Optimising multi-pollutant phytoremediation strategies to sustainably improve raw water quality

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Abstract

Surface waters are vital for supporting people and ecosystems; however, freshwater quantity and quality is under increasing pressure from multiple stressors such as diffuse pollution and climate change that can both impair water quality and reduce ecosystem-service provision. Diffuse pollutant impacted freshwaters can often contain 'cocktails' of multiple pollutants such as nitrogen (N), phosphorus (P), and heavy metals such as iron (Fe), copper (Cu) and Zinc (Zn). However, nature-based solutions (NbS), such as aquatic phytoremediation that capitalises on the ability of macrophytes (aquatic plants) to remove and sequester pollutants from freshwaters whilst simultaneously providing ecosystem services, have the potential to tackle the multi-faceted challenges that are associated with pollutant-impacted waters. The overarching aim of this study was to optimise a series of phytoremediation strategies that could improve water quality and freshwater ecosystems. The research was primarily framed around the plant community paradigm of the 'mass ratio hypothesis' and the plant strategies 'Competitor-Stress tolerator-Ruderal' (CSR) frameworks. A mixed-method approach including a field survey, a mesocosm experiment, and field trail was employed to develop a new plant community-based approach for optimising aquatic phytoremediation.

Firstly, the phytoremediation potential of different inorganic pollutants within wild stands of macrophytes and floating treatment wetlands was quantified. Standing stocks of macronutrient-type pollutants had strong positive significant correlations with sampled plant community biomass, while standing stocks of Cr, Cu and Mo micronutrient-type pollutants were positively correlated with biomass. Conversely, Fe, Mn, and Zn standing stocks were not correlated with biomass. Understanding wild macrophyte communities based on their overall CSR strategy representation with a simulated harvest regimes showed that optimal harvest strategies could be developed.

Secondly, mesocosm experiments demonstrated strong negative correlations between concentrations of Ca, K, P and Zn and maximum root length; although some pollutants were not effectively removed using phytoremediation e.g., Cr and Fe. On average, over 24% of Mn, TIN and P removal was by accounted for by plant uptake, which means it is

an important factor for removal of these pollutants. However, plant uptake mechanisms only removed <4% of the total Zn, K, Cu, Ca, Mg and Na from experimental systems.

Finally, a field trial comprised of floating treatment wetlands (FTWs) planted with various plant communities was used to quantify those ecosystem services provided by phytoremediation systems beyond just pollutant removal. However, there was no significant difference in invertebrate community assembly between monoculture and polyculture FTWs indicating that this service may be equally provided by most community types. However, FTWs can be used to support macroinvertebrate communities suggesting these systems can also be utilised to increase habitat within freshwaters.

Plant community structure in phytoremediation systems was found to be a key determinant of success of phytoremediation and ecosystem service provision. Communities structured by plant height can moderate levels of ecosystem multi-functionality (i.e., number and quantity of ecosystem service provision). Small-emergent communities outperformed all other community types due to their increased provision of both regulation and maintenance, cultural and provisioning services. Conversely, large emergent communities that are more typical candidates for phytoremediation had the highest levels of multifunctionality only when function performance was lower. In terms of pollutant removal, increasing species diversity allowed more pollutants to be targeted overall. However, depending on the pollutant, the removal of single pollutants was more effective when single macrophyte species were used.

This research demonstrates the importance of plant community as a factor in designing phytoremediation strategies for improving water quality and providing ecosystem services. By considering the key outputs of this thesis, phytoremediation can be employed in a range of impacted freshwaters with different issues to remove pollutants and improve habitat quality. Therefore, affirming phytoremediation as a NbS that can be utilised to tackle interconnected challenges including diffuse pollution and resource depletion.

Contents

List of Figures and Tables

List of common abbreviations

AG	Above ground	**MF**	Medium Frequency
A-RE	Adjusted Removal Efficiency	**MA**	*Mentha aquatica*
BG	Below ground	**MEC**	Mixed Emergent Community
Ca	Calcium	**MS**	*Myosotis scorpioides*
Cr	Chromium IV	**NbS**	*Nature-based Solution*
C/S	Competitor/Stress Tolerator	**NO**	*Nasturtium officinale*
Cu	Copper	**NO_3^-**	Nitrate
CW	Constructed wetland	**NO_2^-**	Nitrite
EP	*Eleocharis palustris*	**P**	Phosphorus (P)
FTW	Floating Treatment Wetland	**PA**	*Phragmites australis*
GM	*Glyceria maxima*	**PPCP**	Pharmaceuticals & personal care products
HF	High Frequency	**K**	Potassium
Fe	Iron	**RE**	Removal Efficiency
LEC	Large Emergent Community	**SEC**	Small Emergent Community
LF	Low Frequency	**Na**	Sodium
LS	*Lythrum salicaria*	**NH_3**	Total Ammonia
Mg	Magnesium	**TIN**	Total Inorganic Nitrogen
Mn	Manganese	**TL**	*Typha latifolia*
		Zn	Zinc

1. Introduction

1.1 Background

Freshwater is a fundamental resource for ecosystems, society, and global economies (Boretti and Rosa, 2019). Yet, freshwaters are also primary recipients for the waste materials from heavy industry, agriculture, and sewage discharge. Pollution is one of the key stressors on freshwater ecosystems and significantly reduces water quality, and therefore, is a serious threat to water security (Vardhan et al., 2019). Receiving waters can be negatively influenced by a range of pollutants such a nitrogen (N), phosphorus (P), and heavy metals (e.g. copper, zinc and manganese), with pollutant 'cocktails' an increasingly common phenomenon in receiving waters (Kaushal et al., 2019; Wu et al., 2016). When multiple pollutants enter the environment from disparate diffuse sources, receiving waters can face complex issues. For example, eutrophication from nutrient enrichment can cause harmful algal blooms (HABs) and excessive macrophyte growth, which can have human health, economic and environmental consequences (Briffa et al., 2020; Heathwaite, 2010). Securing a safe and sustainable water supply is critical for providing drinking water and the resources needed for sanitation, food production and industry. The aim of the United Nation's Sustainable Development Goal 6, (*Clean Water and Sanitation*), is to improve global water quality and protect and restore freshwater ecosystems by the year 2030 (Bartram et al., 2018). Therefore, there is an urgent need to develop sustainable solutions that enhance water quality and freshwater environments.

Systems-level thinking is of critical importance for addressing environmental challenges (Bartram et al., 2018). Many key environmental challenges are interlinked, for example, nutrient enrichment due to excessive or poor fertiliser use is also linked with the depletion and scarcity of natural raw resources, and the degradation of ecosystem functions (Jones et al., 2013). Recognition of such interrelationships enable effective and sustainable solutions to be developed. Nature-based solutions (NbS) are those that work with nature to address societal challenges and provide multiple benefits (Raymond et al., 2017). Specifically, they are actions that involve the protection, restoration or management of natural and semi-natural ecosystems, or the creation of novel ecosystems (Faivre et al., 2017; Nesshöver et al., 2017). NbS range widely in form and function but in general they provide 'multiple benefits'

(Liquete et al., 2016). Therefore, within the framework of NbS there is an opportunity to find approaches to tackle the multi-faceted challenges associated with multi-pollutant impacted waters, together with resource depletion, and ecosystem degradation.

1.2 Aquatic phytoremediation

The use of phytoremediation in freshwaters is a NbS that has the potential to improve water quality and provide multiple ecosystem services through an ecological engineering approach (Mitsch, 2012). In the context of freshwaters, phytoremediation involves using macrophytes (aquatic plants) and their associated microbial communities to degrade, and/or uptake and sequester waterborne pollutants into plant tissue. Using macrophytes for phytoremediation is a non-invasive strategy for sustainably improving water quality in impacted waterbodies (Newete and Byrne, 2016). After sequestering pollutants, macrophytes can be harvested and removed from the water and the biomass used as a soil conditioner, animal feed or as feedstock for bioenergy production; alternatively, the pollutant can be recovered and purified from the plant tissue (Quilliam et al., 2015). As phytoremediation can involve natural, semi-natural or novel ecosystems there is scope to provide added value through increased habitat provision, pollination and aesthetic value (and in turn human wellbeing).

Whilst there is great potential for aquatic phytoremediation, published studies are limited by concentrating on single plant species and targeting individual pollutants. There are very few studies that focus on assembling specific plant communities to target multiple pollutant phytoremediation. Studies that do explore the structure of plant communities on pollutant yield have shown inconsistent outcomes as to the most effective means of assembling plant communities for optimised functioning (Ge et al. 2015; Geng et al. 2017). Therefore, it is important to understand how plant communities can be most effectively assembled for guiding environmental management decisions (Colares et al., 2020). Given the ubiquity of the pollution challenges across different water body types there is also a need to consolidate the different reported approaches to phytoremediation and focus on optimising and developing specific strategies. Finally, in contrast to most other methods of pollutant removal, aquatic phytoremediation can provide a range of additional ecosystem services;

however, these wider benefits have not received the same attention as their pollutant removal effectiveness (e.g. Wang et al., 2015).

1.3 Aims and objectives of the thesis

The overarching aim of this project was to optimise a series of strategies that can improve water quality and freshwater ecosystems by exploiting the ability of aquatic plants to assimilate waterborne pollutants and provide ecosystem services. This was achieved using a novel mixed-method approach employing a field survey, an open-air mesocosm experiment and a field-scale trial to understand and develop different phytoremediation strategies. On commencing the project, a detailed critical review of the topic area was carried out and several research priorities were recommended. Therefore, this thesis focuses on three primary objectives:

1) Develop and optimise novel aquatic phytoremediation strategies to maximise pollutant removal (Chapter 3, Chapter 5).
2) Explore how macrophyte community assembly influences the removal of waterborne pollutants and provision of ecosystem services (Chapter 4, Chapter 5, Chapter 6).
3) Quantify the ecosystem service provision of different phytoremediation strategies, particularly those that have been unexplored within existing literature. (Chapter 3, Chapter 6)

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2. Phytoremediation using aquatic plants

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2.1 Water contamination and water security

Surface waters are vital for supporting people and ecosystems; however, freshwater availability is under increasing pressure due to a growing human population requiring access to safe water (Heathwaite, 2010). Global freshwater resources comprise 2.5% of the total global water budget, although only 0.0072 % (93,120 km^3) of the total global waters are available for drinking, energy, food production and the industry sector (Lawford *et al.*, 2013; Zimmerman et al., 2008). Tilman *et al.* (2011) predicts that crop production will need to increase by 100-110 % by 2050 to feed the growing population, leading to a global freshwater deficit of approximately 2, 400 km^3 per year (Rockström *et al.*, 2014).

Many surface waters are currently of sub-optimal standards due to a range of stressors impacting freshwaters such as point source and diffuse pollution, land-use change and climate change, which further compounds the challenge of providing water security (Ormerod *et al.*, 2010; Berger *et al.*, 2017). One of the major pressures on water quality in the United Kingdom is nutrient enrichment from diffuse pollution (Ulén *et al.*, 2007), whereas elsewhere in countries such as China, additional issues of heavy metal pollution are also prominent (Cheng, 2003). Interactions between different stressors in space and time can also lead to additive effects (Heathwaite, 2010), for example, increased land-use change towards intensive agriculture and a potential increase in storm frequency may increase the delivery of nitrogen (N) phosphorus (P) and fine sediment to receiving water (Dunn *et al.*, 2012).

Table 2.1 summarises the surface water pollutants that are of concern and where remediation solutions are being developed. Water pollutants can be broadly categorised as either: organic, e.g. hydrocarbons, pesticides and algal toxins, or inorganic, e.g. metals or synthetic and manure-based fertilisers containing excess amounts of N and P, or biological, e.g. pathogens and algal toxins. The mobilisation and effects of different pollutants have been discussed extensively elsewhere (Heisler *et al.*, 2008; Ohe *et al.*, 2004; Liess & Carsten Von Der Ohe, 2005; Edwards, 2015; Lintelmann *et al.*, 2003). However, different pollutants may have multiple sources, for example, N and P can be released from agriculture, aquaculture and urban waste water streams.

Managing waterborne pollutants through *in-situ* best management practices (BMPs) that target the source of pollution is the principal approach to improving water quality (Lam *et al.*, 2011). However, lag times associated with the improvement of water quality and subsequent ecological recovery of receiving waters following mitigation may range from 1 to > 50 years (Meals *et al.*, 2010). The 'legacy effect' is one such component delaying water quality improvements in spite of BMPs being in place (Haygarth *et al.*, 2014). Water bodies, such as those with long residence times, may become reservoirs for pollutants over time, meaning that although source management is in place, the receiving waters remains high in pollutant levels for significant amounts of time (Meals *et al.*, 2010). Therefore, developing management systems that combine BMPs with other methods of remediating waters with high levels of pollutants, both at source and throughout the catchment, is needed to sustainably improve water quality.

The pollution of water with inorganic elements such as N, P and metals also provides an opportunity to recover elements as part of a 'circular economy' approach (Masi *et al.*, 2017; Quilliam *et al.*, 2015). Energy-intensive mining for macronutrients such as P and potassium (K) are exhausting finite supplies of nutrients for the production of agricultural fertilisers (Jones *et al.*, 2013), whilst liquid fertilisers and nutrient-rich solid manures applied to agricultural land are readily transferred to receiving waters. Coupling systems that remediate water pollution and enable the capture of these resources may help close the loop on nutrient loss (Quilliam *et al.*, 2015). Therefore, macrophyte phytoremediation has the potential to be employed for both the sustainable remediation of surface waters and as a management strategy for recovering nutrients.

Table 2.1: Key pollutants impacting the aquatic environment, organised by pollutant category, type and providing examples of the pollutants, their sources and impacts

Pollutant category	**Pollutant Type**	**Example pollutant**	**Sources**	**Potential impacts**
Organic	Persistent organic pollutants (POPs)/Xenobiotics	Dioxins, organochlorides, Polycyclic aromatic hydrocarbons (PAH), Polychlorinated biphenyls	Industry Agriculture	Toxicity Endocrine disrupting effects
	Pesticides	Glyphosate Hexachlorocyclohexane Fenhexamid Deltamethrin	Agriculture Aquaculture	Toxicity Endocrine disrupting effects
	Pharmaceutical and personal care products (PPCPs)	Antibiotics Hormones Pain relief medication	Domestic Agriculture Aquaculture	Endocrine disrupting effects Antibiotic resistance Destabilising microbial communities
	Algal toxins	Microcystin-LR	Cyanobacterial algal blooms	Acute/chronic toxicity
Inorganic	Nutrients	Nitrogen (N) Phosphorus (P) Potassium (K)	Agriculture Aquaculture Septic tank inputs	Nutrient enrichment/eutrophication
	Metalloid elements	Iron (Fe) Aluminium (Al) Lead (Pb) Nickle (Ni) Cadmium (Cd) Copper (Cu) Uranium (U)	Agriculture Industry (mining and combustion of fossil fuels) Al mobilisation through acid rain	Toxicity Endocrine disrupting effects
Microbial	Pathogens and parasites	*E. coli* O157 *Cryptosporidium parvum*	Agriculture Aquaculture Domestic	Human illness (intestinal infection)

2.2 Aquatic phytoremediation

Aquatic phytoremediation is a phytotechnology used for the removal of pollutants from surface waters and the restoration of impacted water bodies (rivers, streams, lakes, ponds). Within surface waters plants can be cultured to remove pollutants from both the water column and the sediment (Newete & Byrne, 2016; Miretzky *et al.*, 2004), and can be deployed at either the point source, or within waterbodies where diffuse pollution is problematic (Lu *et al.*, 2011). Aquatic phytoremediation specifically uses macrophytes (i.e. freshwater adapted angiosperms, pteridophytes and ferns) for removing and degrading pollutants within aquatic environments (Rai, 2009). This definition does not include microalgae species. Macrophytes can be broadly classified into three primary growth forms: floating, submerged and emergent (Figure 2.1). Floating macrophytes occupy the water surface and include genera such as *Lemna* (duckweeds), *Hydrocharis* (frogbit) and *Nymphaea* (water-lilies) which may be free-floating or rooted. Submerged macrophytes grow primarily below the water surface and may be anchored to the substrate, although *Ceratophyllum* (hornworts) are a widespread genus of unrooted submerged plants. Emergent macrophytes occupy the margins of water bodies and are rooted into the substrate but have significant shoot growth above the water level, e.g. *Typha* (reedmace) and *Phragmites* (common reed). These different growth forms facilitate the removal of pollutants from both the water column and the sediment depending on the way in which they are deployed (Newete and Byrne, 2016).

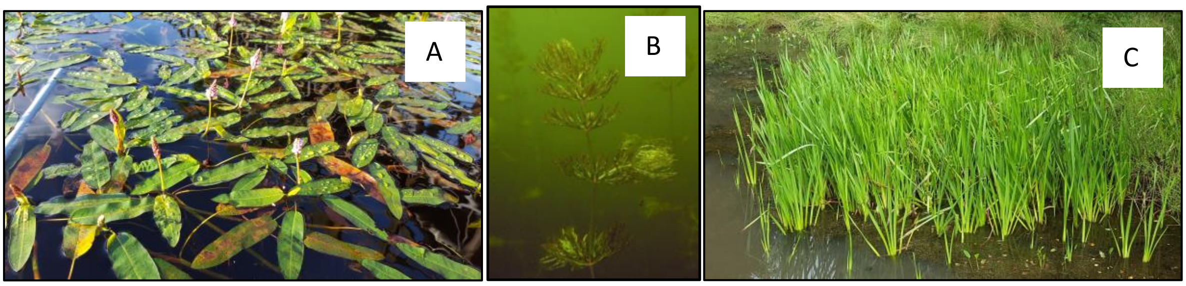

Figure 2.1: Photo examples of floating, submerged and emergent macrophyte life forms. *Persicaria amphibia* (floating) (A), *Ceratophyllum demersum* (Submerged) (B) and *Sparganium erectum* (emergent) (C)

Macrophytes have significant capacity for uptake of nutrients and other substances from their growth medium, and can thus lower the pollution concentration of a target water body (Dhote and Dixit, 2009). Macrophytes can remove and degrade pollutants using the key mechanisms of rhizo/phyto-filtration, phytoextraction, phytovolatilization and phytodegradation (Table 2.2). Emergent and floating macrophytes primarily take up nutrients and other contaminants (whether from the substrate or water column) through their roots, whereas stem tissue can also be an important pathway for removal from the water column for submerged macrophytes (Denny, 1972; Gabrielson, Perkins and Welch, 1984; Dhote and Dixit, 2009). Specific mechanisms for pollutant removal and degradation by macrophytes depend primarily on the type of pollutant (nutrient, heavy metals, organic pollutants, biological), and the location of the pollutant within the surface water body (water column, lake or streambed sediment) (Miretzky, Saralegui and Cirelli, 2004; Padmavathiamma and Li, 2007; Vymazal, 2011; Xing *et al.*, 2013; McAndrew, Ahn and Spooner, 2016; Polechońska and Samecka-Cymerman, 2016). Different mechanisms for removing various classes of pollutant from surface water systems by macrophytes are considered below.

2.2.1 Macronutrients

It is important to note that elements targeted for phytoremediation may exist in a dissolved phase, or in a particulate phase adhered to suspended material in the water column or bound to sediment, which means there are different mechanisms for removal (Perk, 2006). Macronutrients, including N and P, are essential elements required in relatively large concentrations for plant metabolism (Hawkesford *et al.*, 2011). Therefore, when aquatic system are enriched with N and P, phytoextraction (uptake and sequestration) is an important mechanism (Eid *et al.* 2012; Mkandawire & Dudel, 2005). Particulate pollutants in the water column, such as P, can be stabilised by phytofiltration (Tanner and Headley, 2011a; Olguín and Sá Nchez-Galvá, 2012), where plant roots may excrete exudates that assist phytoextraction of adsorbed elements (Jackson, 1998; Verkleij *et al.*, 2009; Akeel, 2013). For N removal, phytodegradation may also be important in the water column and sediment as the oxygen and energy supplied to the root zone from macrophytes may support nutrient-degrading microbial communities, including the simultaneous presence of both nitrifying and denitrifying bacteria (Table 2.2) (Lu *et al.*, 2018).

Table 2.2: Phytoremediation mechanisms, adapted from Dhir (2013) and Rezania *et al.* (2016).

Mechanism	Medium	Contaminant category	Description	Accumulation Part	Example genera
Rhizofiltration/phytofiltration	Water	Organics/inorganics /heavy metals	Extraction from contaminated water by adsorption/absorption	Shoots/roots	*Lemna, Hydrocharis, Eichhornia*
Phytoextraction/phytoaccumulation	Soil/water	Inorganics/heavy metals	Uptake by roots and translocation to upper parts	Shoots	*Juncus, Schoenoplectus*
Phytostablisation	Soil/sediment	Inorganics/heavy metals	Rendering contaminants immobile within soil matrix due to plant root action	Reduction in rhizosphere	*Chenopodium*
Phytovolatilization	Soil/sediment/ water (less common)	Organics	Conversation of containments to volatile form	Atmospheric release	*Phragmites*
Phytodegradation	Soil/sediment/ Water	Organics/inorganics /microbiological	Degradation in Rhizosphere through microbial degradation or by metabolism within plant	Degradation in rhizosphere/pollutant degraded in plant to less harmful metabolite	*Typha, Phragmites, Myriophyllum*

2.2.2 Micronutrients/metals

Micronutrients are essential elements that are required by plants in relatively small quantities, e.g. to regulate redox reactions, metabolism and cell integrity (Broadley *et al.*, 2011). Essential micronutrients include iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Md) and boron (B); beneficial but non-essential micronutrients include sodium (Na), silicon (Si), cobalt (Co), selenium (Se); while there are elements that can be found in plant tissue but are not thought to be beneficial such as aluminium (Al) vanadium (V), titanium (Ti), lanthanum (La) and cerium (Ce) (Broadley *et al.*, 2011) (Table 2.1). Some of these elements may be enriched by industrial pollution but can be reduced by phytoextraction through repeated harvesting of plant tissue, following uptake in the water column through hydroponic growth (e.g. in FTWs) or where plants are rooted in sediment (Ali *et al.*, 2013) (Figure 2.2). The efficiency of phytoextraction as a phytoremediation strategy depends upon the specific degree of essentiality of each element for plant metabolism and is determined by specific mechanisms for uptake and translocation into plant tissue (Dhir, 2013). Hyperaccumulators are plants that have a high affinity for certain elements and through enhanced phytoextraction can sequester high concentrations of metals (Sarma, 2011; van der Ent *et al.*, 2013). Phytofiltration is important for soluble and particulate pollutants with absorption/adsorption to plant roots (Olguín and Sá Nchez-Galvá, 2012), and in some cases metals can be bound and/or precipitated on the plant roots (Xian *et al.*, 2010; Gomes *et al.*, 2016) (Figure 2.2).

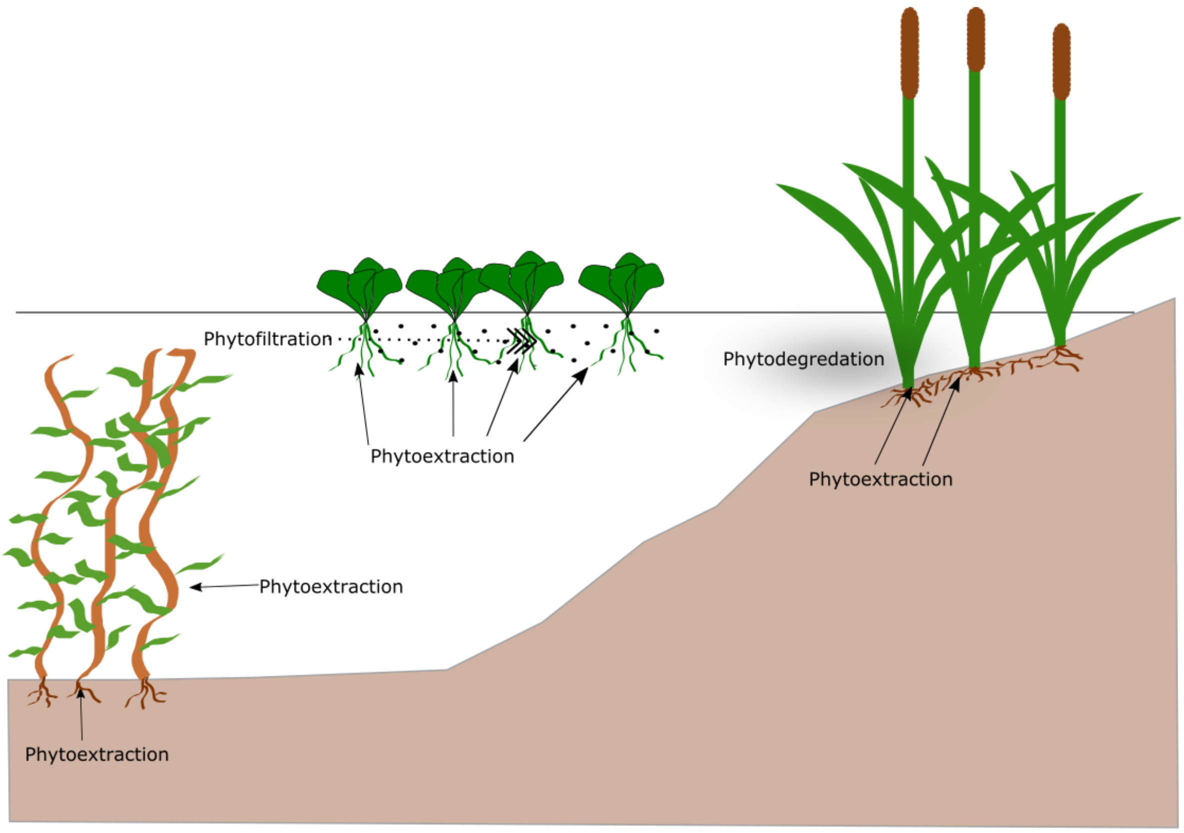

Figure 2.2 Phytoremediation mechanisms used to degrade/remove waterborne pollutants, by growth form.

2.2.3 Organic pollutants

Organic pollutants are compounds containing carbon that are primarily synthetic, environmentally persistent and potentially toxic. They include products such as pesticides, solvents and pharmaceuticals and personal care products (PPCPs) (El-Shahawi *et al.*, 2010)(Table 2.1). Phytometabolism and rhizodegredation within the water column and sediment are integral processes in the aquatic phytoremediation of organic compounds (Reinhold *et al.*, 2010). Phytometabolism can occur if organic compounds are more hydrophilic meaning they pass more readily through the plant epidermis into plant cells (Lintelmann *et al.*, 2003; Dettenmaier, Doucette and Bugbee, 2009; Yamazaki *et al.*, 2015) (Figure 2.2). Sequestered compounds undergo chemical modification through oxidation, reduction or hydrolysis which makes them chemically more reactive within plant cells; the less harmful metabolite is then conjugated/bound to sugars, amino acids or glutathione to reduce its toxicity and hydrophobicity (Macek *et al.*, 2000; Geissen *et al.*, 2015). These bound metabolites may then be either stored within the vacuole or excreted from the plant, or can become insoluble by being covalently bound within the cell wall (Zhang *et al.*, 2014). Rhizodegradation can take place within sediment, and more hydrophobic compounds can serve as a microbial carbon source where emergent macrophytes supply oxygen to the root zone (Figure 2.2). The advantage of these two phytoremediation processes is that there is no need for repeated harvests to extract the pollutant and thus disturbance to the aquatic system is reduced.

2.2.4 Microbial pollutants

Microbial water pollutants such as the bacteria *Escherichia coli* O157, the protozoan parasite *Cryptosporidium* spp. and viruses such as norovirus can cause harm to humans and animals (Haack *et al.*, 2016; Fuhrimann *et al.*, 2017) (Table 2.1). The ability of plants to directly take up microbial pollutants is limited; however, there are some accounts of pathogens entering plant tissue through the process of internalisation, although whether this is an active or passive process is unclear and likely depends on the type of pathogen, plant and the local abiotic conditions (Hirneisen et al, 2012). The primary mechanisms for removal of microbial pollutants from water are either, chemical, e.g. oxidation, photodegradation, exposure to plant root biocides and adsorption to organic material and biofilms; physical, e.g. through filtration and sedimentation; or biological, e.g. predation, natural die-off, antibiosis and other biolytic processes (Decamp and Warren, 2000; Karathanasis et al, 2003; Karim *et al.*, 2004; Wand *et al.*, 2006; Makvana and Sharma, 2013). Macrophyte planting systems, particularly CWs, may promote these mechanisms and thus facilitate the degradation of microbial pollutants.

2.3 Macrophytes used in aquatic phytoremediation

2.3.1 Macronutrients

Macrophytes uptake and sequester N primarily in the form of nitrate (NO_3^-) and ammonium (NH_4^+), while P is taken up as phosphate (PO_3^{4-}). Studies vary in their focus on total amounts (i.e. including particulate) versus the dissolved fraction of macronutrients, which makes comparing optimal macrophyte accumulator species challenging (Table 2.3). Macrophytes that have the greatest biomass production and/or fastest growth rates are some of the most effective nutrient phytoremediators (Keenen and Kirkwood, 2015), for example, *Eichhornia crassipes*, *Lemna sp*. and *Typha latifolia* have growth rates of 60-110 t/ha/yr, 6-26 t/ha/yr and 8-61 t/ha/yr, respectively (Gumbricht, 1993).

Emergent species have received considerable attention in nutrient phytoremediation and are often deployed in CWs, with *Canna* spp. and *Cyperus* spp. showing some of the highest removal efficiencies for ammonium (NH_4^+) of between 74 - 100 % (Table 2.3). *Typha latifolia*, *Lolium multiflorum* and *Polygonum hydropiperoides* showed high TP removal efficiency of 81 - 90 % (Table 2.3). For floating macrophytes *Eichhornia crassipes*, *Lemna gibba* and *Pistia stratiotes* show good potential for nutrient removal: *E. crassipes* can remove up to 92 % NO_3^- and 81 % NH_3^- whilst *L. gibba* can remove 100 % $NO_3^-$and 82 % NH_3^- (Table 2.3). The same two species were also effective at removing total phosphorus (TP) (Table 2.3). Submerged plants have received less attention for their nutrient phytoremediation capacity (Table 2.3). This may reflect the difficulty of cultivating and harvesting submerged macrophytes, and the potentially lower biomass generated compared to emergent plants (Du *et al.*, 2017). *Ceratophyllum demersum* and *Myriophyllum aquaticum* are potential candidates for the targeting of total nitrogen (TN) and TP with removal rates > 41 % (Table 2.3). *Potamogeton crispus* was deployed as part of a hybrid FTW experiment and was found to have enhanced effects over the FTW comprised of only emergent plants; however, the individual removal contribution from *P. crispus* was not quantified (Guo *et al.*, 2014). Most submerged species are rooted in sediment and may also remove nutrients from the water column through foliar absorption (Eichert and Fernández, 2011). Hence they offer the dual ability to remove nutrients from water and sediment, allowing the simultaneous remediation of sediments that have a pollutant legacy and which may continue to release nutrients to the water column via internal loading even after external loads have been reduced. However, the disturbance caused during harvesting can re-suspend sediment-bound elements, and alter the macrophyte-equilibrium state to a potentially undesirable phytoplankton-dominated state (Kuiper *et al.*, 2017).

Table 2.3: Removal efficiencies (%) of macrophyte species investigated in this review of nutrients phytoremediation

Species	**Life Form**	**Removal Efficiency (%)**						**Macrophyte Deployment**	**Experiment**	**Reference**
		Total Nitrogen	**Nitrate (NO_3)**	**Ammonia (NH_3)**	**Ammonium (NH_4)**	**Total Phosphorus**	**Phosphate**			
Canna sp.	Emergent	50			100			FTW	Mesocosm	Sun et al (2009)
					42			FTW	Mesocosm	Ayaz & Saygin (1996)
Cyperus sp.	Emergent				33			FTW	Mesocosm	Ayaz & Saygin (1996)
		72			75			Constructed wetland	Constructed wetland	Kyambadde *et al.* (2004)
		57			63	54.09		FTW	Microcosm	Kansiime *et al.* (2005)
Polygonum hydropiperoides	Emergent	74				81		Direct planting	Mesocosm	Lang Martins *et al.* (2010)
Echinodorus cordifolius	Emergent		45		49.9	10.85		Direct planting	Mesocosm	Moore *et al.* (2016)
Ipomoea aquatica	Emergent	76						FTW	Mesocosm	Karnchanawong (1995)
		36-46				36-47		FTW	Mesocosms	Li *et al.* (2010)
		61.94			48	62		FTW	Mesocosm	Li *et al.* (2010)
Juncus effusus	Emergent	48		50		63		Constructed wetland	Constructed wetland	Coleman *et al.* (2001)
Leersia oryzoides	Emergent					51		Direct planting	Mesocosm	Tyler *et al.*(2012)
Limnocharis flava	Emergent			92			96	Constructed wetland	Constructed wetland	Kamarudzaman & Ismail (2011)
Lolium multiflorum	Emergent	81				90		FTW	Mesocosm	Xian *et al.* (2010)
Miscanthidium violaceum	Emergent	57			47	41		Constructed wetland	Constructed wetland	Kyambadde *et al.* (2004)

Species	Life Form	Removal Efficiency (%)						Macrophyte Deployment	Experiment	Reference
		Total Nitrogen	**Nitrate (NO_3)**	**Ammonia (NH_3)**	**Ammonium (NH_4)**	**Total Phosphorus**	**Phosphate**			
Oenanthe javanica	Emergent	91		97		76		FTW	Mesocosm	Zhou & Wang (2010)
Panicum hemitomon	Emergent		60		54	28		Direct planting	Mesocosm	Moore *et al.* (2016)
Phragmites	Emergent				98			FTW	Mesocosm	Kintu Sekiranda & Kiwanuka, (1997)
Saururus cernuus	Emergent		35		-3	-13		Direct planting	Mesocosm	Moore *et al.* (2016)
Scirpus atrovirens	Emergent			91			82	Constructed wetland	Constructed wetland	Kamarudzaman & Ismail (2011)
Scirpus validus	Emergent	25		25		48		Constructed wetland	Constructed wetland	Coleman *et al.* (2001)
Sparganium americanum	Emergent					14		Direct planting	Mesocosm	Tyler *et al.* (2012)
Thalia dealbata	Emergent		46		31	4		Direct planting	Mesocosm	Moore *et al.* (2016)
Typha angustifolia	Emergent	57				23		FTW	Mesocosm pots	Keizer-Vlek *et al.* (2014)
Typha latifolia	Emergent	62		62		81		Constructed wetland	Constructed wetland	Coleman *et al.* (2001)
						53		Direct planting	Mesocosm	Tyler *et al.* (2012)
			32		17	12		Direct planting	Mesocosm	Moore *et al.* (2016)
Vetiveria zizanoides	Emergent	49		50		21		FTW	Mesocosm	Boonsong & Chansiri (2008)
Eichhornia crassipes	Floating		61-83					Direct planting	Mesocosm	Ayyasamy *et al.* (2009)
			92	81		67		Direct planting	Mesocosm	Kutty *et al.* (2009)

Species	Life Form	Removal Efficiency (%)						Macrophyte Deployment	Experiment	Reference
		Total Nitrogen	Nitrate (NO$_3$)	Ammonia (NH$_3$)	Ammonium (NH$_4$)	Total Phosphorus	Phosphate			
Pistia stratiotes	Floating	50				14-31		Direct planting	Ponds (storm water detention)	Lu *et al.* (2010)
			31-51					Direct planting	Mesocosm	Ayyasamy *et al.* (2009)
Salvinia molesta	Floating		18-36					Direct planting	Mesocosm	Ayyasamy *et al.* (2009)
Lemna gibba	Floating	97				99		Direct planting	mesocosm-wastwater	Körner & Vermaat (1998)
			100	82			64	Sewage water system	Sewage water system	El-Kheir *et al.* (2007)
Ceratophyllum demersum	Submerged	42			65	73		Direct planting	Mesocosms	Dai *et al.* (2012)
Myriophyllum aquaticum	Submerged	88				94		Direct planting	Mesocosm	Souza *et al.* (2013)
			45		35	7		Direct planting	Mesocosm	Moore *et al.* (2016)

Species	Life Form	Removal Efficiency (%)						Macrophyte Deployment	Experiment	Reference
		Total Nitrogen	Nitrate (NO$_3$)	Ammonia (NH$_3$)	Ammonium (NH$_4$)	Total Phosphorus	Phosphate			
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				42				FTW	Mesocosm	Ayaz & Saygin (1996)
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		72		75				Constructed wetland	Constructed wetland	Kyambadde *et al.* (2004)

Species	Life Form	Removal Efficiency (%)						Macrophyte Deployment	Experiment	Reference
		Total Nitrogen	Nitrate (NO_3)	Ammonia (NH_3)	Ammonium (NH_4)	Total Phosphorus	Phosphate			
		57		63		54.09		FTW	Microcosm	Kansiime *et al.* (2005)
Polygonum hydropiperoides	Emergent	74				81		Direct planting	Mesocosm	Lang Martins *et al.* (2010)
Echinodorus cordifolius	Emergent		45		49.9	10.85		Direct planting	Mesocosm	Moore *et al.* (2016)
Ipomoea aquatica	Emergent	76						FTW	Mesocosm	Karnchanawong (1995)
		36-46				36-47		FTW	Mesocosms	Li *et al.* (2010)
		61.94		48		62		FTW	Mesocosm	Li *et al.* (2010)
Juncus effusus	Emergent	48		50		63		Constructed wetland	Constructed wetland	Coleman *et al.* (2001)
Leersia oryzoides	Emergent					51		Direct planting	Mesocosm	Tyler *et al.*(2012)
Limnocharis flava	Emergent			92			96	Constructed wetland	Constructed wetland	Kamarudzaman & Ismail (2011)
Lolium multiflorum	Emergent	81				90		FTW	Mesocosm	Xian *et al.* (2010)
Miscanthidium violaceum	Emergent	57		47		41		Constructed wetland	Constructed wetland	Kyambadde *et al.* (2004)
Oenanthe javanica	Emergent	91		97		76		FTW	Mesocosm	Zhou & Wang (2010)
Panicum hemitomon	Emergent		60		54	28		Direct planting	Mesocosm	Moore *et al.* (2016)
Phragmites	Emergent			98				FTW	Mesocosm	Kintu Sekiranda & Kiwanuka, (1997)
Saururus cernuus	Emergent		35		-3	-13		Direct planting	Mesocosm	Moore *et al.* (2016)
Scirpus atrovirens	Emergent			91			82	Constructed wetland	Constructed wetland	Kamarudzaman & Ismail (2011)

Species	Life Form	Removal Efficiency (%)						Macrophyte Deployment	Experiment	Reference
		Total Nitrogen	Nitrate (NO_3)	Ammonia (NH_3)	Ammonium (NH_4)	Total Phosphorus	Phosphate			
Scirpus validus	Emergent	25		25		48		Constructed wetland	Constructed wetland	Coleman *et al.* (2001)
Sparganium americanum	Emergent					14		Direct planting	Mesocosm	Tyler *et al.* (2012)
Thalia dealbata	Emergent		46		31	4		Direct planting	Mesocosm	Moore *et al.* (2016)
Typha angustifolia	Emergent	57				23		FTW	Mesocosm pots	Keizer-Vlek *et al.* (2014)
Typha latifolia	Emergent	62		62		81		Constructed wetland	Constructed wetland	Coleman *et al.* (2001)
						53		Direct planting	Mesocosm	Tyler *et al.* (2012)
			32		17	12		Direct planting	Mesocosm	Moore *et al.* (2016)
Vetiveria zizanoides	Emergent	49		50		21		FTW	Mesocosm	Boonsong & Chansiri (2008)
Eichhornia crassipes	Floating		61-83					Direct planting	Mesocosm	Ayyasamy *et al.* (2009)
			92	81		67		Direct planting	Mesocosm	Kutty *et al.* (2009)
Pistia stratiotes	Floating	50				14-31		Direct planting	Ponds (storm water detention)	Lu *et al.* (2010)
			31-51					Direct planting	Mesocosm	Ayyasamy *et al.* (2009)
Salvinia molesta	Floating		18-36					Direct planting	Mesocosm	Ayyasamy *et al.* (2009)
Lemna gibba	Floating	97				99		Direct planting	mesocosm-wastwater	Körner & Vermaat (1998)
			100	82			64	Sewage water system	Sewage water system	El-Kheir *et al.* (2007)

Species	**Life Form**	**Removal Efficiency (%)**						**Macrophyte Deployment**	**Experiment**	**Reference**
		Total Nitrogen	**Nitrate (NO_3)**	**Ammonia (NH_3)**	**Ammonium (NH_4)**	**Total Phosphorus**	**Phosphate**			
Ceratophyllum demersum	Submerged	42			65	73		Direct planting	Mesocosms	Dai *et al.* (2012)
Myriophyllum aquaticum	Submerged	88				94		Direct planting	Mesocosm	Souza *et al.* (2013)
			45		35	7		Direct planting	Mesocosm	Moore *et al.* (2016)

The phytoremediation potential of a macrophyte is influenced by biotic factors such as competition, predation and developmental stage (Quilliam *et al.*, 2015), and abiotic factors such as temperature, pH, light availability, seasonality and nutrient loading (Ansari et al, 2014). For example, Ayyasamy *et al.* (2009) found that the removal efficiency of by *E. crassipes* increased between concentrations of 100 mg/l to 300 mg/l of NO_3^-, but decreased at higher concentrations of 400 and 500 mg/l of NO_3^-. Similarly, a mesocosm-based study of the effect of different temperature regimes on N and P removal by *Nasturtium officinale* and *Oenanthe javanica* found that maximum net accumulation of TN and TP occurred at an air temperature of 22 °C but deteriorated thereafter (Hu *et al.*, 2010). Given the wide range of factors that may influence the ability of macrophytes to remove contaminants, understanding the performance of some of the key macrophyte accumulators under different environmental conditions is prudent in order to optimise species selection.

2.3.2 Metals

Macrophytes can also remove micronutrients (henceforth referred to as metals (Rai, 2009)) from water and sediments, and hyperaccumulators are most appropriate for the phytoremediation of metals (Ali *et al.*, 2013). The search for hyperaccumulator species has been one of the primary foci within the field given the widespread prevalence of past and current metal industrial effluents and the ecological risks they carry (van der Ent *et al.*, 2013); however, metal bioavailability can be reduced by sedimentation and adsorption to clay particles (Kumar *et al.*, 2008). Studies based on mesocosm-scale CW experiments have been carried out on synthetic solutions with elevated metal concentrations in domestic and industrial wastewaters to assess the potential of macrophytes of different growth forms to act as hyperaccumulators (Fu & Wang, 2011; Kamal *et al.*, 2004; Rai, 2009; Rezania *et al.*, 2016) (Table 2.4). Many species also have the capacity to take up multiple types of metals meaning that some species could be more beneficial in phytoremediation (Table 2.4).

Table 2.4: Key macrophyte metal accumulators reported in the literature

Species	Life Form	Metals	Reference
Ceratophyllum submersum	Submerged	Ni	Kara (2010)
Ceratophyllum demersum	Submerged	Cr, Pb	Osmolovskaya and Kurilenko (2005)
Potamogeton natans	Submerged	U	Pratas *et al.* (2014)
Myriophyllum spicatum	Submerged	Co,Cu, Mn, Pb, Zn	Wang *et al.* (1996); Sivaci *et al.*, (2004); Lesage *et al.* (2008)
Potamogeton pectinatus	Submerged	Cd, Cu, Mn, Pb, Zn	Rai *et al.* (2003); Singh *et al.* (2005)
Hydrilla verticillata	Submerged	As, Cu	Srivastava *et al.* (2011)
Lemnocharis flava	Emergent	Cu, Fe, Hg, Pb, Zn	Anning *et al.* (2013)
Glyceria maxima	Emergent	Cu, Zn	Parzych *et al.* (2016)
Typha latifolia	Emergent	As, Cu, Ni, Zn	Ye *et al.* (1997); Ha *et al.* (2009); Manios *et al.* (2003); Qian *et al.* (1999)
Typha angustifolia	Emergent	Pb	Panich-pat (2005)
Elodea densa	Emergent	Hg	Molisani and Lacerda (2006)
Phalaris arundinacea	Emergent	Fe, Mn, Ni	Parzych *et al.* (2016)
Phargmites australis	Emergent	As, Hg	Windham *et al.* (2003); Afrous *et al.* (2011)
Scirpus maritimus	Emergent	As,	Afrous *et al.* (2011)
Spartina alterniflora	Emergent	As,	Carbonell *et al.* (1998)
Spartina patens	Emergent	Cd	Zayed *et al.* (2000)
Azolla filiculoides	Floating	Cd, Cr, Ni, Pb, Zn	Oren Benaroya *et al.* (2004); Arora *et al.* (2006): Taghi *et al.* (2005); Zayed *et al.* (1998)
Azolla caroliniana	Floating	As, Cr, Cu, Hg	Rahman and Hasegawa (2011); Bennicelli *et al.* (2004)
Pista stratiotes	Floating	Cr, Cu, Hg	Miretzky *et al.* (2004); Molisani *et al.* (2006); Maine *et al.* (2004)
Salvinia cucullata	Floating	Cd, Pb	Phetsombat *et al.* (2006)
Salvinia natans	Floating	Cr, Zn	Dhir *et al.* (2008)
Spirodela polyrhiza	Floating	As	Zhang *et al.* (2011)
Eichhornia crassipes	Floating	Cd, Cr, Cu, Hg, Ni, Zn	Zhu *et al.* (1999): Hu *et al.* (2007); Molisani *et al.* (2006); Low *et al.* (1994)
Lemna gibba	Floating	As, Cd, Ni	Mkandawire and Dudel (2005); Mkandawire *et al.* (2004); Mkandawire *et al.* (2004)

Macrophytes that have often been cited as hyperaccumulators with high biomass potential are free-floating plants, such as members of the Lemnaceae (e.g. *Lemna minor*), *Pista stratiotes*, *Eichhornia crassipes* and those from the genera *Salvinia* (Table 2.4). For example, *L. gibba* has been reported to concentrate between 14,000 mg/kg dry weight of Cd, whilst *E. crassipes* can concentrate 10,000 mg/kg Zn (Low *et al.*, 1994; Mkandawire *et al.*, 2004). Furthermore, *Typha latifolia* and *Cetatophyllum demersum L.* have also shown good potential (Osmolovskaya & Kurilenko, 2005; Sunita *et al.*, 2015). The main limitation for macrophyte metal uptake is the toxicity of the target metal pollutant at higher concentrations (Landesman *et al.*, 2011). However, detoxification mechanisms also allow species to avoid the negative effects of these metals (Deng *et al.*, 2004); for example, more than 50 % of the Ca, Cd, Co, Fe, Mg, Mn, and Zn recovered in the roots of *Pistia stratiotes* were actually attached to the external surfaces indicating the ability of the plant to exclude metals and thus maintain tolerable levels internally (Lu *et al.*, 2011). Newete & Byrne (2016) also state that the extent of the root system affects the ability of macrophytes to remove metal pollutants, with fibrous root systems being superior due to their large surface area. Physio-chemical factors are also important for uptake and accumulation of metals with temperature, light, pH and salinity all having been shown to influence remediation performance (Rai, 2009).

2.3.3 Organic pollutants

Table 2.5 shows the wide range of studies that have been carried out in relation to the phytoremediation of organic pollutants and some of the key macrophytes that may be utilised. For pesticides, *Lemna minor* removed 95 % of 2, 4, 5-trichlorophenol, whereas for isoproturon and glyphosate *L. minor* its removal efficiency was poor (25 % and 8 % respectively; Table 2.5). *Eichhornia crassipes* also shows good phytoremediation potential, removing up to 81 % of ethion within a water mesocosm experiment (Table 2.5). The removal of DDT by macrophytes shows promise. For the DDT isomers *o,p′*-DDT and *p,p′*-DDT: *Spirodela oligorrhiza* can remove 66 % and 50 % respectively; whilst *Myriophyllum aquaticum* can remove 76 % and 82 % respectively (Gao *et al.*, 2000). *Elodea canadensis* also has the ability to remove 48% to 89% of *p,p′*-DDT (Gao *et al.*, 2000; Garrison *et al.*, 2000). *Lemna gibba, L. minuta* and *Potamogeton crispus* have been demonstrated to be very efficient at removing phenols from water (Barber *et al.*, 1995; Hafez *et al.*, 1998). However, *P. crispus* is less efficient at removing two PAHs, phenanthrene (removal 18 - 34 %) and pyrene (removal 14 - 24 %) (Meng *et al.*, 2015).

There is great potential for phytoremediation of a wide variety of PPCPs such as anti-inflammatory, hormonal replacement and anticonvulsant products (Zhang *et al.*, 2014). CWs (section 2.7.1) planted with *Phragmites australis* demonstrated very efficient removal of the hormones Estrone, 17 beta-estradiol and 17 alpha-ethinylestradiol from water (Table 2.5). In CWs the water column/plant sediment matrix a depth of *c*.7.5 cm provided more efficient

PPCP removal than deeper depths of 30 cm (Zhang *et al.*, 2014). This highlights the importance of oxygen for the removal of waterborne hormone pollutants with vertical mixing from the surrounding atmosphere increasing the aeration of plant roots and (Zhang *et al.*, 2014). Plants such as *Typha latifolia* with more extensive roots and rhizomes system may be favourable for deployment due to their capacity to oxygenate water (Makvana and Sharma, 2013).

Scirpus validus displays mixed ability to remove anti-inflammatory pharmaceuticals with very efficient removal of naproxen, compared to very poor removal of diclofenac (Zhang *et al.*, 2012; Zhang *et al.*, 2013a). *Typha angustifolia* removed 27 - 91 % of anti-inflammatory drugs in a study by Zhang *et al.* (2011). Chen *et al.* (2016) found that there is large variability in planted rural CWs in terms of their removal efficiency of PPCPs with 11 - 100 % removal of anti-inflammatories, 37 - 99 % for β-blockers and 18 - 95 % for diuretics. Understanding this variability and identifying macrophytes for the removal of PPCPs through laboratory studies and at the field-scale is important given the need for low cost removal solutions, especially in developing countries. There has been little focus on the use of novel macrophyte planting systems (e.g. FTWs) for the removal of organic chemicals, and future work on these systems would build flexibility into the deployment of different aquatic phytoremediation schemes for tackling the problem of PPCP pollution. Importantly, the distribution and storage of organic chemicals within plants, especially for PPCPs, requires further study in order to avoid the problem of transferring pollutant from one place to another (sections 2.8 and 2.9)

Table 2.5: Removal efficiencies of macrophyte species investigated in phytoremediation studies of organic pollutants

Organic Pollutant	Species	Life Form	Target pollutant	Experimental situation	Removal (%)	Reference
Pesticides	*Canna x generalise*	Emergent	Isoxaben, oryzalin	Mesocosm	n/a	Fernandez *et al.* (1999)
	Pontaaderia cordata	Emergent	Isoxaben, oryzalin	Mesocosm	n/a	Fernandez *et al.* (1999)
	Iris L.x'Charjoys Jan'	Emergent	Isoxaben, oryzalin	Mesocosm	n/a	Fernandez *et al.* (1999)
	Eichhornia crassipes	Floating	Ethion	Mesocosm	81	Xia & Ma (2006)
	Juncus effusus	Emergent	Atrazine, Lambda-cyhalothrin	Mesocosm	n/a	Bouldin *et al.* (2006)
	Ludwigia peploides	Emergent	Atrazine, Lambda-cyhalothrin	Mesocosm	n/a	Bouldin *et al.* (2006)
	Lemna minor	Floating	2,4,5-trichlorophenol	Mesocosm	95	Tront & Saunders (2006)
			Isoproturon, Glyphosate	Mesocosm	25, 8	Dosnon-Olette *et al.* (2011)
	Spirodela oligorrhiza	Floating	DDT (OP,PP-DDT)	Mesocosm	66, 50	Gao *et al.* (2000)
	Elodea canadensis	Submerged	DDT (OP,PP-DDT)	Mesocosm	31, 48	Gao *et al.* (2000)
	Mariophyllum aquaticum	Submerged	DDT (OP,PP-DDT)	Mesocosm	76, 82	Gao *et al.* (2000)
			Trifluralin, cycloxidim, Atrazine, Terbutryn	Mesocosm	n/a	Turgut (2005)
	Elodea canadensis	Submerged	DDT (OP,PP-DDT)	Mesocosm	89	Garrison *et al.* (2000)
POP	*Lemna gibba*	Floating	Phenol	Mesocosm	90	Barber *et al.* (1995)
	Lemna minuta	Floating	Phenol	Mesocosm	100	Paisio *et al.* (2018)
	Potamogeton crispus	Submerged	Phenol	Mesocosm	70-100	Hafez *et al.* (1998)
			PAHs (phenanthrene and pyrene)	Mesocosm (sediment pots included)	18−34 , 14.-24	Meng et al (2015)

PPCP	*Phragmites australis*	Emergent	Estrone, 17 beta-estradiol, 17 alpha-ethynylestradiol	Constructed wetland	68-84	Song *et al.* (2009)
	Scirpus validus	Emergent	Diclofenac	Mesocosm	1-7%	Zhang *et al.* (2012)
			Naproxen, Carbamazepine,	Constructed wetland	97-99,53-60	Zhang *et al.* (2013a)
			Caffeine	Mesocosm	>99.7	Zhang *et al.* (2013b)
	Typha angustifolia	Emergent	Carbamazepine, Naproxen, Diclofenac, Ibuprofen	Constructed wetland	27, 91, 55,80	Zhang *et al.* (2011)
	Pontederia cordata	Emergent	Troclosan, methyl-triclosan & Triclocarbon	Constructed wetland	n/a	Zarate *et al.* (2012)
	Sagittaria graminea	Emergent	Troclosan, methyl-triclosan & Triclocarbon	Constructed wetland	n/a	Zarate *et al.* (2012)
	Typha latifolia	Emergent	Troclosan, methyl-triclosan & Triclocarbon	Constructed wetland	n/a	Zarate *et al.* (2012)

Note

1. n/a refers to studies where the removal efficiencies are not reported

2.3.4 Microbial pollutants

Most studies on the removal of microbial pollutants and their indicators of the presence (e.g. *E. coli*, faecal coliforms and faecal streptococci) are focused on macrophytes within CWs, therefore the following examples will mainly refer to this planting type (see section 2.7.1). Furthermore most studies show that CW planting systems remove microbial pollutants from water via a combination of chemical, biological and physical mechanisms. A study of 12 CWs found that over a year vegetated CWs removed between 95 - 97 % of faecal coliforms and 93 - 98 % of faecal streptococci (Karathanasis *et al.*, 2003). Similarly, in an experimental CW system, Makvana & Sharma (2013) demonstrated removal rates of 94 %, 87 % and 94 % for *Salmonella*, *Shigella* and *Vibrio,* respectively. However, the removal of *Salmonella* and *E. coli* from water in unplanted control mesocosms versus mesocosms containing *Typha latifolia*, *Cyperus papyrus*, *Cyperus alternifolius* and *Phragmites australis* showed no significant difference in the removal rates (> 98 %) between the two treatments; furthermore, in general, unplanted mesocosms reached their maximum removal rate before the planted mesocosms (with the exception of the *C. alternifolius* mesocosm) suggesting that plants provide little additional benefit for removing biological pollutants over and above the effect of standing water conditions (Kipasika *et al.*, 2016). Similarly, a review comparing *Lemna sp*. treatment ponds against unplanted treatment ponds showed that the latter had greater removal rates of *E. coli* facilitated by the greater exposure of the water to UV light and the subsequent photodegradation and microbial die-off (Ansa *et al.*, 2015). However, Decamp & Warren (2000) have shown that gravel beds planted with *Phragmites australis* remove *E. coli* more quickly compared to unplanted soil beds, possibly as a result of the impact of antagonistic root exudates from *P. australis* on *E. coli* survival.

The variability of the results obtained between planted and unplanted experiments suggests that for each treatment system different mechanisms of microbial pollutant removal become dominant. Within unplanted facultative systems or lagoons it is likely that oxygenation and phytodegradation from UV light are the dominant methods of removal (Ansa *et al.*, 2015). Conversely, biological and chemical process may become more important within planted systems, for example, *Pistia stratiotes* facilitates presence of protozoa by providing structural habitat, which can increase predation on *Salmonella* (Awuah, 2006). Conversely, predation from protozoa seemed to have a negligible effect in systems planted with *Spirodela* *polyrhiza* (greater duckweed), highlighting that removal mechanisms are probably related to below-ground morphological attributes, with more extensive roots/rhizomes providing superior habitat for grazers (Awuah and Gyasi, 2014). Increased root zone surface area also facilitates greater microbial biofilm growth which is thought to be a key removal structure for bacterial adsorption and predator microbial proliferation (Decamp and Warren, 2000). Therefore, smaller grasses such *Festuca arundinacea* may have limited potential for microbial pollutant removal compared to large emergent such as *Typha latifolia* (Decamp and Warren, 2000). Future research investigating the ability of different macrophytes to remove microbial pollutants from water, especially outside of CW systems, is clearly merited. Direct deployment of macrophytes for pathogen removal would be highly beneficial in developing countries where low-cost options for remediation could provide accessible water treatment.

Of the few experimental studies investigating potential for macrophyte removal of microbial pollutants outside of CWs, Saeed *et al.* (2016) demonstrated a 72 % reduction of *E. coli* in FTWs planted with *Phragmites australis* and *Canna indica*. However, during times of high *E. coli* loading, induced by experimental 'shock phases' where hydraulic loading was increased between 5 to 14-fold to simulate low frequency and high magnitude discharge events, the removal of *E. coli* was reduced significantly to levels varying between 6 - 45 %. The effect of hydraulic retention time is also important for pathogen survival and die-off (Reinoso *et al.* 2008) and may have implications for the use of phytoremediation (with FTWs) in lakes and rivers given the difference in hydraulic retention times.

2.4 Macrophyte phytoremediation communities

There has been considerable work focusing on the ability of individual plant species to remove single pollutants from water (e.g. Zhou & Wang 2010), with the design of CWs also focusing on monocultures of macrophytes (Kadlec, 2009). Conversely, there has been a lack of studies that explicitly explore the ability of mixed plant assemblages to simultaneously take-up and degrade multiple pollutants (Koelbener *et al.*, 2008). A plant community-based approach provides the opportunity to enhance the removal of both single pollutants, but also target multiple contaminants. Studies that have looked specifically at phytoremediation using plant communities have shown encouraging results (Fraser *et al.*, 2004; Zhang *et al.*, 2007; Liang *et al.*, 2011; Türker *et al.*, 2016). For example, an experiment testing the removal of N and P from four different emergent macrophytes in parallel (*Carex lacustris*, *Scirpus validus*, *Phalaris arundinacea* and *Typha latifolia*) found that microcosms planted with all four macrophytes in equal proportion, either matched or outperformed microcosms planted with a single species (Picard *et al.* 2005). Earlier studies also suggest that plant polycultures have a greater removal potential for heavy metals and can reduce biochemical oxygen demand (BOD) (Karpiscak *et al.*, 1996; Scholes *et al.*, 1999). However, Türker *et al.* (2016) reported that boron removal from mine effluent was more effective in native emergent monocultures compared to polycultures, although the opposite was true for NO_2^- removal. These results suggest that there are probably optimal plant combinations for particular pollutants and further experiments designed to identify these combinations would help to optimise the efficiency of phytoremediation.

To assemble appropriate plant combinations there are several important factors to consider including the functional diversity of the community. It has been reported that simply increasing species diversity in a plant assemblage can increase nutrient removal, although polycultures containing more than three species showed no further benefit (Ge *et al.* 2015; Geng *et al.* 2017). A common theme among these studies is the importance of species identity in explaining variation in nutrient removal, where specific combinations can more effectively remove pollutants. Therefore, assembling appropriate plant communities based around the complementary phytoremediation potential of individual species, and the interaction of those plants with others in the assemblage is potentially more important than simply increasing species richness per se. However, the effect of competition between plants is important to recognise as this may impact the community composition, and therefore the ability to remove the targeted pollutants from water (Zhang *et al.* 2007). In a mesocosm experiment, containing the submerged macrophytes *Stuckenia pectinata* (Sago pondweed), *Potamogeton natans* (broad-leaved pondweed), *Potamogeton crispus* (curled pondweed) and *Zannichellia palustris* (horned pondweed), it was found that *S. pectinata* reduced the biomass of the other species (Engelhardt & Ritchie, 2001). Reducing the biomass of certain species will not necessarily compromise overall removal efficiency as uptake and sequestration potential will vary with species. However, this highlights the need to understand interspecific interactions in order to enhance removal efficiency, especially when considering targeting water bodies in a non-equilibrium state where conditions favour the dominance of one particular species (Engelhardt & Ritchie, 2002).

A field study employing plant communities revealed some of the benefits of combining multiple macrophytes (Wang *et al.*, 2009; Zhao *et al.*, 2011). Nine macrophytes species (five floating, one submerged and three emergent) deployed on FTWs and planted on river banks outside Jiaxing City, China, demonstrated removal rates of TN and TP at 16 % - 37 % and 26 % - 43 % respectively (Zhao *et al.*, 2011). Although the removal rates were relatively low, it was also highlighted that the plant community-based approach allows for species within the community to compensate for deficits in uptake of other species (Zhao *et al.*, 2011). For example, the average P content of floating macrophytes was *ca.* 5.9 g/m^2, whereas, emergent species including *Canna indica and Pontederia cordata* with higher biomass accumulation, stored P at a level of *ca.* 7.3 g/m^2.. Similarly, a phytoextraction study with emergent species (*Carex flava*, *Centaurea angustifolia* and *Salix caprea*) allowed the impact of facilitation across increasing concentration gradients to be seen (Koelbener *et al.*, 2008). Here, the willow *S. caprea* attenuated the toxic effect of Zn on the relative growth rate of *C. flava* by lowering the availability of Zn, thus mitigating the negative effect of Zn on the sedge (Koelbener *et al.*, 2008). This highlights that competitive effects may not always be negative and may produce positive effects through 'over yielding'. The consequences of competitive interactions between candidate macrophytes evidently deserve particular attention within the field of plant community-based phytoremediation.

As well as the potential enhanced removal of pollutants from plant communities with macrophytes of different life forms (Koelbener *et al.*, 2008) there may also be the potential for generating ecosystem services from polycultures. A 2-year study by Wang *et al.* (2009) explored the potential restoration of Lake Taihu and Lake Machou by using a mosaic of macrophytes in successional stages highlighting the potential for spatial and temporal diversity in macrophyte deployment, and the provision of ecosystem services. Floating and emergent macrophytes were first introduced to reduce light availability for algal growth, facilitating the introduction of submerged species leading to removal rates of TN and TP of 60 % and 72 % (Wang *et al.*, 2009). The provision of ecosystem services due to the different plant life forms was highlighted as an advantage by Wang *et al.* (2009) as increased patches of vegetation provided refuge for zooplankton that subsequently grazed phytoplankton. The added value of diverse plant communities is a factor that requires quantification to espouse the benefits of aquatic phytoremediation over and above water treatment.

Plant community-based approaches provide the opportunity to build temporally more consistent treatment into phytoremediation by exploiting the differing phenology of plant species; polyculture systems can thus offer the most consistent water treatment option with least susceptibility to seasonal variation (Karathanasis *et al.*, 2003). However, the temporal dynamics of plant communities within the context of phytoremediation are under-researched, and there is a need to explore the assembly of plants, e.g. in terms of differing phenologies, to extend the growing season, especially in temperate regions where water treatment potential declines after senescence.

2.5 Issues in utilising invasive macrophytes

The most effective phytoremediators have fast growth rates and high biomass accumulation; however, outside of their native range macrophyte species with these traits are often considered to be invasive, and given their potential for rapid colonisation they can quickly outcompete native macrophytes (Chambers *et al.*, 2008). Species that are invasive in the UK, such as *Azolla filiculoides* and *Hydrocotyle ranunculoides*, can clog waterways and have serious ecological impacts on native flora and fauna (Schultz and Dibble, 2012). In the UK, the combined cost of controlling invasive plants, together with their economic impact, is estimated to be £1.7 billion per annum (The Great Britain Non-native Species Secretariat, 2015). Therefore, there is a significant juxtaposition between using species of invasive plants in phytoremediation, and management strategies to control invasive species (Rodríguez *et al.*, 2012). Given that in many cases the complete eradication of invasive aquatic macrophytes such as *Eichhornia crassipes* is unlikely, it may be more appropriate to exploit these macrophytes as part of an integrated management strategy that controls the spread of these species whilst at the same time effectively removing nutrients and metals, capturing suspended sediment, and harvesting the biomass for economic gain (Patel, 2012; Yan *et al.*, 2017). A similar parallel can be drawn with non-native and invasive zebra mussels (*Dreissena polymorpha*) which are often considered detrimental (Matsuzaki *et al.*, 2009), but have also widely been reported to stabilise the clear-water state of shallow lakes through filtering phytoplankton and removing harmful cyanobacteria (Gulati *et al.*, 2008).

Water bodies where invasive species are already present may be targeted for active harvesting allowing periodical regrowth for continued phytoremediation (Xu *et al.*, 2014).

However, there are important factors to consider including the containment of macrophytes to avoid transfer to other water bodies (e.g. via contaminated harvesting equipment or through downstream spread of fragments), including the most appropriate harvesting technique, and the sustainability of exploiting such an ecological engineering systems (Rodríguez *et al.*, 2012; Yan *et al.*, 2017). The site-specific context will likely determine the appropriateness of active harvest of invasive aquatic plants (Yan *et al.*, 2017). In terms of introducing macrophytes into a freshwater system for phytoremediation, it is inappropriate, and indeed possibly illegal, to deploy invasive species given the potential for ecosystem damage and long terms effects. In these circumstances non-invasive or native plants should therefore be employed, unless containment of invasive plants can be ensured, such as in engineered CW systems.

2.6 Macrophyte planting systems

Macrophyte planting systems are effectively planting strategies that are employed to facilitate targeted phytoremediation of waters in different contexts in terms of point source and diffuse source treatment and restoration. The following section details the key aspects of the three main aquatic phytoremediation planting systems that have been developed; CWs, wild macrophyte harvesting and planting, and FTWs.

2.6.1 Constructed wetlands

Phytoremediation has primarily been optimised for point source wastewater treatment in the form of CWs. CWs have been used for the treatment of a variety of effluents including urban storm water, sewage, mine tailing drainage, storm water treatment, landfill leachate treatment systems and for wastewater polishing (Kivaisi, 2001; Nivala *et al.*, 2007; Tanner, 1996; Vymazal, 2009; Vymazal, 2011). CWs also show potential for treating wastewater containing emerging contaminants of concern including pharmaceuticals and other endocrine disrupters (Vymazal, 2009).

CWs can be categorised as free water surface flow wetlands (FWSF) or sub-surface flow (SSF) wetlands (Dhir, 2013) (Figure 2.3). FWSF wetlands contain emergent, floating and submerged macrophytes growing in shallow ponds or lagoon waters over sandy or organic soils, which allows the influent contaminated water to slowly flow through the emergent macrophyte stems for maximum pollutant uptake and UV degradation (Kadlec, 2009).

