Variation in dissolved organic matter (DOM) stoichiometry in U.K. freshwaters: Assessing the influence of land cover and soil C:N ratio on DOM composition

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Abstract

Dissolved organic matter (DOM) plays an important role in freshwater biogeochemistry. To investigate the influence of catchment character on the quality and quantity of DOM in freshwaters, 45 sampling sites draining subcatchments of contrasting soil type, hydrology, and land cover within one large upland-dominated and one large lowland-dominated catchment were sampled over a 1-yr period. Dominant land cover in each subcatchment included: arable and horticultural, blanket peatland, coniferous woodland, and improved, unimproved, acid, and calcareous grasslands. The composition of the C, N, and P pool was determined as a function of the inorganic nutrient species (NO$_3^-$, NO$_2^-$, NH$_4^+$, and PO$_4^{3-}$) and dissolved organic nutrient (dissolved organic carbon [DOC], dissolved organic nitrogen [DON], and dissolved organic phosphorus [DOP]) concentrations. DOM quality was assessed by calculation of the molar DOC : DON and DOC : DOP ratios and specific ultraviolet absorbance (SUVA$_{254}$). In catchments with little anthropogenic nutrient inputs, DON and DOP typically composed > 80% of the total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) concentrations. By contrast, in heavily impacted agricultural catchments DON and DOP typically comprised 5–15% of TDN and 10–25% of TDP concentrations. Significant differences in DOC : DON and DOC : DOP ratios were observed between land cover class with significant correlations observed between both the DOC : DON and DOC : DOP molar ratios and SUVA$_{254}$ ($r_s$ = 0.88 and 0.84, respectively). Analysis also demonstrated a significant correlation between soil C : N ratio and instream DOC : DON/DOP ($r_s$ = 0.79 and 0.71, respectively). We infer from this that soil properties, specifically the C : N ratio of the soil organic matter pool, has a significant influence on the composition of DOM in streams draining through these landscapes.

Global flux estimates of dissolved organic carbon (DOC) suggest that river networks are responsible for the transport of 0.25 Pg C yr$^{-1}$, the largest transfer of reduced carbon from the terrestrial to marine environment (Cauwet 2002). In addition to acting as a significant store and transporter of reduced carbon, dissolved organic matter (DOM) also plays a pivotal role in the complexation of trace metals (Christensen et al. 1996), mobilization of pollutants (Aiken et al. 2011), and controls instream biotic exposure to ultraviolet radiation (Kelly et al. 2001), impacting heavily on autotrophic and heterotrophic production (Lindell et al. 1995). In addition, DOM is responsible for the delivery of significant quantities of dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP), which have been shown to dominate export from...
natural and seminatural systems (Johnes and Burt 1991; Campbell et al. 2000; Durand et al. 2011; Perakis and Hedin 2002).

In most natural or seminatural catchments, allochthonous sources dominate the aquatic DOM pool, derived mainly from the degradation of vascular plant material and soils and its incorporation into animal and microbial biomass, with soil biogeochemical processes acting to mediate the delivery of this material to aquatic systems (McDowell and Likens 1988; Mattsson et al. 2005). However, catchments impacted by agricultural intensification (Graeber et al. 2015; Yates et al. 2016), subject to urbanization (Aitkenhead-Peterson et al. 2009), or heavily impacted by sewage treatment works (STWs; Sickman et al. 2007; Yates et al. 2019), have demonstrated increases in both DOM concentration and its relative nutrient richness (measured as DON and DOP). Not only is DOM concentration therefore known to vary in relation to catchment character (Palviainen et al. 2016) but also a wide range of studies have observed compositional differences in DOM related to specific catchment sources (Mattsson et al. 2005; Spencer et al. 2007; Hernes et al. 2008; Yates et al. 2016). These differences reflect the influence of land use and management, soil type, and hydrological function in controlling the rates of microbial decomposition, nutrient cycling, uptake within the soil, and the net flux of DOM from different soil horizons to adjacent waters (Austnes et al. 2010). Autochthonous production through both autotrophic and heterotrophic pathways is also a significant source contributing N- and P-rich organic compounds to the aquatic DOM pool (Roberts and Mulholland 2007; Lutz et al. 2012; Evans et al. 2017).

