

# Methanogen activity in relation to water table level in two boreal fens

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**Abstract** We studied methanogen activity—measured by *in vitro* methane production potential and by detection of the messenger RNA (mRNA) of a functional gene—in two boreal fens under high and deep water table (WT) level conditions resulting from a rainy growing season and a dry growing season. The depth of the highest CH<sub>4</sub>-producing layers differed between the years. In the wet year, the highest CH<sub>4</sub> production rate was around 20 cm below the mean WT. In the dry year, the highest rates were measured close to the peat surface, well above the mean WT. The distribution of activity in the peat profiles of the two fens appeared to be site specific. Under deep-WT conditions, CH<sub>4</sub> production potential was generally lower

than that under high-WT conditions. Detection of the mRNA of the methanogen-specific *mcrA* gene indicated *in situ* methanogenesis in both water-saturated peat (below the WT) and unsaturated peat (above the WT). Analyses of DNA-derived and mRNA-derived methanogen community structures showed greater similarity between those two in water-saturated peat than in unsaturated peat. This suggested that favorable conditions promoted the activity of most members in methanogen communities, but unfavorable conditions showed differences between distinct community members in adaptation to adverse conditions.

**Keywords** Peat · Methane production potential · *mcrA* · T-RFLP

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## Introduction

Methanogenic archaea (methanogens) produce methane (CH<sub>4</sub>) in the terminal step of the anaerobic decomposition of organic matter. Anaerobic environments favorable for methanogenesis are typically provided in water-saturated environments such as mires. In mires, methanogenic activity is controlled by several factors such as availability and quality of substrates, pH, temperature, and water table (WT) level (e.g., Bergman et al. 1998; Nykänen et al. 1998; Sundh et al. 1994; Valentine et al. 1994). Changes in environmental factors controlling methanogenesis and in methane oxidation activity reflect variations in CH<sub>4</sub> emissions from the ecosystem to the atmosphere (e.g., Furukawa et al. 2005; Inubushi et al. 1998, 2003; McLain and Ahmann 2008; Rinne et al. 2007; Tuittila et al. 2000). Fluctuating WT is characteristic of northern mires due to seasonal and short-term changes in weather conditions.

Generally, anaerobic conditions are present below WT level; consequently, WT level is a major factor regulating methanogenesis, which is a strictly anoxic process. However, due to the heterogeneous, porous structure of peat (Weiss et al. 1998), waterlogged microenvironments in smaller pores may also remain above the WT, providing niches for methanogens to survive under unsaturated, and thereby more aerated, conditions.

Methanogens have adapted to the changing moisture conditions in northern mires. Kettunen et al. (1999) suggested that methanogens stay attached to peat in certain pores rather than float along the WT. Molecular studies have shown stratification of methanogenic taxa in peat profiles (Galand et al. 2002, 2003; Juottonen et al. 2006; Merilä et al. 2006; Putkinen et al. 2009). Vertical distribution of methanogenic activity can be assessed by measuring the anaerobic in vitro CH<sub>4</sub> production potential in different layers of peat profiles. By this method, the highest production rates have frequently been observed in a zone 0–20 cm below the WT (Galand et al. 2002; Juottonen et al. 2005; Kettunen et al. 1999; Merilä et al. 2006; Sundh et al. 1994). Lower CH<sub>4</sub> production potentials in deeper peat layers likely reflect the availability of less suitable substrates for methanogens (Valentine et al. 1994). However, these patterns of vertical distribution of methane production rates and methanogen stratigraphy have mostly been obtained from studies based on one sampling. Therefore, information on the impact of fluctuating WT level on methane production and methanogen stratigraphy is sparse. Under changing climatic conditions, understanding the link between fluctuating WT level and methanogen activity is becoming increasingly important.

In this study, we aimed to assess the impact of WT conditions on CH<sub>4</sub> production potential and to describe the in situ active methanogen community structure under high-WT and deep-WT conditions in two boreal fens. We hypothesized the following:

- Hypothesis 1 The location of the highest CH<sub>4</sub> production potential would follow the position of the WT with decreasing WT level.
- Hypothesis 2 CH<sub>4</sub> production potential would diminish under deep-WT conditions compared to that under high-WT conditions due to the prevailing drought stress in upper peat layers and the poorer substrate quality in deeper layers.
- Hypothesis 3 The structures of an active methanogen community are different under high-WT and deep-WT conditions.

