

RESEARCH ARTICLE

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Key Points:

- ^{13}C NMR used to show changes in functional group composition of different organic matter sources from peat
- DOM is derived from the oxidation of biomass and the degradation of lignin
- POM is derived by erosion of the peat profile

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A Molecular Budget for a Peatland Based Upon ^{13}C Solid-State Nuclear Magnetic Resonance

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Abstract Peatlands can accumulate organic matter into long-term carbon (C) storage within the soil profile. This study used solid-state ^{13}C nuclear magnetic resonance (^{13}C -NMR) to investigate the transit of organic C through a peatland ecosystem to understand the molecular budget that accompanies the long-term accumulation of C. Samples of biomass, litter, peat soil profile, particulate organic matter, and dissolved organic matter (DOM) were taken from the Moor House National Nature Reserve, a peat-covered catchment in northern England where both the dry matter and C budget for the ecosystem were known. The results showed that: The interpretation of the ^{13}C -NMR spectra shows that polysaccharides are preferentially removed through the ecosystem, while lignin components are preferentially retained and come to dominate the organic matter accumulated at depth in the profile. The DOM is derived from the oxidation of both biomass and the degradation of lignin, while the particulate organic matter is derived from erosion of the peat profile. The DOM is differentiated by its proportion of oxidized functional groups and not by its aromatic content. The changes in functionality leading to DOM production suggest side chain oxidation resulting in C-C cleavage/depolymerisation of lignin, a common reaction within white rot fungi. The ^{13}C -NMR budget shows that O-alkyl functional groups are disproportionately lost between primary production and accumulation in the deep peat, while C-alkyl functional groups are disproportionately preserved. The carbon lost as gases (CO_2 and CH_4) was estimated to be composed of 93% polysaccharide-derived carbon and 7% lignin-derived carbon.

1. Introduction

Globally, Northern Hemisphere peatlands cover only ~3% of the Earth's total land area but are the most important carbon (C) store within the terrestrial biosphere (Rydin & Jeglum, 2013), storing an estimated 500 ± 100 Gt C (Gorham, 1991; Loisel et al., 2017; Yu et al., 2014). The peatland coverage of Europe is estimated at $593,727 \text{ km}^2$, 5.4% of the total surface area; in the UK peatlands cover 11% of the land area (Tanneberger et al., 2017), where their C budgets have been extensively studied (e.g., Billett et al., 2004).

The C budgets of peatlands have been studied in order to better understand the fate of organic matter and the ecosystem's future, initially using long-term accumulation rates, by dating the depth profile (e.g., Turetsky et al., 2004). However, this approach assumes accumulation and cannot account for short periods of net loss or estimate the species of C being lost. The C can be lost to the atmosphere as carbon dioxide (CO_2) or as methane (CH_4) (Houghton et al., 1995; IPCC, 2013), or in water as dissolved and particulate organic carbon (DOC and POC). DOC is a major water quality limitation in many northern countries (Bieroza et al., 2009), while POC can cause loss of water resource via reservoir sedimentation (Evans & Warburton, 2010).

An alternative approach to estimating C budgets of peatlands is to measure the fluxes of all C species in and out of the peat ecosystem, as in Billett et al. (2004), Nilsson et al. (2008), Roulet et al. (2007), and Worrall et al. (2003). If C is accumulating in peat soils, then the other elements in organic matter must also be accumulating, and so nitrogen (N) (e.g., Hemond, 1983; Worrall et al., 2012), organic oxygen (O) (Worrall et al., 2016), and phosphorus (P) (Richardson & Marshall, 1986; Wang et al., 2015) budgets have also been calculated for peatlands. Additionally, the impact of land management interventions on C biogeochemistry have been studied, for example, the impact on the C budget due to drainage (e.g., Rowson et al., 2010), and these often reflect the influence of water table on the decomposition processes in the peat profile (e.g., Clay et al., 2010). The

budgets based on the inputs and outputs of C from peatlands consider the species of the gaseous emissions from peatlands (e.g., CO₂ or CH₄) but use the lumped terms of DOC and POC without further characterization. Primary productivity is simplified as the process of sequestering CO₂ from the atmosphere into plants, but it is more complex: CO₂ is sequestered into glucose which is then transformed through metabolic cycles and combined with nutrients to build the components of plants—lignin, carbohydrates (cellulose and hemicellulose), proteins, and lipids (McDermitt & Loomis, 1981). Different peatlands have different dominant vegetation types, and so the plant community is composed of different proportions of these components (Huang et al., 1998). The vegetation is transformed back to CO₂ through root respiration, falls as litter, released as plant exudates into the soil pores, or transformed into soil organic matter, resulting in the formation of CO₂, CH₄, DOC, or POC. As contemporary C budgets have advanced our understanding of processes and drivers, and quantified gas, particulate, and dissolved fluxes (and have been one step forward from the black box of the long-term accumulation approach), the purpose of this paper was to consider the molecular changes occurring as organic matter is created and then moves through the peatland ecosystem into long-term storage or it is released by considering the composition of the organic matter present in a peatland.

Numerous methods have been used to study the composition of organic matter: colorimetric methods from ultraviolet/visible spectroscopy (e.g., Blackford & Chambers, 1993); coal petrology techniques such as rock eval (e.g., Carrie et al., 2012), elemental ratios (e.g., C/N, Kuhry & Vitt, 1996), stable isotopes (e.g., Jones et al., 2010); Fourier transform infrared spectroscopy (e.g., Artz et al., 2008), and pyrolysis gas chromatography mass spectrometry (e.g., Buurman et al., 2006). These approaches have commonly been focused on characterization of peat profiles for the purpose of understanding humification (e.g., Zacccone et al., 2008) or palaeohistoric reconstructions (e.g., McClymont et al., 2011). Worrall et al. (2017) have used thermogravimetric analysis to explore the processing of C fixed in primary productivity through a peatland into long-term accumulation.

Solid-state ¹³C nuclear magnetic resonance (¹³C-NMR) provides information on functional groups in naturally occurring organic macromolecules and has been widely used to characterize organic matter from different sources. The technique has been used to examine the link between the chemical composition of biopolymers and peat soils (Orem et al., 1986). In peatlands ¹³C-NMR has been used to characterize peat humic matter for its macromolecular composition (Kaal et al., 2007), measure susceptibility of organic matter across climate gradients (Sjögersten et al., 2003), find the fate of lignin within peats (Schellekens, Buurman, & Kuyper, 2012), understand changes in biomarkers (Schellekens et al., 2015), and most importantly for this study understand the fate of *Calluna vulgaris* in UK peatlands (Huang et al., 1998). However, none of the above techniques have been used within the context of a C budget of a peatland, or to measure the molecular and functional group changes that actually occur to make both accumulation and release possible. Therefore, the purpose of this study was to use ¹³C-NMR to trace organic matter compositional changes through a peatland ecosystem where the C, elemental, and mass budgets were already well constrained.

