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*Article*

# Microbial Diversity of Arctic Soils with Long-Standing Pollution by Petroleum Products and Heavy Metals

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**Abstract**

Long-standing and chronic soil pollution in the Polar Regions is the most persistent. Simultaneous contamination with petroleum products and heavy metals puts additional load on the soil microbial community. The purpose of this work was to determine the composition of microorganisms in the soils of Mount Kaskama with long-standing contamination with petroleum products and heavy metals (Murmansk region, Russia) and outside this zone and the potential ability of microorganisms to participate in the self-purification of these soils. Using high-throughput sequencing of 16S rRNA gene V3–V4 fragments an increase in the proportion of bacteria of the phyla *Pseudomonadota*, *Verrucomicrobiota*, *Cyanobacteriota*, and *Bacillota* was shown with an increase in soil contamination. Bacteria of the genera *Bacillus*, *Caballeronia*, *Cytobacillus*, *Paenibacillus*, *Paraburkholderia*, *Pseudomonas*, and *Rhodanobacter* were isolated from soil samples. Bacteria of the genus *Paenibacillus* capable of hydrocarbon oxidation and iron reduction were isolated from the subsurface contaminated layers. Under aerobic conditions, Fe(II) oxidation by bacteria of the genus *Pseudomonas* and biodegradation of hydrocarbons by isolated bacteria are possible. The isolated strains grew at low temperatures, used diesel fuel components, and were resistant to Cu(II), Ni(II), and Pb(II). The data obtained indicates the adaptation of microbial communities to environmental conditions and the ability to participate in the process of soil self-healing.

**Keywords:** arctic soil; long-standing pollution; hydrocarbons; heavy metals; microbial community; high-throughput sequencing; the 16S rRNA gene; hydrocarbon-oxidizing bacteria

## 1. Introduction

The Arctic is often called the "World's treasure trove" due to the vast reserves of mineral resources hidden here. Oil, gas, rare earth metals, diamonds, etc. are recovered in Russian Arctic region. Intensive industrial activity leads to emergency situations and the accumulation of environmental damage [1]. The elimination of pollution from petroleum products and heavy metals is an important task for the preservation of this vulnerable region. Modern technologies of soil purification include physical and chemical methods, which are not always applicable in the conditions of the North due to harsh climate and imperfect logistics [2]. Bioremediation is considered an eco-friendly, economical, and sometimes the only possible method to restore polluted soils in the Polar regions [3]. It is the most effective method, leading to the complete mineralization of

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hydrocarbons [4]. The possibilities of bioremediation, including biostimulation (fertilization, moistening, aeration) and bioaugmentation (introduction of additional oil-degrading bacteria), are described in detail in reviews [5,6]. Adverse environmental conditions, a short growing season, and poor soils determine the persistence of anthropogenic pollution and extremely low rates of soil self-purification in the Polar regions. Decreasing temperature leads to an increase in the viscosity of spilled oil and delayed evaporation of low-molecular-weight toxic compounds, which slows down the microbial degradation of hydrocarbons [7]. Although microbial activity has been proven even at subzero temperatures, the rate of hydrocarbon biodegradation under such conditions is extremely low [8–10]. The ability of soil microbiota to resist oil pollution depends on the type of pollutant and the duration of exposure [11]. Over time, the pollutant penetrates into the pores of soil aggregates, its polymerization occurs, and it reacts with soil humus, complicating the biodegradation process [12,13]. In Antarctic soils, hydrocarbons persisted and were detected more than 40 years after contamination [14].

When soils are contaminated simultaneously with petroleum products and heavy metals (HMs), microorganisms are subjected to double stress and are forced to adapt to several additional aggressive factors. The simultaneous effect of petroleum products and heavy metals on the microbial community has been poorly studied. While hydrocarbons can be completely degraded by microorganisms to carbon dioxide and water, heavy metals remain in the soil. The mechanisms of interaction between microorganisms and metals can be different, associated with cellular metabolism and specific enzymes (intracellular separation and extracellular precipitation) or simple physicochemical interactions (adsorption on cell wall components) [15,16]. Microorganisms are capable of participating in the accumulation and sorption of heavy metals, affecting their solubility, toxicity, and mobility [17,18]. The combined use of bacteria and plants promotes the conversion of heavy metals into safer forms and their removal from soils *in situ*, which is important for soil restoration in hard-to-reach places [19]. Iron-reducing microorganisms possess enzymes that reduce heavy metals and metalloids such as U(VI), Tc(VIII), Cr(VI), and Co(III), using acetate, lactate, pyruvate, and aromatic hydrocarbons as substrates [20–22]. The ability to grow on hydrocarbons with the reduction of heavy metals has been shown for bacteria of the genera *Geobacter* and *Rhizobium* [23,24].

The presence of anthropogenic pollutants in the soil leads to changes in the composition of the microbial community, an increase in the proportion of groups tolerant to it, and the disappearance of sensitive ones. Soil pollution with heavy metals leads to the inhibition of microorganism growth and hydrocarbon biodegradation [25–27].

The choice of remediation technology used depends on the characteristics of the soils, the pollutant, and environmental conditions [28,29]. The rate of polycyclic aromatic hydrocarbon (PAH) degradation depends on the type of hydrocarbons, the target oil-degrading strain, or consortium of strains [30]. The selection of fertilizers for Northern soils, initially poor in nitrogen and phosphorus, has shown that oligotrophic biostimulation is more effective than eutrophic approaches [31].

Under laboratory conditions was shown that the most effective method for cleaning soil from PAHs was a combination of biostimulation and bioaugmentation [32]. In addition to fertilizers, an enrichment culture of oil-oxidizing bacteria isolated from the same soil was introduced, which allowed for the removal of 87% of the pollutant in 80 days, while self-purification achieved only 42%. Heavy metals had less effect on the microbial community than PAHs in soils with aged PAHs and HMs contamination [33].

The goal of the present work was to determine the composition of microorganisms in Russian Subarctic soil with long-standing pollution by petroleum products and heavy metals and outside this zone, and the potential ability of microorganisms to participate in the self-purification of the soil.