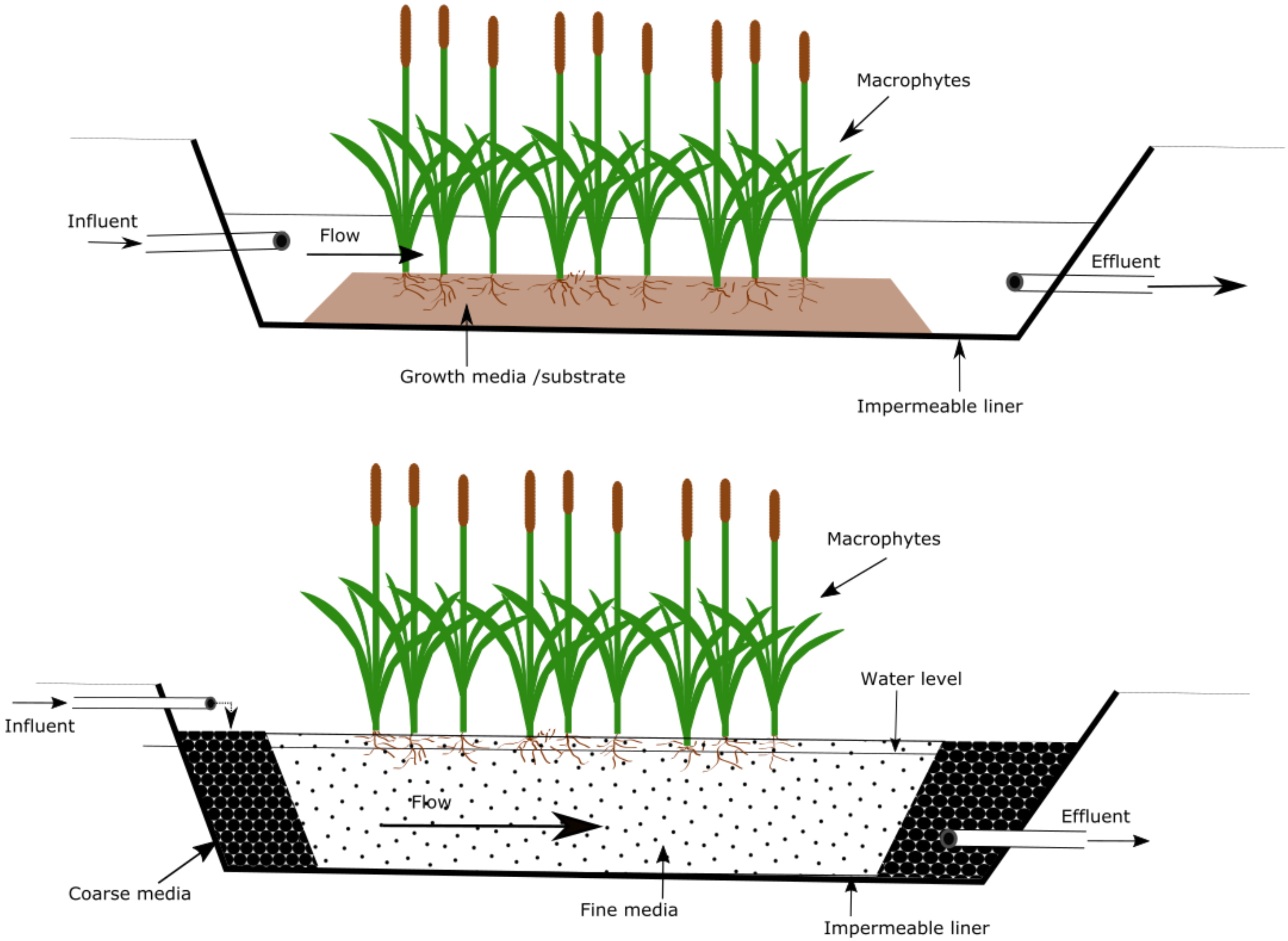

Figure 2.3: Top: Key elements of a free water surface flow wetlands (FWSF) constructed wetland. Bottom: Key elements of a or sub-surface flow (SSF) constructed wetland.

SSF wetlands are the most common type of CW and comprise emergent macrophytes growing over a substrate of stone or gravel matrix enabling water to come in direct contact with plant roots, rhizomes and biofilms, which promote aerobic conditions (Vymazal, 2011). Several processes including physical filtering of the water, biological processing of water by plants and microbial biofilms, and chemical changes due to redox state can assist in pollutant removal in SSF systems (Faulwetter *et al.*, 2009). The average SSF CW system is 100 times smaller than the FWSF CW system (Kadlec, 2009), therefore, FWSF are more common in North America and Australia where a larger surface is available, whilst SSF wetlands are more common in Europe where land availability is more limited (Vymazal, 2011). SSF wetlands are frequently used to ameliorate the concentration of biologically derived organic material as indicated by the lowering of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) from waste waters (Vymazal & Kröpfelová, 2009).

CWs are the most advanced form of macrophyte deployment within the umbrella of aquatic phytoremediation (Kennen and Kirkwood, 2015). However, these systems can require high investment costs and they are restricted primarily to pollutant point sources where there is wastewater treatment such as tertiary sewage treatment and wastewater polishing before water enters a natural waterway (Patiño Gómez and Lara-Borrero, 2012). This restricts the application of CWs for the treatment of water containing pollutants from diffuse sources. Although CWs have the potential to be utilised for treatment of a wide range of contaminants, their most widespread application has been for sewage wastewater-related contaminants, including BOD, COD, N and P, and often they are set up with crop monoculture to maximise plant uptake (Kadlec & Wallace, 2009; Sundaravadivel & Vigneswaran, 2001; Vymazal, 2009).

CWs vary in level of design and engineering required for their development; FWSF wetlands are generally low tech gravity-fed systems, whereas, SSF require more construction and management to import the stone/gravel matrixes, and also may include bunds to separate different treatments then requiring the use of electric pumps (Kadlec and Wallace, 2009). In both types of CWs there are high investments in construction and operational costs. CW can also become clogged with sediment, which impacts the functioning of the system and imposes additional costs for excavation and removal of contaminated sediments, and the subsequent reinstatement of macrophytes (Machado *et al.*, 2016). According to design guidance for the treatment of urban waste water and sewage, SSF CWs may require an area of around 5 m^2 to 10 m^2 of CW per person equivalent for adequate water purification (Tilley *et al.*, 2014). Therefore, given the potentially large area required, CW-based phytoremediation may be unable to compete for limited land availability with other more profitable land uses. Furthermore, in countries where vector-borne diseases, such as malaria or dengue, are a public health issue the creation of open shallow wetland environments may be undesirable as it has the potential to provide ideal conditions for the propagation of mosquitoes and other disease vectors (Mwendera *et al.*, 2017).

From both industry-based observations and from the available literature, the primary purpose of CWs is water treatment and wastewater polishing. This however, ignores their potential to offer ecosystem services such as sequestering and harvesting nutrients for reuse, provisioning for biodiversity, pollination and carbon sequestration, and thus underplays the overall value of CWs. There is great potential to develop different post-remediation 'streams' which have been relatively unexplored, and which emphasise support for different ecosystem services (see section 2.10.2). Aquatic phytoremediation is a promising technology for the treatment and remediation of polluted water with the operational point-source based CW systems in place, but given the limitations of these systems, including the lack of application for diffuse pollutants, investment costs and lack of ecosystem focus there is an opportunity to further develop context-specific, sustainable phytoremediation that provides ecosystem services within wider environmental systems.

2.6.2 Wild macrophyte harvesting

Most aquatic phytoremediation planting systems involve the deliberate deployment (FTW) or engineering of planted systems (CWs). Harvesting of existing wild macrophytes from water bodies such as shallow lakes can also be a phytoremediation strategy, and relies upon the opportunistic and timely removal of macrophyte biomass in order to manage waterborne pollutants such as N and P (Huser *et al.*, 2016). A study of an urban shallow lake, showed that harvesting an annual amount of 3,600 kg dry weight of *Elodea canadensis* led to 16.4 kg P being removed from the system, equating to around 53 % of the TP load removed (Bartodziej *et al.* , 2017). Although the estimated cost of removal was $670 per kg of TP, which was more expensive than chemical flocculating treatment, this was still considerably less expensive than many catchment best management practices (Bartodziej *et al.*, 2017). Macrophyte harvesting is often carried out in lakes and waterways ostensibly to relieve navigation, drainage, aesthetic or recreational problems, rather than for phytoremediation purposes, but is notable that nutrient export may be a collateral benefit of such harvesting. Other case studies have shown that macrophyte harvesting for nutrient removal does not reduce nutrient loading quite as favourably (Carpenter and Adams, 1977; Morency and Belnick, 1987), with Peterson *et al.* (1974) estimating that plant harvesting only removed 1.4% of TP loading.

The variation between these case studies is possibly a result of the levels of nutrient loading, with waters that receive extremely high inputs of nutrients leading to a poor offset by removal from plant harvesting (Bartodziej *et al.*, 2017). Another source of variability for nutrient removal is the coverage of macrophytes across the particular water body; the reported optimal coverage of macrophytes ranges from 5 % to 40 % (Portielje and Van der Molen, 1999; Dai *et al.*, 2012; Xu *et al.*, 2014). For environmental managers considering macrophyte harvesting as a mechanism for in-water nutrient management, it is crucial that a scoping study is carried out to determine the base balance of nutrient input/output and plant removal capacity, and to identify the need for upstream best practices as part of an integrated management strategy.

The harvesting method itself is also an important element of harvesting wild macrophytes, e.g. removal by hand, or mechanically via specialised boats equipped with cutting or raking apparatus (Quilliam *et al.*, 2015). Hand removal is labour and time intensive, although it allows targeted macrophyte removal and minimises disturbance. Conversely, mechanical removal allows more rapid and extensive removal but is non-selective and can lead to high levels of turbidity due to the re-suspension of sediments. This can impact invertebrates and fish by removing structural habitat and may ultimately drive the system from a desirable clear water macrophyte-dominated state to a potentially unfavourable phytoplankton- dominated state (Dawson *et al.*, 1991; Sayer *et al.*, 2010; Habib and AR, 2016).

In some circumstances it may be necessary to establish macrophytes in waterbodies by direct planting through seeding or transplanting propagules (e.g. tubers/root crowns) if there are no existing macrophytes, or if a particular species is required to target certain pollutants (Smart *et al.*, 1998; Hilt *et al.*, 2006). In addition to plant establishment there is also scope to enhance macrophyte growth and biomass by engineering interventions such as the assembly of polytunnels over vegetation, or enclosures to reduce grazing losses.

2.6.3 Floating treatment wetlands

Within aquatic phytoremediation one such novel ecological engineering solution that has been developed is the FTW. The premise of this system is that highly productive emergent macrophytes such as *Typha latifolia* are planted within a growth medium, which is supported by a buoyant frame allowing the roots of the emergent macrophytes to be submerged in the water, thus enabling rhizofiltration, phytoextraction and phytodegradation to take place hydroponically (Nichols *et al.*, 2016; Kiiskila *et al.*, 2017) (Figure 2.4). Root uptake associated with FTWs is primarily applicable to water-soluble contaminants within the water column only, although sediment-bound pollutants can be physically filtered from the water column by plant roots (Tanner and Headley, 2011b). FTWs have recently gained increased attention and may also be referred to in the literature as artificial floating islands, integrated ecological floating beds, floating plant bed system and hydroponic root mats (Yeh *et al.*, 2015).

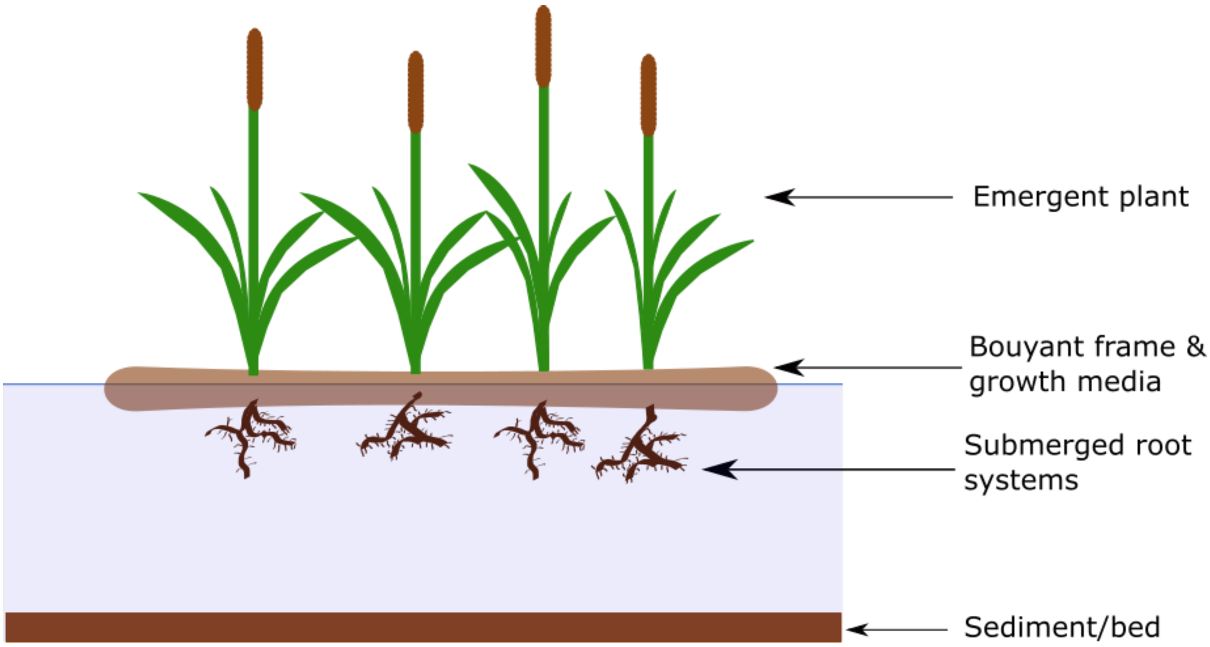

Figure 1.4: Schematic view of a floating treatment wetland (FTW)

FTWs can accommodate fluctuations in water levels, and the stability of materials used to construct the buoyant frame may include items such as polyvinyl chloride (PVC) pipes, foam sheets, bottles and bamboo (Ladislas *et al.* 2013; Wang *et al.* 2015; Pavlineri *et al.* , 2017). However, it would be useful within the literature if qualitative information and design challenges were also reported to provide an idea of performance and usability of FTWs in practice, and although there are no reported incidences of FTWs capsizing or other failures during pilot tests, this may simply reflect publication bias.

Netting material or foam is generally used to support the growth medium in which the macrophytes are grown (Yeh *et al.*, 2015). Material previously used as substrate includes peat, soil, cotton and coir fibre (Pavlineri *et al.*, 2017). Furthermore, FTWs comprising foam with gaps to support pots have also been designed (Lynch *et al.*, 2015). Growth media physically supports the planted macrophytes and provide nutrition, but the substrate can also enhance pollutant removal through the stimulation of microbial activity (Tanner & Headley, 2011a). Macrophytes may be established by transplanting of seedlings, cuttings or whole plants (Yang *et al.*, 2008; Ning *et al.*, 2014). An advantage of using FTWs rather than direct planting of macrophytes is the ease in which the biomass can be harvested from the frame, instead of having to remove plants from the sediment. The quick and simple method of harvesting afforded by growing plants in FTW facilitates recovering pollutants from plant biomass (Bartodziej *et al.*, 2017). There is potential for quick re-planting of the FTW for continued remediation and biomass removal (Wang *et al.*, 2015; Ge *et al.*, 2016).

FTWs have been studied principally for their capacity to remove nutrients, but there have also been attempts to assess heavy metal, pathogen and phytoplankton removal (Borne, 2014; Yeh *et al.*, 2015; Jones *et al.*, 2017; Kiiskila *et al.*, 2017). FTWs have been deployed at a variety of different scales including microcosms, mesocosms, and as pilot trials within lagoons (Headley and Tanner, 2008; Ladislas *et al.*, 2013; Chang *et al.*, 2014; McAndrew *et al.*, 2016; Nichols *et al.*, 2016; Kiiskila *et al.*, 2017). Here the experimental polluted water used has included storm water, lake water, river water, sewage effluents, domestic wastewaters, refinery wastewater, acid mine drainage, and livestock effluents (Zhu *et al.*, 2011; Li *et al.*, 2012; Borne, 2014; Wang and Sample, 2014a; Abed *et al.*, 2017; Kiiskila *et al.*, 2017). Mesocosm-scale studies are the most prominent form of exploration into the effectiveness of FTW thus far (Chen *et al.*, 2016) , although there have been a few examples of deployment at field-scale, such as Zhao *et al.* (2012) who demonstrated that TN and TP concentrations could be reduced in a polluted Chinese river. Mesocosm studies with synthetically produced experimental water allows full control of all input parameters. However, they may not be representative of the real remediation performance given that polluted waters contain a multitude of chemicals and microbes which may influence remediation (Javadi *et al.*, 2005).

Therefore, further studies would benefit from testing the remediation of water sourced from the environment.

Only a small handful of field-scale experiments have been carried out that assess the usefulness of FTWs in successfully remediating pollutant-impacted waters (Zhu *et al.*, 2012; McAndrew *et al.*, 2016; Nichols *et al.*, 2016; Olguín *et al.*, 2017). Of the available studies that assess FTW performance within water bodies, including streams, urban and rural ponds, results focus on plant tissue element accumulation rather than the arguably more pertinent issue of water quality improvement (Zhu *et al.*, 2012; Olguín *et al.*, 2017; McAndrew *et al.*, 2016; Nichols *et al.*, 2016). Although plant tissue sequestration is extremely important for assessing the bioaccumulation potential of macrophyte species it does not explicitly demonstrate water quality improvement; this can only be proven through monitoring water chemistry. Scaling up mesocosm scale experiments to assess actual field-scale water quality improvement is challenging given the ideal of a control site with comparable water chemistry and abiotic and biotic conditions, or high-temporal resolution baseline water quality data for the experimental water body, both of which may be unavailable. Where there is a clear opportunity for upstream and downstream water quality sampling near the experimental FTWs, such as a stream, water quality changes are more likely to be attributed to the FTW intervention between these points (Olguín *et al.*, 2017). Similarly, more field studies longer than 2 years, ideally up to 5 to 10 years, would lead to a better understanding of the longer-term performance of FTWs and, crucially, reveal the actual remediation time (Yang *et al.*, 2006). Furthermore, the influence of inter-annual hydrological variability on FTW performance in terms of precipitation and evaporation could also be evaluated. Despite the paucity of scientific studies at the field scale, commercial companies now commonly offer FTWs as a water treatment solution, and as part of the aesthetic enhancement of urban rivers. The phytoremediation research community must aim to keep pace with the private sector to corroborate industry-advocated benefits of FTWs and avoid any potential reputational damage to aquatic phytoremediation where expectations of these systems from stakeholders are not met (Keenen and Kirkwood, 2015).

The remediation performance of FTWs is highly variable with reported minimum and maximum removal efficiencies for TN values being 0.71 mg/l (4 %) and 51 mg/l (91 %) and 0.06 mg/l (1 %) and 18.85 mg/l (90 %) for TP (Figure 2.5). This high variability may be due to differences in FTW design, macrophyte species employed, and the chemical composition of the experimental water. A further example of variation in removal efficiency comes from Lynch *et al.* (2015) who compared two commercial FTWs (Beemat and BioHaven®) planted with the rush *Juncus effusus* that had been designed to treat storm water. It was found that Beemat FTW outperformed BioHaven® in both TN and TP removal (Lynch *et al.* 2015). The difference in removal may have been due to the difference in substrate (coir matting vs. sphagnum peat) or the physical design of FTW (Lynch *et al.* 2015). The growth medium is indeed an important source of variability within FTW design. Rice straw used as growth medium was found to enhance removal of TN, NH_4^+ and NO_3^- compared to plastic filling (Cao and Zhang, 2014). Similarly, the FTW with straw filling had a greater total density of nitrifying and denitrifying bacteria which suggests that this organic material was providing both a habitat and a source of C for the growth of microorganisms, which were able contribute to pollutant metabolism (Cao and Zhang, 2014). Commercial FTWs are still an expensive management option, and there is currently a demand for more low-cost growth media that both provides a suitable substrate for macrophytes and enhances pollutant removal; such examples include biochar, activated carbons, coffee waste and green compost (Tran *et al.*, 2015). To date there has been no research incorporating these materials into FTWs to assess the potential for enhanced remediation and the potential value post-remediation.

Hybrid FTW planting systems are being developed in an attempted to enhance pollutant removal and ecosystem restoration (Guo *et al.*, 2014; Li *et al.*, 2010; Lu *et al.* 2015). Such systems integrate a new layer beneath the floating platform containing submerged macrophytes such as *Potamogeton crispus*, and/or bivalves such as freshwater clams (*Corbicula fluminea*) (Guo *et al.*, 2014; Li *et al.*, 2010) (Figure 2.6). Photovoltaic solar panels have also been attached to the frames of FTW to power a submerged aerator to enhance oxygenation in the vicinity of the plant roots and associated microorganisms, thus increasing the nutrient degradation process (Lu *et al.*, 2015) (Figure 2.6). While these hybrid systems appear to enhance pollutant removal from the water column compared to their macrophyte-only counterparts (Guo *et al.*, 2014; Li *et al.*, 2010), the added complexity may impact on the utility of FTW as a phytoremediation system. With increasing complexity of FTW design there is an increase in pollutant removal efficiency, cost and maintenance, but a decrease in user uptake given the added management of submerged plants or solar PV systems. A focus on maximising removal efficiency over the simplicity of the system may create barriers for uptake by stakeholders such as farmers, land managers and government organisations looking for low-cost low maintenance treatment options, especially within developing countries. A useful exercise might be to compare the economics, maintenance requirements and user experience of hybrid versus conventional FTWs to determine when increasing FTW complexity is appropriate.

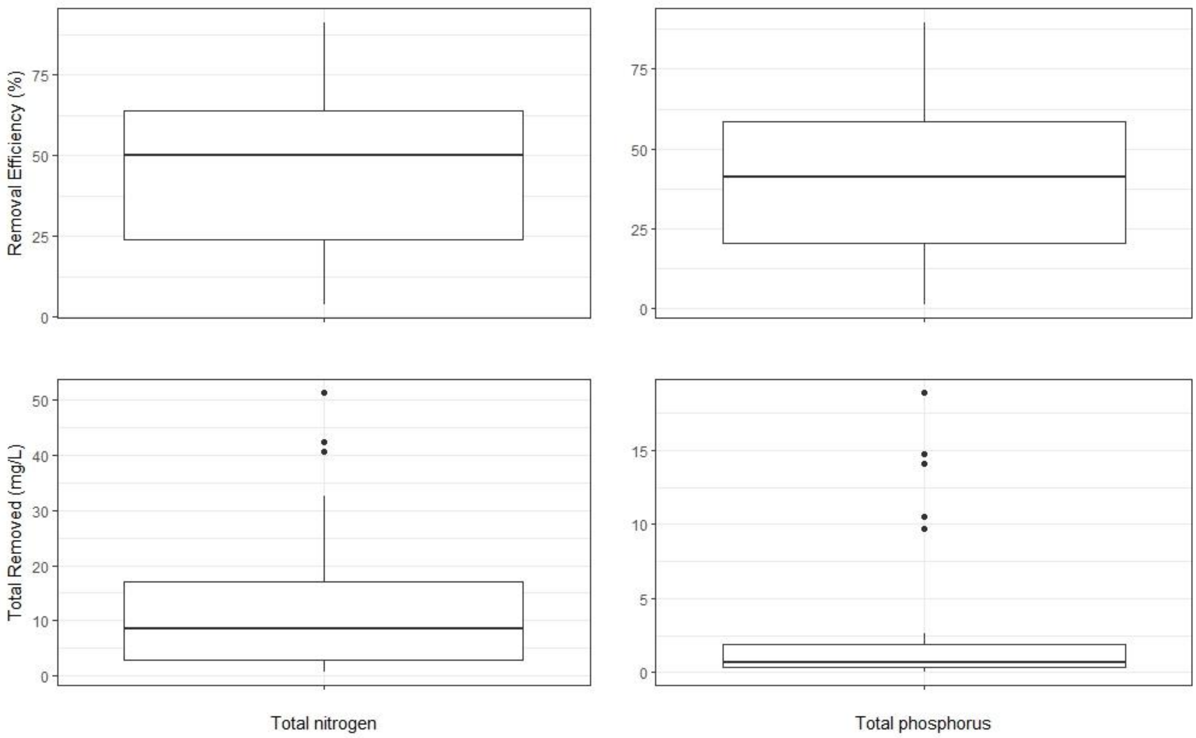

Figure 2.5: Boxplots of removal efficiencies (%) and total removed (mg/l) of Total Nitrogen (TN) (*n*=44) and Total phosphorus (TP) (*n*=28), raw data taken from literature reviewed by Pavlineri *et al.* (2017).

The coverage of FTW over the target water body is also important, as indicated by a meta-analysis showing that vegetation cover is significantly correlated with the removal of NH_4^- (Pavlineri *et al.*, 2017). Although increasing FTW coverage reduces atmospheric diffusion, oxygen is supplied to water by emergent plants via root oxygenation (Xiao *et al.*, 2016; Yeh *et al.*, 2015). Furthermore, in eutrophic waters this coverage may inhibit algal primary productivity, which may be beneficial for mitigating the potential for occurrences of large algal blooms (Jones *et al.*, 2017). The optimal coverage of FTWs has been reported as 10 -

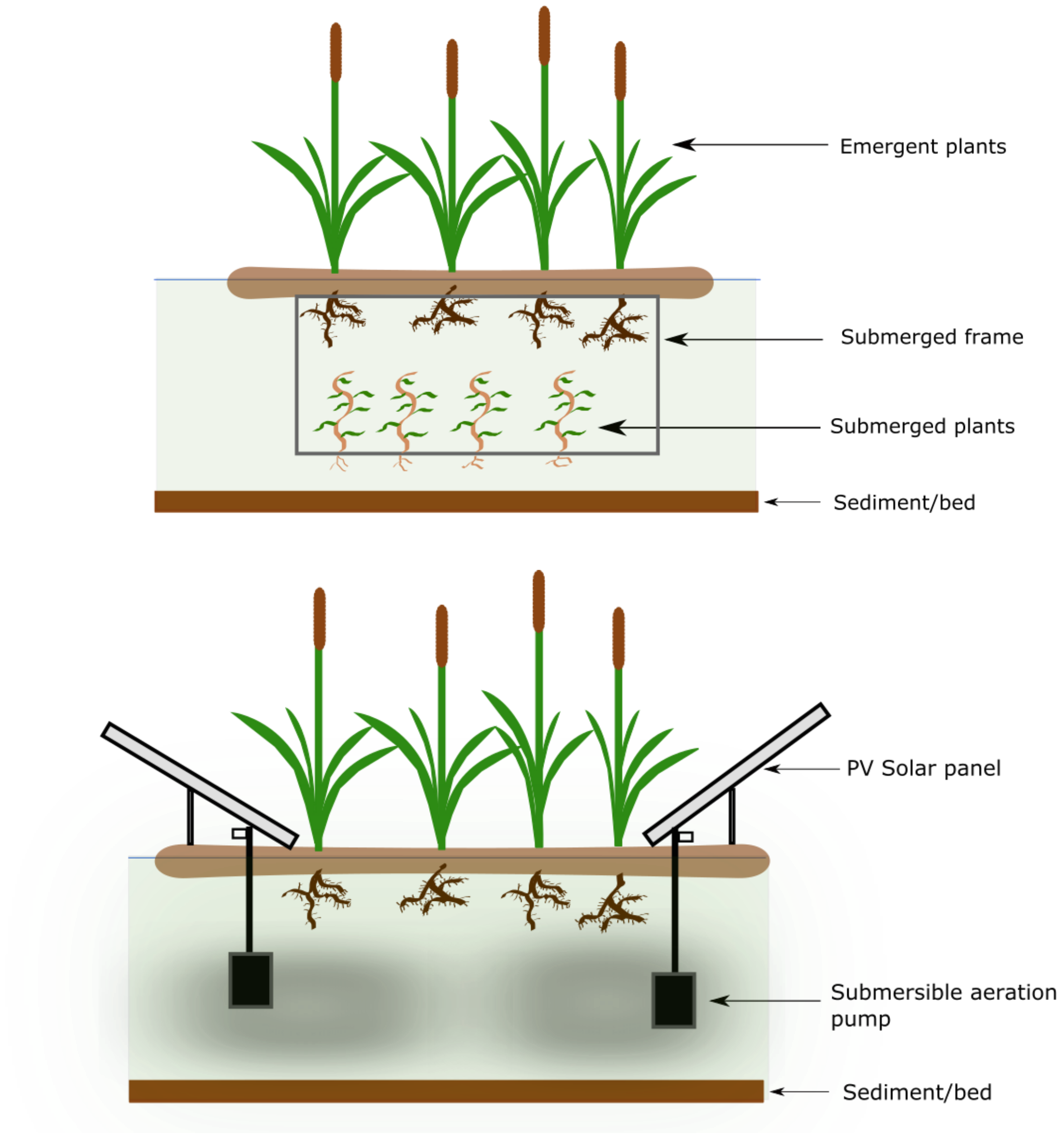

Figure 2.6: Top, a schematic reprentation of a hybrid FTW including submerged vegetatation. Bottom schematic reprentation of a FTW incorporating solar technolgy to power an areation device.

25% (Marimon *et al.*, 2013), although generally there is wide variation in the literature with values of between 100 %, 50 % and 5 - 8 % being reported as acceptable for water treatment (Pavlineri *et al.*, 2017). McAndrew & Ahn (2017) also note that hydraulic retention time and plant productivity are important for determining removal efficiency. Surface cover therefore needs to be considered in tandem with hydrology and macrophyte selection. As the focus within the literature is on coverage, there has been no clear attempt to look at the different surface arrangements of FTW on the water surface. For example, targeting of an area, such as water inlet or outlet to a lake may be more beneficial than increased FTW coverage over the target water body. Clearly, the coverage and area of FTW treatment is context-specific but there is likely to be significant potential in investigating spatially targeted phytoremediation.

Finally, the poor design and management of FTWs is a topic that is rarely discussed within the literature. FTWs have the potential to be pollutant sources should the biomass not be continually harvested and removed, or if water birds attracted to the FTWs defecate into the water inputting nutrients and microbial contaminants (guanotrophication). Nutrient-rich growth media such as peat may also leach nutrients into the target water body compared to more inert coir fibre (Lynch *et al.*, 2015). The placement of FTWs in watercourses must also be given full consideration as water birds and recreational users may also use the target waterbody. FTWs potentially slow the velocity of water in small water bodies such as ditches, which may conflict with farming interests where good drainage is required. As with any good catchment management practice, appropriate consultation with stakeholders is important for success.

2.7 Translocation and element storage in macrophytes

Understanding how and where nutrients and other pollutants are distributed within macrophyte tissues is important to inform plant harvesting for removal of pollutants. The recovery of nutrients is crucial for the value of post-harvest plant biomass, whilst ensuring correct plant parts are harvested for effective removal of heavy metal and organic pollutants from the planting system. Allometry of pollutants within plants varies according to species, but is also influenced by the environmental conditions in terms of nutrient availability (Barrat-Segretain, 2001; Demars and Edwards, 2007).

Typha domingensis, Eichhornia crassipes, Pistia stratiotes and *Myriophyllum aquaticum* preferentially store N and P in the shoot compared to the roots or rhizome (Table 2.6), although nutrients can be translocated through the plants leading to temporal dynamism in element distribution driven by plant phenology and diurnal metabolism (Masclaux-Daubresse *et al.*, 2010; Hawkesford *et al.*, 2011; Eid *et al.*, 2012). More than 50 % of N can be stored in below-ground plant parts by the end of a growing season (Vymazal, 2007). *Phragmites* *australis* grown in either natural waters or a waste water infiltration pond demonstrated a clear seasonal pattern in the translocation of nutrients from above-ground to below-ground parts as the end of the growing season approached (Meuleman *et al.*, 2002). Early in the growing season N and P concentrations are higher due to sink demand during active growth before concentrations decrease gradually through the season as plants begin to senesce.

Table 2.6: Plant allocations of pollutants in selected emergent, floating and submerged macrophytes

Species	**Growth form**	**Plant allocation of pollutant**		**Reference**
		Above-ground	**Below-ground**	
Cyperus riparia	Emergent	Cd, Ni, Zn		Ladislas *et al.* (2013)
Cyperys esculentus	Emergent	Cd, Cr, Cu, Fe, Mn, Ni	Pb	Chandra & Yadav (2011)
Glyceria maxima	Emergent	Cu, Fe, Mn, Ni, Zn		Parzych *et al.* (2016)
Juncus effusus	Emergent	Cd, Ni	Zn	Ladislas *et al.* (2013)
Phalaris arundinacea	Emergent	Cu, Fe, Mn, Ni, Zn		Parzych *et al.* (2016)
Phargmites australis	Emergent	Cu, Fe, Ni, Zn	Mn	Parzych *et al.* (2016)
		Cr, Cu, Mn, Ni, Zn		Duman *et al.* (2007)
Phragmites australis	Emergent	Cd, Cu, Zn	Cr, Fe, Mn, Pb	Chandra & Yadav (2011)
Schoenoplectus lacustris	Emergent	Cu, Ni, Pb, Zn		Duman *et al.* (2007)
Typha angustifolia	Emergent	Cd, Cr, Cu, Fe, Mn, Ni, Pb	Zn	Chandra & Yadav (2011)
Typha domingensis	Emergent	Ca, Cu, Fe, P, Zn	N	Eid *et al.* (2012)
Typha latifolia	Emergent	Cu, Fe, Ni, Zn	Mn	Parzych *et al.* (2016)
Eichhornia crassipes	Floating		N, P	Polomski *et al.* (2009)
Pistia stratiotes	Floating		N, P	Polomski *et al.* (2009)
	Floating	Al, Cd, Co, Cr, Cu, Fe, K, Mg, Na	Ca	Lu *et al.* (2011)
Micranthemum umbrosum	Submerged	Cd	As	Islam *et al.* (2013)
Myriophyllum aquaticum	Submerged		N, P	Polomski *et al.* (2009)

Coinciding with the decrease in nutrient concentrations in above-ground biomass, below-ground concentrations of N and P increase, representing the preparation for plant senescence with nutrient storage in the roots and rhizomes for the following season's growth (Garver *et al.*, 1988). Meuleman *et al.* (2002) suggested that harvesting during the winter meant that only 9 % of N and 6 % of P associated with nutrient loading was removed, whereas, harvesting above-ground parts during peak nutrient storage in summer enhanced removal to

40 - 50 % of N and P. Seasonality is important, although seasonal effects will differ between temperate, subtropical and tropical zones with macrophytes in the latter two zones showing less element translocation and therefore enabling multiple annual harvests (Vymazal, 2007). Macrophytes may perform poorly if nutrient translocation to the rhizome is inhibited by harvesting during the active growing period (Tanaka *et al.*, 2017), although the issue of nutrient allocation is less problematic for floating macrophytes and emergent macrophytes deployed in FTWs as the full plant can then be harvested (Wang *et al.* 2014).

Studies on element allocation tend to report absolute concentrations to determine if a species is a better above-ground or below-ground accumulator. The potential for pollutant uptake and removal by harvesting the areal parts is a function of both concentration and the biomass produced (Polomski *et al.*, 2009). For example, although shoot concentration of N in *Pistia stratiotes* (13.93 mg/g) was greater than in *Eichhornia crassipes* (10.16 mg/g) in a study of nutrient recovery, the total areal shoot storage of N for *Eichhornia crassipes* was over four times higher due to its greater biomass (Polomski *et al.*, 2009). This demonstrates that it is more effective to harvest plants with greater above-ground biomass and moderate tissue concentrations of the pollutant of interest, rather target plants with lower biomass but higher tissue concentrations (Duman *et al.*, 2007; Vymazal, 2016).

In eutrophic waters light is commonly the limiting factor for growth and plants therefore tend to allocate nutrients to above-ground growth to maintain efficient light capture, while excessive nutrient availability negates the requirement for belowground storage (Polomski *et al.*, 2009; Lynch *et al.*, 2015); this also maintain intra-specific competitive advantages in these environments and can be exploited as part of a phytoremediation management strategy . Where non-hyperaccumulator plants are grown in a substrate where high concentrations of heavy metals and organic pollutants are present, physiological mechanisms within these plants often limit the transport of these compounds to above-ground tissue to mitigate damage to important cells, such as those responsible for photosynthesis (Zhu *et al.*, 1999; Verkleij *et al.* 2009).

The preference for below-ground storage by emergent macrophytes has been demonstrated in multiple studies, as listed in Table 2.6. However, there are some occasions where metals are found at greater concentration in aerial parts, such as Pb in *Cyperus esculentus*, Zn in *Glyceria maxima*, Mn in *Phragmites australis* and Cu in *Phragmites australis*

(Table 2.6), which suggests that specifically classing species as above-ground or below-ground accumulators of specific pollutants may be inappropriate. Furthermore, not all studies capture the full seasonal dynamics of nutrient or pollutant translocation and allometry under different concentration regimes, and therefore, to enable sound recommendations on harvesting during phytoremediation projects, further studies to characterise chemical allocation over time of key species should be carried out to ensure pollutant removal is appropriately targeted.

2.8 The role of microbial activity in aquatic phytoremediation

There is debate within the phytoremediation literature as to the relative importance of macrophytes in removing pollutants compared to the independent microbial degradation. This perspective primarily comes from observations showing that unplanted CWs can match or outperform planted CWs in terms of pollutant removal (Cardinal *et al.*, 2014). In addition to microbial activity, processes such as sedimentation in P stabilisation and removal, and the photodegradation of PPCPs have also been noted as important (Cardinal *et al.*, 2014; Tanner & Headley, 2011; Zhang *et al.*, 2014). Microbial activity is also an important factor for enabling phytodegradation of pollutants, however, the independent role of microbial communities is now receiving much more attention (Houda *et al.*, 2014). Improved understanding of how microbial activity contributes to pollutant degradation is essential because it not only influences removal rates but may have implications for the value of harvesting plant biomass and post-remediation resource recovery if the actual plant uptake and sequestration (phytoextraction) of target pollutants is low.

There is an abundance of microorganisms associated with macrophyte roots that influence the removal and degradation of pollutants (Stottmeister *et al.*, 2003; Faulwetter *et al.*, 2009). These include bacteria that assist in nitrification and denitrification for the transformation and removal of excess N, and biological mineralization of organic P (Valipour and Ahn, 2016). These processes are integral to the efficient functioning of CWs but the role of macrophytes in facilitating and enhancing the metabolic processes of these microorganisms is still not well understood, although it is likely that the rhizosphere provides an energy source for microorganisms (Thijs *et al.*, 2016). Redox state, dissolved oxygen content and temperature are common limiting factors for different microorganisms (Truu *et al.*, 2009), and the potential for macrophytes to oxygenate the substrate surrounding their below-ground organs can also facilitate the growth of microbes in the rhizosphere (Pavlineri *et al.*, 2017).

CWs are highly engineered, with multiple design elements that may influence the abundance and diversity of microorganisms. Consequently carefully designed experiments are required to explore the potential role of the plant microbiome in phytoremediation. Applying this knowledge is particularly important for developing novel environmental engineering solutions such as FTWs. The formation of microbial biofilms on the underside of FTWs and plant roots has been suggested as a key removal pathway for nutrients and heavy metals (Tanner *et al.*, 2011). Wang & Sample (2014) found that unplanted FTWs had similar removal efficiencies compared to those planted with monocultures of *Pontederia cordata* and *Schoenoplectus tabernaemontani* (Figure 2.7). In this study, and elsewhere, temperature was a key factor in the performance of FTW which has been related to changes in microbial activity (Van de Moortel, 2011; Wang & Sample, 2014b). In contrast, Zhang *et al.* (2014) were unable to link microbial community traits associated with FTWs biofilm such as ribotype number and diversity index to the removal efficiency of pollutants.

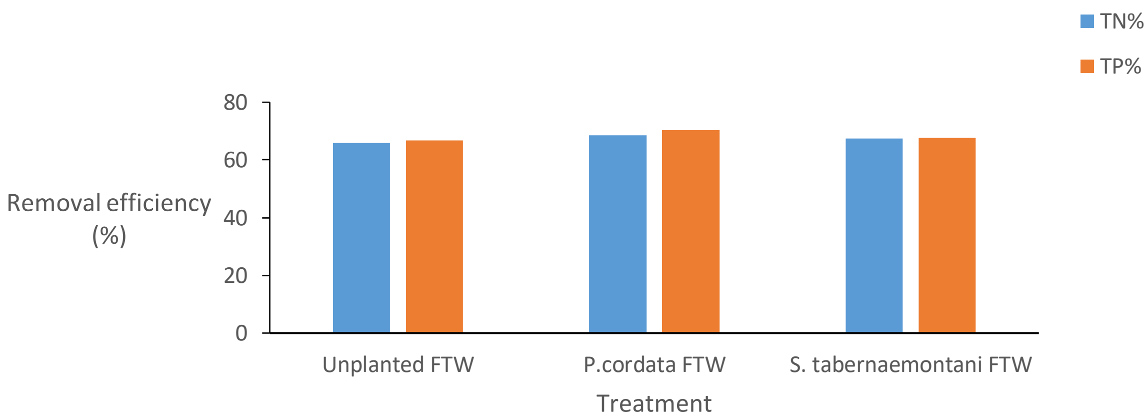

Figure 2.7: Removal efficiencies of TN and TP for an unplanted FTW, a *P. cordata* planted FTW and an *S. tabernaemontani* FTW. Raw data taken from Wang & Sample (2014)

Given the conflicting evidence on the relative importance of plants and biofilms in phytoremediation, a 'meta-organism' approach to phytoremediation is now required to appreciate the multitude of factors and process at work (Thijs *et al.*, 2016; Feng *et al.*, 2017).

Further studies are required in these areas that employ suitable control treatments, along with adequate spatial and temporal characterisation of microbial communities for different macrophytes in monoculture and polyculture, and growth media. Furthermore, within these studies the mass balance of pollutant allocation should be investigated to fully assess where and how pollutants are being stored and translocated. Radio-labelled isotopes have been successfully employed to quantify cycling of nutrients within CWs (Truu *et al.*, 2009). However, such techniques have not been employed during FTW studies, where the application of radio-labelled isotopes would provide an opportunity to understand the biochemical cycling with these novel systems. Finally, after adequate characterisation of microbial communities and their relation to the plant and associated abiotic environment, there may be new opportunities to enhance the microbial community to promote pollutant removal (Glick, 2003; Thijs *et al.*, 2016).

2.9 Added value of aquatic phytoremediation

2.9.1 Ecosystem services

The process of phytoremediation has primarily been concerned with maximising the efficiency of water treatment, whilst the benefits of phytoremediation over and above remediation have essentially been overlooked. Clearly, water treatment is the primary ecosystem service in the provision of safe and clean water; however, the planting of vegetation within the environment creates new habitats for organisms (Zhu *et al.*, 2011). For example, the presence of artificial floating islands improved chick productivity of Black-throated Divers (*Gavia arctica*) by 44 % in waterbodies with these structures (Hancock, 2000), indicating a potential combined role for FTWs in water treatment and improved habitat connectivity. Similarly, a 15 year project investigating the environmental benefits of creating treatment wetlands to ameliorate mine tailing effluents found that there was a high abundance and diversity of protozoa, higher plants, terrestrial animals, and birds (Yang *et al.*, 2006).

In addition to habitat provisioning there is also the potential for facilitating pollination and carbon sequestration (Nesshöver *et al.*, 2017). The capacity for the latter may depend on the post-remediation stage and the reuse of the biomass. Cultural services can also be provided by an improvement in the aesthetic appeal of an area with increased vegetation (Masi *et al.*, 2017). This is most likely in urban waterways where FTW might provide attractive green infrastructure (Olguín *et al.*, 2017). There is a need to quantify and assess ecosystem services associated with phytoremediation projects in order to better appreciate the multiple benefits generated from this form of water treatment.

2.9.2 Resource recovery

The potential to generate large volumes of biomass through phytoremediation means that there are opportunities for resource recovery within the process (Gomes, 2012). Post-remediation biomass re-use streams (PBRSs) are the disposal process and utilisation of the harvested plant tissues of macrophytes used for phytoremediation (Gomes, 2012). As macrophytes are able to remove and assimilate metals there is certainly potential for the recovery of metals such as gold, Cu and Ni (phytomining) (Anderson *et al.* 2005). To date, most research in this area has focused on terrestrial plants and soils contaminated through industrial mining (Rosenkranz *et al.*, 2017). However, there may be potential to explore metal-contaminated waters and sediments of wetlands used to treat mine-tailing effluents. The usefulness of this process depends on the current market value of target metals and the economic benefits associated with this form of phytoremediation (Sheoran *et al.*, 2009).

The use of macrophytes as biofuels is another possibility and is a feasible option to increase the value of phytoremediation if there is a market for biomass. An economic assessment by Jiang *et al.* (2015)found that high biomass production plants are required to make this a profitable venture. However, different options need to be considered in pre-treatment, such as de-wetting and briquetting, since fresh plant biomass comprises up to 90% water (Newete and Byrne, 2016). Macrophyte biomass may also be used for animal feed, or to make compost or biochar (Quilliam *et al.*, 2015; Tanaka *et al.* 2017). Quilliam *et al.* (2015) discussed in detail the issues with these PBRSs in terms of the transfer of pathogens, bio-magnification of heavy metals and propagation of invasive species. A phytoremediation decision-making system that couples the target pollutants and the PBRS would allow the resource recovery options to be established early in the process (Song and Park, 2017). For example, the remediation of a eutrophic lake would seem to link well with composting or animal feed PBRS given the potential for high nutritional content. However, if heavy metal or pesticide contamination also is identified, then a biofuel or phytomining PBRS may be more appropriate. Larger scale pilot tests of aquatic phytoremediation are required, and these should explore the feasibility of using produced biomass in PBRSs.

2.10 Summary and future perspectives

This chapter has outlined the potential of aquatic phytoremediation to provide efficient, multi-targeted and sustainable remediation solutions for polluted waters. A summary of a proposed research agenda required to fulfil the potential of these systems is presented in Table 2.7. Given the wide range of organic, inorganic and biological pollutants that can impact surface waters there is a need to steer phytoremediation towards a context-specific approach that allows the remediation of multiple water body types, and waters affected by a range of pollutants.

With the development of novel ways to deploy macrophytes, such as by FTWs, there are emerging options for spatial flexibility of applying phytoremediation, which are relatively inexpensive. Larger scale pilot studies are required in this respect to assess the realistic opportunities for use. At present there are a wide range of macrophytes of different growth forms that have been established as efficient accumulators of pollutants. A further focus is required to investigate the remediation potential of submerged species and to establish new accumulators that may be used. Importantly, some of the key hyperaccumulators are considered invasive and would be unsuitable to be deployed in natural surface waters. A proposed advancement for phytoremediation systems is to consider the benefits of a plant community based-approach that assembles polycultures of macrophytes with good accumulation capacity for different pollutants, enabling multi-targeted remediation. Here, the need for a logical system of macrophyte selection based on plant removal efficiencies and environmental tolerances, and target pollutant specifications, requires development.

The process of macrophyte phytoremediation still requires a deeper understanding of how to enhance removal efficiency and ensure sustainable harvesting of macrophytes. Understanding the spatial and temporal dynamics of pollutant translocation within macrophytes is crucial for permanent pollutant removal from water and for maintaining the economic value of different PBRSs. Furthermore, a ‘meta-organism’ approach needs to be considered in future phytoremediation studies to establish the role of plant-associated microbial communities. There may be untapped potential in manipulating these microbial communities for enhanced performance.

Finally, the focus of phytoremediation has been on the water treatment aspect, whilst there is growing recognition of the capacity of these ecological engineering strategies to provide ecosystem services such as carbon sequestration and biodiversity support. These benefits need to be better quantified to determine the added-value of phytoremediation. With the waste management sector shifting towards a life-cycle approach, there are clear opportunities for resource recovery through identifying PBRSs such as composting, biofuel production and animal feed. These PBRSs require further exploration in terms of their safety, value and ability to link directly with the target pollutants removed (Figure 2.8). A life-cycle approach needs to embedded in prospective aquatic phytoremediation projects, to ensure that target pollutant(s) are being considered in tandem with the PBRS, whilst the frequency of harvest and replacement/regrowth of macrophytes is properly linked into the remediation of the target pollutant (Figure 2.8).

Table 2.7: Summary of the aquatic phytoremediation research agenda required to deliver efficient, multi-targeted and suitable phytoremediation. Research areas, specific lines of investigation and their priority are highlighted.

Research area	Lines of investigation	High priority (0-2 years)	Medium priority (2-5 years)	Low priority (5-10 years)
Identify new macrophyte accumulators for emerging pollutants	To what extent can macrophytes assimilate and degrade PPCPs and pathogens?			
Plant community-based remediation	Evaluate potential for multi-targeted remediation in plant polyculture incorporating temporal/phonological differences and asses plant competitive effects			
Investigate the role of microbial communities on pollutant uptake/ removal	Adopt a 'Metaorganism' approach to address the role of microorganisms and biofilms in phytoremediation by ensuring studies have suitable control treatments, assess spatial and temporal variation in microbial communities in order to fully characterise the bacteria by their functions. Investigate how microbes can maximise the phytoremediation process by different plant associations and FTW growth media. Mas balance studies required, potentially incorporating radiolabelled tracers.			
Assess provision of phytoremediation to provide ecosystem services	Identify and quantify ecosystem services associated with phytoremediation to appreciate the value of method over and above water treatment.			
Develop a system for macrophyte selection	Develop a suitable system for macrophyte selection to provide context-specific phytoremediation as a tool for environmental agencies and stakeholders.			
Identify accumulation zones of pollutants within macrophytes	Further studies into the allocation and translocation of pollutants within plants with temporal assessments of the optimum time to harvest biomass.			
Explore novel ways of deploying macrophytes in the environment for phytoremediation	Explore new ways to deploy macrophytes into aquatic environment, especially by developing aquatic-aquatic attenuation and inducing growth in native flora. Undertake large scale studies of FTWs that assess remediation and FTW surface spatial arrangement.			

Research area	Lines of investigation	High priority (0-2 years)	Medium priority (2-5 years)	Low priority (5-10 years)
	Assess stakeholder usability of novel phytoremediation methods.			
Determine the effect of different growth media on pollutant removal	Assess influence of different FTW growth media e.g. biochar.			
Determine post-remediation re-use streams for resource recovery	Investigate feasible options for resource recovery and identity context-specific post-remediation biomass re-use streams that link with target pollutants e.g. biomass as fertilizers.			
Testing macrophytes for individual accumulators	Continue testing new macrophytes for phytoremediation for inorganic, organic and biological pollutants. Focus on finding non-invasive plants.			

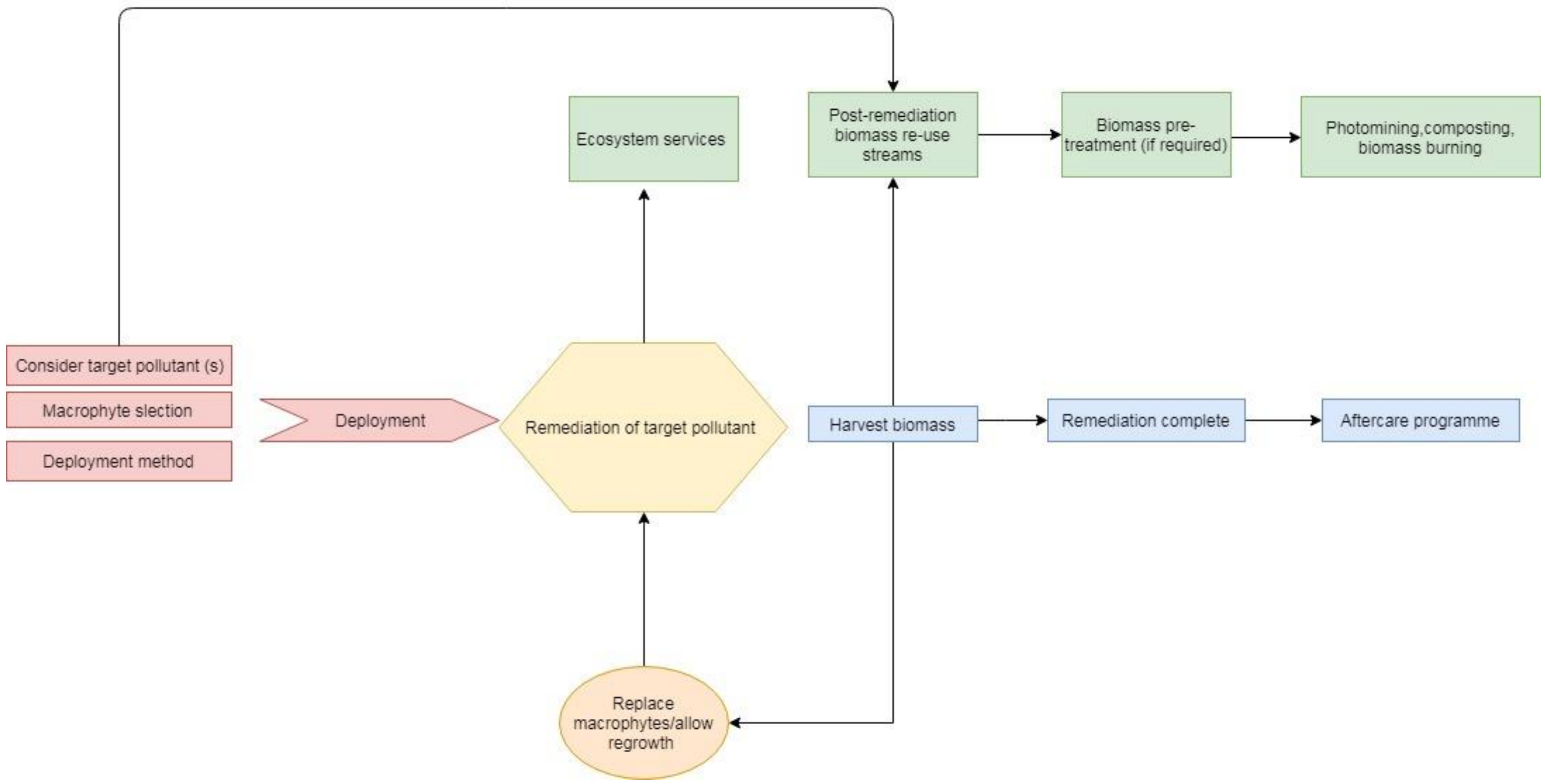

Figure 2.8: Process diagram illustrating the proposed phytoremediation process in its entirety

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3. Resource recovery and freshwater ecosystem restoration – prospecting for phytoremediation potential in wild macrophyte stands

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Highlights

- Harvesting freshwater macrophytes can improve water quality.
- Recovering nutrients from macrophytes has significant circular economy potential.
- Resource recovery can be optimised by using easily identifiable plant growth strategies.
- Fast-growing ruderal plants yield greater amounts of recoverable nutrients.
- Harvest regime is critical for maximising returns from macrophytes.

3.1 Introduction

Freshwaters are under increasing pressure from diffuse pollutants such as phosphorus (P), nitrogen (N) and potentially toxic metals (Berger et al., 2017). These pollutants can impair ecosystem health, decrease water quality for domestic and agricultural use, and reduce access to water for recreation (Berger et al., 2017; Posthuma et al., 2019). Following rainfall, agricultural and semi-rural landscapes often generate run-off contaminated with synthetic fertilisers, soil, sediment, manure, and pesticides (Foley et al., 2005). Vegetated buffer zones and nutrient management planning (including appropriately-timed fertiliser application and loading) are best management practices that are critically important for reducing contaminant transfer and delivery to freshwaters (Lam et al., 2011). However, if mitigation measures become compromised and/or there are stores of legacy pollutants in the catchment, remedial systems to remove pollutants are required (Jarvie et al., 2013).

Aquatic phytoremediation is a promising nature-based solution (NBS) that capitalises on the ability of macrophytes to take-up and sequester pollutants from water and sediments and thus improve water quality (Newete and Byrne, 2016). The primary foci of aquatic phytoremediation as an application has been the construction of artificial wetlands, i.e., the deliberate planting, and deployment of macrophytes in shallow freshwater (Chapter 2). An alternative approach is to harvest existing stands of wild macrophytes in locations where diffuse, point source, and legacy pollution is a problem. Here, the aim is to harvest and remove pollutants sequestered in above-ground plant tissue, therefore reducing the return of nutrients back to the water following plant die-back (Zhou et al., 2017). Pollutant sequestration by macrophytes from sediment and/or the water column is recognised as an important pathway for pollutant removal from freshwater (Preiner et al., 2020; Zhang et al., 2007), e.g., harvesting macrophyte biomass can offset incoming nutrient inputs into water bodies by over 50 % (Bartodziej et al., 2017; Huser et al., 2016). Furthermore, through systematic harvesting and removal of macrophyte biomass there are opportunities to rebalance nutrient losses from terrestrial ecosystems as part of a circular agronomic model (Quilliam et al., 2015). This represents a crucial opportunity to reduce demands on raw materials given that many of these pollutants are derived from compounds manufactured in an energy and resource intensive way (Jones et al., 2013), or their supply is finite. Phytoextraction is the specific phytoremediation process whereby targeted pollutants are taken-up and assimilated into macrophyte tissue, which is then harvested to enable the pollutants to be disposed of or targeted for resource recovery.

Our current understanding of phytoextraction potential of macrophytes comes from quantifying tissue concentrations from either controlled experiments or surveys where specific species or locations were targeted (Kuiper et al., 2017; Petrů and Vymazal, 2018; Vinten and Bowden-Smith, 2020). While these approaches provide important information for specific phytoremediation candidates, they do not necessarily enable larger-scale harvesting strategies to be explored at multiple locations in a catchment or landscape where water body characteristics, pollutant types and sources, and vegetation will naturally vary. Furthermore, focusing on tissue concentrations alone to measure phytoextraction can give a false impression of the quantity of pollutant that can be removed via plant harvesting as it does not account for the amount of biomass available (Vymazal, 2016). Standing stock measures of pollutants give a more representative quantity of the total phytoextraction potential from aquatic systems. However, this can also lead to the misunderstanding that species with larger biomass should always be the *de facto* choice when selecting plants for phytoextraction. When harvesting is employed as a long-term strategy, cross-cutting factors such as pollutant type, harvest strategy and dominant plant growth strategies can impact the total yield of target chemicals over several years. Disentangling the importance of biomass and tissue concentration when determining standing stocks for different pollutants is an important consideration for pollutant removal and resource recovery. Plant life strategies within the CSR plant growth framework proposed by Grime (Grime, 1977; Pierce et al., 2012) closely related to nutrient recovery from harvested plants because of contrasting standing biomasses and nutrient acquisition between competitor, stress-tolerator, and ruderal dominated communities (Grime, 1977; Pierce et al., 2017). Therefore, classifying plant communities using this approach helps to generalise results across different communities and environments. It has been recognised that macrophytes are generally not well represented by strongly stress-tolerator selected species (i.e., with conservative resources economics) (Pierce et al., 2012). In this study, most of the studied communities were predominantly competitor dominant with some species that exhibited variation towards the stress tolerance strategy which ultimately meant communities were grouped by competitor and stress tolerators together (C/S), versus ruderals (R).

The primary aim of this work was to understand the factors, e.g., harvest frequency and plant community type, that can help optimise phytoextraction in wild macrophyte communities as part of a strategy for water-quality improvement and resource recovery. This was achieved by surveying wild macrophyte communities and quantifying standing stocks of key pollutants. Scaling-up the pollutant/nutrient export potential over a decade can enable an estimate of which plant community types can provide the greatest levels of nutrient export depending on the chosen harvest strategy. Investigating the relationship between macrophyte tissue concentrations of pollutants and biomass will increase our understanding of how these two factors influence the standing stock concentrations of a suite of pollutants and facilitate the targeting of natural vegetation for harvesting.

3.2 Methodology

3.2.1 Survey sample strategy

A field survey was designed to capture a wide range of freshwater sites (including both lotic and lentic systems) and characterise macrophyte phytoextraction potential within an area dominated by lowland agriculture and urban land uses. In total, 21 individual sites were selected using aerial photography and Ordnance Survey maps to identify areas of macrophyte coverage over a range of land uses (Figure 3.1; Table A1.1). Sampling was undertaken between July and September 2017, to coincide with peak biomass. A stratified random sample strategy was used where visually homogenous stands of macrophytes greater than 1 m^2 were targeted (the average stand size was 150 m^2). To avoid sampling terrestrial plants, only macrophytes growing in at least 3 cm of standing water were sampled.

A 0.25 m^2 quadrat was placed within each stand and all macrophytes were identified, the total coverage recorded, and the Domin scale used to estimate community composition. Simulating a realistic harvesting regime, only the more accessible above ground plant parts were removed from within each quadrat for biomass and tissue concentration analyses. This included stems and leaves for emergent and submerged plants, whilst for free-floating plants that lack substrate anchorage the whole plant was harvested. The total area of the stand was estimated, either on site or, for larger stands, using aerial photographs (Google Earth Pro 3.0). Harvested plant tissue was washed with tap water to remove sediment and oven dried at 75°C until a constant dry weight was achieved.

At each sample quadrat location, a 60 ml water sample was taken inside the quadrat at a depth of approximately 5 cm for subsequent water quality analyses including a range of parameters detailed in section 2.2. A second 60 ml water sample was taken either upstream or in an open water location to compare water quality in an area not directly influenced by the macrophyte stand. All water samples were vacuum filtered through 1 µm pore-size Whatman glass microfiber filters (Whatman PLC, Buckinghamshire, UK), to maximise representation of dissolved element fractions, within 4 hours of collection to remove particulate material. Filtered samples were then preserved for bulk analysis by freezing at -20° C. Unfiltered samples were used to determine turbidity using a LP2000 turbidity meter (Hanna Instruments, Bedfordshire, UK). Conductivity, pH and water temperature were quantified in the field using a Hanna HI 98129 combi-meter (Hanna Instruments, Bedfordshire, UK).

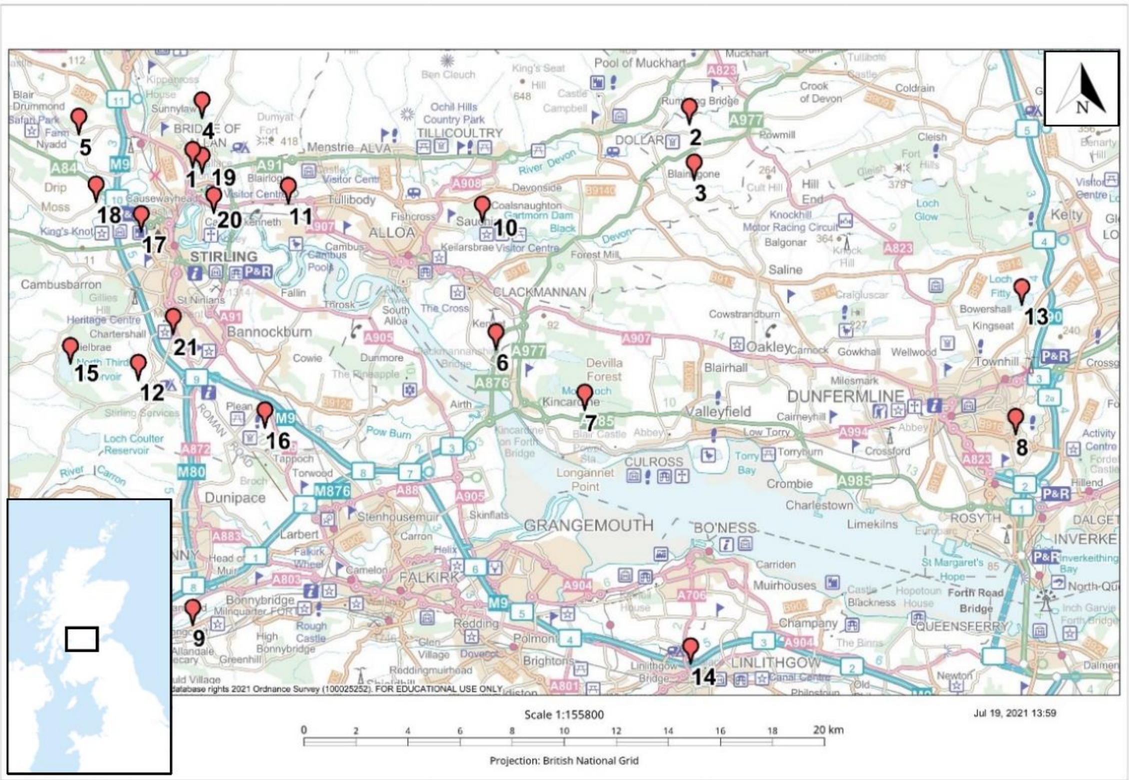

Figure 3.1: Location of sample sites across central Scotland, numbers correspond to site information in Table A1.1. The map extents North 55°58’13.7’’ - 56°11’14.1”, and West 04°02’20.3” - 03° 20’ 58.1”.

3.2.2 Determination of pollutant concentrations in water and plant tissue samples

A SEAL Analytical AA3 Continuous Segmented Flow Autoanalyzer was used for determination of nitrogen species in the water samples (NH_3, NO_2^-, NO_3^-) using SEAL analytical method No. G-171-96 Revision 8 and No. G-172-96 (Revision 9). Total P (< 1 µm particle size) and metalloid elements in water were quantified by inductively coupled plasma spectrophotometry (ICP-Optical Emission Spectrometer, Thermo Scientific iCAP 6000 Series ICP; Thermo Scientific, UK). Dried plant tissue was weighed to quantify total biomass per quadrat; from this, approximately 10 % by weight of each sample was selected and pulverised using a RETSCH RS200 vibratory disk mill (RETSCH, Germany) to obtain material for tissue concentration analysis. Milled subsamples were either analysed for total C and N using a C: N analyser (FlashSmart NC ORG, ThermoFisher Scientific, UK), or microwave-digested with 70 % nitric acid and analysed for P and metalloid element concentration using ICP spectrophotometry. Calcium (Ca), potassium (K), magnesium (Mg), nitrogen (N), and phosphorus (P) are collectively referred to here as 'macronutrient-type pollutants' while micronutrients including copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn), sodium (Na) and chromium (Cr) are collectively referred to as 'micronutrient-type pollutants'.

2.2.3 Community ecology variables and nutrient standing stocks

Plant communities were categorised by the dominant plant growth strategy following Grime's C (competitor), S (stress tolerator) and R (ruderal) (CSR) plant growth strategy framework (Grime, 1977). The component plants from each community were assigned to their primary growth strategies (i.e. C, S and R) on a continuous scale (Pierce et al., 2017). Of the 30 species recorded, only four could not be matched directly with those documented by Pierce et al. (2017); therefore, these species were matched to a closely related species with a similar growth form, habitat, and life history. For each quadrat, the proportion of the primary growth CSR strategies within the community, weighted by the Domin cover scores of the component species, were calculated following the approach of Willby et al. (2001).

Once the proportion of C, S or R strategies in each community were known, each community quadrat sample was assigned a category for the purpose of analysis: competitor/ stress tolerator (C/S) or ruderal (R). These were chosen as categories because these life strategies relate strongly to harvest potential. Ruderals are adapted to disturbance and are fast growing which means there is the potential for multiple harvests per year, whereas for C/S species any regeneration after loss of biomass is likely to be much slower, e.g., taking several seasons growth to reach a similar biomass. Where the proportion of ruderals was greater than 50 % these samples were assigned to the ruderal category. Where there were similar proportions of each strategy a decision was made on what plant species would determine the harvest regime. For example, if abundance of tall macrophytes (e.g., *Glyceria maxima*) was greater than 30 % then these were assigned to the C/S, as the harvesting strategy would normally be defined by the greater presence of larger statured plants because of their higher contribution of biomass.

The total standing stock of each pollutant per 0.25m^2 quadrat was calculated by multiplying the community nutrient tissue concentration (mg/g) by the total community biomass (g). Standing stocks were multiplied by 4 to present results on a g/m^2 basis so they could easily compared with existing literature. Total standing stock is used as the main indicator of phytoextraction potential as it incorporates the realised measure of pollutant export potential (Vymazal, 2016). To assess the interaction between harvest frequency and pollutant removal/nutrient export the standing stocks from the survey were multiplied-up over a 10-year time scale with three harvesting scenarios considered: (1) High Frequency (HF) in which C/S communities are harvested every three years (to allow recovery time), and ruderal communities harvested three times annually; (2) Low frequency (LF) in which C/S communities are harvested every three years and ruderal communities are harvested only once annually; and (3) Medium frequency (MF) with C/S communities harvested every year but with a 40 % decline in productivity applied to all values after the first harvest, and ruderal communities harvested once annually to simulate a scenario where one annual harvesting is carried out for all community types (Table 3.1). These scenarios have been informed by existing studies of plant re-growth following harvesting and the number of harvests, and productivity decline of C/S communities in the MF is a realistic parameter for the harvest strategies (Avellán and Gremillion, 2019; Jakubowski et al., 2010; Nassi o Di Nasso et al., 2010; Niemiec et al., 2019). Apart from the C/S communities in the MF scenario, a constant standing stock return is assumed in each post-harvest period to fit with observations of the effects of plant harvesting or other management activities (e.g., ditch dredging and weed-cutting).