2. Approach and Methodology

The approach taken here was to analyze the composition of the organic matter in a series of carbon pools within the context of a known carbon budget (Figure 1). The composition was analyzed using ¹³C solid-state NMR and elemental analysis (for C, H, N, and O).

2.1. Study Site

The peatland ecosystem chosen for this study was the Cottage Hill Site catchment, which is a 0.2 km² subcatchment of the Trout Beck catchment, within the Moor House National Nature Reserve, a terrestrial and freshwater site monitored as part of the UK Environmental Change Network (Rennie et al., 2017; Sykes & Lane, 1996). Between 1993 and 2013 the median pH of Cottage Hill Sike was 4.36, the conductivity was 42.94 μS/cm, and the DOC concentration was 18.1 mg C/L (Rennie et al., 2017). The Trout Beck catchment is an 11.4 km² blanket peat catchment in the headwater of the River Tees (N54°41'18"–W2°22'45"; Figure 2) and lies largely above an altitude of 500 m; the highest point is the summit of Great Dun Fell at 848 m. The underlying geology is a succession of Carboniferous limestones, sands, and shales with intrusions of the doleritic Whin Sill (Johnson & Dunham, 1963). This solid geology is covered by glacial till whose poor

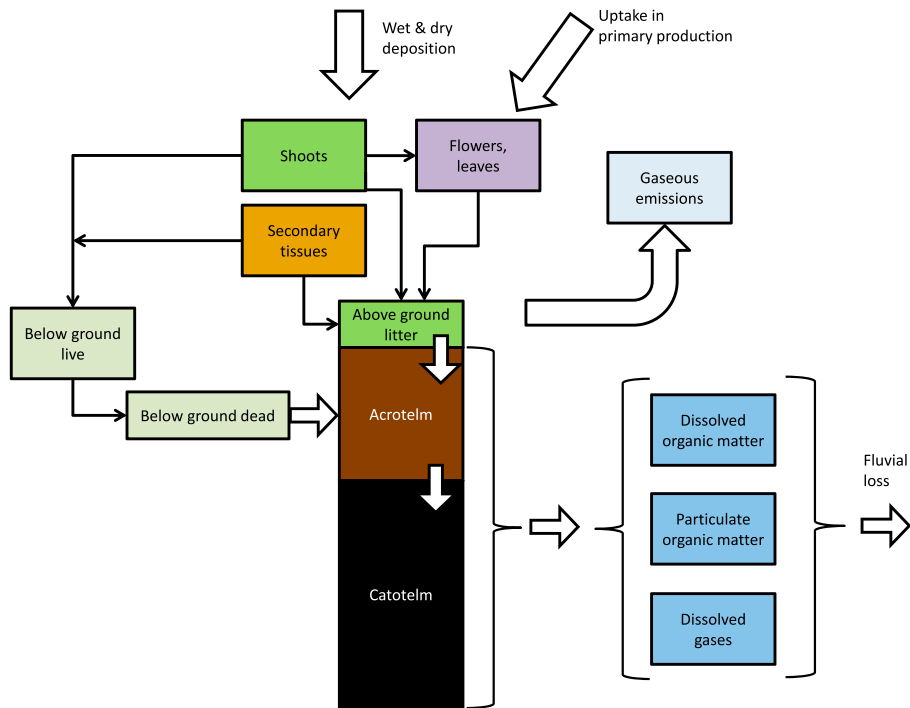


Figure 1. Schematic diagram of organic matter pools and fluxes considered by this study.

drainage facilitated the development of blanket peat during the Holocene. Blanket peat covers 90% of Trout Beck catchment, and 100% of the Cottage Hill Sike catchment (Evans et al., 1999). The recent climate of the site has been summarized by Holden and Rose (2011): between 1991 and 2006 the mean annual temperature was 5.8°C; air frosts were recorded on 99 days in a year; and the mean number of days with snow cover was

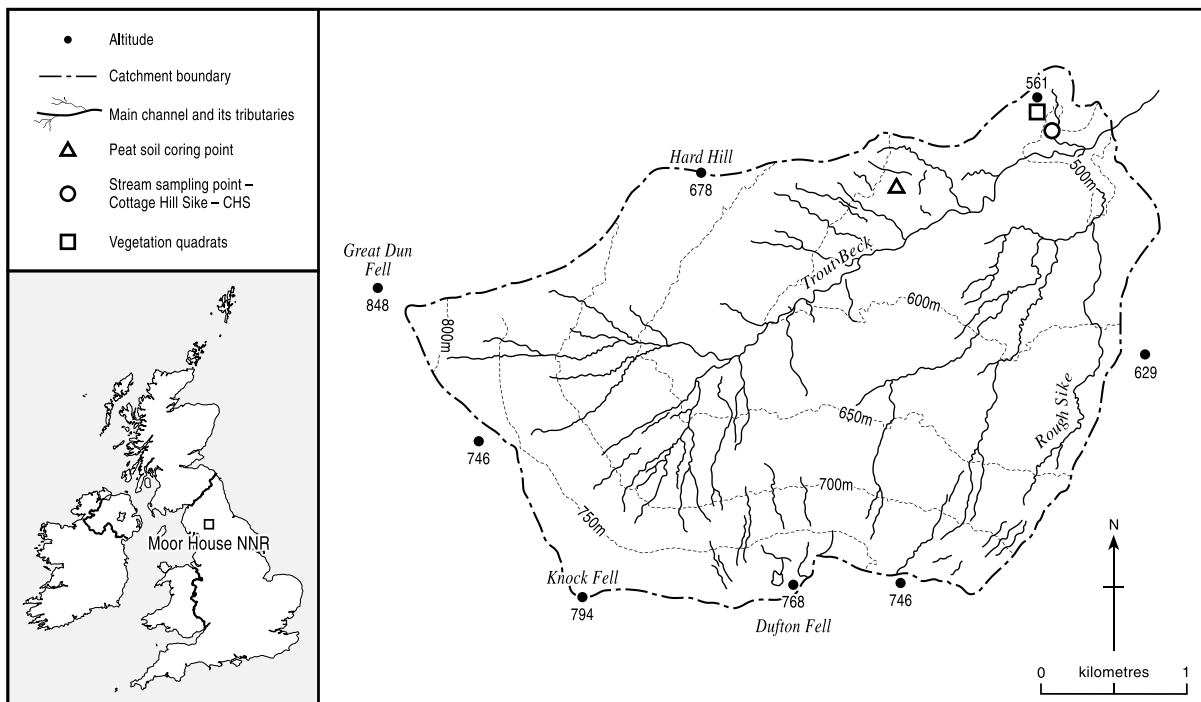


Figure 2. Location of the study site used in this study.

