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Stormwater treatment in constrained urban spaces through a hybrid Sequential Sedimentation Biofiltration System

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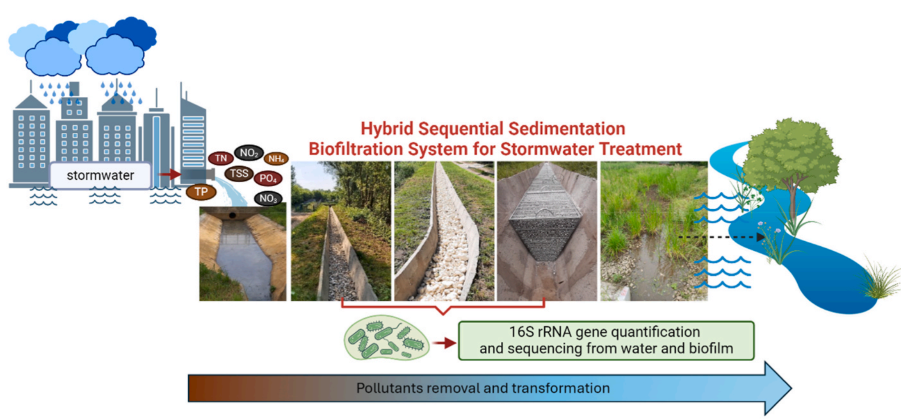
HIGHLIGHTS

- A hybrid SSBS was implemented for stormwater treatment within an urban area.
- The system effectively removed TSS, N, and P despite space constraints.
- Permeable Reactive Barriers maintained their efficiency after 110 days of operation.
- Biofilms with potential nutrient-cycling bacteria developed on sorption materials.

GRAPHICAL ABSTRACT



ABSTRACT

Urban areas face increasing pressures on water resources, necessitating innovative approaches to climate adaptation and water quality management. Nature-based Solutions (NbS) offer a sustainable pathway, yet their integration with existing infrastructure in urban settings remains occasional. This study presents a novel hybrid system—Sequential Sedimentation Biofiltration System (SSBS)—designed for stormwater treatment within confined urban spaces. The system was adjusted to the existing stormwater infrastructure by integrating a sedimentation tank (SED), three Permeable Reactive Barriers (PRBs), and a biofiltration zone (BIO). The SSBS was evaluated for its efficiency in removing nutrients and sediments, focusing on the performance of PRBs. Our findings showed limited sediment removal in SED and PRBs due to spatial constraints and a high Hydraulic Loading Rate (HLR = 1.31 m/d), achieving an average of 13.6% Total Suspended Solids (TSS) removal. However, PRBs demonstrated effective removal of ammonium (43.4%) and phosphate (59.3%), potentially due to sorption and biofilm activity, with dominant microbial communities including Proteobacteria, Bacteroidetes, and nutrient-transforming taxa such as Nitrospirae. Interestingly, PRBs increased nitrite levels (57.1%) but did not significantly impact nitrate, chloride, or TSS. The BIO zone further enhanced nutrient retention (56% PO_4–P) and served as a sink for TSS (52%). This study underscores the potential for integrating traditional urban infrastructure with NbS in a sequential stormwater treatment system, demonstrating its effectiveness in space-constrained urban environments.

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1. Introduction

Climate change, along with more intense and variable precipitation patterns, represents a significant threat to urban water resources and exacerbates the pollution-related issues (Whitehead et al., 2009). At the global level, cities are becoming more vulnerable as a result of unsustainable development, the expansion of impermeable surfaces, and the deterioration of urban green spaces (Willems et al., 2012; Zalewski et al., 2021; Zhou et al., 2019). In particular, urban drainage systems are not equipped to handle prolonged periods of water scarcity and unpredictable flooding events causing the direct release of polluted waters into receiving aquatic systems (e.g., rivers, lakes, reservoirs), which serve as fundamental water supplies and recreational areas (Gogien et al., 2023; He et al., 2021).

Untreated runoff and stormwater contain high loads of chemical and microbiological contaminants (e.g., nutrients, heavy metals, pesticides, microplastics, fecal bacteria) (Kang et al., 2024; Khan et al., 2021; Lange et al., 2023), which affect the environmental quality of urban waters and sediments (Chen et al., 2019; Simpson et al., 2022). Increased pollution levels and temperatures can promote eutrophication and harmful algal blooms, directly impacting biodiversity, local communities, and economies (Heil and Muni-Morgan, 2021).

Traditional stormwater infrastructure, such as sedimentation tanks, is widely used in urban drainage systems to remove Total Suspended Solids (TSS). However, their performance is highly variable, depending on factors like particle size and rainfall characteristics (Falco et al., 2020). Additionally, sedimentation tanks often exhibit limited efficiency in removing dissolved nutrients, particularly nitrogen and phosphorus compounds, and may even become sources of nutrient release from accumulated sediments (Jurczak et al., 2018; Tondera et al., 2018). As the element of new approach for stormwater management, Nature-based Solutions (NbS) have emerged as a promising alternative to traditional systems, offering the potential to mitigate hydrometeorological and pollution risks in urban areas (Biswal et al., 2022; Moghadam et al., 2023; Zalewski et al., 2021). Examples include Constructed Wetlands (Masi et al., 2018), biofiltration and bioretention systems (Betz et al., 2023; Moghadam et al., 2023), and Sequential Sedimentation Biofiltration Systems (SSBS) (Jurczak et al., 2018; Szklarek et al., 2018). These NbS not only enhance the removal of both solid and dissolved pollutants but also provide additional ecosystem services, such as supporting biodiversity. However, their adoption in urban areas remains limited due to uncertainties around their long-term performance and integration with existing infrastructure.

The efficiency of NbS in treating stormwater depends on the effective immobilization and transformation of pollutants, which are influenced by factors such as adsorbing materials (Okaikue-Woodi et al., 2020), the activity of microbial communities (Font-Nájera et al., 2020), and the hydraulic loading regime (Tanner et al., 1998). Larger surface areas and extended water residence times are known to improve treatment efficacy by promoting microbial growth and bioremediation processes (Font-Nájera et al., 2020; Rahman et al., 2019; Stefanakis and Tsihrintzis, 2012). Yet, the integration of NbS in dense urban environments is challenging due to space constraints and the need to adapt to specific local conditions (Jarosiewicz et al., 2022; Walaszek et al., 2018).

Despite the increasing focus on NbS, significant research gaps persist. One key area is understanding how to retrofit these systems into existing stormwater infrastructure, especially in space-limited urban settings. Questions remain about how system maturation affects performance over time and how different configurations can optimize pollutant removal.

A particularly promising approach is the use of Sequential Sedimentation Biofiltration Systems (SSBS). This technology integrates a sequence of processes—including sedimentation, filtration, and adsorption through Permeable Reactive Barriers (PRBs) coupled with biofiltration—to achieve high treatment efficiency (Jurczak et al., 2018; Szklarek et al., 2018; Zalewski et al., 2012). SSBS is notable for its flexibility, allowing it to be retrofitted into existing stormwater retention ponds (Szklarek et al., 2018), which are often limited in their capacity to remove nutrients (Yang and Lusk, 2018). However, further research is required to optimize NbS design, scalability (Na Nagara et al., 2024; Tansar et al., 2024), and, importantly, to demonstrate the advantages of sequential treatment processes over conventional methods (Jarosiewicz et al., 2022).

