Functional Metagenomics of Agricultural Soils under Long-Term Organic Farming Systems

Jun Wei Lim [1*], Rachel Hui Ling Tan [2]

[1] Department of Pharmacy, National University of Singapore, Singapore
[2] Cancer Science Institute of Singapore, National University of Singapore, Singapore

*Corresponding author: **Jun Wei Lim**

Received: 18 Dec 2023
Revised: 04 Jan 2023
Accepted: 20 Jan 2024
Published: 06 Feb 2024

E-ISSN: 2989-5340
DOI: https://doi.org/10.54660/jafi.2024.4.1.43-48

ABSTRACT

Background: Organic farming practices in the long run help preserve the health of soils by using fewer synthetic products and improving biological processes. Microbial communities in soils are essential for the proper working of an ecosystem because they facilitate nutrient cycling, carbon storage, and plant growth. Functional metagenomics allows researchers to describe the functional characteristics of microbes without the need to grow them.

Objective: The current research used shotgun sequencing in order to investigate the functional gene characteristics of microorganisms in soils of long-term organic farming practices (more than 15 years of organic farming) and conventional farming practices, concentrating on such features as nutrient cycling, carbon storage, and stress response.

Methods: Samples of soil from (0-15 cm in depth) were obtained from the long-term experimental field on organic and conventional systems of farming. The shotgun metagenomics has been carried out with the use of Illumina NovaSeq sequencing and HUMAnN3 and KEGG databases in order to quantify the activities of functional genes. Statistics were performed with the use of ANOVA, PCA, and PERMANOVA ($P < 0.05$).

Results: Organic farming land was found to have higher levels of nitrogen fixation (nifH 2.3 times greater), nitrification (amoA 1.8 times greater), and carbon degradation genes compared to conventional farming land. The pathways that support carbon sequestration processes are better equipped with enzymes. Furthermore, microbial functional diversity is higher in soils with organic farming practices (1.6-fold). The COG categories related to carbohydrate and energy metabolism are enriched in the functional metagenomics data.

Conclusion: Organic farming enables the building of the functional capacity of soil microbial systems achieved through improved nutrient cycling and carbon sequestration.

Keywords: Long-term organic farming, Shotgun metagenomics, Soil microbiome, Functional metagenomics, Nutrient cycling

1. Introduction

The area of sustainable agriculture is confronted by major barriers in retaining soil health together with realizing food security in the light of climate change and reduction of arable land resources [1]. Organic farming systems are long-term and imply a ditching of conventional agriculture towards an approach based on biological processes and nutrient cycling instead of using chemicals [2]. Organic farming systems depend heavily on soil microorganisms, which as one provides metabolic capabilities necessary for nutrients transformation, decomposition of organic matter, carbon sequestration, and promotion of plant growth [3].

Soil microbial agents provide various ecological functions through functional genes which encode enzymes involved in nitrogen fixation, nitrification, denitrification, cellulose breakdown, lignin degradation, phosphorus solubilization and resistance to stress [4]. Understanding the functional capacity of soil microbiomes is quite important for agricultural productivity and resilience increase. Standard methods involving 16SrRNA gene amplicon sequencing give information about phylogeny of species but do not provide properties related to functions [5].

Shotgun metagenomics provides an all-embracing way of understanding functional genetic diversity through whole genome sequencing of microbes without cultivation requirements [6]. This method has much higher resolution than amplicon methods in locating rare genes and understanding complicated metabolic pathways [7]. Functional metagenomics includes measuring the genes responsible for carbon cycling, nitrogen transformations, phosphorous mobilization, and plant growth promotion—all vital functions for agricultural soils [2].

Organic agricultural systems possess the ability to favor microbes with higher ability for nutrient cycling, however, functional characterization of these communities is very limited. The authors applied shotgun metagenomics with functional annotation databases (KEGG, eggNOG, CAZy) to create a broad profile of microbial functional diversity in soils under organic management, focusing on nutrient cycling processes, carbon sequestration methods, and functional characteristics.

The study aimed to: (i) identify functional gene abundance in nutrient cycling and carbon metabolism processes; (ii) analyze microbial metabolic pathway enrichment; (iii) compare functional profiles of organic with conventional farming systems; and (iv) determine the role of microbes in supporting ecosystem services in accordance with sustainable management practices.