41, while for the period 1953–2006 the mean annual precipitation was 2,012 mm. The vegetation is dominated by *Eriophorum sp.* (cotton grass), *Calluna vulgaris* (heather), and *Sphagnum sp.* (moss).

2.2. Sampling

Given the range of carbon pools and fluxes identified in Figure 1, the following sampling was carried out. First, the peat profile was sampled. A location in active, deep peat within the Cottage Hill Sike catchment was selected and a core taken to 1 m depth with a gouge auger. The depth was selected to ensure coverage of both the acrotelm and catotelm (Worrall et al., 2012). The peat core was sampled at five depths (0–2, 10–12, 35–40, 45–50, and 95–100 cm depth below the surface). Peat core samples were dried at 105°C overnight and checked for no further mass loss, and their bulk density measured prior to further processing.

Second, vegetation samples were collected from six quadrats. The six quadrats (0.25 m², 0.5 × 0.5 m) were located in the Cottage Hill Sike catchment and chosen to cover the three main plant functional groups: shrubs (dominantly *Calluna vulgaris*, henceforward referred to as Calluna); grasses, and sedges (dominantly *Eriophorum spp.*, henceforward referred to as grass/sedge); and mosses (including *Sphagnum spp.*, henceforward referred to as mosses). In three of the quadrats, the entire aboveground biomass was quantitatively recovered. For the three other quadrats, the total aboveground biomass was collected but separated by functional group. From within these quadrats, samples of litter and belowground biomass were recovered but not quantitatively. Quantitative biomass samples were dried to 105°C and weighed so that an estimate of total aboveground biomass, and the contribution from the dominant functional plant groups, could be estimated. This estimate was then used to confirm the dry matter budget reported by Forrest (1971). Samples of the litter and belowground biomass were not recovered quantitatively and were dried to 105°C. The dried samples were then homogenized and powdered as described below. In this way we were able to consider bulk measures of the vegetation carbon pool (above and belowground biomass) and assess the contribution of its components (plant functional types: Calluna, grasses/sedge, and mosses). It would be expected that the aboveground biomass would be weighted average of its components.

Third, dissolved organic matter (DOM) samples were taken from the first-order stream Cottage Hill Sike. Large-volume water samples (at least 25 L) were collected monthly from October 2011 to September 2015, except for months where winter conditions precluded taking flowing water samples (40 samples taken in total). On return of these samples to the laboratory, the samples were allowed to settle and the next day the water tapped off from above the sediment layer and evaporated to dryness (between 60 and 80°C) to collect the total dissolved solids. By using settling as a means of separation, no filtration cutoff was being applied; rather, this study was defining DOM as being that component which was either colloidal or truly dissolved.

Fourth, from June 2013 to January 2014 the settled sediment from the large volume samples used to gather samples of DOM was recovered as a sample of the particulate organic matter (POM). The settled sediment was dried to 105°C (overnight, checked for no further mass loss) and retained for subsequent analysis.

Finally, four standard materials were included in the analysis: lignin (Aldrich, CAS 8068-05-1), humic acid (Alfa-Aesar, CAS 1415-93-6), cellulose (Whatman, CAS 9004-36-4), and protein (Sigma, CAS 100684-25-1). The lignin, cellulose (taken as representative of polysaccharides, including hemicellulose), and protein represent the three most abundant components of plants found in a peatland system (McDermitt & Loomis, 1981). The humic acid standard was analyzed for comparison to the DOM samples.

The considerable advantage of studying peatland ecosystems is that soil, total dissolved solids, and suspended sediment would be expected to be predominantly organic matter, but nevertheless, the ash content of the samples was analyzed. A subsample of the biomass, litter, peat, DOM, and POM collected was ashed at 550°C for at least 4 h and the residual mass recorded. All subsequent analysis of vegetation, peat, DOM, and POM was corrected so that all elemental analysis are quoted on an ash-free basis.

All samples of the vegetation, litter, and peat soil were dried to 105°C and milled to a submillimeter powder using a Spex 6770 Freezer Mill. For the samples of DOM and POM cryomilling was not necessary.

2.3. ¹³C Solid-State Nuclear Magnetic Resonance

The ¹³C solid-state NMR data were obtained at the EPSRC UK National Solid-state NMR Service at Durham University. Solid-state ¹³C-NMR spectra were recorded at 100.56 MHz using a Varian VNMRs spectrometer and a 4 mm magic angle spinning probe. The spectra were obtained using cross polarization with a 0.5 s

Table 1
The Median Composition of the Carbon Pools Considered by the Study

Carbon pool/type	C _{ox}	OR	Degree of unsaturation (Ω)	Median stoichiometry	C-alkyl		N-alkyl/methoxyl		O-alkyl		O ₂ -alkyl/ acetal C		Aromatic/unsaturated		Phenolic C		Carboxyl/amide		Aldehyde/ketone	
					0–45	45–65	65–95	95–110	110–145	145–160	160–190	190–220								
DOM	0.40	0.92	2.42	C ₃₁ H ₃₈ NO ₂₄	14.6	8.8	11.8	10.9	12.4	9.6	23.1	7.3								
POM	–0.21	1.09	1.76	C ₂₂ H ₃₅ NO ₁₄	17.0	11.8	37.9	14.6	5.9	4.0	8.2	0.5								
Aboveground biomass	–0.26	1.08	1.98	C ₅₇ H ₈₆ NO ₃₅	13.4	8.2	37.5	16.2	8.0	7.6	7.7	1.4								
Belowground biomass	–0.25	1.08	2.12	C ₄₉ H ₇₃ NO ₂₉	14.3	11.6	32.8	16.1	8.1	7.7	7.5	1.9								
Grass and sedge	–0.19	1.07	1.95	C ₃₃ H ₅₂ NO ₂₁	10.4	14.1	37.8	17.2	5.2	5.7	8.7	0.9								
Mosses	–0.19	1.06	1.92	C ₉₅ H ₁₄₅ NO ₅₆	8.8	11.6	42.0	15.6	8.1	5.9	6.4	1.5								
Calluna	–0.32	1.09	2.03	C ₅₅ H ₈₆ NO ₄₆	11.2	14.5	36.2	17.3	5.6	7.3	7.8	0.2								
Litter	–0.05	1.05	2.30	C ₂₅ H ₃₅ NO ₁₅	16.2	11.6	30.4	15.3	9.0	7.5	8.9	1.7								
Peat soil	–0.15	1.06	2.20	C ₄₃ H ₆₂ NO ₂₆	34.2	11.7	13.7	7.3	9.8	7.2	10.2	3.8								
Lignin	–0.43	1.12	3.14	C ₈₇ H ₁₀₃ NO ₃₁	7.0	28.5	6.2	13.0	19.1	23.7	1.8	0.8								
Cellulose	0.05	0.99	1.11	CH ₂ O	30.2	12.8	40.4	13.6	1.2	0.5	0.5	0.8								
Humic acid	0.19	0.97	2.18	C ₄₉ H ₆₀ NO ₃₃	26.0	9.5	3.1	6.8	25.9	10.5	15.1	3.0								
Protein	0.18	0.96	3.63	C ₅ H ₅ NO	27.5	19.7	10.9	2.4	8.6	2.3	27.6	0.9								

