# **РОЗДІЛ 2.** БІОЛОГІЧНА БЕЗПЕКА ТА ГРОМАДСЬКЕ ЗДОРОВ'Я В УМОВАХ ВОЄННОГО ЧАСУ

# **CHAPTER 2.** BIOLOGICAL SAFETY AND PUBLIC HEALTH IN WARTIME

## CHANGES IN THE SOIL MICROBIOME OF A MILITARY TRAINING GROUND

## ЗМІНИ МІКРОБІОМУ ҐРУНТІВ НАВЧАЛЬНОГО ВІЙСЬКОВОГО ПОЛІГОНУ

**Solomiia Komplikevych,** PhD in Biology, Ivan Franko National University of Lviv, solomiia.komplikevych@lnu.edu.ua, https://orcid.org/0000-0002-9774-7113

**Olha Maslovska,** PhD in Biology, Associate Professor, Ivan Franko National University of Lviv, olha.maslovska@lnu.edu.ua, https://orcid.org/0000-0002-0177-1419

**Andriy Hnatush,** PhD in Biology, Security Service of Ukraine, andrew.gnatush@gmail.com, https://orcid.org/0009-0007-7237-3913

**Yeva Zaritska**, State Research Control Institute of Veterinary Medicinal Products and Feed Additives, eva7e7@gmail.com, https://orcid.org/0000-0002-6963-5560

**Oleksiy Telehuz,** PhD in Geography, Associate Professor, Ivan Franko National University of Lviv, oleksiy.telehuz@lnu.edu.ua, https://orcid.org/0000-0002-7828-634X

**Svitlana Hnatush,** PhD in Biology, Professor, Ivan Franko National University of Lviv, svitlana.hnatush@lnu.edu.ua, https://orcid.org/0000-0002-5353-102X

**https://doi.org/10.32447/bcet.2026.08**

**Abstract.** An analysis of the heavy metal content in the soil samples taken from the military training ground revealed that the maximum permissible concentrations for Cu, Cr, and Zn had been exceeded. The levels of other metals in the soils studied were within acceptable limits. As a result of the ammunition explosions, metals such as Cu, Cr, Hg, and Se were released into the soil, as the concentration of these metals in the sample taken from the craters was statistically higher than in the soil sample taken from areas without visible signs of damage. In particular, copper levels increased 2.4-fold ($p<0.001$), chromium levels 8.3-fold ($p<0.001$), mercury levels 4.0-fold ($p=0.015$), and selenium levels 2.1-fold ($p=0.001$). No statistically significant differences in the content of other metals were found between the samples. Among the 10 most abundant families identified in the metagenome of the soils sampled from the military training ground, the relative abundance of *Mycobacteriaceae* was 3.06 times higher in soil samples from craters (ranging from 0.81% in a sample of undamaged soil to 2.49% in soil from a crater). An increase was also observed in the proportion of sequences identified as belonging to the families *Xanthobacteraceae* (1.87-fold), *Micrococcaceae* (1.13-fold) and *Solirubrobacteraceae* (1.08-fold). In contrast, a sharp decline was observed in the relative abundance of the *Nitrososphaeraceae* family, whose proportion in the crater sample decreased by 18.23-fold (from 3.48% to 0.19%). In addition, there was a decrease in the proportion of families such as *67-14* (2.38-fold), *Gemmatimonadaceae* (1.72-fold), *Nocardioidaceae* (1.45-fold), *Streptomycetaceae* (1.4-fold) and *Pseudomonadaceae* (1.29-fold). Among the 10 most abundant genera identified in the metagenome of the studied soils, the relative abundance of sequences identified as members

of the genus Candidatus *Solibacter* was 3.23 times higher (from 0.46% in a soil sample without visible damage to 1.50% in a soil sample from a crater), and *Mycobacterium* was 3.06 times higher (from 0.82% to 2.49%, respectively). The proportion of sequences belonging to the genera *Bradyrhizobium* (2.60 times higher) and *Candidatus Udaeobacter* (2.25 times higher) was also greater. In contrast, the sample from the crater showed a decline in the relative abundance of several taxa, particularly the genus *RB41*, whose proportion decreased 3.29-fold (from 1.87% to 0.57%). In addition, there was a decrease in the relative abundance of genera such as *Pseudonocardia* (2.45-fold), *Gaiella* (2.28-fold), *Streptomyces* (1.75-fold), *Nocardioides* (1.51-fold), and *Pseudomonas* (1.29-fold).

**Keywords:** impact of hostilities, soil composition, soil microbiome.

**Анотація.** У результаті аналізу вмісту важких металів у досліджених зразках ґрунту з навчального військового полігону виявлено перевищення ГДК для Cu, Cr, Zn. Вміст інших металів у досліджених ґрунтах був у допустимих межах. У результаті вибухів боєприпасів у ґрунт потрапляли такі метали, як Cu, Cr, Hg, Se, оскільки вміст цих металів у зразку з вирв був статистично вищим, порівняно зі зразком ґрунту без видимих ознак руйнування. Зокрема, вміст купруму зростав у 2,4 раза ($p<0,001$), хрому – у 8,3 раза ($p<0,001$), меркурію – у 4,0 раза ($p=0,015$), селену – у 2,1 раза ($p=0,001$). Не виявлено статистично значущих відмінностей вмісту інших металів між зразками. Серед 10-ти найчисельніших родин, ідентифікованих у метагеномі досліджених ґрунтів з навчального військового полігону, у зразках з вирв відносна чисельність представників родини *Mycobacteriaceae* була вищою у 3,06 раза (з 0,81 % у зразку без видимих ознак руйнування до 2,49 % у вирві). Також спостерігали зростання частки послідовностей, ідентифікованих як представники родин *Xanthobacteraceae* (у 1,87 раза), *Micrococcaceae* (у 1,13 раза) та *Solirubrobacteraceae* (у 1,08 раза). Натомість зафіксовано різке зниження відносної чисельності родини *Nitrososphaeraceae*, частка якої у зразку з вирв зменшилася у 18,23 раза (з 3,48 % до 0,19 %). Окрім того, знизилася частка таких родин, як 67-14 (у 2,38 раза), *Gemmatimonadaceae* (у 1,72 раза), *Nocardioidaceae* (у 1,45 раза), *Streptomycetaceae* (у 1,4 раза) та *Pseudomonadaceae* (у 1,29 раза).Серед 10-ти найчисельніших родів, ідентифікованих у метагеномі досліджених ґрунтів, у зразках з вирв суттєво більшою була відносна чисельність послідовностей, ідентифікованих як представники родів *Candidatus Solibacter* – у 3,23 раза (з 0,46 % у зразку без видимих ознак руйнування до 1,50 % у зразку з вирв) та *Mycobacterium* – у 3,06 раза (з 0,82 % до 2,49 % відповідно). Також більшою була частка послідовностей представників родів *Bradyrhizobium* (у 2,60 раза) та *Candidatus Udaeobacter* (у 2,25 раза). Натомість у зразку з вирв зафіксовано зниження відносної чисельності низки таксонів, зокрема роду RB41, частка якого зменшилася у 3,29 раза (з 1,87 % до 0,57 %). Окрім того, знизилася відносна чисельність представників таких родів, як *Pseudonocardia* (у 2,45 раза), *Gaiella* (у 2,28 раза), *Streptomyces* (у 1,75 раза), *Nocardioides* (у 1,51 раза) та *Pseudomonas* (у 1,29 раза).

**Ключові слова:** вплив військових дій, мікробіом ґрунту, склад ґрунту.

## INTRODUCTION

Armed conflicts are one of the key factors in human-induced ecosystem disruption, as they lead to soil degradation, loss of biodiversity, water pollution, and adverse effects on the climate system[1,2]. The russian-Ukrainian war is leading to a significant deterioration in the state of the environment and a rise in

[1] M Solokha, O Demyanyuk, L Symochko, S Mazur, N Vynokurova, K Sementsova and R Mariychuk, 'Soil degradation and contamination due to armed conflict in Ukraine' (2024) 13(10) *Land* 1614 https://doi.org/10.3390/land13101614.

[2] Державна екологічна інспекція України, 'Збитки довкіллю внаслідок збройної агресії РФ: актуальна інформація' (*ДЕІ*, 2026) https://www.dei.gov.ua/ accessed 19 May 2026.

greenhouse gas emissions across Europe, with Ukraine bearing the greatest environmental burden[3]. Over the past century, large-scale armed conflicts have had a significant impact not only on the socio-economic development of nations, but also on the functioning of the planet's natural systems[4]. The cumulative impact of military operations creates additional obstacles to achieving the Sustainable Development Goals (SDGs) and implementing strategies for the conservation and sustainable use of soil resources in the context of food security[5]. The SDGs in the field of biodiversity are proving difficult to achieve at the regional level in Ukraine, russia, neighboring countries, and the countries of the European Union. They are influenced at both the local (e.g. SDG 3 – Good Health and Well-being; SDG 4 – Quality Education) and global (e.g. SDG 2 – Zero Hunger) levels. Undoubtedly, the russian-Ukrainian armed conflict has global implications for the economic SDGs[6].

The use and disposal of explosives in the military and mining sectors are among the main sources of soil contamination, posing a threat to the environment and human health[7]. The most commonly used explosive organic compounds are 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). Once released into the environment, these compounds—along with toxic gases, munitions debris, fuel and lubricants, and the remains of military equipment—contribute to the degradation of ecosystems. Consequently, combat zones, military training grounds, and ammunition disposal sites are regarded as the primary sources of man-made pollution of terrestrial ecosystems[8,9].

It has been established that soil in military areas can remain contaminated with toxic compounds for decades. The main contaminants identified include antimony (Sb), lead (Pb), uranium (U), 2,4-dinitrotoluene, TNT, RDX, and other toxic substances[10,11]. Most of them are highly resistant to degradation, which contributes to their accumulation in the biosphere and the emergence of long-term environmental risks[12]. The migration of heavy metals (Pb, Cu, Cd, Sb, Cr, Ni, Zn) into water bodies and their accumulation in plants, invertebrates, and vertebrates also poses a significant danger[13, 14]. High concentrations of toxic

[3] I Appiah-Otoo and X Chen, 'Russian-Ukrainian war degrades the total environment' (2023) 16 *Letters in Spatial and Resource Sciences* 32.
[4] Imperial War Museums, 'Timeline of 20th and 21st century wars' (*IWM*, 2024) https://www.iwm.org.uk/history/timeline-of-20th-and-21st-century-wars accessed 19 May 2026.
[5] T Ben Hassen and H El Bilali, 'Impacts of the Russia-Ukraine war on global food security: Towards more sustainable and resilient food systems?' (2022) 11 *Foods* 2301.
[6] P Pereira, W Zhao, L Symochko, M Inacio, I Bogunovic and D Barcelo, 'The Russian-Ukrainian armed conflict will push back the sustainable development goals' (2022) 3 *Geography and Sustainability* 277.
[7] FJ Flores, E Mena, S Granda and J Duchicela, 'Microbial community composition of explosive-contaminated soils: A metataxonomic analysis' (2025) 13(2) *Microorganisms* 453 https://doi.org/10.3390/microorganisms13020453.
[8] RC Wilhelm, JP Amsili, KSM Kurtz, HM van Es and DH Buckley, 'Ecological insights into soil health according to the genomic traits and environment-wide associations of bacteria in agricultural soils' (2023) 3 *ISME Communications* 1.
[9] CPB de Mesquita, L Vimercati, D Wu, MK Childress, A Danz, AC Grupe, D Haelewaters, NM Hyde, T Kossmann, C Oliver et al, 'Fungal diversity and function in metagenomes sequenced from extreme environments' (2024) 72 *Fungal Ecology* 101383.
[10] M Solokha, O Demyanyuk, L Symochko, S Mazur, N Vynokurova, K Sementsova and R Mariychuk, 'Soil degradation and contamination due to armed conflict in Ukraine' (2024) 13(10) *Land* 1614 https://doi.org/10.3390/land13101614.
[11] C Fernandez-Lopez, R Posada-Baquero and J-J Ortega-Calvo, 'Nature-based approaches to reducing the environmental risk of organic contaminants resulting from military activities' (2022) 843 *Science of the Total Environment* 157007.
[12] C Fernandez-Lopez, R Posada-Baquero and J-J Ortega-Calvo, 'Nature-based approaches to reducing the environmental risk of organic contaminants resulting from military activities' (2022) 843 *Science of the Total Environment* 157007.
[13] A Kicińska, R Pomykała and M Izquierdo-Diaz, 'Changes in soil pH and mobility of heavy metals in contaminated soils' (2022) 73 *European Journal of Soil Science* e13203.
[14] L Gnatyshyna, V Khoma, V Martinyuk, T Matskiv, V Pedrini-Martha, M Niederwanger, O Stoliar and R Dallinger, 'Sublethal cadmium exposure in the freshwater snail Lymnaea stagnalis meets a deficient, poorly responsive metallothionein system while evoking oxidative and cellular stress' (2023) 263 *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 109490 https://doi.org/10.1016/j.cbpc.2022.109490

chemical compounds disrupt the physiological and biochemical processes of living organisms; in particular, they inhibit the vital functions of microorganisms [15,16].

In recent years, there has been a significant increase in studies examining the environmental consequences of military operations in Ukraine, particularly the impact of armed aggression on soil cover and the assessment of damage to land resources[17,18]. The scientific literature suggests identifying a new type of soil degradation – degradation caused by armed aggression, which encompasses mechanical, physical, chemical, physico-chemical, and biological change[19]. Despite the issue's relevance, comprehensive studies of the multifaceted impact of military operations on soil ecosystems remain insufficient.

One promising approach to remediation of contaminated sites is natural attenuation, which relies on the ability of physical, chemical, and biological processes to reduce the concentrations of toxic substances in soil. Microorganisms, particularly bacteria and fungi, play a key role in these processes by transforming or degrading toxic compounds[20]. These compounds are susceptible to degradation by *Pseudomonas*, *Bacillus*, *Klebsiella*, *Arthrobacter*, and *Acinetobacter* bacteria, some of which are associated with *Arabidopsis* and *Populus* root[21]. In addition, certain representatives of the *Ascomycota* and *Basidiomycota*, as well as the genera *Aspergillus*, *Fusarium*, and *Mortierella*, are considered promising agents for bioremediation[22,23]. Transgenic *Arabidopsis* plants, modified with the AfSSB gene from the bacterium *Acidithiobacillus ferrooxidans*, demonstrate increased tolerance to TNT and cobalt and improved phytoremediation efficiency[24].

At the same time, the impact of explosives significantly alters the structure of soil microbial communities, reducing their diversity and functional activity. Analysis of alpha diversity showed that bacterial diversity was significantly higher in uncontaminated root and soil samples, whereas fungal diversity did not differ significantly between sites. An analysis of beta-diversity revealed that location, year, and sample type significantly influenced microbial community structure, with location being the most influential factor. Microbial taxa with differential distribution, including bacteria such as *Pseudarthrobacter* and fungi such as *Paraleptosphaeria* and *Talaromyces*, may contribute to natural degradation processes in soils contaminated with explosives[25].

---

[15] M Solokha, O Demyanyuk, L Symochko, S Mazur, N Vynokurova, K Sementsova and R Mariychuk, 'Soil degradation and contamination due to armed conflict in Ukraine' (2024) 13(10) *Land* 1614 https://doi.org/10.3390/land13101614.

[16] O Maslovska, S Komplikevych and S Hnatush, 'Oxidative stress and protection against it in bacteria' (2023) 17(2) *Studia Biologica* 153 https://doi.org/10.30970/sbi.1702.716.

