

Supporting Information for:

Impact of wastewater on the microbial diversity of periphyton and its tolerance to micropollutants in an engineered flow-through channel system

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50 **I. Maiandros channel system**

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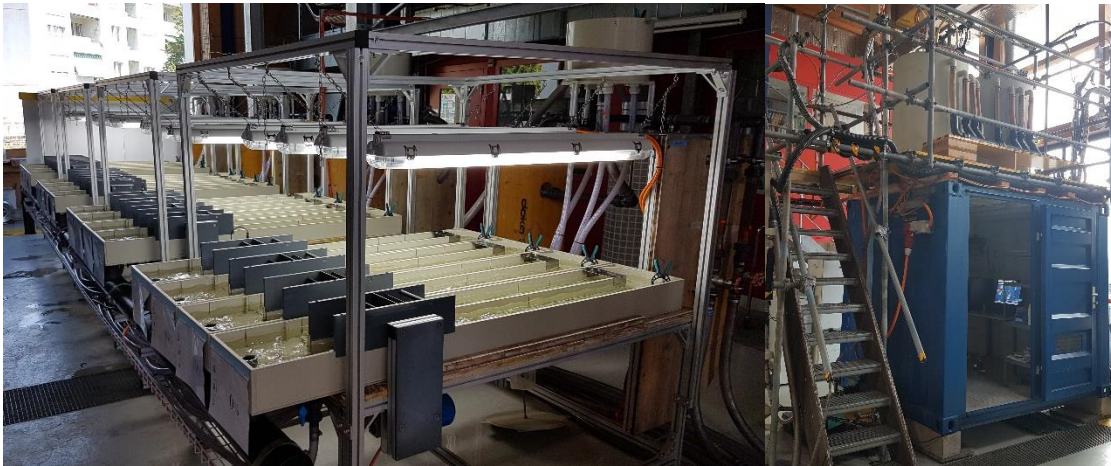
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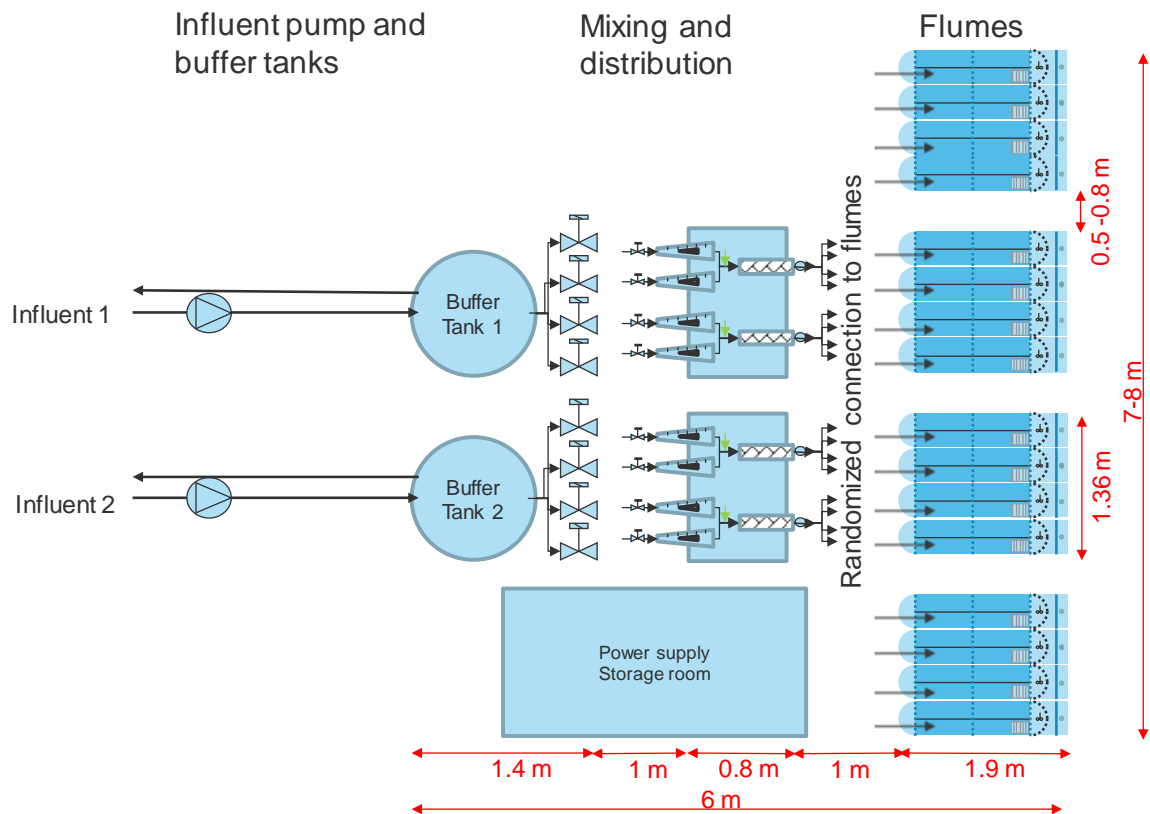
76 (A)

(B)



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78 Figure 1: Overview of the Maiandros flume system. (A) 4 mixing units (in the background) and 16 flumes. (B) the dark blue
79 container with the controlling unit and two buffer tanks on the roof.



80

81 Figure 2: Schematic of the elements composing Maiandros.

82

83 2. Scope of the installation

84 The goal of the Maiandros system is to allow controlled mixing of different waters for
85 comparative experiments in flumes. Up to four mixing ratios can be controlled and distributed

on totally 16 flumes (Figure 1 and Figure 2). Typically, wastewater is mixed with surface waters from a nearby river or creek.

The installation offers the following possibilities:

- Pumping of two different inflows
- Four mixing units with two manual flow control valves each as well as a flow splitter for distributing each mixture to the flumes
- Nozzle for spiking test solutions
- Randomized connection of mixing units with flumes
- Each flume is equipped with a paddle wheel for internal recirculation (flow ca. 0.25 m/s)

3. Description of the elements composing the installation

3.1. Influent pumps and buffer tanks

Two influent pumps (frequency controlled with a maximal flow of 10 m³/h each) feed water into the two buffer tanks of 1.2 m³ volume each (1.0 m diameter, 1.5 m height).

Because the pumping from the creek was prone to failure due to debris, especially during storm weather, two redundant submerged centrifugal pumps were installed. The active pump was chosen manually as required. The pumps were anchored to the ground behind a V-shaped screen for protection from materials transported by the current.

The two buffer tanks are equipped with stirrer to avoid settling of particulates

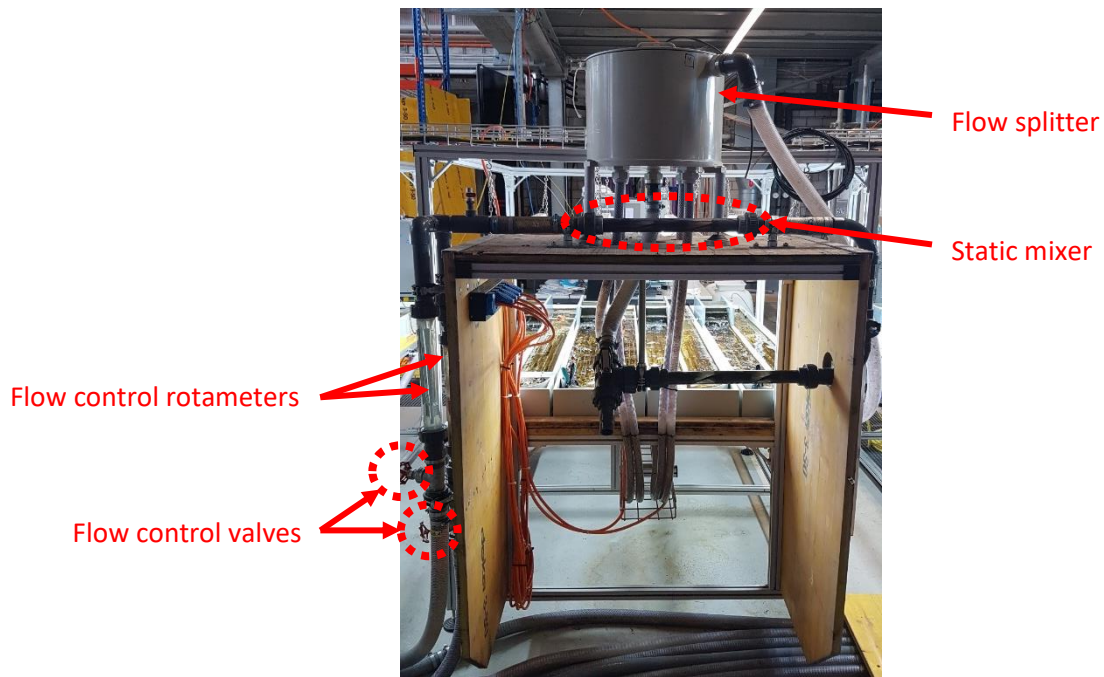


Figure 3: Elements composing the mixing unit and flow splitter.

