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Temperature, phytoplankton density and bacteria diversity drive the biotransformation of micropollutants in a lake ecosystem

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ABSTRACT

For most micropollutants (MPs) present in surface waters, such as pesticides and pharmaceuticals, the contribution of biotransformation to their overall removal from lake ecosystems is largely unknown. This study aims at empirically determining the biotransformation rate constants for 35 MPs at different periods of the year and depths of a meso-eutrophic lake. We then tested statistically the association of environmental parameters and microbial community composition with the biotransformation rate constants obtained. Biotransformation was observed for 14 out of 35 studied MPs for at least one sampling time. Large variations in biotransformation rate constants were observed over the seasons and between compounds. Overall, the transformation of MPs was mostly influenced by the lake's temperature, phytoplankton density and bacterial diversity. However, some individual MPs were not following the general trend or association with microorganism biomass. The antidepressant mianserin, for instance, was transformed in all experiments and depths, but did not show any relationship with measured environmental parameters, suggesting the importance of specific microorganisms in its transformation. The results presented here contribute to our understanding of the fate of MPs in surface waters and thus support improved risk assessment of contaminants in the environment.

1. Introduction

European lakes are subject to the input of a large range of micropollutants (MPs), ranging from pesticides to pharmaceuticals, artificial sweeteners and personal care products. These chemicals can reach surface waters through diffuse sources (e.g., runoff or leaching of pesticides from soils (Sandin et al., 2018)) or point sources (e.g., discharge from wastewater treatment plants after incomplete removal (Eggen et al., 2014)). As a consequence, MPs may be found in lake environments at concentrations in the range of several hundred ng/L. For instance, the three common pharmaceuticals, carbamazepine, diclofenac, and sulfamethoxazole, have been measured in Lake Tegel (Germany) in concentrations of 750, 950 and 1200 ng/L, respectively (Schimmelpfennig et al., 2016). In a lake used as major drinking water supply, i.e., Lake Mälaren in Sweden, a total of 46 organic MPs have been detected, with the highest concentration found being 140 ng/L for the anti-epileptic lamotrigine (Rehrl et al., 2020). MPs can harm aquatic organisms, especially in complex mixtures where they might potentially show synergistic effects (Shao et al., 2019). In molluscs, for instance,

antidepressants have been shown to affect spawning and larval release (Fong and Ford, 2013). Additionally, the presence of antibiotic residues, even at low concentrations, could lead to an increase in antibiotic-resistant bacteria (Novo et al., 2013). Finally, these MPs can eventually reach drinking water plants (Luo et al., 2014), where they may still be present in considerable concentrations (e.g., up to 600 ng/L carbamazepine in finished drinking water (Kleywegt et al., 2011)).

Once they reach lake ecosystems, these MPs can be dissipated through several known pathways, such as flushing, sorption and phototransformation (Tixier et al., 2003). The role and importance of biotransformation as a relevant removal process, however, has received little attention. Li and McLachlan (2019) showed that some pharmaceuticals were removed at different rates in two different lakes, being up to 4.5 times slower in Lake Mälaren compared to Lake Norra Bergundasjön. The authors speculated that this discrepancy was due to a much lower microbial activity in Lake Mälaren. The same study also showed that the pharmaceuticals already present in lake water were transformed faster and without any lag phase compared to those spiked into lake water at elevated concentrations of 50 μg/L (Li and

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McLachlan, 2019). Conversely, MPs dissipated faster in lake water close to the effluent discharge point of a wastewater treatment plant than further away from the facility where their concentrations were diluted (Blunt et al., 2018). In this case, the higher biotransformation rates correlated with more active microbial communities. Together, these studies suggest that there might be adaptation of local microbial communities to degrade MPs that have a constant input into a lake environment. Microbial diversity has been identified as another factor potentially influencing MP biotransformation. In different contexts, e.g., activated sludge, biofilms or phytoplankton cultures, the biotransformation rate constants have been found to be higher when the microorganism community was more diverse (Johnson et al., 2015; Stravs et al., 2019; Torresi et al., 2016). Yet, it may be possible that a few specific groups of microorganisms are particularly active in the transformation of specific compounds. For instance, cyanobacteria were suspected to play an important role in the dissipation of the pesticide glyphosate in Lake Greifen (Huntscha et al., 2018).

Despite these new findings, there are still large knowledge gaps regarding the potential for biotransformation in lakes for most MPs. Particularly, with a few exceptions (Blunt et al., 2018; Li and McLachlan, 2019), there is hardly any systematic investigation of the spatial and temporal variation of MP biotransformation and how it relates to variation in environmental parameters and microbial community density and composition. While concentrations of MPs in lake waters have previously been observed to decrease during summer months when the lake is warmer and the microorganisms more abundant (Huntscha et al., 2018; Rehrl et al., 2020), the question whether this phenomenon is due to increased microbial biotransformation or rather sunlight-induced phototransformation could not be resolved in most cases (e.g., Huntscha et al. (2018); Tixier et al. (2003)). In addition, the effects of many physico-chemical parameters (e.g., temperature, pH, dissolved oxygen, nutrients) on biotransformation rates have been studied in the past (see review by Fenner et al. (2016)), but rarely in combination. Hence, increased knowledge about the extent, variation and factors influencing biotransformation rate constants of MPs in lakes would enable a better prediction of MP dissipation or persistence in natural surface waters, and thus, improved risk assessment. In addition, as there is increasing interest in microalgae-based wastewater treatment (e.g., da Silva Rodrigues et al. (2020)), our study would also inform on the optimal conditions for MP biotransformation, and hence foster a better management of contaminated effluents prior to their discharge into the environment.

This study therefore had two main objectives: (i) To evaluate the importance and variation of biotransformation for a diverse set of relevant MPs in a lake ecosystem during different seasons of the year and at different water depths, and (ii) To identify associations between the biotransformation rate constants and different physico-chemical parameters of the water samples and microbial community characteristics such as microorganism density, composition and diversity. By contrasting the effects of the environment, including microorganism density and composition, at each sampling time and depth, we aim to identify the most important drivers of MP biotransformation in a lake ecosystem. To this end we sampled a study lake five times between May and October at 3 and 6 metres depth and spiked the lake water with a mixture of 40 MPs. Biotransformation rate constants were derived from the decrease in spiked MP concentrations over time. A machine learning approach (Random Forest) was used to rank the most important environmental or microbial community variables predicting biotransformation rate constants.