Studies involving quantification of DOC, together with both DON and DOP as part of the total dissolved C (TDC), total dissolved N (TDN), and total dissolved P (TDP) pool in waters are limited. Nevertheless, the stoichiometry of organic matter has proved a useful tool in assessing compositional changes in the complex and dynamic pool of organic compounds that comprise DOM (Mattsson et al. 2009; Austnes et al. 2010; Inamdar et al. 2012). What is not clear in the existing literature is whether DOM composition in streams can be reliably predicted from a knowledge of the landscape stores of DOM in soils and biota in landscapes of different character. This presents a particular challenge where waters drain through heavily modified, urbanized, or intensively agricultural landscapes, receiving diffuse and point source discharges of animal manures and slurries, fertilizers, and human sewage effluent. Here, plant-derived and soil-derived DOM may no longer be the dominant sources of DOM in streams. A detailed understanding of the landscape drivers of DOM compositional differences is therefore required, along with a thorough understanding of the natural and anthropogenic sources of stream DOM, to generate strategies to deal with the impact of increasing nutrient loading on freshwater ecosystems. Here, the findings of a study undertaken to assess the relative importance of catchment character (land cover and its management, population density, and soil C : N ratio) as a control on stream DOM flux rates and composition are reported. We hypothesize that (1) stoichiometric ratios will differ significantly between catchments of differing dominant land cover classifications and (2) soil C : N (as an indicator of terrestrial ecosystem fertility) will act as a control on DOM stoichiometric ratios at the landscape scale.

Materials and methods

Catchment selection

Forty-five sampling sites were selected within the catchments of two large rivers (Table 1), the Conwy (N. Wales; 53°00′02.92″ N 3°49′10.1″ W) and Nadder (S. England; 51°07′34.4″ N 2°16′37.5″ W). The sites span gradients of nutrient enrichment status, population densities, and geoclimatic character, with seven distinct dominant land cover types. Land classification data were produced and supplied by the Centre for Ecology and Hydrology (LCM 2007). Dominant land classifications sampled during this study include: blanket peatland (BP), improved grassland (IG), coniferous woodland (CW), acid grassland (AG), low-productivity grassland (LPG), arable and horticultural (AH), and calcareous grassland (CG).

The River Conwy, North Wales (Fig. 1a,b), has a catchment area of 580 km² and drains northward to the Irish Sea. It has its source in one of the largest areas of BP in Wales, the Migneint. Major tributaries include the Merddwr, Machno, Lledr, and Llugwy, which drain a diverse range of landscapes including the montane ecosystems of the Snowdonia massif, extensive areas of conifer plantation forest, upland AG and heathland, and fragmentary broadleaf woodland. The eastern part of the catchment contains a higher proportion of IG, supporting moderately intensive sheep and cattle production. The lower Conwy valley occupies a glacial trough, supporting more intensive agricultural land and the small towns of Betws-y-Coed, Conwy, and Deganwy. The maximum elevation of the catchment is 1050.6 meters above ordnance datum (mAO), and mean annual average rainfall (AAR) above the tidal limit is 2042 mm (1961–1990; station: Conwy at Cwmlanerch). Underlying geology is bounded to the east by Silurian mudstone with harder Cambrian mixed igneous and sedimentary rocks to the west. The Conwy catchment has a population of 78,000, giving an average density of 135 people km⁻². However, the population is unevenly distributed, with most people living in the towns located along the lower river valley.

