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EXPERIMENTAL ARTICLES

Biological Activity of Ancient Cultivated Soil with Buried Horizons (the Iverskii Monastery, XVII Century)

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Abstract—The biological activity of an ancient cultivated soil that has been in intense agricultural use since approximately the first half of the XVII century was studied. The potential biological activity of the buried horizon of the ancient cultivated soil was higher than that of its modern horizon or that of the noncultivated soil of an adjacent territory occurring under similar lithological and geomorphological conditions. A decreased rate of oxidative processes (decreased rates of CO_2 production and CH_4 oxidation) and an increased rate of reductive processes (denitrification and nitrogen fixation) were found in the buried horizon. A high potential denitrification activity (with predominant formation of nitrous oxide) was found in the buried horizon; in the upper horizon, the end product was molecular nitrogen.

Key words: potential biological activity, methane production, methane consumption, denitrification, nitrogen fixation, ancient cultivated soil, burried horizon

The peculiarity of the soils used in agriculture is a change in their biological activity, which is connected with the application of various agrotechnologies, such as plowing, irrigation, the introduction of organic fertilizers, etc. These influences lead to the rearrangement of the whole complex of soil microorganisms, as well as to changes in the biogeochemical cycles of the most important biogenic elements [1]. Soils have been involved in agricultural use for many centuries. In particular, monastery soils have been exposed to a continuous anthropogenic effect. The studies of the peculiarities of microbial transformation of N and C in ancient cultivated soils, including those conserved at present as buried horizons, are of great interest.

This work was carried out with soil samples selected on the territory of the Iverskii monastery (Novgorod region, XVII century). These soils are unique, because it is possible to exactly trace back the history of their agricultural use with the documents retained and to determine their age.

The aim of this work was to determine the number and biomass of microorganisms and the rates of soil respiration, methane production, methane oxidation, nitrogen fixation, and denitrification in different horizons of the ancient cultivated soil and the adjacent noncultivated soil.

MATERIALS AND METHODS

The soil of the Iverskii monastery, which has been in the cultivated state for more than three centuries, was the object of this investigation. According to the modern classification [2], this soil is *agrozem* (ancient cultivated soil) with a buried horizon. The upper horizon P and the buried ancient cultivated horizon A_{buried}, occurring at a depth of 55 to 75 cm, were compared. The soil occurring near the monastery under similar lithological and geomorphological conditions but not subjected to agricultural treatment (superficial–podzolic illuvial–humus–ferruginous sandy soil on fluvioglacial deposits) was chosen as the control. The AO organomineral horizon, the AE humus–eluvial horizon, and the B_{hf} illuvial humus–ferruginous horizon were studied. The general characteristics of the soils are presented in Table 1.

The number of microorganisms in the soil samples was determined by inoculating solid media (20 g agar per 1 l) of the following compositions (g/l). (1) Czapek medium, used to determine the fungal CFU number: KCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; K_2HPO_4, 1.0; $NaNO_3$, 2.0; $FeSO_4 \cdot 7H_2O$, 0.01; and sucrose, 30.0 (pH 6.5). (2) Gauze medium, used to determine the CFU number of actinomycetes: K_2HPO_4, 0.5; KNO_3, 1.0; NaCl, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; $FeSO_4 \cdot 7H_2O$, 0.01; and starch, 20.0 (pH 7). (3) GPA medium, used to determine the bacterial CFU number: glucose, 5.0; peptone, 5.0; yeast extract, 1.0; and casein hydrolysate, 1.0 (pH 7).

