

# Comparison of Soil Microbiome Diversity in Establishing an Agronomically Valuable Association for Agricultural Applications

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**Abstract.** The article presents the data of metagenomic sequencing of several samples of fertile soils (the World Soil Bank of Unitsky Farm Enterprise and the arboretum of the Belarusian State Agricultural Academy). The analysis was performed using NovaSeq 6000. The authors carried out a comprehensive assessment of the microbial potential of soils and the presence of agronomically valuable microorganisms for further use in organic farming. We also studied the taxonomic composition of fertile soil samples and determined the predominant genera of microorganisms. Based on the obtained data, we selected and isolated an association of agronomically valuable bacteria, which was introduced into the complex organic fertilizer for plants – fertility elixir "uTerra".

## 1 Introduction

The ever-increasing need to maximize the production of food, fuel and other resources to meet global demand and eliminate hunger is putting significant pressure on the soil and its fertile layer. This is primarily due to the use of intensive agricultural industrial technologies involving increased doses of mineral fertilizers, herbicides, pesticides and other agrochemicals, which, if applied irrationally, cause soil degradation and disturbance of its biospheric balance.

Thus, degraded and marginal lands with significant agricultural limitations are the result of inadequate intensive land cultivation with insufficient attention to soil fertility conservation and climate change [1, 2]. Continued use of soils in agricultural production often requires greater and greater reliance on external inputs such as mineral fertilizers and herbicides, which further exacerbates degradation and impedes nutrient recycling and conservation. Soil degradation represents not only an accelerated decomposition of natural humus, leaching of micro- and ultramicroelements, but also a reduction in soil biodiversity.

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Soil microorganisms play a critical role in agricultural systems, especially plant growth-promoting microorganisms (PGPM) [3]. Stimulation of plant growth by such microorganisms is mainly explained by the effects of three biomechanisms:

- they act as biofertilizers (nitrogen-fixing and phosphate-solubilizing bacteria) and promote nutrient uptake by plants by providing fixed nitrogen or other nutrients;
- they act as phytostimulants (microorganisms expressing phytohormones, e.g. bacteria of the genus *Azospirillum*) can directly stimulate plant growth;
- they are plant biological defense agents (e.g. *Trichoderma*, *Pseudomonas* and *Bacillus*).

With a better supply of biological nitrogen to plants, a larger assimilative surface is formed, photosynthetic potential, net photosynthetic productivity, dry matter accumulation by all plant organs and, ultimately, yield, protein and micronutrient productivity of crops increase. Biological fixation of nitrogen ( $N_2$ ) is the process of converting atmospheric nitrogen into a bioavailable form such as ammonium ( $NH_4^+$ ), which is used by organisms (bacteria, algae, fungi and plants) to synthesize organic compounds including proteins and nucleic acids [4].

Most agronomic soils contain large reserves of total phosphorus, but fixation and deposition cause phosphorus deficiency and, in turn, severely limit crop growth. Phosphorus replenishment, especially in sustainable production systems, remains a major concern as it depends mainly on the mineral composition of the soil. Therefore, there has been an increased interest in finding a technology that can provide plants with sufficient amounts of mobile phosphorus. Among heterogeneous and naturally occurring microorganisms inhabiting the rhizosphere, phosphate solubilizing microorganisms occupy dominant positions.

Understanding soil ecology is essential for building sustainable ecosystems as well as restoring disturbed habitats. Soil microorganisms are a key element of natural soil fertility and play an important role in soil ecosystem processes such as nutrient cycling, organic matter decomposition and bioremediation. Therefore, the study of the microbiome community of fertile soils is an urgent problem in organic farming and in the search for new approaches to quality and affordable food.

## 2 Materials and methods

Sequencing was performed at CeGat (Tunebengen, Germany) using NovaSeq 6000. The accuracy of correct base reads was 93.44%. Relative sequence abundance for taxonomic units and functions, as well as Bray-Curtis diversity and dissimilarity indices were calculated using R.

We sent two samples of the soil microbiome obtained from:

1. The World Soil Bank (S3067Nr1 Soil bank) – Maryina Gorka, Unitsky Farm Enterprise. The World Soil Bank contains samples of natural fertile soils from 110 regions of 5 continents of the planet.

The World Soil Bank, created in the territory of Unitsky Farm Enterprise, has a total mass of about 100 thousand kilograms and contains the entire natural diversity of living microorganisms.

This Soil Bank is created from fertile soils that have not participated in agricultural crop rotation and have not been exposed to pesticides, herbicides and insecticides. The World Soil Bank is designed for scientific and practical purposes, to study the symbiosis of agronomically valuable microorganisms and the subsequent selection of target consortia capable of restoring the fertility of depleted soils and effectively used in organic farming.