In order to target the effect of WT level, we studied methanogenic activity in two boreal fens under high-WT and deep-WT conditions at the end of the rainy growing

season (2004) and at the end of the dry growing season (2006), respectively. We measured the anaerobic in vitro CH<sub>4</sub> production potential in peat profiles and detected methanogen communities by molecular methods. We assessed methanogens that are active in situ by detecting the messenger RNA (mRNA) of the methanogen-specific gene *mcrA* for the  $\alpha$ -subunit of methyl coenzyme M reductase, which catalyzes the last step in methanogenesis (Thauer 1998). The methanogen community structures were determined by terminal restriction fragment length polymorphism (T-RFLP) analysis of both DNA-derived and mRNA-derived polymerase chain reaction (PCR) products.

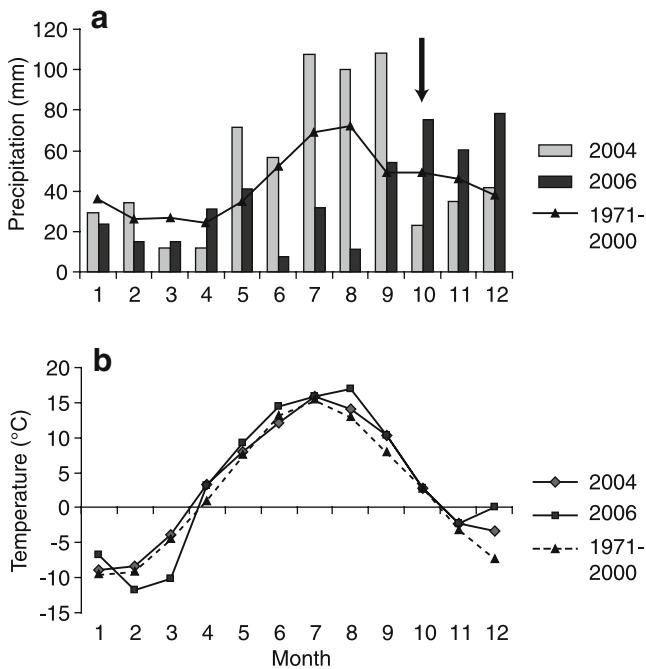
## Materials and methods

### Experimental design

The sampling of peat profiles was carried out in two mires, fens SJ3 and SJ4, located on the Finnish land uplift coast of the Gulf of Bothnia (64°45'N, 24°42'E) at Siikajoki. The sites are characterized by fen vegetation consisting mainly of sedges (*Carex chordorrhiza*, *Carex rostrata*, and *Carex canescens*). The dominant forbs are *Potentilla palustris* and *Menyanthes trifoliata*. The moss layer of both sites is rather dense and consists of *Warnsdorffia* and *Sphagnum* spp. The mean peat depths of fens SJ3 and SJ4 are 50 and 75 cm, respectively.

In order to assess the impact of WT conditions on methanogen activity (determined by the anaerobic in vitro CH<sub>4</sub> production potential) and to describe the structure of an active methanogen community, we collected peat samples from fens SJ3 and SJ4 in October 2004, the wet year (after a rainy growing season), and in October 2006, the dry year (after a dry growing season) (Fig. 1). The peat profiles ( $n=3$  for each mire) were taken with box samplers (8×8×100 and 4×7×70 cm), and WT level was determined at each spot. Sampling was designed to cover moisture-related variation within both mires. In the wet year, the peat profiles were analyzed to a depth of 40 cm, as deeper layers could not be pulled up due to extensive wetness. In the dry year, the profiles from fens SJ3 and SJ4 were analyzed to depths of 50 and 70 cm, respectively. pH was measured in the laboratory from samples suspended in sterile water (peat/water 1:5 vol/vol).

In order to determine the bulk density and organic matter content of peat layer, we collected a set of peat profiles ( $n=10$  for SJ3;  $n=9$  for SJ4) from both mires with box samples in August 2007. The peat profiles were cut at 10-cm intervals, and each volumetric sample was measured for dry mass (105°C) and loss on ignition (content of organic matter, OM%).



