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Ecological Engineering

Published: 01/02/2017

Peer reviewed version

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

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

Jones, T., Willis, N., Gough, R., & Freeman, C. (2017). An experimental use of floating treatment wetlands (FTWs) to reduce phytoplankton growth in freshwaters. *Ecological Engineering*, 99, 316-323.

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**An experimental use of floating treatment wetlands (FTWs) to reduce
phytoplankton growth in freshwaters**

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Keywords – Phytoplankton, Chlorophyll, Dissolved Organic Carbon (DOC),
Eutrophication, Floating Treatment Wetlands (FTWs), Nitrate, Phosphate, *Phragmites*
australis

Abstract

Eutrophication and the formation of phytoplankton blooms in freshwaters can be detrimental to water quality and biological health and produce organic matter that can be difficult to remove during water treatment processes. With the frequency of phytoplankton blooms increasing, remediation solutions are becoming increasingly popular. This study investigated the use of a peat-based floating treatment wetland (FTW) for reducing phytoplankton growth in eutrophic waters. Over a four-week period, the FTWs were able to reduce chlorophyll a concentrations by 80%, through sequestration of nitrate and phosphate and possibly due to the direct inhibitory properties of phenolic compounds. Although there are concerns about the leaching of dissolved organic carbon (DOC) from the FTWs, this may be more than offset by the beneficial suppression of phytoplankton growth and the resulting reduced input of ‘untreatable’ low molecular weight DOC.

Introduction

The eutrophication of freshwaters is currently a major global environmental issue, particularly in lowland lakes and reservoirs (Smith, 2003). The major driver has been widespread nutrient enrichment of freshwaters, specifically of nitrogen (N) and phosphorus (P) derived from anthropogenic sources, principally fertiliser use in the agricultural sector (Herath, 1997; Carpenter *et al.*, 1998; McDowell *et al.*, 2009; Withers, *et al.* 2014) and the expansion of urban areas and resulting discharges of sewage (Jenny *et al.* 2016). Environmental standards now exist for P (as orthophosphate) in freshwaters in the EU under the Water Framework Directive (WFD) and it is estimated that a half to two thirds of lakes in England and Wales are failing to meet good ecological status due to elevated concentrations of P (Carvalho *et al.* 2005; Duethmann *et al.* 2009). Excess N (as nitrate) and P (as phosphate) in freshwaters can lead to excessive growth of macrophytes and phytoplankton, reduced water quality (most significantly dissolved oxygen concentrations) and loss of aquatic fish life. Some phytoplankton species (cyanobacteria or blue-green algae) can be harmful due to toxic effects (Osborne *et al.*, 2001; Johnk *et al.*, 2008; Paerl & Otten, 2013). The frequency of occurrence of phytoplankton blooms in freshwaters has increased over the last few decades (Van Dolah *et al.*, 2001; Moore *et al.*, 2008) and climate change, specifically rising temperatures, is expected to lead to elevated phytoplankton growth in water bodies that currently do not experience such issues (Ritson *et al.*, 2014).

Excess phytoplankton can be particularly problematic in reservoirs used as sources of drinking water. Algogenic organic matter can cause odour and taste problems in potable water sources (especially when bacteria decompose labile compounds) and increase coagulant and chlorine demand, lead to membrane fouling and elevate disinfection by-product concentrations during water treatment processes (Bernhardt *et al.*, 1991; Knappe *et al.*, 2004; Nguyen *et al.*, 2005; Li *et al.*, 2012). As phytoplankton enter the senescence phase, decomposing cells release low molecular weight organic matter which is virtually untreatable by conventional water treatment processes (Cheng and Chi, 2003).

Eutrophication may therefore cause elevated levels of low molecular weight carbon in raw and final waters and potentially lead to increased bacterial re-growth in the drinking water distribution systems (Jjemba, *et al.* 2010).

Tackling the issue of excess nutrients leaching into freshwaters is best achieved at source (Withers et al. 2014) but some studies have attempted to reduce phytoplankton blooms in freshwaters by more direct means (Lurling et al. 2016). One common technique is to utilise the inhibitory properties of straw and deciduous litter to directly suppress the growth of phytoplankton (Welch *et al.*, 1990; Murray et al. 2010). Inhibition has been linked to the release of phenolic compounds derived from the oxidation of lignin (Ridge & Pillinger 1996) and these compounds have been described as xenobiotic due to their effects on algae and cyanobacteria (Laue et al. 2014). Recent work has demonstrated that polyphenolic compounds released from decomposing barley straw can produce hydrogen peroxide in the presence of UV radiation and this can be inhibitory towards some phytoplankton species (Iredale, et al. 2012). Despite the possible benefits, the use of barley straw requires considerable management effort and the long term ecological safety is not known (Martin and Ridge, 1999; Ball *et al.*, 2001).

