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1 **Evaluation of algal bloom mitigation and nutrient removal in floating constructed**
2 **wetlands with different macrophyte species**

3

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8 **Abstract**

9 Algal blooms resulting from the eutrophication of surface waters represent a significant
10 ecological and water treatment issue. The potential for wetland systems to act as sinks for
11 various types of pollutants indicates their potential for mitigating algal blooms. Although
12 nutrient uptake in terrestrial treatment wetland systems has received substantial attention in
13 the literature, relatively little is known about the mechanisms involved in floating constructed
14 wetland (FCW) function for algal control and whether plant species can optimise
15 performance. Here, the effect of FCWs on water quality including nutrient levels and algal
16 biomass was investigated, along with the effect of planting with different species of
17 macrophyte. All the planted FCWs showed significant potential for algal bloom mitigation in
18 both hypereutrophic and mesotrophic systems; algal biomass control is proposed to be due to
19 the direct uptake of nitrate and phosphate *via* macrophyte roots, rather than algaecidal effect
20 of phenolic compounds. Dissolved organic carbon (DOC) release was found to differ
21 between species, with implications for drinking water treatment.

22 **Keywords** - Algae, Chlorophyll, *Phragmites australis*, *Juncus effusus*, *Iris pseudacorus*

23 **Introduction**

24 In both freshwater and marine systems, the leaching of nutrients from the surrounding
25 terrestrial environment can lead to high nutrient loading (McDowell & Wilcock 2008). This
26 can result in eutrophication, a process known to have deleterious effects on ecosystem goods
27 and services. Pollutants containing nitrogen and phosphorus can drive increases in primary
28 production. In aquatic systems this often results in the formation of algal blooms, whilst in
29 wetland habitats, species composition shifts can be observed with increasing trophic level.
30 Many studies on lakes and rivers have found a direct correlation between nutrient levels and
31 algal biomass (Smith et al. 1999).

32 Several factors influence the degree of eutrophication including flow rate, retention time and
33 degree of water inundation. Reviews on pollutant concentrations required in order for
34 eutrophic conditions to arise in lentic (standing) and lotic (flowing) systems are discussed by
35 Nürnberg (1996) and Dodds et al. (1998), respectively. Evidence presented in Dodds et al.
36 (1998) indicates clearly that lotic, fast moving water bodies require extremely concentrated
37 nitrogen and phosphorus inputs in order for eutrophic conditions to prevail. The slow
38 movement of water in lakes, ponds and wetland sites make them more susceptible to the
39 effects of enrichment due to higher water retention times.

40 Wetlands are biologically, geologically and chemically unique ecosystems (Kadlec &
41 Wallace 2008). These systems are regarded as hydrological buffers, stabilizing flow rates and
42 ameliorating flooding and drought by recharging aquifers (Mitsch & Gosselink 2000). The
43 ability of natural wetlands to act as sinks for chemicals has encouraged researchers to
44 investigate the possibility of using constructed wetlands (CW) to treat wastewater and water
45 of high nutrient or pollutant content, with increasingly diverse applications being seen.

46 The use of floating constructed wetlands (FCW), where buoyancy is engineered within the
47 design, is a possible solution to the problem of eutrophic water bodies. These systems allow
48 the roots of the macrophytes to be suspended in the water column, resulting in direct uptake
49 of nutrients and therefore greater uptake rates than in benthic sediments (Headley et al. 2006).
50 The FCW can be constructed and designed in order to deal with varying amounts of pollutant
51 loading and the ability of the floating systems to track the water table is also advantageous in
52 circumventing issues that can reduce performance in conventional CW, such as lowered flow
53 rate or water volume.

54 The consensus is that phosphate is generally the most limiting nutrient in aquatic ecosystems
55 and wetlands. It can be removed in CWs through various pathways including sorption,
56 biomass storage and cycling, microbial phosphorus in flocs, longer term accretion in soils and
57 sediments (Kadlec & Wallace 2008). Primary removal methods, in conservation site CWs in
58 particular, are precipitation with other compounds, dissolution resulting in sedimentation and
59 peat accretion (Vymazal 2007). Plant-bound phosphorus will cycle in the wetland from plants
60 to soils to microbes, some of which will be lost from the system during biomass degradation.

61 Nitrate is often the dominant form of nitrogen-based pollutant in waters affected by
62 agricultural activity due to its high solubility. Complete removal of nitrate can be achieved by
63 microbial denitrification (Shapleigh 2013). This process converts nitrate to nitrogen gas *via* a
64 number of intermediate phases. Denitrification is facilitated by microbial communities
65 through the production of reductase enzymes (Knowles 1982). However, in many cases N₂O
66 (nitrous oxide), a potent greenhouse gas, is emitted from the CW before complete
67 transformation to N₂ (Kadlec & Wallace 2008).

68 Although it has been shown that FCWs offer significant potential in the mitigation of algal
69 blooms through nutrient uptake (e.g. Jones et al. 2017), to date, the effect of planting with
70 different macrophyte species has not been investigated. Thus, this paper presents the results
71 of a study designed to test the effectiveness of FCWs planted with different macrophyte
72 species for nutrient removal and algal bloom mitigation and the potential mechanisms
73 involved in this process.

74 **Materials and methods**

75 Water quality, including nutrient concentrations and algal biomass, was assessed over a 10
76 week period in water tanks under 5 different treatment regimes. The treatments included
77 FCWs planted with 3 different plant species, an unplanted FCW treatment and a control (no
78 FCW present).

79 **Experimental mesocosm set-up**

80 Thirty 80 L plastic tanks were set up in a roof top research compound (Bangor University,
81 Wales, UK). After filling with 70 L of tap water, the water in the tanks was vigorously mixed
82 in order to drive off any dissolved chlorine gas added to the water during treatment and then
83 allowed to equilibrate for 2 days.

