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Quantifying Dissolved Organic Carbon Concentrations in Upland Catchments Using Phenolic Proxy Measurements

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Abstract

Concentrations of dissolved organic carbon (DOC) in soil and stream waters in upland catchments are widely monitored, in part due to the potential of DOC to form harmful by-products when chlorinated during treatment of water for public supply. DOC can be measured directly, though this is expensive and time-consuming. Light absorbance in the UV-vis spectrum is often used as a surrogate measurement from which a colour-carbon relationship between absorbance and DOC can be derived, but this relationship can be confounded by numerous variables. Through the analysis of data from eight sites in England and Wales we investigate the possibility of using the concentration of phenolic compounds in water samples as a proxy for DOC concentration. A general model using data from all the sites allowed DOC to be calculated from phenolics at an accuracy of 81-86%. A detailed analysis at one site revealed that a site-specific calibration was more accurate than the general model, and that this compared favourably with a colour-carbon calibration. We therefore recommend this method for use where estimates of DOC concentration are needed, but where time and money are limiting factors, or as an additional method to calculate DOC alongside colour-carbon calibrations. Tests demonstrated only small amounts of phenolic degradation over time; a loss of 0.92 mg L^{-1} after 8 months in storage, and so this method can be used on older samples with limited loss of accuracy.

Keywords: Dissolved organic carbon, phenolics, absorbance, peatland, water colour,

1. Introduction

Dissolved organic carbon (DOC) is a fluvial export from organic rich soils. Its concentration is affected by various factors, such as soil carbon pool, peat cover (Aitkenhead *et al.*, 1999), hydrology (Dawson *et al.*, 2004), and vegetation (Palmer *et al.*, 2001), as well as autochthonous production (Hope *et al.*, 1994). DOC concentrations have been increasing

in waters draining upland catchments in the UK (Freeman *et al.*, 2001a), with similar trends being observed in waters in North America (Stoddard *et al.,* 2003) and Scandinavia (Skjelkvåle *et al.,* 2005). One hypothesis is that these increases are driven by a recovery from atmospheric deposition (Monteith *et al.*, 2007, Ekström *et al.*, 2011, Evans *et al.*, 2012) although experimental studies also demonstrate that DOC loss can be strongly affected by climate (e.g. Fenner & Freeman, 2011), and other factors such as hydrology, land management, and atmospheric carbon dioxide concentration (Clark *et al.,* 2010). Rising DOC concentrations have implications for human health, as harmful by-products can be formed when DOC is chlorinated during water treatment (Chow *et al.,* 2003). Additionally, high levels of DOC result in increased water treatment costs due to the use of a higher coagulant dose, increased filter backwashing, and the production of larger amounts of sludge (McDonald *et al.,* 1991). DOC cycling is also of interest to those studying carbon budgets, and significantly affects aquatic ecosystem functioning via its influence on light penetration, mobility and form of toxic substances, and the supply of energy and nutrients.

DOC is typically measured by high temperature combustion using infra-red detection either as 'non-purgeable' organic carbon (i.e. that part of the total dissolved carbon that is not removed following acidification of the sample and sparging with oxygen gas), or by calculating and then subtracting inorganic carbon from total carbon. These methods are expensive and time-consuming, and require access to specialist analytical equipment. A second method is to use absorbance at certain wavelengths in the ultraviolet-visible (UV-vis) range as a proxy for DOC. Wavelengths used include 254 nm (e.g. Edzwald, 1985), 330 nm (e.g. Moore, 1987), 360 nm (e.g. Collier, 1987) and 400 nm (e.g. Gibson *et al.,* 2009). Routinely, a calibration curve is established between the chosen wavelength and a limited series of DOC measurements, so that further DOC concentrations can be calculated from the calibration. Wallage and Holden (2010) demonstrate that caution must be used when using

absorbance as a proxy for DOC, as relationships between DOC and absorbance change over time, with depth, and with management practices. Tipping *et al.* (2009) created a DOC model for non-polluted waters, using absorption at 254 nm and 340 nm, but Grayson & Holden (2012) argued that wavelengths under 300 nm are unsuitable as DOC proxies, as they display rapid fluctuations in absorbance and a lack of differentiation between wavelengths. However, wavelengths in the 400 nm region can sometimes be unsuitable as iron can interfere with absorbance readings (Kritzberg & Ekström, 2012) Other colorimetric methods exist to measure DOC, whereby the chemically-induced colour change of a sample is measured with a spectrophotometer, such as that proposed by Bartlett & Ross (1988). Finally, fluorescence spectroscopy can be used as a method to characterise DOC, but not to measure total DOC. This approach is valuable due to its high specificity and sensitivity (Chen *et al.*, 2003). An alternative method, rather than UV-vis, may therefore prove useful as a surrogate DOC measure.

One feature of waters draining from wetlands, including peatlands, is the presence of recalcitrant phenolics (Wetzel, 1992), which are secondary plant metabolites (Hättenschwiler & Vitousek, 2000). Their concentrations vary seasonally (Kaiser *et al.*, 2001) and are controlled by plant characteristics (Wetzel, 1992), and physical and chemical factors such as photodegradation (Faust & Holgne, 1987). They accumulate due to a lack of oxygen in waterlogged soils, which limits the activity of the extracellular enzyme phenol oxidase (Freeman *et al.*, 2004). Phenolics are part of the coloured component of DOC (Toberman *et al.*, 2008). They are aromatic, but DOC also includes aliphatic compounds (Leenheer & Croué, 2003). Relationships between DOC and phenolics have been noted previously (Kang *et al.*, 2002, Hagedorn & Machwitz, 2007). The aim of this analysis is therefore to determine if an empirical relationship exists between the concentrations of DOC and phenolic-OH (hydroxyl group) in upland waters, and under what conditions such a relationship might exist:

whether it is the same for different sites, soils and samples types, and how stable it is in the long term. Based on the results of this analysis, the potential for using phenolics as a surrogate measure for DOC is critically evaluated.

2. Materials and Methods

2.1. Study Sites

A total of 2020 water samples were taken from eight sites in northern Wales and northern England, UK, summarised in table 1. At Ffynnon Eidda 192 samples were from ditch water and 132 samples were from pore water. The Migneint site was split into three sub-sites: pore waters from two different soil types (blanket peats and peaty podzols) and soil leachate samples. The Peaknaze site was split into two sub-sites (again with pore water samples from blanket peat and peaty podzols). For each peat and podzol sub-site approximately 600 data points were available, but random selections of 300 were taken so as not to bias the model towards these sites. Other samples were taken from either standing water bodies or pore water (using piezometers or Rhizon samplers at 10 cm depth), or were generated from soil samples (from 10 cm or 30 cm depth) in the laboratory (leachate). At all sites, sampling was repeated at fixed locations on a number of occasions.

Table 1. Location of field sites (ordered by sample type), including soil type, sample type, and the time period over which sampling took place. For pore waters, P indicates a piezometer sampler, and R indicates a Rhizon sampler. The fen mesocosms consisted of rafts of vegetation floating in individual pools.

2.2 Phenolics Assay

Samples were filtered through Whatman 0.45 μm cellulose nitrate filters, and phenolic concentrations were determined using a method adapted from Box (1983). 0.25 ml of sample

was added to a clear microplate well. 12.5 µl of Folin-Ciocalteau reagent was added (using a pipette calibrated to 1.98% accuracy with a covariance of imprecision of 0.57%), followed by 37.5 µl of Na_2CO_3 (200 g L^{-1}). After 1.5 hours the absorbance was measured at 750nm on a BMG Fluostar Galaxy or Molecular Devices M2e Spectramax plate-reader. Phenolic concentrations were then derived from the preparation of a standard curve using laboratory-prepared standards of known concentration (0, 1, 2, 4, 6, 8, 10, 15, 20 mg L^{-1}). Additional standards (0.2, 0.5, 0.75, 1.5 mg L^{-1}) were used for the analysis of samples from Llyn Cwellyn, Llyn Conwy and Llyn Teyrn as phenolic concentrations from these sites were frequently found to be < 1 mg L^{-1}. Box (1983) cited a limit of detection of 6 µg phenol L^{-1} and a standard deviation of 4.1% at 1 mg phenol L^{-1} for this assay, although more recently the limit of detection has been cited as 25 µg L^{-1} (Thoss *et al.*, 2002).

