BMC Plant Biology

RESEARCH Open Access

Metagenomic insights reveal the impact of natural farming on soil nutrients, enzyme activities, microbial communities, and yield in turmeric cultivation

Kartik Gajjar[1], Sweta Patel[2], Mahendra Chaudhary[2], Dhanvanti Agrawal[3], Rajiv Maniyar[3], Doongar Chaudhary[3], C. K. Patel[2], Chaitanya Joshi[1*], Madhvi Joshi[1*] and Darshan Dharajiya[1*]

Abstract

Background Turmeric (*Curcuma longa* L.) is a key spice, medicinal and industrially important crop that is increasingly being cultivated under sustainable practices such as natural farming system (NFS). This study compares NFS and conventional/chemical farming system (CFS) in terms of soil physicochemical properties, enzyme activities, microbial diversity, and yield parameters, providing a comprehensive understanding of their ecological and agronomic impacts.

Results Field experiments evaluated nine NFS treatments alongside CFS for high throughput amplicon (16S rRNA and ITS) metagenomics, soil physicochemical properties, enzyme activities, and yield parameters. NFS treatments with ≥ 5 t/ha mulching and 4 t/ha *Ghanjeevamrit* (i.e. NFS-T6 and NFS-T9) significantly enhanced soil organic carbon (~ 0.25%), nitrogen (~ 239.9 kg/ha), and phosphorus (~ 38.16 kg/ha), alongside elevated enzyme activities like alkaline phosphatase (ALP; ~112.11 µg PNP/g/hr) and protease (PR; ~16.22 µg Tyrosine/g/hr). These treatments also exhibited significantly higher microbial richness and evenness (Shannon index: ~5.09; Simpson index: ~0.981). NFS enriched beneficial bacterial (e.g., *Priestia*, unclassified Acidobacteria, etc.) and saprophytic fungal genera (e.g., *Humicola*, *Mortierella*, etc.), enhancing soil nutrient cycling and soil health. Conversely, CFS enriched chemical-resilient and pathogenic taxa (e.g., *Alternaria*, *Curvularia*, etc.). NFS-T5 (40.66 t/ha) and NFS-T9 (40.47 t/ha) yielded over twice the fresh rhizome compared to CFS. NFS treatments recorded higher net returns and Benefit-Cost Ratios (BCRs) of 7.51 to 10.57. Microbial profiling of natural farming (NF) inputs (*Beejamrit*, *Jeevamrit*, and *Ghanjeevamrit*) showed distinct bacterial and fungal communities influencing soil microbiome structure. Co-occurrence network analysis of NF soils revealed that microbial taxa introduced via NF inputs had limited integration into native soil communities, indicating selective incorporation governed by competitive ecological interactions.

*Correspondence:
Chaitanya Joshi
joshi.chaitanya@gmail.com
Madhvi Joshi
madhvimicrobio@gmail.com
Darshan Dharajiya
darshanbiotech1@gmail.com; darshan.scib@gbrc.res.in

Full list of author information is available at the end of the article

Conclusions Natural farming practices significantly enhanced soil fertility, microbial community structure, enzymatic activity (ALP and PR), and turmeric productivity with superior BCRs. These findings provide scientific evidence supporting natural farming as a viable and sustainable agricultural approach, contributing to improved soil health and crop performance in turmeric cultivation.

Keywords Natural farming, Turmeric, Soil metagenomics, Sustainable agriculture, *Ghanjeevamrit*, *Jeevamrit*, *Beejamrit*, Soil health

Introduction

Turmeric (*Curcuma longa* L.), a medicinal and spice crop widely cultivated in tropical and subtropical regions, plays a significant role in traditional medicine, culinary applications, agro-industrial sectors, textile, pharmaceutical and cosmetics industries [1]. The growing demand for turmeric, particularly for its bioactive compound curcumin, has led to increased cultivation intensity, often relying on chemical fertilizers and pesticides. However, excessive use of agrochemicals in conventional farming (CF) depletes soil health, disrupts microbial diversity, and increases environmental pollution [2]. There is a growing emphasis on sustainable farming approaches such as natural farming over CF among smallholder farmers to mitigate these impacts [3, 4]. Natural farming (NF) is an ecological approach that eliminates synthetic fertilizers and pesticides, instead relying on locally prepared cow-based traditional bioformulations such as *Jeevamrit*, *Ghanjeevamrit*, *Beejamrit*, and the practice of mulching [3]. It enhances soil health, crop yield, support beneficial microbial communities with reduced cost of inputs [3, 5]. NF inputs are widely applied across various crops, including cereals, pulses, oilseeds, vegetables, and fruits [6]. *Beejamrit* serves as a seed treatment that promotes germination, protects against seed-borne pathogens, and supports early seedling vigor [7]. *Jeevamrit* and *Ghanjeevamrit* function as biofertilizers that improve soil fertility, stimulate beneficial microbes, and enhance nutrient uptake efficiency in plants [3]. Additionally, mulching improves soil moisture retention, aeration, and temperature regulation while reducing erosion and nutrient loss, thereby maintaining long-term soil health [8].

Soil microbiomes play a crucial role in nutrient transformation, organic matter decomposition, disease suppression, and plant growth promotion [9]. The turmeric rhizosphere harbors a diverse microbial community, as assessed using cultivable techniques, which plays a role in influencing crop productivity [10]. Studies have shown that NF promotes beneficial microbial populations, including plant growth-promoting rhizobacteria (PGPR) such as *Bacillus* and *Pseudomonas*, which enhance nutrient availability and pathogen resistance in turmeric [11]. Furthermore, metagenomic studies have revealed shifts in microbial diversity and function in response to different agricultural practices, highlighting the importance of understanding soil microbial dynamics under NF [3].

Unlike CF, NF practices encouraged the proliferation of beneficial microbes that enhance plant defense mechanisms and improve nutrient cycling efficiency in apple and rice fields [12, 13]. Additionally, NF supported the growth of beneficial microbial phyla involved in organic matter decomposition and nutrient cycling in rhizosphere soil [14]. Research suggested that turmeric rhizomes grown in organic soil exhibited better growth and development compared to those in CF and organic farming (OF) positively influence the growth and quality of black turmeric [15]. Study related to turmeric cultivation under OF is limited and scientific studies on the comparative impact of NF and CF on turmeric-associated microbial communities remain unexplored. Thus, the present study aimed to evaluate the impact of NF and CF on soil microbial diversity, nutrient properties and enzyme activity in turmeric cultivation. The findings will contribute to understanding the microbial mechanisms underlying sustainable turmeric farming and provide insights into the potential benefits of NF for improving soil health, soil nutrients, and rhizome yield.

Materials and methods

Experimental design and field sampling

A field experiment on turmeric (*Curcuma longa* var. GNT-3) was conducted under a semi-arid climate at the Centre for Organic and Natural Farming Research, Centre for Natural Resources Management, Sardarkrushinagar Dantiwada Agricultural University (SDAU), Sardarkrushinagar, Banaskantha, Gujarat, India (24°19′ N, 72°19′ E) from June 2023 to January 2024. Before the experiment, the NF plot had followed organic practices (applications of FYM and plant mulch) for five years without chemical inputs, while the CF plot was managed with recommended fertilizer and pesticide applications. In previous crop season both plots remained fallow, and no prior turmeric cultivation was done. The crop was sown simultaneously under the conventional farming system (CFS) and natural farming system (NFS). Recommended agronomic practices were followed for CFS to serve as a control for comparison with NFS treatments (Table 1). The NFS experimental design and treatment details are shown in Supplementary Figure S1. Sampling details under CFS and NFS are provided in Supplementary Table S1.

Table 1 Details of treatments used under natural and conventional farming practices in turmeric cultivation

Particulars	Details
Spacing	55-20 cm × 20 cm (Paired row)[#]
Seed rate	2200–2500 kg/ha[#]
Conventional Farming System (CFS) control	
Fertilizer	Urea – 79.50 kg/ha in 2 split doses at before sowing and 90 DAS DAP – 130.4 kg/ha before sowing MoP – 100 kg/ha before sowing
Natural Farming System (NFS) treatments	
NFS-T1	No Mulch + *Ghanjeevamrit* @ 2.5 t/ha[*]
NFS-T2	No Mulch + *Ghanjeevamrit* @ 3.5 t/ha[*]
NFS-T3	No Mulch + *Ghanjeevamrit* @ 4.0 t/ha[*]
NFS-T4	Wheat straw mulch @ 5.0 t/ha + *Ghanjeevamrit* @ 2.5 t/ha[*]
NFS-T5	Wheat straw mulch @ 5.0 t/ha + *Ghanjeevamrit* @ 3.5 t/ha[*]
NFS-T6	Wheat straw mulch @ 5.0 t/ha + *Ghanjeevamrit* @ 4.0 t/ha[*]
NFS-T7	Wheat straw mulch @ 7.5 t/ha + *Ghanjeevamrit* @ 2.5 t/ha[*]
NFS-T8	Wheat straw mulch @ 7.5 t/ha + *Ghanjeevamrit* @ 3.5 t/ha[*]
NFS-T9	Wheat straw mulch @ 7.5 t/ha + *Ghanjeevamrit* @ 4.0 t/ha[*]

[#]Spacing and seed rate were same for both farming system

[*]Seed treatment with *Beejamrit* (200 ml/kg) + *Jeevamrit* @500 l/ha at sowing, 30 and 60 DAS + *Jeevamrit* spray @ 7.5% at 90 and 120 DAS

In CFS plot, soil samples were collected in a zig-zag pattern from nine points to prepare composite samples for both before sowing (BS) and at harvest (AH) as shown in Supplementary Figure S2. In NFS, BS samples were collected similarly to form a composite sample. After pre-sowing collection, nine NFS treatments (NFS-T1 to NFS-T9) were applied in four replications, and post-harvest composite soil samples were collected from each replicated plot (one per treatment per replication) as shown in Supplementary Figure S3. Accordingly, CFS-BS, CFS-AH, and NFS-BS samples were composite samples analyzed in triplicate, whereas NFS-AH samples (NFS-T1 to NFS-T9) represented composite samples analyzed in four biological replicates. Natural farming inputs, *Jeevamrit*, *Ghanjeevamrit*, and *Beejamrit* were used as NFS treatment components and were also collected for amplicon metagenomics. All soil samples were stored at − 20 °C until analysis of physico-chemical properties, enzyme activities, and microbial profiles. For plant nutrient and yield parameter analysis, in NFS, five plants per treatment per replication were randomly selected and mean values were calculated, whereas in CFS, five plants were randomly selected and averaged for analysis.

Assessment of soil physicochemical properties

Standard analytical methods were used to assess soil chemical and nutrient properties. Soil pH and electric conductivity (EC) were measured in a 1:2.5 soil-to-liquid suspension using 1 M KCl for pH and distilled water for EC, analyzed with a potentiometric method and a conductivity TDS meter (Systronics, India). Organic carbon (OC) was determined using the Walkley-Black wet oxidation method [16]. Available nitrogen (N) was estimated by the alkaline permanganate method [17], available phosphorus (P_2O_5) by spectrophotometric method [18], and available potassium (K) using flame photometric method [19]. Sulphate (SO_4) content was determined using the turbidimetric method [20]. Available micronutrients (Fe, Mn, Zn, and Cu) were extracted using diethylene-triaminepentaacetic acid (DTPA) extraction method [21] and quantified via Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, SPECTROBLUE FMX 36, Kleve, Germany).

Evaluation of soil microbial enzyme activities

Soil enzyme activities indicative of carbon, nitrogen, phosphorus, and sulphur cycling were analyzed. β-glucosidase (BGL) activity was measured following the protocol described previously [22] with some modifications [23]. Dehydrogenase (DHA), Urease (URE), Protease (PR), Alkaline Phosphatase (ALP), and Sulphatase (STS) activities were determined using protocols described previously [24–28].

Analysis of plant nutrient content and yield parameters

Total nitrogen and phosphorus in straw samples were estimated using the micro Kjeldahl's method and Vanadomolybdophosphoric acid method with HNO_3, respectively [29]. Total potassium was determined using a flame photometer (Cole Parmer, Chicago, USA) following [30]. Total sulphur and micronutrients (Fe, Mn, Zn, and Cu) were analyzed using the same methods as those applied for soil nutrient analysis. At harvest, plant population, plant height (cm), number of primary and secondary rhizomes per plant, and fresh rhizome yield (t/ha) were recorded for both CF and NF. The cost of total inputs used in CF and NF for two conditions (farmer owns a cow and farmer doesn't own a cow) were calculated (Supplementary Table S2).

