3.4 Microbial process studies on methane fluxes from permafrost environments

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3.4.1 Introduction

The wet lowland areas of Arctic permafrost landscapes are natural sources of the climate relevant trace gas methane. The strength of permafrost, which cover nearly one fourth of the Earth's land surface (Zhang et al., 1999), as a source of methane is a still insufficiently estimated size in the global trace gas cycle.

Subarctic and arctic tundra located above 60° N covers a global area of 1.5 10^9 km^2 (Harris et al. 1993). They represent the largest grouped of natural wetlands with an area of about 26 %. The reported methane emissions of wet tundra varied between 1 to 42 Tg CH_4 per year (Christensen et al. 1995). About 14 % of the global organic carbon is accumulated in permafrost soils (Post et al. 1982). The importance of this carbon pool is discussed regarding an expected climate warming. Especially, the carbon fixation in permafrost soils and the release of climate relevant trace gases like CH_4 and CO_2 due to the carbon decomposition are important for the global carbon budget.

The soil microbiological studies are focused on the seasonal variability of the modern carbon fluxes (CH_4, CO_2), the quantification of the fundamental processes (methane production and oxidation) and the structure and functioning of the microbial communities in permafrost affected soils of the Lena Delta.

During the sixth Expedition to the Lena Delta in summer 2003 the long-term studies on methane emissions from different polygonal tundra sites could be continued. The microbial methane production and oxidation of permafrost soils was studied by additional field experiments. Furthermore soil samples were taken for molecular ecological and geochemical analyses.

3.4.2 Methane emission and microbial methane production

3.4.2.1 Methods and field experiments

The investigation of methane emission as well as process studies of methane fluxes were carried out on Samoylov, a representative island in the Lena Delta.

Daily measurements of trace gas emission (CH_4), thaw depth, water surface and soil temperature were determined from July 13 to October 25, 2003 at a low-centred polygon site. Additional measurement of CH_4 release from the floodplain site on Samoylov was monitored. The used method and the main investigation sites were described previously (Wagner et al. 2003a).

The *in situ* CH_4 production was investigated considering the natural soil temperature gradient and different substrates (H_2, Acetate). At two different times, end of July and end of August, fresh soil material (20 g and 30 g,

respectively) from different soil horizons of the polygon centre and the floodplain site, respectively, was weight into 100-ml glass jars, closed gas-tight with a screw cap with septum and flushed with pure N_2. The prepared soil samples were re-installed in the same layers of the soil profile from which the samples had been taken. Gas samples were taken from the headspace with a gastight syringe and analysed for the concentration of methane by gas chromatography in the field laboratory.

Dissolved organic carbon was extracted at two different times from soil samples of three vertical profiles (polygon centre and border, floodplain). The first extraction occurred at the end of July and the second extraction at the end of August. About each 5 cm fresh soil material (9 g) was taken in July to a depth of 30 cm for the polygon centre, to a depth of 21 cm for the polygon border and to a depth of 38 cm for the floodplain. In August soil samples were taken to a depth of 41 cm for the polygon centre, to a depth of 41 cm for the polygon border an to a depth of 60 cm for the floodplain site.

The samples from each layer were weight into glass flasks (50 ml) and mixed with 45 ml distilled water. The flasks were closed and shaken for 1 h in darkness. Afterwards the suspension was filtered (mesh 0.45 µm, Gelman Science) and the clear solution was inactivated by the addition of sodium acid.

CH_4 and CO_2 concentrations were determined with a Chrompack (GC 9003) gas chromatograph in the field laboratory. The detailed configuration was described previously (Wagner et al. 2003b).

4.2.2.2. Preliminary results

The weather in summer 2003 was changeable from warm and dry to cold and stormy, with strong rain. Therefore, the measuring sites, especially the polygon centre and the floodplain were temporary water-filled and flooded, respectively. The water level for the polygon centre and the floodplain site is shown in Figure 3.4-1 and in Figure 3.4-2. As a result of the wet summer the methane emission of the polygonal tundra site showed varying methane fluxes over the vegetation period with a maximum of about 200 mg CH_4 d^{-1} m^{-2} for the polygon centre, while the dryer polygon border had a relatively constant rate with an average of about 4 mg CH_4 d^{-1} m^{-2} (Figure 3.4-3).

