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Elena Fernández Pascual and Jens Lange

Technical report on the design and construction of a laboratory-scale experimental set-up

A model constructed wetland system designed to perform high vertical-resolution sampling and monitoring

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List of abbreviations

Br	Bromide
CW	Constructed wetland
Fi	Flowmeter at the inlet
Fo	Flowmeter at the outlet
Gf	Glassfilter
HrP	High-resolution pipes
Met-ESA	Metazachlor sulfonic acid
Met-OA	Metazachlor oxalic acid
OP	Observation pipes
P. australis	*Phragmites australis*
Pk	Piezometer in the gravel
Ps	Piezometer in the sand
r	Platinum redox electrode
Re	Reference electrode (Ag:AgCl)
SRB	Sulforhodamine B
T. latifolia	*Typha latifolia*
TP	Transformation product
UR	Uranine

1. Introduction

Constructed wetlands (CWs) are cost-effective treatment systems designed and constructed to mimic processes found in natural wetlands. These systems have been used to treat, among other pollutants, pesticides from intensive agriculture. CWs typically consist of a shallow basin filled with filter material, generally sand or gravel, which serves as a substrate where vegetation adapted for life in saturated soil conditions is planted (USEPA, 2000). The distribution and collection of contaminated water in CWs is usually achieved by a controlled arrangement of the inlet and outlet. There are several types of CWs, and they can be classified according to the absence or presence of free water surface, the direction of the flow and the type of macrophytes used (Vymazal and Brezinová, 2015). The combination of different types of CWs is gaining popularity in recent years due to the advantages that they offer (Vymazal and Brezinová, 2013). For instance, both aerobic and anaerobic processes can be created from the combination of vertical and horizontal CWs, which often results in an increase in contaminant removal efficiency. The removal in CWs is accomplished by physical, chemical, biological and biochemical processes. The large number of these processes functioning simultaneously and influencing each other makes CWs quite complex systems (Langergraber & Šimůnek, 2012).

A variety of experiments in CWs have been implemented to study transport and dissipation of pesticides, at both laboratory (e.g. Zhao et al., 2014; Chen et al., 2017; Gikas et al., 2018) and field scale (e.g. Passeport et al., 2010; Lange et al., 2011; Imfeld et al., 2013; Maillard et al.,2016). Laboratory experiments offer the advantage of allowing detailed monitoring and extensive sampling under known boundary conditions. An example of laboratory experimental systems for wetland investigations are mesocosms. These systems have been shown to provide important insight and quantitative information about processes of dissipation of pollutants (Mazanti et al., 2003; van Wijngaarden et al., 2005). Moreover, mesocosm experiments allow for precise high-resolution measurements, which at field full scale would be much more difficult to achieve. The information provided by this type of measurements is important, particularly when studying parameters (e.g. redox potential) whose fluctuations in CWs may be very steep and depth-dependent (Seybold et al., 2002) and crucial for the control of dissipation of numerous contaminants (Borch et al., 2009).

Most of the studies in CWs have treated the systems as “black boxes” where only the influent and effluent were measured. Consequently, the mechanisms that dominate solute transport and dissipation are still not fully understood. Recent investigations have shown that the application of specific methodologies, such as high-resolution multi-level wells, may assist in providing information about the behavior of pollutants with high vertical-resolution (Anneser et al., 2008; Birkigt et al., 2018). However, to the best of our knowledge, no similar approaches have been implemented to investigate pesticide transport and dissipation processes.

This report provides an overview of the design and construction of a model CW system equipped with a structure to perform high vertical-resolution sampling and monitoring on a long-term basis. Here, we

explain how the set-up was designed and built according to the purpose of our study. This report also presents the performance evaluation of the experimental set-up by means of thermal camera tests and an overview of the experiment results. The last sections provide the conclusion of the study and further recommendations for better addressing the challenges posed by the construction of viable experimental set-ups to study transport and dissipation of contaminants.

2. Definition of objectives and design of the experimental set-up

Previous studies with similar approaches were consulted before designing the experimental set-up (e.g. Anneser et al., 2008; Beketov et al., 2008; Durst et al., 2013; Maillard et al., 2016; Chen et al., 2017; Birkigt et al., 2018). Among them, the approach carried out by Maillard et al. (2016) was particularly interesting, given the objectives of our investigation (see below). However, unlike their study, our experiments were carried out under laboratory conditions in order to achieve detailed monitoring and extensive sampling under known boundary conditions.

The laboratory-scale experimental set-up described in the present report consisted of a model CW system capable of performing high vertical-resolution sampling and monitoring on a long-term basis.

The objectives of our study were:

- Identify temporal and spatial transport and dissipation processes by applying a multi-tracer approach.
- Compare the temporal and spatial behavior of the applied tracers (bromide, uranine and surforhodamine B) with three selected pesticides (boscalid, penconazole and metazachlor) and evaluate their main dissipation pathways.
- Assess the influence of the vegetation and saturation conditions on transport and dissipation processes.

Thus, according to our objectives, we defined the main requirements of our experimental set-up as follows:

- ✓ The experimental set-up must contain the main structural elements of a CW (substrate, vegetation and water).
- ✓ The experimental set-up must be able to:
    - Create subsurface vertical up- and down- flow and surface-flow.
    - Function as both a static (without inputs and outputs) and dynamic system (with inputs and outputs).
- ✓ The experimental set-up must have a water storage tank connected to the inlet and a safe storage tank connected to the outlet where to direct the contaminated water exiting the system.
- ✓ The experimental set-up must have a system to control the inputs and outputs (of clean or contaminated water).

- ✓ The experimental set-up must have a system to collect water at the outlet and perform pore water sampling with high vertical-resolution.
- ✓ The experimental set-up must have a system that allows to measure and monitor on a long-term basis and with high vertical-resolution the following parameters:
    - Water table
    - Temperature
    - Conductivity
    - Soil moisture
    - Redox potential

3. Construction of the experimental set-up

3.1 Inlet and outlet reservoirs and sediment bed

Material: *Aquarium made of glass (47.6 x 56.8 x 177.4 cm); 10 square aluminum tubes (1 x 1 x 177.3 cm); 1 stainless steel wire mesh (0.27mm mesh size, 0.1 mm thickness); 1 aluminum perforated sheet (0.15 x 47.3 x 177.3 cm, 3 mm diameter round holes, 5 mm offset pitch); 2 glass plates (1 x 45 x 47.3 cm); 2 glass plates (1 x 10 x 47.3 cm); 2 Plexiglas tubes (15/13 mm x 2000 mm); 1 aluminum perforated sheet (0.15 x 10 x 100 cm, 5 mm diameter round holes, 8 mm offset pitch).*

A tank made of glass (aquarium) served as a structure on which we built the model CW system (Fig. 1A). In order to create vertical water flows we had to devise a system that could inject water from the bottom to the top. To do this, we first started creating an empty space of one centimeter height in the bottom of the aquarium by placing square tubes made of aluminum (Fig. 1B). The square tubes were then covered with an aluminum perforated plate and then topped with a stainless steel wire mesh (Fig. 1C). The whole structure was intended to hold the sediments, prevent sediment outwash, enhance drainage and allow an homogeneous injection of water from the bottom.