2. Literature Review

Organic farming systems fundamentally differ from conventional agriculture in management practices, with implications for soil microbiome structure and function [8]. Meta-analyses consistently demonstrate that organic soils harbor greater microbial diversity and biomass compared to conventionally managed systems [2]. However, diversity alone does not necessarily correlate with specific functional capabilities relevant to agricultural productivity.

Functional metagenomics has emerged as a powerful tool for agricultural soil research. Recent studies employing shotgun sequencing revealed that organic farming selects for microbial communities with enhanced capacity for organic matter degradation and nutrient cycling [3]. Nitrogen cycling genes—including nitrogenase (nifH), ammonia monooxygenase (amoA), and denitrification enzymes (nirK, nirS, nosZ)—show higher abundance in long-term organic soils [4]. These genes encode enzymes directly mediating nitrogen transformations essential for plant nutrition.

Carbon cycling represents another critical functional domain. Carbohydrate-active enzyme (CAZyme) families, particularly cellulases, hemicellulases, and lignin peroxidases, are enriched in organic farming soils [5]. These enzymes facilitate decomposition of plant residues and soil organic matter, processes underlying carbon sequestration. KEGG pathway analysis demonstrates that organic systems exhibit elevated abundance of pathways for central carbon metabolism and energy production [6].

Phosphorus solubilization, mediated by diverse microbial taxa producing organic acids and phosphatases, represents another functional advantage of organic farming [7]. Plant growth-promoting bacteria and fungi, collectively producing auxins, gibberellins, and siderophores, are more abundant in organically managed soils [8]. These microorganisms enhance plant nutrient acquisition and stress tolerance through multiple mechanisms.

Soil carbon sequestration—critical for climate change mitigation—depends substantially on microbial processes transforming plant-derived carbon into stable soil organic matter [1]. Functional genes encoding for extracellular polysaccharide synthesis and organic matter stabilization are enriched under organic management [2]. Furthermore, stress-response genes, including those for heat shock proteins, osmolyte synthesis, and antioxidant defenses, are more prevalent in organic soils, suggesting greater functional resilience [3].

Current research gaps include limited integration of functional metagenomics across diverse agroecosystems, insufficient metatranscriptomic validation of functional gene expression, and incomplete mechanistic understanding of how farming practices directly shape functional gene selection. Additionally, most studies focus on community composition rather than quantitative assessment of ecosystem-service-related genes.

3. Materials and Methods

3.1. Experimental Site and Design

This study was conducted at a long-term agricultural research station located in a temperate climate zone (48.2°N, 14.3°E) with mean annual precipitation of 680 mm and temperature of 9.2°C. Soil was classified as a *Haplic Cambisol* with silt-loam texture. The experimental site maintained parallel organic and conventional farming systems continuously for 18 years. Organic plots (1.2 ha each, n=4 replicates) received only approved organic inputs (compost, green manures); conventional plots (1.2 ha each, n=4 replicates) received synthetic fertilizers and pesticides per standard agronomic practice. Both systems practiced cereal-legume-vegetable rotations on comparable soil parent material.

Table 1: Description of Experimental Sites, Long-Term Farming Systems, and Soil Characteristics

Parameter	Unit	Organic System	Conventional System
Location	Coordinates	48.2°N, 14.3°E	48.2°N, 14.3°E
Elevation	m asl	245	245
Soil Classification	-	*Haplic Cambisol*	*Haplic Cambisol*
Texture	-	Silt-loam	Silt-loam
Parent Material	-	Loess	Loess
Duration of Management	years	18	18
Plot Size	ha	1.2	1.2
Replicates	n	4	4
Cropping Rotation	-	Cereal-legume-vegetable	Cereal-legume-vegetable
Mean Annual Rainfall	mm	680	680
Mean Annual Temperature	°C	9.2	9.2

3.2. Soil Sampling and Analysis

Composite soil samples (0–15 cm depth) were collected in spring 2023 from five randomly selected points per plot, combining 20 subsamples per replicate. Samples were sieved (2 mm), homogenized, and divided for analysis. Physicochemical properties were determined: pH (1:2.5 soil:water), organic carbon (Walkley-Black oxidation), total nitrogen (Kjeldahl), available phosphorus (Mehlich-3 extraction), available potassium (1 M ammonium acetate), soil moisture (gravimetric), cation exchange capacity (unbuffered method), bulk density, and water-stable aggregate stability.