The objective of this study was to assess the performance of a novel hybrid SSBS integrated into an existing urban stormwater infrastructure. Specifically, we aimed to (i) quantify the removal efficiency of suspended solids and nutrients across different system components (sedimentation tank, PRBs, and biofiltration zone), and (ii) examine the development and composition of microbial communities within the PRBs. We hypothesize that the sequential treatment process will significantly enhance stormwater quality, offering effective nutrient removal driven by microbial biofilms and providing a potential model for scalable urban stormwater management.

2. Materials and methods

2.1. Study site and the hybrid sequential sedimentation biofiltration system

The hybrid SSBS was prototyped in Radom (51.385109, 21.115966), within the Mleczna River catchment area (Fig. 1), on an existing channel that previously discharges polluted stormwater directly into the river. This was implemented as a novel approach to improving water quality in river and reservoir ecosystems, aimed at reducing nutrients responsible for eutrophication (Fig. 1). The system is part of city-scale, climate change adaptation concept assigned to the Global Network of Ecohydrology Demonstration Sites of UNESCO's Intergovernmental Hydrological Program.

The urban stormwater is supplied by an underground channel (A0 - pipe diameter = 1800 mm) that collects the runoff from an urban area of 371 ha. Uptake is by gravity at the dammed part of the channel, through a steel pipeline that directs the water to the stabilizing tank at the maximum ratio of approximately 0.2 m^3/s. There is the possibility of regulating the gravity flow from A0 by closing the valve. Later, the stormwater is pumped to the expansion chamber with deflector and horizontal sedimentation function, followed by gravity flow into a pre-existing open-air sedimentation tank (ca. 30 m^3) and concrete channel (length = 131 m, width = 0.6 m, slope = 1.12 ‰). To accommodate limited space for water treatment and meet hydrological load demands, the operation was restricted to one of four available pumps, resulting in a hydraulic loading rate (HLR) of 1.31 m/d. A single pump, with an efficiency of 0.043 m^3/s, was activated when the stabilizing tank reached a specific level. Initially, the gravity-fed water from A0 was regulated with the valve, limiting pumping to 5 min followed by a 15-min resting period, yielding an average discharge of 930 m^3/d. Flow in the A0 pipe was observed throughout the study period, including dry episodes, possibly due to groundwater drainage, though this was not field-verified. This pumping station was designed to supply treated stormwater, following sedimentation, to the River Mleczna and its downstream recreational reservoir, compensating for evapotranspiration, infiltration and outflow losses during summer months and stabilizing the water level in this reservoir.

To create the sequence of processes used in the concrete channel, three Permeable Reactive Barriers (PRBs) were constructed with dolomite rock (DOL – 42 m with a fraction of 64 mm), limestone rock (LIM - 42 m with a fraction of 64 mm), and low-mass adsorbent – bioker (BK - 300 kg m^{-3}, 30 m with a fraction of 10–16 mm) (Jarosiewicz et al., 2022). In this study, BK was modified with the addition of calcite and provided in containers adjusted to the shape of the concrete channel (Fig. 1). The PRBs were separated by substrate-free sections (approx. 4.5 m) suitable for water sampling. The materials were selected to initiate treatment with coarser fractions (DOL and LIM) and conclude with finer filtration by BK. The use of different materials in each PRB allowed for comparison and informed recommendations for future applications.

Finally, a sand-based surface-flow wetland (BIO - 600 m^2) was constructed at the river floodplain as a biofiltration zone. This zone was planted with macrophytes including *Typha angustifolia*, *Typha minima*, *Glyceria maxima*, *Carex acutiformis* L., *Acorus calamus* L., *Iris pseudacorus* L., and *Scirpus sylvaticus* L. Overflow into the River Mleczna occurs via side banks of the biofiltration zone, which were reinforced with dolomite rocks.

2.2. System monitoring and sampling

Physical-chemical parameters such as temperature (°C), specific conductance (SPC, mS/cm), dissolved oxygen (mg/L), and pH were monitored on-site using a YSI multiparameter probe (Xylem Inc., Yellow Springs, USA). Water samples (n = 134) were collected during five sampling campaigns conducted in 2022 (June, July, August, September, and October). These campaigns were not linked to specific rainfall events, as this study was intended to evaluate the efficiency of the proposed solution under conditions where the water flow was regulated by pump operation. Samples were collected from the locations specified in Fig. 1. All samples were stored in 1-L PET bottles, refrigerated, and analyzed in the laboratory within 48 h of collection.

2.3. Water chemistry

Water samples (300–1000 ml) were filtered onto 0.45-μm glass fiber filters (Whatman GF/C). Total Suspended Solids (TSS) were determined by weighing dried filters (4h at 105 °C). For the analysis of Particulate Organic Matter (POM), the filters underwent carbonization (loss on ignition method) at 500 °C for 4 h and were then weighed. The determination of total nitrogen and phosphorus (TN, TP) were performed on unfiltered water samples. The quantitative determination of TP was carried out using a modified molybdenum method with ascorbic acid (Murphy and Riley, 1962). Mineralization was conducted using Oxisolve reagent (Merck, Darmstadt, Germany) in a mineralizer (Merck MV 500 Microwave Digestion System). Absorbance measurements were taken with a spectrophotometer (Thermo Scientific BioMate 3S UV/Vis) at a wavelength of 690 nm. TP values in the sample were determined based on a calibration curve. TN was determined using the sulfate mineralization method and the formation of a colored complex with chromotropic acid, with absorbance measured at 410 nm using a spectrophotometer (DRB200, Hach, United States). A ready-to-use reagent kit for TN determination was used according to the method protocol 10071 (available on the Hach company website).

In filtered waters, the content of dissolved ions, including calcium (Ca), chloride (Cl), sodium (Na), sulfate (SO_4), ammonium (NH_4), nitrite