The aquarium was divided into three parts. This was achieved by gluing two glass plates on top of the base formed by the square tubes and metal meshes. Inlet and outlet reservoirs with a capacity of about 23 liters were formed at both ends of the aquarium, while the larger center part constituted the main sediment bed. Inlet and outlet reservoirs were sealed at the bottom with additional glass plates. These plates were afterwards drilled to contain one Plexiglas tube that would be connected to the empty space in the bottom (Fig. 1D). Two additional pairs of tubes were attached and inserted into the reservoirs to fill or empty the water contained in them.

Figure 1. Different stages in the construction of the inlet and outlet reservoirs and sediment bed: A) front view of the aquarium before starting the construction of the model CW system, B) top view of the system with the square tubes made of aluminum, C) top view of the system with the aluminum perforated plate and D) perspective view of the system with the stainless steel wire mesh, the inlet and outlet reservoirs and the Plexiglas tubes.

A plastic support was fixed on top of the glass wall at the inlet reservoir to hold an aluminum perforated sheet (Fig. 2A). The purpose of this structure was to perform two functions: 1) laminate the water when entering the sediment bed from the surface and 2) act as a barrier preventing the water from entering the inlet reservoir. The plastic support created a height difference between the inlet and the outlet glass walls of about 1.3 cm (see Fig. 3), thus facilitating a unidirectional flow towards the outlet reservoir. A second structure was built on the glass wall at the outlet reservoir for sampling purposes. It consisted of a water collector gutter-like glued to the glass wall with a small drain at one end (Fig. 2B). This structure was glued with a small tilt angle to facilitate the collection of water after overflowing the sediment bed.

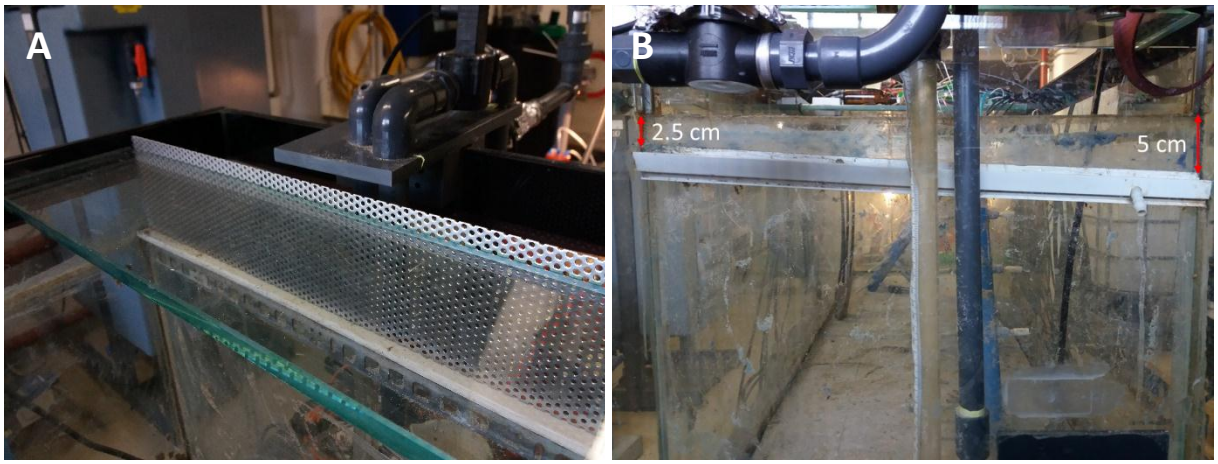

Figure 2. A) Aluminum perforated sheet placed on top of the glass wall at the inlet reservoir and B) water collector gutter-like with a small drain at one end for sampling purposes glued to the glass wall of the outlet reservoir.

3.2 Observation pipes and high vertical-resolution sampling and monitoring system

Material: *8 PVC pipes (pipe DN: 35-40 mm); 8 glass filters (12.5 mm diameter, pore-diameter: 40-100 µm, VitraPOR®, ROBU, Germany); stainless steel capillaries (OD: 1/16 inches, ID: 0.020 inches, Swagelok, Germany); TYGON® peristaltic 2-stop tubing (ID: 1.02 mm x WD: 0.85 mm, Proliquid, Germany); 8 5TE sensors (Decagon 5TE Em50 serie, Campbell Scientific); 8 redox electrodes (Pt, 8 mm diameter, 8 cm length, 3 m PUR cable, Paleo Terra, The Netherlands); 1 reference electrode (Epoxy, Gel, Ag/AgCl, 4 mm, QiS, The Netherlands); 24 nylon cable glands; 1 multichannel peristaltic pump (Pulse-free flow peristaltic pump, Gilson, France); 1 datalogger (CR1000, Campbell Scientific); sand and gravel (grain size of 0.01-2 and 4-8 mm, respectively, Kepes Handelsgesellschaft GmbH, Germany); 6 water level probes; 2 flexible tape measures.*

The arrangement of the observation and multi-level pipes is shown in Figure 3. The sediment bed was filled with a 10 cm layer of gravel. Following this, six observation pipes were placed symmetrically forming two parallel lines in the longitudinal direction of the sediment bed. One half of the pipes were intended to measure the water level in the gravel, therefore they were introduced in the gravel layer. These tubes were only drilled in the first 10 cm at the base. The other half of the pipes were placed on the other side on top of the gravel and were intended to measure the water level in the sand (Fig. 4A). These tubes were fully drilled. Six water level probes were then inserted inside each observation pipe. The water level was additionally monitored with a tape measure attached to the inlet and outlet Plexiglass tubes.

Two additional PVC pipes were placed into the gravel at the central part of the sediment bed to function as multi-level pipes (Fig. 4B). These pipes were designed to perform vertical monitoring and sampling with a resolution of 12 cm. A total of four sampling depths were created. Each multi-level pipe was equipped with four small glassfilters located at each sampling depth. The glassfilters were intended to perform water

sampling, therefore, they were connected to a multichannel peristaltic pump via capillaries made of stainless steel that were directly inserted into TYGON tubes. Four 5TE sensors, intended to perform temperature, conductivity and soil moisture measurements, were also installed in each multi-level pipe and sampling depth. The 5TE sensors were arranged horizontally at 45 degrees of separation from the glassfilters (see Fig. 4B). In addition, four redox electrodes were installed at the same multi-level pipes and sampling depths, but at 45 degrees of separation from the 5TE sensors. The glassfilters and sensors were fixed to the multi-level pipes by means of nylon cable glands. After this, the gravel layer was topped with a 32 cm layer of sand (Fig. 4C). Then, a reference electrode connected to the redox electrodes was inserted in the sand between the multi-level pipes.

When the observation and multi-level pipes were arranged, all the water level probes, sensors and electrodes were connected to a datalogger (Fig. 4D). The datalogger was programmed to record the measurements of the different sensors and probes with an interval of 2 minutes. Direct plug connection facilitated measurements on a long-term basis. Also, it was possible to monitor the system in real-time by connecting the logger to a computer.

