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Research Letter

Carbon, Nitrogen, and Phosphorus Stoichiometry and Eutrophication in River Thames Tributaries, UK

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Core Ideas

- A novel graphical method for displaying C/N/P stoichiometry is presented.
- Five River Thames tributaries were used to assess the utility of stoichiometric plots.
- <13% total dissolved P limited chlorophyll a to <30 µg L^{-1}.

Abstract: Primary productivity in aquatic systems relies on carbon (C), nitrogen (N), and phosphorus (P) availability, with a reference stoichiometric ratio of 106 C/16 N/1 P, known as the Redfield ratio. This paper presents a methodology to visualize river water C/N/P stoichiometry and examine phytoplankton response. Redfield total dissolved C/N/P concentration ratios (TDC/TDN_R/TDP_R) from five River Thames tributaries were plotted in a ternary diagram, allowing relationships between nutrient stoichiometry, total P concentrations, and chlorophyll a, as a surrogate for phytoplankton biomass, to be explored. Chlorophyll a concentrations above 100 µg L^{-1} were not observed below 14% TDP_R, and concentrations above 30 µg L^{-1} were not observed below 13% TDP_R. This indicates a potentially lower TDP_R limit for highly eutrophic waters. These rivers are C and N rich, and this methodology should be applied to a wider range of rivers to explore C, N and P thresholds across different river typologies.

Eutrophication is a global issue threatening the water security of millions, with aquatic primary productivity responding to increasing concentrations of the major macronutrients: nitrogen (N), phosphorus (P), and carbon (C) (King and Novak, 1974; Rabalais et al., 2001; Jarvie et al., 2015). The Redfield ratio (106 C/16 N/1 P) (Redfield, 1958) is widely used as an optimum for algal growth, although other factors, such as light or temperature, often also influence algal growth (Hecky et al., 1993). Identifying the possible occurrence of nutrient limitation or co-limitation needs to take into account both individual stoichiometric ratios (C/N, C/P, and N/P) and the absolute nutrient concentrations present, as well as how these concentrations and ratios change throughout the ecologically active growing season. Presentation of individual stoichiometric ratios can be difficult to visualize since each element varies independently. Here, we present a ternary graphical system to simultaneously visualize the Redfield C/N/P ratios in aquatic systems. We apply this visualization tool to tributaries of the River Thames, UK, to explore links between nutrient stoichiometry and phytoplankton biomass, a means of assessing algal growth limitation.

Presentation of Concept

Similar to a soil textural triangle, use of a ternary diagram places C/N/P stoichiometric ratios into various "regions" (Fig. 1). A sample with C/N/P at the Redfield ratio would appear at the center of the triangle (33.3% C, 33.3% N, and 33.3% P). Samples that plot in the central ("balanced") region (delineated here by an arbitrary cut-off of >20% of the sum of C, N, and P) have nutrient ratios approaching optimal for algal uptake. Samples that plot in the regions along

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Abbreviations: Chl-a, chlorophyll a; TDC, total dissolved carbon; TDN, total dissolved nitrogen; TDN_R, Redfield transformed total dissolved nitrogen; TDP, total dissolved phosphorus; TDP_R, Redfield transformed total dissolved phosphorus; TP, total P.