The Nadder catchment (Fig. 1c,d) covers an area of 673 km² and is located in southern England. It is a major tributary of the Hampshire Avon catchment which drains southward to the English Channel. The headwaters of the Nadder are underlain by clay, while a major tributary, the River Wyllye, is groundwater dominated and underlain by chalk. In marked contrast to the acidic soils and geology of the Conwy, the Nadder catchment drains land underlain by base-rich sedimentary rocks, supporting intensive arable production on the chalk to the middle and north of the catchment and intensive cattle production on heavy clay soils to the west of the catchment.
| Site code | Land classification | Dominant class (%) | Catchment | Soil C:N | Area (km²) | Population density (people km²) | No. samples | Latitude | Longitude |
|----------|---------------------|--------------------|-----------|----------|------------|-------------------------------|-------------|----------|-----------|
| 1        | Mixed –             | Conwy              | 24.57     | 51.5     | 203*       | 53.039                         | –3.709      |
| 2        | Mixed –             | Conwy              | 12.17     | 15.3     | 278        | 53.020                         | –3.835      |
| 3        | IG 50.6             | Conwy              | 19.58     | 364      | 53.002     | –3.797                         |
| 4        | Mixed –             | Conwy              | 20.47     | 364      | 53.090     | –3.799                         |
| 5        | Mixed –             | Conwy              | 35.6      | 1.36     | 53.064     | –3.781                         |
| 6        | BP 53.6             | Conwy              | 25.39     | 1.36     | 53.076     | –3.817                         |
| 7        | AG 78.8             | Conwy              | 18.21     | 3.33     | 53.061     | –3.706                         |
| 8        | IG 90.4             | Conwy              | 25.39     | 5.08     | 53.171     | –3.800                         |
| 9        | Mixed –             | Conwy              | 20.44     | 5.08     | 53.106     | –3.750                         |
| 10       | BP 100              | Conwy              | 35.6      | 1.36     | 53.086     | –3.817                         |
| 11       | BP 85.2             | Conwy              | 35.6      | 1.36     | 53.076     | –3.706                         |
| 12       | IG 78.8             | Conwy              | 18.21     | 3.33     | 53.061     | –3.706                         |
| 13       | IG 84.1             | Conwy              | 16.26     | 7.16     | 53.090     | –3.799                         |
| 14       | IG 90.4             | Conwy              | 25.39     | 5.08     | 53.171     | –3.800                         |
| 15       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 16       | Mixed –             | Conwy              | 15.23     | 3.33     | 53.061     | –3.706                         |
| 17       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 18       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 19       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 20       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 21       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 22       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 23       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 24       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 25       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 26       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 27       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 28       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 29       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 30       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 31       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 32       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 33       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 34       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 35       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 36       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 37       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 38       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 39       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 40       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 41       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 42       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 43       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 44       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |
| 45       | Mixed –             | Conwy              | 20.46     | 115      | 53.086     | –3.750                         |

BP, blanket peatland; IG, improved grassland; CW, coniferous woodland; AG, acid grassland; LPG, low-productivity grassland; AH, arable and horticultural; CG, calcareous grassland.

*Daily sampling.
†Weekly sampling.
‡Monthly sampling.
§Bi-weekly sampling.
The Nadder catchment also receives significant nutrient and organic matter input from treated wastewater discharges from large urban conurbations and riverside villages (Yates et al. 2019). Elevation across the Nadder catchment ranges between 51 and 283 m AOD and has a mean AAR of 875 mm (1961–1990; station: Nadder at Wilton). The Nadder catchment has a population density of 202 people km$^2$.

Sample collection and storage

Samples were collected at varying frequencies over a 1-yr period between October 2015 and December 2016 across a range of flow conditions (for sampling frequency and numbers, see Table 1). Samples were collected in acid washed (5% HCl) high density polyethylene bottles and stored in the dark at 4°C during transport to the University of Bristol for analysis. Samples were filtered through 0.45 μm prewashed cellulose nitrate filters (Whatman GF/C). An aliquot of unfiltered sample was decanted for total N (TN) and total P (TP) analysis. A second filtered aliquot was collected for determination of TDN and TDP, inorganic N and P species, and DOC concentrations and UV absorbance spectra, with analyses completed within 24 h of collection.

Analytical methodologies

Dissolved organic carbon

Concentrations of DOC were determined by coupled high-temperature catalytic oxidation using a Shimadzu TOC-L series analyzer (Shimadzu Corp.), measured as nonpurgeable organic carbon following sample acidification with HCl. The mean of three to five injections of 150 μL, where the coefficient of variance for the replicate injections was < 2%, is presented here.

