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## **Phosphorus enrichment of the oligotrophic River Rede (Northumberland, UK) has no effect on periphyton growth rate**

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### **Abstract**

Reducing phosphorus (P) loading to rivers is seen as a key mitigation measure to improve aquatic ecology and control excessive algal growth, as P is widely assumed to be the limiting nutrient in most rivers. Nutrient enrichment experiments using within-river flume mesocosms were conducted in the oligotrophic River Rede, to determine how periphyton accrual was affected by increasing P concentrations. Increasing the soluble reactive phosphorus (SRP) concentration from the ambient concentration of 15  $\mu\text{g L}^{-1}$  to concentrations ranging from 30  $\mu\text{g L}^{-1}$  to 130  $\mu\text{g L}^{-1}$  had no significant effect of periphyton growth rate, demonstrating that the periphyton was not P limited, even in this nutrient poor river. However, at SRP concentrations greater than 100  $\mu\text{g L}^{-1}$ , diatom communities shifted to species that were more tolerant of higher nutrient concentrations. Elemental analysis showed that there was a positive linear relationship between biofilm P content and the SRP concentration in the overlying water. This ability to store P suggests that periphyton growth is being limited by a secondary factor (such as nitrogen (N)) and may provide a mechanism by which future periodic increases in N concentration may stimulate periphyton growth. Flow velocity, light, and invertebrate grazing pressure also have important roles in controlling periphyton biomass in the River Rede.

## Introduction

The anthropogenic elevation of phosphorus (P) concentrations of rivers is widely believed to reduce ecological status and ecosystem services across the world (Smith et al. 1999), leading to excessive periphyton and macrophyte growth, changes in species composition, low dissolved oxygen concentrations, and even fish kills (Mainstone and Parr 2002, Dodds 2003, Smith 2003, Gold and Sims 2005). Reducing P loading to rivers is seen as a key mitigation measure to improve aquatic ecology (Gold and Sims 2005, Smith and Schindler 2009), as P is widely assumed to be the limiting nutrient in most rivers, thereby constraining primary production. This conviction drives policy at both the national and international level. For instance, the introduction of UK-based schemes, such as the Catchment Sensitive Farming Initiative has attempted to reduce diffuse, agricultural nutrient inputs to rivers, while the European Union's Urban Waste Water Treatment Directive (EEC 1991) has imposed P reduction targets on all large sewage treatment works (STW). STW improvements in particular have resulted in significant reductions in P concentrations, and improved water quality in many rivers across the world over the last decade (Foy 2007, Haggard 2010, Neal et al. 2010a, Bowes et al. 2011). However, there is little evidence that these reductions in river P concentration are delivering a significant improvement in ecological status (Neal et al. 2010b, Bowes et al. 2012)

Even in relatively pristine, low nutrient rivers, the reduction of P inputs remains one of the primary mitigation options to improve ecological status. An example of this is the River Rede, Northumberland, UK. Its water quality is classified as Very Good by the Environment Agency, with dissolved oxygen concentration > 90 %, nitrate concentration < 0.5 mg L<sup>-1</sup> (Baker and Inverarity 2004), and soluble reactive phosphorus (SRP) concentrations < 20 µg L<sup>-1</sup>. The river is classified as being oligotrophic (Dodds et al. 1998) and is of national and

international importance, as it is one of the few remaining sites in the UK where the freshwater pearl mussel (*Margaritifera margaritifera*) can be found.

Large quantities of periphyton biomass (a complex mixture of algae, heterotrophic microbes, cyanobacteria and detritus attached to submerged substrata within aquatic ecosystems) have been identified as the key driver in the ecologically-damaging processes associated with eutrophication (Hilton et al. 2006). A series of flume mesocosm experiments have been conducted in a variety of rivers across the south of England over recent years, to identify the impact of changing P concentration on periphyton growth rates (Bowes et al. 2007, Bowes et al. 2010, Bowes et al. 2012). These experiments have all shown that increases in P had no effect on periphyton growth rate in rivers with SRP concentrations ranging from  $60 \mu\text{g L}^{-1}$  to  $230 \mu\text{g L}^{-1}$ , indicating that P was in excess for primary production. In this study, we aim to apply this flume mesocosm methodology to a river that would be expected to be strongly phosphorus limited: the oligotrophic River Rede ( $10\text{-}15 \mu\text{g L}^{-1}$ , N:P ratio of 50:1). This experiment aimed to identify how periphyton growth rate responds to increasing SRP concentrations, which should lead to identifying the P limiting concentration (i.e. the concentration at which P becomes in excess, and periphyton growth rate no longer increases with increasing SRP concentration). Knowing this P limiting concentration is key information for P concentration target setting and effective catchment management (Bowes et al. 2007). This study will identify if the present P concentrations in the River Rede have an impact on primary production, and thereby establish if the nutrient mitigation strategies presently employed in the catchment are having a beneficial effect on ecological status.

## *Catchment description and study site*

The River Rede is a 58 km long tributary of the North Tyne River, rising within the Cheviot Hills, north-east England and entering the North Tyne at the village of Redesmouth (Figure 1). The upland catchment has a total area of 343.8 km<sup>2</sup>, and is underlain by Carboniferous limestone and sandstone formations, overlain by superficial deposits of boulder clay, alluvium, and peat (Lawrence et al. 2007, Marsh and Hannaford 2008). Mean annual rainfall in the catchment is 1026 mm and the river has a particularly flashy nature with a base flow index of 0.33 and an average discharge of 5.89 m<sup>3</sup> s<sup>-1</sup> with a high flow of 14.1 m<sup>3</sup> s<sup>-1</sup> (Marsh and Hannaford 2008).