The maximum thaw depth of the permafrost soil was reached in August 2003: The thaw depth of the center was in average 46 cm, the border had a depth of about 47 cm (Figure 3.4-4) and the floodplain of about 66 cm (Figure 3.4-5).

The investigation of *in situ* activity of methanogenic archaea showed CH_4 production at the bottom of the active layer at temperatures around 1°C. Figure 3.4-6 shows the activity from samples of the polygon centre at the end of August, while Figure 3.4-7 and 3-8 demonstrate the activity from samples of the floodplain at the end of July and August.

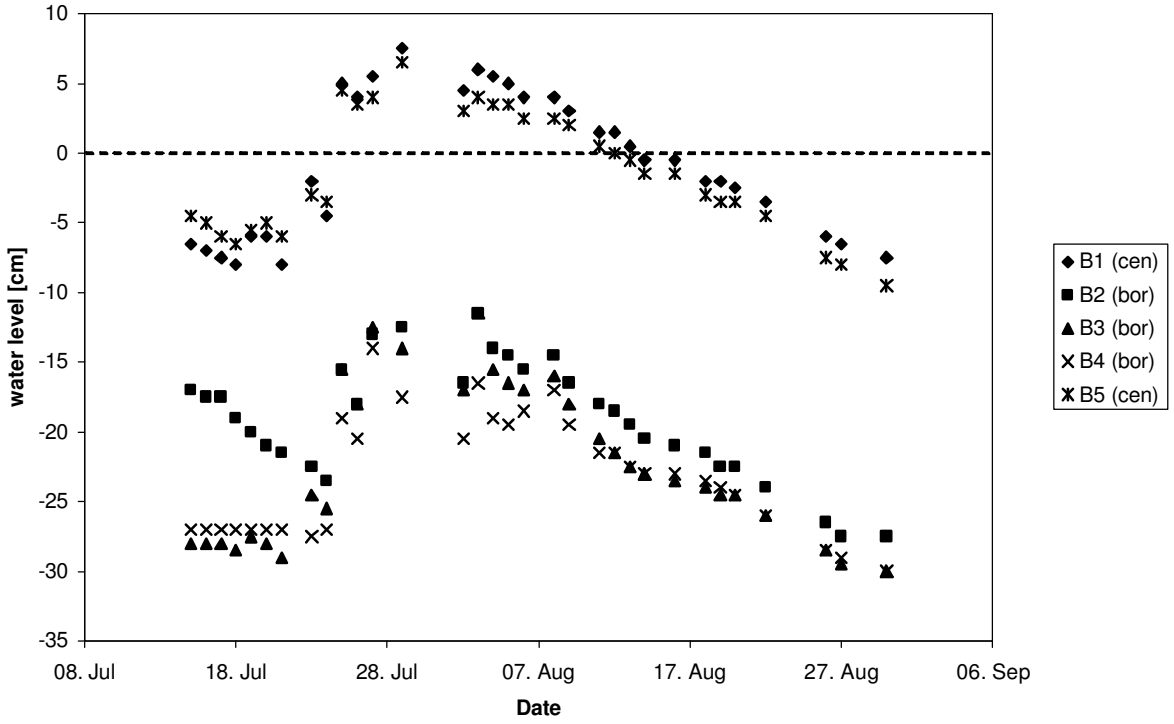

Figure 3.4-1: Water level of a low-centred polygon in summer 2003 (B1 to B5: measuring sites; bor – border, cen – centre)

