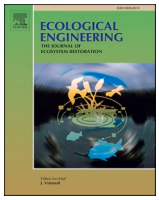
Ecological Engineering 215 (2025) 107598

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng



Biodiversity assessment in a floating treatment wetland established in a stormwater pond

Cristina S.C. Calheiros [a,*], Martina Ilarri [a], Mariana Godinho [b], Paula M.L. Castro [b], Sofia I.A. Pereira [b]

[a] CIIMAR/CIMAR LA, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
[b] CBQF—Centro de Biotecnologia e Química Fina—Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

ARTICLE INFO

Keywords:
Nature-based solutions
Plant growth-promoting bacteria
Macroinvertebrates
Dragonflies
Damselflies
Phytoremediation
Nature restoration
Ecosystem services
Water management

ABSTRACT

Floating treatment wetland (FTW) are a nature-based solution delivering a wide range of ecosystem services when applied in water bodies, such as lakes and rivers. They are recognized for delivering biodiversity, aesthetic integration, and water quality enhancement through phytoremediation processes, although research is still needed to go deeper into the processes underlying the performance of these systems and evaluate the extent of biodiversity promotion especially on pond ecosystems. This study aimed to assess the plant establishment and biodiversity associated with an FTW set up in a rural artificial stormwater pond, with a polyculture comprising *Iris germanica*, *Acorus gramineus*, *Caltha palustris*, and *Typha latifolia* set in a cork agglomerate platform. For this, it was assessed the culturable bacterial communities associated to the floating platform and the rooting system, and the macroinvertebrates associated to the FTW and to the pond margin. Culturable bacterial communities colonizing the floating platform biofilm and the plant rhizosphere were isolated, identified by 16S rRNA, and characterized for their ability to produce plant growth-promoting substances (e.g., indole-acetic acid, siderophores). There was a high bacterial genera diversity associated with the FTW and with the ability to produce plant growth-promoting substances. Bacterial strains with outstanding growth-promoting traits can be used in the future to support phytoremediation strategies or plant resilience to climate change-related abiotic stresses. Regarding the biodiversity of macrofauna, namely macroinvertebrates, associated with FTW, they were mostly from the order Odonata. The FTW attracted mainly individuals of the genus *Coenagrion*, which represented more than 80 % of the associated fauna. The full life cycle of dragonflies and damselflies occurred in the FTW. These systems proved to be a hotspot of biodiversity supporting water and landscape management plans, besides aesthetics integration. This study gives new insights into broadening the FTW applications in stormwater or prospects to polluted water.

1. Introduction

Floating treatment wetland (FTW) are a nature-based solution (NBS) that gained ground in the last decades providing an effective, low-cost, and low-maintenance approach to improve water quality for a wide range of applications. This system comprises a buoyant structure vegetated with aquatic plants, usually macrophytes, that float on the surface of water bodies (Colares et al., 2020). Ensuring long-term effective buoyancy and support for the plants are two priorities for the material chosen for the floating platform. Besides that, it must be resistant to abiotic stresses (such as temperature oscillation), allow for an anchoring system, be economically feasible, and of easy implementation. A variety of FTW materials have been reported in the literature, such as polyethylene-based, polypropylene mats, foam materials (Barco et al., 2021), or cork (Calheiros et al., 2020). They have been consistently considered to be used for wastewater treatment, nutrient removal from eutrophicated waters (Sharma et al., 2021), urban stormwater runoff treatment (Colares et al., 2020), namely from highways (Borne et al., 2013), and commercial parking lots (Winston et al., 2013), and fewer for saline or marine environments (e.g. Calheiros et al., 2020).

* Corresponding author.
E-mail addresses: cristina.sc.calheiros@gmail.com, ccalheiros@ciimar.up.pt (C. S.C. Calheiros), sapereira@ucp.pt (S. I.A. Pereira).

https://doi.org/10.1016/j.ecoleng.2025.107598
Received 10 November 2024; Received in revised form 24 February 2025; Accepted 5 March 2025
Available online 11 March 2025

Biodiversity promotion provided by the FTW is also a valuable feature with the potential to be explored and targeted as an accountable ecosystem service, by creating an adequate habitat for species to thrive as also a stepping stone (Calheiros et al., 2023; Karstens et al., 2021). FTW can improve ecosystem conditions by establishing a more stable and diverse habitat for a variety of fauna (e.g., invertebrates, fishes, amphibians, reptiles, and birds) (Calheiros et al., 2023). They can be a promising habitat for macroinvertebrates (Salmon et al., 2022), leading to an increase in interstitial space that provides shelter and refuge from predators and also serves as a food source linked to the particulate matter that gets trapped in plant roots (Prashant and Billore, 2020). Additionally, the roots and rhizomes of plants and the floating platforms provide a vast surface for the attachment of pollutant-degrading microbial communities, which can enhance the removal efficiency of the system (Calheiros et al., 2020). Furthermore, rhizospheric microbial communities are known to be of utmost importance to foster plant growth and resilience under adverse conditions. In particular, it is widely accepted that plant growth-promoting bacteria (PGPB) benefit plants through a plethora of well-known mechanisms including phytohormone and siderophore production and phosphorous (P) solubilization ability (Basu et al., 2021). For instance, siderophores, which are low molecular-weight molecules, are able to chelate ferric iron and increase Fe supply to plants promoting their growth (Timofeeva et al., 2022). However, the best-studied PGPB are those associated with terrestrial plants, while little is known about the growth-promoting mechanisms of PGPB colonizing the rhizosphere of aquatic plants (Makino et al., 2022). Several authors reported that plants in FTW inoculated with PGPB and/or pollutant-degrading bacteria further enhanced the efficiency of the system (Fahid et al., 2020; Nawaz et al., 2020; Rehman et al., 2018; Tusief et al., 2022). For instance, the inoculation of hydrocarbon-degrading bacteria in *Cyperus laevigatus* plants improved the diesel oil remediation significantly while increasing plant growth (Fahid et al., 2020). According to Rehman et al. (2018) the inoculation of *Brachiara mutica* and *Phragmites australis* with an inoculum containing the hydrocarbon-degrading bacteria *Bacillus subtilis* LORI66, *Klebsiella* sp. LCRI87, *Acinetobacter Junii* TYRH47 and, *Acinetobacter* sp. LCRH81, also significantly improved the efficiency of the FTW in removing oil from water. Similar results were obtained by Nawaz et al. (2020) where the inoculation of bacterial strains with pollutant degrading ability and plant growth-promoting traits enhanced the removal of metals and color dyes from a dye-enriched synthetic effluent.

Currently, most research on FTW focuses on its use for bioremediation, while studies focused on biodiversity enhancement are less common (Vo et al., 2023; Arivukkarasu and Sathyanathan, 2023; Colares et al., 2020; Yeh, N. et al., 2015). In addition, few studies have addressed biodiversity with emphasis on the macroinvertebrate communities. The present work aims to investigate the establishment and biodiversity associated to an FTW, with a polyculture in a cork agglomerate platform, in an artificial stormwater pond integrated in a rural area. This was achieved by identifying macrofauna, namely macroinvertebrates, and the culturable bacterial communities colonizing the platform (biofilm) and the rhizosphere of FTW plants. Bacterial strains were also characterized for their ability to produce plant growth-promoting substances (e.g., indole-acetic acid (IAA), siderophores) for further application in bioaugmentation strategies. To the best of our knowledge, this is the first study highlighting the biodiversity associated to an FTW concerning bacteria and respective plant growth-promoting traits and macroinvertebrates' life cycle. This research brings important outcomes to support further the implementation of the Nature Restoration Law (European Union Parliament, 2024). A key component of this law is the emphasis on NBS, which are recognized for their potential to achieve environmental goals while providing socio-economic benefits. The Restoration Law and NBS share a common ground based on leveraging natural processes to address environmental challenges by aiming to restore ecosystems, improve biodiversity, and reverse environmental degradation. Nature-based solutions seek to harness the power of nature to tackle issues such as climate change, biodiversity loss, water management, and urban resilience, offering a complementary pathway to achieve more sustainable and resilient territories.

2. Materials and methods

2.1. Study area and floating wetland island implementation

The study site is focused on a constructed pond, that has an impermeable geomembrane liner, coated with a layer of granite stones incorporated in hardened mortar, with no vegetation in the margin. The pond has a perimeter of 48 m with a depth of approximately 1.2 m (Calheiros et al., 2017). The site is located in a rural area dominated by agriculture and forests, within a farm with a tourism guest house – Paço de Calheiros (41.80555384924214, −8.565179785400563). The pond has an inlet and outlet, being fed, on the north side, by an upper artificial pond and when full, the water runs off through a pipe on the south side. The upper artificial pond is fed by a temporary stream that is mixed with stormwater leachate from the woods and agricultural fields.

