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Microcosm experiments for simulation of freeze-thaw cycles and studying methane dynamics in permafrost-affected soils

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Abstract

Long-term methane flux studies in the Lena Delta (Siberia) indicated that back-stored methane adds to the emission of newly produced methane at the beginning of the vegetation period. Further field analysis showed that microbial methane production already occurred at *in situ* temperatures of around -6 °C in the perennially frozen ground. The microbial process of methane production during the back freezing of permafrost soils in autumn and the future fate of produced methane in the thawing phase of the following spring are not well understood so far. Therefore, a permafrost microcosm is developed to simulate the influence of the annual freezing-thawing cycles on the methane fluxes in the active layer of permafrost soils. Two cryostats ensure an independent freezing and thawing from the top and from the bottom of the microcosm to simulate different field conditions. The methane concentration, the soil temperature and the soil water content are analysed in different depth of the microcosm during the simulation as well as the concentration of emitted methane in the headspace of the microcosm. The simulation studies will contribute to the understanding of microbial processes and methane fluxes in permafrost environments in the scope of a warming Arctic.

Introduction

Arctic permafrost environments play an important role within the global carbon cycle because one third of the worldwide soil carbon pool is stored in ecosystems of the northern latitudes (Gorham 1991). Permafrost-affected soils can function both as a source and as a sink for carbon dioxide and methane (see also Chapters 52 and

103). Large areas of permafrost environments are dominated by moist to wet ecosystems as recently shown for the Siberian Lena Delta (Schneider et al. 2009). Under these conditions the mineralization of organic matter can be only realized stepwise by specialized microorganisms of the so-called anaerobic food chain (Schink and Stams 2006) with methane as the final end product.

Methane fluxes from natural wetlands are basically caused by two microbiological processes: (i) methane production by methanogenic archaea in the anaerobic soil horizons (compare Chap. 52) and (ii) methane oxidation by methane oxidizing proteobacteria (compare Chap. 103) in the aerobic soil horizons. In permafrost habitats microbial activity is influenced by extreme gradients in temperature, moisture and chemistry (Wagner 2008 and references within there). In spite of the extreme conditions of permafrost soils, methane production was revealed in incubation experiments with Holocene permafrost deposits down to -6 °C (Wagner et al. 2007).

Nevertheless, there are large uncertainties of methane emissions from tundra environments showing variations ranging between 17 and 42 Tg CH_4 yr-1 (Whalen and Reeburgh 1992, Cao et al. 1996, Joabsson and Christensen 2001). Winter methane fluxes have been estimated so far only in North America and West Siberia (Whalen and Reeburgh, 1988; Dise, 1992; Melloh and Crill, 1996; Panikov and Dedysh, 2000). The reported winter emission rates amounted from about 4 to 41 % of the annual methane fluxes. Friborg et al. (1997) observed a drastic increase of methane release from subarctic mires during the thawing period, which reached approximately 25 % of the mid-summer flux. Recently observed findings suggested that permafrost-associated freeze-in bursts of methane emissions from tundra regions could be an important and so far unrecognized component of the seasonal distribution of methane fluxes from high latitudes (Mastepanov et al. 2008).

However, methane production and oxidation rates during back freezing of the active layer in autumn and the future fate of produced methane in the thawing phase of the following spring are not well understood so far. Thus, the relevance of microbial processes for the Arctic methane budget during autumn and winter time needs further clarification as well as their future development in the scope of a warming Arctic. Therefore, a permafrost microcosm was developed to simulate the influence of the annual freezing-thawing cycles on the methane fluxes in the active layer of permafrost soils. Such experiments can give insights into the natural ecosystem and may yield important clues for the understanding of microbial life under extreme permafrost conditions. The use of undisturbed soil cores keep the original structure, pore system and stratification of the natural soil. The temperatures and water contents measured during the simulation can be adjusted with field data. Thus a direct correlation to the field conditions and the involved processes can be derived. Under this premise, the interaction between microorganisms and soil matrix can be assessed in a reasonable scope. The permafrost microcosm simulates the two freezing fronts (one from the top, one from the bottom), while the centre of the active

layer remains unfrozen and methane production can still continue. Such freezing-thawing experiments can help to answer questions how the microbial population will be influenced by the natural permafrost system and how microorganisms interact with the combined environmental conditions.