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## 2. Materials and Methods

### *2.1. Site Description and Soil Sampling*

Soil samples were collected on October 07, 2022, and August 02, 2023, on the southeastern slope of Mount Kaskama (N 69° 16' 44''; E 29° 28' 33''; Pechengsky District, Murmansk Region, Russia). Mount Kaskama is located in the subarctic climate zone, and the sampling site, considering altitudinal zonation, can be classified as a mountain tundra zone [34]. The height of the sampling point was about 300 m; it was located 50 m downhill from the top.

As a result of anthropogenic impact, a zone of long-standing contamination (over 20 years) with petroleum products (presumably fuels and lubricants), scrap metal, and fragments of equipment had formed. Four sites were selected – from the center of the visible fuel pollution towards the periphery, arranged in a horizontal line approximately 10 m apart from each other (Figure 1). Sites No. 1 and No. 2 were located directly within the contaminated area, site No. 3 was on the border of the contaminated and conditionally clean zone, and site No. 4 was on the conditionally clean zone (with no visible fuel contamination and with preserved vegetation cover). In 2022, samples were collected from depths of 0–10 cm and 10–15/20 cm from the surface (at the boundary with the underlying rocks) aseptically into sterile containers using the X-shaped sampling method. In 2023, samples were collected from a depth of 0–20 cm. The collected samples were stored at +4°C until analysis.

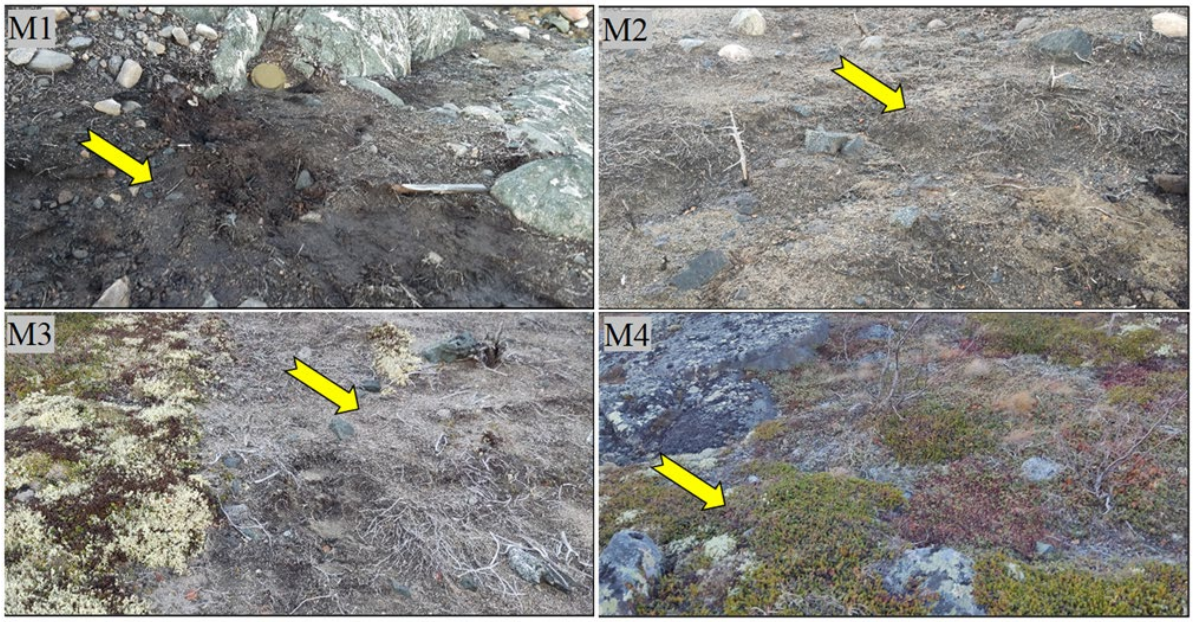


**Figure 1.** General view of the sampling sites M1–M4 in 2023.

### *2.2. Culture Media Composition*

The number of microorganisms was determined by the tenfold serial dilution method on selective media. The average weight of the soil sample (10 g) was mixed with 90 ml of sterile tap water and shaken for 30 min at 110 rpm. After precipitation of large particles for 1–2 minutes, the resulting aqueous suspension was used for inoculation of selective media. Aerobic organotrophic bacteria (AOB) were enumerated in a liquid R2A medium (per liter distilled water): 0.5 g peptone; 0.5 g yeast extract; 0.5 g glucose; 0.5 g casein hydrolysate; 0.5 g starch; 0.3 g Na pyruvate; 0.3 g $K_2HPO_4$; 0.024 g $MgSO_4 \cdot 7H_2O$; 1.0 g NaCl; pH 6.0 ± 0.2 [35]. Oligotrophic bacteria (OL) were enumerated on a medium (per liter distilled water): 0.8 g $Na_2HPO_4$; 0.5 g $KH_2PO_4$; 0.5 g $NH_4Cl$; 0.2 g $MgSO_4$; 0.1 g $CaCl_2 \cdot 2H_2O$; 1.0 g NaCl; 0.05 g yeast extract, pH 6.0 ± 0.2. Hydrocarbon-oxidizing bacteria (HOB) were analyzed on a medium (per liter distilled water): 0.75 g $KH_2PO_4$; 1.5 g $K_2HPO_4$; 1.0 g $NH_4Cl$; 1.0 g NaCl; 0.1 g KCl; 0.2 g $MgSO_4 \cdot 7H_2O$; 0.02 g $CaCl_2 \cdot 2H_2O$, pH 6.0 ± 0.2.. After

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sterilization, 0.1% (*v*/*v*) sterile diesel fuel was added to the medium as a carbon source. To determine the number of iron-oxidizing bacteria (FeOx), a medium [36] was used (per liter distilled water): 0.5 g $K_2HPO_4$; 0.5 g $(NH_4)_2SO_4$; 0.5 g $NaNO_3$; 0.5 g $MgSO_4 \cdot 7H_2O$; 5.9 g $FeSO_4 \cdot 7H_2O$; 10.0 g citric acid; 2.0 g sucrose; 1.0 g tryptone; pH 6.6–6.8.

