# New Microprofiling and Micro Sampling System for Water Saturated Environmental Boundary Layers

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S Supporting Information

ABSTRACT: The spatial high resolution of a microprofiling system was combined with the multi element capability of ICP-MS to enable a better understanding of element distributions and related processes across environmental boundary layers. A combination of a microprofiling system with a new micro filtration probe head connected to a pump and a fraction collector (*microprofiling and micro sampling system, missy*) is presented. This enables for the first time a direct, dynamic, and high resolution automatic sampling of small water volumes (<500 μL) from depth profiles of water saturated matrices (e.g., sediments, soils, biofilms). Different membrane cut-offs are available, and resolutions of a few (matrices with a high physical resistance) to a submillimeter scale (matrices with low physical resistance) can be achieved. In this Article, (i) the modular setups of two *missy*s are presented; (ii) it is demonstrated how the micro probe heads are manufactured; (iii) background concentrations and recoveries of the system as well as (iv) exemplary results of a sediment water interface are delivered. On the basis of this, potentials, possible sources of errors, and future applications of the new *missy* are discussed.
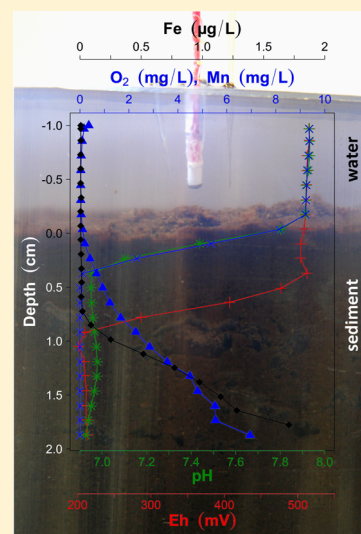
INTRODUCTION

Understanding biogeochemical processes at the boundary layer of sediment water interfaces (SWI) requires the investigation of physicochemical parameters (like the O_2 concentration, redox potential, or pH value) in parallel to the distribution of different analytes (e.g., trace metals, nutrients, or organic compounds) in the pore water at a spatial high resolution. Due to the development of different measurement and sampling techniques, the possibilities to study these heterogeneous and dynamic environments were improved considerably during the last decades.[1] In particular, the availability of numerous microsensors and -electrodes (e.g., O_2, redox potential, pH value, H_2S, N_2O) enables the analysis of several parameters/analytes at a high resolution and provides a better understanding of processes at boundary layers and micro niches of the SWI or biofilms.[2,3] Apart from that, the analysis of chemical species at the SWI is performed on the basis of pore water samples.[1] Commonly applied sampling techniques can be categorized[4,5] as either *ex situ* methods, like centrifugation or squeezing,[6,7] or *in situ* methods like dialysis[8,9] (including diffusive gradients/equilibration in thin film (DGT/DET) or peepers)[3−5,10] and suction-based techniques.[11−13] Drawbacks of these approaches are, on the one hand, the requirement of either the installation of the sampling devices at the sampling site and/or elevated sample preparation procedures (e.g., slicing, centrifugation, or re-elution from/digestion of accumulation gels) that may influence the environmental conditions at the area studied and/or the characteristics of the samples. On the other hand, only few techniques allow for a spatial resolution at a millimeter or submillimeter scale and hence do not capture fine-scale differences of heterogeneous (micro)-environments even though some DGT-based methods are reported that enable a high resolution sampling.[1,10,14,15]

The investigation of trace metal distributions at the SWI, particularly in relation to different physicochemical parameters, is important to gain a holistic understanding of biogeochemical processes determining the mobility and transformation of chemical species and to assess potential risks arising from contaminated sediments (of, e.g., industrial,[8] urban,[14,16] or mining areas,[6] deltas[17] or fish farms[18]). One of the most common and powerful analytical techniques in environmental analytical chemistry of metals and metalloids (metal(loid)s) is inductively coupled plasma-mass spectrometry (ICP-MS). Multi element capabilities combined with low detection limits led to a widespread dissemination of ICP-MS systems within the last decades.

To combine the analytical benefits of ICP-MS analyses with the spatial information on microprofiling, a *microprofiling and micro sampling (filtration) system (missy)* was developed. Two
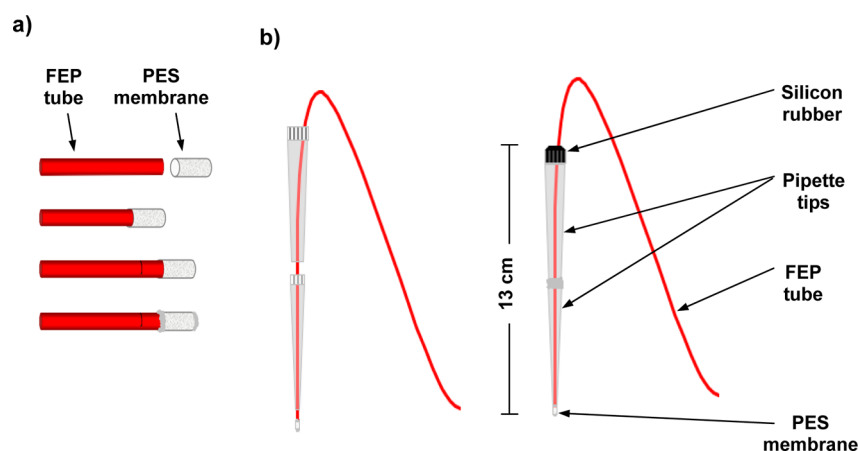
Figure 1. Schematic drawing of the manufacturing of a micro sampling probe. (a) Connecting the tube with the PES membrane and sealing of the membrane. (b) Connection of two pipet tips and fixing of the tube by silicon rubber (a detailed description is given in the text).

different setups of a *missy* were tested for background concentrations and recoveries and their applicability to investigate metal(loid) distributions at the SWI at a spatial high resolution. Therefore, proof-of-principle experiments were conducted, studying the distributions of five exemplary chosen metal(loid)s along a transect of a sediment core in correlation to the redox potential, O_2 concentration, and pH value. With the aim to enable other scientists to create and install their own *missy* setup or to adapt the ones presented to their scientific questions/needs, detailed information on the system and on potential applications is delivered. On the basis of the results of the first applications, the potentials and possible sources of error and suggestions for improvements of the system are discussed.

EXPERIMENTAL SECTION

Terms and Definitions. Since some of the terms addressed in this Article are not sufficiently defined or have several meanings, a short overview of basic terms/concepts, focusing on environmental analytical sciences and sampling of liquids, is given.