The total biomass of the microorganisms was determined according to the method of Domcsh modified by West and Sparling [3]. To do this, 1 g of the naturally moist soil to be studied was placed in 15-ml flasks, and 2 ml of glucose solution was added (2 mg/g soil). Thirty minutes later, after the glucose was uniformly distributed in the soil sample, the flasks were closed with rubber stoppers and incubated in a thermostat (25°C) for 1 h. The CO_2 concentration increment in the gas phase was determined using a gas chromatograph. The CO_2 evolution rate was averaged over 10 replicate flasks, and the

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Table 1. Physicochemical characteristics of the soils studied

Characteristic	Soil, horizon				
	agrozem with buried horizons		superficial–podzolic illuvial–humus–ferruginous sandy soil		
	P	A_{buried}	AO	AE	B_{hf}
Horizon depth, cm	0–30	55–75	5–8	8–10	10–17
Saturation with bases, %	93	91	48	47	49
Density, g/cm^3	1.11	1.48	0.22	0.99	1.46
pH of aqueous extract	6.55	7.05	3.8	4.05	5.25
Humus, %	3.45	1.65	39.84	6.12	1.58

Table 2. Microbial number and biomass

Characteristic	Soil, horizon				
	agrozem with buried horizons		superficial–podzolic illuvial–humus–ferruginous sandy soil		
	P	A_{buried}	AO	AE	B_{hf}
The number of fungi, 10^3 CFU/g	17 ± 2	10 ± 0.5	96 ± 1	30 ± 3	17 ± 0.5
The number of actinomycetes, 10^3 CFU/g	380 ± 2.5	628 ± 3	19 ± 2	20 ± 0.8	35 ± 1
The number of bacteria, 10^6 CFU/g	18 ± 0.3	13 ± 0.1	20 ± 0.4	3 ± 0.3	5 ± 0.2
Biomass of microorganisms, μg C/g	487.3 ± 7.2	165.9 ± 8.3	590.7 ± 6.4	149.1 ± 7.1	202.6 ± 4.3

total microbial biomass was calculated: $C = 433 \ln A + 40.3$ (C is the biomass carbon, μg C/g soil, and A is the rate of CO_2 evolution, μl of CO_2/(g h)).

To determine the rates of methane production, methane consumption, denitrification, and nitrogen fixation, soil samples (5 g; 20% moisture content) were placed in 15-ml flasks and incubated in a thermostat at 28°C.

When determining the rate of methane production, the headspace was filled with argon to create the anaerobic conditions required for methanogenesis [4].

To study the methane-oxidizing activity of the soils, methane was introduced in the gas phase (10 μl of CH_4/l) [4].

The nitrogen-fixing activity of the soils was determined by the acetylene method [4].

The methane and ethylene contents in the gas phase were measured on a Chrom-41 gas chromatograph equipped with a flame ionization detector and a 2.2-m column filled with Spherosil. The thermostat temperature was 30°C. Argon was used as the carrier gas at a flow rate of 30 ml/min. The flow rates of hydrogen and oxygen were 20 and 10 ml/min, respectively.

The potential activity of denitrification in soils was determined in the following way. A solution of 1.2% glucose and 0.4% potassium nitrate solution (1 ml) was added to the flasks containing 5 g of soil. The flasks were flushed with argon and incubated in a thermostat at 28°C for 6 days [4]. The denitrification activity was assessed from the accumulation of N_2O in the presence of acetylene or N_2 in the absence of acetylene in the gas phase.

The activity of soil respiration was determined from the rate of CO_2 accumulation in the gas phase of the flasks.

Measurements of the N_2O and CO_2 concentrations in the gas phase were carried out using a 3700/4 gas chromatograph with a heat conductivity detector (the Chromatograph Moscow Experimental Plant). The columns were of stainless steel; the inner diameter was 2.0 mm, and the length was 3.2 m. The adsorbent was Polysorb-1. The katharometer temperature was 100°C. The measuring element current was 148 mA. The thermostat temperature was 30°C. The injector temperature was 40°C. The flow rate of the carrier gas (helium) was 30 ml/min. The samples (0.5 cm^3) were injected with a medical syringe.

