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Removal of micropollutants, facultative pathogenic and antibiotic resistant bacteria in a full-scale retention soil filter receiving combined sewer overflow†

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Combined sewer systems collect surface runoff as well as wastewater of industrial and domestic origin. During periods of heavy rainfall the capacity of the sewer system is exceeded and the overflow is discharged into receiving waters without any treatment. Consequently, combined sewer overflow (CSO) is considered as a major source of water pollution. This study investigates the effectiveness of a retention soil filter (RSF) for the removal of micropollutants as well as facultative pathogenic and antibiotic resistant bacteria from CSO. The removal of organic group parameters like total organic carbon was excellent and the removal efficiency for micropollutants of the RSF and the wastewater treatment plant (WWTP), which treats wastewater of the same origin during dry and normal weather conditions, was comparable. Compounds of high environmental concern like estrogens or certain pharmaceuticals, *e.g.* diclofenac, were completely eliminated or removed to a high degree during RSF passage. RSF treatment also reduced the number of *E. coli*, enterococci and staphylococci by 2.7, 2.2 and 2.4 log-units (median values), respectively. Obviously, some *Staphylococcus* species can better adapt to the conditions of the RSF than others as a shift of the abundance of the different species was observed when comparing the diversity of staphylococci obtained from the RSF influent and effluent. RSF treatment also decreased the absolute number of antibiotic resistant bacteria. The percentage of antibiotic resistant *E. coli* and staphylococci isolates also decreased during passage of the RSF, whereas the percentage of resistant enterococci did not change. For *E. coli* ampicillin and for enterococci and staphylococci erythromycin determined the antibiotic resistance level. The results demonstrate that RSFs can be considered as an adequate treatment option for CSO. The performance for the removal of micropollutants is comparable with a medium sized WWTP with conventional activated sludge treatment. The number of facultative pathogenic and antibiotic resistant bacteria was considerably decreased during RSF passage. However, as RSF effluents still contained antibiotic resistance genes and traces of micropollutants; receiving waters may still be at risk from negative environmental impacts.

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Environmental impact

During periods of heavy rainfall the capacity of combined sewer systems can be exceeded and the overflow is discharged without treatment into receiving waters. Consequently, water treatment options for such events are highly desirable as combined sewer overflow is considered as a major source of surface water pollution. The use of retention soil filters is a growing technology and first studies showed a great potential for the removal of single micropollutants and pathogens. This study proves the good efficiency for numerous micropollutants and facultative pathogenic bacteria and demonstrate that the removal in retention soil filters is comparable with that in activated sludge treatment in wastewater treatment plants. Furthermore, retention soil filters are a good treatment option for the removal of antibiotic resistant bacteria and the overall risk on autochthonous organisms in receiving waters can be reduced.

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1 Introduction

Pollution of surface waters poses a threat for the aquatic environment. For this reason the European Union Water Framework Directive (EUWFD) was passed in 2000 to reduce acute and chronic toxicity of aquatic organisms, to minimize the accumulation of pollutants in ecosystems and to protect biological diversity and human health.[1] Environmental quality standards were established for an initial set of 33 priority substances, which must not be exceeded for a good surface water chemical status. In 2013 the list was extended by twelve new compounds. Additionally, three compounds (diclofenac, 17-β-estradiol and 17-α-ethinylestradiol) were put on a watch list, for which monitoring data are to be collected to support a decision on potential prioritization in the future.[2] Some chemicals listed in the EUWFD are supposed to be mainly discharged by point sources like wastewater treatment plants (WWTPs). This holds true not only for compounds on the EU list of priority substances, but also for numerous other chemicals as well as facultative pathogenic and antibiotic resistant bacteria which might also affect the aquatic environment or human health.

One successful approach to minimize the discharge of micropollutants and microorganisms (bacteria, viruses, protozoan pathogens) by point sources is upgrading WWTPs by additional treatment technologies like ozonation or powdered activated carbon filtration.[3] In Germany, however, two thirds of the sewer systems collect wastewater with domestic and industrial origin together with storm water runoff.[4,5] During heavy rainfall up to seven times more water is collected in combined sewer systems than under dry weather conditions.[6] As a consequence, the capacity of the sewer system and of WWTPs can be insufficient during heavy rainfalls as most facilities have a maximum treatment capacity equivalent to twice the dry weather discharge.[4]

It is therefore assumed that 30% to 50% of the annual storm water runoff is discharged as untreated combined sewer overflow (CSO) into receiving waters,[5] including readily biodegradable or nonpolar micropollutants which would have been removed by microbial degradation or by sorption onto the sewage sludge under dry weather conditions. One measure to cope with high flows in the sewer system are storm water overflow basins (SOBs), which provide an interim storage capacity for excess water, that is transferred to the WWTP after the rain event. However, no microbial degradation of nutrients or micropollutants is achieved in the SOBs and when their storage capacity is exceeded, untreated storm water overflow is also a source for contamination with micropollutants and potentially pathogenic microorganisms. This chemical and bacterial pollution can be a threat to human health when water is used for drinking water production, recreational activities or irrigation. Antibiotic resistance genes within the released microorganisms even increase this health threat and may contribute to their dissemination to the autochthonous microbial community. Therefore, CSO is an important source of pollution for receiving waters as it may represent large loads of microbial and chemical contaminants.[7,8]

For combined sewer systems retention soil filters (RSFs) are a treatment technology that helps to safeguard WWTPs and receiving waters from hydraulic, chemical and hygienic stress caused by the feed of excess water.[6] RSFs are soil filters with a vertical flow-through passage that also provide additional intermediate storage and retention capacity. Basically, RSFs are constructed wetlands that only have an intermittent inflow and receive overflows from combined sewers, storm water or highway runoff and not predominantly wastewater.[9] They provide water treatment by filtration, sorption and biological processes. In previous studies, RSFs showed excellent retention or removal efficiencies for nutrients like ammonium or phosphate as well as for suspended solids and oxidizable organic matter, expressed *e.g.* as chemical oxygen demand (COD) and biochemical oxygen demand after 5 days (BOD_5).[4,6]

The interest in RSF technology is growing, but with many newly deployed filters there still is little experience with regard to long term performance. A survey among RSF operators in Germany ($n = 83$) revealed that only 21% of all filters have been in service for more than five years.[10]

The RSF analyzed in this study treats CSO of two SOBs and discharges into the river Schussen, a densely populated catchment area in Southwest Germany. The Schussen is a tributary of Lake Constance, which is an important drinking water reservoir and a popular recreation area.[11] The elimination of nutrients, organic trace pollutants and facultative pathogenic bacteria by a RSF downstream of two SOBs is presented. For numerous micropollutants, facultative pathogenic and antibiotic resistant bacteria this study presents the first baseline data for their retention by RSF.