3.2. Mixing units and flow splitters

The four mixing units allow controlling the mixing via the following elements:

- Two manual flow reduction valve
- Two rotameter for visual inspection of the two inflows
- A nozzle for dosing of chemical solutions
- A static mixer to homogenizes the mixture

The flow splitter is implemented as a horizontal circular overflow edge divided into four 90° sectors each one connected to a flume.

3.3. Flumes

The 16 flumes are subdivided into four groups of four flumes. The pipe connection from the flow splitters to the flumes allows randomizing the flumes to reduce the risk of systematic artefacts. Each group of flumes consists of the following elements:

- 4 flumes shaped as a ‘circular race-track’ with a total channel length of 2.6 m, 150 mm wide and 100 mm height

- 123 - a paddle wheel for each flume providing 0.2 m/s horizontal flow speed
- 124 - one motor for four paddle wheel with sensor for supervising the rotation speed
- 125 - a removable pipe as effluent overflow to control the fill level in the channels (typically
- 126 50 to 80 mm)

127 3.4. Lightening system

128 The lightening system (Philips Master LEDtube HF 1200 mm) consist in LED tube of 2500
129 lumen (colour temperature 6500 K) and is installed as follow:

- 130 - 2 LED tubes per flume (i.e. in total 32 LED tubes for the 16 flumes)
- 131 - Distance between LED tubes and water level: 50 cm

132

133 4. *Operation*

134 4.1. Influent pumps and buffer tanks

135 The influent pumps in the creek required weekly visual inspection (i.e. after every rain event)
136 for debris and branches getting tangled in pumps or screen. Centrifugal pumps of sufficient size
137 (relevant is the free water path) proved significantly more reliable than other pump types for
138 clogging with debris.

139 4.2. Mixing units and flow splitters

140 The manual flow control valves required daily adjusting of the flows.

141 The flow splitters required cleaning of the overflow edge twice per week.

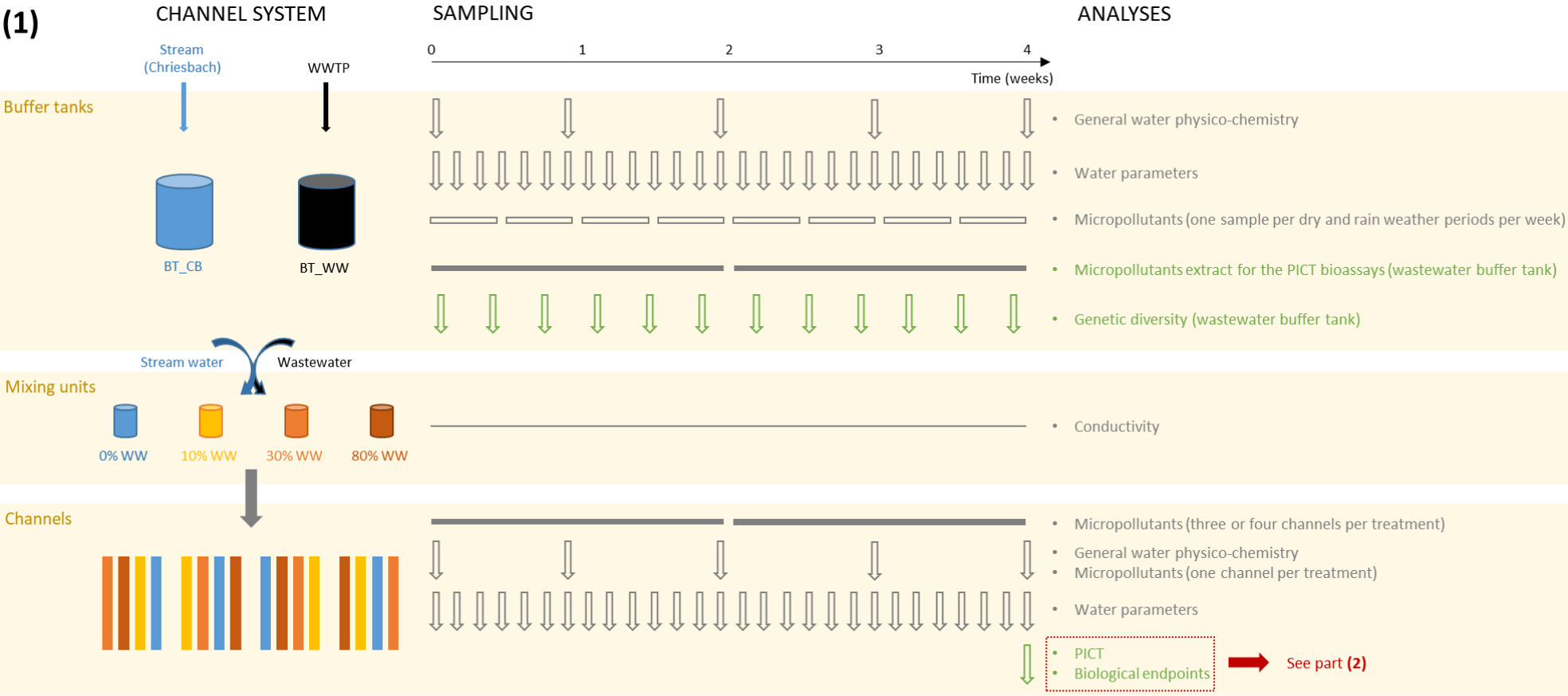
142 The position of the manual flow control valves was corrected daily according to the reading of
143 the rotameter.