A pilot FTW was implemented in the pond in June 2018 being the present study carried out in September 2022. Between July and September 2022, monthly visual observations of the FTW were carried out to inspect the fauna inhabiting the platform.

The FTW system was constituted by an agglomerate cork-based platform (Cork Floating Island®, Supplier: Bluemater, S.A., Porto, Portugal) with the following technical characteristics: cork agglomerate (density of 0.2); a size of 100 × 50 × 10 cm (l x w x h) with frustoconical holes of two different sizes (8 cm and 16 cm diameter for the small and the larger types, respectively); capacity to support a weight of 16 kg of plants/m^2, and up to 24 plants/m^2, according to the manufacturer (Amorim, 2018). The FTW was anchored to the margin by a rope, allowing 3 m of distance from the margin. Plant species were selected based on:

(i) presence in the country; (ii) presence in the region of the experimental site; (iii) potential to survive in hydroponic mode; (iv) perennial species, and (v) commercial availability. Fig. 1 presents the cork platform with a polyculture of mostly ornamental plants, where *Iris germanica* (4 plants), *Caltha palustris* (2 plants), *Acorus gramineus* (4 plants), and *Typha latifolia* (2 plants) were placed in coconut fiber vases, fixed with rockwool and adjustable plastic clamps (to reinforce the vases between the holes), being afterward inserted into the holes of the platform in a random distribution of the plant species. This FTW pilot intends to provide a baseline of knowledge related to biodiversity establishment and promotion associated with this NBS. To characterize pond's water, 4 samples were collected from distinct zones of the pond surface (at the level of the FTW) and subsequently analyzed in laboratory following the methodology presented in (Calheiros et al., 2015). For that, a single sampling took place in parallel with the sample collection described in section 2.2 for, culturable bacterial communities assessment and in section 2.3 for macroinvertebrate communities' studies.

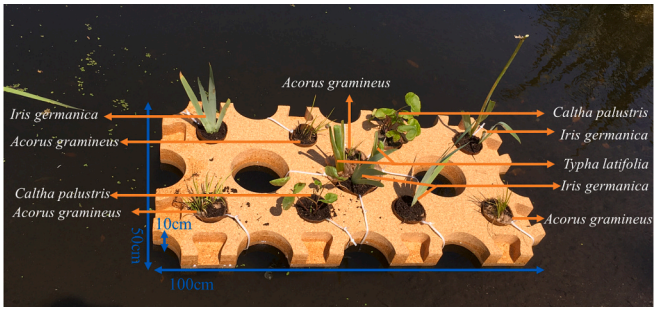


Fig. 1. Floating treatment wetland configuration setup with platform dimensions (100 × 50 × 10 cm) and plant species: *Iris germanica* (4 plants), *Caltha palustris* (2 plants), *Acorus gramineus* (4 plants), and *Typha latifolia* (2 plants).

The physiochemical characteristics of the water body where the FTW was implemented showed mean ± SD (n = 4) of pH of 6.81 ± 0.05, temperature of 14.68 ± 0.15 °C, electrical conductivity (EC) of 56 ± 0 µS/cm, total dissolved solids (TDS) of 28 ± 0 mg/L, PO_4^-P of 0.06 ± 0.03 mg/L, NH_4^+-N below the detection limit <0.03 mg/L, NO_3^--N of 1.1 ± 0.1 mg/L, NO_2^--N 0.04 ± 0.01 mg/L and chemical oxygen demand (COD) of 7 ± 2 mg/L.

2.2. Culturable bacterial communities colonizing the platform and the rhizosphere of plants inhabiting the FTW

In order to identify the culturable bacteria colonizing the platform and the rhizosphere it was necessary to proceed with the biofilm collection associated with each matrix, followed by bacteria cultivation, enumeration, and species identification. For that, a single sampling took place in parallel with the sample collection described in section 2.1 for water analysis and section 2.3 for macroinvertebrate communities' studies. The approach of cultivating the bacteria is justified by the fact that, further on, the production of plant growth-promoting substances by bacterial strains was going to be assessed and the most promising bacteria could be used as inoculants in future phytoremediation strategies.

2.2.1. Sampling

The FTW was pushed with a rope to the pond margin (that acted as an anchoring system, based on section 2.1 description) in order to facilitate access for the collection of the biofilm adherent to the platform, as well as the roots of plants, to further study the bacterial communities associated to the rhizosphere of the FTW plants. FTW was divided into 4 different quadrants to have a representative sampling of the whole platform, for biofilm and root collection. Concerning biofilm collection, for further isolation and evaluation of the bacterial communities the procedure was: for each quadrant, a scalpel was used to scrape off the biofilm adhering to the platform (face in contact with water) in 3 different locations to form a composite sample.

Concerning root collection, for the isolation and evaluation of the bacterial communities, the procedure was: in each quadrant, 3 bulk root areas, each forming a 4 × 4 cm square, were collected and pooled to form a composite sample. The bulk roots were cut using a sterilized scissor, leaving 2 cm of root below the platform. The roots had an average elongation of 22 cm, and each bulk root square weighted approximately 50 g (fresh weight). Samples were kept in refrigerated boxes until arriving at the laboratory.

2.2.2. Enumeration and bacterial isolation

Twenty-five grams (fresh weight) of each composite sample of roots were retrieved in duplicate in sterile bottles, mixed with 225 mL of sterile saline solution (0.85 % NaCl), and shaken using a vortex mixer for 30 min at room temperature. A similar approach was used for the platform-associated biofilm, by mixing 100 mg of sample with 900 µL of sterile saline solution (0.85 % NaCl) and shaking for 3 min at room temperature in a vortex mixer. Serial dilutions were made in duplicate, and 0.1 mL of each dilution was spread in trypticase soy agar (TSA; Biokar) for heterotrophic bacteria enumeration and isolation. Plates were incubated at 30 °C for 5 days after which colony-forming units (CFU) were determined. Bacterial colonies were isolated based on microscope observed morphological differences (size, color, and shape). Isolates were then purified by sub-culturing on TSA medium and stored at −80 °C in modified Luria-Bertani (Merck) broth supplemented with 15 % (v/v) glycerol.

2.2.3. BOX-PCR fingerprinting

Extraction of DNA was performed according to the method described in Calheiros et al. (2010). The BOX-PCR fingerprinting was performed to group similar isolates using the primer BOXA1R (5-CTACGGCAAGGC-GACGCTGACG-3). The reaction mixture (10 µL) consisted of 6.3 µL of ultrapure water, 0.8 µL of BOXA1R primer (10 µmol/L, NZYTech), 2.5 µL of NZYTaq II 2× Green Master Mix (NZYTech) and 0.4 µL of DNA template. Amplification was done in a iCycler Thermal Cycler (Bio-Rad Laboratories) using the following PCR conditions: initial denaturation at 95 °C for 7 min, followed by 30 cycles of denaturation at 94 °C for 1 min, primer annealing at 53 °C for 1 min, and extension at 65 °C for 8 min, with a final extension at 65 °C for 16 min. PCR products along with a 1 kb ladder (GeneRuler DNA Ladder Mix, Thermo Scientific) were run in 1.5 % (w/v) agarose gel electrophoresis in 1× Tris–Acetate–EDTA (TAE) buffer and stained with SYBR Safe (Invitrogen) for 180 min at 80 V. Fingerprinting of bacterial isolates were analyzed and compared using Bionumerics software (Applied Maths, St-Martens-Laten, Belgium). Isolates displaying different profiles were selected for identification.

2.2.4. 16S rRNA gene sequencing

Identification of the selected bacterial isolates was done through amplification of the 16S rRNA gene region using the universal primers 27F (5'-GAGTTTGATCCTGGCTCAG-3′) and 1492R (5'-ACCTTGTTAC-GACTT-3′). The PCR mixture (25 µL) consisted of 8.5 µL of ultrapure water, 1.5 µL of each primer (10 µmol/L, NYZTech), 12.5 µL of NZY Taq 2× Green Master Mix (NZYTech), and 1 µL of DNA template. The amplification was done in an iCycler Thermal Cycler (Bio-Rad Laboratories) using the following conditions: initial denaturation step 95 °C for 5 min, followed by 30 cycles: denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min, and a final extension step at 72 °C for 10 min. Amplicons were visualized in 1.5 % agarose gel stained with SYBR Safe (Invitrogen). PCR products were purified using GRS PCR & Gel Band Purification Kit (GRiSP), following manufacturer's instructions, and sequenced by Eurofins Genomics (Germany). The sequences were edited using BioEdit software and subjected to nucleotide BLAST search against the National Centre for Biotechnology Information (NCBI) database to identify the closest strains. The 16S rRNA gene sequences of bacterial strains isolated in this study are deposited in NCBI database under the accession numbers OR756618 to OR756647.