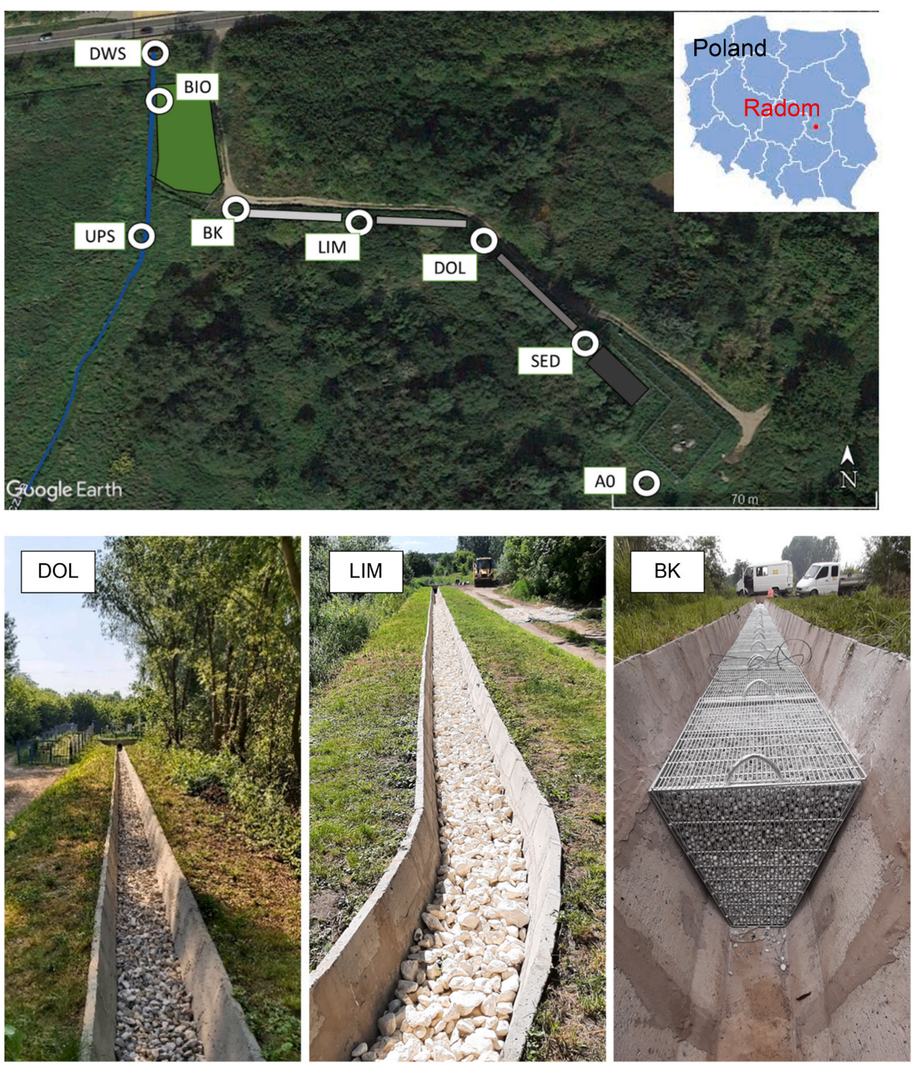

Fig. 1. Study site and the hybrid Sequential Sedimentation Biofiltration System (SSBS) with sampling points. Permeable Reactive Barriers (PRBs) were placed within a pre-existing concrete open channel. A0 – sampling point at the underground channel; SED – sedimentation zone; DOL – dolomite; LIM – limestone; BK – bioker; BIO – biofiltration zone; UPS – upstream; DWS- downstream of River Mleczna.

(NO_2), nitrate (NO_3), and phosphate (PO_4), was determined using liquid ion chromatography (Dionex ISC-1000). Anions were analyzed on a JonPac AS22 2 × 250 mm column, while cations were analyzed on a Dionex JonPac CS16 5 × 250 mm column. The system was equipped with guard columns (CG18 for cations and AG22 for anions) and suppressors CSRS-ULTRA II for cations and ASRS-ULTRA II for anions. The eluents used were 16 mM methanesulfonic acid for cation analysis and a mixture of 4.5 mM sodium carbonate and 1.4 mM sodium bicarbonate for anion analysis. Isocratic elution was carried out at a temperature of 30 °C for anions, and at a temperature of 40 °C for cations. For both columns, the flow rate was 1 ml/min. Calibration standards for anions and cations (DIONEX) were used to establish calibration curves. The detection limit was 1 μg/L and the quantification limit was 10 μg/L for the analyzed anions and cations.

2.4. Microbial community structure

This analysis was performed for water and biofilm samples collected at the three PRBs (DOL, LIM and BK). Water samples were collected in sterile containers directly after each barrier. To study the biofilm, materials were collected in sterile containers transported in cold (4 °C) and dark conditions to the laboratory. Water samples were collected during three consecutive times: July 17th, August 16th and October 10th, while DOL, LIM and BK samples were collected on August 16th and October 10th to allow sufficient time for biofilm formation.

Water samples were filtered (100 mL) through a 0.22 μm mixed cellulose membrane (MCE) to retain the cells, and the filters were subjected to DNA extraction with the specifications from the DNeasy Power Water Kit (Qiagen, USA). Biofilm was scraped from the surface of PRBs materials (DOL, LIM and BK) with a sterile scalp. An aliquot of 500 mg was used for DNA isolation with the specifications from the FastDNA™ Spin Kit for Soil (MP Biomedicals).

The total abundance of bacteria was assessed with the use of qPCR and the primers BAC1369F (CGGTGAATACGTTCYCGG) and PROK1492R (GGWTACCTTGTTACGACTT) targeting a fragment of 123bp (Suzuki et al., 2000), according to the specifications in Mankiewicz-Boczek et al. (2022). A standard curve containing six serial dilutions was prepared from genomic DNA obtained from the bacterial culture of *Pseudomonas mandelii* Str21, a bacterial strain isolated from SSBS sediment and described in our previous research (Font-Nájera et al., 2021a, 2021b). Calculations were standardized to a total genome of 6.7 Mb according to a similar strain of the same species described in the work of Formusa et al. (2014).

Bacterial communities were described by amplicon sequencing of the gene 16S rRNA (region V3–V4) with the primers S-DBact-0341-b-S-17/S-D-Bact-0785-a-A-21 (464 bp) according to Klindworth et al. (2013). Sequencing was performed on an Illumina MiSeq platform (2x250 bp) with an expected amount of 100,000 pair-end reads per sample. Sequence editing, including ASV taxonomical assignation, was performed using the SILVA 138 database in R environment. Original datasets as FASTQ files were uploaded to the NCBI Sequence Read Archive under the project PRJNA1053261. Three water samples were sequenced in July (DOL-WJ = SAMN38851733, LIM-WJ = SAMN38851734, and BK-WJ = SAMN38851735), August (DOL-WA = SAMN38851736, LIM-WA = SAMN38851737, and BK-WA = SAMN38851738), and October (DOL-WO = SAMN38851739, LIM-WO = SAMN38851740, and BK-WO = SAMN38851741). Three biofilm samples were sequenced in August (DOL-BA = SAMN38851742, LIM-BA = SAMN38851743, and BK-BA = SAMN38851744), and October (DOL-BO = SAMN38851745, LIM-BO = SAMN38851746, and BK-BO = SAMN38851747). Data visualization was performed with the phyloseq package.

Additionally, the total microbial load was assessed by flow cytometry (A50-micro, Apogee Flow System, Hertfordshire, England) on formaldehyde-fixed water samples using the fluorochrome SYBR Green I (1:10,000 dilution; Molecular Probes, Invitrogen). By following a harmonized protocol (Amalfitano et al., 2018), light scatter and green fluorescence (530/30 nm) signals were acquired to identify and quantify microbial cells in water samples collected from two sampling campaigns (July, August).