144 4.3. Flumes





145 The walls of the flumes have been cleaned manually daily to limit biofilm growth.

146 The inflow to each flume was 120 L/h resulting in a hydraulic residence time of 10 minutes.

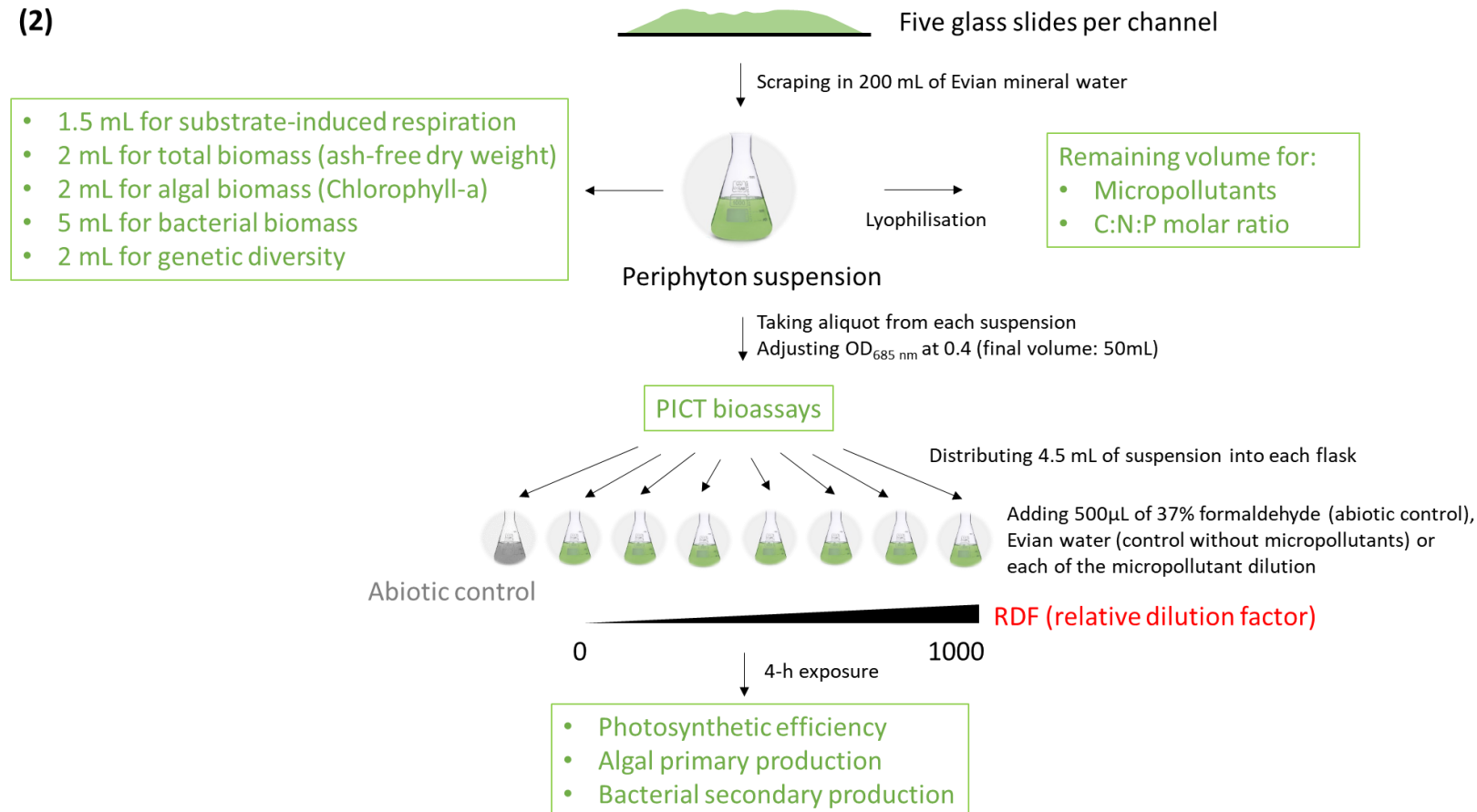
II. Overview of the experimental design



Legend

Sampling/measurement method		Analyses
	One-time sampling/measurement	Water physico-chemistry
	Composite sampling	Biology
	Passive sampling	
	Continuous measurement	

(2)



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150 **II. Overview of the experimental design. (1)** Sampling during the 4-week colonization phase. Periphyton was grown on glass slides submerged in the
151 artificial streams. A total of 40 glass slides were installed in each channel. WW, wastewater; WWTP, wastewater treatment plant; CB, Chriesbach;
152 BT, buffer tank. **(2)** Biological analyses carried out with periphyton, including pollution-induced community tolerance (PICT) assays. The relative
153 dilution factor value refers to the relative concentration of micropollutants extracted from passive samplers. The pure extract corresponds to an RDF
154 of 1000.

III. Supplemental materials and methods

1. *Extraction of micropollutants from passive samplers*

The recovered SDB disks (in acetone) were shaken on a rotary shaker for 30 min. The acetone was transferred to a new vial and 6 mL of methanol was added to the SDB disk and shaken again for 30 min. The methanol was then transferred to the new vial (containing acetone) as well. From the vials containing PS extracts from 0% and 10% waste water mixture, 1/10 of the solvent was transferred to a centrifugal vial. For the PS extracts from 30% and 80% waste water, 1/50 and 1/100 of the solvent were transferred respectively. All centrifugal vials were spiked with 20 μL (0.1 mg L^{-1}) isotope labelled internal standard (ISTD). The acetone/methanol fraction was then reduced to approximately 50 μL using a N_2 -evaporator (TurboVap[®], Biotage, Sweden). 950 μL nanopure water were added to the reduced extracts, they were vortexed and centrifuged. From each centrifugal vial, approximately the top 800 μL were transferred to a new LC-vial. The samples were stored at 4 °C until analysis.

2. *Extraction of micropollutants from periphyton*

Micropollutants were extracted by a QuEChERS-based method as described by Munz et al. (2018) with some modifications. Fifty mg of freeze-dried biofilm sample per channel were spiked with 20 μL (0.01 mg L^{-1}) ISTD and stored over night at 4 °C. The samples were then homogenized in a Fast Prep bead beater (MP Biomedicals, Switzerland) with 500 mg of 1mm zirconia/silica beads (Biospec Products, Inc., U.S.A.) in 500 μL acetonitrile (ACN) and 500 μL nanopure water. After homogenization, the samples were centrifuged and 800 μL of the supernatant were transferred into clean tubes with 300 mg of QuEChERS salts (4:1, $\text{MgSO}_4\text{:NaCl}$, Agilent Technologies). The samples were vortexed, centrifuged and the top 400 μL of supernatant were transferred to clean tubes. Five hundred μL ACN was added to the tubes containing used QuEChERS salts and the procedure was repeated. A clean-up was performed to remove lipids by adding 500 μL of heptane. After vortexing and centrifugation, the heptane was removed and 700 μL of the ACN phase (bottom layer) was transferred to a LC-vial. The

ACN was then evaporated (not to dryness) under a gentle stream of nitrogen and 1 mL of nanopure water was added. Finally, 200 μ L were transferred to a clean LC-vial and diluted with 750 μ L of nanopure water and 50 μ L MeOH. The samples were stored at -20 °C until analysis.

3. Chemical analysis with HPLC-MS/MS

Before MP analysis, LC-vials of the samples (grab water, composite water, diluted PICT-extract, diluted biofilm-extract) were thawed and centrifuged. From each LC-vial, the top 1 mL was transferred to a new LC-vial. The samples (grab water, composite water, diluted PICT-extract) were spiked with ISTD (20 μ L, final conc. 200 ng L⁻¹), the biofilm-extract and PS-extract from the channels being already pre-spiked. The LC-MS-method used is described by Hagemann et al. (2020), with some modification. Briefly, direct injection of 100 μ L per sample was performed on an Agilent 1290 Infinity LC System equipped with an Acquity UPLC HSS T3 (1.8 μ m, 3.0x100 mm, Waters) column for chromatographic separation, coupled to a triple quadrupole MS (Agilent TQ6495C) for detection. For PS-extracts of the channels, only 10 μ L per sample were injected. The electrospray ionization was operated with a capillary voltage of 3500 V in positive and 3000 V in negative mode and a dynamic MRM with 650 ms cycle time. The LC System was operated at a flow rate of 0.5 mL min⁻¹ with a gradient of 100% eluent A (nanopure water plus 0.1% formic acid) to 95% eluent B (methanol plus 0.1% formic acid) in 18.5 min. Hold for 3.5 min, go to 100% eluent A in 0.5 min and hold for 4.5 min.

The quantification of MPs was performed by using ISTD and a standard calibration curve between 0.5 - 7500 ng L⁻¹. For PS-extracts of the channels, a standard calibration curve between 1.0 - 100'000 ng L⁻¹ was used. The applied software was MassHunter Quantitative Analysis Version B.08.00 for QQQ (Agilent Technologies). Two transitions were analysed for quality control. The qualifier recovery was calculated as the ratio between the quantifier transition and qualifier transition with a tolerance between 80 – 120 %. For relative recovery calculation, nine samples in total were spiked with a known concentration of the analysed compounds. For PS extracts of the channels, four samples were spiked. The relative recovery was calculated with

