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Activity and Species Composition of Aerobic Methanotrophic Communities in Tundra Soils

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Abstract. The low-temperature, methane-oxidizing activities and species composition of methanotrophic communities in various tundra bog soils were investigated by radioisotopic and immunofluorescent methods. Methanotrophic bacteria carried out the methane oxidation process through all horizons of seasonally thawed layers down to permafrost. The highest activity of the process has been observed in the water surface layer of overmoistured soils and in waterlogged moss covers. Up to 40% of $^{14}CH_4$ added was converted into $^{14}CO_2$, bacterial biomass, and organic exometabolites. By immunofluoresecent analysis it was demonstrated that the representatives of I + X (*Methylomonas, Methylobacter,* and *Methylococcus*) and II (*Methylosinus, Methylocystis*) methanotrophic groups occurred simultaneously in all samples at 61.6% and 38.4%, respectively. The number of methane-oxidizing bacteria in the ecosystems studied was 0.1–22.9 × 10^6 cells per gram of soil. Methanotrophic organisms ranged from 1% to 23% of the total bacterial number.

Peat bog soils from various regions including tundra zone are not only the sources of methane, but also peculiar filters on the way of this gas to the atmosphere [5, 7, 8]. It is clear that the active agents of these filters must be methanotrophic bacteria, since only these organisms are capable of oxiding methane. However, earlier studies on the methane oxidation process in overmoistured soils involved neither revelation of the microorganisms nor investigation of the methanotrophic community taxonomic structure. Moreover, lack of information on the nature of methanotrophic microorganisms in this ecosystems led to misunderstanding of the phenomenon.

Complex studies of methane oxidation in tundra bog soils and of microorganisms responsible for the process appear to be urgent aspects in current research.

Materials and Methods

Methane-oxidizing activity was estimated by a radioisotopic method [4]. Water samples, soil, and mosses were incubated with $^{14}CH_4$ dissolved in sterile, degazated water (overall radioactivity 2.5–6.8 μKu) at sampling temperatures. After an incubation period (72 h), the samples were fixed with 1 ml of 2 *N* NaOH. Distillation and quantitation of ^{14}C converted from $^{14}CH_4$ into CO_2, bacterial biomass, and exometabolites were performed as previously described [1, 4]. The r/R (%) ratio of ^{14}C found in CO_2, biomass, and exometabolites to $^{14}CH_4$ added was used to evaluate the activity of methanotrophic communities.

Desorbtion of bacteria from soil samples was performed by the method of Boohlol and Schmidt [2].

Total bacteria count. The suspension of desorbed bacteria was filtered through a nonfluorescent polycarbonate filter with a pore diameter of 0.2 μm (Bio-Rad, USA). Bacteria retained by filters were stained with fluorescein isothiocyanate (FITC; Serva, Germany) and counted in a luminescent microscope [3].

Quantitation of species composition in methanotrophic microflora. Methanotrophs were directly evaluated by the immunofluorescent method [3]. Filters with bacteria were coated with a mixture (1 : 1) of specific rabbit antiserum (diluted 1 : 4 to 1 : 16 by buffered 0.85% NaCl) and rodamine-labeled bovine albumin (diluted 1 : 8). The filters were placed into a moist chamber for 20 min and then washed twice with buffered 0.85% NaCl solution (pH 7.4) to remove unbound antiserum. Dried filters were coated with FITC-labeled antiserum against rabbit globulins (diluted 1 : 16), placed in a moist chamber for 20 min, and finally washed twice with NaCl solution. Filters with stained methanotrophs were examined and counted in a luminescent microscope.