The term micro sampling (also micro-sampling) is used either for samples <1 mg (e.g., in laser ablation[19]) or <1 mL or for sampling with low flow rates in the μL/min range (e.g., for ICP-MS analyses[20]). In this study, micro sampling addresses a volume <1 mL per sampling point as well as sampling with a flow rate of 5 μL/min.

Microprofiling (also micro-profiling or micro profiling) is most commonly used to describe studies performed with microsensors/-electrodes, e.g., redox potential, N_2O, oxygen, or multi analyte arrays.[21] For high resolution depth profiling (on, e.g., sediments or biofilms), experiments are often conducted using laboratory stands equipped with micromanipulators enabling a precise operation of the microsensors.[22,23]

For filtration approaches, different devices are applied like rhizon samplers[11,24] or comparable probes[12,13] with membranes made of, e.g., poly(ether sulfone) (PES), with different cut-offs (e.g., 0.1 or 0.45 μm). Beside this, micro dialysis can be applied to quantify metal(loid)s in liquids. However, most of the micro dialysis studies address in vivo metabolization[25] and only few with an environmental focus are available (e.g., Torto and Mogopodi[26]). For the experiments conducted in this study, filtration was found to be more suitable than micro dialysis, because the latter can be hampered by, e.g., uncertainties in

dialyzing efficiency with changing media compositions or by challenges in calibration.[25]

Chemicals and Materials. Ultrapure water was produced using an USF ELGA Purelab Plus system (ELGA LabWater, Germany). ICP-element standards (1 g/L) and nitric acid (HNO_3, 65% w/w, for analysis) were purchased from Merck GmbH (Germany). The acid was sub-boiled using a dst-1000 (Savillex, USA). Prior to use, all vessels and the 96-microwell plates (Riplate RW, 1 mL, PP, Ritter medical, Germany) were rinsed >24 h with HNO_3 (1.3%). All tubes and connectors used were made of fluorinated ethylene propylene (FEP) or from polyether ether ketone (PEEK). The sediment examined in the experiments was a mixture of 80% sieved (20−63 μm) and freeze-dried natural freshwater sediment (information on the general characteristics of the sediment is given in Tables SI1−SI3, Supporting Information) and of 20% sand (Spielsand, Silex GmbH, Germany) to produce an idealized, homogeneous sediment core. The freshwater sediment was sampled in 2012 at the river Lahn (stream km 136, water gate Lahnstein, Germany, 50°18′29.47″ N, 7°36′46.24″ O). After homogenizing the mixture, the sediment was placed in an aquarium (glass tank, 20 × 15 × 20 cm) filled with demineralized water (electric conductivity ~0.5 μS/cm; demineralization system by Grünbeck Wasseraufbereitung GmbH, Germany) and left untreated for 9 weeks to allow the gradients at the SWI to develop. If required, evaporated water was refilled.

Sample Probes. The manufacturing of the probes used within the experiments is presented in Figure 1. They consisted of a PES porous hollow fiber (0.45 μm) head connected to a tube which was stabilized by pipet tips. However, also other materials and cut-offs are available and can be used for probe manufacturing. To manufacture a probe, the hollow fiber was cut in 3 mm pieces using a scalpel. A piece of ~25 cm of (FEP) tube (inner diameter 0.125 mm Upchurch, USA), with an outer diameter that fits tightly into the fiber, was inserted fully in the fiber piece (refer to Figure 1a).

Using a binocular (SZB 300, VWR, Germany), the FEP tubing was marked at the end of the fiber piece by means of a scalpel. Subsequently, the tubing was removed (2 mm) using the scalpel scratch as the mark, resulting in an overlap of the tube and the fiber piece of ~1 mm. The distances were measured using an objective micrometer. The PES piece and the FEP tube were connected using molten (by means of a lighter) polypropylene (PP) pipet tips (Finntip, Thermo
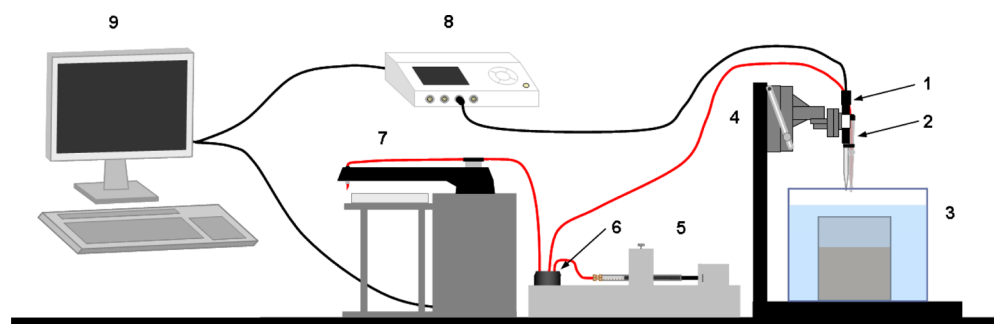
Figure 2. Schematic drawing of the *missy* setup 1. 1, microelectrode; 2, sample probe; 3, aquarium with sediment core; 4, laboratory stand with two profiling motors (*X*- and *Z*-axis); 5, syringe pump; 6, Rheodyne valve; 7, fraction collector: positioning system with a microwell plate; 8, microsensor multimeter; 9, PC.

Scientific, Germany). After sealing the open end of the piece of hollow fiber with molten PP, the probe head was tested for leak tightness of the connection between tube and fiber as well as of the end of the fiber. Therefore, a syringe (Hamilton, Switzerland, Gastight #1750) filled with water was connected to the tube via a Finger Tight Fitting (PEEK, F-120X) and a Luer adapter (PEEK, P-659, both Upchurch, USA) and water was pressed extremely carefully through the PES membrane to avoid ruptures. After the probe head was found to be leak proof (water penetrates only through the membrane, visible under the binocular), the tube was inserted into two connected pipet tips (Finntip 1000, Thermo Scientific, Germany; Figure 1b) and fixed with molten PP as described above. Finally, the FEP tube was fixed at the pipet tip using a self-setting silicone rubber (Sugru, FormFormForm Limited, United Kingdom) to stabilize the probe.

