



# Multi-pollutant removal dynamics by aquatic plants in monoculture or mixed communities

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## ARTICLE INFO

### Keywords:

Eutrophication  
Floating treatment wetlands  
Freshwater restoration  
Nature-based solutions  
Phytoremediation: water quality

## ABSTRACT

Much of our knowledge about the phytoremediation potential of floating treatment wetlands (FTWs) comes from studies focusing on the removal of single pollutants, often by a single plant species. Here, we quantify the potential of FTWs planted with varying proportions of the emergent monocots *Typha latifolia*, *Glyceria maxima*, and *Phragmites australis* to simultaneously remove a suite of eleven nutrient/metalloid pollutants. Pollutants most readily removed from water included total inorganic nitrogen (TIN), K and Mn, whilst P, Zn and Cu showed a moderate removal efficiency, and Mg, Ca, Na, Cr, and Fe were poorly removed. Root length within a FTW was correlated with lower concentrations of Ca, Mg, K, P, and Zn remaining in the water, whilst plant uptake and tissue sequestration was more important for reducing concentrations of Mn, TIN, P, and Fe. The effect of community composition over time was greatest for the removal of Zn, with FTWs containing *T. latifolia* having the strongest effect; community type was less important for the removal of TIN, Mg, K, and Na. Plant tissue sequestration was important for reducing concentrations of Mn, TIN, P and Fe in the water, with median uptake values all greater than 12.5%. Importantly, the removal of some pollutants (e.g., Cu) increased with retention time. Therefore, depending on the management objective, FTWs generally perform better where and when residence times are longer e.g., in ponds or streams under low flow, and assembling FTW communities with varying traits and associated removal mechanisms can allow several pollutants to be remediated at once.

## 1. Introduction

The volume of global freshwater available for drinking, food production, energy and the industrial sector is increasingly impacted by current or legacy pollution, e.g., from nutrients and heavy metals (Cantoni et al., 2023). With a rapidly growing human population and increases in living standards and urbanisation, there is an urgent need to maintain and improve water quality (Scanlon et al., 2023). Therefore, regulating pollution in order to protect freshwaters and promote best practice is essential for minimising sub-optimal freshwater quality (Flitcroft et al., 2023; van Rees et al., 2023). However, regulation cannot provide a safeguard against non-compliance, legacy pollution issues, or diffuse pollution arising from disparate land use practise (e.g., fertiliser run-off) where problems are complex and involve multiple stakeholders (Patterson et al., 2013; Wiering et al., 2023). Therefore, remedial solutions that can improve water quality, but can also be combined with regulatory and best management practice approaches (e.g., nature-based solutions), are an important strategy for tackling the freshwater crisis.

Floating treatment wetlands (FTWs) are a form of phytotechnology that are increasingly employed to improve water quality and support freshwater restoration (Colares et al., 2020; Vo et al., 2023). These systems comprise emergent macrophytes rooted in floating platforms growing hydroponically in the water, and facilitate the removal of waterborne pollutants via plant uptake and sequestration, or degradation in the root zone (Fletcher et al., 2020). Owing to their flexibility in deployment, they have been studied and used as remedial solutions in a variety of urban (e.g., ponds, canals, sustainable drainage systems) and rural settings (e.g., rivers, drainage ditches, lakes) for waste-water treatment, water polishing and general water quality improvement (Fletcher et al., 2023; Ma et al., 2021; Shahid et al., 2018). FTWs can be deployed more flexibly into water bodies without the need for significant landscape engineering (which is required for constructed wetlands) and can become an important part of the vegetation on and around waterbodies, providing added value for a range of ecosystem services (Fletcher et al., 2023). Consequently, FTWs have the potential to be incorporated into both 'green' urban waterscapes and used as part of decentralised water treatment systems (Sharma et al., 2021;

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<https://doi.org/10.1016/j.envres.2024.120041>

Received 3 July 2024; Received in revised form 18 September 2024; Accepted 19 September 2024

Available online 20 September 2024

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**Table 1**  
Macrophyte species combinations used in experimental FTWs.

Treatment	Plant community	Treatment Code
1	<i>Typha latifolia</i>	TL
2	<i>Glyceria maxima</i>	GM
3	<i>Phragmites australis</i>	PA
4	<i>T. lat</i> + <i>P. aus</i>	TL + PA
5	<i>T. lat</i> + <i>G. max</i>	TL + GM
6	<i>G. max</i> + <i>P. aus</i>	GM + PA
7	<i>T. lat</i> + <i>P. aus</i> + <i>G. max</i>	TL + PA + GM
8	Open water control	OWC
9	Unvegetated FTW	uFTW

*T. lat*, *Typha latifolia*; *G. max*, *Glyceria maxima*; *P. aus*, *Phragmites australis*.

Stefanatou et al., 2024).

Understanding how to optimise the performance of FTWs for pollutant removal is important for promoting the widespread uptake of this technology and improving its value in enhancing freshwater quality (Pavlineri et al., 2017; Vo et al., 2023). Variables such as macrophyte community selection, hydraulic retention time, vegetation maturation (and associated root growth) are critical factors for the removal of nutrients and heavy metals by FTWs (Fletcher et al., 2023; Pavlineri et al., 2017). However, optimising these factors for the removal of single pollutants is often studied in isolation, with little consideration for the potential of FTWs to target multiple pollutants. Modifying plant community composition (and thus increasing plant functional diversity), or assembling optimal combinations of accumulator species, can enhance the removal of pollutants (e.g., Fletcher et al., 2024; Ge et al., 2015; Geng et al., 2017; Han et al., 2018). Therefore, a mechanistic understanding of how different macrophyte communities perform temporally in terms of multi-pollutant removal and hydraulic retention times could be used to maximise the performance of FTWs by optimising plant community combinations (García Chanc et al., 2019). Importantly, the inclusion of negative controls in experiments (i.e., inclusion of unvegetated FTWs) are also necessary to help identify additional pathways for removing pollutants, such as shading, and surface area provision from microbial biofilm development (Shahid et al., 2020; West et al., 2017).

