table, in agreement with bulk density. ESI FT-ICR MS of PW-DOM showed higher variation throughout the core than previously reported, demonstrating again the need to examine multiple layers.

A comparison of cores taken from drained and drain-blocked cores from Red Moss of Balerno, indicated that differences do occur on a molecular level, however both ATR-FTIR and solid state ^{13}C NMR data showed that for the solid phase samples there are some differences between each of the cores even from the same site with depth, which hindered clear differences due to health status. Nevertheless, it was possible to detect an increase in alkyl compounds in the restored area at bottom layers, while the damaged site was more characterised by a higher number of O-alkyl fractions until a depth of 15 cm depth. Differently, the liquid state ^1H NMR data showed a clearer difference between the two sites in terms of molecular composition, precisely aromatics are more prevalent in the damaged area, while the restored area is more abundant in carbohydrate compounds. This was explained by specific metabolites, which are able to drive the main differences in the NMR spectra. The ESI FT-ICR MS on PW-DOM demonstrated, using the modified aromaticity index, that the restored site has higher plant-derived polyphenols, highly unsaturated and phenolic compounds than the damage area, while there is an opposite trend for aliphatics.

The ATR-FTIR, solid state ^{13}C NMR and LDI MS results about cores taken from the near natural, drained and drain-blocked sites in the Flow Country show a higher content of aliphatic compounds, increasing with depth, in the restored area, while carbohydrates decreased in both near natural and drained sites throughout the cores. Using both liquid state ^1H NMR and ESI FT-ICR MS, the cores extracted from these three sites compared to Red Moss indicated that both these kind of peatlands show a similar rising trend of condensed hydrocarbons with depth in PW-DOM on restored sites, precisely an increase of them going down the cores. Then, it can be noted that the lipid content in the solid state phases demonstrated a clear increasing pattern with depth in both restored sites from Flow Country and Red Moss.

Overall, the difference between those two different sites might be attributed to their different nature in which some variables such as pH, vegetation, microbial activities, water content and/or water level play a role in the composition at molecular level. However, despite the investigation of peatlands at molecular level was able to give a great contribution in understanding their status, future work needs to focus on monitoring other key factors that can affect the carbon storage in these ecosystems.

Lay Summary

Peatlands are organic matter rich soils that are mainly found in the Northern latitudes where they formed under cold and wet conditions.. Peat bogs are vital ecosystems to life on this planet and rich in unique plants and animals. In addition, peatlands are a great carbon sink since they are able to actively take carbon dioxide from the atmosphere and store twice as much carbon than all the Earths' biomass. Moreover, they act as a sponge storing and filtering water, preventing floods and safeguarding our drinking water supply. However, peatlands need to be in optimal conditions to conduct all these important services, but unfortunately the majority of peatlands are in a state of degradation. This is a global concern as peatlands in damaged states release larger amounts of the stored carbon mainly to the atmosphere, which affects our daily life and brings more complications on tackling the greenhouse gas emissions. One of the major causes of peatland damage is drainage and in order to restore the damaged sites, they have been rewetted (drainage) by installing dams. In order to monitor if restoration is working, some key factors such as vegetation, gas fluxes and water table are usually measured. However the question that arises is whether restoration is having countable effects even below the peatlands surface. In this PhD project, we investigated the peat properties of near natural, damaged and restored sites of a Scottish raised and blanket bog at the molecular level. We compared the differences between these sites and characterised the changes happening with depth. To do this, we extracted 50 cm peat cores from locations near drains or blocked drains as well as sites identified as having no historic human activity. We examined the physical properties of each core such as colour, volume, bulk density, water and ash content which also helped to determine how much organic matter each peat core contains with depth. Finally, to get a

deeper insight we studied both the solid phase and the pore water, plus labile part coming out from the solid phase, at the molecular level to directly assess changes in peat composition. In order to examine all these different samples at the molecular level, we applied very powerful techniques such as Fourier Transform Ion Cyclotron Resonance mass spectrometry, to find out the molecular formulae of the major compounds present in peat, and Nuclear Magnetic Resonance spectroscopy which uses radio waves to fingerprint the different compound classes within samples placed in a strong magnetic field. This project involved measuring hundreds of samples and created a very complex data set. Therefore, in order to visualise the changes, multivariate statistical analysis techniques such as principle component analysis (PCA) have been used. By using this statistical approach in combination with physical-chemical and molecular differences between sites, it was possible to describe the differences between near natural, damaged and under restoration sites, to determine if rewetting peatlands is helping to bring these kind of soils back to a healthy conditions.

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Abbreviation list

AI: aromaticity index

AI_{mod}: modified aromaticity index

APPI: atmospheric pressure photoionisation

ATR-FTIR: attenuated total reflectance Fourier transform infrared

BBOT: 2,5-Bis(5-tert-butyl-2-benzo-oxazol-2-yl) thiophene

C: carbon

CHN: carbon, hydrogen, nitrogen

CI: chemical ionisation

CP-MAS: cross polarisation- magic angle spinning

DBE: double bond equivalence

DOC: dissolved organic carbon

DOM: dissolved organic matter

ESI: electrospray ionisation

FA: fulvic acid

FC: flow country

FID: free indication decay

FT-ICR MS: Fourier Transform ion cyclotron resonance mass spectrometry

HA: humic acid

HPDEC: high power decoupling

HW-E: hot water extract/ion

IAT: ion accumulation time

NaOH-E: NaOH extract

NOM: natural organic matter

OM: organic matter

PCA: principle component analysis

PC: principle component

PPL: priority pollutant

PW: pore water

RM: Red Moss

RSPB: Royal Society for the Protection of Birds

SNR: signal to noise ratio

SNV: standard normal variate

SOM: soli organic matter

SPE: solid phase extraction

SRFA: Suwannee River fluvic acid

SWT: Scottish Wildlife Trust

TOF: time of flight

VK: van Krevelen

WEOM: water extracted organic matter

1. Introduction

1.1 Peatlands: definition, global distribution and importance

Peatlands are organic matter (OM) rich soils composed of dead and partially decomposed plant remains that has accumulated in situ under water-logged conditions. (1) In such conditions, peat starts to accumulate as the rate of input of organic material from the surface exceeds the rate of decomposition of the new material. The OM fraction in peat material is exceedingly high and varies from above 20-25% OM for peaty soil, to more than 50-60% for peat. (5) Due to the high OM content, peat is usually less dense than mineral soils, (4) with most of its volume being occupied by water. Thus peatlands are considered as transitional environments between terrestrial and aquatic ecosystems. (6)

Globally, peatlands cover 2-3% of the Earth's landmass, (2) covering large areas of North America, northern Europe, western Siberia, Indonesia and south-east Asia. (3) Peatlands in the Northern Hemisphere were formed due to changing climatic conditions. For instance, circum-arctic peatlands started to develop approximately 16,500 years ago, after the last glacial maximum, with a period of expansion between 8,000-12,000 years ago due to the increasing temperature. This estimation has been done from the analysis of more than 1500 radiocarbon dates. (3) However, after the thermal maximum during the Holocene (~8,000 years ago), climate cooling slowed peat formation. (3) In the UK, peat formation is proven to have started at the end of the last ice age (9500-10,000 years ago). (7)

Peatlands provide many ecological, biogeochemical and hydrological functions. First and foremost they are powerful agents of carbon capture, in fact has been estimated that peatlands in the UK can store 3.2 billion of tones of carbon. (7) (8)

In their natural state, peatlands are also able to catch carbon dioxide (CO₂), drawing it down from the atmosphere through plants. (8) Peatlands are also important for atmospheric pollutant interception as well as flood prevention. (9) 70% of our drinking water is filtered through peatlands (10) and water derived from active bogs is naturally of high quality. (11) (12)

Peatlands are also habitats for rare flora and fauna as well as places of historical and cultural significance. (13)

1.2 Scottish peatlands

The focus of this study is Scottish raised and blanket bogs, therefore this section provides a general introduction to Scottish peatlands and more specific details on these two kind of bogs, including their formation and typical vegetation. In addition, the ecosystem services provided by Scottish peatlands and their current status, in terms of damage and restoration processes, will be described.

1.2.1 Definition of peat in Scotland

Peat is defined by OM content and depth. While the OM content should be > 50% w/w, there is no single formal clarification of what depth of OM is required to classify a soil as peat in Scotland. (10) It is stated by the Soil Survey for Scotland that peat should have an organic layer or layers that exceed 50 cm from the soil surface. (5) However it should be noted that ecologists state a minimum peat depth of 30 cm, while geologists use 1 m as the threshold. (5) (10) (11) Figure 1.1 shows a map of average peat depth in Scotland. This map shows that there is areas of Scotland where the peat depth is over 2 meters.

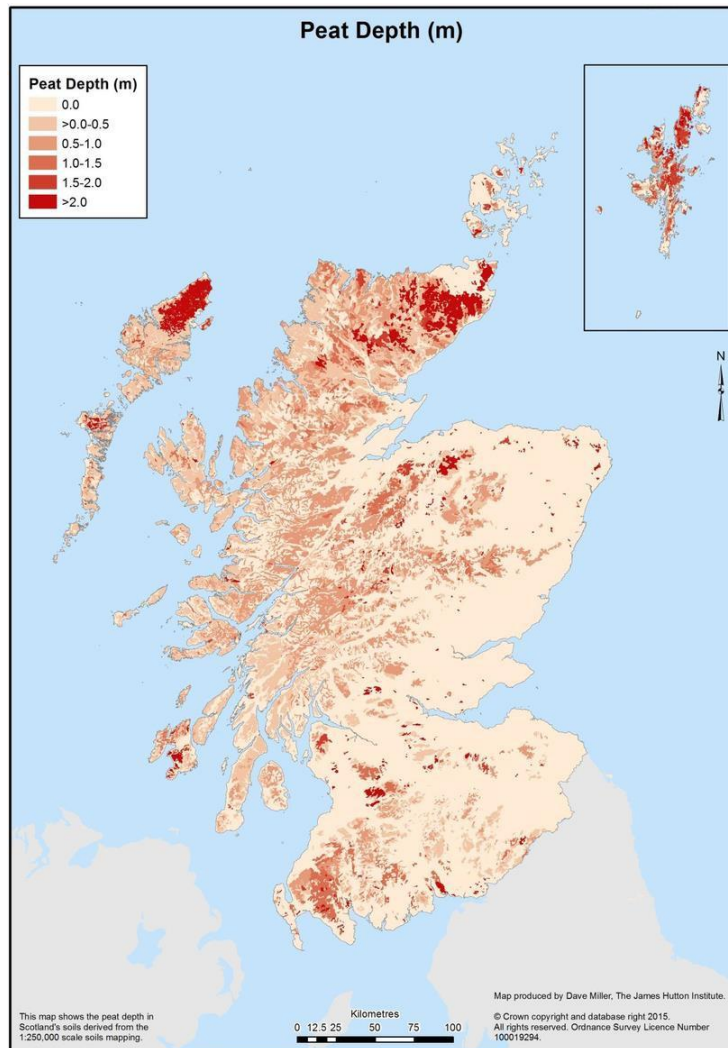


Figure 1.1 Average estimated peat depths in Scotland. (176)

Taking the minimum peat depth as 0.5 m, Scotland has around 2 million ha of peatland. From the map in Figure 1.2, these peatlands are mainly located in the western and northern parts of the country, where suitable conditions of high rainfall, cold temperatures and glaciated landscape enabled there formation. (5) (11)

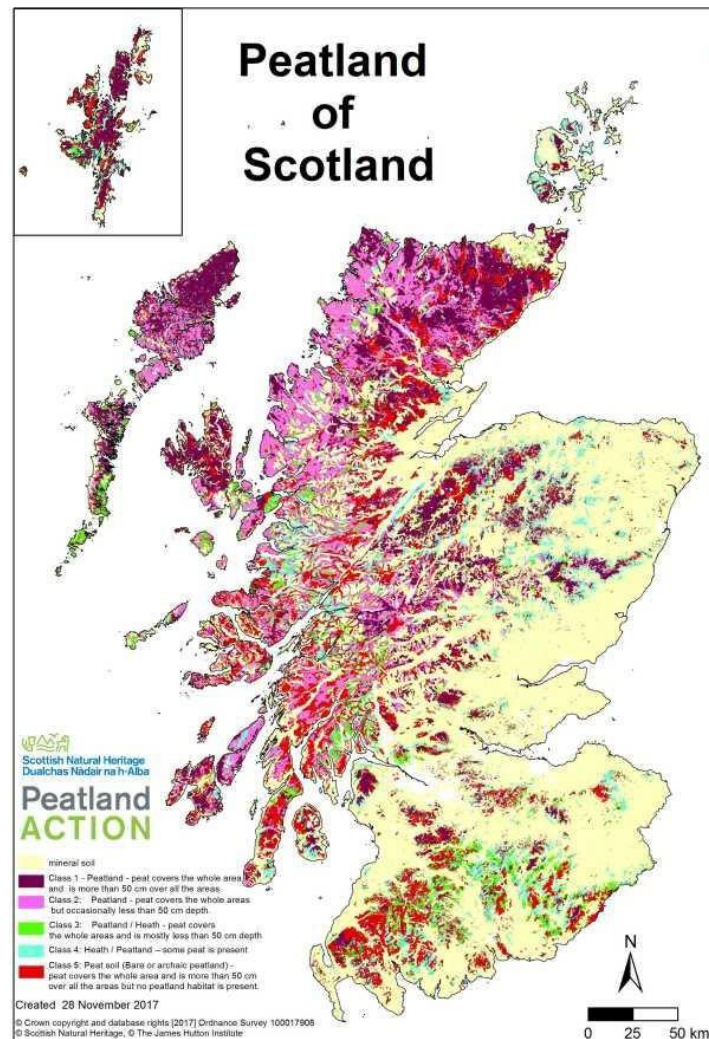


Figure 1.2 Distribution of peat and peaty soils in Scotland. (5)

1.2.2 Scottish Peatland types and formation

According to literature, there are four main natural peatland types in Scotland: blanket bog, raised bog, fen and bog woodland. (5)

Peatlands are classified as fens or bogs based on landform, water chemistry, nutrient status, vegetation and hydrology. (5) (10)

Bogs receive all their water and nutrients from precipitation and are termed ombrotrophic. In contrast, fen formation is influenced by

inflowing ground and/or surface water supply. Thus fens receive nutrients from both atmospheric deposition as well as percolating ground waters and are termed minerotrophic. (10) (12) Fens are therefore nutrient-rich environments that can be acidic or alkaline with pH values ranging from 4.0 - 9.0. (15) Bogs are on the other hand are nutrient-poor, acidic environments with pH values ranging from 3.5 - 4.5. (16)

Although, the distinctions can sometimes be blurred, bogs and fens usually have very different vegetation, due to the different nutrient status and pH. For instance, bogs tend to be dominated by a range of *Sphagnum* mosses, shrubs and pockets of sedges such as cotton grass (Figure 1.3). Fens tend to be dominated by sedges, grasses, rushes, and brown moss species.

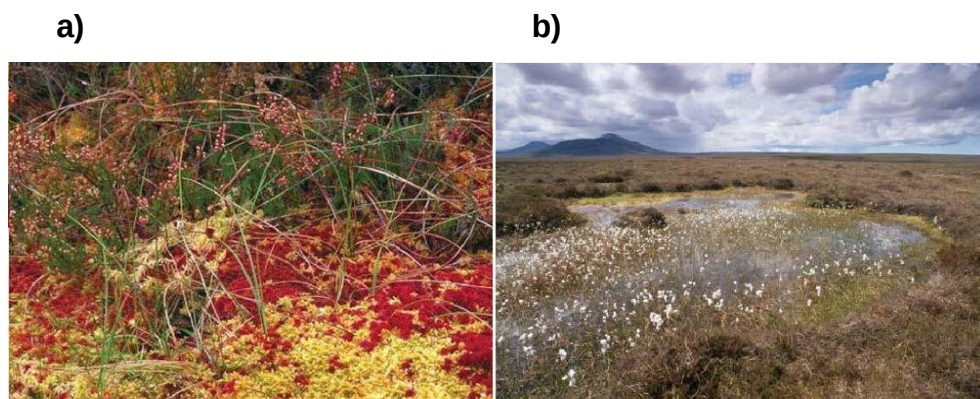


Figure 1.3 Typical flora in peatlands, a) *Sphagnum* moss, (178); b) cotton grass and moss on a bog pool in Forsinard. (20)

Bogs are the main focus of this focus of the project and will be discussed in more detail here. Overall, bogs can be divided between blanket and raised bogs, depending on their different formation and other environmental factors.

Bogs which form in upland colder areas with high rainfall and poor drainage are known as blanket bogs (Figure 1.4b). (10) (11)

In lowland areas, bogs that form on wet floodplains or in basins, and that rise up slightly above the surrounding landscape, are classified as raised bogs (Figure 1.4a). Raised bogs formed in depressions occupied by shallow lakes characterized by anaerobic conditions, after un-decomposed plant material generated a thick layer of peat rising above the lake. (19) Their surface peat layers are raised above the elevation of surrounding mineral soils.

Blanket bogs are vast unconfined areas that cover the ground like a blanket (Figure 1.4b). They develop in areas that are constantly wet with low evapotranspiration which allows accumulation of peat on the sloping ground. They also form on top of mineral soils that have become leached over time as a result of heavy rainfall. (3)

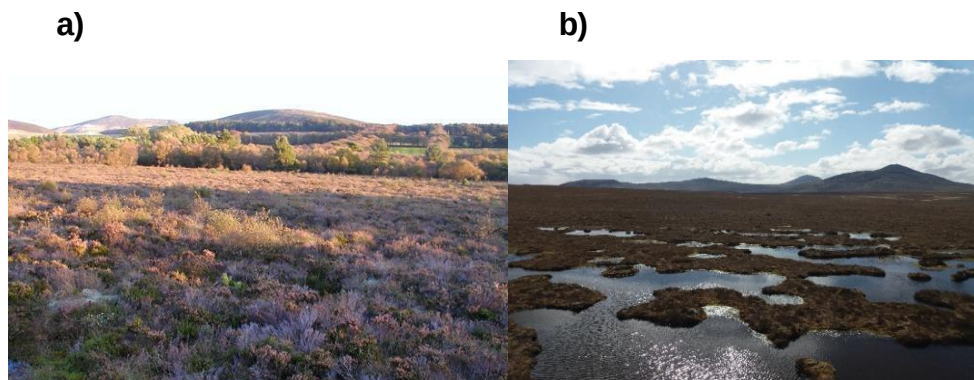


Figure 1.4 a) Raised bog (19) and b) blanket bog. (10)

In general, Scottish bogs are able to store approximately 1620 Mt of carbon, (7) (10) which represents a very large proportion of the global blanket bog carbon content. There are 475 separate palaeoecological records about Scottish peatlands (11) which are a valuable resource in terms of environmental change in carbon accumulation from the

past. (11) Many changes occurred across the Scottish peatland landscape at different stages throughout the Holocene, for instance the expansion of *Pinus sylvestris* trees across previously treeless peatland have been noted, (12) (122) together with the sudden regional extinction of species such as *Sphagnum austinii* (formerly known as *Sphagnum imbricatum*), which were previously widespread. (13) (122)

1.2.3 Blanket and raised bogs in Scotland

Scotland is covered by about 60% of the UK's peatlands, which represents 4% of Europe's total peat carbon store.

Scottish peatlands are predominantly blanket bogs, covering 1.9 million ha, 23% of the land area. (5) (12) The largest blanket bogs are found in the Highlands and Western Isles (Figure 1.5). In contrast, the total area of Scottish raised bog is estimated at only ~28,000-35,000 ha, (10) and are mostly confined to the Central Belt and Grampian Plain. (10) (12)

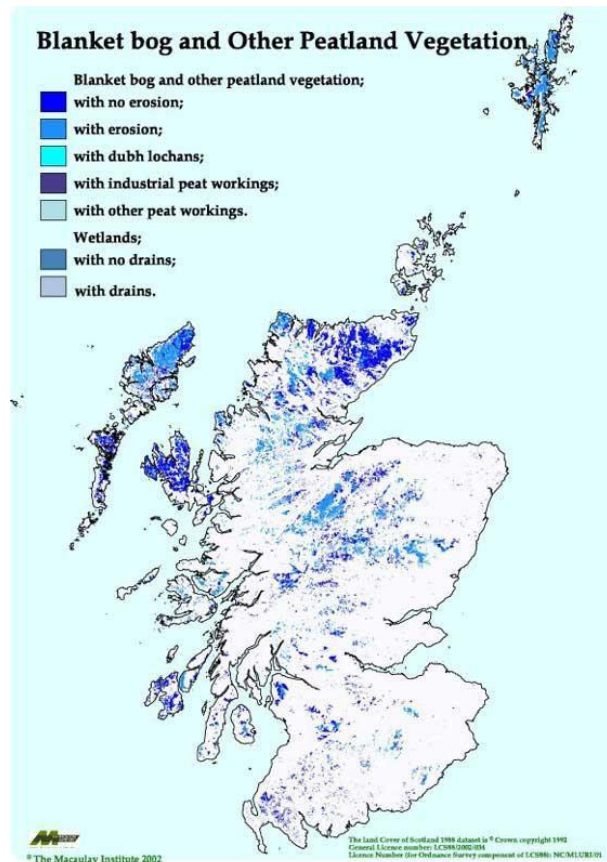


Figure 1.5 Distribution of blanket bogs in Scotland. (177)

1.3 Near natural bogs: above and below ground features

The main factor controlling peat accumulation is the ratio between peat formation and decay of OM and this is dependent on the climatic or hydrological conditions, level of erosion and redox conditions. Because the nutrient supply in ombrotrophic bogs is limited to atmospheric deposition, the most crucial factor controlling peat decomposition is the oxygen availability controlled by the water table. Considering the role of the water table (24) a bog's profile could be described, historically, as separated into two sections: the first, permanently waterlogged (*catotelm*) which is characterised by anoxic conditions and slow peat decay, while the second is temporally aerated (*acrotelm*), where decomposition is fast and most of the initial plant mass is mineralised.

The boundary between these two layers (*mesotelm*) is characterised by the transition of peat decaying from the *acrotelm* to the *catotelm* and this zone is controlled by the water table. These layers and the decomposition processes and outputs are shown in the peatland carbon cycle (Figure 1.6).

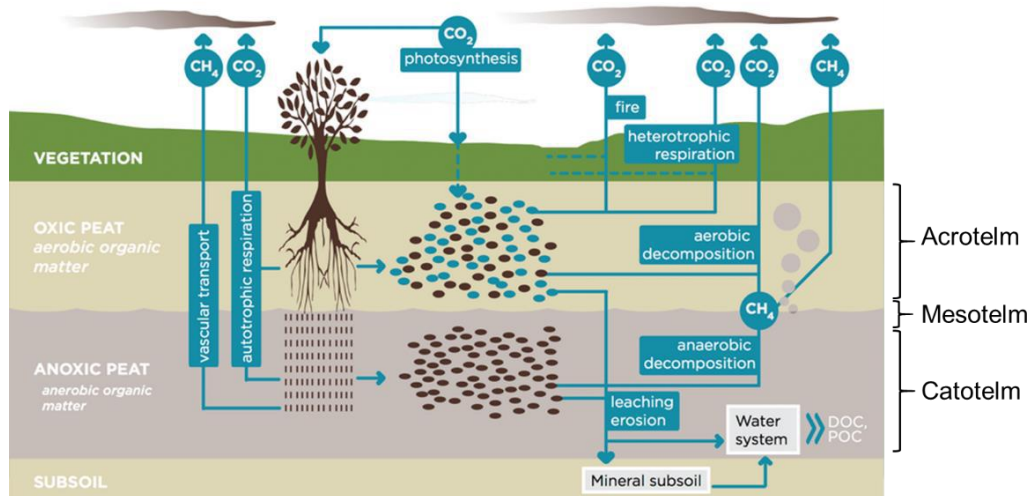


Figure 1.6 The peatland carbon cycle adapted from Jesse F. Abrams PhD. (179)

The highest rates of decomposition occur in the *acrotelm* releasing CO₂ to atmosphere as a result of aerobic decomposition. (25) Under water logged anoxic conditions, CH₄ is produced by anaerobic decomposition.

The water table drawdown into the anoxic layers allows the introduction of oxygen, with a consequent enhancement in rates of aerobic decomposition and subsequent increase and decrease in

CO₂ and CH₄ flux, respectively. (26) In addition, dissolved forms of carbon can be released as a result of leaching or erosion.

The degree of peat decomposition in the historic profile reflects the hydrological conditions during the time of peat formation, or more exactly at the time of peat burial in the *catotelm*. However, repeated drying and re-wetting cycles might also affect deeper and older peat layers and modify the historic record of peat decomposition. This latter process, named secondary decomposition, can therefore result in a decoupling of the age versus depth relationship between peat formation and peat decomposition. (27)

However, the diplotelmic scheme can result to an inflexible model, hiding many ecohydrological phenomena. An alternative approach, which tries to account for horizontal spatial heterogeneity, has been proposed under the name of hot spot and cold spot theory. (180) Generally, hot spots represent areas of a peatland which show faster processing rates in terms of mechanistically linked hydrological, ecological and biogeochemical processes. On the other hand, the concept of cold spots refers to slower mechanisms acting in peatlands, which could also be useful in describing peatland ecohydrology, especially when talking about the vulnerability of peatlands.

A variability in precipitation patterns has been observed in the Northern Hemisphere over the past years (125), with subsequent reduction in peatland water table levels and increased frequency and intensity in mid-summer water table downsizing. (126) As a consequence of water table lowering and its direct impact on C cycling, peatlands transit from C sinks to sources. (127)

Water table regulation of decomposition can be explained by the fact that, due to lack of oxygen (O₂) as a necessary reactant, activity of oxidative enzymes (e.g., phenol oxidase) is suppressed under

saturated water table conditions as postulated by the enzymic 'latch' hypothesis. (128) Firstly, these oxidative enzymes play a crucial role in C cycling as being involved in the breakdown of phenolic compounds that suppress hydrolytic enzymes responsible for carbon and nutrient cycling. Therefore, a suppression of their activity provoked by low oxygen availability in the peat soil leads directly to an increased amount of phenolics, which bind to hydrolytic enzymes and inhibit function. (128) This so-called enzyme 'latch' theory demonstrates the role that molecules, in this case phenolics, play in peatland health.

In addition to the mechanism illustrated above, an alteration of the plant functional groups arises from sustained modifications in peatland water table (129) due to the ability of the dominant vascular plants, such as sedges and ericaceous shrubs, to regulate soil microbial processes by influencing litter quality (130), O₂ availability and nutrient competition (130).

1.3.1 Erosion, peat extraction, drainage and afforestation

It is estimated that 70% of the blanket bogs and 90% of the raised bogs in Scotland are damaged through a range of land management practices. (5) In this section, land use practices on peatlands are briefly described, highlighting damage they have caused.

Peat erosion has been recognized as one of the main problems in Scotland. Following an investigation carried out by Scottish Natural Heritage, climate factors, such as changes in rainfall or extreme fluctuations in temperature, represent the principal drivers of erosion, together with vegetation type and animals, e.g. deer, living in peatlands. (28) Peat erosion in natural, especially blanket bogs, could extend over large areas and remove noticeable amounts of peat material. (7)

According to a review by Holden J. (29), erosion results in three fundamental issues: export of carbon, reduction of water storage

capacity and transport of pollutants such as lead. This would not only have an impact on the ecosystem services of peatland but lead to additional downstream costs as well.

Peat extraction has historically removed the surface layers of peatlands. The practice consists of cutting the peat by hand or machinery, drying on site just before collection and transport for use. (30) Historically, peat has been extracted for different uses, including as organic fertiliser, raw materials for chemistry (e.g. agriculture, medicine, cosmetics, polygraphy), (31) as fuel and as a filter and absorbent material. (32) In Scotland, the amount of extracted peat has been estimated to be around $440,000 \text{ m}^3 \text{ y}^{-1}$ over the past decade and every year, around 20,000 tonnes of peat are still cut for fuel use. (33)

Drainage of peatlands was encouraged in UK from ~1847 with the aim to increase land for agricultural activities, such as grazing. Between the 1930s and 1960s, a series of state-driven land drainage schemes were set in place, (34) which reached a peak between 1940s and 1950s, when $\sim 20,000 \text{ ha y}^{-1}$ was drained. (35) Drainage results in lowering the water table, leading to a loss of vegetation, reducing the stability of peat structure and increasing DOC in water flowing out of peatlands. (5) A recent survey of 58 lowland raised bogs, found that $\sim 97\%$ have been affected by drainage ditches.

Finally, in the UK, it is reported that around 200,000 ha of peat bog have been afforested. (36) A significant increase in the number of forests after the Second World War has been carried out by several human interventions. In the 80s, this was particularly encouraged by the UK government which offered tax incentives for private companies to reduce the cost of afforestation, increase jobs and reduce wasteland. (37) (38) To establish forestry on

peatlands, drainage and ploughing were required which has resulted in peat exposure, decomposition and erosion. (5)

1.4 Peatland Restoration

In recent decades, the value of peatlands has been increasingly recognised. The UK government has realised that meeting national and international targets for preserving biodiversity and carbon emission reduction is not achievable without reversing the damage sustained by peatlands and maintaining them in an active state. For example, without restoration the carbon loss can be so high, with estimates of 10 million tonnes of CO₂ loss per year from damaged peatlands in UK. (10) As a result, restoration programmes and schemes have been set-up and established across the country. For example, the Scottish Government funded '*Peatland action*' project that supports on-the-ground peatland restoration activities through funding, assessing area for restoration feasibility to guiding landowners through the restoration process.

In this section, the aims, methods, current status and monitoring of peatland restoration are described.

1.4.1 Restoration aims and current methods

Peatland restoration aims to return damaged peatlands to a stable state where they can perform ecosystem functions and be more resistant to future changes. Initially this requires stopping the removal or use of peat (e.g. for fuel) before re-establishing the peat forming conditions. This can be achieved by re-establishing the hydrological conditions by raising the water table to near surface levels. (10)

The main restoration measures include drain blocking (43) re-vegetation, (44) and tree removal. (45) (46)

The first UK upland peat drain dams were installed in the late 1980s (43) and since then many different types have been used, mainly differing in permeability. Permeable dams include heather bails to trap water and encourage re-vegetation. Impermeable dams are typically made from plastic and used to raise the water table and create peat pools. (194)

In addition, on sites where there is no longer any source of *Sphagnum* mosses, which represent the most important peat-forming species, both biologically and structurally in the restoration of peatlands (39), mosses have been propagated offsite and inoculated on the degraded peat to build up a plant community able to retain water on the peatland. (10) Another technique used to revegetate bare peat is to spread heather brash with moorland seed, lime and fertiliser as required, to stabilise the peat surface and creates microclimates for bog vegetation. (40)

1.4.2 Current restoration status in UK

In the UK, over 20,000 ha of blanket bog, compared with less than 2000 ha of lowland raised bog has been restored so far. (41)

Based on the survey of 58 Scottish lowland raised bogs during 2010-2011, 68% of surveyed sites are considered restorable, but the main limitation is funding and maintaining the restoration measures. Restoring 50 sites will cost £1,481,000 and the management about £46,000 every year. To restore all 851 recorded raised bogs alone in Scotland, the total cost would be between 20-32 million pounds (42) or around £3,000,000 ha. (46)

Considering the expense, it is crucial to have the correct restoration measures and to monitor the outcome closely.

1.4.3 Restoration monitoring

Monitoring restoration is necessary to assess if the activities on the ground are successful and if alternative methods need to be sought. (45)

In one study conducted by Bardsley, it was suggested to follow three main steps: a) pre-restoration: monitoring the site before restoration, to obtain an idea of the baseline condition or control; b) post-restoration: monitoring after a period time (years) of restoration; c) long-term management to maintain the established state. (47) Because there is limited understanding of the composition, structure and function of peatland ecosystems, monitoring the success of restoration is challenging. (48)

For example, the fundamental conditions required to create or maintain a functioning peat bog against further climate or fabricated changes, are not fully understood at this current time. (45) This issue is augmented by the fact there is a lack of intact or near natural peatland sites in the UK for comparison. Thus, in many instances we must rely on indicators that are considered evidence of an active or non-degrading state. Indicators include hydrology (e.g., water table level, evapotranspiration rates), biogeochemistry (e.g., peat quality, gas fluxes), biota (e.g., vegetation cover and types) and physical integrity (e.g., bulk density, OM content). (45) While the list is extensive, this also brings the issue that to monitor all indicators requires a variety of expertise as well as equipment and funding. Usually, restoration is monitored using vegetation and/or species surveys, water table depth and gas fluxes, which represent the easiest methods.

The evidence from these above ground monitoring methods to date is conflicting. For example a study of two raised bogs in Ireland showed adequate recovery of water tables after rewetting, however

the vegetation recovered only in one bog but not in the other. (193) It has also been shown that drain blocking cannot give reliable results in short time scales since it is not clear whether drain-blocking is able to reduce the organic carbon loss as peat undergoes significant physical and chemical change when degraded. (184)

Time after rewetting is an important variable to consider and studies of Swedish bogs 15 years after rewetting did show return of hydrology and vegetation. It is clear that research is required to monitor peat below the surface, in particular the chemical properties, which are going to be discussed in the next sections, since their potential modification are harder to evaluate in short period. The Swedish bog study for example examined DOC and nutrient concentrations within the peat pore waters below the surface. It has been shown that neither reached post-drainage levels, demonstrating that simple rewetting in this case did not change the chemistry of the pore waters and it is possible this prevents the vegetation from changing. The impact of peat vegetation is one important aspect of peat chemistry however many believe that it is also key to peat formation and stability. The most renowned theory to explain the carbon storage mechanism in peatlands is the so called enzyme latch theory. (185)

The theory states that the oxygen limitation prevents the activity of phenol oxidases which are responsible for phenolics decomposition. This allows the build of phenolics which in turn reduce the hydrolytic enzymes activity, considered as the main responsible of organic matter decomposition in inpeat. If this theory is correct then examining the molecular composition could give a more sensitive and accurate description of peatland health status. The only other hindrance is time, it is not possible to conduct research examining the same site over 15 years. Thus examination of the impact of drainage and restoration should be conducted on sites under these different health statuses that are close to one other.

1.5 Main techniques used to assess below-ground peatland status

In principle, examination at a molecular level provides greater insight about peat decomposition. In the next paragraph it will be discussed what kind of techniques have been used to obtain a deeper evaluation of below-ground peatlands.

1.5.1 Physicochemical properties to assess peatlands status

Several physicochemical properties have traditionally been linked to the hydrology and degree of decomposition, which in turn can be related to status. These consist of quantitative laboratory-based measurements including bulk density, water content, ash content and elemental (CHN) analysis.

1.5.1.1 Bulk density

Peat bulk density is a measure of the dry weight per unit volume of peat and it is a function of changing rates of peat accumulation and compaction. Differences in bulk density with depth have been used to evaluate the degree of total peat decomposition, with higher bulk density indicating higher decomposition as they reflect a peatland with low porosity and poor compaction. (75) Usually, peat bulk density in high-latitude peatlands in near natural conditions, fall in a range between 0.05 and 0.2 g cm⁻³. (76)

1.5.1.2 Water content

Oven drying of peat at 100-110°C using either gravity-convection or forced-draft ovens have been demonstrated to be acceptable for routine water content analysis. (77) The hydraulic properties are affected by the porosity and pore-size distribution of peat. These physical properties change with depth, influencing the water storage and the water flow. (78) Usually, the more the peat is humified (especially at depth) the less the permeability of water is allowed,

limiting the exchange with surface layers, thus creating a water-logged system at depth.

1.5.1.3 Ash content

Ash content is a measure of the total inorganic content of peat, which consists of both atmospheric mineral input, such as particulate matter containing trace metals, and mineral input from plants present in the peat. It is quantified as mass of residual ash after peat combustion, which removes all OM present. Usually, peat contains less than 25% of ash and ombrotrophic peat bogs keep values less than 15% (79) although ash content is variable from bog to bog and with depth. (80)

1.5.1.4 CHNO elemental analysis

The elemental composition is considered as one of the most important indicators of peat properties. The major elements present in peat are hydrogen (H), carbon (C), nitrogen (N) and oxygen (O). Out of these elements, N content is often used to assess peat decomposition and nutrient content. (53) (81)

N abundance can change with depth over the peat profile depending on microbial biomass, surface vegetation and high N-content substrates like fulvic and humic compounds (defined later). (66)

During decomposition, N percentage can increase from 0.3 to 4%. (82) Typically, N content is assessed as C/N ratio, which is highly informative for peat decomposition. (83) A low C/N ratio is usually associated with a high humification or decomposition degree. (82) (84)

However, N content of the soil surface is hugely affected by rainfall input, the surrounding environment and productivity of surface vegetation. (85) Therefore, it can be expected that the depth profiles of N and the resulting C/N ratio depend on location and environmental conditions. For example, the analysis of peat profiles from Marcell Experimental Forest in Minnesota (USA) showed a

higher C/N ratio at the peat surface, which was around 50 %, and much lower C/N ratio at deeper depths, precisely between 20 % and 30 %. (82) In contrast, three oligotrophic (where peat was poorly supplied with nutrient) peat cores from the Wester Ross region of North West Scotland, showed the lowest C/N ratio at the surface. (84)

1.6 The molecular composition of peat natural organic matter (NOM)

Peat is typically > 60% natural organic matter (NOM) with high water content (> 90%). (49) (50) NOM is a heterogeneous complex mixture of organic compounds ubiquitous in waters, sediments, and soils. (49) (50) Solid phase NOM found in soils like peat is termed soil organic matter (SOM), while the solution phase NOM in any natural system is defined as dissolved organic matter (DOM). Due to its complexity, SOM is not typically studied as a whole; instead, it is separated by means of chemical extraction into operational fractions. The next sections will define peat SOM and DOM present in peat pore waters in detail as well as the main extractable fractions of SOM.

1.6.1 Soil organic matter (SOM)

Soil organic matter can split into a series of fractions. The two main fractions are recalcitrant and labile organic matter (OM). (51)

Recalcitrant OM is also known as Humic substances, which can be divided into further operational fractions based on their solubility in acid or alkaline solutions. Humic substances can be differentiated into humic acids, fulvic acids and humin (88) and the resulting peat has different proportions of these humic substances. As the proportion of these substances is different among different peat types (80) and

changes with peat depth, the diagenetic transformations that take place are overly complex.

The water extractable organic matter (WEOM) was defined as readily decomposable organic matter which can be extracted by water under various laboratory conditions. (52, 54) WEOM has been the focus of many investigations as this component is acknowledged to be a good indicator of soil quality (53) and has been used to study the effects of management practices for restoration. (54)

1.6.2 Dissolved organic matter (DOM)

Dissolved organic matter (DOM) is defined by McFee *et al.* as the organic matter which is present in the soil pore waters and can pass a filter pore size of 0.4-0.6 μm . (55) 56)

Most DOM is a product of microbial metabolism and a decomposition product of surface litter. (57) It comprises of a wide range of CHO molecules, ranging from 150 Da to 700 Da, comprising simple acids, sugars, aromatics and complex humic substances with large molecular weights. (58)

DOM is the most mobile form of organic matter in soils (59) and it has been reported as a sensitive soil and water quality indicator by its fluxes and concentration. (60) It contributes to carbon turnover, the distribution of carbon and nutrients within ecosystems (61), to soil-forming processes and microbial metabolism in aquatic systems. (62) In peatlands, the supply of DOM is controlled by various variables, including the water table, hydraulic gradients, temperature, and vegetation type. (63) Therefore, any change in the flux of DOM may cause a regional redistribution of soil carbon. (64) For example, increased evapotranspiration or decreased water table levels caused by peat damage, which led to increased peat oxidation, could also affect the flux and composition of DOM.

1.6.3 Techniques for the Molecular characterization of peat organic matter

1.6.3.1 Attenuated Total Reflectance (ATR)-Fourier Transform Infrared spectroscopy (FTIR)

The ATR-FTIR spectroscopy is type of IR that can be directly applied to solid or liquid samples without any sample preparation. ATR operates by measuring the changes in a totally internally reflected infrared beam after the infrared beam encounters a sample. Specifically, the infrared beam is directed onto an optically dense crystal with a high refractive index at a specific angle. Then, the internal reflectance creates an evanescent wave which extends beyond the surface of the crystal in contact with the sample. Therefore, evanescent wave is attenuated in regions of the infrared spectrum where the sample absorbs energy and this attenuated energy is passed back to the infrared (IR) beam. Finally, the IR beam is passed to the detector, generating an infrared spectrum. (18)

1.6.3.2 ATR-FTIR application to peat organic matter

ATR-FTIR is useful to examine a possible change in molecular composition of solid peat samples without the need for any pretreatment. The marked absorption bands in IR spectra report on the main functional groups of peat molecules, which have been assigned to polysaccharides, fatty acids, lipids and lignin. (71) (82) (95)

It should be noted, however, that as peat OM is a complex mixture of thousands of compounds, IR spectra should be interpreted with caution, since a particular stretch in the spectrum is a superposition of many types of molecules. For example, not only will the carbohydrate

stretch consist of a mixture of small and large carbohydrates, which cannot be differentiated, other types of molecule, such as flavonoids, that have a carbohydrate moiety will also show absorbances in the same region. The same argument can apply to aliphatic C-H stretches, which will arise from a mixture of small and long chain aliphatic acids and aromatic stretches, from a wide range of sources. Therefore, if one or another functional group dominates the spectra, it cannot be classified as belonging to a particular molecule based exclusively on IR data. For this reason, IR data must be interpreted alongside other techniques, such as NMR, to draw structural information.

1.6.3.3 NMR spectroscopy

NMR spectroscopy is one of the most powerful tools for the determination of molecular structures and can be used to analyse samples in various states, including solutions, solids and gels. (96) This technique is able to use radiofrequency radiation to extract molecular information about NMR- active atomic nuclei present in samples placed in a strong magnetic field. With regard to soils, the main nuclei of interest are ^1H , ^{13}C and less frequently ^{15}N and ^{31}P . There are thousands of experiments, ranging from one-dimensional to more complex multidimensional experiments that can be applied to access specific, molecular-level information. Over the last decade the application of NMR in environmental science has increased with numerous examples spanning the atmospheric, soil and water research areas. (97)

To understand the molecular composition of DOM, water extractable organic matter (WEOM) and how the composition of these fractions responds to external changes, various NMR experiments have been used to characterise both. One dimensional (1D) NMR experiments

are most frequently used to assess the major compound classes present in soil organic matter. (98) (99)

The simplest 1D NMR experiment is a one pulse experiment that shows the chemical shifts of the nuclei of interest as a 1D trace.

In terms of soil DOM, 1D ^1H (100) (101) and ^{13}C (102) (103) NMR spectroscopy have been used to provide a fingerprint of the types of compounds present in DOM. For example, Tfaily et al. applied ^1H NMR spectroscopy on DOM from deep bog. (41) Overall, they observed that over 80% of CHO molecular formulas appearing in DOM from the surface are also present at 2.9 m depth, indicating that most of DOM in the bog is resistant to microbial degradation.

Overall, ^1H (104) (105) ^{13}C (106) ^{15}N (107) ^{31}P (108) have been used for structural analysis. For instance, Santos *et al.* applied solution state ^1H NMR spectroscopy for analysing WEOM from soil samples heated to different temperature as a means of understanding the effect of fire on the composition of OM exported from soils to rivers. They found that WEOM extracted from soil heated 350°C was more enriched in aromatic C structures compared to WEOM extracted at lower temperatures. (104)

1D ^1H NMR experiments are the easiest and fastest experiment to run on DOM and WEOM compared to other nuclei. However, ^{13}C provides information about the backbone of the molecules present, spectra can take many hours to acquire compared with minutes for ^1H . This is due to the much lower natural abundance of ^{13}C (only 1.1%) and the fact that its overall sensitivity is only 1.6% that of ^1H . Therefore, 1D ^1H NMR experiment is the most practical method for fingerprinting DOM and WEOM samples.

Solid state ^{13}C NMR unlike solution state NMR can be applied directly to solid peat samples without any pre-treatment. The main issue with solid state ^{13}C NMR is spectra is that if acquired on static samples,

anisotropic effects and orientation-dependent interactions broaden the spectral lines significantly, degrade the signal-to-noise-ratio and produce overlap of lines that makes their an analysis impossible . (109)

At the beginning, the solid state NMR experiments were focused on the measurement of ^1H spin-lattice relaxation times as a function of temperature for the investigation of methyl group rotations in solids. (110) However, it was discovered that anisotropic dipolar interactions can be suppressed by rotating the sample around an axis oriented at 54.74° with regard to the external magnetic field. This technique is known as magic-angle spinning (MAS). In order to be successful, spinning must occur at a rate equal to or greater than the dipolar linewidth or chemical shift anisotropy (which can be many kHz). Magic-angle spinning introduces artificial motion which averages both dipolar and chemical shielding interactions, characterized by the $3 \cos^2 \theta - 1$ term (the term $3 \cos^2 \theta - 1 = 0$ when $\theta = 54.74^\circ$). (111)

MAS can be combined with polarization transfer from abundant nuclei (^1H , ^{19}F and ^{31}P) to dilute nuclei (^{13}C , ^{15}N , ^{29}Si), enhancing the signal to noise ratio. This technique is referred to as cross polarization (CP); it is able to enhance the signal of the dilute spins by a factor of γ_I/γ_S , where γ is the gyromagnetic ratio, I is the abundant spin and S is the dilute spin.

The CP time or the spin lock period (the contact time) is typically of the order of few hundreds of micro seconds. As the high gyromagnetic ratio spins have a strong dipolar coupling, they are subsequently affected by large fluctuating magnetic fields resulting from motion more than e.g. ^{13}C , inducing a more rapid spin-lattice relaxation at the abundant nuclei. This means that the relaxation time can be shortened, which reduces the time between individual scans allowing to accumulate many more scans per unit of time. (90)

1.6.3.4 Fourier Transform-Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS)

It is widely recognized that Fourier Transform Mass Spectrometry has the highest resolving power and mass accuracy of any mass spectrometry technique.(128) For more detailed discussion on the theory and application of FT-ICR, an excellent review by Nikolaev *et al.* (2014) is available. (195)

In general an FT-ICR instrument can be described as consisting of a ionisation source, an ICR cell and a superconducting magnet. In terms of magnetic field, FT-ICR can be performed at low fields, but higher fields (e.g. 7T or 12T) are able to provide higher performance. In order to generate the ions, different ionisation sources exist with the most common for ionisation of solutions being, electrospray ionization (ESI), chemical ionization (CI) and Atmospheric Pressure Photoionization (APPI). Alternatively, a solid state sources such as laser desorption ionization (LDI) can be used under vacuum, therefore the access chamber can be taken out to atmospheric pressure, when there is the need to change samples, and pumped down to vacuum again. Further discussion regarding the theory behind the ionisation techniques used in the study can be found in Chapter 3.

In general, upon leaving the source ions are funnelled into the quadrupole which acts as a mass filter, removing ions below a certain threshold (Q1 mass) or within a specific range (isolating masses). At normal operation, it has nominal mass resolution for filtering of ions. After the quadrupole, the filtered ions are then sent to the collision cell where they are accumulated for a period of time (the ion accumulation time (IAT)). Those ions can be fragmented here, if required, typically after isolation of single masses within the quadrupole. The IAT is an important parameter which, along with the original sample, dictates

how many ions will be in the cell. Furthermore, ions are not stable indefinitely, and a longer IAT can yield different spectra for complex mixtures, including NOM.

The accumulated ion packet is then sent into the ion cyclotron resonance (ICR) cell, which is within the centre of the static magnetic field (B_0) and where a charged particle will oscillate due to the Lorentz force. Here, a predefined time of flight (TOF) is set and only ions which travel the distance between plates within this TOF will stay within the ICR cell. This TOF parameter is thus another way to filter ion mass ranges, for instance higher mass species will travel slower and require a longer TOF. For small molecules, a TOF of 500 ms to 700 ms is typical used.

Generally, FT-ICR MS is quite similar to FT NMR. Indeed, particles with charge (ions in MS, nuclei in NMR), have an oscillating motion within a magnetic field, which induces a current in a detector. The recorded sine wave is then Fourier transformed to produce a frequency domain spectrum. Again, multiple transients (scans) are co-added to increase the signal-to-noise ratio (SNR). This approximation lends itself to a better visualisation, however the main difference with NMR is that after Fourier transformation, the mass spectrum has to be converted into a mass-to-charge domain (m/z).

Modern FT-ICR MS instruments are equipped with cryomagnets of various strengths (usually 7-15 T). Once ions are trapped in the ICR cell under the influence of a homogeneous magnetic field, they circulate at a frequency characteristic of their m/z value. Cyclotron frequency is inversely proportional to m/z , as shown in equation 1:

$$f_c = B_0 (z/m) \tag{1}$$

In equation 1, f_c is the cyclotron frequency, B_0 is the magnetic field strength, z is the charge of the ion, and m is the mass of the ion. Ions

in orbit in the ion cyclotron resonance (ICR) cell are excited by a radiofrequency (RF) pulse, which increases the radius of their orbit. Then, these ions circulate closer to the detector plates, inducing an image current on the receiving electrode. Subsequently, field inhomogeneities cause the ions to lose coherence and orbit radius, which leads the ions to collapse towards the central core of the ICR cell, producing an image current trace that is called a free induction decay (FID).

Over the last decade, FT-ICR MS has emerged as a valuable tool for the characterisation of NOM and DOM, (74) (68) (112) providing very precious information with regard to the composition. For example a typical FT-ICR MS spectrum of DOM contains thousands of molecular ions between 146 and 702 Da.

Peat DOM has been previously characterised as having molecular formula confined between O/C 0.2 to 1.0 and H/C 0.5 to 1.6 or 1.9 (113) as shown in Van Krevelen diagrams (VKs), which are cross-plots of H/C vs O/C, from literature. (114) However, this spread of compounds described in previous studies could be due to the number of compounds assigned, different experiment conditions, different peat types or due to the removal of part of the sample by solid phase extraction (SPE) during the sample pre-treatment. SPE, using PPL columns that are selective for organic acids/alcohols, has been used in many previous studies to desalt the samples. In the case of peat DOM, the salt content is negligible and therefore this step is unnecessary.

In addition, it is important to reiterate that the use of SPE will naturally inflict some bias in the data. In particular, SPE cartridges are poor at retaining carbohydrates (115) and considering that terrigenous-sourced DOM contains a considerable proportion of carbohydrates, it would be preferable to avoid the use of SPE on peat DOM.

Since hot water extraction of peat is a new method, there is little evidence in the literature that FT-ICR MS has been applied to those samples. It is possible, however, to compare with MS data from peat samples extracted in water at room temperature by Han et al. (2019), who also recorded spectra of fulvic acids extracted from the same samples for comparison. The authors found that the water extraction led to an increased number of assigned formulae in comparison with FA, with the additional formulae being predominantly lignin-like. Finally, LDI has been shown to be suitable for ionisation of natural organic matter samples without the need for a matrix as the substantial number of aromatics is enough to promote ionization. (116) (117) (118) As there are no existing studies on LDI of peat, the data shown in this work can only be compared with the limited examples of LDI of Suwannee river fulvic acid (SRFA, (116) (117)) and bulk, humic and fulvic acid extracts from a silt loam soil. (118)

Both studies demonstrate the prevalence of aromatic or condensed aromatic molecules (e.g., 92% in LDI of SRFA with 40% laser power), similar to the peat sample (85%) discussed in this work.

1.6.3.5 Examination of peat restoration at the molecular level

There has been only a handful of studies that have examined the impact of peatland restoration using IR and FT-ICR MS. This section will summarise the main findings. (71)

ATR-FTIR spectroscopy has been used to monitor the quality of OM of restored peatland from four European countries (Finland, France, Switzerland and Scotland) (71), showing a decrease of the polysaccharide band intensities and an increase of lignin-like and aliphatic band intensities with depth. These results confirmed the higher level of humification characterizing deep peat.

The only study to compare near natural, drained and rewetted sites was conducted on Irish blanket bog cores using IR. (22)

The authors reported slight differences in the C-O stretch and C=C stretch maxima between damaged and near natural/restored. They also reported that the FTIR absorption spectra of the drained site have less intense absorbances at all depths than the other two sites, while the highest absorbance occurred at different depths in the restored and near natural site. The authors concluded that this indicates different rates of decomposition due to exposure to oxygen. (22)

Other studies were supposed to evaluate chemical changes during peat decomposition, but they are not often clearly defined.

In terms of liquid phases, one study conducted FTIR analyses on porewater samples from drained and near natural peatland sites in Canada, however they did not find differences between the composition of both these kind peatlands. (196)

This study contrasts with the only FT-ICR MS study of peat pore water DOM from pristine and rewetted peatland sites in Central Germany. (91) DOM quality was less variable in all the cores from the pristine sites compared to those from the rewetted site. In terms of composition, the rewetted site had greater abundance of oxygen-rich unsaturated compounds, which the authors related to polyphenol-like components.

These studies demonstrate that different phases may require different techniques to probe the differences in molecular composition and that peat depth could be an important variable to include.

1.6.4 Statistical analysis

Considering the large data sets acquired from both physical and chemical analyses, multivariate statistical techniques represent a useful way to understand possible correlations between physical and

molecular variables. Therefore, two different statistical methods were applied to the data sets obtained from the peat core:

- 1) Principal Component Analysis (PCA).
- 2) UpSet plots.

When dealing with a significant number of datasets, PCA is a useful method to reduce dimensionality, facilitate visualisation, analyse the interrelationships among many variables and explain these variables in terms of a smaller number of variables, called principal components, with a minimum loss of information (119).

In order to compare multiple sets of MS data, UpSet plots are used using the assigned molecular formulae. (197) The UpSet method plots the intersections of different sets of molecular formulae as a matrix, showing an accurate number of molecular formulae in each set, and stating which samples are in that set. The sets are ordered according to the number of formulae, meaning those on the left hand side are those which have the greatest overlap in composition. If the sets on the left hand side consist of single samples, then there is generally not much overlap between samples, whilst if they consist of many samples there is large overlap.

2. Aim and objectives

The main aim of this project is to use complimentary analytical techniques to understand both physical and chemical properties of peatbogs from Scotland and try to determine if health status (near natural, damaged and restored) of a peatland is reflected in the these properties. This represents a great challenge due to the high complexity and heterogeneity of peatlands, and thus advanced analytical techniques are required to provide a detailed examination of the molecular composition of peat. The information acquired from peatlands molecular composition could be vital to the environment in terms of both carbon storage and water management.

Therefore, the principal objectives of this project are:

- To create a set of advanced solid and liquid state analytical methods for the examination of the molecular composition of peat.
- To apply these advanced methods alongside traditional physicochemical methods to a whole core of peat to evaluate the information content obtained from each method and determine any relationships between the advanced molecular and traditional properties.
- To examine the physicochemical and molecular differences between drained, rewetted and near natural sites of two different Scottish peat bogs types (raised and blanket bog).
- To evaluate if peatland status is reflected by molecular indicators.

