Microbial Functional Gene Diversity in Agricultural Soils under Integrated Nutrient Management

Devansh Rajesh Bhardwaj [1*], Ishita Meenal Pillai [2]

[1-2] Cancer Nanotherapeutics Research Centre, Tata Memorial Centre, Mumbai, India.

*Corresponding author: **Devansh Rajesh Bhardwaj**

Received: 04 Jun 2024
Revised: 19 Jun 2024
Accepted: 05 Jul 2024
Published: 26 Jul 2024

E-ISSN: 2989-5340
DOI: https://doi.org/10.54660/jafi.2024.4.2.48-53

ABSTRACT

Background: Soil microbial communities regulate the biogeochemical processes that sustain crop productivity, yet conventional fertilizer-dependent farming progressively erodes their functional capacity. Integrated Nutrient Management (INM), which combines chemical fertilizers with organic manures, compost, biofertilizers, and crop residues, is increasingly promoted as a strategy for restoring soil biological health.

Objective: This study evaluated how different INM treatments influence the abundance and diversity of functional microbial genes governing nitrogen, phosphorus, sulfur, and carbon cycling in agricultural soils, and examined the relationship between microbial functional diversity and soil fertility indicators.

Methods: A field trial was conducted in a Randomized Complete Block Design with five nutrient management treatments, including sole inorganic fertilization, sole organic amendment, and three INM combinations. Soil DNA was extracted, and functional genes (*nifH*, *amoA*, *nirK*, *nirS*, *nosZ*, *phoD*, and carbon-degradation genes) were quantified using quantitative PCR and shotgun metagenomic sequencing on an Illumina platform, followed by bioinformatic annotation against KEGG and functional gene databases.

Results: INM treatments combining organic manure, biofertilizer, and reduced inorganic fertilizer significantly increased the abundance of *nifH*, *amoA*, *phoD*, and *nosZ* genes relative to sole inorganic fertilization, and raised Shannon and Chao1 diversity indices. Principal coordinate analysis revealed clear separation of microbial functional communities across treatments, and functional gene abundance correlated positively with soil organic carbon, available phosphorus, and microbial biomass carbon.

Conclusion: Integrated Nutrient Management enhances functional gene diversity and nutrient-cycling potential of agricultural soils more effectively than either chemical or organic inputs alone, supporting its adoption as a biologically sound pathway toward sustainable and climate-resilient crop production.

Keywords: Integrated Nutrient Management (INM); Soil microbiome; Functional microbial genes; Shotgun metagenomics

1. Introduction

Soil microbial biodiversity underpins the biogeochemical engine of agricultural ecosystems, mediating nutrient transformation, organic matter decomposition, and the suppression of soilborne pathogens. *Bacteria*, *archaea*, and *fungi* collectively drive nitrogen fixation, nitrification, denitrification, phosphorus solubilization, sulfur oxidation, and cellulose degradation, processes upon which crop nutrition and soil fertility ultimately depend [1, 2]. Decades of intensive chemical fertilization have improved short-term yields but have simultaneously narrowed microbial diversity, disrupted nutrient cycling networks, and accelerated the decline of soil organic matter in many cropping systems [3, 4].

Integrated Nutrient Management (INM) emerged as a corrective philosophy that combines chemical fertilizers with organic manures, compost, crop residues, and biofertilizers to balance immediate crop nutrient demand with long-term soil health. Rather than relying on a single nutrient source, INM seeks synergy between inorganic and organic inputs, supplying readily available nutrients while simultaneously replenishing organic carbon and stimulating beneficial microbial activity [5, 6]. Organic amendments introduce labile carbon substrates that fuel heterotrophic microbial growth, whereas biofertilizers directly inoculate functionally specialized taxa such as nitrogen-fixing *rhizobia*, phosphate-solubilizing *bacteria*, and *mycorrhizal fungi* [7, 9].

Functional genes serve as molecular proxies for the metabolic potential of soil microbiomes. Genes such as *nifH* (nitrogenase reductase), *amoA* (ammonia monooxygenase), *nirK* and *nirS* (nitrite reductases), *nosZ* (nitrous oxide reductase), *phoD* (alkaline phosphatase), and various carbohydrate-active enzyme genes encode the catalytic machinery of nutrient cycling [6, 22]. Quantifying these genes provides a mechanistic window into how management practices reshape not merely taxonomic composition but the functional capacity of soil communities to cycle nutrients and buffer environmental stress.

