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# The removal of micropollutants from treated effluent by batch-operated pilot-scale constructed wetlands

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ABSTRACT

Micropollutants (MPs), such as pharmaceuticals and antibiotics, are present in the environment at low concentrations (ng/L-µg/L). A constructed wetland (CW) is a nature-based wastewater treatment technology, which can be used to remove MPs from wastewater treatment plant effluent. This study aimed to improve MP removal of CWs by optimizing the design of batch-operated CW. Three pilot-scale CWs were built to study the effect of two design-features: the use of a support matrix (a mixture of bark and biochar) and continuous aeration. The use of bark-biochar as support matrix increased the removal of 11 of 12 studied MPs compared to the CW filled with conventional material sand. The highest improved removal by the addition of bark-biochar was more than 40% (median) for irbesartan, carbamazepine, hydrochlorothiazide and benzotriazole. Aerating the bed of the bark-biochar CW did not change MP removal. Besides, the presence of bark-biochar also enhanced the removal of total nitrogen during 10 months of operation, but no improvement was observed on the total organic carbon and total phosphorus removal. Considering the application in a batch-operated CW, MP removal can be greatly enhanced by replacing sand with bark-biochar that will act as MP adsorbing matrix.

## 1. Introduction

Micropollutants (MPs), such as pharmaceuticals and personal care products, are often detected in the aquatic environment (including wastewater, surface water, groundwater and drinking water) at trace concentrations from ng/L to µg/L (Luo et al., 2014). The discharge of effluent from wastewater treatment plants (WTTPs) is considered as a major path to introduce MPs to surface water, as current WTTPs are not designed to specifically remove MPs (Kasprzyk-Hordern et al., 2009; Luo et al., 2014). The discharge of unremoved MPs to receiving water bodies may lead to potential risks to ecosystems and humans after short-term and/or long-term exposure, such as the presence of antibiotic resistance genes in microorganisms (Wu et al., 2015).

In Europe, some strategies have been implemented to monitor the occurrence and spread of MPs in the aquatic environment. For example, in 2020, the European Commission updated the list of priority substances to monitor MP presence in the surface water, including nineteen compounds, such as the antibiotic sulfamethoxazole and trimethoprim, and the antidepressant venlafaxine and its metabolite O-desmethylvenlafaxine (Decision 2020/1161/EU, 2020). Upgrading the existing WWTPs by adding an additional treatment step (i.e., post-treatment) is a possible solution to reduce the spread of MPs to the aquatic environment. For example, in the Netherlands, the Ministry of Infrastructure and Water Management, the Foundation for Applied Water Research STOWA and the joint Dutch water authorities financed an 'Innovation Program Micropollutants Removal' (IPMV) since 2019 to assess the performance of several tertiary treatment technologies for removal of 19 guide MPs from wastewater effluent (van Weeren et al., 2021). These 19 guiding MPs, such as carbamazepine and diclofenac, were selected since they are insufficiently removed in Dutch WWTPs and often present in the Dutch water bodies (Dutch Ministry of Infrastructure and Water Management et al., 2020).

Physical/chemical technologies, such as activated carbon and ozone, are tested or already used as a post-treatment step for MP removal in Switzerland, Germany and Netherlands (Athing et al., 2018; Boehler et al., 2012; Hollender et al., al.,2009; Margot et al., 2013; van Weeren et al., 2021). Compared with the physical/chemical technologies, biological technologies have the potential to treat wastewater in an eco-friendly and sustainable manner, such as constructed wetlands (CWs). CWs are a natural-based wastewater treatment technology, which mainly consists of support matrix, plants, and microorganisms (Gorito et al., 2017). In CWs, various physical, chemical and biological

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processes take place to remove organic compounds, such as photochemical oxidation, sorption, plant uptake and biodegradation (Imfeld et al., 2009; Susarla et al., 2002).

CWs can be classified into Free Water Surface CWs (FWS CWs) and Subsurface flow CWs (including horizontal flow CWs (HF CWs), vertical flow CWs (VF CWs) and French VF CWs) (Dotro et al., 2017). In subsurface flow CWs, the water flows through the support matrix, which is often sand or gravel. The support matrix of subsurface flow CWs promotes more adsorption and interaction between wastewater, support matrix, plants and microorganisms, resulting in a higher potential to remove biodegradable MPs compared to FWS CWs where the water flows on top of the support matrix (Gorito et al., 2017). CWs are either operated in a batch, continuous or intermittent feeding mode (Meng et al., 2014). Batch feeding mode consists of alternating cycles of filling and draining, which brings atmospheric air into the CW bed, thereby promoting aerobic biodegradation. Aerobic biodegradation is generally a more efficient MP removal pathway in CWs than anaerobic biodegradation (Ilyas and Hullebusch, 2020). For example, Zhang et al. (2012) found that CWs with batch feeding mode removed ibuprofen, caffeine, salicylic acid, ketoprofen and diclofenac more efficiently than that with continuous feeding mode. Some MPs are recalcitrant to biodegradation and other removal processes are needed to further remove them, such as carbamazepine with sorption as its preferable removal pathway (Matamoros et al., 2005). Optimizing the design of batch-operated CW can be a solution to improve the removal of this MP.

Possibilities for enhancing MP removal efficiency of batch-operated CWs by small technological adjustments are the implementation of enhanced adsorption support matrix or aeration. Sorption is a dominant removal pathway for hydrophobic MPs, such as carbamazepine (Matamoros et al., 2005). Sorption of MPs in CWs can be improved by adding a support matrix with high adsorption capacity. Previous studies with columns and small-scale indoor CWs showed that the re-use of natural materials (bark and biochar) could enhance the removal of MPs through enhanced adsorption, such as carbamazepine (Lei et al., 2021, 2022).

