Supporting Information

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SI Materials and Methods

To assess whether indigenous CH_4 was formed during the incubations, we sampled a subset ($n = 3$) of the moss vials in the $^{15}N_2$-only treatment (no CH_4 added) for the headspace CH_4 concentration after the incubation period of 45 h. An air sample of outdoor air on site was also drawn to serve as a control for initial headspace conditions. The gas samples were analyzed by using gas chromatography as described in *Materials and Methods*. In the vials, the final CH_4 concentrations ranged from 0 to 0.5 ppm, indicating that during the incubation the headspace CH_4 concentration did not increase but decreased from the initial atmospheric concentration of 2.0 ppm (outdoor air). This change in the headspace CH_4 concentration corresponded to net CH_4 oxidation rates of 0.17–0.24 nmol·g^{-1} of moss biomass·h^{-1}. Thus, it is unlikely that possible indigenous methane had a major contribution to the N_2 fixation rates.

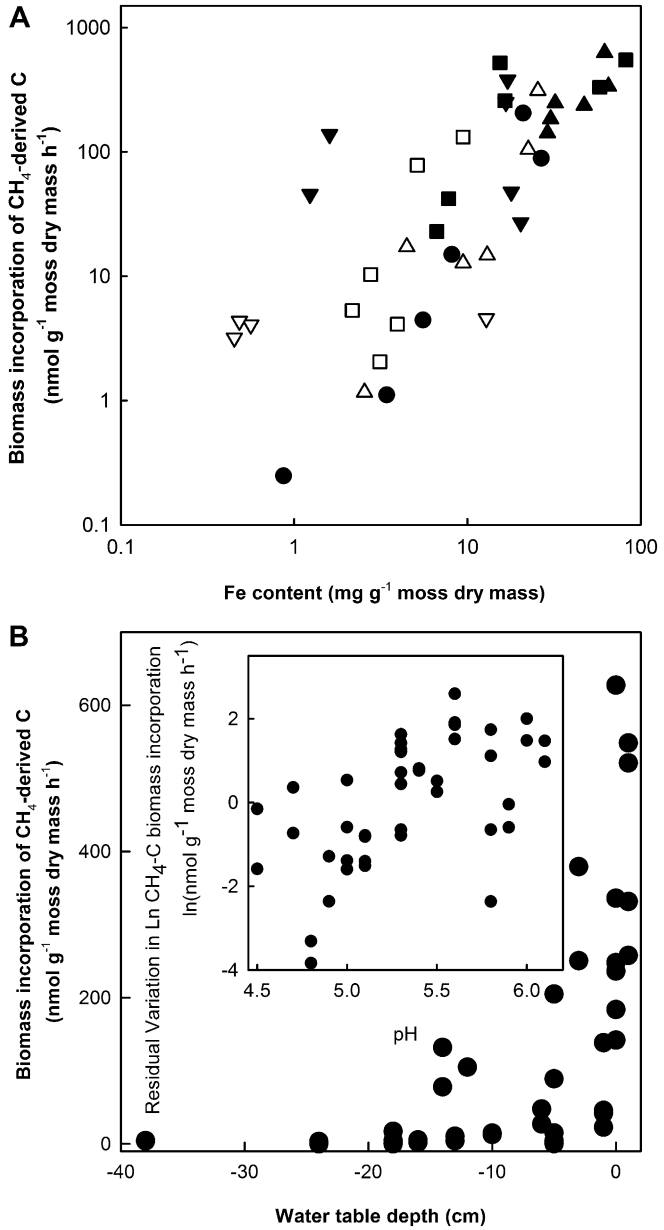

Fig. S1. The relationship between the rate of CH_4 oxidation (measured as biomass incorporation of CH_4-derived C) and selected environmental variables. (*A*) The rate of biomass incorporation of CH_4-derived C and Fe content in moss samples of different successional peatland stages (●, meadow; ■, mesotrophic fen; ▲, oligotrophic fen, and ▼, fen-bog transition). The samples collected from flark microhabitats are indicated by filled symbols. (*B*) The rate of the biomass incorporation of CH_4-derived C (shown untransformed) and water table depth of *Sphagnum* moss samples. Negative values indicate depth of the water table below the moss surface. *Inset* shows the relationship between pH and variation in the biomass incorporation of CH_4-derived C [natural log (ln) transformed] not related to the water table depth (ln CH_4-C biomass incorporation = WT + pH, $R^2 = 0.58$, $df_{reg,\ res}$ 1, 39).

