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EXPERIMENTAL ARTICLES

Seasonal Dynamics of Atmospheric Methane Oxidation in Gray Forest Soils

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Abstract—Seasonal fluctuations in the methane fluxes in the soil–atmosphere system were determined for gray forest soils of Central Russia. Consumption of atmospheric methane was found to exceed methane emission in gray forest soils under forest and in the agrocenosis. The average annual rates of atmospheric methane consumption by the soil under forest and in the agrocenosis were 0.026 and 0.008 mg C-CH_4/(m^2 h), respectively. The annual rate of atmospheric methane oxidation in the gray forest soils of Moscow oblast was estimated to be 0.68 kton. Seasonal fluctuations in the methane oxidation activity were due to changes in the hydrothermal conditions and in the reserves of readily decomposable organic matter and mineral nitrogen, as well as to changes in the activity of methane oxidizers.

Key words: methane oxidation, gray forest soil, correlation analysis.

The main feature of the present-day biogeochemical methane cycle is the annual 0.8–1.0% increase in methane concentration in the earth's atmosphere, which suggests increasing methane emission from natural and anthropogenic sources with unchanged or even decreasing rates of its consumption. The main sinks for atmospheric methane are its photochemical oxidation by hydroxyl radicals in the troposphere (490 ± 85 Tg/year) and consumption by soil microorganisms as a source of carbon and energy (30 ± 15 Tg/year) [1]. These values may need correction as new data on the methane-oxidizing capacity of soils in different climatic zones are obtained.

Despite the active study of different aspects of biological methane consumption [2–4], the ecological representativeness of the data available for soils remains insufficient. This does not allow a complete quantitative concept of the role of soil in the global methane sink to be developed, which makes it impossible to explain and predict the tendency of methane concentration to change in the atmosphere. It is especially important to understand the role of the soils of Russia, which are usually not included in the estimates of the global sinks of atmospheric methane on the territory of Europe [5] due to the lack of published data on seasonal changes in the methane-oxidizing capacity of Russian soils, which occupy vast territories and occur in a zone characterized by sharp variability of environmental factors. Extremely scarce are complex studies on methane oxidation that combine field and laboratory methods of soil science and microbiology. More detailed understanding is needed of the changes in the methane-oxidizing capacity of soils depending on their physicochemical and biological properties that change under the action of ecological and anthropogenic factors.

The aim of this work was (1) to estimate the annual net methane flux for the gray forest soil of the forest biocenosis and agrocenosis and (2) to determine the influence of the physicochemical and biological parameters of the soil on the magnitude of this flux.

MATERIALS AND METHODS

The subject of study was the gray forest soil of stationary plots of forest biocenosis and agrocenosis (Experimental Field Station, Institute of Physicochemical and Biological Problems of Soil Science, Russian Academy of Sciences, Pushchino, Moscow oblast), located 300–400 m apart. The forest plot was in a secondary mixed coniferous–broad-leaved forest with rarefied grass cover. The agrocenosis plot was a part of a three-field crop rotation (fallow–winter wheat–barley) cultivated since 1988 without the introduction of mineral fertilizers. In the study period, winter wheat was

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grown in the plot, with subsequent fall plowing after harvesting.

Performing field measurements. The magnitude of the methane flux in the soil–atmosphere system was determined using the static chamber method [6] every month from December 1999 through December 2000. In each experimental plot, there were three chambers (a steel 32- × 32-cm bottom cut to a depth of 20 cm and connected with a hydraulic valve to a 15-cm-high chamber made of organic glass). The gas was sampled with a syringe and introduced into 20-ml flasks filled with 1 N NaCl. Sampling was performed between 10 and 12 a.m.; the exposure time was 30 min in the frost-free period and 1 h in winter. The magnitude of the methane flux was calculated proceeding from the change in methane concentration in the chamber during the exposure period.