2.2.5. Phylogenetic analysis

The CLUSTALW software was used to align sequences (Thompson et al., 1994). 16S rRNA gene sequences of the closest neighbors of each isolate were used. Trees construction was performed with MEGA 11.0 (Tamura et al., 2021), using the maximum likelihood estimation method based on the Tamura–Nei sequence evolution model (including Gamma distribution rates) and 1000 bootstrap resamplings of the sequences.

2.2.6. Production of plant growth-promoting substances by bacterial strains

All bacterial isolates were tested (3 replicates) for their ability to produce plant growth-promoting traits, including the production of siderophores and IAA and the ability to solubilize P. For siderophore production, bacterial strains were inoculated in Chrome Azurol S (CAS) agar medium (Schwyn and Neilands, 1987) for 5 days at 30 °C. Yellow-orange halos around the colonies on CAS agar were indicative of siderophore production. The phosphate solubilizing activity was evaluated in a modified National Botanical Research Institute's Phosphate (NBRIP) medium amended with 0.5 % tricalcium phosphate (Nautiyal, 1999). Strains were incubated in NBRIP at 30 °C for 15 days. The presence of a hyaline halo around the colonies is indicative of P solubilization and the solubilization index was calculated as follows: (colony diameter + halo zone diameter)/colony diameter. The amount of IAA produced by bacterial strains was determined according to the method of (Gordon and Weber, 1951) in the presence of 1 % of L-tryptophan as described in (Pereira and Castro, 2014).

2.3. Macroinvertebrates communities

To assess the biodiversity associated with the FTW established in the pond, the macroinvertebrate communities were assessed and diversity indexes calculated. For that, a single sampling took place in parallel with

the sample collection described in section 2.1, for water analysis, and section 2.2, for culturable bacterial communities assessment.

The study focused on the FTW rhizosphere and the non-vegetated pond margin to enable a comparative analysis of communities present with and without vegetation. For this purpose, macroinvertebrates were sampled at two distinct sites of the pond at the same water depth: (1) FTW (samples were collected 20 cm below the platform, comprising the rhizosphere), and (2) pond margin (samples were collected about 20 cm below the water surface in the pond margin). Adapted from Khudhair et al. (2019), the associated macroinvertebrates were collected using hand-net trawls for two minutes (individuals/2 min). This allowed determining the abundance as individuals captured per unit of effort (CPUE) (ind/2 min). The hand net had a mesh size of 500 μm. After the collection of the associated macroinvertebrates, the organisms were sorted and fixed in 70 % ethanol. Then, all organisms were identified to the lowest practical taxonomic level according to Tachet et al. (2000), and counted. A total of three replicates ($n = 3$) per treatment (i.e. FTW and pond margin) were collected.

2.4. Statistical analyses

Differences in the abundance of the associated macroinvertebrates (all the taxa collected associated to the treatments), total abundance, and diversity indices were tested using Welch's *t*-tests, with treatments (two levels: pond margin and FTW) represented by the type of area in which the samples were collected as a fixed factor. Welch's t-test assumptions about the normal distribution was checked, and whenever the data did not satisfy these conditions the non-parametric Mann-Whitney *U* test was used (this was mainly for the comparisons with the taxa collected associated to the treatments). Assumptions about the normal distribution of the residuals were tested using the shapiro.test function from the package car (Fox and Weisberg, 2011). The diversity indices, Shannon-Wiener diversity index, and species richness were calculated using the diversity and specnumber functions of the package vegan (Oksanen et al., 2019). Pielou's evenness index was calculated by dividing the Shannon-Wiener index by the log-transformed species richness. Diversity indices are statistical representations of biodiversity in different aspects (richness, evenness, and dominance). A diversity index is a quantitative measure that indicates how many different species there are in a community. The Shannon-Weiner diversity index takes into account both species richness and evenness. Species evenness refers to how close in numbers each species in an environment, and can be represented by Pielou's evenness index (Bouwer et al., 2024). All analyses were performed using R software (version 4.0.2; R Core Team, 2020). When applicable, data was presented as means ± standard deviations (mean ± SD).

3. Results and discussion

3.1. Floating treatment wetland establishment

The FTW platform used in this study was agglomerate cork-based, being cork a naturally occurring material with a negative carbon footprint, extracted from the *Quercus suber* L. tree, thus considered a promising material as a net carbon sink (Demertzi et al., 2018). In the first years since its implementation, the FTW preserved its structural integrity, although after six years the platform was 20 cm below the water surface and the cork agglomerate particles were being released in the edges. It is hypothesized that, because the platform is made of a cork agglomerate (obtained from selected cork granules, compressed and bound with specially developed binders), instead of cork oak bark, it may absorb water and thus compromise future buoyancy, for long-term use.

One of the objectives of establishing an ornamental polyculture was to evaluate if a certain species was dominant and if there was a beneficial cohabitation with enhanced aesthetic integration. The results show that *A. gramineus* fully dominated the platform in the presence of *T. latifolia*. Although the system was completely vegetated, with a well-established root system, the flowering ornamental purpose was not achieved (Supplementary material S1). Besides the natural dominance of a certain species, Barco et al. (2021) stated that wastewater physicochemical composition and plant adaptability to living in hydroponic conditions may influence the establishment of plants and thus showing species-specific behavior.

3.2. Assessment of culturable bacterial communities associated with FTW

FTW have been studied regarding their efficiency for water purification, however little is known about the bacterial communities associated to the roots of macrophytes and the artificial floating platform. The culturable bacterial communities constituted a small fraction of the bacteriome associated to the FTW, however, the isolation and characterization of these strains may allow their use in bioaugmentation strategies to foster plant growth and the efficiency of the system. In the present work, the number of heterotrophic bacteria found in biofilm and rhizosphere were quite similar, being 5.78 ± 0.14 $\log_{10}$ g^{-1} fresh weight (f.w.) for biofilm and 5.59 ± 0.11 $\log_{10}$ g^{-1} f.w. for rhizosphere. In total, 80 bacterial strains were isolated from the FTW, 46 from the rhizosphere of plants, and 34 from the biofilm adherent to the platform. According to BOX-PCR, 42 different profiles represented by a single isolate were identified. Although it was not possible to obtain BOX profiles for 34 strains, according to their distinct morphological characteristics, 12 strains were also selected for further identification by the 16S rRNA partial gene. Among the 54 strains, only 30 strains were identified, 19 from the rhizosphere and 11 from the biofilm. The values of 16S rRNA gene sequence similarity between the bacterial strains and the closest relatives were generally high (97–100 %) (Table 1, and Supplementary material S2 and S3). It is worth mentioning that this study was carried out four years after the FTW was set up, being considered an established system, since acclimation periods can be of 2 months (Calheiros et al., 2020).

Phylogenetic analysis showed that most of the bacterial strains belong to the classes *Bacilii* and γ-*Proteobacteria*, being *Priestia* (former *Bacillus*) the most predominant genus in plant rhizosphere, while in the biofilm were the genera *Enterobacter*, *Pseudomonas,* and *Priestia*. These findings agree with Srivastava et al. (2017), who reported that these taxa are commonly associated to freshwater systems. On the other side, a study conducted by Urakawa et al. (2017) showed differences in the composition of microbial bacterial communities attached to the submerged roots and the biofilm adhering to the floating platform in a FTW installed in a manmade stormwater pond. The class α-*Proteobacteria* was the most dominant in plant root microbiome being the genera *Rhizobium* and *Rhodobacter* the most representative, while in biofilm communities the class γ-*Proteobacteria* and the genus *Pseudomonas* were the most represented.

Plants colonizing FTW form associations with microorganisms that help in both pollutant degradation/removal and plant growth (Demarco et al., 2023; Rehman et al., 2018). However, although PGPB associated with terrestrial plants are well studied, little is known about the phylogeny and growth-promoting mechanisms of PGPB associated with aquatic plants. Several PGPB isolated from aquatic environments have been reported to increase the growth of the aquatic plant *Lemna minor* (Yamakawa et al., 2018). Makino et al. (2022) also reported the beneficial effect of 4 bacterial strains, previously isolated from Japanese loosestrife plants, with the ability to produce IAA and siderophores, to fix N_2 and solubilize P on the number of *L. minor* fronds, biomass, and chlorophyll content. In the present study, the bacterial isolates retrieved from the FTW were also tested for their ability to produce siderophores and IAA and to solubilize P (Table 2).