Note. Median stoichiometry is expressed relative to nitrogen content except for cellulose which expressed relative to carbon. The range of each chemical shift (ppm) for ¹³C NMR is from Chadwick et al. (2004).

recycle delay, 1 ms contact time, at ambient probe temperature (~25°C) and at a sample spin rate of 14 kHz. Between 50,000 and 100,000 repetitions were accumulated. The pulse sequence is a Durham-modified version of a generic provided by the manufacturer. The pulse sequence was a direct excitation one with “DEPTH” background suppression and high-power spinal-64 proton decoupling. Radiofrequency fields equivalent to 75 kHz were used on both channels. The free induction decay (FID) was zero filled to 16 k data points and transformed with 200 Hz of exponential line broadening. The phased spectrum was baseline corrected using the Bruker “abs” correction with a polynomial of degree 5. Spectral referencing was with respect to an external sample of neat tetramethylsilane (carried out by setting the high-frequency signal from adamantane to 38.5 ppm). Between 50 and 60 mg of each sample was used for each analysis. The chemical shift ranges used were adapted from Chadwick et al. (2004) and are shown in Table 1. The maximum peak height in each range was divided by the molar carbon concentration of the sample (from elemental analysis described below), to get a relative peak height for each type of carbon observed. Peak integration on the basis of the ranges shown in Table 1 did not give a significantly different result at a probability of at least 95% of being different from zero.

2.4. Elemental Analysis

The samples were subject to carbon, hydrogen and nitrogen (CHN) and separately to O analysis on a Costech ECS 4010 Elemental combustion system with pneumatic autosampler. Computer software used was EAS Clarity (DataApex Ltd, Prague, Czech Republic). For both CHN setup and the separate O setup, calibration curves of $r^2 > 0.999$ were created using acetanilide as the standard. Samples of acetanilide were included within each run as unknown samples to act as internal quality control checks. Each sample was analyzed in triplicate, that is, three times on the CHN setup and a further three times on O setup, and a mean calculated for C, H, N, and O. All samples were corrected for their measured ash content.

The elemental analysis results were converted to molar percentage and considered as elemental ratios and also as measures of oxidation state (C_{ox} and oxidative ratio (OR)—Masiello et al., 2008) and the degree of unsaturation (Ω—McMurry, 2004). The value of C_{ox} can be related to the nominal oxidation of the carbon as developed by LaRowe and van Cappellan (2011). As noted by Gallagher et al. (2014), OR not only is a property of organic matter (e.g., peat soil) but also can be a property of an ecosystem and its biosphere-atmosphere exchanges. The CHNO elemental analysis for this site and these samples has described and analyzed in detail in Worrall et al. (2016).

2.5. Statistical Analysis

An analysis of variance was carried out on each functional group to identify differences between the sample types (e.g., between vegetation and peat soil), and to look for differences between sampling months of the DOM. The changes in the proportions of each C functional groups range with depth in the peat core were modeled using a linear regression.

The interpretation of the spectra into the proportion of certain functionalities means that the spectra were transformed into a multivariate data set. Principal component analysis (PCA) was used to assess the differences between organic matter pools (including standards) and fluxes on the basis of the proportion of functionalities. In the analysis, the components with an eigenvalue >1 were considered for further examination, as these components are those which represent more of the data set variance than any of the original variables (Chatfield & Collins, 1980). An analysis of variance was carried out on the results of the PCA, using the principal component values and sample/standard type as a factor. To aid the interpretation, the PCA was repeated including the elemental analysis. Furthermore, stepwise multiple and logistic regression were used to understand the elemental and functional group composition. The logistic regression was fitted using a maximum likelihood approach rather than least squares fit as for the stepwise multiple regression. For both logistic regression and stepwise multiple regression selection of variables was based upon those with a probability of not being $0 < 0.05$ for inclusion. All statistical analysis was judged at a probability 95% of being different from 0. Stepwise multiple regression was applied using both forward and backward addition of variables, and the importance of variables within any significant logistic regression was assessed using the odds ratio.

2.6. Carbon and Dry Matter Budget

This study considers organic matter in the ecosystem as transferring through a series of carbon pools and fluxes (Figure 1). The fluxes of gases (N_2O , CO_2 , and CH_4) have fixed and known molecular composition and as such were not analyzed as part of this study.

The dry matter budget of the catchment was first recorded by Forrest (1971) who studied the productive ecology of the dominant species present in the catchment. Forrest (1971) did include biomass production (both above and belowground production) and divided production estimates between the dominant functional plants types within the catchment (Calluna, grass/sedge, and mosses). Forrest (1971) did include litter production rates but not their subsequent decay and peat formation rates nor the loss of production via fluvial pathways. Therefore, this study will combine the study of dry matter production with a carbon budget. This study used the most up-to-date and longest carbon budget information for the site based upon the approach of Worrall et al. (2009, 2012), which summarized the carbon budget as:

$$100C_{\text{pp}} = 35C_{\text{R}} + 26C_{\text{DOC}} + 4C_{\text{CH}_4} + 4C_{\text{dCO}_2} + 9C_{\text{POC}} + 22C_{\text{RES}} \quad (1)$$

(6) (14) (0.5) (1) (3) (17)

where C_x = carbon from the following uptake or release pathways, where x is pp = primary productivity, R = net ecosystem respiration, DOC = dissolved organic carbon; CH_4 = methane; dCO_2 = dissolved CO_2 ; POC = particulate organic carbon; and RES = residual carbon stored in the soil. The values in brackets below equation (1) are the 95% confidence interval on the coefficients which was calculated given the variation of the carbon budget over the 13 years (Worrall et al., 2012).

