Soil Microbial Diversity under Conservation Agriculture Using 16S rRNA Gene Sequencing

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ABSTRACT

Background: Microorganisms in soils are vital to the ecosystem, nutrient cycles, and carbon processes. Although we know that conservation agriculture enhances soil biology, there has yet to be a complete understanding of how communities of microorganisms in soils change.

Objective: The study aims to investigate the diversity of soil microorganisms and how communities differ under the effects of different conservation agricultural practices, including the 16S rRNA gene sequencing method, and to find out which bacteria enhance soil health.

Methods: A randomized complete block design was used for the field experiment at research in northern India with the land consisting of silty clay loam soil. Five treatments were tested: conventional tillage (CT), minimum or reduced tillage (MT), zero tillage (ZT), residue retention (RR), and integrated conservation agriculture (ICA). Soil samples were taken at the depth of 0–15 cm from replicated plots for extraction of DNA, amplification of the 16S rRNA gene, and sequencing by Illumina MiSeq. Bioinformatics evaluation involved the use of QIIME2 and DADA2 pipelines and resulted in the generation of amplicon sequence variants (ASVs). The indices of diversity and composition were evaluated using analysis of variance (ANOVA) and PERMANOVA tests ($p < 0.05$).

Results: Application of conservation agriculture methods induced considerable elevation in Shannon indices (4.2–4.8) compared to conventional tillage methods (3.9). Use of zero tillage methods and integrated conservation agriculture brought about maximum bacterial species richness (Chao1 ≥ 2,450 ASVs). Phyla with the highest abundance included Proteobacteria (28%–35%), Actinobacteria (22%–28%) and Acidobacteria (15%–19%). The processes of ammonia oxidation were significantly enhanced under conservation agriculture methods with large spiking of *Nitrospira*e and Nitrospina. Strong correlation between organic carbon contents and Shannon diversity indices was established ($r = 0.78$, $P < 0.01$). Results of function prediction suggest improved capabilities with respect to N cycling and C sequestration.

Conclusion: The application of conservation agriculture techniques will enhance the diversity of soil microbes further increasing the number of organisms which are engaged in nutrient cycling including carbon sequestration processes. The use of 16S rRNA sequencing allows comprehensively studying microflora responses to the application of conservation agriculture techniques.

Keywords: Conservation Agriculture, Soil Microbial Diversity, 16S rRNA Gene Sequencing, Soil Bacterial Communities

Introduction

Microorganisms present in soil play an important role in different environmental processes occurring in agricultural systems, enhancing nutrient cycling, organic matter breakdown, and carbon cycle [1]. Diversity of soil bacteria is of great importance as it affects major agricultural parameters such as productivity of crops, disease suppression, and the resilience of the entire production system [2]. Intensive farming, and in particular the usage of conventional tillage, causes major decline in both abundance and diversity of soil microorganisms thus affecting ecosystem services performed by ecosystems [3].

Conservation agriculture introduces a system of farming management based on three main principles: minimal disturbance of land or no disturbance of soil at all, permanent presence of an organic layer made of crop residues, and crop rotation with inclusion of legumes [4]. This holistic approach does not only contribute to the prevention of soil degradation but also preserves its capability of productivity. There are many mechanisms through which conservation agriculture achieves improved soil health: avoidance in mechanical disturbances prevents disappearance of fungi and development of bacteria; higher presence of organic matter supplies energy and nutrients for microorganisms; and crop rotation diversifies root exudates [5].

The structure and maintenance of soil bacterial communities fulfill essential roles in ecosystems. Nitrogen transformations, including nitrification, denitrification, and assimilation of nitrate, can only take place with specific bacteria such as *Nitrosomonas*, *Nitrobacter*, and heterotrophic organisms [6].

As in the case of nitrogen processes, the processes of carbon cycling/sequestration imply that various organisms have to take part in it. These include cellulose-digesting bacteria, fermentation bacteria, and methanotrophs [7]. The functional redundancy and complementarity provide bacterial communities with the potential for resilience. Therefore, preserving and improving soil bacterial diversity is essential for providing agroecosystem functions.

The field of soil microbial community study has been influenced by the era of high-throughput sequencing technology. Specifically, the recent development of next-generation sequencing (NGS) which uses ribosomal RNA genes has changed the way molecular characterization of soil microbial communities is done. Thanks to the 16S rRNA gene which is a universal marker of bacteria, soil microbial communities can be studied without any culture-dependent schemes. With respect to the V4 and V5 hypervariable regions of the 16S rRNA gene, sufficient phylogenetic resolution is provided for identification of microbes and drawing ecological conclusions. Due to the progressive Illumina sequencing platform, it is possible to perform millions of sequencing reactions in one sample for comparative analysis of soil microbial communities in experimental conditions. The next-generation sequencing technology requires bio-informational processing of data using computer programs such as QIIME2 and DADA2 which greatly simplifies the process of obtaining the right information.

