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Bioremediation of 27 Micropollutants by Symbiotic Microorganisms of Wetland Macrophytes

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Abstract: Background: Micropollutants in bodies of water represent many challenges. We addressed these challenges by the application of constructed wetlands, which represent advanced treatment technology for the removal of micropollutants from water. However, which mechanisms specifically contribute to the removal efficiency often remains unclear. Methods: Here, we focus on the removal of 27 micropollutants by bioremediation. For this, macrophytes *Phragmites australis*, *Iris pseudacorus* and *Lythrum salicaria* were taken from established wetlands, and a special experimental set-up was designed. In order to better understand the impact of the rhizosphere microbiome, we determined the microbial composition using 16S rRNA gene sequencing and investigated the role of identified genera in the micropollutant removal of micropollutants. Moreover, we studied the colonization of macrophyte roots by arbuscular mycorrhizal fungi, which are known for their symbiotic relationship with plants. This symbiosis could result in increased removal of present micropollutants. Results: We found *Iris pseudacorus* to be the most successful bioremediative system, as it removed 22 compounds, including persistent ones, with more than 80% efficiency. The most abundant genera that contributed to the removal of micropollutants were *Pseudomonas*, *Flavobacterium*, *Variovorax*, *Methylotenera*, *Reyranella*, *Amaricoccus* and *Hydrogenophaga*. *Iris pseudacorus* exhibited the highest colonization rate (56%). Conclusions: Our experiments demonstrate the positive impact of rhizosphere microorganisms on the removal of micropollutants.

Keywords: arbuscular mycorrhizal fungi; bioremediation; constructed wetlands; removal of micropollutants; rhizosphere microbiome

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1. Introduction

With rising globalization, industrialization and the world population in general, the use of synthetic chemical compounds continues to grow. Many of these compounds are micropollutants (MPs); released broadly, their removal from bodies of water is an enormous challenge [1]. This is due to the fact that current conventional wastewater treatment plants (WWTPs) are not designed for MPs' removal and, therefore, a high percentage of MPs remains in the WWTPs' effluents, which are then discharged into bodies of water [2]. MPs cause negative effects on aquatic fauna and flora [3], induce mutagenicity [4], contribute to antibiotic resistance [5] and, consequently, have negative impacts on human health [6]. Because of this, the European Commission (EC) decided to require mandatory monitoring of some MPs (i.e., antibiotics, such as azithromycin, clarithromycin and erythromycin) by all European Union (EU) member states, with the ultimate goal of preserving the ecological and chemical status of the surface bodies by 2027 [7–9]. This also contributed to the active research and introduction of advanced treatment technologies in the last twenty years. Advanced oxidation processes, UV photolysis, ozonation and membrane applications are widely used; however, these technologies have high financial requirements and are

challenging to implement (e.g., due to the space requirements) [10–12]. According to the principles of the 2030 agenda for sustainable development, with rising urbanization, there is a growing demand for the presence of nature in urban islands promoting resilience [13,14].

Constructed wetlands (CWs) are a possible solution to these challenges and offer distinct environmental advantages. CWs act as attractive biodiverse enhancements in many urban areas [15,16] and have recently been reported as useful for MPs' removal [17–19]. We investigated CWs in our recent project, EmiSûre (Interreg, N 013-2-03-049), where, for most of the 27 investigated compounds, the overall removal efficiencies of vertical subsurface flow CWs, as a post-treatment step, exceeded 90% [20]. In order to learn from this experiment, a further aim has been developed to quantify the MPs' removal mechanisms in the studied wetlands and understand the individual contributions. These mechanisms can be divided as follows: 1. phytoremediation by wetland macrophytes, 2. adsorption on the soil matrix, and 3. bioremediation by microorganisms. Phytoremediation is important in horizontal configurations but is considered negligible for subsurface flow CWs, especially in vertical configurations where the surface exposed to sunlight is limited [21]. Phytoremediation and adsorption have already been targeted in our previous studies [22]. With the knowledge gained from phytoremediation, we could compare and improve our current set-up and assess the efficiency of pure plants for removing MPs. During the phytoremediation experiments, the roots were immersed into a biocidal solution to exclude the presence of microorganisms in the root zone. With this information, it is possible to develop an innovative experimental set-up for the establishment of bioremediation and appraise the additional contribution of the rhizosphere organisms, which we suppose to be significant, as the rhizosphere is known to be the most reactive zone of a wetland [23]. Bioremediation has gained increased attention in recent years, as it is a non-invasive and natural way of eliminating MPs. There are recent studies on the application of the bioremediation of MPs present in irrigation water. The implementation of biochar in bioremediation, which is an effective substrate for MPs' removal, was also in our previous applications [20,24,25]. The aqueous environment can have bioremediation effects influenced by various factors, such as the presence of commonly occurring MPs (e.g., bisphenol A), which enhance bacterial growth [26] and the beneficial relationships between plants and microbes [27,28].