3.2.4 Data analysis

All data analysis was carried out in R studio version 4.0.3 (R Core Team, 2021). For group comparisons, non-parametric Wilcoxon tests were performed as data did not confirm to the assumptions required for parametric tests. Similarly, Spearman rank correlation coefficients were calculated to assess relationship between biomass, tissue concentrations and standing stocks.

To assess communities that may have hyperaccumulation potential the bioconcentration factor (BCF) was calculated for each community using the following equation:

$$BCF = \frac{Pollutant\ concentration\ in\ plant}{Pollutant\ concentration\ in\ water}$$

The BCF is most applicable to understanding the accumulation of metalloid elements in plant tissue, therefore has only been applied to the micronutrient-type pollutants. The ratio is indicative of phytoextraction potential; critical values of the BCF >25 indicate good accumulation, whilst a BCF > 50 shows potential for hyperaccumulation (van der Ent et al., 2013). Mean standing stocks of the most common macrophytes were also calculated following the approach detailed in the Section 1.0 of appendix 1 to understand which species within each community may be useful for resource recovery.

Table 3.1: Harvest scenarios including frequency of harvest, total harvests per scenario and reduction in productivity for ruderals (R) and competitor/ stress tolerator (C/S) community types

Harvest Scenario	**Frequency of harvest**		**Total harvest per scenario**		**Reduction in C/S productivity**
	Ruderals	**C/S**	**Ruderals**	**C/S**	
High frequency (HF)	3 harvests per year	1 harvest every 3 years	30	4	-
Medium frequency (MF)	1 harvest per year	1 harvest per year	10	10	40%
Low frequency (LF)	1 harvest per year	1 harvest every 3 years	10	4	-

3.3 Results

3.3.1 Co-variation between biomass, pollutant tissue concentrations and standing stocks

Tissue concentrations of most macronutrient-type pollutants had significant weak to moderately strong negative correlations with community biomass (R between 0.28-0.45, $P < 0.05$) (Figure 3.2). Ca had both a weak and non-significant correlation with community biomass ($P > 0.05$). C/S communities had a significantly higher community biomass compared to ruderal communities, with a greater value range ($P < 0.05$) (Figure 3.2; Figure 3.3; Figure

A1.2). Ruderal communities generally had significantly higher tissue concentrations than C/S communities (Figure A1.3 & A1.4). Specifically, Ca, Mg, N, P, Fe and Mn tissue concentrations were significantly greater in ruderal communities compared to competitor/ stress tolerators ($P < 0.05$) (Figures A1.3 & A1.4). Overall, communities with a lower biomass tended to have higher tissue concentrations (Figure 3.2; Figure 3.3). However, for each pollutant, tissue concentration did not significantly correlate with community biomass ($P > 0.05$) (Figure 3.3). Broadly, there was little difference in magnitude between the two community types in micronutrient-type pollutant tissue concentrations (Figure A1.4).

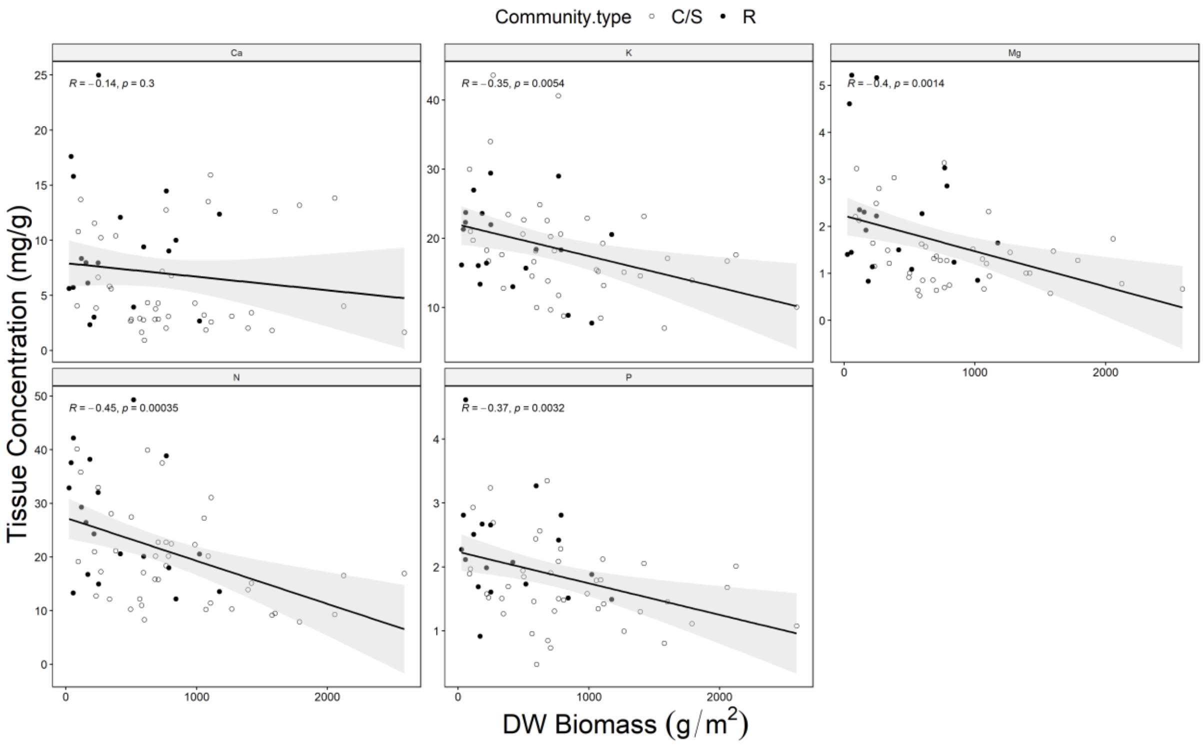

Figure 3.2: Relationship between dry weight biomass (g/m^2) and tissue concentrations of macronutrients (mg/g) (n = 61). Datapoints represent competitor/ stress tolerator (C/S) communities (open circles) and ruderals (R) communities (filled circles). Regression line and 95% confidence intervals is calculated for the whole dataset including both community types.

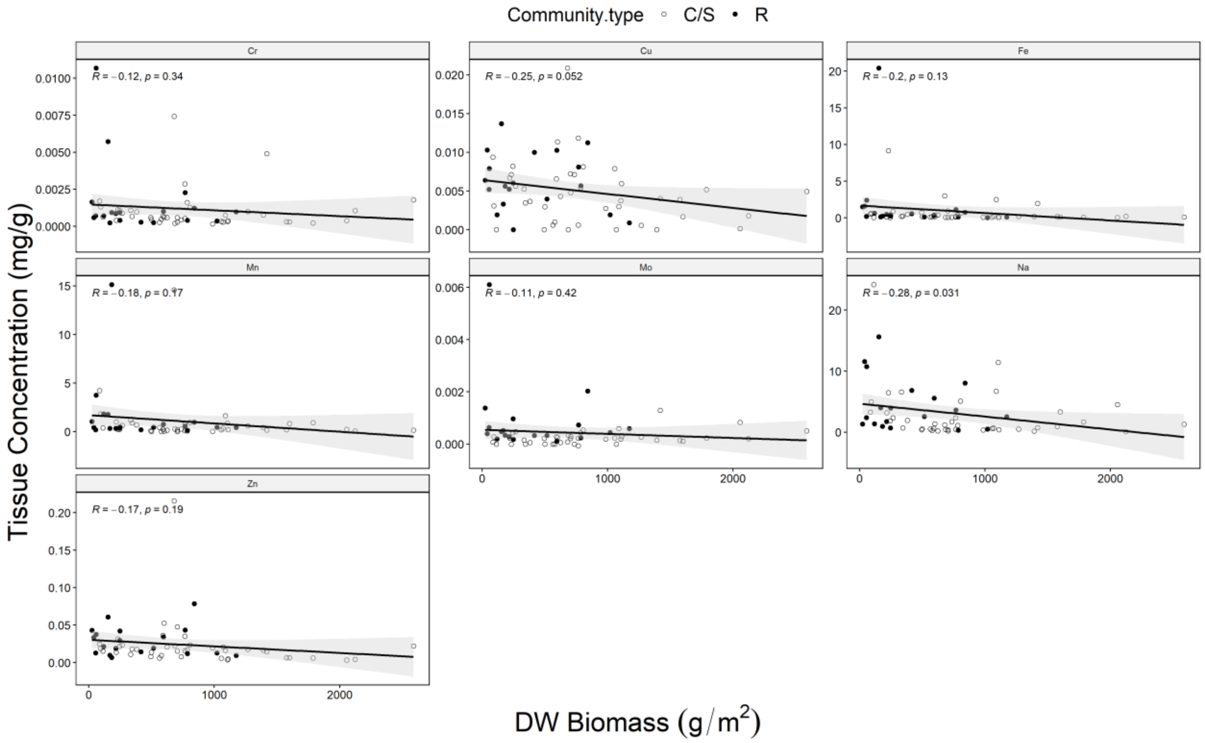

Figure 3.3: Relationship between dry weight biomass (g/m^2) and tissue concentrations of micronutrient-type pollutants (mg/g) (n = 61). Datapoints represent competitor/ stress tolerator (C/S) communities (open circles) and ruderals (R) communities (filled circles). Regression line and 95% confidence intervals was calculated for the whole dataset including both community types.

Standing stocks of macronutrient-type pollutants had strong, and positive, significant correlations with biomass (Figure 3.4). The standing stocks of K, N and P in C/S communities were significantly higher than ruderal communities ($P < 0.05$) (Figure A1.5), although there were no differences between the two community types for standing stocks of Ca and Mg. Standing stocks of Cr, Cu and Mo micronutrient-type pollutants were positively correlated (R= ~0.5) with biomass (Figure 3.5) ($P < 0.05$), while Fe, Mn, and Zn standing stocks were not correlated with biomass ($P < 0.05$). However, Na was an exception, with a weak negative correlation with biomass (Figure 3.5). Furthermore, except for Cr, there were no significant differences in median pollutant standing stocks between C/S tolerator communities and ruderal communities ($P < 0.05$) (Figure A1.6). There were several communities that had a relatively low to medium biomass but still had higher pollutant standing stocks than communities with the highest biomass, e.g., communities with very low biomass also had higher Na, Cu, Mo and Zn standing stocks (Figure 3.4 & 3.5). Where the correlation between biomass and tissue concentration was weak, predicting standing stocks of macro or micronutrients is more difficult. In these cases, it was possible to highlight those communities with phytoremediation potential using the bioconcentration factor in section 3.2.

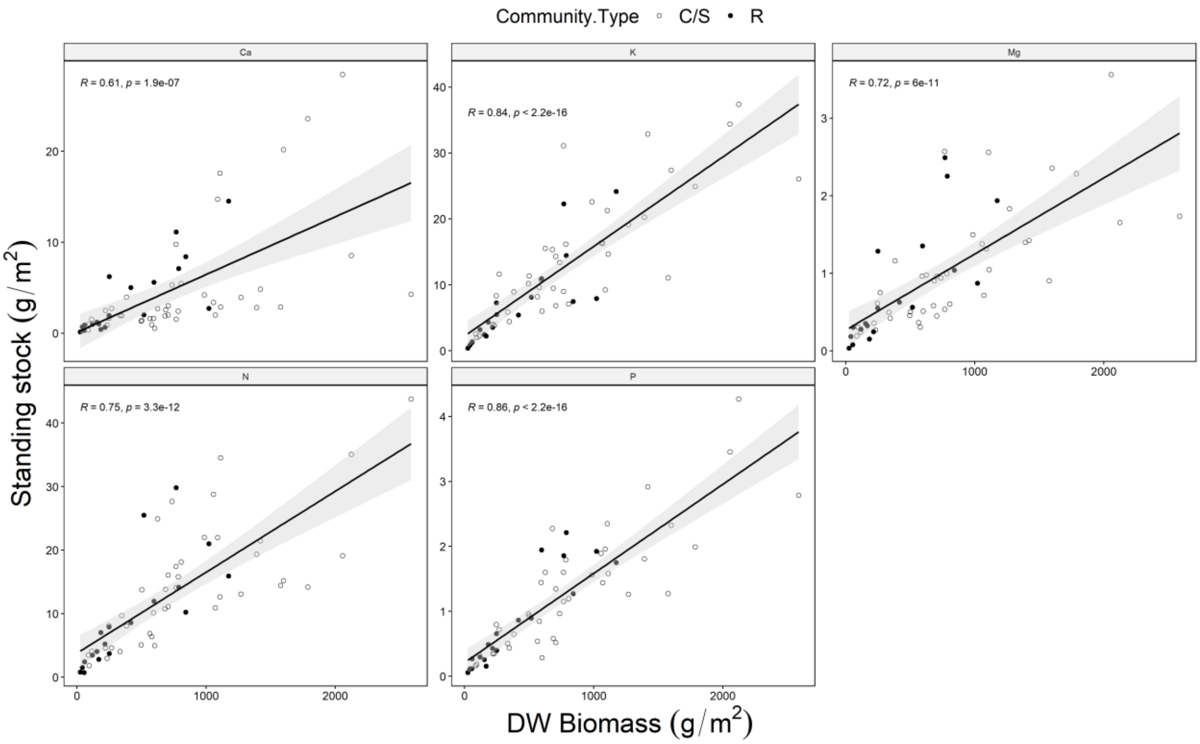

Figure 3.4: Relationship between dry weight biomass (g/m^2) and standing stocks (g/m^2) of macronutrient-type pollutants (mg/g) (n = 61). Datapoints represent competitor/ stress tolerator (C/S) communities (open circles) and ruderals (R) communities (filled circles). Regression line and 95% confidence intervals is calculated for the whole dataset including both community types.

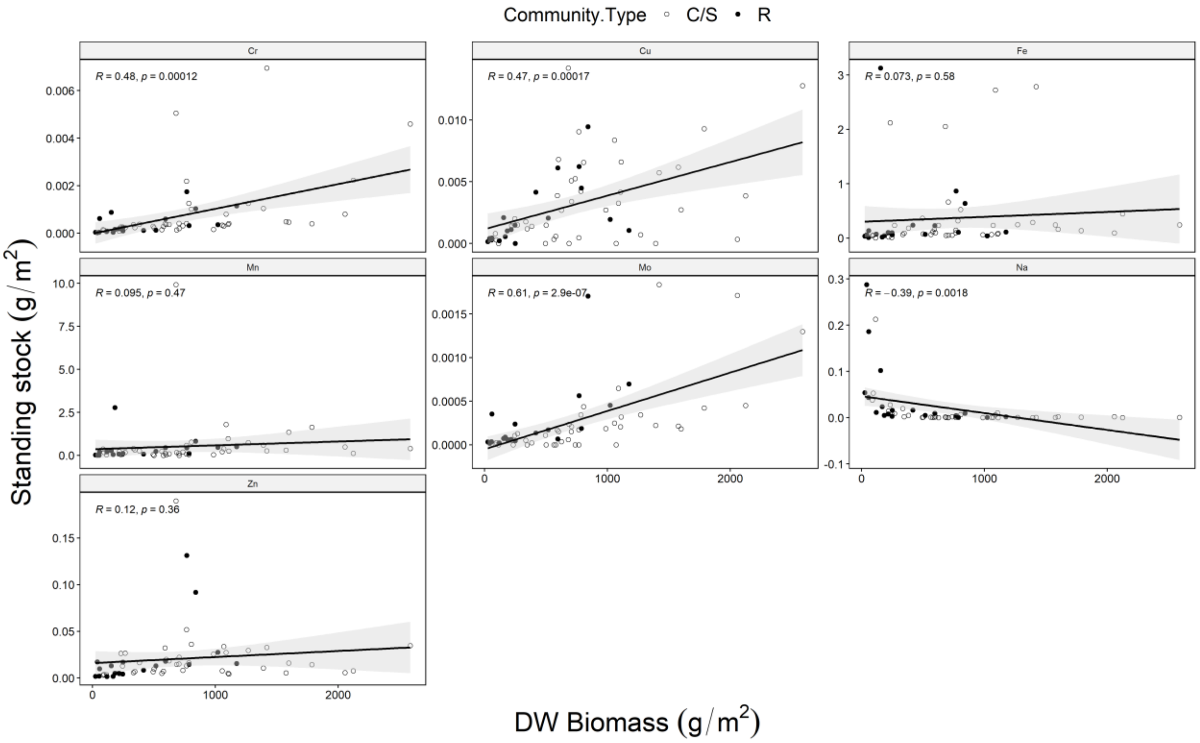

Figure 3.5: Scatterplots showing relationship between dry weight biomass (g/m^2) and standing stocks (g/m^2) of pollutants) (n = 61). Datapoints represent competitor/ stress tolerator (C/S) communities (open circles) and ruderals (R) communities (filled circles). Regression line and 95% confidence intervals is calculated for the whole dataset including both community types.

3.3.2 Bioconcentration factors and common phytoextractor species

For micronutrient-type pollutants, the application of the bioconcentration factor (BCF) suggests that several plant communities could be acting as hyperaccumulators (Table 2). The BCFs of these communities exceeded the critical thresholds as stipulated in Table S3 and contained high tissue concentrations of Fe, Cu and Mn compared to the relative water concentrations (Table 2; Table S3). Importantly, none of these communities contained more than two different macrophyte species; however, overall species richness per community did not have an influence on tissue concentration or nutrient standing stocks (Figure A1.7). Using the most common species found in the survey to generalise specific phytoextractor species indicated that *Phragmites australis* and *Glyceria maxima* ($P < 0.05$) had the greatest standing stocks of P and N ($P > 0.05$) (Figure A1.8). For the micronutrient-type pollutants there was insufficient evidence to determine if any of the species were optimal phytoextractors across the environments studied (Figure A1.9), e.g., although mean standing stocks were generally higher for *P. australis*, *G. maxima* and *T. latifolia* this was not statistically significant.

Table 3.2: Community and species associated with high bioconcentration factors (full Table of BCFs in Table S3)

Element	**Community**
Fe	*Glyceria maxima*
	Azolla filiculoides
	Callitriche stagnalis
	Phalaris arundinacea and *Glyceria maxima* (mixed stand)
Cu	*Azolla filiculoides*
	Typha latifolia and *Lemna minor* (mixed stand)
	Alisma plantago-aquatica and *Equisetum fluviatile* (mixed stand)
	Juncus effusus
Mn	*Glyceria maxima*
	Azolla filiculoides
	Potamogeton natans and *Alisma plantago-aquatica* (mixed stand)

3.3.3 Potential pollutant yields over 10-years by harvest scenario.

In the HF scenario, ruderal dominated communities had significantly higher yields of both N and P compared to competitor/ stress tolerator communities over the 10-year timescale, ($P < 0.05$) (Figure 3.6). Differences between the two community types were most pronounced for this harvest strategy, with ruderal communities providing a mean yield of N and P that was four times greater than competitor/ stress tolerator communities. Conversely, for both the MF and the LF harvest strategies there was no significant difference in median total yield of N and P between the two community types. Across each harvest scenario the patterns described above were similar for all studied pollutants including Ca, Cr, Cu, Fe, K, Mg, Mo, and Zn (Figure A1.10). The magnitude of the difference between the two communities varied, with ruderal communities having 2 – 6.5 times greater mean yields in the HF scenario than the competitor/ stress tolerator communities. Mn was an exception to this as there were no significant differences between the two community types in mean yield across all harvest strategies. Median Na yields were significantly higher in each harvest scenario for ruderal communities ($P < 0.05$) (Figure A1.10), with the HF strategy yielding the most Na over 10 years compared to all the other scenarios.

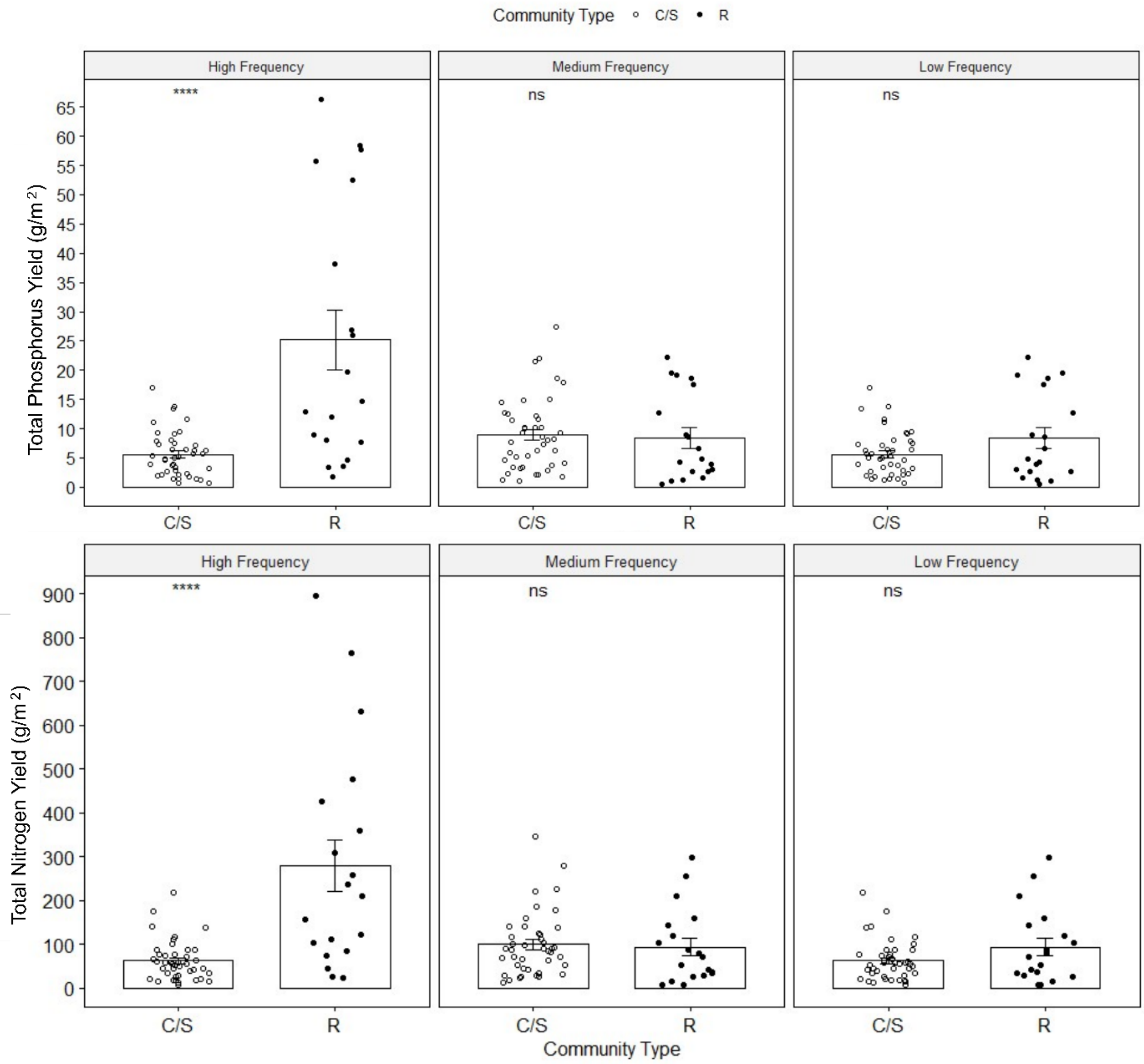

Figure 3.6: Scenario-based differences over ten years in total yield of N and P between competitor /stress tolerators (C/S) (n = 42) and ruderal (R) (n = 19) communities. Error bars show the SE of the mean. $P \leq 0.0001$ (****) and 'ns' indicates no significant difference.

3.4 Discussion

Over a simulated 10-year period, higher frequency harvesting of plant communities comprised primarily of ruderal species provides an opportunity for macro and micronutrient pollutant removal, and the potential for subsequent resource recovery. Harvest frequency is thus a crucial confounding factor that can alter long-term returns from phytoextraction, although it does depend on the plant growth strategy. Reduction in the number of simulated harvests of ruderal-dominated communities in the MF and LF scenarios led to the biomass-driven standing stocks of C/S communities becoming more important for determining returns, therefore there were no significant differences with ruderals in the other scenarios of harvest frequency.

To determine standing stocks (and therefore resource recovery potential) total biomass has more importance for macronutrient-type pollutants, as opposed to micronutrient-type pollutant stocks, suggesting that biomass accumulation is a critical trait for the export and recovery of N, P, K, Mg, Ca and indeed Mo (a micronutrient) (Zhou et al., 2017). This relates to the basic trade-off in plant growth strategies between energy invested in reproduction versus vegetative growth to monopolise light (Craft, 2016). Competitor-dominated communities in this study, such as those including *Typha latifolia*, *Phragmites australis* and *Glyceria maxima* generally have large statures and are more likely to have larger biomass, and therefore higher standing stocks of macronutrients. Conversely, the ruderal plant communities generally have smaller statures leading to a reduced macronutrient standing stocks. Higher tissue concentrations, driven by physiological traits, phenotypic plasticity and concentrations of pollutants in the growth media are likely to be more important in determining standing stocks of micronutrient-type pollutants Cu, Fe and Zn (Ali et al., 2013; Padmavathiamma and Li, 2007). If harvesting regime over time is not considered, macronutrient recovery is more easily achieved simply by harvesting high biomass plants, while for micronutrients closer attention to species-specificity is required, particularly for identifying hyperaccumulators.

By optimising a HF harvest strategy, ruderal communities have the potential to return substantial yields compared to harvesting C/S communities. Wetland ruderal plants are characterised by fast growth rates and leaf turnover, and rapidly proliferating root systems with a high capacity for the absorption of limiting nutrients (Willby et al., 2001). Consequently, they have higher tissue concentrations and can be harvested multiple times annually; thus, previous studies utilising bi-weekly harvests have strongly advocated floating aquatic ruderals such as *Azolla* as ideal phytoextractors (Tang et al., 2017). While extra harvests could theoretically increase nutrient acquisition, an intense harvest regime with a lower recovery time may result in lower biomass gains over the full season and reduce overall nutrient gains (Bal et al., 2017). An early harvest at the start of the growth season can enable biomass to recover in 3 – 6 weeks and facilitate plant recovery time to enable further harvests (Bal et al., 2006; Cooke et al., 1990), and in tropical regions with reduced seasonality there is great potential for multiple annual harvests (Vymazal, 2007).

Less intensive harvest regimes such as the MF and LF scenarios that do not capitalise on the rate of ruderal re-growth reduce the benefit of targeting macrophyte communities or deliberately planting specific communities for pollutant removal and resource recovery. However, the cost-benefit of harvesting multiple times annually is an important consideration and depends on the method of harvest. The approach taken here to harvest only the accessible plant parts means the results are applicable to conventional mechanical harvesting, one of the most economical methods available (Quilliam et al., 2015). C/S communities are less adapted to continual disturbance due to slower relative rates of biomass acquisition and the requirement to aerate a large below-ground biomass. Hence, returns from these more slow-growing plants would likely be diminished by employing an annual harvest (Atkinson et al., 2014). Therefore, wherever high frequency harvests are not planned, other priorities should guide the choice of plant communities for harvesting to generate multiple benefits. Considerations might include the method of harvesting and what other collateral benefits or ecosystem services can be gained. For example, nesting habitat could be provided for waterfowl by planting and periodically harvesting large statured C/S communities containing *Glyceria maxima*.

There are potential negative consequences to harvesting freshwater macrophytes such as the resuspension of nutrients, loss of structural habitat, increased bank erosion, and the risk of shifting lacustrine systems from clear-water to phytoplankton-dominated (Habib and AR, 2016; Sayer et al., 2010; Soana et al., 2018). However, with an appropriate site characterisation and sustainable harvest regime many negative effects can be mitigated (Kohzu et al., 2019; Kuiper et al., 2017). Broadly speaking, the results presented here demonstrate the significant potential for water quality improvements and resource recovery by harvesting macrophytes from aquatic systems. This concept has shown particular success when coupled with forms of sustainable agriculture less reliant on raw resources, such organic arable farming (Stabenau et al., 2018). Harvesting nuisance aquatic plants can also be cost effective compared to other in-lake management schemes, e.g., by reducing the P load by as much as 50%. (Bartodziej et al., 2017). Focusing on harvesting existing invasive plants may therefore offer a sustainable option for joint freshwater habitat improvement and resource recovery schemes (Carson et al., 2018). Caution must be exercised in these cases and a risk assessment must be carried out before harvesting invasive plants to determine the likelihood of facilitating further invasion elsewhere by unintendedly transporting propagules, indeed harvesting some invasive species may also be illegal (Chapter 2).

One difficulty of scaling up phytoextraction is the transporting and processing of material beyond the site, while assessing the costs and benefits can prove challenging (Edgar et al., 2021). Therefore, using the harvested biomass close to its source will help build circular systems within multi-functional landscapes, and is certainly the most promising application of this approach (Atkinson et al., 2014). For example, ideal locations for targeted plant harvesting and re-use could be agricultural lands adjacent to river navigations or recreational waterbodies where existing nuisance plants are periodically removed and deposited at the water side, resulting in nutrients leaching back into the waterway (Boerema et al., 2014). Here it has been highlighted that the different plant community types and harvest regimes that can facilitate the removal of pollutants from waterways. Catchment managers and stakeholders can therefore consider phytoextraction as part of a wider suite of measures that target the sources, mobilisation, delivery, and impacts of pollution. This will help to build circular economy approaches back into existing land management.

3.5 Conclusion

Phytoextraction using macrophytes is a nature-based solution to the dual problem of water pollution and declining supplies of raw materials. Targeting phytoremediators that can be harvested from existing freshwaters or for deliberate planting (and subsequent harvesting) can be challenging because of the different approaches to quantifying phytoextraction potential. By examining the relationship between biomass and tissue concentrations on standing stocks of macronutrient- and micronutrient-type pollutants the influence of these two components have been disentangled. High biomass plant communities, particularly those comprised of competitor or large stress tolerator species, will produce greater standing stocks of macronutrients (such as N and P) for harvesting. Yields of micronutrient-type pollutants are more likely to be influenced by their tissue concentrations (which are determined by plant physiological traits) and targeting these communities for phytoextraction using species-specific knowledge of hyperaccumulation potential is more appropriate than community generalisations. However, by utilising a high frequency harvest regime over a multi-annual time scale ruderals yield far greater amounts of macronutrients and micronutrients for export than competitor or stress tolerator dominated communities. These results can help guide environmental managers in their approach to developing circular economy schemes to improve water quality and export nutrients and emphasise the need to consider both the plant community and the harvesting regime employed.

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4. Floating treatment wetlands - engineering nature-based solutions for ecosystem multifunctionality

Highlights

- The potential for floating treatment wetlands to provide multiple ecosystem services is explored.
- Employing comparative ecology is an effective method for phytoremediation plant community selection.
- Ecosystem multi-functionality increases with increased number of plant traits.
- Successful freshwater restoration should align objectives with plant community type

4.1 Introduction

Surface waters are vital for supporting people and ecosystems; however, freshwater quantity and quality is under increasing pressure from a growing human population that requires access to safe water (Birk et al., 2020). Freshwaters are negatively impacted by multiple stressors such as diffuse pollution, land-use change and increased storm and drought frequency, which can both impair water quality and reduce ecosystem-service provision (Berger et al., 2017). One strategy to mitigate stressors and restore water bodies in a sustainable way is to use nature-based solutions (NbS). Aquatic phytoremediation is an NBS that utilises the capacity of macrophytes to uptake, sequester and/or degrade water-borne pollutants (Newete & Byrne, 2016; Quilliam et al., 2015; Wang et al., 2002). However, most studies on aquatic phytoremediation focus on selecting macrophytes that optimally target single pollutants (Chapter 2); whilst this approach is important it ignores the potential for multiple-pollutant uptake by macrophytes, and the parallel benefits that could be achieved through the additional provision of, for example, biomass production, habitat provision and pollination services.

One key method of deploying macrophytes for freshwater phytoremediation is via floating treatment wetlands (FTWs); these buoyant structures allow emergent macrophytes to grow hydroponically in the water, which facilitates the removal of waterborne pollutants (Chen et al., 2016). FTWs are increasingly used worldwide as a ‘best practice’ management tool for freshwater restoration in both urban and rural settings spanning a range of temperate and tropical climatic zones (Colares et al., 2020). Despite the increased application of FTWs and a general appreciation of the diverse and important roles played by aquatic vegetation in freshwater systems there has been little work to determine how FTWs can be designed to support ecosystem functions beyond just pollutant removal (Wang et al., 2015).

To improve ecosystem functioning and increase the (multiple) ecosystem service provision of phytoremediation, it is important to optimise the composition of the plant community both in terms of its species richness and the functional traits of the component species (Engelhardt and Ritchie, 2002). Generally, in terrestrial ecosystems two hypothesised competing relationships are used to understand how community composition influences ecosystem function: (1) the mass ratio hypothesis which proposes that ecosystem functioning is determined by the traits of the most dominant species (species identity) and (2) complementarity effects which highlights the importance of species and functional diversity leading to reduced competition and increased resource partitioning (Garnier et al., 2015). However, relationships between community structure and functioning are not necessarily transferable across different ecosystem types or contexts (Daam et al., 2019) and therefore may not provide the best guide for how to assemble an optimal macrophyte community for freshwater restoration.

Previous phytoremediation studies that have attempted to quantify the effects of macrophyte composition on the efficiency of nutrient uptake have shown that species diversity was strongly correlated with removal efficiency of nitrogen (N) based pollutants, whilst species identity was more important for the removal efficiency of phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca) (Ge et al., 2015; Geng et al., 2017; Han et al., 2018) . Consequently, focusing on the relative importance of species diversity versus species identity can contribute towards a mechanistic understanding of ecosystem functioning in FTWs. However, employing a comparative ecological approach focusing on a single common plant trait could both enhance understanding of plant community composition on functioning and provide readily transferable applied knowledge across different locations. Therefore, the aim of this study was to determine how plant composition influences the ecosystem service provision by macrophyte communities designed for freshwater restoration. To address this aim and provide a set of principles that can guide community assembly, we focused on plant stature (i.e. plant height) axis, which is recognized as a key dimension of plant strategies with a major influence on the associated ecosystem functioning of plant communities (Butterfield and Suding, 2013; Lavorel and Grigulis, 2012).

Specifically, our objectives were to (1) identify which types of macrophyte communities are most effective at phytoremediation while concurrently providing a range of ecosystem functions and (2), determine what level of ecosystem multifunctionality each community type can achieve. Anticipated outcomes and priorities differ between environmental managers when restoring freshwaters; and understanding how FTWs can maintain levels of multifunctionality under different restoration objectives is important for making decisions on plant community selection. Therefore, three different restoration objectives were employed in this study in order to crosscut measures of multifunctionality and provide targeted information for environmental managers.

4.2 Methods

4.2.1 Plant species and community selection

To determine ecosystem multifunctionality, six indicators of specific ecosystem functions relevant to phytoremediation were measured (Table 4.1).Eight macrophyte species (all native to the UK where they are typical components of the vegetation of fertile freshwaters and often coexist) were selected based on their commonality and differing growth traits. The large-statured emergent monocots (defined as those that can grow over 150 cm at maturity) *Typha latifolia* (TL), *Glyceria maxima* (GM) and *Phragmites australis* (PA) were selected based on their fast growth rate, ability to readily take-up nutrients and their widespread use as phytoremediation candidates (Brisson and Chazarenc, 2009; Vymazal, 2007). Smaller flowering emergent herbs including *Myosotis scorpioides* (MS), *Nasturtium officinale* (NO), *Mentha aquatica* (MA), *Lythrum salicaria* (LS) were selected primarily based on the numerous flowers they produce, which can provide both pollination and aesthetic services, while *Eleocharis palustris* (EP), a reported hyperaccumulator of copper (Sakakibara et al., 2011), was selected based on the predicted function of effective removal efficiency. The eight species were then combined into 11 different community combinations (Table 4.2) spanning three broad community types: large emergent community (LEC), small emergent community (SEC) and mixed emergent community (MEC) - a combination of large and small statured species. This allowed us to test if communities differing in richness of trait diversity provide enhanced ecosystem multifunctionality.

Table 4.1: Ecosystem function, corresponding ecosystem service and the indicator measured in this study*

Ecosystem function	Ecosystem service type	Ecosystem service	Indicator used
Resource pool utilisation	P, R	Water treatment	Removal efficiency (RE)
Above ground biomass production	P	Forage production	Above ground biomass
Nutrient sequestration	P, R	Nutritional value & nutrient retention	Tissue nutrient concentration
Root biomass production	P, R	Anchorage/below ground structure;	Below ground biomass
Dissolved oxygen leakage	R	Provision of (an)aerobic conditions	Dissolved oxygen content
Total visible reproductive organs	C, R	Pollination and aesthetic appeal	Number of flowers per week

Service abbreviations are provisioning (P), Regulation & maintenance (R), Cultural (C)

*Indicators from (Haines-Young and Potschin, 2018)

4.2.2 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 x 2 m x 2 m) (Figure 4.1) to avoid dilution effects from rain, and to maintain the polytunnel temperature (mean experiment air temperature, water temperature and light intensity were 14.4°C, 16.6°C and 29.5 KLux respectively).

Macrophyte communities were planted in experimental mesocosm FTWs, which were designed to be buoyant and allow hydroponic growth into the growth media. Each FTW was constructed from white 40 mm diameter polyethylene pipe (44 cm x 32 cm) with 12 modified hydroponic plant pots (12 cm depth and diameter of 7 cm) joined with plastic cable ties (Figure 4.1). The 12 planting spaces gave a planting density of 85.2 plants per m^2 which would stimulate natural plant interactions (Pavlineri et al., 2017) and was within the range of previous experimental FTW studies (Jones et al., 2017). Each FTW was placed into a clear polypropylene plastic tank (0.56 x 0.39 x 0.42 m) with a maximum volume of 50 l. There were four replicate mesocosms per treatment, and all replicates were randomly assigned to two adjacent open-ended polytunnels (Figure 4.1).

Table 4.2: Community types and the specific community treatments associated with these groupings

Community type	**Treatment**	**Plant communities/treatments**	**Monoculture/mixture**
Large Emergent community (LEC)	1	*T.lat*	Monoculture
	2	*G.max*	Monoculture
	3	*P.aus*	Monoculture
	4	*T.lat + P.aus*	Bi-culture
	5	*T.lat + G.max,*	Bi-culture
	6	*G.max + P.aus*	Bi-culture
	7	*T.lat + P.aus + G.max*	Polyculture
Mixed emergent community (MEC)	*8*	*G.max +E.pal* bi-culture	*Bi-culture*
	9	*G.max + N.off + E.pal L.sal*	Polyculture
Small emergent community (SEC)	*10*	*E.pal*	Monoculture
	11	*M.sco + N.off + M.aqu + L.sal*	Polyculture

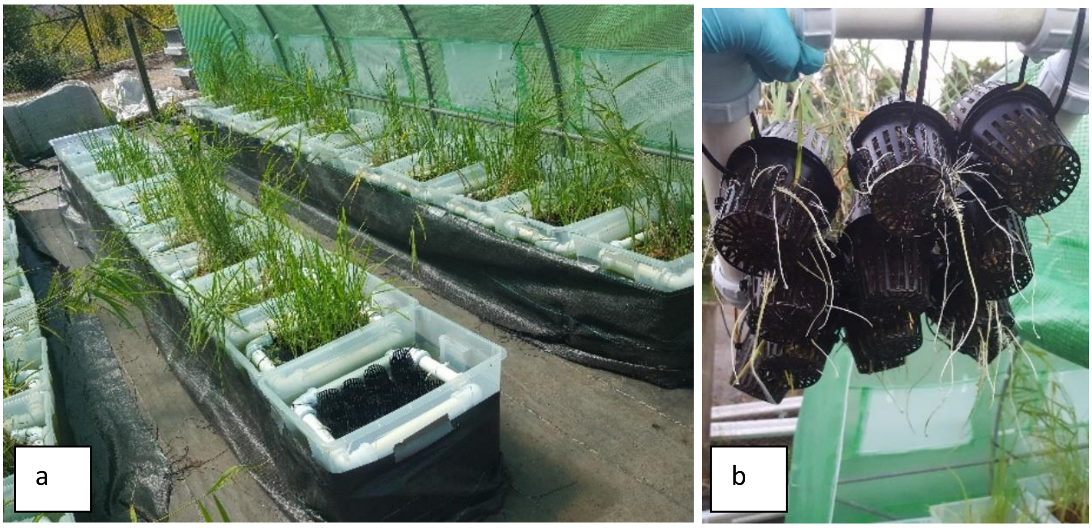

Figure 4.1: Experimental mesocosm set up within polytunnels (a); each FTW was made up of 12 individual plants (b)

Mesocosms were developed to simulate a scenario typical of urban and semi-rural environments impacted by multiple pollutants. Each mesocosm contained modified Hoagland's solution (Table A2.1) (Hoagland and Arnon, 1938), a cocktail of target pollutants (Table 4.3) and were filled with tap water to 50 l. This volume allowed enough space for root growth and 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 measurement, all water was removed from each mesocosm, and the container cleaned; a new supply of Hoagland's solution and water was added as described above. To minimise any edge effects, the innermost two mesocosms from each row 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.

Table 4.3: Final concentration of target pollutant in each experimental mesocosm

Constituent	Concentration (µg/L)
Ammonia (NH_3)	254
Nitrite (NO_2)	9
Nitrate (NO_3)	2,311
Calcium (Ca)	7,707
Chromium IV (Cr)	74
Copper (Cu)	34
Iron (Fe)	2,289
Potassium (K)	10,619
Magnesium (Mg)	6,152
Manganese (Mn)	358
Sodium (Na)	5,634
Phosphorus (P)	963
Zinc (Zn)	162

T. latifolia, G. maxima, P. australis, M. aquatica, L. salicaria and M. scorpioides were supplied as pre-grown seedlings (www.salixrw.com), individually propagated in a 110 cm^3 plug. The growth media used for propagation (20 % loam and 80 % peat) was carefully washed from the roots to reduce nutrient input into the mesocosms (Figure 4.1). *N. officinale* cuttings were collected from an agricultural ditch (56° 12' 41.4"N 03° 21' 15.9"W) and *E. palustris* from an urban surface flow wetland (56° 07' 26.3"N 03° 57' 17.1"W); both were hydroponically propagated for 10 days in 20 % Hoagland's solution to allow enough root and stem growth to be transplanted.

Individual macrophytes were randomly planted into the experimental FTWs Using a random number generator. The base 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. All FTWs were then placed in 25 % strength Hoagland's solution for 14 days acclimation prior to the experiment commencing.

4.2.3 Ecosystem functioning assessment

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 the initial concentrations of pollutants and then every mesocosms was sampled on day 7 and 14. Within 4 hours of collection all samples were vacuum filtered through 1µm pore-size Whatman glass microfiber filters to remove particulate material. Filtered samples were then preserved for bulk analysis by freezing at -20° C. Dissolved oxygen was quantified in 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_3, NO_2, NO_3) using SEAL analytical method No. G-171-96 Revision 8 and No. G-172-96 (Revision 9) (SEAL Analytical, n.d.). For the analysis of both total phosphate (< 1 µm particle size) and metalloid elements, inductively coupled plasma spectrophotometry (ICP-Optical Emission Spectrometer, Thermo Scientific iCAP 6000 Series ICP; Thermo Scientific, UK) was used. Removal efficiency (RE) was calculated for each batch using Equation 1:

$$\text{Removal efficiency (\%)} = \left(\frac{C_1 - C_2}{C_1}\right) \times 100 \qquad \textbf{[Equation 1]}$$

Where Removal efficiency (%) is the reduction in the concentration of a pollutant C, C_1 being its concentration on day 1 and C_2 its concentration on day 7. Day 7 results were used for this calculation as preliminary trials showed that the greatest amount of pollutants were removed during this time. The mean removal efficiency from each batch was used to calculate an average for each replicate to assess this continuous function.

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) plant parts for each species within each replicate 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). Subsamples 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 a community replicate, and a dry biomass-weighted mean per replicate was calculated to generate a representative tissue nutrient concentration. To assess the pollination and potential aesthetic appeal of the plant communities, each week the total number of flower heads in bloom on each individual plant was counted.

4.2.4 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 community type; for biomass measures a treatment mean of the total standing biomass of each replicate, for tissue nutrient concentration a biomass-weighted mean for each replicate, and for flowers a mean of the total number of flowers per replicate per date. Mean removal efficiency (RE) and dissolved oxygen concentration were determined for each replicate across the experiment duration based on the first 7 days of each batch. To compare the RE and concentrations of pollutants in plant tissues between the different plant community types the data were grouped by either nutrient (P, NH_3, NO_2, NO_3) or major ions (Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Zn), in both cases the total mean was used to compute a global average. The large range of concentrations in above and below-ground tissue meant that each dataset was first normalised by calculating a Z-scores for each element before computing the single variable. The data from the experiment did not conform to the assumptions required to carry out parametric statistical analysis so non parametric Kruskal–Wallis tests were used to compare groups and post-hoc Dunn tests employed to identity significant differences with the Bonferroni adjustment used to correct p values (Dinno, 2017).

Multifunctionality was calculated for each plant community type using a threshold-based approach (Allan et al. 2015). Each ecosystem function for every replicate was scored on whether it exceeded a threshold of 25 % (low), 50 % (medium) and 75 % (high) of the maximum value across all the replicate mesocosms. To avoid the influence of outlier values, the maximum value was based on the mean of the highest five results. This method for assessing multifunctionality assumes that environmental managers can accept a reduced level of ecosystem functioning - but below each threshold, this loss is not acceptable. These three thresholds were chosen to cover a range of performance levels across the dataset. Multifunctionality was calculated using the function ‘multidiv’, available from git-hub.com/eric-allan/multidiversity. In the assessment of multifunctionality, all data input was mean-centred and Z-scored. To quantify multifunctionality according to different freshwater restoration objectives the ecosystem functions were weighted appropriately (Table 4.4). The equal importance objective was based on there being no preference for any service, with the phytoremediation objective prioritising removal efficiency of both the nutrient and major ions-type of pollutants and above-ground harvestable plant parts (biomass and above-ground tissue concentration). The regulating and cultural objective weights more heavily flower production (pollination and aesthetics services), dissolved oxygen and below ground biomass.

Table 4.4: Ecosystem functions used in the calculation of ecosystem multifunctionality with the weighted proportion of each for the restoration objective

Ecosystem function	**Equal importance**	**Phytoremediation**	**Regulation and cultural**
RE nutrients	0.125	0.3	0.05
RE major ions	0.125	0.3	0.05
AG tissue	0.125	0.15	0.025
BG tissue	0.125	0.01	0.025
Dissolved oxygen	0.125	0.05	0.1
AG biomass	0.125	0.15	0.05
BG biomass	0.125	0.03	0.1
Flowers	0.125	0.01	0.6

AG: above ground; BG: below ground; RE: removal efficiency

4.3 Results

4.3.1 Ecosystem functioning

The composition of macrophyte communities (based on stature) intended for phytoremediation has an impact on ecosystem service provision. Large emergent communities (LECs) removed more nutrients from the water column with an average RE of 76 % compared to 45 % in Small emergent communities (SECs). Mixed emergent communities (MECs) were intermediate, with an average RE of 60 %, and were not significantly different from their large or small statured counterparts (Figure 4.2). In contrast, there was limited difference in the capacity of the three different community types to remove major ions from the water column (Figure 4.2). MECs removed 45 % of major ions while LECs and SECs only removed a further 2 - 3 %, and only LECs showed a significantly higher RE (Figure 4.2) ($P < 0.05$). However, there was a degree of specificity in the removal of specific nutrients and major ions for each plant community type: LECs were more efficient at removing nutrients (P and N species) and in general more inorganic elements, including both micro and macronutrients (A2.1). The unplanted control mesocosms had the highest levels of dissolved oxygen while MECs had the highest DO concentrations of the planted treatments, which was significantly higher than the LECs (Figure A2.2) (P < 0.05).

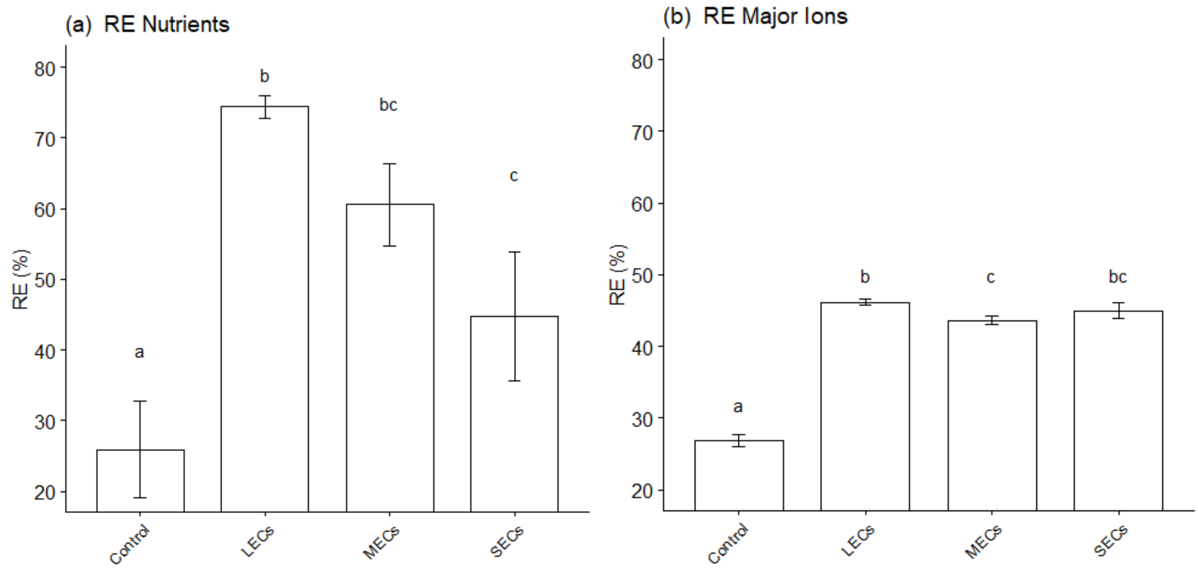

Figure 4.2: Overall mean removal efficiency (%) for large statured emergent communities (LECs) (*n* = 80), mixed statured communities (MECs) (*n* = 40), small statured communities (SECs) (*n* = 40) and unplanted controls (*n* = 40) by (a) Nutrients and (b) RE Major Ions after 7 days (50 % of HRT). Error bars show the standard error of the mean, and bars with different letters are significantly different from each (P> 0.05).

There was no significant difference between LECs and SECs in above-ground mean normalised nutrient and major ion tissue concentrations (P > 0.05) (Figure 4.3). This is despite LECs having numerically higher mean values, implying that these two community types have a similar capacity to sequester and translocate pollutants to above-ground tissue. Conversely, both SECs and LECs had significantly higher above-ground mean normalised nutrient and major ion tissue concentrations compared to MECs (P < 0.05). Mirroring these collective results there were no significant differences between LECs and SECs for specific pollutants (A2.3) (P < 0.05). Below-ground mean normalised tissue concentrations of both nutrients and major ions were significantly higher by two orders of magnitude in LECs compared to MECs and SECs (P < 0.05) (Figure 4.3). However, there was no significant difference between MECs and SECs in below-ground mean normalised tissue concentrations (P > 0.05) implying that uptake capacity and below-ground storage were similar (Figure 4.3).

In terms of plant allometry and associated element storage, SECs and MECs had a significantly higher shoot to root (S:R) tissue ratio for most elements including Ca, Cr, Cu, Fe, P, Na and Zn (P < 0.05) compared to LECs (A2.5). However, above-ground biomass was not significantly different between the three community types (Figure 4.4) despite LECs having a significantly higher below-ground biomass than SECs (P < 0.05). MECs were intermediate and not significantly different from either LECs or SECs. Finally, SECs and MECs produced significantly higher numbers of flowers than LECs (A2.6) (P < 0.05).

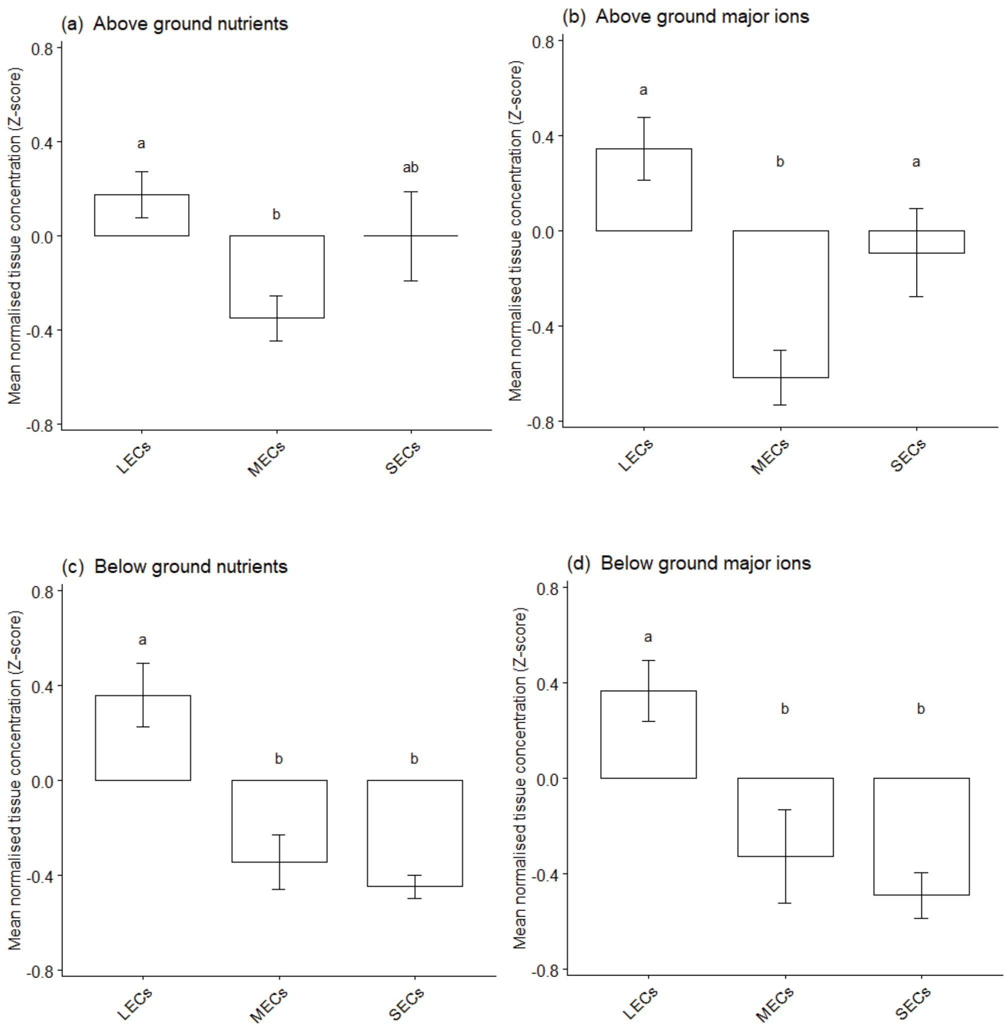

Figure 4.3: Mean normalised above (a, b) and below tissue concentrations (c, d) (normalised by z-scores) for large statured emergent communities (LECs) (*n* = 28), Mixed statured communities (MECs) (*n* = 8) and small statured communities (SECs) (*n* = 8) for nutrients and major ions at the end of the experiment. Error bars show the standard error of the mean, and bars with different letters are significantly different from each (P > 0.05).

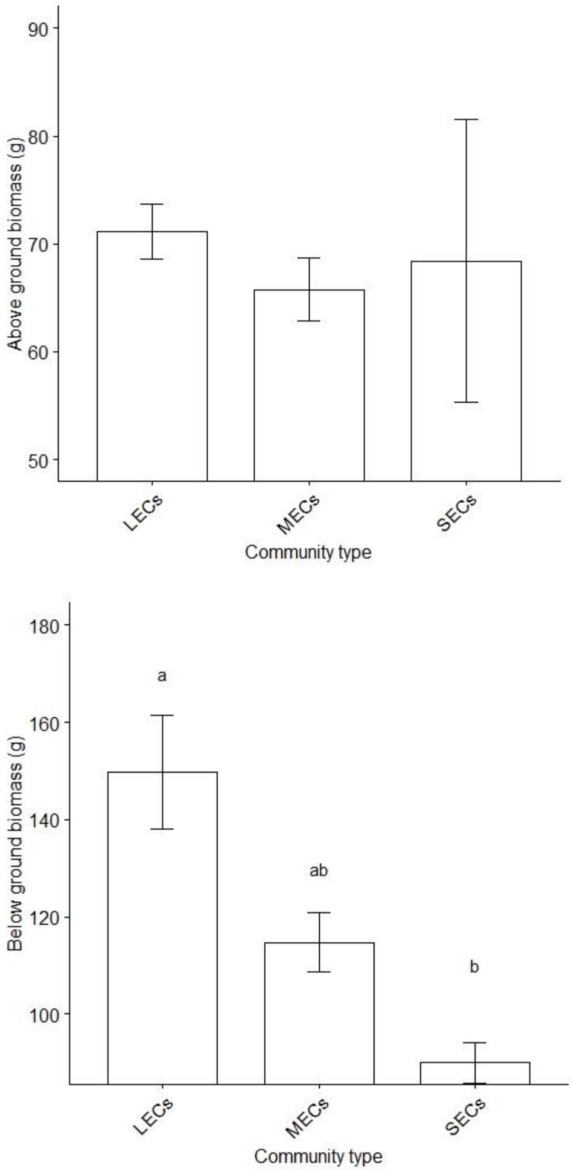

Figure 4.4: Above ground biomass and below ground biomass large-statured emergent communities (LECs) (*n* = 28), Mixed-statured communities (MECs) (*n* = 8), small-statured communities (SECs) (*n* = 8) by pollutant type at the end of the experiment. Error bars show the standard error of the mean, and bars with different letters are significantly different from each ($p > 0.05$).

4.3.2 Ecosystem multifunctionality

Structuring plant communities in FTWs by stature led to differences in ecosystem multifunctionality (EM) at varying performance thresholds. At the high-performance threshold (75 %) the EM values for SECs were significantly higher than all other plant community types regardless of objective ($P < 0.05$) (Figure 4.5). SECs also showed significantly higher levels of EM in the medium performance threshold in the cultural and regulation objectives ($P < 0.05$). Conversely, LECs performed multiple ecosystem services most optimally at lower performance thresholds. This was indicated by the significantly higher levels of EM compared to SECs and MECs in the 25 % (low) and 50 % (medium) thresholds in the equal importance objective ($P < 0.05$) (Figure 4.5). Similarly, under the 25 % (low) threshold of the phytoremediation objective the EM value for LECs was significantly higher than other community types ($P < 0.05$). Across all objectives and performance thresholds the EM values for MECs were significantly lower than other community types ($P < 0.05$), or not significantly different from the LECs ($P > 0.05$) (Figure 4.5).

In general EM values decreased with increasing performance thresholds indicating a reduction in the total amount of functions delivered at higher levels (Figure 4.7) ($P < 0.05$, for all pairwise comparisons). Within plant community types there was variation in the EM values due to the influence of specific species combinations (Figure A2.8). Under the equal importance and phytoremediation objectives, TL+PA, TL, GM+PA, TL+PA+GM from the LEC had the highest EM values among all plant combinations for low to medium performance thresholds (Figure A2.8). In the high-performance threshold under these objectives, the TL community remained the highest of the LECs and comparable to the polyculture SEC. The polyculture community MS+NO+MA+LS consistently demonstrated higher levels of EM compared to its monospecific small emergent counterpart community EP and exceeded all communities at all performance thresholds in the culturing and supporting objective.

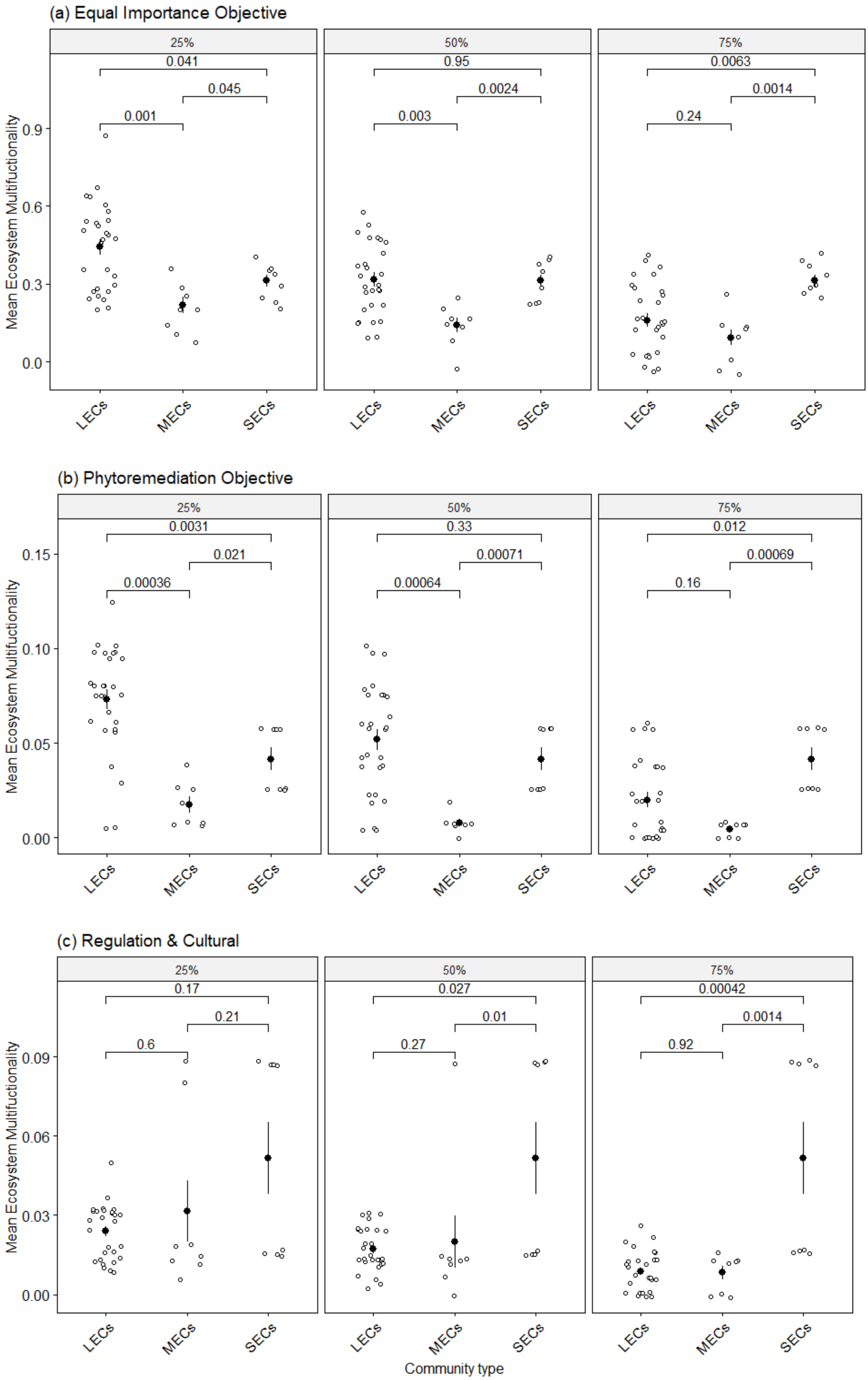
(a) Equal Importance Objective
25%
50%
75%
0.041
0.045
0.001
0.95
0.0024
0.003
0.0063
0.0014
0.24
Mean Ecosystem Multifunctionality
0.9
0.6
0.3
0.0
LECs
MECs
SECs
(b) Phytoremediation Objective
25%
50%
75%
0.0031
0.021
0.00036
0.33
0.00071
0.00064
0.012
0.00069
0.16
Mean Ecosystem Multifunctionality
0.15
0.10
0.05
0.00
LECs
MECs
SECs
(c) Regulation & Cultural
25%
50%
75%
0.17
0.21
0.6
0.027
0.01
0.27
0.00042
0.0014
0.92
Mean Ecosystem Multifunctionality
0.09
0.06
0.03
0.00
LECs
MECs
SECs
Community type

Figure 4.5: Mean ecosystem multifunctionality calculated by different objectives (equal importance, phytoremediation and regulation and supporting) for LECs (*n* = 28), MECs (*n* = 8) and SECs (*n* = 8). Each objective is split by ecosystem performance thresholds of 25 %, 50 % and 75 % of the maximum of each service. Unfilled circles represent spread of data, filled circles with error bars show the mean ±1SE. Post-hoc pairwise comparisons with P level are shown in lines between each treatment comparison.

4.4 Discussion

We hypothesised that structuring plant communities by stature would result in differences in ecosystem functions and capacity for multifunctionality. Arranging macrophyte communities for phytoremediation this way has a clear impact on the outcomes of freshwater restoration and on the (multiple) ecosystem service provision potential of these FTW systems. Our results suggest that SECs maintain the best EM at higher levels compared to both LECs and MECs. SECs are characteristically different from LECs by their ability to produce numerous flowers, and this function consistently results in an overall higher EM value. LECs were unable to achieve the same high-performance thresholds in key ecosystem functions, including nutrient and major ion RE likely leading to the lower EM. While there were clearly some individual plant communities that have a high level of multifunctionality within the LEC community type, generally this grouping produces higher EM only at low to medium performance levels. Although the SECs performed better *per se*, the overall trend of decreasing ecosystem multifunctionality with increased threshold level, suggests that fewer functions are being performed at a high level for all community types. Therefore, an expectation of a trade-off between an overall high multifunctionality and optimum performance of some individual functions, such as pollutant removal, should be anticipated. This is consistent with terrestrial-based studies which show that some ecosystem functions can be negatively impacted by increases in performance thresholds (Allan et al., 2015).

The higher EM values for LECs at lower performance thresholds suggests that more functions are performed, but at the cost of overall effectiveness. If more regulatory and cultural functions had been considered, such as support for invertebrate biodiversity, and/or a more comprehensive assessment of cultural value, then these types of functions may perform at higher threshold levels allowing EM to remain higher in LECs. The same patterns were mirrored in the phytoremediation objective despite RE being weighted strongly.

However, the lack of a difference in the RE of major ions, above ground biomass and mean normalised above-ground tissue concentrations between the LECs and SECs means that these measures, which are all important indicators of phytoremediation success, did not allow the LECs to show enhanced multifunctionality at a higher performance threshold. Under the regulating and cultural services objective SECs maintained the highest level of multifunctionality throughout all performance levels, most likely due to the importance of flower production. Despite containing plants with traits from both LECs and SECs, structuring plant communities as MECs did not lead to enhanced ecosystem functioning and suggested that combining extremes of plant stature negatively impacts on ecosystem functioning and may represent a 'worst of both worlds' strategy.

LECs that are comprised of multiple species demonstrated higher levels of multifunctionality at low-medium levels than monocultures, due to the advantage of including species that are effective in several ecosystem functions (Riis et al., 2018) and complementarity in timing of growth that ensures functions are better delivered (Manolaki et al., 2020). However, as the performance threshold became higher (75 %) the monoculture plant community (e.g. *Typha latifolia*) was most effective. It is probable that a small set of specific ecosystem functions allowed this monoculture to maintain an overall higher level of multifunctionality. The more intermediate performance of the polyculture communities was less effective and therefore less likely to achieve the higher threshold requirements. Consideration of both the stature of communities and their diversity is also therefore important. In contrast, for SECs the more species diverse community had higher levels of multifunctionality than the associated monoculture in the same group. This was mainly due to the more diverse community having species that produced conspicuous flowers, while the monoculture did not.

Some of the macrophyte community types can be characterised by their ability to perform specific ecosystem functions based on their broad traits, allowing a species selection based on comparative ecology rather than species specificity. For example, the increased capacity of LECs to remove nutrients was related to their ability to maintain larger biomass and thus creating a higher demand for uptake and sequestration (Brisson and Chazarenc, 2009; Vymazal, 2007). However, stature is not always an overriding factor for removal efficiency of pollutants and other species-specific physiological traits can be more important, e.g. root growth and associated biofilm attachment, and the possession of specific uptake transport proteins (Printz et al., 2016; Tanner and Headley, 2011).