3. Sampling sites and Methods

3.1 Study sites

This section describes the two Scottish peatlands that were the focus of this study, Red Moss of Balerno and Crocach, Flow Country.

3.1.1 The Red Moss of Balerno

The Red Moss of Balerno raised bog extends approximately 14 km south-west of Edinburgh and 2 km south of the village of Balerno. Located on the north side of the Pentland Hills next to Threipmuir reservoir, it is considered as the largest ombrotrophic raised peat bog (area 23 ha) in the Midlothian region (Figure 3.1). (52)

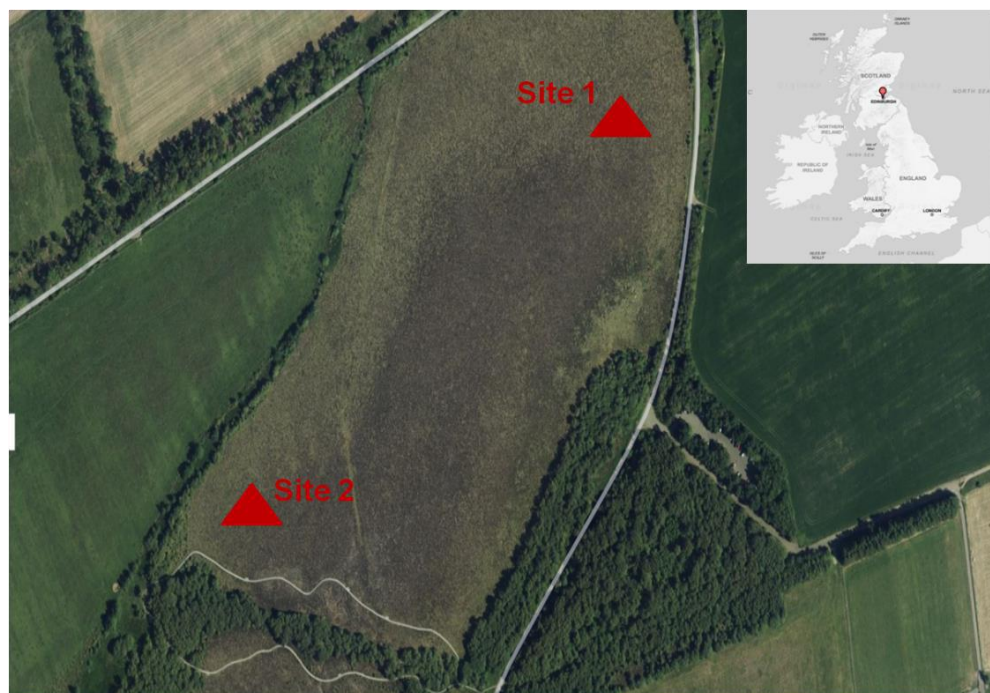


Figure 3.1 Red Moss of Balerno location. Site 1 (damaged) and Site 2 (restored), map created in Edina Digimap, scale 1:2,500.

The Red Moss of Balerno was a shallow lake dominating a basin depression in the boulder clay sheet, which extends over much of the Balerno area and is underlain by a series of shales, mudstones and cement stone bands. (53) The site is characterized by two distinct sections; a southern section of woodland and scrub with some underlying *Sphagnum* moss and a northern section dominated by *Sphagnum* (*S. capillifolium* and *S. papillosum*), feather moss (*Pleurozium schreberi*), heather (*Calluna vulgaris*) and cotton grass (*Eriophorum vaginatum*). The northern section of the bog is approximately up to 6 m deep and has all the characteristics of an ombrotrophic peat bog. (53)

The nearest weather station in Edinburgh recorded an average annual rainfall of 62.6 cm y⁻¹ for the period 1951-1980 A.D., in which the wettest months were July and August and the driest months February, March and April. Usually, the highest average maximum temperature can reach 23.4°C in June, while the lowest average minimum temperature is able to decrease till -6.6°C in January. (53)

Most of the Red Moss of Balerno was enclosed in 1868 A.D., although part of it remained as a common used by local villagers. The enclosed bog was cut away, currently forming pastoral land but lightly used for grazing, shooting and some domestic peat cutting. Lint holes are also present at the site, indicating that it was used for flax-retting. (54) (55) Parts of the site have been severely damaged by drainage ditch running from north to south and an aqueduct was built on the south of the site in the late 19th century A.D. The aqueduct ran across the reserve from east to west and was used to carry water from the Harperrig reservoir (Pentlands) to the Harelaw reservoir to supply the city of Edinburgh with water. (5) In 1986, The Red Moss

of Balerno became part of the designated Pentlands Regional Park, protected as a Scottish Wildlife Trust wildlife reserve.

At the beginning of 1980's, the main drainage ditch was dammed with peat and rubble, however this caused peat contraction and a water seepage formed. In 1993, two large elm board dams were installed trying to tackle the drainage, but this was not successful as they were not located in the right position. In 2004, multiple plastic dams were installed, the locations of these installation are shown in Figure 3.2.

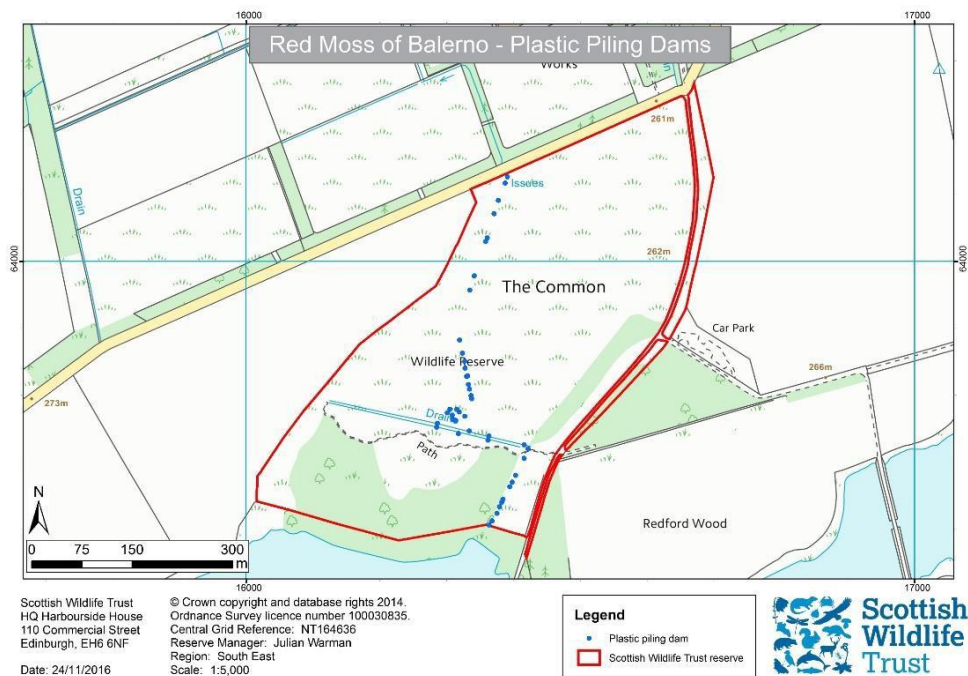


Figure 3.2 Location of plastic dams (blue dots) installed in Red Moss raised bog. Map created by Julian Warman, Scottish Wildlife Trust using Edina Digimap.

At present the site is surrounded by areas of pastoral land and open forest or woodland, with a water treatment works situated to the north of the bog. Restoration monitoring is conducted by the Scottish Wildlife Trust mainly in the form of water table monitoring. A series of dipwells, 4 walrags and 2 automatic dataloggers are located on site. A

vegetation survey of the whole bog was also conducted in 2016. Invading birch, pine and scrubs are removed each year to preserve the natural state of the bog. To examine the impact of the restoration work, this study will examine two areas of Red Moss of Balerno; one area still believed to be experiencing drainage and another area close to the drains blocked by plastic dams.

3.1.2 The Flow Country

The Flow Country is located in Caithness and Sutherland and is Europe's largest blanket bog; covering an area of 4000 km². It is estimated to store more than three times the amount of carbon of all the UK's woodlands. (55) The Flow Country contains a diverse range of vegetation types covering various microtopographies, thus it is an area of international importance in terms of conservation as well as scientific interest. While there are areas considered to be in near natural conditions, unfortunately large areas of the peatland have been subject to land-use change and damage. Between 1970s and 80s, the Scottish government gave tax incentives to plant non-native forestry plantations in the Flow Country to produce more timber, unfortunately leading to peat drying, carbon loss and disappearance of special wildlife. (55) In addition, in the past areas were damaged by drainage for agriculture purposes.

Parts of the Flow Country are now being restored by RSPB Scotland, land managers and other organisations, involving the removal of the forestry plantations and blocking drainage ditches.

This study examines three areas of the Flow Country, which were suggested by the RSPB. Two are located north (known as Nam Breac) and one south of a road (known as Crocach). All three sites are located west from the town of Forsinard (Figure 3.3).



Figure 3.3 Flow Country map. S1 (near natural), S2 (restored), S3 (damaged). Map produced using Edina Digimap, scale 1:10,000.

The northern areas consist of a near natural site where no human interventions have taken place and a restored site, which was drained for local forestry before being restored in 1998 by wooden dams. The area south of the road, is damaged by a number of drains. Note this is the first scientific study of these three areas so there is no historic record of vegetation, water table or peat depth. On each area, three automatic water table dataloggers have been installed after samples were taken for this project to record the water table.

3.2. Peat sampling equipment and strategy

This section will describe the peat sampling equipment and strategy at each of the study sites.

3.2.1 Red Moss sampling

Prior to the outset of the project, a total of 22 cores were collected across two areas (damaged by drainage and restored) within the Red Moss site (Figure 3.4) in Dec 2016.

11 cores were taken from the north-east side, which has remained unrestored. Herein referred to as RMI with cores labelled a-k. Another set of 11 cores were taken from the south-west side of the bog, which is in close proximity to the old aqueduct and two drains and has been the primary target for restoration efforts. This location is referred to herein as RMII with cores labelled A-K. In each area, three cores were taken from the central area, with eight more extending out within a 20 m radius.

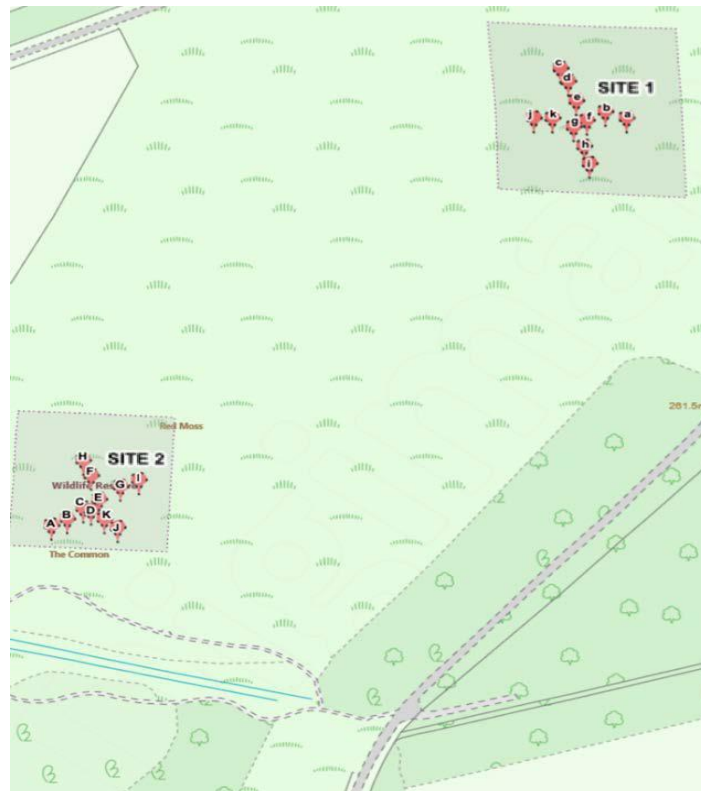
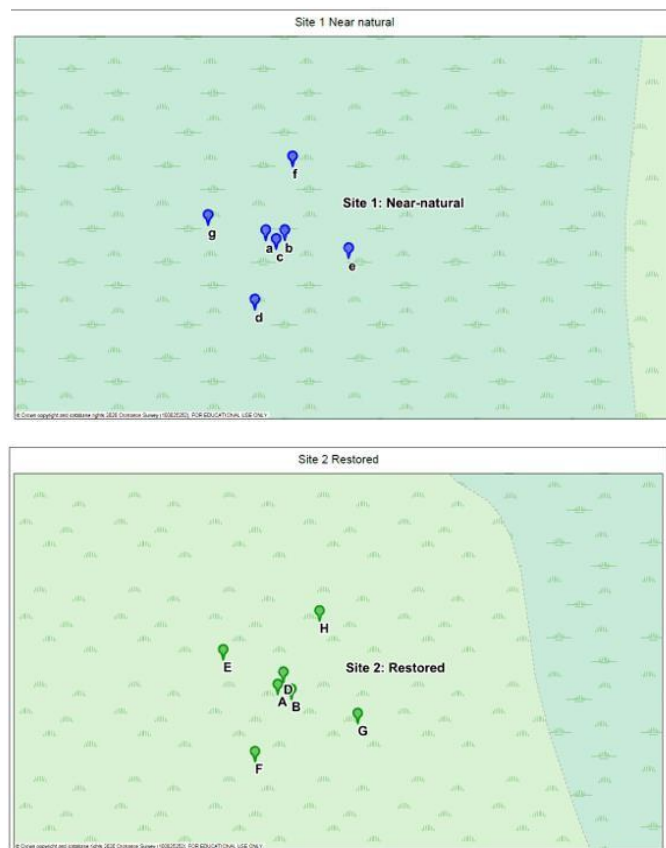


Figure 3.4 Map of the Red Moss site, showing sampling locations: site 1 (RMI, degraded) and site 2 (RMII, restored). Map created in Edina Digimap, scale 1:2,000.

3.2.2 Crocach, Flow Country sampling

Twenty-one cores across three areas (near natural, drained and restored). These were obtained during separate visits in August 2017 and May 2018, respectively.

Three cores were taken from the near natural, 3 cores from the damaged site and the other 3 cores from the restored site during the first trip. Then, another 4 cores from the near natural site, 4 cores from the damaged site and 4 cores from the restored site were taken during the second trip. The near natural site was named 1 (S1) and cores were labelled a-g. The damaged site was named 3 (S3) and cores were labelled J-N. The restored site was named 2 (S2) and cores were labelled A-H (Figure 3.4).



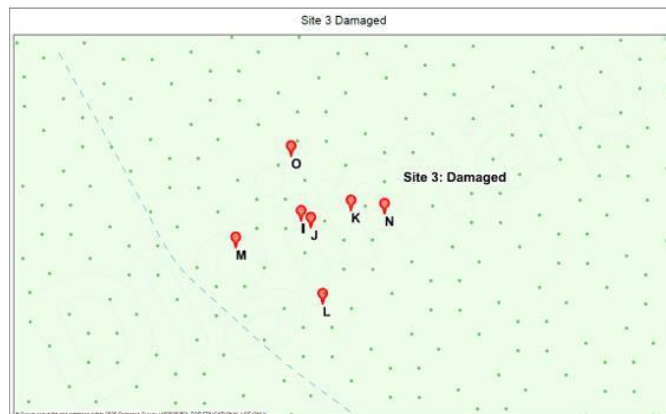


Figure 3.5. Map of the Flow Country sites, showing sampling locations: site 1 (Near Natural) site 2 (Restored) and site 3 (Damaged). Map created in Edina Digimap. The drain is shown on the bottom image, scale 1:1,000.

3.2.3 Peat core sampling

From all sampling locations, cores (50 x 12 x 12 cm) were collected using a Wardenaar peat sampler (Eijkelkamp). This sampler was chosen as it is the only sampler available that collects an undisturbed core. (181). Where possible, core samples were taken from between hummocks and hollows to take into account the variations due to microtopography.(181) An example of a core extracted using the Wardenaar peat sampler is shown in Figure 3.5.

Each core was cut into 13 sections (2 cm intervals between 0-10 cm in depth, then 5 cm intervals from 10-50 cm) on site. Reasoning for the smaller sectioning of the top 10 cm is as follows. As peat is estimated to grow at a rate of a few mm to a cm per year, 2 cm represents decades-years of accumulation, which covers the time of drainage (e.g. 60/70s for Flow Country) and restoration (~40-12 and ~19 years ago for Flow Country and RM, respectively). This is justified from the

^{210}Pb dating conducted on peat cores from RM that shows that age of 50 cm depths is 1000 ± 35 years old. (53)



Figure 3.5 Photo of 50 cm core extracted from the restored Flow Country site.

A portion of each section of known volume was packed into polyethylene bags for air drying. The rest of each section was stored under Helium in a WhirlPakTM bag and stored in a cool box at 4 °C for transportation back to the University of Edinburgh, where they were stored at -80 °C for further study.

3.2 Sample preparation

In this section it will be discussed about the sample preparation used for the different kind of analyses carried out during this PhD project.

3.2.1 Dry peat samples

The wet peat sections were weighed and subsequently air-dried at room temperature for 30 days on drying trays and then they were re-weighed (dry weights). In addition, large roots were removed from each sample before being ground using a mallet and, when necessary, a ceramic mortar and pestle. Then, a ball mill mixer (Mixer Mill MM 200, Retsch) was used for 2 minutes at 25 Hz for each sample to

create homogenous powders. Between each sample the ball mixer has been cleaned with methanol and distilled water. Subsequently, each sample was stored in labelled petri-dishes for physical and chemical analysis.

3.2.2 Pore-water dissolved organic matter (PW-DOM)

Pore-water dissolved organic matter (PW-DOM) samples were extracted by directly squeezing wet peat samples. Samples were then centrifuged to remove solid material, frozen at -80 °C and freeze dried, without the use of a filter. The decision to omit the use of a filter was made to avoid the addition of contaminants in FT-ICR MS spectra. A number of filters were investigated and all added unknown contaminants to the sample. An example of such contamination is shown for the 0.45 micron Whatman cellulose nitrate filter in Figure 3.6. While the large contaminants are obvious and could be removed by post processing, there were many additional contaminants of varying ionisation efficiency throughout the spectrum, hence affecting the quality of the formulae assignments.

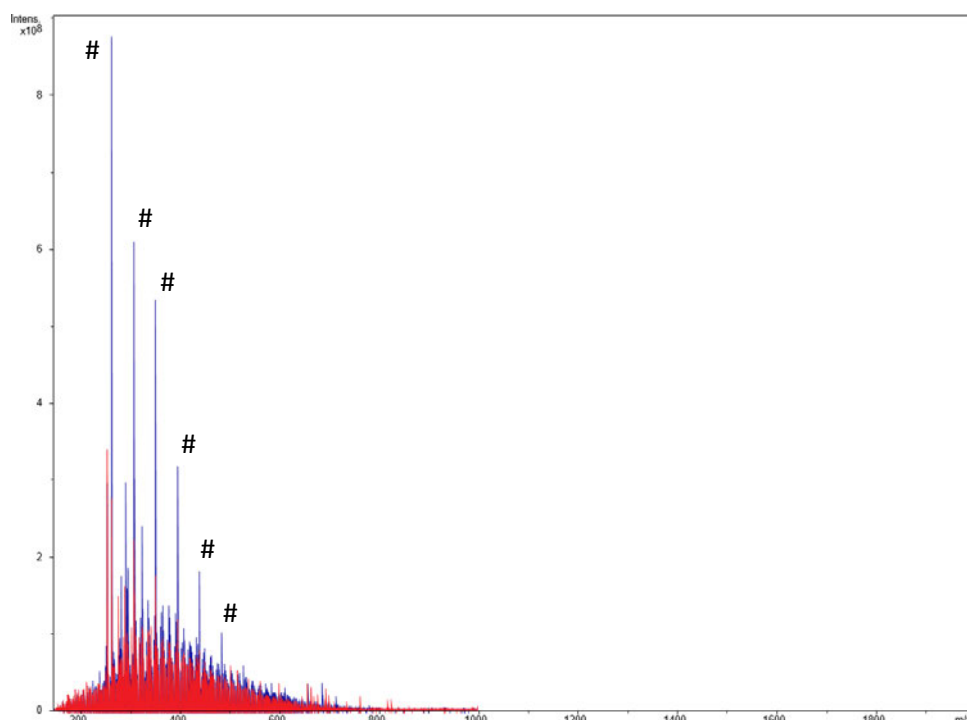


Figure 3.6 FT-ICR MS spectra of PW-DOM samples by using cellulose nitrate filter (blue, major contaminants marked with hashtags) and without using it (Red).

3.2.3 Hot water extracted (HW-E) peat

Hot water extraction (HW-E) was performed by heating ball-milled peat (250 mg) in distilled (Milli-Q) water (40 ml) at 80 °C, while stirring for 1 h. After cooling to room temperature, the solution was centrifuged at 8000 rpm for 10 minutes. The supernatant was frozen at -80 °C and freeze dried.

3.2.4 Alkaline extracted (NaOH-E) peat

The alkaline extraction (NaOH-E) of peat samples, performed by PhD student Richard York only on samples from core S2D from Flow Country site, was applied to frozen (-80 °C) peat samples. Frozen peat soil (50 g) was shaken in NaOH (250 ml, 0.5 M; analytical reagent

grade, Fisher Scientific) under Nitrogen for 4 h. Solid material was removed via centrifugation at 8000 rpm for 10 min. The supernatant was decanted and then dialysed against distilled (Milli-Q) water using cellulose acetate dialysis membrane (Spectrum Labs, molecular weight cutoff of 100-500 Da) until a dialysate conductivity of $10 \mu\text{S cm}^{-1}$ was reached. Samples were then frozen at -80°C and freeze dried to obtain solid extract.

3.2.5 Bulk physicochemical methods

Bulk physicochemical properties, such as bulk density, water content and ash content detailed below, were recorded for all cores extracted from both peatlands. Any additional measurements only conducted on samples from one location are stated.

The dry bulk density was calculated for each peat section using Equation 2:

$$\text{Bulk density (g cm}^{-3}\text{)} = \text{mass of air-dried peat section (g)} / \text{volume of peat section (cm}^3\text{)} \quad (2)$$

The volume of each peat section was based on the cross-sectional area of the core and peat section thickness. Please note that bulk density could not be calculated on peat samples from RMI because the volumes were not taken accurately for this site.

Using the wet weight and air-dried weight of each peat section, the water content for each sample was determined using the Equation 3:

$$\text{Water content (\%)} = \frac{[(\text{mass of wet peat (g)} - \text{mass of air-dried peat (g)}) \times 100]}{\text{mass of wet peat (g)}} \quad (3)$$

The ash content was determined by weighing 1 g of air-dried ball-milled peat into pre-weighed beakers (in duplicate) and heating them in an oven at 105°C for 24 hours. These were then cooled to room temperature in a desiccator and re-weighed. Subsequently, the 105°C dried peat samples were ashed by placing them into a Eurotherm Carbolite muffle furnace for four hours at 450°C before cooling them to room temperature in a desiccator and re-weighing.

The ash content of each peat section was determined using the equation 4:

$$\text{Ash content (\%)} = \text{mass of ash (g)} \times 100 / \text{mass of peat dried at 105}^\circ\text{C (g)} \quad (4)$$

Finally, the elemental composition (C, H, N, S) of oven-dried ball-milled peat samples (10 ± 0.5 mg) was determined by dry combustion in an elemental analyser (ThermoScientific FlashSmart) calibrated using 2,5-Bis(5-tert-butyl-2-benzo-oxazol-2-yl) thiophene (BBOT) and cysteine. S was negligible in peat samples, therefore O content was estimated by subtracting C, H and N content from 100% in most of the samples apart for one set of samples from Flow Country (S1a, S2E and S3N), in which it was able to determine the O content precisely.

3.2.6 ATR-FTIR spectroscopy

The attenuated total reflectance (ATR)-FTIR spectra were obtained from ~0.1 mg air-dried ball-milled peat samples. The spectra were recorded in the 450-4000 cm^{-1} range on an ATR-FTIR PerkinElmer Spectrum Two Spectrophotometer (Perkin-Elmer Instruments, Norwalk, CT). Each spectrum was recorded in triplicate with 32 scans at a resolution of 2 cm^{-1} and data interval equal to 0.5 cm^{-1} . The force constant was set to 60 Nm^{-1} . Between each sample, the ATR crystal

was cleaned with methanol and dried. Background spectrum was run before starting the acquisition and between every 3 different samples. All spectra were baseline corrected using the adaptive baseline correction, normalised, smoothed using the Savitzky-Golay filter and finally spikes removed by using SpectraGryph 1.2.8 Software. (198) This kind of pre-processing of IR spectra in this study has been evaluated as the best option for peat samples.

3.2.7 ^1H Liquid state NMR spectroscopy

PW-DOM, HW-E and NaOH-E extracted peat samples (2.5 mg) from each depth were dissolved in D_2O (99.9% D, Sigma-Aldrich). 1D ^1H NMR spectra were acquired at 600 MHz Bruker UltraShield 4 channel HD instrument with a 5 mm TCI $^1\text{H}/^{13}\text{C}/^{15}\text{N}$ CryoProbeTM with SampleXpress Lite 16 holder sample changer.

About the 1D ^1H spectra, they were acquired using the lc1gppnps NOESY water presaturation sequence with the following parameters: time domain points = 66560, sweep width = 40 ppm, acquisition time = 1384 ms, relaxation delay = 9 s, number of scans = 64, 90° pulse length = 8.310 μs , total experiment time = 12 min.

3.2.8 ^{13}C Solid state NMR spectroscopy

For acquisition of solid state NMR spectra, the optimization of the main parameters has been chosen following the paper by Mao *et al.*, 2017 as a reference. (25)

Ball-milled air-dried samples (70 mg) were packed tightly into 4 mm ZrO_2 MAS rotors. Subsequently, 1D ^{13}C CP-MAS semi-quantitative spectra were obtained at a spin rate of 10 kHz on two spectrometers. Samples from both Red Moss and Flow Country were recorded on a 300 MHz Bruker AV, with MAS unit using the following parameters: time domain points = 2048, sweep width = 592 ppm, acquisition time

= 23 ms, relaxation delay = 5 s, received gain = 203, number of scans = 512, total experiment time = 42 min.

In order to obtain more quantitative spectra, a high power decoupling experiment (HPDEC) was applied to the core S2D 45-50 cm sample on the same spectrometer. This is displayed in chapter 4, along with the CP-MAS spectrum of the same sample for comparison. It is clear that the aromatic region is far more intense in the HPDEC spectrum; as previously discussed, this is as would be expected using direct polarisation techniques, given that cross polarisation underrepresents signals from quaternary carbons, which have no nearby protons for transfer to occur from.

When the intensity of the alkyl region (0-45 ppm) in each spectrum is calibrated to 1, the aromatic intensity (110-160 ppm) increases from 0.1791 (CP-MAS) to 0.8576 (HPDEC); this represents an almost 5-fold increase. Whilst the improvement is clear, this spectrum was recorded over a period of 12 hours in order to obtain a similar signal-to-noise ratio to the CP-MAS spectra (42 minutes). The reason for this increase is the use of a much longer (50 s) relaxation delay between scans, which is necessary as ^{13}C returns to equilibrium very slowly in the solid state. Analysis of large numbers of samples in this fashion would therefore be immensely time consuming, and it could be argued that the use of CP-MAS is adequate, provided comparisons are not attempted between the absolute intensities of different regions, but rather how these intensities change between different spectra. Thus for this study only CP-MAS spectra were recorded.

3.3 FT-ICR MS

3.3.1 Electrospray ionisation FT-ICR MS

Electrospray Ionisation (ESI) is the most commonly used ionisation source and considered a soft technique (limited fragmentation).

A dilute sample is injected by a syringe, at a low flow rate (1-20 $\mu\text{L}/\text{min}$), into a nebuliser through which a neutral gas (often nitrogen) flows at high pressure (2-3 bar). The neutral gas contributes to nebulise the sample solution into solvent droplets. A very high voltage of around 2-6 kV is applied across the outlet (i.e. the end of this nebuliser), adding a charge on the solvent droplets. (195) At this stage, the charged droplets, attracted into the spectrometer vacuum, pass through a drying gas which concentrates the charge further. At the end of the process, the charge remains no longer only on the solvent, but is passed on to the analyte of interest, generating ions. Functional groups possessing labile protons, such as carboxylic acids or amines, are needed for ESI, as it will not produce ions from non-polar species. (119) (195)

ESI can be performed either in positive and negative mode. In the case of negative mode ESI, ions are formed by the loss of an H^+ ion, which implies the parent ion (M) detected is an M^- ion. Positive mode ESI results implies the addition of another ion, usually a proton or for example sodium, to produce $[\text{M}+\text{H}]^+$ or $[\text{M}+\text{Na}]^+$, respectively. However the inclusion of these adducts increases the complexity of spectra, so longer transient lengths are required for unambiguous assignment. Therefore positive mode is not routinely performed for complex mixture analysis. It should be underlined that adduction can also occur in negative mode (although less common), usually in the form of chloride adducts, $[\text{M}+\text{Cl}]^-$, this is one reason complex mixture samples, particularly marine NOM, are often desalted as part of the sample preparation. (195)

ESI creates a stable spray conditions, leading to stable introduction of ions into the spectrometer. In addition, it allows a consistent number of ions being introduced to the detection cell from transient to transient, allowing the user to tune the instrument for improved resolution. It is for all these reasons that ESI is the ionisation technique of choice in the field of complex mixtures. (119) (195)

In this study, Suwannee River Fulvic Acid (SRFA, International Humic Substances Society, USA), peat DOM, HWE and NaOH samples were prepared using 1 mg in 50:50 MeOH/H₂O by volume (both LC-MS grade), diluted to a final concentration of 0.1 mg ml⁻¹. Analysis was performed using a 12T Solarix (Bruker Daltonics) with an Infinity cell using an optimised method.

Negative mode (-) ESI source was used with a capillary voltage of 4.0 kV and flow rate of 200 µl h⁻¹. The ion accumulation time and time of flight were set to 0.1 s and 0.55 ms, respectively. A total of 200 transients at 4 MW were summed. (195)

3.3.1.1 Calibration of ESI FT-ICR MS spectra

As previously mentioned, space charge effects, ion cloud stability and inhomogeneous electric fields can all have an impact on the frequency of ions within the cell. To minimise these issues, FTMS spectra need to be calibrated. For FT-MS spectra implementation of external and internal (re)calibrations are performed during the acquisition.

In regards with internal calibration, a selection of a series of known species across the mass range of the spectrum is carried out and a correction function is fitted to their mass error; this calibration method for complex mixtures can result in a spectrum with extremely high mass accuracy (<0.5 ppm).

On the contrary, external calibration requires creating an internal calibration for a sample by using an external list of known molecular

formulae as reference, then creating that correction function and applying this function to another spectrum.

After calibration, the resulting masses can be assigned to molecular formulae, usually on the basis of accurate mass alone. By convention, ^{12}C has been assigned a mass of exactly 12 Da and is used as the basis for all other atoms, the majority of which have been assigned a highly accurate mass. Therefore, in order to assign a molecular formula, the calculation of the combination of elements is the only thing required.

In order to externally calibrate the liquid state MS spectra, Suwannee River Fulvic Acid (SRFA), an internationally recognised IHSS standard was used. (199)

3.3.2 Laser desorption ionisation FT-ICR MS

Laser desorption ionisation FT-ICR MS was performed on ball-milled air dried samples. Initially the analysis was conducted by placing samples on electrically conductive non-porous copper tape mounted on a MALDI plate. However due to larger background signal coming from the tape itself, a very high laser power was required to achieve as much signal as possible (the laser power was optimised to 35% and each spectrum was exposed to 100 laser shots at 1000 Hz.). To remove the background and reduce the required laser power a conductive double sided copper tape was used instead. Therefore, it was possible to optimize a laser power to 20% using 200 laser shots at 1000 Hz on a minimum laser focus, leading to a better quality spectra, in terms of signal to noise ratio (SNR) and resolution.

The analyses were performed using negative mode (-) LDI 12T SolariX (Bruker Daltonics) with an Infinity cell and using a Smartbeam-II ND:YAG laser (wavelength 355 nm). The broadband spectra were

acquired between m/z 100 and 2000. A total of 200 transients at 4MW were summed.

3.3.3 Calibration of LDI FT-ICR MS spectra

The calibration of LDI FT-ICR MS spectra has been carried out by using the same list of known molecular formulae mentioned on liquid state section.

3.3.3.1 Assignment of FT-ICR MS spectra

The calibrated spectra with a mass accuracy of ± 0.5 ppm, for singly charged ions in an m/z range between 200-700 Da, with a signal to noise ratio > 4 were used to assign molecular formula in Formularity (120), with the elemental limits $C_{1-60}H_{1-150}O_{1-30}N_{0-1}$. N_1 was confirmed by the isotopic fine structure, also the elemental analysis confirmed a percentage of N_1 in those samples. Each negative ion exact mass was assigned a calculated neutral mass with the addition of $^1H^+ = 1.007276$ Da.

3.3.3.2 FT-ICR MS spectra analysis and visualisation

The data obtained from FT-ICR MS are very complex, with a typical DOM sample usually having more than 10,000 peaks. Therefore, in order to compare datasets, a number of analysis tools were applied to these data, which are described in this section.

3.3.3.3 Double bond equivalents (DBE)

Double bond equivalents (DBE) is a measure of the saturation, where a DBE value of zero describes a fully saturated molecule (Eqn. 8).

$$DBE = c - h/2 + 1 \quad (8)$$

c and h defines the number of carbon and hydrogen in a molecular formula respectively. Generally, the addition of a ring or double bond is accompanied by a loss of two hydrogens with corresponding DBE values calculated to be greater than zero.

3.3.3.4 Van Krevelen and compound class analysis

A van Krevelen diagram is scatter plot that displays the H/C vs O/C from assigned FT-ICR MS molecular formulae. (119) They have been used as a means to visualize and compare NOM data. Different regions of the van Krevelen diagram are assigned to compound class based on the H/C and O/C boundaries. An example of a typical Van Krevelen diagram showing the distribution of CHO molecular formulae in a DOM sample can be found on Figure 3.7.

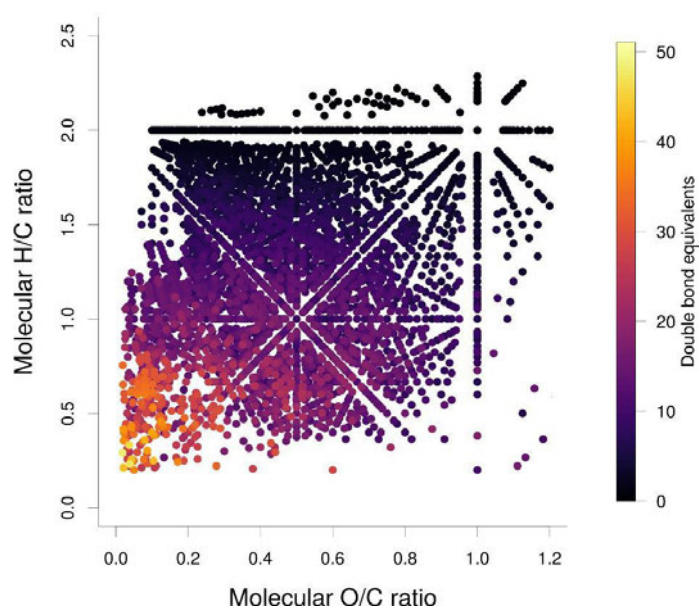


Figure 3.7. Example of a Van Krevelen diagram, colour by different DBE, of CHO molecular formulae in a DOM sample.

Each compound class is described as ‘like’, as such compound class assignments based on elemental ratios are putative; more experimental evidence would be required to unambiguously categorise a given molecular formula to a specific compound class. In addition, many molecular formulae could represent structural isomers belonging to different compound classes. However the use of elemental ratios to describe compound class is still the best means to date to explore the chemical diversity of FT-ICR MS data. In this work, the H/C and O/C boundaries are denoted in Formularity and automatically assigned to each molecular formula. As the van Krevelen diagram does not allow facile determination of the number of compounds assigned to a given compound class, bar charts were used in this study to make such comparisons.

3.3.3.5 Oxygen numbers and aromaticity index modified.

To examine if the number of oxygens per molecular formula change with peat depth of condition oxygen class plots have been produced and displayed as histograms.

Since many formulae assigned to complex mixtures are abundant in oxygen which is not considered in the DBE equation, a more accurate classification of saturation/unsaturation was created in the form of the aromaticity index (AI), Equation 5.

$$AI = \frac{1+C-O-S-0.5H}{C-O-S-N-P} \quad (5)$$

This parameter provides the C-C double bond density for a given molecule and was later modified (AI_{mod} , Equation 6) to take into account the fact that it is believed that half of the oxygens in NOM are present as carbonyl functional groups.

$$AI_{\text{mod}} = \frac{1+C-0.5O-S-0.5H}{C-0.5O-S-N-P} \quad (6)$$

The modified aromaticity index is used to assign formulae into one of either non-aromatics, aromatics or condensed aromatic species (182). However, in this thesis four distinct categories are used based on literature (182): polycyclic aromatics ($AI_{\text{mod}} > 0.66$), plant-derived polyphenolics ($0.5 < AI^{\text{mod}} \leq 0.66$), highly unsaturated and phenolic species ($AI_{\text{mod}} \leq 0.5$ and $H/C < 1.5$) or aliphatic species ($AI_{\text{mod}} \leq 0.5$ and $H/C \geq 1.5$). (182)

3.4 Multivariate analysis

PCA was applied to ATR-FTIR, solid state CP-MAS ^{13}C NMR, liquid state ^1H NMR and MS data using the SIMCA 16 software. Before PCA, all IR (intensity values), ^1H and ^{13}C NMR (integral values) using bin intervals of 0.5 cm^{-1} (excluding $4000\text{--}3200\text{ cm}^{-1}$ and $2800\text{--}1800\text{ cm}^{-1}$), 0.01 ppm between $0\text{--}10\text{ ppm}$ (excluding $4.65\text{--}4.75\text{ ppm}$), and 0.1 ppm between $0\text{--}200\text{ ppm}$, respectively.

For NMR data each integral was treated as a single variable. These integrals were then normalised using the standard normal variate (SNV) method and scaled using Pareto scaling. A model was fitted to the processed data and a score plot of principal components 1 and 2 produced. PCA of MS data was conducted by treating each assigned formula as a variable, with a value of either 1 or 0 indicating presence or absence in each spectrum.

4. Comprehensive physicochemical and molecular analysis of a single peat core

This chapter provides a detailed examination of the physicochemical and molecular properties of a 50 cm peat core. The core was chosen at random from those extracted from the Flow Country site, S2D. The whole peat as well as pore water, labile and recalcitrant fractions is examined using ATR-FTIR, solid and liquid state NMR as well as solid and liquid state FT-ICR MS. Physicochemical properties such as bulk density, ash content, CHON were also examined. The aim of this chapter is to compare the techniques and the information they provide with respect to peat depth.

This chapter is the subject of the following publication:

Trifiró, G., York, R., & Bell, N. G. (2021). High-Resolution Molecular-Level Characterization of a Blanket Bog Peat Profile. *Environmental science & technology*, 56, 1, 660–671.

4.1 Bulk density, water content, ash content and elemental analysis

The bulk density, averaged ash content, C/N and O/C ratios of the peat core are presented in Figure 4.0. Further details about the percentage of carbon, hydrogen, oxygen and nitrogen can be found in Appendix A, Table A1.

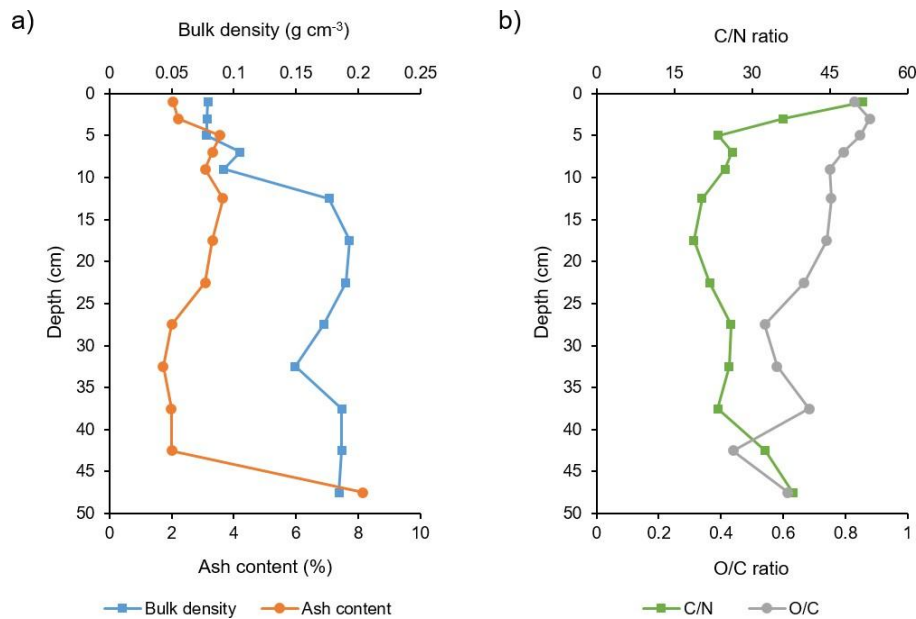


Figure 4.0 The bulk density, averaged ash content a), C/N and O/C ratios b) of the peat core D from the Flow Country site.

Bulk density increases from 0.07 - 0.09 g cm^{-3} in the top 10 cm to 0.17- 0.18 g cm^{-3} in the lower layers from 10 cm to 50 cm, with a small decrease between 25 and 35 cm. The bulk density values show a very similar trend to those reported in other studies of peat bogs in high latitude regions. (1) (2) (3) Bulk density is reported to increase with depth due to increased decomposition, reduction in porosity and compaction. (4) The presence of a step change at a depth of 10 cm could mark the transition from acrotelm to catotelm and appears at around the expected depth for such sites. (5) The ash content ranges from 1.7-3.5% until 45 cm, after which there is a steep increase to 8.1% for the 45-50 cm sample. Excluding the first two samples, the ash content is higher in the upper layers (4-25 cm) than the lower layers. Malawska *et al.* (2006) and Weiss *et al.* (2002) showed similar decreasing ash content with depth for blanket bogs from Canada. (7)

A combination of these factors may lead to increased ash content in the upper layers, thus outweighing the expected increase with depth. (7) Ash content decreasing with depth could also be explained by increased OM loss through mineralisation. (8) Additionally, it has been reported that elevated ash content follows increased bulk density, (9) and while there is no overall increase, the increase in ash content between adjacent samples does follow the increase in bulk density to an extent. The sharp increase at 45 cm could also be attributed to atmospheric inputs of pedogenic dust which accumulated as a result of organic matter decomposition or by precipitation of new diagenetic solid phases. (10) However, upon examining the peat depth at the collection site, rocky material was observed below 50 cm, and it is therefore likely that they have contributed to the increased inorganic content at the bottom of the core. (183) As previously discussed, the core was slightly grey in colour between 40-50 cm depth, which supports this hypothesis. Finally, the C/N ratio initially declines sharply with depth, roughly halving between 0 and 6 cm, but then remains relatively constant until a depth of 40 cm at which it increases slightly. The C/N ratio is generally thought to decrease, along with H/C and O/C, with decomposition, (11) (12) (13) (14) (15) as total N is enriched relative to C due to preferential decomposition of high C compounds, together with an increase in more recalcitrant compounds. (16) (17) The O/C ratio does decrease with depth as expected, but the absolute C content in fact shows a general increasing trend (49-63%), in accordance with previous studies conducted by Artz *et al.* (18) It is clear that C does not universally decrease with depth; although an enrichment of N content is typical, C content in fact increases as well. (18) It has been argued by Zacccone *et al.* (2018) that there is a notable lack of correlation between C/N ratio and humification index, and that ash content (and by consequence bulk density) is too dependent on

the rate of supply of dust particles, so none of these traditional humification proxies are adequate for use alone in investigating peat decomposition. (19) Additionally, the authors noted that C/N ratios varied significantly between different sites by up to a factor of 5, showing that absolute values of these measurements cannot be used to quantify the extent of humification. It is clear that although the bulk properties discussed here can provide an indication, more sophisticated methods are required to truly understand the composition and decomposition of peat.

4.2 ATR-FTIR spectroscopy

4.2.1 ATR-FTIR spectral observations

ATR-FTIR stacked spectra obtained from an average of triplicates at each depth of the core are provided in Figure 4.1.

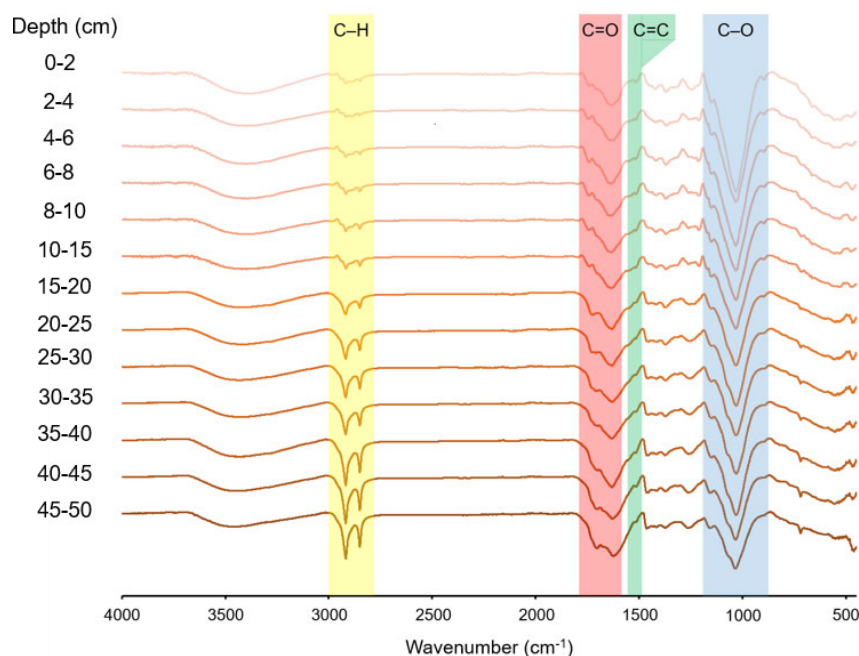


Figure 4.1 ATR-FTIR stacked spectra of core S2D (13 depths) from the Flow Country with the main functional groups highlighted.

Overall, we can note a broad band at 3400 cm^{-1} (most likely the O-H stretch from carboxylic acids), two sharp peaks at 2920 and 2850 cm^{-1} (sp^3 C-H stretches of alkanes), a wide band between 1650 - 1725 cm^{-1} containing four distinct resolved peaks shifting in wavenumber with depth at 1650 and 1675 cm^{-1} (C=C alkene/aromatic stretch or C-O carboxylate stretch) and 1702 cm^{-1} , 1725 cm^{-1} (C=O stretches, most likely from saturated acids), 1510 cm^{-1} (C=C stretch of aromatic molecules) and 1030 cm^{-1} (C-O alkoxy stretch, e.g. from carbohydrates). The relatively intense peaks (not shown) below 500 cm^{-1} are due to possible mineral interferences. In addition, spectra contain a number of minor peaks between 1500 and 1200 cm^{-1} . The observations described are typical of peat samples and linked to polysaccharides, fatty acids, lipids and lignin. (18) (20) (21) (19) (2) (1) (3) It should be noted, however, that as peat OM is a complex mixture of thousands of compounds, IR spectra should be interpreted with caution, since a particular stretch in the spectrum is a superposition of many types of molecules. For example, not only will the carbohydrate stretch consist of a mixture of small and large carbohydrates, which cannot be differentiated, other types of molecule, such as flavonoids, that have a carbohydrate moiety will also absorb IR radiation in the 1030 cm^{-1} region. The same argument can apply to aliphatic C-H stretches, which will arise from a mixture of small and long chain aliphatic acids and aromatic stretches, from a wide range of sources. Therefore, if one or another functional group dominates the spectra, it cannot be classified as belonging to a particular molecule based exclusively on IR data. For this reason, IR data must be interpreted alongside other techniques, such as NMR, in order to draw structural information.

4.2.2 Statistical analysis

In order to examine the changes in ATR-FTIR spectral features with depth, PCA was conducted on the data set. The PCA score plot is shown in Figure 4.2.

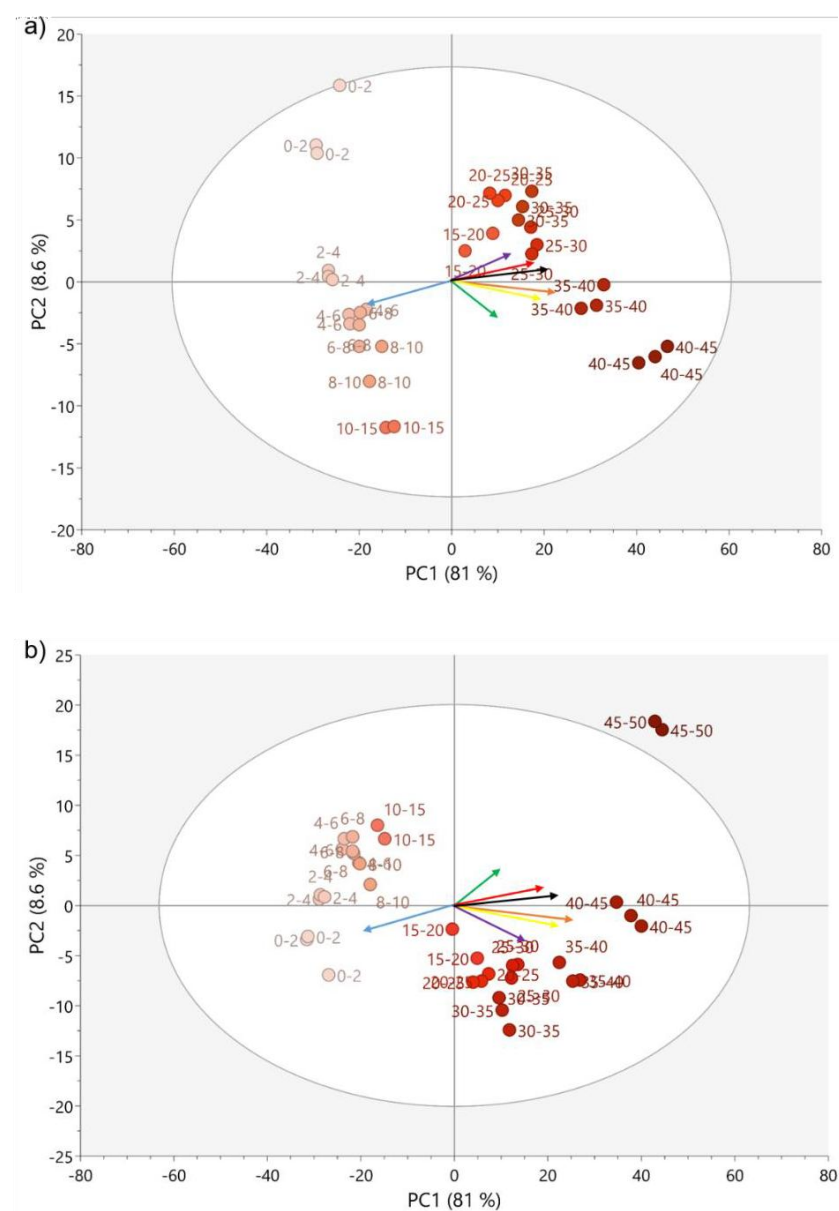


Figure 4.2 PCA score plot of ATR-FTIR data core D a) excluding 45- 50 layer and b) including 45-50 layer. The scores are colour coded and

labelled according to depth. The loadings are relative to C-H stretch (2920 cm^{-1} , orange arrow), C-H stretch (2850 cm^{-1} , yellow arrow), C-O stretch (1030 cm^{-1} , blue arrow), C=O stretch (1630 cm^{-1} , black arrow), C=O stretch (1702 cm^{-1} , red arrow), C=O stretch (1725 cm^{-1} , purple arrow) and C=C stretch (1510 cm^{-1} , green arrow). Ellipse shown represent Hotelling's T^2 (95%).

The PCA score plot of ATR-FTIR data (Figure 4.2a) excludes the three points from the 45-50 cm layer as these samples were shown to be outliers, falling outside the Hotelling's T^2 ellipse in Figure 4.2b. PCA analysis also excluded the regions without major peaks ($2800\text{-}1800\text{ cm}^{-1}$), and the O-H stretch at 3400 cm^{-1} as this region could be affected by moisture. In both plots, a clear trend with depth is observable, with samples from lower depths having higher PC1 values. There is a negative correlation between PC1 (81%) and the C-O stretch loading (1030 cm^{-1}), which is likely to arise from carbohydrates, indicating that the top layers are higher in carbohydrate content. The lower layers show a positive correlation between PC1 and the C-H, C=C and C=O stretch loadings (1510 , 1630 , 1702 , 1725 , 2850 and 2920 cm^{-1}), suggesting that there are more aromatic and aliphatic molecules in the lower layers. Similar trends were reported by Artz *et al.* (2008) and Tfaily *et al.* (2014) from Scottish and US blanket bogs; Artz *et al.* defined polysaccharide markers as O-H and C-O bands at 3400 and 1040 cm^{-1} which were found to decline with depth, whilst all other major bands increased. McAnallen *et al.* (22) investigated a Northern Irish blanket bog, using a humification index calculated from the ratio of intensities of the carboxylic or aromatic (1420 or 1510 cm^{-1}) to polysaccharide (1070 or 1040 cm^{-1}) peaks, and finding that this generally increased with depth, in agreement with this study. (22) Whilst polysaccharides such as cellulose are clearly the most labile

components, degradation of these will produce smaller carbohydrate molecules which will exhibit similar IR stretches. Meanwhile, recalcitrant molecules are likely to be formed through metabolic production or transformation of plant material, but also produced directly from plants, such as the phenolic molecules derived from *Sphagnum* mosses. (23) Additionally, from the PCA scores, there are two clear clusters (0 to 15 cm and 15 to 45 cm) separated along PC1. This indicates a boundary at a depth of around 15 cm, at which the composition changes; this is close to the boundary of 10 cm identified by bulk density analysis, and provides further support for the acrotelm/catotelm model. It is clear that ATR-FTIR data is closely linked to, and likely influenced by, the bulk properties; from the identification of a step change at intermediate depths to the observation of the 45-50 cm spectra being an outlier, there are several similarities between the two types of data. This observation has also been reported in the literature, including by Artz *et al.* (2008), who observed a correlation between carbon content and polysaccharide IR markers. A point to note is that the triplicate IR spectra are not identical. This is due to the nature of ATR measurements, where one cannot control the amount of material directly pressed onto the surface of the crystal. Therefore, triplicate measurement of IR spectra should be acquired as a minimum. This is not required for other techniques such as NMR or MS, and should be considered when evaluating the time available to conduct molecular analysis.

