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Effect of sawdust and polonite addition to multi-module biochar filters for the enhanced removal of nitrate and phosphorus

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ABSTRACT

A high level of nutrient removal in onsite wastewater treatment systems is critical for avoiding eutrophication and groundwater contamination, especially in phosphorus- and nitrogen-sensitive areas. This study evaluates modifications to a pilot-scale Multi-module Biochar Filter (MmBF) system aimed at enhancing nitrogen and phosphorus removal through material amendments. A preliminary column experiment identified sawdust as an efficient carbon source for supporting denitrification, motivating its use in anoxic modules containing biochar and 10 wt% and 30 wt% sawdust. Polonite, a calcium-silicate material, was also tested at the same dosages in the final aerobic module for phosphate removal. The results showed that biochar with 30 wt% sawdust significantly enhanced nitrate removal (up to 83%) during the initial three weeks of operation, while biochar with 30% polonite addition achieved an average phosphate removal of 38%, with a peak of 95%. However, these improvements were not sustained over the full sampling period (199 days), suggesting the need for periodic media replacement. The MmBF system maintained high COD and nitrogen removal efficiencies even under doubled hydraulic loading (100 L m^{-2} day^{-1}), though phosphate removal declined under these conditions. These findings support the potential of MmBF systems with targeted media amendments as a flexible and modular onsite wastewater treatment.

Key words: biochar, multi-module biochar filter, nitrogen, onsite wastewater treatment, phosphorus, sawdust, solid carbon source

1. INTRODUCTION

Onsite wastewater treatment systems (OWTSs) play a critical role in managing wastewater, particularly in areas where centralized systems are not feasible. Their main purpose is to protect the aquatic environment and, consequently, human health by removing organic matter and nutrients such as phosphorus (P) and nitrogen (N), which are key contributors to water eutrophication. Nutrient removal standards for OWTSs in sensitive recipient areas, developed by the Swedish Agency for Marine and Water Management, require a 90% removal of total P and 50% removal of total N (SwAM 2016; Envall *et al.* 2020). Comparable rules appear internationally. The other European nations issue strict N and P effluent caps in sensitive zones (Preisner *et al.* 2020), and some U.S. states require OWTS designs that achieve total N concentrations under 20 mg L^{-1} (Washington State 2013).

Despite their role in wastewater management, OWTSs often fail to achieve sufficient nutrient removal, leading to significant environmental challenges. Phosphorus accumulation in soil treatment systems can lead to leaching into water bodies, deteriorating water quality (Eveborn *et al.* 2014). Similarly, nitrogen, particularly in the form of nitrate, is a major concern, as it is highly mobile and often inadequately removed from septic tank effluent (Hu *et al.* 2019). If released into receiving waters, excess nitrates can contribute to eutrophication, promoting algal blooms and oxygen depletion (Ghafari *et al.* 2008). Additionally, nutrient leaching from OWTSs can contaminate groundwater, posing risks to drinking water supplies and ecosystem sustainability (Mechtensimer & Toor 2017; Herren *et al.* 2021). Eutrophication remains a major environmental concern in Sweden, with nutrient pollution significantly affecting freshwater bodies and marine ecosystems

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(HELCOM 2021). The Baltic Sea, in particular, has suffered widespread algal blooms and oxygen-deficient zones due to excessive phosphorus and nitrogen loads (Elmgren 2001; Iho *et al.* 2023).

To address these concerns, a multi-module biochar filter (MmBF) system has been developed and tested as an alternative OWTS (Shigei *et al.* 2024). The MmBFs demonstrated high efficiency in removing organic matter and nitrogen under pilot conditions, achieving >95% COD (chemical oxygen demand) removal and >70% total inorganic nitrogen removal. However, there is potential for increasing nitrogen removal in these systems. Also, phosphorus removal remains a challenge, indicating the need for design improvements, especially in phosphorus-sensitive areas (Shigei *et al.* 2024).

A range of technological and management strategies have been developed to mitigate nutrient releases from OWTSs. These include chemical precipitation and biological filters, which can achieve high levels of nutrient removal (Weiss 2007; Thynell 2011; Rout *et al.* 2021). The systems incorporating media filters or aerobic units demonstrated significantly improved nutrient attenuation performance. However, higher operational costs and energy demands are primarily associated with systems relying on active aeration, such as aerobic treatment units, rather than with passive media-based filters (Diaz-Elsayed *et al.* 2017). OWTSs require an affordable solution to meet removal performance standards. MmBF is an alternative system that can be constructed using cost-effective materials, including biochar; however, its performance needs improvement for nitrate and phosphorus removal. One way to enhance MmBF's efficiency is by incorporating additional filtration media into its modules.

Advancements in filtration media for enhanced denitrification have led to the exploration of diverse solid carbon sources, ranging from traditional compounds to alternative and novel materials (Ahmed *et al.* 2022). In particular, carbon sources derived from agricultural by-products (e.g., peanut shells, sawdust, peat, and corncob) have gained attention for their sustainability and cost-effectiveness, offering a slow-release mechanism for prolonged carbon availability (Chen *et al.* 2021). Additionally, plant-based materials such as woodchips, sawdust, and macrophytes have been successfully implemented to enhance denitrification (Zhang *et al.* 2014; Hang *et al.* 2016). These solid carbon source can also work as a biofilm carrier in solid-phase denitrification (Healy *et al.* 2006; Hu *et al.* 2019). The effectiveness of these carbon sources is influenced by key factors such as the carbon-to-nitrogen (C/N) ratio, dosing methods, and pre-treatment techniques.

For phosphorus removal, various media have been investigated. Polonite®, a calcium-carbonate-based material containing silica, offers one of the highest reported sorption capacities (94 mg g^{-1}) and showed 90% removal (Karczmarczyk & Bus 2014; Karczmarczyk *et al.* 2017). Other materials, such as expanded clay, (5.1 mg g^{-1}), autoclaved aerated concrete, steel slag, and recycled concrete aggregate, have shown varying efficiencies, with some exceeding 90% removal (Hedström 2006; Tran *et al.* 2023; Drummond *et al.* 2024). Natural options like shell sand and limestone are low-cost but have lower capacities ($\leq$48 mg g^{-1}) (Karczmarczyk & Bus 2014; Das 2022). Cost considerations differ significantly across these materials, with natural filter media generally being more affordable, while synthetic and specialized media entail higher production costs (Karczmarczyk & Bus 2014; Das 2022).

This study aims to enhance the nutrient removal efficiency of MmBFs through the integration of supplementary materials, such as Polonite for improved phosphorus adsorption, and organic solid carbon sources to support denitrification. To address phosphorus removal, we tested two different dosages of Polonite under varying hydraulic conditions and evaluated their impact on treatment performance. To explore options for enhancing denitrification, a preliminary study assessed two organic by-products (sawdust and bark) as potential solid carbon sources. Findings from the preliminary study informed the subsequent integration of a selected material into the pilot MmBF system. The study addresses the following research questions: (1) Which of the tested solid carbon sources shows the most sustainable and effective support for denitrification? (2) How does the dosage and integration of the selected carbon source in MmBFs affect nitrogen removal? (3) How does the dosage of Polonite in MmBFs affect phosphorus removal?

2. MATERIALS AND METHODS

To determine the most suitable carbon source, a bench test for COD and phosphate release was followed by a column experiment for evaluating the denitrification efficiency of various carbon source options. The selected carbon source was subsequently applied in the pilot-scale MmBF system.

