Remediation and Control Technologies

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# Microbiome triggered transformations of trace organic chemicals in the presence of effluent organic matter in managed aquifer recharge (MAR) systems

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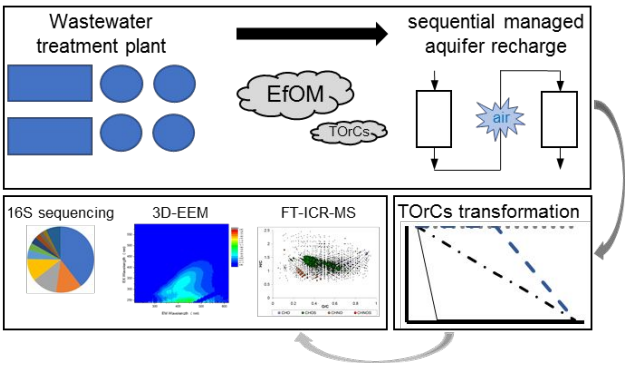
## Abstract

It is widely assumed that biodegradation of trace organic chemicals (TOrCs) in managed aquifer recharge (MAR) systems occurs via a co-metabolic transformation with dissolved organic carbon serving as primary substrate. Hence, the composition facilitating bioavailability of the organic matter seems to have a great impact on TOrCs transformation in MAR systems. The aim of this study was to elucidate the character of effluent organic matter present in the feed water of a simulated sequential MAR system throughout the infiltration by use of FT-ICR-MS analyses as well as spectroscopic methods. Furthermore, compositional changes were correlated with TOrCs targeted throughout the system as well as the abundance of different microbial phyla. Based on their behavior throughout the infiltration system in which different redox and substrate conditions prevailed, TOrCs were classified in four groups: easily degradable, redox insensitive, redox sensitive, and persistent. Masses correlating with persistent TOrCs were mainly comprised of CHNO containing molecules, but also of CHO which are known as carboxyl-rich alicyclic molecules, while CHOS and CHNOS can be neglected. Easily degradable TOrCs could be associated with CHNO, CHO and CHOS containing compounds. However, a shift of molecular compounds to mostly CHOS was observed for redox insensitive TOrCs. 338 masses correlated with removal of redox sensitive TOrCs, but no distinct clustering was identified.


## Table of content/Abstract Art




## 1. Introduction

Trace organic chemicals (TOrCs) usually occur in wastewater treatment plants (WWTP) effluents as well as in the aquatic environment in a concentration range of nanogram to microgram per liter (ng-µg/L), whereas the concentration of the dissolved organic carbon (DOC) is orders of magnitudes higher - typically at several milligrams per liter (mg/L)[1–4]. It is widely assumed that ambient concentrations of TOrCs are not sufficient to support microbial growth[5]. Thus, additional organic matter or DOC is needed as a primary growth substrate[5]. This transformation of rarely available substrates without any direct benefit to bacteria in the presence of growth supporting substrates is known as co-metabolism[6]. Recent studies indicated that the removal of TOrCs in the natural environment or engineered biological treatment systems commonly follows such co-metabolic mechanisms[7,8]. A key parameter for co-metabolic transformation of TOrCs is the availability of biodegradable DOC (BDOC) as primary substrate, both with respect to its quality and quantity[1,9]. Previous studies revealed that both the composition and the concentration of BDOC affect the total amount of biomass and the structure of the microbial community[10–12] as well as their functionality[13] and, therefore, TOrCs removal[9]. It is well established that the transformation of TOrCs is enhanced under carbon-limited conditions[3,13,14]. Primary substrate comprised of refractory organic compounds, such as humic acid like organic matter, led to an increased TOrCs removal compared to systems

using a high fraction of easily degradable compounds like peptone/yeast[9,13]. However, it still remains unclear, which functional characteristics of the natural organic matter serving as primary substrate determine the efficacy of TOrCs biotransformation. To unravel the composition of the dissolved organic matter (DOM), several analytical tools were previously applied[15,16]. DOM can be separated based on operationally defined polarity gradients using XAD-resins fractionation or according to its size using size-exclusion chromatography[17,18]. Furthermore, spectroscopic methods like UV absorbance at 254 nm ($UVA_{254}$) and 3D-fluorescence (3D-EEM) were widely used[19–21]. However, applied alone these approaches have the disadvantage of deciphering only chromophoric DOM[16]. In addition, analytical tools allowing for the assignment of functional groups, compound classes and molecular formulas were commonly applied[16]. This includes Fourier transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectroscopy[16–18] as well as more lately Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS)[16,22,23]. Because of its high resolution and mass accuracy, FT-ICR-MS has a great capability to unravel the molecular complexity of DOM and is suitable for assigning molecular formulas for complex structures[24,25].

The aim of this study was to decipher the composition of effluent organic matter (EfOM) potentially serving as primary substrate in a simulated sequential managed aquifer recharge (MAR) system using FT-ICR-MS and spectroscopic methods and to correlate potential changes throughout the infiltration with the transformation of selected TOrCs as well as the abundance of microbial phyla. The sequential MAR technology (SMART) is defined as a combination of two infiltration steps in series with an intermediate aeration step to establish highly controlled oxic and carbon-limited conditions in the second infiltration system which are favorable for the removal of many TOrCs[26].

## 2. Materials and Methods

### *2.1 Laboratory-scale column experiment*

The laboratory-scale column experiment consisted of two sequential infiltration systems operated in downward flow direction under fully saturated conditions (Figure 1) for a period of approximately eight months. Tertiary treated effluent of the WWTP in Garching, Germany was continuously fed into the first column. The influent to the system was stored at ~4°C and filled up twice a week without an additional spike of TOrCs due to their immediate presence in the tertiary treated wastewater. Prior to sampling within this study, the column system was continuously operated with tertiary treated WWTP effluent for more than six months. Both systems were connected with an intermediate aeration step using pressurized air to simulate SMART and therefore, providing a series of different redox conditions and substrate availability. The columns of the first infiltration system (B01, B02; height (h): 50 cm, inner diameter (ID): 14 cm) were filled with technical sand (grain size ranged from 0.2 to 1.0 mm; Euroquarz GmbH, Germany), the columns of the second infiltration system (b1-b4; h: 30 cm, ID: 9 cm) with aquifer material ($d_{50}$ = 0.8 mm, $f_{oc}$ = 0.003%), which was taken from from previousl column experiments that were continuously operated for more than 5 years[1,14]. The flow rate of 60 mL/h (B01, B02) and 30 mL/h (b1-b4) resulted in a hydraulic retention time (HRT) of 2.1 days/column or 0.9 days/column, respectively an overall HRT of 7.8 days. The HRT was determined based on the C-peak method[27] using the conservative tracer potassium bromide (data not shown). Columns were composed of polymethylmethacrylate and could be opened on top for soil sampling. All columns were equipped with oxygen sensor spots (SP-PSt3, PreSens, Germany) for non-invasive oxygen measurements along the length of the columns. Water samples to characterize the bulk organic carbon (DOC; $UVA_{254}$, 3D-EEM, FT-ICR-MS) and prevailing redox conditions (dissolved oxygen (DO); ammonium- and nitrate-

106 nitrogen) as well as for quantifying TOrCs were taken (bi-)weekly in the influent (0.0 days)
107 and in the effluent of each column with respect to the HRT (2.1; 4.2; 5.1; 6.0; 6.9 and 7.8 days).

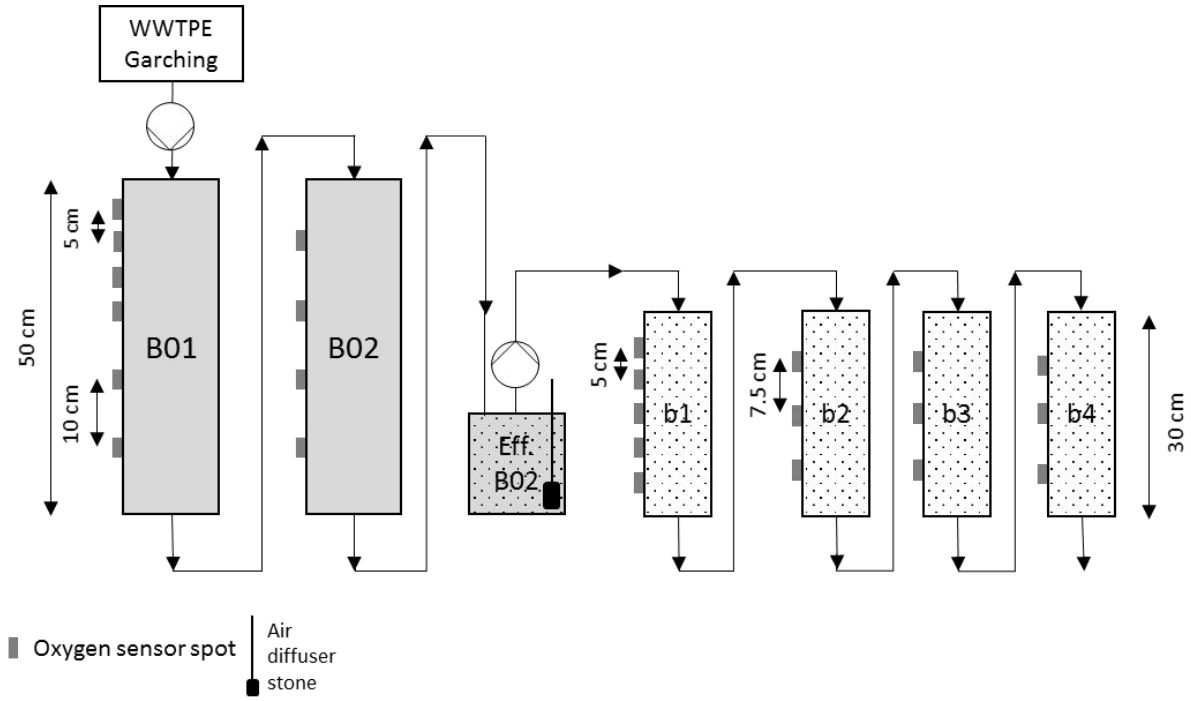



**Figure 1: Laboratory-scale column experiment consisting of two infiltration steps (system 1, grey: columns B01, B02; system 2, patterned: columns b1-b4) operated in series with an intermediate aeration (Eff. B02, grey patterned). The system was fed with effluent of the WWTP Garching, Germany (WWTPE) with a flow rate of 60 mL/h (system 1) and 30 mL/h (system 2), respectively.**

108

109 *2.2 Analytics*

110 ***Sampling:*** Soil samples for 16S rRNA amplicon sequencing were collected once,
111 approximately 1.5 months after the initial start of the experiment, from the top of each column.
112 Water samples for bulk organic carbon and redox characterization as well as for TOrCs
113 quantification were collected in 200 mL amber bottles and were filtered through 0.45 µm with
114 cellulose-nitrate filters (Sartorius AG, Germany). To characterize the EfOM, > 50 mL sample
115 were filtered (Whatman GF/F filter, Germany) followed by acidification to pH 2 using
116 hydrochloric acid (32%, Merck KGaA, Germany) and stored at 4 °C pending further analysis.