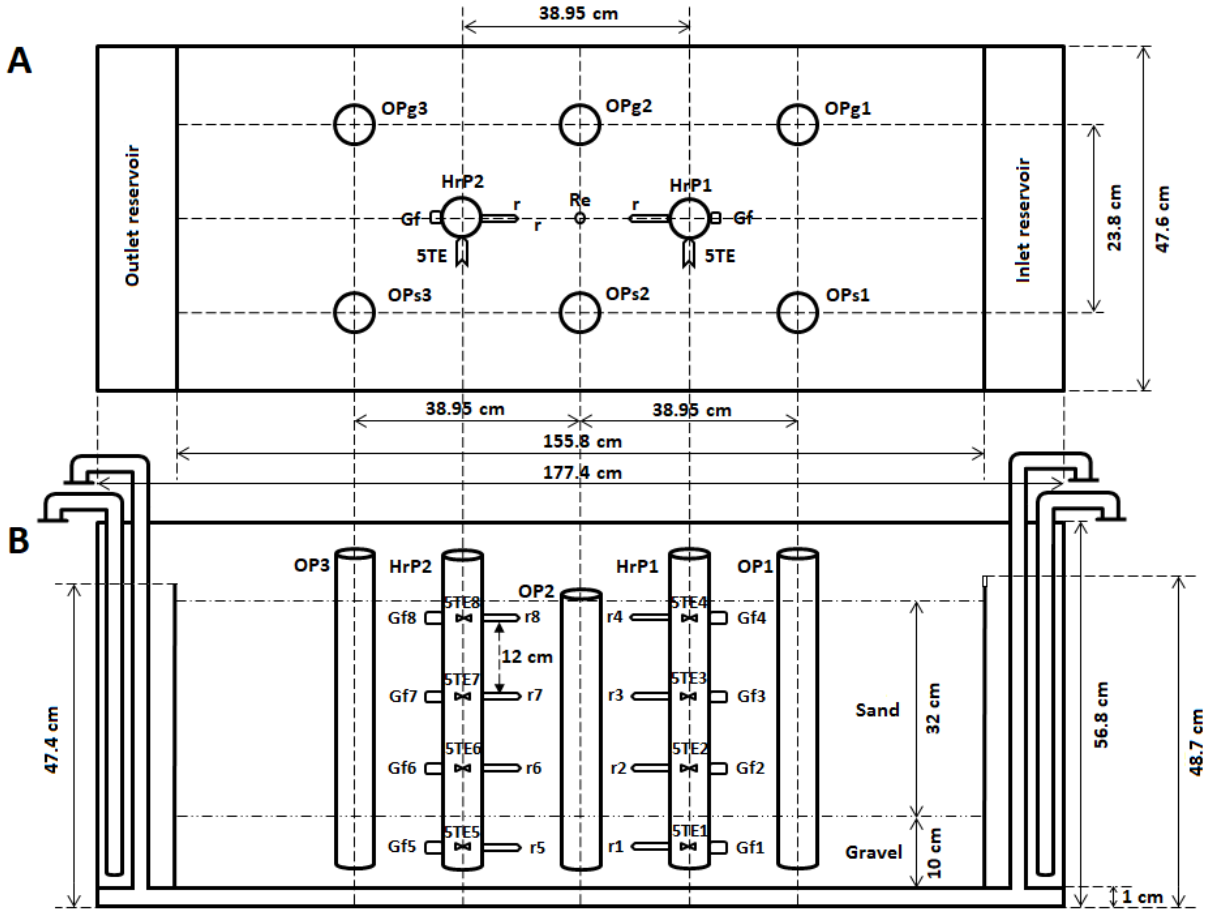

Figure 3. Arrangement of the observation and multi-level pipes. A) Top and B) front view. Legend: OP= observation pipes; HrP= high-resolution pipes; 5TE= soil moisture, temperature and electrical conductivity sensor; Re= Reference electrode (Ag:AgCl); r= Platinum redox electrode; Gf= Glassfilter.

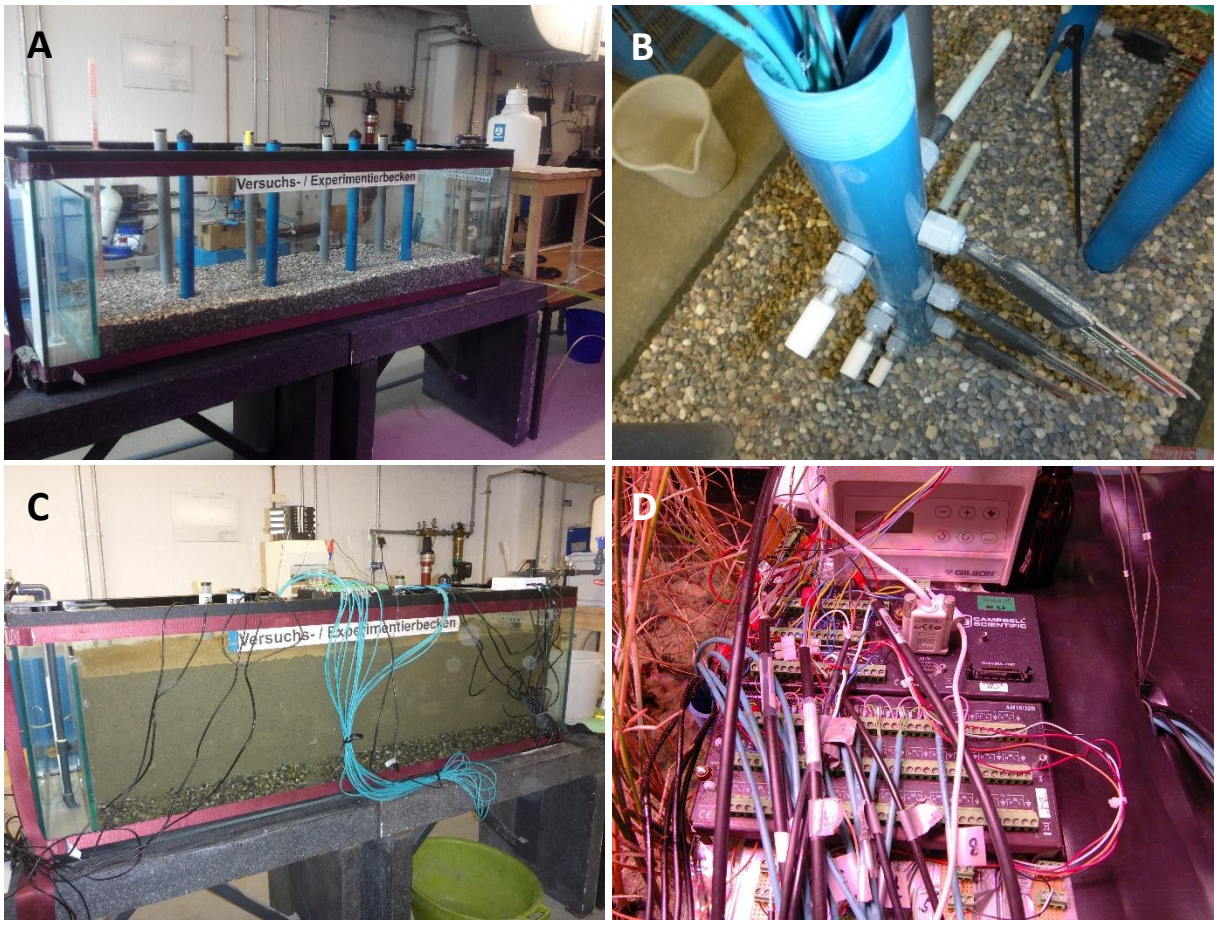

Figure 4. A) Front view of the system with the layer of gravel and the observation pipes, B) top view of the multi-level pipe, C) front view of the system with the layer of gravel and sand and D) top view of the datalogger with all sensors and probes connected.

