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ECOBIOLOGICAL ASSESSMENT OF THE CURRENT STATE OF MICROBIOMES

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SUMMARY

An evaluation of quantitative variations in the main taxonomic groups of the microbial community during long-term use of the direct seeding technology in Ismayilli Region, Azerbaijan, was the aim of this research. The assessment focused on the quantitative characteristics of the microbial community in agrocenoses using no-till and traditional plowing conducted for variants with various agricultural crops and with or without mineral fertilizer application. The abundance of ecotrophic microbial groups (nitrogen-fixing bacteria, ammonifiers, denitrifiers, amylolytics, and cellulolytics) received evaluation using the classical method of inoculation on selective media, while assessing the ribosomal genes of archaea, bacteria, and fungi used real-time PCR (polymerase chain reaction). The traditional plowing displayed an enhanced abundance of cultivable aerobic ammonifiers, denitrifiers, aerobic cellulolytics, actinomycetes, and micromycetes. However, the direct sowing with no tillage occurred with numerous anaerobic cellulolytics and nitrogen fixers, aerobic diazotrophs, and amylolytics.

Keywords: Bacteria, archaea, actinomycetes, micromycetes, traditional plowing, no tillage

Key findings: Microbial parameters provided valuable information about soil health under different management practices, contributing to the development of effective sustainable land use strategies. Their ratio can also serve as bioindicators of agricultural greening during the transition from traditional plowing to no tillage.

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INTRODUCTION

Alongside traditional agricultural practices, organic farming, involved in the development of an ecologically balanced agroecosystem, is rapidly growing. The sustainability of such agroecosystems is consistent by minimizing negative effects on the agroecosystem while maintaining mandatory agroecological monitoring (Akbarova and Mammadova, 2024; Amanova *et al.*, 2025). This also requires a minimum set of parameters that correlate with the natural processes occurring in the agroecosystem found to be relatively easy to employ in the field and sensitive to variations. Therefore, for assessing the overall sustainability of field phytocenoses, key indicators should include biological (microbial activity, organic matter decomposition, and nutrient cycling) and biochemical parameters (Ahmad *et al.*, 2026; Bunyatova *et al.*, 2025; Hasanova and Abasova, 2024).

It is usual that the soil microbial community is a sensitive indicator of the degree of anthropogenic pressure on the ecosystem. The disturbances in its composition and structure become apparent before variations in the soil's physicochemical properties (Ismayil *et al.*, 2025). Therefore, microflora is a vital informative indicator of the variations occurring in the soil and proves it advisable to include the same in the agroecological monitoring systems. Soil micromycetes (microscopic fungi, including molds and yeasts) are key components of the biota, directly associated with soil formation and nutrient cycling in northern ecosystems.

Knowledge of the fungal flora diversity is doubtlessly important for the soil's ability to regenerate after anthropogenic effects. The utilization of various types of mineral fertilizers and the chemicals used for crop protection considerably affect the chemical, physicochemical, and biological properties of the soil (Ismayilova *et al.*, 2025; Ibrahimova and Imanov, 2025; Mammadova *et al.*, 2024; Mammadova *et al.*, 2025a). It has also been well established that under the influence of anthropogenic factors, soil micromycete

communities undergo alteration, restructuring their species diversity and considerably reducing their overall diversity.

Crop cultivation methods largely determine the physical and chemical characteristics of the soil, plant growth and development, and overall productivity of the agroecosystems (dominated by cultivated crops and accompanied by weeds, microorganisms, and animals) (Allahverdi *et al.*, 2026). Crop cultivation techniques significantly alter the composition and functioning of microbial communities inhabiting the soil and rhizosphere. The prevalence of pathogenic and symbiotic microorganisms also depends on the physical properties of the soil, such as density, porosity, aeration, water-air, and thermal conditions. Information on the microbiological state of the arable soils using various methods is only fragmentary (Macnunlu *et al.*, 2025; Mammadova *et al.*, 2024, 2025b).