RESULTS AND DISCUSSION

The determination of the number of microorganisms in the soil samples studied showed that there were considerable differences between the agrozem and the superficial–podzolic soil of the adjacent territory (Table 2). Thus, the number of fungi in the upper horizon (AO) of the superficial–podzolic soil was almost an order of magnitude higher than in the upper horizon (P) of the agrozem. The numbers of actinomycetes were also sig-

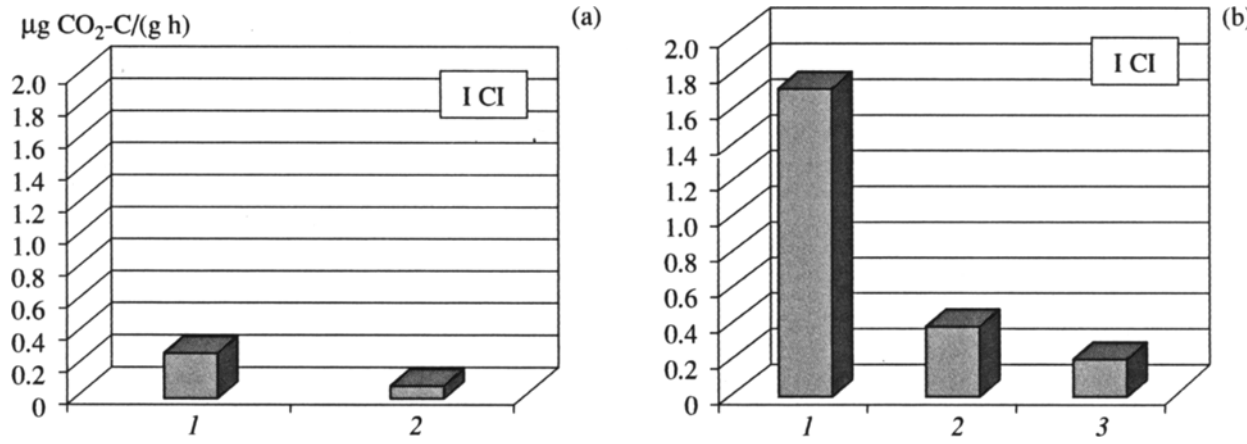

Fig. 1. Soil respiration (a) in agrozem horizons (*1*) P and (*2*) A_{buried} and (b) in horizons (*1*) AO, (*2*) AE, and (*3*) B_{hf} of the superficial–podzolic soil (CI is the confidence interval).