Nitrogen species and phosphorus fractions

Inorganic nutrient analyses were conducted using a Skalar multichannel continuous flow autoanalyzer (Skalar Analytical B.V.) set up for simultaneous determination of total oxidized nitrogen (TON, comprising nitrate as NO$_3$-N, plus nitrite as NO$_2$-N) hereafter referred to as NO$_3$-N (as NO$_2$-N accounted for
<1% TON), total ammonium (NH$_3$-N + NH$_4$-N), and soluble reactive phosphorus (measured as PO$_4$-P) concentrations. TDN and TDP fractions were determined in the form of TON and PO$_4$-P following digestion of filtered samples with K$_2$S$_2$O$_8$, using the protocol modified by Johnes and Heathwaite (1992), whereas TN and TP concentrations were similarly determined following digestion of an unfiltered sample. DON and DOP concentrations were then determined by difference (DON = TDN − TON − NH$_4$-N; DOP = TDP − PO$_4$-P). Particulate organic N (PON) and particulate P (PP) fractions were calculated by difference (PON = TN − TDN; PP = TP − TDP). Quality control standards were made from an independent stock solution of mixed standards at low (0.2 mg L$^{-1}$) and high concentrations (0.8 mg L$^{-1}$) for all determinands and run randomly throughout analysis. Analytical and digest blanks were run, in addition to quality control standards, to monitor instrument performance.

**Optical measurements of DOM**

Absorbance spectra were scanned using a Varian Cary 60 UV/Vis spectrometer (Agilent Technologies) on each sample over the wavelength range 200–800 nm at 1 nm intervals, with samples brought to a constant temperature (20°C) prior to analysis. Specific ultraviolet absorbance (SUVA$_{254}$) was calculated by dividing the decadic absorbance at 254 nm by DOC concentration (mg L$^{-1}$) for each sample, with all absorption data presented in this manuscript expressed as absorption coefficients, as calculated in

$$a(\lambda) = \frac{2.303A(\lambda)}{l}$$  \hspace{1cm} (1)

where $a(\lambda)$ is the absorption coefficient in units of reciprocal length (m$^{-1}$), $A(\lambda)$ is raw absorbance, and $l$ is the cuvette path-length (m).

**Statistical analysis**

Prior to statistical analysis, all data were assessed for normality using the Shapiro–Wilk test, with homogeneity of variance evaluated by the Levene statistic. Spearman’s rank correlation coefficients were calculated to determine the strength of relationships between catchment descriptors and instream chemical determinands. To examine the differences in stoichiometric ratios between different land cover classifications, sites were grouped by dominant land cover class and in-stream chemical determinands. To enable multiple statistical comparisons across groups, the nonparametric Kruskal–Wallis test, with subsequent Mann–Whitney tests applied to assess statistical differences between land cover classifications. All statistical analyses were conducted using SPSS (IBM SPSS Statistics for Windows, version 25.0; IBM Corp.) with plots generated using SigmaPlot (version 13.0; Systat Software). Processing and analysis of absorbance spectra including calculation of SUVA$_{254}$ was conducted using R (R Foundation for Statistical Computing).

**Catchment delineation, land classification, and population density estimation**

Catchment reach structures and land cover were determined using ArcGIS Hydrology toolbox (ESRI 2018. Version 10 Redlands) based upon digital elevation models (10 × 10 km grid squares) and land cover mapping (LCM 2007) provided by the Centre for Ecology and Hydrology. Due to the rural location of the study catchments, official population census data could not be used to generate robust population density estimates. Population densities were, instead, calculated for delineated catchment reaches using Address Base Premium, the most accurate geographic database of U.K. addresses, properties, and land areas, provided by the U.K. Ordnance Survey. Total building numbers classified as residential and occupied were multiplied by the average number of people per household (data provided by the Office for National Statistics) to generate a robust population estimate. This was then divided by the catchment area to provide a population density estimate (population per km$^2$).

**Modeled soil C : N ratios**

Estimates of topsoil C : N ratios for each sampling locations catchment area were extracted from a modeled data set for the United Kingdom (see Henrys et al. 2012) using ArcGIS. Sampling and analysis methodologies are discussed in detail by Emmett et al. (2008). Briefly, 1024 soil cores were analyzed from 256 1 km × 1 km grid squares across Great Britain in 2007. Samples were air dried and sieved (< 2 mm) and then analyzed by CEH Lancaster using a total elemental analyzer (UKAS accredited method SOP3102). Soil C : N data were then modeled for the United Kingdom using both land classification data produced by CEH (LCM 2007) and soil parent material data provided by the British Geological Survey.

