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Impacts of phosphorus concentration and light intensity on river periphyton biomass and community structure

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Abstract

Periphyton growth rate has been identified as the key process that leads to river eutrophication. Effort has focused on reducing phosphorus concentrations to control periphyton biomass, but other factors, such as light, are also important. Within-stream flume mesocosms were deployed in the River Lambourn, UK, to investigate how light intensity and phosphorus concentrations affect periphyton biomass and community structure. Soluble reactive phosphorus (SRP) concentrations were tripled in some flumes, and decreased in others by dosing of $FeCl_3$. Increasing SRP concentrations from the ambient concentration of 49 µg L^{-1} to 155 µg L^{-1} had no effect on biomass, but community composition (by flow cytometry) shifted from diatom to cyanobacterial dominance. Reducing light levels (equivalent to riparian tree shading) decreased biomass by 40 %, showing that the biofilms were light limited at SRP concentration ≥ 49 µg L^{-1}. Periphyton were phosphorus / light co-limited when SRP concentrations were reduced to 33 µg L^{-1}. Further reductions in SRP concentration (23 µg L^{-1}) resulted in phosphorus limitation of periphyton biomass and increased dominance of diatoms and chlorophytes within the biofilm. Reducing light intensity through providing riparian tree shading could be an important management tool to reduce periphyton biomass and improve ecological status.

Keywords

- Eutrophication
- Phosphorus-threshold
- Algal biofilms
- Flow cytometry
- Trophic diatom index
- Multiple stressors

Introduction

The growth rate of periphyton (the complex mixture of algae, cyanobacteria, detritus and heterotrophic organisms attached to substrata) in rivers has been identified as a key process that drives the ecological problems associated with eutrophication, such as macrophyte loss and habitat degradation (Hilton et al. 2006). Periphyton biomass is thought to be controlled by multiple factors including nutrient concentration, light, invertebrate grazing, water temperature and flow velocity (Bernhardt and Likens

2004; Flynn et al. 2002; Francoeur et al. 1999; Lewis and McCutchan 2010), and manipulating these factors is seen as essential to controlling algal blooms and the effects of river eutrophication.

In the UK and much of the developed world, most catchment management resources are usually focussed on reducing nutrient concentrations, and phosphorus (P) concentrations in particular, as P is assumed to be the limiting nutrient for algal growth. European Union directives such as the Urban Wastewaters Treatment Directive (Council of European Communities 1991b), Nitrates Directive (Council of European Communities 1991a; Kelly et al. 2005), and Water Framework Directive (Council of European Communities 2000) have led to wide-scale nutrient concentration reductions across the UK in recent decades (Bowes et al. 2010b; Kinniburgh and Barnett 2010). However, these improvements in water quality have not produced the expected ecological responses, with many river ecosystems failing to achieve the 'good' ecological status required by the Water Framework Directive. Despite major reductions in phosphorus concentrations, many rivers across the UK still experience phytoplankton blooms (Bowes et al. 2012a) and high levels of periphyton biomass (Kelly and Wilson 2004; Neal et al. 2010), implying that phosphorus concentrations are still in excess for periphyton growth.

Numerous studies have examined the effects of increasing phosphorus concentration on periphyton biomass through river nutrient enrichments (Greenwood and Rosemond 2005), nutrient diffusing substrata (Sanderson et al. 2009; Tank and Dodds 2003) and mesocosm experiments (McCall et al. 2014; Stelzer and Lamberti 2001; Wagenhoff et al. 2013). These approaches have increased ecosystem understanding, particularly in low-nutrient environments. However, in countries with high populations and intensive agriculture where nutrient concentrations are greatly in excess of those needed to achieve a good ecological status, experiments with the capability to *reduce* phosphorus concentrations are urgently needed to advance scientific knowledge and inform the impacts of catchment management decisions.

Reductions in light intensity, through riparian shading, have been shown to result in significant reductions in periphyton biomass and community shifts in a number of studies (Bowes et al. 2012b; Halliday et al. 2016; Hill et al. 2009; Johnson and Almlof 2016; Sturt et al. 2011). Specifically, the study of the River Thames (UK) by Bowes et al. (2012b) showed that reducing light intensities to mimic riparian tree shading reduced periphyton biomass by up to 56 % . Furthermore, recent modelling studies on the River Thames basin have shown that in headwater streams, light reduction had a greater potential impact on reducing phytoplankton biomass than phosphorus concentration reduction (Hutchins et al. 2010). Therefore, reduction of light levels by increasing riparian shading offers an alternative means of controlling excessive algal biomass in rivers.

To achieve good ecological status in rivers, and comply with legislative requirements, it is essential to understand how the manipulation of multiple environmental variables affects biomass responses and community structure. The effects of these two key factors; light and nutrients, have been examined previously (Hill and Fanta 2008; Hill et al. 2009; Sturt et al. 2011), although these studies only investigated the impact of increasing nutrient concentrations on periphyton biomass. This present study investigated how periphyton biomass and community composition were affected by the simultaneous manipulation of light intensity and phosphorus concentrations in a relatively low nutrient-impacted tributary of the River Thames; the River Lambourn. Within-river flume mesocosms were used to produce a wide range of SRP concentrations, at two different light levels. The novel aspect of this study was that river phosphorus concentrations were both increased, *and decreased* (using a P-stripping methodology previously developed by Bowes et al (2007). The ability to reduce river soluble reactive phosphorus concentration was used to determine the P concentration threshold at which periphyton biomass became phosphorus-limited. This information is vital for target setting and the effective management of periphyton growth and community structure in the River Lambourn and other similar chalk rivers.

Materials and Methods

Catchment description and site location

The River Lambourn is a second-order chalk stream located in the county of Berkshire, southern England (Figure 1). The source of the river is located 152 m above sea level in Lynch Wood, to the north of the village of Lambourn. The 25 km long river flows in a south easterly direction with a total

catchment area of 234 km^2 (Marsh and Hannaford 2008). The perennial head of the river is located at Great Shefford, approximately 8 km downstream from the source. The River Lambourn is a tributary of the River Kennet which itself is a tributary of the River Thames. The confluence of the River Lambourn and the River Kennet is to the east of the town of Newbury while the River Kennet joins the River Thames at Reading. The high base flow index of the river (0.97) is due to the groundwater dominance of this permeable Chalk catchment. Mean river flow is 1.7 $m^3 s^{-1}$ and mean annual rainfall is 745 mm (Marsh and Hannaford 2008). The land use within the catchment is predominantly rural, with arable and grassland comprising 51.8 and 33.8 % of the land cover respectively, with only 2 % urban or semi-urban development (Bowes et al. 2014).

The experiments for this study were located at the Centre for Ecology & Hydrology's (CEH) River Lambourn Observatory research site (Figure I), just upstream of the village of Boxford. The river is typically 9 m wide and 0.4 m deep at this point (Old et al. 2014). This site has been monitored for river water quality at weekly interval since June 2008 (Bowes et al. 2014).

In-stream flume mesocosms

The in-stream flume mesocosms were constructed from polyvinyl chloride sheeting, supported by an aluminium frame. The flumes were arranged in sets of three (five sets of three flumes were used in this experiment; fifteen flumes in total), with each flume being 5 m long and 0.3 m wide (Figure 2). Adjustable gates at the upstream end of each flume allowed the flow velocity of the incoming river water within each flume to be standardised to 0.15 $m^{-1} s^{-1}$ at the start of the experiment. Cylindrical plastic floats were attached to either side of each set of three flumes to allow them to float at a constant depth of 5 cm in the river. The flumes were secured in place using metal poles that had been pile-driven into the river bed. The flumes were not in contact with the river bed, thereby minimising potential grazing of periphyton by benthic invertebrates. Unglazed ceramic tiles (approximate area of 49 cm^{-2} each) were placed in the downstream end of each flume (termed the periphyton monitoring area; Figure 2) to act as artificial substrata for periphyton growth. A more detailed description of the flume mesocosm design is given elsewhere (McCall et al. 2014). The fifteen flume mesocosms were installed along a 200 m stretch of river at the River Lambourn Observatory in May 2012 (Figure 1). Flumes were located on relatively straight, uniform sections of river that were unshaded by riparian vegetation and exposed to full sunlight throughout the day.

A wide range of phosphorus concentrations were simultaneously produced across the flumes. A concentrated solution of potassium dihydrogen orthophosphate was dripped into the upstream section of three replicate flumes, to aim to triple the phosphorus concentration in the incoming river water from 50 µg SRP L^{-1} to approximately 150 µg SRP L^{-1}. The SRP concentration in the incoming water was reduced in six flumes, using the methodology developed by Bowes et al. (2007). An iron (III) chloride solution was added at two different rates, to produce three replicate flume treatments of approximately 25 and 35 µg SRP L^{-1}. The added iron solution reacted with dissolved phosphate ions present in incoming river water to rapidly form an insoluble, non-bioavailable precipitate ($Fe_3(PO_4)_2$), thereby reducing the SRP concentration in the lower sections of the flumes. One flume received a combined phosphorus (P) and nitrogen (N) treatment. This treatment was included to confirm that there was no P and N co-limitation of periphyton biomass. Co-limitation was not expected due to the extremely high ambient nitrate-N concentration at the study site (average NO_3-N from 2008 to 2012 was 7.77 mg L^{-1}; data provided by CEH River Lambourn Observatory), therefore only one flume was used for this treatment. Chemical additions were controlled via multi-channel peristaltic pumps (205S; Watson-Marlow, Falmouth, UK). Within each set of three flumes, one flume received no chemical addition and therefore had unmodified river water flowing through it for the duration of the experiment, thereby acting as a control (five controls in total). The choice of nutrient treatment within each flume and the position of controls in each set of three flumes were randomly assigned. A summary of the treatments and target phosphorus concentrations in each of the flumes is given in Figure 2.