In order to complete the understanding of the mechanisms' removal, the characterization of the present microorganisms, namely bacteria and fungi, was performed. In order to characterize the bacterial community and to determine the role of selected genera in the studied systems, 16S rRNA gene amplicon sequencing [29] was applied to samples of roots and soil. Wetland bacteria are known for the degradation of an expansive variety of nutrients and inorganic and organic compounds. Most bacteria degrade broad groups of compounds, e.g., *Reyranella* or *Rhodobacter* decompose organic matter and, therefore, play major roles in the removal of petroleum pollutants [30]. For instance, *Hydrogenophaga* is a genus of known general benzene degraders [31]. Other bacterial genera that target specific compounds are *Massilia,* which decompose tris (1-chloro-2-propyl) phosphate (TCIPP) [32], and *Sphingobium,* which include known diclofenac degraders [33].

Besides bacteria, we also studied arbuscular mycorrhizal fungi (AMF), which are commonly present in wetlands [34]. The symbiotic relationship of these soil-borne fungi with plants belongs to the most important ones on Earth (Bucking et al., 2012), as they are found in over 80% of all plant species [35]. AMF can also enhance phytoremediation by creating an underground network from mycelium, which acts as a bridge between plant roots, soil and microorganisms in the rhizosphere. The hyphae of AMF can significantly increase the access area of the plant to nutrients and contaminants. Therefore, AMF contribute to bioremediation because they considerably increase the active root area for the uptake of pollutants [36]. AMF provide host plants with nutrients, such as phosphorus and nitrogen; host plants transfer 4 to 20% of photosynthetically fixed carbon to fungi. The presence of AMF spores generally decreases with soil depth, and the spores are normally absent below the root zone [37]. AMF colonization can be influenced by environmental parameters, such as, (1) flooding conditions [38], (2) temperature (the colonization rate

increases with the growth of the temperature from 10 to 30 °C) [39], (3) level of oxygen (the decrease of colonization is between 21 and 3% of oxygen and concentration of oxygen below 3% cases abrupt decrease of the colonization) [40], and (4) pH (the maximum spore germination occurs between pH 6 and 8) [39]. For the contribution of the AMF to the phyto- and bioremediative activity of wetlands, a colonization of the plant roots by the AMF has been examined in this work.

Overall, the main aim of this work is to understand the bioremediation process and its contribution in a CW environment to the removal of MPs. The hypotheses are (1) the rhizosphere is the most active area in which the removal of MPs occur, and (2) fungi and bacteria in the rhizosphere are crucial in the removal process. Thus, in order to better understand this, we designed a new experimental set-up. Consequently, it will be possible to: (1) evaluate the bioremediative potential of the wetland macrophytes with organisms present in the rhizosphere for the removal of MPs; (2) characterize the available bacterial microbiome, aiming to understand their function better; and (3) relate the MPs' removal of bacterial genera with the presence of AMF determined by the colonization of plant roots. Ultimately, it will be possible to offer advice on how to enhance the potential of the rhizosphere in the removal of MPs via CWs.

2. Materials and Methods

2.1. Design of a Bioremediation Experiment

Three common wetland macrophytes (*Lythrum salicaria* (A), *Iris pseudacorus* (B) and *Phragmites australis* (C)) previously purchased at re-natur GmbH (Ruhwinkel, Germany) were taken from an established pilot-scale CW. Our usage of the plants did not disregard any of the legal conservation guidelines. In the CW, bentonite sand and a 15% activated biochar admixture acted as a substrate. The wetland was tested in the WWTP Echternach (20,000 PE equivalent capacity, Luxembourg) as a post-treatment step. When removing the plants, the excess soil was removed, leaving just the soil present in theroot area (rhizosphere). This was due to the preservation of the rhizosphere microbiome, which should contribute to the removal of MPs. The samples from the rhizosphere soil, with the roots of the macrophytes, were sampled with sterilized tools and immediately put in a liquid nitrogen dry shipper (Voyageur–Dry Shippers (2–Plus) AIR LIQUIDE Medical GmbH, Düsseldorf, Germany). The "systems" (plants with present rhizosphere microbiome) were placed into special hydroponic pots (Growrilla Hydroponics, Ciriè, Italy) with tap water for one day for conditioning. The pots contained an aeration unit, which ensured the sufficient oxygenation of the plants' roots and constant recirculation of the liquid medium in the pot (Figure 1).

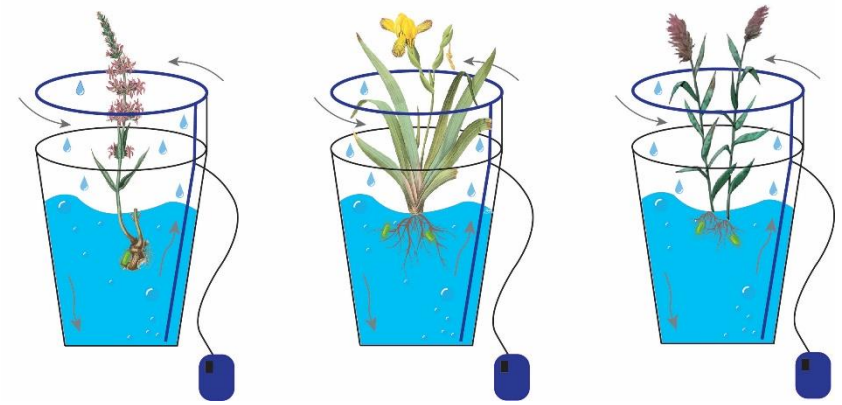

Figure 1. Scheme of the bioremediation experiment.

