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Catchment Controls on the Use of Dissolved Organic Nutrients by River Phytoplankton in the United Kingdom

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ABSTRACT

1. Dissolved organic matter (DOM) affects the structure and function of aquatic ecosystems and can supply nitrogen and phosphorus to meet the stoichiometric needs of biota. Anthropogenic alteration of the nature and rates of DOM inputs to freshwaters may be increasing the risk of eutrophication, but most of our understanding of DOM use in phytoplankton is based on site specific assessments; little is known about landscape-scale controls on DOM bioavailability to river phytoplankton communities, across varying landscape character. It is important to test whether our understanding of DOM use at individual sites is generalisable at larger scales and if simple measures of catchment characteristics can be used as an effective proxy to predict growth responses of riverine phytoplankton to DOM.
2. We used bioassays to investigate the ability of phytoplankton to use dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) compounds to support growth in rivers in the United Kingdom (UK). We then assessed the extent to which statistical models built upon these data can be used to provide generalisable insights into phytoplankton growth responses at new sites based on riverine nutrient concentrations and catchment land cover.
3. We found the greatest phytoplankton growth responses to DOM compounds occurred at lower concentrations of inorganic nutrients, although organic nutrients continued to stimulate growth even at higher inorganic nutrient concentrations. Water quality models based on bioassay responses developed in two catchments of contrasting character could adequately predict growth responses across the UK ($r^2 = 0.56$ for DON; $r^2 = 0.33$–0.51 for DOP). However, better model fit and performance was generated by models developed at the national scale using national monitoring and bioassay datasets (overall $r^2 = 0.90$, fixed effects $r^2 = 0.82$ for DON and overall $r^2 = 0.82$–0.92, fixed effects $r^2 = 0.39$–0.51 for DOP). Models using only a lowland to upland land cover gradient could adequately predict DON use by river phytoplankton (overall $r^2 = 0.90$, fixed effects $r^2 = 0.56$) but by contrast, their DOP use was influenced more strongly by site- and DOM compound-specific factors (overall $r^2 = 0.83$–0.92, fixed effects $r^2 = 0.17$–0.25).
4. Growth stimulation of river phytoplankton by organic nutrient enrichment was widespread across the UK, even at nutrient concentrations considered non-limiting to growth. The extent to which catchment land cover could predict DON versus DOP growth responses differed, but nutrient concentrations remained a key driver in predicting growth stimulation in UK rivers.
5. Human-induced perturbations to organic matter inputs to freshwaters may therefore be altering these ecosystems and changing the composition of the communities that they support. The models generated here are useful tools to identify which rivers

may be more at risk from the eutrophication potential of dissolved organic nutrient inputs, such as those resulting from predicted future changes in nutrient management and land cover.

1 | Introduction

Dissolved organic matter (DOM), a diverse mixture of compounds, is a key structuring component of aquatic ecosystems (Thomas 1997; Creed et al. 2018; McDowell 2022; Chen et al. 2023). In addition to carbon, many DOM molecules also contain the essential macronutrients nitrogen (N) and phosphorus (P), as dissolved organic N (DON) and P (DOP) compounds, respectively. Interest in DOM has grown as research suggests that human activities are increasingly perturbing the global carbon cycle, affecting decomposition rates, nutrient immobilisation (Costello et al. 2022; Tiegs et al. 2024) and leading to rising concentrations (Monteith et al. 2007). The changing fluxes of DOM, including altered element stoichiometry (Yates et al. 2019; Wymore et al. 2021; Rodríguez-Cardona et al. 2022), are likely to impact the functioning of aquatic ecosystems.

In particular, there is a growing recognition that different DOM compounds are bioavailable to primary producers and the microbial community (Flynn and Butler 1986; Berman and Chava 1999; Bronk et al. 2007; Brailsford, Glanville, Golyshin, Johnes, et al. 2019; Mackay et al. 2020; Johnes et al. 2023). In addition to using DOM compounds, different groups of primary producers are known to use DON selectively over other N sources, indicating that DOM composition plays a role in shaping community composition (Berman and Bronk 2003; Glibert et al. 2004; Altman and Paerl 2012; Reinl et al. 2022). However, while land cover type and nutrient enrichment have been shown to regulate DOM turnover in fresh waters (Brailsford, Glanville, Golyshin, Marshall, et al. 2019; Brailsford et al. 2021) and phytoplankton growth on organic nutrients was found to be inversely related to stream inorganic nutrient concentrations (Mackay et al. 2020), much less is known about wider landscape controls on DON and DOP composition (e.g., Yates et al. 2022) and how its shifting composition influences its use by the freshwater biota.

Understanding the controls and conditions that affect DON and DOP use at larger scales could potentially assist in identifying the risk it poses to the ecological health of freshwater bodies. For example, at a global scale, organic matter decomposition rates are closely related to latitude, being influenced by temperature and nutrient availability (Tiegs et al. 2019). Many DOM compounds are anthropogenic in origin (Lloyd et al. 2023) and originate from a variety of different sources including sewage and agricultural runoff (Lloyd et al. 2019; Yates et al. 2019, 2022). These organic nutrient sources, combined with the anthropogenic perturbation of natural catchment nutrient cycling through land use change, could result in eutrophication of the aquatic environment.

Much of our current understanding of the factors that control DON and DOP bioavailability comes from a few sites in a limited number of catchments. Additionally, little is known about how generalisable these catchment scale relationships are over larger scales, which may be important for managing eutrophication risk. Previous work to predict DON and DOP effects on the growth of river phytoplankton has highlighted that the relative nutrient enrichment status of the water body is a key control on relative growth responses in different rivers (Mackay et al. 2020). Nutrient concentrations, the fractionation of the nutrient load into inorganic and organic nutrient forms and the composition of the DOM pool in rivers are often closely associated with the geology and land use of the catchment (Yates et al. 2016, 2019, 2022; Fuß et al. 2017; Lloyd et al. 2019; Djodjic et al. 2021) and therefore at a large scale, these factors may be useful proxies to predict growth responses to organic nutrients. However, the influence of site-specific factors on biological communities is also widely recognised (Leland and Porter 2000; Tsai et al. 2013; Lange et al. 2016) and the interaction between the two could confound efforts to upscale predictions of DON and DOP use by river phytoplankton.

Currently, clear knowledge gaps exist with respect to the differing bioavailability of DON and DOP as this varies according to landscape character. We know little of the generalisability of nutrient drivers for phytoplankton growth over large spatial scales, or the landscape controls on DON and DOP use by river phytoplankton. Here, using nutrient chemistry and organic nutrient bioassay incubations from sites across Great Britain, we upscale dissolved organic nutrient–phytoplankton growth response (bioavailability) relationships from a catchment scale study (Mackay et al. 2020; herein referred to as Tier 1 sites), additional data sets based on bioassays on samples collected from twenty-four sites spanning the range of nutrient concentrations in UK rivers, herein referred to as Tier 2 sites. These were then used in combination to assess the generalisability of these relationships across the broader range of catchment characteristics as this varies at a national scale. Using landscape scale data on nutrient speciation and DOM chemistry (Yates et al. 2022) we test whether DON and DOP bioavailability to phytoplankton can be predicted from simple, widely available data on catchment character. Specifically, we address the following questions: (1) What are the relationships between ambient nutrient concentrations and nutrient limitation, and between nutrient limitation and DON and DOP growth responses in riverine phytoplankton? (2) How well do local-scale relationships between organic nutrient use and ambient nutrient concentrations predict DON and DOP use at a national scale? (3) Are the predictors of organic nutrient use similar at local and national scales? (4) Can land use and geology datasets available nationally provide robust predictions of organic nutrient use, when reliable data of nutrient chemistry and fractionation are unavailable?