All bacterial strains were able to produce siderophores, however, most of them (>60 %) showed weak and intermediate production. P solubilization index also varied among bacterial strains. Five strains (R3,

Table 1
Phylogenetic affiliation of bacterial strains isolated from the rhizosphere (R) and the biofilm (B) of the floating treatment wetland.

Source	Strain	Gram	NCBI acession No.	Phylogenetic affiliation	Closest Blastn match (NCBI acession No.)	Similarity (%)
	R2	−	OR756618	Pseudomonadota	*Erwinia endophytica* SH-3 (MN220597.1)	99.35
	R3	+	OR756619	Bacilli	*Bacillus* sp. (OQ874296.1)	99.82
	R5	+	OR756620	Bacilli	*Rossellomorea marisflavi* L2 (ON337508.1)	99.90
	R8	+	OR756621	Bacilli	*Bacillus zanthoxyli* (NR_1648821)	99.07
	R11	−	OR756622	g-Proteobacteria	*Citrobacter freundii* E3–4 (KY938091.1)	99.73
	R12	+	OR756623	Bacilli	*Priestia aryabhattai* M2C (OP890302.1)	99.65
	R13	−	OR756624	g-Proteobacteria	*Enterobacteriaceae bacterium* SB690 (MG491579.1)	98.17
	R15	+	OR756625	Bacilli	*Priestia megaterium* APBSDSB23 (MG705569.1)	99.45
	R18	+	OR756626	Bacilli	*Priestia koreensis* NES-CTC-3 (MF079292.1)	99.82
Rhizosphere	**R20**	+	OR756627	Bacilli	*Bacillus altitudinis* CPO MNS18-Y28 (OP897613.1)	99.73
	R21	+	OR756628	Acnitomycetes	*Curtobacterium herbarum* I-S-L1–2 (MK398004.1)	92.56
	R22	+	OR756629	Bacilli	*Priestia aryabhattai* M2C (OP890302.1)	97.38
	R26	−	OR756630	g-Proteobacteria	*Dickeya solani* NCPPB 4479 (KY190324.1)	99.72
	R28	+	OR756631	Bacilli	*Bacillus cereus* cz49 (OP090466.1)	99.62
	R42	−	OR756632	g-Proteobacteria	*Kluyvera intermedia* NBRC 102594 (MN901963.1)	98.50
	R44	+	OR756633	Bacilli	*Priestia megaterium* APBSDSB23 (MG705569.1)	99.17
	R74	+	OR756634	Acnitomycetes	*Micrococcus luteus* SCH0405 (AY881238.1)	99.46
	R75	+	OR756635	Bacilli	*Paenibacillus glebae* LMG 23880 T (AM745264.1)	98.63
	R80	+	OR756636	Bacilli	*Bacillus safensis* Rb1S3 (MH844969.1)	99.54
	B49	−	OR756637	g-Proteobacteria	*Pseudomonas moraviensis* KR3M-28 (MN752870.1)	99.05
	B55	−	OR756638	g-Proteobacteria	*Enterobacter* sp. H297 (MH669343.1)	99.14
	B60	+	OR756639	Bacilli	*Bacillus tequilensis strain* BT31 (OP905597.1)	99.82
	B61	−	OR756640	g-Proteobacteria	*Enterobacter* sp. H297 (MH669343.1)	99.14
	B65	−	OR756641	g-Proteobacteria	*Enterobacter* sp. H297 (MH669343.1)	99.14
Biofilm	**B67**	+	OR756642	Bacilli	*Bacillus cereus* cz49 (OP090466.1)	99.71
	B69	−	OR756643	g-Proteobacteria	*Pantoea vagans* ASH38 (OM585545.1)	95.50
	B71	+	OR756644	Bacilli	*Priestia megaterium* APBSDSB23 (MG705569.1)	99.63
	B77	−	OR756645	b-Proteobacteria	*Chromobacterium rhizoryzae* LAM1188 (NR 152068.1)	99.73
	B78	−	OR756646	g-Proteobacteria	*Pseudomonas hibiscicola* ATCC 19867 (MT780275.1)	99.82
	B79	+	OR756647	Acnitomycetes	*Microbacterium oleivorans* AT1 (MG016448.1)	99.65

Table 2
Characterization of bacterial strains isolated from the rhizosphere (R) and the biofilm (B) for colony morphology and multiple plant growth-promoting traits: siderophore and indole-acetic acid (IAA) production and P solubilization ability.

Source	Strains	Colony morphology	Siderophore production	P solubilization index	IAA (mg/L) (mean ± SD)
Rhizosphere	**R2**	beige, milky, translucent, shiny	++++	2.8	408.0 ± 63.9
	R3	white, opaque	++++	–	22.5 ± 8.8
	R5	yellow, translucent	++	–	11.8 ± 6.5
	R8	beige, milky, translucent	+++	2.0	81.0 ± 11.6
	R11	white, opaque, irregular	+	2.0	244.7 ± 49.3
	R12	yellow, opaque, viscous	+++	2.3	170.8 ± 29.0
	R13	beige, irregular, translucent	++++	2.6	275.5 ± 38.9
	R18	yellow, translucent, irregular	++	1.9	140.2 ± 83.1
	R20	beige, opaque, irregular	+	2.3	10.2 ± 6.2
	R21	salmon, shiny	+++	2.6	38.4 ± 15.8
	R22	beige, irregular	+	1.7	45.8 ± 23.6
	R26	beige, translucent, shiny	++	2.0	229.7 ± 50.1
	R28	yellow, translucent, shiny	++	1.5	25.3 ± 18.1
	R42	yellow, shiny, viscous	++	4.1	70.7 ± 3.2
	R44	beige, opaque	++	1.2	80.0 ± 11.6
	R74	yellow, shiny, viscous	+	+	169.2 ± 26.2
	R75	white, milky, opaque	+	–	94.5 ± 9.7
	R80	beige, opaque	++	1.7	15.5 ± 15.5
Biofilm	**B49**	white, opaque, shiny	+++	2.1	112.6 ± 53.4
	B55	yellow, shiny, viscous	++	4.3	118.6 ± 30.2
	B60	white, translucent	++++	+	9.7 ± 7.8
	B61	white, translucent, viscous	+	3.1	186.1 ± 57.6
	B65	yellow, shiny, viscous	+	6.0	144.2 ± 47.7
	B67	yellow, translucent, shiny	+	3.6	23.7 ± 7.8
	B69	yellow, translucent	+	–	107.8 ± 40.1
	B71	beige, opaque, shiny	+	2.4	134.0 ± 49.7
	B77	light pink, rough, opaque	++++	+	47.4 ± 17.4
	B78	beige, milky, translucent	++	–	100.8 ± 19.0
	B79	yellow, translucent, shiny	+	1.3	87.7 ± 24.0

Note: (−) negative, (+) positive/weak, (++) intermediate, (+++) strong, very strong (++++) siderophore production. P solubilization index: (colony diameter + halo zone diameter)/colony diameter. IAA is expressed as means ± SD (n = 3–5).

R5, R75, B69, B78) showed negative results for this trait, while the strains R42, B55, and B65, belonging to *Enterobacter* genus presented the highest (>4) P solubilization indexes. Different results were obtained by Makino et al. (2022), as all strains were negative for P solubilization. The production of IAA in the presence of L-tryptophan was observed for all PGPB, with several strains producing high amounts (>150 mg/L) of

this phytohormone, which may promote root elongation (Etesami and Glick, 2024). Like terrestrial PGPB, bacterial strains isolated from FTW showed to produce several plant growth-promoting substances. However, further studies are needed to assess its effect on plants and water purification.

3.3. Macrofauna assessment

3.3.1. Macroinvertebrate communities

A total of 245 individuals corresponding to 12 macroinvertebrate taxa (Table 3) were collected: 11 taxa in the pond margin (85 specimens), and 7 taxa in the FTW area (160 specimens).