Table S1. Study sites at Siikajoki peatland chronosequence

Successional stage	Terrestrial age (1), y	Elevation (1), m a.s.l.	Peat thickness, cm	No. of peatlands	No. of *Sphagnum* samples	*Sphagnum* species
Meadow	200	1.5–2	12 (0.4)	3	3	*sub, squ*
Mesotrophic fen	700	7	58 (3)	3	6	*pla, maj, fim, pap*
Oligotrophic fen	1,070–1,300	12	86 (5)	3	6	*sub, pap, obt*
Fen-bog transition	2,410–2,520	25	117 (37)	3	6	*sub, maj, obt, fus*

Values in parentheses show SDs. Species are *fim*, *Sphagnum fimbriatum*; *fus*, *Sphagnum fuscum*; *maj*, *Sphagnum majus*; *obt*, *Sphagnum obtusum*; *pap*, *Sphagnum papillosum*; *pla*, *Sphagnum platyphyllum*; *squ*, *Sphagnum squarrosum*; *sub*, *Sphagnum subsecundum*. a.s.l., above sea level.

1. Tuittila E-S, et al. (2013) Wetland chronosequence as a model of peatland development: Vegetation succession, peat and carbon accumulation. *Holocene* 23(1):25–35.

Table S2. Nested ANCOVAs on the effects of terrestrial age and treatments on N_2 fixation and biomass incorporation of CH_4-derived C

	LnN_2 fixation			Ln CH_4-C biomass incorporation		
Source	*df*	*F*	*P*	*df*	*F*	*P*
Corrected model	14	9.5	<0.001	13	17.9	<0.001
Intercept	1	356.8	<0.001	1	727.6	<0.001
Water table	1	53.8	<0.001	1	113.1	<0.001
Successional stage	3	6.8	<0.001	3	10.1	<0.001
Site (successional stage)	8	7.3	<0.001	8	11.8	<0.001
CH_4 addition	1	4.4	0.039	NA	—	—
Light	1	17.2	<0.001	1	0.2	0.668
Error	64	—	—	28	—	—
Total	79	—	—	42	—	—
Corrected total	78	—	—	41	—	—
R^2	0.60	—	—	0.84	—	—

All interactions among factors were nonsignificant and excluded from the final models. Five observations with high N_2 fixation rates were excluded as outliers.

Table S3. Rates of N_2 fixation in different successional stages

	N_2 fixation, nmol·g^{-1} of moss dry mass·h^{-1}					
	$^{15}N_2$ treatment			$^{15}N_2$+$^{13}CH_4$ treatment		
Successional stage (*microhabitat*)	Mean (SEM)	Min	Max	Mean (SEM)	Min	Max
Under prevailing light conditions						
Meadow	0.8 (0.5)	0.2	1.8	3.3 (0.5)	2.3	3.9
Mesotrophic fen *flark*	35 (26)	5.8	87	49 (39)	7.7	126
hummock	5.5 (2.8)	0.2	9.2	9.1 (5.8)	0.3	20
Oligotrophic fen *flark*	3.0 (1.9)	0.9	6.8	6.5 (3.2)	1.6	12
hummock	1.8 (0.4)	0.9	2.3	2.9 (0.3)	2.4	2.4
Fen-bog transition *flark*	6.0 (3.6)	0.1	13	5.7 (3.0)	0.4	11
hummock	0.6 (0.1)	0.5	0.9	0.7 (0.4)	0.1	1.5
In dark						
Meadow	2.0 (1.1)	0.01	4.1	2.5 (0.6)	1.4	3.5
Mesotrophic fen *flark*	11 (7.4)	2.9	25	16 (13)	2.8	43
hummock	1.2 (1.0)	0	3.3	3.5 (3.3)	0.2	10
Oligotrophic fen *flark*	2.0 (0.2)	1.6	2.3	4.6 (3.0)	1.4	11
hummock	0.9 (0.1)	0.6	1.1	0.8 (0.2)	0.5	1.1
Fen-bog transition *flark*	2.0 (1.0)	0.2	3.7	2.5 (1.3)	0.2	4.5
hummock	0.3 (0.1)	0	0.4	0.1 (0.04)	0	0.1

