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Soil Microbial Dynamics across Conventional, Organic, and Natural Farming Systems in a Rice-Wheat Agroecosystem of the Indo-Gangetic Plains

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Abstract

Soil microbial processes are key drivers of agroecosystem functioning, regulating carbon turnover, nutrient cycling, and long-term soil resilience under varying management regimes. This study evaluated the influence of conventional (CON), organic (ORG), and natural farming (NAT) systems on soil microbiological health in a rice–wheat cropping system of the Indo-Gangetic Plains during 2023–2024. Soil samples collected from two depths (0–15 and 15–30 cm) were analyzed for basal respiration rate (BRR), microbial biomass carbon (MBC), soil organic carbon (SOC), nitrogen mineralization rate (NMR), and phospholipid fatty acid (PLFA)-based microbial community composition.

The results revealed significant improvements in soil biological properties under organic and natural farming systems, particularly in the surface soil (0–15 cm). Compared with conventional farming, organic farming increased MBC, SOC, BRR, and NMR by 451%, 99%, 39%, and 119%, respectively, while natural farming increased these parameters by 168%, 36%, 19%, and 44%, respectively. PLFA profiling showed bacterial-dominated communities under conventional farming, whereas organic and natural farming systems supported greater fungal, actinomycetal, anaerobic, and eukaryotic biomass, indicating higher microbial diversity and ecological stability.

The findings demonstrate that soil microbial activity and community composition are sensitive indicators of soil health and agroecosystem resilience. Enhanced microbial functioning under organic and natural farming systems supports nutrient cycling, carbon sequestration, and long-term sustainability. These emphasise the importance of biologically sustainable farming practices as policy-relevant pathways for strengthening resilient food systems and sustainable food security in the Indo-Gangetic Plains under increasing climatic and resource stresses.

Introduction

Healthy soil can be considered one of the critical components of sustainable farming practices and depends to a great extent on soil microbial population abundance and functionality. Soil microorganisms strongly affect the decomposition process, mineralization cycle, and even plant growth and yield (1, 2). Agricultural practices strongly influence this process since the degree of management, residues, and types of inputs significantly affect soil biological quality (3, 4).

Although conventional agricultural practices result in increased productivity through the use of synthetic fertilizers, pesticides, and intensive tilling practices, such methods negatively affect the quality of soil, reduce microbial biomass, decrease microbial diversity, and impair nutrient cycling (5, 6). Organic agricultural practices reduce these adverse effects by employing biological alternatives for soil management, such as composting, crop waste recycling, green manure, and biopesticides, which help improve the quality of soil by promoting microbial activity and carbon stabilization (7, 8). Furthermore, natural farming practices take this concept forward by focusing on on-farm resource recycling and minimal disturbance to land through mulching, microbial inoculation, and cow dung-based applications, among other practices (9, 10).

In the Indo-Gangetic Plains, particularly in Haryana, Uttar Pradesh, and Punjab, India's rice–wheat cropping system, which covers approximately 10 million hectares, is crucial for national food security (11) Haryana cultivated more than 95% of its agricultural land under this crop rotation, which underscores the region's heavy dependence on intensive cereal-based agriculture (12). Rice is grown during the kharif season in flooded or puddled soil conditions, followed by wheat in the rabi season, when the soil is relatively dry and tilled. Wheat (*Triticum aestivum* L.) is India's second most important staple crop after rice. Since IGPs produce most of the country's wheat, they have a large effect on calorie consumption, food safety, and the survival of farmers (10, 13). Cropping systems for wheat production in this region involve the heavy use of fertilizers, traditional tillage operations, and a small window period between the harvesting of rice crops and the planting of wheat crops, all of which affect soil microbial activities. In recent decades, the heavy dependence on conventional practices such as the burning of residue, excessive tillage, and the indiscriminate use of fertilizer has resulted in a decline in soil structural and functional stability. This has led to the depletion of soil organic carbon (SOC), disturbances in soil microbial community composition, and changes in nutrient cycling pathways (14, 15). After the rice is harvested, crop residue burning is frequently performed to facilitate rapid wheat growth. However, this approach led to the depletion of vital organic matter and increased pollution in the atmosphere (16), which ultimately suppressed the microbial consortia's biomass that is responsible for nitrogen and carbon transformations (17). Tillage activities associated with the establishment of wheat lead to increased oxidation of organic material, deterioration of soil aggregation, and weakening of rhizosphere relationships. These effects ultimately decrease microbial stability and nutrient efficiency (18, 19, 20, 21). Research conducted recently on wheat-based farming systems in the IGPs suggests that soil biological deterioration in conventional agricultural practices is positively related to decreased productivity and greater dependency on external inputs (10, 22). These results highlight the urgent need for alternative farming systems that can sustain wheat productivity through enhanced microbial activity, carbon storage, and nutrient recycling in highly productive rice–wheat agricultural landscapes. Resilient food systems depend not only on crop productivity but also on the ecological integrity of soils that sustain agricultural production over time. Soil microbial communities play a foundational role in maintaining this resilience by regulating nutrient cycling, organic matter decomposition, carbon sequestration, and soil structural stability (12). Since microbial biomass and activity respond rapidly to agricultural disturbances and management interventions, they are increasingly recognised as early indicators of soil health and agroecosystem resilience. In intensive rice–wheat systems of the Indo-Gangetic Plains, declining soil biological quality threatens long-term productivity and resource-use efficiency, thereby posing risks to regional food security. Therefore, evaluating microbial dynamics under different farming systems is essential for identifying sustainable management approaches and policy interventions that can support resilient food systems under climate variability, soil degradation, and increasing food demand.

The evaluation of soil biological quality involves multiple parameters, such as the microbial biomass carbon content, soil organic carbon content, basal respiration rate, and nitrogen mineralization rate. The parameter microbial biomass carbon is considered an indicator of living microorganisms contained in

soil organic matter, which rapidly respond to management modifications (23, 24, 25). The indicator basal respiration rate is associated with metabolic processes and is linked to substrate utilization efficiency; soil organic carbon not only sequesters carbon but also serves as a niche for microorganisms (26, 27). NMR tracks essential nitrogen transformations driven by microbes needed for healthy crop growth (28, 29, 30, 31, 32). Recent studies emphasize that these indicators differ significantly between low-disturbance farming systems and chemical-intensive agriculture (9, 22).

Phospholipid fatty acid (PLFA) profiling enhances biochemical indicators by characterizing microbial community composition and distinguishing among functional groups, including gram-positive bacteria, gram-negative bacteria, fungi, and actinomycetes (33, 34, 35, 36). In recent years, assessments based on PLFAs (phospholipid fatty acids) across Indian R–W systems have shown that retaining crop residues and practicing reduced tillage significantly improve microbial diversity, increase the dominance of fungi, and increase actinomycete activity. Conversely, practices such as residue burning and chemical intensification lead to a decrease in fungal and eukaryotic markers while increasing signals of microbial stress (22, 37).

Despite the growing recognition of the role of alternative farming systems in restoring soil biological health, comparative, field-based evidence that integrates microbial biomass, biochemical indicators, and PLFA-derived community composition across conventional, organic, and natural farming systems in the rice–wheat agroecosystem of Haryana is lacking. Existing research tends to focus on individual management practices—such as tillage, irrigation, or residue management—rather than assessing how entire farming systems influence microbial abundance, diversity, and function.