Results and Discussion

Methane oxidation proceeded through the seasonally thawed layer of tundra bog soils under weak

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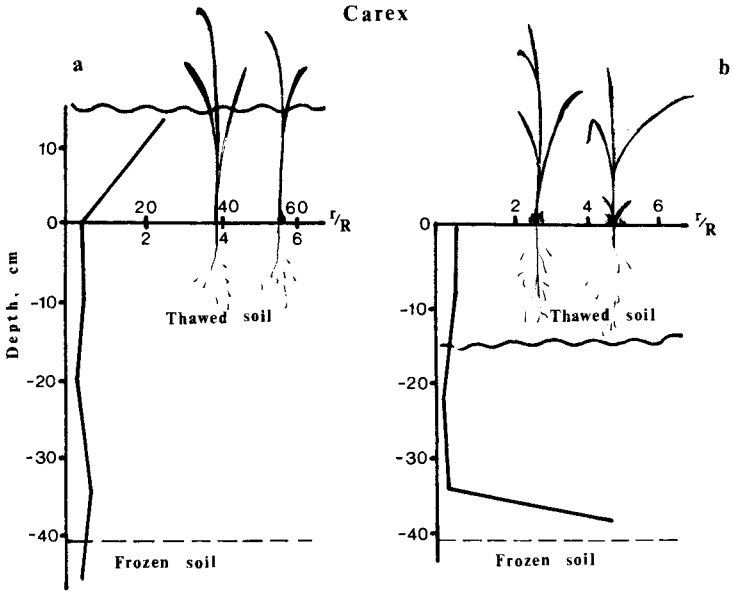

Fig. 1. Activity of $^{14}CH_4$ oxidation in tundra bog soils with various water table levels (r/R-radioactivity, %).

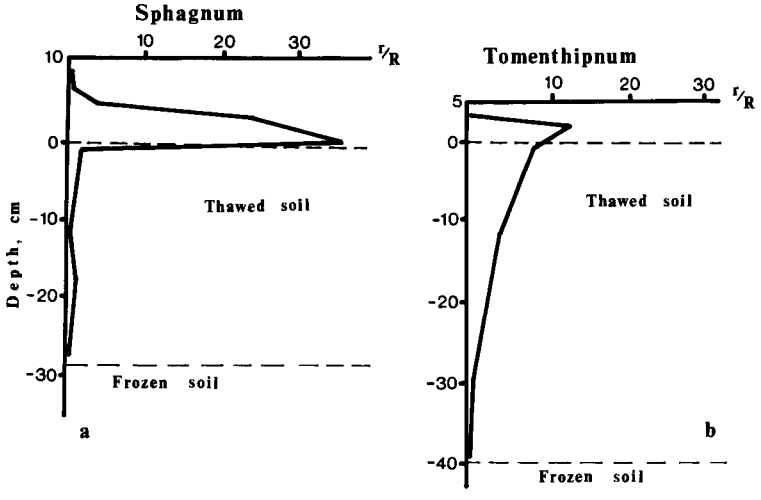

Fig. 2. Activity of $^{14}CH_4$ oxidation in tundra bog soils occupied by different moss associations (r/R-radioactivity, %).

acidic conditions (pH 4.95–5.70) at low temperatures (+7.5° to +9.0°C in surface layers and +0.5°C in above-frozen layers of the soils). This process was strongly affected by the extent of hydromorphism of the soils and the nature of the floristic association. Figure 1 shows the methane oxidation activity profiles of two sites with tundra peat bog soils with different extents of hydromorphism and occupied by vascular plant associations. A direct correlation was demonstrated between methane-oxidizing activity and the water table level. Methane-oxidizing activity in the soil with surface water (Fig. 1a) exceeded that in the soil without a surface water layer (Fig. 1b) by an order of magnitude. In the first case, up to 30% of $^{14}CH_4$ added was converted into $^{14}CO_2$, bacterial biomass, and exometabolites.

Comparison of three overmoistured sites occupied by associations of *Carex* (Fig. 1a) and mosses *Sphagnum* and *Tomenthipnum* (Fig. 2) allows the conclusion that the methanotrophic bacterial filter was disposed in surface water and moss top layers.