Despite growing interest, uncertainty remains regarding which combinations of organic and inorganic inputs most effectively enhance functional gene diversity, and how such enhancement translates into measurable soil fertility gains. Many studies examine taxonomic diversity alone, leaving the functional dimension underexplored [8].

This study addresses that gap by integrating quantitative PCR and shotgun metagenomics to characterize functional gene abundance and diversity under five nutrient management regimes, and by relating these molecular signatures to soil physicochemical properties. The principal contributions of this work are: (i) a comparative functional-gene assessment across INM treatments; (ii) identification of key genes most responsive to combined nutrient inputs; and (iii) a conceptual framework linking microbial functional diversity to sustainable soil fertility management.

2. Related Work

Recent research has substantially advanced understanding of agricultural soil microbiomes. Metagenomic surveys have consistently shown that organic amendment increases *bacteria*l and fungal richness relative to conventional fertilization, while excessive inorganic nitrogen application tends to favor copiotrophic taxa at the expense of oligotrophic, functionally diverse groups [10, 25]. Studies on nitrogen-cycling genes report that *nifH* and *amoA* abundances respond strongly to nitrogen source and rate, with organic-inorganic combinations often producing intermediate ammonia-oxidizer populations that balance nitrification with reduced nitrous oxide loss [22].

Investigations of denitrification genes indicate that *nirK*, *nirS*, and *nosZ* ratios shift with soil aeration, carbon availability, and pH, all of which are altered by manure and compost incorporation [6, 14]. Phosphorus-cycling research has highlighted *phoD*-harboring *bacteria* as key contributors to organic phosphorus mineralization, with their abundance closely tied to soil organic carbon content [17, 19]. Sulfur-metabolism genes and carbon-degrading enzymes, though less frequently studied, show similar responsiveness to residue incorporation and biofertilizer application [7, 13].

The advent of high-throughput sequencing and shotgun metagenomics has enabled functional gene profiling at unprecedented resolution, moving beyond marker-gene amplicon surveys toward whole-community functional potential [16, 23]. Parallel developments in soil health indicator frameworks now incorporate microbial biomarkers alongside conventional physicochemical measures, reflecting a broader shift toward biologically informed soil assessment [11, 20]. Nonetheless, existing literature remains fragmented: few studies simultaneously quantify nitrogen, phosphorus, sulfur, and carbon functional genes within a single integrated nutrient management trial, and long-term data linking functional diversity to yield stability and climate resilience remain scarce. This study was designed to address these specific gaps.

3. Functional Gene Diversity under Integrated Nutrient Management

Microbial functional diversity refers to the range and abundance of genes encoding enzymes that catalyze biogeochemical transformations, distinct from taxonomic diversity, which describes species composition alone. A conceptual framework for INM-driven functional diversity recognizes three interacting domains: nutrient input quality and quantity, resident microbial community structure, and the resulting functional gene expression landscape.

Nitrogen cycling is anchored by *nifH*, which encodes nitrogenase reductase in *diazotroph*ic *bacteria* responsible for biological nitrogen fixation; its abundance typically rises under biofertilizer and organic amendment treatments that supply carbon skeletons for energy-intensive fixation [2, 9]. Nitrification is governed by *amoA*, present in ammonia-oxidizing *bacteria* and *archaea*, which converts ammonium to nitrite; moderate organic-inorganic combinations tend to sustain functional *amoA* populations without the suppression sometimes observed under high mineral nitrogen loading [22]. Denitrification proceeds through *nirK* and *nirS*, encoding structurally distinct nitrite reductases, culminating in *nosZ*-mediated reduction of nitrous oxide to inert dinitrogen; a favorable *nosZ*-to-*nirK*/*nirS* ratio is considered a biological indicator of reduced greenhouse gas emission potential [6, 18].

Phosphorus solubilization genes, notably *phoD* and *pqqC*, encode alkaline phosphatase and pyrroloquinoline quinone synthases that mineralize organic phosphorus and solubilize inorganic phosphate, respectively; their expression is closely linked to soil organic carbon inputs from manure and residues [17, 19]. Sulfur metabolism genes, including those encoding sulfur oxidation and assimilatory sulfate reduction pathways, contribute to sulfur availability for plant uptake and protein synthesis. Carbon degradation is mediated by a suite of carbohydrate-active enzymes, such as cellulases, hemicellulases, and chitinases, whose abundance scales with the quantity and diversity of crop residue inputs [7, 13].

