Constructed wetlands operated as bioelectrochemical systems for the removal of organic micropollutants

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Abstract

The removal of organic micropollutants (OMPs) has been investigated in constructed wetlands (CWs) operated as bioelectrochemical systems (BES). The operation of CWs as BES (CW-BES), either in the form of microbial fuel cells (MFC) or microbial electrolysis cells (MEC), has only been investigated in recent years. The presented experiment used CW meso-scale systems applying a realistic horizontal flow regime and continuous feeding of real urban wastewater spiked with four OMPs (pharmaceuticals), namely carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBU) and naproxen (NPX). The study evaluated the removal efficiency of conventional CW systems (CW-control) as well as
CW systems operated as closed-circuit MFCs (CW-MFCs) and MECs (CW-MECs).

Although a few positive trends were identified for the CW-BES compared to the CW-control (higher average CBZ, DCF and NPX removal by 10-17% in CW-MEC and 5% in CW-MFC), these proved to be not statistically significantly different. Mesoscale experiments with real wastewater could thus not confirm earlier positive effects of CW-BES found under strictly controlled laboratory conditions with synthetic wastewaters.

Keywords
Emerging contaminants, meso-scale setup, microbial electrolysis cells, microbial fuel cells, real urban wastewater

1. INTRODUCTION
Micropollutants can be defined as organic and inorganic substances of anthropogenic origin, with the potential of causing negative effects for the environment already at very low concentrations, i.e. in the order of micro, nano or pico-grams (Chapman, 1992; Stamm et al., 2016). Organic micropollutants (OMPs) include a large array of substances, such as pharmaceuticals, personal care products (PPCPs), hormones, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), endocrine-disrupting chemicals (EDCs) or pesticides (Bolong et al., 2009).

The four OMPs investigated in this study, i.e. diclofenac (DCF), ibuprofen (IBU), naproxen (NPX) and carbamazepine (CBZ), were all pharmaceuticals and chosen due to their high occurrence in raw wastewaters and/or their low or moderate removal rates in conventional activated sludge (CAS) wastewater treatment plants (WWTPs) (Gros et al.,
2012, 2010; Mamo et al., 2018). So far there are no legal discharge limits for OMPs, but some regulations such as the European Decision 2015/495/EU of 20 March 2015 (amending earlier regulations), which included DCF as well as several antibiotics, hormones and pesticides (Barbosa et al., 2016).

A potentially more efficient and less energy intensive alternative to CAS WWTPs are Constructed Wetlands (CWs), which showed promising results regarding OMPs removal (Ávila et al., 2014b, 2014a; Matamoros et al., 2015). The removal efficiency of OMPs in CWs varies with design, operation and type of CW (e.g. surface, subsurface vertical/horizontal flow) employed. In the case of subsurface horizontal flow (HF) CWs, the removal of OMPs ranges from poor to very efficient, depending on characteristics such as bed depth, media size, loading frequency or potential clogging (Ávila et al., 2014b; Matamoros and Bayona, 2006). Various CW intensification strategies have been developed over the last decades and were also tested for the treatment of OMPs, with promising results especially for biodegradable OMPs, but further research is still needed (Ávila et al., 2014b; Nivala et al., 2019; Zhang et al., 2014).

A relatively recent development in the field of wastewater treatment is based on coupling CWs with bioelectrochemical systems (BES) such as Microbial Fuel Cells (MFCs) (Villaseñor et al., 2013; Yadav et al., 2012) and Microbial Electrolysis Cells (MECs) (Ju et al., 2014) called CW-MFC and CW-MEC, respectively, from here on. MFCs use electrochemically active bacteria (EAB) (also known as exoelectrogens, electrogens, electricogens, exoelectrogenic or anode respiring bacteria) as catalysts in order to produce current from the oxidation of organic/inorganic compounds (Logan et al., 2006). These EAB are able to transfer electrons in and out of their cell in a process called
extracellular electron transfer (EET) and need a redox-gradient between the MFC electrodes to produce a current. Such a gradient occurs naturally in CW systems. MECs are a modification of MFCs, with the main difference that an external power source is supplied to control the potential between the electrodes, i.e. the anode and cathode, and thereby they are able to achieve thermodynamically reactions, which are otherwise unfavourable (Rozendal et al., 2006). Another advantage of MECs is that only an additional voltage of 0.2-0.8 V is required for water electrolysis to occur (usually 1.8-3.5 V are required), due to the current produced through the activity of EAB at the anode. Both technologies, MFCs and MECs, are able to use wastewater as a substrate and remove a variety of contaminants in the process, showing promising results for the removal of OMPs (Cecconet et al., 2017).

Up to date, there are only a few publications dealing with the use of CW-MFC for OMP removal, and to the authors’ knowledge, there are none on the use of CW-MEC systems. Generally, CW-MFC systems have been described to increase the microbial activity (determined by means of fluorescein diacetate (FDA) hydrolysis) in CW-MFC (Hartl et al., 2019), and some studies showed that CW-MFC enhance microbial community richness and diversity as compared to an open-circuit control (Song et al., 2018; F. Xu et al., 2018a). Earlier studies of CW-BES or BES systems for OMP removal used artificial wastewater, which is advantageous for the study of fundamental processes, but less realistic than real urban wastewater (Li et al., 2019; Pun et al., 2019; Wang et al., 2015). The present experiment used CW meso-scale systems which, despite being unplanted, were intended to give additional information on OMP removal in larger scale CW-BES systems with a more realistic horizontal flow, continuous feeding of real urban
wastewater and realistic spiking concentration levels of OMPs. Additionally, to the best
knowledge of the authors this is the first publication on OMP removal in CW-MEC, and
consequently also the first one to compare OMP removal efficiency of CW-MFC and CW-
MEC side by side. To this end, duplicate systems with conventional CW (CW-control),
closed-circuit CW-MFC (CW-MFC) as well as CW-MEC (CW-MEC) configuration have
been used.

The hypothesis was that CW-MEC and CW-MFC will improve organic micropollutants
removal as compared to the CW-control system.

2. MATERIALS AND METHODS

2.1 General design

For the purpose of this work, six unplanted meso-scale horizontal subsurface flow (HF)
systems were used (a duplicate of systems per treatment: two systems per CW-control,
CW-MFC and CW-MEC). The setup of these systems is detailed in a previous study (Hartl
et al., 2019). Briefly, the systems consisted of a PVC reservoir of ca. 0.2 m² (55 cm length
x 35 cm width) surface area filled up with 4/8 mm granitic riverine gravel. The systems
were not planted to avoid an additional influencing parameter and further increase the
experimental complexity. Wetted depth was set to be 25 cm. At the inlet and around
the drainage of the outlet 7/14 mm granitic riverine gravel was used.

Both, the CW-MFC and CW-MEC were designed as three independent BES (MFCs or
MECs), respectively, along the length of each system (see Figure 1). Each BES electrode
consisted of a gravel-anode with four stainless steel mesh rectangles (Figure 1, C) (SS
marine grade A316L, mesh width=4.60 mm, Øwire=1.000 mm, S/ISO 9044:1999) in series acting as electron collectors (4 cm away from each other). The anode area considered for current density calculations was the surface area (0.04 m²) of the anode.

Each metal mesh covered nearly the whole cross-sectional area (0.08 m²) of the CW.

Each cathode consisted of a carbon felt mat (Figure 1, D) (1.27 cm thick, with a surface area of 0.03 m², 99.0% carbon purity). A layer of glass wool was placed underneath the cathodes in order to avoid any oxygen leaking from the cathode down to the anode as recommended elsewhere (Venkata Mohan et al., 2008).
Figure 1. Section- (top) and plan-view (bottom) of the CW-BES systems. A: Pump; B: Inflow; C: Anode; D: Cathode; E/F: Anode/Cathode connection to datalogger; G: Inflow barrier to avoid water short-circuiting on surface; H: Gravel core sampling tubes; I: Liquid sampling tubes; J: Water level; K: Standing pipe effluent; L: Drainage; M: Effluent collection tube, N: Reference electrode.

CW-MFC were operated at closed-circuit by connecting the anode and cathode over a wire and a 220Ω resistor following the recommendations of Corbella and Puigagut (2018). The voltage across the external resistor for each electrode was continuously monitored by means of a datalogger (Campbell Scientific CR1000, AM16/32B Multiplexor). The other two CW-BES systems were operated as CW-MEC by connecting potentiostats to each electrode. CW-MEC systems had the same setup as the CW-MFC.
but with an additional reference electrode placed near the respective transects anode (Figure 1, N). Each MEC was poised at a potential of 0.3 V vs Ag/AgCl at the anode using a potentiostat (nanoelectra NEV 4). The conventional HF CW-control systems contained no anode metal meshes or electrical connections, but only the cathode carbon felt in order to not confuse physical filtration effects of the carbon felt with BES effects.

2.2 Operational conditions

The experimental CWs were mature at the time this work was conducted. The systems of all three treatments were in operation and fed with real urban wastewater already for about 18 months. CW-MFC and CW-control were in the same operation mode throughout the whole time, but CW-MEC systems were operated in CW-MEC mode for the last 9 months leading up to the experiment (the 9 months before that, CW-MEC systems were also run as CW-MFC systems during earlier experiments). During the experiment, the systems were fed with fresh pre-settled urban wastewater every weekday. Influent wastewater was spiked with the target OMPs at a final concentration of 4 µg/L for 4 weeks. Samples for OMP analyses were taken after one week of the start of daily OMP dosing (which represents a bit less than two times the nominal HRT in order to ensure that the OMPs had reached the outlet of the CW during sampling).

Further details on pre-treatment (settling for 3 hours) and operation are given in Hartl et al. (2019). The average hydraulic loading rate (HLR) applied during the experiment was 28 mm/d, resulting in a nominal HRT of 3.6±0.3 days and an average organic loading rate (OLR) of 8.7±2.5 g COD/m² day.
2.3 Sampling and analysis

2.3.1. Water quality parameters

Eight sampling campaigns for the characterization of conventional wastewater quality parameters were conducted during 12 weeks. These campaigns were conducted already 3 weeks before OMP sampling started, and continued during the OMP sampling period, whereas conventional wastewater samples were taken just before the OMP dosing on weekdays. Conventional wastewater parameters were measured for the influent, after the first and second third of the wetland length, and as also at the effluent. All samples were analysed for total suspended solids (TSS), volatile suspended solids (VSS) and total chemical oxygen demand (COD) according to Standard Methods (APHA-AWWA-WEF, 2012); NH$_4^+$-N, according to Solórzano method (Solórzano, 1969); NO$_2^-$-N, NO$_3^-$-N, SO$_4^{2-}$-S and PO$_4^{3-}$-P by ion chromatography (ICS-1000, Dionex Corporation, USA). Physical parameters such as water temperature, dissolved oxygen (DO) concentration and pH were measured directly in the influent, using portable devices after the first and second transect, as well as in the effluent (EcoScan DO 6, ThermoFisher Scientific, USA and CRISON pH/mV – meter 506, Spain, respectively). Further details are given in Hartl et al. (2019).

2.3.2. OMP analysis

High purity standards (>99%) of the parent compounds and the isotopically labelled compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA). Detailed information on their physical and chemical characteristics is given in Table S1 of the Supplementary Information (SI). Standard solutions of the mixtures of the four compounds were made at the appropriate concentrations and used to dope the influent...
wastewater. Five OMP sampling campaigns were conducted during 3 weeks. Grab samples were taken from the CW influent and effluent sampling points (see Figure 1, points B and K, respectively). All water samples were filtered and processed using an adapted methodology by Matamoros and Bayona (2006). Briefly, 50 mL of influent and 100 mL of effluent samples were filtered (0.7 μm Whatman™ glass microfiber filters GF/F), acidified to pH 2-3 with HCl (0.02M) and spiked with a mixture of surrogate standards to a final concentration of 50 ng L⁻¹ (atrazine-d₅, mecoprop-d₃, tonalide-d₃, and dihydrocarbamazepine). Solid phase extraction was then performed, using 200 mg Strata™-X polymeric cartridges from Phenomenex (Torrance, CA, US), previously conditioned with 3 mL of hexane, 3 mL of ethyl acetate, 5 mL of MeOH and 5 mL of acidified milli-Q water. Elution was performed with 10 mL of hexane/ethyl acetate (1:1, v:v). The eluted extract was evaporated under a gentle nitrogen stream to a volume of 100 μL, and triphenylamine was added as an internal standard (20 ng). Finally, vials were reconstituted to 300 μL and analysed by GC-MS/MS as described by Matamoros et al. (2017).

### 2.4 Data Analysis

Contaminant removal efficiencies were calculated on a mass balance basis taking into account the wastewater flow and pollutant concentration. Statistical analyses were conducted for comparison of the relevant parameters COD, NH₄⁺-N, SO₄²⁻-S, PO₄³⁻-P, pH and current density as well as the OMPs CBZ, DCF, IBU and NPX across the three treatments. Since Shapiro-Wilk tests showed a normal distribution for all data, single-factor analysis of variance (ANOVA) could be used on all the above mentioned
parameters. Post-hoc Tukey HSD and Scheffé multiple comparison tests were performed for all parameters but only presented when relevant, i.e. in the case that ANOVA reported significant differences between treatments, thus only in the case of pH. The software used for calculations and statistical analysis was Microsoft® Excel® 2016 and the included Analysis ToolPak add-in.

3. RESULTS AND DISCUSSION

3.1 Electrical behaviour

Table 1 shows average and maximum measured cell voltages (E_{cell}) as well as consequent current and power densities per surface area and anodic compartment volume for CW-MFC treatments in all 3 transects.

Table 1. Average, standard deviation and maximum for E_{cell} as well as current density and power density of closed-circuit CW-MFC systems. Note: The surface area of each electrode was used for current density calculations.

| Transect | E_{cell} (mV) | Current Density per Area (mA/m²) | Current Density per Volume (mA/m³) | Power Density per Area (mW/m²) | Power Density per Volume (mW/m³) |
|----------|---------------|----------------------------------|-----------------------------------|-----------------------------|-------------------------------|
|          | Avg ± SD      | Max                              | Avg ± SD                          | Max                         | Avg ± SD                      | Avg ± SD                      |
| 1        | 372±119       | 552                              | 40±13                             | 60                          | 183±59                        | 273                          |
| 2        | 378±81        | 577                              | 41±9                              | 62                          | 186±41                        | 282                          |
| 3        | 372±128       | 711                              | 40±14                             | 77                          | 183±64                        | 350                          |

Average current densities per surface area for CW-MFC (all transects considered) resulted in 40 mA/m². Differences in current density between transects were not statistically significant according to ANOVA. A polarization curve (PC) analysis (see SI, Figure S1) showed that maximum power densities of 30, 11 and 24 mW/m² in transect
1, 2 and 3 of CW-MFC mode, respectively, were achieved at current densities of 79, 35 and 66 mA/m$^2$, respectively, which is higher than that described by Saz et al. (2018) (ca. 20 mA/m$^2$) under comparable conditions.