2 Materials and methods

2.1 Study site and sampling protocol

The RSF was put into operation in 2002. It receives the overflow of two SOBs, which serve the city of Tettnang in Baden-Württemberg, Germany (Fig. 1). The effluent of the SOBs passes a cross-flow screen with a gap size of 5 mm. The wastewater solely originates from the two residential catchments of Tettnang (2450 population equivalents) as the wastewater from the local hospital is separately discharged to the WWTP. The effluent of the RSF is discharged into the Tobelbach, which flows into the Schussen river, one of the main tributaries of Lake Constance. The RSF has a surface area of 2000 m^2 which is planted with common reed (*Phragmites communis*). The formation of a colmation layer and the risk of surface clogging are reduced by the reed's stable rootstock growth. At the study site no further measures against clogging are necessary. The rooting and intermittent flooding also enables the penetration of oxygen into the subsurface, which stimulates the microbial activity. Once a year the reed is mown and removed from the filter surface. The filter layer has a thickness of about 80 cm and rests on a gravel drainage system (40–60 cm). At its base the RSF is furnished with a 2 mm non-permeable lining. On average, 40 to 60 impounding events occur per year. In more than one third of the events the influent volume exceeds the capacity of the RSF of 2000 m^3. Excess wastewater (storm water) is discharged to the

Tobelbach without treatment. The RSF's effluent discharge point is controlled by a throttle valve limiting the outflow to 10 L s^{-1}. Complete drainage takes about 60 h. The removal of group parameters, micropollutants, facultative pathogenic and antibiotic resistant bacteria presented here is based on the difference between concentration values determined for the influent of the RSF after SOBs and for the effluent after the throttle valve.

The WWTP in Eriskirch which serves the two catchment areas depicted in Fig. 2 was used for comparison of treatment efficiencies. This WWTP treats a population equivalent of 50 000 per day and the maximum dry weather flow is 17 300 m^3 per day. Treatment consists of a screen, sand trap and a primary clarifier, followed by biological treatment comprised of zones of denitrification and nitrification combined with simultaneous phosphorus elimination. The water is then directed to a secondary sedimentation basin and iron(III) chloride sulfate is added as a precipitating agent. The flocs are removed by filtration through layers of sand and anthracite before the water is discharged into the Schussen river. Results presented here are based on values determined for the influent of the WWTP after mechanical treatment and for the wastewater after activated sludge treatment.

At the RSF there were five sampling events between June 2012 and November 2013. The WWTP Eriskirch was sampled up to 11 times between May 2012 and August 2014. Though 187 micropollutants were measured, only a small number were defined as indicative parameters based on their nearly ubiquitous presence in WWTP influents and CSO and were measured more frequently. Some more hydrophobic micropollutants like

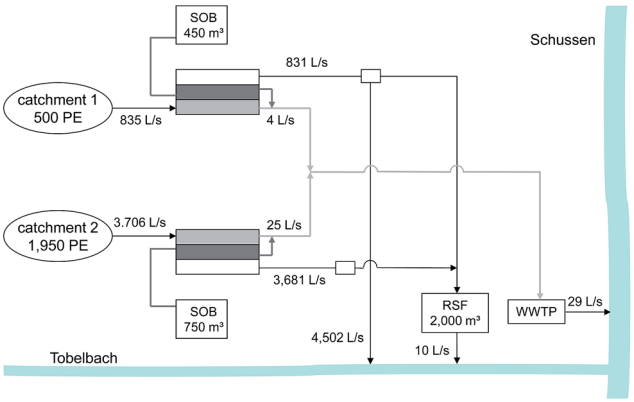

Fig. 2 Flow diagram of the study site (adopted from Dr.-Ing. Jedele and Partner GmbH, Stuttgart, Germany).

polybrominated diphenyl ethers (PBDE) and polycylic aromatic hydrocarbons (PAHs) were only screened at the RSF site. Furthermore, heavy metals and group parameters were measured.

During the sampling events, 250 mL of RSF influent were sampled every 3 min and for the effluent 250 mL were sampled every 10 m^3 until the required total sample volume for all analyses was collected. In case of the WWTP samples, 24 h composite samples were analyzed. All samples were cooled down immediately with icepacks and transported to the laboratories, where they were stored at 4 °C until sample preparation and analysis.