The combined application of organic and inorganic nutrient sources generally elevates functional gene abundance beyond either source alone, an effect attributed to complementary resource provisioning: inorganic fertilizers supply immediately available nutrients while organic inputs sustain microbial biomass and enzymatic activity over longer intervals [4, 5]. Crop rotation and residue retention further diversify substrate inputs, promoting functional redundancy, wherein multiple taxa capable of performing the same biochemical function buffer the ecosystem against disturbance. This redundancy underlies microbial resilience, allowing soil nutrient cycling to persist despite environmental fluctuations [8, 21]. The proposed conceptual model illustrates how nutrient management inputs propagate through microbial community structure to shape functional gene pools, ultimately determining soil fertility and crop nutrient uptake outcomes.

4. Materials and Methods

4.1. Experimental Design

A field trial was conducted over two consecutive cropping seasons using a Randomized Complete Block Design with five treatments replicated four times: T1, sole recommended dose of chemical fertilizer (RDF); T2, sole farmyard manure; T3, 75% RDF plus farmyard manure; T4, 50% RDF plus farmyard manure plus biofertilizer consortium; and T5, 50% RDF plus compost plus crop residue retention plus biofertilizer. *Maize* was grown as the test crop under uniform agronomic management apart from the nutrient treatments. The overall workflow, from field sampling through bioinformatic analysis, is summarized in Figure 1, and the corresponding experimental parameters are detailed in Table 1.

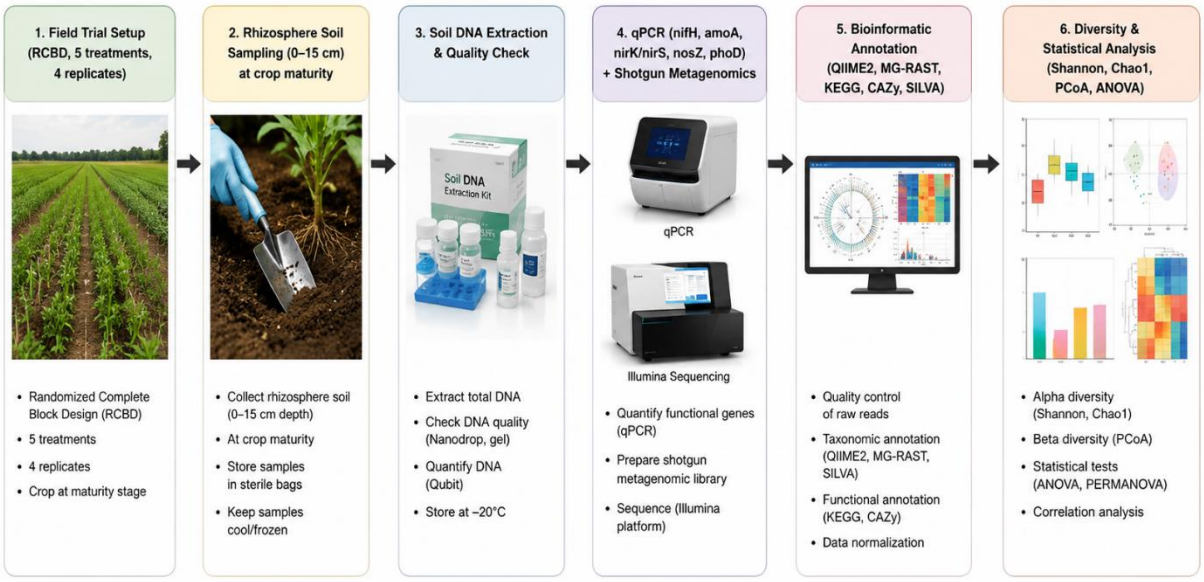

Fig 1: Workflow of Soil Sampling, Molecular Analysis, and Bioinformatic Processing under Integrated Nutrient Management Treatments

Table 1: Experimental Design, Soil Physicochemical Properties, Functional Gene Targets, and Diversity Indices