Treatment wetlands offer a low cost green approach for minimising phytoplankton growth in freshwaters, mainly by reducing concentrations of N and P within a body of freshwater rather than direct effects on phytoplankton. A consequence of the high levels of biological productivity within wetlands is that pollutants which enter through run off, especially nitrogen-rich compounds contained in domestic and agricultural wastewater, are easily broken down into substrates for the plants and microorganisms (Mitsch and Gosslink, 2000). Wetlands also act as chemical sinks, storing large amounts of carbon (Jenkinson *et al.*, 1991) and nutrients in the soil matrix and water (Vymazal, 2007). The characteristic of carbon storage is largely attributed to waterlogging of the soil, creating anaerobic conditions and inhibiting enzymic decomposition of organic matter through an ‘enzymic latch mechanism’ (Freeman *et al.*, 2001; 2004). An additional benefit of wetland soil is the presence of plant derived phenolic material (Wetzel, 1992) which, studies have indicated, suppress algal blooms (Pillinger *et al.*, 1994; Everall and Lees, 1997; Ferrier, *et al.*, 2005).

It is therefore possible that the nutrient absorbing capabilities of wetland plants and microbes in conjunction with their ability to store large amounts of soil phenolic carbon may provide a unique method for controlling phytoplankton blooms. Whilst a fixed constructed wetland installed within the catchment of a lake can be used to reduce point

sources of N and P such as from inflowing streams (Scholz et al. 2016), such systems are less effective at targeting non-point (diffuse) sources of pollution. A series of small, floating wetlands may be more suitable for treating this type of pollution and have shown to be effective in a small number of previous studies. FTWs could be installed when phytoplankton blooms are known to occur rather than all year round and removed during the winter months, and the *Phragmites* harvested, to prevent potential re-release of nutrients during plant senescence (Toet, et al. 2005). FTWs are also beneficial through not needing to have water diverted to them from inflowing streams or the lake itself and they are particularly suitable for treating event-driven discharges such as during storm events (Van de Moortel, et al. 2012). Whilst a number of studies have demonstrated the effectiveness of floating treatment wetlands (FTWs) in nutrient removal (e.g. Vymazal, 2007; De Stefani *et al.*, 2011; Keizer-Vlek *et al.*, 2014; Lynch *et al.*, 2015; Saeed, et al. 2016) and some have considered the role of algae either as a mechanism for nutrient assimilation (Keizer-Vlek *et al.*, 2014) or as a biological indicator of water quality (Lu *et al.*, 2015), to date, none have directly investigated the potential of FTWs for mitigating against phytoplankton blooms.

The aim of this experiment is to examine the potential of FTWs planted with *Phragmites australis* for controlling phytoplankton blooms in eutrophic water bodies. Phytoplankton blooms were artificially generated in small pond systems and phytoplankton biomass and pond water hydrochemistry compared between control and FTW treatments over a four-week period.

Materials and methods

FTW and pond designs

Phragmites australis (Cav.) Trin. ex. Steud was the chosen plant species for both phase 1 and 2 due to its ability to sequester nitrate and phosphate in freshwaters and its widespread use in remediation wetlands (Massacci *et al.*, 2001). The *Phragmites australis* plants were grown to a height of 30 cm in a greenhouse prior to being planted in the FTWs. The average leaf length was approximately 15 cm. The healthiest looking plants were selected from the original stock and then divided out between the treatments for both the phase 1 and 2 experiments.

