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Methane cycling in a drained wetland soil profile

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Abstract
Purpose Peatlands have an important role in methane cycling in the natural environment. Methane emissions as a result of methanogenesis and methanotrophy in soil are affected by several environmental factors such as temperature, oxygen and groundwater level. The objective of this study was to analyse methane cycling as a function of soil depth.
Materials and methods In this study, methane cycling and soil organic matter mineralization were investigated in a drained fen grassland area of Ljubljana marsh, Slovenia that has been subjected to reclamation strategies for several centuries. Potential mineralization, methane production and methane oxidation rates were measured in slurry incubation experiments with soil samples from 10 sampling depths of a 1-m profile. In addition, the extent of iron reduction in the soil was determined.
Results and discussion The potential for methane production was low in the investigated soil profile, even in constantly flooded layers below the water table fluctuations. During anaerobic incubations, the highest accumulated concentrations and production rates of methane were observed in the upper 10-cm layer and the lowest in deeper soil layers, indicating that plant exudates are the main source of energy for heterotrophic soil microbes and that methanogenesis in deeper layers is limited by the availability of appropriate organic substrates. Methane oxidation was on the other hand active throughout the soil profile, suggesting that the potentially active methane oxidizing community is present despite low methane production. The highest abundance and activity of methanotrophs was detected in the water table fluctuation layers.
Conclusions Together, these findings have implications for understanding the biogeochemical function of drained peat soils and emphasize the influence of drainage on quality of soil organic matter and consequently on methane production even in flooded soils.

Keywords Gas emissions · Methane cycling · Methanotrophs · Peat soil · Soil profile · Wetland

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1 Introduction

Natural temperate wetlands, e.g. fens, are flat areas defined by a high level of soil water, supplied by groundwater, and by high quantity of preserved organic matter accumulated as peat (Amon et al. 2002). Undisturbed wetlands are recognized as important reservoirs of carbon and significant participants in greenhouse gas (GHG) emissions. They are generally characterized as sinks for CO_2 and source of CH_4; however, the GHG emission budget of a wetland depends on several biochemical factors, e.g. temperature, pH, climate and agricultural use (Smith et al. 2003; Danevčič et al. 2010). Methane production in undisturbed wetlands is limited to anoxic soil layers, where it is produced microbially by methanogenic archaea. Substrates for methanogenesis in wetlands are mainly final fermentation products of soil organic carbon and methane production, therefore, depends on all preceding steps in soil organic matter (SOM) mineralization. Up to 80% of the produced methane can be oxidized as it diffuses to the soil surface by methane oxidizing bacteria with oxygen as the

terminal electron acceptor (Holzapfel-Pschorn et al. 1985). Net methane emission from a particular wetland is, therefore, regulated by factors affecting both methane production and methane consumption (Stępniewska et al. 2014).

While natural wetlands are important contributors to methane emissions, reclaimed areas emit considerable amount of CO_2 due to the aerobic mineralization of organic matter (Petrescu et al. 2009). Many wetlands were initially exploited for peat and were later reclaimed for agriculture and settlement. Reclaimed wetland soils were highly valued for their productivity but exposure of soil layers to oxygen resulted in higher rates of mineralization leading to soil impoverishment (Hogg et al. 1992). The positive functions of natural wetlands were recognized after the majority of European lowland wetlands were already lost and are only today, together with increasing ecological awareness regaining attention and are subjected to restoration tendencies (Mitsch et al. 2013). Raising the water table generally reduces CO_2 emissions and can lead to an increase in CH_4 release (Wilson et al. 2009). This is, however, not necessarily true in individual cases, what is comprehensively summarized in the paper of Maljanen et al. (2010).

Ljubljana Marsh represents a former lowland raised bog and temperate fen that has been exploited for agriculture, fuel and human encroachment for more than two centuries. The area is still partially and seasonally flooded, while drainage resulted in aerated upper layers of soil and in peat degradation (Mandic-Mulec et al. 2014). Seasonal changes in GHG emissions on Ljubljana Marsh were studied on four sites and showed that overall soil respiration was predominantly controlled by groundwater levels and was comparable to other European wetlands (Danevčič et al. 2010). Methane emissions in this area were very low with seasonally exchanging small net sinks and fluxes. Further, we have found that methane production rarely occurred in the upper soil layer due to the high content of iron oxides and rare periods of anoxia (Jerman et al. 2009). However, preliminary results indicated active methane oxidation in the upper 30-cm layer suggesting methane production at greater soil depths. We predicted that active methane production does occur in deeper soil layers below the water table and methane oxidation prevails in the water table fluctuation zone, where methane and oxygen are available and above. The aim of this study was to define the potential of this soil for CO_2 and CH_4 production and their consequent emission. The majority of studies on drained wetlands explore the upper soil layer; however, when studying mineralization in organic soils, it is important to take into account the contribution of the entire soil profile to overall emissions of GHG. Therefore, we analysed SOM mineralization with emphasis on methane cycling in the complete organic vertical soil profile. Methane cycling was analysed as a function of soil depth by determining potential activities of methanogenic archaea and methane oxidizing bacteria in separate soil layers of one soil profile.