***DNA extraction and 16S rRNA amplicon sequencing:*** DNA was extracted in triplicate by use of the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, USA) according to manufacturer`s guidelines with the following exceptions: i) 1.0 g of soil was used as starting material, ii) a FastPrep®-24 cell disrupter (MP Biomedicals, USA) was used at 6 m/s for 40 s for disruption of the cells. The eluted DNA was pooled prior to downstream analyses. To increase the purity of each DNA extract, the Genomic DNA Clean & Concentrator Kit (Zymo Research Europe, Germany) was used with an input of 100 µL sample and 200 µL binding buffer. Illumina library preparation was operated following the 16S Metagenomic Library Preparation workflow (# 15044223 Rev. B; Illumina, USA) targeting the V3 and V4 region using Nextera XT Index Kit (Illumina, USA) for indexing as previously described by Engel et al. (2017)[28]. Flash v.1.2.11[29] was used to merge MiSeq forward and reverse paired end reads with a maximum overlap of 300 bp. All merged reads were combined to a single file and further processed using USEARCH v9.2.61[30] as provided in the supporting information (SI). Taxonomy assignment of identified operational taxonomic units (OTUs) was obtained by use of Ribosomal Database Project (RDP) classifier[31].

***Bulk organic carbon and redox parameters:*** For the DOC analysis, samples were acidified with hydrochloric acid to a pH of 2 and stored at 4 °C prior to the measurement in triplicate using a Vario TOC CUBE analyzer (Elementar, Germany). $UVA_{254}$ was photometrically determined in duplicate using a BioSpec UV-1601 (Shimadzu Europa GmbH, Germany). As the ratio of $UVA_{254}$ and the DOC the specific UV absorbance ($SUVA_{254}$) was calculated which could be used as parameter representing the aromatic content of the DOC[32]. To avoid quenching effects of the fluorescence signal, all samples with a DOC > 2 mg/L were diluted to 2 mg/L with ultrapure water prior to 3D-fluorescence spectroscopy[33] using a Aqualog® fluorescence spectrometer (Horiba Scientific, USA). More information about the 3D-

fluorescence spectroscopy measurements are provided in the SI. The dissolved oxygen (DO) concentration was monitored with the Fibox 4.0 (PreSens, Germany) by use of the oxygen sensor spots within the columns as well as flow-through cells (FTC-PSt3, PreSens, Germany) in the influent and the effluents of each column (detection limit = 0.015 mg/L). Hach cuvette tests LCK304 (0.015 – 2.0 mg/L $NH_4^+$ -N) and LCK340 (5 – 35 mg/L $NO_3^-$ -N) (Hach Lange GmbH, Germany) were used to determine the concentration of ammonium- and nitrate-nitrogen, respectively.

***TOrCs quantification:*** Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was used to quantify TOrCs[2]. Measurements were performed in positive electrospray ionization mode. All investigated TOrCs and their corresponding limits of quantification (LOQ) are listed in Table 1. If a measured TOrC concentration was lower than the stated LOQ, the detected value was set as half of the LOQ for further calculations.

***FT-ICR-MS analysis:*** Prior to FT-ICR-MS, a solid phase extraction (SPE) was performed as previously described by Dittmar et al. (2008)[34]. Therefore, 50 mL of each sample were extracted with 5 mL methanol (LC-MS ChromasolV®, Sigma-Aldrich, Germany) using PPL cartridges (Bond Elut PPL, 1 g, 3 mL; Agilent, USA) followed by a dilution of 1:50 (v/v %) with methanol. The analysis was conducted on a solariX FT-ICR-MS (Bruker Daltonik GmbH, Germany) equipped with a 12 Tesla superconducting magnet (Magnex Scientific Inc., GB) and an APOLLO II electrospray ionization (ESI) source (Bruker Daltonik GmbH, Germany) in the negative ionization mode. Further information about the mass spectra acquisition are provided in the SI. To elucidate masses, which were assigned to molecular formulas based on the elements $^{1}H$, $^{12}C$, $^{16}O$, $^{14}N$ and $^{34}S$, van Krevelen diagrams[35] are used representing masses by means of their hydrogen to carbon (H/C) and oxygen to carbon (O/C) ratio. Molecules

164 comprised of the elements H, C and O are referred as CHO in the following. The same counts
165 for CHNO, CHOS as well as CHNOS.

166 *2.3 Statistical analyses*

167 ***Parallel factor analysis (PARAFAC):*** PARAFAC is a statistical method, which is often
168 applied to identify and quantify components of 3D-fluorescence spectroscopy data[36].
169 Therefore, normalized 3D-EEM data were exported with adjusted excitation wavelengths of
170 239-599 nm from the Aqualog® software and further processed using the SOLO software
171 (Eigenvector Research Inc., USA)[33]. Details of the PARAFAC analysis are given in the SI.

172 ***Multivariate analyses:*** Multiple co-inertia analysis (MCIA) as well as orthogonal partial least
173 squares (OPLS) regression were used in order to describe the correlation between the masses
174 extracted by use of FT-ICR-MS, the microbiome in terms of OTUs identified by applying 16S
175 rRNA amplicon sequencing and in case of OPLS, metadata (Table S 2). For the correlation of
176 masses (and metadata) with the microbiome, the OTUs from the top of the first column (B01)
177 were assigned to the system`s influent B0 and OTUs from the top of each of the following
178 columns (B02, b1-b4) were equalized to the effluent of its previous column (B01, B02-b3).
179 Since no distinct differences in microbial community diversity in deeper sediments were
180 observed[12], OTUs from the top of column b4 were not only accorded to effluent b3 but also to
181 the final effluent of the system. Prior to analyses, all data (masses, microbiome and metadata)
182 were stored in one matrix and a unit variance (UV) scaling was applied. The MCIA was
183 calculated with the purpose of integrating the two different datasets: masses and OTUs.
184 Therefore, the MixOmics package (RStudio Version 1.0.136 – © 2009-2016 RStudio, Inc.)
185 was used. In order to describe the relation that link the metadata to the masses together with
186 the OTUs an OPLS was calculated in SIMCA 13.0.3.0 (Umetrics, Umeå, Sweden). Therefore,

a logarithmic transformation was applied, and the final model was built only with the significant metadata ($p<0.05$). The model's goodness of fit was tested by the p-values calculated with the CV-ANOVA (Cross Validation ANOVA). The procedure to select masses and OTUs, which were most relevant to describe the experimental design, followed the method previously described in Adrian et al. (2017)[37].

## 3. Results and Discussion

### *3.1 Long-term performance characterization of column systems*

***Bulk organic and redox parameters:*** The initial DOC concentration of 7.6 ± 1.9 mg/L was reduced to 4.6 ± 0.9 mg/L within the first 4.2 days of infiltration with the majority of removal occurring within the first column (Figure 2). After reaeration, the DOC was further depleted in the second infiltration system resulting in a final concentration of 3.6 ± 0.6 mg/L after 7.8 days.

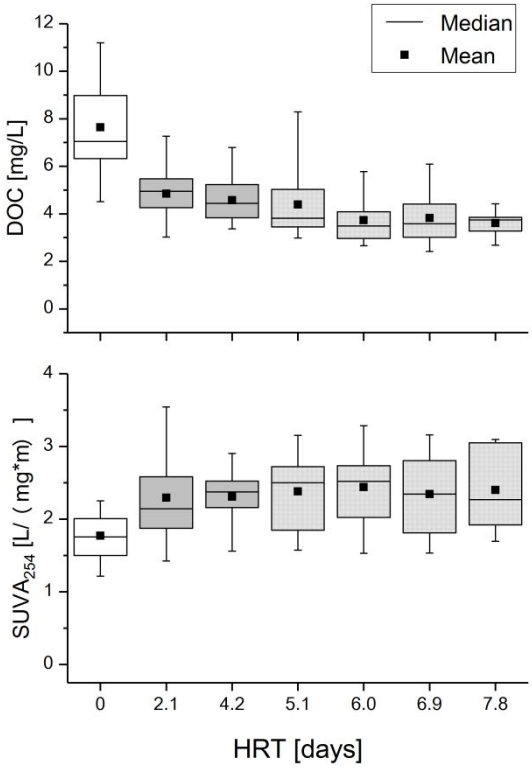



**Figure 2: DOC concentration ($n \geq 12$) and $SUVA_{254}$ ($n \geq 11$) throughout the system with respect to the HRT. The box represents the 25 – 75 percentiles, the whiskers indicate the maximum and minimum values.**

A significant increase of $SUVA_{254}$ in the first system (student`s *t*-test, two-tailed, paired, $\alpha < 0.05$) indicates a preferred removal of easily degradable aliphatic structures changing the character of the DOC to more aromatic. No significant change in $SUVA_{254}$ was observed after reaeration (Figure 2). Results of $UVA_{254}$ measurements are presented in Figure S 1.