Even though literature is available about conservation agriculture and soil microbiomes, the assessment of the response of bacterial diversity across various methodologies of conservation is limited. Furthermore, the functional characterization of bacteria and their contribution in ecosystem services requires integrating of sequencing and biogeochemical analysis data. This study intends to fill these gaps through the evaluation of soil bacterial communities under various conservation agriculture systems. The specific objectives of this study are: (1) to assess the alpha and beta diversity of soil bacteria under conservation agriculture, (2) to identify the most significant bacterial taxa and their response to conservation management practices, (3) to explore the relationship that might exist between microbial diversity and soil properties, and (4) to investigate the functional capabilities.

Literature Review

Conservation agriculture has demonstrated consistent benefits for soil quality, productivity, and environmental sustainability across diverse agroecosystems [4]. Foundational studies established that minimum or zero tillage reduces soil organic matter oxidation, increases carbon retention, and promotes soil aggregation compared to conventional tillage [13]. Crop residue retention—a critical component—directly increases soil carbon inputs, enhancing organic matter levels and creating favorable conditions for microbial proliferation [5]. Legume-based crop rotation diversifies nitrogen sources through biological fixation and modulates root exudate composition, selecting for distinct bacterial assemblages [6].

Soil bacterial diversity encompasses community structure (taxonomic composition and relative abundance), alpha diversity (within-sample richness and evenness), and beta diversity (compositional differences among samples). Culture-independent molecular surveys utilizing 16S rRNA gene amplicon sequencing have revealed substantial bacterial diversity in agricultural soils—typically encompassing 500–3,000 bacterial operational taxonomic units (OTUs) or amplicon sequence variants (ASVs) per gram of soil [2].

Dominant bacterial phyla across agroecosystems include Proteobacteria (typically 30–40% relative abundance), Actinobacteriota (20–30%), Acidobacteriota (10–20%), and Firmicutes (5–15%) [14]. Phylum-level composition responds predictably to agricultural management: Acidobacteria favor acidic, undisturbed soils; Proteobacteria abundance correlates with nutrient availability; and Actinobacteria proliferate under water-limited conditions [15].

Rhizosphere microbiology reveals intimate associations between plant roots, root exudates, and soil bacteria. The rhizosphere represents an extreme microhabitat where root-derived carbon supports up to 100-fold increases in bacterial abundance relative to bulk soil [9]. Specific bacterial taxa form plantmicrobe associations promoting nutrient acquisition (e.g., phosphate-solubilizing bacteria, nitrogen-fixing *Azospirillum*) or pathogen suppression through production of antimicrobial metabolites [7]. Conservation agriculture practices, by maintaining intact root systems through reduced tillage and diversifying root exudates via crop rotation, select for plant-beneficial bacterial consortia.

16S rRNA amplicon sequencing has become the standard methodology for assessing soil microbial communities. The technique involves PCR amplification of specific 16S rRNA gene regions (commonly V3–V4 or V4–V5), library construction, and high-throughput sequencing using Illumina, PacBio, or Ion Torrent platforms [11]. Sequencing depths of 50,000–100,000 reads per sample provide robust quantification of community composition with rarefaction-adequate coverage [12]. DADA2 and other bioinformatics pipelines replace traditional OTU clustering with amplicon sequence variant (ASV) inference, increasing reproducibility and resolution [16]. Taxonomic assignment using reference databases such as SILVA, RDP, or Greengenes enables phylogenetic classification to family or genus levels [12].

Alpha diversity indices—including Shannon diversity, Simpson index, and Chao1 richness—quantify within-community diversity. Shannon index integrates richness and evenness, increasing with both abundance and equitable distribution of taxa. Chao1 estimates total richness accounting for rare OTUs/ASVs. Conservation agriculture typically increases alpha diversity 10–20% relative to conventional tillage [5]. Beta diversity, assessed through principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS), evaluates compositional differences among samples. Beta diversity responses reveal treatment clustering and degree of community assembly predictability [2].

Soil enzyme activities—including β-glucosidase, acid phosphatase, urease, and dehydrogenase—reflect bacterial community function and organic matter transformation capacity [17]. Conservation agriculture increases enzyme activities, indicating metabolic enhancement. PICRUSt2 functional profiling uses reference genomes to predict metabolic pathways (KEGG Orthologs) from 16S sequences, inferring pathways for nitrogen cycling, carbon degradation, and stress response without requiring shotgun metagenomics [16].

Carbon sequestration under conservation agriculture operates through increased soil organic carbon accumulation and reduced heterotrophic respiration. Long-term field experiments demonstrate 0.2–0.6 Mg C ha^{-1} $year^{-1}$ accumulation, with microbial-mediated processes governing rates [13]. Bacterial taxa associated with carbon decomposition (cellulolytic Actinobacteria, Firmicutes) and methanotrophy (*Methylobacteriaceae*) directly influence carbon cycling efficiency [18].