Although the area has a low human density (< 1 % of the catchment is classified as urban), the upper reaches of the river are heavily modified due to impoundment by Catcleugh Reservoir (built 1905), which covers 40 km<sup>2</sup> (11 %) of the catchment, maintaining low flows of 0.158 m<sup>3</sup> s<sup>-1</sup> (Petts et al. 1993). The main land uses within the catchment are agricultural grazing (39 %), and coniferous forestry (31 % of the catchment) (Fuller et al. 2002).

The flume mesocosms were installed in the River Rede near the village of Otterburn (grid reference NY 890 926). At this point, the river is *ca.* 8 m wide with a maximum depth (at the time of the experiment) of 0.96 m. Mean flow at Redesmouth (*ca.* 25 km downstream of the study site) for the duration of the experiment was 1.46 m<sup>3</sup> s<sup>-1</sup>, with a maximum and minimum value of 3.66 and 0.79 m<sup>3</sup> s<sup>-1</sup> respectively. Potential small point source nutrient inputs to the river upstream of the study site arise from a minor STW located at Byrness (population estimate (P.E.) of 168), and a water treatment works at Rochester. Diffuse nutrient inputs arise from individual septic tanks, and agricultural and forestry activities. Otterburn STW (P.E. of 550) was 50 m downstream of the study site, and there are 2 further STW

discharging treated final effluent into the lower river at West Woodburn (P.E. of 128) and Redesmouth (P.E. of 45) (Figure 1).

## **Methodology**

### *Flume mesocosm experiments*

Twelve through-flow flume mesocosms were installed along a 40 m straight, unshaded section of the River Rede at Otterburn. Each flume was 5 m long and 0.3 m wide, with adjustable gates at the upstream end to allow the velocity of the incoming river water within each flume to be standardised at  $0.11 \text{ m s}^{-1}$  (Figure 2). The flumes were constructed from PVC sheeting and each set of 3 flumes was supported within an aluminium frame to prevent deformation. Floats attached to the side of each set of 3 flumes allowed them to float at a constant depth of 5 cm in the river. Because the floating flumes were not in contact with the river bed, potential grazing of periphyton by benthic invertebrates was minimised. A sump was located two-thirds of the way down the flume to collect any debris entering the flume, so that it would not disturb the periphyton in the downstream monitoring section of the flumes. Temperature and light levels in the river and flumes were measured hourly using HOBO pendent loggers (Onset Computer Corporation, Massachusetts, USA).

### *Experimental treatments*

A range of nutrient concentrations were simultaneously produced in the 12 flumes by the addition of concentrated nutrient solutions to the incoming river water. To identify the P limiting concentration, 5 flumes received different levels of P additions. This concentration-

effect approach was chosen over treatment replication to accurately identify the concentration at which P became limiting in the River Rede (Guckert 1993, Bowes et al. 2012). Another flume was dosed with iron (II) sulphate solution ( $\text{FeSO}_4$ ), with the aim to reducing the river's SRP concentration, using the P-stripping methodology that has been successfully used in similar previous experiments (Bowes et al. 2007, Bowes et al. 2012). However, this iron-dosing treatment was ineffective at reducing SRP, possibly due to chemical interferences in this highly-organic, peaty river water, and therefore this treatment was stopped and has not been included in this paper. To investigate if the periphyton was limited or co-limited by N, one flume received N addition and one received a combined P+N addition. Nutrients were dripped into the upstream end of each flume from P and N stock solutions on the river bank, using peristaltic pumps. The target nutrient concentrations are given in Table 1. Stock solutions of potassium orthophosphate ( $\text{KH}_2\text{PO}_4$ ) and sodium nitrate ( $\text{NaNO}_3$ ) were made up by dissolving 20 g, 50 g and 100 g of  $\text{KH}_2\text{PO}_4$  and 1000 g of  $\text{NaNO}_3$  in 25 L of deionised water. One flume in each set of 3 received no chemical addition, thereby acting as a control with unaltered river water flowing through it for the duration of the experiment. The choice of nutrient treatment in each flume and position of controls in each set of 3 flumes was randomly assigned.

Once the required nutrient concentrations had been achieved (Table 1), the flumes were thoroughly scrubbed to remove any periphyton that had accumulated during the set-up stage. Unglazed ceramic tiles (approximate area 7 x 7 cm) were placed in the downstream section of each flume on the 24<sup>th</sup> June 2011, to act as artificial substrates for periphyton growth (Figure 2).

## *Sampling and analysis*

For the duration of the experiment, water samples (25 ml) were taken 2 to 3 times per day from the area immediately above the tiles in each flume. These were immediately filtered through sterile 0.45 µm cellulose nitrate membrane filters (WCN grade; Whatman Ltd., Maidstone, UK) and analysed within 20 minutes in the field for SRP concentration using the phosphomolybdenum blue method of Murphy and Riley (1962) and a portable spectrophotometer (model DR2800; Hach Lange, Düsseldorf, Germany). The daily nitrate concentrations of the flumes were determined by colorimetry through the addition of 2, 6-dimethylphenol (Hach 2012). These analyses informed the altering of nutrient drip rates and concentration of stock solutions to maintain stable nutrient concentrations in each flume throughout the experiment.