In the Ismayilli Region, Azerbaijan, some arable areas using no tillage with direct seeding minimize the effect of agricultural tools used on the soil and keep a layer of plant residue from the previous crop on the soil surface (Mammadzada *et al.*, 2025). However, the no-till technology has acquired criticisms from experts for several reasons, one of which is the frequent outbreaks of phytopathogenic diseases in fields using this type of tillage. Microorganisms (primarily fungi and actinomycetes) are modular organisms, which predetermine their reproduction not only by specialized cells but also by vegetative cells (including parts of the mycelium) (Nasirova *et al.*, 2026). Traditional plowing loosens and mixes the soil, breaking up soil aggregates, and consequently, disrupting the colonies and hyphae of the microbiota. Some of these microbiota appeared to be capable of active growth, facilitated by optimal laboratory conditions on nutrient media.

Therefore, microbiological seeding typically revealed a considerably greater colony-forming unit (CFU) micromycetes in soils of plowed fields than those where the soil had no mechanical cultivation from any agricultural machinery. The mycelial

organization of the body of some microorganisms allows them to occupy leading positions in ecosystems with a low content of organic matter, which probably predetermined the dominance of actinomycetes and fungi in the γ -glutamic acid (GA) environment (Makki *et al.*, 2025; Shukurov *et al.*, 2025). Based on the above discussion, the following study aimed to evaluate the quantitative variations in the main taxonomic groups of the microbial community during long-term utilization of the direct seeding technology in Ismayilli Region, Azerbaijan (Ahmad *et al.*, 2026).

MATERIALS AND METHODS

Experimental site and procedure

Numerous soil samples from the experimental fields of ordinary, medium-deep, low-humus, and heavy-loamy chernozem on loess-like carbonate loams entailed collection and studies at a private experimental farm in the village of Basgal, Ismayilli Region, Azerbaijan (40.75556°N 48.39639°E). The arable horizon of this slightly acidic (pH 6.32) soil under conventional tillage contained 2.24% C_{org} and 11.9 mg/kg N-NO₃, as well as 18.7 mg/kg of labile phosphorus (determined using the Machigin method) and 245 mg/kg of exchangeable potassium (Allahverdi *et al.*, 2026). With increasing depth in the soil profile, values of these parameters considerably decreased. Horizon C, identified at the depth of 127–175 cm, comprised characteristics of the following parameters: C_{org} = 0.38%; N-NO₃ = 0.5 mg/kg; P₂O₅ = 3.4 mg/kg; K₂O = 155 mg/kg; and pH = 8.0. The duration of agricultural treatments was five years on the studied soils, with the field experiments repeated three times. The experimental plot area was 300 m² (50 m × 6 m), and the sample plot area was 90 m². Collected samples were in triplicate from the 0–15 cm layer in October 2020, with possible measures to prevent contamination. Before analysis, samples for classical microbiological analyses received storage at +4 °C, and for molecular biological studies, at -70 °C (Verdiyeva *et al.*, 2025; Mammadova *et al.*, 2026).

Microbiological culture

Ecological groups of microorganisms underwent identification in five replicates by culturing soil suspensions on solid elective media: meat-peptone agar (MPA-ammonifying bacteria), starch-ammonia agar (SAA-amylolytics), starvation agar (GA-oligotrophic bacteria, actinomycetes, and micromycetes), Czapek medium (CA-microscopic fungi), Hutchinson's medium with cellulose disks (aerobic cellulolytics), and Ashby's medium (aerobic nitrogen fixers). Using limiting dilutions on liquid media served to identify anaerobic diazotrophs (Winogradsky's medium), the nitrate respiration group, and anaerobic cellulolytics (Hutchinson's medium with ash-free filter paper strips). For CA, GA, and SAA, the inoculum dilutions were 1:1000, 1:10,000, and 1:100,000, while for GE the dilutions were 1:10, 1:100, and 1:1000. A 100 μ l aliquot of the soil suspension obtained placement on the surface of sterile agar media in 90 mm Petri dishes (Mammadova *et al.*, 2024; Nazim and Oqtay, 2024).

Suppressing bacterial growth employed the addition of streptomycin powder (100 mg/l) to the Czapek and Hutchinson culture media. The number of colony-forming units (CFU) of micromycetes grown entailed counting on the 7th, 14th, and 20th days. Morphological types' isolation from the cultures continued into pure cultures based on macro- and micro-cultural characteristics. All microscopic isolates sustained preliminary identification using cultural and morphological characteristics (Mammadzada *et al.*, 2025). All initial detections of microbial isolates used molecular biological characteristics (based on plot analysis) (Nazim and Oqtay, 2024).

