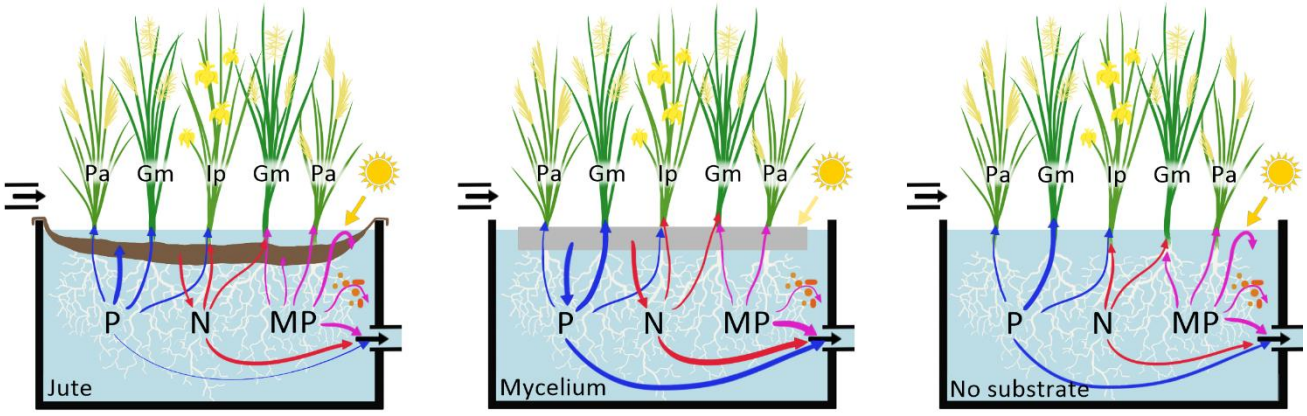
Nature-based nutrient and micropollutant removal by sustainable plant-diverse floating treatment wetlands

Hazel van Waijjen, 25 October 2022 *Internship report*

Abstract

The Netherlands is a country full of water challenges. Among these are the decrease in water quality due to excess nutrients and micropollutants, and the subsequent decline of water ecology and biodiversity. Much work has been done to mitigate these issues separately, while nature-based solutions offer the possibility to solve multiple water-related problems with one integrated approach. In this research, we explore the application of floating treatment wetlands, a nature-based water treatment technology, to treat the Zijdewetering, a waterway with poor chemical and biological water quality. For this, mesocosms were used with bio-based substrates jute and mycelium, in combination with plant species *Phragmites, Glyceria,* and *Iris*. High removal was achieved for ortho-phosphate (80-100% with jute and with *Glyceria*), ammonium (70-90%), nitrite (100%), and nitrate (100%). Total nitrogen was removed less (50-80% without substrate), due to assimilation of organic nitrogen. Micropollutant removal varied depending on compound, substrate and month, but high removal (>70%) was achieved in August for 11 out of 16 compounds. Jute and *Glyceria* performed best overall, while mycelium often decreased water quality. Practically, FTWs are low-cost, low-footprint and low-maintenance, but require a large area (4 ha), have lower efficiency in winter and need to be harvested twice per year for optimal removal. Implementing floating wetlands with jute and non-fossil absorbents in the Zijdewetering would remove nutrients and micropollutants in an efficient and season independent manner. Moreover, these multifunctional nature-based solutions will decrease water ecotoxicity, forming an ecological buffer and enhancing water quality and biology.

Graphical abstract



Pa = Phragmites australis, Ip = Iris pseudacoris, Gm = Glyceria maxima, P = Phosphate, N = Nitrogen, MP = Micropollutants

Keywords: Floating treatment wetland (FTW), Surface water, Nutrients, Micropollutants, Substrate, Mesocosm

1. Introduction

1.1 Climate urgency: Dutch water quality

We live in an era of environmental challenges. The Netherlands, one of the most densely populated countries in the world, copes with extreme droughts, heavy rainfalls, increasing water pollution, and biodiversity loss [1-3]. Intensive land use and various anthropogenic activities pollute our waters with a wide range of constituents [RIVM][4], making Dutch surface waters among the most polluted of Europe [5]. Less than 25% of surface waters reach the chemical standards of the Water Framework Directive[1] (WFD) [2000/60/EG], and only 1% has sufficient ecological quality [5]. Nutrients (phosphorous and nitrogen) from agriculture runoff and waste water treatment plants (WWTPs) cause water eutrophication and subsequent algae blooms, harming local wildlife and threatening human health [6, 7]. Additionally, extreme rainfalls have increased stormwater overflows and agricultural runoff containing industrial chemicals and pesticides [4, 8, 9]. Furthermore, pharmaceuticals (e.g. antibiotics, beta blockers, pain killers) are increasingly present in urban waste waters [10, 11], while hardly removed by WWTPs [9]. Consequently, these micropollutants[2] end up in our surface waters, severely threatening wildlife by accumulating in food webs [6, 7, 9], and polluting our drinking water

[1] Water framework directive (WFD) - *Dutch: Kaderrichtlijn water*

[2] Micropollutants - *organic contaminants present in the environment at low concentrations of ng-µg/L*

sources by entering our water cycle [4, 6-8]. Therefore, there is an increasing concern about the future quality of our surface waters [4, 12, 13].

Many attempts are being made to solve these rising issues. Legislations like the WFD [2020/1161/EU], and the "Zero pollution ambition" action plan [European commission, 2021] aim to stimulate improvement of water quality; while innovation programs like the Dutch IPMV[3] try to find high-tech solutions for pollutant removal [14, 15]. Several such high-tech solutions exist that remove phosphorus [16, 17], ammonium [16-19], nitrite [19], or micropollutants [20-22] from waste water. However, each of these remove just one contaminant group and together these techniques demand much energy and resources [23]. With regard to our current climate-, energy-, and resource crises, there is a clear demand for *integral* water remediation techniques that emit little CO_2, require little input, remove both nutrients and micro-pollutants, and synchronously enhancing local water ecology and biodiversity [SDG6.6].

1.2 Nature-based solutions

Nature-based solutions are such integrated approaches to mitigate climate change, remove contaminants, and stimulate biodiversity [24]. They are becoming increasingly popular as sustainable solutions to broad environmental challenges [24]. Correspondingly, constructed wetlands have gained scientific and social interest as natural planted systems for post-treatment of waste water effluent [25-27]. These wetlands remove nutrients and micropollutants from effluent [27-30]. They are generally considered to be sustainable, effective, environment-friendly, low-cost, low-maintenance, and energy-efficient [25, 28-30]. Accordingly, the use of such nature-based water treatment systems would not only improve Dutch surface water quality in a sustainable manner, but also has the potential to restore water ecology [28].

1.3 Casus: Floating treatment wetlands for the Zijdewetering

The Zijdewetering, a waterway situated in Veenendaal (Fig. 3), is an example of the Dutch water challenges. It has one of the worst chemical and biological water qualities of province Gelderland [31, 32]. However, the waterway is WFD-protected with high quality standards (TP <0,11 mgP/L; TN <2,3 mgN/L; oxygen 5,5-8,5 mgO/L) [32], and contributes one fifth of the water of the protected Valleikanaal (Fig. 3). The Zijdewetering is mainly fed by the water treatment plant of Ede (±37.000 m^3/day), which uses a combined aerobic and anaerobic treatment system with high phosphorus and nitrogen removal efficiency (88-95%) [33]. Despite high nutrient removal, the effluent is still rich in phosphorus (0,84 mgP/L), and nitrogen (7,67 mgN/L) [33], resulting in high nutrient concentrations in the Zijdewetering [32, 34]. These nutrients stimulate growth of water plants and filamentous algae, which cause nightly oxygen concentration to decrease below the standard of 5,5 mg/L [32, 34]. Together, nutrients, low oxygen, and micropollutants cause poor biological quality [32], underlining the need to improve the water quality in the Zijdewetering.

***Figure 1**, Floating treatment wetland (FTW) with Glyceria maxima in the Zijdewetering.*

For waters polluted with various compounds, nature-based approaches like wetlands present a sustainable multifunctional solution. Yet, conventional sand-based constructed wetlands require a considerable land area for the throughput of large water volumes and the treatment of high constituent loads [28]. On the other hand, floating treatment wetlands[4] (FTWs) can be implemented in existing water ways with varying water levels, treating large quantities of water without obstructing the water flow (Fig. 1, Fig. 2). These FTWs are especially suitable for on-site remediation of slow streaming polluted surface waters [25, 26], like the Zijdewetering.

1.4 Background: Design and function of floating treatment wetlands

FTWs consist of macrophytes grown on the water surface using a buoyant frame or substrate (e.g. foam, coconut fibre, peat, cotton [28, 35]), allowing the plant roots to grow directly in the polluted water (Fig. 1, Fig. 2). Plant roots provide habitat, oxygen, and chemical root exudates for microbes, which form a microbiome[5] [36, 37]. Altogether, the five most important pathways of pollutant removal in FTWs are: 1) aerobic and anaerobic biodegradation by microbes; 2) photodegradation by sunlight; 3) sorption to the substrate and rhizosphere; 4) sedimentation aided by the root system; and 5) uptake by the plants, followed by translocation, degradation, or accumulation in the plant tissue (Fig. 1) [28, 38].

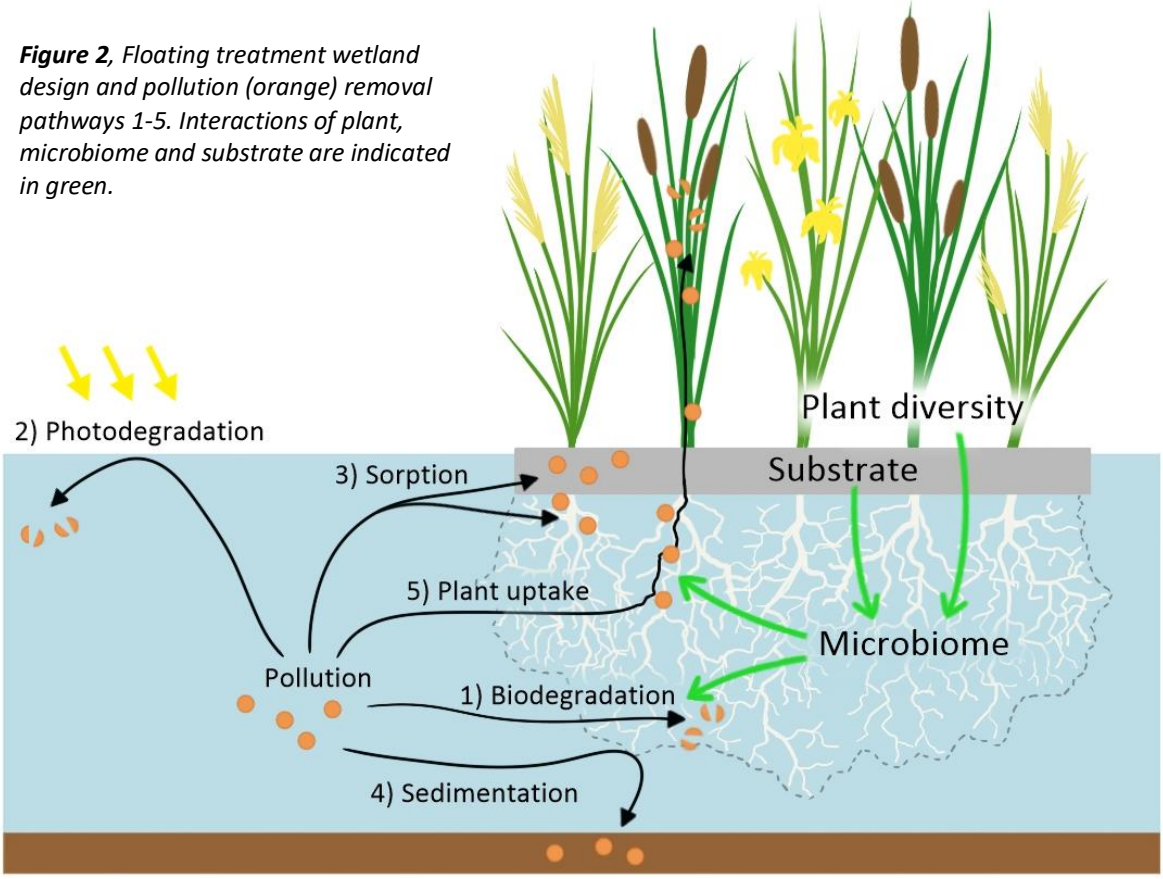
[3] IMPV - *the Innovation Program Removal of Micropollutants at wastewater treatment plants*
[4] Floating treatment wetlands (FTW) - *constructed floating islands, consisting of a buoyant mat with wetland plants*
[5] Microbiome - *the entirety of bacteria, archaea, algae, and fungi in the rhizosphere and root endosphere*

These processes are strongly influenced by plant species, substrate selection, and the microbiome [25, 29, 39]. Microbes degrade pollution and enhance uptake and breakdown of distinct pollutants by plants [25, 40-43]. Which microbes are abundant in the microbiome is greatly influenced by plant identity [44-48], and by the substrate, which functions as physical and chemical support [29, 39, 49-52].

Due to species-specific plant-microbe interactions, increasing plant diversity would strongly diversify the microbiome. This could provide a dynamic multi-target approach, removing a broad spectrum of (micro-)pollutants. Additionally, plant diversity in FTWs offers an extended growing season in temperate zones; robustness against (a)biotic stresses; increased biodiversity; and higher aesthetic value. However, the majority of FTW studies focus on single plant species [25, 26]. Concurrent, there is a scientific debate whether plant diversity enhances nutrient removal [53-58], while this remains to be investigated for micropollutants. Understanding how plant identity and diversity influences pollutant removal can enable us to select plant communities optimal for location-specific water treatment.

Furthermore, sustainable bio-based floating materials are needed to support FTWs. Much research has been done on the effect of substrate on pollutant removal in both constructed and floating wetlands [38, 39, 49, 51, 52, 59]. Jute[6], is one of the more commonly used bio-based substrates for FTWs [60-62]. Jute has a large surface area due to its woven structure, offering attachment sites for microbes that enhance pollutant breakdown. It consists mainly of cellulose and lignin [63], which are broken down slowly and are therefore durable. Another biobased substrate is mycelium, a firm buoyant material made from the mycelium of white rot fungi grown on wood waste streams. Different species of white rot fungi are able to degrade micropollutants like pharmaceuticals and pesticides [23, 64-67]. Furthermore, remnants of wood waste from a carbon source that can stimulate denitrification [23, 68]. Mycelium could therefore be the ideal substrate for FTWs [69]. However, to our knowledge, floating mycelium has not been used as an FTW substrate before.

***Figure 2**, Floating treatment wetland design and pollution (orange) removal pathways 1-5. Interactions of plant, microbiome and substrate are indicated in green.*



1.5 This research: Are FTWs suitable to treat the Zijdewetering?

This research investigates the application of floating treatment wetlands for improving the water quality of the Zijdewetering. The effect of substrate (jute and mycelium) and plants (*Phragmites, Glyceria,* and *Iris*) on nutrient and micropollutant removal is investigated using 39 mesocosms[7] with water from the Zijdewetering. For nutrients, ortho-phosphate, ammonium, nitrite, nitrate, and total nitrogen were measured. For micropollutants we measured four beta blockers, three antibiotics, five pharmaceuticals, three crop protection products, one insect repellent, two corrosion

[6] Jute - *rough fibre fabric made from the dried stems of Corchorus plants, also known as burlap*

[7] Mesocosm - *outdoor experimental system under controlled conditions*

inhibitors, and one stimulant (Table 2). The goal of this set-up is to find the best suitable substrate and plant community for treatment of the Zijdewetering. Based on the best functioning system, an estimation is made for the FTW-area needed to reach the chemical WFD standards of the Zijdewetering.

Beside the mesocosm set-up, a few FTWs made of jute and mycelium were placed in the Zijdewetering to investigate the structural integrity and durability of these two substrates in practice. In this field set-up, also the plant growth in streaming water was monitored to determine the best suitable plant species. Lastly, a literature study was done to answer practical questions concerning environmental impact, costs, maintenance, practical limitations, seasonal influence, surface area, and added benefits.

This research is, to our knowledge, the first to test the effect of plant diversity on micropollutant removal. We are also the first to test the added value of floating mycelium, as an innovative bio-based material, on nutrient and micropollutant removal. Lastly, we are one of few who investigate the application of FTWs for slow streaming water instead of stagnant ponds, stimulating FTW application for polluted streams.

2. Methods

2.1 Location: The Zijdewetering

The experimental location is situated at the end of the Zijdewetering in Veenendaal (52°02'27.1"N 5°31'42.5"E) (M in Fig. 3). Water for the mesocosms was retrieved 20 meters before the Zijdewetering meets the Valleikanaal.

***Figure 3**, Geographic location of the Zijdewetering, the Valleikanaal, water treatment plant Ede (WWTP), connecting waterway of WTP and Zijdewetering (Connection), water streaming direction (arrows), FTWs in Zijdewetering (FTW), mesocosms (M), and plant retrieval location (R).*

2.2 Mesocosms

Adopting commonly used mesocosm set-ups [33, 36-41, 44], 39 small mesocosms of 48 litres (55cm length x 35cm width x 25cm height, Fig. 4A) were used. In each mesocosm either jute, floating mycelium, or no substrate was placed (Fig 4B-D). The jute consisted of 100% jute fibres and was attached to the sides of the mesocosms with additional loose jute (Fig. 4B). The mycelium substrate exists of wood fibres on which the fungus *Ganoderma* was grown for two weeks, after which the substrate was dried for two weeks and stored for nine months (Mycelium Materials Europe B.V., Hedel, The Netherlands). These mycelium units (50cm length x 30cm width x 10cm height, Fig. 4C) floated freely in the mesocosms. In the jute and no-substrate mesocosms, plants were attached to iron netting for stability (Fig. 4B&D).

