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Microbiological Transformation of Organic Matter in Oil-Polluted Tundra Soils after Their Reclamation

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Abstract—The influence of oil pollution caused by the accident of 1994 on the biological activity of Histic Cryosols is considered. Despite the use of complex mechanical (oil removal), physicochemical (washing on special devices), and agrotechnical (fertilization, plowing, grass sowing) reclamation techniques, the residual content of oil hydrocarbons in the soils can reach high values. The disturbance of the natural peat soil horizon during the reclamation procedures reduces the respiratory activity, as well as the potential rate of consuming the substrates, plant residues and water-soluble organic matter, which decreases the carbon cycle rate.

Keywords: soil respiration, microbial biomass, Histic Cryosols, pollution of terrestrial ecosystems, reclamation, Bolshezemel'skaya tundra

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INTRODUCTION

Soil pollution caused by spills of oil and oil products, including spills during their transportation, is the major environmental problem in regions of developing oil and gas industry [3, 14, 15]. Entering the soil, oil and oil products exert a toxic effect on plants and animals, as well as inhibit the activity of soil microbiota. Due to the high sorption capacity of the soil (especially peat soil) in relation to oil and its components, oil hydrocarbons can accumulate and stay in the soil for a long time significantly changing the chemical, physical, and biological properties of the soil. During the last century, the main areas of oil production in Russia moved to the north causing a high risk of long-term pollution of the soils, which, due to severe bioclimatic conditions, are characterized by a relatively low ability for self-purification [5, 7, 10]. The most complex and objective indicator, which allows one to assess quickly the functional activity of soil microbial communities, is the respiratory activity of the soil [2, 21, 27]. This index is believed to characterize indirectly the intensity of microbiological decomposition of hydrocarbons (HCs) [23].

The use of a complex of mechanical, physicochemical and agrochemical methods for the reclamation of oil-polluted soils can cause significant changes in their biological activity and the ability of soil microbial communities to perform ecosystem functions—first, to maintain the carbon cycle. It is also important to evaluate the effect of the "old" HC contamination on the functioning of soil microbial complexes.

The aim of this work was to evaluate the microbiological transformation of organic carbon in the tundra soils polluted with oil because of the 1994 emergency spill and reclaimed by various methods.

MATERIALS AND METHODS

The studies were performed in the "Vozei–Golovnye Sooruzheniya" (66°25′ N, 67°18′ E, the Republic of Komi) area, where in 1994, an accident occurred on the main pipeline in 1994. Overall, 60 to 270 thousand tons of crude oil were spilled [16]. Reclamation works in this area were carried out using different ways, among which we considered four variants (Table 1). To date, the key areas have passed all stages of reclamation.

The soil cover of the background territory is represented by Histic Cryosols [20]. The names of technogenic surface formations (TSF) on the reclaimed areas are given in accordance with the soil classification of the Dokuchaev Soil Science Institute.

The surface layer of the background (reference) soil is a peat horizon with a high content of total carbon (C_{tot}, 42.0 ± 2.4%), and the surface layers of the soils and TSF in different reclaimed areas have the high content of total carbon (C_{tot})–5–20%. The distribution of C_{tot} in the profiles of the background and reclaimed soils (variants 2 and 4) was characterized by its sharp decrease with depth. In the TSF (variants 1 and 3), the distribution of the C_{tot} along the profile is more even, which is associated with mixing the soil and sediments in the course of remediation.

Table 1. Key characteristics of the studied objects

No	Reclamation variant	Soil / TSF	Reclamation measures	Note
1.1	Cleaning of soil on an installation for solid oil slime	Replantozem	Harrowing, application of dolomitic meal (1.9 t/ha), potassium chloride (0.2 t/ha), urea (0.35 t/ha), sawing of cereals	Watering close to background
1.2				Draining due to landfilling
2	Washing of oil from surface and plowing of its remains	Haplic Cryosols	Application of dolomitic meal (1.1 t/ha) and Azofoska (0.26 t/ha)	Plant cover is sparse
3.1	Replacement of polluted layer by a sandy one with arrangement of sewage sludge on its surface	Artifimostrat	Harrowing, application of dolomitic meal (0.5 t/ha), Azofoska (1.38 t/ha), sawing of cereals, and planting of pine trees	Automorphic position without vegetation
3.2				Automorphic position with vegetation
3.3				Hydromorphic position without vegetation
3.4				Hydromorphic position with vegetation
4	Partial removal of oil from the surface	Haplic Gleysols	Without fertilizers	Oil spots on surface, organic horizon is damaged

