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Methane-Cycling Microbial Communities and Methane Emission in Natural and Restored Peatlands

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We addressed how restoration of forestry-drained peatlands affects CH_4-cycling microbes. Despite similar community compositions, the abundance of methanogens and methanotrophs was lower in restored than in natural sites and correlated with CH_4 emission. Poor establishment of methanogens may thus explain low CH_4 emissions on restored peatlands even 10 to 12 years after restoration.

Boreal peatlands have extensively been drained for forestry, agriculture, and peat mining. In Finland, for instance, more than half of the original peatland area has been lost (26). Restoration of drained peatlands involves raising the water table position and facilitating the return of peat-forming vegetation. With drainage, previously anoxic peat has been exposed to oxygen, which reduces CH_4 production by methanogenic archaea and may limit CH_4 consumption by aerobic methanotrophic alpha- and gammaproteobacteria through substrate depletion (9, 12, 16, 40). The objective of restoration is to revitalize the processes typical for pristine peatlands, including their function as carbon sinks and thus the microbial activity controlling carbon cycling. In restored mined peatlands, CH_4 production and oxidation potentials have recovered and even exceeded those of natural sites in 4 to 30 years (1, 4, 10). On the other hand, the relatively few studies on CH_4 emissions of restored drained peatlands indicate that although restoration increases the emissions (37), they may remain lower than on pristine mires during the first 2 to 3 years after restoration (18, 34). The low rates have been proposed to result from slow establishment of methanogens after prolonged aerobic conditions (34), but the effects of restoration on CH_4-cycling microbial communities have not yet been demonstrated.

We addressed the response of methanogens, methanotrophs, and CH_4 emission rates to restoration by comparing three natural peatlands and three restored forestry-drained peatlands in Finland (Table 1). The sites receive outflow water from the surrounding forested catchment (29, 36). Restoration of the drained peatlands was carried out 10 to 12 years before our study by filling in the drainage ditches with peat. The natural sites had vegetation typical of wet fen habitats, whereas species typical to drier and forestry-drained peatlands still occurred in the restored sites (see Fig. S1 in the supplemental material). In peat chemistry, site R2 differed from the other sites, because it had the lowest pH and the lowest Ca, K, and Mg levels (see Fig. S1).

Methane emission rates were measured at eight sampling points four or five times from May through September 2007 by the closed-chamber technique (11). The emission rates of the natural sites were at the higher end of the range reported for pristine boreal mires (Table 1) (25, 38). The restored sites had substantially lower CH_4 emission rates (nested analysis of variance [ANOVA], $P = 0.001$). Even 10 to 12 years after restoration, their rates were more similar to those of drained, nutrient-poor, and forested peatlands (25, 28, 31). The emission rates in August 2007, when peat was sampled, were consistent with the rates for the whole growing season (data not shown).

Peat samples for microbial community analyses were taken from depths of 0 to 7.5 cm (aerobic surface peat for methanotrophs) and 7.5 to 15 cm (anaerobic layer for methanogens) next to the gas sampling positions ($n = 8$) at each site. The layer sampled for methanogens was below the water table level in all sites except R2 (Table 1). Samples were stored at −20°C. DNA was extracted with the Powersoil DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA) with cell lysis in a FastPrep instrument (Thermo Savant, Holbrook, NY) with 3 rounds of 20 s at 4 m s^{-1}. Quantitative PCR for methanogens with primers for methyl-coenzyme M reductase gene *mcrA* (30) (see the supplemental material) showed marginally larger *mcrA* copy numbers in natural than in restored sites (nested ANOVA, $P = 0.065$) (Fig. 1A). Methanogen abundance correlated with log-transformed CH_4 emission rate ($r = 0.884$, $P = 0.019$) (Fig. 1A). Methanogen community composition was analyzed by *mcrA* terminal restriction fragment length polymorphism (T-RFLP) (40) (see Table S1 in the supplemental material). Communities between natural and restored sites differed only moderately (Fig. 2A) (analysis of similarity [ANOSIM] based on relative abundance of *mcrA* terminal restriction fragments [T-RFs], $R = 0.20$; presence/absence of T-RFs, $R = 0.14$; $P < 0.001$ in both) (see the supplemental material for details on statistical analyses). However, the communities of the restored sites showed significantly higher within-site heterogeneity (ANOVA of multivariate dispersion [3], $P = 0.034$) (Fig. 1). Community heterogeneity correlated negatively with CH_4 emission rate ($r = -0.967$, $P = 0.002$) (Fig. 1C). For identification of T-RFs, we constructed and screened one clone library per site and

