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'Save Soil' by managing soil nutrient losses, agronomic practices and crop-microbial interaction: World Soil Day 2022

Edited by
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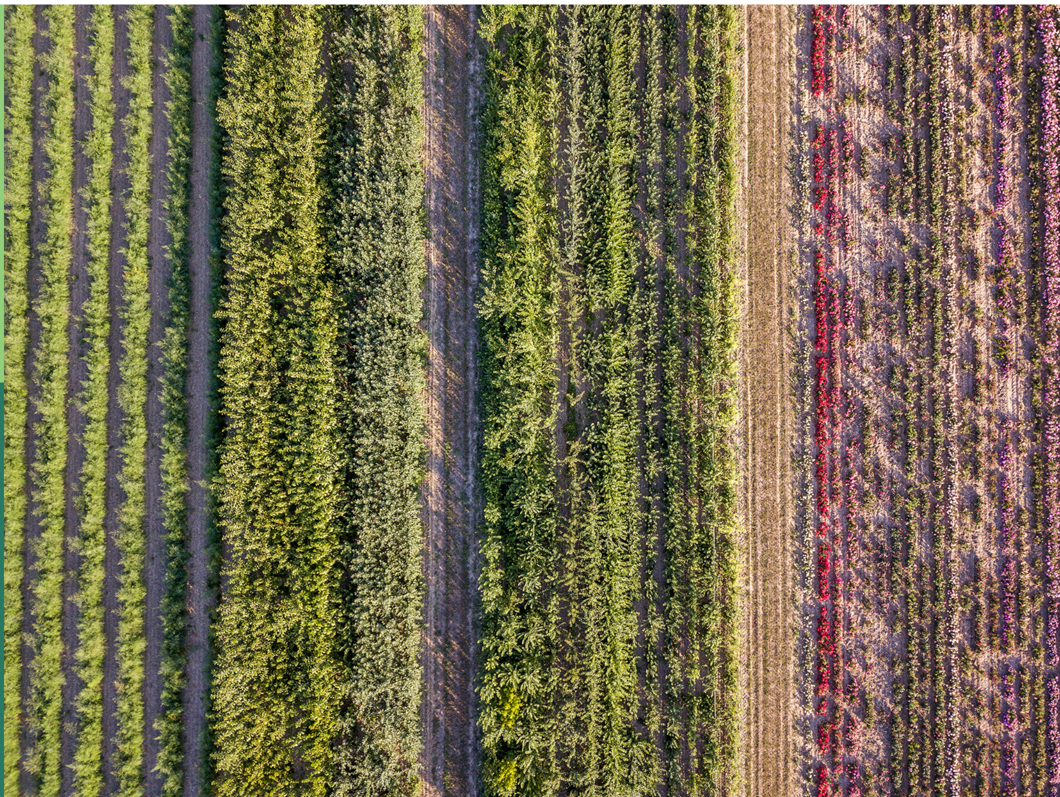

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‘Save Soil’ by managing soil nutrient losses, agronomic practices and crop-microbial interaction: World Soil Day 2022

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Editorial: 'Save Soil' by managing soil nutrient losses, agronomic practices and crop-microbial interaction: World Soil Day 2022

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Editorial on the Research Topic
'Save Soil' by managing soil nutrient losses, agronomic practices and crop-microbial interaction: World Soil Day 2022

Soil and its variable forms are the primary source of all terrestrial life and the soil is considered to be living for its dynamic nature (Pattnaik et al., 2023). Soil is a fundamental source for all living organisms and impacts non-living factors (water, air, minerals, etc.), making it an invaluable and finite resource (Kaur et al., 2023). The importance of soil expands from agronomy through to industrialization; thus, it is crucial to understand the impact of human activity on soil quality (Singhal et al., 2023). To address several global issues related to pollution, food security, and health, the United Nations promotes the Sustainable Development Goals (SDGs) with targets to "save soil" by minimizing nutrient loss and pollution load from the soil. Due to increasing anthropogenic pollution load, many soil pollution control measures are failing, therefore, new technologies and eco-friendly solutions are needed to balance and restore soil health (Majumdar et al., 2021, 2023). Soil-crop interactions are essential considering the crop yield and productivity under different soil statuses (Upadhyay and Majumdar, 2022; Sarkar et al., 2023). These processes, including nutrient release or soil detoxification, are mediated by soil-inhabiting microbes. The intrinsic role of soil parameters, including the different classes of soil, control soil microbiota which in turn modulate soil nutrient contents and make these bioavailable. Different crops, especially cereals, are constantly interacting with these soil microbes, thus the relationship between soil, crops, and microbes is complex (Majumdar et al., 2022).

This Research Topic has been enriched with some of the crucial aspects of soil quality management, crop plant intensification and microbial community involvement in soil-crop betterment. Diverse academicians around the world have shared their current research findings within this Research Topic. Primarily, three categories have been highlighted by the articles accepted under this Research Topic—soil amendments and quality management, crop productivity and nutrient dynamics, and soil microbial diversity with intricate involvements. In a study, it has been demonstrated that a combination of conventional fertilizers and nano-fertilizers can increase the bioavailability of N, Cu, and

Zn along with microbial biomass C and soil dehydrogenase enzyme activity (Upadhyay et al.). The application of nutrients from fertilizer mixes at the right time and right ratio is an essential contributing factor for better soil quality maintenance and the proposed method can increase soil NPK content and plant uptake (Kumar et al.). As a potential soil amendment modulator and nutrient management tool, an optical sensor-based application in-season estimate of yield (INSEY) and grain yield (GY) has been developed which will help to estimate the nitrogen and other soil nutritive parameters (Mitra et al.). Another integrated nutrient management (INM) practice has been reported for the measurement of active and passive soil organic content and nutrient enrichment which proved to be efficient in microbial metabolisms and soil enzymatic activities (Bamboriya et al.). Management of soil organic content has been tested with the tea plantation approach where proposed practice along with the conventional management strategy have been compared and a higher microbial community associated with *N*-metabolism and mycorrhizal fungi are noted (Huang et al.). In recent times, tillage practice has become a center of focus for many research groups to understand different aspects of agronomic situations that may arise when variable tillage practices are implemented. For sustainable soil management, conservation tillage practices supplemented with organic fertilizers are suggested for the long-term benefit of wheat-soybean productivity and quality enhancement (Meena et al.). For the rice-lentil cropping system, a minimal tillage practice with implemented INM has been proven to be efficient in field trials (Bhattacharya et al.) whereas zero tillage and short-duration varieties of lentils are found more growth-oriented and nutrient-conserving compared to the conventional tillage practice (Mukherjee et al.). During a 29-year long-term field trial of a rice-wheat cyclic cropping system, variable rates of NPK has been used along with or without Zn supplementation, at a fixed ratio. This improved the soil enzymatic activities along with greater microbial diversity and organic matter release making the crop productivity higher (Bhatt et al.). For some crops like soybeans, the conventional method of cultivation might be deteriorating which can be compensated for implementing system of crop intensification (SCI) approach. This boosts higher yield and soil nutrition balance where root nodules associated with soybeans are larger, resulting in a bulk plant growth and root density with greater NPK uptake (Singh et al.). Microbial communities are one of the primary modulators of soil nutrient dynamics and these microbial communities might get influenced with changing plant species. This theory has been proved by considering a soil microbial community analysis where apple orchard associated fields are inter-planted with leguminous forage red clover and natural mixed herbs, resulting in an increase of species richness, metabolic activities, soil fertility and land use efficiency (Jiao et al.). The conservation practices for soil quality with proper crop intensifications and associated microbial interventions are well-portrayed in these articles.

While there is no limit to research in soil quality management in urban, rural and agro-climatic areas, the focus should be more engaged toward sustainable development goals (SDGs) promoted by the United Nations (Lal et al., 2021). This Research Topic certainly elucidated some promising research themes that trigger some more future aspects of environmental sustainability and "Save Soil" motto including eco-friendly amendments and seed-priming techniques, water-saving irrigation approaches, molecular assessments of microbial involvement in plant growth promotion, and the fate of natural soil contaminants. It is believed these future research sections will be explored in due time.

Author contributions

AM: Conceptualization, Project administration, Writing – original draft, Writing – review & editing. DM: Project administration, Validation, Writing – review & editing. SS: Project administration, Validation, Writing – review & editing.

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Growth, nodulation, yield, nitrogen uptake, and economics of lentil as influenced by sowing time, tillage, and management practices

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Crop management practices and variety are two very important parameters that decides the crop performance. A field experiment was carried out during the two consecutive *rabi* seasons of 2018–19 and 2019–20 to determine the impact of sowing timing, tillage operation, and variety on the growth, development, yield characteristics, and nitrogen uptake in lentil crops. The experiment was conducted in a split-split plot design with 3 replications comprising two different sowing conditions (S_1: early sowing after harvesting of short duration *kharif* rice, S_2: delayed sowing after harvesting of long duration *kharif* rice) in main plots, three different tillage operations (T_1: Relay cropping, T_2: Zero tillage, T_3: Conventional tillage) in subplots and two different varieties (V_1: short duration: L4717, V_2: long period: *Moitri*) in subplots. The findings demonstrated a substantial interaction between sowing time, tillage, and variety on various growth and yield parameters of lentil crops. The early sowing of lentil crops (early November) yielded 4.8% more (1,105 kg ha^{-1}) than late November sowing and adapting to the short-duration variety L4717 over the long-duration cultivar *Moitri* resulted in a yield increase of 5.9% (1,086 kg ha^{-1}). Apart from providing a higher yield, it also provided an opportunity to take another crop like leafy vegetables. Among the three tillage practices adopted, conventional tillage produced the lowest yield (1,017 kg ha^{-1}) in both experimental years. In contrast, a yield increase of 6.9% and 26.9% in relay cropping and zero tillage systems was observed, respectively. Early-sown lentils with no-tillage and a short-duration variety reached a certain phenophase faster than other combinations (life cycle: 96.2 and 98.7 days for lentils in both years). For both the sowing times, the growth parameters and the number of nodules $plant^{-1}$ were highly correlated with nitrogen uptake at different stages of the life cycle. High net returns (Rs. 51,220 and 59,257) leading to higher benefit-cost ratios were observed under the treatment combination of early sowing + zero tillage + short duration variety. Therefore, the study found that short-duration lentil cultivars in combination with early sowing in the zero-tillage system are the best agronomic approach for the sustainability of lentil production after the monsoon rice harvest.

KEYWORDS

lentil, nitrogen uptake, nodule, sowing time, tillage, variety, LAI

1. Introduction

Several grain legumes have been found to be crucial for meeting food requirements and for food security as they are primary plant protein sources. Besides being a rich source of proteins and essential amino acids, pulses can serve as a 'mini nitrogen factory', reducing the requirement for chemical fertilizers and maintaining environmental balance. Legumes have become recognized for their extensive contribution to crop diversity and sustainable agriculture. In light of urgent challenges such as climate change, population explosion, and agricultural land degradation, legumes have a bigger and more important role to play. Chickpeas, lentils, and faba beans are three food legumes whose production has decreased over the past decade. Though there are many high-yield varieties and advanced production technologies that should result in higher and more stable yields, production trends regarding food legumes have mostly been static or have declined in developing countries. In developing countries like India, food legumes are as equally important as food grains and oilseeds (Choudhary, 2009).

In India, chickpea, pigeon pea, mungbean, urdbean, lentil, and field pea are the important legume crops grown (Saxena et al., 2000; Ali and Gupta, 2012), which form an integral part of the different cropping systems for sustainable agriculture. Lentil (*Lens culinaris*) is amongst the oldest domesticated crops cultivated in the world. The world's total lentil cultivated area is estimated at ∼6.10 million ha, with annual production and yield of 6.33 million tons (MT) and 1,038 kg ha^{-1}, respectively (FAOSTAT, 2019). Lentil is India's second most important *rabi* pulse crop (Singh et al., 2014) with a rich source of protein and essential amino acids (Tripathi, 2016). Lentil cultivation in India is characterized by low yields with high variability and is grown mainly by marginal farmers. There are studies that have indicated that replacing age-old lentil varieties and adjusting sowing time in the crop sequence may help improve yield and expand the area in potential regions (Sen et al., 2016).

Adjusting sowing time may be an important factor in lentil production as it is an essential factor that plays a pivotal role in crop growth, phenological development, nodulation, pod development, and productivity (Pradhan et al., 2018). The plant environment such as temperature, photo-period, and moisture availability (in dryland/rainfed conditions), significantly differs from the sowing date. Therefore, shifting the *kharif* crop sowing dates to accommodate the next cool-season crop (*rabi*) in the cropping system can ensure more system productivity (Maji et al., 2019). In the lower Gangetic plains of India, late transplanting of monsoon rice with long-duration varieties along with erratic rainfall in October or November delays the sowing of post-rice CSFLs, making them susceptible to terminal heat/drought stresses and eventually lowering productivity. In the future, the change in rainfall is going to be drastic. According to Yadav et al. (2017) and Chandran et al. (2022) the mean percent change in projected rainfall compared to baseline during the lentil growing period was −7%, −8.5%, +1.7%, and −6.8%, respectively under mid_rcp4.5, mid_rcp8.5, end_rcp4.5 and end_rcp8.5 scenarios.

Along with the proper time of sowing, a high-yielding short-duration variety is equally important. Days to maturity of a crop have a significant role in finding a fit, especially after the rice harvest. During the last three decades, lentil breeders have made tireless efforts to increase the productivity of lentil crops across different global locations by developing short-duration varieties with high and stable yield advantages for diverse agro-climatic conditions. An early maturing variety may escape from drought or flooding and allow the cultivation of multiple crops in a year. At Indo Gangetic plains of India, lentil varieties like Ranjan, Subrata, and *Moitri* (duration: 125–130 days) are popular across agro-climatic zones. But, many location-specific and widely adaptable high-yielding short-duration varieties of pulses have already been developed (Erenstein and Laxmi, 2008), which also need to be popularized across different lentil growing locations to provide tolerance against biotic and abiotic stresses.

Tillage is a well-known agricultural practice that has a larger impact on creating better soil conditions for ensuring satisfactory crop growth and development (Singh et al., 2022). Conservation or zero tillage, which leads to less disturbance of soil and permits residue retention, has gained momentum across the globe (Nuttonson, 1955). No-till or zero tillage is a critical component of conservation agriculture to produce crops cheaply with a profound effect on natural resources. In many parts of the world, conservation agriculture has become increasingly popular because soil degradation and the sustainability of agriculture have become increasingly apparent. Proper tillage management can be a better solution for the soil degradation processes documented in parts of Indo-Gangetic plains.

Keeping a view of the literature above, an experiment was designed to study the effects of different sowing times, tillage, and varieties on growth, nodulation pattern, yield parameters, phenophase attainment, total N uptake and economics in lentils grown after monsoon rice. We believe this study will also be important for a future in which we expect a change in the climate. The changes in rainfall and maximum and minimum temperatures can be effectively managed with this agronomic management.

2. Materials and methods

2.1. Details of experimental site

The present experiment was carried out during two successive *rabi* seasons (October to March) of 2018–19 and 2019–20 at the District Seed Farm (22°56′ N latitude, 88°32′ E longitude, and an altitude of 9.75 m above mean sea level), AB Block, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India. The soil was well-drained Gangetic alluvial (order: Inceptisol), which belongs to the class of sandy loam with medium fertility, almost neutral in reaction (pH 7.0), low in organic carbon (0.52 and 0.57%), available nitrogen (132.59 and 138.19 kg ha^{-1}), phosphorus (24.86 and 29.66 kg ha^{-1}), and potassium (157.82 and 159.16 kg ha^{-1}) for both the years.

2.2. Weather conditions

The day-to-day meteorological data during the period of the experiment was collected from AICRP on Agro Meteorology, Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal. The maximum and minimum temperatures during the cropping period ranged between 24.5 and 32.4°C and 10.1–22.5°C in 2018–19 and 23.4–32°C and 11.2–23.3°C in 2019–20. The daily temperature during the experiment period of both years started to decline in November and reached a minimum in January. The relative humidity in these cropping seasons was perceived between 96.0–42.5% and 97.5–40.1% during 2018–19 and 2019–20, respectively. The crop was grown exclusively under rainfed conditions. The total amount of rainfall received during the experiment period was 133.7 mm in 2018–19 and 189.6 mm in 2019–20.

2.3. Experimental design and treatment details

The investigation was laid out in a split-split plot design with three replications comprising 12 treatment combinations (Supplementary Figure S1). In the study, the main plots included two sowing conditions (S_1: Early sowing, i.e., 1st week of November after harvesting of short duration *kharif* rice, i.e., Sahabhagi and S_2: Delayed sowing, i.e., last week of November) after the harvesting of long period *Kharif* rice, i.e., Swarna, 3 tillage practices in the subplots (T_1: Relay cropping, T_2: Zero tillage, T_3: Conventional tillage), and two varieties of lentil (V_1: short duration L4717, V_2: long duration Moitri) that were laid on sub-sub plots. The L4717 variety used in the experiment is an early maturing (105 days), powdery mildew and Fusarium wilt disease-resistant lentil variety released by the Indian Agriculture Research Institute, New Delhi, India, with a yield potential of 12–13 q ha^{-1}. On the other hand, *Moitri* is a long-duration (120 days), rust disease-resistant popular lentil variety released by the Pulses and Oilseed Research Station, Berhampore, West Bengal, India, with a yield potential of 10–12 q ha^{-1}. There was a difference of at least 20 days between the early and late sowing period. There was a visible difference noted in the moisture content of the soil between the early November sowing (initial moisture content was 25.24% at 0–15 cm and 27.07% at 15–30 cm) and the last week of November sowing (initial moisture content was 24.12% at 0–15 cm and 25.58% at 15–30 cm) of the lentil crops.

2.4. Crop management

Lentil (60 kg ha^{-1}) seeds were broadcasted on the standing rice crop for both dates of sowing in relay cropping. On both dates of sowing, residues of previously grown rice crops were left in the field. A zero till drill was used to implement zero tillage in the plots. One deep plowing with a disc plow followed by 2 harrowing and 1 planking was provided for conventional tillage treatment. After the final land preparation, the seeds were sown at a distance of 25 cm row to row by opening furrows and placing seeds in a depth of 3–4 cm in both zero-tilled and conventionally-tilled plots in both the sowing dates.

A recommended fertilizer dose of 20:40:40 kg N-P_2O_5-K_2O ha^{-1} for lentils was applied as basal dose. The whole quantity of nitrogen, phosphorous, and potassium was applied at the time of sowing of the crop. No external irrigation was provided to the crops and the crop survived by utilizing the residual soil moisture. Two-hand weeding at 25 DAS (days after sowing) and 50 DAS for the lentil crops was done to minimize crop weed competition. The crop was harvested manually when the leaves became yellow, and ∼85–90% of green pods turned to golden straw color. After threshing, cleaning, and drying, the grains of each plot were weighed separately, and the yield was determined in terms of kg ha^{-1}.

2.5. Methodology used

For growth analysis, five randomly selected lentil plant samples from the experimental plots were collected at respective stages of observation, and plant height (45, 75 DAS and at harvest), dry aerial biomass accumulation (45, 75 DAS, and at harvest), the number of nodules $plant^{-1}$ (45, 60, and 75 DAS), and leaf area index, i.e., LAI (45, 60, and 75 DAS), were determined by following the standard procedure. The yield components and grain yield of the lentil crops were determined at maturity. The different phenophases (viz., days to flower initiation, flowering to pod initiation and pod initiation to maturity, sowing to maturity) of lentil crops planted at other dates were noted using the regular field inspection method. The degree days were obtained as the difference between the mean daily temperature and the base temperature of the crop (AOAC, 1995).

$$GDD = \sum_{i=1}^{n}[(\frac{T_{max}+T_{min}}{2})-T_b]$$

where Tmax. and Tmin. were the maximum and minimum air temperatures of a day, Tb was the base temperature (50 C for pulses) and n was the number of days to attain a phenophase. The degree days for different phenophases of lentil varieties were calculated, which were summed up from the sowing to the maturity of the crop.

The micro-Kjeldahl method was used to determine the total nitrogen (N) in the lentil plant samples as per the procedure suggested by Gomez and Gomez (1984). The uptake of N by the plant on a dry weight basis (seed and stover) was calculated by the following formula:

$$\text{Uptake in Kg ha}^{-1} = \frac{\text{Nutrient \% (seed or stover) XYield (seed orstover)}}{100}$$

For the economics of lentil cultivation, the cost of each treatment was calculated based on the then-prevailing market price of the inputs. The cost of cultivation was worked out with the following formula:

$$\text{Cost of Cultivation (Rs ha}^{-1}) = \text{Variable Cost (Rs. ha}^{-1}) + \text{Fixed Cost (Rs. ha}^{-1})$$

The gross return was obtained by converting the harvest into monetary terms at the prevailing market rate during the study period. The net return was calculated by deducting the cost of production from the gross return and expressed as:

$$\text{Net Return (Rs. ha}^{-1}) = \text{Cost of Cultivation (Rs. ha}^{-1}) - \text{Gross Return(Rs. ha}^{-1})$$

The benefit-cost ratio was calculated with the help of the following formula:

$$\text{B : C ratio} = \frac{\text{Gross return (Rs ha}^{-1})}{\text{Cost of Cultivation (Rs ha}^{-1})}$$

The data obtained on the various parameters under study were analyzed statistically using the variance analysis method (ANOVA) for a split–split plot design. The significance of the treatments was tested at a 5% least significant difference and was computed by the "F" test (Wickham, 2016).

2.6. Correlation

The Pearson correlation coefficients with significance levels presented in a scatterplot and correlogram were done with the help of ggplot2 (Schloerke et al., 2021) and GGally (Edalat and Naderi, 2016) package in R v4.1.2.

3. Results and discussion

As lentil is an indeterminate crop, growth parameters such as plant height and dry aerial biomass accumulation tend to increase with the advancement of the crop growth and reach a peak at harvest irrespective of different treatments.

3.1. Impact on growth and nodulation

The significantly highest plant height in the lentil crops was found in the early sown treatment (first week of November) as compared to the delayed sowing (last week of November) in all the different growth stages, as shown in Supplemenatry Table S1 and Figure 1.

These results are in line with the findings of Sen et al. (2016) and Venugopalan et al. (2022) where he has reported a reduction in plant height when the lentil was sown late (last week of November). This might be due to its congenial weather condition during the first sowing date of the lentil crops which provided a favorable environment for the growth and development of the crop. Among the various tillage conditions, maximum plant height was noted in the zero tillage plots in both years (Figure 1). Zero tillage perhaps provided an advantage to crop over weeds, resulting in better resource utilization and greater suppression ability of weeds than the other treatments that indirectly led to better growth of the crop. Among the various varieties studied, lentil variety L4717 resulted in the significantly tallest plant at various growth stages. The genetic makeup of the short-duration varieties may have resulted in taller plants compared to the longer-duration varieties. An earlier experiment showed that the simultaneous improvement of yield and earliness was possible because of high heritability for important traits, including crop growth rate, efficient partitioning of photosynthetic assimilates, and reproductive duration (Jogloy et al., 2010; Edalat and Naderi, 2016). However, the interaction effect of sowing condition, tillage, and variety did not significantly affect the plant height of the lentil crop.

A significant influence of sowing time was noted on the dry aerial biomass accumulation of lentils at 75 DAS during 2018–19 in the experiment. The early sowing of lentils at various stages resulted in superior dry aerial biomass accumulation (Supplementary Table S2 and Figure 2), which may be due to proper weather conditions.

The experiment results found similarity with the results of Tyagi (2014) and Visha Kumari et al. (2021). Maximum aerial biomass was noted in the treatment plots with zero tillage during both years. Improved soil moisture and nutrient availability at the crop root zone, sufficient aeration facility, and greater transpiration surface area could all contribute to better results in lentil plots with zero tillage. During both years of experimentation, the short-duration variety L4717 produced maximum dry aerial biomass accumulation (Supplementary Table S2 and Figure 2) compared to the long-duration variety *Moitri*. In the case of plant height and dry aerial biomass accumulation of the lentil crops, the 2nd year of the study recorded superior values due to receiving of excess amount of rainfall (almost 56 mm) and there were also improved soil physical properties in response to winter pulse cultivation in the previous year which contributed to better vegetative growth of the lentil crops.

Early sowing resulted in a significantly higher number of nodules plant^{-1} throughout the growth stages (13.0 and 14.3 at 45 DAS, 23.2 and 27.2 at 75 DAS, and 11.7 and 14.5 at 75 DAS) compared to delayed sowing of lentil (12.4 and 13.6 at 45 DAS, 22.5 and 26.5 at 75 DAS, and 11.2 and 13.8 at 75 DAS) for both the years of experimentation (Table 3 and Figures 3–5). The results of the experiment are

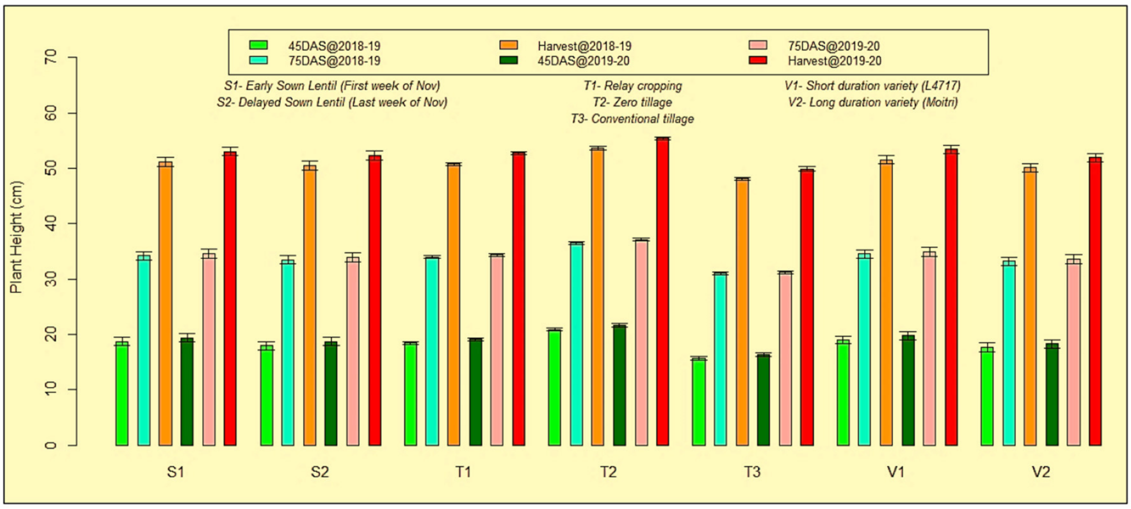

FIGURE 1
The effect of sowing time, tillage, and variety on the plant height of the lentil crops sown after the harvesting of monsoon rice. S_1, Early sowing (1st week of November) after the harvesting of short-duration *Kharif* rice; S_2, Delayed sowing (last week of November) after the harvesting of long-duration *Kharif* rice. T_1, Relay cropping; T_2, Zero tillage; T_3, Conventional tillage; V_1, Lentil short duration: L4717; V_2, Lentil long duration: Moitri. Significant at $p \leq 0.05$. NS- Non-significant at $p \geq 0.05$.

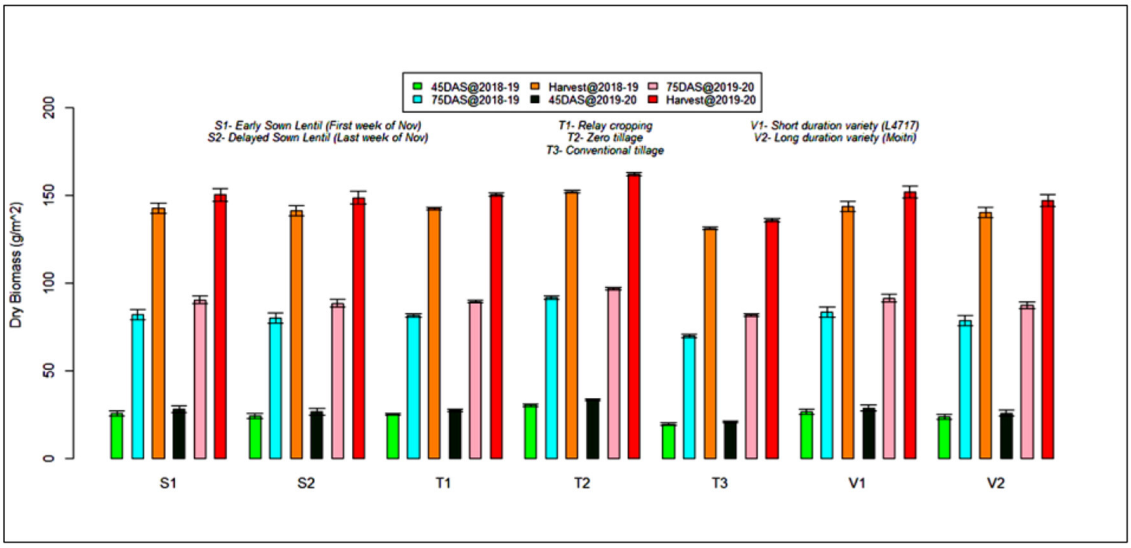

FIGURE 2
Effect of sowing time, tillage, and variety on dry aerial biomass accumulation of lentil crops sown after monsoon rice harvesting. S_1, Early sowing (1st week of November) after harvesting of short-duration *Kharif* rice; S_2, Delayed sowing (last week of November) after harvesting of long-duration *Kharif* rice. T_1, Relay cropping; T_2, Zero tillage; T_3, Conventional tillage; V_1, Lentil short duration: L4717; V_2, Lentil long duration: Moitri. Significant at $P \leq 0.05$. NS- Non-significant at $P > 0.05$.

close to the results of the experiment (Sah et al., 2019) in chickpeas.

A significant influence of earlier sowing date on the nodules plant^{-1} of lentils was also observed by Visha Kumari et al. (2019). Among the different crop establishment methods, the maximum number of nodules plant^{-1} was recorded in the zero tillage plots followed by the relay cropping plots during both the years of the experiment (Table 3 and Figures 3–5). This may be attributed to better seedbed preparation, which might have facilitated better root growth in the no-till plots. These findings could be supported by the previous observations of Quddus et al. (2020) in various growth parameters of winter chickpeas with various tillage operations. Among the different lentil varieties studied, the maximum number of nodules plant^{-1} was found in the short-duration variety L4717 at all the growth stages (Supplemenatry Table S3 and Figures 3–5) in both years. The difference in growth attributes may be due to the varietal characteristics and their crop duration.

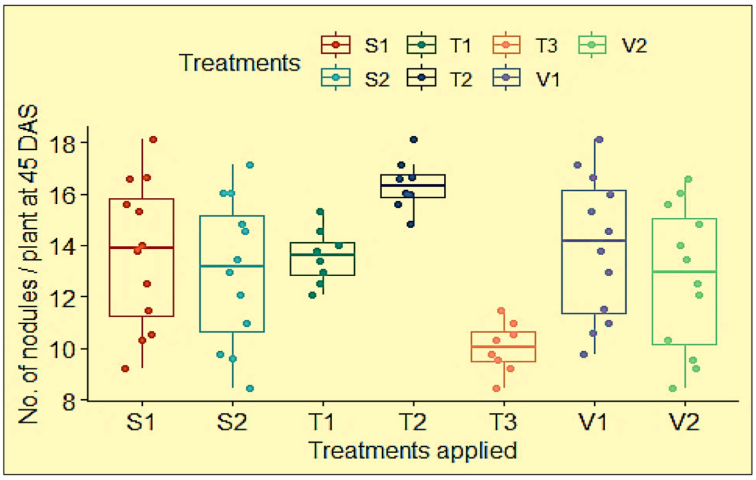

FIGURE 3
Effect of sowing time, tillage, and variety on a number of nodules plant^{-1} at 45 DAS of lentil crops sown after monsoon rice harvesting (S_1, Early Nov sown Lentil; S_2, Late Nov sown Lentil; T_1, relay cropping; T_2, zero tillage; T_3, conventional tillage; V_1, L4717; V_2, Moitri).

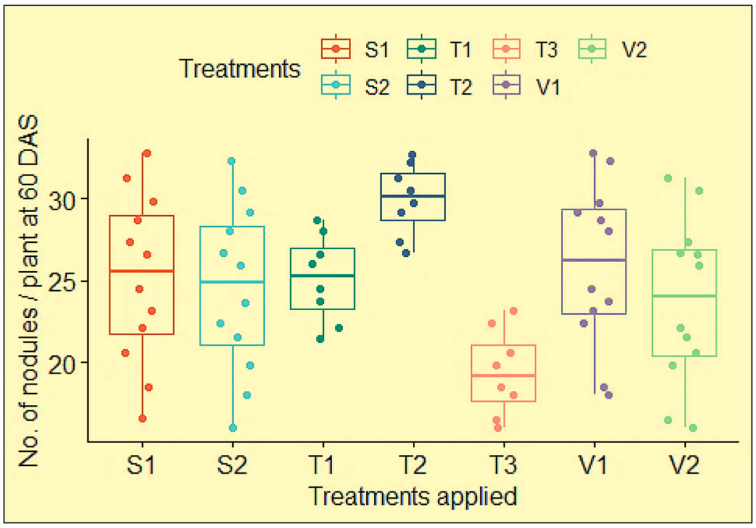

FIGURE 4
Effect of sowing time, tillage, and variety on the number of nodules plant^{-1} at 60 DAS of lentil crops sown after monsoon rice harvesting (S_1, Early Nov sown Lentil; S_2, Late Nov sown Lentil; T_1, relay cropping; T_2, zero tillage; T_3, conventional tillage; V_1, L4717; V_2, Moitri).

The trend remained the same for LAI too. Sowing in the first week of November resulted in a significantly higher LAI throughout the growth stages compared to sowing in the last week of November for both the years of investigation (Supplementary Table S4 and Figure 6). For the various tillage treatments, LAI could be arranged as zero tillage > relay cropping > conventional tillage (Supplementary Table S4 and Figure 6). There are few studies which show that no-tillage and minimum tillage performed better in terms of LAI in lentil crops in new alluvial soil (Bandyopadhyay et al., 2018). These results can be explained by the fact that the soil's physical properties such as organic carbon content, aeration facility, and fertility status might be more favorable in zero tillage than in other tillage-based systems. During both years of the experiment, lentil variety L4717 attained the highest LAI at all observed stages as compared to the long-duration lentil variety Moitri due to faster genetic expressions under favorable conditions.

3.2. Impact on grain yield

Among the different yield traits observed, the early sown lentil crops showed the highest number of plants m^{-2} (28.7 and 27.6), while the late sown lentil crops showed fewer plants (25.7 and 25.3). At the lower Gangetic plains of India, the first fortnight of November is considered optimum (Sen et al., 2016; Visha Kumari et al., 2019) and our results are in line with the earlier studies. As

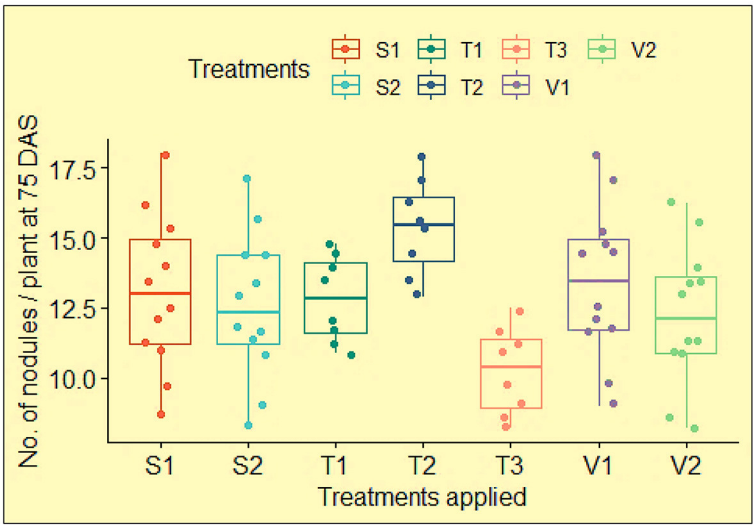

FIGURE 5
Effect of sowing time, tillage, and variety on the number of nodules plant^{-1} at 75 DAS of lentil crops sown after monsoon rice harvesting (S_1, Early Nov sown Lentil; S_2, Late Nov sown Lentil; T_1, relay cropping; T_2, zero tillage; T_3, conventional tillage; V_1, L4717; V_2, Moitri).

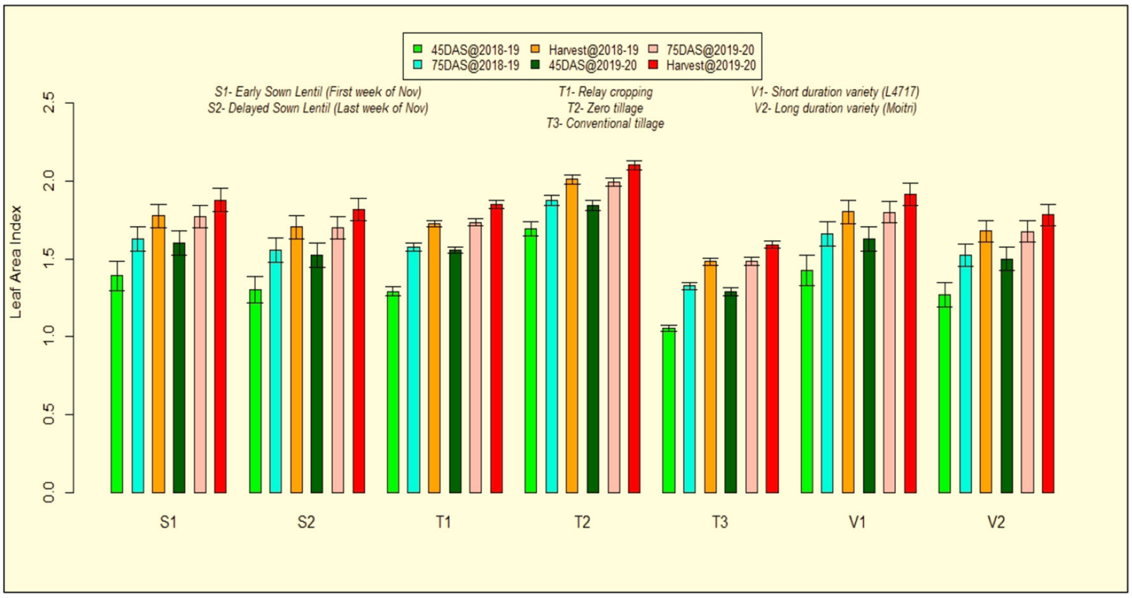

FIGURE 6
Effect of sowing time, tillage, and variety on leaf area index (LAI) of lentil crops sown after monsoon rice harvesting.

per the results, tillage treatments can be significantly arranged as zero tillage (35.3 and 35.8) > relay cropping (25.8 and 24.9) > conventional tillage (20.4 and 18.6) regarding this parameter during both consecutive years of study (Table 1).

The short-duration variety L4717 produced the significantly highest number of plant stand m^{-2} in both years, followed by the long-duration variety *Moitri* (Table 1). The interaction effect of sowing condition, tillage, and variety, however, did not significantly affect the plant population of the lentil crops. Among the different treatments, a significant difference in the number of pods plant^{-1} in the lentil crops was observed during the study (Table 1). Early sowing of lentils resulted in more pods (89.2 and 105.4) due to favorable temperatures during the crop growth period and higher growth attributes, which may be responsible for a better source-sink relationship. Zero tillage-treated plots recorded a higher number of pods plant^{-1} (105.7 and 107.3), followed by relay cropping (87.4 and 97.2) and conventional tillage (70.9 and 82.4) in both years. Due to the compound effects of reduced soil erosion, suppressed nutrient removal by weeds, better soil physical health, improved water regimes, and restoration of soil moisture, there were more

TABLE 1 Effect of sowing time, tillage, and variety on yield parameters of lentil crops sown after monsoon rice harvesting.