Three native wetland species were used: *Phragmites australis* (common reed) [70, 71], *Iris pseudacorus* (yellow iris) [70, 71], and *Glyceria maxima* (reed sweet-grass) [50, 72]. *Phragmites* and *Glyceria* were attained from the local water borders (R in Fig. 3), while Iris was attained from 'Biovijver' (Otterlo). Plants were distributed in three diversity levels (one to three species per tank) with every possible combination (7 combinations in total). Each combination was planted on each substrate with some duplications (Table 1, Fig. 4E, Sup. A). Per mesocosm, 12 individual plants were planted. Unplanted mesocosms were used as negative controls.

The mesocosms were filled with water from the Zijdewetering using a pump. 24 litres of water was replaced every 10 days (June-July 2022), which was later shortened to every 5 days (August-September 2022).

***Table 1**, Overview of all mesocosm substrate and plant-combinations. First mesocosm number, then which substrate (J = Jute, M = Mycelium, N = No substrate), lastly plant species (P = Phragmites, I = Iris, G = Glyceria maxima, O = no plants).*

4 N - O	**8** M - IG	**12** J - PIG	**16** N - IG	**20** M - PIG	**24** N - PG	**28** M - P	**32** M - G	**36** J - I	**39** J - PIG
3 N - PI	**7** M - I	**11** N - I	**15** M - G	**19** J - IG	**23** J - PIG	**27** N - G	**31** N - P	**35** M - PG	**38** N - IG
2 J - G	**6** J - P	**10** J - O	**14** M - O	**18** N - I	**22** J - G	**26** N - PI	**30** J - PG	**34** J - O	**37** N - P
1 M - P	**5** N - PIG	**9** J - PG	**13** J - PI	**17** N - PG	**21** M - PI	**25** N - PIG	**29** J - P	**33** N - G	

***Figure 4**, Mesocosm set-up September 19th. **A.** Mesocosm size, influent from yellow hose, effluent tap indicated with yellow arrow; **B.** Mesocosm with Jute substrate (including algae growth); **C.** Mesocosm with Mycelium substrate; **D.** Mesocosm without substrate; **E.** Overview of all mesocosms, first 8 numbered and coded as illustration (Table 1).*

2.3 Experiments

Over the summer of 2022, water samples from influent and effluent were taken four times (Sup. A2). During *experiment 1*, from the July 8th to 18th, nutrient removal was measured in all mesocosms for a treatment time of 10 days (Sup. A2). Following, in the *time experiment*, from July 29th to August 1st, nutrient removal was measured for a treatment time of 0, 8, 24, 30, 48, and 72 hours after water replacement in mesocosms 2, 27, and 32 (Sup. A2). Based on these results, in *experiment 2*, from August 22nd to 23rd, nutrient and micropollutant removal was measured in all mesocosms with a treatment time of 24 hours (Sup. A2). Lastly, in *experiment 3*, September 5th to 6th, micropollutant removal was measured in all mesocosms for a treatment time of 24 hours (Sup. A2).

2.4 Sampling and monitoring

The first day of all experiments, 24 litres of water was replaced with fresh water from the Zijdewetering between 9p.m. and 11p.m.. At 9p.m., 10p.m. and 11p.m., influent samples were taken in a large clean 50 ml centrifuge tube. At this time, water characteristics (pH, conductivity, oxygen concentration, and temperature) of the influent were noted. After 1 to 10 days, water characteristics and evaporation were measured in the mesocosms, while effluent samples were taken from the mesocosm tap in 15 ml centrifuge tubes. Both influent and effluent samples were directly put on ice in a cooling

bag. For *experiment 1* and *experiment 2*, these samples were filtered later the same day over a 2µM nylon mesh and stored in the freezer for analysis. For the *time experiment*, both influent and effluent samples were filtered on location before put on ice and stored in the freezer [73].

For *experiment 3*, a different sampling procedure was used in order to prevent micropollutant sorption by either the tubes or the filter [74]. First, 44 clean 15 ml centrifuge tubes were prepared containing 0,526 ml of acetonitrile [74]. Influent and effluent samples were taken by rinsing a 30 ml syringe six times with mesocosm effluent from the mesocosm tap, filling the syringe to exactly 10 ml, and adding the effluent directly to the 15 ml tube with acetonitrile (5%). An additional tap water sample was taken in the same way to verify that samples were not contaminated through the syringe. These samples were put on ice until stored in the freezer. The samples were centrifuged before use, not filtrated.

Plant growth was monitored during the experiments by taking pictures of each mesocosm with a white screen behind the mesocosm (Fig, 5A-D, Sup. B1). Additionally, weather (temperature, rainfall, humidity, wind, etc.) was monitored for the whole season using METEO NEDER-BETUWE for Kesteren Centrum and rain radar FEWS_RADAR_315 (Sup. B2).

2.5 Sample analysis

Before analysis, samples were brought to room temperature. Total phosphorus, ammonium, and total nitrogen were measured using HACH Lange kits on a DR 3900 spectrophotometer (HACH, Germany) [75, 76]. Anions (fluoride, chloride, nitrite, bromide, nitrate, sulphate and ortho-phosphate) were measured using ion chromatography (IC) with a Dionex ICS2100 (Dionex, Breda, The Netherlands) equipped with a Dionex IonPac AS10 column [76, 77]. A diverse set of persistent micropollutants was selected based on their varying chemical characteristics and frequent occurrence in the environment (Table 2, Sup. D1). These were measured using Ultra High Performance Liquid Chromatography (UPLC-MS) (AB SCiex, MA, USA) with a standard calibration line of 50 to 1000 ng/L and an internal standard of 500 ng/L for (Caffeine-D3, Sulfamethaxole-D4, Propanolol-D7, CBZ-D10, Furosemide-D5, DCF-D4, MCPP-D6) as described by Van Gijn *et al.* 2021 [76].

***Table 2**, Micropollutants measured. The IMPV guide compounds are underlined [23].*

Group	Substance
Beta blockers	metoprolol, propranolol, atenolol, sotalol
Antibiotics	erythromycin, clarithromycin, sulfamethoxazole, trimethoprim
Other medicine	irbesartan, carbamazepine, dimetridazole, diclofenac, furosemide
Crop protection products	mecoprop (MCPP), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,6-dichlorobenzamide (BAM), desphenyl chloridazon
Insect repellent	diethyl-3-methylbenzamide (DEET)
Corrosion inhibitors	sum of 4 and 5 methylbenzotriazole (4(5)-methylbenzotriazole), benzotriazole
Stimulant	caffeine

2.6 Data analysis

IC data was analysed using Chromeleon, and LC-MS data was analysed using the ScieX analyst software. Thereafter, data was processed using Microsoft Excel. Concentrations were corrected for evaporation. Standard deviations were calculated using the Excel STDEV.P function and significant differences were calculated with the two-sided, heteroscedastic Excel T.TEST function. Removal efficiencies were calculated using formula 1. Plant effect was calculated using a weighted average based on the number of plants present per species (formula 2). Graphs were made using Excel.

Formula 1:

$$Removal = \frac{average\ influent\ concentration\ -\ mesocosm\ effluent\ concentration}{average\ influent\ concentration} \times 100\%$$

***Formula 2**:*

$$Plant\ effect\ on\ removal = \frac{\sum_{M=1}^{M} Removal\ efficiency\ of\ M * number\ of\ P\ present\ per\ M}{total\ number\ of\ P\ in\ all\ mesocosms}$$

M = Mesocosm number, P = Individual plants per species

2.7 FTWs in the Zijdewetering

To test the durability of the substrates, two jute FTWs were build and put in the Zijdewetering on June the 29th (Fig. 3). These consisted of PVC tubes with PET-bottles inside for buoyancy and jute wrapped around as planting medium. The FTWs were planted with a combination of *Phragmites australis*, *Iris pseudacorus*, and *Glyceria maxima*, and monitored regularly by making pictures (Fig. 16). On August 19th, 16 small mycelium FTWs were attached to the two jute FTWs and half was planted with *Glyceria maxima*, while the other half was left unplanted (Fig. 16C).

2.8 Literature study

A literature study was done investigating FTW environmental impact, costs, maintenance, practical limitations, seasonal influence, area, and added benefits.

3. Results

3.1 Plant growth, substrate degradation, and algae occurrence

Plants were transferred to the mesocosms in June (Fig. 5A), after which most *Phragmites* and *Glyceria* plants started to wither (Sup. B1). After two weeks, new *Phragmites* and *Glyceria* sprouts started to form from the withered parent plants

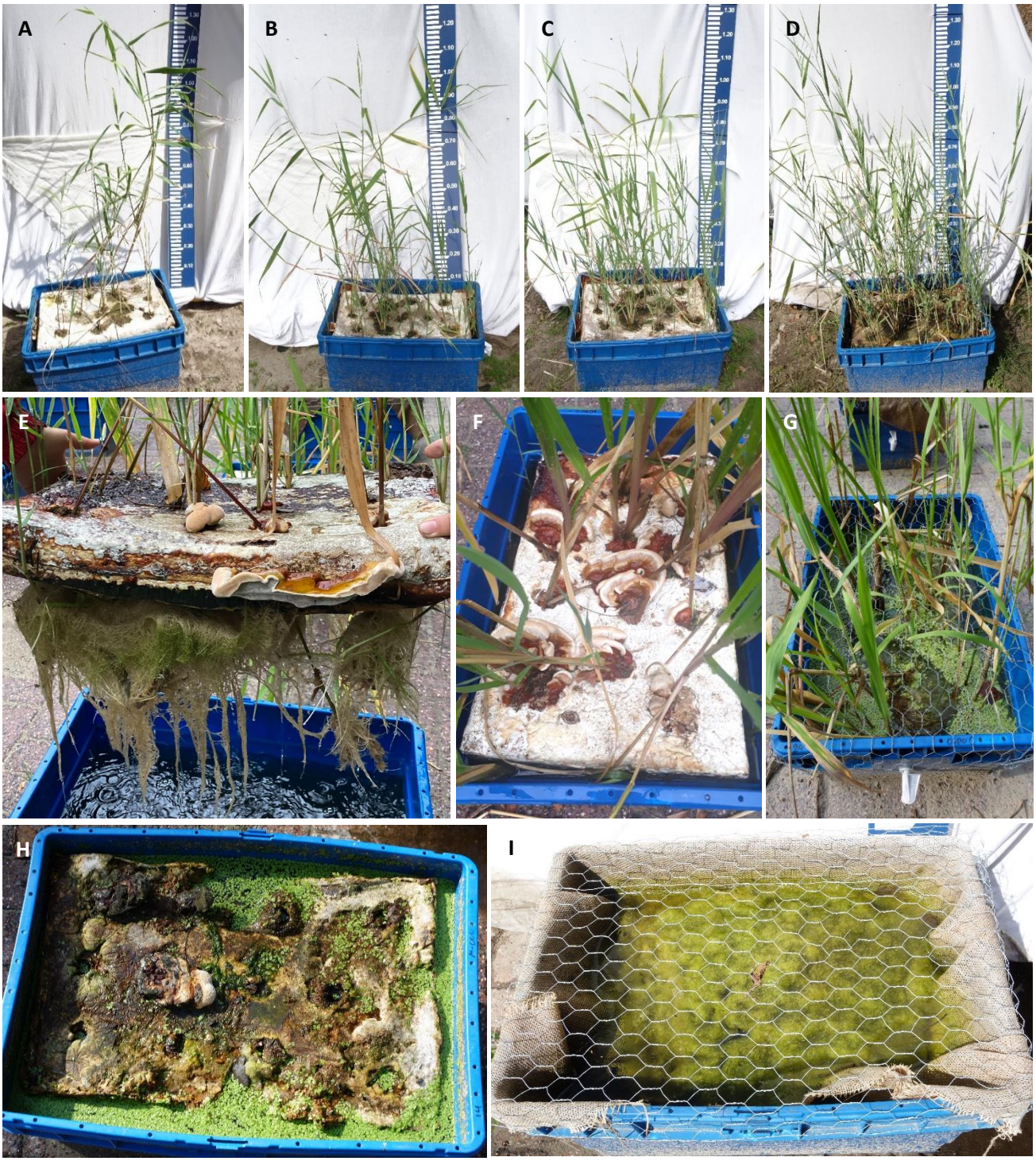


***Figure 5**, Set-up development. **A-D**, Plant growth in mesocosm 1 (M-P) for June 29th, July 12th, July 29th, and August 22nd; **E.** Roots in mesocosm 28 (M-P) July 12th; **F.** Mushrooms in mesocosm 32 (M-G) July 21st; **G.** Duckweed in mes. 24 (N-PG) on July 29th; **H.** Rotting mycelium and duckweed in mes. 14 on September 5th; **I.** Algae in mes. 10 (J-O) on August 22nd.*

(Fig. 5B, Sup. B1). Growth was fastest during the end of July till the end of August (Fig. 5B-D, Sup. B1), after which growth was halted and plants started yellowing in September (Sup. A1). *Iris* plants stayed relatively unchanged during the whole experimental period (Sup. AB). Major *Iris* growth was only seen in one mesocosms (7 M-I; Sup. B1), while in other mesocosms iris was often overgrown by *Phragmites* and *Glyceria* (Sup. B1).

Root development was most remarkable for *Phragmites*, which formed dense root systems within the first month (Fig. 5E). *Glyceria* also formed extensive root systems, with thicker roots and less 'hairy' roots (Sup. B1), while *Iris* formed short and thick roots (Sup. B1).

The mycelium substrate started to form mushrooms after one month in the mesocosm (Fig. 5F, Sup. B1), with no apparent effect on mesocosm performance. Some mycelium units stayed firm and white for two months, before becoming brown and soft (Fig. 5 A-D). Other mesocosms (7 and 15) turned brown and started rotting half-July (Sup. B1), after which gaps formed in the mycelium (Fig. 5H).

Filamentous algae formed during the second half of August in mesocosms with jute and no substrate (Fig. 5I). Underneath the mycelium, little algae growth was seen because of light-shortage. Duckweed grew quickly during the second-half July and in August in some mesocosms (Fig. 5G, Sup. B1). The influence of algae and duckweed could not be isolated from the over-all mesocosm performance and no direct correlation between their presence and mesocosm performance was found.

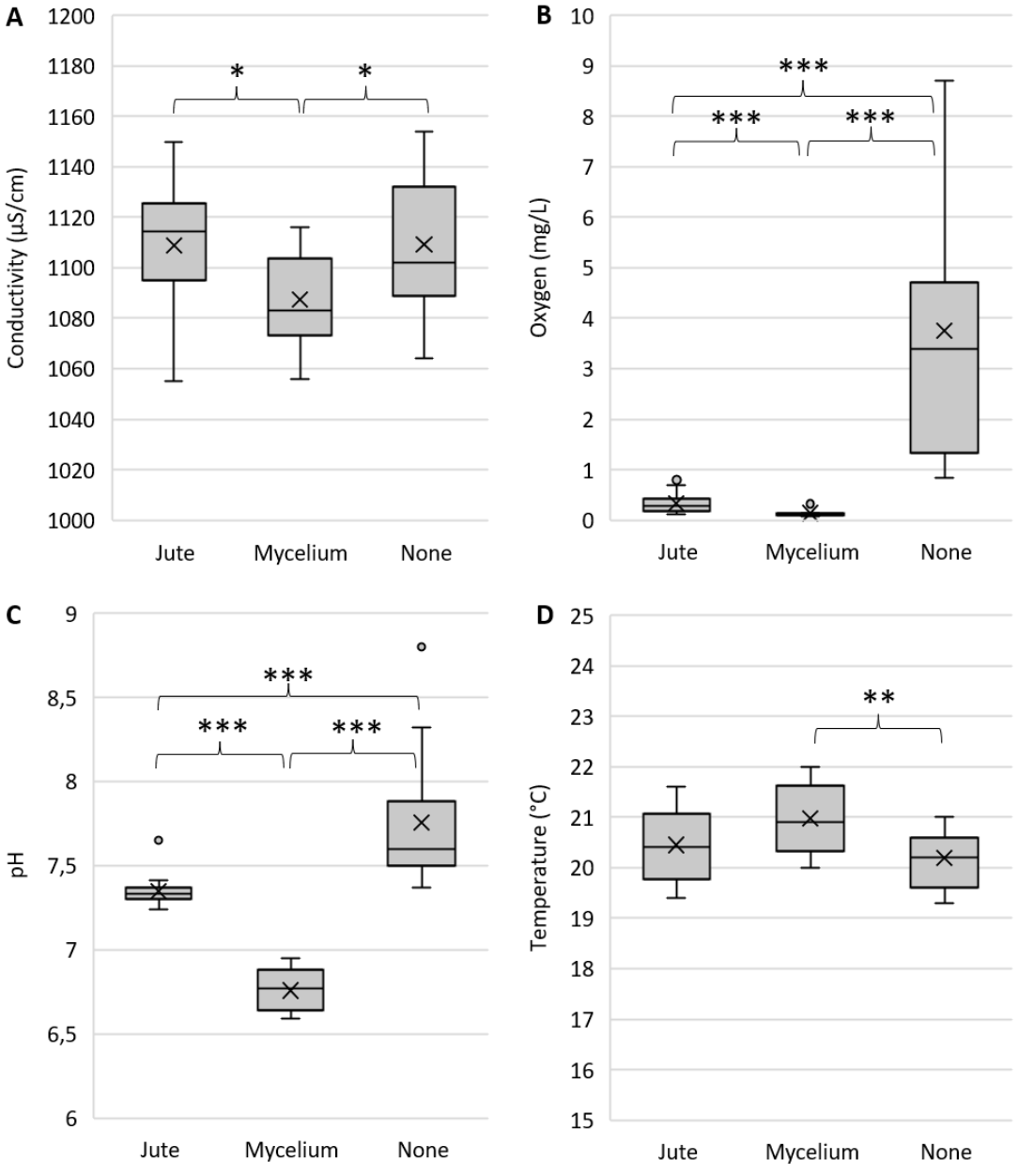


***Figure 6**, Effluent water characteristics of experiment 2 (August 23th) for all mesocosms, groups divided on substrate. 'X' indicates average. Significant group differences indicated with asterisks (* $p < 0,05$, ** $p < 0,01$, *** $p < 0,005$) **A.** Conductivity corrected for temperature, **B.** Oxygen concentration between 9p.m. and 11p.m., **C.** pH, **D.** Temperature between 9p.m. and 11p.m..*

3.2 Water characteristics

Effluent water characteristics (conductivity, oxygen concentration, and pH) were mainly dependent on the substrate used (Fig. 6). Conductivity was high (Fig. 6A), due to high chloride concentrations from the Zijdewetering (±150 mg/L; Sup. C1-3). The differences in pH and conductivity for mycelium, jute, and no-substrate (Fig. 6B-C), can be ascribed to the identity of the substrate [50, 78, 79], due to the presence of different acids, bases and ions in the substrates. The anaerobic conditions for mycelium and jute can be ascribed to both the substrate composition [50, 52], and oxygen-demanding microbial processes such as degradation and nitrification [52].