**Results**

**Variations in inorganic and organic nutrients**

NO$_3$-N and PO$_4$-P concentrations in this study ranged between < 0.001 and 11.07 mg N L$^{-1}$ (mean = 3.40 mg N L$^{-1}$) and < 0.001 and 479 μg P L$^{-1}$ (mean = 44.5 μg P L$^{-1}$), respectively. TN and TP concentrations ranged between 0.177 and 11.98 mg N L$^{-1}$ (mean = 103 μg P L$^{-1}$) and < 0.001 to 1557 μg P L$^{-1}$ (mean = 103 μg P L$^{-1}$), resulting in a wide range of trophic conditions from oligotrophic in the headwaters of the Conwy catchment to eutrophic in the lower reaches of the Nadder catchment (Fig. 2; see also Table 1). DOC concentrations demonstrate significant variation with concentrations ranging between 0.76 mg C L$^{-1}$ in the headwaters of the Wylde chalk catchments to 26.1 mg C L$^{-1}$ in the peatland headwaters of the Conwy catchment (mean = 4.4 mg C L$^{-1}$). When sites are ranked according to TDN (Fig. 3a) and TDP (Fig. 3b) concentration, a clear pattern emerges in the proportion of the TDN and TDP present in the water column in the form of DON.
and DOP, respectively. DON proportion decreases from >80% of TDN in seminatural systems to <10% in intensively farmed arable catchments underlain by chalk ($r^2 = 0.90; p < 0.001$). A similar trend is evident in the dissolved P fractionation data for these sites. On average, DOP concentrations account for >90% of TDP concentration in oligotrophic sites, decreasing to <15% TDP in hypertrophic streams draining from intensively farmed arable catchments ($r^2 = 0.76; p < 0.001$). The dominant nitrogen fraction in the highly enriched sites, based on this quantitative assessment, is NO$_3$-N, whereas PO$_4$-P can comprise up to 50% of the TP concentration. In arable farming systems, PP is the dominant fraction of TP concentrations in these nutrient enriched sites (Fig. 2c,d).

**Site discrimination based on catchment character and chemical variables**

Spearman’s rank analysis demonstrated significant correlations between land cover classification and chemical variables. For example, as seen in Table 2, % AH land correlates positively with TN concentration ($r_s = 0.942; p < 0.01$) and negatively with SUVA$_{254}$ ($r_s = -0.807; p < 0.01$). Significant negative correlations are also observed with the molar DOC : DON ($r_s = -0.732; p < 0.01$) and DOC : DOP ($r_s = -0.835; p < 0.01$) ratios, variables which also show a positive correlation with % BP (DOC : DON: $r_s = 0.808; p < 0.01$; DOC : DOP: $r_s = 0.777; p < 0.01$).

A Kruskal–Wallis test ($p < 0.05$) demonstrated there were significant differences observed in the molar DOC : DON stoichiometric ratios for each dominant land cover classification group (Fig. 4a). Catchments dominated by BP were found to have distinct, elevated DOC : DON (Mann–Whitney $U$-test; $p < 0.05$) ratios from catchments with a dominance of agricultural inputs (land cover classifications IG, AH, and CG). CW was found to demonstrate a DOC : DON ratio distinct from all other dominant land cover classifications except for those sites with a high percentage of AG and LPG. There was a significant difference in DOC : DOP ratios between land classifications...
ANOVA $F_{6588} = 133, p < 0.01)$. Post hoc testing revealed the statistical differences shown in Fig. 4b. In summary, as observed with DOC : DON ratios, agriculturally impacted land classifications demonstrated a significant difference ($p < 0.05$) from all other land classifications.

Of the 10 landscape descriptors evaluated here, only soil C : N ratio had a value that could be applied across all sampling locations. The range of modeled soil C : N ratios varied considerably between the land cover classes included in this study. For example, IG soil C : N ranged between 11 and 14.6, with catchments sampled from the U.K. uplands, classified as bog, ranging between 24.4 and 32.6. Similarly, both instream DOC : DON and DOC : DOP molar ratios demonstrated considerable variation across sites and between sampling occasions (Supporting Information Table S2). Soil C : N ratio showed a significant positive correlation with DOC : DON ($r_s = 0.768; p < 0.01$), DOC : DOP ($r_s = 0.707; p < 0.01$), and SUVA$_{254}$ ($r_s = 0.825; p < 0.01$). When sites are grouped by dominant land classification, a relationship between mean modeled soil C : N ratio and mean instream DOC : DON and instream DOC : DOP is suggested (Fig. 5).