In the soil of the background area, the content of hydrocarbons is low (300–500 mg/kg) with the maximum in the top 20-cm layer of the peat horizon, which is mainly related to their biogenic nature. All the studied soils and TSF, despite the reclamation measures applied, contain high amounts of oil hydrocarbons. The maximum HC content in the surface layer was noted for the areas, where oil was removed from the surface (variant 4), or oil was removed from the surface and plowed into the soil (variant 2). This procedure shifted the maximum HC content from the 0–10-cm layer to the 10–20-cm one. In the cases, when only oil was only removed from the surface (without plowing), the HC concentration in the soil decreased with the depth. The HC distribution over the area was uneven: in microdepressions, oil accumulated as pools (formed due to squeezing out of oil-containing soil water in cold seasons), and the HC content of hydrocarbons in the soil under them was more than 60000 mg/kg. The heterogeneity of the microrelief results in a significant spatial variation of the total HC content in the surface layers of artifabricats (variant 3). Thus, the maximum HC content (5000–29000 mg kg) was recorded for the hydromorphic areas as compared with the automorphic ones (600–5000 mg/kg).

The soil of the background area is poor in mineral nitrogen compounds. The organic nitrogen compounds prevail in the extracted N pool. Such a ratio of mineral and organic forms in the N pool is generally characteristic of tundra soils [11]. The reclamation measures associated with the disturbance of the peat horizon and application of nitrogen fertilizers caused changes in the extractable nitrogen pool: its total content decreased, but the proportion of mineral forms increased. The main form of mineral nitrogen in the reclaimed soils was ammonium (up to 80 mg/kg). However, when applying sewage sludge, the share of nitrates increased (up to 7 mg/kg), which is related to their formation in the course of heterotrophic nitrification developing under the sufficient amounts of $N\text{-}NH_4^+$ and organic matter.

In each plot, key soil pits were made with sampling by layers every 10 cm. The results obtained for the layers of 0–10, 10–20, and 45–55 cm were analyzed.

Labile organic compounds (C_{extr}) were extracted with 0.05 M K_2SO_4 [8] and determined using a TOC-V CPN (Shimadzu) automatic analyzer. Carbon of the microbial biomass (C_{micr}) was analyzed using the modified [9] fumigation–extraction method [26], it corresponded to the difference between the C_{extr} concentration in the fumigated and nonfumigated soil samples.

Basal respiration (BR) of the soils was determined from the production of $C\text{-}CO_2$ after incubating the samples at 60% of the total water capacity at +22°C for 24 h. The rate of substrate-induced respiration (SIR) was measured in the same way in 3 h after the introduction of 0.2 mL of 10% glucose solution to the soil. The concentration of released $C\text{-}CO_2$ was determined using an Agilent 6890N chromatograph (Hewlett-Packard, USA) equipped with a flame ionization detector and methanator (Supelco 10182004 column with an inner diameter of 3.175 mm and a length of 1828.8 mm; adsorbent is 80/100 Porapak Q, column thermostat temperature is 60°C, the carrier gas (helium) flow is 20 mL/min, detector temperature is 300°C, rear port temperature is 375°C, hydrogen flow is

30 mL/min, air flow is 400 mL/min, volume of injected gas sample is 1 mL).

The substrate profile of microbial communities was determined according to the C-CO_2 production after 24 h of soil incubation with 1% solutions of differently available substrates (glucose, amylose, sodium citrate, leucine, urea, mannitol, glycerin, hydroquinone), as well as ground plant residues (annual falloff of the aboveground biomass of the *Empetrum hermaphroditum* dwarf shrub, C : N = 34). The amount of the isolated C-CO_2 was calculated per atom of introduced carbon.