[17] А Сплодитель, О Голубцов, С Чумаченко та Л Сорокіна, 'Вплив війни Росії проти України на стан українських ґрунтів' (Звіт, *Громадська організація «Центр екологічних ініціатив "Екодія"»* 2023) https://ecoaction.org.ua/wp-content/uploads/2023/03/zabrudnennia-zemel-vid-rosii2.pdf

[18] МБ Галкін, ІВ Страшнова та АВ Андрющенко, 'Використання мікроорганізмів у біоремедіації ґрунтів, забруднених внаслідок бойових дій' (2024) 2(61) *Мікробіологія і біотехнологія* 28 https://doi.org/10.18524/2307-4663.2024.2(61).310553.

[19] СА Балюк, АВ Кучер, МО Солоха та ВБ Соловей, 'Оцінювання впливу збройної агресії РФ на ґрунтовий покрив України' (2024) 1 *Ukrainian Geographical Journal* 7 https://doi.org/10.15407/ugz2024.01.007.

[20] FJ Flores, E Mena, S Granda and J Duchicela, 'Microbial community composition of explosive-contaminated soils: A metataxonomic analysis' (2025) 13(2) *Microorganisms* 453 https://doi.org/10.3390/microorganisms13020453.

[21] FJ Flores, E Mena, S Granda and J Duchicela, 'Microbial community composition of explosive-contaminated soils: A metataxonomic analysis' (2025) 13(2) *Microorganisms* 453 https://doi.org/10.3390/microorganisms13020453.

[22] FH Crocker, CM Jung, KJ Indest, SJ Everman and MR Carr, 'Effects of chitin and temperature on sub-Arctic soil microbial and fungal communities and biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 2,4-dinitrotoluene (DNT)' (2019) 30 *Biodegradation* 415.

[23] F Ye, D Gong, C Pang, J Luo, X Zeng and C Shang, 'Analysis of fungal composition in mine-contaminated soils in Hechi City' (2020) 77 *Current Microbiology* 2685.

[24] JJ Gao, RH Peng, B Zhu, YS Tian, J Xu, B Wang, XY Fu, HJ Han, LJ Wang, FJ Zhang et al, 'Enhanced phytoremediation of TNT and cobalt co-contaminated soil by AfSSB transformed plant' (2021) 220 *Ecotoxicology and Environmental Safety* 112407.

[25] FJ Flores, E Mena, S Granda and J Duchicela, 'Microbial community composition of explosive-contaminated soils: A metataxonomic analysis' (2025) 13(2) *Microorganisms* 453 https://doi.org/10.3390/microorganisms13020453.

The aim of our study was to characterize the microbiomes of areas damaged by explosions and areas with no visible damage within the military training ground, and to determine changes in the physico-chemical and granulometric composition of these soils.

## METHODOLOGY

### 2.1. Subject of the study and sampling procedure

For the analysis, a site was selected within the Yavoriv military training ground district (Lviv region, Ukraine). Soil samples were taken directly from the site of the artillery firing (craters) and from an area located 50 meters from the craters, without any visible damage. Soil samples were collected in March 2024. At each site, samples were taken from five points (at a depth of 0–10 cm) using the diagonal method; these were thoroughly mixed to produce a representative composite sample in accordance with current standards[26]. The samples were transported and stored at 8±2 °C in thermal containers, and genetic analysis began no later than 48 hours after collection.

### 2.2. Analysis of the physical and chemical properties of soil

Soil moisture was determined by drying samples at 105±2 °C until constant weight was reached. Particle size analysis was performed using the pipette method (Kachinsky modification), with a 4% sodium pyrophosphate solution for particle digestion[27]. The humus content was determined using the Tyurin method (Nikitin modification), which involves oxidation with potassium dichromate in an acidic medium, followed by spectrophotometric measurement at 590 nm. The carbon content was calculated using a calibration curve based on a standard glucose solution. The mass fraction of humus in the soil was obtained by multiplying the carbon content (in %) by a factor of 1.724[28]. The values for active and metabolic acidity (pH) were measured potentiometrically in aqueous and salt (KCl) suspensions[29]. The total amount of absorbed bases was determined using the Kappen-Gilkovich method.

The concentrations of heavy metals and metalloids (Cd, Co, Cr, Cu, Mn, Pb, Se, Hg, Zn, As) were measured using atomic absorption spectrophotometry on a Varian Zeeman AA240Z equipped with a GTA 120 graphite furnace (Made in Australia) under the conditions specified in Table 1. For sample preparation, the wet acid digestion method was used in sealed autoclaves. Soil samples were mineralized in a 'START D' (Milestone, Italy) using a mixture of concentrated $HNO_3$ (Merck, Millipore) and deionized water (Milli-Q, Millipore) under a stepwise heating program (up to 195 °C). After cooling, the resulting hydrolysates were quantitatively transferred to volumetric flasks, made up to 25 ml with deionized water, and, where necessary, diluted with 1 % $HNO_3$ to concentrations within the working range of the calibration curves.

**Table 1**

Parameters of heavy metals determination

| | Wavelength of absorption (resonance) lines, nm | Argon flow was, L/min | Voltage of resonance radiation lamp, mA | Slit width, nm | The temperature of ashing stage, °C | The temperature of atomization stage, °C |
|---|---|---|---|---|---|---|
| Mn | 279.5 | 0.3 | 10 | 0.2 | +700 | +2400 |
| Cu | 327.4 | 0.3 | 10 | 0.5 | +800 | +2300 |
| Zn | 307.6 | 0.3 | 9 | 1.0 | +300 | +1900 |

[26] *DSTU ISO 10381–2:2004. Soil quality — Sampling — Part 2: Guidance on sampling techniques* (State Housing and Municipal Economy Committee of Ukraine 2004).
[27] *DSTU ISO 11464–2007. Soil quality — Pretreatment of samples for physico-chemical analysis* (State Consumer Standard of Ukraine 2007).
[28] *DSTU ISO 14235:2005. Soil quality — Determination of organic carbon by sulfochromic oxidation* (State Consumer Standard of Ukraine 2005).
[29] *DSTU ISO 10390:2022. Soil quality — Determination of pH* (State Agency for Standardisation of Ukraine 2022).

**Continued from Table 1**

| | Wavelength of absorption (resonance) lines, nm | Argon flow was, L/min | Voltage of resonance radiation lamp, mA | Slit width, nm | The temperature of ashing stage, °C | The temperature of atomization stage, °C |
|---|---|---|---|---|---|---|
| Cr | 357.9 | 0.3 | 10 | 0.2 | +1000 | +2600 |
| Se | 196.0 | 0.3 | 8 | 1.0 | +1000 | +2600 |
| Cd | 228.8 | 0.3 | 10 | 0.5 | +250 | +1800 |
| Pb | 283.3 | 0.3 | 10 | 0.5 | +400 | +2100 |
| Hg | 253.6 | - | 10 | 9 | +200 | +550 |

### 2.3. Isolation of metagenomic DNA and PCR amplification

Total DNA was extracted from 100 mg soil samples using the commercial DNeasy PowerSoil Pro Kit (Qiagen). The quality and concentration of the isolated genetic material were assessed using a fluorescence-based method (Denovix) and by visualization in an agarose gel.

The target regions of the 16S rRNA gene (the V3–V4 region) were amplified using the specific primers 341F and 806R. The reaction was carried out using high-precision Q5 DNA polymerase according to an optimized temperature protocol: initial denaturation (98 °C, 2 min), 20 amplification cycles, and final extension. The purified amplicons (QIAquick Gel Extraction Kit) were used to prepare libraries by ligating individual barcodes.

### 2.4. Sequencing and bioinformatic data analysis

High-throughput sequencing of the prepared libraries was performed on the Illumina MiSeq platform (2×250 bp) at a target read depth of 100,000 sequences per sample. Initial data processing (demultiplexing and removal of technical sequences) was carried out using the Cutadapt software. The FLASH and vsearch tools were used to merge paired-end reads and filter out chimeric sequences. Sequence clustering into operational taxonomic units (OTUs) was performed using the UPARSE algorithm. The taxonomic classification and calculation of alpha-diversity metrics (Shannon, Simpson, and Chao1 indices) were performed using QIIME 1.9.1.

The metagenomic sequences have been deposited in the NCBI SRA as Bioproject “Microbiome of the Sand soil affected by military operations” (Accession: PRJNA1218905).

### 2.5. Statistical analysis

The results were analyzed mathematically to determine the mean values and standard deviation (M±SD). The statistical significance of differences between the samples was assessed using Student’s t-test ($p \leq 0.05$).

## RESULTS AND DISCUSSION

### 3.1. Changes in the physical, chemical composition, and particle size distribution of soils

Soil contamination resulting from the detonation of munitions is a complex, multi-component process involving the accumulation of both inorganic and organic toxicants[30,31]. Military training grounds and combat zones are becoming ‘hotspots’ for the accumulation of potentially toxic elements and energetic

[30] H Zhang, Y Zhu, S Wang, S Zhao, Y Nie, X Liao, H Cao, H Yin and X Liu, ‘Contamination characteristics of energetic compounds in soils of two different types of military demolition range in China’ (2022) 295 *Environmental Pollution* 118654

[31] A Rodríguez-Seijo, D Fernández-Calviño, M Arias-Estévez and D Arenas-Lago, ‘Effects of military training, warfare and civilian ammunition debris on the soil organisms: An ecotoxicological review’ (2024) 60 *Biology and Fertility of Soils* 813 https://doi.org/10.1007/s00374-024-01835-8.

compounds. The main sources of contamination are products of shell casing fragmentation, the detonation of explosives, and the weathering of munitions remnants[32].

Lead (Pb) is the most widely studied and relatively common inorganic contaminant identified in studies of the environmental impact of military operations, accounting for over 90% of the mass of most bullets and shells[33]. Although it was long believed that lead remained inert, in soil it gradually oxidizes into mobile forms ($Pb^{2+}$ or $Pb^{4+}$), which become available to plants and microorganisms and therefore easily enter food chains[34].

In addition to lead, detonation of munitions releases metals such as antimony (Sb), used to harden alloys, as well as copper (Cu), zinc (Zn), cadmium (Cd), mercury (Hg), and arsenic (As). These elements enter the soil through the corrosion of fragments and the detonation of fuses, resulting in persistent contamination of the area[35]. Studies at military firing ranges show that in areas where live-fire exercises occur, soil mercury concentrations can be 10 times higher than background levels[36].

Modern 'eco-friendly' ammunition often uses alternatives to lead, such as tungsten (W) and bismuth (Bi)[37]. However, research shows that they are not inert: tungsten can be toxic to soil organisms, accumulate in plant biomass, and negatively impact microbial community structure[38].

Depleted uranium, which is widely used in the manufacture of armor-piercing shells, poses a particular danger[39]. Its presence in soil following shell detonation significantly impairs the reproductive functions of soil fauna, particularly earthworms, and the level of its accumulation in organisms' tissues is directly dependent on its concentration in the soil[40].

Organic contamination is usually in the form of explosive compounds such as TNT, RDX, and HMX[41]. These substances enter the soil through leaks caused by detonation or the fragmentation of unexploded ordnance, resulting in long-term toxic effects[42,43]. The highest levels of contamination are observed during low-order detonations, when a significant proportion of the explosive is dispersed into the

[32] A Rodríguez-Seijo, D Fernández-Calviño, M Arias-Estévez and D Arenas-Lago, 'Effects of military training, warfare and civilian ammunition debris on the soil organisms: An ecotoxicological review' (2024) 60 *Biology and Fertility of Soils* 813 https://doi.org/10.1007/s00374-024-01835-8.

[33] A Reigosa-Alonso, R Lorenzo Dacunha, D Arenas-Lago, FA Vega and A Rodríguez-Seijo, 'Soils from abandoned shooting range facilities as contamination source of potentially toxic elements: Distribution among soil geochemical fractions' (2021) 43 *Environmental Geochemistry and Health* 4283 https://doi.org/10.1007/s10653-021-00900-7.

[34] P Sanderson, R Naidu, NS Bolan, M Bowman and S McLure, 'Effect of soil type on distribution and bioaccessibility of metal contaminants in shooting range soils' (2012) 438 *Science of the Total Environment* 452 https://doi.org/10.1016/j.scitotenv.2012.08.014.

[35] AJ Barker, JL Clausen, TA Douglas, AJ Bednar, CS Griggs and WA Martin, 'Environmental impact of metals resulting from military training activities: A review' (2021) 265 *Chemosphere* 129110 https://doi.org/10.1016/j.chemosphere.2020.129110.

[36] A Rodríguez-Seijo, D Fernández-Calviño, M Arias-Estévez and D Arenas-Lago, 'Effects of military training, warfare and civilian ammunition debris on the soil organisms: An ecotoxicological review' (2024) 60 *Biology and Fertility of Soils* 813 https://doi.org/10.1007/s00374-024-01835-8.

[37] P Sanderson, F Qi, B Seshadri, A Wijayawardena and R Naidu, 'Contamination, fate and management of metals in shooting range soils—a review' (2018) 4 *Current Pollution Reports* 175 https://doi.org/10.1007/s40726-018-0089-5.

[38] AJ Kennedy, DR Johnson, JM Seiter, JH Lindsay, RE Boyd, AJ Bednar and PG Allison, 'Tungsten toxicity, bioaccumulation, and compartmentalization into organisms representing two trophic levels' (2012) 46 *Environmental Science & Technology* 9646 https://doi.org/10.1021/es300606x.

[39] AV Skalny, M Aschner, IP Bobrovnitsky, P Chen, A Tsatsakis, MMB Paoliello, A Buha Djordevic and AA Tinkov, 'Environmental and health hazards of military metal pollution' (2021) 201 *Environmental Research* 111568 https://doi.org/10.1016/j.envres.2021.111568.

[40] JK Stanley, JG Coleman, SM Brasfield, AJ Bednar and CY Ang, 'Environmental assessment of depleted uranium used in military armor-piercing rounds in terrestrial systems' (2014) 33 *Environmental Toxicology and Chemistry* 1308 https://doi.org/10.1002/etc.2551.

[41] J Luo, Y Li, H Cao, Y Zhu, X Liu, H Li and Liao X, 'Variations of microbiota in three types of typical military contaminated sites: Diversities, structures, influence factors, and co-occurrence patterns' (2023) 443(B) *Journal of Hazardous Materials* 130290 https://www.sciencedirect.com/science/article/abs/pii/S0304389422020842.

[42] J Luo, Y Li, H Cao, Y Zhu, X Liu, H Li and Liao X, 'Variations of microbiota in three types of typical military contaminated sites: Diversities, structures, influence factors, and co-occurrence patterns' (2023) 443(B) *Journal of Hazardous Materials* 130290 https://www.sciencedirect.com/science/article/abs/pii/S0304389422020842.

[43] H Zhang, Y Zhu, S Wang, S Zhao, Y Nie, X Liao, H Cao, H Yin and X Liu, 'Contamination characteristics of energetic compounds in soils of two different types of military demolition range in China' (2022) 295 *Environmental Pollution* 118654 https://www.sciencedirect.com/science/article/abs/pii/S0269749121022363.

soil in its original form[44]. Polycyclic aromatic hydrocarbons are also common contaminants at military sites[45]. They may originate from fuel spills or be present in shooting targets that break apart during training, further compromising soil environmental conditions[46]. Chemical warfare agents, such as mustard gas or polychlorinated biphenyls, are being detected in some military areas. A new type of contamination is perfluorinated and polyfluorinated substances, which are used in special munitions and are released as aerosols following an explosion[47]. Areas where munitions are destroyed (by detonation or incineration) are the most heavily contaminated sites[48]. In such areas, concentrations of energy-releasing compounds can be extremely high, and toxic residues can migrate to depths of up to 100 cm, posing a threat to groundwater[49].

Shell explosions and the movement of heavy machinery cause mechanical disturbance to the soil, known as "bombturbation"[50]. This leads to changes in landscape structure, soil compaction, and disruption of natural nutrient cycles, which negatively impact underground biota[51] .

The particle-size distribution of soil plays a decisive role in munition fragmentation. When shells strike coarse-grained and stony soils, this results in significantly greater projectile fragmentation and metal abrasion[52].