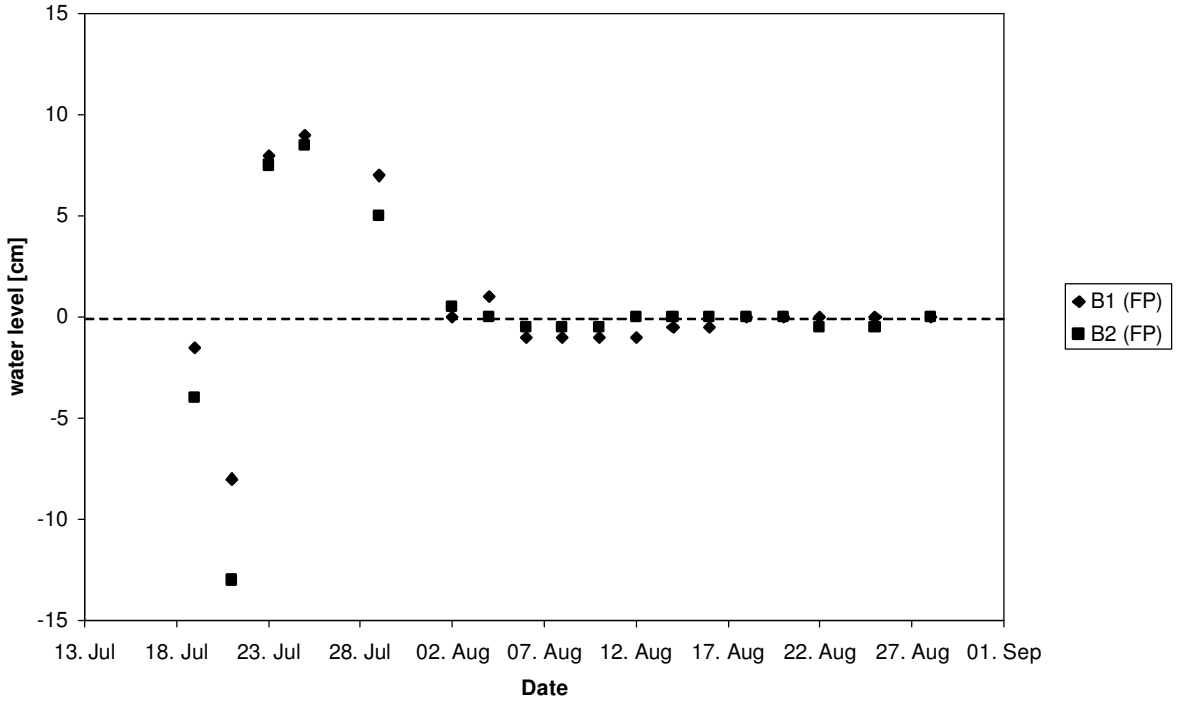

Figure 3.4-2: Water level of the floodplain site in the northern part of Samoylov Island in summer 2003 (B1 to B2: measuring sites; FP – floodplain)

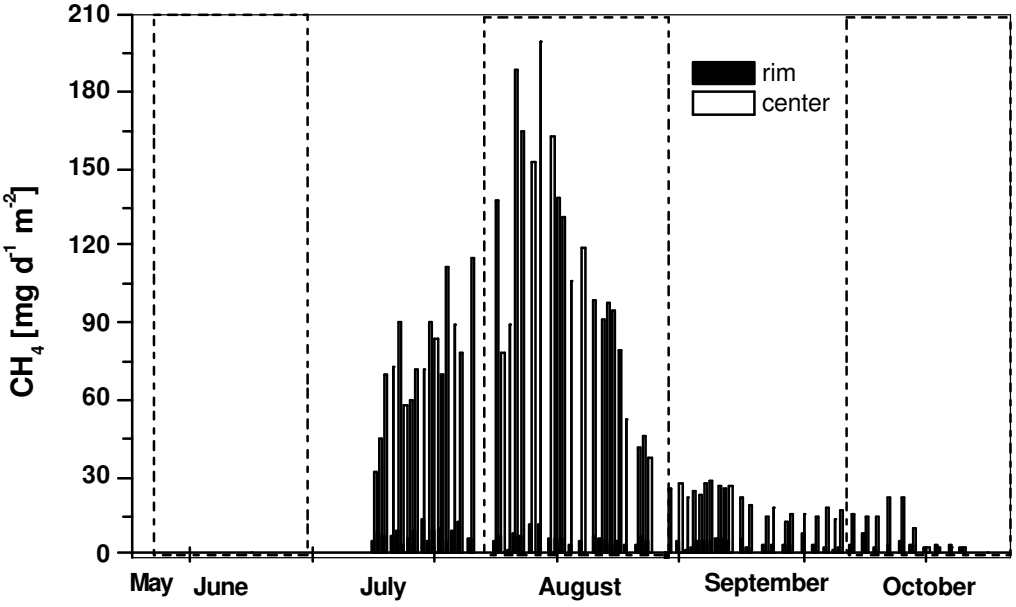

Figure 3.4-3: Methane emission of the low-centred polygonal tundra from July to October 2003.