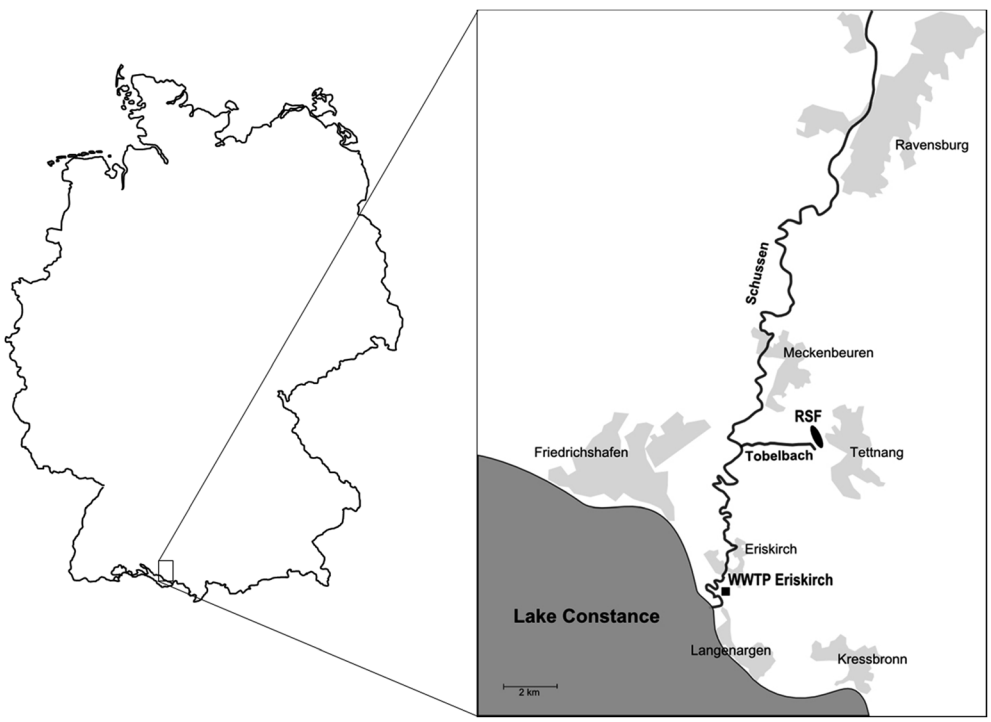

Fig. 1 Map of Germany and scheme of the study area in the southwestern federal state Baden-Württemberg (by courtesy of Katharina Peschke, University of Tübingen, Germany. The map of the study area is based on OpenStreetMap. Mapdata: ©OpenStreetMap contributors, license: http://opendatacommons.org/licenses/dbcl/1.0/).

2.2 Analysis of micropollutants, heavy metals and water-chemical group parameters

For the analyses of micropollutants water samples were spiked with internal standards prior to extraction. Solid-phase extraction (SPE) or liquid–liquid-extraction (LLE) was used for pre-concentration. Gas chromatography or liquid chromatography were coupled to different detector techniques ((tandem) mass spectrometry, nitrogen–phosphorus detection, diode array detection). Concentrations of micropollutants, heavy metals and group parameters were analyzed in influent and effluent waters of the RSF. A complete list of all parameters with their respective limit of quantification (LOQ) can be found in the ESI (Table S1†). Standard methods used for analysis are listed in Table 1. Non-standard methods are described briefly in the text below.

Pharmaceuticals and some of their metabolites, artificial sweeteners, iodinated X-ray contrast agents, benzotriazoles and antibiotics were pre-concentrated by SPE using SDB (J. T. Baker, Philipsburg, USA), Strata-X (Phenomenex, Aschaffenburg, Germany) or PPL Bond Elut (Agilent Technologies, Santa Clara, USA) polymeric adsorbent materials. The very polar antidiabetic drug metformin and its degradation product guanylurea were enriched with a cationic exchange adsorbent material (Strata-X-CW from Phenomenex) as described in Scheurer *et al.*[12]

Sample pH, the water volume used for pre-concentration, elution solvents and the established liquid chromatographic methods were optimized for each substance group. The analytes were quantified using a 1290 HPLC system (Agilent Technologies) coupled to an API 5500 mass spectrometer (AB Sciex, Framingham, USA).

Polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and chlorinated insecticides were extracted by LLE with 20 mL cyclohexane. The organic solvent was evaporated to a final volume of about 0.5 mL and the compounds were measured by gas chromatography coupled to a tandem mass spectrometer (GC/MS/MS).

PBDEs were extracted with 25 mL cyclohexane. Residual water was removed with a sodium sulfate filled cartridge. The extract was evaporated to a volume of 0.2 mL and measured with GC/MS with negative chemical ionization.

Trialkylphosphates were enriched with a polymeric adsorbent (SDB) and cartridges were eluted with dichloromethane. GC/MS-MS was performed for separation and quantification using a TRACE GC Ultra gas chromatograph coupled to a TSQ Quantum XLS Ultra mass spectrometer (both Thermo Scientific, Waltham, USA).

For the SPE of phthalates self-packed glass SPE cartridges filled with Chromabond C18 Hydra material (Macherey Nagel, Düren, Germany) were used. Phthalates were analyzed using a Autosystem XL GC coupled to a Turbo Mass Gold MS (both Perkin Elmer, Waltham, USA).

Endocrine disrupting chemicals were also pre-concentrated by SPE with a polymeric adsorbent material (Strata-X from Phenomenex, Aschaffenburg, Germany). After elution of the analytes with acetone the extracts were evaporated to dryness and reconstituted with a derivatization mixture (MSTFA (*N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide), trimethylchlorosilane and pyridine). After silylation (80 °C for 45 min) a keeper was added and the derivatization reagent was removed in a gentle stream of nitrogen. The residue was reconstituted in cyclohexane and measured by a Trace GC TSQ Quantum XLS Ultra GC-MS/MS (Thermo Scientific).

Pesticides were enriched using 1 g IST Isolute C18 adsorbent (Biotage, Uppsala, Sweden) and analyzed after elution with acetone by GC-MS using a 6890 5973 GC-MS system (Agilent Technologies).

Aliphatic amines were derivatized in the water sample with fluorenylmethyloxycarbonyl chloride (FMOC) and pre-concentrated using 200 mg LiChrolut EN adsorbent material (Merck, Darmstadt, Germany). Measurements were performed with LC coupled to a fluorescence detector (both Agilent Technologies).

2.3 Analysis of microbiological parameters – enumeration, isolation and identification of *E. coli*, enterococci and staphylococci

Depending on the probable contamination level, either the membrane filtration method (according to ISO 7704H) or direct plating for *E. coli*, enterococci and staphylococci was used.[19] *E. coli* were grown on ECD-agar (Merck, Darmstadt, Germany). After incubation at 37 °C for 20–24 h, under UV light blue fluorescent colonies on the membrane (cellulose nitrate, pore size 0.45 μm, Ø 50 mm (Sartorius, Göttingen, Germany)) or on the agar surface (resulting from the beta-glucuronidase activity that hydrolyzes MUG present in the media) were counted as *E. coli*. Presumptive *E. coli* isolates were further tested for tryptophanase activity with Kovac's reagent.[20]

For *Enterococcus* spec., membrane filters with appropriate dilutions were incubated on azide nutrient pads (Sartorius) for 40–48 h at 37 °C according to Slanetz and Bartley, followed by incubation on kanamycin-aesculin-agar (Merck) for 1 h at 44 °C.[21] Red, pink and reddish brown colored colonies with positive aesculin reaction were counted based on ISO 7899-2.[22]

Table 1 Standard methods used for the analysis of water samples. Non-standard methods are explained in more detail in the text

Parameter	Standard method
Perfluorinated compounds	German standard method DIN 38407-42 (ref. 13)
Chelating agents	DIN EN ISO 16588 (ref. 14)
Metals	DIN EN ISO 17294-2 (ref. 15)
Chemical oxygen demand (COD)	ISO 6060 (ref. 16)
Total organic carbon (TOC) and dissolved organic carbon (DOC)	DIN EN 1484 (ref. 17)
Spectral absorption coefficient	German standard method DIN 38404-3 (C3) (ref. 18)

For analysis of staphylococci, Chapman-Stone agar containing 0.05 g L^{-1} sodium azide was used. After incubation for 48 h at 37 °C, colonies were counted and streaked on Mannitol-Salt agar.