Temperature was dependent mainly on the measuring time, with higher temperatures measured later in the day (Sup. B2). Additionally, mycelium insulated the mesocosms, causing the water to be slightly warmer in the mornings (Fig. 6B). Temperatures remained mostly between 18°C and 25°C, which is around the same temperature as in the Zijdewetering at the same time. Temperatures could reach 30°C in the afternoon on warm days.

For plant species and combination, no significant effect was seen on water characteristics. Slightly higher oxygen concentrations could be seen with *Phragmites* (Sup. C1), which can be expected based on the fact that different plant species have different oxygen transporting capacities [80].

3.2.1 Sulphate

Effluent sulphate (SO_4) concentrations were significantly lower in mesocosms with mycelium or jute compared to mesocosms without substrate (Fig. 7), and compared to the influent concentration of 36 mg/L. In anaerobic conditions, sulphate is reduced by sulphate-reducing bacteria to sulphide (S_2^-) or gaseous sulphide (H_2S), a process strongly enhanced by the presence of a carbon source [81]. It is likely that mycelium and jute function as this carbon sources for sulphate reduction, stimulating sulphate reduction compared to the no-substrate mesocosms [81]. The increase in sulphate seen in the no-substrate mesocosms can be ascribed to the presence of oxygen, causing oxidation of sulphide to sulphate [81]. Sulphate decrease by mycelium and jute is, in that sense, also in line with the absence of oxygen over longer time periods.

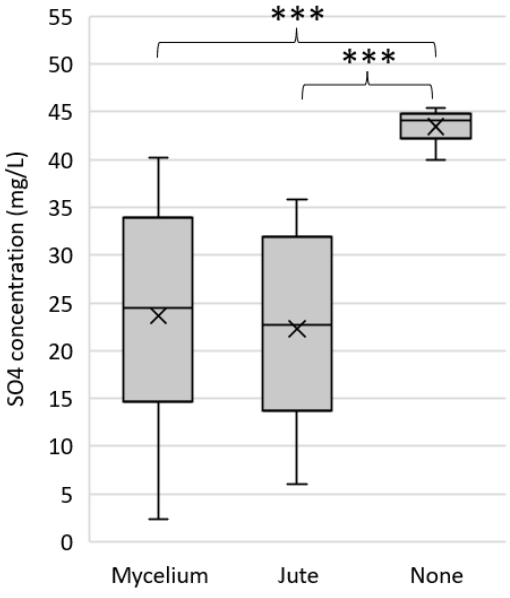


Figure 7**, Sulphate concentration in all mesocosms during experiment 2 (August 23th) divided on substrate. 'X' indicates average. Significant group differences indicated with asterisks (p < 0,005).*

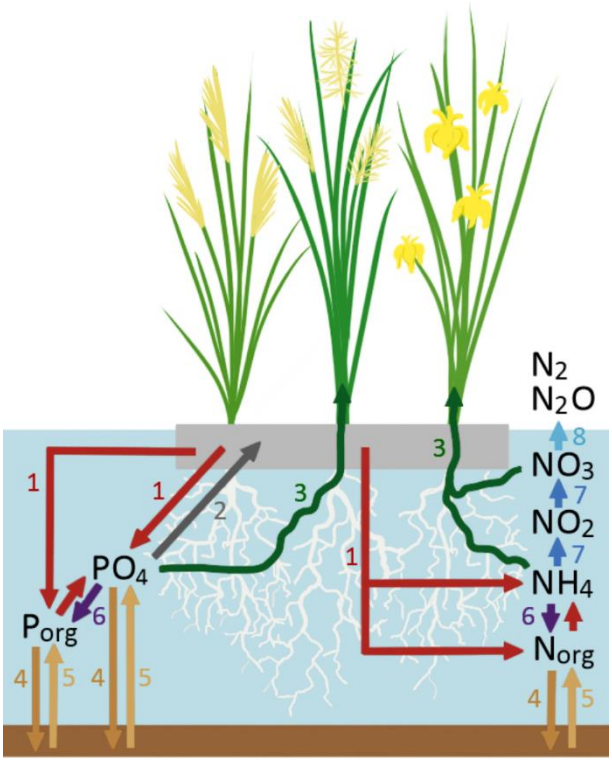


***Figure 8**, Nutrient removal pathways: 1) degradation, 2) sorption, 3) uptake by plants, 4) sedimentation, 5) release by sediment, 6) assimilation by microbes, 7) nitrification, 8) denitrification.*

3.3 Nutrients

Nutrients in the mesocosms were removed or increased via multiple pathways: 1) degradation of substrate and organic compounds by microbes, 2) sorption by substrate, 3) uptake by plants followed by assimilation, 4) sedimentation, 5) release by sediment, 6) assimilation by microbes, 7) nitrification by microbes (aerobic), and 8) denitrification by microbes (anaerobic) (Fig. 8).

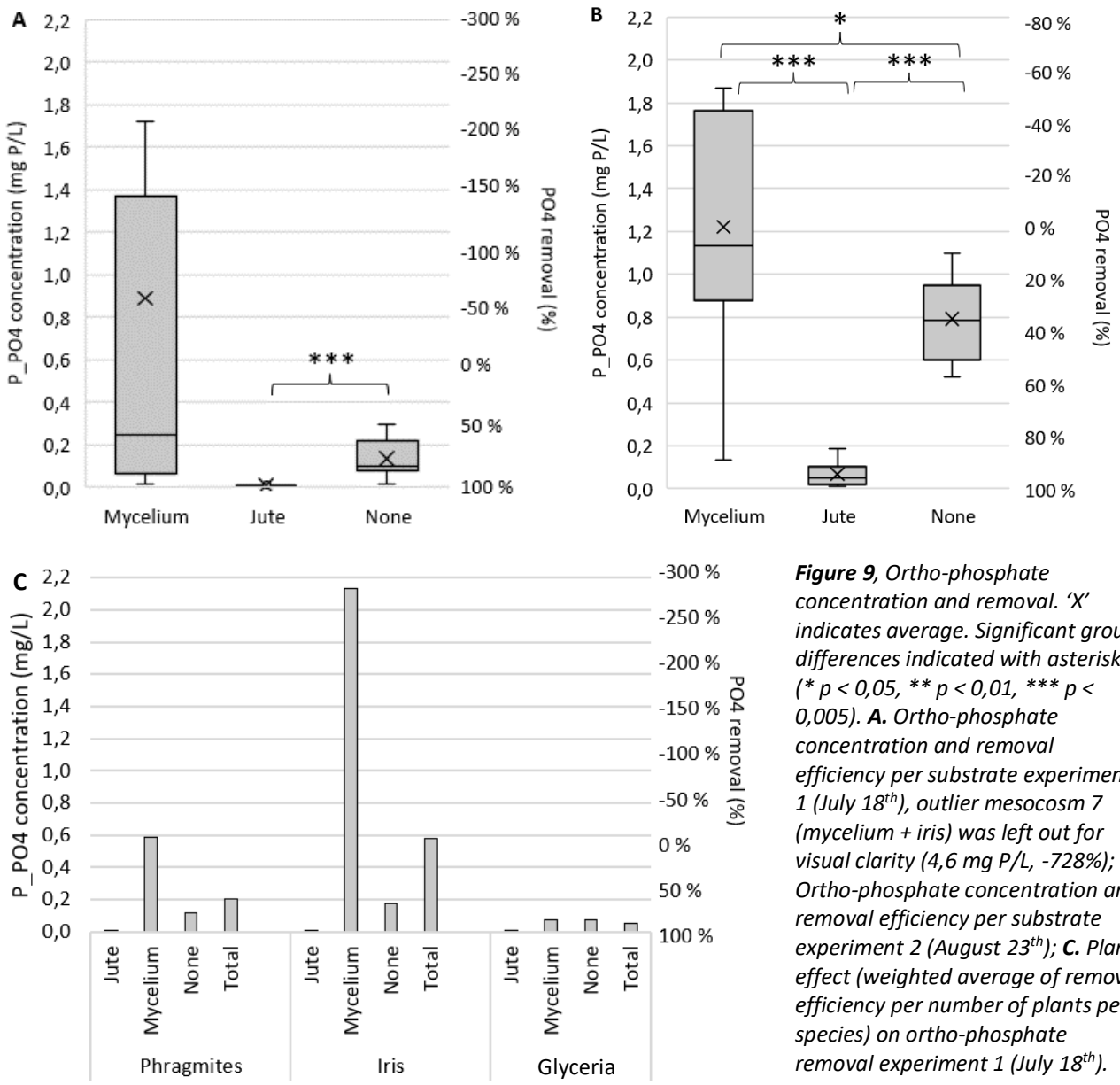


***Figure 9**, Ortho-phosphate concentration and removal. 'X' indicates average. Significant group differences indicated with asterisks (* p < 0,05, ** p < 0,01, *** p < 0,005). **A.** Ortho-phosphate concentration and removal efficiency per substrate experiment 1 (July 18th), outlier mesocosm 7 (mycelium + iris) was left out for visual clarity (4,6 mg P/L, -728%); **B.** Ortho-phosphate concentration and removal efficiency per substrate experiment 2 (August 23th); **C.** Plant effect (weighted average of removal efficiency per number of plants per species) on ortho-phosphate removal experiment 1 (July 18th).*

3.3.1 Phosphorus

Ortho-phosphate (PO_4) concentrations are high in the Zijdewetering and fluctuate strongly over time [33, 34]. In *experiment 1* and *3*, the influent contained 0,56 and 1,19 mgP/L ortho-phosphate respectively, which is far above the WFD standard of 0,11 mgP/L [32].

After treatment in the mesocosms, jute removed almost all ortho-phosphate to well below the standard (Fig. 9A-B). This is likely because of absorption into the jute (Fig. 8), since also mesocosms with only jute and no plants showed this removal (Sup. C1-3). Mesocosms without substrate showed ortho-phosphate removal percentage between 50 and 100% in *experiment 1* (Fig. 8A), and between 10 and 60% in *experiment 3* (Fig. 8B). Mycelium showed very poor ortho-phosphate removal, and in many a phosphate increase was seen (Fig. 9A-B). Ortho-phosphate concentrations were much higher in mesocosms with degrading mycelium (mesocosms 7: 4,6 mgP/L, and 14: 1,7 mgP/L, Sup. B1, Sup. C1-3), which indicates that the increase is caused by degradation of phosphor-containing organics inside the mycelium substrate.

During *experiment 1*, a clear effect of plant species was seen (Fig 9C). *Glyceria* showed far higher ortho-phosphate removal efficiencies compared to *Phragmites* and *Iris*, especially for mycelium containing mesocosms (Fig. 9C). It seems that *Glyceria* takes up enough phosphate to compensate for the leakage of the mycelium. However, this trend was not seen in *experiment 2* (August), when plants were turning yellow (Sup. B1).

Total dissolved phosphorus (TDP) showed the same removal trends as ortho-phosphate (Sup. C3). However, often the total phosphorus concentration was measured lower than the ortho-phosphate concentration in mgP/L (Sup. C3), while

ortho-phosphate is part of TDP. This discrepancy can be caused by the different methods used (HACH Lange for phosphorus, and IC for ortho-phosphate). Since total phosphorus was measured manually, it is less reliable than the IC-measured ortho-phosphate, and therefore only the ortho-phosphate data is presented.

Three mesocosms with high ortho-phosphate removal were selected for the *time experiment*. These contained only *Glyceria* and the different substrates. The mesocosm starting (t=0h) ortho-phosphate concentrations were about half the influent concentration of 0,27 mgP/L (Fig. 10), because little ortho-phosphate was left in the mesocosms before replacing half the treated water with influent. Most ortho-phosphate is removed within the first 24 hours after refreshing the mesocosms, with the fastest removal in the first 8 hours (Fig. 10). After two days, ortho-phosphate concentrations remain low for jute and no substrate, but starts to rise for mycelium. This supports the theory that breakdown of mycelium causes the ortho-phosphate increase. Leakage of phosphorus from substrates has been reported before [75].

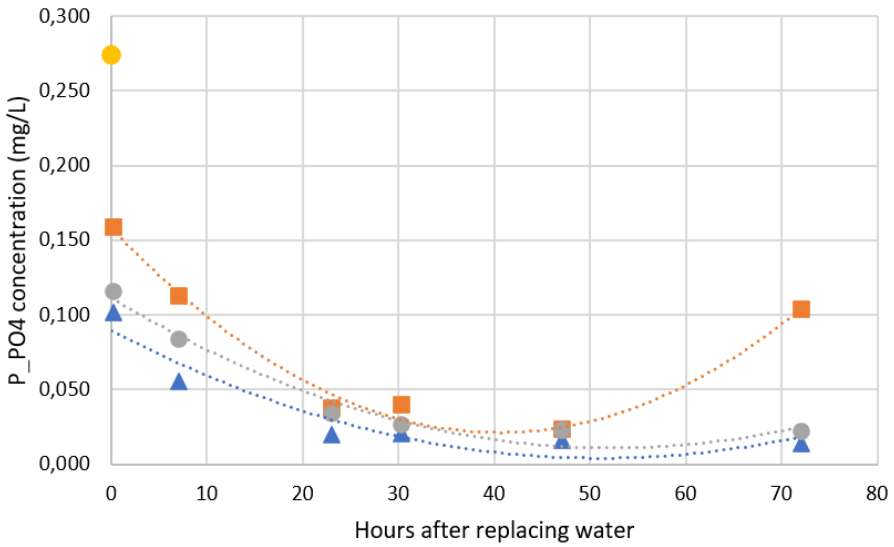


***Figure 10**, Ortho-phosphate concentration (mgP/L) over time from 'time experiment'. Each data-set represents a mesocosm over time with only Glyceria plants and one of the three substrates.*

3.3.2 Nitrogen

Nitrogen was measured in different forms: ammonium (NH4), nitrite (NO2), nitrate (NO3), and total dissolved nitrogen (TDN). Other nitrogen compounds (NOx) were assumed to be zero [36, 82]. Dissolved organic nitrogen was calculated as *N_org = TDN - N_NH4 - N_NO2 - N_NO3* [36, 82]. The concentrations of all forms of nitrogen are expressed in their nitrogen content (mgN/L). The environmental WFD standard is expressed in total dissolved nitrogen as: TDN < 2,3 mg N/L [32], while the Zijdewetering has an average TDN concentration of 4-6 mg/L [34].

For all mesocosms, in all experiments, nitrite and nitrate were completely removed within 8 hours and remained under the quantification limit (Sup. C5), which is very efficient [36, 82, 83]. This indicates complete denitrification [36, 83] (Fig. 8), which is in line with the low oxygen concentrations found [36, 82, 84]. Only mesocosm 14 (M-O) in *experiment 1* showed increased nitrite (0,059 → 0,272 mgN_NO2/L) and detectable nitrate concentrations (0,047 mgN_NO3/L).

Ammonium removal is high for most mesocosms (80-100% *exp. 1* & 50-90% *exp. 2*, Fig. 11A&C), in line with other FTW set-ups [26, 35, 36]. The removal of ammonium was also fast: within 8 hours most ammonium is removed (Fig. 12A). This is partially by plant uptake [85], but mostly by nitrification into nitrite under aerobic conditions [36] (Fig. 8). Nitrification explains the higher efficiency found in the mesocosms without substrate, which had higher oxygen concentrations (Fig. 6B). After 24 hours, the ammonium concentration started rising (Fig. 12A). Ammonium increases because of breakdown of organic nitrogen-containing compounds [86] (Fig. 8). Consequently, the rotting mycelium mesocosms 7 and 14 have a low, or even negative, ammonium removal efficiency (Fig. 11A&C, Sup. C1&3).

For TDN, most mesocosms meet the standard of 2,3 mg/L (Sup. C4), though the removal is less efficient: a maximum of 50-80% (Fig. 11B&D). This is mainly due to an increase in organic nitrogen after ± 30 hours (Fig. 11C). Dissolved organic nitrogen is the nitrogen containing fraction of dissolved organic matter and consists of various compounds (e.g. proteins, amino acids, urea, humic and fulvic substances) [86, 87]. Mostly, organic nitrogen forms a small fraction of TDN [82, 83, 86], but in this research organic nitrogen forms the majority of the total nitrogen. In warm stationary water, highly active microbes produce and release large amounts of organic nitrogen during growth and decay [86, 88], which explains this large portion of organic nitrogen.

In conclusion, like phosphorus, all nitrogen forms are removed most efficiently with a retention time of 24 hours.