Current research gaps include: (1) incomplete characterization of bacterial diversity responses across multiple conservation practices simultaneously; (2) limited functional validation of predicted metabolic pathways; (3) insufficient temporal datasets of soil microbiome dynamics; and (4) underexplored integration of bacterial diversity with biogeochemical process rates. This study addresses these gaps through comprehensive 16S rRNA profiling, functional prediction, and correlation with soil properties.

Materials and Methods

Experimental Site

A field experiment was conducted at the Agricultural Research Station, Pantnagar (29.01°N, 79.47°E), Uttarakhand, India, at 244 m elevation. The soil was classified as silty-clay loam (*Inceptisol*) with baseline (year 0) properties: pH 6.8, organic carbon 18 g kg^{-1}, available nitrogen 320 mg kg^{-1}, available phosphorus 14 mg kg^{-1}, and available potassium 145 mg kg^{-1}. The climate is subtropical monsoon with mean annual rainfall 1,420 mm and annual temperature range 10–38°C. The field had been under conventional tillage maize-wheat rotation for >15 years prior to experiment initiation.

Experimental Design

A randomized complete block design (RCBD) with 5 treatments and 4 replications was established in 2020. Total experimental area was 1,200 m^2 (5 treatments × 4 blocks × 60 m^2 $plot^{-1}$). Treatments were:

1. **Conventional tillage (CT)**: Annual moldboard plowing (20 cm depth) + conventional seedbed preparation + crop residue removal
2. **Minimum tillage (MT)**: Shallow tillage (8–10 cm) with seed-cum-fertilizer drill + partial residue retention (50%)
3. **Zero tillage (ZT)**: No-till direct planting with zero-till drill + full residue retention (100%)
4. **Residue retention (RR)**: Zero-till direct planting + full residue retention + in situ mulching
5. **Integrated conservation agriculture (ICA)**: Zero-till + full residue retention + crop rotation with legume (50% area under pulses annually)

All treatments used maize-wheat crop sequence over 4 years (2020–2024), with ICA including maize-legume-wheat-legume rotation. Standard fertilization (120:60:40 kg N:P:K ha^{-1} maize; 100:50:40 kg N:P:K ha^{-1} wheat) and pest management protocols were applied uniformly across treatments.

Table 1: Experimental Conservation Agriculture Treatments, Management Practices, and Soil Characteristics

Treatment	Soil Disturbance	Residue Management	Crop Rotation	Tillage Depth (cm)	Annual Organic Matter Input (Mg ha^{-1})	Bulk Density Year 4 (g cm^{-3})
Conventional Tillage (CT)	Annual moldboard plow	Complete removal	Maize-Wheat monoculture	20	2.1	1.38 ± 0.09
Minimum Tillage (MT)	Shallow annual tillage	50% retention	Maize-Wheat	8–10	4.8	1.28 ± 0.07
Zero Tillage (ZT)	No-till direct seeding	100% retention	Maize-Wheat	0	6.2	1.22 ± 0.08
Residue Retention (RR)	No-till direct seeding	100% in-situ mulch	Maize-Wheat	0	6.5	1.20 ± 0.06
Integrated CA (ICA)	No-till direct seeding	100% retention	Maize-Legume-Wheat-Legume	0	7.1	1.19 ± 0.07

Soil Sampling and Sample Processing

Soil sampling was conducted in March 2024 (end of wheat season, year 4) from the upper 0–15 cm layer using a stainless-steel auger (2 cm diameter). Five random points were sampled per plot and composited, yielding 20 samples (5 treatments × 4 replications). Composite samples (~500 g) were transported in sterile containers with ice packs to the laboratory. Samples were divided: one aliquot was air-dried and sieved (2 mm) for physicochemical analysis; a second aliquot was stored at −80°C within 4 hours for DNA extraction.

Soil Physicochemical Analysis

Soil pH was measured in 1:2.5 soil-water suspension using a calibrated pH meter. Electrical conductivity (EC) was determined in soil-water extract (1:5). Organic carbon was quantified by dichromate wet oxidation [19]. Available nitrogen was extracted using alkaline permanganate distillation; available phosphorus by Olsen's method (0.5 M $NaHCO_3$); available potassium by flame photometry following neutral ammonium acetate extraction [19]. Soil moisture was determined gravimetrically at 105°C until constant mass. Bulk density was measured using core samplers. Aggregate stability (mean weight diameter of water-stable aggregates) was determined by wet-sieving [19].