2 | Methods

2.1 | Study Design and Field Methods

The study design comprised two groups of sites selected to assess the transferability of relationships between water chemistry and nutrient bioavailability between scales. Six Tier 1 sites were chosen to span five major land cover types (arable, improved grassland, coniferous forest, peatland and mixed agriculture) in two river catchments and a UK dataset of twenty-four Tier 2 sites was selected to represent a wider gradient of natural and semi-natural habitats to intensively farmed land across the geoclimatic regions found in the UK (Figure 1) (Emmett et al. 2008; Greene et al. 2015). The national Tier 2 dataset was a subset from a larger study of 51 sites chosen to span a range of geologies, soil types, land cover and nutrient enrichment as described in Yates et al. (2022) (Table S1). Those selected for this study comprised six sites on upland moorland habitats underlain by impermeable bedrock, nine sites on lowland, poorly draining clay soils underlain by mudstones and nine sites on permeable, free-draining soils underlain by calcareous sedimentary rock. A principal components analysis (PCA) of land cover and geology using both Tier 1 and Tier 2 sites demonstrated that the Tier 1 sites would provide a representative selection of sites with which to assess the transferability of water chemistry and nutrient bioavailability across scales (Figure S1). Stream water samples were collected at each location on one occasion in late summer or early autumn 2017 and returned to the laboratory within 24 h for nutrient and bioassay analyses. Context for these spot samples was provided by weekly or daily monitoring to determine all N species, P fractions and dissolved organic carbon (DOC), and the fluorescent characteristics of stream DOM, as previously reported in Yates et al. (2019, 2022). Summary data from this parallel study of the Tier 2 sites are reported here to provide context for the interpretation of the subset of sites

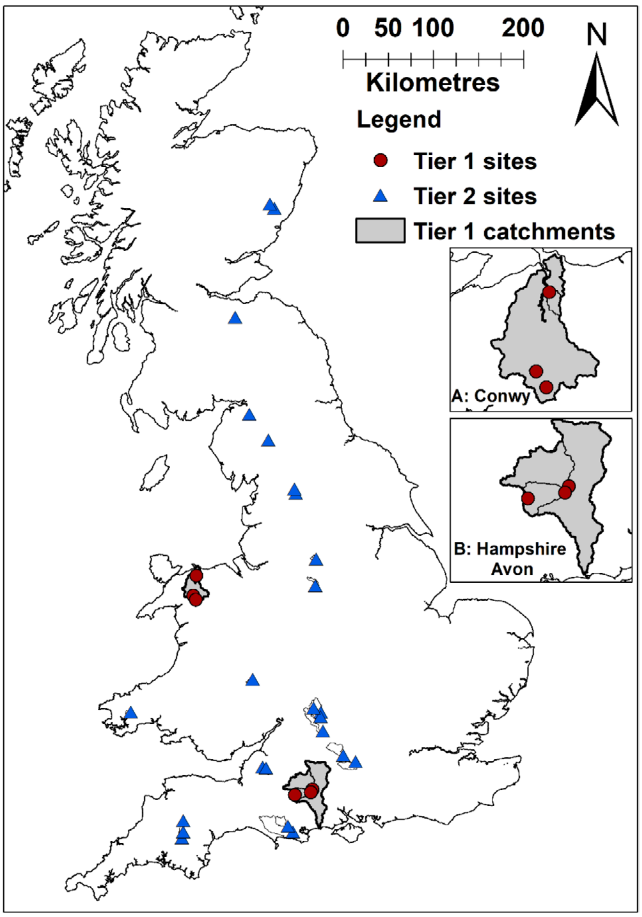


FIGURE 1 | Locations of Tier 1 and Tier 2 sites. Inset A: Tier 1 sites on the Conwy in North Wales and B: Tier 1 sites on the Hampshire Avon in Southern England.

investigated for organic nutrient bioavailability to river phytoplankton reported in this paper.

Tier 1 data on land cover, geology, nutrient concentrations and bioassay responses for three sites within each of two catchments, the upland River Conwy and lowland Hampshire Avon catchment were taken from Mackay et al. (2020). The three sites in each catchment spanned locations from the headwaters to larger tributaries. The soil types across the Tier 1 sites ranged from peat soils and brown earths in the Conwy catchment, Wales, to thin organic mineral soils underlain by chalk, a porous sedimentary rock underlying much of the Hampshire Avon catchment in England. Sampling for water chemistry analysis at these sites took place on at least a fortnightly basis from February to November 2016 and water samples for nutrient bioassays were collected on six occasions during this period (Yates et al. 2019; Mackay et al. 2020).

2.2 | Laboratory Methods for Tier 1 and 2

2.2.1 | Water Chemistry

Full details of the chemical methods used are provided in Yates et al. (2022). Briefly, a Skalar^{++} multi-channel continuous flow autoanalyser (Skalar Analytical B.V., The Netherlands) was used to carry out inorganic nutrient analysis (nitrate, NO_3-N; nitrite, NO_2-N; total ammoniacal N measured as ammonium, NH_4-N; soluble reactive phosphorus measured as orthophosphate, PO_4-P), and analysis of sample digests to determine total and total dissolved N and P (TN or TP, TDN or TDP) following persulphate digestion of unfiltered and filtered (pre-leached 0.45 μm cellulose nitrate) samples respectively (Johnes and Heathwaite 1992; Yates et al. 2016). Dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP), were determined by difference between TDN and DIN or TDP and SRP, respectively. Particulate organic N (PON) and particulate P (PP) fractions were determined by difference between TN and TDN and TP and TDP, respectively.

2.2.2 | Organic Nutrient Bioassay

The bioassay method used was the same for both Tier 1 and Tier 2 sites: a laboratory-based approach aimed to balance environmental control, replication and system realism, following Maberly et al. (2002). The aim was to assess the overall bioavailability of the different organic nutrient compounds by comparing differences in algal growth across treatments. In all cases, raw water samples were kept at 4°C in the dark and transported to the laboratory within 48 h. On arrival they were acclimatised overnight in a constant temperature room at 20°C, representative of temperate summer conditions. Water for the bioassays was initially filtered through a 100 μm mesh to remove zooplankton or large detritus. Following this, 35 mL of sample was transferred to 50 mL boiling tubes for the nutrient additions. Tubes were stoppered with foam bungs enabling gas exchange and incubated for 14 days at 20°C under a photon irradiance of 80–120 $\mu mol\, m^{-2}\, s^{-1}$ (photosynthetically available radiation, Macam Q102) and an 18 h light, 6 h dark cycle, emulating optimal summer growing conditions in this climate zone.