84 The experiment included 2 trophic states, namely a hypereutrophic nutrient balance (25 mg/L
85 nitrate, 2 mg/L phosphate) and a mesotrophic nutrient balance (2.5 mg/L nitrate, 1 mg/L
86 phosphate). For each trophic state, 5 different treatments were applied, with 3 replicates per
87 trophic state/treatment combination. The 5 treatments comprised a FCW planted with
88 *Phragmites australis*, *Juncus effusus*, *Iris pseudacorus*, an unplanted FCW and a control
89 system (no FCW present).

90 The experimental design employed the random assignment of trophic state and treatment type
91 to different tanks with the tanks positioned in 3 rows of 10. Although randomly assigned, in
92 order to achieve robust experimental design, each trophic state and treatment type occurred in
93 each of the 3 rows. Each treatment type was then randomly positioned within the row in order
94 to reduce any potential environmental effects due to tank position.

95 The nutrient concentrations required to achieve the 2 trophic states were based upon analysis
96 carried out by Wetzel (2001), Smith et al. (1999), Nürnberg (1996) and Dodds et al. (1998)
97 on freshwater bodies. The desired nutrient levels were achieved by the addition of
98 concentrated KNO_3 , $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ solutions. The nutrient solutions
99 were added to the tanks at the start of the experiment (7th June). A secondary nutrient
100 replenishment was performed on the 1st of August.

101 **FCW Design and Addition to Mesocosms Systems**

102 The FCWs were constructed using plastic coated wire (30 cm width, 15 cm height, 0.0706 m²
103 surface area) supported by an inert foam floating ring around the circumference. A rooting
104 media mixture of equal measures of peat, coir (coconut fibre) and finely chopped heather
105 were used to replicate organic matter used in Welch et al. (1990). Porous liners were used in
106 order to prevent loss of the organic media into the water column.

107 Systems were planted with equal quantities of plants by biomass. *Phragmites australis*,
108 *Juncus effusus* and *Iris pseudacorus* species were used. When planting out the new FCWs,
109 attached material was washed from the root zone of the plants in order to avoid

110 contamination. FCWs containing *Juncus effusus* were planted 9 months in advance of the
111 testing period. This was done in order to achieve a pseudo-control which aimed to investigate
112 how performance might change with established systems.

113 Once fully planted, each system was rinsed with 50 L of tap water in order to remove any
114 residual nutrient within the organic material added to the substrate material. This allowed for
115 more accurate quantification of plant nutrient assimilation. Once the nutrients within the
116 tanks had been manipulated to the appropriate levels, the FCWs were placed in the relevant
117 tanks, with the outer floating ring allowing the vegetation to sit above the water surface.

118 **Sample collection**

119 Sampling was carried out weekly. The water in the tanks was thoroughly mixed before a 50
120 mL sample was collected from the water column. The pH and conductivity were measured on
121 raw, unfiltered samples. Dissolved oxygen was measured *in situ* using a dissolved oxygen
122 probe, calibrated prior to each sample run. Pore water dissolved oxygen measurement was
123 carried out *in situ* from a 10 cm porous tube inserted into the system rhizosphere and sealed
124 with a cap. Unless otherwise stated, water chemistry parameters refer to water column
125 samples rather than the pore water.

126 Following measurement of pH and conductivity using probes, water samples were filtered
127 through GF/C 1.2 µm filter paper in order to extract an algal sample. Secondary filtration of
128 the same samples through 0.45 µm membrane filters was performed in order to allow matched
129 sampling and prevent sample degradation. Samples were stored at 4°C until analysis.

130 **Sample analyses**

131 Dissolved organic carbon (DOC) concentration was measured using an Analytical Sciences
132 Thermalox TOC/TN analyser. In order to measure DOC the samples were acidified to
133 between pH 2 and 3 and sparged with oxygen for 2 minutes in order to remove inorganic
134 carbon compounds. The instrument was calibrated using potassium hydrogen phthalate
135 standards (0, 5, 10, 15, 20, 30 and 40 mg/L). The concentration of phenolics was determined
136 using the spectrometric method described by Box (1983) and adapted for 96-well
137 microplates.

138 The concentrations of nitrate and phosphate were measured using an 850 Professional Ion
139 Chromatograph (Metrohm, UK Ltd., Runcorn, UK) and 858 autosampler equipped with a
140 Thermo Fisher AS14A anion column and a Metrohm C4 cation column. Multi-ion standard
141 were used separately for anions and cations.

142 Algal biomass was monitored by measuring chlorophyll-*a* concentration using the method
143 described by Jespersen & Christoffersen (1987) with 100% methanol as the solvent and an
144 incubation time of 30 minutes in a 60°C water bath.

145 **Statistical Analyses**

146 Analysis of variance (ANOVA) was conducted to investigate differences in water quality
147 parameters between treatments. Normality and homogeneity of variance in the data could not
148 be accurately assessed due to small sample sizes. However, ANOVA is robust to violations of
149 these assumptions. One-way ANOVA was performed at 3 different time points: the
150 beginning of the experiment (7th of June), just before the second nutrient replenishment (26th
151 of July) and the end of the experiment (16th of August). Where significant results for

152 ANOVA were identified, a Tukey HSD post-hoc test was performed. Statistical analyses
153 were conducted using version 19 of the SPSS statistical package.