After one day, the tap water was withdrawn from the pots. Then, a mixture of 27 MPs in concentrations of 1–5 µg/L (Techlab, purity >99.99%) was added to the pots. This concentration is typical for small-to-medium sized WWTP effluents and hydroponic

nutrients in water (15 L) (Flora Series, General Hydroponics—the detailed composition of the nutrient solutions is available in Supplementary Materials). The list of MPs is shown in Table 1.

Table 1. MPs studied in this work.

Application	Compound	CAS Number	Therapeutic Group/Use
Pharmaceuticals and metabolites	Atenolol	29122-68-7	Beta Blocker
	Bezafibrate	41859-67-0	Lipid regulator
	Carbamazepine	298-46-4	Psychiatric drug
	Clarithromycin	81103-11-9	Antibiotic
	Ciprofloxacin	85721-33-1	Antibiotic
	Cyclophosphamide	50-18-0	Cytostatic
	Diclofenac	15307-86-5	Analgesic/anti-inflammatories
	Erythromycin A	114-07-8	Antibiotic
	Ketoprofen	22071-15-4	Analgesic/anti-inflammatories
	Lidocaine	137-58-6	Anaesthetic
	Metoprolol	51384-51-1	Beta Blocker
	Propranolol	525-66-6	Beta Blocker
	N4-acetylsulfamethoxazole	21312-10-7	Metabolite of Sulfamethoxazole
	Sulfamethoxazole	723-46-6	Antibiotic
Pesticides/Herbicides	Carbendazim	10605-21-7	Fungicide
	DEET	134-62-3	Insect repellent
	Diuron	330-54-1	Herbicide
	Isoproturon	34123-59-6	Herbicide
	Terbutryn	886-50-0	Herbicide
	Mecoprop (MCPP)	7085-19-0	Herbicide
	Tolyltriazole	29385-43-1	Fertilizer
	Glyphosate	1071-83-6	Herbicide
	Aminomethylphosphonic acid (AMPA)	1066-51-9	Degradation product
Fluorosurfactants	Perfluorooctanesulfonic acid (PFOS)	1763-23-1	Surfactant
	Perfluorooctanoic acid (PFOA)	335-67-1	Surfactant
Corrosion inhibitor	Benzotriazole	95-14-7	Corrosion inhibitor/Antiviral
Flame retardant	Tris(2-chloroisopropyl)phosphate (TCPP)	13674-84-5	Flame retardant

The pots were lighted with a LED lamp for hydroponic plants, which included 96 LED chips (32 yellow beads, 32 blue beads, and 32 red beads), and a wavelength of 380–800 nm at 36 watts (Lovebay International Limited, Bristol, England), for 12 h per day. The duration of the experiment was 30 days, with sampling on days 0, 1, 2, 5, 7, 14, and 30 (analogous to our phytoremediation experiment [22]). The volume of each sample was 100 mL. The samples were, subsequently, filtered through a 0.45 µm syringe (Carl Roth, GmbH, Karlsruhe, Germany), and the content of the macronutrients and values of the general parameters were analyzed on-site (COD, TN, NO_3-, NH_4+, PO_4-P (Hach Lange cuvette text box), electrical conductivity, oxidation-reduction potential (ORP), dissolved oxygen (DO), and pH (multi-portable parameter meters by Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim in Oberbayern, Germany)). The concentrations of the MPs were measured at the Luxembourg Institute of Science and Technology (LIST) [22].

2.2. Microorganisms

In order to determine the bacterial composition, samples of the plants' roots and the rhizosphere were taken and immediately placed in a liquid nitrogen dry shipper (Voyageur–Dry Shippers (2–Plus) AIR LIQUIDE Medical GmbH, Düsseldorf, Germany). Next, root and soil samples were prepared for DNA extraction at the Luxembourg Centre for Systems Biomedicine (LCSB). First, the samples were milled and homogenized under cryogenic conditions at −196 °C (6875D Freezer/Mill® Dual-Chamber Cryogenic Grinder SPEXSamplePrep). After homogenization, the DNA was extracted according to

standardized procedures (DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany) and PowerSoil DNA Isolation Kit (MOBIO Laboratories, Inc., Berlin, Germany). The extracted DNA was concentrated and purified. The DNA's quality and quantity were assessed using a nanophotometer (Nanodrop) and fluorometer (Qubit dsDNA HS Assay Kits, Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the sample preparation, sequencing (Oxford Nanopore Technologies MinION sequencer) and data analysis, including the taxonomic classification, were carried out by the LCSB Sequencing Platform (RRID SCR_021931) at the University of Luxembourg using the protocols provided by the manufacturer.

The roots were examined for the presence of AMF. First, the roots (more than 100 pieces per plant species) were cleaned under a water stream and cut into 1 cm pieces. Next, the roots were cleaned in a 10% KOH solution [41] and stained in an ink and vinegar solution [42]. Then, the colonization of the macrophytes' roots by AMF, before and after the targeted experiment, was evaluated with the help of the grid-line intersect method and microscopical observation (LMS Leica DM1000, Düsseldorf, Germany, zoom 10×).

3. Results

3.1. General Parameters and Macronutrients

We observed a rapid increase in the removal efficiency of the COD (chemical oxygen demand) within the first days (Figure 2). The efficiency of system A dropped slightly towards the end of the experiment on day 30. The efficiency of system B continued to increase, reaching 84% on day 30 slowly. It was also the highest removal efficiency that we observed across the three plant species studied herein.