In total, 12 different treatments were applied at approximately Redfield ratio [N: 90 μmol L^{-1} (1.261 mg N L^{-1}) and P: 6 μmol L^{-1} (0.186 mg P L^{-1})] in triplicate. The treatments included a control with no addition of nutrients, three inorganic treatments (N only, P only and N and P), four organic N treatments and four organic P treatments. To avoid false negative responses of nutrient limitation by the alternative nutrient, the organic addition of N or P was combined with the inorganic addition of the other nutrient, resulting in organic N being added alongside inorganic P and organic P being added alongside inorganic N. Details of the treatments and specific compound choices are provided in Table S2. Compound choices were based on our understanding of catchment land use and activities and direct measurement of urea at Tier 1 sites and their likely presence in fresh waters based on previous studies (Reay et al. 2019; Pemberton et al. 2020; McIntyre et al. 2020; Lloyd et al. 2023). Growth was determined as a change in the concentration of chlorophyll *a* in the sample relative to the relevant control. Following the incubations, tube contents including any biofilm were resuspended by scraping and vortex mixing before being filtered onto a Whatman GF/C glass fibre filter (nominal pore size 1.2 μm) and frozen. Filters were later defrosted, and chlorophyll *a* was extracted in hot methanol and measured spectrophotometrically according to Talling (1974). Chlorophyll *a* concentrations were calculated using equations in Ritchie (2008).

2.3 | Data Analyses

2.3.1 | Quantifying Nutrient Limitation

Growth response in the bioassays was assessed for each treatment replicate and then averaged, via a natural log response ratio following Elser et al. (2007):

$$GR_x = \ln\left(\frac{Ct_x}{Cc_x}\right), \quad (1)$$

where: GR is the growth response ratio for nutrient addition *x*, Ct the chlorophyll *a* concentration of the nutrient addition and Cc is the relevant control chlorophyll *a* concentration.

The control treatment selected depended on the type of nutrient addition. For inorganic nutrients, this was the treatment with no nutrients added. For the organic nutrient treatments, the control was the contrasting inorganic nutrient treatment alone. Specifically, for DON + inorganic P, the control was inorganic P alone and for DOP + inorganic N, the control was inorganic N alone.

To determine inorganic nutrient limitation at all sites, we used the critical effect size criteria from Harpole et al. (2011). See Mackay et al. (2020) for more details. Briefly, where the growth response to the inorganic nutrient treatment of a site exceeded that in the control by the critical threshold, it was assigned as being either nitrogen, phosphorus, or co-limited by nitrogen and phosphorus, depending on whether the exceedance was for the single or dual nutrient addition. No overall limitation was assigned where the threshold was not exceeded.

2.3.2 | Upscaling Models to Predict Organic Nutrient Use

We assessed how well local-catchment scale models of organic nutrient growth responses (developed for Tier 1 sites, as reported in Mackay et al. (2020)), predicted growth responses at a national scale for the Tier 2 sites. To achieve this, we adopted two complementary approaches. *Modelling approach 1* took the models developed in Mackay et al. (2020) from Tier 1 sites and applied them to the water chemistry data from the Tier 2 sites, to predict growth responses in those locations. *Modelling approach 2* used only the Tier 2 data from the bioassays and Tier 2 water chemistry data to independently develop new national-scale models to predict organic nutrient use across those sites and identify dominant drivers.

2.3.2.1 | Modelling Approach 1. Using the linear mixed effects (LME) model structure identified by Mackay et al. (2020), which included log TN and log ammonium in the DON growth response model and log SRP and DOC in the DOP growth response model, we made new predictions of phytoplankton growth in response to DON or DOP additions respectively using Tier 2 data. This was done for all compounds tested, combined and for individual compounds. We also compared the DOP model with and without the methylphosphonate treatment, as this treatment exhibited a different response to the other DOP treatments in both this and previous work (Mackay et al. 2020). To evaluate the performance of these models, we used linear models of the predicted vs observed DON or DOP response ratios and calculated r^2, Root Mean Square Error (RMSE) and Nash Sutcliffe efficiency (NSE) statistics to assess the goodness of fit.

2.3.2.2 | Modelling Approach 2. This approach replicated the original model selection procedure carried out by Mackay et al. (2020), using the observed organic nutrient growth responses and water chemistry data from Tier 2 sites. Briefly, this comprised a two-stage statistical modelling approach using LME models with a Gaussian error distribution. Initially, visual inspection of the relationships between water chemistry data and the organic nutrient response ratios was used to assess the need for data transformation. Where non-linear relationships were found, we added a small constant (0.001) to prevent zeros in the original data and then log-transformed the independent variable.

Firstly, we fitted a null model to assess variability in organic nutrient response ratios across sites and treatments. This had only a random intercept term for site and organic nutrient treatment. Secondly, to identify the extent to which water chemistry variables could account for the variation in the organic nutrient growth response, we added these predictors to this model as fixed effects. Owing to the high likelihood of correlation among water chemistry variables, assessed using a correlation matrix, we only included variables in candidate models where the Pearson correlation coefficient between each water chemistry variable was < 0.65. To assess the balance between goodness of fit and model simplicity, the comparison among the null models and these water chemistry candidate models was carried out using Akaike Information Criterion (AIC) values. The model with the lowest AIC value was judged to be the best water chemistry model. Further simplification of this model was then carried out by removing water chemistry variables with the lowest F statistic and using AIC and r^2 to compare the new model with the previous models. The model with the fewest parameters, lowest AIC and highest r^2 was then identified as being optimal. Visual assessment of the model residuals was made to ensure conformity to the underlying model assumptions of independence of observations, normality, and homogeneity of variance. LME analyses were carried out using the lme4 package in R (Bates et al. 2014).

2.3.3 | Predicting Organic Nutrient Growth Response Using Land Cover and Geology

Since routine water chemistry data are not ubiquitously available, particularly in upland environments in the UK (Jarvie et al. 2018) or including the dissolved and particulate N and P fractions (Lloyd et al. 2019; Johnes et al. 2023), we assessed how well proxies for water chemistry in the form of data on land cover and geology, as reported by Yates et al. (2022) would perform in comparison to our water chemistry models in providing predictions of organic nutrient growth response.

Catchment delineation, % land cover and % geology cover were derived from Yates et al. (2022). Classifications for land cover and geology were simplified into six land cover classes (deciduous woodland, coniferous woodland, upland, arable, urban, grassland) and four geology classes (chalk, igneous/metamorphic, sandstone/mudstone/unconsolidated, and limestone). Using % coverage for several land cover and geology classes is potentially problematic for statistical modelling, as they are inherently non-independent. Therefore, before modelling, we carried out separate PCAs of the land cover and geology class data. Scores on the first two PCA axes for both land use and geology were then used as the fixed effects within the LME model selection procedure outlined above to enable comparison with the water chemistry models. All analyses were carried out in R version 3.5.1 (R Development Core Team 2011).