Significant relationships were also observed between instream DOC : DON and SUVA$_{254}$ ($r^2 = 0.84; p < 0.001$; Fig. 6a) and DOC : DOP and SUVA$_{254}$ ($r^2 = 0.75; p < 0.001$; Fig. 6b). Mineral catchments with little soil organic matter demonstrated significantly lower SUVA$_{254}$ values when compared to organic-rich upland catchments.

**Discussion**

The data presented here support the hypothesis that DOM stoichiometric ratios differ significantly between land cover classifications and demonstrate that soil C : N ratios can be a useful metric in assessing DOM stoichiometric ratios at a landscape scale. The low DOC : DON and DOC : DOP ratios observed in catchments where agricultural and heavily fertilized land classifications dominate (IG and AH) is a pattern consistent within the wider literature (Aitkenhead-Peterson et al. 2009; Graeber et al. 2015; Heinz et al. 2015; Williams et al. 2016). Removal of crop residues and well-maintained field drainage systems in areas with intensive arable production have been found to reduce soil organic matter content while also reducing contact time between water and soil organic material, thus reducing organic matter dissolution rates (Mattsson et al. 2009; Palviainen et al. 2016). In addition, physical disturbances from agricultural practices and higher soil pH have been shown to increase soil DOM turnover rates (Leifeld et al. 2013). Sites with intensive livestock production on IG, however, contribute N- and P-rich DOM to the soil organic matter pool, which is then exported to adjacent waters. This is reflected in the lower DOC : DON and DOC : DOP molar ratios and SUVA$_{254}$ values reported here, alongside higher DON and DOP concentrations in the water body.

DOC : DON ratios in water exported from BP were found to be significantly greater than all other land cover classifications, while CW was found to be different from both BP and IG and arable/horticultural land. DOM sourced from the degradation of terrestrial vegetation, as is the case in Histosol soils, typically have a high DOC : DON ratio that comprises high-molecular-weight compounds, generating elevated SUVA$_{254}$ values (Weishaar et al. 2003). This is seen in the data collected from the upper reaches of the Conwy catchment, where BP and CW dominate the landscape. Regression analysis found a strong positive relationship between both the DOC : DON and DOC : DOP molar ratios and SUVA$_{254}$, reflecting the higher aromatic content and lower N and P content of DOM exported from soil organic matter in systems where the organic matter pool is dominated by leachate from BP. Similar patterns are observed in a wide range of studies into DOM composition draining BP across Europe (Mattsson et al. 2005; Broder et al. 2017). Similar mean DOC : DON
|                | DON (mg L\(^{-1}\)) | DOP (μg L\(^{-1}\)) | DOC (mg L\(^{-1}\)) | DOC : DON | DOC : DOP | TN (mg L\(^{-1}\)) | TP (μg L\(^{-1}\)) | SUVA\(_{254}\) (mg C L\(^{-1}\) m\(^{-1}\)) | Soil C : N | Catchment area (km\(^2\)) | Population density (people km\(^2\)) |
|----------------|---------------------|---------------------|---------------------|-----------|-----------|---------------------|---------------------|---------------------|-----------|-----------------------------|-------------------------------------|
| AG (%)         | -0.445*             | -0.621*             | 0.515*              | 0.575*    | -0.726*   | -0.675*             | 0.659*              | 0.610*              | -0.422*             | -0.694*                     | -0.646*                            |
| AH (%)         | 0.565*              | 0.767*              | -0.468*             | -0.732*   | -0.835*   | 0.942*              | 0.833*              | -0.807*             | -0.776*             | 0.517*                      | 0.761*                             |
| BP (%)         | -0.625*             | -0.737*             | 0.497*              | 0.808*    | 0.777*    | -0.796*             | -0.781*             | 0.844*              | 0.899*              | -0.739*                     |                                    |
| Urban (%)      | 0.584*              | -0.556*             | -0.620*             | -0.701*   | 0.641*    | 0.649*              | -0.658*             | -0.578*             | 0.627*              | 0.887*                      |                                    |
| CW (%)         | -0.322†             |                      |                     |           |           |                     |                     |                     |                     |                            |                                    |
| IG (%)         | 0.681*              | 0.812*              | -0.546*             | -0.598*   | 0.635*    | 0.734*              | -0.434*             | -0.649*             | 0.638*              |                             |                                    |
| LPG (%)        | 0.437*              | 0.778*              | -0.504*             | -0.820*   | 0.796*    | 0.783*              | -0.714*             | -0.664*             | 0.519*              |                             |                                    |
| Catchment area (km\(^2\)) |                  |                     |                     |           |           |                     |                     |                     |                     |                            |                                    |
| Soil C : N     | -0.608*             | -0.671*             | 0.454*              | 0.768*    | 0.707*    | -0.780*             | -0.734*             | 0.825*              | -0.664*             | -0.664*                     |                                    |
| DON (mg L\(^{-1}\)) |                    |                     |                     |           |           |                     |                     |                     |                     |                            |                                    |
| DOP (μg L\(^{-1}\)) |               |                     |                     |           |           |                     |                     |                     |                     |                            |                                    |
| DOC (mg L\(^{-1}\)) |                  |                     |                     |           |           |                     |                     |                     |                     |                            |                                    |
| DOC : DON      |                      |                     |                     |           |           |                     |                     |                     |                     |                            |                                    |
| DOC : DOP      |                      |                     |                     |           |           |                     |                     |                     |                     |                            |                                    |
| TN (mg L\(^{-1}\)) |                    |                     |                     |           |           |                     |                     |                     |                     |                            |                                    |
| TP (μg L\(^{-1}\)) |                    |                     |                     |           |           |                     |                     |                     |                     |                            |                                    |
| SUVA\(_{254}\) (mg C L\(^{-1}\) m\(^{-1}\)) |              |                     |                     |           |           |                     |                     |                     |                     |                            |                                    |