The estimated internal resistances derived from the polarization curves were around 108 Ω, 220 Ω and 124 Ω for first, second and third transect, respectively. Principally, the potential maximum power is achieved when internal and external resistances are close to each other (Lefebvre et al., 2011). Coincidentally, the external and internal resistance were exactly the same in transect 2. However, for the current experiment and its primary goal contaminant removal the lower external resistances in transects 1 and 3 could have been beneficial, since lower external resistances increase the generated current and studies have also shown that consequently organic matter removal was increased (Aelterman et al., 2008; Gil et al., 2003; Katuri et al., 2011).

The coulombic efficiency (CE) is the proportion of the produced current to the carbohydrates which are theoretically derived from oxidation, indicated by the change of COD from transect to transect (Scott, 2016). The resulting average CE values amounted to 1.4±2.4%, 9.5±7.6% and -29.4±4.6%, for transects 1, 2 and 3, respectively. Note that CE can have a negative value when COD concentrations were increasing from the influent to the end of transect 1 or from one transect to the other. Generally, it can be assumed that only the CE value measured in transect 1 gives a good indication since not only organic matter from the influent can contribute to the MFC signal but also accumulated organic matter within the gravel bed is a fuel source for MFC (Corbella et al., 2016). Therefore, CE in transect 2 could be higher than transect 1 and transect 3 CE.
even negative on average. Comparable CW-MFC studies produced CEs from 0.01‰ (Wang et al., 2016b) up to 16.4% (Xie et al., 2018).

Table 2 shows the poised potential and the resulting achieved average current as well as average current and power densities per electrode surface area and anodic compartment volume for each transect in CW-MEC systems.

Table 2. Poised potential (at Anode vs. Ag/AgCl reference electrode) as well as resulting average current applied also expressed in current and power density per surface area and volume in CW-MEC (MEC)

| Transect | Poised Potential (V) | Current (mA) Avg ± SD | Current Density per Area (mA/m²) Avg ± SD | Current Density per Volume (mA/m³) Avg ± SD | Power Density per Area (mW/m²) Avg ± SD | Power Density per Volume (mW/m³) Avg ± SD |
|----------|----------------------|------------------------|-------------------------------------------|-------------------------------------------|----------------------------------------|----------------------------------------|
| 1        | 0.3                  | 23±11                  | 535±263                                   | 2434±1197                                 | 161±79                                 | 730±359                                 |
| 2        | 0.3                  | 10±5                   | 223±112                                   | 1015±510                                  | 67±34                                  | 304±153                                 |
| 3        | 0.3                  | 5±3                    | 120±74                                    | 545±334                                   | 36±22                                  | 163±100                                 |

The poised potential of 0.3 V vs. Ag/AgCl reference electrode at the anode, was chosen on the basis of experiences showing that poised potential around this value benefit the growth of electrochemically active bacteria (EAB) genera such as Geobacter in mixed bacterial cultures (Fricke et al., 2008; Liu et al., 2008). The average current density in CW-MEC was more than double in transect 1 compared to transect 2, and transect 2 was again roughly double of transect 3, assumingly because the organic matter concentration was decreasing along the flow path through the systems.

The CW-MEC current densities in all three transects were low when compared to other similarly built CW-MEC systems which showed values ranging from 200 to 24500 mA/m² (Gao et al., 2017; Srivastava et al., 2018; Xu et al., 2017; Zhang et al., 2018). Authors
believe that the use of non-conductive media (gravel) as anode material is the main cause of the lower observed current densities in the CW-MEC systems because this increases the internal resistance of the systems.

3.2 Removal efficiency of conventional wastewater quality parameters

Results on the removal of conventional contaminants in all three treatments (CW-control, closed-circuit CW-MFC and CW-MEC systems) are summarized in Table 3. All results were obtained during 8 weeks of intensive sampling (5 weeks before the OMP sampling campaigns and the three weeks during the OMP sampling campaign). Data is shown as average mass loading rate at the system inlet (influent), after the first and second transects and effluent, as well as mass removal rate from influent to effluent based on the average mass and percentage. During this period, all systems received continuous flow with an average OLR of 8.7±2.5 g COD/m².day.
Table 3. Results for COD, ammonium, sulphate and orthophosphate for CW-control, closed-circuit CW-MFC and CW-MEC systems during the 8 sampling weeks, expressed as average mass loading rate at influent, after first transect, after second transect and effluent as well as removal from influent to effluent based on the average mass removal rate and percentage.

|                  | Influent | 1/3     | 2/3     | Effluent   | Removal from Influent to Effluent |
|------------------|----------|---------|---------|------------|-----------------------------------|
|                  | (g/m².d) | (g/m².d)| (g/m².d)| (%)        |                                   |
| COD (n=8)        |          |         |         |            |                                   |
| CW-control       | 8.6±2.6  | 4.4±1.9 | 3.7±2.2 | 3.7±1.6    | 4.9±1.4                           | 57                     |
| CW-MFC           | 8.9±2.4  | 4.4±2.3 | 3.8±2.3 | 4.0±1.5    | 4.9±0.5                           | 55                     |
| CW-MEC           | 8.7±2.5  | 3.9±2.3 | 2.5±1.4 | 2.6±1.0    | 6.1±0.8                           | 70                     |
| NH₄-N (n=7)      |          |         |         |            |                                   |
| CW-control       | 1.2±0.4  | 1.0±0.4 | 0.9±0.3 | 1.1±0.3    | 0.1±0.2                           | 10                     |
| CW-MFC           | 1.3±0.4  | 1.0±0.3 | 0.8±0.2 | 1.0±0.3    | 0.3±0.2                           | 24                     |
| CW-MEC           | 1.2±0.4  | 0.9±0.3 | 0.7±0.2 | 0.9±0.2    | 0.3±0.3                           | 28                     |
| SO₄²⁻ (n=6)      |          |         |         |            |                                   |
| CW-control       | 2.0±1.3  | 0.5±0.5 | 0.4±0.3 | 0.8±0.6    | 1.1±0.9                           | 58                     |
| CW-MFC           | 2.1±1.4  | 0.6±0.4 | 0.6±0.4 | 1.1±0.9    | 1.0±0.3                           | 48                     |
| CW-MEC           | 2.2±1.4  | 0.8±0.7 | 1.0±0.8 | 1.1±0.9    | 1.1±0.8                           | 51                     |
| PO₄³⁻-P (n=6)    |          |         |         |            |                                   |
| CW-control       | 0.09±0.05| 0.09±0.06|0.09±0.05|0.09±0.05 | 0.09±0.05                        | 2                      |
| CW-MFC           | 0.09±0.05| 0.09±0.06|0.08±0.05|0.08±0.05 | 0.08±0.05                        | 7                      |
| CW-MEC           | 0.09±0.05| 0.07±0.06|0.06±0.05|0.08±0.05 | 0.08±0.05                        | 7                      |

* Some experimentation weeks could not be considered due to highly diluted influent or technical analysis problems

In contrast to previous studies done on the same experimental systems study (Hartl et al., 2019), ANOVA reported no statistically significant differences for general wastewater quality parameters, with the exception of pH (see SI, Table S2 and S5). NH₄⁺-N removal was generally low, and decreased towards the end of the study period, which was also observed for COD, although to a lesser extent. It is assumed that ageing and possible partial clogging of the carbon felt cathodes might have limited removal performance of CW-MFC systems.

Indeed, results from MFC studies (Kim et al., 2008; Yan et al., 2012; Yuan et al., 2021) suggested that their single-chamber air-cathode MFC system provided a suitable microenvironment for biological ammonia oxidation whereas the ammonia oxidizing bacteria (AOB) found in the cathode biofilm derived oxygen via diffusion through the...
cathode. So the observed partial clogging of the cathode could have limited the activity of AOB at the cathode, and as a consequence also have reduced nitrification and NH$_4^+$-N removal. In the case of NH$_4^+$-N, it could also be assumed that due to the system ageing and the accompanying development and establishment of the microbial communities, the systems turned more anaerobic, which in turn would have lowered nitrification rates.

For both COD and NH$_4^+$-N removal, the lack of plants in the meso-scale systems could have had an effect as well on overall treatment efficiencies, since the presence of plants has shown to improve treatment efficiency in HF CWs (Tanner, 2001). NO$_2^-$-N and NO$_3^-$-N were generally below the limit of detection. A recent study showed that planted CW-MFC systems show higher power density and contaminant removal, however, dead plant parts in turn also reduced the power production (Yang et al., 2019).

Generally, PO$_4^{3-}$-P removal efficiency was lower when compared to current literature regarding CW-MFC (Corbella and Puigagut, 2018; Saz et al., 2018; Xu et al., 2018; Yakar et al., 2018) or CW-MEC (Gao et al., 2018; Ju et al., 2014; Zhang et al., 2018) systems. However, many studies were conducted only over a short-time and it is generally known that phosphorus storage in subsurface flow CWs has a finite capacity and therefore removal by sorption normally decreases over time (Kadlec and Wallace, 2009), as could have been the case in this study as the wetlands were operated for about 18 months.

The average values for pH measurements at each sampling point are shown in SI, Table S2. The ANOVA results for influent and average pH values of all sampling points were statistically not significantly different across treatments. However, after the first transect, CW-MEC systems showed a lower pH than other treatments on average, being
significantly different from CW-MFC as well as CW-control according to ANOVA, with both, post-hoc Tukey HSD and Scheffé pairwise comparison showing CW-MEC to be either significantly or very significantly different to CW-MFC and CW-control (for more details see SI, Figures S3 and S4). After the second transect, pH values of all three treatments were significantly different from each other according to ANOVA, with CW-MEC showing the lowest pH, followed by a higher pH in CW-MFC and the highest in CW-control (meaning the smallest change since the influent inlet in the system). Post-hoc Tukey HSD and Scheffé pairwise comparison of pH after the second third showed very significant differences between all treatments (for more details see SI, Figures S3 and S4). pH values at the effluent were generally higher than in the previous two transects within the systems, and the difference between treatments was again only statistically different in the CW-MEC systems. Post-hoc Tukey HSD and Scheffé pairwise comparison of pH at the effluent showed CW-MEC to be very significantly different to CW-MFC and CW-control (for more details see SI, Figures S3 and S4).

Changes in pH within the system might affect the activity of bacteria, and influence the charge state as well as hydrophobicity of certain OMPs (Wang et al., 2015). While the measured pH in solution showed some significant differences between treatments, the changes seemed not big enough to alter the charge state and hydrophobicity of the investigated OMPs significantly, especially in the case of CBZ with its high pKₐ of 13.9 (see SI, Table S1). However, pH at the micro-scale, e.g. near the cathode or anode, might have changed more drastically, and could have created micro-environments in which charge state and/or hydrophobicity were influenced. Unfortunately, it was not possible to measure these changes in pH on a micro-scale with the presented setup.
3.3 Removal efficiency of organic micropollutants

Table 4 shows the removal of the four targeted OMPs for all three treatments (see also SI, Figure S2 for box- and whisker plots).

| OMP (n=5) | Background (µg/L) | Influent (µg/L) | Treatment | Effluent (µg/L) | Effluent (µg/m².d) | Removal (µg/m².d) (%) |
|-----------|-------------------|----------------|-----------|----------------|-------------------|----------------------|
| CBZ       | 3.5±2.2           | 5.3±2.2        | CW-control | 4.6±1.4        | 123±41            | 26                   |
|           |                   | 149±61         | CW-MFC    | 4.3±1.0        | 116±26            | 33                   |
|           |                   |                | CW-MEC    | 3.7±0.8        | 99±24             | 50                   |
| DCF       | 0.6±0.3           | 4.2±1.9        | CW-control | 2.7±1.4        | 73±17             | 65                   |
|           |                   | 137±56         | CW-MFC    | 2.2±1.0        | 65±20             | 72                   |
|           |                   |                | CW-MEC    | 2.2±0.8        | 59±16             | 79                   |
| IBU       | 12.6±3.6          | 18.6±8.8       | CW-control | 12.0±2.0       | 321±53            | 202                  |
|           |                   | 523±202        | CW-MFC    | 12.6±1.7       | 341±40            | 182                  |
|           |                   |                | CW-MEC    | 12.0±2.2       | 320±52            | 202                  |
| NPX       | 3.8±0.7           | 10.2±1.4       | CW-control | 7.6±2.4        | 203±62            | 70                   |
|           |                   | 273±29         | CW-MFC    | 7.1±2.0        | 191±50            | 82                   |
|           |                   |                | CW-MEC    | 6.1±1.5        | 163±37            | 109                  |

Table 4. Results for OMPs carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBU) and naproxen (NPX) in CW-control, closed-circuit CW-MFC and CW-MEC systems during the 5 sampling campaigns, expressed as average background, influent and effluent concentration, average mass loading rate at influent and effluent as well as removal from influent to effluent based on the average mass removal rate and percentage. (Concentration variability in the influent concentrations is due to the background concentration of the urban wastewater for each of the compounds).

Similar as for the general wastewater parameters, removal differences across treatments were not statistically significant for any of the four compounds (see SI, Table S6). Nevertheless, few tendencies were visible which are discussed further below.
Carbamazepine

The CW-control system removal of 17% is in accordance with results of previous studies on treatment capacity in conventional HF CW systems (not operated as BES), reporting removals of 13% (Nivala et al., 2019) and 21% (Matamoros et al., 2017). These results show that CBZ can be removed to a certain degree in HF CWs (supposedly due to anaerobic processes), however, CBZ is not biodegradable in aerobic conditions and therefore VF CWs show lower removal rates (Hai et al., 2011; Jekel et al., 2015; König et al., 2016; Nivala et al., 2019).

The only other study looking at CBZ removal in CWs operated as BES resulted in removal of more than 99% from synthetic wastewater (Pun et al., 2019). However, this system was operated in short-circuit and used a bed of highly porous and electroconductive media (graphitized coke), in which anodic and cathodic processes were uncontrolled (comparable to a CW-MFC but without solid state electrodes or external connection).