3 | Results

3.1 | Among-Site Gradients in Nutrient Concentration, Nutrient Limitation and Organic Nutrient Growth Responses

Total nitrogen and total phosphorus concentrations across the Tier 2 sites varied over 27-fold for TN (0.35 to 9.57 mg N L^{-1}, average 4.32 mg N L^{-1}) and over 100-fold for TP (0.01 to 1.05 mg P L^{-1}, average 0.23 mg P L^{-1}) (Figure 2). The organic nutrient concentrations across sites were less variable for DON concentration, varying by 4.7-fold (0.32 to 1.51 mg N L^{-1}), but similarly variable for DOP concentration which varied by over 44-fold (~0 to 0.44 mg P L^{-1}). The proportion of the total nutrient concentration made up of organic compounds ranged between 9%–99% for DON and 0%–100% for DOP.

The inorganic nutrient response ratio varied across sites from below 0 in the Upper Chew catchment, where no nutrients were limiting phytoplankton growth, to >4 on the East Dart where the site was co-limited by both N and P (Figure 3a), based on the critical effect size. Across the 24 sites, P limitation occurred at half the sites ($n=12$), eight sites were co-limited by both N and P, three sites were N-limited and one site had no overall limitation. Phosphorus limitation of the sites tended to occur where stream TP concentrations were <0.25 mg L^{-1}, while the occurrence of co-limitation and N limitation lacked a clear pattern across the

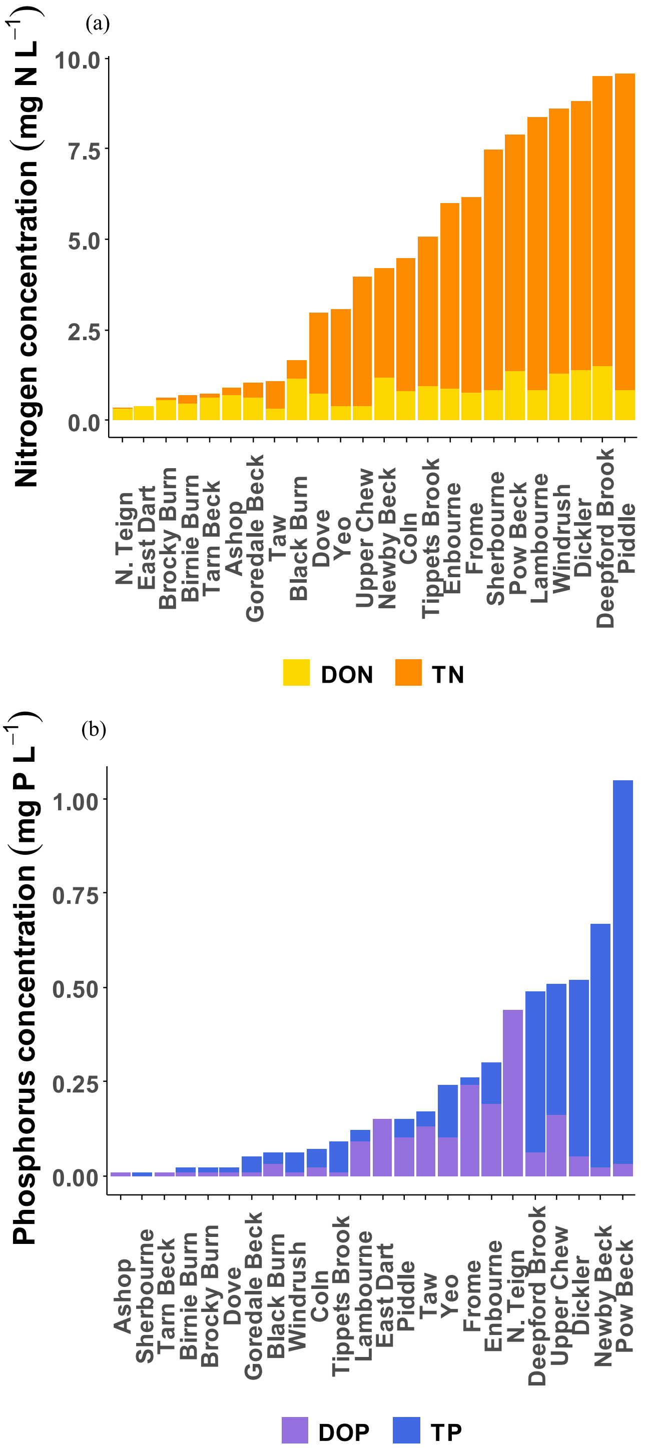
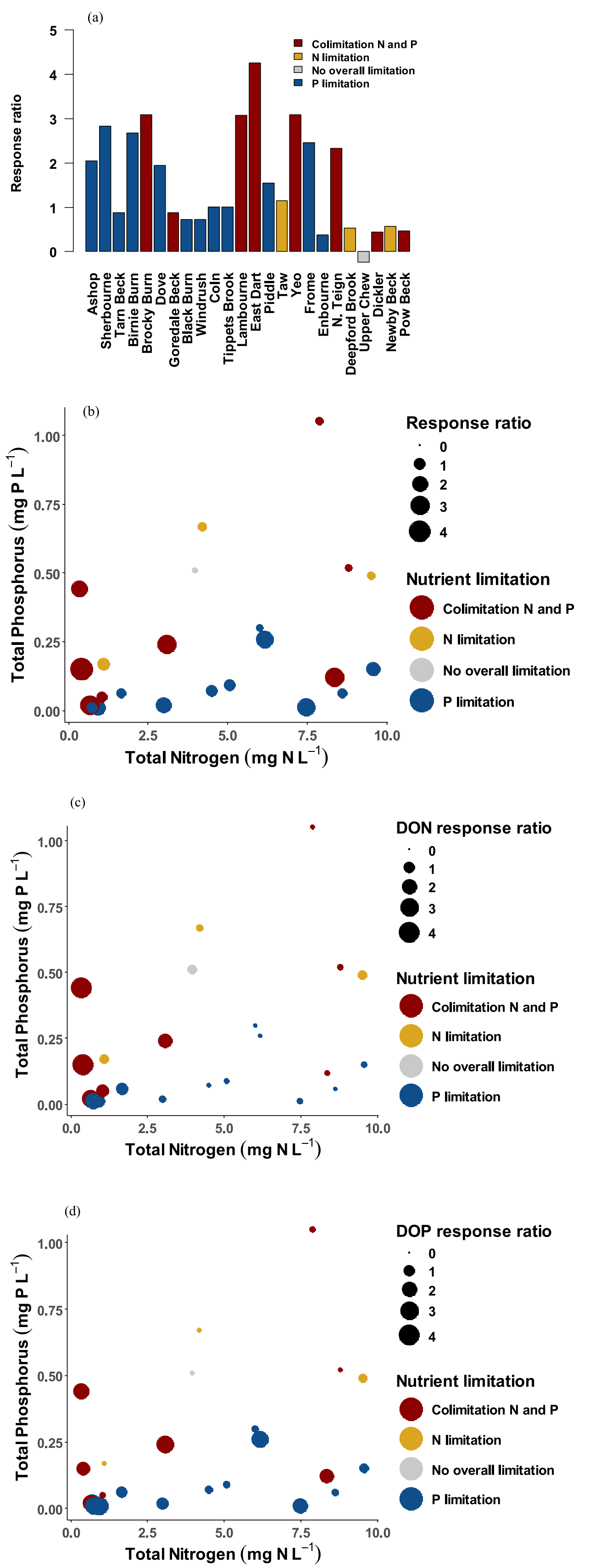


FIGURE 2 | Nutrient concentrations at Tier 2 sites. (a) Total and organic nitrogen concentrations and (b) total and organic phosphorus concentrations. Sites are ordered from lowest to highest total nutrient concentration, which is represented by the total height of the bar.