Amplicon (16S rRNA and ITS) metagenomics of soil and natural farming inputs

DNA was extracted from soil and NF input samples using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) with vortexing for 15 min to ensure thorough homogenization and lysis. The DNA quality and integrity were assessed, and 16S rRNA gene (V3-V4 region) was amplified via polymerase chain reaction (PCR) using

Illumina-barcoded primers (ILL_HP-16S-F and ILL_HP-16S-R) [31]. The ITS region was amplified using ITS1 region specific primers (ILL_HP-FN-F and ILL_HP-FN-R) [32]. The resulting amplicons were purified using QIAseq beads (QIAGEN, Hilden, Germany), followed by index PCR with IDT-compatible index (Integrated DNA Technologies, Coralville, IA, USA). Purified libraries were normalized to equimolar ratios (4 nM), and their quality was assessed using QIAxcel advanced (Qiagen, Hilden, Germany). Sequencing was performed on the Illumina NovaSeq 6000 platform using paired-end chemistry (250 × 2), following the manufacturer's guidelines (Illumina, San Diego, CA, USA).

Bioinformatics and statistical data analysis

Quality control (QC) of raw FASTQ sequences from 16S rRNA and ITS amplicon metagenomics were performed using FASTQC and analyzed on the Illumina BaseSpace platform (https://basespace.illumina.com). Taxonomic classification of amplicon sequence variants (ASVs) was conducted using the RDP Classifier 2.14 for bacteria and the UNITE database v10 for fungi. Samples with low-quality data and outliers were removed. The filtered sequencing data were normalized to an even sequencing depth based on the minimum library size. Filtered sequencing data were normalized to even sequencing depth based on the minimum library size. Downstream statistical analysis and data visualization were performed using the Microeco R package [33]. Alpha diversity was assessed using Observed and Chao1 indices (species richness) and Shannon and Simpson indices (species richness and evenness), and for comparison between groups and significance, one-way ANOVA followed by Tukey's HSD post hoc test was used. Beta diversity was evaluated using Bray-curtis indices for principal coordinates analysis (PCoA) and non-metric multidimensional scaling (NMDS). Outlier samples were not considered for data analysis. Linear discriminant analysis (LDA) Effect Size (LEfSe) was used to identify discriminant taxonomic features between soil groups, applying an LDA score >3.0 and p-value ≤0.01 as thresholds. Correlation analysis between amplicon metagenomics data, soil chemical properties, and enzyme activities were performed using redundancy analysis (RDA) and Pearson correlation coefficients. Core microbiome analysis was conducted using MicrobiomeAnalyst 2.0 [33], identifying taxa with a relative abundance threshold of >1% and a prevalence cutoff of 50%. Comparative bar plots of bacterial and fungal genera were analyzed using one-way ANOVA followed by Tukey's HSD post hoc test for significance, visualized with ggplot2 [34]. Venn diagrams were generated to identify common and unique genera between NF inputs and soil samples [35]. To generate a combined microbial co-occurrence network for NFS and NF input, individual networks were constructed using SparCC for correlation analysis, implemented via the SpiecEasi method in R [36]. Taxa with relative abundance >0.1% and correlation coefficients >0.6 (adjusted p <0.05) were retained based on random matrix theory method. Data of nodes and edges were merged by identifying common nodes and edges between NF soils and NF inputs. The merged data were imported into Gephi for visualization. Networks topological properties were analyzed, and visualization was performed in Gephi (ver. 0.10.1) [37].

Results

Soil physicochemical properties, nutrients, and enzyme activities

The Supplementary Figure S4 (A-K) provides a comparative analysis of key soil physicochemical parameters, including pH, electrical conductivity (EC), organic carbon (OC), and essential macro- and micronutrients (N, P, K, S, Fe, Mn, Zn, and Cu), across different soil groups under CFS and NFS. Significant variations in available soil nutrients and chemical properties were observed among different soil groups. Soils of CFS and NFS exhibited significant reduction in OC levels e.g. 0.13% (CFS-AH) from 0.17% (CFS-BS) and 0.18–0.25% (NFS-AH) from 0.35 (NFS-BS), respectively. Available nitrogen levels were relatively higher in NFS treatments (167.8 (NFS-T9)-239.9 (NFS-T7) kg/ha) compared to CFS groups (120.7 (CFS-BS)-144.3 (CFS-AH) kg/ha). Notably, available P was significantly higher in NFS-BS (64 kg/ha) and reduced in NFS-AH ranged from 38.2 (NFS-T6) to 27.9 kg/ha (NFS-T9). In CFS also, it was significantly reduced to 23.0 kg/ha (CFS-AH) from 27.9 kg/ha (CFS-BS). A significant increase in available K was observed in CFS, rising from 146.4 kg/ha (CFS-BS) to 164.9 kg/ha (CFS-AH). In contrast, K levels declined across NFS treatments (126.6 (NFS-T3)-142.5 (NFS-T6) kg/ha) compared to NFS-BS (183.6 kg/ha). Moreover, Mn and Cu levels showed a noticeable decline in NFS treatments compared to NFS-BS whereas, available S, Fe and Zn remained relatively stable with minor variations across NFS treatments.

The results of soil enzyme activity analysis are illustrated in Supplementary Figure S5 (A-F). BGL activity significantly increased in CFS-AH (33.33 µg PNP/g/hr) compared to CFS-BS (14.26 µg PNP/g/hr). However, in NFS treatments, BGL activity decreased (30.14 (NFS-T1)-39.51 (NFS-T6) µg PNP/g/hr) compared to NFS-BS (60.39 µg PNP/g/hr). ALP activity showed a significant increase in certain NFS treatments, with the highest activity observed in NFS-T3 (112.11 µg PNP/g/hr), followed by NFS-T4 (104.12 µg PNP/g/hr) and NFS-T5 (98.41 µg PNP/g/hr). In CFS, ALP activity was increased to 71.9 µg PNP/g/hr (CFS-AH) from 47.48 µg PNP/g/hr (CFS-BS), remained very low from NFS treatments.

For STS, DHA, and URE activities were relatively stable across NFS treatments (NFS-T1 to NFS-T9), which remained at par with CFS-AH. PR activity increased significantly in some NFS treatments, with NFS-T9 exhibited highest value (16.22 µg Tyrosine/g/hr), followed by NFS-T7 and NFS-T5 (14.50 and 14.39 µg Tyrosine/g/hr, respectively), increased from 12.56 µg Tyrosine/g/hr (NFS-BS). The PR activity in NFS was significantly higher than CFS (6.4 µg Tyrosine/g/hr (CFS-BS) and 10.6 µg Tyrosine/g/hr (CFS-AH)). Interestingly, PR activity displayed a dose-dependent increase in activity with higher applications of *Ghanjeevamrit* and mulch.

Plant nutrient profiles and yield attributes

Plant nutrient analysis revealed significant variations among different treatments (Supplementary Figure S6 (A-H)). Certain NFS treatments exhibited significantly higher levels of total N, P, and Fe compared to CFS. However, remaining nutrients were relatively stable across NFS treatments and were comparable to CFS. Yield-related parameters showed no significant differences between CFS and NFS treatments for plant population, number of primary rhizomes per plant and number of secondary rhizomes per plant (Supplementary Figure S7 (A-E)). However, plant height increased with higher doses of *Ghanjeevamrit* and mulch. Fresh rhizome yields were significantly higher (34.2–40.7 t/ha) in all NFS treatments except NFS-T1 than yield of CFS (19.3 t/ha). The highest yield recorded in NFS treatments was for NFS-T5 (40.66 t/ha), followed closely by NFS-T9 (40.47 t/ha).

Based on the cost of inputs and rhizome selling price, Benefit-Cost Ratios (BCRs) was calculated for CFS and NFS. Economic analysis revealed the clear superiority of NFS treatments over CFS in turmeric cultivation. If "a farmer owns a cow", net returns under NFS treatments ranged between 750,630 INR (~9,040 USD) and 1,104,360 INR (~13,300 USD) per hectare (Supplementary Table S2). If "a farmer doesn't own a cow", net returns under NFS treatments ranged between 719,980 INR (~8,675 USD) and 1,050,710 INR (~12,655 USD) per hectare, significantly surpassing the net return of 361,477 INR (~4,355 USD) per ha recorded under CFS. Among the NFS treatments, NFS-T5 recorded the highest net returns of 1,104,360 INR (~13,300 USD) per ha followed by NFS-T9 with 1,061,430 INR (~12,780 USD) per ha, under the "farmer owns a cow" scenario, with BCRs of 10.57 (NFS-T5) and 10.20 (NFS-T9). Even in the "farmer doesn't own a cow" condition, these treatments remained economically superior over CFS, showing BCRs of 7.22 (NFS-T5) and 6.52 (NFS-T9) - substantially higher than CF's BCR of 3.99. Notably, the lowest-performing NFS treatment (NFS-T1) still outperformed CFS economically, achieving a BCR of 5.93 (without cow).

Association between soil physicochemical properties and microbial enzyme activities

The correlation analysis between soil enzyme activities (BGL, ALP, STS, URE, DHA, and PR) and soil physicochemical parameters, including macronutrients (pH, EC, OC, N, P, K) and micronutrients (S, Fe, Mn, Zn, and Cu) is shown in Supplementary Figure S8 and S9. A strong positive correlation was observed between OC and BGL activity, while OC also displayed moderate positive correlations with ALP, STS, DHA, and URE activities. Among macronutrients, available N exhibited a moderate positive correlation with BGL, STS, and PR, whereas available P showed a strong positive correlation with BGL and DHA and a moderate positive correlation with STS and URE. Additionally, available K correlated strongly with DHA and moderately with BGL and URE. Correlation between enzyme activities and micronutrients revealed significant interactions affecting nutrient dynamics. BGL, ALP, and STS exhibited strong to moderate positive correlations with Fe and S, while DHA correlated positively with Mn, Zn, and Cu. Moreover, URE and BGL showed positive correlation with Mn, whereas PR and ALP were negatively correlated with Cu.

Soil bacterial community profiling based on 16S rRNA amplicon metagenomics

16S rRNA amplicon metagenomics data revealed valuable insights into microbial diversity of turmeric soil samples. A total of 16,774,762 reads were obtained from 45 soil samples (Supplementary Table S3), with 14,872,549 reads (88.66%) successfully classified into 3,295 unique features. The reads were normalized to the minimum library size of 1,00,759 reads to mitigate biases towards varying sequencing depths with high average Good's coverage value (99.68%) across all samples.

The bacterial community composition varied between farming systems, with differences in the relative abundance of specific taxa. The microbial composition at the genus level showing the top 25 genera is summarized in Fig. 1A, while a detailed sample-wise taxa distribution is presented in Supplementary Figure S10. Few genera such as Gp6, *Priestia*, and Gp4 exhibited a notable increase in NFS (NFS-T1 to NFS-T9) compared to CFS (Supplementary Figure S11). Specifically, Gp6 significantly increased in NFS from 2.98% (NFS-BS) to 7.60–8.30% in NFS treatments, which are higher than CFS-AH (6.23%). Similarly, NFS treatments showed significantly higher proportions of *Priestia* (2.31–3.03%) and Gp4 (2.48–2.7%) compared to 1.78% and 2.07%, respectively in CFS-AH. In contrast, *Domibacillus*, *Neobacillus*, and *Ammoniphillus* were less abundant in NFS samples compared to CFS.

Alpha diversity analysis was conducted across soil treatments using four different diversity indices (Observed, Chao1, Shannon and Simpson) (Fig. 1 (B-E)).