In the pond margin, the most abundant taxa were the *Ophiogomphus* (31.76 %), *Caenis* (25.88 %), and Chironomidae (22.35 %), while the remaining 8 taxa contributed to 20.0 %. In the FTW the most abundant taxa were *Coenagrion* (84.38 %), *Boyeria irene* (5.63 %), and Hydrophilidae (5.0 %), while the remaining 3 taxa contributed to 4.99 %. Comparison of the associated macroinvertebrate communities among the different treatments indicated that the FTW (mean ± SD) (53.33 ± 13.05) had a higher abundance of individuals compared to the pond margin (28.33 ± 22.55) (Fig. 2). However, concerning the diversity indices, Shannon-Wiener diversity (Welch's *t*-test = 3.77; $p = 0.019$) and Pielou's evenness (Welch's *t*-test = 4.64; $p = 0.020$) were significantly higher in the pond margin (mean ± SD) (Shannon-Wiener diversity: 1.47 ± 0.28; Pielou's evenness: 0.87 ± 0.08) compared to the FTW (Shannon-Wiener diversity: 0.60 ± 0.29; Pielou's evenness: 0.37 ± 0.17) (Fig. 3).

The *Coenagrion* genus of the Odonata order was the taxonomic group with the highest abundance. Despite the higher abundance values of *Coenagrion* associated with the FTW (mean ± SD) (45.33 ± 12.12) compared to the pond margin (0.66 ± 1.15), no significant differences were found in the comparison per treatment (*U* test = 0; $p = 0.072$) (Fig. 4).

The results of the FTW-associated biodiversity indicated that the macrozoobenthic community varied significantly in some aspects compared to the pond margin. The treatment attracted a significantly more diverse and even associated community, while the FTW group exhibited a more abundant macrozoobenthic community dominated by Odonata larvae of the genus *Coenagrion*.

In the present study, an increase in abundance and diversity of the FTW-associated community was expected, but only an increase in abundance was observed. This increase is closely associated with the higher abundance of *Coenagrion* larvae, suggesting that the structure provided by FTW may have helped to attract *Coenagrion* larvae in some way. Aquatic macrophytes are generally associated with a reduction in predation and a decreased likelihood of detection by potential predators of Odonata larvae (Andersson, 2006). In this case, it is possible that the plants colonizing the FTW played a role in attracting *Coenagrion* larvae. Previous studies have already suggested a potential association between some of the selected plants used in the FTW, such as *T. latifolia* and *C. palustris*, and Odonate specimens of the genus *Coenagrion* (Bouwman et al., 2008; Fidalgo, 2018; Martens, 2000). In previous studies, it was also observed that *Coenagrion* larvae prefer microhabitats near the water surface (Johansson, 2000), as provided by FTW. These microhabitats are associated with lower predation and competition, as well as lower temperatures compared to surrounding areas (Johansson, 2000). The structure provided by FTW is thus probably used primarily to avoid predators and competitors, to regulate temperature, and possibly for feeding purposes. However, detailed studies on the influence of FTW structure (e.g. plant species, plant roots, and FTW materials) on *Coenagrion* larval attraction should be conducted in order to provide more comprehensive and definitive conclusions.

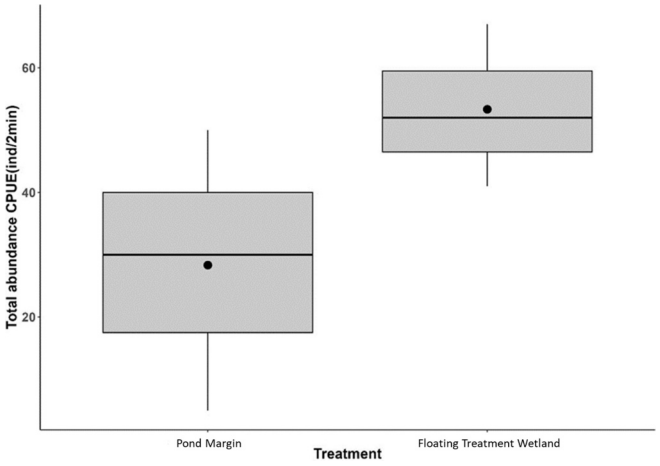


Fig. 2. Box plot of the total abundance of macroinvertebrates associated with the pond margin and floating treatment wetland. Total abundance is set as individuals captured per unit effort (CPUE) using hand-net trawls for two minutes (individuals/2 min). The intervals represent the standard deviation, the black line represents the median, and the black dot is the mean.

Table 3
Total abundance (mean ± SD) of the macroinvertebrates taxa recorded associated with the pond margin and the floating treatment wetland (FTW).

Taxonomic group Arthropoda (Phylum) Insecta (Class)	Pond margin total abundance (mean ± SD)	FTW total abundance (mean ± SD)
Ephemeroptera (Order)		
Baetidae (Family)	2.00 ± 2.00	not found
Caenis (Genus)	7.33 ± 6.43	not found
Odonata (Order)		
Boyeria (Genus) (*Boyeria irene*)	not found	3.00 ± 2.65
Coenagrion (Genus)	0.67 ± 1.15	45.00 ± 12.12
Ophiogomphus (Genus)	9.00 ± 9.17	1.33 ± 0.58
Hemiptera (Order)		
Hemiptera sp.	0.33 ± 0.58	0.66 ± 0.58
Corixidae (Family)	0.33 ± 0.58	not found
Vellidae (Family)	0.66 ± 0.58	not found
Diptera (Order)		
Ceratopogonidae (Family)	0.33 ± 0.58	not found
Athericidae (Family) (*Atrichops crassipes*)	0.33 ± 0.58	0.33 ± 0.58
Chironomidae (Family)	6.33 ± 8.50	0.33 ± 0.58
Coleoptera (Order)		
Hydrophilidae (Family)	1.00 ± 1.00	2.66 ± 2.52

3.3.2. Odonata associated to the floating treatment wetland

Odonata are considered a flagship group in freshwater conservation, being often used as cost-effective biological indicators of habitat integrity and condition. This is because insects in general, have a rapid response to environmental stress compared to most other taxonomic groups. Odonata are important predators in both aquatic and terrestrial food webs, displaying considerable taxonomic diversity with various species needing distinct environmental conditions. Their life cycles, though short, are complex, spanning the aquatic-terrestrial boundary, enabling them to swiftly adapt to changes in both aquatic and nearby terrestrial environments (Manu et al., 2023; Vilenica et al., 2024).

The FTW hosted at least 10 different Odonata species, belonging to 4 families, based on systematic observation (Table 4, Fig. 5). Although none of these species is considered threatened under the IUCC red list (IUCN, 2023), is of great importance to preserve the habitat and prevent further degradation. Huikkonen et al. (2020) studied 20 constructed agricultural wetlands in Central Finland accounting altogether for 17 Odonata species. Further on, Odonata abundance was positively associated with increasing aquatic vegetation diversity and growth forms in the wetlands.