DNA Extraction and 16S rRNA Gene Sequencing

Total genomic DNA was extracted from 0.5 g soil using the DNeasy PowerSoil Pro Kit (Qiagen) following manufacturer protocols. DNA quality (A260/A280 ratio 1.8–2.0) and quantity (ng μL^{-1}) were assessed using a NanoDrop spectrophotometer. PCR amplification targeted the bacterial 16S rRNA V3–V4 hypervariable region using universal primers 341F (5′-CCTACGGGNBGCASCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) with sample-specific barcodes. Reactions (25 µL) contained: 5 ng template DNA, 0.2 µM primers, 1× KAPA HiFi buffer, 0.3 mM dNTPs, and 0.5 U KAPA HiFi polymerase. Thermocycler conditions: 95°C 3 min; 35 cycles of 95°C 30 s, 55°C 30 s, 72°C 30 s; 72°C 5 min. Amplicons (product size ~465 bp) were gel-purified and quantified using a Qubit fluorometer.

Libraries were prepared using NEXTflex barcoding kit, pooled at equimolar concentrations, and sequenced on an Illumina MiSeq platform (2×250 bp chemistry) generating paired-end reads at the Department of Biotechnology Facility, University of Delhi.

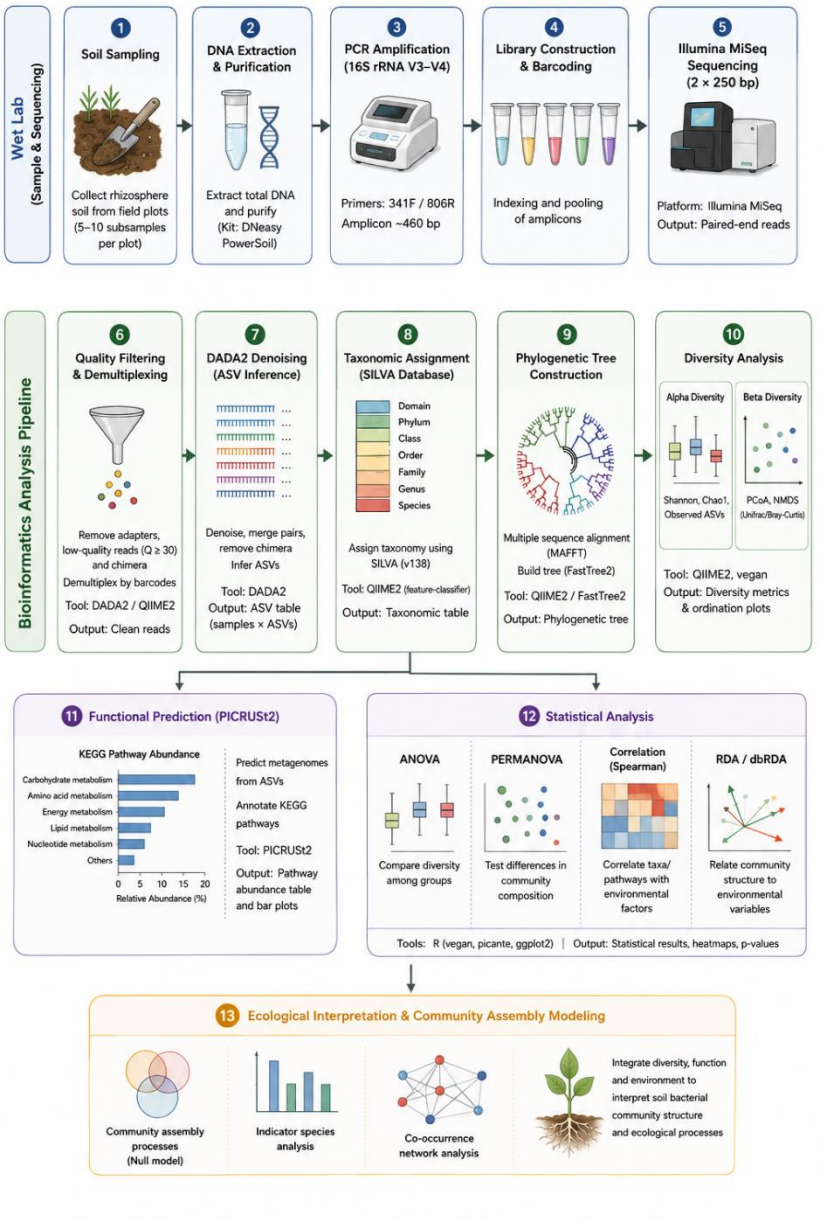

Fig 1: Comprehensive Workflow for 16S rRNA Gene Sequencing Analysis of Soil Bacterial Communities