Based on the 3D-fluorescence measurements and the subsequent PARAFAC analysis, two characteristic components were identified

(Figure S 2). The accuracy of the PARAFAC model was confirmed by i) a core consistency of 100 %, ii) 98 % total variance explained, and iii) 99 % as a result of the split half analysis. The first component had a maximum fluorescence intensity at 239/451 nm ($\lambda_{Ex}/\lambda_{Em}$) and the second component at 239/376 nm, respectively. Based on previously published studies, Chen et al. (2003)[38] classified five characteristic regions for organic compounds. Following this approach, component 1 was assigned to region III, fulvic acid-like substances and component 2 to region II, aromatic protein II (tryptophan-like substances). The intensity of these two components decreased throughout the infiltration, while a strong decline was observed in the first column. This is in accordance to the removal of DOC and the reduction of $UVA_{254}$, which were also mainly removed or rather declined during the first two days. After reaeration, a further decrease of both components was detected in the second infiltration system showing a similar trend as the $UVA_{254}$ measurements (cf. Figures S 1 and S 3). Due to the high amount of easily degradable DOC in the influent, the oxygen ($DO_{Influent}$ = 5.9 ± 1.0 mg/L) was rapidly consumed during subsurface treatment (Figure S 4). In addition, $NO_3^-$-N concentration decreased insignificantly from 13.3 ± 2.4 mg/L to 12.0 ± 2.2 mg/L indicating the prevalence of suboxic to anoxic conditions within 4.2 days of infiltration. An electron balance assuming average oxidation number of zero for organic carbon resulted in a consumption of 2.2 mg/L DOC from influent oxygen (2.7 mg $O_2$/mg C). A decrease of $NO_3^-$-N by 0.7 mg/L could be further explained due to the oxidation of 0.8 mg/L DOC to $CO_2$ (0.9 mg N/mg C) which confirms prevailing anoxic conditions in the first infiltration system. After reaeration, the DO immediately decreased within the first centimeters of infiltration which could not solely be explained by residues of easily degradable DOC in the influent to the second system. Therefore, redox conditions changed from oxic (DO (5.1 days) = 1.1 ± 1.3 mg/L) to suboxic defined by DO < 1.0 mg/L and $\Delta NO_3^-$ -N < 0.5 mg/L[14]. Ammonium ($NH_4^+$ -$N_{Influent}$ = 0.10 ± 0.12 mg/L)

was removed mainly below the LOQ (0.015 mg/L) after the first two days of infiltration (data not shown).

***Trace organic chemicals:*** TOrCs were classified into four groups based on their behavior throughout the system (Table 1), namely i) persistent compounds exhibiting less than 10% removal throughout the system; ii) easily degradable compounds being removed below LOQ within 4.2 days, even under anoxic redox conditions; iii) redox insensitive compounds exhibiting removal in both systems before and after aeration, and iv) redox sensitive compounds being persistent in the first infiltration system (4.2 days) but efficiently transformed after reaeration. Relative removal of all targeted TOrCs throughout the system are given in Figure S 5. Since limited contribution of sorption has been reported previously for similar column systems after long-term operation by Alidina et al. (2014), biotransformation can be assumed as major mechanism for TOrCs removal in this study[9].

**Table 1: List of TOrCs investigated during this study ($n \geq 7$).**

| TOrCs | LOQ [ng/L][a] | Molecular formula | Log P (log D pH 7.4)[d] | $c_0$ [ng/L] | Removal [%] | |
|---|---|---|---|---|---|---|
| | | | | | 4.2 days | 7.8 days |
| *Persistent (≤ 10% after 7.8 days)* | | | | | | |
| Carbamazepine | 10; 5 | $C_{15}H_{12}N_2O$ | 2.77 (2.77) | 393 ± 98 | < 10 | < 10 |
| Primidone | 25; 25 | $C_{12}H_{14}N_2O_2$ | 1.12 (1.12) | 72 ± 26 | < 10 | < 10 |
| *Easily degradable (removal below LOQ within 4.2 days)* | | | | | | |
| Citalopram[b] | 5; 5 | $C_{20}H_{21}FN_2O$ | 3.76 (1.41) | 154 ± 48 | >98 | - |
| *Redox insensitive (continuous removal within 7.8 days)* | | | | | | |
| Climbazole[c] | 5; 5 | $C_{15}H_{17}ClN_2O_2$ | 4.34 (4.30) | 111 ± 35 | 78 ± 21 | > 98 |
| Metoprolol[c] | 2.5; 2.5 | $C_{15}H_{25}NO_3$ | 1.76 (-0.47) | 309 ± 104 | 80 ± 16 | > 99 |
| Diclofenac | 25; 5 | $C_{14}H_{11}Cl_2NO_2$ | 4.26 (1.10) | 1326 ± 534 | 47 ± 19 | 94 ± 5 |
| Gabapentin | 10; 2.5 | $C_9H_{17}NO_2$ | -1.27 (-1.27) | 2246 ± 727 | 58 ± 15 | 92 ± 4 |
| Sotalol[c] | 5; 5 | $C_{12}H_{20}N_2O_3S$ | -0.40 (-2.12) | 41 ± 41 | 65 ± 46 | > 95 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Sulfamethoxazole | 10; 5 | $C_{10}H_{11}N_3O_3S$ | 0.79 (0.00) | 131 ± 72 | 29 ± 41 | 55 ± 36 |
| *Redox sensitive (persistent in first infiltration system, removal after reaeration)* | | | | | | |
| Benzotriazole | 50; 50 | $C_6H_5N_3$ | 1.30 (1.28) | 4302 ± 1187 | < 10 | 35 ± 33 |
| Tramadol | 5; 5 | $C_{16}H_{25}NO_2$ | 2.45 (0.62) | 265 ± 66 | < 10 | 71 ± 17 |
| Venlafaxine[c] | 2.5; 2.5 | $C_{17}H_{27}NO_2$ | 2.74 (1.22) | 343 ± 74 | < 10 | > 99 |

[a]The LOQ was adjusted during the experimental period: the first number gives the LOQ of the first five months, the second number of the last two months of operation.
[b]Removed < LOQ after 2.1 days of infiltration for most samples
[c]Removed < LOQ after 7.8 days of infiltration for most samples
[d]Calculated by use of Chemicalize (https://chemicalize.com/; accessed on 13.10.2018) developed by ChemAxon (http://www.chemaxon.com)

246

247 The persistent behavior of carbamazepine and primidone in MAR systems is well documented
248 from field- as well as laboratory-scale investigations[3,39–41]. Removal up to 70 % of citalopram
249 in a carbon-rich environment has recently been shown from batch experiments using activated
250 sludge[42]. Climbazole as well as metoprolol were removed > 75 % even under anoxic redox
251 conditions within 4.2 days of infiltration. A removal of > 75 % (HRT ≈ 12 days) under anoxic
252 redox conditions for metoprolol was also observed in laboratory-scale column experiments[41].
253 Muntau et al. (2017)[43] reported a removal below the LOQ (5 ng/L) via soil-aquifer treatment
254 (HRT < 1 day) for climbazole confirming the results of this study. Diclofenac, gabapentin, and
255 sotalol were removed < 70 % in the first, but > 90 % in total after 7.8 days of infiltration.
256 However, for sotalol 7 out of 22 measured influent concentrations were below the LOQ and
257 therefore, due to the low influent concentrations, further discussions about its removal
258 efficiency are not expedient. Diclofenac is known to be biodegradable in MAR systems and
259 particularly under oxic and carbon-limited conditions[14,26,41]. In addition, a significantly
260 enhanced transformation using the SMART approach in comparison to a conventional MAR
261 treatment was previously reported for gabapentin[3]. In case of the antibiotic drug
262 sulfamethoxazole, moderate to poor transformations of approximately 30 % were detected

after 4.2 and approximately 55 % after 7.8 days of infiltration. In the literature, several studies focusing on sulfamethoxazole and its behavior in MAR systems are reporting ranges of biotransformation from partially and slowly removable during bank filtration and artificial recharge[4] to resistant to microbial biotransformation in a pilot-scale riverbank filtration system[44]. Three out of 12 investigated TOrCs, namely benzotriazole, tramadol and venlafaxine, persisted in the first infiltration system under mostly anoxic redox conditions but were transformed throughout the system at approximately 35 % (benzotriazole), 70 % (tramadol), and below the LOQ (venlafaxine) after reaeration with air under prevailing oxic to suboxic redox conditions. The high redox sensitivity and favored degradation under carbon-limited conditions were previously reported for benzotriazole[2,3,41]. However, removal up to 35% is not very efficient and therefore it is postulated that oxic to suboxic redox conditions are not sufficient. Tramadol was not degraded in the first 0.9 days after reaeration, however, interestingly, a steady transformation up to approximately 70 % could be detected from 5.1 to 7.8 days of infiltration. It seems that the HRT had a significant influence on tramadol removal as it was previously shown to be persistent in a single-stage as well as sequential biofiltration system with an empty bed contact time of 290 to 2,090 minutes[2]. A transformation of > 99 % after reaeration was observed for venlafaxine emphasizing the benefit of predominant oxic and carbon-limited conditions for its removal. Previous results on venlafaxine degradation varied from persistent behavior in single stage as well as sequential biofiltration[2] to degradation of > 50 % even under anoxic redox conditions[41]. However, removal seemed most efficient under oxic conditions[41].