On the contrary, soils with a high clay content act as a 'cushion', minimising the deformation of projectiles and the depth to which they penetrate. Under such conditions, lead often remains in the form of large fragments, which limits the rate at which it weathers and transforms into soluble forms[53].

Silt and clay particles have the highest specific surface area, which facilitates the intensive adsorption of dissolved metals and organic toxins[54]. This means that the finest soil particles are often the most toxic because they contain higher concentrations of accumulated pollutants[55].

Mechanical impact energy can pulverize the soil itself, particularly if it contains fragile materials such as dredged sand with shell fragments or mica. This artificially increases the proportion of fine particles, which are more easily carried by the wind as toxic dust[56].

---

[44] H Zhang, Y Zhu, S Wang, S Zhao, Y Nie, X Liao, H Cao, H Yin and X Liu, 'Contamination characteristics of energetic compounds in soils of two different types of military demolition range in China' (2022) 295 *Environmental Pollution* 118654 https://www.sciencedirect.com/science/article/abs/pii/S0269749121022363

[45] A Rodríguez-Seijo, D Fernández-Calviño, M Arias-Estévez and D Arenas-Lago, 'Effects of military training, warfare and civilian ammunition debris on the soil organisms: An ecotoxicological review' (2024) 60 *Biology and Fertility of Soils* 813 https://doi.org/10.1007/s00374-024-01835-8.

[46] A Rodríguez-Seijo, D Fernández-Calviño, M Arias-Estévez and D Arenas-Lago, 'Effects of military training, warfare and civilian ammunition debris on the soil organisms: An ecotoxicological review' (2024) 60 *Biology and Fertility of Soils* 813 https://doi.org/10.1007/s00374-024-01835-8.

[47] BJ Ruyle, CP Thackray, CM Butt, DR LeBlanc, AK Tokranov, CD Vecitis and EM Sunderland, 'Centurial persistence of forever chemicals at military fire training sites' (2023) 57 *Environmental Science & Technology* 8096 https://doi.org/10.1021/acs.est.3c00675.

[48] A Rodríguez-Seijo, D Fernández-Calviño, M Arias-Estévez and D Arenas-Lago, 'Effects of military training, warfare and civilian ammunition debris on the soil organisms: An ecotoxicological review' (2024) 60 *Biology and Fertility of Soils* 813 https://doi.org/10.1007/s00374-024-01835-8.

[49] J Luo, Y Li, H Cao, Y Zhu, X Liu, H Li and Liao X, 'Variations of microbiota in three types of typical military contaminated sites: Diversities, structures, influence factors, and co-occurrence patterns' (2023) 443(B) *Journal of Hazardous Materials* 130290 https://www.sciencedirect.com/science/article/abs/pii/S0304389422020842.

[50] PS Althoff, TC Todd, SJ Thien and MA Callaham, 'Response of soil microbial and invertebrate communities to tracked vehicle disturbance in tallgrass prairie' (2009) 43 *Applied Soil Ecology* 122 https://doi.org/10.1016/j.apsoil.2009.06.011.

[51] PS Althoff, SJ Thien and TC Todd, 'Primary and residual effects of Abrams tank traffic on prairie soil properties' (2010) 74 *Soil Science Society of America Journal* 2151 https://doi.org/10.2136/sssaj2009.0091.

[52] SL Larson, CL Teeter, VF Medina and WA Martin, *Treatment and management of closed or inactive small arms firing ranges* (U.S. Army Corps of Engineers 2019).

[53] D Dermatas and M Chrysochoou, 'Lead particle size and its association with firing conditions and range maintenance: Implications for treatment' (2007) 29(4) *Environmental Geochemistry and Health* 347 https://doi.org/10.1007/s10653-007-9092-2.

[54] SL Larson, CL Teeter, VF Medina and WA Martin, *Treatment and management of closed or inactive small arms firing ranges* (U.S. Army Corps of Engineers 2019).

[55] K Gębka, J Bełdowski and M Bełdowska, 'The impact of military activities on the concentration of mercury in soils of military training grounds and marine sediments' (2016) *Environmental Science and Pollution Research* https://doi.org/10.1007/s11356-016-7436-0.

[56] D Dermatas and M Chrysochoou, 'Lead particle size and its association with firing conditions and range maintenance: Implications for treatment' (2007) 29(4) *Environmental Geochemistry and Health* 347 https://doi.org/10.1007/s10653-007-9092-2.

Vertical migration of contaminants following ruptures can be significant. Organic compounds and metals can penetrate to a depth of up to 100 cm, posing a risk of groundwater contamination, particularly in soils with high hydraulic conductivity[57].

Landfill management practices also influence the rate of weathering. For example, the use of water spraying to suppress dust at enclosed firing ranges can accelerate the corrosion of metal fragments and the formation of secondary minerals, such as cerussite ($PbCO_3$)[58].

The bioavailability of contaminants depends directly on their form: metals in the form of large fragments (shrapnel) have low bioavailability, but over time, they transform into more bioavailable ionic forms[59]. This process is significantly influenced by soil pH and organic matter content[60].

Based on our analysis of soil samples taken from the crater and from a visually undamaged area within the military training ground, we have determined that the explosions affected the particle-size distribution (mechanical composition) of the soils examined. Physical mixing (pedoturbation processes of military origin) has altered the distribution of grain-size fractions, shifting it from coarse sand to silt. The greatest changes occurred in the silt content: from 0.4 to 0.8%; medium silt, from 1.2 to 2.4%; and clay, from 0.4 to 0.8% (Table 2). The clay content of soils damaged by explosions is slightly higher (3.2%) due to mixing with transitional, heavier illuvial horizons. To some extent, this increases the soil's mass-specific surface area, thereby affecting its physical and chemical properties, particularly its absorption capacity. In general, changes in particle-size distribution do not affect the classification of these soils; they remain sandy.

**Table 2**

Particle size distribution analysis of soil samples from a crater and from a visually undamaged area at a military training ground

| Area | Particle size (mm), quantity (%) | | | | | | The sum of particles less than 0.01 mm (fraction of clay) (%) | Soil name based on particle size distribution |
|---|---|---|---|---|---|---|---|---|
| | sand | | silt | | | clay | | |
| | 1–0.25 | 0.25–0.05 | 0.05–0.01 | 0.01–0.005 | 0.005–0.001 | <0.001 | | |
| Without visible damage | 38.2 | 52.2 | 6.8 | 1.2 | 0.8 | 0.8 | 2.8 | Sandy |
| Crater | 35.6 | 54.4 | 6.8 | 2.4 | 0.4 | 0.4 | 3.2 | Sandy |

The mechanical impact of the explosion altered the particle size distribution, which, in turn, affected the main physical and chemical properties. Specifically, the humus content decreased by 0.15% compared to the control; the hydrogen pH decreased by 0.7, while the salt pH increased by 0.3; accordingly, the hydrolytic acidity increased by 0.2 mg/eq/100 g of soil; the sum of absorbed bases decreased by 1.2 mg/eq/100 g of soil (Table 3).

[57] H Zhang, Y Zhu, S Wang, S Zhao, Y Nie, X Liao, H Cao, H Yin and X Liu, 'Contamination characteristics of energetic compounds in soils of two different types of military demolition range in China' (2022) 295 *Environmental Pollution* 118654 https://www.sciencedirect.com/science/article/abs/pii/S0269749121022363.

[58] D Dermatas and M Chrysochoou, 'Lead particle size and its association with firing conditions and range maintenance: Implications for treatment' (2007) 29(4) *Environmental Geochemistry and Health* 347 https://doi.org/10.1007/s10653-007-9092-2.

[59] P Sanderson, R Naidu, NS Bolan, M Bowman and S McLure, 'Effect of soil type on distribution and bioaccessibility of metal contaminants in shooting range soils' (2012) 438 *Science of the Total Environment* 452 https://doi.org/10.1016/j.scitotenv.2012.08.014.

[60] A Rodríguez-Seijo, D Fernández-Calviño, M Arias-Estévez and D Arenas-Lago, 'Effects of military training, warfare and civilian ammunition debris on the soil organisms: An ecotoxicological review' (2024) 60 *Biology and Fertility of Soils* 813 https://doi.org/10.1007/s00374-024-01835-8.

**Table 3**

Physical and chemical properties of soils from the crater and from a visually undamaged area within the military training ground

| Area | The sum of particles less than 0.01 mm (fraction of clay) (%) | Humus, % | $pH_{H2O}$ | $pH_{KCl}$ | Hydrolytic acidity, mg-eq./100 g of soil | The amounts of adsorbed bases, mg-eq./100 g of soil |
|---|---|---|---|---|---|---|
| Without visible damage | 2.8 | 0.65 | 7.5 | 7.2 | 0.35 | 2.0 |
| Crater | 3.2 | 0.52 | 6.8 | 7.5 | 0.53 | 0.8 |

The observed changes in the acid-base properties and particle-size distribution of the soils are primarily caused by mechanical soil disturbance and the thermodegradative effects of the explosion.

Analysis of the heavy metal content in the soil samples taken from the undamaged area and the crater revealed that the maximum permissible concentrations (MPCs) for soil were exceeded for Cu, Cr and Zn (Table 4). The levels of other metals in the soils studied were within acceptable limits. As a result of the munitions explosions, metals such as Cu, Cr, Hg and Se were introduced into the soil, as the concentration of these metals in the soil from the crater was statistically higher than in the soil from the nearby area. In particular, copper levels increased 2.4-fold ($p<0.001$), chromium levels 8.3-fold ($p<0.001$), mercury levels 4.0-fold ($p=0.015$) and selenium levels 2.1-fold ($p=0.001$). No statistically significant differences were found in the content of other metals compared with the soil from the undamaged area.

**Table 4**

Metal contents and contamination indices of the soil samples

| **Metal** | **Soil from the undamaged area** | **Soil from the crater** | **MPC[1]** |
|---|---|---|---|
| | Metal content, $mg \cdot kg^{-1}$ | | |
| **Cu** | 3.87±0.45 | 9.27±0.55*** | 3.0 |
| **Cr** | 11.1±1.1 | 91.6±2.9*** | 6.0 |
| **Zn** | 46.5±2.2 | 41.9±2.8 | 23.0 |
| **Pb** | 4.23±0.65 | 3.37±0.45 | 32.0 |
| **Cd** | 0.113±0.005 | 0.087±0.004** | 1.5 |
| **As** | 1.69±0.05 | 1.83±0.06 | 2.0 |
| **Hg** | 0.0004±0.0001 | 0.0016±0.0002* | 2.1 |
| **Mn** | 205.3±2.4 | 207.2±2.1 | 1500 |
| **Co** | 2.00±0.60 | 2.35±0.12 | 5.0 |
| **Se** | 0.22±0.03 | 0.47±0.04** | 3.0 |

Note: [1] Order of MHO of Ukraine № 1595 from 14.07.2020;* – $p < 0.05$, ** – $p < 0.01$; *** – $p < 0.001$ – probable differences in metal content compared to the soil from the control area

The 8.2-fold increase in chromium (Cr) levels (to 91.6 mg/kg) and the 2.4-fold increase in copper (Cu) levels we detected in the soil of the military training ground demonstrate that the chemical load varies depending on the type of ammunition used. By way of comparison, in a similar study we conducted using soil samples taken in the Sumy region (the village of Rudak), we recorded a 2.3-fold increase in cadmium levels and a 2.1-fold increase in copper levels in the crater left by an 82-mm mortar shell[61]. In another study

[61] O Maslovska, S Komplikevych, D Zinchuk, A Hnatush, Y Zaritska, O Telehuz and S Hnatush, 'Bacteriome of the soil affected by hostilities as a source of isolation of plant growth-promoting metal-resistant bacteria' (2025) 75(1) *Annals of Microbiology* https://doi.org/10.1186/s13213-025-01807-9.

of soil samples collected in the Sumy region, conducted by researchers from the Institute of Soil Protection of Ukraine, the average zinc (Zn) content was 3.9 times the background level, and the cadmium content was 1.4 times the background level. Furthermore, at the sites where aerial bombs had fallen, the lead content reached 113.5% of the maximum permissible concentration[62, 63] . In the Lviv region (sites of missile strikes), researchers used ICP-OES chemical analysis to establish the following order of concentration: Cd > Cu > Pb > Cr > Zn > Ni[64] . In the Kharkiv region, cadmium levels in some water samples exceeded background levels by 2–18 times, and lead levels by 2.6–22 times[65]. The most critical levels were recorded in soil samples taken in the Luhansk region, where extreme concentrations of lead (up to 14,000 mg/kg) and copper (up to 330 mg/kg) were detected[66], which is significantly higher than our results in this study.

Such significant spatial variation in contamination confirms that military soil degradation is random in nature and depends on the intensity of fighting and the types of munitions used. Whereas the soil at the Yavoriv Military Training Ground is most heavily contaminated with chromium, lead and zinc are the predominant contaminants in the eastern regions of Ukraine, which calls for a tailored approach to the selection of bioremediation methods.

**3.2. The impact of hostilities on the diversity of microorganisms in soil samples**

Changes in the microbial composition of soils caused by the detonation of munitions and military activities result from a complex combination of chemical toxic stress and physical habitat degradation[67]. The main chemical stressors for the microbiota are energetic compounds (TNT, RDX, HMX) in extremely high concentrations, as well as heavy metals, which in some cases reach tens of thousands of mg/kg, causing a significant reduction in microbial biodiversity and functional stability[68,69,70].

Studies show that high levels of potentially toxic elements, in particular Pb, Cu, Sb and Hg, have an inhibitory effect on microbial biomass. Soil enzymatic activity is a particularly sensitive indicator. For example, dehydrogenase activity is used as an indicator of overall soil biological activity; its suppression correlates with increasing levels of contamination[71,72].

---

[62] 'Vplyv aviabomb ta zghoriloi tekhniky na zabrudnennia gruntiv: doslidzhennia' (*SuperAgronom.com*, 2024) https://superagronom.com/blog/987-zabrudnennya-gruntiv-vajkimi-metalami-v-mistsyahpadinnya-aviabomb-ta-zgoriloyi-tehniki-doslidjennya-v-sumskiy-oblasti accessed 20 May 2026.

[63] A Yakymchuk, O Balanda and M Bzowska-Bakalarz, 'Assessment of Soil Contamination of Ukraine with Heavy Metals During the War' (2024) 196 *Scientific Papers of Silesian University of Technology. Organization & Management*.

[64] K Petrushka, MS Malovanyy, D Skrzypczak, K Chojnacka and J Warchoł, 'Risks of Soil Pollution with Toxic Elements During Military Actions in Lviv' (2024) 25(1) *Journal of Ecological Engineering* 195 https://doi.org/10.12911/22998993/175136.

[65] 'Heokhimičnyj stan gruntiv u zoni bojovych dij: imovirni ryzyky dlia ahrosektoru ta shliachy vyrishennia' (*AgroPortal*, 2024) https://agroportal.ua/blogs/geohimichniy-stan-gruntiv-u-zoni-boyovih-diy-imovirni-riziki-dlya-agrosektoru-ta-shlyahi-virishennya accessed 21 May 2026.

[66] A Splodytel, O Holubtsov, S Chumachenko and L Sorokina, 'The impact of Russia's war against Ukraine on the state of the country's soil. Analysis results' (Report, *Ecoaction - Centre for Environmental Initiatives* 2023).

[67] H Zhang, Y Zhu, S Wang, S Zhao, Y Nie, X Liao, H Cao, H Yin and X Liu, 'Contamination characteristics of energetic compounds in soils of two different types of military demolition range in China' (2022) 295 *Environmental Pollution* 118654 https://www.sciencedirect.com/science/article/abs/pii/S0269749121022363.

[68] A Rodríguez-Seijo, D Fernández-Calviño, M Arias-Estévez and D Arenas-Lago, 'Effects of military training, warfare and civilian ammunition debris on the soil organisms: An ecotoxicological review' (2024) 60 *Biology and Fertility of Soils* 813 https://doi.org/10.1007/s00374-024-01835-8.