The aim of this study was to use a temporally high-resolution approach to quantify the removal of a suite of nutrient and metalloid-based pollutants by FTWs in contaminated waters and determine how plant community composition interacts with temporal and plant biophysical factors. Specifically, our objectives were to: (1) quantify and compare the removal efficiencies of macrophyte communities at two different hydraulic retention times and with different plant combinations; (2) assess the temporal, physiochemical and ecological factors influencing pollutant removal efficiency; and (3) calculate the mass balance of pollutant transfer to identify the influence of plant uptake on pollutant removal.

2. Methods

2.1. Plant selection

Large-statured emergent monocots *Typha latifolia*, *Glyceria maxima* and *Phragmites australis* were selected based on their commonality, fast growth rate, ability to readily take-up nutrients and their widespread use as phytoremediation candidates (Brisson and Chazarenc, 2009; Vymazal, 2007). These species are all native to the UK where they are typical components of the vegetation of fertile freshwaters and often coexist. Assembling communities with different proportions of each species enabled species interactions and influences of species-specific traits to be investigated (Table 1). A more detailed description of the phytoremediation potential of these species combinations is described in Fletcher et al. (2024).

**Table 2**  
Final concentration of target pollutant in each experimental mesocosm.

	Concentration (µg/L)
Total Ammonia (NH <sub>3</sub> )	254
Nitrite (NO <sub>2</sub> <sup>-</sup> )	9
Nitrate (NO <sub>3</sub> <sup>-</sup> )	2311
Calcium (Ca)	7707
Chromium IV (Cr)	74
Copper (Cu)	34
Iron (Fe)	2289
Potassium (K)	10,619
Magnesium (Mg)	6152
Manganese (Mn)	358
Sodium (Na)	5634
Phosphorus (P)	963
Zinc (Zn)	162

2.2. Experimental design

Experiments were carried out in the growing season between July and September 2018 and were housed in two open-ended polytunnels (3 m × 2 m x 2 m) to avoid dilution effects from rain, and reduce variations in temperature. Macrophyte communities were planted in experimental mesocosm FTWs, which were designed to be buoyant and allow hydroponic growth of roots into the growth media. Each FTW was constructed from white 40 mm diameter polyethylene pipe (44 cm × 32 cm) with 12 modified hydroponic plant pots (12 cm depth and diameter of 7 cm) joined with plastic cable ties (illustrated in Fletcher et al. (2024)). The 12 planting spaces gave a planting density equivalent to 85.2 plants per m<sup>2</sup> and was designed to simulate natural plant interactions (Pavlineri et al., 2017; Jones et al., 2017). Each FTW was placed into a clear polypropylene plastic tank (0.56 x 0.39 × 0.42 m) with a maximum volume of 50 l. There were four replicate tanks per treatment, and all replicates were assigned to two adjacent open-ended polytunnels. To understand the plant-specific removal of pollutants, two types of control FTWs were used, (1) an unvegetated control (uFTW) that had no plants but still contained the frame structure of the FTW, and (2) an open water control (OWC) that contained neither plants nor the FTW frame. Over the duration of the experiment the mean air temperature was 14.4 °C; water temperature 16.6 °C; and light intensity 29.5 Klux.

Mesocosms were developed to simulate a scenario typical of urban and semi-rural environments impacted by multiple pollutants and the concentrations used were informed by preliminary field sampling of freshwaters (Fletcher et al., 2022). Each mesocosm contained modified Hoagland's nutrient solution containing a combination of target pollutants (Table 2) topped-up with tap water to a total volume of 50 l, which allowed enough space for root growth but avoided hypoxia. The experiment was designed to simulate a batch-fed wetland with a two-week hydraulic retention time (HRT); therefore, over the ten-week experimental period there were five batches in total. At the start of each batch period, all water was removed from each mesocosm, and the container cleaned; a new supply of Hoagland's solution, target pollutants, and tap water was added as described above. To reduce potential edge effects, the innermost two mesocosms from each row within the polytunnel were re-positioned to the outside end of the row at the beginning of each new batch period; this allowed all mesocosms to occupy a different part of the polytunnel over the course of the experiment.

*T. latifolia*, *G. maxima*, and *P. australis* were supplied as pre-grown seedlings (www.salixrw.com), individually propagated in 110 cm<sup>3</sup> plugs. The growth media used for propagation (20 % loam and 80 % peat) was carefully washed from the roots to reduce nutrient input into the mesocosms. A random number generator was used to allocate individual macrophytes to the twelve spaces of each experimental FTW. The hypocotyl area of each plant was wrapped with 2.6–3.4 g of coir fibre to

provide support for the stem and protect the roots from direct sunlight. The fresh weight, maximum stem height, and number of stems were recorded for each individual plant at the time of planting. To allow for acclimatation, all FTWs were subsequently placed in 25 % strength Hoagland's solution for 14 days prior to the experiment commencing.

### 2.3. Sampling strategy

Water samples were taken from the centre of the mesocosm at a depth of approximately 10 cm. On day 1, four random mesocosms were sampled to obtain a mean of initial concentrations of pollutants and then every mesocosm was sampled on day 7 and 14. Within 4 h of collection, all water samples were vacuum filtered through 1 µm pore-size Whatman (Whatman PLC, Buckinghamshire, UK) glass microfiber filters to remove particulate material. Filtered samples were then preserved for bulk analysis by freezing at -20 °C. Dissolved oxygen was quantified directly in the water of each mesocosm on day 1, 7 and 14 for each of the five batches using a HACH LDO101 Field Luminescent/Optical sensor (HACH, UK).