Natural farming is being increasingly promoted in India, yet it remains underrepresented in peer-reviewed studies on soil microbial ecology. Few studies integrate microbial biomass carbon, soil organic carbon, basal respiration rate, nitrogen mineralization rate and PLFA profiling within a single analytical framework, particularly in the Indo-Gangetic Plains, where agricultural intensification is most pronounced. To address these gaps, the present study undertakes a side-by-side, multiparameter evaluation of soil microbial functioning and community composition across conventional, organic, and natural farming systems in a rice–wheat rotation system, providing evidence to guide the development of resilient and ecologically sustainable management strategies for the region.

Materials and methods

3.1. Study area

The investigation was conducted in three intensively cultivated rice–wheat (R–W) growing districts of Haryana, India—Karnal, Kurukshetra, and Kaithal—between 29°27'–30°15' N latitude and 76°08'–77°15' E longitude. These districts constitute a major part of the Upper Yamuna Basin of the Indo-Gangetic Plains, representing the core of the cereal-based production system in the region. The total geographical areas of the Karnal, Kurukshetra, and Kaithal districts are approximately 2,520 km², 1,530 km², and 2,317 km²,

respectively (38). Sandy loam alluvial soil is the dominant soil type across the study sites, which is typical of the Indo-Gangetic alluvial tract.

The study region is characterized by a subtropical continental monsoon climate with hot summers and cool winters. From 2020 to 2024, t-he average annual rainfall in the districts of Karnal, Kurukshetra, and Kaithal was 704.7 mm, 601.7 mm, and 515.6 mm, respectively (39). During the monsoon season, approximately 80% of this precipitation occurs, which lasts from June to September. The mean daily maximum temperatures in May and June often reach approximately 40°C, whereas in winter, the temperatures start to decrease from mid-November onwards, with January being the coldest month.

A representative long-term rice–wheat rotation system was selected in each district, where the associated farming practices had been continuously implemented for at least five years without the inclusion of any intermediate crops. In this system, rice is cultivated during the Kharif season under warm, humid monsoon conditions, followed by wheat during the Rabi season under cooler and relatively drier winter conditions. Both crops are grown within a single annual cycle.

Table 1
Study area and experimental design

District	Villages	Latitude	Longitude
Kurukshetra (C)	Mehra	30.03767	77.06637
Kurukshetra (O)	Dera Fateh Singh	29.96466	76.9142
Kurukshetra (N)	Mehra	30.03735	77.06771
Karnal (C)	Khanda Kheri	29.57738	76.64884
Karnal (O)	Chirao	29.67623	76.9142
Karnal (N)	Khanda Kheri	29.57716	76.64769
Kaithal (C)	Karora	29.65038	76.55415
Kaithal (O)	Chandlana	29.87251	76.62837
Kaithal (N)	Kheri Rai Wali	29.87918	76.56756

3.2 Experimental design

The field experiment was conducted using a completely randomized design (CRD), which included three farming systems as treatments: conventional farming (CON), organic farming (ORG), and natural farming (NAT). In each district, three independent study sites were chosen, each representing one of the three farming systems, resulting in a total of nine sites across the study area. The experimental units were plots of 1 acre (220 ft × 199 ft) each. To capture within-site variability, triplicate soil samples were collected from each site during each sampling. This experimental design provided a robust framework

for systemwise and districtwise comparisons of soil microbial and biochemical responses under different farming system regimes within the rice–wheat agroecosystem of Haryana.

3.3 Soil sampling and analysis

Soil sampling was conducted over two consecutive cropping years, specifically in April 2023 and April 2024 after the wheat harvest, as well as in October 2023 and October 2024 following the rice harvest. Soil samples were collected at each site from two depths, 0–15 cm and 15–30 cm, using an acore sampler with a diameter of 7 cm. For each sampling, three replicate soil samples were collected from each site. This resulted in a total of 54 samples for each crop season (9 sites × 3 replicates × 2 depths) and a combined total of 216 samples across both crops and years. The samples used for microbial parameter analyses were transported to the laboratory in insulated ice boxes and stored at 4°C. Subsamples designated for phospholipid fatty acid (PLFA) analysis were preserved at -20°C to prevent lipid degradation, following standard protocols.

The following soil microbial parameters were analysed: soil organic carbon (SOC), microbial biomass carbon (MBC), nitrogen mineralization (NMR), and the basal respiration rate (BRR) via standard laboratory procedures. For SOC, we used the dichromate oxidation method of (40), which is widely used to determine soil organic carbon for determining farm fertility (41). Recent work continues to report the effectiveness of this method in the monitoring of agricultural soil, particularly in rice–wheat systems (10, 42). MBC was determined by the fumigation–extraction method followed by UV absorbance analysis, with calculations based on MBC = 2.64 × EC, which is the difference in extractable carbon between fumigated and nonfumigated samples (25, 43). Recent studies report the increased sensitivity of this method in conservation and organic farming systems, which in turn proves its importance in contemporary soil biological assessments (44, 45). BRR is a measure of microbial activity analysed via the alkali absorption method. In this case, the CO2 released from microbial respiration is absorbed in NaOH and then titrated with HCl (46). Recent studies have focused on the effectiveness of this method in differentiating microbe responses to different agricultural inputs (47, 48). NMR was evaluated using the Kjeldahl method by (49). This relates to recent findings that show shifts in mineralizable nitrogen under organic and residue-retentive management (50, 51). These methodological frameworks accurately characterize soil biological functioning in conventional, organic, and natural farming systems.

Soil microbial community composition and biomass were assessed by phospholipid fatty acid (PLFA) profiling, a method for evaluating viable microbial communities (52, 53). Lipids were extracted from the freeze-stored samples using modified Bligh–Dyer extraction. The extracted phospholipids were separated by solid-phase extraction and converted into fatty acid methyl esters (FAMEs).

PLFAs were analysed via gas chromatography using the MIS Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA). The MIDI PLFAD1 calibration mix and naming table were used for biomarker identification. FAME groups were categorized into straight-chain, branched-chain, hydroxylated, monounsaturated (MUFA), polyunsaturated (PUFA), and dimethyl acetal (DMA) fatty acids. The total PLFA concentration is expressed in nmol g^{-1} soil (54).

The high-throughput PLFA protocol improved processing efficiency for large sample sets, as documented by (54), and strongly agreed with standard protocols while reducing solvent use and preparation time.

3.4 Statistical Analysis

The data were analysed separately for all the factors. For depth and cropwise variations in different farming systems, a t test was performed. For site-wide variation, one-way ANOVA was performed. One-way analysis of variance (ANOVA) was also used to test the statistical significance of the effects of different farming practices on soil microbial parameters and soil microbial community structure (PLFA profile). Least significant difference (LSD) tests and Duncan's multiple range test (DMRT) were used to determine the significance of the treatment means. Data analysis was carried out using SPSS 21.0. Principal component analysis (PCA) was performed to identify relationships between soil PLFA indicators and different farming indicators, and a 2nd biplot generated from PCA was used to determine relationships between microbial parameters and farming systems.

Principal component analysis (PCA) and Pearson correlation analysis were performed to quantify the correlations between soil microbial parameters and soil microbial community structures using XLSTAT 2025.1.3 (1431).

