

Land cover and nutrient enrichment regulates low-molecular weight dissolved organic matter turnover in freshwater ecosystems

Brailsford, Francesca L.; Glanville, Helen C.; Marshall, Miles R.; Yates, Christopher A.; Owen, Alun T.; Golyshin, Peter N.; Johnes, Penny J.; Jones, Davey L.

Limnology and Oceanography

DOI:

[10.1002/lno.11852](https://doi.org/10.1002/lno.11852)

Published: 01/08/2021

Publisher's PDF, also known as Version of record

[Cyswllt i'r cyhoeddiad / Link to publication](#)

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):

Brailsford, F. L., Glanville, H. C., Marshall, M. R., Yates, C. A., Owen, A. T., Golyshin, P. N., Johnes, P. J., & Jones, D. L. (2021). Land cover and nutrient enrichment regulates low-molecular weight dissolved organic matter turnover in freshwater ecosystems. *Limnology and Oceanography*, 66(8), 2979-2987. <https://doi.org/10.1002/lno.11852>

Hawliau Cyffredinol / General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Land cover and nutrient enrichment regulates low-molecular weight dissolved organic matter turnover in freshwater ecosystems

Francesca L. Brailsford^{1,2,3*,†} Helen C. Glanville^{1,4*,†} Miles R. Marshall,¹ Christopher A. Yates^{1,5},
Alun T. Owen,^{5,6} Peter N. Golyshin,¹ Penny J. Johnes,⁵ Davey L. Jones^{1,2,3}

¹School of Natural Sciences, Bangor University, Bangor, UK

²SoilsWest, Murdoch University, Murdoch, Western Australia, Australia

³SoilsWest, The University of Western Australia, UWA School of Agriculture and Environment, Perth, Western Australia, Australia

⁴School of Geography, Geology and the Environment, Keele University, Keele, UK

⁵School of Geographical Sciences, University of Bristol, Bristol, UK

⁶Faculty of Health and Applied Sciences, University of the West of England, Bristol, UK

Abstract

Dissolved organic matter (DOM) is a complex mixture of carbon-containing compounds. The low-molecular weight (LMW) fraction constitutes thousands of different compounds and represents a substantial proportion of DOM in aquatic ecosystems. The turnover rates of this LMW DOM can be extremely high. Due to the challenges of measuring this pool at a molecular scale, comparatively little is known of the fate of LMW DOM compounds in lotic systems. This study addresses this knowledge gap, investigating the microbial processing of LMW DOM across 45 sites representing a range of physicochemical gradients and dominant land covers in the United Kingdom. Radioisotope tracers representing LMW dissolved organic carbon (DOC) (glucose), dissolved organic nitrogen (DON) (amino acid mixture), dissolved organic phosphorus (DOP) (glucose-6-phosphate), and soluble reactive phosphorus (SRP, measured as orthophosphate) were used to measure the microbial uptake of different DOM compounds in river waters. The amount of DOM biodegradation varied between different components (DON ≥ DOC > DOP), with the rate of turnover of all three increasing along a gradient of N and P enrichment across the range of sites. Conversely, the uptake of SRP decreased along this same gradient. This was ascribed to preferential utilization of DOP over SRP. Dominant land cover had a significant effect on DOM use as a resource, due to its control of nutrient enrichment within the catchments. We conclude that nutrient enrichment of river waters will lead to further DOM removal from the water column, increased microbial growth, and a decrease in stream oxygen saturation, exacerbating the effects of eutrophication in rivers.

Dissolved organic matter (DOM) is a complex mixture of chemicals, traditionally defined as organic carbon (C)-containing compounds that can pass through a 0.45 μm filter (Thurman 1985; Akkanen et al. 2012). DOM constitutes a key form in which terrestrially derived C is transported from headwaters, through the catchment hydrological network and into the marine environment. It has been estimated that ~ 33% of terrestrial C is exported to the ocean in this way (Stutter et al.

2018). In addition to C, DOM also contains nitrogen (N) and phosphorus (P), which are together considered to be the three major nutrients required by freshwater organisms (Tipping et al. 2016). DOM therefore represents a key source of nutrients for microorganisms and plants along the aquatic gradient (Kirchman 2003; Cuss and Guéguen 2015). Further, recent research has shown that DOM quality and quantity can change from source to sea suggesting that components of the DOM pool are abiotically and biotically transformed during transit (Battin et al. 2008; Massicotte and Frenette 2011; Ejarque et al. 2017).

DOM is a highly complex mixture composed of tens of thousands of individual compounds differing in size, charge, and solubility. Broadly, DOM can be divided into two size categories, namely those compounds that are of high molecular weight (HMW; > 1000 Da) and those that are of low molecular weight (LMW; < 1000 Da) (Cui and Choo 2013). High-molecular weight DOM, typically lignin and lignin-

*Correspondence: fran.brailsford@uwa.edu.au (F.L.B.); h.c.glanville@keele.ac.uk (H.C.G.)

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Additional Supporting Information may be found in the online version of this article.

†These two authors contributed equally.

derived breakdown products from woody debris, is considered to be relatively recalcitrant and not readily degraded by the microbial biomass (Zhang et al. 2017). Therefore, it is often present at high concentrations, particularly in peat-rich headwater catchments. It can, however, have an abiotic function, reducing light attenuation in the water column due to its chromophoric properties, shielding the microbial biomass, and extracellular compounds from UV degradation (Fellman et al. 2010) and is readily broken down through photodegradation to release lower MW compounds to the river ecosystem as the material moves downstream (Cory and Kling 2018; Bowen et al. 2019). In general, LMW DOM (e.g., sugars, organic acids) is present in lower amounts (ca. 20% of the total DOM pool) in rivers draining from natural and seminatural landscapes, rising in waters draining through urbanized and intensively farmed agricultural landscapes (Yates et al. 2019b) but is often more labile and rapidly metabolized by the microbial biomass (Dawson et al. 2001). This makes the measurement of this fraction both important, as the LMW DOM pool is an important component of in-stream C, N, and P processing and biotic uptake, as well as technically challenging (Lutz et al. 2011; Salcher et al. 2013; Parr et al. 2014). In natural and seminatural systems, individual LMW DOM compounds (e.g., amino acids) are present at very low concentrations in freshwaters (1–10 nmol L⁻¹; Marie et al. 2015; Horňák et al. 2016). These concentrations are close to the influx-efflux equilibrium point for microbial transport systems (i.e., point of zero net flux). It is unclear, however, if this is a reflection of low rates of LMW DOM input or whether it is due to high rates of consumption, especially for a wide range of compound classes and freshwater types.