After 9 days (3<sup>rd</sup> July 2011), significant quantities of periphyton had accrued on the tiles in some of the flumes and sloughing appeared to be imminent. As a result, the experiment was terminated and five tiles were removed from each flume. Four of these tiles were stored at -20 °C for later determination of chlorophyll-*a* (chl-*a*) concentration, ash free dry mass (AFDM) and phosphorus concentration of the biofilm. The remaining tile was scrubbed with a toothbrush and the resulting suspension was preserved in neutralised 40 % formalin solution for analysis of diatom communities (Kelly et al. 1998).

Three tiles were defrosted in the laboratory and periphyton removed from each tile by scrubbing and washing in deionised water. Aliquots of the biofilm suspension were filtered through ashed (500°C; 2 hours), pre-weighed GF/C grade glass microfibre filter papers (Whatman Ltd., Maidstone, UK). The filters were dried overnight at 105 °C to constant mass and reweighed to determine dry mass. The samples were then incinerated in a muffle furnace (model AAF 1100; Carbolite Ltd., Hope, UK) at 500 °C for 2 hours, and then reweighed and

AFDM determined by subtracting ashed mass from dry mass. A second aliquot was filtered and placed in 90 % (v/v) acetone for over-night extraction of chl-*a* in the dark at 4 °C. The light absorption of each sample was measured at 665 and 750 nm using a spectrophotometer. The quantity of chl-*a* per tile ( $\mu\text{g cm}^{-2}$ ) was then back-calculated using the equations of Parsons and Strickland (1963) (APHA. 2005). Chl-*a* and AFDM were normalised for tile surface area and used to calculate the Autotrophic Index (AI).

$$\text{AI} = \text{AFDM (mg m}^{-2}\text{)} / \text{Chl-}a \text{ concentration (mg m}^{-2}\text{)}$$

The phosphorus concentration of the periphyton biofilm on the final day of the experiment was also analysed. Frozen periphyton from one tile in each flume was removed using a scalpel and dried to constant mass at 105 °C. The sample was then ashed at 500 °C for 2 hours and AFDM determined. The resulting ashed biofilm sample was ground to a homogenous powder before triplicate subsamples of approximately  $3 \text{ mg} \pm 0.1 \text{ mg}$  were taken. Samples were diluted to 60 ml using deionised water and autoclaved with acidified potassium persulphate at 121 °C. P concentration was determined using the colorimetric method of Eisenreich et al. (1975).

To analyse diatom communities, 5 – 10 ml of biofilm suspension from each flume was digested using 30 % hydrogen peroxide (Kelly et al. 2001). Permanent slides of cleaned diatom frustules were mounted in Naphrax (refractive index= 1.74) (Brunel Microscopes Ltd., Chippenham, UK) and at least 300 undamaged valves were counted for each sample using a DMLB2 microscope (Leica Microsystems Ltd., Milton Keynes, UK) at 100 x oil immersion under phase contrast. Identification of diatom assemblages was carried out following the diatom key developed by Kelly et al. (2005) for constructing the Trophic Diatom Index (TDI) (Kelly et al. 2001) and species abundance. All taxa were identified to the highest possible resolution, usually species or variety.



### *Longitudinal water quality surveys*

In order to relate the results from the flume experiments to water quality along the River Rede, a longitudinal water quality survey of the River Rede, 2 of its major tributaries and the final sewage effluent from Otterburn STW was conducted under base flow conditions (Figure 1). Samples were collected from the main flow of the river on the 1<sup>st</sup> July 2011. Aliquots of each sample were immediately filtered through a 0.45 µm cellulose nitrate membrane filter and analysed for SRP within 20 minutes of collection using the phosphomolybdenum blue method of Murphy and Riley (1962). All other samples were kept refrigerated and returned to CEH Wallingford's chemistry laboratory for analysis.

Total phosphorus (TP) and total dissolved phosphorus (TDP) concentrations were determined on unfiltered and filtered samples respectively, by acid persulphate digestion in an autoclave at 121 °C followed by a reaction with acid ammonium molybdate reagent (Eisenreich et al. 1975). Dissolved reactive silicon was determined colorimetrically by reaction with acid ammonium molybdate and oxalic acid (Mullin and Riley 1955). Ammonia concentration was also determined by colorimetry using a Seal autoanalyser (AA3; Seal Analytical, Fareham, UK) (Chaney and Marbach 1962). Ion chromatography (Dionex DX500; Thermo Scientific, Sunnyvale, USA) was employed to determine nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ) concentrations (APHA. 2005). Boron concentration (a sewage tracer) (Neal et al. 1998) was determined by inductively coupled plasma optical emissions spectrometry (Optima 2100 DV; Perkin Elmer, Waltham, USA). All analysis was quality control checked against accredited external reference standards (LGC Aquacheck, Lancashire, UK).

## *Data analysis*

Relationships between chl-*a* concentration, AFDM and internal phosphorus concentration of the biofilm and water SRP concentrations were quantified using model II regression, the specific test employed being ranged major axis (RMA) regression (Legendre and Legendre 2012). Model II regression was run within a specific Fortran program (Legendre 2001). As with many other large-scale, in-situ experiments, full treatment replication was not feasible, as it would greatly constrain the number of nutrient treatments that could be investigated, due to the practical constraints of the number of available flumes and the time taken to continually monitor the nutrient concentrations within each one. Previous work has cited treatment diversification to be a reasonable alternative to replication in such cases (McIntire 1993). Also, due to the difficulties in maintaining consistent nutrient concentrations in each flume, the ability to produce true replicates (i.e. a specific P concentration) was not plausible. As N and P+N treatments were not replicated (by a gradient approach), the results from these flumes did not form part of the regression analysis.