The bark and biochar are nature-based materials, which have potential to be biodegraded after use. Bark can be biodegraded by fungi to providing nutrients for fungal growth in the fungal bioremediation (Valentín et al., 2010). Biological mineralization of biochar has been studied by incubating biochar under diverse conditions, such as sand, soil, inoculum solutions or nutrients (Liu et al., 2013). When applying bark and biochar as support matrix in a CW, a higher oxygen consumption within the system can be expected due to active biological activities compared to a CW with sand. To maintain aerobic conditions in the bark-biochar batch-operated CW and promote aerobic biodegradation of MPs, aeration can be used to maintain aerobic conditions in the CW, which is reported as an efficient method to increase an oxidizing condition in batch-operated CWs (Stefanakis and Tsihrintzis, 2012; Wu et al., 2016).

The aim of this study is to determine the effect of two CW design-features on the removal efficiency of MPs in a batch-operated CW: the use of an enhanced adsorption support matrix or aeration. Three pilot-scale CWs were studied, a control with sand as support matrix, and two with bark-biochar as support matrix, of which one was continuously aerated. A year-round monitoring was performed to evaluate the MP removal in the pilot CWs under varying climatic conditions.

## 2. Materials and methods

### 2.1. Chemicals

Trimethoprim was purchased from Sigma- Aldrich (US). Metoprolol, benzotriazole, irbesartan, carbamazepine, propranolol, sulfamethoxazole, furosemide, diclofenac, sum 4 and 5 methyl-1H-benzotriazole, clarithromycin and hydrochlorothiazide were purchased from TOKYO CHEMICAL INDUSTRY (Japan). Internal standards sulfamethoxazole-D4, furosemide-D5 and diclofenac-D4 were purchased from LGC (Germany), propranolol-D7 from MERCK (the Netherlands), carbamazepine-D10, irbesartan-D6 and benzotriazole-D4 from Toronto Research Chemicals (Toronto, Canada). The physicochemical properties of these chemicals are present in Appendix A. Formic acid was bought from MERCK (the Netherlands). Ultra-pure water, methanol and acetonitrile were purchase from ACTU-ALL CHEMICALS (the Netherlands).

### 2.2. Wastewater effluent

The wastewater effluent used was obtained from Bennekom WWTP (Bennekom, the Netherlands). The population equivalent of Bennekom wastewater treatment plant is 20,000 (de Wilt et al., 2018). The raw wastewater is treated by primary screening, activated sludge, clarifier and sand filtration. Afterwards, the treated wastewater effluent is discharged to the surface water. Average pH and conductivity of the wastewater effluent were 7.2 and 537 μS/cm, respectively. Average total organic carbon (TOC), average total nitrogen (TN) and average total phosphorous (TP) of wastewater effluent were 8.4 TOC mg/L, 7.5 mg TN/L and 0.2 mg TP/L, respectively. The concentration of MPs in the treated WWTP effluent is presented in Appendix B.

### 2.3. Pilot constructed wetlands

Three pilot-scale batch-operated subsurface flow CWs were built in March 2020 at the campus of Wageningen University and Research (the Netherlands): a sand CW and two bark-biochar CWs, see Fig. 1. The CWs were built in 11.6 $m^2$ polyethylene basins (3.96 m* 2.94 m* 1 m) that were embedded into the ground. The support matrix in the sand CW consisted of 10 cm gravel (bottom, 10–60 mm), 85 cm sand (0.2 mm) and 5 cm gravel (top). The support matrix in the bark-biochar CW 1 and 2 consisted of 10 cm gravel (bottom), 20 cm sand, 60 cm of a mixture of bark (5–25 mm, LENSLI, the Netherlands) and biochar made from cow manure (0.5–5 mm, MAVITEC, the Netherlands) with ratio 9:1 (wet v:v), 5 cm sand and 5 cm gravel (top). Influent and effluent pipes were installed at the top and bottom of the studied CWs. The aeration pipe in the bark-biochar CW 2 was installed at the middle of 10 cm gravel (bottom) and 20 cm sand layers (Fig. 1). The CWs were planted with *Typha angustifolia* (Directplant, the Netherlands) in March 2020 and the invasion of other plant species was prevented manually.

Each CW was connected to its own 1 $m^3$ polyvinyl chloride influent tank (Fig. 2). This influent tank was used to make a homogeneous influent for each batch feeding cycle. The WWTP effluent was continuously pumped into each influent tank before feeding it into the corresponding CW. All CWs were run in a semi-batch mode with an 8-hour per cycle, which was repeated continuously during the operation. Each cycle consisted of three phases: influent feeding, treatment and drainage. First, 0.737 $m^3$ of WWTP effluent was pumped from the influent tank to the CW at a flow rate of 0.05 $m^3$/min in 0.25 h. Then, the wastewater remained in the CW for 7.25 h. Thereafter, the treated wastewater was discharged within 0.5 h. The operation mode used in this study, batch feeding combined with drainage after a certain period, is also used in other MP removal oriented batch-operated CWs, such as Dan et al., 2017 and Marcelino et al., 2020. The feeding and discharge cycle was controlled by a Siemens LOGO! automation system (Siemens, Germany) that governed the feeding times, and opening and closing of the valves.

The CWs were started in March 2020, and fed with mainly rainwater and occasionally wastewater effluent (only in summer due to high water evaporation rate) to promote the growth of microorganisms and plants. After that, the research and addition of MPs to the CWs started on December 21$^{th}$ 2020 with two phases.

In phase 1 (December 21$^{th}$ 2020 to February 21$^{th}$ 2021), the three CWs were fed with WWTP effluent to test the operation of set-ups. WWTP effluent was continuously pumped into the influent tank with 1535 m/min, and subsequently fed to the CWs according to the feeding cycle.