Experimental approach

Procedure

Back freezing and thawing of the active layer in permafrost soils and its effect on the microbial carbon turnover can be studied with a so-called permafrost microcosm. The instrument can be used to analyse the in situ methane concentration (or the concentration of other relevant gases like carbon dioxide) in different core depth and the concentration of the emitted methane in the headspace of the microcosm during simulated freezing-thawing cycles. Obtained data are useful for the calculation of methane turnover rates.
The permafrost microcosm can be prepared from undisturbed soil cores including the plant cover, taken from the active layer of permafrost soils by an edged stainless steel tube. The soil core is characterized by the natural structure, pore system and water content. Different states of the active layer (frozen, partly frozen, unfrozen) and varying freezing times can be simulated. The variations of the duration of freezing-thawing cycles and their influence on the microbiological activity can be studied. During the experiment the temperature, moisture and methane concentration should be determined at 5-cm intervals of the microcosm.

Preparing the permafrost microcosm

The permafrost microcosm consists of a Plexiglas tube accommodating the undisturbed soil core (Figure 1). The tube is closed gastight by an upper and lower flange plate. The Plexiglas tube has a length of 30 cm, an inner diameter of 10 cm and a wall thickness of 0.5 cm (all dimensions can be adapted to the corresponding conditions and specific research interests). One side of the tube wall was cut open and the slot was filled with a polyurethane elastomer. In this way, the tube wall can be exactly adjusted to the undisturbed permafrost core by using pipe clamps without influencing the gastightness of the microcosm. The flanges consist of two aluminium plates with a cooling coil in between. The aluminium plates facing the soil core incorporate a rubber seal to make a gastight connection to the Plexiglas tube.