AG, acid grassland; AH, arable and horticultural; BP, blanket peatland; CW, coniferous woodland; IG, improved grassland; LPG, low-productivity grassland. Blank space denote no statistical significance.

*Correlation is significant at the 0.01 level.
†Correlation is significant at 0.05 level.
ratios were observed previously from the same catchment (Austnes et al. 2010).

Compositional differences in DOM have been shown to affect the bioavailability of DOM to stream biota in both laboratory and field-based studies (Wiegner et al. 2006; Petrone et al. 2009; Asmala et al. 2013; Hosen et al. 2014; Berggren and del Giorgio 2015). For example, the quality of DOM, as determined by the DOC : DON ratio, has been shown to be a major determinant of bacterial growth efficiency, demonstrating an inverse relationship, with lower DOC : DON ratios resulting in greater conversion of substrate material to bacterial biomass (Kroer 1993). Studies such as these demonstrate how the quantification of instream stoichiometric ratios is not only a useful tool in the assessment of DOM compositional differences but may also act as an indicator of the relative bioavailability of organic material between catchments with differing landscape character, as well as the capacity of river systems to assimilate inorganic N and P from anthropogenic sources.

Intensification of agricultural practices have been observed to shift natural DOM composition while also increasing DON bioavailability (Petrone et al. 2009; Quaranta et al. 2012; Sun et al. 2017). Asmala et al. (2013) studied into DOM exported from three Baltic sea estuaries and found DOM exported from catchments dominated by agricultural land to have a higher pool of bioavailable DON relative to DOC than contrasting sites draining forested and peatland sites. DOC : DON ratios were also lowest and more variable in estuaries draining agricultural catchments, similar to data reported for freshwater sites in this study.

In addition to inputs of DOM from agricultural land, lower DOC : DON ratios observed in our study could be explained by the presence of effluent discharge from septic tank systems.
often associated with rural riverside properties, as well as larger STWs where these were present within these two study catchments (Yates et al. 2019). Due to regulation surrounding septic tank system design in the United Kingdom, such systems are often indistinguishable from diffuse nutrient sources. Source-specific studies into the bioavailability of treated effluent discharged from these systems are becoming more common in the literature. Following a 14-d study into the bioavailability of DON derived from treated wastewater effluent, Urgun-Demirtas et al. (2008) reported DON derived from STWs to be, either directly or indirectly, available to algae and/or bacteria as seen from a decrease in DON concentration, an increase in chlorophyll $a$ and biomass concentration, and a decrease in DOC : DON ratios. Although studies on the bioavailability of DOP discharged from STWs are rare, due to the difficulty associated with its quantification, it has also been found to be highly labile material (Qin et al. 2015).