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TABLE 1 Characteristics of peatland sites

Site	Location	Yr constructed (age [yr] at sampling)	Water table (cm)[a]	Methane emission rate (mg m^{-2} day^{-1})[a]	pH[b]	Bulk density (g cm^{-3})[b]
Natural sites						
N1, Asusuo	60°26′N, 23°38′E	1997 (10)	2.3 ± 1.0	139 ± 67	4.6 ± 0.1	0.13 ± 0.02
					4.7 ± 0.0	0.58 ± 0.13
N2, Kallioneva	62°16′N, 23°48′E	1998 (9)	−0.9 ± 0.6	707 ± 477	5.1 ± 0.0	0.08 ± 0.01
					4.9 ± 0.1	0.07 ± 0.01
N3, Hirsikangas	64°04′N, 26°40′E	1999 (7)	12.1 ± 0.7	227 ± 45	5.3 ± 0.1	0.12 ± 0.02
					5.1 ± 0.1	0.15 ± 0.03
Restored sites						
R1, Murtsuo	61°01′N, 28°19′E	1997 (11)	3.6 ± 0.7	6.9 ± 3.7	5.1 ± 0.0	0.25 ± 0.02
					5.2 ± 0.0	0.14 ± 0.00
R2, Konilamminsuo	61°48′N, 24°17′E	1995 (12)	−14.8 ± 2.1	3.0 ± 1.3	4.0 ± 0.1	0.08 ± 0.02
					4.0 ± 0.1	0.08 ± 0.01
R3, Vanneskorpi	61°51′N, 23°42′E	1996–1997 (10–11)	−8.9 ± 1.1	10.8 ± 3.2	4.9 ± 0.1	0.35 ± 0.12
					4.6 ± 0.1	0.15 ± 0.02

[a] Growing season mean of eight sampling points ± SE. Water level values are negative below the peat surface.
[b] For each site, the top row represents a depth of 0 to 7.5 cm and the bottom row represents a depth of 7.5 to 15 cm (mean ± SE).

sequenced a total of 52 clones (40). The main components of communities in both restored and natural sites were *Methanoregulaceae* (T-RFs 293, 470, 278, and 254 bp), *Methanobacteriaceae* (T-RFs 468, 275, and 251 bp), and *Methanosarcinaceae* (T-RFs 489 and 272 bp) (see Fig. S2 in the supplemental material). Several sequences were highly similar (>97% DNA identity) to those from peatlands and wetland soils (13–15, 23), but we also detected novel sequence types (<92% DNA identity to database sequences) associated with *Methanobacteriales*, *Methanoregulaceae*, and *Methanocellales*.

The small and heterogeneous methanogen communities at the restored sites could be an effect of drainage that restoration has