A shading treatment was also applied to each flume to reduce light intensity and mimic full riparian tree shading (Figure 2). The method used was the same as that of Bowes et al. (2012b). Greenhouse shade cloth was positioned over the periphyton monitoring area so that half the tiles were shaded and half were in full sun (unshaded). The position of the shade cloth (i.e. in the upstream or downstream end of the periphyton monitoring area) was randomly assigned for each flume (Figure 2). Water temperature and light intensity in both the shaded and unshaded section of the flume were recorded

hourly throughout the experiment, using HOBO pendant loggers (Onset Computer Corporation, Massachusetts, USA).

Once nutrient concentrations were within the desired range and the shading treatment had been applied, the flumes were thoroughly scrubbed to remove any periphyton that had colonised during set-up. Clean ceramic tiles were then placed within the periphyton monitoring area at the downstream end of each flume, on the 11th May 2012, and the shading cloths were positioned to shade half the tiles (Figure 2). SRP concentrations in the lower section of each flume were monitored in the field twice per day, using a Hach Lange DR2800 portable spectrophotometer, using the colorimetric method of Murphy and Riley (1962). This allowed the drip rates of the potassium dihydrogen orthophosphate and iron chloride solutions to be adjusted to maintain the desired SRP concentration in each flume. After ten days (21st May), maximum periphyton biomass had accrued on some of the tiles and sloughing appeared to be imminent. At this point, the experiment was terminated for all flumes. Three tiles from both the unshaded and shaded treatments of each flume were removed and placed in a bag and stored at -20 °C for later analysis of chlorophyll *a* concentration and ash free dry mass (AFDM). Biofilms from two further tiles were sampled immediately by scrubbing each with a toothbrush into separate plastic trays. The resulting suspension from one tile was preserved with Lugol's iodine for determination of diatom communities and the suspension from the other was left unpreserved for microbial community characterisation by flow cytometry.

Periphyton biomass characterisation

Tiles were defrosted in the dark and the biofilm removed with a toothbrush into a plastic tray. The resulting suspension was decanted into a 500 ml Duran bottle (Fisher Scientific, Loughborough, UK) and made up to 300 ml using deionised water. The toothbrush, tray and storage bag were thoroughly washed to ensure all the periphyton biofilm had been transferred into the glass bottle..

After homogenisation by vigorous stirring, a 40 ml sub-sample was taken from the biofilm suspension and vacuum filtered through a 0.45 µm glass fibre filter (GF/C grade; Whatman Ltd., Maidstone, UK). Filters were placed into vials and chlorophyll *a* was extracted overnight by the addition of 90 % (volume/volume) acetone/water at 4 °C. The absorbance of each sample was measured spectrophotometrically at wavelengths of 630, 645, 665 and 750 nm and the total quantity of chlorophyll *a* on each tile was then back-calculated (APHA. 2005).

A further 40 ml sub-sample was taken from the homogenised biofilm suspension for analysis of AFDM. The sub-sample was vacuum filtered through an ashed, pre-weighed GF/C filter. The filters were then dried at 105 °C until constant mass was attained, after which they were cooled to room temperature in a desiccator and reweighed to determine the dry mass of each sample. Samples were then incinerated at 500 °C for two hours in a muffle furnace (model AAF1100; Carbolite Ltd., Hope, UK). After cooling in a desiccator, filters were reweighed and the AFDM of each sample was back-calculated (APHA. 2005).

From the chlorophyll *a* concentration and the AFDM, the autotrophic index was calculated. This is the ratio of heterotrophic to autotrophic organisms within each biofilm and was calculated by dividing AFDM by chlorophyll *a* concentration. A higher autotrophic index is representative of a periphyton community dominated by heterotrophic organisms.

Flow cytometry

Flow cytometry is a laser-based analytical technique that can be used to discriminate algal and bacterial cells based on size, shape, cell structure and their constituent photosynthetic pigments (Read et al. 2014). To prevent cell degradation, analyses were run on the flume biofilms within 24 hours of sample collection. The biofilm suspension was homogenised by vortex mixing before 500 µl was passed through a 40 µm cell strainer (BD Biosciences, Oxford, UK) to remove large sediment particles, after which 20 µl of the filtrate was added to 980 µl of ultrapure water. The sample was vortex-mixed again to disaggregate colonial algae, and then analysed using a Gallios flow cytometer (Beckman-Coulter, High Wycombe, UK) equipped with blue (488 nm) and red (638 nm) solid state diode lasers. Samples were run for six minutes and scatter plots were used to delineate and count the major periphyton groups (diatoms, chlorophytes, cryptophytes and cyanobacteria) (Read et al. 2014).

Diatom identification and Trophic Diatom Index

Approximately 5 ml of each preserved biofilm was digested using the hot peroxide method of Kelly et al. (2001). The measured suspension was placed in a beaker with *ca*. 40 ml of hydrogen peroxide. This was then gently heated to 90 ± 5 °C for three hours until all organic matter had been removed. After cooling, a few drops of hydrochloric acid were added to remove carbonates and the samples were centrifuged at a speed of 3000 rpm for 5 minutes. The centrifuging was repeated twice more with the supernatant decanted and deionised water added each time until all traces of hydrogen peroxide had been removed.

Permanent slides of diatom frustules for unshaded treatments in seven of the flumes (covering the range of SRP concentrations, plus the flume receiving combined P and N additions) were mounted using Naphrax (refractive index 1.74) (Brunel Microscopes Ltd., Chippenham, UK). Approximately 300 frustules were counted per slide using a DMB2 microscope (Leica Microsystems Ltd., Milton Keynes, UK) at 1000 x oil immersion under phase contrast. Identification, to species level, took place using the diatom key of Kelly et al. (2005) and allowed the Trophic Diatom Index (TDI) for each nutrient treatment to be calculated (Kelly et al. 2001).

Water quality analysis

A longitudinal water quality survey of six sites along the River Lambourn was conducted at monthly intervals in the 12 months following the experiment, to provide seasonal context for the outcomes of this study, and to allow these outcomes to be applied to the catchment as a whole. Soluble reactive phosphorus was analysed using the molybdenum blue method of Murphy and Riley (1962), while total phosphorus and total dissolved phosphorus were analysed spectrophotometrically following acid persulphate digestion (Eisenreich et al. 1975) on unfiltered and filtered samples respectively. Dissolved reactive silicon concentrations were determined colorimetrically by reaction with acid ammonium molybdate and oxalic acid (Mullin and Riley 1955). Nitrate-N concentrations were measured using ion chromatography (Dionex DX500; Thermo Scientific, California, USA) and total dissolved nitrogen was determined by high temperature catalytic oxidation (Vario TOC select; Elementar, Hanau, Germany).

Data processing and analysis

Each batch of field SRP measurements were run against fresh calibration standards, which were traceable to standard reference materials (LGC Standards, Teddington, UK). All statistical analyses were run on mean data from the three tiles in each flume, using SPSS 22 statistical software. Prior to analysis, the data were investigated for normality and homogeneity of variances. SRP and AFDM data were not normally distributed and were $\log_{10}$ transformed to improve normality and these data were used in all subsequent analyses. A one-way ANOVA was used to compare mean SRP concentrations between P-treatments. Chlorophyll *a* data met the assumptions above, so parametric tests were used to analyse these data.

To confirm that N was not limiting or co-limiting periphyton biomass, a single sample t-test was run to compare the mean Chlorophyll *a* and AFDM from the single flume treated with the combined N and P addition, with the mean data from each of the three replicate flumes where only P was added, for each of the shaded and unshaded light treatments. To investigate the effects of P-treatment and light regime on periphyton biomass (as quantified by mean Chlorophyll *a* and AFDM in each flume), two-way ANOVA tests were run using Type III sums of squares to account for the unbalanced experimental design (Keppel and Wickens 2004). Where significant differences were observed, a post-hoc Tukey test was used to determine which P-treatments were significantly different from each other.

Results

Nutrient treatments

The soluble reactive phosphorus (SRP) concentrations measured in each flume over the course of the ten day experiment are presented in Table 1 and Figure 3. The mean SRP concentration of the individual control flumes ranged from 45 to 55 µg P L^{-1} (with a mean concentration of 49 µg P L^{-1}), showing that there was some small variation in phosphorus concentrations between flumes across the 200 m study reach. Throughout the experiment, the mean SRP concentrations observed in the five control flumes

(equivalent to unaltered river water) declined from 55 µg P L^{-1} on the first day of the experiment to a relatively stable concentration of between 36 and 42 µg L^{-1} over the last five days.

All P-treatments significantly altered the mean SRP concentrations (One-way ANOVA, $F_{3,10} = 514.8$, $p < 0.001$) compared to those in the control flumes and compared to each other (Tukey HSD, $p < 0.001$). The two different rates of iron (III) chloride addition significantly lowered SRP concentrations, varying from 30 to 35 µg P L^{-1} for the low-rate iron addition (mean = 33 µg P L^{-1}) and between 22 and 25 µg SRP L^{-1} (mean = 23 µg L^{-1}) for the high-rate $FeCl_3$ additions, equivalent to reductions of the ambient river concentrations of between 30 and 50 %. The flumes receiving phosphorus additions had a significant increase in SRP, ranging from 147 to 162 µg L^{-1} (mean = 155 µg L^{-1}), which was equivalent to a 3 to 3.5-fold increase of the ambient river concentration. To investigate potential N–limitation or P and N co-limitation, the SRP concentration in Flume 2 was increased to 122 µg P L^{-1} and nitrate-N concentration was increased from 6.9 to 7.9 mg NO_3-N L^{-1}. Results from this single flume treated with P and N confirmed that N did not limit periphyton biomass (as measured by chlorophyll *a* and AFDM) in either the shaded or non-shaded flumes (One-sample t-tests, $p>0.179$) (Figures 4a and 5a). This outcome was expected due to the very high ambient nitrate-N concentration of the river (with an average of 7.8 mg NO_3-N L^{-1}).