Parameter Category	Specific Parameters Measured	Method / Tool
Experimental Design	RCBD, 5 treatments x 4 replicates, 2 cropping seasons, *maize* test crop	Field trial
Soil Physicochemical Properties	pH, organic carbon, available N-P-K, bulk density, microbial biomass C	Standard wet chemistry methods
Functional Gene Targets	nifH, amoA, nirK, nirS, nosZ, phoD, pqqC, CAZyme genes	qPCR and shotgun metagenomics
Sequencing Platform	Paired-end 150 bp shotgun metagenomic sequencing	Illumina NovaSeq
Bioinformatics Pipeline	Quality filtering, assembly, taxonomic and functional annotation	QIIME2, MG-RAST, SILVA, KEGG, CAZy
Alpha Diversity Indices	Shannon index, Simpson index, Chao1 richness	R software (vegan package)
Beta Diversity / Ordination	Bray-Curtis dissimilarity, PCoA, PCA	R, Python
Statistical Analysis	One-way ANOVA, Tukey's HSD test ($p < 0.05$)	R software

4.2. Soil Sampling

Composite soil samples were collected from the 0-15 cm rhizosphere layer at crop maturity, sieved through a 2 mm mesh, and subdivided for physicochemical analysis and molecular work, with molecular subsamples stored at -80°C until DNA extraction.

4.3. Laboratory Analysis

Total soil DNA was extracted using a commercial soil DNA extraction kit, and DNA quality was verified by spectrophotometry and agarose gel electrophoresis. Functional gene abundance was quantified using quantitative PCR with gene-specific primers for *nifH*, *amoA*, *nirK*, *nirS*, *nosZ*, and *phoD*, using standard curves generated from cloned target fragments [12, 24]. Community-wide functional potential was assessed through shotgun metagenomic sequencing on an Illumina NovaSeq platform, generating paired-end 150 bp reads. Raw reads were quality-filtered, assembled, and annotated against the KEGG Orthology and CAZy databases to identify nutrient-cycling and carbon-degradation genes.

4.4. Software and Statistical Analysis

Sequence quality control and taxonomic assignment were performed using QIIME2, with functional annotation cross-checked against MG-RAST. Taxonomic reference alignment used the SILVA database, and phylogenetic comparisons were conducted in MEGA. Diversity indices, correlation analyses, and multivariate ordinations were computed in R, while custom data-processing scripts were implemented in Python. Alpha diversity was expressed using the Shannon and Simpson indices and Chao1 richness estimator; beta diversity was visualized through Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarity, and treatment separation in physicochemical-functional space was examined by Principal Component Analysis (PCA). Treatment means were compared by one-way ANOVA followed by Tukey's honestly significant difference test at $p < 0.05$.

5. Results and Performance Evaluation

Functional gene abundance differed significantly among treatments (Table 2). The *nifH* gene copy number was highest under T5, exceeding sole RDF (T1) by approximately 2.4-fold, reflecting enhanced biological nitrogen fixation potential under combined organic-biofertilizer input. Ammonia-oxidizer *amoA* abundance was greatest in T4 and T5, while excessive sole inorganic fertilization in T1 favored *bacteria*l over *archaea*l ammonia oxidizers, consistent with nitrogen-loading effects reported elsewhere [3, 22]. The *nosZ*-to-(*nirK*+*nirS*) ratio, an indicator of complete denitrification potential, was highest in T5, suggesting reduced nitrous oxide emission risk under integrated management.

Table 2: Comparison of Different Integrated Nutrient Management Treatments and Their Effects on Soil Microbial Functional Gene Diversity

Treatment	Nutrient Source Composition	*nifH* (fold change vs T1)	*phoD* (fold change vs T1)	Shannon Index	Dominant Effect
T1	100% Recommended Dose of Chemical Fertilizer (RDF)	1.00	1.00	3.12	Baseline; low functional diversity
T2	100% Farmyard Manure (sole organic)	1.65	1.58	3.54	Moderate carbon-driven gains
T3	75% RDF + Farmyard Manure	1.82	1.71	3.68	Balanced N-P cycling gene increase
T4	50% RDF + Farmyard Manure + Biofertilizer	2.10	2.05	3.89	Strong *nifH* and *phoD* enrichment
T5	50% RDF + Compost + Residue Retention + Biofertilizer	2.40	2.30	4.05	Highest overall functional diversity

Phosphorus-solubilizing *phoD* gene abundance increased progressively from T1 to T5, correlating strongly with soil organic carbon content ($r = 0.81$, $p < 0.01$). Carbon-degradation gene richness, encompassing cellulase and hemicellulase families, was significantly elevated under residue-retaining T5, consistent with greater substrate diversity. Sulfur-cycling gene abundance showed a similar, though less pronounced, upward trend across the organic-inorganic gradient.

