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TECHNICAL NOTE

A combined flow cell–respiration box to quantify carbon transformation processes in undisturbed soil

Henrik Redweik[1] | **Moritz Rahlfs**[2] | **Jörg Bachmann**[1]

[1] Institute of Soil Science, Gottfried Wilhelm Leibniz Univ. Hannover, Herrenhäuser Straße 2, Hannover 30419, Germany

[2] LBEG- Niedersachsen, Stilleweg 2, Hannover 30655, Germany

Correspondence
Henrik Redweik, Institute of Soil Science, Gottfried Wilhelm Leibniz Univ. Hannover, Herrenhäuser Straße 2, 30419 Hannover, Germany.
Email: redweik@ifbk.uni-hannover.de

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Abstract

A new experimental laboratory design is introduced to analyze and quantify transport and turnover processes in top- and subsoils. The proposed technique allows the monitoring of transformation processes in an undisturbed soil matrix while preserving entirely intact soil particle interfaces. The experimental setup with undisturbed soil samples, which are embedded into two coupled gas sealed chambers, allows on-time measurement of soil respiration processes under realistic moisture, water flux, and temperature conditions.

1 | INTRODUCTION

Numerous processes in soil as the transport and sorption of solvents and colloids involve the physical and specific interaction of soil particles with soil solution. Generally, the soil water dynamics are controlled by soil pore architecture as well as by pore interfacial interfaces, which are often coupled with biological processes and thus termed as biogeochemical interfaces (Totsche et al., 2010). As a consequence, diffusive and convective limitations of solute and nutrient transport are, in turn, strongly coupled to the responses of soil biota on several spatial scales.

To unravel single processes and their interrelations, it is apparently needed to evaluate real soil microcosms under conditions as close to reality as possible. Corresponding datasets of controlled systems are seldom reported but are required, for example, for the development or calibration of mathematical models simulating transport or complex and coupled transformation processes like soil carbon and nutrient cycling under realistic micro hydraulic conditions. Hence, understanding how processes change over time and over scales can be considered one of the greatest challenges in soil science by analyzing model systems observed on adequate special scales.

There is still a considerable discrepancy in results between in vitro laboratory artificial models and in situ field studies in the area of material and element cycling research such as nutrient, pollutant, carbon, and nitrate cycling (Hallett et al., 2013; van der Weerden et al., 2012). Of course, these methodological approaches have their justification, but the simplicity of some artificial model systems might interfere with the complexity of natural structures and processes given in field studies. Vice versa, field experiments lack apparently the controllability of a laboratory environment. For example, the use of repacked soil columns to study physical and biological interaction (Sleutel et al., 2012) has been well established due to its better repeatability and control of major structural features, such as bulk density, aggregate size, or macroporosity, in comparison with the structure of undisturbed field soil (Carstens et al., 2018; Weigand & Totsche, 1998). Complex structural features as found in undisturbed soil horizons such as intact

Abbreviations: DOM, dissolved organic matter; FC, flow cell; PTFE, polytetrafluoroethylene; PVC, polyvinyl chloride.

pore space topology and pore surface properties are definitely destroyed. Such features cannot be simulated quite accurately in artificial systems, which leads to discrepancies in comparison with undisturbed soil (Carstens et al., 2018). One example recently reported by Krueger et al. (2018) is the formation of micro-preferential flow pathways leading to heterogeneous nutrient supply in the transport domain with consequences for the microbial activity, which has been visualized by microbial exoenzyme mapping. A similar experimental approach under exclusion of transport phenomena has been reported by (Heitkötter & Marschner, 2018a, 2018b). In addition, further significant environmental factors such as temperature, humidity, and ambient gas composition are often neglected or simplified in column-based percolation studies. If applied, such as in mineralization survey experiments with or without intact soil columns, percolation dynamics, flow interruption, and other precipitation effects are not taken into account—factors that are crucial to mimic environmental field conditions in detail. Some studies (i.e., Carstens, 2016; Carstens et al., 2017) show the significant importance of flow interruption processes in percolation studies for the transport and sorption of solutes and colloids such as dissolved organic matter (DOM).