2.1. Selection of carbon source for enhanced denitrification

A shaken flask test was conducted to assess COD and phosphate release from different carbon sources, to inform carbon availability and potential phosphorus release. The carbon source candidates were bark (Plantagen Sverige AB, Sweden),

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pine and spruce sawdust (Setra Group AB, Sweden), and corncob. The corn kernels were dried in the oven at 150 degree for 3 hours and crushed into small pieces. All materials were sieved and kept for less than 1 cm. Each material (2 g) was submerged in 200 mL of Milli-Q water in flasks and agitated on a shaking table (215 rpm) at 20 °C for 96 hours. COD concentrations were measured periodically to assess carbon release at 0, 0.25, 1, 3, 6, 8, 24, 48, 72, and 96 hours. Following the COD test, phosphate release was evaluated under the same condition, with phosphate concentrations measured at 0, 1, and 24 hours.

Based on the results of the COD and phosphate release test, only bark and sawdust were selected for further evaluation in the column experiment, while corncob was not investigated further. A column experiment was subsequently conducted to evaluate which would be the more efficient carbon source, bark or sawdust. Four acrylic columns, each 60 cm in height and 4.35 cm in diameter, were packed with 100 g of a filter media, consisting of 25 wt% bark or sawdust and 75 wt% biochar. Granitic pebbles were placed at the top and bottom of each column to ensure even flow distribution. Two replicate columns were prepared for each material.

The column experiment was operated for a total of 70 days, including an initial start-up period of 31 days with municipal wastewater feed to promote biofilm development. Each column was manually fed with 150 mL of influent per day, and effluent was collected from the bottom of the columns.

Following the start-up period, the experiment consisted of two operational phases. During the **performance assessment phase (day 36–60; 25 days)**, a synthetic nitrate solution was applied at two concentration levels (10 and 45 mg L^{-1}) to evaluate denitrification performance using the solid carbon sources. During the **sustained denitrification phase (day 64–70; 7 days)**, carbon-rich water obtained from the prior shaking tests of the respective materials was added to the influent to maintain denitrification at a nitrate concentration of 45 mg L^{-1} as solid-phase carbon release declined. Influent and effluent samples were analysed for COD, nitrate, nitrite, ammonium, and pH. Sampling was conducted every three days during the performance assessment phase and five times during the sustained denitrification phase.

2.2. Pilot-scale MmBF pilot setup and operational conditions

The experimental setup used in this study followed the same configuration described in a previous study (Shigei *et al.* 2024), utilizing three identical MmBFs (Figure S1). Each filter consists of six sequentially arranged modules: three aerobic (M1–M3), two anaerobic (M4–M5), and one final polishing module (M6). The same biochar has remained in use since the initial system start-up on January 25th, 2021. Although there were intermittent periods without water flow (March to July 2024), the total elapsed time with the biochar in place reached 1,357 days by October 13th, 2024, the day before the material amendments were introduced. To minimise any potential effects of the standing period on microbial activity, the MmBFs were restarted on August 2nd, 2024, and operated under their original, unmodified configuration for approximately ten weeks before the material amendments were introduced. This reactivation period was intended to re-establish stable treatment performance before the experimental evaluation. On October 14th, 2024, the carbon source and Polonite were added, marking day 1 of this study, which focuses on performance evaluation under the updated conditions. The HLR was controlled by pump timers, delivering five intermittent doses per day, 2 L per dose to each filter, corresponding to a total of 10 L per day per filter and an initial HLR of 50 L m^{-2} day^{-1} (Shigei *et al.* 2025, 2024). This HLR was later doubled to 100 L m^{-2} day^{-1} between April 9th and April 30th, 2025 (days 178 to 199), to evaluate performance under higher flow conditions.

To assess the effect of material amendments on nutrient removal, specific dosages of carbon source and Polonite were added to the MmBFs (Figure 1). Sawdust was selected as the carbon amendment based on the preliminary screening and column experiments, while corncob was not investigated further due to its substantial phosphate release. Previous studies have shown that woody biomass effectively supports denitrification in biofilters, with amendment levels of approximately 20–30 vol% providing a balance between carbon availability and operational stability (Schipper & Vojvodić-Vuković 1998; Hang *et al.* 2016). Accordingly, sawdust was incorporated at 10 and 30 wt%, corresponding to approximately 6 and 20 vol%, respectively. The higher dosage was selected to achieve a volumetric proportion closer to that reported in the literature. Polonite was selected as a phosphorus sorption medium because of its documented phosphate adsorption capacity (1–2 g PO_4-P kg^{-1}) and its established use as a polishing medium in onsite wastewater treatment systems (Heistad *et al.* 2006; Renman & Renman 2010; Vidal 2018). It was incorporated into M6, the final treatment stage, in accordance with common practice in onsite wastewater treatment systems. Two amendment levels (10 and 30 wt%) were applied to evaluate moderate and high amendment scenarios for phosphorus removal.

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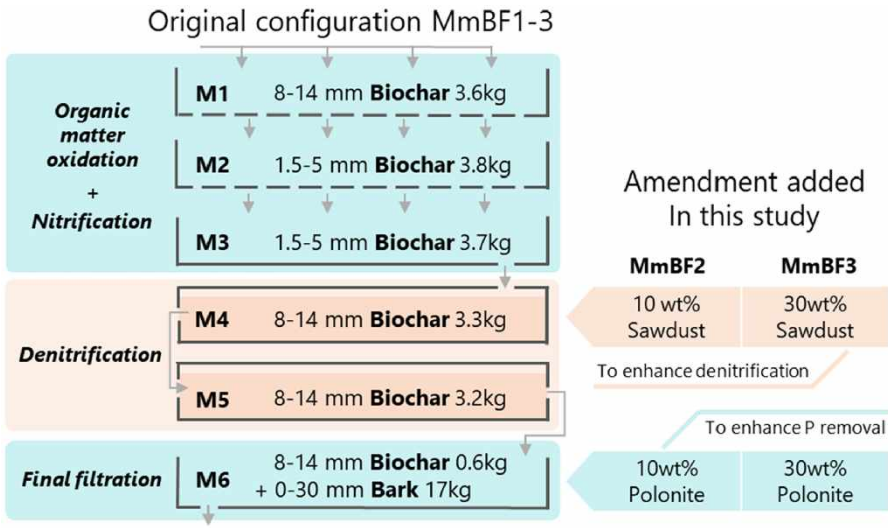

Figure 1 | Filter media composition across three identical Multi-module Biochar Filters (MmBF1–3). The original configuration included six modules filled with biochar (M1–M5) and bark (M6). This study introduced amendments to MmBF2 and MmBF3 to enhance denitrification and phosphorus removal while keeping MmBF 1 as a control. Grey arrows indicate the water flows.

Previous studies (Shigei *et al.* 2025, 2024) found no significant treatment differences among the filters, which allowed this study to assign specific configurations to each filter for comparative testing. MmBF1 functioned as the control with no material additions. MmBF2 – 10% CP was amended with 10 wt% of the selected carbon source in anoxic module M4 to enhance denitrification, and 10 wt% polonite in M6 for phosphate adsorption. MmBF3 – 30% CP received higher dosages, 30 wt% of the selected carbon sources in M4 and 30 wt% polonite in M6. All systems treated the same influent under identical operational conditions. Descriptions of each system's configuration are detailed in Figure 1 and Table 1.

