

Development of methanogen communities during a primary succession of mire ecosystems

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Approach and aim of the study

Due to post-glacial rebound, the coastline along the Gulf of Bothnia between Finland and Sweden is continuously rising at a rate of 8-9 mm yr⁻¹. Consequently, primary successional series of mire ecosystems from young sites in the first step of primary peatlandification to

Using this approach, we studied the development of

- potential methane production and
- methanogen *Archaea* communities along a successional transect of peatlands.

The transect and sampling

The ca. 8 km long transect is located in Siikajoki, Northern Ostrobothnia consisting of five peatland sites (Fig. 1). From each site, two or three peat (or peat/mineral soil) profiles were collected with box samplers in September 2003. Peat or mineral soil samples were taken each 10 cm (± 1 cm) of the core under the current water level table (in maximum -0, -10, -20, -30, -40 cm samples per core; Table 1).

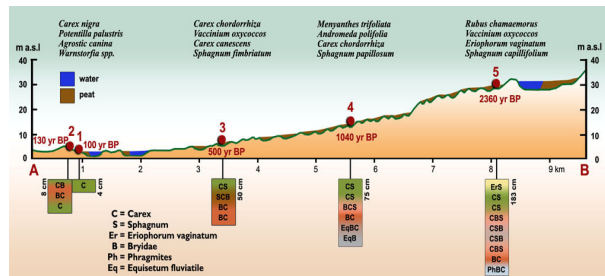


Fig. 1. The profile of the primary successional transect of peatlands consisting of five peatland sites.

Table 1. Loss on ignition (OM%), bulk density and pH of the soil samples analysed (P = peat, M = mineral soil). The values are means of 2-3 observations measured 0, 10, 20, 30 and 40 cm below the water table at the time of sampling.

depth under water table, cm	successional age increases →					← successional age increases					
	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5	SITE 5	SITE 4	SITE 3	SITE 2	SITE 1	
0	M 0.3 2.80 4.8	M 0.5 2.85 8.30	M 0.79 1.94 4.90	P 88.0 0.60 5.80	P 97.8 0.50 4.35	+	+	+	+	+	+
10	-	-	M 0.31 2.22 4.91	P 81.0 0.15 5.81	P 94.1 0.50 4.40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
20	-	-	P 88.8 0.15 5.80	P 97.7 0.50 4.40	-	-	-	-	-	-	-
30	-	-	-	P 80.7 0.12 5.50	P 90.1 0.07 4.95	-	-	-	-	-	-
40	-	-	-	P 90.7 0.09 5.93	P 92.6 0.07 4.40	-	-	-	-	-	-

Table 2. Abundance of the PCR-product obtained from the DNA extracted from the peat/mineral soil samples by amplification with methyl coenzyme M-reductase gene specific primers. ++ = abundant, + = minor, - = none, n.d. = not detected.

depth under water table, cm	successional age increases →					← successional age increases				
	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5	SITE 5	SITE 4	SITE 3	SITE 2	SITE 1
0	+	+	+	+	+	+	+	+	+	+
10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Analysis of methanogen communities

The methanogen communities were analysed by extracting and purifying DNA from peat and mineral soil samples. Purified DNA was PCR-amplified by methyl coenzyme M-reductase (mcr) gene specific primer pair with a fluorescently labeled forward primer. The resulting amplicons from the water table level (-0, all 5 sites included) and 40 cm below (-40, applicable for sites 4 and 5) were selected for further analysis. The amplicons were purified by using WIZARD PCR purification columns (Promega) and aliquots of amplicons were digested with MspI restriction enzyme (+37°C, 2 h). The T-RFLP (terminal restriction fragment chain length polymorphism) fingerprints of three replicates of each community were determined by electrophoresis with a model 310 automated sequencer (Applied Biosystems Instruments). The electropherogram analysis was performed with the Genescan analysis software. The T-RFLP data were ordered by non-metric multidimensional scaling (NMS).

Results

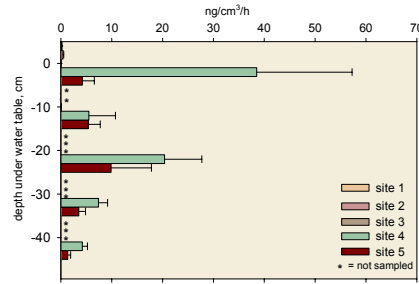


Fig. 2. Potential methane production at depths of 0-40 cm below water table in peat profiles taken along a successional transect of peatlands. The successional age increases from site 1 to 5. Error bars depict SEM (n = 2-3 per site).

CH₄ production was mainly detected just in sites 4 and 5 (Fig. 2) and showed the highest rates at depths of 0-20 cm under the water table at the time of sampling. The younger sites 1, 2 and 3 showed very low if any CH₄ production during the incubation experiment (Fig. 2).

The PCR-amplification resulted in a detectable PCR-product at every depth sampled (Table 2). The T-RFLP fingerprinting resulted in 15 terminal restriction fragments (T-RFs, groups a-o). Their relative abundance along the transect is presented in Fig. 3. The T-RFLP fingerprint patterns of methanogen communities in the two youngest sites were very similar at water table level and consequently, they were grouped together in NMS configuration (Figs. 3 and 4). The fingerprint patterns from the water table level (sites 4-0 and 5-0) and 40 cm below (sites 4-40 and 5-40) were distinctively different from each other showing a depth-related distribution. The upper layer of ombrotrophic site 5 showed very low diversity of methanogens and was the most separated sample in NMS configuration. The dominant T-RF group in site 5-0 is likely to be the same as the one found in an other ombrotrophic site in Lakkasuo in

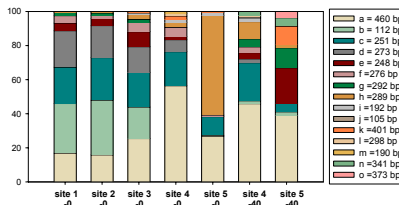


Fig. 3. The relative abundance of terminal restriction fragments of PCR amplified methanogen communities obtained by digestion with MspI restriction enzyme. The samples were taken along a successional transect of peatlands at the depth of water level table at the time of sampling (-0) and 40 cm below (-40). The successional age increases from site 1 to 5. bp = length of terminal fragment in base pairs.

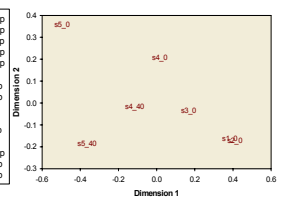


Fig. 4. Two-dimensional solution of NMS ordination of T-RFLP fingerprints of the samples (cf. Fig. 3). Bray-Curtis distance was applied as a measure of dissimilarity. Stress factor of the solution = 0.1071.

Measurement of potential methane production

For measurement of the potential CH₄ production (= the maximum CH₄ production measured in the laboratory under defined temperature conditions), peat or mineral soil samples were added to infusion bottles containing distilled water. The bottles were flushed with nitrogen in order to obtain anoxic conditions, sealed and stored in +4 °C. Before the start of incubation experiment, the bottles were flushed with nitrogen and kept unshaken in +14°C in the dark for 22 h. During the incubation experiment of 70 hours, four subsamples were taken from the headspace and analysed for methane concentration by gas chromatograph. The rate of CH₄ production was calculated from the slope of the linear regression given by the CH₄ concentration increase over time.