The extraction of water-soluble organic matter (WOM) (soil: solution is 1 : 5) was carried out from samples of the upper horizons (0–10 cm) of the background soil in the site reclaimed using sewage sludge (variant 3.2) and the site with oil pollution (variant 4). The soil suspension was shaken on a rotator for an hour, then centrifuged (4500 rpm, 20 min) and filtered through a membrane filter (0.45 micron). The extracts were inoculated with 100 μL of the soil suspension (1 : 10) obtained from the horizons investigated and incubated shaking continuously for 42 days at +22°C. On the 1^{st}, 3^{rd}, 7^{th}, 14^{th}, 28^{th}, and 42^{nd} days, the concentration of extracted organic carbon was determined in the samples. The processing of the data was performed using a two-component first-order exponential regression equation.

All determinations were carried out in three replicates. The tables and graphs present average values ± standard deviation; the values were calculated on the absolutely dry soil (105°C, 8 h). When processing the data, the statistical package of Microsoft Excel 2007 was used. The correlation analysis was performed separately for each layer studied. The one-factor analysis of variance (ANOVA) was carried out using the STATISTICA 13 program for the data obtained for the surface soil layer.

RESULTS AND DISCUSSION

The content of labile and microbial carbon in the soils reclaimed using different methods. The surface peat horizon of the background soil is characterized by the high content of extractable carbon (1405 ± 157 mg/kg). The concentration of C_{extr} in the upper layer of the soils and TSF in the variants with reclamation carried out by different methods was significantly lower than in the background soil and varied from 270 to 800 mg/kg (Fig. 1a). The minimum C_{extr} concentrations were in the soil of variant 4; they were associated with the removal of a larger part of the organic horizon together with the removal of oil from the soil surface. The maximum C_{extr} content was characteristic for artifimostrats (variant 3) formed in automorphic positions.

In the background soil, the maximum C_{extr} content was found in the 10–20-cm layer, which is related to the transformation of plant residues in the peat horizon. The C_{extr} content in the profile of the reclaimed soils decreased with the depth. For the 10–20-cm soil layer, as well as for the surface horizon, it is still possible to disclose the same regularities of the spatial distribution of labile carbon. However, at a depth of 45–55 cm, no significant differences between the soils of different sites were found.

The soils and TSF differ strongly in the C_{extr} content (Fig. 1b). Significant differences were characteristic not only for the background and reclaimed soils, but also for the soils reclaimed in different ways. Thus, in the reclaimed areas, the highest C_{extr} content in the surface soil layer was in the automorphic series of artifimostrats (variant 3.1 and 3.2) and replantozem (variant 1.1). The high C_{micr} content in these areas is associated with the best thermal and water–air regimes, which prevents the stagnation of moisture and provides surface and lateral outflow of hydrocarbons, and with the application of organic substrates (artifimostrats) or mineral fertilizers (replantozem). The main part of microbial carbon in the studied soils and TSF concentrates in the upper 20-cm soil layer.

Soil respiration indices. The method of site reclamation had a significant influence on the intensity of basal respiration in microbial communities (Fig. 2a). Most soils were characterized by the low BR values; the soils and TSF formed in the automorphic positions had higher rates of respiratory activity. Our results allow one to conclude that the intensity of organic matter mineralization was approximately the same in the soils of all types of reclamation, but it was significantly lower than in the background soil. The rate of mineralization depended on the soil position in the relief. The regularities revealed are characteristic not only for the 0–10-cm layer but also for the 10–20-cm one. However, the BR decreased sharply with the depth. For the surface layer of the soils and TSF, the BR value correlated with the content of total ($r = 0.834$, $p < 0.05$) and extractable ($r = 0.864$, $p < 0.05$) carbon in the soil. There was no statistically significant correlation of basal respiration with the HC content. The one-factor analysis of variance also showed the absence of the statistically significant difference in BR of the soils with low, medium and high HC contents ($F = 3.24$, $p = 0.540$, $F_{crit} = 3.34$). Fresh oil pollution is known to enhance soil respiration [2, 4]. Twenty years after the introduction of hydrocarbons into the soil, their biological effects on the microbial communities decreased significantly, which was associated with both evaporation and biodegradation of the most toxic *n*-alkanes and with changes in the structure of the microbial communities themselves.