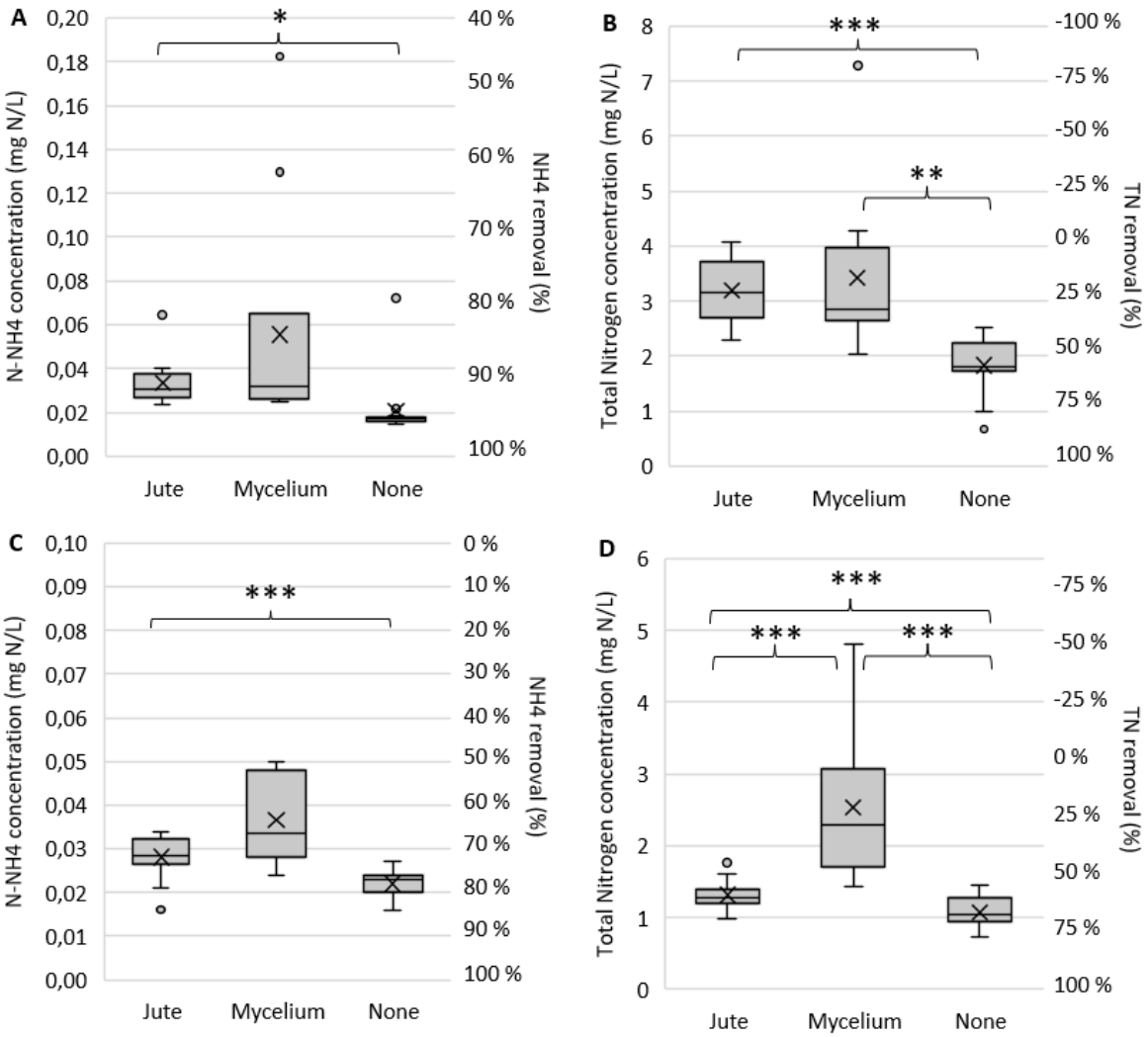


***Figure 11**, Boxplot of nitrogen concentration (left Y axis) and removal (right Y axis) per substrate. 'X' indicates average. Significant group differences indicated with asterisks (* p < 0,05, ** p < 0,01, *** p < 0,005). **A.** Ammonium removal experiment 1 (July 18th); **B.** Total nitrogen concentration experiment 1 (July 18th), **C.** Ammonium removal experiment 2 (August 23th), outlier mesocosms 7 (mycelium + iris) and 14 (mycelium + no plants) were left out for visual clarity (7: 0,41 mgN/L, -728%; 14: 1,02 mgN/L, -%); **D.** Total nitrogen concentration experiment 2 (August 23th).*

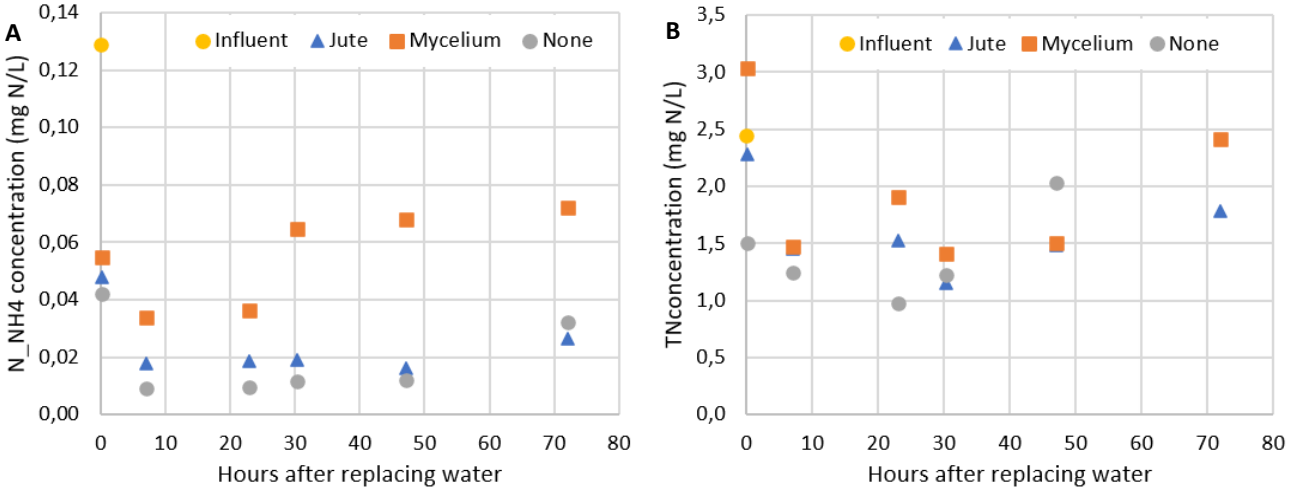


***Figure 12**, Nitrogen concentration over time for mesocosms 2, 27, and 32 from time experiment (July 29th-August 1st). **A.** Ammonium, **B.** Total dissolved nitrogen (TDN = TN). Total nitrogen concentration 'no substrate' 72 hours was not measured. After 24 hours, dissolved organic nitrogen concentrations were approximately the same as total nitrogen.*

3.3.3 Upscaling nutrient removal

Upscaled nutrient removal for the Zijdewetering can be calculated based on the best-performing mesocosms. The best ortho-phosphate removal was seen in *experiment 2* for mesocosms 29 (J-P): 1,18 mgP/L/day, which calculates to 28,32 mgP/mesocosm/day, or 147 mgP/m^2/day (Sup. C6). This is half of the phosphorus removal reported in comparable FTW set-ups (Sup. C7) [59, 89], and half to a fourth of the removal by constructed wetlands (Sup. C7) [90, 91], depending on the substrate [92]. Each day, 31,3 kilograms of ortho-phosphate is discharged from the WWTP Ede [33]. Following the WFD standard of 0,11 mg/L and an average daily water discharge of 37391 m3, WWTP Ede discharges 27 kg excess phosphor per day (Sup. C6). Consequently, we would need about one million mesocosms (18 hectares of 25 cm deep water) to clean the Zijdewetering from excess phosphorus.

During the *time experiment*, the best removal is 1,2 mgN/L in 8 hours, which calculates to 28,8 mgN/mesocosm/8h (Sup. C6). For streaming water that remains one day in the system, this means that one mesocosm removes 86,4 mgN/mesocosm/day, or 449 mgN/m^2/day (Sup. C7), which is quite high for nitrogen removal in small FTW set-ups [93, 94], and comparable to constructed wetlands (Sup. C7) [68, 95, 96]. Each day, 287 kilograms of nitrogen is discharged at the WWTP Ede [33]. Based on the WWTP flow rate (average 37391 m3/day), and the WFD nitrogen standard of 2,3 mg/L, 201 kg of excess nitrogen is discharged daily (Sup. C6). Following this calculation, we would need about 2,3 million mesocosms (45 hectares of 25 cm deep water) to clean the Zijdewetering from excess nitrogen.

However, upscaling small pilot mesocosms will not translate directly to larger systems, since an upscaled system is much deeper, and larger systems have been reported to be more efficient for both phosphorus and nitrogen (Sup. C7) [59, 68, 89]. Especially for nitrogen removal, it is likely that an upscaled streaming system will show less increase in organic nitrogen, but a decrease of about 25% [97]. FTWs can treat water efficiently up to 1,5 meter deep [59] using *Phragmites* roots (90 cm [37]) combined with biofilm carriers [26, 38, 51, 59]. This is 6 times deeper than the mesocosms used. Plant biomass and uptake will not increase 6 times, but microbial processes play a more significant role in pollutant removal [25, 40-43]. Therefore, it is predicted that an upscaled set-up of 1,5 m deep will be about 5 times more efficient per area then reported in this research, calculating to removal rates of 735 mgP/m^2/day and 2244 mgN/m^2/day (or 1318 mgN/m^2/day for inorganic nitrogen). Following this prediction, about 3,6 hectares is needed for ortho-phosphate removal, and 9 or 6,7 hectares for nitrogen removal, depending on the increase or decrease of organic nitrogen (Sup. C6). Since plant growth will be limited by low phosphorus concentrations, using 3,6 hectares would be enough to battle harmful algae and water plants. This area is small compared to other wetland systems [23].

3.4 Micropollutants

In this research, 19 of the 21 micropollutants were detected (Fig. 13, Table 2, Sup. D1-3). Of these, metoprolol, sotalol, irbesartan, carbamazepine, diclofenac, benzotriazole and 4(5)-methylbenzotriazole are guide compounds in micropollutant removal (Table 2) [23]. Trimethoprim and desphenyl chloridazon were not detected.

We have used the predicted no-effect concentration (PNEC) as a standard [98], although for some micropollutants there is an environmental standard available (Sup. D2) [99]. PNECs are often used instead of environmental standards because of a lack of official standards and due to uncertain environment effects [98]. Additionally, PNECs are more suitable when working towards the 'Zero pollution ambition' [European commission, 2021].

We found that micropollutant concentrations were mostly (16/21) below the (PNEC) (Fig. 13, Sup. D2). For carbamazepine, propranolol, and sulfamethoxazole, influent concentrations were above their PNEC, while effluent reached below the PNEC in August (Fig. 13, Sup. D2, Sup. D7). 4(5)-methylbenzotriazole and diclofenac remained above their PNEC in both in- and effluent (Fig. 13). Of these five substances, especially carbamazepine and diclofenac are known to have severe adverse effects on the environment [100, 101].

3.4.1 Micropollutant removal

The removal efficiencies for sotalol, irbesartan, sulfamethoxazole, carbamazepine, diclofenac, DEET, benzotriazole, and 4(5)-methylbenzotriazole were relatively high compared to literature (Sup. D4) [88, 102-109]. The negative efficiency for DEET in *experiment 3* was caused by a low influent concentrations compared to remaining DEET concentrations in the mesocosms. For metoprolol, propranolol, atenolol, erythromycin, clarithromycin, and furosemide, the removal efficiencies were comparable to literature (Sup. D4) [102, 103, 109, 110], despite the retention time of 24 hours being relatively short [21, 70, 104, 109]. Caffeine and MCPP, however, had lower removal efficiencies than literature (Sup. D4) [103, 111]. For caffeine this is likely due to sample contamination. For MCPP, this is likely due to the short retention time of 24 hours, as this compound is removed slowly [70]. Clarithromycin, dimetridazole, BAM, and 2,4-D had concentration of below the quantification limit of 50 ng/L, making their removal incomparable to literature.

From the seven guide compounds detected, six were removed with an efficiency above 70% in august. In September, these six were removed with an efficiency of 30-50%. This means that these floating wetlands comply with the removal standard of the IMPV during the summer months only [23].

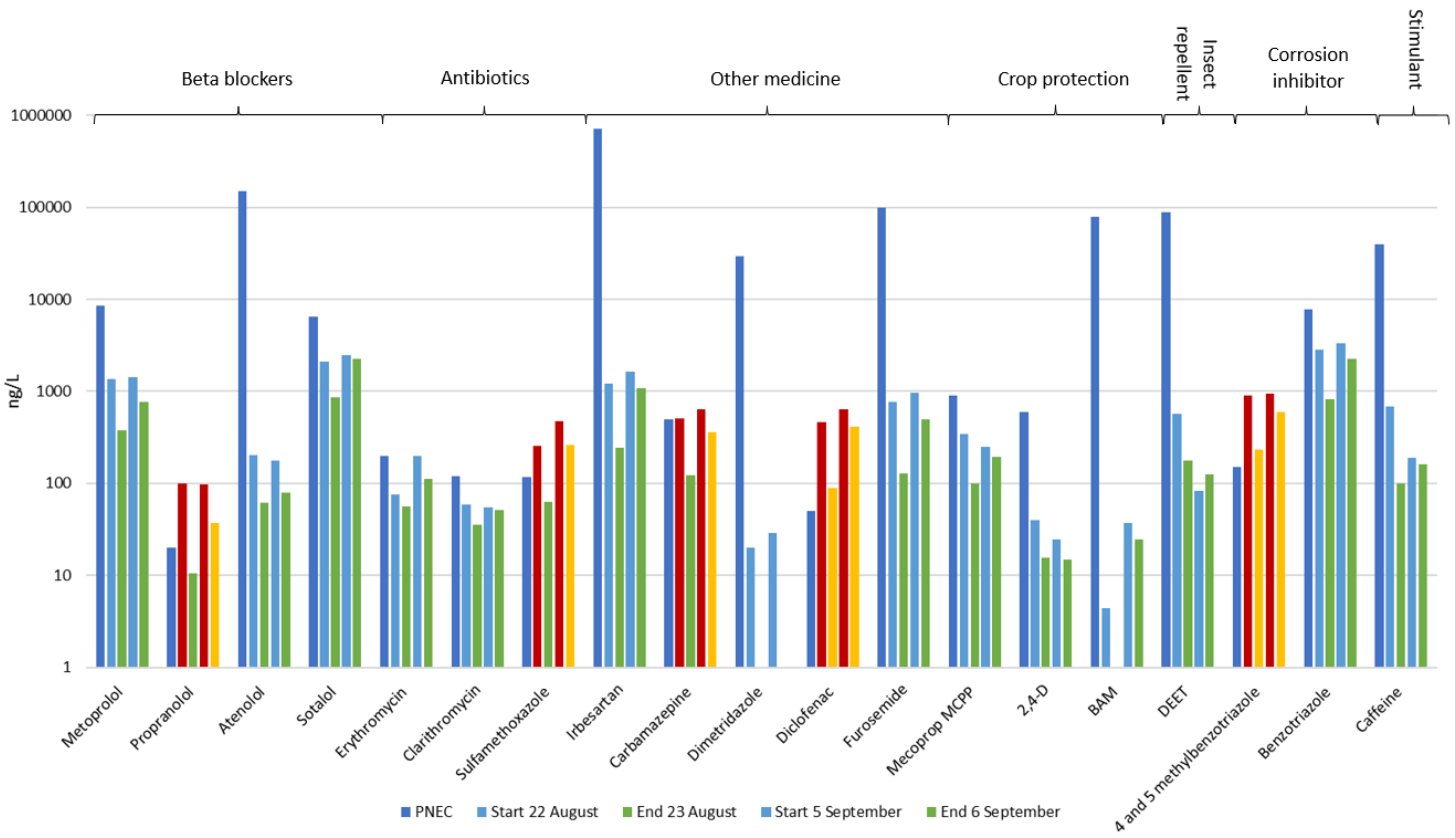


***Figure 13**, Predicted no-effect concentration (PNEC), average influent (start) concentrations, and average effluent (end) concentrations for jute mesocosms in experiment 2 and 3. Influent concentrations exceeding the PNEC are indicated with red, effluent concentrations above PNEC are indicated with yellow. Note the logarithmic scale for concentrations.*

3.4.2 Substrate influence on removal

Jute is the most reliable substrate for micropollutant removal, because its large surface area supports an active microbiome that degrades micropollutants, and because it is able to absorb some pollutants. The hydrophobic compounds metoprolol, propranolol, and carbamazepine are known to be retained by bark and compost substrates (Sup. D4) [78]. However, comparing jute to no-substrate mesocosms shows that sorption is not the main removal pathway in this set-up (Fig. 14). The only compounds for which sorption seems to be relatively important are carbamazepine, a pharmaceutical known to be recalcitrant for biodegradation [75, 112, 113], and dimetridazole [114].

Mycelium did not stimulate micropollutant removal (Fig. 14). The fungus did not break down micropollutants because it needs a pH of 4,5 to do so [65]. It is likely that the organic compounds in the remnant wood waste were preferred by microbes as an energy sources [65]. Besides, the mycelium covered most of the water surface, thereby inhibiting potential photodegradation of compounds. Propranolol, sotalol, sulfamethoxazole, irbesartan, diclofenac, furosemide, MCPP, and benzotriazole degrade readily (>50%) under influence of UV [21, 107]. For these compounds, sulfamethoxazole exempted, indeed the average efficiency of mycelium-mesocosms is lower than the other mesocosms (Fig. 14). It must be noted, that during these measurements, many of the no-substrate mesocosms were overgrown with duckweed, which also limit sunlight penetrating the water. For some overgrown mesocosms the efficiency for these photo-degradable compounds is indeed lower (Sup. D7-8), but no significant correlation could be found.

3.4.3 Plant influence on removal

There is no significant effect of plant species or combinations on removal efficiency found (Sup. D6). However, the micropollutant experiments were done after the first yellowing of plants, and for nutrients only *experiment 1* showed a clear plant species difference (Fig. 8C). Therefore, there is a likely undetected season-dependent plant effect.