For each sampling event, randomly selected colonies of the genera *Enterococcus* and *Staphylococcus* were selected for identification on species-level by their physiological reactions on Micronaut-Staph®- and Micronaut-Strep2®-microtiter plates (MERLIN Diagnostika GmbH, Bornheim-Hersel, Germany) according to manufacturer's instructions (for details: http://www.merlin-diagnostika.de/micronaut-identifizierung.html).

2.4 Antibiotic susceptibility testing

The agar diffusion test according to the German standard method DIN 58940 was performed with 50 enterococci to test the antibiotics ampicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), and erythromycin (15 μg).[23] To detect vancomycin resistant *E. faecium* and *E. faecalis* (VRE), the susceptibility of these isolates against vancomycin was tested as described by the Clinical and Laboratory Standards Institute (CLSI).[24] Intrinsic low-level vancomycin resistance of *E. gallinarum* and *E. casseliflavus* (vanC1- and vanC2-type) were not considered.

Susceptibility of 76 *E. coli* isolates was tested against the antibiotics ampicillin (10 μg), ciprofloxacin (5 μg), cotrimoxazol (1.25 μg trimethoprim/23.75 μg sulfamethoxazol) with agar diffusion tests according to DIN 58940 and against cefotaxim (5 μg) using the clinical breakpoints listed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[23,25] All ampicillin resistant isolates were additionally tested against ceftazidim (30 μg) and cefpodoxim (10 μg) according to CLSI;[24] the inhibitory effect of clavulanic acid on the β-lactamase was checked as described by Bradford.[26] According to the definition of the Robert-Koch-Institute, extended-spectrum-β-lactamase producer phenotypically showed resistance against cefpodoxim as well as ceftazidim and/or cefotaxim and the inhibition of the enzyme by clavulanic acid.

Susceptibility of the 144 obtained *Staphylococcus* isolates against oxacillin (5 μg), ciprofloxacin (5 μg) and erythromycin (15 μg) was tested by the disc-diffusion test with Mueller-Hinton agar according to DIN 58940 and to clindamycin (2 μg) according to CLSI.[23,24]

2.5 Data analysis

Changes in the number of living *E. coli*, enterococci and staphylococci and in the percentages of antibiotic resistant isolates during passing the RSF were tested respective their statistical significance using bilateral *t*-test. Bacterial numbers represent the median values of five parallels. Removal of micropollutants was determined by calculating the average removal of all single sampling events. The variability of the data is expressed by the standard deviation. In the case of effluent values below the LOQ, the value half of the LOQ was used for calculation. Data are only presented if for at least half of the sampling events concentrations above the LOQ were measured.

3 Results and discussion

In the years 2012 and 2013, the volume of CSO treated in the RSF per month varied in a wide range between less than 100 m^3 up to more than 12 000 m^3. The same holds for the number of days when a measureable amount of drainage could be observed in the RSF's effluent. The total number of drainage events at the RSF in Tettnang can be considered as rather high. A survey by Roth-Kleyer *et al.* showed that almost 60% of RSFs in Germany had less than twelve events per year.[10] However, it has to be pointed out that such small numbers of events have to be regarded as contradictory to the establishment of reed vegetation. The intermittent loading of RSFs is important for the aeration of the subsurface. It has been demonstrated that sufficient oxygen supply in the soil filter enhances the removal of COD, ammonia and total nitrogen and that other factors like vegetation or temperature variations between 2 °C and 20 °C had only little effect on removal rates.[27]

3.1 Group parameters

DOC represents the total carbon concentration of all dissolved organic compounds. It is especially important for the depletion of oxygen and thus affects prevailing redox conditions and the degradation of inorganic nitrogen compounds like ammonia. The UV absorption of dissolved organic matter is measured by the parameter $SAC_{254\ nm}$. As mainly unsaturated organic compounds absorb radiation at 254 nm, the parameter is used as a proxy for aromatic dissolved organic matter. Both, DOC and $SAC_{254\ nm}$ can be used to calculate the specific UV absorption (SUVA) coefficient ($SAC_{254\ nm}$ divided by DOC). A high SUVA indicates a high portion of aromatic compounds in the water. The good DOC-removal of more than 80% (Fig. 3) during a short residence time in the filter reflects a high portion of readily biodegradable dissolved organic matter in the influent of the filter. The average DOC concentration in the RSF influent of 6.9 mg L^{-1} is in a concentration range typically measured in the effluent of municipal WWTPs, where readily biodegradable DOC has already been assimilated during activated sludge treatment. The $SAC_{254\ nm}$ is reduced in the RSF by about 50%.

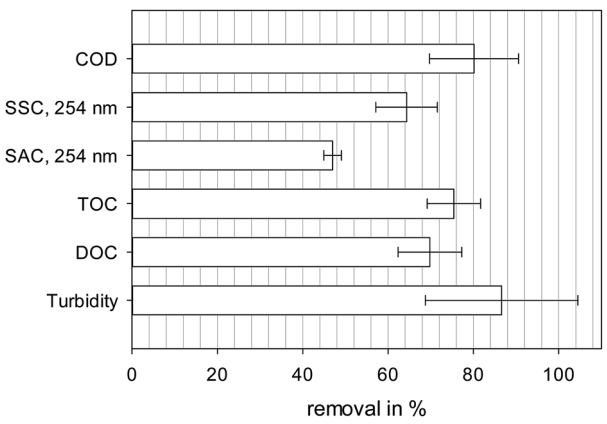

Fig. 3 Mean removal of physical–chemical and group parameters by RSF treatment. Error bars represent standard deviation.