The motivation for this note is to connect conceptionally the best advantages of both worlds (i.e., the complexity of field experiments combined with defined laboratory conditions). Our proposed experimental laboratory design aims to add the complexity of heterogeneous soil into controllable settings. In consistent continuation of the novel soil flow cell (FC) concept (Krueger & Bachmann, 2017), which already emphasized the potential of a special designed soil sample geometry to observe physical and biological properties and processes with high spatial resolution, we implemented in the present project a set of undisturbed FC soil samples into a fully controllable laboratory mesocosm with respect to light, temperature, ambient gas atmosphere, and water flow conditions. The laboratory-scale continuous-flow-through chamber design ("flow cells") as proposed by Starek et al. (2011) for fractured groundwater systems is in the present paper extended to capture soil-specific undisturbed samples. This approach resembles the micromodel system strategy that has been developed for the direct observation of bacteria or micromodel transport (Baumann & Werth, 2004; Bos et al., 1999) or in situ biofilm formation (Starek et al., 2011). In this respect, it is worth mentioning that soil structure and heterogeneity is not destroyed during sampling as micro-computed tomography scans suggest (Figure 1).

Regarding the functionality of intact pore geometries and pore surface properties that governs transport and transforma-

Core Ideas

- We introduce improved column breakthrough experiments with the advantage of visual monitoring processes.
- The stated device combines laboratory precision and controllability with field conditions such as temperature regime.
- Due to its design, it is possible to combine soil horizons, which allows us to simulate soil profiles.
- In combination with labeled C, small-scale mass balancing of the C cycle is possible.
- The controllability allows flow in unsaturated conditions with control over water content and flow velocity column breakthrough experiments with the advantage of visual monitoring processes.

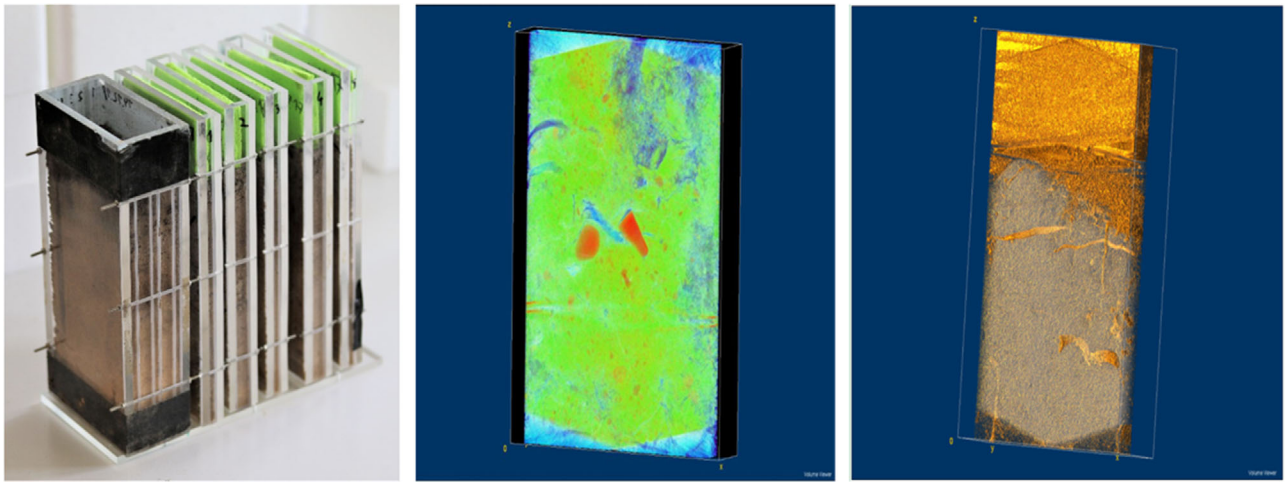

FIGURE 1 Flowcells after field extraction and in sliced state and two computed tomography scans in false-color display (Helmholtz Center for Environmental Research [UFZ] Halle, Vogel & Köhne)

tion processes in soils, we combine advantages of soil monoliths (Lewis & Sjöstrom, 2010) with the accessibility of different section slices and with the advantage of physically highly similar sample replicates (Krueger & Bachmann, 2017). Conventional experimental systems that do not provide comparable flow rates including possible stagnation phases may introduce a different flow field pattern with little relevance in situ. Now, with the installation of the FC into gas sealed chambers, different sorption and transport processes can be monitored during percolation and breakthrough experiments. Simultaneously, it is possible to retrace, recover, and balance the total input in the three phase system gas–solid–liquid. By controlling temperature and percolation rates, it is possible to simulate environmental conditions (e.g., winterly drainage periods during phases without transpiration) at defined subsoil temperatures. The flat and transparent design of the FC allows instantaneous analysis of undisturbed flowpaths according to the natural heterogeneous structure and under unsaturated hydraulic conditions, allowing visual evaluation options that were not provided by standard column experiments.