The Odonata (Dragonflies and Damselflies) have three

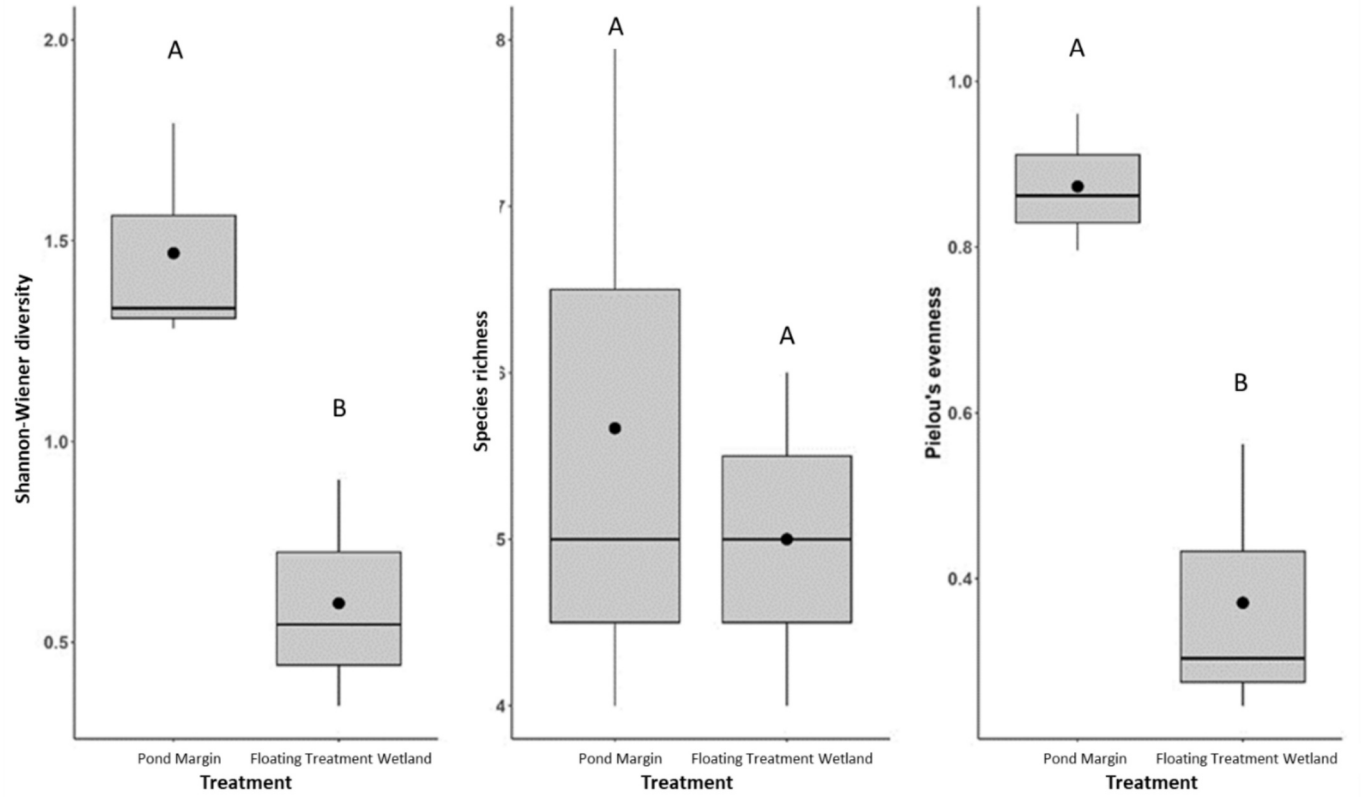


Fig. 3. Box plot of the Shannon-Wiener diversity, species richness, and Pielou's evenness of the macroinvertebrates community associated to the pond margin and floating treatment wetland. The intervals represent the standard deviation, the black line represents the median, and the black dot is the mean. Different letters (A, B) indicate significant differences among treatments (Welsh's *t*-test; $p < 0.05$).

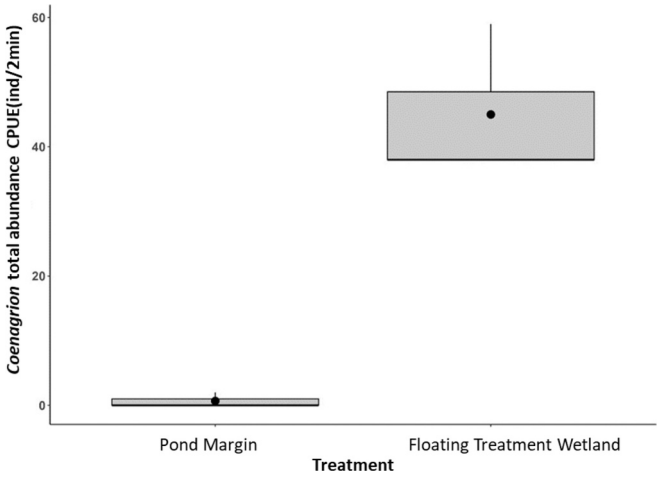


Fig. 4. Box plot of the total abundance of *Coenagrion* associated with the pond margin and floating treatment wetland. Total abundance is set as individuals captured per unit effort (CPUE) using hand-net trawls for two minutes (individuals/2 min). The intervals represent the standard deviation, the black line represents the median, and the black dot is the mean.

developmental stages: the egg, larva, and adult, being hemimetabolous, which means that they have external wing pads, aquatic larvae, and terrestrial adults (Abbott, 2009). Through systematic observation and identification of adult Odonata organisms, it was possible to conclude that they use the FTW as support for the complete life cycle (Supplementary material S4): laying the eggs, eggs laid associated to the root system, nymph (larvae), molting, and female and male mating forming a wheel. Besides that, in supplementary material S5, it is shown an aquatic larva, called naiad or nymph, that was collected from the FTW rooting system; two adult insects in copula, mating in the"wheel position", on the FTW plant leaves; and the exuvia, that results from the Odonata

Table 4
Odonata classification of the 10 species associated to the floating treatment wetland, according to the International Union for Conservation of Nature (IUCN) Red List status of threatened species (IUCN, 2023).

Family (common name)	**Species** (common name)	**Status of threatened species**	**Population trend**
Libellulidae (Dragonflies)	*Crocothemis erythraea* (Scarlet dragonfly)	Least concern	Increasing
	Libellula depressa (Broad-Bodied Chase)	Least concern	Stable
	Orthetrum coerulescens (Keeled Skimmer)	Least concern	Stable
	Sympetrum striolatum (Common Darter)	Least concern	Unknown
Aeshnidae (Dragonflies)	*Anax imperator* (Blue Emperor)	Least concern	Stable
Coenagrionidae (Damselflies)	*Ceriagrion tenellum* (Small Red Damsel)	Least concern	Decreasing
	Ischnura graellsii (Iberian Bluetail)	Least concern	Unknown
	Pyrrhosoma nymphula (Large Red Damsel)	Least concern	Stable
Calopterygidae (Damselflies)	*Calopteryx haemorrhoidalis* (Copper Demoiselle)	Least concern	Unknown
	Calopteryx virgo (Copper Demoiselle)	Least concern	Stable

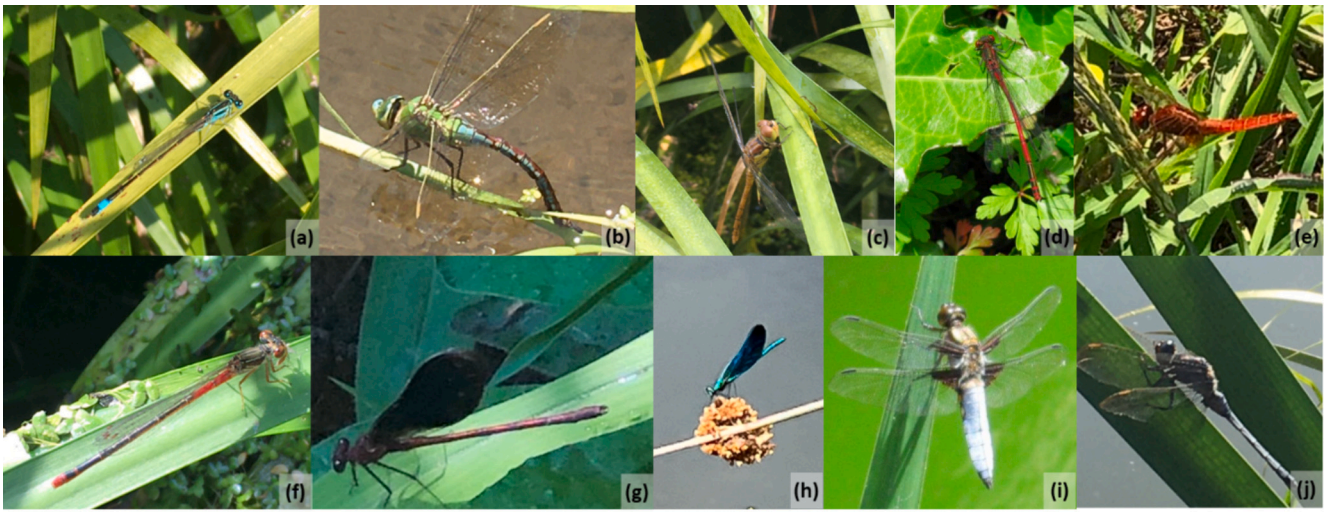


Fig. 5. Odonata species observed at the floating treatment wetland vegetation: (a) *Ischnura graellsii*, (b) *Anax imperator*, (c) *Sympetrum striolatum*, (d) *Pyrrhosoma nymphula*, (e) *Crocothemis erythraea*, (f) *Ceriagrion tenellum*, (g) *Calopteryx haemorrhoidalis*, (h) *Calopteryx virgo*, (i) *Libellula depressa*, (j) *Orthetrum coerulescens.*

action of liberate the larval exoskeleton in order to grow in a process called molting.

3.3.3. *Aquatic and terrestrial fauna*

Floating wetland islands are expected to support populations of aquatic organisms by providing a platform for the growth and maintenance of aquatic fauna (de Moraes et al., 2023). They provide benefits such as shelter, shade, and food, thus maintaining the diversity of fauna (e.g., invertebrates, fishes, amphibians, reptiles, and birds) (Calheiros et al., 2023; Karstens et al., 2021). Organisms observed in the FTW under study comprised amphibians, like *Pelophylax perezi*, reptiles like *Natrix maura,* and several arthropods, specifically spiders. Calheiros et al. (2023) have summarized the main species and groups associated with FTW established in different environments, covering reptiles, birds, fishes, crustaceans, mollusks, bivalves, annelids, insects, and amphibians. Wang et al. (2015) mentioned 8 classes of organisms inhabiting, foraging, breeding, nursing, or resting in the FTW, based on systematic observation. In the present study, 9 families of organisms were identified to be associated with the FTW, with different species of individuals, being mainly from amphibia, reptilia, and insect classes.

