

**Figure S1.** (A) Detrended correspondence analysis (DCA) of vegetation and principal component analysis (PCA) of peat chemistry at the depths of (B) 0-7.5 cm and (C) 7.5-15 cm of natural (N1-N3) and restored (R1-R3) peatlands. Environmental variables with >0.5 correlation

with the axes are included (grey arrows) but do not affect the placement of the study sites. WT, water table level; BD, bulk density; DOC, dissolved organic carbon; temp, peat temperature at the depth of 5 cm; CH<sub>4</sub>, mean emission rate for the growing season . AP *Andromeda polifolia*; BN *Betula nana*; CP *Calamagrostis purpurea*; CS *Calliergon stramineum*; CC *Carex canescens*; CH *C. chordorrhiza*; CL *C. lasiocarpa*; CI *C. limosa*; CM *Carex magellanica*; CR *C. rostrata*; DC *Deschampsia cespitosa*; DP *Dicranum polysetum*; EF *Equisetum fluviatile*; EA *Eriophorum angustifolium*; EV *Eriophorum vaginatum*; JF *Juncus filiformis*; LP *Ledum palustre*; LT *Lysimachia thysiflora*; MT *Menyanthes trifoliata*; PA *Picea abies*; PS *Pleurozium schreberi*; PC *Polytrichum commune*; PP *Potentilla palustris*; RC *Rubus chamaemorus*; Salix SA *Salix aurita*; SH *Salix phylicifolia*; SZ *Scheuchzeria palustris*; SY *Sciuro-hypnum* spp.; SN *Sphagnum angustifolium*; SF *S. fallax*; SG *S. girgensonii*; SM *S. magellanicum*; SP *S. papillosum*; SR *S. riparium*; SU *S. russowii*; TR *Trichophorum cespitosa*; VO *Vaccinium oxycoccus*; VU *V. uliginosum*; VVV. *vitis-idaea*.