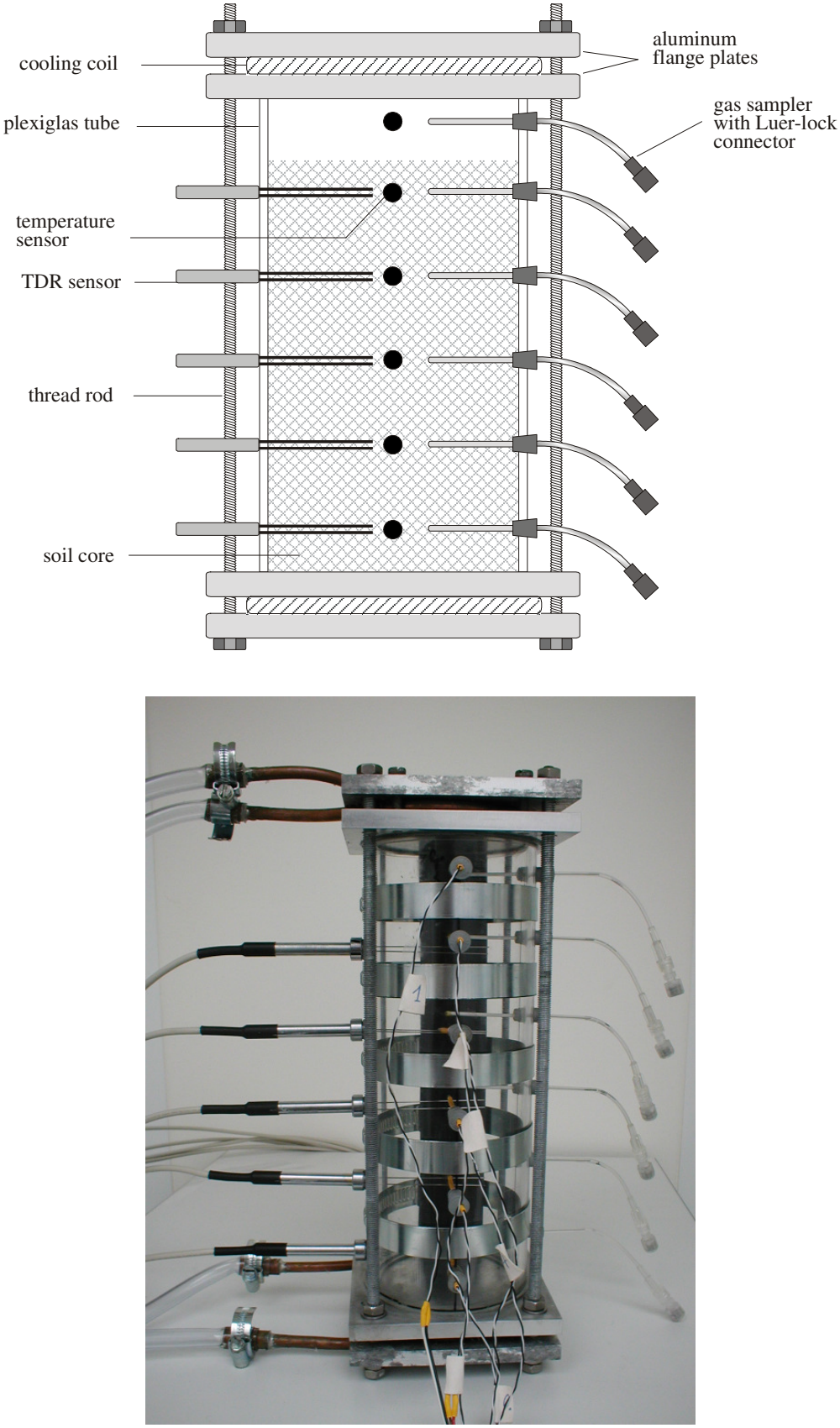

Figure 1: (a) Schematic view of the microcosm for simulation freezing-thawing cycles in permafrost ecosystems (height = 30 cm, inner diameter = 10 cm). (b) Photograph of the microcosm.

For the insertion of temperature sensors, water content probes (TDR), and gas samplers, the Plexiglas tube is prepared with compatible holes. The holes are placed in a distance from 120° around the tube, while the vertical distance between the holes is 5 cm. In order to establish a gastight connection to the Plexiglas tube, the temperature sensors and gas samplers are glued into conical rubber stoppers which are plugged into the holes. The TDR probes have special thread flanges which are screwed into the tube wall and sealed with a polyurethane elastomer.
The installation of the permafrost microcosm is carried out in a cooling chamber at 4 °C. The soil core is transferred to the Plexiglas tube in frozen condition. The core is arranged exactly aligned with the bottom side of the tube and then clamped by reducing the tube's diameter with metal clamps. The flange plates and the tube are then closed gastight using four threaded bolts.
Five temperature sensors and gas samplers are inserted into the frozen soil, after adequate holes were drilled through the openings in the Plexiglas. Additionally, one temperature sensor and one gas sampler are installed in the headspace above the soil core. Finally, the microcosm is left in the cooling chamber to achieve thawing of the soil core. Afterwards, TDR probes are plugged into the soft soil substrate.