Soil sampling. Simultaneously with gas sampling, four soil blocks of intact texture were cut out from the 5- to 10- and 10- to 15-cm soil layers at a distance of 5–10 m from the chambers. Two blocks were used for determining the moisture content, chemical composition, and microbiological characteristics of the soil, and the remaining two were used for the assessment of the potential methane-oxidizing capacity of the soil. Soil temperature was registered at the moment of sampling.

Determination of methane-oxidizing activity. Fresh soil (50 g) was introduced into 500-ml flasks. The methane-oxidizing activity was judged from the dynamics of the loss of methane, whose initial concentration in the gas phase constituted 5–6 nl CH_4/ml. Samples (0.5 ml) were taken from the air phase of the flasks for 120 h at an interval of 8–24 h. The methane concentration was measured using a model 3700 gas chromatograph (Moscow) equipped with a flame-ionization detector and 2-m columns with Porapak Q; the carrier gas was helium at a flow rate of 30 ml/min; the column temperature was 70°C. Changes in the methane concentration were described using the equation $d[CH_4]/dt = k_1[CH_4]$.

Enumeration of bacteria in soil was carried out by luminescence microscopy. The cells were desorbed from the surface of soil particles using a UZDN-A ultrasonic unit (Russia) for 3 min at 22 kHz with the addition of Tween 80 detergent (Serva, Germany) and M-30 silicone antifoaming agent (Serva, Germany). A coagulating mixture of $Ca(OH)_2$ and $MgCO_3$ (2 : 5) was then added and allowed to stand for 5 min for the soil particles and colloids to settle. The supernatant containing bacteria was filtered through nonfluorescent polycarbonate filters (Poretix, USA), stained with 4% fluorescein isothiocyanate (Serva, Germany), and examined under a Lyumam-I2 epiluminescence microscope (Russia).

Analytical determinations. Chemical analyses were performed in four replicates. The NH_4^+-N and NO_3^--N contents were determined using the phenolate–hypochlorite method [7] directly in the soil extract and after reducing nitrates to ammonium with a zinc powder and 10% $CuSO_4$. The soil content of salt-soluble carbon and nitrogen compounds was determined, and the carbon and nitrogen of microbial biomass were calculated according to the technique described earlier [8]. The organic carbon content and the total nitrogen content were assessed by Tyurin's and Kjeldahl's methods, respectively, in a mixture of samples taken from the 5- to 10- and 10- to 15-cm soil layers in June 2000.

Statistical processing of the results was carried out using the Excel XP and Origin 7.0 software.

RESULTS AND DISCUSSION

Methane cycling in the soil–atmosphere system is determined by the processes of mineralization of organic matter in anaerobic microzones and ascending and descending diffusion in the pore space of the methane produced in the soil and the methane arriving from the atmosphere, as well as by methane oxidation by methane oxidizers. Methane production in aerobic soils is related to the granulometric composition; the presence of large-sized aggregates, inside which anaerobic conditions may be created; and overconsolidation of the upper-horizon lower layers, especially in arable soils [9, 10]. In most forest soils, the highest methane oxidation activity was revealed in the upper 4- to 18-cm soil horizon [2, 11]. The methane produced under this layer is partially or completely oxidized by soil microorganisms. Thus, in aerobic soils, the closed-chambers method measures the resultant of the processes, which characterizes the net gas flux and gross methane oxidation.

Figure 1 shows the results of in situ determination of the methane flux in the soil–atmosphere system. The gray forest soil of both the forest biocenosis and the agrocenosis is a sink for atmospheric methane, with the magnitude of the flux varying substantially during the annual cycle. In both soils, the highest rate of methane oxidation was recorded in the warm period of the year (May–October), constituting 0.048–0.06 mg C-CH_4/(m^2 h) for the forest area and 0.03–0.037 mg C-CH_4/(m^2 h) for the agrocenosis. In the cold season (November–April), the capacity of the forest soil for methane oxidation was retained; however, its rate decreased 3- to 4-fold. In the agrocenosis soil, methane oxidation in this period reduced significantly or ceased completely, resulting in methane emission from the soil into the atmosphere being recorded by us in February and March.