[69] J Luo, Y Li, H Cao, Y Zhu, X Liu, H Li and Liao X, 'Variations of microbiota in three types of typical military contaminated sites: Diversities, structures, influence factors, and co-occurrence patterns' (2023) 443(B) *Journal of Hazardous Materials* 130290 https://www.sciencedirect.com/science/article/abs/pii/S0304389422020842.

[70] H Zhang, Y Zhu, S Wang, S Zhao, Y Nie, X Liao, H Cao, H Yin and X Liu, 'Contamination characteristics of energetic compounds in soils of two different types of military demolition range in China' (2022) 295 *Environmental Pollution* 118654 https://www.sciencedirect.com/science/article/abs/pii/S0269749121022363.

[71] K Gębka, J Bełdowski and M Bełdowska, 'The impact of military activities on the concentration of mercury in soils of military training grounds and marine sediments' (2016) *Environmental Science and Pollution Research* https://doi.org/10.1007/s11356-016-7436-0.

[72] IJC Rijk and A Ekblad, 'Carbon and nitrogen cycling in a lead polluted grassland evaluated using stable isotopes (δ13C and δ15N) and microbial, plant and soil parameters' (2020) 449 *Plant and Soil* 249 https://doi.org/10.1007/s11104-020-04467-7.

In addition to direct chemical effects, the microbiota is also affected by mechanical stress. Explosions of munitions trigger a process known as "bombturbation". The use of heavy tracked vehicles in combat zones causes soil compaction, which has a negative impact on microbial growth[73, 74]. However, it is interesting to note that, over time, compaction can alter the structure of the micropores, leading to a predominance of Gram-positive bacteria over Gram-negative bacteria due to changes in the availability of moisture and oxygen[75].

Changes in microbial composition also depend on soil pH and organic matter content. In alkaline soils, metals often convert into insoluble forms (oxides, carbonates), which may somewhat mitigate their toxic effects on microorganisms, whereas in acidic soils their mobility and toxicity increase[76, 77].

Military contamination causes the vertical migration of toxicants. The structure of bacterial communities at different depths is controlled by a combination of energy compound levels and soil moisture[78].

Despite the overall negative impact, prolonged contamination promotes the selection of tolerant strains. Bacteria of the genera *Pseudomonas*, *Bacillus* and *Klebsiella* have been identified in landfill soils; these have adapted to high concentrations of toxicants and are capable of degrading TNT and RDX[79,80,81]. These microorganisms have the potential to be used in bioremediation schemes at military sites[82].

Changes in microbial diversity have a chain effect on higher trophic levels. For example, a reduction in microbial activity can reduce the availability of nutrients to plants and suppress populations of soil fauna, such as nematodes and earthworms[83].

Modern ammunition introduces a new factor of impact: microplastics resulting from the fragmentation of plastic components in projectiles[84]. These polymer particles can alter the structure

[73] A Rodríguez-Seijo, D Fernández-Calviño, M Arias-Estévez and D Arenas-Lago, 'Effects of military training, warfare and civilian ammunition debris on the soil organisms: An ecotoxicological review' (2024) 60 *Biology and Fertility of Soils* 813 https://doi.org/10.1007/s00374-024-01835-8

[74] J Luo, Y Li, H Cao, Y Zhu, X Liu, H Li and Liao X, 'Variations of microbiota in three types of typical military contaminated sites: Diversities, structures, influence factors, and co-occurrence patterns' (2023) 443(B) *Journal of Hazardous Materials* 130290 https://www.sciencedirect.com/science/article/abs/pii/S0304389422020842.

[75] A Rodríguez-Seijo, D Fernández-Calviño, M Arias-Estévez and D Arenas-Lago, 'Effects of military training, warfare and civilian ammunition debris on the soil organisms: An ecotoxicological review' (2024) 60 *Biology and Fertility of Soils* 813 https://doi.org/10.1007/s00374-024-01835-8.

[76] J Luo, Y Li, H Cao, Y Zhu, X Liu, H Li and Liao X, 'Variations of microbiota in three types of typical military contaminated sites: Diversities, structures, influence factors, and co-occurrence patterns' (2023) 443(B) *Journal of Hazardous Materials* 130290 https://www.sciencedirect.com/science/article/abs/pii/S0304389422020842.

[77] SL Larson, CL Teeter, VF Medina and WA Martin, *Treatment and management of closed or inactive small arms firing ranges* (U.S. Army Corps of Engineers 2019).

[78] J Luo, Y Li, H Cao, Y Zhu, X Liu, H Li and Liao X, 'Variations of microbiota in three types of typical military contaminated sites: Diversities, structures, influence factors, and co-occurrence patterns' (2023) 443(B) *Journal of Hazardous Materials* 130290 https://www.sciencedirect.com/science/article/abs/pii/S0304389422020842.

[79] MÁ Cabrera, SL Márquez, CP Quezada, MI Osorio, E Castro-Nallar, FD González-Nilo and JM Pérez-Donoso, 'Biotransformation of 2,4,6-trinitrotoluene by Pseudomonas sp. TNT3 isolated from Deception Island, Antarctica' (2020) 262 *Environmental Pollution* 113922 https://doi.org/10.1016/j.envpol.2020.113922.

[80] J Li, X Yang, J-L Lai, Y Zhang, X-G Luo, S-P Zhao and Y-B Zhu, 'Characteristics of RDX degradation and the mechanism of the RDX exposure response in a Klebsiella sp. strain' (2021) 176 *Biochemical Engineering Journal* 108174 https://doi.org/10.1016/j.bej.2021.108174.

[81] J Luo, Y Li, H Cao, Y Zhu, X Liu, H Li and Liao X, 'Variations of microbiota in three types of typical military contaminated sites: Diversities, structures, influence factors, and co-occurrence patterns' (2023) 443(B) *Journal of Hazardous Materials* 130290 https://www.sciencedirect.com/science/article/abs/pii/S0304389422020842.

[82] JA Siles and R Margesin, 'Insights into microbial communities mediating the bioremediation of hydrocarbon-contaminated soil from an Alpine former military site' (2018) 102 *Applied Microbiology and Biotechnology* 4409 https://doi.org/10.1007/s00253-018-8932-6.

[83] M Lu, Li H, Li Y, Lu Y, Wang H and Wang X, 'Exploring the toxicology of depleted uranium with *Caenorhabditis elegans*' (2020) 5 *ACS Omega* 12119 https://doi.org/10.1021/acsomega.0c00380.

[84] G Rotter, C Correzzola, VF Del Ángel, E Daminato and V Causin, 'Characterisation of plastic wads: A useful approach for elucidating shooting accidents and homicides involving shotguns' (2022) 332 *Forensic Science International* 111194 https://doi.org/10.1016/j.forsciint.2022.111194.

of microbial biofilms in the soil and interact with chemical pollutants, creating additional environmental pressure[85,86,87].

To assess microbial community species richness and evenness, alpha-diversity indices were calculated for soil samples from the crater and an undamaged nearby area (Table 5). High alpha-diversity indices were recorded in the soil from both plots, indicating a high level of complexity and stability in the soil bacterial community. At the same time, a general trend of a slight decrease in all alpha-diversity metrics was observed in soil samples from the crater compared with those from the undamaged nearby area. The high values of the Shannon index (>8.9) and Simpson's index (>0.99) in both samples indicate a well-established, stable, and ecologically flexible soil bacterial community. A slight decrease in alpha-diversity indices (Observed OTUs, Chao1, and Shannon) in the soil from the pit suggests that the tested factor exerted moderate selective pressure on the microbiota. This pressure likely suppressed sensitive or rare groups of microorganisms, whilst the core taxonomic group retained its functional stability.

**Table 5**

Alpha diversity indices

| Alpha Diversity | Soil from the undamaged area | Soil from the crater |
|---|---|---|
| **Observed species, OTUs** | 3119 | 2944 |
| **Shannon's diversity index** | 9.177 | 8.981 |
| **Simpson's index of diversity** | 0.995 | 0.992 |
| **Chao1 index** | 3255.005 | 3010.333 |

To gain a deeper understanding of the differences between the microbial communities in the soil from the crater and the undamaged area nearby, a comparative analysis of OTUs composition was carried out, with the results visualized using a Venn diagram (Fig. 1).

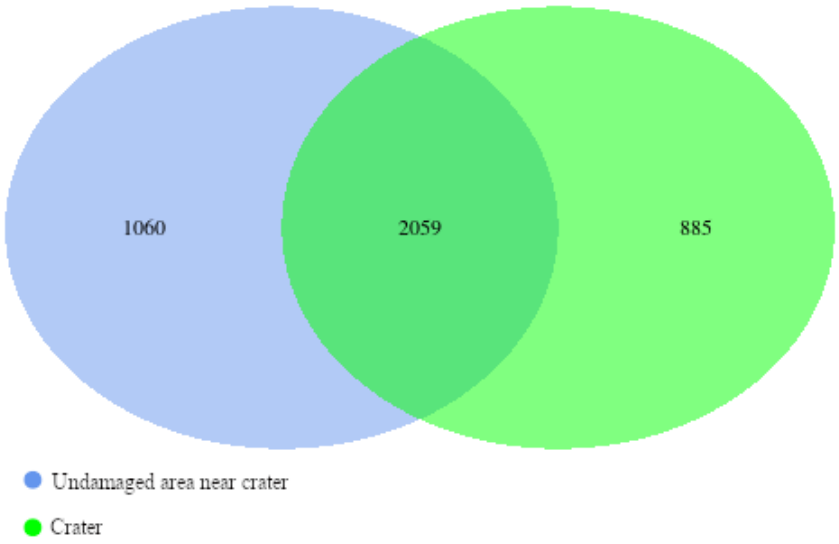



**Figure 1.** Similarity (or dissimilarity) and overlaps are shown in a Venn diagram, which shows the OTUs distribution in the soil samples

[85] L Zimmermann, A Dombrowski, C Völker and M Wagner, 'Are bioplastics and plant-based materials safer than conventional plastics? In vitro toxicity and chemical composition' (2020) 145 *Environment International* 106066 https://doi.org/10.1016/j.envint.2020.106066.
[86] E Liwarska-Bizukojc, 'Effect of (bio)plastics on soil environment: A review' (2021) 795 *Science of the Total Environment* 148889 https://doi.org/10.1016/j.scitotenv.2021.148889.
[87] NN Nik Mut, J Na and J Jung, 'A review on fate and ecotoxicity of biodegradable microplastics in aquatic system: Are biodegradable plastics truly safe for the environment?' (2024) 344 *Environmental Pollution* 123399 https://doi.org/10.1016/j.envpol.2024.123399.

The high proportion of common OTUs (2059), accounting for approximately 66% of the total taxa in the soil metagenome from the undisturbed site and 70% from the artillery firing range, indicates significant functional stability of the soil microbiome. This core group likely drives the major biogeochemical cycles and ensures the stability of the soil ecosystem. The presence of a significant number of unique OTUs in each sample confirms the ecological plasticity of the microbiota and its sensitivity to changes in environmental conditions. The reduction in the number of unique OTUs in the soil microbiome from the craters to 885 suggests that the explosions of munitions led to the elimination of some rare or sensitive species, while simultaneously promoting the survival or, possibly, the emergence of specific taxa adapted to the new conditions. These unique taxa are potential indicators of specific soil ecological niches.

In this study, we identified 38 phyla, 96 classes, 199 orders, 248 families, and 377 genera of prokaryotic microorganisms. Among the OTUs of prokaryotic microorganisms detected in the metagenome of both soil samples, 96.4–99.7% belonged to bacteria (35 phyla), and 0.3–3.5% to archaea (Table 6).

**Table 6**

Relative abundance of prokaryotic OTUs at the phylum level in soil samples from the crater and from a visually undamaged area within the military training range

| Kingdom | Phylum | Phylum, relative abundance | |
|---|---|---|---|
| | | Near crater | Crater |
| *Archaea* | *Crenarchaeota* | 3.49 | 0.27 |
| | *Thermoplasmatota* | 0.05 | 0.002 |
| | *Nanoarchaeota* | 0.004 | 0.005 |
| *Bacteria* | *Proteobacteria* | 24.29 | 36.72 |
| | *Actinobacteriota* | 35.75 | 28.87 |
| | *Acidobacteriota* | 11.90 | 15.34 |
| | *Chloroflexi* | 7.47 | 4.85 |
| | *Gemmatimonadota* | 4.28 | 2.46 |
| | *Methylomirabilota* | 2.90 | 1.06 |
| | *Bacteroidota* | 0.96 | 2.31 |
| | *Verrucomicrobiota* | 1.36 | 1.97 |
| | *Myxococcota* | 1.42 | 1.70 |
| | *Firmicutes* | 1.37 | 1.34 |
| | *Nitrospirota* | 0.77 | 0.20 |
| | *Latescibacterota* | 0.66 | 0.24 |
| | *RCP2-54* | 0.59 | 0.58 |
| | *Desulfobacterota* | 0.40 | 0.29 |
| | *Armatimonadota* | 0.11 | 0.29 |
| | *Bdellovibrionota* | 0.19 | 0.21 |
| | *Entotheonellaeota* | 0.17 | 0.07 |
| | *Patescibacteria* | 0.11 | 0.17 |
| | *MBNT15* | 0.15 | 0.07 |
| | *Planctomycetota* | 0.10 | 0.13 |
| | *NB1-j* | 0.13 | 0.02 |
| | *Elusimicrobiota* | 0.05 | 0.10 |
| | *WPS-2* | 0.007 | 0.07 |
| | *Cyanobacteria* | 0.05 | 0.06 |
| | *Dependentiae* | 0.05 | 0.03 |
| | *Fibrobacterota* | 0.02 | 0.05 |
| | *GAL15* | 0.05 | 0.03 |

**Continued from Table 6**

| Kingdom | Phylum | Phylum, relative abundance | |
|---|---|---|---|
| | | Near crater | Crater |
| *Bacteria* | *Dadabacteria* | 0.003 | 0.04 |
| | *Sumerlaeota* | 0.02 | 0.02 |
| | *SAR324_clade(Marine_group_B)* | 0.02 | 0.009 |
| | *FCPU426* | 0.006 | 0.01 |
| | *Zixibacteria* | 0.008 | 0 |
| | *WS2* | 0.006 | 0.003 |
| | *Margulisbacteria* | 0.003 | 0 |
| | *Deinococcota* | 0 | 0.0006 |
| *Others* | | 1.04 | 0.38 |

Explosions altered soil composition, thereby altering the abundance of archaea. The OTUs belonging to *Archaea* were identified as *Crenarchaeota*, *Thermoplasmatota*, and *Nanoarchaeota*. In the crater soil, the relative abundance of Archaea OTUs was lower than in the nearby area soil, which was not visibly affected. In soil samples from the crater and the nearby area, OTUs of *Crenarchaeota* were predominant; however, the relative abundance of *Crenarchaeota* OTUs in the soil from the crater was 13 times lower than in the soil from the nearby area. The relative abundance of *Thermoplasmatota* OTUs in the soil sample from the crater was also lower compared to that in the undisturbed soil. No significant changes in the abundance of *Nanoarchaeota* OTUs were observed between the soil from the crater and that from the unaffected area (Table 7).

Among the *Crenarchaeota*, the most abundant OTUs belong to the order *Nitrososphaerales*, particularly the family *Nitrososphaeraceae*. At the genus level, *Candidatus Nitrocosmicus* accounted for the largest number of identified OTUs in both the soil from the crater and the nearby area. The relative abundance of OTUs of *Candidatus Nitrocosmicus* in the soil from the crater was 3.5 times lower than in the soil from the nearby undamaged area. OTUs of *Candidatus Nitrososphaera* and *Candidatus Nitrosotenuis* were detected in soil samples taken near the crater, but were not detected in soil samples taken from the crater. In contrast, OTUs of *Candidatus Nitrosotalea* were detected in soil samples from the crater but not in those from the nearby area (Table 7).