Results

Soil organic carbon (SOC)

Significant differences in soil organic carbon (SOC) were observed among the three farming practices at both soil depths (0–15 cm and 15–30 cm), with the most pronounced variation occurring in the surface layer (0–15 cm). The highest SOC content was observed in organic farming (11.01 g/C/kg^{-1}), which was significantly greater than that in natural farming (7.57 g/C/kg^{-1}) and conventional farming (5.55 g/C/kg^{-1}) across both the crops and years in the surface layer.

At the 0–15 cm soil depth, the SOC differed significantly between wheat and rice across years and farming practices. The SOC content of wheat was substantially greater than that of rice under organic farming (82.7% greater), whereas under natural farming, a 4.7% greater SOC content was observed for wheat. In contrast, under conventional management, compared with wheat (35.6%), rice had higher SOC content in 2023. Compared with those under rice, the SOC under conventional, organic, and natural farming scenarios are 73.6%, 49.8%, and 34.4% greater, respectively, in 2024. Overall, these results indicate a consistent advantage in terms of SOC accumulation in the surface layer, particularly under organic management, while the differences in crop-specific characteristics were less pronounced under natural farming and reversed under conventional management in 2023.

Microbial Biomass Carbon (MBC)

Significant differences in MBC were observed among the three framing practices at both soil depths (0–15 cm and 15–30 cm). In the surface layer, the highest MBC was observed in the organic farming treatment (0.331 mg C kg^{-1}), which was significantly greater than that in the natural farming (0.161 mg C kg^{-1}) and conventional farming (0.060 mg C kg^{-1}) treatments across both years and crops. A similar pattern was observed in the subsurface layer, where organic farming (0.262 mg C kg^{-1}) was much greater than natural farming (0.113 mg C kg^{-1}) and conventional farming (0.049 mg C kg^{-1}) across both years and crops.

For the MBC, no significant differences were detected between the rice and wheat crops ($p > 0.05$). In the surface layer, compared with those in the other treatments, the values in the organic farming treatment (0.372 mg C kg^{-1}) were greater than those in the rice treatment (0.289 mg C kg^{-1}), whereas the values in the natural farming and conventional farming rice treatments (0.167 mg C kg^{-1} and 0.066 mg C kg^{-1}, respectively) were greater than those in the wheat treatment (0.155 mg C kg^{-1} and 0.054 mg C kg^{-1}, respectively) across both years. In the subsurface layer, compared with the organic, natural and conventional soils, the organic and conventional soils (0.302 mg C kg^{-1}, 0.118 mg C kg^{-1}, and 0.068 mg C kg^{-1}, respectively) had higher MBCs (0.221 mg C kg^{-1} and 0.108 mg C kg^{-1}, respectively) in both years. No significant differences were observed in the MBC across the two years ($p > 0.05$).

Table 2
Microbial soil properties (means± SEss) of different farming practices under the wheat–rice cropping system.

Crop type	Farming type	Year	MBC (mg/C/kg^{-1})	SOC (g/C/kg^{-1})	BRR (µg CO_2-C g^{-1} soil hr^{-1})	NMR (µg N g^{-1} month^{-1})
			0–15			
Wheat	CON	2023	.01 ± 0.02^{c}	4.01 ± 2.11^{c}	123.18 ± 24.74^{c}	18.65 ± 11.20^{b}
		2024	.09 ± 0.05^{c}	7.58 ± 2.02^{b}	138.41 ± 14.90^{b}	24.23 ± 8.4^{c}
	ORG	2023	.31 ± 0.08^{a}	16.40 ± 3.05^{a}	205.53 ± 18.17^{a}	59.73 ± 7.41^{a}
		2024	.43 ± 0.13^{a}	11.21 ± 1.61^{a}	170.55 ± 8.85^{a}	59.08 ± 17.37^{a}
	NAT	2023	.10 ± 0.11^{b}	8.48 ± 3.25^{b}	167.75 ± 26.53^{b}	27.34 ± 10.17^{b}
		2024	.21 ± 0.08^{b}	7.84 ± 2.08^{b}	151.55 ± 16.17^{b}	38.54 ± 10.76^{b}
Rice	CON	2023	.05 ± 0.05^{b}	6.23 ± 1.85^{b}	153.67 ± 3.62^{b}	38.58 ± 12.03^{c}
		2024	.08 ± 0.04^{b}	4.37 ± 2.66^{b}	85.96 ± 47.01^{b}	39.82 ± 11.35^{c}
	ORG	2023	.30 ± 0.28^{a}	8.97 ± 1.50^{a}	174.26 ± 12.69^{a}	73.42 ± 13.27^{a}
		2024	.28 ± 0.13^{a}	7.48 ± 2.43^{a}	146.25 ± 13.35^{a}	72.80 ± 16.33^{a}
	NAT	2023	.17 ± 0.17^{ab}	8.10 ± 1.26^{a}	160.81 ± 7.15^{b}	54.76 ± 9.61^{b}
		2024	.16 ± 0.13^{b}	5.83 ± 2.20^{ab}	117.99 ± 34.95^{ab}	53.51 ± 10.52^{b}
		15–30				
Wheat	CON	2023	.02 ± 0.02^{c}	3.25 ± 1.77^{c}	127.41 ± 27.48^{b}	12.63 ± 9.23^{c}

Crop type	Farming type	Year	MBC (mg/C/kg^{-1})	SOC (g/C/kg^{-1})	BRR (µg CO_2-C g^{-1} soil hr^{-1})	NMR (µg N g^{-1} month^{-1})
			0–15			
		2024	.03 ± 0.02^{b}	3.66 ± 1.99^{b}	130.37 ± 10.38^{c}	23.61 ± 9.74^{b}
	ORG	2023	.32 ± 0.12^{a}	15.05 ± 3.10^{a}	234.51 ± 47.44^{a}	47.28 ± 9.05^{a}
		2024	.13 ± 0.04^{a}	6.72 ± 1.31^{a}	168.25 ± 9.42^{a}	46.63 ± 9.70^{a}
	NAT	2023	.16 ± 0.12^{b}	8.73 ± 3.19^{b}	155.01 ± 14.95^{b}	27.78 ± 8.32^{b}
		2024	.06 ± 0.03^{b}	4.82 ± 1.90^{b}	149.72 ± 10.82^{b}	27.34 ± 7.81^{b}
Rice	CON	2023	.07 ± 0.06^{b}	3.60 ± 0.93^{c}	137.03 ± 19.91^{c}	31.73 ± 10.10^{b}
		2024	.07 ± 0.13^{a}	1.06 ± 0.61^{c}	90.24 ± 45.51^{b}	29.87 ± 7.92^{c}
	ORG	2023	.46 ± 0.32^{a}	6.25 ± 0.51^{a}	176.96 ± 11.37^{a}	58.49 ± 10.88^{a}
		2024	.14 ± 0.10^{a}	4.76 ± 1.80^{a}	138.61 ± 15.68^{a}	56.00 ± 13.72^{a}
	NAT	2023	.17 ± 0.11^{b}	5.14 ± 0.89^{b}	160.56 ± 4.26^{b}	39.82 ± 11.00^{b}
		2024	.07 ± 0.05^{a}	2.54 ± 1.64^{b}	113.61 ± 33.56^{ab}	41.69 ± 6.33^{b}