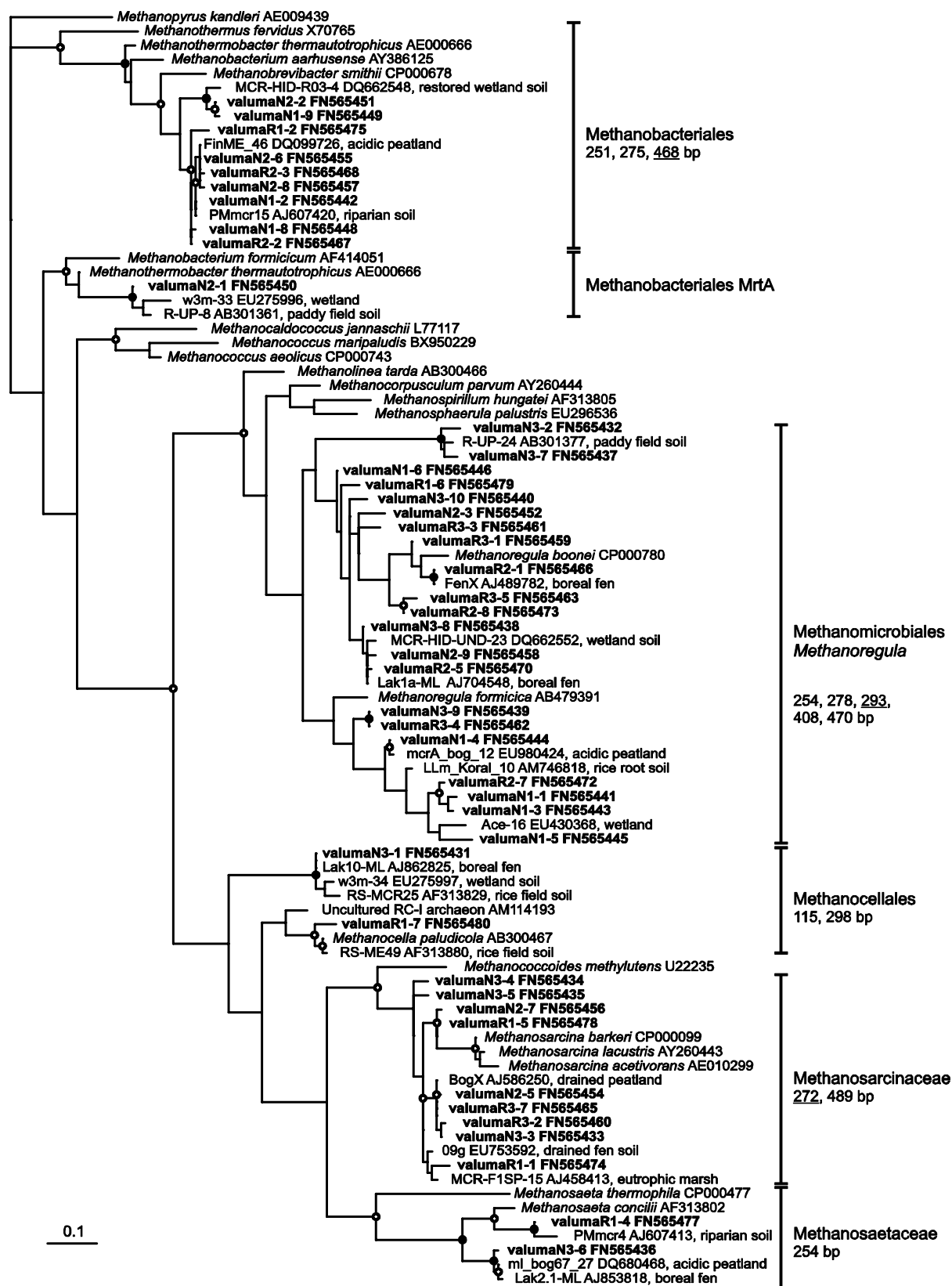
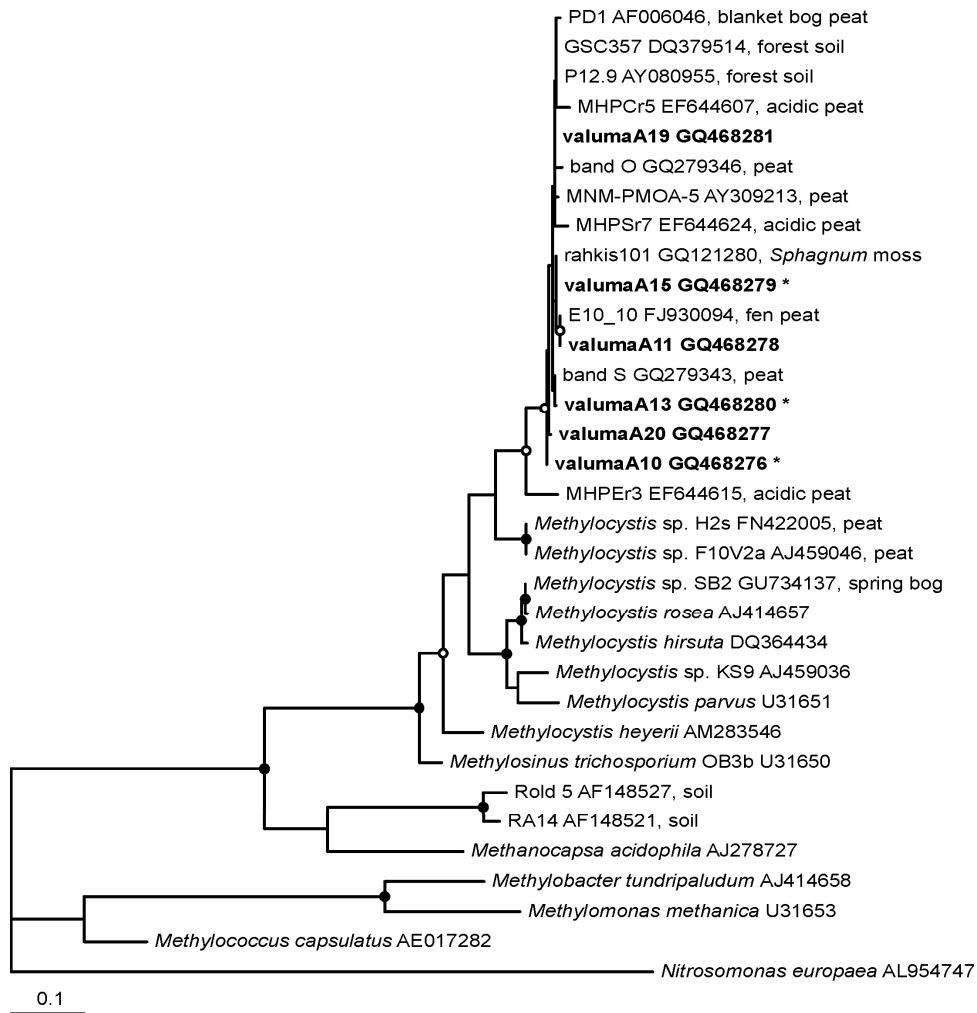


Figure S2.

