Physical and Chemical Characterisation of Natural Organic Matter in Upland *Ombrotrophic* Peat by Tangential Flow Ultrafiltration and Pyrolysis - Gas Chromatography - Mass Spectrometry

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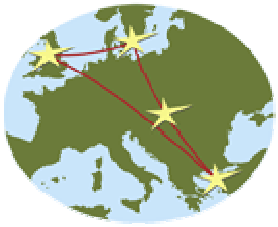
MESPOM



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Abstract

This study examines the potential of physical (particle size distribution) and chemical characterisation of natural organic matter in natural waters from upland *ombrotrophic* peat using Tangential Flow Ultrafiltration and Pyrolysis - Gas Chromatography - Mass Spectrometry (in the presence of tetramethylammonium hydroxide) techniques, respectively.

The characterisation methods were performed on water samples from three sampling sites of different degree of erosion in the catchment of Crowden Great Brook of Peak District National Park, UK. Particle size distribution results were shown varied spatially and positive relationship was found between log discharge and log dissolve organic carbon (DOC). Long-termed DOC flux was calculated, however no clear temporal trend was observed due to background variability. The method of chemical characterisation using Pyrolysis - Gas Chromatography - Mass Spectrometry was found feasible in identifying and quantifying aquagenic/pedogenic components of natural organic matter, which was shown to vary spatially and under different discharge conditions. Concentrations of such components also seemed to decrease as particle size fractions decreased.

Declaration

The dissertation entitled “Physical and Chemical Characterisation of Natural Organic Matter in Upland *Ombrotrophic* Peat by Tangential Flow Ultrafiltration and Pyrolysis - Gas Chromatography - Mass Spectrometry” is an original research work. For material obtained from other sources has been duly acknowledged in the report.

I declare that no portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Quynh Thu Nguyen

MESPOM

26th May 2009

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Abbreviations

C/G	Cinnamyl/Guaiacyl
COC	Colloidal Organic Carbon
DIW	Deionised Water
DOC	Dissolve Organic Carbon
EC	Electrical Conductivity
EI	Electron Ionization
FA	Fulvic Acid
FAME	Fatty Acid Methyl Ester
HA	Humic Acid
IC	Inorganic Carbon
MC	Methylated Carbohydrate
NOM	Natural Organic Matter
OC	Organic Carbon
POM	Particulate Organic Matter
PS	Polysaccharide
PSD	Particle Size Distribution
Py-GC-MS	Pyrolysis - Gas Chromatography - Mass Spectrometry

S/G	Syringyl/Guaiacyl
SOM	Soil Organic Matter
TC	Total Carbon
TMAH	Tetramethylammonium hydroxide
TFU	Tangential Flow Ultrafiltration
THM	Thermally assisted Hydrolysis and Methylation
TIC	Total Ion Current
TOC	Total Organic Carbon
UV	Ultra Violet

Chapter 1 - Introduction

1.1. Peat and upland *ombrotrophic* peat

Peat which is broadly defined as the organic waterlogged deposits of partially decayed remains of plants (Bragg and Tallis, 2001) covers quite a significant area of about 8% of the United Kingdom's, 10.4% of Scotland's (Taylor, 1983) and on average approximately 3% of the total landmass of the earth (Holden, 2005).

Peatlands which are also referred to as "organic wetlands" (Charman, 2002) can be classified based on their landscape position, hydrology, nutrition status or even associated plant community depending on the area of interest. For instance, classification based on hydrology may divide peatlands into *ombrotrophic* (rain-fed blanket peatlands with nutrients derived solely from the atmosphere), and *minerotrophic* and *rheotrophic* peatlands which receive nutrients and water from a groundwater resource. Meanwhile, peatlands classified based on their nutrition status include *oligotrophic* (nutrient-poor), *eutrophic* or *minerotrophic* (nutrient-rich) and *mesotrophic* (nutrient status between *oligotrophic* and *eutrophic*) peatlands (Charman, 2002).

Among the various types of peatlands, upland *ombrotrophic* peat is considered a crucial form offering a diverse range of services to the economy, society and people in

addition to their own intrinsic values. This type of landform serves as a unique landscape with *Sphagnum* mosses being the dominant form of plantation (Gorham, 1991; Zaccone *et al.* 2008). Upland peat offers recreational values and also provides a habitat for many rare and threatened species *e.g.* Golden Plover, *Pluvialis apricaria* (Evans *et al*., 2006) and various agriculture uses (Labadz *et al*., 1991). It also contains about 20-30% of the world's total soil carbon (Freeman *et al*., 2001a) and thus the movement of carbon inside upland *ombrotrophic* peat is considered a major link in the global carbon (Hope *et al*., 1997). For large parts of UK and in many other countries, runoff from upland catchments covered by *ombrotrophic* peat is a major source for local drinking water supply which is partly due to increasing amount of rainfall at high altitude and also from impervious bedrock of poor upland aquifers (Newson *et al*., 2001). Changes in these upland catchments therefore can have profound impacts on the global climate in addition to regional and local peatland conservation and water supply issues.

Reported changes on these upland catchments involved changes in vegetation, increasing erosion and increasing particle loads in runoff water. It was found that vegetation communities of the northern Peak District National Park uplands have changed significantly between 1931-1979, with enormously increasing grassland areas and declination of heather and bilberry moorland (Anderson and Yalden, 1981). Anderson and Tallis (1981 cited in Yeloff *et al.* 2002) also found two thirds of *ombrotrophic* peatlands as being impacted by erosion. Peat erosion in turn may lead to increased flux of materials from its natural organic matter (NOM), which derives from

dead materials of animals, vegetation and microbes (Knicker, 2004). Such release of organic carbon would elevate CO_2 production and provide a positive feedback to climate change (Evans *et al.*, 2006). For example, it was reported that dissolved organic carbon (DOC) in British upland surface waters had increased by 65% during 1988-2000 (Freeman *et al.*, 2001b).

Meanwhile, an unequivocal consensus on the causes of peat erosion is not yet to be realised. Peat erosion has been attributed to catchment management activities, such as overgrazing and moorland-burning, leaving peat denuded and susceptible to erosion (Tallis, 1997). Climate change has also been identified as another potential cause which might intensify in the future as a consequence of peat desiccating and weakening during drier summers and erosion due to increasing precipitation during wetter winters according to current UK Climate Impacts Programme scenarios (Tallis, 1997; Evans *et al.*, 2006, Hulme *et al.*, 2002).

There is thus a requirement for catchment managers, water providers, farmers and those concerning about the values of upland *ombrotrophic* peatlands and their conservation to understand the changes occurring in the system and to predict future scenarios for upland peat, as such appropriate resource management strategy could be formulised. A full understanding of such processes may also justify the cause of peat erosion. As a contribution to such study demands, the remainder of this work will focus on the characteristics of natural organic matter runoff from upland *ombrotrophic* peat.

1.2. Characterisation of natural organic matter

Studies on NOM runoff from peatlands have focused on the quantification and monitoring of sediment flux draining peat catchments over time (Francis, 1990; Labadz *et al.*, 1991; Evans *et al.*, 2006). This indeed is a rational approach as such quantification may provide crucial information on the state of peat and peat erosion.

Meanwhile, the physical and chemical characteristics of NOM which are also indicative of the processes occurring in upland peat catchments remain largely unattended. Chemical characterisation of peat may convey valuable information on its formation and transport and has in fact been used to identify its original materials (McKnight *et al.*, 2001) and runoff flow pathways (Newson *et al.*, 2001). Physical characteristics of NOM in regards of its variability in size distribution may also record similar information (Gaffney, 2007). For example, a smaller particle can be potentially transported over larger distances as it can avoid gravitational settling and remain in suspension (Buffle and Leppard, 1995a). Information on the size distribution of NOM may also be useful in understanding change in carbon storage, as for instance particulate load was found increasing during a discharge event in an eroded catchment area (Evans *et al.*, 2006).

There is thus a potential for chemical and physical (particle size) characterisation of NOM in natural waters in order to understand occurring processes within upland peat catchments. Given the topography of these sites, investigations should also combine hydrological monitoring of discharge at the site.

1.2.1. Physical characterisation of natural organic matter

Previous studies on particle size distribution (PSD) of NOM in natural waters often involved pressure filtration through a 0.45 or 0.2 µm membrane, thereby dividing NOM into particulate organic matter (POM) (>0.45 or 0.2 µm) and dissolved organic carbon (DOC) (<0.45 or 0.2 µm). For instance, Ritchie and Perdue (2003) defined DOC as that which passes through a 0.45 µm membrane, while POM is retained by such membrane. However, as colloidal material is also present in the <0.45 or 0.2 µm range of fraction, DOC is now often further divided into two sub-groups of colloidal organic carbon (COC) (<0.2 µm, >10 kD) and DOC (<10 kD). NOM in natural waters is mainly comprised of COC and DOC and a minor proportion of POM with the ratio ranging from 6:1 - 10:1 respectively (Wetzel, 1983). Thus in order to investigate the largely unknown group of COC and DOC, there is a requirement to determine particle size in a greater resolution than the traditional 0.45 or 0.2 µm membrane rudimentary scale.

Ultrafiltration, ultracentrifugation and laser light scattering are available analytical techniques which have been used for particle size determination. Each technique has its own advantage and disadvantage, mainly involving the preservation of particle size fractions during the course of analysis. For example it was thought that ultracentrifugation might increase particle agglomeration during the settling process (Lead *et al*., 1997). The technique is also disadvantageous in processing large volume of sample which is required for natural water samples as their concentration of analyte is usually low and thus requiring amplification (Gaffney, 2007).

Laser light scattering has a relatively high detection limit and thus often requires pre-concentration of samples which can result in alteration to PSD (Wagoner and Christman, 1999). Reported standard deviation in determining PSD of heterogeneous samples using this technique was also poor (Wagoner and Christman, 1999). Furthermore, it does not allow separation of fractions for analysis and thus considerably limits PSD fraction range analysis.

Ultrafiltration technique can be performed using either pressure or tangential flow filtration. While pressure filtration uses pressures to force sample through a membrane, tangential flow ultrafiltration (TFU) allows analyte particle to pass tangentially over the membrane of specific pore size. If the analyte particle is of correct size, it will be filtered by gravity.

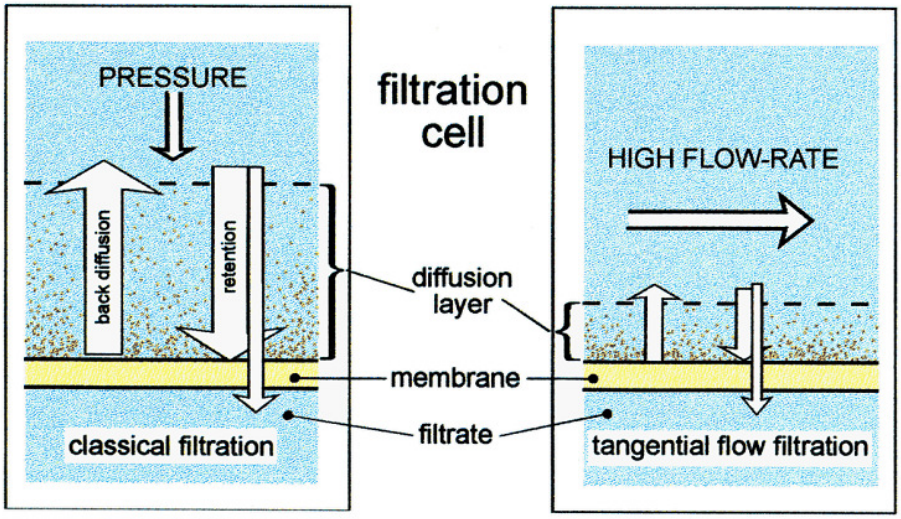

Figure 1 Comparison between classic pressure filtration and tangential flow ultrafiltration. (Figure was adapted from Buffle and Leppard, 1995b).

The design of TFU applies less force compared to the classic pressure filtration, thus minimising distortion of particle shape which enhances precision in separating fractions. Furthermore, as the height at the diffusion layer on top of the membrane is significantly reduced as shown in **Figure 1** slow back diffusion which could facilitate coagulation and create a barrier of high concentration of particles at the surface can be minimised (Buffle and Leppard, 1995b). In fact, TFU has been used to fractionate both marine (Wen *et al.*, 1996) and freshwaters (Ross and Sherrell, 1999) and separated fractions could be retained for subsequent analysis. Recently the technique has also been used for determination of PSD of NOM (Gaffney, 2007) with promising results. TFU was thus chosen as the analytical technique for determination of PSD of NOM in the work herein.

As mentioned earlier, the key reason for using TFU is to examine PSD in a greater resolution other than the traditional cut-off of 0.45 µm/0.2 µm using pressure filtration through a membrane of that size. In order to separate sample into fractions of useful particle size, Gaffney (2007) used a TFU membrane of 0.2 µm as the coarsest pore size, a 50 kDa membrane as the middle size to divide the colloidal fractions and a finest pore size of 10 kDa to divide the colloidal and the soluble. A pre-treatment by pressure filtration through a pore size of 1 µm was performed as such pore size would be unlikely to create any artefacts (Gaffney, 2007). This method has been proved to be feasible in fractionating NOM in natural waters.

1.2.2. Chemical characterisation of natural organic matter

As mentioned above, NOM in natural waters is mainly comprised of COC, DOC and POM. While POM usually enters the water stream from branches and leaves of overhanging canopies, most COC and DOC originates from terrestrial sources such as by leaching of soil horizons and bankside plants (Wetzel, 1983). COC and DOC contents therefore often involve plant and animal products at different decomposing stages and also matters arising from those biological and chemical degradation processes (Wetzel, 1983). Such products can be broadly divided into two groups of humic and non-humic substances.

Humic substances are comprised of dark-coloured and acidic compounds of large molecular weight of hundreds to thousands of Dalton (Wetzel, 1983) which are very resistant to microbial breakdown. As a consequence, humic substances are very persistent in aquatic system. Humic acid (HA) and fulvic acid (FA) are the major components of this group with the former usually having larger molecular weight than the latter (Wetzel, 1983). Humic substances are often leached from terrestrial soil into the aquatic system and thus are usually referred to as pedogenic (terrestrial origin).

Non-humic substances include a range of components such as carbohydrates, amino acids and other organic compounds of low molecular weight. This group of NOM can be utilised easily by micro-organisms, resulting in a rapid turnover and smaller proportion in DOC and COC concentrations (Wetzel, 1983). Meanwhile, polysaccharides are produced by microbes to incorporate into their own cell wall or

excreted to create an extra-cellular layer surrounding the cell (Schultze-Lam *et al.*, 1996). As they are produced within waters, microbial polysaccharides are referred to as aquagenic components.

It has been thought that fulvic and humic acid are the main components of aqueous NOM (Parsi *et al.*, 2007), contributing 60-80% of DOC in natural waters (Reuter and Perdue, 1977). However, they were shown not always the dominant forms of DOC and the dominance of any actually depends on the characteristic of the water body (Boult *et al.*, 2001). For example, polysaccharide can also be a major component in standing waters which are favourable to algal and microbial activity. In fact, it was reported that the fraction of polysaccharide found in DOC varied temporally from 18-72% in a eutrophic lake (Biber *et al.*, 1996). In contrast, in upland streams where NOM is largely derived from water draining nutrient-poor *ombrotrophic* peat, pedogenic NOM might be the dominant form. Therefore, it might be more appropriate to subdivide aqueous NOM into two classes: aquagenic and pedogenic. The former refers to fresher biota products with polysaccharide (PS) as the main component whereas the latter refers to older products mainly comprised of humic substances. Knowledge of chemical components of NOM in regards of these two broad groups is also required to achieve an understanding of change in carbon storage in upland peat. A high amount of younger components in aqueous NOM is less alarming than if older components are dominant as prevalence of older components could indicate the inadequate formation of new peat (from freshly decomposed materials) and thus certain degree of erosion and lack of living materials of an 'unhealthy' peat site.