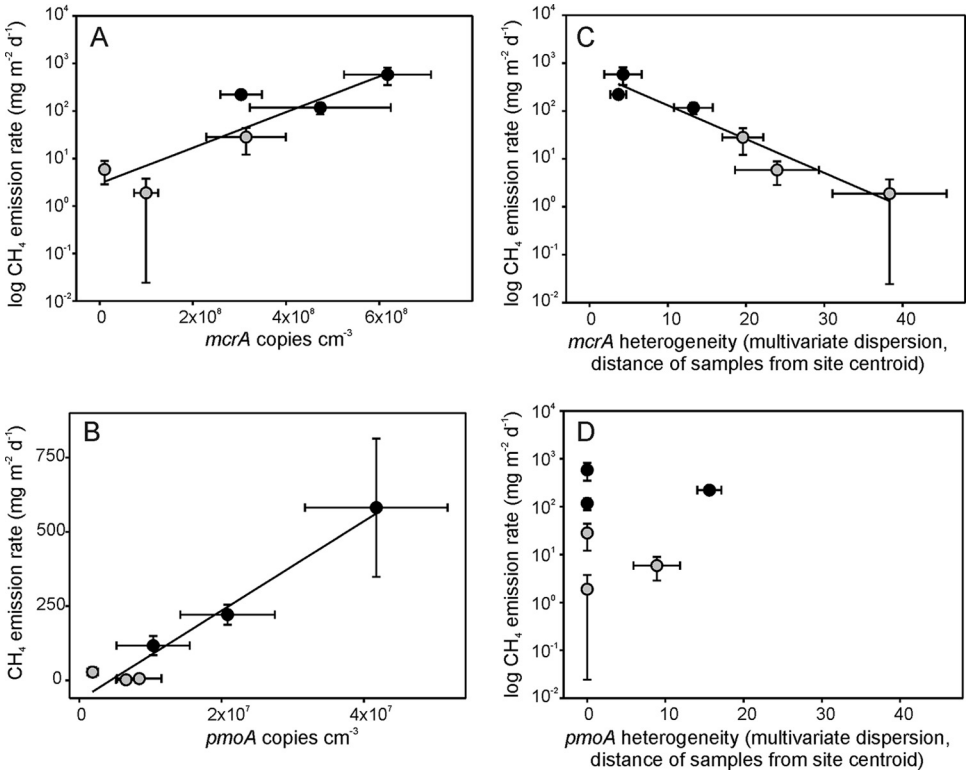

FIG 1 Abundance (A and B) and within-site community heterogeneity (C and D) of methanogens (*mcrA*) and methanotrophs (*pmoA*) in six peatlands in relation to CH_4 emission rate at the time of sampling. Black symbols represent natural sites, and gray symbols represent restored sites. Each data point represents the average of 7 or 8 field replicates ± standard error (SE). Microbial abundance was measured by quantitative PCR (gene copies cm^{-3} peat). Community heterogeneity was determined as multivariate dispersion based on T-RFLP or DGGE fingerprints. Note the logarithmic scale on the *y* axis in panels A, C, and D. The regression line is shown for data sets with significant relationships.