2. Experimental methods

2.1. Chemical and reagents

Micropollutants were selected to be environmentally relevant (i.e., commonly measured in European watercourses) and to cover a variety of chemical structures and potential pathways of transformation. The full list is available in Table S1. Five compounds were excluded from further data processing because of instrumentation/analytical issues (i. e., acesulfame, DEET, gemfibrozil, metformin and ranitidine).

Reference standards were obtained from Sigma-Aldrich (Seelze, Germany), Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Toronto Research Chemicals (North York, Canada). The deuterated and carbon-labelled internal standards were purchased from Toronto Research Chemicals. Each compound was diluted to a concentration of 1 g/L in ethanol or methanol. Stock solutions of the spiking mixture were prepared in ethanol at a concentration of 2 mg/L. These stock solutions were further diluted to 100 µg/L in Milli-Q water just before each experiment. The spiking solution contained 0.2% EtOH.

2.2. Study site

Lake Greifen is an alpine meso-eutrophic lake of about 8.46 km^2 and a depth of 32 m at its deepest point. At the sampling point, the lake is 20 m deep. The sampling point is located next to an automated monitoring station (47.3663 °N, 8.665 °E), where a device called Aquaprobe provides daily measurements of temperature, pH, turbidity, dissolved oxygen, conductivity, chlorophyll a and phycoerythrin along the whole water column (Pomati et al., 2011). Three wastewater treatment plants equipped for nitrification, denitrification and phosphorous removal are discharging directly into Lake Greifen. The mean residence time of water is 408 days (Ulrich et al., 1994).

2.3. Water sampling and characterisation

Lake water was collected on May 22, July 9, August 23, September 11 and October 16, in 2018, at around 9 am. Dates were selected to be about one month apart. This study focused on the warmer months of the year so the microorganism's density and activity would be the largest. Lake water was collected next to the monitoring station, in 3 replicates, at a depth of 3 m and 6 m using a 5 L Niskin bottle. Water was filtered through a 80 µm mesh membrane to exclude zooplankton and the filtrate was collected directly in an autoclaved glass bottle that was then kept in a cooler box until all samples were brought back to the laboratory, less than 2 hours later. All samples were processed that same day.

A subsample of lake water was used for chemical analysis at the analytic and training laboratory of Eawag (AUA – Analytik- und Ausbildungslabor). Ammonium, nitrate, nitrite, ortho-phosphate, and total phosphorous were measured spectrophotometrically (Varian Cary 50 Bio, Melbourne, Australia). Dissolved organic carbon, total organic carbon and total nitrogen were measured using a TOC-analyzer (Shimadzu Corporation, Kyoto, Japan). Sulphate was measured by ion chromatography (Metrohm Schweiz AG, Zofingen Switzerland). The total suspended solid (TSS) content was determined by filtering 100 mL of the lake water on a pre-dried, pre-weighted GF/F filter (Sigma-Aldrich, Darmstadt, Germany), which was then dried again at 95°C for 2 hours, cooled down in a desiccating jar, and weighted again.

A 10 mL subsample of each lake water sample was spiked with a filter-sterilised fixing solution (final concentration 0.01% para-formaldehyde, 0.1% glutaraldehyde, pH 7) and kept at 4°C until flow cytometric analysis. Phytoplankton cells counting and characterisation was performed using a scanning flow cytometer Cytobuoy (Woerden, The Netherlands) that uses 2 laser wavelengths (488 nm and 635 nm) in a time-resolved mode to measure length and pigment fluorescence of particles (Pomati et al., 2017). Bacteria cell counts were done using a CytoFlex flow cytometer (Beckman Coulter, Fullerton, USA) after incubating the cells for 10 min with 5 µL of SYBR green stain (Molecular Probes, Basel, Switzerland).

2.4. Experimental setup

An experimental chamber was used to replicate the conditions (e.g.,

temperature, light intensity) observed in Lake Greifen at each sampling time (See Supplementary Information (SI) section S5). A light setup using a combination of large spectrum lights (Philips Master LED tube UO 24W 830 T8) and green filters (LEE filters) were used to replicate the light intensity and light spectrum measured in Lake Greifen at 3 m and 6 m depths (Fig. S2-S3). The temperature of the chamber was set as the average between the 3 m and 6 m lake water temperature on each sampling date.

For each triplicate of lake water samples, a subsample of 400 mL of the raw lake water was used for the biotransformation experiment, in a 500 mL wide-opening beaker. Another subsample of 400 mL of the lake water was filtered at 0.2 μm to exclude microorganisms and used, in similar beakers, as abiotic controls. All beakers were covered with a plastic film to reduce evaporation and penetration of dust and micro-organisms, but not completely sealed to allow air exchange. They were placed on a shaker at 120 rpm, under a light intensity of 70 μmol photons $m^{-2}\,s^{-1}$ for the 3 m depth samples and 10 μmol photons $m^{-2}\,s^{-1}$ for the 6 m depth and a photoperiod of 14 h light: 10 h dark. Another 150 mL subsample was autoclaved at 120°C for 20 min in a glass Nalgene bottle and used as sorption control. The sorption controls were kept in tightly closed bottles in the dark, also on a shaker at 120 rpm. All glassware used for sampling and experiments were muffled at 550°C for 4 hours prior to the experiment to remove any trace of organic contaminants.

The biotic, abiotic and sorption flasks were spiked with the micropollutant spiking mix to a final concentration of 100 ng/L (containing 0.0002% v/v EtOH). Beakers and bottles were gently swirled and the first sample (1 mL) for time 0 was taken immediately after. Further samples were then taken after 4 h, 24 h, 3 d, 7 d, 10 d, 14 d and 21 d (19 d in the case of the August experiment). The October experiment had an additional sample taken after 28 d. In the July and August experiments, a non-spiked control containing lake water and 0.0002% v/v EtOH was added to the sets of controls to monitor the transformation of MPs already present in Lake Greifen water.