## **Results**

### *Flume water chemistry*

The control flumes had average SRP concentrations of between 14 and 17  $\mu\text{g L}^{-1}$  during the 9 day experiment (Figure 3; average concentrations in Table 2). The average nitrate-N concentration in the control flumes during the experiment was 0.76  $\text{mg L}^{-1}$ . This was increased at the start of the experiment to 1.30 and 1.37  $\text{mg L}^{-1}$  in 2 of the flumes so that N concentrations were increased by approximately 80 % (Table 2). In addition, the flume that had its N concentration increased to 1.30  $\text{mg L}^{-1}$  simultaneously had its P concentration

increased to  $134 \mu\text{g L}^{-1}$ . P was added to five flumes successfully producing a continuum of SRP concentrations ranging from 30 to  $130 \mu\text{g L}^{-1}$  (Figure 3). The resulting N: P ratios ranged from 45 to 54: 1 in the control flumes to 6: 1 in the flume receiving the largest P addition. The flume receiving only N addition had an N: P ratio of 91: 1 while the flume receiving a combination of P+N had a ratio of 10: 1 (Table 2).

### *Periphyton accrual and P storage in response to P enrichment*

Statistical analysis (RMA regression), of the quantities of periphyton accrued on the tiles on day 9 of the experiment showed that up to a 9-fold increase in river SRP concentration ( $15 \mu\text{g L}^{-1}$  to  $130 \mu\text{g L}^{-1}$ ) had no significant effect on chl-*a* concentration or AFDM (chl-*a*:  $p = 0.09$ , statistic = 0.43; AFDM:  $p = 0.12$ , statistic = 0.55) (Figure 4 and 5). There was, however, a significant linear relationship between periphyton P concentration and the SRP concentration in each flume ( $p = 0.001$ , statistic = 0.79) (Figure 6). At ambient SRP concentrations, stored P concentration within the periphyton biofilm was  $2.90 \mu\text{g.mg AFDM}^{-1}$ . A 9-fold increase in SRP concentration to a mean of  $130 \mu\text{g L}^{-1}$  resulted in periphyton phosphorus concentration increasing 3-fold to  $8.65 \mu\text{g.mg AFDM}^{-1}$ .

An 80 % increase in ambient N concentration gave a 48 % increase in periphyton biomass accrual, compared to the relevant control treatment (Figure 4 and 5), indicating some degree of N limitation. Adding both P ( $134 \mu\text{g L}^{-1}$ ) and N ( $1.30 \text{mg L}^{-1}$ ) simultaneously resulted in a 3.5-fold increase in chl-*a* concentration (Figure 4) and a 62 % increase in AFDM (Figure 5). The biofilm grown in the N addition treatment had a mean periphyton P concentration that was 15 % less than the mean of the control treatments (N treatment –  $2.42 \mu\text{g.mg AFDM}^{-1}$ , S.E. = 0.07; control treatment –  $2.84 \mu\text{g.mg AFDM}^{-1}$ , S.E. = 0.06) despite having similar SRP concentrations for the duration of the experiment. The mean periphyton P concentration of

the biofilm in the flume receiving P+N addition was also slightly lower than when P was added alone at a similar concentration ( $7.30 \mu\text{g}\cdot\text{mg AFDM}^{-1}$ , S.E. = 0.15 for the PN addition flume, compared to  $8.65 \mu\text{g}\cdot\text{mg AFDM}^{-1}$ , S.E. = 0.53 for the P addition flume) (Figure 6).

### *Periphyton community composition*

On the final day of the experiment, the AI values from the 4 control flumes were between 356 and 410. The addition of P did not affect this with values ranging from 292 to 401. AI values between 100 and 400 are representative of a periphyton community with a balanced population of autotrophic and heterotrophic organisms. AI values of less than 100 indicate communities dominated by autotrophs while values greater than 400 are said to represent communities dominated by heterotrophs (Ameziane et al. 2002). Adding P+N resulted in a much lower AI of 167, suggesting a shift towards a community dominated by autotrophs.

The lowest TDI values (47 to 54) were observed in the control treatments (Figure 7). Nutrient enrichment only affected TDI values when P was in excess of  $100 \mu\text{g L}^{-1}$  (TDI increased to 60), showing that at such high P concentrations there was a growing proportion of more nutrient-tolerant diatom species. Diatoms classed by the TDI as group 5 sensitivity (i.e. tolerant to high nutrient loads) were 23.8 % of the total count at higher SRP concentrations ( $130 \mu\text{g L}^{-1}$ ) and dropped to between 9.6 and 14.6 % at all other nutrient concentrations. One species in particular (*Achnantheidium minutissimum*), known to be sensitive to pollutants, showed a marked decline in abundance with increasing SRP concentrations (from 16 % to 6.5 % of the total count at SRP  $15 \mu\text{g L}^{-1}$  and  $130 \mu\text{g L}^{-1}$  respectively). A further indication of change in species composition was given by the percentage of motile species (Figure 7), whose abundance increased at all phosphorus concentrations above the ambient concentration. An average of 50 species were identified per sample and the most commonly

identified species were *Nitzschia acicularis*, *Achnanthydium minutissimum*, *Fragilaria vaucheriae*, *Nitzschia palea* and *Encyonema minutum*. Assemblage differences were observed between the different treatments, though the dominance of *A. minutissimum* and *F. vaucheriae* throughout all samples is an indication of the overall high ecological status of the River Rede.