### *3.2 Microbial community structure*

285 The microbiome was elucidated throughout the system based on 16S rRNA amplicon
286 sequencing resulting in a median sequencing depth of approximately 60,000 reads. Based on
287 this analysis, the most dominant phyla were *Proteobacteria* (40 - 48 %), *Planctomycetes* (5 –
288 13 %), *Acidobacteria* (12 – 22 %), and *Bacteroidetes* (1 - 11 %) (Figure S 6). This is
289 confirming previous studies reporting that these organisms are, amongst others, highly
290 abundant in soil[45]. *Proteobacteria* were detected in all depths with the strongest contribution
291 to the overall microbial community structure. Whereas in the top of the first column *α*- and *γ*-
292 *Proteobacteria* dominated, after six days of infiltration *β*-*Proteobacteria* mainly occurred.
293 *Planctomycetes* as well as *Bacteroidetes* were also enriched in the shallow sediments and in
294 contrast, *Acidobacteria* and *Nitrospirae* primarily prevailed in the second system characterized
295 by carbon-limited ($\Delta DOC = 1.1 \pm 1.2$ mg/L) and oxic (DO > 1 mg/L) to suboxic
296 (DO <1 mg/L; $\Delta NO_3^-$ -N < 0.5 mg/L) conditions. This characterization focused at the phyla
297 and class level in order to provide a general overview of the microbial community structure in
298 MAR systems.

299 *3.3 Characterization of effluent organic matter (EfOM)*

Based on the (-)ESI FT-ICR-MS measurements, masses were mainly detected up to 600 m/z with a resolution of > 450,000 at 320 m/z. The EfOM was characterized by more than 1,000 CHNO containing molecules and a lower number (n = 572) of masses assigned to CHOS molecular composition with approximately 20 high intense signals (Figure 3). The high number of nitrogen containing compounds could be linked to the peak of protein-like substances identified by the 3D-fluorescence measurements (Figure S 2), which are known to be specific for EfOM in comparison to natural organic matter (NOM)[18,46]. CHNO containing molecules assigned to proteins and lignin-like substances from WWTP effluents have been identified previously[47]. However, based on compound classes and their location within van Krevelen plots[48] most intense signals of CHNO containing molecules were in the area of lignin-like substances and only few masses were detected in the area of proteins. Molecules containing a sulfur atom (CHOS) were previously shown to be dominant in EfOM, which could partly be attributed to NOM reaction products of sulforeduction and substances from anthropogenic origin like anionic surfactants such as linear alkyl benzene sulfonates and their transformation products[22,49]. Well known surfactants, which may be present in water and wastewater[22,50], were plotted in Figure S 7 and those which were detected in the EfOM of this study were highlighted. The most detected masses in EfOM could be assigned to transformation products of surfactants pointing to a highly efficient removal of surfactants in the WWTP Garching. Throughout the infiltration, the relative abundance of masses assigned

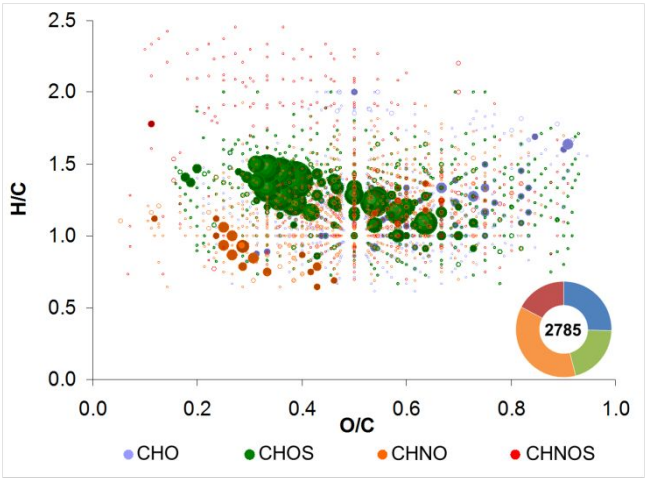



**Figure 3: Van Krevelen plot of the effluent organic matter (EfOM) measured in column feed water showing the H/C and O/C ratio of each mass assigned to a specific elemental composition (CHO, CHNO, CHOS, CHNOS). Bubble sizes depict the absolute intensity of each mass.**

to CHO containing molecules increased and a decrease of molecules comprised of CHNOS was observed (Figures S 8 and S 9). However, even if shifts regarding the number of molecular formulae comprised of different elements were observed, the general pattern of elemental compositions elucidated from van Krevelen plots did not clearly change showing that only a small fraction of the EfOM was affected by the process.

### *3.4 Fate of the EfOM and correlation with microbial community*

***Multiple co-inertia analysis (MCIA):*** To elucidate the co-inertia between two datasets, masses assigned using FT-ICR-MS and OTUs identified via 16S rRNA amplicon sequencing, a MCIA was performed. Thereby, the influence of the organic matter on the microbial community composition at the phyla level could be revealed. A distinct separation between the influent, the first and the second infiltration system was shown by a clear clustering of masses and OTUs (Figure 4). Masses which could be detected throughout the system or even increased in the second infiltration system are further assigned as persistent (Figure S 10, persistent). More than 50% of these persistent masses were composed of CHO containing molecules, followed by CHNO (34 %) and only some CHOS and CHNOS compounds. CHO and CHNO containing molecules seem to be more resistant against microbial degradation than molecules containing sulfur atoms. Based on the van Krevelen diagram of such persistent CHO containing molecules, compounds could be designated as carboxyl-rich alicyclic molecules (CRAM), which are known as highly abundant and refractory dissolved organic matter[51]. Furthermore, the regions in van Krevelen diagrams in which persistent CHO and CHNO containing molecules were observed are characteristic for lignin- and tannin-like substances[48]. Some of the high intense CHOS peaks could be assigned to biodegradation intermediates of dialklyltetralin sulfonates and additionally, persistent compounds comprised of C, H, O and S were also located in the

region of lignins. These refractory substances have previously been found in soils and sediments[52] but also in WWTP effluents[53]. Hence, the persistent backbone after natural treatment seem to share similarities with organic matter found in the environment regarding elemental compositions which confirms results of a study previously performed by Drewes et al. (2006)[18]. Masses which were generally detected in the influent but with less intense signals in different effluents throughout the system (Figure 4: Influent B0; Figure S 10: easily degradable to redox insensitive), were characterized by a large proportion of sulfur containing molecules (CHOS). Three of the most intense signals (H/C ≈ 0.3-0.4) derived from sulfophenyl carboxylate compounds, also known as degradation products of linear alkylbenzenesulfonates[54]. CHNO and CHO appeared similarly abundant, whilst most intense signals of CHNO were obtained in the region of lignin-like substances, but CHO in the area of proteins and cellulose. Masses of the third group representing molecules, which had the highest intensities in the effluents of columns B01 and B02 coupled with efficient removal in the second system, were strongly dominated by sulfur containing compounds (Figure 4: B01, B02; Figure S 10: redox sensitive). The relative contribution of CHNO remained constant in comparison to easily degradable and redox insensitive compounds, CHNOS slightly increased but the number of CHO became negligible. Hence, we assume compounds comprised of C, H and O do not respond to changing redox conditions, whereby CHOS containing molecules seemed to be sensitive with respect to oxygen availability in MAR systems.

Microbial taxa such as *Acidobacteria, Proteobacteria (α-, β-, γ-Proteobacteria), Planctomycetes* and *Verrucomicrobia* were dominant (relative abundance > 1 %) in all three groups obtained by elaborating MCIA (Figure 4). This suggests that these phyla do not essentially need specific growth substrates in MAR as they grow with any carbon source as primary substrate available within the system. *Bacteroidetes*, *Chloroflexi* but also *Candidatus*

371 *Saccharibacteria* and *candidate division WPS-1* were primarily correlated with masses
372 showing an immediate removal in the first infiltration system which may indicate that those
373 bacterial phyla prefer easily degradable organic matter as growth substrate. *Firmicutes* had a
374 relative abundance of > 1 % within the cluster of persistent masses or those which have an
375 increased intensity in the second system, but they were less abundant (< 1 %) in the other two
376 groups (easily degradable/redox insensitive and redox sensitive). Thus, the majority of species
377 belonging to *Firmicutes* seem to preferentially settle in MAR systems if the organic matter is
378 characterized by a high refractory content.