3.3 Inlet and outlet pumping system and external water storage tanks

Material: *2 flow meters (0.50- 25.0 L/min, Vision 2000, B.I.O-TECH e.K., Germany); 2 flow meters (0.50-30.0 L/min, FCH-C-PA-N, B.I.O-TECH e.K., Germany); peristaltic pump tubing (diameter:); 3 peristaltic pumps; 1 laboratory power supply (Quat Power LN-3003XIII, 2x 0 – 30 V/0 – 3 A 5 V/3 A); 2 water storage tanks (350 and 1000 liters, respectively).*

Two pairs of flow meters were installed at the inlet and outlet pipes of the system. With this arrangement we ensured that each connection to either the reservoirs or the sediment bed had a flow meter. All flow meters were connected to the same datalogger where the other sensors and probes were connected (Fig. 4D).

The water was pumped both inside and outside the system by peristaltic pumps. A set of three peristaltic pumps was arranged, one at the inlet and two at the outlet. The peristaltic pumps were connected to the

pipes by means of peristaltic pump tubing. The inlet peristaltic pump was easily detachable and depending on the type of flow (vertical up- or downward), we attached it to the pipe at the inlet reservoir or to the Plexiglas tube connected to the sediment bed. The voltage and current of the peristaltic pumps were controlled by a laboratory power supply system (Fig. 5A).

The inlet water was pumped from a 350 liters storage tank connected directly to the tap water with a hose (Fig. 5B). A water level probe connected to the datalogger was installed inside the storage tank in order to control the water inlets and outlets. The water exiting the CW system was pumped to a second storage tank of 1000 liters capacity (Fig 5C). Both the inlet and outlet storage tanks were connected to the peristaltic pumps by means of peristaltic pump tubing.

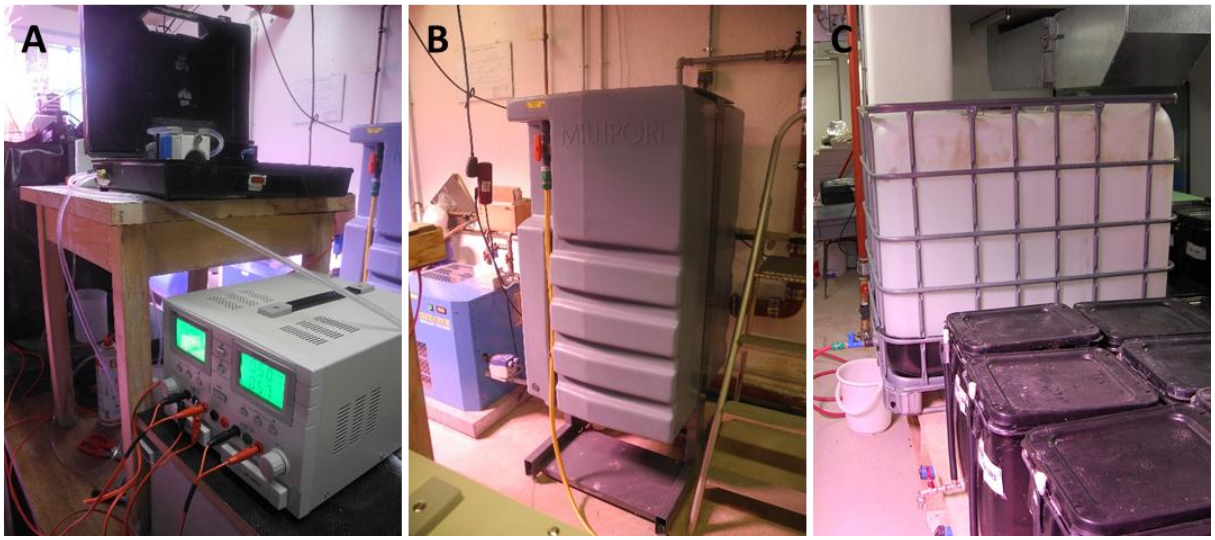

Figure 5. A) Peristaltic pump and laboratory power supply, B) tap water storage tank and C) wastewater storage tank.

3.4 Plants and related devices

Material: *7 plants of P. australis and 4 plants of T. latifolia (Kaiserstühler Staudenhof Menton, Freiburg); 4 LED lamps for plant growth (64 OSRAM SSL 3W light-emitting diode, Purple Alien 2.0, LED Grow Shop, Germany); 1 programmable lamp timer (Diehl variomat-e, Diehl GmbH & Co. Schaltsysteme, Germany); 1 PVC pond liner (thickness: 0.5 mm); 2 HOBO data loggers (Onset HOBO Pendant Event Data Loggers UA-003-64).*

Two species of widespread and ubiquitous wetland plants (*T. latifolia* and *P. australis*) were planted in one half of the system (Fig. 6A). The plants were purchased from a local garden center. Prior to planting, several tests with a thermal camera (see section 4) were performed. The unplanted half and the glass walls of the aquarium were covered with a PVC pond liner sheet to prevent direct exposure of the system to light.

Four light-emitting diode lamps for plant growth were installed on the roof above the planted area (Fig. 6B). Daily photoperiods of 11 hours were controlled with a programmable lamp timer.

In addition, two HOBO data loggers were placed under both the illuminated and the unlit area in order to record the temperature and light intensity during the experiment.

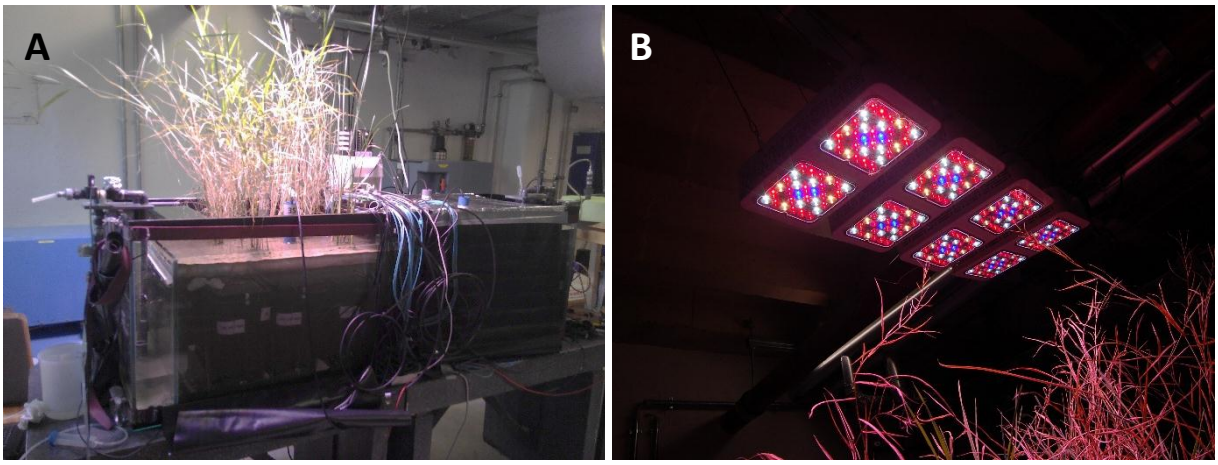

Figure 6. A) Front view of the model CW system with the vegetated and non-vegetated half and B) lamps for plant growth.