The more pronounced reduction of the DOC relative to the $SAC_{254\ nm}$ is expressed by an increase of the SUVA from 1.7 L $(mg\ m)^{-1}$ to 3.1 L $(mg\ m)^{-1}$. Therefore, it can be concluded that less UV light absorbing saturated organic compounds are preferentially degraded in the RSF.

In similar subsurface treatment processes like soil aquifer treatment (SAT) the same observations have been made.[28,29] For a SAT field in Israel a preferential degradation of non-aromatic organic compounds was reported as an increase of SUVA from the percolation basin to a vertically located observation well (detention time 1.5 months) was observed.[30] In contrast to the $SAC_{254\ nm}$, the $SSC_{254\ nm}$ takes the scattering of light due to suspended matter into account.[31] As expected, a higher removal rate of $SSC_{254\ nm}$ in the RSF Tettnang could be observed (Fig. 3) due to the removal of suspended particles in the RSF by physical filtration processes. Higher removal rates of TOC compared to DOC and the pronounced improvement in turbidity can also be attributed to the removal of suspended solids by the RSF.

The COD removal of 80 ± 10% is in the range found in literature for other RSFs in Germany. Mean removal rates from 40% up to more than 90% have been reported.[4,32]

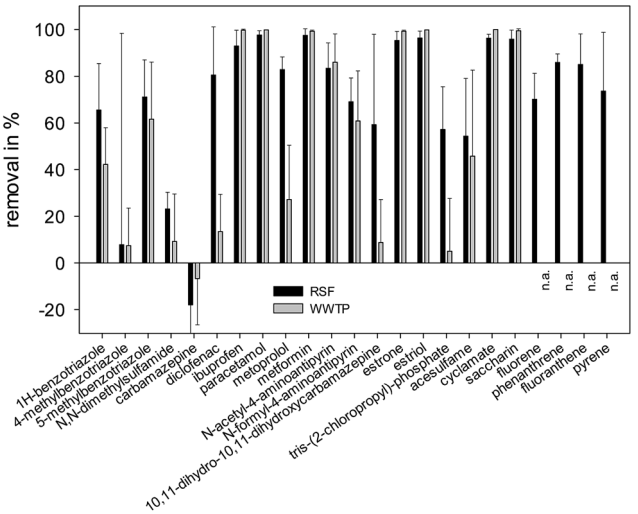

Fig. 4 Comparison of removal rates of organic trace pollutants in a retention soil filter and a wastewater treatment plant after activated sludge treatment, n.a. = not analyzed. Error bars represent standard deviation of removal.

3.2 Micropollutants

Most of the 187 micropollutants analyzed were not detected in the RSF influent or effluent on a regular basis. Micropollutants with wastewater origin are diluted by storm water and consequently if present their concentrations often decreased below the respective LOQ.

Pharmaceuticals and their metabolites. From a total of 79 pharmaceuticals (including antibiotics and some metabolites and excluding X-ray contrast media) only 24 were regularly found in more than half of the WWTP influent samples analyzed within this study. Only nine out of those 24 compounds were detected in the RSF influent in more than 50% of the samples. Generally, the pharmaceutical residues with the highest concentrations in the WWTP influent were also detected in the RSF influent and are depicted in Fig. 4. Pharmaceuticals additionally found in the influent of the WWTP Eriskirch belonged to several therapeutic classes with betablockers (atenolol, bisoprolol, sotalol) and antibiotics (clarithromycin, sulfamethoxazol, trimethoprim, ciprofloxacin, ofloxacin) as the most prevailing compound groups.

For most pharmaceuticals removal efficiencies were similar for activated sludge treatment in the WWTP and in the RSF. Pharmaceutically active compounds which are commonly readily biodegradable in WWTPs were also removed in the RSF to a high degree. Examples are paracetamol (98%) and ibuprofen (94%). For the latter substance good removal from 0.5 μg L^{-1} to a concentration below the LOQ has been reported for another RSF site.[33]

For diclofenac, metoprolol and 10,11-dihydro-10,11-dihydroxycarbamazepine, a metabolite of the antiepileptic drug carbamazepine, removal rates were slightly higher than in the WWTP. Varying removal rates have been reported for diclofenac.[34–37] The fact that diclofenac is not readily biodegradable is attributed to its chlorine group. Wastewater characteristics as well as treatment conditions also influence removal rates in WWTPs.[37] The mean removal of diclofenac in the WWTP Eriskirch was 14% ($n = 11$) after activated sludge treatment and 37% post sand filter at the end of the treatment train, whereas the RSF removed 89% on average (all effluent values < LOQ). This is in agreement with good diclofenac removal rates reported for other RSFs. In Altendorf (Germany) the mean concentration of diclofenac (0.14 μg L^{-1}) was reduced to below the LOQ.[33] Tondera *et al.* reported a diclofenac removal of more than 70% for two RSF in North-Rhine-Westphalia (Germany).[9,38]

The reported elimination for metoprolol in WWTPs is also highly inconsistent and ranges between 0% and more than 80%.[36,39–41] Sorption in water-sediment systems revealed negligible sorption tendency for metoprolol.[42] The removal of 36% and 84% in the WWTP Eriskirch and the RSF Tettnang, respectively should be attributed mainly to biodegradation although metoprolol is present in its cationic form at environmental pH values and might also be retained by negatively charged solid particles.

The negative elimination of carbamazepine (see Fig. 4) can be presumably explained by the cleavage of glucuronide conjugates, which are formed in the human body, back to carbamazepine. The fact that carbamazepine is not removed at all may reflect the prevailing redox conditions in the RSF, as the compound is persistent in an aerobic environment but can be degraded under anaerobic conditions.[43]

For the antidiabetic drug metformin concentrations in WWTP influents up to 100 μg L^{-1} have been reported and as a rule removal rates are higher than 90%.[12,44] This was also the case for the WWTP Eriskirch, but due to high influent concentrations still several hundred ng L^{-1} could be detected after biological wastewater treatment. However, as for most micropollutants no complete mineralization takes place during biological treatment, but degradation products are formed. In

the case of metformin, transformation into guanylurea (diaminomethylideneurea) takes place.[44,45] Consequently, a maximum concentration of 96 μg L^{-1} guanylurea was measured in the effluent of the WWTP Eriskirch. Removal of metformin was high and comparable to the WWTP Eriskirch, but again traces of metformin were detected in the RSF effluent. Interestingly, no guanlyurea was detected in the effluent of the RSF. The residence time and biological activity of the RSF appear to be sufficient to degrade or retain both metformin and its transformation product and therefore, the RSF has a better performance compared to conventional activated sludge treatment in WWTPs.