The methane oxidation rate values agree well with the data obtained by other authors for similar soils under similar ecological conditions. Thus, for example, methane oxidation by the soils of natural ecosystems of the state of Iowa (USA) constituted 0.027–1.046 mg CH_4/(m^2 day), whereas in agricultural ecosystems it constituted 0.077 mg CH_4/(m^2 day) and alternated with methane emission [12]. In subarctic and temperate forest soils, the rate of methane oxidation was equal to

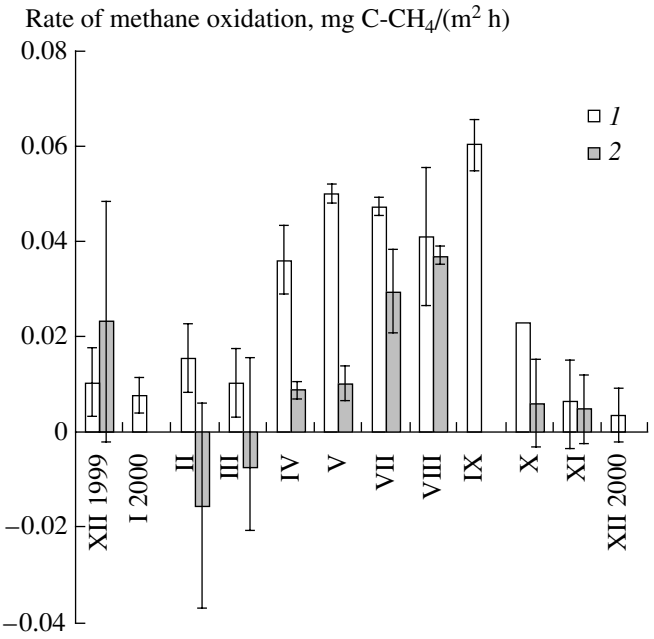

Fig. 1. Methane fluxes in the soil–atmosphere system measured by the static chambers method in forest biocenosis and agrocenosis: (*1*) forest; (*2*) agrocenosis.

1–3 mg CH_4/(m^2 day) [2] or 2.1–6.9 mg CH_4/(m^2 day) [13]. The average annual rate of methane consumption by the gray forest soil in the forest was 0.026 mg C-CH_4/(m^2 h) and 0.008 mg C-CH_4/(m^2 h) in the agrocenosis, which corresponds to approximately 2.3 and 0.8 kg of C-CH_4/(ha year), respectively. Thus, the annual rates of atmospheric methane sink due to its oxidation in the gray forest soils of Moscow oblast may be estimated as 0.68 kton.

The incubation experiments showed that the methane-oxidizing activity of the gray forest soil sampled over the year underwent significant changes under controlled laboratory conditions (Fig. 2). The highest values were revealed in the May–October period, the activity in the 10- to 15-cm horizon samples being higher than in the 5- to 10-cm horizon samples. In arable soil, the methane oxidation activity of the samples virtually did not vary and was very low at all determination times. The total bacterial number was at its maximum in the summer months both in the forest and the arable soil and decreased significantly in spring and winter (Fig. 3).

The markedly pronounced seasonal dynamics of methane oxidation activity in situ testifies to a considerable dependence of the activity of the microbial community responsible for this process on the soil hydrothermal conditions and physicochemical properties. During the observation period, significant changes in the soil temperature and moisture content and slight pH changes were noted (Fig. 4). The difference between the minimal and maximal values of soil temperature in the agrocenosis and in the forest was 13–18 and 18–25°C, respectively. The changes in the 5- to 10-cm soil layer were more pronounced than at a depth of 10–15 cm. As distinct from the arable lot, the snow-covered forest soil virtually did not freeze, retaining its loose texture. In the opinion of several authors [3, 11, 14], methane consumption by soil ceases at 0°C; this process is most active at 20°C and slows down with a temperature rise to 30°C. Our data are consistent with this conclusion. We consider the decline in methane oxidation activity to be the main cause of methane emission from frozen arable soil into the atmosphere in February and in March.