2.5. Chemical analysis

Samples from the experimental beakers and bottles were transferred to a 1.5 mL centrifuge tube and centrifuged at 20 000 g for 15 min to remove cells and suspended particles. 500 μL of the supernatant was transferred in a glass LC-vial and spiked with 20 μL of the internal standard solution (final concentration of 80 ng/L for each analyte). The samples were kept at 4°C until they were directly injected on an Agilent 6495 Triple Quad LC-MS/MS with electrospray ionisation. Analytes were separated on an Acquity UPLC HSS T3 column (3.0 mm x 100 mm, particle size of 1.8 μm) connected to a VanGuard pre-column (2.1 × 5.0 mm, particle size of 1.8 μm) and kept at 30°C. For all samples, 100 μL were injected on the column at a flow rate of 0.5 mL/min.

Calibration curves were obtained by a weighted (1/x) linear least-squares regression of 10 calibration standards (0.5 – 500 ng/L) prepared in Evian water and spiked with the same amount of internal standard as the samples. Blanks (Milli-Q water or Evian water only), blinds (blanks with the internal standard) and one calibration standard were injected every 10-12 samples. Data processing was done with MassHunter Workstation Quantitative or Qualitative software version B.08.00 (Agilent Technologies Inc., Santa Clara, USA). The limit of quantification (LOQ) calculation is detailed in SI section S2, and ranges of LOQ as determined for each compound and each of the five experiments separately are presented in Table S1. For the seven compounds without matching labelled internal standards, a labelled standard with similar molecular mass and LC retention time was used for quantification and determination of LOQ. The calculation of the LOQ is slightly different for these compounds and detailed in SI section S3.

2.6. DNA extraction and isolation

On each sampling date (section 2.3), biomass was also collected for DNA extraction. To that end, lake water (400 mL) was filtered on a 0.22 μm Sterivex™ cartridge (Millipore Corporation, Billerica, USA) in a biological cabinet, using sterile syringes. The cartridge was immediately cracked open using a clean PVC-pipe cutter and the filter was delicately removed from its support using a scalpel and forceps according to the method of Cruaud et al. (2017). When the biomass of the lake water was too high and blocking the Sterivex, a prefiltration on a Whatman 47 mm GF/A (Sigma-Aldrich) was done. All filters (Sterivex and GF/A) were placed in individual 2 mL screw cap centrifuge tubes containing ~ 0.5 g of glass beads (≤106 μm, Sigma-Aldrich). The filters were covered with 1.5 mL of RNA later solution (Sigma-Aldrich) and kept at -20°C until extraction. The total genomic DNA was extracted according to AllPrep DNA/RNA Mini Kit (Qiagen Cat No. 80204) protocol, with the modifications detailed in SI (section S7). Two sets of 16S (variable regions V1-V3 and V3-V4) and 18S primers (variable regions V4 and V8-V9) were used to amplify the samples' genomic DNA (Sigma-Aldrich).

Details of library preparation are available in SI (section S7). Polymerase chain reaction (PCR) was run on a thermal cycler (Sensoquest, Applied Biosystems) and amplified regions were cleaned with AMPure Beads (Beckman Coulter). After quality checks and DNA concentration quantification, samples were normalized and pooled before sequencing using the paired-end 2 × 300 (600 cycle) protocol v3 on a MiSeq platform (Illumina) at the Genomic Diversity Centre at the ETH in Zurich, Switzerland. The demultiplexed raw read data is available at the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB41377.

2.7. Sequencing data processing

Sequencing data preparation was performed on the ETH Euler cluster. The workflow used to process the demultiplexed raw data into (Z) OTU tables is described in more details in the Supplementary Information section S15.1. In short, after a data quality evaluation to determine the optimal workflow parameters, in a first processing step, the read ends were trimmed and the read pairs merged. We used an in-silico PCR approach to remove the primer site on both ends. The merged and trimmed reads were quality filtered and size selected. We used the classical OTU clustering approach (UPARSE2) with a 97% identity threshold and the newer zero OTU (ZOTU) amplicon sequence variant approach (UNOISE3). Both methods are implemented in USEARCH and well documented in SI (section S15.1). We used SINTAX and the Silva SSU version 128 (16S) and PR2 version 4.10.0 (18S) references to predict taxonomic associations for (Z)OTUs.

2.8. Kinetic analysis

From the resulting MP concentration-time series, first-order biotransformation rate constants were derived from the linear regression applied to the logarithmic concentrations of MPs against experimental time, using the software R (version 3.5.1). Biotransformation rate constants were only considered valid if the degradation percentage was higher than 10% and the r^2 of the linear regression was > 0.6. Below these values, the decrease in MP concentrations was considered hidden in the analytical uncertainty, or not following a first-order kinetic. These considerations led to a "limit of detection" for biotransformation rate constants in our current system of 0.005 day^{-1} (which is equivalent to a half-life of approx. 140 days). Any transformation slower than that could not be reported. A micropollutant multifunctionality score (MPMF) was calculated by scaling the rate constants of the individual compounds to an average value of 0 and a standard deviation of 1. MPMF was then calculated as the average of all the biotransformed micropollutants' scaled biotransformation rate constants for each sampling time and depth separately (Johnson et al., 2015). This MPMF represents the

relative biotransformation efficiency of each water sample across all MPs exhibiting a quantifiable degree of transformation.

2.9. Association analysis

All environmental variables recorded for the lake at each sampling time are reported in Table S2. For downstream analysis, they were scaled to a mean of 0 and a standard deviation of 1 to account for the large differences in unit size between the different variables before performing a principal component analysis (R-package "vegan").

The annotated (Z)OTU count tables obtained after data processing were further analysed using R and various R-packages (see SI sections S15.2 and S15.3). Only the region V3-V4 of the 16S rRNA amplicon and region V8-V9 of the 18S rRNA amplicon were kept for further analysis. The 16S-V3-V4 had more taxonomic assignments than the 16S-V1-V3 region, and the 18S-V4 region primers did not amplify the selected 18S region for many samples.