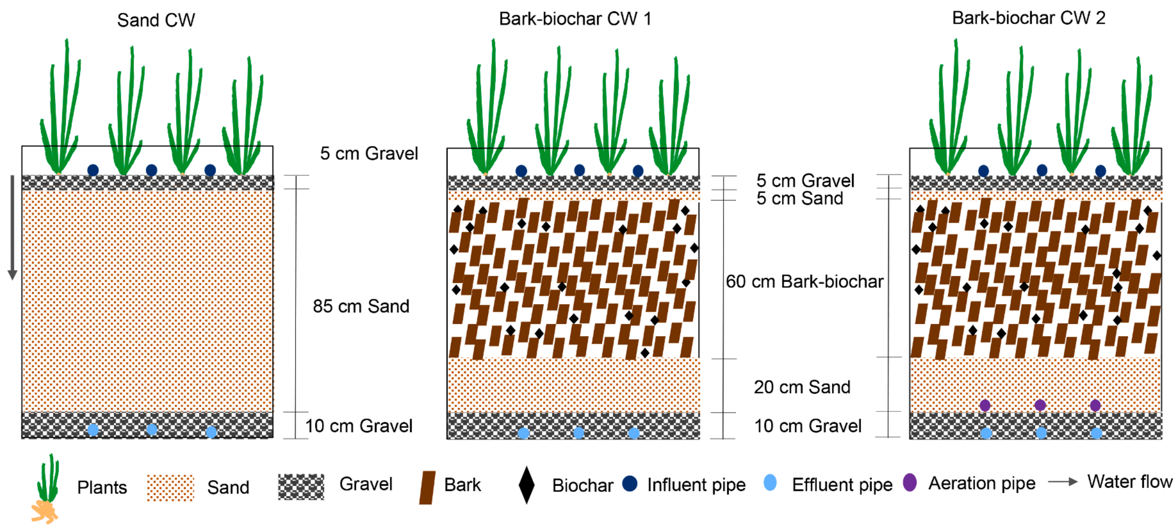


**Fig. 1.** Composition of the sand CW and bark-biochar CWs.

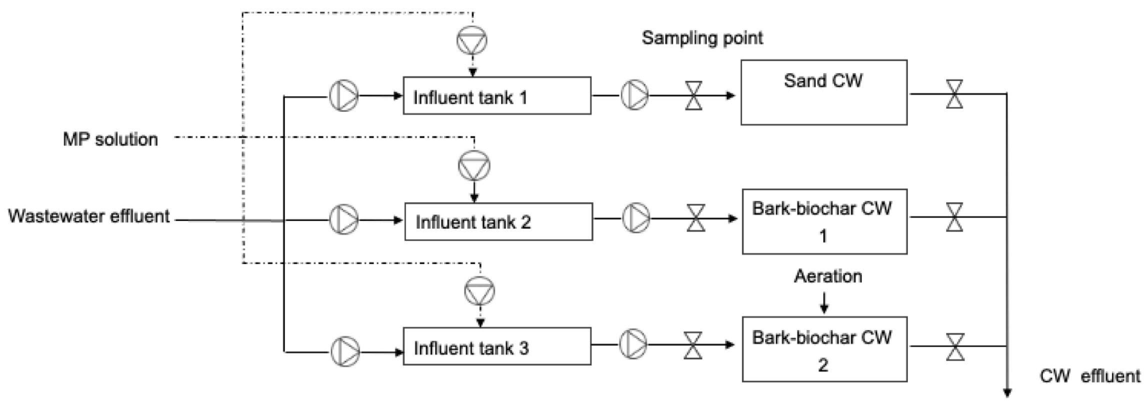


**Fig. 2.** Flowchart of the pilot CWs.

In phase 2 (February 23th 2021 to December 24th 2021), a stock solution of 29 mg/L of target MPs (each compound) was spiked to WWTP effluent at a flow rate of 0.305 mL/min to obtain the targeted MP influent for the CWs. This MP solution was prepared in demi-water, diluted from a 400 mg/L (each compound) solution prepared in methanol. The final concentration of MPs in the CW influent was the sum of the actual WWTP effluent concentration and the spiked MPs, which was around 5 μg/L (Appendix C). The WWTP effluent was spiked with MPs to achieve a stable concentration of target micropollutants (around 5 μg/L of each compound) during 10 months. In this way, the observed removal efficiencies in the constructed wetlands at the same climate conditions are due to different configurations of the tested constructed wetlands. The MP solution and WWTP effluent were homogeneously mixed in the influent tank, as tested in separate experiments (Appendix D). From October 20th 2021 to December 24th 2021, the bed of the bark-biochar CW 2 was aerated with a continuous air flow (180 mL/min) controlled by a Brooks 5850E Massflow controller (Brooks, Veenendaal, the Netherlands) during each cycle.

During the operation, at least two complete influent/effluent cycles (8 h per cycle) were sampled per month. Liquid samples were manually collected at the influent ($t = 0$ h) and effluent ($t = 8$ h) (Fig. 2), and stored at −20 °C prior to chemical analysis.

### 2.4. Chemical analysis

#### 2.4.1. Water quality parameters

pH value and electrical conductivity were measured with a multi-digital meter (HACH HQ40d, Germany). Total nitrogen (TN), total phosphorus (TP) and ammonia ($NH_4^+$) were measured with HACH Lange GMBH kits on a DR 3900 spectrophotometer (HACH, Germany). Total organic carbon (TOC) was measured by a TNM-L TOC analysed (Shimadzu, US), as described by Wagner et al. (2020a). $NO_3^-$ and $NO_2^-$ were measured by ion chromatography with a Dionex ICS2100 (Thermo Fisher Scientific, US) based on the method of Wagner et al. (2020a). Dissolved oxygen of influent, effluent and inside the CWs was measured by oxygen probe with Pst3 sensorspots (PreSens, Germany) and an Oxy-10 mini readout unit.