### *Stream water chemistry*

The water quality data from the longitudinal survey of the River Rede on 1<sup>st</sup> July 2011 are presented in Table 3 (site locations are shown in Figure 1). There was a general increase in nutrients (P and N) with distance downstream. TP concentration was 6  $\mu\text{g L}^{-1}$  upstream of Catcleugh Reservoir (Site 1), increasing to 22  $\mu\text{g L}^{-1}$  at West Woodburn (Site 7) and Redesmouth (Site 8). A spike in SRP of 30  $\mu\text{g L}^{-1}$  was observed 100 m downstream of the Otterburn STW (Site 5), due to effluent inputs (TP and SRP concentration in the final effluent were 6270  $\mu\text{g L}^{-1}$  and 4000  $\mu\text{g L}^{-1}$  respectively). Such a spike would not be expected to impact significantly on river ecology as river concentrations returned to 13  $\mu\text{g L}^{-1}$  (750 m downstream) owing to rapid sequestration by sediment and biota (Bowes and House 2001, Jarvie et al. 2012). Between Otterburn and the confluence with the North Tyne at Redesmouth, P and N concentrations remain relatively stable with SRP concentration increasing from 13 to 15  $\mu\text{g L}^{-1}$ . The nitrate-N concentration increased from 0.2  $\text{mg L}^{-1}$  upstream of Catcleugh Reservoir (Site 1) to 0.7  $\text{mg L}^{-1}$  at West Woodburn (Site 7). The boron concentration (an indicator of sewage input) of the river also increased downstream from 11.2  $\mu\text{g L}^{-1}$  to 19.4  $\mu\text{g L}^{-1}$ .

## Discussion

Increasing the ambient SRP concentration of the water from  $15 \mu\text{g L}^{-1}$  to  $130 \mu\text{g L}^{-1}$  had no significant effect on periphyton accrual rate. Therefore, the P limiting concentration for the River Rede was at or below the ambient SRP concentration of  $15 \mu\text{g L}^{-1}$ . In comparison to the Redfield ratio of 16: 1 (Redfield 1958), N: P ratios calculated in the control flumes and low phosphorus addition treatments (i.e.  $\text{SRP} < 40 \mu\text{g L}^{-1}$ ) (Table 2) suggest ambient summer P concentrations could limit to periphyton growth. Yet, even in this nutrient poor system, this study has demonstrated that P concentration did not limit periphyton growth, indicating that the N: P ratio is not an effective means of predicting nutrient limitation.

However, P enrichment of the river water did have an effect on the biofilm. There was a shift in the diatom community when SRP concentrations were  $>100 \mu\text{g L}^{-1}$  (Figure 7), and excess P was being sequestered and stored within the periphyton cells through the process of luxury consumption (Figure 6). This P-storing activity suggests that the periphyton was partially limited by P availability (i.e. that the ambient P concentration is at or near the P limiting concentration), but periphyton must also be limited by another factor.

The addition of N led to a small increase in chl-*a* concentration (Figure 4) and AFDM (Figure 5), indicating some limitation by N. The simultaneous addition of P+N resulted in a 3.5-fold increase in periphyton biomass, suggesting that periphyton growth in the River Rede is co-limited (sequential limitation) by P and N. This is the first time that these flume-based nutrient limitation experiments (previously applied to English rivers with SRP concentrations ranging from  $60 \mu\text{g L}^{-1}$  to  $230 \mu\text{g L}^{-1}$ ) have shown a periphyton growth response resulting from any nutrient enrichment. As only one flume was exposed to each of the N and P+N treatments, further work would need to be undertaken to confirm the co-limitation of the system. However, the possible co-limitation of periphyton biomass concurs with recent

studies that indicate that occurrences of P and N co-limitation were significantly greater than limitation by P or N individually (Davidson and Howarth 2007, Elser et al. 2007, Harpole et al. 2011). Co-limitation was also determined to be more common in environments where ambient concentrations of P and N were low (Harpole et al. 2011), as in the case of the River Rede.

If the system is P and N co-limited (Figure 4 and 5) and the biofilm was able to store P (Figure 6), periphyton may be able to increase its accrual rate when N was supplied. This conclusion is further supported by the biofilm P concentrations in the N addition treatment being 15% less than the biofilms in the control flumes (Figure 6), showing that P was utilised, along with the N in the overlying water to produce new biomass.

These observations indicate that individual spikes in P concentration in the River Rede catchment would not immediately result in a benthic algal bloom. However, if this excess P was being stored within the periphyton cells, subsequent spikes in N concentration may be likely to cause enhanced periphyton growth rates. This important observation should be investigated in future replicated experiments, to determine how periphyton responds to intermittent P and N spikes of different concentrations and durations.

Results from the longitudinal survey of the River Rede and some of its major tributaries show that although nutrient concentrations increase along the length of the river, these concentration increases are low and insignificant when compared to the nutrient treatments produced in the flume experiment (Table 2). This suggests that nutrient concentrations are likely to co-limit periphyton growth rate along the entire length of the River Rede.