2.3. Sampling and analysis from MmBF experiment

Water samples (40 mL each) were collected weekly from all modules of the three MmBFs between October 14th 2024 and April 30th 2025. Influent and final effluent samples were collected on 19 sampling occasions, whereas complete module-by-module sampling was conducted on 14 occasions. Only influent and final effluent samples were collected on days 31, 47, 117, 150, and 157. Consequently, Figures S3–S5, which present module-specific results, contain gaps at these time points. Sampling was not conducted on days 67 and 162. Between these two sampling events, an operational pause occurred from day 118 to day 149. The analysed parameters included pH, COD, dissolved oxygen (DO), ammonium, nitrate, nitrite, total nitrogen, and phosphate. To ensure consistency and comparability, the same test kits for spectrophotometric analysis and analytical procedures were used as those in the previous study by Shigei *et al.* (2024). DO was measured from each sampling tube after sample collection, except for M4 and M5, where measurements were taken directly in the module (M4 at the back end and M5 at the front), using a portable optical DO meter (Orion Star A223; Thermo Fisher Scientific, USA).

Removal efficiency for different parameters was calculated, using the formula:

$$\text{Removal efficiency (\%)} = \left(1 - \frac{C_{\text{eff}}}{C_{\text{in}}}\right) \times 100$$

where C_{eff}: effluent concentration, C_{in}: influent concentration.

For samples with ammonium concentrations below the analytical detection limit of 2 mg L^{-1}, a value of 2 mg L^{-1} was used for conservative estimation. The inorganic nitrogen (Inorg-N) to total nitrogen (Tot-N) ratio was calculated by summing NH_4-N, NO_3-N, and NO_2-N to obtain Inorg-N. The calculation was applied to each module's average values over the focus period (day 2–24, $n = 5$), and the corresponding results are presented in Table 2 in section 3.2. The focus period (day 2–24) was determined based on consistently elevated COD concentrations observed in the M4 effluent of MmBF3 – 30% CP

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Table 1 | Configuration of each module in the multi-module biochar filter (MmBF) in this study

	MmBF1				MmBF2 – 10% CP				MmBF3 – 30% CP			
Module	**Type of Material**	**Particle size [mm]**	**Bulk density [kg m^{-3}]**	**Porosity[a] [cm^3 cm^{-3}]**	**Type of Material**	**Particle size [mm]**	**Bulk density [kg m^{-3}]**	**Porosity[a] [cm^3 cm^{-3}]**	**Type of Material**	**Particle size [mm]**	**Bulk density [kg m^{-3}]**	**Porosity[a] [cm^3 cm^{-3}]**
1 (top)	Biochar	8–14	112	0.75	Biochar	8–14	111	0.75	Biochar	8–14	111	0.75
2	Biochar	1.5–5	117	0.85	Biochar	1.5–5	118	0.85	Biochar	1.5–5	118	0.85
3	Biochar	1.5–5	116	0.85	Biochar	1.5–5	116	0.85	Biochar	1.5–5	117	0.85
4	Biochar	8–14	101	0.78	Biochar + Sawdust (10%)	8–14 (biochar)/ < 10 (sawdust)	112	0.77	Biochar + Sawdust (30%)	8–14 (biochar)/ < 10 (sawdust)	132	0.80
5	Biochar	8–14	101	0.78	Biochar	8–14	100	0.78	Biochar	8–14	100	0.78
6 (bottom)	Biochar +Bark	8–14 (biochar)/ 0–30 (bark)	546	0.29	Biochar + Bark + Polonite (10%)	8–14/0–30/2–6 (polonite)	601	0.29	Biochar + Bark + Polonite (30%)	8–14/0–30/2–6	710	0.30

[a]Porosity values for each module were determined based on the method described in the supplementary material of previous study (Shigei *et al.* 2024). For modules with mixed materials (e.g., biochar and sawdust; biochar, bark, and polonite), the bulk density was measured, and porosity was calculated using the weighted average particle density of the individual components.

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Table 2 | Calculated functional performance (%), DO (mg L^{-1}), COD to total nitrogen ratios (COD/Tot-N), and inorganic nitrogen fractions (Inorg-N/Tot-N, %), based on focus period data (day 2–24; $n = 5$ for each parameter)

	MmBF1				MmBF2 – 10% CP				MmBF3 – 30% CP			
Module	**Functional Performance [%]**	**DO ± SD [mg L^{-1}]**	**COD/ Tot-N[a] ± SD**	**Inorg-N/ Tot-N ± SD [%]**	**Functional Performance [%]**	**DO ± SD [mg L^{-1}]**	**COD/ Tot-N[a] ± SD**	**Inorg-N/ Tot-N ± SD [%]**	**Functional Performance [%]**	**DO ± SD [mg L^{-1}]**	**COD/ Tot-N[a] ± SD**	**Inorg-N/ Tot-N ± SD [%]**
Influent	–	0.6 ± 0.5	4.6 ± 0.4	85 ± 14	–	0.6 ± 0.5	4.6 ± 0.4	85 ± 14	–	0.6 ± 0.5	4.6 ± 0.4	85 ± 14
1 (top)	90 ± 13†	3.5 ± 1.6	4.2 ± 1.3	93 ± 10	92 ± 6†	4.3 ± 1.3	4.3 ± 1.4	113 ± 37	92 ± 5†	4.3 ± 1.5	3.8 ± 0.4	87 ± 18
2		5.0 ± 1.3	2.5 ± 1.6	91 ± 28		4.8 ± 1.8	1.8 ± 0.8	99 ± 37		5.5 ± 0.4	2.7 ± 1.2	97 ± 23
3		3.5 ± 0.7	1.7 ± 0.7	68 ± 6		5.5 ± 0.7	2.3 ± 1.6	82 ± 22		4.3 ± 0.6	3.2 ± 3.1	75 ± 6
4	49 ± 14††	1.8 ± 1.0	0.8 ± 0.3	66 ± 19	72 ± 16††	0.6 ± 0.4	3.9 ± 2.6	70 ± 19	80 ± 14††	0.8 ± 0.4	12.7 ± 11.0	55 ± 8
5		1.3 ± 0.8	0.9 ± 0.6	76 ± 37		1.7 ± 0.8	3.0 ± 2.1	66 ± 25		1.2 ± 0.5	6.1 ± 5.1	58 ± 25
6 (bottom)	2 ± 19†††	9.2 ± 0.5	1.9 ± 1.8	82 ± 10	13 ± 12†††	9.7 ± 0.5	3.0 ± 2.4	80 ± 15	31 ± 19††	9.0 ± 1.0	2.8 ± 1.1	85 ± 20

Functional performance is defined as: nitrification efficiency (% NH_4-N removal) for across M1–M3†, denitrification efficiency (% NO_3-N removal) for over M4–M5††, and phosphate removal efficiency (% PO_4-P removal) for M6†††. Values are mean ± SD. A dash (–) indicates no data.

[a]All ratios and efficiencies were calculated per sampling date for each module (or functional unit), and then the mean and SD were calculated ($n = 5$).

compared to the control filter MmBF1 (Figure S2), with effluent values up to 15 times higher, suggesting leaching of organic matter for denitrification.

Statistical analyses were performed to evaluate differences in performance across the experiments. A summary of the statistical tests, group structures, and assumptions checked is provided in Supplementary Table S1. In brief:

(i) COD release from carbon sources in the shaking experiment was analysed with repeated-measures ANOVA followed by Tukey's test.
(ii) Column experiment nitrate removal was assessed using paired *t*-tests.
(iii) Functional performance in the MmBF units was compared among filters with one-way ANOVA, using Tukey's test for pairwise comparisons; both short- and extended-period data were analysed.
(iv) Phosphorus removal under different hydraulic loading rates was compared with independent *t*-tests.
(v) Pearson correlation was used to test relationships between influent COD and nitrate removal in M4.
(vi) Influent characteristics between the MmBF system and the municipal WWTP were compared with two-tailed t-tests.