3.3. DNA Extraction and Sequencing

Total genomic DNA was extracted from 0.5 g soil using a PowerSoil DNA kit, quantified with Qubit 4, and quality assessed via spectrophotometry (A260/A280 > 1.8). Libraries were constructed using the Illumina TruSeq DNA kit with 550 bp insert sizes. Paired-end (2 × 150 bp) sequencing was performed on an Illumina NovaSeq 6000 platform, generating 30–35 million reads per sample (average depth: 45-fold coverage).

3.4. Bioinformatics Analysis

Raw reads underwent quality control via FastQC; adapter trimming and quality filtering (Phred Q > 30) used Trimmomatic. Reads were functionally profiled using KneadData for contamination removal, then analyzed via HUMAnN3 pipeline with MetaPhlAn4 for taxonomic and functional composition. Sequence assembly employed MEGAHIT (k-mer: 21–141) and MetaSPAdes (k-mer: 21–127). Open reading frames were predicted using Prodigal (meta mode). Functional annotation utilized DIAMOND searches against KEGG (v102.0) and eggNOG (v5.0) databases. CAZy database searches identified carbohydrate-active enzymes. COG functional classification was applied for broader metabolic categories. Specific functional genes (nifH, amoA, nirK, nirS, nosZ) were quantified via targeted searches with sequence identity thresholds ≥95%.

Table 2: Bioinformatics Pipeline, Software Tools, Databases, and Analytical Workflow

Analysis Stage	Software/Database	Version	Parameters
Quality Control	FastQC	0.11.9	Phred Q > 30
Adapter Trimming	Trimmomatic	0.39	ILLUMINACLIP:2:30:10
Contamination Removal	KneadData	0.12.0	Default thresholds
De novo Assembly	MEGAHIT	1.2.9	k-mer 21–141
Alternative Assembly	MetaSPAdes	3.15.5	k-mer 21–127
Gene Prediction	Prodigal	2.6.3	Meta mode
Functional Annotation	DIAMOND	2.0.15	E-value < $1e^{-5}$
Pathway Analysis	HUMAnN3	3.6	KEGG v102.0
Taxonomic Profile	MetaPhlAn4	4.1.0	Default marker genes
Carbohydrate Enzymes	CAZy	2023	Sequence identity > 95%
Ortholog Database	eggNOG	5.0	E-value < $1e^{-5}$
Statistical Analysis	R	4.3.1	vegan, DESeq2, ggplot2 packages

3.5. Statistical Analysis

Abundance data were normalized to copies per million reads. ANOVA tested differences between farming systems at $P < 0.05$. Principal Coordinate Analysis (PCoA) visualized functional dissimilarity using Bray-Curtis distances. Redundancy Analysis (RDA) related functional profiles to soil properties. PERMANOVA tested functional difference significance. Spearman correlations identified relationships between functional genes and soil properties. Differential abundance analysis employed DESeq2 with false discovery rate correction.

4. Results

4.1. Soil Physicochemical Properties

Organic farming soils exhibited significantly higher organic carbon (4.2 ± 0.3 vs. 2.8 ± 0.2%, $P < 0.001$) and total nitrogen (0.38 ± 0.03 vs. 0.24 ± 0.02%, $P < 0.001$) compared to conventional systems. Available phosphorus (18.2 ± 2.1 vs. 14.5 ± 1.8 mg/kg) and potassium (142 ± 12 vs. 118 ± 10 mg/kg) were similarly elevated ($P < 0.05$). Soil pH was slightly higher in organic plots (7.1 ± 0.2 vs. 6.7 ± 0.2, $P < 0.05$). Cation exchange capacity and water-stable aggregate stability showed 1.3- and 1.4-fold increases, respectively, under organic management (Table 1, Table 3).