### *Advantages of using within-river flumes*

The newly developed within-river flume mesocosms used in this study have a number of advantages over traditional methods of studying nutrient – periphyton relationships. They allow multiple nutrient concentrations to be simultaneously studied at a single location, whereas traditional river fertilisation experiments usually only allow one nutrient manipulation. The flume methodology also allows the effect of major nutrient increases to be investigated, without causing any ecological damage to the river, which is unethical / not possible in river fertilisation studies. The flumes allow other factors that affect periphyton growth, such as light, flow, temperature, and invertebrate grazing, to be largely controlled. Because these flumes float at a constant depth, this eliminates the need to readjust the position of the flumes in response to storm events, which is an issue with other flume mesocosm designs (Kjeldsen 1996). The portable nature of these within-stream flumes, with minimal power requirements, allows for deployment at sites of particular scientific or environmental interest, which means that they are much more flexible than streamside flume / artificial stream facilities.

One of the major advantages of using this flume methodology over the more commonly used nutrient diffusing substrata (NDS) approach is that the nutrient concentrations experienced by the flume biofilms can be directly controlled, maintained, and accurately quantified throughout the experiment. NDS and periphytometer experiments are purely qualitative, as the increase in nutrient concentration to the biofilm is unknown (Brown et al. 2001). This nutrient supply will often change throughout the monitoring period (Corkum 1996a), and the results obtained from NDS approaches have been shown to be very unreliable (Capps et al. 2011). These within-river flumes allow gradients in nutrient concentration to be simultaneously produced, allowing researchers to identify threshold concentrations, such as



the P Limiting Concentration, which are vital for effective catchment management, and nutrient target setting.

### *Differences between flumes and river system*

The excessive periphyton growth that was observed in the control flumes after 9 days of the experiment was not representative of that observed in the main river channel, despite the water chemistry (and thus nutrient concentrations) being the same. There are 3 possible reasons for this. Firstly, periphyton biomass in the river channel could be regulated by top-down control due to the influence of grazers (Feminella and Hawkins 1995, Hillebrand 2002) (which were largely excluded from the flume mesocosms). Secondly, the periphyton on the river bed could be limited by light (Corkum 1996b, Hill et al. 2009, Hill et al. 2011). A light / depth profile of the River Rede during the flume experiment showed light intensity to be 20,937 Lx at a depth of 5 cm below the water's surface (the same water depth as the flumes), decreasing rapidly to 4,846 Lx at the river bed (a depth of 85 cm). The rapid attenuation of light levels with river depth is a result of the high coloration of the water (derived from the peaty soils within the upper catchment) and could play a major role in limiting benthic algal growth within the river. Finally, the water velocity within the flume mesocosms was approximately half of the mean velocity measured in the main river channel during base flow conditions. Therefore the influence of scouring of periphyton biomass would be greatly reduced in the flumes.

## *Conclusions*

This study clearly demonstrated that even in a river with some of the lowest nutrient concentrations in England, a sustained 9-fold increase in P concentration had no significant effect on periphyton accrual rates, and P concentration was therefore not the primary limiting factor of periphyton accrual. Similar experiments on a range of English rivers have all shown that an increase in phosphorus concentration has never caused a corresponding increase in periphyton growth rate. This poses serious questions for the current national and international mitigation strategies that are very much focussed on P reduction. There is clearly a need to consider other abiotic variables known to affect periphyton growth, including flow regime, light intensity, food-web interactions and sedimentation.

The present work suggests the need for future management of the River Rede catchment to take a balanced approach to the abatement of both P and N. As this study has shown, P is not limiting algal growth in the river, but elevated concentrations of both P and N resulted in an increase in periphyton biomass. It may be particularly important to control N concentrations downstream of STW, as the peaks in P caused by waste-effluent discharge into the river and the ability of periphyton to store excess P make this part of the river ecosystem particularly vulnerable to increased periphyton growth.

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## Tables and figures

Table 1: Target nutrient concentrations applied during the nutrient manipulation (flume) experiment. Increases are based on ambient soluble reactive phosphorus concentrations of  $15 \mu\text{g L}^{-1}$  and an ambient nitrate-N concentration of  $0.70 \text{ mg L}^{-1}\text{-N}$ .

Table 2: Nutrient treatments applied during the flume experiment. Numbers in brackets are inferred, rather than measured.

Table 3: Water chemistry data from the longitudinal survey conducted on 1<sup>st</sup> July 2011.

Figure 1: Map of the River Rede catchment, Northumberland showing the location of the flume experiment at Otterburn. Numbers denote river sampling sites as part of a longitudinal survey.

Figure 2: A photograph showing two sets of three flumes at the Otterburn site.

Figure 3: Top: soluble reactive phosphorus (SRP) concentrations observed in each flume for the duration of the nutrient manipulation experiment. Dashed lines with no symbols = control flumes which received no addition (i.e. contained unmodified river water). Alternate dashed line = flume receiving N addition only. Solid line with open symbols = flume receiving P+N treatment and solid lines with closed symbols = flumes receiving a range of P additions.

Bottom: Nitrate concentrations observed in the two flumes receiving N treatment and one control flume (no N addition). Alternate dashed line = flume receiving N addition only. Solid line with open symbols = flume receiving P+N treatment and dashed line = control flume.