**Fig. 1** Monthly average values of **a** precipitation and **b** temperature in the years 2004 and 2006 (data from the Finnish Meteorological Institute) and over a long-term period (1971–2000) (Drebs et al. 2002), collected at the Siikajoki-Revonlahti observation station (WMO 02803, 64°41'N, 25°05'E) located close to study sites SJ3 and SJ4

#### Measurement of in vitro methane production

We measured the in vitro  $\text{CH}_4$  production potential in peat profiles, collected in 2004 and 2006, by gas chromatography, as described by Merilä et al. (2006). In brief, the peat profiles were cut at intervals of 10 cm ( $\pm 1$  cm) and, from each layer, a 15-ml portion was suspended in 30 ml of distilled water in a 120-ml glass bottle. To obtain anoxic conditions, we flushed the peat–water suspensions twice for 1 min with  $\text{N}_2$ , and we sealed the bottles with rubber septa and stored them at 4°C for 3 days. On the fourth day,  $\text{N}_2$  flushing was repeated to remove residual  $\text{CH}_4$  before beginning the incubation experiment. During incubation at 14°C for 168 h,  $\text{CH}_4$  concentrations in headspace were monitored six times (including the starting point) by injecting a 0.5-ml subsample into a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector and a 2-m-long column (inner diameter of 1.5 mm, packed with HayeSep Q 80/100 mesh, 75 ml, no. 483015; Chromatography Research Supplies, Inc., Louisville, KY). Helium was used as carrier gas at a flow rate of 20 ml/min. The temperatures of the injector, detector, and oven were 120, 250, and 50°C, respectively. The rate of  $\text{CH}_4$  production was calculated from the slope of the linear regression given by the increase in  $\text{CH}_4$  concentration over time, and the final results were calculated on volume basis.

#### Analyses of methanogen communities

We targeted the molecular analyses of methanogens to the layers exhibiting the highest in vitro  $\text{CH}_4$  production potential in each peat profile. The *mcrA* gene was chosen as a functional molecular marker for detecting methanogen communities that are present (DNA) and active (mRNA) in the samples.

#### Nucleic acid extraction

Total nucleic acids were extracted from frozen but otherwise untreated peat samples, as described by Korkama-Rajala et al. (2008), with some modifications. Peat samples (0.4 g fresh weight) were homogenized, and cells were disrupted in 400  $\mu\text{l}$  of lysis buffer [75 mM Tris (pH 7.4), 75 mM ethylenediaminetetraacetic acid (pH 8.0), 4.5% sodium dodecyl sulfate, and 1.5%  $\beta$ -mercaptoethanol] by beating with quartz sand for 30 s at 4.5 m/s in a FastPrep homogenizer (FP120A model; Qbiogene, Illkirch, France) and by incubating for 30 min at 65°C. The nucleic acids were extracted from the suspension with phenol–chloroform–isoamylalcohol (50:49:1) and filtered through a polyvinylpolypyrrolidone column to remove impurities such as phenolic compounds. The nucleic acids were precipitated with 0.6 $\times$ vol of solution containing 20% polyethylene glycol and 2.5 M NaCl. The pellet was resuspended in 50  $\mu\text{l}$  of TE buffer and stored at  $-70^\circ\text{C}$ .

#### Reverse transcription

Reverse transcription (RT) was performed as reported by Juottonen et al. (2008). In brief, RNA templates for RT were prepared by degrading DNA from the total nucleic acid extracts with RQ1 DNase (Promega, Madison, WI). Thereafter, 2  $\mu\text{l}$  of RNA preparation was used as template for RT in a 20- $\mu\text{l}$  reaction containing 0.2  $\mu\text{g}$  of the random hexamer primer p(dN)<sub>6</sub> (Roche, Mannheim, Germany), 1 mM dNTPs (Fermentas, Vilnius, Lithuania), 200 U of RevertAid™ H-Minus M-MuLV Reverse Transcriptase (Fermentas), and reaction buffer. The RT amplification procedure followed the protocol suggested by the reverse transcriptase manufacturer.

#### PCR

The partial sequence (ca. 460–480 bp) of the methanogen-specific *mcrA* gene was amplified by PCR with the primer pair of Luton et al. (2002): 5'-GGTGGTGTMGGATTCA CACARTAYGCWACAGC-3' (forward primer MLf) and 5'-TTCATTGCRTAGTTWGGRTAGTT-3' (reverse primer MLr). The forward primer was fluorescently labelled with 5'-carboxyfluorecin to detect the 5'-containing fragments

through T-RFLP. PCRs (50  $\mu$ l) contained 15 pmol of each primer, 200  $\mu$ M dNTPs, 1 U of DNA polymerase, and 1–5  $\mu$ l of total DNA or RT product. The amplification procedure included denaturation at 94°C for 3 min, followed by 40 cycles at 94°C for 45 s, 52°C for 1 min, and 72°C for 1 min. The final extension was 7 min at 72°C.