Missy Setups. Two different *missy* setups were tested; both combining a microprofiling with a micro sampling system. In the case of the first setup (*missy* setup 1, presented in Figure 2), the micro sampling system consists of a low pressure high precision syringe pump (neMESYS), a Rheodyne valve (Qmix EX), and a miniature positioning system (rotAXYS, all three Cetoni, Germany). In the alternative setup (*missy* setup 2), the syringe pump and the valve were replaced by a micro annular gear pump (mzr-2542) connected to a console drive module (mzr-S06, both HNP Microsystems, Germany). By synchronizing the software of the profiling (SensorTrace PRO, Unisense, Denmark) and the sampling system (QmixElements, Cetoni GmbH, Germany), it was possible to measure profiles of oxygen and redox potential or pH value in parallel to the sampling of water and pore water.

Microprofiling System. Measurements of the oxygen concentration, the redox potential, and the pH value were conducted by means of a microprofiling system equipped with a computer controlled motorized micromanipulator (Unisense, Denmark). Oxygen was measured using a Clark-type O_2 microsensor (OX-100, Unisense Denmark), profiles of redox potential and pH value were obtained by application of microelectrodes (standard hydrogen potential; RD-100, pH-100 glass electrode, Unisense, Denmark) connected to an Ag/AgCl-reference electrode (REF321, Radiometer Analytical, Denmark). To facilitate the reading, the term "redox potential" is used for the potential measured by the platinum electrode even though this represents the operationally determined value and not the thermodynamic potential. Sensors/electrodes were connected to a microsensor multimeter that transferred the data to the mircroprofiling software (SensorTrace PRO). The pH/redox microelectrode and the O_2 microsensor were

calibrated previous to the experiments in accordance to the instructions given in the manuals.

System Characterization: Background Concentrations and Recoveries. Prior to the experiments, the metal(loid) background concentrations of the sampling system were determined for ultrapure water and the experiment matrix (aquarium water). The latter was taken as a reference for the profiling experiments and was additionally compared to samples filtered by syringe filters (Minisart NML Syringe Filters 16555 K, surfactant-free cellulose acetate, pore size 0.45 μm, Sartorius, Germany). Analyses of ultrapure water were conducted after cleaning the *missy* and after the profiling experiments. Moreover, the recoveries for the two systems were determined in ultrapure water and nitric acid (1.3%). A detailed description and discussion of the background and recovery experiments are given in the Supporting Information (page 4 et. seq). All values presented were determined on the basis of ten, in the case of the syringe filter approach five, replicates and were tested for outliers (see the section Data Analyses).

Micro Sampling System. The sediment profiles of pore water, exemplary presented, were obtained by application of *missy* setup 1. Therefore, an intended volume of 500 μL was sampled with a flow rate of 5 μL/min and transferred (25 μL/min) to the well plate on the positioning system. To reduce evaporation of the samples, the well plate was covered by a self-adhesive foil (adhesive polyethylene film for ELISA incubation, nonsterile, VWR, Germany). In agreement with the general convention, the elemental concentrations of the samples, filtered by the PES membrane (cutoff <0.45 μm) of the probe, were defined as "dissolved", even though also colloids can pass the pores. At the beginning of each experiment, all parts of the sampling system were filled with aquarium water. The volume of each sample (depending on the gas to water ratio at the sampling point ~50−450 μL) was determined by pipetting the sample from the well plate into centrifugal tubes (15 mL, PP, VWR, Germany). After diluting the samples to 3−4 mL using 1.3% HNO_3, the metal(loid) concentrations were determined by means of ICP-MS. Setup 2 including the micro annular gear pump was only tested for general comparability.

Profiling Experiments. In total, a transect of five profiles was measured from 1 cm above the sediment surface to 2 cm within the sediment. The sediment surface (0 cm) of the first profile was identified by the help of the O_2 electrode (beginning of the O_2 decline) and verified by an USB microscope (DNT DigiMicro 2.0, Germany). To map the microstructures of the sediment surface and to provide the comparability between the profiles, the surface setting was also kept for the following profiles. The profiles were approximately

Table 1. Background Concentrations (μg/L) of the Microwell Plates and the Sampling Systems of the Two *Missy* Setups (without the Microwell Plate) Determined for Aquarium Water[a]

aquarium water	Mn	Fe	Co	Zn	Sb
direct sampling	1.16 ± 0.01	1.97 ± 0.16	0.14 ± 0.01	67.67 ± 1.05	14.86 ± 0.13
well plate	3.44 ± 0.29	5.87 ± 1.82	0.18 ± 0.01	60.89 ± 4.02	16.15 ± 0.20
missy setup 1	1.61 ± 0.26	6.37 ± 3.14	0.17 ± 0.01	70.71 ± 6.03	15.85 ± 0.43
missy setup 2	1.81 ± 0.23	5.44 ± 2.39	0.16 ± 0.01	61.19 ± 9.49	14.43 ± 0.24

[a]Values given represent the mean concentrations of ten replicates and the corresponding confidence interval (CI; $\alpha = 0.05$) after testing for outliers.

Table 2. Background Concentrations (μg/L) of the Microwell Plates and the Sampling Systems of the Two *Missy* Setups (Without the Microwell Plate) Determined for Ultrapure Water[a]

ultrapure water	Mn	Fe	Co	Zn	Sb
well plate	0.26 ± 0.03	1.85 ± 0.79	<0.01	2.41 ± 0.50	<0.17
missy setup 1					
before	1.46 ± 0.88	0.87 ± 0.69	<0.01	8.37 ± 2.35	<0.17
after	17.90 ± 2.96	3.83 ± 1.08	0.12 ± 0.05	19.49 ± 2.86	<0.17
missy setup 2					
before	1.90 ± 0.38	13.28 ± 3.89	0.05 ± 0.01	16.07 ± 9.43	<0.17
after	0.42 ± 0.08	3.29 ± 1.07	<0.01	28.80 ± 27.03	<0.17

[a]In case of the sampling systems, background concentrations were analyzed before and after the profiling experiments. Values given represent the mean concentrations of ten replicates and the corresponding confidence interval (CI; $\alpha = 0.05$) after testing for outliers.