ANOVA		Plants m^{-2}		Pods $plant^{-1}$		Test weight (g)	
		2018–19	2019–20	2018–19	2019–20	2018–19	2019–20
Sowing condition (S)	S_1	28.7 ± 1.2a	27.6 ± 2.1a	89.2 ± 12a	105.4 ± 12a	18.75 ± 0.2a	18.93 ± 0.2a
	S_2	25.7 ± 1.4b	25.3 ± 1.3b	86.8 ± 15b	85.9 ± 12b	18.57 ± 0.5a	18.70 ± 0.4b
Tillage (T)	T_1	25.8 ± 1.4b	24.9 ± 2.2b	87.4 ± 18b	97.2 ± 15b	18.70 ± 0.8b	18.64 ± 0.2b
	T_2	35.3 ± 2.1a	35.8 ± 2.0a	105.7 ± 17a	107.3 ± 17a	19.30 ± 0.1a	19.95 ± 0.5a
	T_3	20.4 ± 1.2c	18.6 ± 1.4c	70.9 ± 21c	82.4 ± 0.8c	18.00 ± 0.4c	17.85 ± 0.4c
Variety (V)	V_1	29.7 ± 1.1a	28.6 ± 1.2a	93.1 ± 24a	98.4 ± 14a	18.90 ± 0.1a	19.05 ± 0.5a
	V_2	24.7 ± 1.0b	24.3 ± 1.5b	82.9 ± 25b	92.8 ± 15b	18.45 ± 0.5b	18.60 ± 0.8b

S_1, Early sowing (1st week of November) after the harvesting of short duration Kharif rice; S_2, Delayed sowing (last week of November) after the harvesting of long duration Kharif rice, T_1, Relay cropping; T_2, Zero tillage; T_3, Conventional tillage; V_1, Lentil short duration: L4717; V_2, Lentil long duration: Moitri. Significant at $P \leq 0.05$. NS, Non-significant at $p \geq 0.05$. Values are means ± SEM ($n = 3$). Different letters indicate significant differences between means.

TABLE 2 Effect of sowing time, tillage, and variety on seed yield and total *N* uptake of lentil crops sown after monsoon rice harvesting.

ANOVA		Seed yield (kg ha^{-1})		Total *N* uptake (kg ha^{-1})	
		2018–19	2019–20	2018–19	2019–20
Sowing condition (S)	S_1	1,131 ± 85a	1,185 ± 115a	80.28 ± 87a	87.24 ± 42a
	S_2	1,108 ± 105b	1,102 ± 148b	71.25 ± 25b	76.07 ± 18b
Tillage (T)	T_1	1,081 ± 124b	1,092 ± 135b	73.38 ± 15b	77.80 ± 45b
	T_2	1,271 ± 154a	1,310 ± 128a	103.16 ± 18a	115.09 ± 25a
	T_3	1,006 ± 118c	1,028 ± 147c	50.75 ± 26c	52.08 ± 12c
Variety (V)	V_1	1,154 ± 154a	1,177 ± 102a	79.35 ± 12a	85.95 ± 14a
	V_2	1,084 ± 112	1,109 ± 98b	72.18 ± 10b	77.36 ± 16b

S_1, Early sowing (1st week of November) after the harvesting of short duration Kharif rice; S_2, Delayed sowing (last week of November) after the harvesting of long duration Kharif rice; T_1, Relay cropping; T_2, Zero tillage; T_3, Conventional tillage; V_1, Lentil short duration: L4717; V_2, Lentil long duration: Moitri. Significant at $P \leq 0.05$. NS, Non-significant at $p \geq 0.05$. Values are means ± SEM ($n = 3$). Different letters indicate significant differences between means.

yield attributes and yield in the zero tillage plots. There were varietal differences in the number of pods $plant^{-1,}$ too, owing to the genetic potential (Table 1).

A similar observation regarding the test weight of lentils was found regarding sowing time, tillage, and variety in lentils where the early November sowed crop resulted in higher test weight (18.75 and 18.93 g) (Table 1). Tillage treatments could be arranged as zero tillage (19.30 and 19.95 g) > relay cropping (18.70 and 18.64 g) > conventional tillage (18.00 and 17.85 g) regarding this parameter during 2018–19 and 2019–20. The higher yield attributes in zero tillage plots of lentil crops are also similar to the results of earlier work (Bandyopadhyay et al., 2018; Chauhan et al., 2019; Quddus et al., 2020). The L4717 variety recorded a higher test weight (18.90 and 19.05 g) in both the years of study than the long-duration variety *Moitri* (18.45 and 18.60 g).

Lentil yield also varied significantly with the time of sowing, tillage, and variety. For 2018–19 and 2019–20, early sowing recorded a 2.08 and 7.53% increase over the delayed sowing of lentils in the investigation, respectively (Table 2). The results of delayed sowing were in accordance with Sita et al. (2017) where the high sensitivity of lentil crops to high temperature resulted in a negative impact on the reproductive stage followed by a yield reduction. Zero tillage resulted in a higher yield among the tillage treatments followed by relay cropping (Table 2).

Zero tillage had the advantage of better depth and moisture availability. The L4717 variety produced 6.46 and 5.34% higher seed yields than Moitri during the study period of the year 2018–19 and 2019–20, respectively. It is reported that lentil genotypes with a short duration can produce higher seed yield if they efficiently partition the photosynthetic assimilates into economic profit (Kumar and Srivastava, 2015; Mukherjee et al., 2020). This may be the reason for the higher yield in L4717. Further, the yield attributing parameters and seed yield of lentil was found to achieve far superior values in 2019–20 as compared to 2018–19 probably due to improved organic carbon content (0.05%) as well as receiving a greater amount of rainfall that increased the soil moisture reserve in the 2nd year of study which helped in better utilization of water resource attributing to better reproduction and yield advantages.

3.3. Impact on total *N* Uptake

Total N uptake also varied significantly following a similar pattern of higher total N uptake (80.28 and 87.24 kg ha^{-1}) in early sown lentils during both years. Similar findings were reported previously by experimental results (Kumar et al., 2016; Neenu et al., 2017). Higher nutrient content and uptake with the early planted

TABLE 3 Effect of sowing time, tillage, and variety on the phenology of lentil crops sown after monsoon rice harvesting.

ANOVA		Days to flower initiation		Flowering to pod initiation		Pod initiation to maturity		Sowing to maturity	
		2018–19	2019–20	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20
Sowingcondition (S)	S1	43.2 ± 0.2b	43.9 ± 0.5	20.3 ± 0.1b	20.9 ± 0.5b	39.0 ± 0.2b	39.2 ± 0.3b	110.4 ± 0.1b	112.1 ± 0.2b
	S2	43.9 ± 0.1a	44.7 ± 0.2a	21.0 ± 0.2a	21.7 ± 0.4a	39.8 ± 0.1a	39.9 ± 0.5a	113.0 ± 0.2a	114.7 ± 0.1a
Tillage (T)	T1	43.3 ± 0.1b	43.6 ± 0.2b	20.6 ± 0.1b	21.1 ± 0.2b	39.6 ± 0.2b	39.6 ± 0.1b	111.2 ± 0.2b	112.4 ± 0.1b
	T2	39.6 ± 0.2c	40.4 ± 0.2c	18.0 ± 0.1c	18.4 ± 0.1c	36.0 ± 0.2c	36.4 ± 0.2c	100.1 ± 0.1c	102.1 ± 0.2c
	T3	47.8 ± 0.1a	48.9 ± 0.1a	23.4 ± 0.2a	24.4 ± 0.1a	42.6 ± 0.1a	42.7 ± 0.1a	123.7 ± 0.2a	125.7 ± 0.1a
Variety (V)	V1	42.8 ±	43.6 ±	20.0 ±	20.5 ±	38.7 ±	38.8 ±	109.3 ±	110.9 ±
	V2	44.2 ±	45.0 ±	21.3 ±	22.1 ±	40.2 ±	40.3 ±	114.1 ±	115.9 ±

S_1, Early sowing (1st week of November) after the harvesting of short duration Kharif rice; S_2, Delayed sowing (last week of November) after the harvesting of long duration Kharif rice; T_1, Relay cropping; T_2, Zero tillage; T_3, Conventional tillage; V_1, Lentil short duration: *L4717*; V_2, Lentil long duration: Moitri. Significant at $P \leq 0.05$. NS, Non-significant at $P > 0.05$. Values are means ± SEM ($n = 3$). Different letters indicate significant differences between means.

crop were due to the more extended vegetative phase of the product, leading to the efficient use of growth resources and hence higher dry matter production. Zero-tilled plots (103.16 and 115.09 kg ha^{-1}) were noted to have maximum total N uptake by lentil grain and stover, followed by relay cropped and conventionally tilled plots during 2018–19 and 2019–20, respectively (Table 2). An earlier finding also documented the highest total N uptake in zero tillage as compared to other tillage practices (Bandyopadhyay et al., 2018). Higher nutrient content and uptake were observed under zero tillage might be because it can promote root growth and development and improve soil macro-aggregates and soil microbial activities as it endorsed good soil conditions through less soil disturbance. Similar observations regarding total N uptake by lentils among the varieties tested were found where the L4717 variety resulted in a significantly superior value (79.35 and 85.95 kg ha^{-1}) during both years due to the difference between the varieties regarding the number of nodules plant^{-1} (Table 2).

3.4. Impact on phenology and GDD

The duration of the lentil crop significantly increased with the delay in sowing from the 1st week of November to the last week of November in the experiment. On average, the early sowing took relatively fewer days from planting to maturity (110.4 and 112.1 days) and went through different phenophases, such as days to flowering, flowering to pod initiation, and pod initiation to maturity compared to the late sowing, which needed more days (113.0 and 114.7 days) to reach maturity in both survey years (Table 3).

The early November sown crops' accelerated phenological stages could be caused primarily by the temperature difference between them. For the crop planted in late November, when temperatures during vegetative growth were much colder and closer to the base temperature of the crop, growth was temporarily halted, delaying the attainment of phenophases at the optimum time. The results follow the results of earlier works (Islam et al., 2008; Sen et al., 2016) across different locations. Among the tillage operations followed, zero tillage treated plots took the lowest number of days for attaining sowing to maturity (100.1 and 102.1 days), followed by relay cropped plots in both the years of study. The minimal soil disturbance that reduces soil water loss through lower evaporation may account for faster growth in zero tillage systems. The softened seedbed helped in rapid emergence (Basir et al., 2011; Edalat and Naderi, 2016). The short-duration lentil variety L4717 recorded the minimum number of days in each of the growth stages noted (days to flower initiation, 42.8 and 43.6 days, flowering to pod initiation, 20.0 and 20.5 days, pod initiation to maturity, 38.7 and 38.8 days) in both years as compared to the long-duration variety *Moitri* (Table 3) due to their faster growth habit and good climate advantages. The interaction effect of sowing condition, tillage, and variety was not significant on the phenophase attainment of the lentil crops during two consecutive years.

Among the different treatment combinations, the $S_1T_1V_2$ (early sowing + relay cropping + *Moitri*) and $S_1T_3V_2$ (early sowing + conventional tillage + Moitri) combinations were noted to have

TABLE 4 Growing degree days (°C day) of lentil canopy at different phenophases grown under different combinations of sowing time, tillage, and variety after monsoon rice harvesting.

Treatment	Days to flower initiation		Flowering to pod initiation		Pod initiation to maturity		Life cycle sowing to maturity	
	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20
$S_1T_1V_1$	797.50	794.00	256.40	284.35	480.50	456.05	1694.82	1715.88
$S_1T_1V_2$	804.50	811.00	259.95	296.60	524.60	489.80	1762.88	1792.50
$S_1T_2V_1$	641.25	738.60	190.80	196.55	466.55	432.45	1426.72	1501.95
$S_1T_2V_2$	646.05	772.30	204.80	205.45	485.00	470.80	1470.13	1594.98
$S_1T_3V_1$	661.15	878.20	287.30	283.15	667.50	627.60	1794.72	1958.10
$S_1T_3V_2$	677.05	893.70	313.90	320.45	718.10	674.20	1900.15	2077.70
$S_2T_1V_1$	556.15	582.40	262.65	264.65	648.40	607.10	1600.35	1592.80
$S_2T_1V_2$	568.15	598.45	291.95	278.60	723.65	672.90	1734.65	1700.73
$S_2T_2V_1$	464.75	480.30	256.15	223.95	673.80	622.45	1494.30	1445.25
$S_2T_2V_2$	496.15	504.95	269.40	238.05	723.50	660.40	1597.88	1527.53
$S_2T_3V_1$	612.25	588.15	386.85	378.80	952.40	928.20	2073.07	2018.43
$S_2T_3V_2$	625.15	606.45	424.25	437.90	1017.45	1007.75	2196.60	2189.08

S_1, Early sowing (1st week of November) after the harvesting of short duration Kharif rice; S_2, Delayed sowing (last week of November) after the harvesting of long duration Kharif rice; T_1, Relay cropping; T_2, Zero tillage; T_3, Conventional tillage; V_1, Lentil short duration: L4717; V_2, Lentil long duration: Moitri. Significant at $P \leq 0.05$. NS, Non-significant at $P > 0.05$.

the highest accumulated GDD (804.50 and 893.70°C day) from emergence to flower initiation period during the two consecutive years. Mean gathered GDD from flowering to pod initiation (424.25 and 437.90°C day), pod initiation to maturity (1017.45 and 1007.75°C day), and life cycle, i.e., sowing to maturity (2196.60 and 2189.08°C day), recorded the highest value in the $S_2T_3V_2$ (delayed sowing + conventional tillage + *Moitri*) combination for both the years, respectively (Table 4).

The mean air temperature was higher for the late November sown crop than the early November sown crop, which staggered the phenological development of the delayed planted crop at the vegetative growth phase due to the unavailability of favorable weather parameters. Hence the accumulated GDD was higher in the late November sown crop than in the early November sown one. Notably, under both the sowing conditions, zero-tilled plots of lentils recorded the lowest accumulation of GDD compared to the other tillage treatments. This might be due to the lower time requirement of zero-tilled plots of lentils to attain different phenophases and complete their life cycle as compared to relay cropped and conventional tillage plots. That the short-duration varietal combination was less superior regarding GDD accumulation than the longer-duration varietal mix might be due to fewer days required for attaining phenophases across their lifecycle during the winter season of both years.

3.5. Relationship between growth, yield parameters, and total *N* uptake

The correlation studies among the different parameters of lentils (plant height, dry aerial biomass accumulation, LAI, and no. of nodules plant^{-1} at periodic observations) with total N uptake and seed yield depicted a significant positive correlation between themselves in the present investigation (Figure 7). The findings from the correlogram revealed that no. of nodules plant^{-1} at 45 DAS (0.962***), plant height at 45 DAS and 75 DAS (0.979***, 0.978***), dry aerial biomass accumulation at harvest (0.885***), and LAI at 75 DAS (0.977***) possessed the highest positive significant correlation values concerning the total N uptake of the lentil crops among all the observations. The no. of nodules plant^{-1} at 45 DAS (0.913***), plant height at 45 DAS and 75 DAS (0.944***, 0.943***), dry aerial biomass accumulation at 45 DAS (0.839***), and LAI at 75 DAS (0.960***) possessed the highest positive significant correlation values concerning seed yield in the study during 2018–19 and 2019–20. The seed yield also showed a significant positive correlation (0.973***) with the total N uptake of the lentil crops in the experiment, as assumed to be a highly dependent factor mentioned by earlier researchers (Kumar et al., 2016). From the above findings, it is evident that the number of nodules plant^{-1} at the vegetative stage is an important determining factor for lentil crops' N uptake. N uptake occurs throughout the vegetative and reproductive phases, reflecting its effect on plant height and LAI. The highest correlation value regarding dry aboveground biomass accumulation and seed yield in the vegetative stage indicates that better vegetative growth due to better plant vigor leads to higher lentil seed yield.

3.6. Impact on economics

The results indicated a particular trend of highest gross return (Rs. 82,660 and 93,237), net return (Rs. 51,220 and 59,257), and B:C ratio (2.63 and 2.74) under the treatment combination of earlier sowing + zero tillage + short duration variety in the

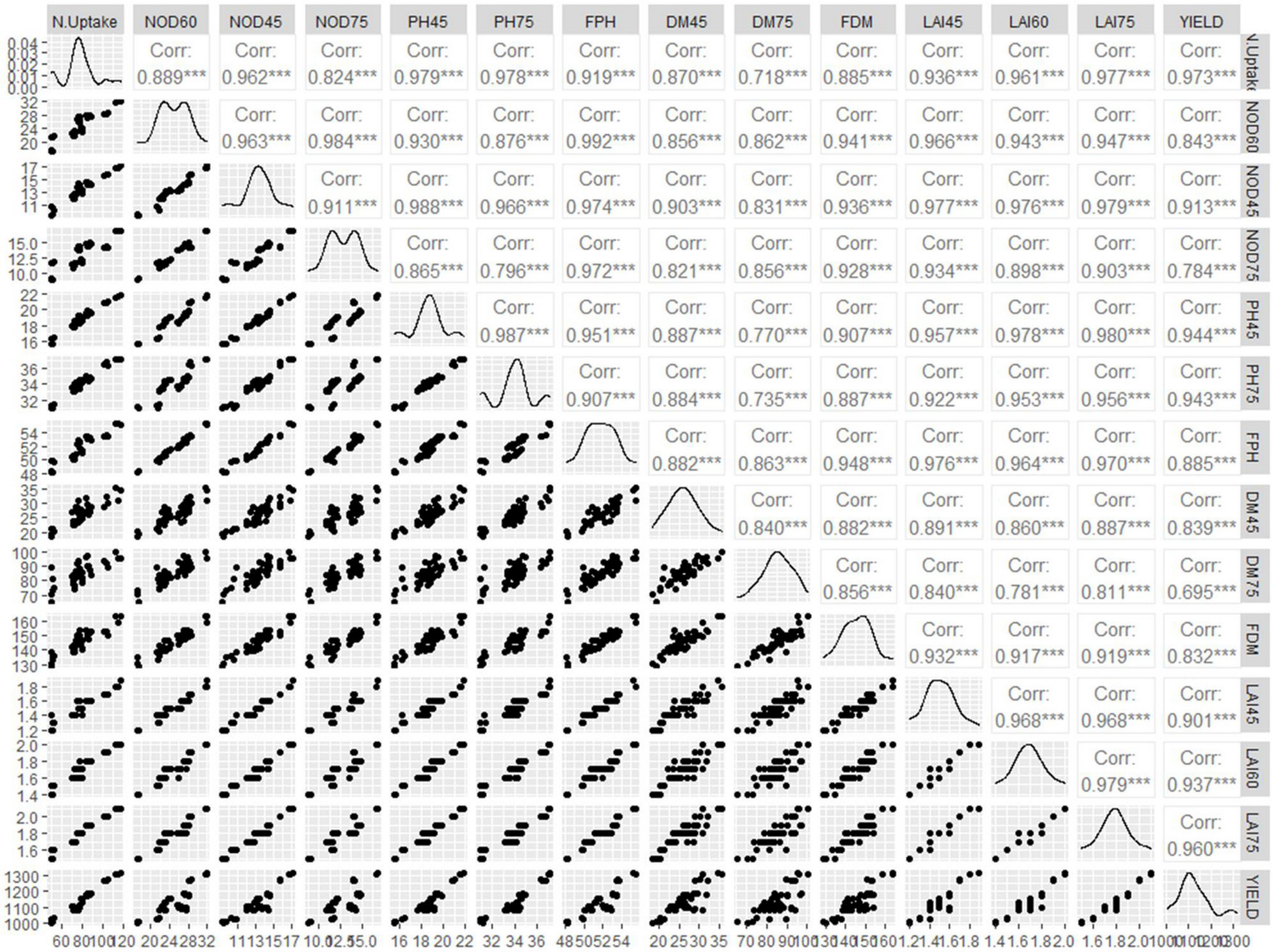

FIGURE 7
Correlogram showing Pearson correlation coefficient of growth and nodulation parameters with nitrogen uptake and seed yield. NOD 45, number of nodules plant^{-1} at 45 DAS; NOD 60, number of nodules plant^{-1} at 60 DAS; NOD 75, number of nodules plant^{-1} at 75 DAS; PH 45, Plant height at 45 DAS; PH 75, Plant height at 75 DAS; FPH, Plant height at harvest; DM 45, Dry aerial biomass accumulation at 45 DAS; DM 75, Dry aerial biomass accumulation at 75 DAS; FDM, Dry aerial biomass accumulation at harvest; LAI 45, Leaf Area Index at 45 DAS; LAI 60, Leaf Area Index at 60 DAS; LAI 75, Leaf Area Index at 75 DAS.

experiment with a near lowest cost of cultivation (Rs. 31,440 and Rs. 33,980) for the lentil crops during both the years of investigation (Table 5). Similar findings regarding sowing time were found in previous research by Islam et al. (2008) and Rao et al. (2016), who reported that optimum sowing time ensures a lesser cost of cultivation along with a much higher return in winter pulses across different locations of India. The findings of Visha Kumari et al. (2019) and Sasode et al. (2020) regarding the economic analysis of winter pulses also supported the results of the present investigation regarding the economic benefits of zero tillage among different tillage operations.

4. Conclusion

With the prevailing emissions scenario, within this century, the Indo-Gangetic plains are going to experience a 3–30% decrease in winter rainfall as revealed by CMIP6 model simulations. A temperature increase of 3°C is indicated under the lower Shared Socioeconomic Pathways (SSPs 1–3) whereas an increase of 4.8°C is indicated under the higher SSP conditions. This emphasizes the importance of introducing improved cultural practices along with the selection of suitable drought-tolerant varieties. The present study clearly indicated that the introduction of reduced or zero tillage and short-duration lentil cultivars helps in regaining moisture within the profile and significantly contributes to the overall productivity of lentils in terms of ensuring superiority in growth, nodulation, phenophasic development, yield, nitrogen uptake, and the economics of cultivation in lentil crops. Early November is the ideal planting time for lentils cultivated under residual moisture conditions. These combinations of various treatments can help the farmers, especially in the rainfed/dryland region to have more yield by mitigating early cessation of the monsoon season along with sparse winter rainfall.

TABLE 5 Effect of sowing time, tillage, and variety on the economics of lentil crops sown after monsoon rice harvesting.

Treatment	Cost of cultivation (Rs.)		Gross return (Rs.)		Net return (Rs.)		B:C ratio	
	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20
$S_1T_1V_1$	30,940	33,480	68,773	74,286	37,833	40,806	2.22	2.22
$S_1T_1V_2$	31,240	33,780	66,350	71,439	35,110	37,659	2.12	2.11
$S_1T_2V_1$	31,440	33,980	82,660	93,237	51,220	59,257	2.63	2.74
$S_1T_2V_2$	31,615	34,155	75,924	87,078	44,309	52,923	2.40	2.55
$S_1T_3V_1$	32,815	35,480	65,688	70,028	32,873	34,548	2.00	1.97
$S_1T_3V_2$	32,990	35,655	61,532	66,348	28,542	30,693	1.87	1.86
$S_2T_1V_1$	30,940	33,480	67,883	73,667	36,943	40,187	2.19	2.20
$S_2T_1V_2$	31,240	33,780	65,759	70,598	34,519	36,818	2.10	2.09
$S_2T_2V_1$	31,440	33,980	80,950	89,523	49,510	55,543	2.57	2.63
$S_2T_2V_2$	31,615	34,155	73,827	83,871	42,212	49,716	2.34	2.46
$S_2T_3V_1$	32,815	35,480	63,195	66,495	30,380	31,015	1.93	1.87
$S_2T_3V_2$	32,990	35,655	60,184	64,127	27,194	28,472	1.82	1.80

Price of lentil grain and stover = Rs. 60 kg^{-1} and Rs. 1 kg^{-1} (2018–19), Rs. 65 kg^{-1} and Rs. 1.50 kg^{-1} (2019–20).
S_1, Early sowing (1st week of November) after the harvesting of short-duration kharif rice; S_2, Delayed sowing (last week of November) after the harvesting of long-duration kharif rice; T_1, Relay cropping; T_2, Zero tillage; T_3, Conventional tillage; V_1, Lentil short duration: L4717; V_2, Lentil long duration: Moitri.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

Conceptualization, investigation, and methodology: BM, MK, RN, KA, and KP. Validation, writing—original draft preparation, and visualization: BM, MK, RN, KA, VV, PB, SA, and KP. Formal analysis: BM and AH. Data curation: BM, VV, SA, AL, and AH. Writing—review and editing: BM, VV AH, SA, MS, and AL. Funding acquisition: AH and SA. All authors have read and agreed to publish the current version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Optical sensor-based nitrogen management: an environmentally friendly and cost-effective approach for sustainable wheat (*Triticum aestivum* L.) production on Eastern plains of India

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An optical sensor like Green Seeker (GS) is an emerging tool for site-specific in-season fertilizer nitrogen management strategy. The objective of this study was to establish an in-season estimate of yield (INSEY)–grain yield (GY) relation in wheat grown under Eastern plains of India using normalized difference vegetation index (NDVI) at 45 and 65 days after sowing (DAS). Data revealed lower NDVI values at 65 DAS over 45 DAS in no-nitrogen (N), phosphorus (P), and potassium (K) applied control plots as well as in N-rich plots (225 kg ha^{-1} N); on the contrary, the values were higher at 65 DAS over 45 DAS in treatments where some N fertilizers were added based on NDVI readings at 45 DAS. Response index (RI) showed higher chances of response to external application of N in NDVI-based treatments. The INSEY–GY relation for wheat at 45 and 65 DAS was worked out as a power function of $y = 64265x^{1.171}$ and $y = 46949x^{1.036}$ (y is the attainable yield in kg ha^{-1} and x is INSEY), respectively. The yields could fairly be predicted through this relation even at 45 DAS, though the relationship was more robust at 65 DAS ($R^2 = 0.94$). A prescriptive dose of 60 kg N ha^{-1} as basal + 60 kg N ha^{-1} at crown root initiation (CRI) stage followed by NDVI sensor-guided N application (at 45 and 65 DAS) brought about a significant improvement in yield performances, N use efficiencies with higher net returns, and benefit-to-cost ratio. The results proved the reliability of the NDVI sensor as an important tool for the optimization of fertilizer nitrogen in wheat grown under the Eastern plains of India. The new INSEY–GY relation developed through this trial could successfully be used for yield prediction in the Eastern plains of India under changing climate.

KEYWORDS

NDVI, wheat, optical sensor, nitrogen, yield

1. Introduction

Rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) are a staple food for a large population in South Asia, and their assured supply is essential for the food security of this region. Thus, this rice–wheat cropping is one of the most important systems in the entire Indo-Gangetic plains (IGP), covering countries such as India, Bangladesh, Nepal, and Pakistan. Only in the Eastern Gangetic plains, the rice–wheat cropping system is practiced on over 6.22 million hectares (Timsina et al., 2018), and the farmers of the entire IGP continue to intensify growing wheat as a winter season crop due to high subsidies for power, fertilizer, and irrigation water, and well-developed production and marketing systems (Saharawat et al., 2009). The crop yields of the Eastern Gangetic plains are, however, low due to a range of biophysical (poor soil fertility status, imbalanced fertilization, water and temperature stress, pest and disease infestation, and high weed pressure) factors (Islam et al., 2019). In the rice–wheat system, sub-optimal nutrient management in these tracts results in low nutrient use efficiency, nutrient mining, as well as monetary loss (Dhawan et al., 2021). Soils of India, Eastern plains in particular, are inherently deficient in N supply because of the topographical location in the subtropical climate that hinder the accumulation of soil organic matter, a primary source of soil N (Dwivedi et al., 2017). Again, N is highly mobile in the soil–plant system and prone to losses resulting in reduced recovery of applied N by the crop, making it difficult for its efficient management (Ladha et al., 2005). The contribution of N fertilizer to increased global food production cannot be ignored for feeding the ever-growing human population. On the contrary, the soils of Eastern India are not so deficient in P and K in general. Thus, better nutritional management, nitrogen in particular, needs immediate attention for maintaining the sustainability of the rice–wheat system which suffers from production fatigue.

As a general cultivation practice, all phosphatic and potassic fertilizers along with 50% of N are applied as basal at final land preparation just before the seeding of wheat; the rest of 50% N is top-dressed either one or two times based on irrigation events (Pathak et al., 2006). Due to the large-scale blanket application of fertilizers, particularly N fertilizers over the region, the nitrogen use efficiency suffers to a great extent (Majumdar et al., 2013; Mitra et al., 2019), and as reported by Dobermann et al. (2003), there is large spatial and temporal variability of soil N which exists in this region contributing to poor fertilizer use efficiency, N in particular.

As reported from various experiments carried out over these Eastern plains, split application of N fertilizers was found to perform much better toward the enhancement of crop productivity as well as nitrogen use efficiency (NUE) (Rahman et al., 2002; Majumdar et al., 2013; Singha and Mitra, 2020). However, the number of splits and quantities of N to be applied in each split is a debatable issue in fertilizer management for maximizing wheat yield with better NUE as there are contradictory references. We know NUE is affected by soil type, temperature regimes, the application rate of N fertilizer, soil moisture conditions, and crop rotation (Halvorson et al., 2002). We have tried using a leaf color chart (LCC) and Soil Plant Analysis Development (SPAD) chlorophyll meter for better synchronization of N application in wheat, as these are simple handheld devices that are used for rapid, non-destructive measurement of leaf chlorophyll. It was found that these handheld diagnostic tools are very reliable in real-time N management in certain crops (Ladha et al., 2005), but these tools have the greatest limitations in that we are simply taking into account the leaf color, not taking into account the photosynthetic rates or total biomass production of the crop or even expected yields in working out fertilizer requirement, especially N. Under the circumstances, the use of Green Seeker (GS), an optical sensor, could measure the spectral vegetation index—the normalized difference vegetation index (NDVI) which is linked with leaf area as well as green biomass (Raun et al., 2002). An index of an in-season estimate of yield (INSEY) could be worked out as a measure of the accumulated biomass per day from the time of seeding to the day of taking observation through the sensor. INSEY could help guide N application, and this INSEY–grain yield (GY) relation could optimize N scheduling in wheat successfully (Singh et al., 2011) as witnessed in the northwestern plains, the wheat bowl of India. We strongly believe that there is a huge difference in crop expression over the entire Eastern plains in comparison to the northwestern plains for which the GY-INSEY relation would be different for this zone; however, no such study has yet been conducted in the Eastern plains of India to precisely manage fertilizer N in wheat using NDVI sensor. Keeping these in the background, the present experiment has been planned to optimize N scheduling in wheat through the use of NDVI sensor-GS, and we have tried to establish a new equation for in-season prediction of wheat yield using NDVI sensor for this Eastern plains of India.

2. Materials and methods

2.1. Experimental site

The study was conducted in the research field of 'Uttar Banga Krishi Viswavidyalaya' (UBKV), Cooch Behar, West Bengal, India (26°24'02.2"N latitude, 89°23'21.7"E longitude, 43 msl), in two consecutive wheat seasons during 2017–2018 and 2018–2019. The soil, on which the experiment was carried out, was sandy loam in texture with a good drainage facility having pH 5.78 with 0.83% organic C, 188.16 kg ha^{-1} mineralizable nitrogen, 27.05 kg ha^{-1} available phosphorus, and 141.90 kg ha^{-1} available potassium.

2.2. Agroclimatic conditions

Being located in a subtropical humid climate, the experimental site receives higher annual rainfall (3,000 mm); however, a major proportion of the total rainfall (2,200–2,500 mm) is received during monsoon months (June–September), keeping the winter season almost dry. The temperature began to rise from February to March and reached its peak from April to May. The relative humidity remained very high almost throughout the year except during the winter season. During the wheat crop growing period, there was not much variation in minimum and maximum temperatures (data not given) during both years and fair cool weather during vegetative growth and a bit of warm weather during later stages prevailed suggesting an overall favorable temperature regime for the crop during both the seasons. In both years, the crop received rainfall (36 mm and 9.62 mm during 2017–2018 and 2018–2019, respectively) during March when the crop was almost at maturity.

2.3. Treatment details

The experiment was laid out in a randomized block besign (RBD) comprised of 10 treatments of different N scheduling including the use of NDVI sensor-GS. The treatments comprising of T1 = absolute control (no application of NPK); T2 = 150 kg ha^{-1} N (1/2 as basal + 1/4thCRI + 1/4th active tillering); T3 = 120 kg ha^{-1} N (1/2 as basal + 1/4thCRI + 1/4th active tillering); T4 = 150 kg ha^{-1} N (1/2 as basal + 1/2 CRI); T5 = 120 kg ha^{-1} N (1/2 as basal + 1/2 CRI); T6 = 30 kg N ha^{-1} as basal +30 kg N ha^{-1} at CRI + NDVI sensor-based N; T7 = 30 kg N ha^{-1} as basal +60 kg N ha^{-1}at CRI + NDVI; T8 = 60 kg N ha^{-1} as basal +60 kg N ha^{-1}at CRI + NDVI; T9 = 60 kg N ha^{-1} as basal +30 kg N ha^{-1}at CRI + NDVI; and T10 = N-rich 225 kg ha^{-1} N (1/2 as basal + 1/2 CRI). The NDVI data were taken at 45 and 65 days after sowing (DAS) coinciding with the second and third irrigation events. Each treatment was replicated three times. The sizes of each experimental plot were 8 m x 2 m.

2.4. Crop management practices

The land preparation started with plowing twice using a tractor-drawn cultivator. The soil was kept open for the next 10 days for bringing down the soil moisture. Afterward, a rotavator was used criss-cross for bringing the desirable tilth. The phosphorus (60 kg ha^{-1} P_2O_5) and potassium (40 kg ha^{-1} K_2O) were supplied uniformly in all the treatments except control (T1) through single super phosphate and muriate of potash, respectively. HD 2967, a recommended high-yielding variety for the timely sown irrigated condition under this zone (Eastern plains of India) having a yield potential of 6.2 t ha^{-1}, was used in this experiment. This variety 'HD 2967' was developed by the Indian Agricultural Research Institute, New Delhi, India, and the pedigree of the variety is ALONDRA/CUCKOO/URES-81/HD-2160-M/HD-2278[4251][4282]; HD-2733/K-9423/K-9351 [4281]. After treating the seeds with carbendazim @ 2.5 g kg^{-1} of seeds, the seeds were sown in lines 20 cm apart at 2–3 cm depth with a seed rate of 100 kg ha^{-1}. Metribuzin at 300 g ha^{-1} was used as pre-emergence herbicides to keep the experimental plots free from weeds during initial growth, while the later flashes of broad-leaved weeds were controlled through post-emergence Carfentrazone at 20 g ha^{-1}, applied 4 weeks after sowing. Considering the B and Zn deficiency in this zone, soluble B (0.2%) and chelated Zn (0.1%) were applied as foliar spray twice at 35 and 55 DAS. The crop received four irrigations at CRI, active tillering, jointing, and milking stage in both years. The crop was harvested manually, and the yield was estimated based on the net plot of 12.8 m^2 (8 inner lines, each of 8 m length with 20 cm spacing) excluding the border rows. Grain yield was recorded at 12% moisture content.

2.5. Soil and plant analyses

The pH of the experimental soil was determined with Sorensen's pH meter (1909), using soil–water suspension (1:2.5) by the potentiometric method. Mineralizable nitrogen and available phosphorus and potassium were analyzed using the hot alkaline potassium permanganate method (Subbaih and Asija, 1956), Olsen's method (Olsen et al., 1954), and the neutral ammonium acetate-flame photometer method (Brown and Warncke, 1988), respectively; the wet digestion method was followed for determining organic C (Walkley and Black, 1934). Aerial plant parts were separated into grain and straw. After crushing, samples were prepared for grain and straw nitrogen content separately. The total N content in plants was determined by the modified micro-Kjeldahl method (Jackson, 1973). The total uptake of nitrogen by wheat at harvest was determined on a dry weight basis by multiplying the total dry matter of the crop with its corresponding content of nitrogen. It was expressed in kg ha^{-1}.

2.6. Observations based on NDVI sensor

Normalized difference vegetation index (NDVI), a good indicator of green biomass, was determined by dividing the difference between near-infrared and red wavebands by the sum of these two wavebands. In general, NDVI values range from −1.0 to 1.0, with negative values indicating clouds, water surfaces, rocks, snow, etc; bare soils usually have lower positive values (0.10–0.15), and plants will always have higher positive values above 0.2 (sparse vegetation within 0.4–0.5 while dense vegetation has 0.5 and above). Several studies have shown that the NDVI value of a matured wheat crop ranges between 0.4 and 0.8 depending on variety and climatic conditions (Thapa et al., 2019; Gozdowski et al., 2020). In the present experiment, GS was calibrated based on the value of NDVI reading in the N-rich treatment where 225 kg N ha^{-1} was applied. NDVI sensor-GS was used for the splitting of nitrogen in four treatments (T6, T7, T8, and T9) in the present experiment. The sensor was passed over the crop at a height of approximately 0.9 m above the crop canopy and oriented so that the 0.6 m sensed width was perpendicular to the row and centered over the row. With the advancing stage of growth, sensor height above the ground increased proportionally. Nitrogen doses using NDVI-GS were calculated as per the following discrete components:

2.6.1. Normalized difference vegetation index

Normalized difference vegetation index (NDVI) measurements made by Green Seeker were based on the following formula as given by Raun et al. (2002) and Singh et al. (2011):

$$NDVI = [(NIR_{ref}/NIR_{inc})-(Red_{ref}/Red_{inc})]/\ [(NIR_{ref}/NIR_{inc}) + (Red_{ref}/Red_{inc})]$$

where NIR_{ref} and Red_{ref} = magnitude of reflected near-infrared and red lights; NIR_{inc} and Red_{inc} = magnitude of the incident near-infrared and red lights.

2.6.2. In-season estimation of yield

In-season estimation of yield (INSEY) was expressed as the measure of the daily accumulated biomass from the time of planting to the day of sensing. It was measured based on the following formula as given by Raun et al. (2002) and Singh et al. (2011):

$$\text{INSEY} = \text{NDVI/days of sensing}$$

2.6.3. Yield potential with no added inputs (YP_0)

Yield potential (YP_0) with no added fertilizer was calculated from the following power equation model as given by Raun et al. (2002) and Singh et al. (2011):

$$YP_0 = a\,(\text{INSEY})^b$$

where the values of constants 'a' and 'b' were used as depicted in graphs; the Coefficient of Determination (R^2) value was also determined using MS Excel.

2.6.4. Response index (RI_{NDVI})

The magnitude of response to N fertilization was predicted from RI_{NDVI} as per the following formula given by Raun et al. (2002) and Singh et al. (2011):

$$RI_{NDVI} = \text{NDVI of N rich strip/NDVI of the test plot}$$
$$RI_{Harvest} = \text{Maximum yield/yield of the test plot}$$

One N-rich strip (NRS) was laid out along with experiment (T10) as a prerequisite for precisely working out the N requirement based on the possible response of that crop to applied N.

2.6.5. Predicted attainable yield (YP_N)

The yield potential achievable with applied N fertilizer (YP_N) was predicted based on RI and YP_0.

For a particular treatment before any mid-term N application, yield could be predicted by YP_N/RI as RI represented the percent yield increment to achieve the attainable grain yield (5 t ha^{-1} for the present experiment).

2.6.6. Fertilizer N recommendation

The fertilizer N dose was calculated based on the following formula as given by Singh et al. (2011) and Ratanoo et al. (2018a):

$$\text{Fertilizer N dose (kgha}^{-1}) = [1.8\,\text{x}\,(YP_N - YP_0)/100\,\text{x}\,0.5]$$

The average percentage of N in grain at harvest was 1.8% over IGP. The divisor 0.5 represents the fertilizer N use efficiency factor of 50% for wheat.

In the third year, GS-based nitrogen recommendations were put under farmers' participatory demonstration in which five doses (60, 90, and 120 kg N ha^{-1} upto CRI followed by GS at 45 and 65 DAS along with absolute control and N-rich strip at 225 kg N ha^{-1}) were validated.

2.6.7. NUE indices

The following NUE indices were worked out as per the standard formula:

- Agronomic N-use efficiency (kg grain kg^{-1} nutrient applied over control) = [yield under test treatment (kg ha^{-1})–yield under control (kg ha^{-1})]/[unit of nitrogen applied in the treatment (kg ha $^{-1}$)].
- Physiological efficiency (kg biomass kg^{-1} nutrient uptake) = [(biomass yield under fertilizer treatment (kg ha^{-1}) – biomass yield under control (kg ha^{-1})]/[(N uptake of nutrient in test treatment (kg ha^{-1}) – N uptake of nutrient in control (kg ha $^{-1}$)].

2.7. Production economics

To identify the most remunerative treatment, the economic parameters *viz*., the total cost of cultivation (₹ha^{-1}), gross return (₹ha^{-1}), net return (₹ha^{-1}), and benefit-to-cost ratio (B:C) were calculated for wheat grown under various N scheduling and interpreted accordingly. In the calculation of the economics of cultivation, the purchase rates of inputs and the selling rates of outputs were assumed as per the prevailing local market rates.

2.8. Statistical analyses

The data were analyzed on an individual year basis using the analysis of variance (ANOVA) method for RBD. The significance of various sources of variation was tested by the mean square error by Fisher–Snedecorst "F" test at probability levels of 0.05 (Cochran and Cox, 1955; Panse and Sukhatme, 1967). Mean separation for different treatments under different parameters was performed using Tukey's honestly significant difference (HSD) test ($p \leq 0.05$).

The functional relation between INSEY and grain yield was expressed in a power function as follows: yield without further addition of N = a (INSEY)^b; the values of constants a and b were depicted from graphs; and coefficient of determination (R^2) value was also determined using MS Excel.