Materials and methods

Two Thermo Haake cryostats (C10-K15 and WKL26; Karlsruhe, Germany) are used to freeze the permafrost microcosm by flowing a coolant through the coils in the flanges. Two cooling circuits ensure different cooling rates and temperatures for the flanges at the top and bottom of the microcosm.
Measurements of volumetric water content are carried out by the Campbell Scientific TDR (Time Domain Reflectometry; Longborough, UK) system, which consists of a CR1000 data logger, a TDR 100 reflectometer and a SDM50x multiplexer. The TDR probes used for the simulation are 2-rod, 75 mm LP/ms laboratory probes from EASY TEST Ltd. (Lublin, Poland; Fig. 2a). The distance between the rods is 4.3 mm and the rod diameter is 0.8 mm.
Gas samples are taken with Rhizon soil moisture samplers (Rhizosphere Research Products, Wageningen, The Netherlands; Fig. 2a). The sampler consists of a porous polymer tube of 50 mm length and an outer diameter of 2.5 mm connected to a PVC tube and a septum, which is handmade from Luer-lock components. A stainless steel wire inside the polymer and PVC tubes provides support. The dead volume of the sampler is 0.5 ml. Gas samples can be taken with a gastight syringe through the septum for direct analysis by gas chromatography.
Temperature measurements are made with thin film platinum RTDs (Honeywell HEL-705-U; Fig. 2a)). These sensors have cylindrical ceramic cases of 2.2 mm in diameter and 5 mm in length.