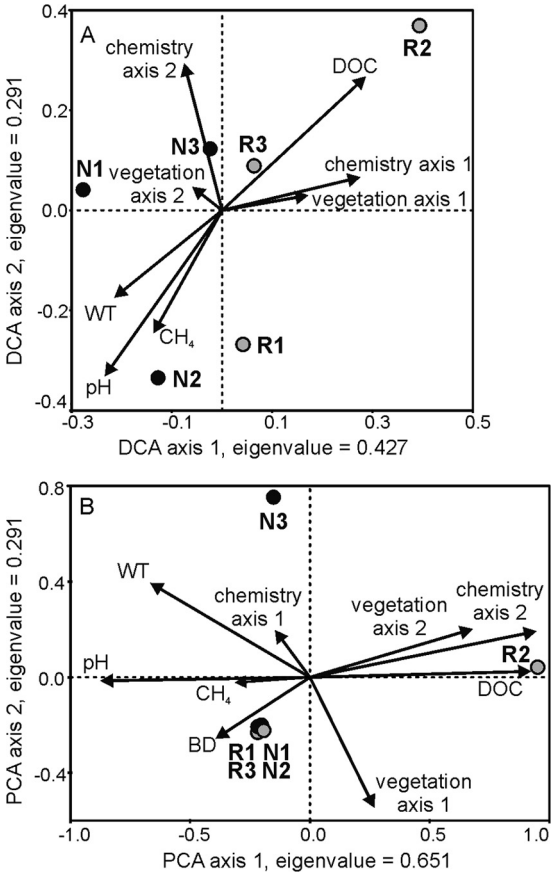

FIG 2 (A) Detrended correspondence analysis (DCA) of methanogen communities and (B) principal component analysis (PCA) of methanotroph communities in natural (N1 to N3) and restored (R1 to R3) peatlands ($n = 7$ to 8; only the site centroid is shown). The first two axes accounted for 42% (methanogens) and 87% (methanotrophs) of community variation. Communities were analyzed by *mcrA* T-RFLP and *pmoA* DGGE of peat samples. Arrows represent environmental variables. WT, water table level; DOC, dissolved organic carbon; CH_4, mean emission rate for the growing season. Vegetation axis 1 refers to DCA axis 1 (wet-dry gradient) and vegetation axis 2 to DCA axis 2 (variation between restored sites related to plant species benefitting from disturbance) in Fig. S1 in the supplemental material. Chemistry axes 1 and 2 refer to PCA axes in Fig. S1C for methanogens and Fig. S1B for methanotrophs.

not yet reversed, indicating incomplete restoration succession. Drainage restricts methanogenesis to anoxic microenvironments, and after restoration, proliferation of species selected by differing microenvironments could create large heterogeneity. The initially dominant species would shape the course of succession, similarly to models of vegetation succession (8, 39). Community heterogeneity could be further promoted by patchy vegetation formed with drainage (24). Studies on mined peatlands indicate that restored hydrology (1), but also recently formed peat or herbaceous vegetation, providing a good supply of labile carbon compounds, appears to be required to support pristine-like rates of methanogenesis (2, 4, 10, 21, 37). Since our restored sites did not have consistently low water table levels, but their vegetation still contained species typical of drained peatlands (see Fig. S1 in the supplemental material), the factor limiting the abundance and activity of CH_4-cycling microbes could be peat quality (19, 32, 33). Resolution of this issue requires assessing the availability of labile carbon compounds for methanogenesis in restored peatlands.

Methanotroph communities were analyzed by denaturing gradient gel electrophoresis (DGGE) and band sequencing of *pmoA* coding for particulate methane monooxygenase using primers A189f and A621r with GC clamp (12, 20, 35). The reverse primer has been developed for amplification of type II methanotrophs when primer A682r yields no products (35). The restored and natural sites were not differentiated based on community composition (Fig. 2B) (ANOSIM $R = 0.095$, $P = 0.027$) or multivariate dispersion (Fig. 1D) ($P = 0.730$). Four out of six sites showed identical DGGE patterns of three bands, which were identified as type II methanotrophs grouping with *Methylocystis* spp. (see Fig. S3 and Table S2 in the supplemental material). Two bands unique to site R2 yielded sequences of the same sequence cluster, which contains several nearly identical *pmoA* sequences from wetlands and forest soils (5, 7, 20, 22, 27, 35, 40). No overall difference in methanotroph abundance was observed between natural and restored sites (nested ANOVA, $P = 0.118$), but the highest numbers occurred at natural sites (Fig. 1B). The *pmoA* copy numbers correlated with CH_4 emission rate ($r = 0.980$, $P = 0.001$). Since we focused on *pmoA*-containing proteobacterial methanotrophs, our study cannot provide insight into methanotrophs lacking *pmoA* such as *Methylocella*. However, our results support the wide occurrence of *Methylocystis* in *Sphagnum* peat (5, 6, 17, 20). Drainage has been shown to affect distribution of *Methylocystis* (12, 40), but our results suggest that the community composition of methanotrophs may either respond to restoration in 10 years or may not always be affected by drainage. Methane oxidation has been observed to be less sensitive to peatland drainage than CH_4 production (28), and similarly to our results, CH_4 oxidation was suggested to be restricted by CH_4 production in restored peat-mining sites (4).

In conclusion, our results suggest that unresponsiveness of methanogens to restoration rather than enhanced methane oxidation is behind the low CH_4 emission of restored peatlands. Methanotroph abundance appeared to depend on CH_4 supply. Methanogens typical of natural sites were present in restored peatlands, but clarification of the factors that limit their abundance and activity requires further studies. Nevertheless, our results indicate that time scales of over 10 years or additional restoration measures may be required for pristine-like microbial processes to return to forestry-drained peatlands.

Nucleotide sequence accession numbers. The *mcrA* and *pmoA* sequences have been submitted to the EMBL database under accession numbers FN565431 to FN565482 (*mcrA*) and GQ468276 to GQ468281 (*pmoA*).

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