Light treatments

Throughout the experiments, the day length was ca. 15 hours, between 06:00 and 21:00 hr. The effects of the shade cloth on light intensities reaching the tile substrata are shown in Supplementary Figure S1. In the unshaded treatment, the mean light intensities during daylight hours were 28,000 Lx, while in the shaded treatment, mean light intensities during daylight hours were reduced by 78 % to 6500 Lx (Supplementary S1). The reduction in light intensity achieved by shading was equivalent to the 79 % average reduction in light intensity under full tree canopy shading, measured at multiple sites along the Boxford study reach prior to the commencement of the experiment. The different light treatments had no significant effect on stream water temperature (two-sample T-test: $T = -1.690$, $p = 0.090$), with mean hourly temperature recorded ranging between 11.1 and 11.3 °C. Temperatures in the flumes and river showed almost identical diurnal fluctuations, varying between 8.5 and 14 °C during the period of the study (Supplementary S2).

Periphyton biomass response

The mean chlorophyll *a* concentrations and AFDM at the end of the ten day experiment are shown in Figures 4 and 5 respectively, plotted against the mean SRP concentrations during the study period. Chlorophyll data are displayed for each individual flume (Figure 4(a), acknowledging that there was variation in SRP concentrations within replicate treatments that produced a gradient in SRP concentrations), and also as mean data from treatment replicates (Figure 4(b)).

The addition of P did not significantly increase chlorophyll *a* concentrations (Tukey HSD, $p = 0.676$), demonstrating that at ambient SRP concentrations (49 µg P L^{-1}) and above, SRP concentrations did not limit periphyton biomass in the River Lambourn, and that sustained phosphorus pollution incidents are not likely to lead to excessive periphyton growth. Reducing SRP concentrations led to significant decreases in chlorophyll *a* compared to the controls (Tukey HSD, $p < 0.001$). A 30 % decrease in SRP concentration (to approximately 33 µg SRP L^{-1}) reduced chlorophyll *a* concentrations by *ca*. 45 and 50 % in the unshaded and shaded treatments, respectively (Figure 4). Further reductions in SRP concentration to 23 µg L^{-1} resulted in further decreases in chlorophyll *a* concentration under both light conditions. When SRP concentration was reduced by 50 %, to ~ 25 µg L^{-1}, chlorophyll *a* concentrations were reduced by 75 and 60 % in the unshaded and shaded treatments, respectively (Figure 4). Results from the two-way ANOVA testing for the effects of both P-treatment and shading on mean chlorophyll *a* concentration confirmed a significant interaction between the two factors ($F_{3,20} = 3.687$, $p < 0.001$), indicating that the effect of shading was different for different P-treatments. Shading had no significant effect on mean chlorophyll *a* concentrations in the flumes where mean SRP concentrations were reduced to below ambient concentrations (23 and 33 µg SRP L^{-1}). However, in control flumes and in flumes where SRP was increased 3.5-fold relative to the control (to 155 µg SRP L^{-1}), shading significantly reduced chlorophyll *a* concentrations by up to 35 % (Figure 4b).

There was a significant correlation between the chlorophyll *a* concentration and AFDM measured in each flume (Spearman's rho = 0.616, $p < 0.001$). However, the AFDM of periphyton had a greater

variation between the flume sets than chlorophyll *a* concentration (Figure 5a) and therefore, the AFDM data were normalised to the values of the unshaded control in each set of three flumes (i.e. the mean AFDM values from the three replicate tiles in each flume were divided by the mean AFDM for the unshaded control flume, in that flume set) (Figure 5(b)). The phosphorus treatments were all significantly different from each other (Two-way ANOVA, $F_{2, 12} = 60.04$, $p < 0.001$), with mean normalised AFDM reduced by 50 % and 73 % in flumes where mean SRP was reduced to 33 and 23 µg P L^{-1}, respectively. As the data were normalised, we were unable to directly compare the responses in the treatments with the unshaded control flume in the two-way ANOVA. Instead, one-sample t-tests were used to determine whether the mean responses in selected treatments were significantly different to 1. There was no significant effect of increasing the SRP concentration to 155 µg L^{-1} (One-sample t-test, $t_2 = -0.266$, $p = 0.815$) (Figure 5b), providing further evidence that the River Lambourn was not nutrient limited in unshaded conditions over the ten day study period.

Shading significantly affected mean normalised AFDM (Two-way ANOVA, $F_{1,12} = 7.660$, $p < 0.001$), and reduced the mean normalised AFDM by 20 % in the flumes where mean SRP was increased to 155 µg L^{-1}. Similarly, normalised AFDM was reduced by 28 % in the shaded controls (One-sample t-test, $t_4 = -9.019$, $p = 0.001$) (Figure 5b). Where mean SRP concentrations were reduced to 33 and 23 µg L^{-1}, shading did not have a significant effect. Therefore, the periphyton biomass, as determined by both chlorophyll *a* and AFDM, became phosphorus-limited when the SRP concentrations were reduced to below the current ambient river concentration of 49 µg P L^{-1}, and became light limited when the SRP concentrations were at or above the ambient concentration observed during the study period.

The biofilms in the shaded flumes generally had a higher autotrophic index compared to the unshaded sections of the same flume, particularly at increased SRP concentrations (Table 1), indicating that shaded periphyton communities contained more heterotrophs relative to autotrophs. Reducing light intensity is likely to have limited autotrophic biomass accrual by reducing photosynthesis, while heterotrophs were relatively unaffected, and so were able to dominate the biofilm community.

Periphyton community response

Flow cytometry

The relative proportions of the phenotypically-distinct groups within the autotrophic periphyton community (as determined by flow cytometry) after ten days are shown in Figure 6. All biofilms consisted predominantly of nano-and pico-chlorophytes and cyanobacteria, accounting for 80 to 90 % of the total autrotrophic cell count. There was relatively little differences in community composition between light treatments, but there were major shifts in periphyton community with changing SRP concentration. The proportion of cryptophytes was greatest at SRP concentrations of 23 µg L^{-1} for both unshaded (5 %) and shaded (8 %) treatments, but declined to a mean of 2 % of the total autotrophic cell abundance in the flumes receiving P additions. Cyanobacteria constituted ca. 50 % of the mean autotrophic cell abundance in the control and P-addition flume biofilms, but only 25 to 28 % in the flumes where SRP concentrations were reduced. In the shaded flumes, there appeared to be a threshold at 30 µg SRP L^{-1}, below which cyanobacterial cells only constituted between 14 to 21 % of the total autotrophic cell counts.

The proportion of diatoms / large chlorophytes did not change in response to changing SRP concentration, with mean proportions from the P-reduction, control and P-addition flumes remaining at between 10 to 14 % under both light treatments. The nano- and pico-chlorophytes showed the greatest compositional change in response to flume SRP concentrations, exhibiting a negative relationship with SRP concentration. Relative abundances of nano- and pico-chloroplasts were highest within the biofilms (54 to 65 % of all autotrophic cells) when SRP concentrations were lowest (~23 µg L^{-1}). In contrast, the proportion of nano- and pico-chloroplasts was as low as 8 % when SRP concentrations were highest (~ 155 µg L^{-1}).

Diatom assemblage and trophic diatom index

As TDI is purely a nutrient based index (Kelly et al. 2001), biofilm samples for diatom identification were only taken from unshaded biofilms. Biofilm samples were taken from seven of the fifteen flumes, covering the full range of SRP concentrations across the treatments. The control flumes had TDI scores of 46 (Flume 3) and 54 (Flume 11) (Table 1), with *ca.* 50 % of the diatom species identified falling

within TDI sensitivity category 1 or 2 (Supplementary Table S1), indicating they favoured very low or low nutrient concentrations (Kelly et al. 2001). These biofilms contained high abundances of *Synedra ulna* (Nitzsch) Ehrenberg and *Fragilaria capucina* Desmaziere (approximately 20 and 14 % of the diatom population respectively). Tripling the SRP concentration resulted in an increase in the TDI to 57 in the two flumes analysed (Table 1) with a corresponding decrease in the proportion of sensitivity category 1 and 2 species from ca. 50 % to *ca.* 28 %. *Cocconeis pediculus* Ehrenberg (11 %) and *Cyclotella meneghiniana* Kützing (9 %) were the more abundant diatom species at higher SRP concentrations (Supplementary Table T1). Both of these species have a sensitivity score of 4, suggesting they favour high concentrations of nutrients (Kelly et al. 2001). The addition of nitrogen in combination with phosphorus (Flume 2) did not lead to further increases in TDI compared to the increases in phosphorus concentration alone (Table 1). This further confirms the results from total periphyton biomass analysis that N was not limiting or co-limiting periphyton communities in the River Lambourn. Decreasing SRP concentration to ~ 23 µg P L^{-1} reduced the TDI to 37 and 39, with the proportion of species tolerant of low and very low nutrient concentrations increasing from 50 to 63 %. As with the control treatment, *Synedra ulna* (27 % in Flume 9 and 24 % in Flume 4) and *Fragilaria capucina* (13 % in Flume 9 and 15 % in Flume 4) dominated the diatom flora.

River water quality

Mean values of water quality parameters from the longitudinal survey for the year following the in-stream flume mesocosm experiment are presented in Supplementary Table T1. The mean SRP concentrations across all six sites (Figure 7) were consistently below the 49 µg SRP L^{-1} threshold identified within this study (ranging from 18 to 38 µg SRP L^{-1}), due to higher than average rainfall diluting point source phosphorus inputs. These low SRP concentrations may well have led to phosphorus limitation of periphyton biomass along the entire length of the River Lambourn throughout the period from June 2012 to April 2013. The nitrate concentration along the entire the River Lambourn was high throughout this period, suggesting that periphyton communities in the River Lambourn were unlikely to be nitrogen limited or nitrogen and phosphorus co-limited. At these relatively low SRP concentrations, the influence of light on controlling periphyton biomass within the River Lambourn would likely have been relatively minor. However, as explained above, this monitoring period was very wet, particularly through the summer of 2012. Under more usual spring – autumn rainfall and flow conditions, SRP concentrations would be higher and light would likely be the dominant control on periphyton biomass.