In contrast, as systems become nutrient enriched through anthropogenic activity LMW DOM compound concentrations increase, this will exceed the equilibrium point and stimulate higher rates of microbial consumption (Sirivedhin and Gray 2005). The aim of this study was therefore to: (1) compare the rate of microbial uptake of LMW forms of DOC (sugars), DON (amino acids), and DOP (sugar phosphates) at different times of the year in 45 individual rivers draining through landscapes with a range of physicochemical properties and land covers; (2) determine which physicochemical parameters best correlated with DOC, DON, and DOP uptake, and (3) compare the rates of DOP and inorganic P (SRP) use by the microbial community. The results of the study were then used to evaluate how DOM and SRP are processed across catchment-scale gradients of disturbance and nutrient enrichment.

Materials and methods

Field site and sampling

Samples were collected from 45 rivers across five contrasting land covers (arable, grasslands, conifer forests, peatland, and mixed agricultural) in the Conwy catchment, North

Wales, and the Nadder Catchment, a subcatchment of the well-researched Hampshire Avon catchment in Southern England (Jarvie et al. 2005; Pirani et al. 2016; Fig. 1). This was undertaken alongside regular monitoring of all sites between February 2015 and December 2016, detailed methods are given in Yates et al. (2019b). The sites selected encompass a wide range of chemical and physical gradients and contain a range of dominant land covers spanning from natural and seminatural ecosystems to moderately and intensively farmed improved agricultural grassland with settlements discharging septic tank and sewage effluent to the rivers (Emmett et al. 2016; Lloyd et al. 2019; Yates et al. 2019a).

The Conwy catchment (Fig. 1a) covers an area approximately 580 km², draining a wide range of land-cover types, and is underlain by Silurian siltstones, mudstones and slate to the east and older, more resistant Ordovician rocks to the west. The lithology has a low permeability and soils are acidic. Land cover in this part of the catchment comprises the Migneint, one of the largest areas of upland blanket peat bog in Wales in its headwaters, together with acid grasslands, coniferous plantations, and broadleaf forests. The lower half of the catchment is underlain by Ordovician mudstones, siltstones, and sandstone with a lower relief, with acidic to neutral soils supporting lowland improved grassland used for moderately intensive agricultural production primarily sheep farming, before entering the Irish Sea (Cooper et al. 2014; Emmett et al. 2016; Brailsford et al. 2019a). The Nadder catchment covers a larger area (673 km²; Fig. 1b). In its headwaters, the Nadder is underlain by Gault Clay and Upper Greensand, supporting intensive cattle production on its neutral to slightly alkaline clay soils, while the Wylze, its major tributary, is underlain by Cretaceous Chalk, is heavily influenced by groundwater recharge, and supports intensive arable production on calcareous brown earths (Yates and Johnes 2013; Yates et al. 2019b). This range of landscape characteristics and resulting land cover and use provided a gradient of sites ranging from C-rich, highly acidic soils to mineral calcareous soils, from high natural organic matter (NOM) to low NOM systems, and from low N and P to high N and P flux in either dissolved organic, particulate, or soluble inorganic form. Land cover similarly varied from woodland to heathland, and acid grassland to intensively fertilized and manured improved grassland, and mineral soils supporting arable production with no crop cover for much of the year. Each would generate a different DOM profile from its contributing sources to the rivers draining each landscape type (Yates et al. 2019b). Sites for this suite of experiments were selected to reflect these gradients. Details on site characteristics are given in the Supporting Information Table S1.

At each site, 1 L midstream samples were manually collected in acid-washed, high density polyethylene bottles. The pH, electrical conductivity (EC), and water temperature were measured at the time of collection. For the laboratory studies, the samples were kept cool (ca. 4°C) in the dark from the