To evaluate and rank the most important environmental or microbial community variables predicting biotransformation rate constants we used a Random Forest model as it seemed appropriate for the type and amount of data generated in our study. Random Forest is a robust machine learning algorithm, able to afford non-parametric regression analysis of ecological data (Feld et al., 2016; Grizzetti et al., 2017). It can handle a large number of correlated variables and low number of observations, and is suited to analyse non-linear relationships and complex interactions, even with numerous missing values (Breiman, 2001). It provides variable importance measures highlighting the most important predictors, which was used in this study to identify the environmental parameters that were influencing MPs' biotransformation most. The Random Forest model was developed and validated using the method described in Supplementary Information (section S15.4).

3. Results & Discussion

3.1. Transformation of micropollutants in lake water

Out of the 35 compounds analysed in the biotransformation experiments, 15 compounds (listed in the legend of Fig. 1, except chlorpyrifos) showed a decrease in concentration of more than 10% over the experimental time period (i.e., 19-28 days) in at least one of the experiments (Fig. S6). The experimental conditions tried to mirror the lake conditions as much as feasible (see Supplementary Information sections S5-S6 for

Fig. 1. Arithmetic mean rate constants (three replicates per sampling time) for the 14 compounds showing biotransformation in at least one experiment at 3 m and 6 m depth between May and October 2018 (average values with their standard deviation are reported in Table S4).

more details). To distinguish between the effect of biotic and abiotic processes in the biotransformation experiments, abiotic degradation and sorption controls were run in parallel with the lake water experiments. Chlorpyrifos showed dissipation in the abiotic and sorption controls to the same extent as in the biotic beakers (Figs S8-S11), and its dissipation in all beakers was thus ascribed to abiotic hydrolysis and/or evaporation. It has been shown previously that chlorpyrifos is susceptible to hydrolysis, and its half-life depends on pH but also salinity and metal concentration, e.g., copper, in surface water (Liu et al., 2001). For gabapentin, slow dissipation was observed in the different controls at a similar rate (average of $0.005 \, d^{-1}$). This abiotic dissipation rate constant was lower than the lake water (biotic) dissipation rates, and therefore subtracted from the lake water biotransformation rate constants to yield estimated biotransformation rate constants. Verapamil was strongly sorbing during the first day after spiking, however, the sorbed fraction was very similar for all experiments ($\pm$ 15% variation between day 1 concentration values). Verapamil then showed a first order decrease until day 14, after which its concentrations fell below the limit of quantification. Since this dissipation could still be compared between the different experiments, biotransformation rate constants were calculated between day 1 and day 14 for this compound. The remaining 12 compounds did not show any significant dissipation in the controls, as shown in Figs S8-S11, and their first-order rate constants were used without further modification for the following analyses.

In two experiments (July and August), non-spiked controls were used to determine the biotransformation of MPs already present in Lake Greifen. The rate constants obtained were generally not significantly different between spiked and non-spiked microcosms (Fig. S12).

The biotransformation rate constant values and the TSS-normalized rate constants for the 14 compounds showing biotransformation are presented in Fig. 1 and Tables S4-S5. The rate constant values obtained were often very low, in the $0.005 – 0.2 \, d^{-1}$ range, suggesting generally slow biotransformation in lake water. This might be related to the low biomass concentration in the water column (TSS values of $0.5 – 8 \, mg/L$ obtained in lake Greifen, compared to $1 – 12.4 \, g/L$ typically used in activated sludge experiments (Achermann et al., 2018; Helbling et al., 2012; Men et al., 2017)). However, once normalized to the TSS content, rate constant values for the compounds with observable degradation in the lake water are on the same order of magnitude as those observed in activated sludge from wastewater treatment plants (Achermann et al., 2018; Falås et al., 2016) (for a comparison of values, see Table S5 in the SI).

The half-lives for the biotransformed compounds are presented in Table 1. The transformation rates and calculated half-lives varied by one order of magnitude for the same compound under different conditions (i. e., month sampled, or depth). For instance, the half-life of valsartan varies between non-detectable in May and June and only 5.6 days in the September experiment. Metolachor biotransformation was only quantifiable in the August experiment at 3 m, but non-detectable in all the other experiments. Gabapentin's half-lives measured were 121 days at 3 m, but only 19.4 days at 6 m, in the August experiment. Many compounds showed higher biotransformation rates during the August or September period (Fig. 1) when the phytoplankton was growing or around its peak density in Lake Greifen (Fig. 2). These results qualitatively support the notion that biomass concentration is an important factor in the transformation of these compounds. However, biomass concentration is not explaining the higher transformation rate constants of some compounds, such as mianserin and asulam, in May or July, or the depth-related differences in half-lives. Other parameters, such as microbial composition, thus need to be considered.

For the 20 compounds that did not show detectable biotransformation in our study, some of them are usually considered persistent in surface waters, such as carbamazepine and venlafaxine (Boix et al., 2016; Li and McLachlan, 2019; Löffler et al., 2005; Tixier et al., 2003). Others have been found to be transformed in some studies but not in others, such as trimethoprim (Jasper et al., 2014; Jewell et al., 2016).
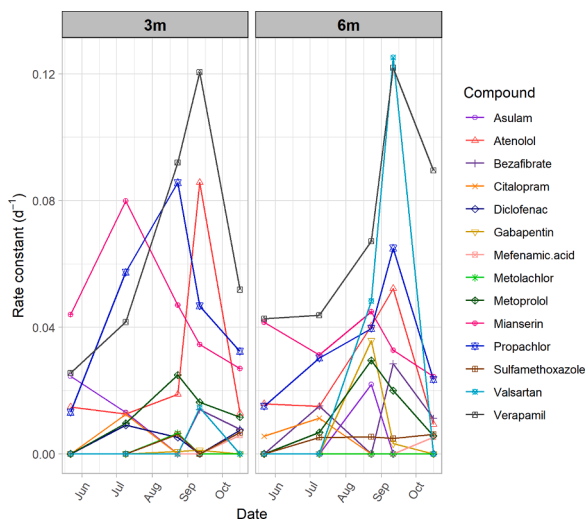
Table 1

Half-lives (days) for the compounds showing detectable biotransformation over the experimental time period. Values are presented as arithmetic means $\pm$ their standard deviation