154 Results and discussion

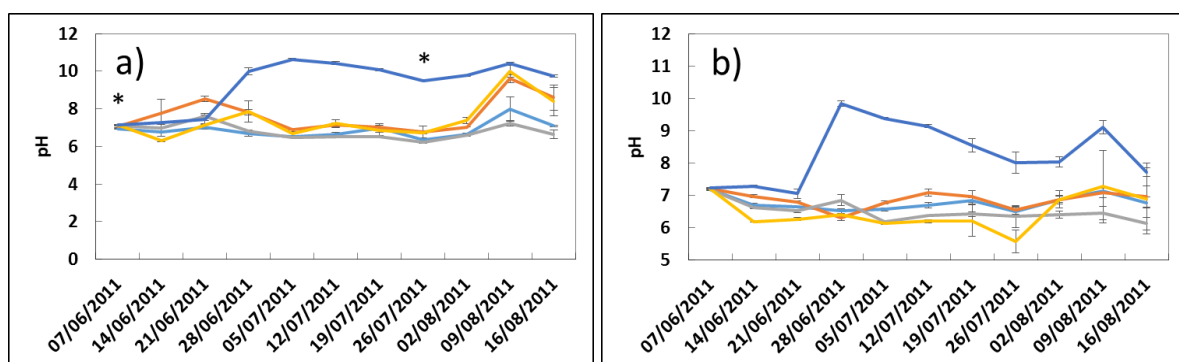
155 **Routine Hydrochemistry**

156 The pH in the hypereutrophic control system increased dramatically on 28/6/2011 (Figure
157 1a). This can be linked to a rapid increase in algal biomass observed at the same time (see
158 below). An increase in pH for all treatments was also observed following nutrient
159 replenishment on the 01/8/2011, although for the *Phragmites* and *Iris* treatments the increase
160 was less dramatic. The main driver for this effect is likely to be the growth of phytoplankton,
161 which assimilate carbon into biomass, thereby decreasing CO₂ concentrations in the water
162 column and, in turn, increasing the pH (Willoughby 1976; Schippers et al. 2004).

163 In the mesotrophic situation (Figure 1b), pH in the control peaked at a similar time to the
164 hypereutrophic control suggesting a consistent mechanism. The planted FCWs show a small
165 amount of variation in pH over time, but remain slightly acidic throughout the experiment.
166 ANOVA analysis revealed no significant differences in pH between treatments for the
167 mesotrophic system for the time points analysed (Table 1), suggesting similar
168 biogeochemical controls on pH.

169 A number of significant differences were, found at the beginning of the experiment for the
170 hypereutrophic system (Table 1), although percentage differences were modest (Figure 1a).
171 The only other significant difference in the hypereutrophic system was for the 26th of July,
172 between the control treatment and the *Iris* treatment. These differences are likely to relate to
173 differential nutrient uptake via plants and algae thereby affecting conductivity and in turn pH
174 (Figure 2a).

175 The most striking overall effect, however, was that the presence of FCWs (either planted or
176 unplanted) mitigated against the increase in pH observed in the control treatments at both
177 trophic levels (Figure 1a and b).



178
179 *Figure 1. Water column pH for hypereutrophic (a) and mesotrophic (b) systems over 10 week*
180 *experimental period showing Phragmites (light blue), Juncus (orange), Iris (grey), unplanted*
181 *(yellow) and control (dark blue) treatments. Error bars represent the standard error of the*
182 *mean (n = 3).*

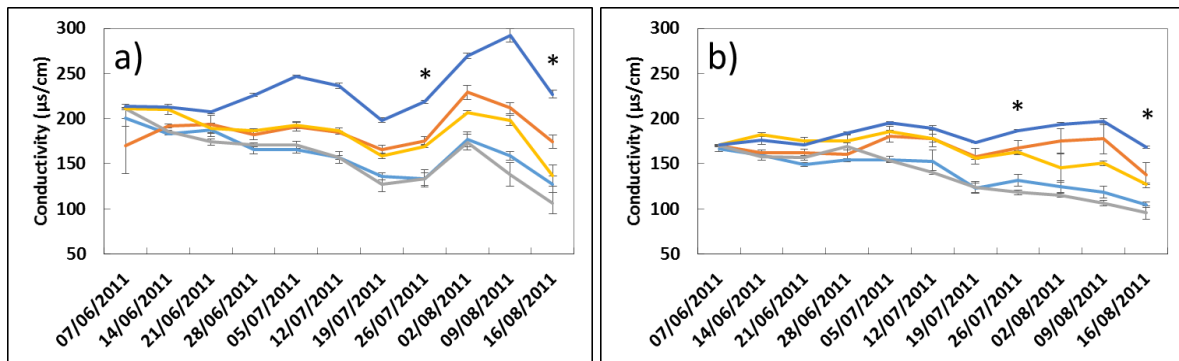
183
184

185 *Table 1. Statistically significant differences between the Phragmites (P), Juncus (J), Iris (I),*
 186 *unplanted (U) and control (C) treatments indicated by Tukey HSD post-hoc tests for Time 1*
 187 *(7th of June), Time 2 (26th of July) and Time 3 (16th of August).*

Parameter	Significant differences Time 1 (7 th of June)		Significant differences Time 2 (26 th of July)		Significant differences Time 3 (16 th of August)	
	Hypereutroph c	Mesotroph c	Hypereutroph c	Mesotroph c	Hypereutroph c	Mesotroph c
pH	PvJ, PvI, PvU, PvC		IvC			
Conductivity			PvJ, PvU, PvC, JvI, JvC, IvU, IvC, UvC	PvJ, PvC, JvI, IvU, IvC, UvP, UvC	PvJ, PvC, JvI, JvC, IvC, UvC	PvC, JvI, IvC, UvC
Phenolics	UvC		PvJ, PvC, JvI, IvC, UvJ, UvC	PvJ, PvC, JvI, JvU, IvC, UvC	PvJ, PvC, JvI, JvU, IvC, UvC	PvC, JvI, JvU, IvC, UvC
DOC	PvC		PvJ, PvC, JvU, IvJ, IvC, UvC	IvC, UvC	JvU, JvC	UvC
Chlorophyll -a			JvU, JvC	PvF, JvU, IvU, UvJ, UvC	PvF, JvU, IvU	PvU, JvU, IvU, UvC
Phosphate	PvU, PvC, JvU, JvC, IvU, IvC				PvC, JvC, IvC, UvC	
Nitrate			JvC, UvC			PvI, PvU

188 In the hypereutrophic treatments, conductivity (assumed to be a measure of total dissolved
 189 nutrients) showed a general decrease over time, except in the control, where conductivity
 190 generally increased (Figure 2a), probably due greater accumulation rates of nutrients
 191 compared with algal uptake and other losses from the water column. An increase in
 192 conductivity was observed in all treatments following nutrient replenishment on the 1st of
 193 August, as might be expected.