The mineralization potential of microbial biomass determined by the SIR intensity was 2–4 times higher in the background soil compared to the reclaimed ones (Fig. 2c). For the soils reclaimed by different methods, this index was almost the same. It is worth noting that the microbial communities of the auto-

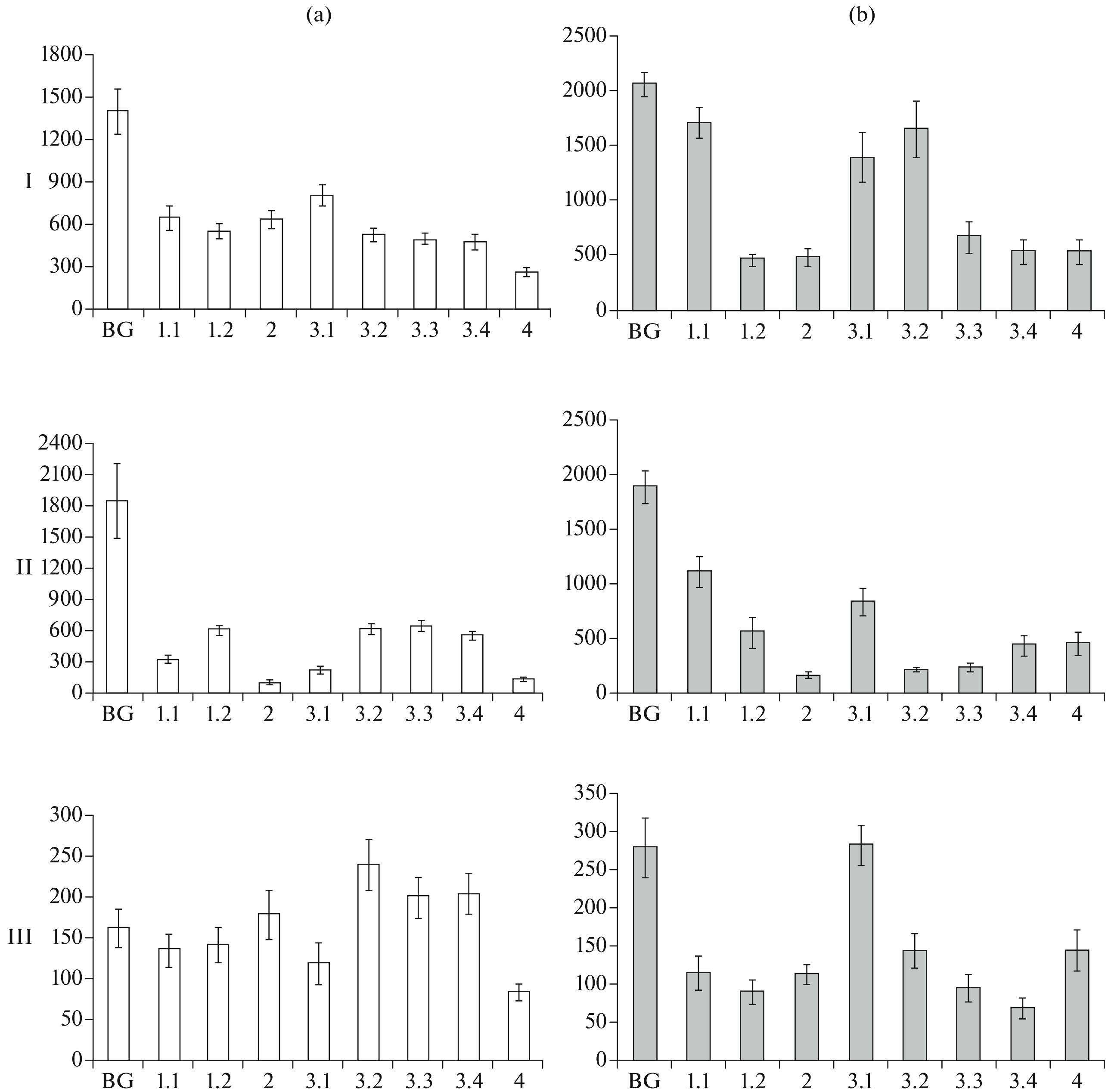

Fig. 1. The contents (mg/kg) of (a) extractable carbon and (b) carbon of microbial biomass in the soil layers (here and in Fig. 2): (I) 0–10 cm, (II) 10–20 cm, and (III) 45–55 cm; BG—background.