3.4.4 Upscaling

Based on these two experiments, it is not possible to calculate the area needed for a 70% micropollutant removal [23]. Treating all effluent with the upscaled system of 1,5 m deep and a retention time of 24 hours, we would need an area of 2,5 ha (37391 m^3 / 1,5 m = 24927 m^2). Following, the pollutant concentrations in the effluent from Ede need to be studied in more detail, and we need a better understanding of the factors influencing micropollutant removal in the mesocosms.

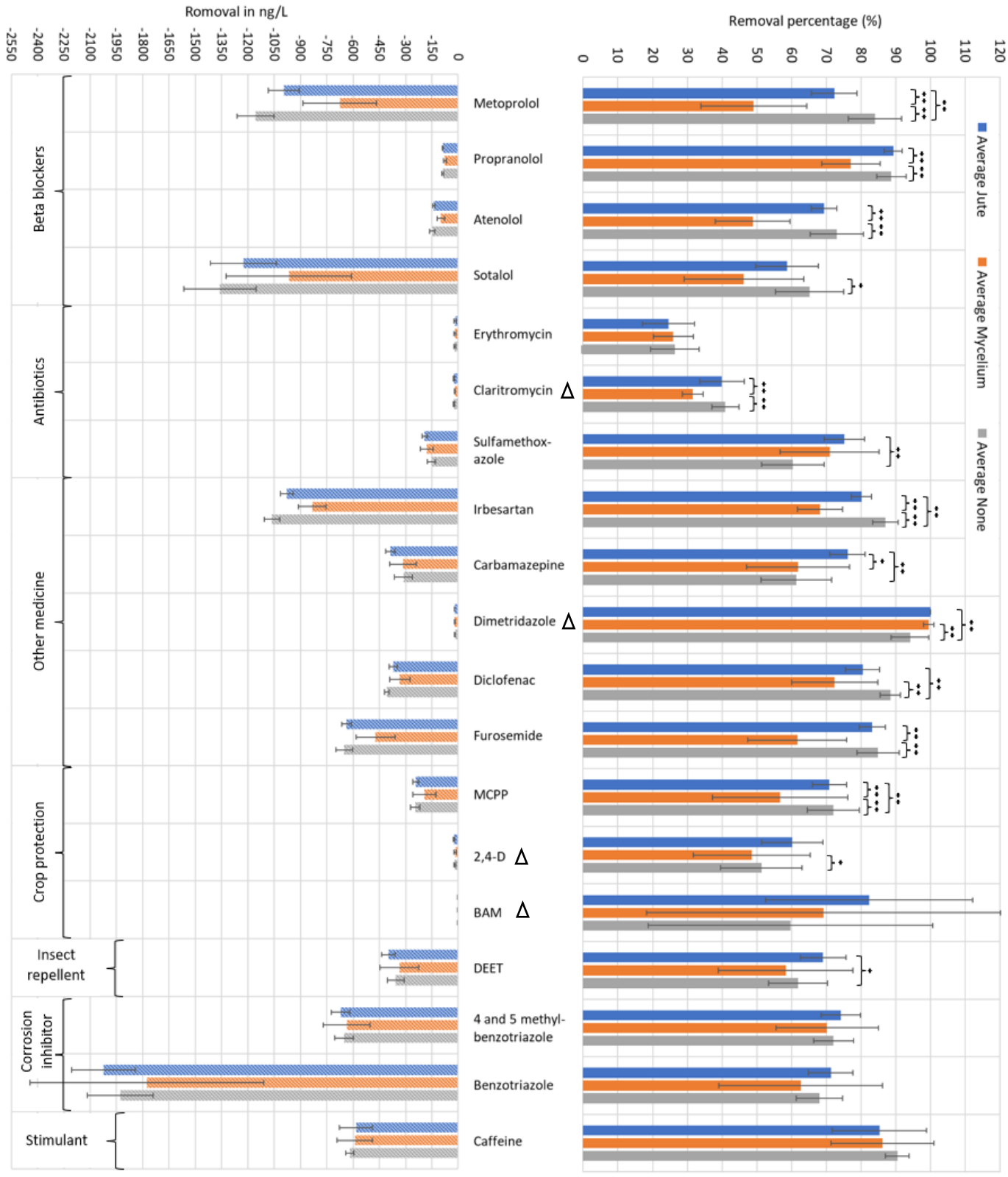


***Figure 14**, Micropollutant removal experiment 2. **Left** pollutant removal from influent to effluent in ng/L, **Right** in percentage (influent-effluent/influent*100%). Bars represent average removal per substrate (blue jute, orange mycelium, and grey no substrate). Error bars represent standard deviations. Significant group differences indicated with asterisks (* p < 0,05, ** p < 0,005). Trimethoprim and desphenyl chloridazon were measured, but not present (<1ng/L) in influent or effluent samples. Clarithromycin, Dimetridazole, 2,4-D, and BAM are indicated with a triangle Δ, for they had concentration of below the quantification limit of 50 ng/L and are therefore unreliable. (Sup. D7)*

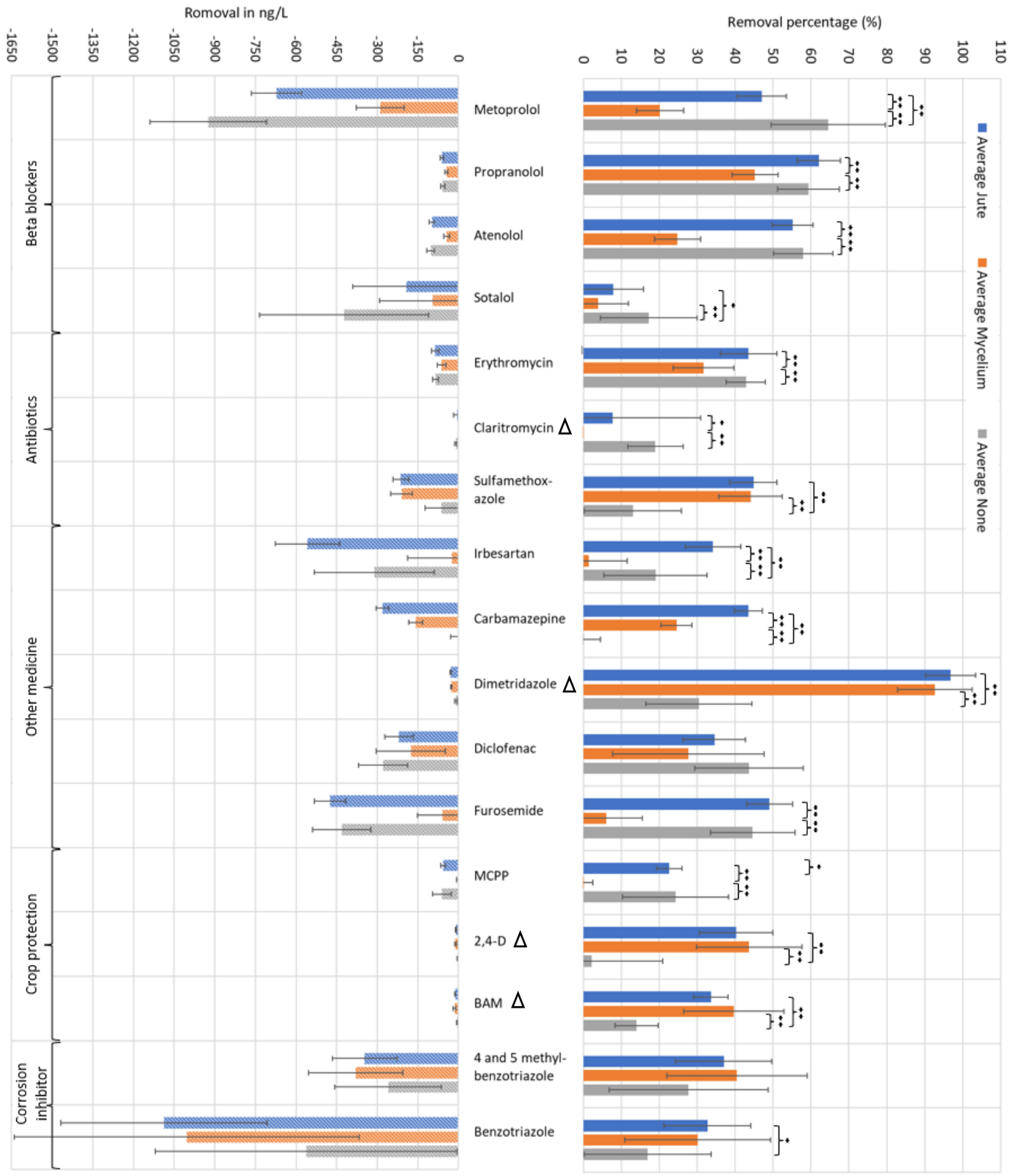


***Figure 15**, Micropollutant removal experiment 3. **Left** pollutant removal from influent to effluent in ng/L, **Right** in percentage (influent-effluent/influent*100%). Bars represent average removal per substrate (blue jute, orange mycelium, and grey no substrate). Error bars represent standard deviations. Significant group differences indicated with asterisks (* p < 0,05, ** p < 0,005). Trimethoprim and desphenyl chloridazon were measured, but not present (<1ng/L) in influent or effluent samples. Clarithromycin, Dimetridazole, 2,4-D, and BAM are indicated with a triangle Δ, for they had concentration of below the quantification limit of 50 ng/L and are therefore unreliable. Caffeine is not represented due to high eluent contamination. DEET is not represented due to coincidental low influent concentration. (Sup. D8)*

3.5 FTWs in the Zijdewetering

The FTWs build in this study were not durable over longer time (Fig. 16A-E). Jute was ripped from the PVC frame after one and a half month, leaving the plants floating freely in the frame (Fig. 16 C-E). The buoyancy of the frame remained good, in contrast to other jute-based FTWs from literature [60] (Fig. 16F). The mycelium units started to degrade very quickly (Fig. 16C-D), and many sank (Fig. 16D-E). The units that did not sink were covered with bird faeces (Fig. 16E) from visiting ducks (Fig. 16D). Only well-planted units stayed relatively clean (Fig. 16E).

Phragmites and *Iris* had difficulty establishing in the streaming water due to the poor substrate integrity. *Glyceria*, on the other hand, managed to stay afloat and thrived well in the polluted stream (Fig. 16 C-D).

***Figure 16**, FTW durability. FTWs in the Zijdewetering on **A.** June 29th (start), **B.** July 28th, **C.** August 19th (start mycelium), and **D.** September 1st; **E.** Detail of mycelium unit on September 26th; **F.** A jute-based FTW after 1 year, derived from Strosnider et al. 2017 [60].*

3.6 Practicalities, possibilities and limitations

Many papers report that wetlands are an environmental friendly and cost-effective option as a tertiary treatment for WWTP effluent [28, 81, 115, 116]. However, they also have some practical disadvantages: need for harvesting of biomass, potential toxicity of the biomass, low winter-efficiency, and large area needed [23, 28]. Still nature-based solutions offer additional off-target benefits, since they remove various compounds from water, absorb CO2, increase biodiversity, retain water for dry periods, and function as an eco-buffer zone between the WWTP and receiving surface waters [81].

3.6.1 Environmental impact: Life cycle assessment comparison

Life Cycle Assessments (LCAs) have determined the environmental impact of different types of wetlands for nutrient removal from wastewater, and found them to be environmentally friendly compared to conventional methods with the same treatment performance [117, 118]. Recently, the STOWA analysed micropollutant removal and environmental

impact of multiple nature-based techniques based using LCAs [23, 115]. They showed that constructed wetlands, though somewhat less efficient, have a smaller CO_2 footprint compared to ozonation and activated carbon [23].

FTWs are usually simpler in structure than constructed wetland and do not need energy demanding water pumps. Therefore, the resources, energy use, and Global Warming Potential of FTWs may be significantly lower than other wetlands. Yao *et al*. (2021a) performed an LCA comparing floating wetlands to constructed wetlands for nutrient removal and found that the floating wetlands had higher environmental impact due to a complicated construction using PVC, floating beds, planting pots, steel wire and concrete anchors [119]. They did not include the pumping of water into or out of the wetland, which is the main energy usage for constructed wetlands [115]. Takavakoglou *et al*. (2021) and Yao *et al*. (2021b) also found that raw material production and acquisition contributed to the majority of the FTW's environmental impact [62, 120]. Therefore, the choice of material can largely improve the FTW environmental impact, by using waste streams or biobased materials.

3.6.2 Costs

Nature-based solutions are cost-compatible compared to other post-treatment techniques [121, 122], with 0,06 to 0,42 €/m3 depending on materials used and whether land area needs to be bought [23]. Construction cost are the main costs for FTWs [62, 120]. These vary depending on the choice of material and can be reduced substantially by using second-hand materials or waste streams. With the current energy crisis and shortage of activated carbon, it is likely that these techniques will become increasingly expensive, making nature-based solutions an economically feasible alternative.

Maintenance costs consist of harvesting plant biomass twice per year and processing the biomass (Section 3.6.3). For the Zijdewetering, this can be done concurrent with the mowing of the water boarders, instead of mowing the water plants that are replaced by FTWs. Thus, no additional maintenance costs are generated by FTWs in the Zijdewetering.

3.6.3 Maintenance: system care and biomass harvesting

FTWs require little maintenance [25, 122], and can stay outside the whole season. For this, it is important that the FTWs remain stable and buoyant for a long period (10-20 years) to support plant growth [60]. Some FTW implementation had difficulties in generating durable FTW designs [60, 123] (Fig. 16F). Other studies did not have these issues [61, 121], and multiple companies exist now-a-days that produce durable floating planting beds (e.g. BioHaven®, Aqua-Flora® or Aqua Biofilter™). In cooperation with companies, durable sustainable FTW designs need to be made before implementation.

The only FTW maintenance required is the harvesting of plants. Plants take up nutrients in spring and summer, but release the majority of these nutrients again in autumn and winter [85, 124]. Thus, there is little nutrient removal by plants when they are not harvested. If harvested correctly, plant uptake can contribute 20-25% of N and P removal [124]. Above ground plant biomass should be harvested at the end of July, when the nitrogen and phosphorus content is optimal [85, 124], and ecological disturbance is relatively low [125]. In autumn, nutrient content is higher in roots [85, 124]. Contrary to constructed wetlands, it is possible to harvest these plant roots or whole plants in FTWs, thereby optimising nutrient removal in autumn [25, 26].

Practically, FTWs in the Zijdewetering could be harvested from the water boarder using an mowing machine with arm. This can be done while mowing the water boarder, and should be done conform the ecological mowing protocols [125]. It is suggested to mow in patches [125], leaving about one third of vegetation to protect and stimulate ecology. Flowering plant should not be mown for ecological and aesthetic reasons.

3.6.4 Biomass application and pollutants content

Costs and environmental impact of treatment wetlands can be substantially lowered by finding an application for the harvested plant biomass [26, 28]. Low-end purposes like energy generation by burning or fermenting biomass, can yield bio-ore that can be used for the recovery of phosphorus [126, 127] or valuable metals [26, 28]. For high-end products like building materials or livestock feed, micropollutant accumulation in plant material must first be investigated [25, 28]. Whether pollutants accumulate in biomass, are transported within the plant, or are degraded, dependents on the compound and plant species. For example, propranolol is taken up by *Phragmites australis, Typha angustifolia*, and *Juncus effusus*, and is transported from root to shoot only in *Phragmites*, thereby contaminating the biomass, while *Typha* and *Juncus* degrade propranolol within their tissues [70]. For many pollutants and plants it is known whether or not contaminants are taken up, accumulate or degrade [21, 73], still this remains unknown for most contaminants. Therefore, the pollutant content of plant material should always be investigated before choosing high-end biomass application.

3.6.5 Winter efficiency

Wetland efficiency is season dependent for both nutrient [51, 96], and micropollutant removal [23, 128-132]. Low nutrient removal in winter is mainly caused by nutrient leakage from senescing plants [85, 124], while low micropollutant removal is caused by lower microbial activity at low temperatures [23, 51]. In this research, micropollutant removal was also clearly season dependent (Fig. 14, Fig. 15, Sup. D4, Section 4.1.2).

There are several techniques that can increase pollutant removal in the winter up to 98% [51]. Firstly, biofilm carriers function as artificial roots that remain present in winter to support the microbiome [26, 38, 51]. Secondly, absorbents like biochar (non-fossil absorbent made from charred biomass) retain pollutants which can be broken down or assimilated in spring [23, 51, 78]. Thirdly, artificial aeration enhances aerobic pollutant breakdown and increases both summer and winter efficiency [51, 122]. Energy use for aeration also increase the FTW footprint, which can be compensated by using solar panels or green energy [51, 133]. Fourthly, selecting multiple plants with different growth-seasons extends the functional season of FTWs [53], while adding cold-resistant plants can increase winter efficiency specifically [51]. Lastly, bioaugmentation with efficient cold-resistant microbes stimulates microbial breakdown of pollutants in the winter [51]. These options can be combined and the most suitable enhancement is dependent on location and application.

3.6.6 Land area

The main issue with constructed wetlands is the area needed for treatment of effluent [23, 115]. Empty land is not present in abundance in a densely populated country as the Netherlands [115]. Three main solutions for this problem are: 1) making wetlands more efficient (Section 3.6.5) [23, 26, 38, 51, 78]; 2) finding alternative wetland locations like rooftops [134-136], or 3) re-directing agricultural land to produce valuable biomass with waste water [Aqua-farm]. However, floating wetlands do not need empty land area, but free water area. The Netherlands has plenty of open surface waters that are suitable for FTW application. Because of this abundance, floating wetlands could be *the* solution to the Dutch area-issue in treatment wetlands.