#### 2.4.2. Micropollutants

For the analysis of MPs in the influent and effluent of the CWs, liquid samples were taken and centrifuged at 15,000 rpm for 10 min and the supernatant was collected for analysis. The concentration of 9 target MPs was measured with a Triple quad 5500+ QTRAP Ready liquid chromatography-mass spectrometry (LC-MS) (SCIEX, the Netherlands), except for the sum of 4 and 5 methyl-1H-benzotriazole, clarithromycin and hydrochlorothiazide. The used column was a Kinetex® 1.7 μm Phenyl-Hexyl 100 A (Phenomenex, USA) and its operation temperature was 35 °C. The injection volume was 25 μl per sample. Two mobile phases were used; eluent A (0.1% fumaric acid in water) and eluent B (0.1% fumaric acid in acetonitrile), at a flow rate of 0.4 mL/min. Samples were analysed with a gradient elution: 95% eluent A and 5% eluent B were used from 0 to 0.5 min; the composition of two eluents was changed to 20% eluent A and 80% eluent B from 0.5 to 3.5 min; this composition was kept from 3.5 to 7.5 min; the composition of two eluents was changed again to 95% eluent A and 5% eluent B from 7.5 to 8.5 min; this composition was kept constant until 12.4 min. A detection range from 50 ng/L to 900 ng/L was used. The internal standards (sulfamethoxazole-D4, furosemide-D5, diclofenac-D4, propranolol-D7, carbamazepine-D10, irbesartan-D6 and benzotriazole-D4) were used to correct for matrix effects. The calibration standards (50 ng/L to 900 ng/L) showed a good linearity ($R^2 > 0.99$).

The concentration of sum 4 and 5 methyl-1H-benzotriazole, clarithromycin and hydrochlorothiazide was measured by AQUALYSIS waterlaboratorium (Zwolle, the Netherlands).

## 3. Results and discussion

### 3.1. Micropollutants

#### 3.1.1. Bark-biochar constructed wetland

The bark-biochar CW 1 and the sand CW were able to remove all 12 target MPs after start-up (Fig. 3). The bark-biochar CW 1 showed a high removal (median >80%) for trimethoprim, metoprolol, propranolol, furosemide, the sum of 4 and 5 methyl-1H-benzotrizaole and clarithromycin, followed by a moderate removal (median from 40% to 80%) of benzotriazole, irbesartan, carbamazepine, sulfamethoxazole, diclofenac and hydrochlorothiazide. These removal efficiencies were similar in the bark-biochar CW 2 prior to the aeration period (Appendix E), showing that these two CWs were actual duplicates, of which one of the two could be used as a control in phase 2 to determine the effect of aeration on the MP removal efficiency.

By replacing sand with bark and biochar, the highest improvement in removal, i.e., with more than 40% (median), was seen for irbesartan, carbamazepine, hydrochlorothiazide and benzotriazole (Fig. 3). During 10 months, higher removal efficiencies of irbesartan, carbamazepine and hydrochlorothiazide were observed in the bark-biochar CW 1 (40% to 90%) over time than in the sand CW (<40%) (Fig. 4). This positive effect of bark-biochar on the removal of irbesartan and carbamazepine is in line with the findings reported in mesocosm batch-operated CWs (Lei et al., 2022). In this mesocosm CW study, irbesartan and carbamazepine had higher removal efficiencies in the bark-biochar CW (>80%) than in the sand CW (45% and 34%). One important reason to explain the improved removal is the higher adsorption capacity of bark-biochar to these two MPs than the sand, as demonstrated by Lei et al. (2021) in abiotic column tests. Sorption is likely to be an important removal mechanism for irbesartan in a CW, which is firstly reported by the present study. Carbamazepine is recalcitrant to biodegradation, and adsorption is its main removal pathway (Matamoros et al., 2005). The recalcitrant behaviour of carbamazepine was also reported in other sand/gravel based batch-operated CWs <30% (Nivala et al., 2019).

In the present study, using adsorptive material bark-biochar significantly improved the removal of hydrochlorothiazide (>60%) compared to the sand CW (<40%), indicating that adsorption is likely an important removal mechanism for hydrochlorothiazide in a CW. To date, this study is the first research to explore the removal of hydrochlorothiazide in a batch-operated CW. The removal of hydrochlorothiazide has been studied in continuous-operated CWs, with reported removal varying from with 18% – 91% by Chen et al. (2016) and 44% - 83% by Vymazal et al. (2017).

Unlike irbesartan, carbamazepine and hydrochlorothiazide, the bark-biochar CW1 showed higher removal efficiencies of benzotriazole (>70%) in the start-up phase of operation compared to the sand CW (<20%) (Fig. 4). After that, its removal efficiencies in the sand CW increased over time and were comparable to the bark-biochar CW. This