Small plants in natural wetlands tend to be have ruderal plant strategies in which rapid growth and reproduction necessitates a need to acquire and transport nutrients efficiently to above ground tissue (Vymazal, 2016; Willby et al., 2001). Although small statured plants can have higher pollutant tissue concentrations due to their lower biomass (i.e. less dilution effects), hydroponic emergent macrophyte growth in the FTWs may alter the normal plant allometry, particularly for LECs, for example due to increased root growth at the expense of stem height and girth. Such a reduced above-ground biomass likely reduced the 'dilution effect' leading to an increased above-ground tissue concentration in the LECs. However, preferential storage in the roots and rhizomes of plants in LECs in preparation of overwintering also allows greater competitor and stress tolerance and can lead to higher below-ground tissue concentrations (Ge, Feng, Wang, & Zhang, 2016).

Assembling plants in mixed stature communities appears to negatively influence above ground tissue concentrations of nutrients/major ions, likely due to antagonistic interactions between large emergent species and smaller species, such as competition for light (Ervin and Wetzel, 2002). Additionally, the water in the mesocosm containing the MECs had comparatively higher levels of dissolved oxygen suggesting lower productivity and root turnover. Despite MECs being functionally more diverse in terms of the stature trait than LECs and SECs, this did not lead to enhanced functioning, in contrast to previous studies on emergent macrophytes (Ge et al., 2015). MECs are generally intermediate in their ecosystem functioning capacity which suggest that the traits inherent in LECs and SECs community types become proportionally reduced when intermixed. Although this study did not explicitly explore the effects of individual species, our results have shown that the mass ratio hypothesis (Garnier, Navas, & Grigulis, 2015) is probably more important for understanding how ecosystem functioning is affected by the inclusion of species with differing traits in a community (Mokany et al., 2008).

This research has clear practical implications for the design of macrophyte communities employed for freshwater phytoremediation, particularly on FTWs. Assembling mixed communities by stature can enable practitioners to gain non-species dependent transferable knowledge on expected performance of each community type. This provides opportunities to use native flora and potentially reduce costs by transplanting local species into FTWs or propagating from existing stands. While using stature as a trait provides an opportunity to generalise performance expectations, it is not advocated to abandon selection of specific species where there are very targeted project aims, for example, where the removal of a single pollutant is required. Maximising EM at a high-performance level means that environmental managers should also consider trade-offs that might occur with other services. Therefore, the restoration objectives should be clear from the onset as to which, how many and what levels of performance are expected from services derived from a phytoremediation installation. FTWs have the highest overall EM where performance is at low to medium levels, and while this suggests that phytoremediation has the capacity to be a ‘multi-tool’ application it also underscores that expectations must be proportionate and contextualised to the specific restoration project.

The variable performance of MECs compared to other community types highlights the importance of community assembly and of understanding how different plant combinations can influence performance. Conversely, assembling similar functional types may later lead to interspecific competition for resources (Cadotte, 2017). However, selecting species that are functionally diverse, e.g. in root zone morphology or phenology, may promote niche partitioning and thereby overall performance. Therefore, at the level of plant stature, competition and antagonistic interactions are not necessarily inevitable. Finally, this study has not explicitly considered the effect of each negative outcomes from functions interacting in the EM measure. For example, the harvesting of biomass for provisioning services or pollutant export (Quilliam et al., 2015) may impact on the delivery of other services such as RE or future tissue concentration gains. Therefore, building in trade-off measures would be a useful avenue for further study.

4.5 Conclusion

Combining concepts of comparative ecology and ecosystem multifunctionality is an effective approach for determining how macrophyte communities can be assembled for optimal performance in FTWs. By focusing on the key plant trait of stature, environmental managers can more easily align objectives for freshwater restoration with plant selection as some key ecosystem functions are more likely to be associated with each community type. For the removal of nutrients from water LECs may be more suitable than other community types, while SECs are likely to be appropriate when increased flower production for pollination and aesthetic value is desired. Furthermore, ecosystem multifunctionality is likely to be maintained at a higher threshold when the community performs more diverse functions, in this case the SECs. However, within the context of phytoremediation multifunctionality is higher where the expected performance of functions is lower, which means environmental managers must recognise a potential trade-off between these outcomes. In other words, there is greater confidence of effective pollutant removal and less confidence of multiple functions including pollutant removal with increased performance expectations. There is clear potential for aquatic phytoremediation to be a 'multi-tool' in the freshwater restoration tool kit; combining measures of ecosystem multifunctionality and plant community assembly provides a framework for enhancing the value of FTW systems as a nature-based solution.

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5. Understanding multi-pollutant removal dynamics in mesocosms with mixed or monoculture floating treatment wetlands

Highlights

- Pollutant removal mechanisms for floating treatment wetlands are explored.
- Plant community composition, temporal and biophysical factors influence pollutant removal.
- Effective removal of pollutants is primarily attributed to plant root structure, and plant uptake.
- The contribution of plant uptake to pollutant removal varies between pollutant but it is generally low.
- Optimisation of community composition can enable effective removal of pollutants.

5.1 Introduction

Global freshwater resources comprise 2.5 % of the total global water budget but only 0.0072 % (93,120 km^3) of this is available for drinking, energy and food production, and for use by the industry sector (Lawford et al., 2013; Zimmerman et al., 2008). This small portion of available freshwater is often impacted by current or legacy pollution, e.g., from nutrients and heavy metals used in agriculture or industry (Berger et al., 2017). With a rapidly growing human population, increases in living standards and urbanisation there is a need to maintain and improve water quality to support these societal developments (Birk et al., 2020). Pollution-based regulation to protect freshwaters and promote best practice is essential for minimising sub-optimal freshwater quality (Pan and Tang, 2021). However, regulation cannot provide a complete safeguard against non-compliance, legacy pollution issues, or diffuse pollution arising from disparate negative land use practise (e.g., fertiliser run-off) where problems are complex and involve multiple stakeholders (Patterson et al., 2013). Remedial solutions are important for supporting good water quality and can be combined with regulatory and best management practice approaches.

Floating treatment wetlands (FTWs) are a form of phytotechnology under the nature-based solutions (NBS) umbrella and are increasingly employed to improve water quality and support freshwater restoration (Colares et al., 2020). 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 (Chen et al., 2016). Owing to their deployment flexibility, they have been studied and used as remedial solutions in a variety of urban (ponds, canals, storm water retention pond retrofits) and rural settings (rivers, drainage ditches, lakes) for waste-water treatment, water polishing and general water quality improvement (Shahid et al., 2018). There is great potential for FTWs to be used as both 'green' urban waterscapes and for decentralised water treatment; particularly at a low cost and maintenance if designed appropriately (Chapter 2).

Understanding how to optimise performance of FTWs for pollutant removal is important for widespread uptake of this technology and ultimately for improving its value in enhancing freshwater quality. Research on FTWs has shown that factors including macrophyte community selection, hydraulic retention time, vegetation maturation (and associated root growth) can be crucial in determining good performance for removing nutrients and heavy metals (Pavlineri et al., 2017). These variables are often studied compartmentally for a individual pollutants and the potential to target multiple pollutants is often overlooked. Recent developments have shown that modifying plant community composition (and thus increasing plant functional diversity), or assembling optimal combinations of accumulator species, can enhance removal of pollutants (Ge *et al.*, 2015; Han *et al.*, 2016, 2018; Geng *et al.*, 2017). There is, therefore, an opportunity to design plant communities that can enhance pollutant removal, target multiple pollutants, or show more intra-seasonal stability in performance. Understanding how different macrophyte community combinations perform temporally in terms of hydraulic retention times and across the season can feed into plant community design and FTW system management (Garcia Chanc et al., 2019). Mechanistic knowledge of macrophyte pollutant removal is key to determining the factors that maximise performance of FTWs. Importantly, the inclusion of negative controls in experiments (i.e., with unvegetated FTWs) are necessary to help identify additional pathways for removing pollutants, such as shading and surface area provision from microbial biofilm development (West et al., 2017).

This study aimed to understand the key mechanisms of pollutant removal by FTWs in multi-polluted waters by determining how plant community composition interacts with temporal and plant biophysical factors. The key temporal factors that influence phytoremediation are hydraulic retention time (HRT), the time a soluble compound remains in a system, and the experiment duration. To our knowledge, this is the first temporally high-resolution study to quantify removal of such a large suite of nutrient and metalloid-based pollutants, including total inorganic nitrogen (TIN), calcium (Ca), chromium IV (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P) and zinc (Zn). Our objectives were three-fold: (1) quantify and compare the removal efficiencies of macrophyte communities at two different hydraulic retention times and by different plant combinations and controls; (2) analyse the temporal, physiochemical and ecological factors influencing removal efficiency such as HRT, experiment duration, root development and community composition; and (3) calculate the mass balance of pollutant transfer in the systems to identify the influence of plant uptake on pollutant removal. By teasing apart these factors and understanding their interactions, new knowledge of plant community selection and maintenance can aid real-world application of these systems to a variety of locations and pollutants.

5.2 Methods

5.2.1 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 with each other. Assembling communities with different proportions of each species enabled species interactions and influence of species-specific traits to investigated (Table 5.1). This chapter was based on the same experiment as Chapter 4 with a subset of species as detailed above.

Table 5.1: Different treatment and plant combinations used in these experiments

Treatment	**Plant communities/treatments**	**Monoculture/mixture**	**Treatment Code**
1	*T.lat*	Monoculture	TL
2	*G.max*	Monoculture	GM
3	*P.aus*	Monoculture	PA
4	*T.lat + P.aus*	Bi-culture	TL+PA
5	*T.lat + G.max,*	Bi-culture	TL+GM
6	*G.max + P.aus*	Bi-culture	GM+PA
7	*T.lat + P.aus + G.max*	Polyculture	TL+PA+GM
8	Open water control	-	UN
9	Unvegetated FTW	-	FTW

5.2.2 Experimental design

Experiments were carried out in the growing season between July and September 2018 and were housed in open-ended polytunnels (3 m x 2 m x 2 m) (Figure 5.1) to avoid dilution effects from rain, and to maintain the polytunnel temperature (mean air temperature, water temperature and light intensity were 14.4°C, 16.6°C and 29.5 Klux respectively).

Macrophyte communities were planted in experimental mesocosm FTWs, which were designed to be buoyant and allow hydroponic growth into the growth media. Each FTW was constructed from white 40 mm diameter polyethylene pipe (44 cm x 32 cm) with 12 modified hydroponic plant pots (12 cm depth and diameter of 7 cm) joined with plastic cable ties (Figure 5.1) . The 12 planting spaces gave a planting density of 85.2 plants per m2 which would simulate natural plant interactions (Pavlineri et al., 2017) and was within the range of previous experimental FTW studies (Jones et al., 2017). Each FTW was placed into a clear

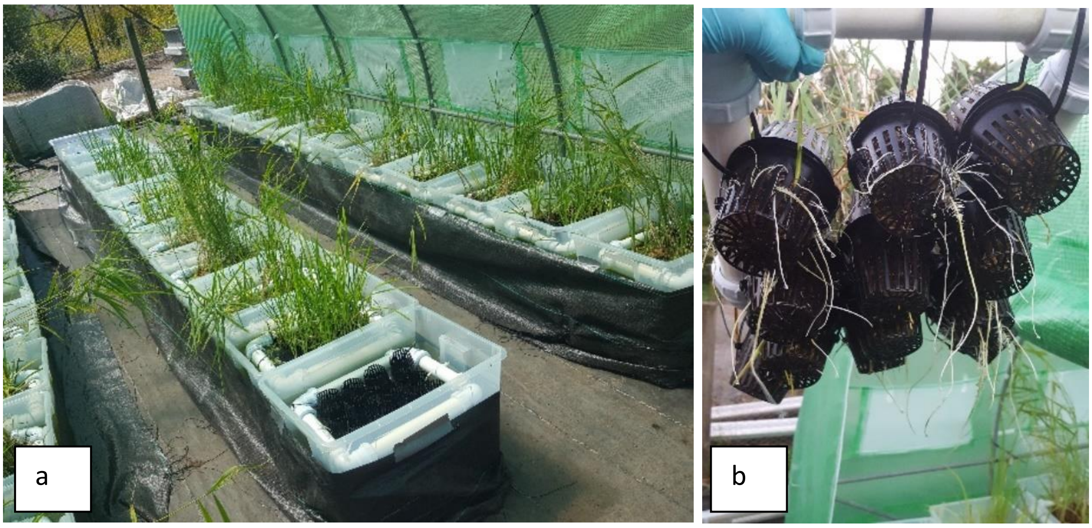


Figure 5.1: Experimental mesocosm set up within polytunnels (a); each FTW was made up of 12 individual plants (b).

polypropylene plastic tank (0.56 x 0.39 x 0.42 m) with a maximum volume of 50 l. There were four replicate mesocosms per treatment, and all replicates were assigned to two adjacent open-ended polytunnels (Figure 5.1).

Mesocosms were developed to simulate a scenario typical of urban and semi-rural environments impacted by multiple pollutants and concentrations used were informed by preliminary field sampling of freshwaters. Each mesocosm contained modified Hoagland's solution (Hoagland and Arnon, 1938), a cocktail of target pollutants (Table 5.2) and were filled with tap water to 50 l. This volume allowed enough space for root growth and avoided hypoxia. The experiment was designed to simulate a batch-fed wetland with a two-week 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 and water was added as described above. To correct for potential edge effects, the innermost two mesocosms from each row 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.

Table 5.2: Final concentration of target pollutant in each experimental mesocosm

Constituent	Concentration (µg/L)
Total Ammonia (NH_3)	254
Nitrite (NO_2^-)	9
Nitrate (NO_3^-)	2,311
Calcium (Ca)	7,707
Chromium IV (Cr)	74
Copper (Cu)	34
Iron (Fe)	2,289
Potassium (K)	10,619
Magnesium (Mg)	6,152
Manganese (Mn)	358
Sodium (Na)	5,634
Phosphorus (P)	963
Zinc (Zn)	162

T. latifolia, G. maxima, and P. australis were supplied as pre-grown seedlings (www.salixrw.com), individually propagated in a 110 cm^3 plug. The growth media used for propagation (20 % loam and 80 % peat) was carefully washed from the roots to reduce nutrient input into the mesocosms (Figure 5.1). Individual macrophytes were randomly planted into position within the experimental FTWs using a random number generator. The base 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. All FTWs were then placed in 25 % strength Hoagland's solution for 14 days acclimation prior to the experiment commencing.

5.2.3 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 four hours 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 in 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_3, NO_2, NO_3) using SEAL analytical method No. G-171-96 Revision 8 and No. G-172-96 (Revision 9) (SEAL Analytical, n.d.). Total inorganic nitrogen (TIN) was calculated by the summation of each three determined nitrogen species (NH_3, NO_2, NO_3) for use in the analysis as it is a more stable metric than each component individually due to gas fluxes such as ammonia. For the analysis of both total phosphate (< 1 µm particle size) and metalloid elements, inductively coupled plasma spectrophotometry (ICP-Optical Emission Spectrometer, Thermo Scientific iCAP 6000 Series ICP; Thermo Scientific, UK) was used. Removal efficiency (RE) was calculated for each batch using Equation 1:

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

Where Removal efficiency (%) is the reduction in the concentration of a pollutant, with C_1 being its concentration on day 1 and C_2 its concentration on day 7 or day 14. Relative growth rate was sampled and calculated following the approach detailed in Appendix 3.

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) plant parts 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). Subsamples 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 a community replicate, and the mean dry weight biomass per replicate was 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 seedlings 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 each species per community.

5.2.4 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 that accounted for removal by the control treatments to provide a realistic overview of the benefits of vegetated floating treatment wetlands.

$$Adjusted\ Removal\ Efficiency\ (\%) = RE(Replicate) - \bar{X}\ RE(Open\ Water\ Control\ treatment) \qquad \text{[Equation 2]}$$

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 open water control 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 analysis used pollutant concentration values from each treatment replicate per week rather than percentage values. Correlation analysis was employed using square root-transformed concentration values with Person’s Product correlation coefficient to quantify relationships between selected variables.

To understand the effects of treatment type and time in weeks on pollutant removal, linear regression 2-way interaction models between treatment and experiment duration (Weeks) were constructed for each pollutant to calculate the effect sizes. All pollutant concentrations were Z-scored and mean-centred to standardise units across all pollutant types. As treatment type was a categorial variable, dummy data was calculated using the controls as the reference variable in the model. The resultant 95 % confidence interval parameter estimates (i.e., effect size) for each pollutant was used to quantity the effect of these variables and their interaction on pollutant concentrations. The effect sizes were plotted as a forest plot per treatment, per pollutant in order of mean effect size, per pollutant.

Mass balance of pollutants for each treatment replicate was calculated to understand the removal mechanisms for each element and treatment:

$$Total\ pollutant\ input\ per\ replicate\ (mg) = T_c + \sum T_t + \sum T_b$$ [Equation 3]

Where T_c is the total quantity of pollutant in the pre-experiment conditioning phase, T_t is the total quantity of pollutant input during tap top-ups and T_b is the total intended quantity of pollutant input for all batches via modified Hoagland's solution. Pollutant sequestered per community was calculated as follows:

$$Total\ pollutant\ squestered\ (mg) = \big((B_e \times TC_e) + \cdots\big) - \big((n \times (\bar{X}\,B_s \times \bar{X}\,TC_s)) + \cdots\big)$$ [Equation 4]

Where B_e is the mean of the biomass of all of one particular species in a replicate, TC_e is tissue composite concentration of one particular species in a replicate. $\bar{X}\,B_s$ is the mean biomass of the reference plants (*n* = 4) at the start of the experiment (before the conditioning phase), $\bar{X}\,TC_s$ is the mean tissue concentration of a pollutant 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 GM+PA there are six GM individuals and six PA individuals. To calculate the total pollutant removal from each mesocosm Equation 5 was used:

$$Total\ pollutant\ removed\ by\ plants\ (mg) = Total\ pollutant\ squestered\ (mg) - Total\ pollutant\ input\ per\ replicate\ (mg)$$

[Equation 5]

Allowing the plant uptake mechanism contribution to be compared between different pollutants, the direct contribution of plant uptake to pollutant removal was calculated as a percentage following Equation 6:

$$Proportion\ of\ uptake\ by\ plants\ (\%) = \frac{Total\ pollutant\ squestered\ (mg)}{Total\ pollutant\ input\ per\ replicate\ (mg)} \times 100$$

[Equation 6]

5.3 Results

5.3.1 Removal efficiencies of vegetated and unvegetated FTW systems relative to controls

There was a significant difference between treatment values and controls for all pollutants ($P < 0.05$) except Cr which was not significantly different (Figure 5.2). For all FTW systems there was wide variation in the adjusted-RE (%) at a 7-day HRT between and within each individual pollutant (Figure 5.2). Average values for pollutant removal within each mesocosm ranged from 55 % adjusted-RE for TIN to -9 % adjusted-RE for Fe. The pollutants that were most readily removed by the vegetated and unvegetated treatments, had the highest adjusted-RE (ranging between 50 % and 70 %) were TIN, K and Mn. Pollutants that showed a moderate adjusted-RE (ranging from 12 % to 26 %) were P, Zn and Cu while Mg, Ca, Na, Cr and Fe were pollutants that were poorly removed or increased within the vegetated mesocosms relative to the controls as indicated by low or negative adjusted-REs. The pollutants with the highest rate of removal also showed the highest variation. Treatment type was likely the main source of variation as demonstrated by analysis of each specific community (Figure A3.1). For example, for Zn treatments containing *T. latifolia* were between 40 and 45 % mean RE while treatments containing *P. australis* were significantly lower at 25-30% RE (Figure A3.1).

There was a significant difference between treatment values and controls for all pollutants at a 14-day HRT ($P < 0.05$; Figure 5.3). The descending order of pollutants in Figure 5.3 (from the those that showed the highest adjusted-RE to those lowest) was different to that of the 7-day HRT indicating that at 14 days there were different A-RE profiles for some pollutants. After 14 days K was removed from the simulated polluted water 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, showing that there were very small differences in the vegetated treatments compared to the open water control at 14-days. K and Cu were removed from the simulated polluted water more readily after 14 days compared to 7 days, while TIN, P and Mn all had reduced adjusted-RE. There was wider variation in the removal of K, Cu and Zn by vegetated and unvegetated treatments which also coincided with significant differences in removal rates between communities (Figure A3.1).

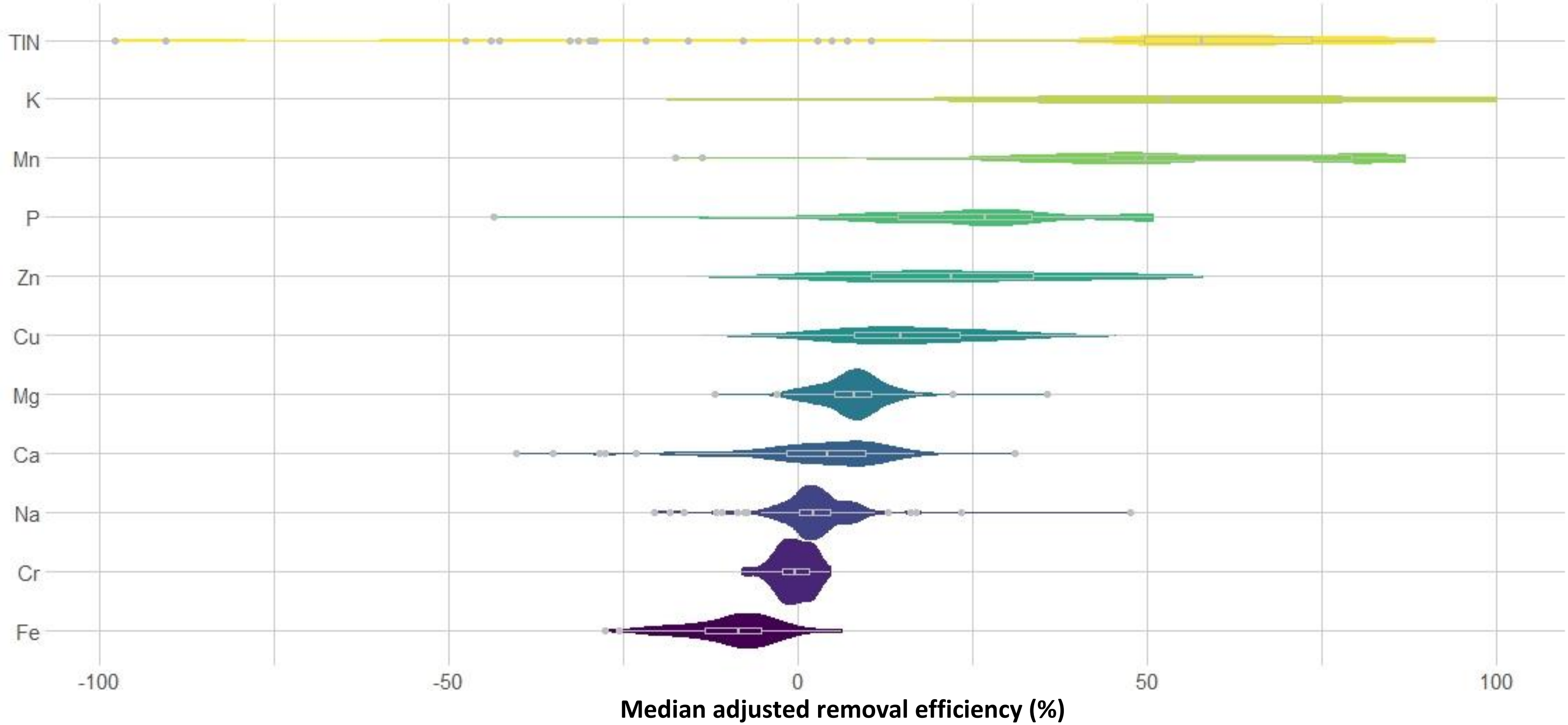


Figure 5.2: Adjusted removal efficiencies (A-REs) at 7-day HRT for each studied pollutant, violin plots show variation around the mean and box plots show median and interquartile range and outliers (>-100, <100). Each element is ranked from top to bottom by order of greatest mean removal efficiency relative to open water control treatment.

5.3.2 Modelling and characterisation of factors influencing removal of pollutants from simulated polluted water

5.3.2.1 Pollutant removal by experiment duration and community type interaction

This section considerers the factors influencing pollutant removal within the mesocosms through an analysis of the pollutant concentrations within the simulated polluted water. The interaction effects between macrophyte community and experiment duration (weeks) were both negative and positive depending on pollutant. The former indicates that with increasing experiment duration pollutant concentrations beyond a HRT of 7 days were progressively lower (i.e. increased removal efficiency), and vice versa for positive effect sizes. Effect sizes close to 0 indicated a weak combined influence of treatment and time on removal efficiency. Pollutant concentrations of Zn, Ca, Mn and P in the water were predominantly negative suggesting a weak inverse relationship with experiment duration (Figure 5.4). The polyculture treatment containing *T. latifolia*, *P. australis* and *G.maxima*, and *T. latifolia*-containing bi-culture communities had the strongest interaction with time on Zn removal with effect size ranges below 0.

The *P. australis* monoculture, followed by all other communities and treatments, had the strongest overall negative effect size for Ca removal. There were only small differences in effect sizes at the treatment level for Mn and P indicating plant community type did not play a large role in their removal over time. With effect sizes centring around 0, and uncertainty ranges spanning positive and negative scales, the interaction between time and plant community was likely not important for determining TIN, Mg, K, Na removal.

Concentrations of Fe, Cu and Cr within the mesocosms were positively related to the experiment duration and treatment interaction, meaning that removal efficiency decreased with time. Treatments containing T. latifolia as part of the community had particularly strong effect sizes (>0.25). For the unvegetated FTW treatment (FTW) the effect size confidence intervals were generally centred on 0 with uncertainty ranges spanning both positive and negative scales, indicating that experiment duration did not have a strong influence on removal of pollutants in these mesocosms.

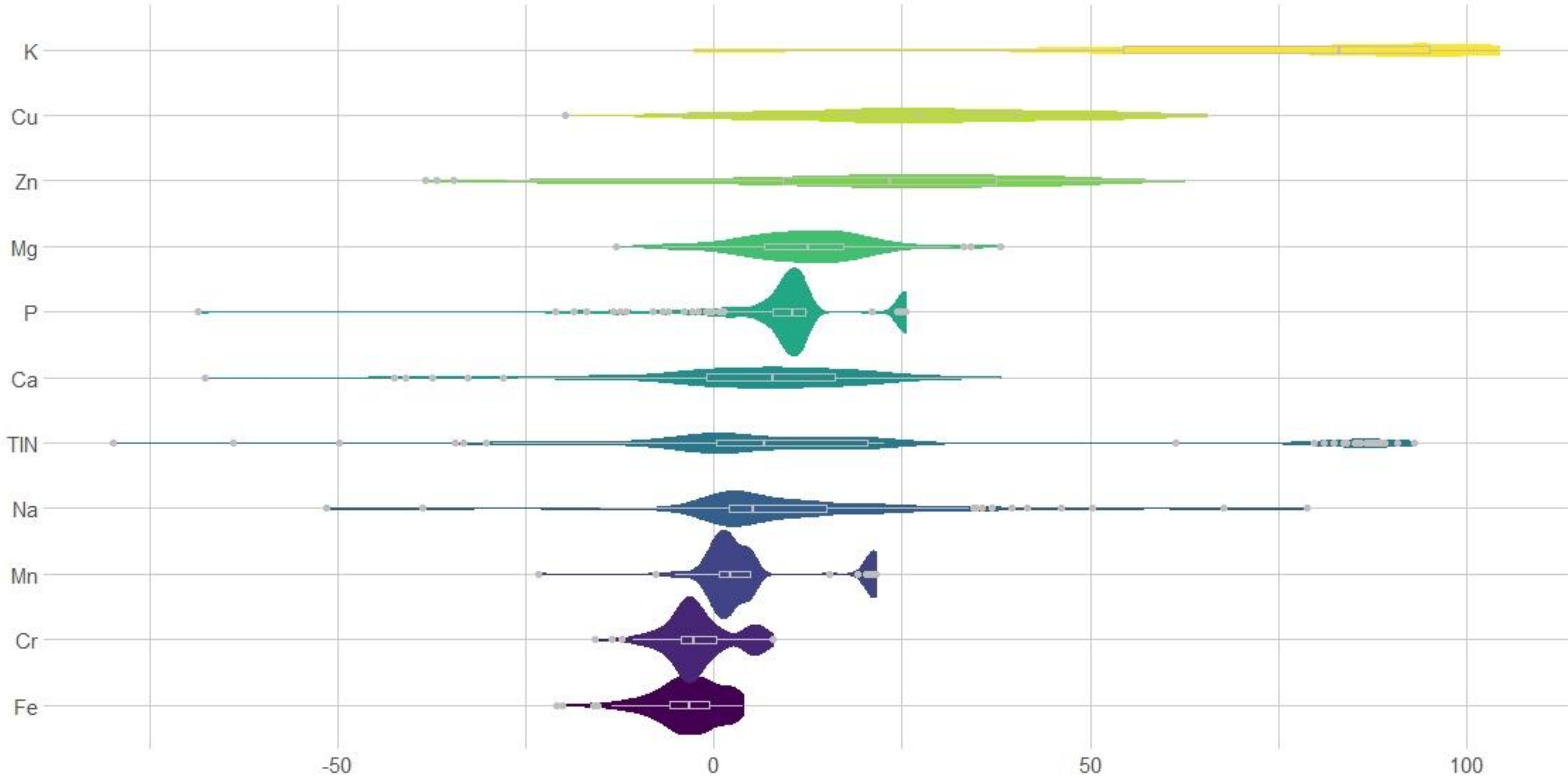


Figure 5.3: Adjusted removal efficiencies (A-REs) at 14-day HRT for each studied pollutant, violin plots show variation around the mean and box plots show median and interquartile range and outliers. Each pollutant ranked from top to bottom by order of greatest mean removal efficiency relative to open water control treatment.

5.3.2.2 Pollutant removal from water versus biological, ecological and physicochemical factors

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 (Figure 5.5; $P < 0.05$; R^2 between -0.52 and -0.61). Mg and Mn water concentrations correlated weakly and negatively with maximum root length (Figure 5.5; R^2 -0.4 and -0.3 respectively; $P<0.05$). Cr concentrations in water had a strong and positive relationship with maximum root length (R^2 0.67; $P<0.05$) and there were very weak relationships for Cu, Fe, Na and TIN. The positive correlation between experiment duration and maximum root length suggested that the temporal effects seen for Ca, K, P, Mg and Mn in Figure 5.5 may also be related to root growth (Figure A3.2). Relative growth rates and ambient air temperature did not correlate well with pollutant concentrations in the simulated polluted water across the experiment duration (Figure A3.3; Figure A3.4). Absolute levels of turbidity in the mesocosms were low and there were no significant differences at a 7-day HRT for turbidity (Figure A3.5). At a 14-day HRT, the open water control (UN) had significantly higher levels of water turbidity than all other treatments (Figure A3.5). 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 open water control and the unvegetated FTW (UN and FTW respectively) had a higher pH (ranging from pH 8-11) across the experiment compared to the vegetated treatments (Figure A3.6).

Plant community composition and species identity of vegetated FTWs influenced the concentration of Zn, P, Mn, Cu, Cr, Ca, K and Mg in the water within each mesocosm, while there were no differences observed between community composition for TIN, Fe and Na (Figure 5.6; Figure A3.7). These differences were primarily driven by the presence and total proportion of T. latifolia and P. australis within each community (Figure 5.6; Figure A3.7). Concentrations of Zn and P remaining in the water after 7 days were significantly lower in mesocosms with FTWs planted with communities containing T. latifolia ($P < 0.05$) compared to those without T. latifolia ($P > 0.05$). Although mean pollutant water concentrations were lower between FTWs communities planted with 100 % T. latifolia and mixed cultures (communities with 33 % and 50 % of T. latifolia) this difference was not significant.

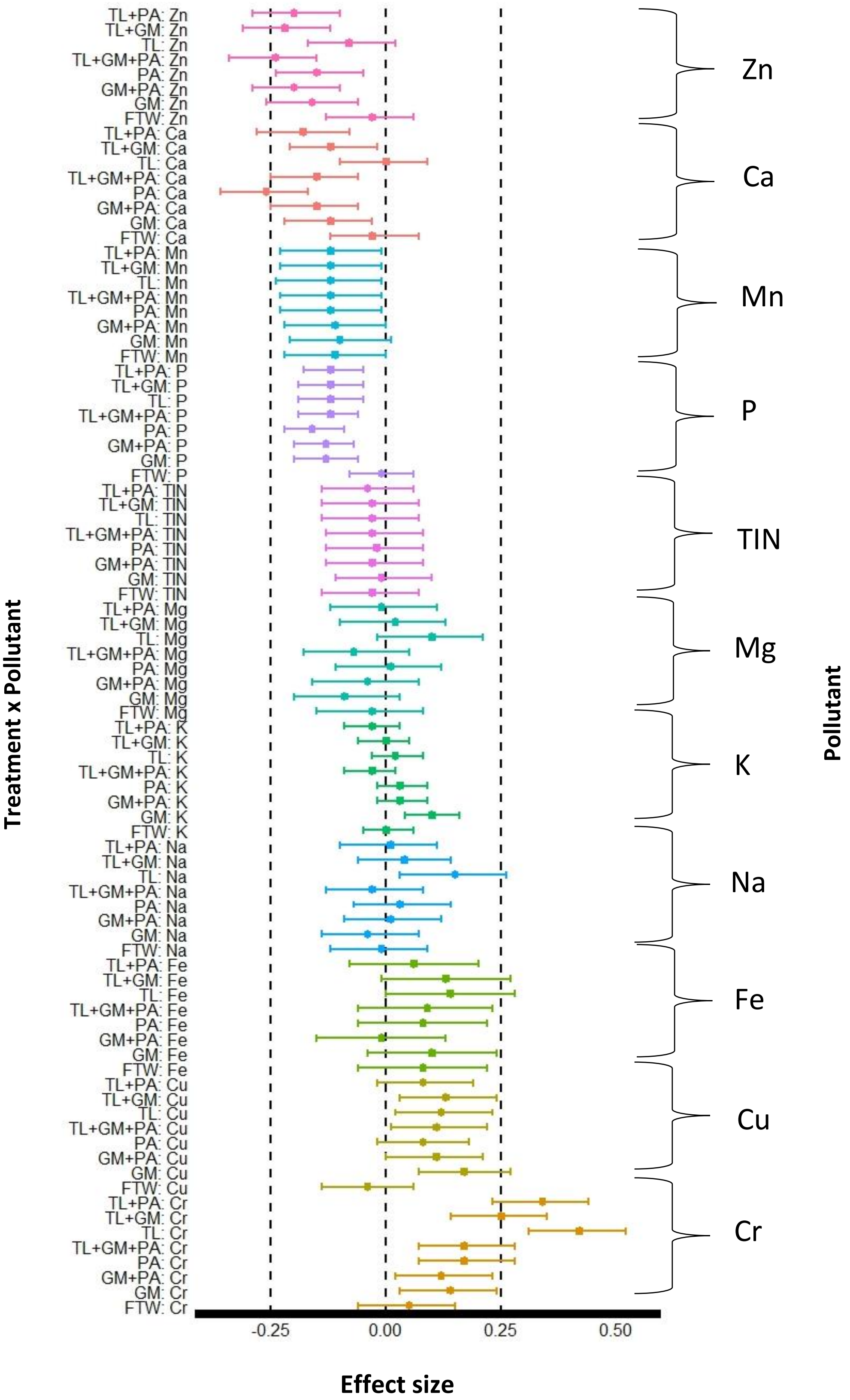


Figure 5.4: Forest plot of the effect sizes and associated 95 % confidence interval for the relationship between *treatment x experiment duration (weeks)* for each pollutant and treatment, per pollutant in order of mean effect size per pollutant. Full model outputs associated with each pollutant are contained within Table A3.1.

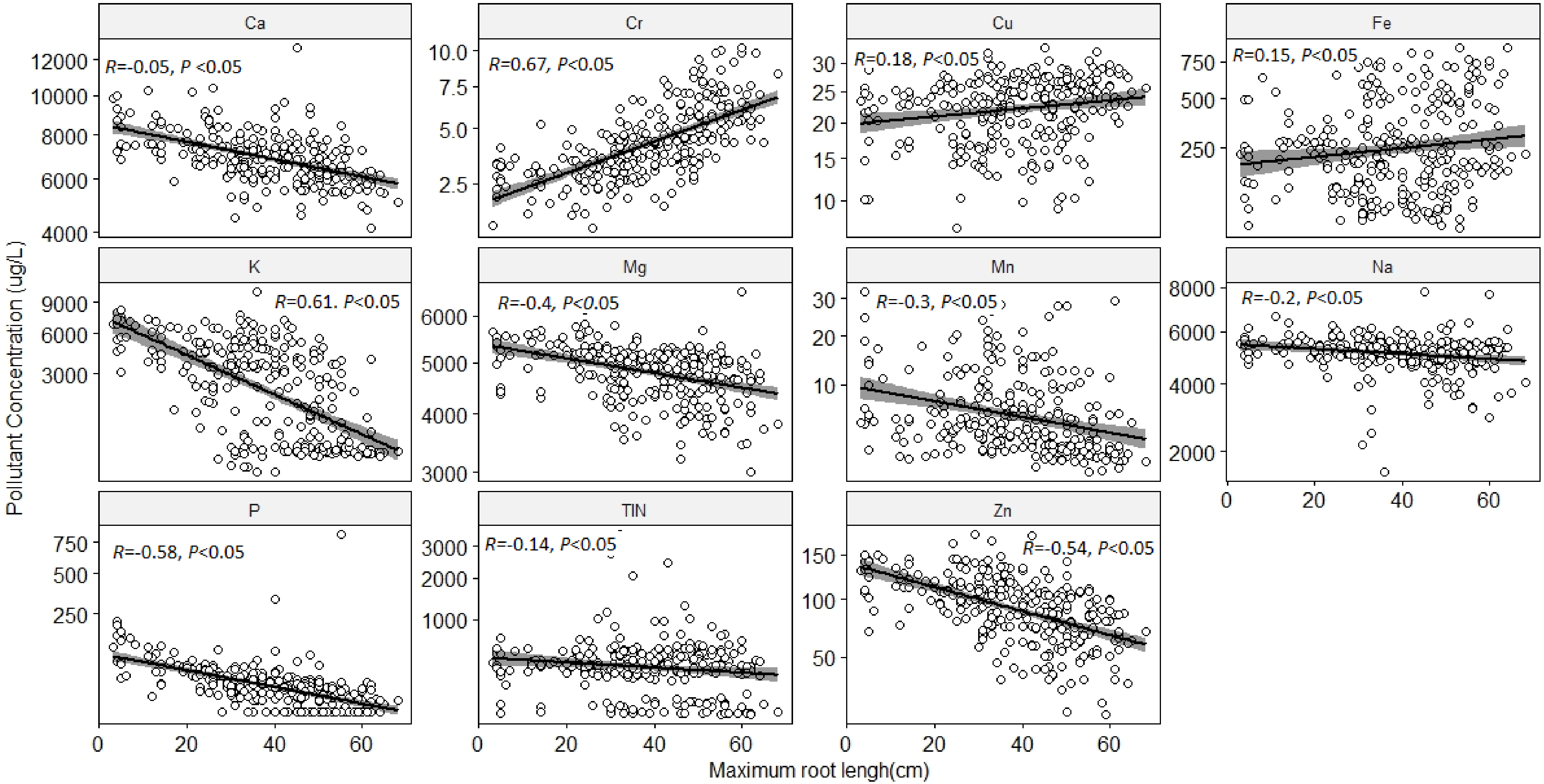


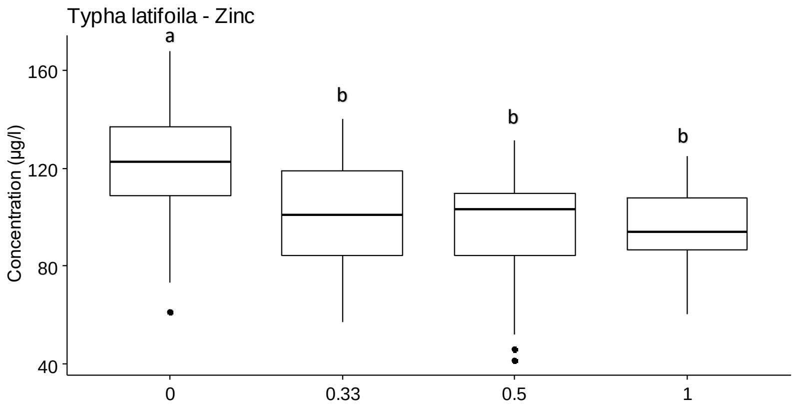
Figure 5.5: Scatter plots with a square root transformation on the y axis showing the relationship between maximum root length and pollutant concentrations in mesocosm after 7 and 14 days. Correlation coefficients and p-values are given for each plot. Fitted line based on a simple linear regression.

FTWs vegetated with a monoculture of *T. latifolia* (100 %) had a significantly lower concentration remaining of Ca compared to all other mixed communities (with 50 % and 33 % *T. latifolia*; Figure 5.6; P < 0.05) indicating a stepped effect of *T. latifolia* proportion on Ca removal from the water. This trend with communities containing *T. latifolia* was also observed in the removal of Mn, K and Mg (Figure A3.7). Water in mesocosms with vegetated FTWs including *P. australis* had a lower concentration of Cu compared to mesocosms with FTWs that were not planted with *P. australis* (Figure 5.6; 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) (Figure 5.6). The species richness of the vegetated FTWs mostly did not have an impact on pollutant removal from the water as there were no significant differences between each species number and pollutant concentration (Figure A3.8). The only exception was K where an increase in species number coincided with significant decreases in concentration of K in the water (Figure A3.8; P<0.05).

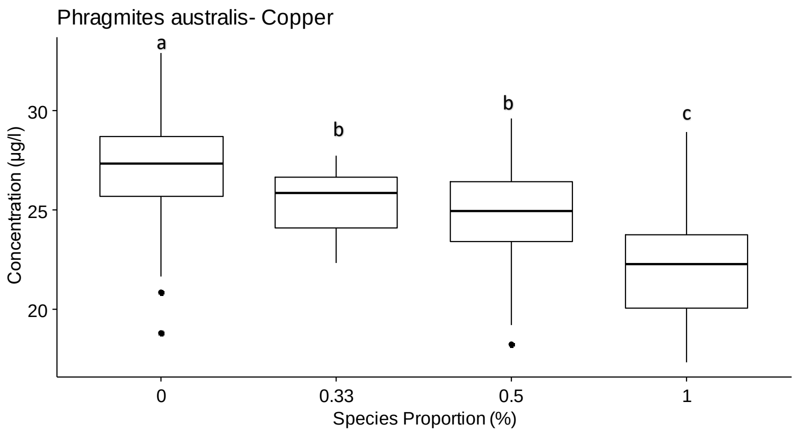
5.3.3 Pollutant mass balance and plant update contribution to pollutant removal

Plant uptake (%) was responsible for average pollutant removal ranging from median values of 30 % to 0.5 % depending on the pollutant (Figure 5.7; Table A3.2). Plant uptake was an important control on concentrations of Mn, TIN, P and Fe with median uptake values greater than 12.5 %. 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 (<4%). In terms of pollutant sequestration, the largest gains were in the below ground parts (roots and rhizomes) with 3 – 8 times greater storage compared to above ground plant parts (stems and leaves) (Figure A3.9). For the P. australis monoculture and in some cases mixed cultures containing P. australis there was a net loss in overall aboveground storage for all pollutant apart from TIN (Figure A3.9 and Figure A3.10). The variation in levels of plant uptake was primarily related to treatment. There were significant differences between treatments for plant uptake of Ca, K, Na, P and Zn (P < 0.05, Figure A3.11) where treatments containing T. latifolia were the most effective accumulators. Communities containing P. australis had the highest uptake of Cu and Fe (P < 0.05, Figure A3.10), while the mixed community had the highest uptake of Mg (P < 0.05). There were no significant differences in plant uptake of TIN, Cr and Mn between any of the treatments (Figure A3.11).

(A)



(B)



(C)

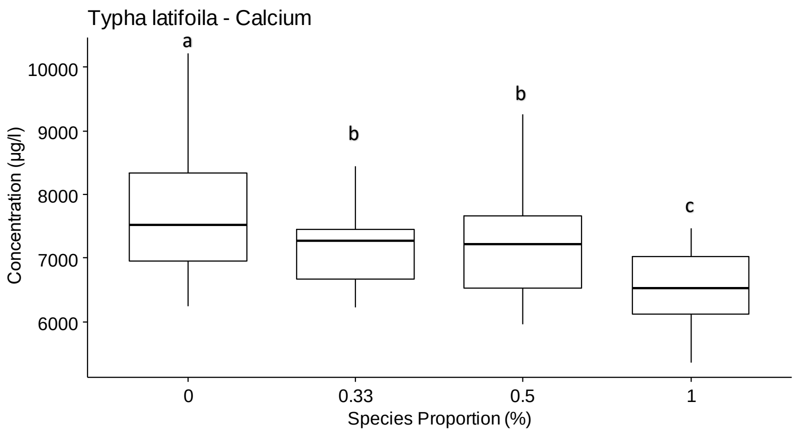
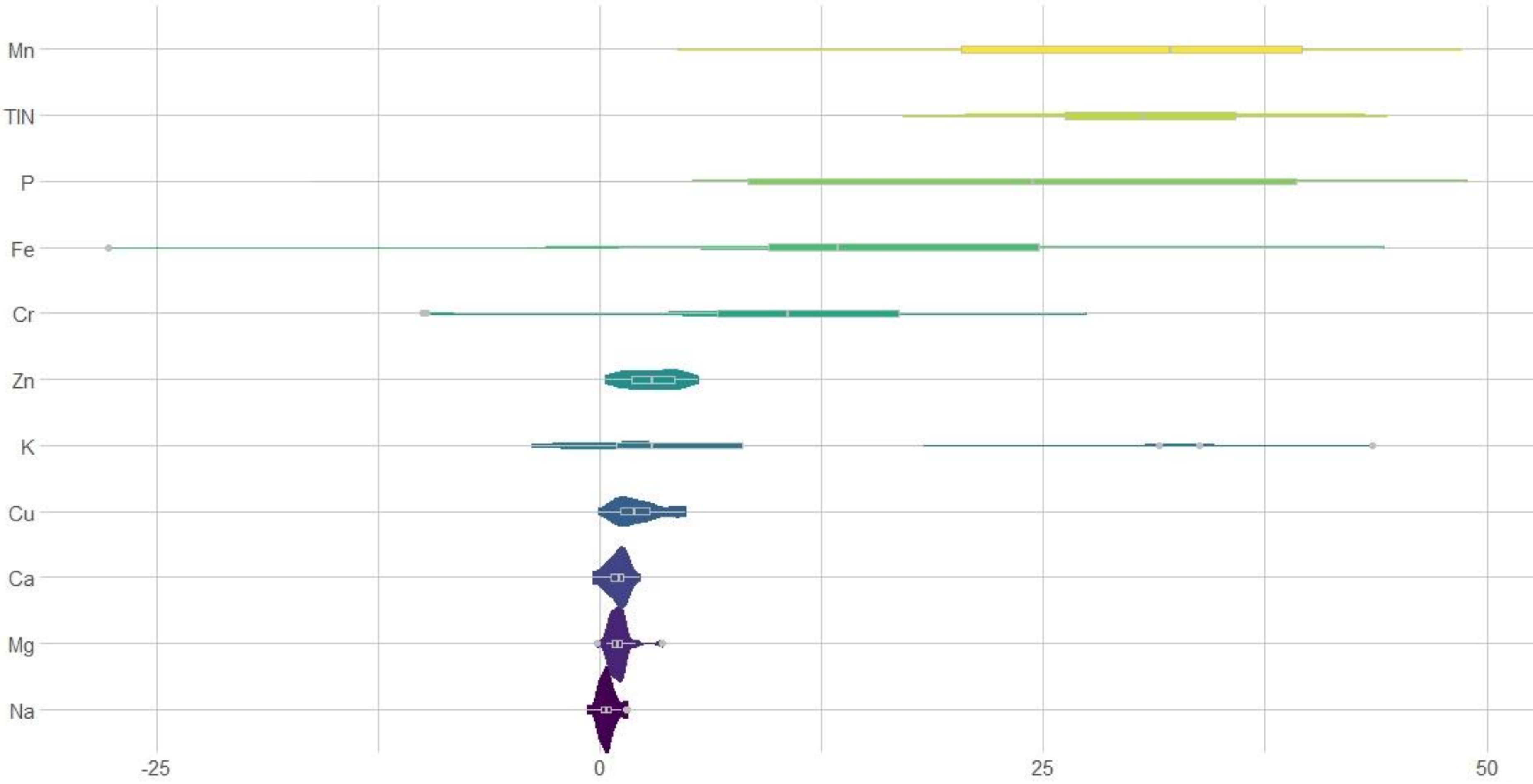


Figure 5.6: Water pollutant concentration by proportion of species for selected pollutants and species including (A) *Typha latifolia* and zinc, (B) *Phragmites australis* and copper, and (C) *Typha latifolia* and calcium. Letters denote significantly different groups ($P < 0.05$) by post-hoc Tukey test. Full results for all species and pollutants can be found in Figure A3.6.



Percentage pollutant removal accounted for by plant uptake and sequestration (%)

Figure 5.7: Percent of pollutant removal accounted for by plant uptake, 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 median percent of removed pollutant accounted for by plant uptake

5.4. Discussion

5.4.1 Effectiveness of FTWs for pollutant removal and sequestration

Floating treatment wetlands planted with macrophytes can effectively remove waterborne pollutants including TIN, K, Mn, and can also contribute well to removing P, Zn and Cu from multi-contaminated water. These results compare favourably with the removal efficiencies reported in existing FTW-based studies (Zhao *et al.*, 2012; Ladislas *et al.*, 2013; Keizer-Vlek *et al.*, 2014; Han *et al.*, 2018). Ca, K , Mg and Cu removal was between 20 – 40 % lower than those reported elsewhere and was likely due to the higher pollutant concentrations used in these other studies meaning they were more readily sequestered (Han et al. 2018; Tanner and Headley 2011). Based on the results of this study the pollutants that could be targeted most readily as part of FTW system are TIN, K, Mn, P, Zn and Cu. HRT was a factor that variably influenced the removal of each pollutant with K, Cu and Zn most positively benefiting from a HRT of 14 days. For removal of pollutants such as TIN, Mn and P increased HRT was not beneficial in the vegetated treatments suggesting that removal mechanisms in the open water control reduced pollution levels close to those of plants. It has been demonstrated that for some pollutants shorter timescales are ultimately more effective as pollutant removal maxima can be achieved quickly, in 7 days or less (Van de Moortel et al., 2010). Ultimately for increased benefit of vegetated wetlands a shorter HRT is more important and preferable from an operational perspective (Zhang et al., 2011), unless specific elements are being targeted that take longer to remove (e.g., K, Cu and Zn).

Rates of plant sequestration of Mn, TIN, P, Fe and Cr demonstrate the potentially positive role of harvesting for removing these pollutants and achieving a net loss from polluted water. However, as sequestered pollutants were mainly stored in below-ground plant parts, the opportunity to recover pollutants is more challenging and whole-plant harvesting would be required (Garcia Chanc et al., 2019). Harvesting belowground is practically more difficulty and may disturb root zone pollutant removal processes, which often continue during plant dormancy in winter (Soana et al., 2018), as well as mobilising pollutants in sediments. Harvesting above-ground plant parts is a more viable strategy as an average of 100 mg and 500 mg (taking as m^2, unit squared equivalent) of P and N respectively was sequestered in above ground tissue. Plant uptake and sequestration of Zn, K, Cu, Ca, Mg and Na represents only a small fraction of the removal process, so harvesting FTWs specifically for these elements for the sole purpose of water quality improvement is not recommended. In such circumstances, passive management of the FTW may be more appropriate as opposed to active management; however, if a net loss from the system is important then plant biomass should be harvested periodically to avoid cycling of pollutants back into the system (Quilliam et al., 2015). Furthermore, employing known metal hyperaccumulators may also make this process more viable (Verkleij et al., 2009).

5.4.2 Mechanisms for pollutant removal

5.4.2.1 Temporal and developmental factors

Time and plant development (root growth) were significantly correlated suggesting that root length was an important co-varying factor and the underlying trait driving removal process for Ca, Mg, K, P and Zn. The optimal range for biological activity and removal efficiency is above 15 °C (Akratos and Tsihrintzis, 2007) and despite a general air temperature decrease through the experiment it never decreased below this. Therefore, it is likely that temperature did not play a significant role in determining pollutant removal compared to root length. Furthermore, relative growth rate as measured in above-ground biomass development did not correlate with any pollutant removal suggesting below ground processes were more important. Rhizosphere effects are influential in removing waterborne pollutants due to interactions between water and plant (Urakawa et al., 2017), for example, plants with the greatest root length are more effective phytoremediators of P (Wang et al., 2015). Longer and denser roots can more effectively scavenge nutrients because of enhanced contact with the growth media (Colares et al., 2020). Microorganisms attach to root and rhizome surfaces forming biofilms where fungi, bacteria and algae can metabolise free elements (Masters, 2012). Finally, sorption of pollutant molecules to organic material produced by plant roots which may then settle is also a removal pathway (Tanner and Headley, 2011b). It is likely that a combination of the above mechanisms aided the removal process in the experimental mesocosms. However, the low contribution of plant uptake to pollutant removal suggests that plant-mediated processes associated with biofilm development on roots were most important (Borne et al., 2014). Therefore, if targeting the removal of Ca, Mg, K, P and Zn plant species with rapid and extensive fibrous root growth should be selected.

5.4.2.2 Plant uptake & environmental factors

Plant uptake contributed to a large portion of the TIN and Fe removal as demonstrated by the mass balance analysis. As there were no significant differences between the vegetated treatments in A-RE for TIN it suggests that all plants had similar affinity and removal mechanisms for N. Plant uptake accounted for a significant portion of TIN removal (between 12.5% to 45%) suggesting that sequestration was a key mechanism for removal. Accounting for the additional mechanisms in TIN removal is more challenging due to the fluxes in N species. Denitrification is one of most important processes for removal of nitrate from aquatic systems. However, dissolved oxygen levels in the water were above the threshold for a switch to anaerobic conditions (i.e., below 1mg/l) and therefore the dominant N cycle processes would have been nitrification of the ammonia fraction (and thus increasing plant-available N). Sampling error with the dissolved oxygen probe is unlikely as the mesocosm were not particularly large and water was well homogenised. After 14 days TIN removal in the open water controls was equal to all vegetated treatments. The significantly elevated levels of turbidity and visible algal mass within the open water control means it is likely that algal growth accounted for a significant uptake of TIN. While algal growth was far less prevalent in the vegetated treatments, towards the end of each 2-week batch visible algal growth on the sides of the tank and in the water could be observed. Immobilisation of N due to algal growth was therefore a likely a mechanism for N removal in vegetated FTW systems alongside macrophyte uptake. Unvegetated FTW treatments had low and negative adjusted removal efficiencies suggesting that shading of the water reduced algal growth, and with no plant-driven mechanisms availability there was less TIN removal than both open water controls and vegetated FTW treatments. In terms of TIN removal from the system shorter HRT was most effective to take advantage of plant uptake.

pH levels combined with plant uptake were most likely the combination of factors that contributed to removal of Fe. It is an essential micronutrient so elevated levels available in the mesocosms would have been readily sequestered, as evidenced by the high contribution of plant uptake (Kirkby, 2011). Fe also can precipitate rapidly in neutral to alkaline conditions (Bassez, 2018). Both control treatments had alkali pH levels throughout the experiment stimulating Fe precipitation from the water column. Control treatments had higher pH levels likely increasing Fe removal while concentrations in vegetated FTW treatments took longer due to the more acidic pH due to the release of protons through plant roots (Rai, 2009). The process influencing Cu removal is more challenging to understand; in previous studies sorption to plant roots or induced settlement have been proposed as key mechanisms (Borne et al., 2013). However, root length did not correlate well with Cu, and *P. australis* treatments (the species with the lowest root growth and length) had the greatest Cu removal. While it did not contain the longest roots *P. australis* did have the largest and thickest rhizomes; this plant species may release more organic material and promote flocculation of Cu. *P. australis* may also have specific transporter proteins (Printz et al., 2016) and hence the removal of Cu was improved with HRT and was significantly higher than the control. Cr removal was partially though plant uptake and likely settlement as there was little difference between vegetated treatment and the controls at 7-day HRT. Therefore, macrophyte phytoremediation of Cr is not recommended as, based on these results, vegetated treatments did not have a significant influence on Cr concentration.

5.4.3 Plant community ecology

The influence of species-specific plant traits on removal mechanisms can be affected by the community composition (Brisson and Chazarenc, 2009). For example, the extensive root growth of *T. latifolia* can have a positive impact when it is included in an assemblage, particularly for removing P, Zn, Ca, Mn, K and Mg. The mass ratio hypothesis postulates that ecosystem functioning is related to the proportion of a species within a community (Grime, 1998; Mokany et al., 2008). There was evidence of proportionality between the removal of Ca, Mg, K and Mg and the occurrence of *T. latifolia* in a community, with significant differences between communities containing *T. latifolia* in bi and mixed-cultures, versus monocultures. Removal of Cu was also similarly related to the proportion of *P. australis* within the vegetated FTW communities. The lack of a significant difference in removal of P and Zn between the different communities containing *T. latifolia* suggests that in some cases inclusion of the important plant trait is key, rather than the overall proportion of that species. There is evidence that increased species diversity is the most important factor in enhancing pollutant removal (Ge *et al.*, 2015; Han *et al.*, 2016, 2018; Geng *et al.*, 2017). In this study the most diverse plant community did not show the greatest absolute removal suggesting that species, or at least trait identity, is most important, although it did only consider richness over the range of 1-3 species. While diversity does not necessarily enhance removal of specific pollutants, assembling communities with multiple species with varying traits and associated mechanisms may allow several pollutants to be remediated at once. Such a flexible remediation systems, that offers a variety of other ecosystem services, may be desirable in multi-pollutant waters.

5.4.4 Applied use of FTWs and further work

The above sections have highlighted some of the important factors in relation to the implementation and management of FTWs that should be considered when utilising FTWs in freshwater remediation. Based on the results these systems have application potential in surface waters impacted by diffuse pollution including those with nutrient enrichment (P and TIN), and/or where there are other pollution issues due to legacy (Mn due to former mining activities), or ongoing pollution issues (e.g. high Zn and Cu levels due to pesticide use). As all the pollutants were studied within a multi-polluted water context, FTWs can simultaneously remove multiple pollutants. The community composition of vegetated FTWs strongly moderates the ability to target multiple pollutants as the proportion or inclusion of certain species can influence removal efficiency. Therefore, attention to community assembly is necessary when targeting a specific set of pollutants. Considering plant traits is also of key importance as root length is evidently a crucial factor in removal, particularly for Ca, Mg, K, P and Zn. Therefore, plant selection should also consider and give preference to species with strong fibrous root growth where a wide range of pollutants are targeted for removal.

Plant uptake was the most important mechanism for TIN removal and therefore the greatest resource recovery potential may be from focusing on nitrogen export from aquatic systems. However, as noted in section 4.1 there are benefits and disbenefits from active versus passive management strategies relating to harvesting FTWs. The temporal element associated with pollutant removal can also be critical for successful phytoremediation. For pollutants that were more readily removed with an increasing HRT such as Cu, it means that the residence time of the target water body will influence removal rates. Therefore, FTWs might generally perform better where and when residence times are longer e.g., in ponds or in streams during slow flows. Removal efficiency across the growth season varied depending on the element, but as this study was primarily based on a new installation FTWs, longer term (3-5 year) studies including different community combinations would lead to a further understanding on intra and inter seasonal and successional effects.

5. 5 Conclusion

This study aimed to better understand the mechanisms underpinning the improvement of water quality in multi-polluted freshwater using FTWs. 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). By understanding how species-specific plant traits can maximise pollutant removal, the most appropriate plant species can be selected for FTWs. It was also determined that plant update is generally 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. Plant community ecology is also an important moderator of pollutant removal and sequestration, and our results suggest that that the proportionality or presence of certain traits represented within a community is crucial to enhanced performance. This understanding can be used to aid the develop of specific plant communities for FTWs that can target multiple pollutants and predict performance more readily.

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6. Field-scale floating treatment wetlands: quantifying ecosystem service provision from monoculture vs polyculture macrophyte communities

Highlights

- Floating treatment wetlands can demonstrably provide ecosystem services over and above water treatment.
- Plant community composition changed substantially and may impact ecosystem service provision.
- Root morphology did not play a significant role in shaping associated belowground macroinvertebrate communities.
- Planted communities showed little difference in resource recovery potential.
- Floating treatment wetlands can support freshwater habitat restoration.

6.1 Introduction

Freshwater environments are under increasing pressure due to a range of stressors such as point source and diffuse pollution, land-use change and associated habitat loss, and climate change (Berger et al., 2017; Ormerod et al., 2010). Improving freshwater environments and water quality to support these ecosystems, and the people that rely on them, is a key priority and global policy aim through SDG 6 (Bunn, 2016). Enhancing water and habitat quality are strongly linked to the ability to provide multiple ecosystem services such as carbon sequestration, water flow regulation and habitat provision (Grizzetti et al., 2016; Keeler et al., 2012). Utilising so-called 'nature-based solutions' (NbS) to enhance freshwaters and support ecosystem functioning is one strategy to improve habitats and promote ecosystem service provision (Nesshöver et al., 2017).

Floating treatment wetlands (FTW) are a form of phytotechnology and a type of NBS that can assist in freshwater restoration (Shahid et al., 2018); these buoyant structures allow emergent macrophytes to grow hydroponically in the water, and after deployment in freshwater bodies can facilitate the removal of waterborne pollutants (Chen et al., 2016). FTWs can demonstrably improve water quality and are increasingly used worldwide as a 'best practice' management tool for freshwater restoration in both urban and rural settings, spanning a range of temperate and tropical climatic zones (Colares et al., 2020). However, existing studies of FTW are primarily focused towards understanding pollutant removal dynamics of specific pollutants; therefore, there is a clear knowledge gap in understanding the added value of FTWs and how plant ecological processes influence the provision of ecosystem services (Chapter 2).

As a nature-based solution, FTWs systems are systems governed by the key ecological engineering principle of 'self-design', where plant successional processes introduce an element of unpredictability to the eventual outcome (Mitsch, 2012). Ecosystem service provision from FTWs is strongly related to specific community compositions (Storkey et al., 2015; Chapter 4). This means that there can be unintended outcomes (positive and negative) from utilising such an NBS, together with associated impacts on restoration objectives. Given the increasing popularity of FTW as a restoration tool, there is a need to understand how the 'self-design' aspect is manifested in these systems.

The influence of discrete FTW plant communities with correspondingly varying root morphologies on habitat provision for macroinvertebrate communities is not well understood. The physical structure provided by macrophyte belowground structures such as roots and rhizomes are important for supporting macroinvertebrate communities and thus higher trophic levels such as fish populations (Yofukuji et al., 2021). More complex macrophyte root morphology is thought to increase macroinvertebrate diversity and abundance due to niche stratification and an increase in microhabitats (Hansen et al., 2010). Such abundant and diverse macroinvertebrate communities may also support higher trophic levels. Species-rich plant communities are more likely to have more diverse root morphologies compared to monocultures (Hassall et al., 2011), although there are currently no studies that have explored the macroinvertebrate community composition associated with FTWs.

FTWs are often viewed as a potentially low-cost intervention and an ideal NBS for decentralised water treatment (Shahid et al., 2018). However, challenges of deploying and maintaining FTW are not reported in the scientific literature which means that without a specialist (e.g., a paid consultant) it may be difficult for communities to initiate the use of FTWs as an economically sustainable solution. In addition, the potential benefits from resource recovery in real field-deployment is also a useful aspect in appreciating the added-value of these system. Given the above information-gaps in the application of FTWs as tools for freshwater restoration, the overarching aim of this study was to quantify ecosystem service provision (e.g., macroinvertebrates, biomass, tissue nutrient concentrations) and multi-annual change in vegetation in FTWs planted with different vegetation community types. This was achieved by setting up a field-scale trial of FTWs with either plant polycultures or a monoculture, and sampling over three years. By using vegetation community type as a variable, this approach will increase our understanding of how ecosystem services are influenced by plant community type. In addition, by reporting on the logistical challenges of deployment and maintenance encountered during this field trial the information provided here will aid in the future design and management of these systems.

6.2 Methods and materials

6.2.1 Experimental design

Field site and FTWs

The location for the field trial was Airthrey Loch (56° 8' 51.6252" N, 3° 55' 0.3278" W), which is an artificial water body with an area of 8.67 hectares and a residence time of 0.4 years (Figure 6.1). Airthrey Loch is primarily fed by a single stream carrying high levels of nitrogen and phosphorus from diffuse sources upstream, although there are numerous other point source inflows containing grey water from the surrounding residences. Nutrient-rich inflows have led to the eutrophication of Airthrey Loch and recently there have been large cyanobacterial blooms. These conditions also assist in facilitating the persistence of invasive non-native macrophytes such as *Azolla filiculoides* (water fern) and *Elodea Canadensis* (Canadian pondweed) in some locations. The loch itself is classified under the Water Framework Directive criteria as a small, lowland, high alkalinity, very shallow lake with moderate, bordering poor, water quality. Therefore, Airthrey Loch provides a model opportunity for deploying FTWs as a freshwater restoration tool and case study for ecosystem service generation in impacted waters. An accessible 40 m stretch of the northern shore of Airthrey Loch was utilised for this study. This field trial was carried out from May 2019 to September 2021 and allowed the establishment of the FTWs within this impacted system and provided enough time for root system development.

The FTWs were composed of HDPE 20 cm diameter hollow tubes, extruded at each end to allow nut and bold fixtures to produce a modular design (2 m x 2 m). Each FTW when fully constructed was 2 m x 4 m and comprised two modules with each having a 2 m x 2 m zinc coated iron grid underneath (held together by strong cable ties) to support macrophytes and substrate. Fences were attached to the FTWs to discourage herbivory from waterfowl. The polyculture and monoculture FTWs were arranged alternately along the shoreline to account for variation in the water depth and sediment conditions along the shoreline. Underwater separation fences were constructed using HDPE semi-rigid plastic mesh with a 2 mm x 2 mm diamond hole size, secured to the floor of the loch using fence posts, to facilitate the development of distinct microhabitats between the floating wetlands and help secure the floating wetlands in place. In total six FTWs were deployed following the planting arrangements in Figure 6.1.