379

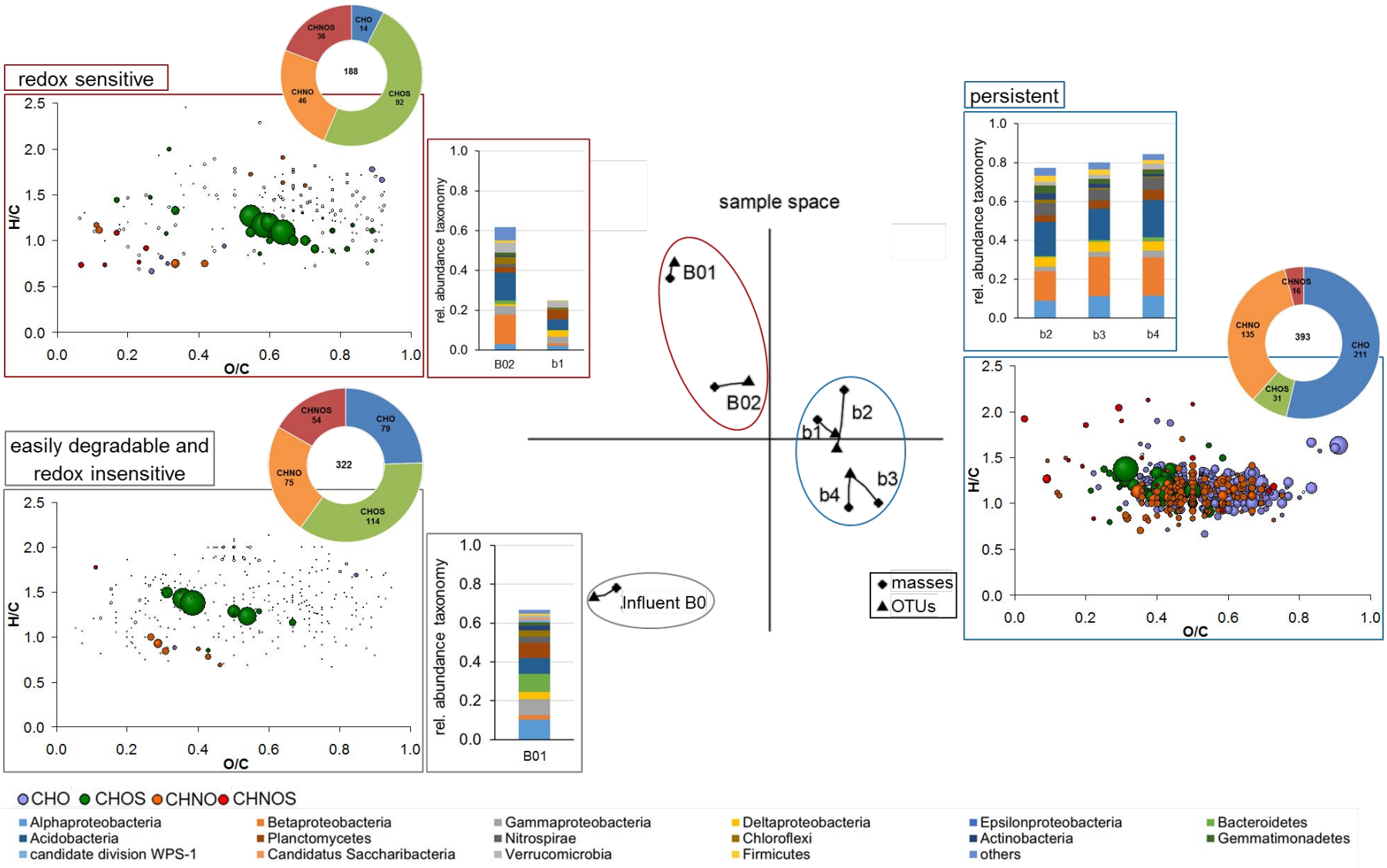

redox sensitive
CHNOS 36
CHO 14
188
CHNO 46
CHOS 92
H/C
O/C
rel. abundance taxonomy
B02
b1
easily degradable and redox insensitive
CHNOS 54
CHO 79
322
CHNO 75
CHOS 114
sample space
B01
B02
b1
b2
b3
b4
Influent B0
masses
OTUs
persistent
CHNOS 16
CHNO 135
393
CHO 211
CHOS 31
CHO
CHOS
CHNO
CHNOS
Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Deltaproteobacteria
Epsilonproteobacteria
Bacteroidetes
Acidobacteria
Planctomycetes
Nitrospirae
Chloroflexi
Actinobacteria
Gemmatimonadetes
candidate division WPS-1
Candidatus Saccharibacteria
Verrucomicrobia
Firmicutes
others

**Figure 4: Based on multiple co-inertia analysis three distinct clusters of masses (diamonds) and OTUs (triangles) were identified according to the influent (Influent B0), the first (B01, B02) and the second (b1-b4) infiltration system. Corresponding OTUs (B01 – b4) and masses are connected by a line, which length is proportional to the divergence between the data from the same sample. Van Krevelen diagrams, absolute numbers of masses and their elemental compositions as well as taxonomy assignments representative for each cluster are shown. The total number of masses are given within each circle; the abundance of microbial phyla or classes, respectively, are shown as relative values.**

***Orthogonal partial least squares analysis (OPLS):*** As a co-metabolic transformation of TOrCs in MAR systems is widely assumed[7], a correlation between masses assigned by use of FT-ICR-MS and detected TOrCs may be evident. The establishment of the microbial community on phyla level at different depths based on receiving feed water should be elucidated. In addition, the microbiome which could be involved in the transformation processes of the primary substrate and therefore, the TOrCs had to be considered. Hence, an OPLS was performed to emphasize masses as well as OTUs, which correlate with targeted TOrCs. Bulk organic parameters (DOC, $UVA_{254}$) as well as redox parameters (DO, $NO_3^-$ -N) were also included in the model. However, $NO_3^-$-N will not be considered in further discussions as its actual concentration will be biased especially after reaeration. As shown in Figure 5, masses and OTUs were successfully clustered with respect to the four distinct groups: easily degradable, redox insensitive, redox sensitive and persistent compounds (Table 1), which indicates there is a relation between the behavior of TOrCs in MAR and primary substrate. The persistent compounds primidone and carbamazepine strongly correlated with masses dominated by CHNO and CHO containing molecules, whilst CHOS and CHNOS ones can be neglected (Figure S 11). In accordance to results obtained by applying MCIA, CHO and CHNO containing molecules could be assigned to refractory dissolved organic matter such as CRAM[51–53]. It could be assumed, that TOrCs correlating with such refractory organic matter may also be incorporated in the environmental organic backbone. With respect to the easily degradable TOrC citalopram, relative abundances of CHO and CHOS were approximately 30 %, whilst CHNO containing molecules were most frequently detected (38 %; Figure 5). For CHO, most intense signals were observed from oxidized compounds (O/C > 0.5) within a H/C range of 1.0 - 1.5. In case of CHNO, signals with O/C < 0.4 and H/C 0.75 – 1 but also with O/C > 0.5 and H/C 1.0 - 1.5 were detected. Single highly intense CHOS signals could be

assigned to sulfophenyl carboxylates and intermediates of dialklyltetralin sulfonates. The largest group of TOrCs was those showing a redox independent behavior, namely, transformation throughout the system under anoxic as well as oxic to suboxic redox conditions (Table 1, Figure S 5). From easily degradable to redox insensitive compounds a shift from 38 % to 50 % regarding the relative abundance of CHOS was observed. In contrast, the number of CHO and CHNO containing molecules decreased from approximately 30 % (easily degradable) to approximately 20 % (redox insensitive). While CHO containing molecules were mainly characterized by most intense signals in the area of O/C > 0.5 and H/C > 1.0, CHNO exhibited intensive signals in the range of O/C < 0.5 and H/C < 1.0. The fourth group of major interest within this study contained the TOrCs benzotriazole, tramadol and venlafaxine due to their redox sensitive behavior (Table 1). There are 338 masses in total which could be unambiguously assigned to molecular formulas containing C, H, O and/or N and/or S following this trend. In this group, molecules comprised of CHO were less abundant (17 %), CHOS and CHNOS occurred to approximately 25 % each. CHNO containing molecules were the most abundant group with more than 30%. In all four molecular groups, no distinct clustering was obtained in the van Krevelen plots, they were all detected in a range of O/C 0.2 – 1.0 and H/C of 0.5 - 2.0.

Microbial phyla imply highly diverse characteristics regarding metabolic functions as they are comprised of a variety of species having specialized strategies to utilize growth substrate. In accordance to the results observed by use of MCIA, the microbiome which seems to not prefer a specific growth substrate was mainly comprised of *α-Proteobacteria*, *Acidobacteria* and *Planctomycetes* (relative abundance > 1 % at all four groups; Figure 5*)*. *Firmicutes* and *Gemmatimonadetes* could be correlated with persistent masses having a relative abundance of > 1 %, but < 1 % in correlation with easily degradable, redox insensitive or redox sensitive

compounds. A higher abundance of *Firmicutes* in simulated MAR receiving primary substrate mainly shaped by refractory humic acid in comparison to easily degradable peptone/yeast has previously been reported[55]. A relative abundance of > 1 % in correlation with easily degradable and redox insensitive masses, TOrCs as well as the DOC and $UVA_{254}$ but also with redox sensitive masses, TOrCs and DO was observed for *Bacteroidetes* (Figure 5). As this phylum was also correlated to easily degradable masses using MCIA it may be suggested that *Bacteroidetes* remarkably contribute to degradation of organic matter and therefore, co-metabolic transformation of TOrCs. Similar results were observed by Li et al. (2013) who proposed a link between *Bacteroidetes* abundance and BDOC reduction[12]. Microbial phyla with an abundance of > 1 % solely correlated to either easily degradable, redox insensitive or redox-sensitive compounds could not be identified. However, based on phylum level, it will not be possible to derive a deeper understanding of metabolic pathways and their associated metabolites. Nevertheless, a correlation between compounds comprised of CHOS with easily degradable and redox insensitive masses was figured out, while persistent organic matter mainly correlated with highly refractory CRAM. To further elucidate the correlation between TOrCs transformation and the composition of organic matter, NMR analysis would be expedient to emphasize not only compound classes but also functional groups which correlate between organic matter and TOrCs.