TN and TP nutrient gradients (Figure 3b). The size of the inorganic response ratio declined for all limitation types as the TP concentration increased (Figure 3c,d).

FIGURE 3 | Nutrient limitation of sites and growth response ratios for inorganic and organic nutrients. (a) Inorganic nutrient response ratio is plotted for sites ordered by increasing TP concentration. Growth response ratios are then plotted for (b) inorganic nutrients and (c) organic N compounds against the site concentration of TN and (d) organic P compounds, against the site concentration of TP.

The magnitude of the organic nutrient growth response ratio also corresponded with the respective total nutrient concentration in the stream water. Average DON response ratios were greatest at sites where the TN concentration was $<3\,\text{mg N L}^{-1}$ and DOP response ratios tended to be higher at sites where TP was $<0.25\,\text{mg P L}^{-1}$ (Figure 3c,d). For DOP, this pattern was clearer for sites that were P-limited. For DON, the nutrient limitation of the site appeared to be less important in determining the growth response to DON compounds, however the largest growth responses occurred at sites that were co-limited. DON growth response ratios at N-limited sites were smaller than at co-limited sites.

Based on null models for growth, which included only random effects of site and organic nutrient treatments, the variability among sites was greater than the variability among the different organic nutrient compounds for DON (site variance = 1.26 vs. compound variance = 0.01) and DOP (site variance = 1.13 vs. compound variance = 0.34).

3.2 | Prediction of UK-Scale Phytoplankton Growth Responses to Organic Nutrients Using a Local Scale Model From Tier 1 Sites (Modelling Approach 1)

The linear model comparison of the DON predicted growth responses to the observed growth responses performed adequately in fitting the Tier 2 observations, although it tended to overpredict growth at high response ratios and underpredict at low response ratios (Table 1, Figure S2a). The NSE of 0.28 implies that the model performed better than simply taking the mean of the observed data, but it deviated substantially from a perfect fit of the observations (NSE = 1.0). The model fit for individual compounds was very similar to that of the combined model (Table 1, Figure S2b–e).

The predicted growth responses for all compounds performed less well for DOP than for DON, despite the slope of the DOP fit being closer to one. The poorer fit was associated with a much larger standard error than for the DON predicted growth responses (Table 1, Figure S3a). The model fit for individual DOP compounds was also more variable than DON compounds, with the model slopes for glucose-6-phosphate and methylumbelliferyl-P being >1, while those for phytic acid and methylphosphonate were <1 (Table 1, Figure S3b–e). The model fit for the methylphosphonate model was substantially poorer than for the other compounds and this compound was also found to be poorly bioavailable in Mackay et al. (2020), so we refitted the combined compound model, excluding those values and the overall fit to the observed data improved, with the model $r^2 = 0.51$ being comparable to the

TABLE 1 | Linear model comparisons of observed growth response to DON and DOP compounds at Tier 2 sites with modelled growth response based on Tier 2 water chemistry and the model derived from Tier 1 catchments (Mackay et al. 2020).

Explanatory variables	Growth response model	Coefficient	Standard error	r^2	RMSE	NSE
log ammonium and log TN	Model for urea	0.57	0.09	0.64	0.95	0.17
	Model for glycine	0.52	0.11	0.47	1.00	0.04
	Model for glutamic acid	0.65	0.12	0.55	1.08	0.41
	Model for acetyl glucosamine	0.64	0.12	0.55	1.00	0.38
	DON model for all compounds	0.60	0.05	0.56	1.00	0.28
log SRP and DOC	Model for glucose-6-phosphate	1.18	0.19	0.61	0.91	0.56
	Model for phytic acid	0.77	0.21	0.36	0.99	0.24
	Model for methylumbelliferyl-P	1.19	0.20	0.60	0.92	0.57
	Model for methylphosphonate	0.19	0.15	0.02	1.48	−3.73
	DOP model for all compounds	0.83	0.12	0.33	1.10	0.30
log SRP and DOC	DOP model excluding methylphosphonate	1.04	0.12	0.51	0.94	0.51

TABLE 2 | Organic nutrient growth response model structure and fit.

Model	Explanatory variable	Coefficient	Standard error	*F* statistic	r^2 full model (fixed effects)
Organic nitrogen growth response ratio (all compounds)	Log TON	−0.37***	0.04	85.87	0.90 (0.82)
	Log Ammonium	0.11 ns	0.07	2.51	
	Log DON	−0.56 ns	0.27	4.16	
Organic phosphorus growth response ratio (all compounds)	Log TP	−0.60**	0.17	12.84	0.82 (0.39)
	Log Ammonium	0.25 ns	0.14	3.07	
	DOP	5.18 ns	2.69	3.71	
	DOP:TP	1.10 ns	0.68	2.62	
Organic phosphorus growth response ratio (excluding methylphosphonate)	Log SRP	−0.37**	0.10	17.28	0.92 (0.51)
	DOP:TP	1.04 ns	0.60	4.54	

Note: Statistics: ns, not significant; **, $p < 0.01$; ***, $p < 0.001$.

DON model, with a lower RMSE = 0.94 and larger NSE = 0.51 (Table 1, Figure S3f).

3.3 | Comparing Water Chemistry to Organic Nutrient Responses at Catchment and UK Scales (Modelling Approach 2)

The Tier 1 catchment models were able to explain just over half of the variability in the Tier 2 UK-scale growth responses to organic nutrient addition. To determine whether the drivers of organic nutrient use at this larger scale were similar to the Tier 1 models, or if additional drivers became important across environmental gradients, the model selection process was carried out with all the water chemistry data from the Tier 2 sites.

The most parsimonious model for DON growth response, based on AIC, included TON, ammonium and DON as explanatory variables and had an r^2 of 0.90 (0.82 for the fixed effects) (Table 2). Growth on DON compounds was significantly negatively associated ($t = -9.28$, df = 23.94, $p < 0.001$) with TON concentration, while the other two terms in the model had positive (ammonium) and negative (DON) coefficients, but they were not significant. The inclusion of ammonium in the final model was very marginal.