2.3 DOC Analysis

All samples were filtered through Whatman 0.45 µm cellulose nitrate filters and analysed using an Analytical Sciences Thermalox Total Carbon analyser. Samples were acidified (pH < 3) and sparged with oxygen to remove any inorganic carbon, and DOC concentrations calculated using a seven point calibration curve (plus a quality control sample), with additional standards to check for drift, and several samples (1-3 per run) duplicated to check for reproducibility. Each individual sample was injected 5 times, and the result accepted if the coefficient of variation of the five injections was less than 3%.

Plynlimon samples were analysed differently. They were diluted with sulphuric acid and purged with oxygen (to remove inorganic carbon), after which a digestion reagent (consisting of 0.044 M $K_2S_2O_8$, 0.089 M $Na_2B_4O_7$ and H_2O) was added. Following exposure to a UV source, radicals react with the organic material in the sample, which is converted into CO_2 and H_2O. By gas dialysis the CO_2 is lead into a colour reagent. Colour intensity

147 (measured at 550 nm) then decreases proportionally to the change in pH caused by the CO_2,
148 and this decrease is in relation to the DOC.
149
150 *2.4 UV-vis analysis*
151 UV-vis analysis was conducted on 192 samples from the Ffynnon Eidda site using a
152 Molecular Devices M2e Spectramax plate-reader. Light absorbance at the 254 nm and 400
153 nm wavelengths was measured.
154
155 *2.5 Statistics*
156 Phenolic and DOC values were paired together in order to examine any relationship between
157 them, and statistical analysis carried out using SPSS v16.0.1 (IBM Corporation, http://www-
158 01.ibm.com/software/analytics/spss/products/statistics/). Different sites and samples were
159 compared using t-tests and ANOVAs or, where data were not normally distributed (identified
160 by Kolmogorov-Smirnov Test), Mann-Whitney and Kruskal Wallis tests, with Bonferroni-
161 adjusted p values. The Bonferroni correction is a method to control the familywise error rate,
162 but does increase the probability of missing real differences in the data.
163 **3. Results**
164 *3.1 General model*
165 The linear regression gave the fit shown in Figure 1.
166
167 **Figure 1. Observed relationship between phenolic concentrations (mg L^{-1}) and DOC concentrations (mg**
168 **L^{-1}) for all 2020 water samples. $r^2 = 0.84$, residual variance = 72.051, $p < 0.001$.**
169
170 This linear regression allowed DOC concentrations to be calculated directly from phenolic
171 concentrations, according to the formula:

$$DOC = (5.68 \times Phenolics) + 1.99 \quad (1)$$

where DOC is calculated in mg L^{-1}, and Phenolics is the measured phenolic concentration, also in mg L^{-1}. Standard errors of the model parameters are respectively (5.68) $^{+}$/- 0.06 and (1.99) $^{+}$/- 0.32. Confidence intervals at 95% were 2.24 (lower) and 2.33 (upper).

This general model was then tested using phenolic and DOC data from other sites in north Wales (figure 2). These were stream samples from the Nant y Brwyn (an upland stream in a peat catchment, 410 m ASL), leachate samples from Alwen Reservoir (an upland forested peat catchment, 390 m ASL), and pore water samples from Llyn Serw (an upland peat catchment, 460 m ASL). Fits were generally good ($R^2 \geq 0.75$) although the model tended to overestimate DOC concentrations at the Nant y Brwyn and underestimate them at Llyn Reservoir and Llyn Serw. The model calculated DOC to a mean accuracy of 86% (modelled values were on average 1.69 mg L^{-1} different to measured, standard error 0.32 mg L^{-1}) at the Nant y Brwyn, 81% (mean difference of 2.21 mg L^{-1}, SE = 0.36 mg L^{-1}) at Alwen Reservoir, and 86% (mean difference of 7.65 mg L^{-1}, SE = 0.94 mg L^{-1}) at Llyn Serw.

Figure 2. Regression between measured DOC and modelled DOC (mg L^{-1}) in Nant y Brwyn stream water, *n*=24, r^2=0.90 (A), Alwen Reservoir leachate samples, *n*=25, r^2=0.88 (B), and Llyn Serw pore water samples, *n*=44, r^2=0.75 (C). p<0.001 for each relationship. Dashed line shows 1:1 relationship.

Despite the strength of the model, there was variation in the relationship between DOC and phenolics at the different sites. Figure 3 shows the median ratio of phenolic to DOC concentrations at each site, which ranged from 0.14 : 1 to 0.27 : 1. Differences in the ratios were tested using the Kruskal Wallis test, followed by Mann-Whitney tests with Bonferroni corrections to control the probability of false positive results. A total of 26 tests were performed (table 2). The highest mean phenolic:DOC was found at Llyn Teyrn but there is no significant difference when compared to the other two lakes Llyn Cwellyn and

Llyn Conwy. The lowest mean phenolic:DOC was in the Peaknaze podzol and the fen mesocosms. It can be noted that spatial proximity of sampling sites is sometimes, but not always, associated with a similar response between DOC and phenolics. For instance, the peat and podzol sub-sites at Peaknaze are approximately 200 m apart and have no significant difference in their ratios. However, the Migneint peat and podzol pore water sample sites which are 500 m apart do show a significant difference.

Figure 3. Median phenolic concentrations (mg L^{-1}) per 1 mg L^{-1} DOC concentrations for each site used in the model.

Table 2. Results of Mann Whitney tests to compare for site differences in the median ratio of phenolics to DOC. Asterisks indicate a significant difference at a Bonferroni corrected p value <0.05. NS indicates no significant difference. A blank space shows where no comparison was carried out. It is unfeasible to run all possible pairwise comparisons as the Bonferroni correction would then produce a critical value of significance that is too restrictive. Sites along the top are abbreviated, but are in the same order as those down the side.

A further investigation of different samples types is useful. For instance, there is no significant difference between the two podzol soils at Peaknaze and the Migneint. Figure 4 displays this amalgamated podzol data against its peat equivalent. The mean ratio of phenolics to DOC is significantly different between the two soil types: 0.15 : 1 in the podzol, and 0.18 : 1 in the peat. Additionally, the concentrations of DOC and phenolics cover a larger range and increase to higher values in the peat soil. Phenolic concentrations had a range of 21.05 mg L^{-1} with a maximum of 21.53 mg L^{-1} in the two peat soils, compared with a range of 15.83 mg L^{-1} and maximum of 16.27 mg L^{-1} in the podzols. There is also a difference between surface water and pore water when all sites are considered (figure 5). The mean proportion of phenolics to DOC is 0.20 : 1 in pore water compared to 0.17 : 1 in surface water. The three lakes all possessed a high proportion of phenolics but their relatively small

sample sizes compared to other surface waters reduced their influence on the mean. Concentrations of phenolics and DOC ranged more in the pore water and reached higher levels. Maximum pore water phenolic concentration was 21.53 mg L^{-1}, whilst the highest surface water value was 12.71 mg L^{-1}.

Figure 4. Regression between phenolic and DOC concentrations (mg L^{-1}) for the Migneint and Peaknaze podzol (white circles) and peat (black circles) sites. *n*=600 for each soil type. Podzol r^2=0.71. Peat r^2=0.79. For both soils p<0.001.

Figure 5. Regression between phenolic and DOC concentrations (mg L^{-1}) for surface waters (from Ffynnon Eidda, Llyn Cwellyn, Llyn Conwy, Llyn Teyrn, and fen mesocosms – *n*=608) and pore waters (from Migneint peat, Migneint podzol, Peaknaze peat, Peaknaze podzol, and Plynlimon – *n*=767). Surface waters r^2=0.88. Pore waters r^2=0.84. For both samples types p<0.001.