The FC implementation into a gas sealed compartment paper allows the measurement of microbial induced soil respiration to quantify carbon dioxide release into the atmosphere. The design of two vertically installed chambers as reported here allows monitoring flow behavior and respiration of two FCs sampled in two different depths, hence simulating the flow of soil solution through a soil profile with contrasting (soil horizon-dependent) properties. Through minor design changes, individual monitoring of each FC is also possible to distinguish coupled and cumulative soil horizon specific transformation processes during percolation into greater depths.

In the present paper, we will introduce this respiration box setup in its function as a flow-controlled reactor and we will describe its properties, advantages, and multipurpose use by presenting trial testing experiments for its suitability. For this purpose, pilot tests were conducted with DOM to evaluate its applicability for mass balancing carbon transport and turnover processes in simulated conditions of a northern European forest soil under winterly conditions. Results are presented below.

2 | MATERIALS AND METHODS

2.1 | Respiration box

The respiration box consists of two vertically attached sealable chambers in which the FCs are positioned. The volume of each chamber is 1,590 cm^3 (Figure 2). Each component is built of acrylic glass with a thickness of 1 cm. The transparency of the material was chosen for monitoring purposes (i.e., photography during the experiment). A removable front plate is attached with 24 metal screws, allowing direct access to spatial information (i.e., for multispectral photography or mapping of contact angle, diffuse reflectance infrared Fourier transform spectroscopy [DRIFT-IR], or microbial exoenzyme mapping). Gas sealing was provided by a rubber inlay and silicone grease.

2.2 | Sensors and ports

Both chambers are similar in design in regard of sensor placement and other devices. On the lower right side of the chamber two gas sealed silicone septa for gas sampling are installed. Soil respiration is measured and controlled by two cable sensors, one temperature sensor and one oxygen optode. (i.e., Fibox 4 trace, PreSens Precision Sensing). Under aerobic conditions inside the chambers, the measurable decrease in oxygen concentration is proportional to the increase of carbon dioxide, allowing hereby an assertion about soil respiration. A Voltcraft multipurpose logger for temperature, air pressure, and humidity (Voltcraft DL-220THP, Conrad Electronic) is situated inside each chamber for hourly routine measurements. To assure homogeneous gas distribution, a small PC ventilator is built behind each FC. In order to avoid the growth of algae and to simulate soil conditions during winter, the typical percolation period in Middle Europe, the entire respiration box compartment is placed inside a refrigerator, ensuring darkness and controlled temperature during the experiment including pump and reservoir for soil solution. The FCs are attached on four brackets glued on the backside wall of the chamber.

2.3 | Flow cells

Soil samples were prepared according to the FC design method established by Krueger and Bachmann (2017). Each FC has the dimensions of 20 × 7.5 × 1 cm. Alternatively, slices with 0.5-cm thickness can also be prepared. The soil material originates from the Grinderwald beech forest stand (52°34′22.3“ N, 9°18′48.0″ E) in Lower Saxony, Germany. The Dystric Cambisol is characterized by a homogeneous sandy substrate in the topsoil horizon and locally gravel layers in around 40-cm depth followed by a heterogeneous subsoil with few loamy-silty lenses (Krueger & Bachmann, 2017). Basic soil properties are given in Table 1. To simulate top and subsoil, samples from 15-cm and 150-cm depth were taken according to the sampling protocol (Krueger & Bachmann, 2017). Soil pH and electrical conductivity for both samples were measured, being 32.5 μS cm^{-1} at pH 4.5 in 15-cm depth and 27.4 μS cm^{-1} at pH 4.2 in 150-cm depth (inoLab pH 720, WTW). To evaluate soil hydraulic functions, conventional steel cylinder soil cores (e.g., those provided by the HYPROP