Benzotriazoles. The corrosion inhibitor 1*H*-benzotriazole is used in dishwashing liquids, anti-freezing and deicing fluids. Its elimination in the WWTP Eriskirch was incomplete (mean value 42%) and an average value of 5.1 μg L^{-1} could still be measured in the treated wastewater. This is in accordance with the incomplete removal reported in literature, where effluent concentrations of several μg L^{-1} were detected.[46–48] The mean removal of 1*H*-benzotriazole in the RSF was better (66 ± 20%) but not significantly different. The compound was found in production wells at river bank filtration sites after a residence of several months in the saturated subsurface.[49] However, sorption onto soils is negligible[50] and the decrease in concentration during RSF passage can most likely be attributed to a slow biodegradation. Greater persistence in aquatic environments has been reported for the 4-methyl analogue 4-methylbenzotriazole.[48] A decreasing 1*H*-benzotriazole/4-methylbenzotriazole ratio with increasing residence time was observed in the partially closed water cycle in Berlin, Germany.[49] In comparison to 1*H*-benzotriazole, the removal for 4-methylbenzotriazole was lower at both sites, the WWTP and the RSF.

Estrogens. The main sources for estrogenic activity in wastewater are natural hormones and synthetic compounds like 17α-ethinylestradiol (EE2) and, to a smaller extend, alkylphenols. The removal of such compounds during conventional wastewater treatment is usually good, but often incomplete. Both, biodegradation and sorption onto sludge contribute to the elimination of these compounds.[51] A good compilation of estrogenic compounds, their effects and fate during wastewater treatment can be found in Teske and Arnold.[52] Estrone (E1), 17β-estradiol (E2) and estriol (E3) were detected in every influent sample of the WWTP Eriskirch with mean concentrations of 140 ± 130 ng L^{-1}, 37 ± 16 ng L^{-1} and 180 ± 48 ng L^{-1}, respectively. The removal during wastewater treatment was in almost all cases complete and only once E1 was detected with 2.1 ng L^{-1} above the limit of quantification (LOQ = 0.5 ng L^{-1}) after activated sludge treatment. Only E1 and E3 were detected on a regular base in the RSF influent but due to dilution with lower concentrations of 22 ± 19 ng L^{-1} and 25 ± 17 ng L^{-1}. In Fig. 4 the removal of both compounds appears to be slightly lower than in the WWTP, but concentrations were lower than the detection limit. The apparent lower removal rate is simply due to lower influent concentrations, which were compared with the same effluent values as above (half of the LOQ).

Artificial sweeteners. For the artificial sweeteners saccharin and cyclamate, good removals were observed for both the WWTP and RSF. This is in accordance with high removal rates for both compounds during wastewater treatment reported in literature.[53] Acesulfame was retained with a mean value of 46% in the WWTP Eriskirch. However, the removal rate varied over a wide range from −5% to 90% with no obvious trend or seasonal correlation like it was reported for some pharmaceuticals in the past.[54] The results are against expectation as in previous publications acesulfame was reported to be recalcitrant and was only poorly removed in WWTPs.[53,55] An adaptation of microorganisms cannot be the only reason for the degradation as after sampling events with high removal a pronounced stability of acesulfame was observed again. However, recent studies have questioned the compound's stability and report a partial degradation in certain environmental compartments like river bank filtration sites.[56] The reason for the heterogeneous stability of acesulfame is yet unknown. Tran *et al.* reported a poor removal of acesulfame in batch tests with nitrifying sludge, but removal was increased with increasing ammonium concentrations.[57] Maximum acesulfame concentrations after activated sludge treatment or in the effluents of the RSF were 19 μg L^{-1} and 1.3 μg L^{-1} respectively and thus among the highest values observed for micropollutants.

Antibiotics. Within this study 37 antibiotics comprising different classes (macrolides, tetracyclines, penicillins, sulfonamides, fluoroquinolones) were analyzed. Only two of them (sulfamethoxazole and trimethoprim) were detected once in the influent of RSF Tettnang with a maximum concentration of 0.12 μg L^{-1} for sulfamethoxazole. Beside these two antibiotics azithromycin, clarithromycin, roxithromycin, ciprofloxacin and ofloxacin were detected in the influent of the WWTP. With two exceptions the concentration levels were below 1 μg L^{-1}, but all antibiotics mentioned before could be found at least once above the LOQ after activated sludge treatment. No conclusions about the RSF performance in terms of the removal of antibiotics can be drawn as values below LOQ can be a matter of dilution and are no proof for the complete absence of a certain compound. Assuming similar elimination processes and removal rates in the WWTP and the RSF, traces of antibiotics might end up in receiving waters. Other studies have pointed out that presence and long-term exposure even to rather low concentrations of antibiotics might be a reason for the selection of resistant bacteria and their spreading in the environment.[58,59]

The source of a compound is an important factor for its presence in combined sewers. If compounds which derive mainly from domestic wastewater are biodegradable, their absolute amount released to the environment will increase due to the absence of treatment of CSO overflow. However, there will be no change in the absolute amount of recalcitrant compounds.

In contrast, the absolute amount of micropollutants which are only re-mobilized, washed off or introduced by surface runoff during rain events will increase in any case.[60] Biodegradable micropollutants are discharged by CSO overflow, while recalcitrant substances are released by both CSO overflow and WWTP effluent to receiving waters.

One example for the appearance of a micropollutant in runoff is mecoprop, which is used as pesticide but also as

biocide, *e.g.* in roof sealings. As a consequence it can be washed off and released into the environment during rainfall events.[61] The compound was not detected in samples from the WWTP influent in Eriskirch. In contrast, in two RSF influent samples mecoprop was measured with a maximum concentration of 1.4 $\mu g\ L^{-1}$. However, mecoprop was efficiently removed during RSF passage to a final concentration below the LOQ.