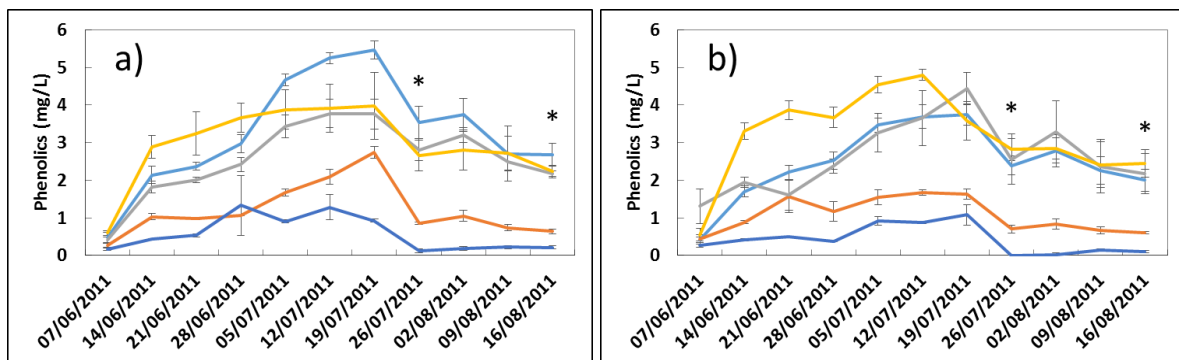
194 Although conductivity values were generally lower (by 30-40 $\mu\text{s}/\text{cm}$) in the mesotrophic
 195 treatments, a similar pattern of decreasing conductivity over time was observed (Figure 2b),
 196 again suggesting similar control mechanisms. Indeed, in both hypereutrophic and
 197 mesotrophic systems, a divergence in mean conductivity levels was evident as the experiment
 198 proceeded, which was confirmed by the large number of significant differences observed
 199 between treatments on the 26th of July and at the end of the experiment (Table 1). In both
 200 systems the following order of conductivity was observed by the end of the experiment:
 201 control > *Juncus* > unplanted > *Phragmites* > *Iris*. Decreasing conductivity in both systems is
 202 likely to be caused by nutrients being removed and bound in the FCW both biologically and
 203 physico-chemically. However, plant species is clearly important since, *Iris* and *Phragmites*
 204 reduced the ions in the water column to the greatest degree, possibly due to enhanced direct
 205 nutrient uptake.



206
 207 *Figure 2. Water column conductivity for hypereutrophic (a) and mesotrophic (b) systems*
 208 *over 10 week experimental period showing Phragmites (light blue), Juncus (orange), Iris*
 209 *(grey), unplanted (yellow) and control (dark blue) treatments. Error bars represent the*
 210 *standard error of the mean (n = 3).*

211 Phenolics and DOC

212 Overall, phenolics concentrations tended to increase during the early part of the experiment,
 213 and decrease during the latter stages (Figure 3a and b). In both the hypereutrophic systems
 214 and mesotrophic systems, the highest phenolics concentrations were detected in the *Iris*,
 215 *Phragmites* and unplanted systems, with intermediate concentrations observed in the *Juncus*,
 216 and the lowest concentrations occurring in the control treatment (Figures 3a and 3b).
 217 ANOVA analysis indicated few significant differences in phenolics levels at the beginning of
 218 the experiment (Table 1) but the difference between *Juncus* and control treatments versus the
 219 *Iris*, *Phragmites* and unplanted systems in the latter part of the experiment was statistically
 220 significant.



221
 222 *Figure 3. Water column phenolics concentration for hypereutrophic (a) and mesotrophic (b)*
 223 *systems over 10 week experimental period showing Phragmites (light blue), Juncus (orange),*
 224 *Iris (grey), unplanted (yellow) and control (dark blue) treatments. Error bars represent the*
 225 *standard error of the mean (n = 3).*

226 Given that release of phenolics from both the unplanted and planted treatments was observed,
 227 and that the control treatment (no FCW) had the lowest phenolics levels, it appears that the
 228 FCW substrate acted as a source of phenolic compounds. High phenolics levels in the
 229 unplanted treatments may also be linked with lower nutrient assimilation (due to the absence
 230 of plants) which increases algal biomass production and hence the release of algal phenolics
 231 (Willoughby 1976).

232 Variations in phenolics levels between planted treatments may be attributed to differences in
 233 root exudates release. Considerable evidence supports differences in chemical composition
 234 and concentration of plant root exudates between different species. For example, Larue et al.

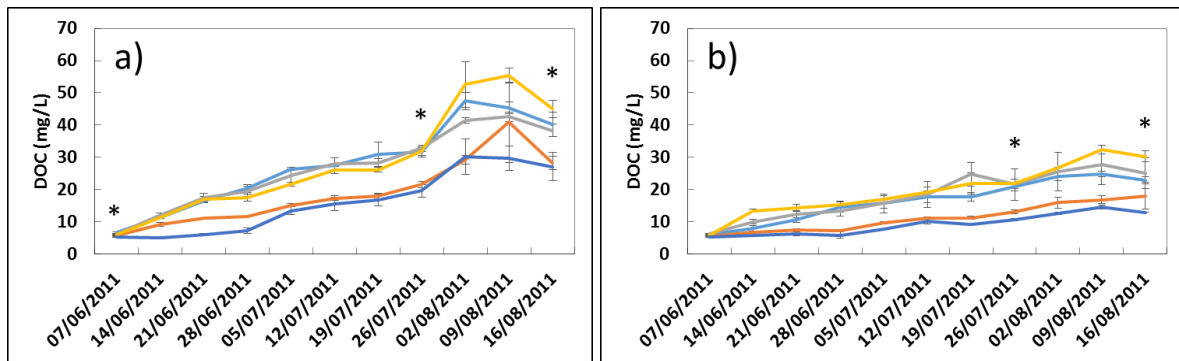
235 (2010) describe how *Iris*, *Typha* and *Phragmites* exhibit varying concentrations of
236 intracellular root tissue phenolics. This is especially evident in *Iris* during the spring, where
237 intracellular phenolic concentrations are found to be more than 10 times higher than in
238 *Phragmites*. However, here, in the hypereutrophic planted systems, higher water column
239 phenolic concentrations were observed in *Phragmites* compared to other species suggesting
240 this phenomenon may not be species specific but may relate to precise environmental
241 conditions related to season, trophic level or plant microbe interactions. Indeed, under
242 mesotrophy, *Iris* produced more concentrated phenolic concentrations than *Phragmites*.