Their own sorption experiments showed that ca. 30% of the compound was removed solely by abiotic sorption onto the highly porous media. Also in conventional MFC and MEC (poised potential of -0.4 V vs Ag/AgCl at the anode) systems, Werner et al. (2015) identified hydrophobic sorption as the dominant mechanism for CBZ removal, attributing the removal (>80%) mainly to the large anode areas provided by the graphite fibre brushes (material with high sorption propensity) and the attached biofilm. However, graphite has a high sorption propensity as well, unlike the used gravel in the presented study. Although CBZ can actually not be considered hydrophobic (log D of 2.77, see SI, Table S1), it is less polar than the other three tested OMPs, and therefore the contribution of sorption to CBZ removal is potentially higher than in the three other
tested OMPs. Generally, CBZ is considered a recalcitrant due to its low removal in conventional CAS, which rarely exceeds 10% (Joss et al., 2005; Zhang et al., 2014).

Despite being non-significant according to the performed ANOVA (see SI, Table S6) – probably the result of working with real wastewater with variable composition – the results obtained for CW-MEC and CW-MFC show a tendency for improvement compared to CW-control (see Table 4 and SI, Figure S2a). There are several processes which could potentially play a role here.

Electrosorption and hydrophobic sorption could have influenced CW-MFC and CW-MEC by offering additional sorption sites at the electrodes and the biofilm, and thereby improved the removal. However, these sorption sites are finite and longer term investigations using BES incorporated in CWs for CBZ removal are suggested. An effect of pH changes (see SI, Table S2) on hydrophobicity and charge in the different treatments is unlikely in the case of CBZ due to the high pKₐ of 13.9 (see SI, Table S1).

However, an increase in microbial activity observed in CW-MFC in an earlier study (Hartl et al., 2019), could have led to an improved biodegradation and at least partly explain the tendency for higher removal in CW-MFC and possibly CW-MEC as compared to the CW-control. Although no microbial activity studies in CW-MEC are known to the authors it could be assumed that it is affected in a similar way as in CW-MFC. Further investigation of the microbial communities, especially of CW-MEC, are suggested.

**Diclofenac**

DCF removal of 47% in CW-control was higher than in other publications on conventional HF CW systems, reporting 25% (Nivala et al., 2019) and 19±21% removal
There are no publications yet on DCF removal by CW-MFC or CW-MEC systems. DCF removal rates in the presented CW-MFC were high even when compared to conventional MFC systems fed by synthetic wastewater, which reached only 4-8% in a single-chamber closed-circuit MFC and up to ca. 23% and 45% in the anode and cathode chamber of a double chamber MFC, respectively (Wang et al., 2015).

De Gusseme et al. (2012) applied biogenic Pd nanoparticles as a biocatalyst to a conventional MEC (voltage of -0.8 V applied to the circuit) for the catalytic dechlorination of DCF (from synthetic wastewater with 1 mg/L DCF) and achieved full removal while no significant removal was achieved without the use of the nanoparticles.

In conventional CW systems (not operated as BES), vertical flow (VF) CW systems are more efficient in removing DCF through aerobic processes, with performances ranging from 50-70% (Ávila et al., 2014a, 2014b; Matamoros et al., 2007; Nivala et al., 2019), while the removal in HF CWs is lower and thought to happen through anaerobic degradation (Ávila et al., 2010). The biological removal of DCF is not fully understood and results are usually very variable (Zhang et al., 2008). DCF is also a recalcitrant (though not as strongly as CBZ), thus removal rates in conventional WWTPs can be also relatively low and variable with elimination values in the range of 7-75% (Zhang et al., 2014).

Although the log $K_{ow}$ of DCF is high with 4.26, it gets deprotonated and becomes highly hydrophilic at the pH range of 6.6 to 7.6 of the presented systems, with a log D of 1.70 to 1.04 (see SI, Table S1), resulting in a low sorption propensity. Given the charge and sorption characteristics of DCF, conventional sorption and pH effects seem unlikely to influence the DCF removal to a great extent. However, in theory, electrosorption at the
electrode with opposite charge (i.e. at the positively charged cathode, since DCF has a negative charge, see SI, Table S1) (Kong et al., 2013; Yang et al., 2015) could have resulted in an increased tendency for DCF removal (see Table 4 and SI, Figure S2b). Apart from that, CW-MFCs have been proven to enhance microbial activity (Hartl et al., 2019). Additionally, potential electrolysis of water in CW-MEC could produce oxygen and H⁺ at the anode and H₂ at the cathode. The produced oxygen could increase the aerobic biodegradation of DCF in CW-MEC. However, these effects could not be (statistically) confirmed according to the ANOVA (see SI, Table S6).

Ibuprofen

As for all other OMPs, also an ANOVA of IBU removal was not statistically significantly different across treatments (see SI, Table S6), and showed also in relative comparison the smallest differences between treatments with 39% removal in CW-control and CW-MEC, and 35% in CW-MFC systems (see Table 4 and SI, Figure S2c). Anyway, the here reported removal rates were comparable to those found in two exemplary HF CW systems amounting to 28% (Matamoros et al., 2017; Nivala et al., 2019). To the knowledge of the authors, there are no publications yet on IBU removal by CW-MEC systems and just one other publication which currently addresses IBU removal using a CW-MFC; Li et al. (2019) reported IBU removal rates of 82-96% from synthetic wastewater in a CW-MFC, which was 9% higher than their open-circuit control, with 63-79% of the removal happening in the anodic section.
Removal rates in conventional MFC systems reached values of 18-20% in single-chamber closed-circuit systems, and up to ca. 40% and 87% in anode and cathode chambers of a double-chamber MFC, respectively (synthetic wastewater was used) (Wang et al., 2015). In general, IBU is highly hydrophilic and therefore sorption is low, with a log D of 1.16 to 2.10 in the measured pH range (see SI, Table S1). Aerobic conditions favour its biodegradation (Monsalvo et al., 2014; Quintana et al., 2005), hence VF CWs show removal rates above 88% (Ávila et al., 2010; Nivala et al., 2019; Vystavna et al., 2017). This is probably also why plants – known to provide oxygen to the systems via their roots (Kadlec and Wallace, 2009) - improved IBU removal in HF CWs (Li et al., 2016). Removal rates in conventional WWTPs are usually high (41-100%) due to the prevalent aerobic removal mechanisms (Zhang et al., 2014). In general, the authors suggest to confirm the obtained results of all OMPs in planted CWs operated as BES.

In summary, IBU removal was not notably improved through CW-MFC or CW-MEC, although other studies on CW-MFC or conventional MFC were able to achieve that in comparison to control systems. In terms of charge, sorption propensity and biodegradability, IBU has similar characteristics as DCF and NPX, therefore other factors seem to be responsible for the even clearer lack of difference between treatments. Further investigation shall be carried out to confirm and possibly explain the results reported here.

Naproxen

The 25% NPX removal in the CW-control was lower than in comparable HF CW systems showing 32% (Nivala et al., 2019) and 66% removal (Matamoros et al., 2017). The short-
circuit CW-BES by Pun et al. (2019) removed more than 95% of NPX from synthetic wastewater; only a fraction (13.1-18.5% according to abiotic sorption tests) of that was retained within the material and therefore unrelated to biological activity of bacteria. Removal rates in conventional MFC systems by Wang et al. (2015) reached ca. 12-19% in single-chamber closed-circuit systems and up to ca. 40% and 84% in the anode and cathode of double-chamber MFC, respectively (all using synthetic wastewater).

Sorption of NPX is low, with a log D of 0.61 to -0.18 at the pH range of 6.6-7.6 (see SI, Table S1). Generally, NPX is mainly removed by biodegradation, and preferably under aerobic conditions (Kahl et al., 2017), hence VF CWs show high removal rates above 88% (Ávila et al., 2010; Nivala et al., 2019; Vystavna et al., 2017). Again as for IBU, removal rates in conventional WWTPs are relatively high and in the range of 40-98% (Zhang et al., 2014).

As for DCF, the positive tendencies seen in CW-BES (see Table 4 and SI, Figure S2d) could be possibly due to electrosorption. Also an increase in microbial activity and/or a potential increase in oxygen through electrolysis at the anode could have led to the observed slight NPX removal improvement.

In general, according to Cecconet et al. (2017), BES are theoretically more efficient in removing OMPs which are hydrophobic and positively charged. The former due to the better adsorption onto charged electrodes and the latter due to the better interaction with the negatively charged biofilm. The four OMPs presented in this study are all hydrophilic at neutral pH and either negatively charged (DCF, IBU and NPX), or neutrally charged (CBZ) under the pH range of the systems (see SI, Table S1), which could be
additional reasons for the statistically not significant results.

As mentioned above, in the case of DCF and NPX the observed insignificant but yet positive tendencies could be due to electro sorption to the positively charged electrode in CW-BES. These OMPs are present in the form of charged ions or polar molecules and could therefore have been adsorbed after migrating to the system´s electrode with opposite charge (Kong et al., 2013; Yang et al., 2015). Apart from that, MFCs seem to offer a beneficial environment for the growth of non-electrochemically active bacteria and increasing the metabolic rate of anaerobes due to the artificial presence of an insoluble electron acceptor, i.e. an anode (Fang et al., 2013). Another important factor to consider - apart from charge, sorption effects, microbial activity and direct impact on microbial communities - is the biodegradability of the compound (Wang et al., 2015). The BES itself might have influenced environmental conditions, especially on a micro-scale (e.g. at the electrodes or adjacent pore spaces) changing factors like pH (with statistically significant differences) and DO, which in turn could have indirectly affected microbial communities and their degradation of OMPs in the systems. Unfortunately, as mentioned above it was not possible to measure these parameters on such a small scale in the present study. However, similar studies reported electrolysis at the anode of CW-MEC systems (Gao et al., 2017) which would cause oxygen and hydrogen to be released and consequentially increase aerobic and hydrogen consuming microbial processes which could increase the removal of OMPs which show high removal rates in aerobic processes such as DCF and NPX.
4. CONCLUSIONS

The investigation of meso-scale CWs operated as BES (CW-BES) resulted in the following conclusions:

- Contrary to the hypothesis, no statistically significant effect of CW-BES on OMP removal could be found. A potential reason could have been the use of real urban wastewater which is more variable than synthetic wastewater. In general, the authors suggest careful consideration of results based on artificial conditions and recommend continued research with application of real urban wastewater and conditions as realistic as possible.

- However, some tendencies of increased OMP removal were noted in CW-MEC and CW-MFC when compared to CW-control for three out of the four investigated pharmaceuticals, namely CBZ, DCF and NPX with an average increase of 10-17% in CW-MEC and 5% in CW-MFC systems, compared to the CW-control. These tendencies could be due to various reasons such as increased microbial activity, or indirect effects through an electrolysis induced increase of DO and subsequent aerobic degradation (at least in the case of DCF and NPX in CW-MEC mode). Hydrophobic (and electro-) sorption might have played an additional role in the removal of CBZ, and electrosorption effects in the case of DCF and NPX. More long-term observation periods are recommended in order to take into account the inherent limitation of (electro-)sorption sites.

- In contrast to earlier research, no statistically significant removal was found regarding conventional wastewater parameters such as COD and NH₄⁺-N, potentially due to ageing effects of the systems, especially clogging of cathodes,
which could have also influenced the BES performance and consequently OMP removal. Thus, further investigations into the long-term ageing and clogging effects on electrodes are suggested, which would ideally lead to practical recommendations regarding system design, maintenance and regeneration.

• Finally, pH variations within the CW-BES systems - which were statistically significantly different in this study when compared to CW-control - are suggested to be investigated further with equipment allowing for observations at the micro-scale near the cathode and anode.

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Figure S1. Power density and polarization curves for each transect of one of the closed-circuit CW-MFC replicates measured during sampling week 4.
Figure S2. Specific removal from influent to effluent for all four OMPs (a; CBZ, b; DCF, c; IBU and d; NPX) comparing CW-control, CW-MFC and CW-MEC treatments (n=5). The box- and whisker plots show the minimum and maximum (lower and upper whiskers), first and third quartile (lower and upper end of box), median (horizontal line in box) and average (marked as an “x”) values.
**Table S1.** Chemical structure and characteristics of the selected OMPs used in this study and their respective hydrophobicity and charge states estimated from the compound’s Log D and pKₐ, respectively (relative to the experimental pH of 7 – 7.5). Log Kₐ describes the octanol-water partition coefficient which is a compound’s measure of the ratio of concentrations in octanol and water (Schwarzenbach et al., 2003). Log D is the partition coefficient for a compound at a specified pH.

| Compound       | Classification   | Structure                                      | Log Kₐ  | Log D (pH 6.6-7.6) | Hydrophobicity       | pKₐ | Charge state |
|----------------|------------------|------------------------------------------------|---------|--------------------|----------------------|-----|--------------|
| Carbamazepine  | Anticonvulsant    | ![Image](https://chemspider.com/808)            | 2.45 b  | 2.77               | hydrophilic          | 13.9| neutral      |
| Diclofenac     | Anti-inflammatory| ![Image](https://pubchem.ncbi.nlm.nih.gov)      | 4.51 d  | 1.70 to 1.04       | hydrophilic          | 4.15| negative     |
| Ibuprofen      | Anti-inflammatory| ![Image](https://chemicalize.com)               | 3.97 b  | 2.10 to 1.16       | hydrophilic          | 5.30| negative     |
| Naproxen       | Anti-inflammatory| ![Image](https://avdeef.com)                   | 3.18 b  | 0.61 to -0.18      | hydrophilic          | 4.15| negative     |

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*a* chemspider.com  
*b* [https://pubchem.ncbi.nlm.nih.gov](https://pubchem.ncbi.nlm.nih.gov)  
*c* chemicalize.com (data has been obtained from the empirical model)  
*d* Avdeef et al. (1998)
Table S2. Results for pH for CW-control, CW-MFC and CW-MEC systems during the OMP spiking and sampling weeks at the influent, after first transect, after second transect and effluent as well as overall average.

|       | Influent | 1/3    | 2/3    | Effluent | Average |
|-------|----------|--------|--------|----------|---------|
| pH (-) |          |        |        |          |         |
| CW-control | 7.50±0.00 | 7.35±0.05 | 7.35±0.00** | 7.70±0.01 | 7.48±0.02 |
| CW-MFC  | 7.45±0.05 | 7.09±0.02 | 7.05±0.07** | 7.66±0.07 | 7.32±0.05 |
| CW-MEC  | 7.54±0.07 | 6.69±0.09** | 6.60±0.05** | 7.15±0.03** | 7.00±0.06 |