#### T-RFLP analysis

PCR products were gel purified with Wizard® SV Gel and PCR Clean-Up System (Promega) and digested with *Msp*I (Fermentas) at 37°C overnight. T-RFLP analysis of the digested PCR products was performed as described by Juottonen et al. (2008). Each sample was electrophoresed two or three times. Results are expressed as the percentage of each terminal restriction fragment (T-RF) of the total peak area of the sample. T-RFs that represented >2% of all T-RFs were included in the fingerprints.

#### Statistical analyses

We used linear mixed models to test Hypotheses 1 and 2. In both cases, the fixed part of the model consisted of intercept, year, depth, and site, as well as their interactions (Year  $\times$  Depth, Year  $\times$  Site, Depth  $\times$  Site, and Year  $\times$  Depth  $\times$  Site). Profile, nested within site, was included in the random part, along with error variance. Year was used as a fixed variable because our years described two different WT situations and therefore did not represent random between-year variation. Site was also included in the fixed part since we sampled only two sites and, technically, estimating their difference as random variation would make no sense. Year, depth, and site were applied as categorical variables. Only the depths sampled in both sites were included in the test data.

To test if the location of the highest CH<sub>4</sub> production potential varies between the two years with different WT levels (Hypothesis 1), we used the relative CH<sub>4</sub> production potential as the response variable. To form the relative CH<sub>4</sub> production potential, we first gave a value of 1 for the layer of highest production in each profile. The CH<sub>4</sub> production potential of other layers was expressed as a proportion of the highest production. If our hypothesis would hold, the interaction term Year  $\times$  Depth would have a significant effect on the relative methane production potential.

To test if the CH<sub>4</sub> production potential diminishes under deep-WT conditions compared to high-WT conditions (Hypothesis 2), we used the measured in vitro production values, (ln-transformed to remedy nonnormality), as response variables. If our hypothesis would hold, year would have a significant effect on CH<sub>4</sub> production.

We used SYSTAT 12 for Windows for these analyses. The parameters were estimated with the restricted maximum likelihood method suited for small materials.

To explore variation in methanogenic communities, we applied detrended canonical analysis (DCA), which showed compositional variation to be small (length of gradient, 1.929 SD units). We therefore used a method applying linear response, namely redundancy analysis (RDA), to test whether methanogen mRNA and DNA communities are similar under favorable high-WT conditions and differ under unfavorable deep-WT conditions (Hypothesis 3). We performed RDA using the interactions 2004  $\times$  DNA, 2004  $\times$  RNA, 2006  $\times$  DNA, and 2006  $\times$  RNA as explanatory variables. The significance of the explanatory variables was assessed using the Monte Carlo permutation test. DCA and RDA were performed using the program package CANOCO 4.5 (ter Braak and Smilauer 2002). To quantify the difference between DNA-derived and mRNA-derived T-RFLP data from the same peat samples, we compared them by using the Bray–Curtis similarity index with the program package PAST software version 1.75b (Ø. Hammer and D.A.T. Harper; <http://folk.uio.no/ohammer/past/>). This similarity index describes the similarity between two community samples in a numerical scale from 0 to 1 (a value of 1 indicates 100% identity).

## Results

### Environmental conditions

The yearly precipitation sum was 631 mm in 2004 (dry year) and 442 mm in 2006 (wet year), which both deviate from the long-term average of 521 mm (Fig. 1). The mean WT levels at the time of sampling in the wet year were 6  $\pm$  8 and 5  $\pm$  5 cm in SJ3 and SJ4, respectively. In the dry year, the respective WT levels were  $-39.3 \pm 7.4$  and  $-32.7 \pm 5.8$  cm. The positive and negative values indicate the WT position above and below the peat surface, respectively. Organic matter content, bulk density, and the pH of peat samples are presented in Table 1.

### CH<sub>4</sub> production

The depth of the highest CH<sub>4</sub>-producing layers differed between the years (Table 2). In the wet year, the highest CH<sub>4</sub> production rate in SJ3 was observed 20 cm below the peat surface (~25 cm below the mean WT); in SJ4, the highest CH<sub>4</sub> production rate was observed 10 cm below the peat surface (~15 cm below the mean WT). In the dry year, the highest rates were measured at the peat surface (~39 cm above the mean WT) in SJ3, and 10 cm below the peat surface (~22 cm above the mean WT) in SJ4 (Fig. 2).