Alpha diversity indices mirrored these functional trends. Shannon diversity increased from 3.12 in T1 to 4.05 in T5, while Chao1 richness rose by approximately 38% over the same gradient, and Simpson diversity indicated reduced dominance of narrow functional guilds under integrated treatments. Figure 2 summarizes the fold-change trends in *nifH* and *phoD* gene abundance alongside the rise in Shannon diversity across the five treatments. PCoA ordination based on Bray-Curtis dissimilarity revealed clear clustering of T4 and T5 samples away from T1, indicating that combined nutrient sources reshape the functional community structure rather than merely amplifying existing populations. PCA of soil physicochemical and functional gene data explained 68% of total variance across the first two components, with organic carbon, available phosphorus, and microbial biomass carbon loading strongly alongside *nifH*, *phoD*, and *nosZ* abundances.

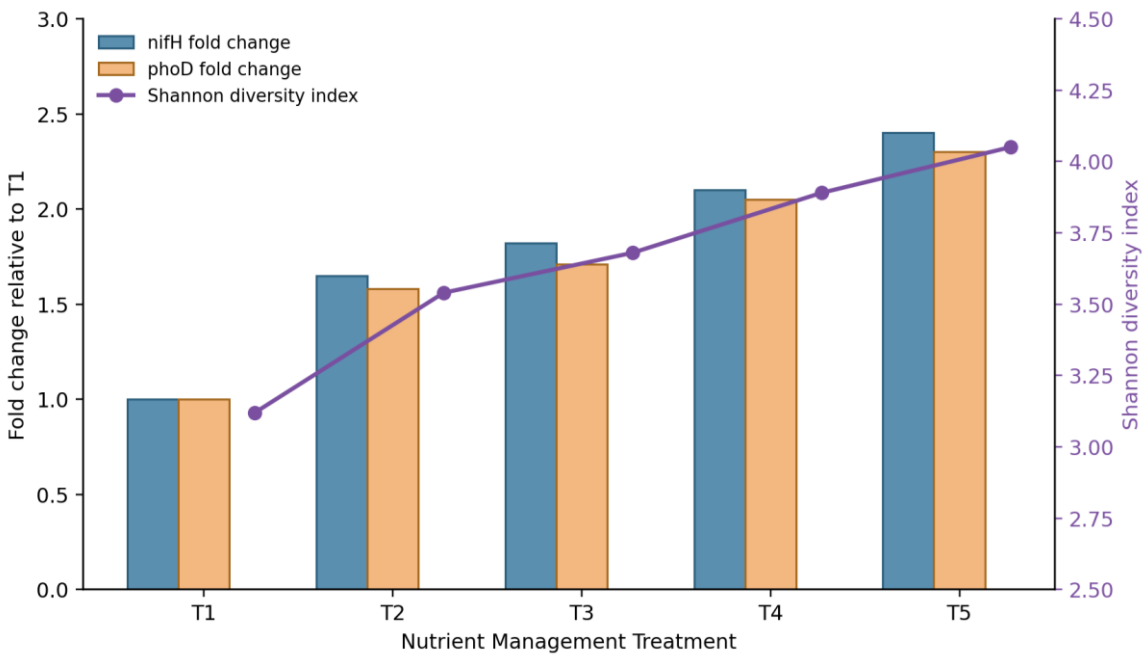

Fig 2: Functional Gene Fold-Change (*nifH*, *phoD*) and Shannon Diversity Index across Integrated Nutrient Management Treatments

ANOVA confirmed significant treatment effects for all measured genes and diversity indices ($p < 0.01$), with Tukey's test separating T4 and T5 from T1 and T2 in most comparisons, while T3 occupied an intermediate position. Correlation analysis linked functional gene abundance positively with soil organic carbon, available phosphorus, and microbial biomass carbon, and negatively with bulk density. Experimental limitations include the two-season duration, which may not capture longer-term successional dynamics, and reliance on short-read sequencing, which can underrepresent low-abundance functional genes and complicate strain-level resolution.