It was found that in the *Sphagnum* layer (Fig. 2a) at the boundary with the turf horizon, methane oxidation proceeded most intensively. Up to 40% of labeled carbon was found in CO_2, biomass, and exometabolites. It should be mentioned that high methane-oxidizing activity proved to be typical for various moss associations. Although *Tomenthipnum*

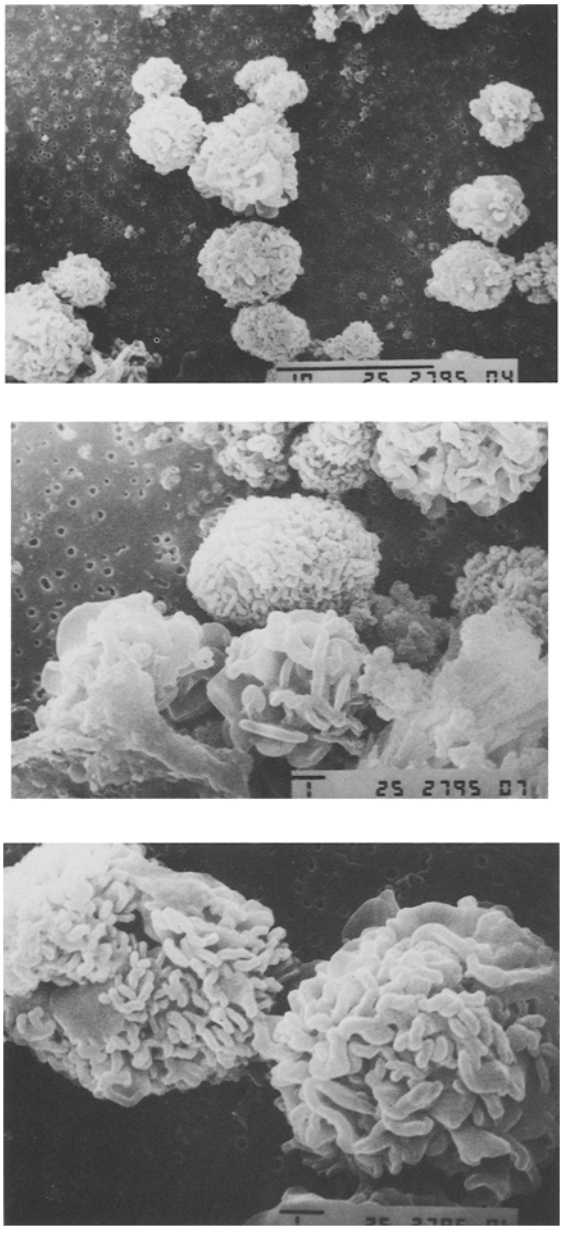

Fig. 3. Scanning electron micrographs of methanotrophic cells that form microcolonies on the particles of half-destroyed organic matter. Bar = 10 μm (*top panel*) or 1 μm (*middle and bottom panels*).

mosses form less thick cover (2–3 cm) than *Sphagnum*, the methane-oxidizing activity is rather high—up to 15% from $^{14}CH_4$ added converted into $^{14}CO_2$, bacterial biomass, and exometabolites. It should also be noted that in all the cases the share of methane carbon oxidized to CO_2 and the share of methane carbon assimilated into bacterial biomass and exometabolites were equal.

The scanning electron microscope method was used to investigate water samples taken from sites occupied by *Carex* as well as from *Sphagnum* layers. Figure 3 shows bacterial microcolonies on organic residues. Immunofluorescent analysis confirmed that these microorganisms belong to methanotrophs. Bacteria adsorbed on particles gave positive reaction with antisera against species: *Methylocystis parvus*, *Methylosinus trichosporium*, *Methylobacter bovis*, and *Methylococcus capsulatus*. The highest methane-oxidizing activities were observed in the same samples. Most intensive methane oxidation takes place at the interfaces where the methane–oxygen ratio is optimal for methanotrophs. Besides, activity of the process is probably affected by the extent of organic matter destruction. The particles of organic matter suspensions serve for formation of methanotrophic microcolonies and gas adsorption. It appears that in this case we observe immobilization and a gas adsorption effect that markedly increase the activity of methanotrophs.