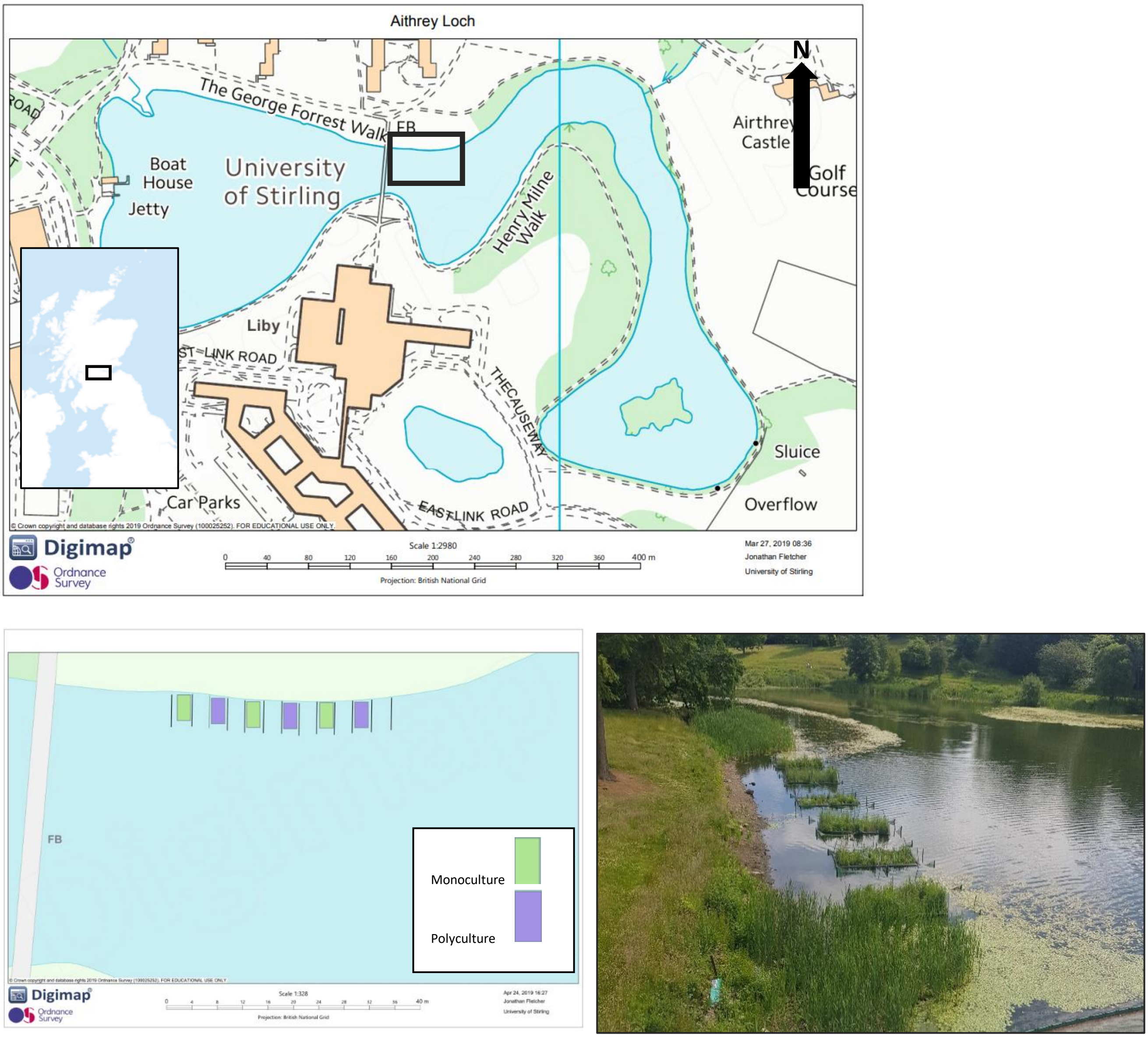

Figure 6.1: Field trial location at Airthrey Loch, Stirling (top, site marked with black square), field trial set-up with site map and configuration (left) and site photo (right)

Plant communities

To test the hypothesis that different plant communities lead to varying outcomes in ecosystem services, two different plant communities were chosen: (1) a *Phragmites australis* monoculture, and (2) a polyculture comprised of a diverse community including (but not limited to) *Alisma plantago-aquatica*, *Juncus effusus*, *Lythrum salicaria* and *Myosotis scorpioides*. In total, there were three monoculture FTWs and three polyculture FTWs. Coir-mattering was placed within the floating treatment wetlands, supported by the attached grids, enabling the hydroponic growth of the macrophytes and development of root systems within the water. Macrophytes growing in the FTWs were surveyed two months after deployment to assess which species had established. Despite changes in community composition during the duration of the trial, for ease of reference both communities are referred henceforth as either 'monoculture' or 'polyculture'.

6.2.2 Sample strategy

Field notes and photos were taken frequently and as required to highlight operational and maintenance aspects of FWTs. Plant surveys were carrier out three times over the course of the field experiment to track changes in the establishment of the floating wetlands (Table 6.1). Each wetland was treated as a distinct 2 m x 4 m quadrat, with the Domin scale approach used to estimate coverage of each vegetation type. After one year of root establishment (August 2020), waterborne macroinvertebrates were sampled from each FTW replicate. For each individual FTW replicate, a 1 mm mesh pond net was passed once for a 1.5 meters transect underneath the FTW and used to scrape back against the plant roots to sample for associated organisms. The content of the net was placed in a plastic zip-lock bag and immediately preserved by adding 70 % industrial methylated spirits (IMS). For each FTW, a 1.5 meter transect scrap was done four times starting from different locations to generate four pseudo-replicates per FTW replicate. Macroinvertebrate species were sorted and identified to the lowest taxonomic level.

Table 6.1: Sampling frequency during field trial

Ecosystem service	Measurable variable	Sample timing/frequency		
		Year 1 (2019)	**Year 2 (2020)**	**Year 3 (2021)**
Habitat provision	Macroinvertebrates (abundance and diversity)	-	August (summer)	-
Plant community stability	Abundance (Domin scale)	June (summer)	August(summer)	September (summer)
Biomass	Dry weight biomass	-	September (late summer)	September (late summer)
Resource recovery	Tissue concentration & standing stocks	-	September (late summer)	

Above ground biomass from each FTW was quantified by using a 0.25 m^2 quadrat placed within the FTW. All plant biomass within the quadrat was harvested to water level. For each FTW, three random quadrats were used to gain a representative biomass for each FTW and therefore each community type. This was undertaken on two occasions to relate biomass to community composition over time (Table 6.1).

To understand the resource recovery gains from harvesting macrophytes growing on FTWs in multi-polluted waters, the concentration of a range of recoverable pollutants (calcium (Ca), potassium (K), magnesium (Mg), nitrogen (N), and phosphorus (P), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn), sodium (Na) and chromium (Cr)) were determined in the above-ground biomass samples collected in Year 2. Plant tissue was oven dried at 75°C until a constant dry weight was achieved, and then pulverised using a RETSCH RS200 vibratory disk mill (RETSCH, Germany) to obtain material for tissue concentration analysis. Milled subsamples were either analysed for total C and N using a C: N analyser (FlashSmart NC ORG, ThermoFisher Scientific, UK), or microwave-digested with 70 % nitric acid and analysed for P and metalloid element concentration using inductively coupled plasma spectrophotometry (ICP-Optical Emission Spectrometer, Thermo Scientific iCAP 6000 Series ICP; Thermo Scientific, UK).

6.2.3 Data Analysis

All data analyses were carried out in R studio version 4.0.3 (R Core Team, 2021). For group comparisons, non-parametric Wilcoxon tests were performed as data did not confirm to the assumptions required for parametric tests due to small sample sizes. Where there were pseudo replicates from the FTWs (e.g., for biomass, tissue concentrations, and invertebrate species composition) a replicate mean was taken before a treatment-mean was taken. The total standing stock of each pollutant per 0.25 m^2 quadrat was calculated by multiplying the community polluant tissue concentration (mg/g) by the total community biomass (g). Standing stocks were multiplied by four to present results on a g/m^2 basis for comparability with existing literature.

The Shannon Weiner Index was used for comparison between communities as it accounts for diversity and species evenness. This was applied to both plant survey data and macroinvertebrate datasets. To compliment these approaches, functional representation was sought within these datasets for macrophytes and macroinvertebrates. For macroinvertebrates this included categorising each species by its functional feeding group, which included collectors, filterers, predators, scrapers, shedders and others (e.g. parasites) (Cummins, 2016). Using the approaches detailed by Cummins (2016), ratios between functional feeding groups were calculated to help understand community composition and environmental background (Table 6.2). For example, differences between functional feeding groups can relate to availability of fine particulate organic matter (FPOM) and coarse particulate organic matter (CPOM) or habitat stability. Habitat stability index, Shredder index, Filtering Collector index and Top-down predator index were the ratios chosen to understanding the habitat provision for macroinvertebrate communities by FTWs.

To assess the plant community composition in a summarised form, each surveyed FTW community was categorised by the dominant plant growth strategy following Grime's C (competitor), S (stress tolerator) and R (ruderal) (CSR) plant growth strategy framework (Chapter 3). The component plants from each community were assigned to their primary growth strategies (i.e., C, S and R) on a continuous scale. For each quadrat, the proportion of the primary growth CSR strategies within the community, weighted by the Domin cover scores of the component species, were calculated following the approach of Willby et al. (2001).

Table 6.2: Functional feed group calculations based on (Cummins, 2016) with an explanation of thresholds and possible interpretations as a result*.

Ratio name	**Ratio**	**Thresholds and explanations**	**Interpretations**
Habitat stability index	$\frac{Filterers + scrapers}{shredders + \ gatherers}$	Ratio bigger than 0.5 indicates suspended fine particulate organic matter is greater than entrained fine particulate matter	Filtering collectors require stable locations for attachment and construction of capture nets and scrapers requires surfaces that remain in a stable position facing up
Shredder index	$\frac{Shredders}{Collectors + filterers}$	A ratio of > 0.5 in autumn-winter of 0.25 in spring-summer, indicates that course particular organic matter avaibility for shredders is greater than fine particular organic matter avaibility for collectors	CPOM food support for shredders is greater than FPOM for collectors.
Filtering Collector index	$\frac{Filterers}{Collectors}$	A ratio of < 0.50 indicates suspended FPOM load < storage (entrained) FPOM	FPOM food for collectors at higher density and/or better quality than storage FPOM
Top-down predator index	$\frac{Predators}{All\ functional\ feeding\ groups}$	Predator to prey ratio 0.10 – 0.20 to total macroinvertebrate population Interpretations	This level of predator population density (or biomass) allows for sufficient prey to support them. If predators > 20 % probably indicates populations of rapid turnover (polyvoltine prey populations present Proposed

*Assuming that all shredders are herbivore shredders

6.3 Results

6.3.1 Macrophyte community composition changes

One year after deployment, the monoculture FTWs were almost completely dominated by *Phragmites australis,* while the polyculture contained an even mixture of *Juncus effusus*, *Lythrum salicaria*, *Alsilma-plantago*, *Iris pseudacorus* with numerous minor species including *Phalaris arundinacea*, *Caltha palustris* and *Lycopus europaeus* (Figure 6.2; Table A4.1). Consequently, the polyculture FTWs had significantly higher species richness and levels of diversity in year 1 (Figure 6.3; Figure 6.4; $P<0.05$). In the following year, there were shifts in both community types with simultaneous increases in species richness and diversity by an order of 1 - 2 times (Figure 6.3; Figure 6.3; $P < 0.05$). For example, the proportion of *Phragmites australis* decreased from near dominance in the monoculture FTWs to around 30 - 40%, with *Lycopus europaeus*, *Epilobium hirsutum* and *Myosotis scorpioides* increasingly represented (Table A4.2). The composition of the main species in the polyculture FTWs remained stable, although there was an increase in the proportion of *Myosotis scorpioides*, *Mentha aquatica*, *Lycopus europaeus*, *Epilobium hirsutum* and *Iris pseudacorus* (Table A4.2). This was primarily at the expense of the originally planted *Alsima-plantago* and *Juncus effusus* (Table A4.1; Figure 6.2). Overall, both species richness and community diversity remained higher in the polyculture FTWs although the difference was considerably smaller (Figure 6.3 and 6.4).

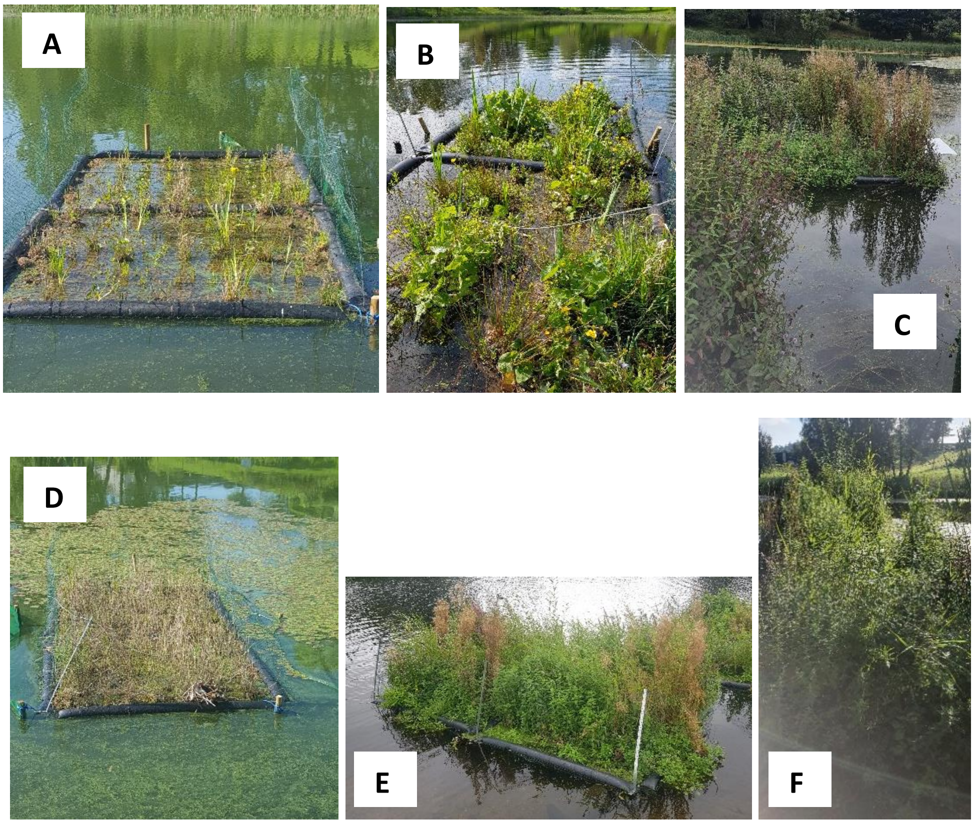

Figure 6.2: Site photographs showing an example of each plant community in different phases of development. Polyculture in Year 1 (spring/summer) (A), Year 2 (spring/summer) (B) and Year 3 (summer) (C), monoculture in Year 1 (spring/summer) (D), Year 2 (spring/summer) (E) and Year 3 (summer) (F). A video showing experiment set-up in Year 2 can be found here: https://www.youtube.com/watch?v=rCUP5r-Dj5U&t=6s

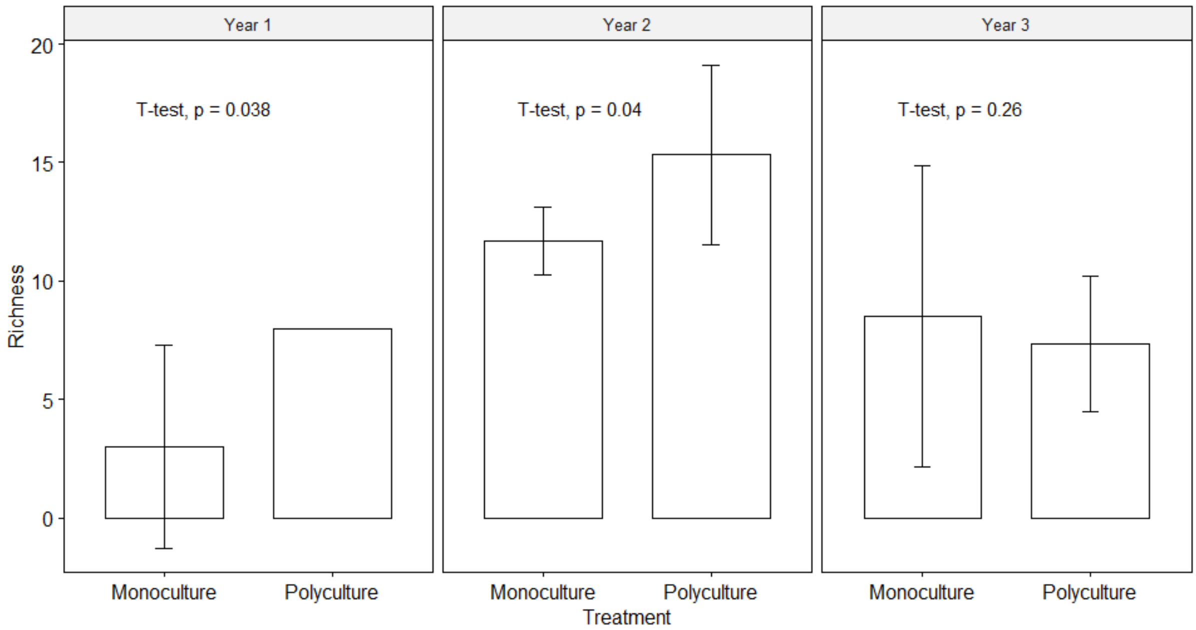

Figure 6.3: Mean plant community species richness for the monoculture versus polyculture communities from year 1 to 3.

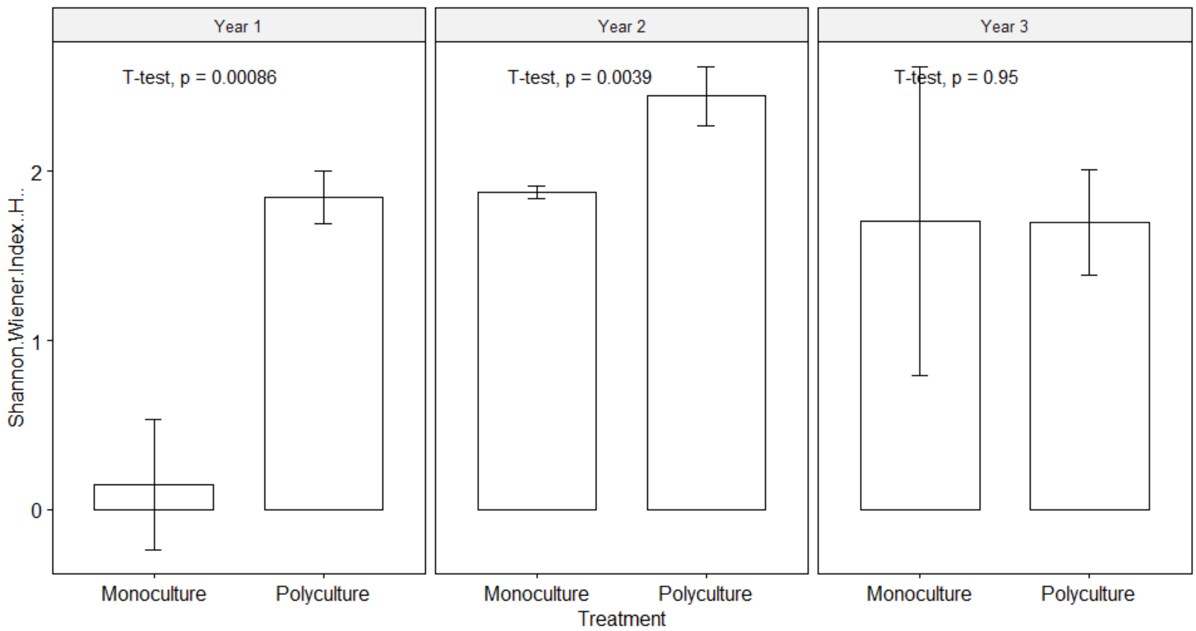

Figure 6.4: Mean plant community species diversity (Shannon-wiener index) for the monoculture versus polyculture communities from year 1 to 3.

The *Phragmites australis* component of the monoculture was small in year 3 with *Lycopus europaeus*, *Mentha aquatica* and *Lythrum salicaria* the most dominant species (Figure 6.2; Table A4.3). For the polyculture FTWs in year 3, the most dominant species were *Lycopus europaeus*, *Epilobium hirsutum*, *Mentha aquatica* and *Lythrum salicaria*. However, by year 3, there were no differences in species richness or species diversity between the two communities and overall, both species richness and diversity were lower (Figure 6.3; Figure 6.4). Both communities showed signs of community assemblages merging with several common major species between them including *Lycopus europaeus*, *Mentha aquatica* and *Lythrum salicaria* (Table A4.3). Reinforcing this observation is the progression of each plant growth strategy represented in both communities with an increased proportion of ruderal species at the expense of stress tolerators species (Figure 6.5). Overall, there was little difference in the overall representation in growth strategy between the two community types (Figure 6.5).

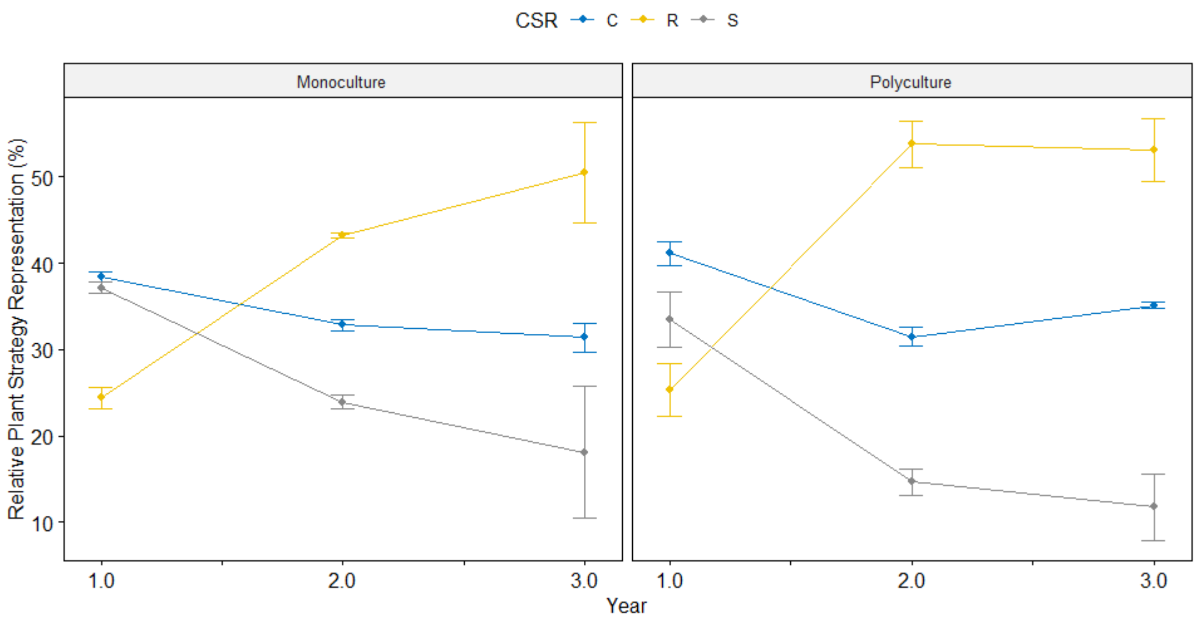

Figure 6.5: Mean percentage plant strategy representation (competitors, ruderals, stress tolerators) for monoculture and polyculture FTWs from years 1 to 3. Error bars show 1 standard deviation.

6.3.2 Macroinvertebrate community composition

Across both monocultures and polyculture FTWs, the macroinvertebrate assemblages inhabiting the root zones were mostly populated by molluscs (snails), malacostraca (crustaceans), diptera (fly larvae); and to a lesser extent trichoptera (caddisfly larvae), coleoptera (beetles) and hemiptera (true bugs) (Table A4.4). There were no significant differences between the two types of FTWs for macroinvertebrate richness ($P > 0.05$; Figure 6.6). Equally, there was also no significant difference in mean abundance levels of identified macroinvertebrates, although abundance was slightly higher from the polyculture FTWs ($P > 0.05$; Figure 6.6). The most represented macroinvertebrate groups in both FTW community types were *Planorbidae* (ramshorn snails), *Pisidium tenuilineatum* (pea mussel), *Radix balthica* (wandering snail), *Asellidae* (isopod crustaceans), Crangonyx (amphipod shrimp), Chaoborus (glassworm, insect larvae), chironomidae (nonbiting midge larvae) and Leptoceridae (long-horned caddisflies larvae) (Table A4.4). However, there were no significant differences between these groups of macroinvertebrates (Figure A4.2).

The functional feeding groups from each type of FTWs were similar (Figure 6.7). In relative order of greatest representation, these were predators (40 – 45 %), shredders (25 – 35 %), collectors (10 – 22 %), scrapers (4 -5 %), filterers (c. 4 %) and others (e.g. parasites) (< 1 %) (Figure 6.7). Functional feeding group representation between the two FTW types were not significantly different in each category. Although not significant, there were c. 10 % more collectors in the monoculture than the polyculture FTWs, and slightly more shedders represented in the polyculture FTWs (Figure 6.7).

As detailed in in section 2.3 the macroinvertebrate communities and calculated ratios can help understand the habitat type and avaibility and type of organic matter (Table 6.1). Between the monoculture and polyculture FTWs there were no significant differences ($P < 0.05$) in all functional feeding group ratios (Table 6.3). For all FTWs, the mean habitat stability index was < 0.5, indicating a greater presence of entrained fine particulate organic matter (Table 6.3, see also Table 6.2 for further information on ratio thresholds). A large mean shedder index indicated that course particular organic matter avaibility for shredders is greater than FPOM avaibility for collectors. Similarly, a mean filtering collect index is < 0.5 indicates suspended FPOM load is less than storage (entrained) FPOM. The mean top-down predator index is larger than the ideal window, which indicates high predator presence (0.2 - 0.4) were sustained.

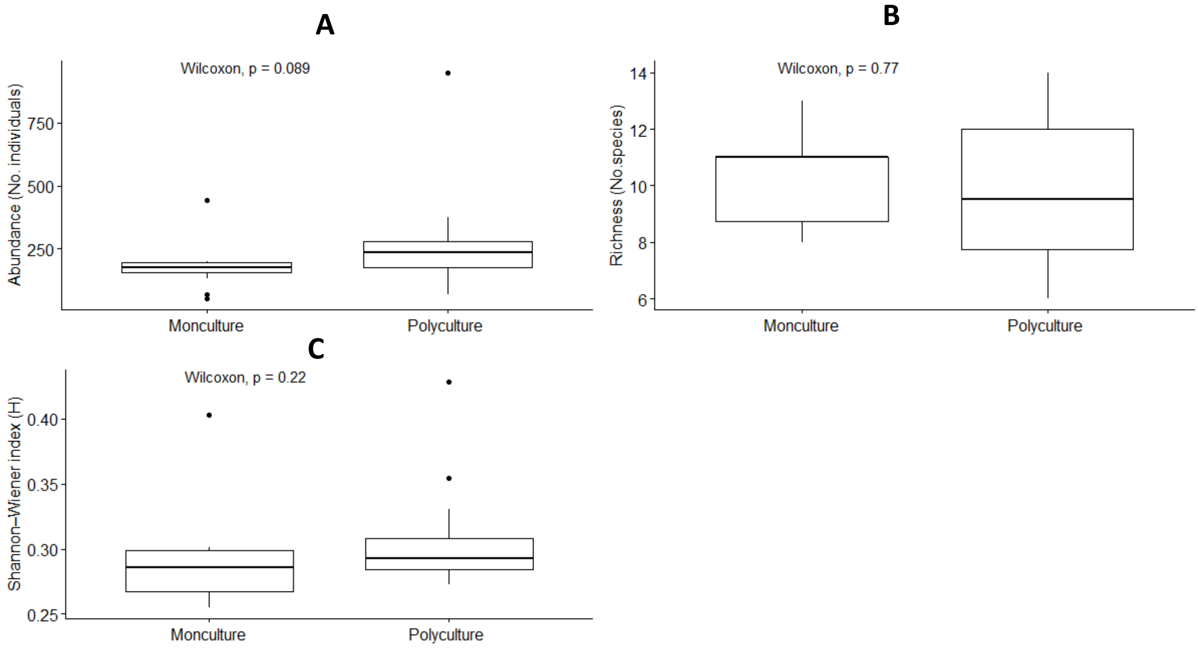

Figure 6.6: Macroinvertebrate community abundance (A), richness (B) and diversity as measured by the Shannon-Wiener index (C), associated with the roots of monoculture versus polyculture for year 2.

Table 6.3: Mean values of indices for each calculated macroinvertebrate index for monoculture, polyculture FTWs, and both combined.

	Mean Habitat stability index	**Mean Shedder index**	**Mean Filtering collector index**	**Mean Top-down predator index**
Monoculture	0.19	8.36	0.23	0.42
Polyculture	0.21	9.95	0.24	0.44
All Communities	**0.20**	**9.16**	**0.24**	**0.43**

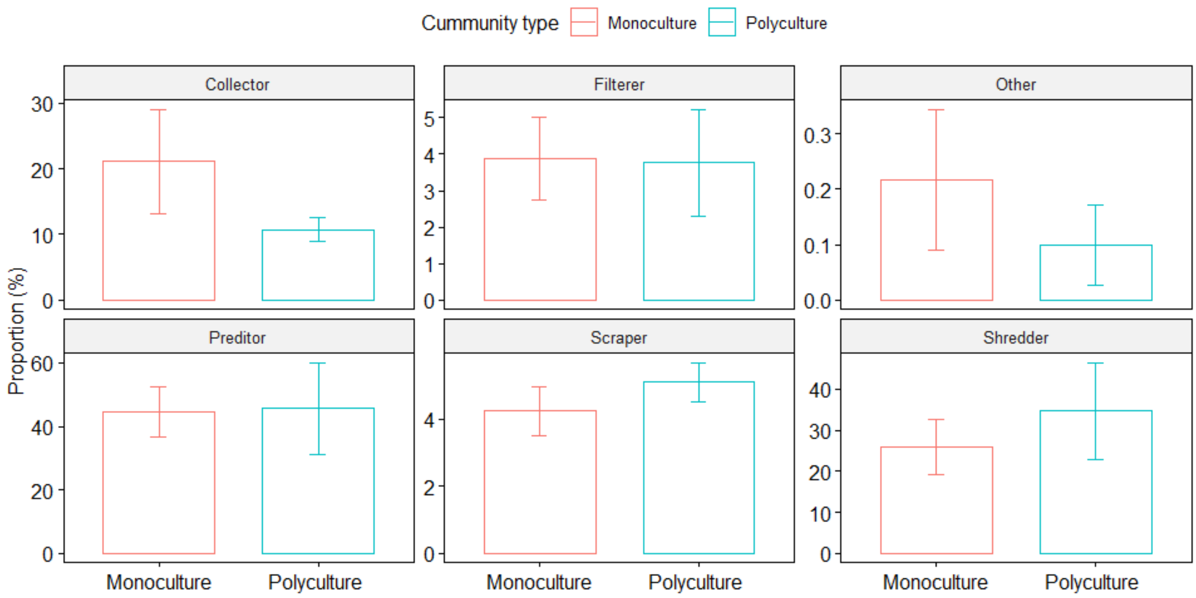

Figure 6.7: Proportional representation of macroinvertebrate functional feeding groups collected from monoculture and polyculture FTWs

6.3.3 Tissue concentration and standing stocks

In year 2, biomass was not significantly different between the monoculture and polyculture FTWs (Figure 6.8). However, in year 3 the biomass from the polyculture FTWs was significantly lower. Polyculture FTWs had higher tissue concentrations of Ca, Cr, Mg and Mn in year 2, although there were no differences in N and P concentrations between the two community types (Figure 6.9). In terms of standing stock, the only element which was significantly different was Cu, where it was higher in the biomass of the monoculture FTW communities.

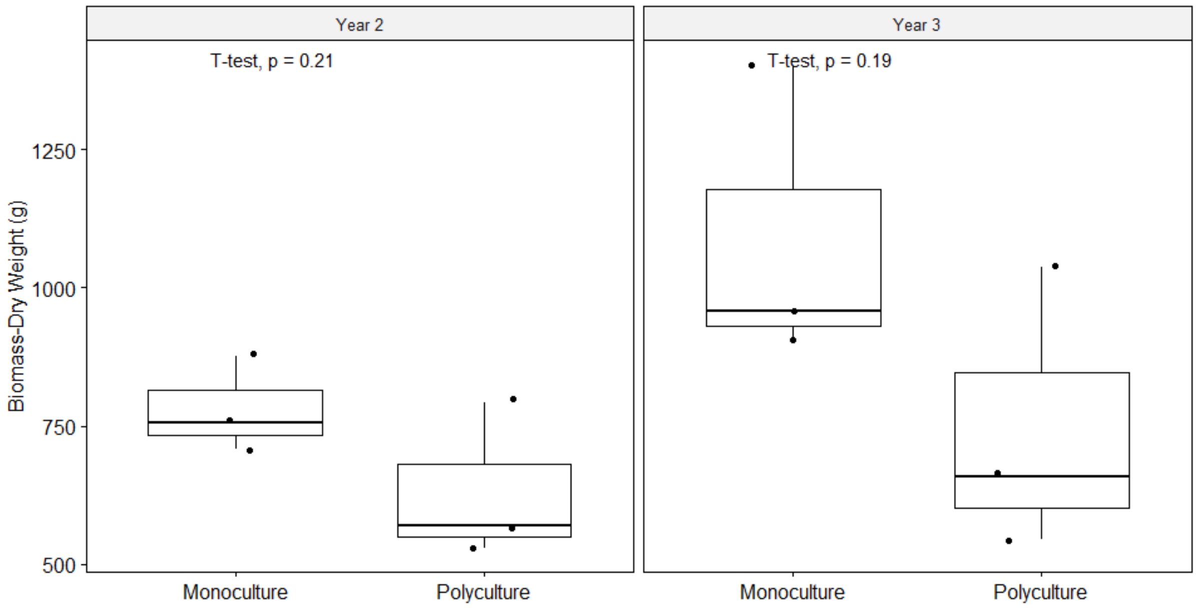

Figure 6.8: Plant community dry weight biomass for monoculture versus polyculture FTW communities for year 2 and year 3

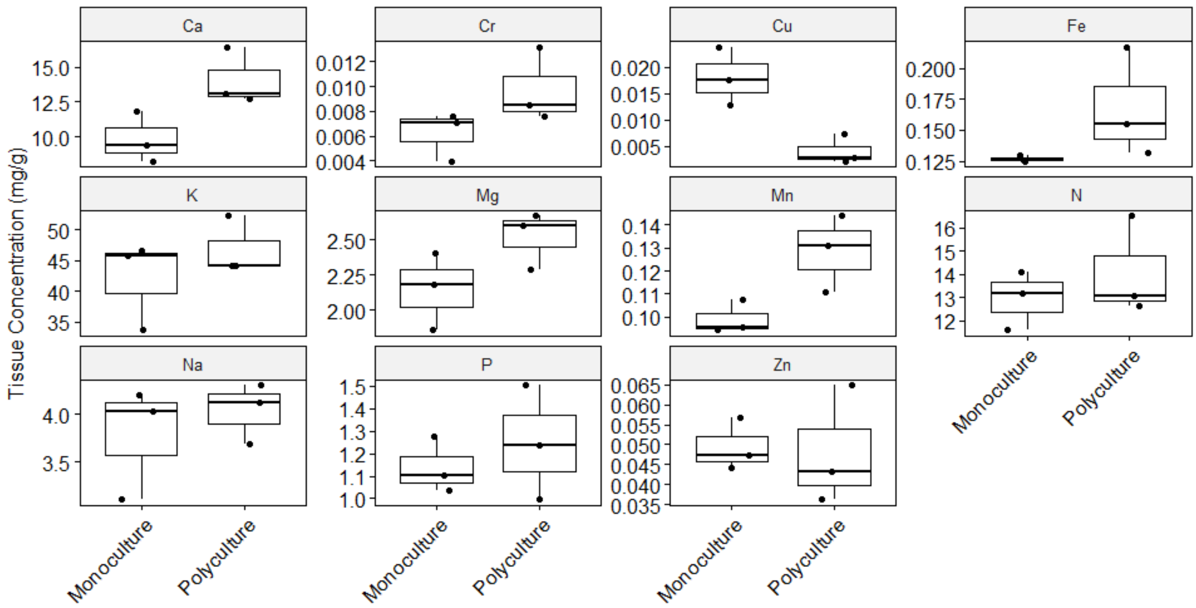

Figure 6.9: Plant community above-ground tissue concentration per element for monoculture versus polyculture FTW for year 2.

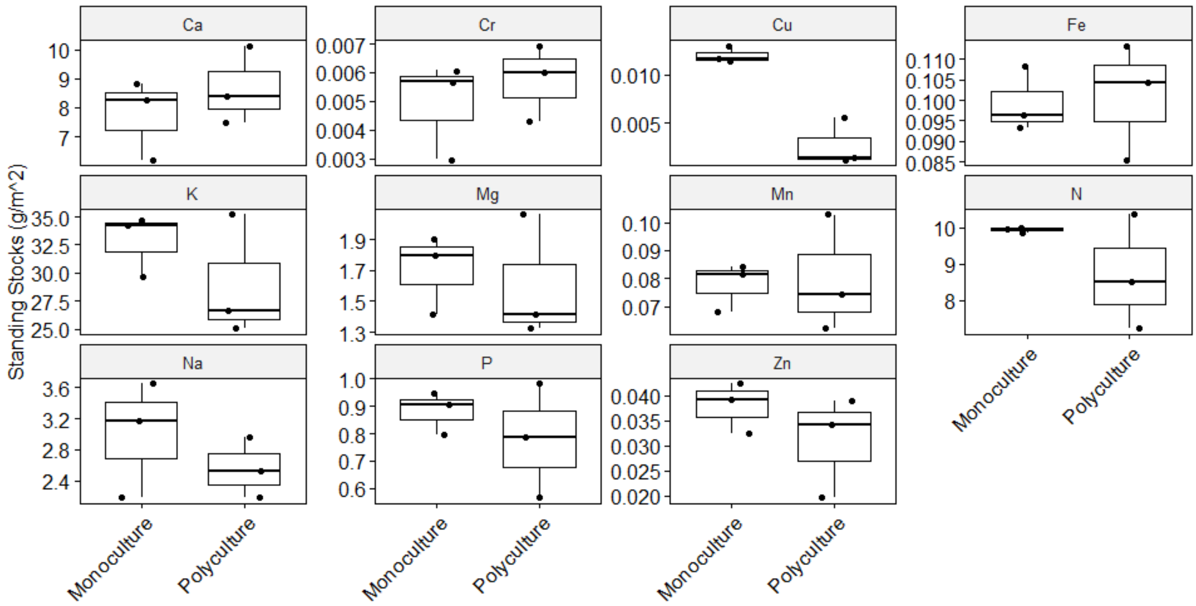

Figure 6.10: Plant community median stand stock storage per element for monoculture versus polyculture FTW for year 2.

6.4 Discussion

6.4.1 Plant community succession

Plant community secession is an inevitable feature of natural systems, and this study is the first to report on multi-annual changes in plant community composition in FTW following their deployment in the field. The progression in community composition of both plant community types away from their original assemblages demonstrates the importance of considering 'self-design' in utilising FTWs. However, the passive management strategy used in this study and the resultant compositional changes, highlights the risk of deploying FTWs with set expectations in the performance of these systems for pollutant removal and/or ecosystem service provision. This is because performance is often tied to the service provision of certain plant species of specific community assembles that has been derived from previous FTW studies (Williams, 2010; Yuan and Huang, 2010). Arresting succession through active management, such as removing selective species, would protect the original assemblage but increase the expense of time and labour cost (Thrippleton et al., 2018); although could be offset by additional benefit of resource recovery potential (Williams, 2010). The change in plant succession and impact on performance in phytoremediation systems, particularly FTWs, is a knowledge-gap, and given these results, suggests that within the time frame of two full years there is considerable deviation from the original planted communities. This is important to consider given the increasing use of these FTW systems as tools for restoration of pollutant removal.

The increase in similarity between the communities with *Lycopus europaeus*, *Mentha aquatica* and *Lythrum salicaria* becoming prominent in both FTW community types suggests a convergence in community composition was taking place (Matthews and Spyreas, 2010). Given that both communities were situated in the same environment with the same pressures, convergence or stability in community composition were the most likely outcomes. The loss of stress-tolerant species and increase of ruderal types of species, including *Lycopus europaeus* and *Mentha aquatica*, suggests that FTWs can be disturbance-prone habitats. This concurs with observations of FTW physically moving around and how exposed the FTWs were to wave action. While site specific conditions can vary, it is reasonable to suggest that in cooler and windier conditions, such as those in high latitude countries, FTWs may be more disturbance prone and therefore ruderals that are better adapted as macrophyte communities.

The prevalence of some species in both types of FTWs may have resulted from cross-colonisation (e.g., *Myosotis scorpioides* from the polyculture FTW), or may have been from marginal vegetation, for example, *Lycopus europaeus* and *Epilobium hirsutum* were present on the banks of the site and likely colonised the FTWs from here. Therefore, FTWs, like any other ecosystem can be sinks for colonising-plants and may eventually subsume surrounding appropriately adapted plant species. The opposite scenario may also occur, when FTWs are installed in areas of bare vegetation, e.g., newly constructed stormwater ponds, they may act as sources of propagules. Using FTWs as a source and facilitator of plant vegetation is an interesting concept and this type of approach with terrestrial vegetation has been found to be useful in unvegetated environments (Munford and Al, 2021). The concept of FTW as both sources and sinks in ecosystem restoration is unexplored, but the results highlight additional applied benefits and risks associated with FTWs. For example, if the surrounding environment contains diverse macrophytes then it may be possible to plant the FTWs with a single species (or just planting with substrate) to commence the colonisation process, rather than planting diversity initially, which could potential reduce both effort and costs. However, initially planting with a diverse community may ultimately led to increased diversity later in other wetland systems (Williams and Ahn, 2015). Although in this study, the decreases in diversity and species number suggested that in FTW there are limits to the number of species that can be supported in these engineered systems.

While the above discussion on community dynamics has primarily focused on the potential risk to restoration and ecosystem service provision provided by FTWs, there are also possible benefits of 'self-designed' systems, e.g., the increase of existing habitats, or the provision of desirable ecosystem services associated with the developed community. It is important for environmental managers to be aware that plant succession, particularly towards ruderal types may influence the outcomes of projects employing FTWs.

6.4.2 Macroinvertebrate communities and habitat provision by FTWs

In year 2 of the study, although there were differences in the macrophyte composition between the two types of FTWs, the macroinvertebrate communities remained similar in all key variables including diversity, abundance, key species, and functional feeding groups. While there is debate in the literature about the importance of macrophyte species richness on individual indicators including number of invertebrate taxa, abundance and diversity, most studies demonstrate that increases in macrophyte diversity and/or structural diversity leads to significant differences in at least one of these metrics (Gallardo et al., 2017; Hansen et al., 2010; Yofukuji et al., 2021). It is possible that plant root structure for each community type was not significantly different to drive differences in invertebrate communities, despite polycultures having a visually denser root network (often due to the higher density of *Mentha aquatica*) compared to the *Phragmites* dominated monocultures.

The ratios of invertebrate functional feeding groups help construct a picture of the bio-physical environment which is available to (and potentially limits) the studied macroinvertebrate communities. The low habitat stability index, with a predominant prevalence of shredders and gatherers consuming entrained fine particulate organic matter, is indicative of a habitat that is unstable and unsuitable for organisms that require a firm and calm substrate (e.g., filterers and scrapers). The large mean shedder index supports this indicating that course particular organic matter is more widely available (e.g., from decaying plant matter) rather than fine particulate organic matter that would settle in more calm and undisturbed areas. A positive design feature of FTWs is that they move with the water level and current, but this is the first study to find an impact of the habitat type provided by the FTWs on associated invertebrate communities. Compared with the study of rooted, floating, and emergent macrophytes by Yofukuji et al. (2021), the similar levels of plant diversity in this study does not lead to similarly high levels of diversity, richness and abundance of invertebrates. The instability and stress of the habitat provided for macroinvertebrates by FTWs may be the key limitation on abundance, richness, and diversity in these systems, overriding any effects driven by differences driven by macrophyte community composition. However, it is noteworthy that macroinvertebrate predators, as a functional feeding group, were well represented suggesting that there was a sufficient turnover of prey. It is possible these organisms were supported by an ample supply of course particulate organic matter from the dense macrophyte roots and turnover of aboveground parts. Furthermore, a higher proportion of macroinvertebrate predators suggests that predation by higher order carnivores such as fish was not a significant environmental pressure in this system.

While it is likely that the habitat provided by the roots of FTWs is different and possibly less stable than other freshwater habitats the evidence above does demonstrate that a FTW can provide good habitat for certain macroinvertebrate communities. As FTWs can be strategically positioned it would be possible to create and expand habitats across freshwaters, especially in recently constructed water bodies. Given the expansion of blue-green spaces there are clear opportunities for using FTWs to assist the establishment of macroinvertebrate communities and enhance the associated ecosystem, e.g., by creating habitat corridors and supporting higher order predators such as fish (Urban, 2017; Hassall et al., 2011; Hyseni et al., 2021).

6.4.3 Resource recovery

In year 2 of the experiment, despite some key differences remaining in the macrophyte community composition between the two types of FTWs, such as diversity and a greater proportion of *Phragmites australis*, few differences in the tissue concentrations of pollutants were found. The convergence in composition of the two community types - with several common macrophyte species between them - means that it is probable that for most of the pollutants studied increased the likelihood of similar tissue nutrient concentrations in either community. The differences in the proportion of some plant species between the communities may have led to some of the differences between the monoculture and polyculture FTWs for Ca and Mn concentrations. The higher concentration of Ca in the above-ground plant biomass of the polyculture FTWs may be because although Ca is required to build structural tissue, it is lower in monocots than dicots due to their low concentration of cell wall pectate (Hawkesford et al., 2011). The polyculture FTW contained more dicotyledonous species compared to the monoculture FTW because *Phragmites australis* (a monocot) remained a large component of the community (almost 40 %). There is also evidence to suggests that dicotyledonous species possess more effective Mn transporter proteins for transporting this pollutant to aboveground tissue (Alejandro et al., 2020), potentially explaining the pattern of increased Mn tissue concentration in the plants growing in the polyculture FTWs.

In terms of nutrient standing stocks of key recoverable pollutant such as N and P, there was no difference between the two FTW community types. Given that biomass is likely to be the main driver of pollutant standing stocks for macronutrient-type pollutants (Chapter 3) ,it is unsurprising that there was no differences in these pollutants as the biomass between the two community types was similar. Higher standing stocks of Cu in the monoculture FTW containing *Phragmites australis* as a large community component affirms previous work (Printz et al., 2016, Chapter 5), that suggests that this species may have an enhanced ability for Cu sequestration and translocation to aboveground tissues.

There are only a small number of published field-scale studies of the performance of FTWs and the results compare both favourably and unfavourably with these for standing stocks (Karstens et al., 2021; Olguín et al., 2017). Of comparable pollutants N and P, standing stocks are similar to Karstens et al. (2021), whilst the N standing stocks of the FTWs in this study were lower than Olguín et al. (2017) although P was substantially higher. Differences in climate (tropical versus temperate), community compostion, and variable water chemistry of available studies makes an overall apriasal of standing stocks in scale FTWs challangeing. A more useful evaluation is to compare FTWs with existing stands of plant communities with similar background environmental variables. By comparing the results from this study to a survey of existing wild plant stands in proximity to our study site we found broad similarity in standing stocks of most pollutants at communities with a similar biomass range of 500-1000g/m^2 (Chapter 3). However, some stands of wild macrophytes had a much higher standing stocks when comparing communities suggesting that macrophytes rooted in sediment may be able to achieve a higher above ground biomass. Nevertheless, harvesting FTWs can allow nutrients to be exported from freshwaters but the cost-benefit of an active management regime may depend on multiple site-specific factors such as harvesting costs, proximity to biomass reuse location (transportation), biomass quantity related to FTW coverage and specific plant communities (Quilliam et al., 2015).

6.5 Conclusion

Floating treatment wetlands (FTWs) are novel ecological engineering systems that can assist the remediation of polluted waters, and when used at scale can provide ecosystem services in the form of habitat provision and resource recovery. Through a field-scale experiment it was found that plant community composition in passively managed FTWs can change substantially, and may impact ecosystem service provision. The two initially different plant community types in the polyculture and monoculture FTWs converged in similarity in their community composition and macrophyte diversity. There were few substantial differences between both FTW communities for habitat provision for macroinvertebrates and diversity suggesting root morphology did not play a significant role in shaping macroinvertebrate communities. Calculated macroinvertebrate indices suggested that habitat provided by FTWs was unstable without the adequate provision of fine particulate organic matter, which may have been the main limiting factor over root morphologies. However, a high incidence of predators means that FTWs can support macroinvertebrate communities suggesting these systems can also be used to increase habitat within freshwaters. Resource recovery between the two FTW communities in terms of nutrient standing stocks were also similar although compared favourably to similar naturally wild growing plant communities. Therefore, depending on the cost-benefit scenario, FTWs in practice can be used to export nutrients from eutrophic waters. With existing extensive laboratory and controlled mesocosm experiments, this timely field-scale study has demonstrated that ‘self-design’ is an important practical factor to consider when employing these nature-based solutions in freshwater restoration projects. In the absence of many field-scale FTWs studies on the possible added value in ecosystem service provision, this work provides new insights to help inform freshwater management decisions.

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7. Synthesis and key findings

7.1 Introduction

Nature based solutions (NbS) are increasingly employed to mitigate the impacts of human activities due to their perceived (and evidenced) effectiveness and ability to generate multiple benefits for nature and society (Raymond et al., 2017). There are existing and emerging policy strategies at national and international level advocating, supporting and developing NbS as they interface with multiple priorities including biodiversity and human well-being (European Commission, 2022; Liquete et al., 2016). Phytoremediation has not received the same level of attention compared with other NbS strategies, such as peatland restoration, reafforestation, or wetland restoration. This may be because research has generally focused on single species for targeting remediation of single pollutants, together with a lack of demonstrable field studies (Chapter 2). There are clear opportunities to explore the use of aquatic phytoremediation as a fully multi-functional NbS that can tackle water quality issues, including diffuse pollution with multiple pollutants, while simultaneously enhancing freshwater habitats and contributing to circular economy approaches.

The focus of this thesis has been on optimising a series of strategies that can improve water quality and freshwater ecosystems by exploiting the ability of aquatic plants to assimilate waterborne pollutants and simultaneously provide ecosystem services. By focusing research effort on ecologically engineered phytoremediation strategies for multifunctionality, it has provided new approaches and insights for practitioners, researchers, and policy makers so that aquatic phytoremediation can become a more widespread and utilised NbS. The research in this thesis builds upon existing phytoremediation studies and focuses on the relatively unexplored ecological dimension of community composition through the lens of two different plant growth strategies, and with experimentally different approaches (mesocosm and field trails, and a natural vegetation survey). The research outputs provide practical and fundamental information for planning and designing phytoremediation interventions, validate the provision of added-value ecosystem services, and help to understand how these services can contribute to better environmental management decision-making. The findings address the research priorities for aquatic phytoremediation identified in Chapter 1, which were to (1) develop and optimise novel aquatic phytoremediation strategies to maximise waterborne pollutant removal; (2) explore how macrophyte community assembly influences the removal of waterborne pollutants and provision of ecosystem services; (3) quantify the ecosystem service provision of different phytoremediation strategies, particularly those that have been unexplored within existing literature. Table 7.1 summarises the research findings that map onto these aims and directions for future research. The following section discusses the scientific implications of the key findings and identifies future research needs and policy opportunities.

7.2 Develop and optimise novel aquatic phytoremediation strategies to maximise pollutant removal

The three main phytoremediation options identified in Chapter 2, i.e., constructed wetlands, floating treatment wetlands (FTWs) and wild macrophyte harvesting, are NbS for remediating polluted water. By exploring the use of FTWs and wild macrophyte harvesting strategies this research has identified that phytoremediation is effective for a range of waterborne inorganic pollutants. Understanding the mass balance of pollutants within these strategies has demonstrated that plant uptake and sequestration directly from the water column is generally low, apart from a small number of inorganic pollutants including N (Chapter 5). Therefore, harvesting plants does not necessarily enhance water quality (Borne et al., 2013; Garcia Chanc et al., 2019). For optimal phytoremediation the choice of strategy and system should be designed around target pollutants and specific plant traits, e.g., root growth, structure, and plant stature which are all important for process optimisation (Chapters 4 and 5). Additionally, how pollutants are delivered to receiving waters, and if there are pollutant reservoirs in sediments should also inform strategy choice: constructed wetlands are most appropriate for point-source pollution, whereas wild macrophyte harvesting and floating treatment wetlands can be more easily adapted for diffuse pollution (Chapter 2). For removal of legacy pollution, wild macrophyte harvesting has potential because rooted emergent, floating and submergent macrophyte communities can access pollutant reservoirs in sediments (Eichert and Fernández, 2011; Chapter 3). Floating treatment wetlands with hydroponic root growth can intercept dissolved and particulate-bound pollutants in the water column so are most effective for exporting active diffuse pollutants, or where there is re-suspension of pollutants (Chapter 4-5). Importantly, where hydraulic retention time is short, phytoremediation systems may fail to effectively remove

Table 7.1: Summary of thesis research priorities, outcomes, and future work and practical/policy implications

Research priorities	Outcomes	Future work and practical/policy implications
1. Develop and optimise novel aquatic phytoremediation strategies to maximise waterborne pollutant removal	**Chapter 3**: Resource recovery of both macronutrient and micronutrient-type pollutants in wild macrophyte stands can be optimised using identifiable plant growth strategies, based on the Grime's CSR framework. **Chapter 3**: Harvest regime is critical, and the timings and frequency of harvest has important implications for the recovery of pollutants. For example, ruderal species are generally smaller in stature but due to high tissue concentrations and rapid regrowth, greater amounts of nutrients can be exported in the long term. **Chapter 3**: Standing stocks of macronutrient-type pollutants (e.g., N and P) are related to plant biomass (i.e., better to target larger plants for phytoremediation). Standing stocks of micronutrient-type pollutants (e.g., Cu and Zn) are more related to tissue concentrations which means hyperaccumulator species are more effective than plants that can simply accumulate high biomass. **Chapter 5**: Mechanisms for pollutant uptake in floating treatment wetlands varies depending on pollutant type. Root structure and rhizosphere interactions are related more to removal of Cu, Mg, K, P, Zn. Plant uptake more likely has an effect for TIN, Fe and Cu. **Chapter 5**: Plant uptake generally is not the main driver for removing waterborne pollutants.	<u>Scientific</u> Research into the optimal time to harvest above-ground plant tissue would help maximise nutrient recovery. A pan-regional approach to studying aquatic phytoremediation, similar to existing approaches employed for terrestrial phytoremediation development. Knowledge exchange programmes could build relationships between stakeholders to promote integrated resource management and help support phytoextraction potential. <u>Practical/Policy</u> In the immediate term, utilising macrophyte biomass close to its source is strongly advocated to be used as either a fertiliser or soil conditioner Co-ordinated inter sectorial development of infrastructure to support resource recovery from phytoremediation and reduce cost of transporting material. For example, use of biomass in biorefineries and anaerobic digestion plants.

	Chapter 5: Macrophyte harvesting may be better recognised as a mechanism for removal of pollutants in wild stands where root growth is in sediments to provides a circular economy route. Conversely, harvesting floating treatments may not be cost beneficial. **Chapter 5**: Temporal variation in both hydraulic retention times and season influences the removal efficiencies of some pollutants.	
2. Explore how macrophyte community assembly influences the removal of waterborne pollutants and provision of ecosystem services	**Chapter 3**: Ruderal species have higher tissue concentrations of inorganic pollutants than macrophytes belonging to other growth strategies **Chapter 4**: A comparative ecology approach showed differences in multi-ecosystem service provision along the axis of plant stature. Small emergent macrophytes outperformed large, and mixed statured, communities. Large emergent communities had higher multi-functionality levels only when specific function performances were lower. **Chapter 5**: Community structure is likely a moderator for pollutant removal – proportionality of species or traits within a community mainly determine effectiveness (in line with the mass ratio hypothesis). Communities can be structed to remove multiple pollutants, but monocultures remain more effective at targeting single pollutants **Chapter 6**: Plant communities can rapidly change in composition on floating treatment wetlands, which means that associated ecosystem services may also be impacted	<u>Scientific</u> Understanding how ecosystem service provision in phytoremediation changes with changes in community composition urgently requires attention for sound long term management of these systems Testing the community combinations studied in different pollutant concentrations would help adapt the approach to different water types. <u>Practical/Policy</u> There is potential for floating treatment wetlands to act as novel ecosystems and be included as part of local biodiversity action plans, with potential to include rare species

		Practitioners should be mindful of plant community shifts and the impacts of this on the dynamics of associated ecosystem service provision. Maximising ecosystem multifunctionally at a high-performance level means that environmental managers should also consider trade-offs that might occur with other services when designing communities for maximum ecosystem service provision or multi-targeted pollutant removal.
3. Quantify the ecosystem service provision of different phytoremediation strategies, particularly those that have been unexplored within existing literature	**Chapter 4**: Large emergent macrophytes are better at specific functions such as provision services. It is possible to achieve multi-functionality but at different thresholds and quality. There is a decline in multi-functionality as the threshold increases. **Chapter 6**: Floating treatment wetlands can provide good quality habitat for macroinvertebrates, but this is not affected by community composition. **Chapter 4**: Phytoremediation can also provide cultural and regulating ecosystem services e.g., pollination. **Chapter 6:** Resource recovery did not differ between two different plant communities.	<u>Scientific</u> Assess the trade-offs between different ecosystem services using a framework approach to understand impact on ecosystem multi-functionality. Carry out ecosystem valuation of the range of services provided by phytoremediation to assist policy development. GIS mapping could help identify appropriate phytoremediation installation locations and economically scale-up aquatic phytoremediation. <u>Practical/Policy</u> If valued, phytoremediation could be included in biodiversity net gain metrics where value is assigned and used to create or enhance habitat on or off the site of new developments including production sites

		(particularly those with pollutant emissions) and new housing. Ecosystem valuation approaches combined with GIS mapping could help identify appropriate phytoremediation installation locations and economically scale-up aquatic phytoremediation. Trade-offs between plant biomass harvesting and the reduction in other ecosystem services must be considered e.g., reduced water treatment vs. habitat availability.

waterborne pollutants (Colares et al., 2020; Pavlineri et al., 2017). In contrast to constructed wetlands, the scientific basis for the appropriate scale needed for effective FTWs and wild harvesting is lacking, and there is an urgent need for large field-scale studies to adequately address this knowledge gap. A pan-regional approach might be appropriate where catchments with similar physiochemical characteristics are studied with the same phytoremediation interventions at different scales (PhytoSUDOE, 2008).

Subsequent harvesting and export of macrophytes may not contribute significantly to pollutant removal in the water column but it does remain an important pathway for the permanent removal of pollutants from freshwater systems (Zhang et al., 2007), particularly in wild harvested planting systems (Chapter 3). Targeting macronutrient-type pollutants and some micronutrient-type pollutants including Cu, Zn and Mn from water receiving multi-diffuse pollutants shows the most promise and viability. Therefore, realistic aquatic phytoremediation of multi-polluted waters would likely focus on key macronutrients including N and P, and the micronutrient-type pollutants such as Fe, Cu, Zn, Mn (Chapters 3-6). It was determined that pollutant standing stocks were higher in larger plants, particularly of macronutrient-type pollutants such as P and N (Chapter 3 and 4). However, the harvest strategy employed has a considerable impact on the resource recovery and optimisation of phytoremediation for pollutant removal for all planting systems (Verhofstad et al., 2017), so smaller statured plants (e.g. ruderals) with rapid nutrient acquisition and high tissue concentrations can have greater gains if managed appropriately (Chapter 3). Whilst harvesting macrophytes on FTWs for resource recovery can be equally effective in terms of pollutant export as harvesting wild growing or seeded macrophytes, when compared with communities with a similar biomass, the effort of harvesting FTWs platforms may not be cost effective (Pavlineri et al., 2017). Furthermore, trade-offs between harvesting and reduction in other ecosystem services must also be considered e.g., reduced water treatment or habitat availability (Habib and AR, 2016). Generally, it was found that pollutant standing stocks were more likely to be stored in greater quantities in below-ground plant parts (Chapters 4-6). This makes resource recovery from root and rhizome compartments much more challenging and potentially disruptive to plant communities and ecosystems (Soana et al., 2018). Therefore, focusing on maximising above-ground stores should be the focus both in practical terms, and in research effort for understanding translocation of pollutants above-ground.

This work was focused on a suite of diffuse pollutants which limits its application to predominantly point source-impacted freshwaters where single pollutant concentrations are significantly higher. However, regulation, better water governance and best practices for land management continue to be key mechanisms for the control of point source pollution, and so the main and future challenges will involve managing diffuse pollution sources (Ji et al., 2022). The planting of or targeting hyperaccumulators for removal of metalloid pollutants can aid removal and recovery of pollutants; importantly, in freshwater systems these plants are often be considered as invasive (Chapter 2). Therefore, developments in enhancing phytoremediation potential of native plants, such as enhancing uptake and removal using biostimulation (e.g., microbial inoculation of plant roots), or genetic selection, could provide novel and more sustainable routes in which to enhance phytoremediation (Ali et al., 2020; Tondera et al., 2021). However, to realise the potential of phytoremediation as a scalable, widespread and integrated nature-based solution, less technically-dependent enhancements such as community selection will probably be more effective.

Transporting and processing biomass post-harvest can be costly and logistically challenging if appropriate facilities or end product-use are not co-located. Either infrastructure or investment in facilities for processing (e.g., existing waste streams for anaerobic digestion) need to be established and developed (Quilliam et al., 2015). In the immediate term, utilising macrophyte biomass close to the source is strongly advocated, for example as either fertiliser or soil conditioner (Stabenau et al., 2018). The developments presented in this thesis support advances in circular economy approaches within nutrient-impacted freshwaters, particularly within rural areas. Knowledge-exchange programmes to build relationships between stakeholders for integrated resource management (potentially tied to existing integrated catchment management approaches) of macrophytes would be useful. For example, in the UK a useful project would be to bring canal authorities and farmers together to remove excessive macrophyte growth and provide organic-based materials for soil amendment.

7.3 Explore how macrophyte community assembly influences the removal of waterborne pollutants and provision of ecosystem services.

Community ecology is a relatively unexplored theme within aquatic phytoremediation, although data from this thesis has found this to be an important consideration for pollutant removal and ecosystem service provision. While the small number of available studies demonstrate improvements in function through the lens of plant species diversity (Ge et al. 2015; Geng et al. 2017), the present work has developed a framework based around comparative ecology and structuring communities based on species and trait identity (Chapters 3-5). This framework is centred on (1) plant growth strategies and understanding opportunities for optimised harvest strategies (Chapter 3); (2) comparative ecology using basic plant traits to assemble communities and to explore use of phytoremediators elsewhere in the world (Chapters 4-6); and (3) appropriate representation of species or traits (i.e., the mass ratio hypothesis) to achieve desired ecosystem service provision. For example, through an analysis of CSR plant growth strategies in wild growing stands effective harvest regimes could be optimised by changing harvest frequencies depending on the growth strategy (Chapter 3). Structuring plant communities by species and trait attributes (following Grimes mass ratio hypothesis (Mokany et al., 2008)) appears to be the most effective way of maximising multi-functional phytoremediation, as there were rarely gains in functioning simply by increasing richness (Chapter 4 and 5).

This thesis has developed a novel approach to understanding ecosystem service provision in aquatic phytoremediation: using plant stature as a plant trait axis can determine multi-functionality and therefore the number and quantity of services that can be provided (Chapter 4). Plant community structure is also a key moderating factor for removing pollutants with there being direct proportionality between the plant species (Chapter 5) or plant trait types (Chapter 4) and the removal of specific pollutants. It was possible to target multiple pollutants by structuring communities with different species, but removal efficiency of the specific pollutants was reduced as a function of the abundance of the associated phytoremediator. Ultimately, monocultures were most effective at removing a single pollutant. The advantages of assembling more diverse plant communities include supporting a greater number of ecosystem services (Chapter 4, Chapter 6); however, stakeholders employing this approach need to be aware that specific pollutant removal might be less effective (and require additional management solutions) or take longer.

Temporal variation in community structure can occur naturally or be induced via the macrophyte harvesting process (Chapters 3 and 6). Harvesting is a disturbance and may impact the community structure, perhaps more so on FTW planting systems given they have been established as potentially disturbance and stress prone environments for organisms (plants and macroinvertebrates) that have colonised them (Chapter 6). This is more likely to benefit ruderal species (Chapters 3 and 6), which over time may result in community shifts towards species that have shorter life cycles, higher turnover and lower biomass, thus increasing the necessity to harvest more frequently for phytoextraction. The associated changes in ecosystem service provision with changes in community composition requires attention for the long-term management and understanding of phytoremediation systems (Chapter 6). There is also a need to test these theories in different environments and with different plant species, with the potential for FTWs to act as novel 'ecosystems' to be included as part of local biodiversity action plans when appropriate.

7.4 Quantify the ecosystem service provision of different phytoremediation strategies, particularly those that have been unexplored within existing literature.

This thesis sought to establish phytoremediation as a multi-functional tool to support freshwater improvement focusing on a range of services over and above pollutant removal. Natural wetlands have received much attention in terms of their ecosystem service value (Mitsch et al., 2015; Tondera et al., 2021), and our understanding of these systems can inform the potential for added value of phytoremediation approaches. Chapter 2 underpinned the need to quantify additional ecosystem services to understand how the management of phytoremediation systems can impact on ecosystem service delivery. By applying the ecosystem service framework to this approach, it was possible to expand the multiple benefit understanding of phytoremediation in terms of plant community trait diversity and phytoremediation using scenario development (Chapter 4).

The provision services provided by phytoremediation include the production of plant biomass and resource recovery. These are highly dependent on factors such as plant community composition (Chapters 3, 5 and 6) and harvest regime (Chapter 3). Regulation and maintenance services include water treatment, provision of anchorage and flow interruption (depending on plant system). In addition, if plant species are appropriately chosen phytoremediation installations can support pollinators and provide aesthetic value (Chapter 4). FTW also have capacity to provide habitat for waterfowl, and macroinvertebrate communities that are adapted to more disturbance prone environments (Chapter 6), although this does not appear to depend on community type or diversity. While not explicitly measured in person-perception terms, anecdotally these systems can also provide cultural value through improved aesthetic and educational value.

This research presented here is the first to establish a framework for phytoremediation as a multifunctional ecosystem. This was achieved by developing a scenario-based approach linked to goals for phytoremediation where it was found that increasing multifunctionality normally means that the strength of individual service provision decreases (Chapter 4). This has implications for the design and understanding of phytoremediation for the provision of multiple services. The scenario modelling is an approach that could be further improved by including additional services, e.g., habitat provision and importantly, trade-offs between different services. For example, harvesting my have negative impacts on the provision of other services and such an understanding would help practitioners make decisions on levels of ecosystem service provision (Soana et al., 2019).

The ‘added value’ in ecosystem services provided by aquatic phytoremediation makes it an important tool for enhancing impacted freshwaters. For it to be more widely adopted, ecosystem services need to be valued and scaled-up to the catchment level to allow decision makers to understand the cost-benefits of this NbS. Ecosystem valuation approaches combined with GIS mapping could help identify appropriate phytoremediation installation locations and inform the economic viability of scaling-up. There are also relevant policy avenues in which aquatic phytoremediation could play a role. For example, it could be included in biodiversity net gain metrics where value is assigned and used to create or enhance habitat on or off site of new developments such as production sites (e.g. factories) or housing (Defra and Natural England, 2021; Scottish Government, 2021). Given the deployment flexibility of FTWs there are opportunities for supporting more sustainable approaches to new developments. Furthermore, when phytoremediation projects are undertaken in partnership with communities with engagement strategies there is also the opportunity to gain collateral benefits such as raising awareness of environmental issues and increasing inclusion in community activities (Conte et al., 2020; Riley et al., 2021).

7.5 Conclusion

Nature-based solutions have the potential to tackle interlinked environmental issues including multi-diffuse pollution of freshwaters, resource depletion and biodiversity loss. This thesis has examined the potential to optimise aquatic phytoremediation as a multi-functional tool to improve water quality whilst providing ecosystem services to support freshwater enhancement and derive ‘added value’. By examining plant community dynamics across different phytoremediation planting types, new insights have been presented such as harvesting strategies and plant community design. These findings highlight the importance of embedding concepts of plant strategies, community structure and trait identification and the interplay between species into aquatic phytoremediation strategies. These factors can play a moderating role in ecosystem functioning, and by extension ecosystem service provision including pollutant removal and resource recovery. Phytoremediation of pollutants in freshwaters can also provide regulation and cultural services such as habitat provision and pollination. It is advocated that for phytoremediation to become a widely adopted nature-based solution the technology needs to be recognised as multi-functional with the potential to co-deliver.