2.5. Statistics

Summary statistics were computed per each parameter, including mean with standard deviation, median, minimum, and maximum values. The normality distribution of variables was evaluated using the Shapiro-Wilk test, with a significance level set at $p < 0.05$. Log-transformation was implemented where required. Student's t-test or the non-parametric Mann-Whitney *U* test were used for group comparison. One-way analysis of variance (ANOVA) at 95% significance level was used to examine the effect of various treatment materials. Post-hoc Tukey test was used to identify significant difference between sample groups. Welch correction was used when the assumption of equal group variance was violated through the Levene's test. Correlation analysis was conducted using Pearson and Spearman tests. Principal Component Analysis (PCA) was applied to analyze the microbiome development on different materials in PRBs. Statistical analyses were performed using the Past program (version 3.20) (Hammer et al., 2001) and Orange Data Mining (version 3.35.0).

3. Results and discussion

3.1. Stormwater quality along the treatment system

The physical-chemical parameters of the studied stormwater remained relatively stable throughout the monitoring period (temperature = 12.4 ± 0.63 °C; pH = 7.74 ± 0.10) (Table S1). This stability can be attributed to groundwater infiltration, which sustained a continuous flow in the A0 pipe, even during periods without precipitation. Along the treatment system, temperature increased gradually, while pH and EC slightly decreased ($p < 0{,}001$) (Table 1). PRBs did not influence pH, despite the use of calcium-based materials with anticipated alkalizing potential.

The concentration of DO was close to saturation levels, because of low temperatures and the turbulent flow at A0. Surprisingly, it did not decrease after the SED, although the anoxic conditions inside the sedimentation tanks can be anticipated (Jurczak et al., 2018). Subsequentially, DO decreased by passing through PRBs, particularly after BK ($p < 0.05$) after 3 months of system operation, indicating the growing oxygen consumption in barriers (Fig. 2). The biofiltration zone significantly increased the oxygen level by 20% ($p < 0.01$). Shallow depth supported development of algae on the bottom, and oxygen levels in the outflow reached the mean value of 10.82 ± 2.34 mg/L.

The maximum nutrient concentrations measured at A0 (PO_4–P = 0.104 mg/L, TP = 0.423 mg/L, NH_4–N = 0.809 mg/L) did not exceed the legal limits in Poland (Regulation of the Minister of Maritime Economy and Inland Navigation, 2019). However, these values suggest the necessity of treating stormwater before discharge into downstream environments, such as urban recreational reservoirs (Table S1). The mean concentrations of TP, NH_4–N, and PO_4–P were similar to those reported elsewhere for stormwater, whereas TSS values were relatively low (Li et al., 2012;Adyel et al., 2017; Khan et al., 2021; McIntyre et al., 2023) likely due to the presence of sedimentation tanks at the entry points of Radom City's drainage system. The average concentration of TSS in the stormwater was 17.3 mg/L, with POM contributing 5.23 mg/L, indicating that the majority of the suspended solids were mineral-based (70%). The SED zone removed only 6% of TSS ($p > 0.05$) and increased POM by 8% ($p > 0.05$), demonstrating limited effectiveness, potentially due to suboptimal design. The PRBs provided an additional 8.5% reduction in TSS, but POM increased by 11.3%, likely from the introduction of allochthonous material through the open channel and biomass detachment from the PRB materials. At the BIO zone, 50.2% of

Table 1
Average values and standard deviations (n = 18) of physical-chemical parameters (T - temperature, pH, EC - electrical conductivity, DO - dissolved oxygen, PO_4–P – phosphates, NO_3–N – nitrates, NO_2–N – nitrites, NH_4–N – ammonium, TP – total phosphorus, TN – total nitrogen, TSS – total suspended solids, POM – particulate organic matter) at the sampling points along the treatment system and in the river upstream and downstream from discharge (UPS and DWS). n.a. – not analyzed.

	T	pH	EC	DO	PO_4–P	NO_3–N	NO_2–N	NH_4–N	TP	TN	TSS	POM
	°C		mS/cm	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
A0	12.4 ± 0.6	7.74 ± 0.10	0.82 ± 0.07	8.70 ± 0.79	0.023 ± 0.027	1.415 ± 0.508	0.008 ± 0.015	0.245 ± 0.198	0.159 ± 0.115	3.7 ± 1.6	17.30 ± 5.60	5.23 ± 2.79
SED	12.8 ± 0.7	7.48 ± 0.13	0.85 ± 0.07	9.28 ± 0.54	0.027 ± 0.034	1.284 ± 0.430	0.014 ± 0.015	0.401 ± 0.182	0.151 ± 0.117	3.6 ± 1.9	16.32 ± 2.59	5.66 ± 2.05
DOL	13.5 ± 2.0	7.48 ± 0.11	0.85 ± 0.08	9.02 ± 0.50	0.017 ± 0.023	1.335 ± 0.448	0.016 ± 0.013	0.332 ± 0.170	0.168 ± 0.142	3.2 ± 1.8	n.a.	n.a.
LIM	13.9 ± 2.2	7.50 ± 0.12	0.84 ± 0.07	8.95 ± 0.56	0.013 ± 0.018	1.335 ± 0.452	0.016 ± 0.012	0.298 ± 0.175	0.204 ± 0.122	3.0 ± 1.3	n.a.	n.a.
BK	14.1 ± 2.3	7.52 ± 0.12	0.84 ± 0.08	8.50 ± 0.67	0.011 ± 0.014	1.400 ± 0.466	0.022 ± 0.010	0.227 ± 0.142	0.129 ± 0.091	3.3 ± 1.2	14.94 ± 2.79	6.30 ± 3.05
BIO[a]	16.7 ± 1.0	7.66 ± 0.13	0.73 ± 0.08	10.82 ± 2.34	0.003 ± 0.001	0.999 ± 0.131	0.008 ± 0.002	0.072 ± 0.035	0.160 ± 0.107	3.5 ± 1.6	7.44 ± 1.89	5.30 ± 2.12
UPS	17.9 ± 3.6	8.02 ± 0.12	0.61 ± 0.02	8.12 ± 0.76	0.027 ± 0.017	0.354 ± 0.050	0.007 ± 0.005	0.030 ± 0.042	0.162 ± 0.102	2.4 ± 1.8	9.63 ± 3.21	4.51 ± 2.97
DWS	17.7 ± 3.4	7.95 ± 0.12	0.64 ± 0.03	8.22 ± 0.73	0.022 ± 0.013	0.445 ± 0.079	0.009 ± 0.004	0.043 ± 0.044	0.179 ± 0.099	2.3 ± 2.2	9.32 ± 2.35	4.37 ± 1.27

[a] Monitoring conducted on August (n = 5) and September (n = 3).