While detailed understanding of chemical characterisation of NOM is crucial to investigate their source materials, formation and various NOM-involving processes (Parsi *et al*., 2007), knowledge on detailed chemical structure of NOM is limited due to its high heterogeneity. Traditional chemical characterisation of NOM is often labour intensive as it requires organic acids to be isolated from bulk sample volumes. However, recently advances have been achieved using modern instrumental techniques such as ^{13}C NMR, ^{1}H NMR and analytical pyrolysis (Malcolm, 1990).

Among these techniques, pyrolysis coupled with analytical instruments such as gas chromatograph and mass spectrometer (Pyrolysis - Gas Chromatography - Mass Spectrometry (Py-GC-MS)) has been shown as a useful technique in analysis of bio-polymers (Fabbri *et al*., 1999) and environmental samples (Parsi *et al*., 2007). The technique employs thermolytic conditions to break macromolecules into small fragments which are separated by gas chromatograph and detected by mass spectrometer. Such fragments may provide information on the composition and structure of the original macromolecules. Py-GC-MS however suffers from thermally induced secondary reactions, which can result in modification of the original material and thus biased interpretation of pyrolysis data (Knicker, 2004; Fabbri, 1996). Pyrolysis in the presence of tetramethylammonium hydroxide (TMAH) can partly overcome this issue by methylation reaction (or also referred to as simultaneous derivatisation) converting polar functional groups to less polar derivatives, thus enhancing separation efficiency of the gas chromatograph (Knicker, 2004, Parsi *et al.* 2007). Py-GC-MS in

the presence of TMAH is also referred to as Thermally-assisted Hydrolysis and Methylation - Gas Chromatography - Mass Spectrometry (THM-GC-MS).

Py-GC-MS in the presence of TMAH has been widely applied to study chemical characteristics of soil organic matter (SOM) (Mason *et al*., 2009) and its various components such as humins, humic acid and fulvic acid (Fabbri, 1996, 1998), lignin (Vane *et al*., 2001) and carbohydrates (Fabbri, 1999). (Smith 1984). It has also gained momentum in analysing composition of aqueous NOM (Christy *et al*., 1999; Guo *et al*., 2003).

In analysis of aqueous NOM, Py-GC-MS requires sample to be separated as sediment from the bulk volume of water, which has been attained by several techniques, such as reverse osmosis and evaporation (Christy *et al*., 1999). Meanwhile, the design of TFU allows continuous reduction in volume of retentate while filtrate (including particles of the correct size) is passed through the membrane, thus offering a simple concentration method for sediment separation from the bulk aqueous volume. In addition, the potential of combining chemical characterisation of aqueous NOM with its PSD which offers another level of information on size fraction of chemical components and thus contribute to the understanding of origin and fate of NOM remains poorly characterised. This potential thus will be explored in this work.

1.3. Available sampling site and characterisation records

Recent work has been performed on the upland catchment of Crowden Great Brook of Peak District National Park, about 30 km east of Manchester (**Figure 2**) (Todman,

2005; Gaffney, 2007). The brook drains from the slopes of Black Hill from an elevation of 530 m AOD into Torside reservoir, which is part of the Longdendale reservoir series (220 m AOD) (Todman, 2005).

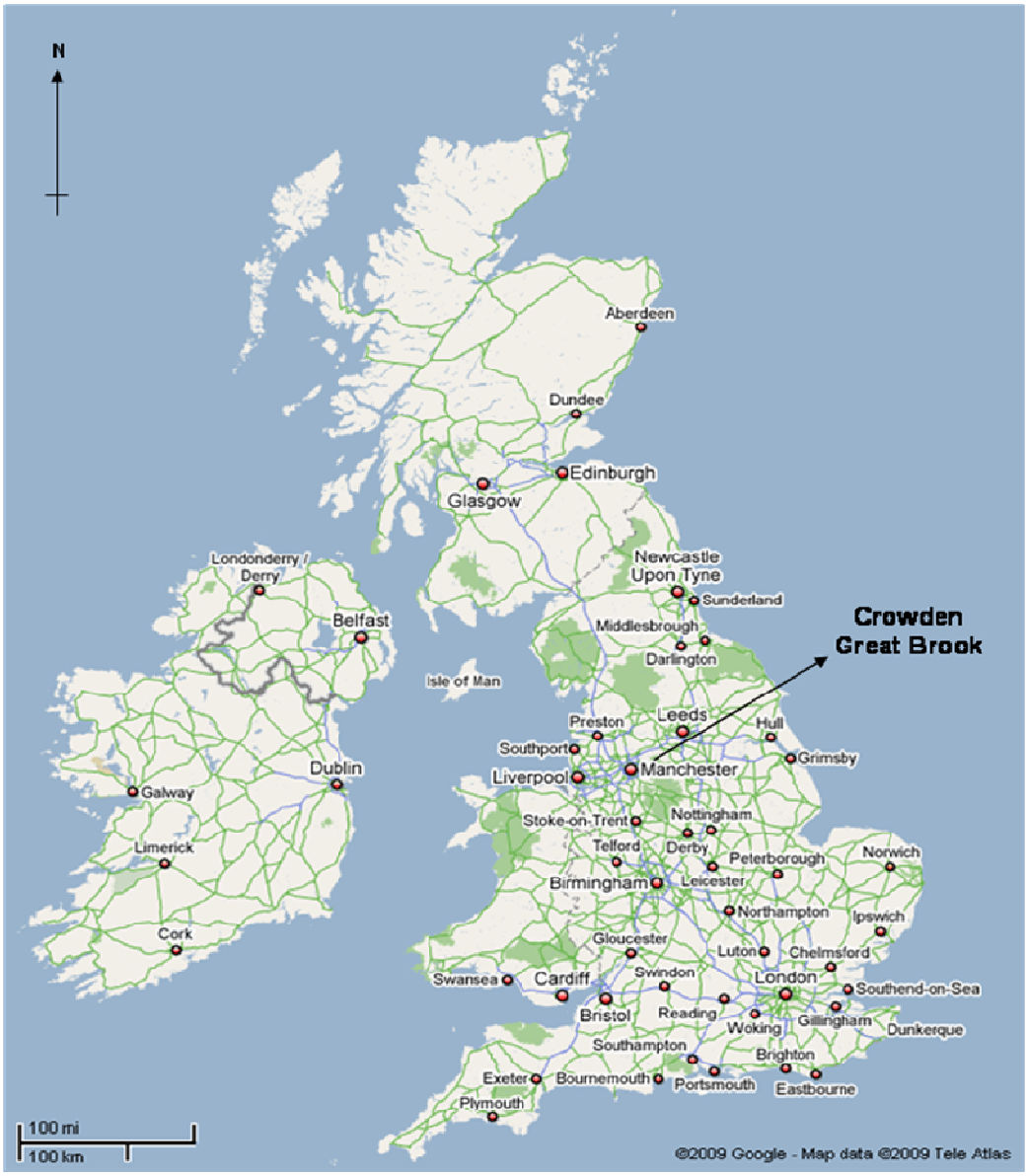

Figure 2 Location of Crowden Great Brook.

(Figure adapted from ©2009 Google – Map data ©2009 Tele Atlas).

The geology underlying Crowden Great Brook catchment area is dominantly comprised of Carboniferous sandstone, shale, mudstone and kinder grit (Todman, 2005).

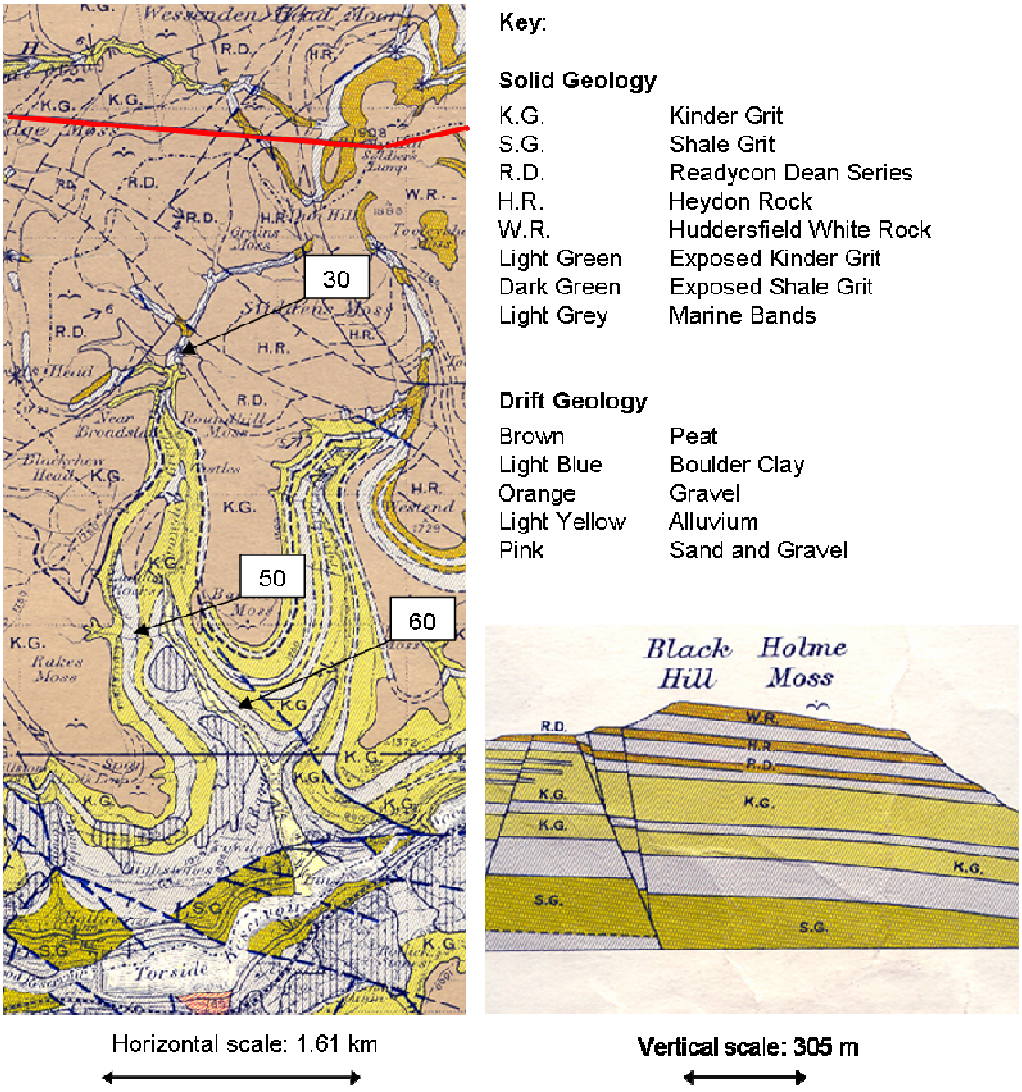

Figure 3 Geology of the Crowden Great Brook catchment. Three main sampling locations shown by a) map b) cross section with red line indicating cross-sectional position. Ordnance Survey Geological Mapping Sheet 86. Crown Copyright. Figure was taken from Todman (2005).

The catchment is largely covered by *ombrotrophic* peat which overlays approximately 70% of the catchment surface. Different parts of the site have been impacted by erosion to different degree (Todman, 2005). Peat at headwaters of the main stream is vegetated while downstream (to the west side of the stream) the sites are quite denuded (**Figure 4**).

Figure 4 The vegetated (left) and the eroded (right) peat sub-catchment of Crowden Great Brook. (Photos were taken from Gaffney (2007)).

Three main sampling sites were selected based on their different position within the catchment and degree of erosion incurred, with site 30 draining the vegetated sub-catchment, site 50 the denuded sub-catchment and site 60 the whole catchment (Todman, 2005; Gaffney, 2007). The total catchment area is 7 km^2, with the area of the vegetated sub-catchment covering 3 km^2 and the bare peat sub-catchment 0.5 km^2 (**Figure 5**). As these sub-catchments have different degree of erosion and vegetation change, certain sites can potentially be used as a future model for the whole area.

Thus in charactering NOM flux from each sub-catchment, it is also important to realise their spatial variability.

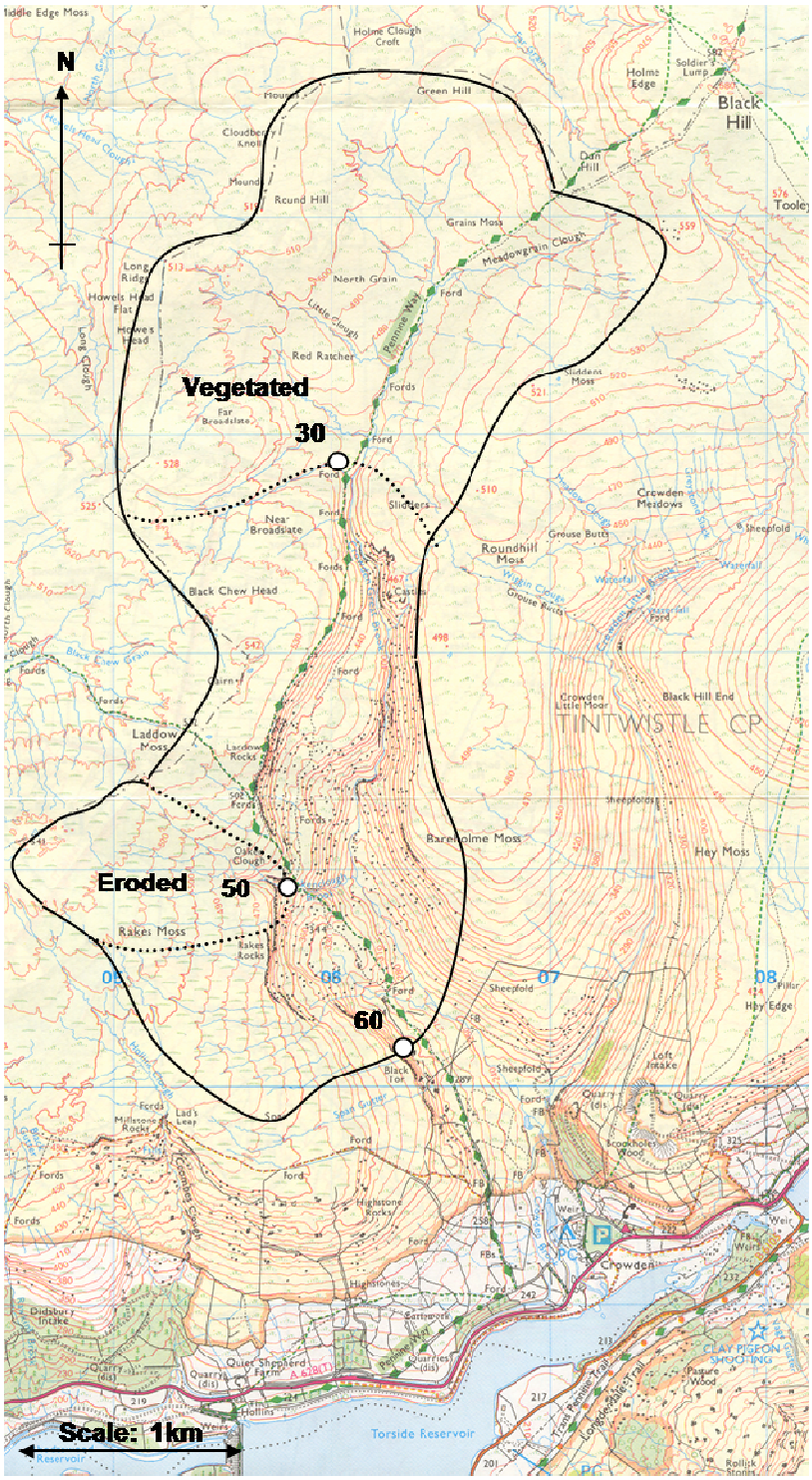

Figure 5 Location of the three monitoring sites on Crowden Great Brook. Site 30 drains the vegetated sub-catchment, site 50 drains the eroded eroded sub-catchment and site 60 drains catchment as a whole (Figure adapted from Todman (2005) and reproduced from Ordnance Survey map data by permission of Ordnance Survey, © Crown copyright).