4. Performance evaluation of the experimental set-up: thermal camera tests

Several tests with different configurations were carried out in order to evaluate the performance of the experimental set-up. Cold water was used as a tracer and its movement was recorded with a thermal camera (VarioCAM high resolution, InfraTec, Dresden, Germany). The cold water was generated by introducing blocks of ice in the water contained in the tap water storage tank. This water was injected immediately after into the system with a fixed pumping rate of 60 L/h. The thermal camera was installed on the roof above the experimental set-up to cover the entire surface of the model CW system. The program of the camera was adjusted to take images with the default colour palette (VarioCAM) and a frequency/recording rate of 2.5 seconds. Images were later processed with the software IRBIS3.

Two tests were carried out before planting. Then they were repeated after planting to check the performance of the experimental set-up under the influence of the plants. The effectiveness of the measures applied to mitigate preferential flow paths was also evaluated. The first test consisted of the surface injection of cold water from the inlet reservoir and the second test of the injection of cold water from the bottom of the system. Both injections were performed in a system that was saturated with water at room temperature.

The information obtained from the thermal camera tests was taken into account in the execution of the experiment and the subsequent analysis of the results. The different tests (with and without plants) are summarized below.

4.1 Tests without plants

During the first test (surface injection of water), cold water moved fast and dominated on the surface after approximately 11 minutes. No preferential flow paths were observed (Fig. 7).

The arrival of cold water to the surface during the second test (injection of water from the bottom) was captured about 40 minutes after the start of the injection (Fig. 8). Cold water dominated on the surface after approximately two hours. Several preferential flow paths along the observation pipes installed in the sand were captured with the thermal camera. In order to avoid this, the pipes were clogged with sand.

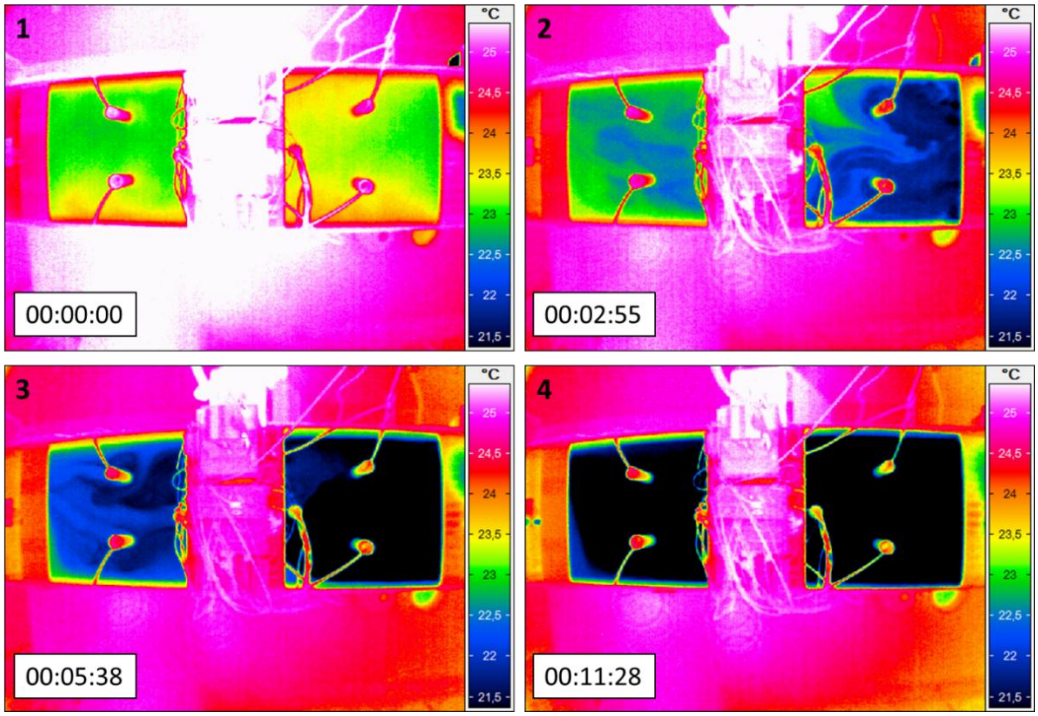

Figure 7. Selected frames recorded during the surface injection of water without plants. Temperature scale 21.5 - 25°C. Each frame represents a top view of the sediment bed. On the right is the inlet and on the left the outlet.

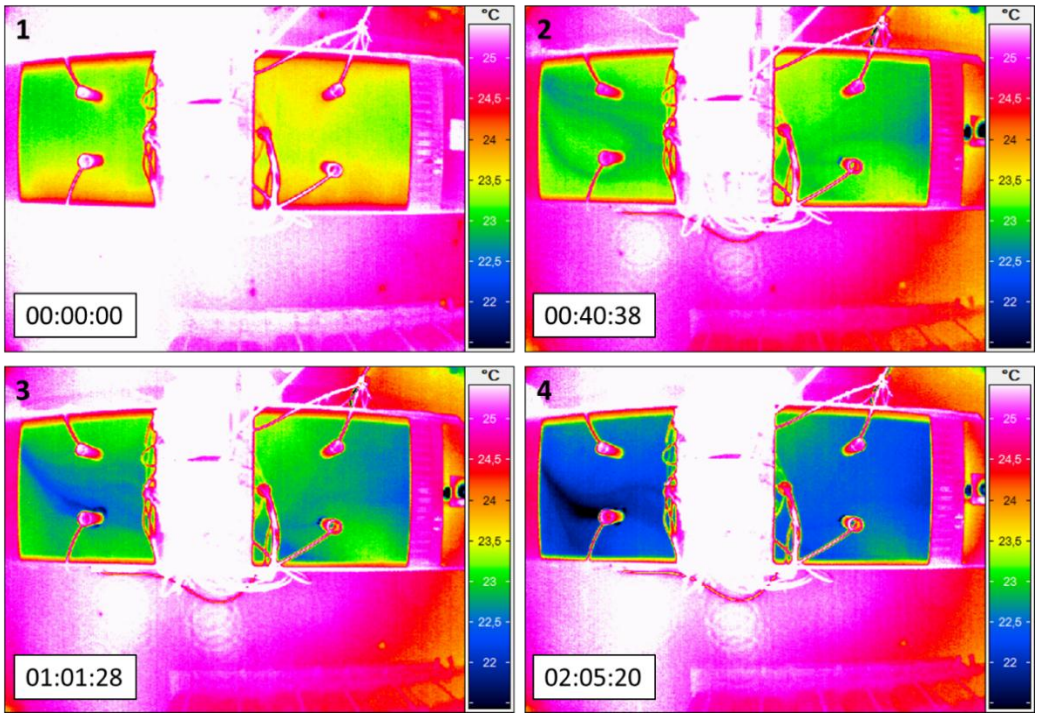

Figure 8. Selected frames recorded during the injection of water from the bottom without plants. Temperature scale 21.5 - 25°C. Each frame represents a top view of the sediment bed. On the right is the inlet and on the left the outlet.

4.2 Tests with plants

During the first test after planting (surface injection of water), cold water moved fast through the non-vegetated surface, whereas a slower transport through the vegetated part was observed (Fig. 9). Cold water dominated on the surface approximately 40 minutes after the injection. No preferential flow paths were detected.