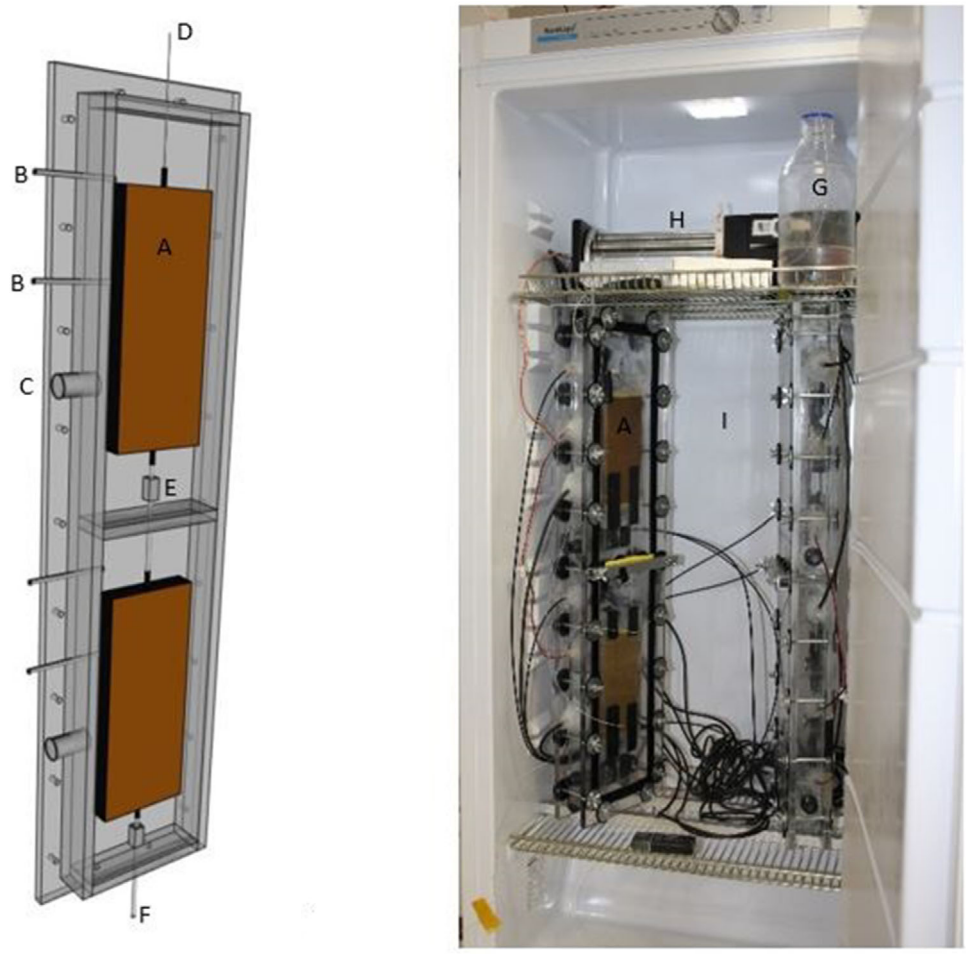

FIGURE 2 Technical drawing and final application setup of the respiration chamber: A = flow cell; B = sensor ports; C = gas sampling port; D = polytetrafluoroethylene (PTFE) influent tube; E = siphon; F = effluent tube; G = liquid reservoir; H = peristaltic pump; I = refrigerator

TABLE 1 Characterization of the Dystric Cambisol at the Grinderwald site

Soil horizon	Depth	pH ($CaCl_2$)	SOC	Sand	Silt	Clay
	cm		%			
AE (Ahe)	0–2	3.3	27	70	26	4
Bsw (Bsv)	2–12	3.4	17	65	30	5
Bw (Bv)	13–36	4.4	7	67	29	4
BwC (Bv/C)	36–65	4.5	3	73	24	3
C (C)	65–125	4.4	0.4	95	4	1
2C (IIC)	125–150	4.1	0.1	81	11	8
2Cg (IICg)	150–180	4.2	0.8	72	19	9
3C (IIIC)	>180	4.2	<0.1	95	4	1

Note. Data were taken from Bachmann et al. (2016). SOC, soil organic carbon.

system, Meter Group) can be taken in corresponding depths close to the sampling locations of the FC core samples.

For testing gas sealing of the respiration chamber, two FCs were prepared with disturbed soil material. Soil bulk density was adjusted in coherence to excavated sampling cores of the same depth around the FC sampling spots (Table 2). The soil was air dried and sieved to 2 mm. Carbon content before and after the experiment was measured by a CNS analyzer (vario EL III Element Analyzer, Elementar Analysensysteme). Preferential bypass alongside the acrylic glass walls, as mentioned by several authors (Corwin, 2000; Ghodrati et al., 1999; Sentenac et al., 2001), was no concern due

TABLE 2 Comparison of simulated parameters in the laboratory with measured data from the sampling site