** ANOVA very significant difference (p < 0.01)

Table S3. Post-hoc Tukey HSD pairwise comparison results for pH in CW-control, CW-MFC and CW-MEC systems during the OMP spiking and sampling weeks after first transect, after second transect and effluent.

|       | 1/3    | 2/3    | Effluent |
|-------|--------|--------|----------|
| Tukey HSD results |        |        |          |

| Pair                   | Q statistic | p-value | Inference | | Q statistic | p-value | Inference | | Q statistic | p-value | Inference |
|------------------------|-------------|---------|-----------|-----------------|-------------|---------|-----------|-----------------|-------------|---------|-----------|
| CW-control vs CW-MFC   | 4.1187      | 0.06    | insignificant | 13.39          | 0.001  ** | p<0.01 | ** p<0.01 | 0.586  | 0.9    | insignificant |
| CW-control vs CW-MEC   | 10.6203     | 0.001   ** p<0.01 | 33.51      | 0.001  ** | p<0.01 | ** p<0.01 | 7.633  | 0.004  ** p<0.01 |
| CW-MFC vs CW-MEC       | 6.5016      | 0.009   ** p<0.01 | 20.12      | 0.001  ** | p<0.01 |           | 7.047  | 0.006  ** p<0.01 |

* significant difference (p < 0.05)
** very significant difference (p < 0.01)

Table S4. Post-hoc Scheffé pairwise comparison results for pH in CW-control, CW-MFC and CW-MEC systems during the OMP spiking and sampling weeks after first transect, after second transect and effluent.

|       | 1/3    | 2/3    | Effluent |
|-------|--------|--------|----------|
| Scheffé results |        |        |          |

| Pair                   | TT-stats | p-value | Inference | | TT-stats | p-value | Inference | | TT-stats | p-value | Inference |
|------------------------|----------|---------|-----------|-----------------|-------------|---------|-----------|-----------------|-------------|---------|-----------|
| CW-control vs CW-MFC   | 2.9124   | 0.071   In-significant | 9.468      | 2E-04  ** | p<0.01 |          | 0.414  | 0.919  In-significant |
| CW-control vs CW-MEC   | 7.5097   | 9E-04   ** p<0.01 | 23.7       | 1.18E-06 ** | p<0.01 |          | 5.398  | 0.005  ** p<0.01 |
| CW-MFC vs CW-MEC       | 4.5973   | 0.011   * p<0.05 | 14.23      | 2.39E-05 ** | p<0.01 |          | 4.983  | 0.007  ** p<0.01 |

* significant difference (p < 0.05)
** very significant difference (p < 0.01)
Table S5. One-factor ANOVA (with replication) results for the comparison of conventional wastewater parameters between the electric connections during the sampling period, for the total system from inlet to outlet and each of the three transects separately (statistically significant different if p-value < 0.05).

| One-factor ANOVA | Inlet-Outlet | Transect 1 | Transect 2 | Transect 3 |
|------------------|--------------|------------|------------|------------|
| COD F (2, 8)     | 0.37         | 0.84       | 0.42       | 0.97       |
| NH₄-N F (2, 7)   | 0.20         | 0.21       | 0.93       | 0.99       |
| SO₄²⁻ F (2, 6)   | 0.97         | 0.98       | 0.16       | 0.36       |
| PO₄-P F (2, 6)   | 0.96         | 0.76       | 0.57       | 0.20       |

Table S6. One-factor ANOVA (with replication) results for the comparison of the four tested OMPs between the electric connections during the sampling period, for the total system from inlet to outlet and each of the three transects separately (statistically significant different if p-value < 0.05).

| One-factor ANOVA | Inlet-Outlet | Comparing Electric Connections |
|------------------|--------------|--------------------------------|
| Carbamazepine (CBZ) F (2, 5) | 0.48 |
| Diclofenac (DCF) F (2, 5) | 0.48 |
| Ibuprofen (IBU) F (2, 5) | 0.75 |
| Naproxen (NPX) F (2, 5) | 0.47 |
Abstract

The removal of organic micropollutants (OMPs) has been investigated in constructed wetlands (CWs) operated as bioelectrochemical systems (BES). The operation of CWs as BES (CW-BES), either in the form of microbial fuel cells (MFC) or microbial electrolysis cells (MEC), has only been investigated in recent years. The presented experiment used CW meso-scale systems applying a realistic horizontal flow regime and continuous feeding of real urban wastewater spiked with four OMPs (pharmaceuticals), namely carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBU) and naproxen (NPX). The study evaluated the removal efficiency of conventional CW systems (CW-control) as well as...
CW systems operated as closed-circuit MFCs (CW-MFCs) and MECs (CW-MECs).

Although a few positive trends were identified for the CW-BES compared to the CW-control (higher average CBZ, DCF and NPX removal by 10-17% in CW-MEC and 5% in CW-MFC). Generally, these proved to be not statistically significantly different.

Mesoscale experiments with real wastewater could thus not confirm earlier positive effects of CW-BES found under strictly controlled laboratory conditions with synthetic wastewaters - possibly also due to the use of real wastewater. However, comparatively higher removal rates were obtained for three out of the four compounds (CBZ, DCF and NPX) with an increase of 10-17% in CW-MEC and 5% in CW-MFC systems, compared to the CW-control. However, no statistically significant differences were found. IBU removal was similar amongst treatments.

Keywords
Emerging contaminants, meso-scale setup, microbial electrolysis cells, microbial fuel cells, real urban wastewater

1. INTRODUCTION

Micropollutants can be defined as organic and inorganic substances of anthropogenic origin, with the potential of causing negative effects for the environment already at very low concentrations, i.e. in the order of micro, nano or pico-grams (Chapman, 1992; Stamm et al., 2016). Organic micropollutants (OMPs) include a large array of substances, such as pharmaceuticals, personal care products (PPCPs),...
hormones, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), endocrine-disrupting chemicals (EDCs) or pesticides (Bolong et al., 2009). This study focuses on the removal of pharmaceuticals, which enter our environment mainly via human intake and consequent excretions, but can also originate from livestock excretions, domestic animals or manufacturing operations. In general, the human excretions arrive via sewer systems to wastewater treatment plants (WWTP) or septic systems and reach the wastewater effluent due to incomplete degradation along the way. Unfortunately, there is still a big gap of knowledge regarding the potential ecotoxicological effects of the active pharmaceutical ingredients (APIs) and their metabolites to the environment and human health (Barbosa et al., 2016; Garcia-Rodríguez et al., 2014). Traces of APIs were as well already found in drinking water, however, the low concentrations indicate that the risk to human health is low (Leung et al., 2013; Schwab et al., 2005). Some of these pollutants are also being considered as contaminants of emerging concern (CECs) or emergent contaminants, due to their continuous release, presence in water bodies and potential ecotoxicological effects. Although there are no legal discharge limits for OMPs, some regulations have been published for several of them, in the case of European policy for example in the Decision 2015/495/EU of 20 March 2015 amending earlier regulations, which included OMPs such as namely diclofenac (DCF) as well as several antibiotics, hormones and pesticides (Barbosa et al., 2016). The four OMPs, pharmaceuticals investigated in this study, i.e., diclofenac (DCF), ibuprofen (IBU), naproxen (NPX) and carbamazepine (CBZ), were all pharmaceuticals and chosen due to their high occurrence in raw wastewaters and/or their low or moderate removal rates in conventional activated sludge (CAS) wastewater.
treatment plants (WWTPs) (Gros et al., 2012, 2010; Mamo et al., 2018). They include three non-steroidal anti-inflammatory drugs (NSAIDs), namely diclofenac (DCF), ibuprofen (IBU) and naproxen (NPX), and a psychiatric drug, carbamazepine (CBZ). CBZ, an anticonvulsant and sedative drug also used to treat epilepsy and bipolar disorder, is one of the most frequently detected pharmaceuticals in basically all kind of environments at concentrations reaching the µg L⁻¹ level (Hai et al., 2018; Tran et al., 2018). CBZ is resilient to degradation in CAS treatment systems, showing a low tendency to sorption in sewage sludge and to photodegradation (Andreozzi et al., 2003; Calisto et al., 2010; Hai et al., 2011). Furthermore, it is not eliminated from sewage sludge by anaerobic digestion (Carballa et al., 2007). Even tertiary treatments such as advanced oxidation processes (UV/chlorination) have resulted in incomplete removal of CBZ (Wang et al., 2016; Zhou et al., 2016). Its ubiquity and resilience has led to consider it as a potential marker of anthropogenic contamination in water (Hai et al., 2018). The anti-inflammatories DCF, IBU and NPX also occur in comparatively high concentrations in WWTP effluents (Gros et al., 2012, 2010; Mamo et al., 2018) and therefore may pose a risk to aquatic ecosystems and different non-target species (Huerta et al., 2016; Ruhí et al., 2016). Furthermore, IBU and NPX belong to the most detected NSAIDs in WWTP influents worldwide due to their widespread use as over-the-counter pharmaceuticals (i.e. sold directly to a consumer without a prescription from a healthcare professional). For instance, IBU is one of the most consumed anti-inflammatories in Europe, with estimated yearly consumptions of 250 tons in Spain alone (Ortiz de García et al., 2013). Since DCF is an NSAID which often requires a prescription, it is found to a lesser extent in WWTP influent compared to IBU and NPX. Still, it is one of the most widely prescribed
anti-inflammatories and shows lower and more variable removal rates (7-75%) than IBU and NPX in WWTPs (40-100% and 40-98%, respectively) (García-Galán et al., 2016; Gros et al., 2012; Mamo et al., 2018). Similarly to CBZ (which rarely exceeds 10% removal in conventional WWTPs (Joss et al., 2005; Zhang et al., 2014)), DCF is usually classified as a recalcitrant compound (Hai et al., 2018; Osorio et al., 2016; Zhang et al., 2008). Due to the resulting high concentrations in WWTPs discharges, this NSAIDs may pose a risk to aquatic ecosystems and different non-target species (Huerta et al., 2016; Ruhí et al., 2016). So far there are no legal discharge limits for OMPs, but some regulations have been published for several of them, in the case of European policy for example in such as the European Decision 2015/495/EU of 20 March 2015 (amending earlier regulations), which included OMPs such as namely diclofenac (DCF) as well as several antibiotics, hormones and pesticides (Barbosa et al., 2016).

For instance, DCF has been found in larvae of caddisflies and leeches at concentrations up to 183 ng g⁻¹ (Huerta et al., 2015), and it was responsible for the drastic decline of vulture populations in Pakistan which fed on cattle carcasses treated with this drug (Oaks et al., 2004). Considering these data, the need and search for alternative and more efficient treatments is evident. In particular, nature-based, low-cost treatment systems such as microalgae-based systems or constructed wetlands (CWs) are currently being intensively investigated and, so far, showed promising results regarding OMPs removal (Ávila et al., 2014b, 2014a; García-Galán et al., 2018; Matamoros et al., 2015). Specifically, CWs are well-established systems for wastewater treatment and have been successfully applied in different climate zones worldwide (Langergraber and Haberl, 2001; Molle et al., 2005). A potentially more efficient and even less energy intensive
alternative to CAS WWTPs are Constructed Wetlands (CWs), which showed promising results regarding OMPs removal (Ávila et al., 2014b, 2014a; Matamoros et al., 2015).

The removal efficiency of OMPs in CWs varies with design, operation and type of CW (e.g. surface, subsurface vertical/horizontal flow) employed. In the case of subsurface horizontal flow (HF) CWs, the removal of OMPs ranges from poor to very efficient, depending on characteristics such as bed depth, media size, loading frequency or potential clogging (Ávila et al., 2014b; Matamoros and Bayona, 2006). Various CW intensification strategies have been developed over the last decades and were also tested for the treatment of OMPs, with promising results especially for biodegradable OMPs, but further research is still needed (Ávila et al., 2014b; Nivala et al., 2019; Zhang et al., 2014).

A relatively recent development in the field of wastewater treatment is based on coupling CWs with bioelectrochemical systems (BES) such as Microbial Fuel Cells (MFCs) (Villaseñor et al., 2013; Yadav et al., 2012) and Microbial Electrolysis Cells (MECs) (Ju et al., 2014) called CW-MFC and CW-MEC, respectively, from here on. MFCs use exoelectrogenic microorganisms, electrochemically active bacteria (EAB) (also known as exoelectrogens, electrogens, electricegens, exoelectrogenic or anode respiring bacteria) as catalysts in order to produce current from the oxidation of organic/inorganic compounds (Logan et al., 2006). These EAB are able to transfer electrons in and out of their cell in a process called extracellular electron transfer (EET) and they need a redox-gradient between the MFC electrodes to produce a current, such a gradient occurs naturally in CW systems. MECs are a modification of MFCs, with the main difference that an external power source is supplied to control the potential between the electrodes.
i.e. the anode and cathode, and thereby they are able to achieve thermodynamically reactions, which are otherwise unfavorable (Rozendal et al., 2006). Another advantage of MECs is that only an additional voltage of 0.2-0.8 V is required for water electrolysis to occur (usually 1.8-3.5 V are required), due to the current produced through the activity of EABs at the anode. Both technologies, MFCs and MECs, are able to use wastewater as a substrate and remove a variety of contaminants in the process, showing promising results for the removal of OMPs (Cecconet et al., 2017).

Up to date, there are only a few publications dealing with the use of CW-MFC for OMP removal, and to the authors’ knowledge, there are none on the use of CW-MEC systems. Generally, CW-MFC systems have been described to increase the microbial activity (determined by means of fluorescein diacetate (FDA) hydrolysis) in CW-MFC (Hartl et al., 2019), and some studies showed that CW-MFC enhance microbial community richness and diversity as compared to an open-circuit control (Song et al., 2018; F. Xu et al., 2018a). Earlier studies of CW-BES or BES systems for OMP removal used artificial wastewater, which is advantageous for the study of fundamental processes, but less realistic than real urban wastewater (Li et al., 2019; Pun et al., 2019; Wang et al., 2015).