A SEAL Analytical AA3 Continuous Segmented Flow Autoanalyzer was used for determination of nitrogen species (NH<sub>3</sub>, NO<sub>2</sub>, NO<sub>3</sub>) using SEAL analytical method No. G-171-96 (Revision 8) and No. G-172-96 (Revision 9; SEAL Analytical), and total inorganic nitrogen (TIN) was calculated by the summation of these three nitrogen species (NH<sub>3</sub>, NO<sub>2</sub>, NO<sub>3</sub>). Total phosphate (<1 µm particle size) and metalloid elements were quantified by inductively coupled plasma spectrophotometry (ICP-Optical Emission Spectrometer, Thermo Scientific iCAP 6000 Series ICP; Thermo Scientific, UK). Removal efficiency (RE), was calculated for each batch as the reduction in the concentration of each pollutant, using Equation (1):

$$\text{Removal efficiency (\%)} = \left( \frac{C_1 - C_2}{C_1} \right) \times 100 \quad (1)$$

where  $C_1$  is the concentration of pollutant on day 1 and  $C_2$  the concentration of pollutant on day 7 (or day 14).

To understand the contribution of direct plant growth on pollutant removal across each week of the experiment, the following growth indicator (G) was used to estimate relative growth:

$$\text{Growth Multiple (G)} = \frac{\text{Total No. of live stems}}{\text{Maximum plant height (cm)}} \quad (2)$$

Relative growth rate (RGR) of each individual plant was then calculated from the start of each week by the following equation:

$$\text{RGR} = \frac{\ln G_2 - \ln G_1}{T_2 - T_1} \quad (3)$$

where  $G_2$  is the growth indicator at time 2 and  $G_1$  is the growth indicator at time 1.

At the end of the experiment, all above-ground and below-ground plant material was harvested separately, and oven dried at 75 °C to achieve a constant dry weight. Representative composite samples of dried above-ground (shoots and leaves) and below-ground (roots and rhizomes) biomass for each species within each replicate tank were pulverised using a RETSCH RS200 vibratory disk mill (RETSCH, Germany). The resultant powder was analysed for total C and N using a C:N analyser (FlashSmart NC ORG, ThermoFisher Scientific, UK). Sub-samples were also microwave-digested with 70 % nitric acid and analysed for P and metalloid element concentration using ICP spectrophotometry. Tissue nutrient concentration was quantified for each species within each replicate community, and the mean dry weight biomass per replicate calculated (weighted by proportion of biomass) to generate a representative tissue nutrient concentration. To calculate the total net gain of pollutants in plant tissue across the full experiment, the initial standing stocks of each community were quantified. At the start of

the experiment, five juveniles of each species were weighed and processed following the above approach to determine biomass and tissue concentration for a representative sample of each species. These standing stocks were multiplied up for each community depending on number of individuals of each species per community.

### 2.4. Statistical analysis

All statistical analyses were undertaken using R version 3.5.3 (R Core Team, 2019). Treatment means were calculated for each variable per replicate or by treatment depending on the subsequent analysis. An adjusted-removal efficiency (A-RE %) was calculated using Equation (4) to account for any pollutant removal by the open water control (OWC) treatments:

$$\text{Adjusted Removal Efficiency (\%)} = \text{RE(Replicate)} - \overline{\text{RE(Open water control treatment)}} \quad (4)$$

where Adjusted Removal Efficiency (%) (Adjusted-RE) is the difference between the RE (%) of each treatment replicate per week and the mean of the RE (%) of the OWC treatment replicates per week.

T-tests were carried out when comparing treatment means to open water controls and ANOVA was used to compare multiple mean values (followed by a post-hoc Tukey Test). Where data did not conform to parametric assumptions, non-parametric equivalents were employed including Wilcoxon test and Kruskal-Wallis (with post-hoc Dunn test). Aside from the analysis of the adjusted removal efficiencies, all other statistical analyses used pollutant concentrations from each treatment replicate per week rather than percentages. Relationships between selected variables were quantified using Pearson's Product correlation coefficient applied to square root-transformed concentration data.

To understand the effects of treatment and time on pollutant removal, general linear models including treatment, time (i.e., experiment duration in weeks) and their interaction were constructed for each pollutant. In this model the interaction term is of primary interest as it focuses on differences in the rate of change of pollutant concentrations between treatments. All pollutant concentrations were Z-scored and mean-centred to standardise units across pollutant types. As treatment type was categorical, the open water control (OWC) served as the reference level in the model. The interaction effect sizes and their confidence interval were plotted as a forest plot ranked in order of mean effect size per pollutant.

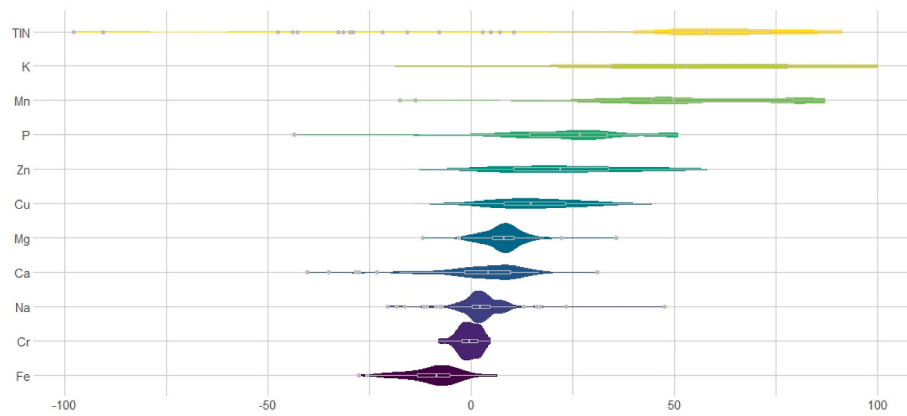
Mass balance of pollutants for each treatment replicate was calculated using Equation (5), to understand the removal mechanisms for each element and treatment:

$$\text{Total pollutant input per replicate (mg)} = T_c + \sum T_t + \sum T_b \quad (5)$$