Data on PSD of NOM flux in natural waters and discharge was available since 01/02/03 at all three monitoring sites though quite scattered due to the piloting nature of previous works (Todman, 2005; Gaffney, 2007). Nevertheless, such data is important given the requirement of extensive record for a more complete understanding of the processes occurring in an upland catchment. This should also allow the quantification of carbon mass exiting the stream using carbon flux data, which should serve as an indication on change of carbon storage within the catchment. The confidence with which the change can be recognised will also be enhanced due to the length of data set which should reduce noise from background variability.

1.4. Aim and objectives

The thesis aims to characterise NOM fluxes in natural waters draining upland *ombrotrophic* peat in regards of their physical (PSD) and chemical characterisation using two potential techniques TFU and Py-GC-MS respectively in correlation with hydrological condition (discharge) at the three monitoring sites of Crowden Great Brook. In addition, given available data on PSD and discharge since 2003, the thesis also has the aim to investigate temporal and spatial variability of such physical characteristic of NOM. Furthermore, it is also aimed to obtain initial investigation on chemical characteristics of NOM using Py-GC-MS. Such knowledge on physical and chemical characteristics of NOM will contribute to the understanding of change of carbon storage and processes occurring in upland *ombrotrophic* peat.

In order to achieve these aims the following objectives will be met:

(1) To confirm that OC concentration of NOM in Crowden Great Brook catchment can be measured;

(2) To confirm if NOM is variable within the catchment;

(3) To investigate whether carbon storage of the catchment has changed over the years of available records by (a) quantifying dissolved organic carbon flux, (b) examining response of system to discharge events;

(4) To determine if there is a relationship between discharge and physical characteristic (PSD) and if such relationship varies spatially;

(5) To determine if chemical components of NOM can be identified using Py-GC-MS;

(6) To determine if such components can be quantified;

(7) To determine whether there is a relationship between aquagenic/pedogenic components of NOM and PSD;

(8) To determine if such relationship varies spatially within the catchment and under different discharge conditions.

Chapter 2 – Materials and Method

2.1. Total Organic Carbon analysis

2.1.1. Materials

Total carbon (TC) stock solution was prepared by dissolving 2.125 g potassium hydrogen phthalate (Shimadzu, Japan) in 1 L of deionised water (DIW) (15 MΩ, ultra violet (UV) treated, Elga, Option 4, water purifier). Inorganic carbon (IC) stock solution was prepared by dissolving 3.5 g sodium hydrogen carbonate (Shimadzu, Japan) and 4.41 g sodium carbonate (Shimadzu, Japan) in 1 L of DIW (15 MΩ, ultra violet (UV) treated, Elga, Option 4, water purifier).

Standard solutions were prepared freshly before measurement by diluting the stock solution using DIW (15 MΩ, ultra violet (UV) treated, Elga, Option 4, water purifier). TC and IC were calibrated using TC and IC standards. TC standards included 0, 1, 5 and 20 mgL^{-1} standards. IC standards included 0, 0.5 and 5 mgL^{-1} standards. Standards were run prior to and after each period of analysis. After every 5 samples a TC standard and an IC standard of closest concentration to the samples were run.

2.1.2. Determination of discharge

Discharge was determined using the dilution gauging technique (Wood and Dykes, 2002) and adapted work by Gaffney (2007). A four pole electrical conductivity (EC)

probe (measurement range 0-2500 µS) pre-calibrated with NaCl standards was connected to a Sentry II data logger (Intelisys, UK) and placed into the stream. A known amount of NaCl was dissolved in river water in a plastic bag and released into stream water about 10 m upstream from the position of the probe. EC was measured at both NaCl dilution point and the measurement point until a stable consistent background reading was obtained.

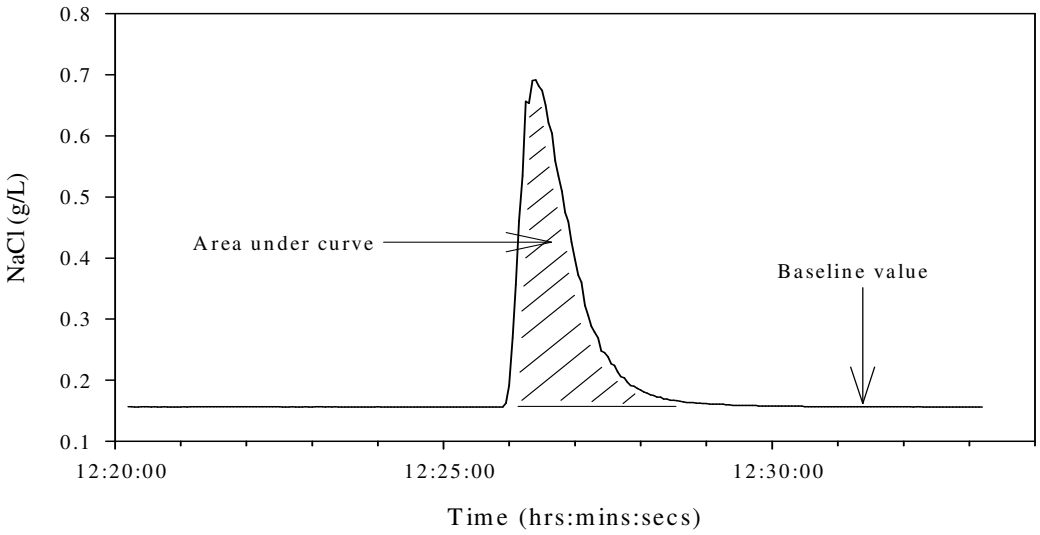

Figure 6 Illustration of a dilution gauging curve. (Figure was taken from Gaffney (2007)).

In the laboratory data was downloaded from the logger to the computer. Discharge was calculated based on the EC value, mass of NaCl and time as follows.

$$Q = \frac{m}{A}; A = (t \times m) - (t \times b) \times n$$

where Q = discharge /Ls^{-1}; m = mass of NaCl /g; A = Area under curve /$gL^{-1}s$; t = time interval /s; b = baseline value /gL^{-1}; n = number of data point.

2.1.3. Field sampling and size separation analysis

Every three weeks natural water was sampled at site 30, 50 and 60 of Crowden Great Brook (Figure 5) using polypropylene 500 mL bottles pre-washed with HNO_3 (10%, Analytical grade, Fisher chemicals, UK). On each sampling occasion, discharge at each sampling site was measured using dilution gauging technique detailed above. The size separation of samples in the laboratory was performed within 24 hours of collection following available protocol (Gaffney, 2007), detailed as follows.

Samples were filtered through a 1.0 µm cellulose nitrate membrane (Whatman, UK). Filtrate of this sample was size separated using tangential flow ultrafiltration (TFU) plates with pore size cut-off of 0.2 µm, 50 kDa and 10 kDa on a Vivaflow 50 system (Viva Science, UK) with masterflex pumphead (Sartorius, Germany). The sample was circulated through the plate until the left retentate volume is about 50 mL. All of the retentate was kept for analysis whereas 25 mL of the filtrate was kept for analysis before being further size separated on the next plate. DIW adjusted to pH 2 (using HNO_3 (70%, Analytical grade, Fisher chemicals, UK) and then DIW were circulated through each TFU plate for 10 min and cleaning solutions were kept for analysis.

After each sample run the TFU plates were cleaned with 250 ml of 0.5 mM NaOCl in 0.5 M NaOH (Fisher Chemicals, UK) for 30 minutes (this solution was recommended by the supplier Vivaflow for cleaning PES TFU filter membranes). Then about 2 L of 15MΩ DIW was passed through each membrane before a portion of 500 mL of 15MΩ

DIW was concentrated, collected and measured to ensure that the plates are free of carbon before being reused.

2.1.4. Total Organic Carbon measurements

After size separation, the samples were analysed for total organic carbon (TOC) on a TOC analyser (Shimadzu, 5050A, Japan).

Differing instrument performance between sets of standards was attributed to linear drift of the TOC analyser, which was corrected using a programme of linear drift correction developed by Boult (2000). This programme allows correction of data according to both standard concentration and time assuming that the time and thus the drift interval between each measurement is the same. It draws a polygonal surface between standard concentration and the next standard in the period of analysis and sample concentration can be determined as a point on this surface by performing linear interpolation between known concentrations of standard.

2.2. Pyrolysis - Gas Chromatography - Mass Spectrometry analysis

2.2.1. Materials

Chemical reagent used for THM-GC-MS included *n*-hexane (Riedel-de Haën), tetramethylammonium hydroxide (TMAH) (Sigma - Aldrich) and 5α-androstane (Sigma - Aldrich).

2.2.2. Preparation of samples for Py-GC-MS

Natural water samples were collected under two separate normal and higher discharge events. Under higher discharge condition, it was only possible to collect sample from the most accessible site (site 60). Under the normal discharge conditions, it was possible to collect samples from all three monitoring sites (site 30, 50 and 60). Volume of collected water sample is approximately 10L for the most accessible site (site 60) and 3 L for two other sites.

Water samples were separated into different fractions on a Vivaflow 50 system (Viva Science, UK) with masterflex pumphead (Sartorius, Germany) using different TFU membrane sizes. For the sample under higher discharge conditions, two membrane sizes of 0.2 µm and 50 kDa were used. Firstly, the untreated water sample was passed through a blanked 0.2 µm membrane. The retentate was collected into the same sample container to re-circulate through the membrane and was thus continuously concentrated. Meanwhile, the filtrate was passed through a 50 kDa membrane. Retentate from this 50 kDa membrane was similarly collected and concentrated while the filtrate was discarded. At normal discharge condition, an additional membrane size of 10 kDa was able to be employed (**Figure 7**). Similar procedure was performed for the 0.2 µm and 50 kDa membranes; however the filtrate that passed through the 50 kDa membrane was further passed through a 10 kDa membrane. The retentate of the 10 kDa membrane was then collected and concentrated, while the filtrate was discarded. Ideally concentrated volume of samples should be less than 10 mL to allow for efficient freeze-drying in the subsequent step.

Such concentrated volume and TOC of concentrated samples (after being diluted into the calibrated range of measurement) were measured on the TOC analyser as detailed in section **2.1.4**.

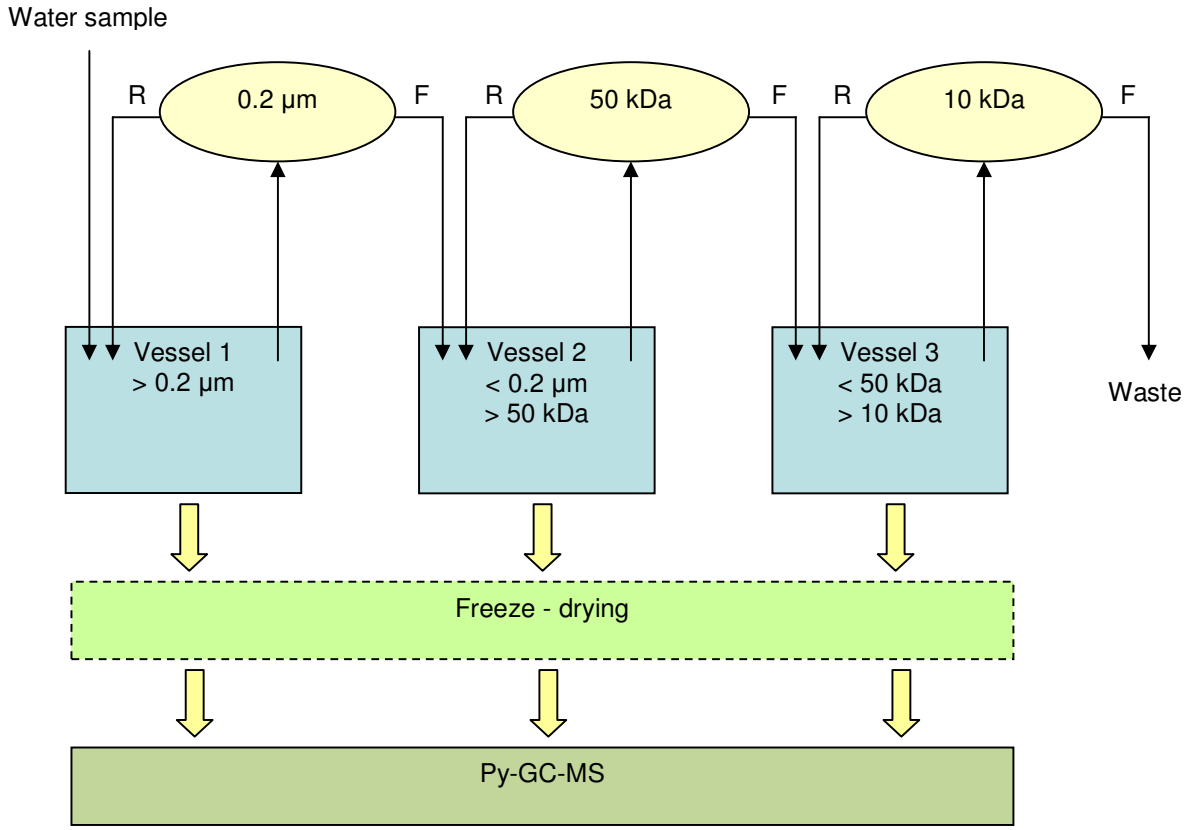

Figure 7 Schematic of NOM size fractionation using TFU plates (0.2 µm, 50 kDa, 10 kDa).

Water sample was led to vessel 1, then passed through 0.2 µm membrane. Retentate (R) was recollected into vessel 1, while Filtrate (F) is led to vessel 2, then passed through 50 kDa membrane. R was recollected into vessel 2, while F is led to vessel 3, then passed through 10 kDa membrane. R was recollected into vessel 3, while F is discarded. Circulation is continued until volume of sample in vessel is *ca.* 10 mL. Samples were then freeze-dried before being analysed on Py-GC-MS.

After each sample run the TFU plates were cleaned with 300 ml of 0.5 mM NaOCl in 0.5 M NaOH (Fisher Chemicals, UK) for 30 minutes (this solution was recommended by the supplier Vivaflow for cleaning PES TFU filter membranes). Then about 2 L of

15MΩ DIW was passed through each membrane before a portion of 500 mL of 15MΩ DIW was concentrated, collected and measure to ensure that the plates are free of carbon before being reused.

Concentrated samples were frozen using liquid nitrogen and then freeze-dried using an integrated freeze-drying pumping system (Edwards High Vacuum International Model RV12). Dried sample obtained was then evenly grounded to ensure homogeneity before being analysed on Py-GC-MS in the presence of TMAH.

2.2.3. Pyrolysis - Gas Chromatography - Mass Spectrometry measurements

Online thermally assisted hydrolysis and methylation (THM) in the presence of TMAH was performed based on a published method (Mason *et al.*, 2009) on a pulsed mode open integrated pyrolysis system. The system employs a CDS 5200 pyroprobe unit (Analytical Inc., Pyroprobe 5000 series) fitted with an inner platinum coil which was interfaced with an 7890A gas chromatograph (Agilent Technologies) coupled to a 5975C MSD (Agilent Technologies) mass spectrometer operated in electron ionization (EI) mode (scanning a range from m/z 50-600 at 2.7 scan s^{-1}, ionisation energy 40 eV).