**Figure S2.** Phylogenetic tree of partial McrA sequences (133 aa) from peatland clones (in bold) and reference sequences. In bold sequence names, R refers to restored sites (R1-R3) and N to natural sites (N1-N3). Size of terminal restriction fragments is indicated for each group, and the most abundant T-RFs are underlined. No T-RFs were detected corresponding to Methanobacteriales MrtA for the isoenzyme methyl-coenzyme M reductase II. Scale indicates 10% sequence divergence. Nodes with bootstrap values  $\geq 95\%$  are marked with filled circles and  $\geq 75\%$  with open circles. The maximum likelihood tree was rooted with *Methanopyrus kandleri*.



**Figure S3.** Phylogenetic tree of partial *pmoA* sequences (435 bp) from peatland DGGE bands (in bold) and reference methanotroph sequences. Scale indicates 10% sequence divergence. Nodes with bootstrap values  $\geq 95\%$  are marked with filled circles and  $\geq 75\%$  with open circles. The maximum likelihood tree was rooted with *Nitrosomonas europaea amoA*. Bands common to samples with identical banding patterns are marked with an asterisk.

1 Supplementary materials and methods

2 **Vegetation and peat chemistry.** The vegetation of the sites was inventoried from 12 to 69 plots  
3 (2 m × 2 m) in 2009, the number of plots depending on the size of the site. The field and bottom  
4 layer vegetation was determined visually as a percentage cover of each species (scale 0; 0.1, 0.2,  
5 0.3, 0.5; 1, 2, 3...98, 99, 100%). Tree and shrub saplings less than 50 cm in height were included.  
6 The peat profiles in the upstream parts of sites N1, N3, R1, and R3 were mixed with mineral soil.  
7 The layers had probably been formed by sedimented suspended solid material that had eroded  
8 from the ditches of the upstream drained peatlands and had been deposited in the buffer area.  
9 Water table levels were measured from plastic tubes inserted next to the gas sampling points, but  
10 redox conditions and oxygen concentrations were not determined. Peat chemistry is presented as  
11 an average of eight replicate samples of the 0-7.5-cm and the 7.5-15-cm layer of each site. Total  
12 carbon and total nitrogen content of peat (%) were determined by LECO CHN-1000 analyzer  
13 (ISO 10694:1995). P, Ca, Mg, and K content of peat (mg kg<sup>-1</sup>) was analyzed by inductively  
14 coupled plasma atomic emission spectrometer (ICP-AES) after microwave wet digestion in  
15 HNO<sub>3</sub>/HCl. Peat pH was measured in water (ISO 10390: 1994). Dissolved organic carbon  
16 (DOC) content of water (mg l<sup>-1</sup>) was determined with Shimadzu TOC-5000 analyzer (SFS-EN  
17 1484:1997), total nitrogen of water with Lachat Quickchem 8000 FIA analyzer (SFS-EN ISO  
18 11905-1:1998), and pH of water with Denver 20 pH-meter (SFS 3021:1979).

19

20 **Quantitative PCR.** The abundance of methanogens and methanotrophs was compared by  
21 quantitative PCR (qPCR) of *mcrA* and *pmoA*. Reactions (20 µl) were run in duplicate in Rotor-  
22 Gene 6000 (Corbett Research, Australia) and contained 1 × qPCR master mix (Maxima qPCR  
23 kit, Fermentas, Lithuania), 0.375 µM of primers and 1 µl of template DNA. The primers for