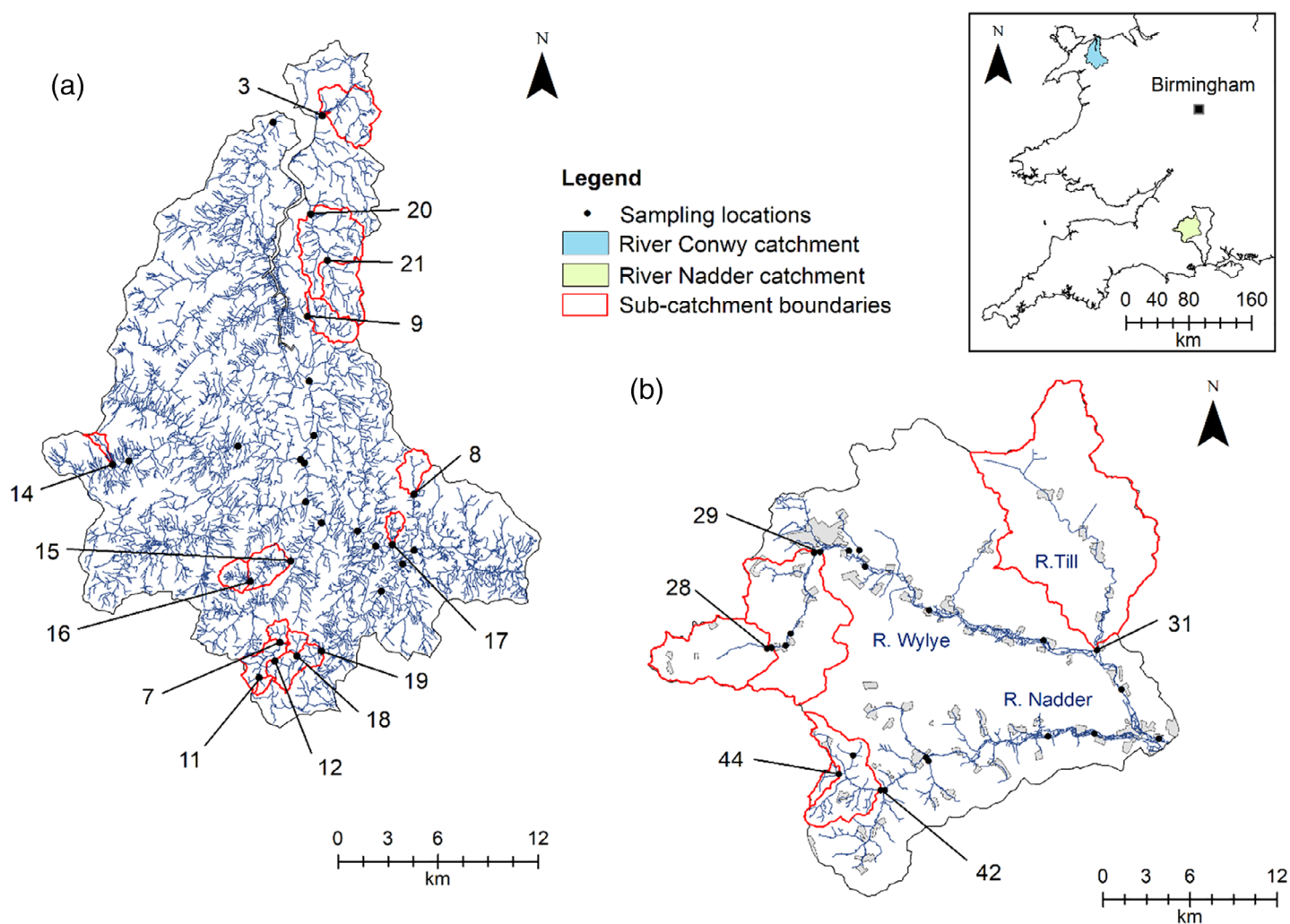


Fig. 1. Sampling locations across (a) Conwy and (b) Nadder catchments. Inset shows catchment locations in relation to the UK. Red boundaries represent catchments with > 50% dominance of a single land cover. Catchment reach structures were determined using ArcGIS Hydrology toolbox (Version 10, Redlands, California, U.S.A.) based upon digital elevation models provided by EDINA Digimap.

point of collection and during transportation to the laboratory where they were placed in cold storage. Chemical analyses were conducted, and experiments commenced within 24 h of sample collection (*see* Yates et al. 2019b for detailed methods).

Nutrient depletion experiment

To evaluate DOC, DON, DOP, and SRP depletion across the samples, individual samples were spiked with either: (1) ^{14}C -labeled glucose; (2) a mixture of ^{14}C -labeled free amino acids; (3) ^{14}C -labeled glucose-6-phosphate; or (4) ^{33}P -labeled PO_4^{3-} , respectively. For each treatment, three independent replicate 25 mL samples from each of the 45 sampling sites were added to sterile 50 mL polypropylene centrifuge tubes (Corning, New York, U.S.A.) and spiked with individual radioisotope (0.2 mL, 0.2 kBq mL $^{-1}$ final activity). The radioisotopes were added at low concentrations (< 1 nmol L $^{-1}$) which would be unlikely to increase the background pool of the

target compound or change its pH (Brailsford et al. 2017). After sealing with sterile caps, samples were incubated on an orbital shaker (200 rev min $^{-1}$) in the dark at 10°C for the duration of the experiment.

After incubation for 2, 5, 24, 48, 72, 144, or 168 h, 1 mL subsamples were removed, centrifuged to remove microbial cells (20,817 \times g, 5 min), and 0.5 mL supernatant added to a plastic 7 mL scintillation vial (Meridian Biotechnologies, Tadworth., UK). The subsamples were then acidified with 0.1 mol L $^{-1}$ HCl (50 μL), vortexed, left to stand for 3 h and then vortexed again to remove any dissolved $^{14}\text{CO}_2$ present. The subsample was then mixed with Optiphase HiSafe 3 scintillation cocktail (4 mL; PerkinElmer, Waltham, Massachusetts, U.S.A.) and the ^{14}C or ^{33}P quantified on a Wallac 1404 liquid scintillation counter with automated quench correction (Wallac EG&G, Milton Keynes, UK).

Statistical analysis

The physicochemical parameters used in the analysis included DOC (mg C L^{-1}), total nitrogen (TN) (mg N L^{-1}), DON (mg N L^{-1}), $\text{NO}_3\text{-N}$ (mg N L^{-1}), $\text{NO}_2\text{-N}$ (mg N L^{-1}), $\text{NH}_4\text{-N}$ (mg N L^{-1}), total phosphorus (TP) (mg P L^{-1}), DOP (mg P L^{-1}), SRP (mg P L^{-1} , measured as $\text{PO}_4\text{-P}$), pH, and EC ($\mu\text{S cm}^{-1}$). All physicochemical parameters were tested for normality using the Shapiro–Wilk test. Nitrate and DOC were \log_{10} transformed to pass the normality test. The percentage depletion at 24 h was used for comparison as this captured the linear phase of the degradation curve for the majority of sites and radioisotope tracers used. A one-way ANOVA with Tukey pairwise comparisons was undertaken for the 24 h depletion data using Minitab v18.0 with $p < 0.05$ as the cutoff for statistical significance (Minitab, State College, Pennsylvania, U.S.A.). Canonical correspondence analysis (CCA) of physicochemical parameters for the 45 sites and mean radioisotope tracer depletion data was conducted using the *envfit* function in the *Vegan* package (R version 4.0.3; Oksanen et al. 2018; R Core Team 2019). Environmental data were scaled to unit variance when applying the CCA. To note, the results from the experiments repeated over the four seasons were averaged together as season was not found to be a major driver of the differences between sites during initial analyses and many sites did not exhibit distinct variation between seasons (Supplementary Document 1). The significance of the ordination was tested using a permutation test. The coordinates resulting from the CCA analysis were plotted using the *ggplot2* package (Wickham 2016). Arrow/vector length and position provide information about the relationship between the environmental variables and the axes, with arrows parallel to axis orientation indicating a correlation and the length of the arrow inferring strength of that correlation. Permutational multivariate analysis of variance (PERMANOVA) was used to test the effect of land cover type on the dispersal of sites within the CCA. Prior to this, homogeneity of multivariate dispersions throughout the sample was confirmed using a beta dispersion test.

Results

Physicochemical properties

Significant variation was apparent between the chemistry of the river waters across the 45 sites (Supporting Information - Table S1; Yates et al. 2019b). Comparison of the rate of substrate depletion over 24 h across all samples also showed differences in substrate depletion rate following the series: glucose \geq amino acids \geq glucose-6-phosphate $>$ SRP ($p < 0.001$; Table 1; Supporting Information Table S2). In addition, there were major differences in substrate depletion between land cover for all three organic substrates ($p < 0.001$); however, no difference in SRP depletion was observed across the land covers ($p = 0.613$). Overall, rates of substrate depletion were greatest in catchments draining agricultural land (arable and

grassland in both catchments) in comparison to sites draining peat moorland and coniferous forest in the Upper Conwy catchment. Notably, SRP depletion was only greater than DOP depletion rates in sites draining from the peat moorland and coniferous forest landscapes where P concentrations are naturally extremely low (Supplementary Document 1).