For all ANOVAs, assumptions of normality and variance homogeneity were checked. All reported *p*-values are two-tailed with significance set at 0.05. Analyses were performed in Excel and GraphPad Prism version 10.5.0 (GraphPad Software, San Diego, CA, USA).

3. RESULTS AND DISCUSSION

3.1. Selection of carbon source for enhanced denitrification

3.1.1. Carbon and phosphorus release test

Results showed that corncob released significantly more organic carbon, measured as COD, compared to sawdust and bark throughout the shaking experiment ($p < 0.0001$), with a rapid initial release (Figure 2(a)). Sawdust and bark showed moderate and comparable carbon release. Although corncob showed the highest release of organic carbon, it also released high concentration of phosphate (Figure 2(b)), while the phosphate concentration of bark and sawdust were below the detection limit after both 1 and 24 hours. Therefore, only sawdust and bark were used for further testing in the column experiment.

3.1.2. Column experiment for bark and sawdust

During the start-up period with municipal wastewater influent (Step 1), ammonium concentrations decreased over time, whereas effluent nitrate concentrations increased, indicating the development of nitrifying activity in the columns (Figure 3(a)). Therefore, nitrate removal efficiency was not calculated for this period, as nitrate was produced within the columns through nitrification rather than being supplied as the main influent nitrogen species.

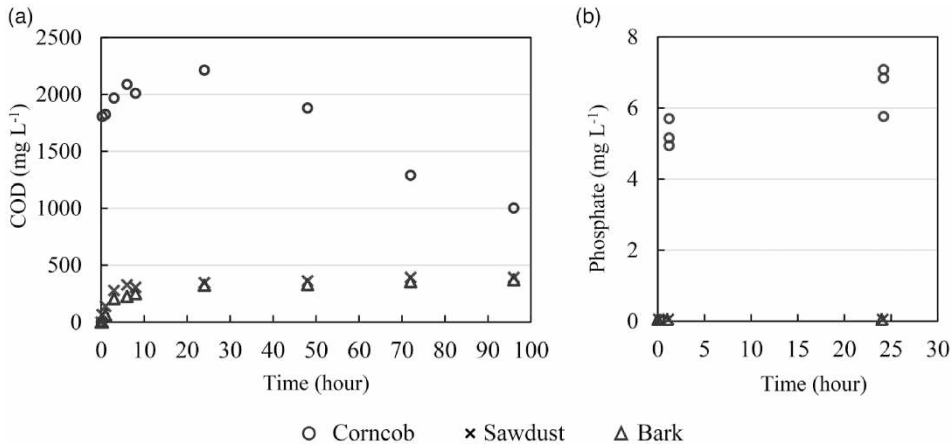

Figure 2 | Carbon and phosphorus release from three carbon sources in a shaking flask experiment at 20 °C. (a) COD concentrations (mg L^{-1}) over 96 hours from a single flask per material ($n = 1$); (b) phosphate concentrations (mg L^{-1}) at 0, 1, and 24 hours from triplicate flasks per material ($n = 3$). Each point in (b) represents an individual replicate.

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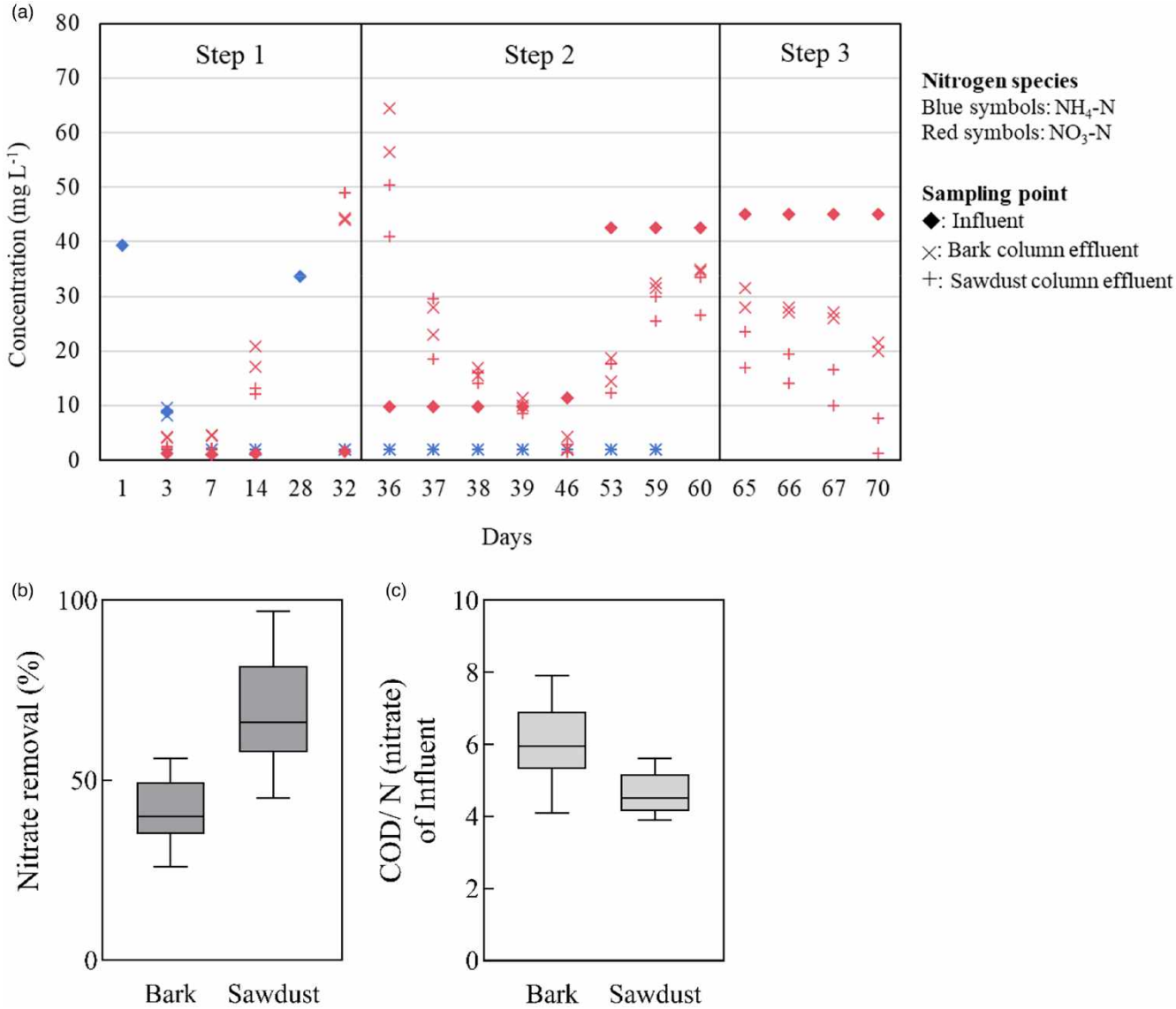

Figure 3 | Nitrate transformation and removal performance in bark- and sawdust-amended columns. **(a)** Concentrations of NH_4-N and NO_3-N in the influent and column effluents during the start-up period with municipal wastewater (Step 1), nitrate-fed phase (Step 2), and nitrate-fed phase with carbon-rich water addition (Step 3). Blue symbols indicate NH_4-N and red symbols indicate NO_3-N. Diamonds represent influent, crosses represent bark column effluent, and plus signs represent sawdust column effluent. **(b)** Nitrate removal efficiency (%) during the sustained denitrification phase (days 64–70; n = 8) and **(c)** influent COD to nitrate ratio during the sustained denitrification phase (days 64–70; n = 8).