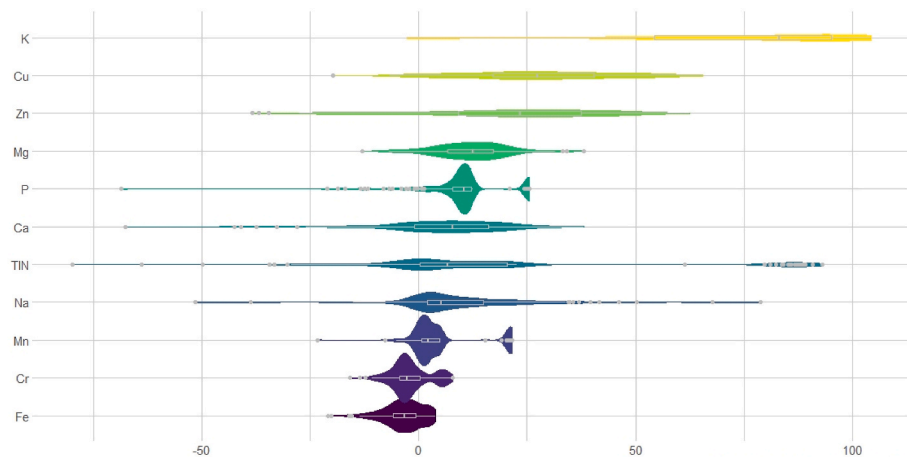
Where  $T_c$  is the total quantity of pollutant in the pre-experiment conditioning phase,  $T_t$  is the total quantity of pollutant after top-up with tap water, and  $T_b$  is the total intended quantity of pollutant input for all batches supplied via modified Hoagland's solution. Pollutant sequestered per community was calculated using Equation (6):

$$\text{Total pollutant sequestered (mg)} = ((B_e \times TC_e) + \dots) - ((n \times (\overline{X} B_s \times \overline{X} TC_s)) + \dots) \quad (6)$$

Where  $B_e$  is the mean of the combined biomass of each species within a replicate,  $TC_e$  is tissue composite concentration of each individual species within a replicate,  $\overline{X} B_s$  is the mean biomass of the reference plants ( $n = 4$ ) at the start of the experiment (before the conditioning phase),  $\overline{X} TC_s$  is the mean tissue concentration of a specific pollutant in the reference plants ( $n = 4$ ) at the start of the experiment (before the conditioning phase).  $n$  references the number of individuals of a certain species in a specific community treatment e.g., in the treatment "*G. max* + *P. aus*" there are six *G. maxima* individuals and six *P. australis*



**Fig. 1.** Adjusted removal efficiencies for a 7-day hydraulic retention time for each pollutant. Violin plots show variation around the mean and box plots show median and interquartile range and outliers. Each pollutant is ranked from top to bottom by order of greatest mean removal efficiency relative to the open water control treatment.



**Fig. 2.** Adjusted removal efficiencies for a 14-day hydraulic retention time for each pollutant. Violin plots show variation around the mean and box plots show median and interquartile range and outliers. Each pollutant is ranked from top to bottom by order of greatest mean removal efficiency relative to the open water control treatment.

individuals.

Equation (7) was used to calculate the total pollutant removal from each mesocosm:

$$\begin{aligned} \text{Total pollutant removed by plants (mg)} \\ = \text{Total pollutant sequestered (mg)} \\ - \text{Total pollutant input per replicate (mg)} \end{aligned} \quad (7)$$

To allow the plant uptake of different pollutants to be compared between treatments, the direct contribution of plant uptake to pollutant removal was calculated as a percentage following Equation (8):

$$\begin{aligned} \text{Proportion of uptake by plants (\%)} = \frac{\text{Total pollutant sequestered (mg)}}{\text{Total pollutant input per replicate (mg)}} \\ \times 100 \end{aligned} \quad (8)$$

### 3. Results

#### 3.1. Removal efficiencies of vegetated and unvegetated FTW systems relative to controls

There was a significant difference between treatments and open water controls for all pollutants ( $P < 0.05$ ) except Cr (Fig. 1). For all FTW systems there was wide variation between and within each individual