Discussion

The chlorophyll and AFDM data reveal that periphyton biofilms at ambient nutrient concentrations were not phosphorus limited or phosphorus and nitrogen co-limited. However, when SRP concentration was reduced to *ca.* 33 µg L^{-1}, there were significant decreases in periphyton biomass. This suggests that the phosphorus-limiting threshold of the River Lambourn was at or below 49 µg SRP L^{-1}; the current ambient river concentration of the River Lambourn and indicates that the threshold of *ca.* 100 µg L^{-1} suggested by Bowes et al. (2007) and Bowes et al. (2012b) is not universal to all rivers across the UK. Similar flume studies of the adjacent River Kennet (with an ambient SRP concentration of 55 µg P L^{-1}) also identified that the river was at the phosphorus limiting threshold (Bowes et al. 2010a). At ambient and increased nutrient concentrations, shaded biofilms were primarily light limited, with a 40 % increase in biomass in unshaded treatments, relative to shaded treatments. As SRP concentrations were reduced (to 33 µg L^{-1}; low $FeCl_3$ addition treatment), the effects of light limitation between the unshaded and shaded treatments were less marked, as light limitation became secondary to phosphorus limitation. At this point, periphyton communities in the shaded treatment were likely co-limited by phosphorus concentration and light intensity. As SRP concentration was decreased further to 23 µg P L^{-1}, there was no difference in chlorophyll *a* concentration between the shaded and unshaded flumes. Therefore, at these low SRP concentrations, periphyton biomass was phosphorus limited to the point where light intensity had no effect. This finding is similar to studies by Hill et al. (2009) and Stephens et al. (2012), who also found light to have a great impact in controlling periphyton accrual in low P concentration streams. Whether light intensity or nutrient concentration is more important in controlling periphyton biomass therefore depends on the ambient nutrient concentration and whether or not this is below the nutrient-limiting threshold for a particular river.

There was relatively little differences in autotrophic community composition between light treatments, although shaded biofilms developed a greater proportion of heterotrophs, as revealed by the autotrophic

index data. However, there were major shifts in periphyton community with changing SRP concentration, particularly with cyanobacteria, which constituted 50 % of the total periphyton cell abundances in the control and P-addition treatments. Previous lake research has suggested that cyanobacteria dominate periphyton communities at high phosphorus concentrations due to the ability of certain species to fix atmospheric nitrogen (Schindler 1977). Increasing SRP concentration can result in a reduced N: P ratio and potential nitrogen limitation of periphyton communities. Therefore, cyanobacteria are thought to have a competitive advantage over other autotrophic groups as their ability to fix nitrogen eliminates this resource limitation (Schindler et al. 2008; Vrede et al. 2009). However, our study found that nitrogen did not limit periphyton biomass in the River Lambourn, and suggests that changes in community composition were in response to the high phosphorus concentration (rather than low nitrogen concentration or low N: P ratio). This may indicate that chlorophytes and diatoms are better able to sequester nutrients under ambient conditions, but when the nutrient stress is removed, cyanobacteria may be able to outcompete other autotrophic functional groups and gain a competitive dominance within the biofilm. This study has demonstrated that phosphorus concentrations can play a major role in structuring periphyton communities, as has been shown in other studies. For example, a mesocosm study conducted by Van der Grinten et al. (2004) also showed that under nitrogen-replete conditions, cyanobacteria dominated the periphyton community at higher phosphorus concentrations. The increase in cyanobacteria with increasing SRP concentration could have important implications for water quality and water resources / supply, as well as impacting recreation, ecosystem integrity and human and animal health due to the toxicity of some cyanobacterial blooms (Downing et al. 2001). The flow cytometry data showed that biofilm community composition became dominated by nano- and pico-chlorophytes when SRP concentrations were reduced to 23 µg L^{-1}, accounting for between 54 and 65 % of the total autotrophic cell count. The increased dominance of these small algae within the biofilm as P concentrations were reduced suggests that the community may have become nutrient stressed. The larger surface area to volume ratio of these small autotrophs may have provided a competitive advantage for the cells to take up nutrients when in short supply (Lewis 1976).

The flow cytometry and TDI results both show changes in community structure and species composition when ambient SRP concentration in the River Lambourn was both increased and decreased. However, analysis of total periphyton biomass led to the conclusion that the phosphorus-limiting threshold of the River Lambourn is at or below the 49 µg L^{-1} ambient concentration, in terms of periphyton biomass. This concurs with the work of Bowes et al. (2012b) which found that although the phosphorus-limiting threshold of the River Thames was *ca.* 100 µg L^{-1}, it was only when SRP concentrations were reduced and maintained at concentrations of *ca.* 30 µg L^{-1} that there was a change in diatom community structure and the TDI. This suggests that it is plausible that rivers across Britain have two phosphorus thresholds, one which affects periphyton biomass accrual rate (phosphorus-limiting threshold) and a lower threshold which affects the ecology and periphyton community structure. Further work is necessary to confirm this important observation and to identify the P threshold that would deliver improvements in ecological status (TDI) through a shift in diatom community.

The subsequent monthly water quality monitoring surveys along the length of the River Lambourn showed that SRP concentrations were relatively uniform along the entire river, and during the March to September period (when significant algal growth can potentially occur), they were all lower than the 49 µg SRP L^{-1} concentration observed during the flume study. Therefore the results on this algal limitation study are likely to be applicable to the entire River Lambourn.

Conclusions

This experiment on the River Lambourn has shown that SRP concentration is important in determining whether light intensity or nutrient concentration exert a greater influence on periphyton biomass. At an ambient SRP concentration of ca. 49 µg P L^{-1}, the river appears to be at the phosphorus-limiting threshold. This result provides evidence that despite being recently revised (and lowered) (UKTAG 2013), the UK targets to achieve good water quality status, in terms of mean annual SRP concentration, may still be too high, with the current phosphorus standard to achieve WFD good ecological status for this stretch of the River Lambourn set at an annual average of 76 µg P L^{-1}.

Increased shading did not alter the phosphorus threshold concentration. At ambient phosphorus concentration and above, light exerts a critical influence on periphyton biomass. In the River Lambourn, it is light intensity that controls periphyton biomass at SRP concentrations > 49 µg L^{-1}. As SRP concentrations are reduced to *ca.* 33 µg L^{-1}, light and nutrients appear to co-limit periphyton biomass.

Further reductions in SRP concentration (~ 23 µg L^{-1}) resulted in phosphorus limitation of periphyton growth. As such low SRP concentrations would be difficult to achieve in many lowland rivers across the highly populated south of England, manipulating light intensity through the addition of riparian shading could be a viable alternative catchment management tool to reduce periphyton accrual and meet the requirements of European legislation. Similar conclusions have been reached in field and modelling studies in both the UK (Bowes et al. 2012b; Hutchins et al. 2010) and the USA (Bernhardt and Likens 2004; Greenwood and Rosemond 2005; Mebane et al. 2014; Schiller et al. 2007).

Despite periphyton biomass (determined by chlorophyll *a*) not changing when SRP concentrations were increased above the ambient SRP concentration, community structure shifted, with cyanobacteria dominating the periphyton community at higher SRP concentrations. Alongside this, diatom communities shifted to be dominated by less nutrient-sensitive species at increased SRP concentrations and more nutrient sensitive species at reduced SRP concentrations. This study has shown that despite the River Lambourn being at the phosphorus-limiting threshold for periphyton growth rate, ambient SRP concentrations must be reduced further to improve community structure and ecological status.

List of Figure captions

Table 1: Average soluble reactive phosphorus concentrations measured in each flume at each site over the course of the experiment, with average chlorophyll *a*, ash free dry mass, and autotrophic index of the biofilms after ten days. Trophic diatom index data were calculated for unshaded biofilms in selected flumes covering the full range of SRP concentrations.

Fig. 1. Map of study site, showing location of Thames basin (a), location of River Lambourn within the Thames basin (b) and (c) River Lambourn catchment showing the location of the flume studies (green circle) and water quality monitoring sites (red circles).

Fig. 2. Experimental design, showing treatments and soluble reactive phosphorus concentrations in each flume. Grey areas represent shaded areas of the flume. Black arrow indicate direction of flow of incoming river water. The inset photograph shows a set of 6 flumes deployed within the study reach, facing downstream. The green shading cloth can be seen covering half of the tiles in the downstream sections of each flume

Fig. 3. Soluble reactive phosphorus (SRP) concentrations monitored in each flume during the course of the experiment. Average SRP concentrations (in µg L^{-1}) for each flume presented in the legend.

Fig. 4. Relationship between soluble reactive phosphorus (SRP) concentration and chlorophyll *a* concentration at the end of the 10 day flume experiment at both light levels. (a) Data points are mean chlorophyll *a* values based on analysis of three tiles from each individual flume ± one standard error. Filled black symbols = shaded tiles, unfilled symbols = unshaded tiles. (b) Boxplots of the mean chlorophyll concentrations of the four replicate treatments; (P-addition, control, and two levels of P-reduction). The Y axis is the same for both graphs, to show reduction in biomass due to shading.

Fig. 5 Relationship between soluble reactive phosphorus (SRP) concentration and ash free dry mass after 10 day experiment (a) and (b) after data normalised to the mean AFDM observed in the control flume of each set of three flumes, at both light levels. Data points are mean AFDM values based on analysis of three tiles from each flume ± one standard error. Filled black symbols = shaded tiles, unfilled symbols = unshaded tiles.

