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EXPERIMENTAL ARTICLES

Coupling of Microbial Processes of Methane and Ammonium Oxidation in Soils

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Abstract—The effect of ammonium ions on the activity of methane oxidation in soils was studied. The degree of inhibition of the methanotrophic activity in the presence of ammonium in the soil solution was quantitatively assessed as dependent on ammonium concentration and the properties of different types of soils of the European part of Russia.

Key words: methane oxidation, ammonium, inhibition, methanotrophs, nitrification.

Methane and ammonium are important intermediates of the biological cycles of nitrogen and carbon. The ecological role of methane is determined by its capacity to absorb infrared radiation reflected from the Earth's surface, which allows methane to be assigned to the greenhouse gases which are of key significance in global climate change [1].

It has long been believed that the process of methane oxidation in soils is driven solely by methanotrophic bacteria possessing methane monooxygenase. However, it was suggested in a number of recent publications [2, 3] that the group of ammonium-oxidizing bacteria, earlier thought to be highly specific, is able, for lack of the basic substrate, to switch over to methane oxidation. This is explained by the similarity of the key enzymes, methane monooxygenase and ammonium monooxygenase [3]. Several researchers [4, 5] revealed the ability of methane-oxidizing bacteria to switch over to the oxidation of ammonium ions. High methane concentrations (6%) in the soil gaseous phase are also known to decrease the nitrification activity. The inhibition of nitrifying activity by methane can attain 40 to 50% [6].

The aim of this study was to determine the degree of inhibition of methanotrophic activity upon the introduction of ammonium into soils of different types.

MATERIALS AND METHODS

The work was carried out with samples of different soils of the European part of Russia. The general characteristic of soil samples is given in Table 1.

To determine the methane consumption rate, freshly selected soil samples (5 g) were placed in 15-ml flasks; methane at a rate of 10 μl CH_4/l was introduced into the gas phase of the flasks, which were then incubated at 28°C [7].

Methane content in the gas phase was measured using a Chrom-41 gas chromatograph equipped with a flame–ionization detector and a 2.2-m column packed with Spherosil. The thermostat temperature was 30°C; the flow rate of the carrier gas (argon) was 30 ml/min; the flow rate of hydrogen was 30 ml/min; the flow rate of oxygen was 10 ml/min. The samples (0.5 cm^3) were injected with a medical syringe.

The inhibitory effect of ammonium was determined from the decrease in methanotrophic activity of soils after the addition of an aqueous solution of ammonium chloride (0.083 mg N/g of soil) to the flasks [8].

The coupling of the processes of methane and ammonium oxidation in soil samples was assessed from the relative contribution of methanotrophic and nitrifying bacteria to these processes. For this purpose, we used a method based on the different rates of restoration of the initial activity of methanotrophs and nitrifiers after acetylene treatment [3]. This distinction is due to the different sensitivity of the enzymes, methane monooxygenase and ammonium monooxygenase, the to acetylene, which was confirmed by several researchers [2, 4]. Exposure to acetylene results in the inhibition of the de novo synthesis of ammonium monooxygenase, which allows temporary exclusion of nitrifiers from being involved in the processes of methane and ammonium oxidation.

To assess the contribution of nitrifiers to methane oxidation in soils, two experimental variants were staged. In the first variant, the total contribution of methanotrophs and nitrifiers to methane oxidation was assessed. For this purpose, methane (10 to 15 vol %) was introduced into the gas phase of the flasks containing 5-g soil samples with a natural humidity [7]; the flasks were incubated at 28°C for 48 h. In the second variant, the participation of nitrifiers in methane oxidation was excluded and the contribution of only methan-

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Table 1. Main characteristics of soil samples used in this work

Soil	The region of sampling	Horizon, depth (cm)	pH of aqueous extract	Humus content, %
Leached medium-thick sandy-loam chernozem on carbonate loess-like loam	Lipetsk oblast	Al, 2–10	6.5	6.9
Well-cultivated peat soil on herbaceous–ligneous peat	Moscow oblast	A_{arable} 0–20	5.4	30%*
Soddy podzolic medium-loam soil on the loam of moraine deposits	Moscow oblast	Al, 3–15	5.2	4.2
Gray forest medium-thick medium-loam soil on carbonate-free loam	Tula oblast	Al, 3–15	6.1	5.3

* The organic substance content.

otrophs was assessed. To do this, acetylene (1 vol %) was introduced into the flasks with soil samples [7], which were then incubated for 24 h. After acetylene removal, methane (10 to 15 vol %) was introduced into the gas phase [7], and the flasks were incubated at 28°C for 48 h. After a preliminary soil incubation in the presence of acetylene, the nitrifiers remained inactive for at least three days, while the activity of methane monooxygenase was restored in 24 h [3]. The relative contribution of nitrifiers to methane oxidation was calculated using the following formula:

$$\mathrm{AmOx} = (A - B)/A \times 100\%,$$

where AmOx is the relative contribution of ammonium-oxidizing bacteria to methane oxidation in the soil, %; A is the average rate of total methane oxidation in the soil, nmol CH_4/(g h); and B is the average rate of methane oxidation by methanotrophs in the soil, nmol CH_4/(g h).