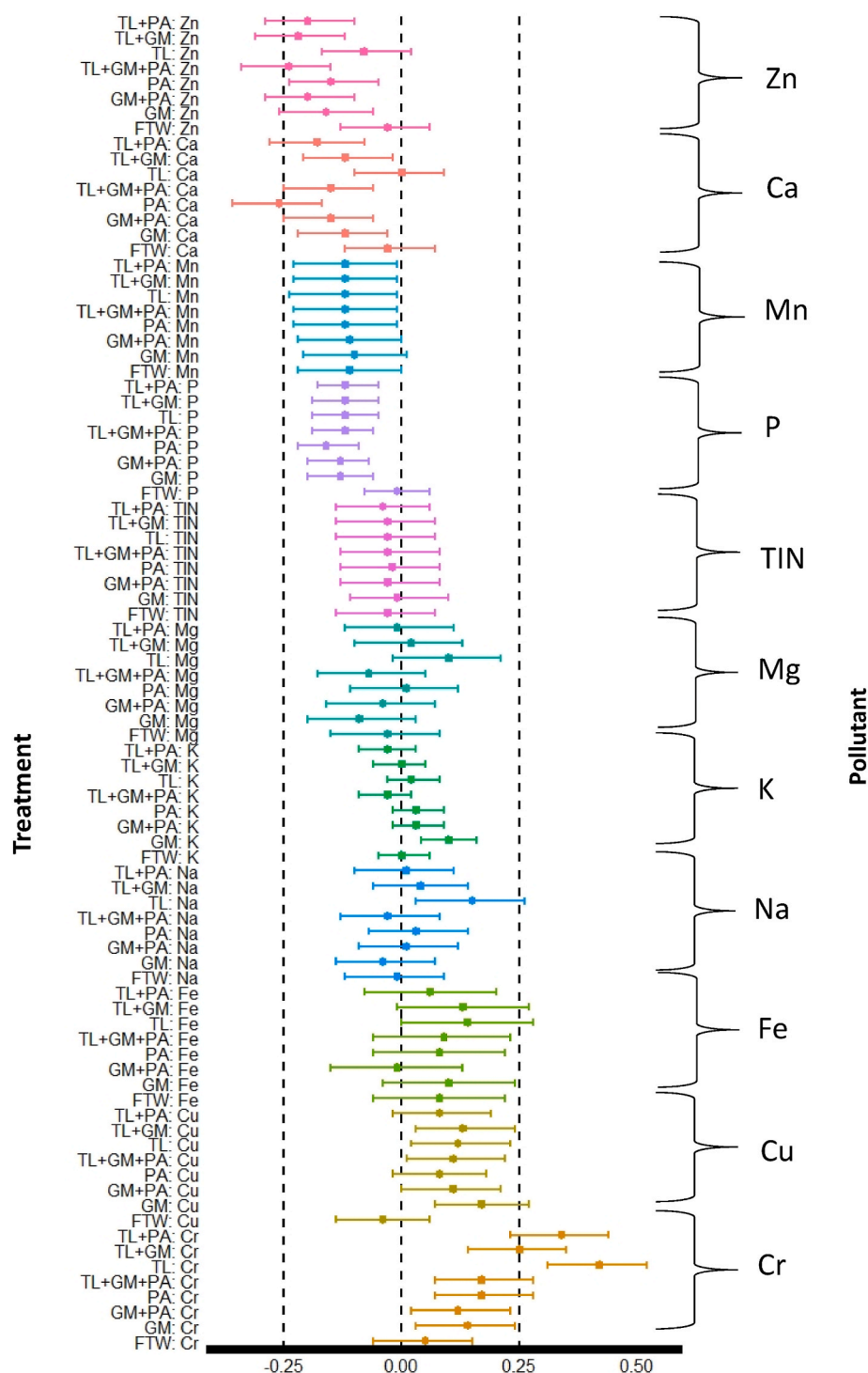
pollutant (Fig. 1) for adjusted-removal efficiency (a-RE) with a 7-day HRT. Average a-RE values for pollutant removal within each mesocosm ranged from 55 % (most readily removed) for TIN to –9 % for Fe. Pollutants most readily removed by either the vegetated or unvegetated FTWs included TIN, K and Mn. Pollutants such as P, Zn and Cu showed a moderate a-RE, whilst Mg, Ca, Na, Cr, and Fe were poorly removed or even increased within the vegetated mesocosms relative to the controls as indicated by low or negative a-RE.

There was also a significant difference ( $P < 0.05$ ) between treatment and controls for all pollutants with a 14-day HRT (Fig. 2). The descending order of pollutants (i.e., those that showed the highest adjusted-RE to those lowest) was different at 14-day HRT compared to a 7-day HRT (Figs. 1 and 2), indicating a different a-RE profile for certain pollutants. After 14 days, K was removed most readily, followed by Cu and Zn at 80 %, 26 % and 23 %, respectively. The average adjusted-RE for all other pollutants was 12.5 % or below, demonstrating that after 14-days there were only small differences between the vegetated treatments and open water control in these cases.

#### 3.2. Relationship between time and macrophyte community type on the effect size of pollutant removal

For each pollutant, there were both negative and positive interactions between the macrophyte community type and the duration of the experiment (Fig. 3). A negative interaction indicated that with



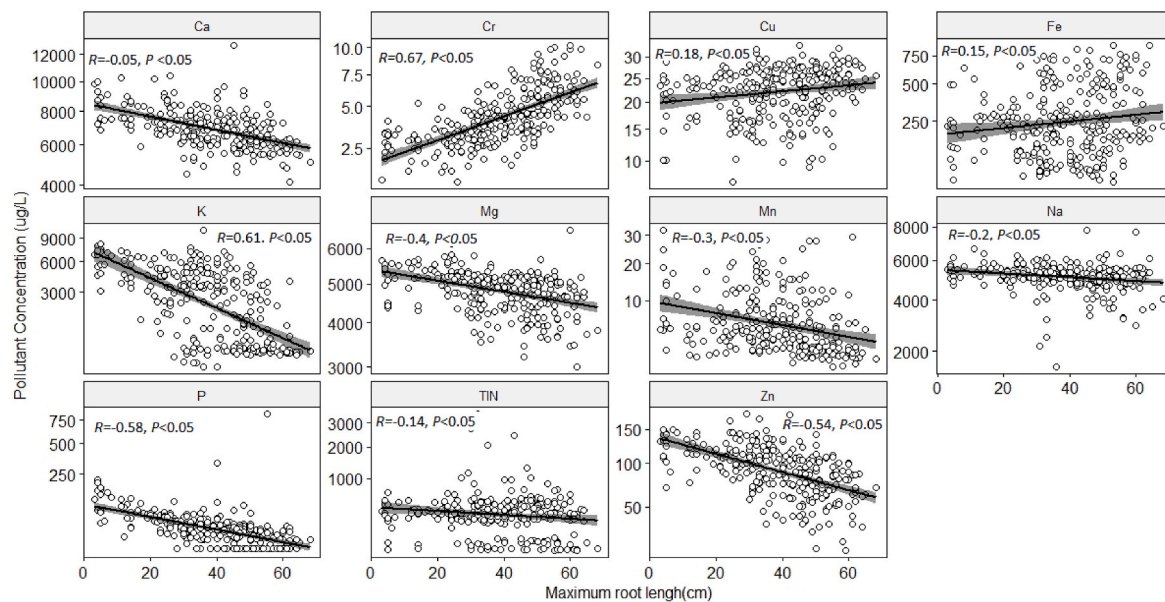


**Fig. 3.** Forest plot of the 'treatment x experiment duration' effect sizes and associated 95 % confidence interval for each pollutant. Vegetated treatments are ranked in order of the mean effect size per pollutant. Effect sizes below 0 indicate declines in pollutant concentration from 0 to 14 days, those above 0 indicate increases.

increasing experimental duration pollutant removal increased from 0 to 14 days (i.e., concentrations declined), whilst a positive relationship showed the reverse. An effect size close to zero indicated a weak combined influence of treatment and time on removal efficiency. The strongest interaction of community type with time was seen for the removal of Zn (Fig. 3). Effect sizes were also predominantly negative for Ca, Mn, and P, implying increased pollutant removal over time, but, by contrast, positive effect sizes for Cr, Cu, and Fe revealed a loss of removal capacity (Fig. 3).