0.5 cm separated from each other (manually positioned with the micromanipulator). Per profile, 480 data points were measured for O_2 concentration, pH, and redox potential (step size of 63 μm) and 24 water samples were taken, each sampled over a distance of 1.1 mm. The time required per profile was 44 h (Table SI5, Supporting Information). To avoid influences of the sampling procedure on the sediment parameters measured, the electrodes/sensors and the sample probe were fixed in such a manner that the tips were at the same height. The difference between the measurements and sampling depths was considered in the depth correction after the experiments. To avoid biases of the results by a potential carryover from the last sample of a profile (2 cm in the sediment) to the first samples of the following profile (overlying water; see also below), two dummy samples were inserted (first two samples) in each profile. Further details on the measurement settings of the two systems are given in the Supporting Information (Tables SI5 and SI6). In order to test the comparability of different sample probes, a new probe was used after three profiles (refer to Figure 3c−g). Since for some elements a carryover from the last sample of a profile (e.g., very high concentrations of Mn) to the first samples of the following profile (e.g., very low Mn concentrations in the overlaying water) was observed, an additional experiment was conducted to quantify the maximum amount of elements potentially carried over. Therefore, after a profiling experiment was finished, seven additional samples were taken from the water phase (1 cm above the sediment surface). Sample preparation and measurements were carried out as described (see the section on the Micro Sampling System). Since only a sensor for redox potential or pH measurements can be run, three profiles of the pH value were measured after the experiments described above. To avoid an unfavorable stratification of the water column, the overlying water was slightly disturbed by a soft air stream on the surface, generated by an aquarium pump (MARINA 50, Model 11110, Germany) connected to a pipet tip.

ICP-QMS-Analyses. The metal(loid) concentrations of the pore water samples were determined by means of ICP-MS (Agilent 7700 series), equipped with a PFA-ST Micro Flow nebulizer (ES − 2040) and a PFA inert sample introduction kit with a sapphire injector (inner diameter 2.5 mm, all Agilent Technologies, Germany). Measurements were conducted at a RF power of 1550 W and carrier and dilution gas flows of 0.95 and 0.1 L/min, respectively. Details on the isotopes analyzed and the measurement modes as well as the certified reference materials (CRMs) used are given in the Supporting Information (Table SI7). External calibration was conducted using multielement standard solutions.

Data Analyses. Data analyses (calculations and statistical tests) and plotting of the profiles were performed using R (version 3.0.2; 2013-09-25);[27] for 2D-plots, the R package "gplots"[28] was applied. The R-package "outliers"[29] was used to test for outliers within the replicates of the blank and recovery experiments. Values were excluded if identified as outliers (p-value of <0.01) by the results of two tests (Grubb's and a Dixon-Test[30]).

To correlate the element concentrations of the pore water samples with the microprofiling parameters (O_2, redox potential, and pH), the dead volume of the sampling system was considered (Table SI8, Supporting Information) causing a shift of the element profiles upward in relation to the parameters measured by electrodes/sensors (0.3 and 0.2 mm for *missy* setups 1 and 2, respectively; Table SI9, Supporting Information).

RESULTS AND DISCUSSION

System Characterization: Background Concentrations and Recoveries. The background concentrations determined for the aquarium water by the different sampling approaches were comparable and were clearly above the values found for ultrapure water (Tables 1 and 2). Hence, an influence of the backgrounds of the *missy* on the results of the profiling experiments can be excluded.

Nevertheless, the comparison of the analyses of ultrapure water before and after the profiling experiments as well as the results of the recovery experiments (Table SI4, Supporting Information) indicate potential sources of error that may bias the results if trace metal analyses (ng/L to μg/L) are required:
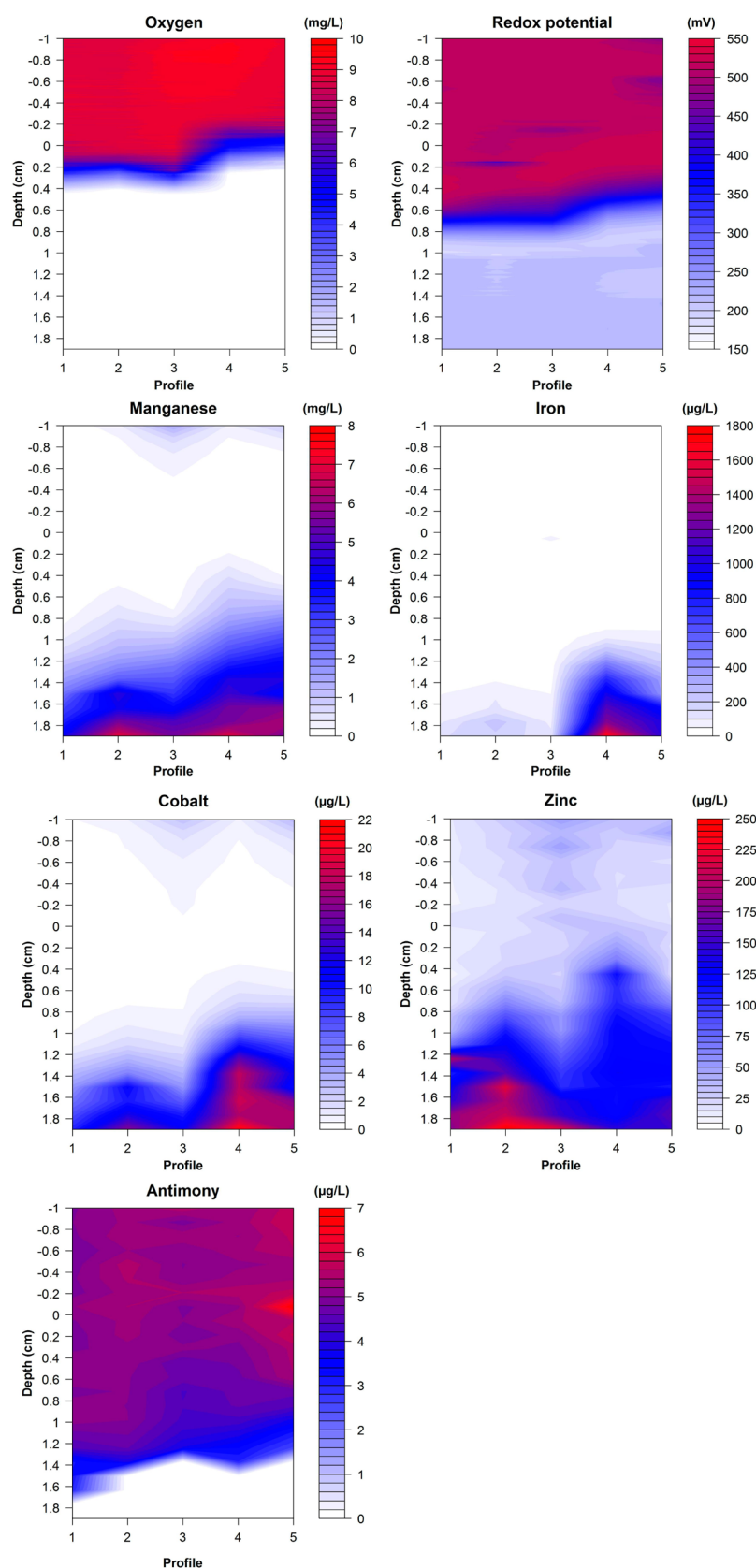
Figure 3. 2D-plots of five profiles of oxygen concentration and redox potential as well as the element concentrations of Mn, Fe, Co, Zn, and Sb of the freshwater sediment (1 cm above to 2 cm in the sediment). The color gradient (white equals minimum, blue is medium, and red is maximum values) refers to the element/oxygen concentration and the values of the redox potential, respectively. The labels of the x-axis represent the sampling points at a distance of ∼0.5 cm from each other.