2 Materials and methods

2.1 Sampling site

Soil samples were collected on an experimental field at the Ljubljana Marsh near Tomišelj, Slovenia (45° 58′ N, 14° 28′ E) that has been for more than a decade used for studying nitrogen mineralization, GHG emissions and microbial communities and is described in more detail by Hacin et al. (2001), Jerman et al. (2009), Höfferle et al. (2010) and Danevčič et al. (2010). Briefly, the experimental field is a part of a grassland area of a degraded fen located in the southern region of the marsh, representing a second most common type of soil use after agriculture. Grassland has been established five decades ago and was not fertilized since. Climate in the region is continental with an average annual temperature of 10 °C and an average annual precipitation of 1400 mm. Groundwater is on average 50 cm below the surface, with pronounced seasonal fluctuations between 30 and 90 cm. Redox potential of the soil profile was reported previously (Table 1, Höfferle et al. 2010) and it is in the range of 200–600 mV in the upper soil layers, while it decreases to the range between −230 and 120 mV below 30-cm depth. Ammonium, nitrate and nitrite concentrations are below or at the detection limit throughout the soil profile (Table 1, Höfferle et al. 2010). Soil temperature ranges from 1 to 20 °C annually in the upper 30 cm, while temperature differences in deeper soil layers are less pronounced and vary from 5 to 17 °C below 60 cm. Soils are classified as histosols with 27 to 40% of soil organic material. Peat remains are present in the upper layers while below 1 m mineral and clay strata predominate (Pavšič 1989).

2.2 Soil pore air sampling

Soil air sampling was done in summer months, when the groundwater level was below 1 m. Pore air samples were taken with custom made steel auger (d = 2 cm, inner d = 0.2 cm, h = 2 m; Kambič d.o.o., Slovenia) with attachment for syringe on one end. Samples were collected at each 10-cm soil layer to 1-m depth with 10-ml syringe and transferred to 2-ml vials with rubber septum. Five samplings were done for each depth. Methane concentration in pore air was determined within the same day with gas chromatography as described before (Danevčič et al. 2010).

2.3 Soil sampling and soil management

To reduce the spatial variability across the site, simple sampling strategy was applied. In July 2007, 10 soil cores were collected randomly on a 10 m × 10 m area. The water table was 41 cm below ground level on the sampling day. One metre of the soil profile was sampled with steel gouge augers and divided into 10-cm soil layers. In soil microbial

Table 1 Soil characteristics of 10-cm soil layers in 1-m soil profile

Depth interval (cm)	Water content ($g\ g^{-1}_{dry\ soil}$)	pH	Eh (mV)[a]	Bulk density ($g_{dw}\ cm^{-3}$)	Humus (%)	C_{tot} (%)	$CaCO_3$ (%)	C_{min} (%)	C_{org} (%)	N_{tot} (%)	C_{org}:N_{tot} ratio	S (%)
0–10	0.78 (±0.02)	7.5	384 ± 23	0.53 (±0.06)	34.1	20.2	2.9	0.4	19.8	1.6	12.1	0.11
10–20	0.81 (±0.04)	7.4	10 ± 24	0.61 (±0.01)	30.7	18.3	4.0	0.5	17.8	1.5	11.8	0.13
20–30	1.02 (±0.15)	7.4	1 ± 10	0.49 (±0.05)	29.3	17.7	5.3	0.6	17.0	1.4	12.0	0.09
30–40	1.37 (±0.12)	7.4	−36 ± 10	0.35 (±0.06)	31.2	18.8	5.6	0.7	18.1	1.4	12.6	0.16
40–50	1.93 (±0.04)	7.3	−113 ± 9	0.28 (±0.03)	38.5	22.8	3.9	0.5	22.3	1.5	15.1	0.20
50–60	2.33 (±0.08)	7.3	−175 ± 7	0.28 (±0.02)	41.3	24.5	4.2	0.5	23.9	1.5	16.0	0.23
60–70	2.13 (±0.10)	7.3	39 ± 10	0.28 (±0.02)	37.0	22.1	5.0	0.6	21.5	1.3	17.0	0.20
70–80	2.21 (±0.08)	7.2	−134 ± 8	0.39 (±0.02)	36.6	22.0	6.4	0.8	21.2	1.2	17.2	0.17
80–90	1.88 (±0.03)	7.1	−7 ± 8	0.22 (±0.06)	28.9	18.4	13.4	1.6	16.8	1.1	16.0	0.12
90–100	1.34 (±0.03)	7.2	−25 ± 9	0.18 (nd)	29.4	18.2	9.8	1.2	17.0	1.2	14.2	0.12