**Table 7**

Relative abundance of *Crenarchaeota* OTUs in soil samples from the crater and from a visually undamaged area within the military training ground

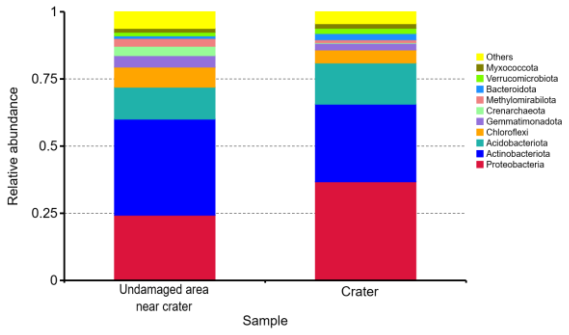
| Class | Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|---|
| | | | | Near crater | Crater |
| *Nitrososphaeria* | *Nitrososphaerales* | *Nitrososphaeraceae* | *Candidatus_ Nitrocosmicus* | 0.39 | 0.11 |
| | | | *Candidatus_ Nitrososphaera* | 0.15 | 0 |
| | *Nitrosopumilales* | *Nitrosopumilaceae* | *Candidatus_ Nitrosotenuis* | 0.009 | 0 |
| | *Nitrosotaleales* | *Nitrosotaleaceae* | *Candidatus_ Nitrosotalea* | 0 | 0.003 |

The top 10 phyla of *Bacteria* included *Proteobacteria, Actinobacteriota, Acidobacteriota, Chloroflexi, Gemmatimonadota, Methylomirabilota, Bacteroidota, Verrucomicrobiota, Myxococcota,*

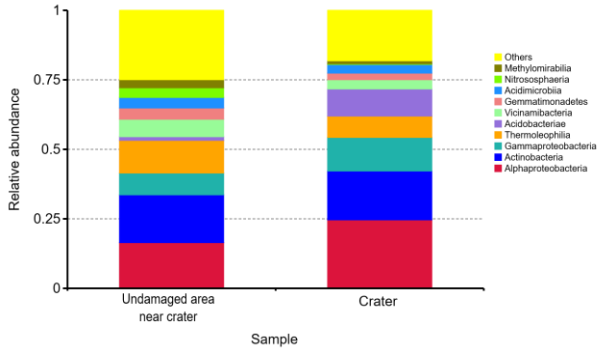
*Firmicutes*. The *Proteobacteria*, *Actinobacteriota*, and *Acidobacteriota* accounted for the largest proportion of identified bacterial OTUs. OTUs from other phyla were fewer in number (Fig. 2).

The relative abundance of OTUs of *Proteobacteria*, *Actinobacteriota*, and *Acidobacteriota* in soil samples from the crater and from the undamaged area differed: the relative abundance of OTUs of *Proteobacteria* and *Acidobacteriota* in soil from the crater was higher than in soil from the nearby area, while that of *Actinobacteriota* was lower (see Table 4).

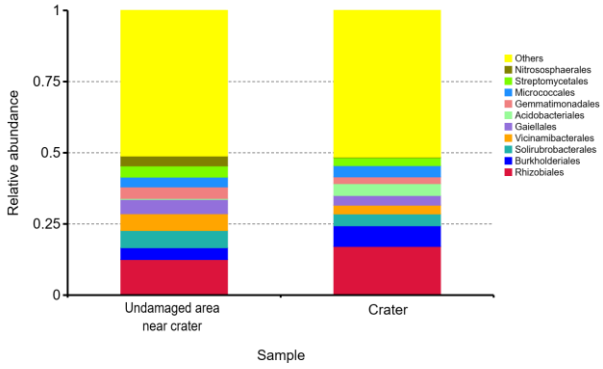
OTUs belonging to the Actinobacteriota accounted for approximately 35% of all transcriptional units in the soil metagenome from the visually undamaged area. In soil from the crater, their relative abundance decreased to nearly 29%. This is the most abundant group of bacteria in the undamaged soil. Among the OTUs of *Actinobacteriota*, 5 classes were identified (the most numerous and diverse being the class *Actinobacteria*), comprising 21 orders: *Solirubrobacterales*, *Gaiellales*, *Micrococcales*, *Streptomycetales*, *Propionibacteriales*, *Frankiales*, *Corynebacteriales*, *Pseudonocardiales*, IMCC26256, *Micromonosporales*, *Microtrichales*, *Streptosporangiales*, *Actinomarinales*, *Kineosporiales*, FFCH16263, *Rubrobacterales*, *Catenulisporales*, 0319-7L14, *Acidimicrobiales*, *Euzebyales*, KIST-JJY010 (Table 8 lists 14 orders). The most numerous order was *Solirubrobacterales*. The relative abundance of OTUs of *Solirubrobacterales* in soil from the crater was lower than in soil from the undisturbed area (6.06% and 4.17%, respectively). Slightly smaller in relative abundance was order *Gaiellales*. The relative abundance of OTUs of *Gaiellales* was also lower in the soil from the crater. *Euzebyales* and *KIST-JJY010* were the least abundant (relative abundance less than 0.002%) in the soil from the undamaged area, and were not detected in the soil from the crater.



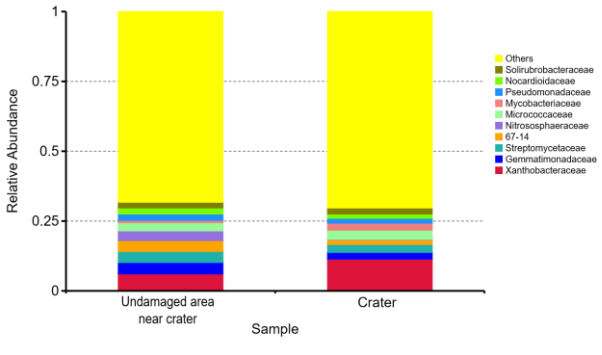

a



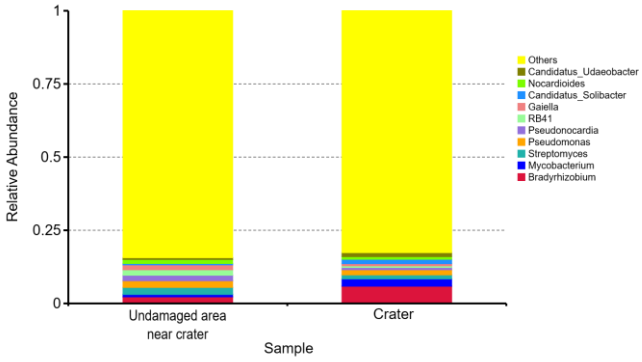

b




c




d




e

**Figure 2.** The relative abundances of the top 10 prokaryotic taxa at the phylum (a), class (b), order (c), family (d), and genus (e) levels in the metagenomes of the studied soils

During the metagenomic analysis of the studied soils at the family level, it was found that in the soil from the undamaged area, the most abundant families were *Streptomycetaceae* (relative abundance 3.94%), *67-14* – unclassified OTUs belonging to *Solirubrobacterales* (relative abundance 3.90%), *Micrococcaceae* (relative abundance 2.94%), *Nocardioidaceae* (relative abundance 2.20%), *Solirubrobacteraceae* (relative abundance 2.02%), and *Pseudonocardiaceae* (relative abundance 2.04%). In soil from the crater, the relative abundance of *Streptomycetaceae*, *67–14* and *Nocardioidaceae* decreased, while that of *Micrococcaceae* and *Solirubrobacteraceae* increased slightly; therefore, the most abundant families were *Micrococcaceae* (relative abundance 3.32%), *Streptomycetaceae* (relative abundance 2.82%), *Mycobacteriaceae* (relative abundance 2.49%), and *Solirubrobacteraceae* (relative abundance 2.18%).

**Table 8**

Relative abundance of *Actinobacteriota* OTUs in soil samples from the crater and from a visually undamaged area within the military training ground

| Class | Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|---|
| | | | | Near crater | Crater |
| *Actinobacteria* | *Corynebacte-riales* | *Mycobacteriaceae* | *Mycobacterium* | 0.81 | 2.49 |
| | | *Nocardiaceae* | *Rhodococcus* | 0.02 | 0.07 |
| | | | *Smaragdicoccus* | 0.005 | 0.02 |
| | *Streptomycetale* | *Streptomycetaceae* | *Streptomyces* | 2.41 | 1.37 |
| | *Pseudonocar-diales* | *Pseudonocardiaceae* | *Pseudonocardia* | 1.90 | 0.77 |
| | | | *Crossiella* | 0.003 | 0.11 |
| | | | *Actinomycetospora* | 0.08 | 0.04 |
| | | | *Amycolatopsis* | 0.02 | 0.06 |
| | | | *Actinophytocola* | 0.009 | 0.02 |
| | | | *Kibdelosporangium* | 0 | 0.001 |
| | *Propionibacte-riales* | *Nocardioidaceae* | *Nocardioides* | 1.37 | 0.91 |
| | | | *Marmoricola* | 0.43 | 0.36 |
| | | | *Kribbella* | 0.14 | 0.15 |
| | | | *Aeromicrobium* | 0.07 | 0.04 |
| | | | *Actinopolymorpha* | 0.02 | 0.001 |
| | | *Nocardiaceae* | *Nocardia* | 0.006 | 0.06 |
| | | *Propionibacteriaceae* | *Microlunatus* | 0.86 | 0.16 |
| | | | *Luteococcus* | 0 | 0.002 |

**Continued from Table 8**

| Class | Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|---|
| | | | | Near crater | Crater |
| *Actinobacteria* | *Frankiales* | *Acidothermaceae* | *Acidothermus* | 0.06 | 1.36 |
| | | *Frankiaceae* | *Jatrophihabitans* | 0.21 | 0.58 |
| | | *Geodermatophilaceae* | *Blastococcus* | 0.06 | 0.27 |
| | | *Geodermatophilaceae* | *Modestobacter* | 0.03 | 0.12 |
| | | | *Geodermatophilus* | 0.02 | 0.03 |
| | | | *Antricoccus* | 0 | 0.004 |
| | | *Nakamurellaceae* | *Nakamurella* | 0.09 | 0.23 |
| | | *Sporichthyaceae* | *Candidatus_Planktophila* | 0 | 0.07 |
| | | | *Sporichthya* | 0.007 | 0.02 |
| | *Micromonosporales* | *Micromonosporaceae* | *Luedemannella* | 0.40 | 0.79 |
| | | | *Catellatospora* | 0.28 | 0.21 |
| | | | *Dactylosporangium* | 0.16 | 0.11 |
| | | | *Virgisporangium* | 0.11 | 0.05 |
| | | | *Rhizocola* | 0 | 0.03 |
| | | | *Actinoplanes* | 0.03 | 0.02 |
| | | | *Allocatelliglobosispora* | 0.02 | 0.02 |
| | *Streptosporangiales* | *Streptosporangiaceae* | *Streptosporangium* | 0.21 | 0.09 |
| | | | *Sphaerisporangium* | 0.16 | 0.04 |
| | | | *Nonomuraea* | 0.09 | 0.05 |
| | | | *Acrocarpospora* | 0.05 | 0.02 |
| | | *Thermomonosporaceae* | *Actinoallomurus* | 0.20 | 0.17 |
| | | | *Actinocorallia* | 0.13 | 0.02 |
| | | | *Actinomadura* | 0.04 | 0.05 |
| | | *unidentified* | *Motilibacter* | 0 | 0.0006 |
| | *Micrococcales* | *Microbacteriaceae* | *Galbitalea* | 0.05 | 0.14 |
| | | | *Agromyces* | 0.09 | 0.03 |
| | | | *Microbacterium* | 0.02 | 0.05 |
| | | | *Leucobacter* | 0 | 0.01 |
| | | | *Leifsonia* | 0.001 | 0.003 |
| | | *Intrasporangiaceae* | *Terrabacter* | 0.09 | 0.06 |
| | | *Cellulomonadaceae* | *Cellulomonas* | 0.01 | 0.07 |
| | | *Micrococcaceae* | *Arthrobacter* | 0 | 0.01 |
| | | | *Kocuria* | 0.01 | 0.002 |
| | | *Dermacoccaceae* | *Flexivirga* | 0.0006 | 0 |
| | *Catenulisporales* | *Catenulisporaceae* | *Catenulispora* | 0.08 | 0.06 |
| | | *Actinospicaceae* | *Actinospica* | 0.007 | 0.01 |
| | *Kineosporiales* | *Kineosporiaceae* | *Angustibacter* | 0.02 | 0.03 |
| *Thermoleophilia* | *Gaiellales* | *Gaiellaceae* | *Gaiella* | 1.68 | 0.74 |
| | *Solirubrobacterales* | *Solirubrobacteraceae* | *Solirubrobacter* | 1.04 | 0.53 |
| | | | *Conexibacter* | 0.81 | 0.94 |
| | | | *Ilumatobacter* | 0.003 | 0.002 |
| | | *Iamiaceae* | *Iamia* | 0.16 | 0.16 |
| | *IMCC26256* | *unidentified_IMCC26256* | *unidentified_IMCC26256* | 0 | 0.03 |
| | *Actinomarinales* | *unidentified* | *unidentified* | 0.003 | 0 |
| *Rubrobacteria* | *Rubrobacterales* | *Rubrobacteriaceae* | *Rubrobacter* | 0.14 | 0.008 |

**Table 9**

Relative abundance of *Alphaproteobacteria* OTUs in soil samples from the crater and from a visually undamaged area within the military training ground

| Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|
| | | | Near crater | Crater |
| *Rhizobiales* | *Xanthobacteraceae* | *Bradyrhizobium* | 2.27 | 5.91 |
| | | *Rhodoplanes* | 0.95 | 0.72 |
| | | *unidentified* | 0.006 | 0.06 |
| | | *Afipia* | 0.01 | 0.05 |
| | | *Pseudolabrys* | 0.01 | 0.03 |
| | | *Starkeya* | 0.02 | 0.007 |
| | *Hyphomicrobiaceae* | *Pedomicrobium* | 1.25 | 0.51 |
| | | *Hyphomicrobium* | 0.23 | 0.02 |
| | *Beijerinckiaceae* | *Methylocapsa* | 0.13 | 0.92 |
| | | *Microvirga* | 0.46 | 0.39 |
| | | *Roseiarcus* | 0.002 | 0.23 |
| | | *Bosea* | 0.009 | 0.05 |
| | | *Methylobacterium-Methylorubrum* | 0.02 | 0.04 |
| | | *FFCH5858* | 0.04 | 0.02 |
| | | *Psychroglaciecola* | 0.005 | 0.008 |
| | | *1174-901-12* | 0.001 | 0.0006 |
| | *Rhizobiaceae* | *Mesorhizobium* | 0.23 | 0.64 |
| | | *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* | 0.34 | 0.41 |
| | | *Phyllobacterium* | 0.34 | 0.25 |
| | | *Ensifer* | 0.003 | 0.003 |
| | *unidentified* | *Nordella* | 0.57 | 0.21 |
| | | *Bauldia* | 0.16 | 0.12 |
| | | *Phreatobacter* | 0.006 | 0.001 |
| | *Labraceae* | *Labrys* | 0.10 | 0.38 |
| | *Devosiaceae* | *Devosia* | 0.10 | 0.21 |
| | *Rhodomicrobiaceae* | *Rhodomicrobium* | 0.04 | 0.08 |
| | *Kaistiaceae* | *Kaistia* | 0 | 0.008 |
| | *Pleomorphomonadaceae* | *Chthonobacter* | 0.001 | 0.002 |
| *Reyranellales* | *Reyranellaceae* | *Reyranella* | 0.38 | 1.20 |
| *Sphingomonadales* | *Sphingomonadaceae* | *Sphingomonas* | 0.86 | 0.72 |
| | | *Novosphingobium* | 0.05 | 0.13 |
| | | *Plot4-2H12* | 0.07 | 0.06 |
| | | *Altererythrobacter* | 0.02 | 0.004 |
| | | *Sphingobium* | 0 | 0.003 |
| *unidentified* | *unidentified* | *unidentified* | 0.18 | 0.74 |
| *Tistrellales* | *Geminicoccaceae* | *Candidatus_Alysiosphaera* | 0.22 | 0.14 |
| | | *Geminicoccus* | 0.003 | 0.004 |
| *Caulobacterales* | *Caulobacteraceae* | *Phenylobacterium* | 0.09 | 0.22 |
| | | *Caulobacter* | 0.02 | 0.05 |
| | | *Asticcacaulis* | 0.004 | 0.02 |
| | | *Brevundimonas* | 0.002 | 0.004 |
| | *Hyphomonadaceae* | *SWB02* | 0.07 | 0.09 |
| | | *Hirschia* | 0.04 | 0.05 |