The most parsimonious model for DOP growth response where all organic compounds were included, contained TP, ammonium, DOP and DOP:TP, although TP was the only statistically significant term (Table 2). Growth on DOP compounds was negatively related to TP concentrations ($t = -3.58$, df = 23.36, $p < 0.01$). When methylphosphonate growth responses were excluded from the model, the model fit to the remaining data improved, particularly in terms of fixed effects; this model identified SRP with a significant negative coefficient ($t=-3.57$, df = 23.67, $p<0.01$), with only DOP:TP as an additional, but not significant term (Table 2). The overall r^2 of these models (0.82–0.92) was similar to the DON growth response model, but fixed effects explained a much lower proportion of the total variance in the DOP (0.39–0.51) model than the DON model (0.82).

3.4 | Comparing Water Chemistry, Land Cover and Geology With Organic Nutrient Responses at the UK Scale

A principal components analysis showed that the first two principal components for land cover explained nearly three-quarters of the variance among sites. Axis one explained 55.2% of the variance and differentiated among sites with upland (acid grassland, bog, burnt heather, heather, inland rock) and more lowland land covers (arable, urban, deciduous woodland, including broadleaf woodland and scrub) (Figure 4a). Axis two explained 19.1% of the variance and separated coniferous woodland from grassland land cover. The first two geological principal components explained 71% of the variance among sites. Axis one explained 38.9% of the variance and separated sedimentary (sandstone, mudstone) from igneous and metamorphic geologies (Figure 4b). Axis two explained 31.9% of the variance, separating chalk from Carboniferous limestone geologies (Yates et al. 2019). These axes were then used as independent measures of land cover and geology gradients to assess how well these catchment features could explain the observed patterns in organic nutrient use.

The first axes of the PCAs for land cover and geology provided the most parsimonious model to predict DON growth responses based on the AIC value, with an r^2 of 0.90 (0.56 fixed effects) (Table 3). The model coefficient was negative for PCA1 of land cover and positive for PCA1 of geology (although the significance of this variable was marginal). This implies that increased DON growth responses occurred as catchment land cover varied from lowland intensive arable and urban areas to land cover dominated by semi-natural upland habitats underlain by hard, impermeable igneous and metamorphic geologies.

The fit of the land cover and geology-only model was overall much stronger for the DON compounds than for the DOP compounds (considering the variability explained by the fixed effects) (Table 3). The best models based on AIC for growth responses from all DOP compounds and DOP compounds excluding methylphosphonate only included the first PCA land cover axis, with a significant negative coefficient. Although overall model fit was similar to the nutrient-only models, very little of the variance in the model was explained by the land cover axis, suggesting that site-specific and compound-specific differences were more important in this model.

4 | Discussion

Nutrient enrichment is a major pressure on fresh waters worldwide but dissolved organic N and P are widely overlooked nutrient resources for freshwater biota (Johnes et al. 2023). It is vital, therefore, that we advance our understanding of the role of dissolved organic nutrients in impacting freshwater ecosystems. Here, we assessed how well local-scale understanding of phytoplankton responses to such nutrients generalises to new systems, over larger spatial scales and across ecological gradients at national scale in the UK. To do this, we evaluated how well data on in situ nutrient concentrations, and more widely available data on land cover and geology can account for variation in the bioavailability of organic nutrients for river phytoplankton.

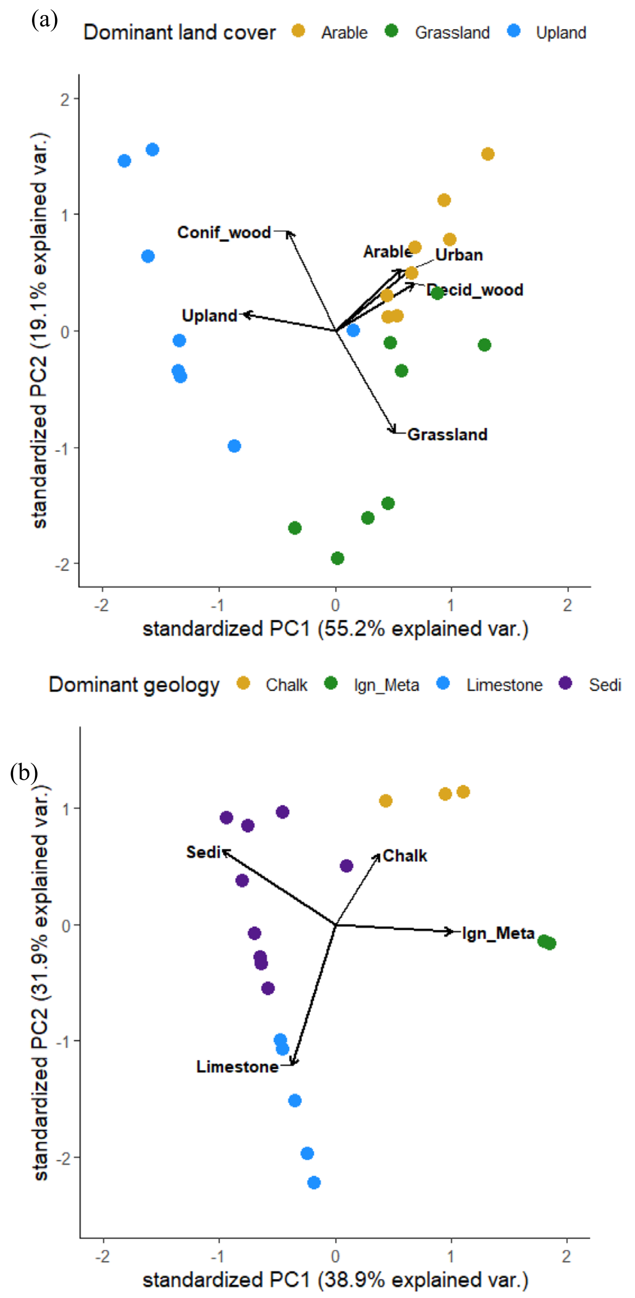


FIGURE 4 | Principal components separating (a) land cover and (b) geology of the Tier 2 sites. Sites are coloured according to the land cover or geology category which has the highest percentage cover in the catchment. Land cover abbreviations: Conif_wood, coniferous woodland; Decid_wood; deciduous woodland. Geology abbreviations: Ign_Meta = igneous and metamorphic; Sedi, sedimentary.

TABLE 3 | Land cover and geology model of organic nutrient growth responses.

Model	Explanatory variable	Coefficient	Standard error	*F* statistic	r^2 full model (fixed effects)
Organic nitrogen growth response ratio (all compounds)	Land cover PC1 scores	−0.37***	0.09	16.02	0.90 (0.56)
	Geology PC1 scores	0.28 ns	0.14	4.14	
Organic phosphorus growth response ratio (all compounds)	Land cover PC1 scores	−0.30**	0.11	7.85	0.83 (0.17)
Organic phosphorus growth response ratio (excluding methylphosphonate)	Land cover PC1 scores	−0.38**	0.12	9.32	0.92 (0.25)

Note: Statistics: ns, not significant; **, $p < 0.01$; ***, $p < 0.001$.