4.3 NMR spectroscopy

4.3.1 ^{13}C Solid state spectral observations

Stacked ^{13}C CP-MAS NMR spectra with the characteristic spectral regions labelled are shown in Figure 4.3a. The relative integrals of each of these regions are plotted against depth in Figure 4.3b.

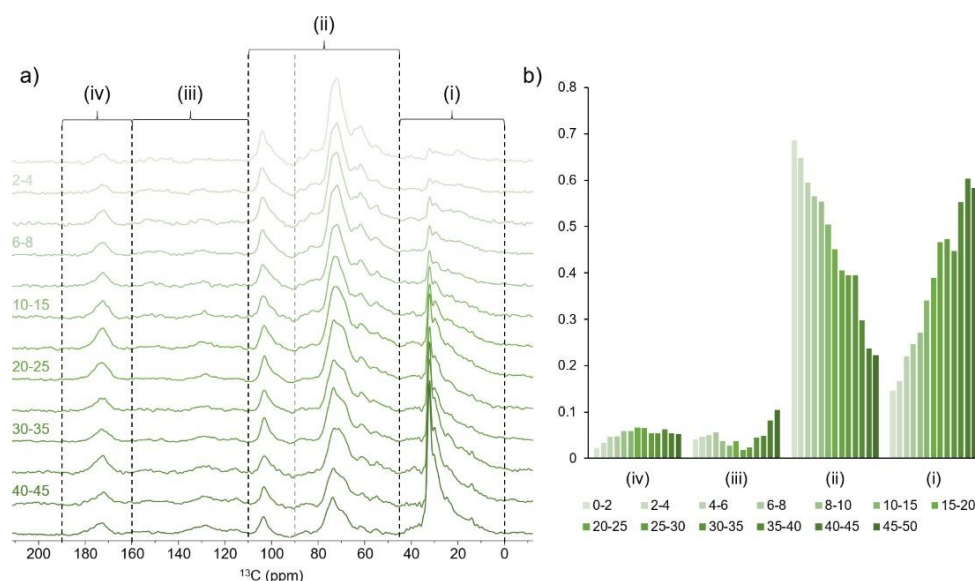


Figure 4.3 a) Solid state CP-MAS ^{13}C spectra of dried peat samples, with characteristic regions labelled: (i) Alkyl (0-45 ppm), (ii) O-alkyl (45- 110 ppm, with anomeric region 90-110 ppm), (iii) Aromatic (110-160 ppm), (iv) Carboxyl (160-190 ppm) and b) bar chart showing relative intensities of the same four regions against depth.

These spectra are typical of peat samples, (24) (25) showing broad resonances throughout due to overlap of large numbers of resonances from the many compounds in peat. Regions are assigned based on observed literature assignments summarised by Baldock *et al.* (24) From the spectra, the carboxyl peak that appears between 170 and 175 ppm can be assigned to aliphatic esters or aromatic acids or

amides, based on the ^{13}C chemical shifts of some standard compounds, such as methyl pentanoate (170 ppm), valine (171 ppm), benzoic acid (173 ppm). (24) (26) Aliphatic acids or many long chain fatty acids, however, have carbonyl ^{13}C chemical shifts above 175 ppm, and therefore are unlikely to be the source of these peaks. Previous studies using dipolar dephasing experiments with varying dephasing times to peat samples to elucidate the source of non-protonated carbons, concluded that the peak at 107 ppm arose from aromatic carbons but was not well separated from a polysaccharide peak at 104 ppm. (26) This complicates the use of cleanly divided integrals so the region from 160 to 190 ppm was removed from further analysis. CP-MAS spectra, in part due to the nature of cross polarisation, are only semi-quantitative; cross polarisation is far less efficient for carbon atoms not directly bonded to protons, i.e. quaternary carbons. For this reason, very low signal is observed in the aromatic region, even though peat samples would be expected to contain significant quantities of aromatic molecules. This implies that the aromatic molecules present in these samples are highly substituted. A solution to this lack of signal from substituted aromatics is the HP-DEC experiment. A comparison between CP-MAS and HP-DEC is shown for one sample in Figure 4.4.

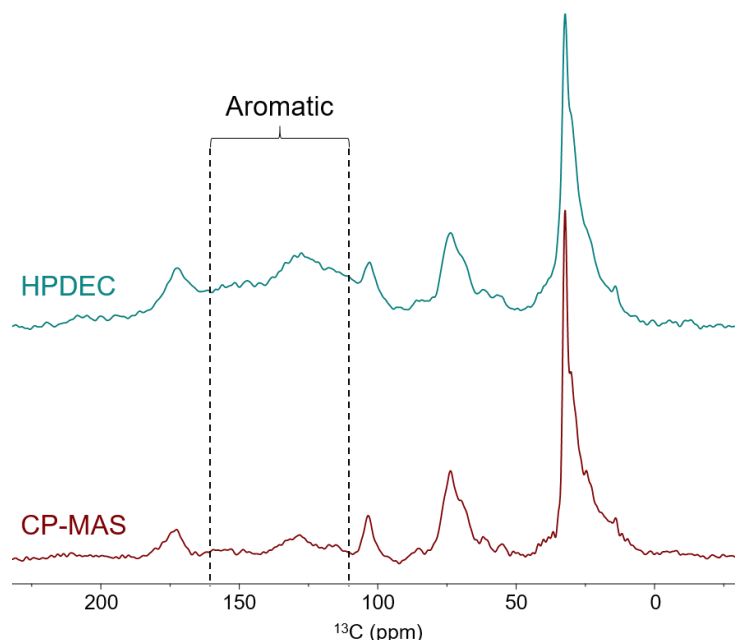


Figure 4.4 Solid state ^{13}C CP-MAS and HPDEC spectra of the dried peat sample from a depth of 45-50 cm.

It is clear that the aromatic region is far more intense in the HPDEC spectrum; as previously discussed, this is as would be expected using direct polarisation techniques, given that cross polarisation underrepresents signals from quaternary carbons, which have no nearby protons for transfer to occur from. When the intensity of the alkyl region (0-45 ppm) in each spectrum is calibrated to 1, the aromatic intensity (110-160 ppm) increases from 0.1791 (CP-MAS) to 0.8576 (HPDEC); this represents an almost 5-fold increase. Whilst the improvement is clear, this spectrum was recorded over a period of 12 hours in order to obtain a similar signal-to-noise ratio to the CP-MAS spectrum (42 minutes). The reason for this increase is the use of a much longer (50 s) relaxation delay between scans, which is necessary as ^{13}C returns to equilibrium very slowly in the solid state.

Analysis of large numbers of samples in this fashion would therefore be immensely time consuming and therefore it is unsurprising that many studies examining peat have applied CP-MAS quantitatively. (26) (27) (28) (29) (30) According to Krosshavn *et al.* (28),s it is reasonable to make quantitative comparisons between such spectra as long as the parameters, such as contact time, remain the same, as was done in this study. Examining the relative intensities with depth in Figure 4.3b there is a clear increase in intensity of the alkyl region with depth, whilst the O-alkyl resonances are characterised by a slightly decreased intensity at lower depths. The aromatic region shows a slight increase with depth. CP-MAS spectra of peat samples of varying depths have been acquired in several studies of Canadian raised peat bog/swamps/fens (unspecified depths from 0-2 m), (26) (27) Norwegian bogs (unspecified bog type, 2-12 cm), (28) Swiss raised bog (0-2 m), (29) and a British blanket bog (0-1 m). (30) All studies report a decrease in O-alkyl region intensity with depth, while some report an increase in the aromatic region intensity. (29) (30) The composition of the peat has been related to the predominant vegetation and level of decomposition. (26) (27) (28) For example, Hammond *et al.* (1985) reported that peat dominated by mosses and softwoods had higher polysaccharide (O-alkyl) and lower lignin (aromatic) content than those composed of hardwoods and sedges. A comparison of the spectra with those of heather, sedge and *Sphagnum* derived peat indicate that indeed the core samples presented here are typical of sedge/*Sphagnum* derived peat. (200) Whilst site-specific observations differ, the literature generally shows that the alkyl:O-alkyl intensity ratio is a good marker of extent of decomposition, given that this ratio almost universally increases with depth. The explanation for this is that hydrophilic and oxygen-rich polysaccharides decompose preferentially in anoxic conditions, where

molecules such as lignin are only degraded in the presence of oxygen, leading to a decrease in O-alkyl intensity. The increase in alkyl intensity is caused by the build-up of hydrophobic aliphatic molecules as products of microbial decomposition. (29) A plot of alkyl:O-alkyl ratio against depth was produced (Appendix A, Figure A1), showing the same trend. Similar to the trends in the data of Leifeld *et al.* (29) (59), reported that the greatest increase in this ratio with depth occurred between roughly 0-25 cm depth. However, the reliability of comparisons drawn between different studies is questionable, given that the parameters used for acquiring CP-MAS spectra are not identical, nor are the spectral regions used for assignments. It would be beneficial for a comprehensive set of guidelines to be agreed upon for acquisition and analysis of such spectra from peat, and other NOM samples.

4.3.2 ^1H Liquid state spectral observations

Liquid state ^1H NMR spectroscopy with suppression of the HOD signal at 4.7 ppm was conducted on the HW-E, NaOH-E and PW-DOM solutions obtained from each depth. Stacked ^1H NMR spectra from the three extraction methods with the characteristic spectral regions labelled are shown in Figure 4.5.

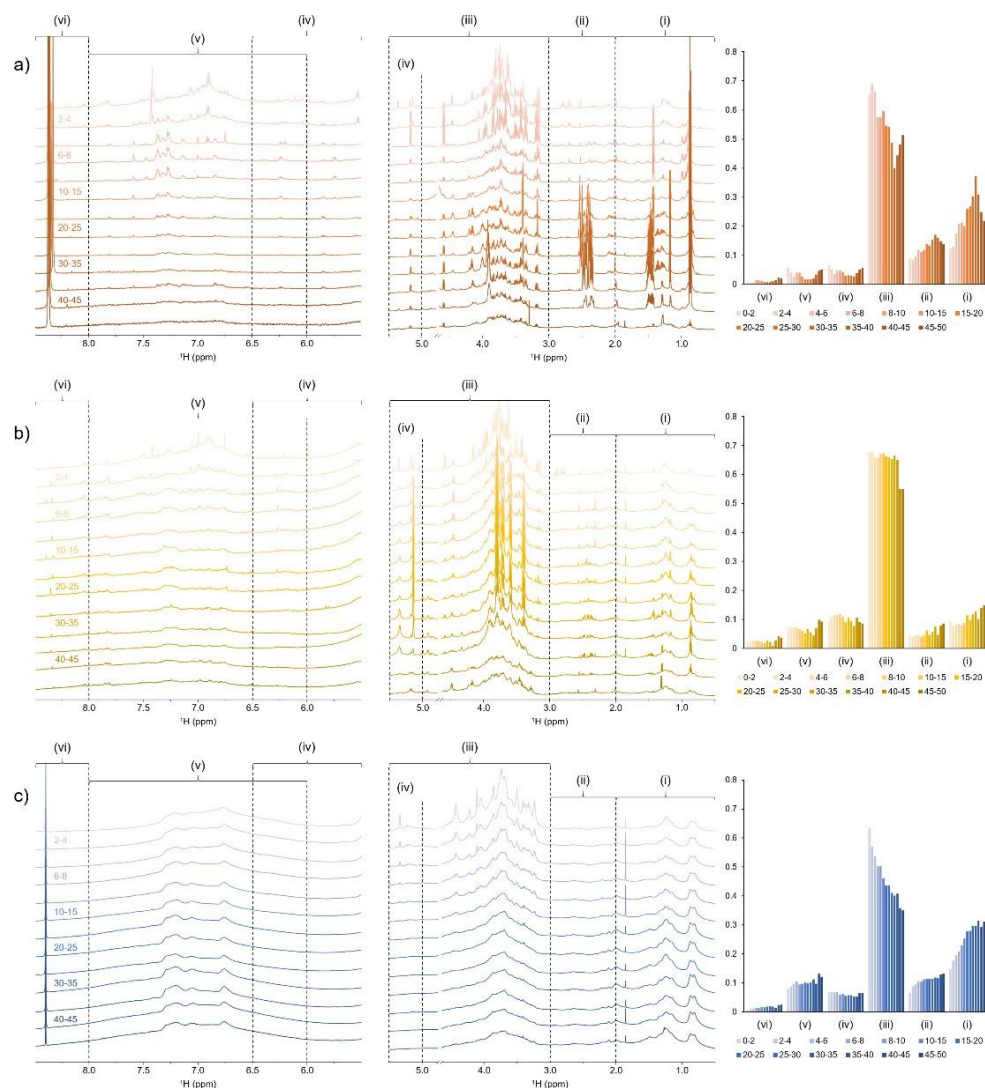


Figure 4.5 Liquid state ^1H NMR spectra, and bar charts showing relative intensities of various regions of a) PW-DOM, b) HW-E, and c) NaOH-E peat samples. Aromatic regions are scaled to 5x intensity for NaOH and 10x intensity for HW-E and PW-DOM. Characteristic regions are labelled: (i) Alkyl (0-2 ppm), (ii) Functionalised alkyl (2-3 ppm), (iii) O-alkyl (3-5.5 ppm), (iv) Alkene (5-6.5 ppm), (v) Aromatic (6-8 ppm), (vi) Carbonyl (8-10 ppm). Regions below 0.5 and above 8.5 ppm are excluded due to a lack of resonances. The residual HOD signal has been removed from all spectra, and the aromatic region has

been plotted separately at increased scale in order for greater detail to be observed.

The spectra obtained from the different sample types all show similar features. The regions with the largest intensity of resonances are the O-alkyl (3-5.5 ppm), followed by the alkyl region (0-3 ppm). The aromatic region (6-8 ppm) has a much lower signal intensity again due to the lack of aromatic protons relative to the other compound classes. The alkyl region may be split into two regions, assigned to terminal CH₃ groups away from any electronegative group (0-2 ppm) and CH₂ or CH₃ groups attached to a functional group, such as carbonyls (2-3 ppm). It must be reiterated that when defining spectral regions of complex mixtures there is a great deal of overlap, and those given are only a rough guide; for example, the majority of O-alkyl signals will occur between 3-5.5 ppm, but it is possible that other ¹H environments may be present within this region. There are a series of sharp peaks superimposed on the spectra, which indicate the presence of small molecules in high enough concentration for their resonances to be resolved. They are likely the metabolites of plant material, root exudates or decay products in the core. Major compounds identified in the literature include formic acid at 8.44 ppm, acetic acid at 1.90 ppm and lactic acid at 1.33 ppm, by Woods *et al.* (2011). (31) Sharp resonances at 8.4 ppm and 1.9 ppm are particularly prevalent in the NaOH-E and PW-DOM spectra. These small aliphatic acids are known root exudates, (32) and precursors for methanogenesis in peat. (33) (34) The presence of acetic acid in PW-DOM is unsurprising as porewaters have been reported to accumulate acetic acid from anaerobic metabolism and it can be found in all levels due to diffusion. (35) Whilst there is no literature evidence for the use of extracted peat samples to track changes with depth, spectra are similar in overall

shape to those obtained by Hertkorn *et al.* (2002) for extracted peat HA and FA, with the NaOH-E spectra (especially those from lower depths) being almost identical to the HA spectra from this study. (36) However, it has been argued by Chen *et al.* (2019) that extraction using NaOH-E leads to undesired chemical modification of peat samples. (37) They reported a significant increase in carboxyl content and decrease in O-alkyl content as a result of the extraction, measured using solid state NMR. (37) The oxidation of organic matter when exposed to air under alkaline conditions has been well understood since 1950, (38) and hence extractions were carried out in this study under nitrogen, but the authors argue that such changes occur upon exposure to air as part of the dialysis step. (37) Also, smaller molecules may be lost during dialysis. For these reasons, caution should be made when comparing NaOH-E extracted samples. However, in this study the resemblance between the trends for NaOH-E solution state and bulk peat solid state NMR spectra indicate that NaOH-E spectra are representative of the bulk recalcitrant fraction. HW-E spectra bear more resemblance to FA with a greater number of sharp resonances, but notably the aromatic region is much less intense than observed by Hertkorn. (36) These observations are unsurprising, given that extraction using NaOH-E by definition isolates both HA and FA; HA generally represents a larger fraction of peat, (39) and thus will dominate the spectra. On the other hand, extraction using hot water is likely to extract a fraction similar to FA given HA is soluble only at high pH. It is not known exactly how the compounds extracted by HW-E relate to these well-defined operational fractions, but from the NMR spectra it is likely that this process is less successful at extracting aromatic compounds. Examining the spectral changes with depth in all samples, the carbohydrates decrease in concentration with depth, although the rate of decline differs depending on sample type. A

number of sharp carbohydrate resonances are observed in surface layers, which rapidly disappear with depth, although in HW-E and PW-DOM spectra these are later replaced by other sharp resonances. The spectra contrast with the solid state spectra in that the alkyl region does not drastically increase with depth; the extraction process only yields a fraction of the overall peat sample, whilst the solid state spectra examine the whole sample, so it can be deduced that the alkyl compounds present in the dried sample are much less soluble and hence not observed in the extracted samples. Examining each sample type in turn, the NaOH-E spectra possess fewer sharp resonances, which indicates fewer small concentrated molecules in this extract compared to PW-DOM and HW-E. The NaOH-E extraction would be expected to yield the majority of material present in both the HW-E and PW-DOM fractions, given the extraction is performed on frozen samples with pore waters intact. Since there are a larger subset of molecules present, it is likely that the relative concentration of these small molecules is much lower and hence their signal is swamped by other resonances. The sample from depth 30-35 cm stands out as having the largest alkyl and functionalised alkyl, lowest O-alkyl intensity, aromatic and alkene intensity compared to the other depths, indicating some clear hot spot in decomposition. In contrast, HW-E spectra exhibit these sharp resonances in all spectra. The HW-E procedure is suitable for the extraction of more labile compounds from peat, (40) and thus would be expected to represent a smaller fraction of the overall peat sample than the NaOH-E extraction. Interestingly, these spectra possess several sharp peaks which appear only at intermediate depths, primarily in the O-alkyl region. These may arise from small sugars, for instance glucose. In terms of change in composition with depth, it is clear that in each region the intensity is relatively similar with a slight increase in alkyl compounds with depth.

However the last two samples stand out as having the lowest O-alkyl and highest alkyl and aromatic intensity, indicating a change in composition of the labile component in these two layers. Finally, the NMR spectra from PW-DOM samples reveal some differences in terms of the carbohydrate content; the aliphatic peaks follow an increasing trend until a depth of 40 cm is reached, and the O-alkyl peaks the opposite. The sample at 45-50 cm possesses far fewer sharp resonances. Trends in general are less clear, which can be in part explained by the fact that the pore waters are freer to move between upper and lower depths of the peat column, leading to the transfer of water-soluble compounds. The major sharp peaks in the O-alkyl region can be assigned to glucose (by additional 1D/2D experiments by Bell group, data not shown) and vary wildly between different samples, being most clear in the 4-6 cm sample, indicating a peak in metabolism at this depth. In addition, there are a series of sharp resonances that appear only between 20-45 cm in the functionalised alkyl region (2-3 ppm). Another set of sharp resonances also appear in the alkyl region at the same depths. These are likely to arise from the same compound or group of compounds since their intensities vary together. These sharp resonances are likely to be the cause of the lack of clear trend in integrals with depth. It should also be noted that the chemical shift of formic acid (along with ^{13}C satellites) at around 8.4 ppm changes slightly between the different spectra. This can be attributed to small changes in pH if the pH is close to the pKa of formic acid, 3.75. The trend observed for the alkyl region of all sample types almost mirrors that of the O-alkyl region; layers with the highest O-alkyl compounds have lowest alkyl compounds. Taking the integrals of the carbohydrate and alkyl regions again allowed for comparison using the alkyl:O-alkyl ratio of each spectrum. Plots of alkyl:O-alkyl ratios against depth were produced (Appendix A, Figure

A1), in which it is clear that for each of the three extraction methods, the carbohydrate region becomes comparatively less intense with depth. This fits with similar trends observed in solid state spectra. The NaOH-E spectra in particular show the most significant changes in alkyl:O-alkyl ratio in the uppermost 20 cm, which is very similar to the trend observable in the data of Leifeld *et al.* (2012). (29)

4.4. Statistical analysis of the solid and liquid state NMR data

PCA score plots for the CP-MAS spectra and each of the three liquid-state spectra are shown in Figure 4.6.

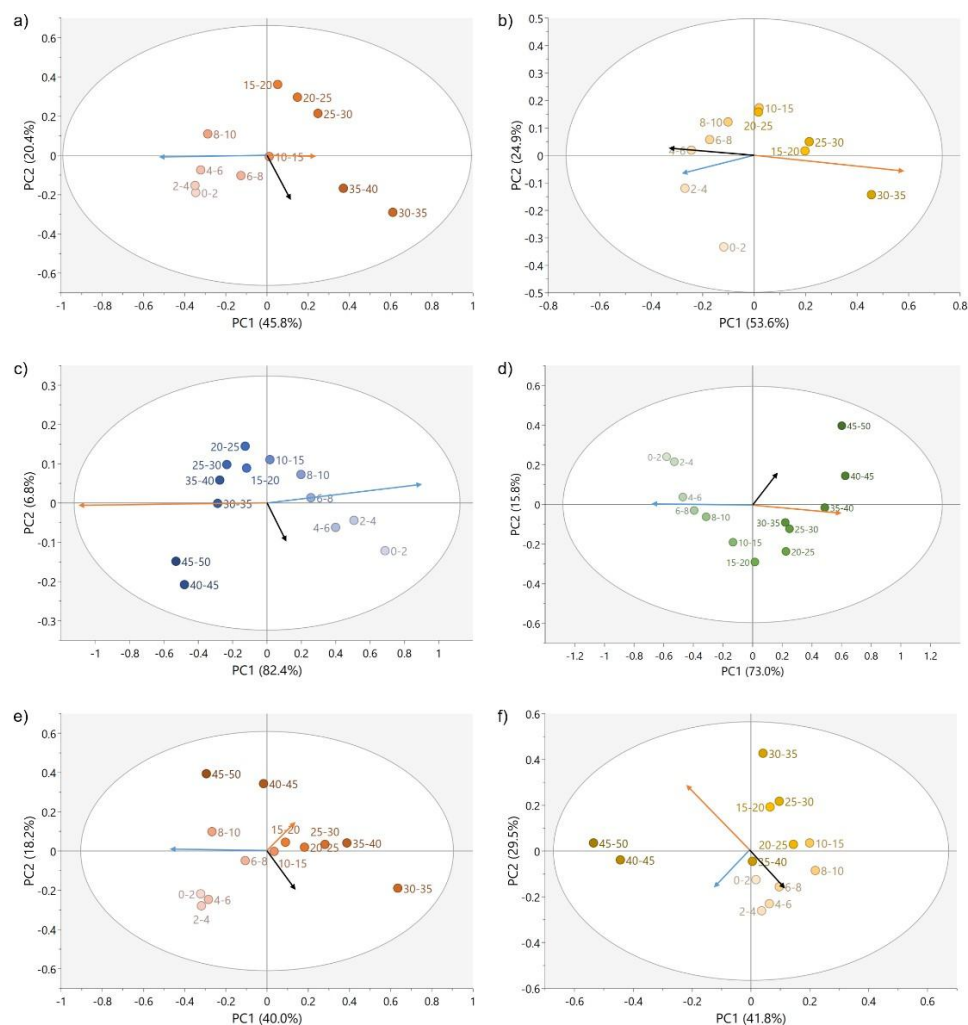


Figure 4.6 PCA score plots of a) PW-DOM NMR data with 40-50 cm points removed, b) HW-E NMR data with 35-50 cm points removed, c) NaOH-E NMR data, d) CP-MAS NMR data, e) PW-DOM NMR data with 40-50 cm points included and f) HW-E NMR data with 35-50 cm points included. The points are coloured and labelled by depth. The averaged loadings are relative to: alkyl and functionalised alkyl (orange arrow), O-alkyl and carbohydrates (blue arrow), and aromatic (black arrow) with chemical shift regions as defined in previous sections for ^1H and ^{13}C respectively. Ellipses shown represent Hotelling's T^2 (95%).

The PCA score plots shown in Figure 4.6 exhibit similar patterns, with the first principal component capturing well the variation with depth. Contrary to the IR data, it is possible to follow a continuous trend along PC1 with depth, rather than the points being separated into two distinct groups. It is therefore likely that NMR spectra are less strongly influenced by bulk properties. They also generally follow a U-shaped curve; this “horseshoe effect” is commonly observed as a result of dimensionality reduction. (43) It is worth noting, however, that the lowest three depths in the HW-E spectra and the lowest two depths in the PW-DOM spectra had to be excluded before such trends were observable. Through closer examination of the spectra, it is clear that these are visually different from those obtained from the rest of the core, primarily due to a lack of the sharp resonances observed in the upper levels. For each of the PCA score plots, the averaged loadings of the alkyl region, consistently, show a strong correlation with the lower depths along PC1, indicating that the alkyl intensity increases with depth. Conversely, the loadings representing the O-alkyl region exhibit a correlation with the upper depths, showing that the O-alkyl region intensity decreases with depth. The aromatics generally show

a stronger loading along PC2, indicating that the aromatic region does not consistently show a particular trend with depth. These trends are consistent with those noted in the intensity bar charts for the four types of NMR spectra. Overall, the PCA score plots show little information that cannot be obtained from other means, but the loadings plots (Figure 4.7) may be used to determine whether the sharp peaks observed in some spectra show any correlation with depth.

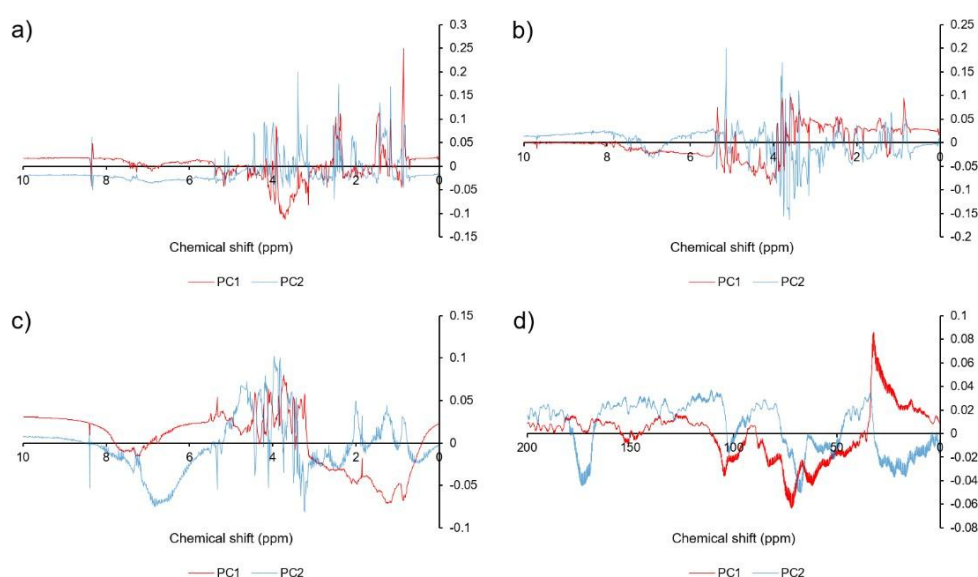


Figure 4.7 PCA loadings plots showing the first two principal components of a) PW-DOM, b) HW-E, c) NaOH-E and, d) CP-MAS NMR data.

It is clear from the loadings that for both HW-E and PW-DOM that sharp resonances influencing separation on the PCA score plots. Further NMR experiments would be required to confirm what these resonances belong too, however it does show that one should interpret the PCA of liquid state NMR with caution as only a handful of molecules could be driving the trends.

4.5 (-) ESI and (-) LDI FT-ICR MS

ESI FT-ICR MS spectra for pore water PW-DOM, HW-E and NaOH-E extracted samples and LDI FT-ICR MS of whole peat samples were acquired. Tables listing the number of peaks, number of CHO and CHON assigned formula and % assignment from each spectrum can be found in Appendix A, Table A2. Each spectrum contains thousands of peaks, with up to 18 per nominal mass, as demonstrated in Figure 4.8.

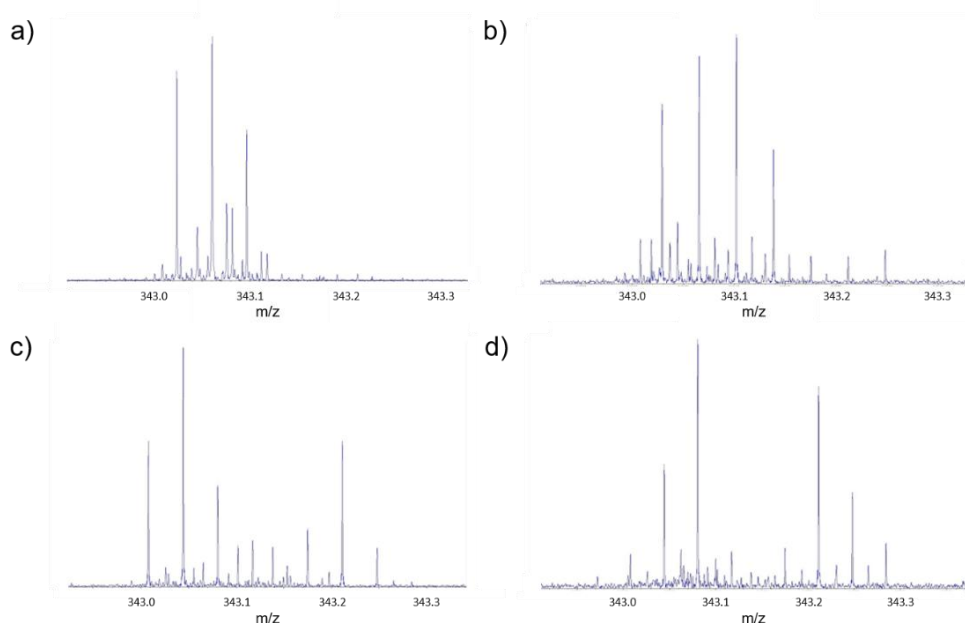


Figure 4.8 a) LDI, b) PW-DOM, c) HW-E and, d) NaOH-E FT-ICR MS spectra windowed at 343 m/z. Table of assigned peaks can be found in Appendix A, Table A3.

4.5.1 Comparison of the sample types and ionisation methods

To compare the sample types (PW-DOM, HW-E and NaOH-E) and ionisation methods (LDI vs ESI), PCA was again conducted. The score plot is shown together with van Krevelen (VK) diagrams (colour coded

by double bond equivalents (DBE)) constructed from all the $C_xH_yO_z$ molecular formulae assigned across all depths, in Figure 4.9. Each VK is split into regions denoting compound class.

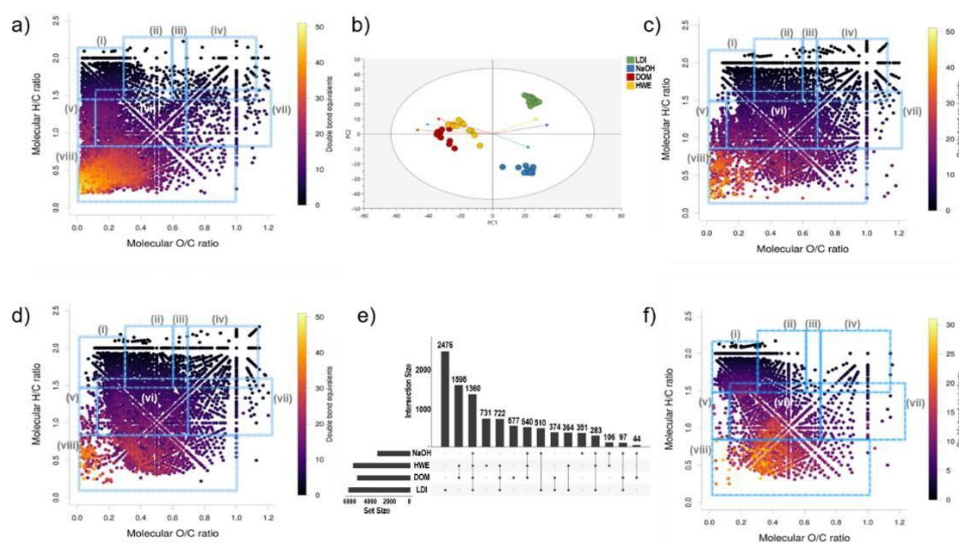


Figure 4.9 VK plots of a) LDI (8394 molecular formulae), c) PW-DOM (8279 molecular formulae), d) HW-E (8241 molecular formulae), f) NaOH-E (3865 molecular formulae) assigned molecular formulae from all core depths colour coded by DBE. Regions shown marked in dashed lines are the assigned compound classes regions denoted in Formularity, namely: (i) lipid-, (ii) protein-, (iii) amino sugar-, (iv) carbohydrate-, (v) unsaturated HC-, (vi) lignin-, (vii) tannin-, and (viii) condensed HC-like. b) PCA of the FT-ICR MS data. e) UpSet plot of all LDI, PW-DOM, HW-E and NaOH-E MS $C_xH_yO_z$ molecular formulae.

To further aid the comparison, an UpSet plot (Figure 4.9e) was also produced, showing 15 intersections following the $2^k - 1$ distribution, where $k = 4$ (LDI, PW-DOM, HW-E, NaOH-E). From a total of 15,355 assigned $C_xH_yO_z$ molecular formulae, 66% are considered in these UpSet intersections; 41% represented unique formulae. In addition,

the first 10 intersections represented more than 90% of the 1360 molecular formulae shared between all MS data under investigation. The UpSet plot including CHON compounds can be found in Appendix A, Figure A2. From a comparison of the different sample types, it can be noted a similarity between HW-E (5701 CHO compounds) and PW-DOM (5309 CHO compounds), sharing 4217 compounds. Both show predominantly lignin-like, protein-like and carbohydrate-like compounds. The main difference between the two is the condensed hydrocarbon-like region (HW-E > PW-DOM) and unsaturated hydrocarbon (PW-DOM > HW-E) regions. It is clear that while the absolute numbers of condensed hydrocarbons are greater in the HW-E sample, the VK shows that PW-DOM has a greater variety of compounds in this region. LDI and NaOH-E samples are clearly different in composition from the other sample types. Considering NaOH-E samples, fewer compounds (3291 CHO compounds) were found in all apart from the lipid-like region of the VK. The LDI samples show a much larger number of condensed hydrocarbon-like as well as lipid-like compounds compared to other samples. The PCA score plot (Figure 4.9b) confirms that NaOH-E samples are characterised by the lipid-like compounds. Compounds with high O/C are considered to be the most labile compounds in a given sample, so it is not surprising that PW-DOM and HW-E samples, both of which are expected to contain the labile component of organic matter, have a higher proportion of these compounds than the NaOH-E extract, which is expected to contain the more recalcitrant fraction. The differences between the samples agree largely with the NMR data, but their relative compositions do not, particularly the large number of aromatic molecules. This difference is due to ionisation efficiency and MW cut off. Carbohydrates, which dominate the NMR/IR spectra do not ionise well, while at the same time many of the small resolved compounds in

the NMR spectra are below the MW cut-off typically used to acquire such data. Therefore, it is crucial that both methods are utilised in order to avoid inaccurate interpretations of MS data. Examining the similarity between the methods using UpSet analysis (Figure 4.9e) indicates that there are 1360 CHO molecular formulae shared by LDI, NaOH-E, HW-E and PW-DOM sample sets, which mainly lie in the lipid-like and lignin-like regions (Figure 4.10).

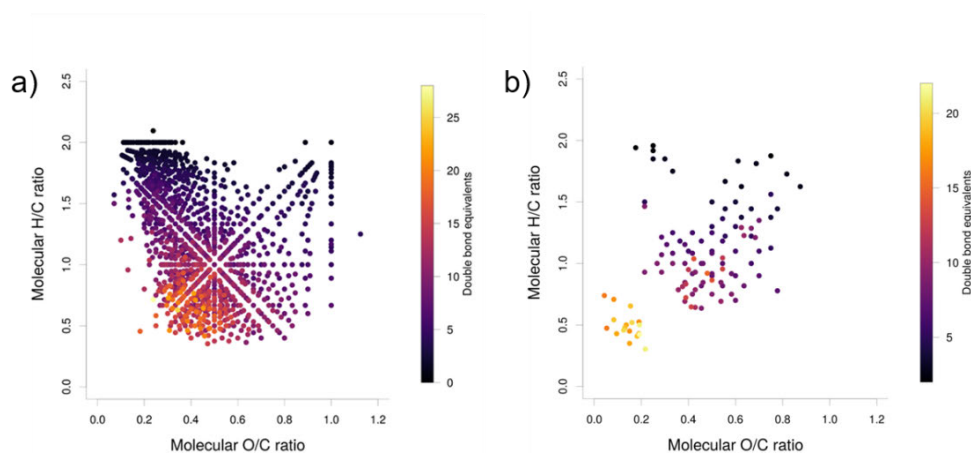


Figure 4.10 Van Krevelen diagram coloured by DBE of the a) 1360 CHO and b) 114 CHON molecular formulae shared between LDI, PW- DOM, HW-E and NaOH-E MS data.

Upon examination of the UpSet plot which includes the nitrogen-containing compounds (Appendix A, Figure A2), there are just 114 additional shared formulae (1474 compared to 1360), but the number of formulae that are unique to NaOH-E, HW-E, PW-DOM and LDI increases by 121, 460, 833 and 1260, respectively. In NaOH-E, HW-E, LDI there are almost half the unique CHON compounds compared to CHO, but for PW-DOM there are more unique CHON compounds. According to the VK showing only the unique formulae (Appendix A, Figure A3, A4, A5 and A6), these CHO and CHON compounds mainly

fall in similar regions. The comparison between sample types here demonstrates that while there may be some common compounds being ionized there are differences between sample types, particularly those obtained using LDI. Thus, it is clear all four sample types need to be included in the examination of the molecular changes across the whole core.

4.5.2 FT-ICR MS analysis of the molecular changes with peat depth

The numbers of monoisotopic compounds assigned in each sample with depth are given in Appendix A, Table A4, A5, A6 and A7. For whole peat (LDI), PW-DOM, HW-E and NaOH-E, the average assignment with depth was 69, 64, 64 and 62%, respectively. UpSet analysis shows that 1253, 1441, 1193, 723 compounds are found at all depths in DOM, HW-E, LDI, NaOH-E samples, respectively (Appendix A, Figure A7, A8, A9, A10). This equates to around 40% of the formulae assigned and indicates that in general, there is a change in molecular composition with depth.

To examine the changes in molecular composition with depth compound class bar charts were produced, showing a variety of changes in different regions (Figure 4.11).

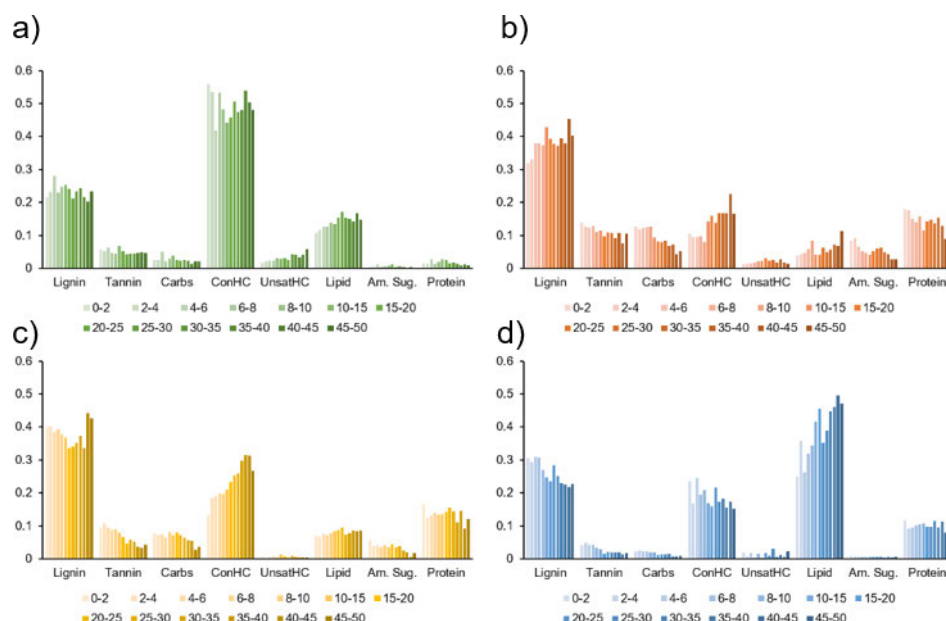


Figure 4.11 Compound class bar charts created using the number of formulae assigned to a particular compound class in each FT-ICR MS spectrum as a proportion of the total number of formulae assigned in that spectrum. a) LDI, b) PW-DOM, c) HW-E, d) NaOH-E.

This study represents the first, to our knowledge, to perform FT-ICR MS on NaOH-E extracted, HW-E peat as well as the first to demonstrate LDI on whole peat samples, hence the only direct comparisons that can be made are with PW-DOM, of which there are a handful of studies. From the bar charts, it is clear that the ionisable compounds in PW-DOM are dominated by lignin-like compounds > condensed hydrocarbon-like > protein-like compounds. Examining each compound class with depth, there is no apparent linear trend for any of the compound classes. Comparing top with bottom, there are greater numbers of lignin-like, condensed-HC-like and lipid-like compounds in the bottom layers, while tannin-like, carbohydrate-like, amino sugar-like and protein-like compounds dominate the upper layers. For both carbohydrate-like and tannin-like,

there is an apparent step-change occurring after 8-10 cm. Overall, regardless of some anomalies present for the top and bottom samples, the trends seen in all those compound class bar charts are: lignin like, tannin-like, carbohydrate-like, amino sugar-like, and protein-like compounds are generally decreasing with depth, while the condensed HC-like, unsaturated HC-like and lipids-like compounds are generally increasing with depth. This could be explained by a general decrease of the plant-derived material and increase in more recalcitrant molecules with depth as a product of abiotic/biotic degradation. However, there are two exceptions to these trends: lignin-like compounds in the PW-DOM samples increase with depth, which may be peatland specific and/or reflect preservation of vascular plant-and Sphagnum moss derived polyphenols, then condensed HC-like compounds showed a decreasing trend with depth in the NaOH-E samples. Peat DOM has been previously characterised as having molecular formula confined between O/C 0.2 to 1.0 and H/C 0.5 to 1.6 or 1.9, (44) however the VKs (Figure 4.12) show the molecular formulae stretch beyond these limits.

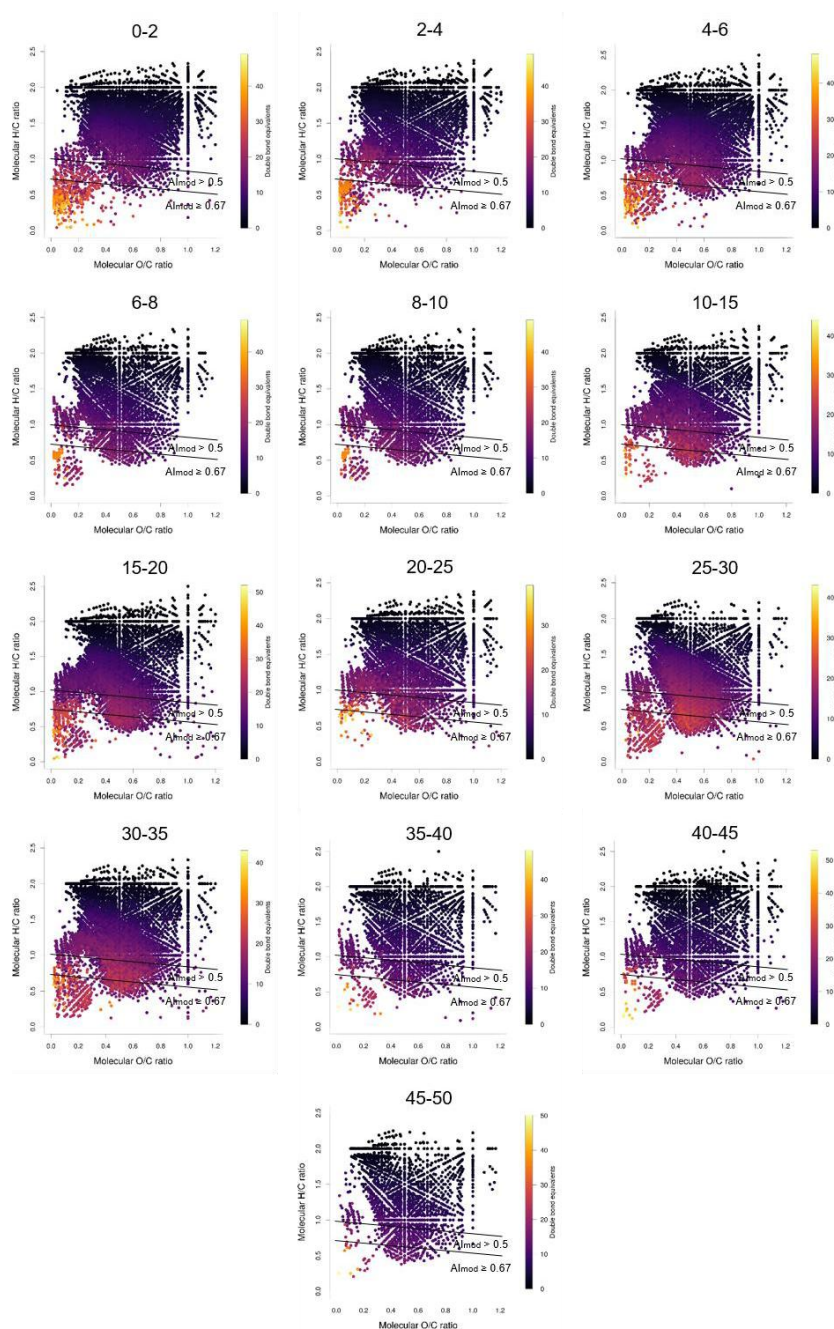


Figure 4.12 van Krevelen (VK) diagrams created from the assigned ESI FT-ICR MS molecular formulae from DOM samples from each depth. Each VK is colour coded by DBE. The solid black lines divide the area of the aromaticity index modified (Al_{mod}) between $Al_{mod} \geq 0.67$ and $Al_{mod} > 0.5$.

In particular, these show that peat DOM samples contain large numbers of condensed HC and aliphatic compounds absent in previous studies. However, the overall appearance of the VKs are similar but fill more of the H/C vs O/C space compared to those in the literature. (45) (49) The lower spread of compounds in previous studies could be due to the number of compounds assigned, different experiment conditions, different peat types or due to the removal of part of the sample by SPE. SPE, using PPL columns that are selective for organic acids/alcohols, has been used in the majority of previous studies to desalt the samples. However in the case of peat DOM, the salt content is negligible and therefore this step is unnecessary. In addition, it is important to reiterate that the use of SPE will naturally inflict some bias in the data. In particular, SPE cartridges are poor at retaining carbohydrates, (46) and considering that terrigenous-sourced DOM contains a large proportion of carbohydrates, the use of SPE on peat DOM should be avoided.

4.5.3 Statistical analysis of PW-DOM with depth

Considering the composition of PW-DOM with depth, UpSet analysis shows that 77% of the CHO assigned molecules (Figure 4.13) were found to be shared at least once in all depths. A similar conservation of compounds was observed for pore waters from the raised bog areas of Red Lake Peatland, Minnesota from two depths, 17 and 250 cm, (47) or 30 and 260 cm, (42) and at 4 depths (10, 100, 200 and 290 cm.). (41) This conservation of compounds with depth was believed to indicate either that the majority of compounds are resistant to microbial degradation, or that DOM is composed mainly of the products of surface decomposition and subsequent transportation by advection downwards below the water table. (41) (42)

PW-DOM

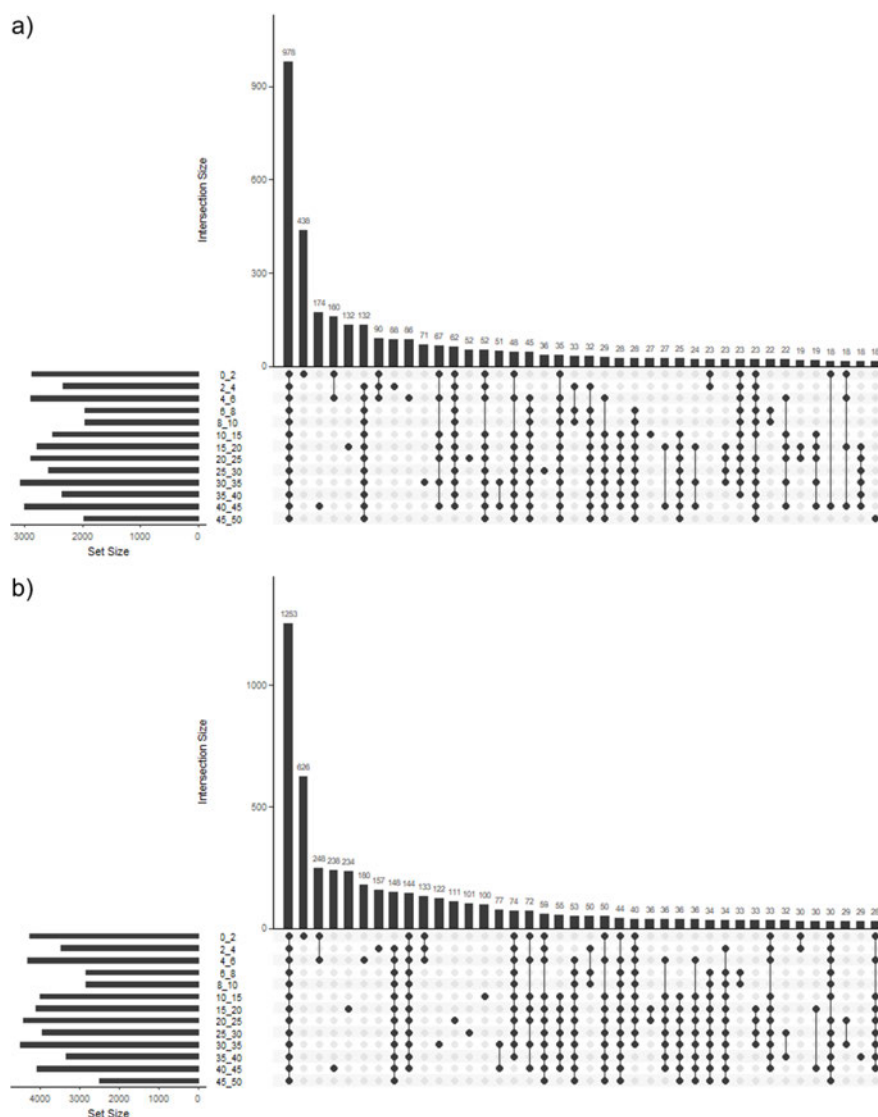


Figure 4.13 Upset plots of PW-DOM a) CHO molecular formulae and b) CHO + CHON molecular formulae.

However, in this study only 40% of the assigned formulae were shared across all depths (specifically, 978 CHO and 275 CHON compounds), which shows that while the vast majority are found in one depth or another, simple advection from the surface cannot account for the molecular variation with depth. The apparent disparity in results

demonstrates the issue with comparing samples from only a few depths. Peatlands have largely been split into two or three vertical zones by water table dynamics, but lateral pipes or hot spots of microbial activity can occur anywhere within the profile, (48) and thus as with peat dating, molecular analysis should be performed at more than a handful of depths. Considering the numbers of unique compounds to each depth (Figure 4.14), it is clear the top layer (0-2 cm) has the largest proportion of unique compounds (18% of the assigned CHO compounds, with the next highest unique set accounting for only 3%). This finding is not unique to DOM, with HWE (11%) and NaOH (13%) samples following the trend.

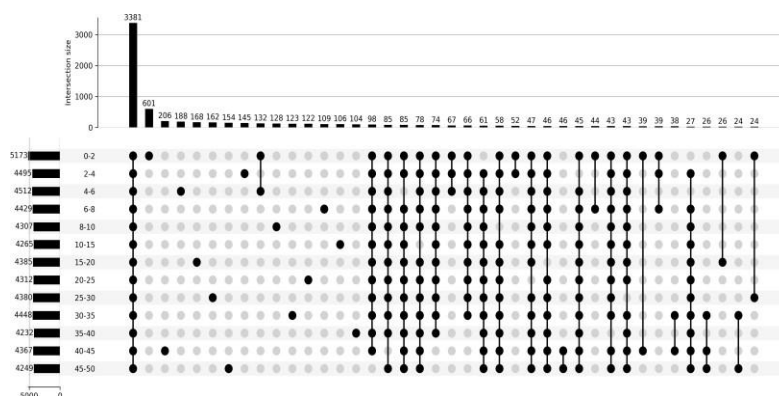


Figure 4.14 Upset plot of the overall unique compounds to each depth.

As shown in Appendix A, Figure A11, these compounds are mostly lignin-like, protein-like and condensed-HC-like (note that this region includes molecules such as flavonoids) and thus indicative of higher biological activity and vegetation in the top. The same increase in unique compounds was not found in the layer 2-4 cm, demonstrating that hot spots in activity can be confined to narrow layers within the peat core. Furthermore, since protein-like compounds would share the

same regions in NMR spectra as O-alkyl and alkyl compounds, this observation was not clear in the NMR spectra of DOM. While this analysis does not take into account quantity of compounds behind these molecular formulae, it suggests that the fluctuating water table leads to variations in the PW-DOM composition with depth. MS analysis of four sample types from 13 core depths using several complementary approaches found evidence for gradual changes in the distribution of small molecules across the peat core. The identified molecular formulae provide a molecular signature, which when rationalized, could allow causative relationships to be drawn with the biochemical processes taking place along the peat core.