2. Arboretum (S3067Nr2 Arboretum) – Gorki (Belarusian State Agricultural Academy). Soils of the arboretum have not been exposed to intensive chemical farming for more than 170 years.

In this study, 10 million raw reads were matched against the RefSeq protein database. Taxonomic distribution was performed using the Lowest Common Ancestor (LCA) algorithm [5] implemented in MEGAN6 Ultimate Edition. Based on the obtained data, the most potentially fertile soil with a diverse microbiome was selected for further isolation of microorganisms for application in agriculture to restore land fertility as well as to stimulate plant growth.

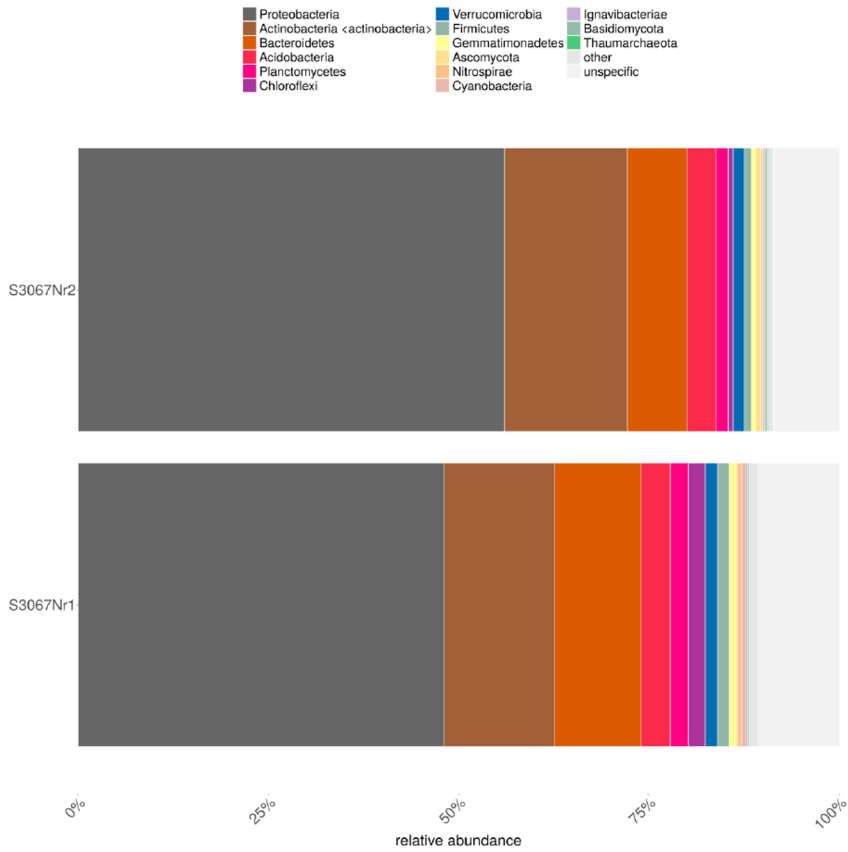
### 3 Results and discussions

The results of the obtained data made it possible to analyze the composition of microorganisms from fertile soil samples. Table 1 shows the distribution of species by kingdoms. Only taxa with relative abundance of sequences over 0.01% were considered.

**Table 1.** Percentage of reads belonging to the kingdoms of Bacteria, Archaea, Eukaryotes, and Viruses

Sample name	Bacteria	Archaea	Eukaryotes	Viruses
S3067Nr1	98.89	0.35	0.24	0.01
S3067Nr2	98.27	0.21	1.16	0.02

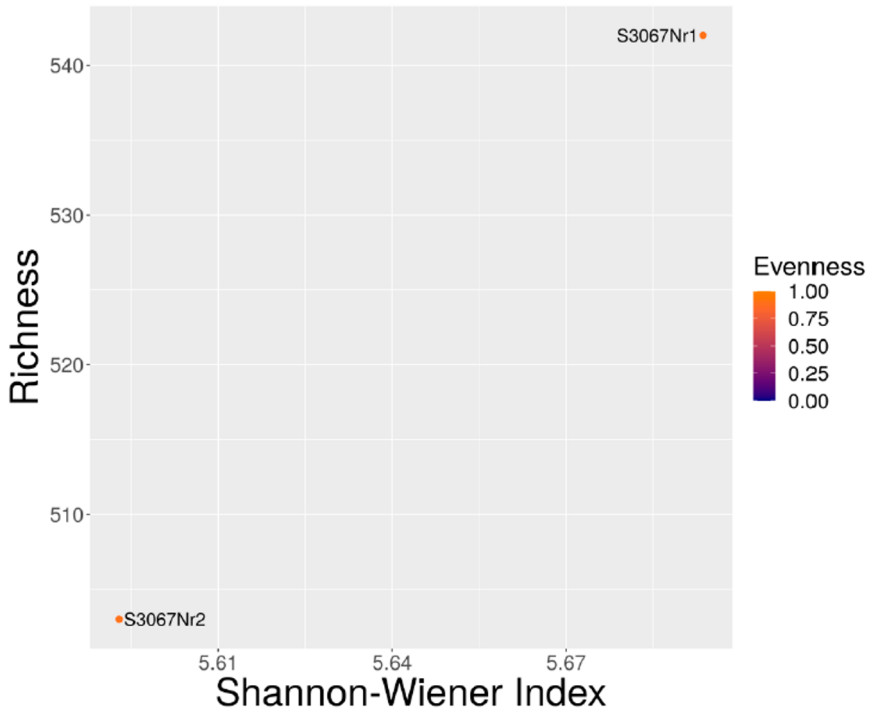
According to the table, bacterial species dominate in the samples, while S3067Nr2 has slightly more representatives of the Eukaryote kingdom than S3067Nr1. This is probably due to the presence in the arboretum of a large diversity of basidial fungi belonging to this kingdom.