Fig. 6 Changes in periphyton biofilm community composition based on cell abundances, at different nutrient concentrations at the end of the 10 day experiment in (a) unshaded and (b) shaded conditions

Fig. 7. Soluble reactive phosphorus concentrations at multiple points along the River Lambourn 2012-2013. Locations of sampling points are shown in Figure 1.

Supplementary data

S1. Light intensities monitored within flumes in full light and under shading cloths, throughout the 10 day experiment. Light loggers were submerged at a depth of 4 cm.

S2. Water temperatures in shaded and unshaded regions of two flumes, and River Lambourn river water during the course of the 10 day experiment.

Table S1. Diatom species list with TDI scores.

Table S2. Average nutrient concentrations from longitudinal surveys along the River Lambourn, conducted at monthly intervals between May 2012 and April 2013.

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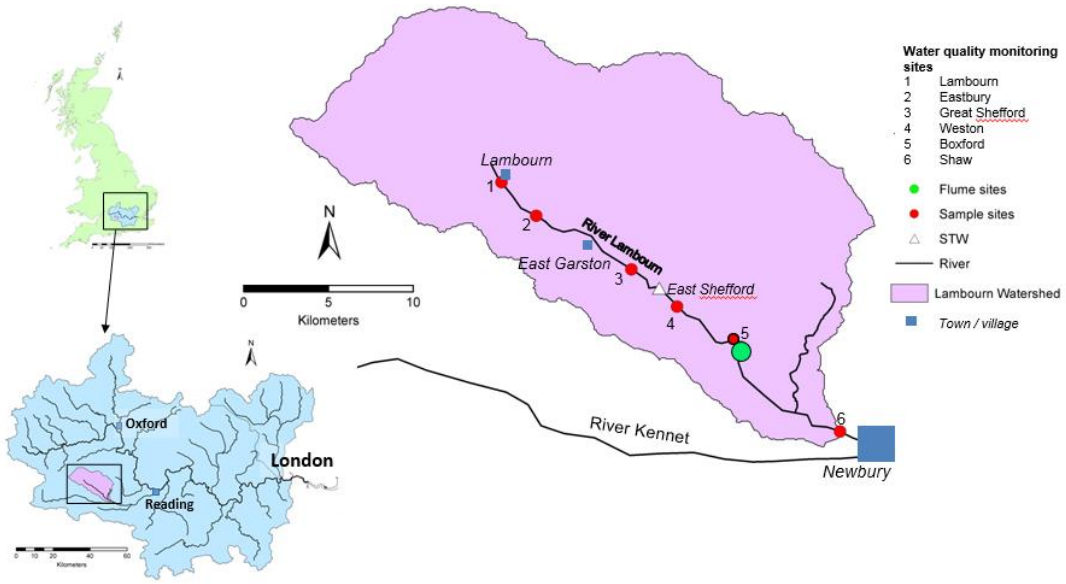

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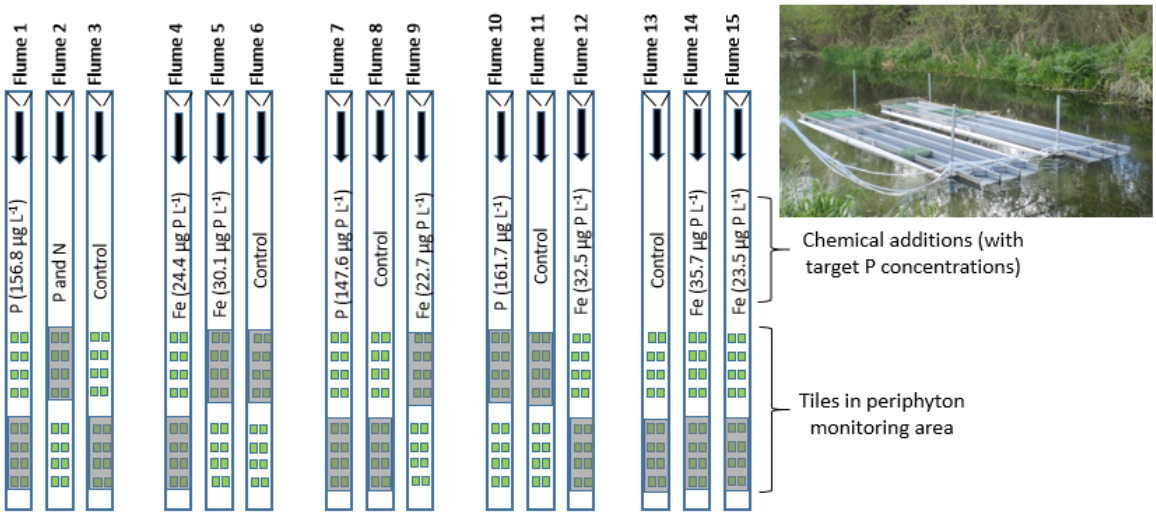

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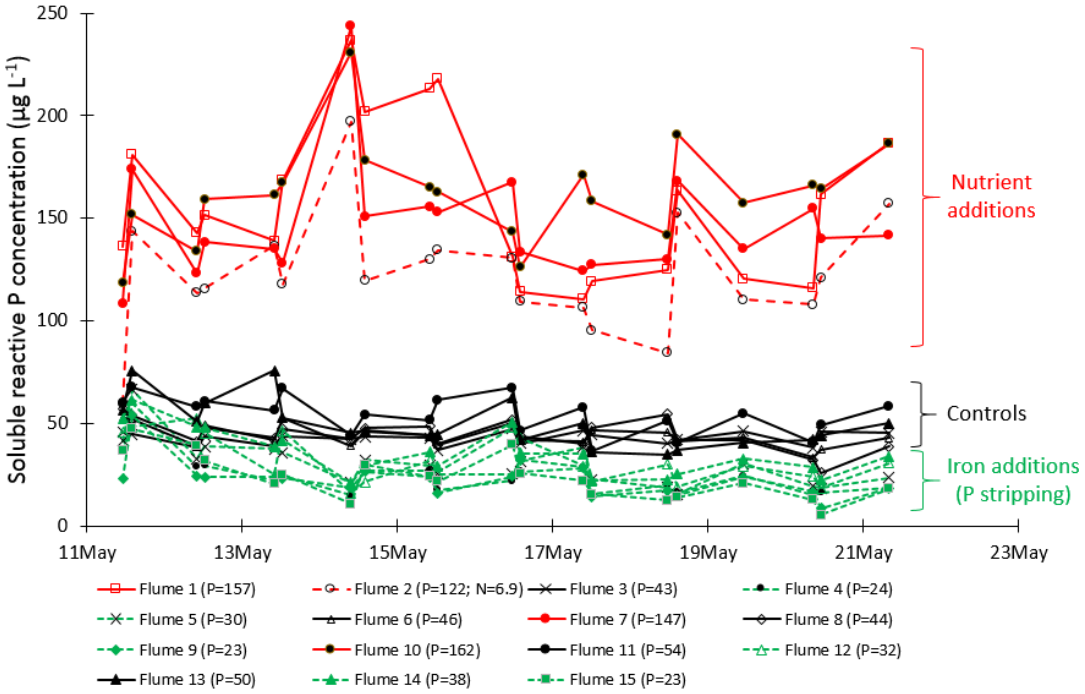

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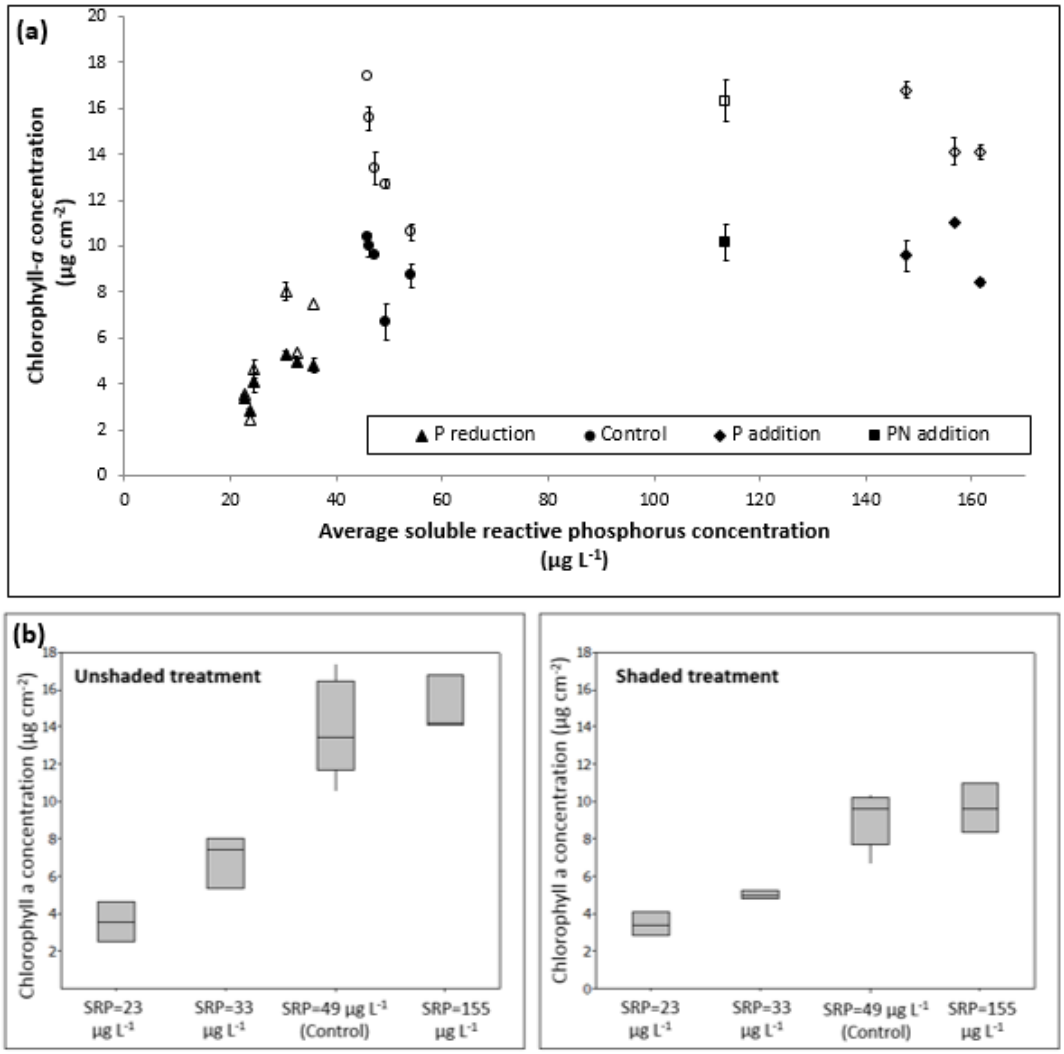