243 Previous research suggests that phenolics release may contribute to algal control due to its
244 inhibitory effect on algal growth thereby acting as a natural algaecide (Pillinger et al. 1994).
245 However, there appeared to be no relationship between phenolics level and algal biomass
246 (below) in this experiment. A number of reasons may explain this: a) high nutrient levels may
247 overcome any inhibitory effect of phenolic compounds on algal growth mediated through
248 nutrient binding (Wetzel, 2001), and b) the type of phenolic compounds, and therefore their
249 effect, may differ depending on the plant and or algal species involved.

250 In both the hypereutrophic and mesotrophic treatments, *Juncus* treatments exhibited
251 significantly lower phenolic concentrations than the other treatments probably due to the fact
252 that these were pre-established; phenolic compounds along with other constituents of the
253 DOC pool (below), leached away *via* rainfall and lateral water flow. This might suggest that
254 over time, phenolics released from FCWs will decline, and thus any algaecidal properties
255 would be lost.

256 In the hypereutrophic systems an increase in DOC over time was observed (Figure 4a). The
257 control and *Juncus* treatments showed very similar and consistently lower concentrations of
258 DOC throughout the experiment, supporting the suggestion that net leaching of dissolved
259 organic carbon constituents will occur over time. Indeed, other factors that change the
260 conditions for nutrient transformation/availability in mature compared with newly planted
261 systems require further research (e.g. detritus and organic carbon build up, biofilm growth)
262 and the incorporation of redox potential measurements could provide useful insight into the
263 such processes over time.

264 A similar trend of increasing DOC concentration over time was observed in the mesotrophic
265 systems (Figure 4b) but with a magnitude of approximately half the concentration observed
266 in the hypereutrophic systems. And, DOC concentrations for individual treatments tended to
267 diverge as the experiment progressed, as evidenced by a number of statistically significant
268 differences on the 26th of July and the 16th of August (Table 1). In the hypereutrophic and
269 mesotrophic systems, a similar order of increasing DOC concentration was observed at the
270 end of the experiment, with the control and *Juncus* treatments showing the lowest levels,
271 followed by *Phragmites* and *Iris*, and the unplanted treatment the highest concentration. The
272 occurrence of highest levels in the unplanted treatment suggests that algal production (which
273 was most pronounced in this treatment) contributed significantly to the DOC pool. An
274 increase in algal biomass (below) could also explain the pulse of DOC observed in both
275 nutrient regimes following nutrient replenishment on the 1st of August. In the planted
276 systems, the nutrient influx may have also increased macrophyte primary production,
277 increasing DOC as exudate production or other associated inputs (e.g. root cell sloughing or
278 decomposition).



279
 280 *Figure 4. Water column DOC concentration for hypereutrophic (a) and mesotrophic (b)*
 281 *systems over 10 week experimental period showing Phragmites (light blue), Juncus (orange),*
 282 *Iris (grey), unplanted (yellow) and control (dark blue) treatments. Error bars represent the*
 283 *standard error of the mean (n = 3).*

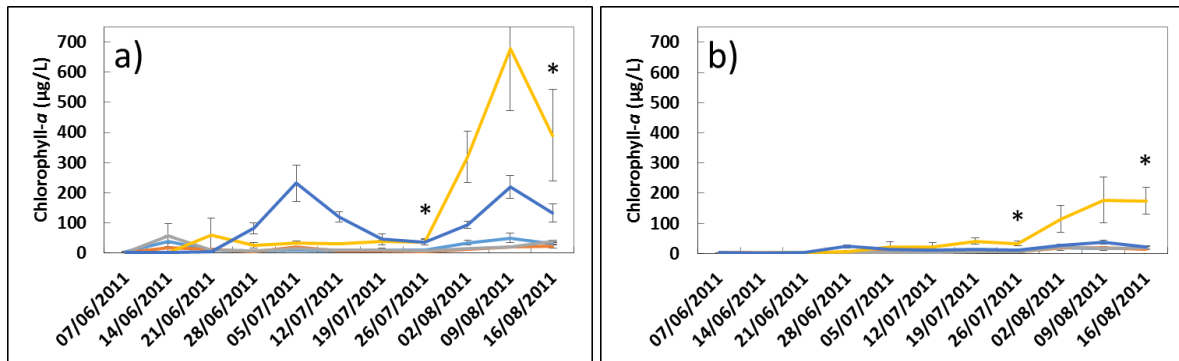
284 Kadlec & Wallace (2008) describe the main processes occurring in a treatment wetland that
 285 contribute to the rhizosphere and pore water DOC pool. The processes included are
 286 solubilisation of chemically bound carbon and the decomposition of structurally bound
 287 carbon. Here there is no reference to the component of the carbon pool produced or removed
 288 by the plants, however, Koretsky & Miller (2008) report that total organic carbon is typically
 289 higher in un-vegetated sites. Jones et al. (2017) also reported a release of DOC from FCWs,
 290 which they attributed to leaching from root exudates and soil organic matter. Smith & Kalin
 291 (2000) report that DOC is released from the rhizosphere of FCWs in significant, and
 292 biologically useful quantities. And, DOC and particularly particulate organic matter released
 293 from such systems could be enough to act as a source of carbon for important remediation
 294 processes such as biomineralisation of metal pollutants and denitrification (see later).
 295 However, high DOC levels can also represent a problem for drinking water, with DOC
 296 removal representing one of the most costly aspects of drinking water treatment.