3.6.7 Additional benefits

Nature-based solutions are multifunctional. Natural post-treatments do not only remove nutrients and micropollutants, they also remove pathogens, suspended solids, and metals [23]. By strongly reducing the general ecotoxicity of effluent, they function as an ecological buffer zone between the WWTP and the receiving surface waters [23]. Moreover, in dry periods, larger natural treatment systems can help retaining water, or make water suitable for agricultural re-use. The latter is especially beneficial when micropollutants are removed but some nutrients remain [137]. Lastly, nature-based solutions are adaptive and robust compared to conventional treatments.

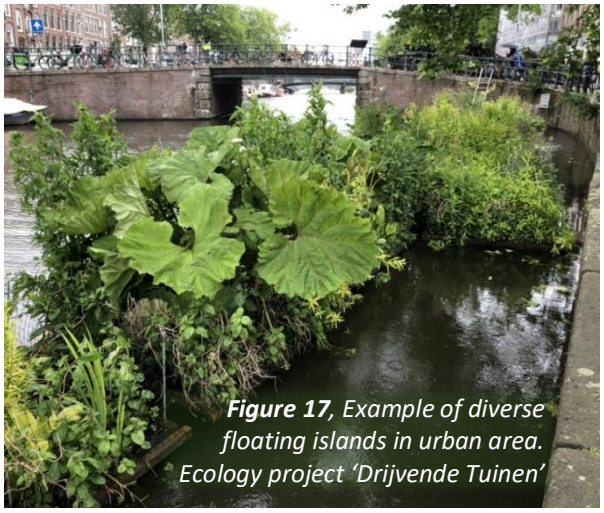


Figure 17, *Example of diverse floating islands in urban area. Ecology project 'Drijvende Tuinen'*

Nature-based solutions can also be used in combination with recreational purposes (The Ceuvel, Amsterdam). Biodiverse FTWs have high aesthetic value, making them suitable for implementation in urban areas (Fig. 17). These wetlands can show people the benefits and beauty of nature-based solutions, while enhancing mental health [138, 139], cooling cities [23], cleaning the environment [139], and creating natural habitats for urban wild-life [139].

Collectively, nature-based solutions, including floating wetlands, tackle multiple climate issues at once and can be considered as an integral "no regret"-solution.

4. Discussion

This research has examined application of floating treatment wetlands (FTWs) for local treatment of the Zijdewetering, a polluted WFD surface water. For this, 39 mesocosms were used with different substrates (jute and mycelium) and different plant species (*Phragmites australis*, *Iris pseudacorus*, and *Glyceria maxima*). Four experiments with different treatment times showed that 24 hours is the optimal hydraulic retention time for this system. High removal rates were achieved for ortho-phosphate (80-100%), ammonium (70-90%), nitrite (100%) and nitrate (95-100%); moderate removal rates for total nitrogen (50-80%); and varying efficiencies for different micropollutants (10-100%). The substrate had most influence on removal rates; while plants had a relatively minor influence.

The following sections will first touch upon some research inaccuracies. Thereafter the substrate and plant suitability for the Zijdewetering is reflected, while giving options for future research. Lastly, the application of FTWs for the Zijdewetering is reflected together with practical limitations and advice is given for proper implementation.

4.1 Research inaccuracies and future improvements

4.1.1 System monitoring and mass balance

Temperature, pH, and oxygen concentration have a strong day-night rhythm. Since we measured only during sampling (between 9 and 11a.m.; two times at 5p.m.), the continuous mesocosm water characteristics remain unknown, whilst

these conditions have major impact on pollutant removal [51, 140]. Moreover, measuring the redox potential and chemical oxygen demand would have given more insight into the processes happening in the mesocosms [141].

Measuring biomass and nutrient content of the plants, algae and duckweed, before and after the experimental period would have given a precise estimation of nutrient removal by biomass production [54]. When also measuring mass and nutrient content of substrate and sediment, a mass balance for nutrients can be made. Such mass balance gives insight into the relative contributions of nutrient removal pathways [38, 103, 142]. Likewise, micropollutant content in substrate and plant biomass should be measured to investigate which removal pathways contribute most, optimising the system.

4.1.2 Micropollutant sorption during sampling

Some micropollutants attach well to different types of plastics [143, 144], among which the sampling tubes and nylon filters used in this research. In *experiment 3*, 5% acetonitrile was directly added to the samples to prevent sorption and samples were centrifuged instead of filter-sterilized [74]. There is a strong difference in removal efficiency between these two experiments (Fig. 14, Fig. 15). However, if this was purely due to micropollutant sorption by tube and filter, the influent concentrations of *experiment 3* would be higher than *experiment 2*, which is often not the case. When correcting for a substantial sorption of 100 ng/L by tube and filter in *experiment 2*, the removal efficiencies are just 10-20% lower for most pollutants (Sup. D3). Only the compounds that were detected around or below quantification limit of 50 ng/L (erythromycin, clarithromycin, dimetridazole, 2,4-D, and BAM) have a dramatically lower efficiency (Sup. D3). Most importantly, other studies that did not take this sampling sorption into account, still found lower removal efficiencies than in this research [73, 103]. Therefore, the results as shown can be used as an strong indication of micropollutant removal with a variance of ± 10%. Further experiments should be performed to verify these high removal efficiencies.

4.1.3 Dilution by refreshing water

In *experiment 2* and *3,* the hydraulic retention time and the treatment time are not the same (Sup. A2). This means that the fresh influent is diluted with water treated for five days, which often has a lower pollutant concentration (ortho-phosphate Fig. 10, ammonium Fig. 12A, nitrite and nitrate Sup. C5). Thus, the measured removal efficiencies (influent-effluent) are different from the actual take up or breakdown in 24 hours. Nonetheless, they do give a good indication of treatment performance compared to other mesocosms over longer time, and the dilution has no influence on the calculated total amount of nutrients removed, which is calculated only for the 24 litres fresh water.

Dilution is especially an issue for micropollutants that vary greatly in influent concentration and remain in the mesocosms longer than 5 days. For example, in *experiment 3*, influent with low DEET concentration (82 ng/L) was diluted with mesocosm water with a higher remaining DEET concentration (± 200 ng/L, based on effluent *experiment 2*), causing the effluent DEET concentration to be higher than the influent (143 ng/L), despite DEET breakdown (160 to 140 ng/L). This is important to take into account when using real waste- or surface water with varying pollution concentrations.

4.1.4 Replicates

Small sized mesocosms are sensitive to biological variations, among which algae, duckweed and plant growth. Therefore, it is common practice to use three to six replicates per treatment [47, 54, 56, 57, 145-147]. In this research, however, just 14 of the 24 treatments were present in duplicate with varying similarities in pollutant removal (Sup. C3). More replicates make it possible to isolate the effect of plant, substrate, algae or duckweed in more detail. Using the same set-up next year, it would be best to replace the mycelium-mesocosms for additional replicates of the jute and no-substrate treatments, including triplicates of the negative control mesocosms J-O and N-O.

4.2 Substrate

Substrates form the foundation of treatment wetlands. They play an important role in supporting plants and biofilm [29, 49], can absorb or retain contaminants [29, 49, 78], and influence general water characteristics like pH and oxygen availability (Fig. 6) [29, 78] which in term determine microbial presence and activity [52, 148]. Therefore, substrate selection is the first and most important step of designing a treatment wetland [29, 39, 49]. In this research, we used and compared two bio-based substrates: jute, a commonly used substrate in floating wetlands [60-62], and mycelium, an innovative floating substrate made from fungi grown on wood waste.

4.2.1 Jute

In terms of nutrient removal, jute achieved high phosphorus removal rates. Jute consisting of mainly cellulose and lignin [63], and is in production process and content comparable to coconut fibres [149]. Coconut fibres have been shown to absorb phosphorus in laboratory-scale wetlands [150]. Therefore, it is likely that phosphorus removal by jute is due to its absorbing capacities, something that has not been reported before. Additionally, jute fibres have been reported to absorb heavy metals [151]. The amount of phosphorus absorbed by jute, the maximum absorption capacity of jute, and the strength of the jute-phosphorus-binding require further studying.

For micropollutants, sorption into the substrate is one of the most important removal pathways [75]. In *experiment 3*, when biodegradation was lowered due to seasonal changes, sorption into jute became a more relevant removal pathway for sulfamethoxazole, carbamazepine, dimetridazole, 2,4-D, BAM, and benzotriazole (Fig. 15). Untreated jute has been shown to absorb 2,4-D with relatively high affinity [152], while jute-based activated carbon cloth can absorb several chemicals and heavy metals [153, 154]. Additional tests should be run with isolated jute to determine its sorption capacity for different micropollutants [78, 155].

Concerning durability, jute in mesocosms stayed unchanged during the experimental period, while the jute FTWs in the Zijdewetering started tearing after a few weeks. Wet jute is clearly unable to withstand streaming water for a longer period. Therefore, a more durable outer frame needs to be designed that can be stuffed with jute and planted.

4.2.2 Mycelium

Mycelium achieved low removal efficiencies for both phosphorous (-60 - 80%) and nitrogen (-50 - 50%) (Fig. 9, Fig. 11). An increase in nutrients was especially seen in mesocosms containing rotting mycelium (7&14), indicating that there is a direct release of nutrients from the break-down of the mycelium, as reported for manure-based biochar [75]. It is likely that also the remnants of wood waste, on which the mycelium is grown, leak organic compounds in the water [49].

Also for micropollutants, mycelium reached low efficiencies, which was surprising since white rot fungi have been reported to degrade a multitude of micropollutants from wastewater [65-67]. However, effective fungal treatment with *Ganoderma* requires a pH of 4,5 and aerobic conditions [65, 66], while the mycelium mesocosms ranged from pH 6,5-7 and oxygen concentrations of 0-0,5 mg/L (Fig. 6). Therefore, most pollutants were broken down by bacteria that use contaminants as growth substrate [65]. In rotting mycelium mesocosms, an abundance of organic compounds was present, compared to low concentrations of micropollutants. It is likely that these 'simple' organic compounds were preferred by bacteria and that therefore micropollutant removal was relatively lower.

In the Zijdewetering, mycelium lasted less than a month before breaking down. The use of different fungal species, fungal growth substrates, treatments or reinforcements could improve mycelium durability in streaming waters.

4.2.3 Advised substrate and FTW design

Concludingly, jute is the most suitable substrate for sustainable bio-based FTWs. To make these jute-FTWs, a more durable outer frame needs to be constructed. To minimise costs and environmental footprint, this frame should be made as much as possible from second-hand materials or waste streams. Additionally, combing jute with non-fossil absorbents like biochar further enhances removal of both nutrients [29] and micropollutants [39, 75], in summer and winter.

4.3 Plant effect

The role of plants in treatment wetlands is a topic of discussion [27, 59]. In constructed wetlands, plants have a major effect during the first two years, after which microbial processes are relatively more important [85]. However, plant presence also has an indirect positive influence on wetland performance, by supporting the microbiome, transporting oxygen, and retaining pollutants [25, 27, 59, 104]. For floating wetland, where substrate is relatively less abundant, plants have a more significant effect on wetland functioning [59, 61].

4.3.1 Plant effect on nutrient removal

Plant diversity can have a positive effect on nutrient removal [54, 57, 58, 146], especially under disturbed conditions [57, 58]. However, in this research, no difference in removal efficiency between plants or plant-combinations was found. Moreover, little effect of plants was found compared to the unplanted controls. The addition of plants was found beneficial for nutrient removal in mesocosms with mycelium or no substrate, but not in jute-containing mesocosms (Sup. C1, Sup. C3). If the main function of plants is to support the microbiome with an attachment site [26, 27, 59, 104], it is possible that jute supports microbes in a comparable manner [26, 38, 51], thereby minimising the added value of plants.

The main effect of plants on nutrient removal is during spring [85]. In this research, mesocosms were planted at the end of spring, giving plants little time to settle. Because of this, plant growth was sub-optimal and all experiments were relatively late in season, making it difficult to measure plant effect. This explains why only *experiment 1* (July 8th - 18th) showed a difference in pollutant removal between plants (Fig. 9C). Additionally, algae and duckweed also remove nutrients, making the effect of the emergent vegetation more difficult to isolate. Using the same set-up for next year, starting in early spring, will make it possible to measure the plant effect more accurately.

4.3.2 Plant diversity for micropollutant removal

Plant species have different qualities with regard to micropollutant removal [70, 73, 156]. Thus, it can be expected that combining different plants will increase the number of contaminants removed. However, this has never been investigated before. In this research no differences between the plant communities was found. In order to accurately determine the effect of plant-diversity, it is essential to measure at the peak of the growing season with much plant biomass present.

4.3.3 Advised plant community for the Zijdewetering

Glyceria is both in nutrient removal and practical application a well suitable plant species to use in the Zijdewetering. However, plant diversity offers more advantages beside pollutant removal, among which system resilience, adaptation, and biodiversity. Therefore a plant community consisting of mainly *Glyceria*, with patches of other treating wetland plants (e.g. *Phragmites, Juncus, Typha*) and flowering wetland plants (e.g. *Iris, Lythrum, Sagittaria*) is advised.

4.4 FTW application for the Zijdewetering

For the Zijdewetering, a plant-diverse FTW with jute and *Glyceria* can remove phosphorus with an area of 3,6 hectares, and nitrogen with an area of 6-9 hectares. The Zijdewetering has a total volume of 49000 m3, an area of 3 hectares, and a hydraulic retention time varying between 11 and 126 hours from WWTP to the Valleikanaal [34]. This means that the current volume and area of the Zijdewetering is large enough to remove ammonium, nitrite, and nitrate, but not large enough to remove total phosphorus and total nitrogen to the environmental standards. For micropollutant removal, the area needed remains uncertain. A larger-scale pilot (1 m broad, 1,5 m deep, 20 m long, retention time 24 hours) can verify whether the calculated upscaled removal efficiencies (both nutrients and micropollutants) comply with reality before conclusively stating whether or not the Zijdewetering is suitable for treatment with floating wetlands.

On the other hand, FTW application in the Zijdewetering would not only remove nutrients and micropollutants, but also function as an ecological buffer zone between the WWTP and the Valleikanaal (Section 3.6.7). When implemented in the connection between WWTP Ede and de Zijdewetering (Fig. 3), a large FTW can transform dead effluent to biologically active surface water, enhancing the ecological status of both the Zijdewetering and the Valleikanaal [23].

4.4.1 Enhanced FTW performance and other treatment options

There are several options to increase the summer and winter efficiency of the FTWs to decrease the area needed and to ensure efficient treatment in cold periods (Section 3.6.5). The most suitable option is to retain pollutants by adding non-fossil absorbents (biochar or active carbon from renewable resources) to the FTW medium [23, 51, 78, 157]. This will make the FTWs comparable in function to biologically enhanced activated carbon [23, 115].

Another option for the Zijdewetering is to add an additional nutrient removal treatment at the WWTP. The most suitable options to remove excess phosphorous and nitrogen are struvite [16, 17] or a nitrifying sand filter [158]. This should then be followed by a floating wetland for the ecological polishing of effluent and removal of micropollutants, or by a Biological Oxygen-Dosed Active Carbon (BODAC) installation for removal of micropollutants [23, 115]. Ozonation is not an option for Ede because of the substantial bromide concentrations (Sup. C1-3), that are oxidated into the toxic compound bromate [159].

4.4.2 Practicalities of FTW implementation in the Zijdewetering

Considering environmental impact, costs and maintenance, (floating) wetlands are a reasonable option. Costs and impact are low compared to technical systems and maintenance consists mainly of plant harvesting which can be done simultaneous with mowing the water boarders. The harvested biomass can be dried or composted and incorporated in the sludge processing to produce biogas, until a more valuable biomass usage can be found.

A challenge with implementing FTWs in the Zijdewetering is the occasional high water flow and occurring floods. Floating wetlands can rise with the water level and allow good water flow underneath the wetland. Still, it is best to implement the FTWs in a broad part of the Zijdewetering to ensure that excess water can pass the system unhindered.

Lastly, like a natural wetland, a large floating wetland could decrease the pre-dawn oxygen concentration in the Zijdewetering [160]. Emerging vegetation can strongly increase day-time oxygen concentrations with oxygen transport through the root system [36, 37, 80]. Although this oxygen transport is limited in the night, emergent vegetation does not actively decrease nightly oxygen concentrations the way submerged water plants do, indicating that FTWs would improve oxygen dynamics. However, in the mesocosms, oxygen concentrations remained very low (Fig. 6B). The amount of oxygen transported into the water by plants is strongly dependent on plant species and environmental factors [80], making it difficult to estimate their large-scale effect on oxygen dynamics. Thus, poor oxygen dynamics remain an unknown risk that needs to be monitored in a larger pilot.

4.4.3 Wetland application at other locations

Recently, treatment wetlands (floating and constructed) have become increasingly popular [25-27]. A beautiful example is the large vertical constructed wetland built for the purification of household waste water from 50 houses (200 citizens) in the urban neighbourhood Giel Peetershof [Wetlantec][23]. FTWs too have been found efficient for large-scale application for wastewater treatment [121] and effluent polishing [161]. Multiple locations in The Netherlands could benefit from FTWs to treat wastewater or effluent in a sustainable manner. Additionally, Gelderland has many surface waters that struggle with nutrient pollution and toxic algae growth, which too are suitable locations for FTW implementation. Thus, many possibilities for FTW application in The Netherlands remain to be investigated.

5. Conclusion

This research has investigated the use of plant-diverse floating treatment wetlands (FTWs) for removing nutrients and micropollutants from contaminated surface water. High removal efficiencies were achieved for ortho-phosphate (80-100%) using a jute and *Glyceria*, as well as for ammonium, nitrite and nitrate (70-90%, 100%, and 100% respectively). Total nitrogen was removed less efficiently (50-80% for jute; -50-60% for mycelium) due to an increase in organic nitrogen by microbial assimilation. Micropollutant removal varied depending on compound, substrate and month. In September, removal efficiencies were drastically lower than in August. Still, removal of guide compounds irbesartan, carbamazepine, diclofenac, DEET, 4(5)-methylbenzotriazole, and benzotriazole was high in both months.