Compound	Depth	2018-05-22	2018-07-09	2018-08-23	2018-09-11	2018-10-16
Asulam	3m	28.5±2.7	53.1±5.1	–	–	–
	6m	–	–	33.4±10.4	–	–
Atenolol	3m	47.0±3.8	55.1±4.2	38.5±10.7	8.2±1.1	53.9±3.1
	6m	44.7±7.4	46.5±3.2	23.5±13.7	14.0±3.7	79.2±24.6
Bezafibrate	3m	–	–	–	49.5±1.6	90.6±15.4
	6m	–	46.6±7.0	–	24.4±0.3	62.2±3.1
Citalopram	3m	–	57.7±13.0	–	–	–
	6m	135.1±50.1	61.8±4.3	–	–	–
Diclofenac	3m	–	76.6±5.2	140.6±41.4	–	93.4±9.9
	6m	–	–	–	–	–
Gabapentin*	3m	–	–	121.0±13.6	132.2±50.9	–
	6m	–	–	19.4±7.5	99.4±41.9	–
Mefenamic acid	3m	–	–	–	–	118.2±7.4
	6m	–	–	–	–	134.0±21.4
Metolachlor	3m	–	–	106.1±4.8	–	–
	6m	–	–	–	–	–
Metoprolol	3m	–	72.9±11.6	28.5±5.5	43.3±8.1	59.7±4.0
	6m	–	104.3±17.8	31.3±16.4	48.2±26.8	121.5±4.4
Mianserin	3m	15.8±0.9	9.2±2.5	15.2±3.1	20.5±3.6	25.8±1.7
	6m	17.1±3.4	22.3±1.4	18.4±8.9	22.2±6.0	28.9±5.1
Propachlor	3m	52.8±3.6	12.1±0.4	8.1±0.6	14.8±0.5	21.4±0.5
	6m	48.7±13.4	23.0±2.2	17.5±0.6	10.7±0.9	29.6±1.5
Sulfamethoxazole	3m	–	–	112.4±8.3	–	102.9±6.5
	6m	–	133.9±3.1	148.6±73.9	190.6±138.6	113.8±12.3
Valsartan	3m	–	–	–	49.9±12.4	–
	6m	–	–	15.4±5.4	5.6±0.6	–
Verapamil	3m	47.2±33.2	16.7±0.4	8.0±2.5	6.6±3.2	14.6±5.7
	6m	18.1±7.5	16.1±2.6	15.5±12.5	6.0±1.7	8.3±3.0

*Half-lives of gabapentin have been calculated with their overall dissipation rate, i.e. that includes abiotic transformation. –: no measured biotransformation.
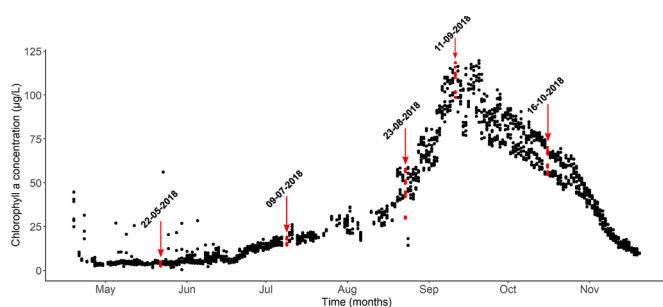

Fig. 2. Chlorophyll a concentration measured at 3 m depth in Lake Greifen over the measuring period of 2018.

Trimethoprim's biotransformation was shown to be taking place at low dissolved oxygen concentrations and is thought to be related to certain heterotrophic microorganisms thriving under sub- or anoxic conditions (Khunjar et al., 2011). Since our microcosms were not tightly closed and continuously shaken, they were operating under aerobic conditions, thus perhaps preventing conditions that would favour its biotransformation. Finally, many compounds are known to be biodegradable, but no transformation rate could be observed or calculated in our study. Caffeine, for example, has been shown to be transformed in Lake Greifen under similar conditions (Buerge et al., 2003). However, the transformation rate they measured was very close to our limit of detection for biotransformation (their calculated value being 0.004-0.006 d^{-1} while our "limit of detection" was 0.005 d^{-1}). For other compounds, a lag phase has previously been observed when 50 μg/L were spiked into experimental systems with surface waters: in one study, for caffeine, it took more than 20 days to start seeing a decrease, after which the transformation was very fast (Li and McLachlan, 2019). It is possible that the duration of the experiments in our study was not long enough to see the adaptation of microorganisms to the spiked MP concentrations and subsequent onset of biotransformation. In the case of gabapentin, for instance, a lag phase of 10 days was observed in the August experiment, followed by a rapid transformation at a rate of 0.112 d^{-1} from day 10 to day 19, concomitantly with an increase in the experimental replicates variation. This phenomenon has also been observed in biotransformation experiments with suspended sediment (Seller et al., 2020) and demonstrates that a differential evolution of the communities in the microcosms could lead to a changing biotransformation potential over time. Concentrating the biomass or running the experiments for a longer time could have helped detecting slower biotransformation but would also have steered the experimental conditions away from the original lake conditions even more, making the microcosms less representative of a real lake ecosystem.

3.2. Environmental and microbial parameters during sampling times

Lake Greifen was closely monitored during the sampling period in terms of environmental parameters, microorganism density and diversity. The summer of 2018 was exceptionally dry and warm in Switzerland, with average temperatures 1.5°C above the average from 1981-2010, and only 59% of the normal precipitation in the northern part of the country (MétéoSuisse, 2019). During the sampling period, there was one major phytoplankton bloom noted, starting in August and ending in November (Fig. 2).