The level of CH<sub>4</sub> production potential showed a significant difference between the years (Table 2). However, the course of change seemed to differ between the fens. Under deep-WT conditions, SJ4 showed a generally lower

**Table 1** The average ( $\pm$ SE) organic matter content (OM%), bulk density, and pH of peat from fens SJ3 and SJ4

Depth (cm)	SJ3				SJ4			
	OM%	Density (g/cm <sup>3</sup> )	pH in 2004	pH in 2006	OM%	Density (g/cm <sup>3</sup> )	pH in 2004	pH in 2006
0–10	83.6 (3.0)	0.03 (0.01)	5.0 (0.2)	5.2 (0.5)	90.0 (1.8)	0.03 (0.02)	5.2 (0.2)	5.0 (0.5)
10–20	68.2 (7.3)	0.12 (0.03)	5.0 (0.1)	4.5 (0.2)	88.3 (1.9)	0.07 (0.02)	5.1 (0.2)	4.9 (0.1)
20–30	54.6 (8.5)	0.24 (0.06)	4.9 (0.1)	4.5 (0.2)	89.8 (0.8)	0.09 (0.02)	5.5 (0.1)	5.6 (0.1)
30–40	33.2 (9.2)	0.62 (0.16)	4.8 (0.1)	4.8 (0.7)	89.0 (1.5)	0.12 (0.00)	5.4 (0.1)	5.7 (0.1)
40–50	10.4 (3.8)	1.03 (0.18)	4.4 (0.2)	4.7 (0.5)	91.0 (1.3)	0.13 (0.01)	5.2 (0.1)	5.5 (0.2)
50–60	–	–	–	3.9 (0.1)	93.4 (0.3)	0.10 (0.01)	–	5.5 (0.2)
60–70	–	–	–	–	84.1 (9.6)	0.11 (0.04)	–	5.7 (0.0)
70–80	–	–	–	–	55.1 (15.1)	0.33 (0.14)	–	5.8 (0.0)

CH<sub>4</sub> production potential compared to that under high-WT conditions (Fig. 2b, d). In SJ3, the trend was not that clear (Fig. 2a, c); depthwise examination showed both greater and smaller CH<sub>4</sub> production potentials under deep-WT conditions than under high-WT conditions. The vertical distribution of the CH<sub>4</sub> production potential for the two fens appeared to be site specific (Fig. 2, Table 2).

T-RFLP analysis of methanogen communities

We detected *mcrA* mRNA by RT-PCR in 7 of 12 samples, among which three were taken above the WT. The samples undetectable for *mcrA* mRNA were mostly from the year 2004. We amplified *mcrA* DNA from the corresponding samples and analyzed both types of amplification products

**Table 2** Fixed-effect test results for the relative and absolute CH<sub>4</sub> production potentials in peat cores sampled to a depth of 40 cm in both fens SJ3 and SJ4 in the wet year (2004) and in the dry year (2006)

Effect	df	Relative production		Absolute production	
		F-ratio	p	F-ratio	p
Year	1, 36	2.148	0.151	<b>5.840</b>	<b>0.021</b>
Depth	4, 36	2.074	0.105	1.043	0.399
Site	1, 4	1.781	0.253	3.906	0.119
Year × Depth	4, 36	<b>2.885</b>	<b>0.036</b>	1.871	0.137
Year × Site	1, 36	0.196	0.660	4.479	0.041
Depth × Site	4, 36	3.228	0.023	3.618	0.014
Year × Depth × Site	4, 36	1.404	0.252	1.321	0.281

The interaction term Year × Depth describes the significance of the difference between the depth of the highest CH<sub>4</sub>-producing layer in the wet year and the depth of the highest CH<sub>4</sub>-producing layer in the dry year (Hypothesis 1). The variable year describes the significance of the difference between the level of CH<sub>4</sub> production potential in the year 2004 and the level of CH<sub>4</sub> production potential in the year 2006 (Hypothesis 2)