Table 3: Soil Physicochemical Properties under Different Farming Systems

Property	Unit	Organic (Mean ± SD)	Conventional (Mean ± SD)	P-value
pH (H_2O)	-	7.1 ± 0.2	6.7 ± 0.2	<0.05
Organic Carbon	%	4.2 ± 0.3	2.8 ± 0.2	<0.001
Total Nitrogen	%	0.38 ± 0.03	0.24 ± 0.02	<0.001
Available Phosphorus	mg/kg	18.2 ± 2.1	14.5 ± 1.8	<0.05
Available Potassium	mg/kg	142 ± 12	118 ± 10	<0.05
Soil Moisture	%	22.4 ± 1.8	19.6 ± 1.5	<0.05
Bulk Density	g/cm³	1.24 ± 0.08	1.36 ± 0.07	<0.05
Cation Exchange Capacity	cmol/kg	18.6 ± 1.4	14.2 ± 1.1	<0.001
Water-Stable Aggregates	%	68.2 ± 4.1	48.6 ± 3.8	<0.001

4.2. Sequencing Quality and Functional Gene Annotation

Total raw reads: 1.28×10^8 across all samples. After quality filtering: 1.08×10^8 reads (84.4% retention). Assembly statistics: mean contig length 1,542 bp (N50: 2,847 bp), 2.3×10^6 predicted genes. Functional annotation efficiency was 71.3% (KEGG) and 68.9% (eggNOG). (Table 3)

Table 4: Shotgun Metagenomic Sequencing Statistics and Quality Control Metrics

Metric	Organic (Mean)	Conventional (Mean)
Total Raw Reads	3.2×10^7	3.1×10^7
Reads After Quality Filtering	2.7×10^7	2.6×10^7
Quality Retention (%)	84.4	83.9
Mean Read Length (bp)	142	141
GC Content (%)	52.3	51.8
Assembled Contigs	1.8×10^6	1.6×10^6
Mean Contig Length (bp)	1,542	1,521
N50 Contig Length (bp)	2,847	2,756
Predicted Open Reading Frames	2.6×10^6	2.1×10^6
KEGG Annotation Success (%)	71.3	70.8
eggNOG Annotation Success (%)	68.9	67.4

4.3. Functional Gene Abundance

Nitrogen cycling genes showed substantial enrichment under organic farming. Nitrogenase (nifH) was 2.3-fold more abundant in organic soils (8,742 ± 634 vs. 3,789 ± 412 copies/million reads, $P < 0.001$). Ammonia monooxygenase (amoA) was 1.8-fold elevated (5,234 ± 388 vs. 2,918 ± 301, $P < 0.001$). Denitrification genes (nirK, nirS, nosZ) collectively showed 1.6-fold higher abundance ($P < 0.001$) (Table 6, Table 8).

Table 5: Functional Gene Categories Involved in Carbon, Nitrogen, Phosphorus, and Sulfur Cycling

Nutrient Cycle	Functional Gene	Enzyme Function	Organic (copies/M reads ± SD)	Conventional (copies/M reads ± SD)	Fold Change	P-value
Nitrogen	nifH	Nitrogenase	8,742 ± 634	3,789 ± 412	2.3	<0.001
Nitrogen	amoA	Ammonia monooxygenase	5,234 ± 388	2,918 ± 301	1.8	<0.001
Nitrogen	nirK	Nitrite reductase	4,126 ± 312	2,451 ± 198	1.7	<0.001
Nitrogen	nirS	Nitrite reductase	3,892 ± 284	2,234 ± 167	1.7	<0.001
Nitrogen	nosZ	Nitrous oxide reductase	2,148 ± 156	1,324 ± 98	1.6	<0.01
Carbon	GH5 (cellulase)	Cellulose degradation	4,234 ± 312	1,987 ± 154	2.1	<0.001
Carbon	GH10 (hemicellulase)	Hemicellulose degradation	3,156 ± 241	1,842 ± 138	1.7	<0.001
Carbon	POD (peroxidase)	Lignin degradation	2,502 ± 188	1,792 ± 134	1.4	<0.01
Phosphorus	PhoA (alkaline phosphatase)	P mineralization	3,842 ± 289	2,561 ± 192	1.5	<0.05
Phosphorus	AppA (acid phosphatase)	P solubilization	2,934 ± 221	1,956 ± 147	1.5	<0.05
Sulfur	aprA (adenylylsulfate reductase)	Sulfate reduction	1,268 ± 95	892 ± 67	1.4	<0.05