Anaerobic microorganisms were cultivated in Hungate's tubes. Argon was used as the gas phase. Fermentative bacteria were enumerated on a medium (per liter distilled water): 10.0 g glucose; 4.0 g peptone; 2.0 g $Na_2SO_4$; 1.0 g $MgSO_4$; 0.5 g $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$; pH 6.0 ± 0.2. For iron-reducing bacteria (FeRed), a medium [37] of the following composition was used (per liter distilled water): 1.5 g $NH_4Cl$; 1.0 g NaCl; 0.75 g $KH_2PO_4$; 1.5 g $K_2HPO_4$; 0.1 g $CaCl_2 \cdot 2H_2O$; 0.6 g $NaH_2PO_4 \cdot H_2O$; 0.1 g $MgCl_2 \cdot 6H_2O$; 0.1 g KCl; 0.005 g $MnCl_2 \times 4H_2O$; 0.001 g $Na_2MoO4$; 2.5 g $NaHCO_3$ 16.2 g $Fe^{3+}$ citrate; 2.0 g Na acetate; 5.0 g yeast extract; pH 7.0±0.2. A trace elements solution (1 ml·$L^{-1}$) [38] was added to each medium after sterilization. All experiments were performed in triplicate. Cultivation was carried out for 14 days at 15 °C.

The growth of AOB and OL was determined by changes in the medium turbidity. The growth of iron-oxidizing bacteria was determined by the color change of the medium from light green to rusty brown. The growth of fermentative bacteria was assessed by the increase in $H_2$ and $CO_2$ in the gas phase. The growth of iron-reducing bacteria was detected by a decrease in $Fe^{3+}$ and an increase in $Fe^{2+}$ content, determined by the method of complexometric titration with sulfosalicylic acid. Microbial growth was also controlled by microscopy.

Pure cultures of AOB were isolated on R2A agar medium. The temperature range for bacterial growth was determined on R2A medium, and the salinity range was determined on R2A medium with varying NaCl content. The growth of iron-reducing bacteria on hydrocarbons was determined on the medium [37] without yeast extract and acetate, supplemented with diesel fuel (2 g·$L^{-1}$). Bacterial growth on crude oil and other substrates was determined on the mineral medium for HOB; sugars and protein substrates were added at a concentration of 5 g·$L^{-1}$, salts of organic acids and alcohols – 2.5 g·$L^{-1}$, crude oil and diesel fuel – 2.0 g·$L^{-1}$. Growth in the presence of heavy metals was determined in liquid R2A medium; heavy metals were added as $NiCl_2 \cdot 2H_2O$, $CuSO_4 \cdot 2H_2O$, and $Pb(NO_3)_2$ to final concentrations of $Ni^{2+}$ – 40 μg·$L^{-1}$, $Cu^{2+}$ – 75 μg·$L^{-1}$, and $Pb^{2+}$ – 100 μg·$L^{-1}$. The effect of metals on bacterial growth was evaluated (in %) by the ratio of turbidity in cultures grown on R2A medium without heavy metals and with metals.

### *2.3. Analytical Methods*

The gas phase composition was determined using a "Kristall 5000.2" chromatograph ("Khromatek", Russia). The actual acidity of the soil and soil moisture were determined as described previously [34]. The total petroleum hydrocarbons (TPHs) were extracted from a soil sample with tetrachlorocarbon; the extract was purified on a column with aluminum oxide and then examined by using the AN-2 analyzer [34]. The biodegradation of petroleum *n*-alkanes was analyzed as described previously [39]. The total content of heavy metals was determined by atomic absorption spectrometry using a spectrometer AAS "Kvant-2M" ("Kortek", Russia) after microwave digestion of the soil sample in a mixture of concentrated hydrochloric acid, nitric acid, and hydrofluoric acid (MVI 80–2008).

### *2.4. DNA Isolation and the 16S rRNA Gene V3–V4 Fragments Sequencing*

DNA from pure cultures was isolated using the Fast DNA Spin Kit (MPBio, Solon, Ohio, USA), followed by amplification of the 16S rRNA gene with universal primers 8–27f and 1492r [40]. Sequencing was performed on a 3730 DNA Analyzer using the BigDye® Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems, Waltham, MA, USA). Assembly and analysis of the obtained sequences were performed using the Bioedit package (http://jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html, accessed on 9 October 2025) and the GenBank database using the BLAST algorithm (NCBI server, www.ncbi.nlm.nih.gov/blast/, accessed on 17 September 2025). For DNA isolation from soil samples, the DNeasy PowerSoil Pro DNA isolation kit (QIAGEN, Hilden, Germany) was used according to the manufacturer's instructions and an average

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weight of the soil sample (10 g). The PCR amplification of 16S rRNA gene fragments comprising the V3–V4 variable regions was carried out using the universal prokaryotic primers 341F_Fr (5′–CCT AYG GGD BGC WSC AG–3′) and 806R_Fr (5′–GGA CTA CNV GGG THT CTA AT–3′) as described previously [41,42].

*2.5. Bioinformatic Analysis*

The quality of the obtained V3–V4 region sequences was analyzed using UPARSE software [43], and then grouped into OTUs with ≥97% similarity using USEARCH [44]. The OTUs were taxonomically identified using SILVA v.138 rRNA sequence database and the VSEARCH v. 2.14.1 algorithm (SILVA release 138.1) [45]. Further sequence analysis was performed as described previously [42]. A heat map of community members at the genus level was constructed using the ClustVis online resource [46]. Canonical correspondence analysis (CCA) was performed in the PAST 4.03 program [47].

*2.6. Nucleotide Sequence Accession Number*

The 16S rRNA gene V3–V4 fragment sequences of M1–M4 microbial communities have been deposited in the NCBI Sequence Read Archive (SRA) and are available via the BioProject PRJNA1346952. Nucleotide sequences of the 16S rRNA gene of pure cultures were deposited into GenBank under accession nos: PX457869, PX457873, PX464108, PX457728, PX462097, PX462100, PX462107, PX462110, PX457785, PX463341, PX463725–PX463727, PX457871, PX457722, PX457868, PX460839, PX457726, and PX463338.