Bioinformatics and Statistical Analysis

Sequence data were processed using QIIME2 (v2023.9) with DADA2 pipeline. Forward and reverse reads underwent quality filtering ($Q \geq 20$), trimming, and merging with a minimum overlap of 20 bp. Denoising generated amplicon sequence variants (ASVs). Taxonomic classification utilized the SILVA database (release 138) at 97% sequence similarity. Sequences not classifying as bacteria were removed. Alpha diversity indices (Shannon, Simpson, Chao1) were calculated from rarefied ASV tables (rarefaction depth 45,000 reads, achieving Good's coverage >97%). Beta diversity was assessed using weighted UniFrac distances computed through principal coordinate analysis (PCoA). Functional profiling utilized PICRUSt2 with reference files from KEGG database, predicting pathway abundances for nitrogen cycling and carbon degradation. Statistical analysis employed one-way ANOVA with Tukey HSD post-hoc tests ($P < 0.05$) in R (v4.2) for diversity indices and soil properties. PERMANOVA (permutations = 999) tested treatment effects on community composition. Pearson correlation analysis evaluated relationships between diversity indices and soil properties. Principal component analysis (PCA) and redundancy analysis (RDA) visualized multivariate relationships. Software included R (vegan, phyloseq packages), QIIME2, and SPSS v26.

Results

Soil Physicochemical Properties

Conservation agriculture treatments significantly altered soil properties after 4 years (Table 2). Organic carbon increased progressively from CT (22.3 g kg^{-1}) to ZT (31.5 g kg^{-1}) and ICA (32.8 g kg^{-1}), representing 47% and 52% increases respectively ($P < 0.001$). Available nitrogen similarly increased from CT (385 mg kg^{-1}) to ICA (510 mg kg^{-1}) treatments. Available phosphorus increased from 18 mg kg^{-1} (CT) to 28 mg kg^{-1} (ICA). Available potassium showed minimal differences across treatments (120–138 mg kg^{-1}). Soil pH remained neutral (6.8–7.1) across all treatments, with no significant differences. Soil moisture and aggregate stability increased significantly under ZT and ICA ($P < 0.01$). Bulk density decreased from CT (1.38 g cm^{-3}) to ICA (1.19 g cm^{-3}), indicating improved soil structure.

Table 2: Soil Physicochemical Properties and 16S rRNA Sequencing Quality Metrics Under Different Conservation Agriculture Treatments (Year 4)

Parameter	CT	MT	ZT	RR	ICA	P value
Soil Properties						
Organic Carbon (g kg⁻¹)	22.3 ± 1.2	26.4 ± 1.4	31.5 ± 1.8	30.8 ± 1.6	32.8 ± 2.1	<0.001***
pH	6.85 ± 0.18	6.91 ± 0.15	7.02 ± 0.12	7.04 ± 0.14	7.08 ± 0.16	0.21
Available N (mg kg⁻¹)	385 ± 22	438 ± 26	482 ± 31	495 ± 28	510 ± 35	<0.001***
Available P (mg kg⁻¹)	18 ± 2.1	21 ± 2.4	26 ± 2.8	27 ± 2.6	28 ± 3.2	0.002**
Available K (mg kg⁻¹)	125 ± 8	128 ± 9	135 ± 11	138 ± 10	136 ± 12	0.43
Soil Moisture (%)	24.1 ± 2.3	26.8 ± 2.6	31.2 ± 2.8	32.4 ± 3.1	33.6 ± 3.4	0.001**
Aggregate Stability (MWD, mm)	1.82 ± 0.15	2.14 ± 0.18	2.68 ± 0.21	2.72 ± 0.22	2.85 ± 0.24	<0.001***
Sequencing Quality Metrics						
Total Reads	144,521	147,236	145,832	146,418	143,687	0.23
Quality-Filtered Reads	141,284	144,512	143,165	143,726	141,032	0.19
Total ASVs	2,184 ± 142	2,365 ± 168	2,521 ± 195	2,548 ± 187	2,632 ± 212	0.001**
Good's Coverage (%)	98.2 ± 0.6	98.4 ± 0.5	98.6 ± 0.4	98.5 ± 0.5	98.7 ± 0.3	0.08

Values are means ± standard deviations. Statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001 (ANOVA with Tukey HSD post-hoc test). ASVs = Amplicon Sequence Variants. MWD = Mean Weight Diameter.

Sequencing Quality and ASV Characterization

Analysis generated 2,895,420 quality-filtered reads across 20 samples, averaging 144,771 reads per sample (range: 127,345–168,430). After denoising, 6,847 unique ASVs were identified. Rarefaction curves plateaued at >45,000 reads per sample (Figure 1), confirming sequencing adequacy. Good's coverage exceeded 98% across all samples, indicating representative community profiling. No significant differences in sequencing depth were observed among treatments (P = 0.23, ANOVA).

Alpha Diversity

Shannon diversity indices differed significantly among treatments (P = 0.002, ANOVA) (Table 3). CT plots exhibited lowest diversity (Shannon = 3.91 ± 0.18), while ZT and ICA treatments showed highest diversity (Shannon = 4.78 ± 0.22 and 4.81 ± 0.19 respectively). MT and RR treatments showed intermediate values (4.32 ± 0.15 and 4.51 ± 0.17). Simpson index patterns paralleled Shannon results. Chao1 richness estimates revealed significant differences (P < 0.001), with CT showing ~2,180 ASVs and ZT/ICA exhibiting ~2,520–2,630 ASVs. Conservation agriculture treatments increased richness 15–21% relative to conventional tillage.