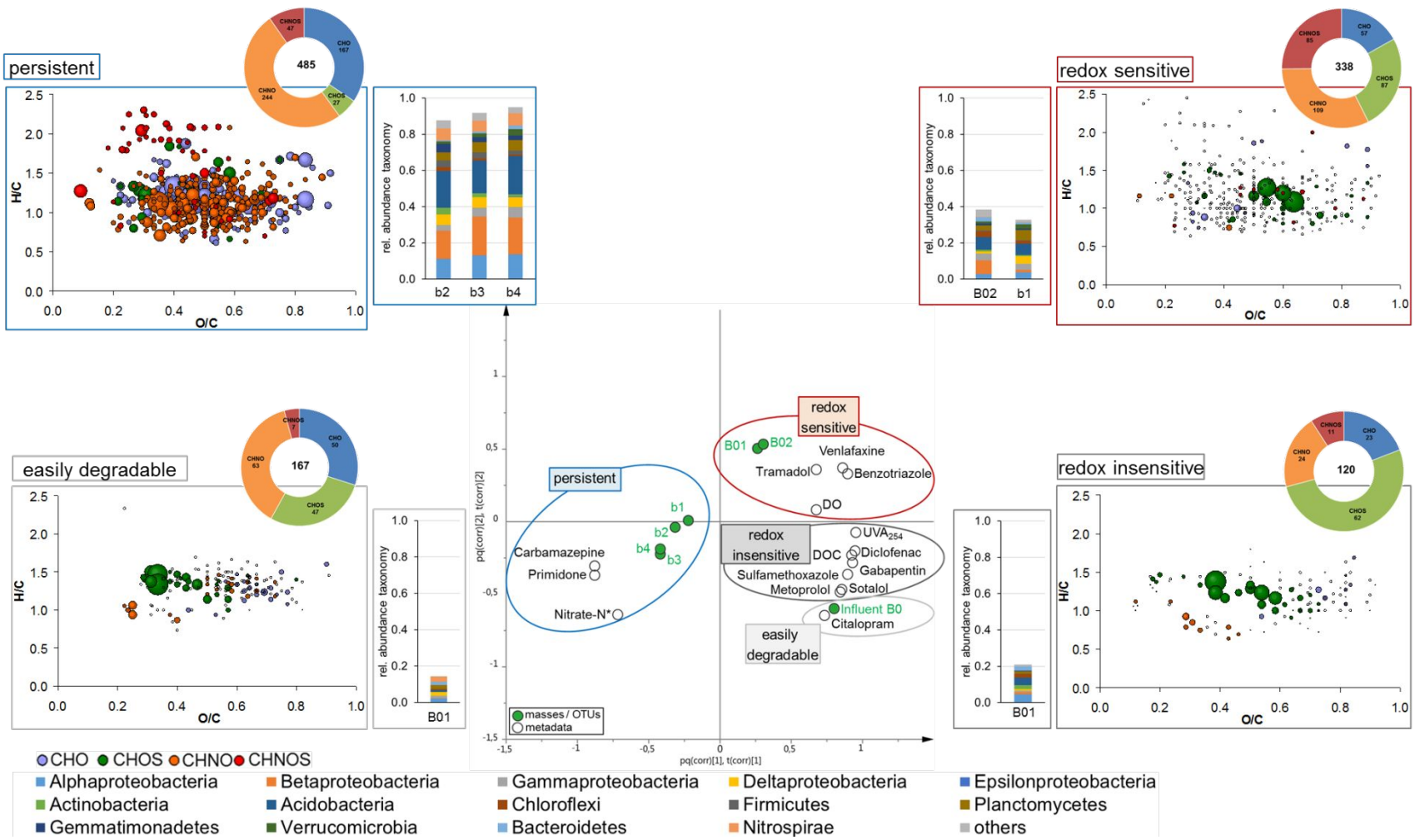



**Figure 5: Based on orthogonal partial least squares (OPLS) regression clusters of masses and OTUs were identified according to the four categories of TOrCs and their behavior in MAR systems: i) persistent, ii) easily degradable, iii) redox insensitive, iv) redox sensitive. Van Krevelen diagrams, absolute numbers of masses and their elemental compositions as well as taxonomy assignments representative for each cluster are shown. The total number of masses are given within each circle; the abundance of microbial phyla or classes,**

**respectively, are shown as relative values; consider that the microbiome of column B01 corresponds to the influent B0 and each column (B02-b4) to the effluent of the previous column (B01-b3). The OPLS model gave the following values for the goodness of its fit and the goodness of the prediction: $R^2Y(cum) = 0.9$ and $Q^2(cum) = 0.9$. *Not considered for further discussions.**

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## Conflict of Interests

The authors declare no competing financial interest.

## Supporting Information

The SI provides further information regarding material and methods (Tables S 1 and S 2) as well as results and discussion (Figures S 1 – S 11).

## 1. References

(1) Rauch-Williams, T.; Hoppe-Jones, C.; Drewes, J. E. The role of organic matter in the removal of emerging trace organic chemicals during managed aquifer recharge. *Water Res.* **2010**, *44*, 449–460.

(2) Müller, J.; Drewes, J. E.; Hübner, U. Sequential biofiltration - A novel approach for enhanced biological removal of trace organic chemicals from wastewater treatment plant effluent. *Water Res.* **2017**, *127*, 127–138.

(3) Hellauer, K.; Karakurt, S.; Sperlich, A.; Burke, V.; Massmann, G.; Hübner, U.; Drewes, J. E. Establishing sequential managed aquifer recharge technology (SMART) for enhanced removal of trace organic chemicals: Experiences from field studies in Berlin, Germany. *J. Hydrol.* **2018**, *563*, 1161–1168.

(4) Grünheid, S.; Amy, G.; Jekel, M. Removal of bulk dissolved organic carbon (DOC) and trace organic compounds by bank filtration and artificial recharge. *Water Res.* **2005**, *39*, 3219–3228.

(5) Tran, N. H.; Urase, T.; Ngo, H. H.; Hu, J.; Ong, S. L. Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants. *Bioresour. Technol.* **2013**, *146*, 721–731.

(6) Dalton, H.; Stirling, D. I.; Quayle, J. R. Co-Metabolism [and Discussion]. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* **1982**, *297*, 481–496.

(7) Alidina, M.; Li, D.; Drewes, J. E. Investigating the role for adaptation of the microbial community to transform trace organic chemicals during managed aquifer recharge. *Water Res.* **2014**, *56*, 172–180.

(8) Tang, K.; Escola Casas, M.; Ooi, G. T. H.; Kaarsholm, K. M. S.; Bester, K.; Andersen, H. R. Influence of humic acid addition on the degradation of pharmaceuticals by biofilms in effluent wastewater. *Int. J. Hyg. Environ. Health* **2017**, *220*, 604–610.

(9) Alidina, M.; Li, D.; Ouf, M.; Drewes, J. E. Role of primary substrate composition and concentration on attenuation of trace organic chemicals in managed aquifer recharge systems. *J. Environ. Manage.* **2014**, *144*, 58–66.

(10) Rauch-Williams, T.; Drewes, J. E. Using soil biomass as an indicator for the biological removal of effluent-derived organic carbon during soil infiltration. *Water Res.* **2006**, *40*, 961–968.

494 (11) Li, D.; Sharp, J. O.; Saikaly, P. E.; Ali, S.; Alidina, M.; Alarawi, M. S.; Keller, S.;
495 Hoppe-Jones, C.; Drewes, J. E. Dissolved Organic Carbon Influences Microbial Community
496 Composition and Diversity in Managed Aquifer Recharge Systems. *Appl. Environ.*
497 *Microbiol.* **2012**, *78*, 6819–6828.
498 (12) Li, D.; Alidina, M.; Ouf, M.; Sharp, J. O.; Saikaly, P.; Drewes, J. E. Microbial
499 community evolution during simulated managed aquifer recharge in response to different
500 biodegradable dissolved organic carbon (BDOC) concentrations. *Water Res.* **2013**, *47*, 2421–
501 2430.
502 (13) Drewes, J. E.; Li, D.; Regnery, J.; Alidina, M.; Wing, A.; Hoppe-Jones, C. Tuning the
503 performance of a natural treatment process using metagenomics for improved trace organic
504 chemical attenuation. *Water Sci. Technol.* **2014**, *69*, 628.
505 (14) Regnery, J.; Wing, A.D.; Alidina, M.; Drewes, J. E. Biotransformation of trace organic
506 chemicals during groundwater recharge: How useful are first-order rate constants? *J. Contam.*
507 *Hydrol.* **2015**, *179*, 65–75.
508 (15) Leenheer, J. A.; Croué, J.-P.; Benjamin, M.; Korshin, G. V.; Hwang, C. J.; Bruchet, A.;
509 Aiken, G. R. Comprehensive Isolation of Natural Organic Matter from Water for Spectral
510 Characterizations and Reactivity Testing. In *Natural Organic Matter and Disinfection By-*
511 *Products;* Barrett, S. E., Krasner, S. W., Amy, G. L., Eds.; ACS Symposium Series 761,
512 American Chemical Society: Washington, DC 2000; pp 68–83.
513 (16) Minor, E. C.; Swenson, M. M.; Mattson, B. M.; Oyler, A. R. Structural characterization
514 of dissolved organic matter: a review of current techniques for isolation and analysis.
515 *Environ. Sci. Process. Impacts* **2014**, *16*, 2064–2079.

(17) Drewes, J. E.; Croue, J.-P. New approaches for structural characterization of organic matter in drinking water and wastewater effluents. *Water Sci. Technol.: Water Supply* **2002**, *2*, 1–10.

(18) Drewes, J. E.; Quanrud, D. M.; Amy, G. L.; Westerhoff, P. K. Character of Organic Matter in Soil-Aquifer Treatment Systems. *J. Environ. Eng.* **2006**, *132*, 1447–1458.

(19) Murphy, K. R.; Butler, K. D.; Spencer, R. G. M.; Stedmon, C. A.; Boehme, J. R.; Aiken, G. R. Measurement of dissolved organic matter fluorescence in aquatic environments: an interlaboratory comparison. *Environ. Sci. Technol.* **2010**, *44*, 9405–9412.

(20) Baghoth, S. A.; Sharma, S. K.; Amy, G. L. Tracking natural organic matter (NOM) in a drinking water treatment plant using fluorescence excitation-emission matrices and PARAFAC. *Water Res.* **2011**, *45*, 797–809.

(21) Li, W.-T.; Chen, S.-Y.; Xu, Z.-X.; Li, Y.; Shuang, C.-D.; Li, A.-M. Characterization of dissolved organic matter in municipal wastewater using fluorescence PARAFAC analysis and chromatography multi-excitation/emission scanA comparative study. *Environmental science & technology* **2014**, *48*, 2603–2609.

(22) Gonsior, M.; Zwartjes, M.; Cooper, W. J.; Song, W.; Ishida, K. P.; Tseng, L. Y.; Jeung, M. K.; Rosso, D.; Hertkorn, N.; Schmitt-Kopplin, P. Molecular characterization of effluent organic matter identified by ultrahigh resolution mass spectrometry. *Water Res.* **2011**, *45*, 2943–2953.

(23) Lavonen, E. E.; Kothawala, D. N.; Tranvik, L. J.; Gonsior, M.; Schmitt-Kopplin, P.; Köhler, S. J. Tracking changes in the optical properties and molecular composition of dissolved organic matter during drinking water production. *Water Res.* **2015**, *85*, 286–294.