Basal Respiration Rate (BRR)

No significant difference was observed in the basal respiration rate (BRR) at either depth. Among the three farming practices, significant differences were observed in the BRR. In the surface layer, the organic farming (174.15 µg CO_2-C g^{-1} soil hr^{-1}) is 38. 9% higher than that in conventional farming (125.31 µg CO_2-C g^{-1} soil hr^{-1}) and 16.4% higher than that in natural farming (149.53 µg CO_2-C g^{-1} soil hr^{-1}). Cropwise analysis revealed significant differences in the basal respiration rate (BRR) at the 0–15 cm soil depth, with wheat crops under organic, conventional and natural farming (188.0 µg CO_2-C g^{-1} soil hr^{-1},

130.8 μg CO_2-C g^{-1} soil hr^{-1} and 159.7 μg CO_2-C g^{-1} soil hr^{-1}) consistently exhibiting higher BRR than rice under organic, conventional and natural farming (160.3 μg CO_2-C g^{-1} soil hr^{-1}, 119.8 μg CO_2-C g^{-1} soil hr^{-1} and 139.4 μg CO_2-C g^{-1} soil hr^{-1}) in both years. A yearwise comparison across all the farming practices indicated significant temporal variation in the BRR. At 0–15 cm, the BRR decreased from 164.2 μg CO_2-C g^{-1} soil hr^{-1} in 2023 to 135.1 μg CO_2-C g^{-1} soil hr^{-1} in 2024 across both the crops and the three farming practices, whereas at 15–30 cm, it decreased from 165.3 μg CO_2-C g^{-1} soil hr^{-1} in 2023 to 131.8 μg CO_2-C g^{-1} soil hr^{-1} in 2024, reflecting a significant reduction in microbial respiratory activity during the second year of the study ($p < 0.05$).

Nitrogen mineralization rate (NMR)

The nitrogen mineralization rate (NMR) varied significantly at both soil depths (0–15 cm and 15–30 cm). In the surface layer, the highest NMR was observed in organic farming (66.26), which was significantly greater than that in natural farming (43.54) and conventional farming (30.32) across both years and crops. A similar pattern was observed in the subsurface layer, where organic farming (52.10) was much greater than natural farming (34.16) and conventional farming (24.46) across both years and crops ($p < 0.05$). Among the three farming practices, significant differences were observed in the NMR ($p < 0.05$). In the surface layer, compared with conventional farming (118.6%) and natural farming (52.5%), organic farming resulted in a higher NMR. Similarly, in the subsurface layer, compared with conventional farming (112.9%) and natural farming (52.5%), organic farming resulted in a higher NMR across both years and crops.

In a cropwise comparison across all the farming practices and at both depths, the average NMR was greater for rice than for wheat. Under organic farming, the NMR in rice was 22.6% greater than that in wheat under natural farming, that in rice was 24.8% greater than that in wheat, and under conventional farming, the NMR was 38.7% greater in rice than in wheat. No significant differences were observed during either year.

PLFA-based Community Structure

Phospholipid fatty acid (PLFA) profiling offers a sensitive and quantitative method to evaluate soil microbial community composition and functional potential, reflecting both taxonomic structure and metabolic activity. In this study, PLFA analysis was used to examine how three distinct farming systems, organic farming (ORG), natural farming (NAT), and conventional farming (CON), influence the composition and functional dynamics of soil microbial communities. The focus included various groups, such as gram-positive ($gram^+$) and gram-negative ($gram^-$) bacteria, fungi, eukaryotes, anaerobes, and actinomycetes. Additionally, the associated fatty acid biomarkers, including straight-chain, branched, cyclopropyl, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), dimethyl acetals

(DMA) and specific fatty acids such as 18:1ω9c, 18:2ω6,9c, and 10-methyl fatty acids, were investigated.

The results in Table 3 indicate that gram-negative bacteria were most abundant in CON soils at 7.94 nmol g^{-1}, compared with 3.54 nmol g^{-1} in NAT and 3.44 nmol g^{-1} in ORG soils. The number of gram-positive bacteria was greatest in the CON soils (28.23 nmol g^{-1}), with lower abundances in the NAT (13.48 nmol g^{-1}) and ORG soils (9.72 nmol g^{-1}). These findings were supported by the PLFA fatty acid profiles, which revealed that branched PLFAs, a biomarker for gram-positive bacteria, were present at the highest levels in CON soils (20.00%), intermediate levels in NAT soils (8.81%), and lowest levels in ORG soils (6.68%), as illustrated in Fig. 2. Straight-chain PLFAs, representing general bacterial biomass, were relatively similar across the three systems, with ORG at 26.8%, NAT at 28.64%, and CON at 27.25%. Cyclopropyl fatty acids were detected only in the CON soils (0.66%). More fungal PLFAs (18:2ω6,9c) were observed in the ORG soils (0.21 nmol g^{-1}) than in the NAT soils (0.24 nmol g^{-1}) but were absent in the CON soils. Eukaryotic PLFAs, representing protozoa and other microfaunal organisms, were highest in the NAT soils (43.08 nmol g^{-1}), followed by the CON soils (32.23 nmol g^{-1}) and the ORG soils (30.99 nmol g^{-1}). PUFA levels, which reflect fungal and eukaryotic biomass, further confirmed these patterns, being highest in the NAT soils (29.02%), moderate in the ORG soils (20.49%), and lowest in the CON soils (19.54%). Anaerobic microorganisms were most abundant in the ORG soils at 54.56 nmol g^{-1}, compared with 37.88 nmol g^{-1} in the NAT and 30.81 nmol g^{-1} in the CON soils, as shown in Table 3. This trend aligned with the DMA content, which was highest in the ORG soils (41.33%), followed by that in the NAT soils (27.62%) and that in the CON soil (24.72%), as shown in Fig. 2. Actinomycetes were most abundant in the NAT soils (1.70 nmol g^{-1}), followed by ORG (1.01 nmol g^{-1}) and CON (0.79 nmol g^{-1}), with the corresponding 10-methyl fatty acid biomarker highest in the NAT soils at 1.55%, compared with 0.73% in ORG and 0.57% in CON.

The functional fatty acid markers shown in Fig. 2 provide additional insights into microbial activity. MUFAs, which are indicators of active bacterial metabolism, were highest in CON soils (5.29%), compared with 2.50% in ORG and 2.32% in NAT soils. The 18:1ω9c fatty acid, a general indicator of bacterial and fungal activity, remained relatively stable across systems (0.48% in ORG, 0.57% in NAT, and 0.57% in CON). Straight-chain PLFAs (ORG 26.8%, NAT 28.64%, CON 27.25%), branched PLFAs (ORG 6.68%, NAT 8.81%, CON 20.00%), cyclopropyl fatty acids (only in CON 0.66%), PUFAs (ORG 20.49%, NAT 29.02%, CON 19.54%), and DMA (ORG 41.33%, NAT 27.62%, CON 24.72%) collectively illustrate the differences in microbial composition and functional potential across the three farming systems.

Table 3

PLFA (phospholipid fatty acid) profiles (nmole g^{-1}) under different farming practices for rice crops.