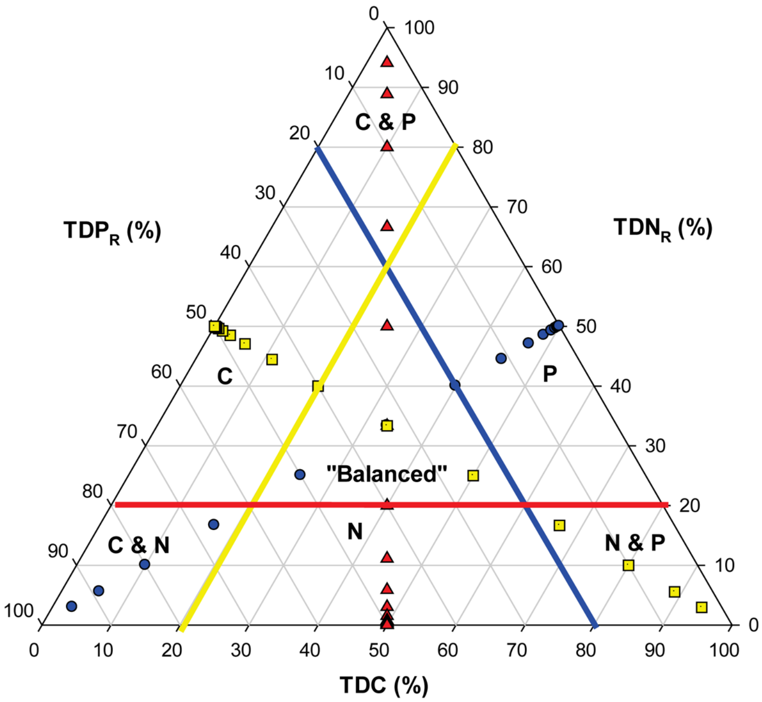

Fig. 1. Conceptual ternary diagram to simultaneously plot the stoichiometric ratios of C/N/P. Blue circles represent a C/N ratio of 6.625 (or 106 C to 16 N) over a range of P concentrations. Red triangles represent a C/P ratio of 106 over a range of N concentrations. Yellow squares represent a N/P ratio of 16 over a range of C concentrations. It is hypothesized that (i) values in the central portion of the graph would be "balanced" relative to each other; (ii) values to the right of the blue line would be P depleted (i.e., <20% TDP$_R$); (iii) values to the left of the yellow line would be C depleted (i.e., <20% TDC); and (iv) values below the red line would be N depleted (i.e., <20% TDN$_R$). The corners would represent codepletion. Values with a "balanced" Redfield ratio (i.e., 106 C/16 N/1 P) would appear at the coordinates 33.3% TDC, 33.3% TDN$_R$, and 33.3% TDP$_R$. TDC, total dissolved C; TDN$_R$, Redfield transformed total dissolved N; TDP$_R$, Redfield transformed total dissolved P.

the edges are "depleted" in one or two nutrients relative to the others, and if concentrations fall below limiting thresholds, this may potentially inhibit algal production. Figure 1 also shows static singular ratios of C/N, C/P, and N/P. The arbitrary value of 20% separating balanced from depleted regions was chosen as a hypothetical cutoff, and this delineation can only be refined using a wide range of river water quality datasets that include C, N and P concentrations, as well as algal biomass.

Procedures

Data from five River Thames tributaries (Rivers Ray, Leach, Cole, Ock, and Pang) were selected. Each river, approximately 30 km in length, had a range of anthropogenic nutrient inputs, with the River Leach being least nutrient impacted and the River Ray most impacted (Table 1), due to sewage effluent inputs. Bowes et al. (2012, 2014) provided full characterization of these tributaries and their watershed characteristics.

Samples were collected weekly and analyzed for dissolved organic C, total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), total P (TP), and chlorophyll a (Chl-a) (Bowes et al., 2012). Dissolved inorganic C was calculated from pH, alkalinity, and water temperature measurements using the THINCARB model (Jarvie et al., 2017). Total dissolved carbon (TDC) was calculated as the sum of dissolved organic C and dissolved inorganic C. Dissolved nutrients were used in this study because analysis protocols did not analyze for particulate N or C and because in these flowing waters the most labile nutrient pools are readily available for algal uptake. Approximately 18 discrete samples from each site were selected from the main algal growth season (1 Apr. 2009 to 1 Aug. 2009) when physical factors such as light and/or temperature are less likely to limit primary productivity. Statistical analysis was performed using ANOVA and Student's *t* test for mean separation in JMP v. 10.

Ternary diagrams were used to plot TDC/TDN/TDP stoichiometric relationships by applying a Redfield normalization to TDN and TDP concentrations through conversion to molar units, then multiplying molar N concentrations by 6.625 and P by 106. Resulting TDC, Redfield transformed TDN (TDN$_R$), and Redfield transformed TDP (TDP$_R$) concentrations were then expressed as relative percentages of their Redfield sum. Stoichiometric ratios do not provide a measure of absolute nutrient concentrations, which determine whether limitation occurs (see Table 1). Therefore, stoichiometric data were categorized on the basis of TP concentrations (Fig. 2A) to provide an additional dimension to the ternary plot. This provides a visualization of how stoichiometry varies based on absolute P concentrations. Stoichiometric data were also categorized by level of primary production, using Chl-a concentrations (Fig. 2b), to show how algal productivity varies with changes in nutrient stoichiometry.

Table 1. Mean stoichiometric constituents and chlorophyll a (Chl-a) concentrations for weekly samples collected from five River Thames tributaries.