Factor	Field	Laboratory
Water flux	1 mm d^{-1} (Liebmann, personal communication, 2018)	3 ml d^{-1} ≙ 4 mm d^{-1}
Volumetric water content	20% in 30 cm10% in 150 cm	20% in 15 cm10% in 150 cm
Temperature	4.9 ˚C mean winter	4.68 ˚C topsoil chamber 4.92 ˚C subsoil chamber
Bulk density	1.36 in 10 cm1.58 in 150 cm	1.36 in 15 cm1.59 in 150 cm
DOC content	72.0 ± 26.4 mg C L^{-1} 10 cm (Leinemann et al., 2016)	100 mg C L^{-1} δ ^{13}C: 164.225‰

Note. DOC, dissolved organic carbon.

to the unsaturated conditions during the experiment, as was proved by preliminary dye tracer experiments and continuous tracing by automatically taking photographs throughout the experiment.

2.4 | Soil solution and DOM

The DOM was produced by an extraction method described by Lamparter et al. (2014) using leaf litter originating from the testing site in the Grinderwald beech forest. One hundred grams of air-dried material was immersed in 1 L of deionized water and shaken for 24 h at 20 ˚C. After centrifugation (20 min at 4,000 rpm), the solution was filtered through a 0.45-µm nitrocellulose filter (Schleicher & Schuell). Organic C concentration was measured via Liquitoc (vario TOC cube, Elementar Analysensysteme), as well as the pH, the electrical conductivity, and the ionic composition of the solution (inoLab pH 720, WTW). Original C content of 1600 mg/l was diluted to 100 mg L^{-1} for the experiments. One hundred milligrams per liter were chosen for further experiments on the basis of mean values of measured in situ concentrations measured by on-site observatories at 10-cm depths (Leinemann et al., 2016). Electrical conductivity was 560.8 µS cm^{-1} at pH 7.2.

2.5 | Membrane box

In order to assure connection for liquid percolation through the FCs, percolation blocks were used on top and at the bottom opening of each FC, below designated as membrane boxes (Figure 3).

Aside to connection purposes, the membrane boxes ensured for a uniform distribution of percolates into the FC. The membrane boxes are interchangeable due to their similar design. Two different sizes in height are available for adjusting the membrane precisely to ensure a close contact to the soil. The connecting side facing the soil of the box contains 13 holes 2 mm in diameter, covered by a membrane with a mesh size of 5 µm. As seen in Figure 2, two loops of synthetic string

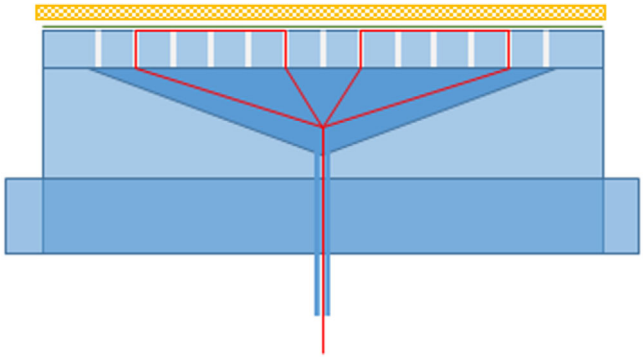

FIGURE 3 Membrane box with mesh (green), string connection (red), and spongecloth (orange)

connect the perforated segment with the solution containing tube. This hydrophilic string consists of a 50% polyamid/50% ployacryl composition and ensures homogeneous liquid distribution to the membrane or draining liquid away from it. In addition, it minimizes the accumulation of water reservoirs and dilution of the effluent during percolation inside the box. Between soil and membrane, a 5-mm-thick sponge layer consisting of a biodegradable resistant mixture of 70% cellulose/30% cotton is situated for membrane protection as well as liquid distribution, ensuring a permanent hydraulic connection between membrane box and soil sample (Figure 4). String and sponge material were tested via ultraviolet–visible spectrophotometry (Varian Cary 50 scan, Agilent Technologies) regarding the outcome of particles or other impurities. No signal alteration in the leachate was detected.