## 3. Results and Discussion

*3.1. Physicochemical Characteristics of the Soil Samples and Culturable Microorganisms*

The physicochemical parameters of the soils sampled in different climatic seasons (October 2022 and August 2023) are shown in Table 1. The measured humidity and temperature values corresponded to the climatic norms of the Murmansk region. The temperature in October was 11–12 °C lower than in August. Soil moisture increased significantly in autumn (and exceeded 80%); this indicator was highest in the area with maximum hydrocarbon pollution (M22-1–M22-3 sites). The pH values of the aqueous soil extracts ranged from 4.33 to 6.39 in October and 5.75–5.80 in August, which may be related to the precipitation regime. Petroleum products were found in all the samples studied, even in a visually clean area (M22-4). In 2022, the total petroleum hydrocarbons (TPH) concentration in the upper layer of Site 4 (M22-4-(0-10)) exceeded the approximate permissible concentration (APC), but was significantly lower than at sites M22-1–M22-3.

**Table 1.** Physicochemical characteristics and the total petroleum hydrocarbons (TPH) and metal content in soil samples.

| Year of sampling, site | Depth of sampling, cm | Temperature, °C | Soil humidity, % | pH of the aqueous soil extract | TPH content, mg/kg | $Cu^{2+}$, mg/kg | $Ni^{2+}$, mg/kg | $Cd^{2+}$, mg/kg |
|---|---|---|---|---|---|---|---|---|
| 2022, October | | | | | | | | |
| M22-1-(0–10)* | 0–10 | 4.8 | >80 | 4.49 | 52729 | 40.11 | 23.85 | 1.423 |
| M22-1-(10–20) | 10–20 | 5.1 | >80 | 4.37 | 64530 | 27.91 | 22.84 | 1.446 |
| M22-2-(0–10) | 0–10 | 5.0 | 39 | 4.65 | 54182 | 55.50 | 35.56 | 1.924 |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| M22-2-(10–20) | 10–20 | 4.8 | 46 | 4.28 | 115079 | 16.55 | 16.07 | 1.244 |
| M22-3-(0–10) | 0–10 | 4.4 | 27 | 4.33 | 29305 | 14.76 | 19.58 | 1.494 |
| M22-3-(10–15) | 10–15 | 4.7 | 20 | 4.46 | 34008 | 19.50 | 24.13 | 0.522 |
| M22-4-(0–10) | 0–10 | 4.9 | 23 | 5.90 | 890 | 75.85 | 40.94 | 1.226 |
| M22-4-(10–15) | 10–15 | 5.0 | 32 | 6.39 | 183 | 21.20 | 15.61 | 0.191 |
| 2023, August | | | | | | | | |
| M1 | 0–20 | 17.6 | 58 | 5.80 | 52500 | 34.01 | 23.34 | 1.44 |
| M2 | 0–20 | 16.0 | 40 | 5.75 | 14900 | 36.02 | 25.82 | 1.584 |
| M3 | 0–20 | 16.1 | 37 | 5.75 | 37700 | 17.03 | 21.86 | 1.008 |
| M4 | 0–15 | 16.3 | 35 | 5.79 | 530 | 48.73 | 28.28 | 0.701 |
| APC** | | | | | 700 | 33.00 | 20.00 | 0.500 |

*–, No data. **APC, approximate permissible concentration.

The content of petroleum products in the lower soil layer was higher than on the surface, with the exception of site M22-4. A similar distribution and seepage of hydrocarbons (HC) deep into the soil has previously been noted by researchers [48]. The binding of HC to the soil matrix decreases their bioavailability and makes soil restoration more difficult. Changes in the TPH concentration in 2022 and 2023 can be caused by the process of soil self-purification, a certain mobility of the pollutant in mountainous conditions, and heterogeneity of pollution.

The content of heavy metals in almost all M1-M4 sites was higher of approximate permissible concentration (APC). The Cd content was more than 3 times higher, and for Cu and Ni it was more than 2 times higher than the corresponding value of the APC. The visually clean site M4 was also significantly polluted with Cu and Ni, and the Cd content in was also higher than APC value in 0-10 cm soil layer. But in the 10-20 cm soil layer of site M4, the content of heavy metals was below the APC.

The number of culturable microorganisms of the main physiological groups (Figure S1) was determined in all selected soil samples. It is known that a change in season leads to changes in the composition and abundance of microorganisms in the soil, with temperature and humidity being the main factors [49,50]. On average, the microbial community was more numerous in soil samples taken in August, when the air temperature was 16-17 °C, than in soils taken in October, at 4-5 °C. In the 2023 samples, the abundance of FeOx and fermentative bacteria was highest in the most polluted M1 sample and decreased as it moved away from the contaminated zone. A high abundance of oligotrophic microorganisms is typical for the soils of the northern regions [51]. The data obtained are consistent with those presented earlier [52]. A change in the sampling depth of 0–10 and 10–20 cm did not lead to a significant change in the number and redistribution of the physiological groups of the studied microorganisms.

### *3.2. Microbial Diversity*

The composition of prokaryotes was determined in soil samples, taken in 2023 year, using high-throughput of 16S rRNA gene V3–V4 fragments sequencing.

Among the studied samples, the alpha diversity was slightly higher in the uncontaminated M4 site, as evidenced by a higher Shannon index compared to other soil samples (Table S1). It is likely that a number of microorganisms are sensitive to contamination by petroleum products and heavy metals. The values of the Shannon index for all samples M1–M4 were comparable and even exceeded the values for other, including Arctic, soils [33,49,53,54]. In general, it seems that the soil community

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has adapted to pollutants. The presence of numerous minor representatives suggests a significant diversity. Rare taxa (minor in the soil community) are able to survive under certain conditions even in polluted sites and may play a role in regulating microbial interactions in response to environmental changes [55].

Bacteria dominated in all soil samples obtained; the proportion of Archaea did not exceed 2% (of the total number of sequences in the library) and was highest in the M4 sample (Figure S2). 16S rRNA gene sequencing confirmed community shift in the contaminated soil. An increase in the content of petroleum products in the soil led to a significant increase in the proportion bacteria of the phyla *Pseudomonadota* (from 23.6 to 45.4–52.5%), *Verrucomicrobiota* (from 13.2 to 15.4%), *Cyanobacteriota* (from 0.7 to 5.7%), and *Bacillota* (from 0.4 to 1.72) and a decrease of *Acidobacteriota*, *Actinomycetota*, *Planctomycetota*, *Thermoproteota*, *Bacteroidota*, and *Candidatus* Patescibacteria (Figure 2). Despite the fact that the phylum *Actinomycetota* contains active hydrocarbon degraders, its share in the M1 sample is significantly lower than that of the phylum *Pseudomonadota*, whose representatives apparently gained an advantage here due to rapid growth and broad metabolic abilities. This distribution was previously detected in soils with long-standing creosote contamination [56]. The predominance of proteobacteria over actinobacteria in the community was noted also in soils in cold regions [57,58]. The predominance of *Pseudomonas* in soil microbial communities, in response to pollution by hydrocarbons and heavy metals has been shown in laboratory conditions [55].