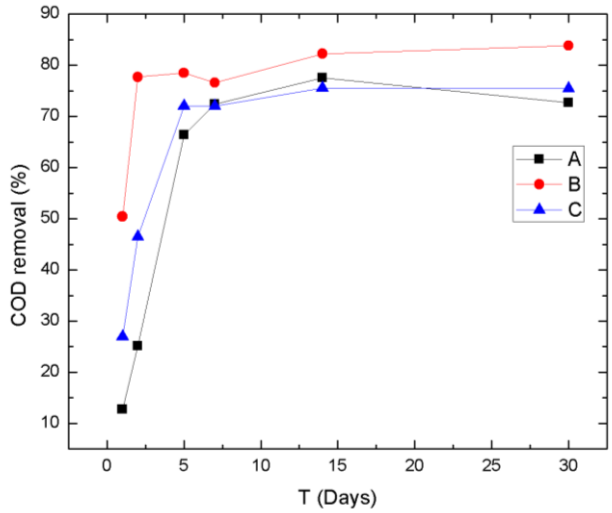

Figure 2. COD removal via bioremediation. A = *Lythrum*, B = *Iris*, and C = *Phragmites*.

The values of TN (NO_3- + NH_4+) (160–220 mg/L) and PO_4-P (38–41 mg/L), which were monitored during the entire experiment, were in line with the recommended values for these nutrients (100–250 mg/L for TN and 30–50 mg/L for PO_4-P [43]. The plants were taken from the CW after the winter season; therefore, they had a comparably low green biomass. During the experiment in semi-hydroponic conditions, the plants underwent a significant increase in healthy biomass (80–100 cm of new stems for each plant and 10–30 cm of roots for each plant). These findings suggest well-established hydroponic surroundings and, therefore, positive prerequisites for an optimal symbiotic relationship between the rhizosphere organisms and the plant roots, which results in a favorable environment for the removal of MPs from the liquid solution.

During the experiment, a constant decrease in the concentration of NH_4+ ions was observed (from 25 to 3 mg/L). The concentrations of NO_3- ions remained constant during

our experiments (60–100 mg/L). Moreover, the values of DO remained stable (6.6–7.9 mg/L corresponding to 71–85% oxygen saturation), considering that the concentration of the DO at a saturation point of 20 °C is 9.1 mg/L [44]. These facts suggest an ongoing nitrification process, where NH_4+ is oxidized to NO_3−. This could help the removal of MPs, as many of the nitrifying bacteria are known for their ability to degrade organic compounds [45]. However, it is not possible to confirm this hypothesis surely, as it is not clear which amount of NO_3− is being up taken by the plants and which amount is oxidized from NH_4+. The measured values of pH and ORP during this experiment are available in the Supplementary Materials.

3.2. Removal of Micropollutants

The ability of the studied systems to remove MPs from the liquid medium is delineated as follows:

The most efficient system for the removal of MPs is macrophyte B, Iris, which removed 22 out of 27 compounds with more than 80% efficiency. The successfully removed compounds were atenolol, benzotriazole, bezafibrate, carbendazim, ciprofloxacin, clarithromycin, cyclophosphamide, DEET, diclofenac, diuron, erythromycin, glyphosate, isoproturon, ketoprofen, MCPP, metoprolol, propranolol, sulfamethoxazole, and its acetyl degradation product, TCIPP, tebutryn, and tolyltriazole. Table 2 shows a comparison of the bioremediation removal of the compounds in the current experiments and the bioremediation experiments described in the literature.

Table 2. Removal of 22 compounds in the current study compared to achieved removals in previous studies.

Compound	Achieved Removal in Current Study (%)	Achieved Removal in Previous Studies	Reference
atenolol	98.8	80%	[46]
benzotriazole	93	complete removal, however conditioned by low concentration of the compound	[47]
bezafibrate	99.9	contribution of the biofilm to removal of 25%	[48]
carbendazim	99.3	41.8%	[49]
ciprofloxacin	99.5	contribution of the biofilm to removal of 22%	[48]
clarithromycin	99.4	75.8–98.6%	[50]
cyclophosphamide	91.8	>20%	[51]
DEET	99.6	no significant removal	[52]
diclofenac	99.7	97 ± 4%	[53]
diuron	99.7	83%	[54]
erythromycin	98.3	75.8–98.6%	[50]
glyphosate	99.2	82.6%	[55]
isoproturon	99.6	complete removal	[56]
ketoprofen	99.9	complete removal	[53]
MCPP	99.5	99%	[57]
metoprolol	91	60%	[46]
propranolol	98.9	60%	[46]
sufamethoxazole	90.5	75.8–98.6%	[50]
N-acetyl-sulfamethoxazole	99.5	no information founded	
TCIPP	89.9	60%	[32]
terbutryn	99.6	complete removal	[58]
tolyltriazole	95.7	complete removal	[47]

From Table 2, it is clear that the previously mentioned experimental set-up could be a solution for the removal of compounds such as beta-blockers, carbendazim, cyclophosphamide, DEET and TCIPP, which were not well-removed by bioremediation before.

Among the plants, Iris did not prove to perform the best during our phytoremediation experiments carried out in the past, probably because it was not very well-developed. A

comparison between the removal efficiency of Iris during the phyto- and bioremediation experiments is shown in Figure 3.