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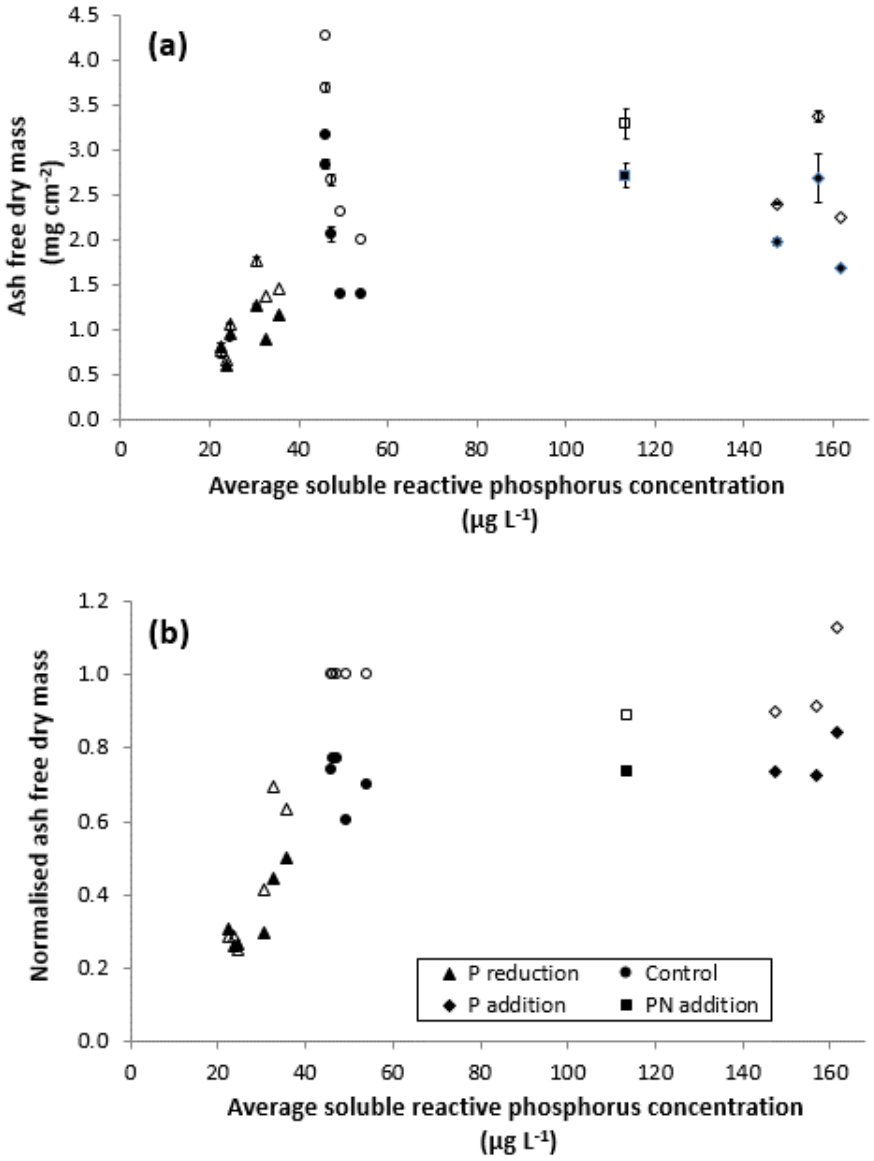

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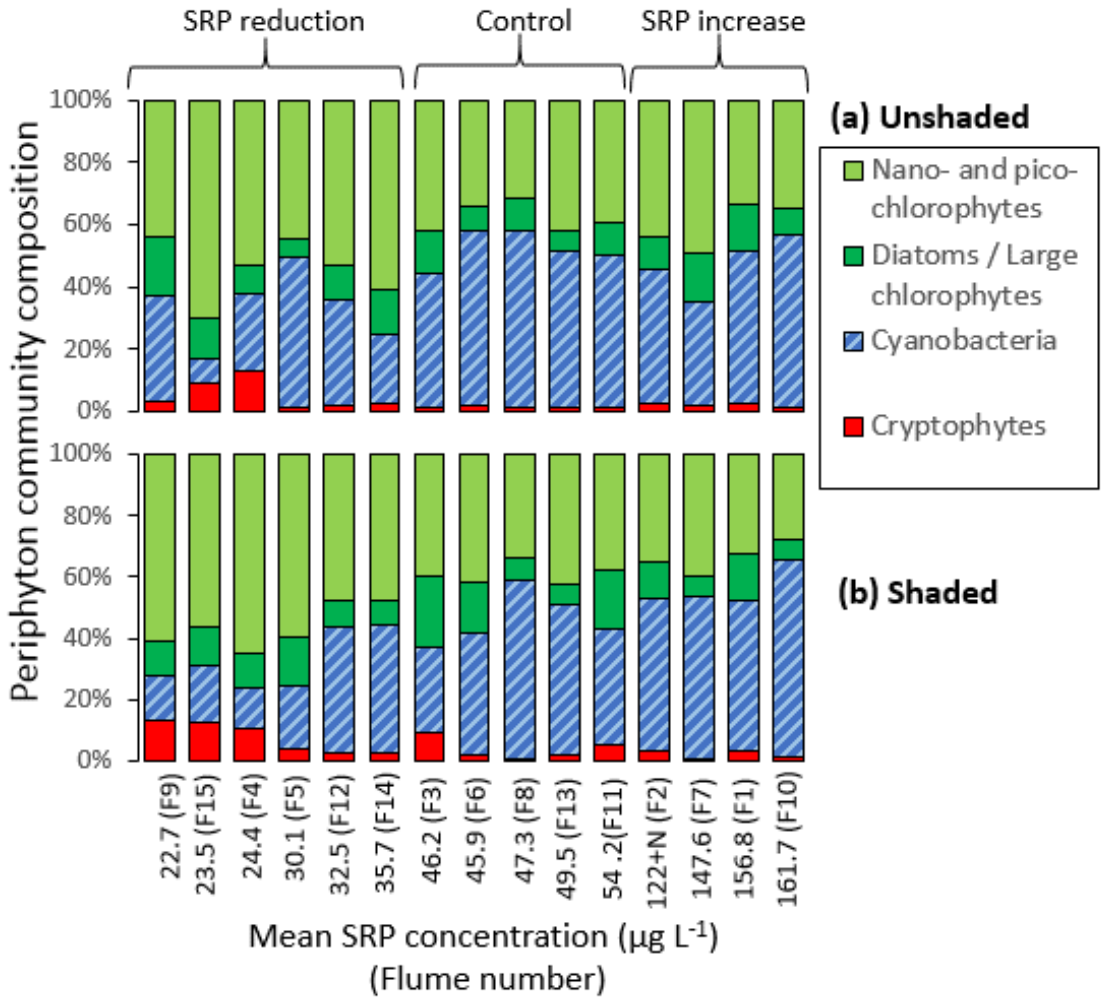

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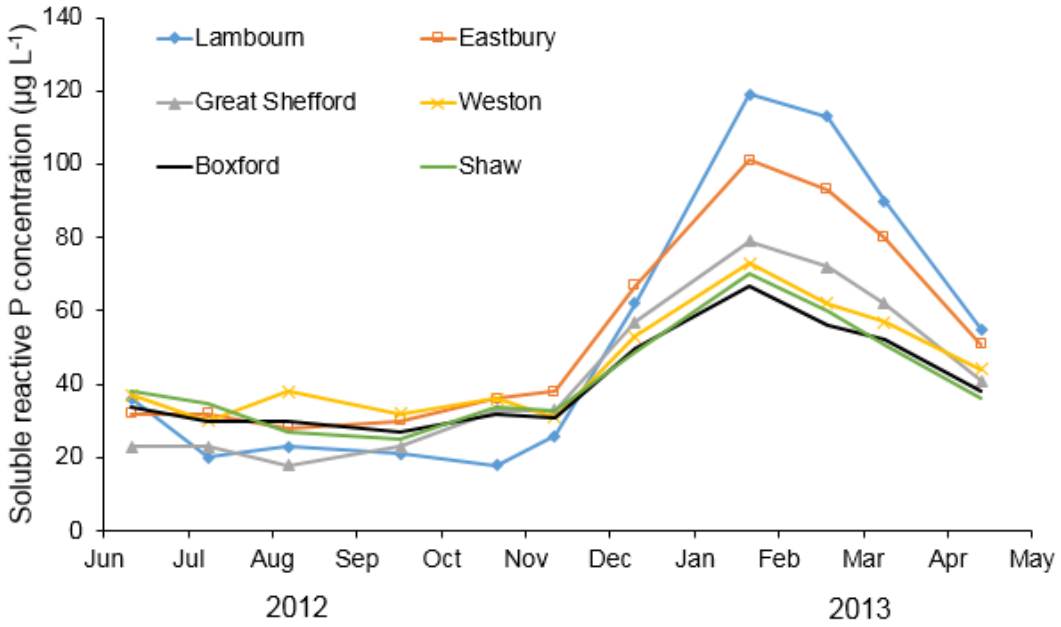