The contribution of methanotrophs to ammonium oxidation was assessed using an indirect method, namely from the methane consumption rate in the presence of ammonium. Thus, we succeeded in assessing the contribution of methanotrophs to the nitrification process in the simultaneous presence of the two substrates, methane and ammonium. From the point of modeling natural processes, this is the most correct approach, because both of the two substrates are normally present in soil. At this step of the work, we also staged two experimental variants. At first, in order to suppress the activity of nitrifiers and to rule out their contribution to ammonium and methane oxidation, the soil samples in both experimental variants were incubated with acetylene. After acetylene removal, methane was introduced into the gas phase of the flasks. In one experimental variant, an aqueous solution of ammonium chloride was introduced in a dose of 0.083 mg N/g soil together with methane [8]. Thus, in one variant, methanotrophs oxidized only methane, while, in the other variant, they participated in methane and ammonium oxidation. The relative contribution of methanotrophs to ammonium oxidation was calculated according to the following formula:

$$\mathrm{MethOx} = (A - B)/A \times 100\%,$$

where MethOx is the relative contribution of methanotrophs to ammonium oxidation in the soil, %; A is the average rate of methane oxidation by methanotrophs in the native soil, nmol CH_4/(g h); and B is the average rate of methane oxidation by methanotrophs after the introduction of ammonium, nmol CH_4/(g h).

The measurement of the acidity of soil samples was carried out in soil suspensions using an EV-74 universal ionometer [9].

The results of the experiments were processed statistically [10]. The maximum confidence intervals are presented in the figures.

RESULTS

The activity of methane oxidation in different types of soils and the changes in the methanotrophic activity after the introduction of ammonium chloride are shown in Fig. 1. The results obtained testify to the fact that, depending on the type of soils, the degree of inhibition of the methanotrophic activity significantly varied. Among the soils studied, the following sequence was traced in order of increasing degree of inhibition: soddy podzolic soil, gray forest soil, leached chernozem, peat soil.

The experiments which determined the contribution of autotrophic nitrifiers to the process of methane oxidation in different types of soils established that the preliminarily treatment of the soil samples with acetylene significantly decreased the methane-oxidizing activity of soils (Fig. 2). A similar decrease in the methane-oxidizing activity in acetylene-treated soil samples was also observed in the experiments with other types of soils. Assuming that such a decrease in the activity is determined by the absence of the contribution of nitrifiers to methane oxidation [3], we tried to approximately assess the contribution of nitrifiers to the methane oxidation process in the types of soils studied. As a result, it was found that the contribution of nitrifiers in different types of soils did not differ significantly (Table 2). This contribution appeared to be somewhat higher in soils with a lower content of organic matter (soddy podzolic and gray forest soils).

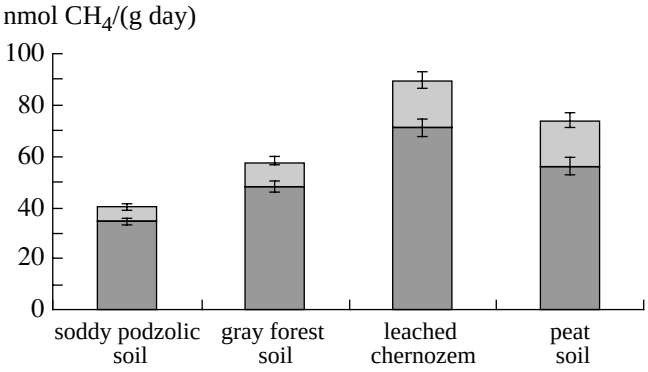

Fig. 1. Decrease in the methanotrophic activity after the introduction of the ammonium salt into soils of different types.

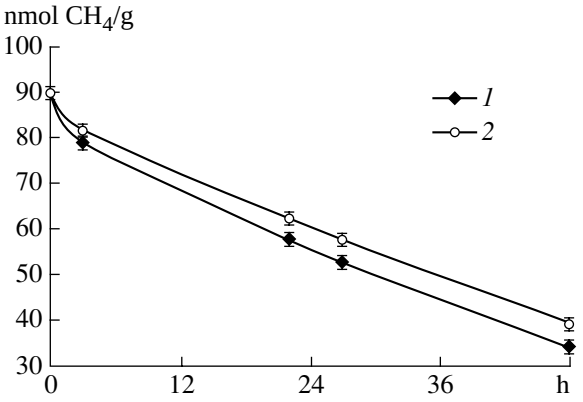

Fig. 2. Dynamics of methane consumption in leached chernozem: (*1*) total consumption; (*2*) methanotroph contribution.

There is a certain discrepancy between the results of our assessment of the contribution of autotrophic nitrifiers to the process of methane oxidation in different types of soils of the European part of Russia and the data obtained by Bodelier and Frenzel, who developed the method we used [3]. This fact can be explained by substantial differences in the biological and chemical properties of the soils of rice check plots [3] and the soils we studied. Bodelier and Frenzel [3] suggested in their work that similar experiments with other types of soils might give different results, and this was confirmed by our work.