For instance, Li et al. (2019) investigated bisphenol A and IBU removal in lab-scale CW-MFC systems fed with synthetic wastewater, obtaining removals of 82-96%, a 9% higher removal than in their control CW. Also Pun et al. (2019) reported high removal rates up to 99% for 11 different OMPs, (including CBZ (99%) and NPX (95%)) in a lab-scale HF CWs with an integrated BES. However, their configuration works in short-circuit mode (no solid-state electrodes) using a highly porous as well as electro-conductive media.
(graphitized coke), which makes it difficult to distinguish the proportion of conventional contaminant and OMP removal effect related to the CW-operated as BES, and that related to the improved physico-chemical factors like sorption. Wang et al. (2015) investigated the removal of a variety of OPMs using conventional MFCs in single-chamber setup (60% CBZ, 4-8% DCF, 18-20% IBU and 12-19% NPX removal) as well as double-chamber setup (ca. 20% and 70% CBZ, 23% and 45% DCF, 40% and 87% IBU, and 40% and 84% NPX removal, in the anode and cathode chamber, respectively) using synthetic wastewater. Regarding other OMPs, apart from the ones addressed in this study, publications using CW-MFCs were published on sulfamethoxazole with 38-50% removal (Li et al., 2018) and >99% removal (Zhang et al., 2016b), one on sulfadiazine removing >99% (Song et al., 2018), one on tetracycline removing >99% (Zhang et al., 2016a), and one on phenanthrene and anthracene with removal ranging from 88.5% to 96.4% (J. Wang et al., 2019).

The present experiment used CW meso-scale systems which, despite being unplanted, were intended to give additional information on OMP removal in larger scale CW-BES systems with a more realistic horizontal flow, continuous feeding of real urban wastewater and realistic spiking concentration levels of OMPs. Additionally, to the best knowledge of the authors this is the first publication on OMP removal in CW-MEC, and consequently also the first one to compare OMP removal efficiency of CW-MFC and CW-MEC side by side. To this end, duplicate systems with conventional CW (CW-control), closed-circuit CW-MFC (CW-MFC) as well as CW-MEC (CW-MEC) configuration have been used.
The hypothesis was that CW-MEC and CW-MFC will improve organic micropollutants removal as compared to the CW-control system.

2. MATERIALS AND METHODS

2.1 General design

For the purpose of this work, six unplanted meso-scale horizontal subsurface flow (HF) systems were used (a duplicate of systems per treatment: two systems per CW-control, CW-MFC and CW-MEC). The setup of these systems is detailed in a previous study (Hartl et al., 2019). Briefly, the systems consisted of a PVC reservoir of ca. 0.2 m$^2$ (55 cm length x 35 cm width) surface area filled up with 4/8 mm granitic riverine gravel. The systems were not planted to avoid an additional influencing parameter and further increase the experimental complexity. Wetted depth was set to be 25 cm. At the inlet and around the drainage of the outlet 7/14 mm granitic riverine gravel was used.

Both, the CW-MFC and CW-MEC were designed as three independent BES (MFCs or MECs), respectively, along the length of each system (see Figure 1). Each BES electrode consisted of a gravel-anode with four stainless steel mesh rectangles (Figure 1, C) (SS marine grade A316L, mesh width=4.60 mm, Øwire=1.000 mm, S/ISO 9044:1999) in series acting as electron collectors (4 cm away from each other). The anode area considered for current density calculations was the surface area (0.04 m$^2$) of the anode. Each metal mesh covered nearly the whole cross-sectional area (0.08 m$^2$) of the CW. Each cathode consisted of a carbon felt mat (Figure 1, D) (1.27 cm thick, with a surface area of 0.03 m$^2$, 99.0% carbon purity). A layer of glass wool was placed underneath the
cathodes in order to avoid any oxygen leaking from the cathode down to the anode as recommended elsewhere (Venkata Mohan et al., 2008).

Figure 1. Section- (top) and plan-view (bottom) of the CW-BES systems. A: Pump; B: Inflow; C: Anode; D: Cathode; E/F: Anode/Cathode connection to datalogger; G: Inflow barrier to avoid water short-circuiting on surface; H: Gravel core sampling tubes; I: Liquid sampling tubes; J: Water level; K: Standing pipe effluent; L: Drainage; M: Effluent collection tube, N: Reference electrode.

CW-MFC were operated at closed-circuit by connecting the anode and cathode over a wire and a 220 Ω resistor following the recommendations of Corbella and Puigagut (2018). The voltage across the external resistor for each electrode was continuously...
monitored by means of a datalogger (Campbell Scientific CR1000, AM16/32B
Multiplexor). The other two CW-BES systems were operated as CW-MEC by connecting
potentiostats to each electrode (the systems were operated in MEC mode for more than
9 months before the present experiment was conducted). CW-MEC systems had the
same setup as the CW-MFC but with an additional reference electrode placed near the
respective transects anode (Figure 1, N). Each MEC was poised at a potential of 0.3 V vs
Ag/AgCl at the anode using a potentiostat (nanoelectra NEV 4). The conventional HF
CW-control systems contained no anode metal meshes or electrical connections, but
only the cathode carbon felt in order to not confuse physical filtration effects of the
carbon felt with BES effects.

2.2 Operational conditions

The experimental CWs were mature at the time this work was conducted. The systems
of all three treatments were in operation and fed with real urban wastewater already
for about 18 months. CW-MFC and CW-control were in the same operation mode
throughout the whole time, but CW-MEC systems were operated in CW-MEC mode for
the last 9 months leading up to the experiment (the 9 months before that, CW-MEC
systems were also run as CW-MFC systems during earlier experiments). They had been
operated under similar conditions for eighteen months before the current experiment
was carried out. During the experiment, the systems were fed with fresh pre-settled
urban wastewater every weekday. Influent wastewater was spiked with the target
OMPs at a final concentration of 4 µg/L for 4 weeks. Samples for OMP analyses were
taken after one week of the start of daily OMP dosing (which represents a bit less than

Commented [M9]: Reviewer #2 comment on L212
L212 - “eighteen months” this is contradictory with the 9 months mentioned above.
The reviewer is right. As it was written before the information seemed contradictory. As also explained in the note on the
comment on L202, the information is now given in more detail and with better overview over all three treatments. See lines 149-154.

Commented [M10]: Reviewer #2 comment on L202
L202 - only the MEC were previously run for 9 months? what about
the MFC?
The reviewer is right. Information about previous operation time was only given partially in section 2.1. Therefore, the information is
now given for all three treatments and in more detail in two additional sentences at the beginning of section 2.2, in lines 152-154 in regards to CW-MEC (see also answer on comment L212).
two times the nominal HRT in order to ensure that the OMPs had reached the outlet of
the CW during sampling.

Further details on pre-treatment (settling for 3 hours) and operation are given in Hartl
et al. (2019). The average hydraulic loading rate (HLR) applied during the experiment
was 28 mm/d, resulting in a nominal HRT of 3.6±0.3 days and an average organic loading
rate (OLR) of 8.7±2.5 g COD/m² day.

2.3 Sampling and analysis

2.3.1 Water quality parameters

Eight sampling campaigns for the characterization of conventional wastewater quality
parameters were conducted during 12 weeks. These campaigns were conducted already
3 weeks before OMP sampling started, and continued during the OMP sampling period,
whereas conventional wastewater samples were taken just before the OMP dosing on
weekdays. Conventional wastewater parameters were measured for the influent, after
the first and second third of the wetland length, and as also at the effluent. All samples
were analyzed for total suspended solids (TSS), volatile suspended solids (VSS)
and total chemical oxygen demand (COD) according to Standard Methods (APHA-
AWWA-WEF, 2012); NH₄⁺-N, according to Solórzano method (Solórzano, 1969); NO₂⁻-N,
NO₃⁻-N, SO₄²⁻-S and PO₄³⁻-P by ion chromatography (ICS-1000, Dionex Corporation,
USA). Physical parameters such as water temperature, dissolved oxygen (DO)
concentration and pH were measured directly in the influent, using portable devices
after the first and second transect, as well as in the effluent (EcoScan DO 6,
Further details are given in Hartl et al. (2019).

### 2.3.2. OMP analysis

High purity standards (>99%) of the parent compounds and the isotopically labelled compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA). Detailed information on their physical and chemical characteristics is given in Table S1 of the Supplementary Information (SI). Standard solutions of the mixtures of the four compounds were made at the appropriate concentrations and used to dope the influent wastewater. Five OMP sampling campaigns were conducted during 3 weeks. Grab samples were taken from the CW influent and effluent sampling points (see Figure 1, points B and K, respectively). All water samples were filtered and processed using an adapted methodology by Matamoros and Bayona (2006). Briefly, 50 mL of influent and 100 mL of effluent samples were filtered (0.7 µm Whatman™ glass microfiber filters GF/F), acidified to pH 2-3 with HCl (0.02M) and spiked with a mixture of surrogate standards to a final concentration of 50 ng L⁻¹ (atrazine-d₅, mecoprop-d₅, tonalide-d₅, and dihydrocarbamazepine). Solid phase extraction was then performed, using 200 mg Strata™-X polymeric cartridges from Phenomenex (Torrance, CA, US—), previously conditioned with 3 mL of hexane, 3 mL of ethyl acetate, 5 mL of MeOH and 5 mL of acidified milli-Q water. Elution was performed with 10 mL of hexane/ethyl acetate (1:1, v:v). The eluted extract was evaporated under a gentle nitrogen stream to a volume of 100 µL, and triphenylamine was added as an internal standard (20 ng). Finally, vials were reconstituted to 300 µL and analyzed by GC-MS/MS as described by Matamoros et al. (2017).
2.4 Data Analysis

Contaminant removal efficiencies were calculated on a mass balance basis taking into account the wastewater flow and pollutant concentration. Statistical analyses were conducted for comparison of the relevant parameters COD, NH$_4^+$-N, SO$_4^{2-}$-S, PO$_4^{3-}$-P, pH, and current density as well as the OMPs CBZ, DCF, IBU and NPX across the three treatments. Since using Shapiro-Wilk tests showed a normal distribution for all data, and single-factor analysis of variance (ANOVA) could be used on all the above mentioned parameters, P as well as post-hoc Tukey HSD and Scheffé multiple comparison tests were performed for all parameters but only presented when relevant, i.e., if necessary in the case that ANOVA reported significant differences between treatments, thus only in the case of pH. The software used for calculations and statistical analysis was Microsoft Excel® 2016 and the included Analysis ToolPak add-in.

3. RESULTS AND DISCUSSION

3.1 Electrical behaviour

Table 1 shows average and maximum measured cell voltages ($E_{cell}$) as well as consequent current and power densities per surface area and anodic compartment volume for CW-MFC treatments in all 3 transects.
Average current densities per surface area for CW-MFC (all transects considered) resulted in 40 mA/m². Differences in current density between transects were not statistically significant according to ANOVA. A polarization curve (PC) analysis (see SI, Figure S1) showed that maximum power densities of 30, 11 and 24 mW/m² in transect 1, 2 and 3 of CW-MFC mode, respectively, were achieved at current densities of 79, 35 and 66 mA/m², respectively, which is higher than that described by Saz et al. (2018) (ca. 20 mA/m²) under comparable conditions.

The estimated internal resistances derived from the polarization curves were around 108 Ω, 220 Ω and 124 Ω for first, second and third transect, respectively. Principally, the potential maximum power is achieved when internal and external resistances are close to each other (Lefebvre et al., 2011). Coincidentally, the external and internal resistance were exactly the same in transect 2. However, for the current experiment and its primary goal contaminant removal the lower external resistances in transects 1 and 3 could have been beneficial, since lower external resistances increase the generated current and studies have also shown that consequently organic matter removal was increased (Aelterman et al., 2008; Gil et al., 2003; Katuri et al., 2011).
The coulombic efficiency (CE) is the proportion of the produced current to the carbohydrates which are theoretically derived from oxidation, indicated by the change of COD from transect to transect (Scott, 2016). The resulting average CE values amounted to 1.4±2.4%, 9.5±7.6% and -29.4±4.6%, for transects 1, 2 and 3, respectively. Note that CE can have a negative value when COD concentrations were increasing from the influent to the end of transect 1 or from one transect to the other. Generally, it can be assumed that only the CE value measured in transect 1 gives a good indication since not only organic matter from the influent can contribute to the MFC signal but also accumulated organic matter within the gravel bed is a fuel source for MFC (Corbella et al., 2016). Therefore, CE in transect 2 could be higher than transect 1 and transect 3 CE even negative on average. Comparable CW-MFC studies produced CEs from 0.01‰ (Wang et al., 2016b) up to 16.4% (Xie et al., 2018).

Table 2 shows the poised potential and the resulting achieved average current as well as average current and power densities per electrode surface area and anodic compartment volume for each transect in CW-MEC systems.

| Transect | Poised Potential (V) | Current (mA) | Current Density per Area (mA/m²) | Current Density per Volume (mA/m³) | Power Density per Area (mW/m²) | Power Density per Volume (mW/m³) |
|----------|----------------------|--------------|-----------------------------------|-----------------------------------|-------------------------------|---------------------------------|
| 1        | 0.3                  | 23±11        | 535±263                           | 2434±1197                         | 161±79                        | 730±359                         |
| 2        | 0.3                  | 10±5         | 223±112                           | 1015±510                          | 67±34                         | 304±153                         |
| 3        | 0.3                  | 5±3          | 120±74                            | 545±334                           | 36±22                         | 163±100                         |
The poised potential of 0.3 V vs. Ag/AgCl reference electrode at the anode, was chosen on the basis of experiences showing that poised potential around this value benefit the growth of electroactive electrochemically active bacteria (EAB) genera such as Geobacter in mixed bacterial cultures (Fricke et al., 2008; Liu et al., 2008). The average current density in CW-MEC was more than double in transect 1 compared to transect 2, and transect 2 was again roughly double of transect 3, assumingly because the organic matter concentration was decreasing along the flow path through the systems. The CW-MEC current densities in all three transects were low when compared to other similarly built CW-MEC systems which showed values ranging from 200 to 24500 mA/m² (Gao et al., 2017; Srivastava et al., 2018; Xu et al., 2017; Zhang et al., 2018). Authors believe that the use of non-conductive media (gravel) as anode material is the main cause of the lower observed current densities in the CW-MEC systems because this increases the internal resistance of the systems.

3.2 Removal efficiency of conventional wastewater quality parameters

Results on the removal of conventional contaminants in all three treatments (CW-control, closed-circuit CW-MFC and CW-MEC systems) are summarized in Table 3. All results were obtained during 8 weeks of intensive sampling (5 weeks before the OMP sampling campaigns and the three weeks during the OMP sampling campaign). Data is shown as average mass loading rate at the system inlet (influent), after the first and second transects and effluent, as well as mass removal rate from influent to effluent based on the average mass and percentage. During this period, all systems received continuous flow with an average OLR of 8.7±2.5 g COD/m².day.
In contrast to previous studies done on the same experimental systems (Hartl et al., 2019), ANOVA reported except for pH, no statistically significant differences were found for general wastewater quality parameters, with the exception of pH (see SI, Table S2 and S35). On average, CW-MEC showed higher COD and NH₄⁺-N removal than the CW-control, with an increase of 13% and 18%, respectively. CW-MFC removed 2% less COD than CW-control, but 18% more NH₄⁺-N on average, which in the case of NH₄⁺-N is slightly higher than previous results measured on the same real wastewater.