**Continued from Table 9**

| Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|
| | | | Near crater | Crater |
| *Acetobacterales* | *Acetobacteraceae* | *Rhodovastum* | 0.01 | 0.19 |
| | | *Craurococcus-Caldovatus* | 0.04 | 0.005 |
| | | *Acidicaldus* | 0 | 0.02 |
| | | *Roseomonas* | 0 | 0.004 |
| | | *Acidiphilium* | 0.002 | 0.003 |
| *Dongiales* | *Dongiaceae* | *Dongia* | 0.11 | 0.10 |
| *Azospirillales* | *Azospirillaceae* | *Skermanella* | 0.08 | 0.09 |
| | *Inquilinaceae* | *Inquilinus* | 0.06 | 0.04 |
| *Rhodobacterales* | *Rhodobacteraceae* | *Amaricoccus* | 0.04 | 0.02 |
| | | *Rubellimicrobium* | 0.003 | 0.007 |
| | | *Cereibacter* | 0.002 | 0.007 |
| | | *Rhodobacter* | 0.006 | 0.001 |
| *Paracaedibacterales* | *Paracaedibacteraceae* | *Candidatus_Paracaedibacter* | 0.003 | 0.01 |
| | | *Candidatus_Captivus* | 0.0006 | 0.001 |
| *Rickettsiales* | *Rickettsiaceae* | *Candidatus_Megaira* | 0.01 | 0.0006 |
| | *Mitochondria* | *unidentified* | 0.0006 | 0.001 |

**Table 10**

Relative abundance of *Gammaproteobacteria* OTUs in soil samples from the crater and from a visually undamaged area within the military training ground

| Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|
| | | | Near crater | Crater |
| *Pseudomonadales* | *Pseudomonadaceae* | *Pseudomonas* | 2.26 | 1.75 |
| | *Cellvibrionaceae* | *Cellvibrio* | 0 | 0.03 |
| | *Moraxellaceae* | *Acinetobacter* | 0.006 | 0 |
| | | *Alkanindiges* | 0 | 0.002 |
| | *Pseudohongiellaceae* | *BIyi10* | 0 | 0.006 |
| | *Halomonadaceae* | *Halomonas* | 0 | 0.0006 |
| *Burkholderiales* | *Oxalobacteraceae* | *Massilia* | 0.58 | 1.09 |
| | | *Pseudoduganella* | 0.17 | 0.18 |
| | | *Duganella* | 0.10 | 0.09 |
| | | *Noviherbaspirillum* | 0.02 | 0.09 |
| | | *Undibacterium* | 0.004 | 0.02 |
| | *Burkholderiaceae* | *BCP-group* | 0.08 | 0.94 |
| | | *Cupriavidus* | 0.005 | 0.19 |
| | | *Limnobacter* | 0.003 | 0.01 |
| | | *Lautropia* | 0.007 | 0.01 |
| | *Nitrosomonadaceae* | *MND1* | 0.40 | 0.19 |
| | | *Ellin6067* | 0.11 | 0.38 |
| | | *IS-44* | 0.08 | 0.09 |
| | | *Nitrosospira* | 0.005 | 0.02 |
| | | *Nitrosomonas* | 0 | 0.006 |
| | *Comamonadaceae* | *Piscinibacter* | 0.20 | 0.28 |
| | | *Rhizobacter* | 0.11 | 0.27 |
| | | *Rhodoferax* | 0.10 | 0.20 |
| | | *Polaromonas* | 0.04 | 0.08 |
| | | *Variovorax* | 0.002 | 0.08 |

**Continued from Table 10**

| Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|
| | | | Near crater | Crater |
| *Burkholderiales* | *Comamonadaceae* | *Ramlibacter* | 0.04 | 0.05 |
| | | *Paucibacter* | 0.02 | 0.04 |
| | | *Pelomonas* | 0.005 | 0.03 |
| | | *Azohydromonas* | 0.01 | 0.02 |
| | | *Ideonella* | 0.01 | 0.02 |
| | | *Caenimonas* | 0.01 | 0.01 |
| | | *Comamonas* | 0 | 0.006 |
| | *Chromobacteriaceae* | *Vogesella* | 0.04 | 0 |
| | *Rhodocyclaceae* | *Dechloromonas* | 0.01 | 0.001 |
| | | *Uliginosibacterium* | 0.005 | 0.008 |
| | | *Ferribacterium* | 0.001 | 0.006 |
| | | *Georgfuchsia* | 0.0006 | 0.006 |
| | | *Sulfuritalea* | 0.003 | 0.0006 |
| | *Chitinimonadaceae* | *Chitinimonas* | 0.01 | 0.01 |
| | *Methylophilaceae* | *Methylotenera* | 0.002 | 0.01 |
| | *Alcaligenaceae* | *Parvibium* | 0.007 | 0 |
| | *Sutterellaceae* | *AAP99* | 0.0006 | 0.005 |
| | *Gallionellaceae* | *Gallionella* | 0 | 0.004 |
| *Steroidobacterales* | *Steroidobacteraceae* | *Steroidobacter* | 0.25 | 0.16 |
| | *Woeseiaceae* | *JTB255_marine_benthic_group* | 0.02 | 0 |
| *Nitrosococcales* | *Nitrosococcaceae* | *wb1-P19* | 0.20 | 0.003 |
| *Xanthomonadales* | *Xanthomonadaceae* | *Arenimonas* | 0.06 | 0.17 |
| | | *Lysobacter* | 0.06 | 0.07 |
| | | *Pseudoxanthomonas* | 0.006 | 0.01 |
| | | *Stenotrophomonas* | 0 | 0.004 |
| | *Rhodanobacteraceae* | *Dyella* | 0.005 | 0.10 |
| | | *Dokdonella* | 0.03 | 0.07 |
| | | *Ahniella* | 0.003 | 0.02 |
| | | *Rudaea* | 0 | 0.01 |
| | | *Luteibacter* | 0.003 | 0.01 |
| *Salinisphaerales* | *Solimonadaceae* | *Polycyclovorans* | 0.02 | 0.05 |
| | | *Nevskia* | 0.001 | 0.02 |
| | | *Panacagrimonas* | 0.006 | 0.01 |
| *Legionellales* | *Legionellaceae* | *Legionella* | 0.03 | 0.03 |
| *Diplorickettsiales* | *Diplorickettsiaceae* | *Aquicella* | 0.02 | 0.03 |
| *Coxiellales* | *Coxiellaceae* | *Coxiella* | 0.02 | 0.02 |
| *Acidiferrobacterales* | *Acidiferrobacteraceae* | *Sulfurifustis* | 0.02 | 0.01 |

A total of 67 genera were identified within the *Actinobacteriota* OTUs. In both the soil from the undamaged area and the soil from the crater, the most abundant genus was *Mycobacterium*. The relative abundance of *Mycobacterium* OTUs in the soil from the crater was three times higher than in the soil from the undamaged area. Other OTUs were also identified in the soil from the crater that were not found in the soil from the undamaged area, including: Candidatus *Planktophila*, *Rhizocola*, *Leucobacter*, *Arthrobacter*, *Antricoccus*, *Luteococcus*, *Kibdelosporangium*, *Motilibacter*. Along with an increase in the relative abundance of OTUs from certain genera in the soil from the crater, we found genera whose relative

abundance of OTUs was lower compared to the soil from the undamaged area. These families included *Streptomyces, Pseudonocardia, Microlunatus, Blastococcus, Modestobacter, Sphaerisporangium* etc.

OTUs of *Proteobacteria* were identified as *Alphaproteobacteria* і *Gammaproteobacteria*. OTUs of *Alphaproteobacteria* were distributed among 19 orders and 41 families. The largest amount of OTUs of *Alphaproteobacteria* was identified as *Rhizobiales* (12.52% of identified OTUs in soil from the undamaged area and 17.11% in soil from the crater). At the family level, OTUs of *Xanthobacteraceae* were dominant. The relative abundance of OTUs of *Xanthobacteraceae* was nearly twice as high in the soil sample from the crater. In both soil samples, the most abundant genus was *Bradyrhizobium*. The relative abundance of *Bradyrhizobium* OTUs was 2.6 times higher in the soil from the crater. Among the smallest families were *Rickettsiaceae, Kaistiaceae, Micavibrionaceae, Sneathiellaceae, Magnetospirillaceae, Pleomorphomonadaceae*. The relative abundance of OTUs belonging to these families did not exceed 0.008%. A total of 60 genera were identified (Table 9).

The OTUs of *Gammaproteobacteria* belonged to 22 orders. Most of the identified OTUs belonged to the *Burkholderiales*. The relative abundance of OTUs belonging to this order was 4.12% in soil from the undisturbed area and 7.25% in soil from the crater. This order was also the most diverse among the *Gammaproteobacteria*, as in both soil samples, *Burkholderiales* OTUs were distributed across 32 families (Table 10 lists only the 11 most abundant) and 40 genera. The majority of OTUs were identified as *Oxalobacteraceae*, with Massilia as the dominant genus. The abundance of OTUs at the genus level differed predominantly between soil samples from the crater and the nearby area; however, the most striking changes in the relative abundance of OTUs were observed in the genera *Cellvibrio, Alkanindiges, BIyi10, Halomonas, Nitrosomonas, Comamonas, Gallionella, Stenotrophomonas,* and *Rudaea,* which were not detected in soil samples from the undamaged area but were identified in soil from the crater. Conversely, no OTUs of *Acinetobacter, Vogesella, Parvibium*, or *JTB255_marine_benthic_group* were detected in the soil from the crater.

The OTUs of *Acidobacteriota* were distributed across 12 classes. Five of them are classified as *Acidobacteriae, Vicinamibacteria, Blastocatellia, Holophagae* and *Thermoanaerobaculia*, and the remaining classes are designated as Subgroup 22, Subgroup 5, Subgroup 25, Subgroup 11, Subgroup 18, AT-s3-28, Subgroup 19, and Subgroup 20. The majority of *Acidobacteriota* OTUs belonged to the *Vicinamibacteria*. In soil from the undamaged area, the OTU count for *Vicinamibacteria* was 5.88%, while in soil from the crater, it was 3.07%. The relative abundance of OTUs belonging to *Blastocatellia,* Subgroup 22, Subgroup 5, Subgroup 25, Subgroup 11, Subgroup 18, and AT-s3-2 in the soil from the crater was also 2–10 times lower. No traces of OTUs of Subgroup_19 і Subgroup_20 were found in the soil of the crater. The relative abundance of *Acidobacteria* OTUs in the soil from the crater increased significantly—from 1.30% in the soil from the undamaged area to 9.76% in the soil from the crater. Although at a slower rate, the relative abundance of *Holophagae* is increasing in the damaged soil (Table 11).

The identified OTUs of *Acidobacteriota* belonged to 21 orders, among which *Vicinamibacterales, Acidobacteriales, Pyrinomonadales, Bryobacterales, Solibacterales, Thermoanaerobaculales, Blastocatellales, Holophagales*were classified. The unclassified OTUs belonged to *Blastocatellia* (11–24, DS-100), *Acidobacteriae* (Subgroup 2, Subgroup_12, Subgroup_13, PAUC26f, Subgroup 15, Elev-16S-1166, AKIW659), *Vicinamibacteria* (Subgroup9, Subgroup 17), *Holophagae* (Subgroup 7).

Most of the OTUs in *Acidobacteriota* belonged to the family *Pyrinomonadaceae* (relative abundance of 1.87%). In soil from the undamaged area, the relative abundance of OTUs of *Pyrinomonadaceae* was 3.3 times higher than in soil from the crater. The *Vicinamibacteraceae* family was slightly less abundant in the soil from the undamaged area (relative abundance 1.73%). In the soil from the crater, the relative

abundance of *Pyrinomonadaceae* OTUs was 4.25 times lower. An increase in the relative abundance of *Solibacteraceae*, *Bryobacteraceae*, *Acidobacteriaceae*_(Subgroup_1), *Blastocatellaceae* was observed in the soil from the crater.

**Table 11**

Relative abundance of *Acidobacteriota* OTUs in soil samples from the crater and from a visually undamaged area within the military training ground

| Class | Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|---|
| | | | | Near crater | Crater |
| *Blastocatellia* | *Pyrinomonadales* | *Pyrinomonadaceae* | *RB41* | 1.87 | 0.57 |
| | *Blastocatellales* | *Blastocatellaceae* | *Aridibacter* | 0.006 | 0.03 |
| | | | *Blastocatella* | 0.0006 | 0.003 |
| | *11-24* | *unidentified* | *unidentified* | 0 | 0.004 |
| | *unidentified_ Acidobacteriae* | *unidentified* | *Paludibaculum* | 0.009 | 0.01 |
| *Acidobacteriae* | *Solibacterales* | *Solibacteraceae* | *Candidatus_Solibacter* | 0.46 | 1.50 |
| | *Bryobacterales* | *Bryobacteraceae* | *Bryobacter* | 0.20 | 0.11 |
| | *Acidobacteriales* | *Acidobacteriaceae_(Subgroup_1)* | *Acidipila-Silvibacterium* | 0 | 0.27 |
| | | | *Granulicella* | 0 | 0.19 |
| | | | *Edaphobacter* | 0.01 | 0.04 |
| | | | *Occallatibacter* | 0.001 | 0.03 |
| | | | *Terriglobus* | 0.006 | 0.02 |
| | | *unidentified* | *unidentified* | 0 | 0.01 |
| | | *Koribacteraceae* | *Candidatus_Koribacter* | 0.01 | 0.09 |
| | | | *Acidicapsa* | 0 | |
| *Thermoanaerobaculia* | *Thermoanaero-baculales* | *Thermoanaerobaculaceae* | *Subgroup_10* | 0.21 | 0.22 |
| *Vicinamibacteria* | *Vicinamibactera-les* | *Vicinamibacteraceae* | *Vicinamibacter* | 0.08 | 0.06 |
| | | | *Luteitalea* | 0.008 | 0.002 |
| | | *unidentified* | *unidentified* | 0.05 | 0.003 |
| *Holophagae* | *Holophagales* | *Holophagaceae* | *Geothrix* | 0 | 0.005 |

The identified OTUs of *Acidobacteriota* were distributed across 20 genera. In the soil from the undamaged area, the most abundant OTUs (relative abundance 1.87%) were those of RB41 (unclassified Acidobacteriota, published in NCBI Taxonomy as Acidobacteria bacterium RB41). In the soil from the crater, their abundance was three times lower. In the soil from the crater, the relative abundance of Candidatus_*Solibacter*, Bryobacter, Candidatus_*Koribacter*, *Edaphobacter*, *Occallatibacter*, *Aridibacter*, *Terriglobus*, *Paludibaculum* increased. In addition, the soil from the crater was found to contain *Acidipila-Silvibacterium*, *Granulicella*, *Acidicapsa*, *Geothrix*, and unidentified_11-24 OTUs that were not detected in the soil from the undamaged area.