0.2 - 1.0 mg of sample (depending on the amount of sample available) was weighed into pre-combusted quartz pyrolysis tube (plugged with pre-combusted silica wool) followed by the addition of 1 - 5 µL of a 0.48 $mgmL^{-1}$ 5α-androstane in hexane solution (depending on the amount of sample used) using a pre-rinsed micro-syringe. The tube was inserted into the platinum coil of the CDS 5200 Pyroprobe and dried at 80 ^{o}C for 20s to remove any trace of solvent. 10 µL of an aqueous solution of

tetramethylammonium hydroxide (TMAH) was added and allowed to soak into the sample for *ca.* 2 minutes. The tube was inserted into the platinum coil, interface temperature was raised from 60 ^{o}C to 300 ^{o}C and thermochemolysis was conducted by heating the sample to 300 for 10 s (20 ^{o}C ms^{-1} temperature ramp). Separation of the thermochemolysis products was achieved using a HP-5MS fused column (J+W Scientific; 5% diphenyl-dimethylpolyolsiloxane, 30 m, 0.32 mm i.d., 0.25 µm film thickness) with helium as a carrier gas (flow rate 1 mLmin^{-1}). A 1:75 split ratio was used for samples less than 1.0 mg or 1:150 ratio for samples containing 1.0 mg of material. The GC oven was programmed to rise at a rate of 3 ^{o}Cmin^{-1} from 50 ^{o}C to 220 ^{o}C followed by 20 ^{o}Cmin^{-1} to 300 ^{o}C were it was kept isothermal for 15 min. The transfer line and injector temperatures were set at 350 ^{o}C, the heated interface at 280 ^{o}C and the MS quadrupole at 150 ^{o}C. Duplicate samples were run for each fraction of each site wherever sufficient amount of sample was available.

Compounds were identified using their characteristic ion fragment patterns and corresponding retention times with reference to previous literature and the NIST 92 spectral library.

2.2.4. Mass yield and analysis parameters

Method of calculating mass yield and analysis parameters was adapted from Mason *et al.* (2009).

Individual amount of each identified thermochemolysis component normalised to 1 mg of OC (µg/mg OC) was calculated using internal standard 5α-androstane and OC concentration (mg OC/mg sediment of sample).

Contribution of individual lignin sub-groups (including syringyl (S), guaiacyl (G) and *p*-hydroxyphenyl (P) sub-group) ΣS, ΣG and ΣP respectively was also obtained as follows. ΣS = Σ(S4 + S5 + S6 + S7 + S8 + S10 + S14 + S15) normalised to 1 mg of OC (µg/mg OC). ΣG = Σ(G5 + G6 + G7 + G8 + G14 + G15 + G18) normalised to 1 mg of OC (µg/mg OC). ΣP = Σ(P6 + P18) normalised to 1 mg of OC (µg/mg OC).

Λ1 was calculated as the sum of total amounts of methylated carbohydrate derivatives Σ(MC1 + MC2 + MC3 + MC4 + MC5 + MC6) normalised to 1 mg of OC (µg/mg OC). *Λ2* was calculated as the sum of the total lignin peaks Σ(P6 + G5 + G6 + S4 + G7 + G8 + P18 + S5 + S6 + G13 + S7 + S8 + G14 + G15 + S10 + G18 + S14 + S15) normalised to 1 mg of OC (µg/mg OC).

The syringyl/guaiacyl (S/G) ratio which can be used to determine the contribution of angiosperm *vs.* gymnosperm vegetation input was determined by dividing Σ(S4 + S5 + S6) (µg/mg OC) by Σ(G4 + G5 + G6) (µg/mg OC).

The cinnamyl/guaiacyl (C/G) ratio which can be used to assess the contribution of non-woody tissues of vascular plant was determined by dividing the cinnamyl amounts Σ(P18 + G18) (µg/mg OC) by the guaiacyl amounts Σ(G4 + G5 + G6) (µg/mg OC).

Chapter 3 – Physical characterisation of natural organic matter in upland *ombrotrophic* peat using Tangential Flow Ultrafiltration

3.1. Performance of Tangential flow ultrafiltration technique

For the convenience of notation, DOC in this chapter refers to <0.2 µm OC, and thus collectively includes COC and DOC defined in **Chapter 1**.

The method of physical (PSD) characterisation of NOM using TFU was performed on three monitoring sites of Crowden Great Brook. OC concentrations of size fractions <1 µm, >0.2 µm; <0.2 µm, > 50 kDa; <50 kDa, >10 kDa and <10 kDa were obtained at measurable quantities.

Table 1 Example of OC (mgL^{-1}) of different PSD fractions. Samples were collected from three monitoring sites during a sampling visit in April 2009. The sites were listed with discharge value (if available).

Site	<1 µm >0.2 µm	<0.2 µm > 50 kDa	<50 kDa >10 kDa	<10 kDa
Vegetated sub-catchment (Q = 5.5 Ls^{-1})	0.44	0.72	1.37	0.72
Eroded sub-catchment	2.30	0.31	0.48	0.32
Whole catchment site (Q = 14.3 Ls^{-1})	1.95	0.27	0.55	0.28

As presented in **Table 1**, OC of different PSD fractions was found varied spatially among different monitoring sites of the catchment. For instance, OC of the <1 µm, >0.2 µm fraction was found highest at the eroded sub-catchment (2.30 mgL^{-1}) and lowest at vegetated site (0.44 mgL^{-1}). However, such data cannot be used for any further speculation due to the short length of the data set and other factors such as rainfall and discharge variability. Instead, the PSD and discharge data obtained during the course of this work will be combined with available data from Todman (2005) and Gaffney (2007) as such potential long-term spatial and temporal change could be observed.

3.2. Dissolve organic carbon flux and discharge

In order to investigate the change of carbon storage of the catchment over the years of available data records, DOC flux (<0.2 µm) at the three monitoring sites was calculated based on the PSD and discharge data from this work and available records from previous works (Todman, 2005; Gaffney, 2007). The <0.2 µm fraction was selected due to the maximum data points available. Results are presented in **Table 2**.

Table 2 Dissolve organic carbon (DOC) flux at three monitoring sites of Crowden Great Brook for the years 2003-2009.

Site	DOC flux /$tkm^{-2}y^{-1}$
Vegetated sub-catchment	5.85
Eroded sub-catchment	4.91
Whole catchment site	8.39

NOM flux was found to vary spatially with the highest accumulated amount at the whole catchment site. This value 8.39 $tkm^{-2}y^{-1}$ is comparable to that reported previously by Gaffney (2007) for the same site in 2003 (9.50 $tkm^{-2}y^{-1}$). It also has the same order of magnitude to DOC flux results reported in similar upland erosion studies, including 31 $tkm^{-2}y^{-1}$ (Evans *et al.*, 2006), 34.4 $tkm^{-2}y^{-1}$ (Francis, 1990), and 38.82 $tkm^{-2}y^{-1}$ (Labadz *et al.*, 1991), which may affirm the validity of the method in quantifying DOC flux. Erosion seems to have occurred to a slightly larger magnitude at the vegetated sub-catchment than the eroded site during the period, as shown by the slightly higher DOC flux value of 5.85 $tkm^{-2}y^{-1}$ compared 4.91 $tkm^{-2}y^{-1}$ respectively.

In addition, it was also attempted to investigate the relationship between discharge and DOC concentration (<0.2 µm) by plotting log discharge against log DOC concentration in the vegetated, eroded sub-catchment and whole catchment site (**Figure 8-10**).

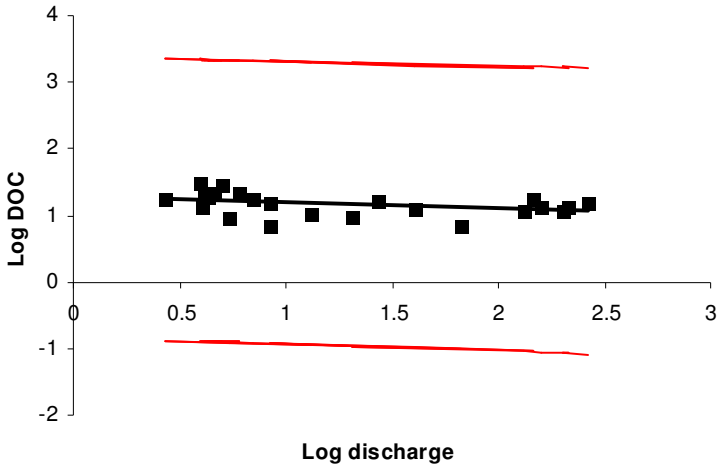

Figure 8 Relationship between Log DOC (<0.2 µm) and Log discharge for the vegetated sub-catchment. Pearsons product moment correlations: R = 0.36, t = 1.81, p = 0.09, slope = -0.09. Red lines indicates SE at 95% confidence.

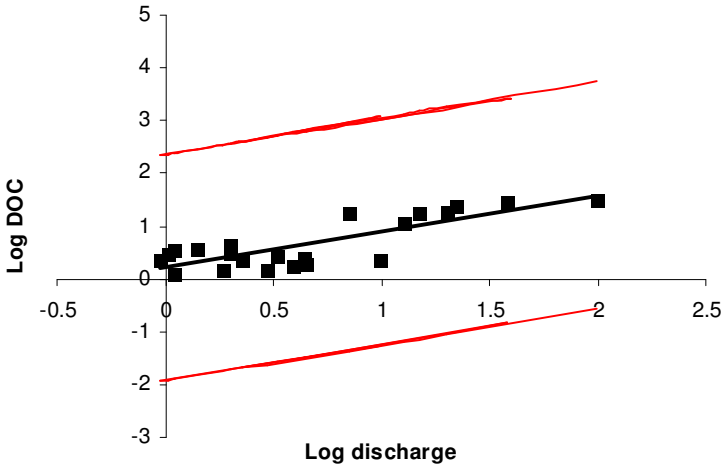

Figure 9 Relationship between Log DOC (<0.2 µm) and Log discharge for the eroded sub-catchment. Pearsons product moment correlations: R = 0.82, t = 6.29, p = 3.8E-06, slope = 0.69. Red lines indicates SE at 95% confidence.

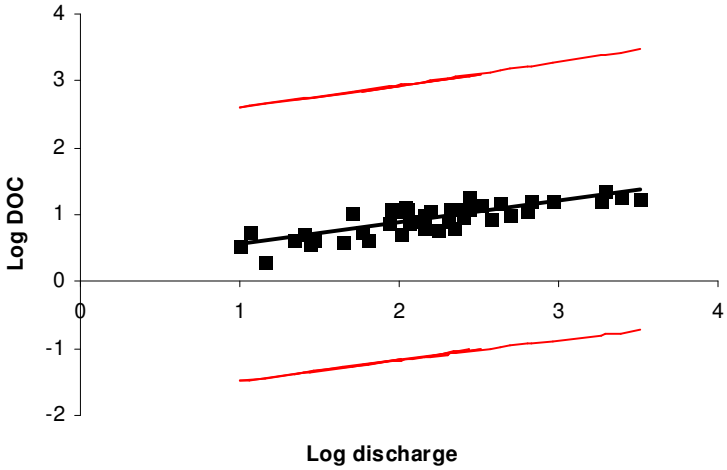

Figure 10 Relationship between Log DOC (<0.2 µm) and Log discharge for the whole catchment site. Pearsons product moment correlations: R = 0.81, t = 8.83, p = 1E-10, slope = 0.32. Red lines indicates SE at 95% confidence.

In the vegetated sub-catchment (**Figure 8**) there was no significant relationship between the two parameters log DOC and log discharge due to low Pearson correlation value (R = 0.36). However in the eroded sub-catchment (**Figure 9**), log DOC was found to increase with log discharge as evidenced by a high Pearson correlation R value of 0.82. This positive relationship is significant at 95% confidence. At the whole catchment site, a positive relationship between log DOC and log discharge was also found significant at 95% confidence (**Figure 10**).

It is interesting to observe such positive relationship at the eroded and whole catchment site since it would be expected that increasing precipitation (or higher discharge condition) would dilute and cause a negative feedback on OC concentration. Hence the relationship observed herein must be caused by another more dominant factor.

That factor may be the degree of erosion and vegetation cover at the site, which could also explain the variability between having no relationship at the vegetated sub-catchment and having a significant positive relationship at both the eroded and whole catchment site. The absence of correlation in the vegetated sub-catchment could be attributed to the 'sponge'-like characteristic of such site (Holden and Burt, 2003). The intact vegetation which has a high capacity of water retention may retain and release water slowly over time. As a result, DOC does not show a direct relationship with discharge. In comparison, the denuded sub-catchment which has much less vegetation cover would be more susceptible to erosion during higher discharge events.

Highest slope value (0.69) of log DOC and log discharge correlation found at this site confirms such rationale. Based on the locations of the three monitoring sites (**Figure 5**), the positive relationship between DOC and discharge at whole catchment site (site 60) may suggest a great contribution of NOM from the eroded peat on the west side of the water stream.

Meanwhile, three possible sources of error exist for such quantification of flux. They include the absence of certain discharge events due to the discontinuous nature of the sampling method, the "pooling" effect created by including a special high discharge event (especially where a positive relationship exists) and the degree of OC concentrations reduced by dilution as a consequence of increased precipitation into the stream. As an example, **Figure 11** was plotted from continuous discharge data obtained *in-situ* at the whole catchment site during 15/12/2008 - 28/02/2009 (Gaffney, 2009) with 12 main discharge events of different magnitude and duration. However during this period, the sites were only visited twice on 26/01/2009 and 26/02/2009, which only covers two single points of the period. Therefore it was not possible to justify any temporal changes of carbon storage of the catchment or to predict any future scenarios based on such DOC flux data.

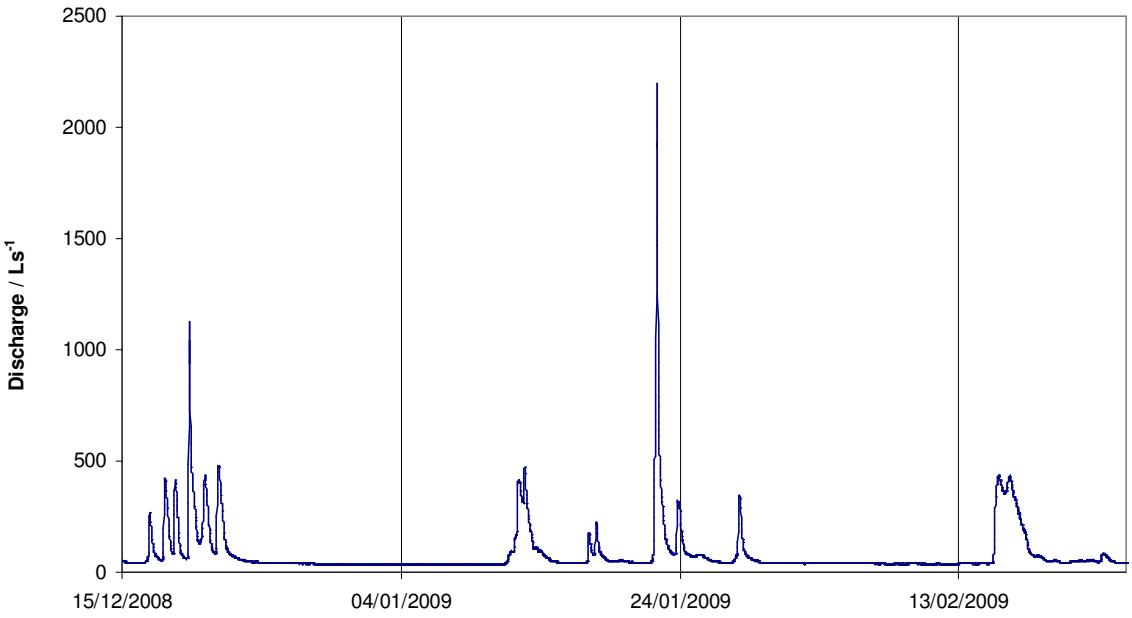

Figure 11 Continuous discharge Q /Ls^{-1} monitored *in-situ* at the whole catchment site during 15/12/2008 - 28/02/2009. Data was obtained from Gaffney (2009).