2.6 | General setup of the breakthrough experiment

To test the suitability of our respiration box, we aimed to simulate exemplarily the DOM percolation during the winter period in northern Germany. For this purpose, two respiration boxes were installed inside a refrigerator at 5 ˚C. By installing two respiration boxes, the option of a repetition under the same climatic circumstances at the same time

FIGURE 4 Membrane boxes installed to the flowcell

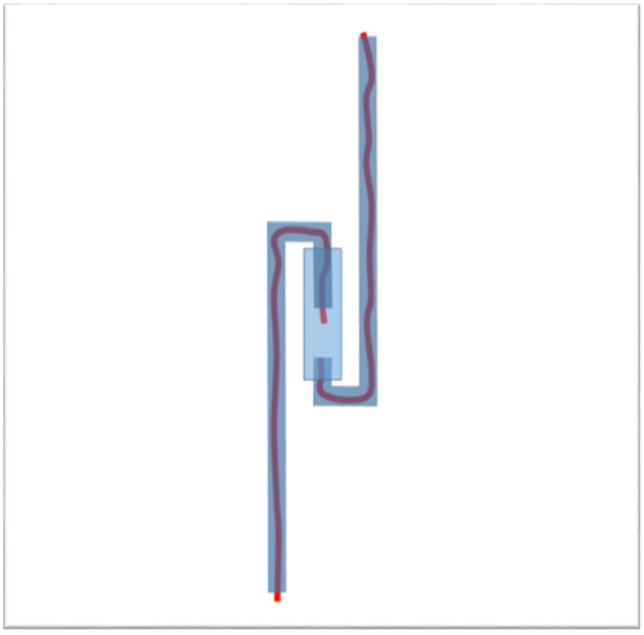

FIGURE 5 Siphon construction. The string (red) is disconnected in a liquid filled tube segment to prevent gas transport

is provided. Additional monitoring of the climate inside the refrigerator was ensured by an additional climate data logger. Prior to the experiment with DOM, a conditioning phase with tap water was performed to assure proper connectivity and inhibit unwanted leakage or water accumulation in the percolation system. After reaching the equilibrium water content and flow stability, the respiration box was sealed.

The DOM solution is stored in small 20-ml vials inside the refrigerator. Small charges of 20 ml were chosen to inhibit or reduce microbial decomposition during the experiment. No changes regarding color and smell of the DOM were notable during the entire duration of the experiment. The DOM was transported in small 0.3-mm-i.d. polytetrafluoroethylene (PTFE) Teflon tubes (Figure 1) to prevent carbon adsorption and minimize transport length and duration. The driving force for DOM transportation was provided by a peristaltic pump (Watson Marlow, Model 202U/1, Watson-Marlow Fluid Technology Group). Yellow Tygon pump tubes suitable for carbon-based liquids, with an inner diameter of 0.25 mm, were used. The small diameter allowed low infiltration rates of 3 ml d^{-1}. The flow rate of 3 ml d^{-1} is four times higher than the average drainage rate at the sampling site; it was decided for this long-term experiment to reduce the experiment duration from the entire winter leaching season to 6 wk. Drainage characteristics as well as water content were monitored from three permanent installed field soil hydraulic measurement stations located closely to the place of soil sampling. After passing the peristaltic pump, the DOM is transported in PTFE tubes into the upper chamber of the respiration box where it infiltrates the upper membrane box and is evenly distributed onto the first FC filled with the topsoil sample. After passing the first FC, the liquid is collected at the lower membrane box and transported via gravitation by the membrane box strings to the following connection tube. To prevent flow interruption by air bubble blockades inside the connecting tubes, polyvinyl chloride (PVC) tubes (3-mm diam.) filled with synthetic string material (50% polyamid/50% plolyacryl) are used. This combination allows liquid to flow through and around the string, which eliminates air blockades, whereas the PVC tube ensures stability and evaporation protection. Since this construct is not gas sealed, a micro-siphon that serves as a liquid trap is attached before each chamber to prevent gas flux (Figure 5). It consists of a string interruption by a thicker tube connection allowing the accumulation of a small amount of liquid on the lower part of this intersection, which blocks gas transport effectively. Through continuous liquid flow from the upper FC, the liquid rises in this connection part until it is in contact with the connecting string located a few millimeters above, permitting the continuing of the flow to lower parts. In an event of higher suction of the lower compartments, the siphon remains filled with liquid right below the