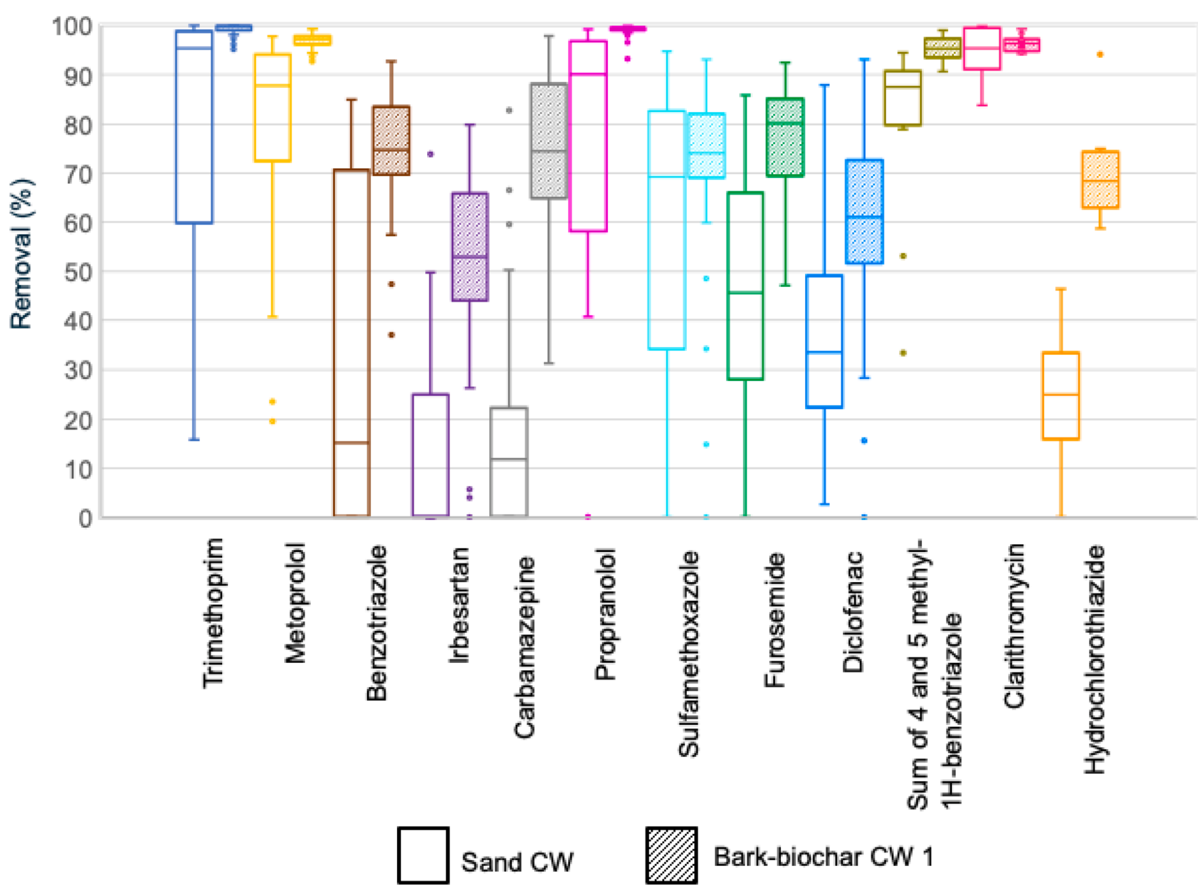


**Fig. 3.** Removal efficiencies of 12 MPs in the sand CW and the bark-biochar CW 1. The box plot shows the values: minimum, first quartile (Q1), median (horizontal line, Q2), third quartile (Q3), maximum and outliers (·). Negative removals were set to zero.

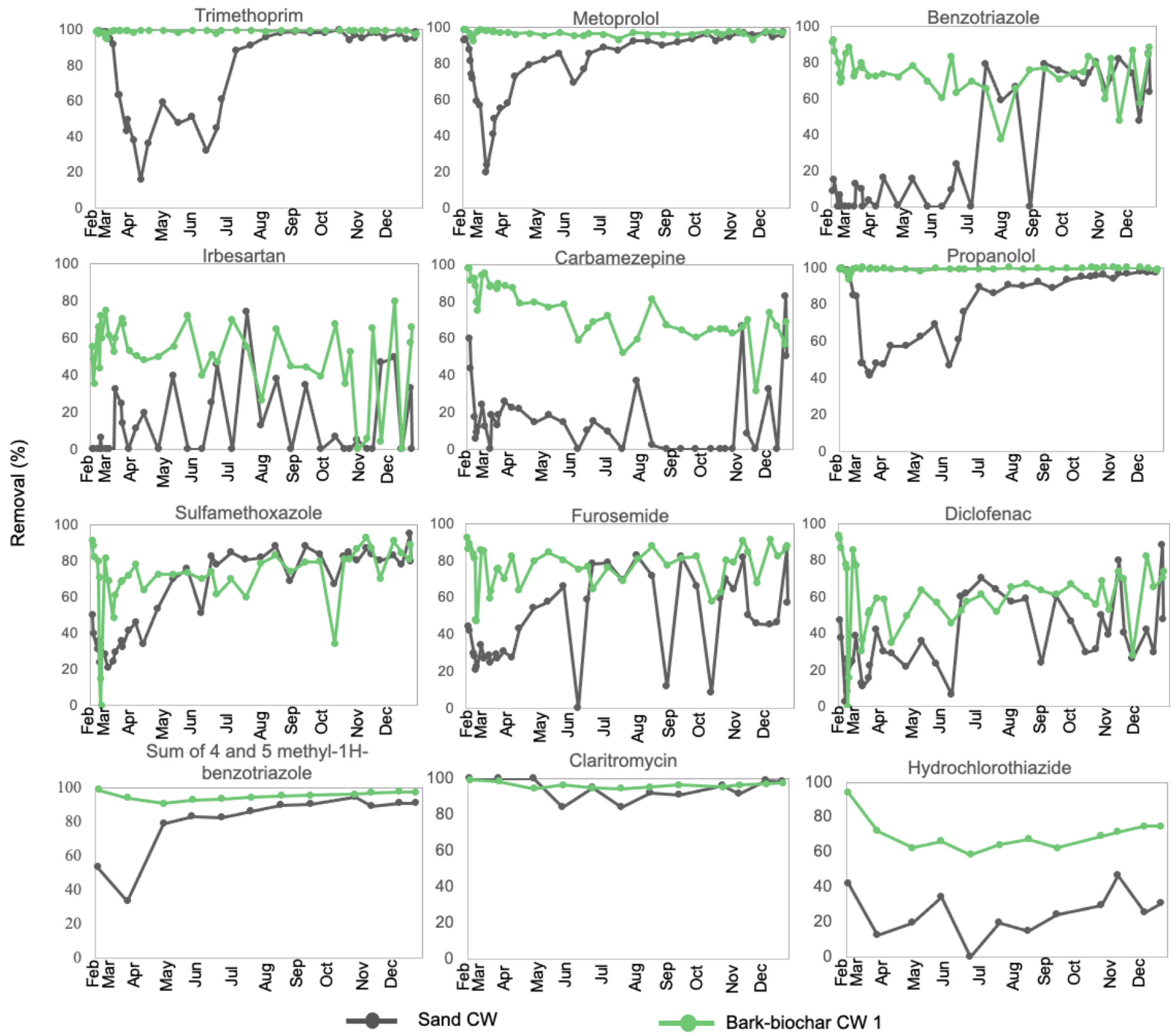


**Fig. 4.** Removal efficiency of 12 MPs in the sand CW and the bark-biochar CW 1. Negative removals were set to zero.

shows that using bark-biochar as support matrix avoids a long adaption period to achieve efficient benzotriazole removal in a CW. The main removal mechanisms of benzotriazole in CWs are adsorption, biodegradation and plant uptake, followed by photodegradation with a minor role (Wagner et al., 2020b). The efficient removal of benzotriazole at the start-up phase probably attributed to the high adsorption of bark-biochar to benzotriazole. Lei et al. (2021) reported the bark-biochar showed higher adsorption of benzotriazole (75%) than sand (8%) in abiotic column tests.