3. Results and discussion

3.1. NDVI values

Normalized difference vegetation index (NDVI) values were taken at two stages of crop growth, *i.e*., at 45 and at 65 days after sowing (DAS). These two stages were coinciding with the second and third irrigation for the wheat crop. For calculating the amount of nitrogen required for topdressing (if any) during the second and third irrigation, NDVI readings were taken during these 2 days. Sixty five days old wheat crop was just prior to or at beginning of jointing stage, the last stage for prescriptive nitrogen management in wheat (Ali et al., 2020). Before taking this reading, only one split of N was applied at the crown root initiation (CRI) stage except for the absolute control. NDVI values varied significantly under various N splitting treatments, and it ranged from 0.29 under absolute control to 0.77 under N-rich plots where 225 kg ha^{-1} of N was applied during 2017–2018 (Table 1). The trend was similar in 2018–2019 also, where NDVI values ranged between 0.29 and 0.78 under absolute control and N-rich plots, respectively (Table 2).

In both the years where 225kg ha^{-1} of N was applied (N-rich treatment), the NDVI value showed a higher value (0.74) as compared with other treatments at 45 DAS. As nitrogen was not applied in control plots (T1), the leaves were lacking greenness; in contrast, the leaves were dark green under N-rich plots (T10) due to excess N application. Even in treatments where 150 kg ha^{-1} of N was applied, NDVI values were fairly higher due to greener leaves. It was noted that the NDVI values at 65 DAS were lesser than NDVI values recorded at 45 DAS in control plots as well as N-rich plots; on the contrary, the values were higher at 65 DAS over 45 DAS in most of the treatments, particularly in treatments where some N fertilizers were added based on NDVI readings at 45 DAS. As there was no further addition of N fertilizers after the CRI stage in N-rich plots (T10) as well as in T4 and T5, the canopy greenness was gradually reduced. The addition of N at 45 DAS helped the crop to improve the canopy greenness afterward reflected through higher NDVI values. In general, NDVI values at 45 days were higher in treatments having higher doses of fixed rate of N at planting and CRI stages; while at 65 DAS, the increase in NDVI values was more in treatments having N application at 45 DAS coinciding with the second irrigation. Ratanoo et al. (2018a) recorded 11.7% to 22.2% increase in NDVI values in treatments receiving N with irrigation events. NDVI measurements at proper growth stages are very important, as the relationship between in-season estimation and grain yields is purely based on NDVI readings.

3.2. Response index (RI)

The response index (RI) was computed based on NDVI values at 45 DAS, and the RI data presented in Tables 1, 2 indicated a significant difference among various treatments comprising various splitting of N applications. RI_{NDVI} was very useful in predicting the response of the crop to added N application. Being the ratio between NDVI-rich N plots and the NDVI-treatment plots, the values were higher in control plots (2.66 and 2.69 during 2017–2018 and 2018–2019, respectively), signifying higher chances of those plots responding to external application of N. It was evident that in treatments where higher amounts of N were added within 45 DAS, there were lower values of RI_{NDVI}, and in contrast, RI_{NDVI} values were higher in treatments with a lesser amount of N application. It indicated that a lesser amount of N was necessary to attain the target yield for plots with higher N fertilization; on the contrary, there might be higher responses from external N application in plots receiving lower N for attaining the yield target.

RI_{NDVI} values at 45 DAS were ranging between 1.04 to 1.24 and 1.05 to 1.26 under fixed N-applied plots (T2-T5) during 2017–2018 and 2018–2019, respectively; whereas it ranged between 1.24 to 1.33 and 1.22 to 1.30 during 2017–2018 and 2018–2019, respectively under the Green Seeker (GS)-guided N application plots (T6–T9) (Tables 1, 2). It signified that a 4–26% yield increment could be possible through N application for fixed N-applied plots while a 22–33% increase in yield could be possible in GS-guided N-treatments. In fixed N-treated plots, the N application rate was quite higher at the initial stages (upto CRI) than in GS-guided N application treatments (T6–T9), where the rate of N application was initially lesser, making an avenue for more application in subsequent stages based on NDVI sensor-provided values. RI_{NDVI} values were closely related to $RI_{Harvest}$ during both 45 and 65 DAS. Johnson and Raun (2003) found a positive correlation between RI $_{NDVI}$ and $RI_{Harvest}$ at Feekes 5–6 (in our experiment, it coincided with 45 DAS) and Feekes 7–8 (in our experiment, it coincided with 65 DAS). Mullen et al. (2003) obtained similar relationships between RI_{NDVI} and $RI_{Harvest}$ with data recorded at different growth stages of winter wheat. This was in line with the findings of Ratanoo et al. (2018a) who showed higher RI in NDVI-treated plots over fixed N-treated plots. It was evident that the values were in general narrower at 65 days over the values at 45 days. With the advancement of crop age, the responsiveness of the crop toward the external application of N was gradually reduced, as the crop had a narrower window to utilize the added N. The crop was moving toward its reproductive phase, and the addition of N fertilizer after 65 days was perceived to be less responsive for the crop. It was evident that in GS-guided treatments where higher amounts of total N (152–155 kg ha^{-1} of N) were added upto 65 DAS (T8), RI value was 1.00 (similar to N-rich plots), indicating no further requirement of N beyond this point for attaining the target yield. Raun et al. (2001) reported higher RI values with the treatment comprising no pre-plant N application as compared with the values resulting from pre-plant N application. It was also evident that the application of comparatively lower doses of nitrogen at early stages of growth and thereafter N application using response index could be a powerful tool to attain the desired grain yield without much increase in the rate of N application. Lower values of response index at later growth stages could be attributed to canopy closure influence on the sensor as noted by Teal et al. (2006) in maize and Ali et al. (2014) in direct-seeded rice. NDVI readings *vis-à-vis* response index, when captured at the jointing stage (65 DAS), could explain the variability in yields at maturity (Ali et al., 2020).

3.3. In-season estimation of yield (INSEY)

In-Season Estimation of Yield (INSEY) was determined by using NDVI values and the crop age at the date of sensing. At 45 DAS during both years, this value was lowest in the control plot, while it was the highest for N-rich plots (Tables 1, 2). As this value was basically an indicator of per day dry matter production of the crop, it was clear that biomass production per day was lowest for control plots and highest under N-rich plots at 45 DAS. Actually, the treatment receiving more N during the initial part of the growth recorded higher INSEY at 45 DAS as this treatment showed higher canopy greenness as reflected through higher NDVI values.

However, this estimation was too early from the overall perspective as the scenario changed afterward through another split application which was still awaited at 45 DAS. For GS-based treatments (T6–T9), the values were comparatively lesser over fixed N-treated (T2–T5) plots at 45 DAS. This was attributed to the lower rate of N application up to 45 DAS in GS-guided plots. At 65 DAS during both years, this value was highest in plots fertilized

TABLE 1 Evaluation of NDVI sensor-based N management in wheat at 45 days after sowing.

Treatments	*N* application up to 45 DAS		NDVI		RI_{NDVI}		$RI_{Harvest}$		INSEY		Yield achievable under current *N* rate (kg ha^{-1})	
	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19
T1	0	0	0.29	0.29	2.66	2.69	2.82	3.75	0.0064	0.0064	-	-
T2	112.5	112.5	0.65	0.65	1.18	1.20	1.05	1.07	0.0144	0.0144	-	-
T3	90	90	0.62	0.62	1.24	1.26	1.15	1.24	0.0138	0.0138	-	-
T4	150	150	0.74	0.74	1.04	1.05	1.09	1.08	0.0164	0.0164	-	-
T5	120	120	0.67	0.67	1.15	1.16	1.20	1.23	0.0149	0.0149	-	-
T6	60	60	0.58	0.60	1.33	1.30	1.20	1.26	0.0129	0.0133	3,766	3,846
T7	90	90	0.60	0.63	1.28	1.24	1.10	1.16	0.0133	0.0140	3,896	4,038
T8	120	120	0.62	0.64	1.24	1.22	1.00	1.00	0.0138	0.0142	4,026	4,103
T9	90	90	0.59	0.63	1.31	1.24	1.11	1.16	0.0131	0.0140	3,831	4,038
T10	225	225	0.77	0.78	1.00	1.00	1.10	1.09	0.0171	0.0173	-	-

T1 = absolute control(No application of NPK); T2 = 150 kg ha^{-1} N (1/2 as basal + 1/4th CRI + 1/4th active tillering); T3 = 120 kg ha^{-1} N (1/2 as basal + 1/4th CRI + 1/4th active tillering); T4 = 150 kg ha^{-1} N (1/2 as basal + 1/2 CRI); T5 = 120 kg ha^{-1} N (1/2 as basal + 1/2 CRI); T6 = 30 kg N ha^{-1} as basal + 30 kg N ha^{-1} at CRI + NDVI sensor-based N; T7 = 30 kg N ha^{-1} as basal +60 kg N ha^{-1} at CRI + NDVI; T8 = 60 kg N ha^{-1} as basal +60 kg N ha^{-1} at CRI + NDVI; T9 = 60 kg N ha^{-1} as basal +30 kg N ha^{-1} at CRI + NDVI; T10 = N rich- 225 kg ha^{-1} N (1/2 as basal + 1/2 CRI). NDVI, Normalized difference vegetation index and INSEY, In-season estimation of yield.

TABLE 2 Evaluation of NDVI sensor-based N management in wheat at 65 days after sowing.

Treatments	*N* application upto 45 DAS		NDVI		RI_{NDVI}		$RI_{Harvest}$		INSEY		Yield achievable under current *N* rate (kg ha^{-1})	
	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19
T1	0	0	0.26	0.26	2.85	2.96	2.82	3.75	0.0040	0.0040	-	-
T2	150	150	0.73	0.73	1.01	1.05	1.05	1.07	0.0112	0.0112	-	-
T3	120	120	0.72	0.72	1.03	1.07	1.15	1.24	0.0111	0.0111	-	-
T4	150	150	0.73	0.73	1.01	1.05	1.09	1.08	0.0112	0.0112	-	-
T5	120	120	0.73	0.73	1.01	1.05	1.20	1.23	0.0112	0.0112	-	-
T6	104	102	0.68	0.70	1.09	1.10	1.20	1.26	0.0105	0.0108	4,595	4,545
T7	130	125	0.70	0.74	1.06	1.04	1.10	1.16	0.0108	0.0114	4,730	4,805
T8	155	152	0.74	0.77	1.00	1.00	1.00	1.00	0.0114	0.0118	5,000	5,000
T9	132	125	0.70	0.75	1.06	1.03	1.11	1.16	0.0108	0.0115	4,730	4,870
T10	225	225	0.74	0.77	1.00	1.00	1.10	1.09	0.0114	0.0118	-	-

Treatments are presented in Table 1.

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with 60 kg ha^{-1} of N as basal plus 60 kg ha^{-1} of N at CRI and another 32–35 kg ha^{-1} at 45 DAS (T8) which was similar or even higher than the INSEY obtained in N-rich plots. A fair amount of N addition through the NDVI sensor at 45 days helped to increase INSEY in T8 signifying higher production of dry matter per day under the treatment. Despite a higher total application of N (225 kg ha^{-1}) under N-rich strips (T10), no addition of N at 45 DAS reduced the chances of further enhancement of INSEY values. The treatment receiving better splitting of N guided through GS was supposed to be a powerful tool for harnessing the yield of wheat with better N management. It was noted that in all the treatments having different N splits, INSEY was much lower during 65 DAS than 45 DAS, despite some improvements in NDVI values in 65 DAS. This was attributed to higher crop age as the crop almost progressed toward its reproductive growth. However, in-season estimation of yield at 65 days of crop age could give a more reliable estimate than its estimation at 45 DAS.

Figures 1A, B shows the INSEY-grain yield (GY) relation for wheat at 45 and 65 DAS, respectively (we have used the station trial data for 2 years as well as the data from the farmers' field during the third year for validation). This relation was expressed as a power function by using the following equations:

$$y = 64265x^{1.171} \text{(at 45 DAS)}$$
$$y = 46949x^{1.036} \text{(at 65 DAS)}$$

In these equations, y is attainable yield in kg ha^{-1}and x is INSEY.

A value of R^2 during 45 DAS (0.90) suggested that even at 45 DAS, the yields could be predicted fairly. This stage actually coincided with Feeks 5–6 stages when the first node appeared on the plant. This relationship was more robust at 65 DAS as reflected by the R^2 value (0.94), which coincided with Feeks 7–8 stage when the flag leaf developed with more visible nodes. These equations were suggestive of the wider applicability of this relation in predicting the midterm yields of wheat, and these equations may be used for this zone while going for N application in wheat through an optical sensor. The robust relationships between in-season sensor-based estimates of yield at Feekes 5–6 and 7–8 stages and actual wheat yields under the northwestern plains of India were previously reported (Singh et al., 2011; Ratanoo et al., 2018a,b). The response of wheat to fertilizer N defined by the sensor was highly correlated with the response index. Gupta (2006) also studied the effect of crop canopy sensors on efficient nitrogen management in Indo-Gangetic plains. Farmers cannot produce the same yields from the same fields every year, despite the same cultivar being planted on the same date with similar management practices. This is due to temporal variability in crop growth which has higher impacts on yield levels through variable responses to applied N fertilizers. However, an in-season response index based on NDVI readings could represent a viable method or approach for identifying environments where the potential to respond to N fertilizer exists (Ali et al., 2020). In fact, optical sensors have opened up a new approach to acquiring crop growth information in a rapid non-invasive manner (Teal et al., 2006; Zhang et al., 2019).

3.4. Calculated *N* doses and actual yields

Considering the attainable/target yield of 5 t ha^{-1}, the achievable yield at 45 DAS was estimated for all GS-guided treatments (T6–T9), and it showed that the treatment in which 60 kg ha^{-1} of N was applied both at basal and at CRI (T8), it recorded the maximum yield (4026 and 4103 kg ha^{-1} during 2017–2018 and 2018–2019, respectively) with the current rate of fertilizer application without subsequent addition. The amount of N to be applied further to attain the target yield of 5 t ha^{-1} was worked out for all these treatments, and it showed that for achieving the desired yield target, more N was required for all these treatments. The estimated amount of N was ranging between 35–44 kg ha^{-1} during 2017–2018 and 32–42 kg ha^{-1} during 2018–2019 for getting the attainable yield of 5 t ha^{-1}. The situation changed a lot for all GS-guided treatments when the doses were calculated at later dates *viz*., 65 DAS. The treatment receiving 60 kg ha^{-1} of N at basal, 60 kg ha^{-1} of N at CRI, and another 32–35 kg ha^{-1} of N at 45 DAS based on GS (T8) reached the attainable yield target of 5 t ha^{-1} itself suggesting no need for further addition of fertilizer to achieve the target yield at 65 DAS. Moreover, for the rest of the GS-guided treatments (T6, T7, and T9), there was still the requirement of some amount of N (lesser than the amount required at 45 DAS) even at this point to achieve the desired yield (Tables 1, 2). However, the application of N at 65 DAS based on the target yield under the other treatments could not achieve the yield target. The crop could not utilize the applied N at later stages, and a portion of it was lost from the soil–plant system as noted by Ali et al. (2015).

Based on the various splitting of N guided through NDVI sensor-GS, there was much variation in overall N management strategies. In some treatments, there were four splitting of N including the basal application during both years of experimentation though the total amount of N added to the crop varied between 118 and 142 kg ha^{-1}. The detailed doses and splitting of N under various treatments, GS-guided treatments in particular, are presented in Table 3.

The estimated yield with GS-based N management was compared with the actual yield obtained under the specified treatment. This comparison could be helpful to assess the actual performance of the crop in relation to various doses and splits of N application, which in turn would validate the findings. Table 4 presents that the maximum grain yield (4.957 and 5.068 t ha^{-1} during 2017–2018 and 2018–2019, respectively) was achieved with the treatment in which 60 kg N ha^{-1} was applied as basal+60 kg Nha^{-1} at CRI +GS-based N application at 45 and 65 DAS (T8). As predicted from the GY–INSEY relation, the actual yield was very close or slightly higher than 5 t ha^{-1}, which validated the robust relation between INSEY and grain yield. In the treatment in which lesser N was applied (T2, T5, and T6), the grain yield was also recorded lower. The actual yields recorded under farmers' fields in the third year also reflected the superiority of GS-guided treatment T8 (data used in INSEY–GY relation Figures 1A, B. It was noted that higher N application during CRI stages followed by further application based on NDVI sensor recorded good yields as compared with lower doses of N application at CRI.

Higher amounts of fertilizer N than the required amount will certainly lead to higher N losses and lower N use efficiency. This GY–INSEY relation suggested that there is a need of

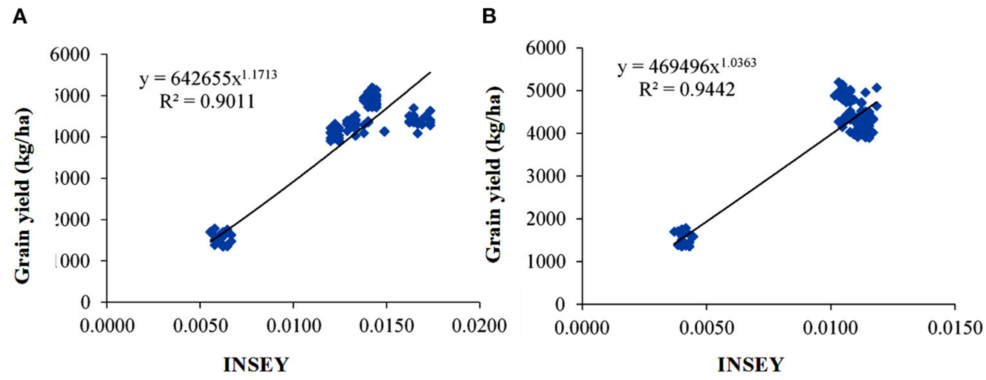

FIGURE 1
*GY–INSEY relationship at **(A)**: 45 and **(B)**: 65 DAS. *2017–2018 and 2018–2019 station data and 2019–2020 farmers' field data were used in developing the relation.

TABLE 3 Nitrogen application at various growth stages under different treatments.

Treatments	2017–18					2018–19				
	Basal	21 DAS	45 DAS	65 DAS	Total	Basal	21 DAS	45 DAS	65 DAS	Total
T1	0	0	0	0	0	0	0	0	0	0
T2	75	37.5	37.5	0	150	75	37.5	37.5	0	150
T3	60	30	30	0	120	60	30	30	0	120
T4	75	75	0	0	150	75	75	0	0	150
T5	40	40	40	0	120	40	40	40	0	120
T6	30	30	44	15	119	30	30	42	16	118
T7	30	60	40	10	140	30	60	35	7	132
T8	60	60	35	0	155	60	60	32	0	152
T9	60	30	42	10	142	60	30	35	5	130
T10	112.5	112.5	0	0	225	112.5	112.5	0	0	225

Treatments are presented in Table 1.

synchronizing fertilizer N application with N demand of the crop and N supply from all sources other than fertilizer. In-season estimation is essential for setting up strategies to optimize nitrogen fertilization and reduced environmental risks associated with fertilizer N use (Yao et al., 2012). Several studies indicated the reliability of spectral measurements in estimating the yields of many crops (Feng and Yang, 2011; Ali et al., 2014, 2015, 2018).

3.5. Total *N* uptake and NUE

Total N uptake varied significantly with respect to various N scheduling during both years of experimentation (Table 4). It was ranging between 31.4 to 103.6 kg ha^{-1} and 23.9 to 108.8 kg ha^{-1} during 2017–2018 and 2018–2019, respectively. The maximum uptake was recorded in N-rich treatment followed by GS-guided T8 treatment (60 kg ha^{-1} applied basal+ 60 kg N ha^{-1} at CRI and two GS-based applications at 45 and 65 DAS), being statistically at par with each other. In general, in the treatment in which higher doses of N were applied, the total uptake was on the higher side. It was due to higher absorption and translocation of the absorbed N, thereby to the grain part under the treatments in which a higher amount of N was applied; due to higher yields as well as higher N percentage in plant parts, the total uptake was higher. Kumar and Yadav (2005) reported that the time of N application had a significant effect on nitrogen uptake by wheat. The split application of N after the first irrigation significantly increased the N uptake. A significant increase in N uptake in 3-split application over 2-splits was noted at Ludhiana, India (Mattas et al., 2011). While studying the relationship between N uptake and grain yield, Ali et al. (2015) found a weak relationship at early stages due to lower N uptake. However, at later stages, they reported a robust relationship.

With increasing rates of nitrogen application, there was a gradual decline in agronomic NUE and physiological efficiency (PE) ensuing in the law of diminishing return. Increasing the doses of N from 120 to 150 kg ha^{-1} as well as N-rich plots showed lower agronomic NUE and PE (Table 4). N application at 120 kg

TABLE 4 Grain yield, total biomass production, and NUE of wheat as influenced by various nitrogen scheduling.

Treatments	Grain yield (t ha^{-1})		Total Biomass (t ha^{-1})		Total *N* uptake (kg ha^{-1})		Agronomic efficiency (kg grain kg^{-1} of *N*)		Physiological efficiency (kg grain kg^{-1} of *N* uptake)	
	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19
T1	1.757d	1.353d	4.92d	3.74d	31.4e	23.9e	-	-	-	-
T2	4.725ab	4.720ab	11.57b	11.32ab	93.9abc	94.8b	19.79ab	22.45ab	106.4b	106.9b
T3	4.296bc	4.098c	11.24bc	10.37b	84.6cd	79.6d	21.16a	22.88ab	118.9a	119.0a
T4	4.527b	4.700ab	11.23bc	11.61ab	89.4bcd	93.9bc	18.47b	22.31b	108.5ab	112.6a
T5	4.140c	4.125c	10.43c	10.52b	80.0d	80.9d	19.86ab	23.10ab	113.3a	119.0a
T6	4.148c	4.030c	10.50c	9.95bc	80.6d	76.9d	20.09ab	22.69ab	113.4a	117.4a
T7	4.523b	4.355bc	11.44c	11.10b	91.0bcd	84.9cd	19.76ab	22.74ab	109.4ab	120.7a
T8	4.957a	5.068a	12.04ab	12.66a	97.6ab	99.9ab	20.65a	24.44a	109.3ab	110.9a
T9	4.450bc	4.385bc	11.22c	11.06b	89.6bcd	84.8cd	18.96b	23.32a	108.2ab	120.3a
T10	4.513b	4.637b	13.11a	13.40a	103.6a	108.8a	12.25c	14.60c	113.3a	113.8a
Significance level	**	**	**	**	**	**	**	**	**	**

Treatments are presented in Table 1.
**Significance at 1% level of probability.
Within a column means followed by the same letter is not significantly different using Tukey's HSD test.

TABLE 5 Production economics of wheat as influenced by various nitrogen scheduling.

Treatments	Total cost of cultivation (₹ha^{-1})		Gross return (₹ha^{-1})		Net return (₹ha^{-1})		B:C ratio	
	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19
T1	42955a	42955a	31626d	24354c	−11329d	−18601d	0.74e	0.57e
T2	45430a	45430a	85050ab	84960ab	39620ab	39530ab	1.87b	1.87b
T3	44935a	44935a	77328bc	73764b	32393bc	28829c	1.72c	1.64cd
T4	45430a	45430a	81486abc	84600ab	36056b	39170ab	1.79bc	1.86b
T5	44935a	44935a	74520c	74250b	29585c	29315c	1.66d	1.65cd
T6	44919a	44902a	74664c	72540b	29745c	27638c	1.66d	1.62d
T7	45265a	45133a	81414abc	78390b	36149b	33257bc	1.80bc	1.74c
T8	45513a	45463a	89226a	91224a	43713a	45761a	1.96a	2.01a
T9	45298a	45100a	80100abc	78930ab	34802b	33830bc	1.77c	1.75c
T10	46668a	46668a	81234abc	83466ab	34566b	36798b	1.74c	1.79bc
Significance level	**	**	**	**	**	**	**	**

1₹ = 0.013 US$; Treatments are presented in Table 1.
**Significance at 1% level of probability.
Within a column means followed by the same letter is not significantly different using Tukey's HSD test.

ha^{-1} recorded maximum agronomic and physiological efficiency whether it was applied in two or three splits; among the GS-guided treatments, T8 was comparable with lower doses of N application in terms of NUE. The increase in N use efficiencies was due to the production of similar yields while using lesser N. GS-guided N fertilizer application could effectively manage to avoid yield losses with moderate rates of N fertilizer application. In N-rich plots, the biological yield was very high; hence, despite very high N uptake, the PE value was higher. Haile et al. (2012) reported that yield increased sharply with increasing N application rates, but NUE significantly decreased with increasing N rates. The values indicated that the conversion of applied N to yields was higher at lower levels of N application. Higher NUE in wheat with lesser N application in the Eastern sub-Himalayan plains of West Bengal, India, was reported by Mondal et al. (2018). The study also revealed that the application of N based on an NDVI sensor with more splits was more efficient as indicated by higher values of agronomic efficiency as compared with recommended doses whether it was applied in two or three splits. The NUE in wheat was improved by more than 15% when N fertilization was based on INSEY calculated from optically-sensed NDVI (Raun et al., 2002).

3.6. Production economics

The total cost of cultivation did not vary significantly among various levels of the N schedule. As all other components of the cost of cultivation except N fertilizer were constant for all the treatments (even in control), the treatment in which maximum N was used resulted in the maximum cost of cultivation, and there was not much variation in the total cost of cultivation between treatments. In our experiment, the treatment, in which 225 kg ha^{-1} of N was applied (T10), recorded the maximum cost of cultivation. There was a significant difference in both gross and net returns achieved with various treatments during both years of experimentation as a huge variation in yields was noted under various treatments. The maximum gross returns, as well as the net returns, were achieved with GS-guided T8 treatment which was significantly higher over all the treatments studied in the experiment (Table 5).

In this split treatment (T8), there was no requirement for extra N application at 65 DAS as reflected by the NDVI sensor though it reflected maximum yields. Mitra et al. (2014) reported higher net return (₹17,955 ha^{-1}) in ZT wheat with 150 kg N ha^{-1} under sub-Himalayan plains of India as compared with the application of 100 kg N ha^{-1} (₹11,545 ha^{-1}) and 125 kg N ha^{-1} (₹14,650 ha^{-1}). As far as the benefit-to-cost ratio (B:C) was concerned, a significantly higher B:C was achieved with T8 (1.96 and 2.01 during 2017–2018 and 2018–2019, respectively). It was followed by the treatment T2 in which the recommended dose of N (150 kg ha^{-1}) was applied in three splits (50% basal and 25% each at CRI and tillering). A dose of 60 kg N ha^{-1} as basal+ 60 kg N ha^{-1} at CRI followed by N application guided through GS could fetch higher profitability over the recommended doses of N application. A higher B: C (1.96) in ZT wheat with 150 kg N ha^{-1}over 100 kg N ha^{-1} (1.64) and 125 kg N ha^{-1} (1.80) under Eastern sub-Himalayan plains was previously reported by Mitra et al. (2014).

4. Conclusion

It can be concluded from the experimental findings that the application of the NDVI sensor could be an important tool for the optimization of fertilizer nitrogen in wheat grown under the Eastern plains of India. A prescriptive dose of 60 kg N ha^{-1} as basal + 60 kg N ha^{-1} at CRI followed by NDVI sensor-guided N application (at 45 and 65 DAS) brought about a significant improvement in yield performances and N use efficiencies with higher net returns and B:C. There should not be any curtailment in N doses at basal and CRI (60 kg N ha^{-1} each); any curtailment in these doses followed by N application through NDVI sensor at later stages could not bring about the target yield (5 t ha^{-1}) of the crop. The new INSEY–GY relation developed through this trial could successfully be used for fair yield prediction in the Eastern plains of India.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

BM, PS, AR, and AS: conceptualization, methodology, writing—original draft preparation, and visualization. MS, AL, SA, and AH: formal analysis, data curation, statistical expertise, and writing—reviewing and editing. BM and AS: supervision. SA and AH: funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Regulation of microbial community structure, function, and nutrient of apple orchard soil by interplanting red clover

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Soil microbial communities are seen to be critical to the maintenance of soil health and quality. Many studies have suggested that soil microbial community composition is affected by different plant species. Based on this theory, we tried to improve the apple orchard soil microorganisms and nutrients by interplanting leguminous forage red clover (RC), meanwhile taking the natural mixed herb (NMH) naturally growing in the orchard as a control. The metagenomic analysis showed that interplanting red clover in the apple orchard markedly improved the soil microbial community composition, especially compared with natural mixed herbs, the abundance of *Nitrospirae* and *Glomeromycota* was higher. Compared with genus level, there are more unique bacteria species in RC, 84 species were unique to treatment (RC) soil and 43 species were unique to control group (NMH). The activities of glycoside hydrolase and carbohydrate-binding modules in RC were significantly higher than those in NMH, while compared with the control group (NMH), the activity of glycoside hydrolase and carbohydrate binding module in the treatment group (RC) was significantly higher, but the activity of glycosyl transferase was lower. In addition, only 5 of the 15 virulence factors they contain were lower in the RC, while the rest were higher in the control group. Based on the above results, we speculate that intercropping red clover can not only significantly improve soil quality, soil microbial community composition and soil fertility, but also effectively improve land use efficiency and reduce the use of chemical fertilizers.

KEYWORDS

interplanting, metagenomic analysis, orchard soil, red clover, soil microorganism

1. Introduction

Microorganisms drive all major biogeochemical cycles on Earth, and have profound effects on all other organisms (and their habitats) due to their different roles as pathogens, symbiotes, biotrophic converters, decomposing agents, and primary food sources (Stott and Taylor, 2016). The enormous metabolic diversity of soil microorganisms means that their activities play a driving or promoting role in the circulation of all major elements (such as C, N, and P), thereby affecting the structure and function of soil ecosystems and the ability of soil to provide services to people (Aislabie and Deslippe, 2013). Soil microbial community composition can be affected by different plant species (Grayston and Campbell, 1996; Grayston et al., 2001). This phenomenon has been demonstrated by a variety of methods, including characterization of microbial biofilms (Bossio et al., 1998; Pierra et al., 2015), DNA gene map (Duarte et al., 1998; Chen et al., 2016), and metabolic characteristics (Degens and Harris, 1997; Bowles et al., 2014). Nutrients required by microorganisms are usually derived from plant litter and root exudates (Grayston et al., 1998). Plants affect soil organic carbon stocks and short-term carbon cycling (Ward et al., 2009) through differences in the quantity and quality of root exudates and litters (Coleman et al., 2000; Deyn et al., 2008), thus affecting microbial community composition and function. Soil enzyme activities related to microbial function are also affected by plant species (Waldrop et al., 2000). The coexistence of different plant functional groups (e.g., gramineae and legume) plays a particularly important role in ecosystem function (De Deyn et al., 2009). Intercropping with leguminous crops can utilize the complementarity between different species to improve the utilization efficiency of water, sunlight, air, and nutrient resources, increase nutrient availability and soil quality, thereby increasing fertility and improving the growth performance of the two intercropping crops in the agricultural system (Tsubo et al., 2005; Monteiro and Lopes, 2007; Massawe et al., 2016). Among mixed grass coverts, leguminous grass species have the greatest influence on microbial function, mainly through roots (Breulmann et al., 2012). According to existing research, utilizing diversity planting such as intercropping can not only change the composition of plant rhizosphere microbial communities, but also help improve the abundance of beneficial microorganisms in the rhizosphere (Zhou et al., 2023). Although soil microbial community structure is generally considered to be limited by carbon nutrition, many studies suggest that it may also be limited by nitrogen nutrition (Chen and Stark, 2000). Schimel et al. (2005) found that nitrogen requirements seem to change the ratio between different microbial populations. Leguminous plants can obtain nitrogen by association with rhizobia or Frankia via differentiation on their respective host plants of a specialized organ, the root nodule (Franche et al., 2009).

Orchard grass mulch is a method of orchard management in which perennial herbs are planted as mulch between rows or throughout the orchard (Almeida et al., 2011; Qian et al., 2015). The soil microbial communities (SMC) in the vast majority of hot and arid regions of the Earth's grasslands are mainly composed of 4 types of feed legumes, namely, alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), underground clover (*T. subterranum* L.), and white clover (*T. repens* L.; Zhou et al., 2017). The related changes of root development and root deposition during the growing season may change the spatial distribution and quality of organic matter (Philippot et al., 2013a,b), thus affecting the dynamics of microbial community over time. Leguminous forage can improve soil microbial community because of its nitrogen fixation function, and nitrogen is the basic element of microbial metabolism. Jiao et al. (2013) found that interplanting red clover in apple orchards could improve the functional diversity of soil microorganisms through Biolog Eco-Plate™. The decomposition of red clover stems and leaves cut into the soil in orchards can affect the abundance and proportion of soil bacteria and fungi (Zhou et al., 2019).

We assumed that planting a leguminous forage variety called red clover (RC) in an apple orchard can improve the soil microbial community and chemical parameters of the orchard. To verify this hypothesis, we planted RC in the orchard and used natural mixed herbaceous plants (NMH) as controls. In addition, the soil microbial community structure, carbohydrate enzyme activity and virulence factor abundance were also analyzed by using the metagenome technology. By comparing the differences between treatment and control, the improvement effect of planting red clover on the composition and function of soil microbial community was evaluated. In addition, we evaluated the differences between stem and leaf yields and soil chemistry parameters. This study explores a method to maintain the basic fertility of orchard soil, reduce fertilizer use, and improve soil ecological environment, laying the foundation for sustainable and efficient production in orchards.

2. Materials and methods

2.1. Experimental site and layout

The experiment was conducted at the National Modern Agriculture Demonstration Base of Heilongjiang Academy of Agricultural Sciences (45°84′N, 126°84′E), located in east of Harbin, Heilongjiang Province, China. The soil type of the apple orchard is an chernozem soil (pH, 6.4; soil organic matter, 5.41%; total nitrogen 1.5 $g\,kg^{-1}$; total phosphorus 332.9 $\mu g\,kg^{-1}$; total potassium 15.2 $g\,kg^{-1}$; available nitrogen 14.6 $mg\,kg^{-1}$; available phosphorus 26.2 $\mu g\,g^{-1}$; available potassium 35.4 $mg\,kg^{-1}$).

The apple cultivar "zhenxing1" seedlings, the rootstock is *Malus baccata*, were planted for orchard construction, and with east–west direction and plant row spacing of 1.5 m × 4.0 m. In the spring of 2019, the red clover (*Trifolium pratense* L.) was sown between rows of fruit trees as experimental treatment (RC). Before sowing, weeds were removed from the test plot, turned 20 ~ 25 cm deep, applied 5 $kg{\cdot}m^{-2}$ of high-quality organic fertilizer, mixed the soil fertilizer evenly, and then used a rake to smooth the land. Meanwhile native mixed herbs (NMH) growing naturally in the orchard, which is a common applied method, was set as the control. Each treatment area was 60 m^2 with four replicates, 240 m^2 in total. Red clover treatment was consistent with other orchard management practices. Other management measures such as fertilizer and water were consistent between the treatment group and the control group.

2.2. Measurement of yield of herb stems and leaves

According to the orchard field management calendar, the herb was mowed three times on June 19, July 27 and September 20, 2020, and the yield of herb stems and leaves was measured at the same time. A 3 m × 1 m square was randomly drawn in the test plot to measure the yield, and the stubble height was 5 cm. The herb stems and leaves cut were collected and weighed to measure the fresh weight. The fresh herb stems and leaves was killed at 105°C for 30 min, and then dried in oven at 70–80°C until the weight did not change, in this way the dry weight was measured. Three repeated measurements were taken.

2.3. Soil sampling and analyses

We collected soil samples in September 2020. Five sampling sites were selected according to "S" shape, respectively, for planting red clover treatment and native mixed herbs control treatment. After removing about 1 cm of the soil surface, samples were collected from different surface treatments at the sampling points using a soil drill that had been sterilized with 70% ethanol. The mixture was evenly mixed, and the soil depths were 0–20 cm, 20–40 cm, and 40–60 cm, respectively. Placed the soil sample in a plastic bag and evenly sieved it (2 mm) to remove debris such as crushed stones, stems, leaves, and dead roots from the soil.

Five gram soil from each sample was extracted into 10 ml sterile centrifuge tubes, stored in liquid nitrogen and brought back to the laboratory, sealed in dry ice, and then sent to MajorBio for metagenomic analysis of soil microbial communities. After DNA extraction, fragmentation, PE library construction and bridge PCR, sequence was performed using Illumina platform. After the quality control, assembly, prediction and redundancy removal of the original data, the non-redundant gene set was obtained. The non-redundant gene set was compared with the selected species or function database to obtain the species or function annotation information corresponding to each sample. Based on the annotated species or functional information, subsequent species composition analysis, samples comparison analysis, species difference analysis, correlation and model prediction analysis, etc. was carried out for these samples.

Setting the ratio of water to soil to 5:2, 10 g soil sample and 25 ml deionized water were added into a 50 ml tall beaker, and fully mixed with a glass rod. After standing for half an hour, the supernatant was poured into a small beaker with a capacity of 20 ml, and the pH meter (Cole-Parmer ph700) was used to determine the pH value of the soil. Each sample was measured 3 times repeatedly. After measuring 5–6 samples, recalibrated the pH meter with standard buffer solution. The $K_2Cr_2O_7$ oxidation method was used for determining the soil organic matter (Nelson and Sommers, 1982). Soil total nitrogen (TN) was measured by the Automatic kjeldahl apparatus method (Meseguer Lloret et al., 2005). Soil total phosphorus (TP) was measured by the Molybdenum blue method (Stauffer, 1983). Soil total potassium was measured by the Sodium hydroxide melting process method. The available phosphorus (AP) in soil was determined by Olsen method (Recena et al., 2022). After extracting NH_4OAc (1.0 mol/L), the available potassium (AK) in the soil is determined by a flame photometer (Xu et al., 2020). Mineral N was extracted from soil with 2 M KCl and quantified using a flow injection analyzer (Westco Scientific Ltd., Brookfield, CT, USA; Engedal et al., 2023).

2.4. Statistical analysis

The data processing and analysis of metagenomic was carried out in the MajorBio data analysis platform.[1] The significance of the differences between the treatment group and the control group analyzed by SPSS 19.0 software (SPSS Co., Ltd., Chicago, Illinois, USA).

3. Results

3.1. Comparison of yield of herb stems and leaves

Herbaceous stems can be used as soil green fertilizers, therefore, their yield can be used as a major indicator for evaluating the quality of herbaceous plants in orchards. In this study, we measured the yield of herb stems and leaves on June 19, July 27 and September 20, respectively, in 2020 (Table 1).

Compared with the first mowing yield measured on June 19, the fresh weight and dry weight of red clover (RC) were significantly ($p < 0.05$) higher (2.1 times) than those of native mixed herbs (NMH). Compared with the second mowing yield measured on July 27, there were no significant ($p > 0.05$) differences in both fresh weight and dry weight between RC and NMH. Compared with the third mowing yield measured on September 20, the fresh weight and dry weight of stems and leaves of RC were significantly ($p < 0.05$) higher (1.8 times) than that of NMH. Compared with the total annual yield, the fresh weight and dry weight yield of RC were significantly ($p < 0.05$) higher (2.4 times) than those of NMH. In summary, compared to NMH, the stem and leaf yield of red clover was higher during the same period. Moreover, according to field observation, red clover has the characteristics of early greening, late dormancy, robust growth and high coverage. These factors combine to make it have the characteristics of high yield of stems and leaves. In addition, according to the results of three yield measurements, red clover showed better growth in spring and autumn, indicating that it is more adaptable to cool climate.

3.2. Comparison of soil microbial community composition at phyla level

Figure 1A showed the annotated column diagram of horizontal species in the NR (RefSeq non-redundant proteins: Non-redundant protein sequences from GenPept, Swissprot, PIR, PDF, P) database. The abundance of bacteria, fungi and viruses in soil samples of red clover treatment and native mixed herbs control were visually studied by using the visualization method of the column diagram.

The results showed that the microbial community composition was significantly different between the red clover treatment group (RC_1, RC_2, and RC_3) and the natural clover control group (NMH_1, NMH_2, and NMH_3), which indicated that the soil microbial community composition was affected by different herb varieties in the orchard. In particular, the abundance of *Nitrospirae* in the red clover soil is 1.4 times higher than that of the native mixed herbs soil. The *Nitrospirae* is a class of Gram-negative bacteria, among which *Nitrospira*, as the *Nitrifier*, can oxidize nitrite into nitrate. Planting red clover in orchard can effectively improve the abundance of soil nitrogen-fixing bacteria. Figure 1B analyzed the significant differences of 15 dominant phyla with the highest abundance between treatment and control ($p < 0.05$). The abundance of 7 phyla was significantly higher in the treatment group, including: *Proteobacteria*, *Gemmatimonadetes*, *Nitrospirae*, *Candidatus_Rokubacteria*, *Bacteroidetes*, *Candidatus_Tectomicrobia*, and *Planctomycetes*. The remaining 8 phyla are more abundant in the control group, including: *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *Thaumarchaeota*, *Firmicutes*, unclassified bacteria, *Verrucomicrobia* and *Euryarchaeota*. In conclusion, planting red clover had significant effects on 15 dominant phyla with the largest abundance.