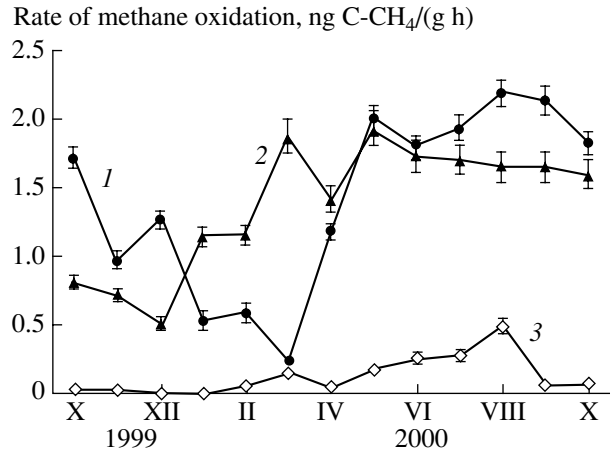

Fig. 2. Change in the potential methane oxidation activity of the gray forest soil of forest biocenosis and agrocenosis: (*1*) forest, 5- to 10-cm layer; (*2*) the same, 10- to 15-cm layer; (*3*) agrocenosis, 10- to 15-cm layer.

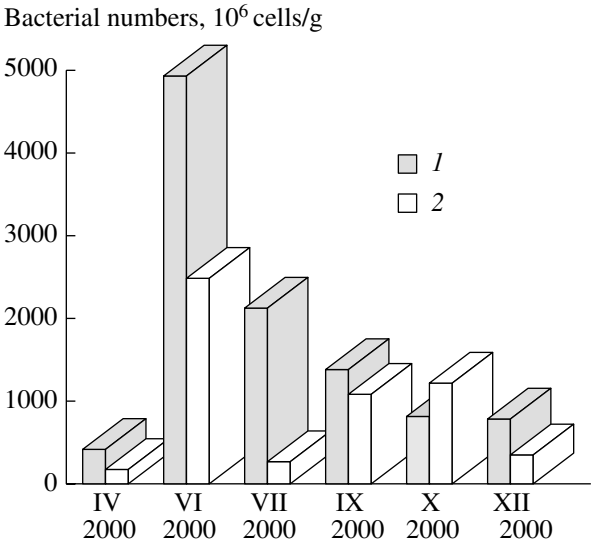

Fig. 3. Bacterial numbers in the gray forest soil of forest biocenosis and agrocenosis in different periods of a year: (*1*) forest; (*2*) agrocenosis.