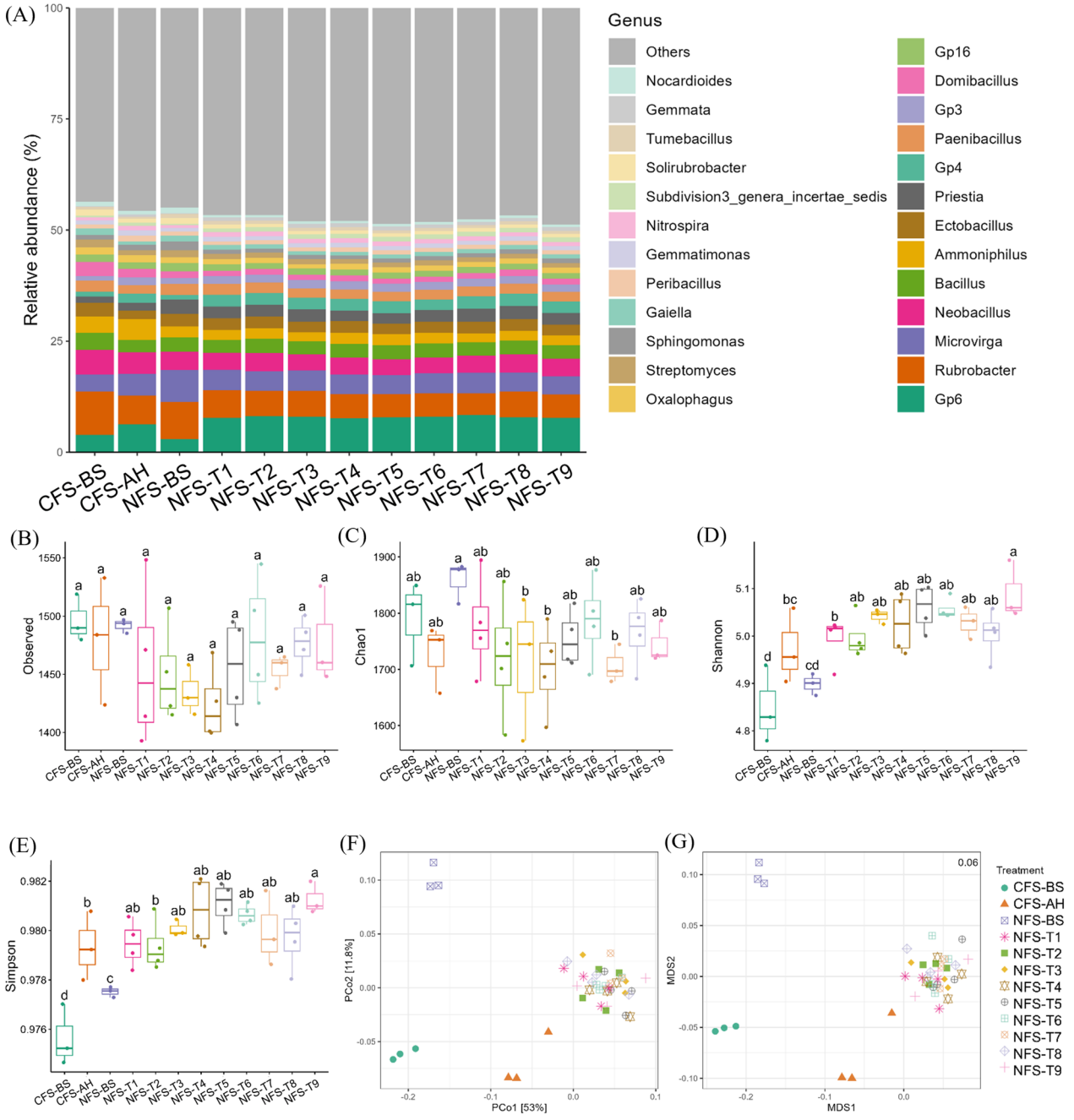

Fig. 1 Soil microbiome profiling through 16S rRNA amplicon metagenomics. **A** Relative abundance of the top 25 bacterial genera illustrating taxonomic composition. Alpha diversity metrics comparing soil treatments based on (**B**) Observed, (**C**) Chao1, (**D**) Shannon, and (**E**) Simpson indices. Beta diversity assessment through (**F**) Principal Coordinates Analysis (PCoA) and (**G**) Non-metric Multidimensional Scaling (NMDS) plots. CFS: Conventional Farming System; NFS: Natural Farming System; BS: Before Sowing; AH: At Harvest. NFS-T1 to T9 were considered under NFS-AH group. Distinct letters denote statistically significant differences among treatments, determined by one-way ANOVA followed by Tukey's HSD post-hoc test ($p < 0.05$)

Observed and Chao1 indices showed no significant differences between CFS and NFS, whereas Shannon and Simpson indices representing richness and evenness, demonstrated a notable increase in NFS treatments compared to NFS-BS. Moreover, in NFS treatments, dose-dependent increase in bacterial diversity was observed with increasing application of Mulching and *Ghanjeevamrit*. The highest diversity was recorded in NFS-T9 (Shannon index: 5.09, Simpson index: 0.981), followed by NFS-T5 and NFS-T6. Beta diversity analysis based on Bray-Curtis dissimilarity was visualized using PCoA and NMDS plots (Fig. 1 (F-G)), showing differences in

bacterial community composition across soil treatments. The substantial variation in data explained by PCo1 (53.0%) and PCo2 (11.8%) with clustering of distinct groups and the low NMDS stress value (0.06) further confirmed statistically significant differences in bacterial community composition.

LEfSe analysis illustrated a cladogram of bacterial taxa from the phylum to genus level, identifying significantly enriched taxa across different soil groups (Fig. 2A). A total of 91 discriminant features (adjusted p-value < 0.01 and LDA score > 3.0) were identified (Supplementary Table S4). CFS-BS was enriched in phylum Actinomycetota, with significant contributions from the orders Rubrobacterales, Solirubrobacterales, and Geodermatophilales. CFS-AH enriched taxa include members from the order Micrococcales, specifically the genus *Arthrobacter*. NFS-BS was dominated by orders, Chloroflexota and Pseudomonadota, and NFS-AH (NFS-T1 to NFS-T9) showed an enrichment of Acidobacteriota and Planctomycetota, particularly from Gemmataceae and Bradyrhizobiaceae family. The LDA score plot further highlighted significantly enriched genera (Fig. 2B). *Rubrobacter*, *Domibacillus*, and *Soliurobacter* were more abundant in CFS-BS, while *Arthrobacter* and *Parifilum* were enriched in CFS-AH. Meanwhile, NFS-BS had higher levels of *Priestia*, *Kallotenue*, *Nocardioides*, and *Brevibacillus*, while NFS-AH exhibited strong enrichment of unclassified acidobacteria (Gp6, Gp4, Gp5), *Gemmata*, and *Acidibacter*. The core microbiome analysis revealed differences in microbial prevalence between CFS and NFS (Fig. 2C). Genera such as *Sphingomonas*, and *Gemmatimonas* were specific to CFS, while Gp3 and *Nitrospira* were unique to NFS.

Redundancy analysis (RDA) demonstrated relationships between soil microbial enzyme activities and the top 10 most abundant bacterial genera across different soil treatments (Fig. 2D). The first two RDA axes accounted for 96.2% of the total variance. Enzymatic activities, including BGL, ALP, PR, and STS positively correlated with taxa such as *Priestia*, Gp6, and Gp4 in NFS treatments. Conversely, *Rubroacter*, *Domibacillus*, *Ammoniphilus*, and *Neobacillus* were negatively associated with these enzymes, particularly in CFS samples. Similarly, RDA plot explaining 90.5% of the total variance, depicted the relationships between soil chemical and nutrient parameters with top 10 bacterial genera in Fig. 2E. Nutrient (OC, N, P, S, and Fe) availability was higher in NFS, corresponding with an enrichment of *Microvirga*, *Sphingomonas*, and *Priestia*. In contrast, *Domibacillus*, *Ammoniphilus*, and *Neobacillus* were negatively associated with these nutrients and were more prevalent in CFS samples.

Soil fungal community profiling based on ITS amplicon metagenomics

Amplicon metagenomics of the ITS region from 45 soil samples generated a total of 18,115,941 reads, of which 14,821,082 (81.81%) were successfully classified into 1,473 distinct features (Supplementary Table S5). To ensure consistency in sequencing depth, the data were normalized to a minimum library size of 91,345 reads per sample. Like bacterial composition, the composition of fungal communities varied significantly between farming systems. The relative abundance of the top 25 fungal genera is illustrated in Fig. 3A, while a comprehensive sample-wise taxa bar plot is presented in Supplementary Figure S12. The prevalence of dominant fungal genera exhibited notable differences across soil treatments. Genera such as *Humicola* (5.28% (NFS-T5)-8.93% (NFS-T1)), *Actinomortierella* (3.42% (NFS-T1)-5.71% (NFS-T7)), and *Amesia* (5.73% (NFS-T9)-12.66% (NFS-T5)) were significantly more abundant in NFS treatments, whereas their presence in CFS-AH was very less with 0.58%, 1.73%, and 1.35% abundance, respectively (Supplementary Figure S13). Additionally, *Fusarium*, *Allocanariomyces*, and *Lennemannia* were enriched in NFS treatments, while *Alternaria* was more prevalent in CFS-BS (2.53%) and CFS-AH (5.74%) but nearly absent in NFS treatments.

In alpha diversity analysis for ITS, Observed and Chao1 indices did not reveal significant differences between farming systems (Fig. 3 (B-E)). However, the Shannon and Simpson indices indicated significantly higher fungal diversity in NFS-T9 compared to NFS-BS. Beta diversity analysis using Bray-Curtis dissimilarity was visualized through PCoA and NMDS plots (Fig. 3 (F-G)). The first two principal coordinates (PCo1: 42.1% and PCo2: 9.1%) together explained 51.2% of the variation, with a low NMDS stress value of 0.08, indicating a good fit. The distinct clustering of NFS samples in both PCoA and NMDS plots suggests a unique fungal community structure, clearly separated from that of CFS.

LEfSe analysis cladogram (Fig. 4A) identified significantly enriched taxa from phylum to genus level and the LDA scores plot (Fig. 4B) at genus level enrichment was represented. A total of 45 taxa were significantly discriminant (adjusted p < 0.01 and LDA score > 3.0) (Supplementary Table S6). The CFS-BS group was enriched for genera such as *Curvularia*, *Ectophoma*, *Coniocessia*, and *Bartalinia*, while CFS-AH exhibited significant enrichment of *Exophiala*, *Cortinarius*, and *Didymocrea*. In NFS, *Alternaria*, *Filobasidium*, and *Plectosphaerella* were enriched in NFS-BS, whereas *Humicola*, *Actinomortierella*, *Fusarium*, *Mortierella*, *Trichoderma*, *Coniochaeta*, and *Olpidium* were significantly higher in NFS-AH. Core microbiome analysis revealed distinct fungal genera prevalent in both farming systems (Fig. 4C). In CFS, the dominant genera included *Alternaria*, *Cyclothyriella*,

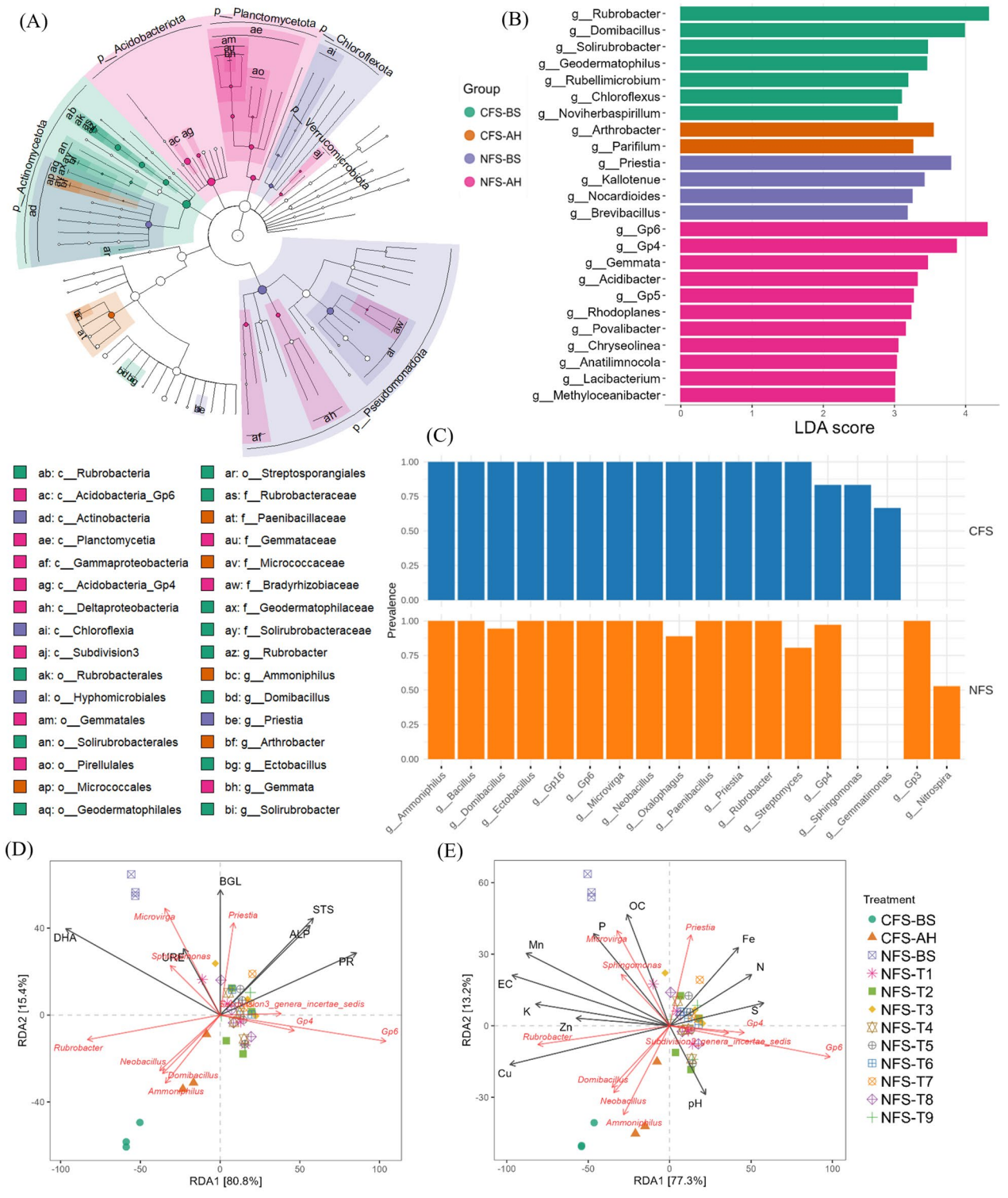

Fig. 2 Discriminant bacterial taxa and their associations with soil health properties. LEfSe analysis across soil groups: (**A**) Cladogram illustrating taxonomic hierarchy from phylum to genus, and (**B**) Bar chart depicting significantly enriched bacterial genera (LDA score > 3.0, $p < 0.01$). **C** Core microbiome comparison between CFS and NFS, based on taxa present in ≥ 50% of samples and with > 1% relative abundance. Redundancy analysis (RDA) plots showing associations between bacterial community composition and (**D**) soil microbial enzyme activities, and (**E**) soil chemical and nutrient parameters across treatments. CFS: Conventional Farming System; NFS: Natural Farming System; BS: Before Sowing; AH: At Harvest. NFS-T1 to T9 were considered under NFS-AH group