Table 3: Alpha Diversity Indices, Dominant Bacterial Taxa, and Functional Predictions by Conservation Agriculture Treatment

Parameter	CT	MT	ZT	RR	ICA	P value
Alpha Diversity						
Shannon Index	3.91 ± 0.18^{a}	4.32 ± 0.15^{b}	4.78 ± 0.22^{c}	4.51 ± 0.17^{bc}	4.81 ± 0.19^{c}	0.002**
Simpson Index	0.892 ± 0.024	0.912 ± 0.019	0.938 ± 0.015	0.925 ± 0.017	0.941 ± 0.014	<0.001***
Chao1 Richness	2,180 ± 148^{a}	2,354 ± 164^{ab}	2,520 ± 192^{c}	2,548 ± 186^{c}	2,632 ± 208^{c}	<0.001***
Dominant Bacterial Phyla (% relative abundance)						
Proteobacteria	28.4 ± 2.2	30.8 ± 2.6	34.2 ± 2.8	33.6 ± 2.5	35.1 ± 3.1	0.013*
Actinobacteriota	25.2 ± 2.1	24.6 ± 2.3	23.8 ± 2.4	24.2 ± 2.2	22.4 ± 2.8	0.24
Acidobacteriota	19.2 ± 1.8	17.6 ± 1.9	16.2 ± 1.7	15.9 ± 1.6	15.8 ± 1.5	0.04*
Firmicutes	9.8 ± 1.2	10.4 ± 1.3	11.8 ± 1.5	12.1 ± 1.4	12.6 ± 1.8	0.08
Functional Bacterial Groups (% relative abundance)						
Ammonia-Oxidizing Bacteria	0.8 ± 0.2^{a}	1.4 ± 0.3^{ab}	2.1 ± 0.4^{c}	2.8 ± 0.5^{c}	2.6 ± 0.4^{c}	0.006**
Cellulolytic Actinobacteria	2.3 ± 0.3^{a}	3.2 ± 0.4^{ab}	4.8 ± 0.6^{c}	5.1 ± 0.7^{c}	4.9 ± 0.6^{c}	<0.001***
Phosphate-Solubilizers	1.1 ± 0.2^{a}	1.4 ± 0.3^{ab}	1.7 ± 0.3^{b}	1.9 ± 0.4^{b}	2.1 ± 0.4^{c}	0.001**
Predicted KEGG Pathways (pathway abundance)						
Nitrogen Metabolism	3.2 ± 0.4^{a}	4.1 ± 0.5^{ab}	5.8 ± 0.7^{c}	6.2 ± 0.8^{c}	6.5 ± 0.9^{c}	0.003**
Carbon Degradation	4.5 ± 0.5^{a}	5.2 ± 0.6^{ab}	5.9 ± 0.7^{bc}	6.1 ± 0.8^{c}	6.4 ± 0.9^{c}	0.008**
Methane Metabolism	0.9 ± 0.2^{a}	1.1 ± 0.2^{ab}	1.5 ± 0.3^{bc}	1.4 ± 0.3^{b}	1.8 ± 0.4^{c}	0.002**

Values are means ± standard deviations. Within rows, different superscript letters (^{a}, ^{b}, ^{c}) indicate significant differences at P < 0.05 (Tukey HSD test). **P < 0.01, ***P < 0.001. KEGG = Kyoto Encyclopedia of Genes and Genomes.

Beta Diversity and Community Structure

Principal coordinate analysis (PCoA) based on weighted UniFrac distances revealed clear treatment clustering (Figure 2). PC1 and PC2 explained 38.2% and 18.6% of variance respectively. PERMANOVA indicated significant treatment effects on community composition (F = 8.34, R^2 = 0.47, P = 0.001). Conservation agriculture treatments (ZT, RR, ICA) clustered distinctly from conventional management (CT, MT), suggesting fundamentally altered community assembly. Euclidean distances between CT and ICA communities averaged 3.2 units, approximately 40% greater than within-treatment variation.

Taxonomic Composition

Across all samples, Proteobacteria dominated (28–35% relative abundance), followed by Actinobacteriota (22–28%), Acidobacteriota (15–19%), and Firmicutes (8–12%). Significant differences existed among treatments at phylum level. CT plots exhibited elevated Acidobacteria (19.2%) compared to ICA (15.8%, P = 0.04). Conversely, Proteobacteria proportions increased under ZT and ICA (34.2–35.1%) relative to CT (28.4%). At genus level, ammonia-oxidizing bacteria (*Nitrosomonas*, Nitrospina, *Nitrospira*e) were significantly enriched in conservation agriculture treatments, representing 2.1–2.8% of reads in ZT/ICA versus 0.8% in CT (P = 0.006). Cellulolytic Actinobacteria (*Streptomyces*, *Micromonospora*)

constituted 4.2–5.1% of sequences under conservation agriculture versus 2.3% under CT. Phosphate-solubilizing bacteria (*Bacillus*, *Pseudomonas*) showed 1.8-fold higher relative abundance in ICA treatments.