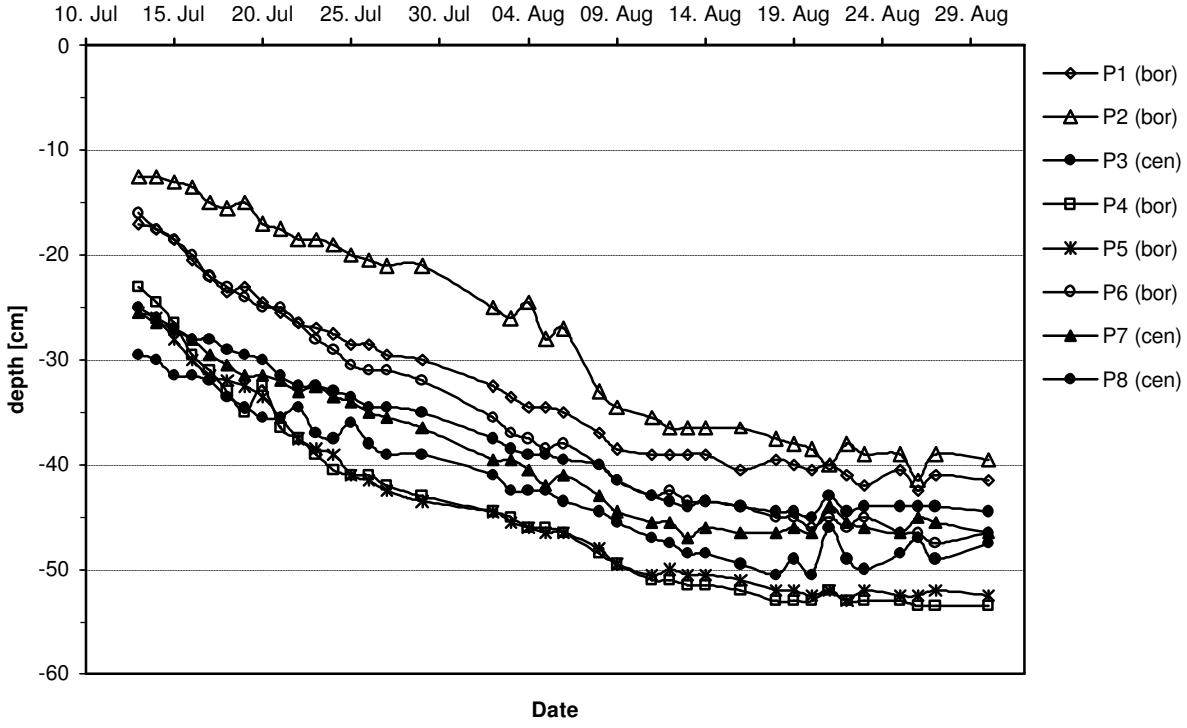

Figure 3.4-4: Thaw depth of a low-centred polygon in summer 2003 (P1 to P8: measuring sites; bor – border, cen – centre)

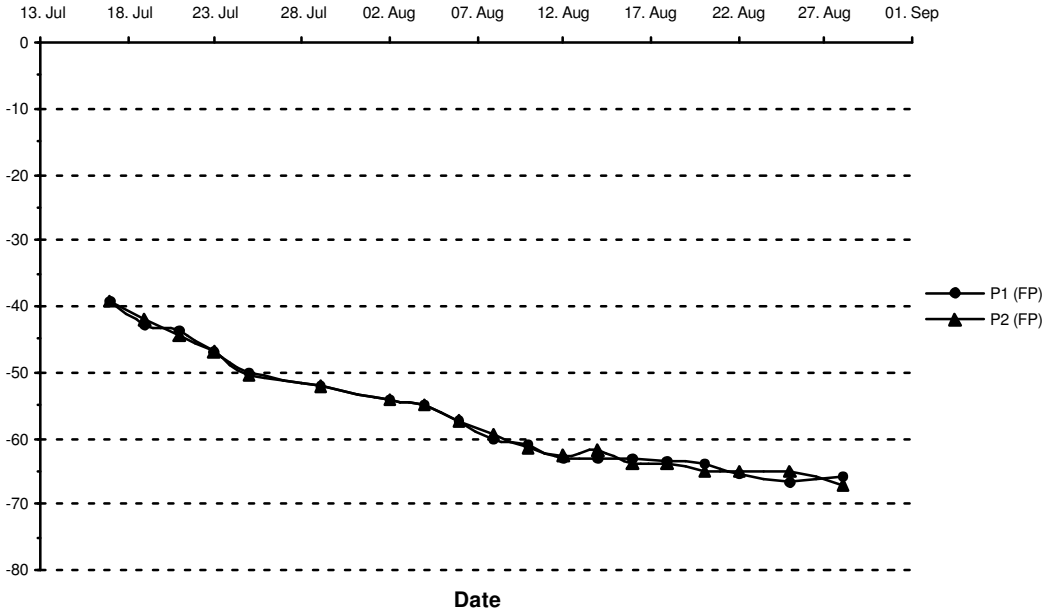

Figure 3.4-5: Thaw depth of a floodplain in the northern part of Samoylov in summer 2003 (P1 and P2: measuring sites; FP – floodplain)