Table 3. Results for COD, ammonium, sulfate, sulfate and orthophosphate for CW-control, closed-circuit CW-MFC and CW-MEC systems during the 8 sampling weeks, expressed as average mass loading rate at influent, after first transect, after second transect and effluent as well as removal from influent to effluent based on the average mass removal rate and percentage.

|                | Influent | 1/3 | 2/3 | Effluent | Removal from Influent to Effluent |
|----------------|----------|-----|-----|----------|----------------------------------|
|                | (g/m².d) |     |     |          | (g/m².d) (%)                      |
| **COD (n=8)**  |          |     |     |          |                                  |
| CW-control     | 8.6±2.6  | 4.4±1.9 | 3.7±2.2 | 3.7±1.6 | 4.9±1.4 | 57% |
| CW-MFC         | 8.9±2.4  | 4.4±2.3 | 3.8±2.3 | 4.0±1.5 | 4.9±0.5 | 55% |
| CW-MEC         | 8.7±1.5  | 3.9±2.3 | 2.5±1.4 | 2.6±1.0 | 6.1±0.8 | 70% |
| **NH₄⁺-N (n=7)** |          |     |     |          |                                  |
| CW-control     | 1.2±0.4  | 1.0±0.4 | 0.9±0.3 | 1.1±0.3 | 0.1±0.2 | 10% |
| CW-MFC         | 1.3±0.4  | 1.0±0.3 | 0.8±0.2 | 1.0±0.3 | 0.3±0.2 | 24% |
| CW-MEC         | 1.2±0.4  | 0.9±0.3 | 0.7±0.2 | 0.9±0.2 | 0.3±0.3 | 28% |
| **SO₄²⁻ (n=6)** |          |     |     |          |                                  |
| CW-control     | 2.0±1.3  | 0.5±0.5 | 0.4±0.3 | 0.8±0.6 | 1.1±0.9 | 58% |
| CW-MFC         | 2.1±1.4  | 0.6±0.4 | 0.6±0.4 | 1.1±0.9 | 1.0±0.3 | 48% |
| CW-MEC         | 2.2±1.4  | 0.8±0.7 | 1.0±0.8 | 1.1±0.9 | 1.1±0.8 | 51% |
| **PO₄³⁻-P (n=6)** |          |     |     |          |                                  |
| CW-control     | 0.09±0.05 | 0.09±0.06 | 0.09±0.05 | 0.09±0.05 | 0.00±0.03 | 2% |
| CW-MFC         | 0.09±0.05 | 0.09±0.06 | 0.08±0.05 | 0.08±0.05 | 0.01±0.03 | 7% |
| CW-MEC         | 0.09±0.05 | 0.07±0.06 | 0.06±0.05 | 0.08±0.05 | 0.01±0.04 | 7% |

* Some experimentation weeks could not be considered due to highly diluted influent or technical analysis problems.

In contrast to previous studies done on the same experimental systems (Hartl et al., 2019), ANOVA reported except for pH, no statistically significant differences were found for general wastewater quality parameters, with the exception of pH (see SI, Table S2 and S35). On average, CW-MEC showed higher COD and NH₄⁺-N removal than the CW-control, with an increase of 13% and 18%, respectively. CW-MFC removed 2% less COD than CW-control, but 18% more NH₄⁺-N on average, which in the case of NH₄⁺-N is slightly higher than previous results measured on the same real wastewater.
The improvement in NH$_4^+$-N removal is similar to that obtained in other studies on CW-MFC (Corbella and Puigagut, 2018) and CW-MEC (Gao et al., 2018). However, NH$_4^+$-N removal was generally low, and decreased towards the end of the study period, which was also observed for COD, although to a lesser extent. It is assumed that aging and possible partial clogging of the carbon felt cathodes might have limited the COD removal performance of CW-MFC systems. Indeed, results from MFC studies (Kim et al., 2008; Yan et al., 2012; Yuan et al., 2021) Kim et al. (2008) suggested that their single-chamber air-cathode MFC system provided a suitable microenvironment for biological ammonia oxidation whereas the ammonia oxidizing bacteria (AOB) found in the cathode biofilm derived oxygen via diffusion through the cathode. So the observed partial clogging of the cathode could have also negatively affected the activity of ammonia oxidizing bacteria (AOB) at the cathode, and as a consequence also have reduced nitrification and NH$_4^+$-N removal in the cathode biofilm, which were described to enhance nitrification in a single-chamber air-cathode MFC by Kim et al. (2008). In the case of NH$_4^+$-N, it could also be assumed that due to the system aging and the accompanying development and establishment of the microbial communities, the systems turned more anaerobic, which in turn would have lowered the nitrification rates. However, NH$_4^+$-N was still removed to a greater extent in CW-MFC and CW-MEC systems. At least for CW-MEC, this could be due to electrolysis happening at the anode, releasing oxygen and hydrogen, which increase aerobic and hydrogen-consuming processes. The increased DO, identified by Wang et al. (2019) as one of the most influential parameters for COD removal in CW-
MFC, could then be assumed to be the main factor for the increased COD removal in the CW-MEC of the presented study.

The bulk DO as measured by the portable meter in the systems’ sampling tubes was always below the detection limit. However, the electrolysis might be effective on a much smaller scale and thus not be reflected in the bulk DO. The higher denitrification capacity in CW-MEC, and possibly to some degree in CW-MFC could be explained by electrolysis induced H₂ serving as electron donor for nitrate reduction to nitrogen gas, and H⁺ could also be involved in autohydrogenotrophic denitrification (Gao et al., 2017). Additionally, in an earlier study, an increase in microbial activity was observed (Hartl et al., 2019), which could have led to an overall improved biodegradation. For both COD and NH₄⁺-N removal, the lack of plants in the meso-scale systems could have had an effect as well on overall treatment efficiencies, since the presence of plants has shown to improve treatment efficiency in HF CWs (Tanner, 2001). NO₂⁻-N and NO₃⁻-N were generally below the limit of detection. A recent study showed that planted CW-MFC systems show higher power density and contaminant removal, however, dead plant parts in turn also reduced the power production (Yang et al., 2019).

Average SO₄²⁻-S removal was lower in CW-MFC and CW-MEC systems than in CW-control, especially after the second transect. This was also observed by Corbella and Puigagut (2018) who found 13% higher SO₄²⁻-S removal in control systems than in CW-MFC systems, likely due to the re-oxidation of sulfides to sulfur and further to sulfate using the MFC anode as electron acceptor (Lovley et al., 2006).

Average PO₄³⁻-P removal was higher in CW-MEC and CW-MFC as compared to CW-control, and again most distinct after the second transect. These results show that the
third transect might have had kind of an equalizing effect when comparing \( PO_4^{3-} \)-P as well as \( SO_4^{2-} \)-S reduction between treatments. Generally, \( PO_4^{3-} \)-P removal efficiency was lower when compared to current literature regarding CW-MFC (Corbella and Puigagut, 2018; Saz et al., 2018; Xu et al., 2018; Yakar et al., 2018) or CW-MEC (Gao et al., 2018; Ju et al., 2014; Zhang et al., 2018) systems. However, many studies were conducted only over a short-time and it is generally known that phosphorus storage in subsurface flow CWs has a finite capacity and therefore removal by sorption normally decreases over time (Kadlec and Wallace, 2009), as could have been the case in this study as the wetlands were operated for over about 18 months.

The average values for pH measurements at each sampling point are shown in see SI, Table S2. The ANOVA results for influent and average pH values of all sampling points were statistically not significantly different across treatments. However, after the first transect, CW-MEC systems showed a lower pH than other treatments on average, being significantly different from CW-MFC as well as CW-control according to ANOVA, with both, post-hoc Tukey HSD and Scheffé pairwise comparison showing CW-MEC to be either significantly or very significantly different to CW-MFC and CW-control (for more details see SI, Figures S3 and S4). After the second transect, pH values of all three treatments were significantly different from each other according to ANOVA, with CW-MEC showing the lowest pH, followed by a higher pH in CW-MFC and the highest in CW-control (meaning the smallest change since the influent inlet in the system). Post-hoc Tukey HSD and Scheffé pairwise comparison of pH after the second third showed very significant differences between all treatments (for more details see SI, Figures S3 and
pH values at the effluent were generally higher than in the previous two transects within the systems, and the difference between treatments was again only statistically different in the CW-MEC systems. **Post-hoc Tukey HSD and Scheffé pairwise comparison** of pH at the effluent showed CW-MEC to be very significantly different to CW-MFC and CW-control (for more details see SI, Figures S3 and S4).

Changes in pH within the system might affect the activity of bacteria, and influence the charge state as well as hydrophobicity of certain OMPs (Wang et al., 2015). While the measured pH in solution showed some significant differences between treatments, the changes seemed not big enough to alter the charge state and hydrophobicity of the investigated OMPs significantly, especially in the case of CBZ with its high pKₐ of 13.9 (see SI, Table S1). However, as for DO, pH at the micro-scale, e.g. near the cathode or anode, might have changed more drastically, and could have created micro-environments in which charge state and/or hydrophobicity were influenced. Unfortunately, it was not possible to measure these changes in pH on a micro-scale with the presented setup.

### 3.3 Removal efficiency of organic micropollutants

Table 4 shows the removal of the four targeted OMPs for all three treatments (see also SI, Figure S2 for box- and whisker plots).
Table 4. Results for OMPs carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBU) and naproxen (NPX) in CW-control, closed-circuit CW-MFC and CW-MEC systems during the 5 sampling campaigns, expressed as average background, influent and effluent concentration, average mass loading rate at influent and effluent as well as removal from influent to effluent based on the average mass removal rate and percentage. (Concentration variability in the influent concentrations is due to the background concentration of the urban wastewater for each of the compounds).

| OMP     | Background (µg/L) | Influent (µg/L) | Treatment | Effluent (µg/m².d) | Removal (%) |
|---------|-------------------|----------------|-----------|-------------------|-------------|
| CBZ     | 3.5±2.2           | 5.3±2.2        | CW-control | 4.6±1.4         | 26          |
|         |                   | 149±61         | CW-MFC    | 123±41           | 17%         |
|         |                   |                | CW-MEC    | 3.7±0.8          | 50          |
|         |                   |                |           | 99±24            | 34%         |
| DCF     | 0.6±0.3           | 4.2±1.9        | CW-control | 2.7±1.4         | 65          |
|         |                   | 137±56         | CW-MFC    | 73±17            | 47%         |
|         |                   |                | CW-MEC    | 2.2±1.0          | 72          |
|         |                   |                |           | 59±16            | 57%         |
| IBU     | 12.6±3.6          | 18.6±8.8       | CW-control | 12.0±2.0        | 202         |
|         |                   | 523±202        | CW-MFC    | 321±53           | 39%         |
|         |                   |                | CW-MEC    | 12.6±1.7         | 182         |
|         |                   |                |           | 341±40           | 35%         |
|         |                   |                |           | 12.0±2.2         | 182         |
|         |                   |                |           | 320±52           | 35%         |
| NPX     | 3.8±0.7           | 10.2±1.4       | CW-control | 7.6±2.4         | 70          |
|         |                   | 273±29         | CW-MFC    | 203±62           | 25%         |
|         |                   |                | CW-MEC    | 7.1±2.0          | 82          |
|         |                   |                |           | 191±50           | 30%         |
|         |                   |                |           | 6.1±1.5          | 109         |
|         |                   |                |           | 163±37           | 40%         |

Similar as for the general wastewater parameters, removal differences across treatments were not statistically significant for any of the four compounds (see SI, Table S6). However, average OMP removals were slightly higher in CW-MEC (by 10-17%) and CW-MFC (by 5%) as compared to the CW-control for CBZ, DCF and NPX. However, these differences were not statistically significant for any of the three compounds (see SI, Table S4). Regarding IBU, the average removal was similar in all treatments, with CW-control and CW-MEC exhibiting the same average removal rates and a 4% lower removal in CW-MFC. Nevertheless, few tendencies were visible which are discussed further below.
Carbamazepine

Average. Although not statistically significantly different, average CBZ removal was higher in CW MEC and CW-MFC as compared to CW-control with values of 34%, 22% and 17%, respectively (see Table 4 and SI, Figure S2a). The CW-control system removal of 17% is in accordance with results of previous studies on treatment capacity in conventional HF CW systems (not operated as BES), reporting removals of 13% (Nivala et al., 2019) and 21% (Matamoros et al., 2017). These results show that CBZ can be removed to a certain degree in HF CWs (supposedly due to anaerobic processes), however, CBZ is not biodegradable in aerobic conditions and therefore VF CWs show lower removal rates (Hai et al., 2011; Jekel et al., 2015; König et al., 2016; Nivala et al., 2019).

The only other study looking at CBZ removal in CWs operated as BES resulted in removal of more than 99% from synthetic wastewater (Pun et al., 2019). However, this system was operated in short-circuit and used a bed of highly porous and electroconductive media (graphitized coke), in which anodic and cathodic processes were uncontrolled (comparable to a CW-MFC but without solid state electrodes or external connection).

Their own sorption experiments showed that ca. 30% of the compound was removed solely by abiotic sorption onto the highly porous media. Also in conventional MFC and MEC (poised potential of -0.4 V vs Ag/AgCl at the anode) systems, Werner et al. (2015) identified hydrophobic sorption as the dominant mechanism for CBZ removal, attributing the removal (>80%) mainly to the large anode areas provided by the graphite fibre brushes (material with high sorption propensity) and the attached biofilm. However, graphite has a high sorption propensity as well, unlike the used gravel in the...
presented study. Although CBZ can actually not be considered hydrophobic (log D of 2.77, see SI, Table S1), it is less polar than the other three tested OMPs, and therefore the contribution of sorption to CBZ removal is potentially higher than in the three other tested OMPs. Generally, low CBZ is considered a recalcitrant due to its low removal in conventional CAS, which rarely exceed 10% (Joss et al., 2005; Zhang et al., 2014).