Gram Negative	Organic	Natural	Conventional
	3.44	**3.54**	**7.94**
Eukaryote	31.0	43.1	32.2
Fungi	0.4	0.48	0
Gram Positive	9.72	13.5	28.2
Anaerobe	54.	37.9	30.8
Actinomycetes	1.00	1.7	0.78

Correlation analysis and principal component analysis between soil PLFA profiles and soil properties

The correlation matrix revealed strong and predominantly positive associations among the microbial, biochemical, and carbon-related soil properties (Table 4). MBC was strongly positively correlated with soil organic carbon (SOC; $r = 0.700$; $p < 0.01$), the net nitrogen mineralization rate (NMR; $r = 0.660$; $p < 0.01$), and the phospholipid fatty acid content (PLFA; $r = 0.679$; $p < 0.01$), indicating that increases in soil carbon pools are closely linked to microbial biomass and functional activity. Its relationship with the basal respiration rate (BRR) was moderate but significant ($r = 0.451$, $p < 0.05$), with metabolic activity increasing as the microbial abundance increased. SOC was significantly correlated with all biological indicators, including NMR ($r = 0.625$, $p < 0.01$), BRR ($r = 0.564$, $p < 0.01$), and PLFA ($r = 0.652$, $p < 0.01$), underscoring its central role in regulating and processing the soil microbial structure. NMR demonstrated the strongest association with PLFAs ($r = 0.755$, $p < 0.01$), suggesting that microbial community size and composition strongly govern N mineralization rate dynamics. BRR also showed significant positive correlations with all the variables, although with relatively low coefficients, suggesting that respiratory activity responds to but is not solely determined by microbial biomass and carbon availability. Overall, the significant interrelationships among SOC, MBC, PLFA, BRR, and NMR indicate a tightly integrated soil biological system in which enhanced carbon pools are linked to greater microbial abundance and nutrient cycling capacity.

Table 4 Correlations between phospholipid fatty acids and soil properties

	MBC	SOC	NMR	BRR	PLFA
MBC	1	.700**	.660**	.451*	.679**
SOC		1	.625**	.564**	.652**
NMR			1	.415*	.755**
BRR				1	.569**
PLFA					1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

The principal component analysis biplot (Figure 3(a)) clearly differentiates between the three farming systems. The first two principal components together account for 97.75% of the total variance—F1 at 90.53% and F2 at 7.22%. Along the F1 axis, conventional farming is strongly associated with gram-positive bacteria, indicating that this group is more abundant under conventional management. At the opposite end of the F1 axis, the organic system related to anaerobes indicates a greater presence of anaerobic microbial communities. The natural system sits in between, reflecting a transition between the other two systems. Additionally, Eukaryotes cluster on the positive side of F1 away from Actinomycetes and gram-negative bacteria, which in turn points to different microbial functional groups based on management practices. Actinomycetes and gram-negative bacteria, which group closely together on the negative side of F1, point to similar community responses and possible sensitivity to intensive management. Overall, the PCA results support that farming practices strongly influence soil microbial structure, with conventional farming favouring gram-positive taxa and organic farming promoting anaerobic and eukaryotic dominance.

In our study, the PCA biplot (Figure 3(b)) shows the relationships between the soil microorganisms and the soil microorganisms in the farming systems at multiple study sites. The first two principal components (F1 and F2), which account for 91.26% of the total variation, F1, which is 84.52%, and F2, which is 6.74%, indicate a robust data structure and are very efficient at reducing dimensions. The F1 axis strongly differed between the ORG and CON farming systems. In the positive area of F1, organic farming systems such as Kkr Org, Krnl Org, and Kthl Org are strongly related to PLFA, NMR, and BRR, which indicates that these systems have high microbial biomass, better community structure and more respiratory-related processes under organic management. In contrast, conventional farming tends to group in the negative area of F1 and is not strongly related to the microbial indicators, as suggested by low biomass and microbial activity. Natural farming lies between the ORG and CON practices, mainly in the positive F1 area. They are strongly associated with SOC and MBC, which implies that natural farming

increases SOC and MBC rather than causing large-scale changes in microbial functional diversity. The F2 axis, which accounts for a smaller variance proportion, differentiates individual sites within identical management categories. SOC and MBC had positive loadings on F2, whereas PLFA and NMR had negative loadings, revealing a secondary environmental gradient that distinguished carbon pool indicators from microbial community composition metrics. The biplot confirms that farming management practices represent the primary determinant of variability in soil microbial characteristics, with organic farming exerting the most substantial positive influence on microbial biomass and community-level parameters, natural farming showing intermediate effects, and conventional farming showing minimal correlation with these biological properties. This pronounced differentiation underscores the ecological advantages of alternative agricultural practices in promoting soil microbial health.

Discussion

Soil organic carbon (SOC)

Soil microbial characteristics are sensitive and early markers of soil disturbance because they directly reflect changes in the environment and management practices (55, 56, 57). The concurrent increase in SOC under organic management compared with that under conventional (98.7%) and natural farming (45.6%) reflects enhanced carbon inputs from crop residues and organic amendments that provide readily decomposable substrates for microbial growth. Similar findings were reported by (58), who reported that compared with chemical fertilizers, organic amendments significantly increased SOC by 17.22%. Compared with those under conventional farming, increases in SOC concentrations reflect the positive effects of inputs such as farmyard manure, compost, and slurry from organic farming on SOC (7, 59). In contrast, conventional systems maintained lower SOC pools and microbial biomass, indicating carbon limitation and reduced microbial turnover due to the use of chemical fertilizers, pesticides, intensive tillage and burning practices. Similar findings (60, 61) have reported that compared with manure, straw-based treatments and reduced tillage practices, chemical fertilizer treatment and intensive tillage resulted in lower soil organic carbon pools. The results of the present study are in line with previous findings that SOC exhibits depth-dependent patterns, with higher values observed in the surface soil layers than in deeper soil horizons (62, 63, 64). SOC (0.60%) and available N, P, and K were greater under organic management than under natural farming and integrated crop management (65). In the present study, the highest SOC content was observed in organic farming compared with natural and conventional farming, which is in line with previous findings (66), which revealed that the highest SOC content was found in organic farming (0.75%), followed by natural farming (0.59%) and conventional treatment (0.48%) in the maize + redgram intercropping system at Bhavikere, Karnataka.

Microbial Biomass Carbon (MBC)

Long-term carbon inputs impact microbial biomass and activity in different farming systems. Microbial biomass is among the most commonly used parameters in soil biology (67) and serves as an important reservoir of plant nutrients (68). In the present study, organic farming surpassed both natural and conventional farming by 105.59% and 451.66%, respectively. This occurred because of the organic C inputs in organically managed soils. These results align with those of (69), who reported that soil microbial biomass increased by 100% to 300% when farms transitioned from conventional to organic practices, demonstrating a clear impact on microbial community size. Although the soil microbial biomass decreased with depth, we detected increased MBC in the ORG at both soil depths (0–15 cm and 15–30 cm), which is important for our study. These findings indicate that a significant portion of the soil microbial community is colonizing the subsurface of the soil. The decrease in soil microbial biomass with increasing depth is consistent with previous findings (70, 71). Nonetheless, the ongoing addition of organic residues to organic farming may promote a greater increase in microbial biomass (72). (73) reported that microbial biomass carbon is significantly greater under zero and reduced tillage than under conventional tillage (e.g., zero tillage ~ 0.024% MBC vs. conventional tillage lower), suggesting enhanced biological carbon fractions under low-disturbance systems.