in case of *missy* setup 1, precipitation of metal(oid)s during sampling under atmospheric conditions caused elevated background concentrations in experiments with ultrapure water after the profiling experiments (Table 2). Due to the small dimensions of the *missy* components, this known challenge of reoxidation during sampling[4,5] can be overcome by placing the devices in a glovebag or glovebox containing an inert, oxygen free atmosphere. Since the pore water has only to be acidified prior to the ICP-MS measurements, the sample preparation can be conducted in a glovebox or bag. A detailed discussion of potential sources of error that should be considered if trace analyses are required is given in the Supporting Information (pages 4 et seq).

Sediment Pore Water Profiles. The results of the oxygen concentration and redox potential as well as the concentrations of Mn, Fe, Co, Zn, and Sb determined along a transect of the sediment core are given in Figure 3. Measurements (O_2 and redox potential) were conducted in parallel to the sampling from 1 cm above to 2 cm below the sediment surface. Since the settings for the vertical position of the micromanipulator were not changed, the SWI varies along the transect displaying the surface micro relief. For a better orientation, the depth scale is given at the *y*-axis ignoring the micro relief of the sediment surface. In addition to the 2D-plots, single profiles of the three parameters (O_2, redox, and pH) are presented in comparison to the concentrations of the major redox-sensitive elements Mn and Fe in Figure 4. The element concentrations of all pore water samples are given in the Supporting Information (Tables SI10–SI14). Individual profiles of the five replicates of the O_2 concentration and redox potential as well as the three replicates of the pH value are given in Figure SI1, Supporting Information. For exemplary results obtained by the second *missy* setup, refer to Figure SI2, Supporting Information.

Oxygen Profiles. The O_2 concentration of the overlaying water was 8.7–9.3 mg O_2/L and decreased rapidly below the limit of detection (0.01 mg O_2/L) within the first millimeter of the sediment. The shape of the single O_2 profiles (Figure SI1, Supporting Information), with high concentration in the water phase and a rapid depletion within the upper ~2−4 mm of the sediment, is characteristic for shallow, stirred waters, where the introduction of oxygen is only driven by diffusion processes.[2] Thicker oxic zones can only be found in the presence of photosynthetic bacteria, intense water movement (e.g., currents, waves), or bioturbation.[2] Moreover, the depletion is enhanced by microbial O_2 consumption during aerobic degradation of organic material and a limited O_2 flux into the sediment.[2] The latter, caused by the diffusive boundary layer above the sediment surface, acts as a barrier for dissolved molecules.[31,32] The results of the third profile indicate a small hole/step in the sediment where oxygen could penetrate deeper into the sediment.

pH Profiles. The decline of the pH value (Figure 4) can be related to (aerobic) respiration processes leading to a release of CO_2 and an increase of the proton concentration.[2,3,33] A slight minimum of the pH was observed at the beginning of the anoxic region probably indicating the O_2/H_2S-interface where H_2S, diffusing from deeper sediment layers, was oxidized to sulfuric acid.[2,3] Besides this, the pH can also be influenced by different processes at the surfaces present (of, e.g., oxides, carbonates, silicates, sulfides, or phosphates).[2,34]

Redox Profiles. In good agreement to the O_2 and pH profiles, a decrease of the redox potential from the water phase (~450 mV) to the lower regions of the sediment (~200 mV)
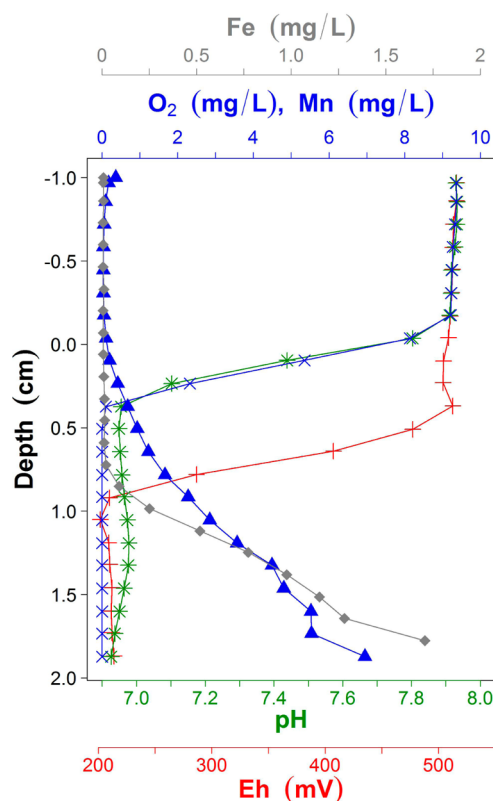

Figure 4. Profiles of oxygen (blue ×), redox potential (red +), and pH value (green *) in comparison to the concentrations of dissolved Mn (blue ▲) and Fe (gray ◆). Measurements of the pH value and the O_2 concentration were conducted in parallel. Profiles of the redox potential and the element concentrations represent the results of the fourth profile. The distance between the data points represents the mean values for the sampling depth (movement of the micro manipulation system during sampling, 1.1 mm) of the respective sample (refer to Table SI5, Supporting Information).

was detected, that was located approximately 1 mm deeper in the sediment (refer to Figures 3 and 4). This decline can be related to the reduced availability of oxygen as electron acceptor in the anoxic layers of the sediment.[2,33] However, the redox potential can also be influenced by other components and processes (e.g., oxidizing capacities of Fe and Mn (oxyhydr)oxides, sulfides, nitrate, or microbial activities)[2,33,34] not further addressed in this study.