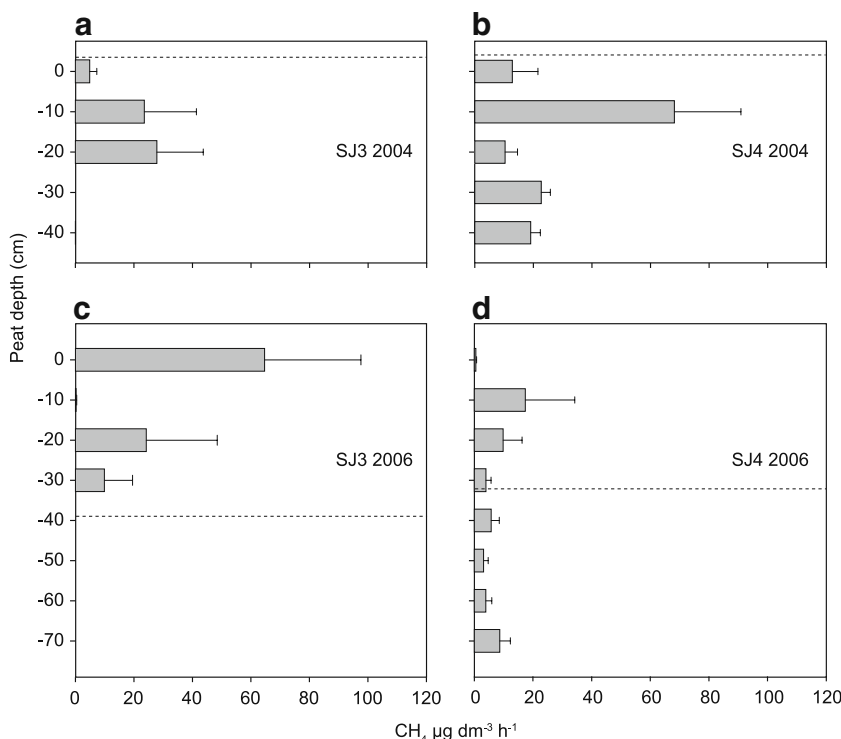
by T-RFLP. The analysis produced 13 T-RFs, with lengths ranging from 105 to 481 bp. mRNA-derived fingerprints constituted of the same T-RFs as the DNA-derived fingerprints, although in different proportional amounts (Fig. 3). This was also seen in the DCA (data not shown), with the first axis being 1.929 SD units in length, quantifying only propositional changes within communities instead of species turnover. Bray–Curtis similarity indices, expressing the combination of qualitative and quantitative similarities between DNA-derived and RNA-derived T-RF fingerprints, were generally higher in samples from water-saturated conditions (SJ3-10a, SJ3-10b, and SJ4-10a in 2004; SJ4-70 in 2006) than in samples from above WT conditions (SJ3-0a, SJ3-0b, and SJ4-10b in 2006) (Fig. 3). According to the RDA of the DNA-derived and RNA-derived T-RF profiles (Fig. 4), the difference between RNA 2006 and the others explained 28.9% of compositional variation. Based on the Monte Carlo permutation test, there was a significant difference ( $p=0.0060$ ) between RNA-derived T-RF profiles from the dry year 2006 and all the other T-RF profiles.

**Discussion**

We studied methanogenic activity in two boreal fens under high-WT and deep-WT conditions in two different years. In the wet year 2004, the mean WT level was above the peat surface in both fens; in the dry year 2006, the mean WT level was several decimeters below the peat surface in both fens at the time of sampling (October). The WT level reflected the weather conditions in the preceding growth period in each year.

Both Hypotheses 1 and 2 (concerning CH<sub>4</sub> production potentials; i.e., under deep-WT conditions, the maximal CH<sub>4</sub> production potential within a peat profile would occur at deeper peat layers compared to that under high-WT conditions, and the overall CH<sub>4</sub> production potential would be lower under deep-WT conditions) were partly supported

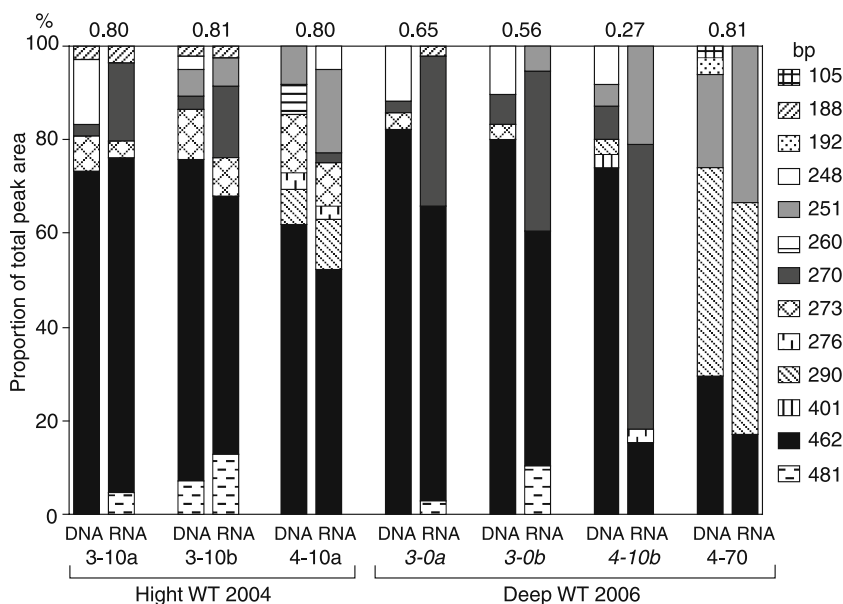
**Fig. 2** Vertical distribution of the in vitro CH<sub>4</sub> production potentials in peat profiles from fen SJ3 (**a** and **c**) and fen SJ4 (**b** and **d**) under high-WT and deep-WT conditions, respectively. The values represent the mean of three replicates in each depth (error bars=SEM). In SJ3, the peat layer is approximately 50 cm thick, and the CH<sub>4</sub> production potential was not determined below that depth. Horizontal dashed lines indicate the average WT level at the time of sampling