3.3. Heatmap analysis

A heatmap analysis of phyla level microorganisms was conducted for the treatment soil samples (RC_1, RC_2 and RC_3) and control

1 https://login.majorbio.com/passport/login/email

TABLE 1 Comparison of yield of herb stems and leaves between red clover and native mixed herbs.

Treatment	The first cut (June 19, 2020)		The second cut (July 27, 2020)		The third cut (September 20, 2020)		Annual total production	
	Fresh weight (kg·m^{-2})	Dry weight (kg·m^{-2})	Fresh weight (kg·m^{-2})	Dry weight (kg·m^{-2})	Fresh weight (kg·m^{-2})	Dry weight (kg·m^{-2})	Fresh weight (kg·m^{-2})	Dry weight (kg·m^{-2})
RC	4.63.6 ± 0.05a	1.56 ± 0.02a	2.14 ± 0.03a	0.72 ± 0.01a	1.49 ± 0.02a	0.50 ± 0.01a	8.26 ± 0.01a	2.78 ± 0.01a
NMH	0.60 ± 0.05b	0.21 ± 0.01b	2.09 ± 0.03a	0.70 ± 0.01a	0.79 ± 0.01b	0.27 ± 0.01b	3.47 ± 0.01b	1.19 ± 0.01b

The data in the table are the mean ± standard deviation of three measurements in different treatments. The different lowercase letter in the same column meant there was a significant difference at the 0.05 level. RC, red clover; NMH, native mixed herbs.

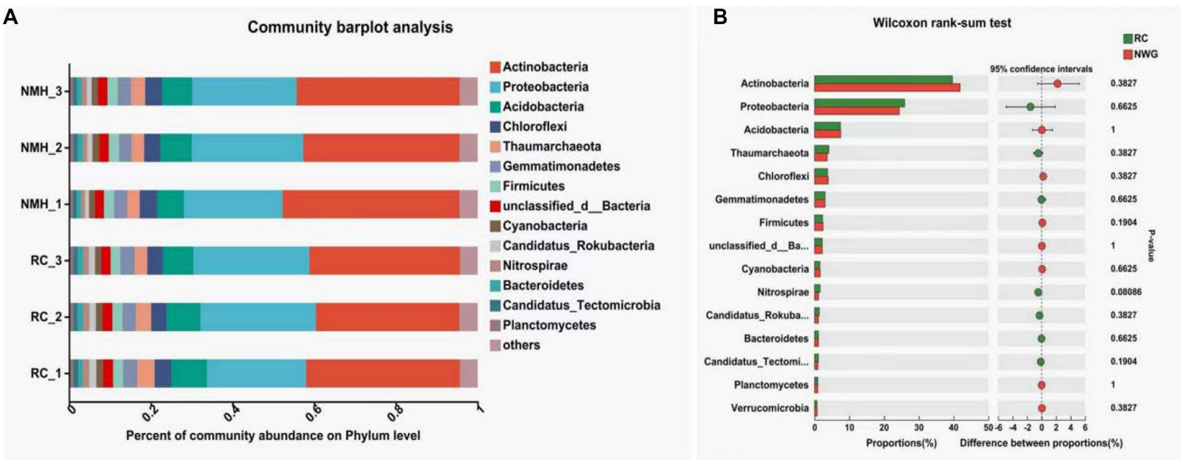

FIGURE 1
Phyla level of microbial community composition of orchard soils. **(A)** Community barplot analysis. **(B)** Wilcoxon rank-sum test.

soil samples (NMH_1, NMH_2 and NMH_3) of the native mixed herbs control (Figure 2). The heatmap uses hierarchical clustering analysis to cluster species with high and low abundance. Color gradient and similarity degree reflect the similarity and difference of species composition in multiple samples communities.

According to Figure 2, the *Glomeromycota* abundance of soil samples planted with red clover for 3 repeats (RC_1, RC_2, and RC_3) had a much higher abundance of Glomeromycota compared to the control group (NMH_1, NMH_2, and NMH_3). Although *Glomeromycota* is a phylum of fungi of which only about 400 species have been found, it can form Arbuscular mycorrhizas with terrestrial plants. This symbiotic structure can assist plants in the absorption of inorganic salts, especially phosphorus, from the soil and is considered to have been an important key for plants to adapt to the terrestrial environments in the early stage. In the present study, planting red clover in the orchard increased the abundance of the *Glomeromycota*, which theoretically indicated that planting red clover can improve the soil microbial community composition and function of the orchard.

3.4. Comparison of soil microbial community composition at genus level

In Figure 3A, Venn diagram was used to analyze the common and unique microbiome genera in different soil treatments, which can intuitively show the similarity and overlap of soil microbial community composition. As it can be seen from Figure 3A, in the treatment group (RC) and the control group (NMH), there were 2,461 species of microorganisms belonging to the same genus, 84 species were unique to treated soil and 43 species were unique to control group. In conclusion, planting red clover in orchards can not only increase the variety of bacteria, but also make orchards have more unique bacterial species. In this study, principal component analysis was used to reflect the degree of difference between the treatment group and the control group at the generic level, which were shown in Figure 3B. The greater the distance between points, the greater the difference. As can be seen from Figure 3B, the distance between the treatment group and control group was significantly different, indicating that planting red clover in the orchard had a great effect on soil microbial community composition.

3.5. Comparison of carbohydrate active enzyme activities of soil microorganisms

Carbohydra-active enZYmes (CAZy) Database[2] is a professional database on the synthesis and breakdown of complex carbohydrate and sugar complex enzymes. According to the similarity of amino acid sequences in protein domain, carbohydrate active enzymes from

2 http://www.cazy.org/

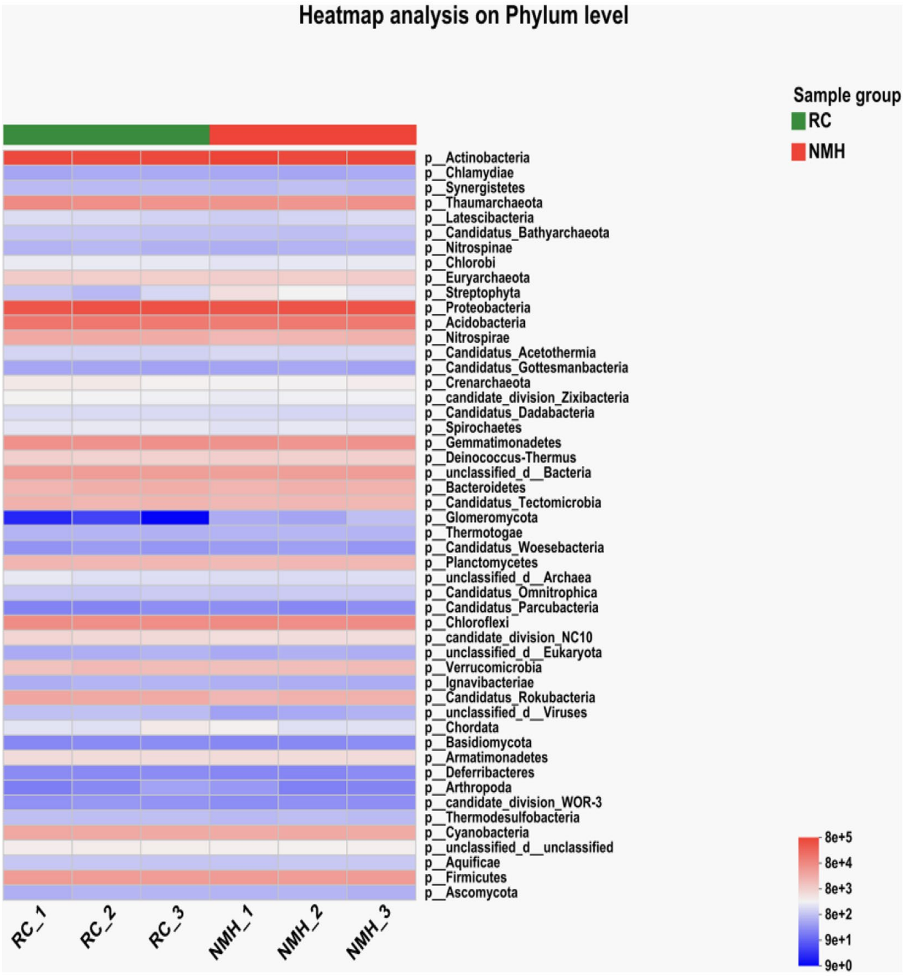

FIGURE 2
Phyla level comparison of soil microbial community composition by heatmap analysis.

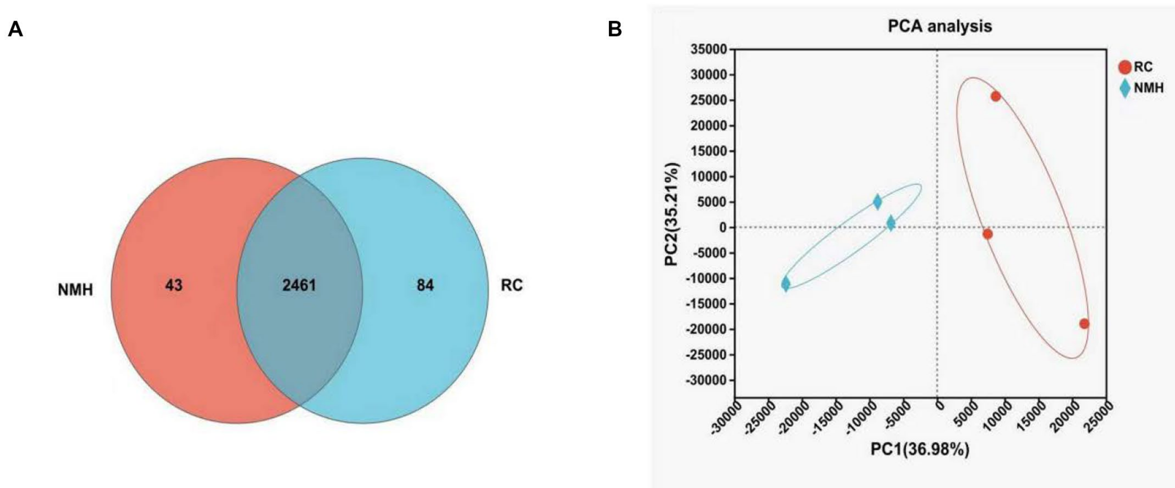

FIGURE 3
Comparison of soil microbial community composition between interplanting red clover and native mixed herbs in orchard at the genus level. **(A)** The similarity and overlap of soil microbial community composition. **(B)** PCA analysis.

different sources could be divided into six types of enzymes, namely glycoside hydrolase, glycosyl transferases, polysaccharide clease, carbohydrate esterase, carbohydrate-binding modules and auxiliary REDOX reductase. Compared the activity of soil carbohydrate active enzymes in the treated group (RC) and the control group (NMH) in the CAZy database. In the soil samples of this study, the activities of three type enzymes, namely glycoside hydrolase, glycosyltransferase and carbohydrate binding module, were higher of all six type enzymes (Figure 4). The activities of glycoside hydrolase and carbohydrate-binding modules in RC were significantly higher than those in NMH, while the activities of glycosyl transferases were significantly lower than those in NMH (Figure 4).

3.6. Comparison of soil microbial virulence factors

In this study, virulence factor genes were counted by comparing virulence factor database,[3] and the difference significance analysis was conducted for the top 15 virulence factors with the highest abundance (Figure 5). Of these, 10 virulence factors were higher in the control group (NMH), and only 5 were higher in the treatment group (RC). In general, planting red clover in orchards has a positive effect on reducing soil microbial virulence factors.

3.7. Comparison of soil nutrients

According to the results shown in Figure 6A, when the soil depth was 0–20 cm and 40–60 cm, the soil pH value of the treatment group and the control group was almost the same, but when the soil depth was 20–40 cm, the pH value of the control group was higher. For soil organic matter content, soil at depths of 0–20 cm and 20–40 cm contained more organic matter in the treatment group, while the organic matter content in soil at 40–60 cm depth were not significantly different from those in the control group (Figure 6B). It can be seen that the organic matter content of the soil in the orchard increased after planting red clover. The total nitrogen content of soil at depths of 0 to 20 cm and 20 to 40 cm was much higher in the treatment group, but the soil nitrogen content in the two groups at depths of 40 to 60 cm was almost the same (Figure 6C). The available nitrogen content in 0–20 cm and 20–40 cm soil depth in treatment group was significantly higher than that in control group, while the available nitrogen content in 40–60 cm soil depth was significantly lower than that in control group (Figure 6D). In general, planting red clover increased the nitrogen content of orchard soil. There was no significant difference in total P content between the treatment group and the control group at three depths (Figure 6E). Soil at a depth of 20–40 cm contained more available P in the treatment group, but at depths of 0–20 cm and 40–60 cm, the two groups of soil contained similar available P (Figure 6F). There was no significant difference in total potassium content between the treatment group and the control group at three depths (Figure 6G). The available potassium content in soil at 0–20 cm and 40–60 cm depth in treatment group was significantly lower than that in control group, while the available potassium content in soil at 20–40 cm depth was not significantly different from that in control group (Figure 6H).

4. Discussion and conclusion

4.1. Improvement of microbial community composition and function

This work provided evidence that growth of red clover has a major impact on microbial community structure in soils (Figures 1, 3). In particular, the abundance of Nitrospirae (Figure 1), which has the effect of nitrogen fixation, and the abundance of the beneficial fungus Glomeromycota were significantly increased (Figure 2). Nitrospirae is important bacteria in soil nitrogen cycle, usually involved in nitrite oxidation (Attard et al., 2010; Florine et al., 2017). Liu et al. (2019) showed that Alpha diversity of surface soil fungi increased significantly 30 years after grassland restoration. In this study, the Glomeromycota abundance in orchard soil after 2 years of intercropping red clover was significantly higher than that in native mixed herbs soil. Although Glomeromycota is a phylum of fungi of which only about 400 species have been found, it can form Arbuscular mycorrhizas with terrestrial plants (Brundrett, 2002). More than 80% of vascular plants have arbuscular mycorrhiza composed of bulbous bacteria, and arboreal mycorrhiza can also be found in bryophytes and other plants without real root structure, indicating the great significance of arbuscular mycorrhizas in terrestrial ecosystems (Schwarzott et al., 2001). It is concluded that intercropping red clover has a significant effect on improving the community and function of fungi. Soil microorganisms

3 https://www.mgc.ac.cn/VFs/

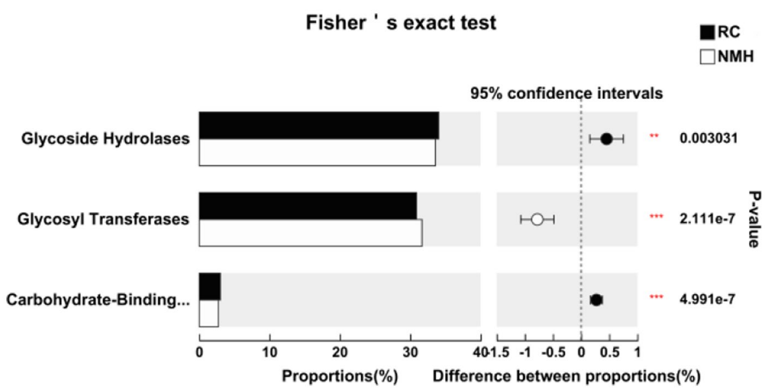

FIGURE 4
Comparison of soil microbial carbohydrate active enzyme activities between planting red clover and growing natural mixed herbs in orchard.

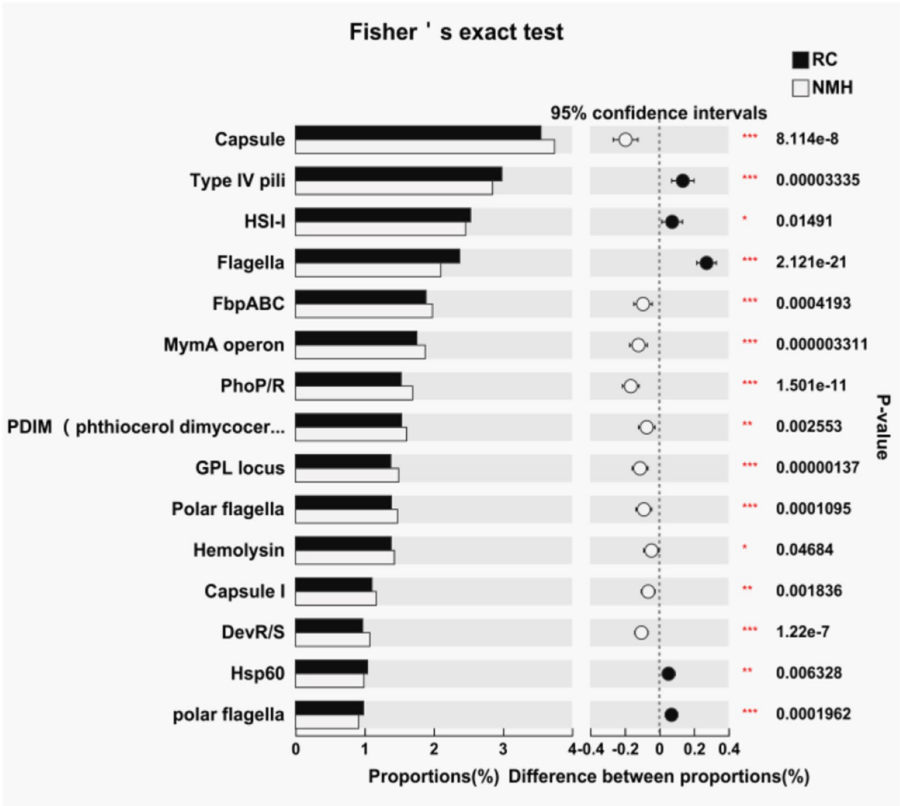

FIGURE 5
Comparison of soil microbial virulence factors between interplanting red clover and native mixed herbs in the orchard.

as indicators of soil quality are an important part of soil (Cui et al., 2021). Existing studies have shown that planting grass in orchards can effectively improve the ground temperature and air relative humidity, enhance soil water retention (Huang et al., 2014), and improve soil physical and chemical properties (Merwin et al., 1994). These changes will directly affect the environmental conditions for the survival of soil microorganisms. At the same time, more plant roots and litters are imported into the soil after planting grass. Their input and decomposition can improve the soil environment, promote the formation and accumulation of soil organic matter, and increase the soil nutrient content (Gomez et al., 2009; Rodrigues et al., 2013). Therefore, the food source and growth environment of soil microorganisms are changed, thus affecting the survival and reproduction ability of soil microorganisms (Zhang et al., 2015). The changes of soil microbial composition and soil enzyme activity were closely related to grass species. It was found that deep root leguminous herbage alfalfa could leave a large amount of root residue in deep soil, which was conducive to improving the urease activity and comprehensive fertilizer supply capacity in deep soil (Manici et al., 2015). Red clover root system was developed and distributed deeply, so it could influence the resource utilization in deep soil (Jiao et al., 2013). On the other hand, the different root exudates of different plants will also have a great influence on the rhizosphere soil microorganisms and enzyme activities (Sturz et al., 2011). Due to the complex physicochemical structure of plant cells, microorganisms usually secrete a large number of degrading enzymes, mainly glycoside hydrolases, lyases, esterases, and helper enzymes to utilize plant residues as an important nutrient source (Gilbert, 2010). In this study, Carbohydrate enzymes were classified according to the CAZy database (Zhong et al., 2018). The activities of glycoside hydrolase and carbohydrate-binding modules in interplanting red clover soils were significantly higher than those in native mixed herbs soils, while the activities of glycosyl transferases were significantly lower than those in native mixed herbs soils (Figure 4). The activity of enzymes related to carbon and nitrogen cycling in soil increased due to grass mulching, usually because the return of organic materials containing carbon and nitrogen to the soil stimulated the activity of these enzymes (Wang et al., 2020). The difference of stem, leaf and root exudates between red clover and native mixed herbs should be the reason for the difference of soil enzyme activity. Virulence factors are derived from microorganisms and can promote their own infection and cause specific host diseases. It mainly includes bacterial toxins, cell surface proteins that regulate bacterial adhesion, proteins that protect bacteria, cell surface carbohydrates and hydrolytic enzymes that are pathogenic to bacteria (Cai et al., 2018). Compared with native mixed herbs, interplanting red clover in orchard effectively reduced the content of virulence factors (Figure 6).

4.2. Improvement of soil nutrients

In this study, interplanting red clover significantly increased soil organic matter content (Figure 6B) and nitrogen fertilizer content (Figures 6C,D). Organic matter has been widely recognized as one of

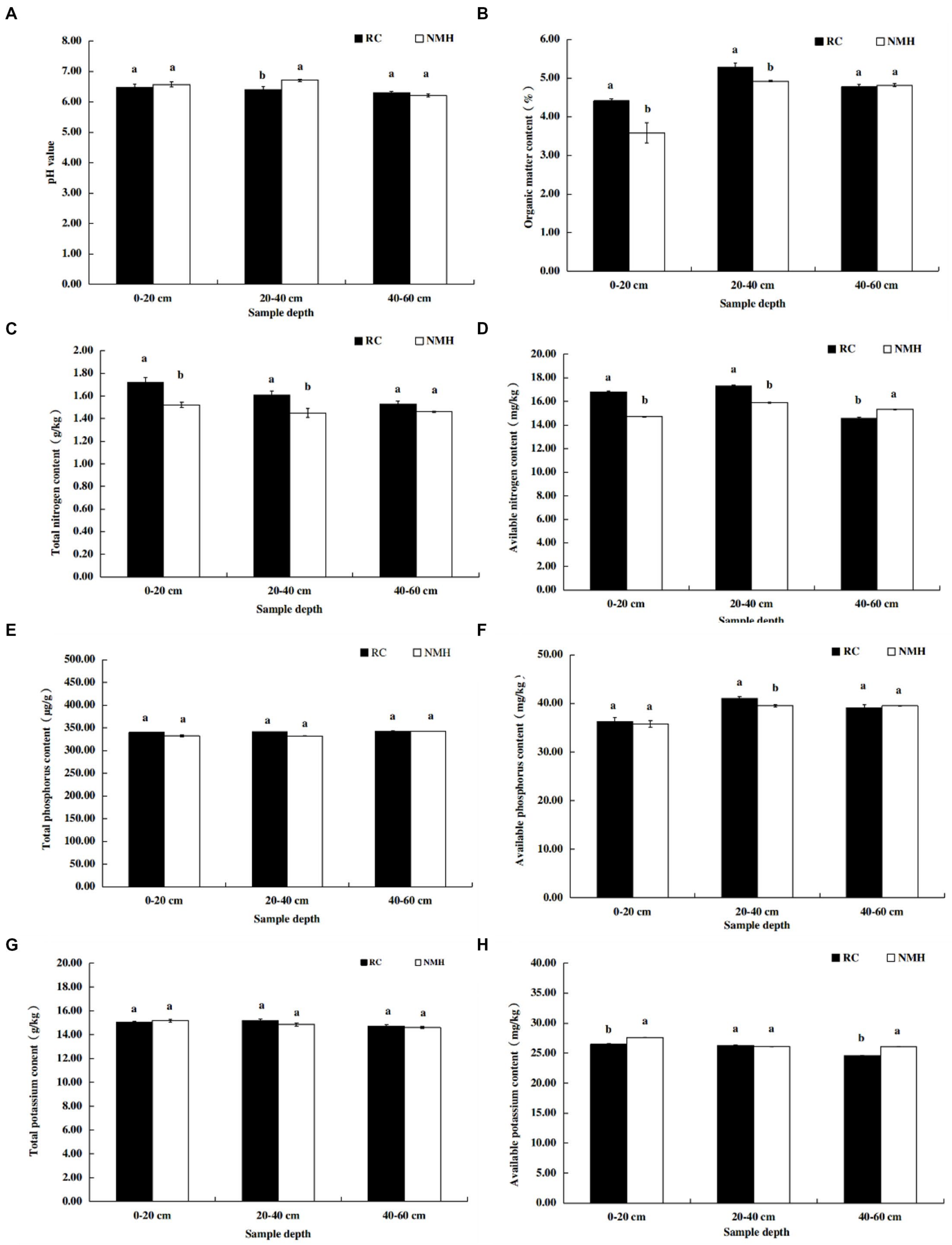

FIGURE 6
Comparison of soil nutrient contents between planting red clover and growing natural mixed herbs in orchard. **(A)** The pH value of soil at different depths. **(B)** Organic matter content of soil at different depths. **(C)** Total nitrogen content of soil at different depths. **(D)** Available nitrogen content in soil layers at different depths. **(E)** Total p content of soil at different depths. **(F)** Available phosphorus content in soil at different depths. **(G)** Total potassium content of soil at different depths. **(H)** Available potassium content in soil at different depths.

the most important indicators of soil quality and farmland fertility (Kleber and Lehmann, 2015), and plays an important role in maintaining ecosystem productivity and sustainability (Liang et al., 2017). Intercropping red clover effectively increased the content of soil organic matter, which may be related to two reasons. On the one hand, red clover has a higher yield of stems and leaves (Table 1), which are decomposed into soil organic matter. On the other hand, the developed root system (Gu et al., 2016) and high quality root exudates (Kong and Six, 2010) of red clover are another reason to increase organic matter content. Interplanting red clover had a positive effect on total and available nitrogen content in apple orchards, consistent with other reported results (Franche et al., 2009; Manici et al., 2015). Stefani et al. (2018) believed that NO_3^- leaching was the main way of nitrogen loss in farmland, and that NO_3^- leaching in soil could be reduced by crop mulching. Nitrogen-fixing rhizobia of red clover can absorb and fix nitrogen in the atmosphere, thereby increasing soil nitrogen levels (Franche et al., 2009).

In summary, the results of this study indicated that intercropping red clover can significantly affect the community structure of soil microorganisms, increase the abundance of Glomeromycota in soil, and improve soil physicochemical properties, which greatly improved the living environment of soil microorganisms. In addition, interplanting red clover can also effectively improve land use efficiency, reduce the content of virulence factor in the soil, increase the content of organic matter, and reduce the use of chemical fertilizers.

4.3. Application prospect and further research

As a special terrestrial ecosystem, soil habitats covered by grass in orchards contain abundant microbial resources. The production of specific microbial functional populations and bioactive substances can play a role in regulating and monitoring the healthy growth of fruit trees and soil quality evaluation. In this study, intercropping of red clover in orchards significantly increased the abundance of *Glomeromycota*, a finding that is worthy of further study. It can be used to isolate, identify and function analysis the fungi and explore the symbiotic relationship between mycorrhizal fungi and fruit roots. In addition, more attention should be paid to the effect mechanism of red clover on soil biological health in orchard. On the one hand, the change characteristics of soil food web and its effect on soil available nutrient supply capacity of this management modes should be studied. On the other hand, it is also necessary to study the mechanism of this management mode on soil pathogenic microorganisms, so as to provide basis for improving the health level of soil and fruit trees. It has guiding significance in improving soil quality, using land efficiently and reducing chemical fertilizer use in terms of application. Breeding industry can be developed based on the excellent herbage production capacity (Table 1) and fertilizer production capacity (Figure 6) of red clover. In order to establish a green production model combining fruit, pasture and livestock, it is necessary to study the carbon cycle and nitrogen cycle mechanism under this model.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

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Author contributions

KJ wrote the draft of the manuscript and contributed to manuscript revision. YW, BG, LZ, YL, and PS contributed to data analysis and visualization. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Soybean crop intensification for sustainable aboveground-underground plant–soil interactions

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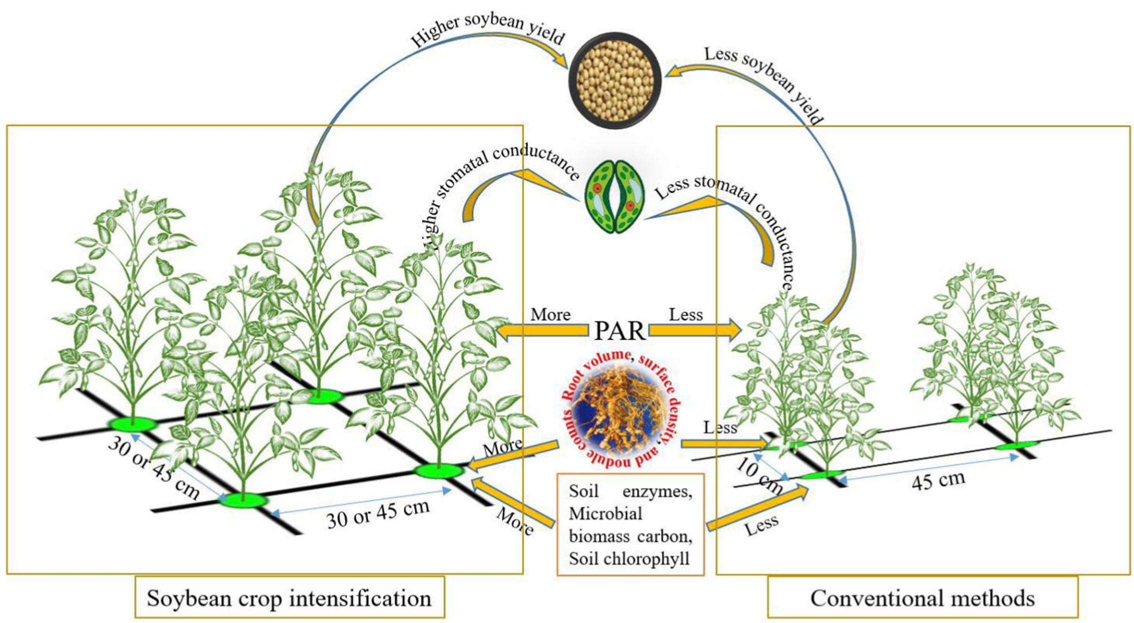
The major challenge of growing soybean, other than unfavorable weather and small farm size, is the non-availability of quality inputs at the right time. Furthermore, in soybean growing regions, crop productivity and soil environment have deteriorated due to the use of traditional varieties and conventional methods of production. Soybean crop intensification or system of crop intensification in soybean (SCI) is an agricultural production system that boosts soybean yields, improves the soil environment, and maximizes the efficiency of input utilization, although the contribution of SCI to crop productivity is not well understood as different genotypes of soybean exhibit different physiological responses. Therefore, a field study was conducted in 2014–2015 and 2015–2016 using three crop establishment methods (SCI at a 45 cm × 45 cm row spacing, SCI at 30 cm × 30 cm, and a conventional method at 45 cm × 10 cm) assisted in vertical strips with four genotypes (Pusa 9,712, PS 1347, DS 12–13, and DS 12–5) using a strip-plot design with three replications. Compared with standard methods of cultivation, the adoption of SCI at 45 cm × 45 cm resulted in a significantly higher stomatal conductance (0.211 mol H_2O m^{-2} s^{-1}), transpiration rate (7.8 mmol H_2O m^{-2} s^{-1}), and net photosynthetic rate (398 mol CO_2 m^{-2} s^{-1}). The implementation of an SCI at 30 cm × 30 cm had significantly greater intercepted photosynthetic active radiation (PAR) (1,249 mol m^{-2} s^{-1}) than the conventional method system, increasing crop yield from 9.6 to 13.3% and biomass yield from 8.2 to 10.7%. In addition, under an SCI at 30 cm × 30 cm, there were more nodules, significantly larger root volume and surface density, and increased NPK uptake compared with the other methods. Significantly greater soil dehydrogenase activity, alkaline phosphatase activity, acetylene-reducing assay, total polysaccharides, microbial biomass carbon, and soil chlorophyll were found with SCI at 45 cm × 45 cm (13.63 g TPF g^{-1} soil $hr.^{-1}$, 93.2 g p-nitro phenol g^{-1} soil $hr.^{-1}$, 25.5 n moles ethylene g^{-1} soil $hr.^{-1}$, 443.7 mg kg^{-1} soil, 216.5 mg kg^{-1} soil, and 0.43 mg g^{-1} soil, respectively). Therefore, the adoption of an SCI at 30 cm × 30 cm and/or 45 cm × 45 cm could provide the best environment for microbial activities and overall soil health, as well as the sustainable productivity of soybean aboveground.

KEYWORDS

seed yield, soil biology, soybean physiology, soybean crop intensification, water productivity



GRAPHICAL ABSTRACT

1. Introduction

The cultivation of traditional/local cultivars and conventional tillage production systems in the Western Indo-Gangetic Plains (IGP) has led to decreased crop productivity (Fatima et al., 2023), soil erosion (Cárceles Rodríguez et al., 2022), nutrient depletion (FAO, 2017; Dutta et al., 2022), and water loss (Jat et al., 2019), resulting in degraded soils (Radosavljevic et al., 2020) with low organic matter content (Singh et al., 2019) and a fragile physical structure (Rajanna et al., 2022). Soybean cultivation using traditional cultivars under conventional crop establishment methods have had the same impact. However, owing to soybean's wide range of uses and health advantages, it is the most widely cultivated oilseed crop worldwide. The production of 369.5 million tons (mt) of soybeans worldwide each year is evidence of the crop's importance on a global scale (USDA, 2018). Owing to its high levels of protein and edible oil content (Dass et al., 2018), soybean products are widely consumed in India and throughout the world. The world's soybean production, yield, and area were 120.4 million hectares, 313.7 million tons, and 2.67 tons ha^{-1}, respectively, in 2015–16 (FAO, 2021). In India, it covered an area of 11.66 million ha, and in the fiscal year 2015–16, an average yield of 737 kg/ha was recorded (Anonymous, 2017). However, soybean's poor average productivity (1.0 Mg ha^{-1}) restricts its wider expansion in this potentially productive region (Dass and Bhattacharyya, 2017). The demand for locally produced soybean oilseed has decreased because of the lower productivity of these traditional production systems.

The System of Rice Intensification (SRI), which evolved from Madagascar (Upadhyay et al., 2022), as well as conservation agriculture (CA), integrated pest management (IPM), agroforestry, and other good combinations of practices that alter crop management, soil, water, and nutrients, are examples of agroecological management. Among other things, these modifications increase soil microbial activity (Adhikari et al., 2018) and abundance in the rhizosphere (root zone) and even the phyllosphere of plants (Uphoff et al., 2013). Soybean crop intensification or system of crop intensification (SCI) in soybean is fundamentally derived from the SRI with slight modifications (Araya et al., 2013). SCI is based on productive efficiencies that are derived from plants (Abraham et al., 2014) with larger more efficient longer-lived root systems (Adhikari et al., 2018) and their symbiotic relationships (Uphoff et al., 2013) with more abundant (Singh et al., 2018), diverse, and active soil biota (Dhar et al., 2016). Crops with more proliferated root systems with profuse and healthy life inside the soil are more resilient (Singh et al., 2018) when exposed to any aberrant weather, such as drought, a sudden increase in temperature, and heavy rainfall (Dass et al., 2015; Adhikari et al., 2018). Therefore, such innovative technology can boost the pace of modern agriculture practices for sustaining crop productivity even under climate change.

Practicing SCI can eliminate the application of agrochemicals on a wider scale and improve the quality of the soil as well as the produce (Upadhyay et al., 2018). Reducing the agrochemical load in the soil positively impacts microorganisms and root proliferation (Gupta et al., 2022). By altering the crop geometry, the symbiotic relationship between microorganisms and the root rhizosphere (Rajanna et al., 2022) can be enhanced which constitutes the sound plants micro-biomes (Kong and Liu, 2022). The SCI offers square planting with wider row-to-row and plant-to-plant spacing along with organic nutrient supplementation, which provides a better environment for the growth and development of above-ground-under-ground plant parts (Singh et al., 2018). Jiang et al. (2015) and Dass et al. (2015) reported that 45-cm row-to-row-spaced soybean sowing resulted in greater stomatal conductance, chlorophyll content, and net photosynthetic and transpiration rate, whereas narrow spacing causes a ceiling effect (Rahman et al., 2004) in the plant canopy, which leads to less growth and development of the plant above and below ground. In summary, proper crop geometry is one of the most important agronomic traits and varies with different cultivars in terms of obtaining optimal productivity with soybean. Therefore, crop intensification, including the alteration of existing crop management practices, is needed to meet the food demand of the ever-increasing population. Considering these facts, the present investigation entitled "Soybean crop intensification for sustainable above-ground-under-ground plant–soil interactions" has been carried out with the following hypotheses in mind: (1) to evaluate the conventional cultivation and SCI methods of soybean genotypes for a better yield, quality, and profitability; and (2) to comparatively analyze the morphophysiological changes in soybean under conventional and SCI methods of cultivation.

2. Materials and methods

2.1. Experimental details and study site

The study was undertaken in two consecutive rainy seasons from 2014 to 2015 to 2015 to 2016 at a research farm belonging to the Indian Agricultural Research Institute (Pusa Institute), New Delhi (28.38° latitude and 77.09° longitude). The soil of the experimental unit had sandy clay loam texture in the upper 30-cm layer with a nearly level to gently sloping topography. The initial soil was slightly alkaline (pH 7.8), low in soil organic carbon (0.39%), low in available nitrogen (N) (155.4 kg ha^{-1}), high in available potassium (K) (311.4 kg ha^{-1}), and had a medium level of available phosphorus (P) (14.2 kg ha^{-1}). The climate of the study site is semi-arid to subtropical, with maximum temperatures ranging from 40 to 45°C during the cropping season. Rainfall of 390.8 mm (2014–2015) and 633.1 mm (2015–2016) was received during the crop growth period.

The study consisted of three crop establishment methods [Conventional (45 cm × 10 cm), SCI: 45 cm × 45 cm, and SCI: 30 cm × 30 cm] assisted in the horizontal strips, and four soybean varieties (Pusa-9712, PS-1347, DS-12-13, and DS-12-5) assisted in the vertical strips under a strip plot design with three replications. After obtaining a proper tilth in the soil, one plowing was carried out using a tractor-drawn double disc followed by harrowing. At the time of sowing, the recommended doses for nutrients for soybean (N, 25 kg; P_2O_5, 60 kg; and K_2O, 40 kg ha^{-1}), were applied through FYM (contained 0.50% N, 0.23% P_2O_5, and 0.56% K_2O) at 5.0 t/ha treated with *Trichoderma* (2.5 kg t^{-1}), and the remaining potassium and phosphorus doses were applied through muriate of potash and single super phosphate (SSP), respectively. The two sprouted seeds per hill were carefully sown at a spacing of 45 × 45 cm and 30 cm × 30 cm spacing without damaging the seed coat, and the respective seed rates of the desired row spacing were 15 and 25 kg ha^{-1}. Seed treatment was carried out according to the SCI protocol (Plate 1). During both study years, the soybean crop was established using the standard agronomic practices listed in Table 1.

2.2. Data collection and analysis

Five soybean plants were randomly selected at intervals of 30, 60, and 90 days after planting (DAP) for the measurement of leaf area index (LAI), crop growth rate (CGR), and net assimilation rate (NAR) from the plant dry matter. Soybean was harvested manually using sickles during the second fortnight of October in both the years of study. After the harvesting bundles were left in the field for 2–3 days for drying, threshing was carried out using a Pullman thresher. Seed and straw yields were calculated from a 15.0 m^2 (5.0 × 3.0 m) plot at crop maturity. Yields were reported on a dry weight basis (12% moisture w/w), and the moisture content of the grain and straw was evaluated on an oven dry basis (70°C). The oil content of the soybean seed was estimated by using a grain analyzer (FOSS Infratec™ 1241) based on the technology of near-infrared transmittance using a non-destructive method of oil estimation. Leaf area (cm^2) was measured with the help of a leaf area meter (LI-3100C) and was further converted into LAI using the following formula (Equation 1):

$$\text{Leaf area index}\left(\text{LAI}\right) = \frac{\text{Leaf area}\left(\text{cm}^2\right)}{\text{Land area}\left(\text{cm}^2\right)} \quad (1)$$

The computation of mean growth rate was performed using Equation 2, as provided by Watson (1952).

$$\text{CGR} = \left(\frac{W_2 - W_1}{T_2 - T_1}\right)\left(\frac{1}{S}\right) \quad (2)$$

Where, W_1 and W_2 are the dry weight (g) of plants at time interval T_1 and T_2, respectively; S is land area (m^2) covered by the plants.