For Zn, the community types containing *T. latifolia* had the most

negative effect sizes. The *P. australis* monoculture, followed by all other communities and treatments, had the strongest overall negative effect size for Ca removal (Fig. 3). There were only small differences in the effect size at the treatment level for Mn and P indicating plant community type had little influence on their removal over time. With effect sizes around zero, and uncertainty ranges spanning both positive and negative values, the interaction between time and type of plant community was likely unimportant for determining removal of TIN, Mg, K, and Na (Fig. 3). For the unvegetated FTW treatment (uFTW) effect sizes were generally close to zero indicating consistently weak effects of the



**Fig. 4.** Scatter plots of the relationship between maximum root length and pollutant concentrations in mesocosms after 7 and 14 days. Correlation coefficients and their p-values are given for each plot. Fitted line based on a simple linear regression. Pollutant concentrations are displayed on a square root scale.

experiment duration on pollutant removal.

### 3.3. Pollutant removal from water as a function of root length and macrophyte combination

There were strong negative correlations between concentrations of Ca, K, P and Zn in the simulated polluted water and maximum root length of vegetated FTWs (Fig. 4;  $P < 0.05$ ) whilst concentrations of Mg and Mn in water correlated weakly and negatively with root length. By contrast, Cr concentrations had a strong and positive relationship with root length ( $R^2 = 0.67$ ;  $P < 0.05$ ), with Cu and Fe being weakly positively correlated to it. The negative correlation between experiment duration and maximum root length suggested that the temporal effects seen for Ca, K, P, Mg and Mn in Fig. 4 may also be related to root growth and development. Turbidity in all mesocosms was low and there were no significant differences at a 7-day HRT; however, at a 14-day HRT, the open water control (OWC) had significantly higher levels of turbidity than all other treatments (data not shown). Between the vegetated treatments there were few differences in the pH of the simulated polluted water, with a median pH of 6–7. However, at 7- and 14-day HRTs both the OWC and the unvegetated FTW (uFTW) had a higher pH (ranging from pH 8–11) across the duration of the experiment compared to the vegetated treatments.

Community composition in the vegetated FTWs influenced the concentration of Zn, P, Mn, Cu, Cr, Ca, K and Mg in the water within each mesocosm (example data shown for Zn, Cu, and Ca in Fig. 5), while there were no effects of community composition on the concentrations of TIN, Fe and Na. Significant differences were mainly driven by the presence and total proportion of *T. latifolia* and *P. australis* within each community (Fig. 5). Concentrations of Zn and P remaining in the water after 7 days were significantly lower in those mesocosms planted with communities containing *T. latifolia* ( $P < 0.05$ ) compared to those without *T. latifolia* ( $P > 0.05$ ). Although the concentrations of pollutants remaining in the water were lower when planted with 100, 50, and 33 % *T. latifolia* there was no significant difference between these three FTWs. The concentration of Ca remaining in the water treated with an FTW containing 100 % *T. latifolia* was significantly lower than those containing either 50 % and 33 % *T. latifolia*; Fig. 5;  $P < 0.05$ ) indicating a cumulative effect on Ca removal from the water with increasing proportion of *T. latifolia*. This trend with communities containing *T. latifolia*

was also observed in the removal of Mn, K and Mg (data not shown).

Water in mesocosms containing *P. australis* had a lower concentration of Cu compared to mesocosms with FTWs not planted with *P. australis* (Fig. 5;  $P < 0.05$ ). Mesocosms planted with *P. australis* monoculture FTWs also had significantly lower water concentrations of Cu compared to communities with bi and polyculture (with 50 % and 33 % *P. australis* respectively) (Fig. 5). There were no significant differences between the number of each species in each FTW and the remaining pollutant concentration in the water, suggesting that macrophyte species richness of FTWs over this range does not impact pollutant removal from the water. The only exception was K where an increase in number of macrophyte species was associated with a significant decrease in the concentration of K in the water ( $P < 0.05$ ).

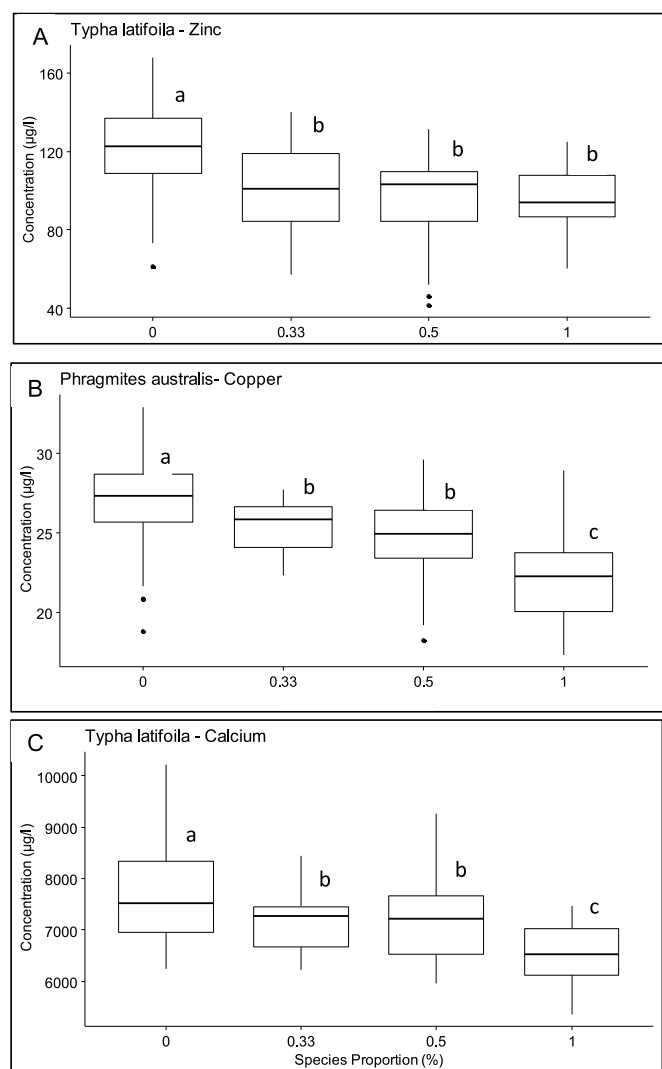
Plant uptake and tissue sequestration was important for reducing concentrations of Mn, TIN, P and Fe in the water, with median uptake values all greater than 12.5 % (Fig. 6). Uptake and sequestration also contributed to around 10 % of removal of Cr; while for Zn, K, Cu, Ca, Mg, and Na the median value was considerably less (i.e., < 4 %).