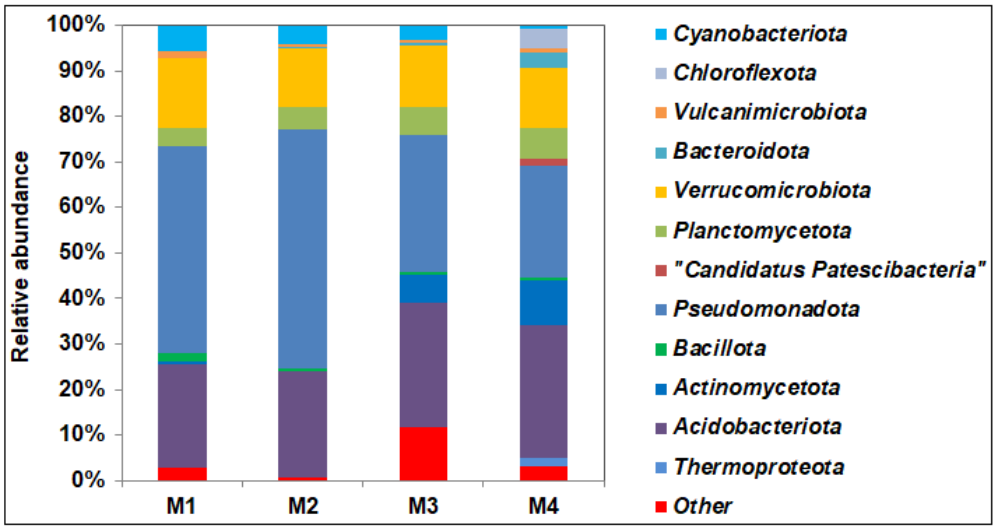


**Figure 2.** Relative abundance of prokaryotic phyla detected by 16S rRNA gene V3–V4 fragments sequencing in M1–M4 soil samples.

In accordance with the slightly acidic conditions in the soils, the bacteria of the *Acidobacteriota* phylum adapted to these conditions prevailed in the microbial community. It is one of the most widespread soil bacterial phyla found worldwide, from tropical agricultural to Arctic soils and sphagnum peat bogs [59–61]. *Acidobacteriota* have been found in microbial communities of bottom sediments of reservoirs contaminated with uranium (U) [62]. For chronically polluted soils around the oldest oil wells in Poland, as well as in the communities we studied, there was a decrease in the proportion of *Acidobacteriota* as pollution increased [63]. The authors found the greatest diversity in communities of samples taken directly near the well, where *Mycobacteriaceae*, *Methylococcaceae*, *Bradyrhizobiaceae*, *Rhizobiaceae*, *Rhodobacteraceae*, *Acetobacteraceae*, *Hyphomicrobiaceae*, and *Sphingomonadaceae* bacteria dominated.

In the M1–M4 soil samples were detected uncultivated bacteria of the families *Acetobacteraceae*, *Acidobacteraceae*, *Methylacidiphilaceae*, as well as bacteria of the genera *Acidisoma*, *Bryobacter*, *Acidothermus*, *Acidocella*, *Acidiphilum* and others (Figure 3).

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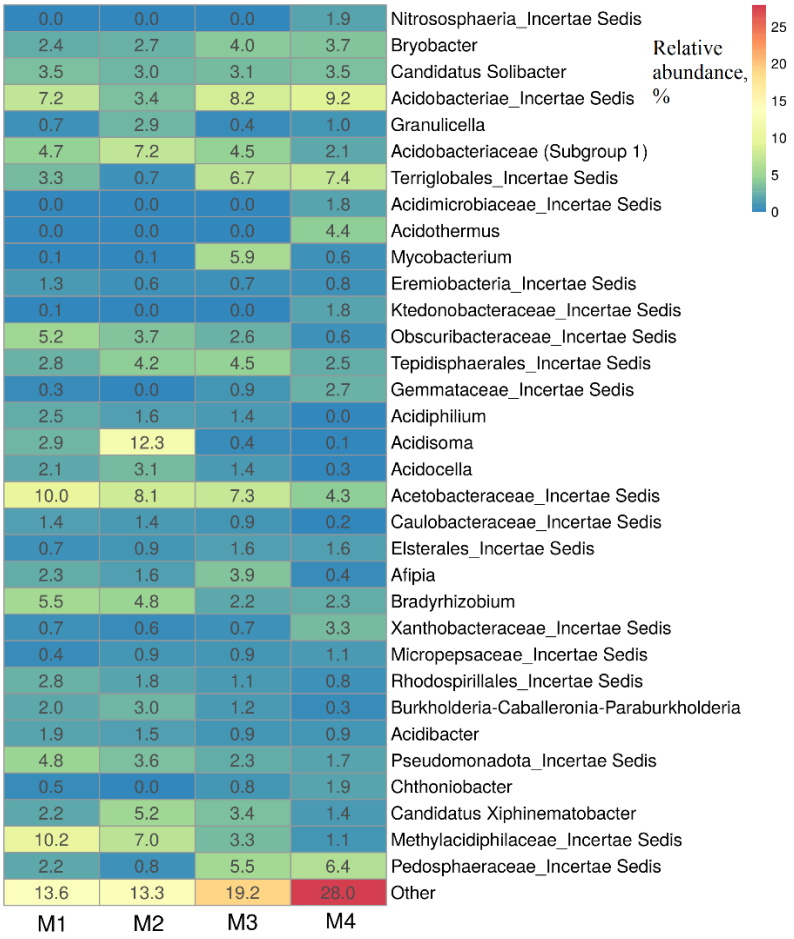


**Figure 3.** A heatmap of the distribution of the most represented genera in the libraries of 16S rRNA gene V3–V4 fragments from prokaryotic communities of M1–M4 soil samples. The numbers in the diagram indicate % of the total number of sequences in the library from each soil sample studied.