Data on temperature, dissolved oxygen, pH, total organic carbon, dissolved organic carbon, total nitrogen, total phosphorous, nitrite, nitrate and ammonium for each sampling time are provided in Table S2. The May samples were mainly characterised by high ammonium and nitrate concentrations and comparably low biomass of microorganisms. The August, September and October samples had high chlorophyll a, total organic carbon and total suspended solids (TSS) content, as expected given the observed phytoplankton bloom. Results from a principal component analysis of environmental variables (PCA; Fig. 3) shows that samples from each sampling time point (e.g., May, July) cluster well together, while each month is clearly separated from the other months. This demonstrates that every sampling time had distinct environmental characteristics. The PCA also shows which environmental conditions most strongly explain the observed variability in the samples (Fig. 3).
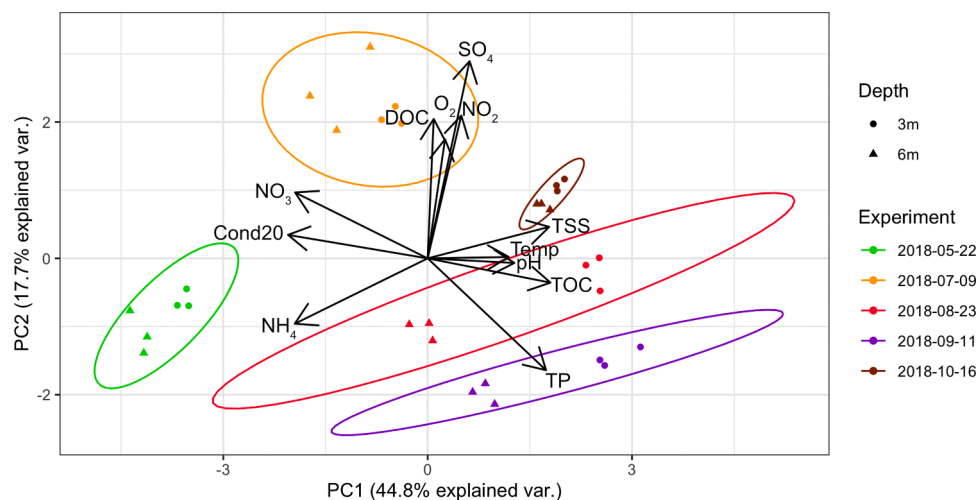
Fig. 3. Principal component analysis of selected environmental parameters measured at 3 m and 6 m for each lake sampling time, with the 95% confidence ellipses of each sampling time point. Cond20: Conductivity corrected at 20°C, DOC: dissolved organic carbon, NH_4: ammonia, NO_2: nitrite, NO_3: nitrate, Temp: Temperature, TOC: total organic carbon, TP: total phosphorous, TSS: total suspended solids, SO_4: sulphate.

The two depths (3 m and 6 m) typically clustered well together for most experiments, demonstrating a good mixing of the water column above the thermocline (see temperature and chlorophyll a profiles in Fig. S1). However, in August and September, a peak of chlorophyll a was observed at 3 m, which is also reflected by higher values of dissolved oxygen, TSS and TOC, hence leading to a larger separation between the two studied depths.

The density of bacteria and phytoplankton cells quantified using flow cytometry are presented in Fig. S13. The phytoplankton cell density increases over the year with its maximal density in September (13 times
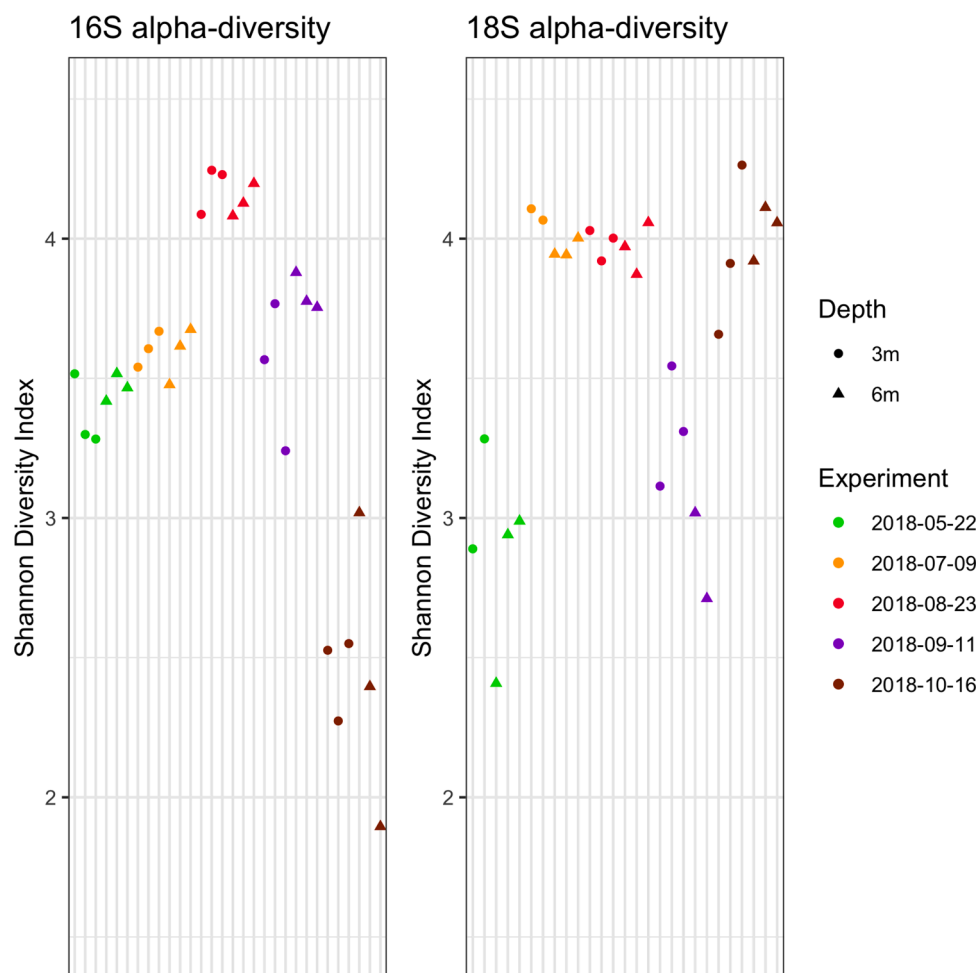

Fig. 4. Alpha-diversity, represented by the Shannon Diversity Index, for the 16S (prokaryotes) and 18S (eukaryotes) rRNA amplicons for each sample at every sampling time.

higher than in May). This result is consistent with the increase in chlorophyll a measured in the lake (Fig. 2). In contrast, the bacteria cell density shows only minor fluctuations over the entire period, with only a 2.7-fold difference between the lowest density (in May) and the highest density (in October) at 3 m depth, while not showing a peak during the phytoplankton bloom of September (Fig. S13). There could be a delayed response in bacteria density to the bloom since the highest densities are observed during the decay of microalgae cells in October. It is not uncommon to find relatively stable densities of bacteria in lakes during summer (Arndt and Nixdorf, 1991). The cell densities for both bacteria and phytoplankton were not significantly different between the 3 m and 6 m samples, except for July and September samples in which bacteria were ~1.3 times more abundant at 3 m than 6 m.