297 **Algal biomass**

298 In the hypereutrophic systems, both the unplanted treatment and control showed peaks of
 299 chlorophyll-*a* above 200 µg/L (Figure 5a). The control showed a peak on the 5th of July and
 300 on the 9th of August following nutrient replenishment. Following both of these peaks
 301 senescence of algae was observed, manifested in the decrease in chlorophyll-*a* concentration.
 302 The unplanted treatment, however, shows only one large increase in chlorophyll-*a*
 303 concentration following the second nutrient addition, reaching a maximum of approximately
 304 700 µg/L. All the planted treatments showed low concentrations of chlorophyll-*a* for the
 305 duration of the experiment, never increasing above 45 µg/L. In the mesotrophic systems a
 306 distinct rise in chlorophyll-*a* concentration for the unplanted treatment was also observed on
 307 the 26th of July following nutrient replenishment, whilst relatively low concentrations were
 308 observed in the planted systems throughout the test (Figure 5b).

309 In both trophic regimes chlorophyll-*a* concentration in the planted treatments was
 310 consistently lower than that observed in the unplanted or control treatments. In a number of
 311 cases these differences were found to be statistically significant (Table 1). Similarly, Jones et
 312 al. (2017) also reported significantly lower chlorophyll-*a* levels after 4 weeks in FCW
 313 systems planted with *Phragmites*, compared with a control treatment. The presence of plants
 314 therefore contributes to algal bloom control. In the mesotrophic systems, lower
 315 concentrations of chlorophyll-*a* suggest that the growth of algae was limited by nutrient
 316 availability.

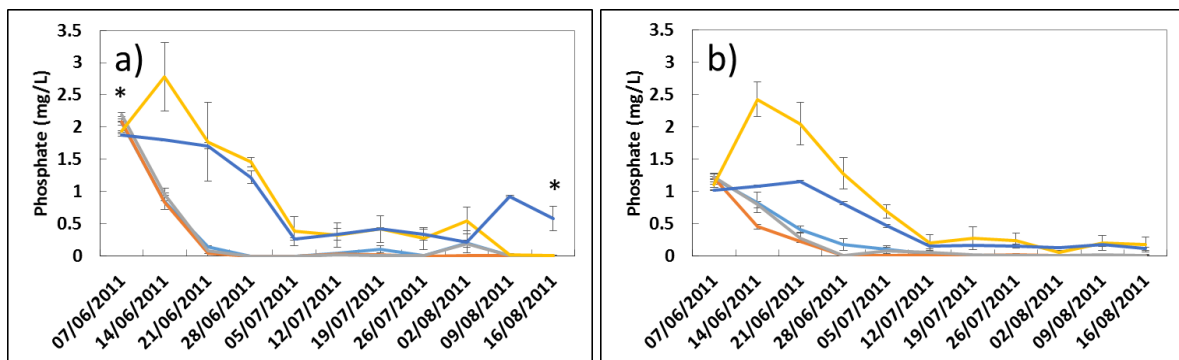
317 Here chlorophyll-*a* is used to provide an estimate of total algal biomass, representing natural
 318 community compositions, however, species identity was not studied here and further research
 319 into the effects of plant species interactions with this component could prove useful in
 320 optimising FCW performance. Along with monitoring temperature and light levels, since
 321 these factors strongly influence algal growth and nutrient uptake. Similarly, investigations
 322 using a specific strain of algae would remove confounding effects of differential algal species
 323 performance, so that results can be compared.



324
 325 *Figure 5. Water column chlorophyll-*a* concentration for hypereutrophic (a) and mesotrophic*
 326 *(b) systems over 10 week experimental period showing Phragmites (light blue), Juncus*
 327 *(orange), Iris (grey), unplanted (yellow) and control (dark blue) treatments. Error bars*
 328 *represent the standard error of the mean (n = 3).*

329 Nutrient cycling

330 For the hypereutrophic systems, all the planted treatments showed a rapid reduction in
 331 phosphate concentration from high concentrations to virtually zero within two weeks of the
 332 start of the experiment (Figure 6a). The control and unplanted treatments both showed a
 333 slower rate of decrease, with concentrations fluctuating around 0.3 mg/L for most of the
 334 remainder of the experiment. Statistical analysis conducted at the beginning of the
 335 experiment showed a number of significant differences between treatments, although the
 336 differences were small (Table 1). At the end of the experiment, the control (no FCW)
 337 treatment showed a significantly higher phosphate level than all the other treatments.



338
 339 *Figure 6. Water column phosphate concentration for hypereutrophic (a) and mesotrophic (b)*
 340 *systems over 10 week experimental period showing Phragmites (light blue), Juncus (orange),*
 341 *Iris (grey), unplanted (yellow) and control (dark blue) treatments. Error bars represent the*
 342 *standard error of the mean (n = 3).*

343 For the mesotrophic systems, phosphate concentrations at the beginning of the experiment
 344 were approximately 50% lower than in the hypereutrophic systems (Figure 6b). However,
 345 similar concentration dynamics occurred; the unplanted FCW rapidly became a source of

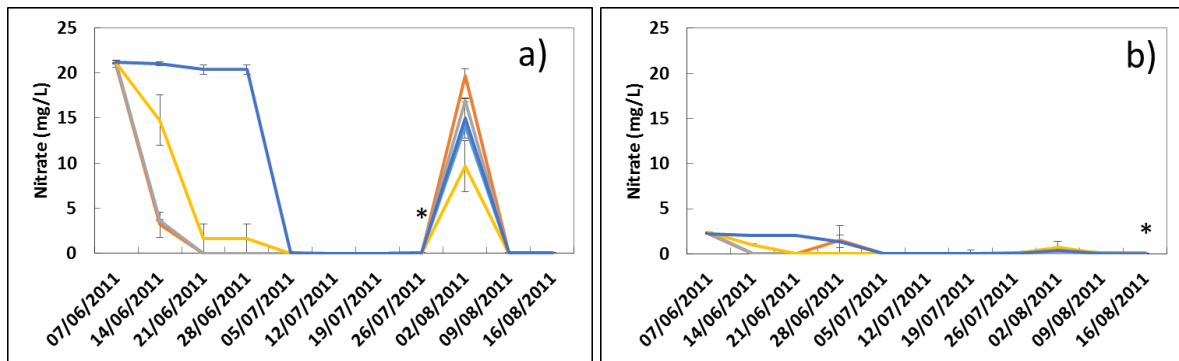
346 phosphorus, before declining, with all treatments showing a dramatic decrease in the first few
347 weeks of the experiment. No statistically significant differences were identified by ANOVA
348 analysis for the time points included.