To identify the major factors associated with the depletion of isotope from solution, mean rates of initial DOC, DON, DOP, and SRP depletion were analyzed alongside physicochemical parameters of the water (Table 1; Fig. 2; Supporting Information Tables S1–S3). Two canonical axes (CCA1 and CCA2) explained 82.5% of the overall variation between the 45 sites (Table 2; Fig. 2). The overall model was statistically significant, with the CCA1 axis being a significant driver of differences between sites (permutation test, $p = 0.001$ for both).

Dominant land cover had a significant effect on sample groupings in CCA analysis; when plotted with coordinates resulting from the CCA ordination analysis, rivers draining peatland (HMW DOC rich, N/P depleted) formed a distinct cluster in the bottom right quadrant (Fig. 2; Table 3). There was also clustering of some separation between catchments, with sites from the along the Wyllye-Nadder catchment, clustering on the left hand side of the plot. In contrast, the majority of sites located within the River Conwy catchment clustered in the top right quadrant (Supporting Information - Table S3). However, improved grassland-influenced rivers (low DOC, N and P enriched) sites from the Conwy catchment rivers were more closely associated with Wyllye-Nadder catchment rivers with a similar level of N and P enrichment, clustering together on the right of the x-axis (sites 2, 10, 20, 21; Supporting Information Table S3), suggesting that their behavior in terms of the utilization of DOM as a resource, is strongly controlled by the level of nutrient enrichment in the catchments.

Discussion

Physicochemical and nutrient controls on processing in rivers

The results presented in this study indicate that both the physicochemical character of the river and its catchment, and its nutrient enrichment status strongly influence how LMW DOM components are biologically processed in freshwaters. Rates of DOM depletion observed in this study were highest in those sites with higher nutrient concentrations, draining from intensively farmed landscapes, and lowest for systems with high background DOC from peaty soils with low N and P concentrations, typically supporting blanket bog, acid grassland, and forestry. This finding confirms the findings of previous studies, including our own, which have shown that dominant land cover is an important determinant of DOM quality and stoichiometry in freshwaters, influencing both the C : N and DOC : DON/DOP ratios (Yates et al. 2019b) and that physicochemical factors control the rate of biodegradation of

Table 1. Percentage depletion of ^{14}C -labeled DOC, DON, and DOP and ^{33}P -labeled SRP from river water across five major land cover types after 24 h. Values represent percentage of tracer depleted within 24 h and are presented as means \pm SEM. The average value across all the sites is also presented at the foot of the table. Significant differences between land use types are shown with lowercase superscript letters while differences between substrates are denoted by uppercase superscript letters ($p < 0.05$).

Land cover	Depletion 24 h (% initial activity added)			
	DOC	DON	DOP	SRP
Arable	92.7 \pm 0.9 ^a	75.6 \pm 0.4 ^a	74.6 \pm 0.5 ^a	32.9 \pm 4.7 ^a
Mixed	81.0 \pm 4.7 ^a	64.6 \pm 4.6 ^a	63.7 \pm 3.5 ^a	32.1 \pm 4.9 ^a
Grassland	78.3 \pm 2.2 ^a	60.6 \pm 1.9 ^a	54.4 \pm 3.1 ^a	21.1 \pm 5.1 ^a
Conifer forest	37.7 \pm 3.6 ^b	31.3 \pm 4.7 ^b	17.6 \pm 1.5 ^b	43.5 \pm 5.2 ^a
Peatland	37.0 \pm 3.9 ^b	38.8 \pm 3.4 ^b	20.2 \pm 2.1 ^b	36.2 \pm 13.2 ^a
Average	74.1 \pm 3.0 ^A	60.0 \pm 2.2 ^{AB}	55.2 \pm 3.1 ^B	30.7 \pm 3.5 ^C

DOM in rivers (e.g., Guillemette et al. 2013; Sleighter et al. 2014; Catalán et al. 2017). Here, both the relative and absolute pool sizes of DOC, TN, and TP appeared to directly influence the separation of sites (Table 2; Fig. 2). Those sites with N and P pools enriched by either inorganic or organic or both forms of N and P show higher rates of depletion of dosed DOC, DOC, and DOP than sites with a lower background concentration of N and P. In this study, it is also clear that controlling factors are intertwined. Those sites in our sample of 45 locations which have lower background N and P

concentrations also have a much higher proportion of HMW DOM relative to LMW DOM, based on optical measurements (Yates et al. 2019b). There was also correlation between land cover, underlying geology, soil acidity, and DOM character at each site, with mountain and moorland sites underlain by impermeable geology supporting acid grassland and peat bog with a higher acidity and higher proportion of HMW DOM along with very low background N and P concentrations. Thus, it is impossible in this experimental framework to entirely attribute the different rates of DOC, DON, and DOP depletion to any one controlling factor. As has been argued in many prior studies, multiple physicochemical and biological controls are exerted on the function of any one biotic group within the ecosystem, controlling the rate of DOM processing and uptake by riverine biota. What is clear from these findings, however, is that the nutrient enrichment status of a waterbody is an additional factor not typically considered in prior work, which also acts to control the rate of biodegradation

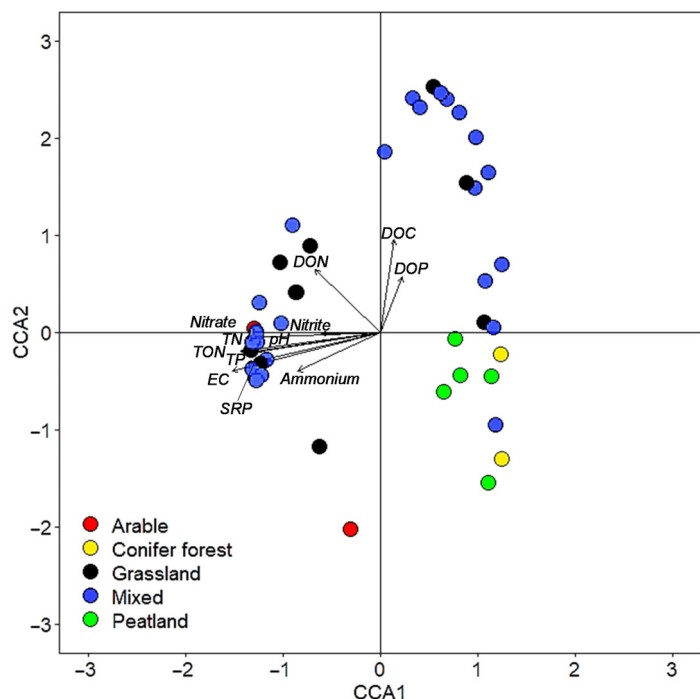


Fig. 2. Correlation bi-plot from the CCA for the main river water chemical variables (total oxidizable nitrogen [TON], nitrate, nitrite, ammonium, DON, TN, soluble reactive phosphorus [SRP], DOP, TP, DOC, pH, and EC) and the mean DOC, DON, DOP, and SRP depletion data for each of the 45 rivers sampled across five different land use types ($n = 4$ per site).