the concentration of the spiked sample minus the concentration of the unspiked sample, divided by the theoretical concentration spiked. The average was taken for each substance. The relative recoveries ranged from 70% to 114% for substances with own ISTD and 44% to 115% for the ones without own ISTD (SI Table 3). For substances without own ISTD, concentrations were corrected by the relative recovery. For PS extracts of the channels, the relative recoveries ranged from 83% to 119%. For biofilm samples, no relative recovery was calculated. For calculation of the limit of quantification (LOQ), the lowest calibration standard found (with S/N 10:1) was divided by the matrix factor. The LOQs ranged between 5 to 587 ng L⁻¹ for water samples and 0.24 to 201.52 ng mg⁻¹ dry biofilm for biofilm samples (SI Table 3). For PS extracts of the channels, the original LOQs of the diluted samples ranged between 26 to 6372 ng L⁻¹. After a back calculation to undiluted samples, these LOQs had to be adjusted by the dilution factors of 10, 50, and 100. The matrix factor was calculated with the area of the ISTD in the sample divided by the average of the areas of the ISTD in the calibration row for substances with an own ISTD. The average was taken for each substance. For substances without own ISTD, the area of an unspiked sample was subtracted from the area of the corresponding spiked sample and the result was divided by the average of the areas of the calibration points with the same concentration. The average was taken for each substance. Three substances (Cyclamate, Mecoprop, and Sucralose-FA) were only analysed semi-quantitatively for water samples and two substances were only analysed semi-quantitatively for biofilm samples (Benzotriazole and Carbendazim) due to calibration- or peak-shape constraints. For PS extracts of the channels, six substances (Cyclamate, Diazinon, Pirimicarb, 4-Formylaminoantipyrine, Clarithromycin, and Lidocaine) were only analysed semi-quantitatively. MP concentrations of PS extracts from the channels were then used to calculate MP concentrations in water with the sampling rates R_S of Moschet et al. (2015).

4. *Periphyton characterization*

4.1. Total, algal and bacterial biomass

Total biomass was determined as ash free dry weight (AFDW) as described in Tlili et al. (2008). Briefly, 2 mL of each suspension were filtered through 25 mm CF/C Whatman glass fibre filters (1.2 µm pore size). Each filter was weighed after drying for 24 h at 105 °C (i.e. total dry matter) and combusting for 1 h at 480 °C (i.e. mineral matter). The AFDW was calculated by subtracting the mineral matter from the total dry matter. Results are expressed as mg cm⁻².

Chlorophyll-a content was additionally used as a proxy for algal biomass (Sartory and Grobbelaar, 1984). Briefly, 2 mL from each periphyton suspension were filtered through a 2.5 cm GF/F glass fibre filter paper (pore size 0.7 µm). The filter was submerged in 5 mL of 96 % ethanol, heated at 80 °C (10 min) and sonicated (5 min). The supernatant was filtered using a Sartorius Minisart NML syringe filter (pore size 1.2 µm), and the chlorophyll-a concentration was determined by measuring the absorbance at 665 nm and 750 nm with a UV/VIS Spectrophotometer (Cary100), using the following equation:

$$C = \frac{e * (A_{665} - A_{750})}{\epsilon * V * l} \quad Eq(1)$$

where C is the concentration of chlorophyll-a (mg mL⁻¹); e is the volume of 96 % ethanol; A_{665} and A_{750} are the absorbance at wavelength 665 and 750 nm, respectively; ϵ is the absorption coefficient in 96 % ethanol (L mol⁻¹ cm⁻¹); V is the volume of biofilm suspension filtered (mL); l is the length of the cuvette (cm). Final concentrations are given as mg g⁻¹ AFDW.

Bacterial biomass was estimated according to Frossard et al. (2012) with few modifications. Briefly, 5 mL from each periphyton suspension were added to 5 mL of phosphate-buffered formalin (2% formaldehyde, 0.2% sodium pyrophosphate, final concentrations). After an ultrasonic treatment for 3 x 20 sec (Branson Digital Sonifier 250, Germany), periphyton suspensions were centrifuged at 2000 g for 15 min in order to spin down larger algae. One mL of the supernatant containing bacterial cells was stained with 10 µL of 100 X SYBR[®] Green I to stain DNA (Promega, Switzerland) and incubated for 15 min in the dark. Fluorescent beads

(Flowcount flurospheres, Beckman Coulter, Switzerland) with a known concentration were spiked to the samples as a standard to determine the cell concentration. Samples were analysed using a Gallios flow cytometer (Beckman Coulter, Switzerland). Bacterial cell numbers were converted to bacterial biomass considering a mean bacterial biomass of 20 fg cell⁻¹ (Norland et al., 1993) and results expressed as mg g⁻¹ AFDW.

4.2. Functional endpoints

Primary algal production was measured via ¹⁴C-carbonate incorporation rate as described in Dorigo and Leboulanger (2001) with few modifications. Briefly, a 2 mL aliquot from each periphyton suspension was put into an 8 mL polyethylene scintillation vial containing 25 µL of NaH¹⁴CO₃ (2.09 GBq mmol⁻¹, Hartmann Analytic GmbH, Germany) 100 times diluted in 5 mM cold carbonate solution. The samples were incubated for 2 h at 16 °C under same lightening as the channel system to allow for photosynthesis. The reaction was stopped by adding formaldehyde (final concentration of 3.7%), followed by 100 mL of glacial acetic acid to remove the inorganic carbon. Periphyton suspensions were then dried overnight at 60 °C before adding 1 mL of DMSO and incubation for 1 h at 60 °C to dissolve the labelled organic matter. Four mL of scintillation cocktail (Ultima Gold LLT, GmbH, Germany) were added, and radioactivity was measured in a Tri-Carb 2810 TR liquid scintillation counter (PerkinElmer GmbH, Germany) with quench correction. Results are expressed as mg C g⁻¹ AFDW day⁻¹.

Secondary bacterial production was measured via ¹⁴C-leucine incorporation into protein according to Buesing and Gessner (2003) with few modifications. Briefly, 2.9 mL from each periphyton suspension were put into a 20 mL glass scintillation vial and incubated at 16 °C for 30 min with 35.5 µL of 4.5 µM ¹⁴C-leucine (12.32 GBq mmol⁻¹; Hartmann Analytic GmbH, Germany) and 64.5 µL of 2.5 mM of cold leucine. Incubations were stopped by adding trichloroacetic acid (TCA) to a final concentration of 5%. Samples were filtered using polycarbonate membrane filters (0.2 µm pore size) and consecutively washed with 1 mL of 5% TCA (twice), 40 mM cold leucine, 80% ethanol and sterile ultrapure water. After the last

washing, the filters were placed in 2 mL screw-cap microcentrifuge tubes and 500 μ L of 0.3% SDS, 75 mM EDTA and 1.5 M NaOH were consecutively added. After heating for 1 h at 90 °C to dissolve proteins and then cooling down to ambient temperature, the tubes were centrifuged for 10 min at 14.000 x g and 1 mL of the supernatant was transferred to an 8 mL polyethylene scintillation vial containing 4 mL Hionic Fluor scintillation cocktail (PerkinElmer GmbH, Germany). The radioactivity incorporated into the dissolved proteins was measured in a Tri-Carb 2810 TR liquid scintillation counter (PerkinElmer GmbH, Germany) with quench correction. Results are expressed as mg C g⁻¹ AFDW day⁻¹.

Microbial substrate-induced respiration (SIR) of the heterotrophic periphyton component was measured using the MicroRespTM technique and glucose as carbon source following the procedure described in Tlili et al. (2011). The system consists of two 96-well microplates placed face-to-face. One is a 1.2 mL deep-well microplate in which each well contains 500 μ L of the periphyton suspension and 30 μ L of D-glucose (6.2 mg of C per well, pH 7). The second microplate contains the detection gel. The two microplates were joined with a silicone seal with interconnecting holes between the corresponding wells. The assembly was clamped together and the system was incubated in the dark at 16 °C for 15 h. Absorbance of the detection gel was measured at 572 nm (Tecan Infinite 200 PRO microplate reader, Tecan Trading AG, Switzerland) immediately before sealing to the deep-well plate and after incubation. Quantities of the produced CO₂ by the microbial communities were calculated using a calibration curve of absorbance values versus CO₂ quantity measured by gas chromatography (MTI 200 thermal conductivity detector). Results were expressed as μ g CO₂ g⁻¹ AFDW day⁻¹.