Data analysis

All the recorded data's analyses used Statistica 10.0 and Microsoft Excel 2020. Visualization of the culture data on selective media employed the heat map method. The presented data were as IgN values (where N is the number of CFU/g of soil of microorganisms from a specific ecological-trophic group), normalized using the Z-transformation principle. Scaling of samples

by the number of ribosomal gene copies of bacteria, archaea, and fungi engaged the principal component (PC) method within the Past 3.25 software package (Bunyatova *et al.*, 2025).

RESULTS AND DISCUSSION

Microbiological seeding

The lowest abundance of aerobic nitrogen fixers (25%–30% of soil clod coverage on Ashby medium) resulted in soil samples grown with beans and hazelnut, which were typical for legume crops (Mammadova *et al.*, 2025b). The highest abundance of nitrogen fixers (81%–84% of soil clod coverage) was evident under lentil crops, a legume whose rhizosphere is always rich in diazotrophs, which considerably enhanced the nitrogen available to crop plants. However, the average *Azotobacter* activity surfaced in fallow soils, accounting for 38% of soil clod coverage. Soil samples with no tillage (excluding fertilized bean and pea) showed the maximum abundance of cultivated nitrogen fixers (10%–35% of soil clod coverage) compared with the arable soil (Mammadova *et al.*, 2025a; Sadigov *et al.*, 2025).

Thus, direct seeding technology with no tillage brings arable soils closer to native and background soils, with the highest abundance of aerobic nitrogen fixers (Mammadzada *et al.*, 2025). The lowest abundance of anaerobic nitrogen fixers appeared in the fertilized fields with no tillage under hazelnut (8.9×10^2 CFU/g of soil). The said crop has a well-developed root system, which provides considerable aeration. The largest number of anaerobic nitrogen fixers (8.3×10^6 CFU/g of soil) occurred under lentil and clover crops, regardless of soil tillage and application of fertilizers (Table 1). The abundance of anaerobic diazotrophs (crucial components of the plant microbiome, directly enhancing plant growth, nutrition, and stress tolerance) was higher (except for the pea variant) in no-tillage plots, where the soil was relatively compact and contained less oxygen. The largest share of the nitrogen cycle

microorganisms achieved occupation by the non-specific group of ammonifiers, and their abundance was a sign of the highest intensity of organic matter mineralization and nitrogen release from the soil. Their CFU count on MPA medium was minimal (4.9×10^6 /g soil) for no-tillage fields under unfertilized clover and hazelnut crops, and the same has also been visible for other gray-brown soils. The maximum (7.2×10^7 /g soil) was for the plowed fields under unfertilized clover and bean crops. Fertilizer application had no positive and negative effects on the number of ammonifier CFUs, except for no-tillage lentil and hazelnut crops, where microorganisms increased with NPK fertilization (Huseynova *et al.*, 2025).

Meanwhile, in fields under lentils (a crop with a higher protein content and a better substrate for this group of microorganisms), tillage technology had a nonsignificant effect on the number of bacteria grown on the meat-peptone agar, or MPA. None of the considered factors (tillage type, fertilizers, and crop) had an effect on relatively high numbers of denitrifiers in all studied samples. The highest number of culturable denitrifiers resulted in no-tillage plots with the hazelnut crop without fertilizers. For other no-tillage variants, the number of denitrifiers was lower than in plowed plots. The number of CFU of oligotrophic microorganisms with the GA medium was practically independent of the type of tillage, the presence, or absence of fertilizers, and the plant species. It ranged from 9.8×10^4 to 5.7×10^6 /g of the soil, with the same found to be typical for the studied soils. However, for both fertilized and unfertilized beans, lentils, and clover crops, the number of microbial colonies was higher in plowed plots than in no-tillage plots. This may be due to the specificity of the research method, which accounted for the modular elements of microorganisms. However, their numbers could rise in the field with mechanical tillage. Oligotrophic micromycetes emerged as dominant on GA, as their propagules were notable for most samples sown on the selective medium, reaching 10^5 – 10^6 CFU/g of soil (Nasirova *et al.*, 2022; Huseynova *et al.*, 2025; Mammadova *et al.*, 2026).