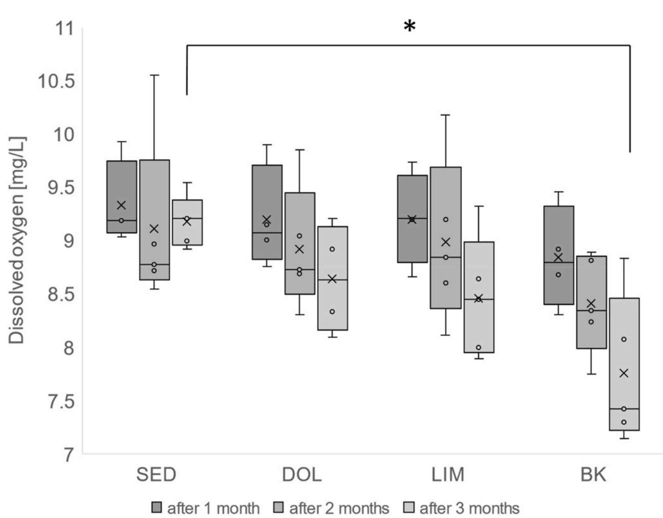

Fig. 2. Oxygen consumption across PRBs at different operation times. (*) Significant differences (ANOVA with Tukey post hoc test; $p < 0,05$) were found between SED and BK zones 3 months from the system start.

TSS and 15.9% of POM were removed ($p < 0.01$) (Fig. 3), highlighting the critical role of the biofiltration process as the final step in the SSBS.

Overall, the implemented SSBS achieved a TSS removal efficiency of 57.0% ($p < 0.01$), while having no significant impact on POM, consistent with other studies where TSS removal rates ranged from 61.4% to 86.0% (Szklarek et al., 2018; Jurczak et al., 2018). Given that HLR is a critical factor for TSS removal (Moghadam et al., 2023), this result can be considered a successful outcome of the filtration process in the PRBs and biofiltration zone, underscoring the importance of the sequential treatment processes. It is important to emphasize that POM removal poses challenges in surface-flow treatment systems, as also observed by Font-Nájera et al. (2021a). We anticipate that this effect will diminish, as vegetation becomes fully established in the BIO zone (Dierberg et al., 2021).

3.2. *Phosphorous removal*

Phosphorus removal from stormwater remains a significant challenge (Adyel et al., 2017; Kill et al., 2022). Therefore, we decided to incorporate an advanced PRBs system using different P-adsorbing materials (DOL, LIM and BK) since the substrate adsorption presents higher efficiency toward P removal compared to the plant uptake and microbial assimilation (Vohla et al., 2011). The positive role of P-sorption

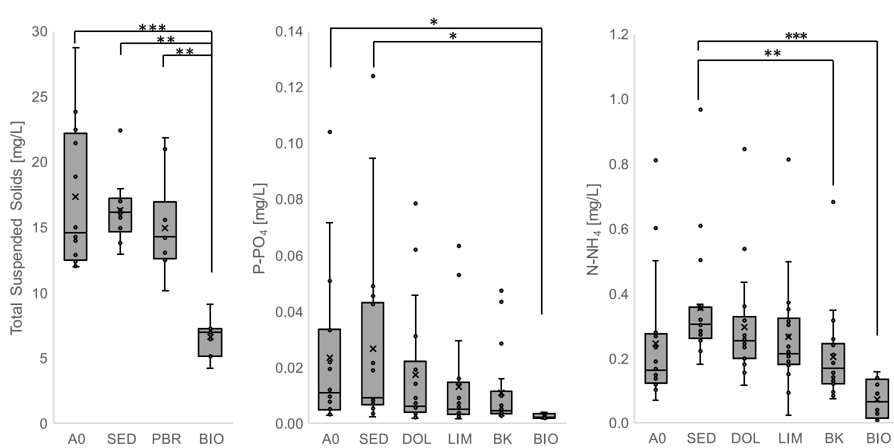

Fig. 3. Distribution of the Total Suspended Solids (TSS), phosphates (PO_4–P), and ammonium (NH_4–N) in the A0 stormwater channel (inlet) and following sampling points after sedimentation tank (SED) and after Permeable Reactive Barriers collectively for TSS (PRB) and separately for ions (DOL, LIM, and BK). Data for biofiltration (BIO) was collected at the outflow to River Mleczna. * for $p < 0.05$; ** for $p < 0,01$; *** for $p < 0.001$.

materials was also confirmed by applying PRBs in different environments (Penn et al., 2016). The effectiveness of sorption materials against dissolved P (mostly phosphates) depends largely on the P concentration (Penn et al., 2016; Sullivan and McDonald, 2022). In this study, stormwater had rather low concentration of PO_4–P (on average 0.023 mg/L). Thus, we assumed that the sorption potential of selected materials was not fully achieved. The highest inflow concentration was 0.104 mg/L, and it was increased to 0.124 mg/L after SED. This effect was observed during the whole study and could be the result of water mixing and anoxic conditions in sediments collected in the sedimentation tank, favoring redox-driven release of P (Christophoridis and Fytianos, 2006). PRBs efficiency at this peak-point reached the level of 65%, evidencing their model of action dependent on the inflow concentration of PO_4–P (Fig. 4). We assumed that with limited urban space and unfavorable HRT for our system, selecting different substrate types may enhance the removal of PO_4–P and create different habitats for microbial communities development, due to the specific surface properties and chemical composition (LeviRam et al., 2023). As demonstrated by Stefanakis and Tsihrintzis (2012), limited contact time may favor microbial consumption and precipitation with calcium, as the dominant removal mechanism, but it depends on the type of adsorbent (Ca-based or Fe-, Al-based) (Scott et al., 2023). Interestingly, we did not observe a significant effect on the removal of TP in the entire system, except for BK which removed, on average, 36.8% of TP ($p < 0.05$). This may be related to the sorption capacity and filtration potential of BK (Jarosiewicz et al., 2022). Overall, all PRBs performed similarly in reducing phosphate concentrations, achieving the average concentration of 0.011 mg/L. While this level may be considered environmentally safe, the final biofiltration zone (BIO) further reduced PO4–P by 56%, bringing the outflow concentration down to an average of 0.003 mg/L. Previous studies, such as Zamorano et al. (2023), noted that a periphyton-based area for stormwater treatment was an effective solution to reduce PO_4–P at low concentrations of (<15 μg/L), therefore, we assumed that the developed periphyton, was responsible for the PO_4–P removal efficiency.

When examining the impact of discharged stormwater on River Mleczna, there was an average decrease of 19% in the concentration of PO_4–P ($p > 0.05$). This decrease could be attributed to either the low concentration of PO_4–P in the treated stormwater, which might result in a dilution effect, or the notable influx of calcium ions (120.9 mg/L in the effluent and 82.8 mg/L in river), which could promote PO_4–P precipitation under favorable conditions (House, 1999).