4.5.4 Summary of the molecular examination of a singular peat core

This is the first study to present a detailed molecular-level profile of the liquid and solid phases of 13 layers of a 50 cm peat core. The methodology and findings presented here are intended to guide future examinations of the molecular composition of peatlands under different conditions. Damaged peatlands need to be restored to reinstate their ability to provide vital ecosystem services. However, our understanding of the outcomes of the ongoing intervention needs to improve. A recent modeling study demonstrated that prompt rewetting of drained peatlands will stop global peatland CO₂ emissions. However, it is impossible to ascertain whether our efforts will be truly sustainable against future perturbations, especially increasing global temperatures. We conjecture that to gain such knowledge requires understanding of peatlands on a deeper level. Phenolics and/or carbohydrates, the source of which may be plant specific have been reported to inhibit microbial decay, and control carbon storage in peatlands. However, questions remain whether specific molecules are

important, what synergies exist between molecules and microbes and what impact damaging actions and restoration activities have on both. To answer these questions, examination of the molecular composition of peat, for which we and others are now laying down foundations, is required. Among the spectroscopic/spectrometric techniques used in this study, FT-IR only showed functional group changes with depth irrespective of the position of the water table, and may not be that useful for molecular level characterization of peat health. Solid state NMR, however, did detect a correlation between compound classes and water table, in agreement with bulk density. While more expensive than FT-IR, it can contribute to our quest for molecular level characterization of peat and importantly, like FT-IR, samples do not require chemical pretreatment. High-resolution NMR and MS are the best tools we currently have to characterize the liquid phases of peat. On a molecular level, FT-ICR MS can determine formulas of small to medium size molecules present in pg to ng quantities. The sensitivity of NMR is much more difficult to evaluate. Depending on the equipment, the limits of detection, and more importantly the ability to solve molecular structures of even purified molecules, vary considerably. We estimate that if using 600–800 MHz 5 mm cryoprobe instruments, the structures of molecules contained in complex mixtures can typically be solved when they are present in quantities around multiple layers need to be examined, as hot spots of microbial activity can occur anywhere within the depth profile. ESI FT-ICR MS of PW-DOM showed greater variation along the core than previously reported, demonstrating again the need to examine multiple layers. Of all the fractions studied here, HW-E and NaOH-E samples are least likely to provide information not captured by PW-DOM or the solid phase; this is important, as reducing the number of measurements and simplifying sample preparation (squeezing peat and ball milling dry peat) are highly desirable. The

less accessible and more costly techniques used in this study are however essential for advancing our knowledge of how peatlands function. Future work aims to examine multiple cores from sites under different land-uses and in different conditions to search for molecular fingerprints or markers of peat health. It is reassuring that, as observed here, solid-state NMR and liquid state NMR can detect differences in peat composition that reflect bulk measurements, such as bulk density and water table. These will continue to be important as for example bulk density is required to determine if the peat column has been compacted, cut, or even flipped. Variations in the PW-DOM composition along the core profile observed here indicate that multiple layers need to be examined, as hot spots of microbial activity can occur anywhere within the depth profile.

5. Results and Discussion: Physicochemical and molecular examination of Drained and rewetted raised bog sites at Red Moss of Balerno

This chapter will examine the impact of drainage and rewetting on the physicochemical and molecular properties of peat cores extracted from a raised bog located at the Red Moss of Balerno. Details regarding the site can be found in chapter 3. Eleven cores were extracted from a drained RM1 site and a rewetted RM2 site as outlined in Chapter 3. The photographs of each core can be found in the Appendix B, Figure B1 and B2.

5.1 Core observations

Cores were extracted from points of different microtopography (between hummock and hollow or in some cases what could be described as a flat area near a hollow). The dominant surface vegetation ranges from lichen, green/red varieties of *Sphagnum* moss and cotton and deer grass. Examination of each 50 cm core visually indicated variations in their profile. Some showing light and dark bands representing highly decomposed peat or partially (not fully) decomposed vegetation, while other cores showed very similar levels of decomposition down the full profile.

5.2 Solid phase physicochemical and molecular examination of drained and rewetted peat samples

This section will discuss the results obtained from air-dried ball-milled peat samples. This will include physiochemical properties, ATR-FTIR, solid state ^{13}C NMR and FT-ICR MS results.

5.2.1 Physicochemical properties

Physicochemical properties were measured, namely ash content, bulk density, water content and elemental content as outlined in chapter 3.

To explore the site variation the average bulk density for each core is plotted in two ways; average value for each depth for each core in Figure 5.1 and the average value for all cores with the standard deviation. Note that the bulk density could only be measured for cores extracted from RM

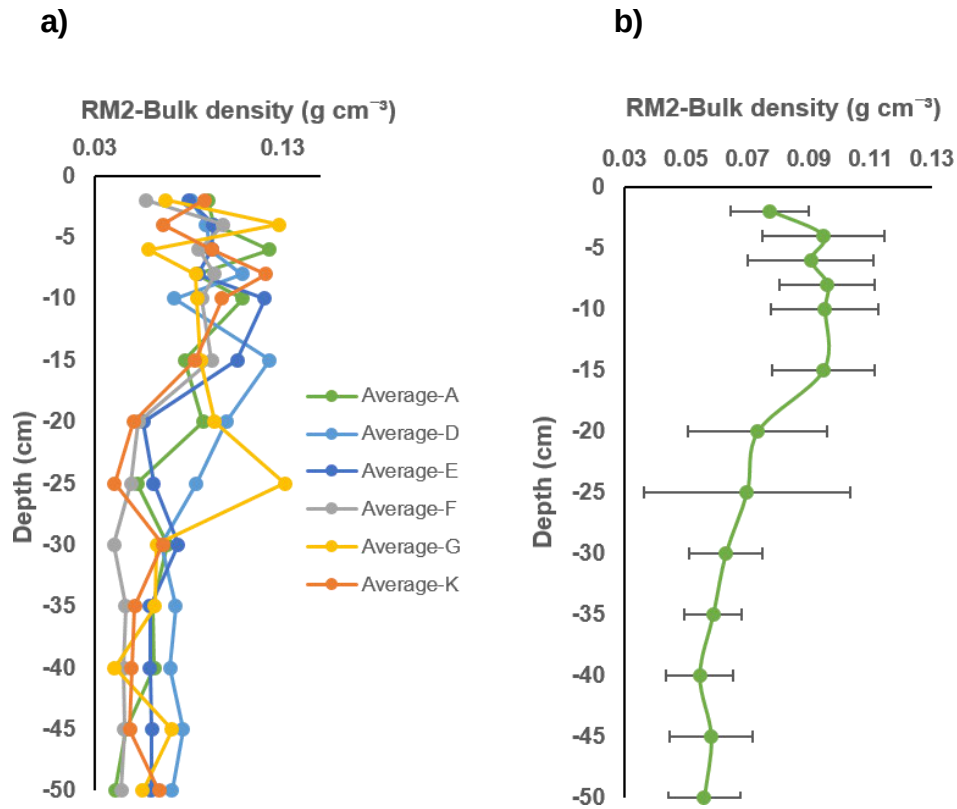


Figure 5.1 a) Average bulk density per depth for each RM2 core, b) Averaged bulk density of RM2 cores with standard deviations plotted against depth.

Examination of Figure 5.1a, it is evident that there is more variation in bulk density in the top 15 cm than at lower depths. Core RM2G stands out with having the highest peak in bulk density at 25 cm of 0.13 g cm^{-3} . Taking all the cores together in Figure 5.1b the average bulk density increases from the surface to 0.11 g cm^{-3} at 8 cm of depth, drops below 0.1 g cm^{-3} at 10 cm depth then rises again to 0.10 g cm^{-3} at 15 cm depth. From 15-50 cm the bulk density then falls rapidly to a more consistent value around $0.06\text{-}0.07 \text{ g cm}^{-3}$ to lower values until 50 cm of depth. The high standard deviation around 15-30 cm is caused by the different trend at those depths between cores A, D, G and E, F and K, indicating already that there is variation with depth between cores from the same site. From the one core examined in Chapter 4, a step change in bulk density was observed and as used to indicate the position of the *catotelm*. As seen in Figure 5.1a there are step changes in bulk density however the exact depth to which they occur differs across the cores in RM2 as summarised in Table 5.1.

Table 5.1 Position of the catotelm determined by bulk density for each RM2 core

Core	Position of Catotelm/ cm
A	20-25
D	15-30
E	15-20
F	15-20
G	25-30
K	15-20

The values demonstrates well the water table variations see across the site. For example, the dipwell closest to the RM2G gives the lowest

water table as 29.9 cm (SWT water table data 2008-2020, unpublished).

The only study to date that includes bulk density measurements from cores from Red Moss of Balerno was conducted by J. Cloy. (53) The two cores from this study were also taken from the highest point of the RM2 site. Comparing profiles, the bulk density differs slightly from those in this study. Cloy reports a higher maximum value of 0.19 g cm^{-3} at around 20 cm after which the bulk density decreases below 0.1 g cm^{-3} and remains steady to the bottom. A surface minimum of 0.014 g cm^{-3} was also reported, which is much lower than the values in this study. Each of the cores in the Cloy *et al* study were taken from hummocks of *Pleurozium schreberi* which differs from the sampling sites in this study, thus this may explain the disparity in the highest and lowest values. However the position of the step change in bulk density and hence position of the *catotelm* (21-23 cm) does largely agree with this study for certain cores.

The general trend in bulk density with depth, matches well with dry bulk density of raised bogs from Scottish soil database (191), where the results of bulk density for layers between 0-30 cm were reported to be higher values (0.136 g cm^{-3} on average) compared to layers below (0.114 g cm^{-3} on average). This trend is not only seen for Scottish raised bogs but Irish as well. For example, a number of cores from Irish Knockroe raised bog report bulk densities between $0.05\text{-}0.6 \text{ g cm}^{-3}$ in layers between 0-15 cm and $0.03\text{-}0.1 \text{ g cm}^{-3}$ in layers below. (171) While the bulk density is not known for cores from RMI, it would be expected from other studies that drainage would cause an increase in bulk density at the surface with values rising well above 0.2 g cm^{-3} . (192) It is clearly not the case for the cores in this study from the restored site, showing the restored site has what would be expected bulk densities for a healthy raised bog.

A comparison between the average water content with depth for RM1 and RM2 and the variation in the water content measurements for the same cores examined above are shown in Figure 5.2.

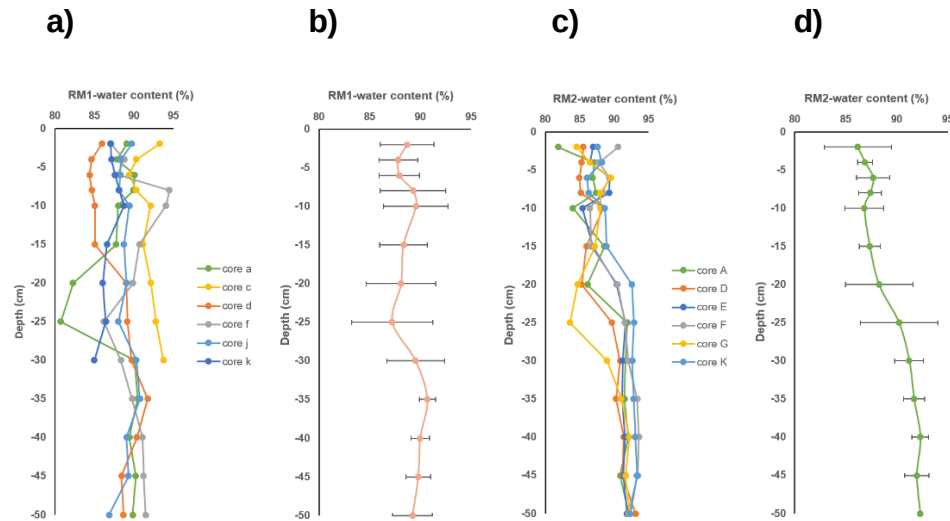


Figure 5.2 Average water content a) per core and b) across all cores with depth for RM1. Average water content c) per core and d) averaged across all cores with depth for RM2.

Considering each site separately, it is clear water content varies between cores from the same sites. RM1 cores differ in absolute values in the top 30 cm with core RM1a showing the lowest values between 20-30 cm and RM1c showing a higher water content with depth than the other cores. RM2 cores vary less in value compared to RM1 cores. The trend with depth varies with core in the top 15 cm but seems to converge below 35 cm. However in the middle depths, a step change in water content and depth at which that step change takes place varies, with RM2G having the lowest step change followed by RM2A/D and then RM2E/F/K. The high percentage of water content at lower depths typically occurs in a waterlogged catotelm, while the

lower values are a general sign of drier acrotelm. According to these results, these differences again reflect the water tables and catotelm boundary and agree with the bulk density data. Comparing the cores with those examined by Cloy (52), the water content at the surface was much lower (60% and 0% for the cores studied) but the trend with depth closely matches with RM2A. Again the disparity could be due to the different vegetation and microtopographies sampled between studies. Examining all the cores together, the average plot for RM1 and RM2, Figure 5.2b and d, shows the water content varies most in the top 30 cm, however, the trend differs. While RMI decreases after 10 cm and then increases after 25 cm, RM2 shows continuous increase after 10 cm with a further step change around 20 cm. Generally, the total porosity of peat decreases with increased decomposition. (50) Therefore, the higher water content in RMII deeper horizons could be related to the presence of a more undecomposed peat, while the lower water content in RMI can be explained by the degradation process which led to a decreased total porosity and thus to a lower water permeability. Further evidence of this is from bulk density which increases with depth for RM2, while the average water content of RM2 decreases. Generally, decomposed peat in raised peat bogs occurring in temperate climates have been shown to exhibit water content values up to 80%, while undecomposed peat can reach values to nearly 100%. (172) It is therefore expected that the water content in healthy peat would be closer to the surface acrotelm (172). Both sites have higher water content than 80% as expected from enhanced decomposition, however cores from RM2 do have lower water content in the top 25 cm than RM1 potentially indicating lower decomposition and hence better health due to restoration. The average ash content from both sites and the variation

in the ash content measurements for each peat core from each site are shown in Figure 5.3.

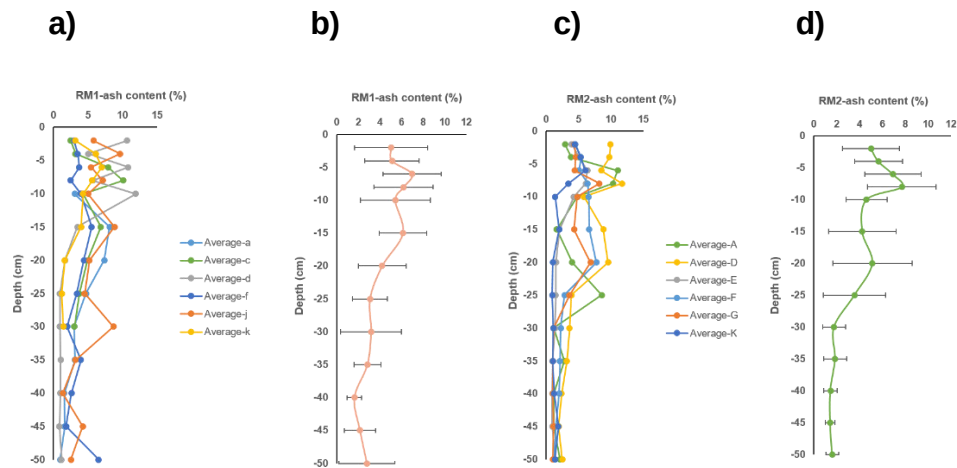


Figure 5.3 Average ash content a) per core and b) averaged across all cores with depth for RM1. Average ash content c) per core and d) averaged across all cores with depth for RM2.

As was observed for both bulk density and water content, the depth profile for ash content varies between cores from the same site. The ash content for the RM1 cores vary in the top 10 cm and slightly between 40 and 50 cm. Apart from RM1j, the ash content for RM1 cores largely decrease after 15 cm, with RM1k and RM1d showing the lowest ash content at a higher depth compared to the others. The ash content for the RM2 cores is more similar in trend with depth. Core RM2A stands out as having a peak in ash content around 20 cm (10%), while the other cores are decrease towards a minimum. Cores RM2K and RM2E drop to a minimum at around 15 cm, while the others has almost identical trend below 15 cm with a step change to lower ash values around 20 cm. The averaged ash content from these sites (Figures 5.3 b and d) show a similar trend until 8 cm in depth, up to 8% of ash content. However, at this point the ash content in RM2 starts

to decrease sharply from 20 cm to the end of the core, reaching values lower than 2%. Differently, the ash content in RM1 never drops below 4%. It is clear that all cores between 0-25 cm are responsible for the large standard deviations. For example core RM1j leads to the higher standard deviation at 30 cm. The ash content values below 25 cm for all cores of RM2 show a very similar trend, resulting in lower standard deviation than RM1. For ombrotrophic peat bogs, ash content can increase either as a result of atmospheric deposition but also due to increasing peat decomposition and mineralisation processes. The higher ash content in surface of both RM1 and RM2 is expected for ombrotrophic peat bogs fed by rain or soil dust influx. (51) The RM2 ash content profile shows the expected decreases of ash content in the lower levels consistent with previous measurements of ash content from Red Moss of Balerno and low values (< 2%) are expected for *Sphagnum* bogs. (52) The higher ash content in RM1 represents an interesting result that could be due to many factors such as hydraulic conductivity occurring in this site which may lead to an increase in ash transportation (51), but further investigations are necessary. As an increase in ash content could also represent a loss in organic matter this could indicate that more OM is being lost with depth in RM1 as a result of drainage. The C/N ratio were determined from the C and N content obtained for each air dried peat sample from each depth. The average C/N ratio with depth for cores from RM1 and RM2 are shown in Figure 5.4.

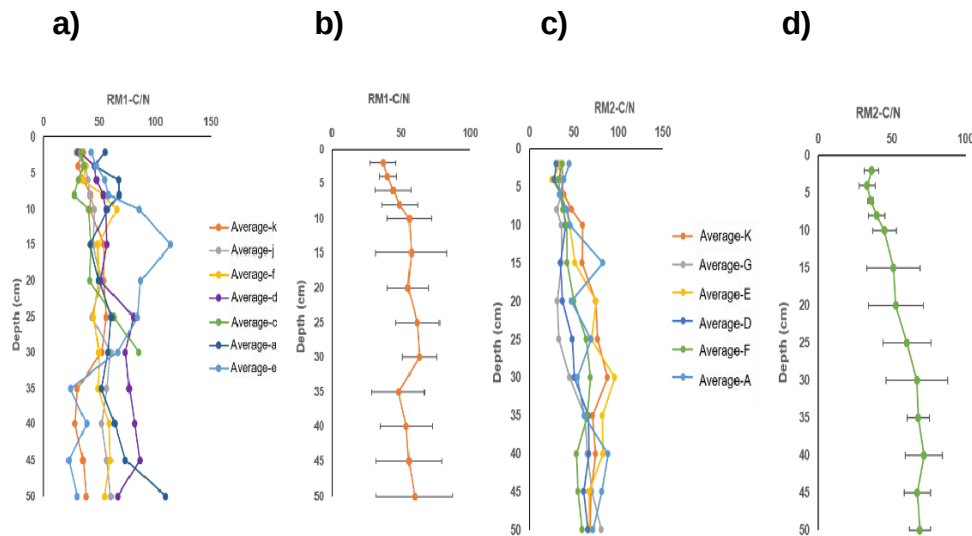


Figure 5.4 Average C/N content a) per core and b) across all cores with depth for RM1. Average C/N content c) per core and d) across cores with depth for RM2.

The average C/N ratio of cores from both sites have similar values in the surface layers, with an increasing trends from 0 to 30 cm. At the bottom layers of RM2 an average C/N ratio of 70 is registered, while for RM1 the value is just over 60. The standard deviation is highest at 15-20 and 35-50 cm depths in RM1, while variations occur between 15 and 30 cm depths in RM2. In detail, the C/N ratio for the RM1 cores is variable from 5 cm onwards with the majority having similar values only between 10-15 cm. From 15 cm onwards cores RM1f and RM1j follow similar trends with depth to 50 cm. Core RM1e and RM1k have similar trends after 35 cm however with much lower C/N values compared to other cores. Core RM1e stands out in the upper layers with the highest values of C/N, close to 100%, which seems an outlier however similar values are observed for the 45-50 cm layer of RM1a, which explains the larger standard deviation observed on Figure 5.3b. Core RM1c also has a different trend to other cores, increasing from below 30 at 6-8 cm to over 80 at 25-30 cm. The RM2 cores are much

less variable. The C/N ratio is similar for all cores below 10 cm after which they deviate into 3 groups (RM2G+D, E+K, A+F) before coming together after 35 cm. The main difference of C/N ratio values between the two groups appears between 15-30 cm, which agrees with the N content and explains the larger standard deviation. The main sources of N in raised bogs comes from deposition from the atmosphere, as well as mineralisation and fixation. (173) According to a study conducted by Damman, during decomposition N content is expected to increase (174) as a result of microbial decay with a consequent storage of N in the microbial biomass. Then, the anaerobic decay in the catotelm occurs where C is preferentially lost. However, an opposite theory found in literature states that hydrological changes may affect the N concentration in peat cores before incorporation within the catotelm. (175) Both N content and C/N ratio for cores in RM1 and RM2 show similar variations with depth, precisely a decreasing N content and moreover an increasing C/N ratio down the RM2 cores, in agreement with the trends reported by Anderson *et al.* (129) Since drainage has occurred on the Red Moss of Balerno, these trends could be explained by substantial hydrological changes occurred on this site. It is interesting to note that the step change of N content and C/N ratio occurs on average around 20-25 cm, which agrees with the acrotelm-catotelm boundaries identified from bulk density and water content.

In summary, the physicochemical properties do indicate differences between cores from the same site. This indicates the necessity to take multiple cores per site. The bulk density, water content and to a lesser extent C/N ratio do indicate differences that reflect the position of the water table. In addition cores from RM2 do show that the average bulk density decrease from 0.13 to 0.06 gcm⁻³ until the bottom layer, with the catotelm defined around 20 cm depth, then the ash content decreases 4% after 30 cm depth. Also, the increase of water content (over 90%) and C/N content (over 70%) indicate that the rewetted site is in better health. This is a promising result and it is now a question of whether these differences are reflected in the molecular data. As many of these properties are determined from air dried samples the results from the solid state molecular techniques were examined first.

5.2.2 Solid state molecular level examination of drained and rewetted site cores from Red Moss

In order to obtain and investigate the molecular changes inflicted due to rewetting of the RM2 site Red Moss molecular level characterization using ATR-FTIR, LDI FT-ICR MS and solid state CP-MAS NMR.

5.2.2.1 ATR-FTIR spectral observations

ATR-FTIR analysis has been conducted on samples from all 13 layers of 6 cores from both RM1 (a, c, d, e, f, k) and RM2 (A, D, E, F, G, K) as outlined in Chapter 3. The spectra obtained are characteristic of peat bogs (18) and appear almost identical in dominant features to the spectra obtained from Chapter 4, with typical stretches for OH (3200-3600 cm⁻¹), CH stretch (2850 cm⁻¹, 2920 cm⁻¹), C=C stretch (1510 cm⁻¹), C=O stretch (1650 cm⁻¹) and C-O stretch (1030 cm⁻¹),

with clear dominance of the last stretch (Appendix B, Figure B3). For all wavenumber regions of interest there are different intensities related to different depth of each core which will be explored by PCA in later sections.

5.2.2.2 ^{13}C Solid state NMR spectral observations

Solid state ^{13}C NMR analysis has been carried out on 13 depths of five cores from RM1 (a, c, d, e, f) and 13 depths of five cores from RM2 (A, D, E, F, G) samples as already outlined in Chapter 3. The spectra obtained are typical of peat samples (25) showing overlapped resonance from 0-200 ppm, dominated by O-alkyl and alkyl resonances. It is clear from the stacked plot (Appendix B, Figure B4) that the spectra are all very similar with a dominance of O-alkyl and C-alkyl resonances. However, differently from the core discussed in Chapter 4, the C-alkyl resonances does not show a clear increasing pattern with depth for all cores. Therefore, further statistical analyses are necessary to better understand the behaviour of the main resonances throughout the cores in those two different sites.

5.2.2.3 (-) LDI MS data observations

LDI MS analysis of RM samples has been carried as outlined in Chapter 3. The analysis was conducted on 6 depths per core on 5 cores from RM1 (a, c, d, e, f) and 6 cores from RM2 (A, D, E, F, G, K). The $79\% \pm 11$ of LDI MS peaks have been successfully assigned with a total molecular formulae assigned of 5541 from RM1 and 5522 from RM2 (80% CHO and 20% CHON in both sites). Due to the high complexity of the spectra, which at first impact look similar to each other, multivariate analysis was performed and Upset plots and compound class bar charts were created in order to analyse the data further.

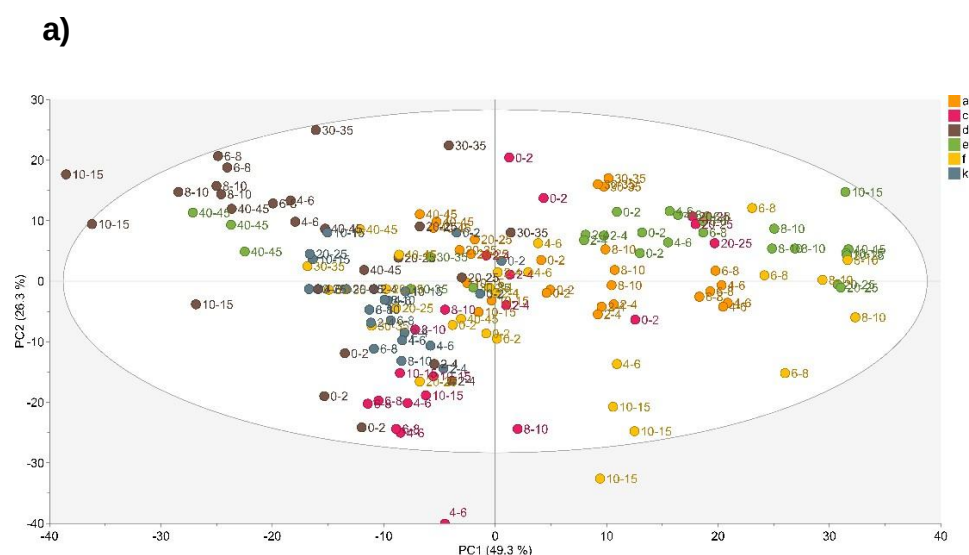
5.2.2.4 Statistical analysis of the solid phase spectroscopic data

In this section, all the statistical analyses that have been carried out on Red Moss data will be discussed.

5.2.2.4.1 PCA analysis of RM ATR-FTIR spectra

The ATR-FTIR spectra showed a characteristic fingerprint of peat samples, but due to the high analytical complexity, the same data has been processed by PCA to explore the differences between the sites, as outlined previously. In order to conduct PCA on the IR data only the regions discussed in Chapter 3 were considered. A total of 13 depths of 6 cores from RM1 (a, c, d, e, f, k) and RM2 (A, D, E, F, G, K) were considered.

PCA was initially calculated using IR spectra from RM1 and RM2 separately to examine the site variation. PCA plots of RM1 cores and RM2 is shown in Figure 5.5 a and b, respectively. It is immediately evident that there is a number of outliers on each plot. The loadings for each plot are shown in Appendix B, Figure B5 and B6.



b)

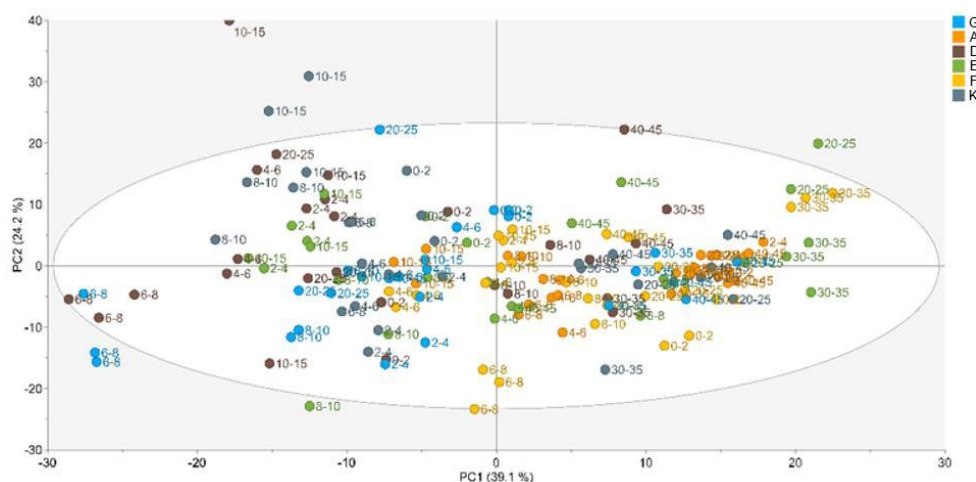
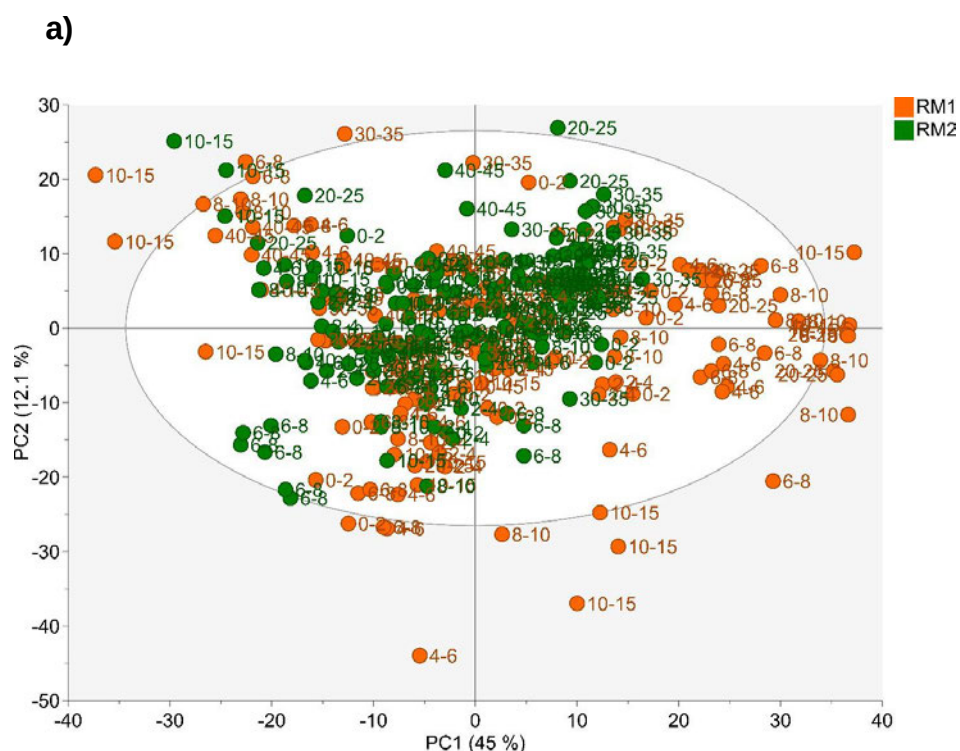


Figure 5.5 PCA score plot of ATR-FTIR cores of RM1 a) and RM2 b) in triplicate for each depth coloured by core. Ellipses shown are Hotelling's T2 (95%).

As was the case for the single core examined in Chapter 4, there is variation in the triplicate spectra which again highlight the disadvantages of using IR. The differences between the triplicate spectra are potentially due to the samples being made up of a mixture of vegetation remains with soil remains despite the ball-milling process or due to slight variations in sample depth. Looking at those two sites separately, the majority of RM1 depths from cores a, e and f show positive correlation with C-O stretch along PC1, while cores c, d and k have an opposite trend with a negative correlation with aliphatic and aromatic stretch loadings. From the same score plot there is no apparent separation due to depth and no cores seem to be overlaying with each other (exact same molecular differences with depth). In addition, neither the outliers, e.g. 10-15 cm RM1d nor the right or left most samples on the score plot agree with any behaviour reported in the physico-chemical properties.

Regarding RM2, most of depths from cores A and F show a positive correlation with C-O stretch loadings along PC1, while D, G and K show negative correlation with aromatic and aliphatic loadings along the same component. Looking at the depths in more detail there the vast majority of samples from 0-15 cm are on the left hand side of the score plot while those below this depth are on the right hand side. Interestingly, this would suggest positive correlation of the bottom samples with C-O stretch, which is opposite to the findings from the one core reported in Chapter 4. To examine the differences between RM1 and RM2, PCA analysis was run using spectra from both sites. The resulting score and loadings plots area shown in Figure 5.6.



b)

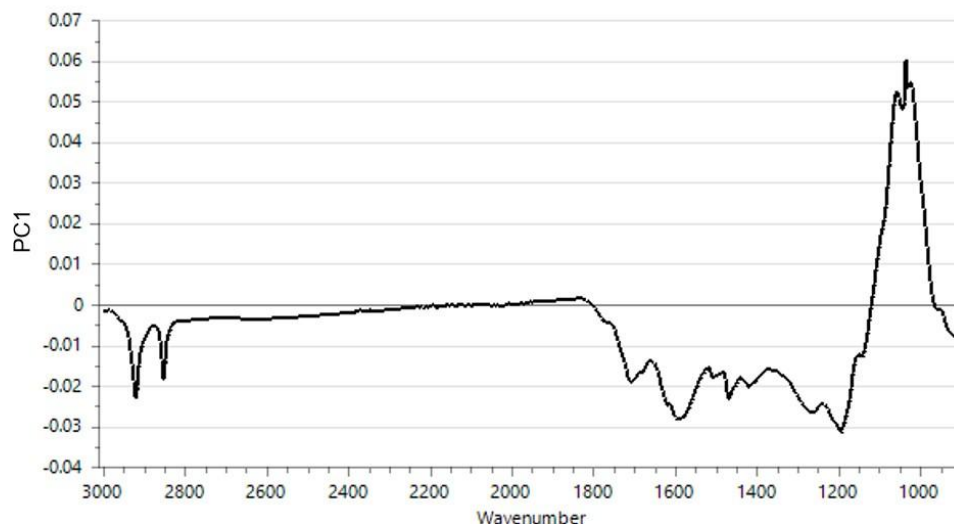


Figure 5.6 a) PCA score plot of ATR-FTIR spectra of RM1 and RM2 core samples in triplicate for each depth, b) loadings plot for PC1. Ellipses shown are Hotelling's T^2 (95%).

Overall, the total variance was 57.1%, where the first component (PC1) described most of the variance (45.00%). As it is shown in Figure 5.6, there is no such a clear separation between samples from RM1 and RM2. However, the major differences observed in the samples come along from 4-15 cm in RM1, at the edges of the score plot, mainly characterized by a positive correlation with C-O stretch band at 1030 cm^{-1} .

5.2.2.4.2 Solid state NMR

In order to investigate the NMR data further PCA has been run using the integral of the signals in the chemical shift range between ppm regions, as previously discussed in Chapter 3, of each solid state ^{13}C NMR spectra from RM1 and RM2. Upon examination of the score plot

obtained by considering depths and cores (Figure 5.7) there was a clear separation of samples from several cores. After close inspection of the data it was found that certain samples have been recorded using a different receiver gain and therefore cannot be included in the PCA.

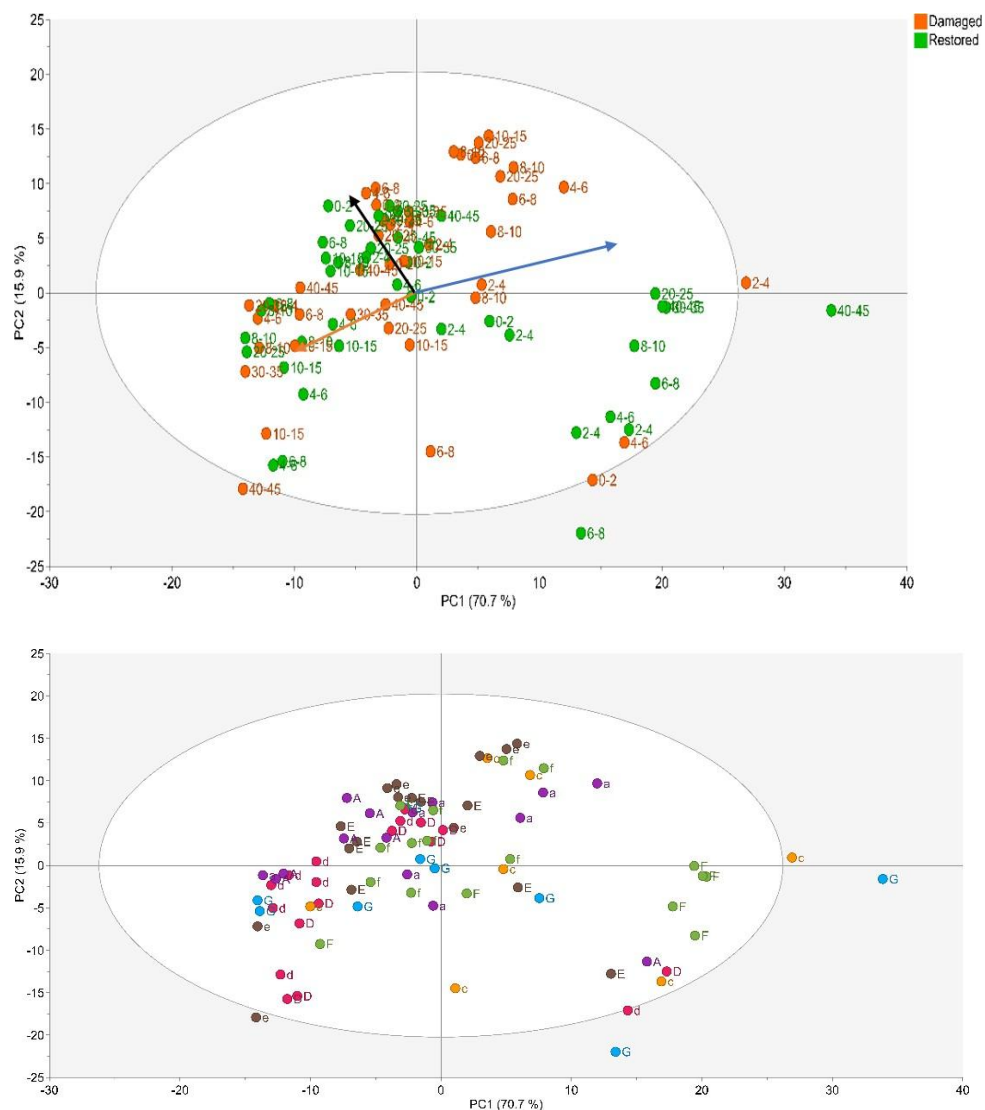
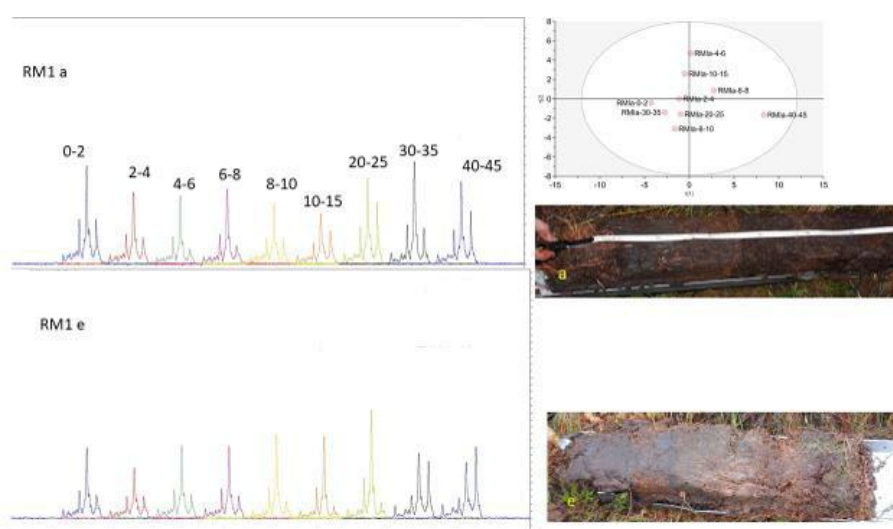


Figure 5.7 PCA score plot of solid state ^{13}C NMR cores of RM1 and RM2 in for each depth (up) with loadings and coloured by core (down). The averaged loadings are relative to: alkyl and functionalised alkyl

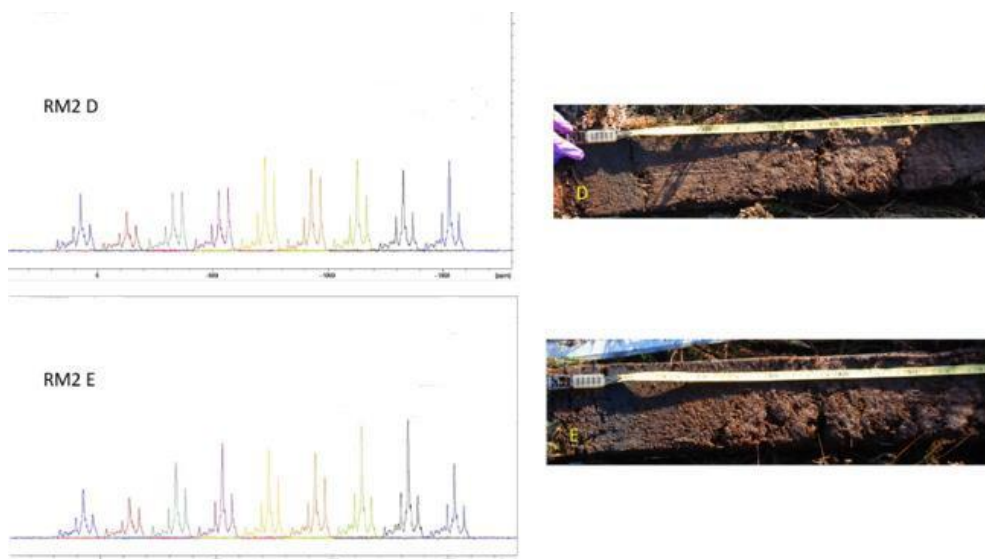
(orange arrow), O-alkyl and carbohydrates (blue arrow), and aromatic (black arrow) with chemical shift regions as defined in previous sections for ^{13}C respectively. Ellipses shown are Hotelling's T2 (95%).

While checking the spectral data visually some differences between RM1 and RM2 with depth, considering specific cores (a, e, D, E) were apparent (Figure 5.8).

a)



b)



c)

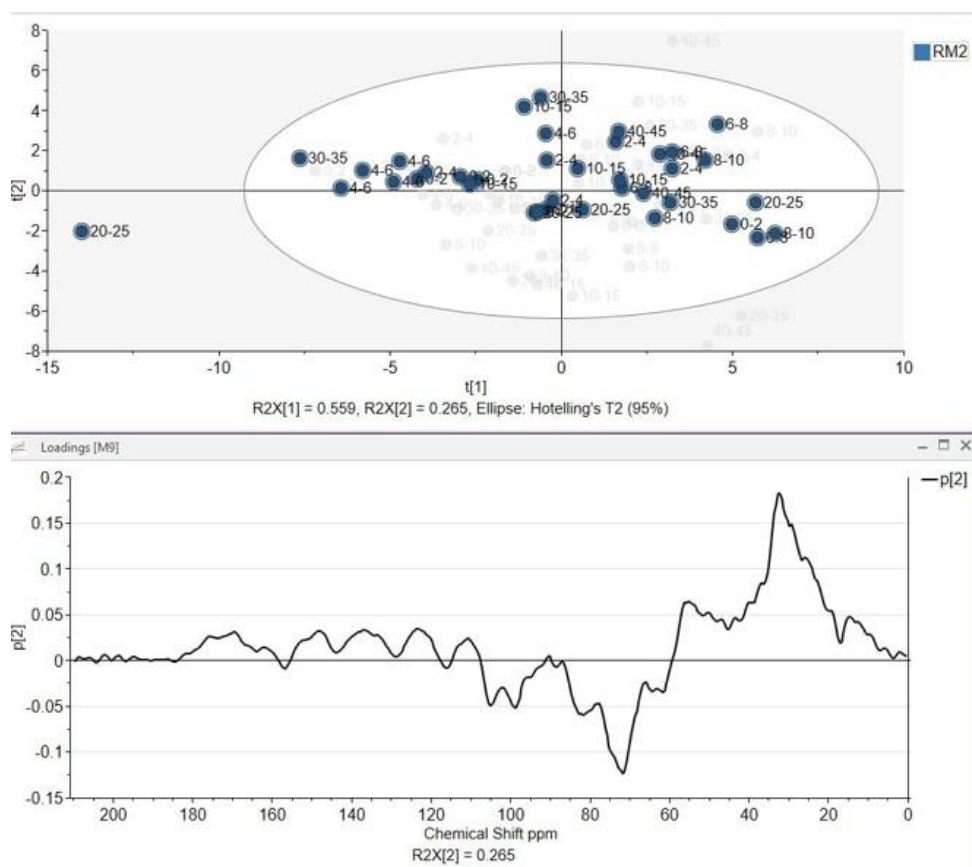


Figure 5.8 Solid state ^{13}C NMR spectra (9 depths) and pictures of cores a) a, e, b), D, E and c) PCA score plot, with RM2 cores underlined, together with the loading plot.

It is clear from Figure 5.8 that there is an increase in alkyl with depth occurs in RM2D and RM2E. Visual variations on the cores can be explained by some dark bands which may be the result of more decomposed peat compared to the lighter bands. The differences with depth for cores from the same site will likely confuse any comparison between RM2 and RM1. This is evident from the updated PCA plot (Figure 5.8c) which only shows a slight difference in alkyl content, especially higher in cores D and E from RM2.

5.2.2.4.3 LDI FT-ICR MS

LDI data have been investigated using PCA in the way described in Chapter 3 Overall, 6 different depths of 5 cores from RM1 (a, c, d, e, f) and 6 cores from RM2 (A, D, E, F, G, K) have been considered. The resulting PCA score plot is shown in Figure 5.9.

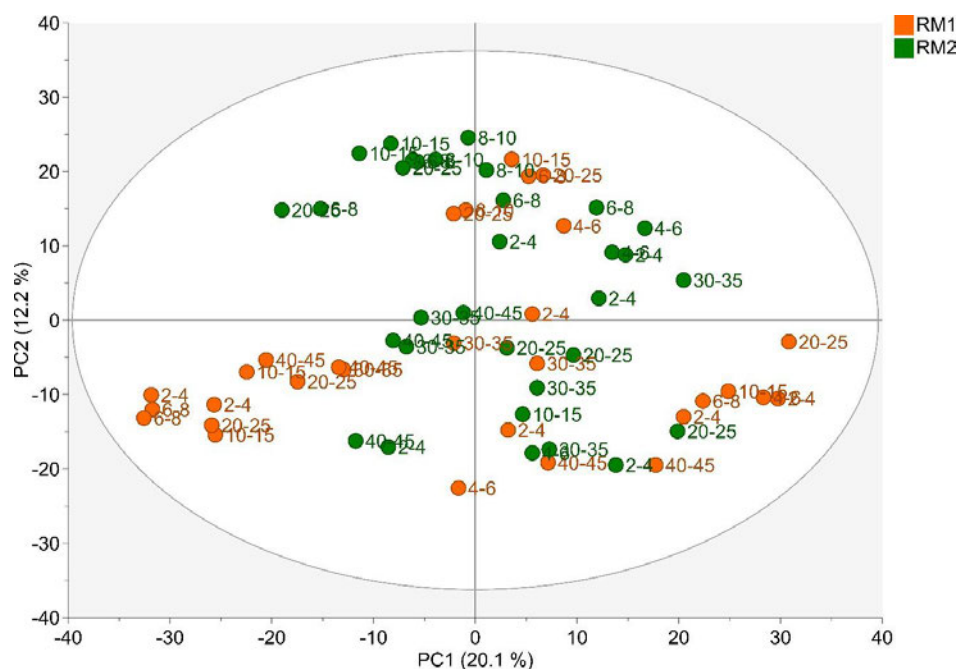


Figure 5.9 PCA score plot of LDI cores of RM1 and RM2 for each depth. Ellipses shown are Hotelling's T^2 (95%).

The total variance of the first components weighs 31.3%, where the first component describes only 20.1% of the initial data variability. The score plot indicates no separation between RM1 and RM2 samples. This may be due to the lack of samples examined or may be due to the nature of LDI data. In order to investigate the data further Upset and bar charts for class compounds and aromaticity index for all molecular formulae assigned in both sites compound were produced (Figure 5.10).

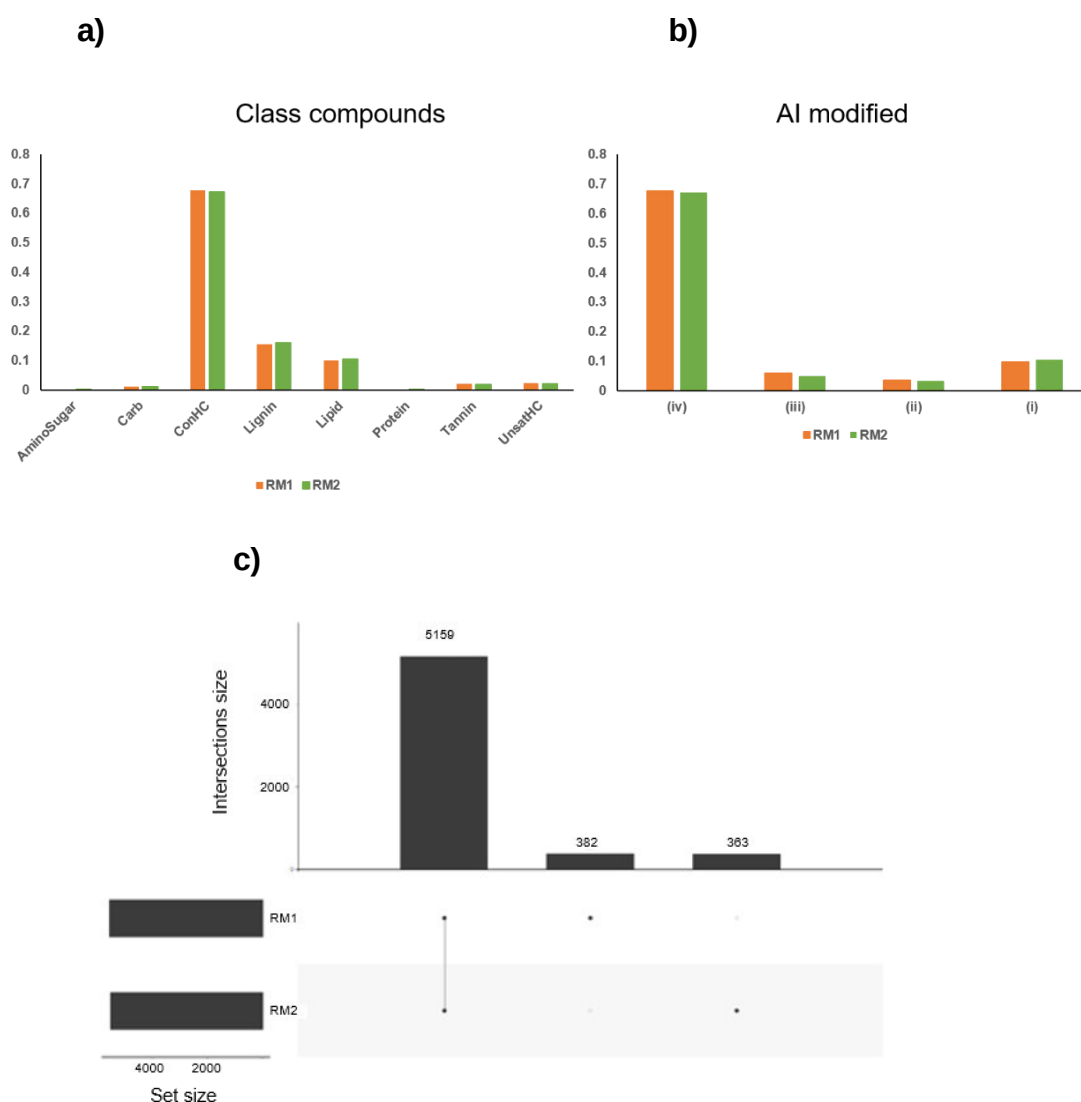


Figure 5.10 a) Class compounds, b) aromaticity index and c) UpSet plots of LDI data for all cores from RM1 and RM2.

As it is shown in Figure 5.10, there is not a substantial difference in terms of class compounds and aromaticity index between the 2 sites, also the UpSet plots underline that on a total of 5904 formulae assigned, 5159 formulae are shared, 382 unique formula from RM1 and 363 from RM2 which are not enough to explain a substantial

difference between those sites. The percentage of formulae assigned was $79\% \pm 11$.

Overall, all the solid phase results suggest that there is not an evident difference between damaged and restored sites in Red Moss, apart from specific cores which showed some deviations in both alkyl and O-alkyl contents. In the next section the results from the liquid state examination of PW-DOM samples from both those sites is examined.

5.3 Liquid state molecular level examination of drained and rewetted site cores from Red Moss (RM)

In this section, the 1D ^1H NMR and ESI FT-ICR MS spectra of PW-DOM samples are discussed, starting with a general inspection of spectra, followed by the analysis of the variance between samples by PCA. For FT-ICR MS data, additional analysis of the assigned molecular formulae is conducted in the form of Upset and compound class plots. HW-E are not included as there were not enough data available while NaOH-E was not conducted on these samples.

5.3.1 Liquid state ^1H NMR spectral observations of RM PW-DOM

As stated in Chapter 3, the top layers from RM were quite dry making it impossible to obtain enough PW-DOM for NMR analysis. DOM was obtained in sufficient quantity from four depths (10-15, 20-25, 30-35, 40-45 cm) from six cores from each site.

Examination of the ^1H spectra (Appendix B, Figure B7) shows that the carbohydrate signals are most intense, while aromatics are weakest in agreement with the spectra obtained from the FC core in Chapter 4. PW-DOM is a very complex mixture resulting in overlapped resonances in the spectra due to the presence of many compounds. Further investigation have indicated that these spectra looked very similar to the ones showed in Chapter 4, with sharp peaks mainly appearing due to presence of glucose for

instance. Overall, the PW-DOM spectra do not show an obvious trend with core depth and all spectra have similar intensity across all the main spectral regions. To further assess the difference across the whole spectra for the PW-DOM samples PCA analysis was performed and described in Chapter 3.

5.3.2 (-) ESI FT-ICR MS of RM PW-DOM observations

Using the optimised method reported in Chapter 3, ESI FT-ICR MS spectra were acquired for PW-DOM samples. In order to examine the quality of the data the peak shape is checked for splitting and a number of peaks should be seen at a given nominal mass, typical of a complex mixture. An overlay MS spectrum obtained from RM2 and SRFA at a nominal mass of 369 Da is shown in Figure 5.11.

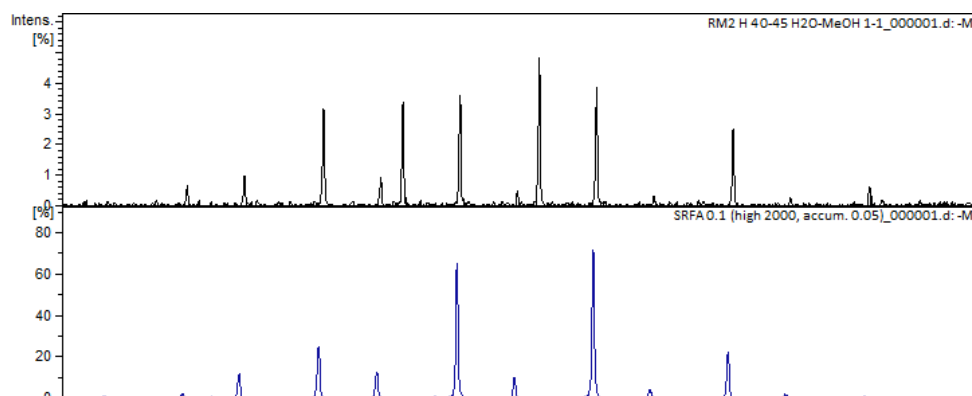


Figure 5.11 PW-DOM sample from RM2 vs SRFA FT-ICR MS spectra.