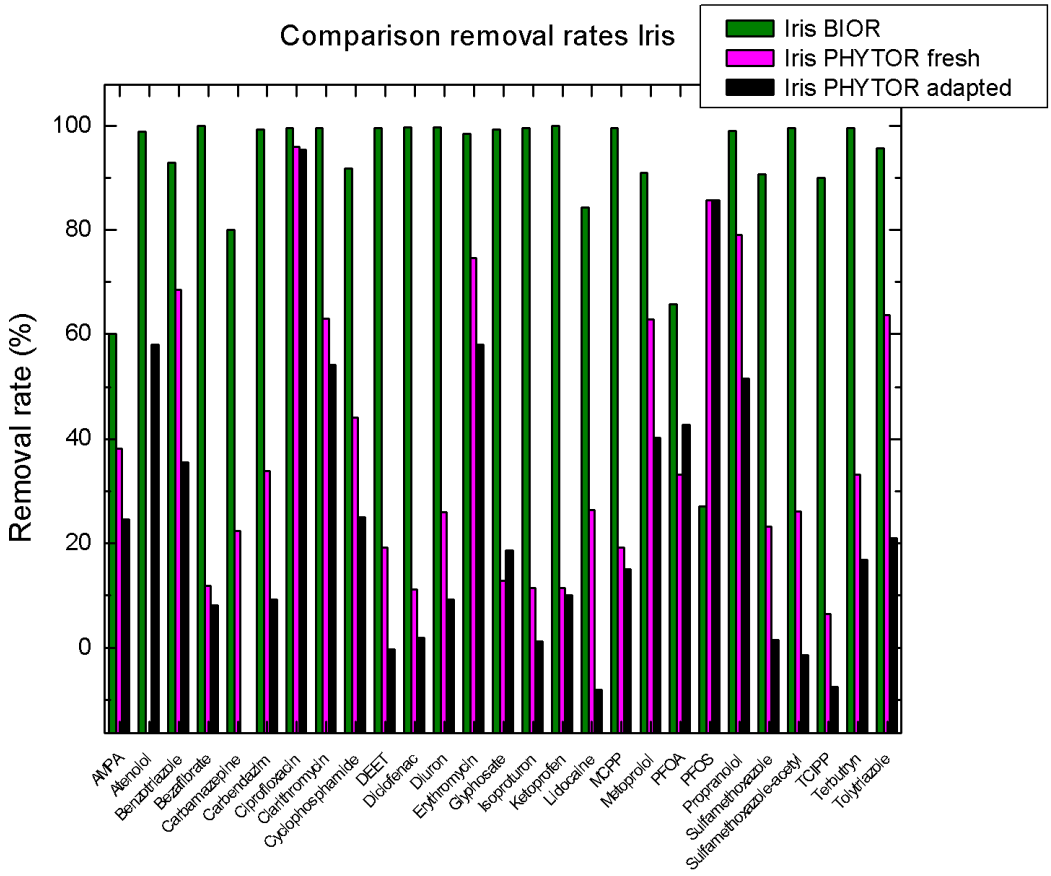

Figure 3. Comparison of the removal efficiency of Iris during bioremediation and fresh and adapted Iris during phytoremediation.

The removal rate (*R. r.*) was calculated using the following equation: $R.r.(\%) = \frac{c_0 - c}{c_0} * 100\%$, where co is the initial concentration of the MPs and c is the concentration on any given day of the experiment. With microorganisms present in the rhizosphere, the remediation system resulted in a higher MP removal than for plants without rhizosphere present. Focusing on the performance of Iris with the presence of the rhizosphere, it is apparent that some compounds are removed with medium-to-poor efficiency (<80%):

- AMPA, which was, notably, not removed from our CWs' installations (Venditti et al., 2022), is a degradation product of glyphosate that tends to retransform back to its maternal compound [59,60].
- Carbamazepine, which is a poorly biodegradable compound, and its metabolites can build back to the parent compound. Therefore, removal is not assumed in conventional WWTPs [51], while in the presented experiments, this compound was removed up to 80%.
- Fluorosurfactants, in this case, PFOA and PFOS, are generally persistent compounds that tend to accumulate in the surrounding media [61] and, in the present study, were removed up to 66% (PFOA) and 27% (PFOS).

We can demonstrate the usefulness of this removal process by providing two insights: First, the adapted method with a continuous oxygen supply (due to aeration) reduces stress in the rhizospheric system (anoxic conditions), and the permanent mixing of the aqueous solution guarantees representative sampling. Therefore, the configuration and design of the experiment represent the bioremediation process and show the importance

of the rhizospheric system. Nevertheless, sufficient oxygen levels seem to be essential for the MPs' removal in the rhizospheric system under real conditions. Additional forced aeration and recirculation of wastewater have previously demonstrated an increase in the aerobic capacity of the system and, thus, could be advantageous for the removal of MPs by CWs [62,63]. Second, poorly biodegradable or persistent MPs, such as metoprolol [64] and lidocaine [65], were removed by 91% and 84%, respectively. TCIPP, which passes through conventional wastewater treatment [66] and persists in treatments by advanced technologies, was removed by our approach up to 90%. The concentration profiles of all the compounds in each system, together with the quantification limits, are available in the Supplementary Materials.