3.3. Nitrogen removal

Transformations of the dissolved nitrogen forms in the stormwater treatment are closely related to the redox potential (Font-Nájera et al., 2021a; Rahman et al., 2019). Removal of nitrates occurred in the sedimentation zone (9.3%), which was in opposition to nitrites and ammonium, increased by 75% and 63.7%, respectively. Although the oxygen level was high in the SED outflow, we cannot exclude the possibility that anoxic conditions developed in the sediments. Nitrates increased by 9% after PRBs, from 1.16 to 1.26 mg NO_3–N/L. Thus, we may conclude that denitrification was not performed at a successful rate in this part of SSBS. This could be the result of high HLR and therefore lack of necessary time to establish anoxic conditions. Nitrate reduction in anaerobic conditions is mainly governed by two dissimilatory processes: denitrification and dissimilatory nitrate reduction to ammonium (Rahman et al., 2019). Due to the pulsating feeding operation of SSBS, oxygen supply could support the nitrification process and mineralization of organic matter, as observed for vertical flow constructed wetlands (Stefanakis and Tsihrintzis, 2012). We have considered that due to the high concentration of ammonium (0.354 mg/L in SED) and its successful removal, effect of nitrate reduction to ammonium could not be observed. After the release of ammonium in SED, from 0.245 to 0.401 mg/L on average, PRBs successfully removed 43.4%. The process of microbiological removal of ammonium ions can take place both in aerobic (nitrification) and anaerobic (annamox) conditions, although due to the high HLR, we assumed that the nitrification process prevailed in PRBs. Biofiltration zone was able to remove 24% of NO_3–N, but we expect its efficiency to grow with time due to the organic matter accumulation, creating more favorable conditions for denitrification (Hernandez and Mitsch, 2007). With media as biochar or woodchip efficiency of denitrification process can be increased to more than 60% efficiency in stormwater bioretention systems (Kong et al., 2022). Nevertheless, high efficiency towards ammonium was achieved, which is not always reached in the other existing SSSB (Font-Nájera et al., 2021a; Negussie et al., 2012). Literature reports revealed that media like compost or sand-based soil can increase the concentration of nitrogen species in stormwater (Betz et al., 2023). We have not observed any significant increase of N-species in the studied SSBS as there was no applied organic-based materials. Biofiltration zone was able to remove NO_3–N and NH_4–N, although NO_2–N did not decrease consistently. Analyzing the total budget for dissolved forms of nitrogen, the PRBs removed 0.378 mg N/L as nitrates and ammonium and released 0.006 mg N/L in the form of nitrites. In total, the system was able to remove 0.372 mg N/L. This underlines the role of PRBs in dissolved nutrients removal, supporting the operation of SSBS.

3.4. PRBs efficiency over time

There is still a gap in understanding the pollutant removal efficiency in wetlands, biofilters and constructed wetlands over varying time scales

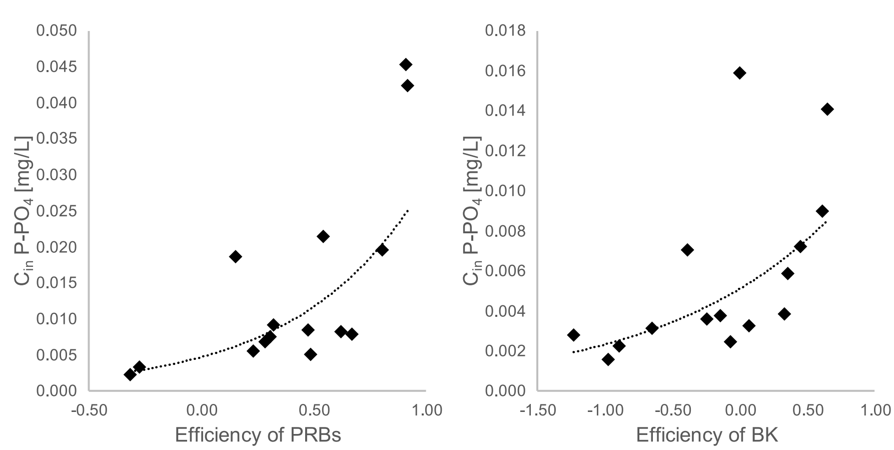

Fig. 4. The effect of inflow concentrations (C_{in}) of PO_4–P on the efficiency of all PRBs on the left, R = 0,60; p < 0,01; and on bioker (BK) on the right, R = 0,78; p < 0,001.

(Yang et al., 2022; Zhao et al., 2024). Usually, the effect of the system maturation is mostly underlined as the limiting factor, with the saturation of P-sorption capacity (Paing et al., 2015; Tanner et al., 1998). In this study, rather than time, we observed the dominant role of the concentration of PO_4–P at the inflow controlling the total efficiency of PRBs (Fig. 4; R = 0,60; p < 0,01), which is a known relation in constructed wetlands (Stefanakis and Tsihrintzis, 2012). Interestingly, while analyzing the PRBs separately, only BK showed similar dependency on the inflow concentration (Fig. 4; R = 0,78; p < 0,001). With progressing P-saturation it is common to observe negative performance of filtering media (Izydorczyk et al., 2013; Paing et al., 2015). We observed two such episodes of PO_4–P release: a 1% increase in BK in August, and a more pronounced 25% increase in the limestone barrier in September (Fig. 5). P-release episodes occurred most probably due to the low concentration in the inflow. As the HLR was almost constant within the study period it cannot be considered as the limiting factor, although it may play an important role in the system maturation (Tanner et al., 1998). Paing et al. (2015) found that constructed wetlands experience an age effect during start-up, but their performances stabilize after 0.5–2 years of operation.

Our study showed that PO_4–P removal persisted even after 110 days, and this was primarily influenced by the concentration flowing in, rather than the treatment time (Fig. 5) suggesting the adsorption process as the mechanism responsible for the efficiency. It was evidenced that PRBs with P-sorption media, could be an important tool in stormwater management and their implementation can be boosted with available models (Penn et al., 2016). Time-dependent development of the PRBs efficiency was visible for NH_4–N removal, when after 7 days efficiency was from 0 to 8% only, but after 45 days it reached values between 25 and 27%, despite similar oxygen and temperature levels (Fig. 5). It is known that microbial nitrifiers require more time to develop in constructed wetlands (Paing et al., 2015), therefore, this could be a visible proof of their metabolic activity. At the beginning, PRBs increased nitrates concentration in the water by 8%, but with time system started to remove nitrates, with efficiency of 11% in July, 13 % in August and 11 % in September. With longer time of operation, anoxic micro-zones may develop in the PRBs and enhance the reduction of oxidized nitrogen forms, but this effect was limited by the high ratio of discharge.

3.5. Microbial community development

Microbial communities play a key role in water treatment and NbS performance. Therefore, our understanding of their development and activity in relation to the local environmental conditions provides knowledge for further design strategies (Font-Nájera et al., 2021a, 2021b). In this study, the total microbial load in water samples ranged between 0.3 and 7.8 × 10^6 cells/ml (1.5 ± 2.3 × 10^6 cells/ml) (Table S2). Higher values were found at LIM, but cell counts did not show significant differences in waters collected along the SBSS. The microbial load in urban runoff and stormwaters can vary greatly depending on several factors, including urbanization (e.g. areas at different impact level, population density, industrial activity), weather patterns (e.g. rainfall intensity and frequency), and pollution sources (Ahmed et al., 2019; Stott et al., 2018). Despite previous studies used cultivation and PCR-based methods to assess microbial contamination levels in stormwater (Hou et al., 2018; Liguori et al., 2021), the novel insights on total cell counts herein obtained by flow cytometry align with previous findings from urban streams and riverbank filtrate (Adomat et al., 2020; Boi et al., 2016).