From the overlay of a section of the MS spectra the method does produce clean spectra with high resolving power and good peak shape. In addition, it is clear that some additional ions are present in RM sample. This is ideal as it means that the formula list created for SRFA can be used to calibrate the formula assignment of peat PW-

DOM. Using DataAnalysis software, a total of 5959 molecular formulae from all samples were assigned ($79.2\% \pm 11$), excluding the large potential contaminant peaks. As multiple MS spectra contain 1000s of molecular formula inspection of diagrams such as Van Krevelen's would be tedious. Therefore, the FT-ICR MS data in later sections was analysed using PCA, Upset and compound class bar charts.

5.3.3 Statistical analysis of ^1H NMR and FT-ICR MS spectra of RM PW-DOM

PCA was conducted on the ^1H NMR and ESI FT-ICR MS spectra as outlined in Chapter 3.

5.3.3.1 PCA of RM1 and RM2 PW-DOM ^1H NMR spectra

The PCA plot produced from the ^1H NMR spectra of PW-DOM from RM1 and RM2 is shown in Figure 5.12.

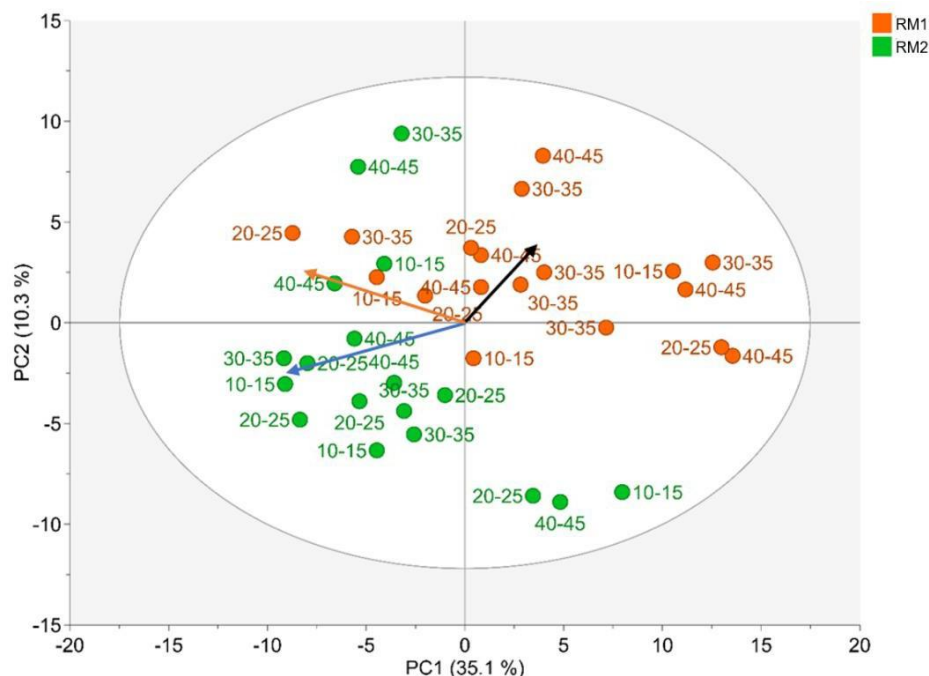


Figure 5.12 PCA score plot of liquid state ^1H NMR of PW-DOM from cores of RM1 and RM2. The averaged loadings are relative to: alkyl

and functionalised alkyl (orange arrow), O-alkyl and carbohydrates (blue arrow), and aromatic (black arrow) with chemical shift regions. Ellipses shown are Hotelling's T² (95%).

As it is shown in Figure 5.12, the total variance of the first components weighs 45.4%, where the first component describe 35.1% of the initial data variability. From the score plot there is a clear separation based on site with most of the samples from RM1 positively correlated to the first component, while the majority of the samples from RM2 have an opposite behaviour. This indicates that there is a clearer difference between the two sites in terms of molecular composition. The average loadings plotted on the score plot indicate that RM1 scores have a positive correlation with PC1 and PC2, mainly aromatics, while the negative PC1 it is more explained by carbohydrate compounds, which are more abundant in RM2, and finally the aliphatic compounds show a negative correlation with PC1 but a positive one with PC2, which is the area populated by both some of the RM1 and RM2 scores. While the score plot shows the compound classes giving rise to the separation, it was observed before in Chapter 4, that PCA was driven by specific metabolites in the NMR spectra. Therefore to examine the cause of the separation further, the loadings plot with chemical shift for PC1 and PC2 (Figure 5.13).

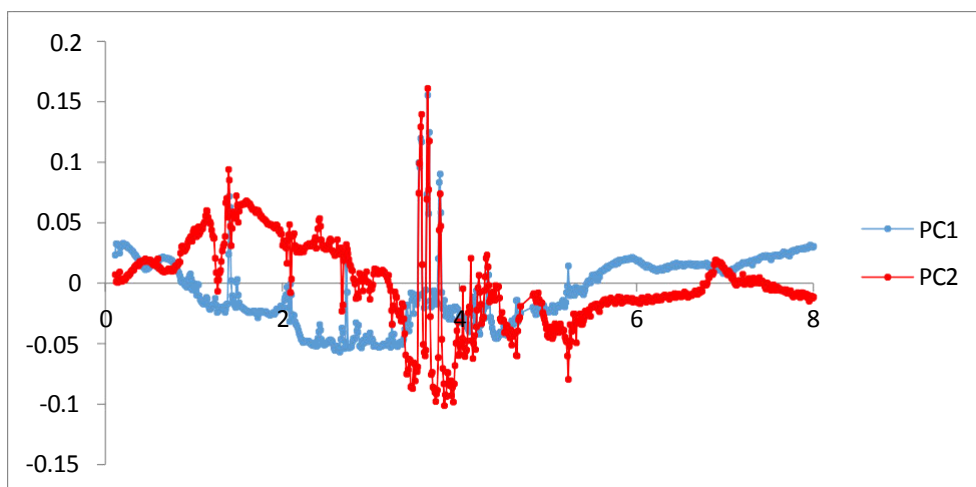


Figure 5.13 PCA loadings plots showing the first two principal components of PW-DOM ^1H NMR data of RM1 and RM2 cores.

The loadings plot also shows sharp peaks which belong to specific metabolites. RM1 samples are negatively correlated with carbohydrate with chemical shifts 4.13, 4.3, 3.77, 3.57, 3.64 ppm, aliphatic compounds with the highest loadings recorded at 1.39 ppm and to an alkene/aromatic between 5.5 and 8 ppm. The chemical shift at 1.39 ppm is either acetate or belongs to fatty acid molecules (or protons beta to acid or ester groups) or both (201), while the carbohydrate molecule is unknown and would require further 2D NMR experiments to explore further. The RM2 samples appear negatively correlated with the same carbohydrate and positively to additional carbohydrate chemical shifts at 5.23, 4.64, 3.83, 3.47 ppm and functionalized aliphatic chemical shifts between 2-3 ppm. The former chemical shifts are from characteristic of glucose. ^1H NMR spectra only show the most concentrated compounds, however it is clear from the PCA that there is a difference between sites. Before examining these differences further the FT-ICR MS data needs to be inspected to take into account those compounds under the detection limit of NMR.

5.3.3.2 Statistical and compound class analysis of PW-DOM FT-ICR MS data

To investigate the site variation within RM1 and RM2 the number of molecules within each compound classes from the only two cores (core e from RM1 and core H from RM2) with enough PW-DOM from at least 6 layers were inspected (Figure 5.14).

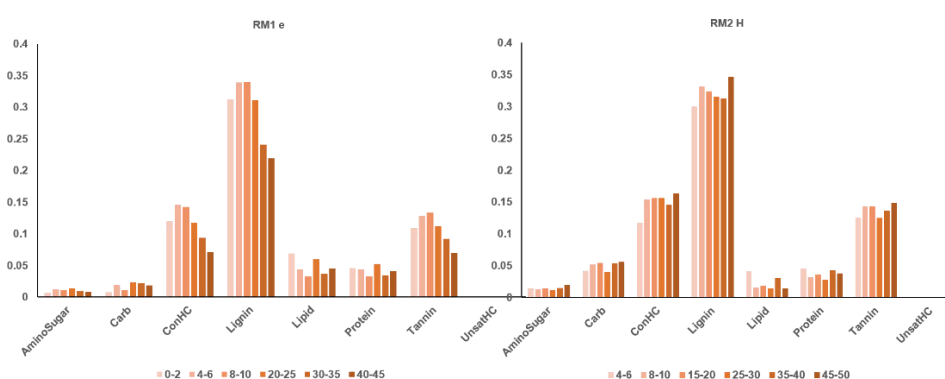


Figure 5.14 Compound class histograms created using the number of formulae assigned to a particular compound class in PW-DOM FT-ICR MS spectrum as a proportion of the total number of formulae assigned in that spectrum. Histograms take in account core e (RM1) and core H (RM2).

After making a comparison with the same kind of histograms made on the whole core discussed in chapter 1, it is possible to identify some differences on both RM1 and RM2. Precisely, RM1 shows a decreasing trend of condensed hydrocarbons, lignin and lipid with depth which is the main difference with the whole core. Also, another difference can be identified by the slight increase of carbohydrates with depth. RM2 also show a different behavior, especially aminosugars, carbohydrates, lipids and tannins increase with depth, while an opposite trend has been seen on the whole, while condensed

hydrocarbons and lignin show a similar trend with the core previously described.

To determine if there is a molecular difference between PW-DOM from RM1 vs RM2, PCA analysis was conducted using 4 cores from RM1 (a, e, f, g) and 3 cores from RM2 (A, B, K) from 6 depths. The PCA score plot of PW-DOM MS spectra from RM1 and RM2 is shown in Figure 5.15 with loadings shown as arrows.

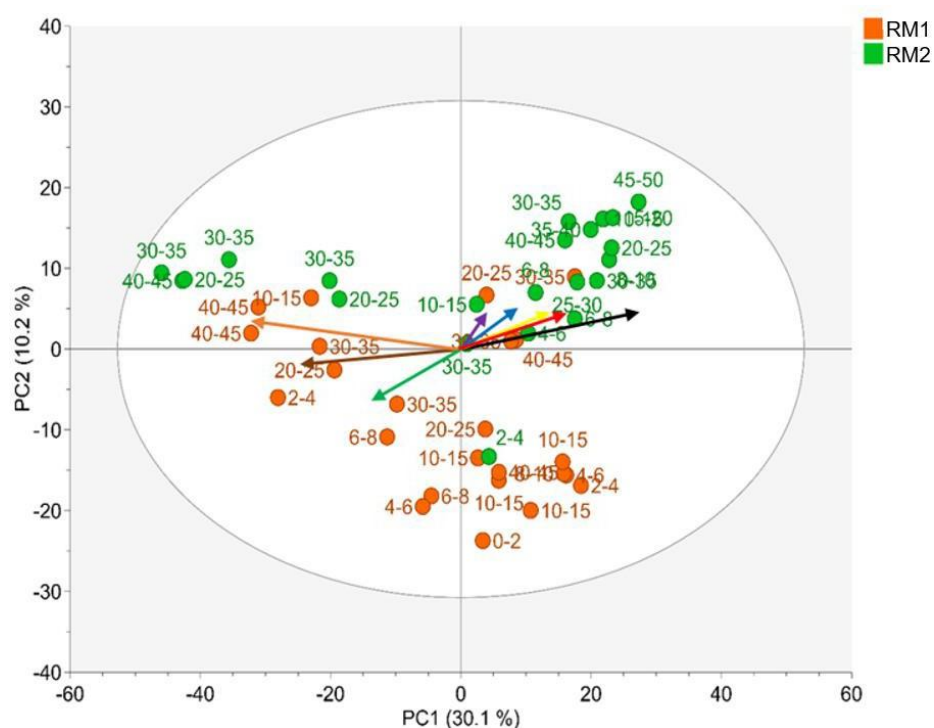


Figure 5.15 PCA score plot of PW-DOM FT-ICR MS data from RM1 and RM2 cores. The scores are coloured by different depths. The loadings are scale relative to carbohydrate-like (blue arrow) and show those of lipid-like (orange arrow), unsaturated hydrocarbons-like (yellow arrow), condensed hydrocarbons-like (black arrow), tannin-like (red arrow), lignin-like (brown arrow), amino acid-like (purple) compounds and protein-like (green).

The total variance of PW-DOM MS PCA represents the 40.3% in which the PC1 explains the 30.1% of it. Similarly to ^1H NMR data, PWDOM MS PCA shows a clear separation between RM1 and RM2, particularly RM2 bottom layers show a positive correlation with PC1, mostly explained by tannins, condensed hydrocarbons, aminosugars, unsaturated hydrocarbons and carbohydrates, while RM1 samples having a negative correlation with both PC1 and PC2 can be attributed to lipid, proteins and lignin. To explore the differences between the sites further compound class histograms, Upset plots and aromaticity index are shown in Figure 5.16.

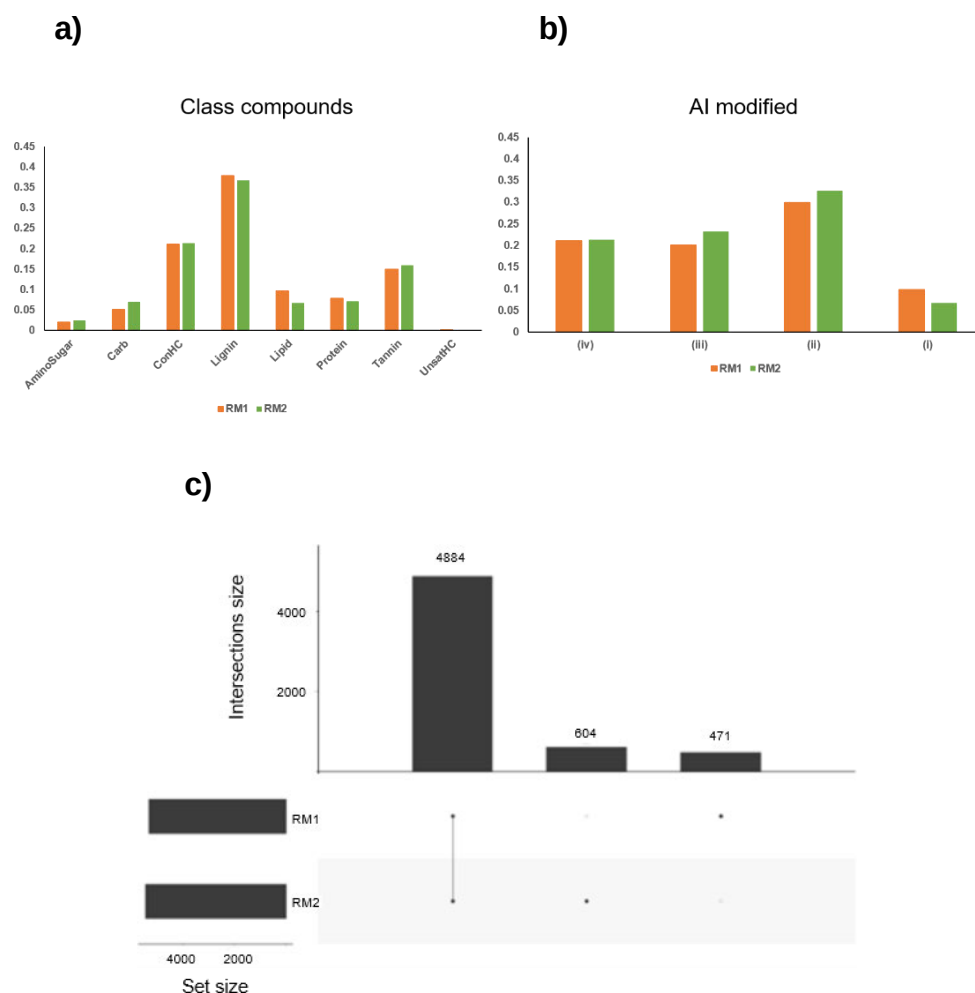


Figure 5.16 a) Class compounds, b) aromaticity index and c) UpSet plots of PW-DOM FT-ICR MS data for 4 cores from RM1 (a, e, f, g) and 3 cores from RM2 (A, E, K).

An interesting result is observed from the modified aromaticity index which shows that RM2 have higher plant-derived polyphenols, highly unsaturated and phenolic compounds than RM1, while there is an opposite trend in terms of aliphatic. However in general there is not a major dominance of one compound class between the sites. The Upset plot shows that a total of 4884 formulae are shared between the 2 sites. However interestingly, it also shows 604 unique formula coming from RM2 while 471 are unique to RM1. The higher number of unique formulae from RM2 could also be explained by the slightly higher number of molecular formulae assigned on this site, precisely 5488 formulae against 5355. However, as there are evidence of different molecular trends on DOM between the 2 sites, the histograms related to unique compounds (Figure 5.17) in PW-DOM samples is able to better represent the difference in classes between the 2 sites.

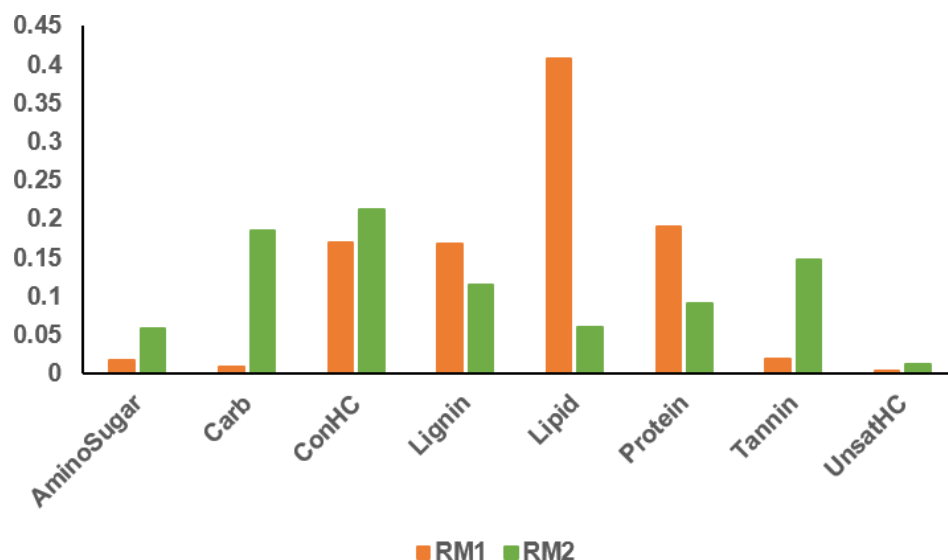


Figure 5.17 Unique class compounds plots of DOM MS data for all cores from RM1 and RM2.

It is clear from a comparison of the unique compounds that there is a higher occurrence of lignin-like, lipid-like and protein-like compounds in RM1 samples, while there are far more carbohydrate-like, tannin-like compounds and slightly more amino sugar-like and condensed hydrocarbon-like compounds unique to RM2. Also ^1H NMR results underlined a positive correlation between RM2 and carbohydrate chemical shifts, precisely at 5.23, 4.64, 3.83, 3.47 ppm, while RM1 samples were negatively correlated with carbohydrate with chemical shifts 4.13, 4.3, 3.77, 3.57, 3.64 ppm. About aliphatic compounds, the unique aliphatic found in RM1 might belong to the highest loadings recorded at 1.39 ppm on ^1H NMR loadings plot. The chemical shift at 1.39 ppm is either acetate or fatty acids molecules.

As previously discussed by D'andrilli *et al.*, "the presence of relatively high levels of aromatic compounds in DOM throughout the bog profile is also consistent with the hypothesis that phenol oxidase activity controls the enzymatic "latch" in peatlands." Also, the higher presence of plant derived compounds in RM2 could be explained by leachates from *Sphagnum* moss due to the rewetting activity, which could lead to an increase in the level of highly unsaturated compounds as secondary products too. (47) However, it is worth to note that the RM1 site shows a lower level of condensed hydrocarbons and tannins, defined by both unique compounds and compound class bar charts for core e, which can be attributed to a higher level of oxidation throughout the core. In general, the differences in composition could be related to the differences in bacterial vs fungal metabolism. Drainage has been shown to alter microbial community structure and diversity. (202) The increase in fatty acids/lipid-like molecular for RM1 could be explained by increased metabolic processes due to the increased oxygen, which have been shown to increase the abundance of fungi derived fatty acids from increased fungal metabolism. (186) In near natural sites fungal metabolism dominates in the surface layers and switches to bacterial metabolism soon after. (186) It would therefore be expected that bacteria would dominate in the rewetted site due to the anoxic conditions and this would have an impact on the metabolic processes going on, which in turn would lead to a difference in the type of compounds that are present in the peat waters. In general it is thought that fungi decompose more recalcitrant molecules compared to bacteria however the exact types of molecules they decompose does depend on the specific fungal group. (203) Further exploration of the microbial abundance and community structure in the drained and rewetted peat samples would be required to confirm this.

As outlined in Chapter 4, the combination of both bulk and physical-chemical properties are able to fully characterize peatlands at molecular scale and with multivariate analysis, it was possible to better visualize the data trends. Precisely, in this study we were able to identify the main difference between damaged and restored area of the Red Moss of Balerno raised bog. Bulk properties of RM2, restored site, mainly showed an increase in both water content and C/N with depth and a decrease in terms of bulk density and ash content as expected. This trend is also occurring in RM1, damaged site, but at lower extent than RM2 and with less steady behavior with depth, potentially due to the drainage process as outlined in the previous chapters. Regarding the molecular insights, both ATR-FTIR and solid state ^{13}C NMR data showed that there are some differences between each core from both sites with depth, which was not particularly evident from PCA. However it was possible to define an increase in alkyl compounds in RM2 at bottom layers, while RM1 was more characterized by O-alkyl fractions till 15 cm depth. However, liquid state ^1H NMR data indicate that there is a clearer difference between the two sites in terms of molecular composition, precisely aromatics occurred more in RM1, while RM2 is more abundant in carbohydrate compounds. This was explained by specific metabolites, which are able to drive the main differences in the NMR spectra. Further 2D experiments would be required to identify these compounds. Finally, about the MS data, LDI technique was not able to show particular evidence of differences between those two sites, while the ESI FT-ICR MS on PW-DOM showed a decreasing trend of condensed hydrocarbons, lignin and lipid with depth in RM1, while a slight increase of amino acids and carbohydrates with depth has been underlined in RM2. However, a very interesting result has been observed from the modified aromaticity index which showed that RM2

have higher plant-derived polyphenols, highly unsaturated and phenolic compounds than RM1, while there is an opposite trend in terms of aliphatic.

6. Results and Discussion: Physicochemical and molecular examination of near-natural, drained and rewetted blanket bog sites at Crocach, Flow Country.

In order to examine the physicochemical and molecular impact of drainage and long term rewetting on blanket bogs, peat cores from the Flow Country sites were examined using the same techniques applied to Red Moss peat samples. Unlike the Red Moss of Balerno, access to a site that has no historic human intervention was possible in the Flow Country and therefore near-natural (S1) peat cores were compared with those from the drained (S3) and rewetted sites (S2).

6.1 Evaluation of site water logger data.

As reported in Chapter 3 nine automatic water loggers were placed on site in May 2019 after cores were taken. The locations of these automatic loggers were deliberately placed near core sampling points. In July 2020, the water table data as well as air temperature and rainfall was downloaded by Alice Martin-Walker, RSPB Forsinard. The average water table, temperature and precipitation recordings for the period May 2019 – July 2020 are shown in Figure 6.1a and 6.1b.

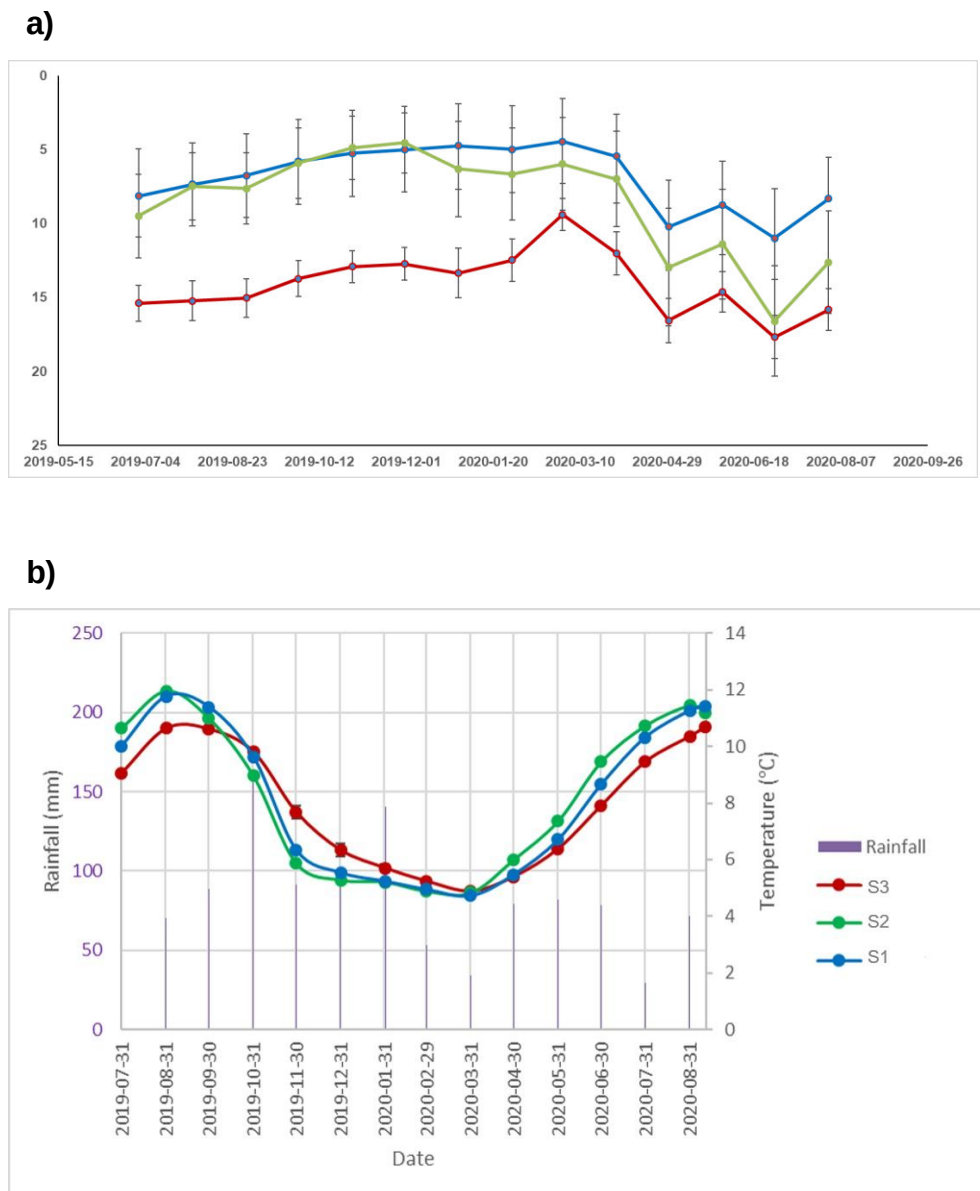


Figure 6.1 a) Mean monthly water table depth, b) temperature and precipitation for the three sites at the Flow Country peatland. Error bars show one standard deviation above and below the mean.

From Figure 6.1, the water table during the observed period was on average $6.87 \text{ cm} \pm 2.05 \text{ (s.d.)}$ for S1, $8.53 \text{ cm} \pm 3.44 \text{ (s.d.)}$ for S2

and $14.066 \text{ cm} \pm 2.05$ (s.d.) for S3. The average water table values followed the expected trend for peatland health status and it is very clear that the drained site, S3, has the lowest water table throughout the year. The restored site, S2, does follow the near natural site, S1, until December 2019 where the average water table deviates, indicating rewetting has not returned the water table to near natural levels.

In all three sites, S1 and S2 water table depths dropped in the period April 2020 – June 2020. Notably the S2's water table depth fell significantly more (to a low of 16.59 cm) than the S1 site (low of 10.98 cm). This coincided with a period of warming temperatures ($6 - > 11 \text{ }^{\circ}\text{C}$) and the lowest rain fall.

For the S3 site, water table depth increased in the period December 2019 – February 2019. This coincided with the period of coldest recorded temperatures ($5 \text{ }^{\circ}\text{C}$). We deduced that this was due to frozen ground reducing the efficacy of existing drainage ditches.

In 2017, Sophie M. Green *et al.* (188) discussed the response of blanket bog vegetation and water tables to ditch blocking. They made measurements on water tables for 4 years from 3 different ditch treatments: open ditches, blocked with closely-spaced dams and partially infilled with peat and blocked with dams. However, they did not find any relevant differences in terms of both vegetation and water tables between these ditch treatments. This can be considered similar to our water content findings, as it has been noted that in all the 3 blanket bogs discussed in this chapter, the step increase in water content occurs after 10 cm in depth, while it is not in line with the water table data showed in figure 5.1a where it is clear that the damaged site (S3) revealed lower water tables ($\sim 14 \text{ cm}$) than S1 and S2 sites ($\sim 6 \text{ cm}$ and $\sim 8 \text{ cm}$ respectively).

For each site, the water content for each core with depth is shown in Figure 6.4a, and the average value for all cores with the standard deviation is shown in Figure 6.4b.

6.2 Site and core observations

Cores were extracted from points of similar microtopography when identifiable. For S1 and S2 sites cores were taken between hummocks and hollows. For the S3 site cores were taken on flat areas near hummocks. The dominant S1 vegetation was varieties of *Sphagnum* moss, cotton grass, sundews, bog asphodel and occasional heather and *Erica tetralix*. The major difference between the S1 and S2 and S3 was the dominance of deer grass and heather, respectively. Unlike the S1 and S2, the ground at S3 was tussocky. Examination of each 50 cm core visually showed characteristic changes with depth due to decomposition, especially for S1. Unusual observations were for cores from S2 where there was a colour change from dark brown to grey near the bottom of several cores and evidence of rocky material. The S3 cores were predominantly roots due to the presence of heather with many showing colour changes from dark to light brown. Pictures of the cores can be found in Appendix C, Figure C1, C2 and C3.

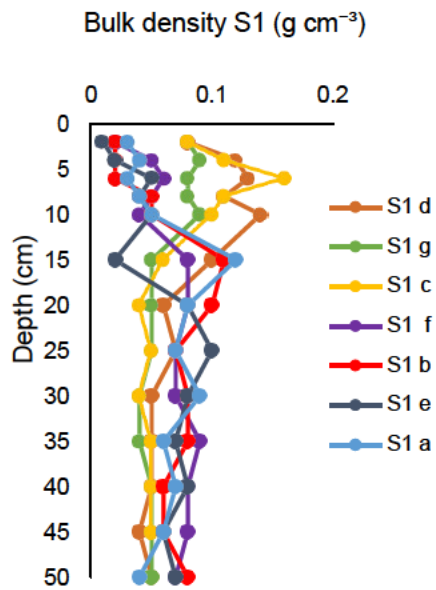
6.3 Solid phase physicochemical and molecular examination of near natural, drained and rewetted peat samples

6.3.1 Physicochemical properties

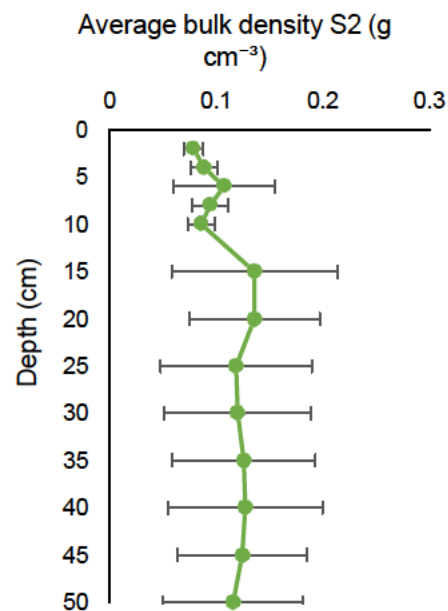
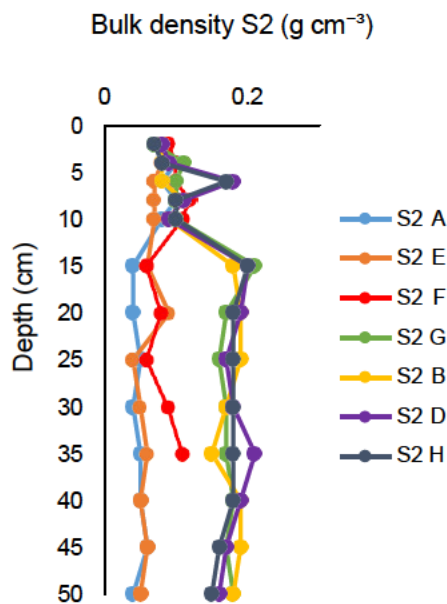
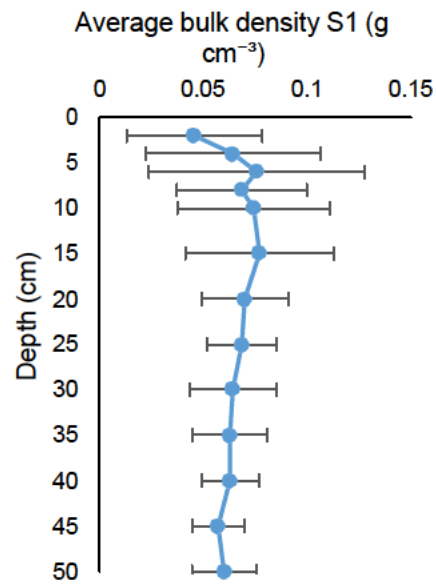
Physicochemical properties were measured, namely ash content, bulk density, water content and elemental content as outlined in Chapter 3. Note as each of these properties was examined in detail for one core from S2 in Chapter 4 only the differences between cores and sites will be examined here. For each site the average bulk density for each

core with depth is shown in Figure 6.2a, and the average value for all cores with the standard deviation is shown in Figure 6.2b.

a)



b)



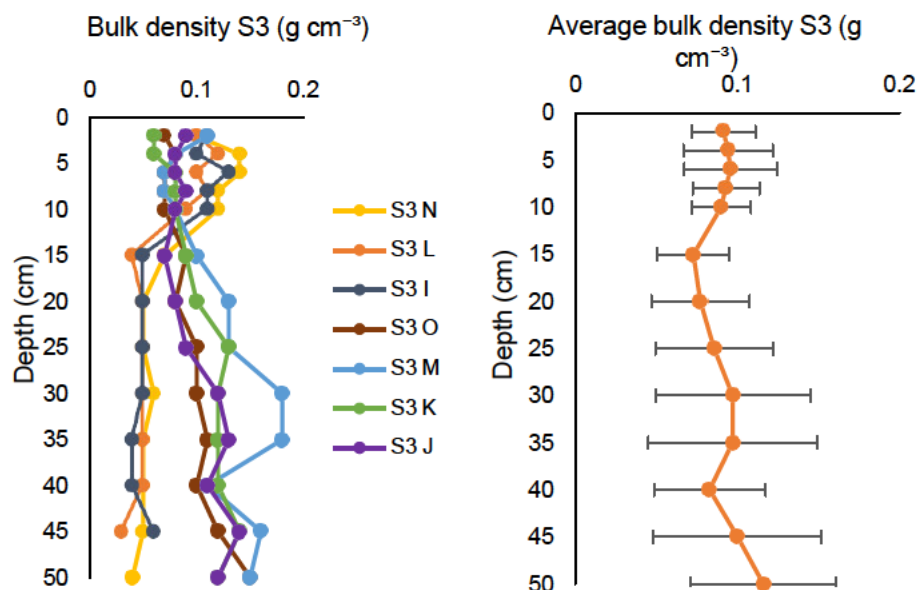


Figure 6.2 a) Bulk density for each core from S1, S2 and S3, b) average bulk density of each site.

From Figure 6.2a, it is evident that the trends with depth divide the cores into groups. The bulk density for cores a, b, e and f from the S1 site show an increase with depth, particularly marked after 15 cm, while cores c, d and g show the completely opposite trend. Bulk density of cores A, E and F from S2 site do not show any particular step change throughout depth, which instead could be noted for cores B, D, G and H where it can be underlined that the bulk density increases until 15 cm. Finally, cores N, L and I from S3 site show an evident decrease in bulk density after 15 cm in depth, while cores J, K, M and O have a regular increase with depth. These variations within each site demonstrate the need to take multiple cores. Despite being taken from similar microtopographies there is clear variations, which would be masked if only a handful of cores were taken. The difference between core bulk densities in the surface layers has been reported

due to different vegetation types capping cores. This is particularly evident here for the S1 and matches the core observations. (187)

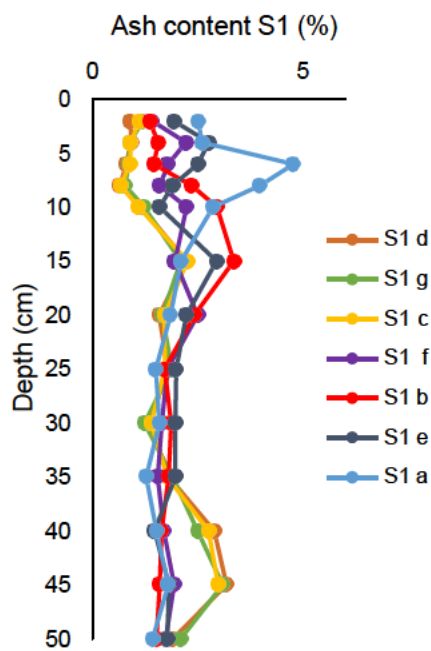
The core groupings described above clearly impact the average bulk density, as indicated by the large standard deviations. However despite this step changes in bulk density are evident for each site. The most evident is for S2 where there is a step change between 10 cm and 20 cm. For S3 there appears to be two at 10-20 cm and 35-45 cm, while for S1 there is a slight change occurring at 8-15 cm. As seen for core D of S2 in chapter 3, these step changes reflect the transitions between oxic and anoxic zones, indicated by the lowest observed water table and agree with water table observations.

Overall, well preserved blanket peat bogs tend to show lower organic matter density with depth than drained peats, due to compression that occurs upon drainage compared to wet conditions that boost a more rapid burial of organic matter. (2) (181) However, despite the fact that the majority of blanket peat bogs have a bulk density from 0.05 g cm^{-3} to 0.2 g cm^{-3} with depth, bulk density values are also affected by the kind of vegetation as well. Morton P.A. *et al.* (187) described the differences in bulk densities between dry and wet cores taken from three English blanket bogs in relation to heather management, dominant vegetation and water table. They showed bulk density values ranging between 0.07 g cm^{-3} to 0.16 g cm^{-3} for 5 cm depth and that the wetter cores had lower bulk density values than drier cores. Interestingly, *Sphagnum* topped dry peat cores were characterized by higher bulk density values than cores with different vegetation on the surface. These results can be considered in line with the results presented here for the S1 site, considered as well preserved blanket peat bog. In contrast, S2 and S3 have higher bulk densities than S1 indicating compression due to damage. However it was not expected for S2 site to have bulk density values similar to S3 site. This could be

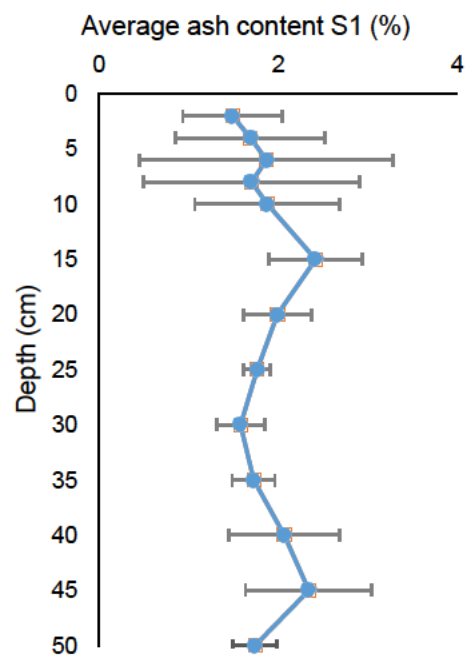
explained by the fact that the restoration activity still did contribute to bulk density levels for some cores (B, D, G and H) while it was the case for cores A, E and F.

For each site, the ash content for each core with depth is shown in Figure 6.3a, and the average value for all cores with the standard deviation is shown in Figure 6.3b.

a)



b)



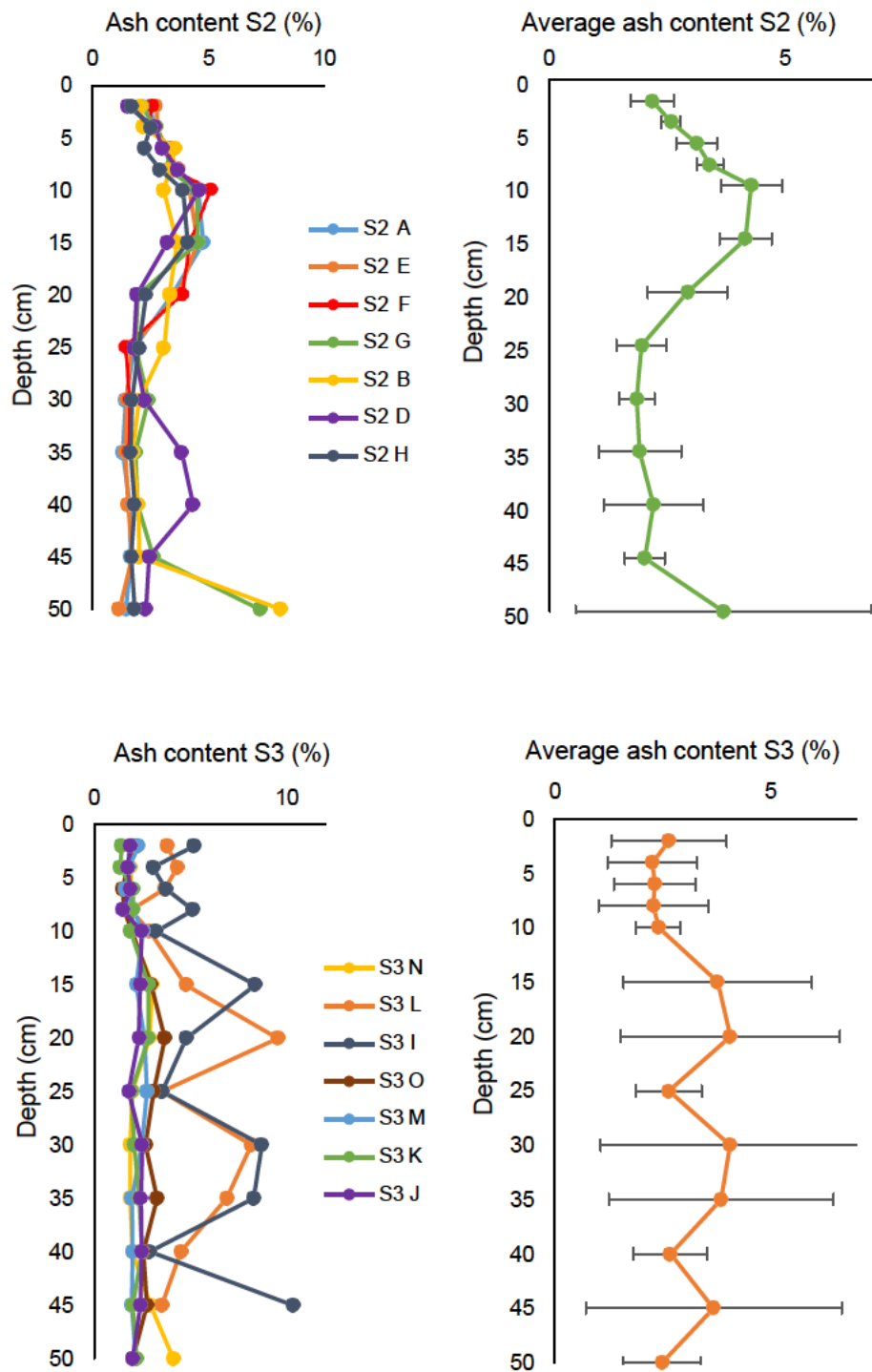


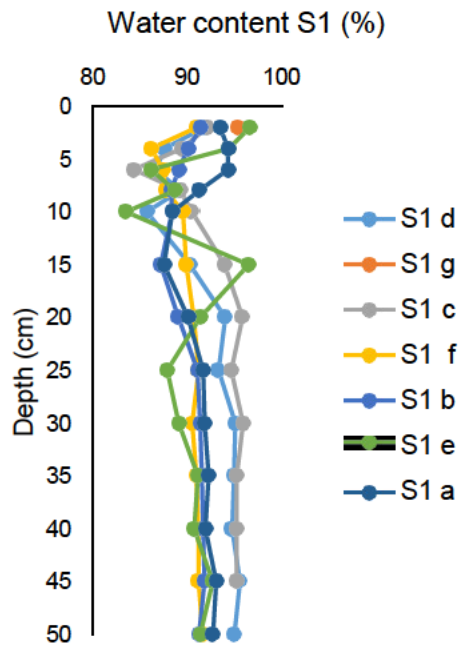
Figure 6.3 a) Ash content for each core from S1, S2 and S3 sites, b) average ash content for each site.

From Figure 6.3a, the ash content for the S1 site cores c, d and g have a slight increase after 40 cm with depth, while in core a decrease with depth. Cores b, e and f instead show a slight increase until 15 cm after that they reach a level of ash content similar to the surface. For the S2 site, the ash content for all the cores increases until 10 cm after which a decrease with depth occurs, apart for cores B, D and G where the ash content increases after 30 cm (core D) and after 45 cm (B and G). Finally, S3 sites show a constant level of ash content with depth for cores J, K, M and O. The same could be said for core N apart from the bottom of the core where there is a slight rise in ash content. Differently, cores I and L do not show a constant trend with depth, instead these two cores have peaks in ash content at 15/30 cm for core I and 20/30 cm for core L.

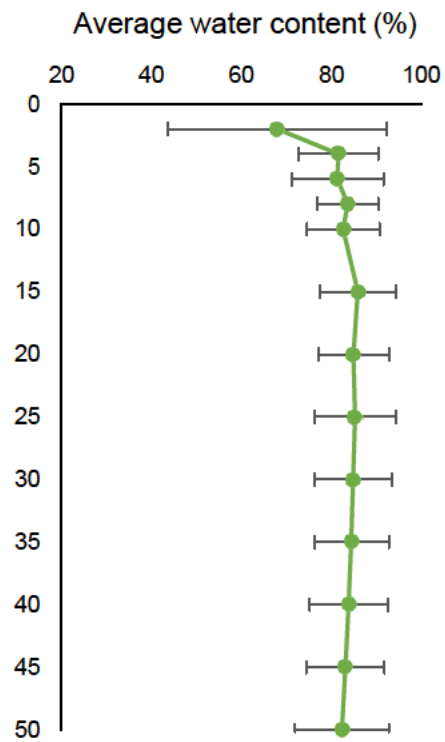
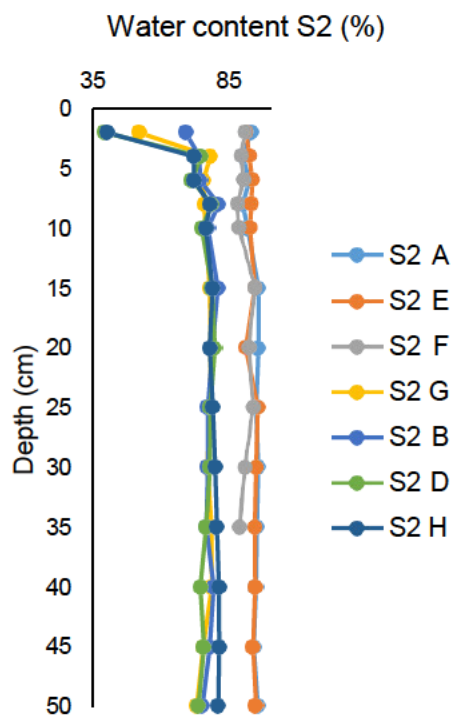
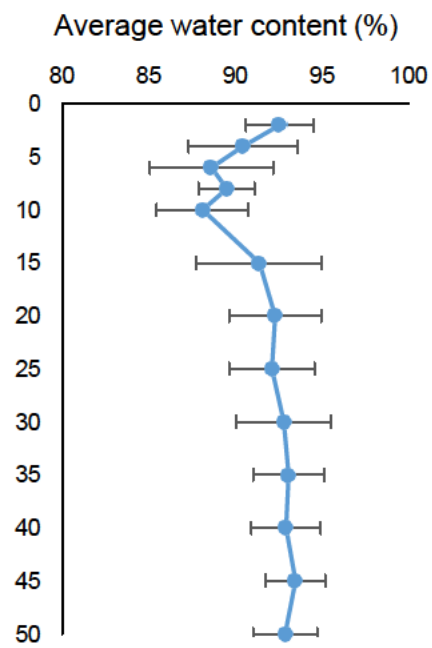
From Figure 6.3b the average ash content shows a non-linear response for all sites with depth, increasing in all three sites from 0 - 20 cm and then levelling out (S2 and S3) while decreasing to 50 cm in S1. It is important to restate here that higher ash content means lower organic matter content. As discussed by Strivins N. *et al.* in 2018 (189), the organic matter content was affected by drainage in a relative long period, specifically it was reduced. Therefore, S2 and S3 sites, in which drainage and restoration activities occurred, have a trend more similar to the one described in Strivin's paper as the organic matter in this case was replaced by an increase in ash content, while the S1 site since it is in near natural condition shows an opposite trend with depth in terms of ash content.

For each site, the water content for each core with depth is shown in Figure 6.4a, and the average value for all cores with the standard deviation is shown in Figure 6.4b.

a)



b)



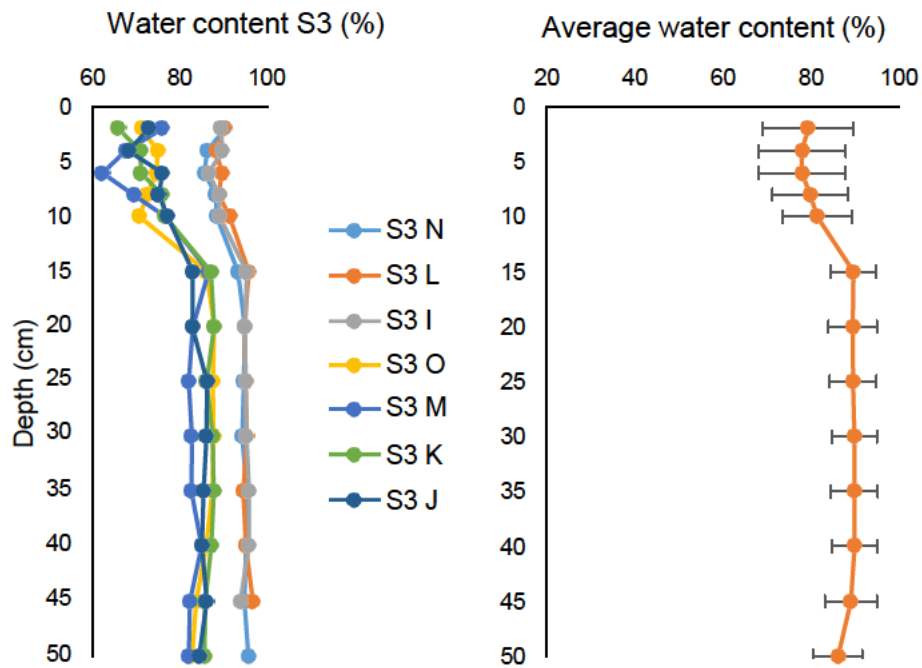


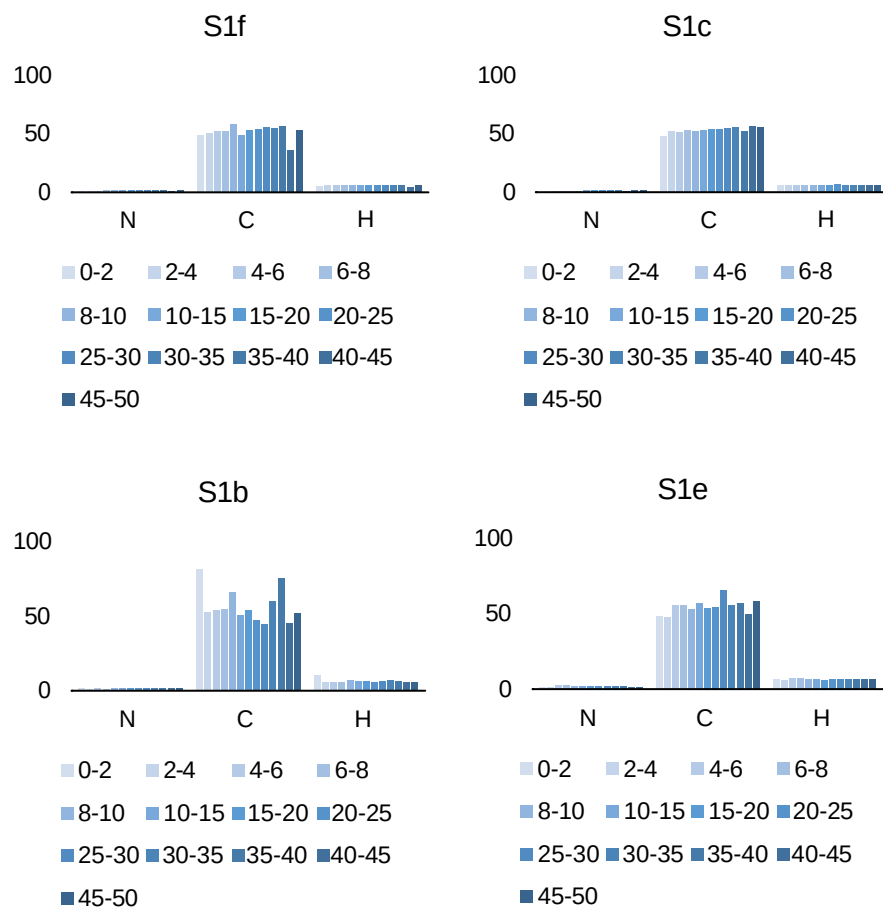
Figure 6.4 a) Water content with depth for each core from S1, S2 and S3 sites, b) average water content with depth for the same sites.