The computation of the mean net assimilation rate was performed using Equation 3, as provided by Watson (1958):

$$\text{NAR} = \left(\frac{W_2 - W_1}{LA_2 - LA_1}\right)\left(\frac{\text{Ln}LA_2 - \text{Ln}LA_1}{T_2 - T_1}\right) \quad (3)$$

Where, W_1 and W_2 are the dry weight (g) of plants at time T_1 and T_2, respectively; Ln is the natural logarithm; T_2- T_1 is the interval of time in days; LA_1 and LA_2 are the leaf area (m^2) covered by plants at time T_1 and T_2, respectively.

2.3. Root attributes

Three plants were selected and pulled from the soil for root nodule examination at the 50% flowering stage of the soybean crop. The rhizosphere soil was then gently shaken from the root systems into a sterilized bag. The number of nodules per plant was then determined after carefully washing the roots with distilled water. Soybean roots were sampled at the 50% flowering stage and root samples were collected from the top 15 cm of soil using a root auger of 8.0 cm in

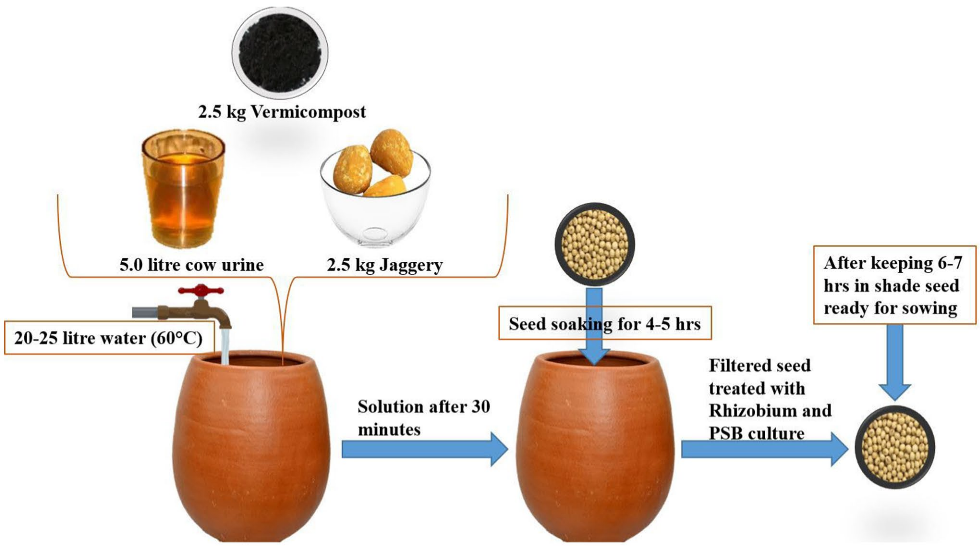


PLATE 1
Seed treatment protocol of SCI.

TABLE 1 Agronomic management practices followed and inputs applied during the experimentation (2014–2016).

Agronomic practices	Crop establishment methods		
	Conventional method	SCI (45cm×45cm)	SCI (30cm×30cm)
Seeding rate	80 kg ha^{-1}	15 kg seed ha^{-1}	25 kg ha^{-1}
Spacing	45 cm × 10 cm	45 cm × 45 cm	30 cm × 30 cm
Planting time	22 and 17 July	22 and 17 July	22 and 17 July
Plot size	5 m × 3 m = 15 m^2	5 m × 3 m = 15 m^2	5 m × 3 m = 15 m^2
Fertilizers	25, 60 and 40 kg N, P_2O_5 and K_2O ha^{-1}, respectively	25, 60 and 40 kg N, P_2O_5 and K_2O ha^{-1}, respectively	25, 60 and 40 kg N, P_2O_5 and K_2O ha^{-1}, respectively
Weed management	One hand weeding at 10–12 DAS and Imazethapyr 10% SL at 0.1 kg *a.i.* ha^{-1} at 30 DAS	Cono-weeder at 20 and 40 DAS	Cono-weeder at 20 and 40 DAS
Pest management	Dimethoate 30 EC (Rogor) at 250 mL *a.i.* ha^{-1} and Mancozeb 75% WP at 0.25%	Dimethoate30 EC (Rogor) at 250 mL *a.i.* ha^{-1} and Mancozeb 75% WP at 0.25%	Dimethoate30 EC (Rogor) at 250 mL *a.i.* ha^{-1} and Mancozeb 75% WP at 0.25%
Harvesting date	11 and 09 November	11 and 09 November	11 and 09 November

DAS, days after sowing. Cultivars were Pusa 9712, PS 1347, DS 12–13, and DS 12–5.

diameter and 15 cm in length (core volume = 754.3 cm^3). The roots were placed in a container with sieves to remove the soil debris and were then stored in a refrigerator at 4°C for preservation. Scanning and image analysis using the WIN-RHIZO system measured the root characteristics, such as root length and root volume (Costa et al., 2000).

2.4. Physiological indicators

An infrared gas analyzer (IRGA) was used to measure physiological parameters, such as photosynthetic rate (photo, mole CO_2 m^2 s^{-1}), stomatal conductance (cond, mole H_2O m^2 s^{-1}), transpiration rate (Tr, m. mol, H_2O m^2 s^{-1}), and intercellular CO_2 concentration (Ci, mole CO_2 mol^{-1}). The physiological indicators were measured at the flowering stage of soybean. Two CO_2 and H_2O infrared gas analyzers each are used in the LI-COR 6400, which provide estimates using the method described by Pandey et al. (2017). Photon (quantum) flux in radiant energy between 400 and 700 nm is used to describe photosynthetic active radiation (PAR). The PAR was determined using a LI-COR Line Quantum Sensor (1 m long) connected to a LI-1000 data logger. PAR was recorded at 1,200- and 1,300-h standard time on a sunny day.

2.5. Nutrient analysis

Grain and straw samples were processed for nutrient analyses. First, samples were oven dried (70°C) until a constant weight was achieved followed by grinding using a Willey mill with stainless steel blades. A modified version of the Kjeldahl method was used to estimate nitrogen (%) in grain and straw (Prasad et al., 2006). Nitrogen (N) uptake was determined using Equations 4 and 5:

$$\text{N uptake in grain / straw}\left(\text{kg ha}^{-1}\right) = \text{\%N in grain / straw} \times \text{grain / straw yield}\left(\text{kg ha}^{-1}\right) \tag{4}$$

$$\text{Total uptake in grain / straw}\left(\text{kg ha}^{-1}\right) = \text{N uptake in grain} + \text{N uptake in straw} \tag{5}$$

Grain and straw phosphorus (P) content was assessed using the vanado-molybdophosphoric acid yellow color method, as suggested by Prasad et al. (2006). Total P uptake (kg ha^{-1}) was determined using the following equations:

$$\text{P uptake in grain / straw}\left(\text{kg ha}^{-1}\right) = \text{\%P in grain / straw} \times \text{grain / straw yield}\left(\text{kg ha}^{-1}\right) \tag{6}$$

$$\text{Total P uptake in grain / straw}\left(\text{kg ha}^{-1}\right) = \text{P uptake in grain} + \text{P uptake in straw} \tag{7}$$

Grain and straw K content was estimated using a flame photometer (Prasad et al., 2006) and uptake (grain/straw and total) was computed using the following equations:

$$\text{K uptake in grain / straw}\left(\text{kg ha}^{-1}\right) = \text{\%K in grain / straw} \times \text{grain / straw yield}\left(\text{kg ha}^{-1}\right) \tag{8}$$

$$\text{Total K uptake in grain / straw}\left(\text{kg ha}^{-1}\right) = \text{K uptake in grain} + \text{K uptake in straw} \tag{9}$$

2.6. Soil microbial dynamics

To estimate soil microbial dynamics, soil samples obtained from a depth of 0–15 cm were collected from each experimental plot using a core sampler. Collected soil samples were processed (air-dried, powdered, passed through a 2 mm mesh sieve, etc.) to estimate microbial parameters, such as soil microbial biomass carbon (MBC), enzymatic activities (dehydrogenase, alkaline phosphatase, and nitrogenase), and total polysaccharides. Soil MBC was evaluated using the method described by Nunan et al. (1998) with 70 mL of 0.5 M potassium sulfate (K_2SO_4). Dehydrogenase enzyme activity was assessed using the processes described by Casida et al. (1964) and a spectrophotometer at a wavelength of 485 nm. Alkaline phosphate activity, which represents free enzymes, was estimated using the technique suggested by Tabatabai and Bremner (1969) and 1.0 mL of p-ntro-phenyl phosphate disodium and 0.25 mL of toluene. Soil nitrogenase activity was estimated using the method suggested by Prasanna et al. (2003) and gas chromatography (GC) (Hewlett Packard 5890 series II) (using ethylene gas) and expressed as acetylene reducing activity (ARA). ARA was calculated using Equation (10):

$$\text{ARA}\left(\text{n Moles ethylene g}^{-1}\text{soil ha}^{-1}\right) = 0.1653 \times \left(\text{Concentartion by GC}\right) \tag{10}$$

Soil chlorophyll was examined using the techniques suggested by Nayak et al. (2004) with pre-weighed soil-cores (0–20 cm soil depth) and acetone: dimethyl sulfoxide (DMSO) in a 1:1 ratio with 4 m L g^{-1} of soil. For soil chlorophyll determination, optical densities at 630, 645, 663, and 775 nm were taken (Equation 11).

$$\text{Soil chlorophyll}\left(\text{mg g}^{-1}\right) = 11.64\left(\text{OD } 663\right) - 2.16\left(\text{OD } 645\right) + 0.10\left(\text{OD } 630\right) \tag{11}$$

2.7. Water productivity

The depth of irrigation water from each plot was measured using a meter scale. Crop water productivity (CWP) and water use efficiency (WUE) were calculated with the help of the formula published by Rajanna et al. (2019).

$$\text{Total water use}\left(\text{mm}\right) = \text{Irrigation water use}\left(\text{mm}\right) + \text{Effective rainfall}\left(\text{mm}\right) \tag{12}$$

$$\textit{Water use efficiency}\left(kg\ ha^{-1}\ mm^{-1}\right) = \frac{\text{Grain yield}\left(\text{kg ha}^{-1}\right)}{\text{Evapotranspiration}\left(\text{mm}\right)} \tag{13}$$

$$\textit{Irrigation water productivity}\left(kg\ m^{-3}\right) = \frac{\text{Grain yield}\left(\text{kg ha}^{-1}\right)}{\text{Irrigation water applied}\left(\text{m}^{3}\right)} \tag{14}$$

2.8. Oil productivity

Soybean seed oil content was estimated using a grain analyzer (FOSS Infratec™ 1241) based on near-infrared transmittance technology using a non-destructive method of oil estimation. It is expressed as a percentage. Oil yield was calculated using Equation (15):

$$\text{Oil yield}\left(\text{kg ha}^{-1}\right) = \frac{\text{Oil content}\left(\%\right) \times \text{Seed yield}\left(\text{kg ha}^{-1}\right)}{100} \tag{15}$$

2.9. Statistical analysis

The research data recorded during the present study was evaluated using the "analysis of variance" (ANOVA) of the strip plot design (SPD) approach, as suggested by Gomez and Gomez (1984). The "F" test was used to determine the significance of treatments (variance ratio). When the "F" test revealed significant differences between means, differences in the treatment means were evaluated using Duncan's multiple range test (DMRT) and least significant difference (LSD) at a 5% probability level. Standard error (SE) was used (in Figures 1–6) to measure the degree of variability between the individual data values.

3. Results

3.1. Growth indices

Growth indices, such as LAI, CGR, and NAR, were significantly influenced by the different cultivation methods and soybean genotypes (Table 2). At 30, 60, and 90 days after sowing (DAS), the conventional method of soybean planting exhibited a significantly higher LAI under both systems of intensification methods (SCI). Similarly, with the conventional method of sowing at 45 × 10 cm, CGR and NAR were considerably greater than both SCI methods from the 0–30-day period to the 30–60-day period. However, for 60–90 days, CGR and NAR were significantly higher in SCI at a spacing of 30 × 30 cm compared with conventional methods and SCI at 45 cm × 45 cm. Among the genotypes, in all phases of development, DS 12–13 had a higher LAI than all the other tested genotypes, followed by DS 12–5 and Pusa 9712. Similarly, DS 12–13 soybean genotypes had the highest CGR across all the time periods (0–30, 30–60, and 60–90 days), while NAR was the highest for PS 1347 and Pusa 9,712 between 30–60 and 60–90 days, respectively.

3.2. Stomatal conductance, transpiration, and PAR

Cultivation methods and soybean genotypes significantly influenced flowering-stage stomatal conductance, intercellular CO_2 concentration, and net photosynthetic rates during 2015–16 (Table 3). The highest intercellular CO_2 concentration among cultivation methods was produced by SCI at 30 cm × 30 cm (260.1 μmol CO_2 mol^{-1}), which was significantly greater than the conventional method of soybean cultivation. Likewise, SCI at 45 cm × 45 cm exhibited significantly higher stomatal conductance (0.211 mol H_2O m^{-2} s^{-1}) and transpiration rate (7.8 m mol H_2O m^{-2} s^{-1}) than other cultivation techniques, whereas the net photosynthetic rate was substantially higher with the conventional method (398 μmol CO_2 m^{-2} s^{-1}) than with SCI techniques. Among the soybean genotypes, DS 12–13 had a higher stomatal conductance (0.22 mol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration (250.3 μ mol CO_2 mol^{-1}), and rate of transpiration (7.5 mmol H_2O m^{-2} s^{-1}) than other soybean genotypes. There was no significant difference between DS 12–13 and DS 12–5. However, the Pusa 9712 genotype had a significantly higher rate of net photosynthesis (353 μmol CO_2 m^{-2} s^{-1}) than the other genotypes.

Intercepted photosynthetically active radiation (PAR) of the soybean genotypes was also significantly influenced by the cultivation method and genotype. The adoption of SCI at 30 cm × 30 cm soybean establishment resulted in a significantly higher intercepted PAR (1,249 μ mol m^{-2} s^{-1}) than other SCI methods (45 cm × 45 cm) and the conventional method of crop establishment. Increases were 14.1 and 8.5% with SCI at 30 cm × 30 cm compared with the conventional method and SCI at 45 cm × 45 cm, respectively. As a result, the PAR interception rate was 83.7% higher in SCI at 30 cm × 30 cm than the other methods. However, soybean genotypes have little impact/no significant effect on the interception of PAR. Comparatively, DS 12–13 intercepted more PAR than DS 12–5, PS 1347, and Pusa 9712.

TABLE 2 Effect of crop establishment methods and cultivars on the leaf area index, crop growth rate (g m^{-2}), and net assimilation rate (g m^{-2} leaf area day^{-1}) of soybean at different growth stages (mean of 2 years).

Treatment	Leaf area index (LAI)			Crop growth rate (m m^{-2})			Net assimilation rate (g m^{-2} leaf area day^{-1})		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Crop establishment methods									
Conventional: (45 cm × 10 cm)	0.755^{A}	3.40^{A}	2.04^{A}	1.27^{A}	5.99^{A}	9.79^{B}	15.40^{A}	4.24^{B}	7.09^{C}
SCI (45 cm × 45 cm)	0.265^{C}	1.76^{C}	1.57^{C}	0.38^{C}	3.12^{C}	10.77^{A}	11.20^{C}	4.39^{AB}	8.64^{A}
SCI (30 cm × 30 cm)	0.567^{B}	2.39^{B}	1.90^{B}	0.84^{B}	4.81^{B}	11.51^{A}	12.85^{B}	4.50^{A}	8.29^{B}
Genotype									
Pusa 9712	0.536^{A}	2.52^{A}	1.82^{B}	0.85^{A}	4.61^{AB}	10.45^{B}	13.35^{A}	4.35^{BC}	9.06^{A}
PS 1347	0.502^{B}	2.32^{B}	1.73^{CB}	0.77^{B}	4.42^{B}	9.59^{C}	12.70^{D}	4.44^{A}	8.58^{AB}
DS 12–13	0.542^{A}	2.65^{A}	1.93^{A}	0.86^{A}	4.78^{A}	11.60^{A}	13.30^{B}	4.33^{C}	8.23^{B}
DS 12–5	0.535^{A}	2.56^{A}	1.87^{AB}	0.85^{A}	4.73^{A}	11.11^{AB}	13.25^{C}	4.40^{AB}	8.82^{A}

DAS, days after sowing. The mean values followed by different capital letter(s) (based on Duncan's multiple range tests) within the row are significantly different at $p \leq 0.05$.

TABLE 3 Effect of crop establishment methods and cultivars on the physiological parameters of soybean at the flowering stage (mean data of 2 years).

Treatment	Intercellular CO_2 concentration (μmol CO_2 mol^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})	Stomatal conductance (mol H_2O m^{-2} s^{-1})	Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	PAR intercepted [μmol (photons) m^{-2} s^{-1}]	% PAR interception
Crop establishment methods						
Conventional: (45 cm × 10 cm)	232.1^{B}	6.4^{B}	0.173^{B}	398^{A}	$1,095^{B}$	73.4
SCI (45 cm × 45 cm)	258.4^{A}	7.8^{A}	0.211^{A}	335^{B}	$1,158^{B}$	77.6
SCI (30 cm × 30 cm)	260.1^{A}	7.2^{A}	0.177^{B}	244^{B}	$1,249^{A}$	83.7
Genotype						
Pusa 9712	243.8	6.6^{B}	0.180^{B}	353^{A}	1,140	76.4
PS 1347	246.0	6.4^{B}	0.160^{C}	340^{B}	1,153	77.2
DS 12–13	250.3	7.5^{A}	0.220^{A}	302^{C}	1,191	79.8
DS 12–5	248.2	7.3^{A}	0.208^{A}	307^{C}	1,186	79.5

PAR, photosynthetic active radiation. Mean values followed by different capital letter(s) (based on Duncan's multiple range tests) within the row are significantly different at $p \leq 0.05$.

TABLE 4 Effect of crop establishment methods and cultivars on the root attributes of soybean (mean data of 2 years).

Treatment	Nodules $plant^{-1}$	Nodule dry weight (mg $plant^{-1}$)	Root length density (cm cm^{-3})	Root surface area density (cm^2 cm^{-3})	Root volume density (mm^3 cm^{-3})	Dry root mass density (mg cm^{-3})
Crop establishment methods						
Conventional: (45 cm × 10 cm)	19.3^{B}	163.8^{B}	1.21^{B}	0.082^{C}	2.43^{C}	1.79^{C}
SCI (45 cm × 45 cm)	41.1^{A}	176.5^{A}	1.58^{A}	0.118^{A}	4.07^{A}	2.10^{A}
SCI (30 cm × 30 cm)	39.4^{A}	175.4^{A}	1.54^{A}	0.103^{B}	4.02^{B}	1.90^{B}
Genotype						
Pusa 9712	33.9^{A}	172.8^{AB}	1.40^{BC}	0.097^{B}	3.48^{A}	1.77^{C}
PS 1347	30.5^{B}	162.7^{B}	1.38^{C}	0.090^{B}	3.07^{B}	1.65^{D}
DS 12–13	35.8^{A}	179.1^{A}	1.52^{A}	0.104^{A}	3.66^{A}	2.30^{A}
DS 12–5	33.7^{A}	173.0^{AB}	1.50^{B}	0.102^{A}	3.51^{A}	2.02^{B}

Mean values followed by different capital letter(s) (based on Duncan's multiple range tests) within the row are significantly different at $p \leq 0.05$.

3.3. Root attributes

Cultivation methods and genotypes significantly influenced nodules per $plant^{-1}$, total nodule dry weight, and root characteristics of soybeans at the blooming stage (Table 4). The SCI method of cultivation at 45 cm × 45 cm produced significantly more nodules per plant (41.1) and higher nodule dry weight (176.5 mg $plant^{-1}$) than the conventional method of cultivation (45 cm × 10 cm); however, the SCI method at 30 cm × 30 cm produced a similar number of nodules. Among the genotypes, DS 12–13 produced the maximum nodules per $plant^{-1}$ (35.8) and nodule dry weight (179.1 mg $plant^{-1}$), making it substantially more productive than the PS 1347 genotype. However, it was on a par with the Pusa 9712 and DS 12–5 genotypes. DS 12–13 had the most characteristics with the highest values, followed by Pusa 9712 and DS 12–5, all of which were comparable with one another.

Similarly, density of root length (1.58 cm cm^{-3}), root volume (4.07 mm^3 cm^{-3}), surface area (0.118 cm^2 cm^{-3}), and dry root mass (2.30 mg cm^{-3}) were significantly higher in the SCI at 45 cm × 45 cm than the conventional method of cultivation. However, differences between both of the SCI methods were not significant. Among the genotypes, DS 12–13 produced a significantly higher density of root length (1.52 cm cm^{-3}), root volume (3.66 mm^3 cm^{-3}), surface area (0.104 cm^2 cm^{-3}), and dry root mass (2.30 mg cm^{-3}) than Pusa 9712 and PS 1347 but it was on a par with DS 12–5.

3.4. Productivity

During the 2014–2015 and 2015–2016 cropping seasons, the method of cultivation and varieties had a significant effect on soybean seed yields, as well as aboveground biomass yields and harvest index (Table 5). Among the cultivation methods, SCI at 30 cm × 30 cm had a considerably greater seed yield (1.99–2.13 t ha^{-1}), above-ground biomass yield (5.82–6.07 t ha^{-1}), and harvest index (34.2–35.1%) than the conventional method of cultivation (45 × 10 cm). The magnitude of increase in seed yields ranged from 9.6 to 13.3%, and the increases in biomass yields ranged from 8.2 to 10.7%. However, compared with the conventional method of cultivation, yields from both SCI methods were almost identical. Among the genotypes, DS 12–13 produced a significantly higher seed yield (1.97–2.15 t ha^{-1}) and aboveground biomass yield (5.86–6.15 t ha^{-1}) than the PS 1347 and Pusa 9,712 genotypes.

The interaction effect of crop establishment methods and genotypes significantly influenced soybean seed and biomass yields (Table 5). The combination of the DS 12–13 genotype with SCI at 30 cm × 30 cm produced a significantly higher seed yield (2.1–2.24 t ha^{-1}) and aboveground biomass yield (6.04–6.31 t ha^{-1}) than other combinations. Interestingly, all the studied genotypes produced significantly higher seed and biomass yields under an SCI at 30 cm × 30 cm than other SCI methods and conventional methods of cultivation.

Irrigation water productivity (IWP) and water usage efficiency (WUE) were considerably influenced by the different cultivation techniques and genotypes (Table 5). SCI at 30 cm × 30 cm yielded significantly higher WUE (3.06–3.34 kg ha^{-1} mm^{-1}) and IWP (1.77–2.22 kg m^{-3}) than conventional practice. Among the genotypes, DS 12–13 recorded significantly higher WUE (3.09–3.30 kg ha^{-1} mm^{-1}) and IWP (1.64–2.01 kg m^{-3}) than Pusa 9,712 and PS 1347. Among the interactions, the combination of DS 12–13 with an SCI at 30 cm × 30 cm produced significantly higher WUE (3.22–3.51 t ha^{-1}) and IWP (1.87–2.33 t ha^{-1}) than other combinations. All genotypes produced significantly higher WUE and IWP with an SCI at 30 cm × 30 cm.

3.5. Oil content and yield

Among the cultivation methods, an SCI at 30 cm × 30 cm had a considerably higher oil yield of 384.1 kg ha^{-1} (2015–2016) and 413.2 kg ha^{-1} (2014–2015) than the conventional method of cultivation (45 cm × 10 cm), whereas soybean genotypes significantly influenced soybean oil content and yield. Cultivation of the DS 12–13 soybean variety resulted in a statistically higher oil yield (394–430 kg ha^{-1}) than that of PS 1347 and Pusa 9712, but it was statistically on a par with DS 12–5, while soybean DS 12–5 produced a significantly higher oil content (20.4–20.5%) than other genotypes, but it was on a par with DS 12–13.

3.6. Nutrient concentration and uptake

Perusal of data revealed that NPK concentration and their uptake in grain and straw of soybean influenced statistically due to cultivation methods during the study period (Table 6). However, the genotypes did not have any significant effect on NPK content. Statistically higher N content in grain (5.94%) and straw (2.61%) was noted in SCI at 45 cm × 45 cm compared with other methods of cultivation. Similarly, grain, straw, and total nitrogen uptake was statistically higher with SCI at 30 cm × 30 cm (108.1, 90.59, and 198.7 kg ha^{-1}, respectively) over the rest of the treatments. By contrast, crop establishment methods had no significant effect on P and K concentrations in soybean plant parts, whereas P and K uptake was significantly influenced by cultivation techniques and genotypes. SCI at 30 cm × 30 cm had a significantly greater P uptake in grain, straw, and total P uptake (9.38, 5.72, and 15.10 kg ha^{-1}, respectively) and K uptake in grain, straw, and total P uptake (15.5, 70.3, and 85.8 kg ha^{-1}, respectively) than other cultivation methods. Among the genotypes, the highest P uptake in grain and straw and total P uptake was recorded with DS 12–13 (9.33, 5.79, and 15.12 kg ha^{-1}, respectively), as was K uptake in grain and straw and total P uptake (15.6, 72.2, and 87.8 kg ha^{-1}, respectively) compared with the other genotypes.

3.7. Soil biological properties

Soil biological properties, such as dehydrogenase activity (DHA) (Figure 1), alkaline phosphatase activity (APA) (Figure 2), acetylene reduction assay (ARA) (Figure 3), total polysaccharides (Figure 4), microbial biomass carbon (MBC) (Figure 5), and soil chlorophyll (Figure 6), were significantly influenced by different methods of cultivation. Significantly higher soil DHA, APA, ARA, total polysaccharides, MBC, and soil chlorophyll were recorded in the SCI at 45 cm × 45 cm (13.63 µg TPF g^{-1} soil $hr.^{-1}$, 93.2 µg p–nitro phenol g^{-1} soil $hr.^{-1}$, 25.5 n moles ethylene g soil $hr.^{-1}$, 443.7 mg kg^{-1} soil, 216.5 mg kg^{-1} soil, and 0.43 mg g^{-1} soil, respectively) compared with the conventional method. Among the genotypes, APA and ARA were considerably impacted by soybean genotypes over the study seasons. However, genotypes had no apparent impact on the levels of DHA, total polysaccharides, MBC, and soil chlorophyll. Among the soybean genotypes, DS 12–13 exhibited significantly higher APA (90.0 µg p–nitro phenol g^{-1} soil $hr.^{-1}$) and ARA (18.8 n moles ethylene g soil $hr.^{-1}$) than the other genotypes.

4. Discussion

4.1. Growth indices

A linear increase in growth indices, such as LAI, CGR, and NAR, was observed with the advancement in the growth stage of the soybean crop under the different cultivation methods and varieties. SCI either at 30 cm × 30 cm or 45 cm × 45 cm exhibited significantly higher LAI, CGR, and NAR over the conventional method (45 cm × 10 cm) due to less competition for growth factors, such as solar radiation interception, root growth, soil aeration, and microbial activity. Ball et al. (2000) reported that an optimum plant population is required for the maximum interception of light for a higher crop growth rate, LAI, NAR, and dry matter accumulation (Berger-Doyle et al., 2014) in the crop.

In the current study, growth indices were higher with the conventional method up to 60 DAS; however, with the advancement of growth stages, LAI, CGR, and NAR increased with the SCI at 30 cm × 30 cm compared with the other cultivation methods. Greater leaf area per plant was observed due to the higher growth performance of the plants that were more widely spaced. A higher population density with the conventional method caused the ceiling effect in the plant canopy, resulting in greater stem elongation leading to increased plant height (Rahman et al., 2004). Rehman et al. (2014) concluded that narrow row spacing (30–40 cm) significantly increased NAR, CGR, RGR, and LAI of soybean compared with wider spacing (70 cm).

TABLE 5 Effect of crop establishment methods and cultivars on soybean crop yields, oil yields, and water use efficiency (mean data of 2 years).

Treatment	Seed yield (Mg ha^{-1})		Biomass yield (Mg ha^{-1})		Harvest index (%)		Oil content (%)		Oil yield (kg ha^{-1})		Water use efficiency (kg ha^{-1}–mm)		Irrigation water productivity (kg m^{-3})	
	2014–2015	2015–2016	2014–2015	2015–2016	2014–2015	2015–2016	2014–2015	2015–2016	2014–2015	2015–2016	2014–2015	2015–2016	2014–2015	2015–2016
Crop establishment methods														
Conventional: (45 cm × 10 cm)	1.98^{B}	1.78^{B}	5.89^{B}	5.42^{B}	33.7^{B}	32.8^{B}	19.0	18.9	376.2	336.4	2.85^{B}	2.98^{B}	1.23^{B}	1.48^{C}
SCI (45 cm × 45 cm)	1.91^{B}	1.73^{B}	5.44^{C}	5.16^{B}	35.2^{A}	33.4^{A}	19.3	19.2	368.6	332.2	2.75^{B}	2.89^{B}	1.60^{B}	1.92^{B}
SCI (30 cm × 30 cm)	2.13^{A}	1.99^{A}	6.07^{A}	5.82^{A}	35.1^{A}	34.2^{A}	19.4	19.3	413.2	384.1	3.06^{A}	3.34^{A}	1.77^{A}	2.22^{A}
Genotype														
Pusa 9,712	1.98^{B}	1.79^{B}	5.78^{B}	5.36^{B}	34.5	33.5	18.6^{B}	18.5^{B}	368.3	331.2	2.85^{B}	3.00^{B}	1.51^{B}	1.83^{B}
PS 1347	1.82^{C}	1.67^{C}	5.32^{C}	5.02^{C}	34.1	33.3	17.7^{B}	17.6^{B}	322.1	293.9	2.61^{C}	2.80^{C}	1.39^{C}	1.71^{C}
DS 12–13	2.15^{A}	1.97^{A}	6.15^{A}	5.86^{A}	35.0	33.6	20.0^{A}	20.0^{A}	430.0	394.0	3.09^{A}	3.30^{A}	1.64^{A}	2.01^{A}
DS 12–5	2.08^{AB}	1.89^{A}	5.95^{AB}	5.64^{A}	35.0	33.6	20.5^{A}	20.4^{A}	426.4	385.6	2.99^{AB}	3.17^{A}	1.59^{AB}	1.93^{A}
Cultivation method × Genotype														
C1 × Pusa 9712	1.97	1.73	5.91	5.26	33.4	32.9	18.5	18.2	364.5	314.9	2.83	2.90	1.22	1.44
C1 × PS 1347	1.77	1.60	5.27	4.89	33.6	32.7	17.5	17.5	309.8	280.0	2.54	2.67	1.10	1.33
C1 × DS 12–13	2.12	1.92	6.28	5.87	33.7	32.8	19.9	19.9	421.9	382.1	3.04	3.22	1.31	1.60
C1 × DS 12–5	2.07	1.86	6.09	5.67	33.9	32.8	20.0	19.9	414.0	370.1	2.97	3.11	1.28	1.55
C2 × Pusa 9712	1.87	1.66	5.15	4.92	36.4	33.9	18.7	18.8	349.7	312.1	2.69	2.78	1.56	1.85
C2 × PS 1347	1.70	1.56	5.08	4.76	33.5	32.7	17.9	17.7	304.3	276.1	2.44	2.61	1.42	1.73
C2 × DS 12–13	2.09	1.89	5.84	5.67	35.8	33.4	20.1	19.7	420.1	372.3	3.01	3.17	1.74	2.10
C2 × DS 12–5	1.99	1.79	5.67	5.30	35.2	33.7	20.6	20.7	409.9	370.5	2.86	2.99	1.66	1.99
C3 × Pusa 9712	2.11	1.98	6.26	5.90	33.7	33.6	18.7	18.5	394.6	366.3	3.03	3.32	1.76	2.20
C3 × PS 1347	1.98	1.86	5.61	5.41	35.3	34.4	17.9	17.8	354.4	331.1	2.84	3.12	1.65	2.07
C3 × DS 12–13	2.24	2.10	6.31	6.04	35.5	34.7	20.1	20.3	450.2	426.3	3.22	3.51	1.87	2.33
C3 × DS 12–5	2.18	2.04	6.09	5.95	35.9	34.2	20.9	20.5	455.6	418.2	3.14	3.41	1.82	2.26
SEm±									–	–				
LSD ($P \leq 0.05$)	NS	NS	0.44	NS	NS	NS	NS	NS	–	–	NS	NS	NS	NS

Mean values followed by different capital letter(s) (based on Duncan's multiple range tests) within the row are significantly different at $p \leq 0.05$.

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TABLE 6 Effect of crop establishment methods and cultivars on NPK concentration and their uptake in soybean (mean data of 2 years).

	N (%)		P (%)		K (%)		N uptake (kg ha^{-1})			P uptake (kg ha^{-1})			K uptake (kg ha^{-1})		
	Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover	Total	Grain	Stover	Total	Grain	Stover	Total
Crop establishment methods															
Conventional: (45 cm × 10 cm)	5.84^{B}	2.52^{B}	0.46	0.15	0.78	1.86	109.8^{B}	94.8^{AB}	204.6^{B}	8.56^{B}	5.56^{AB}	14.1^{AB}	14.6^{B}	69.9^{A}	84.5^{A}
SCI (45 cm × 45 cm)	5.94^{A}	2.61^{A}	0.46	0.15	0.76	1.84	108.1^{B}	90.6^{B}	198.7^{B}	8.33^{B}	5.17^{B}	13.5^{B}	13.7^{C}	63.9^{B}	77.6^{B}
SCI (30 cm × 30 cm)	5.84^{B}	2.49^{B}	0.46	0.15	0.75	1.81	120.3^{A}	97.7^{A}	216.9^{A}	9.38^{A}	5.72^{A}	15.1^{A}	15.5^{A}	70.3^{A}	85.8^{A}
Genotype															
Pusa 9712	5.87	2.56	0.46	0.15	0.76	1.83	110.8	94.1^{A}	204.9^{B}	8.65^{A}	5.44^{B}	14.1^{B}	14.4^{B}	67.4^{B}	81.8^{B}
PS 1347	5.89	2.60	0.46	0.15	0.76	1.84	102.7	88.8^{B}	191.5^{C}	8.00^{B}	5.10^{C}	13.1^{C}	13.3^{C}	62.8^{C}	76.1^{C}
DS 12–13	5.87	2.50	0.45	0.15	0.76	1.83	120.8	98.4^{A}	219.2^{A}	9.33^{A}	5.79^{A}	15.1^{A}	15.6^{A}	72.2^{A}	87.8^{A}
DS 12–5	5.86	2.50	0.46	0.15	0.76	1.83	116.5	94.8^{A}	211.2^{AB}	9.04^{A}	5.62^{AB}	14.7^{AB}	15.1^{Ab}	69.8^{AB}	84.9^{AB}

Mean values followed by different capital letter(s) (based on Duncan's multiple range tests) within the row are significantly different at $p \leq 0.05$.

Among the genotypes, growth attributes were higher in DS 12–13 and DS 12–5 than in other tested cultivars. The growth attributes were significantly influenced by the genetic makeup of plants.

4.2. Stomatal conductance, transpiration, and photosynthetic rate

Leaf is the prime plant part to receive incident solar radiation (PAR) and transform it into photosynthates. Therefore, better soybean growth attributes resulted in better physiological parameters. In the current study, the highest intercellular CO_2 concentration was produced by an SCI at 30 cm × 30 cm (260.1 µmol CO_2 mol^{-1}), and the SCI at 45 cm × 45 cm exhibited a significantly higher stomatal conductance (0.211 mol H_2Om^{-2} s^{-1}) and rate of transpiration (7.8 mmol H_2O m^{-2} s^{-1}) than the other cultivation techniques. The net photosynthetic rate for soybean was substantially higher with the conventional method (398 µmol CO_2 m^{-2} s^{-1}) than with the SCI techniques. A higher transpiration rate and stomatal conductance was observed in the SCI due to the greater leaf surface area and higher temperature (26–36°C). An increased transpiration rate leads to high stomatal conductance, thereby increasing CO_2 influx into the chloroplasts, possibly leading to a higher net photosynthetic rate. These findings are corroborated by Moreira et al. (2015) who reported that low planting densities and N rates increased the greenness index (SPAD value), concentration of intercellular carbon dioxide (Ci), and inherent WUE.

Among the soybean genotypes, DS 12–13 had the highest intercellular CO_2 concentration (250.3 µmol CO_2 mol^{-1}), rate of transpiration (7.5 mmol H_2O m^{-2} s^{-1}), and stomatal conductance (0.220 mol H_2O m^{-2} s^{-1}). There was no significant difference between DS 12–13 and DS 12–5. However, the Pusa 9,712 genotype had a significantly higher rate of net photosynthesis (353 µmol CO_2 m^{-2} s^{-1}) than other genotypes. An increased transpiration rate leads to high stomatal conductance, thereby increasing CO_2 influx into the chloroplasts. A significantly higher chlorophyll content, net photosynthetic rate, and stomatal conductance in JS 95–60 than in JS 97–52 was also reported by Vyas and Khandwe (2014).

4.3. Photosynthetically active radiation

The PAR interception and transmission by soybean in SCI at 30 cm × 30 cm was 14.05 and 8.5%, respectively, and it was greater than the values obtained with the conventional method and the SCI at 45 cm × 45 cm (Table 5). PAR radiation was intercepted more with the SCI due to the green leaf coverage per unit area. Thereby, SCI at 30 cm × 30 cm intercepted 83.65% of incident PAR compared with 73.35% with the conventional method. High foliage surface aids a higher transpiration rate with a high maximum temperature (22–26°C) at the anthesis stage of soybean; furthermore, an increased transpiration rate leads to high stomatal conductance, thus increasing CO_2 entry into the chloroplasts, which might be the reason behind the higher net photosynthetic rate with the SCI method. The density of plants significantly influenced the economic yield in soybean by altering leaf area and therefore light interception and photosynthesis (Wells, 1991) and increasing plant spacing and decreasing row spacing, chlorophyll content (SPAD value), LAI, and the rate of

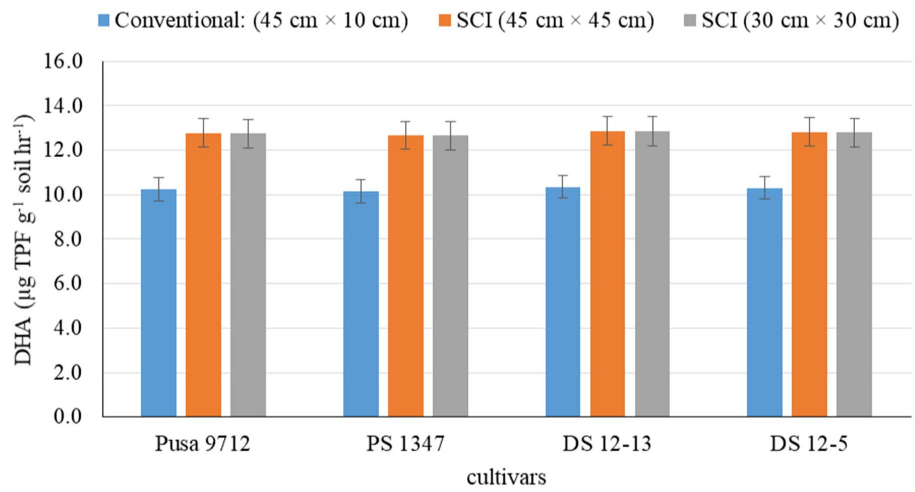

FIGURE 1
Effect of crop establishment methods and soybean cultivars on dehydrogenase activity (DHA) (mean data of 2 years).

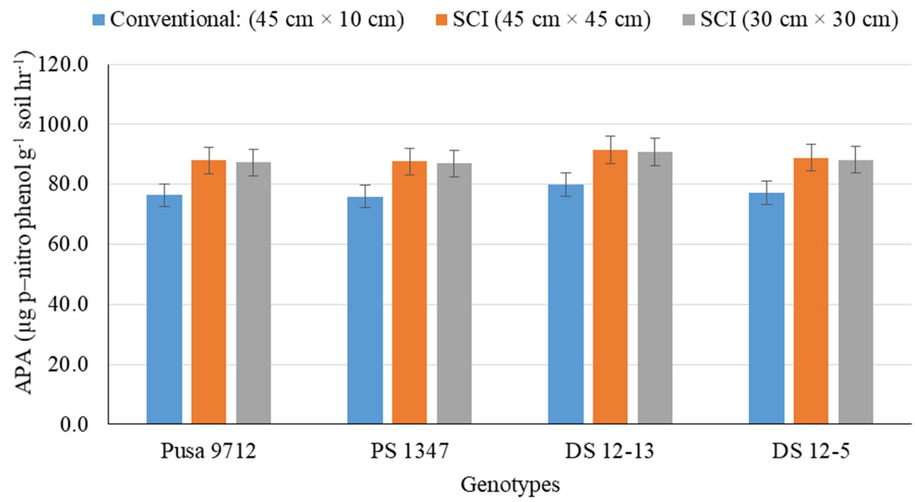

FIGURE 2
Effect of crop establishment methods and soybean cultivars on alkaline phosphatase activity (APA) (mean data of 2 years).

photosynthesis (Jiang et al., 2015). With narrow plant spacing and wider row SPAD, LAI and Pn were higher. The use of wheat straw mulch and wider spacing by Dass and Bhattacharyya (2017) improved leaf SPAD values, PAR interception, the net photosynthetic rate, stomatal conductance, and WUE of soybean in dryland areas. There was a non-significant variation noted among all soybean cultivars with respect to intercepted PAR.