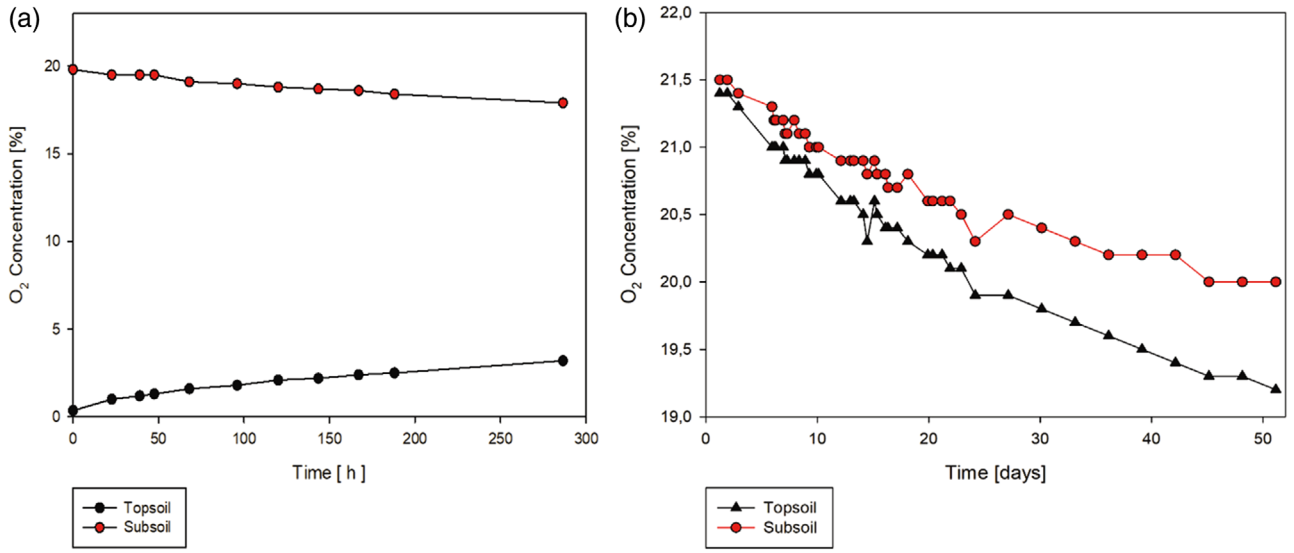

FIGURE 6 (a) Gas sealing test of the respiration box during 287 h and (b) oxygen decrease in both chambers at 5 ˚C

protruding string and still guarantees gas sealing. After the DOM passes into the lower chamber, the abovementioned passing through the FC is repeated with the lower situated subsoil sample. Once the DOM exits the respiration box by a tube string combination, it is collected in parafilm sealed PTFE vials in amounts of 9 ml. The collecting vials are changed every 72 h.

3 | RESULTS AND DISCUSSION

3.1 | Gas sealed test

Both respiration boxes and their corresponding chambers were tested thoroughly for their gas tightness. The difficulty laid down to the sealing onto the surrounding atmosphere as well as the sealing between the two chambers due to the connecting tube. However, regarding the long duration of each experiment, total gas sealing throughout the experiment is not entirely possible. Through every silicone sealed hole as well as the acrylic glass itself, gas exchange can occur during expected months of operation. Nevertheless, a gas tightness status could be achieved apparently suitable enough for proper respiration measurements. In prior gas sealing tests with a maximum concentration gradient of 0.1% oxygen inside the chambers vs. the outer atmosphere of 20.9%, a mean diffusion rate of 0.125 mmol d^{-1} was measured. Related to the inner surface area of the chamber it is equivalent to a diffusion rate of 0.0123 µmol m^{-2} s^{-1} (Figure 6a).

In comparison with the average production of 0.305 µmol m^{-2} s^{-1} carbon dioxide during an experimental run, the maximum loss of 4.03% has to be taken under closer inspection. Figure 6a shows exemplary the slight gas transmission between the topsoil and subsoil chamber with the built in siphon. The topsoil chamber was flooded with gaseous nitrogen while the subsoil chamber remained filled with ambient air. During a period of 22 d, the oxygen amount in the topsoil chamber increased by 2.84% while the oxygen amount in the subsoil chamber fell by 1.9%, resulting in a diffusion rate of 0.129 mmol d^{-1}, which is comparable to the abovementioned sealing tests.

Higher gas tightness might be achieved in further experiments by sealing the siphons tube transitions additionally with silicone, which is subject to further development. Regarding carbon dioxide exchange during real experimental conditions, the loss of carbon dioxide through walls, tubes, and siphons is lower, on average in the range of 0.01% with respect to measured respiration rates. This relates to actual carbon dioxide emissions and considerable lower concentration gradients between the respiration chambers, as well as the gradients to the surrounding open air.