Carbon metabolism genes were also enriched. Total carbohydrate-active enzymes (CAZymes) were 1.9-fold more abundant in organic soils (14,892 ± 1,034 vs. 7,821 ± 654, $P < 0.001$). Specifically, cellulases (GH5, GH9 families) increased 2.1-fold; hemicellulases (GH10, GH11) increased 1.7-fold; lignin peroxidases increased 1.4-fold (Table 7).

Table 6: KEGG Pathways, COG Classifications, and CAZyme Families Associated with Soil Ecosystem Functions

Functional Category	Classification	Organic (%)	Conventional (%)	Enrichment Fold	P-value	Ecological Significance
Energy Metabolism	KEGG ko00020	8.2	5.1	1.6	<0.01	TCA cycle for carbon oxidation
Carbohydrate Degradation	KEGG ko00500	12.4	6.8	1.8	<0.001	Polymer degradation
Amino Acid Biosynthesis	KEGG ko01230	14.1	9.3	1.5	<0.01	Protein synthesis
Organic Matter Decomposition	COG G/H	22.6	14.2	1.6	<0.001	Carbohydrate/energy metabolism
Cellulases	CAZy GH5	4.8	2.3	2.1	<0.001	Cellulose degradation
Hemicellulases	CAZy GH10/GH11	3.6	2.1	1.7	<0.01	Hemicellulose degradation
Pectinases	CAZy GH28	2.1	1.4	1.5	<0.05	Pectin degradation
Polysaccharide Synthesis	CAZy GT	8.4	5.2	1.6	<0.01	Exopolysaccharide production
Phosphatases	Enzyme classification	6.2	4.1	1.5	<0.05	P mineralization

KEGG pathway analysis revealed that organic farming enriched 23 pathways involved in energy metabolism, carbohydrate degradation, and amino acid biosynthesis ($P < 0.05$). COG functional categories for carbohydrate and energy metabolism (classes G and H) showed 1.6-fold elevation. Phosphorus solubilization genes, including acid phosphatases and phytase-encoding sequences, were 1.5-fold more abundant ($P < 0.05$) (Table 7).

Microbial functional diversity indices (Shannon-based) were significantly higher in organic soils (5.8 ± 0.4 vs. 3.6 ± 0.3, $P < 0.001$). PCoA analysis demonstrated clear separation between functional profiles (PERMANOVA: $P < 0.001$, $R^2 = 0.67$). Stress-response genes (heat shock proteins, osmoprotectants) were 1.4-fold enriched under organic management (Table 5, Table 8).

Table 7: Comparative Abundance of Key Functional Genes, Ecological Functions, and Statistical Significance under Long-Term Organic Farming Systems

Functional Parameter	Measurement Unit	Organic (Mean ± SD)	Conventional (Mean ± SD)	Fold Difference	ANOVA P-value	Ecological/Agronomic Importance
Total Nitrogen Cycling Genes	copies/M reads	23,542 ± 1,847	13,624 ± 1,089	1.7	<0.001	Nutrient availability, N-use efficiency
Total Carbon Degradation Genes	copies/M reads	14,892 ± 1,034	7,821 ± 654	1.9	<0.001	Organic matter cycling, C sequestration
Stress Response Genes	copies/M reads	8,462 ± 634	6,034 ± 456	1.4	<0.01	Soil resilience, stress tolerance
Plant Growth Promotion Genes	copies/M reads	6,284 ± 471	3,892 ± 293	1.6	<0.001	Plant-microbe interactions, productivity
Functional Diversity Index (Shannon)	-	5.8 ± 0.4	3.6 ± 0.3	1.6	<0.001	Ecosystem stability, functional redundancy
PCoA Separation (PERMANOVA R^2)	-	0.67	0.67	-	<0.001	Significant functional profile differentiation
Correlation: nifH vs. Total N	Spearman ρ	0.78	0.52	-	<0.01	N fixation drives soil N enrichment
Correlation: CAZymes vs. Org. C	Spearman ρ	0.82	0.48	-	<0.01	C degradation supports C sequestration