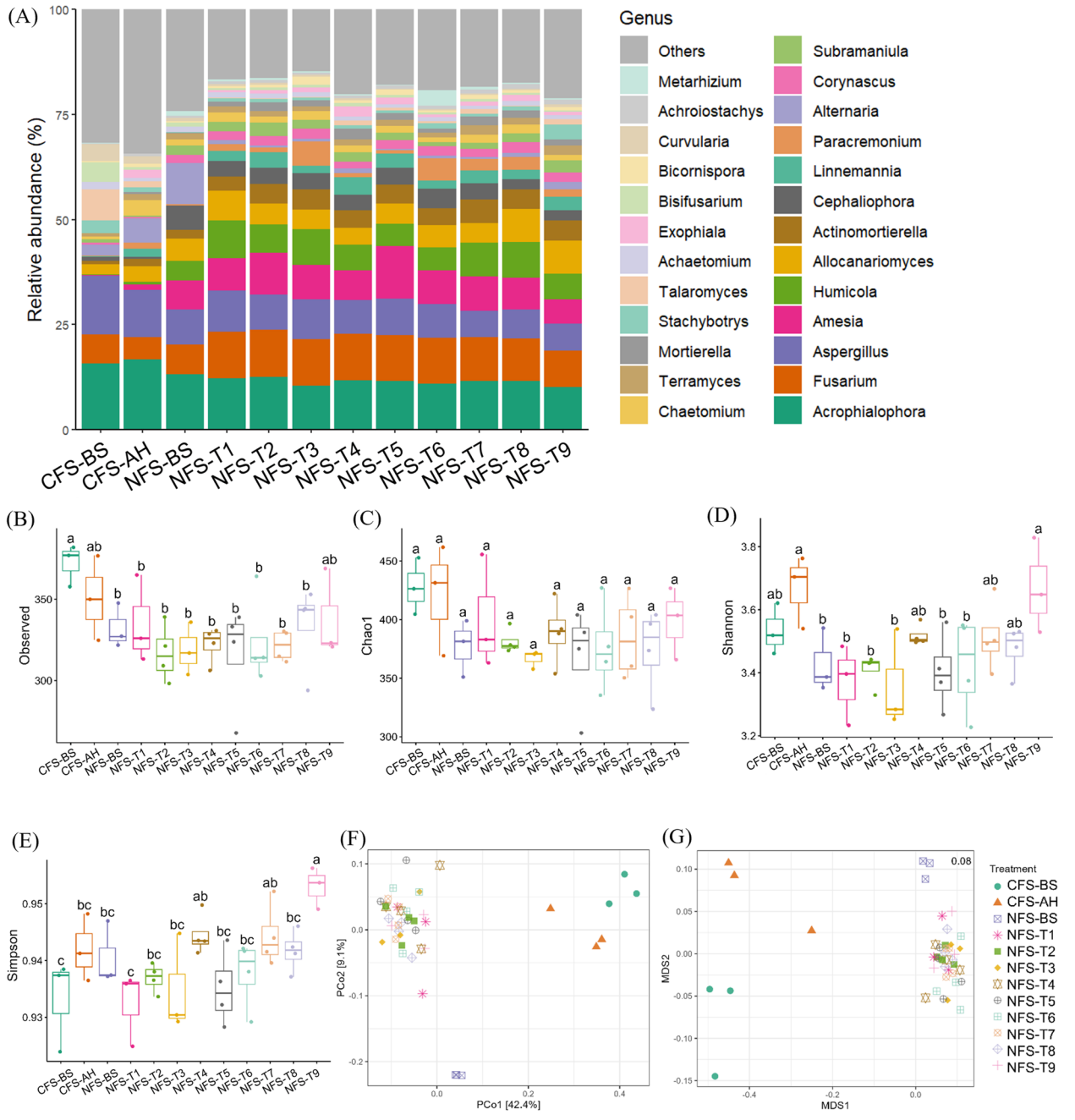

Fig. 3 Soil microbiome profiling analyzed by ITS amplicon metagenomics. **A** Relative abundance of the top 25 fungal genera illustrating taxonomic composition. Alpha diversity metrics comparing soil treatments based on (**B**) Observed, (**C**) Chao1, (**D**) Shannon, and (**E**) Simpson indices. Beta diversity assessment visualized through (**F**) Principal Coordinates Analysis (PCoA) and (**G**) Non-metric Multidimensional Scaling (NMDS) plots. CFS: Conventional Farming System; NFS: Natural Farming System; BS: Before Sowing; AH: At Harvest. NFS-T1 to T9 were considered under NFS-AH group. Distinct letters denote statistically significant differences among treatments, determined by one-way ANOVA followed by Tukey's HSD post-hoc test ($p < 0.05$)

Rhexothecium, *Talaromyces*, *Curvularia*, *Neurospora*, *Stachybotrys*, and *Cortinarius*. Conversely, NFS samples were characterized by dominance of *Actinomortierella*, *Amesia*, *Cephaliophora*, *Chaetomium*, *Corynascus*, *Humicola*, *Linnemannia*, *Mortierella*, *Paracremonium*, *Subramaniula*, and *Terramyces*, all of which exhibited high (> 50%) sample prevalence.

The relationships between soil enzyme activities and fungal communities were examined using RDA analysis (Fig. 4D). The first two RDA axes accounted for 78.1% of the total variance. Genera like *Amesia*, *Actinomortierella*,

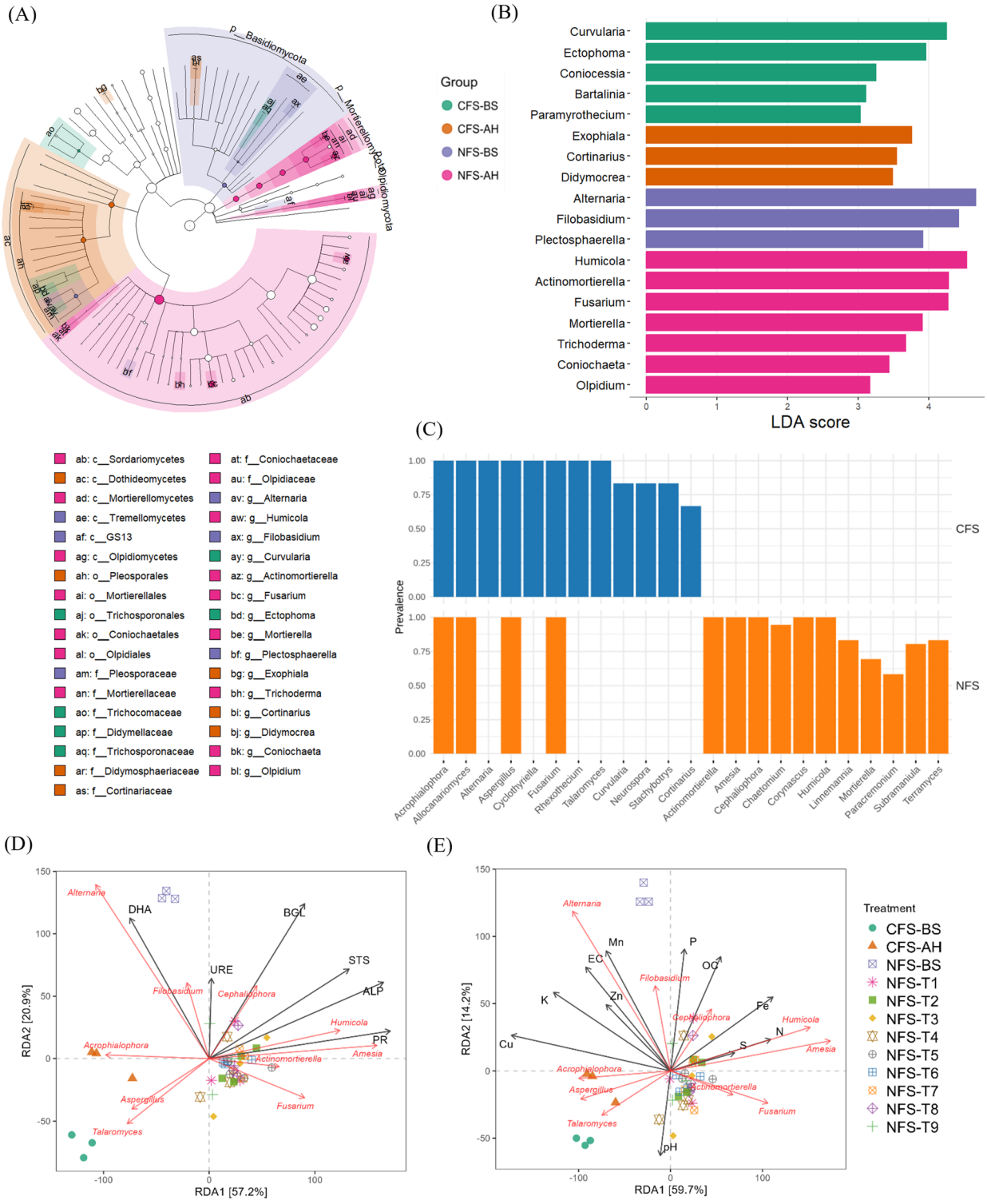

Fig. 4 Discriminant fungal taxa and their associations with soil health properties. LEfSe analysis across soil groups: (**A**) Cladogram illustrating taxonomic hierarchy from phylum to genus, and (**B**) Bar chart depicting significantly enriched fungal genera (LDA score > 3.0, $p < 0.01$). **C** Core microbiome comparison between CFS and NFS, based on taxa present in ≥ 50% of samples and with > 1% relative abundance. Redundancy analysis (RDA) plots showing associations between fungal community composition and (**D**) soil microbial enzyme activities, and (**E**) soil chemical and nutrient parameters across treatments. CFS: Conventional Farming System; NFS: Natural Farming System; BS: Before Sowing; AH: At Harvest. NFS-T1 to T9 were considered under NFS-AH group

Humicola, and *Cephaliophora* were positively associated with enzyme activities (BGL, STS, ALP, and PR) in NFS treatments, whereas *Aspergillus*, *Talaromyces*, and *Acrophialophora* were more strongly linked to CFS. Similarly, another RDA plot (Fig. 4E) highlighted association between soil nutrient parameters and fungal genera with 73.9% of the total variance. Soil nutrients, including available OC, N, P, S, and Fe, were positively correlated with NFS treatments and associated with *Humicola*, *Cephaliophora*, and *Amesia*, indicating their key roles in nutrient cycling and soil health. In contrast, *Aspergillus*, *Talaromyces*, and *Acrophialophora* were more prevalent in CFS and negatively correlated with key nutrients such as N, S, Fe, and OC.