The presence of bark-biochar moderately improved the removal of furosemide and diclofenac in the CW ranging from 20% to 40% (median) compared to the sand CW (Fig. 3), with higher removal efficiencies found in the bark-biochar CW over time than the sand CW (Fig. 4). For furosemide and diclofenac, the positive effect of bark-biochar found in this study is opposed to the conclusion of mesocosm batch-operated CWs with no effect (Lei et al., 2022). This is explained by the effect of operation conditions. In the mesocosm batch-operated CWs, biological processes (e.g. biodegradation and plant uptake) play an important role in the removal of these two MPs due to ideal conditions with the optimal temperature, light and humidity etc. (Lei et al., 2022). In contrast, in an outdoor environment, the biological processes were probably affected by the seasonality and sorption became the main factor to influence the removal of these compounds. The bark-biochar showed better sorption of furosemide and diclofenac than the sand (Lei et al., 2021).

In general, the bark-biochar CW 1 showed only a slight improvement (median <10%) for the removal of trimethoprim, metoprolol, propranolol, sulfamethoxazole and the sum of 4 and 5 methyl-1H-benzotrizaole during 10 months operation compared to the sand CW (Fig. 3). The added value of bark-biochar seems limited for improving the removal of these MPs. However, considering the removal processes in 10 months, the bark-biochar CW showed a higher removal of these MPs in the first months of operation. After that, the removal efficiencies in the sand CW increased and reached a comparable removal efficiency as in the bark-biochar CW (Fig. 4), indicating that biological processes (e.g., biodegradation and plant uptake) play an important role in removing these MPs in the sand CW and need to be built up before high removal can be reached in the sand CW. The bark-biochar CW had immediate high removal for these compounds, most likely by sorption, and on the longer term also biological processes started to contribute to their removal.

Taking trimethoprim as an example, trimethoprim was highly removed by the bark-biochar CW 1 (median 100%) and the sand CW (median 95%) (Fig. 3). The bark-biochar CW 1 showed a stable and high removal of trimethoprim during 10 months while it was only moderately removed by the sand CW at the beginning phase of the operation (Fig. 4). After the beginning phase of the operation, both CWs showed a high removal of trimethoprim (close to 100%), which is in line with a high removal (100%) in the mesocosm batch-operated CW filled with bark-biochar or sand (Lei et al., 2022). A similar high removal of trimethoprim (>95% in summer and 89% in winter) was also reported in a sand continuous-operated CW by Rühmland et al. (2015). Sorption, biodegradation and plant uptake can attribute to trimethoprim removal in a CW. Trimethoprim was adsorbed by various adsorptive materials in abiotic columns (Lei et al., 2021) and on activated sludge (Li and Zhang, 2010). In the present CWs, the batch feeding offered possible aerobic condition. Trimethoprim could be removed by heterotroph nitrifying bacteria, which may degrade trimethoprim to $NH_4^+$ that is subsequently converted to $NO_3^-$ during a nitrification process (Khunjar et al., 2011; Liu et al., 2018). Trimethoprim could be taken up from water phase by plants, such as *Typha angustifolia* of mesocosm batch-operated CWs after treatment (Lei et al., 2022), Cabbage (*Brassica rapa var. pekinensis*) and Wisconsin Fast Plants (*Brassica rapa*) (Herklotz et al., 2010), and Pea (*P. sativum*) (Tanoue et al., 2012). Thus, plants in this study might also have contributed to the removal trimethoprim.

The bark-biochar CW 1 showed a similar removal of clarithromycin with the sand CW (close to 100%) during 10 months (Figs. 3 and 4), showing that using bark-biochar had no added value on the improvement of clarithromycin removal. The removal efficiencies of clarithromycin in this study are higher than that in a sand continuous-operated CW with 0% - 17% in summer and 89% in winter (Ruhmland et al., 2015). To date, the removal mechanisms of clarithromycin in CWs were not reported. In the present study, such high clarithromycin removal efficiencies (close to 100%) also occurred in the studied CWs during periods with non-optimal plant growth (before May and after October, Appendix F), indicating a minor role of plants in the removal of this compound. It is inferred that biodegradation and/or sorption are likely the main removal mechanisms for clarithromycin in the batch-operated CW systems. This study partly validates the speculation of Hijosa-Valsero et al. (2011) on this compound. These authors studied clarithromycin removal in diverse continuous-operated CWs, and suggested that photodegradation, algal interactions, sorption and/or a slow biodegradation are the main removal mechanism of this compound in CWs, with plants playing a minor role for its removal.