24 *pmoA* were A189f (6) and A621r (10) and for *mcrA* mlas and mcra-rev (7, 9). The *mcrA* primers  
25 amplify the same region as the primers used in T-RFLP analysis. The program for *mcrA* qPCR  
26 was 95 °C for 10 min, 40 cycles of 95 °C for 30 s, 55 °C for 45 s and 72 °C for 30 s, followed by  
27 72 °C for 7 min. The program for *pmoA* qPCR was the touchdown program used in end point  
28 PCR (10), but with initial denaturation of 10 min and 42 cycles. Fluorescence was measured at  
29 the end of extension step. Standards were 10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup>, and 10<sup>5</sup> copies of *mcrA* fragment in  
30 plasmid DNA or purified *pmoA* PCR fragment. Standards were quantified with Qubit  
31 fluorometer (Invitrogen, Carlsbad, CA, USA). Primer dimers were not observed based on  
32 melting curves. Efficiency, calculated from the slope of standard curves, was 86.5% for *mcrA*  
33 and 94.3% for *pmoA* assays. Linear regression coefficients of the standard curves were  $r^2 = 0.998$   
34 for both *mcrA* and *pmoA*. Presence of inhibitors was tested by spiking peat DNA samples with  
35 10<sup>6</sup> copies of *mcrA* or 10<sup>5</sup> copies of *pmoA* standard DNA and comparing amplification results  
36 with unspiked samples. Inhibition percentage was calculated with formula  $1 - [(C_{t_{\text{sample}}} -$   
37  $C_{t_{\text{standard}}}) / C_{t_{\text{standard}}}] \times 100$  (3). Three field replicates from sites N3 and R2 were tested with both  
38 *mcrA* and *pmoA* qPCR. Two samples from R2 showed minor inhibition in *mcrA* qPCR (-2.3%  
39 and -0.5%), and the third one together with all samples of N3 and *pmoA* qPCR for both sites  
40 showed no inhibition. Results are given as gene copies per peat volume (cm<sup>3</sup>) instead of dry  
41 weight of soil because of the variation in mineral soil content of the samples (Table 1).

42

43 **Statistical analyses.** Methane emissions rates and numbers of methanogens and methanotrophs  
44 of natural and restored sites were compared by nested analysis of variance (ANOVA) in SPSS  
45 (v. 15.0, SPSS Inc.). Emission rates were log-transformed. Methanogen and methanotroph  
46 community composition was compared by analysis of similarity (ANOSIM) with Past package

47 (5) with significance assessment by 10000 permutations. Within-site community variation of  
48 methanogens and methanotrophs was measured as multivariate dispersion by calculating  
49 distances from a centroid for replicates of each site with PERMDISP2 program (2). The  
50 distances of natural and restored sites were then compared using nested ANOVA. Methanogen  
51 and methanotroph data were analyzed in ANOSIM and multivariate dispersion as binary  
52 matrices based on presence or absence of T-RFs or sequenced DGGE bands using Dice distance  
53 measure. In addition, ANOSIM was carried out with relative abundances of *mcrA* T-RFs based  
54 on peak areas and Bray-Curtis distances. Level of statistical significance in all analyses was  
55  $P < 0.05$ .

56           Multivariate analyses were carried out with Canoco 4.52 (9) to explore variation of  
57 microbial communities, vegetation, and buffer area chemistry and to link the variation to  
58 environmental variables. The soil chemistry was explored using principal component analysis  
59 (PCA) with chemical characteristics as response variables. Initial detrended correspondence  
60 analyses (DCA) where detrending was conducted by segments showed high compositional  
61 variation in vegetation and methanogen communities. In both analyses, the length of first  
62 gradient was over 4, suggesting unimodal response to describe species distribution better than  
63 linear model, and DCA was therefore used to analyze vegetation and methanogen data.  
64 Methanogen data included the relative abundances of T-RFs based on peak areas. DCA of  
65 methanotroph communities showed a short first gradient (3.1), suggesting linear response to be  
66 sufficient to describe variation. We therefore applied PCA to analyze methanotroph  
67 communities. The environmental variables included in the analyses were CH<sub>4</sub> emission rate  
68 (growing season average), peat bulk density, peat carbon content, peat nitrogen content, peat Ca,  
69 Mg, and K content, water table level, dissolved organic carbon (DOC), total nitrogen and total



70 phosphorus of water, and pH of peat and water. In the analyses of microbial communities,  
71 chemical variables were introduced as the first two PCA axes of peat chemistry, and vegetation  
72 as the first two DCA axes of vegetation.