Another compound group typical for the phenomenon of remobilization during rainfall events are polycyclic aromatic hydrocarbons (PAHs). Since their sorption tendency to particulate matter is high, it is of special importance for their elimination that the concentration of solids is reduced to a minimum during treatment. Filterable solids were reduced by more than 97% in the RSF Tettnang. A 93% retention was reported by Tondera *et al.* for a RSF six years in operation.[38] PAHs were analyzed only for the RSF site. When detected in the RSF influent removal efficiency was between 70–90%.

In summary the performance of the RSF for the removal of micropollutants can be most likely attributed to a combination of sorption and biodegradation. Compounds not discussed above were not detected in the RSF influent or the calculation of their removal rate was not feasible due to the lack of frequent detection.

3.3 Removal of microorganisms

Concentrations of all microorganisms studied were significantly lower in the RSF effluent than in the influent (Fig. 5). Removal rates were variable but comparable with those in WWTPs. The number of *E. coli* and enterococci decreased by 2.7 (median value; values for individual samples ranged from 2.1–3.2) and 2.2 log-units (median value; values for individual samples ranged from 0.9–2.8), respectively. The maximum elimination achieved was in a similar range as reported for other constructed wetlands planted with *Phragmites* spec.[33,62,63] The median elimination rate of fecal indicator bacteria (FIB) exceeded the observations made by Tondera *et al.*, who observed removal rates of 1.1 log-units for *E. coli* and enterococci.[9] Comparing the number of staphylococci in the influent and effluent of the RSF, their number significantly decreased by about 2.4 log-units (median value; values for individual samples ranged from 2.2–3.0 log-units). Faria *et al.* reported a removal of about 2 log-units in a conventional activated sludge treatment plant.[64] A higher retention of up to 4 log-units (median value) was detected by Heß and Gallert.[65] Comparing elimination efficiencies of WWTP and RSF, it has to be considered that the cell count of staphylococci was already lower in the influent of the RSF (10^3 CFU per 100 mL, median value) compared to the influent of a WWTP (Faria *et al.*: 10^5 CFU per 100 mL; Heß and Gallert: 2.7×10^4 CFU per 100 mL (median value)).[64,65] In consequence, the removal expressed in log-decades has to be lower. Remarkably, the concentration of staphylococci in the effluent of the RSF (median value: 3.8 CFU per 100 mL) was in the same order of magnitude or lower (Faria *et al.*: 10^2 CFU per 100 mL; Heß and Gallert: 2.3 CFU per 100 mL)[64,65] compared to the WWTP effluents.

Factors like retention time, characteristics of the filter material, organic matter concentration and biocenosis composition differ from site to site, however many authors suggested predation in the colmation layer or the subsurface of the RSF and adsorption as main factors for the retention of bacteria in RSFs.[63,66,67] Bacteria and viruses are attached to solids or in the free water phase[9] and like for hydrophobic micropollutants which are preferentially bound to particles, Passerat *et al.* observed a first-flush effect for *E. coli* in a CSO discharge caused by particle attached cells.[7] Based on the analysis of TOC and SSC, a considerable removal of suspended matter can be assumed and at least partial removal of bacteria attached to particles by filtration is likely. However, Tondera *et al.* observed no correlation between the removal of total suspended solids and the removal of *E. coli* or enterococci.[9] Key factors and mechanisms governing removal processes comprise a complex combination of chemical, physical and biological factors, which was not accounted for in this study and should be investigated by further *in situ* studies. In this context, it would also be interesting to perform long-term experiments investigating whether the RSF sediment is likely to change from sink to source.

Nola *et al.* studied different soil columns with regard to their retention efficiencies.[68] The removal of staphylococci was in the order of 99.99%. Comparing their results with those of the RSF in Tettnang is difficult because of higher staphylococci concentrations in the influent of the columns (2.9×10^7 CFU per 100 mL; RSF, median value: 10^3 CFU per 100 mL) with a retention efficiency of 6.7 log-units (mean value) and different construction and operating factors. Overall, the efficiency of the retention of particles depends on the construction and the operating factors of the RSF. Sidrach-Cardona and Bécares identified a subsurface-flow constructed wetland planted with *Phragmites* spec. as the best combination among several types of construction and reported a removal of total coliforms, *E. coli* and *Enterococcus* spec. between 2.5 and 3 log-units (mean value).[63] The construction of the treatment facility and the removal efficiency are similar to the results obtained in this study.

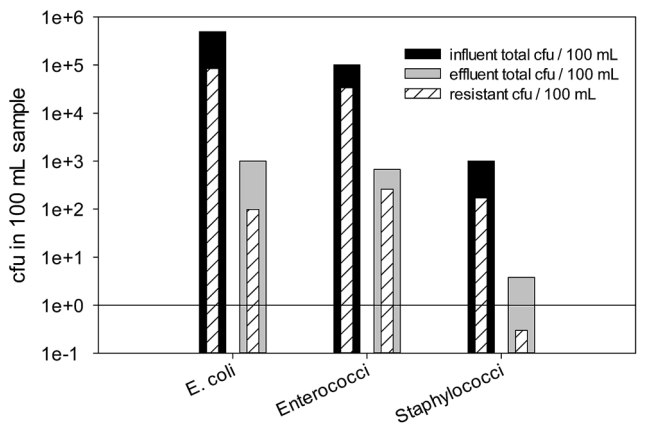

Fig. 5 Colony forming units (cfu) (mean values) of total and resistant *E. coli*, enterococci and staphylococci per 100 mL in the influent and effluent of the RSF Tettnang.