7.6 References

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Appendix 1

Supplementary information for Chapter 3 'Resource recovery and freshwater ecosystem restoration - prospecting for phytoremediation potential in wild macrophyte stands'

Methods

Selection and tissue concentration and standing stocks of calculation of most common macrophytes

To calculate tissue concentrations on a per species basis the contributing proportion of each component species was multiplied by the overall sample biomass to derive individual biomass measures. The same approach was taken to obtain species individual tissue concentrations. Each species could not be separated from the sample due to parts being intertwined, which necessitated this composite approach. Calculations were made for the five most common species in the survey (i.e., those that appeared five times or more).

Supplementary Results

Table A1.1: list of sites and ordnance survey grid reference

Site	Site number	Water body	OS Map grid reference
Airthrey Loch	1	Lake	NS80580 96481
Arndean	2	Lake	NS 99671 98225
Broom Plantations	3	Pond	NS 99846 96088
Cocksburn reservoir	4	Reservoir	NS 80937 98469
Carse of Lecropt	5	Agricultural ditches	NS 76207 97852
Clackmannan	6	Pond	NS 92232 89579
Devilla Forest	7	Lake and ponds	NS 95644 87262
Dunfermline SUDS pond	8	Ponds	NT12200 86331
Forth and Clyde Canal	9	Canal	NS 80582 79007
Gartmorn Dam	10	Reservoir and ponds	NS 91709 94483
Gogar Loan	11	Agricultural ditches	NS 84249 95180
Howietoun Fishery	12	Ponds	NS 278459 688459
Loch Fitty	13	Lake	NT312621 691344
Linlithgow Loch	14	Lake	NS 99712 77529
North Third	15	Reservoir	NS 76824 88579
Plean Country Park	16	Ponds	NS 83357 86586
Raploch Road Suds ponds	17	Ponds	NS 78598 94108
Stirling Carse	18	Agricultural ditches	NS 76870 95251
Stirling University Pond	19	Pond	NS 80942 96354
Wallace monument	20	Agricultural ditches	NS 81466 95160
Whins of Milton	21	Agricultural ditches	NS 80037 90078

Table A1.2: Physicochemical parameters of waters across the study sites

Property	Mean (±1SD)
Ca (mg/L)	24.85 ± 17.16

Conductivity (µS)	297 ± 297
Cu (µg/L)	2.12 ± 2.09
E. coli (CFU/100ml)	1,380 ± 4760
Fe (µg/L)	330 ± 418
K (mg/L)	2.43 ± 2.1
Mg (mg/L)	6.12 ± 4.87
Mo (µg/L)	0.4307 ± 0.474
Na (mg/L)	9.79± 8.82
NH_3 (µg/L)	62.8 ±114
NO_3^- (µg/L)	336.6±540
NO_2^- (µg/L)	11.2±16
P (<1 µm) (µg/L)	60±83
pH	7.04±0.7
Turbidity (NTU)	49.6±142
Zn (µg/L)	45.6±47

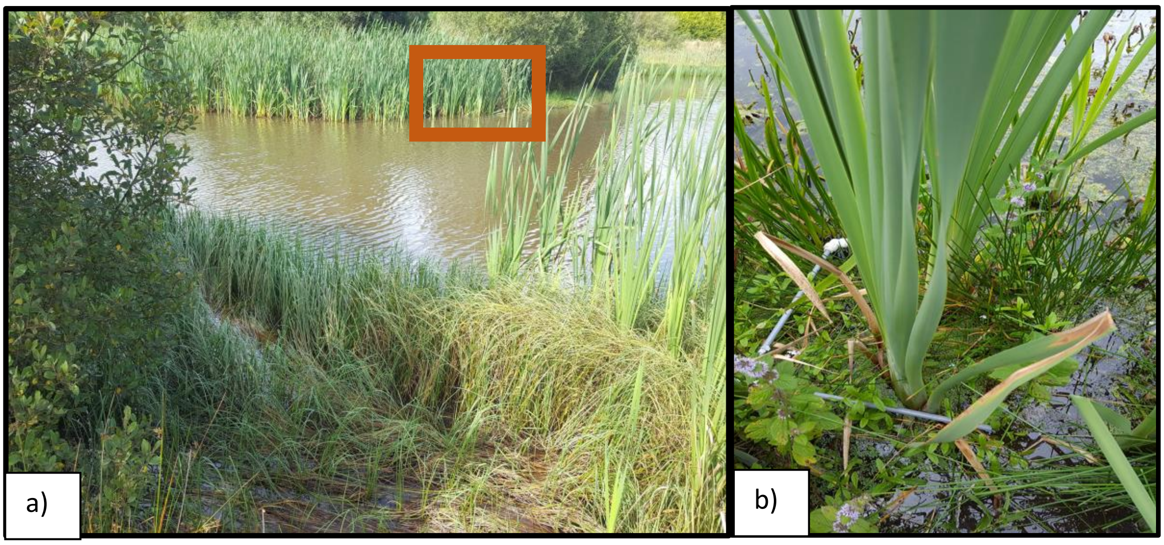


Figure A1.1: Example of survey site, showing (a) macrophyte stand (red box shows sampling location in stand, and (b) macrophytes within a 0.25m^2 floating quadrat

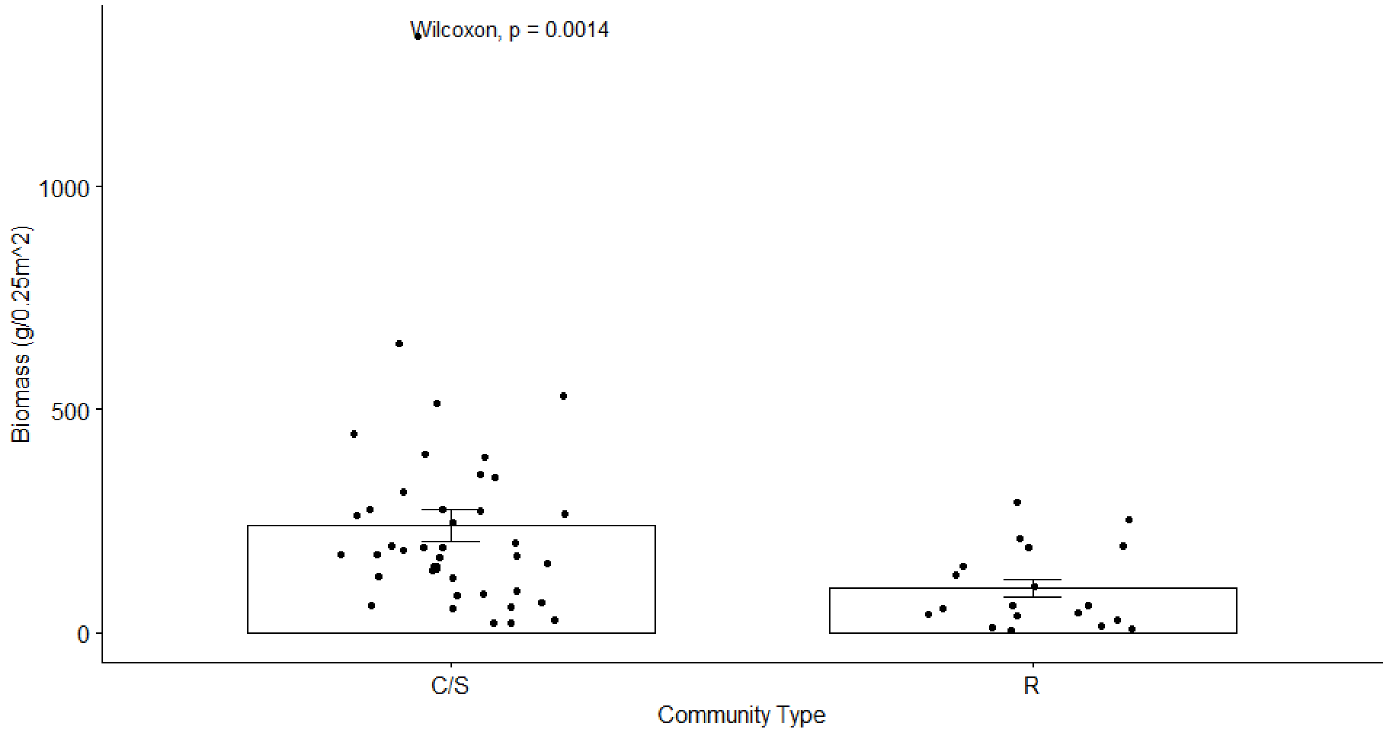


Figure A1.2: Difference in community biomass (g/0.25m^2) between Competitor and Stress-tolerators (C/S) and ruderals (R) type communities.

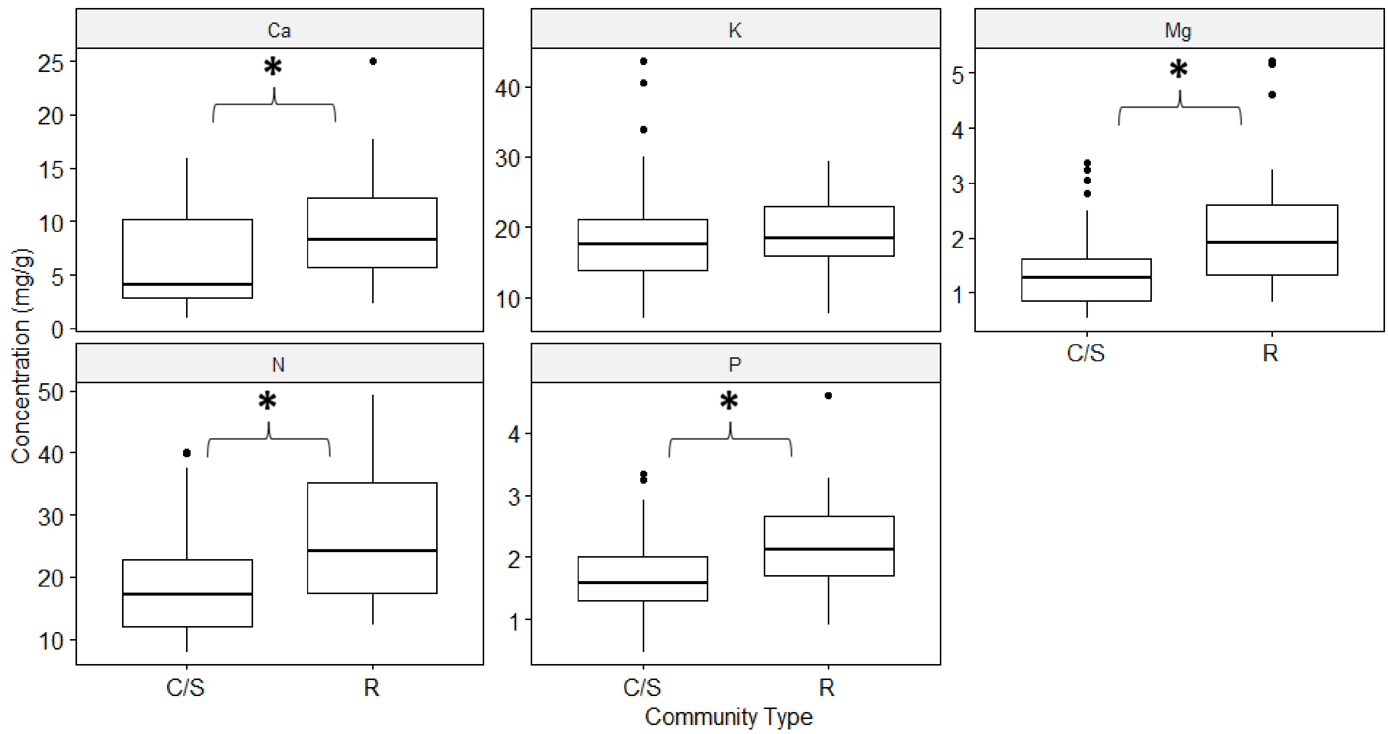


Figure A1.3: Difference in Tissue concentration (mg/g) between Competitor and Stress-tolerators (C/S) and ruderals (R) type communities for each macronutrient.

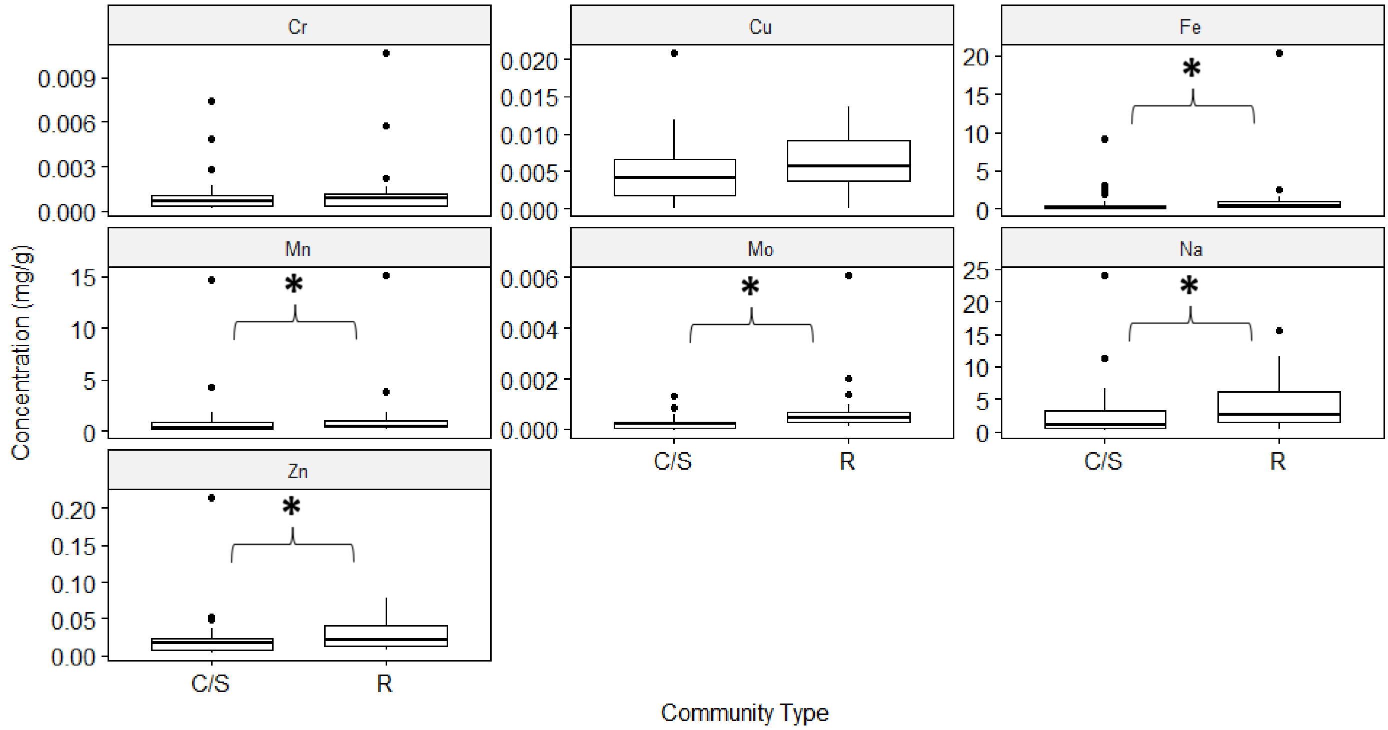


Figure A1.4: Boxplots showing difference in Tissue concentration (mg/g) between Competitor and Stress-tolerators (C/S) and ruderals (R) type communities for each micronutrient-type pollutant. Asterix denotes statistically significant differences (P<0.05)

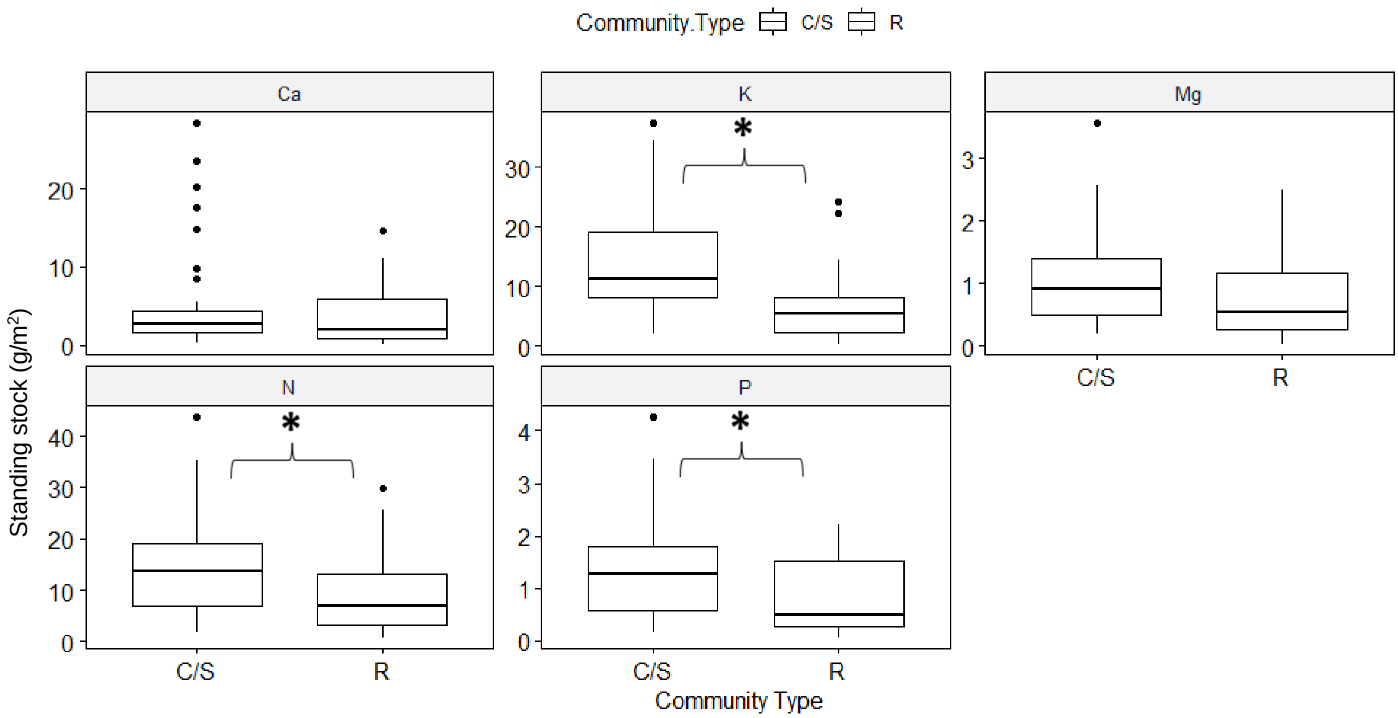


Figure A1.5: Boxplots showing difference in standing stocks (g/m^2) between Competitor and Stress-tolerators (C/S) and ruderals (R) type communities for each macronutrient-type pollutant. Asterix denotes statistically significant differences (P<0.05)

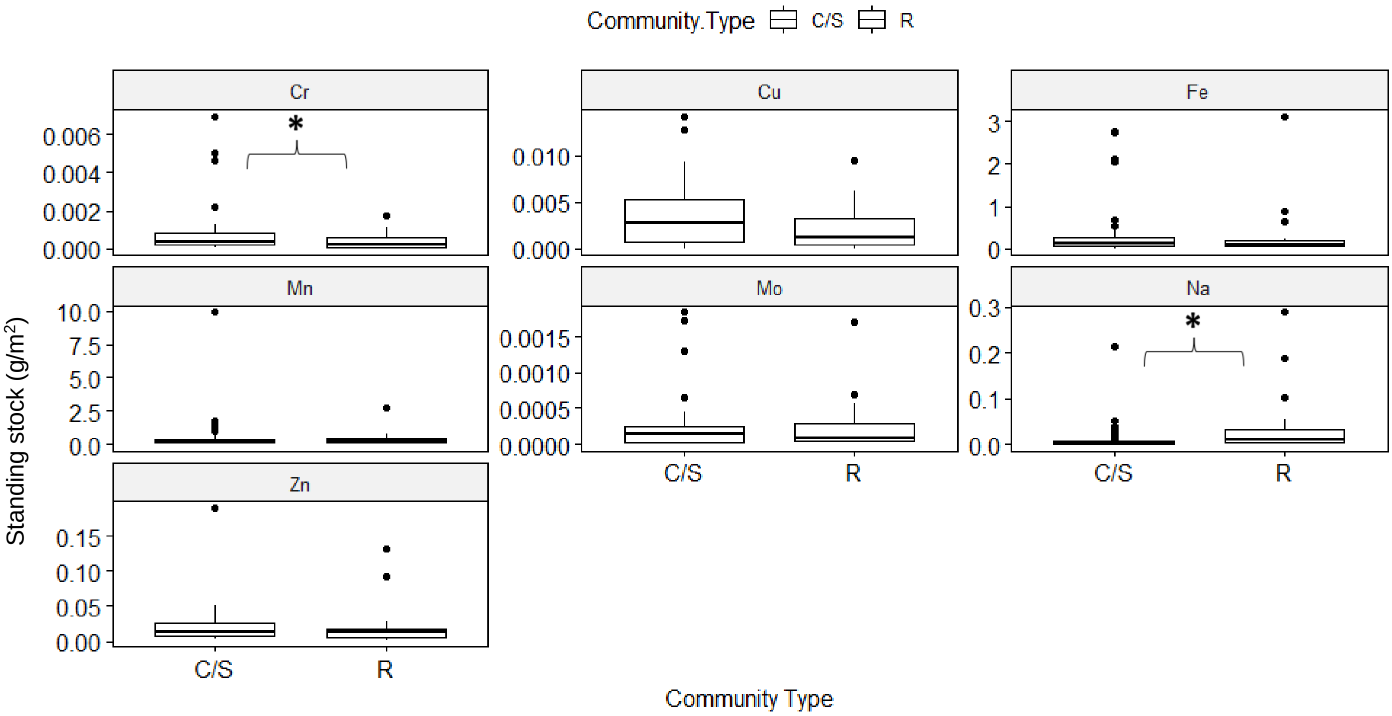


Figure A1.6: Boxplots showing difference in standing stocks (g/m^2) between Competitor and Stress-tolerators (C/S) and ruderals (R) type communities for each micronutrient-type pollutant. Asterix denotes statistically significant differences (P<0.05).

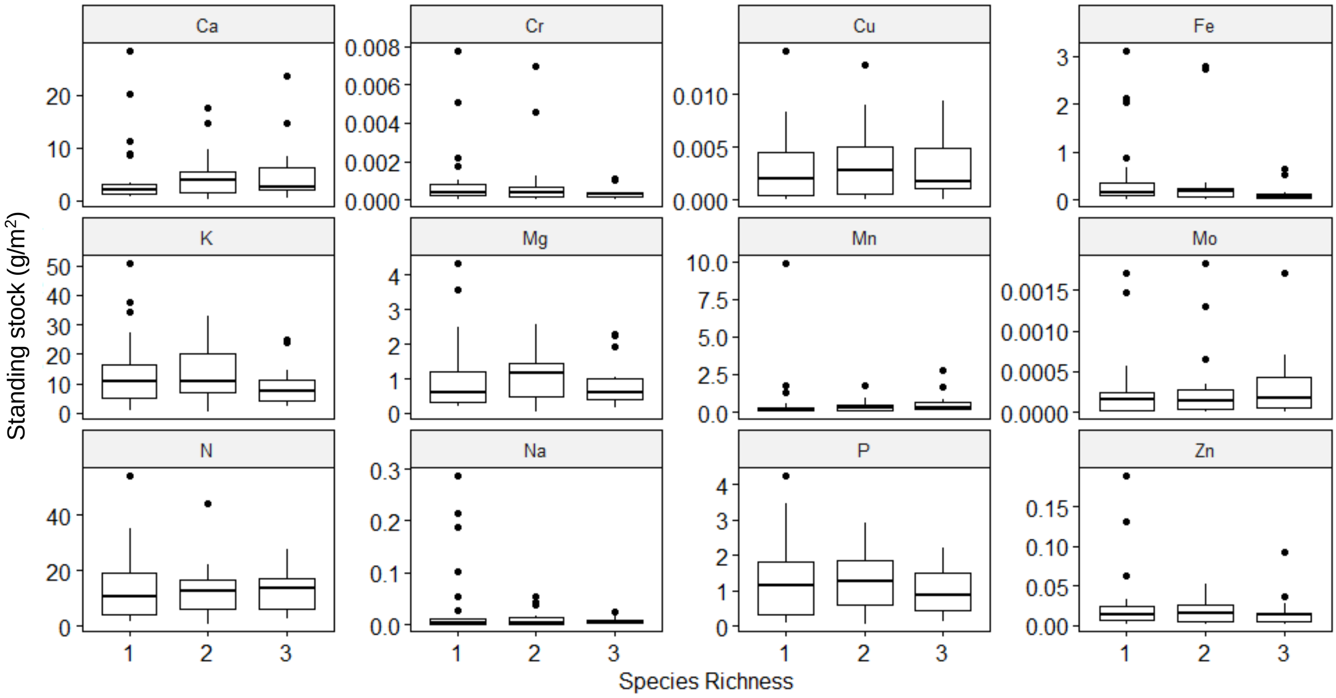


Figure A1.7: Boxplots showing standing stock (g/m^2) per species diversity group 1 (n = 27), 2 (n = 19), and 3 (n = 15) by nutrient. All comparison were not significantly different (P > 0.05)

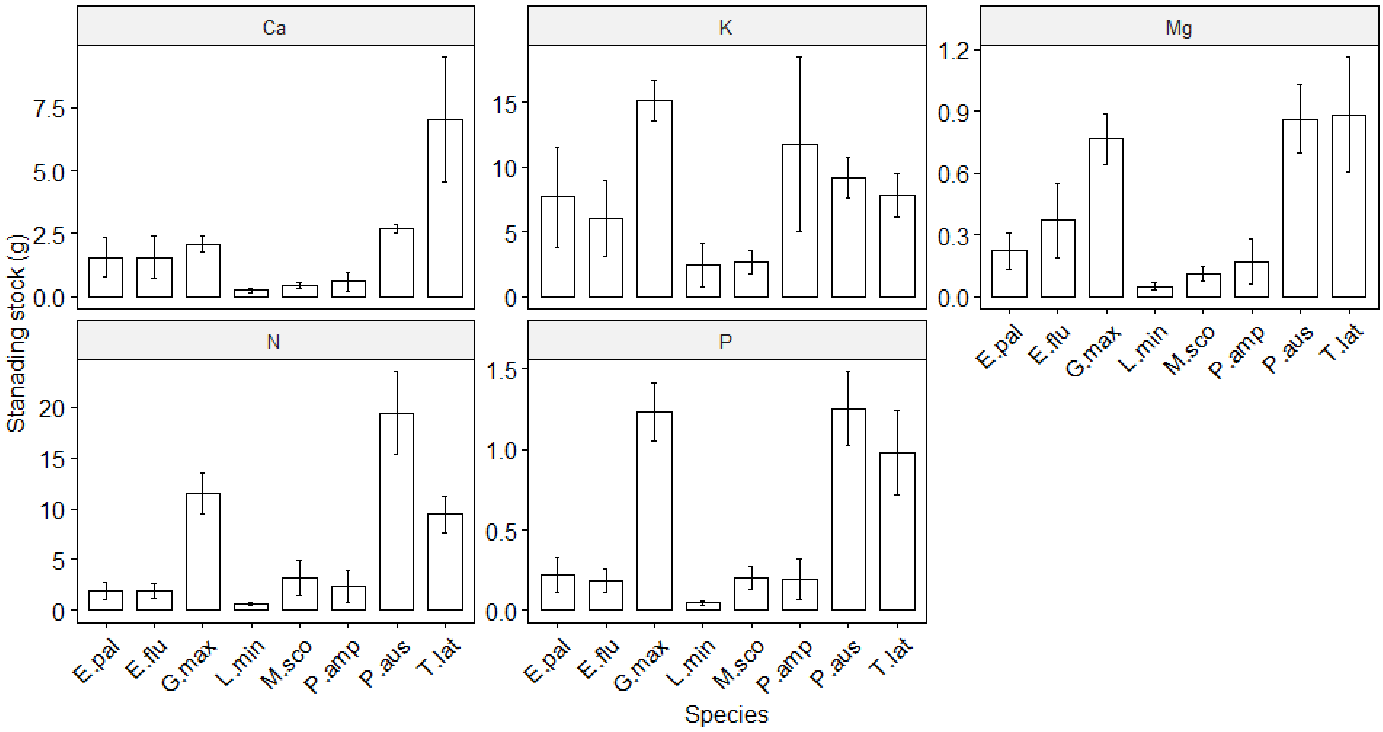


Figure A1.8: Mean standing stocks of macronutrients for *Eleocharis palustris* (n = 5), *Equisetum fluviatile* (n = 7), *Glyceria maxima* (n = 14), *Lemna minor* (n = 12*), Myosotis scorpioides* (n = 6), *Persicaria amphibian* (n = 5), *Phragmites australis* (n = 6) and *Typha latifolia* (n = 13) by nutrient. Error bars are 1 standard errors.

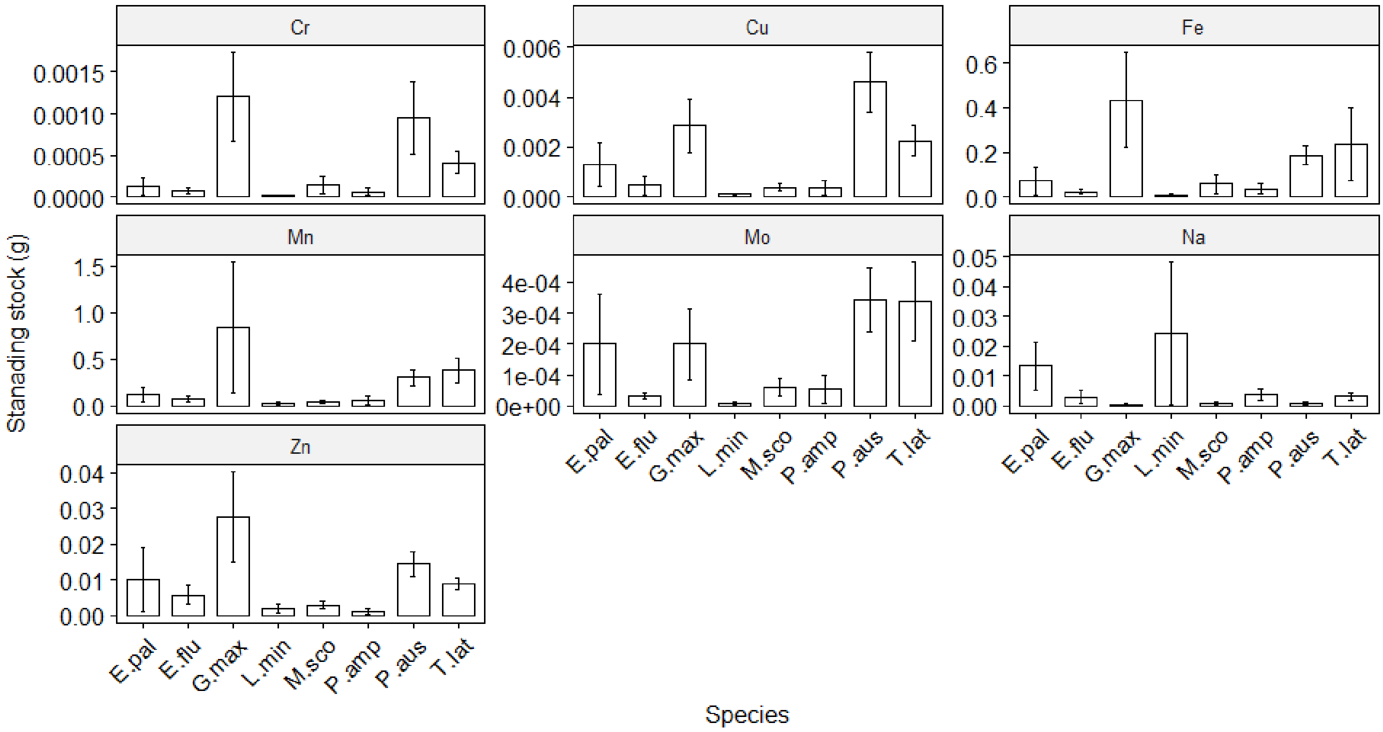


Figure A1.9: Mean standing stocks of micronutrients for *Eleocharis palustris* (*n* = 5), *Equisetum fluviatile* (*n* = 7), *Glyceria maxima* (*n* = 14), *Lemna minor* (*n* = 12*), Myosotis scorpioides* (*n* = 6), *Persicaria amphibian* (*n* = 5), *Phragmites australis* (*n* = 6) and *Typha latifolia* (*n* = 13) by nutrient. Error bars are 1 standard errors.

Table A1.3: Bioconcentration factor (BCF*) per plant community for each studied nutrient. Cells highlighted in red are those with BCFs over the critical value of 50 and those in yellow are for the critical threshold between 25 and 50 (van der Ent et al., 2013). Macronutrient-type elements have been included here for illustrative purposes only and the BCF is generally only applied to micronutrient-type pollutants where tissue concentrations are often very low in comparison

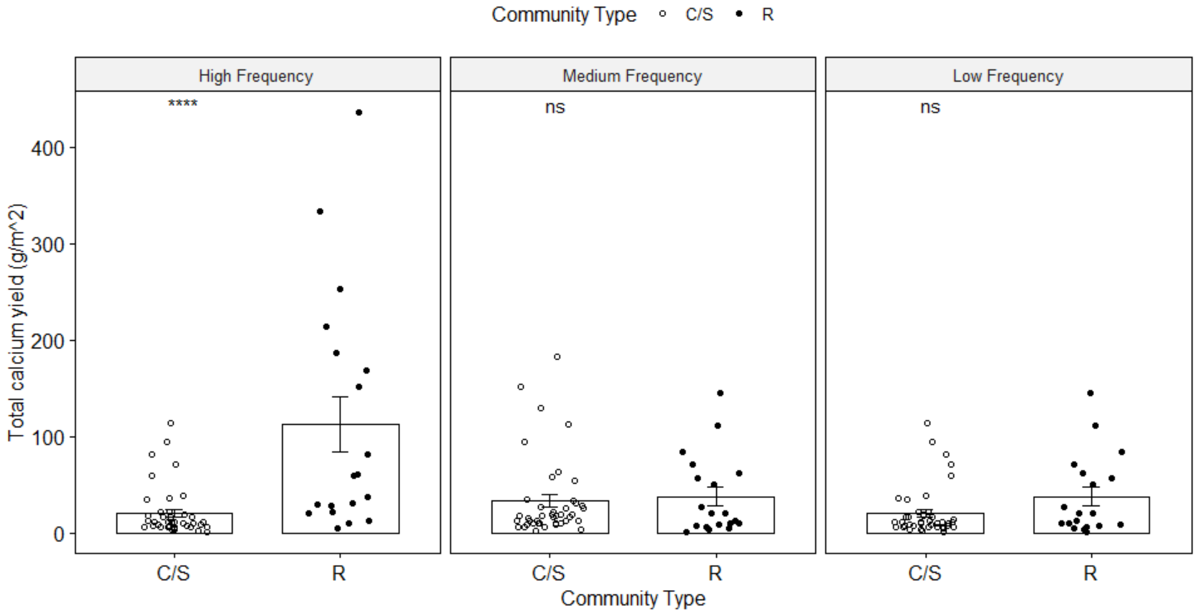
Community	Sample site	Ca	Cu	Fe	N	K	Mg	Mn	Mo	P	Zn
Typha latifolia	**Airthrey Loch**	0.6	0.2	1.4	172.3	27.6	0.4	0.0	1.4	61.9	0.1
Typha latifoliaLeman minor	**Airthrey Loch**	0.3	8.4	75.1	49.1	5.4	0.2	0.0	1.1	34.9	0.4
Azolla filiculoides	**Airthrey Loch**	1.7	29.2	36.7	838.6	20.1	1.1	69.1	27.9	75.7	0.3
Myosotis scorpioidesJuncus effusus Lemna minor	Arndean	0.0	2.9	0.1	835.1	7.8	-161.8	8.9	8.4	49.9	1.4
Potamogeton natans	Arndean	0.9	2.6	0.6	324.7	10.6	2.0	27.6	1.3	60.6	1.5
Typha latifoliaMyosotis scorpioidesEquisetum fluviatilePotamogeton natans	Arndean	1.0	5.7	0.1	355.8	9.2	1.0	15.1	1.1	48.6	1.1
Iris pseudacorus	Arndean	1.0	0.9	0.1	159.4	8.3	0.8	38.2	0.7	43.4	0.3
Carex rostrataEleocharis palustrisLemna minor	Broom Plantations	0.5	0.9	0.4	610.5	6.3	0.6	10.4	0.0	29.3	2.1
Phragmites australisTypha latifolia	Broom Plantations	0.0	1.1	0.2	216.4	3.6	0.1	1.4	0.4	15.4	0.4
Typha latifoliaEleocharis palustrisMyosotis scorpioides	Broom Plantations	0.3	1.4	0.1	86.8	7.1	0.3	8.2	0.8	26.2	0.2
Carex rostrataEquisetum fluviatileLemna minor	Cocksburn reservoir	0.3	0.0	0.2	104.6	15.6	0.4	0.0	1.5	54.3	0.5
Eleocharis palustrisEquisetum fluviatilePersicaria amphibian	Cocksburn reservoir	0.5	3.7	0.4	293.6	22.0	0.5	13.5	1.8	63.3	0.5
Persicaria amphibian	Cocksburn reservoir	0.6	1.8	1.9	460.9	46.5	0.6	46.9	3.0	180.7	1.0
Glyceria maximaMyosotis scorpioides	Carse of Lecropt	0.2	0.7	8.5	13.2	8.5	0.2	2.5	1.0	61.6	0.2
Phalaris arundinacea	Carse of Lecropt	0.2	0.5	0.4	41.1	6.6	0.2	0.9	0.3	20.7	0.2
Phalaris arundinaceaGlyceria maxima	**Carse of Lecropt**	0.1	6.1	29.4	9.4	7.4	0.1	0.0	0.8	64.9	0.9
Epilobium hirsutumGlyceria maxima Callitriche platycarpa	Clackmannan	0.1	1.2	1.8	26.6	2.5	0.2	0.0	0.6	55.5	1.1
Glyceria maxima Callitriche platycarpa	Clackmannan	0.0	0.0	9.9	15.2	2.8	0.1	0.0	0.6	38.4	0.7
Juncus effusus	**Devilla Forest**	1.9	50.5	1.0	259.0	12.0	2.2	4.9	0.0	115.4	0.5
Lemna minor Equisetum fluviatile	Devilla Forest	0.9	0.0	2.5	134.7	6.4	0.6	0.0	4.9	57.8	1.5
Alisma plantago-aquatica Equisetum fluviatile	**Devilla Forest**	0.5	68.2	1.3	205.2	11.8	0.4	0.0	6.0	75.1	1.2
Potamogeton natansAlisma plantago-aquatica	**Devilla Forest**	0.1	23.2	5.3	889.9	9.8	0.3	203.6	0.8	86.8	0.8

Phragmites australis	Dunfermline SUDS pond	0.2	5.1	0.4	198.1	4.6	0.3	0.0	0.9	18.1	3.1
Glyceria maxima	Forth and Clyde Canal	0.1	0.0	1.4	138.5	11.5	0.2	0.0	0.0	99.9	0.6
Glyceria maximaLysimachia thyrsiflora	Forth and Clyde Canal	0.1	0.4	0.7	181.7	10.4	0.1	0.0	0.0	66.7	0.6
Nuphar lutea	Forth and Clyde Canal	0.7	0.0	0.2	711.5	14.5	0.5	0.0	0.0	106.3	0.7
Glyceria maxima	Gartmorn Dam	0.1	0.6	0.2	8.8	1.8	0.3	2.3	0.3	4.9	0.1
Glyceria maxima	Gartmorn Dam	0.1	0.7	4.7	82.6	7.3	0.1	0.0	0.0	147.7	0.1
Eleocharis palustrisPersicaria amphibian	Gartmorn Dam	0.3	3.6	5.8	179.3	9.4	0.3	0.0	2.6	187.0	0.2
Typha latifoliaEquisetum fluviatileLemna minor	Gogar Loan	0.3	0.2	0.5	132.3	5.4	0.1	0.0	0.3	9.9	0.1
Glyceria maximaLemna minor	Howeitoun Fishery	0.4	2.6	0.2	117.5	19.9	0.6	0.0	0.7	32.1	0.4
Elodea canadensisAlisma plantago-aquatica	Howeitoun Fishery	1.1	17.3	1.4	1279.6	272.0	1.1	0.0	1.0	31.6	10.9
Schoenoplectus lacustrisSparganium erectumLemna minor	Loch Fitty	0.2	2.1	0.6	144.8	3.7	0.2	19.2	0.5	46.0	0.7
Carex rostrata Myosotis scorpioidesGlyceria fluitans	Loch Fitty	0.1	2.1	1.6	440.1	3.4	0.1	0.0	0.9	49.4	1.2
Menyanthes trifoliata	Loch Fitty	0.2	3.3	0.2	347.6	6.6	0.2	0.0	0.9	79.6	1.3
Typha latifoliaMentha aquatica Menyanthes trifoliata	Loch Fitty	0.2	2.9	0.3	395.8	3.5	0.1	0.0	0.0	32.2	0.5
Phragmites australis	Linlithgow Loch	0.1	3.9	4.6	142.5	3.2	0.1	0.0	0.5	11.3	0.3
Glyceria maxima	Linlithgow Loch	0.1	4.2	4.4	68.3	3.5	0.1	0.0	0.1	13.5	0.4
Callitriche stagnalis	**North Third**	1.9	5.4	231.8	258.9	48.0	1.2	23.5	2.8	147.1	1.4
Persicaria amphibian	North Third	0.8	12.7	3.2	257.5	58.8	0.8	0.0	4.6	353.3	0.3
Glyceria fluitansPersicaria amphibian	North Third	0.6	13.5	11.6	264.3	32.3	0.5	0.0	6.6	302.6	0.4
Glyceria maximaEquisetum fluviatile	Plean Country Park	0.2	0.1	3.5	81.1	7.1	0.2	20.7	1.3	139.8	0.6
Potamogeton perfoliatusSparganium erectum	Plean Country Park	0.5	1.8	8.8	274.5	12.1	0.4	19.5	0.4	411.8	0.6
Juncus effusus	Plean Country Park	0.1	0.6	1.5	117.9	4.7	0.1	1.4	1.4	118.1	0.8
Nymphoides petula	Plean Country Park	1.0	5.0	40.0	196.2	39.3	1.2	3.3	0.0	64.1	0.3
Phragmites australis	Plean Country Park	0.0	0.0	5.8	9.6	2.6	0.1	0.0	1.0	229.9	0.6
Iris pseudacccorusTypha latifolia	Raploch Road Suds ponds	0.4	1.4	0.0	142.0	19.7	0.3	5.7	0.3	13.1	0.1
Phragmites australisTypha latifolia	Raploch Road Suds ponds	0.0	2.8	0.1	219.7	5.1	0.1	0.9	1.2	6.1	0.5
Callitriche obtusangulaEleocharis palustrisTypha latifolia	Raploch Road Suds ponds	0.4	3.7	6.8	157.8	16.7	0.3	0.0	3.2	25.6	2.6

Callitriche obtusangulaTypha latifolia	Raploch Road Suds ponds	0.4	2.1	3.7	256.2	1.9	0.2	0.0	1.6	11.8	0.4
Callitriche obtusangulaPhragmites australisTypha latifolia	Raploch Road Suds ponds	0.2	5.8	0.7	252.7	1.3	0.1	3.9	1.0	11.4	0.6
Sparganium erectum	Stirling Carse	0.3	0.7	1.9	14.8	9.0	0.5	15.8	0.7	46.0	0.6
Phalaris arundinacea	Stirling Carse	0.1	1.3	6.2	13.2	9.4	0.2	7.6	1.0	91.3	1.2
Typha latifoliaMyosotis scorpioidesLemna minor Mentha aquatica Juncus effusus	Stirling Univeristy Pond	0.7	3.8	0.2	501.5	7.6	0.9	0.0	0.9	7.3	0.4
Callitriche stagnalis Callitriche platycarpaLemna minor	Wallace monument	0.0	5.4	7.3	23.3	9.5	0.1	0.0	0.1	55.2	0.4
Glyceria maxima	**Wallace monument**	0.0	15.6	54.1	10.4	8.5	0.1	2687.2	0.1	52.8	7.1
Mimulus guttatus	**Whins of Milton**	18.1	4.4	4.4	133.7	33.6	77.2	0.0	1.7	48.0	1.3
Phalaris arundinacea	**Whins of Milton**	0.4	4.9	1.6	156.0	32.3	1.0	0.0	0.4	68.3	0.4
Lemna minor	Whins of Milton	1.2	24.3	6.6	217.9	30.5	1.5	0.0	1.6	62.9	0.7
Lemna minor Glyceria maxima	Whins of Milton	0.2	7.0	0.6	159.3	32.5	0.3	0.0	0.0	41.4	0.3
Glyceria maxima	Whins of Milton	0.1	1.7	0.9	73.9	14.9	0.2	0.0	-0.3	39.9	0.4

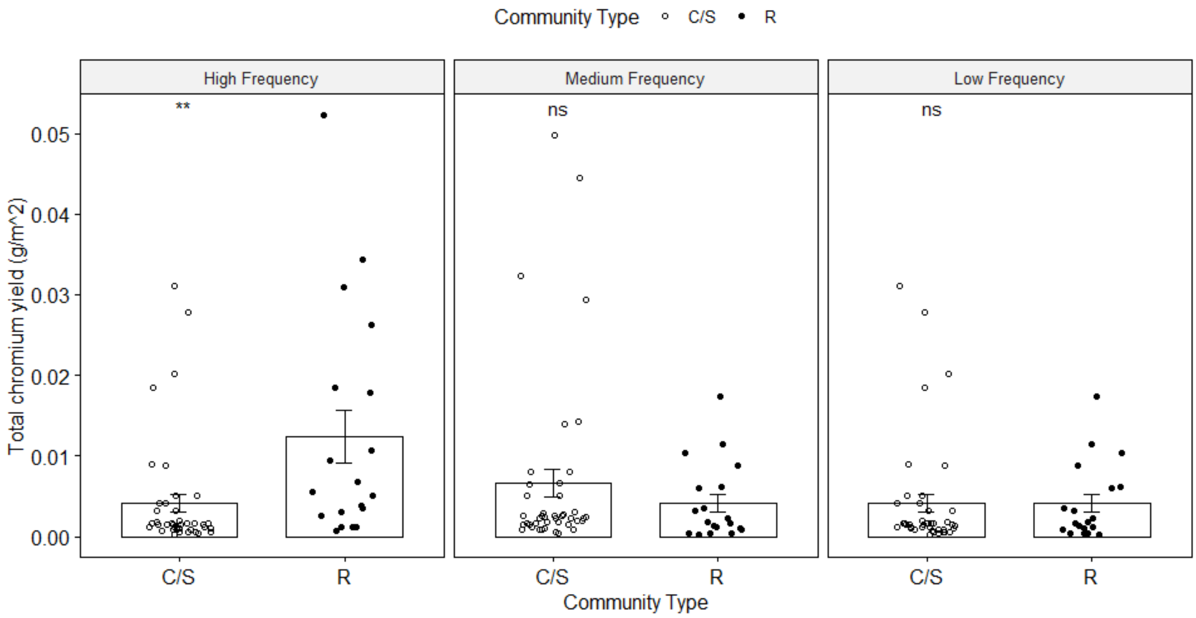
* To assess communities that may have hyperaccumulation potential the bioconcentration factor (BCF) was calculated for each community using the following equation: $BCF = \frac{Pollutant\ concentration\ in\ plant}{Pollutant\ concentration\ in\ water}$

(A)



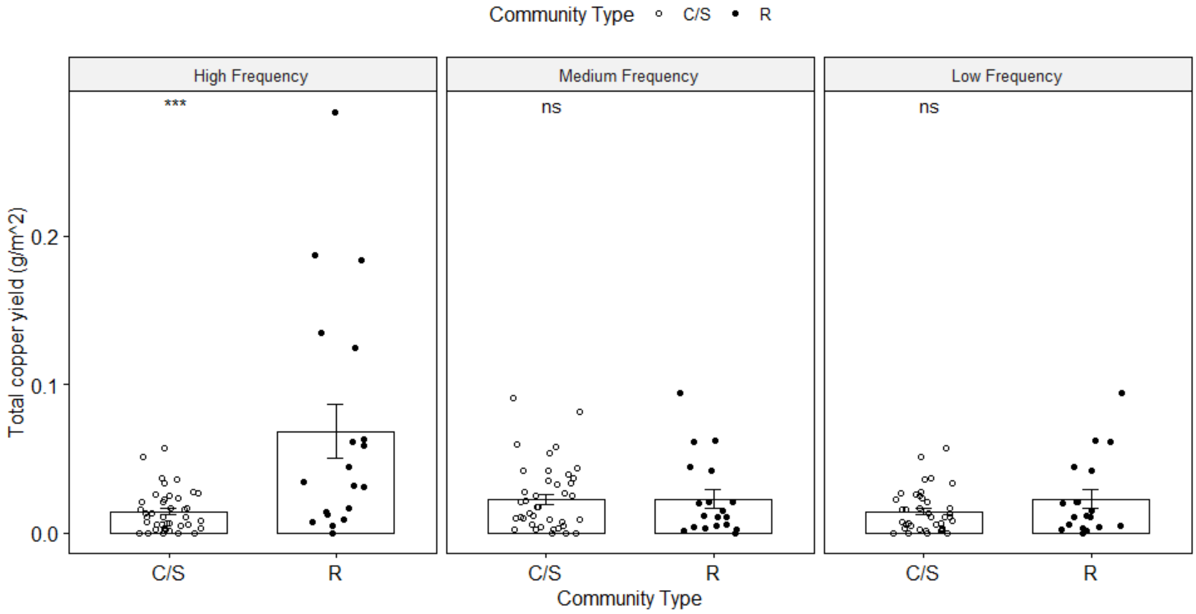
Community Type
C/S
R
High Frequency
Medium Frequency
Low Frequency
****
ns
ns
Total calcium yield (g/m^2)
0
100
200
300
400
C/S
R
Community Type

(B)

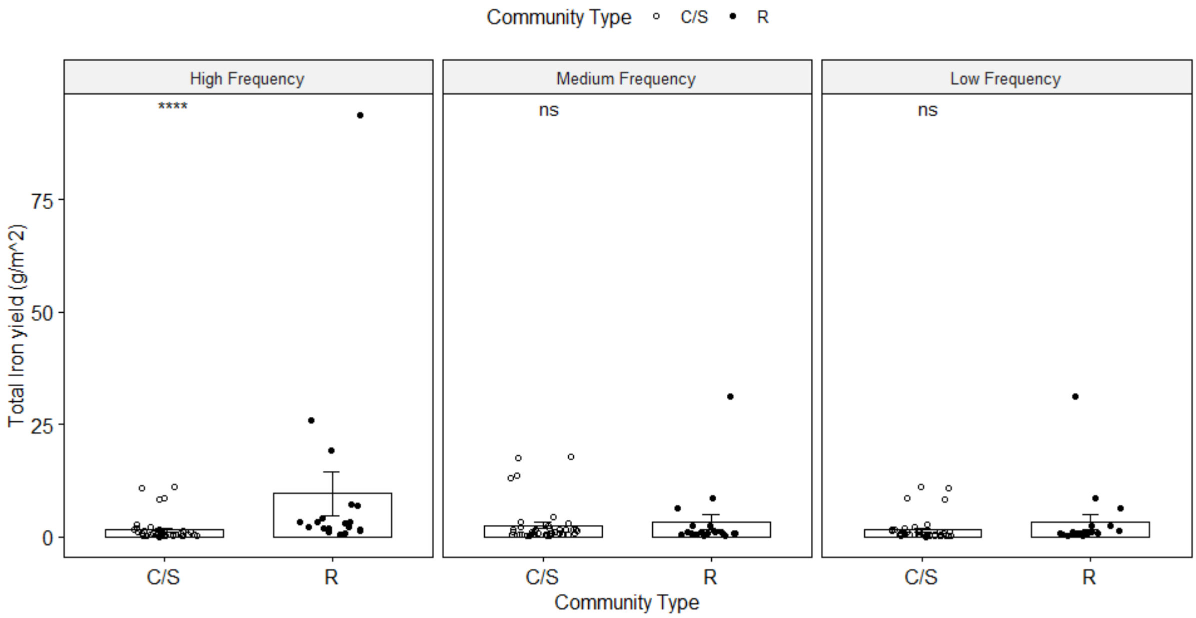


Community Type
C/S
R
High Frequency
Medium Frequency
Low Frequency
**
ns
ns
Total chromium yield (g/m^2)
0.00
0.01
0.02
0.03
0.04
0.05
C/S
R
Community Type

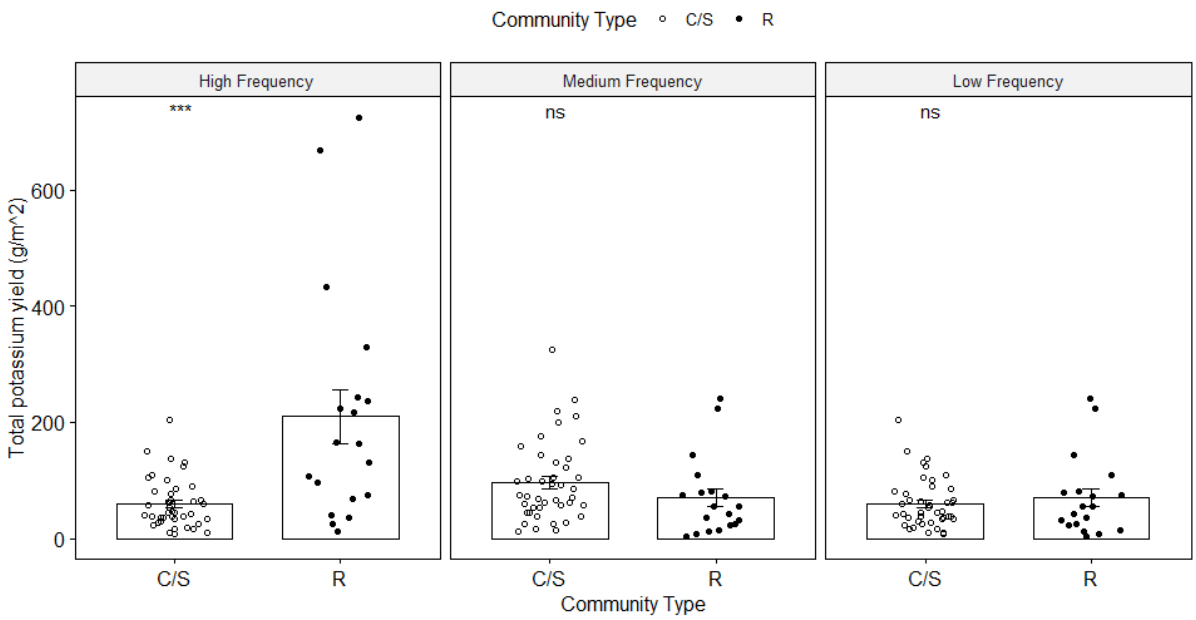
(C)


Community Type
C/S
R
High Frequency
Medium Frequency
Low Frequency
***
ns
ns
Total copper yield (g/m^2)
0.2
0.1
0.0
C/S
R
C/S
R
C/S
R
Community Type

(D)

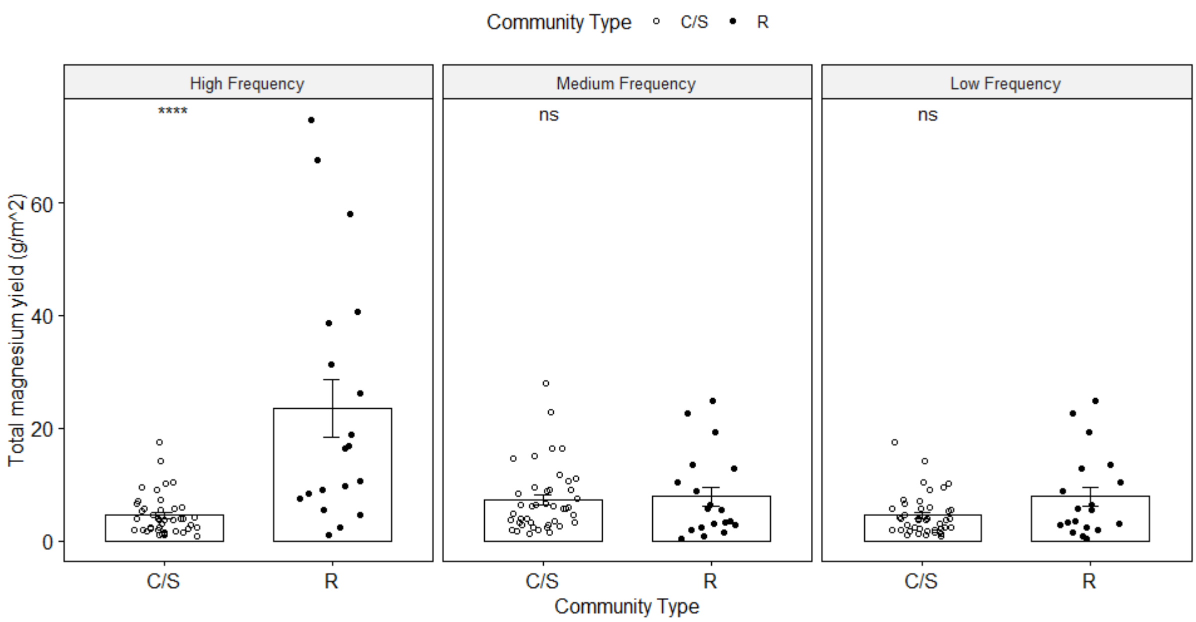

Community Type
C/S
R
High Frequency
Medium Frequency
Low Frequency
****
ns
ns
Total Iron yield (g/m^2)
75
50
25
0
C/S
R
C/S
R
C/S
R
Community Type

(E)



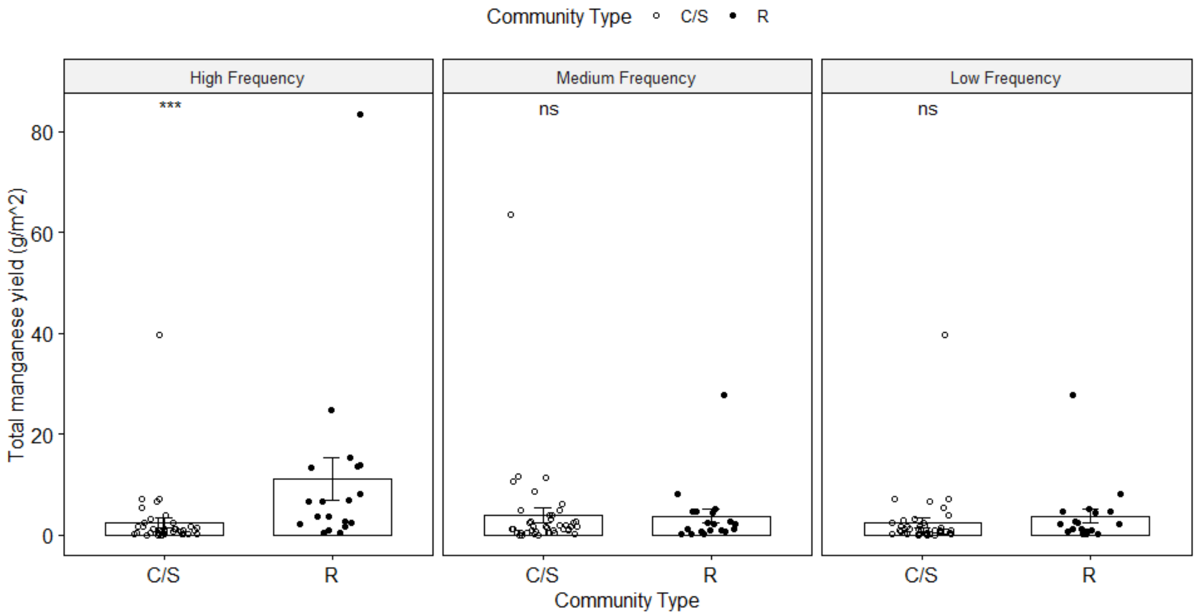
Community Type C/S R
High Frequency
Medium Frequency
Low Frequency
***
ns
ns
Total potassium yield (g/m^2)
0
200
400
600
C/S
R
C/S
R
C/S
R
Community Type

(F)



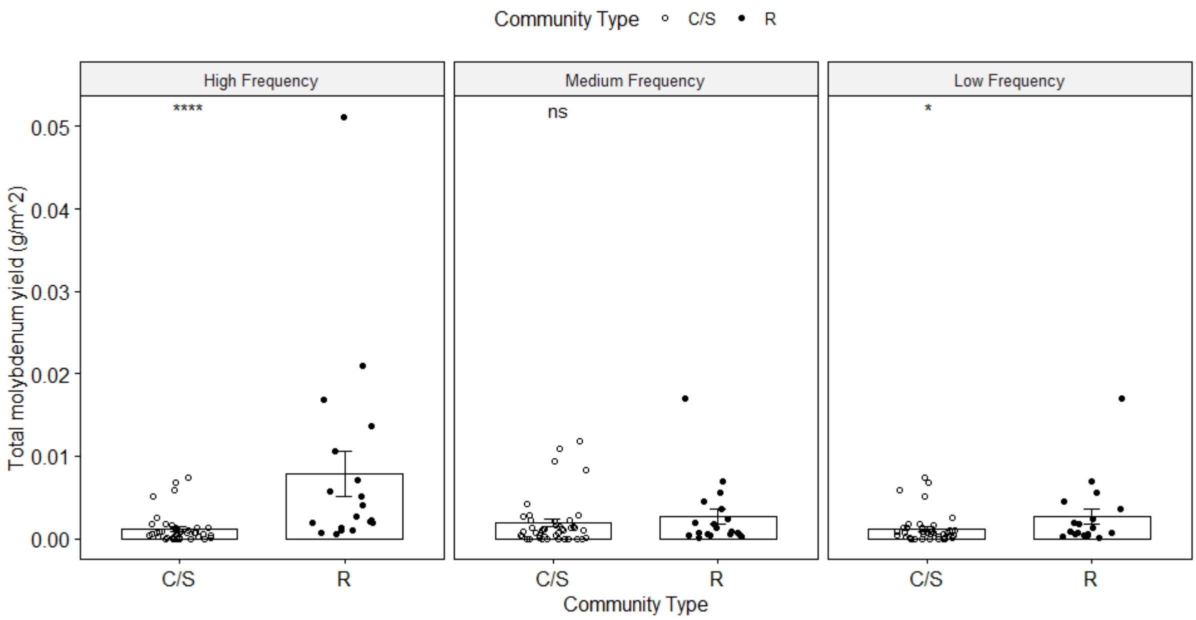
Community Type C/S R
High Frequency
Medium Frequency
Low Frequency
****
ns
ns
Total magnesium yield (g/m^2)
0
20
40
60
C/S
R
C/S
R
C/S
R
Community Type

(G)



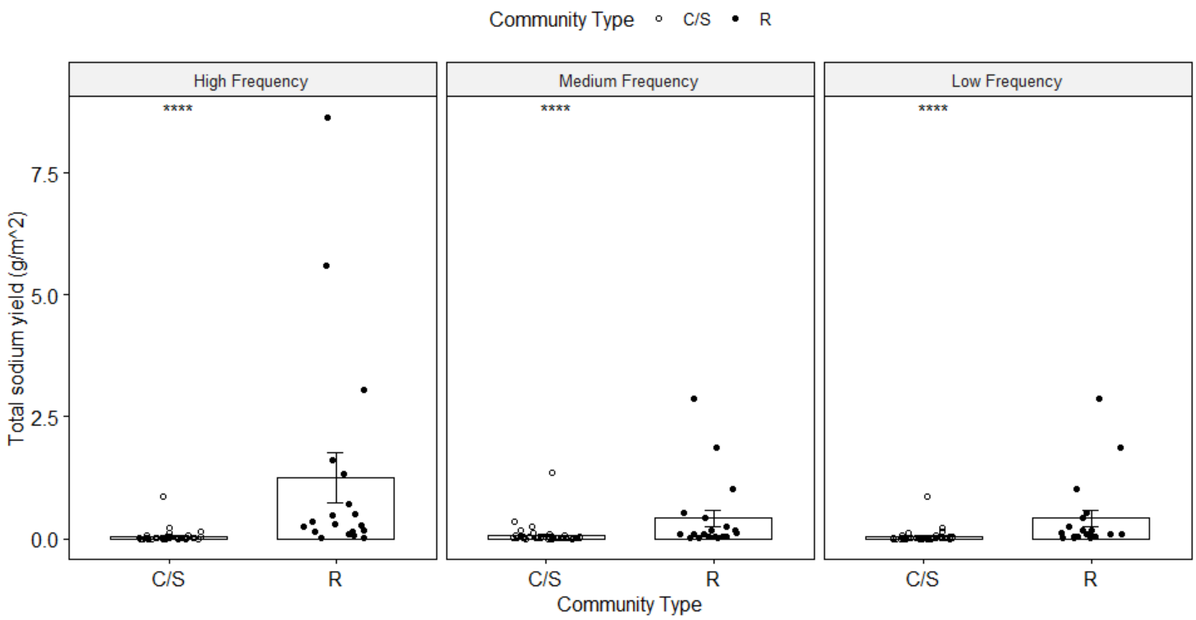
Community Type
C/S
R
High Frequency
Medium Frequency
Low Frequency
***
ns
ns
80
60
40
20
0
Total manganese yield (g/m^2)
C/S
R
C/S
R
C/S
R
Community Type

(H)



Community Type
C/S
R
High Frequency
Medium Frequency
Low Frequency
****
ns
*
0.05
0.04
0.03
0.02
0.01
0.00
Total molybdenum yield (g/m^2)
C/S
R
C/S
R
C/S
R
Community Type

(I)



(J)

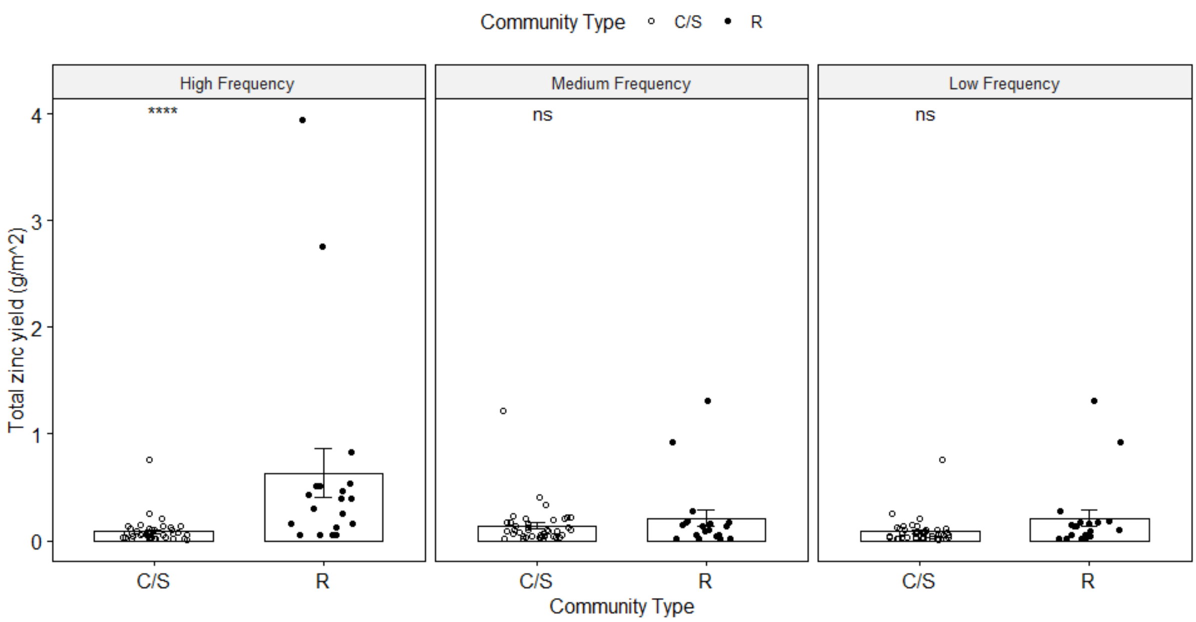


Figure A1.10: Difference over 10 years in total yields of between Competitor and Stress-tolerators (C/S) and ruderals (R) type communities. Calcium (A), Chromium (B), Copper (C), Iron (D), Potassium (E), Magnesium (F) Manganese (G), Molybdenum (H), Sodium (I), Zinc (J). Error bars show the SE of the mean. $P \leq 0.0001$ (****) and 'ns' indicates no significant difference.