Metal(loid) Profiles. For the distributions of dissolved Mn, Fe, Co, and Zn in the pore water, an increase within the deeper, anoxic regions of the sediment with a low redox potential was visible. In contrast to this, dissolved Sb was detected in the overlaying water and the pore water samples of the upper sediment layers but not in the deeper areas. The results of Mn, Fe, Co, and Zn are in agreement with the theory on redox-dependent processes of sediment-water systems and can be explained by the reduction of Mn and Fe (oxyhydr)oxides and a resulting release of Mn^{2+} and Fe^{2+} as well as associated trace metals (like Co and Zn).[34−36] Comparing the pore water concentrations of Mn and Fe, a thicker layer of dissolved Mn was detected, explicable by its reduction at higher redox potentials and a slower (re)oxidation in comparison to Fe.[16,35,36] Thereby, in contrast to Fe^{2+} only released in deeper layers of the sediment, Mn^{2+} can diffuse to regions near the sediment surface (Figure 3). The similarity of the distribution of Co and Mn confirms results of other studies that have shown

a preferential association of Co with Mn-oxides, rather than with Fe-oxides.[17,35] In the case of dissolved Zn, the results demonstrate that also nonredox sensitive elements[16,35] can be (indirectly) related to the redox gradient due to the sorption to Mn- or Fe-(oxyhydr)oxides.[17,35] However, the elevated concentrations of Mn, Co, and Zn in the overlaying water of the third and fifth profile indicate a carryover from the previous experiments in the case of continuously used sample probes with only a short rinsing between two profiles (by excluding the two first samples). This is confirmed by the results of the additional experiment, carried out to quantify the amount of analytes that might (at a maximum) be transferred from the last sample of one profile to the first samples of the next (Table SI15, Supporting Information).

In comparison to the other elements, Sb was inversely distributed, with higher concentrations in the overlaying water and the oxic sediment layers and a rapid decrease below the limit of detection in the deeper anoxic areas. This confirms thermodynamic calculations, predicting the prevalent speciation of Sb as the soluble $Sb(OH)_6^-$ at oxic conditions and a limited availability of dissolved species by $Sb(OH)_3$ formation under anoxic conditions.[37,38] Moreover, studies describing the distribution of Sb along depth profiles of water bodies found comparable results for the transition zone from oxic to anoxic regions.[38,39] Nevertheless, the distribution of dissolved Sb in freshwater systems can vary strongly in relation to the biophysicochemical conditions.[37,40] Hence, the total concentration in pore water samples may also show different or inverse distributions of dissolved Sb species to those presented in this study.[41,42]

Besides the different physicochemical parameters discussed, in the case of Sb and of Zn, the concentration gradient between the water used to (re)fill the aquarium and the sediment was an important factor. In comparison to the background concentrations of the aquarium water (~15 µg Sb/L and 60 to 70 µg Zn/L; Table 1), determined several weeks after the profiling experiments, the respective values found for Sb (~5 µg/L) and Zn (~10 to 20 µg/L) within the profiling experiments were clearly lower, indicating an ongoing flux of the two elements toward the overlying water. In the case of the redox sensitive elements (Mn, Fe, and Co), diffusion toward the overlying water is limited due to the reoxidation of these elements in the oxic zone. However, the results of Sb and Zn demonstrate aging processes of the sediment and raise the need to address in the future also time dependent processes.

General Evaluation of the *missy* Setups. Both *missy* setups equipped with the self-manufactured probes were found to be suitable for a direct micro sampling of sediment pore water. The distributions of Mn and Fe,[36] Co,[43] Zn,[44] and Sb[45] were generally congruent to those reported for freshwater systems demonstrating the direct applicability of the *missy* for analyses in standard concentration ranges of close to natural sediment pore waters (µg/L to mg/L). Nevertheless, for both setups, some aspects were identified that have to be taken into account: in the case of setup 1, the higher dead volume of 121 µL caused a stronger shift of the sampling depth in relation to the microprofiling measurements (Tables SI8 and SI9, Supporting Information). Moreover, the time required to empty the syringe causes a small gap between two samples. The micro annular gear pump (setup 2) maintains a continuous sampling of pore water and a reduction of the dead volume to 82 µL. One remarkable factor of uncertainty is caused by compartments (volume 18.8 µL) included in the pump to

compensate for pressure variations (information given by the manufacturer). This may lead under extreme conditions (very steep concentration gradients) to slight biases of the element concentrations in the water samples. Regarding the replicates analyzed for the backgrounds and recoveries, no remarkable differences were found between the two setups (e.g., for the metal(loid) distributions or outliers). On the contrary, the volumes sampled by the annular gear pump varied, with an average of 7.6%, slightly stronger than those sampled by the syringe pump (2.1%). Concerning the cleaning of the systems required to remove residues after the pore water sampling (see also the discussion in the Supporting Information, pages 5 et seq), the Rheodyne valve (setup 1) can easily be disassembled and enables for a rinsing of all components. The annular gear pump can only be cleaned by pumping acid and/or ultrapure water (no disassembly possible). However, the results obtained by the two systems were in good agreement (Figures 3, 4, and SI2, Supporting Information), even though the slight differences found for the system backgrounds and recoveries (Tables 1, 2, and SI9, Supporting Information) demonstrate the importance of a thorough characterization of all components of a system and a comprehensive validation of the methods applied, especially if trace metal analyses (ng/L to µg/L) are envisaged. Comparing the two setups, setup 1 seems, until now, to provide a more constant and reproducible sampling procedure but is more expansive than setup 2. Both *missy* setups combine the advantages of microprofiling experiments with a direct, high resolution filtration-based method that allows for a multielement analysis via ICP-MS. Although other (static) systems like DGT[14,15] or peepers[46] can also be applied to a (sub)millimeter scale, the dynamic sampling approach provides for the first time the possibility to apply a direct sampling of pore waters at a spatial high resolution.

Future Applications. Besides the application demonstrated in this study, the *missy* may further be improved and adapted to the demands of other scientific questions by modifying the sample probe (e.g., further miniaturization, use of other materials, or filtration cut-offs) or by changing the measurement/sampling settings (e.g., step size, measurement/sampling times). Besides natural processes, the dynamic profiling technique can be applied to investigate the effects of anthropogenic disturbances (e.g., pollutants or mechanical disturbances) and may thus help to assess potential impacts of intended projects in hydraulic engineering or to study the fate of emerging substances at aquatic interfaces. Potential applications are the assessment of dredging activities on contaminated sediments of, e.g., mining sites[6,47] or industrial[48] or urban areas.[16] In addition to trace metal(loid) fractionation and speciation, the fate and behavior of organic compounds/pollutants (like nutrients or pesticides) or emerging substances (e.g., biocides, pharmaceuticals, or nanomaterials) can be studied after an adaptation of the probe heads and the materials used. Since the microprofiling systems are suitable for *in situ* applications,[49] the *missy* approach can potentially be applied not only in laboratory experiments but also under field conditions.