During the second test after planting (injection of water from the bottom), no preferential flow paths along the pipes were observed (Fig. 10). Thus, the mitigation measures were considered successful. The first arrival of water to the surface was captured about two hours after the injection. Cold water dominated on the surface approximately three hours after the injection. Overall, the thermal camera captured a slower movement of water in the system with plants compared to the same system without plants.

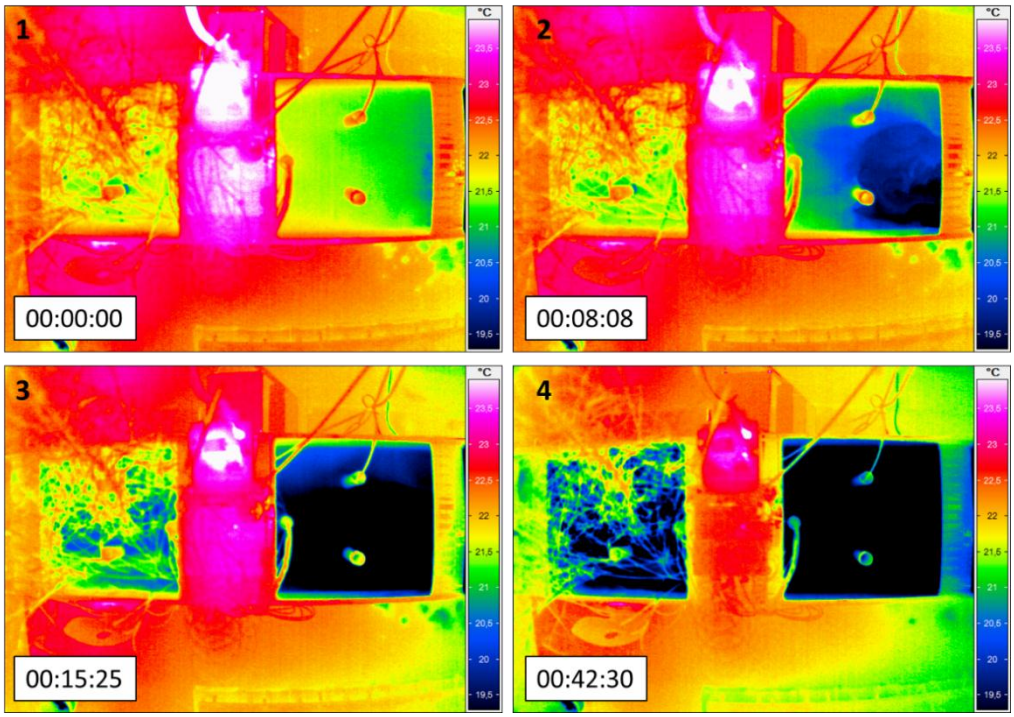

Figure 9. Selected frames recorded during the surface injection of water with plants. Temperature scale 19.5 – 23.5°C. Each frame represents a top view of the sediment bed. On the right is the inlet and on the left the outlet.

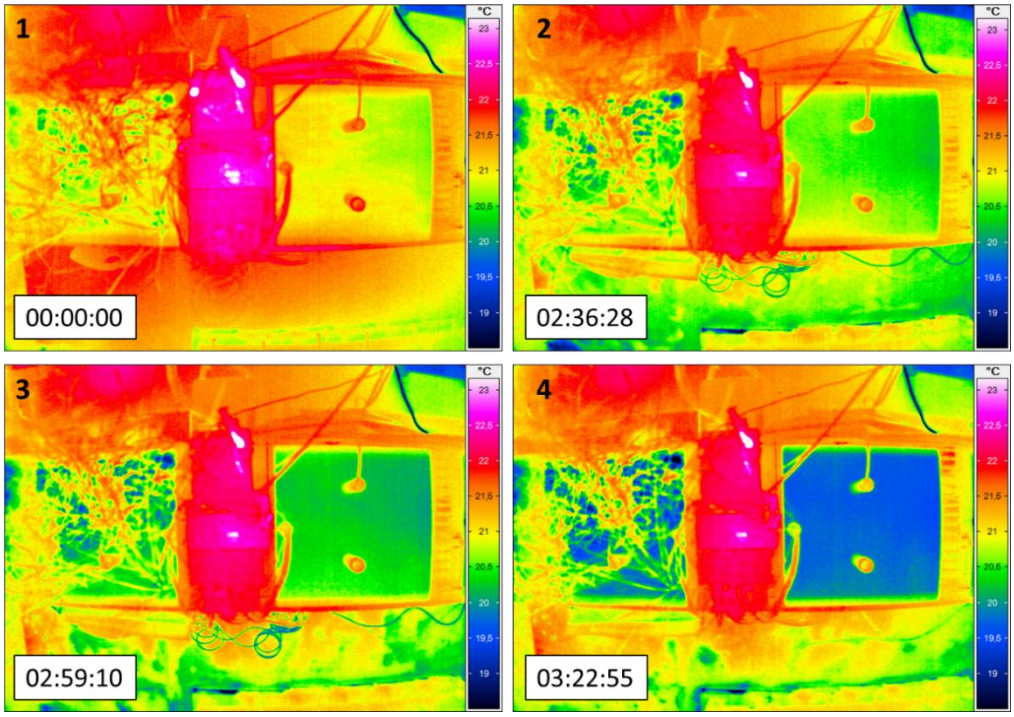

Figure 10. Selected frames recorded during the injection of water from the bottom with plants. Temperature scale 19 - 23°C. Each frame represents a top view of the sediment bed. On the right is the inlet and on the left the outlet.

5. Execution of the experiment and results overview

5.1 Execution of the experiment

Once the set-up of the model CW system was completed and its performance evaluated, the experiment was carried out. The execution of the experiment is summarized in figure 11. First, the target compounds (tracers and pesticides) were injected from the surface into the sediment bed. Prior to this, the system was drained until field capacity was reached. For about a week the system remained saturated with the contaminated water. From then on, no water inlets occurred, and the system was left to dry (by evapotranspiration) until the water level dropped to zero (about three weeks). Then, it was again saturated, but this time with clean water (tap water) that was injected from the bottom. Saturated conditions were maintained for about one month. At the end of the experiment, the sediments were flushed several times with clean water from the bottom. The whole experiment lasted seven months, during which two identical experimental runs and a resting period of three months in between were performed.