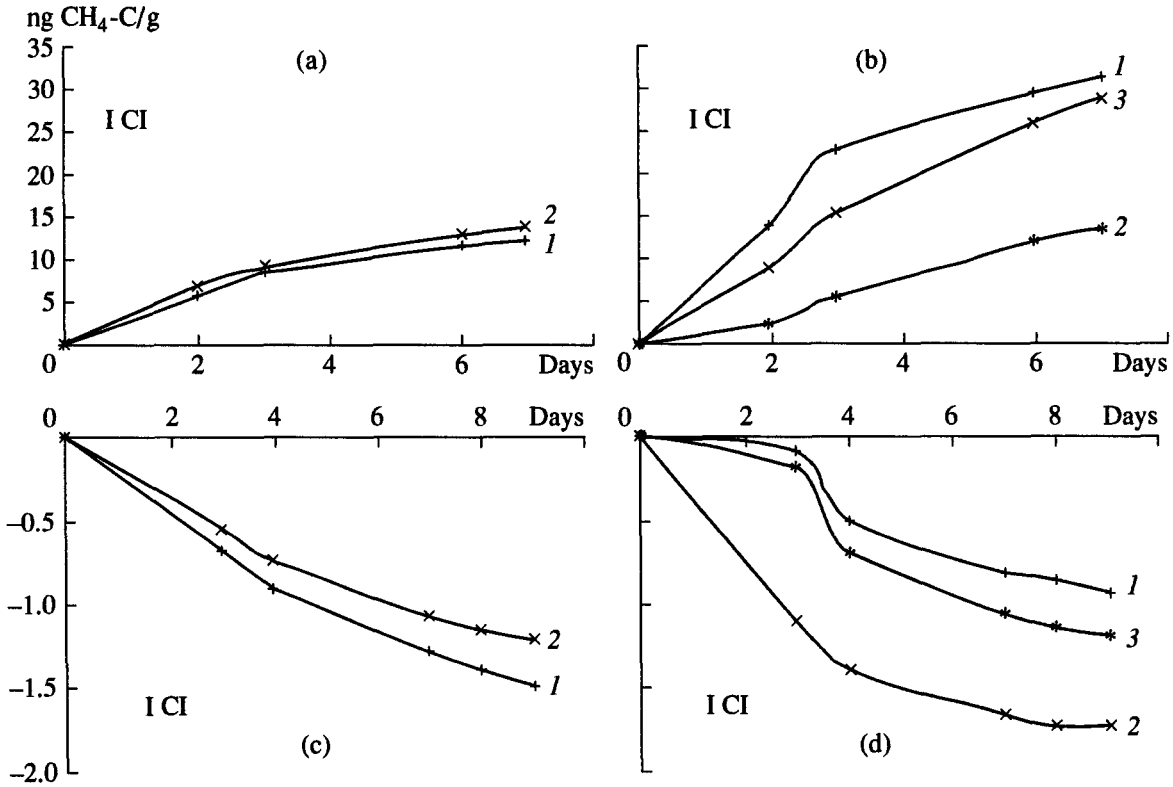

Fig. 2. Methane production (a) in agrozem horizons (*1*) P and (*2*) A_{buried}; (b) in horizons (*1*) AO, (*2*) AE, and (*3*) B_{hf} of the superficial–podzolic soil and methane consumption: (c) in agrozem horizons (*1*) P and (*2*) A_{buried} and (d) in horizons (*1*) AO, (*2*) AE, and (*3*) B_{hf} of the superficial–podzolic soil (CI is the confidence interval).

nificantly different. In the agrozem horizons P and A_{buried}, their number was an order of magnitude higher than in the AO, AE, and B_{hf} horizons of the superficial–podzolic soil of the adjacent territory. The number of bacteria in the agrozem buried horizon (A_{buried}) was 30% higher than in the AE and B_{hf} horizons of the superficial–podzolic soil.

These differences seem to be largely determined by a significant difference in the soil acidity values. In agrozem, pH attains the neutral value, while in the upper horizon of the superficial–podzolic soil of the adjacent territory, pH does not exceed 4.0. Thus, the number of actinomycetes was higher in the soil with a pH close to the neutral value, whereas the number of