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Treatment	Average SRP concentration ($\mu g\ L^{-1}$)	Flume number	Average AFDM ($mg\ cm^{-2}$)		Average chlorophyll *a* ($\mu g\ cm^{-2}$)		Trophic Diatom Index	Average autotrophic Index	
			Unshaded	Shaded	Unshaded	Shaded	Unshaded	Unshaded	Shaded
High $FeCl_3$ addition	22.7	9	0.76	0.81	3.52	3.38	37	217	241
High $FeCl_3$ addition	23.5	15	0.66	0.60	2.48	2.83	39	268	210
High $FeCl_3$ addition	24.4	4	1.07	0.96	4.62	4.07	-	234	235
Low $FeCl_3$ addition	30.1	5	1.76	1.27	8.02	5.26	-	220	242
Low $FeCl_3$ addition	32.5	12	1.38	0.89	5.35	4.99	-	264	177
Low $FeCl_3$ addition	35.7	14	1.46	1.15	7.45	4.80	-	196	240
None (control)	45.9	6	4.3	3.2	17.40	10.42	-	175	238
None (control)	46.2	3	3.70	2.84	15.56	10.02	46	238	285
None (control)	47.3	8	2.67	2.06	13.41	9.62	-	200	214
None (control)	49.5	13	2.30	1.39	12.71	6.69	-	181	210
None (control)	54.2	11	1.99	1.40	10.60	8.72	44	188	161
PN addition	122.1	2	3.29	2.71	16.33	10.13	57	202	269
P addition	147.6	7	2.40	1.97	16.80	9.59	57	143	207
P addition	156.8	1	3.38	2.68	14.12	11.03	-	240	243
P addition	161.7	10	2.26	1.68	14.08	8.41	57	160	200

Supplementary Table S1. Diatom species list with TDI scores.

Flume 10. P addition treatment. Average SRP = 161.7 µg L^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthes	*conspicua*	3	1	4	12
Achnanthes	*oblongella*	4	1	2	8
Achnanthidium	*biasolettiana*	8	3	4	32
Achnanthidium	*minutissimum*	11	4	2	22
Amphora	*libyca*	4	1	4	16
Campylodiscus		3	1		
Cocconeis	*pediculus*	34	11	4	136
Cocconeis	*placentula*	10	3	3	30
Cyclotella	*meneghiniana*	25	8	4	100
Cymbella	*affinis*	6	2	1	6
Cymbella	*lanceolata*	6	2	2	12
Diatoma	*vulgare*	8	3	5	40
Fragilaria	*bidens*	3	1	3	9
Fragilaria	*capucina*	16	5	1	16
Fragilaria	*capucina var. rumpens*	5	2	2	10
Fragilaria	*vaucheriae*	4	1	4	16
Fragilariforma	*virescens*	3	1	3	9
Gomphonema	*truncatum*	6	2	3	18
Navicula	*lanceolata*	14	5	4	56
Navicula	*menisculus*	4	1	5	20
Navicula	*tripuncta*	9	3	4	36
Nitzschia	*amphibia*	11	4	5	55
Nitzschia	*filiformis*	7	2	4	28
Nitzschia	*fonticola*	6	2	4	24
Nitzschia	*linearis*	16	5	4	64
Nitzschia	*recta*	11	4	4	44
Planothidium		8	3	2	16
Pseudostaurosira	*brevistriata*	8	3	4	32
Rhoicosphenia	*abbreviata*	6	2	4	24
Surirella	*angusta*	4	1	3	12
Synedra	*acus*	5	2	3	15
Synedra	*rumpens*	5	2	2	10
Synedra	*ulna*	27	9	2	54
Sum		300			982
Weighted mean sensitivity		3.27			
Trophic diatom index		57			

Flume 11. Control. Average SRP = 54.2 µg L^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthes		10	3	3	30
Achnanthidium	*minutissimum*	3	1	2	6
Amphora		5	2	4	20
Cocconeis	*placentula*	35	12	2	70
Cyclotella	*meneghiniana*	11	4	4	44
Cymbella	*affinis*	9	3	1	9
Diatoma	*vulgare*	4	1	5	20
Fragilaria		10	3	2	20
Fragilaria	*bidens*	7	2	3	21
Fragilaria	*capucina*	32	11	1	32
Gomphonema	*minutum*	4	1	4	16
Gomphonema	*olivaceum*	4	1	3	12
Gomphonema	*truncatum*	4	1	3	12
Navicula	*lanceolata*	9	3	4	36
Navicula	*tripuncta*	4	1	4	16
Nitzschia	*amphibia*	21	7	5	105
Nitzschia	*linearis*	14	5	4	56
Nitzschia	*palea*	11	4	4	44
Nitzschia	*recta*	5	2	4	20
Nitzschia	*sigmoidea*	4	1	3	12
Pinnularia		1	< 1	2	2
Planothidium		13	4	2	26
Reimeria	*sinuata*	7	2	3	21
Staurosirella	*pinnata*	7	2	4	28
Synedra	*acus*	6	2	3	18
Synedra	*parasitica*	4	1	5	20
Synedra	*ulna*	56	19	2	112
Sum		300			828
Weighted mean sensitivity		2.76			
Trophic diatom index		44			

Flume 15. Fe addition. Average SRP = 23.6 µg L^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthes	*conspicua*	3	1	4	12
Achnanthes	*oblongella*	10	3	2	20
Achnanthidium	*biasolettiana*	4	1	4	16
Cocconeis	*pediculus*	17	6	4	68
Cocconeis	*placentula*	16	5	3	48
Cyclotella	*meneghiniana*	7	2	4	28
Cymbella	*affinis*	6	2	1	6
Diatoma	*vulgare*	7	2	5	35
Eunotia	*exigua*	10	3	1	10
Fragilaria	*capucina*	46	15	1	46
Gomphonema	*olivaceoides*	31	10	2	62
Navicula	*cari*	5	2	4	20
Navicula	*cryptonella*	9	3	5	45
Navicula	*lanceolata*	16	5	4	64
Nitzschia	*amphibia*	4	1	5	20
Nitzschia	*capitellata*	3	1	5	15
Nitzschia	*inconspicua*	3	1	5	15
Nitzschia	*linearis*	7	2	4	28
Nitzschia	*sublinearis*	3	1	2	6
Planothidium		7	2	2	14
Rhoicosphenia	*abbreviata*	5	2	4	20
Reimeria	*sinuata*	6	2	4	24
Synedra	*rumpens*	4	1	2	8
Synedra	*ulna*	71	24	2	142
Sum		300			772
Weighted mean sensitivity		2.57			
Trophic diatom index		39			

Flume 2. PN combined addition. Average SRP = 122.1 µg L^{-1}, average NO_3 – N = 7.92 mg L^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthes	*conspicua*	5	2	4	20
Amphipleura	*pellucida*	1	< 1	1	1
Amphora	*pediculus*	4	1	4	16
Cocconeis	*pediculus*	30	10	4	120
Cocconeis	*placentula*	12	4	2	24
Cyclotella	*meneghiniana*	29	10	4	116
Cymbella	*affinis*	4	1	1	4
Cymbella	*cistula*	7	2	2	14
Diatoma	*vulgare*	6	2	5	30
Encyonema	*silesiacum*	3	1	3	9
Fragilaria		12	4	4	48
Fragilaria	*capucina*	20	7	1	20
Fragilaria	*exigua*	6	2	4	24
Gomphonema	*olivaceum*	6	2	3	18
Meridion	*circulare*	3	1	1	3
Navicula	*angustata*	5	2	5	25
Navicula	*cryptocephala*	6	2	4	24
Navicula	*lanceolata*	6	2	4	24
Navicula	*menisculus*	6	2	5	30
Nitzschia	*disputata*	6	2	3	18
Nitzschia	*filiformis*	4	1	4	16
Nitzschia	*fonticola*	9	3	4	36
Nitzschia	*linearis*	26	9	4	104
Nitzschia	*recta*	11	4	4	44
Nitzschia	*sigmoidea*	6	2	3	18
Planothidium	*lanceolata*	7	2	4	28
Reimeria	*sinuate*	5	2	3	15
Staurosirella	*elliptica*	4	1	4	16
Synedra	*acus*	14	5	3	42
Synedra	*ulna*	37	12	2	74
Sum		300			981
Weighted mean sensitivity		3.27			
Trophic diatom index		57			

Flume 3. Control. Average SRP = 46.2 µg L^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthidium	*biasolettiana*	4	1	2	8
Achnanthes	*oblongella*	4	1	2	8
Amphipleura	*pellucida*	2	1	1	2
Cocconeis	*pediculus*	31	10	4	124
Cocconeis	*placentula*	15	5	2	30
Cyclotella	*meneghiniana*	19	6	4	76
Cymatopleura	*librile*	1	< 1	4	4
Cymbella		4	1	2	8
Cymbella	*affinis*	2	1	1	2
Diatoma	*vulgare*	5	2	5	25
Ellerbeckia	*arenaria*	2	1	5	10
Fragilaria		3	1	4	12
Fragilaria	*capucina*	49	16	1	49
Gomphonema	*olivaceum*	7	2	3	21
Gomphonema	*truncatum*	4	1	4	16
Navicula	*gregaria*	2	1	3	6
Navicula	*lanceolata*	8	3	4	32
Navicula	*protracta*	4	1	4	16
Navicula	*tripuncta*	8	3	4	32
Nitzschia	*affinis*	5	2	4	20
Nitzschia	*amphibia*	5	2	5	25
Nitzschia	*fonticola*	6	2	4	24
Nitzschia	*linearis*	21	7	4	84
Nitzschia	*recta*	4	1	4	16
Planothidium	*lanceolata*	6	2	4	24
Psammothidium	*subatomoides*	3	1	2	6
Staurosirella	*pinnata*	3	1	4	12
Surirella	*brebissonii*	2	1	5	10
Synedra	*acus*	6	2	3	18
Synedra	*ulna*	65	22	2	130
Sum		300			850
Weighted mean sensitivity		2.83			
Trophic diatom index		46			