5. DNA Extraction, library construction and sequencing

Two mL from each periphyton suspension were centrifuged at 14,000 x g for 30 min at 4 °C and the pellets kept at -80 °C. In order to compare between the diversity in periphyton and in wastewater, 100-mL wastewater samples were taken regularly (3 times per week) during the

experiment. The samples were also centrifuged at 14,000 x g for 30 min at 4 °C and the pellets kept at -80 °C. Nucleic acid extraction of periphyton and wastewater samples was performed using the Power-Biofilm DNA Isolation Kit (MO BIO Laboratories, CA) following the manufacturer's instructions. Total DNA was then quantified by using a Qubit (1.0) fluorimeter following the recommended protocol for the dsDNA HS Assay (Life Technologies, Carlsbad, CA, USA). Extraction negative control was also created by using an empty DNA-free tube as a starting material and proceeding to the extraction in accordance with the same protocol used for samples.

Library construction consisted in a two-step PCR process. The first PCR amplified the V3-V4 region of the 16S rRNA gene for prokaryotes and the V4-V5 region of the 18S rRNA gene for eukaryotes, using two different primer sets with overhang adapters from Herlemann et al. (2011) and Hugerth et al. (2014), respectively (SI Table 4). The initial conditions of the first PCR, including cycle number, were first determined via quantitative RT-PCR following an internal protocol developed by the Genetic Diversity Center (GDC), Zürich. A minimal cycle number was used to get enough amplicons while limiting PCR-linked bias. Bias was further limited by performing the first amplification in triplicate for each DNA sample. The PCRs were performed in 25-µL volumes with final concentrations of 1x supplied buffer (KAPA HiFi HotStart ReadyMix, Roche, Switzerland) and 0.3 µM of each forward and reverse primer (SI Table 4). In total, 1 µL of extracted DNA was added, ranging in concentration from 16.2 to 70.3 ng/µL. A negative PCR control was carried out in triplicate, by adding 1 µL of PCR grade water instead of DNA sample, as well as positive PCR controls for 16S rRNA and 18S rRNA, consisting in Mock communities (SI Table 5). The PCR program started with 95 °C for 3 min, followed by 21 cycles (16S rRNA) or 30 cycles (18S rRNA) consisting of 95 °C for 20 s, 55 °C (16S rRNA) or 56 °C (18S rRNA) for 15 s, and 72 °C for 15 s. A final extension of 72 °C for 5 min was performed and the PCR products from the three independent reactions for each sample were then pooled and cleaned. Each of the pooled reactions (a total of 75-µL) were

cleaned using a 0.8x bead:sample volume ratio of selfmade SPRI beads and separated with a magnetic stand following the protocol of the Agencourt AMPure XP Kit (Beckman Coulter). The cleaned up PCR was stored at -20 °C until further processing.

The second PCR, consisting in a limited-cycle amplification, was carried out to add multiplexing indices and Illumina sequencing adapters. Each sample was dual-indexed by using the Nextera[®] Index Kit A and D (Illumina, USA). The index PCR was performed in a 20-μL volume with final concentrations of 1x supplied buffer (KAPA HiFi HotStart ReadyMix, Roche, Switzerland) and 0.3 μM of each Nextera Index forward and reverse primer. Two mL of cleaned amplicons from the previous step were added. The PCR program started with 95 °C for 3 min, followed by 10 cycles consisting in 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. A final extension of 72 °C for 5 min was performed and the PCR products were cleaned as described above.

The DNA concentration of the cleaned and indexed libraries was then determined using a Qubit (1.0) fluorimeter following recommended protocols for the dsDNA HS Assay. The libraries were then normalized and pooled at a 1.86-nM concentration. The pooled libraries were cleaned up twice and DNA concentration determined using a Qubit (1.0) fluorimeter. Absence of adapters was checked with a HS 1000 chip on a TapeStation device (Agilent). PHiX control was added at a 1% concentration. Paired end (2 × 300 nt) sequencing was performed on an Illumina MiSeq (MiSeq Reagent kit v3, 300 cycles) at the Genomic Diversity Centre (GDC) at the ETH, Zurich, Switzerland following the manufacture's run protocols (Illumina, Inc.). The MiSeq Control Software Version 2.2 including MiSeq Reporter 2.2 was used for the primary analysis and the de-multiplexing of the raw reads.

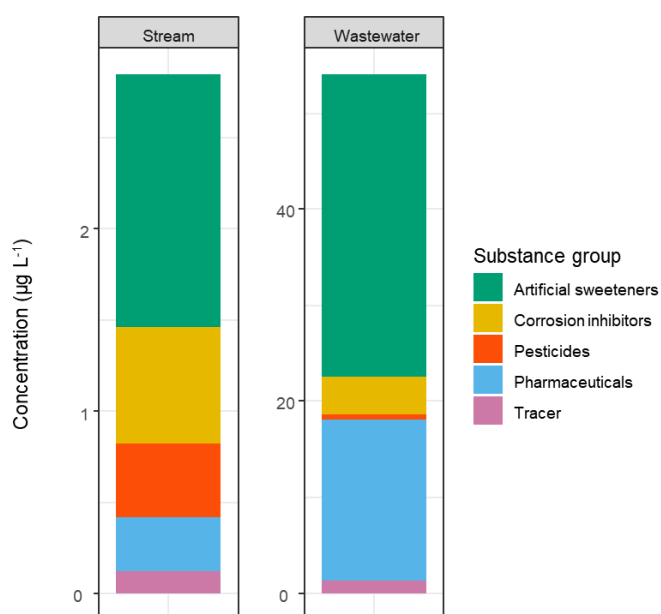
6. NCBI based 18S sequence database

See “SI_III.6.gz” file.

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406 **Supplemental figures**



407
 408 **Figure 1.** Mean concentrations of the organic micropollutants analysed in the composite
 409 samples of stream water and wastewater. Fifty-one substances were analysed in each samples:
 410 artificial sweeteners (n = 3), corrosion inhibitors (n = 2), pesticides (n=21), pharmaceuticals (n
 411 = 24) and tracer (n = 1).
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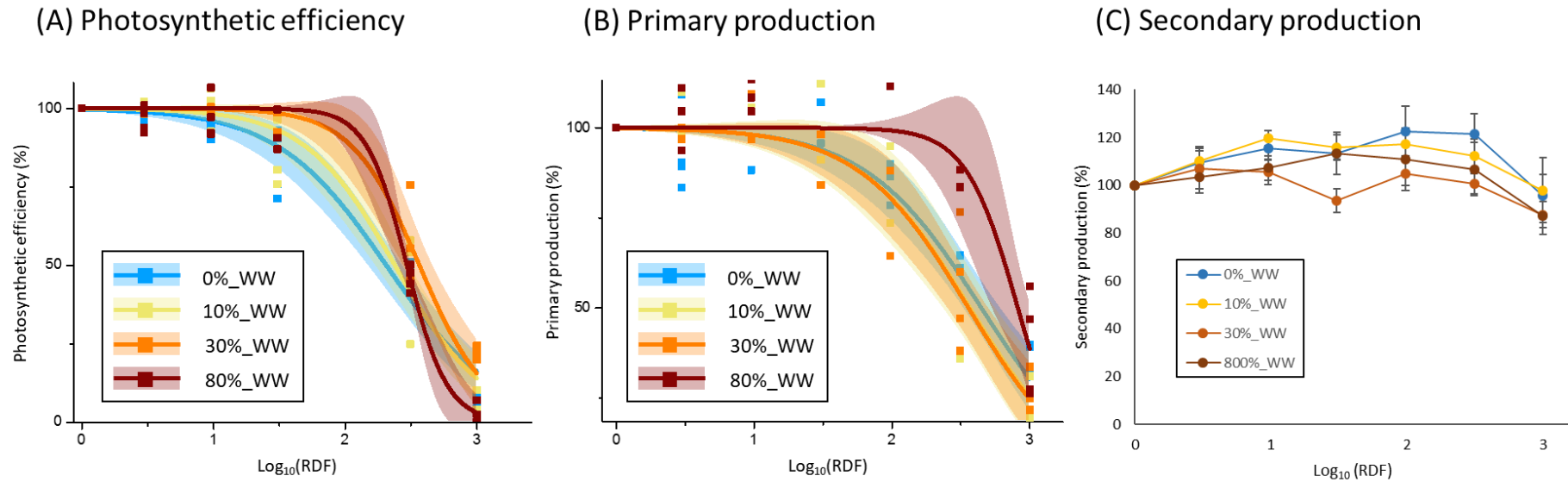


Figure 2. Concentration-effect curves of (A) photosynthetic efficiency, (B) primary production and (C) secondary production after exposure of periphyton to serial dilution of the passive sampler extract during 4 hours. The x-axis is expressed in $\text{log}_{10}(\text{relative dilution factor})$. (A) and (B): four independent replicates are represented together with fitting lines and 95% confidence bands corresponding to the dose-response function described in the Material and Methods section. (C): data are mean \pm SE (n = 4).

