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EXPERIMENTAL ARTICLES

Decline of Activity and Shifts in the Methanotrophic Community Structure of an Ombrotrophic Peat Bog after Wildfire

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Abstract—This study examined potential disturbances of methanotrophic communities playing a key role in reducing methane emissions from the peat bog Tasin Borskoye (Vladimir oblast, Russia), as a result of the wildfire in 2007. The potential activity of the methane-oxidizing filter in the burned peatland sites and the abundance of indigenous methanotrophic bacteria were significantly reduced in comparison to the undisturbed sites. Molecular analysis of the methanotrophic community structure by means of PCR amplification and cloning of the *pmoA* gene encoding particulate methane monooxygenase revealed the replacement of typical peat-inhabiting, acidophilic type II methanotrophic bacteria with type I methanotrophs, which are less active in acidic environments. In summary, both the structure and the activity of the methane-oxidizing filter in burned peatland sites underwent significant changes, which were clearly pronounced even after 7 years of the natural ecosystem recovery. These results point to the long-term character of the disturbances caused by wildfire in peatlands.

Keywords: northern wetlands, wildfire in peatlands, methanotrophic bacteria, methane oxidation, *pmoA* genes

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Regulating the turnover of greenhouse gases, CH_4 and CO_2, is one of the key roles of wetlands in the biosphere (Zavarzin and Dedysh, 2008). This function can be significantly disturbed by wildfires (Zoltai et al., 1998; Wieder et al., 2009). Approximately 90% of the carbon released during phytomass and peat burning occurs in the form of CO_2 (Levine et al., 1993); the total atmospheric CO_2 emission due to wildfires in boreal peatlands is estimated at 8.4 Tg C year^{-1} (Gorham, 1991). Emissions of other gaseous combustion products, CO and CH_4, are estimated at approximately 1.0 and 0.1 Tg C year^{-1}, respectively (Zoltai et al., 1998). Apart from the single-time release of large quantities of greenhouse gases, fires also have important long-term consequences associated with disturbance of the biogeochemical balance in burned peatlands. Enrichment of peat water with ash compounds, increased pH values, accelerated mineralization of organic matter, and changes in the composition of plant communities can have a significant effect on the emission of greenhouse gases (Zoltai et al., 1998). For instance, it was reported that CH_4 emissions from wetlands increased twofold (Levine et al., 1990) and even eightfold (Hogg et al., 1992) following wildfire.

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In our previous studies, it was shown that the structure and functional activity of bacterial communities in the burned sites of a mesotrophic peatland changed considerably: microbial degradation of organic matter accelerated, and the numbers of sulfate-reducing microorganisms increased (Akhmet'eva et al., 2014; Belova et al., 2014). Slowly growing bacteria of the phyla *Verrucomicrobia* and *Planctomycetes*, typical for undisturbed peatlands, were replaced by rapidly growing colonizers from the *Proteobacteria*; in particular, this process involved an outbreak of the phytopathogenic *Agrobacterium tumefaciens*. CH_4 oxidation rates in peat samples from the burned sites were two times lower than in peat from the intact area; that is, the fire depleted the natural methane-oxidizing filter of a mesotrophic peatland (Belova et al., 2014). However, since the latter initially had low methanogenesis rates, disturbance of the methane-oxidizing filter did not lead to an increase in CH_4 emission.

There have been very few studies analyzing the changes that fires cause in the structure and activity of methanotrophic microbial communities. Jaatinen et al. (2004) investigated acidic soils of coniferous boreal forests and found that methane-oxidizing activity of the burned areas increased, whereas the composition of methane-oxidizing communities did not change. Another study focused on acidic peatlands covered with *Calluna vulgaris*, where the aboveground vegetation was burned repeatedly in some

areas (Chen et al., 2008). No significant differences in the methane-oxidizing activity of the peat were observed between burned and intact areas of the peatland; at the same time, the proportion of type I methanotrophs in the communities of burned areas was significantly reduced. Thus, these two studies have reported controversial results. No similar investigations have been performed for ombrotrophic peat bogs. The present study was undertaken in order to fill this gap; its purpose was to perform a comparative analysis of the composition and activity of methane-oxidizing microbial communities of the undisturbed and burned sites of an ombrotrophic peat bog in the European part of Russia.