3.2 | Respiration rate

During the duration of 53 d, oxygen concentrations decreased by 2.2% in the topsoil chamber and 1.5% in the subsoil chamber (Figure 6b). Under the presumption of equimolar carbon dioxide production at given aerobic conditions and atmospheric air pressure, the equal amount of 1.21 mmol carbon dioxide in the topsoil and 0.84 mmol carbon dioxide in the subsoil chamber was produced. The decrease in oxygen concentrations started earlier and was greater in the topsoil chamber, presumably due to earlier access of DOM and higher soil organic carbon amount in comparison with the subsoil sample, as well as a higher abundance of microorganisms. By comparing the respiration rates with recently executed experiments with undisturbed FC, the disturbed FC experiment showed higher respiration rates, on average 28%

higher in the topsoil and 23% higher in the subsoil chamber. This difference between disturbed and undisturbed soil samples is probably caused by the homogenized soil matrix in the disturbed soil sample, which allows the equal distribution of DOM, facilitating its access to microorganisms and promoting their free development and distribution everywhere in the soil sample. In undisturbed FC, however, preferential flowpaths in the soil matrix may additionally bypass potential microbial hotspots and exclude them from the nutritious DOM. Corresponding micro-preferential flow path systems within a similar FC system with undisturbed samples from Grinderwald were reported by Krueger et al. (2018).

3.3 | Effluent rate

Regarding the adjusted and estimated effluent rate of 9 ml per 3 d during the pilot experiment, we observed relatively stable effluent rates despite the very low average rate. The mean value of 26 collected vials was 9.05 ml with a standard deviation of 3.03 ml. Small fluctuations might be induced due to stowage accumulation on sponge string interactions or soil induced matric potential varieties, probably also enhanced by atmospheric pressure changes. It is also likely that these fluctuations have originated for several technical reasons like the change in chemical composition of the effluent. However, the experiment showed that the establishment of a continuous solute flux was possible even on the level of very slow flow rates and that unwanted stagnation phases caused by technical reasons can be avoided. This point seems to be important since especially colloid as well as DOM transport and adsorption processes at solid interfaces might be strongly affected by flow interruption phases during the experiment (Carstens et al., 2017, 2019).

3.4 | Temperature

On average, the temperatures were slightly lower than the adjusted 5 ˚C, being 4.68 ˚C for the topsoil chamber and 4.92 ˚C for the subsoil chamber in mean with a standard deviation of 0.68 ˚C. Short term peaks were caused by opening the refrigerator after sampling or replacement of soil solution. Nonetheless, minor temperature fluctuations during the experimental period were in the range of field measured temperatures and should not affect the respiration rates and the decomposition of DOM.

4 | CONCLUSIONS

The concept of a fully controlled breakthrough experimental setup was tested intensively. Environmental conditions as measured on the sampling site could be simulated to a satisfactory level. The combination of realistic moisture level in combination with realistic flux rates in our system is challenging. In our experiment, we could establish a reasonable combination with flow rates of 3 ml d^{-1} in comparison with the approximately 1 mm d^{-1} in the forest. Mass balance with unlabeled DOM was not possible in this early state of testing due to present carbon in the natural FC soil material. However, testing its suitability for upcoming experiments with undisturbed soil samples and ^{13}C-labeled DOM including partitioning of ^{13}C storage pools in the gas, liquid, and solid phase as well as embedded ^{13}C pools into microbial biomass is a subject of our further research. Assessed against currently available information, this approach to mass balance and quantifying carbon turnover and transformation processes has not yet been realized in such a controllable and accessible setup. Moreover, the transparent and flat design of all compartments allows, in contrast with other undisturbed soil cylinder based breakthrough experiments, the visual evaluation of the breakthrough behavior in the soil, if specific dye tracers are implemented. Due to the block-designed sampling chamber, five to six physically undisturbed flowcells can be sampled separated with minimal distance to get as physically similar samples as possible. This concept allows the investigation of replicate samples almost similar as disturbed samples. Summarized, the full potential of the respiration box as a useful device in environmental and soil sciences for all disciplines of soil science research is promising and will be the subject of further investigations.

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AUTHOR CONTRIBUTIONS

Henrik Redweik: Conceptualization; Data curation; Investigation; Methodology; Writing-original draft; Writing-review & editing. Moritz Rahlfs: Conceptualization; Data curation; Investigation; Methodology. Jörg Bachmann: Conceptualization; Funding acquisition; Investigation; Project administration; Resources; Supervision; Validation; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Henrik Redweik https://orcid.org/0000-0003-4425-1314
Jörg Bachmann https://orcid.org/0000-0002-8269-3116

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