The C_{org}:N_{tot} ratio describes the quality of SOM in this soil

nd not determined

[a] H fferle et al. 2010

community studies, it is preferred to use composite samples instead of merged ones, since microbial communities are highly affected by spatial differences that are very pronounced in organic soils (Baker et al. 2009). Our goal was to gain an insight into the common potential for microbial processes in each soil layer; thus, soil sections from the same depth were merged and sieved through a heat sterilized 4-mm brass sieve. Soil chemical analyses were performed on a freshly sieved soil. Soil pH was measured in dH_2O (1:10 *w*/vol) and the water content was determined by oven drying at 60 °C till constant weight. Soil nitrogen and soil organic carbon were determined by dry combustion on a CNS-analyser (Leco CNS-2000, USA). Reduced iron in soil samples was determined by colorimetric analysis as described before (Jerman et al. 2009). Prior to the setup of experiments, sieved soil was kept at 4 °C. Soil characteristics of each soil layer are listed in Table 1.

2.4 Carbon mineralization and the potential for methane production

The mineralization of SOM and methane production were followed in anoxic soil slurries prepared from homogenized soil and sterile distilled water in the ratio 1:1.5 (vol/vol) to a final volume of 6.5 ml in 15-ml pressure tubes. Additional slurries were prepared containing 40 mM 2-bromoethanesulphonic acid (BES) in dH_2O to inhibit methanogenesis (Chidthaisong and Conrad 2000). Six soil slurries were prepared from each soil layer, three for each treatment. After closing the tubes, the atmosphere was exchanged with N_2 and tubes were incubated vertically at 28 °C in the dark for 120 days. Accumulation of CO_2 and CH_4 in the gas phase was measured weekly by gas chromatography as described by Danevčič et al. (2010). Dissolved CO_2 was calculated as described by Stumm and Morgan (1996). Methane and CO_2 accumulation rates were calculated from the temporal increase in the concentration of both gases in the gas phase, using a one-compartment exponential decay model (Murayama and Bakar 1996). The half-life of both rates was calculated by the equation $t_{1/2} = ln(2)\ k^{-1}$, where k is a rate constant for CH_4 or CO_2 accumulation in the gas phase. After incubation for 120 days, tubes were opened and soil slurries were analysed for Fe(II) content and pH.

2.5 The potential for methane oxidation

Aerobic soil slurries were prepared by mixing 10 g of homogenized soil with 30 ml of dH_2O in 150-ml flasks, which were then closed with gas-tight stoppers with rubber septa and incubated under an atmosphere enriched with CH_4 (Sundh et al. 1995). Methane was added to the gas phase to a final concentration of 1% (vol/vol) and the flasks were incubated in the dark on a rotary shaker at 150 rpm and 28 °C. Three soil slurries were prepared for each soil layer. The decrease in methane concentration in gas phase was followed twice daily by gas phase measurements until the complete methane oxidation (5–8 days). Methane concentrations in gas phase were determined by gas chromatography as described before (Danevčič et al. 2010) and potential methane oxidation rate (MOR) was calculated from the slope of the tangent of logistic regression curve describing CH_4 consumption over time.

2.6 Enumeration of methane oxidizing bacteria

The most probable number (MPN) of MOB was evaluated in microtiter plates (Eller and Frenzel 2001). Soil slurry dilution

series were prepared in nitrate mineral salts (NMS) medium (1:1 ratio) (Bowman 2006). For each soil depth, six replicates were prepared; three microtiter plates were incubated at 10% (vol/vol) methane concentration and three without added methane for 1 month at 28 °C in the dark. MPN values and standard errors were calculated using the BAM-MPN calculator (Garthright and Blodgett 2003). MOB MPN was calculated as the difference between the MPN of cells that grew in the presence of methane and the MPN of heterotrophic bacteria determined from growth in the same media without added methane.

3 Results

3.1 SOM mineralization and the methane production potential

Microbial mineralization was assessed by analysis of CO_2 concentration in anoxic soil slurries. Anaerobic production of CO_2 indicates the availability of organic substrates in peat (Murayama and Bakar 1996) and in 120-day experiment mineralized carbon correlates with the quantity of mineralizable organic pool of this soil. After 4 months, the highest CO_2 concentration was detected in the 0–10-cm soil layer (Fig. 1a or Table 2), while the highest CO_2 production rate of 426 nmol CO_2 g^{-1} dry soil day^{-1} was observed in the 30–40-cm soil layer (Table 2). Mean SOM turnover rate was 324 nmol g^{-1} dry soil day^{-1}. Easily available organic matter was mineralized during the first 60 days (upper 30 cm) to 20 days (below 30 cm) approximately, though clear decrease of the curve slope was observed only in samples from 30- to 100-cm soil layers. In these samples, 50% of CO_2 was produced during this first period (Fig. 1a). Most of the soil properties were, on the other hand, similar across the profile (Table 1). The pH values were alkaline (7.3 on average). The average proportion of organic carbon measured was 19.5%; higher concentration of soil organic carbon (19.8%) was measured in the upper 10 cm, and the highest (22.9% on average) in soil layers between 40 and 80 cm, as indicated also by the high amount of bound water and dense appearance of peat remains. Methane concentration in the pore space of the soil vertical profile was low and increased from atmospheric concentration (1.5 ppm_v) detected in the upper soil layers to a maximum of 4.5 ppm_v of CH_4 90 cm below ground (data not shown). The quantity of Fe(II) in the fresh soil did not exceed 50 μmol g^{-1} dry soil, representing maximum 30% of the Fe(II) detected at the end of experiment. At the end of incubation, reduced iron concentration was the highest in the upper 40 cm of soil and in the 90–100-cm soil layer (on average 236 μmol Fe(II) g^{-1} dry soil, respectively; Table 2), and was lower in 50–90-cm soil layers, with an average of 148 μmol Fe(II) g^{-1} dry soil. In the experiment with added BES, no differences in Fe(II) accumulation were observed.