From Figure 6.4a, it is evident that the water content differs between cores from the same site for S3 and S2, in particular. For S2, cores B, D, G, and H have lower water content than A, E and F. A similar splitting into two groups occurs for S3 where core K, J, M, and O have lower water content than I, L, and N. These differences reflect the core observations where some cores were dry due to roots and the presence of drier vegetation like grasses and heather. The impact of vegetation is very noticeable for the tops of S2 H and G, which have the lowest water content. From the core observations these cores were capped with heather and lichen. From examination of Figure 6.4b, it is possible to state that the S2 site has a lower percentage of water content with depth than the S1 or S3 sites and that the S1 site has a higher percentage of water content than both S2 and S3. On average, all 3 sites underline a step increase in water content after 10

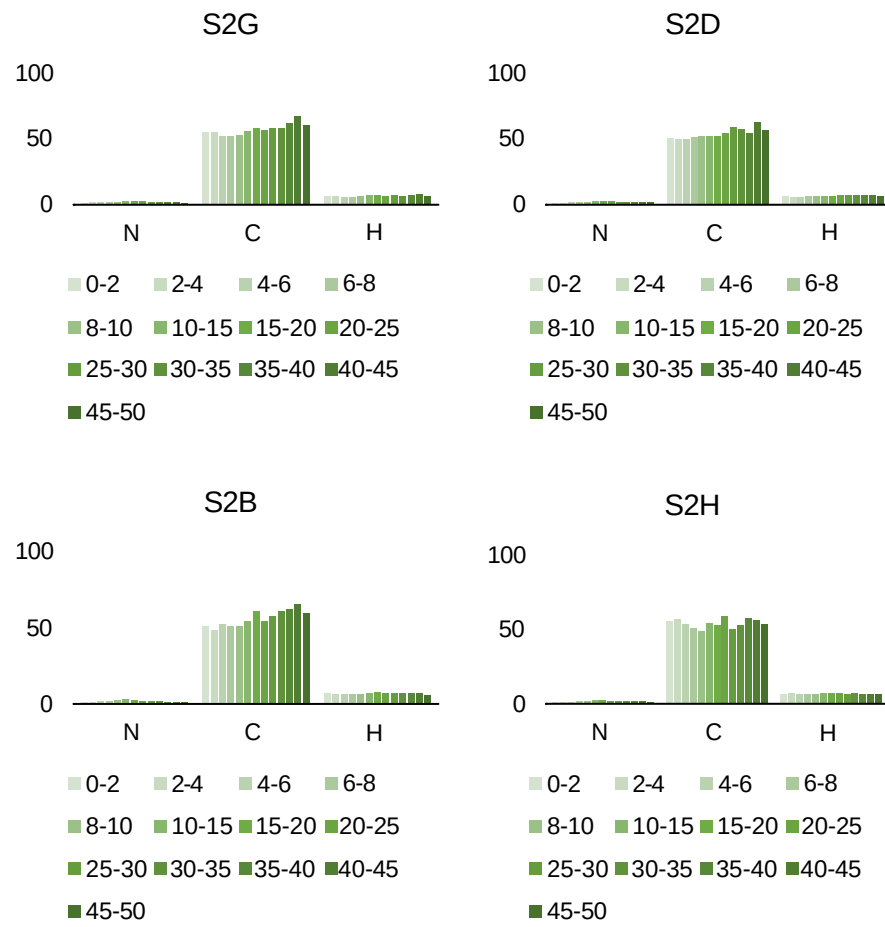
cm, however it is steeper in S1 site. The fluctuation of the water level and other mechanisms connected to drainage trigger considerable water loss. As described by Landry J. and Rochefort N. (190) the water content can decrease by 20 to 29% in the first 10 cm, mostly depending on the distance to the drainage ditch. Also, the drier conditions in the upper peat layers of a drained peatland can change the hydraulic structure by decreasing the size of the pores and therefore favouring the capillary movement of the deep waters towards the surface, which then translates with a consistent loss of water due to evaporation. Hence, the upper layers of core from S3 site looks like to be affected by a notable loss of water content due to drainage and also in the first 10 cm of some cores from S2 site. Despite the restoration activities undertaken, it seems that the water content has been reduced by even more dried conditions.

Differently from Red Moss, bar charts about H %, C % and N % content are shown in Figure 6.5a-c. Cores shown in Figure 6.5 are f, c, b, e from S1, G, D, B, H from S2 and O, M, K, J from S3

a)



b)



c)

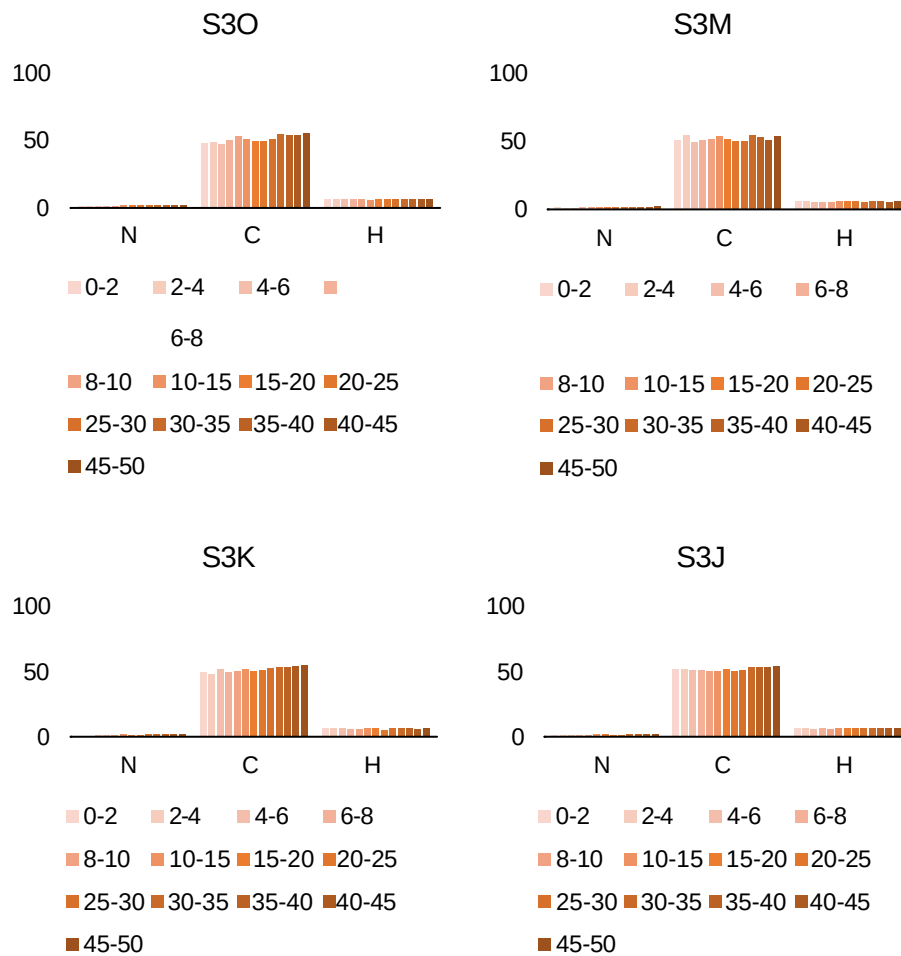


Figure 6.5 Bar charts of H %, C % and N % content for a) S1 cores f, c, b, e, b) S2 cores G, D, B, H and c) S3 cores O, M, K, J.

N (%) content showed a non-linear response with depth which varied particularly between sites. Specifically, in the S1 and S2 sites N (%) increased from 0 to 20 cm from an average of 1% to 1.75 - 2% then it decreased to roughly 1.5% at 50 cm. Differently, for the S3 site N % increased consistently with depth from an average of 0.8% to 2%.

The C (%) content showed a more linear increase with depth across all sites and cores. S1 cores had significantly higher C (%) than S3 or

S2. Also, the C content for the S2 site showed a greater increase with depth than for S1 or S3. Finally, H % did not show any relevant increase with depth across all cores. Overall, H % was significantly higher in S2 peat than in S1 or S3.

As already discussed in Chapter 4, overall the higher C/N occurring in all these sites at the surface can be explained by the presence of vegetation with a low N content, (3) such as *Sphagnum* mosses or partially decomposed cotton grasses. However, high C/N is also associated with high rate of decomposition, while a low C/N is usually a consequence of a high humification degree. (28) (133) Possible explanations for the increase in N % with depth, especially at the mid-depth layers of S1 and S2 cores, can be due to N immobilization/retention in microbial biomass. (29) (43) (133) Instead, the fact that the N % in S3 did not show a similar trend can be explained by the fact that as a consequence of drainage, N immobilization was not possible at middle layers. Finally, the increase in C % with depth in the peat profile is correlated to increased accumulation of organic matter and the absence of inert mineral material to increase in relative concentration, which was more consistent in S1 site than in S2 and S3 sites.

As described in Chapter 3, it was possible to obtain data for O % for one core from each site cores (S1e, S2E, S3N) which are plotted with depth together with N %, C % and H % in Figure 6.8.

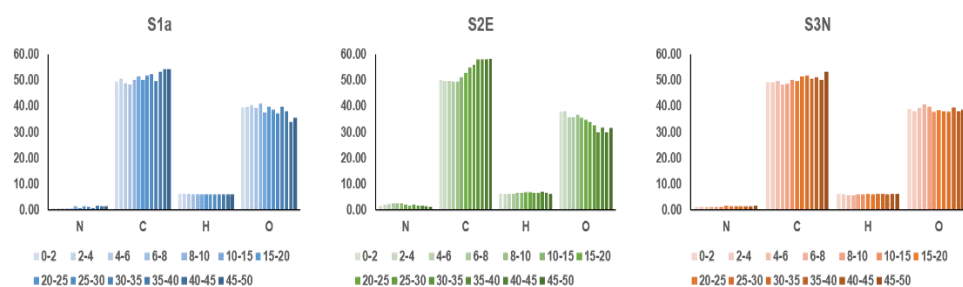


Figure 6.6 Bar charts showing CHNO content from cores S1e, S2E and S3N with depth

For S1a there is a slight decrease in O % with depth and a larger decrease with depth for S2E. S3N does not show any trend with depth. It would be expected that O % would decrease with depth in fully functioning bog and, while only a handful of cores were examined here, there is initial evidence that the lack of decrease observed for S3N indicates a higher level of oxidation through-out this core.

Overall, the bulk properties discussed so far for each site can be summarized in Table 6.1

Table 6.1 Summary of bulk properties from S1, S2 and S3

Site	Average Water table	Bulk density	Ash content	Water content	H%	C%	N%	O%
S1	6.87 cm ± 2.05	Slight step change between 8-15 cm	Increase between 0-20 cm, then decrease to 50 cm	Decrease from 0 to 10 cm, then step change after 10 cm. Highest value: 92% at 0-2 cm	Between 6-7% all depths	Between 50-80%, increasing with depth	Increase between 0-20 cm from 1% to 2%, then decrease till 50 cm	Decrease between 40-30% with depth
S2	8.53 cm ± 3.44	Step change between 10-20 cm	Increase between 0-20 cm, then constant	Increase after 10 cm, top value 86% at 15 cm, then constant	Between 6-7% all depths	Between 50-70%, increasing with depth	Increase between 0-20 cm from 1% to 2%, then decrease till 50 cm	Decrease between 40-30% with depth
S3	14.066 cm ± 2.05	Step change between 10-20 cm and 35-45 cm	Increase between 0-20 cm, then constant	Increase after 10 cm, top value 90% at 15 cm, then constant	Between 6-7% all depths	Between 40-55%, increasing with depth	Linear increase from 0.8% to 2%	Constant values between 30-40%

In summary, the average water table agrees with level of drainage with S1 having the highest water table and S3 the lowest. The ash content follows a similar trend with depth in S2 and S3 sites. The water content shows the same trends as bulk density across all sites. N% and O% showed the same behaviour in both S1 and S2 site, while in S3 site both N% and O% followed on opposite pattern. Whether these bulk properties reflect the molecular composition, will be discussed in the next sections.

6.3.2 Solid state molecular level examination of near natural, drained and rewetted site cores

As it was conducted for cores from Red Moss of Balerno, this section contains the deeper molecular level characterisation of the Flow Country cores.

6.3.2.1 ATR-FTIR spectral observations

ATR-FTIR analysis has been conducted on samples from each layer from each core from S1, S2, and S3 core samples as outlined in Chapter 3. The spectra obtained are characteristic of blanket bogs (18) and appear similar in dominant features to the spectra obtained from Red Moss of Balerno samples with typical stretches for OH ($3200\text{--}3600\text{ cm}^{-1}$), CH stretch (2850 cm^{-1} , 2920 cm^{-1}), C=C stretch (1510 cm^{-1}), C=O stretch (1650 cm^{-1}) and C-O stretch (1030 cm^{-1}), with clear dominance of the last stretch. However, there are some interesting features that need to be underlined. For instance, as exemplified for core S1 b there is a slight shift in wavenumber to the right in the region $1720\text{--}1510\text{ cm}^{-1}$ and from 1030 to 1028 cm^{-1} starting from top layers (Figure 6.7).



Figure 6.7 ATR-FTIR overlay spectra for core b (S1) depths 4-6, 8-10, 15-20, 25-30, 35-40 and 45-50 cm. Most intense wavenumbers are labelled (cm^{-1}).

A stack plot of selected spectra from core O from S3 (Figure 6.8) demonstrates the higher absorbance observed for S3 cores compared to S1 sites in top layers around 3400 cm^{-1} and the lower absorbance observed for the bottom layers at $2920\text{-}2850 \text{ cm}^{-1}$ and between 1720 and 1510 cm^{-1} . Finally, around 1030 cm^{-1} there is no evident shift with depth but the band shows higher absorbance for the top layers of S3 cores than S1 cores (Figure 6.8).

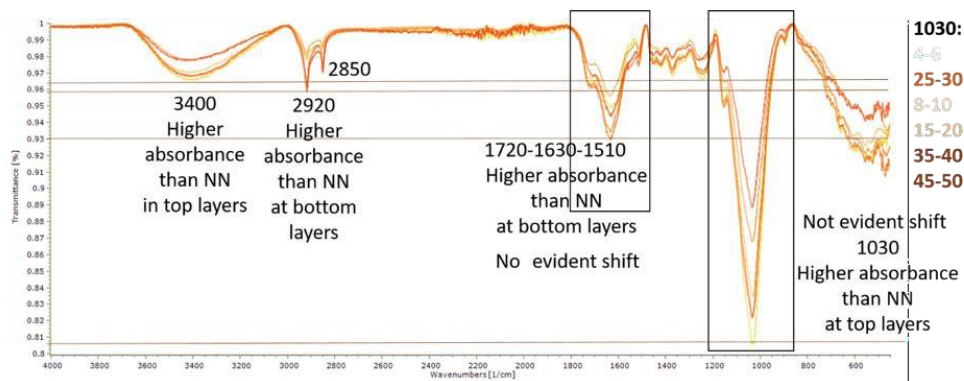


Figure 6.8 ATR-FTIR overlay spectra core O (S3) depths 4-6, 8-10, 15-20, 25-30, 35-40 and 45-50 cm. Most intense wavenumbers are labelled (cm^{-1}).

Finally, the last set of stacked spectra from core S2 B (Figure 6.9) demonstrates the higher absorbance than both S1 and S3 sites observed at around 2920-2850 cm^{-1} , the higher absorbance than S1 sites between 1720 and 1510 cm^{-1} and around 1030 cm^{-1} especially for the top layers. At both regions between 1720 and 1510 cm^{-1} and 1030 cm^{-1} it can be noted a little shift in wavenumber starting from top layers.

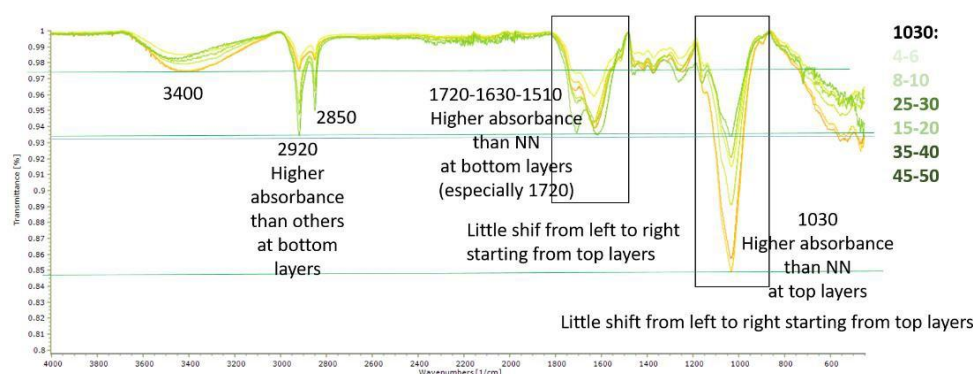


Figure 6.9 ATR-FTIR overlay spectra for core B (S2) depths 4-6, 8-10, 15-20, 25-30, 35-40 and 45-50 cm. Note: In this case, Spectragryph colour palette availability defines the colour light green as orange. Most intense wavenumbers are labelled (cm^{-1}).

Looking at literature, there is only one study to date that has compared FTIR spectra of cores from near-natural, drained and restored peatland sites. McAnallen *et al.* examined a samples from 4 depths (5, 15, 25 and 35 cm) of cores extracted from an Irish blanket bog and noted slight shifts in wavenumbers depending on health status. For example, the wavenumbers assigned to polysaccharides were noted as 1070 cm^{-1} for degrading peat and 1040 cm^{-1} for active and restored peat, which are not the same wavenumbers noted on our samples. (22) Such shifts in wavenumber indicate changes in composition due to peatland health. Similar to the sites studied here,

they also reported changes in intensity of absorbance due to peatland health. For example, the peak at around 3400 cm^{-1} , assigned to cellulose, alcohols or hemicellulose, showed the highest absorbance in damaged cores, in agreement with S3 cores. (22) They also reported an increase with depth of aliphatics (2920 and 2850 cm^{-1}), which mainly occurs in the S2 site, and a decrease of polysaccharides, which is occurring in S1 and S2 cores. The spectral band between $1600\text{-}1650\text{ cm}^{-1}$, generally attributed to aromatic or aliphatic carboxylates, shows higher absorbance at bottom layers in both S2 and S3 sites than S1, in line to McAnallen *et al.*, which demonstrates a further change in organic matter composition with depth. (22) These changes can be explained by an increase in humification, as a consequence of the polysaccharides decomposition under aerobic activity, which leads to the production of less decomposable materials, such as aliphatics or aromatics that accumulate with depth.

While this visual comparison was done by eye for a few depths. To compare the full set of cores multivariate analysis was conducted.

6.3.2.2 Solid state ^{13}C NMR spectral observations

Solid state ^{13}C NMR analysis has been carried out on the same samples as outlined in Chapter 3. The spectra obtained are typical of peat samples (25) showing overlapped resonance from $0\text{-}200\text{ ppm}$, dominated by O-alkyl and alkyl resonances, as previously shown for the whole core described in Chapter 3. From a very initial observation of the spectra from different sites, the C-alkyl resonances are particularly strong in the S2 cores and show a clearer increasing pattern with depth, especially after 20 cm , while the S3 cores are characterized by stronger resonances at chemical shifts related to O-

alkyl throughout all the cores, as exemplified in Figure 6.10 showing cores c (S1), H (S2) and M (S3).

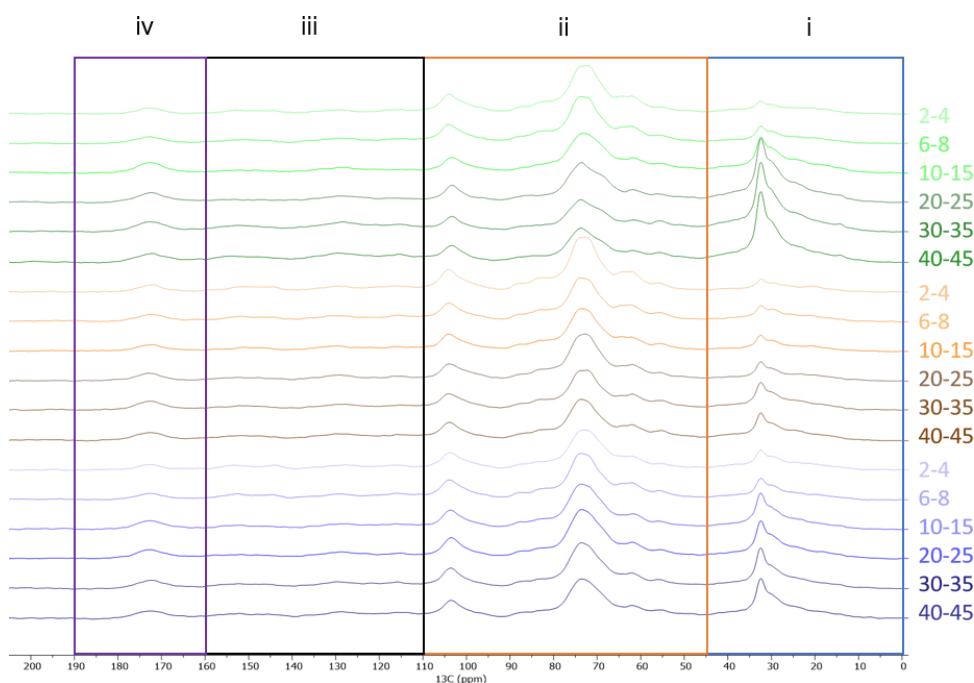


Figure 6.10 ^{13}C NMR stacked spectra of core H (S2, green), core M (S3, orange), core c (S1, blue) depths 2-4, 6-8, 10-15, 20-25, 30-35 and 40-45 cm. Characteristic regions are labelled as follows: (i) Alkyl (0-45 ppm), (ii) O-alkyl (45-110 ppm, with anomeric region 90-110 ppm), (iii) Aromatic (110-160 ppm), (iv) Carboxyl (160-190 ppm).

Overall, by comparing the ^{13}C solid state NMR data from all cores with water table, it results that there is a step increase in alkyl content on S1 and S2 cores starting between 8 cm and 15 cm, in line with water table registered in 2020 of about 8 cm and 13 cm in the S1 and S2 sites respectively. Differently, no evident step change in alkyl content on S3 site, where the water table was registered at around 16 cm in 2020. About O-alkyl content, as noted by the elemental analysis on core N from S3 site, it is evident that O% is constantly higher than in

S1 and S2 sites, reflecting more oxygenated compounds throughout the core which can be considered as representative for all other ones.

6.3.2.3 (-) LDI FT-ICR MS of peat observations

LDI MS analysis of core samples from the three Flow Country sites has been carried as outlined in Chapter 3. The analysis was conducted on 6 depths per core on 7 cores for each site, therefore a total of 21 cores. For all three sites, a total of 7158 molecular formulae (5165 $C_xH_yO_z$ and 1993 $C_xH_yO_zN_n$) have been assigned. A total of 5372 molecular formulae have been assigned for S1 samples, 4825 for S3 samples while 6898 in S2 samples. On average, $66.2 \pm 6.7\%$ of m/z fragments from all the LDI MS spectra were successfully assigned from 149 to 702 m/z .

6.3.2.4 Statistical analysis of the solid phase molecular data

In this section, all the statistical analyses that have been carried out on Flow Country data will be discussed. PCA was applied to the ATR-FTIR, FT-ICR MS and NMR datasets in order to reduce dimensionality to facilitate visualisation and modelling of the datasets. PCA of IR and NMR datasets was performed in SIMCA as discussed previously, while PCA of the MS datasets was conducted using PyKrev by Ezra Kitson (204) in order to show a clearer interpretation of those kind of data. For the LDI FT-ICR MS data additional Upset plots and compound class bar charts were also inspected

6.3.2.4.1 PCA of ATR-FTIR dataset

A PCA score plot with loadings of the ATR-FTIR data is shown in Figure 6.11a and b.

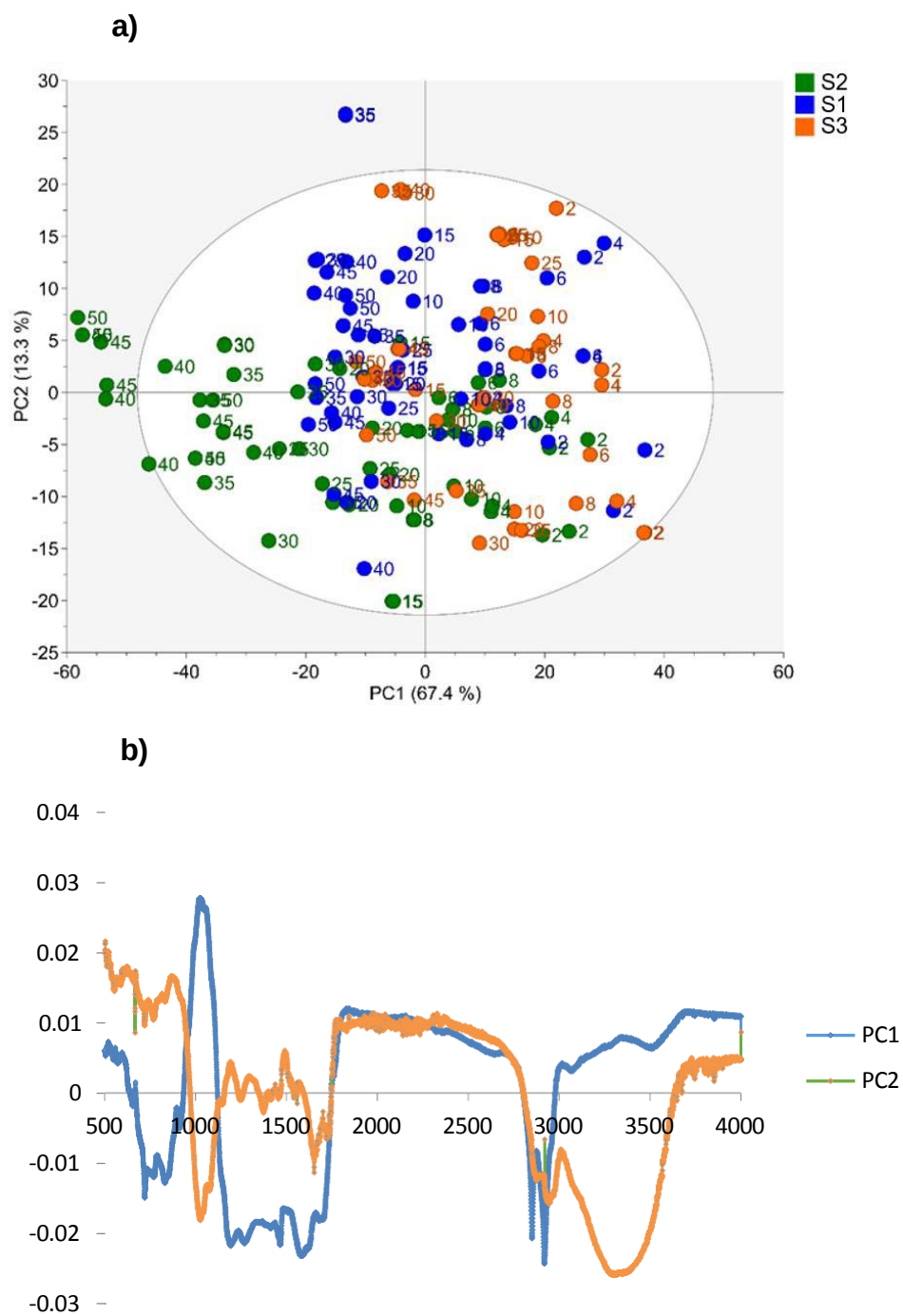


Figure 6.11 a) PCA score plot for the ATR-FTIR average of triplicates data of each depth from S1, S2 and S3 sites and b) PC1 and PC2 loadings.

On PC1, which comprised 67.4% of the variation in the dataset positive scores were associated with the stretch from 1162 – 1668 cm^{-1} , with the greatest increase in this region at 1622 cm^{-1} (aromatic C=C stretching), and a two distinct wavelengths at 2850 and 2920 cm^{-1} (CH stretches). Negative scores were associated with wavelengths at $\sim 1021 \text{ cm}^{-1}$ (C-O stretch). From the scores plot there was considerable separation of the samples both by depth and health status. Comparing S1 and S3 samples, S1 (mean = 4.22, SD = 11.42) had a higher average PC1 score than S3 samples (mean = -4.22, SD = 9.53) (Welch's two-sided T-test: statistic = 5.29, pvalue = 3.61e-07). S2 samples had the highest PC1 score (mean 13.31 = SD: 17.2) (Welch's two-sided T-test: statistic = 4.08/8.28, pvalue = 7.06e-05/1.03e-13). Thus wavelengths associated with C=C and CH stretches were higher in S1 than S3, and highest of all in S2. Conversely, wavelengths associated with C-O stretches were higher in S3 samples than S1, in agreement with literature. (18) (22)

Comparing samples by depth, there was a strongly positive correlation between increasing depth and PC1 score (Spearman's correlation: coefficient = 0.77, pvalue = 6.23e-54). Thus, C=C and CH stretching was higher in the upper samples (0 – 20 cm) while C-O stretching was highest in the deeper samples (20 – 50 cm). (18) (22)

6.3.2.4.2 PCA of Solid State NMR dataset

The PCA score plot with loadings for the ^{13}C solid state NMR CPMAS dataset is shown in Figure 6.12a and b.

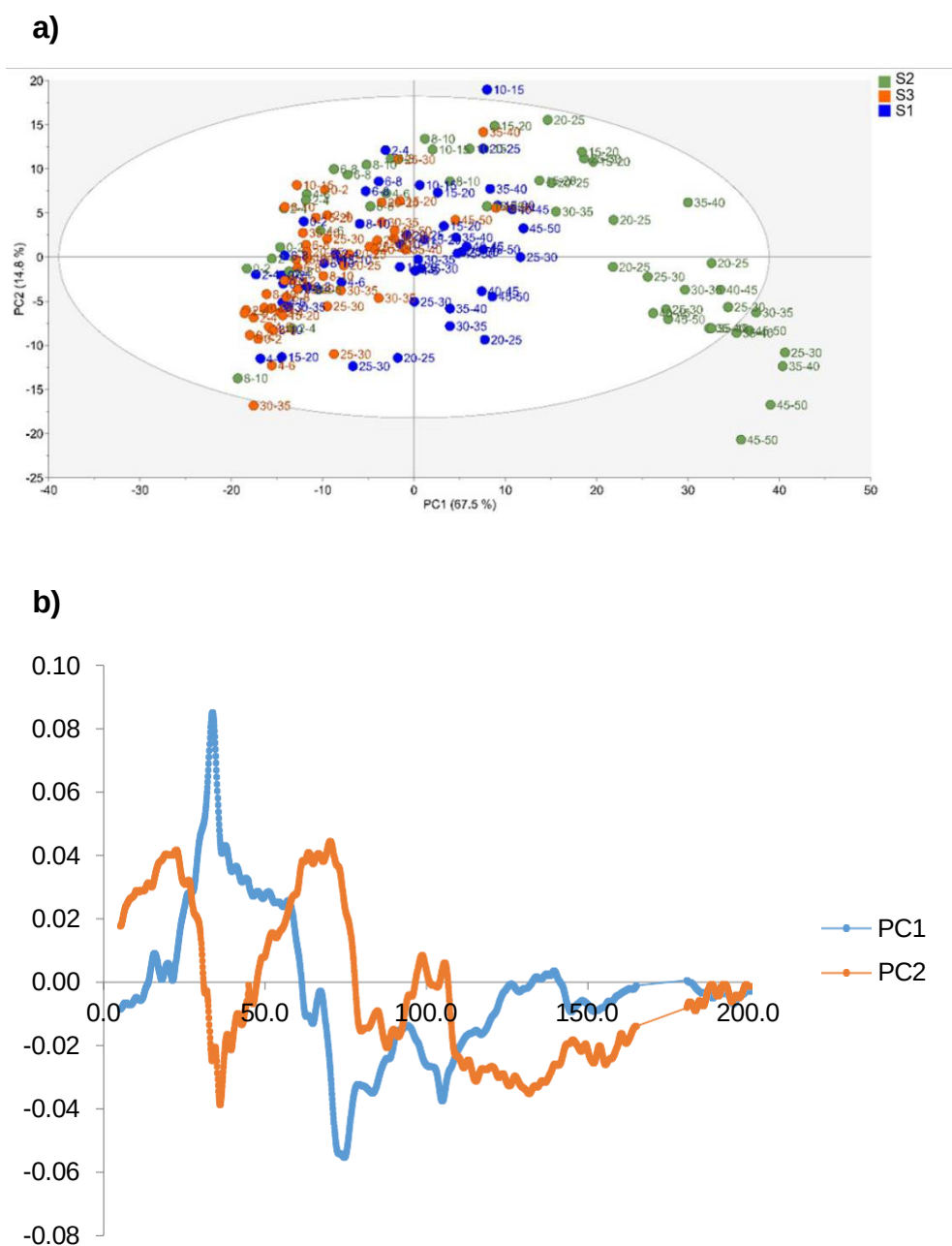


Figure 6.12 a) PCA score plot for the solid state ^{13}C CPMAS NMR data from S1, S2 and S3 sites and b) PC1 and PC2 loadings.

The PCA score plot showed a similar separation of samples to what was observed in the IR PCA score plot (Figure 6.11), and there was a strong correlation (Pearson's correlation: coefficient = 0.81, pvalue = 2.32×10^{-11}) between IR PC1 and solid state NMR PC1. Solid state NMR PC1 comprised 67.5% of the variation in the data. Positive PC1 scores were correlated with a chemical shift at ~ 34 ppm (alkyl chains) while negative scores were correlated with a shift at ~ 70 ppm (carbohydrates or aliphatic alcohols). The samples from S1 site had significantly higher PC1 scores than S3 samples. Thus, chemical shifts associated with alkyl chains were higher in S1 samples compared to S3, and highest in S2. While chemical shifts associated with carbohydrates and aliphatic alcohols were higher in S3 samples compared to S1 and S2. There was no significant correlation observed between PC1 and depth (Spearman's correlation: 0.077, pvalue = 0.39). However, the deeper (20 – 50 cm) S2 samples clearly had the highest PC1 scores and thus were positively correlated with carbohydrates and aliphatics and negatively correlated with carbohydrates.

6.3.4.2.3 Statistical analysis of LDI-FT-ICR MS dataset

The projection PCA model from the LDI MS data is shown in Figures 6.13a, b and c.

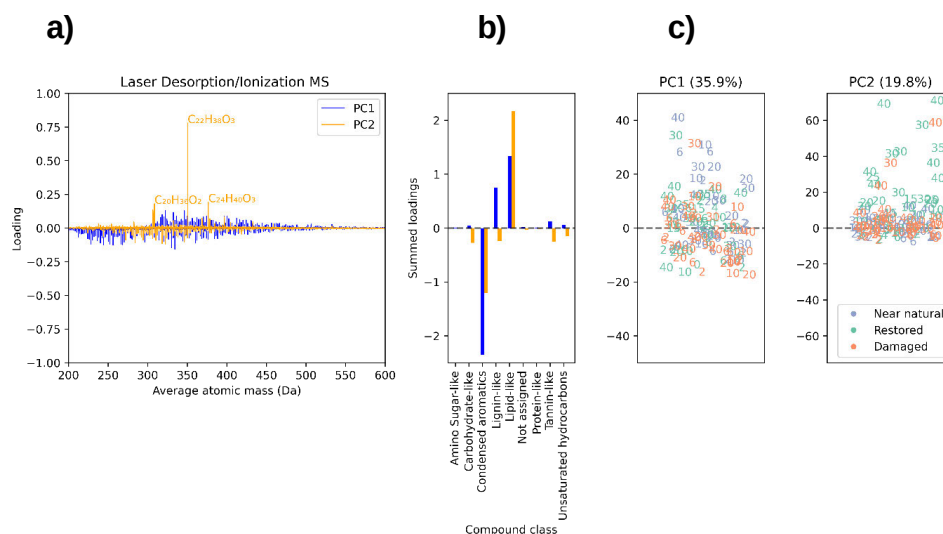


Figure 6.13 Loading plots for the LDI MS PCA model produced by Ezra Kitson in Pykrev. a) The far left plot shows the relative contribution of masses in the spectra to PC1 (blue line) and PC2 (orange line), masses with notably large values are annotated by their corresponding molecular formula. b) The centre left plot shows the relative contribution of compound class to PC1 (blue bar) and PC2 (orange bar). c) The right plots show the separation of samples in the first and second principal component. Samples have randomly jittered x-axis values to avoid overlap, the number represents the depth of the sample was taken at and the colour the restoration status of the sample site.

Figure 6.13b and c showed a slight separation of S1 and S3 samples on PC1 (PC1 comprised 35.9% of the total variation). On PC1, S1 (mean = 3.58, SD = 12.8) had a significantly higher mean than S3 samples (mean = -3.58, SD = 9.60). S2 samples (mean = -0.23, SD = 10.29) were not significantly different from S1 or S3. PC2 (PC2 comprised 19.8% of the total variation) showed a distinct separation of the deeper (30 – 50 cm) S2 samples from S1 and

S3. From Figure 6.13a positive scores on PC1 were associated with a wide range of formula, largely classified as lignin-like and lipid-like compounds. Formulae associated with negative scores were mainly classified as condensed aromatics. Positive scores on PC2 were predominantly caused by the presence of a single molecular formula, $C_{22}H_{38}O_3$, as well as $C_{24}H_{40}O_3$ and $C_{20}H_{36}O_2$, all of which are classified as lipids. Thus, S1 samples were distinguished from S3 samples due to higher lipid like and lignin like compounds and lower condensed aromatics. PC1 showed a marginally significant correlation with depth (Spearman's correlation: coefficient = 0.16, pvalue = 0.058). Thus, deeper samples were also associated with higher lipid and lignin content and lower condensed aromatics. Figure 6.14 shows the Upset plot for all the cores analysed by LDI MS from all 3 sites.

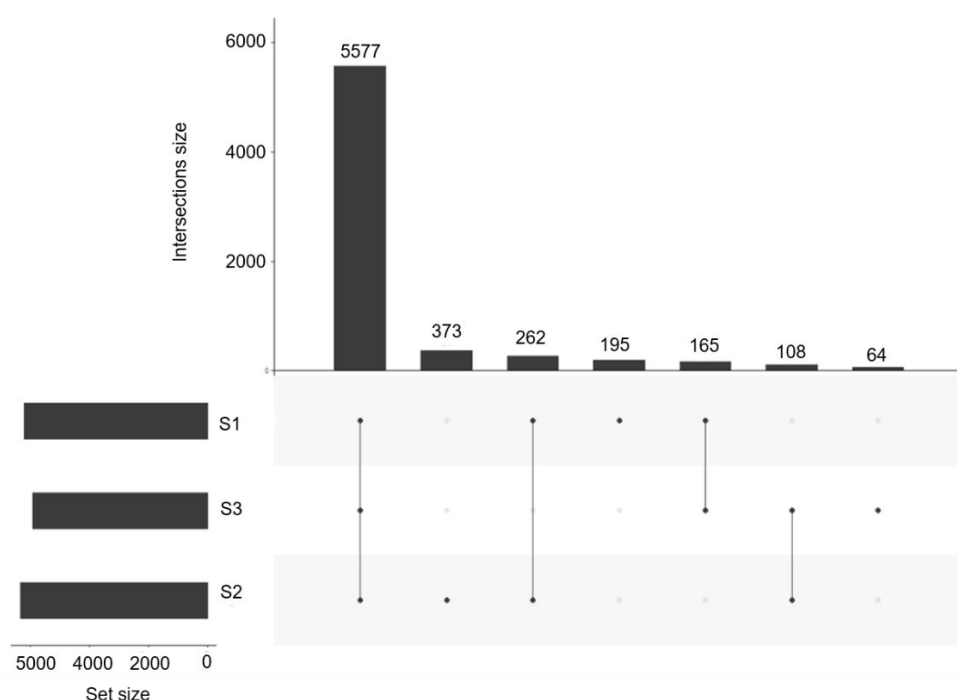


Figure 6.14 Upset plot of CHO and CHON molecular formulae assigned from the LDI MS data of S1, S2 and S3 cores.

From the upset plot, 5577 molecular formulae are shared between all 3 sites, indicating that the difference in compounds ionisable by LDI is not significant amongst the three sites. A total of 373 unique molecular formulae can be found only in S2 cores, 195 in S1 cores and 64 in S3 cores. The higher amount of unique molecular formulae in all S2 cores could be explained by the greater number of new compounds generated as a result of the restoration activity, as shown in the Upset plot of each core (5 cores) at 6 depths for S2 (Appendix C, Figure C4).

To explore the distribution of different class compounds in different sites a bar chart was produced (Figure 6.15).

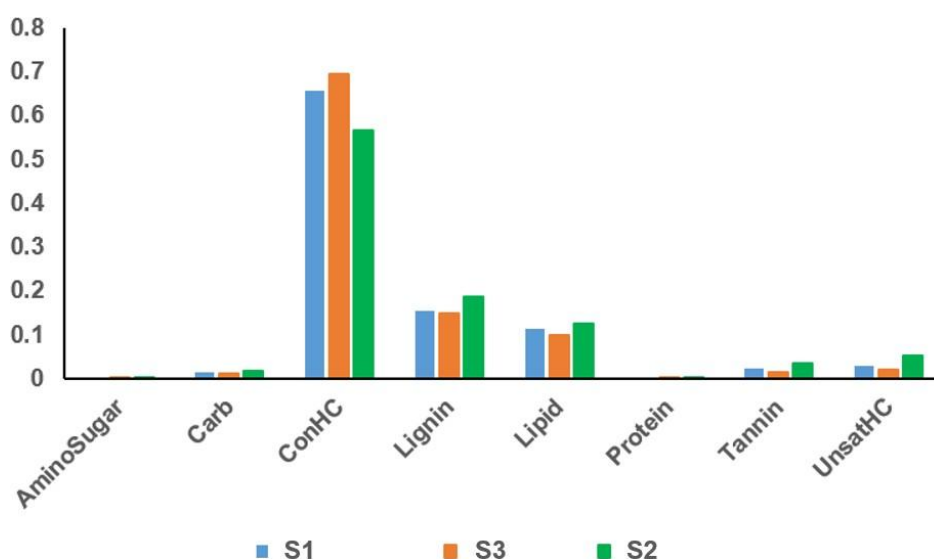


Figure 6.15 LDI MS class compound bar chart of S1, S2 and S3 sites based on assigned CHO and CHON formulae. Note each compound class should have the term 'like' as these categories are only an approximation based on elemental ratios.

Overall, the S3 site shows a higher number of condensed hydrocarbons, while the S2 site is characterized by higher number of

lignin, lipid, tannin and unsaturated hydrocarbon-like compounds. While there is no literature for LDI of blanket bog peat samples the results obtained can be compared to the Red Moss LDI data. Although it has to be noted again that these are two different kinds of peat bogs, the restored site of Red Moss showed a slightly higher amount of lignin and lipid compounds in agreement with the restored site here. Together with the above plots, a plot showing the modified aromaticity index were also produced (Figure 6.16).

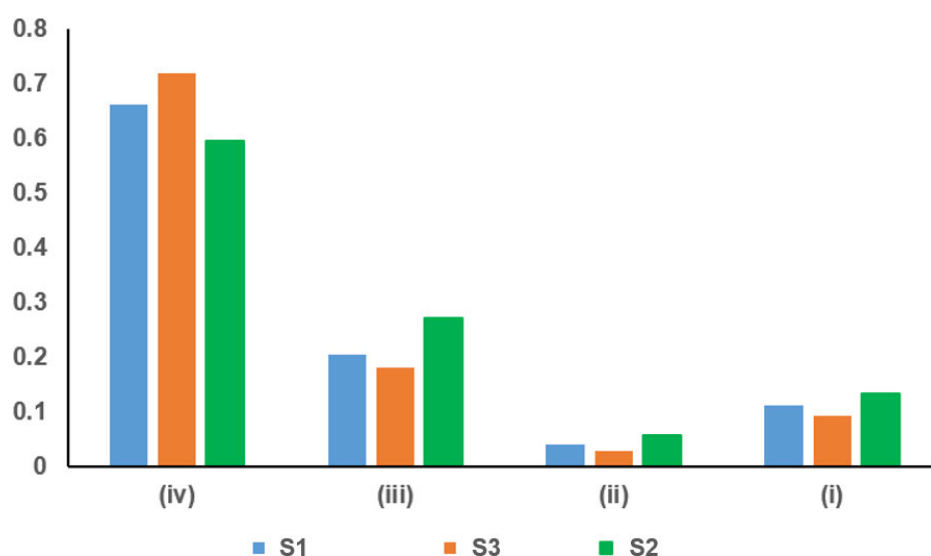


Figure 6.16 LDI MS AI_{mod} bar chart (created using assigned CHO and CHON formulae) of S1, S2 and S3 sites showing the polycyclic aromatic (iv), plant-derived polyphenols (iii), highly unsaturated and phenolic (ii) and aliphatic (i) fractions.

From the AI_{mod} plot it is clear that the S2 site contains a higher amount of plant-derived polyphenols, highly unsaturated and phenolic and aliphatic compounds than S1 and S3 sites. These results match the findings underlined by the FTIR and NMR analyses. It is worth noting

that the S3 site shows a higher amount of polycyclic aromatic compounds, potentially due to chemically less mature humic acids produced after draining activities. (13) (88) (127) While the observed differences between the sites is clear from examining the whole dataset a compound class bar chart of the unique compounds magnifies these differences (Figure 6.17).

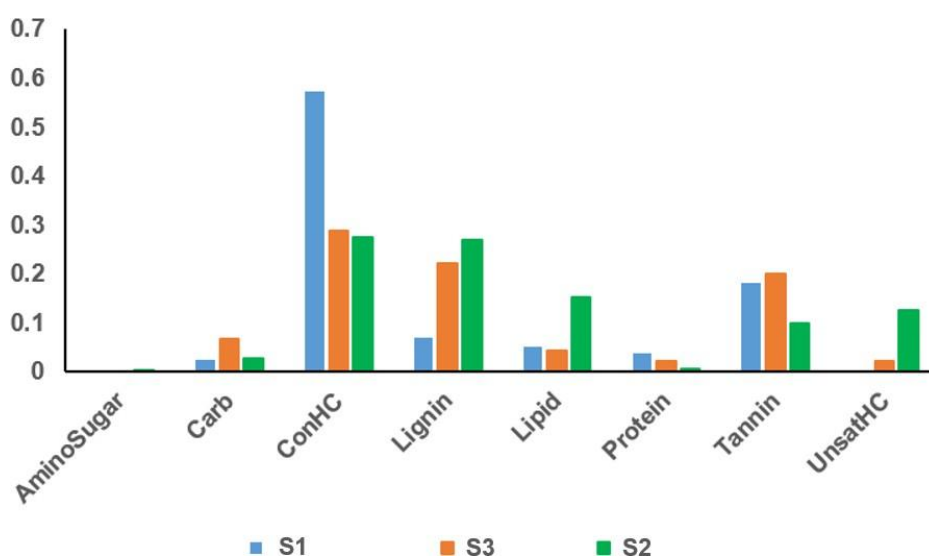


Figure 6.17 LDI MS unique class compound bar chart (created using assigned CHO and CHON molecular formulae) of S1, S2 and S3 sites. Note each compound class should have the term 'like' as these categories are only an approximation based on elemental ratios.

It is interesting to see that S1 site shows a significantly higher amount of unique condensed hydrocarbons, S3 has a slightly higher number of unique tannins and carbohydrates, and S2 site has a greater number of unique lignin, lipid and unsaturated hydrocarbons. While these results are interesting, they are from a handful of molecular formula which could be associated with only certain samples.

6.3.3 Summary of solid state analysis

Overall, the ATR-FTIR, ^{13}C solid state NMR and LDI MS results clearly show a higher number of aliphatic compounds in S2 site, also showing an increasing trend with depth, while carbohydrates exhibited decreasing trend in both S1 and S3 sites with depth. Hence, it is possible to state that modification of surface layers due to restoration activity apparently led to the production of more plant derived polyphenols, aliphatic and aromatic compounds with a consequent increase in the humification rate. (19) (28) (133)

6.4 Liquid state molecular level examination of near natural, drained and rewetted site cores from Flow Country

6.4.1 Liquid state ^1H NMR spectral observations of hot water extracted (HW-E) peat

In contrast to Chapter 5, in this section HW-E ^1H NMR analyses will be discussed too in order to add more relevant information as we were not able to obtain enough PW-DOM samples from the Flow Country cores.

The spectra obtained from the 3 different sites are shown in Figure 6.18a, b and c.

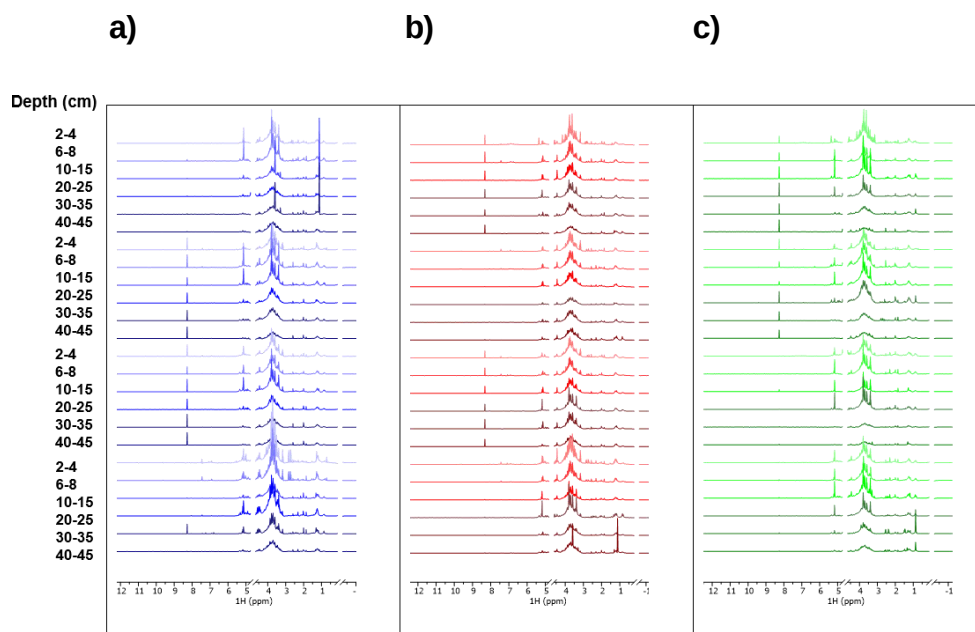


Figure 6.18 Liquid state ^1H NMR spectra of HW-E from 4 cores (a, b, e, f from S1, A, D, E, G from S2 and L, M, N, O from S3) in S1 a), S2 b) and S3 c) at 6 different depths (2-4, 6-8, 10-15, 20-25, 30-35, 40-45 cm).

The spectra show similar regions discussed in the Chapter 4, with the largest intensity of resonances assigned to the O-alkyl (3-5.5 ppm), followed by the alkyl region (0-3 ppm). Then, the aromatic region (6-8 ppm) has a much lower signal intensity again due to the lack of aromatic protons relative to the other compound classes. Finally, the alkyl region can be split into two regions, assigned to terminal CH_3 groups away from any electronegative group (0-2 ppm) and CH_2 or CH_3 groups attached to a functional group, such as carbonyls (2-3 ppm). The sharp peaks superimposed on the spectra indicate the presence of small molecules which are likely to be metabolites of plant material, root exudates or decay products in the different cores. The HW-E spectra have more similarities with fulvic acid, despite the aromatic region is much less intense than observed in literature. (36)

Hence, in our case HWE was less successful at extracting aromatic compounds compared to base-acid extraction used to obtain fulvic acid. As already mention in the Chapter 4, the HW-E procedure is suitable for the extraction of more labile compounds from peat, which would not be possible by base-acid extraction. (40) The spectra from S1 and S2 cores show more sharp peaks appearing at intermediate depths, around the O-alkyl region, which may arise from small sugars like glucose. However, S2 cores show more evidence in higher alkyl and aromatic intensities, indicating a change in composition of the labile component in these cores especially at bottom layers.

6.4.2 Liquid state ^1H NMR spectral observations of PW-DOM

^1H NMR spectra were obtained from 3 cores from S1 (b, e, f,) and S3 (K, M, O) and 4 cores from S2 (B, D, G, H), taking into account S2D from Chapter 3. As an example, S1b, S1e, S3K, S3M, S2B and S2H spectra at 6 different depths (2-4, 6-8,10-15, 20-25, 30-35 and 40-45 cm) are shown in Appendix C, Figure C5.

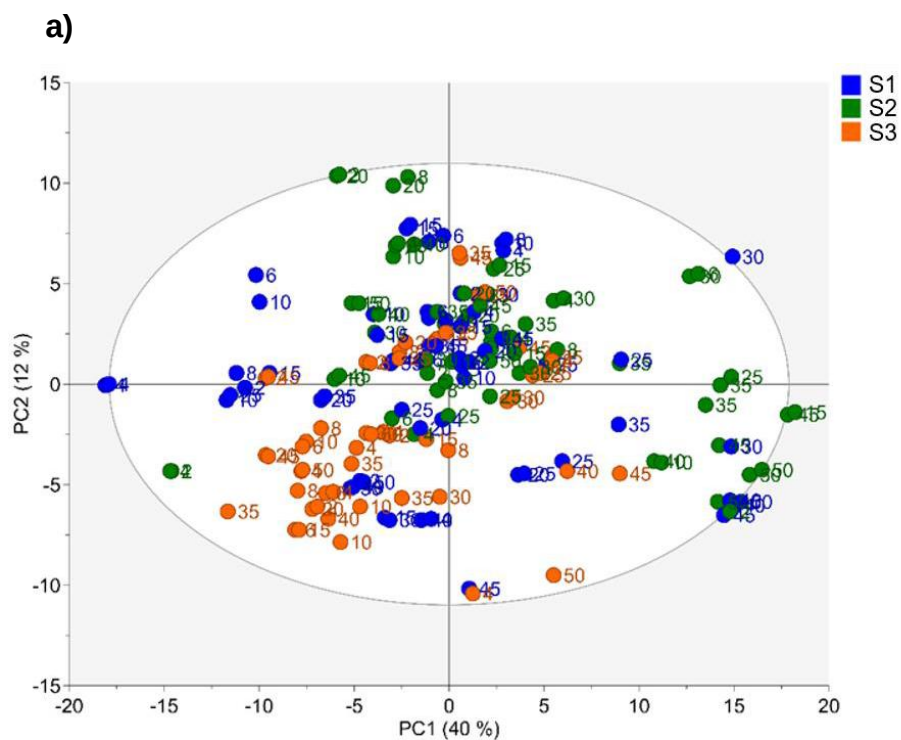
As data acquired for PW-DOM were less than HW-E, it is harder to make more detailed conclusions. However the spectra contained a mixture of sharp resonances in the region from 0.5 to 5.5 ppm. The most intense region was the carbohydrate region which again showed signs of glucose. However, while there were clear differences within individual cores, there was no obvious difference between cores from different sites. S3 cores spectra showed to have more hydrophobic compounds than S1 and S2 cores, potentially due to draining processes which may led to the formation of less chemically mature humic acids. (205) However, deeper statistical analysis following the methods described in Chapter 3 has been carried out to better

understand the composition of PW-DOM, which is going to be discussed in the next sections.

6.4.3 Statistical analysis of ^1H NMR and FT-ICR MS spectra of HW-E

6.4.3.1 PCA of HW-E ^1H NMR data

The PCA score plot with loadings for the HWE NMR data is shown in Figure 6.19a and b.