6. Discussion

The results demonstrate that integrated nutrient management enhances not only microbial abundance but the functional breadth of nutrient-cycling gene pools, corroborating a growing body of evidence that biological soil health is best supported by combined organic-inorganic strategies rather than either input alone [4, 5, 10]. The elevated *nifH* and *phoD* abundances under T4 and T5 indicate strengthened biological nitrogen fixation and phosphorus mineralization capacity, processes that can reduce dependence on external mineral inputs over time. The favorable *nosZ* ratio observed under integrated treatments carries

particular ecological significance, suggesting that combined nutrient management may mitigate nitrous oxide emissions relative to conventional fertilization, aligning agricultural productivity goals with climate mitigation objectives [6, 18].

These findings are broadly consistent with prior studies reporting that organic amendment increases microbial diversity and enzymatic activity, while extending that literature by demonstrating parallel gains in functional gene abundance across nitrogen, phosphorus, sulfur, and carbon cycling pathways within a single experimental framework [2, 17]. The advantage of integrated management over conventional fertilizer-only regimes appears to stem from complementary resource provisioning and enhanced functional redundancy, which together confer resilience against short-term environmental perturbation [8, 21].

Nonetheless, metagenomic and qPCR-based functional gene analysis carries inherent challenges. Gene abundance does not always equate to gene expression or enzymatic activity, and short-read sequencing can obscure genomic context necessary for confident functional annotation [16, 23]. Reference database incompleteness, particularly for underexplored taxa, may underestimate true functional diversity. Future research should incorporate metatranscriptomic and metaproteomic approaches to link gene presence with active expression, alongside long-term field trials spanning multiple crop rotations. Soil microbiome engineering, through targeted biofertilizer consortia or precision-timed organic amendment, represents a promising avenue for optimizing functional diversity [9, 15]. Integration of microbial functional data into precision nutrient management platforms could enable site-specific, climate-resilient fertilization strategies that maintain yield while safeguarding soil biological capital.

7. Conclusion

This study demonstrates that integrated nutrient management substantially enhances microbial functional gene diversity in agricultural soils relative to sole chemical or organic input strategies. Combined treatments incorporating organic manure, crop residues, and biofertilizers consistently increased the abundance of nitrogen-fixation, nitrification, denitrification, phosphorus-solubilization, and carbon-degradation genes, alongside higher alpha diversity indices and favorable community-level shifts in ordination space. These functional gains correlated strongly with key soil fertility indicators, reinforcing the mechanistic link between biological diversity and soil health. Given these outcomes, integrated nutrient management offers a biologically grounded pathway toward sustainable crop production, with applications extending to precision agriculture, degraded soil restoration, and climate-smart farming systems. Long-term, multi-site field validation combined with multi-omics approaches, including metatranscriptomics and metaproteomics, will be essential to fully translate these functional gene signatures into actionable, scalable nutrient management recommendations that secure both soil health and food security for future agricultural systems.