In our previous phytoremediation experiments, Lythrum was the most efficient macrophyte. In the present bioremediation experiments, Lythrum exhibited the lowest MP removal efficiencies. This is probably due to its weakened physiological status after the winter period. A comparison of the medium efficiency of the MPs' removal by the three macrophyte bioremediative systems is shown in Figure 4.

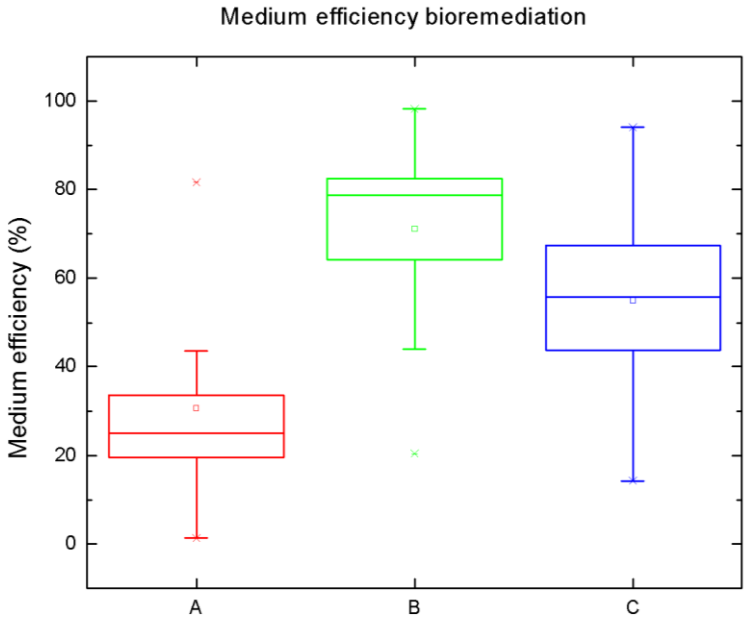

Figure 4. Medium efficiency of MPs' removal of the studied macrophyte systems, (A = *Lythrum*, B = *Iris*, and C = *Phragmites*).

Overall, our results suggest that the role of the rhizosphere in a CW environment could be substantially enhanced if additional aeration conditions and sufficient nutrients are provided.

3.3. Microbial Composition

In order to better understand the rhizosphere microbiome in CWs, we studied the bacterial complement by sequencing the 16S rRNA gene of this microbiome. For simplification, we focused on the most abundant 25 genera (a list of these genera is available in the Supplementary Materials), which represent the majority (>68%) of the overall population. Of these, we focused on bacteria with known potential for MPs' removal. The total abundance of these genera varied from 25–40%. The following figures (Figure 5) show the detailed abundances of genera known for the removal of organic MPs in the studied samples.