■ ASSOCIATED CONTENT

ⓢ Supporting Information
Sediment characterization (metal(loid) contents, TOC, CNS analyses), software settings, details on ICP-MS measurements, calculation of the dead volume, and resulting shift of the sampling depth. Results of the background concentrations and

recoveries, metal(loid) concentrations of profile 1−5, the carryover effect, single profiles of oxygen, redox potential, pH value, and preliminary results obtained by setup 2. This material is available free of charge via the Internet at http://pubs.acs.org/.

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■ REFERENCES

(1) Stockdale, A.; Davison, W.; Zhang, H. Micro-scale biogeochemical heterogeneity in sediments: A review of available technology and observed evidence. *Earth-Sci. Rev.* **2009**, *92* (1−2), 81−97.

(2) Revsbech, N. P.; Jorgensen, B. B. Microelectrodes - Their use in microbial ecology. *Adv. Microb. Ecol.* **1986**, *9*, 293−352.

(3) Tankere, S. P. C.; Bourne, D. G.; Muller, F. L. L.; Torsvik, V. Microenvironments and microbial community structure in sediments. *Environ. Microbiol.* **2002**, *4* (2), 97−105.

(4) Bufflap, S. E.; Allen, H. E. Sediment pore-water collection methods for trace-metal analysis - A review. *Water Res.* **1995**, *29* (1), 165−177.

(5) Mudroch, A. A.; Jose, M. *Manual of Aquatic Sediment Sampling*; CRC Press, Inc.: Boca Raton, FL, 1995.

(6) Lourino-Cabana, B.; Lesven, L.; Billon, G.; Denis, L.; Ouddane, B.; Boughriet, A. Benthic exchange of sedimentary metals (Cd, Cu, Fe, Mn, Ni and Zn) in the Deule River (Northern France). *Environ. Chem.* **2012**, *9* (5), 485−494.

(7) Scholz, F.; Hensen, C.; Noffke, A.; Rohde, A.; Liebetrau, V.; Wallmann, K. Early diagenesis of redox-sensitive trace metals in the Peru upwelling area - Response to ENSO-related oxygen fluctuations in the water column. *Geochim. Cosmochim. Acta* **2011**, *75* (22), 7257−7276.

(8) Rigaud, S.; Radakovitch, O.; Couture, R. M.; Deflandre, B.; Cossa, D.; Garnier, C.; Garnier, J. M. Mobility and fluxes of trace elements and nutrients at the sediment-water interface of a lagoon under contrasting water column oxygenation conditions. *Appl. Geochem.* **2013**, *31*, 35−51.

(9) Lewandowski, J.; Ruter, K.; Hupfer, M. Two-dimensional small-scale variability of pore water phosphate in freshwater lakes: Results from a novel dialysis sampler. *Environ. Sci. Technol.* **2002**, *36* (9), 2039−2047.

(10) Davison, W.; Fones, G. R.; Grime, G. W. Dissolved metals in surface sediment and a microbial mat at 100-[mu]m resolution. *Nature* **1997**, *387* (6636), 885−888.

(11) Seeberg-Elverfeldt, J.; Schluter, M.; Feseker, T.; Kolling, M. Rhizon sampling of porewaters near the sediment-water interface of aquatic systems. *Limnol. Oceanogr.: Methods* **2005**, *3*, 361−371.

(12) Duester, L.; Prasse, C.; Vogel, J. V.; Vink, J. P. M.; Schaumann, G. E. Translocation of Sb and Ti in an undisturbed floodplain soil after application of Sb(2)O(3) and TiO(2) nanoparticles to the surface. *J. Environ. Monit.* **2011**, *13* (5), 1204−1211.

(13) Duester, L.; Vink, J. P. M.; Hirner, A. V. Methylantimony and -arsenic species in sediment pore water tested with the sediment or fauna incubation experiment. *Environ. Sci. Technol.* **2008**, *42* (16), 5866−5871.

(14) Ding, S. M.; Sun, Q.; Xu, D.; Jia, F.; He, X.; Zhang, C. S. High-resolution simultaneous measurements of dissolved reactive phosphorus and dissolved sulfide: The first observation of their simultaneous release in sediments. *Environ. Sci. Technol.* **2012**, *46* (15), 8297−8304.

(15) Ding, S. M.; Wang, Y.; Xu, D.; Zhu, C. G.; Zhang, C. S. Gel-based coloration technique for the submillimeter-scale imaging of labile phosphorus in sediments and soils with diffusive gradients in thin films. *Environ. Sci. Technol.* **2013**, *47* (14), 7821−7829.

(16) Ye, S. Y.; Laws, E. A.; Gambrell, R. Trace element remobilization following the resuspension of sediments under controlled redox conditions: City Park Lake, Baton Rouge, LA. *Appl. Geochem.* **2013**, *28*, 91−99.

(17) Canavan, R. W.; Van Cappellen, P.; Zwolsman, J. J. G.; van den Berg, G. A.; Slomp, C. P. Geochemistry of trace metals in a fresh water sediment: Field results and diagenetic modeling. *Sci. Total Environ.* **2007**, *381* (1−3), 263−279.

(18) Morata, T.; Sospedra, J.; Falco, S.; Rodilla, M. Exchange of nutrients and oxygen across the sediment-water interface below a Sparus aurata marine fish farm in the north-western Mediterranean Sea. *J. Soils Sediments* **2012**, *12* (10), 1623−1632.

(19) Bleiner, D.; Bogaerts, A. Multiplicity and contiguity of ablation mechanisms in laser-assisted analytical micro-sampling. *Spectrochim. Acta, Part B* **2006**, *61* (4), 421−432.

(20) Kang, J. Z.; Duan, T. C.; Guo, P. R.; Wang, C.; Chen, H. T.; Zeng, X. J. Matrix effects of micro-sampling system for inductively coupled plasma mass spectrometry. *Chem. J. Chin. Univ.-Chin.* **2004**, *25* (2), 252−255.

(21) Lee, W. H.; Lee, J.-H.; Choi, W.-H.; Hosni, A. A.; Papautsky, I.; Bishop, P. L. Needle-type environmental microsensors: Design, construction and uses of microelectrodes and multi-analyte MEMS sensor arrays. *Meas. Sci. Technol.* **2011**, *22* (4), 042001.