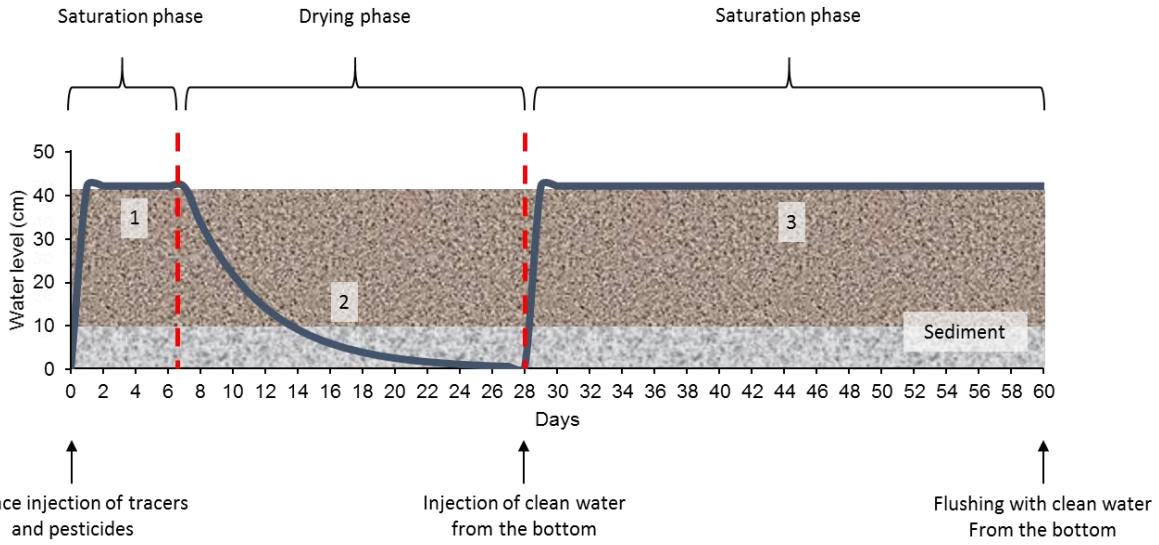

Figure 11. Experimental protocol with the different phases and injections performed during the experiment. The x-axis indicates the duration of the experiment and the y-axis the variation of the water level during the different phases. The water level curve is only schematic and does not correspond to real water level measurements.

5.2 Results overview

Below are the graphs with the results of the measurements performed in the model CW system with high vertical resolution sampling and monitoring over the whole experimental period (from March 9 to October 3, 2017). The resting period was carried out from May 9 to August 1, 2017.

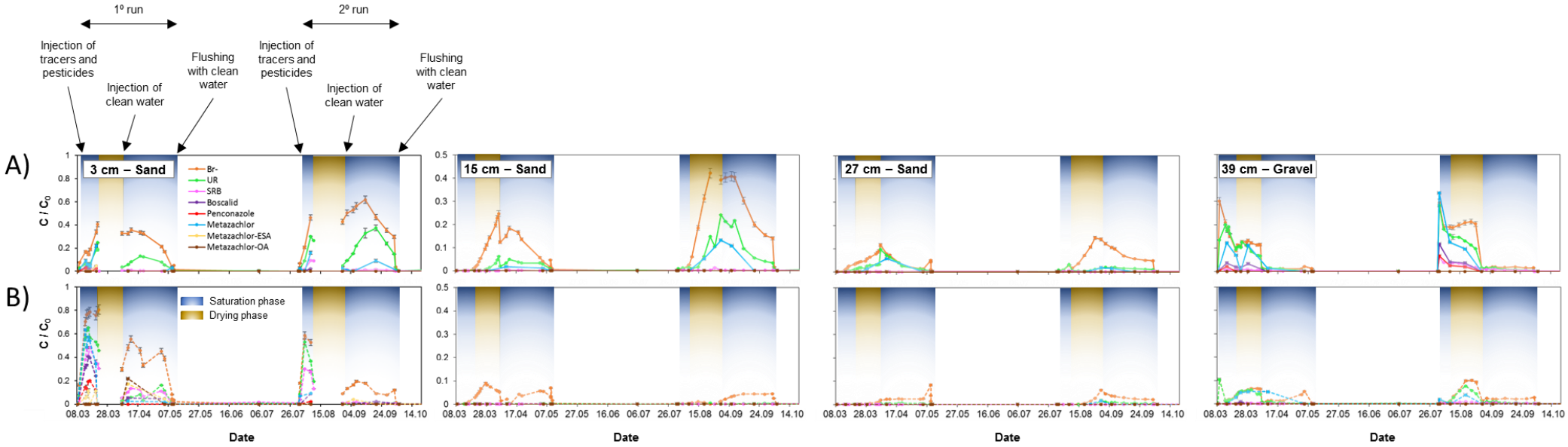

Figure 12. Breakthrough curves of tracers and pesticides expressed as relative concentrations, measured in the pore water of A) the non-vegetated and B) vegetated zone at four different depths (from left to right: 3, 15, 27 and 39 cm) for the first and second run of the experiment. Sampling depths and type of sediment (sand or gravel) are indicated in each graph. The different injections and phases of the experiment (saturation or drying) are also displayed in the figure. Note that the y-axis is different for the graph corresponding to the sampling depth of 3 cm. The missing data during the drying period of the graph corresponding to the sampling depth of 3 cm is due to the complete drying of the top layer.