As an outcome of this research is important to highlight the role of the FTW not just as a phytoremediation and bioremediation tool but also as an ecosystem service provider, as a nature-based solution, in terms of biodiversity promotion, habitat creation, conservation, and restoration. Is thus pivotal to assess the best available strategies and solutions to provide the restoration and regeneration of ecosystems being in alignment with the Nature Restoration Law framework (European Union Parliament, 2024).

4. Conclusions

Our study demonstrated that FTW harbors a diverse array of bacterial genera associated with the platform and the rhizosphere of macrophytes. These bacteria exhibit plant growth-promoting traits, highlighting their potential for use as inoculants in future phytoremediation strategies. Moreover, FTW efficiently enhanced local biodiversity by increasing the abundance of the associated macrozoobenthic community in the selected area. Of particular interest the FTW supports the entire life cycle of common odonate species, considered important freshwater bioindicators with crucial roles as predators in the food web. The results suggest that FTW probably influences the associated macrozoobenthic community by providing refuge from predators, reducing competition, regulating local temperature, and providing food. The FTW-associated area exhibited a more abundant macrozoobenthic community, dominated by larvae of the Odonata genus *Coenagrion*, in comparison with the pond margin.

It is of primary importance, that besides the well-known phytoremediation capacity of the FTW, they can play a pivotal role in maintaining fauna and flora diversity, especially in this case, macroinvertebrate diversity, and supporting the Odonata lifecycle. Having that in consideration, this research brings important outcomes and insights that can contribute to support and widspread the use of FTW, in particular towards the Nature Restoration Law implementation. Therefore, current knowledge on designing and managing FTW to enhance and promote biodiversity envisioning regenerative and restorative action plans, and improving water quality, is critically important.

CRediT authorship contribution statement

Cristina S.C. Calheiros: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Martina I. Ilarri:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Mariana Godinho:** Investigation, Data curation. **Paula M.L. Castro:** Writing – review & editing, Funding acquisition. **Sofia I.A. Pereira:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgments

This research was supported by the National Funds from FCT-Fundação para Ciência e Tecnologia within the scope of projects UIDB/04423/2020, UIDP/04423/2020, LA/P/0101/2020 and UIDB/50016/2020 projects. MII acknowledges support from FCT through the research

contract (DL57/2016/CP1344/CT0018).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2025.107598.

References

Abbott, J.C., 2009. Odonata (Dragonflies and Damselflies). In: Invertebrates. Elsevier Inc., pp. 394–404. https://doi.org/10.2307/j.ctv1xx9cf2.29

Amorim, 2018. Technical bulletin Cork Floating Island®. ACC.851.

Andersson, D., 2006. Effect of the Diversity, Ecology and Composition of Species of Fish on the Odonate Community. Master Thesis in Applied Ecology. Halmstad University.

Arivukkarasu, D., Sathyanathan, R., 2023. Floating wetland treatment an ecological approach for the treatment of water and wastewater – A review. Materials Today: Proceedings 77 (Part 1), 176–181. https://doi.org/10.1016/j.matpr.2022.11.191. ISSN 2214-7853.

Barco, A., Bona, S., Borin, M., 2021. Plant species for floating treatment wetlands: a decade of experiments in North Italy. Sci. Total Environ. 751, 141666. https://doi.org/10.1016/j.scitotenv.2020.141666.

Basu, A., Prasad, P., Das, S.N., Kalam, S., Sayyed, R.Z., Reddy, M.S., El Enshasy, H., 2021. Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. Sustain 13, 1–20. https://doi.org/10.3390/su13031140.

Borne, K.E., Fassman, E.A., Tanner, C.C., 2013. Floating treatment wetland retrofit to improve stormwater pond performance for suspended solids, copper and zinc. Ecol. Eng. 54, 173–182. https://doi.org/10.1016/j.ecoleng.2013.01.031.

Bouwer, N., Connuck, H., Dubniczki, H., Gownaris, N., Howard, A., Olmsted, C., Wetzel, D., Whittinghill, K., Wilson, A., Zallek, T., 2024. Ecology for All!. Licensed CC BY-NC-SA 4.0. Assessed at: 2024/10/04. Available at: https://bio.libretexts.org/Courses/Gettysburg_College/01%3A_Ecology_for_All.

Bouwman, J., Van Bosgroepen, U., Ketelaar, R., 2008. New records of *Coenagrion armatum* in Schleswig-Holstein (Odonata: Coenagrionidae). Libellula 27, 185–190.

Calheiros, C.S.C., Teixeira, A., Pires, C., Franco, A.R., Duque, A.F., Crispim, L.F.C., Moura, S.C., Castro, P.M.L., 2010. Bacterial community dynamics in horizontal flow constructed wetlands with different plants for high salinity industrial wastewater polishing. Water Res. 44, 5032–5038. https://doi.org/10.1016/j.watres.2010.07.017.

Calheiros, C.S.C., Barreira, E., Ramos, N.M.M., Castro, P.M.L., 2017. Water masses surface temperatures assessment and their effect on surrounding environment. Water Sci. Technol. 75 (12), 2916–2925. https://doi.org/10.2166/wst.2017.146.

Calheiros, C.S.C., Bessa, V.S., Mesquita, R.B.R., Brix, H., Rangel, A.O.S.S., Castro, P.M.L., 2015. Constructed wetland with a polyculture of ornamental plants for wastewater treatment at a rural tourism facility. Ecological Engineering 79, 1–7. https://doi.org/10.1016/j.ecoleng.2015.03.001.

Calheiros, C.S.C., Carecho, J., Tomasino, M.P., Almeida, C.M.R., Mucha, A.P., 2020. Floating wetland islands implementation and biodiversity assessment in a port marina. Water 12. https://doi.org/10.3390/w12113273.

Calheiros, C.S.C., Ilarri, M., Pereira, S.I.A., 2023. Biodiversity associated to floating Wetland Islands. In: Xu, H. (Ed.), Proceedings of the 5th International Symposium on Water Resource and Environmental Management, Environmental Science and Engineering. Springer Nature AG 2023, pp. 163–174. https://doi.org/10.1007/978-3-031-31289-2_13.

Colares, G.S., Dell'Osbel, N., Wiesel, P.G., Oliveira, G.A., Lemos, P.H.Z., da Silva, F.P., Lutterbeck, C.A., Kist, L.T., Machado, Ê.L., 2020. Floating treatment wetlands: a review and bibliometric analysis. Sci. Total Environ. 714, 136776. https://doi.org/10.1016/j.scitotenv.2020.136776.

de Moraes, K.R., Souza, A.T., Muška, M., Hladík, M., Čtvrtlíková, M., Draštík, V., Kolařík, T., Kučerová, A., Krolová, M., Sajdlová, Z., Šmejkal, M., Kubečka, J., 2023. Artificial floating islands: a promising tool to support juvenile fish in lacustrine systems. Hydrobiologia 850, 1969–1984. https://doi.org/10.1007/s10750-023-05204-8.

Demarco, C.F., Quadro, M.S., Selau Carlos, F., Pieniz, S., Morselli, L.B.G.A., Andreazza, R., 2023. Bioremediation of aquatic environments contaminated with heavy metals: a review of mechanisms, solutions and perspectives. Sustain 15. https://doi.org/10.3390/su15021411.

Demertzi, M., Paulo, J.A., Faias, S.P., Arroja, L., Dias, A.C., 2018. Evaluating the carbon footprint of the cork sector with a dynamic approach including biogenic carbon flows. Int. J. Life Cycle Assess. 23, 1448–1459. https://doi.org/10.1007/s11367-017-1406-8.

Etesami, H., Glick, B.R., 2024. Bacterial indole-3-acetic acid: a key regulator for plant growth, plant-microbe interactions, and agricultural adaptive resilience. Microbiol. Res. 281, 127602. https://doi.org/10.1016/j.micres.2024.127602.

European Union Parliament, 2024. REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on nature restoration and amending Regulation (EU) 2022/869 2024.

Fahid, M., Ali, S., Shabir, G., Ahmad, S.R., Yasmeen, T., Afzal, M., Arslan, M., Hussain, A., Hashem, A., Allah, E.F.A., Alyemeni, M.N., Ahmad, P., 2020. *Cyperus laevigatus* L. Enhances diesel oil remediation in synergism with bacterial inoculation in floating treatment wetlands. Sustain 12. https://doi.org/10.3390/su12062353.