MATERIALS AND METHODS

Study site. The study was focused on the Tasin Borskoe ombrotrophic peat bog (Meshchera National Park, Vladimir oblast, Russia, 55°36'N 40°06'E), a significant part of which was affected by wildfire in 2007. Peat samples were collected in July 2014 from an undisturbed and a burned site of the bog lying 200 m apart from each other. In the undisturbed area of the peat bog, the plant community was composed of *Sphagnum angustifolium, Sph. fuscum, Carex* spp., *Oxicoccus* sp., and *Andromeda polifolia*. The burned site was covered with young *Betula pubescens* and *Salix cinerea* plants; the grass layer was composed mainly of *Polytrichum commune, Calamagrostis epigeios, Agrostis gigantea*, and *Rumex acetosella*. Peat was collected at the depth of 0–10 cm. The samples were transported to the laboratory in cooled containers and used immediately for measuring methane-oxidizing activity, estimation of methanotroph abundance, and DNA extraction for further use in molecular analyses.

Water chemistry was determined by certified techniques in the Hydrochemical Laboratory of the Ivan'kovo Research Station of the Institute of Water Problems, Russian Academy of Sciences (Konakovo, Tver oblast, Russia).

Rates of methane oxidation by peat samples. Wet peat (10 g) was cut into 10–15 mm fragments and placed into sterile 160-mL glass vials, which were sealed hermetically, supplemented with methane to 1000 ppm, and incubated at room temperature. Gas (0.5 mL) was regularly sampled from the vials, and methane concentrations were determined on a Kristall 5000 chromatograph equipped with a flame ionization detector (Khromatek, Russia). Measurements were continued until methane could no longer be detected. The obtained data were used to calculate the methane oxidation rate for a given peat sample.

Cell numbers of methanotrophic bacteria in peat samples were determined using the most probable number method with serial dilutions in the M2 medium (Danilova et al., 2013) with methane as the carbon source. Methanotrophic bacteria that developed in vials with highest dilutions were identified using fluorescent in situ hybridization (FISH).

FISH. Cells from a 0.5 mL aliquot of an enrichment culture suspension were fixed by adding 1.5 mL 4% formaldehyde solution in phosphate buffer (NaCl, 8.0 g; KCl, 0.2 g; Na_2HPO_4, 1.44 g; NaH_2PO_4, 0.2 g; H_2O to 1 L, pH 7.0) for 1.5 h, collected by centrifugation, and washed with phosphate buffer. Fixed specimens were resuspended in a 1 : 1 (vol/vol) mixture of 100% ethanol with phosphate buffer and stored at –20°C. Suspensions of fixed cells (1–2 μL) were applied to slides with wells and hybridized with the probes at 46°C according to the standard protocol (Stahl and Amann, 1991). Cells of type I and type II methanotrophs were identified by hybridization with an equimolar mixture of Cy3-labeled M705 and M84 probes, and with M450 probe (Eller et al., 2001), respectively. The probes labeled with the Cy3 fluorescent dye were synthesized by Syntol (Russia). Specimens were analyzed using a Zeiss Axioplan 2 epifluorescent microscope (Germany) with Zeiss 20 and Zeiss 02 color filters to identify Cy3-labeled probes and cell autofluorescence, respectively.

Assessment of methanotroph diversity by PCR-based analysis of the *pmoA* genes. Total DNA was extracted from peat samples using a FastDNA SPIN kit for soil (Biol 101, United States) as recommended by the manufacturer and used as a template for PCR. The overall diversity of methane-oxidizing bacteria in peat samples was assessed by means of PCR amplification of the *pmoA* gene fragments with the primers A189f (Holmes et al., 1995) and mb661r (Costello, Lidstrom, 1999); this gene encodes the active center polypeptide of particulate methane monooxygenase (pMMO). Amplification was performed using a PE GeneAmp PCR System 9700 (Perkin-Elmer Applied Biosystems, United States). PCR products were analyzed by electrophoresis in a 1.2% agarose gel, stained with ethidium bromide, and visualized on a UV transilluminator. The obtained amplicons were cloned using a pGem-T Easy Vector System II (Promega), as recommended by the manufacturer. Recombinant clones were selected by amplification of cloned fragments with vector-specific primers T7 and SP6. Plasmid DNA was isolated and purified using a Wizard® Plus Minipreps DNA Purification System (Promega). Nucleotide sequences were determined on an ABI 377A DNA analyzer (Perkin-Elmer Applied Biosystems).