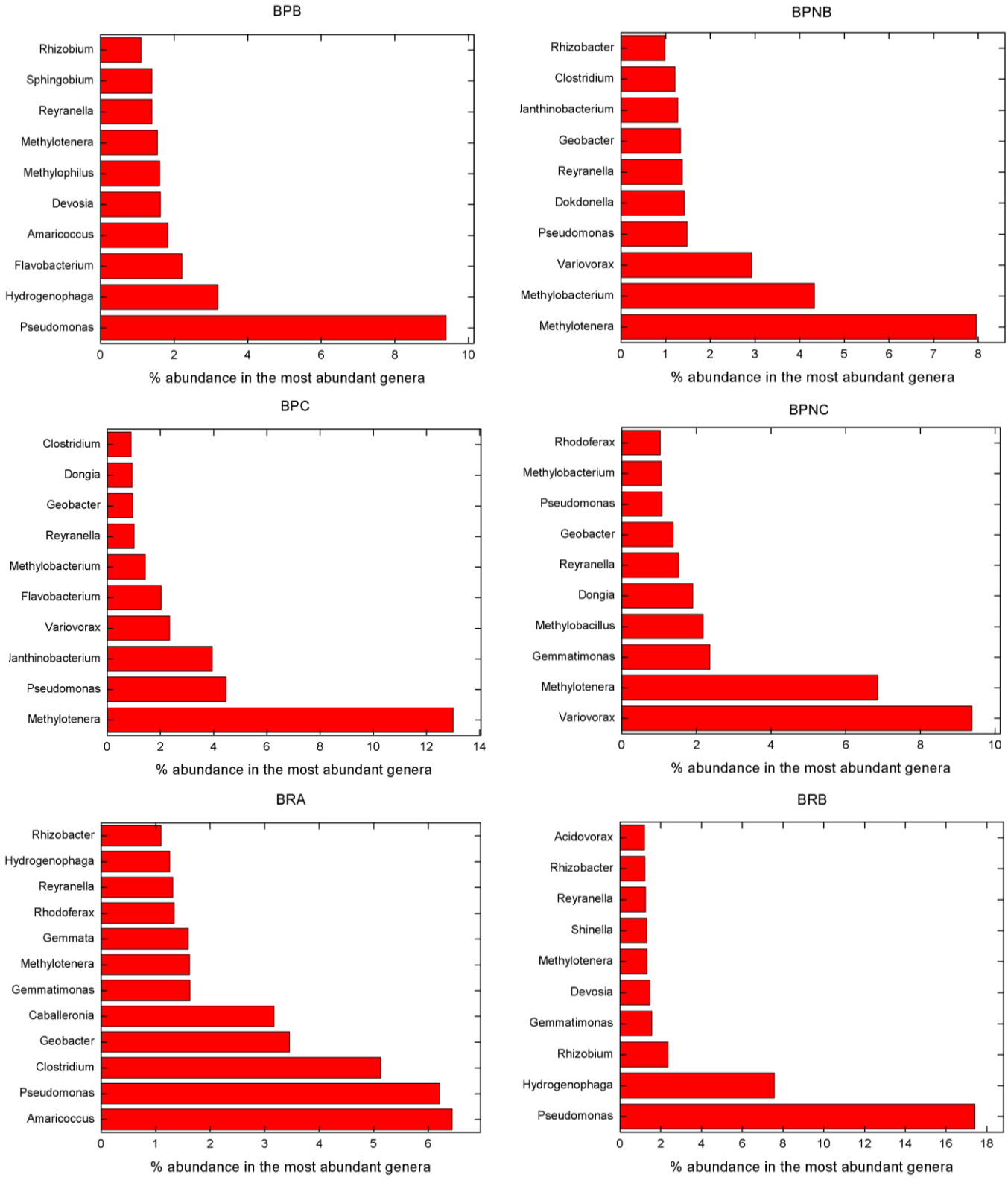

Figure 5. *Cont.*

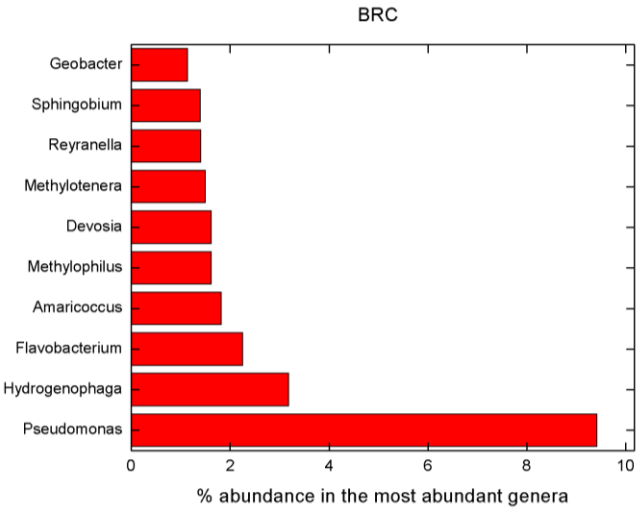

Figure 5. Abundance of the most abundant genera with known organic compound removal potential. BPB = Bioremediation Roots Iris, BPNB = Bioremediation New Roots Iris, BPC = Bioremediation Roots Phragmites, BPNC = Bioremediation New Roots Phragmites, BRA = Bioremediation Rhizosphere Lythrum, BRB = Bioremediation Rhizosphere Iris, and BRC = Bioremediation Rhizosphere Phragmites.

From the genera mentioned in the previous figures, the most abundant ones are shown in Table 3.

Table 3. Relative abundance of the most common genera for organic compound removal.

Sample	*Pseudomonas*	*Flavobacterium*	*Variovorax*	*Methylotenera*	*Reyranella*	*Amaricoccus*	*Hydrogenophaga*
				%			
BPB	9.39	2.22	0	1.56	1.41	1.84	3.2
BPNB	1.48	0	2.93	7.96	1.38	0	0
BPC	4.47	2.03	2.36	13.01	1.02	0	0
BPNC	1.09	0	9.39	6.86	1.54	0	0
BRA	6.22	0	0	1.62	1.31	6.44	1.26
BRB	17.43	2.61	0	1.34	1.26	0	7.58
BRC	9.42	2.26	0	1.5	1.41	1.82	3.19

We could not identify major trends for the abundance of the genera in the studied samples (Table 3). For example, *Flavobacterium* is present in the rhizosphere and root samples of *Iris* and *Phragmites* but not in the rhizosphere of *Lythrum*, and it is not present in the new roots. Hydrogenophaga, similar to *Amaricoccus*, are genera present in most of the rhizosphere samples but only in one root sample (*Iris*). These genera can remove a broad range of organic compounds. *Pseudomonas* is a well-known genus for organic and inorganic pollutants' removal and is commonly present in CWs [67], e.g., herbicides, antibiotics and the anticonvulsant carbamazepine [55,68,69]. *Amaricoccus*, similar to methylotrophs (in this case, *Methylotenera*), is a genus that uses organic compounds as a carbon source [70]. Some genera are targeting specific compounds; for example, halogenated compounds (diclofenac, TCIPP), as it is in the case of *Variovorax* and *Flavobacterium* [71,72].

3.4. Colonization of the Roots by AMF

To broaden the knowledge about a plant roots' microbiome, we observed the presence of the AMF complement by quantifying their colonization in the plant roots using microscopic techniques. AMF belong to endomycorrhizae, meaning that the hyphae penetrate individual root cells of the plant [73]. Thanks to this knowledge and to comparisons with

previously made photographs of AMF, it is possible to detect the fungus' nature successfully (Figure 6).