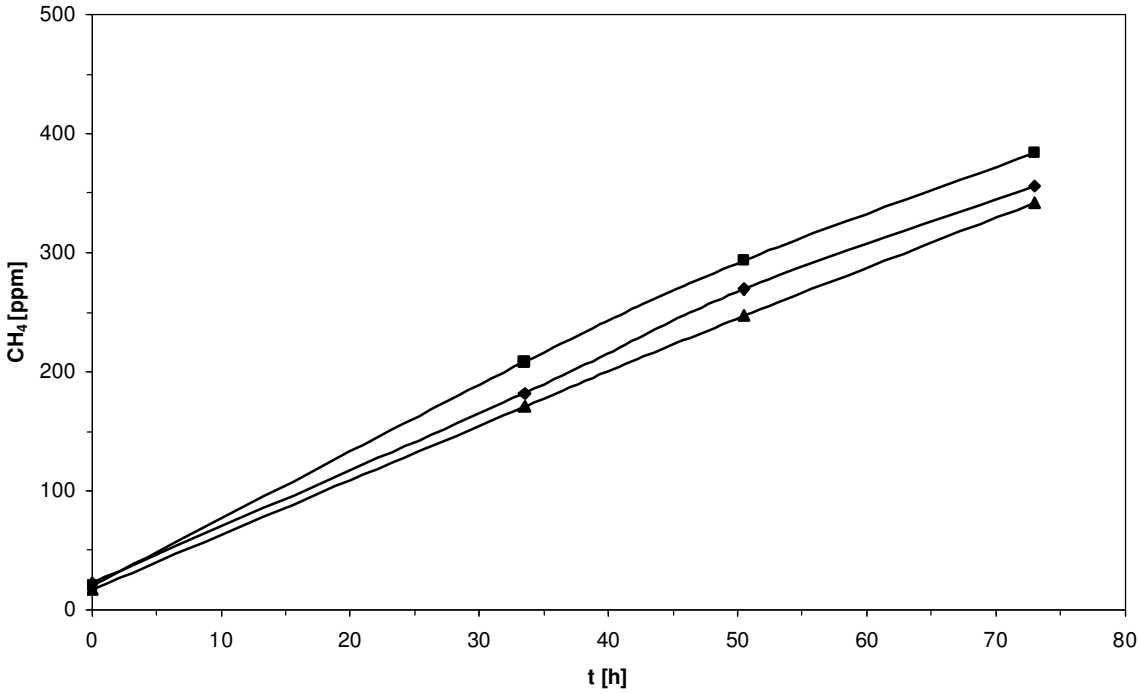

Figure 3.4-6: *In situ* methane production (3 replicates) of the bottom zone of the active layer for the polygon centre at the end of August 2003 (low temperature activity).