Appendix 2

Supplementary information for Chapter 4 'Floating treatment wetlands - engineering nature-based solutions for ecosystem multifunctionality'

Table A2.1: Volume and concentration of stock solution added to each mesocosm at day 1 of each batch to simulate multi-polluted water media

Stock solution	Stock concentration	Volume (ml)
Copper sulfate	0.025	0.070304
Iron(II) sulfate	3	29.88048
Manganese chloride-4-water	0.3	1.0922
Zinc sulfate-7-water	0.12	0.183514
Magnesium sulfate	4.4	9.053498
Ammonium acid phosphate	0.7	1.129032
Potassium nitrate	1.18	4.214286
Calcium nitrate	1	3.571429
Potassium chromate	0.05	0.384615

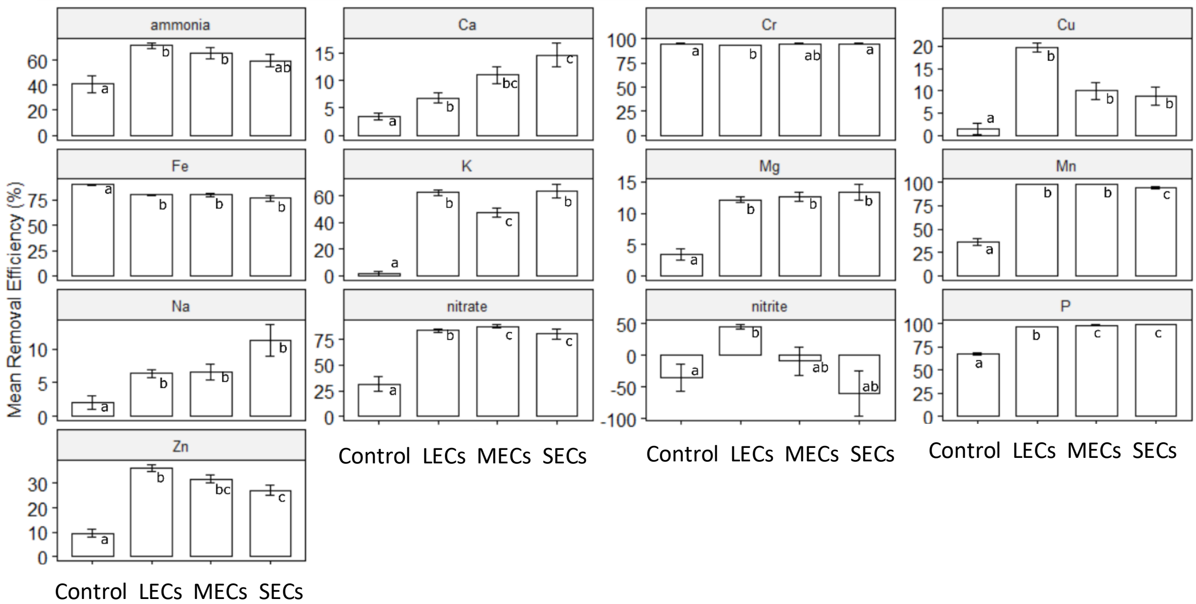


Figure A2.1: Mean removal efficiency (%) for large-statured emergent communities (80) (LECs), Mixed-statured communities (MECs) (n = 40), small-statured communities (SECs) (n = 40) and unplanted controls (n = 40) by pollutant after 7 days (50% of HRT). Error bars are 1 standard errors and bars with the same letter are not statistically significantly different (P > 0.05)

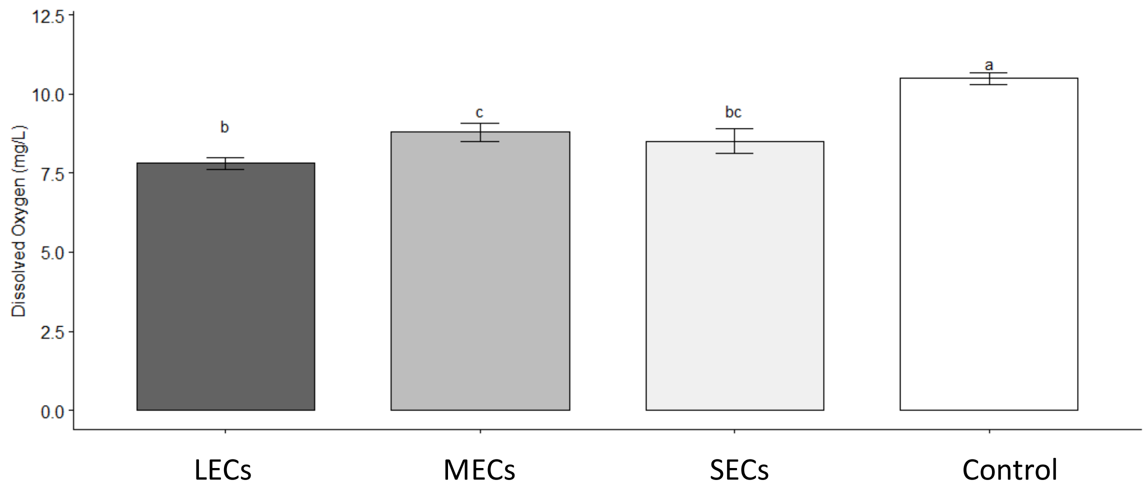


Figure A2.2: Mean dissolved oxygen concentration (mg/L) for large statured emergent communities (80) (LECs), Mixed statured communities (MECs) (*n* = 40), small statured communities (SECs) (*n* = 40) and unplanted controls (*n* = 40) after 7 days (50% of HRT). Error bars are 1 standard errors and bars with the same letter are not statistically significantly different (P > 0.05)

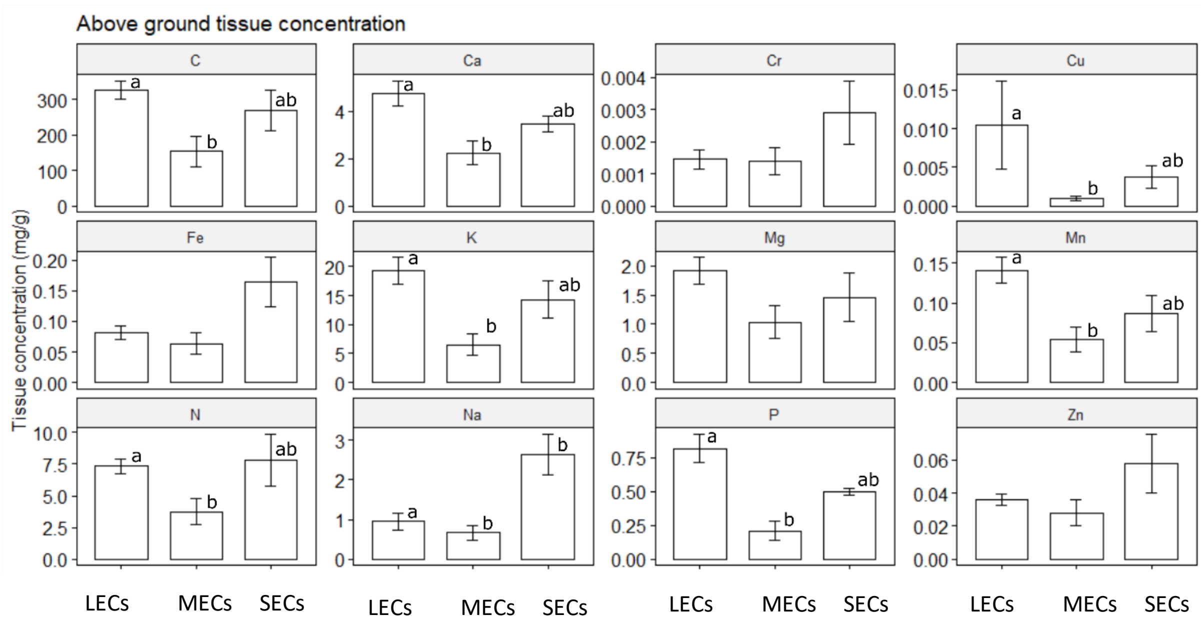


Figure A2.3: Mean above- ground tissue concentration for large statured emergent communities (LECs) (*n* = 28), Mixed statured communities (MECs) (*n* = 8), small statured communities (SECs) (*n* = 8) by pollutant at the end of the experiment. Error bars are standard errors and bars with the same letter are not statistically significantly different (P > 0.05). Plots without letters are not statistically significantly different (P > 0.05).

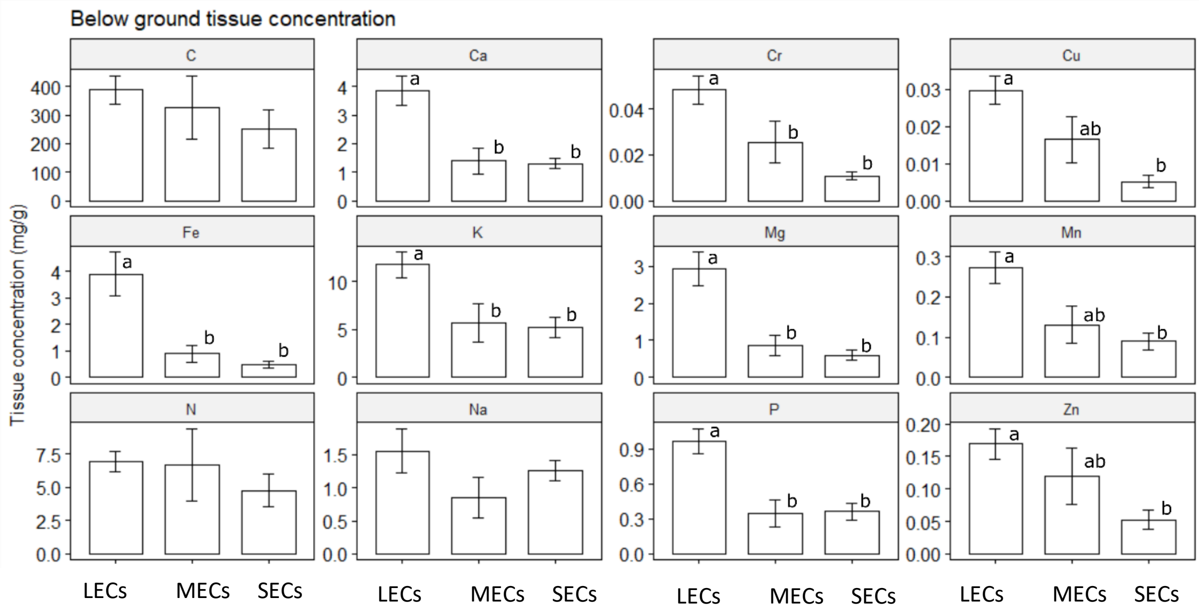


Figure A2.4: Mean below- ground tissue concentration for large statured emergent communities (LECs) (*n* = 28), Mixed statured communities (MECs) (*n* = 8), small statured communities (SECs) (*n* = 8) by pollutant at the end of the experiment. Error bars are standard errors and bars with the same letter are not statistically significantly different (P > 0.05). Plots without letters are not statistically significantly different (P > 0.05).

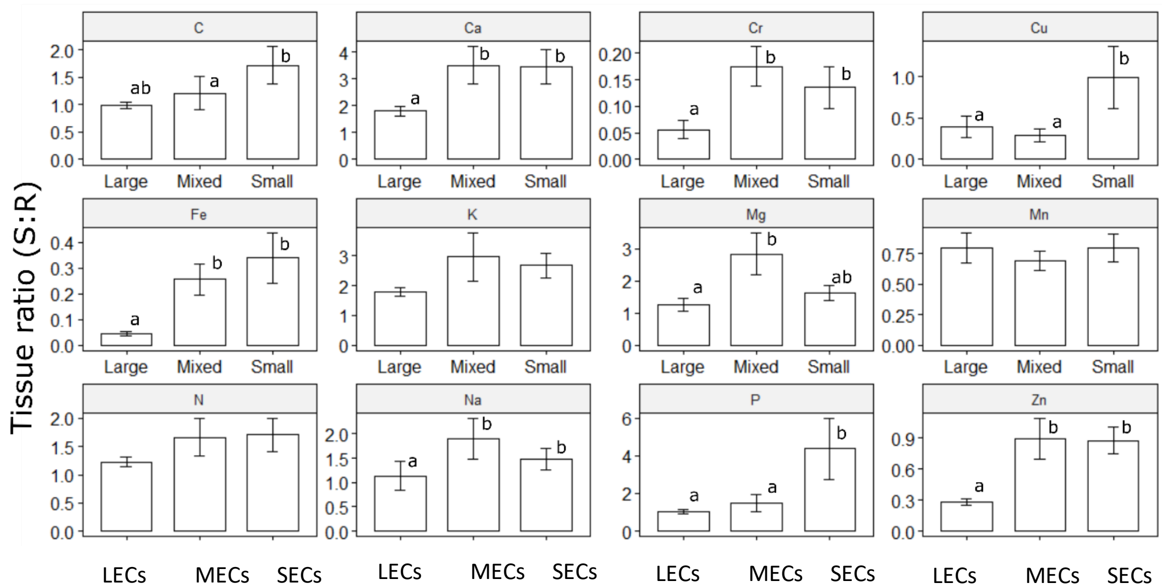


Figure A2.5: Mean tissue ratio (Shoot: Root) for large statured emergent communities (LECs) (*n* = 28), Mixed statured communities (MECs) (*n* = 8), small statured communities (SECs) (*n* = 8) by pollutant at the end of the experiment. Error bars are standard errors and bars with the same letter are not statistically significantly different (P> 0.05). Plots without letters are not statistically significantly different (P > 0.05).

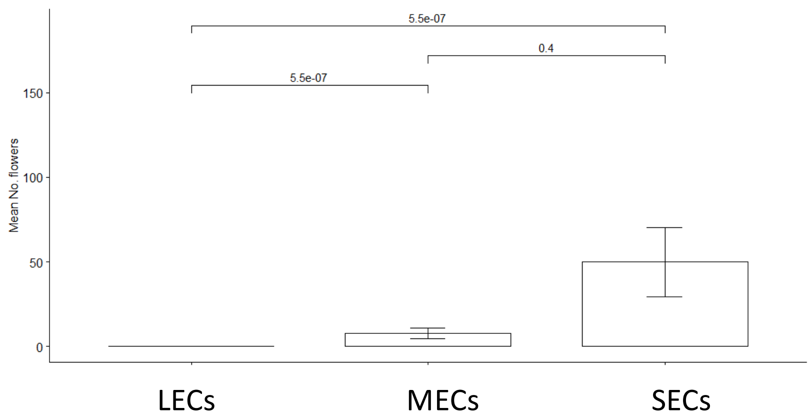


Figure A2.6: Mean number of flowers large-statured emergent communities (n = 28) (LECs), Mixed-statured communities (MECs) (n = 8), small-statured communities (SECs) (n = 8). Error bar (1SE) show variation and pairwise comparisons lines show significance level.

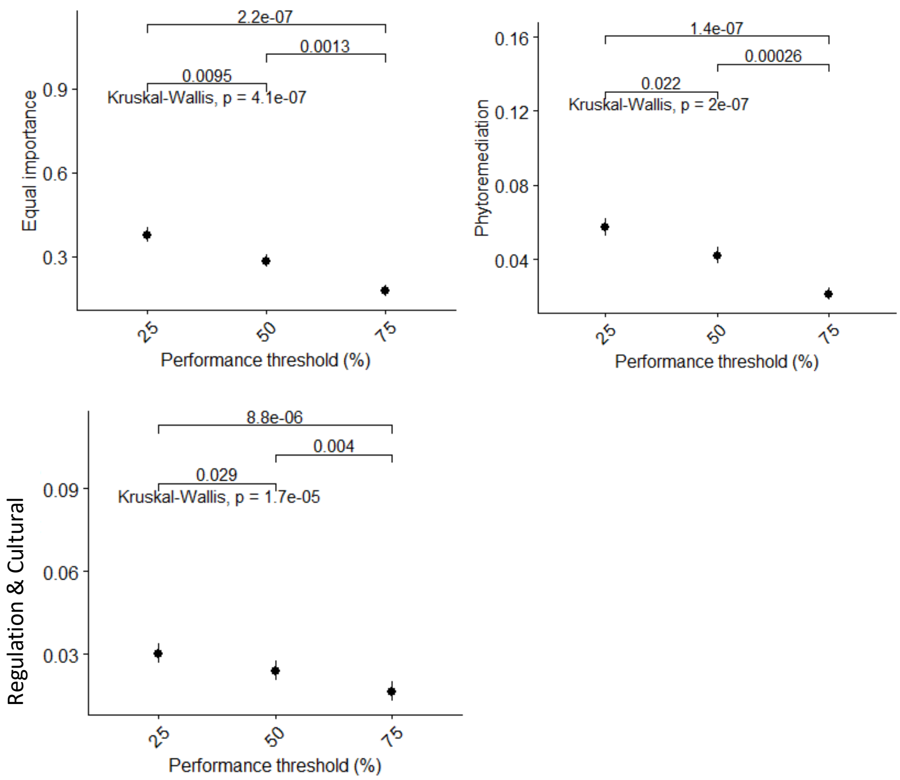


Figure A2.7: Mean ecosystem multi-functionality calculated by different restoration objective scenario (Equal importance, phytoremediation and regulation and cultural) the 25% (n = 44), 50%% (n = 44) and 75% % (n = 44) threshold. Black dot shows mean with error bar (1SE) and pairwise comparisons show significance level.

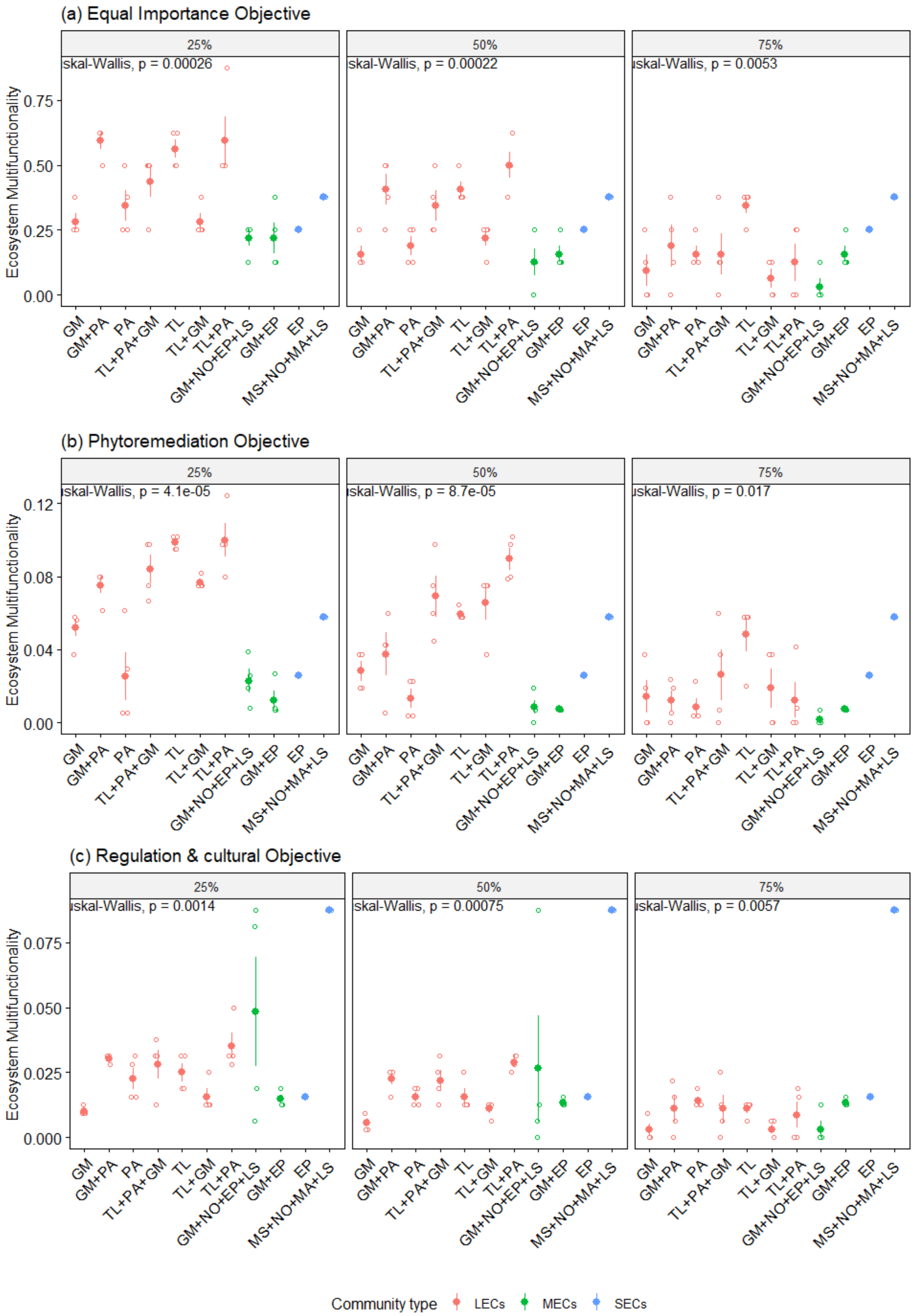


Figure A2.8: Mean ecosystem multifunctionality calculated by different restoration objectives (equal importance, phytoremediation and regulation and cultural) for each plant community (n = 4). Each objective is split by ecosystem performance threshold of 25 %, 50 % and 75 % of the maximum of each service. Unfilled circles represent spread of data, filled circles with error bars shows the mean ±1SE.

Appendix 3

Supplementary information for Chapter 5 ‘Understanding multi-pollutant removal dynamics in mesocosms with mixed or monoculture floating treatment wetlands’

Methods

To understanding the contribution of direct plant growth with pollutant removal week by week across the experiment a growth multiple was used as the basis for a relative growth calculation:

$$Growth\ Mutltiple = Total\ No.live\ stems\ (g) \times Maximum\ plant\ height(cm)$$

Relative growth rate of each individual plant was calculated from the start of the experiment each week was:

$$RGR = \frac{Ln\ G_2 - Ln\ G_1}{T_2 - T_1}$$

Where G_2 is the growth multiple at time 2 and G_1 is the growth multiple at time 1.

Figures and tables

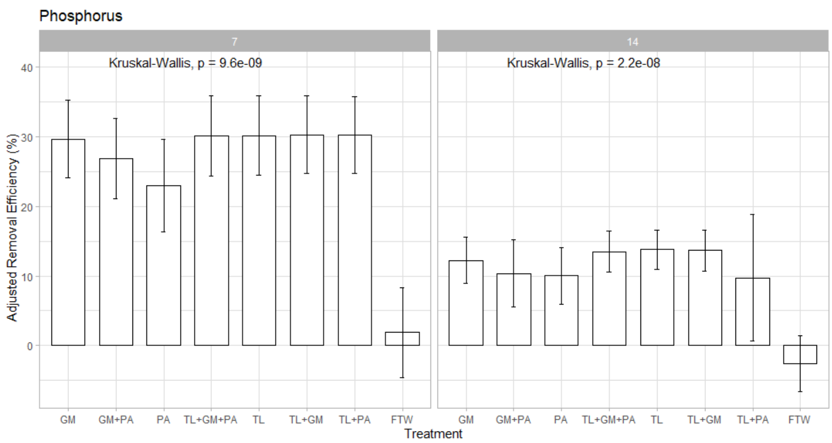


Figure A3.1 (A): Mean Adjusted removal efficiency (%) by treatment at 7 and 14 day HRT

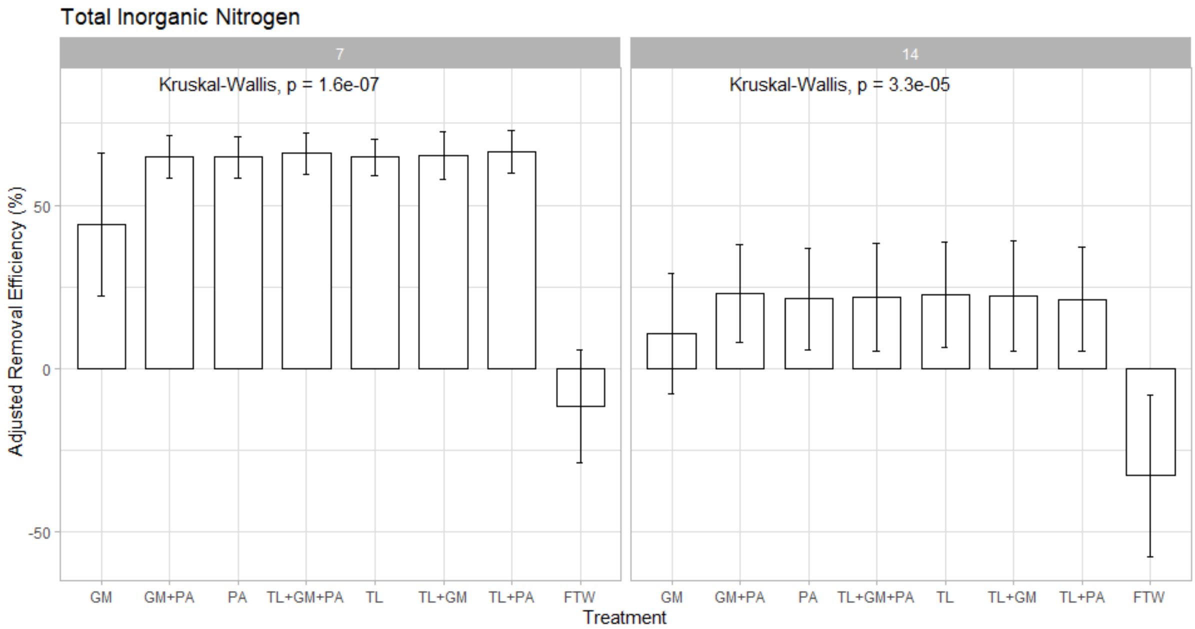


Figure A3.1 (B): Mean Adjusted removal efficiency (%) by treatment at 7 and 14 day HRT

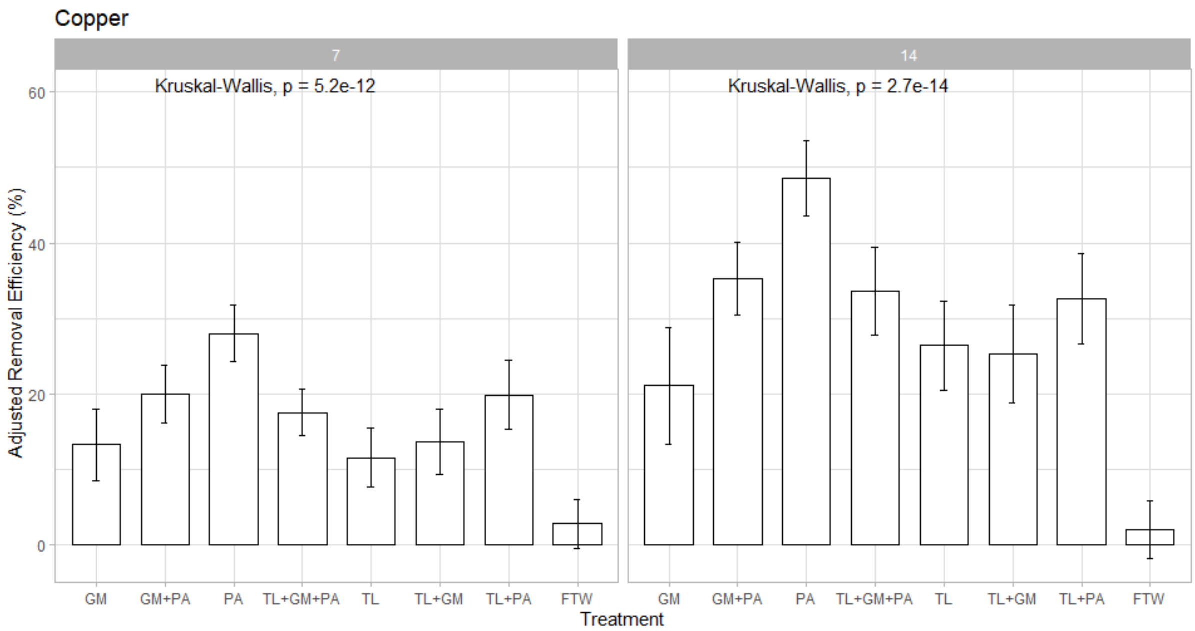


Figure A3.1 (C): Mean Adjusted removal efficiency (%) by treatment at 7 and 14 day HRT

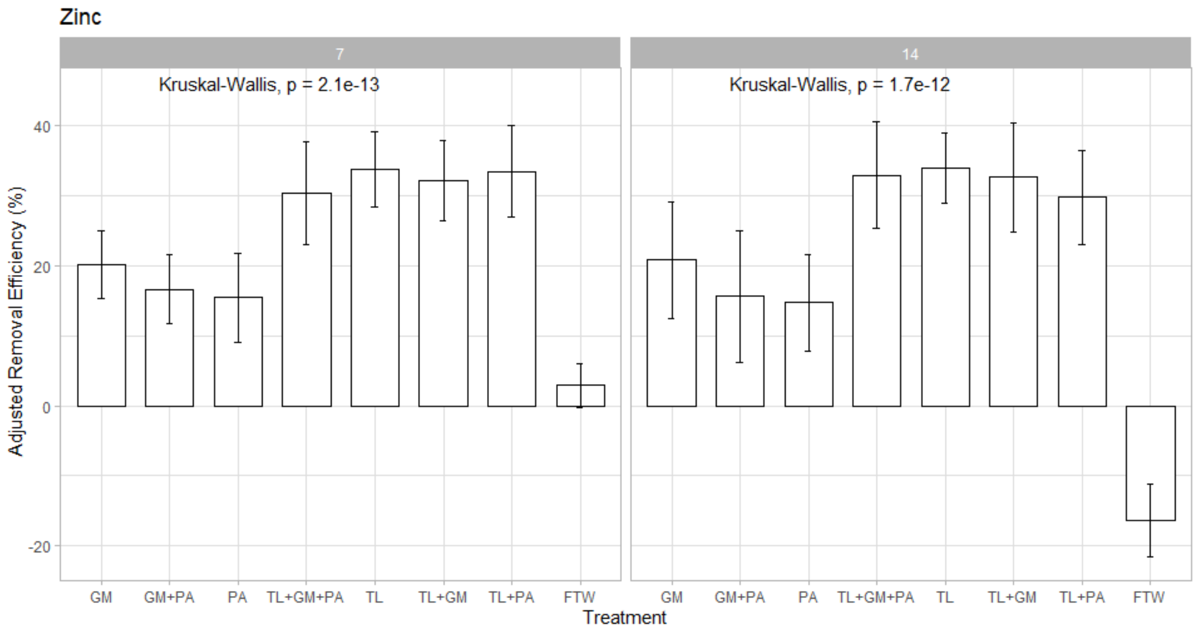


Figure A3.1 (D): Mean Adjusted removal efficiency (%) by treatment at 7 and 14 day HRT

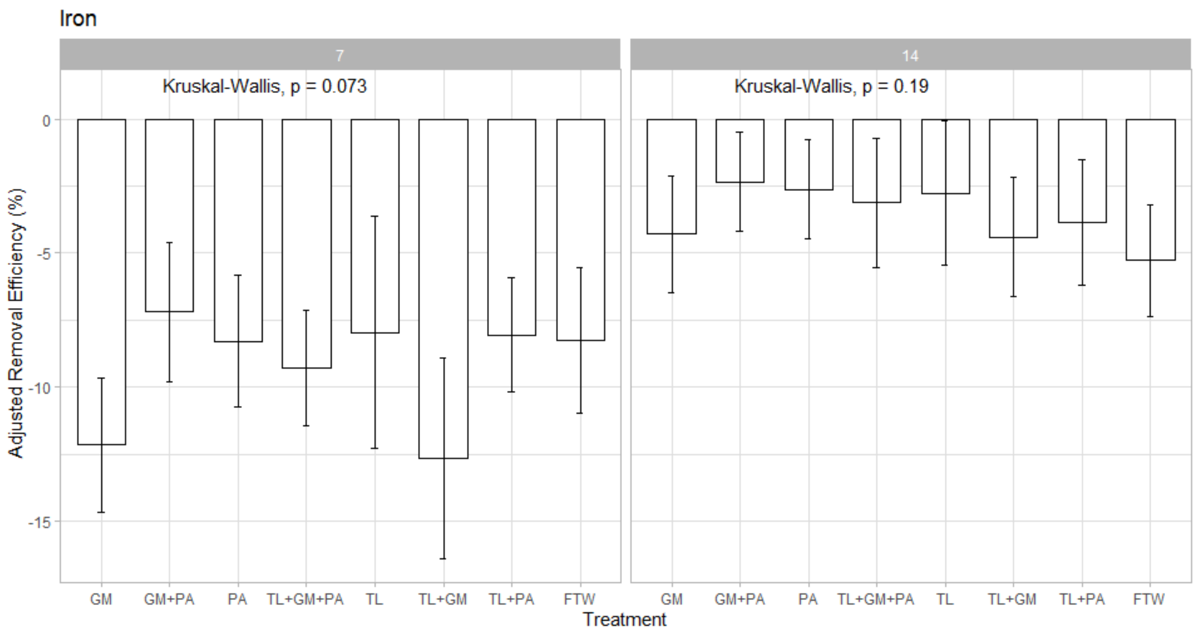


Figure A3.1 (E): Mean Adjusted removal efficiency (%) by treatment at 7 and 14 day HRT

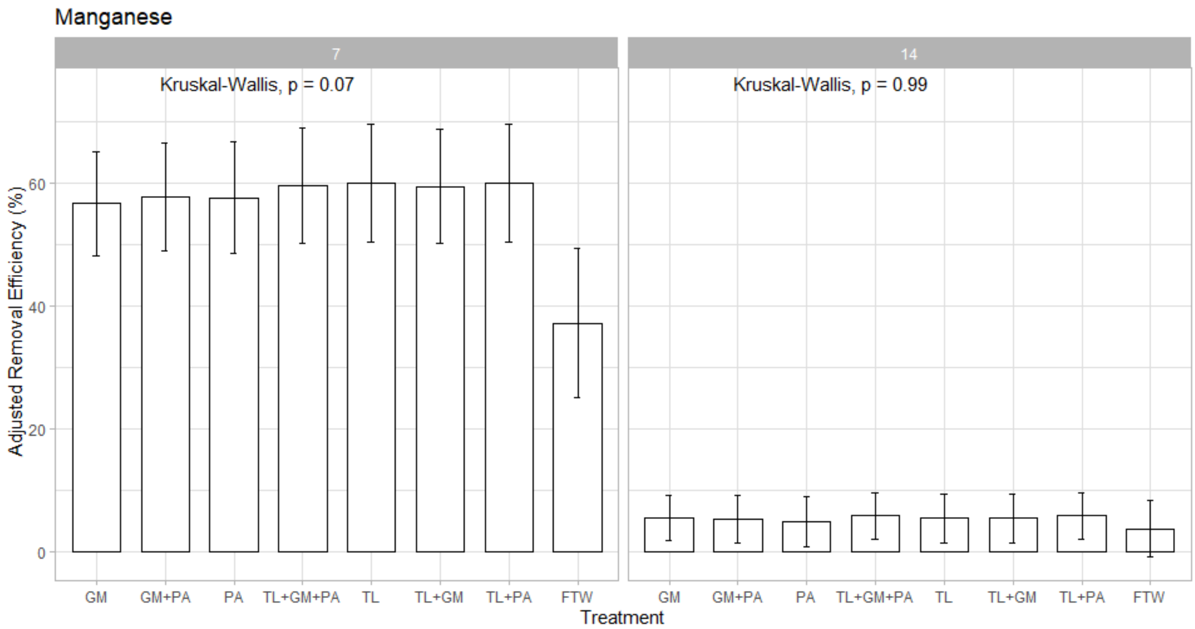


Figure A3.1 (F): Mean Adjusted removal efficiency (%) by treatment at 7 and 14 day HRT

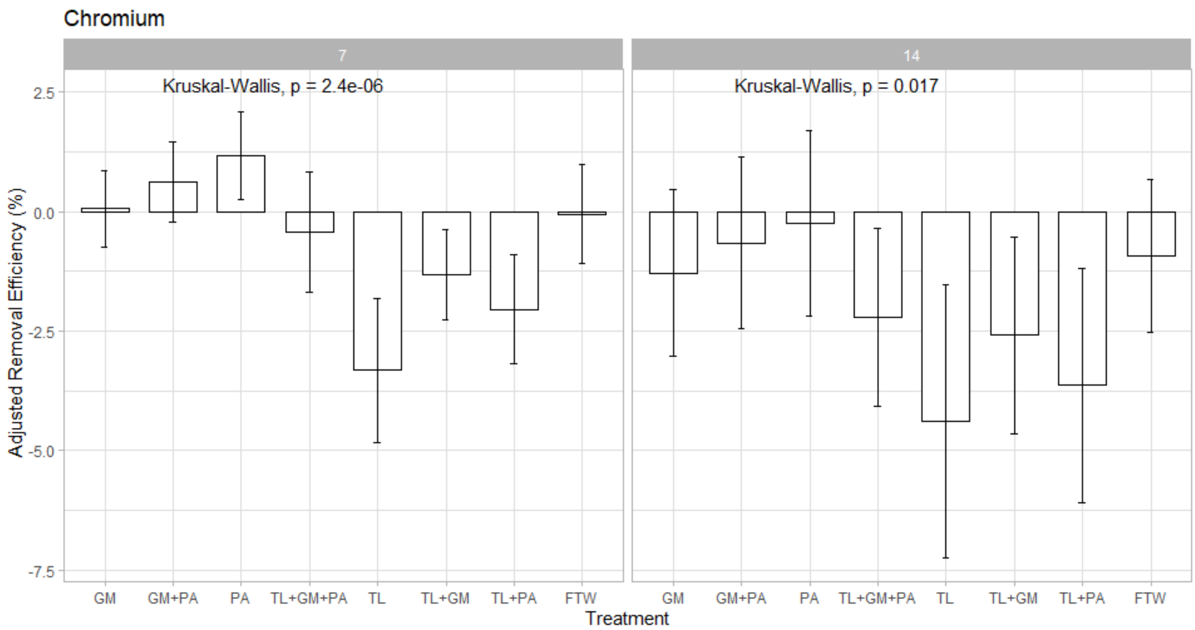


Figure A3.1 (G): Mean Adjusted removal efficiency (%) by treatment at 7 and 14 day HRT

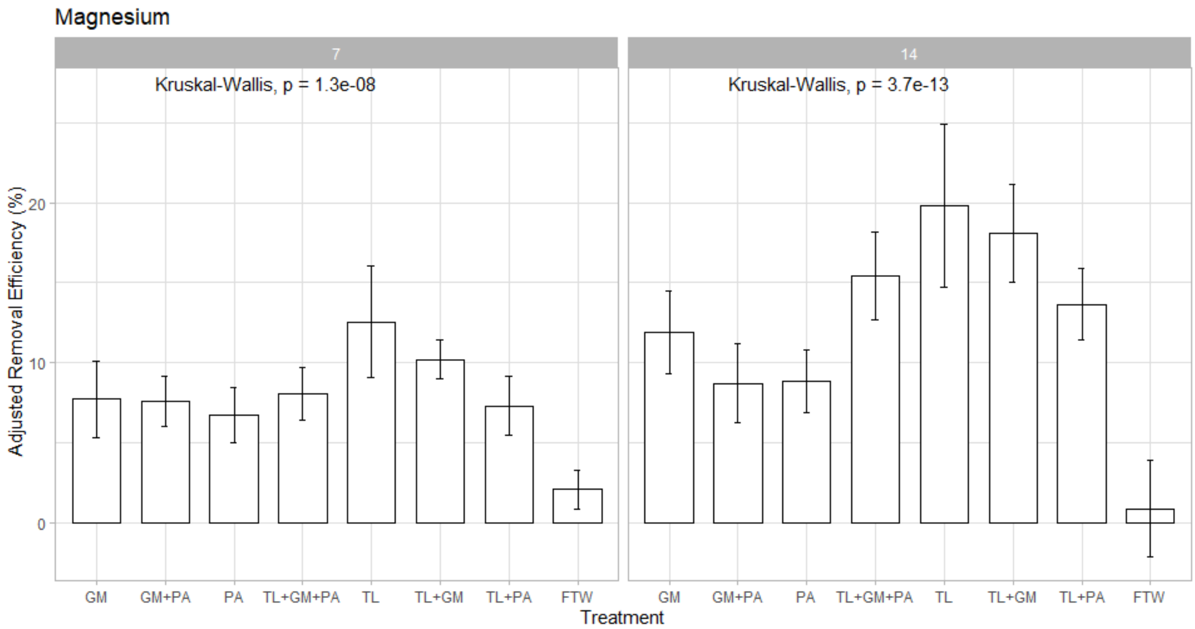


Figure A3.1 (H): Mean Adjusted removal efficiency (%) by treatment at 7 and 14 day HRT

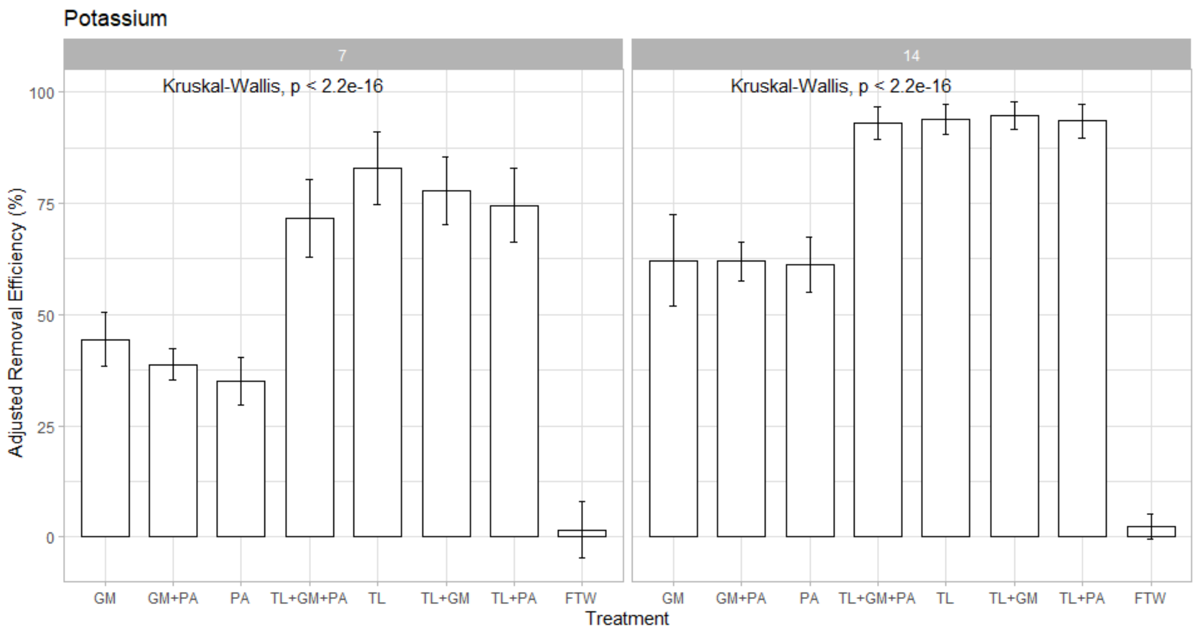


Figure A3.1 (I): Mean Adjusted removal efficiency (%) by treatment at 7 and 14 day HRT

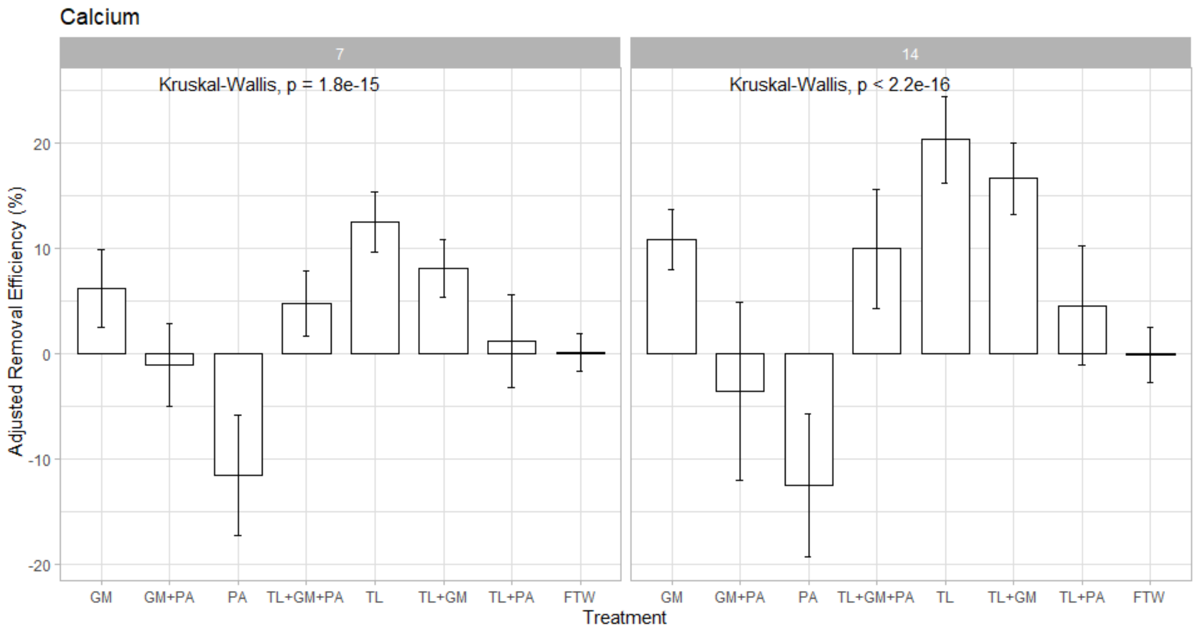


Figure A3.1 (J): Mean Adjusted removal efficiency (%) by treatment at 7 and 14 day HRT

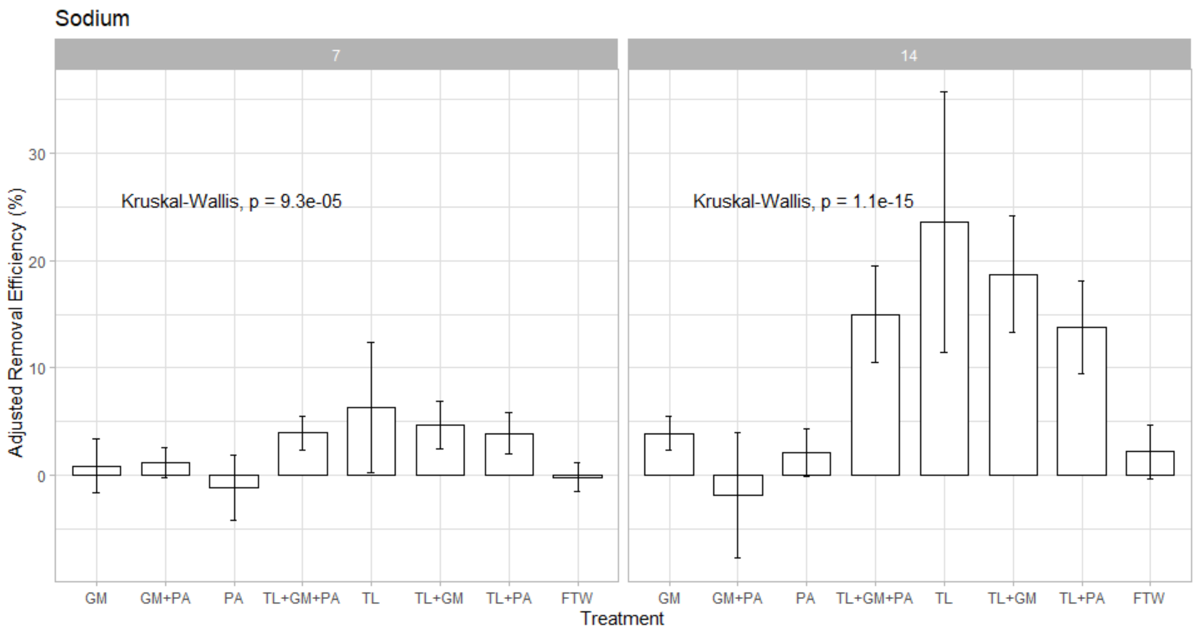


Figure A3.1 (K): Mean Adjusted removal efficiency (%) by treatment at 7 and 14 day HRT

Table A3.1-A: Model outputs for Treatment X Week ~ Zn multiple linear regression model

	Zn		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.65	0.23 – 1.07	**0.003**
treatment [FTW]	0.50	-0.09 – 1.10	0.099
treatment [GM]	-0.18	-0.78 – 0.42	0.550
treatment [GMPA]	0.30	-0.29 – 0.90	0.321
treatment [PA]	0.08	-0.52 – 0.67	0.800
treatment [PC]	-0.20	-0.79 – 0.40	0.519
treatment [TL]	-1.18	-1.77 – -0.58	**<0.001**
treatment [TLGM]	-0.36	-0.95 – 0.24	0.240
treatment [TLPA]	-0.42	-1.02 – 0.17	0.163
Week	0.04	-0.03 – 0.11	0.259
treatment [FTW] * Week	-0.03	-0.13 – 0.06	0.480
treatment [GM] * Week	-0.16	-0.26 – -0.06	**0.001**
treatment [GMPA] * Week	-0.20	-0.29 – -0.10	**<0.001**
treatment [PA] * Week	-0.15	-0.24 – -0.05	**0.003**
treatment [PC] * Week	-0.24	-0.34 – -0.15	**<0.001**
treatment [TL] * Week	-0.08	-0.17 – 0.02	0.117
treatment [TLGM] * Week	-0.22	-0.31 – -0.12	**<0.001**
treatment [TLPA] * Week	-0.20	-0.29 – -0.10	**<0.001**
Observations	358		
R^2 / R^2 adjusted	0.612 / 0.593		
RMSE	0.61		

Table A3.1 B: Model outputs for Treatment X Week ~ Ca multiple linear regression model

	Ca		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.42	-0.01 – 0.84	0.054
treatment [FTW]	0.15	-0.44 – 0.75	0.613
treatment [GM]	0.04	-0.56 – 0.64	0.898
treatment [GMPA]	1.02	0.42 – 1.62	**0.001**
treatment [PA]	2.34	1.74 – 2.94	**<0.001**
treatment [PC]	0.29	-0.31 – 0.89	0.339
treatment [TL]	-1.20	-1.80 – -0.61	**<0.001**
treatment [TLGM]	-0.24	-0.84 – 0.36	0.433
treatment [TLPA]	0.78	0.19 – 1.38	**0.010**
Week	-0.03	-0.10 – 0.03	0.323
treatment [FTW] * Week	-0.03	-0.12 – 0.07	0.583
treatment [GM] * Week	-0.12	-0.22 – -0.03	**0.013**
treatment [GMPA] * Week	-0.15	-0.25 – -0.06	**0.002**
treatment [PA] * Week	-0.26	-0.36 – -0.17	**<0.001**
treatment [PC] * Week	-0.15	-0.25 – -0.06	**0.002**
treatment [TL] * Week	-0.00	-0.10 – 0.09	0.928
treatment [TLGM] * Week	-0.12	-0.21 – -0.02	**0.019**
treatment [TLPA] * Week	-0.18	-0.28 – -0.08	**<0.001**
Observations	358		
R^2 / R^2 adjusted	0.611 / 0.592		
RSME	0.614		

Table A3.1- C: Model outputs for Treatment X Week ~ Mn multiple linear regression model

		Mn	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.85	0.36 – 1.33	**0.001**
treatment [FTW]	-0.51	-1.20 – 0.18	0.145
treatment [GM]	-1.16	-1.85 – -0.47	**0.001**
treatment [GMPA]	-1.14	-1.83 – -0.46	**0.001**
treatment [PA]	-1.06	-1.75 – -0.37	**0.003**
treatment [PC]	-1.17	-1.86 – -0.48	**0.001**
treatment [TL]	-1.13	-1.82 – -0.44	**0.001**
treatment [TLGM]	-1.14	-1.83 – -0.45	**0.001**
treatment [TLPA]	-1.17	-1.86 – -0.48	**0.001**
Week	0.11	0.03 – 0.19	**0.005**
treatment [FTW] * Week	-0.11	-0.22 – -0.00	**0.049**
treatment [GM] * Week	-0.10	-0.21 – 0.01	0.070
treatment [GMPA] * Week	-0.11	-0.22 – 0.00	0.054
treatment [PA] * Week	-0.12	-0.23 – -0.01	**0.030**
treatment [PC] * Week	-0.12	-0.23 – -0.01	**0.039**
treatment [TL] * Week	-0.12	-0.24 – -0.01	**0.030**
treatment [TLGM] * Week	-0.12	-0.23 – -0.01	**0.035**
treatment [TLPA] * Week	-0.12	-0.23 – -0.01	**0.037**
Observations	357		
R^2 / R^2 adjusted	0.407 / 0.377		
RMSE	0.705		

Table A3.1-D: Model outputs for Treatment X Week ~ P multiple linear regression model

		P	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.51	0.21 – 0.81	**0.001**
treatment [FTW]	-0.06	-0.49 – 0.38	0.802
treatment [GM]	-0.83	-1.25 – -0.41	**<0.001**
treatment [GMPA]	-0.68	-1.10 – -0.26	**0.002**
treatment [PA]	-0.35	-0.77 – 0.07	0.102
treatment [PC]	-0.92	-1.34 – -0.49	**<0.001**
treatment [TL]	-0.96	-1.38 – -0.54	**<0.001**
treatment [TLGM]	-0.95	-1.37 – -0.53	**<0.001**
treatment [TLPA]	-0.97	-1.39 – -0.55	**<0.001**
Week	0.09	0.04 – 0.14	**<0.001**
treatment [FTW] * Week	-0.01	-0.08 – 0.06	0.733
treatment [GM] * Week	-0.13	-0.20 – -0.06	**<0.001**
treatment [GMPA] * Week	-0.13	-0.20 – -0.07	**<0.001**
treatment [PA] * Week	-0.16	-0.22 – -0.09	**<0.001**
treatment [PC] * Week	-0.12	-0.19 – -0.06	**<0.001**
treatment [TL] * Week	-0.12	-0.19 – -0.05	**0.001**
treatment [TLGM] * Week	-0.12	-0.19 – -0.05	**0.001**
treatment [TLPA] * Week	-0.12	-0.18 – -0.05	**0.001**
Observations	355		
R^2 / R^2 adjusted	0.687 / 0.672		
RMSE	0.428		

Table A3.1-E: Model outputs for Treatment X Week ~ TIN multiple linear regression model

		TIN	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.85	0.38 – 1.31	**<0.001**
treatment [FTW]	0.57	-0.08 – 1.21	0.086
treatment [GM]	-1.14	-1.79 – -0.49	**0.001**
treatment [GMPA]	-1.18	-1.82 – -0.54	**<0.001**
treatment [PA]	-1.18	-1.82 – -0.54	**<0.001**
treatment [PC]	-1.18	-1.82 – -0.53	**<0.001**
treatment [TL]	-1.15	-1.80 – -0.51	**<0.001**
treatment [TLGM]	-1.14	-1.78 – -0.49	**0.001**
treatment [TLPA]	-1.11	-1.76 – -0.47	**0.001**
Week	0.01	-0.06 – 0.09	0.718
treatment [FTW] * Week	-0.03	-0.14 – 0.07	0.554
treatment [GM] * Week	-0.01	-0.11 – 0.10	0.861
treatment [GMPA] * Week	-0.03	-0.13 – 0.08	0.587
treatment [PA] * Week	-0.02	-0.13 – 0.08	0.646
treatment [PC] * Week	-0.03	-0.13 – 0.08	0.591
treatment [TL] * Week	-0.03	-0.14 – 0.07	0.541
treatment [TLGM] * Week	-0.03	-0.14 – 0.07	0.513
treatment [TLPA] * Week	-0.04	-0.14 – 0.06	0.456
Observations	348		
R^2 / R^2 adjusted	0.485 / 0.459		
RMSE	0.637		

Table A3.1-F: Model outputs for Treatment X Week ~ Mg multiple linear regression model

		Mg	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1.24	0.73 – 1.74	**<0.001**
treatment [FTW]	0.05	-0.67 – 0.76	0.897
treatment [GM]	-0.46	-1.18 – 0.25	0.202
treatment [GMPA]	-0.56	-1.28 – 0.15	0.121
treatment [PA]	-0.81	-1.52 – -0.09	**0.027**
treatment [PC]	-0.77	-1.49 – -0.06	**0.034**
treatment [TL]	-2.20	-2.92 – -1.49	**<0.001**
treatment [TLGM]	-1.39	-2.11 – -0.68	**<0.001**
treatment [TLPA]	-0.99	-1.70 – -0.27	**0.007**
Week	-0.08	-0.16 – 0.00	0.057
treatment [FTW] * Week	-0.03	-0.15 – 0.08	0.564
treatment [GM] * Week	-0.09	-0.20 – 0.03	0.133
treatment [GMPA] * Week	-0.04	-0.16 – 0.07	0.486
treatment [PA] * Week	0.01	-0.11 – 0.12	0.869
treatment [PC] * Week	-0.07	-0.18 – 0.05	0.254
treatment [TL] * Week	0.10	-0.02 – 0.21	0.101
treatment [TLGM] * Week	0.02	-0.10 – 0.13	0.794
treatment [TLPA] * Week	-0.01	-0.12 – 0.11	0.923
Observations	358		
R^2 / R^2 adjusted	0.387 / 0.356		
RMSE	0.733		

Table A3.1- G: Model outputs for Treatment X Week ~ K multiple linear regression model

		K	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1.54	1.29 – 1.79	**<0.001**
treatment [FTW]	-0.10	-0.45 – 0.25	0.582
treatment [GM]	-2.03	-2.39 – -1.68	**<0.001**
treatment [GMPA]	-1.54	-1.90 – -1.19	**<0.001**
treatment [PA]	-1.48	-1.83 – -1.13	**<0.001**
treatment [PC]	-2.03	-2.38 – -1.67	**<0.001**
treatment [TL]	-2.47	-2.83 – -2.12	**<0.001**
treatment [TLGM]	-2.29	-2.64 – -1.94	**<0.001**
treatment [TLPA]	-2.07	-2.42 – -1.72	**<0.001**
Week	-0.04	-0.08 – 0.00	0.083
treatment [FTW] * Week	0.00	-0.05 – 0.06	0.958
treatment [GM] * Week	0.10	0.04 – 0.16	**0.001**
treatment [GMPA] * Week	0.03	-0.02 – 0.09	0.240
treatment [PA] * Week	0.03	-0.02 – 0.09	0.251
treatment [PC] * Week	-0.03	-0.09 – 0.02	0.256
treatment [TL] * Week	0.02	-0.03 – 0.08	0.457
treatment [TLGM] * Week	-0.00	-0.06 – 0.05	0.948
treatment [TLPA] * Week	-0.03	-0.09 – 0.03	0.291
Observations	357		
R^2 / R^2 adjusted	0.857 / 0.850		
RMSE	0.356		

Table A3.1-H: Model outputs for Treatment X Week ~ Na multiple linear regression model

		Na	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.41	-0.04 – 0.87	0.076
treatment [FTW]	-0.01	-0.66 – 0.64	0.974
treatment [GM]	-0.01	-0.66 – 0.64	0.974
treatment [GMPA]	-0.14	-0.79 – 0.51	0.669
treatment [PA]	-0.27	-0.93 – 0.39	0.426
treatment [PC]	-0.56	-1.22 – 0.09	0.092
treatment [TL]	-1.88	-2.60 – -1.15	**<0.001**
treatment [TLGM]	-1.26	-1.91 – -0.62	**<0.001**
treatment [TLPA]	-0.84	-1.50 – -0.19	**0.011**
Week	0.01	-0.07 – 0.08	0.894
treatment [FTW] * Week	-0.01	-0.12 – 0.09	0.789
treatment [GM] * Week	-0.04	-0.14 – 0.07	0.493
treatment [GMPA] * Week	0.01	-0.09 – 0.12	0.792
treatment [PA] * Week	0.03	-0.07 – 0.14	0.561
treatment [PC] * Week	-0.03	-0.13 – 0.08	0.597
treatment [TL] * Week	0.15	0.03 – 0.26	**0.011**
treatment [TLGM] * Week	0.04	-0.06 – 0.14	0.453
treatment [TLPA] * Week	0.01	-0.10 – 0.11	0.893
Observations	346		
R^2 / R^2 adjusted	0.301 / 0.265		
RMSE	0.664		

Table A3.1- I: Model outputs for Treatment X Week ~ Fe multiple linear regression model

	Fe		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	-0.80	-1.42 – -0.19	**0.010**
treatment [FTW]	0.26	-0.60 – 1.13	0.550
treatment [GM]	0.33	-0.54 – 1.19	0.457
treatment [GMPA]	0.46	-0.41 – 1.33	0.295
treatment [PA]	0.08	-0.79 – 0.95	0.861
treatment [PC]	0.18	-0.69 – 1.05	0.691
treatment [TL]	-0.28	-1.15 – 0.59	0.529
treatment [TLGM]	0.21	-0.66 – 1.08	0.635
treatment [TLPA]	0.23	-0.65 – 1.11	0.608
Week	0.04	-0.06 – 0.14	0.432
treatment [FTW] * Week	0.08	-0.06 – 0.22	0.266
treatment [GM] * Week	0.10	-0.04 – 0.24	0.164
treatment [GMPA] * Week	-0.01	-0.15 – 0.13	0.912
treatment [PA] * Week	0.08	-0.06 – 0.22	0.251
treatment [PC] * Week	0.09	-0.06 – 0.23	0.230
treatment [TL] * Week	0.14	-0.00 – 0.28	0.058
treatment [TLGM] * Week	0.13	-0.01 – 0.27	0.067
treatment [TLPA] * Week	0.06	-0.08 – 0.20	0.401
Observations	349		
R^2 / R^2 adjusted	0.197 / 0.155		
RMSE	0.886		

Table A3.1- J: Model outputs for Treatment X Week ~ Cu multiple linear regression model

	Cu		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1.26	0.82 – 1.71	**<0.001**
treatment [FTW]	0.12	-0.51 – 0.75	0.699
treatment [GM]	-1.84	-2.47 – -1.21	**<0.001**
treatment [GMPA]	-2.01	-2.64 – -1.38	**<0.001**
treatment [PA]	-2.39	-3.02 – -1.76	**<0.001**
treatment [PC]	-1.96	-2.59 – -1.33	**<0.001**
treatment [TL]	-1.67	-2.30 – -1.04	**<0.001**
treatment [TLGM]	-1.81	-2.45 – -1.16	**<0.001**
treatment [TLPA]	-1.80	-2.45 – -1.15	**<0.001**
Week	-0.03	-0.10 – 0.04	0.446
treatment [FTW] * Week	-0.04	-0.14 – 0.06	0.403
treatment [GM] * Week	0.17	0.07 – 0.27	**0.001**
treatment [GMPA] * Week	0.11	0.00 – 0.21	**0.041**
treatment [PA] * Week	0.08	-0.02 – 0.18	0.138
treatment [PC] * Week	0.11	0.01 – 0.22	**0.027**
treatment [TL] * Week	0.12	0.02 – 0.23	**0.017**
treatment [TLGM] * Week	0.13	0.03 – 0.24	**0.011**
treatment [TLPA] * Week	0.08	-0.02 – 0.19	0.114
Observations	357		
R^2 / R^2 adjusted	0.495 / 0.470		
RMSE	0.646		

Table A3.1- K: Model outputs for Treatment X Week ~ Cr multiple linear regression model

		Cr	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	-0.29	-0.75 – 0.18	0.227
treatment [FTW]	0.16	-0.49 – 0.82	0.627
treatment [GM]	-0.23	-0.87 – 0.40	0.470
treatment [GMPA]	-0.37	-1.01 – 0.27	0.256
treatment [PA]	-0.84	-1.48 – -0.20	**0.010**
treatment [PC]	-0.10	-0.74 – 0.53	0.750
treatment [TL]	-0.52	-1.16 – 0.12	0.111
treatment [TLGM]	-0.32	-0.96 – 0.32	0.326
treatment [TLPA]	-0.46	-1.10 – 0.17	0.153
Week	-0.08	-0.15 – -0.00	**0.038**
treatment [FTW] * Week	0.05	-0.06 – 0.15	0.385
treatment [GM] * Week	0.14	0.03 – 0.24	**0.009**
treatment [GMPA] * Week	0.12	0.02 – 0.23	**0.018**
treatment [PA] * Week	0.17	0.07 – 0.28	**0.001**
treatment [PC] * Week	0.17	0.07 – 0.28	**0.001**
treatment [TL] * Week	0.42	0.31 – 0.52	**<0.001**
treatment [TLGM] * Week	0.25	0.14 – 0.35	**<0.001**
treatment [TLPA] * Week	0.34	0.23 – 0.44	**<0.001**
Observations	355		
R^2 / R^2 adjusted	0.575 / 0.553		
RMSE	0.632		

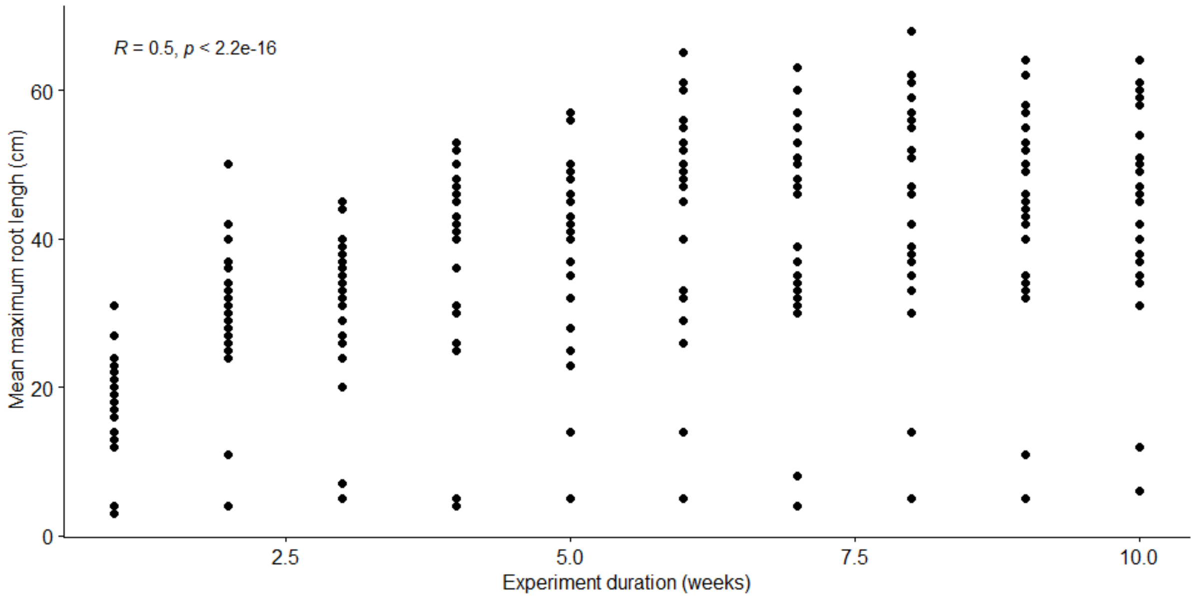


Figure A3.2: Scatter plot showing relationship between experiment duration (weeks) and mean maximum plant root growth per treatment replicate.