Fig. 4 shows the different Shannon α-diversity indices for bacteria and eukaryotes at every sampling time. Even though the bacteria density (abundance of cells) was not strongly influenced by the phytoplankton bloom, the diversity of bacteria (number of taxa identified) found in the water samples was peaking in August, when the phytoplankton cells were on the increase. The α-diversity of bacteria has been shown to be highest during a diatom bloom previously (Zhang et al., 2018). In contrast, while the density of eukaryotes was strongly increasing during that time, the observed diversity of eukaryotes stayed relatively constant, demonstrating that a few species dominated the bloom. Because of these contrasting dynamics, the diversities indices derived from 16S and 18S rRNA amplicon sequences did not correlate with each other. During the bloom of phytoplankton and its subsequent decay (August to October), planctomycetes and verrucomicrobia are particularly relevant in terms of their relative proportions (Fig. S14). These two bacterial

phyla are involved in the degradation of polysaccharides, and thus often abundant during phytoplankton blooms (Fuerst, 2017; Martinez-Garcia et al., 2012). Their large suite of enzymes (Martinez-Garcia et al., 2012) could potentially lead to micropollutant biotransformation as a co-metabolic outcome, although further research is required to confirm this hypothesis.

3.3. Statistical association of biotransformation rate constants with lake environmental parameters, microorganism density and diversity

In order to understand the influence of environmental parameters on the dissipation of MPs in Lake Greifen, a Random Forest model was applied to the biotransformation rates obtained using all the environmental variables including the microorganism densities and diversity indices presented above as descriptors. Random Forest models work by fitting thousands of decision trees to a training data set (a subset of the whole data set) and averaging the prediction. The model then measures the predictive ability of the final decision tree in correctly classifying the outcome (here, the biotransformation rate constants).

When using the Random Forest model to predict the overall MP biotransformation potential (the MPMF), the model obtained explained 72% of the variance. The variable importance (i.e., the best predictors of biotransformation rates) is presented here as the Mean Decrease Accuracy, which is a measure of how much accuracy the model losses by excluding each variable. Hence, the most relevant variables are the lake temperature, conductivity, 16S Shannon Diversity Index, followed by nitrate concentration and indicators of phytoplankton density such as number of phytoplankton cells per mL and chlorophyll a concentration.
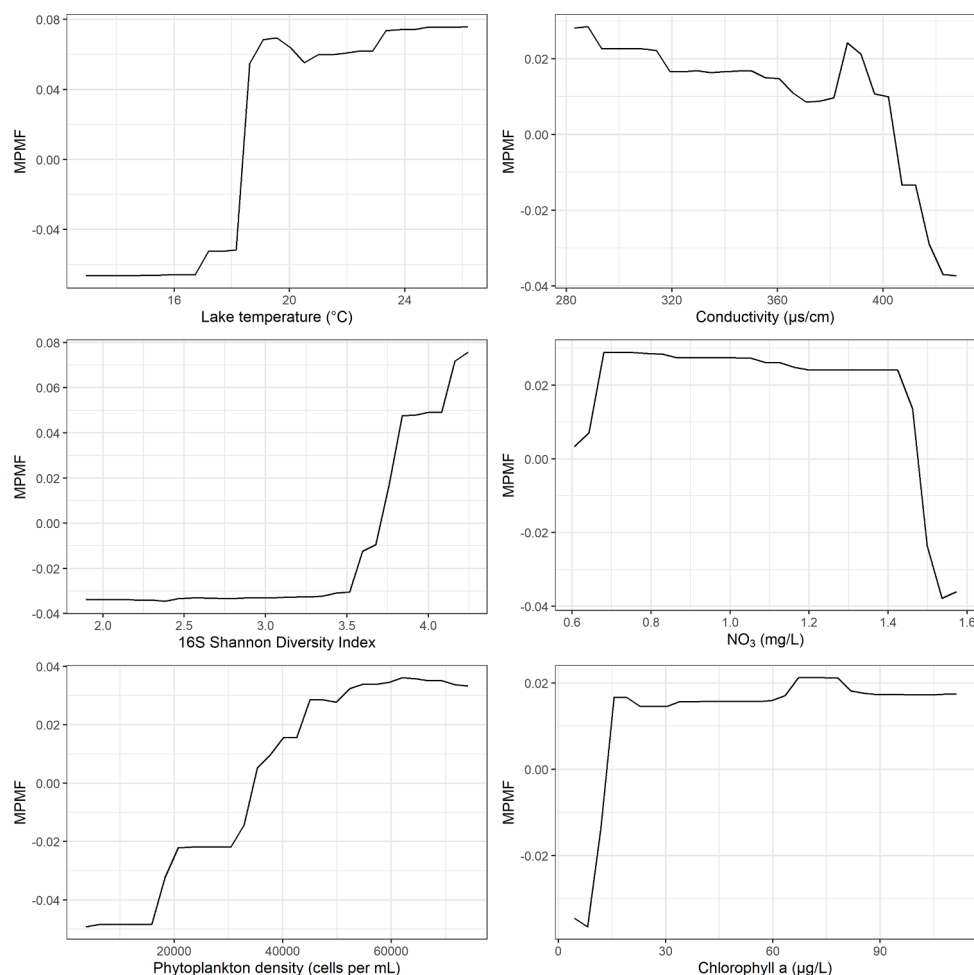

Fig. 5. Partial effects of the Random Forest analysis conducted on the Micropollutant Multifunctionality (MPMF) indices.

The most important variables are presented in Fig. 5 (full ordered list in Fig. S17). The partial dependence plots help understand the causal relationship between specific variables (e.g., temperature, conductivity) and the MPMF prediction.