Table 2. Correlation of TON, nitrate, nitrite, ammonium, DON, TN, SRP, DOP, TP, DOC, pH, and EC with depletion data from 45 sites ($n = 4$) using CCA.

	R^2	p value
TON (mg N L^{-1})	0.73	0.001*
Nitrate (mg N L^{-1})	0.62	0.001*
Nitrite (mg N L^{-1})	0.13	0.236
Ammonium (mg N L^{-1})	0.29	0.032*
DON (mg N L^{-1})	0.26	0.049*
TN (mg N L^{-1})	0.65	0.001*
SRP (mg P L^{-1})	0.68	0.001*
DOP (mg P L^{-1})	0.11	0.322
TP (mg P L^{-1})	0.65	0.001*
DOC (mg C L^{-1})	0.25	0.050
pH	0.56	0.001*
EC ($\mu\text{S cm}^{-1}$)	0.84	0.001*

*Significant p value ($p < 0.05$).

Table 3. Results from PERMANOVA for the effect of land cover.

	df	Sum of squares	R ²	F	p value
Land cover	4	0.219	0.374	6	0.002*
Residual	40	0.365	0.626		
Total	44	0.583	1.000		

*A significant *p* value. The significance level was set at *p* < 0.05.

of labile DOM in river systems. We explore this evidence further below.

Degree of nutrient enrichment as a control of degradation

In this study, the DOC, TN, and TP concentrations in the rivers sampled were correlated with the rate of microbial degradation of DOC, DON, DOP, and SRP (Table 2). Across the 45 sites, the rate of DOC depletion decreased with increasing background DOC concentration, although this may have been a function of the higher proportion of HMW : LMW DOM in the sites with the highest DOC concentrations. The highest observed DOC concentrations were in peatland influenced rivers, where TN and TP concentrations were among the lowest observed (Supporting Information Table S1; Emmett et al. 2016; Yates et al. 2019b). Of note, the proportion of the TN and TP that was in the form of DON and DOP (Supporting Information Table S1) in these sites was the highest, decreasing relative to inorganic N and P as TN and TP concentrations increased, in line with trends in N speciation reported across Europe by Durand et al. (2011). In these headwater sites, where C is abundant, but N and P are scarce, N/P colimitation is likely to be the main physicochemical factor controlling the microbial processing of LMW DOM. Here we observed rapid depletion of our DON and DOP substrates, and argue that this demonstrates the biotic uptake and metabolism of both as a nutrient resource in these nutrient-poor sites. For sites subject to N and P enrichment from human activities in their catchments, we observed an increase in DOC uptake by the microbial biomass, which can be ascribed to the removal of metabolic constraints associated with N/P limitation on microbial growth and therefore labile LMW DOC uptake (Carlson and Ducklow 1996; Creamer et al. 2014; Parr et al. 2015; Fovet et al. 2020). It has been argued that rivers transition from being N/P limited to N/C limited along a gradient from source to sea (Jarvie et al. 2018). However, this would not be a general truth in disturbed river catchments (farming, urbanization) where the nature and location of disturbance would be the key control on nutrient availability and stoichiometry at any particular location rather than any simple measure of distance downstream. Furthermore, it could be argued that increasing N and P enrichment of rivers could potentially increase autochthonous DOC, DON, and DOP synthesis, such that the associated enhancement of microbial growth and consequent increase in the rates of DOM degradation (as observed in the current study) can drive

microbial communities toward C limitation (Stanley et al. 2011; Emmett et al. 2016). This has been observed in the current study, where labile DOC, DON, and DOP depletion in our bioassays increased as background N and P concentrations increased along our gradient of sites, confirming N/P enrichment (Table 2; Supporting Information Table S1). By contrast, SRP depletion from solution decreased with increasing nutrient concentrations; this was in agreement with our study of a nutrient-enriched riparian zone where the addition of SRP, did not lead to an enhancement in microbial activity (represented by ¹⁴C uptake) due to a lack of P limitation, and thus a lack of demand for additional SRP (de Sosa et al. 2018).

Based on the regression analysis parameters, LMW DOM depletion followed the trend: DON ≥ DOC > DOP, even in low nutrient status waters (Table 2). A previous catchment-scale study of DOM metabolism found that DON (in the form of amino acids) degraded quickest in rivers draining through peatland compared to those influenced by other land covers, which is likely influenced by the N limitation of these ecosystems (Berggren and del Giorgio 2015). In this study and in our earlier work, we observed that the microbial degradation of amino acids in oligotrophic peatland rivers was slower than in mesotrophic grassland rivers, and that this amino acid depletion was quicker than that of glucose, organic acids, and phenolic compounds in both river waters and underlying sediments (Brailsford et al. 2019a). This supports the findings set out by Bronk et al. (2007), who suggested that DON can be an extremely important and bioavailable source of N for both bacteria and phytoplankton in aquatic ecosystems.

Nutrient stoichiometry and depletion

In recent years, the influence of stoichiometry of the three major nutrients (C, N, P) has been brought to the forefront of catchment science, with several studies suggesting that modulating nutrient stoichiometry could help tackle eutrophication in freshwaters (Paerl et al. 2016; Stutter et al. 2018; Rankinen et al. 2019). The influence of nutrient stoichiometry on DOM processing in these catchments has been addressed in previous studies (Brailsford et al. 2019b; Yates et al. 2019b). In these studies and others, C : N ratios of soil have also been found to be a good predictor of both DOC : DON ratios and DOM bioavailability (Kroer 1993; Yates et al. 2019b), while N/P addition has been shown to increase the rate of biodegradation of LMW DOC (Creamer et al. 2014; Brailsford et al. 2019b), as in this study. Reduction of nutrient loading to waters ought, therefore, to lead to a reduction in the rate of consumption of labile and semi-labile DOM in the waterbody by stream heterotrophs, reducing the nutrient resource available to support instream biological production.