Bacterial and fungal community profiling of natural farming inputs

Three NF input samples (*Beejamrit*, *Ghanjeevamrit*, and *Jeevamrit*), used as treatment components in NF, were analyzed for their microbial composition through 16S rRNA amplicon metagenomics. A total of 2,296,969 reads were generated, of which 1,767,690 reads (76.96%) were classified (Supplementary Table S7), identifying 2,519 unique features. The sequencing data were normalized to a minimum library size of 1,04,734 reads. The bacterial community composition of NF inputs revealed distinct profiles, highlighting the top 25 genera (Fig. 5A) with detailed sample-wise taxa bar plot given in Supplementary Figure S14. *Beejamrit* was predominantly enriched with *Arcobacter*, *Advenella*, *Anaerocella*, *Falsiporphyromonas*, and *Mediterranea*. *Ghanjeevamrit* displayed a higher abundance of *Povalibacter*, followed by *Planifilum*, *Gelatiniphilus*, and *Galbibacter*. Jeevamrit primarily consisted of *Arcobacter*, *Anaerocella*, *Commamonas*, and *Schlesneria*. Alpha diversity analysis revealed significant differences in bacterial diversity of NF inputs (Fig. 5 (B-E)). *Ghanjeevamrit* exhibited the highest microbial diversity and evenness followed by *Jeevamrit* and *Beejamrit* across all indices. The PCoA plot based on the Bray-Curtis dissimilarity depicted distinct clustering of the three NF inputs (Fig. 5F). PCo1 and PCo2 explained 70.3% and 28.5% of the total variation, respectively, confirming clear separation among the three NF inputs.

Amplicon metagenomic sequencing targeting the ITS region to evaluate fungal community composition of NF inputs generated 1,335,346 total reads, of which 1,082,280 reads (81.05%) were classified (Supplementary Table S8) and resulted in the identification of 572 distinct features. The sequencing depth was normalized to minimum library size of 51,143 reads. The fungal community composition differed significantly among NF inputs, as illustrated in the top 25 genera taxa bar plot (Fig. 5G), with a detailed sample-wise taxa bar plot is given in Supplementary Figure S15. *Beejamrit* was dominated by *Aspergillus* and *Talaromyces*. *Ghanjeevamrit* primarily contained *Aspergillus* and *Myriococcum*, which collectively accounted for a ~ 80% of its fungal composition. In contrast, *Jeevamrit* exhibited a more diverse fungal community with multiple genera contributing to its structure. Alpha diversity indices revealed significant differences in fungal diversity among NF inputs (Fig. 5 (H-K)). *Jeevamrit* exhibited the highest fungal diversity across all indices, making it the most diverse input, followed by *Ghanjeevamrit* and *Beejamrit*. The PCoA plot based on the Bray-Curtis dissimilarity showed a distinct clustering of three NF inputs (Fig. 5L) with two major PCos explaining 96.9% variations.

Microbial co-occurrence network analysis of NF soils and NF inputs

Out of three NF inputs, *Jeevamrit* and *Ghanjeevamrit* were used in the direct field application during the experiment. Cosidering it, Venn diagram (Fig. 6A) of NF inputs (*Jeevamrit* and *Ghanjeevamrit*) and NF soils with > 0.1% relative abundance was generated to find out the common microbes which might be contributed from NF input to soil. *Ghanjeevamrit* and *Jeevamrit* exhibited 27 and 2 common bacterial genera with NF soil samples, respectively and 5 genera were common in all three groups. Similarly, Fig. 6B showed the shared and unique fungal genera between NF soils and inputs. NF soils shared the 11 and 8 fungal genera with *Jeevamrit* and *Ghanjeevamrit*, respectively and 9 genera with both NF inputs. The co-occurrence network analysis revealed microbial interactions between NF soils and NF inputs for bacterial (Fig. 6C) as well as fungal communities (Fig. 6D).

Bacterial communities showed 54.35% of positive and 45.65% of negative correlations, with only 2.99% of nodes represent common genera between NF soils and NF inputs. However, in fungal co-occurrence network, 16.25% of nodes are shared between NF soils and NF inputs, which is higher than bacterial and fungal communities exhibited 56.1% of negative and 43.9% positive correlations, suggesting competitive interactions among fungal taxa.

Discussion

Adoption of natural farming holds significant promise for advancing sustainable agriculture, offering demonstrable benefits in crop productivity, nutrient use efficiency, soil health, and economic viability [3, 4]. Recent studies indicate that NF practices employing inputs such as *Jeevamrit*, *Ghanjeevamrit*, and crop residues mulching can sustain or even enhance yields by 15–20% compared to CF. NF practices can simultaneously improves nutrient cycling and fertilizer-use efficiency through enriched soil microbial activity and nutrient availability [3, 5]. NF

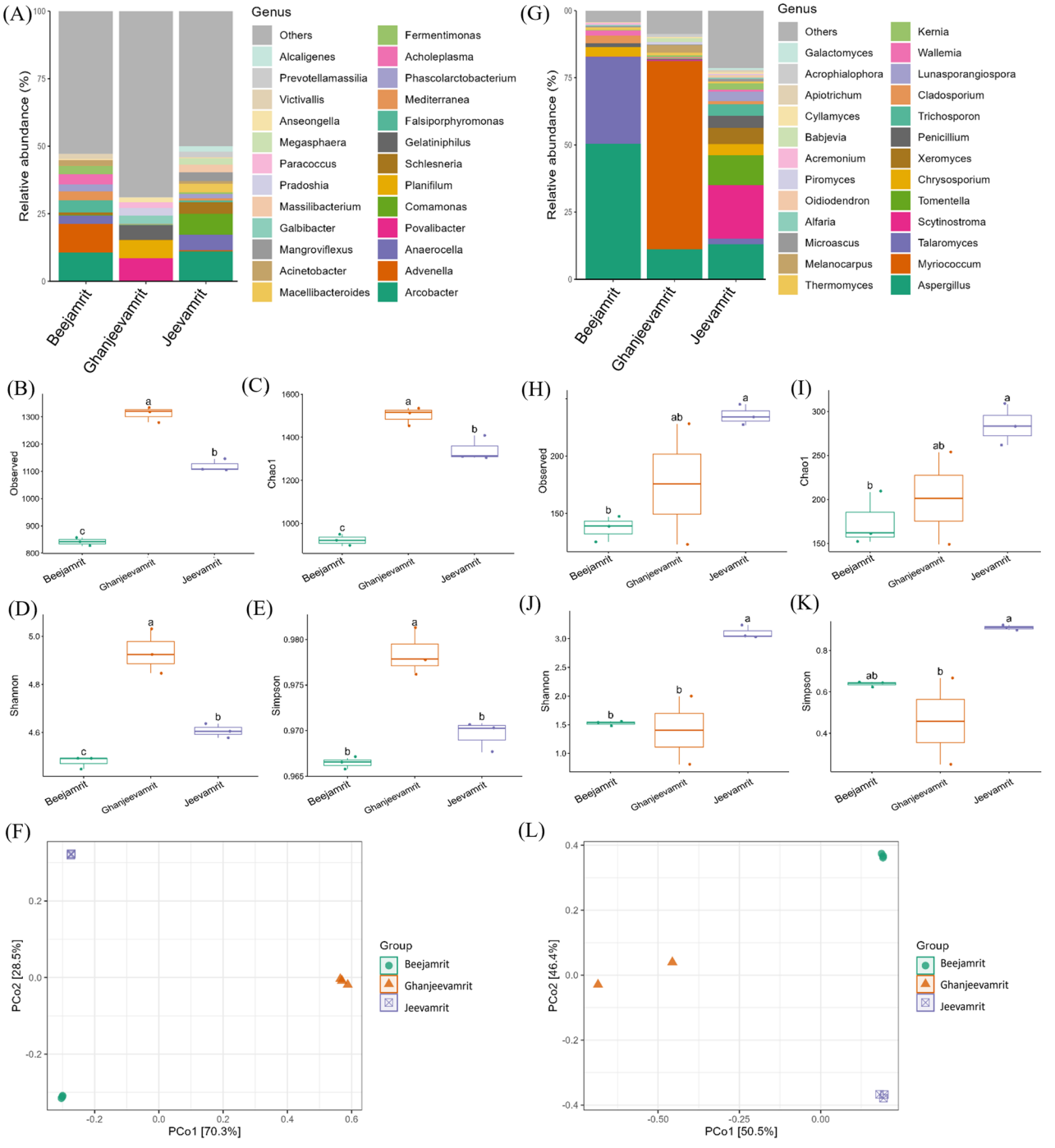

Fig. 5 Microbial profiling of natural farming inputs using 16S rRNA and ITS amplicon metagenomics. **A** Relative abundance of the top 25 bacterial genera identified in NF inputs. Bacterial alpha diversity metrics: (**B**) Observed, (**C**) Chao1, (**D**) Shannon, and (**E**) Simpson. **F** Principal Coordinates Analysis (PCoA) illustrating bacterial beta diversity and community structure among NF inputs. **G** Relative abundance of the top 25 fungal genera detected in NF inputs. Fungal alpha diversity metrics: (**H**) Observed, (**I**) Chao1, (**J**) Shannon, and (**K**) Simpson. **L** PCoA plot depicting fungal beta diversity and community differentiation among NF inputs. Different letters indicate statistically significant differences between inputs, as determined by one-way ANOVA followed by Tukey's HSD post-hoc test ($p < 0.05$)

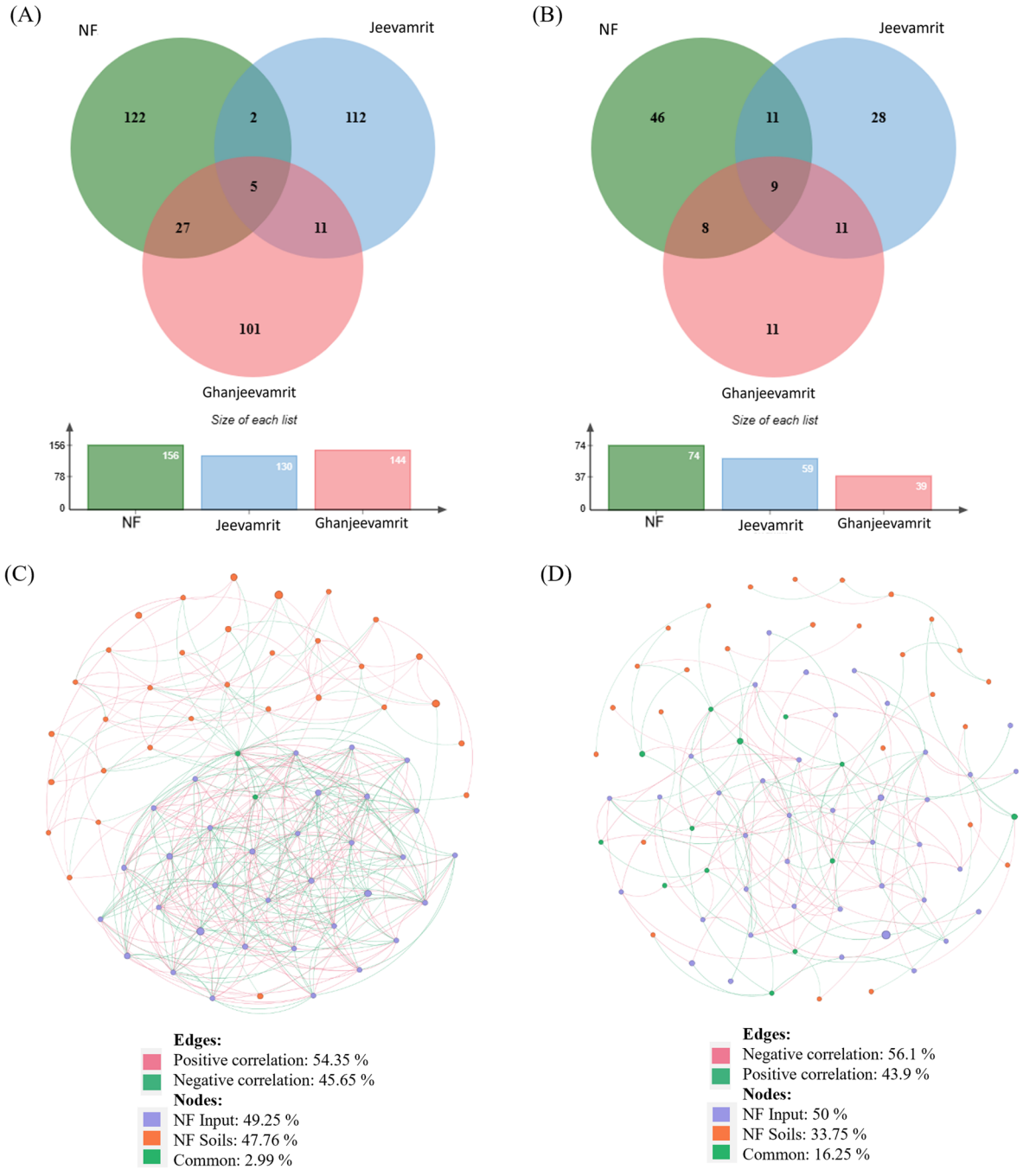

Fig. 6 Microbial community overlap and co-occurrence network analysis of NF soils and NF inputs in turmeric fields. **A** Venn diagram showing common bacterial genera among NF soils and NF inputs. **B** Venn diagram showing common fungal genera among NF soils and NF inputs. Comparative network analysis of microbial co-occurrence in NF inputs and NF soils for (**C**) Bacterial communities and (**D**) Fungal communities. Each node represents the genus. The size of node is proportional to the relative abundance of each genus and color of node represents shared and unique genera. Edges represent the positive and negative correlation between nodes

functions as an integrative approach that harmonizes soil chemistry, nutrient dynamics, and microbial ecology to achieve both agroecological resilience and long-term economic sustainability [38]. The effect of NF practices in turmeric cultivation was evaluated in the present study by analyzing various parameters of soil and plant samples.