4.4. Root attributes

Wide space sowing decreases aboveground competition and ground competitiveness between roots for the absorption of water and nutrients and provides the adequate arena for nodulation and root growth of the crop plants. Therefore, in the current study, wider spacing at 45 cm × 45 cm with an SCI method of cultivation recorded statistically higher nodule counts, higher nodule dry weight (176.5 mg plant^{-1}), better root length, volume, and mass density than the conventional method of cultivation (45 cm × 10 cm). Seed inoculation using *Rhizobium* culture might have enhanced the population of the effective and healthy *Rhizobium* strain in the root nodules, which had a better ability to fix atmospheric N. Improved nodulation with the SCI was due to the slow release of nitrogen from FYM, which tends to form more nodules to meet the nitrogen demand of the crop (Salvagiotti et al., 2008). Mondal et al. (2014) showed that plant population had a significant effect on root length, root surface area, and the number of lateral roots/plant at a broader spacing of 20 cm × 30 cm followed by 15 cm × 30 cm.

Among the genotypes, DS 12–13 genotype produced a significantly higher density of root length (1.52 cm cm^{-3}), root volume (3.66 mm^3 cm^{-3}), surface area (0.104 cm^2 cm^{-3}), and dry root mass (2.30 mg cm^{-3}) Pusa 9712 and PS 1347, but it was on a par with DS 12–5. Varietal root growth performance depends upon the cultivation practice. Additionally, leaf area/plant might be due to the higher growth of individual plants at a wider arrangement than the narrow arrangement. Imtiyaz et al. (2014) observed that DS 9712 had a greater reduction in root dry weight and fresh weight; however, the greatest reduction in shoot fresh and dry weight was noted in PS-1347 and DS-9712, respectively.

4.5. Productivity

The above- and below-ground part of the soybean plant proliferated with the SCI because it provides broader spacing and square planting. Broader spacing leads to the improved growth of specific plants due to the availability of proper space, light, and moisture, which ultimately resulted in higher productivity of the crop (Dass et al., 2015). In the current study, SCI at 30 cm × 30 cm considerably increased seed yields from 9.6 to 13.3% and biomass yields from 8.2 to 10.7% compared with the conventional method of ‘cultivation and the SCI at 45 cm × 45 cm during 2014–2015 and 2015–2016. The increase in yield attributing parameters due to better plant growth and vigor brings out higher yield. More widely spaced soybean sowing resulted in higher seed and straw yield than with narrow row spacing (Pandya et al., 2005). However, compared with the conventional method of cultivation, the yields from both SCI methods were almost identical. Among the genotypes, DS 12–13 produced a significantly higher seed yield (1.97–2.15 t ha^{-1}) and aboveground biomass yield (5.86–6.15 t ha^{-1}) than PS 1347 and Pusa 9712 during both the study seasons. Different genotypes of soybean failed to affect the harvest index significantly during 2014–2015 and 2015–2016. The combination of the DS 12–13 genotype with the SCI at 30 cm × 30 cm produced a significantly higher seed yield (2.10–2.24 t ha^{-1}) and aboveground biomass yield (6.04–6.31 t ha^{-1}) than other combinations. Interestingly, all the studied genotypes produced significantly higher seed and biomass yields with the SCI at 30 cm × 30 cm than other SCI methods and the conventional method of cultivation. The SCI offers wider spacing, square planting, and microbe-loaded organic sources of nutrients, which provide better options for the development of aboveground parts of plants. A similar finding was reported by Abbas et al. (1994) who reported that an optimal plant population boosts the plant to improve yield and yield-attributing characteristics in plants (Abbas et al., 1994).

The SCI at 30 cm × 30 cm yielded significantly higher WUE (3.06–3.34 kg ha^{-1} mm^{-1}) and IWP (1.77–2.22 kg m^{-3}) than the conventional method (Table 5). Among the genotypes, DS 12–13 recorded significantly higher WUE (3.09–3.30 kg ha^{-1} mm^{-1}) and IWP (1.64–2.01 kg m^{-3}) than Pusa 9712 and PS 1347. Among the interactions, the combination of DS 12–13 with the SCI at 30 cm × 30 cm produced significantly higher WUE (3.22–3.51 t ha^{-1}) and IWP (1.87–2.33 t ha^{-1}) than the other combinations.

4.6. Oil yield

Oil yield is the function of its content and seed production. Among the cultivation methods, SCI at 30 cm × 30 cm had a considerably higher oil yield than the conventional method of cultivation (45 cm × 10 cm) and the SCI at 45 cm × 45 cm. Relatively higher oil yields of 19.38 and 19.27% were obtained with the SCI at 30 cm × 30 cm, followed by the SCI at 45 cm × 45 cm with 19.29 and 19.21%, compared with the conventional method during 2014 and 2015, respectively (Table 5). Improvements in oil content and oil yields with the SCI might be due to increased N and S content in grain on account of enhanced soil nitrogen and S through FYM and single super phosphate. Significantly higher oil content and yield were recorded in DS 12–5 (20.5 and 20.4%) and DS 12–13 (431 and 394 kg/ha) compared with other varieties in both years. A similar finding was reported by Moreira et al. (2015) who reported that plant population, row spacing, and nitrogen rates did not influence oil content in soybean seeds. Diep et al. (2016) reported that biofertilizer application in soybean significantly increased seed oil content and oil yield compared with the control, but remained on a par with 400 kg ha^{-1} NPK 15-15-15.

4.7. Nutrient concentration and uptake

Cultivation methods and genotypes significantly affected NPK content in grain and straw, and NPK uptake. Statistically higher NPK content in grain and straw was reported in SCI at 45 cm × 45 cm compared with other methods of cultivation. Similarly, NPK grain and straw and total uptake was statistically higher with SCI at 30 cm × 30 cm. The higher concentration of nutrients in SCI might be due to the microbial breakdown of organic manures, resulting in a release of nutrients into the soil (Prajapati et al., 2014) and, therefore, faster absorption and translocation into plants. Nutrient uptake is a function of nutrient concentration and biomass, and an increase in yield coupled with increased nutrient content resulted in a higher total uptake (Upadhyay et al., 2019) of nutrients with SCI at 30 × 30 cm. The greater microbial and enzymatic activities observed in the present investigation (Table 6) might also be responsible for the release of nutrients, ensuring an adequate supply to the plants. Among the genotypes, DS 12–13 had the highest NPK uptake in grain and straw and total P.

4.8. Soil biological properties

Soil biological properties, such as dehydrogenase activity (DHA) (Figure 1), alkaline phosphatase activity (APA) (Figure 2), ARA (Figure 3), total polysaccharides (Figure 4), MBC (Figure 5), and soil chlorophyll (Figure 6), were significantly higher with the SCI at 45 cm × 45 cm than with the conventional method of cultivation. Improved soil biological properties with the SCI might be due to the application of FYM and split application of vermicompost, which increased soil moisture availability, and the addition of nutrient and organic carbon through the decomposition

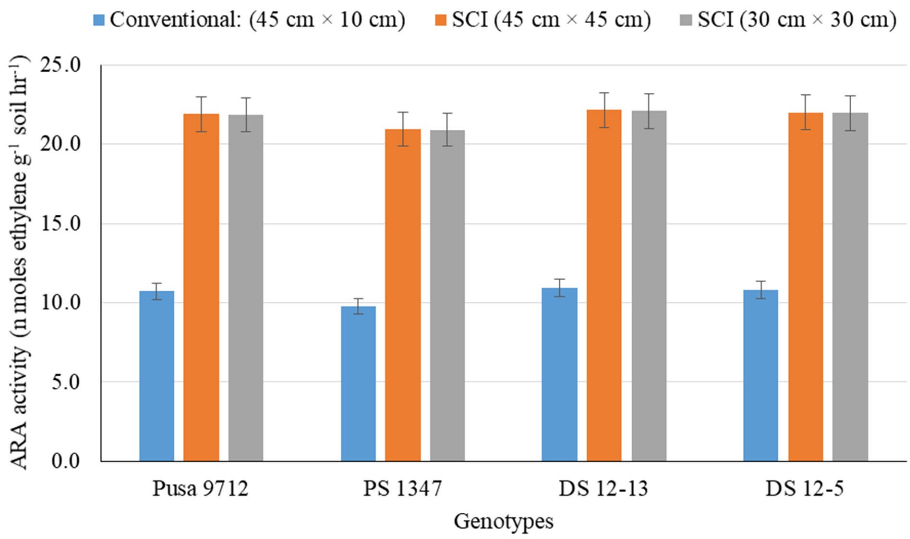

FIGURE 3
Effect of crop establishment methods and soybean cultivars on acetylene reducing assay (ARA) (mean data of 2 years).

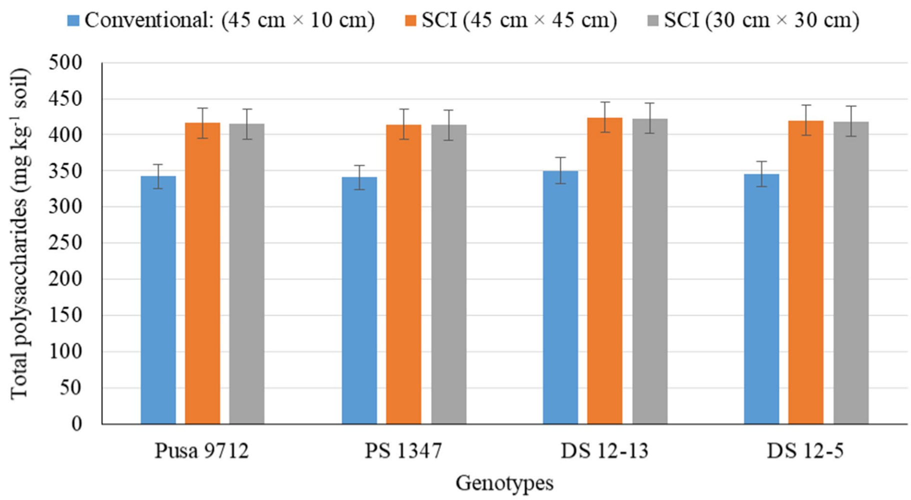

FIGURE 4
Effect of crop establishment methods and soybean cultivars on total polysaccharides (mean data of 2 years).

of organic residue. The availability of easily decomposable carbon (C) sources in soil allows a significant relationship between microbial and enzyme activity and improves the soil water status (Shen et al., 2016). However, carbon is positively correlated with the microbial biomass carbon (Almeida et al., 2011). Hoeing practice in the SCI created a soil mulch, which reduced evaporation and improved soil moisture and, thus, enhanced soil enzymatic activities compared with the conventional method. Among the genotypes, APA and ARA were considerably impacted by soybean genotype. However, genotypes had no apparent impact on the levels of DHA, total polysaccharides, MBC, or soil chlorophyll. Thus, DS12-13 exhibited significantly higher APA (90.0 µg p–nitro phenol g^{-1} soil $hr.^{-1}$) and ARA (18.8 n moles ethylene g soil $hr.^{-1}$) than the other genotypes.

5. Conclusion

From the present study, the following conclusions have been drawn for the farming community, research planners, and policy makers:

- In soybean growing regions, soybean crop intensification (SCI) exhibits higher growth and physiological attributes, such as

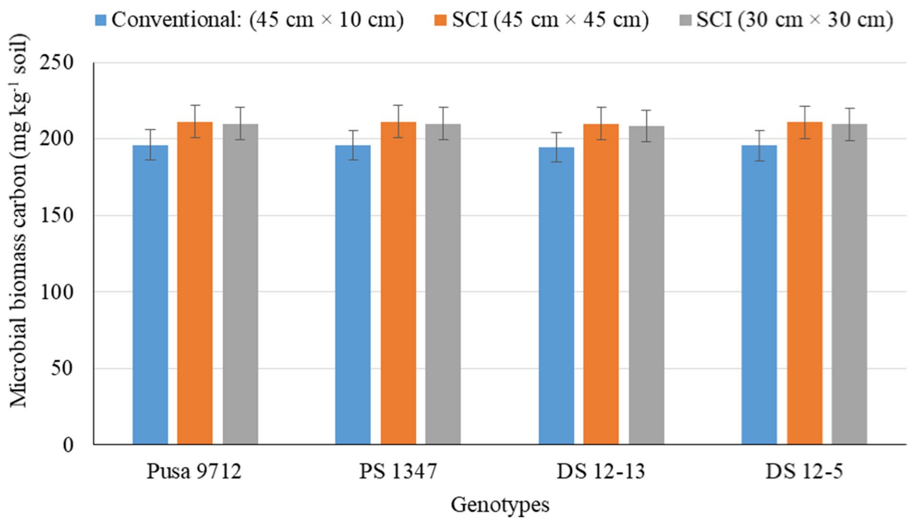

FIGURE 5
Effect of crop establishment methods and soybean cultivars on microbial biomass carbon (mean data of 2 years).

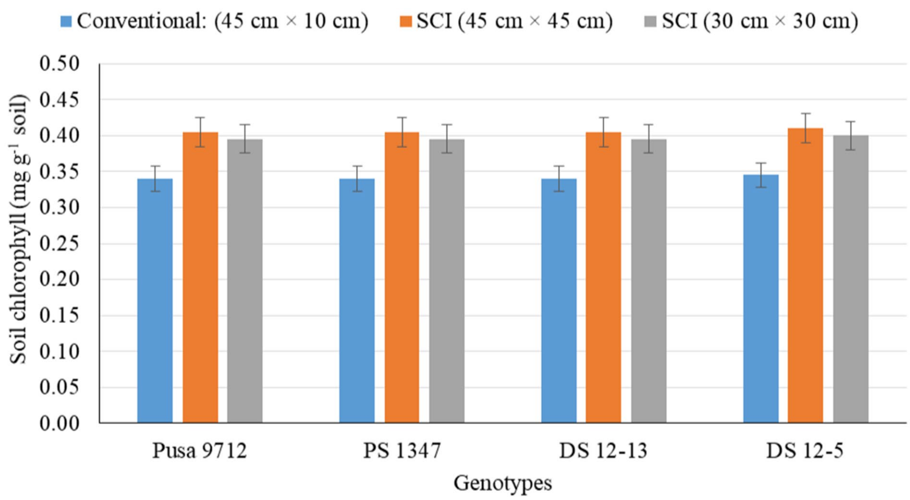

FIGURE 6
Effect of crop establishment methods and soybean cultivars on soil chlorophyll (mean data of 2 years).

stomatal conductance, intercellular CO_2 concentration, and net photosynthetic rates, than conventional methods of cultivation.

- Thus, enhanced physiological attributes increased soybean seed yields from 9.6 to 13.3% and biomass yields from 8.2 to 10.7% through the adoption of SCI at 30 cm × 30 cm over the conventional method of cultivation (45 cm × 10 cm).
- Concurrently, intercoalition operation using a hand hoe in SCI plots resulted in reduced weed infestation, thereby increasing the number of root nodules and improving root attributes and crop water productivity.
- Wider spacing at 30 cm × 30 cm or 45 cm × 45 cm with SCI exhibited higher photosynthetically active radiation (PAR) interception and transmittance by soybean genotypes than with the conventional method.
- Soybean genotypes DS 12–13 and DS 12–5 were superior in increasing soybean yields than the other genotypes.
- Concurrently, soil biological properties, such as dehydrogenase activity (DHA), alkaline phosphatase activity (APA), acetylene reduction assay (ARA), total polysaccharides, microbial biomass carbon (MBC), and soil chlorophyll, were also significantly

enhanced with SCI compared with the conventional method of soybean establishment.

- Therefore, the adoption of SCI either at 30 cm × 30 cm and/or 45 cm × 45 cm could provide the best environment for microbial activities beneath the soil and sustainable productivity of the soybean aboveground.'

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

RamS: methodology, statistical analysis, investigation, writing—original draft. SD and VSi: conceptualization, supervision, writing—review and editing, funding acquisition. PU, GR, and RajS: statistical analysis and writing—original draft. RK and KS: writing—review and editing. SuB: investigation, formal analysis and writing—original draft. SSR: editing final manuscript. AD and SR: data tabulation and statistical analysis. AK: investigation, writing—original draft. GG: statistical analysis of data. GS: statistical analysis and tabulation. VP: statistical analysis. BK and ShB: manuscript editing. VSh: soil analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1194867/full#supplementary-material

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Effects of varied nutrient regimes on soil health and long-term productivity in a rice–wheat system: insights from a 29-year study in the mollisols of the Himalayan Tarai region

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The maintenance of sustainability and quantification of soil health in the rice–wheat system in the Himalayan tarai region is of utmost importance, and a long-term study can properly demonstrate what needs to be done to achieve this. The current study was conducted after the completion of a 29-year crop cycle in the rice–wheat system in 2015 at Pantnagar. Since the beginning of the experiment in 1984, various NPK combinations with or without Zn and farmyard manure (FYM) applications were maintained in a fixed layout along with an absolute control plot without any external nutrition. FYM at 5 mg ha^{-1} and Zn at 5 kg ha^{-1} were applied in only rice, and NPK-chemical fertilizers were applied both in rice and wheat. The results revealed that the application of N at 120 kg ha^{-1} + P at 40 kg ha^{-1} + K at 40 kg ha^{-1} + FYM at 5 mg ha^{-1} + Zn at 5 kg ha^{-1} (NPK + FYM + Zn) resulted in the maximum attainment of long-term system productivity and the sustainable yield index (SYI), which were 22% higher than those with NPK application. NPK + FYM + Zn and NPK + FYM also improved the soil's overall physical, chemical, and biological parameters. Soil organic carbon, dehydrogenase activity, soil available P and K, phosphate solubilizing bacteria, and actinomycetes were found to be the most important soil quality parameters in Mollisols. From this study, it can be concluded that the application of the recommended NPK along with FYM and Zn can improve soil health and sustain the system productivity of the rice–wheat system in Mollisols of the Himalayan tarai region.

KEYWORDS

integrated nutrient management, rice-wheat cropping system, soil quality index, sustainable yield index, system productivity

1. Introduction

The rice–wheat cropping system is an immensely important cropping system in India, especially in the famous Indo–Gangetic plains, (IGP) which cover approximately 12.3 Mha (Kar et al., 2021).

Being cereal-intensive, this system is known to deplete NPK and zinc to the tune of approximately 650 kg ha^{-1} and 0.5–1.0 kg ha^{-1}, respectively, from soil annually (Shah et al., 2011). The continuous practice of this system with excessive chemical fertilizers since the era of the green revolution in India has led to several soil–environment problems, including multi-nutrient deficiencies, loss of soil organic carbon (SOC), environmental pollution, and so on. All these problems have contributed to a yield stagnation of the system (Yadav and Kumar, 2009; Kar et al., 2021; Pramanick et al., 2022). Despite being one of the most heavily fertilized cropping systems in India, it has been repeatedly marked as suffering from poor nutrient management strategies, injudicious application, and a loss in the efficiency of resource use (Kar et al., 2021; Kumar et al., 2021; Laik et al., 2021b; Lakshmi et al., 2021). Long-term application of imbalanced nutrition to the rice–wheat system should be reviewed with respect to soil quality vis-à-vis system productivity. Many previous studies have recommended that a complete organic mode of nutrient management is a plausible option that can improve productivity and soil health (Laik et al., 2021a). However, relying solely on organic nutrient management presents a significant challenge due to the limited organic availability, prompting scientists to explore potential alternatives. Long-term application of farm yard manure (FYM), along with mineral NPK in tropical and sub-tropical soil, can enhance the labile soil organic matter pools (Laik et al., 2021a). Thus, the incorporation of FYM in the nutrient management modules might be a good option in the rice–wheat cropping system. Indian soil is also becoming deficient in Zn, which can be reclaimed through the sequential application of Zn in the rice–wheat system (Lakshmi et al., 2021). The current study was conducted in one of the most fertile lands of India, *viz.,* the Mollisols of the Himalayan tarai region. These soils are known for their high organic matter content and, thus, higher nutrient availability for crops. However, several studies have reported that intensive cultivation of cereal-based cropping has resulted in multi-nutrient deficiencies in these soils, which include universal nitrogen deficiency along with deficiencies of potassium, sulfur, and zinc in widespread areas with the rice–wheat cropping systems for a significant period of time with no or limited organic inputs (Sharma et al., 2014).

Soil quality evaluation is crucial for understanding factors that contribute to crop production in different ecologies (Kumar et al., 2021; Mahapatra and Dey, 2022). A comprehensive soil quality assessment considering the maximum number of soil properties of any soil is much more acceptable for representing soil quality than considering any single important parameter. Traditionally, soil quality is characterized by its physical and chemical attributes (Bünemann et al., 2018). However, recent studies have shown that soil-biological attributes, especially in tropical and sub-tropical soils, are the most sensitive parameters in determining soil quality (Kumar et al., 2021; Laik et al., 2021a). Soil's bio-physico-chemical properties need to be evaluated properly to assess the influence of organic additions on overall soil quality (Min et al., 2003). A soil quality index (SQI) is a useful tool for interpreting data from various soil measures and assessing whether land-use management practices have the desired impacts on production, soil health, and environmental protection (Vasu et al., 2016). Despite the importance of the SQI, the SQI for the rice–wheat system in Mollisol soil has not been quantified properly. Therefore, the quantification of soil quality parameters for the rice–wheat system in Mollisols can provide valuable insights for future research and crop producers, helping to achieve optimal soil management and higher production.

Long-term studies have been recognized as a crucial tool for understanding these impacts. Previous studies conducted in India have investigated the effects of long-term fertilization and manure on soil biological characteristics and the SQI in various agricultural systems and climatic circumstances (Saha et al., 2008; Bedi et al., 2009; Kumar et al., 2021). However, these scientific findings are not enough for the predominant rice–wheat systems in the Mollisols of the Himalayan tarai region, which are considered one of the most fertile soils in the country.

This experiment is distinctive in that it evaluates all soil quality criteria in the Mollisols of the Himalayan tarai region with a special emphasis on the rice–wheat system. This initiative acknowledges the importance of soil biological properties, particularly in tropical and sub-tropical soils, in determining soil quality, whereas earlier studies mainly focused on the physical and chemical characteristics of the soil. In this study, we aimed to construct an SQI unique to the rice–wheat system in the Mollisols by considering a wide variety of soil characteristics. The current study aimed to investigate the impact of long-term nutrient management schedules on soil quality parameters and crop productivity in the Himalayan tarai region of IGP. Furthermore, the project highlights the importance of long-term studies to understand the impacts of nutrient management practices on soil quality and crop productivity. While previous research has investigated similar aspects in different agricultural systems and climatic conditions, there is a lack of specific studies on the rice–wheat systems in the Mollisols of the Himalayan tarai region. Comprehensive soil quality parameters in relation to long-term nutrient management practices in this region have not been studied in detail. This study is expected to provide useful and systematic information on the long-term impact of nutrient schedules on soil quality parameters, soil functions, and crop performance, contributing to the current knowledge gap on the subject.

2. Materials and methods

2.1. Experimental site

The present experiment was conducted after the completion of the 29th cycle of rice–wheat cropping, which was initiated in June 1986. This study was conducted at the Borlaug Crop Research Farm of Pantnagar University, India (29° N, 79°29' E, and 244 m above MSL). The research site was in a sub-humid and sub-tropical zone with hot and humid summers and cold winters. The prevailing

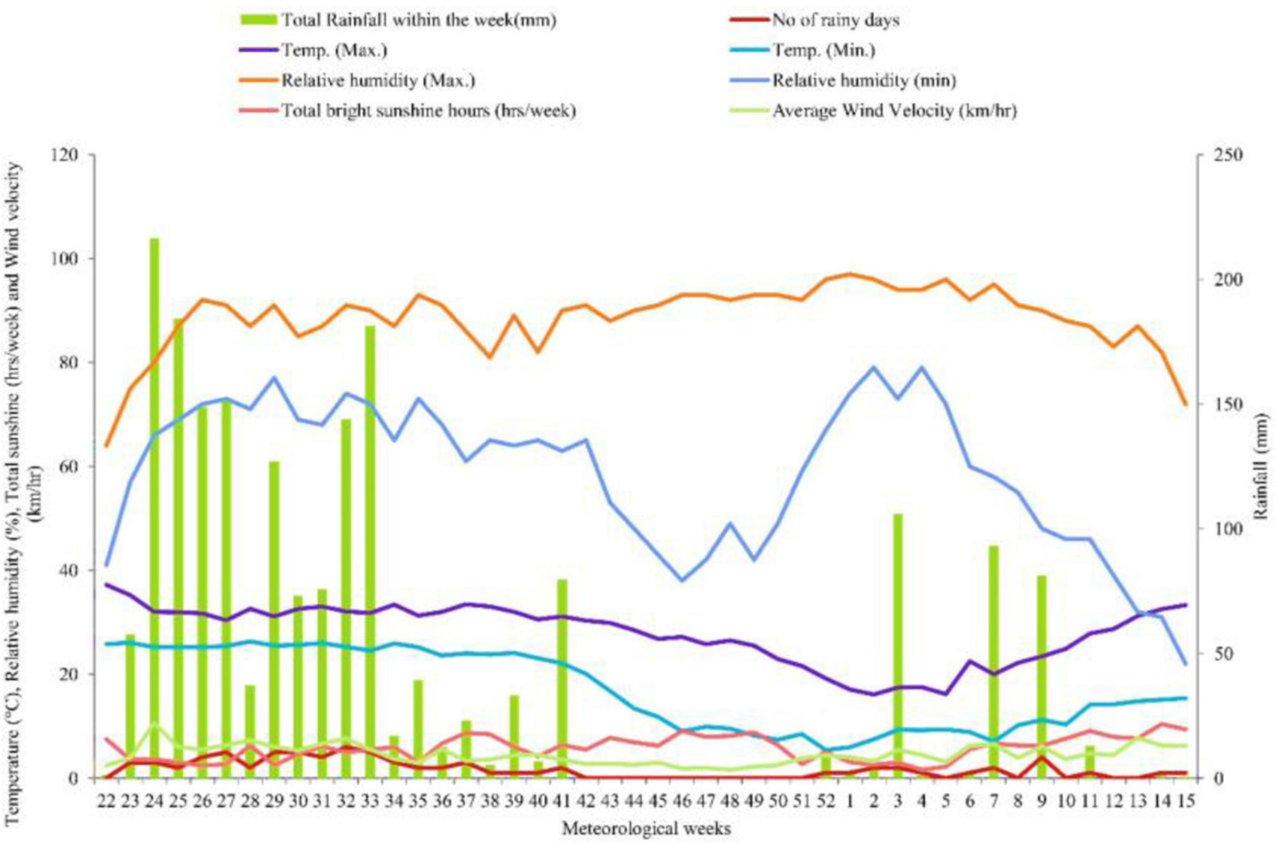

FIGURE 1
Meteorological data during the experimentation period (2015–16).

weather conditions during the current experimental period are reflected in Figure 1. The origin of the experimental soil was alluvial. Study soil falls under the order Mollisols and is classified under the sub-group Aquic hapludoll (Deshpande et al., 1971). The soil texture was clay-loam, which contained sand, silt, and clay of 32, 39, and 29%, respectively. The soil was medium-fine granular in the structure with good water-holding capability. In 1986, when the long-term experiment was initiated, the soil at the experimental site had a pH of 7.8, a cation exchange capacity (CEC) of 20.0 c_{mol} (p +) kg^{-1}, and an electrical conductivity of 0.12 dS m^{-1}. The soil was high in organic carbon (1.2%, or 2688 kg ha^{-1}), with 243 kg ha^{-1} of available nitrogen (alkaline $KMnO_4$ method), 20.0 kg ha^{-1} of available phosphorus ($NaHCO_3$ extracted), 222 kg ha^{-1} of available potassium (NH_4OAC extracted), and 0.8 kg ha^{-1} of available zinc (DTPA extracted).

2.2. Experimental design and treatments

The field experiment was laid permanently in the rainy season of 1986 in a randomized complete block design (RCBD) with four replications and 10 treatments, *viz*., absolute control (no application of any fertilizers); 120 kg ha^{-1} N (N); 120 kg ha^{-1} N + 40 kg ha^{-1} P (NP); 120 kg ha^{-1} N + 40 kg ha^{-1} K (NK); 120 kg ha^{-1} N + 40 kg ha^{-1} P + 40 kg ha^{-1} K (NPK), NPK + FYM (farm yard manure) at 5 mg ha^{-1} (NPK + FYM), NPK + 5 kg ha^{-1} Zn (NPK + Zn); NPK + FYM + 5 kg ha^{-1} Zn (NPK + FYM + Zn); 150 kg ha^{-1} N + 40 kg ha^{-1} P + 40 kg ha^{-1} K (125% N + PK); and 150 kg ha^{-1} N + 80 kg ha^{-1} P + 40 kg ha^{-1} K + 5 kg ha^{-1} Zn (150%N + 200%P + K + Zn). The base treatments were determined based on prevailing fertilization recommendations (NP) and farmers' practices (N). Other treatments, including K and Zn in nutrient schedules, were formulated using crop demand studies and analyzing crop deficiencies. Higher levels of N and P were also included due to the economic response of the system and based on some farmers' practice of over-fertilizing the crop. For the integrated mode, nutrient substitution with FYM has been studied alone and in combination with Zn supplementation. The nutrient sources used for the treatments were commercial-grade urea (46% N), single super phosphate (16% P), fertilizer-grade KCl (60% K), and $ZnSO_4.7H_2O$ (21% Zn). Farmyard manure (FYM) was applied at 5 mg ha^{-1} on a dry weight basis only in the case of rice cultivation at the time of final land preparation. The nutrient contents of FYM were 0.3–0.5% N, 0.1–0.2% P, and 0.3–0.75% K on a dry weight basis. Full doses of P, K, and half of the N in each treatment were applied as basal. The rest of the N was applied in two equal splits. Zn was applied only to rice during secondary tillage operations. Field preparation and establishment techniques for rice and wheat were conventional (puddling-transplanting in rice and conventional cross-harrowing in wheat). The rice variety

Pant Dhan 4 was transplanted in the second week of July and harvested in the first week of November. Wheat (variety UP 2425) was sown in the second fortnight of November.

2.3. System productivity and sustainability parameters

The current experiment comprised two different crops, *viz.*, rice and wheat, which not only have different kinds of yield potential but also fetch a differential price at the market. Thus, the productivity of the system was calculated in terms of rice equivalent yield (REY) using the following formula (Kar et al., 2021):

$$\text{System productivity}(\text{Mg ha}^{-1}) = \text{rice yield}\left(\text{Mg ha}^{-1}\right) + \frac{\text{Wheat yield}\left(\text{Mg ha}^{-1}\right) \times \text{Price of wheat}}{\text{Price of rice}} \quad (1)$$

To calculate the long-term system productivity, the average system productivity over the years was considered. The SYI was calculated with the following formula (Kumar et al., 2021):

$$\text{SYI} = \frac{\overline{Y} - \sigma}{Y_{max}} \quad (2)$$

where $\overline{Y}$ stands for average productivity, σ stands for the standard deviation in the system productivity, and Y_{max} is the maximum witnessed system productivity.

In this manuscript, the current system's productivity has been calculated with the data from the 2015–16 season. While long-term system productivity was calculated using the average of 29 years' system productivity values, changes between long-term and current system productivity were expressed as percentage changes.

2.4. Soil quality parameters

A composite soil sample from each plot was collected using a bucket auger from 0 to 15 cm depth after harvesting wheat (the end of one rice–wheat cycle) in 2015. The soil samples were collected randomly from five spots in each plot and mixed into one composite sample. For chemical analysis, soil samples were processed by shade drying and passed through a 2-mm sieve. For microbiological and enzymatic assays, the collected soil sample was stored at a low temperature of 0–4°C for 1 week. In the current experiment, soil physical parameters such as bulk density (BD), soil chemical parameters, such as organic carbon (OC), available nitrogen (alkaline $KMnO_4$ method), phosphorus ($NaHCO_3$ extracted), and available potassium (NH_4OAC extracted), different soil biological parameters such as soil dehydrogenase activity (DHA) and phosphatase activity, *viz.* alkaline phosphatase (ALK) and acid phosphatase (ACP), and potentially mineralizable nitrogen (PMN) were determined during the year 2015. The bulk density (BD) of soil was determined using a core sampler with a core of 5 cm height and 5 cm diameter. The mass of the collected soil in the core was measured and divided by the volume of the core to obtain the BD in mg m^{-3} (McKenzie et al., 2002). The ground, air-dried soil samples were passed through a 0.2-mm sieve to analyze soil organic carbon (SOC). SOC was determined through the modified Walkley and Black method (Jackson, 1973) using 1N $K_2Cr_2O_7$, conc. H_2SO_4, orthophosphoric acid, sodium fluoride, and ferrous ammonium sulfate solution. Available (mineralizable) nitrogen (N) in the soil was determined by using the alkaline permanganate ($KMnO_4$-N) method (Subbiah and Asija, 1956). Phosphorus was estimated using sodium bicarbonate as an extractant (Olsen et al., 1954). Color development was measured using a spectrophotometer (Double Beam Spectrophotometer AU2703, Systronics (India) Ltd., India) at 720 nm wavelength with known standards. Available potassium (exchangeable + water-soluble K) in soil was determined using a neutral normal ammonium acetate solution using a flame photometer (Systronics (India) Ltd., India) (Jackson, 1973). The soil dehydrogenase activity was estimated using the method described by Casida et al. (1964). Approximately 6 g of soil sample was saturated with 1.0 ml of freshly prepared 3% triphenyltetrazolium chloride (TTC) and incubated for 24 h in the dark. After incubation, methanol was added to stop enzyme activity, and the absorbance of the filtered aliquot was read at the 485 nm wavelength of the spectrophotometer. These results were expressed as μg TPF g^{-1} soil d^{-1}. The soil phosphatase activity was assayed in 1 g of soil saturated with 4 ml of modified universal buffer (MUB) and 1 ml of p-nitrophenolphosphate, followed by incubation at 37°C for 1 h. After incubation, 1 ml of 0.5 M $CaCl_2$ and 4 mL of NaOH were added, and the contents were filtered through a Whatman No. 1 filter paper. The amount of p-nitrophenol in the sample was determined at 400 nm, and the enzyme activity was expressed as μg p-NP g^{-1} soil h^{-1} (Tabatabai and Bremner, 1969). For measuring potentially mineralizable N (PMN), the methods described by Benedetti and Sebastiani (1996) were used. To analyze PMN, 50 g of soil, air-dried and sieved through a 2-RAM screen, was mixed with quartz sand in a 1:1 ratio (sand particle size, 0.2–0.8 mm) and incubated in a Buchner funnel (outer diameter 13 cm) at 60% water-holding capacity and at 30°C for 30 weeks. The mineral N in the soil was leached before incubation by adding 900 ml of CaSO4 solution and 100 ml of nutrient solution without N (0.002M $CaSO_4.2H_2O$, 0.005M $Ca(H_2PO_4)$, 0.0025M K2SO4, 0.002M $MgSO_4$). NO_3^- -N and $NH_4{}^+$ -N concentrations were measured periodically (after 2, 4, 8, 12, 16, 22, and 30 weeks) using an autoanalyzer (Vapodest, Gerhardt, Germany). At the end of the incubation period, exchangeable $NH_4{}^+$ -N was extracted using a 2N KC1 solution following the Bremner (1965) procedure. For microbial counts, collected moist samples were stored at 4°C for less than a month until required for analysis. The pour method and surface spread plate count methods were followed to count the population of the soil microbial community. Thornton's agar medium (Thornton, 1992), Jensen (1930) agar medium, and Martin (1950) Rose Bengal streptomycin agar medium (Martin, 1950) were used to count the total number of viable bacteria, actinomycetes, and fungi, respectively.

2.5. Soil quality index

For the determination of the SQI, a four-step method, as indicated by Masto et al. (2007), was adopted. These four steps

TABLE 1 Principal component analysis of soil parameters.

Parameters	PC-1	PC-2	PC-3	PC-4
Eigenvalue	7.498	1.234	1.085	1.024
Variance	49.99	8.23	7.24	5.83
Cumulative variance	49.99	58.22	65.45	71.28
Eigenvectors				
pH	0.182	−0.095	−0.085	−0.049
BD	0.147	−0.098	0.104	−0.110
OC	**0.844**	−0.226	−0.162	−0.047
N	**0.820**	−0.252	−0.259	−0.102
P	**0.764**	0.027	−0.108	0.407
K	0.758	**0.755**	0.072	0.489
BAC	0.706	0.278	0.382	−0.096
FUN	0.697	−0.316	−0.284	0.170
ACT	0.185	0.223	0.154	**−0.525**
AZO	0.664	0.090	0.217	−0.092
PSB	0.159	**0.681**	0.276	0.057
ALK	0.137	−0.222	0.388	0.322
ACP	0.611	0.212	−0.061	0.178

The bold values explain the most dominant parameters in the individual principal component (PC).

included goal setting, selection of the minimum data set (MDS), indicator scoring, and computation of the SQI based on relative scoring and weights. In the current study, the SQI was calculated to correlate soil indicators with SYI and the system's productivity. A total of 15 soil parameters, *viz.* pH, BD, OC, N, P, K, bacterial count (BAC), fungi count (FUN), actinomycetes count (ACT), Azospirillum, phosphorus solubilizing bacteria (PSB), ALK, ACP, DHA, and PMN, were taken into account, of which only six parameters, *viz.*, OC, DHA, P, K, PSB, and ACT, were selected for the MDS based on their performance in the principal component analysis (PCA), and redundancy in attribute selection was reduced using the procedure described by Andrews et al. (2002b). Principal components (PCs) were extracted based on Eigenvalues of >1 and variation explained by individual PCs of >5% criteria (Andrews et al., 2002a,b).

Further, the number of parameters was reduced using correlation analysis, and factors within the same PC showing significant correlation were identified and simplified. From a wide range of soil quality parameters, the SQI was calculated to properly address the soil quality status in the experimental field so that it can be linked well with the system's productivity and sustainability. The SQI was calculated based on the minimum data set (MDS), which was composed of four principal components (PCs) that had Eigenvalues of >1.0, as presented in Table 1. PCs with higher Eigenvalues were considered better describers of soil quality. Principal components were extracted from a total of 15 soil bio-physico-chemical parameters of importance. The variability in the parameters found in PC 1, PC2, PC3, and PC4 were 49.99, 8.23, 7.24, and 5.83%, respectively. The total variation explained by all four PCs was 71.28%. The factors with the highest absolute loadings and factors lying within 10% of the highest loading factors were retained for the MDS from each PC. If a single factor was selected for multiple PCs, it was assigned to the PC with the highest loading. For PCs, where multiple factors were selected, highly correlated factors (i.e., redundant) were eliminated, and only factors with the highest loading were kept for MDS, whereas factors that were not significantly correlated were kept (Supplementary Figure S1). Thus, the selected indicators were grouped using the soil function to determine whether their higher value was considered 'good' or 'bad.' The parameters in the MDS for the current study were characterized as 'more is better.' For indicators that fell under the 'more is better' category, each observation was divided by the highest observed value so that it received a score of 1. In contrast to the 'less is better' indicators, each observation was divided by the lowest value so that the lowest observed value received a score of 1. A non-linear scoring function was used for scoring the parameters used in calculating the SQI, which was previously used by Kumar et al. (2021):

$$\text{Score(Y)} = \frac{1}{1 + e^{-b(X-A)}} \tag{3}$$

Where, X stands for the value of the indicator, A signifies the baseline value of the soil property where the score is 0.5, and b is the slope.

After the scoring of the variables, weights based on PCA results were assigned. Finally, the SQI was calculated using the following formula, as indicated by Mukherjee and Lal (2014):

$$\text{SQI} = \sum\nolimits_{n=1}^{n} (\text{Wi} \times \text{Si}) \tag{4}$$

Where, n stands for the number of variables in the MDS, Si is the score for soil parameters, and the PCA-derived weighing factor is Wi.