The total carbon budget of this ecosystem varied between -20 and $-91 \text{ t C km}^{-2} \text{ yr}^{-1}$, that is, the ecosystem was accumulating carbon from the atmosphere. By far the biggest single component of the budget was the uptake of carbon by primary productivity (average across the study period of $-176 \text{ t C km}^{-2} \text{ yr}^{-1}$ —the budget status is assessed relative to the atmosphere, and thus, a sink into the soil is negative). The ^{13}C -NMR were weighted according to the carbon budget above, for example, the spectra of the average biomass was weighted by a factor of 1 (the proportion of primary productivity C in equation (1)) as it represents the primary production, and the ^{13}C -NMR for DOM, weighted by a factor of 0.26 (the proportion of DOM C in equation (1)), was subtracted from it. This was then performed for each of the carbon species in the budget as represented by equation (1) for which a ^{13}C -NMR spectra could be measured. The average biomass was calculated as the mix of the spectra of the vegetation samples in the above and belowground biomass mixed in the appropriate ratios as defined by Forrest (1971): this then gives a ^{13}C -NMR spectra of the primary productivity. The two species of carbon for which there was no trace were the CO_2 ($C_{\text{R}} + C_{\text{dCO}_2}$ —equation (1)) and CH_4 ; therefore, the spectra of the primary production once all other species have been subtracted from it represent the spectra of the organic matter that is transformed to and lost in the gaseous form. In this way it was possible to assess the changes between the organic matter compositions as the organic matter transfers through the ecosystem, and so they identify what composition has been lost in the process of humification to

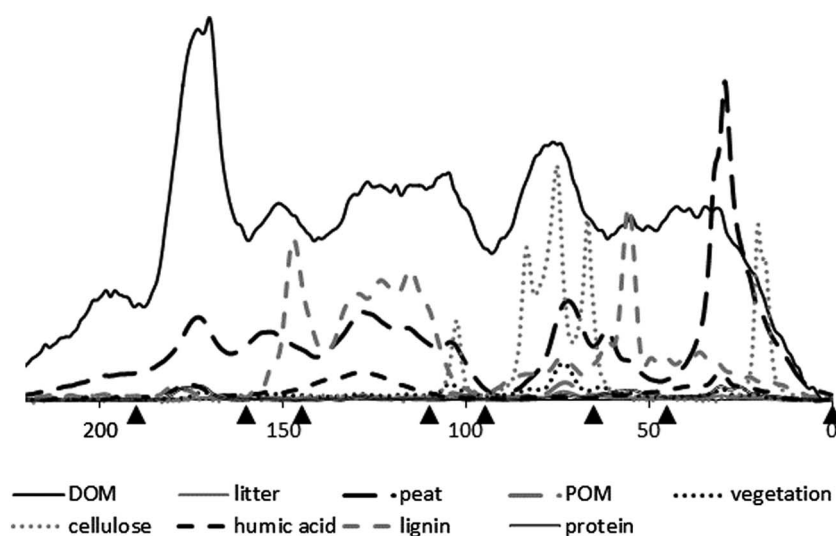


Figure 3. An example NMR spectra for each type of sample analyzed, normalized to carbon content. The triangles on the x axis denote the ranges of chemical shifts (Table 1).

deep peat soil, degradation to gases, and the creation of fluvial organic matter—this reconstructed composition will henceforward be referred to as the lost composition.

3. Results

The median composition of sampled organic matter pools is given in Table 1. Note that given the differing functional group interpretations of the ranges shown in Table 1 means that differing but contrasting interpretations have to be given; for example, a cellulose standard cannot contain amide groups, but a protein standard will, and yet they both have a response in the range 45–65 ppm (Table 1). This contrast illustrates that a singular functional group interpretation of ^{13}C NMR spectra in naturally occurring organic matter such as studied here is impossible. Rather than focus on individual functional groups or ranges, the spectra lends itself to a more holistic interpretation such as PCA. Applying the functional group ranges from Chadwick et al. (2004), the cellulose standard would appear to have a small proportion of the C atoms in the N-alkyl range, which is unlikely as there is no nitrogen in cellulose. However, on closer inspection of the spectra, this “peak” is actually the right-hand side of the O-alkyl peak, and so the N-alkyl range could be better represented by the 45–60 ppm (“cellulose range”) range rather than 45–65 ppm, also changing the O-alkyl range from 65–95 to 60–95 ppm. Changing this range boundary slightly from that of Chadwick et al. to cellulose affected the N-alkyl and O-alkyl proportional relative intensities and found, on average, the N-alkyl decreased from 10.35 to 10.25 for DOM, 11.62 to 8.46 for POM, 11.60 to 7.31 for litter, 11.31 to 9.28 for peat, 12.97 to 7.93 for vegetation, 12.81 to 2.11 for cellulose, 9.54 to 9.44 for humic acid, 28.45 to 27.78 for lignin, and 19.72 to 18.80 for protein; the relative O-alkyl increased from 13.96 to 13.99 for DOM, 25.31 to 26.33 for POM, 30.35 to 31.83 for litter, 13.71 to 14.19 for peat, 36.84 to 38.99 for vegetation, 40.37 to 45.33 for cellulose, 3.14 to 4.18 for humic acid, 6.18 to 8.41 for lignin, and 10.94 to 15.11 for protein. The average changes in the N-alkyl and O-alkyl peaks between the Chadwick range (45–65 ppm) and the “cellulose range” (45–60 ppm) were a decrease of 3% and increase of 1.95% for N-alkyl and O-alkyl, respectively. There were no significant differences between the two different ranges used for the N-alkyl ($p = 0.07$) or O-alkyl ($p = 0.75$), and so for further analysis, the range boundaries of Chadwick et al. (2004) were used. As the peak heights were calculated as proportional relative heights, changing the range boundaries had an effect on the peaks in other six ranges, but this was negligible.

The majority of the carbon atoms in DOM fell into the amide/carboxyl-C range (22%, Figure 3). For the vegetation and litter, the functional group containing the largest proportion of the carbon atoms was the O-alkyl group (37 and 30%, respectively), and for the peat and POM it was the C-alkyl group (37 and 31%, respectively). For the standards, the cellulose had the largest proportion of O-alkyl carbon atoms (40%), the

Table 2
The Regression of the Functional Groups on the Elemental Composition and the Derived Measures

Functional group	C	H	N	O	C _{ox}	C/N	C/O	H/C	Constant	R ²
C-alkyl								14.4	−2.05	13.9
N-alkyl/methoxyl C							16.6	9.6	−17.0	71.1
O-alkyl					−28.0		−15.8		45.4	38.3
O ₂ -alkyl/acetate C	1.5		−9.6						7.1	26.0
Aromatic/unsaturated C				−4.4				−9.7	34.6	39.1
Phenolic C				−4.9		0.08		−9.5	29.7	53.4
Carboxyl/amide			11.7		29.5				1.3	80.8
Aldehyde/ketone					7.9				3.5	60.8

Note. Only terms (coefficient or constant) that were found to be significantly different from zero are reported.

humic acid had 26% of each C-alkyl and aromatic/unsaturated C, the majority of the lignin was N-alkyl (28%), and the protein was equally C-alkyl and amide/carboxyl C, with 28% in each group. For all samples and standards except cellulose the smallest proportion of their carbon atoms fell in the aldehyde/ketone range, ranging from 0.81% in the lignin to 6.64% in the DOM. For cellulose, the lowest proportion of its carbon atoms fell in the phenolic C range; however, 84.27% of the carbon atoms in cellulose fell in only three of the ranges (C-alkyl, O-alkyl, and O₂-alkyl), better reflecting the known composition of cellulose. The DOM had the most varied composition, being the only samples or standard type to have at least 5% of its carbon atoms in each functional group (Table 1).