As assessed by 16S rRNA gene quantification and sequencing, water samples contained between one to four times less bacteria than biofilms (up to 1.49 × 10^6 and 5.40 × 10^6 of 16S rRNA Gene Copy Numbers - 16S GCN per ng of DNA, respectively) (Fig. 6). The bacterial abundance (based on 16S GCN) was an important factor contributing to the distancing and clustering of the samples through the horizontal axis (up to 64.6% variability explained by the PC1; see Fig. 7).

In the case of water samples, bacterial abundance was stable when passing through the PRBs in July, although they showed a visible decreasing pattern in August and October (Fig. 6a). This decrease of 16S GCN was also displayed in the PCA for BK in October, which resulted in a greater distance from the rest of the water samples (12.5% variability explained by the PC2; see Fig. 7). Bacterial taxa in water samples were dominated by Proteobacteria (~67%), Bacteroidetes (~16%), and Epsilonbacteraeota (~7%) (Fig. 6a–Table S3), with the last taxon represented primarily by sequences belonging to *Arcobacter* spp. – a group of bacteria known to contain clinically relevant species that have been isolated from the intestinal tract (Ghaju Shrestha et al., 2022). This observation indicated that input of the stormwater could be contaminated by wastewater including fecal matter, however importantly, the Epsilonbacteraeota were one of the taxa that was greatly reduced from the bacterial assemblages, i.e.: four times lower 16S GCN in BK compared to DOL in October (in absolute values 2.3 × 10^4 and 1.0 × 10^5 respectively) (Fig. 6a). These findings suggested that PRBs may effectively diminish potentially pathogenic bacterial taxa in aquatic environments, benefitting both human and animal health.

In the case of biofilm samples, bacterial communities were monitored after one month of system operation (August) and after three months (October), to allow sufficient time for their development (Fig. 6b). In August, higher bacterial abundances (based on 16S GCN) were registered for DOL and LIM compared to BK (5.19 × 10^6, 5.40 × 10^6, and 3.35 × 10^6 of 16S GCN per ng of DNA, respectively), while the different dynamic was observed for October - DOL and LIM presented lower 16S GCN compared to BK (2.47 × 10^6, 2.92 × 10^6, and 4.45 × 10^6 of 16S GCN per ng of DNA, respectively) (Fig. 6b–Table S2). In our previous research on different SSBSs, bacterial abundance in geochemical zones containing dolomite and limestone was usually higher in

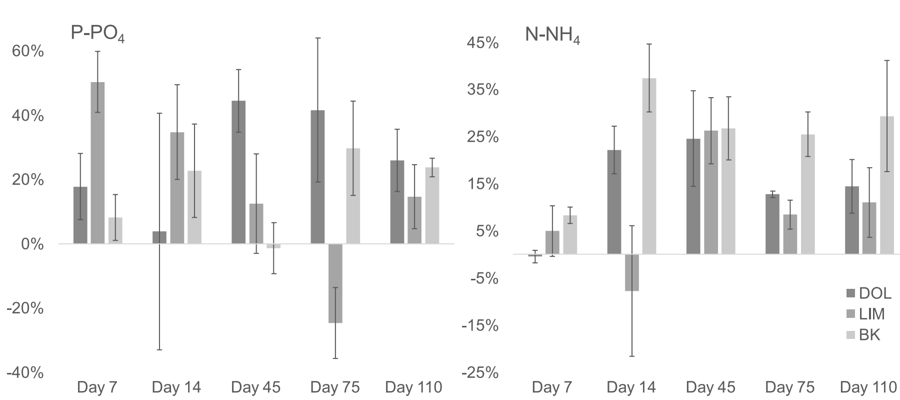

Fig. 5. Mean removal efficiency of the individual barriers (DOL, LIM, BK) after different times with Standard Error of the Mean (SEM). Results presented for PO_4–P and NH_4–N.

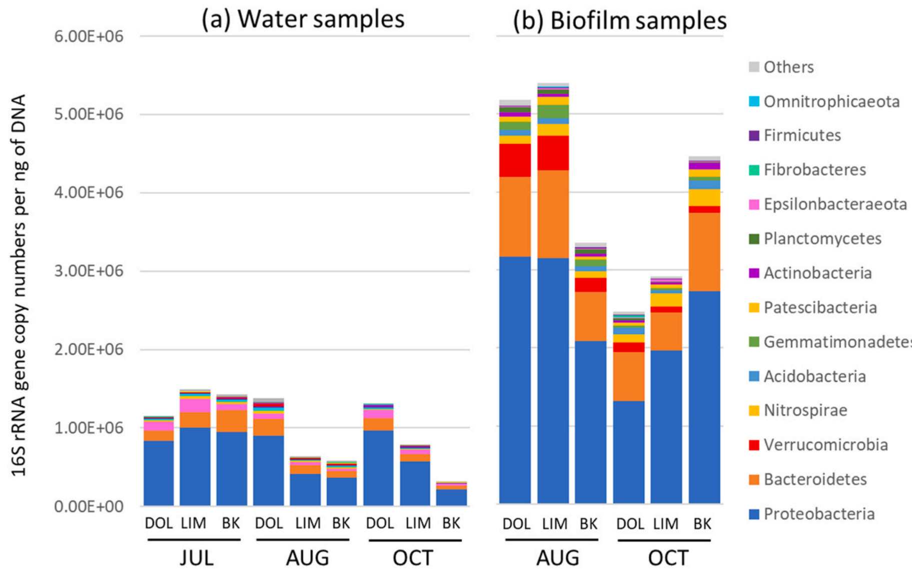

Fig. 6. Bacterial community assemblages in water (a) and biofilm (b) samples from SSBS with the use of 16S rRNA amplicon sequencing. Taxa were described to the level of Phylum. Relative abundance was transformed to absolute values and presented as the gene copy numbers per ng of DNA.

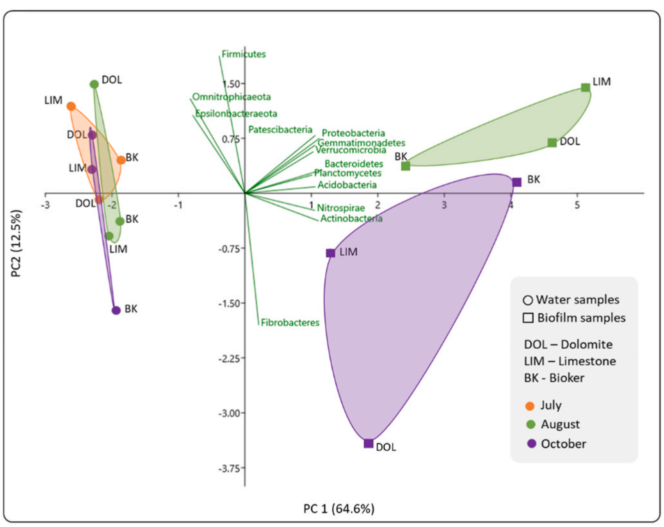

Fig. 7. Principal component analysis (PCA) presenting the spatial and seasonal differentiation of water and biofilm samples based on the bacterial taxa composition at the level of Phylum. The diagram was prepared with the PC1 and PC2 (77.1% variability).