morphic soils (variants 1.1 and 3.1) had a significantly greater mineralization potential compared to their hydromorphic analogues (1.2 and 3.3), which is related to their formation under the more favorable air-water regime and the best heating of the soil surface on the automorphic areas. These factors maintain the development of a larger pool of automorphic microorganisms compared to their hydromorphic analogues (C_{micr} and BR indices, Figs. 1b, 2a), and determine the best conditions for the formation of low-molecular organic compounds (C_{extr}, Fig. 1a). The SIR intensity in the surface soil layers correlated with the content of total ($r = 0.833$, $p < 0.05$), extracted ($r = 0.874$, $p < 0.05$) and microbial ($r = 0.857$, $p < 0.05$) carbon. No statistically significant correlation of SIR with the HC content was revealed. The one-factor analysis of variance also showed the absence of statistically significant difference in SIR in the soils with the low, medium and high HC content ($F = 3.55$, $p = 0.065$, $F_{crit} = 3.34$).

The capacity of soil microbial communities for decomposition of substrates and plant residues. The most intensive consumption of substrates was found for the surface (0–10 cm) soil layers and TSF (Fig. 3).

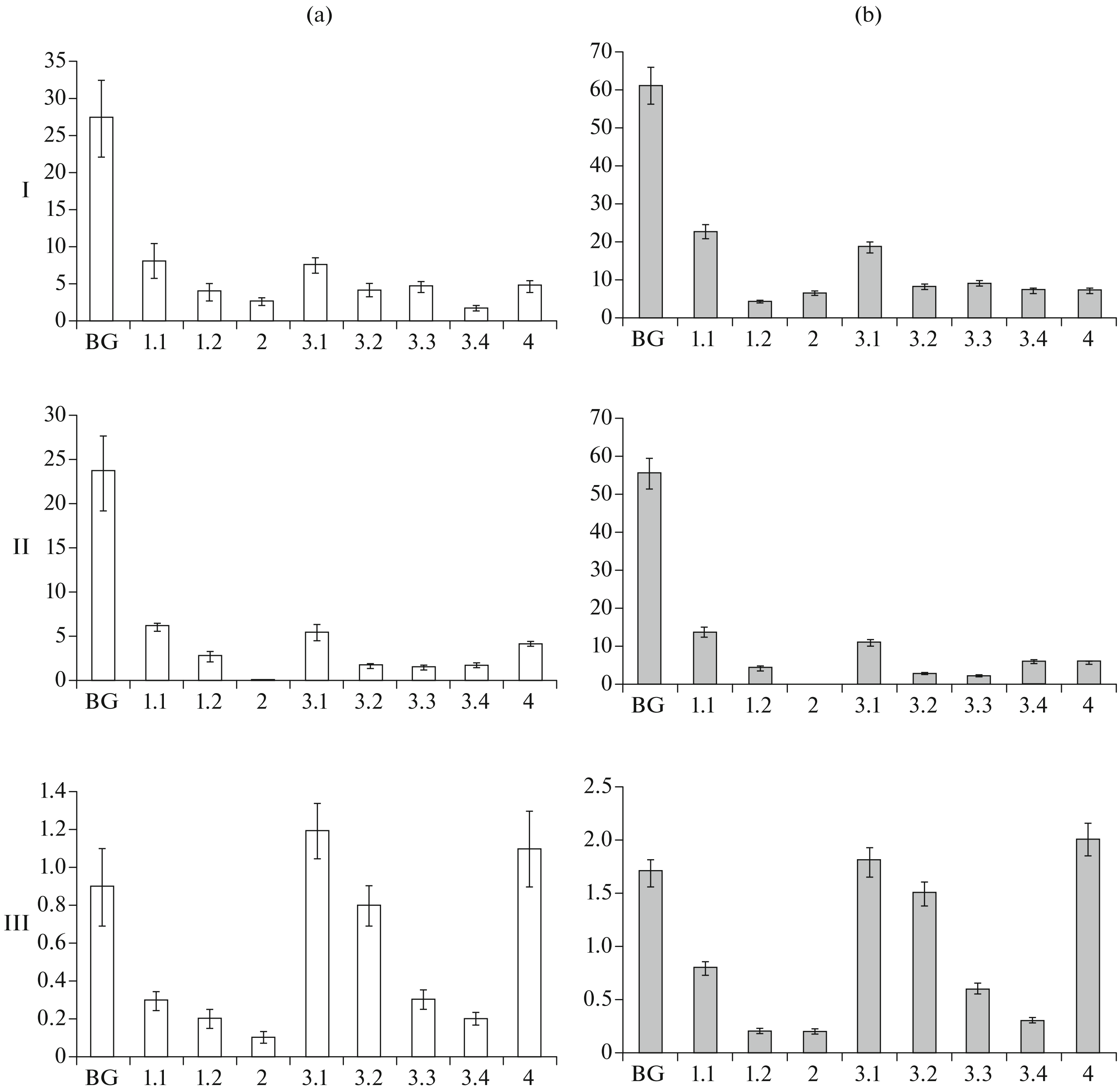

Fig. 2. Characteristics of microbiological transformation of carbon compounds: (a) basal respiration, μg C-CO_2/(g h) and (b) substrate-induced respiration, μg C-CO_2/(g h); BG—background.

At the same time, the microbial community of the background soil was characterized by the highest diversity of consumed substrates, which is related to its more complex functional structure. The intensive consumption of simple substrates (glucose, sodium citrate), as well as of more complicated ones (hydroquinone) and polymers (amylose), was recorded not only for the background soil. This soil was characterized by the intensive consumption of nitrogen-containing substrates (leucine, urea), which indicated a weak saturation of microbial biomass with nitrogen (the C : N ratio in the microbial biomass was 12–14).

In the reclaimed soils and TSF, the microorganisms consume substrates less intensely, which was associated both with reduced C_{micr} content in the surface layers of the reclaimed soils (Fig. 1b) and with the changes in the structure of the microbial community after reclamation. The one-factor analysis of variance showed the absence of statistically significant differences in the rate of substrate consumption in the soils with the low, medium, and high HC contents. At the same time, all the reclaimed soils studied were peculiar by an extremely low mineralization rate of substrates that are difficult to consume (hydroquinone),