The obtained nucleotide sequences were edited using the SeqMan program (Laser Gene 7.0; DNA Star Package) and compared to those in the GenBank database using the Blast software (http://blast.ncbi.nlm.nih.gov). The phylogenetic tree was constructed using the ARB software package (http://www.arb-home.de), and its statistical signifi-

Water chemistry (mg/L) of the Tasin Borskoe ombrotrophic peat bog after the fire of 2007

No.	Parameter	Undisturbed site	Burned site
1	pH	3.7	4.2
2	Mineralization ($\mu S/cm^{-1}$)	50	80
3	C org.	49.9	130.5
4	HCO^{3-}	0	6.1
5	NO^{3-}	0.9	6.9
6	NO^{2-}	0.2	0.2
7	NH_4^+	3.0	3.9
8	SO_4^{2-}	13.7	15.1
9	PO_4^{3-}	0.1	0.1
10	Ca^{2+}	4.9	8.9
11	Fe total	0.8	0.5

cance was assessed by the bootstrap support method with 1000 alternative trees using the Phylip program package.

The obtained nucleotide sequences of the *pmoA* gene fragments from peat-inhabiting methanotrophs were deposited to the GenBank database under accession numbers KR005392–KR005460.

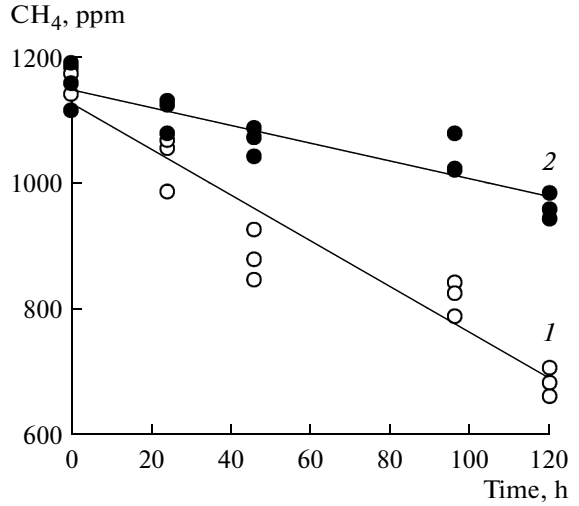

Fig. 1. Dynamics of methane concentrations in experimental vials containing peat from undisturbed (*1*) and burned (*2*) sites of the Tasin Borskoe peat bog.

RESULTS AND DISCUSSION

Water composition. Although natural remediation of the Tasin Borskoe peat bog after the fire has lasted for 7 years, the physicochemical characteristics and the composition of water in the burned site were different from those in the undisturbed area (table). The consequences of the fire included elevated pH and increased mineralization of peat bog water, which was also observed in our previous study performed on the burned Galitskii Mokh mesotrophic peatland (Akhmet'eva et al., 2014; Belova et al., 2014). The levels of nitrate and calcium ions in the water of the burned site were also increased (table).

Rates of CH_4 oxidation by *Sphagnum* peat. The samples of the surface peat layer (0–10 cm deep) collected from the undisturbed peat bog site exhibited methane oxidation rates ranging from 1.8 to 2.2 μg CH_4 g^{-1} peat day^{-1}, which corresponds to the lower range of methane oxidation rates exhibited by the peat of typical boreal peatlands of the European part of Russia (Danilova and Dedysh, 2014). For the burned site of the bog, the corresponding values were significantly lower and constituted 0.7–0.9 μg CH_4 g^{-1} peat day^{-1} (Fig. 1). Thus, in the burned site, the activity of the methane-oxidizing filter was 2.5-fold lower than in the intact sites.