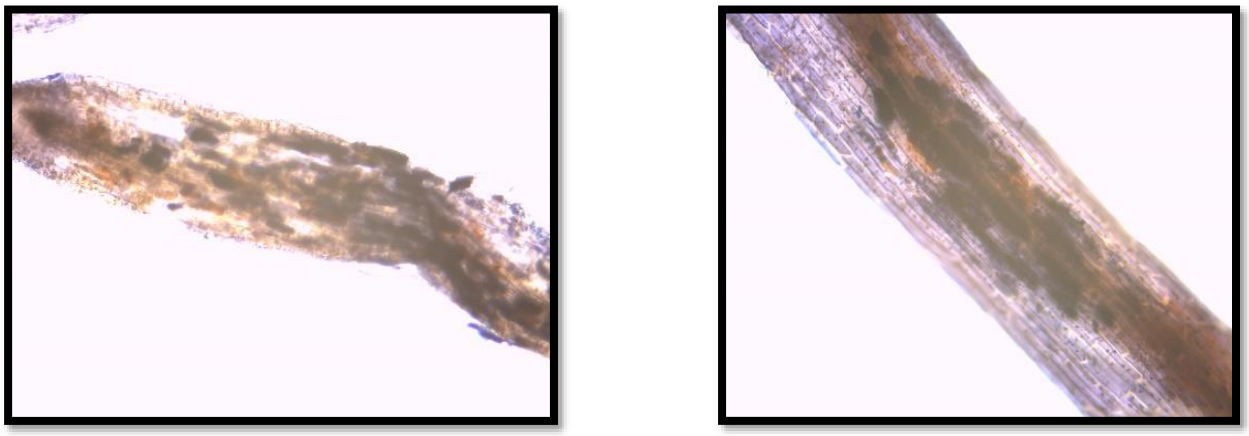

Figure 6. AMF in the plant cells of *Iris* (both photos represent the plant cells of *Iris*). The AMF appear as the very dark, even black spots within the plant cells.

The observed colonization rate of the roots by AMF is shown in the Table 4.

Table 4. Colonization of the plants' roots by AMF in the studied root samples.

Sample	Colonization by AMF (%)
Phragmites before bior. exp.	34
Iris before bior. exp.	56
Lythrum before bior. exp.	36
Phragmites after bior. exp.	10
Iris after bior. exp.	15
Lythrum after bior. exp.	10
Phragmites after bior. exp. new roots	0
Iris after bior. exp. new roots	0
Lythrum after bior. exp. new roots	0

We found that *Iris* consistently exhibited the highest AMF colonization rates. This could be due to *Iris* having a very dense root system compared to the other plants. Unfortunately, the roots suffered some damage during removal from the soil in the semi-hydroponic installation, which resulted, together with the majority of the soil absent, in an overall decreased AMF colonization. During the bioremediation experiment, we also observed fresh root growth. These examined roots showed no evidence of AMF, which may be explained by the fact that these fungi are soil-borne [74–76]. As the roots were not further investigated, the symbiosis between the fungi and the plants was not evaluated further in the present work. Thus, a possible target of future studies could be a deeper analysis of the roots and their associated AMF with possible extraction of the accumulated MPs.

The results acquired in this study indicated that the aforementioned genera are able to contribute to the removal of MPs when the plant roots for the symbiotic AMF are enriched, which is assumed to improve the phytoremediative potential of the plants. This confirms our hypothesis that rhizosphere in CWs has positive impact on the removal of MPs.

4. Conclusions

In this study, experiments determining the bioremediative activity of the studied systems for the removal of MPs were carried out. Next, the rhizosphere microbiome was identified, and the genera responsible for the removal of organic MPs were characterized.

Additionally, the colonization of the plant roots by AMF, which enhanced the removal of the MPs, was determined. The conclusions of the present research are as follows:

- Compared to our previous phytoremediation experiments, the currently described bioremediation experiment in semi-hydroponic conditions showed improved MP removal, which we believe was due to the additional aeration, recirculation of the liquid medium, and commercially bought hydroponic solutions, which favor the growth conditions of the plants and, therefore, enhance the development of the rhizosphere and consequent removal of MPs.
- The most efficient bioremediative system was the system with *Iris pseudacorus*, which removed 22 out of 27 of the MPs with more than 80% efficiency.
- Compounds, which are not well-removed in other bioremediation experiments, were removed here, with more than 90% efficiency (e.g., beta-blockers, carbendazim, cyclophosphamide, and DEET).
- Generally persistent compounds were removed with high efficiency (metoprolol up to 91%, lidocaine up to 84%, and TCIPP up to 90%).
- Possible ongoing nitrification likely enhanced the bioremediative process, as many of the MPs are degraded by nitrifying bacteria.
- *Lythrum salicaria* had the lowest efficiency for removing MPs (contrary to previous phytoremediation experiments). This is probably due to its weak physiological status after the winter season.
- *Pseudomonas, Flavobacterium, Variovorax, Methylotenera, Reyranella, Amaricoccus* and *Hydrogenophaga* belong to genera that are known to be potential MP degraders. High abundances of these organisms were also found in our samples.
- A colonization of the plant roots by AMF was established. This information is valuable, as AMF contribute to phyto- and bioremediation. The macrophyte with the highest colonization was *Iris pseudacorus* (56%).

These conclusions summarize the main outcomes of the discussed research. In the present study, the optimal candidate for bioremediation was found to be *Iris pseudacorus*. It showed an excellent ability to outlast the winter season without considerable loss of its pollutant removal abilities and provided a decisive environment for its advantageous symbiosis with AMF. We believe there is much potential for further investigation of bioremediative systems, their associated microbiomes, and CWs.

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