As phenolic concentrations are affected by factors such as vegetation growth, microbial processes and phenol oxidase activity (Freeman *et al.*, 2001b), their concentrations vary seasonally. Figure 6 details these variations for a time period of just over four years. Although not always consistent, there are occasions when all four sites respond similarly; this is perhaps most pronounced in March 2011 when all sites show a large spike, with a lesser peak following in July/August 2011. There are also occasions where just two sites respond simultaneously, such as peaks for both Migneint sites during October 2009. There is extensive interannual variation, however, with peaks and troughs in the relationship occurring at different times during different years.

Figure 6. Changes in the mean proportion of phenolics to DOC for four sites from September 2007 to January 2012, with an approximate monthly sampling frequency. Sites are: Migneint peat – solid line,

Migneint podzol – dotted line, Peaknaze peat – dashed line, Peaknaze podzol – dotted/dashed line. For each site and each date the mean is generated from *n*=12.

3.2 Site-specific model and comparison with UV-vis method

Results indicate: 1) that the general model calculated DOC to a mean accuracy of 81-86%; 2) that there was considerable difference between sites and soils in the mean ratio of phenolics to DOC. Therefore we investigated the possibility of using phenolic measurements as a proxy for DOC on a specific site basis, with the hope of improving the accuracy and giving more appropriate modelled DOC values. To investigate this a random selection of 100 paired phenolic and DOC measurements were selected from surface water samples from the Ffynnon Eidda site, and a regression fitted to give the site-specific equation (r^2=0.87, $p<0.001$) :

$$DOC = (5.83 \times Phenolics) - 0.59 \qquad (2)$$

where DOC and phenolics are calculated in mg L^{-1}. Equation 2 was then applied to the remaining 92 surface water phenolic measurements from Ffynnon Eidda to calculate DOC, as was equation 1. Equation 1 (the model using data from all sites) calculated DOC to a mean accuracy of 83.67% (standard error = 1.96%) whilst equation 2 (site-specific model) gave a mean accuracy of 86.54% (SE = 1.57%). A paired t-test (after the data was normalised by subtracting each value from 100% followed by square root transformation) showed this difference to be significant ($p<0.05$).

We also compared a site-specific phenolics model against a colour-carbon model: that is, a regression of DOC concentration against light absorbance at a certain wavelength. For this, 192 data points from the Ffynnon Eidda surface water dataset were used, and phenolic concentrations compared against absorbance at 254 nm and 400 nm (figure 7). Absorbance

at 254 nm gave the best fit, closely followed by phenolic concentration, whilst absorbance at 400 nm gave the weakest fit.

Figure 7. Regressions of DOC concentration against A) phenolic concentration, B) absorbance at 254 nm, C) absorbance at 400 nm, for 192 ditch water samples from Ffynnon Eidda. r^2 values A) 0.87, B) 0.9, C) 0.79. For all regressions $p<0.001$.

Finally, if phenolic concentration is to be used as a proxy for DOC it is useful to know if a calibration can be established using a small number of measurements, and how this compares to a colour-carbon calibration. To test this a random sub-sample of 25 measurements was taken from the Ffynnon Eidda data-set and analysed by regression; r^2 and regression equation were noted – to allow a simple comparison the regression was forced through the origin. This method was repeated twenty times for DOC and phenolics, DOC and absorbance at 400 nm, and DOC and absorbance at 254 nm. The mean r^2 values were 0.83 for the phenolics model, 0.71 for the 400 nm model, and 0.85 for the 254 nm model. ANOVA revealed that there was no significant difference in the mean r^2 between the phenolic and 254 nm model, but that the 400 nm model differed significantly from both ($p<0.001$). The mean slope of all twenty regression equations was then compared against the slope of the regression that used all 192 data points; this gives a measure of the magnitude of error that using a small calibration brings. The mean slope difference was 2.65% for the phenolic model, 5.59% for the 400 nm model, and 3.16% for the 254 nm model. The only significant difference was between the phenolic model and the 400 nm model ($p<0.05$).

3.3 Phenolic degradation in stored samples

To investigate how phenolics degrade in stored water samples a small number of samples from the Ffynnon Eidda site were reanalysed for phenolic concentrations. One set of

300 samples had been in storage for 13 months whilst the second set had been stored for 8
301 months. They had been stored in plastic Nalgene® bottles (Thermo Scientific) in the dark at
302 4^{o}C. The site-specific model was then applied to phenolic concentrations that had been
303 measured both before and after storage (table 3). The mean loss of phenolics during storage
304 was 0.74 mg L^{-1} (11.7%) for the 8 month samples and 0.58 mg L^{-1} (8.3%) for the 13 month
305 samples. The smaller value for the 13 month samples is due to the fact that phenolic
306 concentration increased in two samples. Removing these numbers gave a mean of 0.77 mg L^{-1}
307 (12.9%). After 8 months in storage the phenolic measurements calculated DOC, on
308 average, to within a mean of 2.77 mg L^{-1} or 91.4% (compared to 1.87 mg L^{-1} or 93.9% before
309 storage). After 13 months DOC could be calculated to 5.29 mg L^{-1} or 84.6% (compared to
310 3.43 mg L^{-1} or 89.3% before storage). Additional analysis of pore water samples from
311 Ffynnon Eidda revealed that after 8 months the mean loss of phenolics was 0.92 mg L^{-1}
312 (12.4%), but after 13 months there was a mean increase of 0.62 mg L^{-1} (9.4%) (table 4).

313 Table 3. The extent of phenolic degradation in stored water samples taken from ditch water at Ffynnon Eidda.
314 'Phenolics' is the concentration taken immediately after sampling. 'Phenolics^{8}' or 'Phenolics^{13}' is the
315 concentration of the same sample after either 8 or 13 months of storage in the dark at 4^{o}C in plastic Nalgene®
316 bottles. 'Phenolics^{diff}' is the concentration change following storage, - indicates a loss, +indicates a gain. 'Meas
317 DOC' is the measured DOC concentration. 'Mod DOC' is the estimate DOC concentration using the site-
318 specific model, calculated using the original phenolic measurement. 'Mod DOC^{8}' and 'Mod DOC^{13}' are the
319 estimated DOC concentrations using the site-specific model, calculated using the phenolic measurements after
320 either 8 or 13 months of storage. All concentrations are in mg L^{-1}.

321

322 Table 4. The extent of phenolic degradation in stored water samples taken from pore water at Ffynnon Eidda.
323 'Phenolics' is the concentration taken immediately after sampling. 'Phenolics^{8}' or 'Phenolics^{13}' is the
324 concentration of the same sample after either 8 or 13 months of storage in the dark at 4^{o}C in plastic Nalgene®
325 bottles. 'Phenolics^{diff}' is the concentration change following storage, - indicates a loss, +indicates a gain. All
326 concentrations are in mg L^{-1}.

327

4. Discussion

4.1 Using the general phenolic model to calculate DOC

This analysis shows that phenolic concentrations can be used to give an estimate of DOC concentrations for the pore waters and drainage waters of peaty soils. A general model using data from numerous sites allowed DOC to be calculated for three new sites at a mean accuracy of 81-86%; these three sites included pore water, surface water, and leachate samples. For each of the three sites, there was some evidence of small systematic errors in DOC predictions, due to site-specific variations in the ratio of phenolics to DOC, relative to the whole-dataset mean. One of the reasons for the high phenolic concentrations typically observed in wetlands and uplands seems to be due to the occurrence of certain plant species. *Sphagnum* species, *Vaccinium myrtillus*, *Calluna vulgaris*, *Empetrum hermaphroditum*, and *Erica australis* are all phenolic-rich species (Rudolph & Samland, 1985, Gallet & Lebreton, 1995, Kähkönen *et al.*, 1999, Castells, 2008, Carballeria, 1980) and are typical of upland bog vegetation. High water levels that maintain anaerobic conditions constrain phenol oxidase activity and prevent the decomposition of phenolics, causing waters drained from these areas to have high phenolic concentrations (Freeman *et al.*, 2004). Variations in factors such as water table, temperature, soil type and vegetation may therefore explain some of the variability in the relationship between sites. For instance, the Migneint podzol site displays very low concentrations of phenolics per unit of DOC compared to the nearby Migneint peat site and this could be attributed to vegetation; the podzol site is typified by *Festuca ovina* and *Juncus squarrosus* and lacks the *Calluna* species that dominate the peat site. There is therefore less potential for the vegetation to release high concentrations of phenolics. In addition, it is a well drained soil so phenol oxidase activities will be higher, resulting in higher rates of phenolic degradation (Freeman *et al.*, 2001b).