Methanotroph abundance in peat. Large amount of autofluorescent particles present in the samples from the burned site hampered application of FISH for direct enumeration of methanotrophs in peat; therefore, methanotroph abundance was estimated using the Most Probable Number method. In peat samples from the undisturbed bog site, the total number of methanotrophic bacteria was 2.5×10^6 cells g^{-1} wet peat, in agreement with the values typical for boreal *Sphagnum* peat bogs (Dedysh, 2009; Danilova and Dedysh, 2014). In the samples from the burned site, the number of methanotrophs was by an order of magnitude lower, constituting 3.5×10^5 cells g^{-1} of wet peat, indicating that the methane-oxidizing filter of the peat bog was significantly destroyed by the fire. FISH-based identification of methanotrophic bacteria that developed in vials with highest dilutions showed the predominance of type I methanotrophs in peat samples from the burned site (Figs. 2a, 2b), and type II methanotrophs in the peat of the intact area (Figs. 2c, 2d).

Shifts in the methanotrophic community structure. Molecular analysis of methane-oxidizing bacterial communities of the undisturbed and burned sites of the peat bog was performed by means of PCR amplification, cloning, and sequencing of the *pmoA* genes encoding particulate methane monooxygenase, the key enzyme of methanotrophic metabolism. In total, 118 cloned *pmoA* gene fragments from the peat sam-

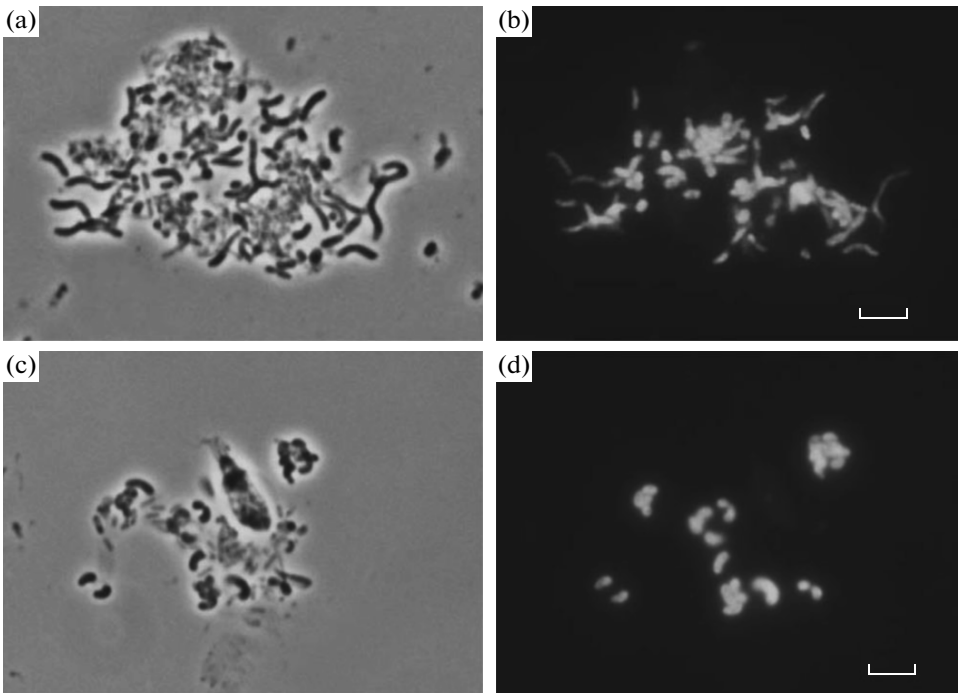

Fig. 2. Detection of type I and type II methanotroph cells in enrichment cultures obtained from peat of the burned (a, b) and the undisturbed (c, d) sites of the Tasin Borskoe peat bog by means of FISH: phase contrast microphotograph (a, c); fluorescent hybridization with M84 + M705 probes specific for type I methanotrophs (b); and fluorescent hybridization with M450 probe specific for type II methanotrophs (d). Scale bar, 5 μm.

ples were obtained and analyzed; their group composition is shown on Fig. 3.