### 3.1.2. Bark-biochar constructed wetland combined with aeration

Continuous aeration at the bottom of the bark-biochar CW 2 was applied during phase 2. The DO of the wastewater was monitored at the outlet of the bark-biochar CW 1 and CW 2 (Fig. 5). After starting the aeration on ay 1, the DO in the effluent of the bark-biochar CW 2 was higher (max. 1.3 mg/L to 1.7 mg/L) than that in the bark-biochar CW 1 without aeration (max. 0 mg/L to 0.1 mg/L) (Fig. 5). This shows that the aeration improved DO in the wastewater of the bark-biochar CW 2. The

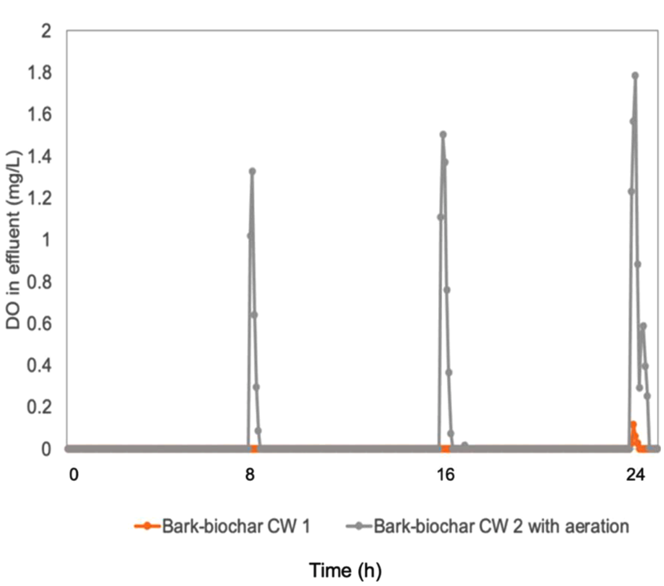


**Fig. 5.** Dissolved oxygen (DO, mg/L) in the effluent of the two bark-biochar CWs on Day 1 after starting aeration (22 October 2022) with 3 feeding cycles.

aeration used did not change the MP removal efficiencies in the bark-biochar CWs (Fig. 6). This is due to that the wastewater in the bark-biochar CW was not completely depleted in DO (the bark-biochar CW1 in Fig. 5) and therefore adding more oxygen had no effect on the aerobic biodegradation of MPs. The positive effect of aeration on improving MP removal from WWTP effluent was reported in gravel-based batch-operated CW, such Auvinen et al. (2017). In the present study, the used batch feeding mode offered an aerobic environment within the bark-biochar CWs, therefore biodegradation of MPs was not dependent on the increased availability of oxygen in the system.

## 3.2. Water quality parameters

The pH and electrical conductivity of the influent and effluent remained similar and stable during the operation of the three CWs (Table 1), indicating the operation stability of the systems.

The bark-biochar CW 1 showed a higher TN removal (50%) than the sand CW (23%) (Table 1). This shows that the addition of bark-biochar had a positive effect on TN removal in a batch-operated CW. Ajibade et al. (2021) reported that incorporating biochar to sand was able to improve nitrogen removal in batch-operated CWs from secondary effluent, as the presence of biochar enhanced the activities of nitrogen removal related microorganisms. Aeration did not result in a significant change of TN removal (Table 1). This is due to that the used batch feeding offered an aerobic environment in the CW system and adding more oxygen did not change TN removal in the bark-biochar CW.

The sand CW removed 51% of the TP, while negative TP removal efficiencies were observed in the bark-biochar CWs (Table 1). Nevertheless, the concentration of TP in the CW effluents (0.1 – 0.8 mg/L) was below the emission standard (2 mg/L) in the Netherlands for WWTPs with 2000 to 100,000 population equivalents (Kenniscentrum InfoMil, 2014). This negative removal means that the bark-biochar released phosphorus to the treated wastewater. Biochar, bark and sand contain phosphorus, respectively 21,473 mg/kg, 117 mg/kg and 57 mg/kg phosphorus (Appendix G), indicating that phosphorus release from biochar can have a big impact on the TP concentration. This release of phosphorus from the bark-biochar CWs reduced over time (Appendix H). A similar phosphorus releasing trend was also reported for biochar made of oak, pine and grass in batch experiments (Mukherjee and Zimmerman, 2013).

All three studied CWs showed a similar performance on TOC removal of 40% (Table 1), showing that bark-biochar and aeration had no significant effect on TOC removal.

## 3.3. Implications for application

A year-round removal performance monitoring is conducive to comprehensively evaluate the highest and lowest treatment performance and the system sensitivity to deal with various operation conditions (Kahl et al., 2017). This study shows that the CW filled with bark-biochar increased the removal of most MPs studied and TN in a long-term operation compared to the CW filled with the conventional material sand. Reproducible removal efficiencies were gained in the bark-biochar CWs, which shows a stable and constant ability to deal with the change of the composition of pollutants in real operations.

A longer monitoring is still needed to further study the interaction between the phosphorus release and the CW functioning. Meanwhile, the biochar made by other biomass without phosphorus release can also be considered as a candidate to replace the current biochar used. Except for animal fecal biochar, biochar also includes other types, such as straw biochar, shell biochar, wood biochar and bamboo biochar (Dai et al., 2019). Adding a post-treatment or replacing the current regular sand to a new type of sand functionalized to enhance binding phosphorus, such as iron oxide-coated sand (Boujelben et al., 2008; Huang et al., 2014), can also be alternative solutions to remove the released phosphorus. Thus, more investigations are still important.