Soil physicochemical properties, nutrients, and enzyme activities

Analysis of soil chemical and nutrient parameters revealed that NFS significantly enhanced OC, N, P, and Fe levels compared to CFS. These results emphasize the positive influence of natural farming on improving soil nutrient status and organic matter content, thereby supporting sustainable agriculture. Previous studies similarly reported improvement in soil nutrient status following three years of NF input application [3]. Although another study found no significant differences between NF and CF treatments for most of the soil nutrients [39]. Increased availability of soil nutrients might be due to increased microbial enzyme activities that improved plant growth parameters [38]. It was suggested that turmeric is highly susceptible to potassium deficiency and requires a large amount of available soil K, which critically improves the size, weight, and color of rhizome [40]. Interestingly, at the time of harvesting, K levels were higher under CFS compared to NFS which indicated more uptake of K in crops grown under NFS. This indicated better availability of K under NF practices. Moreover, it was also reported that NF increased OC and N through microbial activities and resulted in increased plant yield [38, 40].

Employing microbial enzymatic activities as indicators of soil quality is a practical approach for assessing the effects of vegetation and management practices across different land use systems [41]. Elevated nitrogen and phosphorus availability in NFS treatments was associated with enhanced microbial enzymatic activities, particularly ALP and PR, which play crucial roles in phosphorus and nitrogen cycling, respectively. Activities of BGL, STS, DHA, and URE remained comparable between NFS treatments and CFS-AH. ALP is critical for P release from organic matter and is typically boosted by soil health promoting practices such as crop rotation, use of organic amendments, green manures, crop residue management, and application of bio-stimulants containing beneficial microbes [42]. Enhanced PR activity in NF soils suggests improved protein breakdown and nitrogen transformation processes compared to CF soils [43, 44]. The application of NF inputs, rich in microbial populations such as N-fixers, P-solubilizers, actinomycetes, and fungi, contributed to higher enzymatic activity, greater nutrient mobilization, and secretion of plant growth-promoting substances [45]. The applications of NF inputs in the soil might have increased the beneficial microbial activity such as enzyme activity for increased available nutrients in the soil and uptake of such nutrients to plants.

Plant nutrient profiles and yield attributes

Plants nutrient analysis revealed significantly higher concentrations of total N, P, and Fe in certain NFS treatments compared to CFS, while other nutrients remained relatively comparable. Increased plant height observed with higher dosages of *Ghanjeevamrit* and mulching treatments indicated that improved microbial activity and NF input application may enhance plant vigor. In the present study, certain NFS treatments (NFS-T5 showed highest followed by NFS-T9) resulted in significantly higher fresh rhizome yield compared to CFS, supporting the potential of natural farming as a sustainable alternative without compromising productivity. In one of the previous studies, turmeric rhizome yield could increase approximately three-fold under organic farming practices, with profitability improving by three- to four-fold [46]. A study with long-term organic nutrient management reported 48% and 62% increase in yield and net returns, respectively [47]. The higher rhizome yield correlates with greater microbial diversity, suggesting a positive relationship between microbial diversity and crop productivity. This might be due to increased available nutrients (i.e. OC, N, P, etc.) in the soil due to increased microbial enzyme activities and/or increased uplake of nutrients like K into plants. Nonetheless, yield outcomes may vary depending on factors such as climate, preparation and composition of NF inputs, crop variety, and other agronomic practices.

These findings underscore the economic viability of NF approaches, particularly those that combine wheat straw mulching with *Ghanjeevamrit* and *Jeevamrit* applications. Such practices not only improve soil fertility and biological activity but also maximize input-use efficiency and profitability, making NF a robust and sustainable alternative to conventional methods in turmeric production. The higher BCR under the "farmer owns a cow" and "farmer doesn't own a cow" scenarios were recorded for NFS treatments, showing their economical superiority over CFS.

Soil bacterial community profiling based on 16S rRNA amplicon metagenomics

Alpha diversity analysis showed that farming practices significantly influenced soil microbial richness and evenness. Shannon and Simpson indices indicated significantly higher diversity in NFS treatments (NFS-T1 to NFS-T9) than NFS-BS, suggesting that natural farming fosters a more stable and diverse soil microbial ecosystem. Similar trends have been reported, where both NF

and CF practices enhance microbial richness by improving nutrient availability and organic matter turnover [48]. Increased bacterial populations in NF and organic farming relative to CF were also reported, particularly for nitrogen-fixers and phosphate-solubilizing microbes [49]. However, some studies noted no significant differences in microbial richness between soils of NF and CF [38].

Core microbiome composition of bacteria was largely unaffected by farming practices, certain taxa displayed farming system-specific associations where, Gp3 and *Nitrospira* were characteristics of NFS, linked to crucial functions such as nitrogen fixation, plant growth promotion, and stress tolerance [50–52]. LEfSe analysis identified differentially abundant taxa between systems. CFS were enriched with genera like *Rubrobacter*, *Domibacillus*, *Solirubrobacter*, *Geodermatophilus*, and *Arthrobacter*, which are often associated with resilience to chemical inputs. Specifically, *Arthrobacter* found in CFS-AH are known to survive and potentially degrade agricultural pollutants [53, 54]. The relative abundance of *Solirubrobacter* was found to be higher in the soil of CF than that in OF, possibly resistant to chemicals used in CF [55]. Conversely, NFS were characterized by higher abundance of *Priestia*, *Kallotenue*, Unclassified *Acidobacteria* (Gp6, Gp5, and Gp4), *Gemmata*, *Acidibacter*, *Rhodoplanes*, *Povalibacter*, and *Methyloceanibacter*. These taxa contribute significantly to soil health through roles such as organic matter decomposition, nutrient cycling, plant growth promotion and have ability to control soil-borne pathogens [56–59]. Overall, taxa enriched under NFS reflects a biologically active and sustainable system, emphasizing the role of NF practices in fostering a resilient soil microbiome.

Soil fungal community profiling based on ITS amplicon metagenomics

The alpha diversity analysis of fungal communities of NFS and CFS showed no significant overall differences. However, NFS-T9 showed greater diversity in the Simpson index compared to CFS, likely due to high mulch and *Ghanjeevamrit* application. Increased fungal population and higher saprotrophic fungal diversity in NF soils compared to CF was reported [49].

LEfSe analysis revealed enrichment of *Humicola*, *Fusarium*, *Mortierella*, *Actinomortierella*, *Trichoderma*, *Coniochaeta* and *Olpidium* in NFS-AH. Core microbiome showed fungal genera like *Humicola*, *Amesia*, *Corynascus*, *Chaetomium*, *Actinomortierella*, *Terramyces*, *Cephaliophora*, *Mortierella*, *Linnemannia*, *Paracremonium*, and *Subramaniula* were specific to NFS, possibly due to favorable soil conditions. *Humicola*, an endophytic fungus, aids in phosphate solubilization, plant growth and soil fertility [60]. Along with *Humicola*, other saprophytic fungi such as *Amesia*, *Mortierella*, *Actinomortierella*, *Corynascus* and *Chaetomium* are inhabitant of soils rich in organic matter and involved in cellulose degradation and production of bioactive metabolites [61–65]. *Trichoderma* species are widely recognized for their biocontrol potential against soil-borne pathogens and their ability to stimulate plant growth by producing enzymes and secondary metabolites [66]. *Mortierella* species contribute to nutrient cycling, organic matter decomposition, and phosphate solubilization, supporting overall soil fertility [67]. Wheat straw mulching and *Ghanjeevamrit* application, likely promoted the proliferation of these beneficial fungi, enhancing soil organic carbon. Mulching practices are known to reshape microbial communities, encourage saprophytic growth, conserve water, and improve yields in turmeric cultivation [68]. Importantly, *Alternaria* a widely known fungal pathogen has been decreased significantly in NFS treatments from NFS-BS, whereas it was increased in CFS-AH from CFS-BS. *Chaetomium*, enriched in NFS, is a known biocontrol agent against pathogens like *Alternaria* and *Curvularia* [69, 70]. *Talaromyces* efficiently mineralized organic phosphate in the soil and showed plant growth-promoting activities [71, 72].

In contrast, CFS showed enrichment of fungal genera such as *Curvularia*, *Ectophoma*, *Coniocessia*, *Exophiala*, *Cortinarius*, and *Didymocrea* (as shown in LEfSe analysis) and core microbiome identified *Alternaria*, *Stachybotrys*, *Cyclothyriella*, *Curvularia*, *Neurospora*, *Rhexothecium*, *Talaromyces*, and *Cortinarius* were specific to CFS. Pathogenic fungi like *Alternaria*, *Stachybotrys* and *Curvularia* were previously reported to be enriched in CF over OF [73, 74]. Notably, *Curvularia* is associated with major crop diseases [75], while *Neurospora* species can degrade pesticides and herbicides [76, 77], suggesting that CFS associated fungi might adapt to chemical environments and some are pathogenic.

It was hypothesized that the use of wheat straw mulch, combined with NF inputs like *Ghanjeevamrit* and *Jeevamrit*, boosted the abundance of microbes crucial for nutrient cycling, plant growth promotion, and saprophytic activity in NFS. These saprophytic fungi likely decomposed the mulch and organic amendments, enriching soil nutrients (e.g. OC, N, P, and K) and enhancing overall soil health [78]. This microbial-driven nutrient enhancement may have supported robust plant growth and yields in NFS, comparable to CFS, highlighting a promising aspect of sustainable farming. The results of previous study showed that soil organic matter, available N and P amounts, and soil enzyme activities were increased under organic mulch, mainly at a depth of 0 to 20 cm [79] and showed an increase in rhizome yield [80], which is consistent with the present findings.

Bacterial and fungal community profiling of natural farming inputs

The application of *Jeevamrit* to soil is recognized as a potent strategy for enhancing soil fertility, improving soil physicochemical properties, modifying the soil microbial community structure, and promoting plant growth [3]. Combined use of liquid manures such as *Jeevamrit* and *Panchagavya via* foliar sprays, soil drenches, and offers versatile approaches for promoting plant development and soil health while minimizing environmental impact [81]. Similarly, combinational treatments involving *Ghanjeevamrit*, *Jeevamrit*, and mulching have been shown to significantly improve soil fertility, microbial diversity, DHA activity, and nutrient availability compared to conventional farming [5].

16S rRNA and ITS amplicon metagenomics of NF inputs (*Beejamrit*, *Jeevamrit*, and *Ghanjeevamrit*), revealed distinct bacterial and fungal community profiles, respectively. Among bacteria *Arcobacter*, *Anaerocella*, *Commamonas*, and *Schlesneria* were enriched in *Jeevamrit*, while *Povalibacter*, *Planifilum*, and *Gelatiniphilus* were more abundant in *Ghanjeevamrit*. The higher prevalence of functionally diverse bacteria such as *Arcobacter*, *Advenella*, *Anaerocella*, and *Falsiporphyromonas* observed in *Beejamrit*. *Advenella* is associated with crop protection and plant growth under saline stress [82], while *Anaerocella*, isolated from rice straw residues, supports decomposition and nutrient cycling in anaerobic environments [83]. *Povalibacter* and *Commamonas* shows plant growth promoting activities [84, 85]. *Schlesneria* and *Planifilum* is linked to plant growth promotion, nutrient availability, and biocontrol activity [86, 87].

Fungal profiles among NF inputs also showed distinct compositions. Dominant genera included *Aspergillus*, *Myriococcum*, *Talaromyces*, *Scytinostroma*, and *Tomentella*, known for their roles to enhance soil quality and plant growth. *Aspergillus* are multifaceted fungi that benefits the plant in many ways, supports phosphate uptake, produces growth-promoting hormones, purifies soils, and induces systemic resistance [88]. *Myriococcum* and *Talaromyces* promote plant growth, inhibit pathogens and increase stress tolerance [89, 90]. These variations highlight complementary roles of NF inputs in shaping fungal-mediated soil processes favoring soil health and plant growth.