After switching to synthetic nitrate solution in Step 2, nitrate removal could be directly evaluated. Both bark- and sawdust-amended columns supported denitrification following this transition, although high effluent nitrate concentrations were observed immediately after switch, likely reflecting continued nitrification of ammonium retained in the pore water and/or biofilm from the start-up period. From day 46 onwards, sawdust consistently achieved higher nitrate removal than bark. On average, nitrate removal was 54% in the sawdust-amended columns compared with 43% in the bark-amended columns during days 46–60, calculated from the concentrations in Table S2. From day 46 onwards, nitrate removal efficiency was significantly higher in the sawdust columns than in the bark columns (paired t-test, p = 0.0059; Table S2).

In the sustained denitrification phase (days 64–70), the addition of carbon-rich water derived from respective materials further enhanced nitrate removal efficiency in both columns. By day 70, nitrate removal reached 97% in the sawdust column and 56% in the bark column (Figure 3(b)). Although the bark-derived carbon solution supplied a higher influent

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COD concentration than the sawdust-derived solution (260 vs 205 mg COD L^{-1}; Table S2), the sawdust column still achieved higher nitrate removal efficiency. This suggests more efficient carbon utilisation with sawdust, as also reflected by the lower influent COD/N ratios observed for sawdust compared with bark (Figure 3(c)). Overall, both materials functioned as effective solid carbon sources for denitrification in the column experiment, but sawdust supported more efficient nitrate removal after start-up. Based on the literature insights mentioned in Supplementary information S1 and the results of this column experiment, sawdust was selected as an additional material for the MmBF experiment.

3.2. Performance of updated MmBF configurations

3.2.1. Nitrification and denitrification efficiency

During the focus period after sawdust addition (day 2–24), NH_4-N removal in the nitrification unit (M1–M3) did not differ significantly among the three filters (one-way ANOVA, $p = 0.85$; Table S1, S3). Across all MmBFs, 90–92% of influent NH_4-N was removed and largely converted to NO_3-N (Figure 4), indicating stable and comparable nitrification performance. Consequently, similar NO_3-N concentrations were supplied to the downstream denitrification unit (M4) in all systems, allowing differences in denitrification performance to be attributed primarily to the sawdust amendment.

For the entire denitrification unit comprising M4 and M5, NO_3-N removal efficiency differed significantly among the three MmBFs ($p = 0.0165$). Post-hoc analysis showed that MmBF3 achieved significantly higher removal (80%) than the control MmBF1 (49%) ($p = 0.0158$). In contrast, nitrate removal in MmBF2 (72%) did not differ significantly from either MmBF1 or MmBF3 ($p = 0.0749$ and 0.6649, respectively) (Figure 4, Table 2). The improved performance in MmBF3 was likely due to the increased availability of biodegradable carbon, as indicated by the elevated COD/Tot-N ratio of 12.7 and 6.1 for M4 and M5, respectively (Table 2). It supports the intended effect of sawdust addition in promoting denitrification. Dissolved oxygen (DO) concentrations confirmed that M4 and M5 maintained low-oxygen conditions (0.6–1.8 mg L^{-1} across all systems), though not fully anoxic. This suggests that denitrification likely occurred within deeper biofilm layers, where oxygen penetration was limited. High denitrification efficiency (>90%) has generally been reported when DO falls below 0.3 mg L^{-1} (Urbini *et al.* 2015). The M4 and M5 module conditions may have supported aerobic carbon oxidation, which could compete with denitrifying processes (Wei *et al.* 2022). Although COD concentrations decreased across M4 and M5 (Figure 4), indicating active carbon consumption, effluent COD was not significantly correlated with nitrate removal. This suggests that

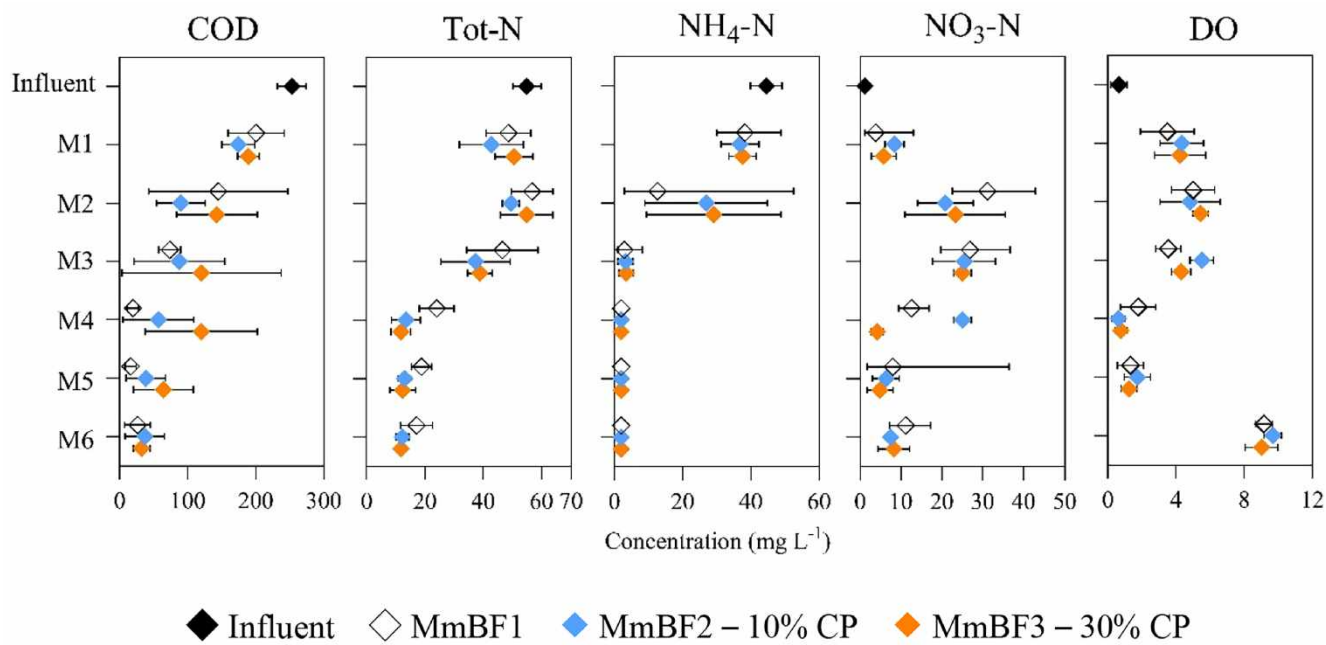

Figure 4 | Concentrations (mg L^{-1}) of COD, NH_4-N, NO_3-N, and Tot-N along the modules (Influent and effluent of M1–M6) during Days 2–24 ($n = 5$). For NH_4-N in modules M4–M6, concentrations were below the detection limit (2 mg L^{-1}); in these cases, values were plotted as the detection limit.

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organic carbon was utilised through multiple pathways, including aerobic oxidation in the presence of residual dissolved oxygen, in addition to denitrification occurring in anoxic micro zones. As a result, bulk effluent COD could not be directly linked to denitrification performance.