A full understanding of site differences is complex, however. Despite the Migneint peat and podzol sites showing differences in the phenolic to DOC ratio, the adjacent Peaknaze peat and podzol sites do not. Like the Migneint sites, the peat site is predominantly comprised of *Calluna* and other bog species, whilst the podzol site largely features *Festuca ovina*, although *Calluna* is present. It therefore seems likely that the presence of *Calluna* could account for the lack of an observed difference at Peaknaze. Alternatively, it is possible that other environmental factors are the primary controller of phenolic concentrations at Peaknaze, such as shared precipitation and temperature. The long-term data sets from the paired Peaknaze and Migneint sites clearly show shared changes in the phenolic to DOC ratio. Some of these will be due to large scale weather events; a severe drought across the UK could stimulate phenol oxidase activity at all sites, thus causing an associated decline in phenolic concentrations. Drought conditions have also been shown to enhance both the abundance and diversity of bacteria that are capable of degrading phenolic compounds (Fenner *et al.,* 2005). On a similar theme, a localised mountain storm on the Migneint would be observed as a spike in the phenolic to DOC ratio as phenol oxidase is suppressed due to aerobic conditions facilitating the accumulation of phenolics (Freeman *et al.,* 2004). Where only one of the four locations shows a change this must be attributable to localised factors, such as vegetation controls.

There was no significant difference in the ratio of phenolics to DOC in the three lakes (Llyn Teyrn, Llyn Cwellyn and Llyn Conwy), and they all showed relatively high proportions of phenolics. This can partly be explained by the fact that all three are humic lakes; Shimp and Pfaender (1985) showed that when microbial communities become adapted to increased levels of humic acids, their capability to degrade phenolics is reduced. Processing of fresh DOC can occur rapidly in lakes (Tranvik *et al.,* 2009) and, coupled with the high dilution effect, differences in phenolic:DOC are unlikely to be observed on the same magnitude as

377 those occurring in soils. Phenolic concentrations and the other fractions of lake DOC will
378 vary throughout the year, due to changing hydrological conditions (Sachse *et al.,* 2001), and
379 differences in the efficiency of photolysis and microbial degradation (Hwang *et al.,* 1986).
380 Leachate samples from the Migneint were not significantly different from pore water
381 samples from the Migneint peat site but the phenolic content of the leachate samples varied
382 by an order of magnitude; the lowest concentration of phenolics to 1 mg L^{-1} of DOC was 0.07
383 mg L^{-1}, whilst the highest was 0.72 mg L^{-1}. Other work from forest ecosystems has
384 demonstrated that one of the main components of fresh leachate is phenolics (Yavitt &
385 Fahey, 1986, Beggs & Summers, 2011) so it seems likely that these differences are driven by
386 the depth of samples from the soil profile, and the availability of phenolics from adjacent
387 vegetation. A comparison of sample types revealed that the ratio of phenolics to DOC was
388 higher in pore water than surface water, and it can be hypothesised that this is due to the
389 increased leaching of phenolics into pore water from fresh litter (Beggs & Summer, 2011).
390 Additionally, precipitation will contribute to surface water, and organic carbon in rainfall has
391 been shown to consist of <1% phenolics (Likens, 1983).
392 Taken together these findings suggest that a general model can be used to calculate
393 DOC, but that variations in sample type, soil type, vegetation, and climate will all contribute
394 a degree of error. Therefore the general model should be a 'last resort' for situations where a
395 site-specific calibration isn't possible. For instance, Worrall *et al.* (2012) applied a general
396 colour-carbon calibration to sites where a site-specific calibration was unavailable. For
397 similar cases, the general phenolics model can be used to provide an additional estimate of
398 DOC concentrations.
399
400 *4.2 Using a site-specific model to calculate DOC*

401 Considering the uncertainty that environmental and climatic factors induce in a
402 general model, it is unsurprising that a site-specific regression of phenolics and DOC at
403 Ffynnon Eidda gave a stronger fit and was significantly more accurate. The exact accuracy
404 of any site-specific model will depend on the extent of phenolic variation throughout the
405 year, which will be controlled by the aforementioned external factors. To generate a robust
406 model, sampling should take place at different times throughout the year (assuming the model
407 will be used on to calculate DOC for an annual data series) and under different climatic
408 conditions. This should allow an 'average' model to be produced, rather than one that
409 systematically over- or underestimates DOC.
410
411 *4.3 Comparison of phenolic-based and absorbance-based DOC estimation*
412 A comparison of the performance of the site-specific phenol model to colour-carbon
413 models indicated that a model based on absorbance at 254 nm produced a slightly better
414 calibration than using phenolics, but that a model based on 400 nm model was not as strong
415 as either. It should be noted that none produced fits that were as good as those produced by
416 Tipping *et al.*, (2009) using a two wavelength (254 nm and 340 nm) model, but this method
417 was not directly investigated here.
418 The models were all created using a large number (192) of data points. A useful
419 model would, in reality, be constructed from as few data points as possible to save on the
420 costs of directly measuring DOC. Repeatedly generating models for each proxy (phenolics,
421 254 nm, 400 nm) using just twenty five randomly selected data points showed that the 254
422 nm model was the strongest on average, with the phenolics model only slightly weaker.
423 Again, the 400 nm model was considerably weaker compared to the other two. However, the
424 phenolic model was the most accurate; on average the twenty five point regression only

deviated from the full (192 point) model by 2.65%. This was significantly better than the 400 nm model (5.59%) but showed no difference to the 254 nm model (3.16%).

These results therefore suggest that a small-dataset, site-specific calibration of phenolics to DOC can be as or more accurate than a colour-carbon calibration, depending on the wavelength of light absorbance used. Accuracy will vary throughout the year as phenolic concentrations fluctuate, but the same problem is true of colour-carbon calibrations, as these also vary seasonally (Watts *et al.*, 2001, Wallage & Holden, 2010). Additionally, this study shows that a colour-carbon calibration at 254 nm is more accurate than one using 400 nm as a proxy, at least for the site examined. Part of the reason for this could be iron interference, as iron can contribute to absorbance measurements at approximately 400 nm (Kritzberg & Ekström, 2012). Wilson *et al.* (2011) found that the best proxy for DOC concentrations from different catchments on blanket bog was either absorbance at 254 nm or 400 nm. The results presented here suggest that studies using colour-carbon calibrations should investigate the potential of both wavelengths, as many just use 400 nm (e.g. Gibson *et al.*, 2009, Wallage & Holden, 2010, Rowson *et al.*, 2010).