3.3. Particle size distribution and discharge

Although the available data set covers a relatively long period, data on some size fractions or discharge records were occasionally unavailable or supply of TFU plate (pore size 0.2 µm) was discontinued for almost one year (personal communication, Gaffney, 2009), thus leading to a smaller number of complete data to examine the relationship between percentage of PSD fractions and discharge. In addition, although the OC concentration of PSD fractions of the eroded sub-catchment was shown measurable in this work, it was found only sufficient for TOC analysis under higher discharge condition (Gaffney, 2007). As a result, the long-term relationship between PSD and discharge was only attempted for site 30 and 60 (**Figure 12-13**).

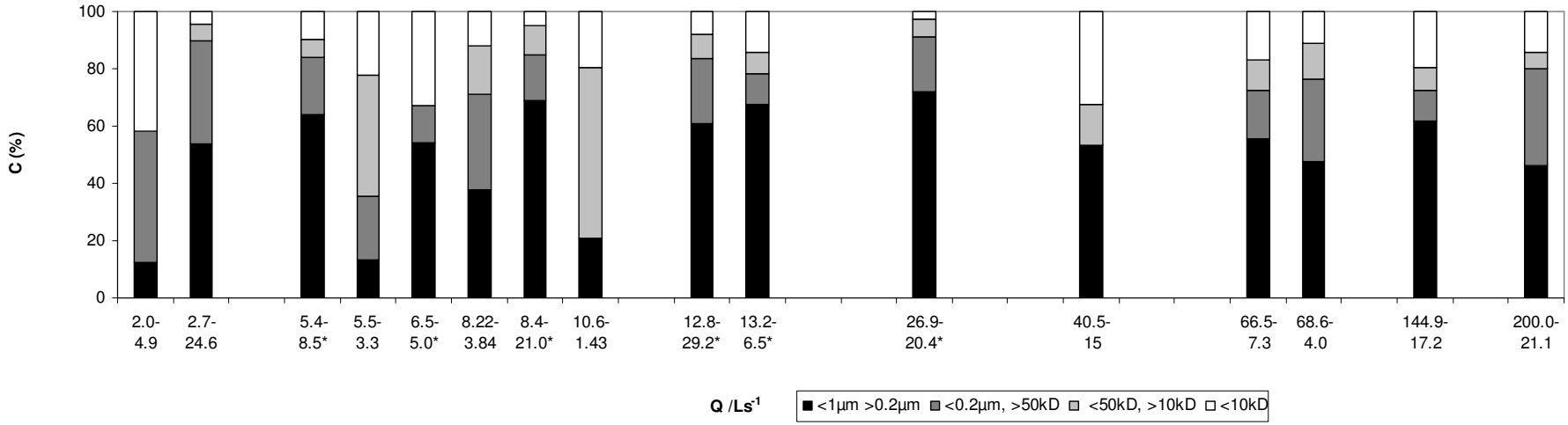

Figure 12 PSD of OC (<0.1 µm) under different discharges (Q) at the vegetated sub-catchment. Beneath each column is the discharge /Ls^{-1} - total carbon concentration /mgL^{-1}. Discharge increases in an approximately linearly manner along the x-axis until a scale break at 68 Ls^{-1}. * indicates results obtained using diafiltration[1] in previous works (Gaffney, 2007).

[1] Gaffney (2007) compared two different approaches in using TFU technique. The first approach which is employed in this work was termed con-filtration. As it involved circulating sample through TFU plates until a small volume of retentate (typically <50 mL) was obtained, it was thought to potentially facilitate coagulation of materials. Thus a second approach was also studied, which involved maintaining a constant volume of retentate in the retentate vessel using airtight sealing with fluid being siphoned from another vessel to replace the collected filtrate. This method was termed diafiltration. Diafiltration was later shown to couple with incomplete separation and thus con-filtration was preferred (Gaffney, 2007).

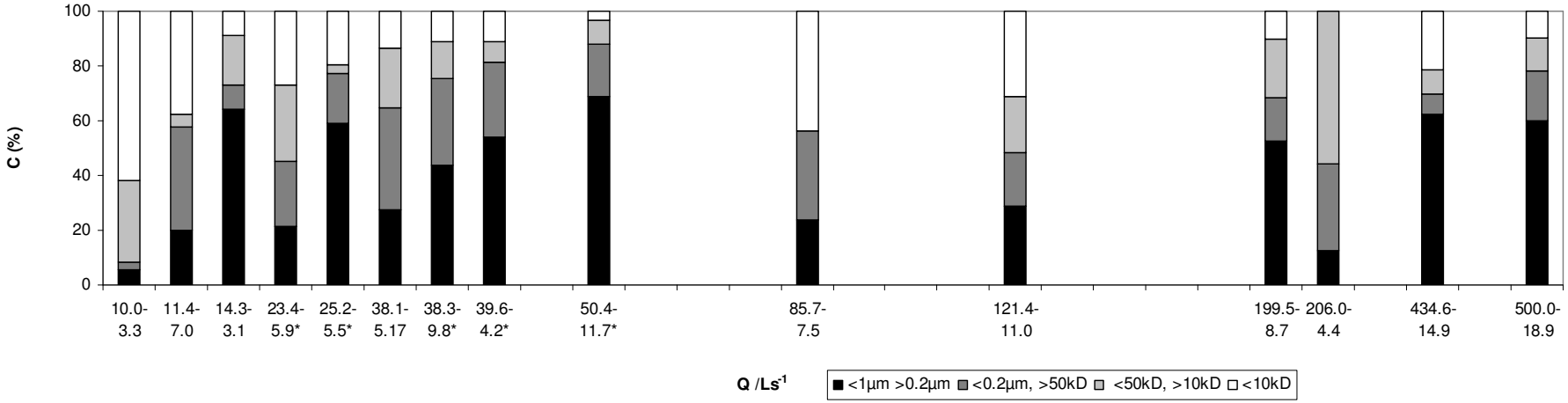

Figure 13 PSD of OC (<0.1 µm) under different discharges (Q) at the whole catchment site. Beneath each column is the discharge (Ls^{-1}) - total carbon concentration (mgL-1). Discharge increases in an approximately linearly manner along the x-axis until a scale break at 206 Ls^{-1}. * indicates results obtained using diafiltration in previous works (Gaffney, 2007).

As can be seen from **Figure 12-13**, the PSD of OC (<1 µm) shows no correlation with discharge at both vegetated and whole catchment site with a high degree of fluctuation (both positive and negative) in all size fractions as discharge increases. Nevertheless, it seems that the vegetated sub-catchment has a higher proportion of the largest fraction (<1 µm, >0.2 µm) with 75% of cases having a proportion of this fraction greater than 50%.

Chapter 4 – Chemical characterisation of natural organic matter in upland ombrotrophic peat using Pyrolysis - Gas Chromatography - Mass Spectrometry

4.1. Identification of thermochemolysis products

Identification of thermochemolysis products from Crowden Great Brook samples using Py-GC-MS was possible. Three main groups of components could be identified including lignin derivatives, methylated carbohydrate derivatives and fatty acid methyl esters moieties. Component and characteristic fragment *m/z* details are presented in **Table 3**.

The group of lignin thermochemolysis products included methylated syringyl (S), guaiacyl (G) and *p*-hydroxyphenyl (P) phenol derivatives (compound notations S, G, P were adopted from previous works by Mason *et al*., 2009; Vane *et al.* 2001). Among these derivatives, G6, P18, S6 and G18 are considered the major building blocks of lignin (Mason *et al.,* 2009). The presence of *p*-courmaric acid (P18) and ferulic acid (G18) which are cinnamyl derivatives suggests the origin in non-woody vascular plants (Mason *et al*., 2009; Vane *et al.* 2001). The presence of G14/G15 and S14/S15 peaks suggest some oxidation of lignin (Mason *et al*., 2009).

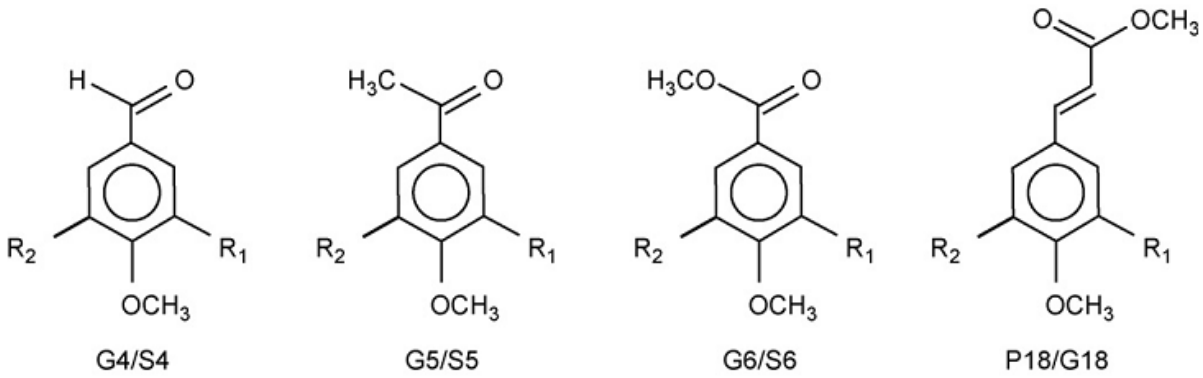

Figure 14 Structure of typical lignin thermochemolysis products. Figure was taken from Mason *et al.* 2009. P group: R_1 = H, R_2 = H; G group: R_1 = OCH_3, R_2 = H; S group: R_1 = OCH_3, R_2 = OCH_3. One of these typical products G_4 was not found in all chromatograms herein, possibly due to the small size of the peak and/or peak overlapping.

The presence of obtained lignin derivatives were very comparable to products obtained from thermally assisted hydrolysis and methylation of soil organic matters (SOM) (Mason *et al*., 2009; Vane *et al.* 2001), suggesting that water draining the catchment and entering the stream carries terrestrial SOM. This however requires further quantification and examination, which will be discussed in the next section.

Many of these guaiacyl, syringyl and *p*-hydroxyphenyl derivatives were previously reported as major compounds found in chromatogram of HA (Fabbri *et al*., 1996) such as P6, G5, G6, S5, P18 and S6. In addition, lignin has also been known as an important component in humic substances, especially in the surface layer of SOM (Chefetz *et al*., 2000). This suggests an apparent overlap of pedogenic compounds containing in lignin and HA of macromolecular weight and relatively resistant to microbial breakdown. Therefore, lignin derivatives will be included in analysis of pedogenic components of NOM.

Another group of prominent peaks was tentatively assigned as aquagenic methylated carbohydrate (MC) derivatives (Mason *et al.*, 2009). As can be seen from **Table 3**, components of this group share some common *m/z* fragments (*e.g.* 101, 129) and therefore without the availability of reference material could not be completely identified. The numbering order MC1 to MC6 was thus based on order of their corresponding retention time. As discussed earlier in **Chapter 1**, such presence of these polysaccharide-derived components can be regarded as microbially-produced polysaccharide signals and suggests a microbial origin. Similar suggestions were also proposed by Mason *et al.* (2009).

Fatty acid methyl esters (FAMEs) derived from linear saturated and unsaturated fatty acids were also found. These FAMEs could be attributed to the cell lipids of bacteria (Saiz-Jimenez and Hermosin, 1999), and are for instance a part of phospholipids (Killops and Killops, 2005).

A number of other components including 1,2,4-trimethoxybenzene (1,2,4-TMB) and 1,3,5-trimethoxybenzene (1,3,5-TMB) were identified. 1,2,4-TMB has been reported as a TMAH thermochemolysis product of cellulose and starch (Fabbri and Helleur, 1999), and also of beech (Fagus sylvatica L.) leaf litter (Hermosin and Saiz-Jimenez, 1999). As such components can be readily consumed by microbe and have a relatively rapid turn-over as discussed earlier, their presence in chemical components of NOM is interesting and merits further investigations.

Table 3 List of TMAH thermochemolysis products.

Fatty acid methyl esters are listed with number of carbon chain atoms : number of double bonds

Label	Tentative assignment	Characteristic m/z
1,2,4 TMB	1,2,4-trimethoxybenzene	125, 153, 168
P6	4-methoxybenzoic acid methyl ester	135, 166
1,3,5 TMB	1,3,5- trimethoxybenzene	125, 139, 168
DP	dimethylphatalate (contaminant)	163, 194
MC1	methylated carbohydrate derivative	101, 129, 161, 191
MC2	methylated carbohydrate derivative	101, 129, 166, 173, 196
MC3	methylated carbohydrate derivative	101, 129, 161, 191
MC4	methylated carbohydrate derivative	101, 129, 161, 163, 194
MC5	methylated carbohydrate derivative	101, 129, 147, 177
MC6	methylated carbohydrate derivative	101, 129, 161, 191
FAME1	fatty acid methyl ester 12:0	74, 87, 143, 214
G5	3,4-dimethoxyacetophenone	137, 165, 180
G6	methyl 3,4-dimethoxybenzoate	165, 181, 196
S4	3,4,5-trimethoxybenzaldehyde	125, 181, 196
G7	*cis*-2-(3,4-dimethoxyphenyl)-1-methoxyethylene	151, 179, 194
G8	*trans*-2-(3,4-dimethoxyphenyl)-1-methoxyethylene	151, 179, 194
P18	*trans*-3-(4-methoxyphenyl)-3-propenoate	161, 192, 133
S5	3,4,5-Trimethoxyacetophenone	195, 210, 139
FAME2	fatty acid methyl ester 14:0	74, 87, 143, 242
S6	methyl 3,4,5-trimethoxybenzoate	226, 211, 195
G13	*trans*-1-(3,4-Dimethoxyphenyl)-3-methoxy-1-propene	91, 177, 208
S7	*cis*-1-(3,4,5-Trimethoxyphenyl)-2-methoxyethylene	209, 224, 181
FAME3	fatty acid methyl ester 15:0	74, 87, 143, 256
S8	trans-1-(3,4,5-Trimethoxyphenyl)-2-methoxyethylene	209, 224, 181
G14	threo/erythro-1-(3,4-Dimethoxyphenyl) -1,2,3-trimethoxypropane	166, 181, 270
G15	threo/erythro-1-(3,4-Dimethoxyphenyl) -1,2,3-trimethoxypropane	166, 181, 270
S10	*cis*-1-(3,4,5-Trimethoxyphenyl)-methoxyprop-1-ene	223, 238, 195
G18	*trans*-3-(3,4-Dimethoxyphenyl)-3-propenoate	222, 207, 191
FAME4	fatty acid methyl ester 16:1	74, 87, 143, 268
S14	threo/erythro-1-(3,4,5-Trimethoxyphenyl) -1,2,3-trimethoxypropane	211, 181, 300
FAME5	fatty acid methyl ester 16:0	74, 87, 268
S15	threo/erythro-1-(3,4,5-Trimethoxyphenyl) -1,2,3-trimethoxypropane	211, 181, 300
FAME6	fatty acid methyl ester 18:1	74, 87, 143, 296
FAME7	fatty acid methyl ester 18:0	74, 87, 143, 298

4.2. Analysis of thermochemolysis products

In order to examine the relationship between thermochemolysis components of NOM and their particle size distribution within the catchment, it was attempted to examine the change of such components as the size fraction decreases for vegetated and eroded sub-catchment (under normal discharge condition) and whole catchment site (under both normal and higher discharge condition).

4.2.1. Overview of total ion current

Figure 15-17 show an overview of total ion current (TIC) showing the position and relative intensity (normalised to 1 mg of sample) of thermochemolysis products.