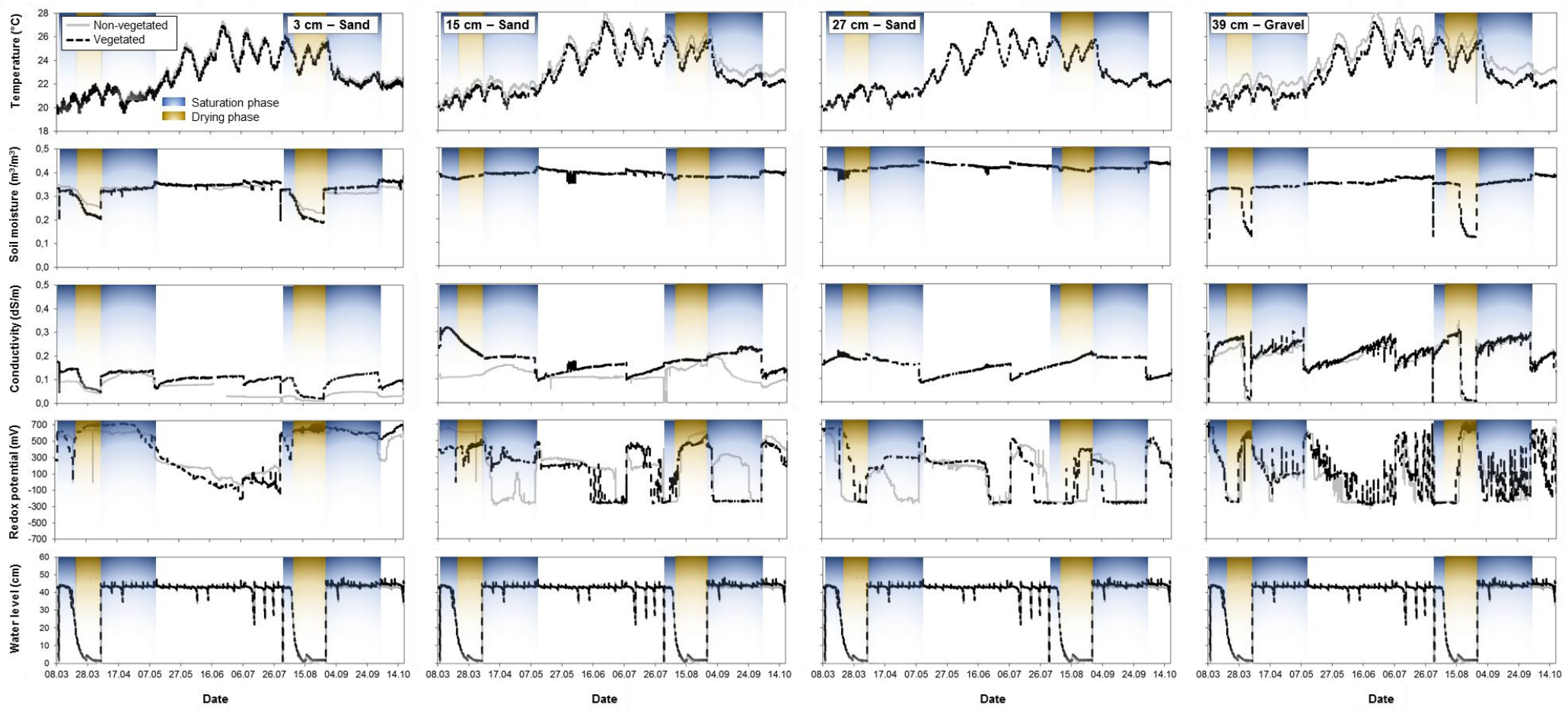

Figure 13. Graphs of the physio-chemical parameters (from top to bottom: temperature, soil moisture, conductivity and redox potential) measured in the sediment at four different depths (from left to right: 3, 15, 27 and 39 cm) for the first and second run of the experiment. Water level (bottom graph) has also been included. Note that it is the same for all depths. The missing data is due to failures in the sensors. The values for the resting period are also shown. Sampling depths and type of sediment (sand or gravel) are indicated in each graph. The different phases of the experiment (saturation or drying) are also displayed.

6. Conclusion

From a functional and structural point of view, the experiment performed well. It was possible to create vertical up-, down- and surface water flow as well as static and dynamic conditions. The target parameters were continuously monitored for seven months and pore water samples were successfully extracted from different depths on a regular basis. Yet, it was not possible to prevent some sensors from having technical malfunctions. In this sense, the sensors that failed the most were the 5TE, the water level probes and the flow meters. The problems arose once the experiment was running, and since these sensors and probes were already installed, they could not be replaced. In the case of the 5TE sensors, only those located in the non-vegetated zone showed failures. Both the conductivity and the soil moisture were the most problematic parameters. As for the water level probes, although they were calibrated and their operation was previously checked, some of them showed inaccurate, missing or flawed data. In addition, since the observation pipes placed in the sand had to be clogged due to the formation of preferential flow paths, the water level could not be monitored there. Instead, we used the data from the probes installed in the gravel. As for the flow meters, the main problem was that they stopped working after few minutes. The data was not reliable due to its low accuracy. A possible cause of such malfunctions was the entrance of bubbles in the pipes due to an error in the design of the inlet and outlet. On the other hand, the flow rate at which the peristaltic pumps operated were often below the measurement range of the flow meters. Thus, the flow rate could not be monitored. However, since the flow meters were calibrated according to the voltage provided by the laboratory power supply, it was possible to estimate the flow rate values.

Other issues arose due to the complexity of the experiment and the limitations of the experimental set-up. In this regard, although the set-up was designed according to the objectives of the study and its performance was previously evaluated, some problems could not be identified. For instance, the possible creation of additional preferential flow paths during the initial injection of the target compounds. Indeed, according to the results, the type of injection performed at the beginning (surface injection) together with the constructional design and the presence of plants resulted in the formation of preferential flow paths.

Further complications were due to the presence and absence of plants in the same experimental unit. This made it difficult to start the experiment under homogeneous conditions. Besides, the long-term character of the experiment and the impossibility of creating natural conditions in the laboratory ended up affecting the plants. Conditions such as dryness, lack of sufficient nutrients and biodiversity led to the infestation of aphids. The pest was present during the whole experiment. Despite efforts to eliminate it with the introduction of general predators such as ladybugs, it could not be removed. As a result of the hostile conditions, and perhaps most sensitive character of the species *T. latifolia*, it did not survive. In contrast, the other species (*P. australis*) not only resisted the conditions, but also grew during the whole experiment. *P. australis* is well known for its invasive character and resilience (Hershner & Havens, 2008). This was a problem because even green shoots of this species were observed in the non-vegetated (and covered) area by the end of the experiment. In addition, there was a rapid emergence and proliferation of algae in the vegetated zone, that introduced additional factors of uncertainty.

7. Further recommendations

Experience has taught us that the design and construction of experiments like the one presented here are rather time-consuming. Hence, it is essential to first determine which hypothesis we want to test and then carefully design the experiment according to our predictions. Before starting the construction, it is advisable to undertake adequate planning. Previous tests and calibrations are crucial to evaluate the performance of the experimental set-up. Especially, if once the experiment starts it is not possible to start over again. However, we must also bear in mind that the performance of previous tests does not guarantee the total elimination of possible sources of errors or uncertainty. If new variables arise that may influence the results, they must be taken into account. Further experimentation after isolating these variables will help to validate our results.

On the other hand, conducting experiments with living organisms may complicate the interpretation of the results. In this context, growing plants for experimental purposes could be a challenge, and it requires great knowledge of the physiological responses of the plants to the environment and proper measurement of the environmental parameters (Poorter et al., 2012). In this case, when designing the experiment, it would be advisable to write a checklist where the environmental factors that will affect the target plants species, such as light quantity and quality, nutrients, air humidity, temperature, etc. are taken into account for their subsequent control during the experiment. This is especially important in indoor studies where the plants may experience different levels of stress.

In addition, when designing an experimental set-up with plants and without plants (control) in the same experimental unit, it is advisable to previously characterize the two zones. Likewise, if we only have one experimental unit and we perform several runs to monitor the performance of the experiment, we must take care when interpreting the results. This is because each run may include variability from different sources, such as changes in the equipment settings or in the environmental factors over time (e.g. room temperature, soil microbial communities and soil porosity). In addition, it should be taken into account that we can only generalize our conclusion to the special case of our experiment. In this regard, it will always be recommendable to repeat the study independently to validate the results. And given the artificial nature and scale of laboratory experiments, it will always be necessary to confirm the results with further experiments in field full-scale systems (Vymazal, 2018).

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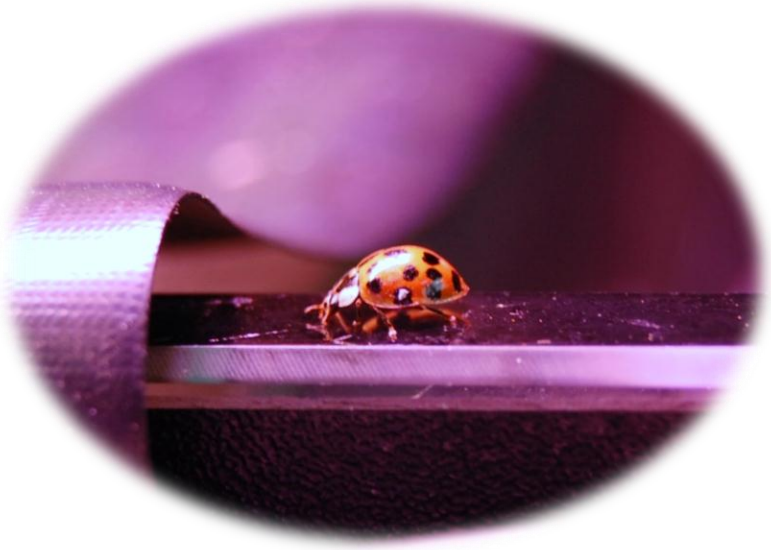

Figure 14. Ladybug on one of the glass walls of the CW.

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