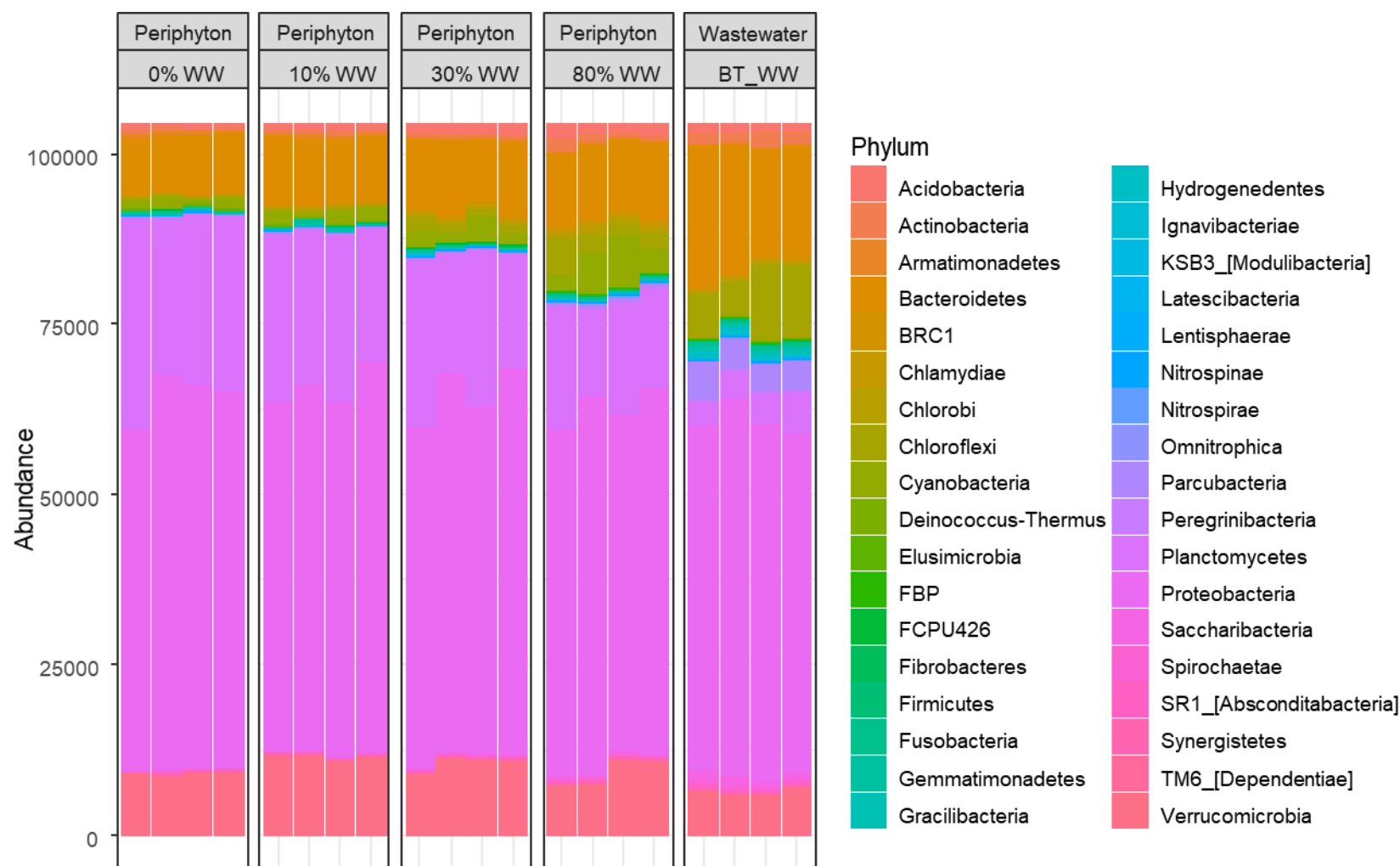


Figure 3. Prokaryotic community composition of periphyton and wastewater at the phylum level. Amplicon Sequence Variants (ASVs) abundance for each replicate (n = 4). The treatments correspond to periphyton grown in the presence of 0% (control), 10%, 30% and 80% wastewater (WW), respectively. BT_WW: community from wastewater in the buffer tank.

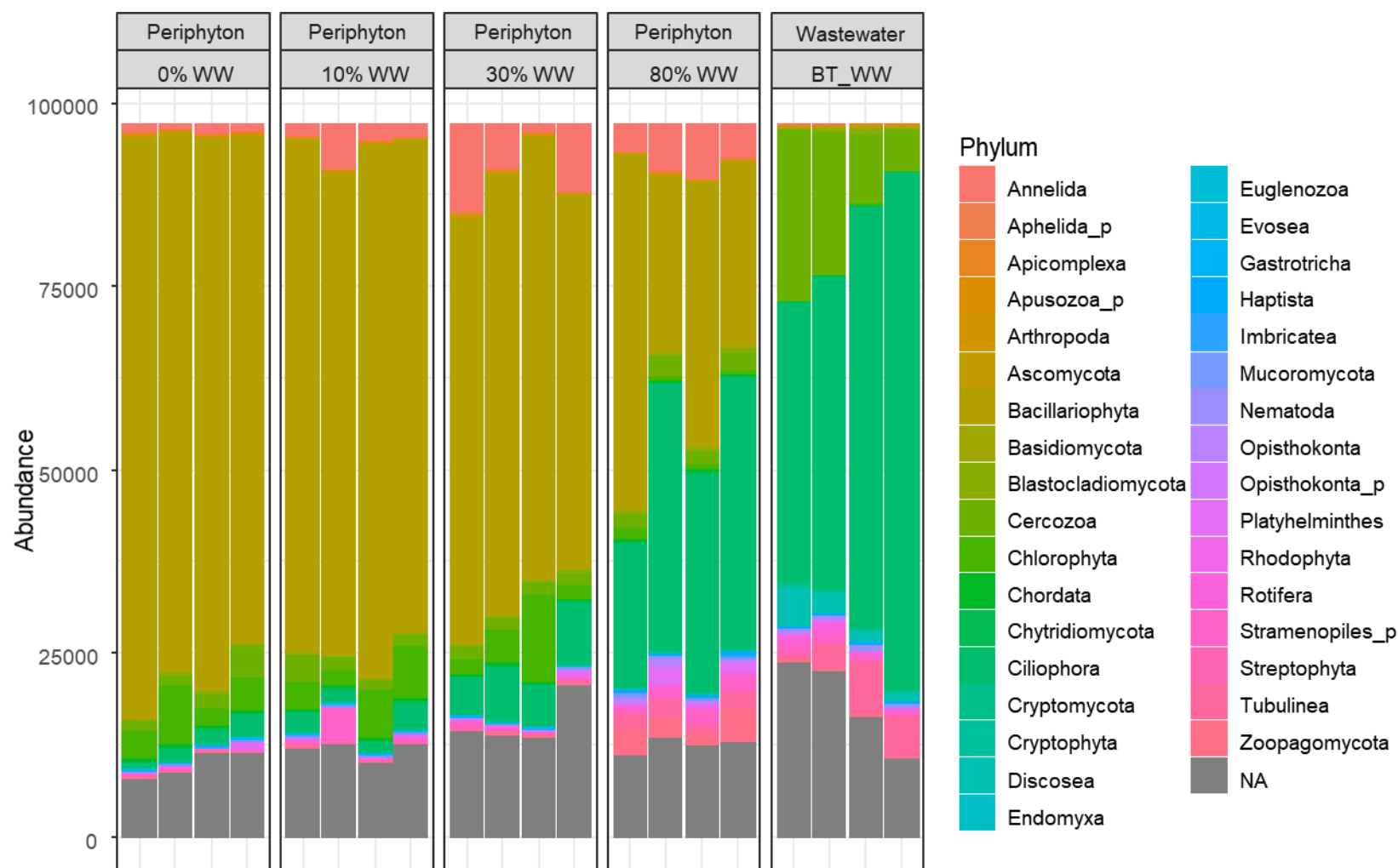


Figure 4. Eukaryotic community composition of periphyton and wastewater at the phylum level. Amplicon Sequence Variants (ASVs) abundance for each replicate (n = 4). NA: not available. The treatments correspond to periphyton grown in the presence of 0% (control), 10%, 30% and 80% wastewater, respectively. BT_WW: community from wastewater in the buffer tank.