summer than in autumn (Font-Nájera et al., 2020, 2021a). A similar seasonal dynamic was observed for DOL and LIM in the present study (Fig. 6b). Additionally, Font-Nájera et al. (2021a) mentioned that the porous and irregular surface of limestone was an important factor contributing to the attachment and development of biofilm – resulting in higher abundances of bacteria – when compared to dolomite. Again, the similar observation was described in the present study, since the LIM generally presented higher 16S GCN when compared to DOL (Fig. 6b). Interestingly, in the BK, the increment in bacterial abundance from August to October could be associated with a higher porosity and irregular surface. In the case of bacterial taxa, the dominant phyla in the water were also found to be dominant in biofilms - Proteobacteria (~61%) and Bacteroidetes (~21%). Nevertheless, they were composed with higher abundances of bacterial taxa belonging to the genera *Crenothrix, Rhodoferax* and *Hydrogenophaga*, and the family Burkholderiaceae for Proteobacteria (up to 3.3% 3.5%, 4.7%, and 6.6%, respectively), and the genus *Flavobacterium* for Bacteroidetes (up to 10.9%). All above-mentioned bacterial taxa are known to play an important role in N-cycling processes, where *Crenothrix* has been associated to nitrification (Stoecker et al., 2006), and *Rhodoferax*, *Hydrogenophaga*, *Flavobacterium* and many strains of Burkholderiaceae to denitrification (Palacin-Lizarbe et al., 2019; Repert et al., 2014; Wang et al., 2016). Interestingly, Font-Nájera et al. (2021a) reported that *Crenothrix* and *Flavobacterium* were also highly enriched in geochemical zones containing limestone, suggesting that they could be important elements in the removal of nitrogen in PRBs treating urban stormwater runoff. Moreover, in the case of Epsilonbacteriota, they were depleted in the biofilm assemblages (<0.4%), indicating that PRBs did not provide a suitable environment for the attachment and persistence of these potential pathogenic bacteria. Most bacterial phyla were oriented towards the biofilm samples in the PCA, while Epsilonbacteriota was oriented towards the water samples (Fig. 7). Other differences were observed in Verrucomicrobia (~5.2%), and two known nutrient-cycling bacteria, i. e.: Nitrospirae (~3.6%) and Gemmatimonadetes (~2.0%) with most sequences belonging to *Nitrospira* and *Gemmatimonas*, respectively. The Nitrospirae (*Nitrospira* spp.) are ubiquitous bacteria that can be found in freshwater ecosystems, and they are known to be important elements in the process of complete nitrification (Daims et al., 2015). The Gemmatimonadetes (*Gemmatimonas* spp.) are also found in freshwater ecosystems and particularly in sludge reactors performing enhanced biological phosphorus removal. Their presence was associated with the removal of phosphorus in wastewater treatment plants (Mujakić et al., 2022). The above results suggested that PRBs may offer a suitable environment for beneficial bacteria involved in nutrient transformations. Their presence within PRBs could be associated to the overall effective removal of ammonium and phosphates.

3.6. Adaptability of the hybrid SSBS

The Hybrid Sequential Sedimentation Biofiltration System (SSBS) offers a flexible approach for stormwater treatment in space-limited urban areas (Jurczak et al., 2018). Its modular design enables customization to address specific pollutant loads, making it a versatile solution for urban stormwater management. The system's sequential treatment processes allow for the removal of a broad spectrum of pollutants: the sedimentation zone effectively captures suspended solids, while PRBs can be optimized with materials that selectively target dissolved pollutants, such as phosphates or organic contaminants (Deng, 2020).

Additionally, the biofiltration zone can be planted with species that are efficient in nutrient uptake, while also serving as a sediment sink, as demonstrated in this study. This modularity allows the SSBS to adapt to diverse pollutant profiles typical of urban runoff, offering a scalable solution for various urban settings where space is limited (Jurczak et al., 2018, 2019; Szklarek et al., 2018; Zalewski et al., 2012; Negussie et al., 2012).

The incorporation of PRBs, a relatively new component in urban stormwater management, can act as a buffer against sudden influxes of pollutants, as observed in this study for phosphates. PRBs also help to reduce overall pollutant concentrations, which is crucial for maintaining the long-term effectiveness of biofiltration systems, where knowledge gaps remain regarding performance over extended periods under varying loads (Yang et al., 2022). Initially, PRBs rely on their sorption potential (Jarosiewicz et al., 2022; Srinivasan et al., 2008), but over time, biofilm formation leads to microbial processes taking a more prominent role in nutrient transformation (Font-Nájera et al., 2020, 2021a). This transition can enhance nutrient cycling but may also impact system performance, especially as biofilm dynamics shift with seasonal variations and pollutant loads. By integrating NbS with existing infrastructure, the hybrid SSBS provides a robust framework for cities aiming to improve climate resilience and water quality. With proper adjustments for local conditions, such as pollutant loads, available space and hydraulic requirements, the SSBS has strong potential for replication as a sustainable urban stormwater management solution.

4. Conclusions

In this study, we introduced and assessed a novel solution for stormwater treatment: a hybrid Sequential Sedimentation Biofiltration System (SSBS) tailored to integrate with existing stormwater infrastructure. The SSBS was tested under full-scale operational conditions, demonstrating significant improvements in the removal of suspended solids, nitrogen, and phosphorus. Utilizing various treatment materials (dolomite, limestone, and bioker), the Permeable Reactive Barriers (PRBs) proved particularly effective in eliminating ammonium and phosphates, maintaining efficiency over 110 days of operation. Additionally, the biofiltration zone effectively reduced dissolved nutrients and retained suspended solids, highlighting the importance of this component as a final factor for stormwater treatment.

Through microbial community analyses of waters and biofilms, we noted that PRBs fostered the microbial proliferation crucial for nutrient transformation processes (i.e., Nitrospirae and Gemmatimonadetes), while simultaneously reducing the prevalence of potentially pathogenic bacteria in water (i.e., Arcobacter spp.).

Overall, the outcomes of this study underscore the relevance of the treatment sequence, including sedimentation, adsorption and filtration through PRBs, and biofiltration in surface-flow wetland in addressing urban water quality challenges, paving the way for more resilient and sustainable urban water systems. Our findings illuminate the potential of hybrid SSBS as a promising tool for enhancing the quality of urban stormwater within constrained urban spaces.

CRediT authorship contribution statement

P. Jarosiewicz: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **A. Font-Najera:** Writing – original draft, Visualization, Methodology, Investigation, Data curation. **J. Mankiewicz-Boczek:** Writing – original draft, Supervision, Resources. **A. Chamerska:** Investigation. **S. Amalfitano:** Writing – review & editing, Writing – original draft, Investigation, Data curation. **S. Fazi:** Writing – review & editing, Writing – original draft, Supervision. **T. Jurczak:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143696.

Data availability

Data will be made available on request.

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