Basal soil respiration rate (BRR)

BRR is an indicator of microbial metabolic activity. Farming practices strongly influence soil respiration (74, 75). In this study, organic farming consistently supported the highest basal soil respiration rate, followed by natural farming and conventional farming. Similar findings were reported by (76), who reported that soil respiration was much greater in an organic farming system (45.51 CO2 mg 50 g-1) than in a conventional farming system (39.30 CO2 mg 50 g-1) of coffee in central western Ghats, India. Other comparisons of conventional and organic systems have also reported increased soil microbial respiration under organic management (77, 78). Increased soil respiration in organic farming results in enhanced microbial activity, caused by the continuous incorporation of decomposable organic matter that stimulates heterotrophic microorganisms (79). This increased respiration also signals vigorous biological processes and rapid organic residue breakdown, which facilitates nutrient availability for plant uptake. In contrast, conventional management restricts microbial metabolism, highlighting the critical role of farming practices in sustaining soil biological function and carbon cycling in intensive rice–wheat systems (24, 80, 81, 82, 24, 83). (84) reported that compared with deeper soil, organic amendments significantly increased soil respiration rates, with the highest rates occurring in the surface soil layers.

Nitrogen mineralization rate (NMR)

NMR responded strongly to farming practices, highlighting the central role of management in regulating nitrogen dynamics. Organic farming and organic amendments promote slower but sustained nitrogen mineralization through enhanced microbial activity and soil organic matter turnover, whereas conventional fertilizers provide rapid inorganic nitrogen with limited contributions to biologically mediated mineralization processes (85, 86, 87). Intensive tillage in conventional farming promotes rapid, short-term nitrogen mineralization by disrupting soil structure and enhancing aeration (88) but leads to

long-term depletion of organic nitrogen pools, whereas reduced tillage or no-tillage in organic farming slows initial mineralization while sustaining biologically mediated nitrogen release through improved soil organic matter protection and microbial functioning (89, 90). In the present study, the NMR was highest under organic farming, followed by natural farming and conventional farming. This finding indicates that chemical fertilizers resulted in rapid nitrogen release, which may not have aligned well with the demand of the crop, leading to lower overall mineralization. In contrast, organic amendments such as green manure, legumes, and crop residues and animal manures resulted in greater net N mineralization rates (91, 92). Natural farming resulted in moderate values, indicating partial restoration of biological processes under reduced chemical inputs, although these processes were constrained by limited organic amendments.

Changes in the composition of the soil microbial community under different farming practices by PLFA analysis

The total phospholipid fatty acid (PLFA) concentration is an indicator of overall microbial biomass in soil samples (93) and provides a valuable tool for evaluating how microbial community composition responds to various agricultural management strategies (94, 95). Farming practices strongly changed the structure of the microbial community; compared with conventional farming, organic and natural farming supported different sets of microbes. In the present study, organic and natural farming resulted in higher levels of anaerobes and eukaryotes, whereas conventional farming resulted in higher levels of gram-positive and gram-negative bacteria. The results of the present study on conventional farming are in line with the previous studies carried out by (96, 97). The PLFA profiles revealed a predominance of gram-negative bacteria under conventional farming, reflecting their copiotrophic strategy and rapid proliferation in nutrient-rich environments created by repeated mineral fertilizer inputs (98, 99). In contrast, the abundance of gram-positive bacteria, which are more stress tolerant and are able to use complex and difficult-to-break carbon sources, is reduced (100, 101). A PLFA-based shift toward fast-growing bacterial communities is a sign of increased short-term nutrient turnover and, at the same time, a trade-off in terms of microbially mediated carbon storage (102). Our results are in line with previous work (103, 4), which reported that in organic farming, there is an increase in species richness but a decrease in evenness and a change in the structure of the soil microbiota compared to those in soils that are managed with only chemical fertilizers.

Moreover, organic farming promoted the growth of fungi and actinomycetes, which are frequently associated with the breakdown of complex carbon compounds and the formation of soil structure, while there were no signs of fungi in conventional farming systems. These findings indicate that the extensive use of inorganic nutrients inhibits fungal populations and their role in soil functions. Such results have been obtained in studies by (104, 105). Monounsaturated fatty acids (MUFAs) were used as rapid-turnover bacteria, whose concentration was relatively high in CON soils, reflecting the high microbial activity as a result of fertilizer addition. The increases in the MUFA-dominant PLFA profile are supported by past research, where it was proven that the availability of easily assimilated inorganic nutrients stimulates the growth of metabolically active gram-negative bacteria (106, 107, 108). In ORG and NAT,

MUFA levels were relatively low because of the more stable metabolism of microorganisms from the consistent release of organic nutrients. This observation is consistent with previous reports on how organic practices contribute more to the stability of microbial community function and structure than to the stimulation of growth responses (109, 103). The microbial communities associated with natural agriculture exhibited an intermediate profile, with relatively high amounts of bacterial and eukaryotic PLFAs, indicating a transitional state between organic amelioration and conventional disturbance.

Principal component analysis (PCA) and correlation analysis between soil PLFA profiles and soil properties

PCA revealed strong multivariate evidence that farming practices strongly affect soil microbial community structure and associated biological processes. Eukaryotic and anaerobic microbes, which are organic, are positively correlated, supporting microbial communities that are complex in structure and diverse in function. Greater inputs of organic matter and less chemical disturbance in organic systems increase substrate availability and microsite heterogeneity, which in turn foster anaerobic niches and support eukaryotes such as fungi and protozoa, which play key roles in residue decomposition and nutrient cycling. Similar results were also shown by (110) for each trophic level, with greater organic biomass than in low-input and conventional systems, and this organic advantage increased with increasing trophic level, which indicates that organic management practices are beneficial to biota. Conventional practices aligned with gram-positive bacteria as indicators of a microbial community adapted to stress and a low-carbon environment. The present study relates to the findings of (111). Gram-positive bacteria are indicators of disturbed soils, as these organisms have thicker cell walls and greater resistance to chemical and physical stress (112). Organic systems clustered with PLFA, NMR and BRR showed enhanced microbial biomass and metabolic activity.

This association suggests that ORG not only increases microbial abundance but also stimulates nutrient turnover and biological efficiency through improved carbon availability. Natural farming systems were more closely linked to SOC and MBC, indicating a greater emphasis on carbon stabilization and microbial biomass accumulation rather than rapid mineralization. CON was weakly linked to PLFA, NMR, and BRR, indicating reduced microbial activity and suppressed biological function. These findings suggest that intensive tillage, inorganic inputs, and limited organic residue return have cumulative effects on soil microbial dynamics. This finding relates to the study of (24), in which zero-tillage with residue retention significantly outperformed conventional tillage in terms of soil enzyme activity. Compared with zero-till systems with residue retention, conventional tillage resulted in lower enzymatic activity during rice growth, reflecting the suppression of soil microbiological activity and nutrient cycling.