Figure 4: Chlorophyll-*a* concentrations after 9 days across the entire range of nutrient concentrations. Upper graph shows mean chlorophyll-*a* concentrations based on analysis of 3 tiles  $\pm$  1 standard error. Lower graph shows data points normalised to the mean chlorophyll-*a* concentrations of the control in each set of 3 flumes.

Figure 5: Ash free dry mass values after the 9 day experiment. Upper graph shows mean ash free dry mass based on analysis of 3 tiles  $\pm$  1 standard error. Lower graph shows data points normalised to the mean ash free dry mass of the control in each set of 3 flumes.

Figure 6: Periphyton phosphorus content for tile substrates after the 9 day experiment across the entire range of nutrient concentrations. Data points are mean values based on analysis of 3 tiles from each flume  $\pm$  1 standard error.

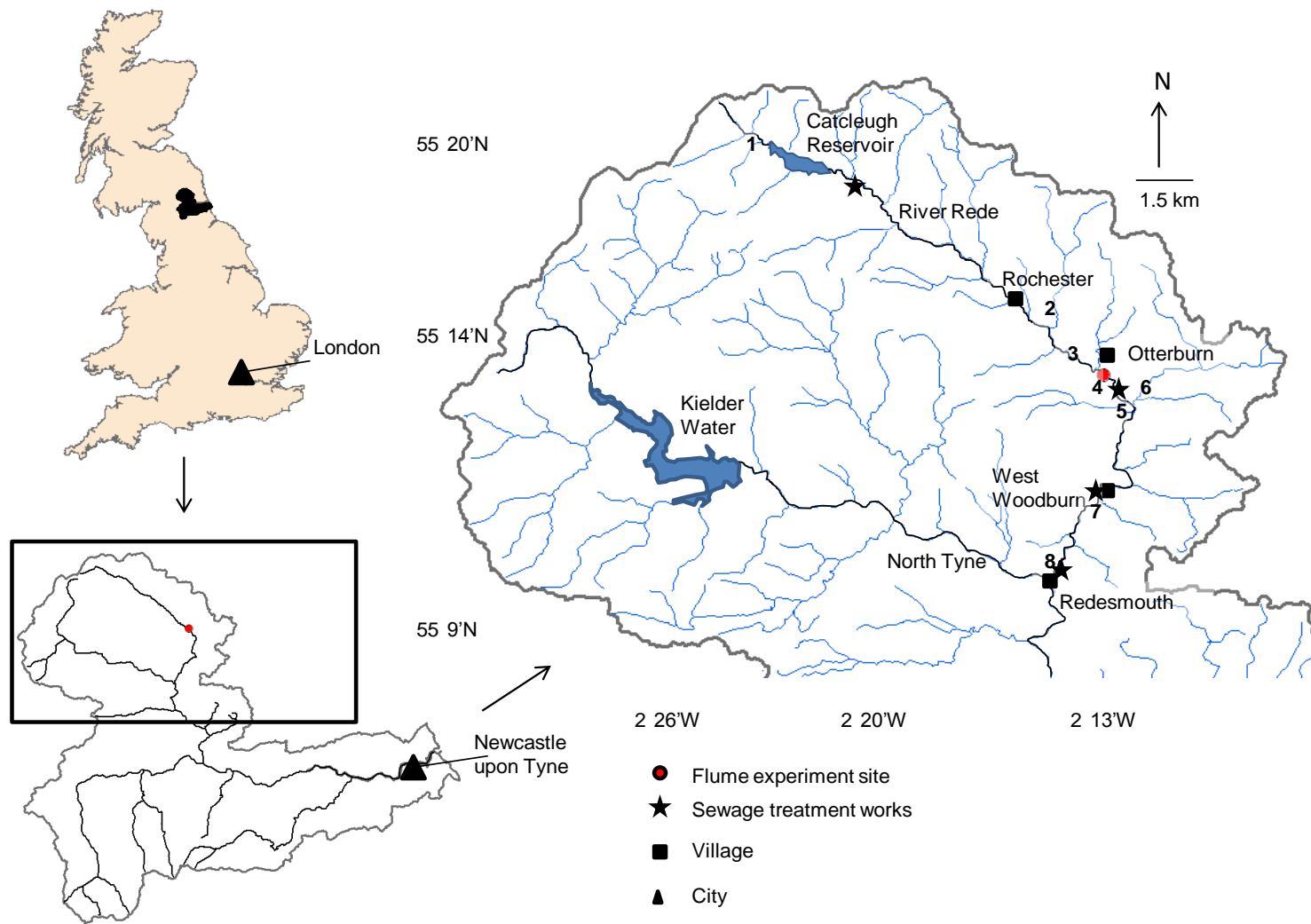
Figure 7: Trophic Diatom Index scores and the percentage motile diatoms present within the biofilm.