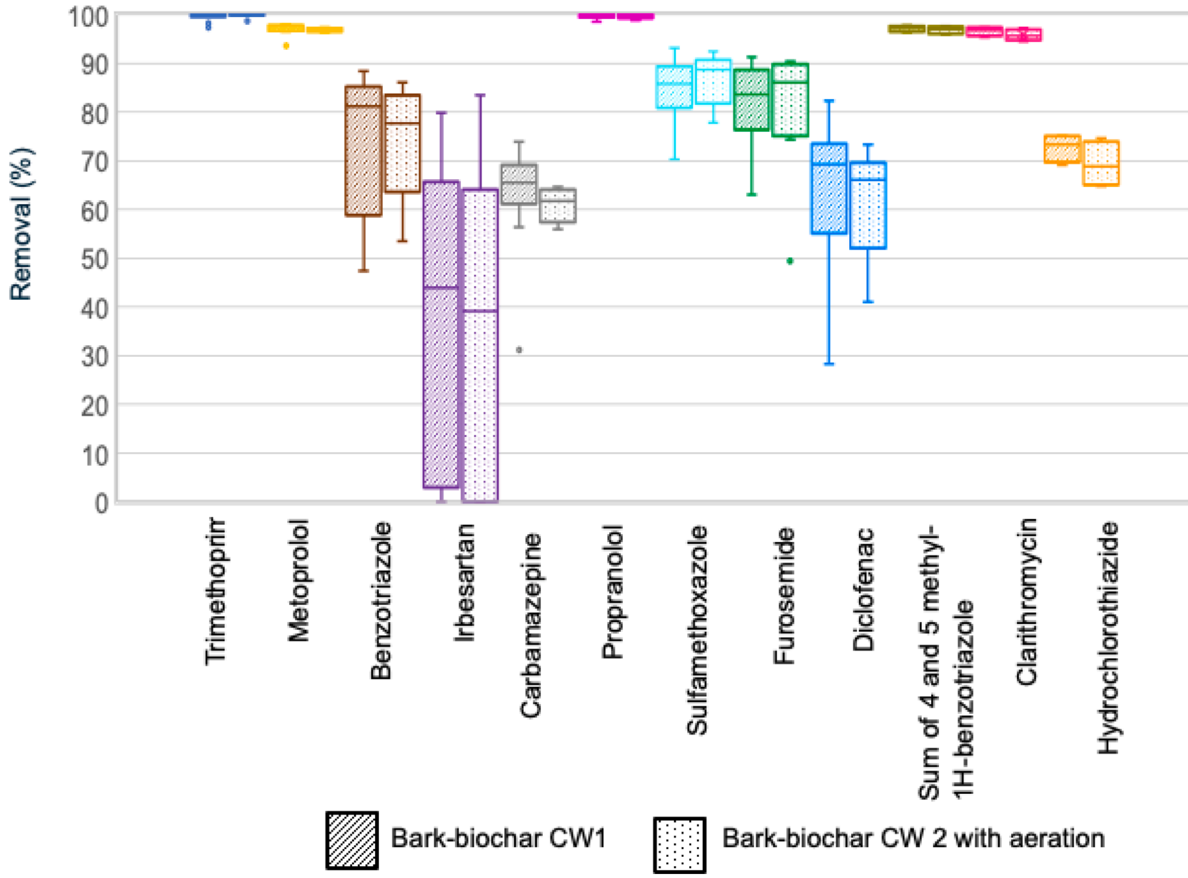


**Fig. 6.** Removal efficiencies of 12 MPs in the bark-biochar CW 1 without aeration and the bark-biochar CW 2 with aeration. The box plot shows the values: minimum, first quartile (Q1), median (horizontal line, Q2), third quartile (Q3), maximum and outliers (·). Negative removals were set to zero.

**Table 1**
Water quality parameters in influent and effluent of the three pilot CWs.

| Parameters | February to December 2021 Influent | | | Effluent | | Average removal efficiency | | October to December 2021 Effluent | | Average removal efficiency | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sand CW | Bark-biochar CW 1 | Bark-biochar CW 2 | Sand CW | Bark-biochar CW 1 | Sand CW | Bark-biochar CW 1 | Bark-biochar CW 1 without aeration | Bark- biochar CW 2 with aeration | Bark- biochar CW 1 without aeration | Bark- biochar CW 2 with aeration |
| **pH** [a] | 7.2 ± 0.2 | 7.2 ± 0.2 | 7.2 ± 0.2 | 7.6 ± 0.2 | 7.5 ± 0.2 | – | – | 7.5 ± 0.2 | 7.3 ± 0.2 | – | – |
| **Conductivity (μS/cm)** [a] | 537 ± 153 | 536 ± 155 | 539 ± 156 | 594 ± 130 | 578 ± 117 | – | – | 493 ± 87 | 477 ± 90 | – | – |
| **TN (mg/L)** [b] | 8 ± 7 | 8 ± 6 | 8 ± 6 | 6 ± 5 | 4 ± 3 | 23 ± 20%[d] | 50 ± 23%[d] | 2 ± 1 | 2 ± 1 | 45 ± 21%[d] | 38 ± 20%[d] |
| **TP (mg/L)** [b] | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.2 | 0.1 ± 0 | 0.8 ± 0.3 | 51 ± 36%[d] | −885 ± 993% | 0.5 ± 0.1 | 0.4 ± 0 | −154 ± 77% | −93 ± 52% |
| **TOC (mg/L)** [c] | 11 ± 4 | 11 ± 7 | 11 ± 4 | 7 ± 3 | 7 ± 3 | 36% | 34% | 5 ± 1 | 5 ± 1 | 48% | 41% |

[a] : $n = 53$.
[b] : $n = 33$.
[c] : $n = 40$.
[d] : Negative removal efficiencies were set to zero.

## 4. Conclusions

The present study demonstrates that the addition of bark and biochar as a support matrix strongly improved the removal of most studied MPs in a batch-operated CW, giving a more stable and robust removal in the start-up phase as well as in the phase with full-grown plants. No additional removal was accomplished by aeration measures, where it should note that the treated effluent had a dissolved oxygen level by itself. From the other water quality parameters monitored in the CW influent and effluent, the addition of bark and biochar to the CW promoted the removal for TN compared to the sand CW, but not for TOC and TP. Additional CW optimization measures are needed to mitigate the leaching of TP out of the bark-biochar matrix.

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## CRediT authorship contribution statement

**Yu Lei:** Funding acquisition, Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft. **Thomas Wagner:** Supervision, Methodology, Visualization, Writing – review & editing. **Huub Rijnaarts:** Supervision, Conceptualization, Methodology, Writing – review & editing. **Vinnie de Wilde:** Methodology, Writing – review & editing. **Alette Langenhoff:** Funding acquisition, Project administration, Supervision, Conceptualization, Methodology, Visualization, Writing – review & editing.

## Declaration of Competing Interest

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

## Data Availability

Data will be made available on request.

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## Supplementary materials

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