by the results, but the CH<sub>4</sub> production potential and its distribution within the peat profile appeared to be site specific. In SJ4, the highest CH<sub>4</sub> production potential receded to deeper layers in 2006 and showed lower CH<sub>4</sub> production values under deep-WT conditions than under

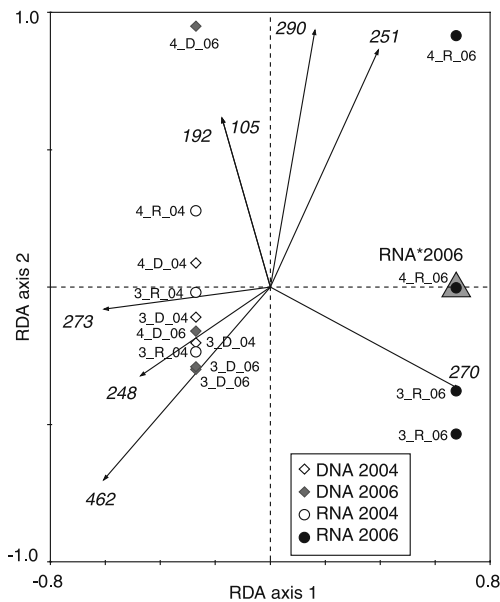
high-WT conditions; in SJ3, both changes occurred in the opposite manner (Fig. 2, Table 2). Thus, the two hypotheses were supported in SJ4, but held weakly in SJ3.

The vegetation providing substrates for decay (litter and root exudates) (Leppälä et al. 2008) and the peat compo-



**Fig. 3** Methanogenic *mcrA* DNA-derived and mRNA-derived T-RFLP profiles from peat layers exhibiting the most active CH<sub>4</sub> production potential under high-WT and deep-WT conditions. Columns represent T-RFs proportional to the total peak area of the T-RFs found in the sample. T-RF sizes are expressed as base pairs. Each column pair comprises the DNA-derived and mRNA-derived profile

of a sample. Decimal numbers over the columns represent the Bray–Curtis similarity indices of each DNA/RNA pair. Samples 3-0a, 3-0b, and 4-10b (*in italics*) were taken from above the WT; other samples were taken from below the WT. Sample names denote the fen (3 for SJ3 and 4 for SJ4) and sample depth (in cm). Letters *a* and *b* denote individual peat cores



**Fig. 4** RDA ordination of methanogen communities in two fens during the wet year (2004) and the dry year (2006). The direction of the *arrow* reflects the increasing relative abundance of T-RF. The relative length of the *arrow* shows how well the ordination describes its abundance

sition in the upper layers (Merilä et al. 2006) are quite similar in both fens. Therefore, variation in vegetation is not a likely course for the differences in CH<sub>4</sub> production potential between the fens. pH values (another important regulator of methanogenesis) were quite similar in both fens (Table 1); in SJ3, pH was, on average, only 0.5 units lower than in SJ4. Hence, we did not consider pH as a probable factor explaining different CH<sub>4</sub> production behaviors between the fens. However, differences in the other physical properties of peat might in part explain the different CH<sub>4</sub> production potential patterns in SJ3 and SJ4 (Table 1). The higher bulk density in SJ3 is due to the relatively high portion of sand mixed in peat. Fens SJ3 and SJ4 have developed as a consequence of primary paludification in a coastal land uplift area. The site of SJ4 has emerged from the sea earlier than SJ3 and has therefore accumulated a thicker peat layer. A higher OM% throughout the peat profile likely contributes to a higher water retention capacity in SJ4 compared to SJ3 and might therefore explain the more evenly distributed CH<sub>4</sub> production profile in the SJ4 peat cores.