Considering the low CBZ removal in conventional CAS (rarely exceeds 10% (Joss et al., 2005; Zhang et al., 2014)), despite being non-significant according to the performed ANOVA (see SI, Table S6) – probably the result of working with real wastewater with variable composition – the results obtained for CW-MEC and especially in the case of CW-MEC MFC show a tendency for improvement compared to CW-control describe a real improvement (see Table 4 and SI, Figure S2a). There are several processes which could potentially play a role here, reason for increased removal in the presented CW-MFC and CW-MEC systems compared to CW-control could be manifold. Electrosorption and hydrophobic sorption could have played a role influenced with CW-MFC and CW-MEC by offering additional sorption sites at the electrodes, and the biofilm, and thereby improved the removal. However, these sorption sites are finite and longer term investigations using BES incorporated in CWs for CBZ removal are suggested. An effect of pH changes (see SI, Table S2) on hydrophobicity and charge in the different treatments is unlikely in the case of CBZ due to the high pKₐ of 13.9 (see SI, Table S1).

However, an increase in microbial activity observed in CW-MFC in an earlier study (Hartl et al., 2019), could have led to an improved biodegradation and at least partly explain the tendency for improved higher removal in CW-MFC and possibly CW-MEC as
compared to the CW-control. Although no microbial activity studies in CW-MEC are
known to the authors it could be assumed that it is affected in a similar way as in CW-
MFC. Further investigation of the microbial communities, especially of CW-MEC, are
suggested.

Diclofenac

Although not statistically significantly different, average DCF removal was higher in
cW-MEC and CW-MEC as compared to CW-control with values of 57%, 52% and 47%,
respectively (see Table 4 and SI, Figure S2b). DCF removal of 47% in CW-control was
higher than in other publications on conventional HF CW systems, reporting 25% (Nivala
et al., 2019) and 19±21% removal (Matamoros et al., 2017). There are no publications
yet on DCF removal by CW-MFC or CW-MEC systems. DCF removal rates in the
presented CW-MFC were high even when compared to conventional MFC systems fed
by synthetic wastewater, which reached only 4-8% in a single-chamber closed-circuit
MFC and up to ca. 23% and 45% in the anode and cathode chamber of a double chamber
MFC, respectively (Wang et al., 2015). De Gusseme et al. (2012) applied biogenic Pd
nanoparticles as a biocatalyst to a conventional MEC (voltage of -0.8 V applied to the
circuit) for the catalytic dechlorination of DCF (from synthetic wastewater with 1 mg/L
DCF) and achieved full removal while no significant removal was achieved without the
use of the nanoparticles.

In conventional CW systems (not operated as BES), vertical flow (VF) CW systems are
more efficient in removing DCF through aerobic processes, with performances ranging
from 50-70% (Ávila et al., 2014a, 2014b; Matamoros et al., 2007; Nivala et al., 2019),
while the removal in HF CWs is lower and thought to happen through anaerobic
degradation (Ávila et al., 2010). The biological removal of DCF is not fully understood
and results are usually very variable (Zhang et al., 2008). **DCF is also a recalcitrant**
(though not as strongly as CBZ), therefore removal rates in conventional WWTPs
are can be also relatively low and variable with elimination values in the range of 7-75%
(Zhang et al., 2014).

Although the log $K_{ow}$ of DCF is high with 4.26, it gets deprotonated and becomes highly
hydrophilic at the pH range of 6.6 to 7.6 of the presented systems, with a log D of 1.70
to 1.04 (see SI, Table S1), resulting in a low sorption propensity. **DCF is also a recalcitrant**
(though not as strongly as CBZ), therefore removal rates in conventional WWTPs are
also relatively low and variable with elimination values in the range of 7-75% (Zhang et
al., 2014). Given the charge and sorption characteristics of DCF, conventional sorption
and pH effects seem unlikely to influence the DCF removal to a great extent. However, in theory, electrosorption at the electrode with opposite charge (i.e.
at the positively charged cathode, since DCF has a negative charge, see SI, Table S1)
could have contributed to the DCF removal (see Table 4 and SI, Figure S2b) (Kong et
al., 2013; Yang et al., 2015). Apart from that, DCF is also a recalcitrant (though not as strongly as CBZ),
therefore removal rates in conventional WWTPs are also relatively low and variable with
elimination values in the range of 7-75% (Zhang et al., 2014). CW-MFCs have been
proven to enhance microbial activity (Hartl et al., 2019). Another factor
could Additionally, the potential electrolysis of water in CW-MEC could, producing
oxygen and H$^+$ at the anode and H$_2$ at the cathode. The produced oxygen could have
increased the aerobic biodegradation of DCF in CW-MEC. However, these effects could not be (statistically) confirmed according to the ANOVA (see SI, Table S6).

Hence, DCF removal was possibly enhanced through an increase in microbial activity in CWs operated as BES, which could have led to the slightly higher observed DCF removal improvement in CW-MFC and CW-MEC as compared to the CW-control. Another factor could be the potential electrolysis of water in CW-MEC, producing oxygen and H⁺ at the anode and H₂ at the cathode. The produced oxygen could have increased the aerobic biodegradation of DCF in CW-MEC and thereby explain the enhancement increase in removal as compared to CW-MFC.

Ibuprofen

As for all other OMPs, also an ANOVA of IBU removal was not statistically significantly different across treatments (see SI, Table S6), and showed also in relative comparison the smallest differences between treatments, very different across treatments, with 39% removal in CW-control and CW-MEC, and 35% in CW-MFC systems (see Table 4 and SI, Figure S2c). Anyway, the here reported removal rates were comparable to those found in two exemplary HF CW systems amounting to 28% (Matamoros et al., 2017; Nivala et al., 2019). To the knowledge of the authors, there are no publications yet on IBU removal by CW-MEC systems and just one other publication which currently addresses IBU removal using a CW-MFC; Li et al. (2019) reported IBU removal rates of 82-96% from synthetic wastewater in a CW-MFC, which was 9% higher than their open-circuit control, with 63-79% of the removal happening in the anodic section.
Removal rates in conventional MFC systems reached values of 18-20% in single-chamber closed-circuit systems, and up to ca. 40% and 87% in anode and cathode chambers of a double-chamber MFC, respectively (synthetic wastewater was used) (Wang et al., 2015). In general, IBU is highly hydrophilic and therefore sorption is low, with a log D of 1.16 to 2.10 in the measured pH range (see SI, Table S1). Aerobic conditions favor biodegradation (Monsalvo et al., 2014; Quintana et al., 2005), hence VF CWs show removal rates above 88% (Ávila et al., 2010; Nivala et al., 2019; Vystavna et al., 2017). This is probably also why plants – known to provide oxygen to the systems via their roots (Kadlec and Wallace, 2009) - improved IBU removal in HF CWs (Li et al., 2016). Removal rates in conventional WWTPs are usually high (41-100%) due to the prevalent aerobic removal mechanisms (Zhang et al., 2014). In general, the authors suggest to confirm the obtained results of all OMPs in planted CWs operated as BES.

In summary, IBU removal was not notably improved through CW-MFC or CW-MEC, although other studies on CW-MFC or conventional MFC were able to achieve that in comparison to control systems. In terms of charge, sorption propensity and biodegradability, IBU has similar characteristics as DCF and NPX, therefore other factors seem to be responsible for the even clearer lack of difference between treatments. Further investigation shall be carried out to confirm and possibly explain the results reported here.

Naproxen

Although not statistically significantly different, Average average NPX removal was higher in CW-MEC and CW-MFC as compared to CW control with values of 40%, 30%
The 25% NPX removal in the CW-control was lower than in comparable HF CW systems showing 32% (Nivala et al., 2019) and 66% removal (Matamoros et al., 2017). The short-circuit CW-BES by Pun et al. (2019) removed more than 95% of NPX from synthetic wastewater; only a fraction (13.1-18.5%) according to abiotic sorption tests) of that was retained within the material and therefore unrelated to biological activity of bacteria. Removal rates in conventional MFC systems by Wang et al. (2015) reached ca. 12-19% in single-chamber closed-circuit systems and up to ca. 40% and 84% in the anode and cathode of double-chamber MFC, respectively (all using synthetic wastewater).

Sorption of NPX is low, with a log D of 0.61 to -0.18 at the pH range of 6.6-7.6 (see SI, Table S1). Generally, NPX is mainly removed by biodegradation, and preferably under aerobic conditions (Kahl et al., 2017), hence VF CWs show high removal rates above 88% (Ávila et al., 2010; Nivala et al., 2019; Vystavna et al., 2017). Again as for IBU, removal rates in conventional WWTPs are relatively high and in the range of 40-98% (Zhang et al., 2014).

As for DCF, the positive tendencies seen in CW-BES (see Table 4 and SI, Figure S2d) theoretically NPX removal differences across treatments were unlikely influenced by differences in charge or sorption but could be possibly enhanced due to by electrosorption in CWs operated as BES. Again, an increase in microbial activity and/or a potential increase in oxygen through electrolysis at the anode could have led to the observed slight NPX removal improvement in CW-MFC and CW-MEC as compared to the CW-control. A potential increase in oxygen through electrolysis at the anode could...
explain the enhanced slightly increased IBU-NPX removal in CW-MES as compared to CW-MFC.

In general, according to Cecconet et al. (2017), BES are theoretically more efficient in removing OMPs which are hydrophobic and positively charged. The former due to the better adsorption onto charged electrodes and the latter due to the better interaction with the negatively charged biofilm. The four OMPs presented in this study are all hydrophilic at neutral pH and either negatively charged (DCF, IBU and NPX), or neutrally charged (CBZ) under the pH range of the systems (see SI, Table S1) and show low removal in WWTPs, which could be additional reasons for the statistically not significant results.

As mentioned above, in the case of DCF and NPX the observed insignificant but yet positive tendencies could be due to Furthermore, the four investigated OMPs are negatively charged (DCF, IBU and NPX), or neutrally charged (CBZ) under the pH range of the systems (SI, Table S1). Therefore, it could be stated that the presented CW-MFC and CW-MEC were even able to improve the removal of theoretically resilient OMPs such as CBZ, DCF and NPX. However, electrosorption to the positively charged cathode electrode could have even improved the adsorption of the negatively charged OMPs in DCF, IBU and NPX in CW-MFC and CW-MECBES. These OMPs are present in the form of charged ions or polar molecules and could therefore have been adsorbed after migrating to the system’s electrode with opposite charge (Kong et al., 2013; Yang et al., 2015).

Apart from that, MFCs seem to offer a beneficial environment for the growth of
electroactive-non-electrochemically active bacteria and increasing the metabolic rate of anaerobes due to the artificial presence of an insoluble electron acceptor, i.e. an anode (Fang et al., 2013). Additionally, CW-MFC mode has shown to increase microbial activity (Hartl et al., 2019) and EAB seem to outperform other microbial communities (Zhang et al., 2015). Some studies claim that CWs operated as MFC enhance microbial community richness and diversity (Song et al., 2018; F. Xu et al., 2018a), however, experiments lasted only for 4 and 2 months, respectively, and microbial communities are known to change over time and might expose different behaviors especially in the initial start-up phase. Another important factor to consider is the biodegradability of the compound (Wang et al., 2015). The BES itself might have influenced environmental conditions, especially on a micro-scale (e.g. at the electrodes or adjacent pore spaces) changing factors like pH (with statistically significant differences) and DO, which in turn could have indirectly affected microbial communities and their degradation of OMPs in the systems. Unfortunately, as mentioned above it was not possible to measure these parameters on such a small scale in the present study. However, similar studies reported electrolysis at the anode of CW-MEC systems (Gao et al., 2017) which would cause oxygen and hydrogen to be released and consequentially increase aerobic and hydrogen consuming microbial processes. The increase in aerobic processes could therefore explain at least partly the higher removal of NPX and DCF in CW-MEC and possibly the improved treatment compared to CW-MFC, which could increase the removal of OMPs which show high removal rates in aerobic processes such as DCF and NPX.
4. CONCLUSIONS

The investigation of meso-scale CWs operated as BES (CW-BES) resulted in the following conclusions:

- Contrary to the hypothesis, no statistically significant effect of CW-BES on OMP removal could be found. A potential reason could have been the use of real urban wastewater which is more variable than synthetic wastewater. In general, the authors suggest careful consideration of results based on artificial conditions and recommend continued research with application of real urban wastewater and conditions as realistic as possible.

- However, some tendencies of increased OMP removal were noted in CW-MEC and CW-MFC when compared to CW-control for three out of the four investigated pharmaceuticals, namely CBZ, DCF and NPX with an average increase of 10-17% in CW-MEC and 5% in CW-MFC systems, compared to the CW-control. These tendencies could be due to various reasons such as increased microbial activity, or indirect effects through an electrolysis induced increase of DO and subsequent aerobic degradation (at least in the case of DCF and NPX in CW-MEC mode). Hydrophobic (and electro-) sorption might have played an additional role in the removal of CBZ, and electrosorption effects in the case of DCF and NPX. More long-term observation periods are recommended in order to take into account the inherent limitation of (electro-)sorption sites.

- In contrast to earlier research, no statistically significant removal was found regarding conventional wastewater parameters such as COD and NH₃-N, potentially due to ageing effects of the systems, especially clogging of cathodes.
which could have also influenced the BES performance and consequently OMP removal. Thus, further investigations into the long-term ageing and clogging effects on electrodes are suggested, which would ideally lead to practical recommendations regarding system design, maintenance and regeneration.

- Finally, pH variations within the CW-BES systems - which were statistically significantly different in this study when compared to CW-control - are suggested to be investigated further with equipment allowing for observations at the micro-scale near the cathode and anode.

- The treatment performance for three out of four investigated OMPs (CBZ, DCF, and NPX) was improved, with removal efficiencies 10-17% higher in CW-MEC and 5% higher in CW-MEC systems than those obtained in the CW-control systems. However, in all three cases no statistically significant differences were found.

- Average IBU removal rates showed no relevant differences when comparing treatments.

- The improved removal of CBZ, DCF and NPX in CW-BES could be due to direct effects of BES, such as increased microbial activity, or indirect effects through an electrolysis induced increase of DO (at least in the case of CW-MEC) and subsequent aerobic degradation, at least in the case of DCF and NPX. Hydrophobic (and electro-) sorption might have played an additional role in the removal of CBZ, and electrosorption effects in the case of DCF and NPX.

- In terms of OMP removal, CWs operated as BES could provide an additional benefit for the removal of the most recalcitrant compounds such as CBZ and
DCF, due to their limited biodegradability and removal in other biological systems.