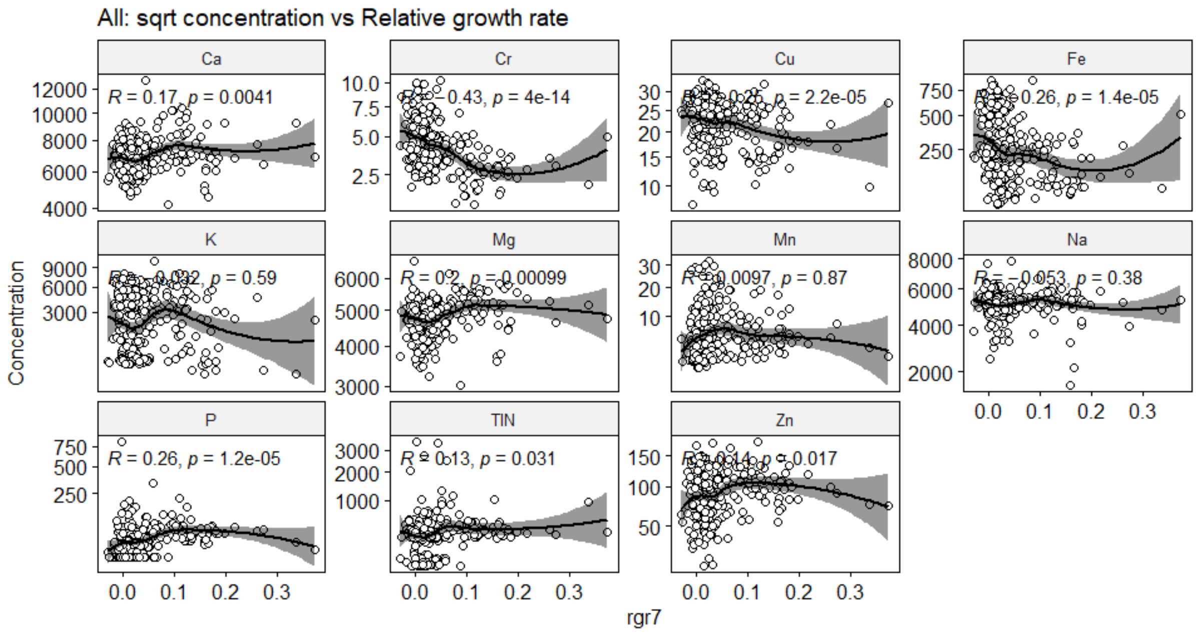


Figure A3.3: Scatter plot series showing relationship between weekly relative growth rate and pollutant concentrations by each pollutant studies. Correlation coefficients and p values are contained within each plot.

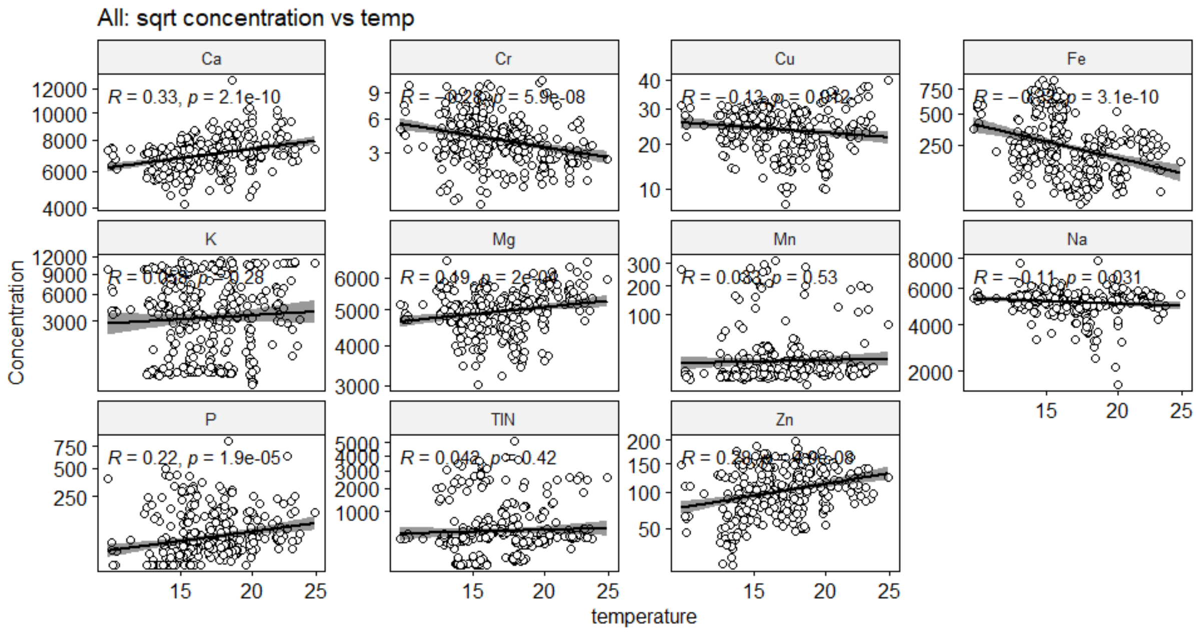


Figure A3.4: Scatter plot series showing relationship between weekly relative growth rate and pollutant concentrations by each pollutant studies. Correlation coefficients and p values are contained within each plot. Fitted line based on a simple linear regression.

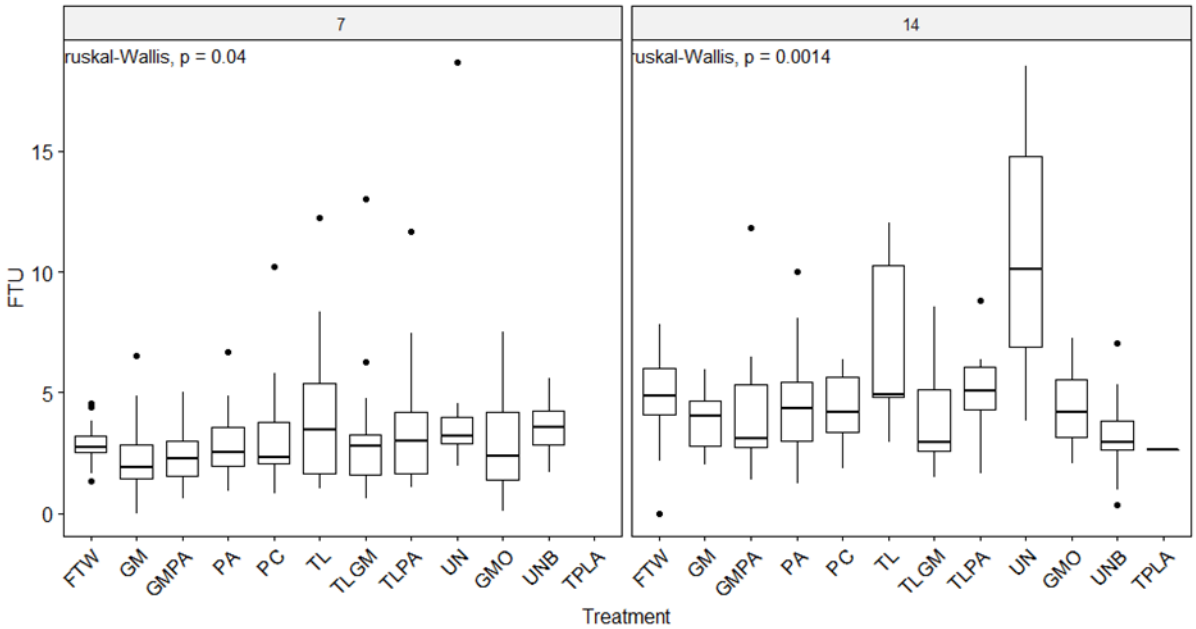


Figure A3.5: Box plots showing turbidity Units (FTU) by treatment aggregated by hydraulic retention times 7 and 14 days.

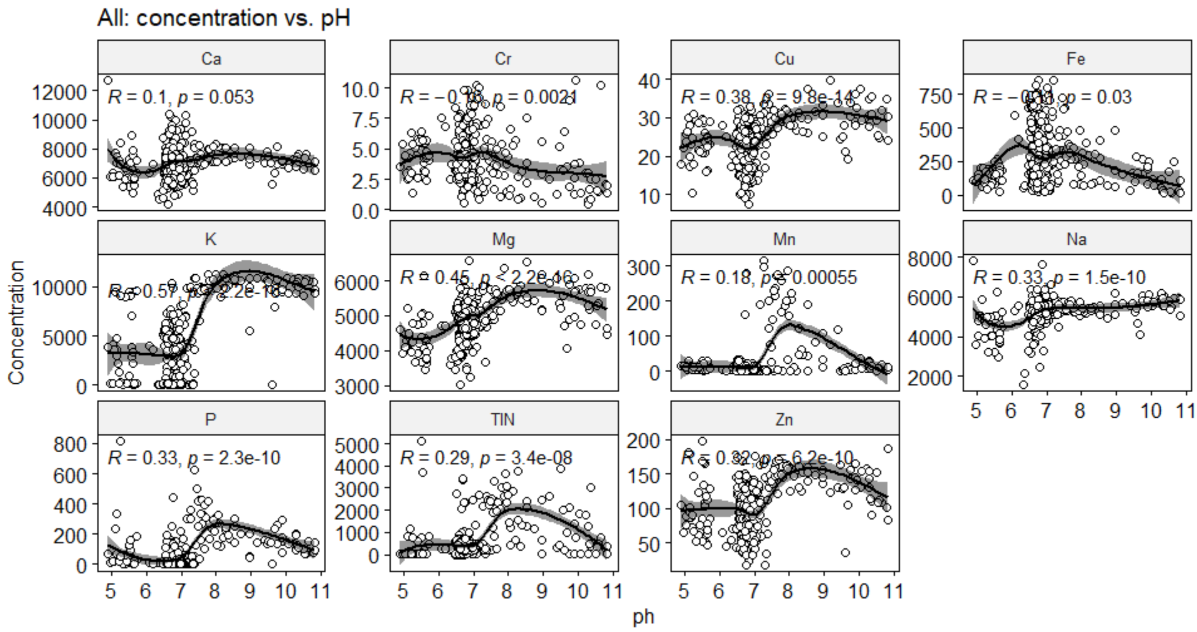


Figure A3.6: Scatter plot series showing relationship between pH units and pollutant concentrations by each pollutant studies. Correlation coefficients and p-values are contained within each plot.

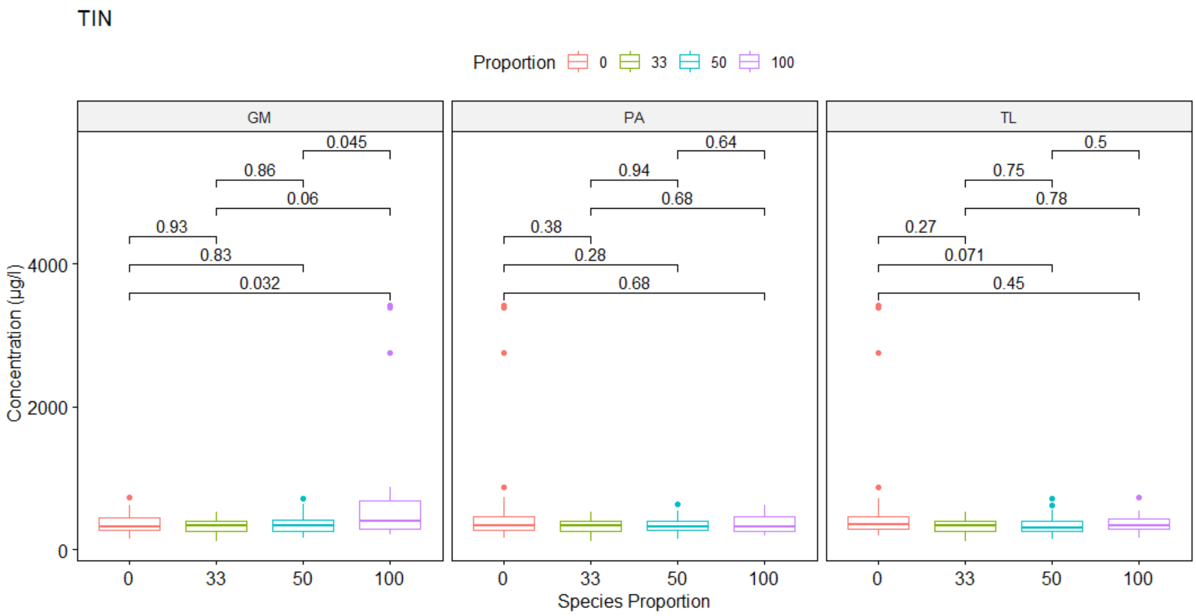


Figure A3.7-A: Pollutant concentration (ug/l) by proportion of each species in the community. P values are displayed for post-hoc test is shown above each.

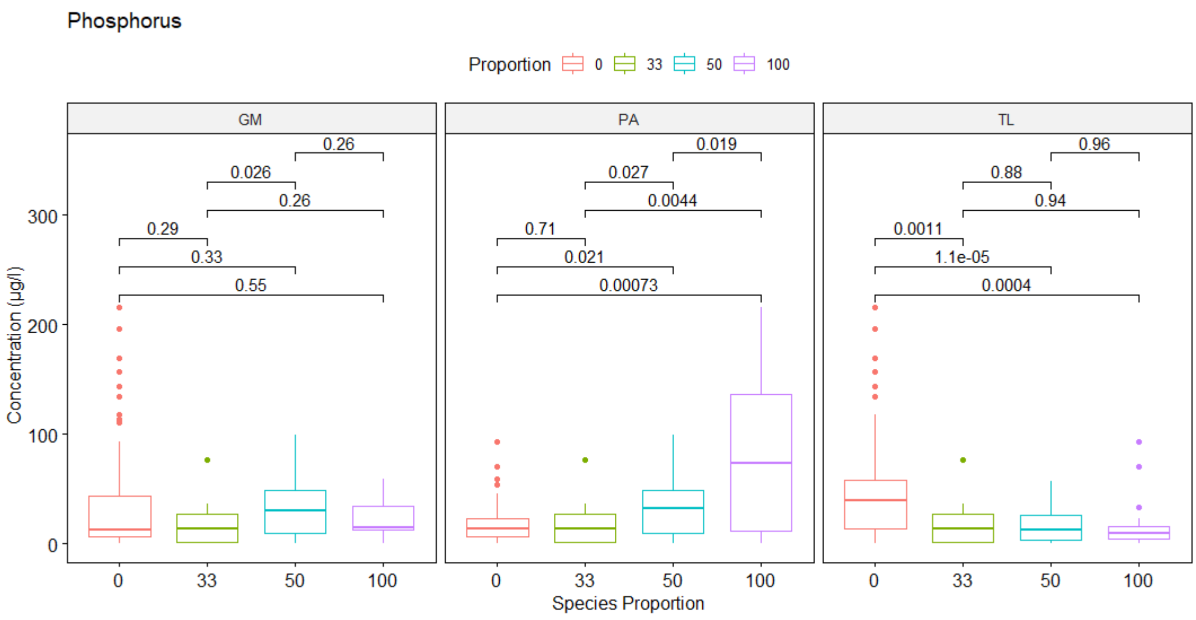


Figure A3.7-B: Pollutant concentration (ug/l) by proportion of each species in the community. P values are displayed for post-hoc test is shown above each.

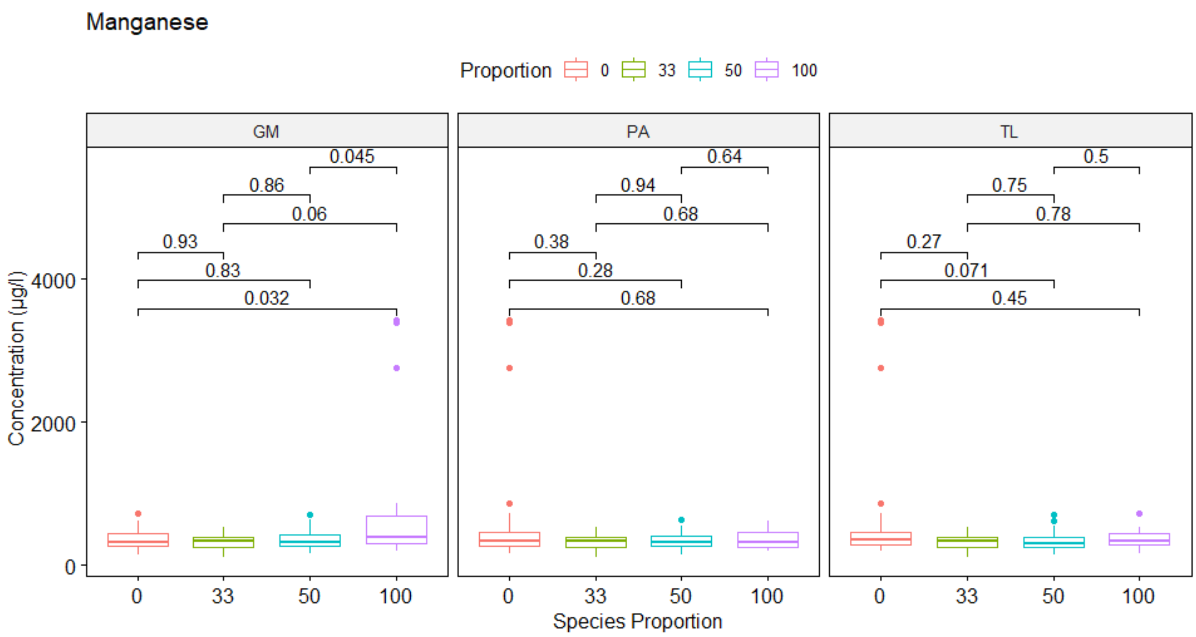


Figure A3.7-C: Pollutant concentration (ug/l) by proportion of each species in the community. P values are displayed for post-hoc test is shown above each.

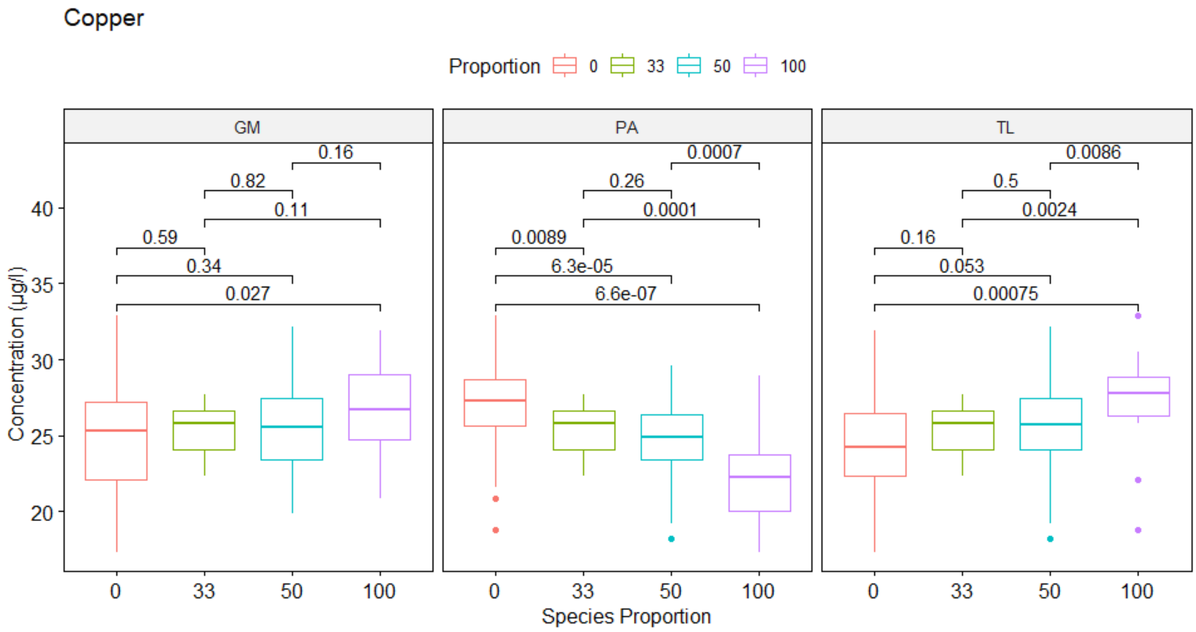


Figure A3.7-D: Pollutant concentration (ug/l) by proportion of each species in the community. P values are displayed for post-hoc test is shown above each.

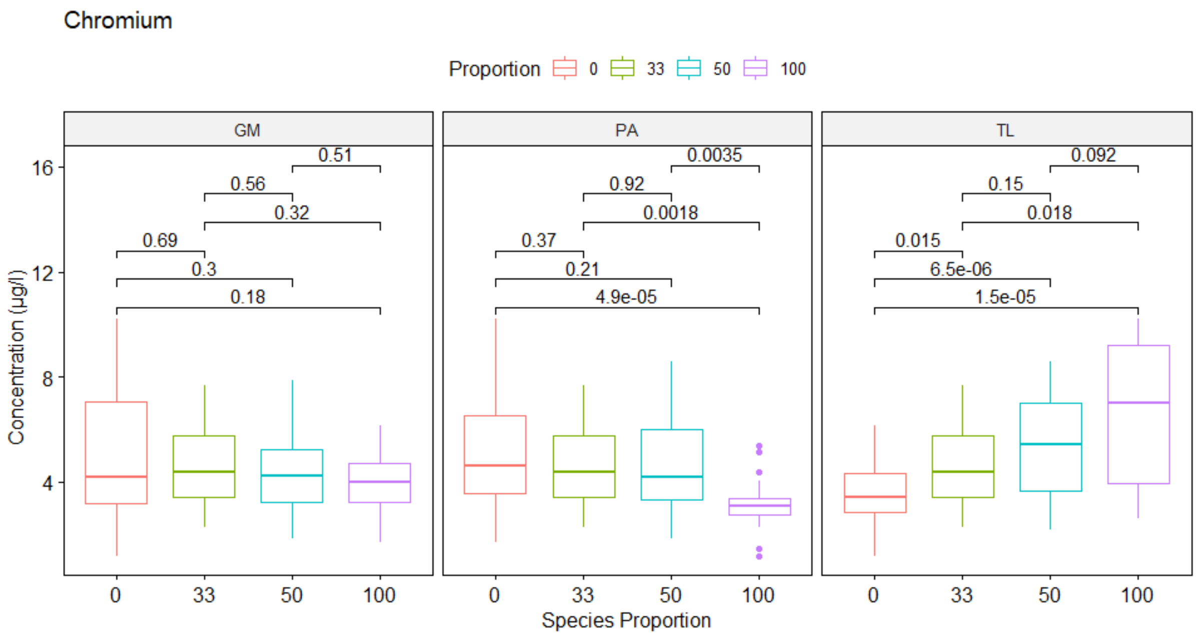


Figure A3.7-E: Pollutant concentration (ug/l) by proportion of each species in the community. P values are displayed for post-hoc test is shown above each.

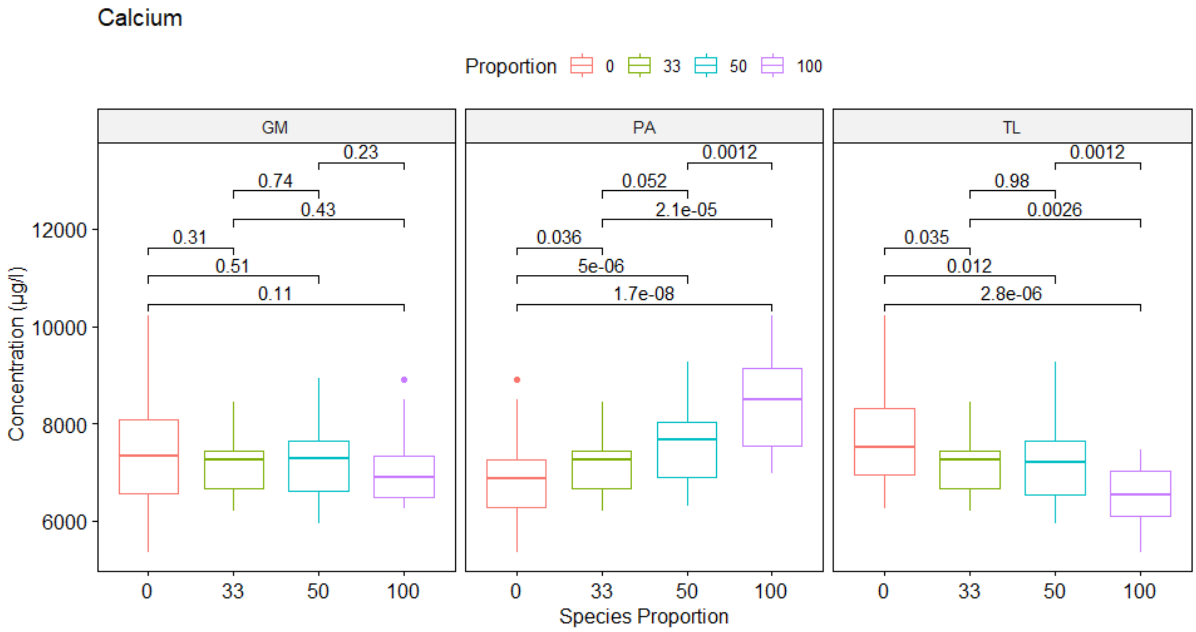


Figure A3.7-F: Pollutant concentration (ug/l) by proportion of each species in the community. P values are displayed for post-hoc test is shown above each.

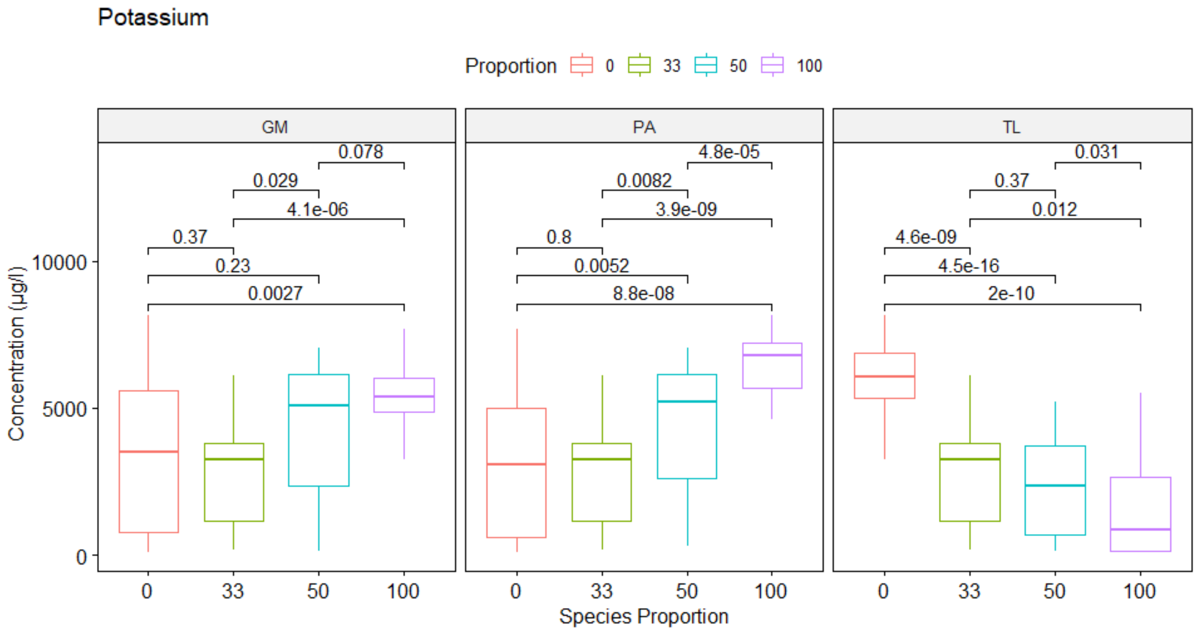


Figure A3.7-G: Pollutant concentration (ug/l) by proportion of each species in the community. P values are displayed for post-hoc test is shown above each.

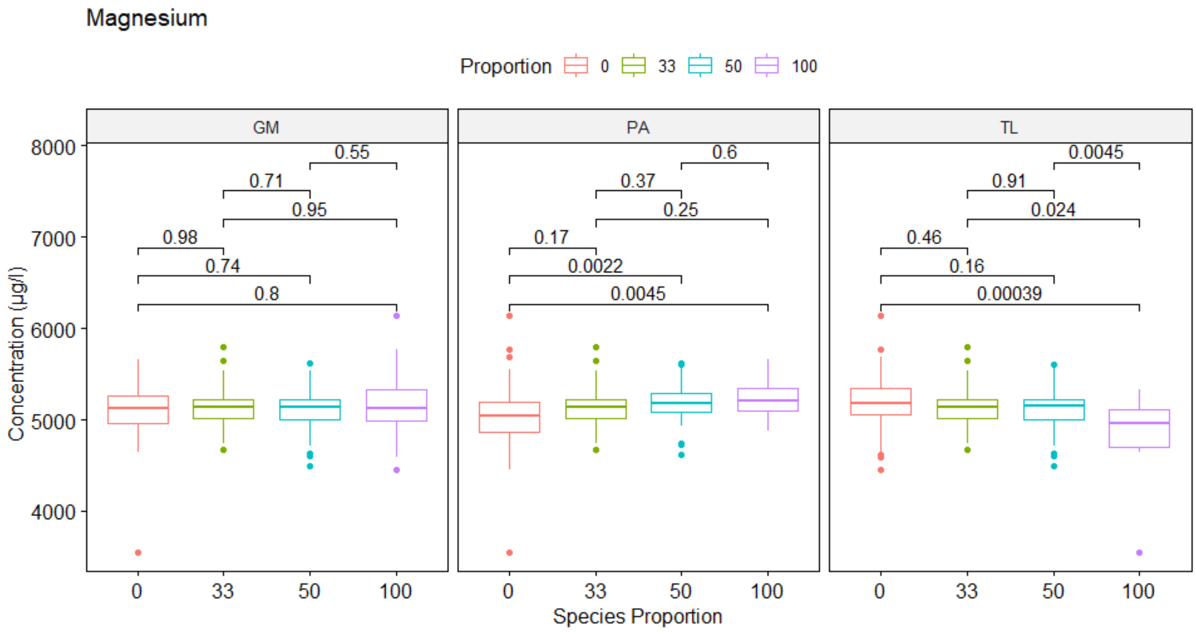


Figure A3.7-H: Pollutant concentration (ug/l) by proportion of each species in the community. P values are displayed for post-hoc test is shown above each.

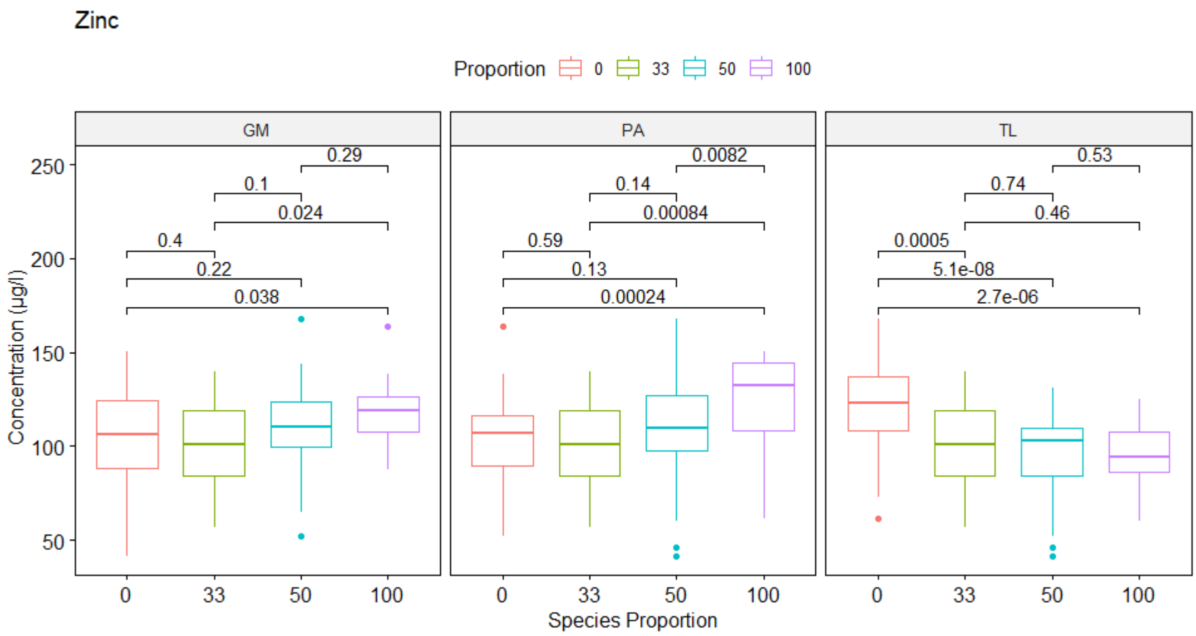


Figure A3.7-I: Pollutant concentration (ug/l) by proportion of each species in the community. P values are displayed for post-hoc test is shown above each.

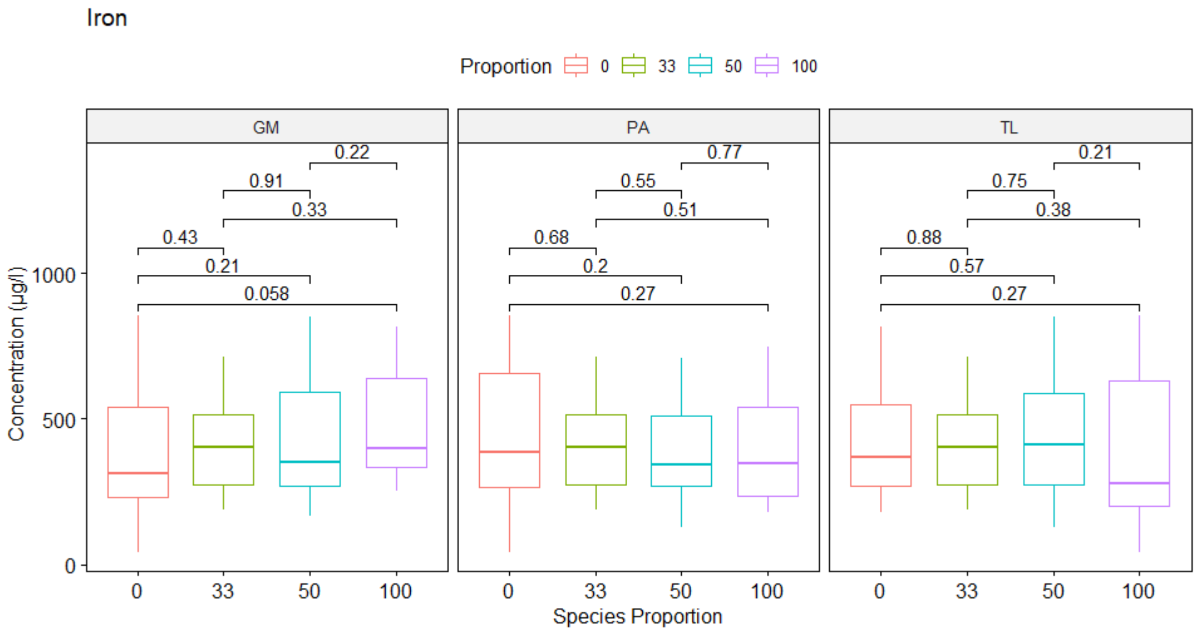


Figure A3.7-J: Pollutant concentration (ug/l) by proportion of each species in the community. P values are displayed for post-hoc test is shown above each.

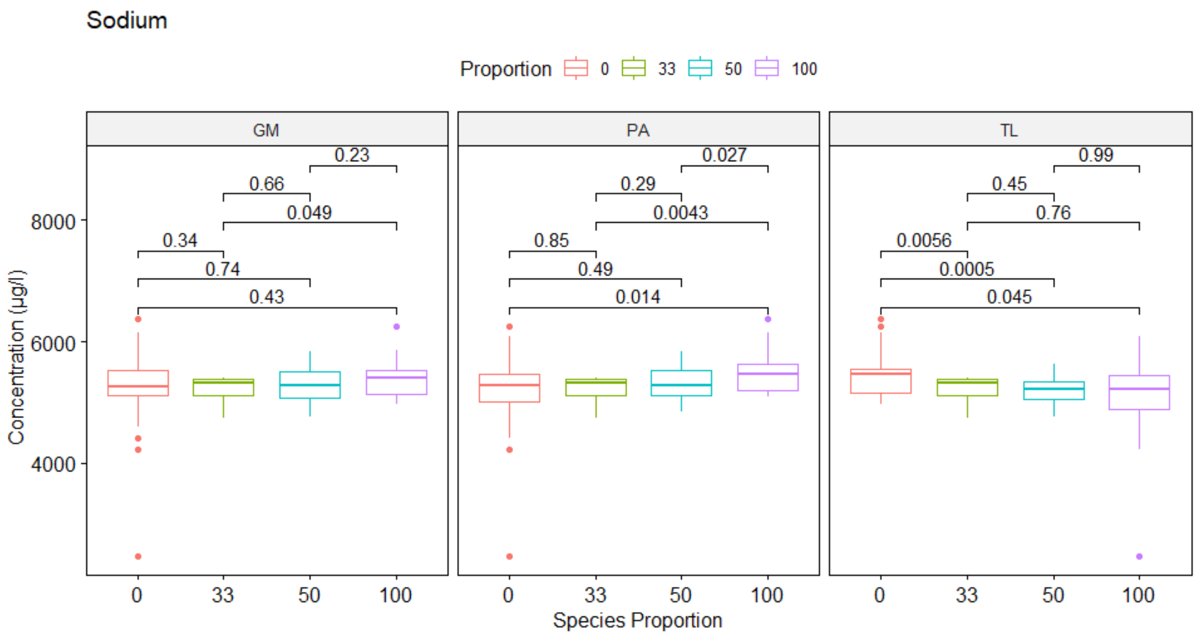


Figure A3.7-K: Pollutant concentration (ug/l) by proportion of each species in the community. P values are displayed for post-hoc test is shown above each.

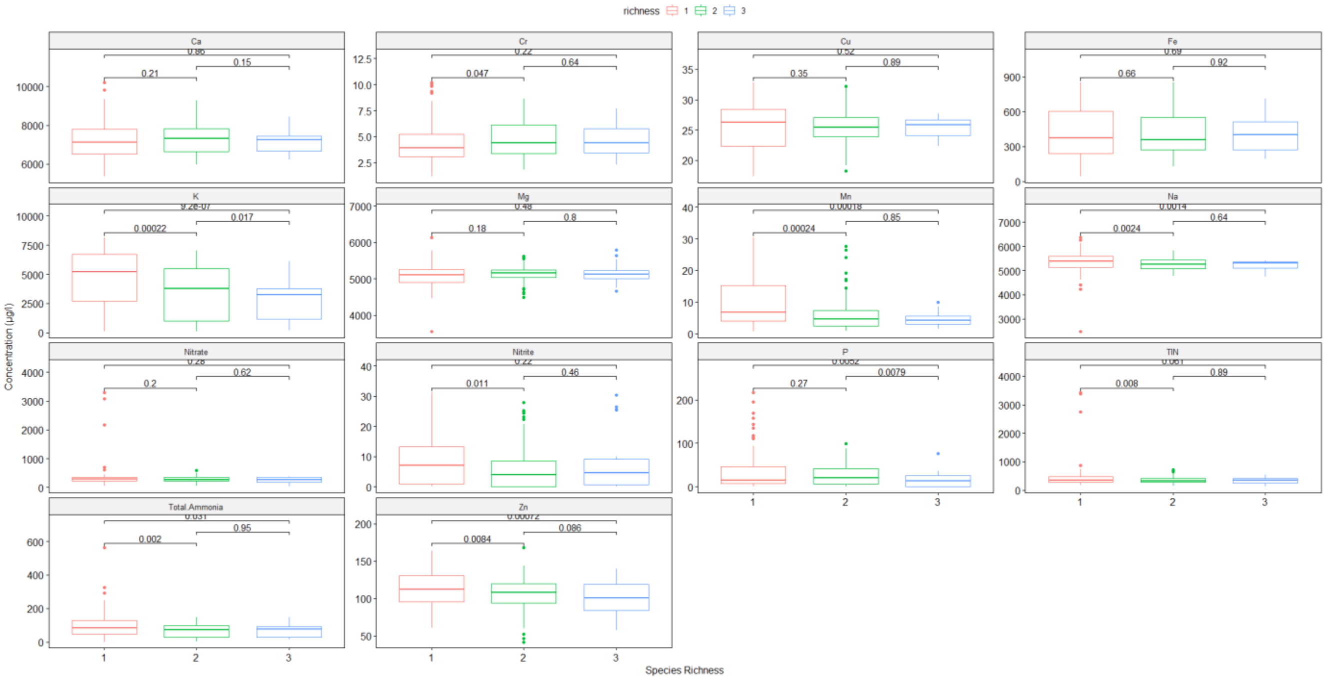


Figure A3.8: Pollutant concentration (ug/l) by species richness for each studied pollutant. P values are displayed for post-hoc test is shown above each.

Table A3.2: Mass balance component by planted treatment for each pollutant

Pollutant	**Treatment**						
P (mg)							
Mass balance	**GM**	**GMPA**	**PA**	**PC**	**TL**	**TLGM**	**TLPA**
Total influent load (mg)	1,359.6	671.6	693.1	518.5	464.2	486.9	1,347.9
Total effluent load (mg)	11.7	17.6	18.7	9.7	8.9	9.2	19.4
Total load reduction (mg)	1,347.9	654.0	674.5	508.8	455.4	477.8	1,328.5
Plant uptake-load (mg)	99.0	79.3	81.5	285.1	217.8	195.3	234.1
Plant uptake-%	7.3	12.1	12.1	56.0	47.8	40.9	17.6
Other processes-load	1,248.9	574.7	593.0	223.8	237.5	282.4	1,094.4
Other processes-%	92.7	87.9	87.9	44.0	52.2	59.1	82.4
TIN (mg)							
Mass balance	**GM**	**GMPA**	**PA**	**PC**	**TL**	**TLGM**	**TLPA**

Total influent load (mg)	6,051.1	4,909.1	5,568.1	5,249.9	5,611.4	4,771.6	5,759.2
Total effluent load (mg)	159.6	74.7	87.8	72.5	69.6	70.6	83.1
Total load reduction (mg)	5,891.5	4,834.4	5,480.2	5,177.5	5,541.8	4,701.0	5,676.0
Plant uptake-load (mg)	1,570.4	1,804.6	2,309.2	1,810.1	1,262.1	1,398.3	1,845.4
Plant uptake-%	26.7	37.3	42.1	35.0	22.8	29.7	32.5
Other processes-load	4,321.1	3,029.8	3,171.0	3,367.4	4,279.6	3,302.7	3,830.6
Other processes-%	73.3	62.7	57.9	65.0	77.2	70.3	67.5
Ca (mg)							
Mass balance	**GM**	**GMPA**	**PA**	**PC**	**TL**	**TLGM**	**TLPA**
Total influent load (mg)	87,479.2	98,562.1	102,862.3	83,063.9	75,845.2	80,160.4	92,367.9
Total effluent load (mg)	1,645.6	1,959.6	2,153.7	1,650.4	1,464.9	1,545.3	1,788.2
Total load reduction (mg)	85,833.6	96,602.5	100,708.6	81,413.5	74,380.3	78,615.0	90,579.8
Plant uptake-load (mg)	389.3	435.1	233.9	1,327.1	1,098.9	858.8	1,083.8
Plant uptake-%	0.5	0.5	0.2	1.6	1.5	1.1	1.2
Other processes-load	85,444.3	96,167.5	100,474.7	80,086.4	73,281.4	77,756.2	89,495.9
Other processes-%	99.5	99.5	99.8	98.4	98.5	98.9	98.8
Cr (mg)							
Mass balance	**GM**	**GMPA**	**PA**	**PC**	**TL**	**TLGM**	**TLPA**
Total influent load (mg)	41.0	38.9	41.5	58.8	67.8	57.4	56.8
Total effluent load (mg)	0.9	0.8	0.7	1.1	1.4	1.1	1.3
Total load reduction (mg)	40.1	38.1	40.8	57.7	66.4	56.3	55.5
Plant uptake-load (mg)	2.7	5.1	3.9	10.3	4.9	5.3	7.2
Plant uptake-%	6.6	13.4	9.5	17.8	7.3	9.4	12.9
Other processes-load	37.5	33.0	36.9	47.4	61.5	51.0	48.4
Other processes-%	93.4	86.6	90.5	82.2	92.7	90.6	87.1
Fe (mg)							
Mass balance	**GM**	**GMPA**	**PA**	**PC**	**TL**	**TLGM**	**TLPA**

Total influent load (mg)	1,686.5	1,538.4	1,602.3	1,669.5	1,788.6	2,192.5	1,550.7
Total effluent load (mg)	38.7	30.4	31.8	34.1	31.9	41.1	38.5
Total load reduction (mg)	1,647.8	1,508.0	1,570.5	1,635.4	1,756.7	2,151.4	1,512.1
Plant uptake-load (mg)	-20.6	292.4	150.2	844.0	237.3	231.5	702.5
Plant uptake-%	-1.2	19.4	9.6	51.6	13.5	10.8	46.5
Other processes-load	1,668.4	1,215.6	1,420.3	791.4	1,519.3	1,919.9	809.7
Other processes-%	101.2	80.6	90.4	48.4	86.5	89.2	53.5
Cu (mg)							
Mass balance	**GM**	**GMPA**	**PA**	**PC**	**TL**	**TLGM**	**TLPA**
Total influent load (mg)	273.5	240.5	182.6	250.1	268.9	273.4	268.1
Total effluent load (mg)	5.6	4.6	3.6	4.8	5.4	5.4	4.9
Total load reduction (mg)	267.9	236.0	179.0	245.3	263.5	268.0	263.2
Plant uptake-load (mg)	2.8	4.5	6.8	6.3	5.1	3.0	6.5
Plant uptake-%	1.0	1.9	3.8	2.6	1.9	1.1	2.5
Other processes-load	265.1	231.5	172.2	238.9	258.4	265.0	256.8
Other processes-%	99.0	98.1	96.2	97.4	98.1	98.9	97.5
K (mg)							
Mass balance	**GM**	**GMPA**	**PA**	**PC**	**TL**	**TLGM**	**TLPA**
Total influent load (mg)	66,340.7	44,218.6	30,260.2	7,903.0	4,646.7	5,558.1	35,782.4
Total effluent load (mg)	800.5	895.9	890.8	90.5	84.2	53.1	89.3
Total load reduction (mg)	65,540.3	43,322.7	29,369.5	7,812.5	4,562.5	5,505.0	35,693.1
Plant uptake-load (mg)	884.1	798.8	-337.2	4,215.3	3,921.6	3,616.8	4,032.5
Plant uptake-%	1.3	1.8	-1.1	54.0	86.0	65.7	11.3
Other processes-load	64,656.1	42,523.9	29,706.7	3,597.1	640.9	1,888.2	31,660.6
Other processes-%	98.7	98.2	101.1	46.0	14.0	34.3	88.7
Mn (mg)							
Mass balance	**GM**	**GMPA**	**PA**	**PC**	**TL**	**TLGM**	**TLPA**

Total influent load (mg)	187.4	72.4	68.3	73.3	50.8	72.5	112.4
Total effluent load (mg)	1.3	1.5	1.9	1.1	1.4	1.4	1.0
Total load reduction (mg)	186.1	70.8	66.4	72.3	49.4	71.1	111.4
Plant uptake-load (mg)	22.3	44.0	22.6	53.5	60.4	45.1	57.9
Plant uptake-%	12.0	62.1	34.0	74.1	122.3	63.4	52.0
Other processes-load	163.8	26.8	43.9	18.7	-11.0	26.0	53.5
Other processes-%	88.0	37.9	66.0	25.9	-22.3	36.6	48.0
Mg (mg)							
Mass balance	**GM**	**GMPA**	**PA**	**PC**	**TL**	**TLGM**	**TLPA**
Total influent load (mg)	61,674.2	61,203.3	60,036.0	56,798.4	53,780.7	55,266.2	61,044.3
Total effluent load (mg)	1,180.1	1,228.8	1,238.6	1,130.4	1,061.6	1,093.4	1,160.0
Total load reduction (mg)	60,494.2	59,974.5	58,797.4	55,667.9	52,719.2	54,172.8	59,884.3
Plant uptake-load (mg)	305.9	582.9	372.4	1,113.4	622.0	484.1	600.0
Plant uptake-%	0.5	1.0	0.6	2.0	1.2	0.9	1.0
Other processes-load	60,188.3	59,391.6	58,425.0	54,554.5	52,097.2	53,688.8	59,284.3
Other processes-%	99.5	99.0	99.4	98.0	98.8	99.1	99.0
Na (mg)							
Mass balance	**GM**	**GMPA**	**PA**	**PC**	**TL**	**TLGM**	**TLPA**
Total influent load (mg)	72,879.6	75,945.1	72,023.6	66,751.6	60,038.3	62,367.2	68,752.9
Total effluent load (mg)	1,421.5	1,506.6	1,476.5	1,270.5	1,146.3	1,216.9	1,292.2
Total load reduction (mg)	71,458.1	74,438.5	70,547.1	65,481.1	58,892.0	61,150.3	67,460.8
Plant uptake-load (mg)	-35.2	-44.9	70.6	257.2	528.0	371.2	538.4
Plant uptake-%	0.0	-0.1	0.1	0.4	0.9	0.6	0.8
Other processes-load	71,493.4	74,483.4	70,476.5	65,223.9	58,364.0	60,779.1	66,922.4
Other processes-%	100.0	100.1	99.9	99.6	99.1	99.4	99.2
Zn (mg)							
Mass balance	**GM**	**GMPA**	**PA**	**PC**	**TL**	**TLGM**	**TLPA**

Total influent load (mg)	1,256.1	1,159.5	1,048.8	901.6	821.9	852.1	1,052.6
Total effluent load (mg)	21.3	23.8	24.6	16.5	16.6	16.9	18.2
Total load reduction (mg)	1,234.8	1,135.7	1,024.2	885.0	805.3	835.2	1,034.4
Plant uptake-load (mg)	21.1	21.3	11.9	30.8	35.5	32.6	28.7
Plant uptake-%	1.7	1.9	1.2	3.5	4.4	3.9	2.8
Other processes-load	1,213.7	1,114.3	1,012.2	854.2	769.9	802.7	1,005.7
Other processes-%	98.3	98.1	98.8	96.5	95.6	96.1	97.2

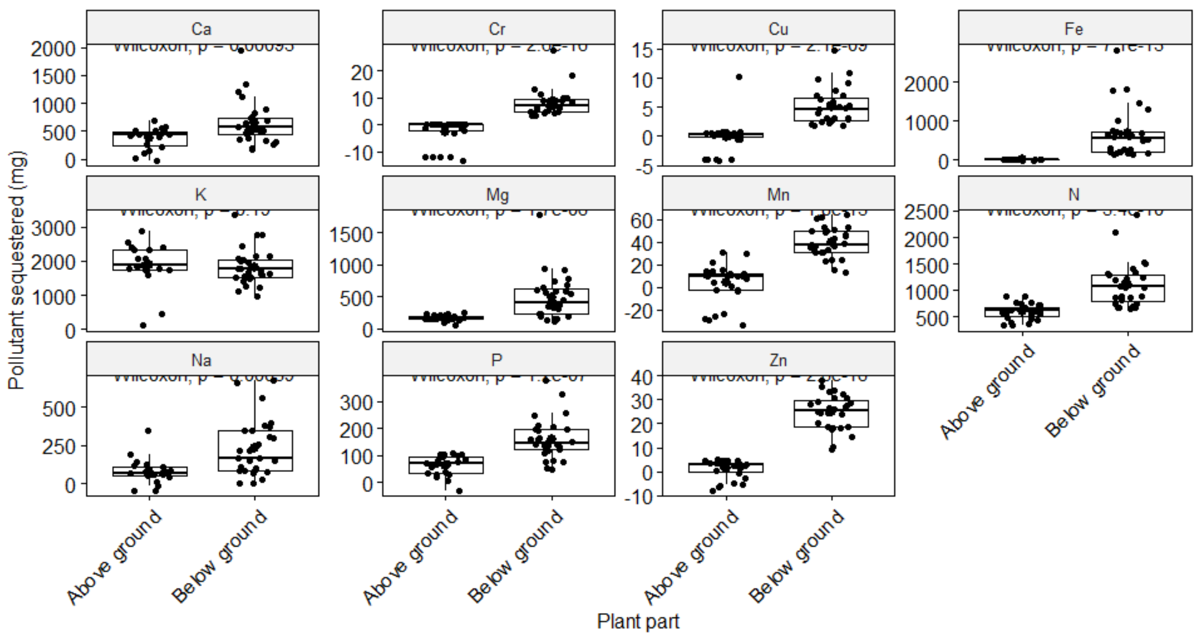


Figure A3.9: Quantity of pollutant sequestered (mg) by plant part per pollutant.

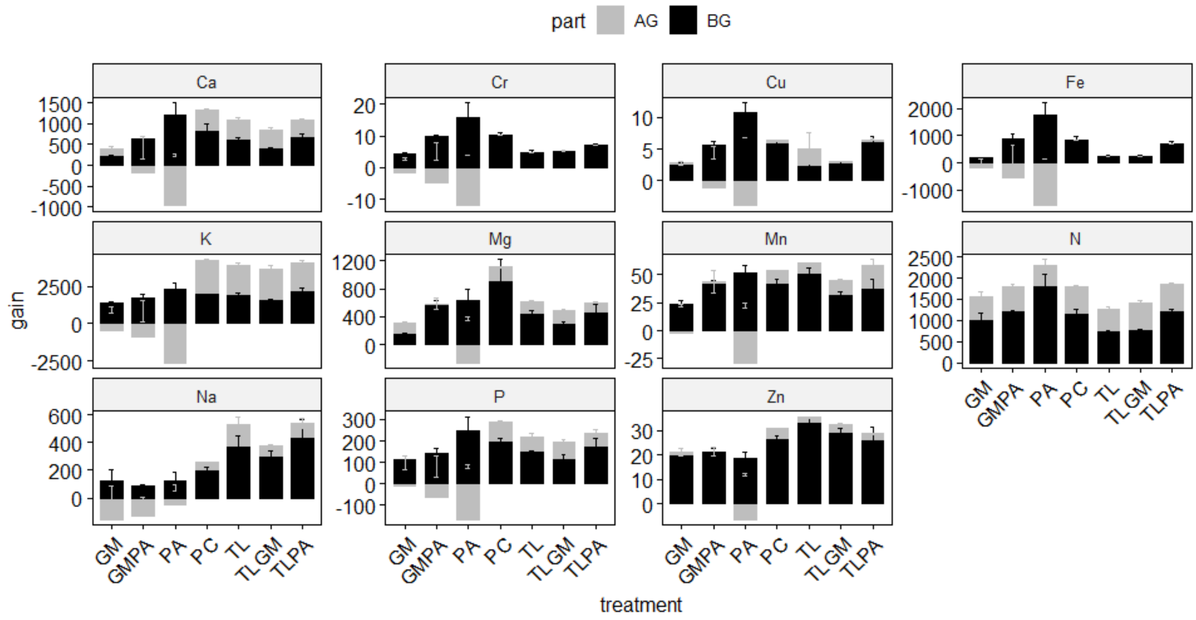


Figure A3.10: Quantity of pollutant sequestered (mg) by plant part per planted treatment for each pollutant

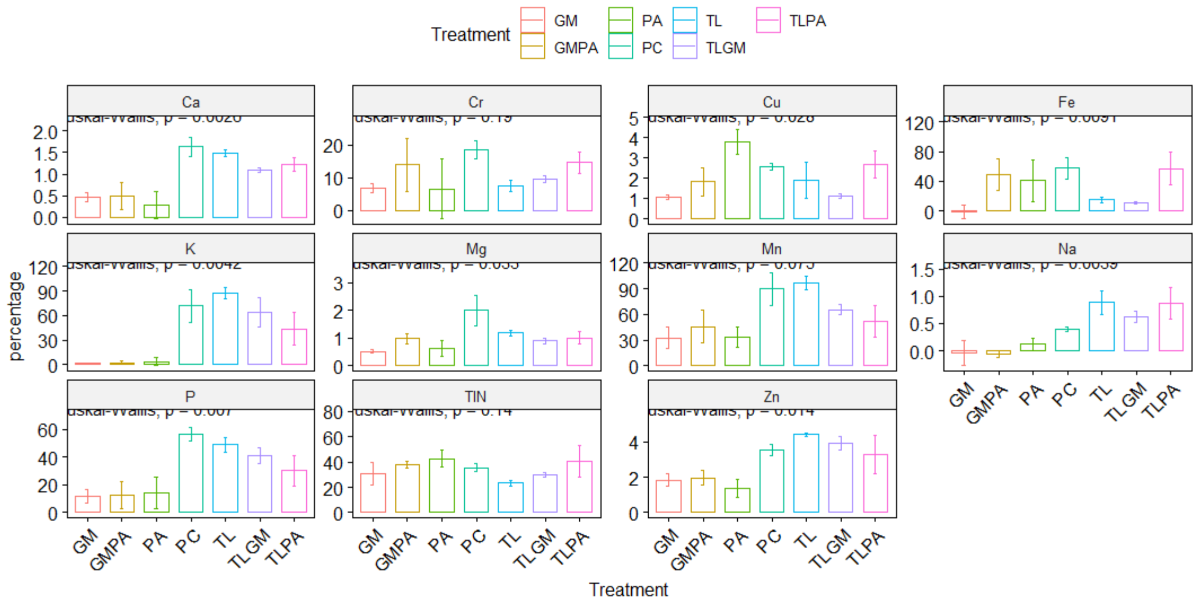


Figure A3.11: Percent contribution (%) of plants to pollutant uptake through the experiment for each studied pollutant by treatment

Appendix 4

Supplementary information for Chapter 6 ‘Field-scale floating treatment wetlands: quantifying ecosystem service provision from monoculture vs polyculture macrophyte communities’

Figure A4.1: Additioan site photos including a floating treatment under ice enhacement in year 2 (left) and underwater photographray beneith a floating treatment wetland in year 2 (right)

Table A4.1: Community composition by percentage Domin scale of each community type and replicate for Year 1 (2019)

	Monoculture			Polyculture		
	Replicate number			Replicate number		
	1	**2**	**3**	**1**	**2**	**3**
Alisma plantago-aquatica				9	10	12
Caltha palustris					1	
Epilobium hirsutum						
Epilobium montanum						
Iris pseudacorus				5	9	9
Juncus acutiflorus						
Juncus effusus				17	20	15
Lemna minor				2	3	4
Lemna minuta	4		1	2	2	3
Lemna trisulca	1					
Lycopus europaeus				3		10
Lysimachia punctata						
Lythrum salicaria	1	1		14	10	12
Mentha aquatica	1					
Myosotis scorpioides						
Nasturtium officinale						
Phalaris arundinacea				5	7	2
Phragmites australis	95	95	90			
Ranunculus flammula						
Salix sup.						
Spirodela polyrhiza						
Veronica beccabunga						

Table A4.2: Community composition by percentage Domin scale of each community type and replicate for Year 2 (2020)

	Monoculture			**Polyculture**		
	Replicate number			Replicate number		
	1	**2**	**3**	**1**	**2**	**3**
Caltha palustris				6	2	5
Epilobium hirsutum	10	9	15	8	10	10
Epilobium montanum		1				
Iris pseudacorus				10	5	10
Juncus acutiflorus					2	
Juncus effusus					2	
Lemna minor	1	1	2	4	2	7
Lemna minuta	1	1	1	1	1	1
Lemna trisulca			1	1		1

Lycopus europaeus	15	15	20	10	15	7
Lysimachia punctata	1	1		10	10	5
Lythrum salicaria	5	5	7	18	6	15
Mentha aquatica	10	5		20	12	15
Myosotis scorpioides	8	10	8	20	15	15
Nasturtium officinale	5		5		15	
Phalaris arundinacea				4	4	5
Phragmites australis	40	30	40			
Ranunculus flammula				1	1	1
Salix sup.	1	2	8			
Spirodela polyrhiza	1		2	4	2	4
Veronica beccabunga		1		10	10	

Table A4.3: Community composition by percentage Domin scale of each community type and replicate for Year 3 (2021)

	Monoculture			**Polyculture**		
	Replicate number			**Replicate number**		
	1	**2***	**3**	**1**	**2**	**3**
Azolla filiculoides	1		1			
Caltha palustris				2		5
Epilobium hirsutum	10		10	30	15	30
Epilobium montanum						
Iris pseudacorus				5	5	2
Juncus acutiflorus						
Juncus effusus						
Lemna minor						
Lemna minuta						
Lemna trisulca						
Lycopus europaeus	20		40	25	35	30
Lysimachia punctata						
Lythrum salicaria	25		1	15		
Mentha aquatica	30		20	20	30	15
Myosotis scorpioides	10		5	5	15	8
Nasturtium officinale			5		4	10
Phalaris arundinacea				5		3
Phragmites australis	5		10			
Ranunculus flammula						
Salix sup.	4		1			
Spirodela polyrhiza						
Veronica beccabunga						

*no sample taken as FTW not accessible

Table A4.4: Count of individual macroinvertebrates where identified to lowest taxonomic level

		Mollusca			Malacostraca		Diptera		Trichoptera				Coleoptera			Hemiptera		Annelida		Odonata	Tricladida	Unknown	
Treatment name	Treatment REP	Planorbidae	Pisidium tenuilineatum	Radix balthica	Asellidae	Crangonyx	Chaoborus	Chironomidae	Leptoceridae	Trichoptera pupa	Polycentropodidae	Hydropsychidae	Dytiscidae	Chrysomelidae	Haliplidae	Corixidae	Gerridae	Piscicolidae	Glossiphoniidae	Agriidae	Planariidae	Unknown 1 (orange)	Unknown 2 (winged)
Mono-1	FTW1.1	25	21	22	30	38	34	8	15				2			1				1			
Mono-1	FTW1.2	8	0	8	9	30	359	3	13				1			4				6		1	2
Mono-1	FTW1.3	10	22	21	20	37	17	26	33				4			1				4			
Mono-1	FTW1.4	13	7	15	1	11	58	10	48							0	1			1			
Mono-2	FTW3.1	14	7	5	4	36	26	58	36														
Mono-2	FTW3.2	1	1	12	1	28	28	27	32										1				
Mono-2	FTW3.3	9	1	2	4	0	41	74	34				1			3		1	1				
Mono-2	FTW3.4	3	2	3	4	51	40	78	10		1					1							1
Mono-3	FTW5.1	2	1	5	2	16	7	9	9														
Mono-3	FTW5.2	7	6	6	24	60	42	9	12							1		1			1		
Mono-3	FTW5.3	3	11	3	11	70	52	5	26							1							
Mono-3	FTW5.4	3		7	3	21	9	13	12							1							
Poly-1	FTW2.1				29	82	3		7				1			2							
Poly-1	FTW2.2	7			6	25	3	1	23						1								
Poly-1	FTW2.3	18	3	24	26	100	31		40														
Poly-1	FTW2.4	7	8	15	25	155	20	4	32											1			
Poly-2	FTW4.1	14	23	9	18	30	811	6	29		10												
Poly-2	FTW4.2	32	1	36	16	49	150	21	58		9		1			2				1			
Poly-2	FTW4.3	10	3	27	19	58	94	28	48		5					1			1		2		
Poly-2	FTW4.4	17	3	20	17	47	55	3	39		2					2							
Poly-3	FTW6.1	18	32	25	28	65	56	9	29			1	1	1					1	4	1		
Poly-3	FTW6.2	11	8	7	8	72	72	13	28				1			1				8			
Poly-3	FTW6.3	6		10	10	62	52	22	20	1	2		1			4			1	1			
Poly-3	FTW6.4	10	1	8	4	25	14	8	55														

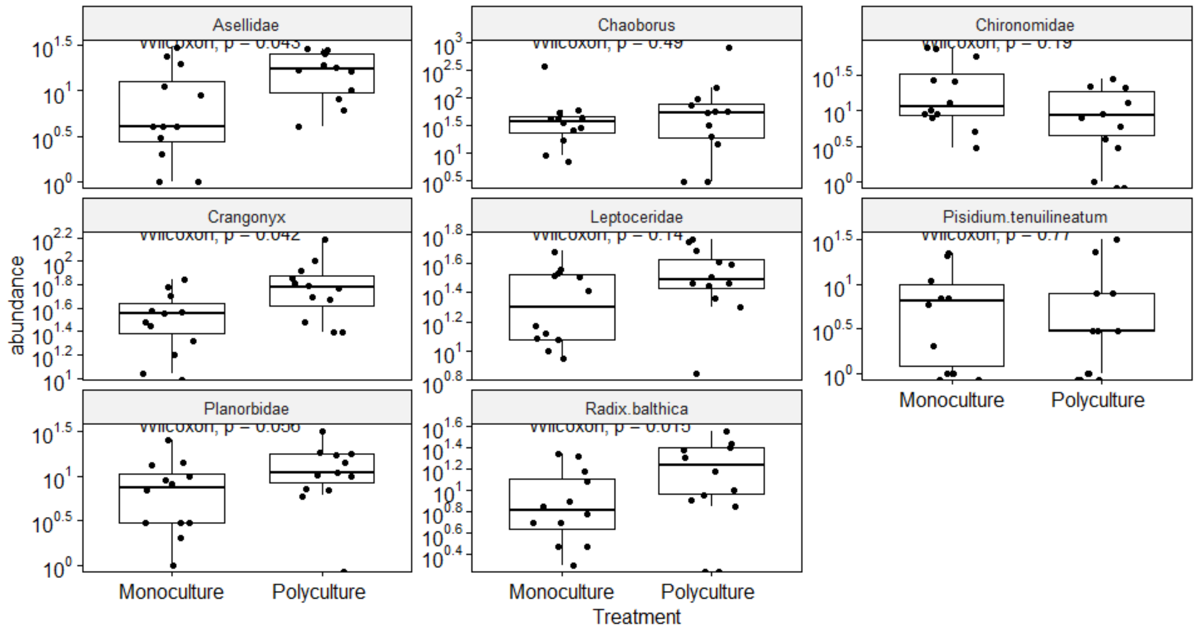


Figure A4.2: Log^10 of 8 most common species in samples comparing monocultures and polyculture communities. *Note this figure requires some tidies and the replicates here for each group are pseudo replicates so I probably need to re-do analysis for this, reducing significance.*

Table A4.5: Details of challenges relating to siting the FTWs, anchorage, and maintenance

Observation	**Observation detail**	**Management recommendation**
Anchorage	-Zinc wire cords tied to a dense hollow block (400x215x215mm) were insufficient as anchorage as FTW moved by an order several meters -Wood posts securing parameter of each FTW failed.	-Tie FTW to a jetty -Use multiple anchors at different sides. Ensure fittings are large enough -Choose locations that are more sheltered (although avoid direct tree cover as this will impact vegetation growth due to shading)
Animal use	-Small waterfowl frequently used the FTWs for loafing or nesting -Noted amphibian use -Pollinators (such as bees, hoverflies, and butterflies) visited the flowering plants from both treatments	
Deployment	-Required several participants to assist deployment as the FTW are cumbersome and heavy. -FTWs required 2-3 days preparation time include pre-assumably and transporting pre-assembled equipment to experiment site	-Ensure there is logistical plan in place with ample support available from at least 3-4 people. - Time delivery or transplant of macrophytes into FTW at the latest time to avoid plants becoming desiccated.
Removal	-FTWs were very heavy following vegetation growth although remained buoyant	-Several people assisting may be required, to keep costs low suggest the use of interested community volunteers. -Use an assistance vehicle such as a tractor
Maintenance	-Water levels at times became low and in anticipation FTWs had to be pushed out a by c.1 meter to ensure they did not dry out	-Ensure a regime for checking FTW, or installation of a camera near the site. Or ensure the siting can accommodate fluctuations in water levels. An assessment of low/high water levels can help placement.
Matting and plant substrate	-Coir matting became loose in some FTWs, mostly plant roots remaining in certain portions of these FTWs.	-Choose locations that are more sheltered (although avoid direct tree cover as this will impact vegetation growth due to shading)

		- research most appropriate design of FTW