In terms of N/P quality, a number of studies have previously demonstrated that although increasing N/P is generally accompanied by increasing DON/DOP concentrations, a decrease in the proportion of DON/DOP components is usually also observed, as the influence of inorganic soluble N and

particulate P increases (Perakis and Hedin 2002; Durand et al. 2011; Berggren et al. 2015; Yates et al. 2019b). In the current study, both DON and DOP degradation were closely related to TN and TP concentrations across our 45 sites (Table 2; Supporting Information Tables S1, S2): the higher the background N and P concentration in the river water, the higher the rate of both DON and DOP depletion. We therefore consider that the increased DON/DOP demand at nutrient-enriched sites could be due to: (1) a demand for labile LMW DON/DOP (Bronk et al. 2007) to meet both nutrient resource and energy needs of organisms; (2) all DOM components (DOC, DON, DOP) flushed downstream from upstream sources being utilized by microbial communities for their carbon content meet their energy needs (Battin et al. 2008); (3) changes in the way compounds are metabolized according to differences in nutrient limitation as this varies in both space and time (Brailsford et al. 2019b).

Limitations and future foci

The current study utilized $^{14}\text{C}/^{33}\text{P}$ -labeled tracers to assess the fate of DOC, DON, DOP, and SRP in river waters from 45 sites covering a range of physicochemical gradients. Apart from DON, a single radioisotope tracer was used in each case. Although LMW DOM may represent a fraction of the total OM in aquatic ecosystems, it is a highly diverse mixture, including sugars, amino acids, peptides, organic acids, carboxylic acids, and nucleic acids (Dawson et al. 2001). Further studies are required using mixtures and stoichiometric ratios of isotopically labeled compounds, in order to provide a more representative view of the microbial uptake kinetics of labile LMW DOM in aquatic ecosystems (similar to Brailsford et al. 2019a). In addition, for DOM compounds only the C was radiolabeled, therefore the ultimate fate of N in DON and P in DOP remains unknown.

Conclusions

Comparison of the depletion of DOM components (DOC, DON, DOP) from solution demonstrated that DOC removal from solution proportionally increases with increases in DON and DOP depletion, with DON removal being slightly more rapid and DOP removal being slightly slower than DOC removal, respectively. The depletion of all three DOM fractions increases as nutrient enrichment increases, and as the proportion of HMW DOM decreases along the river enrichment gradient. This study demonstrates that the nutrient chemistry of waters, which is in turn influenced by land-cover, can be a predictor of the capacity for DOM processing in the aquatic environment, with inorganic nutrient enrichment and carbon limitation stimulating an increase in DOC, DON, and DOP processing by the microbial biomass. By contrast, microbial SRP processing decreases with increasing nutrient enrichment, suggesting that DOP is preferred over SRP in riverine microbial communities, driving many of the adverse

impacts associated with the eutrophication of freshwaters. It is our opinion that downstream “omics” approaches such as primary metabolome analysis could provide more insight into the fate of these compounds following uptake by the aquatic microbial biomass. Programs aiming to control eutrophication in freshwater catchments would therefore need to control both the inorganic and organic N and P loading delivered to waters from their catchments if they are to be effective in realizing improvements in the ecological status of these waters.

References

- Akkanen, J., A. Tuikka, and J. V. K. Kukkonen. 2012. On the borderline of dissolved and particulate organic matter: Partitioning and bioavailability of polycyclic aromatic hydrocarbons. *Ecotoxicol. Environ. Saf.* **78**: 91–98. doi:[10.1016/j.ecoenv.2011.11.010](https://doi.org/10.1016/j.ecoenv.2011.11.010)
- Battin, T. J., and others. 2008. Biophysical controls on organic carbon fluxes in fluvial networks. *Nat. Geosci.* **1**: 95–100. doi:[10.1038/ngeo101](https://doi.org/10.1038/ngeo101)
- Berggren, M., and P. A. del Giorgio. 2015. Distinct patterns of microbial metabolism associated to riverine dissolved organic carbon of different source and quality. *J. Geophys. Res. Biogeosci.* **120**: 989–999. doi:[10.1002/2015JG002963](https://doi.org/10.1002/2015JG002963)
- Berggren, M., R. A. Sponseller, A. R. Alves Soares, and A.-K. Bergström. 2015. Toward an ecologically meaningful view of resource stoichiometry in DOM-dominated aquatic systems. *J. Plankton Res.* **37**: 489–499. doi:[10.1093/plankt/fbv018](https://doi.org/10.1093/plankt/fbv018)
- Bowen, J., L. A. Kaplan, and R. M. Cory. 2019. Photodegradation disproportionately impacts biodegradation of semi-labile DOM in streams. *Limnol. Oceanogr.* **65**: 13–26. doi:[10.1002/lno.11244](https://doi.org/10.1002/lno.11244)
- Brailsford, F. L., H. C. Glanville, M. R. Marshall, P. N. Golyshin, P. J. Johnes, C. A. Yates, A. T. Owen, and D. L. Jones. 2017. Microbial use of low molecular weight DOM in filtered and unfiltered freshwater: Role of ultra-small microorganisms and implications for water quality monitoring. *Sci. Total Environ.* **598**: 377–384. doi:[10.1016/j.scitotenv.2017.04.049](https://doi.org/10.1016/j.scitotenv.2017.04.049)
- Brailsford, F. L., H. C. Glanville, P. N. Golyshin, P. J. Johnes, C. A. Yates, and D. L. Jones. 2019a. Microbial uptake kinetics of dissolved organic carbon (DOC) compound groups from river water and sediments. *Sci. Rep.* **9**: 11229. doi:[10.1038/s41598-019-47749-6](https://doi.org/10.1038/s41598-019-47749-6)
- Brailsford, F. L., H. C. Glanville, P. N. Golyshin, M. R. Marshall, C. E. Lloyd, P. J. Johnes, and D. L. Jones. 2019b. Nutrient enrichment induces a shift in dissolved organic carbon (DOC) metabolism in oligotrophic freshwater sediments. *Sci. Total Environ.* **690**: 1131–1139. doi:[10.1016/j.scitotenv.2019.07.054](https://doi.org/10.1016/j.scitotenv.2019.07.054)
- Bronk, D. A., J. H. See, P. Bradley, and L. Killberg. 2007. DON as a source of bioavailable nitrogen for phytoplankton. *Biogeosciences* **4**: 283–296. doi:[10.5194/bg-4-283-2007](https://doi.org/10.5194/bg-4-283-2007)