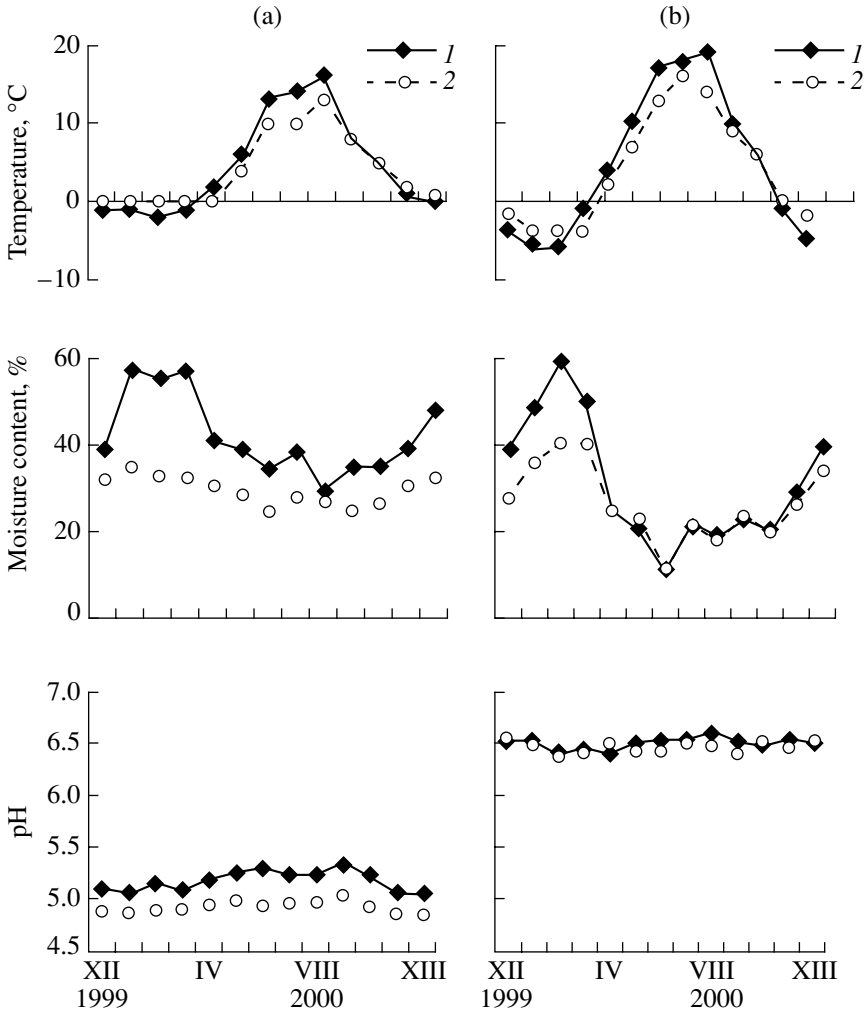

Fig. 4. Seasonal dynamics of temperature, moisture content, and pH in gray forest soil: (a) forest; (b) agrocenosis; (*1*) 5- to 10-cm layer; (*2*) 10- to 15-cm layer.

One of the main factors limiting the methane oxidation activity is the soil moisture content. An excessive moisture content is unfavorable for methane oxidation since the degree of aeration decreases [14]. At the same time, low humidity also results in a substantial decrease in methane oxidation, most probably due to stressful moisture deficiency or the accumulation of mineral nitrogen species in soil [15]. Methane oxidation proceeds most actively when the soil moisture content is 20% [2, 3]. Its is precisely this level of forest soil moisture content in situ that corresponded to the periods of the highest methane oxidation activity (Figs. 1, 4).

The table shows the coefficients of partial correlation between the rate of methane oxidation and the seasonal variability of the soil hydrothermal conditions and physicochemical properties. A positive correlation was revealed between the methane oxidation activity and temperature in both forest (r = 0.735) and arable (r = 0.703) soil. The correlation between the soil moisture content and methane consumption was negative (r = –0.431 for the forest soil and r = –0.634 for the arable soil). Similar conclusions were reached by Swedish investigators when they studied the methane flux in forest biocenosis soils over 1.5 years [16]. The value of the coefficient of correlation between methane oxidation and the moisture content obtained by them (r = –0.671) agrees with our values.

The gray forest soil content of ammonium and nitrate nitrogen in the agrocenosis and forest areas may be considered moderate. In forest soil, ammonium nitrogen prevailed over nitrate nitrogen (Fig. 5), possibly, because of the increased availability of the microbial biomass nitrogen in the forest soil and a pH unfavorable for nitrification. The low content of microbial biomass nitrogen in the arable soil limited the production of mineral nitrogen, predominantly represented by nitrates. The greatest amount of mineral nitrogen in the soil of both areas was discovered in winter, when the biotic utilization of nitrogen was reduced to its minimum. The results of correlation