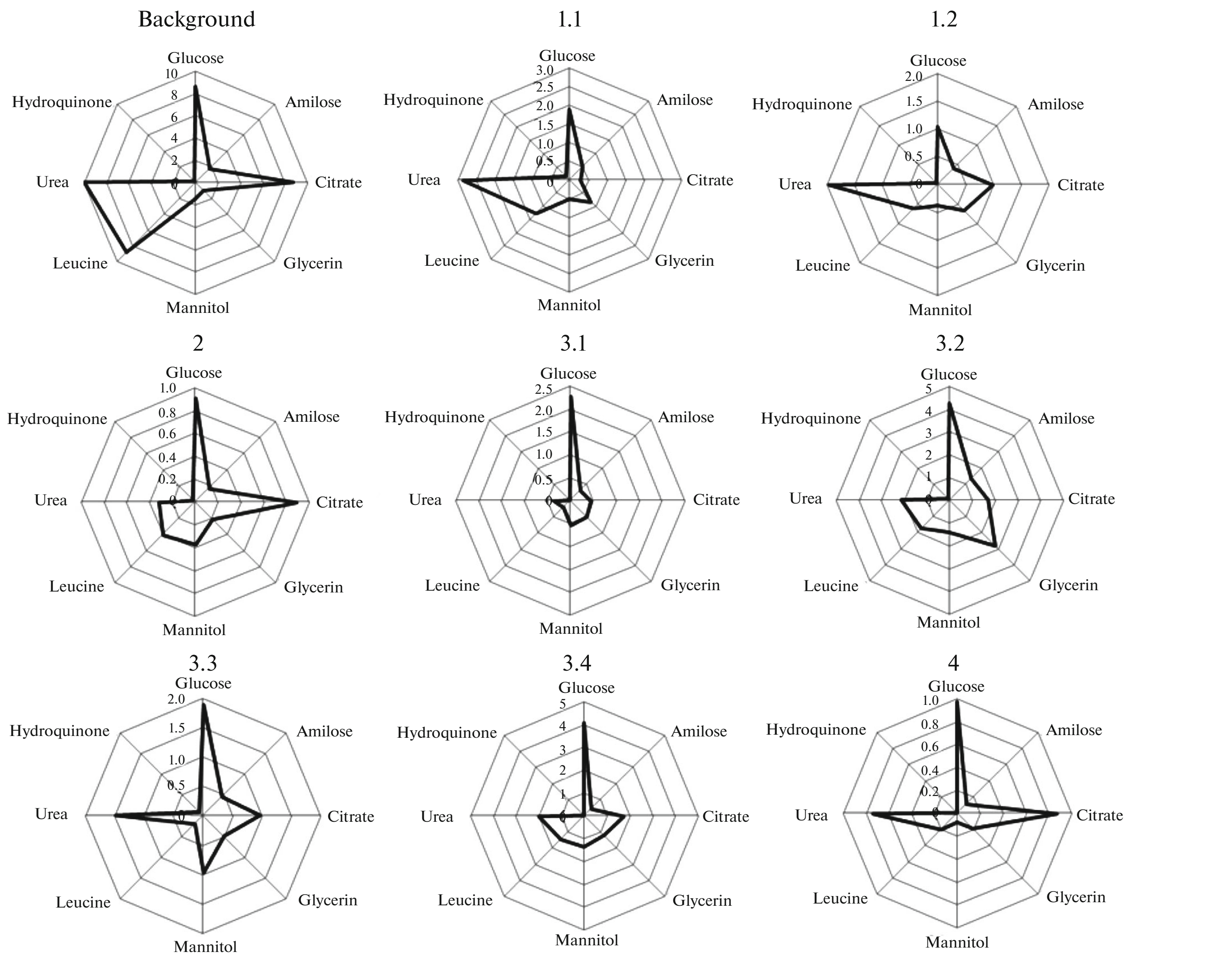

Fig. 3. Spectra for consumption of substrates by microorganisms in the 0- to 10-cm soil layer, μg C-CO_2/(g h $C_{substrate}$).

as well as by a significant decrease in the consumption of polymers (amylose). The decrease in the consumption of substrates by microorganisms indicates a reduction in their ability to decompose oil products, since their oxidation also occurs with participation of microorganisms consuming carbon from carbohydrates, alcohols, fatty acids and other organic compounds [13]. In addition, alcohols and carboxylic acids are products of *n*-alkane oxidation, and diatomic phenols are intermediate products of aerobic degradation of aromatic hydrocarbons [18]. The reduced consumption of products of HC degradation shifts the chemical equilibrium in soils and inhibits the oxidation of oil products.

The ability to decompose plant residues in the reclaimed soils, compared to the background ones was significantly lower (Table 2). The maximum rate of plant residues mineralization was registered in the 0–10-cm layer; it correlated to a great extent with the content of total ($r = 0.900$, $p < 0.05$) carbon. In