These bacteria include acidophilic and acidotolerant heterotrophic bacteria capable of growing at low temperatures, such as *Acidisoma* bacteria isolated from acidic tundra soil [64]. Bacteria of the genus *Bryobacter*, identified in all M1–M4 samples, are chemoorganotrophs, which were previously isolated from acidic sphagnum peat bogs [59].

In the studied soil samples the increase in the *Pseudomonadota* was not due to the rapidly growing *Gammaproteobacteria,* which includes the most famous oil-degrading bacteria of the genera *Pseudomonas*, *Marinobacter*, and *Alkanivorax* but due to *Alphaproteobacteria* (genera *Acidiphilum*, *Acidisoma*, *Acidocella*, and *Bradyrhizobium*). These bacteria are adapted to inhabit acidic soils, mines, and swamps [64]. Bacteria of the genus *Acidiphilum* are resistant to the presence of heavy metals [65]. Strains of *Acidocella aromatica* are able to grow on phenol, reduce $Fe^{3+}$, and resistant to heavy metals [66]. Nodule bacteria of the genus *Bradyrhizobium* are resistant to heavy metals and pesticides, contain genes determining the oxidation of aliphatic and aromatic hydrocarbons, and have been found in microbial communities of oil-contaminated soils [67–70]. Bacteria of the genus *Burkholderia*, belonging to the class *Betaproteobacteria* of the phylum *Pseudomonadota,* are capable of using hydrocarbons, and have been isolated from various sources, including oil-contaminated soils [71]. The *Burkholderia fungorum* FM-2 strain, which oxidizes phenanthrene in a wide range of pH values and resistant to heavy metals, was isolated from the oil-contaminated soil of an oil field in China [27]. Microbial communities of the studied soils also contained uncultivated bacteria. The search for conditions for the isolation and research of such microorganisms will make it possible to better understand the functioning of the entire soil community of Polar soils [72].

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Canonical correspondence analysis showed that a positive correlation can be traced for the content of hydrocarbons in the soil, probably as a substrate used (Figure 4). If we consider the effects of heavy metals, the correlation was positive for Cu and Ni, and negative for Cd, which is associated with its higher toxicity. Previously, for soils chronically subjected to hydrocarbon and polymetallic contamination, it was shown that PAHs rather than heavy metals have a greater impact on the community [33].

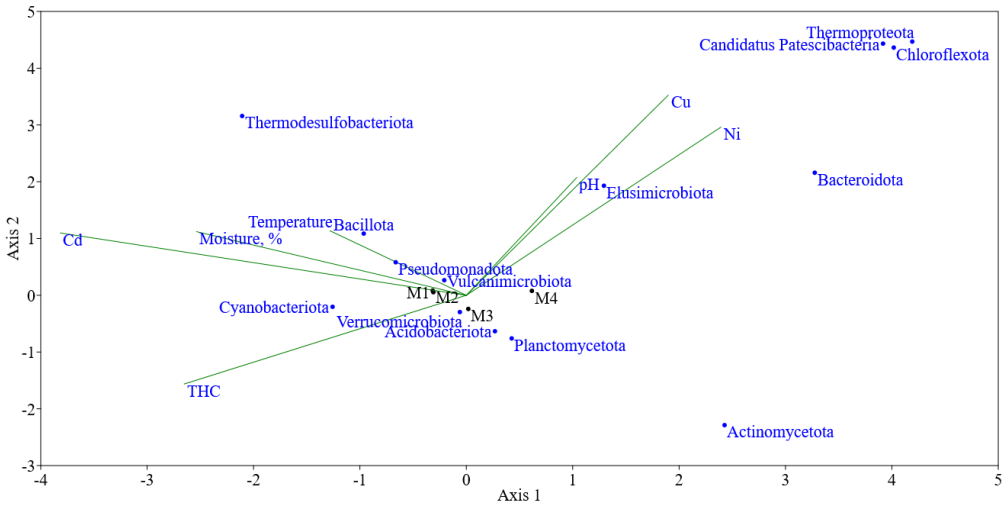


**Figure 4.** Canonical correspondence analysis (CCA) of 16S rRNA gene-based microbial community composition and environmental parameters. Arrows indicate the direction of microbial composition associated with environmental parameters.

### *3.3. Culturable Hydrocarbon-Oxidizing Bacteria Isolated from Contaminated Soil*

A total of 20 strains of aerobic heterotrophic bacteria were isolated from soil samples taken in 2022–2023 years (Table S2). The isolated strains belonged to different taxonomic groups. Most of the pure cultures were gram-negative bacteria, and belonged to the *Pseudomonadota* phylum of the classes *Betaproteobacteria* (genera *Caballeronia* and *Paraburkholderia*) and *Gammaproteobacteria* (genera *Pseudomonas* and *Rhodanobacter*). Isolated gram-positive bacteria of the genera *Bacillus*, *Cytobacillus*, and *Paenibacillus* belonged to the *Bacillota* phylum and were found among the minor components of the M1–M4 soil communities. Most of the isolates belonged to the genus *Pseudomonas*, which can be explained by their high growth rate under specified laboratory conditions.

For further work, 10 strains effectively degrading diesel fuel were selected and their adaptability to the environment was determined, including the temperature range for growth, the use of hydrocarbons and resistance to heavy metals. All the studied strains of the genera *Pseudomonas*, *Caballeronia*, *Rhodanobacter*, and *Paraburkholderia* were psychrotolerant, able to grow at low temperatures and at low optimal NaCl content for growth (0–2%, *w/v*) (Table 2). The substrates used included carbohydrates, alcohols, and volatile fatty acids. The ability to oxidize divalent iron has been shown for *Pseudomonas* strains. The strains were able to grow on diesel fuel and crude oil. Most strains used medium-chain *n*-alkanes; *P. yamanorum* M22-22H and *P. fluorescens* M23-K6fo strains used both medium-chain and long-chain *n*-alkanes from crude oil (Figure S3).