In general, the peak position of thermochemolysis components between different size fractions is very comparable. Complete appearance or disappearance of aquagenic/pedogenic components of interest however was quite limited. An example can be the disappearance of G14 and G15 moving from >0.2 µm to <0.2 µm, >50 kDa fraction at the denuded peat sub-catchment. However, this should not imply any concrete size of such components, as G14 and G15 were still found at both >0.2 µm and <0.2 µm, >50 kDa fractions of the whole catchment site. Instead, this may suggest that such components may have a range of particle size causing their appearance in more than one PSD fraction. Quantifying how their intensity changes as particle size decreases thus can be a better approach.

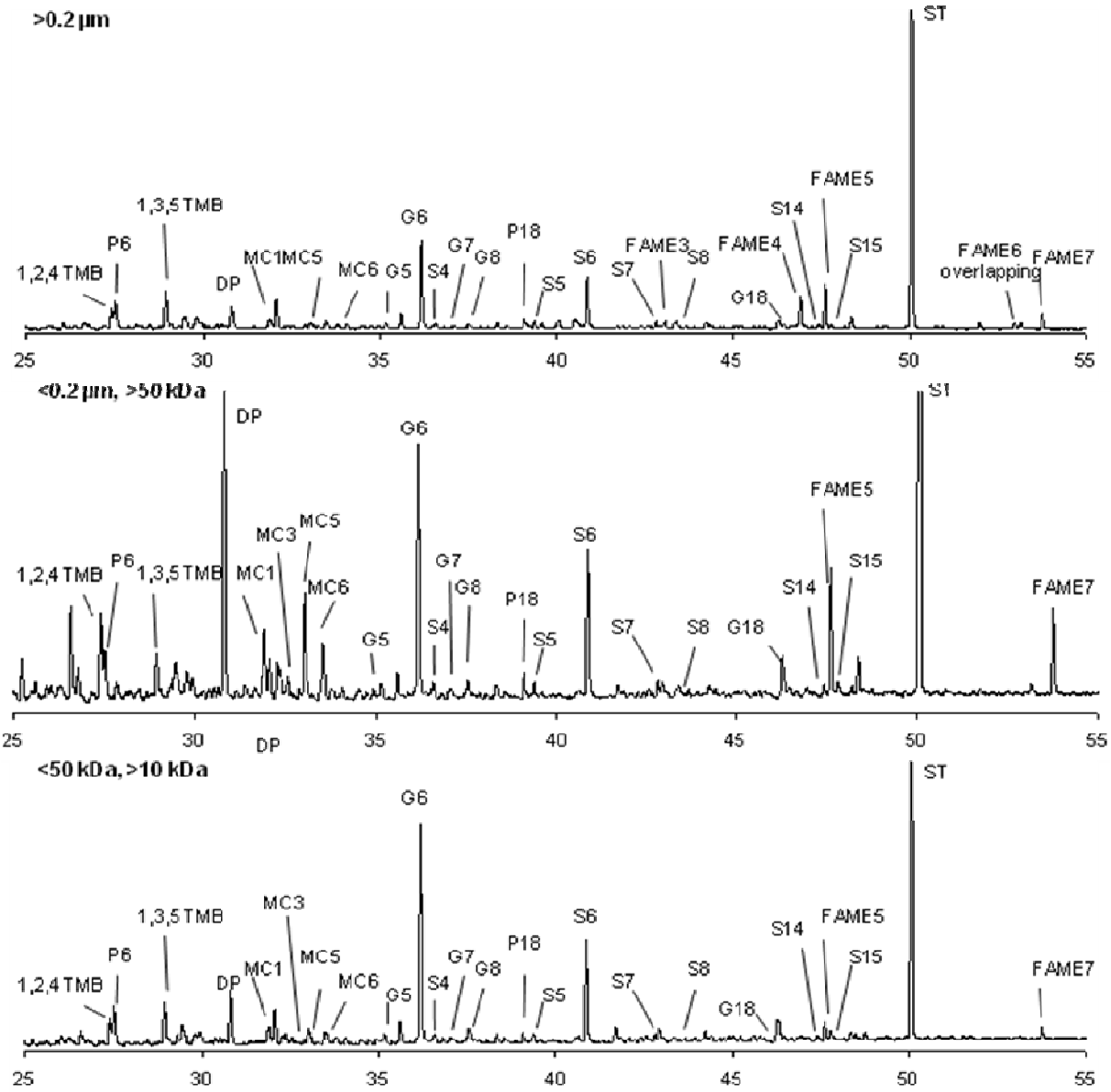

Figure 15 Total ion current (TIC) of thermochemolysis products from different PSD fractions of the vegetated sub-catchment. PSD fractions include >0.2 µm; <0.2 µm, >50 kDa and <50 kDa, >10 kDa. Discharge Q = 5.5 Ls^{-1}. Notations of identified peaks correspond to those compounds listed in **Table 3**.

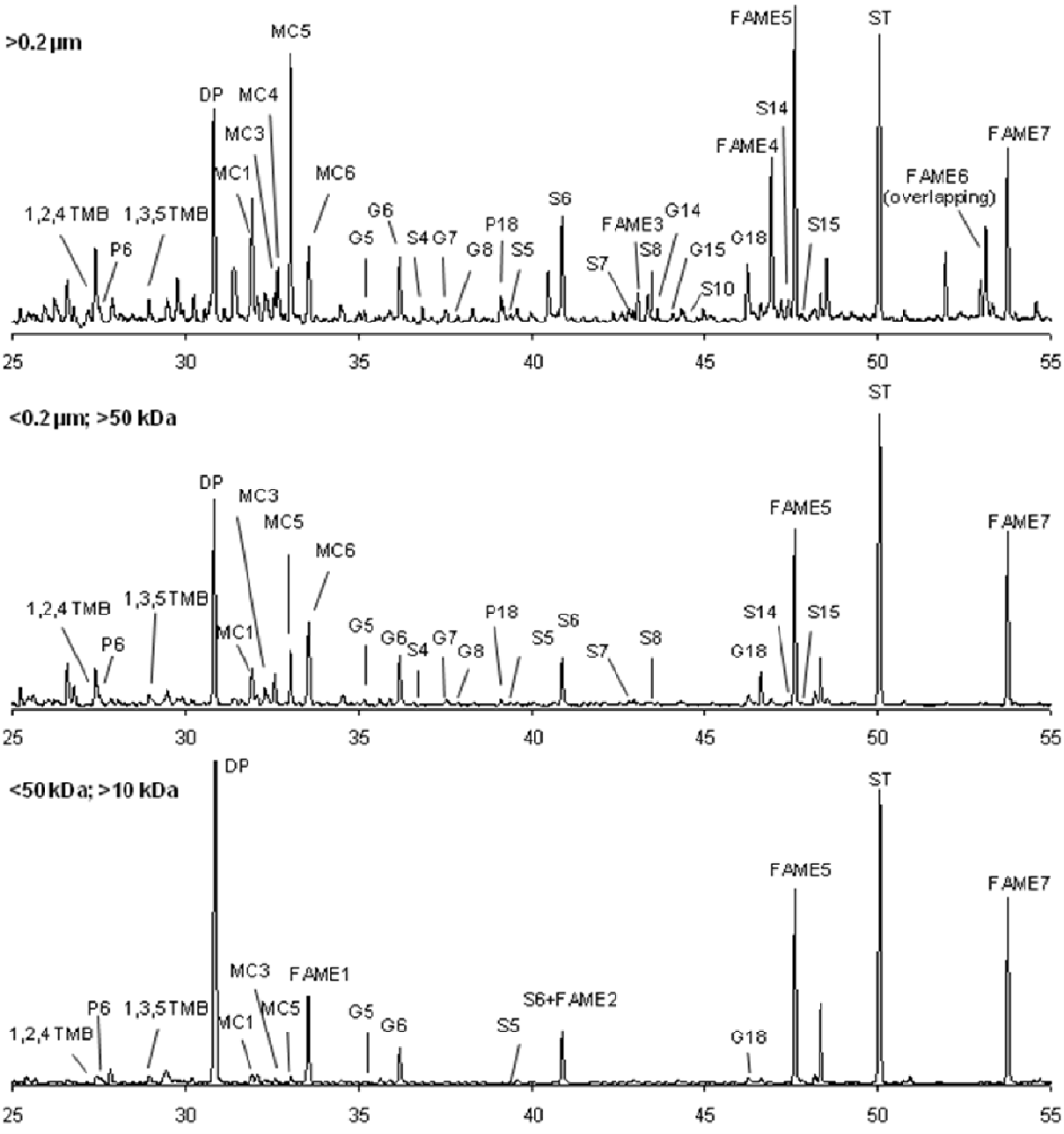

Figure 16 Total ion current (TIC) of thermochemolysis products from different PSD fractions of the denuded sub-catchment. PSD fractions include >0.2 µm; <0.2 µm, >50 kDa and <50 kDa, >10 kDa. Discharge Q = 14.3 Ls^{-1}. Notations of identified peaks correspond to those compounds listed in **Table 3**.

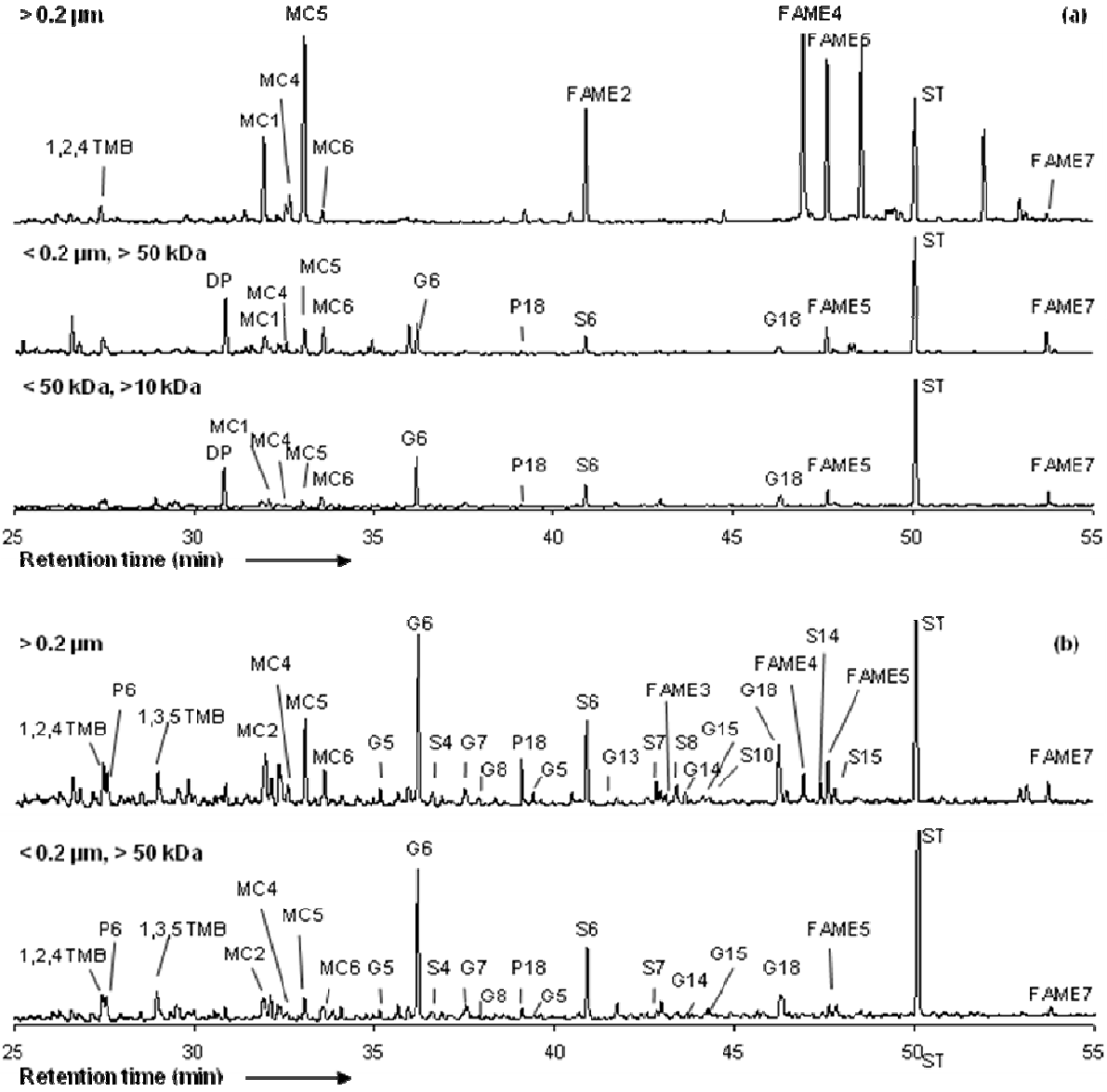

Figure 17 Total ion current (TIC) of thermochemolysis products from different PSD fractions of the whole catchment site under normal (a) and (b) higher discharge condition.

(a) PSD fractions include >0.2 µm; <0.2 µm, >50 kDa and <50 kDa, >10 kDa; Discharge data was unavailable.

(b) PSD fraction includes >0.2 µm and <0.2 µm, >50 kDa. Discharge Q = 44.3 Ls^{-1}.

Notations of identified peaks correspond to those compounds listed in **Table 3**.

4.2.2. Quantification of aquagenic/pedogenic components

As intensity of thermochemolysis products depend upon the concentration of OC available in the sample, in order to obtain a quantitative assessment on the relationship between aquagenic/pedogenic components and the size of fraction, mass yield and analysis parameters were calculated following the method detailed in Section **2.2.4**. A calculation method was adapted from previous published works of Mason *et al.*, 2009. Results are detailed in **Appendix 1**. (**Appendix 2** was tabulated similarly to **Appendix 1** for the other groups of thermochemolysis products for reference purpose). *Λ1* and *Λ2* parameters are illustrated in **Figure 18** and **Figure 19**, respectively.

It was expected that samples from the vegetated site (site 30) would have a higher amount of aquagenic polysaccharide-derived components whereas those from eroded site (site 50) would have a higher amount of pedogenic lignin-derived products, as discussed earlier in **Chapter 1**.

However as can be seen from **Figure 18**, *Λ1* parameter of the carbohydrate moieties from the vegetated site was lowest at all three PSD fractions compared to the eroded and whole catchment site under normal discharge condition and thus requires further investigation. Nevertheless, **Figure 19** shows peak amounts of lignin at the eroded sub-catchment which matches the expectation.

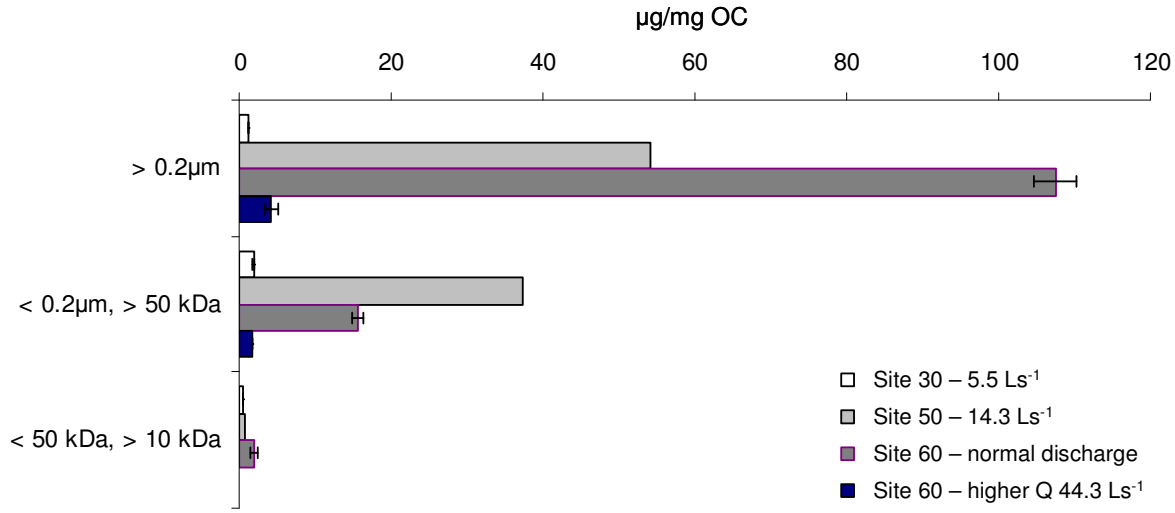

Figure 18 *Λ1* parameter (µg/mg OC) at different PSD fractions and sites. PSD fractions included >0.2 µm; <0.2 µm, >50 kDa and <50 kDa, >10kDa. Site was labelled with discharge (Ls^{-1}). Error bar was calculated as standard deviation of duplicate measurements where applicable. At site 60 (Q = 44.3 Ls^{-1}), fraction <50 kDa, >10 kDa was unavailable.