- Carlson, C. A., and H. W. Ducklow. 1996. Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. *Aquat. Microb. Ecol.* **10**: 69–85. doi:10.3354/ame010069
- Catalán, N., J. P. Casas-Ruiz, D. von Schiller, L. Proia, B. Obrador, E. Zwirnmann, and R. Marcé. 2017. Biodegradation kinetics of dissolved organic matter chromatographic fractions in an intermittent river. *J. Geophys. Res. Biogeosci.* **122**: 131–144. doi:10.1002/2016JG003512
- Cooper, D. M., C. D. Evans, D. Norris, S. Thacker, and M. G. Pereira. 2014. Application of a simple multiplicative spatio-temporal stream water quality model to the river Conwy, North Wales. *Environ. Sci. Process. Impacts* **16**: 1600–1607. doi:10.1039/C3EM00627A
- Cory, R. M., and G. W. Kling. 2018. Interactions between sunlight and microorganisms influence dissolved organic matter degradation along the aquatic continuum. *Limnol. Oceanogr.: Lett.* **3**: 102–116. doi:10.1002/lo2.10060
- Creamer, C. A., D. L. Jones, J. A. Baldock, and M. Farrell. 2014. Stoichiometric controls upon low molecular weight carbon decomposition. *Soil Biol. Biochem.* **79**: 50–56. doi:10.1016/j.soilbio.2014.08.019
- Cui, X., and K.-W. Choo. 2013. Granular iron oxide adsorbents to control natural organic matter and membrane fouling in ultrafiltration water treatment. *Water Res.* **47**: 4227–4237. doi:10.1016/j.watres.2013.04.060
- Cuss, C. W., and C. Guéguen. 2015. Relationships between molecular weight and fluorescence properties for size-fractionated dissolved organic matter from fresh and aged sources. *Water Res.* **68**: 487–497. doi:10.1016/j.watres.2014.10.013
- Dawson, J. J. C., C. Bakewell, and M. F. Billet. 2001. Is in-stream processing an important control on spatial changes in carbon fluxes in headwater catchments? *Sci. Total Environ.* **265**: 153–167. doi:10.1016/S0048-9697(00)00656-2
- de Sosa, L. L., H. C. Glanville, M. R. Marshall, A. Schnepf, D. M. Cooper, P. W. Hill, A. Binley, and D. L. Jones. 2018. Stoichiometric constraints on the microbial processing of carbon with soil depth along a riparian hillslope. *Biol. Fertil. Soils* **54**: 949–963. doi:10.1007/s00374-018-1317-2
- Durand, P., and others. 2011. Nitrogen processes in aquatic ecosystems, p. 126–146. *In* M. A. Sutton, C. M. Howard, J. W. Erisman, G. Billen, A. Bleeker, P. Grennfelt, H. van Grinsven, and B. Grizzetti [eds.], *The European nitrogen assessment*. Cambridge Univ. Press.
- Ejarque, E., A. Freixa, E. Vazquez, A. Guarch, S. Amalfitano, S. Fazi, A. M. Romani, and A. Butturini. 2017. Quality and reactivity of dissolved organic matter in a Mediterranean river across hydrological and spatial gradients. *Sci. Total Environ.* **599–600**: 1802–1812. doi:10.1016/j.scitotenv.2017.05.113
- Emmett, B. A., and others. 2016. Spatial patterns and environmental constraints on ecosystem services at a catchment scale. *Sci. Total Environ.* **572**: 1586–1600. doi:10.1016/j.scitotenv.2016.04.004
- Fellman, J. B., E. Hood, and R. G. M. Spencer. 2010. Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. *Limnol. Oceanogr.* **55**: 2452–2462. doi:10.4319/lo.2010.55.6.2452
- Fovet, O., D. M. Cooper, D. L. Jones, T. G. Jones, and C. D. Evans. 2020. Dynamics of dissolved organic matter in headwaters: Comparison of headwater streams with contrasting DOM and nutrient composition. *Aquat. Sci.* **82**: 29. doi:10.1007/s00027-020-0704-6
- Guillemette, F., S. L. McCallister, and P. A. del Giorgio. 2013. Differentiating the degradation dynamics of algal and terrestrial carbon within complex natural dissolved organic carbon in temperate lakes. *J. Geophys. Res. Biogeosci.* **118**: 963–973. doi:10.1002/jgrg.20077
- Hornák, K., H. Schmidheiny, and J. Pernthaler. 2016. High-throughput determination of dissolved free amino acids in unconcentrated freshwater by ion-pairing liquid chromatography and mass spectrometry. *J. Chromatogr. A* **1440**: 85–93. doi:10.1016/j.chroma.2016.02.045
- Jarvie, H. P., C. Neal, P. J. A. Withers, C. Wescott, and R. A. Acornley. 2005. Nutrient hydrochemistry for a groundwater-dominated catchment: The Hampshire Avon, UK. *Sci. Total Environ.* **344**: 143–158. doi:10.1016/j.scitotenv.2005.02.012
- Jarvie, H. P., and others. 2018. Phosphorus and nitrogen limitation and impairment of headwater streams relative to rivers in Great Britain: A national perspective on eutrophication. *Sci. Total Environ.* **621**: 849–862. doi:10.1016/j.scitotenv.2017.11.128
- Kirchman, D. L. 2003. The contribution of monomers and other low-molecular weight compounds to the flux of dissolved organic material in aquatic ecosystems, p. 217–241. *In* F. SEG and R. L. Sinsabaugh [eds.], *Aquatic ecosystems: Interactivity of dissolved organic matter*. Academic Press.
- Kroer, N. 1993. Bacterial-growth efficiency on natural dissolved organic-matter. *Limnol. Oceanogr.* **38**: 1282–1290. doi:10.4319/lo.1993.38.6.1282
- Lloyd, C. E. M., and others. 2019. Determining the origins of nutrient flux to waters in catchments: Examining the nutrient speciation balance to inform the targeting of mitigation measures. *Sci. Total Environ.* **648**: 1179–1200. doi:10.1016/j.scitotenv.2018.08.190
- Lutz, B. D., E. S. Bernhardt, B. J. Roberts, R. M. Cory, and P. J. Mulholland. 2011. Distinguishing dynamics of dissolved organic matter components in a forested stream using kinetic enrichments. *Limnol. Oceanogr.* **57**: 76–89. doi:10.4319/lo.2012.57.1.0076
- Marie, L., B. Pernet-Coudrier, M. Waeles, M. Gabon, and R. Riso. 2015. Dynamics and sources of reduced sulfur, humic substances and dissolved organic carbon in a temperate