There was a positive relationship between microbial biomass carbon and SOC, suggesting that a higher availability of organic carbon favours an increase in microbial biomass. The results of the current study were similar to those reported by (113), who reported a strong positive relationship between MBC and SOC, which ranged from 700 to 2240 mg kg^{-1}, when the SOC concentration ranged from 25 to 90 g kg^{-1}. This relationship explains why the availability of SOC, particularly the release of water-soluble organic

carbon, is the major factor in the accumulation of microbial biomass. The significant relationships between SOC, NMR, and BRR indicate the importance of organic carbon in stimulating microbial respiration. High SOC levels increase substrate availability, microbial development, and enzyme function, thereby increasing CO_2 production. The role of microbial function, which depends on the presence of organic carbon, has been substantiated by recent research that established significant positive relationships between SOC, microbial biomass, and basal respiration within agroecosystems (114, 115). These findings indicate the importance of SOC as a regulator of nutrient cycles mediated by microorganisms.

Conclusions

This study provides conclusive evidence that farming systems are a dominant regulator of soil carbon dynamics, microbial function, and community structure in the rice–wheat systems of the Indo-Gangetic Plains. The two-year data presented here suggest that SOC, microbial biomass and nutrient cycling processes are consistently enhanced in organic farming, indicating that it is the most effective system for sustaining soil biological health. Natural farming resulted in moderate performance, indicating a functionally resilient, low-disturbance alternative; in contrast, conventional farming was characterized by lower microbial diversity and increased stress indicators. Strong coupling was detected among the SOC, MBC, BRR, NMR and PLFA-derived community structures, highlighting the central role of carbon availability in governing soil ecosystem functioning. Since microbial communities regulate nutrient cycling and maintain soil productivity under environmental stress, their abundance and diversity can be considered key indicators of resilient food systems. The findings suggest that farming systems promoting biological soil health may strengthen long-term food security while reducing dependence on external chemical inputs. Therefore, policies encouraging organic amendments, residue recycling, reduced tillage, and biologically driven nutrient management could play a critical role in improving the resilience and sustainability of cereal-based agroecosystems in the Indo-Gangetic Plains. These management strategies promoting organic inputs and reducing disturbance are critical for maintaining biologically active and resilient soils. Beyond the study region, the results have important implications for sustainable agricultural sustainability and climate change mitigation, as enhanced soil carbon sequestration and improved nutrient use efficiency is central to resilient agroecosystems. However, the study is constrained by its site-specific scope and limited temporal scale and does not incorporate additional functional indicators such as enzyme activities or greenhouse gas fluxes. Long-term, multilocation studies integrating ecological and socioeconomic dimensions are therefore needed to validate and extend these findings. In conclusion, the transition toward organic and natural farming systems is not only beneficial but also essential for restoring the functionality of soil, enhancing resilience, and ensuring the long-term sustainability of agroecosystems under changing environmental conditions.

Declarations

Data Availability Statement: All data generated or analysed during the study are included in this published article.

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Neha Kalonia: Conceptualisation, Data curation, Formal analysis, Methodology, writing original draft, data collection, compilation of manuscript.

Dr Dipti Grover: Supervised the work, manuscript writing, and value addition.

Dr Ajay Kumar Mishra: Conceptualization of work and final editing.

Shakuntla Duman: Manuscript writing and editing.