349 Phosphate is the predominant factor limiting primary production during eutrophication events
350 in freshwater bodies. In both nutrient regimes, a release of phosphate was detected in the
351 early stages in the unplanted FCW system. It is likely organic material is acting as a source of
352 phosphorus, leaching into the water column. Planted systems, in contrast, reduced the
353 phosphate added to the systems at the start, as well as the phosphate leached by the organic
354 material more long term.

355 Whilst phosphate levels in the mesotrophic treatments reached extremely low levels by the
356 22nd of June, in the planted systems, the removal rate in the hypereutrophic system was more
357 rapid. This effect can be explained by the P-k-C* equation developed by Kadlec & Wallace
358 (2009) for the design and scaling of CWs for water pollution control. The method requires
359 knowledge of hydraulic efficiency within the CW and evenness of mixing (referred to as the
360 Tanks in Series model) known by the parameter P, temperature driven compound degradation
361 rates, k, and importantly wetland background concentration given as C*. The latter represents
362 a system cycling and re-release parameter and a potential challenges in CW scaling, due to
363 the fact that as the effluent concentration approaches C* value, it becomes for a pollutant to
364 become removed or degraded. Indeed, manipulating the parameters above could allow more
365 rigorous testing of FCW performance under varied remediation applications.

366 Interestingly, in the hypereutrophic system, an increase in phosphate was observed in week
367 10 for the control system and phosphorus release coincides with the senescence phase of the
368 algal bloom, where chlorophyll a levels begin to decline (Figure 5a). This supports work on
369 algal bloom senescence, post bloom formation where classic effects of eutrophication occur
370 due to the degradation and breakdown of the algal bloom (Wetzel 2001; Schlesinger and
371 Bernhardt 1997; Vitousek et al. 1997; Smith et al. 1999). Zhu et al. (2013) analysed the
372 breakdown of algal blooms from Lake Taihu in the Yangtze River delta with water samples
373 analysed for nutrient pollutant release and effect upon dissolved oxygen. During degradation,
374 phosphate rose rapidly from zero within 15 days of sample collection with a corresponding
375 drop in dissolved oxygen. In both the hypereutrophic and mesotrophic control system then
376 the reduction in phosphate concentration over time is most likely due to algal uptake and
377 subsequent bloom formation, with re-release of immobilised phosphate then occurring during
378 decomposition processes.

379 In contrast, re-release of phosphate was not observed in any of the planted treatments and
380 assuming no substantial algal bloom was formed, there would be no senescence phase during
381 which phosphate could be re-released. Direct plant uptake is therefore likely to be the main
382 driver of phosphate removal in these systems, above that seen due to algae in the control
383 system (no plants or planting medium present) and due physico-chemical phosphate
384 immobilisation along with microbial uptake in the unplanted system.



385
 386 *Figure 7. Water column nitrate concentration for hypereutrophic (a) and mesotrophic (b)*
 387 *systems over 10 week experimental period showing Phragmites (light blue), Juncus (orange),*
 388 *Iris (grey), unplanted (yellow) and control (dark blue) treatments. Error bars represent the*
 389 *standard error of the mean (n = 3).*

390 All hypereutrophic treatments showed initial nitrate concentrations of approximately 22 mg/L
 391 (Figure 7a). For the planted treatments, this decreased to near zero after 2 weeks. The
 392 decrease in the unplanted and control treatments was delayed, particularly in the case of the
 393 control treatments which only started to decrease significantly after 3 weeks. These results
 394 indicate that the algal bloom mitigation observed in the planted treatments was the results of
 395 nutrient removal. Since a dramatic reduction in nitrate was also observed in the unplanted and
 396 control treatment, this cannot be attributed solely to plant uptake. Therefore it is likely that
 397 microbial reduction to nitrite or organic matter binding is also occurring. In the mesotrophic
 398 systems, initial nitrate concentrations were approximately one tenth of the initial
 399 concentration in the hypereutrophic systems (Figure 7b). Although statistically significant
 400 differences in nitrate concentrations were observed between treatments (Table 1), for the time
 401 points analysed, these differences were small.

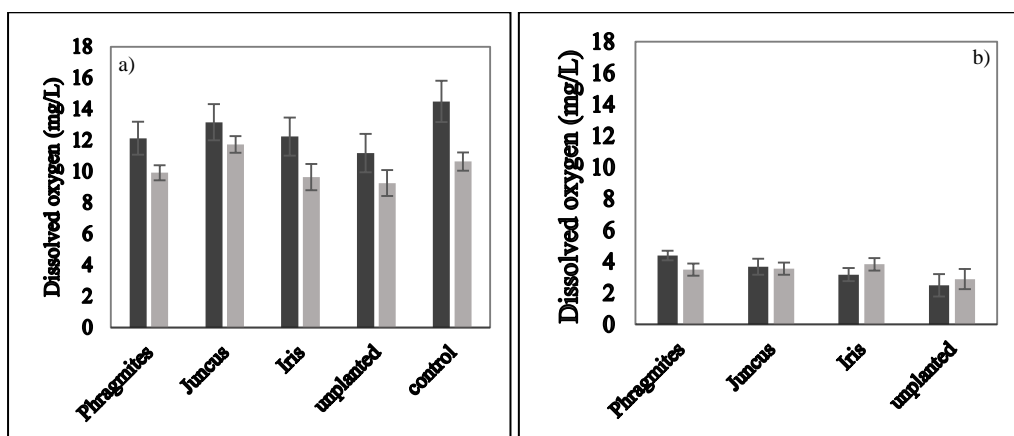
402 Unlike the situation with phosphate where strong limitation may have allowed rapid removal
 403 of replenished phosphate, nitrate concentrations in all treatments peaked sharply following
 404 nutrient replenishment on the 1st of August. It is notable that in all treatments and both
 405 nutrient regimes, rapid nitrate removal was observed following this increase. The fact that
 406 this coincided with substantial increases in algal biomass suggests that assimilation by algae
 407 was responsible.