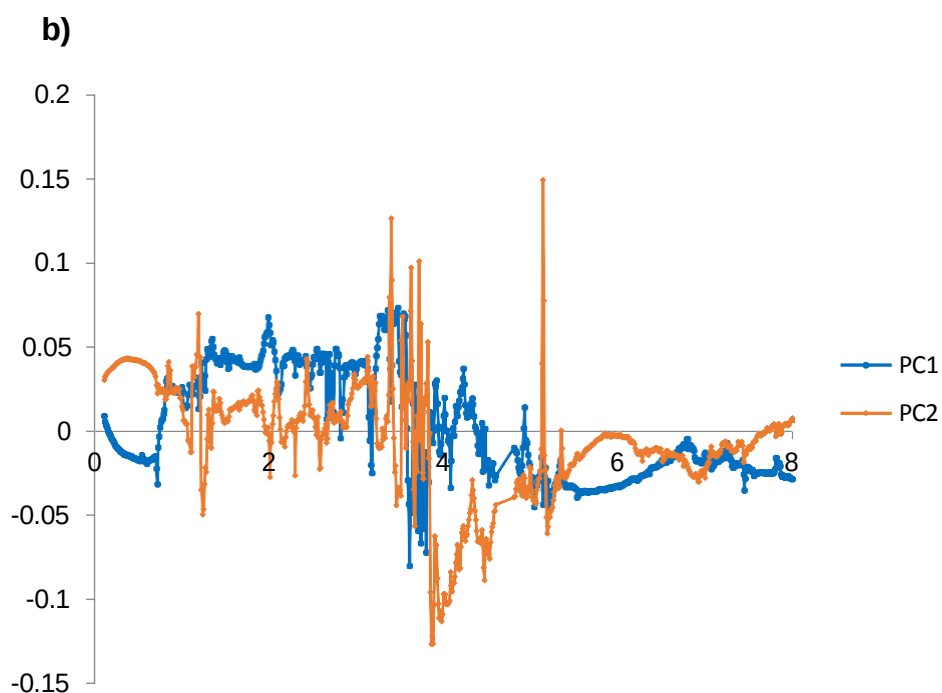


Figure 6.19 a) PCA score plot for the HW-E ^1H NMR data from S1, S2 and S3 sites with b) PC1 and PC2 loadings.

The score plot shows a slight separation based on depth and restoration status was visible on PC2 (15.9% of the variation). Positive scores on PC2 were predominantly driven by a chemical shift at 3.62 ppm and at 3.72 – 3.80 ppm. Negative scores were mainly associated with 3.31 ppm and 3.55 ppm chemical shifts. Further NMR experiments would be required to determine the identity of the compounds associate with these chemical shifts, however they appear in the O-alkyl/carbohydrate regions. S1 (mean = -0.35, SD = 2.76) had on average a lower value than S3 samples (mean = 0.42, SD = 2.55), although this difference was not significant. S2 had the lowest average (mean = -1.53, SD = 2.23), which was

sample was taken at and the colour the restoration status of the sample site.

Figure 6.20 c and d show a separation of samples by depth but not health status on PC1 whereas PC2 (PC2 comprised 15.7% of the variation in the data) showed a slight separation based on health status but not depth. Positive scores on PC2 were associated primarily with lipid-like compounds and to a lesser extent protein-like and tannin like. Positive scores on PC2 were particularly associated with $C_{20}H_{40}O_6$, $C_{18}H_{34}O_2$ and $C_{13}H_{24}O_{13}$. Negative scores on PC2 were associated with condensed aromatics and lignin-like compounds, particularly $C_{16}H_{32}O_2$. S1 (mean=-11.36, SD = 16.22) had a significantly lower mean than S3 samples (mean = 11.36, SD = 18.97) (Welch's two sided T-test: statistic = - 3.75, pvalue = 0.0006). S2 samples (mean=-18.59, SD = 9.19) were significantly lower than S3 samples but not S1 samples (Welch's two sided T-test: statistic = -5.83, pvalue = 4.58e- 06). Thus damaged samples showed higher lipid content and lower condensed aromatics than restored and near natural samples, the opposite of the trend observed with LDI MS. There was a marginally significant negative correlation of PC2 with depth (Spearman's correlation: coefficient = -0.24, pvalue = 0.08). Figure 6.21 shows the Upset plot for all the cores analysed by HW-E MS from all 3 sites.

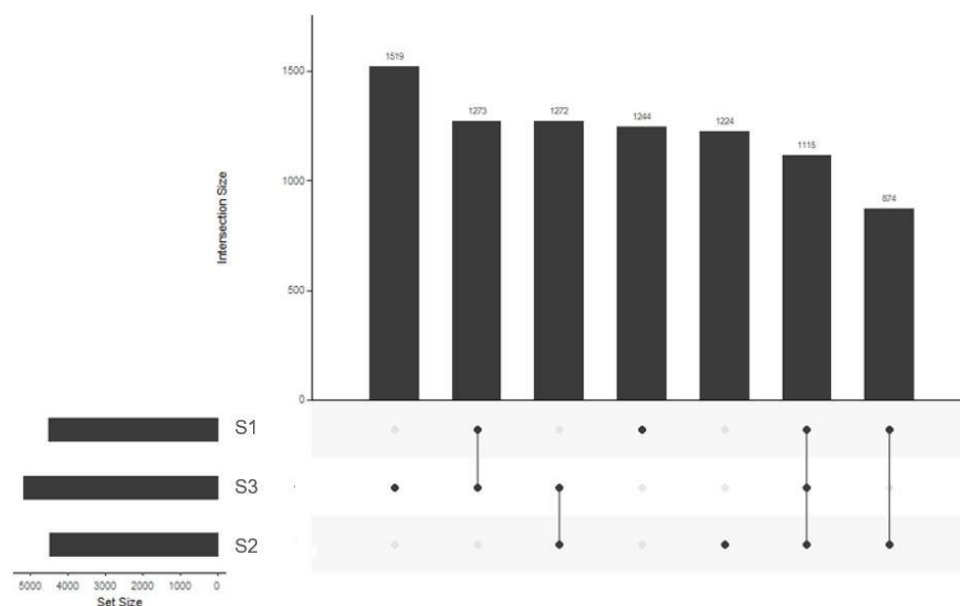


Figure 6.21 Upset plot of CHO and CHON molecular formulae assigned from the HW-E MS data of S1, S2 and S3 cores.

For all three sites a total of 7355 molecular formulae (5516 $C_xH_yO_z$ and 1839 $C_xH_yO_zN_n$) have been assigned. A total of 4506 molecular formulae have been assigned on S1 site, 5179 on S3 site while 4485 in S2 site. On average, $81 \pm 8.0\%$ of m/z fragments (200-702 m/z) from all the HW-E MS spectra were successfully assigned. Overall, 1115 are shared between all 3 sites, a total of 1519 unique molecular formulae can be found only in S3 cores, 1244 in S1 cores and 1224 in S3 cores. Therefore, HW-E analysis showed that this kind of extraction was able to extract more unique labile fractions from S1 site. Compound class and Almod bar charts, produced from the assigned HW-E MS molecular formulae from each site, are shown in Figure 6.22 and 6.23, respectively.

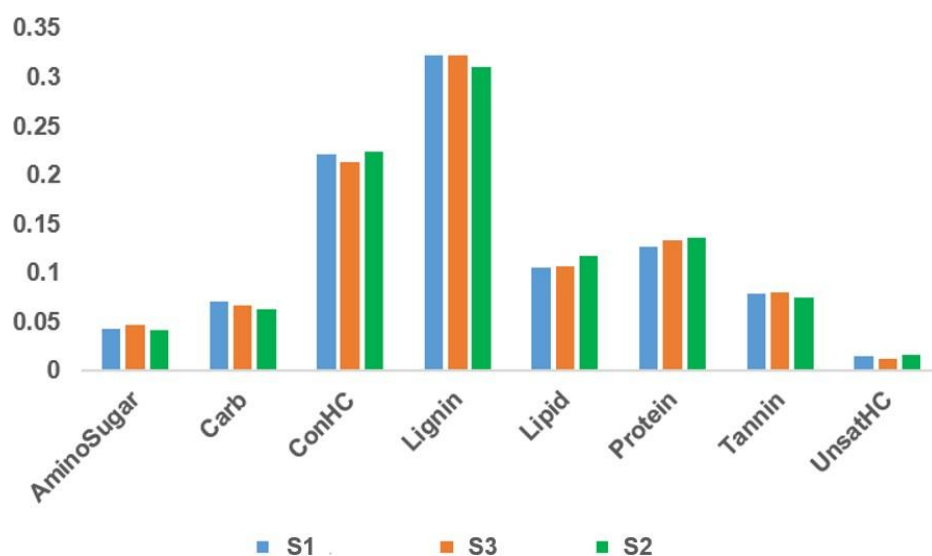


Figure 6.22 HW-E MS class compound (CHO and CHON) bar chart of S1, S2 and S3 sites.

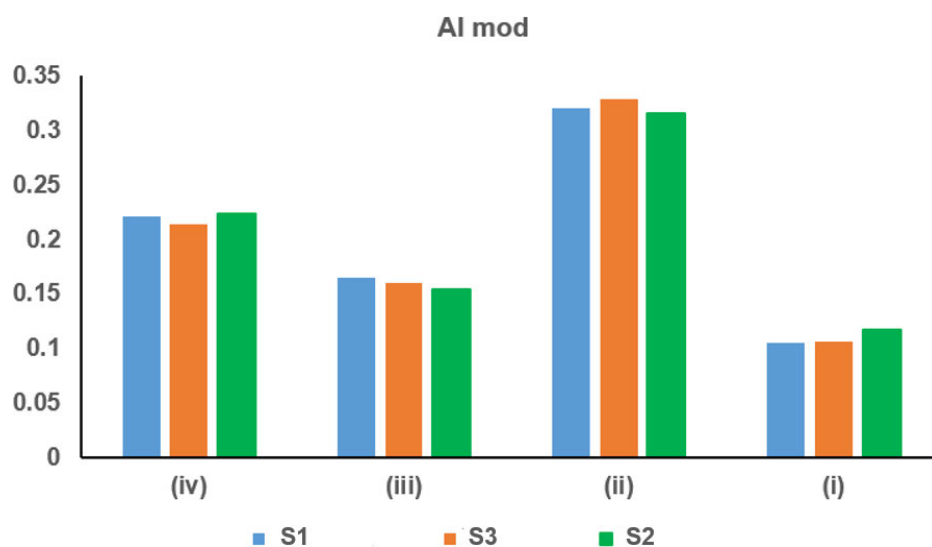


Figure 6.23 HW-E MS AI mod bar chart (CHO and CHON) of S1, S2 and S3 sites showing the polycyclic aromatic (iv), plant-derived polyphenols (iii), highly unsaturated and phenolic (ii) and aliphatic (i) fractions.

From Figure 6.22 there is little evident differences between all 3 sites, even if a slight higher amount of lipids and proteins can be noted in S2 site, while carbohydrates are higher in S1. Condensed hydrocarbons are a bit more pronounced in both S1 and S2 sites, instead lignin in S1 and S3 sites. Apart from a slightly higher amount of aliphatic compounds in core samples from S2, there is neither any clear differences between sites in terms of Al_{mod} . To inspect the large number of unique compounds, compound class bar charts were also produced (Figure 6.24)

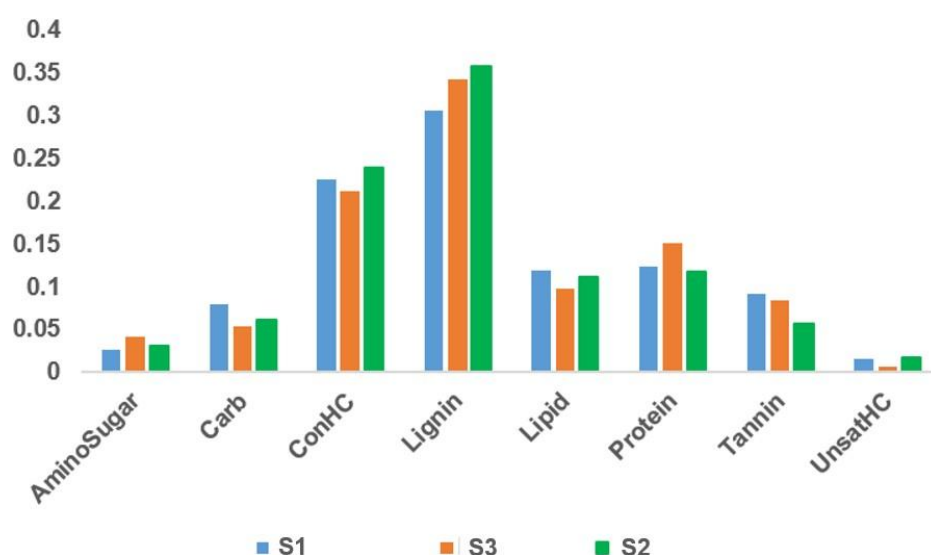


Figure 6.24 HW-E MS unique class compound (CHO and CHON) bar chart of S1, S2 and S3 site.

Unlike for the whole dataset, there are clearly more unique class compound condensed hydrocarbons and lignin in S2 cores, more carbohydrates and tannins in S1 and higher amount of aminosugars and proteins in S3. In terms of lipid compounds, both S1 and S2 sites have a higher number than S3 cores. Unfortunately, there is not enough literature about HW-E to consult, so we cannot make an

extensive discussion of the labile fractions in peat blanket bogs. Overall, by combining of both NMR and MS results, it is possible to state that a higher number of lipid compounds have been detected in both cores from S1 and S2 sites, moreover at lower depth, than cores from S3 site, which matches with the findings discussed on FTIR and solid state NMR data. Then, in terms of sugar like compounds, S1 cores show more presence at upper layers, while it has been noted that more condensed hydrocarbons and lignin occurred in S2 cores. Those results can be explained by the different amount of plant derived metabolites, higher in S1, while polycyclic aromatic and aliphatic are more concentrated in S2 site, probably as a result of the restoration activities.

6.4.3.2 Statistical analysis of ^1H NMR and FT-ICR MS spectra of PW-DOM

6.4.3.2.1 PCA of ^1H NMR PW-DOM data

The projection PCA model for PW-DOM NMR did not show a clear separation of sites by depth or health status (Figure 6.25a and b).

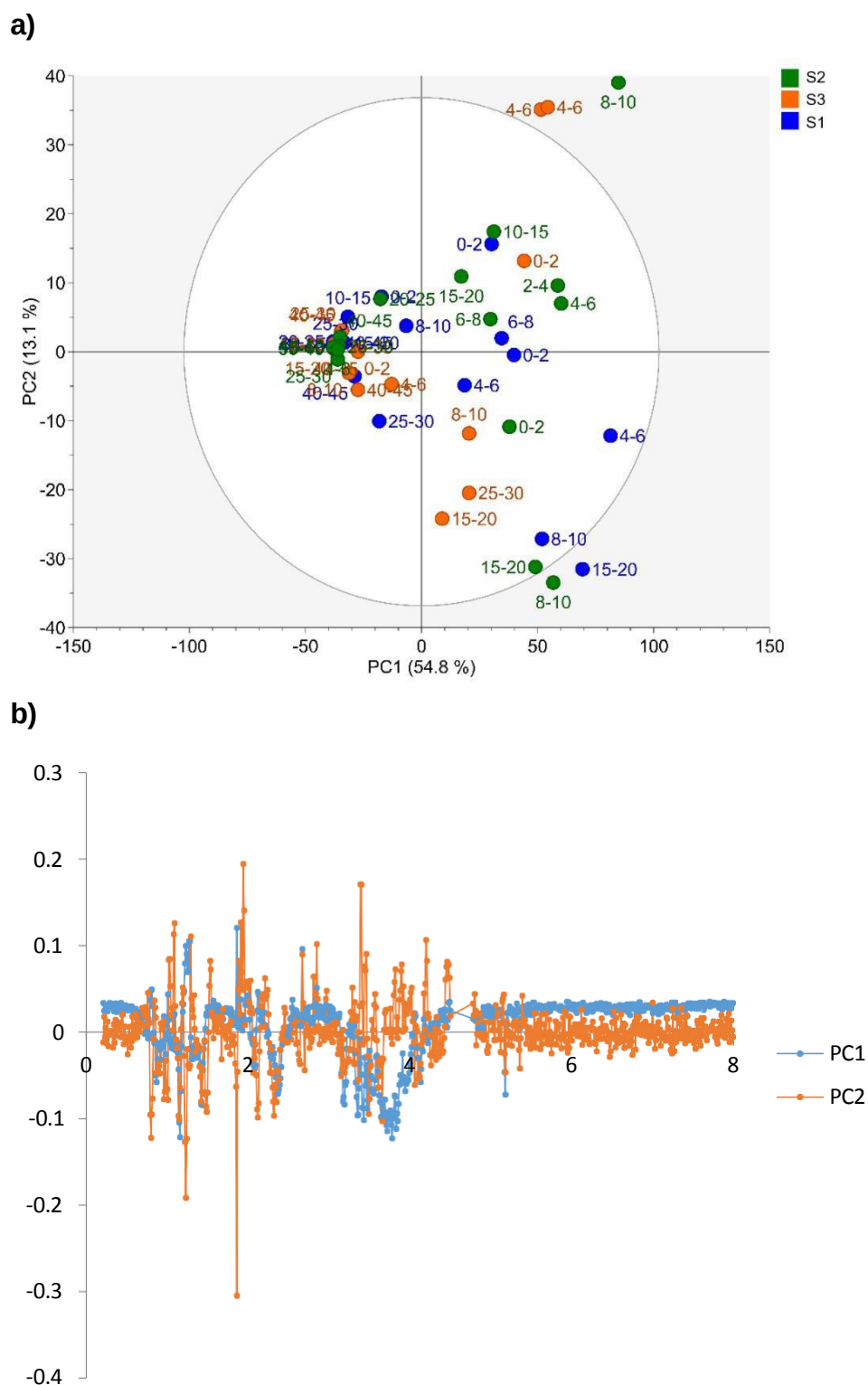


Figure 6.25 (a) PCA plot for the PW-DOM NMR from S1, S2 and S3 sites with loadings (b).

PC1 comprised 44.2% of the variation in the data. Positive scores on PC1 were correlated strongly with a peak at 1.89 ppm and to a lesser extent peaks at 1.23 and 2.68 ppm. Negative scores were correlated with a broad set of peaks in the region 3.10 – 3.84 ppm with the strongest correlation at 3.82 ppm. There was not a significant difference in the scores between near natural (mean = -0.27, SD = 13.72) and damaged samples (mean = 0.27, SD = 13.30) or restored samples (mean = -3.99, SD = 10.91) and there was not a significant correlation with PC1 and depth (Spearman's correlation: coefficient = 0.09, pvalue = 0.48).

6.4.3.2.2 Statistical analysis of PW-DOM FT-ICR MS data

The projection PCA model for PW-DOM MS did not show a clear separation of samples by depth of health status (Figure 6.26a, b and c).

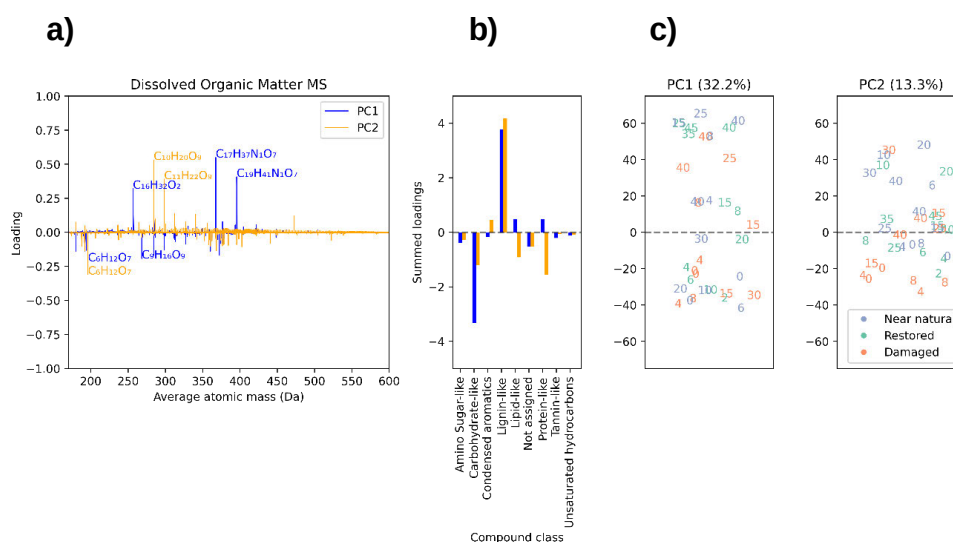


Figure 6.26 Loading plots for the PW-DOM MS PCA model produced by Ezra Kitson.

(a) The far left plot shows the relative contribution of masses in the spectra to PC1 (blue line) and PC2 (orange line), masses with notably large values are annotated by their corresponding molecular formula. (b) The centre left plot shows the relative contribution of compound class to PC1 (blue bar) and PC2 (orange bar). (c) The right plots show the separation of samples in the first and second principal component. Samples have randomly jittered x-axis values to avoid overlap, the number represents the depth of the sample was taken at and the colour the restoration status of the sample site.

PC2, which comprised 13.3% of the variation, showed a slight separation of samples based on health status. Positive scores on PC2 were associated with lignin-like compounds, and the formula $C_{10}H_{20}O_9$ and $C_{11}H_{22}O_9$. Negative PC2 scores were associated primarily with protein like compounds and to a lesser extent with carbohydrate-like and lipid-like compounds. The formula $C_6H_{12}O_7$ was associated with negative PC2 scores. S1 samples (mean = 11.15, SD = 20.43) had significantly higher PC2 scores than S3 samples (mean = -11.15, SD = 21.76). S2 samples (mean = 0.63, SD = 17.79) did not differ significantly from either near natural or damaged. Thus, S1 samples were associated with lignin-like compounds and S3 samples were protein, carbohydrate and lipid like compounds. There was not a significant correlation between PC2 and depth (Spearman's correlation: coefficient = -0.088, pvalue = 0.61). Figure 6.27 shows the Upset plot for all the cores analysed by PW-DOM MS from all 3 sites.

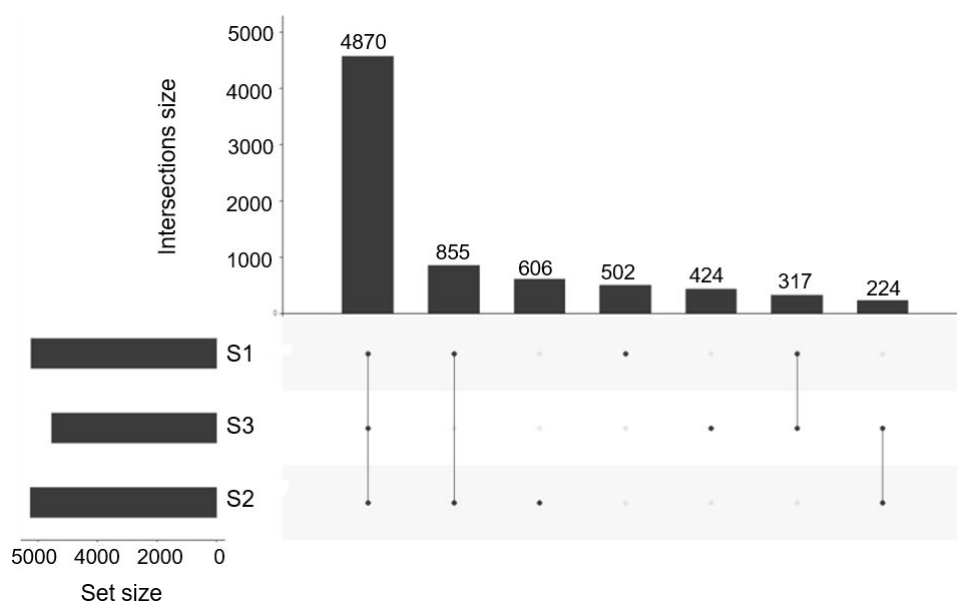


Figure 6.27 Upset plot of molecular formulae (CHO and CHON) assigned from the PW-DOM MS data of S1, S2 and S3 cores.

In general, on 3 cores from S1 (b, e, f.), S3 (K, M, O) and 4 cores from S2 (B, D, G, H), a total of 7775 molecular formulae (5824 $C_xH_yO_z$ and 1951 $C_xH_yO_zN_n$) from DOM MS data have been assigned in all 3 sites, 4811 are shared between all 3 sites. A total of 6866 molecular formulae have been assigned on S1 site, 5577 on S3 site while 6968 in S2 site. On average, $65.5 \pm 4.8\%$ of m/z fragments (200-702 m/z) from all the PW-DOM MS spectra were successfully assigned. Then, a total of 431 unique molecular formulae can be found only in S1 cores, 330 in S2 cores and 189 in S3 cores. Hence, S3 cores showed to have more hydrophobic compounds than S1 and S2 cores, potentially due to draining processes which may lead to the formation of less chemically mature humic acids. (205) The higher number of shared formulae shared between S1 and S2 sites suggest that there are more similar compounds in the cores from those sites, leading to the hypothesis that restoration had effects on PW-DOM able to re-establish a similar

environment than near natural cores. A bar chart showing the compound class distribution of PW-DOM MS data is shown in Figure 6.28.

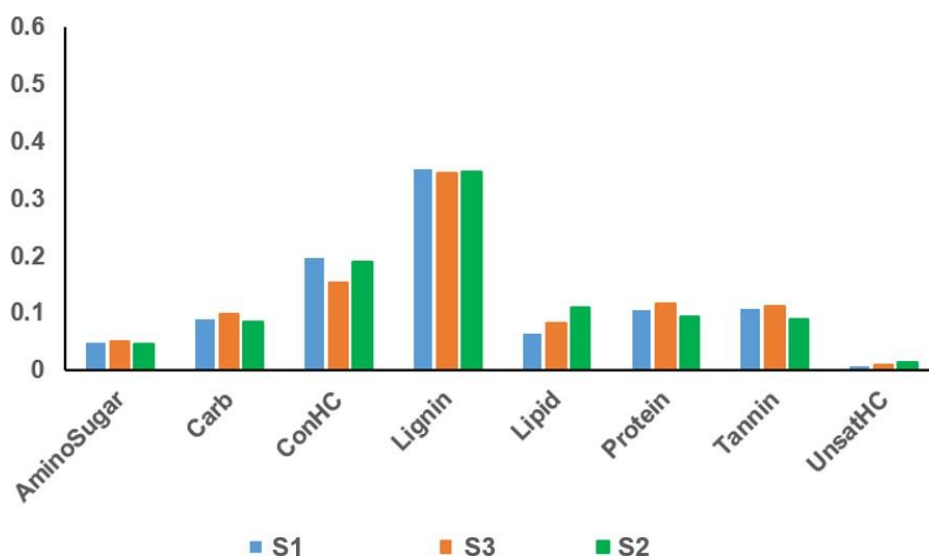


Figure 6.28 PW-DOM MS class compound (based on assigned CHO and CHON molecular formulae) bar chart of S1, S2 and S3 sites.

Both S1 and S2 sites have higher amount of condensed hydrocarbons, than the S3 site, which have higher number of carbohydrates, proteins and tannins. S2 cores are characterized by higher number of lipid and unsaturated hydrocarbon compounds.

A Al_{mod} histogram, shown in Figure 6.29, shows a clear increase of plant-derived polyphenols and highly unsaturated and phenolic in S3 site. Instead a higher number of polycyclic aromatic compounds can be noted for both S1 and S2 sites, while the aliphatic compounds are slightly higher in samples from the S2 site.

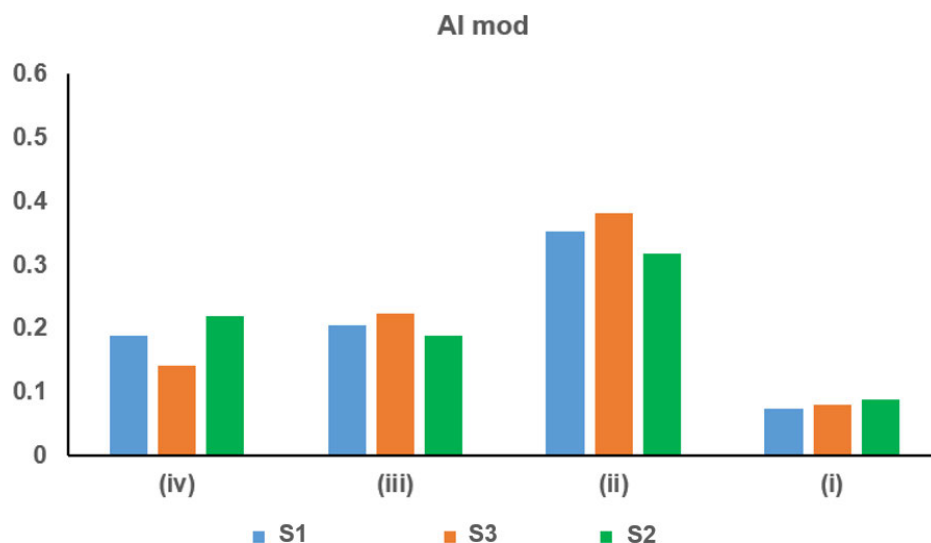


Figure 6.29 PW-DOM MS Al_{mod} (created using assigned CHO and CHON molecular formulae) bar chart of S1, S2 and S3 sites showing the polycyclic aromatic (iv), plant-derived polyphenols (iii), highly unsaturated and phenolic (ii) and aliphatic (i) fractions.

Finally, a unique class compound bar chart of the PW-DOM MS data (Figure 6.30) show a more clear increase of condensed hydrocarbons in S1 cores, also more carbohydrates, aminosugars, tannins, lignin and proteins in both S1 and S3 cores. In terms of lipid and unsaturated hydrocarbon compounds, S2 cores contain a higher number than the other 2 sites.

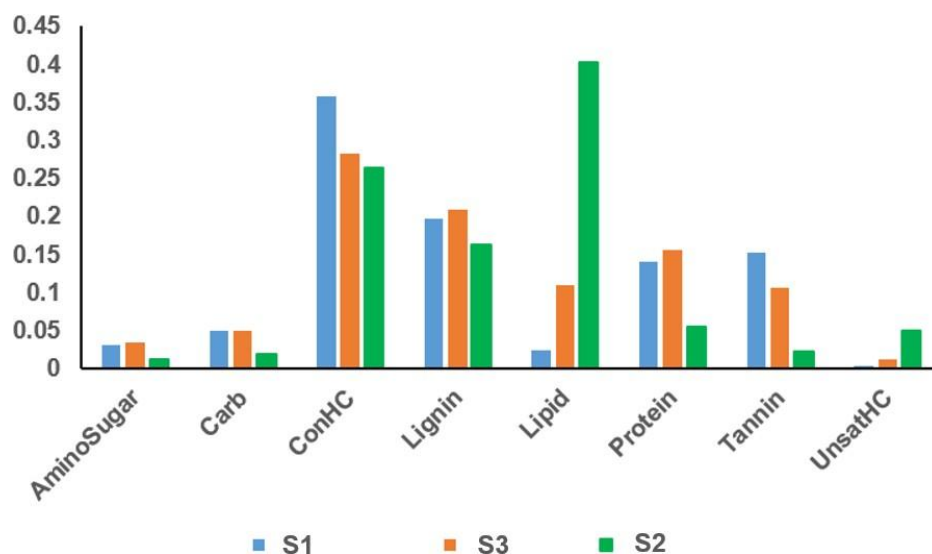


Figure 6.30 PW-DOM MS unique class compound (created using assigned CHO and CHON molecular formulae) bar chart of S1, S2 and S3 sites.

6.5 Summary of the molecular examination of drained, rewetted and near natural blanket bog samples from the Flow Country.

In terms of the solid state phase, the ATR-FTIR, ^{13}C solid state NMR and LDI MS results underlined a higher population of aliphatic compounds in S2 site, particularly characterised by an increasing trend with depth, while carbohydrates showed a decreasing trend in both S1 and S3 sites with depth. It is proposed that a modification of surface layers due to restoration activity could lead to the production of higher amount of plant derived polyphenols, aliphatic and aromatic compounds, affecting the humification rate. (19) (28) (133) It has to be noted that this increasing trend of lipid content in the solid state phases in S2 site is very comparable to one in RM2, despite they are two different kind of peatlands.

Considering the PW-DOM data obtained in comparison with that obtained from Red Moss, both S2 cores from the Flow Country and

RM2 sites show similar trend in terms of condensed hydrocarbons with depth in PW-DOM, precisely an increase throughout the cores. Then, an obvious difference in lipid content can be noted, as those are higher in RM1, the Red Moss (raised bog) damaged site, while it has been shown a clear increasing pattern with depth in S2 cores, which have been collected from the Flow Country (blanket bog) restored site. Sugars plant derived are higher in S3 cores, while it was described a higher amount of those in RM2. In terms of the HW-E results, both NMR and MS results showed a higher number of lipid compounds on cores from S1 and S2 sites, especially at lower depth. These results have been confirmed by FTIR and solid state NMR data. The number of sugar like compounds were higher in S1 cores at upper layers, while more condensed hydrocarbons and lignin were more present in S2 cores. Probably the amount of plant derived metabolites were higher in S1 site, while after the restoration activities the polycyclic aromatic and aliphatic started to accumulate more in S2 site. The difference between those two different sites might be attributed to their different nature (Red Moss is a raised bog, while Flow Country a blanket bog) in which some variables such as pH, vegetation, microbial activity, water content and/or water level play a role in the differentiation of PW- DOM at molecular level. (207) (208) The findings about DOM discussed here are in disagreement with Tfaily study (41) in which DOM from peat bogs was described as uniform in molecular composition and more resistant to microbial decomposition throughout the core. Again, it is important to take under consideration the different nature and environmental conditions of the peatlands under investigation, especially when describing those at such detailed molecular level. Herzsprung *et al* (49) stated that the soil water from the rewetted peatland sites has a higher rate of humification compared to near natural peatlands and that the rewetting technique is coupled with high concentrations of dissolved organic carbon in soil water and its quality is high in aromatic

content, hence we discovered that our findings showed to be similar to the ones described by Herzprung in terms of aromatic content. Finally, the notable higher level of lipids found in S2 site, especially at bottom layers, can be explained by multiple variables. Moreover, lipids could be the result of lignin degradation from plants, which has been boosted by the influence of climate and waterlogged conditions, as a consequence of the rewetting process, together with high rate of precipitation, which may had specific effects in microbial activities and in long period increased the accumulation of lipids at the bottom layers of peat. (206) (207) (208)

7. Conclusion

This is the first research project to provide physicochemical and molecular-level characterisation of both the liquid and solid phases of peat cores from drained, rewetted and near natural peatland sites. The aim of the methodology and findings presented here is to lead future investigations of the molecular composition of peatlands under different conditions (near natural, damaged and restored).

To compare and contrast techniques this study included the first detailed characterisation of 13 layers of one 50 cm peat core using bulk density, CHN content, ash content, FTIR, liquid and solid state NMR as well as liquid and solid state FT ICR-MS. Using NMR and FT ICR-MS it was possible to identify variation along the core, especially for both the solid phase and PW-DOM. Differently, HW-E and NaOH-E did not provide any clear-cut observation with depth and likely can be excluded from depth related molecular studies. Importantly, both solid-state NMR and liquid state NMR variations with depth reflected the bulk density and water table level.

Molecular differences were observed between drained, restored and near natural peatland sites within the Red Moss (raised bog) and Flow Country (blanket bog).

In terms of the cores taken from drained and drain-blocked cores from Red Moss of Balerno, both ATR-FTIR and solid state ^{13}C NMR data showed that there are some differences between each cores from the same site with depth. In addition, an increase in alkyl compounds in the restored area at bottom layers has been detected, while the damaged site was more pronounced with O-alkyl compounds until 15 cm depth. These results matched with the bulk properties variation with depth on each core from the two different sites, which then reflect the water table changes with depth as well.

The ATR-FTIR, solid state ^{13}C NMR and LDI MS results on cores taken from the near natural, drained and drain-blocked sites in the Flow Country were underlined by a higher content of aliphatic in the restored area, showing an increasing trend with depth, while carbohydrate content were characterized by a decreasing trend in both near natural and drained sites throughout the cores. Also in this case, those results followed the bulk properties findings, especially in terms of water table variation, which were useful to understand step changes through the core.

In terms of PW-DOM molecular composition, this was higher in lipid content in RM1 (damaged area) and S2 (restored area), while plant derived sugars were higher in RM2 (restored area) and S3 (damaged area). Therefore, in this study we suggest that some specific sugar like and lipid compounds can be used as molecular markers to understand the peatlands status, since they were the class compounds changing in the peat samples depending on their health conditions. The source of the differences between bog types can be attributed to their different nature, where many variables such as geomorphology, vegetation, microbial activity and hydrology play a role in the differentiation of those two peatlands. To ascertain whether the molecular differences observed between the two peatlands reflect peatland type require future molecular studies on a number of different blanket and raised bogs.

In this study, multiple data sets were examined from different sources largely in isolation (bulk properties, solid and liquid state spectroscopy and spectrometry) using PCA. While an important first step, it was clear that multiple factors were playing a role in some data sets. Specifically the influence of depth vs health status. Suggested future work is to bring the data together using more sophisticated statistical methods. For instance Bayesian models based on probability, which

enhance the representation of all the uncertainty within the model and that it can help to produce predictive models as well. In order to predict the health status of peat, as well as understand the contribution of chemical parameters to the health classification of a peatland, Bayesian hierarchical logistic regression model use a linear model paired to a 'link' function to produce a Bernoulli distributed response (i.e. a probability between 0 and 1). In the case of a logistic regression model trained on chemical data, the response can be interpreted as the probability of a peatland being in near natural, drained or restored conditions. Hence, Bayesian models allow the incorporation of prior beliefs which can reflect a pre-existing understanding of the system, they are less susceptible to falsely significant findings and they can provide a simple way to account for group-level correlations within the population through hierarchical modelling. This could provide a way to account for the depth-dependent effects on chemical parameters on peatland health.

Our understanding about interventions to be carried out on damaged peatlands needs to improve, especially if considering future increasing global temperatures. Many questions about how specific molecules weigh on peatlands status and what synergies exist between these molecules and their sources (e.g. microbial communities, specific vegetation types) still remain. The answer to these questions can only be found after further examinations of peat molecular composition and this study has shown that a combination of molecular methods is required. However the detailed molecular characterization needs to be combined with characterization of both the metabolites from the main vegetation types and microbial community structure. Only by bringing together all factors that play a role in peatland carbon cycling will one be able to uncover the relationships that exist and identify the most

important factors to monitor to keep peatlands healthy and resilient against future changes.

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Appendices

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Appendix A

Table A1. Measured nitrogen, carbon and hydrogen content (w/w %) of the 50 cm restored core. Oxygen contents are calculated by subtracting the total of the other three from 100%.

Depth (cm)	N (%)	C (%)	H (%)	O (%)
0-2	0.99	50.73	6.16	42.12
2-4	1.37	49.38	5.93	43.32
4-6	2.12	49.81	5.87	42.20
6-8	1.96	51.26	6.09	40.69
8-10	2.12	52.50	6.03	39.35
10-15	2.55	51.92	6.34	39.19
15-20	2.79	52.22	6.37	38.62
20-25	2.50	54.43	6.75	36.32
25-30	2.28	59.02	6.82	31.88
30-35	2.25	57.49	6.89	33.37
35-40	2.31	53.99	6.84	36.86
40-45	1.94	63.10	7.28	27.68
45-50	1.51	57.11	6.27	35.11

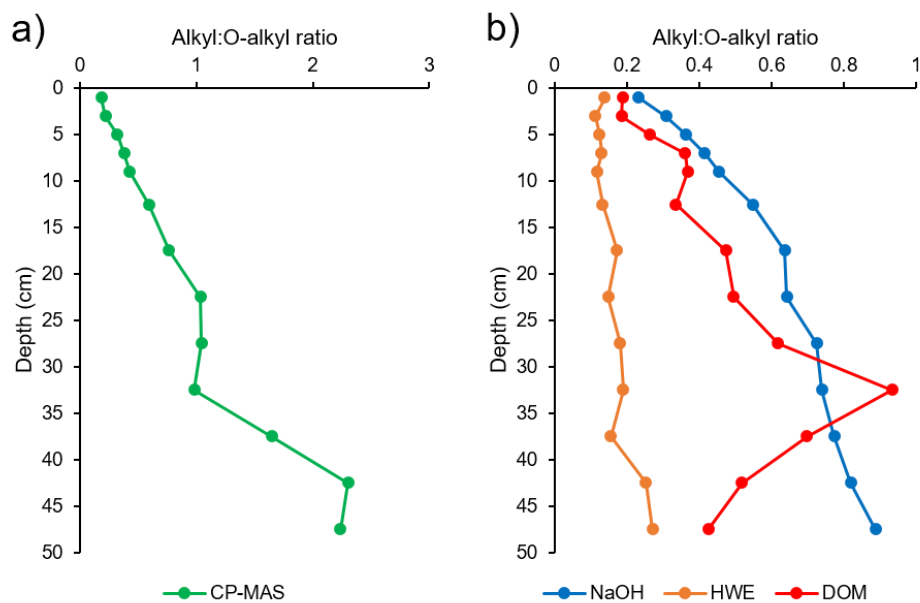


Figure A1. Alkyl:O-alkyl ratios of (a) CP-MAS spectra, and (b) liquid-state DOM, HWE and NaOH NMR spectra plotted against sample depth.

Table A2. Total number formulae assigned on each MS analysis.

MS analysis	Total number of formulae assigned	Total number of CHO compounds	Total number of CHON compounds	% of CHO compounds	% of CHON compounds
LDI	8394	6186	2784	69	31
DOM	8279	5309	2970	64	36
HWE	8241	5701	2540	69	31
NaOH	3865	3291	574	85	15

Table A3. Molecular formula assignments at 343 m/z of the LDI, DOM, HWE and NaOH MS analyses.

Formula	LDI	DOM	HWE	NaOH
C ₁₁ H ₂₀ O ₁₂	✓	✓	✓	
C ₁₂ H ₂₄ O ₁₁	✓	✓	✓	
C ₁₃ H ₁₂ O ₁₁	✓	✓	✓	✓
C ₁₃ H ₂₈ O ₁₀		✓		
C ₁₄ H ₁₆ O ₁₀	✓	✓	✓	✓
C ₁₅ H ₂₀ O ₉		✓	✓	✓
C ₁₆ H ₈ O ₉	✓	✓	✓	✓
C ₁₆ H ₂₄ O ₈	✓	✓	✓	✓
C ₁₇ H ₁₂ O ₈	✓	✓	✓	✓
C ₁₇ H ₂₈ O ₇	✓	✓	✓	✓
C ₁₈ H ₁₆ O ₇	✓	✓	✓	✓
C ₁₈ H ₃₂ O ₆	✓	✓	✓	✓

$C_{19}H_{20}O_6$	✓	✓	✓	✓
$C_{19}H_{36}O_5$		✓	✓	✓
$C_{20}H_{24}O_5$	✓	✓	✓	✓
$C_{20}H_{40}O_4$	✓	✓	✓	✓
$C_{21}H_{12}O_5$	✓		✓	✓
$C_{23}H_{20}O_3$	✓	✓		
$C_{24}H_{24}O_2$		✓		
$C_{25}H_{12}O_2$	✓			
$C_{26}H_{16}O_1$	✓			

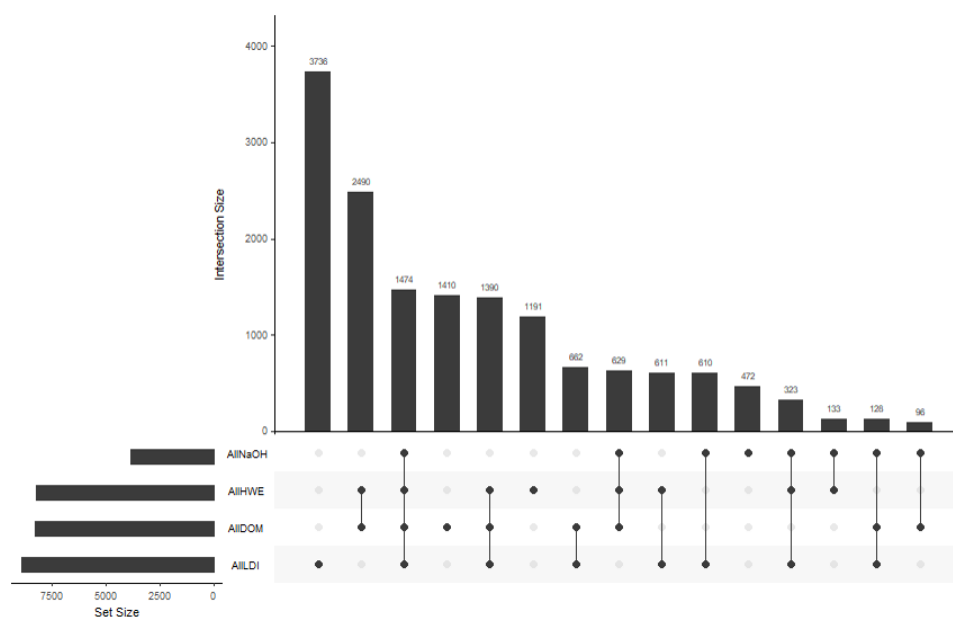


Figure A2. UpSet Plot showing all CHON and CHO molecular formulae observed across the four different methods.

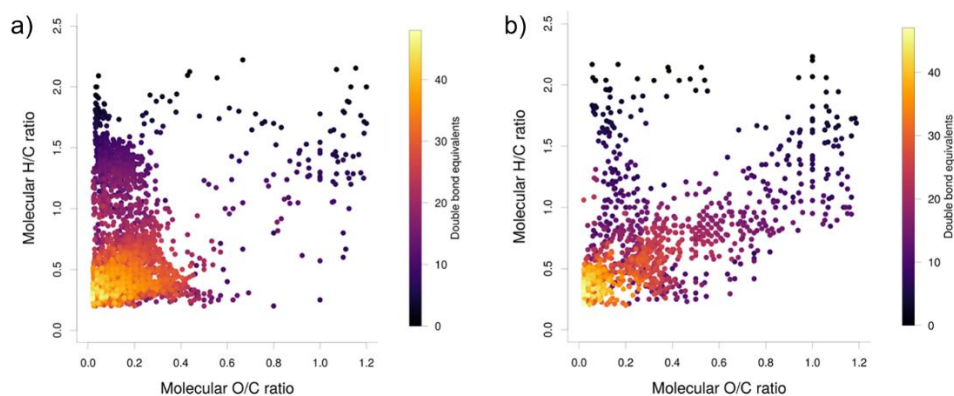


Figure A3. Van Krevelen diagram coloured by DBE of the (a) 2476 CHO and (b) 1260 CHON not shared molecular formulae from LDI MS data.

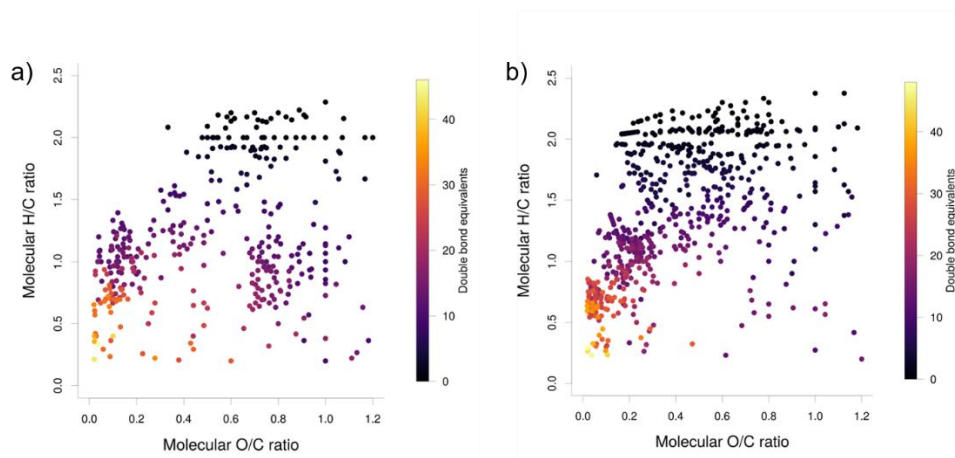


Figure A4. Van Krevelen diagram coloured by DBE of the (a) 577 CHO and (b) 833 CHON not shared molecular formulae from DOM MS data.

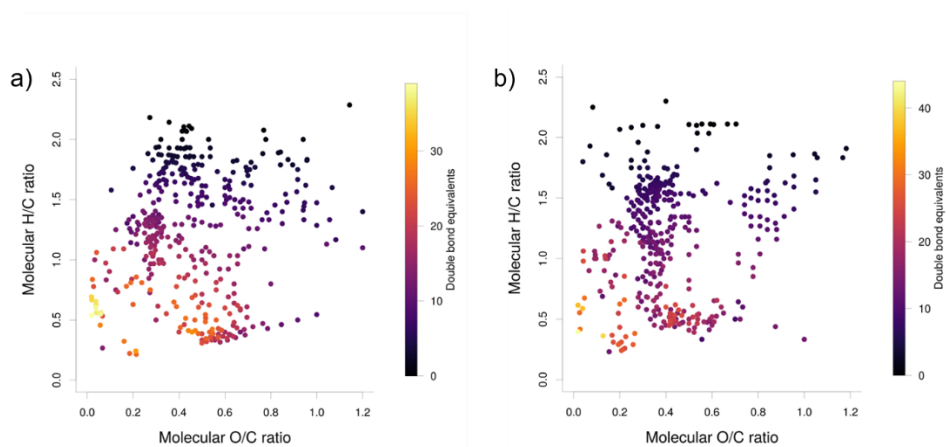


Figure A5. Van Krevelen diagram coloured by DBE of the (a) 731 CHO and (b) 460 CHON not shared molecular formulae from HWE MS data.

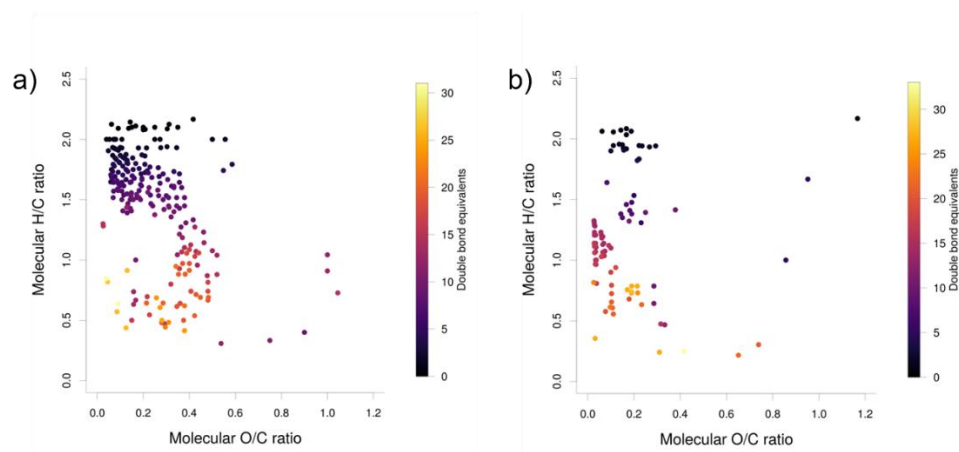


Figure A6. Van Krevelen diagram coloured by DBE of the (a) 351 CHO and (b) 121 CHON not shared molecular formulae from NaOH MS data.

Table A4. Total number of LDI MS picked peaks, formulae assigned and their percentage on each layer of the core.

Depth (cm)	Total number of picked peaks	Total number of CHO monoisotopic formulae assigned	Total number of CHON monoisotopic formulae assigned	% of peaks assigned
0-2	5851	3057	897	67.6
2-4	5736	3104	882	69.5
4-6	5811	2739	895	62.5
6-8	6121	3250	1037	70
8-10	6276	3456	1074	72.2
10-15	5437	2723	1054	69.5
15-20	6235	3129	1058	67.2
20-25	5471	2508	766	59.8
25-30	5893	3217	1034	72.1
30-35	5887	3205	1110	73.3
35-40	5389	3112	982	76
40-45	5132	2617	882	68.2

45-50	5229	2973	898	74
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Table A5. Total number of DOM MS picked peaks, formulae assigned and their percentage on each layer of the core.

Depth (cm)	Total number of picked peaks	Total number of CHO monoisotopic formulae assigned	Total number of CHON monoisotopic formulae assigned	% of peaks assigned
0-2	6553	2882	1369	64.9
2-4	5842	2327	1154	59.6
4-6	6375	2891	1412	67.5
6-8	5051	2019	824	56.3
8-10	5076	1959	882	56
10-15	5737	2520	1467	69.5
15-20	6221	2792	1316	66
20-25	6482	2899	1517	68.1
25-30	5851	2593	1360	67.6
30-35	6291	3080	1429	71.7
35-40	5393	2347	1008	62.2
40-45	6318	2996	1609	72.9

45-50	4871	1971	515	51
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Table A6. Total number of HWE MS picked peaks, formulae assigned and their percentage on each layer of the core.

Depth	Total number of peaks	Total number of CHO formulae assigned	Total number of CHON formulae assigned	% of peaks assigned
0-2	6657	4048	569	69.4
2-4	5981	3409	706	68.8
4-6	6396	2894	1212	64.2
6-8	6251	3680	725	70.5
8-10	5978	2730	1273	67
10-15	5789	2962	769	64.4
15-20	6122	2319	1374	60.3
20-25	6475	2682	994	56.8
25-30	5952	2698	1235	66.1
30-35	6241	2829	1021	61.7
35-40	5387	2205	963	58.8
40-45	6207	2885	667	57.2
45-50	5861	2900	1013	66.8

Table A7. Total number of NaOH MS picked peaks, formulae assigned and their percentage on each layer of the core.