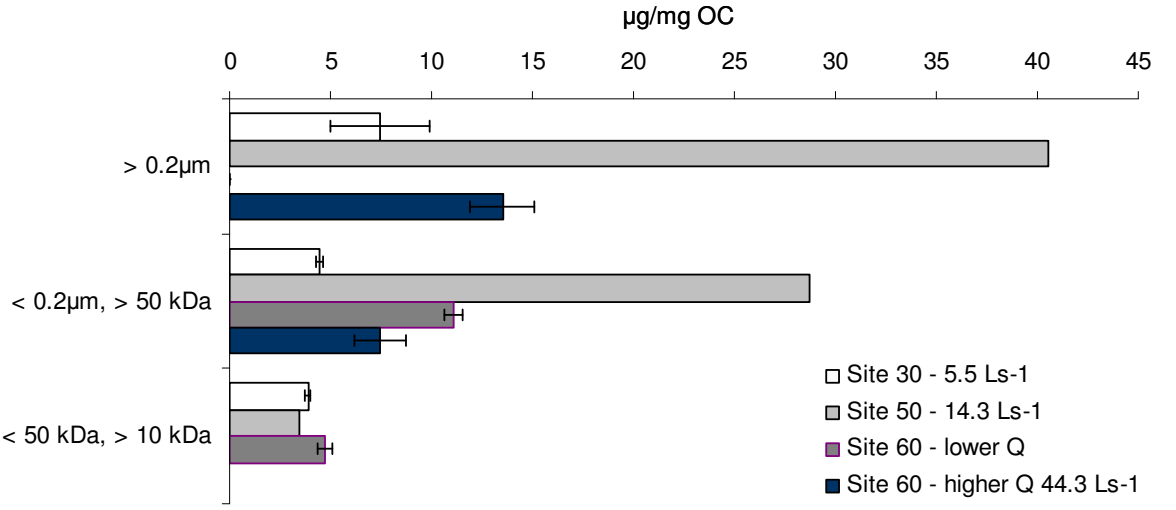

Figure 19 *Λ2* parameter (µg/mg OC) at different PSD fractions and sites. PSD fractions included >0.2 µm; <0.2 µm, >50 kDa and <50 kDa, >10kDa. Site was labelled with discharge (Ls^{-1}). Error bar was calculated as standard deviation of duplicate measurements where applicable. At site 60 (Q = 44.3 Ls^{-1}), fraction <50 kDa, >10 kDa was unavailable.

Both *Λ1* and *Λ2* parameters seem to generally decrease with decreasing particle size fractions. The sharpest decline in polysaccharide-derived components was that of the whole catchment site (site 60) under normal discharge condition, where *Λ2* decreased from 107.49 µg/mg OC (>0.2 µm fraction) to 15.55 µg/mg OC (<0.2 µm, >50 kDa fraction) to 1.96 µg/mg OC (<50 kDa, >10 kDa fraction) (**Figure 18**). Meanwhile, the most distinctive decrease of *Λ1* was observed at the eroded sub-catchment (site 50) where *Λ2* decreased from 40.59 to 28.75 then to 3.44 µg/mg OC moving from the largest to the smallest PSD fraction (**Figure 19**). There was also an exception where *Λ1* increased from 1.26 to 1.85 µg/mg OC moving from larger fraction >0.2 µm to smaller fraction <0.2 µm, >50 kDa at the vegetated site (site 30) though the increasing magnitude and the amounts of polysaccharide found at the site were both small.

Λ1 and *Λ2* parameters were also shown to vary between normal and higher discharge condition. Under higher discharge condition, the PS components seemed to be much lower while the lignin components are much higher and *vice versa*. In particular, the >0.2 µm fraction of sample from site 60 (under normal discharge condition) has the highest amount of polysaccharide (107.49 µg/mg OC) and no lignin (**Figure 18-19**).

4.2.3. Contribution of major lignin derivatives

It was also attempted to understand the contribution of major lignin derivative G6, S6, P18 and G18 to *Λ2* value as this could provide an insight into the dynamic changes of lignin at different fractions and different sampling sites. **Figure 20** was plotted from individual mass yield of each component (µg/mg OC) listed in **Appendix 1**.

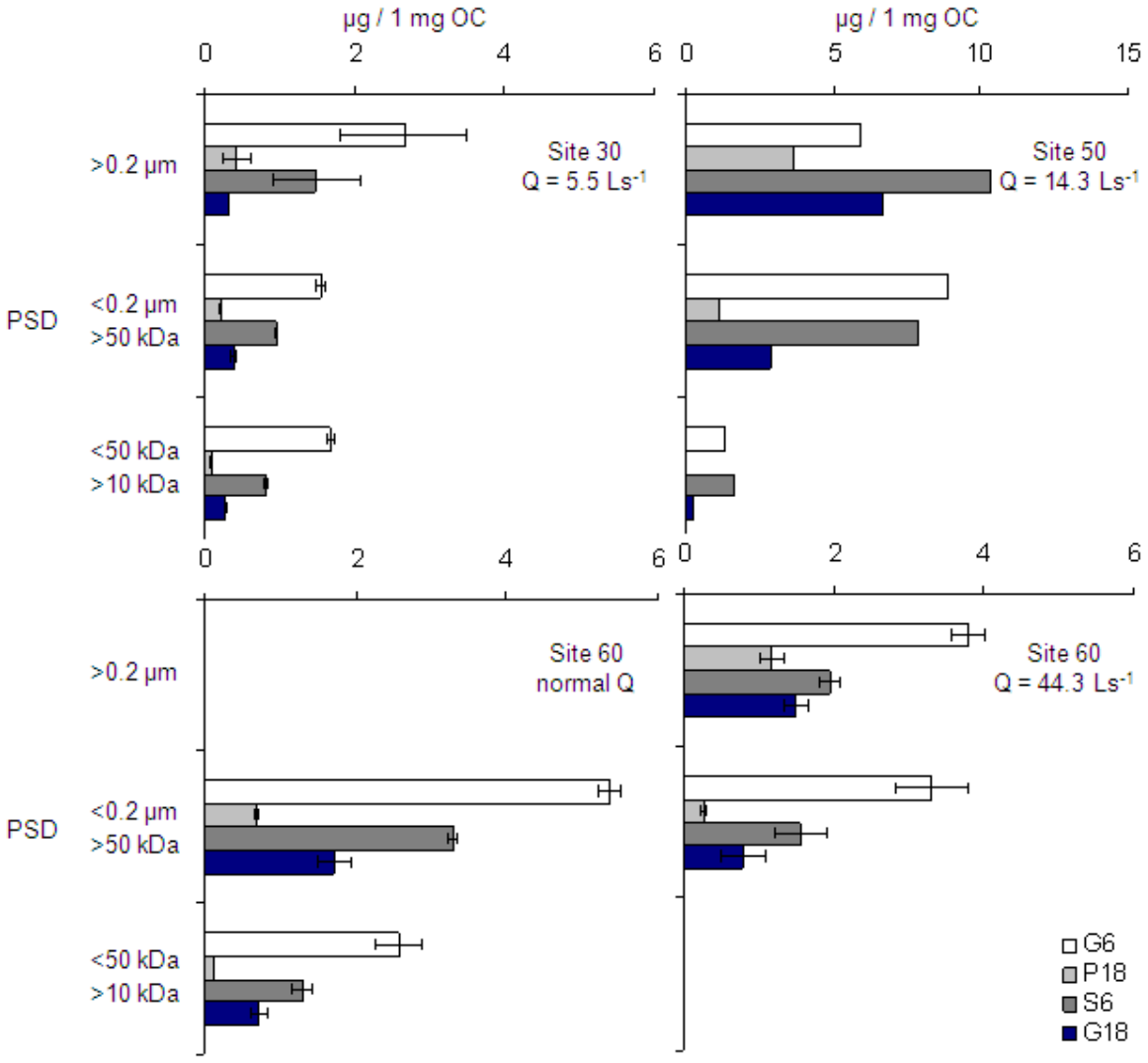

Figure 20 Changes in major lignin thermochemolysis products contributing to *Λ2* parameter. Results were presented for the vegetated sub-catchment (site 30), denuded sub-catchment (site 50) under normal discharge condition and whole catchment site (site 60) under normal and higher discharge condition.

As presented in **Figure 20**, G6 (3,4-dimethoxybenzoic acid methyl ester) seems to be the dominant lignin phenol at all fraction sizes, under both higher and normal discharge conditions for all three sites. Such observation can be correlated to previous published works by Nierop (2001) and Mason *et al.* (2009), where G6 was also found as the major lignin in the soil organic horizon, suggesting a close relationship between components of aqueous NOM and their terrestrial origin. The only exception where G6 was not the dominant derivative was at fraction >0.2 µm of the eroded site, where the amount S6 (3,4,5-trimethoxybenzoic acid methyl ester) was highest amount and magnitudes of all other major contributors (G6, P18, G18) were relatively high. These major derivatives also seem to follow the decreasing trend with decreasing PSD size.

Ferulic acid which is indicative of intact lignin or G18 was highest at the eroded sub-catchment (6.63 µg/mg OC), which accounts for about 35% of lignin derivatives at the site.

4.2.4. Assessment of angiosperm/gymnosperm and vascular vegetation input

The syringyl/guaiacyl (S/G) ratio which is conventionally used in assessing the contribution of angiosperm/gymnosperm was calculated as detailed in Section **2.2.4**, from which **Figure 21** was plotted. Although Mason *et al.* (2009) also used G4 (3,4-dimethoxybenzaldehyde) in their calculation of S/G ratio, G4 was not found in all obtained chromatograms and thus not included in the calculation of S/G ratio. This however should not affect the results, since S6 and G6 are the dominant lignin derivatives which could significantly determine the value of the S/G ratio.

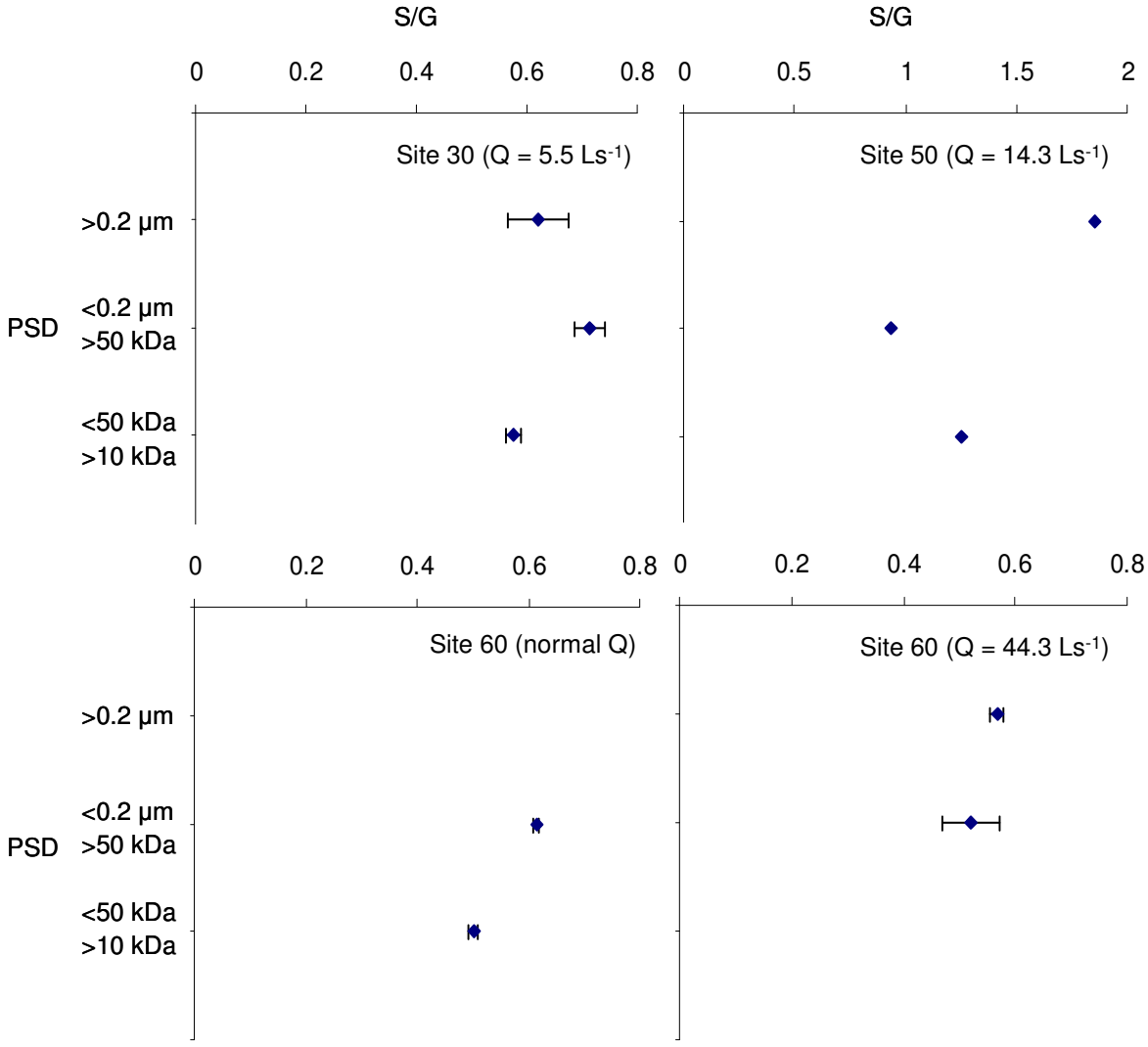

Figure 21 Change in syringyl/guaiacyl (S/G) ratio. Results were presented for the vegetated sub-catchment (site 30), denuded sub-catchment (site 50) under normal discharge condition and whole catchment site (site 60) under normal and higher discharge condition.

There was no clear change of the S/G ratio observed as PSD decreases. At the vegetated sub-catchment (site 30) under normal discharge condition and whole catchment site (site 60) under both normal and higher discharge condition, the ratio is well below 0.8, suggesting a greater magnitude of contribution from gymnosperm. As mentioned earlier, *ombrotrophic* peat is characterised by relatively poor nutrient conditions with abundant mosses (Zaccone *et al.* 2008), such result is promising and assures the value of obtained data. The role of angiosperm was slightly enhanced at site 50, especially at the >0.2 µm fraction, which could be attributed to the relatively high amount of 3,4,5-trimethoxybenzoic acid methyl ester (S6) found at this site as discussed in the previous section.

The cinnamyl/guaiacyl (C/G) ratio which is indicative of the input of vascular plants was calculated as detailed in Section **2.2.4** from which **Figure 22** was plotted. It seems that the smallest particle size fraction (<50 kDa, >10 kDa) tends to couple with the lowest values of C/G ratio. There is also a general decreasing C/G ratio as the particle size fraction decreases, which was observed at the eroded sub-catchment under normal discharge condition and the whole catchment site under both normal and higher discharge condition. Highest C/G ratio was found at the eroded site, which can be attributed to the highest level of the cinnamyl compound ferulic acid (G18) found at this site as mentioned above.