For the Zijdewetering, jute is the most suitable substrate because of its ability to absorb phosphate and enhance micropollutant removal. However, a more durable construction needs to be designed for long-term application in streaming water. These FTWs can be planted with *Glyceria*, the most suitable plant species, and several other species to include biodiversity, enhance ecology, extend the growing seasons, and increase system robustness and adaptivity.

Practically, FTWs have a low environmental footprint, are cost-effective, and easy to maintain. FTWs can be mown simultaneous with the water boarder, conform the ecological mowing protocols. Thereafter, harvested biomass can be used for multiple purposes, as long as the accumulated micropollutants are taken into account. Furthermore, surface area can be reduced and winter efficiency increased by the addition of non-fossil absorbents that retain pollutants. Lastly, FTWs have many added benefits such as reducing ecotoxicity, enhancing ecology, stimulating biodiversity, retaining water, and improving human well-being. Combined, FTWs are considered a “no regret”-solution.

For the Zijdewetering in its current state and the FTWs used, too much area is needed for complete nutrient removal. Creating a big FTW pond with an area of about 3-4 hectares would remove all excess nutrients, function as an ecological transition zone and water buffer for dry periods. Otherwise, an additional treatment step (struvite or a nitrifying sand filter) is needed to remove excess phosphorous and nitrogen. Following, FTWs containing jute and non-fossil absorbents in the Zijdewetering will consistently remove micropollutants and reduce ecotoxicity, thereby transforming the effluent to ecologically healthy water. Concludingly, different options exist for FTW application that will substantially increase water quality in the Zijdewetering, while enhancing nature development and stimulating ecology.

For the near future, the following is advised:

1) using the mesocosm set-up for another year, starting in early spring, and replacing mycelium for jute replicates;
2) investigating the phosphorus and micropollutant absorbing capacity of jute as a wetland substrate;
3) measuring micropollutant content in plant material at the end of the growing season;
4) installing an additional treatment (struvite / sand filter) at WWTP Ede reducing phosphorus and nitrogen discharge;
5) designing and implementing an upscaled pilot for treating the Zijdewetering with jute, non-fossil absorbents, *Glyceria*, and other plants, to verify the predicted upscaled efficiencies in a continuously streaming set-up and to measure and estimate their effect on the water oxygen dynamics;
6) measuring the FTW efficiency in both mesocosms and the upscaled pilot in all seasons to determine their summer and winter efficiency; and
7) investigating other suitable locations for FTW implementation in the region.

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Supplemental data A, Setup

***Table A1**, Mesocosm code, substrate and number of individual plants per mesocosm per species at the start of the experiment.*

Code	#	Substrate	*Phragmites*	*Iris*	*Glyceria*	Code	#	Substrate	*Phragmites*	*Iris*	*Glyceria*
M-P	1	Mycelium	12	0	0	**M-PI**	21	Mycelium	6	6	0
J-G	2	Jute	0	0	12	**J-G**	22	Jute	0	0	12
N-PI	3	None	6	6	0	**J-PIG**	23	Jute	4	4	4
N-O	4	None	0	0	0	**N-PG**	24	None	6	0	6
N-PIG	5	None	4	4	4	**N-PIG**	25	None	4	4	4
J-P	6	Jute	12	0	0	**N-PI**	26	None	6	6	0
M-I	7	Mycelium	0	12	0	**N-G**	27	None	0	0	12
M-IG	8	Mycelium	0	6	6	**M-P**	28	Mycelium	12	0	0
J-PG	9	Jute	6	0	6	**J-P**	29	Jute	12	0	0
J-O	10	Jute	0	0	0	**J-PG**	30	Jute	6	0	6
N-I	11	None	0	12	0	**N-P**	31	None	12	0	0
J-PIG	12	Jute	4	4	4	**M-G**	32	Mycelium	0	0	12
J-PI	13	Jute	6	6	0	**N-G**	33	None	0	0	12
M-O	14	Mycelium	0	0	0	**J-O**	34	Jute	0	0	0
M-G	15	Mycelium	0	0	12	**M-PG**	35	Mycelium	6	0	6
N-IG	16	None	0	6	6	**J-I**	36	Jute	0	12	0
N-PG	17	None	6	0	6	**N-P**	37	None	12	0	0
N-I	18	None	0	12	0	**N-IG**	38	None	0	6	6
J-IG	19	Jute	0	6	6	**J-PIG**	39	Jute	4	4	4
M-PIG	20	Mycelium	4	4	4						

***Table A2**, Experimental planning, retention time and treatment time. Water is refreshed every 5 or 10 days (retention time), and is always refreshed on the first day of the experiment. Effluent is measured after treatment time.*

	from	till	Retention time	Treatment time	Pollutants measured	Mesocosms
experiment 1	8-jul	18-jul	10 days	10 days	Nutrients	All
time experiment	29-jul	1-aug	10 days	0, 8, 24, 30, 48, and 72 hours	Nutrients	2, 27 & 32
experiment 2	22-aug	23-aug	5 days	24 hours	Nutrients and micropollutants	All
experiment 3	5-sep	6-sep	5 days	24 hours	Micropollutants	All

Supplemental data B, Plant growth, substrate development and observations

***File B1**, Plant growth over time (Tab 1 "Groei") and developmental observations (Tab 2 "Observaties").*
"Supplemental file B1_growth and observations"

***Table B2**, Ambient temperature Kesteren centrum (METEO NEDER-BETUWE) and rainfall in the catchment area of the Zijdewetering (FEWS_RADAR_315) during the experiments.*

	Dates		Temperature start			Temperature end			Temperature total			Rainfall (mm)
	from	till	min	average	max	min	average	max	min	average	max	total
experiment 1	8-jul	18-jul	12,5	18,8	25,1	15,8	24,8	33,6	12,1	20,1	33,6	0,29
time experiment	29-jul	1-aug	14,5	20,2	27,2	17,3	19,8	23,9	14,5	20,2	27,2	9,09
experiment 2	22-aug	23-aug	17,2	21,4	27,1	17,7	23,7	30,8	17,2	22,6	30,8	0,16
experiment 3	5-sep	6-sep	16,1	23,5	32,1	17,3	22,6	28,9	16,1	23,1	32,1	1,77

Supplemental data C, Nutrients

***File C1**, Nutrient removal data and water characteristics experiment 1.*
"Supplemental file C1_nutrients experiment 1"

***File C2**, Nutrient removal data and water characteristics time experiment.*
"Supplemental file C2_nutrients time experiment"

***File C3**, Nutrient removal data and water characteristics experiment 2.*
"Supplemental file C3_nutrients experiment 2"

***Figure C4**, Total nitrogen concentration per mesocosm (grey line indicates starting concentration, green line indicates the nitrogen standard of 2,3 mgN/L). Left experiment 1, right experiment 2.*

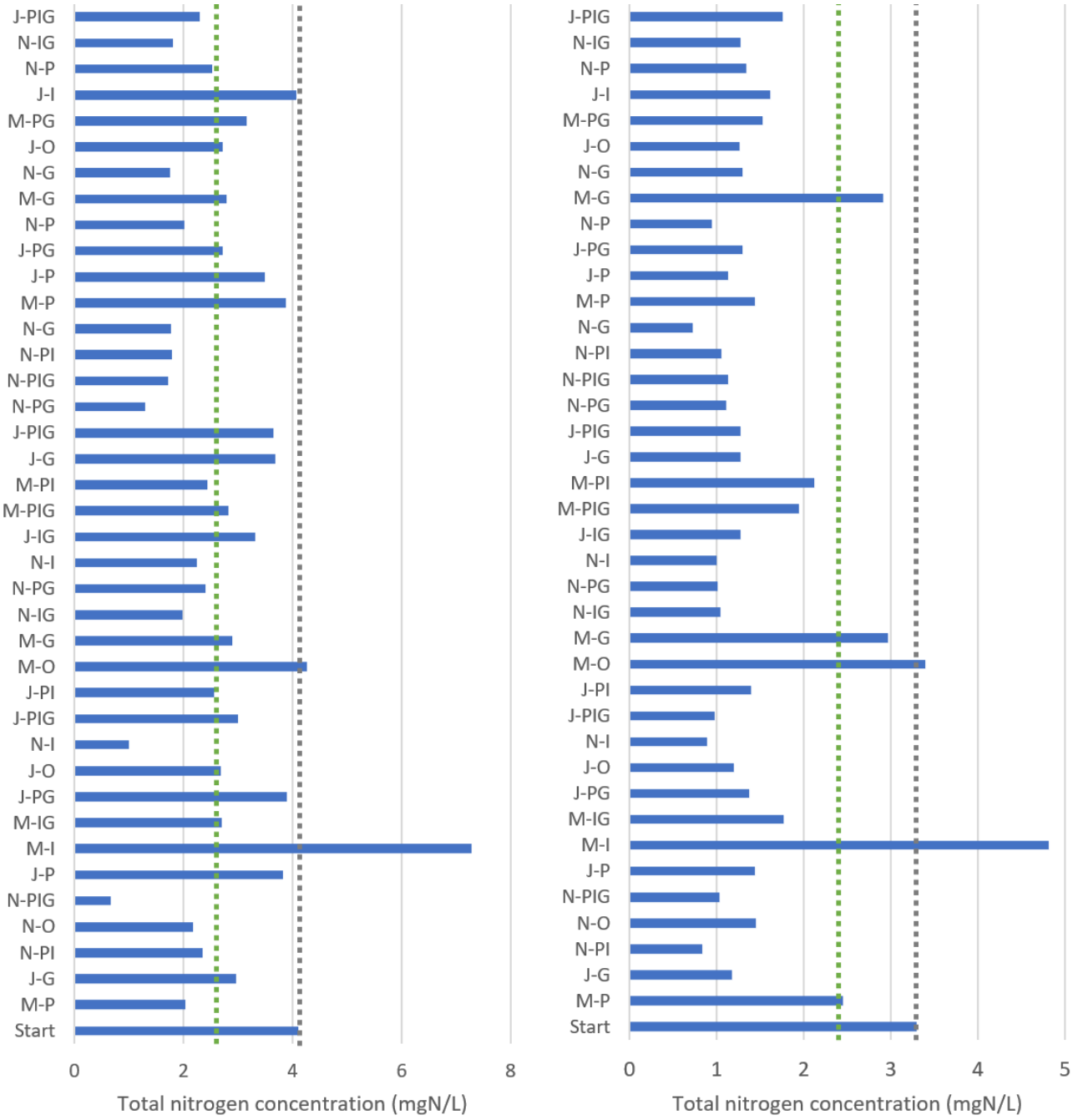

***Figure C5**, Nitrite (left) and nitrate (right) concentration over time for three mesocosms (2, 27, and 32) from time experiment (July 29th-August 1st).*

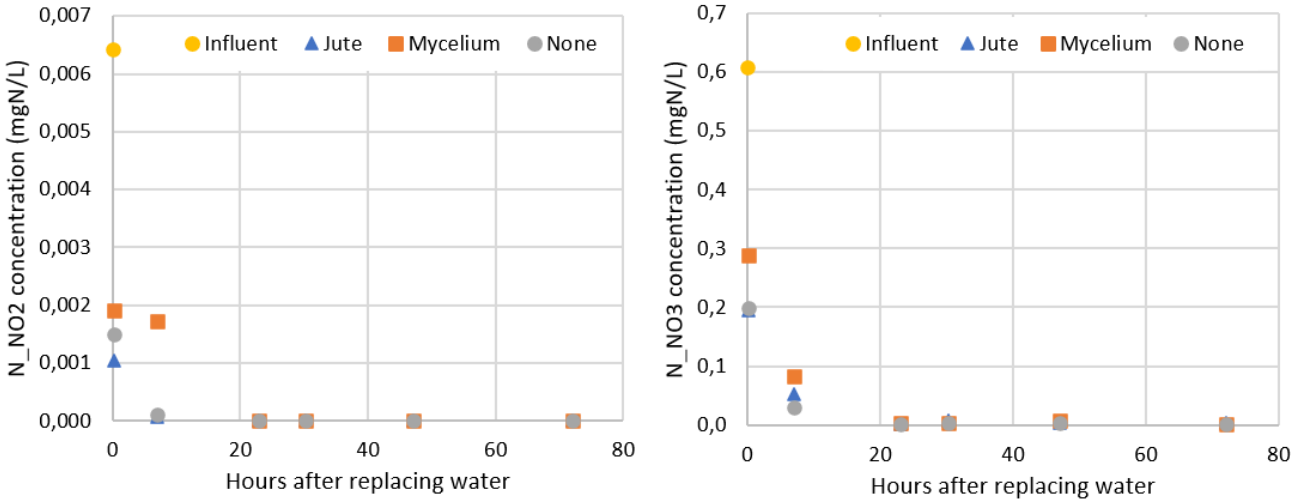

***Table C6**, Calculation of area needed for nutrient removal by floating wetlands for WWTP Ede. Total inorganic nitrogen was based on the sum of NH4, NO2 and NO3. The inorganic nitrogen standard was set to 0,3 mg/L, assuming a stable organic nitrogen concentration of 2 mgN/L (based on the a removal of 25% from the Zijdewetering concentration of 2,5 mg/L).*

	TN	N_inorg	P_PO4	
Starting concentration *time experiment*	2,44	0,744	0,274	mg/L
Ending concentration *time experiment* (best)	1,24	0,039	0,056	mg/L
Maximal removal/mesocosm in 8 hours	1,20	0,71	0,218	mg/L/8h
Maximal removal/mesocosm in 8 hours	28,80	16,92	5,232	mg/8h
From mesocosm	*N-G (27)*	N-G (27)	*J-G (2)*	code (#)
Starting concentration *experiment 2*	3,30	0,66	1,19	mg/L
Ending concentration *experiment 2* (best)	0,73	0,02	0,01	mg/L
Maximal removal/mesocosm in 24 hours	2,57	0,64	1,18	mg/L/day
From mesocosm	*N-G (27)*	*N-OI (3)*	*J-P (29)*	code (#)
Maximal removal/mesocosm in 24 hours	86,40	50,77	28,32	mg/day
Maximal removal/mesocosm in 24 hours	0,0000864	0,0000508	0,00002832	kg/day
Max removal/m2 in 24 hours (25 cm deep)	448,8	263,7	147,1	mg/day
WWTP flow rate/day (average 2022)	37391	37391	37391	m3/day
WWTP flow rate/day (average 2022)	37391000	37391000	37391000	L/day
Allowed concentration in Zijdewetering (WFD)	2,3	0,30	0,11	mg/L
Allowed discharge by WWTP/day (calculated)	85999300	11217300	4113010	mg/day
Allowed discharge by WWTP/day (calculated)	86,0	11,2	4,1	kg/day
Actual average discharge by WWTP/day (2022)	287	99,7	31,3	kg/day
Access discharged (actual-allowed)	201	88	27,2	kg/day
Number of mesocosms needed	2326397	1742421	959993	# mesocosms
Area needed mesocosm scale (25 cm deep)	447831	335416	184799	m2
Area needed mesocosm scale (25 cm deep)	45	34	18	ha
Area needed upscaled (1,5m deep, predicted)	9,0	6,7	3,7	ha

Note C7, *Removal of total phosphorus and total nitrogen in FTWs and CWs.*

Influent concentration of ± 1 mgP/L, depth 0,5-1 meter and retention time of 1 day:

- FTWs
  - 0,6 mgP/L/day for 180L and 0,36m^2, calculating to 300 mgP/m^2/day (Shen, Geng, Li and Lu, 2022)
  - 0,376mgP/L/day for 4420L and 6,21m^2, calculating to 268 mgP/m^2/day (Bu and Xu, 2013)
- Constructed wetlands*
  - 696 mgP/m^2/day for 2059L/day and 5m^2 and 1,7 mgP/L influent (Lin et al. 2002)
  - 384 mgP/m^2/day for 400L, 0,88m^2 and 5 mgP/L influent (Nandakumar et al. 2019)

Influent concentration of 3-8 mgN/L and retention time of 1 day:

- FTWs
  - 3,36mgN/L/day for 4420L and 6,21m^2, calculating to 2392 mgN/m^2/day (Bu and Xu, 2013)
  - 227mgN/m^2/day (84 L and 0,28 m^2) (Keizer-Vlek *et al.*, 2014)
  - 1,77 mgN/L/day for 15L and 0,085m^2, calculating to 312 mgN/m^2/day (Li and Guo, 2017)
- Constructed wetlands*
  - 120-9800 mgN/m^2/day for lab-scale and 3300 and 19600 mgN/m^2/day for pilot scale (Lu *et al.*, 2020)
  - 314 mgN/m^2/day for 120L, 0,3m2, HRT of 2 days, and 5,6 mgN/L influent (Meng et al. 2019)

** Much more literature exists on nutrient removal with wetlands (*Shmaefsky, 2020; Ballantine 2010*), but very few papers use low concentrations with a retention time of 1 day, making accurate comparison difficult.*