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**Table 2.** Physiological characteristics of strains isolated from polluted soils.

| No | Strain | Temperature range (optimum), °C | NaCl range (optimum), % | Substrate* | $Fe^{2+} \rightarrow Fe^{3+}$ |
|---|---|---|---|---|---|
| 1 | *Pseudomonas hamedanensis* M22-18H | 5–37 (15–20) | 0–3.5 (1) | Ac, Eth, Gly, Suc, Pept | + |
| 2 | *Pseudomonas yamanorum* M22-22H | 5–37 (15–25) | 0–8 (1–2) | Ac, Eth, Gly, Pept | + |
| 3 | *Pseudomonas synxantha* M22-62 | 5–37 (20–30) | 0–5 (2) | Ac, Eth, Gly, Suc, Pept | + |
| 4 | *Pseudomonas frederiksbergensis* M23-K5fo | 5–37 (20–25) | 0–7 (0–1) | Ac, Eth, Gly, Suc, Pept | + |
| 5 | *Pseudomonas fluorescens* M23-K6fo | 5–37 (20–25) | 0–8 (0–1) | Ac, Gly, Pept | + |
| 6 | *Pseudomonas synxantha* M23-K7fo | 5–37 (20–25) | 0–5 (2) | Ac, Eth, Gly, Suc, Pept | + |
| 7 | *Caballeronia sordidicola* M23-90 | 5–37 (15–30) | 0–6 (0) | Gly, Pept | – |
| 8 | *Rhodanobacter ginsengisoli* M23-91 | 5–30 (20) | 0–1 (0–0.5) | Eth, Gly, Suc, Pep | – |
| 9 | *Caballeronia udeis* M23-92 | 5–37 (20) | 0–5 (0) | Ac, Gly, Pep | – |
| 10 | *Paraburkholderia domus* M23-93 | 5–37 (25–28) | 0–5 (0) | Ac, Gly, Pep | – |

Designations: *Acetate – Ac; ethanol – Eth; glycerol – Gly; sucrose – Suc; peptone – Pept. **+, positive result; –, negative result.

Previously, the ability of the indigenous soil microfungi and bacteria for hydrocarbon degradation was demonstrated in a field experiment on the western slope of Mount Kaskama [34], where aeration and fertilization of the oil-contaminated soil led to a 47% reduction in total hydrocarbon content. The genus *Pseudomonas* is probably one of the most studied, the bacteria have a flexible metabolism, a wide range of substrates used (including hydrocarbons), resistance to heavy metals, are found in soils of different geographical regions and are applicable in many biotechnologies [73]. Bacteria of the genera *Rhodococcus*, *Pseudomonas*, and *Bacillus* have been repeatedly found in oil-contaminated polar soils of the Arctic and Antarctica [74].

The genus *Caballeronia* was formed through the transfer of a number of bacteria from the genus *Burkholderia*. These bacteria have been isolated from various types of soil and rhizosphere, grow in a wide range of pH values (from 4.0 to 10.0), and are capable of degrading aromatic compounds and xenobiotics [75,76]. A strain of *Paraburkholderia fungorum* JT-M8 is described, capable of self-regulation of Cd ion concentration in the cytoplasm and on the cell surface under conditions of simultaneous contamination with PAHs and heavy metals (Cd) with phosphorus deficiency [77]. Bacteria of the genus *Rhodanobacter* are tolerant to low pH values and to contamination with heavy metals, including nickel, copper, and cadmium [78–80]. They are found in microbial communities that degrade crude oil [81]. In the microbial community of oil-contaminated soils of the subarctic zone, *Rhodanobacter ginsengisoli* was one of the dominant oil degraders [82]. Spore-forming bacteria of the genera *Bacillus* and *Cytobacillus* are common inhabitants of soils, including the soil of the Polar region [5,52,83].

The resistance of the isolates to heavy metals decreased in the range Pb(II)>Ni(II)>Cu(II). The presence of $NiCl_2 \cdot 2H_2O$ stimulated the growth of strains M22–22H and M23–91, and $Pb(NO_3)_2$ – strains M22–18H, M22–22H, M23–K6fo, M23–91 compared with the control without heavy metals (Figure 5). The results obtained are comparable with data from other researchers [83,84]. The studied

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microorganisms are adapted to inhabit soils polluted with heavy metals. These results indicate the adaptation of the isolated strains to the conditions of their habitat and their possible participation in the process of soil self-purification.

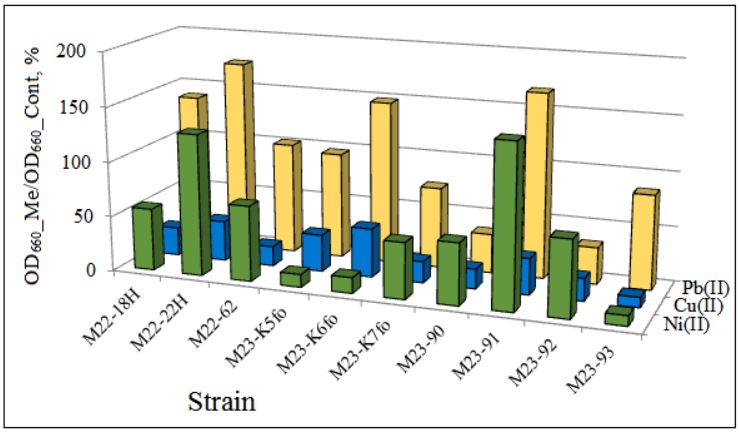


**Figure 5.** The ratio of strain growth in a medium with metals ($OD_{660}$_Me) to the growth in a control medium without metals ($OD_{660}$_Cont) in %.

*3.3. Culturable Fe(III)-Reducing Bacteria*

Both aerobic and anaerobic processes are possible in the 0-20 cm soil layer. An analysis of the number and composition of communities revealed the presence of both aerobic hydrocarbon- and iron-oxidizing, as well as anaerobic or facultatively anaerobic bacteria capable of fermentation and reduction of metals. The processes of metal oxidation and reduction can occur in parallel in microzones with different oxygen contents [85]. Bacteria involved in both the oxidation process and the reduction process in the iron cycle have been described [86]. Bacteria capable of $Fe^{3+}$ reduction can also reduce other metals ($Mn^{4+}$, for example) and vice versa [15,87], therefore $Fe^{3+}$ citrate was chosen as a model compound. The poly-contamination of soils with petroleum products and heavy metals prompted us to check whether HC oxidation is possible in these microbial communities, coupled with the $Fe^{3+}$ reduction. A similar process has been shown for bacteria of the genera *Geobacter* and *Rhizobium* [15,24].