3.4 Species diversity of staphylococci

Comparing the diversity of staphylococci obtained from the influent (71 isolates) and the effluent (73 isolates) of the RSF, a shift of the abundance of the different species could be observed: whereas the percentage of isolates belonging to the Sciuri-group decreased from 59.3% in the influent to 36.0% in the effluent, the percentage of isolates clustered to the Saprophyticus-group remained stable (influent: 37.3%; effluent: 36.0%). However, within the Saprophyticus-group the abundance of the respective species changed: the percentage of *Staphylococcus xylosus* on isolates belonging to the Saprophyticus-group increased from 10.9% in the influent to 66.7% in the effluent. Obviously, some *Staphylococcus*-species can better adapt to the conditions of the RSF than others. Coinciding with Faria *et al.*, Heß and Gallert reported that species of the Saprophyticus-group were dominant in raw sewage (Faria *et al.*: 78.7% of the isolates were representatives of the mentioned group; Heß and Gallert: 51.6% of the isolates were species of the Saprophyticus-group and 31.8% of them were identified as *S. xylosus*).[64,65] Furthermore, the study of Heß and Gallert showed that species of the Sciuri-group were more abundant in river water than in sewage and the percentage of *S. saprophyticus* ssp. *saprophyticus* decreased in favor of *S. xylosus* in receiving water bodies.[65] Using the percentage of isolates belonging to the Sciuri-group as well as the percentage of *S. xylosus* on the abundance of isolates belonging to the Saprophyticus-group as "less-sewage derived" markers, a further characterization of the influent and the effluent of the RSF is possible: based on diversity and abundance of the staphylococci, the influent of the RSF was a mixture of raw sewage diluted with a significant volume of surface runoff. The *Staphylococcus*-species diversity found in the effluent of the RSF was dominated by species rather associated with river water indicating that these species are better adapted to the condition of the RSF.

3.5 Antibiotic resistant *E. coli*, enterococci and staphylococci

The percentage of antibiotic resistant *E. coli* isolates (35 isolates obtained from the influent and 41 strains obtained from the effluent were tested) significantly decreased during passing the RSF from 17% in the influent to 10% in the effluent (level of significance 0.05). If an isolate was resistant, it was resistant against ampicillin. In the influent two isolates were additionally resistant against cotrimoxazole, one of them also against cefotaxim classifying it as an extended spectrum β-lactamase (ESBL) producer. The fact that ESBL-producers can be detected by culture based approaches points out that they are present in respective numbers in sewage. In the effluent, one ampicillin resistant *E. coli* was additionally resistant to cotrimoxazole. Luczkiewicz *et al.* found a comparable resistance pattern for *E. coli* isolated from a municipal wastewater treatment plant, but overall the resistance level was lower.[69]

Similar to *E. coli*, the percentage of antibiotic resistant staphylococci (71 isolates obtained from the influent and 73 strains obtained from the effluent were tested) was significantly lower in the effluent of the RSF (6.8%) than in the influent (16.9%, level of significance 0.05). The percentage of isolates resistant to erythromycin determined the resistance level. None of the obtained staphylococci was constitutively resistant against clindamycin and/or ciprofloxacin. The fact that 5 isolates obtained from the influent were resistant against oxacillin showed that the antibiotic resistance gene *mec*A is present in sewage. The observed resistance pattern was comparable to that described for raw sewage,[64,65] but the overall percentage of resistant isolates was lower. A possible explanation for this observation is based on the species diversity. Some species were more frequently resistant than others: for instance, 24.7% of the *S. saprophyticus* ssp. *saprophyticus* isolates, a species dominating in raw sewage, was resistant against one of the tested antibiotics, whereas "only" 10.8% of *S. xylosus* isolates, which rather could be isolated from river water, was resistant.[65] Consequently, based on the found resistance levels of *E. coli* and staphylococci as well as on the described *Staphylococcus*-species diversity, the influent of the RSF cannot be directly compared to the influent of a WWTP under dry weather conditions.

The percentage of resistant enterococci (32 isolates obtained from the influent and 18 strains obtained from the effluent were tested) did not significantly change during passing the RSF (influent: 34.4%; effluent: 38.9%). This observation might be an effect of the small number of isolates; a higher number is needed to confirm this result. Erythromycin resistance determined the resistance level of enterococci in the respective sample. Two isolates in the influent and one isolate obtained from the effluent were additionally resistant against ciprofloxacin. One *Enterococcus* isolate of the effluent was resistant against the last resort antibiotic chloramphenicol. None of the obtained isolates were high-level resistant against vancomycin and/or ampicillin. Luczekiewicz *et al.*, who isolated enterococci from sewage of a municipal WWTP, also found the described resistance pattern, but as already seen for *E. coli* and staphylococci the overall percentage of resistant isolates was lower.[69]

Even if the resistance level of enterococci did not decrease, the absolute concentrations of antibiotic resistant *E. coli*, enterococci and staphylococci in the effluent of the RSF were about 2.1 and 2.9 log-units lower than in the influent. Nevertheless, antibiotic resistant bacteria and antibiotic resistance genes were released into the receiving Tobelbach. At the moment, the remaining risk and the effect on autochthonous microorganisms in receiving waters cannot be predicted. Some authors discussed the long hydraulic-retention time as well as the exposure to low (not inhibitory) concentrations of different antibiotics as promoting factors for the spread of antibiotic resistance genes.[58,59] Comparing the detected resistance level of the influent and the effluent of the RSF in Tettnang, there were no indices at present for the spread of resistance determinants.

4 Conclusions

The effectiveness of a retention soil filter (RSF) for the removal of micropollutants, facultative pathogenic and antibiotic resistant bacteria from CSO was investigated. The removal efficiency for micropollutans was comparable to activated sludge treatment in a nearby WWTP. WWTP effluent still is an important

source of water pollution with micropollutants. The conventional treatment in WWTPs is especially important for biodegradable mircopollutants or compounds which tend to adsorb onto suspended and settleable solids. First measures for upgrading WWTPs with ozonation or activated carbon treatment units on a full-scale are taken and will improve the overall removal efficiency. However, the whole treatment train is only successfully applied during dry weather conditions. Therefore, a complementary treatment option with an active microbial environment and a reliable adsorption capacity is needed for events of heavy rainfall, when WWTPs and sewage overflow basins cannot cope with the additional amount of water. The results of this study support the extension of the RSF technology as a low cost addition to overflowing combined sewer overflow basins to control the discharge of standard pollutants and micropollutants, and the number of facultative pathogenic and antibiotic resistant bacteria. Detailed investigation of the subsurface of full-scale RSFs should be conducted for a better understanding of the basic processes involved in the removal of micropollutants, facultative pathogenic and antibiotic resistant bacteria.

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