River	TDC	TDN	TDP	TP	Chl-a	C/N	C/P	N/P	TDC	TDN$_R$	TDP$_R$
	—— mg L^{-1} ——		—————— µg L^{-1} ——————						————— % —————		
Ray	58.2 ab†	8.67 a	477 a	534 a	36.7 a	8.0 c	322 c	41.2 c	45.9 c	38.7 b	15.4 a
Leach	53.0 c	7.41 b	23 c	28 c	2.7 c	8.5 c	7630 a	941 a	55.3 b	43.7 a	1.0 c
Cole	56.5 b	4.58 d	244 b	287 b	11.4 b	15.0 a	760 c	54.2 c	61.2 a	28.0 d	10.9 b
Ock	59.5 ab	6.89 bc	278 b	304 b	8.2 b	10.2 b	617 c	61.6 c	54.9 b	35.5 c	10.4 b
Pang	60.7 a	6.49 c	52 c	74 c	4.1 c	11.0 b	3820 b	351 b	60.9 a	37.0 bc	2.1 c

† Values within a column followed by the same letter are not significantly different at $P < 0.05$.

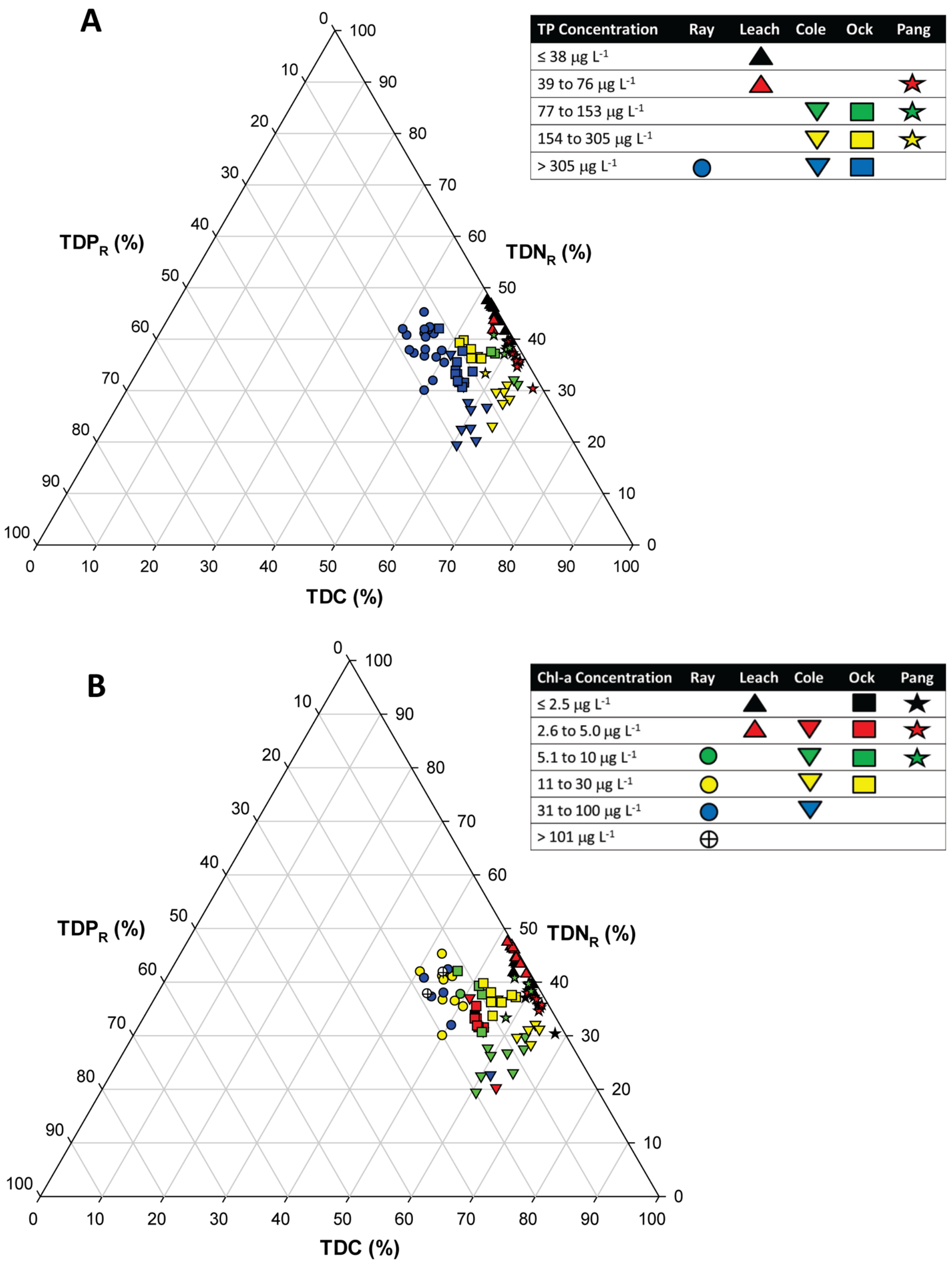

Fig. 2. Ternary plots of total dissolved C (TDC), Redfield transformed total dissolved N (TDN_R), and Redfield transformed total dissolved P (TDP_R) for five River Thames tributaries categorized by (A) the total P (TP) concentration and (B) the chlorophyll a (Chl-a) concentration.