Nutrient treatment	Target SRP concentration	Target nitrate concentration
Fe addition	<10 µg l <sup>-1</sup>	-
Control	-	-
Control	-	-
Control	-	-
Control	-	-
N addition	-	2x increase
P addition	2x increase	-
P addition	3x increase	-
P addition	4x increase	-
P addition	6x increase	-
P addition	10x increase	-
P and N addition	10x increase	2x increase

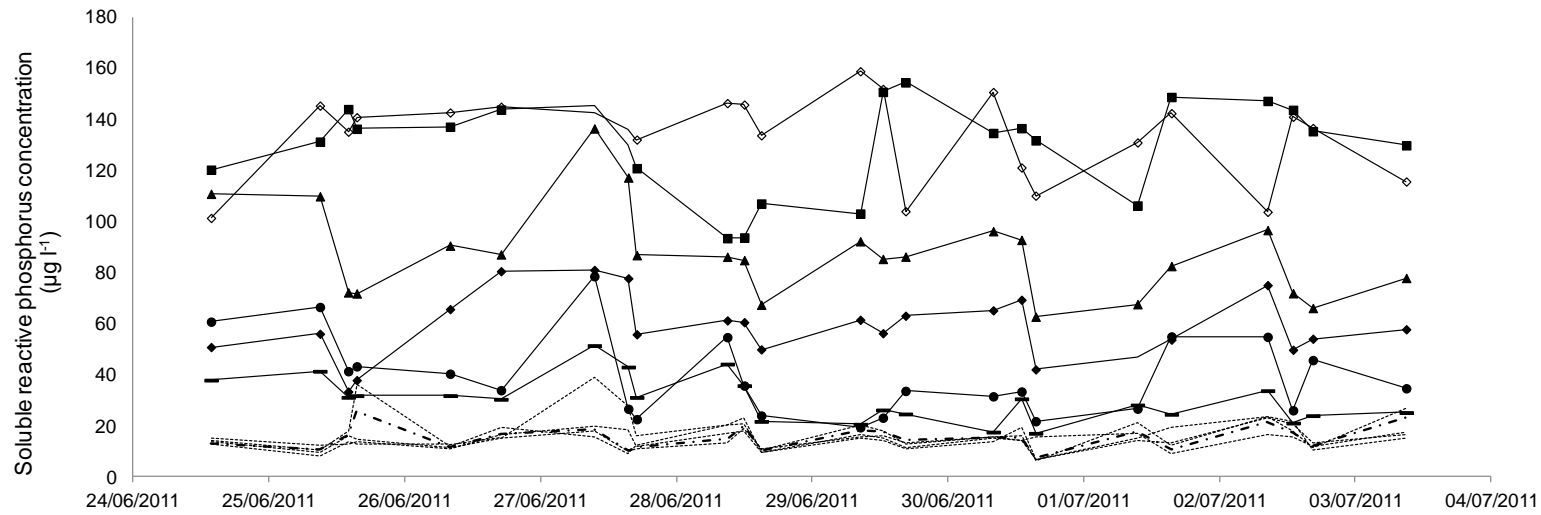


Nutrient treatment	Average nutrient concentration		Percentage increase in nutrient concentration		
	SRP ( $\mu\text{g l}^{-1}$ )	NO <sub>3</sub> - N ( $\text{mg l}^{-1}$ )	P (%)	N (%)	N:P
Control	14	(0.76)	-	-	54:1
Control	15	(0.76)	-	-	51:1
Control	16	(0.76)	-	-	48:1
Control	17	0.76	-	-	45:1
N addition	15	1.37	-	80	91:1
P addition	30	(0.76)	100	-	25:1
P addition	39	(0.76)	160	-	19:1
P addition	58	(0.76)	263	-	13:1
P addition	87	(0.76)	444	-	8:1
P addition	130	(0.76)	829	-	6:1
P and N addition	134	1.30	88	71	10:1

Site	Site location	River	Distance downstream (km)	Soluble reactive P ( $\mu\text{g l}^{-1}$ )	Total dissolved P ( $\mu\text{g l}^{-1}$ )	Total P ( $\mu\text{g l}^{-1}$ )	Ammonium ( $\text{mg l}^{-1}$ )	Nitrate - N ( $\text{mg l}^{-1}$ )	Dissolved reactive silicon ( $\text{mg l}^{-1}$ )	Boron ( $\mu\text{g l}^{-1}$ )
1	Upstream of Catcleugh	Rede	2.6	11	6	6	0.017	0.2	1.8	11.2
2	Rochester	Sills Burn	18.7	19	20	40	0.066	0.1	1.1	24.1
3	Elishaw Bridge	Rede	24.2		9	25	0.000	0.1	0.9	19.4
4	Otterburn	Rede	29.6	13	5	10	0.039	0.4	1.1	17.5
5	Downstream of Otterburn STW	Rede	29.8	30	39	68	0.069	0.7	1.1	17.6
6	Monridge Farm	Elsdon Burn	32.4	7	8	22	0.014	1.2	1.1	22.4
7	West Woodburn	Rede	39.1	6	15	22	0.019	0.7	2.0	18.6
8	Redesmouth	Rede	57.8	15	13	22	0.013	0.5	1.2	19.4
	Otterburn STW	Waste stream	29.7	4000	6270	6840	39	13.6	4.1	57.9







- ◆ P = 58  $\mu\text{g l}^{-1}$
- P = 15  $\mu\text{g l}^{-1}$
- P = 14  $\mu\text{g l}^{-1}$
- - - P = 15  $\mu\text{g l}^{-1}$  N = 1.37  $\text{mg l}^{-1}$
- P = 16  $\mu\text{g l}^{-1}$
- P = 39  $\mu\text{g l}^{-1}$
- P = 130  $\mu\text{g l}^{-1}$
- P = 17  $\mu\text{g l}^{-1}$
- ▲ P = 87  $\mu\text{g l}^{-1}$
- P = 30  $\mu\text{g l}^{-1}$
- ◇ P = 134  $\mu\text{g l}^{-1}$  N = 1.30  $\text{mg l}^{-1}$