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Figures

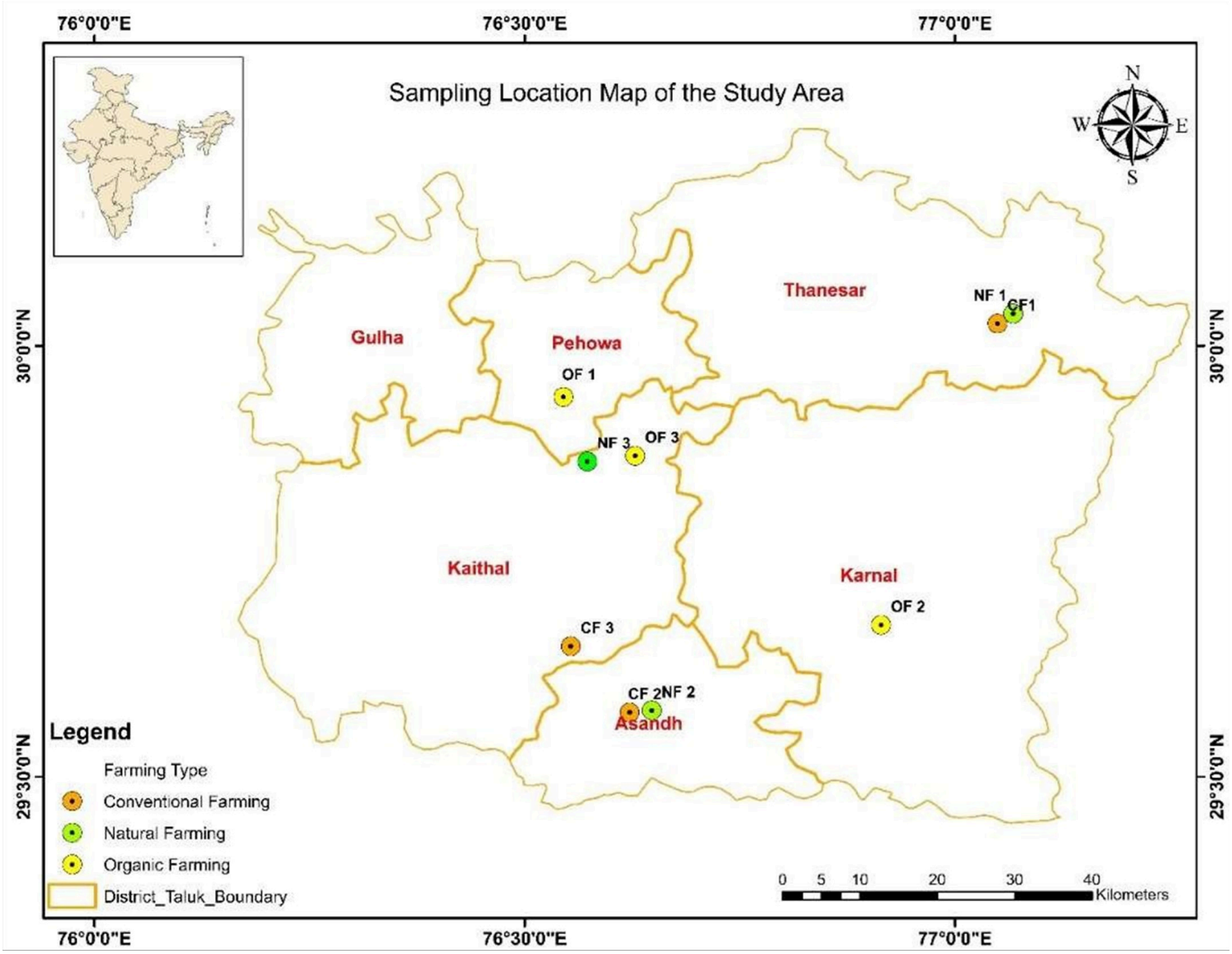


Figure 1

Map of the study sites

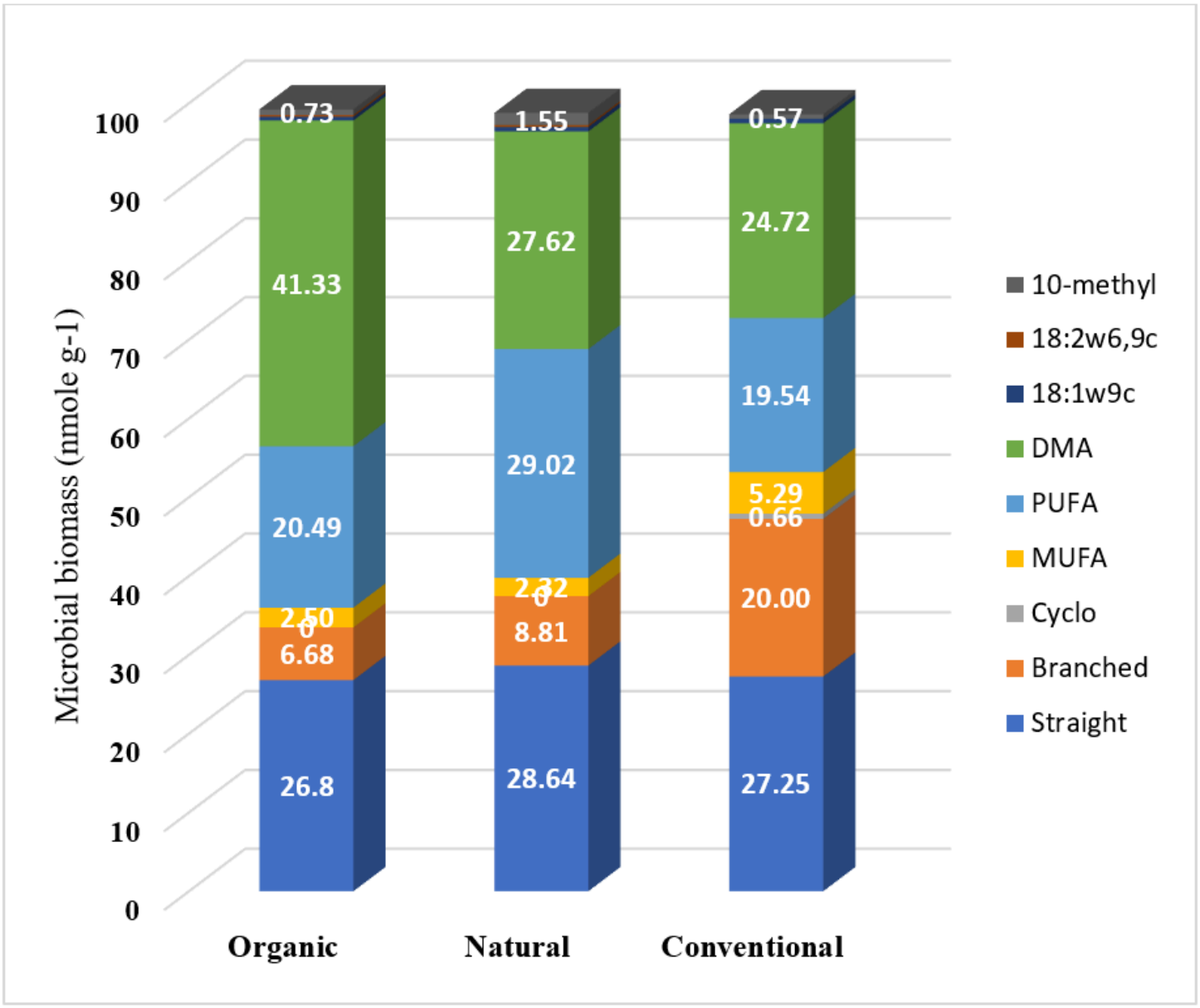


Figure 2

Microbial community structure (biomarkers) under different farming practices for ricecrops

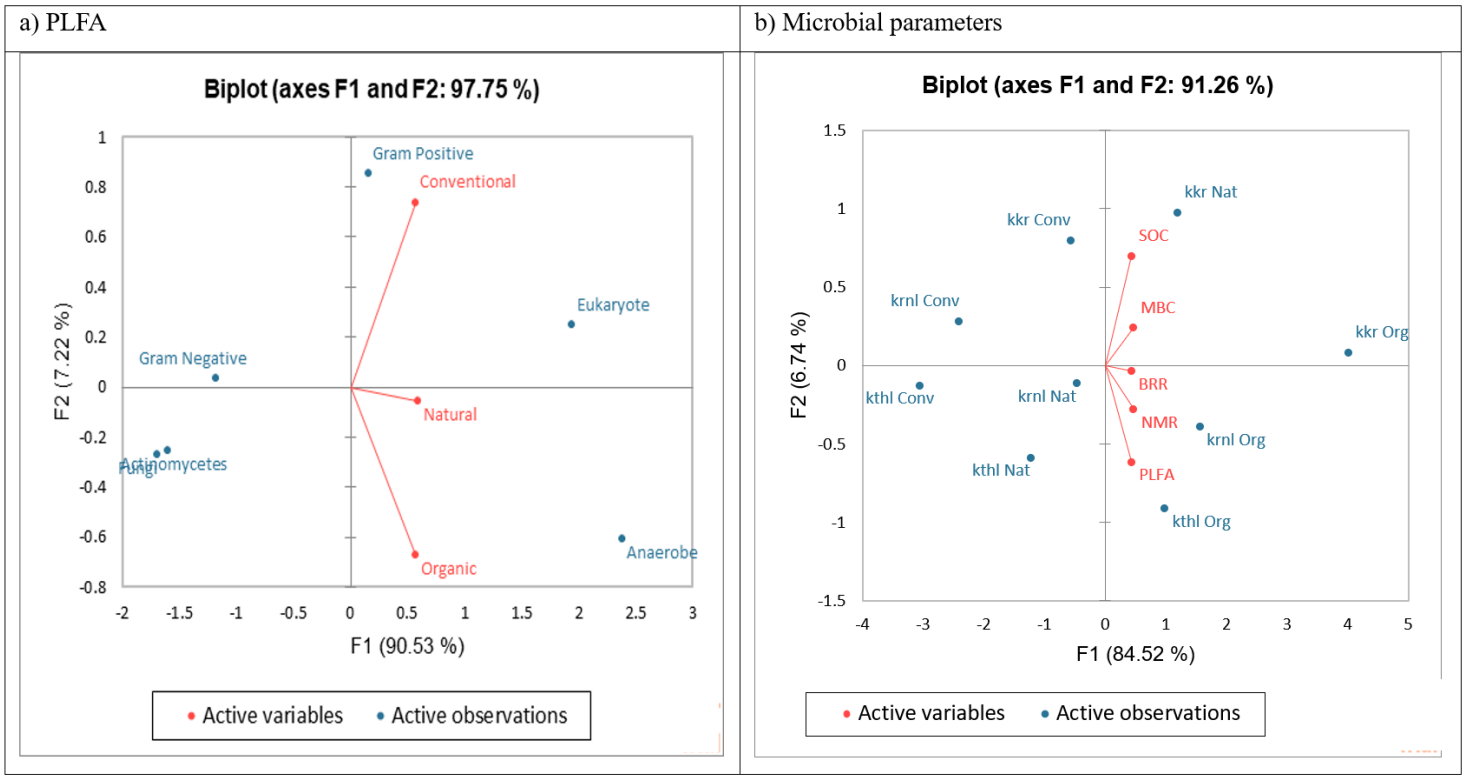


Figure 3

Principal componentanalysis (PCA) between soil PLFA profiles (a) and PLFAs underdifferent agricultural practices (b) for all the samples from different districts with different farming practices.