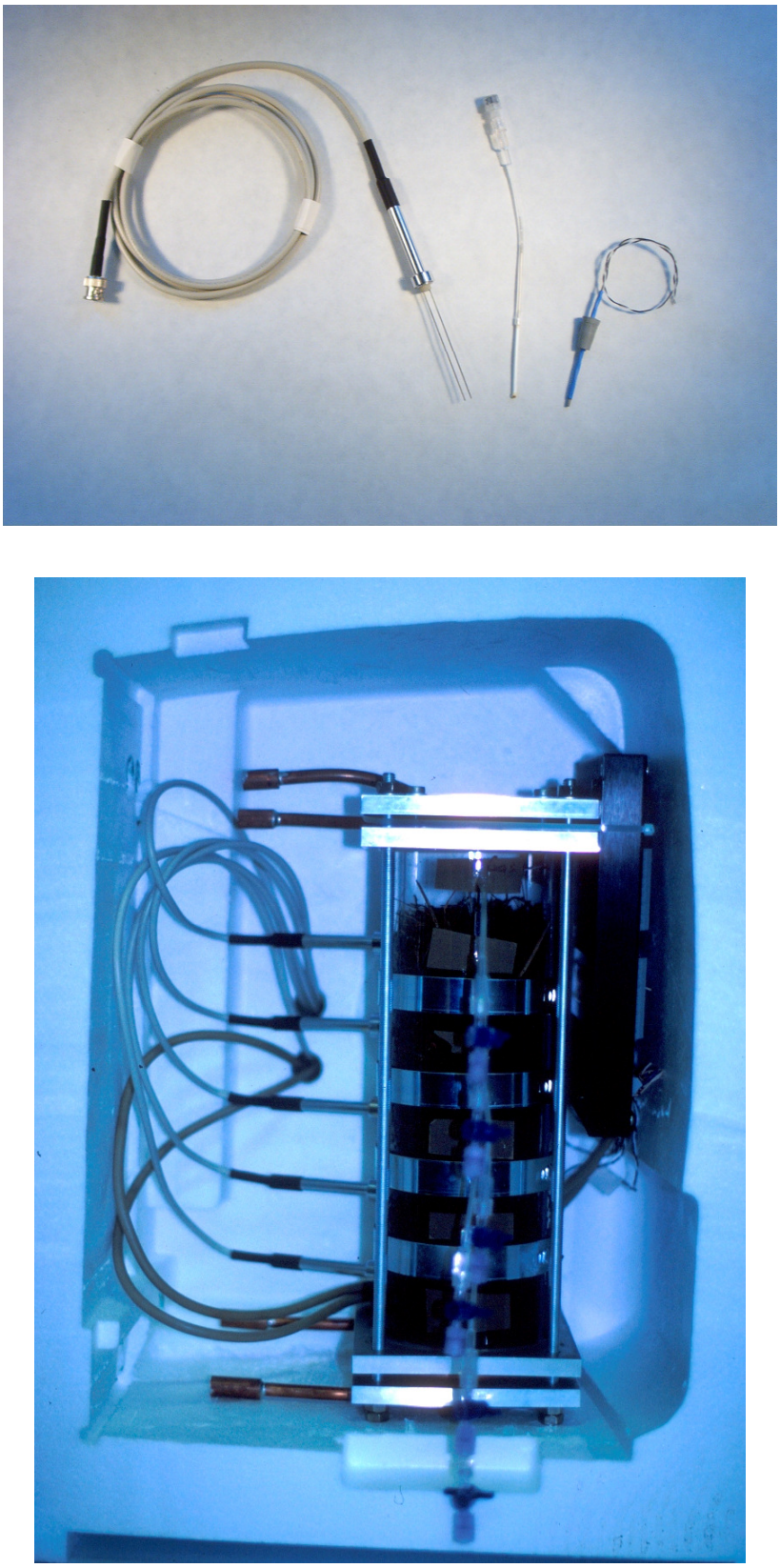

Figure 2: (a) Photographs of the used sensors for moisture, gas analyses, and temperature (from left to right) and (b) the complete system with all installations in the open Styroform box.

In order to simulate the natural freezing-thawing cycles of permafrost soils, the microcosm can be independently frozen downwards and upwards from the top and bottom side. Furthermore, it is possible to adjust the freezing process on the top and on the bottom side of the microcosm to the planned test sequence (see below) by using two separate cooling circuits (Fig. 1).

Time considerations

The simulation experiment begins in unfrozen conditions. Therefore, the permafrost microcosm is incubated in the cooling chamber for about 48 h at 4 °C to calibrate the system. Two different simulation experiments can be performed: (i) the soil is completely frozen and thawed again or (ii) the soil is partly frozen downwards and upwards from the top and bottom side leaving a central zone, which remains unfrozen during the simulation. Another options is to vary the duration of the individual experimental phases. For example, the period of final freezing can be varied, in order to simulate the seasonal influence on the microbial activity during back freezing of the active layer. In general, a simulation cycle consists of three to five freezing-thawing phases to ensure statistical significance of the experiment.
During the experiment in the cooling chamber, the microcosm is kept in a Styrofoam box (Fig. 2b). Measurements of temperature and water content are automatically logged at time steps of 15 minutes. Gas samples are manually taken with a gastight syringe and analysed with a gas chromatograph. A simulation experiment with three freezing-thawing cycles lasts between two and six weeks depending on the soil type and its water content.

Research outlook

Further applications of the permafrost microcosm are the investigation of quasi “in situ” methane production and oxidation activities under different simulated environmental conditions (e.g. variations in soil temperature and soil moisture) by using ^{14}C-labelled substrates (e.g. bicarbonate, acetate or plant material). Furthermore, stable isotope experiments can be accomplished to get more insights into the active part of the microbial communities in permafrost environments.
Apart from the global relevance of permafrost as a large carbon reservoir, this extreme environment is also of particular interest to astrobiological research, as an analogue for extraterrestrial permafrost habitats, which are a common occurrence in our solar system (Wagner et al. 2001). Particularly, the observation of methane in the Martian atmosphere by the current mission of the European Space Agency, Mars Express, (Formisano 2004) has stimulated the debate over possible microbial life on Mars. Recently, it has been shown that methanogenic archaea isolated from Siberian

permafrost environments are more tolerant to environmental stress and simulated thermo-physical Martian conditions than methanogens from temperate ecosystems (Morozova and Wagner 2007, Morozova et al. 2007). In this direction the permafrost microcosm can be used to study the survival and metabolic potential of methanogens or other relevant microorganisms on Martian regolith composed of so-called Martian analog minerals (Poulet et al 2005), which can be filled into the microcosm instead of an undisturbed soil core from permafrost environments.

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