**Fig. 1.** Histogram of the relative abundance of types present in each sample

Figure 2 shows the most dominant groups of microorganisms. The following types were identified in the samples: Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria, Planctomycetes, Chloroflexi, Verrucomicrobia, Firmicutes, Gemmatimonadetes, Ascomycota, Nitrospirae, Cyanobacteria, Ignavibacteriae, Basidiomycota, Thaumarchaeota and other less abundant representatives of taxonomic groups.

Two measures of diversity were calculated to assess sample diversity: the Shannon-Wiener Index (HS) and Evenness (EH). HS is a measure of the total number of species present in each sample (richness) and their frequency of occurrence. The index increases with the number of species [6]. Sampling evenness is estimated from 0 to 1. EH is close to 1 if all species have the same abundance, but is low if there are large differences in the number of species in the sample.



**Fig. 2.** Diversity of species in the samples

Sample S3067Nr1 is much richer in the number of representatives of different species. In two different soils the dominant type was Proteobacteria or Pseudomonadota. This type is the main one in Gram-negative bacteria. Pseudomonadota have a wide variety of metabolic types, and most of them are facultatively or obligately anaerobic, chemolithoautotrophic and heterotrophic organisms [7]. This type includes the broad class Alphaproteobacteria, which is a diverse taxon and includes several phototrophic genera, several genera that metabolize C1 compounds (e.g., *Methylobacterium spp.*), plant symbionts (e.g., *Rhizobium spp.*), arthropod endosymbionts (*Wolbachia*), and others [8].

The Alphaproteobacteria also include various species of nitrogen-fixing bacteria, one such representative is *Azospirillum*, which are aerobic organisms, but many of them can also function as microaerobic diazotrophs, i.e. under low oxygen conditions they can convert inert nitrogen from the air into biologically useful forms. They grow well in an air atmosphere in the presence of a source of bound nitrogen, such as ammonium salts. Metabolism is generally of the respiratory type using oxygen as the final electron acceptor, and in some strains  $\text{NO}_3^-$  [9]. Many *Azospirillae* secrete plant hormones that alter root growth. Affected roots often grow more branches and fine root hairs, which can help plants absorb water and nutrients more efficiently. In addition, *azospirillas* can also change the forms of plant nutrients, such as nitrogen and phosphorus, to make them more available to plants. *Azospirillum*s produce antioxidants that protect plant roots from stresses caused by drought and flooding.

The next numerous represented group of microorganisms is Acidobacteria [10]. Members of this type are particularly abundant in soil habitats and account for up to 52% of the total bacterial community. Most Acidobacteria are considered to be aerobes. Some strains of Acidobacteriota originating from soils have been found to possess the genomic

potential for oxygen respiration at atmospheric concentrations. Members of the Acidobacteriota type are considered oligotrophic bacteria because of their high abundance in environments with low organic carbon content and tolerance to low environmental pH.

## 4 Conclusions

Based on the above results, we should note that soil sample S3067Nr1 from the World Soil Bank has a significant potential for study in organic farming, has a higher diversity of different microorganism species with functional metabolic features even in comparison with a sample of a specific natural soil that has not been used for agriculture for 170 years. Thus, the soil from the World Soil Bank is a donor for isolation of new associations of microorganisms that improve land fertility, plant health and stimulate plant growth and has a high scientific potential for further study of microbiomes.

Using the above data, a liquid organic complex fertilizer for plant fertility elixir "uTerra" has been created [11, 12]. The plant fertility elixir "uTerra" is produced using agronomically valuable soil microorganisms isolated from the World Soil Bank, by bioprocessing of relict fossil raw materials (brown coal, oil shale, peat), crumbly biohumus "uTerra", molasses, honey, natural elicitors, sea salt, mineral and organic elements of plant nutrition and other organic and mineral components.

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