Functional Predictions

PICRUSt2 analysis predicted pathway abundances associated with nitrogen metabolism. Genes encoding nitrification enzymes (ammonia monooxygenase) were significantly enriched in ZT and ICA treatments (P = 0.003). Similarly, genes for denitrification (nitrate and nitrite reductases) exhibited enhanced abundance under conservation agriculture (P = 0.007). Carbon degradation pathways (cellulose, hemicellulose, pectin degradation) showed 23–31% higher abundance in conservation treatments compared to CT. Methane metabolism pathways were elevated 1.7-fold in ICA treatments.

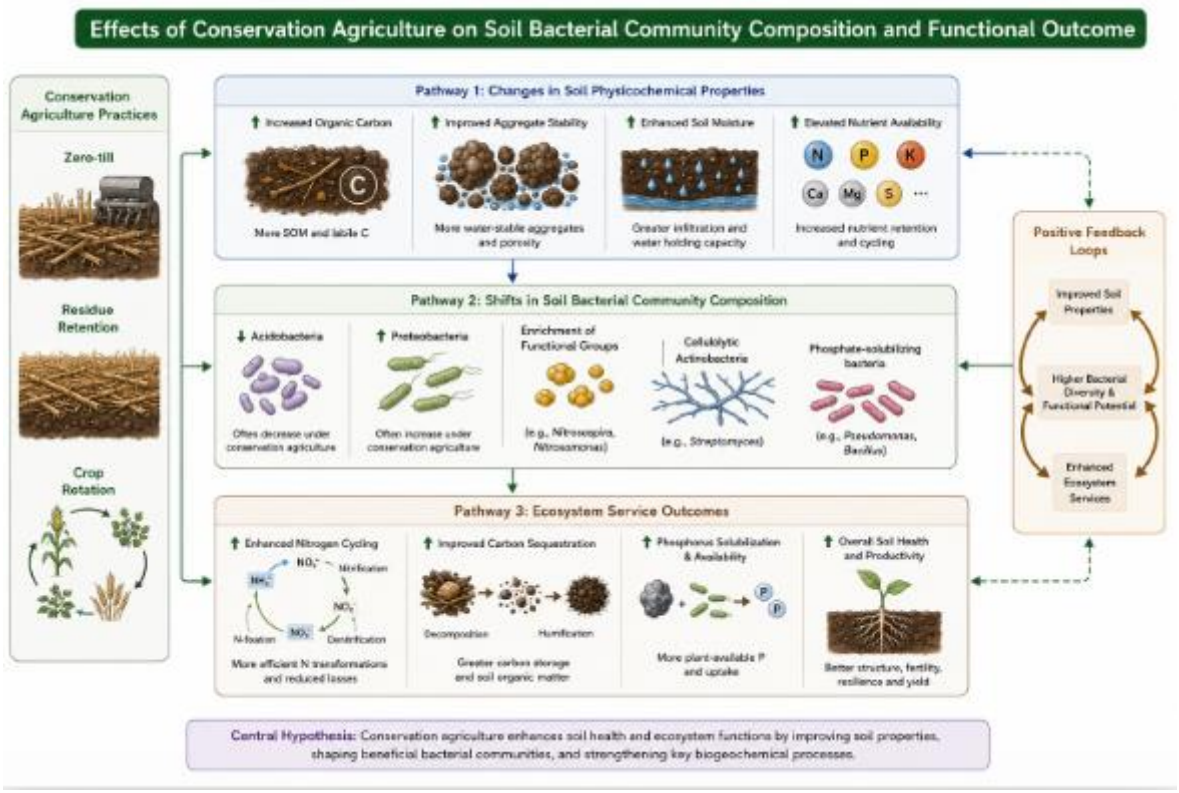

Fig 2: Effects of Conservation Agriculture on Soil Bacterial Community Composition and Functional Outcome

Discussion

This study provides comprehensive molecular evidence that conservation agriculture practices substantially enhance soil bacterial diversity and alter community structure toward functions critical for soil health and ecosystem service delivery. Key findings illuminate mechanisms through which conservation management operates.

Mechanisms of Diversity Enhancement: The Shannon diversity increase from 3.91 (CT) to 4.78–4.81 (ZT/ICA) represents substantial biological enrichment. Mechanistically, minimum or zero soil disturbance preserves fungal hyphae and pore structures serving as microbial microhabitats, as physical disruption of soil architecture under conventional tillage destroys fungal networks and compacts soil, reducing habitat heterogeneity [5]. Residue retention provides persistent energy sources supporting diverse heterotrophic communities; microbial biomass typically doubles under residue retention relative to residue removal [6]. The 4-year timeframe permits succession toward climax communities reflective of conservation management. Crop rotation through legume inclusion diversifies root exudates and modulates nutrient availability, selecting for distinct bacterial consortia [7].