For two functional groups statistically significant relationships with depth in the peat profile were found—the aromatic and the phenolic functional groups

$$\%Aro = 0.04d + 8.2 \quad r^2 = 0.64, p = 0.03, n = 6$$

(0.01) (0.6) (2)

$$\%Phenol = 0.03d + 6.3 \quad r^2 = 0.44, p = 0.09, n = 6$$

(0.01) (0.7) (3)

where d = the depth of the sample from the peat surface (cm), %Aro = the percentage of aromatic-C functional groups, and %Phenol = the percentage of phenolic-C functional groups. The values in brackets below equations (2) and (3) are the standard errors in the coefficient and the constant terms. Both these relationships were positive, indicating that the proportion of C in both aromatic and phenolic groups increases with increasing depth into the peat and both of these groups are in the high shift range (Figure 3). There is insufficient evidence to suggest whether maxima or minima in the down profile functional group composition, as is known for this site in C/N (Worrall et al., 2012). However, a significant increase in the degree of unsaturation with depth in the peat soil profile has been observed for this site (Worrall et al., 2016) and consistently for UK peat soil depth profiles (Clay & Worrall, 2015)—higher degree of unsaturation would lead to higher chemical shifts in ¹³C-NMR spectra.

For the vegetation samples, the aboveground biomass sample represented the median of all five samples in the proportion of the N-alkyl, aromatic, phenolic, and aldehyde functional groups. The composition of the belowground biomass sample was highly correlated with the composition of the aboveground biomass ($r^2 = 0.998$, $n = 6$, $p < 0.01$). A comparison between the functional group composition of the aboveground biomass was compared to that of the lignin, cellulose, and protein, and by linear combination the best fit composition was 49% lignin, 27% cellulose, and 24% protein with a mean percentage error of 53%. Huang et al. (1998) used ¹³C-NMR to measure the composition of *Calluna vulgaris* litter (compared to the analysis of whole *Calluna vulgaris* considered in this study) and found it to be 20% lignin, 46% cellulose, and 28% protein but gave no error estimation.

3.1. Multiple Regression Analysis

A significant linear relationship was found between each of the functional groups defined in Table 1 and the elemental analysis and the parameters derived from the elemental analysis (Table 2). The regression analysis does again highlight that a straightforward interpretation of the NMR spectra as proportions of single functional groups is problematic. For example, the range 45–65 ppm is correlated with elemental measures

Table 3
The Loadings on the Principal Components

Variable	PC1	PC2	PC3
C-alkyl	-0.12	-0.62	0.23
N-alkyl/methoxyl C	-0.24	0.17	0.55
O-alkyl	-0.47	0.20	-0.28
O ₂ -alkyl/acetal C	-0.22	0.58	-0.23
Aromatic/unsaturated C	0.38	0.19	0.42
Phenolic C	0.33	0.41	0.35
Carboxyl/amide	0.46	-0.03	-0.33
Aldehyde/ketone	0.45	-0.02	-0.33
Variance explained (%)	43.9	25.4	18.4

associated with oxygen containing groups and highly oxidized structures and not with N or its ratios. Conversely, the range 160–190 ppm was correlated with the amount of N in the organic matter but also the C_{ox}, a parameter that increases with both N and O content of the organic matter.

3.2. Principal Component Analysis

The analysis of the data set based only on proportion of the functional groups showed three components with an eigenvalue >1 and collectively the three components explained 88% of the variance in the original data set (Table 3). The first component is highly positively correlated with the groups, or ranges, with high parts per million

shifts and contrasts with negative correlation for the low shift. The second component has positive correlations to the midrange chemical shifts with negative correlation to both high and low chemical shifts but particularly low chemical shifts and the 0–45 ppm range ascribed to C-alkyl. The third component is more complex with positive correlation for the ranges 45–65 ppm (N-alkyl/methoxyl-C) and 110–145 ppm (aromatic/unsaturated C) and particular negative correlation for high chemical shift ranges. Examining the values shows that PC3 is dominated by values for the lignin standard.

The comparison of PC1 and PC2 shows a clear differentiation between the samples (Figure 4). The vegetation samples plot at low values of PC1 and positive values of PC2 suggesting that vegetation is dominated by low chemical shift functional groups—the litter sample plotted at the highest PC1. The peat samples plot at low values of PC2 implying that peat is relatively enriched in functionalities at low and high chemical shifts but not the midrange chemical shifts. The shallowest peat samples (2 cm depth) plot at the lowest values of PC1; the peat samples show a positive relationship between PC1 and depth:

$$PC1 = 0.01d - 1.33 \quad r^2 = 0.67, p = 0.0460, n = 6$$

(0.004) (0.21)

(4)

where terms are as described above and values in brackets below equation (4) are the standard error in the coefficient and the constant term. Equation (4) agrees with equations (2) and (3) that the shift with depth is to increase the proportion of unsaturated C and also increased importance of alkyl-C.

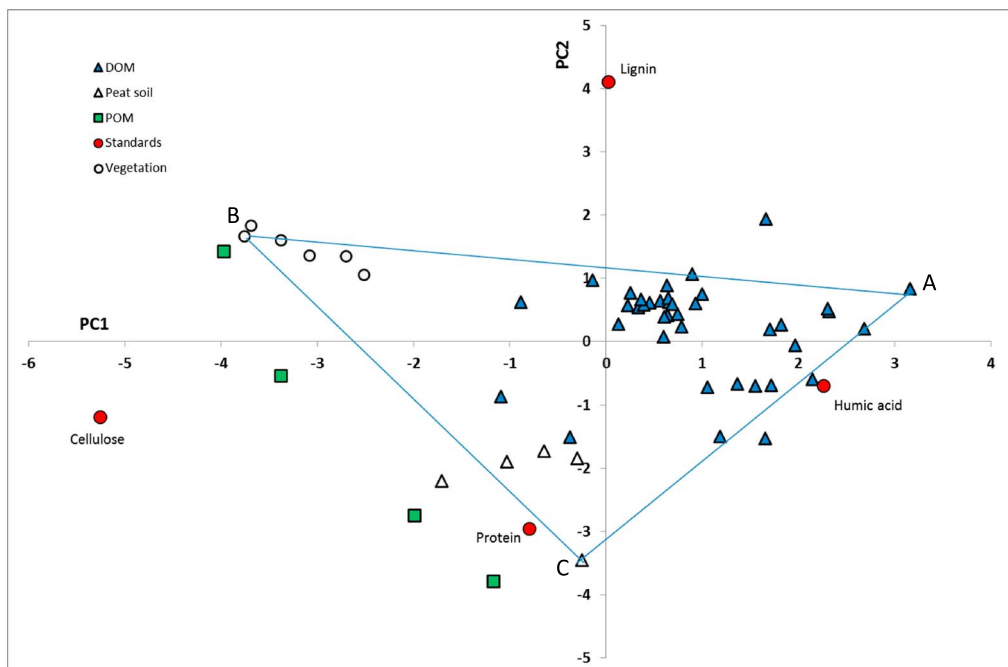


Figure 4. The comparison of PC1 and PC2 for all ¹³C-NMR spectra.