*Chloroflexi* OTUs accounted for 7.47% of all detected and identified transcriptional units. The relative abundance of *Chloroflexi* OTUs in soil from the crater was 1.5 times lower than in soil from the area without visible damage. The microorganisms of the *Chloroflexi* phylum were mainly represented by the unclassified (KD4-96, AD3, TK10, Gitt-GS-136, JG30-KF-CM66, OLB14, P2-11E, SHA-26) and classified (*Chloroflexia*, *Dehalococcoidia*,*Ktedonobacteria*, *Anaerolineae*) classes (Table 12). The class *Chloroflexia*

is represented by three orders (*Chloroflexales*, *Thermomicrobiales*, Elev-1554), *Dehalococcoidia* by two (S085, SAR202_clade), *Ktedonobacteria*—three (*Ktedonobacterales*, B12-WMSP1, C0119), and *Anaerolineae*—five (*Anaerolineales*, *Ardenticatenales*, *Caldilineales*, RBG-13-54-9, SBR1031). Among the 16 orders belonging to *Chloroflexi*, the relative abundance of OTUs in 10 orders decreased. Two orders (*Ardenticatenales* and SAR202_clade) were not detected in soil from the crater; however, OTUs of two currently unclassified orders, B12-WMSP1 and Elev-1554, were identified and were absent from soil from the undamaged area. An increase in the relative abundance of OTUs in the soil from the crater was observed for the *Ktedonobacterales* and *Anaerolineales.* At the family level, a similar trend in changes in the relative abundance of OTUs was observed in soil from the crater and the undamaged area.

**Table 12**

Relative abundance of *Chloroflexi and Gemmatimonadota* OTUs in soil samples from the crater and from a visually undamaged area within the military training ground

| Phylum | Class | Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|---|---|
| | | | | | Near crater | Crater |
| *Chloroflexi* | *Ktedonobacteria* | *Ktedonobacterales* | *JG30-KF-AS9* | *unidentified* | 0 | 0.12 |
| | | | *Ktedonobacteraceae* | *1921-3* | 0 | 0.08 |
| | | | | *1959-1* | 0.003 | 0.06 |
| | | | | *Ktedonobacter* | 0.003 | 0.03 |
| | | | | *JG30a-KF-32* | 0.002 | 0.02 |
| | | | | *1921-2* | 0 | 0.01 |
| | | | | *HSB_OF53-F07* | 0 | 0.009 |
| | | | | *FCPS473* | 0 | 0.006 |
| | | | | *Thermosporothrix* | 0 | 0.004 |
| | *Chloroflexia* | *Chloroflexales* | *Chloroflexaceae* | *FFCH7168* | 0.02 | 0.005 |
| | | | *Herpetosiphonaceae* | *Herpetosiphon* | 0.01 | 0.009 |
| | | *Thermomicrobiales* | *JG30-KF-CM45* | *unidentified* | 0.003 | 0.008 |
| | | | *Thermomicrobiaceae* | *Nitrolancea* | 0 | 0.008 |
| | *Anaerolineae* | *Caldilineales* | *Caldilineaceae* | *Litorilinea* | 0.01 | 0 |
| | *KD4-96* | *unidentified* | *unidentified* | *unidentified* | 0.01 | 0.006 |
| | *TK10* | *unidentified* | *unidentified* | *unidentified* | 0.002 | 0.0006 |
| *Gemmatimonadota* | *Gemmatimonadetes* | *Gemmatimonadales* | *Gemmatimonadaceae* | *Gemmatimonas* | 0.22 | 0.52 |
| | | | | *unidentified* | 0.12 | 0.05 |
| | | | | *Roseisolibacter* | 0.006 | 0.006 |
| | *Longimicrobia* | *Longimicrobiales* | *Longimicrobiaceae* | *YC-ZSS-LKJ147* | 0.02 | 0.03 |
| | | | | *Longimicrobium* | 0 | 0.001 |

The relative abundance of *Gemmatimonadota* OTUs in soil from the crater was 1.7 times lower than in soil from the undamaged area (see Table 6). Like the *Chloroflexi*, the *Gemmatimonadota* are represented primarily by unclassified classes (AKAU4049, S0134_terrestrial_group, BD2-11_terrestrial_group). Only two classes, *Gemmatimonadetes* and *Longimicrobia*, have been validly described. In the soil from the crater, the relative abundance of *Gemmatimonadetes*, S0134_terrestrial_group, and BD2-11_terrestrial_group was lower than in the soil from the undamaged area, OTUs of the AKAU4049 group were not detected, and the relative abundance of *Longimicrobia* increased twofold. At lower taxonomic levels, the detected OTUs belonged to the orders *Gemmatimonadales* and *Longimicrobiales*, and the

families *Gemmatimonadaceae* and *Longimicrobiaceae*, within which the genera *Gemmatimonas*, *Roseisolibacter*, *Longimicrobium*, as well as unidentified_*Gemmatimonadaceae* and YC-ZSS-LKJ147 were identified. Unlike other genera of the *Gemmatimonadota*, *Longimicrobium* OTUs have been found only in soil from the crater (Table 12).

In soil samples from a visually undamaged area, OTUs of *Bacteroidota* were identified as *Bacteroidia* (0.94%), *Kapabacteria* (0.004%), *Kryptonia* (0.006%), *Ignavibacteria* (0.005%), and *Rhodothermia* (0.001%). In the soil from the crater, the relative abundance of *Bacteroidia* OTUs increased 2.5-fold, that of *Kapabacteria* 4-fold, while the relative abundance of *Ignavibacteria* OTUs decreased 3.5-fold. No *Kryptonia* or *Rhodothermia* OTUs were detected in the soil from the crater (Table 13).

Five orders have been identified within the *Bacteroidia*: *Chitinophagales*, *Sphingobacteriales*, *Cytophagales*, *Flavobacteriales*, and *Bacteroidales*. The most abundant order in the soil from the undamaged area was *Chitinophagales* (0.63%). In the soil from the crater, the relative abundance of OTUs of *Chitinophagales* increased threefold. The relative abundance of OTUs of *Sphingobacteriales*, *Cytophagales*, and *Bacteroidales* also increased in this soil. In both samples studied, *Kapabacteria* were represented by *Kapabacteriales*, *Kryptonia* by *Kryptoniales*, *Ignavibacteria* by SJA-28, and *Rhodothermia* by *Rhodothermales*. At lower taxonomic levels, only *Bacteroidia* OTUs were identified.

At the family level, 22 taxonomic units were identified (*Chitinophagaceae*, *Microscillaceae*, *Flavobacteriaceae*, *Sphingobacteriaceae*, *Saprospiraceae*, *Cytophagaceae*, *Spirosomaceae*, *Crocinitomicaceae*, *Hymenobacteraceae*, *Rhodothermaceae*, *Weeksellaceae*, env.OPS_17, AKYH767, 37-13, NS9_marine_group, FFCH9454, KD3-93, BSV26, NS11-12_marine_group, S15-21, *Prevotellaceae*, LiUU-11-161). In both soil samples, the most abundant family was *Chitinophagaceae* (relative abundance of OTUs in soil from the visually undamaged area was 0.59%, and 1.67% in soil from the crater). In addition to the *Chitinophagaceae*, the soil from the crater showed an increase in the abundance of 10 other families (*Microscillaceae*, env.OPS_17, *Flavobacteriaceae*, *Sphingobacteriaceae*, AKYH767, *Saprospiraceae*, *Cytophagaceae*, *Spirosomaceae*, 37-13, *Hymenobacteraceae*). OTUs from the two families Prevotellaceae and S15-21 were detected only in soil from the crater. A decrease in the relative abundance of OTUs in the disturbed soil was observed for NS9_marine_group, FFCH9454, *Crocinitomicaceae*, KD3-93, NS11-12_marine_group, and OTUs of *Rhodothermaceae*, *Weeksellaceae*, BSV26, and LiUU-11-161 were not found. At the genus level, OTUs of Bacteroidia were distributed among 34 genera. Changes in their relative abundance are shown in Table 13.

**Table 13**

Relative abundance of *Bacteroidota* OTUs in soil samples from the crater and from a visually undamaged area within the military training ground

| Class | Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|---|
| | | | | Near crater | Crater |
| *Bacteroidia* | *Chitinophagales* | *Chitinophagaceae* | *Ferruginibacter* | 0.05 | 0.21 |
| | | | *Puia* | 0.02 | 0.17 |
| | | | *Terrimonas* | 0.06 | 0.17 |
| | | | *Edaphobaculum* | 0.01 | 0.08 |
| | | | *Flavisolibacter* | 0.009 | 0.05 |
| | | | *Flavitalea* | 0.02 | 0.04 |
| | | | *Parafilimonas* | 0.009 | 0.04 |
| | | | *Niastella* | 0.03 | 0.03 |

**Continued from Table 13**

| Class | Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|---|
| | | | | Near crater | Crater |
| *Bacteroidia* | *Chitinophagales* | *Chitinophagaceae* | *Sediminibacterium* | 0.0006 | 0.03 |
| | | | *Segetibacter* | 0.005 | 0.03 |
| | | | *Aurantisolimonas* | 0.01 | 0.03 |
| | | | *Chitinophaga* | 0.006 | 0.02 |
| | | | *unidentified* | 0.005 | 0.02 |
| | | | *Heliimonas* | 0.001 | 0.01 |
| | | | *UTBCD1* | 0 | 0.01 |
| | | | *Pseudoflavitalea* | 0 | 0.005 |
| | | *Saprospiraceae* | *unidentified* | 0 | 0.002 |
| | *Flavobacteriales* | *Flavobacteriaceae* | *Flavobacterium* | 0.09 | 0.11 |
| | | *Crocinitomicaceae* | *Fluviicola* | 0.008 | 0.006 |
| | | *Weeksellaceae* | *Chryseobacterium* | 0.0006 | 0 |
| | *Sphingobacteriales* | *Sphingobacteriaceae* | *Mucilaginibacter* | 0.005 | 0.06 |
| | | | *Pedobacter* | 0.0006 | 0.004 |
| | | | *Solitalea* | 0.0006 | 0 |
| | *Cytophagales* | *Microscillaceae* | *Chryseolinea* | 0.008 | 0.05 |
| | | | *Ohtaekwangia* | 0.003 | 0.01 |
| | | | *Hassallia* | 0.0006 | 0.005 |
| | | *Cytophagaceae* | *Sporocytophaga* | 0.002 | 0.008 |
| | | | *Cytophaga* | 0 | 0.006 |
| | | | *Rhodocytophaga* | 0 | 0.002 |
| | | *Hymenobacteraceae* | *Hymenobacter* | 0.002 | 0.006 |
| | | | *Adhaeribacter* | 0.002 | 0.0006 |
| | | *Spirosomaceae* | *Dyadobacter* | 0.007 | 0.01 |
| | | | *Fibrella* | 0 | 0.001 |
| | *Bacteroidales* | *Prevotellaceae* | *Prevotellaceae_NK3B31_group* | 0 | 0.004 |

The relative abundance of *Verrucomicrobiota* OTUs in soil from the crater did not increase significantly compared to soil from the undisturbed area. Similar changes in the relative abundance of OTUs were also observed for *Myxococcota* (see Table 6). Changes in the relative abundance of genera belonging to the *Verrucomicrobiota* and *Myxococcota* in soil samples from the crater and visually undamaged area are shown in Table 14.

**Table 14**

Relative abundance of *Verrucomicrobiota* and *Myxococcota* OTUs in soil samples from the crater and from a visually undamaged area within the military training ground

| Phylum | Class | Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|---|---|
| | | | | | Near crater | Crater |
| *Verrucomicrobiota* | *Verrucomicrobiae* | *Chthoniobacterales* | *Chthoniobacteraceae* | *Candidatus_ Udaeobacter* | 0.61 | 1.36 |
| | | | | *Chthoniobacter* | 0.15 | 0.10 |
| | | | *Xiphinematobacteraceae* | *Candidatus_ Xiphinematobacter* | 0.12 | 0.27 |
| | | | *Terrimicrobiaceae* | *Terrimicrobium* | 0.003 | 0.008 |

**Continued from Table 14**

| Phylum | Class | Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|---|---|
| | | | | | Near crater | Crater |
| *Verrucomicrobiota* | *Verrucomic-robiae* | *Pedosphaerales* | *Pedosphaeraceae* | *ADurb.Bin063-1* | 0.05 | 0.03 |
| | | | | *DEV114* | 0.01 | 0 |
| | | | | *Ellin517* | 0.009 | 0.006 |
| | | | | *Ellin516* | 0.008 | 0.0006 |
| | | | | *Pedosphaera* | 0 | 0.0006 |
| | | *Opitutales* | *Opitutaceae* | *Opitutus* | 0.01 | 0.02 |
| | | | | *Lacunisphaera* | 0.001 | 0.007 |
| | | *Verrucomicro-biales* | *Verrucomicrobiaceae* | *Roseimicrobium* | 0.009 | 0.01 |
| | | | *Rubritaleaceae* | *Luteolibacter* | 0.008 | 0.006 |
| | *Chlamydiae* | *Chlamydiales* | *Parachlamydiaceae* | *Neochlamydia* | 0.01 | 0.004 |
| | | | | *Candidatus_Protochlamydia* | 0.004 | 0.005 |
| | *Omnitrophia* | *Omnitrophales* | *Omnitrophaceae* | *Candidatus_Omnitrophus* | 0.002 | 0.001 |
| *Myxococcota* | *Polyangia* | *Haliangiales* | *Haliangiaceae* | *Haliangium* | 0.61 | 0.86 |
| | | *Polyangiales* | *Phaselicystidaceae* | *Phaselicystis* | 0.11 | 0.01 |
| | | | *Polyangiaceae* | *Pajaroellobacter* | 0.10 | 0.05 |
| | | | | *Sorangium* | 0.03 | 0.002 |
| | | | | *Aetherobacter* | 0.02 | 0.004 |
| | | | | *Minicystis* | 0.004 | 0 |
| | | | | *Labilithrix* | 0 | 0.003 |
| | | | *Sandaracinaceae* | *Sandaracinus* | 0.01 | 0.03 |
| | | *Nannocystales* | *Nannocystaceae* | *Nannocystis* | 0.004 | 0.01 |
| | | *Blfdi19* | *unidentified_Blfdi19* | *unidentified_Blfdi19* | 0.002 | 0.003 |
| | *Myxococcia* | *Myxococcales* | *Anaeromyxobacteraceae* | *Anaeromyxobacter* | 0.02 | 0.09 |
| | | | *Myxococcaceae* | *P3OB-42* | 0.02 | 0.02 |
| | | | | *KD3-10* | 0 | 0.002 |

While the relative abundance of *Verrucomicrobiota* and *Myxococcota* OTUs in the soil from the crater increased slightly compared to the control, the relative abundance of *Firmicutes* in these two soil samples showed almost no difference (Table 15). The *Firmicutes* phylum was represented by the classes *Bacilli*, *Clostridia*, *Desulfitobacteriia*, *Limnochordia*, and *Negativicutes*. The most abundant class in soil from the undamaged area was Bacilli (1.1%). The relative abundance of *Negativicutes* did not change significantly in the soil from the crater. No OTUs of *Limnochordia* were detected in the soil from the crater. In contrast, significant changes in relative abundance were observed for *Clostridia*: of the 14 genera identified in soil from the undamaged area, only 2 were identified in soil from the crater. The relative abundance of *Limnochordia* in soil from an undamaged area is 0.005%, and that of *Clostridia* is 0.2%; therefore, the disappearance or decline in the abundance of these classes likely has no significant effect on the change in the total abundance of *Firmicutes.*

**Table 15**

Relative abundance of *Firmicutes* OTUs in soil samples from the crater and from a visually undamaged area within the military training range

| Class | Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|---|
| | | | | Near crater | Crater |
| *Bacilli* | *Paenibacillales* | *Paenibacillaceae* | *Paenibacillus* | 0.47 | 0.63 |
| | | | *Cohnella* | 0.07 | 0.11 |
| | *Bacillales* | *Bacillaceae* | *Bacillus* | 0.47 | 0.32 |