Total bacterial number in the five sites of tundra bog soils ranged from 14.2×10^6 to 54.0×10^6 cells/g soil. There was no decrease in bacterial number from the soil surface to above the frozen layer. To evaluate the species composition of methanotrophic microflora in the ecosystems studied, we used 14 antisera against the majority of the known methanotrophic species (Table 1). The number of methane-oxidizing bacteria in different horizons of seasonally thawed soils was $0.1–22.9 \times 10^6$ cells/g soil. Methanotrophic organisms ranged from 1% to 23% of the total bacterial number in different horizons. Previously Whittenbury et al. [6] estimated this fraction contribution to be about 10% for lake ecosystems. According to Galchenko et al., [4] in marine ecosystems methanotrophs make up sometimes up to 40% of the total bacterial community.

Using the immunofluorescence method, we have found aerobic methanotrophic bacteria through the whole seasonally thawed layer of the soils studied. Moreover, enrichments of methanotrophic cultures were obtained from some samples. Distribution of aerobic methanotrophs confirms that the methane oxidation process revealed in these peat bog soils is the aerobic one.

Representatives of the *Methylobacter* genus are most prevalent, followed by *Methylomonas* and *Methylocystis* (Table 1). Methanotrophs of I and X groups prevailed over bacteria of II group. A similar ratio of methanotrophic groups has also been demonstrated for other ecosystems [3, 4]. However, we are still unable to explain this phenomenon. There were no correlations between the species (genus,

Table 1. Species and genus compositions of methanotrophic microflora in tundra bog soils accordingly to immunofluorescence analysis (% from total methanotrophs)

Organism	Soil variant				
	I	II	III	IV	V
Methylomonas methanica	1.4	4.9	0.8	—	1.8
Mm. albus	47.3	6.6	—	13.0	41.7
Methylobacter capsulatus	1.3	0.3	1.1	1.2	1.1
Mb. vinelandii	1.5	5.4	2.2	4.6	3.4
Mb. chroococcum	11.6	19.2	31.8	18.5	21.4
Mb. bovis	10.9	14.5	14.3	1.9	1.1
Methylococcus capsulatus	0.7	10.5	0.7	1.2	3.1
Methylosinus trichosporium	—	4.3	5.1	14.0	0.3
Ms. sporium	1.8	3.6	25.2	9.5	11.7
Methylocystis minimus	1.1	2.9	1.7	—	3.8
Mcs. methanolicus	3.5	1.4	—	0.3	4.7
Mcs. pyriformis	1.3	2.1	3.7	9.0	2.8
Mcs. parvus	10.7	13.9	7.2	26.7	0.7
Mcs. echinoides	6.1	10.3	—	—	2.2
METHYLOMONAS	48.7	11.5	0.8	13.0	43.5
METHYLOBACTER	25.3	39.4	49.4	26.2	26.9
METHYLOCOCCUS	0.7	10.5	6.7	1.2	3.1
Group I + X	74.7	61.4	56.9	40.4	73.6
METHYLOSINUS	1.8	7.9	30.3	23.5	12.0
METHYLOCYSTIS	22.7	30.6	12.6	36.0	14.2
Group II	24.5	38.5	42.9	59.5	26.2

group) compositions of these ecosystems and the ecological environments. It might be noted that representatives of I and X groups appear to be somewhat allied to microorganisms of the r-strategy. These organisms are characterized by rapid growth under favorable conditions and low survival (rapid rate of die-off) under unfavorable conditions. On the contrary, methanotrophs of II group are characterized by slower growth but better survival. They may be assigned to microorganisms of the K-strategy. In addition, methanotrophs of II group are capable of diazotrophy under microaerophilic and N-limited conditions.

It appears that in spite of slower growth rate, the features mentioned above give certain advantages for survival to methanotrophs of II group over bacteria of I and X groups. It could be supposed that these peculiarities of different groups of methanotrophs permit them to supplement one another and to live in the same ecosystems.

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