## 4. Discussion

### 4.1. Effectiveness of FTWs for pollutant removal and sequestration

Floating treatment wetlands planted with macrophytes can effectively remove waterborne pollutants including TIN, K, Mn, and enhance the removal of P, Zn and Cu from contaminated water. Based on the results of this study, these pollutants could be targeted most readily as part of FTW systems. However, Ca, K, Mg and Cu removal was between 20 and 40 % lower than reported elsewhere, which is probably a result of other studies using much higher starting concentrations of these pollutants (Han et al., 2018; Tanner and Headley, 2011). Practically, this means that where FTWs are deployed for removal of Ca, K, Mg and Cu then prior assessment of water quality should be established to determine whether concentrations are high enough to make this strategy viable. Alternatively, other forms of remediation could be employed in combination with FTWs, for example, 'hybrid wetlands', which employ bivalves such as freshwater clams or submerged macrophytes to support further pollutant removal (Fletcher et al., 2020).

Increasing the HRT did not improve the removal of pollutants such as TIN, Mn, and P by vegetated FTWs, and for these pollutants a 14-day



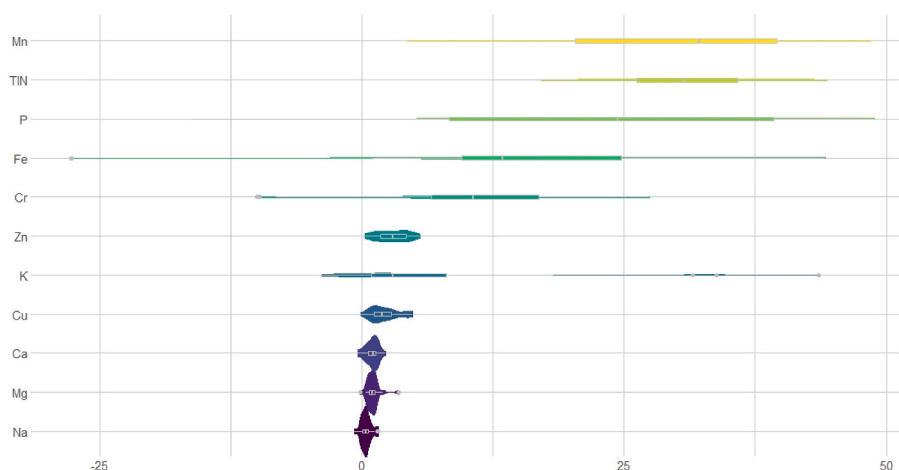
**Fig. 5.** Concentration of three selected pollutants influenced by the proportion of three species of macrophytes in a FTW, (A) *Typha latifolia* and zinc, (B) *Phragmites australis* and copper, and (C) *Typha latifolia* and calcium. Boxes with different letters are significantly different from each ( $P < 0.05$ ; post-hoc Tukey test).

HRT resulted in macrophyte removal dynamics similar to the rate of removal in the unplanted open water control. Previous studies have demonstrated that shorter HRTs are more effective for certain pollutants, e.g., with maximum removal occurring within seven days or less (Van de Moortel et al., 2010). From an operational perspective, and to increase the value of FTWs, exploiting a shorter HRT is important (Zhang et al., 2011), unless specific pollutants are being targeted, e.g., K, Cu and Zn, which do benefit from an increased HRT of 14 days.

The sequestration of Mn, TIN, P, Fe and Cr in plant tissue advocates the periodic harvesting of plants on FTWs to export these pollutants from the freshwater system (Dekle et al., 2024). However, as sequestered pollutants are mainly stored in below-ground plant parts, the opportunity to recover pollutants would require whole-plant harvesting (Garcia Chanc et al., 2019). In practical terms, harvesting belowground plant tissue in a FTW disturbs root zone pollutant removal processes, which often continue during plant dormancy in winter (Soana et al., 2018), and could even mobilise legacy pollutants stored in sediments below the FTW. Therefore, harvesting above-ground plant biomass is a more viable strategy with significant concentrations of P and N sequestered in above ground tissue (Wang et al., 2015). However, if the sole purpose of water quality improvement was to remove Zn, K, Cu, Ca, Mg, and Na, then unless known metal hyperaccumulator plant species were employed (Verkleij et al., 2009), harvesting FTWs is not recommended as these pollutants represent only a small fraction of plant uptake and sequestration. In such circumstances, passive management of the FTW may be more appropriate as opposed to active management, although plant biomass could be harvested periodically to avoid cycling of pollutants back into the system (Quilliam et al., 2015). However, the removal and/or sequestration of pollutants into plant tissue could be further enhanced by various means of bioaugmentation or artificial aeration that can intensify the removal mechanisms and microbial communities that support phytoremediation (Zhang et al., 2023).