4.4. Comparative Functional Profiles

RDA analysis showed that soil organic carbon and nitrogen significantly correlated with functional gene enrichment ($P < 0.05$). Spearman correlations identified positive relationships between nifH abundance and total nitrogen ($\rho = 0.78$, $P < 0.01$) and between CAZyme abundance and soil organic carbon ($\rho = 0.82$, $P < 0.01$). Dominant microbial taxa (*Bacillus*, *Pseudomonas*, *Streptomyces*, *Actinobacteria* phyla) showed functional gene enrichment patterns consistent with previous observations (Table 5).

Table 8: Relative Abundance of Dominant Microbial Taxa Identified through Shotgun Metagenomics

Bacterial Phylum/Taxon	Organic (%)	Conventional (%)	P-value	Functional Role
Actinobacteria	31.2 ± 2.4	18.6 ± 1.9	<0.001	Organic matter degradation, stress tolerance
Firmicutes	22.8 ± 2.1	25.3 ± 2.3	0.14	Diverse metabolic functions
Proteobacteria	24.1 ± 2.5	28.9 ± 2.6	<0.05	Nutrient cycling, plant growth promotion
Bacteroidetes	14.2 ± 1.8	16.8 ± 1.9	0.18	Polymer degradation
Acidobacteria	6.8 ± 0.9	7.2 ± 1.1	0.62	Soil habitant, oligotrophic
Bacillus spp.	8.4 ± 1.2	5.1 ± 0.8	<0.01	N fixation, plant growth promotion
Pseudomonas spp.	6.3 ± 0.9	4.2 ± 0.7	<0.05	Stress tolerance, plant protection
Streptomyces spp.	7.1 ± 1.1	3.8 ± 0.6	<0.001	Antibiotic production, organic matter degradation

5. Discussion

Long-term organic farming fundamentally reshapes soil microbial functional potential, as demonstrated by systematic enrichment of nutrient-cycling and carbon-metabolism genes. The 2.3-fold elevation in nitrogenase (nifH) indicates enhanced biological nitrogen fixation capacity, directly supporting plant nitrogen nutrition and reducing synthetic fertilizer dependency [2]. This enrichment reflects selective pressures from nitrogen limitation under organic management and legacy effects of legume-based rotations.

The elevated amoA and denitrification gene abundance suggests a more complete nitrogen cycle within organic systems. Enhanced nitrification and denitrification capacity may facilitate more efficient nitrogen transformations, reducing losses and improving nitrogen-use efficiency—a critical sustainability indicator [3]. Notably, denitrification gene enrichment potentially indicates enhanced mitigation of nitrogen loss through gaseous pathways, though actual expression requires metatranscriptomic validation.

Carbon metabolism gene enrichment, particularly CAZyme families, reflects organic farming's emphasis on organic residue cycling. The 2.1-fold increase in cellulase abundance directly corresponds to decomposition of crop residues returned under organic systems [5]. This enhanced enzymatic potential accelerates carbon cycling and substrate processing, supporting both microbial growth and soil organic matter accumulation. The relationship between CAZyme abundance and soil organic carbon ($\rho = 0.82$) suggests functional genes directly mediate carbon sequestration [1].

Phosphorus mobilization gene enrichment represents another functional advantage. Enhanced phosphatase abundance compensates for limited soluble phosphorus under organic management, reflecting functional adaptation to nutrient-limited conditions [4]. This mechanism demonstrates microbial metabolic versatility as a response to farming practice constraints [2].

Microbial functional diversity indices reveal organic farming supports communities with greater metabolic breadth. Enhanced diversity implies greater ecological insurance for ecosystem functioning and potentially improves soil resilience to environmental stressors [3]. This aligns with contemporary ecological theory linking diversity to functional redundancy and ecosystem stability [6].

Several limitations warrant consideration. Shotgun metagenomics quantifies gene abundance without directly assessing expression; metatranscriptomic studies are necessary to confirm functional activity. Cross-site validation is needed to generalize findings across diverse agroecosystems. Furthermore, temporal sampling at single timepoint prevents assessment of seasonal functional dynamics. Future research should integrate metatranscriptomics, metaproteomics, and metabolomics to validate functional predictions and elucidate metabolite fluxes [5].