Peat sampled from the undisturbed area of the Tasin Borskoe bog was predominantly inhabited by type II methanotrophic bacteria representing the genera *Methylocystis* and *Methylocapsa*, which is characteristic of acidic *Sphagnum* peat bogs of the boreal zone (Dedysh, 2009). The *pmoA* sequences from these methanotrophs comprised nearly 80% of all clones (Fig. 3), and were mainly represented by *pmoA* and *pmoA2* fragments from *Methylocystis bryophila*, a typical inhabitant of *Sphagnum* peat bogs (Belova et al., 2013) (Fig. 4). The remaining 20% of *pmoA* fragments from the peat of the undisturbed area belonged to type I methanotrophs, most of which are affiliated with the genus *Methylomonas*. These methanotrophs can also develop in acidic peatlands, although they are acid-tolerant organisms and not acidophilic ones (Danilova et al., 2013; Danilova and Dedysh, 2014).

Methanotrophic communities of the peat from the burned site had a different composition. The proportion of the *pmoA* sequences of type II methanotrophs (representing the genus *Methylocystis*) was reduced to 30%, while type I methanotrophs prevailed in the community (Fig. 3). Interestingly, the predominant group of type I methanotrophs were not the *Methylomonas* species but bacteria representing a distinct genus-level phylogenetic lineage within the class *Gammaproteobacteria*, for which no cultured representatives have been described so far (Fig. 4). The *pmoA* sequences belonging to this lineage were also detected in our previous work on *Sphagnum* peat bogs (Danilova and Dedysh, 2014). It is unusual for methane-oxidizing communities of *Sphagnum* bogs to contain a high percentage of type I methanotrophs, so this observation suggests that physicochemical characteristics of the habitat in question have changed significantly. In comparison to type II methanotrophs, methanotrophic *Gammaproteobacteria* are less adapted to acidic environments, which apparently explains the decline in the total methane-oxidizing capacity of the peat from burned sites of the bog.

Thus, our results indicate that the fire had a negative effect on the activity of the bacterial methane-oxidizing filter of an ombrotrophic peat bog and decreased the efficiency of its barrier function, which may result in an increase of methane emission from damaged bog ecosystems.

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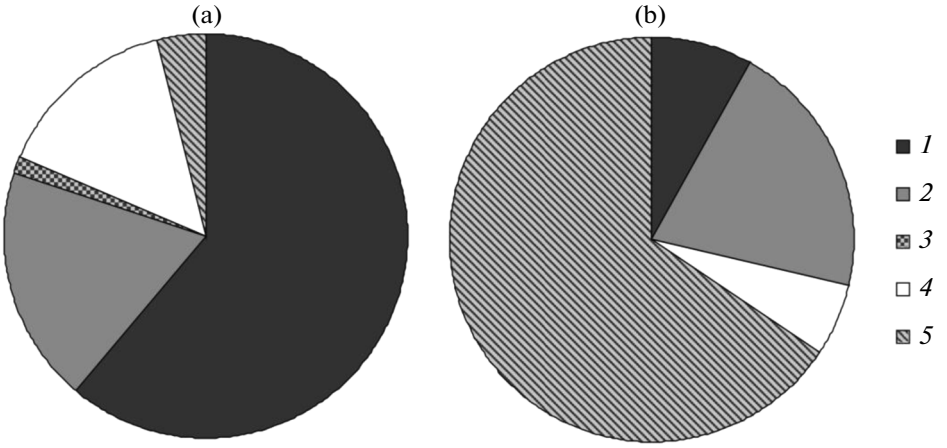

Fig. 3. Composition of methanotrophic communities in peat samples from undisturbed (a) and burned (b) sites of the Tasin Borskoe peat bog based on the analysis of the *pmoA* clone libraries: *pmoA* fragments of *Methylocystis* spp. (*1*); *pmoA2* fragments of *Methylocystis* spp. (*2*); *Methylocapsa* spp. (*3*); *Methylomonas* spp. (*4*); and unidentified type I methanotrophs (*5*).