Lake temperature was thus identified as the descriptor that best explained the variance in MP biotransformation, followed by ions and nutrients indicators such as conductivity and nitrate, biomass indicators such as phytoplankton cell density and TSS, and bacteria diversity. As the metabolic potential for biotransformation resides mostly in enzymes (Krah et al., 2016), it seems logical that temperature would affect transformation rates, either by increasing microorganism growth rate and/or enzymatic activity. There was a large increase in the overall MP transformation rate (MPMF) when the water temperature got over 18°C (Fig. 5), and this might be a tipping point where the activity or abundance of microorganism is high enough to catalyse more transformation reactions (Meynet et al., 2020). It should also be noted that the temperature in the environmental chamber used in this study was kept constant at the temperature measured at the time of sampling. Hence biotransformation rates in Lake Greifen could differ from our study due to daily and weekly changes in temperature.

Conductivity and nitrate have inverse relationships with the MPMF (Fig. 5). In many instances, high conductivity is related to large input of nutrients and ions into a watercourse, often due to the discharge of wastewater or run off from agriculture fields (Gali et al., 2012). In our study, however, the high nutrient concentrations are most likely related to the enhanced nutrient remineralization following the heavy phytoplankton grazing of the spring, and low microorganism density of the May samples (Weithoff and Gaedke (2016); Fig. 3).

Although less abundant than bacteria in terms of cell number, phytoplankton are larger and heavier and represent the bulk of the lake's microorganism total biomass. For micropollutants with non-specific transformation pathways (i.e., transformed by enzymes widely present in microorganisms), crude biomass could be the best predictor of biotransformation. This seems to be the case for sulfonamide compounds whose transformation is either correlating strongly with microorganism density or had similar values when normalised for biomass (Achermann et al., 2018; Meynet et al., 2020).

Analogously to temperature, when the bacteria diversity reaches a certain threshold (here, a Shannon Diversity Index of 3.5; Fig. 5), the overall micropollutant biotransformation increases substantially. Micropollutant biotransformation rate constants in activated sludge sourced from different wastewater treatment plants have been shown to positively correlate with the communities' microbial and functional diversity (Achermann et al., 2018; Johnson et al., 2015). Similarly, it was shown that the diversity of phytoplankton species also had a positive influence on the transformation of several MPs (Stravs et al., 2019). Blunt et al. (2018) studied the biotransformation of 27 pharmaceuticals and endocrine-disrupting substances along a wastewater effluent flume gradient in Lake Mead and found that biotransformation rate constants were higher close to the outlet of the wastewater treatment plant, which is also where the bacterial metabolic capacity (measured by different carbon sources utilization rates with a Biolog EcoPlates™) and species diversity were highest (16S rRNA sequencing). Using Spearman rank's correlation, we can also confirm that the MPMF is significantly correlating with the 16S Shannon Diversity Index, but not with the 18S Shannon Diversity Index (Fig. S16). This finding suggests that procaryote diversity has a stronger impact on biotransformation of micropollutants than eukaryote diversity.

The Random Forest approach was also applied to those individual MPs that had a complete set of biotransformation rate constants (Table S4). When investigating relationships between lake parameters and rate constants for individual micropollutants, some compounds are closely following the MPMF. For propachlor, for instance, temperature and biomass are again the best predictors explaining the observed variance in rate constants (Fig. S18). However, other compounds show some differences in the factors influencing their specific transformation

rate constants. For atenolol, total phosphorous, total nitrogen and dissolved organic carbon are better predictors of their biotransformation than temperature (Fig. S19). Since total phosphorous is measured in unfiltered water, it includes the amount found in biomass, especially the phytoplankton. Indeed, the highest concentration of total phosphorous is concomitant with the peak of phytoplankton bloom (Fig. 3, Table S2). It has been shown by Stravs et al. (2017) that some microalgae and cyanobacteria could transform atenolol to atenolol acid through enzymatic hydrolysis, which potentially explains its larger transformation rate at the peak of phytoplankton density. Finally, the biotransformation rate constants of mianserin are poorly explained by the parameters included in the model (only 32% of explained variance; Fig. S20), and this might be due to specific microorganisms needed for its degradation. Mianserin and asulam have previously been shown to be transformed by ammonia and/or nitrite oxidizing microorganisms (Men et al., 2016; Yu et al., 2018). Both compounds have high biotransformation rate constants in May when Lake Greifen went through a phase called "clear water phase", during which zooplankton, grazing heavily on the spring bloom of phytoplankton, release large quantities of ammonia in the water (Weithoff and Gaedke, 2016). This is consistent with the higher measured ammonia concentrations in May, relative to all other time points (Table S2). Thus, increased activity of ammonia oxidizers might explain the increased transformation potential for mianserin and asulam in May.

4. Conclusions

This study measured the biotransformation of 35 compounds at five different sampling dates and two lake water depths and presents a unique dataset of 78 biotransformation rate constants above our detection limit of 0.005 day^{-1}. This project thus provides valuable data for modelling the fate of MPs in a lake ecosystem, and to support improved risk assessment of contaminants in surface waters. The rate constants obtained were in a similar range as those of other dissipation processes such as sedimentation and phototransformation in lakes (e.g., Tixier et al. (2003)), demonstrating that microbial biotransformation is a non-negligible process for MP fate in large surface water bodies. The rates at which MPs were transformed were largely influenced by the lake temperature, and the phytoplankton bloom (and concurrent increase in bacteria diversity) that started in August. With eutrophication, rise in atmospheric CO_2 concentration and climate warming, blooms of microalgae and cyanobacteria are expected to be more frequent (Gobler, 2020). Warmer lakes might lead to higher microorganism biomass and hence to higher overall MP biotransformation. This suggests that one of the few silver linings of climate change could be a faster degradation of anthropogenic contaminants in lakes.

If the majority of the compounds transformed faster under higher temperature and phytoplankton biomass, divergence from this general pattern was observed for some compounds (e.g., mianserin, asulam). Their biotransformation rate constants seemed to depend more exclusively on specialized – sometimes rare – types of microorganisms. Prior knowledge on involved strains and enzymatic pathways, and a high-throughput combination of field and laboratory experiments are still needed to shed light on the intricacies of MP biotransformation processes in natural environments.

Declaration of Competing Interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.117412.

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