UV-vis scanning of water samples for these models must take place within a week of sampling to ensure accuracy, and it is often desirable to analyse samples within a day of collection (e.g. Wilson *et al.*, 2011), but phenolics are relatively stable to microbial degradation (Chian, 1977) and thus samples do not have to be assayed immediately. There is a lack of information in the literature concerning the exact time samples can be stored for, but Afghan *et al.* (1974) noted no apparent loss after 16 days, provided samples were stored in glass bottles. However, our results demonstrate only a small loss of phenolics from plastic bottles after 8 months in storage in the dark at 4^{o}C. These samples still enabled DOC to be calculated to an acceptable degree of accuracy. Samples stored for 13 months allowed DOC to be calculated accurately, but interestingly two samples showed an increase in phenolics

450 following storage. Theoretically this could be an analytical error, but the fact that pore water
451 samples also showed phenolic increases after 13 months suggests it is a real effect. It may be
452 that the increase is due to phenolic compounds leaching into the sample from the plastic
453 bottle, but it is unknown why only some samples showed increases. More detailed work
454 could focus on the specific rate of phenolic degradation over time which, if known, could
455 then be incorporated into a model to allow DOC to be calculated accurately from older
456 samples. Considering these results, however, and it can be concluded that a phenolics-based
457 model is preferential to a UV-vis-based one if it is not feasible to analyse samples
458 immediately. Where samples can be analysed immediately, it is likely that the two
459 wavelength model of Tipping *et al.* (2009) will be more accurate.
460
461 *4.4 Practical applications*
462 If direct DOC measurements are unavailable or unaffordable then this method can be
463 considered an effective substitute, considering: 1) the equipment needed is minimal,
464 consisting of a few chemicals and access to a spectrophotometer able to determine
465 absorbance at 750nm; 2) preparation time for the samples is quick; 3) a microplate can be
466 used for the analysis, thereby allowing up to eighty four samples to be analysed at once; 4)
467 only a small amount (0.25 ml) of sample is needed; and 5) it can be used on older samples.
468 Some caution may be required in extending this approach to different sample types,
469 for example natural waters draining non-peaty soils, or leachate samples from other types of
470 organic matter. Certain substances will also interfere with the phenolics assay; notably, iron
471 concentrations higher than 2 mg L^{-1}. This was not considered to be an issue for the sites used
472 in this study; monthly samples from the Ffynnon Eidda site taken between September 2006
473 and September 2011 had a mean iron content of 0.86 mg L^{-1}, and only exceeded 2 mg L^{-1} on
474 four occasions out of eighty four sampling dates (CEH unpublished data). None of the

475 incidences of high iron concentrations coincided with high phenolic concentrations. Iron
476 levels for a peatland stream at the Plynlimon site averaged 0.1 mg L^{-1} for the period 1990-
477 2005, with a maximum value of 0.81 mg L^{-1} (Neal *et al.*, 2008). If iron is present in samples,
478 then adding a centrifugation step to the method can remove the error (Box, 1983).

479 This model therefore seems ideal for certain situations, such as those involving
480 practitioners and conservation agencies. For example, in the UK the incidence of drain
481 blocking on peatlands is increasing, often under the stewardship of environmental agencies
482 and land managers (Armstrong *et al.*, 2010). Some of these projects include monitoring of
483 DOC, but are more often focused on other objectives such as restoration of vegetation,
484 biodiversity enhancement and erosion control (Walker *et al.*, 2008). With limited funds and
485 equipment for detailed scientific monitoring, it may not be possible to robustly evaluate the
486 impacts of restoration on water quality. The method described here offers a viable solution to
487 gather data on the effects of restoration on DOC, a key parameter of concern from a water
488 supply and ecological perspective. This approach could replace or augment more commonly
489 used colour-carbon calibrations.

490

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Table 1

Site	Lat	Lon	Soil Type	Sample Type	No. Samples	Altitude (m)	Sampling dates
Ffynnon Eidda	52.97N	3.84W	Peat	Ditch/Pore (P)	326	490	Oct 2010 - Nov 2011
Migneint	52.99N	3.82W	Peat	Pore (R)	300	450	Aug 2007 - Jan 2012
Migneint	52.99N	3.81W	Podzol	Pore (R)	300	480	Sept 2007 - Jan 2012
Peaknaze	53.47N	1.91W	Peat	Pore (R)	300	440	Aug 2007 - Jan 2012
Peaknaze	53.47N	1.91W	Podzol	Pore (R)	300	430	Aug 2007 - Jan 2012
Plynlimon	52.46N	3.74W	Peat	Pore (R)	167	530	May 1992 – Sept 1992
Migneint	52.99N	3.82W	Peat	Leachate	45	450	Sept 2011, Jan 2012
Fen Mesocosms	53.22N	4.13W	Peat	Pool	210	20	June 2011 - July 2011
Llyn Cwellyn	53.07N	4.15W	Peat/Loam	Lake	24	140	Nov 2009 - Oct 2011
Llyn Conwy	52.99N	3.82W	Peat	Lake	24	450	Nov 2009 - Oct 2011
Llyn Teyrn	53.07N	4.03W	Peat	Lake	24	370	Nov 2009 - Oct 2011

Table 2

	Ppe	Mpod	Ppod	Mle	Lcw	Lco	Lt	Fe	Fen	Plyn
Migneint Peat	*	*	*	NS	*	NS	*	*	*	*
Peaknaze Peat		NS	NS					*		*
Migneint Pod			NS	NS		*		*		
Peaknaze Pod										
Migneint Leach								NS		
Llyn Cwellyn						NS	NS		*	
Llyn Conwy							NS		*	
Llyn Teyrn									*	
Ffynnon Eidda										*
Fen Mesocosms										
Plynlimon										

Table 3

Sample	Phenolics	Phenolics[8]	Phenolics[diff]	Meas DOC	Mod DOC	Mod DOC[8]
1	6.13	5.61	-0.52	30.3	32.8	30.2
2	4.99	4.94	-0.05	25.9	27.1	26.8
3	5.76	5.34	-0.43	28.9	31.0	28.8
4	5.71	5.06	-0.65	30.7	30.7	27.4
5	6.41	5.32	-1.09	31.4	34.2	28.7
6	6.35	5.19	-1.17	31.1	33.9	28.1
7	5.66	4.90	-0.76	29.9	30.4	26.6
8	7.09	5.85	-1.24	36.3	37.7	31.4
9	5.97	5.41	-0.56	29.2	32.0	29.2
10	6.52	4.94	-1.58	33.2	34.8	26.8
11	6.30	5.53	-0.77	35.4	33.7	29.8
12	4.77	4.75	-0.02	28.9	26.0	25.9
Sample	Phenolics	Phenolics[13]	Phenolics[diff]	Meas DOC	Mod DOC	Mod DOC[13]
13	6.92	5.54	-1.38	45	36.8	29.8
14	5.21	4.84	-0.37	29.4	28.2	26.3
15	5.46	4.96	-0.50	29	29.4	26.9
16	1.93	2.26	+0.33	14.1	11.6	13.3
17	5.92	5.23	-0.68	32.1	31.7	28.3
18	4.66	5.04	+0.37	30.8	25.4	27.3
19	4.87	4.79	-0.08	33.1	26.5	26.1
20	7.02	6.03	-0.98	42.2	37.3	32.3
21	5.88	5.15	-0.73	31.8	31.5	27.9
22	7.72	5.65	-2.07	35.1	40.8	30.4
23	6.23	5.38	-0.86	33.1	33.3	29.0
24	3.43	3.41	-0.02	24.6	19.2	19.1

763 **Table 4**

Sample	Phenolics	Phenolics^{8}	Phenolics^{diff}
1	5.39	4.53	-0.85
2	7.20	6.39	-0.81
3	8.00	7.22	-0.78
4	6.88	6.52	-0.36
5	6.94	6.61	-0.32
6	5.66	5.14	-0.52
7	9.23	6.71	-2.52
8	7.25	6.85	-0.40
9	7.03	5.41	-1.62
10	8.43	6.36	-2.07
11	8.94	8.55	-0.39
12	5.48	5.05	-0.43
Sample	Phenolics	Phenolics^{13}	Phenolics^{diff}
13	5.54	6.45	+0.90
14	7.40	7.11	-0.29
15	6.10	6.52	+0.42
16	9.61	10.10	+0.49
17	7.57	7.31	-0.26
18	6.72	7.93	+1.21
19	6.95	8.82	+1.87