The POM samples plot between the peat soil and vegetation implying that POM is an admixture of vegetation and peat. Based upon elemental analysis and oxidation state of the organic matter in this catchment, Worrall et al. (2016) showed that POM was a simple admixture of eroded peat soil and vegetation, or litter, washed from the peat surface: this study would concur with that interpretation.

The DOM samples all plot at higher values of PC1 than any of the peat, vegetation, or POM and show that DOM is enriched in the high shift ppm functionalities. Peat DOM has been commonly characterized as highly colored (high light adsorption in the visual range) and that this has been associated with the aromaticity of the DOM (Thurman, 1985), but Worrall et al. (2016) have shown that DOM from this site was more oxidized than any other of the organic matter considered here, and this is consistent with a higher proportion of high shift functionalities such as ketones. The DOM sample with the highest PC1 score of all the samples analyzed as part of this study was collected in August 2013, and it had the second highest proportion of phenolic and aldehyde functionalities from all the samples considered in the study. From the elemental analysis, this sample of DOM taken in August 2013 also had the second highest average carbon oxidation state (C_{ox} , Masiello et al., 2008) of all the samples in this study. Such a highly oxidized sample of DOM can be thought of as the most evolved (the least like the other components) of the DOM samples, and indeed, this sample of DOM contrasts with the DOM sample with the lowest PC1 value which plots toward the vegetation and litter samples, while the DOM sample with the lowest PC2 plots in the direction of the peat samples. Alternatively, the DOM samples can then be interpreted as a mixture of three end-members (Figure 4)—a highly evolved and oxidized DOM (composition A), a biomass source (composition B), and a peat source (composition C). The proportion of the evolved DOM end-member (composition A) varies from 13 to 100%, but the median value was 58%; that is, to be DOM, the organic matter composition must have already evolved to a certain degree. Worrall et al. (2016) showed that DOM did not exist until it has a $C/O < 1.41$, that is, until it had been sufficiently oxidized, and that would appear to be the case here also. There is no significant difference in the proportion of composition A between months and years implying no seasonal cycle in the DOM composition. However, the pattern implies that DOM comes about from two processes. Use of logistic regression gives a significant distinction between DOM and the other sampled organic matter as

$$\begin{array}{r} 2.1\%Ald + 0.3\%Carb > 12.5 \\ (0.9) \quad (0.2) \quad (5) \end{array} \quad (5)$$

where %Ald = percentage of aldehyde functional groups shift range and %Carb = the carboxylic functional groups shift range, and values in brackets represent the standard errors in the coefficients. Equation (5) misclassifies only one of the DOM samples and none of the non-DOM samples. The odds ratio of equation (5) shows that the %Ald term is the most important, and when considered in terms of single variables, then either of the following rules apply, for a greater than 50% probability of becoming DOM:

$$\%Ald > 3.8 \quad (6)$$

$$\%Carb > 16.0 \quad (7)$$

No such significant rule could be found for the other functionalities, and therefore, the distinguishing feature of the DOM is not its aromatic or unsaturated content but the presence of oxidized and acidic groups—very similar to the pattern observed by Huang et al. (1998) for an upland organomineral soil within this study catchment. Further, the trends BA and CA (Figure 4) show that oxidation to DOM could occur from the reaction of either biomass or peat.

3.3. NMR Budget

Weighting the functional group compositions by equation (1) and comparing with the known flux pathways (DOM + POM + residual peat) shows that the greatest loss in processing through the peatland is the O-alkyl functionality, and but that in some cases there was a gain of some functionalities (e.g., aromatic-C—Figure 5). Not only was the greatest loss by mass the loss of O-alkyl but also it was the greatest percentage loss when compared with the amount of each functional group present in the primary productivity (i.e., 75% of the original O-alkyl fixed as primary productivity is lost before 1 m depth). Conversely, the amount of aldehyde functional group has more than doubled between the fixation in primary productivity and 1 m depth. The difference the composition of the primary productivity and the sum of the known flux components represents the composition lost to the gaseous components ($CO_2 + CH_4$) and in which case it is obvious that

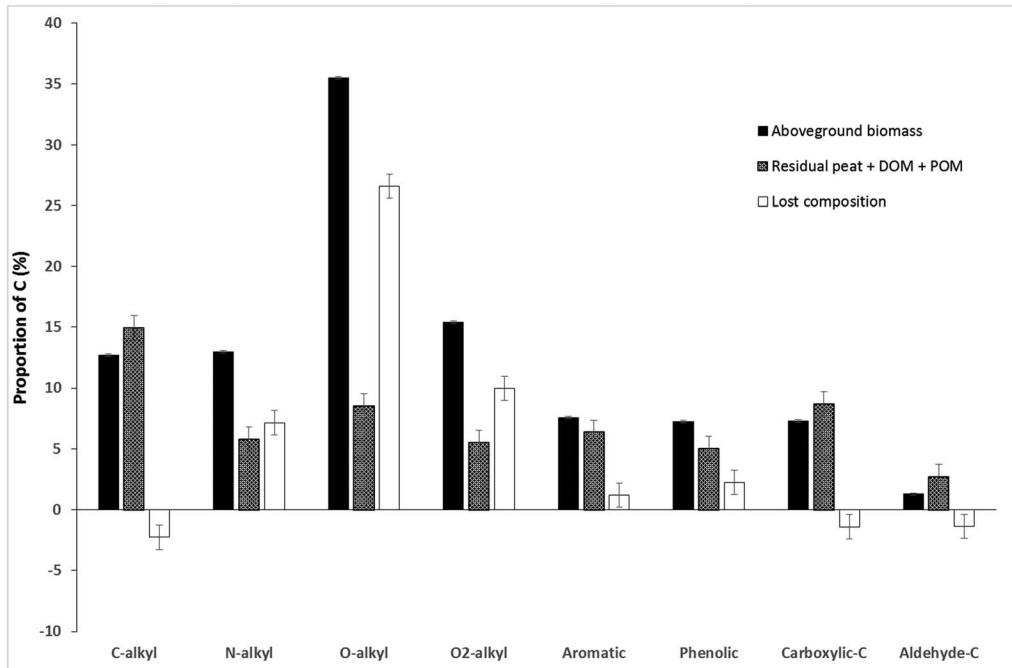


Figure 5. Comparison of composition through the peatland including the estimated lost composition.