73

74 **Analysis of T-RFLP data.** The *mcrA* T-RFLP electropherograms were analysed with  
75 Peakscanner software (v. 1.0, Applied Biosystems). If close T-RFs could not be consistently  
76 aligned among samples, they were combined for further analyses to avoid misinterpretation (T-  
77 RFs 251+254 bp, 468+470 bp). Terminal restriction fragments (T-RFs) were assigned to  
78 phylogenetic groups by determining *in silico* terminal fragments of clone sequences in GeneDoc  
79 software (<http://www.nrbsc.org/gfx/genedoc/>) and by T-RFLP analysis of clones.

80

81 **Phylogenetic analysis.** Deduced McrA amino acid sequences and *pmoA* nucleotide sequences  
82 were screened with NCBI BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>) and aligned with  
83 ClustalW (<http://www.ebi.ac.uk/clustalw>). Evolutionary models were selected with ProtTest (1)  
84 and FindModel (<http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html>).  
85 Maximum likelihood trees were constructed with PhyML (4) with model LG+I+G+F for McrA  
86 and GTR+G for *pmoA*. The *pmoA* tree was constructed with nucleic acid sequences to better  
87 illustrate the differences between closely related sequences. Bootstrap values were generated  
88 from 100 replicates in PhyML.

89

## 90 **References**

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**Table S1.** Distribution of *mcrA* terminal fragments (T-RFs) in analysis of methanogen communities in natural (N1-3) and restored (R1-3) peatlands. Samples are field replicates of peat samples (n=7-8).

site, sample	T-RF (bp), % of total peak area													
	105	115	193	254	258	272	275	278	293	298	408	419	470	489
N1 A1	1.1	0	0	10.5	0	12.5	0	15.2	18.1	1.3	0	0	40	1.3
N1 B1	0	0	0	7.9	0	0	0	30.1	7.4	0	3.1	0	46.9	4.5
N1 B2	1	0	0	7.6	0	6.1	0	7.7	4.9	0	0	0	68.3	4.5
N1 C5	0	0	0	9	0	11.3	0	0	25	0	0	0	43.4	11.3
N1 C4	0	0	0	11.5	0	5.4	0	3.6	28.8	0	0	0	44.2	6.6
N1 D4	1	0	0	8.4	0	1.1	0	30	8.2	1.4	3.8	0	46.1	0
N1 D3	2.8	0	0	7.1	0	0	0	5.2	47.3	0	0	0	37.6	0
N1 E2	0	0	0	8.4	0	7.8	0	3.3	32.3	0	0	0	40.2	7.9
N2 A1	0	0	0	6	0	10.6	0	0	12.1	0	8.9	0	53.9	8.5
N2 B2	0	0	0	7.1	0	10.9	0	0	18.7	0	9	0	50.7	3.7
N2 C2	0	0	0	6.8	0	8.9	0	0	38.2	0	5.5	0	38.4	2.1
N2 C3	0	0	0	4.8	0	12.8	0	0	25.7	0	0	0	51.3	5.3
N2 D1	0	0	0	4.7	0	10.9	0	0	29.2	0	4.6	0	47.1	3.6
N2 D2	0	0	0	0	0	8.3	0	5.5	30.1	0	7.8	0	45.9	2.4
N2 E3	0	0	0	3.9	0	7.8	0	0	32.5	0	17	0	35.7	3
N3 A1	0	0	0	13.2	0	6.5	0	13.7	10.5	1	0	0	52.9	2
N3 B1	0	0	0	14.2	0	9.6	0	2.2	15	0	19.2	0	23.3	16.4
N3 B2	0	0	0	8.6	0	19.1	0	8.8	13.7	0	3	0	45	1.9
N3 C3	0	0	0	13.5	0	5.9	0	7.4	18.2	0	2.3	0	36.8	15.9
N3 C4	0	0	0	15.4	0	16.1	0	9	6.8	3	2.3	0	39.9	7.6
N3 D2	0	0	0	17.7	0	9	0	8.5	6.4	3.7	3.5	0	36.7	14.6
N3 D3	0	0	0	11	0	11.7	0	7.5	11.1	1.3	2.8	0	38.1	16.5
N3 E2	0	0	0	12.2	0	13.4	0	12.2	14.9	1.5	1.6	0	37.1	7.1
R1 A1	0	0	0	3.6	0	39.6	0	0	24.2	0	0	0	31.3	1.3
R1 A2	0	0	0	5.6	0	48.8	2.3	0	16.6	0	0	0	26.7	0
R1 B1	0	0	0	8.2	0	28.2	6.4	0	16.8	0	3	0	37.4	0
R1 B2	0	0	0	0	0	10	0	7.5	71.7	0	0	0	10.8	0
R1 C1	0	0	0	3.7	0	41.8	0	0	1.8	0	8.2	0	44.6	0
R1 C2	0	0	0	2	0	22.1	0	2.4	54.6	0	0	0	19	0
R1 D1	0	3.1	0	45.4	0	49.2	0	0	2.4	0	0	0	0	0
R1 D2	0	0	0	0	0	14.6	0	0	0	0	0	0	0	85.4
R2 A2	0	0	0	2	0	0	0	3.5	2.1	0	33.8	0	53	5.6
R2 B1	0	0	0	58.9	0	0	0	0	0	10.2	0	11.4	19.5	0
R2 B3	0	0	0	18.5	0	0	0	0	0	2.2	29.7	0	42.2	7.4
R2 B5	0	0	0	0	0	0	0	0	0	0	0	0	91.6	8.4
R2 C3	0	0	0	1.9	2.4	0	0	0	0	71.8	0	7.7	0	16.2
R2 C5	0	0	0	0	0	0	0	100	0	0	0	0	0	0
R2 D1	0	0	0	2.2	0	0	0	0	0	0	0	0	61.3	36.5
R3 A1	0	0	0	14.1	0	18.6	0	3.4	16.2	0	0	0	47.8	0
R3 B1	0	0	0	4.5	0	12.6	0	4.7	23	0	5.4	0	46.2	3.7
R3 B2	0	0	0	3.4	0	9.7	0	3	40.5	0	1.4	0	26.1	15.8
R3 C4	0	0	0	0	0	15.6	0	4.8	15.7	1	0	0	59.7	3.3
R3 C3	0	0	2.9	0	0	3	0	2.2	63.6	0	0	0	26	2.1
R3 D2	0	0	0	0	0	0	0	0	51.7	1.7	0	0	18.9	27.6
R3 D3	0	0	1.1	12.1	0	0	0	0	22.4	2.3	2	0	5.5	54.6
R3 E3	0	0	0	0	0	0	0	10.7	59.9	0	0	0	20.2	9.2