6. Conclusion

The functional metagenomic research shows how long-term organic farming helps to increase the number of genes associated with soil microorganisms that are involved in nutrient cycles and

the breaking-down of substances. The processes of nitrogen fixation, nitrification, denitrification, and phosphorus solubilization were observed to take place more frequently with organic farming management. The fact that diversity regarding carbohydrate-active enzymes increased shows how favorable conditions arise for carbon absorption and organic material processing. The functional results from this research help to understand why organic farming improves ecosystem services and how processes connected to microorganisms ensure sustainable agriculture. Functional metagenomics proves that organic agriculture is capable of using microorganisms for effective agricultural production with environmentally safe conditions. It is essential to conduct further studies where several methods are applied simultaneously for a better understanding of soil management and ways of minimizing the

References

1. Lal R. Sequestering carbon and increasing productivity by conservation agriculture. J Soil Water Conserv. 2015;70(5):55A-62A.
2. Mäder P, Fliessbach A, Dubois D, Gunst L, Fried P, Niggli U. Soil fertility and biodiversity in organic farming. Science. 2002;296(5573):1694-7.
3. Van Der Heijden MGA, Bardgett RD, van Straalen NM. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett. 2008;11(3):296-310.
4. Creamer CA, Filley TR, Boutton TW, Bishnoi NC, Wick AF. Agricultural management impacts on soil organic matter molecular composition and turnover in the Texas Blackland Prairie region. Geoderma. 2015;263:86-96.
5. Quince C, Walker AW, Simpson JT, Loman NJ, Segata N. Shotgun metagenomics, from sampling to analysis. Nat Biotechnol. 2017;35(9):833-44.
6. Röthig T, Yap SY, Reiter T, Ochsenkühn MA, Larson H, Gates RD, *et al*. Long-term salinity tolerance is accompanied by major restructuring of the coral bacterial microbiome. Microb Ecol. 2016;71(3):763-77.
7. Bahm M, Hildebrand F, Forslund SK, Anderson JL, Szalay AA, Model F, *et al*. Structure and function of the global topsoil microbiome. Nature. 2018;560(7717):233-7.
8. Caporaso JG, Lauber CL, Costello EK, Berg-Lyons D, Gonzalez A, Stombaugh J, *et al*. Erratum to: moving pictures of the human microbiome. Genome Biol. 2011;12(5):R50.
9. Shade A, Peter H, Allison SD, Baho D, Berga H, Bürgmann H, *et al*. Fundamentals of microbial community resistance and resilience. Front Microbiol. 2012;3:417.
10. Daims H, Lücker S, Wagner M. A new perspective on microbes formerly known as nitrite-oxidizing bacteria. Trends Microbiol. 2016;24(9):699-712.
11. Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. Nucleic Acids Res. 2009;37(Database issue):D233-8.
12. Hug LA, Baker BJ, Anantharaman K, Brown CT, Alexander B, Bhartia AK, *et al*. A new view of the tree of life. Nat Microbiol. 2016;1(5):16048.
13. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, *et al*. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol. 2013;31(9):814-21.
14. McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, *et al*. The comprehensive antibiotic resistance database. Antimicrob Agents Chemother. 2013;57(7):3348-57.
15. Pandit SN, Gopal B, Kullander SO. Fish assemblages of tropical and subtropical Indian lakes. J Fish Biol. 2002;60(4):825-42.
16. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, *et al*. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12(6):R60.
17. Solanas M, Fabregat I, Reverter F, Mora J, Bravo S, Castells A, *et al*. A systems biology approach identifies temporal gene expression changes in BABL mice treated with dietary lipids and its comparison with humans. J Nutr Biochem. 2011;22(8):701-14.
18. Vieira S, Trumbore SE, Camargo PB. Slow turnover rates of soil organic matter in tropical forests. Nature. 2005;437(7056):296-9.
19. Zehr JP, Ward BB. Nitrogen cycling in the ocean: new perspectives on processes and paradigms. Appl Environ Microbiol. 2002;68(3):1015-24.