- river system affected by agricultural practices. *Sci. Total Environ.* **537**: 23–32. doi:[10.1016/j.scitotenv.2015.07.089](https://doi.org/10.1016/j.scitotenv.2015.07.089)
- Massicotte, P., and J.-J. Frenette. 2011. Spatial connectivity in a large river system: Resolving the sources and fate of dissolved organic matter. *Ecol. Appl.* **21**: 2600–2617. doi:[10.1890/10-1475.1](https://doi.org/10.1890/10-1475.1)
- Oksanen, J., and others. 2018. *Vegan: Community ecology package*. Accessed February 2021. Available from <https://CRAN.Rproject.org/package=vegan>
- Paelr, H. W., and others. 2016. It takes two to tango: When and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. *Environ. Sci. Technol.* **50**: 10805–10813. doi:[10.1021/acs.est.6b02575](https://doi.org/10.1021/acs.est.6b02575)
- Parr, T. B., T. Ohno, C. S. Cronan, and K. S. Simon. 2014. *comPARAFAC: A library and tools for rapid and quantitative comparison of dissolved organic matter components resolved by parallel factor analysis*. *Limnol. Oceanogr.: Methods* **12**: 114–125. doi:[10.4319/lom.2014.12.114](https://doi.org/10.4319/lom.2014.12.114)
- Parr, T. B., C. S. Cronan, T. Ohno, S. E. G. Findlay, S. M. C. Smith, and K. S. Simon. 2015. Urbanization changes the composition and bioavailability of dissolved organic matter in headwater streams. *Limnol. Oceanogr.* **60**: 88–900. doi:[10.1002/lno.10060](https://doi.org/10.1002/lno.10060)
- Perakis, S. S., and L. O. Hedin. 2002. Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. *Nature* **418**: 665–665. doi:[10.1038/415416a](https://doi.org/10.1038/415416a)
- Pirani, M., A. Panton, D. A. Purdie, and S. K. Sahu. 2016. Modelling macronutrient dynamics in the Hampshire Avon river: A Bayesian approach to estimate seasonal variability and total flux. *Sci. Total Environ.* **572**: 1449–1460. doi:[10.1016/j.scitotenv.2016.04.129](https://doi.org/10.1016/j.scitotenv.2016.04.129)
- R Core Team. 2019. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.
- Rankinen, K., J. E. C. Bernal, M. Holmberg, K. Vuorio, and K. Granlund. 2019. Identifying multiple stressors that influence eutrophication in a Finnish agricultural river. *Sci. Total Environ.* **658**: 1278–1292. doi:[10.1016/j.scitotenv.2018.12.294](https://doi.org/10.1016/j.scitotenv.2018.12.294)
- Salcher, M. M., T. Posch, and J. Pernthaler. 2013. In situ substrate preferences of abundant bacterioplankton populations in a pre-alpine freshwater lake. *ISME J.* **7**: 896–907. doi:[10.1038/ismej.2012.162](https://doi.org/10.1038/ismej.2012.162)
- Srivedhin, T., and K. A. Gray. 2005. Part I. Identifying anthropogenic markers in surface waters influenced by treated effluents: A tool in potable water reuse. *Water Res.* **39**: 1154–1164. doi:[10.1016/j.watres.2004.11.032](https://doi.org/10.1016/j.watres.2004.11.032)
- Sleighter, R. L., R. M. Cory, L. A. Kaplan, H. A. N. Abdulla, and P. G. Hatcher. 2014. A coupled geochemical and biogeochemical approach to characterize the bio-reactivity of dissolved organic matter from a headwater stream. *J. Geophys. Res. Biogeosci.* **119**: 1520–1537. doi:[10.1002/2013JG002600](https://doi.org/10.1002/2013JG002600)
- Stanley, E. H., S. M. Powers, N. R. Lottig, I. Buffam, and J. T. Crawford. 2011. Contemporary changes in dissolved organic carbon (DOC) in human-dominated rivers: Is there a role for DOC management? *Freshw. Biol.* **57**: 26–42. doi:[10.1111/j.1365-2427.2011.02613.x](https://doi.org/10.1111/j.1365-2427.2011.02613.x)
- Stutter, M. I., D. Graeber, C. D. Evans, A. J. Wade, and P. J. A. Withers. 2018. Balancing macronutrient stoichiometry to alleviate eutrophication. *Sci. Total Environ.* **634**: 439–447. doi:[10.1016/j.scitotenv.2018.03.298](https://doi.org/10.1016/j.scitotenv.2018.03.298)
- Thurman, E. M. 1985. *Organic geochemistry of natural waters*. Martinus Nijhoff/Dr W Junk.
- Tipping, E., J. F. Boyle, D. N. Schillereff, B. M. Spears, and G. Phillips. 2016. Macronutrient processing by temperate lakes: A dynamic model for long-term, large-scale application. *Sci. Total Environ.* **572**: 1573–1585. doi:[10.1016/j.scitotenv.2015.09.129](https://doi.org/10.1016/j.scitotenv.2015.09.129)
- Wickham, H. 2016. *ggplot2: Elegant graphics for data analysis*. Springer-Verlag.
- Yates, C. A., and P. J. Johnes. 2013. Nitrogen speciation and phosphorus fractionation dynamics in a lowland chalk catchment: The Upper Wylde. *Sci. Total Environ.* **444**: 466–479. doi:[10.1016/j.scitotenv.2012.12.002](https://doi.org/10.1016/j.scitotenv.2012.12.002)
- Yates, C. A., P. J. Johnes, and R. G. M. Spencer. 2019a. Characterisation of treated effluent from four commonly employed wastewater treatment facilities: A UK case study. *J. Environ. Manag.* **232**: 919–927. doi:[10.1016/j.jenvman.2018.12.006](https://doi.org/10.1016/j.jenvman.2018.12.006)
- Yates, C. A., and others. 2019b. 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. *Limnol. Oceanogr.* **64**: 2328–2340. doi:[10.1002/lno.11186](https://doi.org/10.1002/lno.11186)
- Zhang, G., and others. 2017. Effects of water table on cellulose and lignin degradation of *Carex cinerascens* in a large seasonal floodplain. *J. Freshw. Ecol.* **33**: 331–325. doi:[10.1080/02705060.2018.1459324](https://doi.org/10.1080/02705060.2018.1459324)

Acknowledgments

We thank Sarah Chesworth, Jonathan Roberts, Laura Lozano de Sosa Miralles, Karina Marsden, William Havelange, Anna Prieto, Mallory Diggins, Lydia Ghuneim, and Sandra Murillo for assistance in field and laboratory work. We also thank Craig Banks, Karen Perrow, and Celia Brailsford for their assistance in the field. Thanks to Rory Shaw for help creating depletion curve graphs using R. Thanks to Paul BL George for help in running canonical correspondence analysis using R. This work was carried out under the DOMAINE project, which is funded by the UK Natural Environment Research Council (NERC) (large grant NE/K010689/1). F.L.B. and P.N.G. would like to acknowledge the support of the Centre of Environmental Biotechnology Project, part-funded by the European Regional Development Fund (ERDF) through the Welsh Government.

Conflict of Interest

None declared.

Submitted 07 January 2020

Revised 04 February 2021

Accepted 02 May 2021

Associate editor: Anna Romani