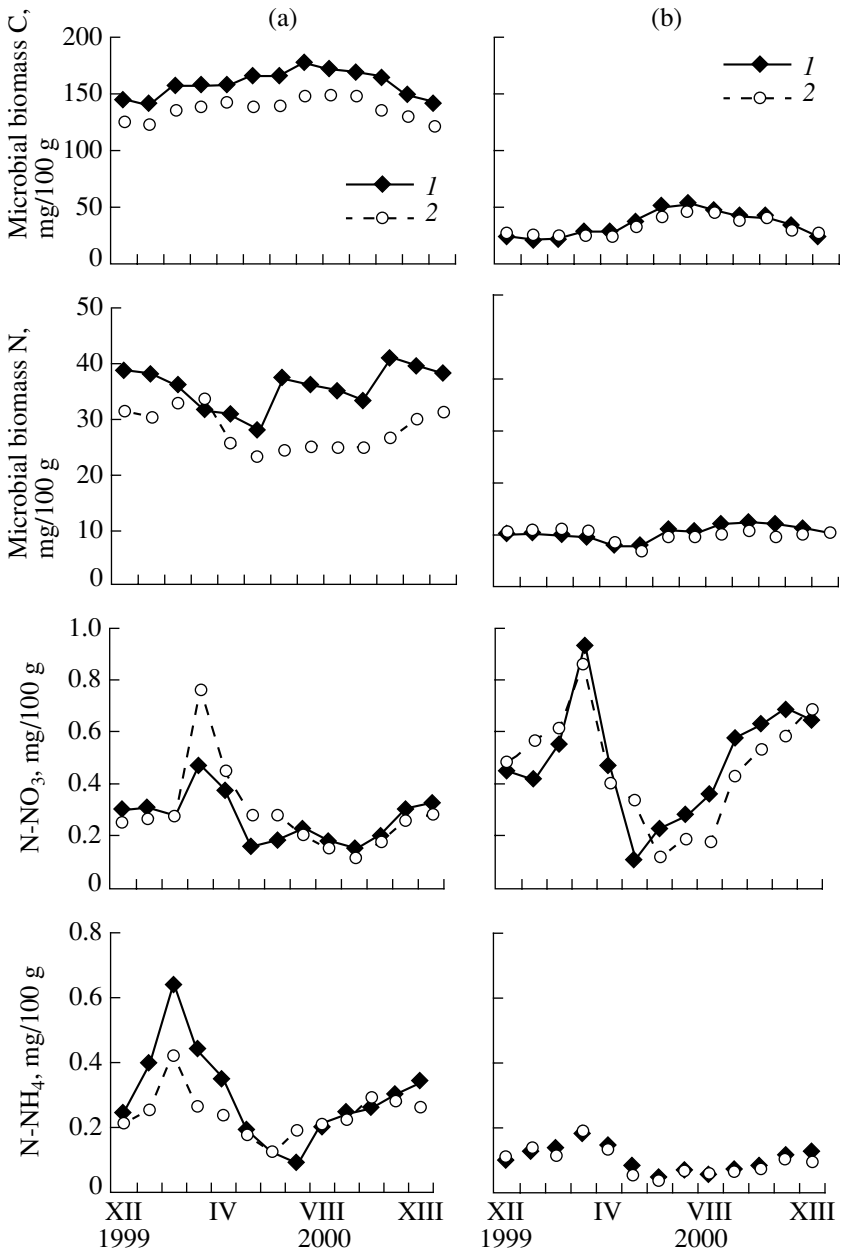

Fig. 5. Seasonal dynamics of carbon and nitrogen contents in gray forest soil: (a) forest; (b) agrocenosis; (*1*) 5- to 10-cm layer; (*2*) 10- to 15-cm layer.

analysis showed a negative influence of mineral nitrogen compounds on the methane-oxidizing capacity of the gray forest soil, and this influence was stronger in the soil of the agrocenosis than in the soil of the forest (table). In addition, a negative correlation between the rate of methane oxidation and the content of microbial biomass nitrogen in the forest soil was established. Thus, the influence of nitrogenous compounds on methane-oxidizing capacity depends on the ratio of the processes of nitrogen mineralization and immobilization in the soil. A similar conclusion was drawn in [13, 17] on the basis of data obtained using other techniques.