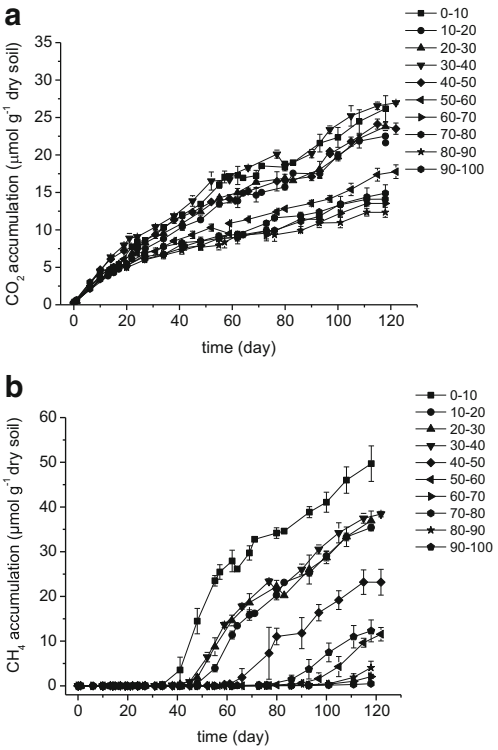

Fig. 1 CO_2 (**a**) and CH_4 (**b**) production in gas phase in soil slurries of different soil depths during 120 days of incubation at 28 °C (±SD, $N = 3$)

Methane accumulation started with a lag period that was the shortest in the upper soil layers and increased with depth to 55 days in 50–60-cm soil layer (Table 2). The highest methane concentrations accumulated in the upper 10 cm (50 μmol CH_4 g^{-1} dry soil) (Fig. 1b or Table 2), where also methane production rates were the highest (1300 nmol CH_4 g^{-1}dry soil day^{-1}) (Fig. 2b). Layers below 50 cm yielded the least methane (5 μmol CH_4 g^{-1} dry soil in 70–80 cm layer) (Fig. 1b or Table 2) with the lowest methane production rates (~250 nmol CH_4 g^{-1}dry soil day^{-1}). The contribution of methanogenesis to the total SOM mineralization was determined by comparing the accumulation of CO_2 with and without an inhibitor of methanogenesis. In soil slurries, where methanogenesis was inhibited, differences in CO_2 production between inhibited and methanogenic samples were significant ($p < 0.05$) in the upper 40 cm of soil (Fig. 3). In these layers, 23 to 26% less CO_2 accumulated in non-methanogenic samples. In deeper layers, the presence of methanogenesis did not affect the production of CO_2. Mineralization rates, however, did not differ between inhibited and methanogenic samples (Fig. 4). When comparing quantities of mineralized carbon to CO_2 and CH_4, twofold more carbon was mineralized to CH_4 in the upper 40 cm; in 40–50 and 90–100 cm soil layer, the quantity of mineralized carbon was equal for CO_2 and CH_4, while in

Table 2 Reduced iron (Fe(II)) content, dissolved and emitted CO_2, CO_2 and CH_4 accumulation rates and lag period prior to methanogenesis for soil slurries of different soil depths during 120-day incubation experiment