Small FTW units were constructed for phase 2. The exterior of the FTW consisted of a plastic-coated wire hanging basket (30 cm width, 15 cm height, 706 cm² surface area), with the interior lined with inert netting to prevent the outward leaching of growth medium. Around the rim of each basket, pipe insulation was fitted to enable the systems to float. The growth medium consisted of equal quantities of peat, coya and shredded heather which was added to just below the rim of the basket. This phenolic-rich substrate was chosen to achieve a low decomposition rate through the enzymic latch mechanism (Freeman *et al.*, 2001), thereby limiting the re-release of nutrients following uptake. Eight *Phragmites australis* plants (as determined by results of Phase 1 experiment) were then planted in each FTW. The FTWs were constructed two weeks prior to the experiment to allow the plants to settle and washed daily with water to minimise the build-up of carbon, nitrate and phosphate that could potentially leach from the FTWs once they were placed in the ponds.

The experiment was performed in clear plastic boxes ('ponds') (59 cm width x 39 cm depth x 42 cm height). The ponds were each filled to 70 L capacity with de-chlorinated tap water. The water was then artificially altered to a eutrophic state through the addition of "Long Ashton nutrient solution", with concentrations taken from Wetzel (2001) and scaled up to generate an extremely eutrophic environment. A highly concentrated 20 mL volume of phytoplankton was then added to the ponds following its culture from water

collected from the naturally eutrophic Llyn Penrhyn on the Isle of Anglesey, Wales, UK (UK grid ref. SH 31382 76921). Initial nitrate, phosphate and chlorophyll *a* concentrations were 12.0, 21.5 mg L⁻¹ and 9.5 µg L⁻¹ respectively. The ponds were placed outside where they could receive full sun for the entire experimental period.

Phase 1 experimental design

This pilot experiment was performed to determine whether the *Phragmites australis* plants were able to sequester N and P under the experimental conditions and, if so, the number of *Phragmites* plants required within a single FCW unit for optimum suppression of the growth of phytoplankton. This was determined by measuring how varying the number of plants reduced the chlorophyll *a* concentration in the ponds. Six ponds were created as described above and 0 (control – no plants), 2, 4, 6, 8 and 10 plants grown hydroponically. The water in each pond was mixed manually every 3 days and topped up with deionised water to replace evaporative losses. After 3 weeks, when there were visible differences between treatments, a 250 ml water sample was collected from just below the surface of each pond.

Phase 2 experimental design

Following the outcome of the pilot phase 1 experiment, eight *Phragmites* plants were planted in each of five new FTW units. This produced a plant density equivalent to 113 per m². Ten new ponds were created with fresh nutrient solution and phytoplankton stock. At the beginning of the experiment, a single FTW was added to five ponds randomly, with the remaining five ponds left empty. The water in each pond was mixed manually for 1 minute three times per week. Water samples were collected on a weekly basis for four weeks starting from the day the FTWs were placed in the ponds. From each pond, 250 mL was extracted from below the surface and transported to the laboratory. After each week's sampling, additional water was added to replace that which had evaporated after sampling. Additional nutrients were added to each pond before sampling in week 3 to replenish those nutrients which had been utilised.

Laboratory analyses

All 250 ml water samples were filtered through GF/A filter paper (Fisher, Leicestershire, UK) and again through 0.45 µm cellulose acetate filters and the solution stored at 4°C until analysis.

The GF/A filter paper was analysed for chlorophyll a (as a proxy for phytoplankton biomass) according to the method of Golterman (1978). The filters were placed in individual 10 ml centrifuge tubes, 5 ml 90% acetone added and the tubes placed on a shaker for 10 minutes. The tubes were then left in the dark at 4°C for 16 hours and then centrifuged at 3,200 rpm for 10 minutes. Absorbance of the supernatant was measured at 665 and 750 nm on a Unikon 943 double beam UV-vis spectrophotometer (Kontron, Chichester, UK) and chlorophyll a concentration was calculated using the following formula:

$$\text{Chlorophyll a } (\mu\text{g L}^{-1}) = 11.9 (\text{Abs}_{665} - \text{Abs}_{750}) \frac{v}{Vp}$$

Here V is the volume filtered (mL), v is the volume of extract (mL), p is the pathlength (cm) and 11.9 the specific absorbance coefficient of chlorophyll a in 90% acetone.

Analyses carried out on the filtered water samples included the determination of concentrations of dissolved organic carbon (DOC), phenolic compounds, nitrate and phosphate and specific UV absorbance (SUVA). DOC concentration was measured using a Thermalox TOC/TN analyser equipped with a non-dispersive CO₂ detector (Analytical Sciences Ltd, Cambridge, UK). UV/visible absorbance measurements were made on the same Unikon 943 spectrophotometer. The concentration of phenolic compounds was determined using the spectrophotometric method described by Box (1984). SUVA (L mg⁻¹ m⁻¹) was calculated as a ratio of UV absorbance at 254 nm (m⁻¹) to DOC (mg L⁻¹); the higher the value the more aromatic and higher molecular weight the DOC (Volk *et al.*, 2002).