To assess the integral effect of the interacting factors, we used the multiple regression method. The coefficients of multiple determination that we obtained showed that, in the forest and in the agrocenosis, 92 and 72% of the methane oxidation activity of the soil was determined by interaction of the factors taken into account (X_1–X_7).

The dependence of the rate of methane oxidation on the hydrothermal and physicochemical factors may be described using the following equations:

$$Y_1 = 0.257X_1 - 0.499X_2 + 0.172X_3 - 2.168X_4 - 10.28X_5 - 1.561X_6 + 90.8X_7 - 363.8, \quad (1)$$

Correlation between methane-oxidizing activity of gray forest soil and physicochemical parameters

Methane oxidation rate, μg C-CH_4/(m^2 h)	Parameters						
	temperature, °C (X_1)	moisture content, % (X_2)	microbial biomass carbon, mg/100 g (X_3)	microbial biomass nitrogen, mg/100 g (X_4)	N-NH_4^+, mg/100 g (X_5)	N-NO_3^-, mg/100 g (X_6)	pH (X_7)
Forest (Y_1)	0.735**	–0.431*	0.578**	–0.508*	–0.496*	–0.454*	0.498*
Agrocenosis (Y_2)	0.703**	–0.634**	0.619**	–0.036	–0.592**	–0.646**	0.594**

* Significant at a 5% significance level.

** Significant at a 1% significance level.

$$R^2 = 0.917,$$
$$Y_2 = 1.054X_1 - 0.128X_2 - 0.251X_3 - 0.155X_4 + 70.86X_5 - 22.77X_6 + 117.7X_7 - 741.3, \quad (2)$$
$$R^2 = 0.725,$$

where Y_1 and Y_2 are the rates of methane consumption in the soils of the forest and the agrocenosis, respectively, mg C-CH_4/(m^2 h); X_1 is soil temperature, °C; X_2 is the moisture content of soil, %; X_3 is the carbon of the microbial biomass, mg/100 g soil; X_4 is the nitrogen of the microbial biomass, mg/100 g soil; X_5 is N-NH_4^+, mg/100 g soil; X_6 is N-NO_3^-, mg/100 g soil; and X_7 is pH.

The coefficients of multiple determination (R^2) characterizing the dependence of the methane oxidation rate on temperature (X_1) and moisture content (X_2) were significantly lower than those obtained when using seven variables (0.546 and 0.511 for the forest and agrocenosis areas, respectively). When the soil chemical properties (X_3–X_7) were used as independent variables, the values of the coefficients of determination for the same samples constituted 0.897 and 0.699, respectively. It can be assumed that the influence of hydrothermal conditions on methane oxidation activity is largely determined by changes in the physicochemical properties of the soil.

Thus, the studies conducted by us show that gray forest soils, both native and liable to an anthropogenic effect, are the sink of atmospheric methane. The methane oxidation values obtained for gray forest soils should be taken into account when calculating the methane balance in the terrestrial ecosystems of Eurasia. The magnitude of the methane flux is subject to considerable seasonal fluctuations caused by the aggregate influence on the activity of methane oxidizers of the hydrothermal soil conditions and availability of organic matter and mineral nitrogen.