Soil depth (cm)	Initial Fe(II) concentration (μmol g^{-1}dry soil)	Final Fe(II) concentration (μmol g^{-1}dry soil)	Dissolved CO_2 (μmol g^{-1}dry soil)	Emitted CO_2 (μmol g^{-1}dry soil	Total CO_2 evolved (μmol g^{-1}dry soil)	Total CH_4 evolved (μmol g^{-1}dry soil)	CO_2 accumulation rate in gas (nmol g^{-1}dry soil day^{-1})	Total CO_2 accumulation rate (nmol g^{-1}dry soil day^{-1})	CH_4 accumulation rate (nmol g^{-1}dry soil day^{-1})	Lag period prior to methanogenesis (days)
0–10	19.3 (±1.5)	239.4 (±33.8)	340 (±12.7)	26.7 (±1.0)	367 (±12.8)	50.8 (±2.1)	366.0 (±6.1)	395 (±6.3)	1353.4 (±179.2)	15.67 (±2.31)
10–20	16.7 (±0.6)	227.8 (±24.5)	249 (±13.4)	24.2 (±1.3)	273 (±13.4)	38.9 (±1.7)	288.0 (±10.1)	316 (±10.4)	858.0 (±44.6)	24 (±0)
20–30	18.6 (±1.6)	234.4 (±31.0)	265 (±12.3)	25.8 (±1.2)	291 (±12.4)	40.3 (±0.6)	314.3 (±13.4)	345 (±13.6)	803.4 (±62.2)	22.67 (±1.15)
30–40	22.4 (±1.9)	241.9 (±26.7)	311 (±3.1)	30.3 (±0.3)	341 (±3.1)	43.7 (±0.2)	388.4 (±22.3)	426 (±22.4)	827.0 (±11.1)	23 (±1.73)
40–50	25.0 (±1.0)	188.6 (±10.6)	222 (±10)	26.6 (±1.2)	248 (±10.1)	26.7 (±3.2)	321.7 (±19.6)	360 (±19.9)	482.1 (±114.5)	40.33 (±4.04)
50–60	33.9 (±1.0)	164.7 (±14.1)	174 (±4.2)	20.9 (±0.5)	195 (±4.2)	15.1 (±1.7)	243.8 (±9.5)	273 (±9.6)	463.8 (±106.1)	56.67 (±4.04)
60–70	38.0 (±0.9)	144.3 (±8.5)	131 (±1.7)	15.7 (±0.2)	147 (±1.7)	6.7 (±0.7)	236.4 (±9.1)	265 (±9.2)	259.8 (±20.2)	45.67 (±16.17)
70–80	41.2 (±3.9)	134.1 (±5.8)	108 (±3.4)	16.0 (±0.5)	124 (±3.4)	5.0 (±1.6)	273.5 (±12.1)	314 (±12.4)	324.0 (±72.9)	55 (±0)
80–90	47.0 (±1.6)	150.2 (±8.2)	77.6 (±4.4)	14.0 (±0.8)	91.6 (±4.5)	8.0 (±0.9)	244.9 (±7.3)	289 (±8.2)	246.7 (±4.9)	38.67 (±4.04)
90–100	27.2 (±2.9)	241.5 (±8.7)	112 (±7.5)	16.6 (±1.1)	129 (±7.5)	16.1 (±2.3)	225.9 (±12.6)	259 (±13.1)	412.3 (±113.3)	43.33 (±4.04)

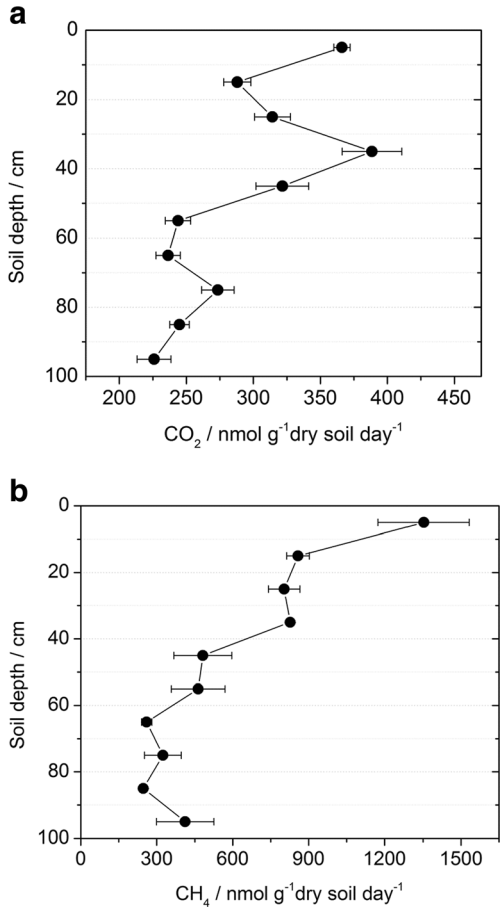

Fig. 2 CO_2 (**a**) and CH_4 (**b**) accumulation rates in gas phase in soil slurries of different soil depths during 120 days of incubation at 28 °C (±SD, $N = 3$)

layers from 50 to 90 cm soil depth, up to three times more organic carbon was mineralized to CO_2. On average, 0.264 mg of carbon was mineralized to CO_2 and 0.299 mg to CH_4 per gramme of soil. The half-life for mineralization of

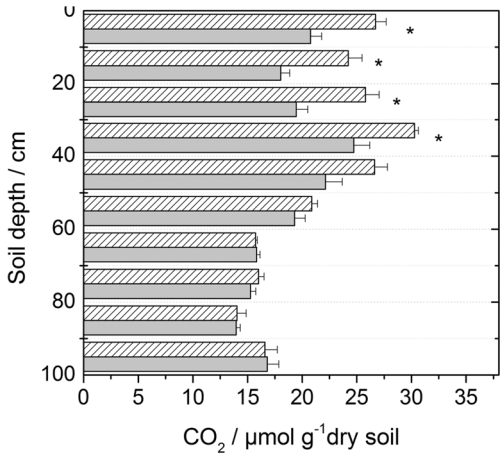

Fig. 3 Net CO_2 accumulation in gas phase in soil slurries after 120 days of incubation at 28 °C in the presence (*striped bars*) and in the absence (*empty bars*) of inhibitor of methanogenesis (BES); $*p < 0.05$