Supplemental data D, Micropollutants

Table D1, *Micropollutant identity.*

	Group	Usage
Metoprolol	Medicine, β-blocker	Treat cardiovascular diseases and migraine
Benzotriazole	Industrial chemical	Corrosion inhibitor, anti-freeze and cleaning
Caffeine	Stimulant	Drinks such as coffee, thee, or soda
Carbamazepine	Medicine	Treat epilepsy and trigeminal neuralgia
Irbesartan	Medicine	Treat mild to moderate hypertension
Propranolol	Medicine, β-blocker	Treat high blood pressure and cardiovascular disease
Sulfamethoxazole	Medicine, Antibiotic	Treat various human infections and animal, such as pneumonia and urinary tract infections
Trimethoprim	Medicine, Antibiotic	
Diethyl-3-methylbenzamide (DEET)	Pesticide	Insect repellent, mainly used against ticks
Erythromycin	Antibiotic	Against gram positive microbes for respiratory infections
Atenolol	Medicine, β-blocker	Treat cardiovascular diseases such as hypertension, angina pectoris, or arrhythmia
Sum 4 and 5 methylbenzotriazole	Industrial chemical	Corrosion inhibitor
2,6-dichlorobenzamide (BAM)	Biocide	Metabolite of pesticide dichlobenil
Clarithromycin	Medicine, Antibiotic	Treat a variety of infections, among which pharyngitis, tonsillitis, acute sinusitis, or chronic bronchitis
Dimetridazole	Medicine	Nitroimidazole, against protozoan infections in livestock
Sotalol	Medicine, β-blocker	Treat arrhythmia
Desphenyl chloridazon	Herbicide	Protection of beat, union, and flower bulbs
Diclofenac	Medicine	Anti-inflammatory drug
Furosemide	Medicine, Diuretics	Treat oedema at heart failure, liver cirrose, kidney failure and nephrotic syndrome
Mecoprop (MCPP)	Herbicide	For lawns and wheat cultivation
2,4-dichlorophenoxyacetic acid (2,4-D)	Herbicide	Against dicotyl weeds

***Table D2**, Predicted no-effect concentration (PNEC) [STOWA], average start and concentrations for experiment 2 and 3 in ng/L. Environmental norm (ng/l) for (1) surface water, (2) drinking water, (3) ground water, and (4) environment [RIVM].* some

	Metoprolol	**Benzotriazole**	**Caffeine**	**Carbamazepine**	**Irbesartan**	**Propranolol**	**Sulfamethoxazole**
PNEC	8600	7770	40000	500	704000	20	118
Norm	760000 (1)		1000 (2)	1000 (2)			
Start 22/8	1376	2837	684	510	1221	100	255
End 23/8	401	913	85	169	247	14	80
Start 5/9	1432	3324	190	644	1630	98	476
End 6/9	761	2459	208	505	1304	43	322

	Trimethoprim	**DEET**	**Erythromycin**	**Atenolol**	**4 and 5 methyl-benzotriazole**	**BAM**	**Clarithromycin**
PNEC	16000	88000	200	148000	150	78000	120
Norm		110 (1)				1000 (1)	
Start 22/8	0	576	75	203	904	4	59
End 23/8	0	210	56	70	250	1	37
Start 5/9	0	82	197	178	937	37	55
End 6/9	0	143	118	91	615	27	51

	Dimetridazole	**Sotalol**	**Desphenyl chloridazon**	**Diclofenac**	**Furosemide**	**Mecoprop MCPP**	**2,4-D**
PNEC	29500	6520	250000	50	100000	900	600
Norm		1000 (2)		1000 (2)		3800 (3)	
Start 22/8	20	2086	0	461	766	341	39
End 23/8	0	875	0	85	1865	110	18
Start 5/9	29	2453	0	637	966	251	25
End 6/9	9	2197	1	406	615	209	18

***Figure D3**, Micropollutant removal experiment 2 corrected for a large hypothetical sorption of 100 ng/L by tube and filter. **Left** pollutant removal from influent to effluent in ng/L, **Right** in percentage (influent-effluent/influent*100%). Bars represent average removal per substrate (blue jute, orange mycelium, and grey no substrate). Error bars represent standard deviations. Lines under the X-axis indicate whether groups are significantly different (T.TEST<0,05). Trimethoprim and desphenyl chloridazon were measured, but not present (<1ng/L) in influent or effluent samples. BAM, Clarithromycin, Dimetridazole, and 2,4-D had concentration of below the quantification limit of 50 ng/L and are therefore unreliable. Note that the order of compounds is different from Fig. 12 and Fig. 13.*

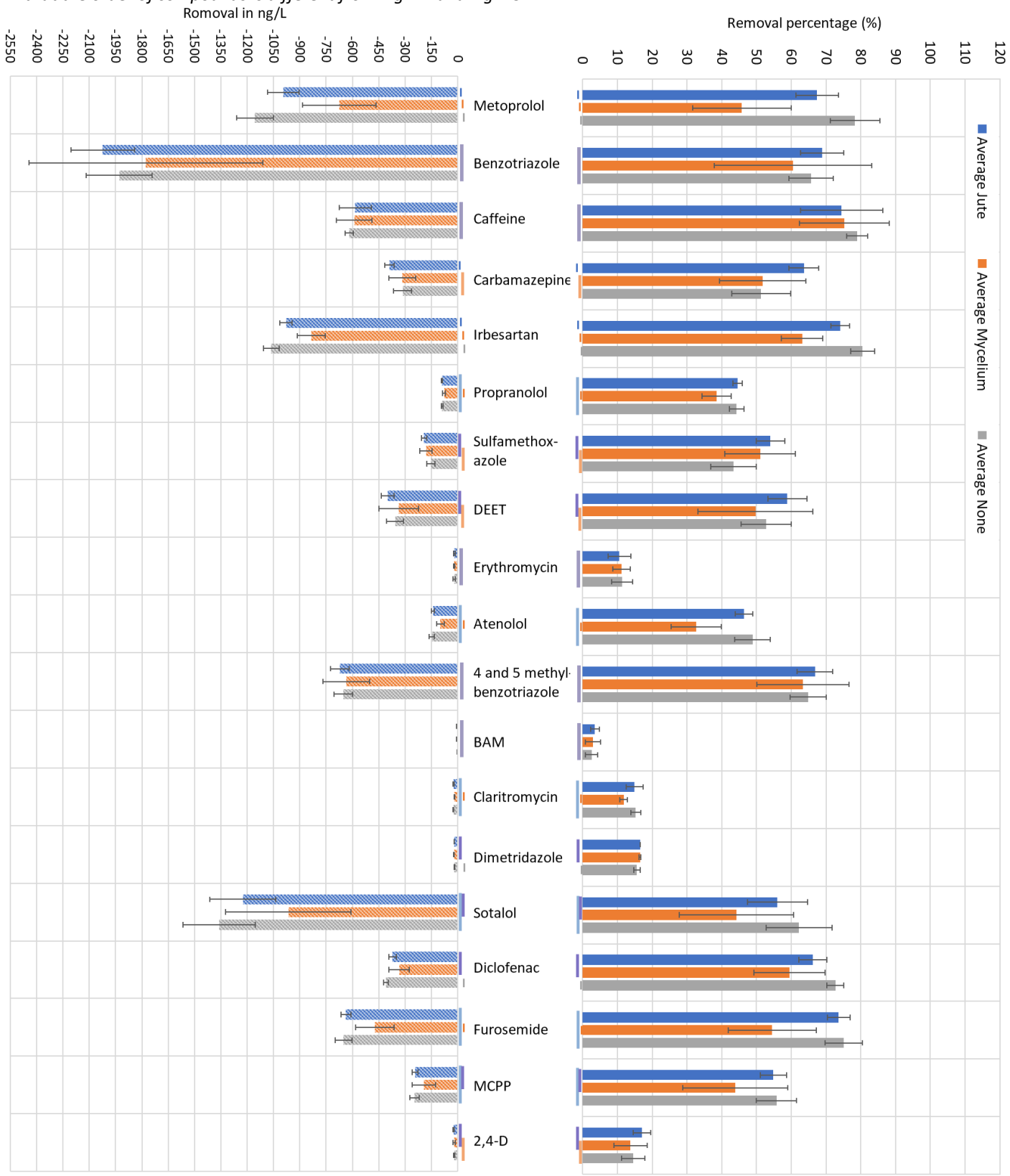

***Table D4**, Micropollutant removal (%) from literature compared to Jute mesocosms. Negative removal efficiencies (accumulation) indicated in dark red. For removal efficiencies either the median (Med.) or single reported efficiencies are given, based on literature availability. Trimethoprim, desphenyl chloridazon, BAM, Clarithromycin, Dimetridazole, and 2,4-D had concentration of below the quantification limit of 50 ng/L and are therefore left out.*

	WWTP		CW		*experiment 2*		*experiment 3*		
	Min-Max	Med.	Min-Max	Med.	Min-Max	Avg.	Min-Max	Avg.	Removal
Metoprolol	3-56 [1]	38 [2], -18 [3]	60-95 [4], 0-90 [17]	85 [4]	57 - 79	72	36 - 56	47	Sorption[18] & indirect photodegr.[17]
Propranolol	29-90 [7]	28 [2], 32 [3]	75-90 [4]	85 [4]	84 - 93	89	49 - 70	62	sorption[18] & photodegr.[19]
Atenolol	<0-85 [1], 30-80 [8]	81 [2], -433[3]	20-97 [13], 92-93 [8]	59 [13], 35 [16]	60 - 74	69	41 - 64	55	biodegradation[21]
Sotalol		22 [5]	15-33 [14]	19 [14]	39 - 71	59	-2 - 24	8	sorp. & biodegr. [17]
Erythromycin	-20-60 [11]	20 [11]	-90-100[11]	50 [11]	14 - 37	25	26 - 56	44	biodegradation[21]
Clarithromycin	-30-70 [11]	30 [11]	0-80 [11]	40 [11]	19 - 47	40	-69 - 28	8	biodegradation[21]
Sulfamethoxazole	-70-80 [11]	40 [11]	-80-100[11]	60 [11]	62 - 83	75	31 - 55	45	photodegr.[19]
Irbesartan		10 [5]	<25 [10, 17]		72 - 84	80	18 - 45	34	sorption[17] & photodegr.[19]
Carbamazepine	0-50 [7], 0-30 [8]	-20 [2], 30 [3]	0-50 [4], 24-28 [9]	10 [4], 28 [9]	67 - 84	76	36 - 50	43	photodegr.[17] & sorption[18, 23]
Diclofenac	<0-81 [1], 7-69 [8]	-76 [2], 22 [3]	30-70 [4], 0-70 [8, 17]	45 [4]	71 - 89	80	22 - 50	35	aerobe biodegr. & photodegr. [1719]
Furosemide	20-96 [13]	48 [2], 51 [5]	20-96 [13]	50 [13]	76 - 90	83	37 - 56	49	photodegr.[19]
Mecoprop		25 [5]	79-91 [15]		61 - 79	71	18 - 29	23	photodegr.[19]
DEET	66-80 [1]	62 [5]	-5-43 [12]	32 [12]	56 - 78	69	~~-66~~ - ~~32~~	~~-51~~	sorption[20]
4&5 methylbenz.		30 [5]	0-50 [6,17]	19 [6]	64 - 84	74	12 - 53	37	unknown[22]
Benzotriazole		26 [5]	0-27 [6]	9 [6]	60 - 82	71	7 - 50	33	aerobe biodegr. [17] & photodegr.[19]
Caffeine	50-100 [1]	94 [3]	75-100 [4]	100 [4]	41 - 96	85	-108 - 57	14	biodegradation[24]

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***Figure D5**, Micropollutant removal (%) August 23th compared to pH per mesocosm. Only the trendline for irbesartan (blue dotted line) has an R^2 above 0,5.*

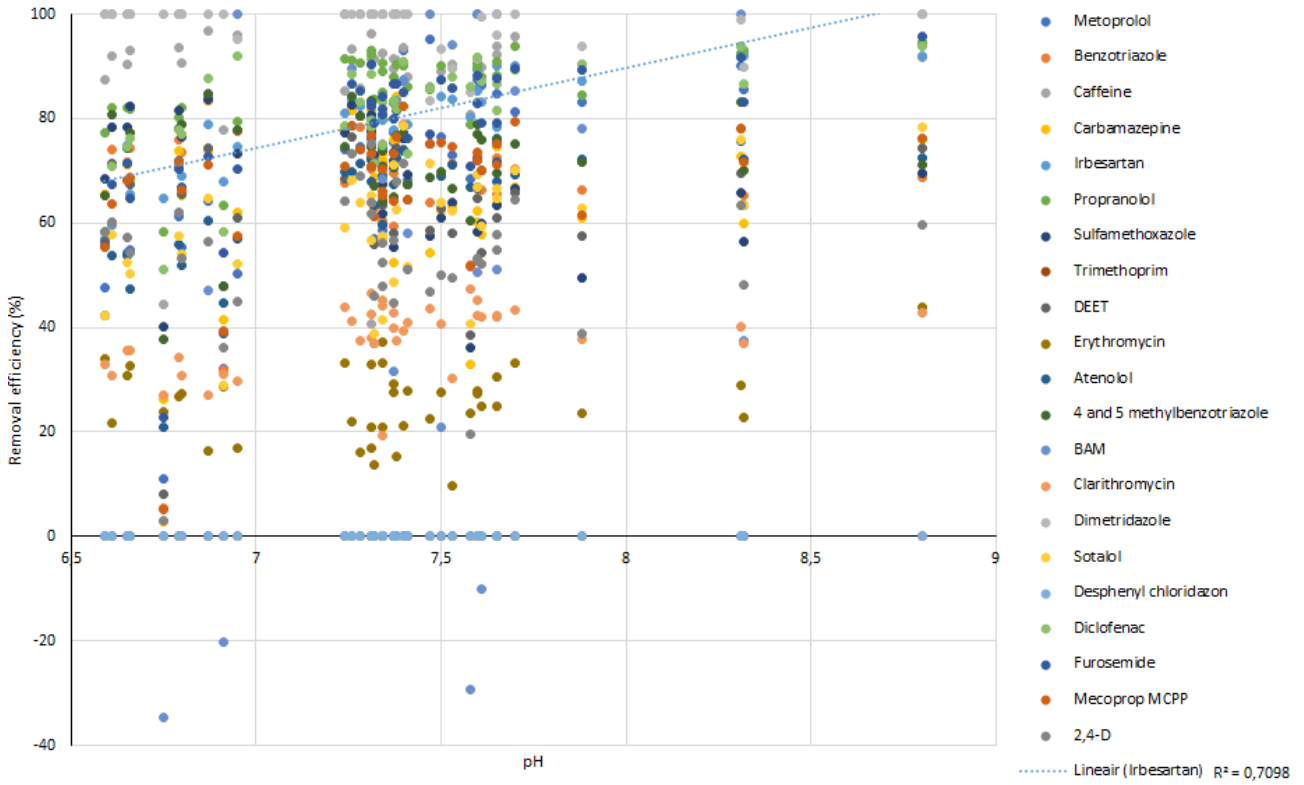
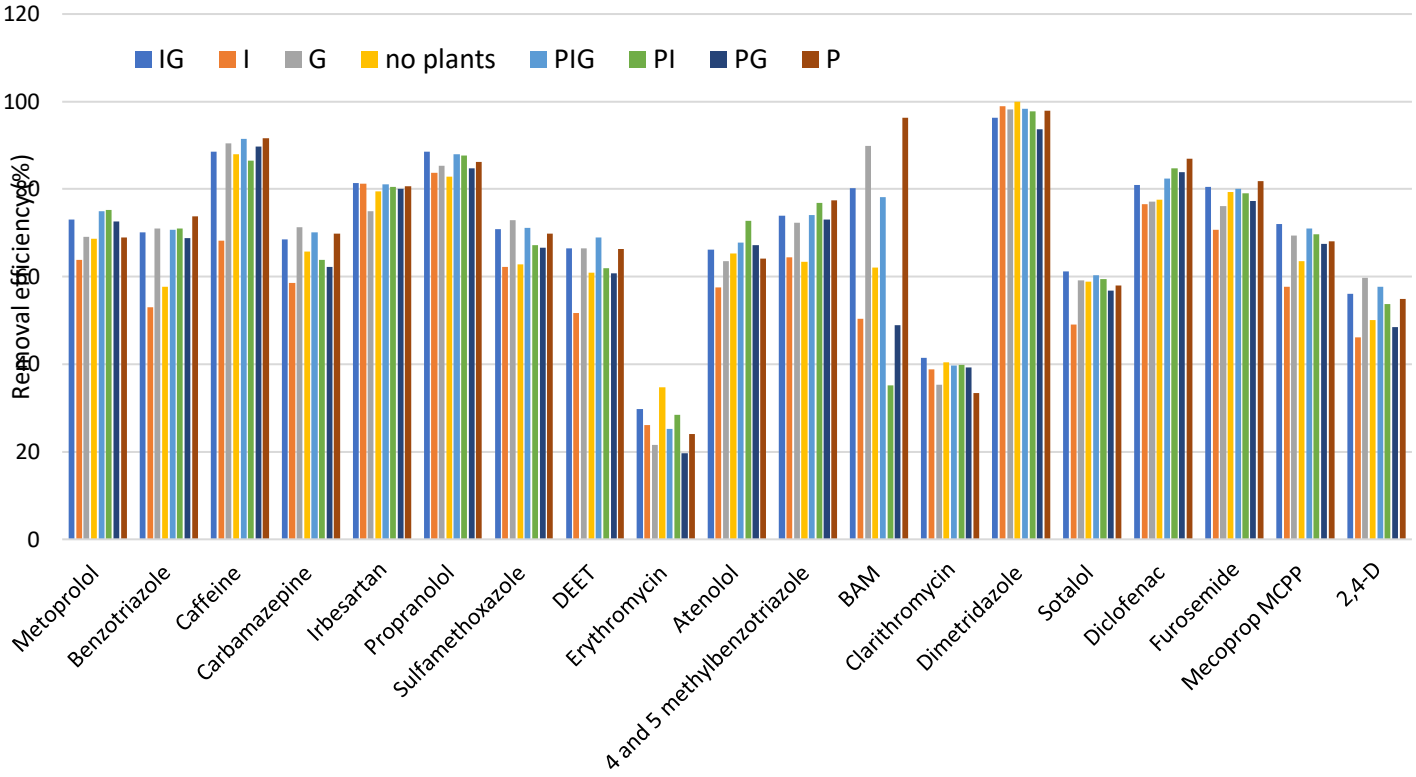


***Figure D6**, Micropollutant removal (%) from August 23th per plant combination (I=iris, G=Glyceria, P=Phragmites).*



***File D7**, Micropollutant data experiment 2.*
"Supplemental file D7_micropollutants experiment 2"

***File D8**, Micropollutant data experiment 3.*
"Supplemental file D8_micropollutants experiment 3"