Nitrate and phosphate were measured using a Dionex DX-120 ion chromatograph fitted with conductivity detection and auto self-regenerating suppression. Separation was achieved using an IonPac AS4A column (Thermo Fisher Scientific Inc., Waltham MA, USA).

Statistical analysis

The data was analysed by simply running t-tests of each time point comparison for each treatment individually for each measured parameter. T-tests were also run to compare between the two treatments at each time point. T-tests were not run when the concentrations of a parameter were below the limit of detection and for DOC, Phenolics or pH because the treatments were significantly different at week 0. The analyses were run in GraphPad InStat (GraphPad Software Inc., CA, USA).

Results

Phase 1

Data from this experiment was used to decide how many plants to use in each FTW for the phase 2 experiment and due to the lack of replication should only be taken as informative. The experiment demonstrated the ability of *Phragmites australis* to reduce chlorophyll a concentrations (by suppressing the growth of phytoplankton), with the control treatment having a chlorophyll a concentration of $133 \mu\text{g L}^{-1}$ and the planted treatments lower values after 3 weeks (Figure 1). The concentration reduced in a near linear manner with increasing plant number up to 8 plants, with 10 plants showing no additional benefit. The treatment with 8 plants had a final chlorophyll a concentration of $35 \mu\text{g L}^{-1}$, 74% less than the control treatment.

Phase 2

In all of the analyses undertaken, differing trends were recorded for the control and planted treatments.

The mean concentration of phosphate (Figure 2) in both treatments declined from approximately 2.6 mg L^{-1} to below the limit of detection ($<20 \mu\text{g L}^{-1}$) over the 4 weeks, only increasing when the nutrient was replenished at week 3. The decline in phosphate was greatest for the planted ponds, falling to undetectable levels by week 2. Phosphate concentrations were significantly higher in the control treatment at weeks 1 and 3 ($p<0.001$).

The mean concentration of nitrate (Figure 3) measured in the both treatments varied significantly from week to week ($p<0.001$). In the control ponds, after an initial increase from week 0 to week 1, the concentration fell to 4.6 mg L^{-1} in week 2, rose to 17.2 mg L^{-1} in week 3 following nutrient replenishment and fell to below the limit of detection ($<20 \text{ } \mu\text{g L}^{-1}$) in week 4. In the planted ponds, the concentration of nitrate fell from 11.6 mg L^{-1} in week 0 to below $20 \text{ } \mu\text{g L}^{-1}$ in weeks 1 and 2. It then rose to 15.0 mg L^{-1} in week 3 and back to an undetectable level in week 4. The control treatment always had the higher concentration and was significantly higher than the planted treatment at week 3 ($p<0.001$).

For chlorophyll a (Figure 4), the mean concentration in the control ponds increased significantly, from $9.5 \text{ } \mu\text{g L}^{-1}$ in week 0 to $128.1 \text{ } \mu\text{g L}^{-1}$ in week 4 ($p<0.001$). In the planted ponds, the mean concentration increased significantly from $9.4 \text{ } \mu\text{g L}^{-1}$ in week 0 to $29.1 \text{ } \mu\text{g L}^{-1}$ in week 1 ($p<0.001$), but then did not change significantly for the remaining three weeks ($p>0.05$). After having almost identical concentrations of chlorophyll a in week 0, the FTW planted ponds had approximately 80% less chlorophyll a than the control ponds by week 4. The control treatment had significantly higher chlorophyll a than the planted treatment at weeks 2, 3 and 4.

Mean DOC concentration (Figure 5) increased over the 4-week period in both the control and planted ponds. The rise was greatest for the planted treatment, increasing significantly from 6.5 mg L^{-1} in week 0 to 16.0 mg L^{-1} in week 4 ($p<0.001$), an average rise of 2.4 mg L^{-1} per week. DOC in the control ponds increased significantly from 4.7 mg L^{-1} in week 0 to 10.0 mg L^{-1} in week 4 ($p<0.001$), an average rise of 1.3 mg L^{-1} per week.