The presence of ammonium in native soil samples does not influence the reliability of the assessment of the contribution of nitrifiers to methane oxidation, since, in this case, the source of energy of the autotrophic nitrifiers is only methane. This is explained by the fact that the oxidation of 1 mol of methane yields more energy than the oxidation of 1 mol of ammonium [11]. Methane cooxidation by nitrifying bacteria is likely to be an important source of energy and cellular material under certain conditions, especially when the optimal pH value is attained. It may be suggested that the capacity for methane oxidation is inherent in all classical chemolithotrophic ammonium-oxidizing bacteria. The ability of ammonium-oxidizing bacteria to switch over from ammonium oxidation to methane oxidation probably serves as a mechanism of survival, when one of these sources of energy is not available. Since methane is always present in small concentrations, the advantage of such a capacity for the use of an alternative substrate is evident. Under the conditions when both ammonium and methane are present at concentrations that are not sufficient for the maintenance of growth, their combination and cooxidation by ammonium-oxidizing bacteria can fulfill the requirements for carbon and energy [11, 12].

Table 2. Inhibitory effect of ammonium on the activity of methane oxidation

Soil	The inhibitory effect (% of the initial methane oxidation rate)
Leached chernozem	17.0 ± 3.0
Peat soil	21.0 ± 3.0
Soddy podzolic soil	24.0 ± 3.0
Gray forest soil	15.0 ± 2.5

Table 3. Contribution of methanotrophs to methane and ammonium oxidation

Soil	Contribution of methanotrophs (%) to the oxidation of	
	methane	ammonium
Leached chernozem	79.0 ± 4.0	21.0 ± 4.0
Peat soil	76.0 ± 4.0	24.0 ± 4.0
Soddy podzolic soil	85.0 ± 3.0	15.0 ± 3.0
Gray forest soil	83.0 ± 3.5	17.0 ± 3.5

The study of the contribution of methanotrophic bacteria to ammonium oxidation in different types of soils revealed a substantial decrease in the methane consumption rate in the presence of ammonium in all types of the soil studied (Fig. 3). As an example of a change in the dynamics of methane oxidation after ammonium chloride introduction, methane consumption rate curves in leached chernozem samples are given in Fig. 2. Based on the measured activity of methane oxidation in the two variants of the experiment, the relative contribution of methanotrophs to ammonium oxidation in different types of soils was calculated (Table 3). In the soils with a high content of organic matter (leached chernozem, peat soil), this contribution appeared to be slightly higher; however, on the whole, this contribution in different types of soils was roughly the same. The probable explanation of this fact is the direct dependence of the activity of methanotrophic and

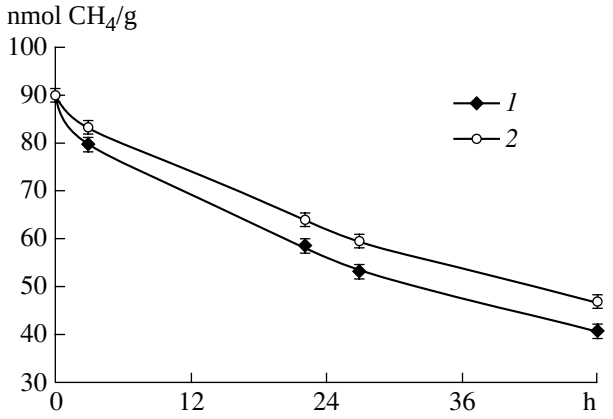

Fig. 3. Dynamics of methane consumption in leached chernozem under the conditions of inhibition of ammonium-oxidizing bacteria: (*1*) native soil; (*2*) after NH_4Cl introduction.

nitrifying microorganisms on the presence of methane and ammonium in the soil and their quantitative ratio.

DISCUSSION

Our study confirmed the inhibitory effect of the ammonium ion on the methanotrophic activity of the soils studied. In terms of an increasing inhibition degree, these soils rank in the following order: soddy podzolic soil, gray forest soil, leached chernozem, peat soil.

The cause of this effect is the involvement of methanotrophic microorganisms in ammonium oxidation, i.e., in the process of nitrification. When ammonium salt is introduced into soil, part of the microbial methanotrophic community is recruited for the nitrification process. This part, depending on the type of the soil, constitutes 12 to 28%.

The assessment of the contribution of nitrifiers to methane oxidation in soils showed that, depending on the type of soil, it reaches 5 to 16%. However, this value may vary within wide limits for various types of soils. This is evidenced by the works published by Kravchenko *et al.* [6].

The effect of ammonium on the methanotrophic activity of soil microorganisms considered in this study emphasizes the special role of ammonium nitrogen in the global nitrogen and carbon cycles. Earlier, we showed the different character of the effect of the ammonium and nitrate forms of nitrogen on the processes of methane release and consumption in soils [13]. There are also certain applied aspects of this work. In particular, the low effectiveness of nitrification inhibitors (for example, N-serve) introduced in soil along with fertilizers can be explained by the ability of methanotrophic bacteria to participate in the nitrification process.

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