2.6. Statistical analysis

The ANOVA method for RCBD was followed to statistically analyze all the data. Fisher's LSD method (least significant difference) was adopted to differentiate the treatment means at 5% probability ($p \leq 0.05$), as stated in the study by Gomez and Gomez (1976). All the statistical computations were conducted using SPSS software version 25 (IBM Corp, 2017) and the R statistical package.

3. Results

3.1. System productivity and sustainability

In the current experiment, the system's performance was evaluated using rice equivalent yields in both the current cycle and the long term. The effect of different long-term nutrient management treatments on the current system's productivity was significant. The highest system productivity was recorded with NPK + FYM + Zn and NPK + FYM (10.5 mg ha^{-1}). The use of NPK + FYM even out yielded 150%N + 200%P + K + Zn, resulting in a 9.3% extra yield with fewer inputs. Adding FYM

TABLE 2 Effect of long-term fertility treatments on current and long-term system productivity, SYI, and change over initial system productivity.

Treatments	Current system's productivity (Mg ha^{-1})	Long-term system productivity (Mg ha^{-1})	SYI	Change over initial system productivity (%)
Control	3.2 ± 0.2^e	4.2 ± 1.7^e	0.35	−42.5
N	4.8 ± 0.9^d	6.2 ± 2.1^d	0.38	−56.2
NP	8.1 ± 1.1^c	8.8 ± 1.3^c	0.65	−25.7
NK	4.6 ± 0.6^d	6.2 ± 2.0^d	0.38	−58.4
NPK	8.6 ± 0.8^c	9.5 ± 1.2^{bc}	0.71	−25.9
NPK + FYM	10.5 ± 1.1^a	10.7 ± 1.0^a	0.79	−3.2
NPK + Zn	8.8 ± 0.9^{bc}	9.6 ± 1.2^b	0.68	−23.2
NPK + FYM + Zn	10.5 ± 1.2^a	10.6 ± 0.9^a	0.79	−5.9
125% N + PK	9.3 ± 1.2^{bc}	9.5 ± 0.9^{bc}	0.73	−11.5
150%N + 200%P + K + Zn	9.6 ± 1.0^b	9.7 ± 0.9^b	0.75	−10.4

Values are followed by the standard deviation (±); different letters followed by values represent statistical significance ($p \leq 0.05$).

with NPK resulted in a 22.1% enhancement in system yield after 29 years (Table 2). A similar trend was observed for long-term system productivity. The same treatments were also found to result in the highest SYI than others, indicating the stability of high-yielding ability over a long duration. All the treatments resulted in lower system yields than the initial system productivity levels in the current year. However, the quanta of reduction ranged between 3.2 and 5.9% for treatments with NPK + FYM and NPK + FYM + Zn. Using NPK alone resulted in a 25.9% reduction in system productivity over the years. Treatments with higher chemical inputs, such as 125%N + PK and 150%N + 200%P + K + Zn, also resulted in 11.5 and 10.5% reductions in system productivity in the long run.

The curve was plotted to understand the system's performance in different treatments over a temporal scale and a time series (Figure 2). Fluctuations between replications under different fertilization regimes in each year are shown in Supplementary Table S1. Figure 2 shows that only after four cycles did the performance of insufficiently fertilized plots (N, NK) decline.

The plots with high inorganic inputs, *viz.* 125%N + PK and 150%N + 200%P + K + Zn, resulted in higher system performance in the initial 3 years, after which the performance started declining, and after 6 years, it stabilized but at a lower level than the initial phase.

Treatments involving integrated nutrient inputs, namely NPK + FYM and NPK + FYM + Zn, initially performed poorly compared to high nitrogen treatments. However, after a period of 5 years, these treatments demonstrated a significant improvement and reached a stable and enhanced yield level.

3.2. Soil physical and chemical parameters

The effect of the different nutrient management regimes on bulk density and soil organic carbon after long-term experimentation was found to be significant (Figure 3). After the completion of the 29th cycle of rice–wheat cropping, treatments with FYM in combination with inorganic fertilizers were recorded to have the lowest BD of soil at 0–15 cm depth. All other treatments resulted in a significant increase in the bulk density from the initial value of 1.26 mg m^{-3} in 1986. NPK + FYM and NPK + FYM + Zn resulted in statistically similar bulk densities even after extensive rice–wheat cropping, in which puddling had been regularly carried out on rice crops. Among inorganic fertilizer-only treatments, 150%N + 200%P + K + Zn resulted in the lowest BD of 1.31 mg m^{-3}. The BD recorded in control and N-only plots were approximately 7.9 and 6.2% higher than the BD recorded in NPK + FYM (Figure 3). At the end of 29 years of rice–wheat crop rotation, NPK + FYM + Zn and NPK + FYM applications were found to have statistically similar organic carbon levels as compared to the initial years (Figure 3).

All the other treatments resulted in a notable reduction in soil organic carbon content. The lowest OC content (0.72%) was recorded for control plots, whereas the highest OC was recorded with NPK + FYM and NPK + FYM + Zn. The use of 150%N + 200%P + K + Zn resulted in a reduction of OC in a 0–15 cm soil profile. Such reductions in OC in inorganically fertilized plots demonstrate the need for supplementary organic inputs to the system to maintain the OC. Treatments such as N, NP, and NK resulted in a reduction in OC by 28.9, 25.4, and 27.2% than NPK + FYM. Additional FYM applications with NPK showed a 32.5% increase in the OC compared to the OC under the application of NPK alone.

Concerning soil chemical status, the highest amounts of soil-available N, P, and K after wheat harvest were recorded from NPK + FYM and NPK + FYM + Zn-applied plots. Except for NPK + FYM, NPK + FYM + Zn, 125%N + PK, and 150%N + 200%P + K + Zn, all other treatments resulted in significantly lower soil available N than the initial years (Figure 4). Figure 4 shows that the application of 125% and 150% N resulted in lower available N in the soil than in treatments where an integrated nutrient management approach was adopted. The amount of available K in soil was observed to be similar in treatments where regular addition of K

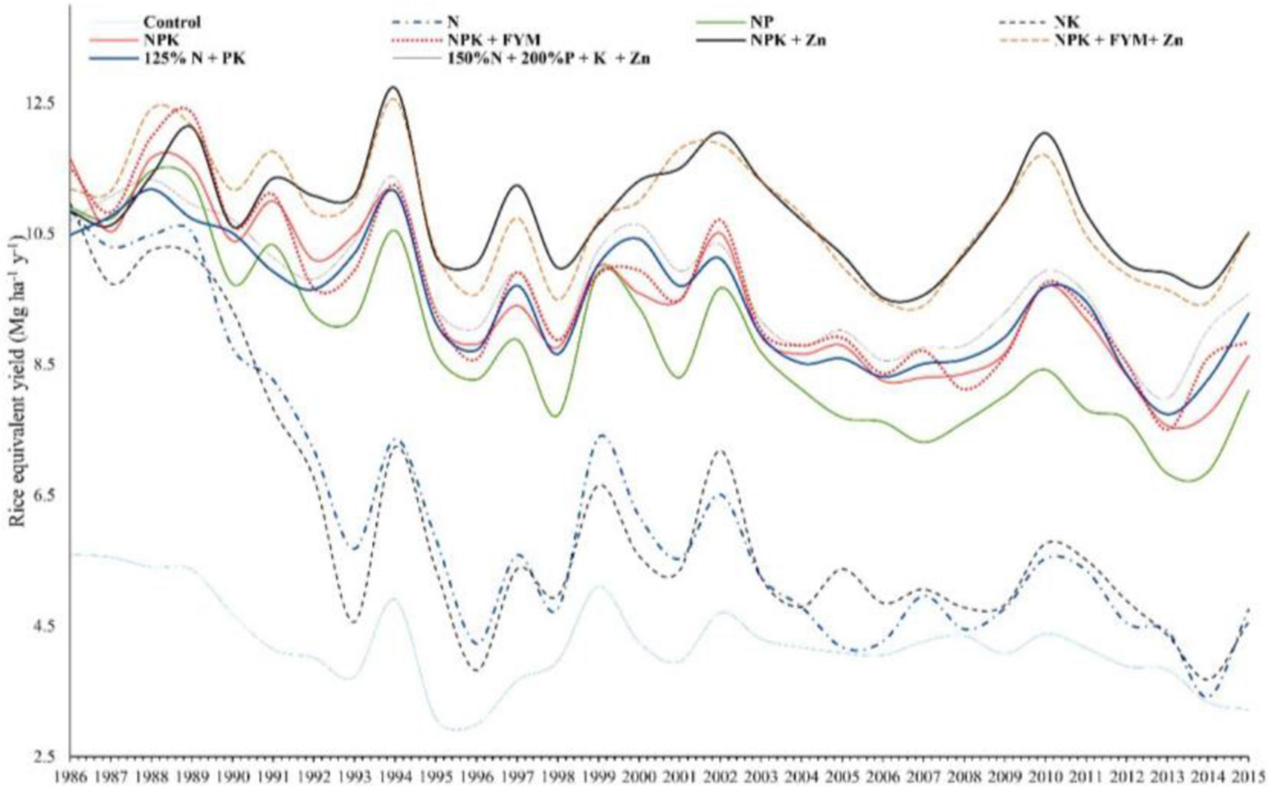

FIGURE 2
Time-series plot of system productivity from 1986 to 2015 under different fertility regimes [treatments are abbreviated as 120 kg ha-1 N (N), 120 kg ha-1 N + 40 kg ha-1 P (NP), 120 kg ha-1 N + 40 kg ha-1 K (NK), 120 kg ha-1 N + 40 kg ha-1 P + 40 kg ha-1 K (NPK), NPK + FYM (farm yard manure) at 5 mg ha-1 (NPK + FYM), NPK + 5 kg ha-1 Zn (NPK + Zn), NPK + FYM + 5 kg ha-1 Zn (NPK + FYM + Zn), 150 kg ha3 N + 40 kg ha-1 P + 40 kg ha-1 K (125% N + PK), and 150 kg ha-1 N + 80 kg ha-1 P + 40 kg ha-1 K + 5 kg ha-1 Zn (150%N + 200%P + K + Zn)].

was performed (Figure 4). In treatments where K was not applied, such as control, N, and NP, a significant reduction in the amount of soil K available was observed. Soil available P was found to be the maximum under NPK + FYM and NPK + FYM + Zn, and these two best-performing treatments were found to be comparable concerning soil available P in 150%N + 200%P + K + Zn applied plots (Figure 4). Soil P availability was found to be considerably lower where organic manure or a higher percentage of P fertilizer was not applied.

3.3. Soil biological parameters

The dehydrogenase activity (DHA) at 0–15 cm soil depths under the rice–wheat cropping system differed significantly among all the treatments. The treatment of receiving no nutrients extraneously for 29 years resulted in the lowest DHA activity (Table 3).

However, the highest DHA activity was obtained due to the combined use of chemical fertilizer and FYM compared to control and other inorganic inputs. The application of Zn was observed to stimulate DHA activity. The combined use of FYM and Zn with NPK fertilizers resulted in the highest DHA activity, which was 31.9% higher than NPK + FYM, where no chemical Zn fertilizers were applied. The effect of different long-term nutrient management treatments on the soil's acid phosphatase (ACP) and alkaline phosphatase (ALK) activity was found to be significant. The highest ACP and ALK activity in the 0–15 cm soil layer was recorded under NPK + FYM + Zn, being statistically at the same level as NPK + FYM. In the case of ACP, the activity of ACP in 125%N + PK and 150%N + 200%P + K + Zn was also found to be statistically similar to that of the best-performing treatment. In both cases, the lowest ACP and ALK activities were detected in unfertilized plots, and insufficiently fertilized plots such as N, NP, and NK were also observed to perform similarly. All other treatments resulted in significantly higher activity of both phosphatases. The highest value of potentially mineralizable nitrogen (PMN) was estimated in NPK + FYM + Zn as being at par with NPK + FYM, and these two treatments were found to be significantly superior to all other treatments. The use of FYM with NPK resulted in 33.2% more PMN than NPK alone. The lowest PMN was recorded in the unfertilized control. The application of 50% additional N over the recommended N fertilizer did not show any significant increase in PMN.

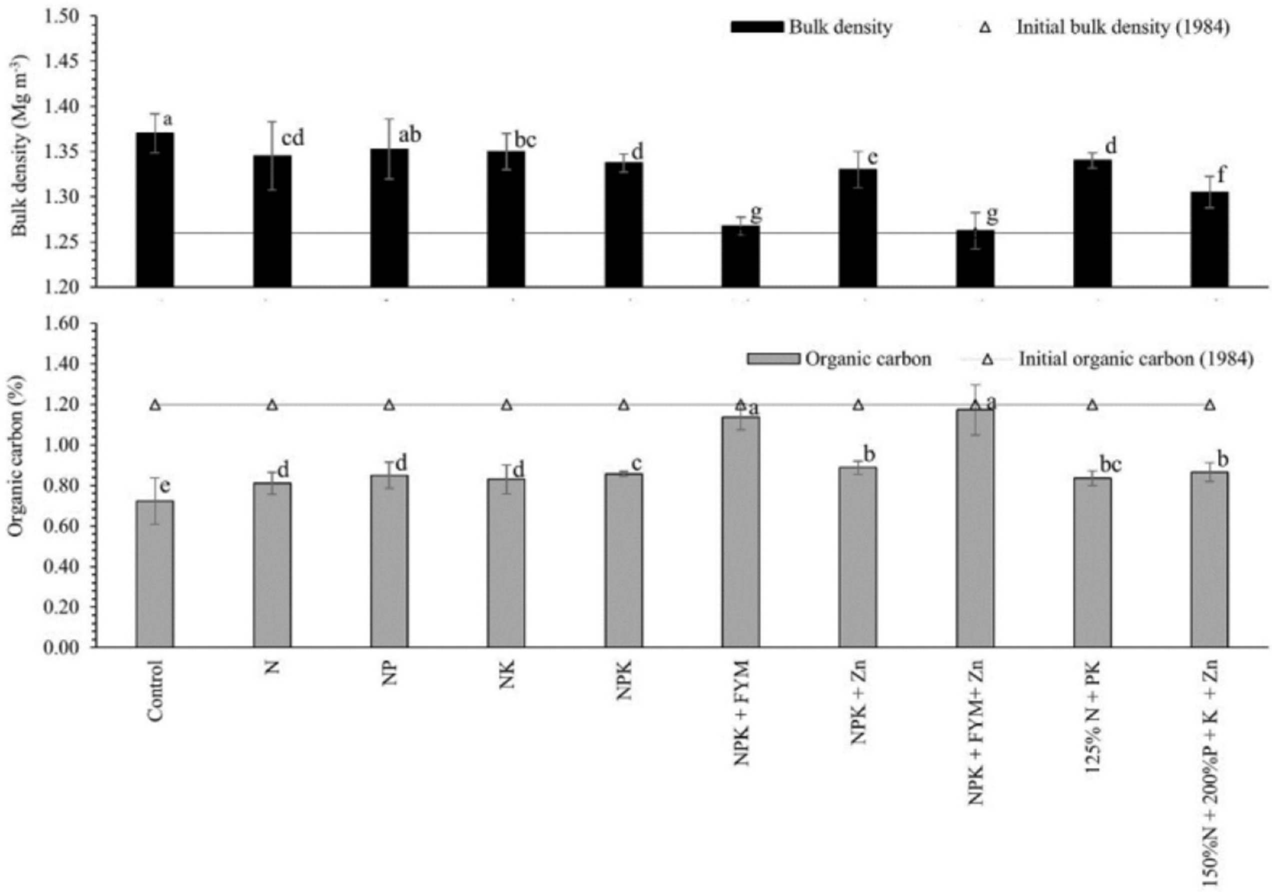

FIGURE 3
Effect of different long-term nutrient management on bulk density of soil and soil organic carbon content [different letters within the treatment pairs followed at the top of the bars represent statistical significance by Fisher's LSD test ($p \leq 0.05$)].

3.4. Soil quality index

In PC-1, OC, available N, and P were loaded, out of which only OC was retained, as a high correlation was observed between OC and N ($r = 0.74$) and OC and P ($r = 0.74$). In PC-2, K and PSB were retained as those factors were not highly correlated ($r = 0.46$). DHA and PMN were selected in PC-3; however, only DHA was retained due to the presence of a significant correlation between DHA and PMN ($r = 0.58$). In PC-4, ACT and K were selected, and both factors were retained due to non-significant correlations ($r = 0.37$). Finally, six soil quality attributes, *viz*., OC, DHA, P, K, PSB, and ACT, were selected for the MDS in the Mollisols of the study region to calculate the SQI.

For the computation of SQI, parameters that are most sensitive, easy to measure, and responsive to soil, crop, and climatic variations are preferred. In the current experiment, MDS contained OC, which has been identified as a very important soil quality indicator, followed by DHA. Soil chemical parameters such as available P and K were also found to be important parameters determining the SQI. Other included parameters were populations of PSBs and actinomycetes. The parameters were scored based on threshold values, and different parameters were noted from various sources presented in Table 4. The factors and their respective scores have been depicted in Supplementary Figure S2.

The SQI for the current experiment ranged from 0.58 to 0.79, depending on the treatments. The highest SQI (0.79) was observed in NPK + FYM + Zn, which was statistically at par with NPK + FYM (0.77). All other treatments resulted in significantly lower SQI than those treatments. SQI in NPK + FYM was 7.8% lower in NPK-only treatments (Figure 5). The use of higher chemical inputs also resulted in lower SQI than those in which regular organic matter was added. The lowest SQI (0.58) was recorded for the control. The average contribution of factors in the SQI across the treatments was to the tune of 75.4, 6.0, 4.4, 5.8, 4.3, and 4.0% for OC, DHA, P, K, PSB, and ACT, respectively. An x-y plot between SQI and system productivity (both current and long-term average) and SYI showed a direct relationship with a significantly high coefficient of determinations (Figure 6). The long-term system productivity has been expressed as SQI with a regression equation (2) with $R^{2=}$ 0.86, whereas the relationship between the current system's productivity and the SQI was expressed in equation (3) with $R^{2=}$ 0.83. The long-term SYI had also been observed to share a similar relationship,

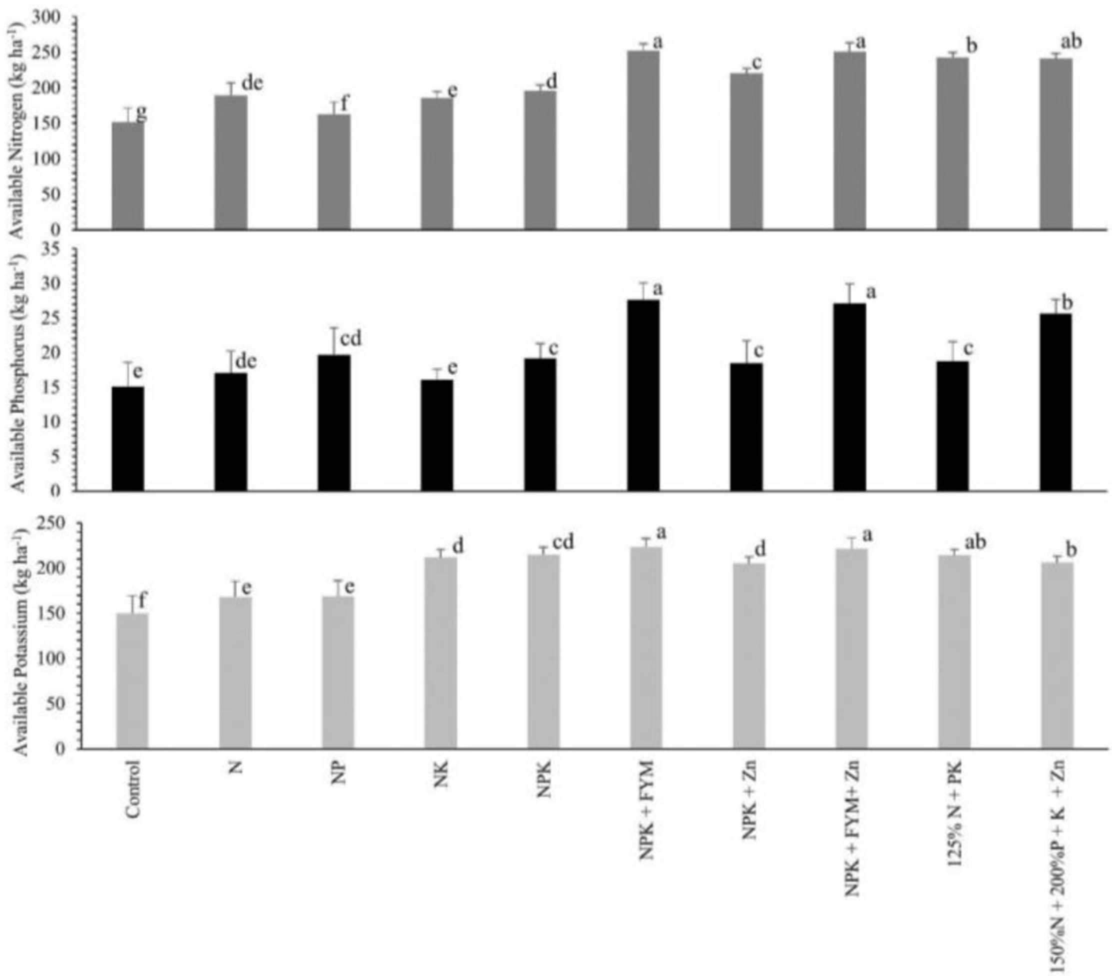

FIGURE 4
Effect of different long-term nutrient management on available nitrogen, phosphorus, and potassium in soil [different letters within the treatment pairs followed at the top of the bars represent statistical significance by Fisher's LSD test ($p \leq 0.05$)].

TABLE 3 Soil enzymatic activities under different long-term nutrition regimes in the rice–wheat cropping system.

Treatments	DHA activity ($\mu g\ g^{-1}\ 24h^{-1}$)	Alkaline phosphatase activity ($\mu g\ g^{-1}\ h^{-1}$)	Acid phosphatase activity ($\mu g\ g^{-1}\ h^{-1}$)	PMN (NH_4-N mg kg^{-1})
Control	168.00 ± 14.9^{d}	32.40 ± 3.5^{c}	44.35 ± 3.2^{f}	85.94 ± 4.0^{c}
N	266.10 ± 15.4^{c}	33.70 ± 2.3^{c}	50.50 ± 6.7^{ef}	100.09 ± 7.4^{bc}
NP	373.20 ± 7.6^{b}	34.20 ± 1.5^{c}	52.00 ± 2.4^{ef}	97.50 ± 6.0^{bc}
NK	231.10 ± 3.4^{c}	33.60 ± 4.2^{c}	45.10 ± 3.2^{f}	100.78 ± 8.2^{bc}
NPK	397.10 ± 37.0^{b}	44.40 ± 5.4^{b}	61.10 ± 6.6^{bcd}	102.03 ± 3.4^{bc}
NPK + FYM	404.90 ± 3.4^{b}	58.50 ± 1.8^{a}	70.20 ± 4.7^{ab}	135.94 ± 12.1^{a}
NPK + Zn	483.30 ± 29.0^{a}	47.90 ± 7.9^{b}	63.60 ± 4.9^{bc}	103.28 ± 5.8^{bc}
NPK + FYM + Zn	534.10 ± 62.7^{a}	59.80 ± 3.7^{a}	76.70 ± 1.6^{a}	147.66 ± 23.4^{a}
125% N + PK	398.60 ± 4.9^{b}	54.20 ± 3.4^{a}	56.30 ± 1.2^{de}	102.50 ± 6.2^{bc}
150%N + 200%P + K + Zn	429.10 ± 75.8^{b}	55.70 ± 4.2^{a}	57.10 ± 6.3^{cde}	114.06 ± 21.3^{b}

DHA and PMN denote dehydrogenase activity and potentially mineralizable nitrogen, respectively; values are followed by the standard deviation (±); and different letters in each column indicate significant differences among the means by Fisher's LSD test ($P < 0.05$).

TABLE 4 Threshold values and curve parameters of selected soil indicators in the minimum dataset.

Parameters	Lower threshold	Upper threshold	A/baseline	Slope	References
OC	0.2	1.27	0.4	4.523	Velmurugan et al., 2009
DHA	125	600	250	0.012	Masto et al., 2008
P	0.0	50	25	0.100	Masto et al., 2008
K	20	300	150	0.035	Masto et al., 2008
PSB	1.2	4.0	1.5	3.900	from the present study
ACT	1.2	3.0	1.5	4.200	from the present study

OC, DHA, P, K, PSB, and ACT denote soil organic carbon, soil dehydrogenase activity, soil available P, soil available K, phosphate solubilizing bacteria, and actinomycetes, respectively.

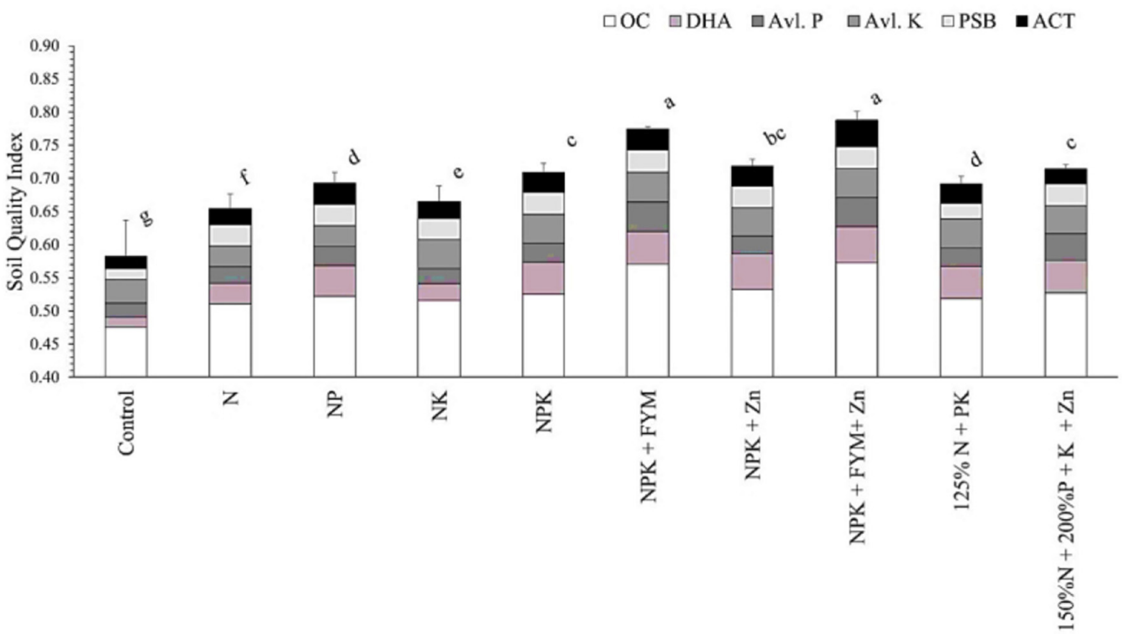

FIGURE 5
The SQI under different long-term fertility treatments [different letters within the treatment pairs followed at the top of the bars represent statistical significance using Fisher's LSD test ($p \leq 0.05$)].

which can be expressed in equation 4 ($R^2 = 0.74$).

$$\text{Long} - \text{term system productivity (kg ha}^{-1}) = 34.6x - 15.7$$
$$\text{Current system's productivity (kg ha}^{-1}) = 40.7x - 20.6$$
$$\text{SYI} = 2.6x - 1.2,$$

Where, x is the SQI.

Thus, the SQI was found to be linearly correlated with the current system's productivity and with long-term system productivity. System sustainability in terms of the SYI was also found to be well correlated with the SQI.

Extraction Method: Principal component analysis. PC stands for the principal component. Parameters are abbreviated as bulk density (BD), soil organic carbon (OC), available nitrogen (N), available phosphorus (P), available potassium (K), total bacterial count (FUN), total actinomycetes count (ACT), total azotobacter count (AZO), total phosphate solubilizing bacteria (PSB), alkaline phosphatase activity (ALK), and acid phosphatase activity (ACP).

4. Discussion

NPK + FYM + Zn- and NPK + FYM-based treatments improved system productivity and the SYI. These nutrient management options even out yielded 150%N + 200%P + K + Zn. These trends might be explained by the fact that the use of inorganic nutrient inputs without any additional organic matter addition may have resulted in systemic deterioration in soil health over a long period due to continuous cropping (Yang et al., 2020). Moreover, the application of balanced NPK coupled with organic input through FYM and the addition of micro-nutrient Zn resulted in better soil health by increasing SOC, lowering the BD, and improving the soil enzymatic activities, which ultimately helped in making the necessary soil nutrients available to the plant, resulting in a sustainable yield in the long run (Kumar et al., 2021; Laik et al., 2021a). This study also found significant correlations between system productivity, SOC, available N, DHA, ACP, and so on.

Only after four cycles did the yield of insufficiently fertilized plots (N, NK) decline, which might be explained by the mining

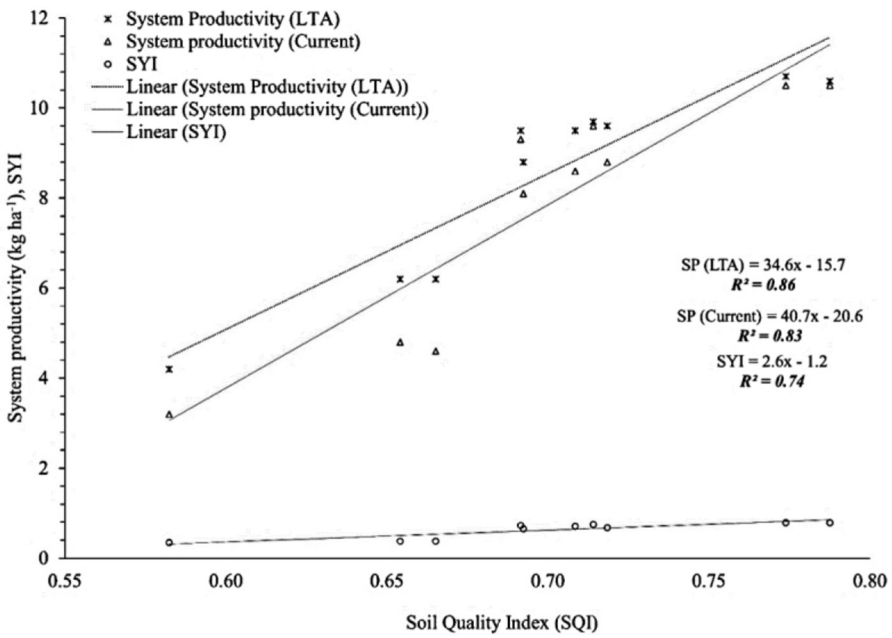

FIGURE 6
Linear relationship of current and long-term system productivity and the SYI with the SQI.

of soil nutrients due to insufficient or ill-balanced nutrition, specifically in an exhaustive system such as the rice-wheat system (Biswas and Naher, 2019). The plots with a high inorganic input showed higher yields only up to the initial 3-year cycle. Afterward, the yield declined. Higher doses of nutrients cannot sustain productivity in the long run due to the continuous depletion of soil carbon and other micronutrients and secondary nutrients and poor soil health (Hubanks et al., 2018). The nutrient regime with balanced inorganic + organic nutrients showed a comparatively poor yield to high nitrogen treatments. However, these integrated approaches stabilized the yield, reaching a significantly improved level only after 5 years.

After 14 years, these treatments significantly diverged from all other treatments and continued to outperform others. Similar trends were also observed by Mangaraj et al. (2022).

Transplanted rice, followed by a conventionally grown wheat system, is a resource-intensive and soil-damaging system, especially due to the destruction of soil structure due to puddling and tillage operations (Alam et al., 2014; Bhatt et al., 2016). Such intensive tillage operations on a long-term basis have significantly increased the soil bulk density, which was also observed in this study except under NPK + FYM + Zn and NPK + FYM, where soil BD was found to be almost similar even after 29 years. An integrated nutrient management mode with the regular addition of organic matter might have stabilized the soil structure to an extent where a change in the BD was greatly buffered (Ramesh et al., 2019). Thus, the BD was found to be almost similar under NPK + FYM and NPK + FYM + Zn, comparing the BD at the initiation of this long-term study. However, BD increased considerably in all other nutrient management regimes.

Soil organic carbon is central to soil health, quality, and sustainability in agroecosystems (Page et al., 2020). The experimental plots were previously forest lands and, thus, initially had a very high organic carbon content of 1.2%, which is rather rare in the cultivated lands of sub-tropical India. SOC was found to be almost at the same level even after 29 years of the rice–wheat cropping system under nutrient management with NPK + FYM and NPK + FYM + Zn. However, the SOC level was found to be considerably lower in all other nutrient management regimes. These results are consistent with the findings by Patel et al. (2018). Laik et al. (2021a) reported that long-term application of FYM with mineral N fertilizer in sub-tropical soil can improve the labile organic matter pools and SOC levels in the soil. Not only the external application of organic matter but also better root biomass and plant growth may contribute to improved soil organic matter, especially in the 0–15 cm soil depth (Ismail-Meyer et al., 2018).

In this study, it was observed that the application of 125% and 150% N resulted in lower levels of the available N in soil than that of under-integrated nutrient management. It might be due to the high losses of N under heavy fertilization (Hou et al., 2021). Treatments with higher fertilization rates without any organic matter also result in lower factor productivity and bear a high risk of becoming unsustainable over time (Meemken and Qaim, 2018). The higher N-availability in the FYM-applied plot might be attributed to higher soil-microbial activity through the FYM application, which facilitates N-mineralization (Hagemann et al., 2016). In this study, a higher correlation between soil available N and DHA was also found. Besides, the addition of organic matter as a source of nutrients also improves soil bio-physico-chemical health, which in turn results in the better availability of nutrients (Bot and Jose, 2005). The production of many organic acids during organic matter decomposition could facilitate phosphate solubilization (Alori et al., 2017). Improvement in the soil's available plant nutrients with the combined use of chemical fertilizers and FYM was also reported by many researchers (Patel et al., 2018). The amount of available K in soil was observed to be similar in treatments where regular addition of K was carried out. This may be justified by the fact that most of the soil K is not heavily dependent upon soil organic matter but depends on the soil mineral composition (Singh et al., 2021).

The soil DHA is an important index indicating the activities of soil microbes (Wolinska and Stepniewsk, 2012). DHA activity was also found to be higher when the addition of FYM was supplemented with the seasonal application of Zn, which might be due to the fact that Zn is a cofactor of many dehydrogenase enzymes and that the availability of Zn with proper carbon-nitrogen balance may have resulted in higher DHA activity (Mathpal et al., 2015). The treatments that have received no external inputs resulted in the lowest microbial activity due to low available nitrogen in the soil and lower microbial activity due to low root activity in crops. In the current study, a higher correlation between soil available N and DHA was found, which justifies this result. The phosphatase activity was also found to be increasing with an increased supply of inorganic nitrogen and phosphates (Widdig et al., 2019). Higher soil phosphatase activity is dependent on the addition of inorganic nutrients. In particular, phosphates had also been noted by Masto et al. (2006). The combined application of organic and inorganic fertilizers provides sufficient carbon and nitrogen sources in the soil and thus raises the activity of soil enzymes (Masto et al., 2006). Potentially mineralizable nitrogen (PMN) is an excellent parameter that can indicate the actual capability of soil microbes to mineralize the organic-residue-bound N into an available NH4 + form for plants (Osterholz et al., 2017). The PMN is strongly related to soil microbial biomass, SOC, and soil organic nitrogen (Osterholz et al., 2017). Increased levels of PMN indicate higher soil biological activity, which may be stimulated by organic matter added to the soil (Masunga et al., 2016). Thus, the nutrient management regimes (NPK + FYM and NPK + FYM + Zn) increased the SOC, soil available N, etc., and also increased the PMN compared to other treatments in this long-term study. A higher, more significant correlation between PMN and SOC was also found in this study.

In the current experiment, MDS contains OC, which has been identified as a very important soil quality indicator in Mollisols and central to the soil's physical, chemical, and biological health by many researchers (Wienhold et al., 2005; Courtney and Mullen, 2008; Bünemann et al., 2018). Soil chemical parameters such as available P and K are also of great importance, especially in the cereal-based cropping systems (Das et al., 2014). Concerning soil biological parameters, DHA was found to be the most important demonstration of other soil microbial activities (Zhang et al., 2010). Integrated nutrient management with regular FYM addition to the soil can thus improve SQI by favorably altering the soil's physical, chemical, and biological parameters, which ultimately resulted in higher system performance in the current year after a 29-year study. Only chemical input, even at high dosage, cannot sustain yield performance, resulting in poor soil quality due to continuous cropping (Röös et al., 2018).

By correlating system productivity (both present and long-term average) with the SYI, the SQI's accuracy was shown. High coefficients of determination were observed in the study to show a direct link between SQI and system performance. The SYI, the present system's productivity, and the long-term system's productivity were all linearly connected to the SQI. Furthermore, the selected soil parameters in the MDS align with their importance in assessing soil quality in the Mollisols. Organic carbon (OC) is a crucial indicator of soil health, and available phosphorus (P) and potassium (K) are essential for the cereal-based cropping systems. The soil biological parameter, dehydrogenase activity (DHA), reflects soil microbial activity. By combining chemical fertilizers with farmyard manure (FYM) and zinc, we found that the integrated nutrient management approach positively affected soil health and system productivity. The SQI used in this study offered an accurate and thorough evaluation of the soil quality criteria, their scoring, and their weighted contribution to the overall index. The SQI's relationship to system productivity and sustainability emphasizes the need to use it to assess soil health and provide the best nutrient management strategies for long-term agricultural sustainability.

5. Conclusion

The purpose of the current study was to assess the effects of various long-term nutrient management techniques on the yield and soil health of the rice-wheat cropping system in the Mollisols of the Himalayan tarai region. The application of chemical nitrogen, phosphorus, and potassium fertilizers coupled with farm yard manure and zinc led to the highest long-term system productivity and the SYI, highlighting the significance of integrated fertilizations. The results also showed that the integrated application of chemical fertilizers such as NPK with farm yard manure could also resist the decline in system productivity as compared to the long-term use of chemical fertilizers alone. Under the combined application of NPK fertilizers with farm yard manure and zinc, the highest levels of soil enzymatic activity and potentially mineralizable nitrogen were found, demonstrating the beneficial effects of organic inputs and zinc fertilization on soil health. In addition, farm yard manure application in an integrated mode with chemical fertilizers enhanced soil organic carbon and available NPK compared to chemical fertilization alone.

Interestingly, neither crop yield nor soil health was improved by applying 50% more nitrogen or 100% more phosphorus supplied through chemical fertilizers over time. According to the study, a long-term improvement in soil health, the SQI, and system sustainability depend on balanced chemical fertilization coupled with regular organic inputs. Together, the findings indicate that it is feasible to increase and sustain the productivity of the rice-wheat cropping system and overall soil quality by applying NPK at 120:40:40 kg ha^{-1} in both rice and wheat, as well as additional applications of 5 mg ha^{-1} FYM + 5 kg ha^{-1} Zn in rice in the Mollisols of the Himalayan tarai region. These results strongly support integrating chemical fertilization with organic inputs to promote system sustainability over time.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

MB, KR, NP, and PD led the research, planned, supervised, conducted field experiments, read, and edited the manuscript. MB, HJ, BP, MK, and PD collected soil/plant samples, performed the chemical analysis, wrote the initial draft of the manuscript, and prepared figures and tables. KR, RC, BP, HJ, AG, AA, AH, and NP supervised the project and reviewed, read, and edited the manuscript with significant contributions. BP, MK, AG, AA, AH, and PD performed the statistical analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1206878/full#supplementary-material

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Implications of minimum tillage and integrated nutrient management on yield and soil health of rice-lentil cropping system – being a resource conservation technology

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Conventional tillage methods and indiscriminate use of chemical fertilizers are causes of edaphic problems like soil degradation and loss of soil fertility which reduces crop yield. Puddling now-a-days, has become a major challenge for farmers due to breaking the soil structure, deficit water regimes, and depletion of soil health. Keeping in view, the absolute need to fulfill food security as well as sustainability, an experiment was conducted for 2 years on a rice-lentil cropping system during 2018–2019 and 2019–2020 in sandy clay loam soil of a new alluvial zone of eastern India to evaluate effects of minimum tillage and integrated nutrient management on yield and soil health. Conventional tillage (CT) direct seeded rice-lentil, Minimum tillage(MT) direct seeded rice-lentil, MT transplanted rice-lentil and MT direct seeded rice-lentil were kept as main plot treatments and control (without any fertilizer), 100% RDF, 75% nitrogen + FYM, 75% nitrogen + FYM + *Azospirillum* and 75% Nitrogen + FYM + *Azospirillum* sp. + Zinc Sulphate were kept as subplot treatments during the study. Though conventional tillage treatments recorded higher LAI, CGR and yield in the first year of study in the case of rice; during the second year, minimum tillage treatments showed significantly ($p \leq 0.05$) better results in the aforesaid aspects with MT_{DSR}-L having a maximum yield of 5.17 t ha^{-1}. In the case of lentil minimum tillage treatments had better results for both years, with MT_{DSR}-L treatment having the highest yield of 8.084 t ha^{-1}. Among the nutrient schedules, the crops had better LAI, CGR and yield during the first year in the case of 100% RDF treatment, but in the second year, 75% Nitrogen + FYM + *Azospirillum* sp. + Zinc Sulphate had the highest respective values. Soil organic carbon was marginally improved by both tillage and nutrient treatments, while soil microbial biomass carbon, dehydrogenase activity and microbial count were significantly influenced. The combination of MT_{DSR}-L and 75% Nitrogen + FYM + *Azospirillum* sp. + Zinc Sulphate (N_5) treatment showed the maximum values for all soil biological parameters leading to improve soil health. The results of the experiment concluded that the practice of minimum tillage and integrated nutrient management may be recommended to enhance the yield and stability of rice-lentil cropping systems in sandy clay loam soil of a new alluvial zone of eastern India by acting as an alternative for the decline of soil health.