(22) Krawczyk-Baersch, E.; Grossmann, K.; Arnold, T.; Hofmann, S.; Wobus, A. Influence of uranium (VI) on the metabolic activity of stable multispecies biofilms studied by oxygen microsensors and fluorescence microscopy. *Geochim. Cosmochim. Acta* **2008**, *72* (21), 5251−5265.

(23) Laverman, A. M.; Meile, C.; Van Cappellen, P.; Wieringa, E. B. A. Vertical distribution of denitrification in an estuarine sediment: Integrating sediment flowthrough reactor experiments and micro-profiling via reactive transport modeling. *Appl. Environ. Microbiol.* **2007**, *73* (1), 40−47.

(24) Meers, E.; Du Laing, G.; Unamuno, V. G.; Lesage, E.; Tack, F. M. G.; Verloo, M. G. Water extractability of trace metals from soils: Some pitfalls. *Water Air Soil Pollut.* **2006**, *176* (1−4), 21−35.

(25) Stenken, J. A. Methods and issues in microdialysis calibration. *Anal. Chim. Acta* **1999**, *379* (3), 337−358.

(26) Torto, N.; Mogopodi, D. Opportunities in microdialysis sampling of metal ions. *TrAC, Trends Anal. Chem.* **2004**, *23* (2), 109−115.

(27) *R: A language and environment for statistical computing*; R Foundation for Statistical Computing: Vienna, Austria, 2013.

(28) Warnes, G. R.; Bolker, B.; Bonebakker, L.; Gentleman, R.; Liaw, W. H. A.; Lumley, T.; Maechler, M.; Magnusson, A.; Moeller, S.; Schwartz, M.; Venables, B. *gplots: Various R programming tools for plotting data*, R package version 2.11.3.; R Foundation for Statistical Computing: Vienna, Austria, 2013.

(29) Komsta, L. *Tests for outliers*, R Package 'outliers'version 0.14; R Foundation for Statistical Computing: Vienna, Austria, 2013.

(30) Kromidas, S. *Handbuch Validierung in der Analytik: Wirtschaft-lichkeit. Praktische Fallbeispiele. Alternativen. Checklisten*, 1 ed.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, 2000.

(31) Jorgensen, B. B.; Des Marais, D. J. The diffusive boundary layer of sediments: oxygen microgradients over a microbial mat. *Limnol. Oceanogr.* **1990**, *35* (6), 1343−1355.

(32) Jorgensen, B. B.; Revsbech, N. P. Diffusive boundary-layers and the oxygen-uptake of sediments and detritus. *Limnol. Oceanogr.* **1985**, *30* (1), 111−122.

(33) Søndergaard, M. Redox Potential. In *Encyclopedia of Inland Waters*; Likens, G. E., Ed.; Academic Press: Oxford, 2009; pp 852−859.

(34) Stumm, W.; Morgan, J. J. *Aquatic Chemistry: Chemical equilibria and rates in natural waters*, 3rd ed.; John Wiley and Sons: Hoboken, NJ, 1995.

(35) Balistrieri, L. S.; Murray, J. W.; Paul, B. The geochemical cycling of trace-elements in a biogenic meromictic lake. *Geochim. Cosmochim. Acta* **1994**, *58* (19), 3993−4008.

(36) Giblin, A. E. Iron and Manganese. In *Encyclopedia of Inland Waters*; Likens, G. E., Ed.; Academic Press: Oxford, 2009; pp 35−44.

(37) Filella, M.; Belzile, N.; Chen, Y. W. Antimony in the environment: A review focused on natural waters II. Relevant solution chemistry. *Earth-Sci. Rev.* **2002**, *59* (1−4), 265−285.

(38) Takayanagi, K.; Cossa, D. Vertical distributions of Sb(III) and Sb(V) in Pavin Lake, France. *Water Res.* **1997**, *31* (3), 671−674.

(39) Cutter, G. A. Dissolved arsenic and antimony in the black sea. *Deep-Sea Res., Part A* **1991**, *38*, S825−S843.

(40) Filella, M.; Belzile, N.; Chen, Y. W.; Elleouet, C.; May, P. M.; Mavrocordatos, D.; Nirel, P.; Porquet, A.; Quentel, F.; Silver, S. Antimony in aquatic systems. *J. Phys. IV* **2003**, *107*, 475−478.

(41) Chen, Y. W.; Deng, T. L.; Filella, M.; Belzile, N. Distribution and early diagenesis of antimony species in sediments and porewaters of freshwater lakes. *Environ. Sci. Technol.* **2003**, *37* (6), 1163−1168.

(42) Shieh, G.-M. Analytical Techniques for Arsenic and Antimony Speciation Studies in Interstitial Water of River Sediments, PhD dissertation, University of Idaho, Moscow, Idaho, 1993.

(43) Schrauzer, G. N. Cobalt. In *Elements and Their Compounds in the Environment*; Wiley-VCH Verlag GmbH: Weinheim, 2008; pp 825−839.

(44) Peganova, S.; Eder, K. Zinc. In *Elements and Their Compounds in the Environment*; Wiley-VCH Verlag GmbH: Weinheim, 2008; pp 1203−1239.

(45) Filella, M.; Belzile, N.; Chen, Y. W. Antimony in the environment: A review focused on natural waters I. Occurrence. *Earth-Sci. Rev.* **2002**, *57* (1−2), 125−176.

(46) Xu, D.; Wu, W.; Ding, S. M.; Sun, Q.; Zhang, C. S. A high-resolution dialysis technique for rapid determination of dissolved reactive phosphate and ferrous iron in pore water of sediments. *Sci. Total Environ.* **2012**, *421*, 245−252.

(47) Percival, J. B.; Outridge, P. M. A test of the stability of Cd, Cu, Hg, Pb and Zn profiles over two decades in lake sediments near the Flin Flon Smelter, Manitoba, Canada. *Sci. Total Environ.* **2013**, *454*, 307−318.

(48) Cathalot, C.; Lansard, B.; Hall, P. O. J.; Tengberg, A.; Almroth-Rosell, E.; Apler, A.; Calder, L.; Bell, E.; Rabouille, C. Spatial and temporal variability of benthic respiration in a Scottish sea loch impacted by fish farming: A combination of in situ techniques. *Aquat. Geochem.* **2012**, *18* (6), 515−541.

(49) Pedersen, O.; Pulido, C.; Rich, S. M.; Colmer, T. D. In situ O_2 dynamics in submerged Isoetes australis: Varied leaf gas permeability influences underwater photosynthesis and internal O-2. *J. Exp. Bot.* **2011**, *62* (13), 4691−4700.