Table 1. Heat map of the influence of studied factors (soil treatment type and crops) on the development of culturable microorganisms.

Processing	Crop	Oligotrophs			I	Amylolytics			II	Nitrogen-fixing bacteria	
		X	Y	Z		X	Y	Z		A	B
Traditional plowing	lentil										
	clover										
	hazelnut										
	beans										
No tillage	lentil										
	clover										
	hazelnut										
	beans										

Note: Data presented as IgN values (where N is the number of CFU/g of soil of microorganisms from a specific ecotrophic group), normalized using the Z-transformation principle. I - Copiotrophic bacteria; II - Cellulolytics; X - Fungi; Y - Bacteria; Z - Actinomycetes; A - aerobic; B - anaerobic.

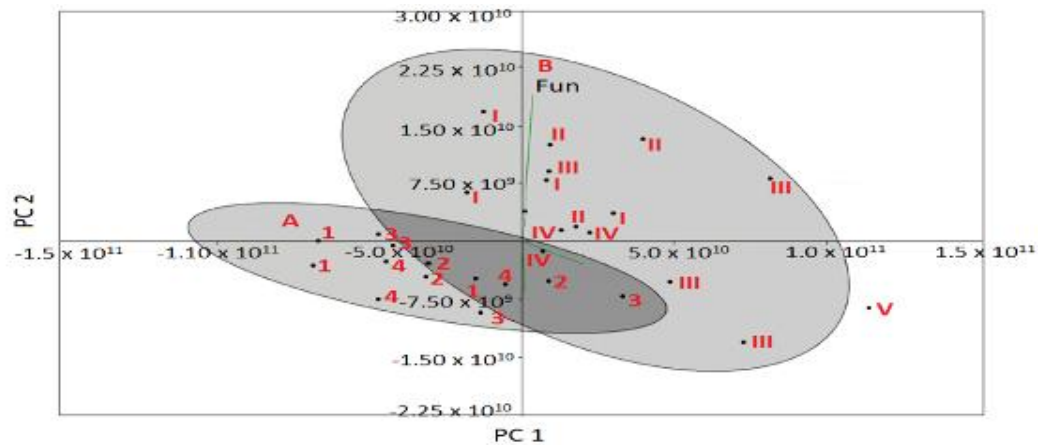


Figure 1. Scaling of samples by the number of copies of ribosomal genes of bacteria, archaea, and fungi using the principal component analysis: bac – bacteria; arc – archaea; fun – fungi. Note: I - no-till clover; II - no-till hazelnut; III - no-till beans; IV - no-till lentils; V - control deposit; 1 - traditional clover; 2 - traditional hazelnut; 3 - traditional beans; and 4 - traditional lentils.

Meanwhile, the count of microscopic fungi in samples with a conventionally cultivated plot under a clover crop did not exceed 10^2 CFU/g of soil, regardless of the presence of fertilizers. For bean crop treatments, only fertilizer application significantly affects the micromycete counts. In the absence of fertilizers, the microscopic fungi count decreased. Soil samples from lentil crop fields with no tillage showed a considerable increase in micromycete counts, which may be because of the abundance of nitrogen

produced by the bacterial symbionts of the legumes. Soil tillage influenced the abundance of individual taxonomic and ecotrophic groups of microorganisms. In turn, no tillage also enhanced the total number of fungi and bacteria (both for ribosomal gene copy number and CFU/g soil), the number of archaea, and the activity of anaerobic cellulolytics, nitrogen fixers, aerobic diazotrophs, and amylolytics (Figure 1) (Mammadova *et al.*, 2024; Sadigov *et al.*, 2026).