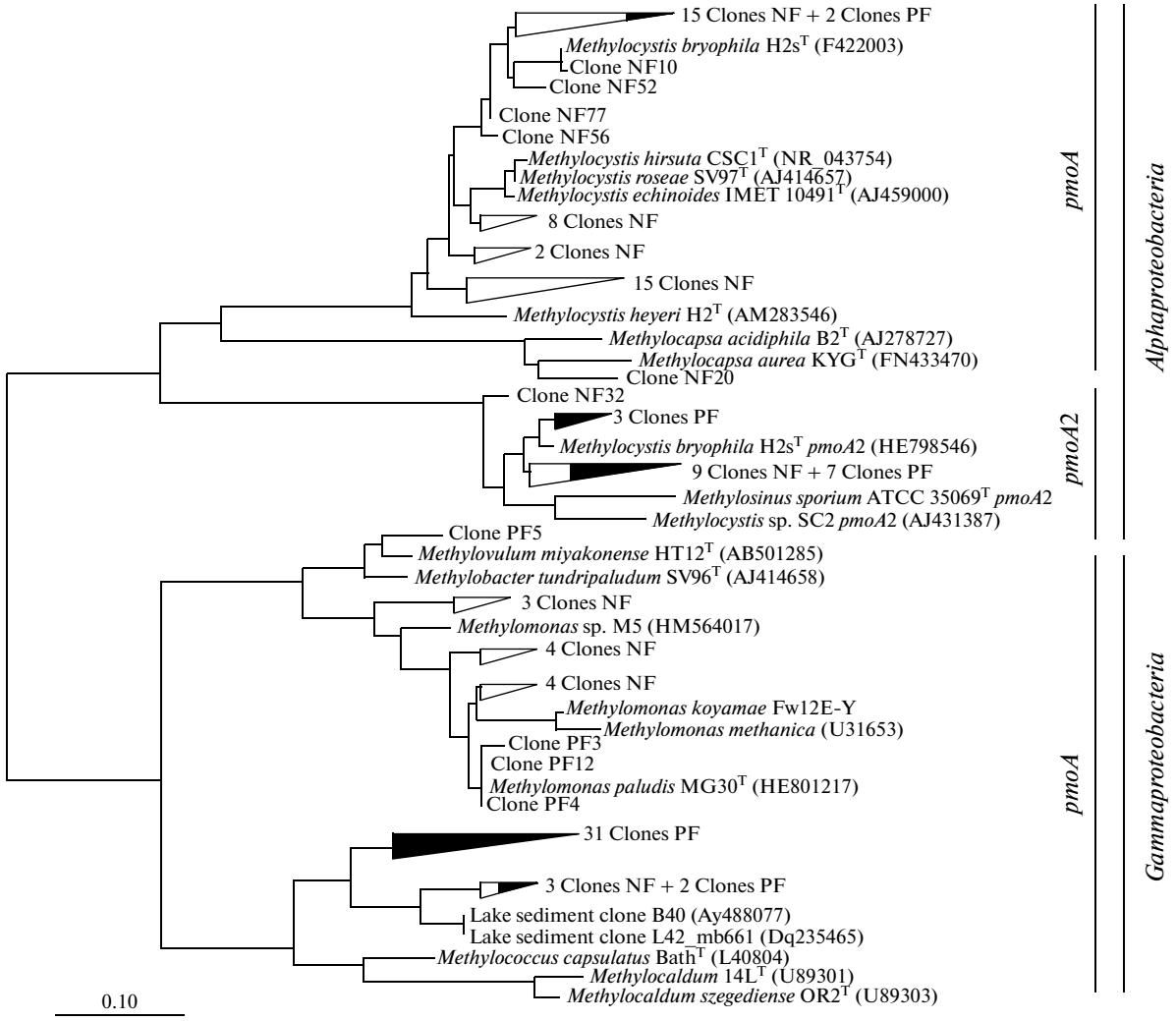

Fig. 4. Phylogenetic tree based on comparison of 145 amino acid sequences of PmoA fragments of methanotrophs detected in peat samples from the burned (PF clones) and undisturbed (NF clones) sites of the Tasin Borskoe bog, as well as of other methanotrophic *Proteobacteria*. The shares of cloned sequences obtained from peat sampled from the burned and the undisturbed sites within clone clusters are shown as filled and open sectors, respectively. The scale bar corresponds to 0.1 substitutions per amino acid position.

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