4.1 | Nutrient Concentration, Limitation and Organic Nutrient Growth Responses

The concentrations of TN and TP at the study sites varied by one to two orders of magnitude. The concentrations of the organic nutrient fractions were less variable; however the proportion of the total made up by organic nutrients varied markedly from a negligible level to being the major nutrient fraction across sites in upland moorland catchments and where the dominant agricultural land use was livestock production. This shift in the composition of nitrogen is seen across the globe in response to changing anthropogenic pressures on freshwater ecosystems (Durand et al. 2011; Wymore et al. 2021). Despite some relatively high total nutrient concentrations in the rivers, approximately half of the sites included in this study were P-limited, while most of the remainder were either co-limited by both N and P, or N-limited. The same pattern of major P limitation followed by co- and then N limitation across UK sites was also seen in the national scale study of Jarvie et al. (2018), who used nutrient ratios and absolute nutrient concentrations to assess the likelihood of N and P limitation in upland and lowland rivers. Interestingly, our bioassays identified nutrient limitation at sites (28% for P limitation, 12% for N limitation) where the nutrient concentrations were in excess of thresholds defined in Jarvie et al. (2018) as either relative (0.05 mg P L^{-1} and 0.4 mg N L^{-1}) or absolute N or P limitation (0.01 mg P L^{-1} and 0.1 mg N L^{-1}). In part, this result may reflect our ex-situ microcosm approach, which will have released the phytoplankton community from light, temperature and flushing controls on growth and loss processes, enabling communities to exhaust available nutrient supplies. However, it also emphasises that growth limitation is not necessarily based on fixed nutrient concentrations of single nutrients over time but is affected by other growth-limiting variables and seasonal changes in limitation factors. The microbial communities present in the water, as investigated by Brailsford, Glanville, Golyshin, Johnes, et al. (2019); Brailsford et al. (2021) will also rapidly adapt to changing conditions to optimise the use of the available resources, and thus identifying growth limitation from snapshot nutrient concentrations, or the application of simple indices such as the Redfield ratio needs to be reappraised, at least in freshwater ecosystems.

Standard nutrient limitation assays and concentration thresholds for inorganic nutrients also do not consider the contribution of organic nutrient forms as bioavailable nutrient resources for freshwater biota. Our organic nutrient assays indicated that growth responses to the addition of DON compounds were largest below TN concentrations of 3 mg N L^{-1}, but that nutrient limitation by N or N and P, judged by inorganic nutrient additions, was not closely associated with the size of the DON growth response. Uptake of DON compounds by freshwater phytoplankton has been found to occur under non-limiting nutrient conditions (Mackay et al. 2020; Meyer et al. 2022), indicating that organic nutrient use does not occur solely in response to scarcity of inorganic nutrients. DOP growth responses appeared to be similarly greatest at lower TP concentrations (< 0.25 mg P L^{-1}) but in contrast to the DON responses, were associated more closely with sites defined as P-limited. Similarly, Thompson and Cotner (2018) also found that bioavailable DOP was drawn down at P-limited aquatic sites across the Upper Midwest United States, while the same principle of P drawdown by the riverine microbial community at P-limited sites was also demonstrated by Brailsford, Glanville, Golyshin, Marshall, et al. (2019); Brailsford et al. (2021) in the UK.

While our work does not provide information on the pathway by which the organic compounds are obtained in these phytoplankton bioassays, since we measure an overall growth response and cannot partition this into direct uptake or indirect use via microbial nutrient recycling, it does illustrate that these compounds can increase the biomass of river phytoplankton, which may have impacts further up the freshwater food web (Kissman et al. 2017). The potential differences seen between the DON and DOP growth response relative to nutrient concentrations and inferred nutrient limitation could relate to variation in mechanisms of acquisition, transport and metabolism (Rofner et al. 2016; Druce et al. 2023) and the associated energetic costs of the processes that organisms use to access the nutrients. Direct uptake of DON including amino acids, lipids and carbohydrates via osmotrophy and other metabolic pathways has been observed, while access to DOP compounds may require upregulation of enzyme activity genes either by the phytoplankton cell (Druce et al. 2025) or the surrounding microbiome (Martinez Soto et al. 2015; Lidbury et al. 2022; Reinl et al. 2022), which may be energetically more costly. However, there is evidence that some DOP compounds may also be taken up directly by bacteria, via different metabolic pathways (Vila-Costa et al. 2013; Rofner et al. 2016), therefore negating energetic differences among the organic nutrients. Organic nitrogen use has also been shown to be enhanced by algal–bacterial interactions in biofilms (Lin et al. 2021) and to be preferentially used by specific groups of phytoplankton (Donald et al. 2011; Glibert et al. 2016; Reinl et al. 2022; Mena-Rivera et al. 2023), which may enhance its more general bioavailability.

4.2 | Water Chemistry Drivers of Organic Nutrient Use Across Scales

Application of the model coefficients from the local-scale Tier 1 nutrient model to the Tier 2 sites across the UK explained around half of the variation in the bioavailability for both DON and DOP compounds. This implies a reasonable level of generalisability between scales of observation and similarity in processes driving organic nutrient use across sites. An improved model fit was found when using the broader range of nutrient concentration data from the Tier 2 sites to predict DON and DOP growth responses at a national scale. While the relatively limited temporal observations used to parameterise the models may not have fully captured seasonal changes in phytoplankton communities or hydrology across sites that could potentially alter organic nutrient uptake, the consistency of the importance of riverine nutrient concentrations among modelling approaches does suggest their role in shaping organic nutrient use by the phytoplankton is important.

The most significant explanatory variables identified in the model selected in each case were similar (Table S3), showing negative relationships with TN or TON for DON growth response, and with soluble reactive phosphorus (SRP) or TP for DOP growth response. Both TN and TON concentrations and TP and SRP concentrations were highly correlated with each other and, as a result, were not included in the same model during the model selection process. This general pattern, confirming the findings of Mackay et al. (2020), shows that the size of the river phytoplankton growth response to organic compounds is linked to ambient nutrient concentrations in the water, and is likely to be higher where inorganic nutrient concentrations are lower. The gradients in river water TN and TP concentrations were similar between the studies. TN concentrations ranged 0.34–8.85 mg N L^{-1} in Mackay et al. (2020) compared to 0.35–9.57 mg N L^{-1} here. TP concentrations ranged 0.005–0.50 mg P L^{-1} in Mackay et al. (2020), compared to 0.01–1.05 mg P L^{-1} here. While the proportion of nitrate within TN from monitoring studies can vary substantially as shown here and from studies in the UK and at a global scale (Yates et al. 2019; Wymore et al. 2021), these concentrations are also comparable to modelled long-term average nutrient concentrations for nitrate (0.61–12.18 mg N L^{-1}) and SRP (0.02–1.65 mg P L^{-1}) in rivers across Great Britain (Tso et al. 2023) and of mean N species concentrations reported for many rivers globally (0.0025–14.0 mg N L^{-1}) Wymore et al. (2021), suggesting that the gradients reported here for UK rivers are representative of conditions reported more widely. Although at a global scale other factors such as temperature and runoff may also influence organic nutrient processing rates (Tiegs et al. 2024).