Stable enrichment cultures were obtained from M2 and M3 soil samples, which carried out the process of $Fe^{3+}$ reduction to $Fe^{2+}$, and oxidation of the alkane fraction (Figure S4). Enrichment culture M2 used medium-chain alkanes to a greater extent, while culture M3 used medium- and long-chain alkanes. The low hydrocarbon biodegradation is probably due to the fact that the rate of anaerobic degradation of hydrocarbons is significantly lower than the aerobic HCs oxidation. Since many iron-reducing bacteria are able to grow aerobically, the resulting enrichment cultures were inoculated on plates with a solid nutrient medium, from which it was possible to isolate the FeRed2 and FeRed3 strains. The isolated strains were able to reduce $Fe^{3+}$, as well as grow aerobically on diesel fuel and crude oil (Figure S5), and were identified as *Paenibacillus pseudetheri* and P*aenibacillus nitricinens*, respectively (Table S2) [88,89].

Bacteria of the genus *Paenibacillus* are able to grow on aromatic hydrocarbons and have previously been isolated from Arctic soils contaminated with petroleum products [52,90]. These bacteria are known for their ability to reduce and sorb heavy metals, form siderophores and form floccules, which allow them to be used for water purification from heavy metals [91–93]. *Paenibacillus* spp. demonstrated high resistance to heavy metals (Cd) [94]. Probably, the bacteria of the genus *Paenibacillus* isolated from the studied soils are able to participate in the process of soil self-purification both in the aerobic and anaerobic zones, using anaerobic respiration for the degradation of hydrocarbons.

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## 4. Conclusions

Remediation of long-standing complex soil pollutants in Polar Regions is an important and difficult task. This work shows that a multifunctional autochthonous microbial community capable of biodegradation of hydrocarbons, oxidation and reduction of metals inhabit the soils of Polar Regions with long-standing pollution by petroleum products and heavy metals. The total content of hydrocarbons and heavy metals influenced the composition of microbial communities. An increase in the content of petroleum products in the soil led to a significant increase in the bacteria of the phyla *Pseudomonadota*, *Verrucomicrobiota*, *Cyanobacteriota*, and *Bacillota* and to a decrease of the *Acidobacteriota*, *Chloroflexota*, *Actinomycetota*, *Planctomycetota*, *Thermoproteota*, *Bacteroidota*, and *Candidatus* Patescibacteria. The increase in the *Pseudomonadota* was due to *Alphaproteobacteria* (genera *Acidiphilum*, *Acidisoma*, *Acidocella*, and *Bradyrhizobium*) apparently adapted to the conditions of poor northern soils. Culturable bacteria capable of degrading hydrocarbons and oxidizing metals were isolated from the upper aerobic zone, while bacteria capable of reducing $Fe^{3+}$ for growth on hydrocarbons were present in the anaerobic subsurface zone. Isolated pure cultures of bacteria of the genera *Bacillus*, *Caballeronia*, *Cytobacillus*, *Paenibacillus*, *Paraburkholderia*, *Pseudomonas*, and *Rhodanobacter* degraded petroleum *n*-alkanes, strains of the genus *Pseudomonas* oxidized $Fe^{2+}$, and *Paenibacillus* spp. we capable of reducing $Fe^{3+}$ to $Fe^{2+}$, which allows them to participate in the process of soil self-purification. The isolated strains are resistant to the used concentrations of heavy metals; their toxicity decreased in the range $Cu^{2+}$>$Ni^{2+}$>$Pb^{2+}$. The data obtained indicate the potential of autochthonous microorganisms for remediation of the studied contaminated soils.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/doi/s1, Figure S1: The number of culturable microorganisms in soil samples in October 2022 (a) and August 2023 (b); Figure S2: Taxonomic classification of prokaryotes at the level of the Bacteria and Archaea domains in soil samples based on the high-throughput sequencing of 16S rRNA genes; Figure S3: Residual content of *n*-alkanes in crude oil (in % relative to the content of *n*-alkanes in the sterile control) degraded by *Pseudomonas hamedanensis* M22-18H (a), *Pseudomonas yamanorum* M22-22H (b), *Pseudomonas synxantha* M22-62 (c), *Caballeronia sordidicola* M23-90 (d), *Caballeronia udeis* M23-92 (e), *Paraburkholderia domus* M23-93 (f), *Pseudomonas frederiksbergensis* M23-K5fo (g), and *Pseudomonas fluorescens* M23-K6fo (h). The bacteria were incubated for 14 days at 15 °C; Figure S4: Residual content of *n*-alkanes in diesel fuel (in % relative to the content of *n*-alkanes in the sterile control) degraded by $Fe^{3+}$-reducing enrichment from M2 (a) and M3 (b) soil samples on the medium with $Fe^{3+}$ citrate and diesel fuel. The enrichments were incubated for 60 days at 15 °C; Figure S5: Residual content of *n*-alkanes in crude oil (in % relative to the content of *n*-alkanes in the sterile control) degraded by strain *Paenibacillus pseudetheri* FeRed2 (a) and *Paenibacillus nitricinens* FeRed3 (b). The cultures were incubated aerobically with sterile crude oil for 21 days at 23 °C; Table S1: Diversity indices in V3–V4 libraries of prokaryotic 16S rRNA gene fragments from the studied soil samples; Table S2: Taxonomic affiliation of the isolated strains based on the analysis of 16S rRNA genes.

**Supplementary Materials:** The following supporting information can be downloaded at: Preprints.org.

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**Data Availability Statement:** The 16S rRNA gene sequences of isolated strains were deposited into GenBank under accession nos: PX457869, PX457873, PX464108, PX457728, PX462097, PX462100, PX462107, PX462110, PX457785, PX463341, PX463725–PX463727, PX457871, PX457722, PX457868, PX460839, PX457726, and PX463338. The raw data generated from 16S rRNA gene profiling of M1–M4 microbial communities have been deposited in the NCBI Sequence Read Archive (SRA) and are available via the BioProject PRJNA1346952.

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