The mean, minimum, and maximum rates as well as SE of mean (SEM, $n = 3$ peatlands in each stage) for the four treatments: $^{15}N_2$ and $^{15}N_2$+$^{13}CH_4$ addition both under prevailing light conditions and in dark are shown.

Table S4. Water table depth (WT) relative to moss surface, water pH, dissolved CH_4 concentration, *Sphagnum* nutrient contents and $\delta^{15}N$ natural abundance in different successional stages.

Successional stage (*microhabitat*)	WT, cm	pH	CH_4, $\mu mol \cdot L^{-1}$	*Sphagnum* C:N:P	*Sphagnum* P, $mg \cdot g^{-1}$	*Sphagnum* $\delta^{15}N$
Meadow	−5 (0)	5.0 (0.1)	7 (2)	515 (167):13 (0):1	1 (0.3)	−1.3 (0.1)
Mesotrophic fen *flark*	0 (1)	5.5 (0.3)	13 (10)	414 (324):15 (13):1	1.6 (1.1)	1.4 (2.2)
hummock	−14 (1)	5.4 (0.3)	69 (18)	733 (441):16 (8):1	0.8 (0.4)	0.8 (0.5)
Oligotrophic fen *flark*	0 (0)	5.6 (0.1)	89 (43)	722 (136):19 (1):1	0.6 (0.1)	−0.6 (0.6)
hummock	−13 (2)	5.3 (0.2)	77 (31)	999 (364):18 (3):1	0.5 (0.2)	−1.6 (0.6)
Fen-bog transition *flark*	−3 (1)	5.3 (0.3)	14 (8)	1376 (493):26 (7):1	0.4 (0.2)	−0.1 (0.1)
hummock	−27 (6)	5.3 (0.4)	20 (9)	1655 (418):23 (6):1	0.3 (0.1)	−0.5 (0.8)

Values in parentheses are SEM.

Table S5. Stepwise linear regression models analyzing the relationships of environmental variables and ln transformed N_2 fixation, CH_4-induced N_2 fixation, and biomass incorporation of CH_4-derived C

	Coefficients, SE					Adj. R^2, df $_{reg, res}$	
Models	P	WT	Fe	pH	Constant	P only or Fe only model	Full model
LnN_2 fixation							
All treatments	1.09 (0.14)	0.03 (0.008)	—	—	0.71 (0.16)	0.49 (1, 82)	0.54 (2, 81)
$^{15}N_2$ light	1.39 (0.34)	—	—	—	0.34 (0.30)	0.45 (1, 19)	—
$^{15}N_2$ dark	0.78 (0.19)	0.03 (0.11)	—	—	0.62 (0.22)	0.50 (1, 19)	0.61 (2, 18)
$^{15}N_2$+$^{13}CH_4$ light	0.96 (0.27)	—	—	—	2.19 (0.69)	0.61 (1, 19)	—
$^{15}N_2$+$^{13}CH_4$ dark	1.12 (0.27)	—	0.02 (0.007)	—	−0.06 (0.22)	0.54 (1, 19)	0.65 (2, 18)
$^{15}N_2$+$^{13}CH_4$- $^{15}N_2$ only	0.55 (0.20)	—	0.01 (0.004)	—	1.12 (0.09)	0.52 (1, 40)	0.59 (2, 39)
$LnCH_4$-C biomass incorporation							
	—	0.10 (0.03)	0.03 (0.02)	1.32 (0.62)	−3.0 (3.0)	0.45 (1, 40)	0.60 (3, 38)

P only and Fe only models were run for LnN_2 fixation and $LnCH_4$-C biomass incorporation, respectively.