Microbial co-occurrence network analysis of NF soils and NF inputs

The microbial co-occurrence network analysis of NF soils and NF inputs (*Jeevamrit* and *Ghanjeevamrit*) offers valuable insights into the integration potential and ecological dynamics of microbes introduced through these natural amendments. NF inputs harbor diverse microbial taxa, only a limited fraction is shared with native NF soils microbial communities. These results imply that although *Jeevamrit* and *Ghanjeevamrit* are microbiologically rich, their microbial overlap with native soil microbiota is modest. This may be due to niche specialization, environmental selection, or competition that restricts the successful establishment of microbes in the soil ecosystem [91, 92]. Only ~ 3% of the nodes represented genera common to both NF inputs and NF soils, underscoring the limited incorporation of NF-derived bacteria into the native NF soils microbial network. In contrast, the fungal network exhibited a greater proportion of shared genera (16.25%) and a predominance of negative correlations (56.1%), indicating stronger competitive or antagonistic relationships among fungal taxa [93]. These findings are consistent with previous studies indicating that microbial inoculants often face significant ecological resistance in native soil microbiomes due to established microbial networks and competitive dynamics [94, 95]. The findings suggest that the primary impact of NF inputs may lie in their transient functional contributions, such as nutrient cycling and plant growth promotion rather than establishing a stable presence within the native microbiome. Overall, the network analysis suggests that NF inputs are associated with changes in soil microbial diversity, and their integration into existing microbial communities appears to be selective and influenced by ecological interactions.

This study presents a comprehensive evaluation of natural vs. conventional farming systems in turmeric cultivation by integrating microbial (16S rRNA and ITS) profiling with soil nutrient dynamics, enzyme activities, and yield attributes. The findings demonstrate that NFS treatments particularly with ≥ 5 t/ha mulching and 4 t/ha *Ghanjeevamrit*, enhance soil fertility, microbial activity, and crop productivity while reducing reliance on synthetic inputs. The enrichment of beneficial bacterial (e.g., *Priestia*, *Acidobacteria*, *Nitrospira*) and fungal (e.g., *Humicola*, *Mortierella*, *Trichoderma*, *Chaetomium*) taxa under NF indicates the formation of a biologically active and functionally diverse soil ecosystem that supports nutrient cycling, organic matter decomposition, and beneficial microflora. Distinct microbial signatures in NF inputs further highlight their complementary role in sustaining soil biological functions.

Conclusion

This study demonstrated that natural farming practices significantly enhanced soil health, microbial diversity, enzyme activity (ALP and PR), and turmeric yield compared to conventional farming. Compared to CFS, NFS treatments showed higher soil available P and K, likely enhancing plant uptake increasing plant vigor and rhizome yield. NFS treatments with ≥ 5 t/ha mulching and 4 t/ha *Ghanjeevamrit* (i.e. NFS-T6 and NFS-T9) exhibited

overall better performance in terms of soil quality, microbial activity, and yield outcomes. NFS also recorded higher net returns with superior (up to 2.68-fold) benefit-cost ratio than CFS. NF inputs enriched beneficial microbial communities crucial for nutrient cycling and plant growth. These findings highlight NF's potential for sustainable turmeric cultivation. Furthermore, the observed variability among treatments highlights the heterogeneity within NFS systems and emphasizes the need for standardized practices to optimize microbial benefits. However, further multi-location, multi-season studies using the best-performing treatments are essential to confirm long-term benefits and optimize practices for broader agricultural applications.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-07781-3.

Supplementary Material 1.

Supplementary Material 2.

Acknowledgements
The authors gratefully acknowledge the financial support provided by the Gujarat State Biotechnology Mission (GSBTM) and the Department of Science and Technology (DST), Government of Gujarat, India, for this research.

Authors' contributions
DC, MJ, CJ, DD, and MC conceptualized the project and the experiment. DD, MC, and DC acquired funding. DD, MC, DC, MJ, and CJ involved in project administration and supervision. SP, MC, and CKP conducted field experiment, collected data for plant, soil physicochemical analysis. RM, DA, and DC involved in soil microbial enzyme activity analysis. KG and DD conducted amplicon metagenomics, data analysis, and drafted the manuscript.

Funding
The authors gratefully acknowledge the financial support provided by the Gujarat State Biotechnology Mission (GSBTM) and the Department of Science and Technology (DST), Government of Gujarat, India, for this research. Project grant number: GSBTM/JD(R&D)/661/2022-23/00172688.

Data availability
Raw data of 16S rRNA and ITS amplicon metagenomics is submitted to IBDC (Study Accession: INRP000314) and INSDC (Project Accession: ERP171280/PRJEB88143). Sample-wise accession numbers are given in Supplementary Table S9.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Gujarat Biotechnology Research Centre (GBRC), Gandhinagar, Gujarat 382011, India
[2]Centre for Natural Resources Management, Sardarkrushinagar Dantiwada Agricultural University (SDAU), Sardarkrushinagar, Banaskantha, Gujarat 385506, India
[3]CSIR - Central Salt and Marine Chemicals Research Institute (CSMCRI), G. B. Marg,, Bhavnagar, Gujarat 364002, India

Received: 8 July 2025 / Accepted: 17 November 2025
Published online: 01 December 2025

References

1. Prasad S, Aggarwal BB. Turmeric, the golden spice. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. 2011.
2. Ozlu E, Arriaga FJ, Bilen S, Gozukara G, Babur E. Carbon footprint management by agricultural practices. Biology. 2022;11:1453.
3. Saharan BS, Tyagi S, Kumar R, Vijay, Om H, Mandal BS, et al. Application of Jeevamrit improves soil properties in zero budget natural farming fields. Agriculture. 2023;13:196.
4. Bharucha ZP, Mitjans SB, Pretty J. Towards redesign at scale through zero budget natural farming in Andhra Pradesh, India. Int J Agric Sustain. 2020;18:1–20.
5. Choudhary R, Sharma RP, Rana N, Dev P, Sharma GD, Kumar R. Effect of natural farming on yield performances, soil health and nutrient uptake in wheat + gram inter cropping system in sub-temperate regions of Himachal Pradesh. J Crop Weed. 2022;18:1–8.
6. Kumar R, Kumar S, Yashavanth BS, Meena PC, Indoria AK, Kundu S, et al. Adoption of natural farming and its effect on crop yield and farmers' livelihood in India. Hyderabad, India: ICAR-National Academy of Agricultural Research Management; 2020. p. 130.
7. Mukherjee S, Sain S, Ali MN, Goswami R, Chakraborty A, Ray K, et al. Microbiological properties of Beejamrit, an ancient Indian traditional knowledge, uncover a dynamic plant beneficial microbial network. World J Microbiol Biotechnol. 2022;38:111.
8. Lakhani H, Bodar N. Natural farming and mulching synergies: enhancing soil health and crop productivity in Indian agroecosystems. Int J Curr Microbiol Appl Sci. 2025;14:12–9.
9. Dubey A, Malla MA, Khan F, Chowdhary K, Yadav S, Kumar A, et al. Soil microbiome: a key player for conservation of soil health under changing climate. Biodivers Conserv. 2019;28:2405–29.
10. Rizvi G, Sinha P. Study of rhizospheric Mycoflora of turmeric (*Curcuma longa* L.) growing areas from Jhansi vicinity. Int J Eng Appl Sci Technol. 2022;11:22–9.
11. Prasad M, Srinivasan R, Chaudhary M, Choudhary M, Jat LK. Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture: perspectives and challenges. PGPR Amelioration Sustainable Agric. 2019;129–57.
12. He Y-H, Adkar-Purushothama CR, Ito T, Shirakawa A, Yamamoto H, Kashiwagi A, et al. Microbial diversity in the phyllosphere and rhizosphere of an Apple orchard managed under prolonged natural farming practices. Microorganisms. 2021;9:2056.
13. Tatsumi C, Lin J, Ishiguro M, Uchida Y. Natural farming diversifies resource-utilisation patterns and increases network complexity in soil microbial community of paddy fields. Agric Ecosyst Environ. 2023;356:108618.
14. Kumar SPL, Latha MR, Janaki P, Parameswari E, Kalaiselvi T, Senthamilselvi D, et al. Sustainable farming practices enhance bacterial diversity and nutrient levels in sorghum rhizosphere soil. Rhizosphere. 2024;32:100967.
15. Jyotirmayee B, Tripathy S, Samal HB, Mahalik G. The impact of organic fertilizers on Growth, Yield, and rhizospheric bacterial diversity in black turmeric (*Curcuma caesia*). J Angiotherapy. 2024;8:1–12.
16. Nelson DW, Sommers LE. Total carbon, organic carbon, and organic matter. Methods Soil Analysis: Part 2 Chem Microbiol Prop. 1982;9:539–79.
17. Subbiah BV, Asija GL. A rapid procedure for the Estimation of available nitrogen in soils. Curr Sci. 1956;25:259–60.
18. Olsen SR. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Department of Agriculture. 1954.
19. Jackson ML. Soil chemical analysis: advanced course: a manual of methods useful for instruction and research in soil chemistry, physical chemistry of soils, soil fertility, and soil genesis. UW-Madison Libraries parallel. 2005.
20. Chesnin L, Yien CH. Turbidimetric determination of available sulfates. Proc Soil Sci Soc Am. 1951;15:149–51.
21. Lindsay WL, Norvell Wa. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci Soc Am J. 1978;42:421–8.
22. Tabatabai MA. Soil enzymes. Methods Soil Analysis: Part 2 Microbiol Biochem Prop. 1994;5:775–833.