#### 4.2. Factors determining pollutant removal by FTWs

The significant correlation between root growth and time suggests that root length is an important co-varying factor and the underlying trait for driving the removal of Ca, Mg, K, P and Zn. Biological and chemical interactions in the rhizosphere can also affect pollutant removal, and plants with longer and denser roots can more effectively scavenge nutrients because of enhanced contact with the growth media (Urakawa et al., 2017; Wang et al., 2015). Microorganisms commonly form biofilms on the surfaces of roots and rhizomes where they can metabolise pollutants (Shahid et al., 2020), whereas suspended pollutants can sorb onto organic matter in the water column (e.g., from root



**Fig. 6.** The percentage of pollutant removal accounted for by plant uptake and sequestration, violin plots show variation around the mean and box plots show median and interquartile range and outliers. Each pollutant is ranked by order of greatest median percent removal.

exudate) leading to flocculation and settlement into the sediment (Tanner and Headley, 2011). It is likely that a combination of these two removal pathways contributed to the removal process in the experimental mesocosms, although the level of pollutant removal via plant uptake and sequestration suggests that plant-mediated processes associated with biofilm development on roots were most important (Tomczyk et al., 2024). If the objective of water quality improvement is to target the removal of Ca, Mg, K, P and Zn, then selecting macrophyte species with rapid and extensive fibrous root growth for inclusion in FTWs would be most advantageous.

The adjusted removal efficiency for TIN was not significantly different between any of the vegetated FTWs, which suggests that all plants had similar affinity and/or removal mechanisms for N. Plant uptake accounted for a significant portion of TIN removal (between 12.5% and 45%) suggesting that sequestration was a key mechanism for removal. Denitrification is one of most important processes for removal of nitrate from aquatic systems; however, dissolved oxygen levels in the water were consistently above the threshold for a switch to anaerobic conditions (i.e., below 1 mg/l) and therefore the dominant N cycle processes would have been nitrification of the ammonia fraction (concurrently increasing the concentration of plant-available N). The short HRT of 7-days was most effective for TIN removal, although after 14 days, the level of TIN removal in the open water controls was equal to that in all the vegetated FTWs. The elevated turbidity and algal mass observed within the open water control signify that algal growth probably accounted for significant uptake of TIN in this treatment. While algal growth was less prevalent in the vegetated treatments, towards the end of each 2-week batch, algal growth was visible on the tank sides and in the water. Immobilisation of N due to algal growth was therefore a likely mechanism for N removal in the vegetated FTWs in combination with macrophyte uptake. Unvegetated FTWs had low and negative adjusted removal efficiencies suggesting that shading by the FTW platform reduced algal growth, which together with the absence of uptake by macrophytes resulted in less TIN removal than either the open water controls or the vegetated FTWs.

Previous studies have proposed sorption to plant roots or induced settlement to be key mechanisms for the removal of Cu (Borne et al., 2013). However, root length did not correlate well with Cu, and FTWs containing *P. australis* (the species with the lowest root growth and shortest root length) had the greatest Cu removal. Importantly, however, *P. australis* did have the largest and thickest rhizomes, and therefore may promote flocculation of Cu by releasing more organic material as exudates, or have increased affinity for Cu due to specific transporter proteins (Printz et al., 2016). Hence the removal of Cu in FTWs containing *P. australis* was improved with HRT and was significantly higher than the control. The pH, combined with plant uptake, also likely contributed to the removal efficiency of certain pollutants, e.g., Fe. The pH of the water in the vegetated FTW treatments was lower (due to the release of protons through plant roots) than in both unvegetated control treatments, which would promote Fe precipitation (Bassez, 2018). Based on the results presented here, macrophyte phytoremediation of Cr is not recommended as the vegetated FTWs did not have a significant influence on Cr concentration.

#### 4.3. Plant community ecology and phytoremediation

The influence of species-specific plant traits on removal mechanisms can be affected by the community composition (Brisson and Chazarenc, 2009; Fletcher et al., 2024). For example, the extensive root growth of *T. latifolia* can have a positive impact on community-based phytoremediation, and there was evidence of proportionality between the removal of pollutants and the occurrence of *T. latifolia* in FTWs, with significant differences between communities containing *T. latifolia* in bi and mixed-cultures, versus monocultures. However, the lack of a significant difference in removal dynamics of P and Zn between the different communities containing *T. latifolia* suggests that in some cases

the overall proportion of a given plant species is less important than the specific trait that it brings to the community. Phytoremediation systems with greater species richness have been considered more optimal in terms of enhanced pollutant removal (Ge et al., 2015; Han et al., 2018; Geng et al., 2017). Yet, in this study, the most diverse plant community (of three plant species) did not show the greatest absolute phytoremediation potential suggesting that species type (or more likely ecophysiological trait), is most important. While diversity does not necessarily enhance removal of specific pollutants, assembling communities comprising species with varying traits and associated removal mechanisms may allow several pollutants to be remediated at once. This study was based on newly installed FTWs, but removal efficiency will vary across the growth season depending on the pollutant, therefore, longer term (3–5 year) studies are needed to understand intra and inter seasonal and successional effects. A dynamic FTW system, that can offer a variety of additional ecosystem services, would be the optimal nature-based solution in multi-pollutant waters (Fletcher et al., 2024).

## 5. Conclusion

This study highlights several important factors that can be used to guide the implementation and management of FTWs in surface waters impacted by diffuse and point-source pollution. Effective removal of specific pollutants by FTWs can be primarily attributed to root structure and development (Ca, Mg, K, P and Zn), and plant uptake (TIN, Fe, Cu). However, in general, plant uptake is not the main mechanism driving pollutant removal for most pollutants, and therefore environmental managers must carefully consider whether active or passive management of FTWs is most appropriate as there are both benefits and dis-benefits linked to harvesting FTWs. The temporal dimension to pollutant removal can be critical for successful phytoremediation, e.g., where pollutants are more readily removed with an increasing HRT (e.g., Cu), as water residence times will influence removal rates. Therefore, depending on the management objective, FTWs might generally perform better where and when residence times are longer e.g., in ponds or in streams under low flows.

## CRedit authorship contribution statement

**Jonathan Fletcher:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Nigel J. Willby:** Writing – review & editing, Methodology, Conceptualization. **David M. Oliver:** Writing – review & editing. **Richard S. Quilliam:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgments

Funding for this work was provided by the Scottish Government Hydro Nation Scholars Programme.

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