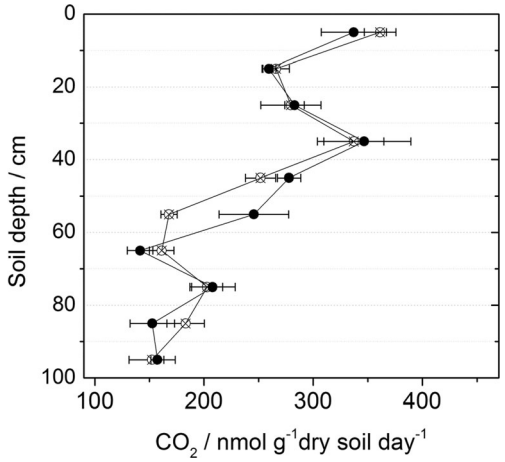

Fig. 4 CO_2 accumulation rates in soil slurries of different soil depths during 120 days of incubation at 28 °C in the presence (*full circles*) and in the absence (*open circles*) of inhibitor of methanogenesis (BES) (±SD, $N = 3$)

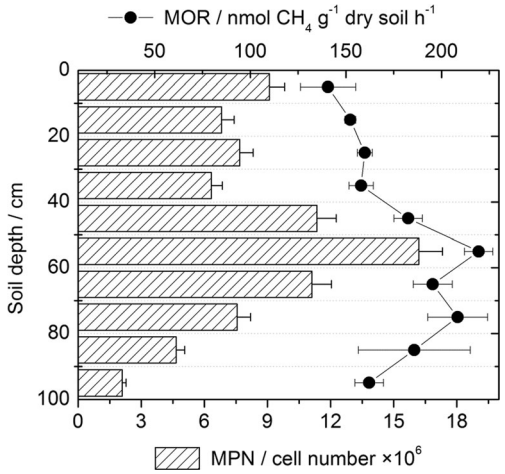

Fig. 6 Methane oxidation rate (MOR; *full circles*) and abundance (MPN; *striped bars*) of MOB in the vertical soil profile (±SD, $N = 3$ (MOR), 8 (MPN))

readily available SOM ranged from 9 to 51 days and through methanogenesis from 6 to 58 days (Fig. 5). When methanogenesis was inhibited, significantly lower half-life values were not characteristic for the upper layers.

3.2 The potential for methane oxidation

In the investigated soil, methane was utilized immediately from the beginning of the incubation; the initial rates of methane oxidation were lower but increased in all soil layers after a maximum of 40 h. Methane oxidation rate was between 150 and 250 nmol CH_4 g^{-1} dry soil h^{-1} (Fig. 6). The potential activity and abundance of methanotrophs were the highest in groundwater fluctuation layer between 50 and 60 cm with 160 nmol CH_4 g^{-1} dry soil h^{-1} of oxidation rate and 1.39 10^7 cells g^{-1} dry soil, respectively (Fig. 6).

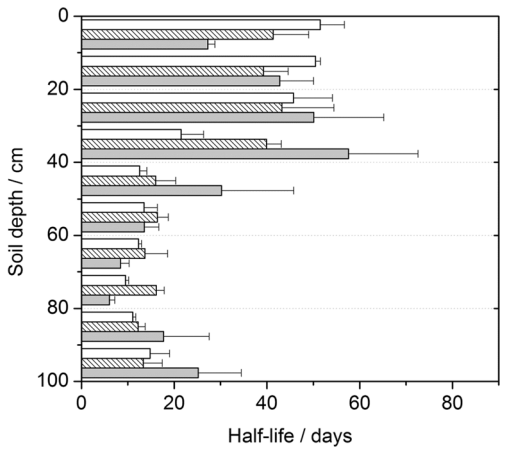

Fig. 5 Half-life for mineralization of readily available SOM calculated from CO_2 and CH_4 accumulation in soil slurries incubated at 28 °C

4 Discussion

Field measurements and previous studies on methane cycling in Ljubljana marsh soil showed low or no methane emissions from the surface soil layer (Jerman et al. 2009; Danevčič et al. 2010). We predicted that methanogenesis takes place in deeper soil layers, where prolonged anoxia supports the complete reduction of available Fe(III) and decreases competition for electron donors by Fe(III) reducers that are abundant in Ljubljana marsh soil (Jerman et al. 2009). However, low methane concentration in the pore space of the soil profile suggested that the majority of methane produced in deeper soil layers was probably oxidized by methanotrophs during the diffusion through the soil profile to the upper soil layers.