Depth (cm)	Total number of picked peaks	Total number of CHO monoisotopic formulae assigned	Total number of CHON monoisotopic formulae assigned	% of peaks assigned
0-2	4118	2489	315	68.1
2-4	2536	1231	64	51.1
4-6	3811	1978	173	56.4
6-8	3121	1565	150	55
8-10	2996	1854	110	65.6
10-15	2437	1492	70	64.1
15-20	2235	1367	65	64.1
20-25	2671	1581	187	66.2
25-30	2893	1749	116	64.5
30-35	2587	1359	59	54.8
35-40	2389	1367	55	59.5
40-45	2132	1369	49	66.5
45-50	2459	1575	91	67.8

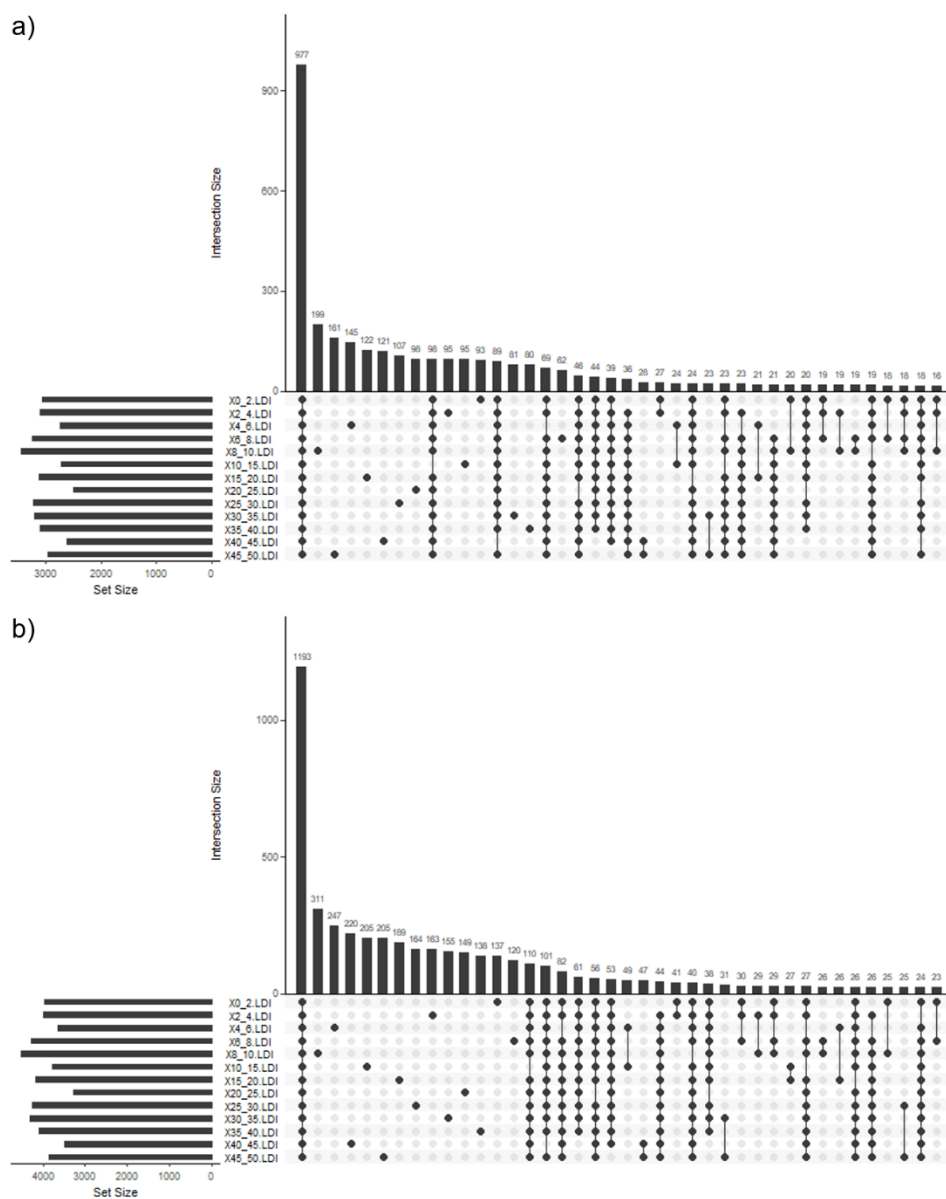


Figure A7. UpSet plots showing (a) all CHO and (b) all CHO and CHON formulae from LDI spectra.

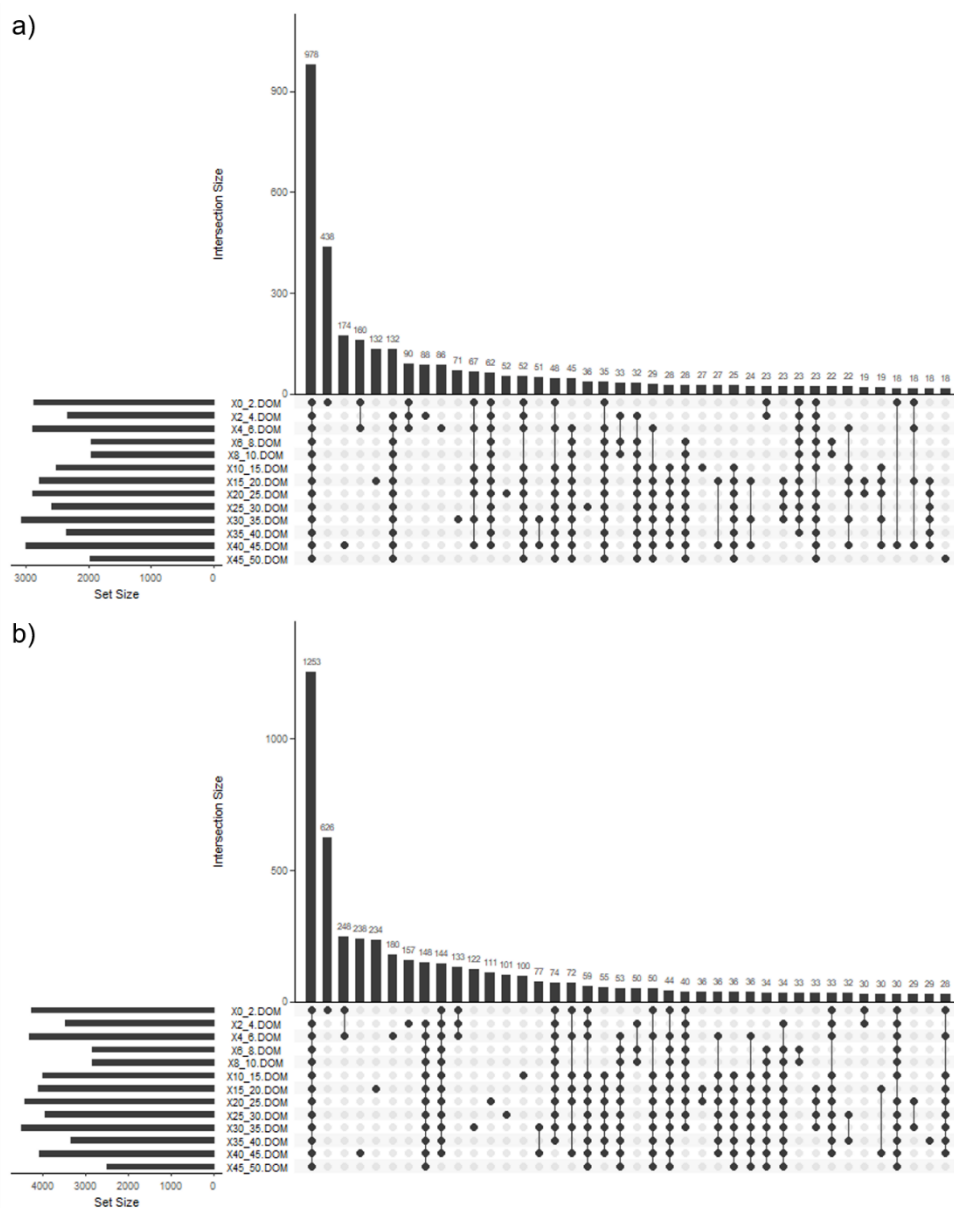


Figure A8. UpSet plots showing (a) all CHO and (b) all CHO and CHON formulae from DOM spectra.

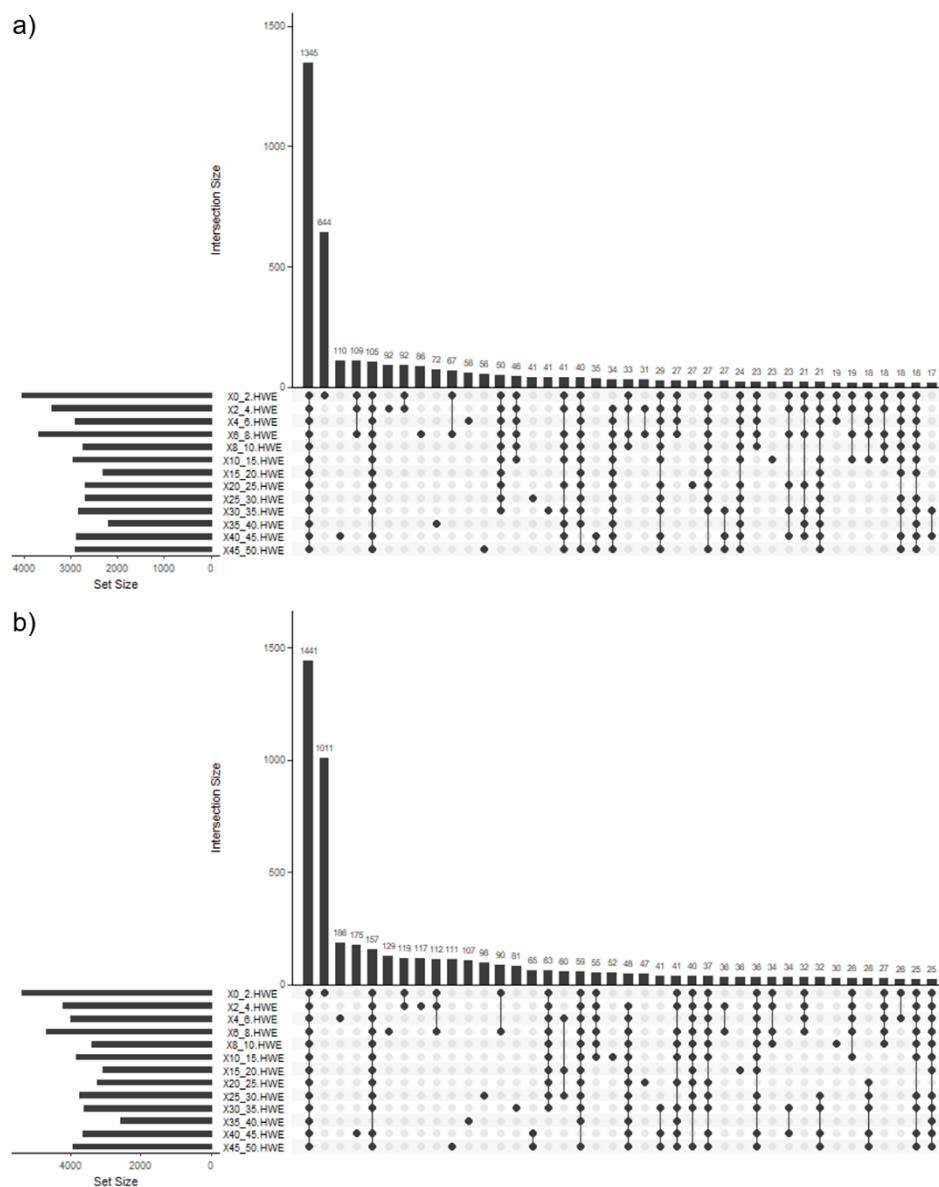


Figure A9. UpSet plots showing (a) all CHO and (b) all CHO and CHON formulae from HWE spectra.

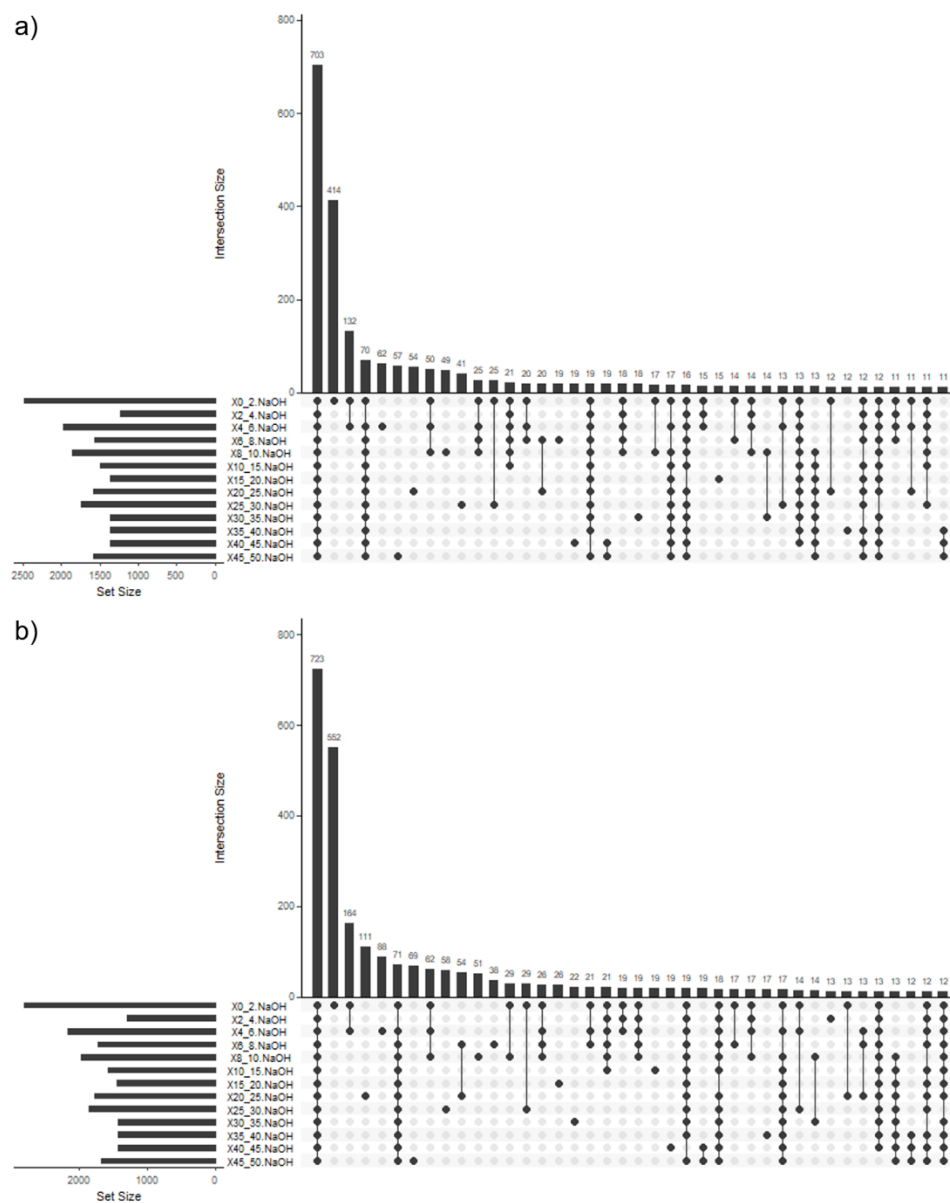


Figure A10. UpSet plots showing (a) all CHO and (b) all CHO and CHON formulae from NaOH spectra.

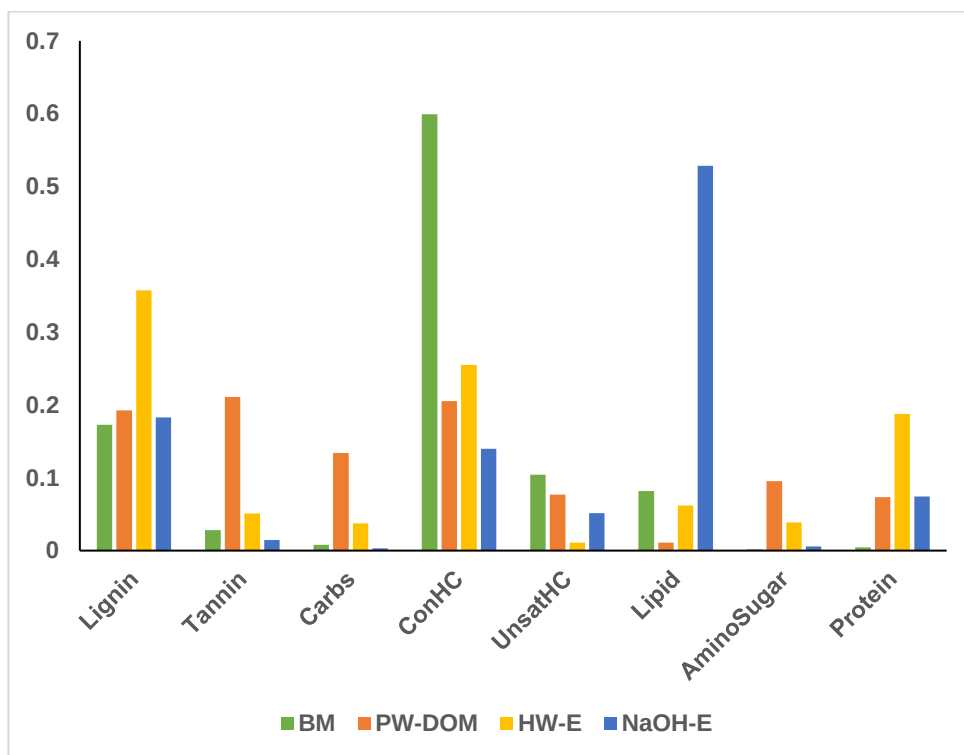


Figure A11. Compound class histogram for unique formulae all methods combined

Appendix B





Figure B1. Pictures of Red Moss damaged cores

RM2A



RM2B



RM2C



RM2D



RM2E



RM2F



RM2G



RM2H



RM2I



RM2J



RM2K



Figure B2. Pictures of Red Moss restored cores

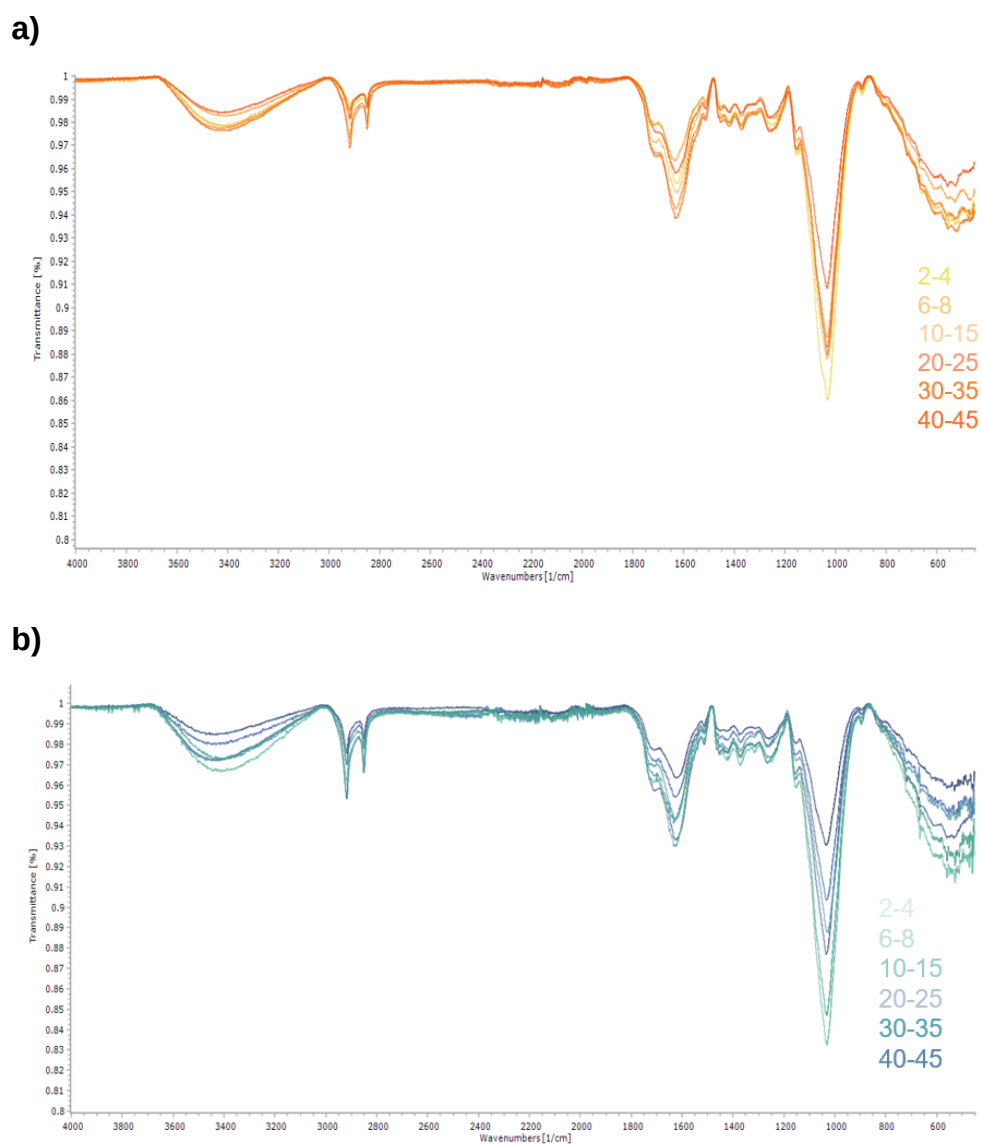


Figure B3. ATR-FTIR overlay spectra of RM1a (a) and RM2A (b) at six different depths (2-4, 6-8, 10-15, 20-25, 30-35, 40-45 cm), colour code highlighted on pictures

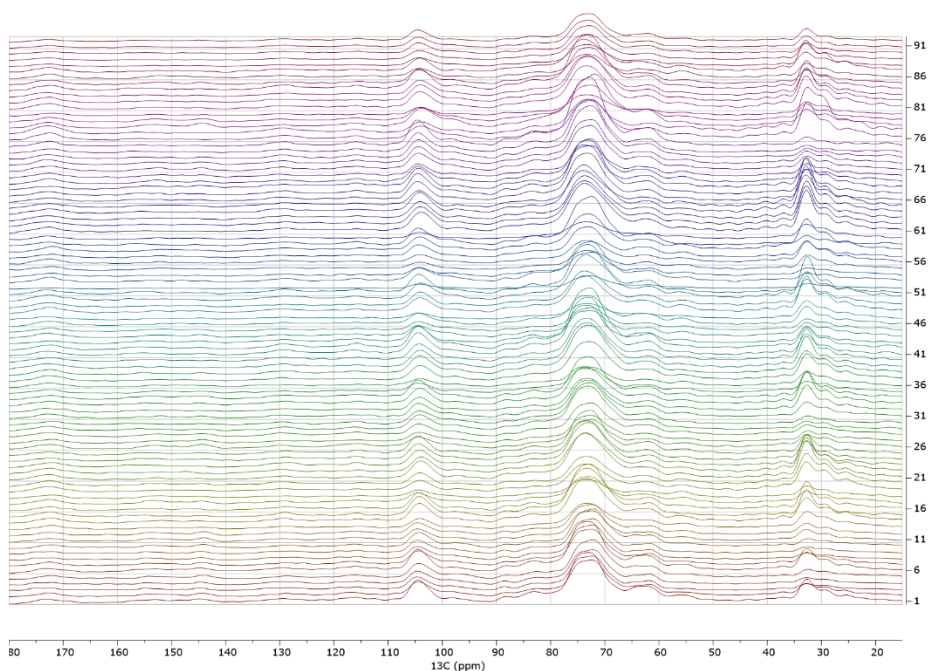
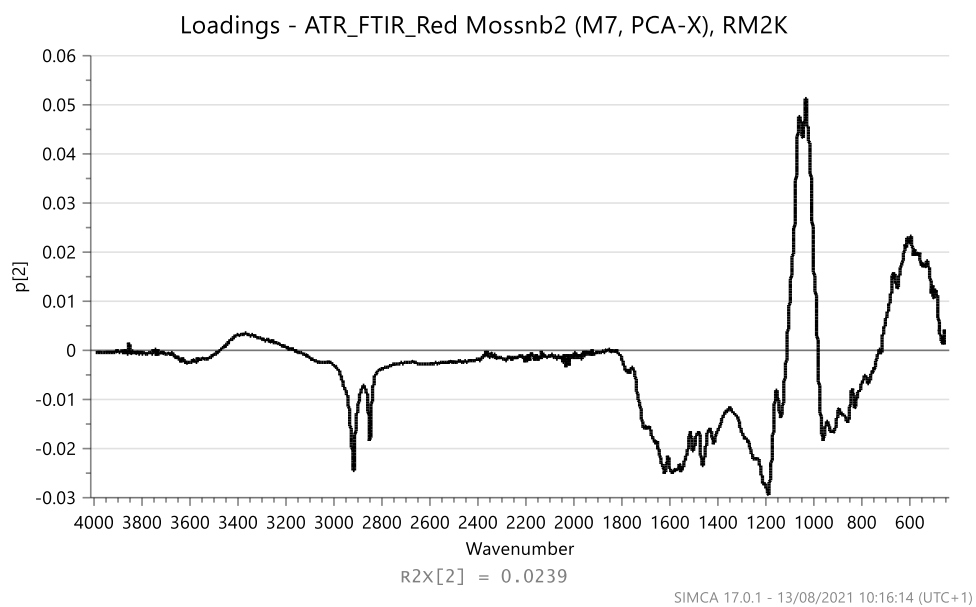
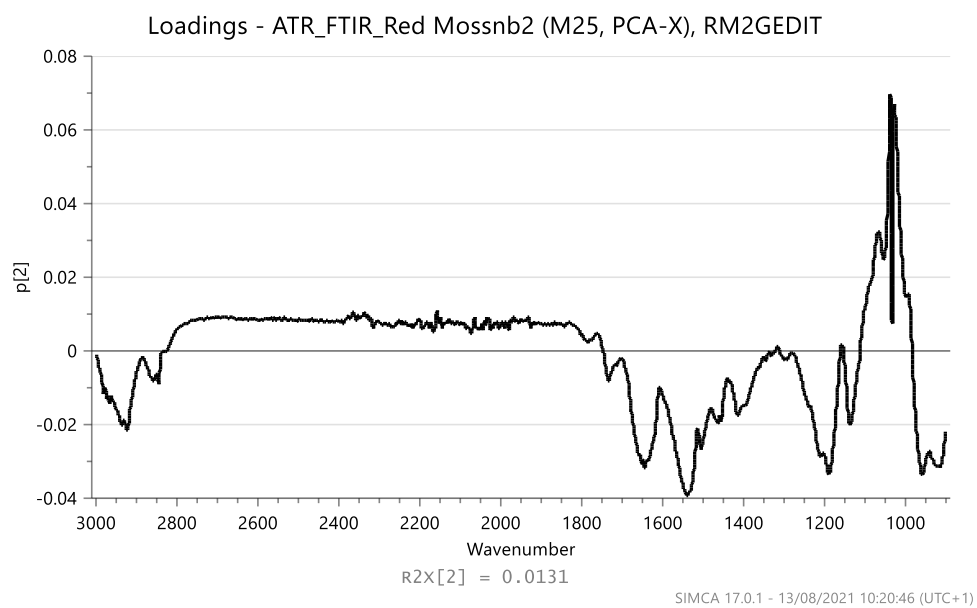
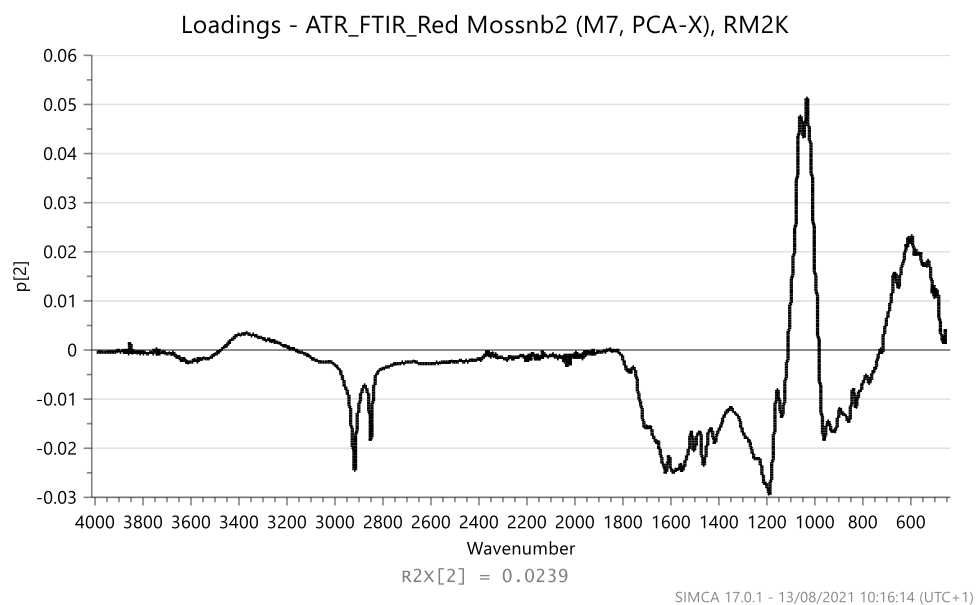
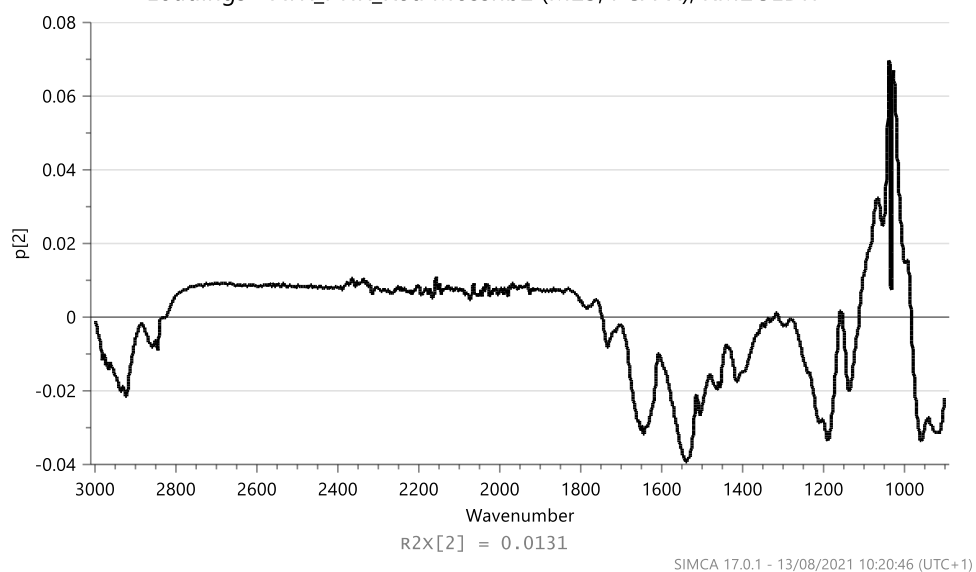


Figure B4. Solid state ^{13}C NMR stacked spectra from Red Moss cores (RM1 and RM2), 13 layers each core

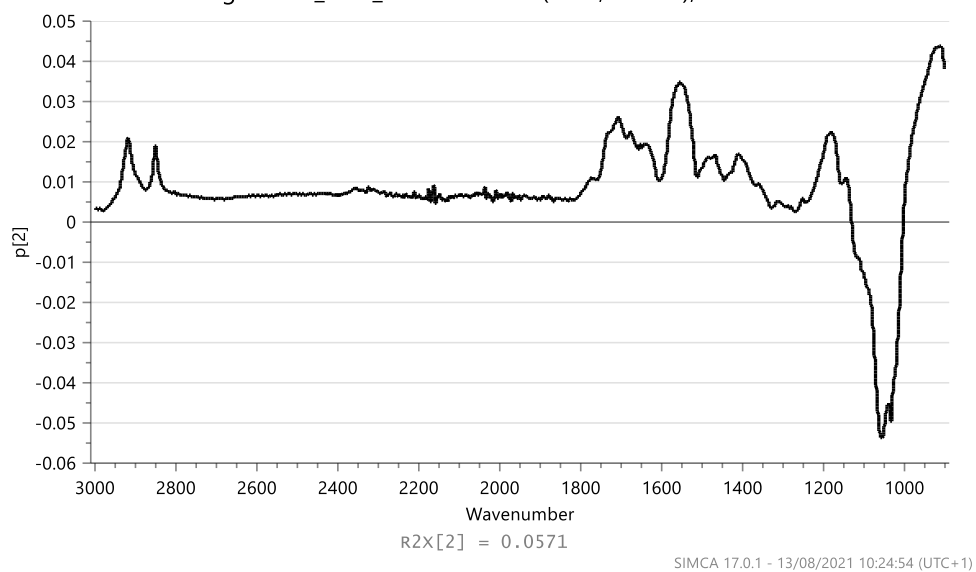




Loadings - ATR_FTIR_Red Mossnb2 (M25, PCA-X), RM2GEDIT



Loadings - ATR_FTIR_Red Mossnb2 (M26, PCA-X), RM2AEDIT



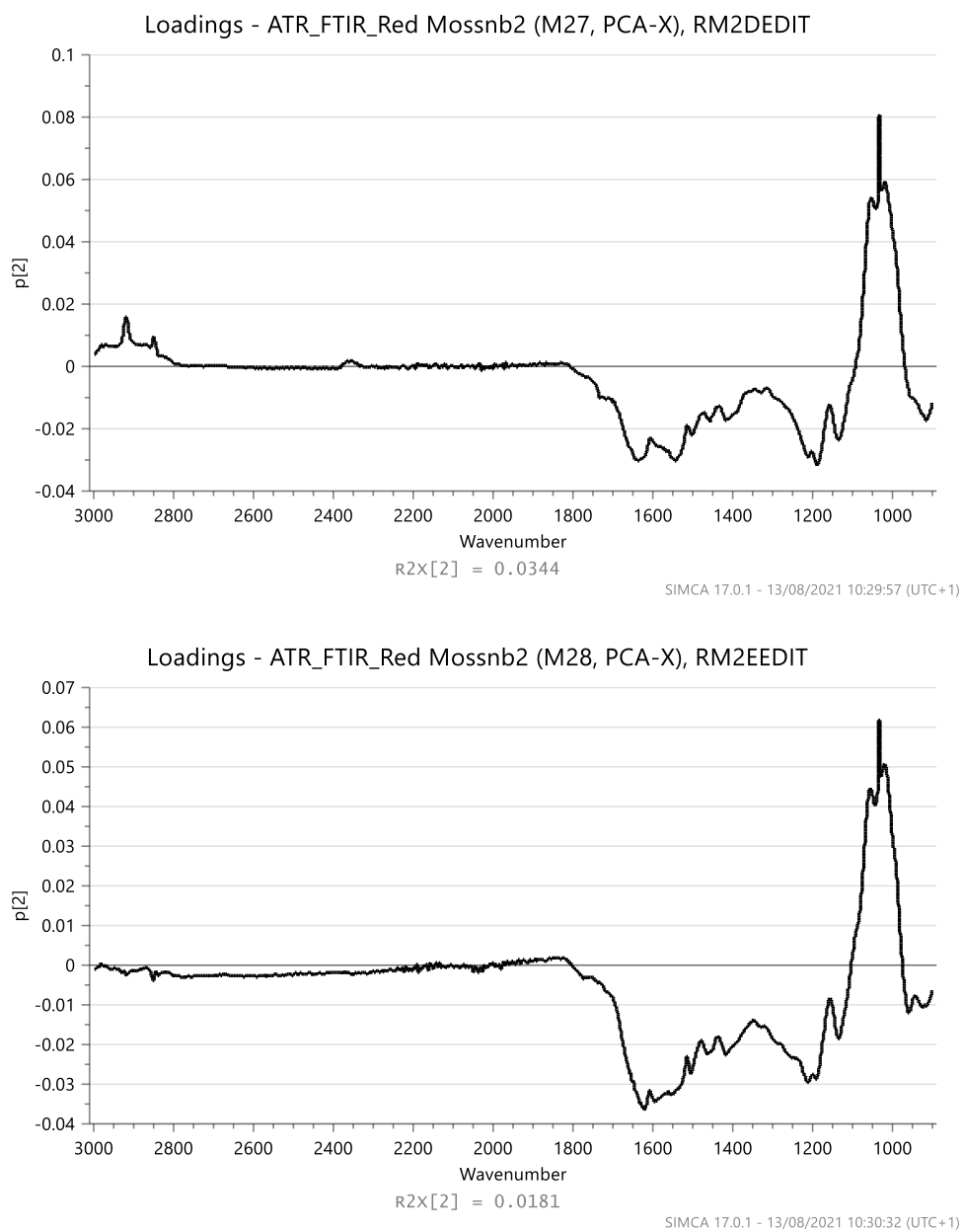
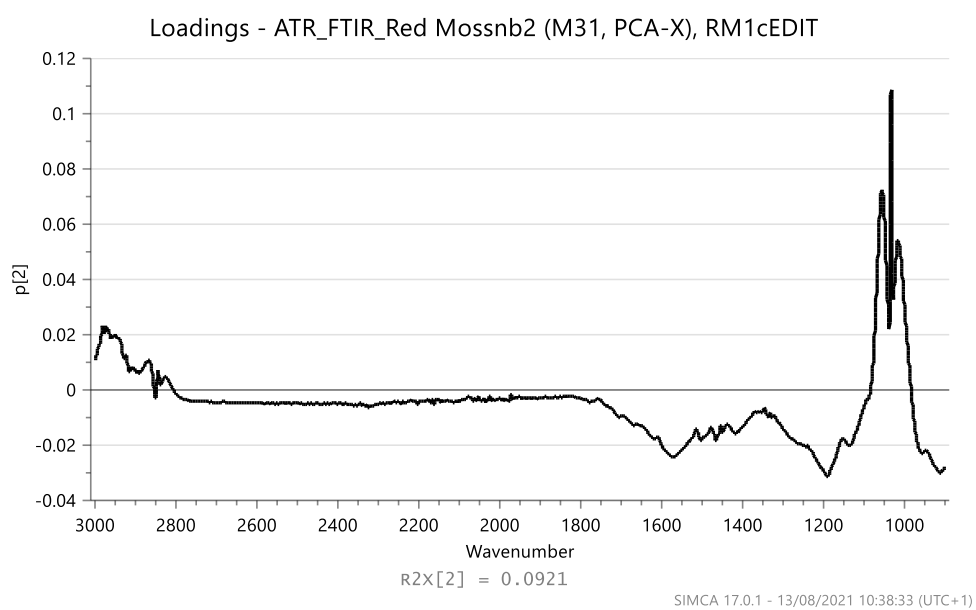
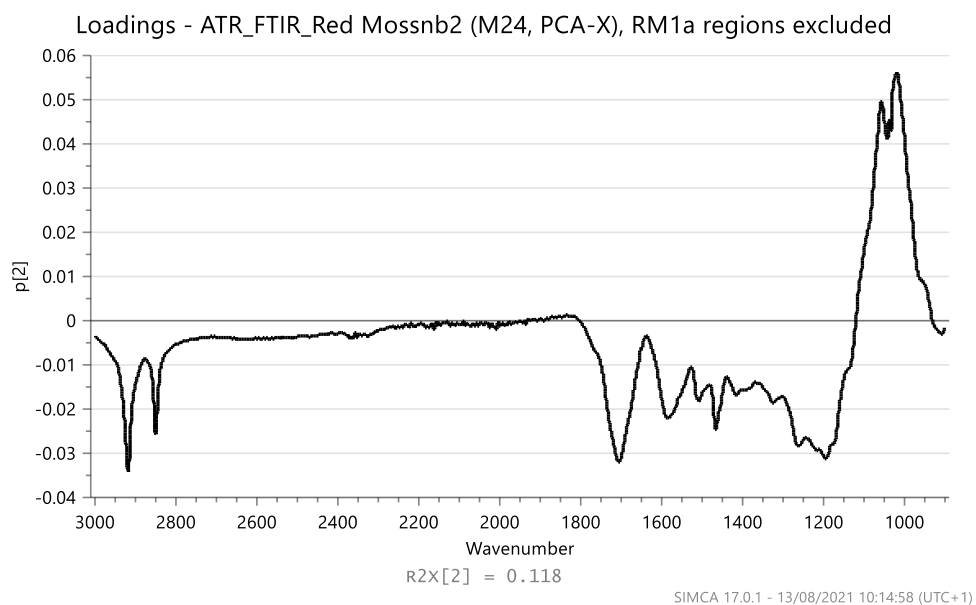
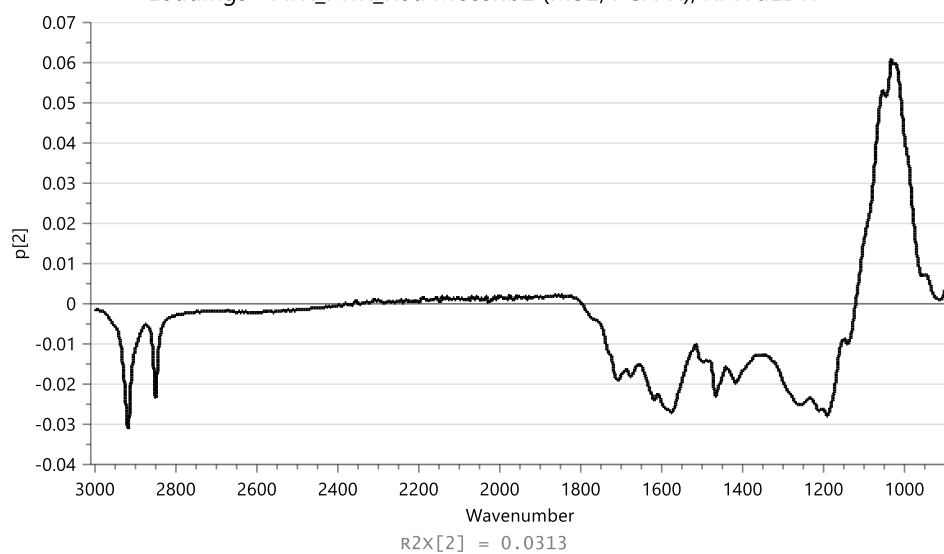


Figure B5. ATR-FTIR loadings of cores RM2K, G, A, D, E in order from top image

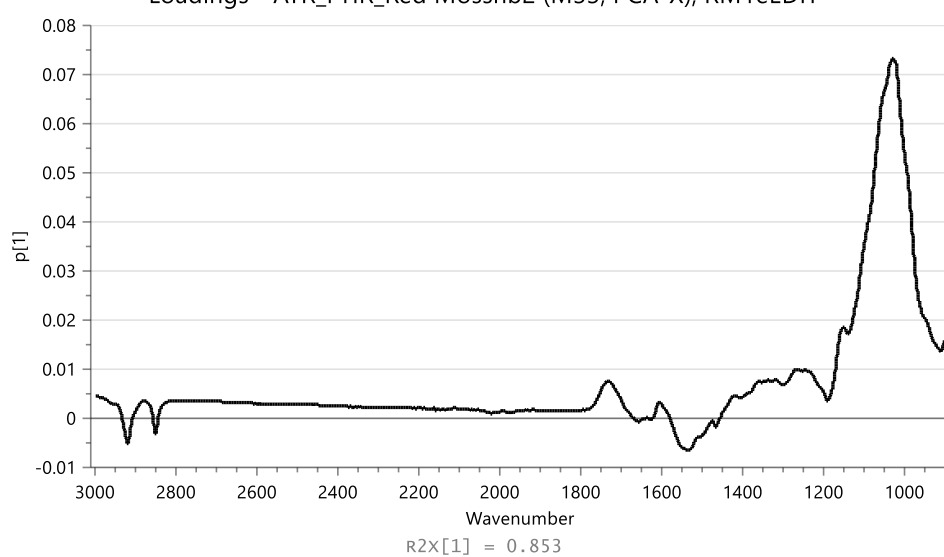


Loadings - ATR_FTIR_Red Mossnb2 (M32, PCA-X), RM1dEDIT



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Loadings - ATR_FTIR_Red Mossnb2 (M33, PCA-X), RM1eEDIT



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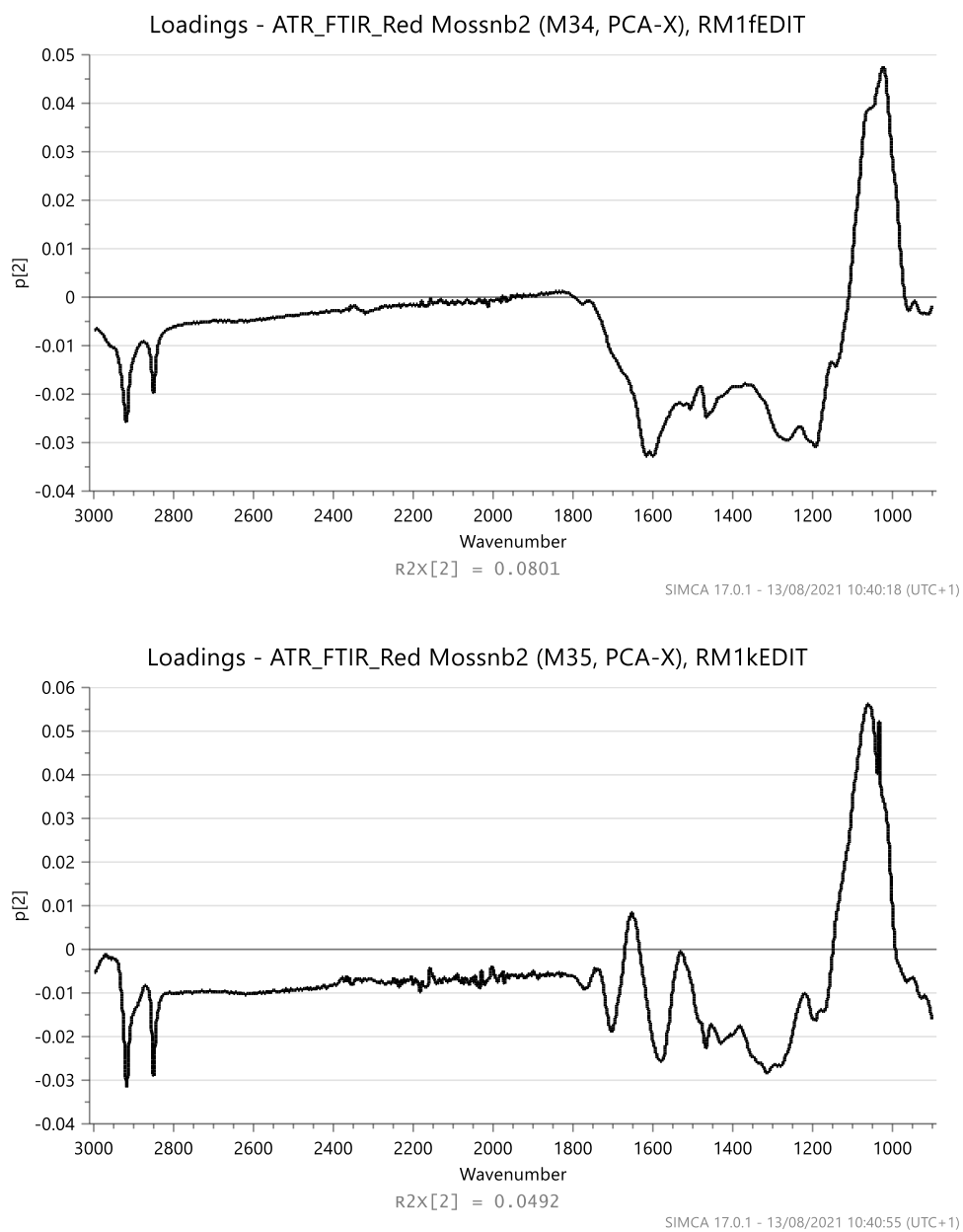


Figure B6. ATR-FTIR loadings of cores RM1a, c, d, e, f, k in order from top image

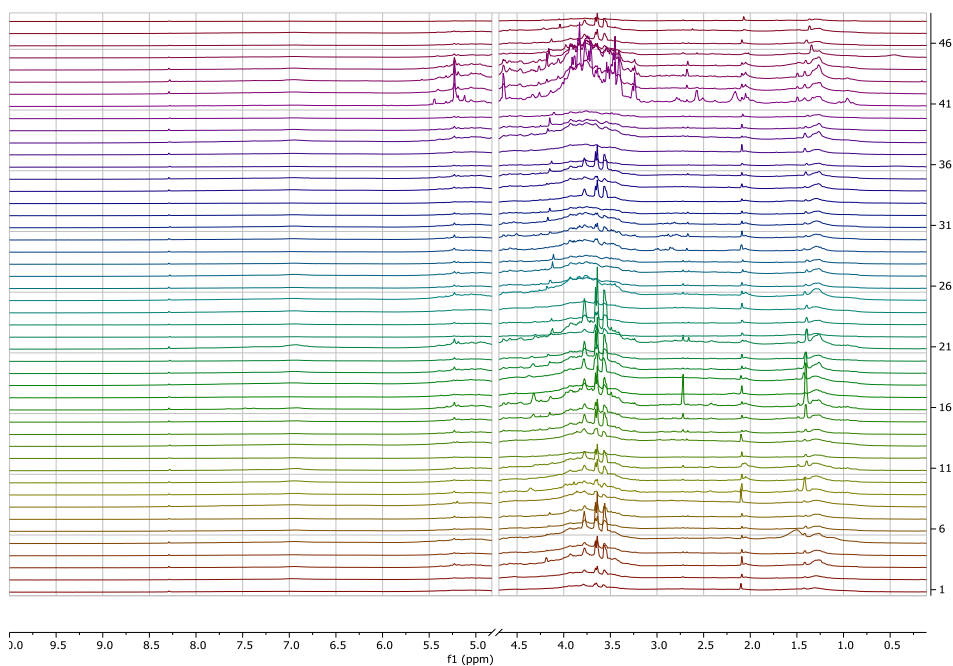


Figure B7. Liquid state ^1H NMR stacked spectra of DOM from Red Moss cores under investigation (10-15, 20-25, 30-35, 40-45 cm depth)

Appendix C

S1a



S1b



S1d



S1c



S1e



S1g

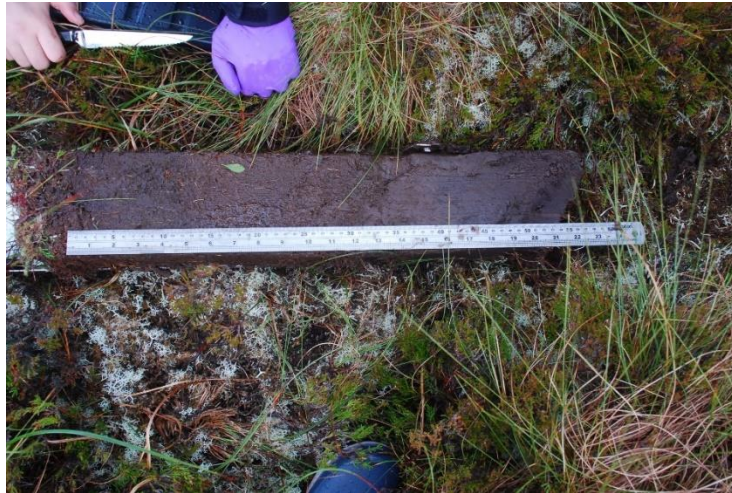


S1f



Figure C1. Pictures of S1 cores

S2A



S2E



S2F



S2G



S2H



S2D





Figure C2. Pictures of S2 cores



S3N



S3L





S3J



S3O

S3M



S3K



Figure C3. Pictures of S3 cores

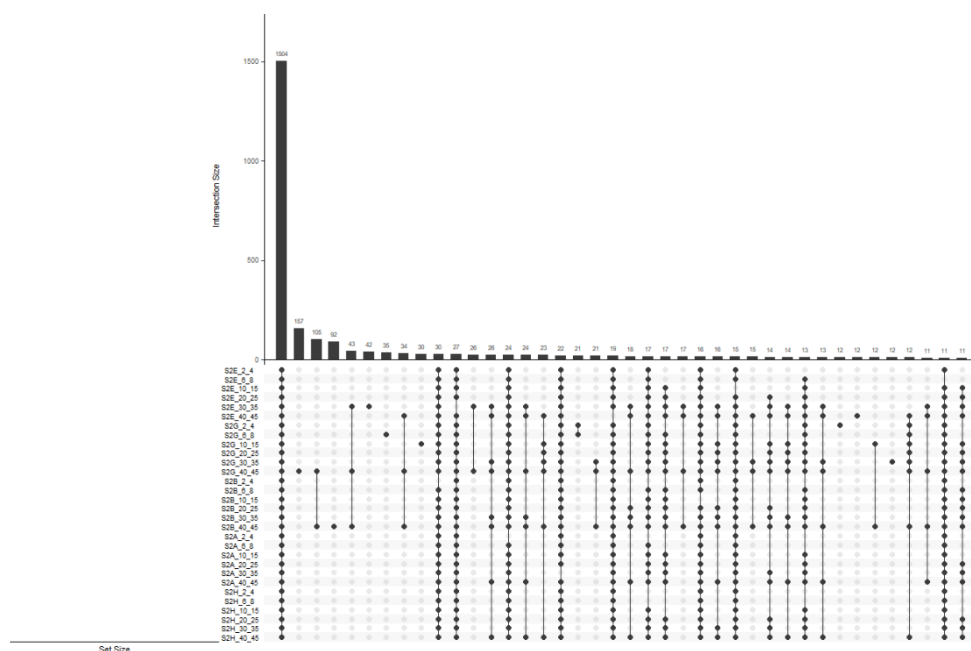


Figure C4. LDI MS Upset plot of each Flow Country core (5 cores) at 6 depths for S2 (CHO + CHON)

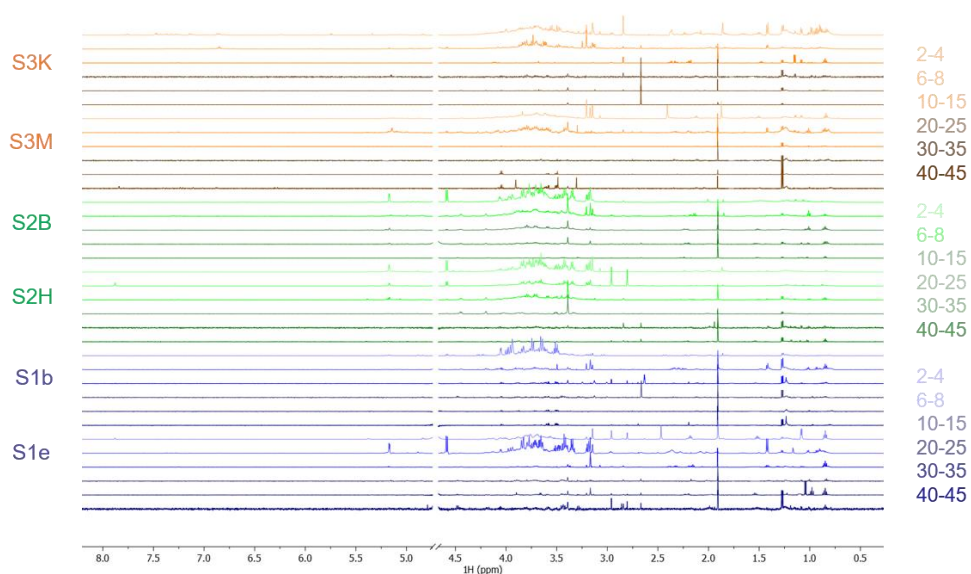


Figure C5. Liquid state ^1H NMR stacked spectra of DOM from S1 (b, e) and S3 (K, M) and S2 (B, H) colour code highlighted on image