**Table S2.** Distribution of *pmoA* DGGE bands in analysis of methanotroph communities in natural (N1-3) and restored (R1-3) peatlands. Samples are biological replicates of peat samples (n=7-8).

site	sample	DGGE band					
		A10	A20	A19	A11	A15	A13
N1	A1	1	0	0	0	1	1
	B1	1	0	0	0	1	1
	B2	1	0	0	0	1	1
	C4	1	0	0	0	1	1
	C5	1	0	0	0	1	1
	D3	1	0	0	0	1	1
	D4	1	0	0	0	1	1
	E2	1	0	0	0	1	1
N2	A1	1	0	0	0	1	1
	B1	1	0	0	0	1	1
	B2	1	0	0	0	1	1
	C2	1	0	0	0	1	1
	C3	1	0	0	0	1	1
	D1	1	0	0	0	1	1
	D2	1	0	0	0	1	1
	E2	1	0	0	0	1	1
N3	A1	1	0	0	0	0	0
	B1	1	0	0	0	0	0
	B2	1	0	0	0	0	1
	C3	1	0	0	0	0	1
	C4	1	0	0	0	0	1
	D2	1	0	0	0	0	0
	D3	1	0	0	0	0	1
	E2	1	0	0	0	0	1
R1	A1	1	0	0	0	1	1
	A2	1	0	0	0	1	1
	B1	1	0	0	0	1	1
	B2	1	0	0	0	1	1
	C1	1	0	0	0	1	1
	C2	1	0	0	0	1	1
	D1	1	0	0	0	1	1
	D2	1	0	0	0	1	1
R2	A2	0	1	1	1	1	0
	B1	0	1	1	1	1	0
	B3	0	0	1	1	1	0
	B5	0	1	1	1	1	1
	C3	0	1	1	1	1	1
	C5	0	1	1	1	0	1
	D1	0	1	1	1	1	1
R3	A1	1	0	0	0	1	1
	B1	1	0	0	0	1	1
	B2	1	0	0	0	1	1
	C3	1	0	0	0	1	1
	C4	1	0	0	0	1	1
	D2	1	0	0	0	1	1
	D3	1	0	0	0	1	1