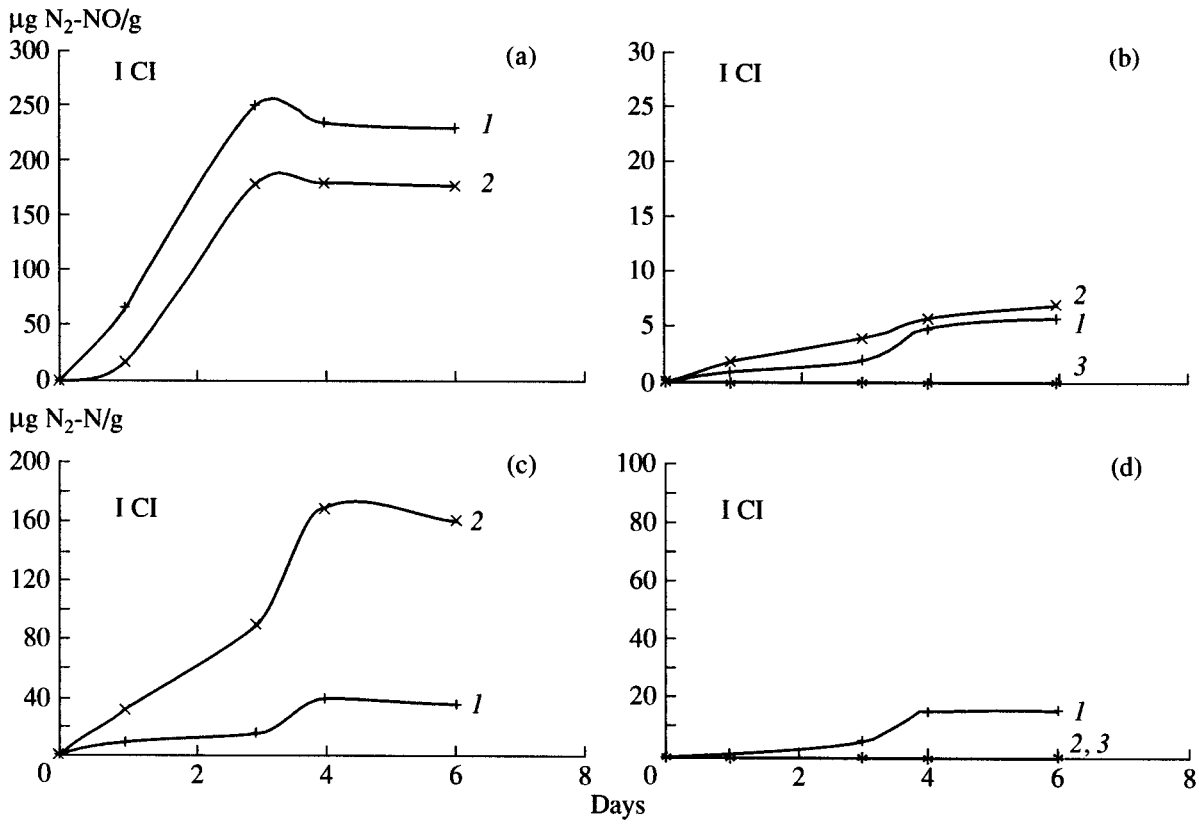

Fig. 3. Denitrification in the soils studied: N_2O emission (a) in agrozem horizons (*1*) P and (*2*) A_{buried}; (b) in horizons (*1*) AO, (*2*) AE, and (*3*) B_{hf} of the superficial–podzolic soil, and N_2 emission (c) in agrozem horizons (*1*) P and (*2*) A_{buried} and (d) in horizons (*1*) AO, (*2*) AE, and (*3*) B_{hf} of the superficial–podzolic soil (CI is the confidence interval).

fungi was greater in the superficial–podzolic soil with acidic pH.

The assessment of the total microbial biomass in the soils examined (Table 2) showed that, in the AO upper horizon of the superficial–podzolic soil, it reached 600 μg C/g, which was 20% higher than in the P upper horizon of agrozem. On the whole, the microbial biomass pool correlated with the content of organic matter in the soils studied.

The results of studying the intensity of soil respiration are given in Fig. 1, from which it follows that the CO_2 evolution rate was the highest in the AO horizon of the superficial–podzolic soil, exceeding the rate of CO_2 evolution in the agrozem arable horizon (P) by a factor of 8. In the AE and B_{hf} horizons of the superficial–podzolic soil, the rate of CO_2 evolution was 3 to 5 times higher than the A_{buried} horizon of agrozem. These results correlate with the data on the microbial biomass pools and on the organic matter content in these soils (a correlation coefficient of 0.99).

The rate of methane production in the superficial–podzolic soil was 1.5 to 2 times higher than in agrozem (Figs. 2a, 2b). The highest rate of methane production was observed in the AO horizon of the superficial–podzolic soil. Apparently, this is connected with the presence of soil aggregates, which are the source of methane [5, 6] in the AO horizon of this soil. In agrozem, these aggregates were absent due to periodical plowing.

The rates of methane consumption in the soils studied differed but slightly (Figs. 2c, 2d); in the buried horizon of agrozem, methane consumption tended to decrease.

The determination of potential denitrification activity showed (Fig. 3) that the rate of this process was an order of magnitude higher in agrozem; the greatest activity was observed in the buried horizon. The denitrification products were different in different horizons of the cultivated soil. Thus, the predominant denitrification product in the P upper cultivated horizon was molecular nitrogen, whereas in the A_{buried} horizon, it was nitrous oxide. This can be explained by a higher content of organic matter in the P horizon. There is evidence [7] that an increase in the organic matter content in the soil results in an increased share of molecular nitrogen in the composition of gaseous denitrification products. The fact that the process of denitrification in the buried horizon mainly yields nitrous oxide can be accounted for by the washout of nitrates from the overlying horizons, due to which the C : N ratio decreases.