The increase in nitrate concentration observed in M6, despite consistently low ammonium levels (Figure 4), is likely due to microbial processing of organic nitrogen within the bark biofilm rather than direct nitrate leaching from the bark material (0.6 wt% Tot-N in bark; Table S4). Given that earlier biochar modules (M1-M3) had already oxidised most ammonium to nitrate, the influent to M6 would have consisted primarily of nitrate and organic nitrogen. Under the aerobic, unsaturated conditions of M6, heterotrophic microbes in the bark biofilm could mineralise this organic nitrogen to ammonium, which would be rapidly nitrified, bypassing detectable ammonium accumulation. The accompanying increase in the inorganic nitrogen to total nitrogen ratio (inorgN/Tot-N) from M5 (56–71%) to M6 (78–88%) in Table 2 further supports this mechanism, as it indicates active conversion of organic nitrogen to inorganic forms within the module. Similar short-term nitrification behaviour has been documented in pine bark substrates under aerobic conditions (Taylor *et al.* 2013), and the long-term persistence of bark observed in boreal forest decomposition studies (Romashkin *et al.* 2018) suggests that the bark matrix can continue to support biofilm activity for years, even decades.

While the sawdust addition in MmBF3 – 30% CP clearly improved denitrification efficiency in M4 during the first three weeks (days 2–24), the time-series data showed that this effect was not sustained beyond day 24 (Figure 5). The COD/Tot-N

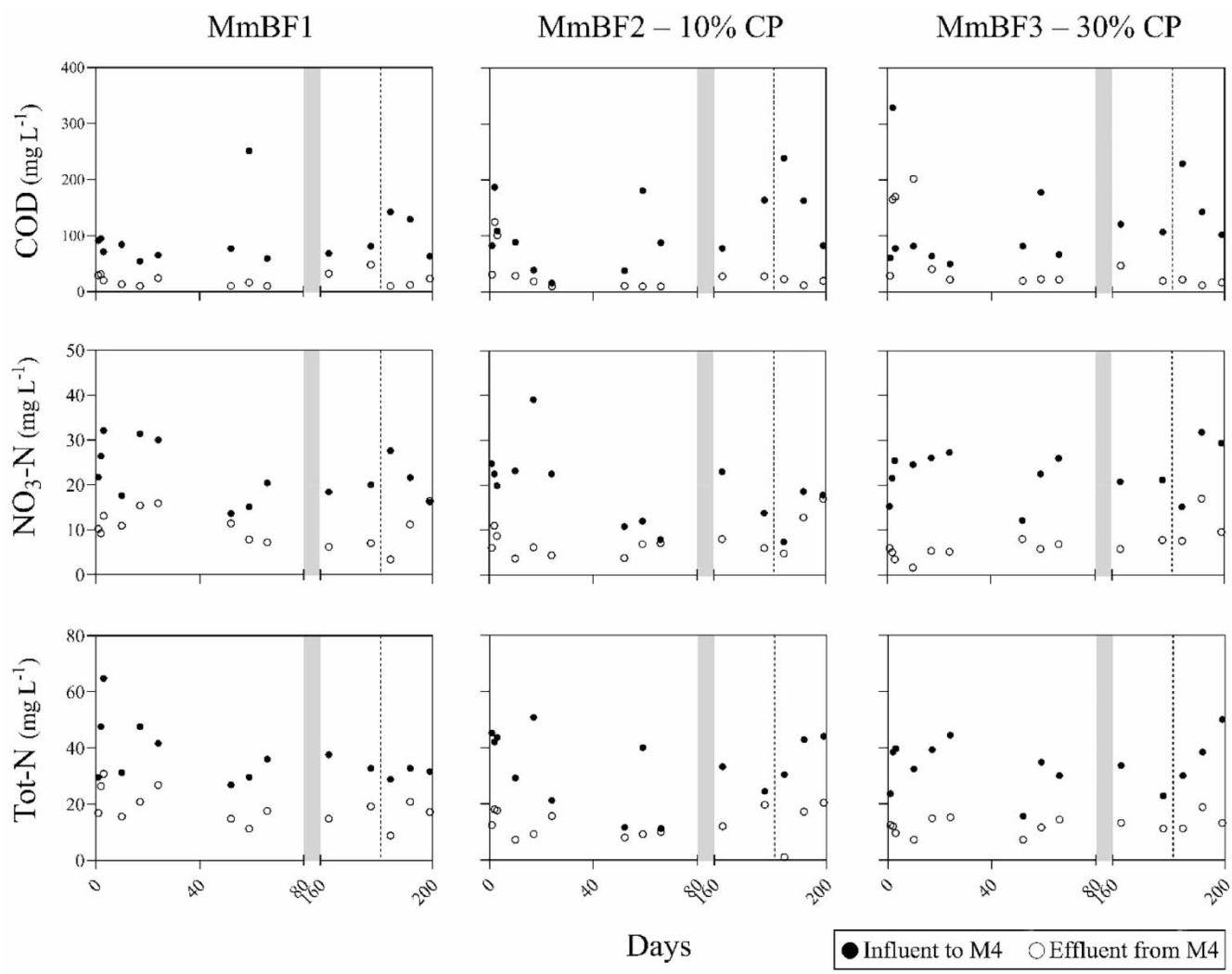

Figure 5 | Concentrations of COD, NO_3-N, and Tot-N in M4 for each MmBF. The grey-shaded area (Day 80–160) was the period without sampling due to technical issues. The dashed line at Day 182 marks the increase in hydraulic loading rate.

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ratio in M4 began to decline after this period, with average COD/Tot-N values during days 52–199 of 1.3 for MmBF1, 1.5 for MmBF2 – 10% CP, and 1.8 for MmBF3 – 30% CP. This suggests that the readily biodegradable carbon fraction in the sawdust was progressively depleted. These findings imply that the 30 wt% dosage used in this study was sufficient only for short-term enhancement of nitrate removal, and that periodic replacement of sawdust would be required to maintain enhanced performance over longer operational periods. This interpretation is supported by the column experiment (Section 3.1), in which 25 wt% sawdust mixed with biochar showed a 93% reduction in effluent COD between day 3 and day 38 (Table S2), reflecting depletion of the readily releasable carbon pool rather than direct evidence of denitrification.

It should also be noted that COD measurements based solely on module effluent may underestimate the actual availability of carbon within the module, as residual biodegradable carbon could remain inside the media matrix or biofilm.

The addition of sawdust enhanced nitrate removal sufficiently to meet the Swedish total nitrogen (Tot-N) standard for onsite systems (50% removal or < 40 mg L^{-1}; SwAM 2016), although the effect was temporary as the labile carbon became depleted. Despite using untreated sawdust, the system still achieved notable performance improvements, indicating that effective denitrification can occur without additional processing. Previous studies have shown that pre-treatment of woody biomass can increase carbon bioavailability by selectively removing lignin and enhancing dissolved organic carbon release (Hu *et al.* 2017). However, Hu *et al.* (2017) emphasised that denitrification performance does not increase monotonically with carbon digestibility, and that excessive DOC release can result in carbon losses and N_2O emissions. Instead, an optimal balance between carbon availability and longevity is required to sustain denitrification over time. In this context, future research should focus on optimising carbon source properties through a combination of material selection, amendment dosage, and replacement frequency, rather than maximising biodegradability alone. Such an approach would allow sustained nitrate removal while limiting operational complexity and costs, which is particularly important for OWTS applications.