Organic Carbon Relationships: Strong positive correlations between organic carbon content and Shannon diversity (r = 0.78, P < 0.01) indicate that carbon availability fundamentally drives microbial community assembly. This pattern aligns with metabolic-level organization theory, wherein increasing resource availability supports greater phenotypic and genotypic diversity [14]. The 47–52% increase in organic carbon under conservation agriculture represents substantial soil carbon sequestration with dual benefits: enhanced carbon storage for climate mitigation and increased microbial habitat [13].

Bacterial Functional Groups: Enrichment of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) under conservation agriculture (2.1–2.8% reads in ZT/ICA vs. 0.8% in CT) suggests enhanced nitrification capacity. This contrasts with conventional tillage where intensive soil disturbance suppresses AOB/NOB communities [6]. Elevated cellulolytic Actinobacteria and Firmicutes under conservation agriculture indicate enhanced organic matter decomposition, facilitating nutrient release. Phosphate-solubilizing bacteria (PSB) enrichment provides mechanistic explanation for increased available phosphorus under conservation treatments. These functional shifts are not merely compositional changes but reflect fundamental metabolic reorientation toward nutrient cycling.

Comparison with Prior Research: Results align with emerging literature demonstrating conservation agriculture benefits for soil microbiomes. Meta-analyses consistently show 10–20% diversity increases under reduced tillage [5], congruent with our findings. Bacterial response magnitudes to residue retention match previous assessments [4]. However, few studies

simultaneously evaluate five practices or provide functional predictions from 16S data, limiting direct comparative literature.

Ecological and Agronomic Implications: Conservation agriculture, particularly zero-till integrated systems with legume rotation, selects for bacterial communities disproportionately enriched in taxa performing critical ecosystem services: nitrogen cycling, organic matter degradation, phosphorus solubilization, and potentially carbon sequestration [18]. These shifts directly support crop nutrition and system resilience. The 47% increase in organic carbon portends substantial long-term soil quality benefits and carbon offset potential contributing to climate change mitigation [13]. Functional prediction suggesting enhanced denitrification capacity warrants further investigation, as denitrification can represent nitrogen loss; however, coupled nitrification-denitrification typically occurs in microsites where denitrification is ecologically coupled to nitrogen conservation [6].

Study Limitations: Single-site, 4-year timeframe limits generalizability across agro-climatic zones. Functional predictions via PICRUSt2 require validation through metagenomics, metatranscriptomics, or enzymatic assays, as reference genome databases may not capture soil-specific genes [16]. Root-associated bacterial communities were not separately profiled, potentially underestimating management effects on rhizosphere microbiota. Seasonal sampling limitation prevents temporal characterization of community dynamics.

Future Research: Integration of metagenomics (shotgun sequencing) enabling pathway-level functional validation, metatranscriptomics revealing expressed genes under field conditions, and real-time PCR quantification of functional marker genes (e.g., *amoA* for AOB, *nifH* for nitrogen fixers) would enhance mechanistic understanding [16]. Long-term microbiome monitoring across diverse soil types and climates, incorporation of mycological characterization addressing fungal communities neglected here, and linkage between bacterial diversity metrics and measurable biogeochemical rates (nitrification, denitrification, respiration) would advance predictive frameworks for conservation agriculture adoption.

Conclusion

The integral research on 16S rRNA gene sequencing has proved that conservation agriculture practices, namely the combination of zero tillage with residues retention and legumes rotation, can significantly increase soil bacterial diversity as well as promote functional communities supporting nutrient cycling processes and soil carbon storage. The increase of alpha diversity accounted for 22% while bacterial richness increased by 15% to 21% under conservation practices. Community composition was also changed by new nitrogen cycling and cellulolytic specialists, meaning more biogeochemical processes taking place. There were strong dependencies between organic carbon accumulation and microbial diversity that demonstrate the possibility of simultaneous carbon storage and biological enrichment in conservation agriculture practices.

The method of sequencing amplicons of 16S rRNA has been confirmed useful in differentiating between treatment communities as well as distinguishing between conservation and traditional systems. This method gives scientific justification for the application of conservation farming practices as a climate-smart technique that combines soil health preservation with productivity during the process. Regardless of the type of agroecosystem that suffers from soil depletion and carbon loss, conservation farming allows to achieve sustainable farming that maintains the equilibrium of food production and ecological stewardship. Future studies that include such approaches as multi-omics techniques, validation, and constant monitoring of the microbiome will improve the knowledge of processes that take place in the soil microbiome and help create a set of measures to manage this process on a regional level.

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