In accordance with our previous study (Jerman et al. 2009), the largest outflow of electrons in the soil was through iron reduction in all soil layers, while nitrate reduction was shown to take place within the first day of anaerobic incubation and sulphate reduction was not detected in this soil. Measured amounts of reduced iron in fresh soil were small even in deeper layers, possible because of groundwater fluctuations, allowing re-oxidation of Fe(II) and persistent soil aeration through summer months. Long-term anoxic incubation allowed microbes to reduce the available iron, which is expected to occur in autumn and spring. In general, iron oxides in soil can be considerably resistant to microbial reduction (Lovley 1987). The mean amount of total iron in the investigated soil was around 490 μmol Fe g^{-1} dry soil (Jerman et al. 2009), and microbes were able to reduce about half of it prior to the onset of methanogenesis.

Methane production in anoxic slurries from all soil layers exhibited a lag period and Fe reduction accounts for a large part of the lag that occurred before CH_4 production began. However, the potential for methane production did not increase with depth and was not correlated with the amount of

Fe(III) that microbes were able to reduce. Methane production rates were the highest in the upper 10 cm and were comparable with methanogenic environments, e.g. bogs and freshwater wetlands, where they range from 10 to 120 nmol CH_4 g^{-1} dry soil h^{-1} (Segers 1998; Updegraff et al. 1998; Bergman et al. 2000). Methane production rate and final methane concentration were up to 10 times lower in deeper layers, suggesting limitation of methanogenesis in deeper soil layers by another factor, most probably by available organic substrate.

Homogenization may have influenced soil aeration; especially methanogenesis is thought to be sensible to small oxygen concentration and aeration could cause a delay in methane production. However, methanogenesis recovered relatively fast after the establishment of anoxic conditions, when taking into account the process of iron reduction. After aerobic stress, expected recovery time for methanogens would be in months (Hahn-Schöfl et al. 2011), while in our study, the highest lag phase of 55 days was observed in deeper layers that were generally flooded. Our previous study also showed high resilience of anaerobic microbes in investigated soil that were able to recover immediately after anoxic conditions were established (Jerman et al. 2009).

Differences in the SOM content between soil layers were minor and cannot explain properly the differences in the extent of mineralization between soil layers, pointing to differences in biological accessibility of SOM with depth. Higher values of organic carbon and the highest mineralization rates in the upper 10 cm were most probably due to exudates of overlying vegetation, while in soil layers, between 40 and 80 cm higher amount of peat is present. Nevertheless, these layers yielded the lowest CO_2 concentration, which is consistent with the prediction of differences in bioavailability of organic carbon between the upper and deeper soil layers. On average, 0.3% of organic carbon mineralization to CO_2 and CH_4 over a period of 4 months was calculated, representing the majority of microbially accessible carbon. This is in accordance with other carbon mineralization studies and suggests that the bulk of SOM in wetlands is poorly degradable and only a small proportion of it is easily accessible (Updegraff et al. 1995; Kluge et al. 2008). However, measured values of emitted CO_2 are rather low with respect to the soil characteristics and higher CO_2 values are expected for such soils. Discrepancies could be due to the fact that at observed soil pH values (7.1–7.5, refer to Table 1), evolved CO_2 is expected to be in the soluble form as bicarbonate ion and would not be measured in the head space. Despite the fact that slurries were vigorously shaken prior to headspace measurements, presented CO_2 accumulation rates could be underestimated. Because of that, the extent of dissolved CO_2 was calculated (Table 2) and represents approximately 90% of CO_2 evolved. This would explain low ratios of emitted CO_2 to evolved CH_4 (ratios below 1.0) and the fact that substantial quantity of CO_2 was derived already from the Fe reduction before methanogenesis started. The amount of Fe(II) created in incubations was as high as ~2 μmol per gramme per day, which is enough to produce about 500 nmol CO_2 per gramme per day. Thus, the majority of the measured CO_2 could have been therefore already derived from the Fe (III) reduction. Further, the CH_4 production rates of over 1000 nmol per gramme per day (or approximately 42 nmol per gramme per hour) are quite high and associated CO_2 production rates should be accordingly higher.

The results of this study suggest that methanogenesis contributed to the total mineralization of organic matter only in the upper 40 cm. These findings support the prevalence of acetoclastic methanogenesis in the upper soil layers (Jerman et al. 2009) and show that only the upper layers have the potential for SOM mineralization through methanogenesis.

Despite the low potential for methane production, active methane oxidation was measured in all soil layers. Most MOB in stressful conditions form cysts or exospores and incubation for, e.g. 300 h at elevated quantities of CH_4 is needed prior to growth for induction of MOB in the resting stage (Bender and Conrad 1995). The immediate utilization of methane in the investigated soil suggests that enzymes needed for methane oxidation were already present and active in the soil. The observed potential methane oxidation rates were higher than the rates generally measured for grassland soils (0.1 to 3.1 nmol CH_4 g^{-1} dry soil h^{-1}) (Knief et al. 2003), and lower than for methanogenic sediments (<500 nmol CH_4 g^{-1} dry soil h^{-1}) (Eller et al. 2005). When compared to a drained peat soils from a study of Andert et al. (2012), potential oxidation rates of Ljubljana marsh soil were up to 100 times higher than observed rates in their most methanotrophically active upper layer.