3.2.2. Phosphorus removal with polonite

Phosphate removal performance in M6 varied across the three filters after Polonite addition, depending on the proportion of Polonite used (Figure 6 and S3). During the whole sampling period from day 2 to 199, the average phosphate removal was

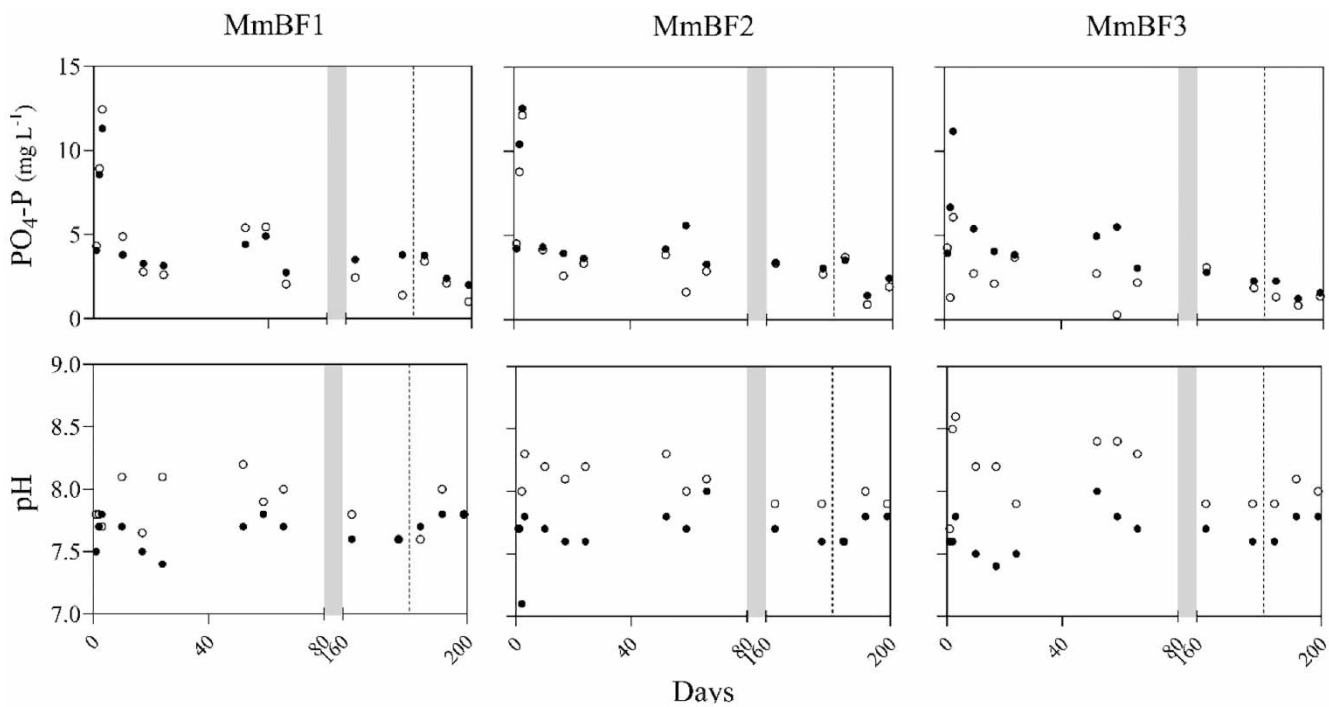

Figure 6 | Concentrations of PO_4-P and pH in module M6 for each MmBF1 (no Polonite), MmBF2 – 10% CP, and MmBF3 – 30% CP. The grey-shaded area (Day 80–160) indicates the no-sampling period, including an operational pause (Day 118–149). The dashed line at Day 182 marks the increase in hydraulic loading rate.

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11.3% in MmBF1 (no Polonite), 17.2% in MmBF2 – 10% CP (10 wt% Polonite), and 37.5% in MmBF3 – 30% CP (30 wt% Polonite). This entire sampling lasted nearly 200 days, which is longer than the initial estimation for the effective period of Polonite, expected to be around one month.

For the short-term focus period (same as N removal, day 2–24), a stable enhanced removal in MmBF3 – 30% CP was observed (Figure 6). However, the overall difference in phosphate removal efficiency among the three MmBFs was not statistically significant (one-way ANOVA, $p = 0.0585$, $R^2 = 0.377$). Post-hoc analysis showed no statistically significant pairwise differences between filters (MmBF1 vs MmBF2: $p = 0.5973$; MmBF1 vs MmBF3: $p = 0.0502$; MmBF2 vs MmBF3: $p = 0.2538$; Table 2).

The phosphate removal efficiencies observed in this study remain lower than those reported in previous investigations using Polonite-based filters, most likely due to the substantially lower proportion of Polonite used in the MmBF system. In earlier studies, much higher Polonite contents were applied, resulting in greater sorption capacity and more sustained phosphorus removal. For example, Gustafsson *et al.* (2008) achieved over 95% phosphate removal in saturated column experiments operated for 68 weeks. Similarly, Renman & Renman (2010) reported an average removal efficiency of 89% over nearly two years in a full-scale OWTS, receiving wastewater from a single household. In both studies, the filter media consisted predominantly of Polonite, mixed only with 10 wt% *Sphagnum* peat, representing a considerably higher Polonite fraction than that used in module M6 of the MmBFs in the present study. The peat addition was included to mitigate clogging associated with calcium carbonate formation. (Gustafsson *et al.* 2008; Renman & Renman 2010). The absence of this modification in the present study may have contributed to the lower removal, as unmodified Polonite may be more prone to pore blockage, reducing reactive surface area and long-term sorption performance. Renman & Renman (2010) also demonstrated a strong correlation between phosphate removal and effluent pH ($R^2 = 0.94$), with efficiency dropping below 70% when pH was below 9. This pH dependency is linked to Polonite's treatment mechanism: the material releases calcium (Ca^{2+}) and hydroxide (OH^-) ions, raising the pH and promoting the solid phase precipitation of calcium phosphates (Nilsson *et al.* 2013).

In the present study, pH in the effluent of M6 was only slightly elevated in the filters containing Polonite. In MmBF3 – 30% CP, pH was higher than in the other filters, at 8.3 (Figure 7), this still indicates limited calcium dissolution (Renman & Renman 2010), which likely constrained calcium phosphate precipitation and phosphorus removal. While mixing Polonite with bark and biochar in M6 may have reduced the risk of clogging, a higher proportion of Polonite would likely be required to achieve phosphorus removal efficiencies comparable to those reported in earlier studies.

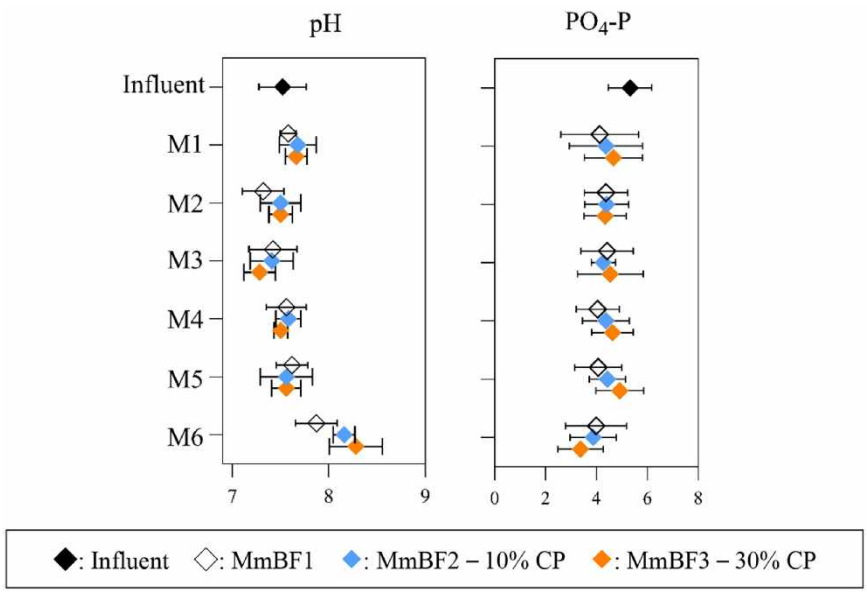

Figure 7 | pH and PO_4-P concentrations in the influent and effluents of the treatment modules (M1–M6) during Days 2–59 ($n = 7$). Error bars represent standard deviations.