This analysis shows that DON growth responses tended to be better predicted by nutrient concentration in rivers than the DOP growth responses. Despite relatively good overall model fits, DOP growth response models always contained greater variance within the random effect of site, implying that unmeasured site-specific factors played an important role in explaining phytoplankton growth responses to DOP compounds. This was particularly the case for methylphosphonate which exhibited a very different growth response and much lower bioavailability than the other DOP compounds tested. Methylphosphonate is used by specific groups of microorganisms in freshwater (Rofner et al. 2016), including filamentous cyanobacteria (Zhao et al. 2022) and has been linked to methane cycling in oxic lakes (e.g., Günthel et al. 2020) with uptake highest when SRP concentrations are lowest, in line with the results reported here. Thus, there is ample evidence for methylphosphonate uptake by the microbiota, but controls on its uptake by phytoplankton may differ from those for other DOP compounds, leading to the different responses observed in this study. Variability in bioavailability of DOP compounds has previously demonstrated that simpler compounds (e.g., nucleic acids, ATP) are nearly entirely bioavailable to algae, while more complex compounds (e.g., phytic acid) are much less bioavailable (Li and Brett 2013). An important external control on DOP bioavailability appears to be the stoichiometry of the organic matter present. For example, a relatively large DOC pool compared to DOP, could result in more DOP being drawn down as bacteria switch from C to P limitation (Thompson and Cotner 2018). The role of heterotrophic bacteria in potentially enhancing bioavailability of DOM for phytoplankton has been suggested for freshwater and marine species, where concentrations of DOC and C:N ratios were important indicators of the algal growth response (Garzon-Garcia et al. 2018). However, enhanced competition for P between bacteria and phytoplankton under P scarcity, resulting in P enrichment of heterotrophs (Stenzel et al. 2017), may therefore decouple the direct association between nutrient enrichment status and resulting phytoplankton growth on organic P substrates. Further work is required to understand the interactions and timescales of responses between autotrophs and heterotrophs in this context.

4.3 | Predicting Organic Nutrient Use to Inform Management Across Landscapes

The ability of land cover and geology data to predict organic nutrient use by river phytoplankton differed strongly between DON and DOP compounds. Over half of the variance in DON growth was explained by the fixed effects in a model containing a gradient that represented upland to lowland land cover types, where upland sites had larger growth responses to the same DON or DOP dose compared to lowland sites. This model also included a term for a geology gradient from hard igneous to sedimentary rocks, which is likely to drive, in part, overlying land cover, although the Pearson correlation of the two PCA axes was only moderate ($r^2 = -0.47$, $p < 0.05$). The ability of these simple land cover models to predict DON growth responses in the river phytoplankton may relate to the more diffuse nature of nitrogen sources across many rural catchments (European Environment Agency 2005), the strong association between landscape character and suitability for intensive farming and variations in whether catchments are used for crop or livestock production, leading to relationships between land cover as an index of nutrient enrichment status of water. It may also hinge on the relative, and potentially ubiquitous use of DON by microbial communities including pico-cyanobacteria (e.g., Glibert et al. 2023; Druce et al. 2025). This suggests that, at least in the UK, information on land use and management may provide a useful surrogate for nutrient data when trying to predict where DON-based growth stimulus is likely to occur and whether increases in its input in the environment may pose risks to aquatic ecosystems.

In contrast, the DOP growth response model containing the same land cover gradient in the fixed effects, performed less strongly. The larger residual variance in the random effects part of the DOP growth response model implies that both compound-specific and site-specific differences are important for understanding when DOP is likely to be used by phytoplankton. This may include issues relating to the specific DOP compounds present and their sources within the catchment. The extent of availability to the microbial community has also been linked to nutrient enrichment status: Rofner et al. (2016) report a greater diversity of DOP use in oligotrophic than in eutrophic sites. Brailsford, Glanville, Golyshin, Marshall, et al. (2019) report greater rates of microbial turnover of DOC and DON, but reduced SRP uptake as nutrient enrichment increases, linked to preferential use of DOP over SRP in more enriched sites. Both inorganic and organic P have been closely related to the presence of sewage effluent discharges, but also with other nutrient inputs including from livestock excreta, fertilisers, and sediment (Lloyd et al. 2019). In addition to differences in nutrient sources, alterations in nutrient demand by changes in the wider microbial community along the river continuum (Read et al. 2015; Chen et al. 2018), including competition for P between bacteria and phytoplankton, may also influence the likelihood of DOP being incorporated into algal growth.

This study investigated the generalisability of water chemistry and catchment controls on DON and DOP uptake and growth responses in river phytoplankton communities. We found that there is widespread bioavailability of DON and DOP compounds to river phytoplankton across a range of inorganic nutrient concentrations, including where these concentrations are considered non-limiting to algal growth. The main nutrient drivers of bioavailability were relatively consistent across geographic scales, from catchment to national scale for predicting both DON and DOP river phytoplankton growth responses. Using land cover data, particularly including a gradient from lowland (arable, grassland, forest) to upland land covers provides good prediction of DON growth by river phytoplankton, while performance for DOP growth was poorer and likely more closely related to site-specific factors. Our work demonstrates that eutrophication risk arises from both inorganic and organic nutrients, and that all nutrient forms or fractions therefore need to be considered when managing and restoring freshwater systems under pressure from nutrient enrichment.

Author Contributions

Conceptualization: E.B.M., P.J.J., S.C.M. Developing methods: E.B.M., S.J.T., C.A.Y., P.J.J., S.C.M. Conducting the research: E.B.M., H.F., S.J.T., N.C., M.M., G.R., C.A.Y. Data analysis: E.B.M., S.J.T. Data interpretation: E.B.M., H.F., S.J.T., P.J.J., S.C.M. Preparation figures and tables: E.B.M. Writing: E.B.M., H.F., S.J.T., N.C., M.M., G.R., C.A.Y., P.J.J., S.C.M.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data are available from the Environmental Informatics Data Centre (EIDC): https://doi.org/10.5285/3ca94e73-57dc-4fcd-afe2-82a4e491970a.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Tier 1 and Tier 2 site names and locations. **Table S2:** Inorganic and organic bioassay treatments applied to Tier 1 and Tier 2 sites. **Table S3:** Comparison of the explanatory water chemistry variables identified during the model selection process for the Tier 1 catchment and Tier 2 GB scale models. Significant terms in the model are indicated with an asterisk. **Figure S1:** Principle components analysis demonstrating the overlap in land cover and geology between sites in Tier 1 and Tier 2. The ellipses represent a normal data ellipse for each group showing the spread and central tendency of the data in each category. **Figure S2:** Growth response ratio predicted vs observed values at Tier 2 sites for the Tier 1 DON model using Tier 2 water chemistry driving data for (a) all compounds combined, (b) urea, (c) glycine, (d) glutamic acid and (e) acetyl glucosamine. **Figure S3:** Growth response ratio predicted versus observed values at Tier 2 sites for the Tier 1 DOP model using Tier 2 water chemistry driving data for (a) all compounds combined, (b) glucose-6-phosphate, (c) phytic acid, (d) methylumbelliferyl-P, (e) methylphosphonate and (f) all compounds excluding methylphosphonate responses.