Previous studies have shown anaerobic in vitro CH<sub>4</sub> production potentials in unsaturated peat (Kettunen et al. 1999) and even in samples taken from typically oxic habitats (Peters and Conrad 1995). These findings, together with our results regarding high CH<sub>4</sub> production potential in unsaturated peat samples, might indicate recovery of dormant methanogens after anoxia has been attained under laboratory conditions and when lack of endogenous

substrate does not limit activity. The outcome would thus reflect a methodological distortion of the natural situation (no in situ CH<sub>4</sub> production due to aerobically unfavorable conditions). However, a laboratory study on <sup>14</sup>C-labelled methane cycling in soil and a field-based study with <sup>13</sup>CH<sub>4</sub> pool dilution technique indicated that CH<sub>4</sub> production took place in oxic soils (Andersen et al. 1998; von Fischer and Hedin 2002, respectively), and anaerobic microsites in soils were proposed as one explanation for these findings. Peat soils are characterized by a heterogeneous pore structure, with part of the smaller pores saturated with water most of the time (Weiss et al. 1998), providing a wealth of microhabitats where anaerobic organisms can survive and even function. Methanogens may tolerate energy stress and survive under unfavorable conditions without forming spores due to their unique physiological and biochemical properties (Valentine 2007). We showed through molecular methods that in our unsaturated peat samples, the methanogens not only survived but also exhibited activity.

We detected *mcrA* mRNA to indicate in situ methanogenesis in peat. As methanogens obtain energy only by methanogenesis (Thauer 1998) and as the *mcr* operon produces the key enzymes involved in this reaction, it could be assumed that mRNA of *mcrA* would have a rather short half-life; thus, detecting it would indicate in situ activity at the time of sampling. The assumption about the short half-life of *mcrA* mRNA (or the mRNAs of archaea in general) gets support from the few studies so far performed on the subject. The only study on methanogenic *mcr* operon mRNA, carried out by Hennigan and Reeve (1994), showed half-life of 15–60 min, depending on treatment, in *Methanococcus vannielii* suspension cultures. In a genomewide study of two *Sulfolobus* species (hyperthermophilic archaea), Andersson et al. (2006) found a 5-min median half-life of all mRNAs and, in general, the transcripts of the genes involved in energy production decayed faster than the transcripts of those involved in maintaining cellular homeostasis.

We did not track *mcrA* mRNA in all samples exhibiting a high CH<sub>4</sub> production potential. This might indicate residuals of inhibitory substances (e.g., phenolic compounds) in nucleic acid preparations (deep peat in SJ4) or degradation of RNA during storage (three samples from 2004), as well as prove the absence of *mcrA* mRNA in those samples. The T-RF profiles showed some shared and distinctive features between fens SJ3 and SJ4 (Fig. 3). T-RF 462 bp dominated (>60%) all DNA-derived profiles, except for SJ4-70 (30%); however, in the mRNA-derived profiles, it showed a smaller proportionality in SJ4 than in SJ3. T-RF 251 bp was more abundant in SJ4 than in SJ3. Some major T-RFs were only found in either fen: 290 bp in SJ4, and 481 bp in SJ3. Sample SJ4-70 from the deepest layer studied deviated from all other samples (T-RF 290 bp dominated, and T-RF 270 bp was absent), in agreement with previous findings of stratified methanogen

communities in mires. T-RF 270 bp was represented in greater proportional amount in the mRNA-derived profiles of the samples taken from above the WT than in the other samples. These observations were condensed by RDA (Fig. 4). The difference between the two sites is seen on the second axis. The first axis distinguishes mRNA communities obtained from year 2006 samples from all other samples, and T-RF 270 bp is an indicator of the difference.

The results supported our third hypothesis that the structures of an active methanogen community are different under high-WT and deep-WT conditions. In agreement with our findings, recent studies in terrestrial environments indicate that distinct members of stable methanogen communities may exhibit different levels of activity under various environmental conditions. In a boreal fen, ribosomal-RNA-derived methanogen communities showed higher fluctuation and were associated with CH<sub>4</sub> production potential, while the corresponding DNA-derived communities remained relatively stable, suggesting taxa-specific metabolic activity under varying environmental conditions (Juottonen et al. 2008). Watanabe et al. (2009) found great seasonal variation in methanogen *mcrA* transcripts in a Japanese paddy field. They suggested that the prevailing methanogen communities were well established and tolerated changing environmental conditions, and that methanogen communities responsible for CH<sub>4</sub> production varied in the annual cycle. They found considerable amounts of *mcrA* transcripts also under drained field conditions. Conclusively, the results point out the adaptation of methanogen communities to varying environmental conditions prevailing in periodically aerobic habitats.

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