the replantozems (variants 1.1 and 1.2), the weak differentiation of the mineralization ability in their profiles is explained by mixing the soil masses in the course of soil formation and uniform distribution of total carbon along the profile. The maximum indices of mineralization capacity in the reclaimed soils and TSF were characteristic of the soils in automorphic positions (variants 1.1 and 3.2).

The decrease in the rate of organic matter mineralization in the soils and TSF of reclaimed areas indicates an insufficient stimulation of their natural microbial community due to the agrotechnical measures applied (Table 1). Further actions to restore these sites can be oriented on biostimulation and bioaugmentation. The efficiency of the natural microbial community in the soils poor in mineral elements (including tundra soils) increases significantly with the application of fertilizers containing nitrogen,

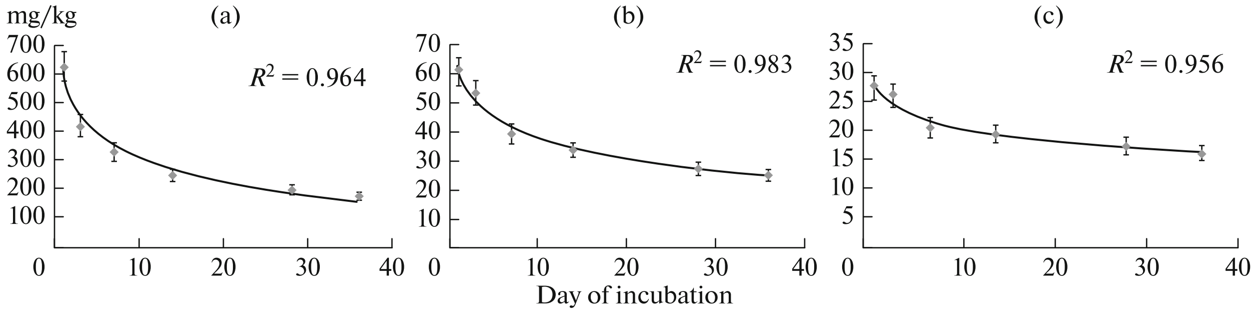

Fig. 4. Dynamics of the biodegradation of water-soluble organic matter (mg/kg): (a) background soil, (b) reclamation using sewage sludge (variant 3.2), and (c) removal of oil from the surface (variant 4).