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Although MmBF3 – 30% CP achieved significantly higher phosphate removal than MmBF1, performance still fell short of the Swedish environmental standard for high protection areas, which requires 90% total phosphorus removal (SwAM 2016). The module M6 could likely have been more effective if the entire media had consisted of Polonite rather than a mixture of Polonite, biochar, and bark.

3.2.3. Overall performance of MmBF and future directions

Even after a five-month pause prior to the present MmBF experiment, the system showed stable performance, comparable to a previous study (Shigei *et al.* 2024), within approximately two weeks after restarting. A similar recovery was also observed following the operational interruption (day 118–149) during this study. These findings indicate the long-term stability of MmBFs, particularly in the removal of organic matter and ammonium oxidation.

While the analysis of the focused period (days 2–24) showed significantly higher nutrient removal efficiency in the amended MmBF3 – 30% CP compared with the control MmBF1, this improvement did not persist over the extended period until the operational pause (days 2–66). For both Tot-N and PO_4-P removal, no significant differences were observed among the filters over the longer period (one-way ANOVA, $p > 0.05$; Dunnett's test; Figure S4). This indicates that the performance gains from sawdust in M4 and Polonite in M6 were most apparent in the early phase of operation (days 2–24). This pattern aligns with performance expectations for Polonite, whereas the effect of sawdust persisted for a shorter period than in the column experiment, likely due to the depletion of the carbon source by the end of performance assessment phase (around day 60). Future research could examine the optimal amendment dosage and operational strategies for material replacement.

In the context of reported effluent concentrations from other OWTSs, the updated MmBF system meets the Swedish EPA standard for effluent COD and nutrient levels (Table 3). This comparison is intended to place the MmBF performance within the range of values reported for other OWTSs rather than to imply direct performance equivalence, as influent wastewater characteristics differ substantially among studies. For example, the septic tank system reported by Pishgar *et al.* (2021) was evaluated using real domestic wastewater, which had higher and more variable nutrient concentrations, while the sand filter study by Kauppinen *et al.* (2014) was based on municipal wastewater. In contrast, the constructed wetland system reported by Wei *et al.* (2025) was operated using synthetic wastewater with defined carbon, nitrogen, and phosphorus concentrations.

Nevertheless, phosphorus removal in the MmBF system remains sensitive to influent composition and media configuration, and further optimisation is required to ensure robust performance under realistic household wastewater conditions. (Lowe *et al.* 2010; Pishgar *et al.* 2021).

To assess the performance of the MmBF system under increased hydraulic stress, the HLR was doubled from 50 to 100 L m^{-2} day^{-1} during day 185–199. Across all three MmBFs, the mean mass removal rates of COD and total nitrogen increased by approximately 14 and 20%, respectively (comparison between day 66–178; $n = 9$, and day 185–199; $n = 9$, Table S6). This suggests that the system effectively managed the higher influent load without a marked decline in treatment efficiency, as both COD and total nitrogen maintained stable removal efficiencies (COD: 96 and 98%; Tot-N: 70 and 71%, respectively; Table S6). In contrast, phosphate removal appeared to decline under the elevated HLR, from 51 to 15% as the mean of three MmBFs (Table S6). Phosphorus removal is likely constrained by the limited sorption capacity of the media,

Table 3 | Effluent concentrations of Swedish EPA standard, MmBF, and other OWTSs

	HLR (L m^{-2} d^{-1})	COD (mg/L)	Nitrogen (mg/L)	Phosphorus (mg/L)	Source
Swedish EPA[a]	–	30	40	3	SwAM (2016)
MmBF[b]	50	28.4 ± 11.8	11.5 ± 3.2	2.7 ± 1.7	this study
Septic tank[c]	51–81	88 ± 50	70 ± 32	10 ± 4	Pishgar *et al.* (2021)
Sand filter	33–167	38–100	46–55	1.7–4.5	Kauppinen *et al.* (2014)
Constructed wetland[d]	90	41.8–66.0	13.9–18.8	1.8–3.3	Wei *et al.* (2025)

[a]COD is reported as BOD_7. Nitrogen values correspond to the higher protection level, and phosphorus values correspond to the normal protection level (Tot-N and Tot-P concentrations).

[b]MmBF data refer to the updated MmBF3 configuration, using mean effluent concentrations from days 2–66 for Tot-N and PO_4-P.

[c]HLR was calculated based on daily wastewater production. COD is reported as BOD_5, nitrogen as Tot-N, and phosphorus as PO_4-P.

[d]Constructed wetland without baffles. The HLR was calculated from the reported flow rate and system dimensions. COD is given as total organic carbon, nitrogen as Tot-N, and phosphorus as Tot-P.

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despite the final polishing module (M6), aimed for phosphate removal, having a longer HRT (168 hours) compared to the anoxic modules (21 hours for M4–M5) and aerobic modules (135 hours for M1–M3) (Shigei *et al.* 2024). It is important to note that these findings are based on a small sample size ($n = 3$ for each HLR condition), and the doubled HLR still represents a relatively low total daily flow (60 L day^{-1} across all three filters), particularly when compared to the average domestic water use of approximately 170 L per person per day (SwAM 2016). Therefore, while this short-term test suggests promising robustness of COD and nitrogen removal under elevated flow conditions, further long-term evaluations under higher loads are necessary to confirm system scalability and to inform future design update.

4. CONCLUSIONS

This study demonstrated that targeted amendments to the Multi-module Biochar Filter (MmBF) system can enhance nutrient removal performance in onsite wastewater treatment applications. Among the tested carbon sources, sawdust was selected for its stable and efficient support of denitrification, showing higher nitrate removal and carbon use efficiency than bark. In pilot-scale MmBF application, 30 wt% sawdust addition to the anoxic module (M4) led to a short-term improvement in denitrification (80% NO_3-N removal), although this effect diminished after approximately three weeks, suggesting the need for periodic replenishment. For phosphorus removal, Polonite addition in the final module (M6) at 30 wt% improved phosphate removal (average 38%, peaking at 95% for day 2–199), outperforming lower dosage and control conditions. However, performance did not meet the Swedish environmental standard of 90% removal for phosphorus-sensitive areas, indicating a need for further investigation for suitable configurations. The study also found that the MmBF system could maintain high removal rates of COD and nitrogen under increased hydraulic loading rate (100 L m^{-2} day^{-1}) under three weeks observation, demonstrating potential robustness for higher flow conditions. Yet, phosphorus removal declined under higher HLR conditions. Long-term stability of the biochar media was also confirmed, as the system rapidly recovered from two operational interruptions and maintained stable treatment performance after four years of total operation. Future work should explore the longevity of media amendments, evaluate cost-effective pre-treatment options for carbon sources, and develop field-adapted system configurations to support implementation at real-world sites.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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