408 Although freshwater system productivity is generally said to be limited by phosphorus inputs,
 409 nitrogen-based compounds also are known to significantly affect the potential for algal bloom
 410 formation, with nitrogen containing compounds being crucial for biomass development.
 411 Wetlands of various types are able to remediate a range of nitrogen containing pollutants.
 412 Primary mechanisms include nitrification and denitrification by microbial communities in the
 413 rhizosphere of the system (Mitsch & Gosselink 2000). Nitrification takes place when oxygen
 414 demands for the process can be met by radial oxygen loss from arenchymous tissues in the
 415 plant root (Sorrell et al. 2000; Armstrong 1980), whereas denitrification occurs when labile
 416 carbon is plentiful and reducing conditions prevail (Vymazal 2007; Sprent 1987). Treatment
 417 wetlands are able to modify the rhizosphere environment allowing for reducing and oxidising
 418 conditions to prevail within the same “reactor” (Wiessner et al. 2006). However, it is also
 419 well known that algae also release both oxygen and labile carbon compounds, potentially
 420 promoting nitrification directly and denitrification indirectly.

421

422 Dissolved oxygen

423 Dissolved oxygen concentrations in the water column were consistently higher in the
424 hypereutrophic treatments compared with the mesotrophic treatments (Figure 8a) and this
425 may relate to the relative stimulation of primary producers (plants and algae). The control
426 showed the highest concentration of oxygen in the water column, suggesting that algal
427 production was dominant, while the unplanted system showed the lowest levels in both
428 trophic systems suggesting that reduction fuelled by organic matter originating in the planting
429 media was offsetting any algal production. However, measurement of redox potential would
430 help further elucidate these biogeochemical processes. Given the high chlorophyll a levels
431 towards the end of the experiment (Figure 5a, b) perhaps argues against any shading effect or
432 lower nutrient levels inhibiting algal production (Figure 6a, Figure 7a, b). No significant
433 differences between plant species were observed in either trophic level, again suggesting
434 similar controlling factors.



435

436 *Figure 8. Summary of mean water column (a) and pore water (b) dissolved oxygen*
437 *concentrations in hypereutrophic (dark grey) and mesotrophic (light grey) systems for*
438 *different treatments over 10 week experimental period. Error bars represent the standard*
439 *error of the mean (n = 10).*

440

441 Within the rhizosphere of the planted FCW the oxygen concentration was at least half that in
442 the watercolumn (Figure 8b), suggesting that algal oxygen production was dominant or that
443 macrophyte production was offset by reduction processes in the waterlogged planting
444 medium. However, planting (irrespective of species) increased oxygen levels compared with
445 the unplanted system. This supports the considerable amount of literature on the adaptations
446 of wetland macrophytes to waterlogging. Wiessner et al. (2006), for example, discuss how
447 aerenchyma tissues, which allow oxygen release into the rhizosphere, can account for up to
448 60% of the tissue volume in wetland macrophytes. And, vascularisation has been proven to
449 enhance the capability of wetland plants to grow in anaerobic and waterlogged conditions
450 (Jackson & Armstrong 1999). Wetland plants are able to withstand varying degrees of
451 saturations and anoxia dependant on the position along the aquatic to terrestrial continuum
452 that they reside and the degree to which root tissues lose oxygen radially through the root
453 surface varies with species. The latter may explain the modest variations between treatments
454 seen here and/or differences in growth observed over the course of the testing despite initial
455 vegetation biomass balancing.

456 Conclusions

457 Headley et al. (2006) describe multiple benefits of using FCWs over more conventional
458 systems including the opportunity to retrofit into aquatic environments and situations where
459 extreme fluctuations in water level are observed, but also, a significantly greater rate of plant
460 uptake due to the direct suspension of the roots of the system. Despite significant potential for
461 nutrient pollution remediation and algal bloom mitigation, relatively little is known about the
462 mechanisms by which FCWs can be used to treat surface waters. Planting of FCWs with
463 macrophytes has been shown to improve their treatment efficiency, but the effects of
464 planting with different species has received little attention.

465 In both hypereutrophic and mesotrophic systems, FCWs were found to limit algal bloom
466 formation and the associated effects on water chemistry (e.g. significantly increased pH) and
467 planted FCWs consistently performed better than the unplanted treatments. This is proposed
468 to be due to the more rapid nutrient reduction observed in the planted treatments, with direct
469 nutrient uptake possibly responsible, although further research on plant-microbial interactions
470 is needed along with the role that higher dissolved oxygen levels play in the rhizosphere of
471 planted FCW treatments. Importantly, unplanted FCWs appeared to release phosphate in the
472 early stages of the experiment, presumably with the organic substrate acting as the source,
473 suggesting planted systems offer more reliable phosphorus removal. Although no significant
474 differences in algaecidal effects were observed between planted treatments, significant
475 differences in DOC release were observed, with the *Phragmites* and *Iris* treatments showing
476 particularly high levels. Higher DOC levels in the rhizosphere may enhance denitrification
477 and therefore nitrate removal efficiency, but where FCW are employed in reservoir
478 applications the drawbacks of high DOC levels in drinking water sources, including increased
479 treatment costs and potential disinfection byproduct formation, should also be considered.

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