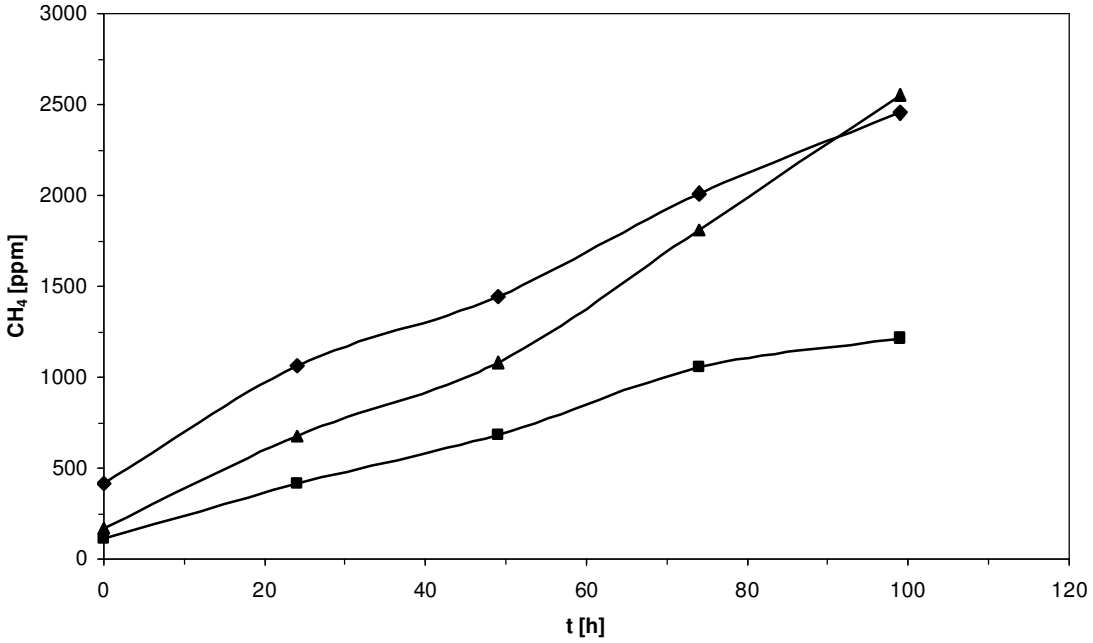

Figure 3.4-7: *In situ* methane production (3 replicates) of the bottom zone of the active layer for the floodplain at the end of July 2003 (low temperature activity).

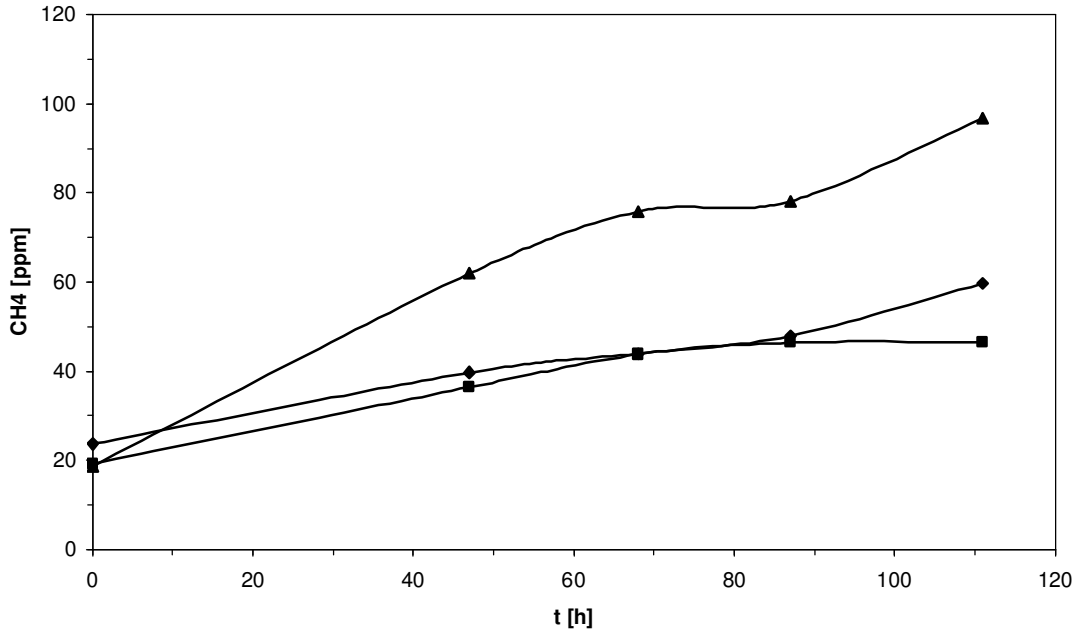

Figure 3.4-8: *In situ* methane production (3 replicates) of the bottom zone of the active layer for the floodplain at the end of August 2003 (low temperature activity).

3.4.3 Process studies on methane oxidation

3.4.3.1 Introduction and objectives

In wetland soils, microbial methane oxidation (methanotrophy) occurs at oxic-anoxic interfaces, which can be found (a) near the water table and (b) in the rhizosphere of vascular wetland plants, where O_2 leaks from the roots into the waterlogged soil. Quantitative estimates of rhizospheric methane oxidation for different northern wetlands vary widely from 0 to 50 % removal of potentially emitted methane (Frenzel and Rudolph, 1998; Moosavi and Crill, 1998; Popp et al., 2000). For wetlands of the Lena Delta, information about plant-associated methane oxidation is still lacking.