The relatively high number of MOB, estimated by the most probable number method, is difficult to correlate with a low potential for methane production in the soil layers investigated. Methane production is not expected below 1 m, where organic matter concentration is low and clay content is relatively high. However, small quantities of methane could arise from the deeper soil layers (below 150 cm), where peat layers are stored and where methanogenesis might have occurred in the distant past, before reformation of a lake (Andrič et al. 2008). The abundance of MOB might be explained by the concept of anaerobic microniches, providing limited formation of methane to this group of lithotrophic organisms. An interesting study was published in 2010 by Askaer and colleagues using planer optodes for quantifying distribution of O_2 in peat soil on cm scale. They found that at fluctuating water levels, oxic and anoxic zones are present below and above the groundwater level, respectively, stressing the variability of methane consumption and production processes on a microscale. It is also possible that soil methanotrophs are mixotrophs and can also exploit other energy sources beside low concentrations of methane (Ward et al. 2004).

Methane concentrations in the deeper soil layers can be potentially consumed also by anaerobic methane oxidation. Although we did not address this question experimentally in this work, very low concentrations of sulphate (below the detection limit) in the upper soil layer imply that these soils are less likely to support anaerobic methane oxidation through sulphate reduction (Valentine and Reeburgh 2000). Methane could be also anaerobically oxidized by nitrate or nitrite, which are better substrates for anaerobic oxidation of methane than sulphate (Nauhaus et al. 2005; Raghoebarsing et al. 2006). In fact, ammonium oxidizing archaea are present throughout the soil profile and these soils show high rates of aerobic ammonium oxidation (Höfferle et al. 2010), which may occasionally provide needed electron acceptors for anaerobic methane oxidation, especially in the upper layers. However, denitrification in these soils is above all limited by nitrate (Stres et al. 2008) and thus competition for nitrate/nitrite as electron acceptors is expected to be fierce.

5 Conclusions

Previous studies of the Ljubljana marsh methane cycle (Danevčič et al. 2010) indicated low methane emissions throughout the year and preliminary experiments confirmed that active methane oxidation occurs in this habitat. This generated the hypothesis that methanogenesis in the deeper layers of the soil profile provides the substrate for methane oxidisers residing in the upper, more oxygenated soil layers.

This study showed that soil layers below the water level have very low potential for methane production and indicated that active methanotrophic community in the soil may thrive on other substrates. Despite the high Fe(III) content, the accessibility of organic substrate was shown to be an important factor in the development of methanogenesis. The bulk of the organic matter in Ljubljana marsh appeared to be poorly degradable and only a small proportion of organic matter is easily accessible. The highest CO_2 and CH_4 emissions and the shortest lag phase for the CH_4 production occurred in the upper 10-cm soil layer, where the most important source of organic matter are plant exudates and plant litter. This is in accordance with observations that readily decomposable plant exudates can be a major drive for carbon cycle in soil, contributing up to half to the carbon pool (Högberg and Read 2006; Sutton-Grier et al. 2011). Marsh soils are rich in organic carbon; however, the quality of organic matter may be the major driving force of carbon cycle. Indeed, deeper layers that contain a higher proportion of SOM than the surface soil contributed lower emissions of CO_2 and CH_4 and showed the longest lag phase for methane production in anaerobic soil slurries. This strongly suggests that SOM in deeper, anoxic layers of Ljubljana marsh is already extensively processed and only a small proportion can be readily mineralized by microorganism. In addition, iron oxides can contribute to formation of soil aggregates with organic matter, making contact between microorganism and the organic substrate difficult, and raising the activation energy of organic matter and lowering the rate of its degradation.

The once rich soil of Ljubljana marsh can thus be classified somewhere between mineral and peat soils and the potential for organic carbon mineralization reflects the consequences of soil exploitation and its alteration from a fen and bog to meadows and fields. An understanding of soil organic matter pool is important for soil management, and the present study stresses the importance to distinguish between the SOM quality and that only readily available SOM should be considered when evaluating organic soils

Several concerns regarding wetland restoration have been exposed in the past, especially the concern that GHG emissions could increase in certain type of reclaimed wetlands. However, now it is generally accepted that benefits wetlands have for the environment are far more complex than the sole GHG aspect of their functioning (Mitsch et al. 2013). Additionally, as also our study implies, specific drained wetlands have poor potential for becoming a significant methane source and different wetland types have different dynamics of carbon storage and emission (Bernal and Mitsch 2012). High SOM soils are after decades of aeration poorly degradable and increased CH_4 emissions after re-wetting such areas are on account of fresh plant carbon (Hahn-Schöfl et al. 2011).

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