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REFERENCES

1. IPCC (Intergovernmental Panel on Climate Change) *Climate Change, 1995: The Science of Climate Change*, Houghton J.T. *et al.*, eds., Cambridge: Cambridge Univ. Press, 1996.
2. Adamsen, A.P.S. and King, G.M., Methane Consumption in Temperate and Subarctic Forest Soils: Rates, Vertical Zonation, and Responses to Water and Nitrogen, *Appl. Environ. Microbiol.*, 1993, vol. 59, pp. 485–490.
3. Castro, M.S., Steudler, P.A., Melillo, J.M., Aber, J.D., and Bowden, R.D., Factors Controlling Atmospheric Methane Consumption by Temperate Forest Soils, *Global Biogeochem. Cycles*, 1995, vol. 9, pp. 1–10.
4. Le Mer, J. and Roger, P., Production, Oxidation, Emission and Consumption of Methane by Soils: a Review, *Eur. J. Soil Biol.*, 2001, vol. 37, pp. 25–50.
5. Smith, K.A., Dobbie, K.E., Ball, B.C., Bakken, R., Sitaula, B.K., Hansen, S., Brumme, R., Borken, W., Christensen, S., Prieme, A., Fowler, D., MacDonald, A., Skiba, U., Klemedtsson, I., Kasimir-Klemedtsson, A., Degorska, A., and Orlanski, P., Oxidation of Atmospheric Methane in Northern European Soils, Comparison with Other Ecosystems, and Uncertainties in the Global Terrestrial Sink, *Global Change Biol.*, 2000, vol. 6, pp. 791–803.
6. Lopes de Gerenyu, V.O., Kurganova, I.N., Rozanova, L.N., and Kudeyarov, V.N., Annual Emission of Carbon Dioxide from Soils of Russian Southern Taiga, *Pochvovedenie*, 2001, no. 9, pp. 1045–1059.
7. Kudeyarov, V.N., Colorimetric Determination of Ammonium Nitrogen in Soils and Plants by the Phenol Method, *Agrokhimiya*, 1965, no. 6, pp. 146–151.
8. Blagodatskii, S.A., Blagodatskaya, E.V., Gorbenko, A.Yu., and Panikov, N.S., The Rehydration Method for Determination of Microbial Biomass in Soil, *Pochvovedenie*, 1987, no. 7, pp. 64–71.
9. Landina, M.M., *Pochvennyi vozdukh* (Soil Air), Novosibirsk: Nauka, 1992.
10. Manucharova, N.A., Stepanov, A.L., and Umarov, M.M., Peculiarities of Microbial Transformation of Nitrogen in Water-Stable Aggregates of Soils of Different Types, *Pochvovedenie*, 2001, no. 10, pp. 1261–1267.
11. Roslev, P., Iversen, N., and Henriksen, K., Oxidation and Assimilation of Atmospheric Methane by Soil Methane Oxidizers, *Appl. Environ. Microbiol.*, 1997, vol. 63, pp. 874–880.

12. Chan, A.S.K. and Parkin, T.B., Methane Oxidation and Production Activity in Soils from Natural and Agricultural Ecosystems, *J. Environ. Qual.,* 2001, vol. 30, pp. 1896–1903.
13. Goldman, M.B., Groffman, P.M., Pouyat, R.V., McDonnell, M.J., and Pickett, S.T.A., CH_4 Uptake and N Availability in Forest Soils along an Urban to Rural Gradient, *Soil Biol. Biochem.*, 1995, vol. 27, pp. 281–286.
14. Sitaula, B.K., Bakken, L.R., and Abrahamsen, G., CH_4 Uptake by Temperate Forest Soils: Effect of N Input and Soil Acidification, *Soil Biol. Biochem.*, 1995, vol. 27, pp. 871–880.
15. Boeckx, P., Van Cleemput, O., and Villaralvo, I., Methane Oxidation in Soils with Different Textures and Land Use, *Nutrient Cycles Agroecosyst.*, 1997, vol. 49, pp. 91–95.
16. Klemedtsson, A.K. and Klemedtsson, L., Methane Uptake in Swedish Forest Soil in Relation to Liming and Extra N-Deposition, *Biol. Fertil. Soils*, 1997, vol. 25, pp. 296–301.
17. Boeckx, P. and Van Cleemput, O., Methane Oxidation in a Neutral Landfill Cover Soil: Influence of Moisture Content, Temperature, and Nitrogen Turnover, *J. Environ. Qual.*, 1996, vol. 25, pp. 178–183.