Results and Discussion

River Leach TDC concentrations were the lowest (Table 1). Highest mean TDP and TDN concentrations were observed in River Ray (TDP, 477 µg L^{-1}; TDN, 8.7 mg L^{-1}). Total dissolved P accounted for between 69 and 91% of TP, showing that dissolved fractions are predominant, which is indicative of the dominance of sewage effluent sources of P under spring and summer baseflow conditions (Neal et al., 2010). The TDN_R was greatest for River Leach (44%) and lowest in the Cole (28%), probably reflecting greater input of nitrate-rich groundwater in the Leach, whereas TDP_R was greatest in the Ray (15%) and lowest in the Leach (1%) and Pang (2%). Chlorophyll a was greatest in the Ray and lowest in the Leach and Pang.

Mean N/P ratios for the River Thames tributaries during the 2009 algal growing season (range, 41–941) exceeded the Redfield ratio of 16:1 (Table 1), indicating stoichiometric potential for P depletion. Annual average N/P in British rivers typically range from 5 to 34 (Jarvie et al., 1998; Christmas and Whitton, 1998), less than the current study, which was limited to the growing season. All samples plotted in the hypothetical zone of P depletion relative to N and C on the ternary diagram (Fig. 2A).

Samples showed a clear transition, with increasing TP concentrations from tributaries shifting values left (i.e., greater representation of TDP_R; Fig. 2A). Most samples above 10% TDP_R contained >305 μg TP L^{-1}. All TP values for the Ray were between 305 and 1000 μg L^{-1}, whereas all River Leach samples were <76 μg L^{-1} (Fig. 2A).

Patterns for Chl-a within the ternary plot were less clearly defined (Fig. 2B). However, the Leach, which had the lowest TP and TDP_R, also had the lowest Chl-a concentrations (<5 μg L^{-1}). The only two samples with Chl-a concentrations above 100 μg L^{-1} were in the Ray, which is characterized by the highest TP and TDP_R. Although these two highest Chl-a samples were P depleted relative to N and C (i.e., <20% TDP_R), they both had absolute TDP concentrations above 430 μg L^{-1} and were thus clearly not P limited. Chlorophyll a exceeded 30 μg L^{-1} in six additional samples, with the lowest TDP_R being 13% for those samples. Below 13% TDP_R, there appears to be a rapid decline in primary productivity, as 91% of the samples had <30 μ L^{-1} Chl-a. The 37 samples <5% TDP_R had a mean Chl-a concentration of 4.2 μg L^{-1}.

Many samples with high TP concentrations and TDP_R above 13% did not yield Chl-a concentrations above 30 μg L^{-1}. Factors other than nutrient concentrations (e.g., light, water temperature, flow/residence time) most likely limited phytoplankton accumulation during these periods (Bowes et al., 2016). All physicochemical thresholds must be met in nonlimiting ranges for phytoplankton blooms to exceed 30 μg Chl-a L^{-1}. The observed reductions in phytoplankton accrual in the River Thames tributaries at ~13% TDP_R may indicate a potential threshold for highly eutrophic waters, and further work is now needed to explore whether similar responses occur across a wider range of river typologies. Further, other river typologies are needed to explore C and N depletion and limiting thresholds for primary production. Given the high background TDN and TDC concentrations in River Thames tributaries, we could not explore C and N limitation thresholds here.

In summary, we demonstrated a methodology to graphically represent C/N/P stoichiometry. By classifying samples according to parameter classes, such as absolute TP or Chl-a concentrations, we were able to examine links between nutrient stoichiometry, limiting P concentrations, and phytoplankton accrual. These categories could be widened to explore other physicochemical pressures and drivers that interact with nutrients in controlling primary production in water bodies. This methodology also offers a new tool for examining influences of land use, nutrient sources, hydrology (e.g., flow paths and flow regimes), and seasonality on coupled cycling of C, N, and P in catchments and the ecological status implications of receiving water bodies.

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