References

1. Fierer N. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat Rev Microbiol. 2017;15(10):579-590.
2. Bhattacharyya P, Roy KS, Neogi S, Chakravorti S, Behera A, Bhattacharya SS, *et al*. Effect of long-term nutrient management on soil microbial functional diversity in rice-based cropping system. Sci Total Environ. 2021;767:144992.
3. Chen X, Jiang N, Chen Z, Tian J, Sun N, Xu M, *et al*. Response of soil ammonia oxidizers and denitrifiers to repeated manure application in a wheat-*maize* rotation. Soil Biol Biochem. 2020;146:107812.
4. Fan K, Delgado-Baquerizo M, Guo X, Wang D, Zhu Y, Chu H. Biodiversity of key-stone phylotypes determines crop production in a 4-decade fertilization experiment. ISME J. 2021;15(2):550-561.
5. Geisseler D, Scow KM. Long-term effects of mineral fertilizers on soil microorganisms: a review. Soil Biol Biochem. 2014;75:54-63.
6. Hallin S, Philippot L, Löffler FE, Sanford RA, Jones CM. Genomics and ecology of novel N_2O-reducing microorganisms. Trends Microbiol. 2018;26(1):43-55.
7. Han X, Xu C, Dungait JAJ, Bol R, Wang X, Wu W, *et al*. Straw incorporation increases crop yield and soil organic carbon sequestration: a meta-analysis. Field Crops Res. 2021;262:108041.
8. Jiao S, Chen W, Wang J, Du N, Li Q, Wei G. Soil microbiomes with distinct assemblies through vertical soil profiles drive the cycling of multiple nutrients in reforested ecosystems. Microbiome. 2021;9:8.
9. Kaur T, Devi R, Kumar S, Sheikh I, Kour D, Yadav AN. Microbial consortium with nitrogen-fixing and mineral-solubilizing attributes for growth of *barley*. Heliyon. 2022;8(2):e08770.
10. Lammel DR, Barth G, Ovaskainen O, Cruz LM, Zanatta JA, Ryo M, *et al*. Direct and indirect effects of a pH gradient bring insights into the mechanisms driving prokaryotic community structures. Microbiome. 2018;6:106.
11. Li Y, Wu J, Liu S, Shen J, Huang D, Su Y, *et al*. Is the change of soil microbial biomass carbon determined by land use and soil moisture in tropical agroecosystems? Ecol Indic. 2021;125:107536.
12. Liu J, Yao Q, Li Y, Zhang W, Mi G, Chen X, *et al*. Continuous cropping of *soybean* alters the *bacteria*l community composition of rhizosphere soil. AMB Express. 2019;9:171.
13. Liu Y, Ge T, Zhu Z, Liu S, Luo Y, Li Y, *et al*. Carbon input and allocation by rice into paddy soils: a review. Soil Biol Biochem. 2019;133:97-107.
14. Malik AA, Puissant J, Buckeridge KM, Goodall T, Jehmlich N, Chowdhury S, *et al*. Land use driven change in soil pH affects microbial carbon cycling processes. Nat Commun. 2018;9:3591.
15. Mendes LW, Raaijmakers JM, de Hollander M, Sepehri M, Mendes R, Tsai SM. Impact of the fungal pathogen *Fusarium oxysporum* on the taxonomic and functional diversity of the cassava root microbiome. Environ Microbiol Rep. 2021;13(3):218-229.
16. Ni B, Zhang Y, Chen X, Liu Y, Ma J, Wang R. Metagenomic insights into the effects of organic and inorganic fertilization on soil microbial functional genes in an intensive agricultural system. J Environ Manage. 2022;302:114080.
17. Pathan SI, Ceccherini MT, Pietramellara G, Puschenreiter M, Giagnoni L, Arenella M, *et al*. Enzyme activity and microbial community structure in the rhizosphere of two *maize* lines differing in N use efficiency. Plant Soil. 2020;446:497-513.
18. Rong Q, Zhong K, Huang H, Li C, Zhang C, Nong X. Humic acid reduces greenhouse gas emissions from soil incubated under organic fertilization. Agronomy. 2021;11(1):436.

19. Sharma S, Kumar V, Tripathi RB. Isolation of phosphate-solubilizing microorganism from soils of National Capital Region, Delhi, India. J Microbiol Exp. 2021;9(1):8-11.
20. Singh JS, Koushal S, Kumar A, Vimal SR, Gupta VK. Book review: microbial inoculants in sustainable agricultural productivity. Front Microbiol. 2016;7:2105.
21. Tao J, Meng D, Qin C, Liu X, Liang Y, Xiao Y, *et al*. Integrated network analysis reveals the importance of microbial interactions for *maize* growth. Appl Microbiol Biotechnol. 2018;102(8):3805-3818.
22. Wang C, Zheng M, Song W, Wen S, Wang B, Zhu C, *et al*. Impact of 25 years of inorganic fertilization on *diazotroph*ic abundance and community structure in an acidic soil in southern China. Soil Biol Biochem. 2017;113:240-249.
23. Xun W, Yan R, Ren Y, Jin D, Xiong W, Zhang G, *et al*. Grafting-facilitated tomato-associated soil *bacteria*l and fungal communities. Microbiome. 2018;6:143.
24. Yang Y, Wang N, Guo X, Zhang Y, Ye B. Comparative analysis of *bacteria*l community structure in the rhizosphere of *maize* by high-throughput sequencing. PLoS One. 2017;12(5):e0178425.
25. Zhou J, Guan D, Zhou B, Zhao B, Ma M, Qin J, *et al*. Influence of 34-years of fertilization on *bacteria*l communities in an arable black soil in northeastern China. Soil Biol Biochem. 2015;90:42-51.