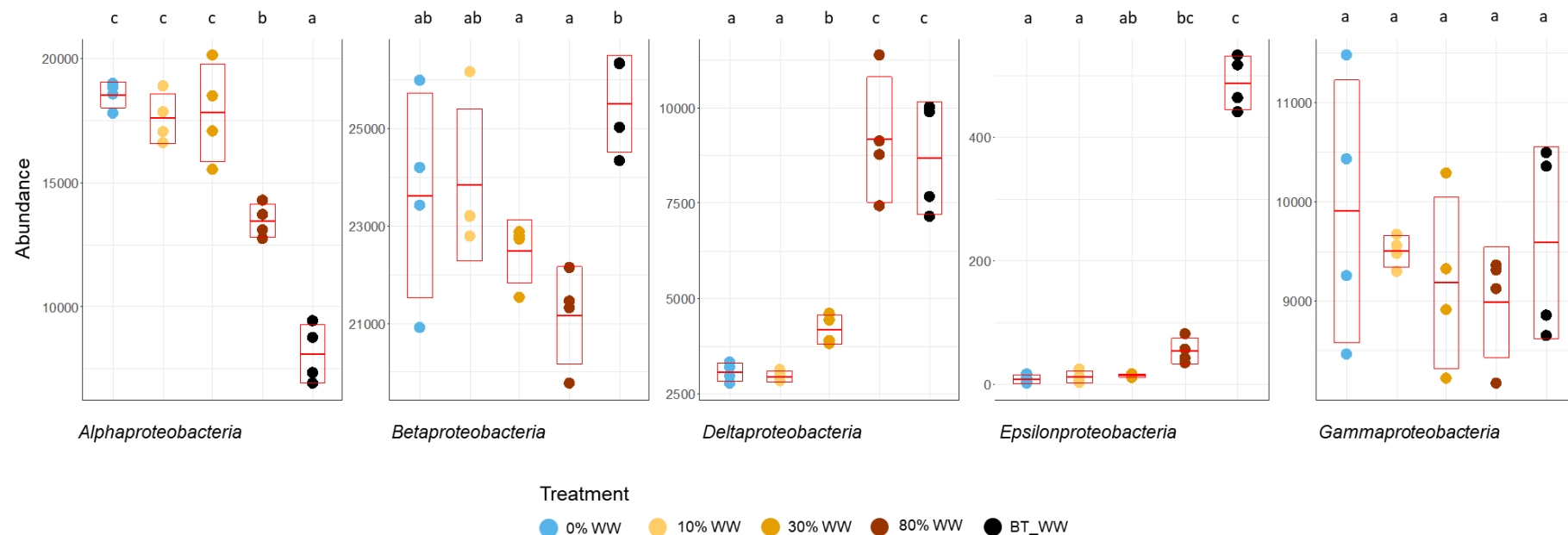


Figure 5. Abundance of the five classes of Proteobacteria in periphyton and wastewater. Amplicon Sequence Variants (ASVs) abundance for each replicate (n = 4). Significant differences are indicated by lowercase letters, $a < b < c$ (Tukey's test, $P < 0.05$). The treatments correspond to periphyton grown in the presence of 0% (control), 10%, 30% and 80% wastewater (WW), respectively. BT_WW: community from wastewater in the buffer tank. Horizontal lines in the red boxes correspond to the average values and the lower and higher limits of the standard deviation.

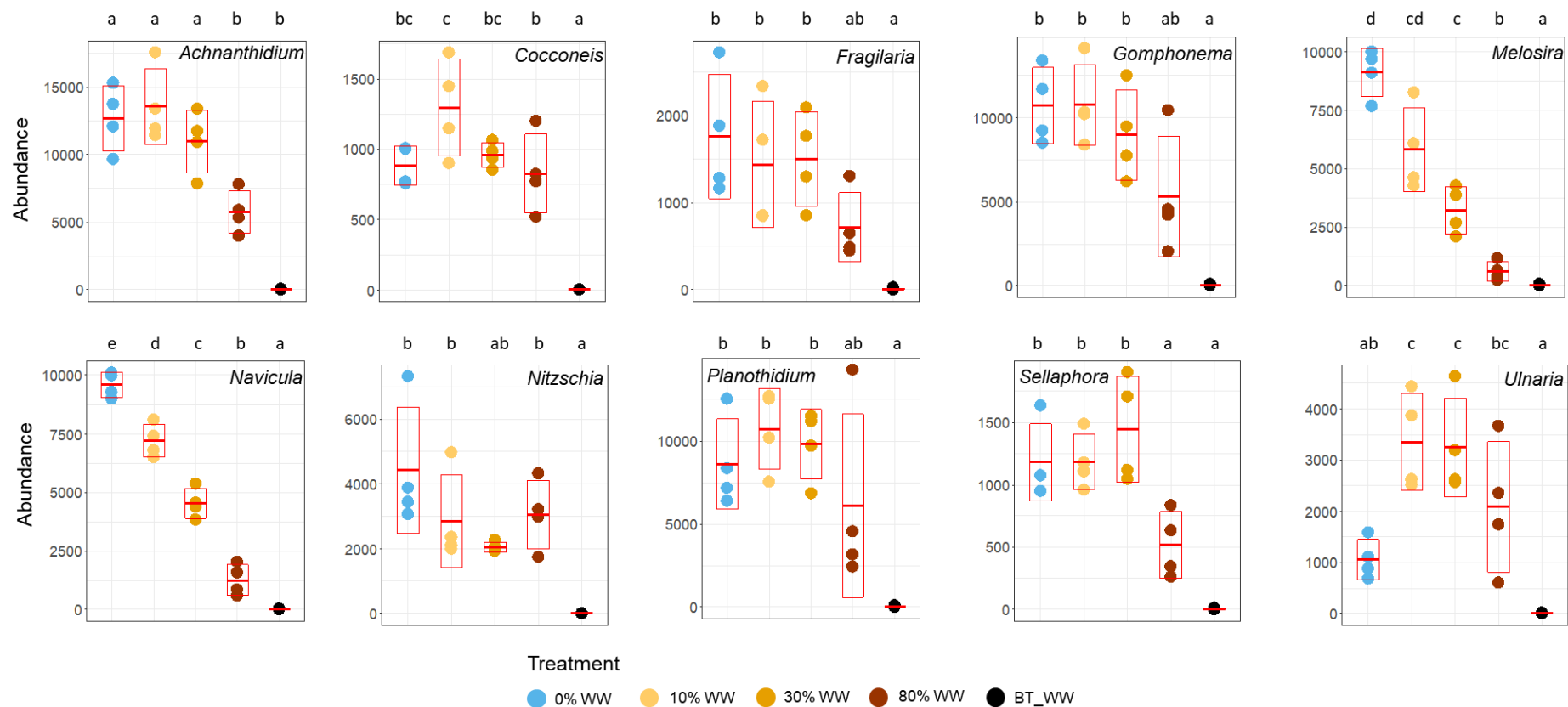


Figure 6. Abundance of the top-ten Bacillariophyta genera in periphyton and wastewater. Amplicon Sequence Variants (ASVs) abundance for each replicate (n = 4). Significant differences are indicated by lowercase letters, a < b < c < d < e (Tukey's test, $P < 0.05$). The treatments correspond to periphyton grown in the presence of 0% (control), 10%, 30% and 80% wastewater (WW), respectively. BT_WW: community from wastewater in the buffer tank. Horizontal lines in the red boxes correspond to the average values and the lower and higher limits of the standard deviation.