The amount of methane oxidation is affected by numerous interrelated factors, one of them being microbial community structure. Therefore, knowledge about the dynamics of methanotrophic population structure in dependence on changing environmental factors helps to understand dynamics of *in situ* methane oxidation rates and consequently methane emissions.

Major questions concerning the microbial methane oxidation were:

- What spatial and temporal differences in methanotrophic population structure can be observed in the course of the summer season, in polygon centre and border?
- How large is the amount of oxidized methane at the roots of wetland plants in waterlogged polygon centers?

3.4.3.2 Methods and field experiments

For the investigation of microbial population structures, soil samples from polygon centre and border were taken in July, August, September and October 2003 (see sample list in Appendix 3-4). These samples were immediately frozen for the transport to Germany. For each soil sampling date important environmental factors were determined, including depth of water table, soil temperatures, soil-pore-water methane concentration and vegetation growth characteristics.

Parts of the samples were prepared in the field for microbial community analyses in Germany: For molecular biological investigations (fluorescent-*in-situ*-hybridization), samples had to be fixed with formaldehyde immediately after collection. Furthermore, parts of the samples were labelled with ^{13}C-enriched methane. A subsequent analysis of ^{13}C-content in biomarker molecules makes it possible to characterize the active methane oxidizing population in soil. For this purpose, fresh soil material (10 g) was incubated with 100 and 1000 ppm of ^{13}C-methane in air in closed 130-ml glass bottles at 0°C (corresponding to *in situ* soil temperature at time of sampling).

Measurements of pore water methane concentration were carried out by placing fresh soil samples together with a saturated NaCl solution into glass jars. After

intensive shaking of the closed jars, methane was forced from the soil solution into the headspace of the bottles and could be analysed by gas chromatography. In the waterlogged polygon centre, soil pore water was additionally collected with a syringe equipped with a steel capillary. Water samples were injected into glass tubes previously filled with saturated NaCl salt solution. Again, methane was forced into the headspace by shaking the tubes and after that analysed by gas chromatography.

In September 2003, root-associated methane oxidation was investigated in a waterlogged polygon centre at the dominant vascular plant species, *Carex aquatilis*. An inhibitor technique was applied as described in chapter 4.5 In addition to a test at single *Carex* culms, closed chambers covering an area of 0.5 x 0.5 m as described by Pfeiffer et al. (1999) were applied for a general test of the technique. For each measurement, 6 chambers were used, 3 of them serving as control. Measurements with the plant flux chambers were carried out with an inhibitor concentration of 0.5 % in the headspace; in the large chambers the inhibitor concentration was varied between 0.5 and 1.5 %.

To investigate methanotrophic population characteristics in late September / beginning of October 2003 (just at the beginning of freeze-back of the soil) potential methane oxidizing activities were determined. For this purpose, samples of polygon centre and rim from different soil depths were analysed. This measurement additionally served as control for a potential activity loss after the frozen samples have been transported to Germany. 5 g of well homogenized fresh soil material was placed in 130-ml glass bottles and incubated with 5000 ppm methane in air at 0°C, which was the approximate *in situ* soil temperature during sampling. Each sample was analysed with 3 replicates. The consumption of added methane was followed by measuring methane concentration in the headspace in regular intervals by gas chromatography.

To consider root-associated methanotrophy, potential activity at fine-root material of *Carex aquatilis* from the depth of 9-15 cm of a polygon centre was determined separately from the remaining soil.

Furthermore, in samples from 3-9 cm depth of a polygon centre the effectiveness of the gaseous inhibitor CH_2F_2, used for the *in situ* inhibitor experiment, was tested at a concentration of 1000 ppm in the headspace, with further incubation conditions as described above for potential activity measurements.