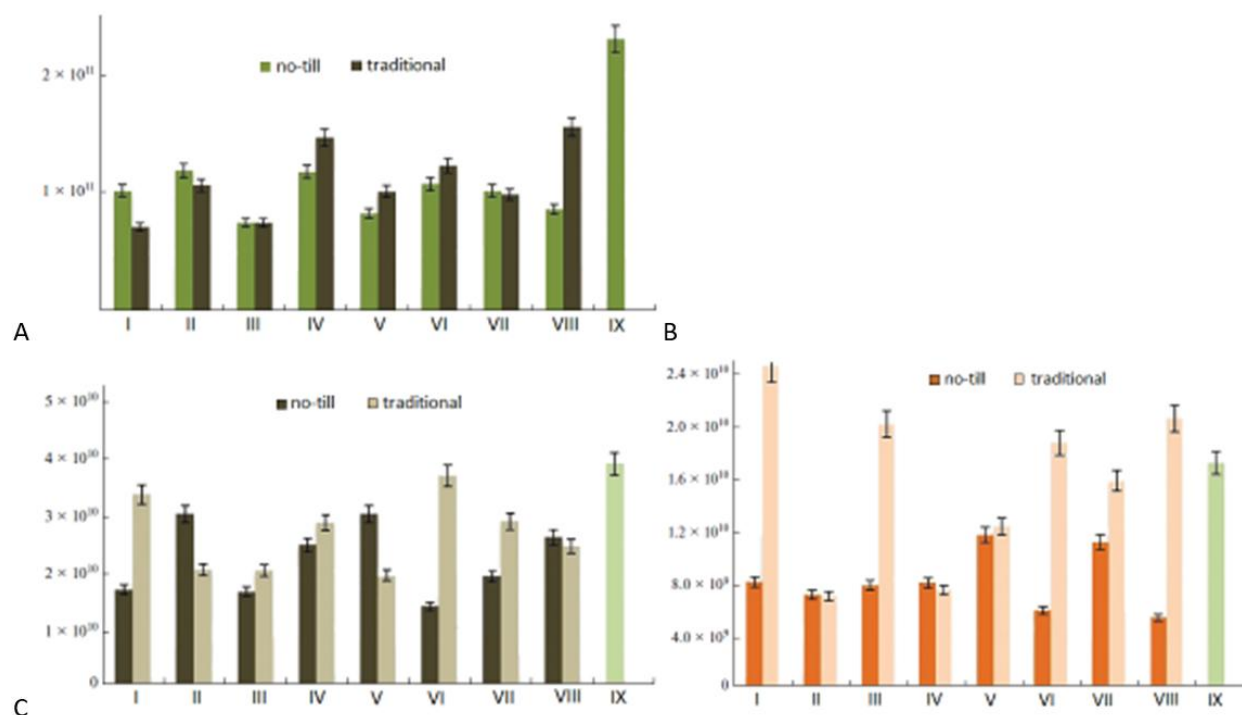


Figure 2. A) Bacterial counts for plots cultivated conventionally (with soil rotation) and no-till, B) the amount of archaea for plots processed traditionally (plowing with soil turnover) and using no-till technology, and C) the number of fungi for plots processed traditionally (plowing with soil turnover) and using no-till technology. I – lentils without fertilizer; II – lentils with mineral fertilizer; III – clover without fertilizer; IV – clover with mineral fertilizer; V – hazelnut without fertilizer; VI – hazelnut with mineral fertilizer; VII – beans without fertilizer; VIII – with mineral fertilizer; and IX – control deposit.

The fertilization factor had no considerable effects on the quantitative parameters of microorganisms in the studied soils, apparently due to the uneven application of mineral fertilizers and the buffering properties of the studied soils. The number of prokaryotic gene copies entailed primary determination by the crop type cultivated in the plots, while determining the number of fungal genes was by the type of soil cultivation (Mammadova *et al.*, 2025a). The number of all considered microbial taxonomic groups (bacteria, archaea, and fungi) was significantly higher in the fallow lands than in the soils under different crops (Figure 2 a-c). Overall, the microbial indicators provided valuable insights into soil health under different management practices, helping in the development of effective strategies for sustainable land utilization.

CONCLUSIONS

The microbial group ratios demonstrated their potential for bioindication, including assessment of the degree of aerobic and anaerobic soil habitat in the steppe zone under minimal tillage conditions. The results revealed significant differences in the composition and structure of the soil microbiome during the transition from plowing to no tillage. Therefore, microorganisms can be useful as a marker for greening of agriculture in the agroecosystems.

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