the lost composition is dominated by O-alkyl and contained little or no C-alkyl, carboxylic-C, or aldehyde-C (Figure 5). When compared to the standards, the best fit combination for this lost composition was 7% lignin, 93% cellulose, and 0% protein, that is, the processing of the peatland is removing carbohydrates which are returning to the atmosphere as CO₂ or CH₄, while the peatland is preferentially retaining lignin. Equation (1) can now be rewritten to account for the changes in polysaccharide, lignin, and protein:

$$\begin{aligned}
 100[0.49C_{\text{cell}} + 0.27C_{\text{lig}} + 0.24C_{\text{pro}}]_{\text{PP}} &= 45[0.93C_{\text{cell}} + 0.07C_{\text{lig}}]_{\text{R+CH}_4+\text{dissCO}_2} \\
 &+ 26[0.16C_{\text{cell}} + 0.37C_{\text{lig}} + 0.47C_{\text{pro}}]_{\text{DOC}} \\
 &+ 9[0.56C_{\text{cell}} + 0.17C_{\text{lig}} + 0.27C_{\text{pro}}]_{\text{POC}} \\
 &+ 22[0.17C_{\text{cell}} + 0.33C_{\text{lig}} + 0.49C_{\text{pro}}]_{\text{RES}} \quad (8)
 \end{aligned}$$

where C_x = carbon from biopolymer x, with cell = cellulose (polysaccharide); lig = lignin; and pro = protein; with other terms as defined for equation (1).

4. Discussion

The PCA of the NMR data within the context of the carbon budget allows a coherent interpretation of the organic matter budget of a peat ecosystem. Primary productivity sequesters CO₂ from the atmosphere which forms biomass as lignin and polysaccharides (here referred to as cellulose). The primary productivity forms litter and exudates which becomes humified into peat soil. The humification process in this ecosystem sees the loss of 78% of the primary productivity, but preferentially, this loss is of cellulose with 91% of the C sequestered into the aboveground biomass as cellulose lost by 100 cm depth but only 70% of the C sequestered in the aboveground biomass as lignin has been lost. Stout et al. (1988) has suggested that the persistence of polysaccharides in soils is actually due to secondary polysaccharides from the in situ microbial population. The loss of carbon can be as any combination of CO₂, CH₄, DOM, or POM. A number of studies have also observed the preferential loss of polysaccharides over lignin in peat soils (Benner et al., 1984; Biester et al., 2014; Bracewell et al., 1980; Van der Heijden & Boon, 1994). In their study of change in the composition of *Calluna vulgaris* litter over 23 years Huang et al. (1998) showed that polysaccharides decomposed faster than lignin although given the initial composition polysaccharide still dominated the litter after 23 years. Using the NMR budget calculated by this study, the peatland net exchange is influenced by the vegetation composition. If 100% of the vegetation in the catchment was *Calluna vulgaris* or *Eriophorum*

spp., the proportion of the lost component attributed to each carbon type would change slightly, but not significantly—the magnitude and direction of each value does not change. However, if all the vegetation in the catchment was moss, such as *Sphagnum spp.*, the loss of O-alkyl groups was much larger and aromatic groups would be lost rather than gained by the ecosystem. Likewise, if all the peat in the catchment was composed of the same proportion of carbon types as the surface peat, then the gain of C-alkyl is much smaller, but there are few other differences. If all the peat was deep peat, the loss of aromatic C and N-alkyl C are smaller than the weighted budget. These show that the budget is sensitive to the peat and vegetation composition, but changing the vegetation drastically only mildly affected the budget values. Changing the aboveground vegetation would affect the composition of the litter layer and the peat, with peat under *Calluna vulgaris* likely to be composed of more N-alkyl C and less O-alkyl C compared to the peat used in this study, taken from under a varied vegetation cover.

The POM composition shows that it is dominantly sourced from the erosion of peat soil and wash off of vegetation (Figure 4). The DOM composition shows that it is predominantly derived from the humification (or degradation) of lignin and protein and not cellulose with 30% of the lignin fixed as primary production lost as DOM. The relationships found by the principle component analysis (Figure 4) can also be used to infer “ages” of the organic matter, with the highly evolved and oxidized DOM resulting from the mixing of relatively “young” vegetation and “old” peat, in consensus with the model of the age of peat-derived DOM by Tipping et al. (2010). They measured the age of DOM samples from the Cottage Hill Sike catchment using ^{14}C isotopes and found it was a maximum of 20 years old but stated that was an average of the various components of which DOM is comprised, with model results showing 50% of DOM was between 1 and 5 years old. Sanderman et al. (2008) showed, using ^{13}C -NMR, that DOM from mineral soils was not representative of either fresh plant residues nor the soil organic matter of the ecosystem from which it derived. Huang et al. (1998) showed that DOM from an UK organomineral soil was richer in lignin and aromatic structures than the soil organic matter they were sampled from. Worrall et al. (2016) studied the C_{ox} values of DOM in the context of this field site and suggested DOM was an intermediary in the complete oxidation of organic matter to CO_2 . However, this study would suggest that the process responsible for the majority of the CO_2 production was oxidation of carbohydrates such as cellulose, while DOM is the product of processing lignin and protein. A commonly reported degradation pathway from lignin is the oxidative cleavage of the alkyl side chain which will result in an increase in oxidized functional groups. Side chain oxidation is a predominant reaction with white rot fungi resulting in C-C cleavage/depolymerisation of lignin (Filley et al., 2000). Previous studies demonstrate that oxidative alteration of the lignin propyl side chain results in the enhanced production of 3,4-dimethoxybenzoic acid and 3,4,5-trimethoxybenzoic acid, and this would be consistent with oxidation leading to increased carboxylic acid functionalities in DOM. The residual peat concentrates the residual lignin components.

5. Conclusions

This study showed the following:

1. DOM existed only once a threshold of functional group composition had been achieved. The critical functionalities for defining DOM were aldehyde and carboxylic acid groups which showed that oxidation state was the defining characteristic of DOM and not its aromaticity.
2. DOM was formed from the oxidation from both biomass and peat, and both sources of DOM mixed and evolved in the streams of the study catchment.
3. The peat ecosystem shows a preferential loss of O-alkyl with a preferential survival of aldehyde and alkyl groups into peat at 1 m depth.
4. The ^{13}C -NMR budget of this peatland showed that carbohydrates fixed by primary productivity are preferentially removed and released as carbon gases, while lignin is preferentially preserved into the organic matter at depth in the peat profile.

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