Fidalgo, M.Á., 2018. Nuevos registros de *Coenagrion caerulescens* (Fonscolombe, 1838) en Asturias (norte de España) (Odonata: Coenagrionidae). BVnPC 7, 70.

Fox, J., Weisberg, S., 2011. An R Companion to Applied Regression, 2nd ed.

Gordon, S.A., Weber, R.P., 1951. Colorimetric estimation of indoleacetic acid. Plant Physiol. 26, 192–195. https://doi.org/10.1104/pp.26.1.192.

Huikkonen, I.M., Helle, I., Elo, M., 2020. Heterogenic aquatic vegetation promotes abundance and species richness of Odonata (Insecta) in constructed agricultural wetlands. Insect Conserv. Divers. 13, 374–383. https://doi.org/10.1111/icad.12396.

IUCN (International Union for Conservation of Nature), 2023. The IUCN Red List of Threatened Species. Version 2023–1. https://www.iucnredlist.org.

Johansson, F., 2000. The slow-fast life style characteristics in a suite of six species of odonate larvae. Freshw. Biol. 43, 149–159. https://doi.org/10.1046/j.1365-2427.2000.00532.x.

Karstens, S., Langer, M., Nyunoya, H., Čaraitė, I., Stybel, N., Razinkovas-Baziukas, A., Bochert, R., 2021. Constructed floating wetlands made of natural materials as habitats in eutrophicated coastal lagoons in the Southern Baltic Sea. J. Coast. Conserv. 25. https://doi.org/10.1007/s11852-021-00826-3.

Khudhair, N., Yan, C., Liu, M., Yu, H., 2019. Effects of habitat types on macroinvertebrates assemblages structure: case study of Sun Island bund wetland. Biomed. Res. Int. 2650678. https://doi.org/10.1155/2019/2650678.

Makino, A., Nakai, R., Yoneda, Y., Toyama, T., Tanaka, Y., Meng, X.Y., Mori, K., Ike, M., Morikawa, M., Kamagata, Y., Tamaki, H., 2022. Isolation of aquatic plant growth-promoting bacteria for the floating plant duckweed (*Lemna minor*). Microorganisms 10. https://doi.org/10.3390/microorganisms10081564.

Manu, M.K., Ashiagbor, G., Seidu, I., Groen, T., Gyimah, T., Toxopeus, B., 2023. Odonata as bioindicator for monitoring anthropogenic disturbance of Owabi wetland sanctuary, Ghana. Aquat. Insects 44, 151–169. https://doi.org/10.1080/01650424.2022.2108844.

Martens, A., 2000. Group Oviposition in *Coenagrion mercuriale* (Charpenter) (Zygoptera: Coenagrionidae). Odonatologica 29, 329–332.

Nautiyal, C.S., 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol. Lett. 170, 265–270. https://doi.org/10.1016/S0378-1097(98)00555-2.

Nawaz, N., Ali, S., Shabir, G., Rizwan, M., Shakoor, M.B., Shahid, M.J., Afzal, M., Arslan, M., Hashem, A., Abd_Allah, E.F., Alyemeni, M.N., Ahmad, P., 2020. Bacterial augmented floating treatment wetlands for efficient treatment of synthetic textile dye wastewater. Sustain 12, 1–17. https://doi.org/10.3390/su12093731.

Oksanen, J., Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P., O'Hara, R., Simpson, G., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., 2019. vegan: Community Ecology Package (2.5-6).

Pereira, S.I.A., Castro, P.M.L., 2014. Diversity and characterization of culturable bacterial endophytes from *Zea mays* and their potential as plant growth-promoting agents in metal-degraded soils. Environ. Sci. Pollut. Res. 21, 14110–14123. https://doi.org/10.1007/s11356-014-3309-6.

Prashant, Billore, S.K., 2020. Macroinvertebrates associated with artificial floating islands installed in River Kshipra for water quality improvement. Water Sci. Technol. 81, 1242–1249. https://doi.org/10.2166/wst.2020.219.

Rehman, K., Imran, A., Amin, I., Afzal, M., 2018. Inoculation with bacteria in floating treatment wetlands positively modulates the phytoremediation of oil field wastewater. J. Hazard. Mater. 349, 242–251. https://doi.org/10.1016/j.jhazmat.2018.02.013.

Salmon, Q., Colas, F., Westrelin, S., Dublon, J., Baudoin, J.M., 2022. Floating Littoral Zone (FLOLIZ): a solution to sustain macroinvertebrate communities in regulated lakes? Ecol. Eng. 176, 106509. https://doi.org/10.1016/j.ecoleng.2021.106509.

Schwyn, B., Neilands, J.B., 1987. Universal assay for the detection and determination of siderophores. Anal. Biochem. 160, 47–56.

Sharma, R., Vymazal, J., Malaviya, P., 2021. Application of floating treatment wetlands for stormwater runoff: a critical review of the recent developments with emphasis on heavy metals and nutrient removal. Sci. Total Environ. 777, 146044. https://doi.org/10.1016/j.scitotenv.2021.146044.

Srivastava, J.K., Chandra, H., Kalra, S.J.S., Mishra, P., Khan, H., Yadav, P., 2017. Plant–microbe interaction in aquatic system and their role in the management of water quality: a review. Appl Water Sci 7, 1079–1090. https://doi.org/10.1007/s13201-016-0415-2.

Tachet, H., Richoux, P., Bournaud, M., Usseglio-Polatera, P., 2000. Invertébrés d'eau douce : systématique, biologie, écologie. In: Bulletin Mensuel de La Société Linnéenne de Lyon, 71^{e} Année, N°3, Mars 2002, p. 99.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol. Biol. Evol. 38, 3022–3027. https://doi.org/10.1093/molbev/msab120.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680. https://doi.org/10.1093/nar/22.22.4673.

Timofeeva, A.M., Galyamova, M.R., Sedykh, S.E., 2022. Bacterial siderophores: classification, biosynthesis, perspectives of use in agriculture. Plants 11. https://doi.org/10.3390/plants11223065.

Tusief, Q., Mohsin, M., Hussan Malik, M., Asghar, H.N., Sardar, S., 2022. Textile wastewater treatment through plant–microbe synergism in a floating treatment wetland system. AATCC J. Res. 9, 182–193. https://doi.org/10.1177/24723444221103679.

Urakawa, H., Dettmar, D.L., Thomas, S., 2017. The uniqueness and biogeochemical cycling of plant root microbial communities in a floating treatment wetland. Ecol. Eng. 108, 573–580. https://doi.org/10.1016/j.ecoleng.2017.06.066.

Vilenica, M., Brigić, A., Štih Koren, A., Koren, T., Sertić Perić, M., Schmidt, B., Bužan, T., Gottstein, S., 2024. Odonata assemblages in urban semi-natural wetlands. Insects 15, 1–18. https://doi.org/10.3390/insects15030207.

Vo, T., Vo, T., Ntagia, E., Amulya, K., Nguyen, N., Tran, P., Ninh, N., Le, S., Le, L., Cong-Sac Tran, The-Luong Ha, Mai-Duy-Thong Pham, Xuan-Thanh Bui, Piet, Lens N.L., 2023. Pilot and full scale applications of floating treatment wetlands for treating diffuse pollution. Sci. Total Environ. 899, 165595. https://doi.org/10.1016/j.scitotenv.2023.165595.

Wang, C.Y., Sample, D.J., Day, S.D., Grizzard, T.J., 2015. Floating treatment wetland nutrient removal through vegetation harvest and observations from a field study. Ecol. Eng. 78, 15–26. https://doi.org/10.1016/j.ecoleng.2014.05.018.

Winston, R.J., Hunt, W.F., Kennedy, S.G., Merriman, L.S., Chandler, J., Brown, D., 2013. Evaluation of floating treatment wetlands as retrofits to existing stormwater retention ponds. Ecol. Eng. 54, 254–265. https://doi.org/10.1016/j.ecoleng.2013.01.023.

Yamakawa, Y., Jog, R., Morikawa, M., 2018. Effects of co-inoculation of two different plant growth-promoting bacteria on duckweed. Plant Growth Regul. 86, 287–296. https://doi.org/10.1007/s10725-018-0428-y.

Yeh, N., Yeh, P., Chang, Yuan-Hsiou, 2015. Artificial floating islands for environmental improvement. Renew. Sustain. Energy Rev. 47, 616–622. https://doi.org/10.1016/j.rser.2015.03.090. ISSN 1364-0321.