(24) Stenson, A. C.; Marshall, A. G.; Cooper, W. T. Exact Masses and Chemical Formulas of Individual Suwannee River Fulvic Acids from Ultrahigh Resolution Electrospray

Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectra. *Anal. Chem.* **2003**, *75*, 1275–1284.

(25) Hertkorn, N.; Ruecker, C.; Meringer, M.; Gugisch, R.; Frommberger, M.; Perdue, E. M.; Witt, M.; Schmitt-Kopplin, P. High-precision frequency measurements: indispensable tools at the core of the molecular-level analysis of complex systems. *Anal. Bioanal. Chem.* **2007**, *389*, 1311–1327.

(26) Regnery, J.; Wing, A. D.; Kautz, J.; Drewes, J. E. Introducing sequential managed aquifer recharge technology (SMART) – From laboratory to full-scale application. *Chemosphere* **2016**, *154*, 8–16.

(27) Schudel B.; Biaggi D.; Dervey T.; Kozel R.; Müller I.; Ross J. H.; Schindler U.. *Einsatz künstlicher Tracer in der Hydrogeologie - Praxishilfe*; Berichte des BWG, Serie Geologie No. 3; *60-67*; Bern, 2002.

(28) Engel, K.; Sauer, J.; Jünemann, S.; Winkler, A.; Wibberg, D.; Kalinowski, J.; Tauch, A.; Caspers, B. A. Individual- and Species-Specific Skin Microbiomes in Three Different Estrildid Finch Species Revealed by 16S Amplicon Sequencing. *Microb. Ecol.* **2017,** DOI: 10.1007/s00248-017-1130-8.

(29) Magoč, T.; Salzberg, S. L. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **2011**, *27*, 2957–2963.

(30) Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **2010**, *26*, 2460–2461.

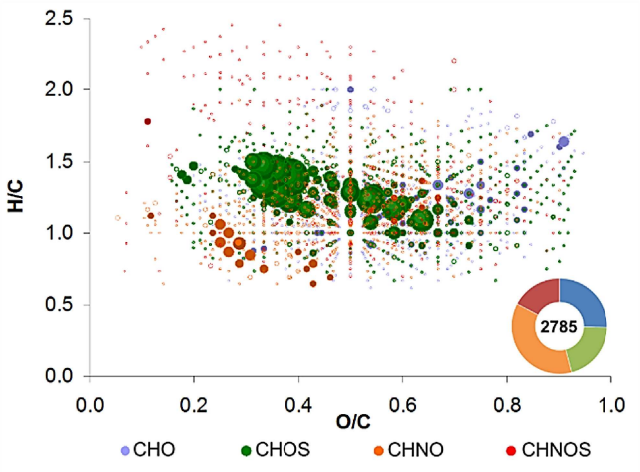
(31) Wang, Q.; Garrity, G. M.; Tiedje, J. M.; Cole, J. R. Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Appl. Environ. Microbiol.* **2007**, *73*, 5261–5267.

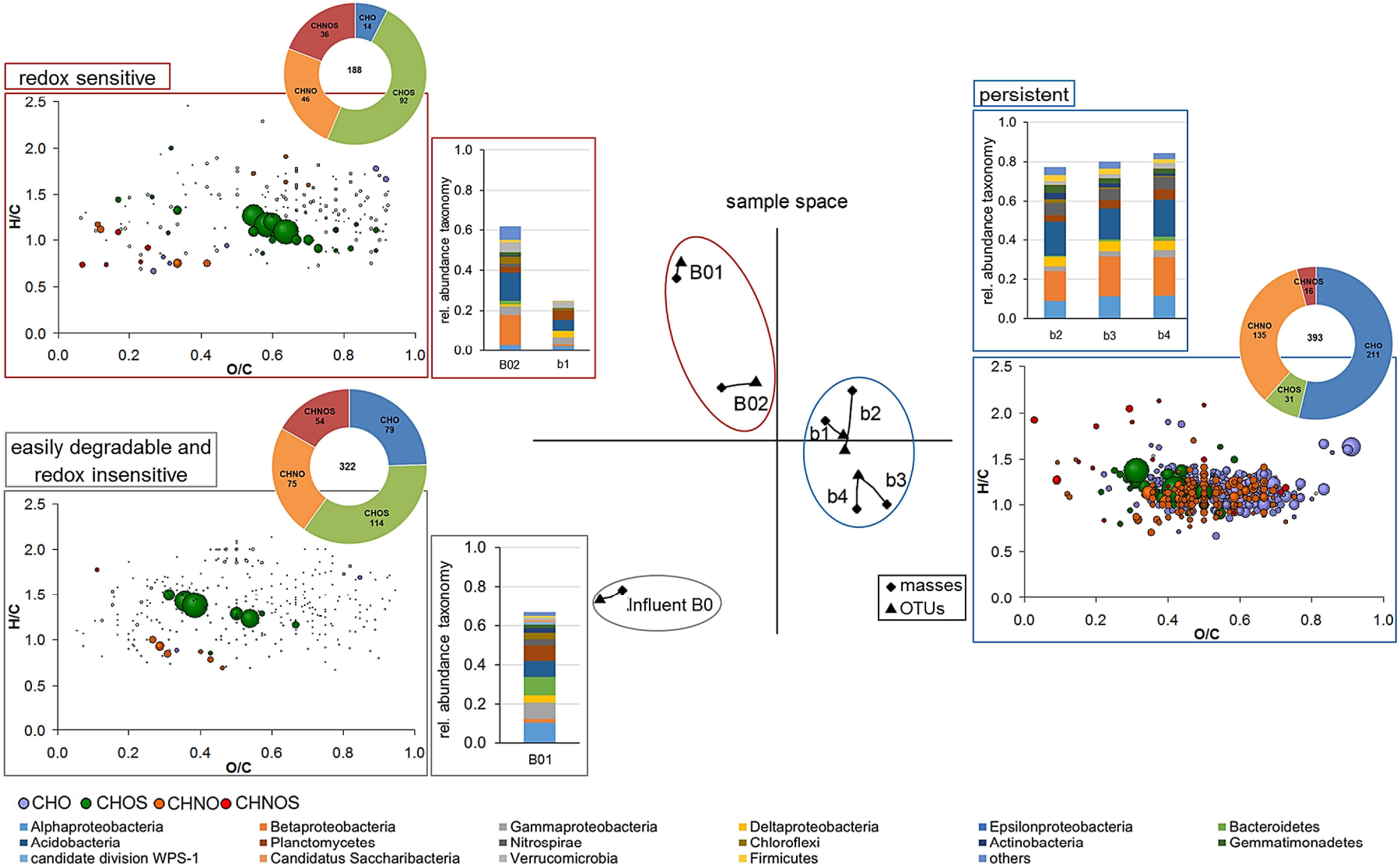
563 (32) Weishaar, J. L.; Aiken, G. R.; Bergamaschi, B. A.; Fram, M. S.; Fujii, R.; Mopper, K.
564 Evaluation of Specific Ultraviolet Absorbance as an Indicator of the Chemical Composition
565 and Reactivity of Dissolved Organic Carbon. *Environ. Sci. Technol.* **2003**, *37*, 4702–4708.
566 (33) Stahlschmidt, M.; Regnery, J.; Campbell, A.; Drewes, J. E. Application of 3D-
567 fluorescence/PARAFAC to monitor the performance of managed aquifer recharge facilities.
568 *J. Water Reuse Desalination* **2016**, *6*, 249-263.
569 (34) Dittmar, T.; Koch, B.; Hertkorn, N.; Kattner, G. A simple and efficient method for the
570 solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnol.*
571 *Oceanogr. Methods* **2008**, *6*, 230–235.
572 (35) Kim, S.; Kramer, R. W.; Hatcher, P. G. Graphical Method for Analysis of Ultrahigh-
573 Resolution Broadband Mass Spectra of Natural Organic Matter, the Van Krevelen Diagram.
574 *Anal. Chem.* **2003**, *75*, 5336–5344.
575 (36) Murphy, K. R.; Stedmon, C. A.; Graeber, D.; Bro, R. Fluorescence spectroscopy and
576 multi-way techniques. PARAFAC. *Anal. Methods* **2013**, *5*, 6557.
577 (37) Adrian, M.; Lucio, M.; Roullier-Gall, C.; Héloir, M.-C.; Trouvelot, S.; Daire, X.;
578 Kanawati, B.; Lemaître-Guillier, C.; Poinssot, B.; Gougeon, R.*; Schmitt-Kopplin, P.*.
579 Metabolic Fingerprint of PS3-Induced Resistance of Grapevine Leaves against Plasmopara
580 viticola Revealed Differences in Elicitor-Triggered Defenses. *Frontiers in plant science*
581 **2017**, *8*, 101.
582 (38) Chen, W.; Westerhoff, P.; Leenheer, J. A.; Booksh, K. Fluorescence
583 Excitation−Emission Matrix Regional Integration to Quantify Spectra for Dissolved Organic
584 Matter. *Environ. Sci. Technol.* **2003**, *37*, 5701–5710.