The mean concentrations of phenolic compounds (Figure 6) followed a similar trend to DOC, increasing from week 0 to week 4 and at a greater rate for the planted ponds. In the control ponds, the concentration rose significantly from 0.54 mg L^{-1} in week 0 to 2.13 mg L^{-1} in week 4 ($p<0.001$); in the planted ponds from 0.73 mg L^{-1} in week 0 to 3.76 mg L^{-1} in week 4 ($p<0.001$).

Values of SUVA (Figure 7) showed markedly different trends for each treatment. For the control ponds, SUVA declined from 2.71 L-mg/m in week 0 to 0.42 L-mg/m in week 3 ($p<0.001$) and did not change significantly in week four ($p>0.05$). In the FTW ponds, the

SUVA did not change significantly throughout the experiment ($p>0.05$), although the mean value declined slightly from 3.20 L-mg/m in week 0 to 2.37 L-mg/m in week 4. The pH (Figure 8) of the pond water increased much more rapidly in the control compared to the planted treatment. In the control treatment the pH increased significantly from 7.53 in week 0 to 10.71 in week 4 ($p<0.001$) with the increase mostly occurring between weeks 0 and 2. In the planted treatment the pH increased slightly but significantly from 7.03 at week 0 to 7.552 at week 4 ($p<0.01$). The sharp rise at week 3 (to pH 9.08) was not sustained in week 4.

Discussion

The FTWs used in this study proved very successful at reducing the growth of phytoplankton in small-scale freshwater ponds, reducing chlorophyll a (used as a proxy for phytoplankton biomass) by 80% compared with the control treatment at four weeks. The dominant mechanism for this was most likely nutrient uptake by the *Phragmites australis*, effectively reducing nitrate and phosphate concentrations to levels that inhibited the growth of phytoplankton. Previous studies have demonstrated the effectiveness of *Phragmites australis* in reducing nutrient levels in both conventional (surface/subsurface flow) (e.g. Vymazal, 2007) and floating treatment wetlands (e.g. Keizer-Vlek, et al. 2014), but to our knowledge ours is the first study to demonstrate its effectiveness for controlling phytoplankton in a small-scale floating system that also utilises a substrate control. Although the concept is still relatively new compared to conventional treatment wetlands, FTW systems have traditionally been employed with rooted plants growing as a floating mat on the water's surface rather than in sediment (Headley & Tanner, 2006). The FTW systems used in this study can be likened to a natural floating wetland, defined in Sasser et al. (1991) as a 'free floating marsh' of vegetation, detritus, peat (De Stefani, et al. 2011).

The small decrease in the chlorophyll a concentration from week 2 to week 3 for the control ponds can be attributed to nutrient limitation and some algal senescence. Once nutrient levels were replenished prior to sampling in week 3, chlorophyll a concentrations rose sharply again in the control by week 4, but continued to be suppressed in the FTW

ponds. Despite evidence of nutrient uptake in the FTW ponds from week 1, our chlorophyll a data also indicate a delay in the suppression of phytoplankton, which was only apparent from week 2. Overall, these data offer encouragement that such systems may be suitable for reducing phytoplankton blooms in nutrient-enriched freshwater lakes but that the initial period of FTW establishment needs to be factored into predictions of the length of time required to reduce nutrient concentrations and phytoplankton densities. The water quality of the pond water with planted FTWs was much improved compared to the control ponds, with much reduced Chlorophyll a, nitrate and phosphate concentrations and a more neutral pH (phytoplankton blooms can lead to very alkaline water due to depletion of inorganic carbon). However, our data show that the use of FTWs utilising a peat/coya/heather based media may increase the concentration of DOC in the water body. Comparing FTW and control data, by week 4 the growth of phytoplankton had contributed approximately 5.3 mg L⁻¹ of DOC in the control ponds, whilst in the FTW ponds, 9.5 mg L⁻¹ of DOC was produced. Therefore the FTWs contributed approximately an extra 4.2 mg L⁻¹ of DOC, presumably due to leaching of DOC from root exudates and soil organic matter. However, the increase in DOC concentration associated with the FTWs should be considered in the context of likely treatment scenario. The occurrence of phytoplankton blooms in freshwater lakes or reservoirs typically occurs during the summer months, when water temperatures and sunlight levels are highest (Johnk *et al.*, 2008). This is also a time of year when DOC concentrations in lakes tend to be low, as the input of allochthonous DOC is reduced due to lower rainfall, lower availability of leachable carbon and greater water usage by vegetation in the lake's catchment (Roberts, 1998). The increased input of DOC from the FTWs may therefore occur at a time when DOC concentrations of the lake in which they are utilised are naturally low.