Flume 7. P addition. Average SRP = 147.6 µg L^{-1}

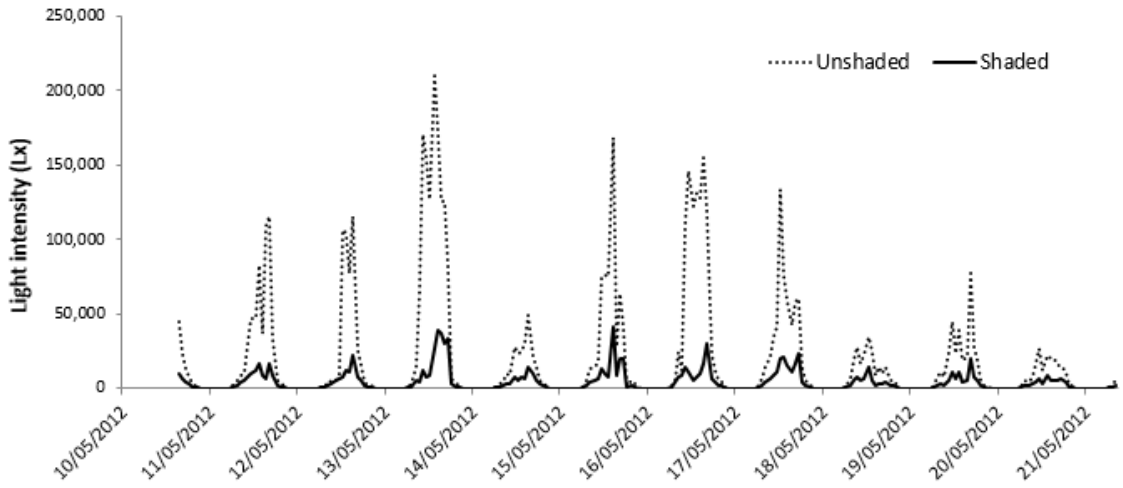
Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthidium	*biasolettiana*	8	3	4	32
Achnanthes	*conspicua*	8	3	4	32
Achnanthes	*oblongella*	8	3	2	16
Amphora	*Libyca*	4	1	4	16
Amphora	*pediculus*	10	3	4	40
Cocconeis	*pediculus*	34	11	4	136
Cocconeis	*placentula*	7	2	2	14
Cyclotella	*meneghiniana*	28	9	4	112
Cymbella	*affinis*	8	3	1	8
Cymbella	*lanceolata*	11	4	2	22
Diatoma	*vulgare*	11	4	5	55
Encyonema	*minutum*	12	4	4	48
Encyonema	*silesiacum*	8	3	3	24
Fragilaria		2	1	2	4
Fragilaria	*capucina*	20	7	1	20
Fragilariforma	*virescens*	3	1	3	9
Navicula	*lanceolata*	8	3	4	32
Navicula	*menisculus*	5	2	5	25
Navicula	*tripuncta*	8	3		0
Nitzschia	*amphibia*	8	3	5	40
Nitzschia	*angustata*	9	3	4	36
Nitzschia	*capitellata*	4	1	5	20
Nitzschia	*disputata*	5	2	3	15
Nitzschia	*filiformis*	7	2	4	28
Nitzschia	*linearis*	12	4	4	48
Nitzschia	*recta*	13	4	4	52
Nitzschia	*sigmoidea*	3	1	3	9
Psammothidium	*didymium*	3	1	5	15
Stephanodiscus		2	1	5	10
Synedra	*acus*	5	2	3	15
Synedra	*ulna*	26	9	2	52
Sum		300			985
Weighted mean sensitivity		3.28			
Trophic diatom index		57			

Flume 9. Fe addition. SRP = 22.7 µg L^{-1}

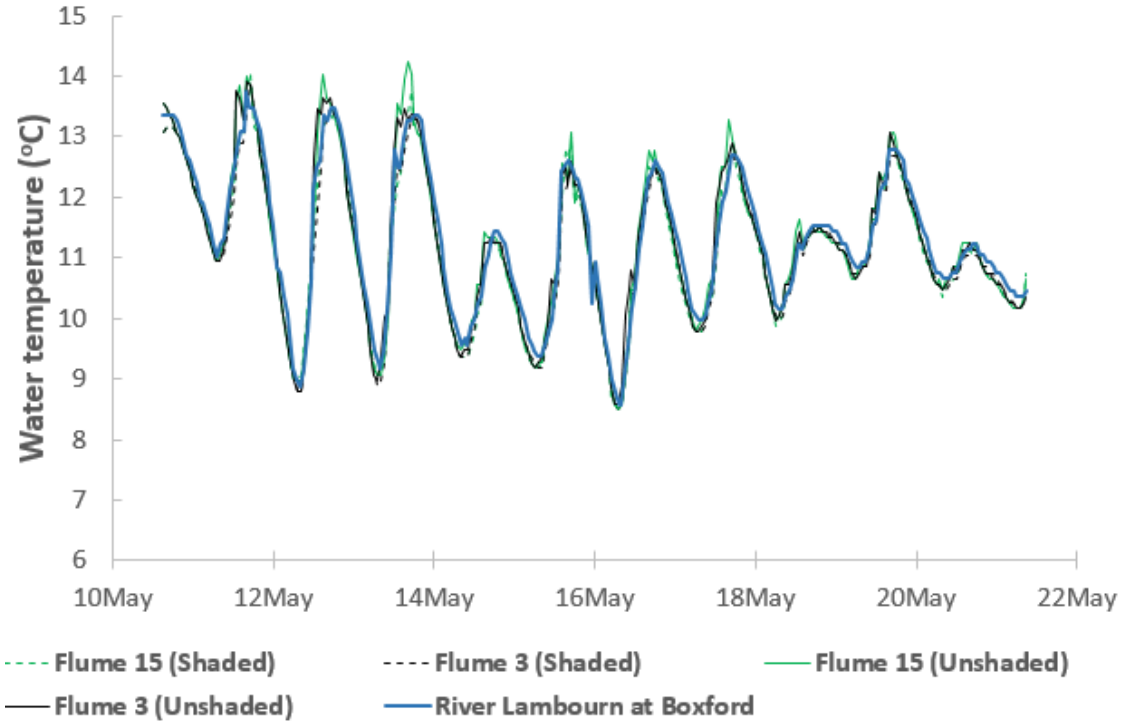
Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthidium	*biasolettiana*	3	1	2	6
Achnanthes	*conspicua*	2	1	4	8
Amphora	*pediculus*	3	1	4	12
Asterionella	*formosa*	14	5	3	42
Cocconeis	*pediculus*	17	6	4	68
Cocconeis	*placentula*	8	3	2	16
Cyclotella	*meneghiniana*	3	1	4	12
Cymbella	*cistula*	3	1	2	6
Diatoma	*vulgare*	3	1	5	15
Encyonema	*minutum*	3	1	4	12
Encyonema	*silesiacum*	12	4	3	36
Eunotia	*exigua*	9	3	1	9
Eunotia	*minor*	5	2	1	5
Fragilaria	*capucina*	40	13	1	40
Fragilariforma	*virescens*	2	1	3	6
Gomphonema	*olivaceoides*	30	10	2	60
Gomphonema	*truncatum*	2	1	4	8
Navicula	*cari*	3	1	4	12
Navicula	*lanceolata*	14	5	4	56
Nitzschia	*angustata*	1	< 1	4	4
Nitzschia	*capitellata*	6	2	5	30
Nitzschia	*linearis*	7	2	4	28
Nitzschia	*filiformis*	2	1	4	8
Planothidium	*lanceolata*	8	3	4	32
Reimeria	*sinuata*	3	1	4	12
Staurosira	*elliptica*	4	1	4	16
Stephanodiscus		1	< 1	5	5
Surirella	*angustata*	1	< 1	3	3
Synedra	*rumpens*	10	3	2	20
Synedra	*ulna*	81	27	2	162
Sum		300			749
Weighted mean sensitivity		2.50			
Trophic diatom index		37			

Table S2. Average nutrient concentrations from longitudinal surveys along the River Lambourn, conducted at monthly intervals between May 2012 and April 2013.

Site Location	Soluble reactive phosphorus	Total dissolved phosphorus	Total phosphorus	Dissolved reactive silicon	Nitrate-N	Total dissolved N
	µg P L^{-1}	µg P L^{-1}	µg P L^{-1}	mg Si L^{-1}	mg N L^{-1}	mg N L^{-1}
Lambourn	53.0	59.7	67.1	7.3	8.9	10.1
Eastbury	53.5	58.3	66.1	6.8	8.7	9.8
Great Shefford	42.2	45.7	58.9	7.4	8.0	9.2
Weston	44.8	50.4	60.9	7.3	8.2	9.5
Boxford	40.6	44.5	54.6	7.5	7.8	8.8
Shaw	41.6	51.5	63.0	7.1	7.6	8.6



S1. Light intensities monitored within flumes in full light and under shading cloths, throughout the 10 day experiment. Light loggers were submerged at a depth of 4 cm.



S2. Water temperatures in shaded and unshaded regions of two flumes, and River Lambourn river water during the course of the 10 day experiment.