3.4.3.3 Preliminary results

The plant-mediated flux as well as the flux in large closed chambers did not change significantly after addition of the gaseous inhibitor of methane oxidation, CH_2F_2. This result confirms measurements at *Carex aquatilis* in waterlogged soils at Mamontovy Klyk in August 2003 (see chapter 4.5.3). For discussion of possible reasons see chapter 4.5.3

The separate activity measurement of fine-roots and remaining soil from 9 – 15 cm depth in a polygon centre resulted in the following: Firstly, the remaining soil without roots showed higher methane oxidation activity (57 ± 6 nmol CH_4 h^{-1} g^{-1} dry material) than the whole sample including roots from the same depth (38 ± 8 nmol CH_4 h^{-1} g^{-1} dry material). Secondly, roots showed a minor activity of 14 ± 1 nmol CH_4 h^{-1} g^{-1} dry material, indicating only small root-associated methane oxidation *in situ.*

Potential methane oxidation rates at 0°C in samples from a polygon centre are shown in Figure 3.4-9. Water table depth at sampling date was 1 cm below the surface. However, potential methane oxidation could be detected up to a depth of 27 cm with maximal activity some cm beneath the water table.

Samples incubated with 1000 ppm CH_2F_2 and 5000 ppm methane did not oxidize added methane, showing that CH_2F_2 inhibits methane oxidizing bacteria in the investigated soil even at lower concentrations than applied in the field experiments.

None of the analysed samples from the polygon border showed methane oxidizing activity at 0°C.

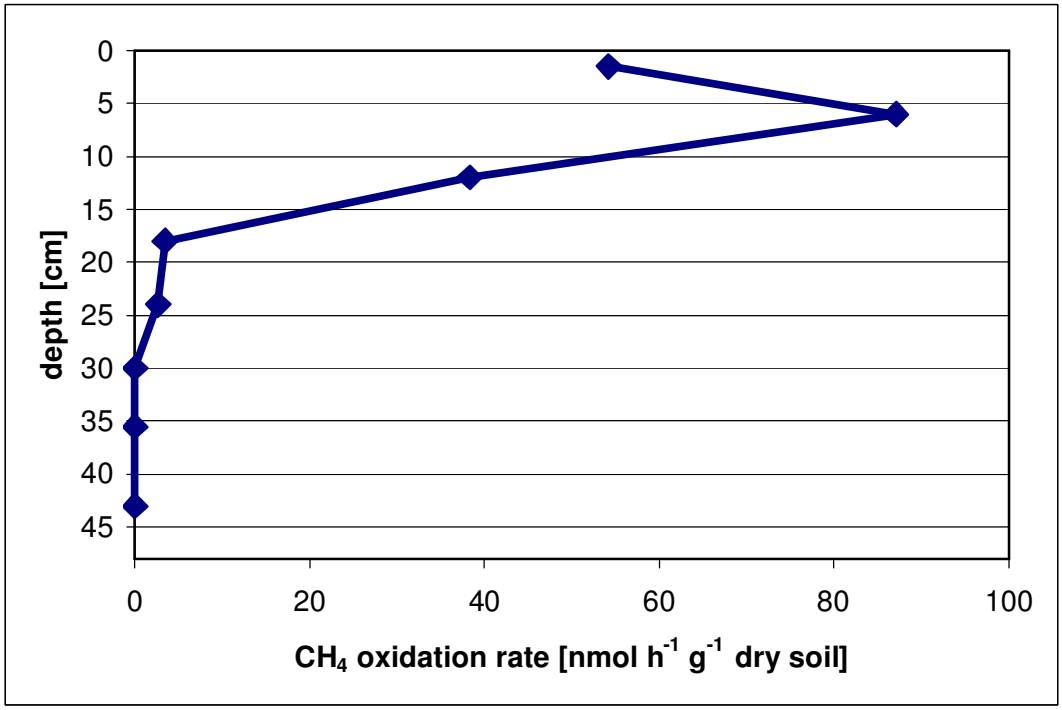

Figure 3.4-9: Vertical profile of potential methane oxidation activity at 0°C, September 2003. *In situ* water table was 1 cm below the soil surface.

3.4.4 Further investigations

The long-term studies on methane fluxes contribute to the understanding of the modern processes of the sensitive tundra ecosystem. They lay the foundation to estimate the impact on possible global climate changes.

The studies will be continued with fresh soil samples from the LENA 2003 Expedition. Especially the analyses of the organic carbon pools, the stable isotope analysis of methane and soil samples as well as the characterization of microbial community structure are still in progress. Furthermore, the isolation and characterisation of methanogenic and methanotrophic microorganisms, which are adapted to the low in situ temperature, is a time-consuming process, which will go on with established and new methods (e.g. fluorescence in situ hybridisation (FISH) and denaturing gradient gel electrophoresis (DGGE), respectively).