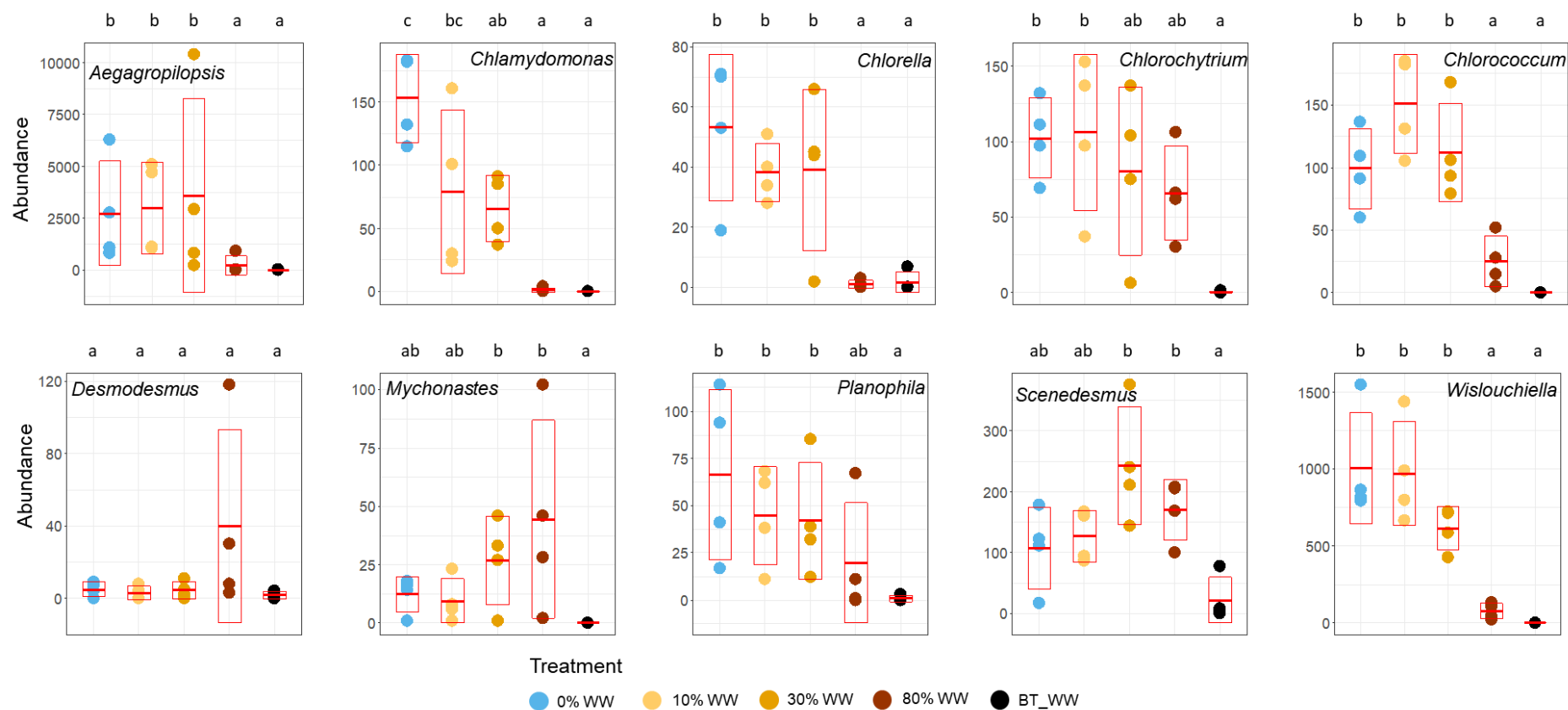
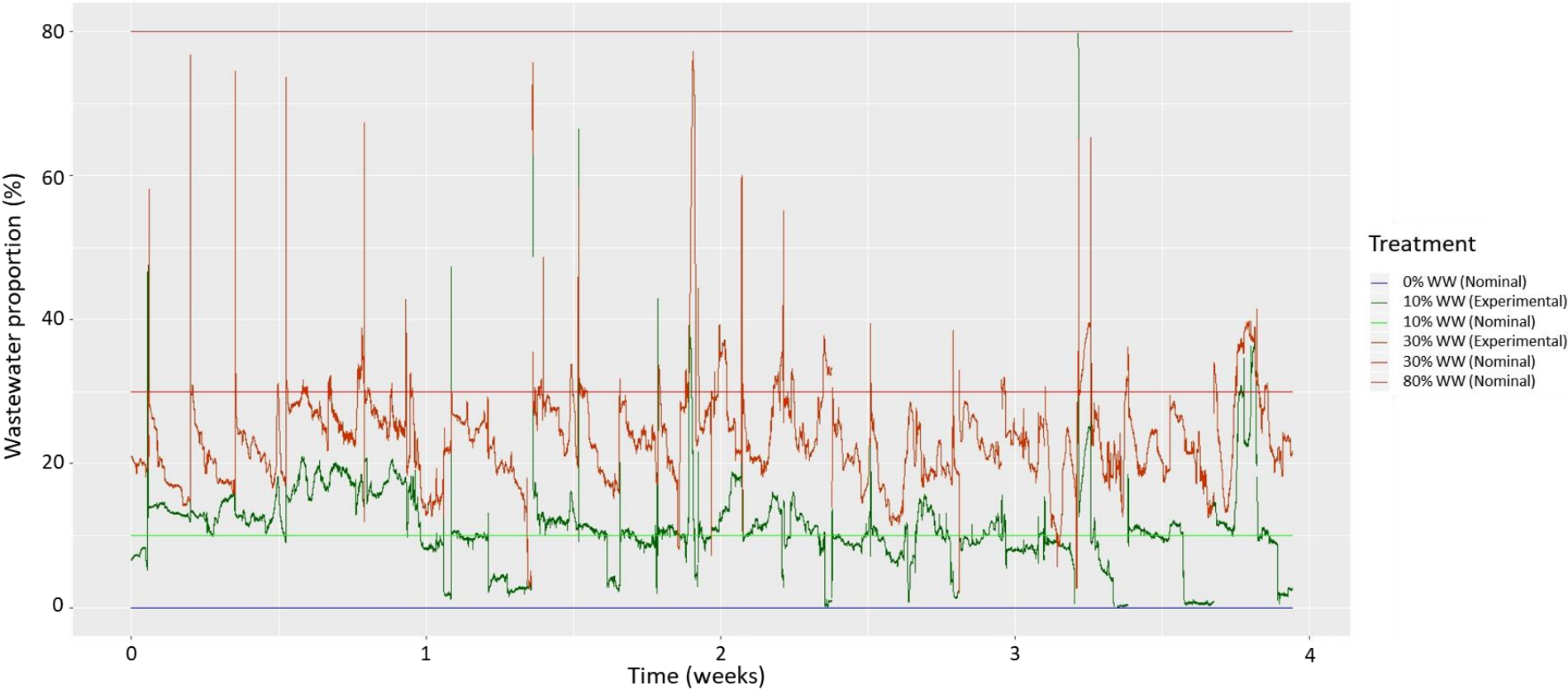


Figure 7. Abundance of the top-ten Chlorophyta genera in periphyton and wastewater. Amplicon Sequence Variants (ASVs) abundance for each replicate (n = 4). Significant differences are indicated by lowercase letters, $a < b < c$ (Tukey's test, $P < 0.05$). The treatments correspond to periphyton grown in the presence of 0% (control), 10%, 30% and 80% wastewater (WW), respectively. BT_WW: community from wastewater in the buffer tank. Horizontal lines in the red boxes correspond to the average values and the lower and higher limits of the standard deviation.

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449

450 **Figure 8.** Nominal and experimental wastewater proportions in the channels based on conductivity measurements. Conductivity was continuously
451 measured in each mixing unit with HOBO[®] conductivity-loggers. The experimental wastewater proportions for the nominal 10 and 30% wastewater
452 proportions were calculated by using 80% wastewater as reference. The treatments correspond to 0% (control), 10%, 30% and 80% wastewater (WW),
453 respectively.

454 **Supplemental tables**

455 See “SI_Tables.xlsx” file.