23. Chaudhary DR, Lorenz N, Dick LK, Dick RP. FAME profiling and activity of microbial communities during *Jatropha Curcas* L. residue decomposition in semiarid soils. Soil Sci. 2011;176:625–33.
24. Casida LE Jr, Klein DA, Santoro T. Soil dehydrogenase activity. Soil Sci. 1964;98:371–6.
25. Keeney DR, Nelson DW. Nitrogen—inorganic forms. Methods of soil analysis: Part 2 chemical and microbiological properties. 1982;9:643–98.
26. Ladd JN, Butler JHA. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol Biochem. 1972;4:19–30.
27. Tabatabai MA, Bremner JM. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem. 1969;1:301–7.
28. Tabatabai MA, Bremner JM. Arylsulfatase activity of soils. Soil Sci Soc Am J. 1970;34:225–9.
29. Jackson ML. Soil chemical analysis, pentice hall of India Pvt. Volume 498. New Delhi, India: Ltd; 1973. pp. 151–4.
30. Toth SJ, Prince AL, Wallace A, Mikkelsen DS. Rapid quantitative determination of eight mineral elements in plant tissue by a systematic procedure involving use of a flame photometer. Soil Sci. 1948;66:459–66.
31. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 2013;41:e1–1.
32. Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kauserud H. ITS as an environmental DNA barcode for fungi: an in Silico approach reveals potential PCR biases. BMC Microbiol. 2010;10:1–9.
33. Liu C, Cui Y, Li X, Yao M. Microeco: an R package for data mining in microbial community ecology. FEMS Microbiol Ecol. 2021;97:fiaa255.
34. Wickham H. ggplot2. Wiley Interdisciplinary Reviews: Comput Stat. 2011;3:180–5.
35. Oliveros JC. VENNY. An interactive tool for comparing lists with Venn Diagrams. http://bioinfogp cnb csic 2007. http://bioinfogp.cnb.csic.es/tools/venny/index.html.
36. Kurtz ZD, Müller CL, Miraldi ER, Littman DR, Blaser MJ, Bonneau RA. Sparse and compositionally robust inference of microbial ecological networks. PLoS Comput Biol. 2015;11:e1004226.
37. Bastian M, Heymann S, Jacomy M. Gephi: an open source software for exploring and manipulating networks. In: Proceedings of the international AAAI conference on web and social media. 2009;3:361–2.
38. Liao J, Xu Q, Xu H, Huang D. Natural farming improves soil quality and alters microbial diversity in a cabbage field in Japan. Sustainability. 2019;11:3131.
39. Duddigan S, Shaw LJ, Sizmur T, Gogu D, Hussain Z, Jirra K, et al. Natural farming improves crop yield in SE India when compared to conventional or organic systems by enhancing soil quality. Agron Sustain Dev. 2023;43:1–15.
40. Datta S, Sarkar A, Rai U. Effect of nitrogen and potassium on yield and quality of turmeric under Terai zone of West Bengal. Environ Ecol. 2022;40:1757–67.
41. Vikram K, Chaudhary H, Rao KS. SOC and TN fluctuations determine the variations in microbial enzymatic activities under diverse land use types in the central Himalaya, India. CATENA. 2024;240:107958.
42. Rocabruna PC, Domene X, Preece C. Relationship among soil biophysicochemical Properties, agricultural practices and climate factors influencing soil phosphatase activity in agricultural land. Agric (Switzerland). 2024;14:288.
43. Greenfield LM, Hill PW, Paterson E, Baggs EM, Jones DL. Do plants use root-derived proteases to promote the uptake of soil organic nitrogen? Plant Soil. 2020;456:355–67.
44. Adetunji AT, Ncube B, Meyer AH, Olatunji OS, Mulidzi R, Lewu FB. Soil pH, nitrogen, phosphatase and urease activities in response to cover crop species, termination stage and termination method. Heliyon. 2021;7:e05980.
45. Yankit P, Chandel RS, Verma S, Sharma PL, Verma SC, Balaso GM, et al. Insights on soil biological properties and crop yields under natural farming in Western himalaya. Indian J Agricultural Sci. 2024;94:89–94.
46. Choudhary AK, Rahi S. Organic cultivation of high yielding turmeric (*Curcuma longa* L.) cultivars: A viable alternative to enhance rhizome productivity, profitability, quality and resource-use efficiency in monkey–menace areas of north-western Himalayas. Ind Crops Prod. 2018;124:495–504.
47. Udhaya Nandhini D, Janaki P, Venkatesan S, Senthilraja K, Somasundaram E, Meena S. Assessing changes in soil quality indicators, turmeric (*Curcuma longa* L.) yield, and monetary returns under different years of organic nutrient management. Org Agric. 2023;13:443–60.
48. Agyekum DVA, Kobayashi T, Dastogeer KMG, Yasuda M, Sarkodee-Addo E, Ratu STN, et al. Diversity and function of soybean rhizosphere Microbiome under nature farming. Front Microbiol. 2023;14:1130969.
49. Mastiholi AB, Sowmya B, Maheswarappa HP, Gondi SP, Shantappa T, Rudresh DL, et al. Organic and natural farming improve microbial diversity and dehydrogenase activity in clusterbean-tomato cropping sequence. Arch Agron Soil Sci. 2023;69:3705–16.
50. Seneviratne M, Gunaratne S, Bandara T, Weerasundara L, Rajakaruna N, Seneviratne G, et al. Plant growth promotion by *Bradyrhizobium Japonicum* under heavy metal stress. South Afr J Bot. 2016;105:19–24.
51. Muleta A, Tesfaye K, Haile Selassie TH, Cook DR, Assefa F. Phosphate solubilization and multiple plant growth promoting properties of *Mesorhizobium* species nodulating Chickpea from acidic soils of Ethiopia. Arch Microbiol. 2021;203:2129–37.
52. Takahashi Y, Fujitani H, Hirono Y, Tago K, Wang Y, Hayatsu M, et al. Enrichment of *comammox* and nitrite-oxidizing *Nitrospira* from acidic soils. Front Microbiol. 2020;11:1737.
53. Zhang Q, Zhu L, Su J, Wang J, Xie H, Wang J, et al. Impacts of nitrogen and phosphorus on atrazine-contaminated soil remediation and detoxification by *Arthrobacter* sp. strain HB-5. Environ Earth Sci. 2014;71:1465–71.
54. Shi X, Tan W, Tang S, Ling Q, Tang C, Qin P, et al. Metagenomics reveals taxon-specific responses of soil nitrogen cycling under different fertilization regimes in heavy metal contaminated soil. J Environ Manage. 2023;345:118766.
55. Li T, Wang R, Zhu J, Cao L, Xia H, Wang M, et al. Effects of organic fertilizer on soil properties and soil microorganisms. J Biotech Res. 2023;15:138–50.
56. Jo H, Lim K, Ibal JC, Kim M-C, Kim H-B, Baek C, et al. Growth increase in the herbaceous plant *Centella Asiatica* by the plant growth-promoting rhizobacteria *Priestia megaterium* HyangYak-01. Plants. 2023;12:2398.
57. Ortega-Urquieta ME, Valenzuela-Ruiz V, Mitra D, Hyder S, Elsheery NI, Kumar Das Mohapatra P, et al. Draft genome sequence of *Priestia* sp. strain TSO9, a plant growth-promoting bacterium associated with wheat (*Triticum turgidum* subsp. *durum*) in the Yaqui Valley. Mexico Plants. 2022;11:2231.
58. Shen Z, Ruan Y, Chao X, Zhang J, Li R, Shen Q. Rhizosphere microbial community manipulated by 2 years of consecutive biofertilizer application associated with banana *Fusarium* wilt disease suppression. Biol Fertil Soils. 2015;51:553–62.
59. Ge Y, Wang Q, Wang L, Liu W, Liu X, Huang Y, et al. Response of soil enzymes and microbial communities to root extracts of the alien *Alternanthera Philoxeroides*. Arch Agron Soil Sci. 2018;64:708–17.
60. Radhakrishnan R, Khan AL, Kang SM, Lee I-J. A comparative study of phosphate solubilization and the host plant growth promotion ability of *Fusarium verticillioides* RK01 and *Humicola* sp. KNU01 under salt stress. Ann Microbiol. 2015;65:585–93.
61. White WL, Downing MH. Humicola grisea, a soil-inhabiting, cellulolytic hyphomycete. Mycologia. 1953;45:951–63.
62. Thakkar AT, Pandya DC, Bhatt SA. Optimization of laccase enzyme production by *Amesia atrobrunnea* A2: A first report. Biosci Biotechnol Res Asia. 2020;17:65–72.
63. Abdel-Azeem AM. Recent developments on genus *Chaetomium*. Springer; 2019.
64. Wani ZA, Kumar A, Sultan P, Bindu K, Riyaz-Ul-Hassan S, Ashraf N. *Mortierella alpina* CS10E4, an oleaginous fungal endophyte of *Crocus sativus* L. enhances apocarotenoid biosynthesis and stress tolerance in the host plant. Scientific reports. 2017;7:8598.
65. Hutchinson MI, Powell AJ, Herrera J, Natvig DO. New perspectives on the distribution and roles of thermophilic fungi. In: Sonia M, Tiquia A, Martin G, editors. Fungi in Extreme Environments: Ecological Role and Biotechnological Significance. 2019. p. 59–80.
66. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat Rev Microbiol. 2004;2:43–56.
67. Ozimek E, Hanaka A. *Mortierella* species as the plant growth-promoting fungi present in the agricultural soils. Agriculture. 2020;11:7.
68. Choudhary VK, Kumar PS. Weed suppression, nutrient leaching, water use and yield of turmeric (*Curcuma longa* L.) under different land configurations and mulches. J Clean Prod. 2019;210:795–803.
69. Khalil MII, Youssef SA. Physiological and biochemical responses of *Alternaria alternata* infected tomato to *Trichoderma harzianum* and *Chaetomium globosum* application. S Afr J Bot. 2024;166:116–25.
70. Cadelina MAS, Divina CC. In-Vitro screening on the interaction of *Chaetomium cellulolyticum* Chachal & D. Hawksworth with *Aspergillus flavus* link Ex Fr. and *Curvularia lunata* (Wakker) Boedijin. J Microbiol Exp. 2015;2:20–7.
71. Kharkwal AC, Joshi H, Shandilya C, Dabral S, Kumar N, Varma A. Isolation and characterization of a newly discovered plant growth-promoting endophytic fungal strain from the genus *Talaromyces*. Sci Rep. 2024;14:6022.

72. Sun X, Liu F, Jiang W, Zhang P, Zhao Z, Liu X, et al. *Talaromyces purpurogenus* isolated from rhizosphere soil of maize has efficient organic Phosphate-Mineralizing and plant Growth-Promoting abilities. Sustainability. 2023;15:5961.
73. Żukiewicz-Sobczak W, Cholewa G, Krasowska E, Zwoliński J, Sobczak Pawełand Zawiślak K, Chmielewska-Badora J, et al. Pathogenic fungi in the work environment of organic and conventional farmers. Adv Dermatology Allergology/Postepy Dermatologii I Alergologii. 2012;29:252–62.
74. Zhang H, Zheng X, Bai N, Li S, Zhang J, Lv W. Responses of soil bacterial and fungal communities to organic and conventional farming systems in East China. Environ Microbiol Biotechnol. 2019;29:441–53.
75. Khan NA, Asaf S, Ahmad W, Jan R, Bilal S, Khan I, et al. Diversity, lifestyle, genomics, and their functional role of *Cochliobolus*, *Bipolaris*, and *Curvularia* species in environmental remediation and plant growth promotion under biotic and abiotic stressors. J Fungi. 2023;9:254.
76. Wang Y, Li H, Feng G, Du L, Zeng D. Biodegradation of diuron by an endophytic fungus *Neurospora intermedia* DP8-1 isolated from sugarcane and its potential for remediating diuron-contaminated soils. PLoS ONE. 2017;12:e0182556.
77. Yada GM Jr, Shiraishi IS, Dekker RFH, Schirmann JG, Barbosa-Dekker AM, de Araujo IC, et al. Soil and entomopathogenic fungi with potential for biodegradation of insecticides: degradation of flubendiamide in vivo by fungi and in vitro by laccase. Ann Microbiol. 2019;69:1517–29.
78. Yang L, Wang C, He X, Liang H, Wu Q, Sun X, et al. Multi-year crop rotation and quicklime application promote stable peanut yield and high nutrient-use efficiency by regulating soil nutrient availability and bacterial/fungal community. Front Microbiol. 2024;15:1367184.
79. Liu F, Yang J, Zhang Y, Yang S, Zhang Y, Chen Y, et al. Mulches assist degraded soil recovery via stimulating biogeochemical cycling: metagenomic analysis. Appl Microbiol Biotechnol. 2024;108:20.
80. Aulakh CS, Walia SS, Gill RS. Management of weeds with rice straw mulch in organic turmeric (*Curcuma longa* L). Annals Agricultural Res New Ser. 2019;40:193–8.
81. Naskar S, Kumari M. others. A review on effect of organic conditioner on Physico-chemical and Microbiological properties of soil. Int J Plant Soil Sci. 2024;36:570–7.
82. Kuzmina LY, Gilvanova EA, Galimzyanova NF, Arkhipova TN, Ryabova AS, Aktuganov GE, et al. Characterization of the novel plant growth-stimulating strain *Advenella kashmirensis* IB-K1 and evaluation of its efficiency in saline soil. Microbiology. 2022;91:173–83.
83. Abe K, Ueki A, Ohtaki Y, Kaku N, Watanabe K, Ueki K. *Anaerocella delicata* gen. nov., sp. nov., a strictly anaerobic bacterium in the phylum bacteroidetes isolated from a methanogenic reactor of cattle farms. J Gen Appl Microbiol. 2012;58:405–12.
84. Yang J, Shi J, Jiang L, Zhang S, Wei F, Guo Z, et al. Co-occurrence network in core microorganisms driving the transformation of phosphorous fractionations during phosphorus recovery product used as soil fertilizer. Sci Total Environ. 2023;871:162081.
85. Zhou X, Yang Y, Yin Q, Zhang X, Li M. Application potential of *Comamonas testosteroni* ZG2 for vegetable cultivation in nickel and cadmium polluted soil. Environ Technol Innov. 2021;23:101626.
86. Peruzzi E, Franke-Whittle IH, Kelderer M, Ciavatta C, Insam H. Microbial indication of soil health in Apple orchards affected by replant disease. Appl Soil Ecol. 2017;119:115–27.
87. Zhou B, Zheng X, Zhu Z, Qin Q, Song K, Sun L, et al. Effects of fertilizer application on phthalate ester pollution and the soil microbial community in plastic-shed soil on long-term fertilizer experiment. Chemosphere. 2022;308:136315.
88. Nayak S, Samanta S, Mukherjee AK. Beneficial role of *Aspergillus* sp. in agricultural soil and environment. In: Suraja KN, Bibhuti BM, editors. Frontiers in Soil and Environmental Microbiology. CRC Press; 2020. p. 17–36.
89. Ran Q, Dong C, Zhang Q, Shao Q, Zhang Y, Long X, et al. Seed endophytes reshape rhizosphere microbiota to promote the growth of *Eucommia ulmoides* seedlings. Appl Soil Ecol. 2024;201:105487.
90. Zapata D, López JE, Saldarriaga JF. Plant growth-promoting and biocontrol potential of *Aspergillus tubingensis* and *Talaromyces Islandicus*. J Soil Sci Plant Nutr. 2024;24:2354–70.
91. Hartmann M, Frey B, Mayer J, Mäder P, Widmer F. Distinct soil microbial diversity under long-term organic and conventional farming. ISME J. 2015;9:1177–94.
92. Fierer N. Embracing the unknown: disentangling the complexities of the soil Microbiome. Nat Rev Microbiol. 2017;15:579–90.
93. Peay KG, Kennedy PG, Talbot JM. Dimensions of biodiversity in the Earth mycobiome. Nat Rev Microbiol. 2016;14:434–47.
94. Mallon CA, Poly F, Le Roux X, Marring I, van Elsas JD, Salles JF. Resource pulses can alleviate the biodiversity-invasion relationship in soil microbial communities. Ecology. 2015;96:915–26.
95. Toju H, Peay KG, Yamamichi M, Narisawa K, Hiruma K, Naito K, et al. Core microbiomes for sustainable agroecosystems. Nat Plants. 2018;4:247–57.

Publisher's note