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Figure 1

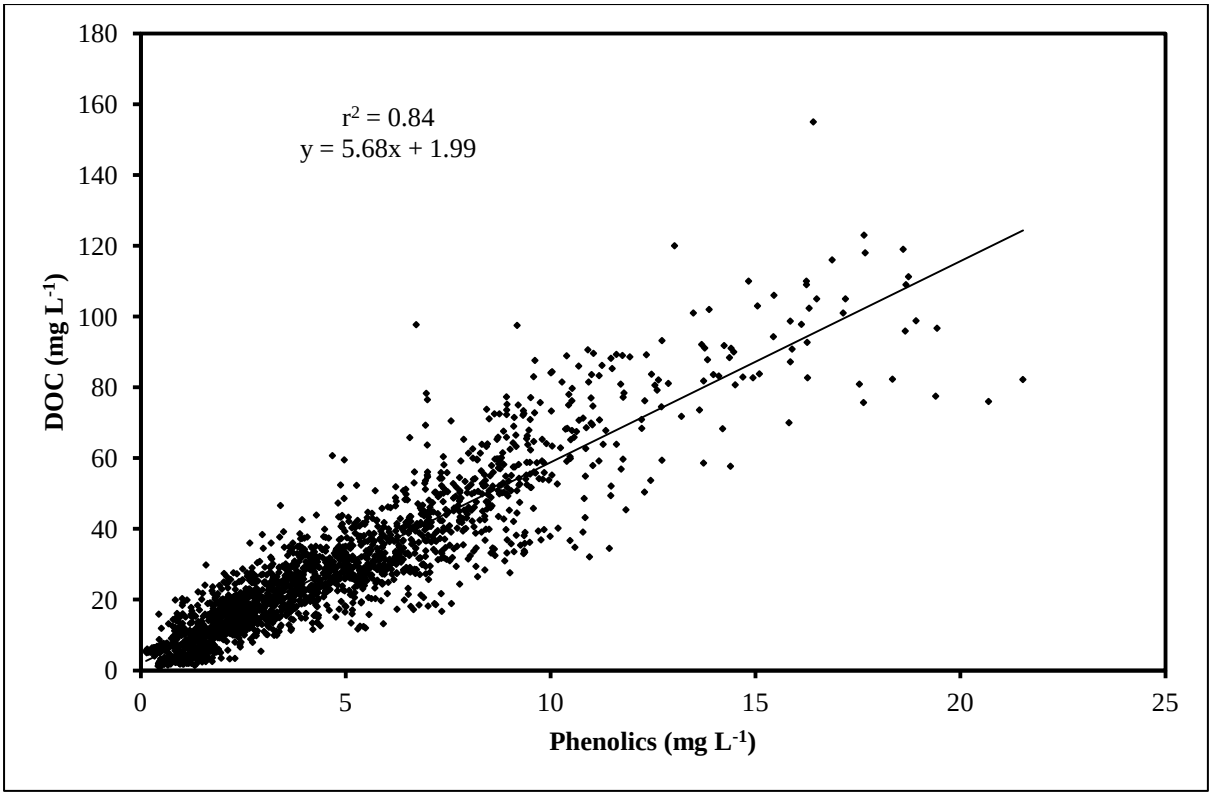

Figure 1. Observed relationship between phenolic concentrations (mg L^{-1}) and DOC concentrations (mg L^{-1}) for all 2020 water samples. $r^2 = 0.84$, residual variance = 72.051, $p < 0.001$.

Figure 2

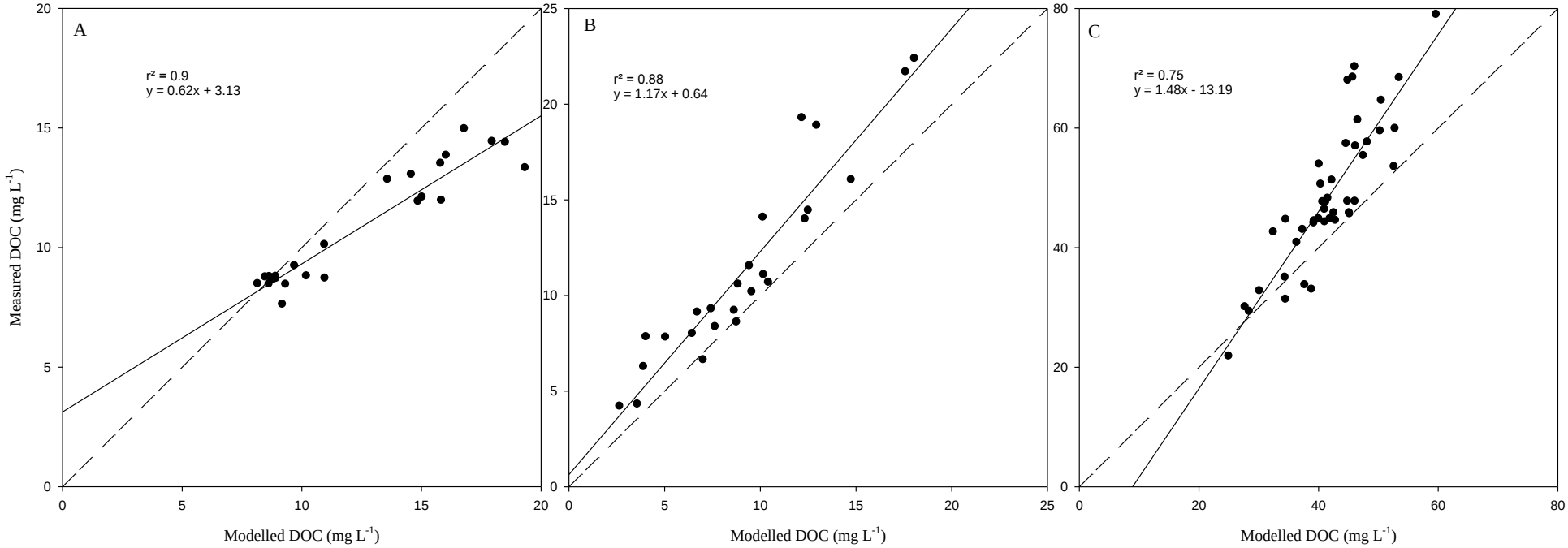

Figure 2. Regression between measured DOC and modelled DOC (mg L^{-1}) in Nant y Brwyn stream water, *n*=24, r^2=0.90 (A), Alwen Reservoir leachate samples, *n*=25, r^2=0.88 (B), and Llyn Serw pore water samples, *n*=44, r^2=0.75 (C). p<0.001 for each relationship. Dashed line shows 1:1 relationship.