Relationships between organic and inorganic nutrient concentrations

The environmental relevance of DOM is a function not only of its composition but also with the relative form and abundance of inorganic nutrient fractions available for biotic uptake. In this study, DON and DOP concentrations were found to increase as TN and TP concentrations increased across the nutrient enrichment gradient but found to decrease as a proportion of TN and TP concentrations decreased. Similar results were found by Perakis and Hedin (2002) who observed DON to dominate N budgets across temperate forests in south American streams, whereas Durand et al. (2011) found similar trends spanning 87 European rivers across a gradient of nutrient enrichment from ultra-oligotrophic to hypertrophic status. In both cases, although DON decreased in its importance relative to total catchment N losses, absolute concentrations of DON increased with high nutrient enrichment. In heavily modified catchments supporting high human population densities and intensive arable production, TN and TP concentrations instream are dominated by NO$_3$-N and PP, and although DON and DOP are quantitatively significant components of the TN and TP load available to the stream biota, they typically comprise $\leq$ 20% of TN and TP concentration. Lithology, SOM stores, and the generation of DOM-rich animal effluents all influence the flux of DOM from land to stream and the ultimate composition, character, and ecosystem functional role of DOM in waters draining through these landscapes. Here, DON and DOP concentrations correlate positively with % agricultural and horticulture in the catchment suggesting anthropogenic export of both DON and DOP to these streams along with inorganic nutrient fractions, leading to an increase in stream DOM concentrations and a shift in DOM composition.

Using soil C : N as an indicator of instream DOM stoichiometry

Modeled soil C : N ratios from earlier work by Henrys et al. (2012) together with land cover classification (LCM2007) and population density statistics have been used in this study to evaluate their relative importance as predictors of DOM stoichiometry in streams. Land cover data have been used extensively in the past to explain variations in DOM composition (Kothawala et al. 2015; Lambert et al. 2017; Singh et al. 2017). Outcomes from this study indicate that the SOM pool is important across a range of environments in controlling riverine DOM composition. However, although this is apparent when examining soil C : N and stoichiometric ratios at catchment scale, it may not hold true for intracatchment variations in DOM chemistry, as nutrient inputs from STWs and septic tank systems have been shown to act as locally important source areas contributing to the stream nutrient pool (Withers et al. 2011; Withers et al. 2014). It is also possible that some of the observed relationship is not directly causative, for example, fertile lowland catchments are likely to have both low soil C : N ratios and high population and/or

![Fig. 6. Regression analysis between (a) mean DOC : DON and SUVA$_{254}$ and (b) mean DOC : DOP and SUVA$_{254}$ for all sites. Error bars show mean ± SEM.](image-url)
livelock densities, each of which produces DOM exports with a low DOC : DON ratio to streams.

Although land cover can be used to differentiate DOM stoichiometry, soil C : N ratio is a better predictor of DOC : DON and DOC : DOP ratios and SUVA254 characteristics. This supports conclusions drawn by Aitkenhead and McDowell (2000), who described soil C : N as an effective descriptor as it incorporates the composite influence of the key variables contributing to the soil DOM pool in a single metric. In heavily modified catchments, allochthonous inputs of N- and P-rich DOM from both point and diffuse sources, along with DOM from the soil matrix and overlying vegetation, and autochthonous DOM produced within the aquatic system due to high N and P availability, collectively influence DOM concentration and composition instream. Understanding differences in DOM stoichiometry is important as this controls the relative bioavailability of DOM to stream biota and its likely ecosystem functional role.

**Conclusion**

This study demonstrates significant variation in DOM composition in catchments relative to environmental character. The data presented support the hypothesis that DOM composition is strongly influenced by the size and quality of SOM. The relationships between soil C : N ratios and instream DOM compositional metrics suggest that modeled soil C : N ratios may be used, with caution, to estimate the likely stoichiometric composition of instream DOM. This relationship is strongest in natural and seminatural catchments with little human disturbance, becoming weaker in systems draining highly modified landscapes, supporting intensive agricultural production and high human population density. In such systems, our evidence points to a lower quantitative significance of DON and DOP in the TN and TP pool but also indicates a lower MW composition and potentially higher bioavailability of DOM to support autochthonous production instream. As systems become nutrient enriched, although soil C : N ratio is still a good predictor of DOM composition instream, it is increasingly reflective of new, N- and P-rich DOM exported to streams from diffuse agricultural and septic tank sources and point source effluent discharges to the water course from STW systems in the catchment.

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Conflict of Interest

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