It is noteworthy that the main product of denitrification in the superficial–podzolic soil was molecular nitrogen.

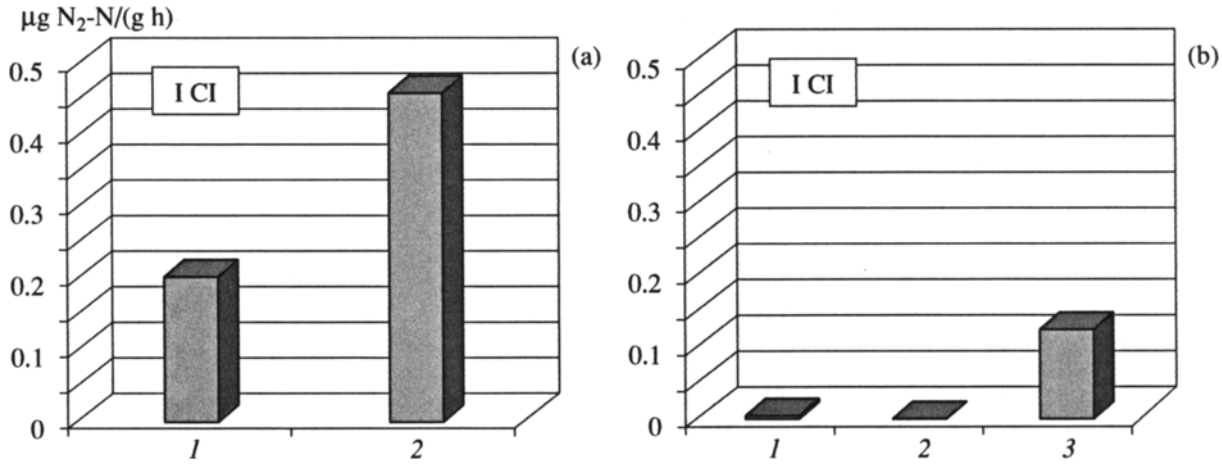

Fig. 4. Potential nitrogen-fixating activity (a) in agrozem horizons (*1*) P and (*2*) A_{buried} and (b) in horizons (*1*) AO, (*2*) AE, and (*3*) B_{hf} of the superficial–podzolic soil (CI is the confidence interval).

The studies of the potential nitrogen-fixing activity of the soils showed that it was considerably higher in agrozem in comparison with the adjacent noncultivated soil (Fig. 4), attaining the greatest value in the agrozem buried horizon. This is likely to be linked to a combination of the neutral pH value, the high number of bacteria, and a considerable content of organic matter in this horizon.

From the foregoing, it can be concluded that, in the buried horizon of the ancient cultivated soil, the number of bacteria and actinomycetes increases compared with the adjacent territory. The ancient cultivated soil showed substantial differences in the intensity of microbial transformation of nitrogen and carbon compounds as compared to the adjacent soil. Thus, in the buried horizon of the ancient cultivated soil, the oxidation processes are decelerated (the rates of CO_2 formation and CH_4 oxidation are decreased) with a simultaneous intensification of reductive processes (denitrification and nitrogen fixation). The only exception is the process of methane production, which, in the agrozem buried horizon, proceeds at a low rate due to the confinement of this process to the structured soil aggregates destroyed in this horizon in the process of plowing. The intensity of respiration in the buried horizon is drastically decreased in comparison with the superficial–podzolic soil, where fungi actively destroy organic matter. A dramatic increase in the potential activity of denitrification (with nitrous oxide as the main product) was observed in the buried horizon. The potential nitrogen-fixing activity in the ancient-cultivated soil also appeared to be significantly higher than in the adjacent soil. Thus, the buried horizon of agrozem has a high biological activity, which, by certain parameters, considerably exceeds the activity of the modern soil horizon.

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