Figure 3

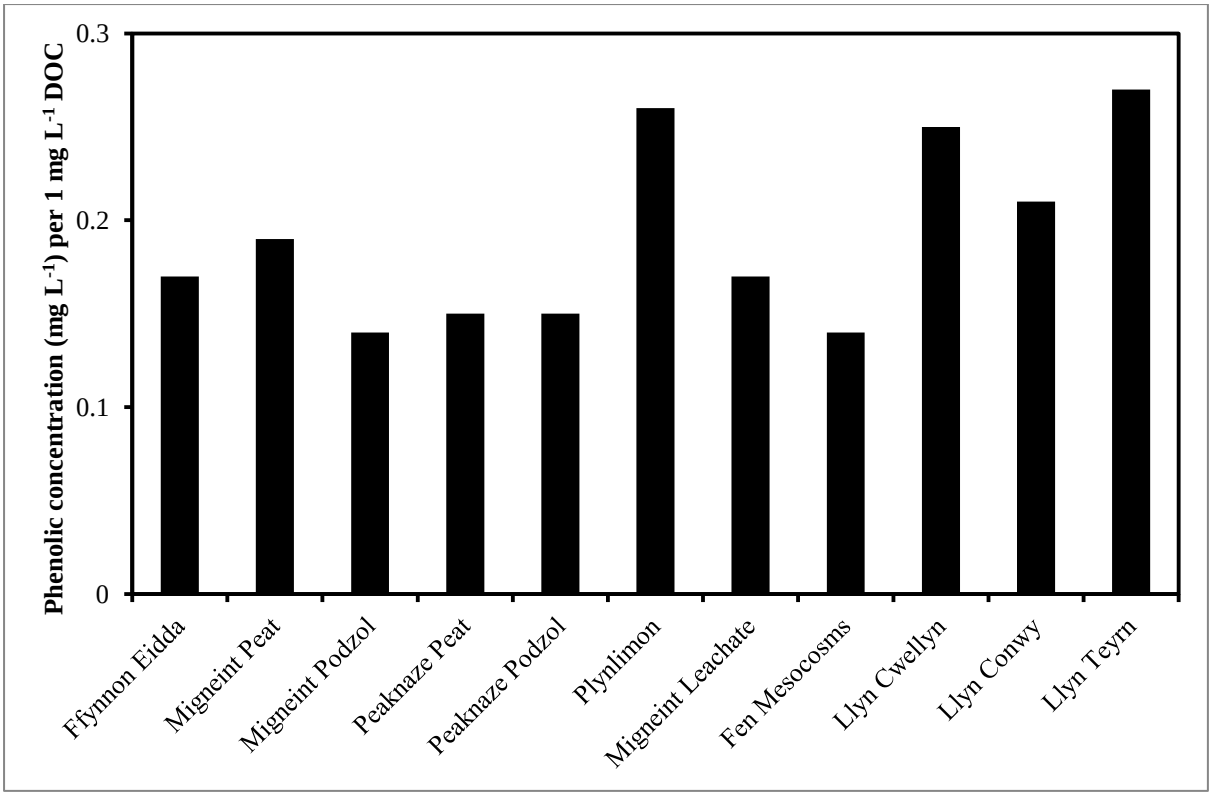

Figure 3. Median phenolic concentrations (mg L^{-1}) per 1 mg L^{-1} DOC concentrations for each site used in the model.

Figure 4

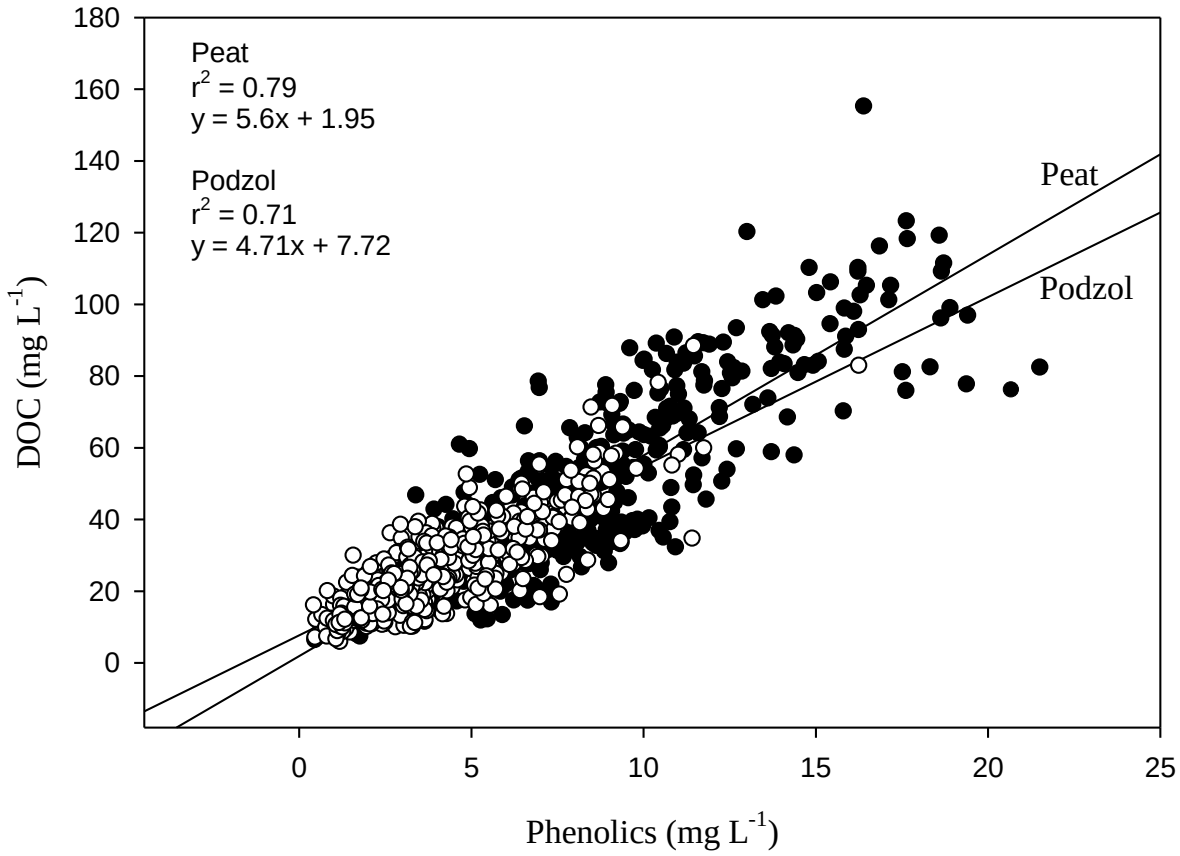

Figure 4. Regression between phenolic and DOC concentrations (mg L^{-1}) for the Migneint and Peaknaze podzol (white circles) and peat (black circles) sites. *n*=600 for each soil type. Podzol r^2=0.71. Peat r^2=0.79. For both soils p<0.001.