**Continued from Table 15**

| Class | Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|---|
| | | | | Near crater | Crater |
| *Bacilli* | *Bacillales* | *Planococcaceae* | *Paenisporosarcina* | 0.008 | 0.04 |
| | | | *Sporosarcina* | 0.02 | 0.03 |
| | | | *Lysinibacillus* | 0.01 | 0.008 |
| | | | *Domibacillus* | 0.002 | 0.004 |
| | | | *Solibacillus* | 0.003 | 0.002 |
| | *Alicyclobacillales* | *Alicyclobacillaceae* | *Tumebacillus* | 0.03 | 0.04 |
| | *Thermoactinomycetales* | *Thermoactinomycetaceae* | *Shimazuella* | 0.004 | 0.02 |
| | | | *Pasteuria* | 0.004 | 0.005 |
| | *Erysipelotrichales* | *Erysipelotrichaceae* | *Turicibacter* | 0.004 | 0.004 |
| | *Brevibacillales* | *Brevibacillaceae* | *Brevibacillus* | 0.002 | 0.003 |
| *Clostridia* | *Clostridiales* | *Clostridiaceae* | *Clostridium_sensu_stricto_13* | 0.13 | 0.07 |
| | | | *Clostridium_sensu_stricto_2* | 0 | 0.001 |
| | | *Caloramatoraceae* | *Fonticella* | 0.007 | 0 |
| | | *Oxobacteraceae* | *Oxobacter* | 0.002 | 0 |
| | *unidentified* | *Gracilibacteraceae* | *Gracilibacter* | 0.01 | 0 |
| | | | *Lutispora* | 0.003 | 0 |
| | *Eubacteriales* | *Alkalibacteraceae* | *Alkalibacter* | 0.005 | 0 |
| | *Lachnospirales* | *Lachnospiraceae* | *Anaerocolumna* | 0.004 | 0 |
| | | | *Mobilitalea* | 0.004 | 0 |
| | | | *Lachnospiraceae_NK4A136_group* | 0.003 | 0 |
| | | | *Lachnoclostridium* | 0 | 0.002 |
| | *Oscillospirales* | *Hungateiclostridiaceae* | *Ruminiclostridium* | 0.003 | 0 |
| | | | *Anaerobacterium* | 0.002 | 0 |
| | | *Oscillospiraceae* | *Papillibacter* | 0.002 | 0 |
| | *Christensenellales* | *Christensenellaceae* | *Christensenellaceae_R-7_group* | 0.002 | 0 |
| | *Peptostreptococcales-Tissierellales* | *Peptostreptococcaceae* | *Sporacetigenium* | 0.0006 | 0 |
| *Desulfitobacteriia* | *Desulfitobacteriales* | *Desulfitobacteriaceae* | *Desulfosporosinus* | 0.02 | 0.005 |
| *Negativicutes* | *Veillonellales-Selenomonadales* | *Sporomusaceae* | *Pelosinus* | 0 | 0.003 |
| | | | *Anaerosinus* | 0.0006 | 0.001 |

Table 16 shows the distribution of OTUs among genera belonging to phyla whose share of OTUs in the soil metagenome does not exceed 1%. The OTUs of *Methylomirabilota, Latescibacterota, Margulisbacteria, Elusimicrobiota, Zixibacteria* and unclassified *RCP2-54, MBNT15, NB1-j, WPS-2, Dependentiae, SAR324_clade (Marine_group_B), FCPU426* were identified only at the phylum level.

**Table 16**

Relative abundance of OTUs from other phyla in soil samples collected from the crater and from a visually undamaged area within the military training ground

| Phylum | Class | Order | Family | Genus | Genus, relative abundance | |
|---|---|---|---|---|---|---|
| | | | | | Near crater | Crater |
| *Nitrospirota* | *Nitrospiria* | *Nitrospirales* | *Nitrospiraceae* | *Nitrospira* | 0.77 | 0.20 |
| *Desulfobacterota* | *Desulfuromonadia* | *Geobacterales* | *Geobacteraceae* | *Citrifermentans* | 0.01 | 0.004 |
| | *Desulfovibrionia* | *Desulfovibrionales* | *Desulfovibrionaceae* | *Desulfovibrio* | 0.0006 | 0.003 |
| *Armatimonadota* | *Chthonomonadetes* | *Chthonomonadales* | *Chthonomonadaceae* | *Chthonomonas* | 0.01 | 0.09 |
| | *Fimbriimonadia* | *Fimbriimonadales* | *Fimbriimonadaceae* | *Fimbriimonas* | 0.003 | 0 |
| *Bdellovibrionota* | *Bdellovibrionia* | *Bdellovibrionales* | *Bdellovibrionaceae* | *Bdellovibrio* | 0.03 | 0.06 |
| | | | | *OM27_clade* | 0.03 | 0.01 |
| | *Oligoflexia* | *0319-6G20* | *unidentified* | *unidentified* | 0.008 | 0.009 |
| *Entotheonellaeota* | *Entotheonellia* | *Entotheonellales* | *Entotheonellaceae* | *Candidatus_ Entotheonella* | 0.001 | 0 |
| *Patescibacteria* | *Parcubacteria* | *Candidatus_Jorgensenbacteria* | *unidentified* | *unidentified* | 0 | 0.004 |
| | | *Candidatus_ Liptonbacteria* | *unidentified* | *unidentified* | 0.002 | 0 |
| | | *Candidatus_ Zambryskibacteria* | *unidentified* | *unidentified* | 0.002 | 0 |
| | | *Candidatus_ Yanofskybacteria* | *unidentified* | *unidentified* | 0.001 | 0 |
| | *Saccharimonadia* | *Saccharimonadales* | *Saccharimonadaceae* | *TM7a* | 0.0006 | 0.004 |
| | | | *unidentified* | *unidentified* | 0.0006 | 0.004 |
| | *WWE3* | *unidentified_ WWE3* | *unidentified_WWE3* | *unidentified_WWE3* | 0.001 | 0 |
| *Planctomycetota* | *Planctomycetes* | *Isosphaerales* | *Isosphaeraceae* | *Aquisphaera* | 0.0006 | 0.007 |
| | | | | *Singulisphaera* | 0.006 | 0.004 |
| | | | | *Tundrisphaera* | 0.002 | 0 |
| | | *Pirellulales* | *Pirellulaceae* | *Pir4_lineage* | 0.003 | 0.001 |
| | | | | *Pirellula* | 0.002 | 0 |
| | | *Planctomycetales* | *Rubinisphaeraceae* | *SH-PL14* | 0.0006 | 0 |
| | *Phycisphaerae* | *Phycisphaerales* | *Phycisphaeraceae* | *SM1A02* | 0 | 0.002 |
| | | *Tepidisphaerales* | *WD2101_soil_group* | *unidentified* | 0.002 | 0.001 |
| *Cyanobacteria* | *Cyanobacteriia* | *Chloroplast* | *unidentified* | *unidentified* | 0.006 | 0.01 |
| | | *Cyanobacteriales* | *Coleofasciculaceae* | *Microcoleus_ SAG_1449-1a* | 0.001 | 0.002 |
| | | | *Phormidiaceae* | *Tychonema_ CCAP_1459-11B* | 0.0006 | 0.0006 |
| *Fibrobacterota* | *Fibrobacteria* | *Fibrobacterales* | *Fibrobacteraceae* | *possible_ genus_04* | 0.008 | 0.03 |
| | | | | *unidentified* | 0.008 | 0.02 |
| *Sumerlaeota* | *Sumerlaeia* | *Sumerlaeales* | *Sumerlaeaceae* | *Sumerlaea* | 0.03 | 0.03 |
| *Deinococcota* | *Deinococci* | *Deinococcales* | *Deinococcaceae* | *Deinococcus* | 0 | 0.0006 |

A visual analysis of the distribution of microbial populations indicates a significant restructuring of microbial communities: the metagenome of soil from the crater shows a marked dominance of the genera *Mycobacterium*, *Acidothermus*, Candidatus *Solibacter*, and *Bradyrhizobium*. At the same time, the soil metagenome from a visually undamaged area revealed a significantly higher proportion of sequences belonging to the RB41 group, the genera *Nocardioides* and *Gaiella*, and the archaeon *Nitrososphaera* (Fig. 3).

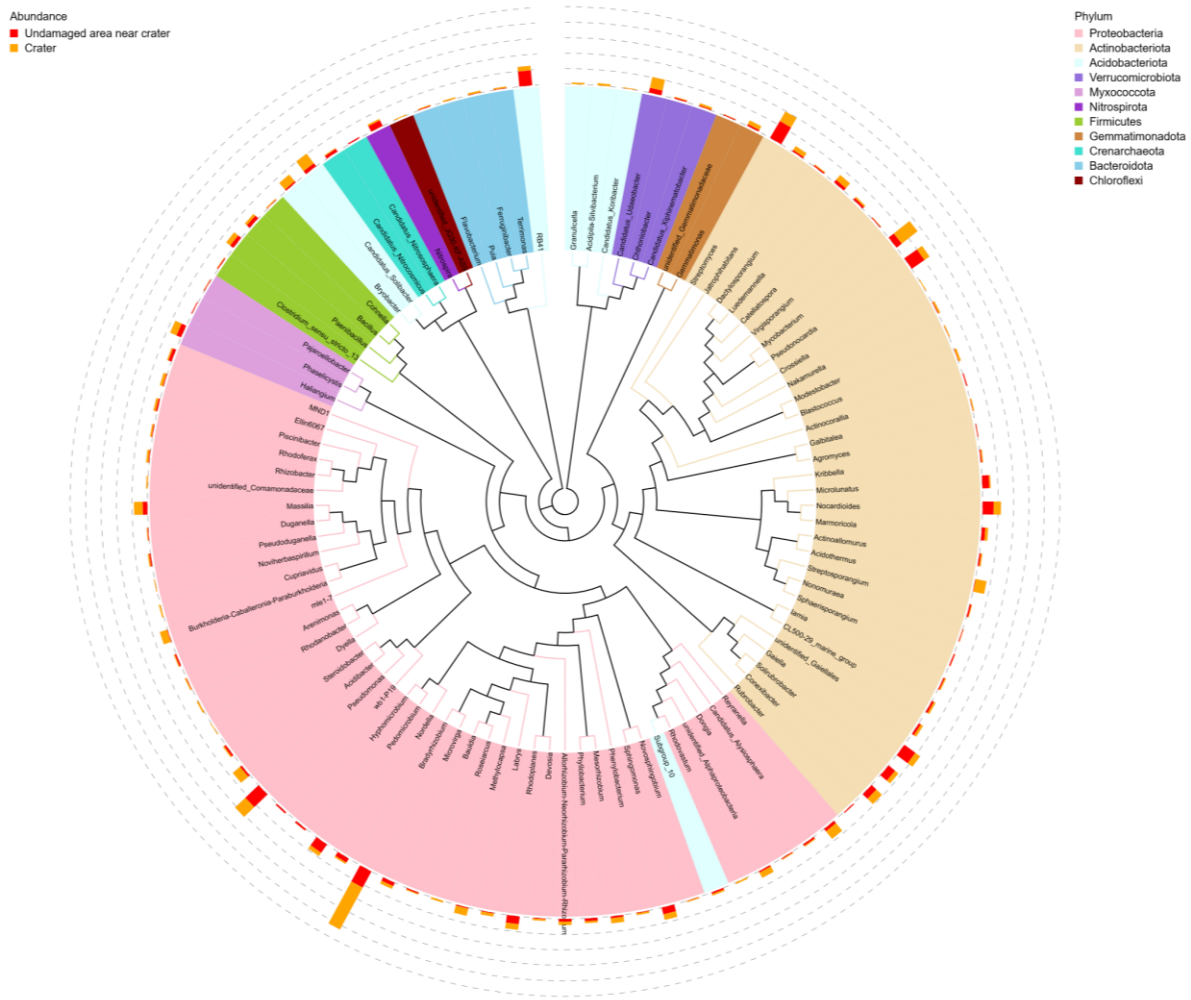



**Figure 3.** Taxonomic structure and comparative analysis of the bacterial communities in the studied soils at the genus level. The colors of the sectors correspond to phyla, and the outer histograms represent the relative abundance of taxa

This phylogenetic tree structure clearly demonstrates not only the high biodiversity of the studied soils but also a shift in the dominant groups. The displacement of typical soil taxa and the accumulation of stress-tolerant representatives of *Actinobacteria* and *Proteobacteria* underscore the formation of a specific adaptive complex of microorganisms in the studied soils.

## CONCLUSIONS

Studies have shown that ammunition explosions at military training grounds significantly affect the physical, physicochemical, and biological properties of the soil. Mechanical mixing of the layers and thermal degradation alter their grain size distribution (the proportion of silt increases, while that of clay decreases). Although the soils remain sandy, an increase in their specific surface area may subsequently

alter their absorption capacity. Physical and chemical degradation is manifested by a decrease in humus content, an increase in hydrolytic acidity, and a reduction in the amount of absorbed bases, which impairs soil fertility and suppresses soil ecosystems.

Heavy metal contamination was also detected: MPCs for Cu, Cr, and Zn were exceeded, and concentrations of Cu, Cr, Hg, and Se were significantly higher than in visually undamaged soil. The concentrations of chromium and copper showed the greatest increase, indicating the specific nature of the munitions. The spatial variability of military contamination in Ukraine depends on the intensity of combat and the types of weaponry used, which requires a tailored approach to reclamation and bioremediation.

In addition, microbial communities have undergone changes: sensitive groups of microorganisms have been displaced by stress-resistant bacteria, a phenomenon typical of industrial areas that poses a threat to biogeochemical processes and soil recovery. Metagenomic analysis showed that ammunition explosions alter the microbiome structure, but it generally retains high diversity and functional stability. High Shannon and Simpson indices indicate the ecological plasticity of the bacterial community, which can sustain biogeochemical processes. At the same time, the decrease in alpha diversity in the crater indicates the selective elimination of sensitive and rare microorganisms. Analysis of OTUs revealed a significant proportion of shared taxa, confirming the presence of a stable “core” of the microbiome. Unique OTUs indicate adaptation to new conditions; however, the decrease in their number in the crater demonstrates the vulnerability of rare members of the microbiota to explosive impacts.

Bacteria were the dominant group among the prokaryotes; the percentage of archaea was lower and decreased significantly in the crater (particularly among representatives of the *Crenarchaeota* and *Thermoplasmatota*). The most sensitive organisms were nitrifying archaea of the family *Nitrososphaeraceae* (Candidatus *Nitrocosmicus*, Candidatus *Nitrososphaera*, Candidatus *Nitrosotenuis*), indicating a risk of disruption to the nitrogen cycle. Among the dominant bacteria in the crater, the proportion of *Proteobacteria* and *Acidobacteriota* increased, while the abundance of *Actinobacteriota* decreased. An increase in the abundance of *Rhizobiales*, *Burkholderiales*, *Bradyrhizobium*, and *Massilia* indicates adaptation to stress and the potential degradation of pollutants. Conversely, the suppression of typical actinobacteria (*Streptomyces*, *Pseudonocardia*) may inhibit humus formation processes.

A notable feature is the increase in the abundance of *Mycobacterium* in the crater and the emergence of genera not detected in the soil from the undamaged area, confirming the creation of specific ecological niches for stress-resistant microorganisms. Thus, military activity significantly alters the soil microbiome. These findings are important for assessing the ecological condition of disturbed areas and developing methods for their bioremediation.

**Acknowledgements** The authors are grateful to the Explogen employee for performing the metagenomic analysis of the soil samples.

**Funding** This study was performed and funded under the Ministry of Education and Science of Ukraine project “Development of an approach to restore soil fertility in soils affected by hostilities using microbial biological preparations” (Grant number 0124U000931).