Our data also show that the composition of the additional DOC in the FTW ponds was distinct. The DOC in the FTW ponds contained proportionally more phenolics and the SUVA data suggests that the DOC was characterised by higher molecular weight, more aromatic constituents (Volk *et al.*, 2002). The low molecular weight, aliphatic DOC produced by algae is reported to be difficult to remove during conventional coagulation-flocculation (Cheng and Chi, 2003) and in the distribution system may lead to harmful

bacterial growth (Volk *et al.*, 2000). Higher removal efficiencies are reported for higher molecular weight, more aromatic (high SUVA) DOC (Sharp *et al.*, 2006; Gough *et al.*, 2014) such as that associated with the FTW treatment. Therefore, the addition of FTWs may actually favour DOC removal during water treatment processes. Furthermore, it is possible that the leaching of phenolics from the FTWs contributed to the suppression of phytoplankton growth since these compounds have been demonstrated to have inhibitory properties towards algae (Pillinger *et al.*, 1994; Ferrier *et al.*, 2005) and photo-degradation of phenolics can produce hydrogen peroxide which has been linked to inhibition of phytoplankton growth (Iredale, et al. 2012).

When assessing the results of this study it is also important to consider the scale of this experiment in relation to the use of an FTW system in a real scenario and to stress the need for follow-up work. This experiment was a pilot-scale feasibility study, directly assessing the ability of a specific FTW design to mitigate phytoplankton blooms through sequestering the key nutrients nitrate and phosphate. Although it is envisaged that the size of an individual FTW would be much larger when used in a freshwater lake, the FTW:water volume would certainly be much smaller than in this study, which could affect the efficiency of phytoplankton bloom control. However, unlike in this study, the use of FTWs in a real situation is expected to take place for many months, whereby the systems can slowly and continuously take up N and P for the times of the year when sunlight levels and water temperatures are sufficiently high to allow for the growth of phytoplankton. Under this scenario, there would not be the demand for the FTWs to rapidly reduce N and P concentrations from a high starting position and the systems could keep the nutrient levels in check. If our FTW system were to be up-scaled the lower FTW:water volume ratio would also likely lead to a much lower net increase in DOC concentrations in the water body being treated. Nevertheless a pilot study would be required to accurately assess the ability of FTWs to control phytoplankton growth at larger scales. It is likely that such a system would not be suitable for large lakes where bed sediment can be an important source of P (Wu, et al. 2014) and one not easily controlled by FTWs. It is also suggested that the FTWs should be removed from the treated water body at the end of the growing season since the senescence of the

Phragmites australis vegetation would likely input large amounts of carbon, nitrogen and phosphorus into the lake (Polomski *et al.*, 2009).

Conclusions

This study demonstrated the potential of a peat-based floating treatment wetland (FTW) to sequester nitrate and phosphate in a small-scale freshwater pond, thereby reducing the growth of phytoplankton. After a period of four weeks, phytoplankton biomass (as indicated by chlorophyll a concentration) was reduced by 80% in the FTW treatment. DOC concentration in the FTW treated ponds was elevated compared with the control treatment, presumably due to leaching from root exudates and soil substrate in the FTWs, however the character of the DOC (more high molecular weight and aromatic) is likely to facilitate effective removal during conventional water treatment compared to waters dominated by phytoplankton-derived DOC. Furthermore, in a real treatment scenario this DOC release is likely to coincide with low ambient DOC levels. The potential benefits in terms of phytoplankton suppression are therefore likely to outweigh the additional DOC release although it is suggested that further study be undertaken to assess the precise impacts of FTW treatment on a larger scale.

Acknowledgements

This work received funding from a Royal Society Mercer Feasibility Award and Industry Fellowship (CF), the European Social Fund, and Dwr Cymru Welsh Water. The authors are grateful to ‘Reeds from Seeds’ for donating the *Phragmites australis* and to Mari Whitelaw, Nina Menichino and Emma Johnstone for their assistance in the laboratory.

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