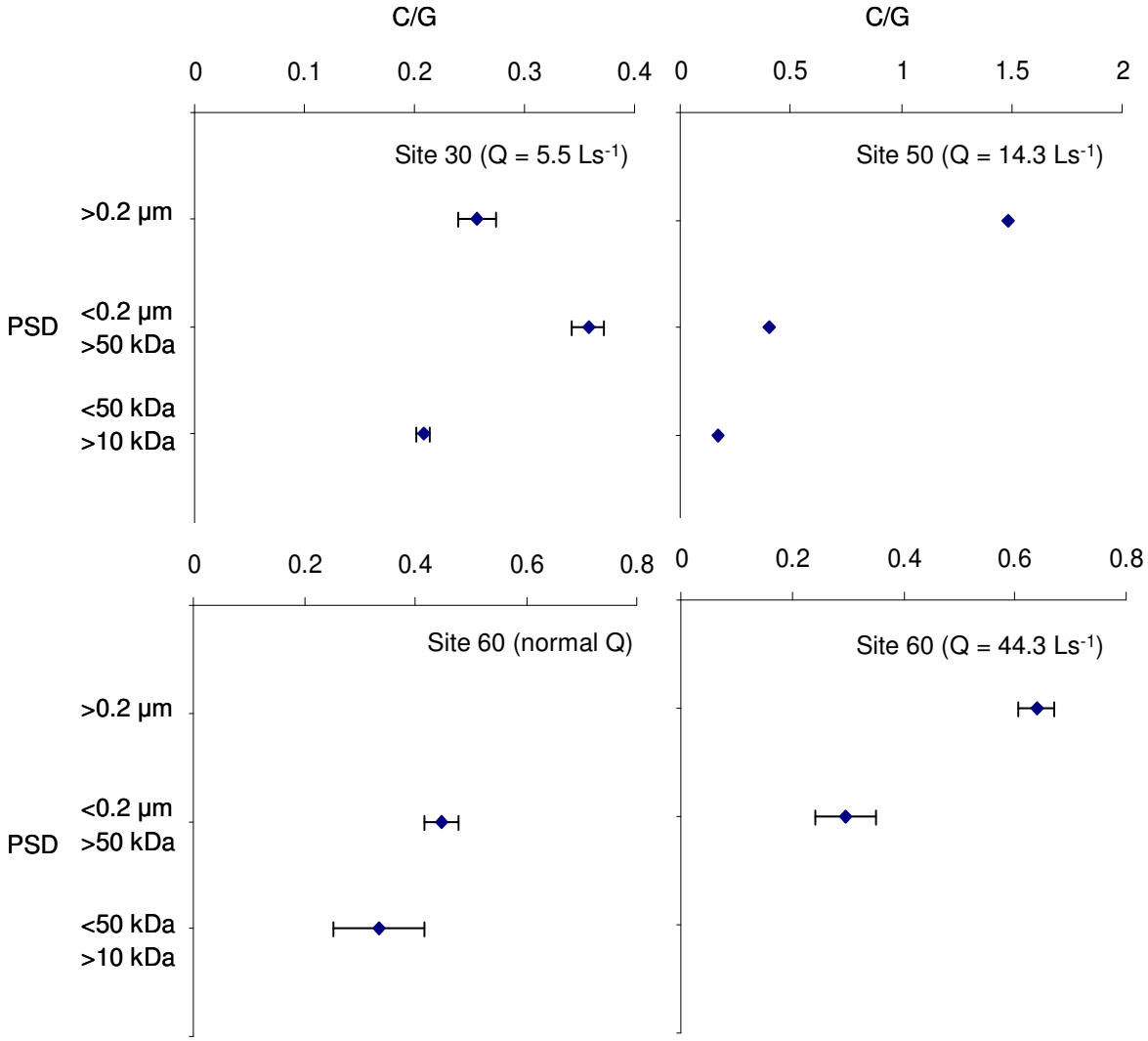

Figure 22 Change in cinnamyl/guaiacyl (C/G) ratio. Results were presented for the vegetated sub-catchment (site 30), denuded sub-catchment (site 50) under normal discharge condition and whole catchment site (site 60) under normal and higher discharge condition.

Chapter 5 – Conclusions

5.1. Method assessment and optimisation

The method of physical (PSD) characterisation of NOM in upland *ombrotrophic* peat using TFU has been developed over certain time and adopted successfully in this project. Improvement of this method could involve addressing the issue of negligible OC concentration of PSD fractions at the eroded sub-catchment under low discharge condition as reported by Gaffney (2007), which would affect the applicability and robustness of the method though such observation was not the case herein. Furthermore, in order to obtain meaningful data from PSD data, additional records over long time are also required.

Meanwhile, the method of chemical characterisation of NOM using Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS) in the presence of TMAH is a newly developed method which has only been performed on samples from three monitoring sites (under normal discharge condition) and only one whole catchment site (under higher discharge condition), thus indeed requiring further records to be obtained.

At the same time, limitation of this method is the potential coagulation of materials on the membrane of TFU plates during concentration prior to Py-GC-MS analysis. This risk of sediment coagulation may not only reduce the speed of particles being passed through the membrane but may also lead to settlement/clogging during the

concentration process may alter the actual pore size of the plate and limit the separating precision achieved. The issue is even more relevant as in this work samples were not pre-treated using the 1 µm membrane in order to generate enough materials for Py-GC-MS measurements, leading to a larger amount of materials being circulated through the plate. In addition, the concentrate volume required was very small (typically <10 mL) to reduce freeze-drying time, hence the amount of materials retained would be higher.

In order to minimise this, sample under higher discharge condition (*e.g.* storm events) is recommended to be pre-filtered using 1 µm membrane prior to concentration since this size of membrane would be unlikely to create any artefacts to the size of particle (Gaffney, 2007) and because high discharge was shown to positively correlate to DOC concentration (at eroded and whole catchment site). For sample under lower discharge condition, higher retentate volume could be accepted though this would require longer freeze-drying time.

5.2. Understanding current processes and future implications

Prediction of carbon storage in the catchment of Crowden Great Brook was attempted in quantifying DOC fluxes from all three monitoring sites over the years 2003-2009. The flux values obtained were comparable to reported results from similar upland system studies. The confidence in quantifying such values however is limited by background variability. Nevertheless such data would be very useful for future work.

The potential of sub-catchments to serve as models for prediction of future scenarios was also recognised. In the eroded sub-catchment, log DOC concentration was found to have a positive relationship with log discharge. Such observation is particularly relevant in eroded peat remediation for Crowden Great Brook and other *ombrotrophic* upland peat catchments.

Although the PSD of NOM did not show any systematic change as the discharge decreased, such relationship might be realised over a longer time frame, which is potential provided that method of analysis using TFU has been proven to be quite characterised, useful and easily used.

Aquagenic/pedogenic components were earlier noted as being able to indicate the relative age of NOM and the health of peat. Therefore, the found possibility of identifying and quantifying aquagenic and pedogenic components of aqueous NOM from upland peat catchment using Py-GC-MS is very crucial. Such components can potentially be characterised to become bio-markers of the processes occurring within the catchment.

In addition, the high amount of pedogenic components found at the eroded sub-catchment (which matches the expectation of an eroded site in a nutrient-poor *ombrotrophic* peat catchment), the decreasing trend in amount of both aquagenic and pedogenic components (as PSD decreased) and their variability under different discharge conditions are an encouraging start in understanding the spatial and temporal variability of NOM chemical characteristics as well as their inter-relationship

with physical properties. In contrast, there was unexpected observation such as the lowest level of polysaccharide at the vegetated sub-catchment. Further extensive investigation is thus required for all such results to be fully understood.

Lastly, the combination of well-developed NOM chemical and physical characterisation method could offer other undiscovered dimensions. A small catchment of Crowden Great Brook if can be fully characterised and understood could contribute significantly to the understanding of *ombrotrophic* upland peat in particular and global carbon storage in general, and thus appropriate management and conservation strategy could be formulised.

Appendix 1 Quantification of lignin and carbohydrate thermochemolysis products (µg/ mg OC).

S = Syringyl; G = Guaiacyl; P= p-hydroxyphenyl. Method of calculations is detailed in section 2.2.4.

			Vegetated site (Q = 5.5 Ls^{-1})						Denuded site (Q = 14.3 Ls^{-1})			Whole catchment site (normal Q)						Whole catchment site (Q = 44.3 Ls^{-1})			
			>0.2 µm	SD	<0.2 µm >50 kDa	SD	<50 kDa >10 kDa	SD	>0.2 µm	<0.2 µm >50 kDa	<50 kDa >10 kDa	>0.2 µm	SD	<0.2 µm >50 kDa	SD	<50 kDa >10 kDa	SD	>0.2 µm	SD	<0.2 µm >50 kDa	SD
Lignin moieties	S	S4	0.18	0.11	0.13	0.02	0.13	0.00	1.65	0.97	-	-	-	-	-	-	-	0.43	0.07	0.31	0.07
		S5	0.19	0.05	0.09	0.01	0.08	0.01	0.78	0.41	-	-	-	-	-	-	-	-	-	-	-
		S6	1.49	0.58	0.96	0.01	0.81	0.02	10.35	7.89	1.64	-	-	3.30	0.07	1.29	0.14	1.94	0.14	1.56	0.35
		S7	0.18	0.04	0.10	0.00	-	-	1.00	0.62	-	-	-	-	-	-	-	0.40	0.10	0.11	0.07
		S8	0.32	0.07	0.10	0.02	-	-	0.55	0.63	-	-	-	-	-	-	-	0.53	0.03	-	-
		S10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.17	0.02	-	-
		S14	0.16	0.02	0.09	0.02	0.04	0.00	1.55	0.72	-	-	-	-	-	-	-	0.41	0.13	-	-
		S15	0.11	0.01	0.09	0.01	0.07	0.01	0.54	0.29	-	-	-	-	-	-	-	0.32	0.07	-	-
		∑S	2.61	0.82	1.57	0.06	1.14	0.03	16.43	11.53	1.64	-	-	3.30		1.29		4.19	0.58	1.98	0.49
	G	G5	0.28	0.09	0.11	0.00	0.10	0.01	0.98	0.99	0.00	-	-	-	-	-	-	0.36	0.07	0.24	0.02
		G6	2.67	0.85	1.55	0.07	1.68	0.06	5.92	8.94	1.31	-	-	5.36	0.16	2.57	0.33	3.80	0.22	3.32	0.48
		G7	0.22	0.05	0.16	0.03	0.15	0.02	2.47	1.07	-	-	-	-	-	-	-	0.61	0.10	0.02	0.01
		G8	0.08	0.04	0.03	0.00	0.07	0.01	0.85	0.38	-	-	-	-	-	-	-	0.20	0.03	0.11	0.02
		G13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.12	0.03	-	-
		G14	-	-	-	-	-	-	1.17	-	-	-	-	-	-	-	-	0.26	0.04	-	-
		G15	-	-	-	-	-	-	0.97	-	-	-	-	-	-	-	-	0.14	0.04	0.05	0.02
		G18	0.32	0.00	0.38	0.03	0.28	0.01	6.63	2.87	0.23	-	-	1.72	0.21	0.72	0.11	1.49	0.15	0.80	0.30
		∑G	3.55	1.02	2.23	0.13	2.27	0.08	18.97	14.25	1.54	-	-	7.08	0.37	3.29	0.21	6.98	0.68	4.54	0.77
	P	P6	0.77	0.37	0.36	0.03	0.34	0.02	1.54	1.84	0.26	-	-	-	-	-	-	0.94	0.06	0.66	0.03
		P18	0.43	0.19	0.21	0.01	0.09	0.01	3.64	1.13	-	-	-	0.68	0.02	0.12	0.01	1.17	0.17	0.26	0.03
		∑P	1.20	0.56	0.57	0.01	0.43	0.03	5.19	2.97	0.26	-	-	0.68	0.02	0.12	0.01	2.12	0.23	0.93	0.06
		Λ2	7.47	2.45	4.42	0.18	3.88	0.13	40.59	28.75	3.44	0.00	0.00	11.06	0.46	4.70	0.36	13.54	1.59	7.45	1.32
Carbohydrate moieties		MC1	0.44	0.05	0.51	0.03	0.18	0.01	12.53	6.67	0.27	29.60	0.78	4.49	0.19	0.43	0.07	-	-	-	-
		MC2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.43	0.26	0.55	0.01
		MC3	-	-	0.10	0.01	0.03	0.00	2.56	5.05	0.13	-	-	-	-	-	-	-	-	-	-
		MC4	-	-	-	-	-	-	5.22	-	-	6.93	1.24	1.37	0.06	0.10	0.04	0.30	0.09	0.13	0.00
		MC5	0.36	0.03	0.73	0.06	0.17	0.01	24.81	9.37	0.27	66.76	2.88	4.44	0.25	0.54	0.18	1.70	0.31	0.56	0.01
		MC6	0.46	0.02	0.50	0.04	0.07	0.01	9.02	16.22	-	4.20	0.33	5.25	0.22	0.89	0.22	0.83	0.20	0.41	0.02
		∑MC	1.26	0.04	1.85	0.13	0.45	0.01	54.14	37.31	0.67	107.49	2.76	15.55	0.73	1.96	0.52	4.27	0.86	1.65	0.03
		Λ1	1.26	0.04	1.85	0.13	0.45	0.01	54.14	37.31	0.67	107.49	2.76	15.55	0.73	1.96	0.52	4.27	0.86	1.65	0.03

Appendix 2 Quantification of fatty acid methyl ester and other thermochemolysis products (µg/ mg OC).

Method of calculations is detailed in section 2.2.4

		Vegetated site (Q = 5.5 Ls^{-1})						Denuded site (Q = 14.3 Ls^{-1})			Whole catchment site (normal Q)						Whole catchment site (Q = 44.3 Ls^{-1})			
		>0.2 µm	SD	<0.2 µm >50 kDa	SD	<50 kDa >10 kDa	SD	>0.2 µm	<0.2 µm >50 kDa	<50 kDa >10 kDa	>0.2 µm	SD	<0.2 µm >50 kDa	SD	<50 kDa >10 kDa	SD	>0.2 µm	SD	<0.2 µm >50 kDa	SD
Fatty acid methyl ester moieties	FAME1	-	-	-	-	-	-	-	-	3.01	-	-	-	-	-	-	-	-	-	-
	FAME2	-	-	-	-	-	-	-	-	0.43	40.34	2.73	-	-	-	-	-	-	-	-
	FAME3	0.17	0.07	-	-	-	-	2.65	-	-	-	-	-	-	-	-	0.16	0.01	-	-
	FAME4	0.80	0.47	-	-	-	-	15.04	-	-	83.66	1.56	-	-	-	-	0.76	0.10	-	-
	FAME5	1.13	0.51	0.73	0.02	0.15	0.01	29.61	27.39	6.48	59.08	4.30	4.73	0.63	0.80	0.17	0.91	0.13	0.24	0.09
	FAME6	0.15	0.10	-	-	-	-	8.79	-	-	-	-	-	-	-	-	-	-	-	-
	FAME7	0.48	0.20	0.53	0.05	0.10	0.00	16.86	27.40	6.24	0.11	0.01	3.98	0.50	0.68	0.11	0.28	0.01	0.18	0.01
	∑FAME	2.74	1.34	1.26	0.07	0.25	0.02	72.95	54.80	16.16	183.19	8.59	8.72	1.13	1.48	0.28	2.12	0.25	0.42	0.10
Others	1,2,4 TMB	1.26	0.77	0.57	0.07	0.22	0.03	7.98	7.12	0.34	8.03	0.98	-	-	-	-	1.06	0.06	0.63	0.07
	1,3,5 TMB	1.02	0.39	0.24	0.02	0.31	0.01	3.27	1.37	0.26	-	-	-	-	-	-	0.91	0.11	0.76	0.02
	∑others	2.28	1.16	0.81	0.09	0.53	0.04	11.26	8.49	0.60	8.03	0.98	0.00	0.00	0.00	0.00	1.91	0.09	1.39	0.10

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