Figure 5

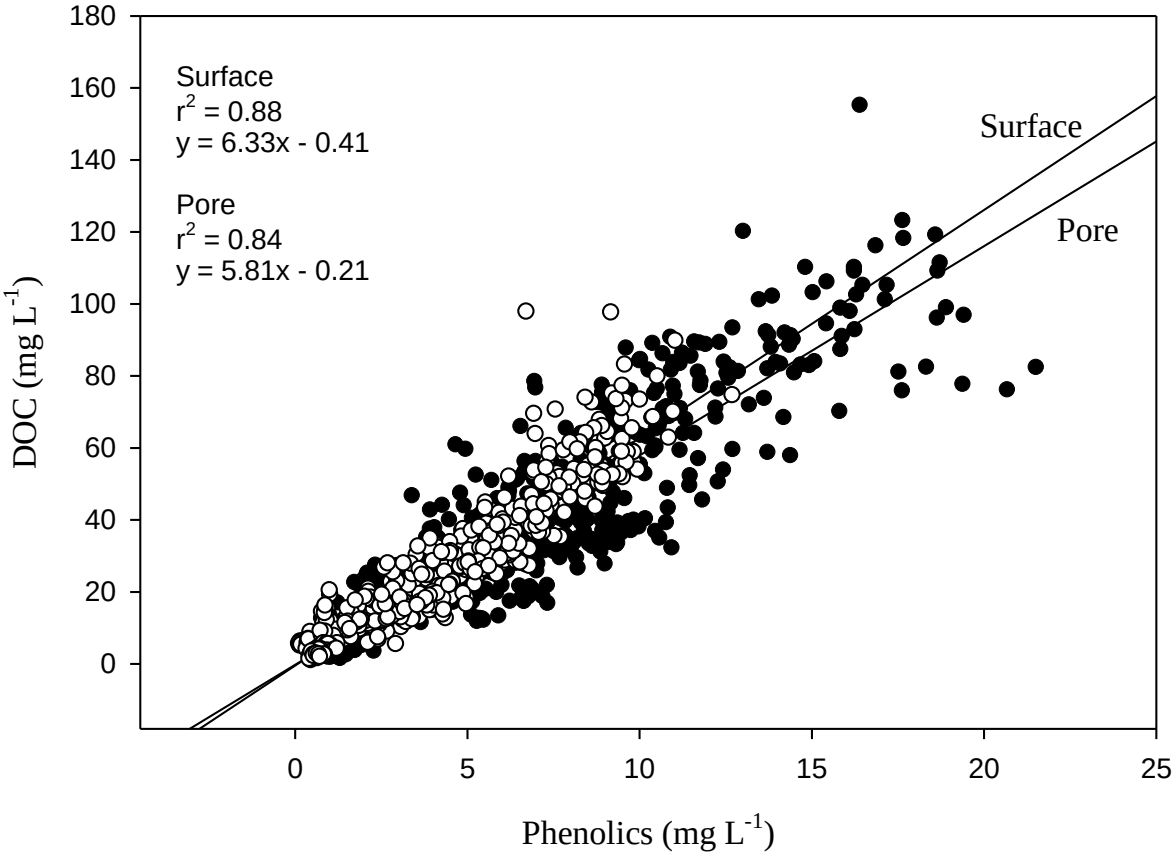

Figure 5. Regression between phenolic and DOC concentrations (mg L^{-1}) for surface waters (from Ffynnon Eidda, Llyn Cwellyn, Llyn Conwy, Llyn Teyrn, and fen mesocosms – *n*=608) and pore waters (from Migneint peat, Migneint podzol, Peaknaze peat, Peaknaze podzol, and Plynlimon – *n*=767). Surface waters r^2=0.88. Pore waters r^2=0.84. For both samples types p<0.001.

Figure 6

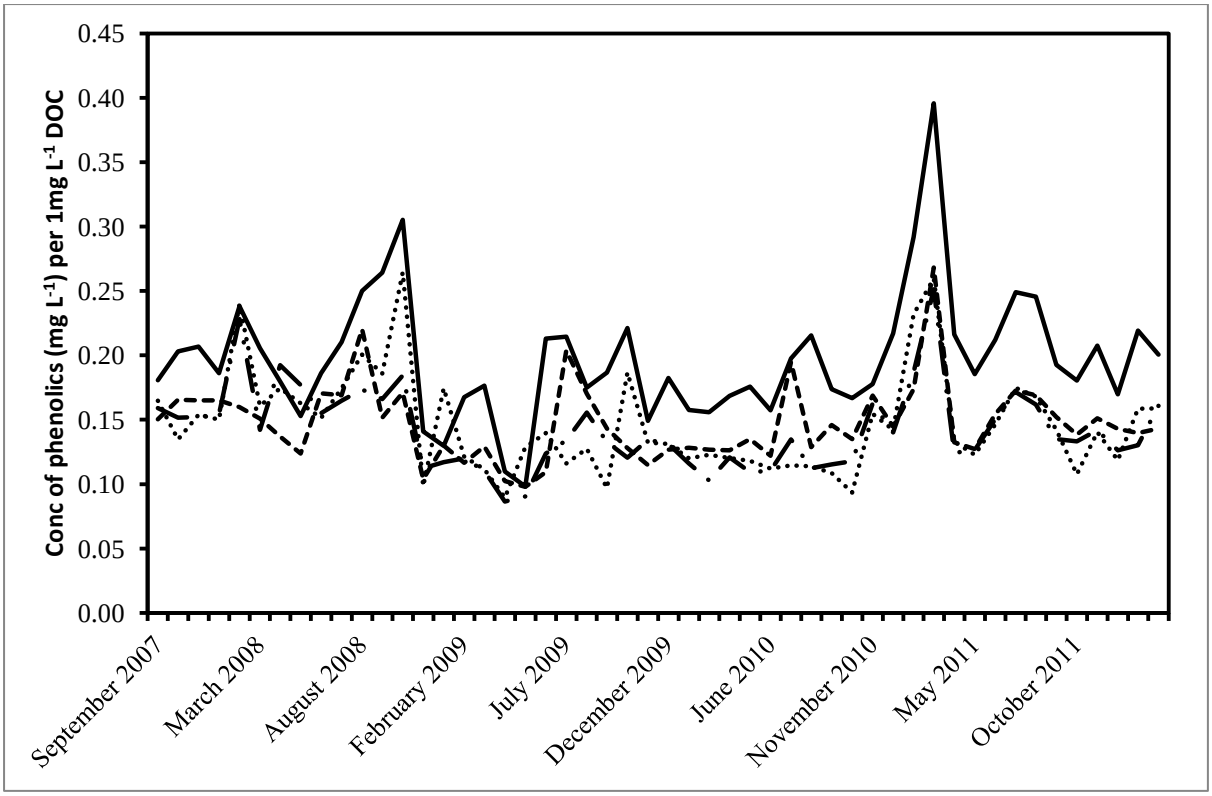

Figure 6. Changes in the mean proportion of phenolics to DOC for four sites from September 2007 to January 2012, with an approximate monthly sampling frequency. Sites are: Migneint peat – solid line, Migneint podzol – dotted line, Peaknaze peat – dashed line, Peaknaze podzol – dotted/dashed line. For each site and each date the mean is generated from *n*=12.

Figure 7

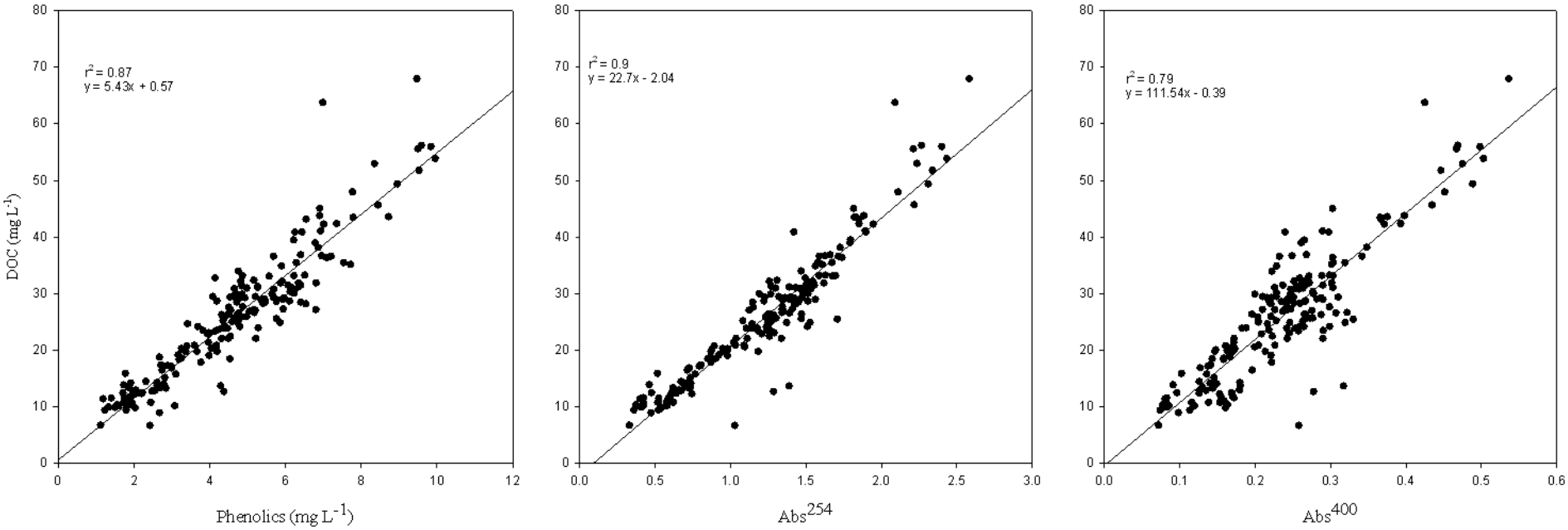

Figure 7. Regressions of DOC concentration against A) phenolic concentration, B) absorbance at 254 nm, C) absorbance at 400 nm, for 192 ditch water samples from Ffynnon Eidda. r^2 values A) 0.87, B) 0.9, C) 0.79. For all regressions $p<0.001$.