phosphorus and potassium [17, 19, 22, 24, 25]. However, additional studies are necessary to identify the optimal doses of fertilizer, since high doses of nitrogen and phosphorus can inhibit the activity of microbial community [1, 22]. In [12], the positive effect of bioaugmentation of oil-polluted tundra soils with several microbiological preparations adapted to the soil and climatic conditions of the northern European territory of Russia on the HC biodegradation and restoration of the ecosystem was shown.

Decomposition of water-soluble organic matter. The background soil had a high content of water-soluble organic matter (630 ± 95 mg/kg).The disturbance of the organic horizon in the tundra soil and its substitution for a sandy layer with sewage sludge resulted in the great WOM reduction to 61 ± 12 mg/kg. The oil contamination of the soil and associated inhibition of plants and microorganisms, as well as damage to the organic soil horizon when oil was removed from the surface (variant 4) also led to a significant decrease of WOM concentrations to 28 ± 5 mg/kg.

The rate of WOM decomposition in the soils of all experimental variants was relatively high in the first 7 days of incubation and significantly decreased further (Fig. 4). In this case, in the extract from the surface horizon of the background soil, 60% of carbon was mineralized; in the extract from the soil reclaimed using sewage sludge, 36% and only 25% of WOM carbon was mineralized in the oil-contaminated soil. The obtained equation of the biodegradation kinetics shows that WOM of the soils consists of two pools, different in the decomposition constant (k) and time (MRT). An increase in the MRT time and decrease in the k constant by 1.3 times when polluted with oil products and by 1.1 times under the sewage sludge reclamation were typical for the "fast" WOM pool. The "slow" WOM pool was different in its characteristics as compared with the "fast" pool. Thus, in the control, the MRT value of the "slow" pool was 42 days, increasing to 58 days under the use of sewage sludge and to 253 days under oil pollution.

Thus, reclamation of oil polluted tundra soils leads to a decrease in the content of organic matter in the surface horizon due to the disturbance of the peat horizon. The soil microbial communities formed after reclamation are less capable to metabolize WOM compared to the microbial community of the background soil. The differences between the background and reclaimed areas are most pronounced in the decomposition of the "slow" WOM pool, and result in slowing down the carbon cycle in the soil.

Table 2. Mineralization (μg C-CO_2 /g h $C_{substrate}$) of annual falloff of aboveground *Empetrum hermaphroditum* biomass in the soils

Reclamation variant	Mineralization		
	0–10	10–20	45–55
Background	1.73 ± 0.20	0.53 ± 0.10	0.08 ± 0.01
1.1	0.26 ± 0.05	0.19 ± 0.03	0.10 ± 0.03
1.2	0.17 ± 0.03	0.16 ± 0.02	0.11 ± 0.03
2	0.17 ± 0.03	0.08 ± 0.01	0.02 ± 0.01
3.1	0.12 ± 0.03	0.10 ± 0.02	0.04 ± 0.01
3.2	0.56 ± 0.08	0.26 ± 0.08	0.07 ± 0.01
3.3	0.25 ± 0.03	0.11 ± 0.02	0.05 ± 0.01
3.4	0.26 ± 0.05	0.18 ± 0.03	0.06 ± 0.01
4	0.10 ± 0.01	0.06 ± 0.01	0.04 ± 0.01

CONCLUSIONS

Despite the reclamation measures, the soils were characterized by the high concentration of hydrocarbons. However, there was no statistically significant effect of their residual amounts on the respiratory activity of the soils and technogenic soil formations. The microbial carbon and biological activity of soils with different levels of residual hydrocarbon content were most related to the concentration of total and labile carbon. For 20 years after the accidental spill of oil, the microbial cenosis has adapted, but its functional structure was simplified, which was reflected in the decreasing rate of plant residues decomposition and in destroying water-soluble organic matter in the soil.

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