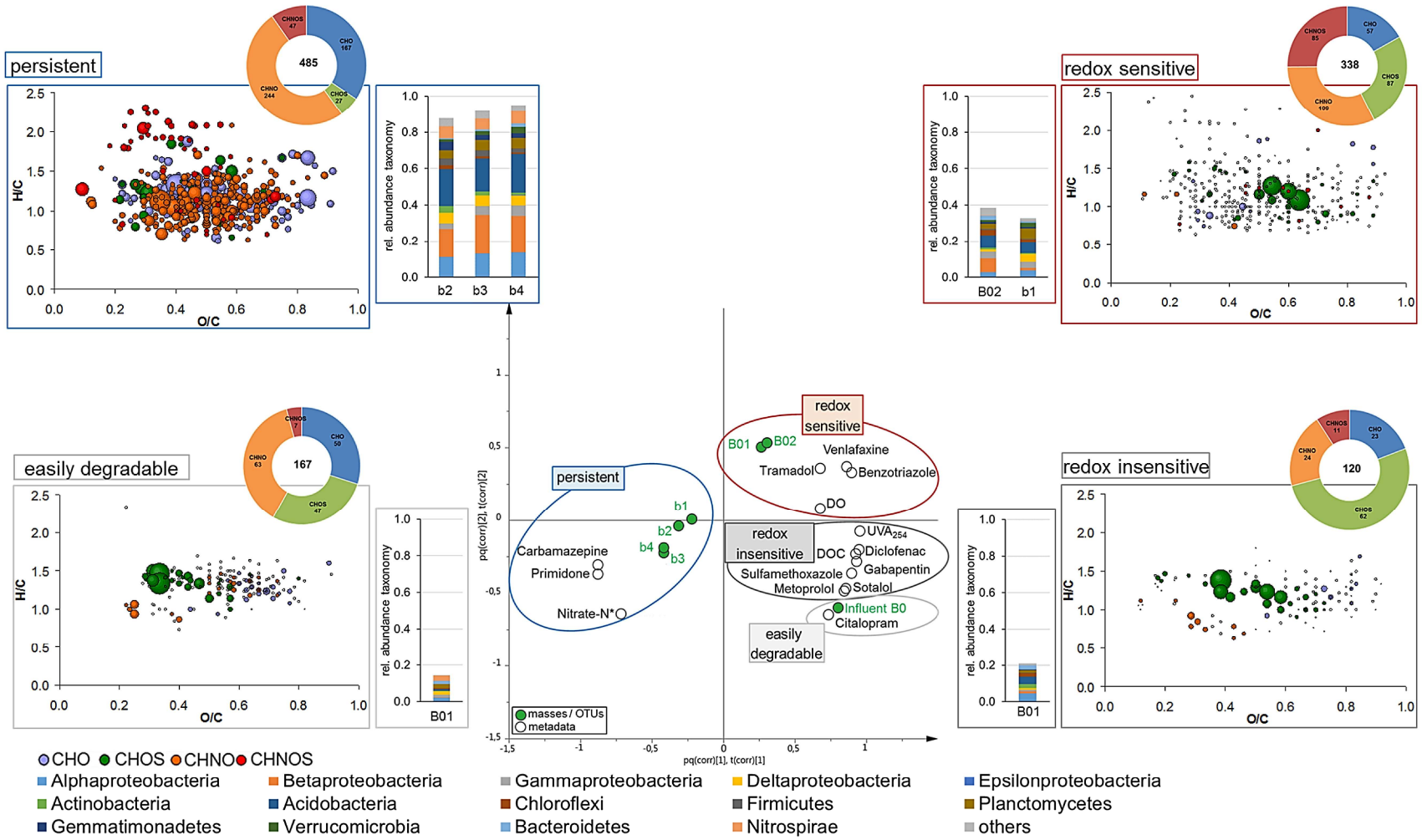
585 (39) Clara, M.; Strenn, B.; Kreuzinger, N. Carbamazepine as a possible anthropogenic
586 marker in the aquatic environment: investigations on the behaviour of Carbamazepine in
587 wastewater treatment and during groundwater infiltration. *Water Res.* **2004**, *38*, 947–954.
588 (40) Hass, U.; Dünnbier, U.; Massmann, G. Occurrence and distribution of psychoactive
589 compounds and their metabolites in the urban water cycle of Berlin (Germany). *Water Res.*
590 **2012**, *46*, 6013–6022.
591 (41) Hellauer, K.; Mergel, D.; Ruhl, A.; Filter, J.; Hübner, U.; Jekel, M.; Drewes, J. E.
592 Advancing Sequential Managed Aquifer Recharge Technology (SMART) Using Different
593 Intermediate Oxidation Processes. *Water* **2017**, *9*, 221.
594 (42) Beretsou, V. G.; Psoma, A. K.; Gago-Ferrero, P.; Aalizadeh, R.; Fenner, K.; Thomaidis,
595 N. S. Identification of biotransformation products of citalopram formed in activated sludge.
596 *Water Res.* **2016**, *103*, 205–214.
597 (43) Muntau, M.; Schulz, M.; Jewell, K. S.; Hermes, N.; Hübner, U.; Ternes, T.; Drewes, J.
598 E. Evaluation of the short-term fate and transport of chemicals of emerging concern during
599 soil-aquifer treatment using select transformation products as intrinsic redox-sensitive tracers.
600 *Sci. Total Environ.* **2017**, *583*, 10–18.
601 (44) Benotti, M. J.; Song, R.; Wilson, D.; Snyder, S. A. Removal of pharmaceuticals and
602 endocrine disrupting compounds through pilot- and full-scale riverbank filtration. *Water Sci.*
603 *Technol.: Water Supply* **2012**, *12*, 11.
604 (45) Janssen, P. H. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and
605 16S rRNA genes. *Appl. Environ. Microbiol.* **2006**, *72*, 1719–1728.
606 (46) Nam, S.-N.; Amy, G. Differentiation of wastewater effluent organic matter (EfOM)
607 from natural organic matter (NOM) using multiple analytical techniques. *Water Sci. Technol.*
608 **2008**, *57*, 1009–1015.

609 (47) Maizel, A. C.; Remucal, C. K. The effect of advanced secondary municipal wastewater
610 treatment on the molecular composition of dissolved organic matter. *Water Res.* **2017**, *122*,
611 42–52.
612 (48) Sleighter, R. L.; Hatcher, P. G. The application of electrospray ionization coupled to
613 ultrahigh resolution mass spectrometry for the molecular characterization of natural organic
614 matter. *J. Mass Spectrom.* **2007**, *42*, 559–574.
615 (49) Tseng, L. Y.; Gonsior, M.; Schmitt-Kopplin, P.; Cooper, W. J.; Pitt, P.; Rosso, D.
616 Molecular characteristics and differences of effluent organic matter from parallel activated
617 sludge and integrated fixed-film activated sludge (IFAS) processes. *Environ. Sci. Technol.*
618 **2013**, *47*, 10277–10284.
619 (50) Terrabase Inc. Surfactants. http://www.terrabase-inc.com/ (accessed June 29, 2018).
620 (51) Hertkorn, N.; Benner, R.; Frommberger, M.; Schmitt-Kopplin, P.; Witt, M.; Kaiser, K.;
621 Kettrup, A.; Hedges, J. I. Characterization of a major refractory component of marine
622 dissolved organic matter. *Geochim. Cosmochim. Acta* **2006**, *70*, 2990–3010.
623 (52) Derenne, S.; Largeau, C. A Review of Some Important Families of Refractory
624 Macromolecules: Composition, Origin, and Fate in Soils and Sediments. *Soil Sci.* **2001**, *166*,
625 833–847.
626 (53) Mesfioui, R.; Love, N. G.; Bronk, D. A.; Mulholland, M. R.; Hatcher, P. G. Reactivity
627 and chemical characterization of effluent organic nitrogen from wastewater treatment plants
628 determined by Fourier transform ion cyclotron resonance mass spectrometry. *Water Res.*
629 **2012**, *46*, 622–634.
630 (54) Riu, J.; Gonzalez-Mazo, E.; Gomez-Parra, A.; Barceló, D. Determination of parts per
631 trillion level of carboxylic degradation products of linear alkylbenzenesulfonates in coastal

632 water by solid-phase extraction followed by liquid chromatography/ionspray/mass
633 spectrometry using negative ion detection. *Chromatographia* **1999**, *50*, 275–281.
634 (55) Li, D.; Alidina, M.; Drewes, J. E. Role of primary substrate composition on microbial
635 community structure and function and trace organic chemical attenuation in managed aquifer
636 recharge systems. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 5747–5756.
637


2.5
2.0
1.5
1.0
0.5
0.0
H/C
0.0
0.2
0.4
0.6
0.8
1.0
O/C
2785
CHO
CHOS
CHNO
CHNOS


redox sensitive
CHO 14
CHOS 92
CHNO 46
CHNOS 36
188
H/C
O/C
rel. abundance taxonomy
B02
b1
easily degradable and redox insensitive
CHO 79
CHOS 114
CHNO 75
CHNOS 54
322
B01
Influent B0
sample space
B01
B02
b1
b2
b3
b4
persistent
CHO 211
CHOS 31
CHNO 135
CHNOS 16
393
masses
OTUs
CHO
CHOS
CHNO
CHNOS
Alphaproteobacteria
Acidobacteria
candidate division WPS-1
Betaproteobacteria
Planctomycetes
Candidatus Saccharibacteria
Gammaproteobacteria
Nitrospirae
Verrucomicrobia
Deltaproteobacteria
Chloroflexi
Firmicutes
Epsilonproteobacteria
Actinobacteria
others
Bacteroidetes
Gemmatimonadetes


persistent
485
CHO 167
CHOS 27
CHNO 244
CHNOS 47
H/C
O/C
rel. abundance taxonomy
b2
b3
b4
redox sensitive
338
CHO 57
CHOS 87
CHNO 100
CHNOS 85
B02
b1
easily degradable
167
CHO 50
CHOS 47
CHNO 63
CHNOS 7
B01
redox insensitive
120
CHO 23
CHOS 62
CHNO 24
CHNOS 11
pq(corr)[2], t(corr)[2]
pq(corr)[1], t(corr)[1]
redox sensitive
B01
B02
Venlafaxine
Tramadol
Benzotriazole
DO
persistent
b1
b2
b4
b3
Carbamazepine
Primidone
Nitrate-N*
redox insensitive
UVA254
DOC
Diclofenac
Gabapentin
Sulfamethoxazole
Metoprolol
Sotalol
Influent B0
Citalopram
easily degradable
masses / OTUs
metadata
CHO
CHOS
CHNO
CHNOS
Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Deltaproteobacteria
Epsilonproteobacteria
Actinobacteria
Acidobacteria
Chloroflexi
Firmicutes
Planctomycetes
Gemmatimonadetes
Verrucomicrobia
Bacteroidetes
Nitrospirae
others