- However, further research should be carried out in order to discern the underlying mechanisms leading to the OMP removal improvement and also to use this information to refine and upgrade the design and operation of CW-BES systems, also including detailed effects of vegetation.

- The increased removal of conventional wastewater parameters COD and NH$_4^+$-N could be due to direct effects of the BES such as an increased microbial activity as well as indirect effects through an electrolysis induced increase of DO (CW-MEC) and subsequent aerobic degradation; in the case of NH$_4^+$-N, hydrogen resulting from the electrolysis might have additionally enabled autohydrogenotrophic denitrification.

In summary the results could not confirm the hypothesis that CW-MEC and CW-MFC+ will improve organic micropollutants removal as compared to the CW-control system, since the differences in removal were not statistically significantly different. However, the results indicated a higher OMP removal in CW-MEC and CW-MFC+ in the case of three out of four OMPs.

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Figure S1. Power density and polarization curves for each transect of one of the closed-circuit CW-MFC replicates measured during sampling week 4.
Figure S2. Specific removal from influent to effluent for all four OMPs (a; CBZ, b; DCF, c; IBU and d; NPX) comparing CW-control, CW-MFC and CW-MEC treatments (n=5). The box- and whisker plots show the minimum and maximum (lower and upper whiskers), first and third quartile (lower and upper end of box), median (horizontal line in box) and average (marked as an "x") values.
**Table S1.** Chemical structure and characteristics of the selected OMPs used in this study and their respective hydrophobicity and charge states estimated from the compound’s Log D and pKₐ, respectively (relative to the experimental pH of 7 – 7.5). Log K_{ow} describes the octanol-water partition coefficient which is a compound’s measure of the ratio of concentrations in octanol and water (Schwarzenbach et al., 2003). Log D is the partition coefficient for a compound at a specified pH.

| Compound      | Structure * | Classification | Log K_{ow} | Log D (pH 6.6-7.6) | Hydrophobicity | pKₐ b | Charge state |
|---------------|-------------|----------------|------------|---------------------|----------------|-------|--------------|
| Carbamazepine | ![Carbamazepine](image) | Anticonvulsant | 2.45 a     | 2.77                | hydrophilic   | 13.9  | neutral      |
| Diclofenac    | ![Diclofenac](image) | Anti-inflammatory | 4.51 d   | 1.70 to 1.04        | hydrophilic   | 4.15  | negative     |
| Ibuprofen     | ![Ibuprofen](image) | Anti-inflammatory | 3.97 b   | 2.10 to 1.16        | hydrophilic   | 5.30  | negative     |
| Naproxen      | ![Naproxen](image) | Anti-inflammatory | 3.18 b   | 0.61 to -0.18       | hydrophilic   | 4.15  | negative     |

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*a* chemspider.com  
*b* https://pubchem.ncbi.nlm.nih.gov  
*c* chemicalize.com (data has been obtained from the empirical model)  
*d* Avdeef et al. (1998)
Table S2. Results for pH for CW-control, CW-MFC and CW-MEC systems during the OMP spiking and sampling weeks at the influent, after first transect, after second transect and effluent as well as overall average.

| pH (−) | Influent | 1/3 | 2/3 | Effluent | Average |
|--------|----------|-----|-----|----------|---------|
| CW-control | 7.50±0.00 | 7.35±0.05 | 7.35±0.00** | 7.70±0.01 | 7.48±0.02 |
| CW-MFC | 7.45±0.05 | 7.09±0.02 | 7.05±0.07** | 7.66±0.07 | 7.32±0.05 |
| CW-MEC | 7.54±0.07 | 6.69±0.09** | 6.60±0.05** | 7.15±0.03** | 7.00±0.06 |

** ANOVA very significant difference (p < 0.01)

Table S3. Post-hoc Tukey HSD pairwise comparison results for pH in CW-control, CW-MFC and CW-MEC systems during the OMP spiking and sampling weeks after first transect, after second transect and effluent.

| pH (−) | Tukey HSD results |
|--------|--------------------|
| | 1/3 | 2/3 | Effluent |
| CW-control vs CW-MFC | 4.1187 | 0.06 | insignificant |
| CW-control vs CW-MEC | 10.6203 | 0.001 | ** p<0.01 |
| CW-MFC vs CW-MEC | 6.5916 | 0.001 | ** p<0.01 |

* significant difference (p < 0.05)
** very significant difference (p < 0.01)

Table S4. Post-hoc Scheffé pairwise comparison results for pH in CW-control, CW-MFC and CW-MEC systems during the OMP spiking and sampling weeks after first transect, after second transect and effluent.

| pH (−) | Scheffé results |
|--------|----------------|
| | 1/3 | 2/3 | Effluent |
| CW-control vs CW-MFC | 2.9124 | 0.071 | insignificant |
| CW-control vs CW-MEC | 7.5097 | 9E−04 | ** p<0.01 |
| CW-MFC vs CW-MEC | 4.5973 | 0.011 | * p<0.05 |

* significant difference (p < 0.05)
** very significant difference (p < 0.01)
One-factor ANOVA (with replication) results for the comparison of conventional wastewater parameters between the electric connections during the sampling period, for the total system from inlet to outlet and each of the three transects separately (statistically significant different if p-value < 0.05).

| One-factor ANOVA | p-value Comparing Electric Connections |
|------------------|----------------------------------------|
|                  | Inlet-Outlet   | Transect 1 | Transect 2 | Transect 3 |
| COD              | F (2, 8)       | 0.37       | 0.84       | 0.42       | 0.97       |
| NH$_4$-N         | F (2, 7)       | 0.20       | 0.21       | 0.93       | 0.99       |
| SO$_4^{2-}$      | F (2, 6)       | 0.97       | 0.98       | 0.16       | 0.36       |
| PO$_4$-P         | F (2, 6)       | 0.96       | 0.76       | 0.57       | 0.20       |

One-factor ANOVA (with replication) results for the comparison of the four tested OMPs between the electric connections during the sampling period, for the total system from inlet to outlet and each of the three transects separately (statistically significant different if p-value < 0.05).

| OMP             | F (2, 5) | p-value |
|-----------------|---------|---------|
| Carbamazepine   | F (2, 5)       | 0.48       |
| Diclofenac      | F (2, 5)       | 0.48       |
| Ibuprofen       | F (2, 5)       | 0.75       |
| Naproxen        | F (2, 5)       | 0.47       |
Marco Hartl: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Visualization

María Jesús García-Galán: Conceptualization, Methodology, Investigation, Formal analysis, Writing - Original Draft, Resources, Funding acquisition

Victor Matamoros: Methodology, Investigation, Formal analysis, Writing - Review & Editing, Resources

Marta Fernández-Gatell: Formal analysis, Investigation

Diederik P.L. Rousseau: Conceptualization, Writing - Review & Editing, Supervision

Gijs Du Laing: Writing - Review & Editing, Supervision, Project administration, Funding acquisition

Marianna Garfí: Writing - Review & Editing, Supervision, Funding acquisition

Jaume Puigagut: Conceptualization, Methodology, Writing - Review & Editing, Supervision
Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
CWs operated as MEC / MFC
Respective effect on removal of Organic Micropollutants

- Carbamazepine: +17% / +5% removal
- Diclofenac: +10% / +5% removal
- Ibuprofen: Similar to CW control
- Naproxen: +15% / +5% removal

CWs operated as MEC / MFC
Respective effect on removal of Organic Micropollutants

- No stat. sig. differences
- CBZ, DCF & NPX tendentially higher removal (5-17%)
- Earlier reports using synthetic wastewater not confirmed
Figures for manuscript entitled:
 Constructed wetlands operated as bioelectrochemical systems for the removal of organic micropollutants

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Figure 1. Section- (top) and plan-view (bottom) of the CW-BES systems. A: Pump; B: Inflow; C: Anode; D: Cathode; E/F: Anode/Cathode connection to datalogger; G: Inflow barrier to avoid water short-circuiting on surface; H: Gravel core sampling tubes; I: Liquid sampling tubes; J: Water level; K: Standing pipe effluent; L: Drainage; M: Effluent collection tube, N: Reference electrode.
Tables for manuscript entitled:

**Constructed wetlands operated as bioelectrochemical systems for the removal of organic micropollutants**

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**Table 1.** Average, standard deviation and maximum for \(E_{\text{cell}}\) and current density of closed-circuit CW-MFC systems. Note: The surface area of each electrode was used for current density calculations.

| Transect | \(E_{\text{cell}}\) (mV) | Current Density per Area (mA/m\(^2\)) | Current Density per Volume (mA/m\(^3\)) |
|----------|----------------|------------------------------------------|------------------------------------------|
|          | Avg ± SD | Maximum | Avg ± SD | Maximum | Avg ± SD | Maximum |
| 1        | 372±119 | 552      | 40±13    | 60       | 183±59   | 273      |
| 2        | 378±81  | 577      | 41±9     | 62       | 186±41   | 282      |
| 3        | 372±128 | 711      | 40±14    | 77       | 183±64   | 350      |

**Table 2.** Pozied potential (at Anode vs. Ag/AgCl reference electrode) and resulting average current applied also expressed in current density per surface area and volume in CW-MEC (MEC)

| Transect | Pozied Potential (V) | Current (mA) | Current Density per Area (mA/m\(^2\)) | Current Density per Volume (mA/m\(^3\)) |
|----------|----------------------|--------------|------------------------------------------|------------------------------------------|
|          | Avg ± SD | Avg ± SD | Avg ± SD | Avg ± SD     |
| 1        | 0.3       | 23±11    | 535±263 | 2434±1197   |
| 2        | 0.3       | 10±5     | 223±112 | 1015±510    |
| 3        | 0.3       | 5±3      | 120±174 | 545±334     |
Table 3. Results for COD, ammonium, sulfate and orthophosphate for CW control, closed-circuit CW-MFC and CW-MEC systems during the 8 sampling weeks, expressed as average mass loading rate at influent, after first transect, after second transect and effluent as well as removal from influent to effluent based on the average mass removal rate and percentage.

|                | Influent 1/3 | Influent 2/3 | Effluent | Removal from Influent to Effluent |
|----------------|--------------|--------------|----------|----------------------------------|
|                | (g/m²·d)     | (g/m²·d)     | (%)      | (g/m²·d)                         |
| **COD** (n=8) |              |              |          |                                  |
| CW-control     | 8.6±2.6      | 4.4±1.9      | 3.7±2.2  | 3.7±1.6                          | 4.9±1.4 | 57%
| CW-MFC         | 8.9±2.4      | 4.4±2.3      | 3.8±2.3  | 4.0±1.5                          | 4.9±0.5 | 55%
| CW-MEC         | 8.7±2.5      | 3.9±2.3      | 2.5±1.4  | 2.6±1.0                          | 6.1±0.8 | 70%
| **NH₄-N** (n=7) |              |              |          |                                  |
| CW-control     | 1.2±0.4      | 1.0±0.4      | 0.9±0.3  | 1.1±0.3                          | 0.1±0.2 | 10%
| CW-MFC         | 1.3±0.4      | 1.0±0.3      | 0.8±0.2  | 1.0±0.3                          | 0.3±0.2 | 24%
| CW-MEC         | 1.2±0.4      | 0.9±0.3      | 0.7±0.2  | 0.9±0.2                          | 0.3±0.3 | 28%
| **SO₄²⁻** (n=6) |              |              |          |                                  |
| CW-control     | 2.0±1.3      | 0.5±0.5      | 0.4±0.3  | 0.8±0.6                          | 1.1±0.9 | 68%
| CW-MFC         | 2.1±1.4      | 0.6±0.4      | 0.6±0.4  | 1.1±0.9                          | 1.0±0.3 | 51%
| CW-MEC         | 2.2±1.4      | 0.8±0.7      | 1.0±0.8  | 1.1±0.9                          | 1.1±0.8 | 51%
| **PO₄³⁻-P** (n=6) |              |              |          |                                  |
| CW-control     | 0.09±0.05    | 0.09±0.06    | 0.09±0.05| 0.09±0.05                        | 0.00±0.03| 2%
| CW-MFC         | 0.09±0.05    | 0.09±0.06    | 0.08±0.05| 0.08±0.05                        | 0.01±0.03| 7%
| CW-MEC         | 0.09±0.05    | 0.07±0.06    | 0.06±0.05| 0.08±0.05                        | 0.01±0.04| 7%

*Some experimentation weeks could not be considered due to highly diluted influent or technical analysis problems.
Table 4. Results for OMPs carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBU) and naproxen (NPX) in CW control, closed-circuit CW-MFC and CW-MEC systems during the 5 sampling campaigns, expressed as average background, influent and effluent concentration, average mass loading rate at influent and effluent as well as removal from influent to effluent based on the average mass removal rate and percentage. (Concentration variability in the influent concentrations is due to the background concentration of the urban wastewater for each of the compounds).

| OMP (n=5) | Background | Influent | Treatment | Effluent | Removal |
|-----------|------------|----------|-----------|----------|---------|
|           | (µg/L) | (µg/L) | (µg/m².d) | (µg/L) | (µg/m².d) | (µg/m².d) | (%) |
| CBZ       | 3.5±2.2 | 5.3±2.2 | 149±61 | CW-control | 4.6±1.4 | 123±41 | 26 | 17% |
|           |          |          |         | CW-MFC | 4.3±1.0 | 116±26 | 33 | 22% |
|           |          |          |         | CW-MEC | 3.7±0.8 | 99±24 | 50 | 34% |
| DCF       | 0.6±0.3 | 4.2±1.9 | 137±56 | CW-control | 2.7±1.4 | 73±17 | 65 | 47% |
|           |          |          |         | CW-MFC | 2.2±1.0 | 65±20 | 72 | 52% |
|           |          |          |         | CW-MEC | 2.2±0.8 | 59±16 | 79 | 57% |
| IBU       | 12.6±3.6 | 18.6±8.8 | 523±202 | CW-control | 12.0±2.0 | 321±53 | 202 | 39% |
|           |          |          |         | CW-MFC | 12.6±1.7 | 341±40 | 182 | 35% |
|           |          |          |         | CW-MEC | 12.0±2.2 | 320±52 | 202 | 39% |
| NPX       | 3.8±0.7 | 10.2±1.4 | 273±29 | CW-control | 7.0±2.4 | 203±62 | 70 | 25% |
|           |          |          |         | CW-MFC | 7.1±2.0 | 191±50 | 82 | 30% |
|           |          |          |         | CW-MEC | 6.1±1.5 | 163±37 | 109 | 40% |