KEYWORDS

agro-ecosystem stability, integrated nutrient management, minimum tillage, soil health, yield

Introduction

The conventional farming system, which previously increased food productivity, has now stagnated at the expense of the environment and natural resources. The population growth, which is anticipated to reach 9.7 billion people in 2050, makes the existing situation worse (FAO, 2019). Injudicious use of chemical fertilizers and different types of farm machinery for the aforesaid reasons has jeopardized the subtle balance of agro-ecosystems causing various ecological stresses and exploitation of natural resources (Sharma et al., 2019; Bisht and Chauhan, 2020). Similar to fertilizers, conventional tillage is also reported to have a huge impact on soil physical, chemical and biological properties which further are closely related to crop yield. Conventional tillage methods have given rise to edaphic problems like soil degradation, soil erosion and loss of soil fertility (Wani et al., 2023). Furthermore, the present issues with climate change, which cause abrupt floods, droughts, increases in temperature, and other changes, have been a huge burden for agriculture, because it is heavily dependent on the weather. So, developing a more resilient, climate-smart alternative to traditional agriculture is crucial for ensuring food security in a way that is economical and long-term so that all farm species and natural resources are protected (Pretty, 2008; Venugopalan et al., 2022).

Continuous use of conventional tillage has been found to degrade soil causing soil erosion and loss of soil fertility. The main objective of conservation tillage is to arrest such soil degradation by retaining crop residues in soil which in turn improves soil organic carbon. Conservation tillage maintains proper pore distribution and stability of the soil, unlike the conventional tillage practices that destructs the soil aggregates causing a hard pan (Das et al., 2020). Minimum tillage is one such conservation tillage practice where tillage is restricted to the minimum level necessary for a good seedbed preparation ensuring satisfactory crop stand and favorable growing conditions. Better nutrient cycling, energy transfers, and soil enzymatic activity have been reported in long-term conservation tillage systems and enzymatic activity and soil organic carbon are said to have a close relationship with soil health (Dick, 1994). Accumulation of crop residues increases the soil microbial biomass, carbon, dehydrogenase activity, and respiration activity of microbes gradually improve soil fertility in the long run (Hungria et al., 2009). Though soil has the innate ability to provide plants with the necessary nutrients, external application of nutrients in the form of organic or inorganic nutrient sources is necessary to supplement the soil sink for more crop production. A balanced approach can promote soil health in harmony with the agro-ecosystem, along with improving the production of high-quality crops without depleting natural resources (Sarkar et al., 2020). Integrated nutrient management refers to the maintenance of soil fertility and plant nutrient supply at an optimum level for sustaining the desired productivity through optimization of the benefits from all possible sources of organic, inorganic and biological components in an integrated manner. Implementing ecological principles like conservation practices and integrated nutrient management can improve species diversity in soil which further makes agro-ecosystem more sustainable (Lal, 2023).

India has the largest area of 45.07 Mha, in the world under rice cultivation of which West Bengal is the highest rice-producing state having production of 16.65 million tonnes (Directorate of Economics and Statistics, 2021; Moulick et al., 2021). Puddling is a common practice for rice cultivation. With the scarcity of water looming in the future due to uneven rainfall patterns, cultivating rice in puddled conditions is becoming a challenge. Minimum tillage in this case can act as an alternative since it reduces the time and water required for land preparation of the succeeding crop as well as reducing the use of tillage implements and thereby saving soil's physical structure from deterioration. Considering the nutritional security and soil fertility, the cereal-cereal cropping system does not fulfill the sustainability aspect of the agroecosystem. Incorporation of a pulse crop in a cropping sequence is essential in terms of crop diversification, soil health enhancement, nitrogen economy and carry-over effects (Praharaj et al., 2021). The introduction of legumes in the cropping system highly improves soil aggregation due to the presence of its intricate roots and the organic root exudates. This results in the formation of newer soil aggregates. Hence a proper crop rotation helps in maintaining soil aggregation dynamics providing a healthy resilient soil environment (Hillel, 2004). After Chickpea, lentil is the second most important *rabi* pulse crop in India (Ahmed et al., 2018), having a production and productivity of 1.45 million tonnes and 1,001 kg ha^{-1}, respectively, (Directorate of Economics and Statistics, 2021). West Bengal is the third-highest lentil-producing state in India. It is the most widely grown rabi pulse crop after *Aman* (June–November) rice in West Bengal having a productivity of 855 kg ha^{-1} (Directorate of Economics and Statistics, 2021). Growing profitable lentil on residual soil moisture and nutrients from preceding rice cultivation is an important resource conservation technology. With this background, a study was initiated to understand the impact of different conservation tillage practices and integrated nutrient management schedules on growth, yield and soil microbes.

Materials and methods

Experimental site

The field experiment was conducted during the *kharif-rabi* seasons of 2018–2019 and 2019–2020 at the Instructional Farm of Bidhan Chandra Krishi Viswavidyalaya, Jaguli, Nadia, West Bengal, India. The farm is located at 22° 93′ N latitude, 88° 53′ E longitude and

9.75 m above mean sea level (MSL). The experimental study site is medium land with good irrigation and drainage facilities. The soil is typical new alluvial, Entisol and sandy clay-loam in texture with moderate water holding capacity. The soil was having 26.19% silt (0.002–0.02 mm), 32.45% clay (<0.002 mm), 36.17% fine sand (0.02–0.2 mm) and 5.19% coarse sand (0.2–2 mm) with pH 6.59. The soil of the experimental field had low organic carbon (0.51%) (Walkley and Black method), medium available nitrogen (alkaline permanganate-oxidizable) (243 kg ha^{-1}), high available P_2O_5(Olsen'P) (28.5 kg ha^{-1}) and K_2O (NH_4OAC-extractable) (253.63 kg ha^{-1}).

Climatic condition

The place under study lies in the sub-humid and subtropical zone since it's situated near the Tropic of Cancer. It falls in the alluvial Indo-Gangetic agro-ecological zone. The average rainfall annually in this region is 1,440 mm. The mean monthly temperature ranges from 37.4°C (maximum) to 9.7°C (minimum). Generally, the average temperature ranges from 37.6°C to 25.4°C during summer months and 23.7 to 8.5°C during winter months. May is the hottest month in this region whereas winter here is quite mild and short. The temperature gradually increases from March and reaches its highest by the end of May. Generally, the second week of June is the onset of monsoon and it extends till the last of September to the middle of October. Pre-monsoon rain is quite common from March to May due to Nor'wester showers. The rainfall is erratic and irregular. During the winter months, rainfall was sufficient during both years of experiments due to the 'depression' occurring in the Bay of Bengal. For both the years of the experiment, maximum rainfall was received during the monsoon season. Maximum humidity was observed in July 2018 (89.18%) and in August 2019 (88.94%) while the minimum was recorded during the winter months of both years. The monthly mean meteorological data for the 2 years of the experiment are given in Supplementary Figure S1.

Experimental design and treatments

The design used in the experiment was a split-plot design, having four main plots, five subplots and three replications. The main plots included: i) Conventional tillage (CT) direct seeded rice –lentil ii) Minimum tillage(MT) direct seeded rice-lentil iii) conventional tillage (CT) transplanted rice-lentil iv) Minimum tillage(MT) transplanted rice-lentil whereas subplots included i) Control (no application of nutrients) – lentil, ii) 100% RDF (80:40:40) (N: P_2O_5:K_2O) kg ha^{-1} (As per government of West Bengal recommendations) – lentil, iii) 75% Nitrogen + FYM (Blanket dose of 10 t ha^{-1}) – lentil, iv) 75% Nitrogen +FYM (Blanket dose of 10 t ha-1) + *Azospirillum* sp. (2 kg ha^{-1}) – lentil, v) 75% Nitrogen + FYM (Blanket dose of 10 t ha^{-1}) + *Azospirillum* sp.(2 kg ha^{-1}) + Zinc Sulphate Heptahydrate (ZnSO4,7H_2O) @ 20 kg ha^{-1}) – lentil. The treatment description and abbreviations used are shown in Table 1.

Crop management practices

Each experimental plot was having a size of 6 × 4 m^2. In CT, two ploughings by tractor-drawn disc-harrow were followed by two ploughings by a harrow with the help of a rotovator and one planking for uniform land leveling. The CT practices were the same for both direct seeded and transplanted rice. In MT, one plowing was followed by one harrowing. MT practices were the same for both the direct seeded and transplanted rice. No further land preparation was done for sowing lentil. Fertilizer P2O5 and K2O were applied at the same rate, i.e., @ 40 kg ha^{-1} each in all subplots as basal dose except the control plot before sowing of rice. Nitrogen was applied @ 0/80/60 kg ha^{-1} as per treatment requirement. No fertilizers have been added for lentil cultivation. The experiment used the rice variety Satabdi (IET-4786) and the lentil variety HUL-57.

TABLE 1 Details of the experiment.

Treatments	Abbreviated forms
CT Direct seeded rice – lentil	CT_{DSR}-L
MT Direct seeded rice – lentil	MT_{DSR}-L
CT Transplanted rice – lentil	CT_{TPR}-L
MT Transplanted rice – lentil	MT_{TPR}-L
No application of nutrients (Control)	N_C
100% RDF	N_1
75% Nitrogen + FYM	N_2
75% Nitrogen + FYM + *Azospirillum*	N_3
75% Nitrogen + FYM + *Azospirillum sp.* + Zinc Sulphate	N_4

Growth and yield parameters

For studying various growth characteristics of plants (rice and lentil), ten random plants were selected from each treatment from one-meter row length leaving two rows from all sides of the border, in each phenotypical stage (vegetative, flowering and maturity stage) as plant samples. For calculation of LAI and CGR, samples were dried under the sun for one to two days before drying them in the oven at a regulated temperature of 65° C ± 1°CThree random plants per treatment were measured to determine the leaf area and the following equation (Eq. 1) was used to determine the LAI:

$$\text{LAI} = \frac{\text{Leaf area per plant}\left(\text{m}^2\right) \times \text{number of plants}}{\text{Ground area}\left(\text{m}^2\right)} \quad (1)$$

The crop growth rate is the rate of increase in dry matter accumulation per unit of time per unit of ground area. It is expressed in g m^{-2} day^{-1} and calculated by the formula:

$$\text{CGR} = \frac{\left(w_2 - W_1\right)}{\left(t_2 - t_1\right)} \text{ g m}^{-2}\text{day}^{-1} \quad (2)$$

Where, W_2 is the dry weight of plant (g m^{-2}) at time t_2 and W_1 is the dry weight of plant (g m^{-2}) at time t_1, t_2–t_1 is the time interval in days.

Both the yield of rice and lentil were recorded when crops achieved physiological maturity. After cutting from the ground, they were dried under the sun for 3–5 days on the threshing floor. Then the grains from the panicle were threshed and grain yield and straw yield from individual plots were taken and expressed as t ha^{-1}. In the case of lentil the pods were threshed, and the seeds were collected and weighed for each treatment plot. The seed yield and stalk yield were further converted on a hectare basis.

Soil biological parameters

Soil upto 0–15 cm depth was collected with the help of an auger from each experimental plot prior to sowing and after harvesting of each crop for both years of experiment. Thereafter the soil samples were sealed in plastic packets and stored at 4°C in the fridge for different soil biological parameters analysis. The analysis was done within 1 month of soil sample collection. Organic carbon content in the soils was estimated by the modified Walkely and Black method as described by Jackson (1973). Soil microbial biomass carbon was calculated by the following equation as described by Jenkinson (1966):

$$B = \frac{(X - Y)}{k}. \tag{3}$$

Where, "B" is the soil "microbial biomass carbon, "X" is the amount of CO_2-C which evolved from fumigated soil and "Y" is the amount of CO_2-C evolved from non-fumigated control soil. "k" is the fraction of the killed biomass-C that decomposed to CO_2 during the 10 days after fumigation. It was expressed in µg of CO_2 evolved per g of soil.

For dehydrogenase activity fresh soil sample of 5 gm was treated with TTC (2,3,5-Triphenyltetrazolium chloride) and incubated with or without electron donating substrate for 96 h in the presence of glucose at 27°C (Klein et al., 1971). The dehydrogenase activity was measured in the form of formazan which was expressed in µg per gm of oven-dried soil.

The microbial population occurring in the soil samples were determined by serial dilution technique and pour plate method (Prammer and Schmidt) using agar plates having appropriate medium. Thornton's agar medium (Thorton, 1922), Martin's rose Bengal streptomycin agar medium (Martin, 1950) and Jensen's agar medium (Jensen et al., 2012) were used for counting the total number of viable bacteria, fungi and actinomycetes, respectively.

Statistical analysis

Statistical analysis of all data was done by ANOVA technique for split-plot design and the least significant values were tested by Duncan's Multiple Range Test (DMRT) ($p \le 0.05$) among various tillage and nutrient treatments to find out the trend and variations of different parameters. Pearson correlations were presented in a correlogram with significant levels ($p \le 0.05$, $p \le 0.001$, $p \le 0.01$) and were deduced with the help of 'psych' package in R to evaluate relationships between various soil microbial parameters with rice and lentil yield. Multiple linear regression ($p \le 0.05$, $p \le 0.001$, $p \le 0.01$ and $p \le 0.1$) was also conducted to identify the relationship between the microbial count data with organic carbon under different tillage systems.

Results

Impact of tillage and nutrients on LAI and CGR of rice and lentil

LAI and CGR are considered to be important growth parameters of any crop. In both the years of experiment, they varied significantly across various tillage treatments and nutrients. LAI and CGR increased with the advancement of age for both crops being maximum during the peak period of vegetative growth, i.e., 60 DAS. They showed a decreasing trend as the crops neared their maturity due to the effect of senescence. It was observed that the first year of transplanted rice grown in CT plots (Figures 1A, 2A) attained the highest LAI and CGR, followed by MT_{TPR}-L (Figures 1B, 2B) at all the dates of observations. Rice grown in CT_{TPR}-L plots recorded (6.361) 9.13% higher LAI and 10.74% more CGR (7.95 g m^{-2} day^{-1}) compared to CT_{DSR}-L plots which recorded the lowest LAI (5.829) and CGR (7.27 g m^{-2} day^{-1}) among all tillage levels at maturity stage. Total rainfall recorded during the rice growing season in the first year was found to be lesser (641.1 mm) (Supplementary Figure S1) as compared to the second year. In the next year of experimentation, MT_{DSR} plots had the highest LAI at all stages, having 4.68% more LAI (6.563) and 3.42% more CGR (7.80 g m^{-2} day^{-1}) compared to rice grown in CT_{TPR}-L plots.

LAI and CGR of residual lentil grown on MT_{DSR}-L plots (Figure 3B) were found to be maximum, which was statistically similar to MT_{TPR}-L plots (Figure 4B), for all the dates of observation during the first year of study. At the maturity stage treatment of MT_{DSR}-L obtained 1.878 as the LAI value which was 1.10% higher as compared to CT_{DSR}-L treatment (Figures 3A,B), which recorded the lowest LAI for all stages of observation. During the flowering to maturity stage of the crop, CGR for MT_{DSR}-L plots was 1.274 g m^{-2} day^{-1}. In the next year of study, MT_{DSR}-L treatment maintained its superiority over other treatments for both LAI & CGR values. At the maturity stage, in MT_{DSR}-L plots the CGR value was 1.393 g m^{-2} day^{-1} which was 9.85 and 15.69% more than CT_{DSR}-L plots (Figure 3A) and CT_{TPR}-L plots (Figure 4A), respectively. At the maturity stage, MT_{DSR}-L plots recorded a maximum LAI of 2.115. N_4 treatment recorded the highest LAI values of 2.101 and 2.138 at the maturity stage, respectively, for both years. During the flowering to maturity stage, N_4 plots recorded 1.492 g m^{-2} day^{-1} and 1.463 g m^{-2} day^{-1} as CGR, respectively, in the first and second year of study.

There were significant changes across the various nutrient levels in terms of LAI and CGR for both rice and lentil. In the first year, the highest LAI and CGR for rice were observed in N_1 plots (Figures 5A–D) throughout their entire growth period. At the maturity stage of rice, N_1 plots recorded 2.47% more LAI (6.51) than N_3 plots (6.353) and it maintained statistical superiority over other treatments at all stages where it recorded CGR (9.51 g m^{-2} day^{-1}) during the late tillering stage to reproductive stage, indicating highest crop growth rate throughout the entire season. In the second year, N_2, N_3 and N_4 treatments recorded statistically higher LAI and CGR than N_c and N_1 treatments throughout all growth stages. N_4 plots gave the highest LAI values compared to all treatments. It

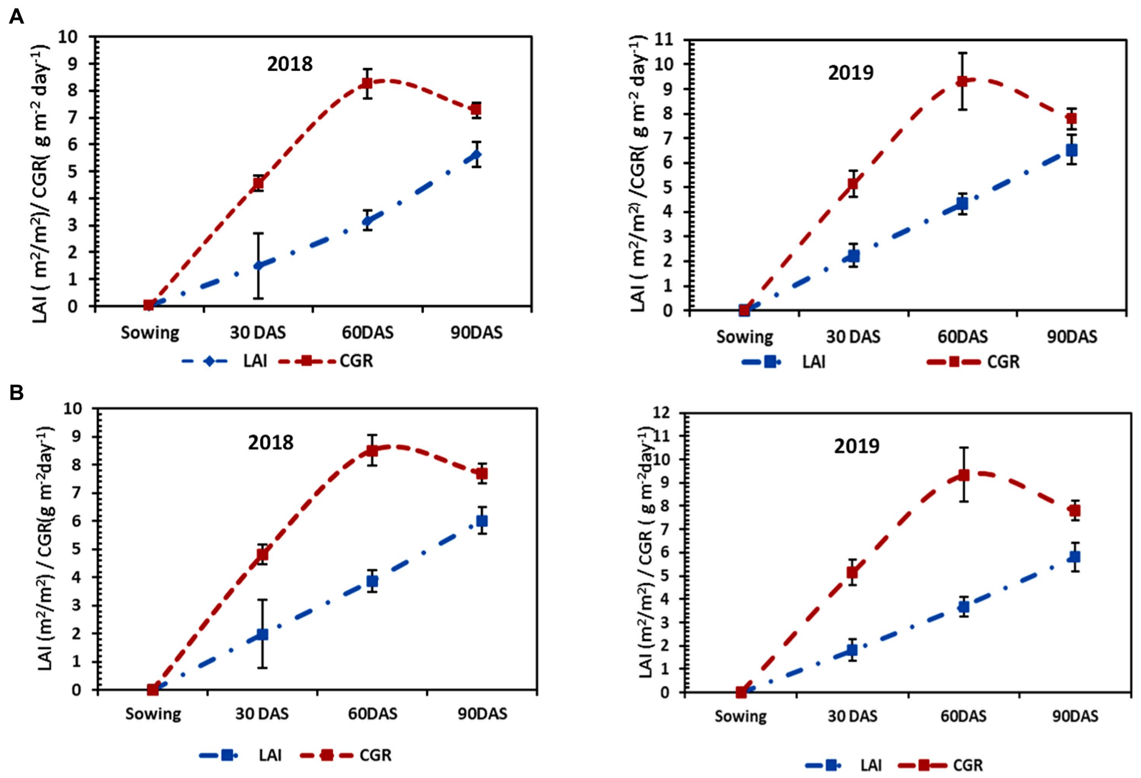

FIGURE 1
Growth parameters [i.e., leaf area index (LAI) and Crop Growth Rate (CGR)] of rice under **(A)** CT_{DSR}-L and **(B)** MT_{DSR}-L systems in both years (2018 & 2019). The error bar represents the standard deviation.

recorded 2.65, 4.70 & 6.995 LAI values during tillering, flowering and grain formation stages and maximum crop growth rates at all stages having 9.96 g m^{-2} day^{-1} during the late tillering stage to reproductive stage.

Impact of tillage and nutrients on yield of rice and lentil

The yield of rice was significantly affected by different tillage treatments for the entire period of the experiment. In the first year, grain and straw yields were found to be the highest in CT_{TPR} plots, resulting in 4.84 t ha^{-1} (Table 2) and 6.46 t ha^{-1}, respectively.

Rice grown in MT_{TPR} plots recorded 3.64% less grain yield than CT_{TPR} (4.84 t ha^{-1}) plots. In the second year, the trend was found to be different, maximum grain yield and straw yield of 5.17 t ha^{-1} and 6.62 t ha^{-1}, respectively, were observed in MT_{DSR} plots, which was 8.15 and 5.58% higher than rice yield in CT_{TPR} plots, respectively. Different nutrient schedules had a significant impact on rice yield for the entire period of the experiment. Rice grain (5.17 t ha^{-1}) and straw yield (6.56 t ha^{-1}) were maximum in N_1 plots during the first year of the study. But in the second year, N_4 treatment recorded the maximum rice grain and straw yield (5.65 t ha^{-1} and 7.17 t ha^{-1}) respectively, which was 10.13% superior to N_1 plots.

Residual lentil grown on MT_{DSR} plots had the highest seed yield of 8.084 q ha^{-1} (Table 2) and stalk yield of 17.846 q ha^{-1} during the first year. In the second year, a similar trend was observed, with residual lentil yielding 12.10 and 10.33% higher seed yield (8.817 q ha^{-1}) and stalk yield (18.852 q ha^{-1}) respectively in MT_{DSR} plots than CT_{TPR} plots. Grain (9.165 & 9.816 q ha^{-1}) and stalk yield (20.081 & 20.074 q ha^{-1}) of residual lentil were found to be maximum in treatment of N_4 treatment for both the years of study, respectively. N_4, N_3 and N_2 performed better than N_1 plots and N_0 plots, during the second year which indicated that FYM-treated plots showed an improving trend of yield-attributing characters. The results concurred with the findings of Das et al. (2019). Several researchers found that organic manure is capable of providing a residual impact on two or more crop seasons in terms of nutrient supply (Dey et al., 2019).

Impact of tillage and nutrients on organic carbon and microbial biomass carbon

Different tillage levels and nutrient levels had a significant effect on the organic carbon content of soil, at the end of each year's rice-lentil cropping system. In, 2019 highest carbon content of 0.5236% (Table 3) in soil was observed in the treatment of MT_{DSR}-L, though it was statistically similar to MT_{TPR}-L treatment.

These were followed by conventionally tilled treatments, recording much lesser carbon values in soil. In 2020, the maximum carbon value in soil was observed with the treatment of MT_{DSR}-L (0.5395%) (Table 3) followed by MT_{TPR}-L treatment. In 2019 and 2020, the highest carbon content of 0.5294 and 0.5422% were observed in N_3 which was statistically at par with N_4. Though all the FYM treatments have close soil carbon values, but they were pronouncedly superior statistically over treatments of N_1 and N_0 for both years.

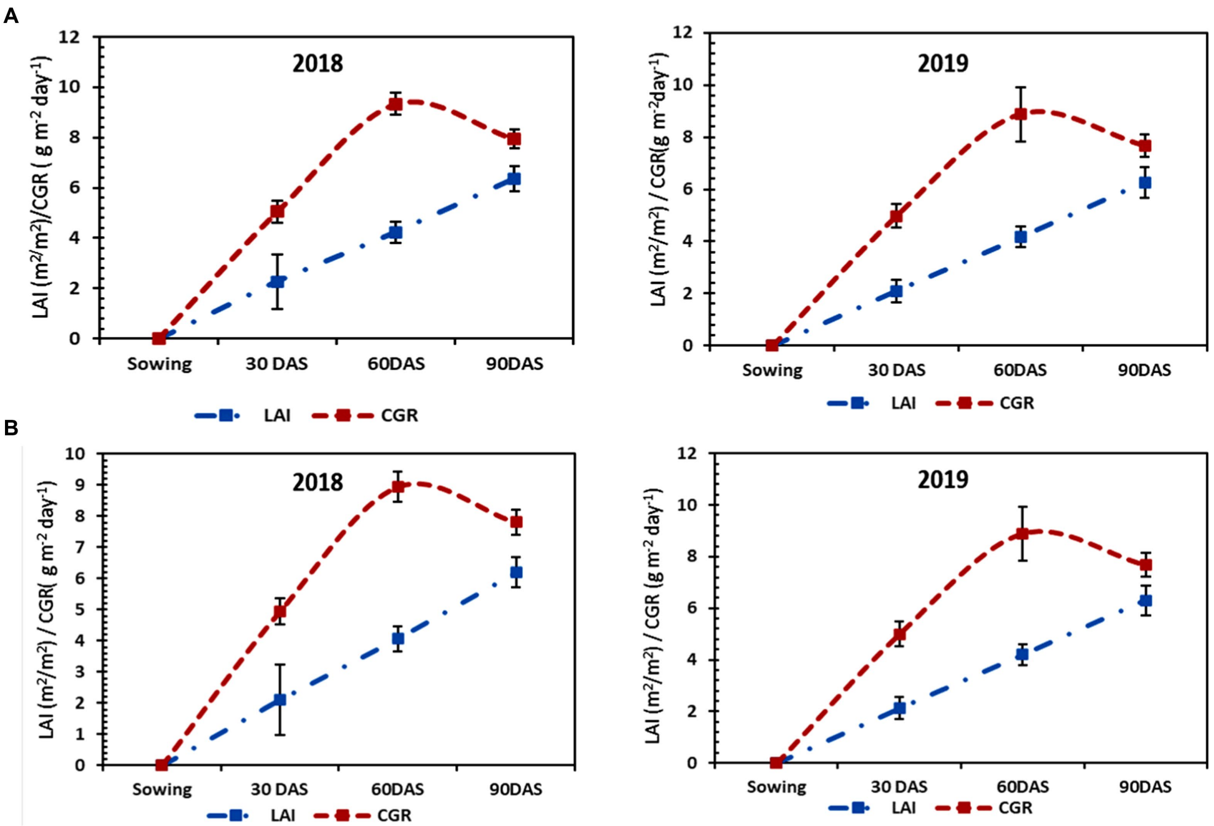

FIGURE 2
Growth parameters [i.e., leaf area index (LAI) and Crop Growth Rate (CGR)] of rice under **(A)** CT_{TPR}-L and **(B)** MT_{TPR}-L systems in both years (2018 & 2019). The error bar represents the standard deviation.

Microbial biomass carbon followed a similar trend as that of organic carbon in soil throughout the experiment. The values improved from the first to the second year. The highest microbial biomass carbon (316.66 µg C g $^{-1}$ oven dry soil) was found in MT_{DSR} plots (Figure 6A) which were 1.93 and 6.90% more than CT_{TPR} plots, respectively for both the years of study. However, MT_{TPR} plots were found to be statistically at par with MT_{DSR} plots in the first year and were closely following MT_{DSR} plots in the second year.

At the end of each year's rice-lentil cropping system, microbial biomass carbon in soil was significantly affected by different nutrient treatments. Maximum soil microbial biomass carbon of 339.07 µg C g^{-1} oven-dry soil (Figure 6A) and 354.22 µg C g^{-1} oven-dry soil, respectively were attained by N_3 which was statistically at par with N_4. All FYM-treated plots recorded more soil microbial biomass carbon than N_0 and N_1 plots, which was due to the presence of higher soil organic carbon content in all FYM-treated plots.

Impact of tillage and nutrients on dehydrogenase activity

Dehydrogenase activity in soil was significantly affected by different tillage treatments at the end of each year's rice-lentil cropping system. For the first year of study, MT_{DSR}-L attained the highest value of dehydrogenase activity (3.251 µg TPF g^{-1} oven dry soil $hr.^{-1}$) (Figure 6B) which was 2.07% more than CT_{DSR}-L and statistically at par with MT_{TPR}-L. In the second year, MT_{DSR}-L outperformed all other treatments recording dehydrogenase activity of 3.439 µg TPF g^{-1} oven-dry soil $hr.^{-1}$ which was 5.16 and 7.46% more than CT_{DSR}-L and CT_{TPR}-L, respectively, and was closely followed by MT_{TPR}-L.

Maximum dehydrogenase activity of 3.521 µg TPF g^{-1} oven dry soil $hr.^{-1}$ & 3.649 µg TPF g^{-1} oven dry soil $hr.^{-1}$ was obtained in N_3 (Figure 6B) respectively in both the years, though it was statistically at par with N_4. Dehydrogenase activity was 20.14 and 21.78% higher in N_3 compared to N_1 in respective years.

Impact of tillage and nutrients on microbial population

Highest bacterial, fungal and actinomycetes population (74.47×10^5 CFU g^{-1} soil and 87.60×10^5 CFU g^{-1} soil; 32.47×10^3 CFU g^{-1} & 39.67×10^3 CFU g^{-1}; 50.67×10^3 CFU g^{-1} soil and 61×10^3 CFU) (Figures 7A–C) was found in the treatment of MT_{DSR}-L, respectively, for both the years, which was statistically at par with MT_{TPR}-L. The bacterial population was found to be 21.21% higher in MT_{DSR}-L compared to CT_{TPR}-L in the final year of the study (Figure 7A). CT_{TPR}-L recorded 22.27 and 22.81% less total fungal and actinomycetes population compared to MT_{DSR}-L treatment in the last year of the experiment (Figures 7B,C). Results from this study reveal that bacterial, fungal and actinomycetes count increased from the first

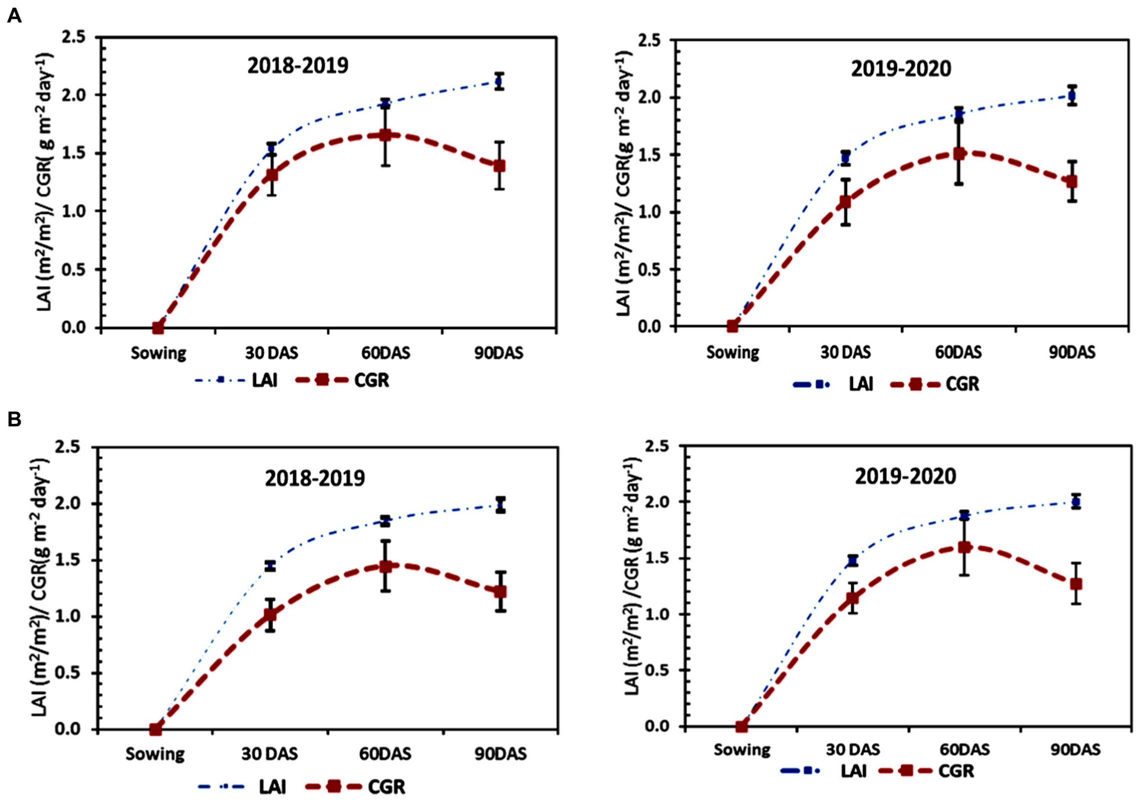

FIGURE 3
Growth parameters [i.e., leaf area index (LAI) and Crop Growth Rate (CGR)] of lentil under **(A)** CT_{DSR}-L and **(B)** MT_{DSR}-L systems in both years (2018–2019 & 2019–2020). The error bar represents the standard deviation.

year to the second year in minimum tillage treatments. Total bacterial, fungal and actinomycetes count was significantly affected by different nutrient treatments, after each year's rice-lentil cropping system. Maximum bacterial, fungal and actinomycetes population of 78.58×10^{5} CFU g^{-1} soil & 88.50×10^{5} CFU g^{-1} soil, 35.67×10^{3} CFU g^{-1} & 40.58×10^{3} CFU g^{-1} and 53.42×10^{3} CFU g^{-1} & 62.25×10^{3} CFU g^{-1} were found in N_3, respectively, for both the years (Figures 7A–C and Supplementary Tables S1, S2, S3). FYM-treated treatments had higher values which are attributed to higher C input. All the FYM-treated plots had closer values for microbial population, while N_1 and N_0 had pronouncedly inferior statistical values.

Relation between different soil microbial parameters with rice and lentil yield

Different correlograms have been constructed according to different tillage practices for 2 years of experiment. In each correlogram, significant positive relationships have been observed between different soil microbial parameters with rice and lentil yield along with LAI values of 60 DAS of rice and lentil (Figure 8).

Rice yield and lentil yield were significantly correlated with organic carbon present in the soil. Under all tillage systems, a strong positive correlation ($R > 0.6$) was observed between all parameters. In Figure 8A, CT_{DSR}-L system, the correlation was found to be strongest among MBC and DEHY, OC and BACT, OC and ACT, BACT and ACT, RY and RLAI60, LY and L LAI60. It implies that the interdependence of microbial populations is determined by the presence of organic carbon in the soil.

Whereas correlation coefficients for all the parameters with respect to direct seeded rice yield under conventional tillage were significant except for dehydrogenase activity (0.62). The lentil yield and 60 DAS LAI value were found to be significantly positively correlated with the microbial parameters. This indicates that conventional tillage results in lower activity of microbial population which is quite obvious due to the disturbance occurring through the tillage operations. From Figure 8B, depicting the MT_{DSR}-L system, it was clear that the microbial characteristics in the soil were highly correlated with each other and they were found to be highly significant.

In the minimum tillage practiced in direct-seeded rice, the dehydrogenase activity was significantly correlated with the rice ($R = 0.64$) and lentil yield ($R = 0.79$) which clearly is the indicator of the microbial oxidation activity. The lentil yield and 60 DAS LAI value were significantly positively correlated with the microbial parameters. The strongest relationship was observed between MBC and DEHY, BACT and OC, FUN and OC, BACT and ACT, and RY and RLAI60. In the CT_{TPR}-L system (Figure 9A) high positive correlation was observed in rice yield with other parameters except for MBC which indicates that the heavy tillage practiced in transplanted rice does not significantly contribute to proper microbial growth which might be the reason for later compaction of soil in this system resulting in lower MBC value. Interestingly in the MT_{TPR}-L system (Figure 9B), rice yield was found to be only correlated with bacterial (0.65*) and fungal population (0.68*) which indicates that the system is not that

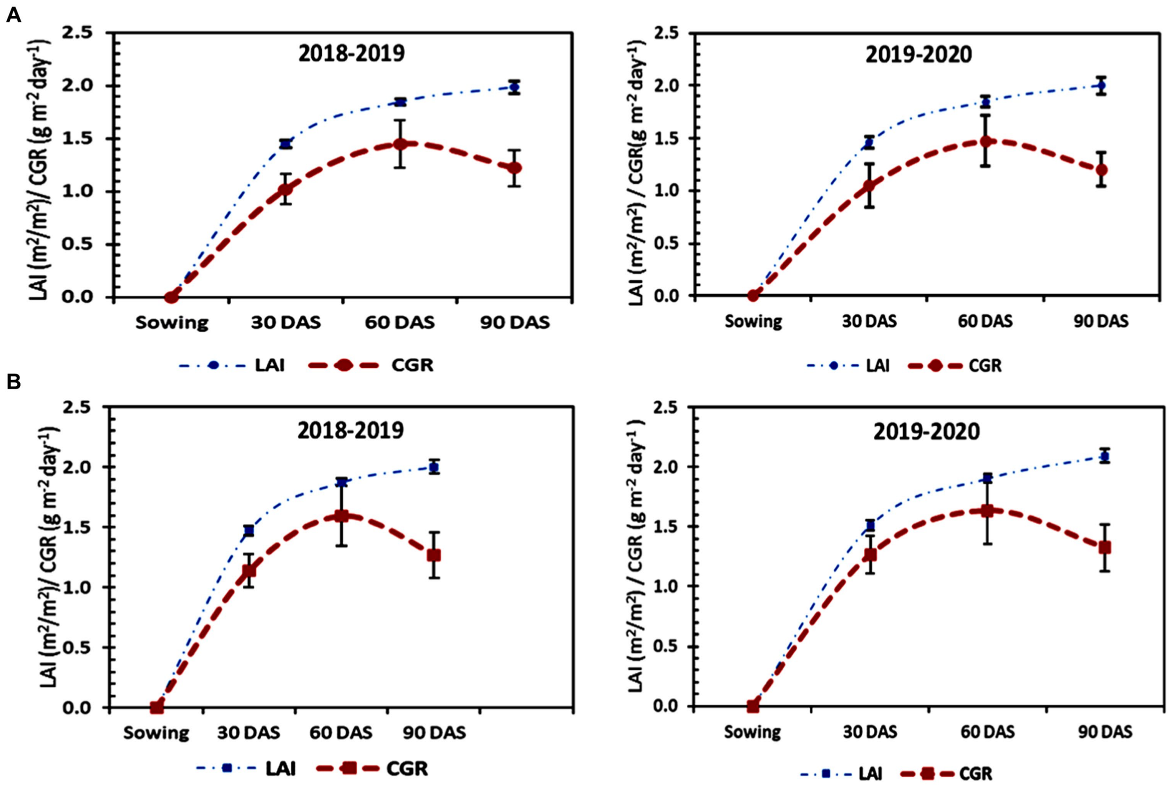

FIGURE 4
Growth parameters [i.e., leaf area index (LAI) and Crop Growth Rate (CGR)] of lentil under **(A)** CT_{TPR}-L and **(B)** MT_{TPR}-L systems in both years (2018–2019 & 2019–2020). The error bar represents the standard deviation.

suitable for soil health. The minimum tillage practiced in the puddled condition might have hindered the oxidation of organic matter leading to poor microbial growth affecting rice yield whereas in the case of lentil yield all the other soil biological parameters were found to be highly interdependent giving rise to better yield performance. LAI values at 60 DAS for both rice and lentil had higher significant coefficient values (R from 0.62 to 0.97) with most of the soil microbial parameters, for all tillage systems.

Relationship between the organic carbon (OC %) in soil with microbial population count under various tillage systems

The relationship between the OC (%) in soil with microbial population count under various tillage systems was deduced in Table 4. The relationship established between the organic carbon of the second year and the microbial population in both years can provide an in-depth idea about the intricate relationship between soil OC with various microbial populations. According to experiment results, soil organic carbon being the key indicator of soil quality, had significant relation with both years of bacteria, fungi and actinomycetes population as a whole, while individual microbial count did not have any significant relation with organic carbon.

The individual relation between microbial count and OC might be more pronounced if the experiment is furthered more which is indicated in the regression table. The microbial population is high in the minimum tillage system but the OC had a relatively significant relation under the conventional tillage system. The organic matter is oxidized faster in conventional tillage systems and thus exhausts the soil capacity for microbial activity in the long run. This may affect the yield in the long term reducing soil productivity and making it barren and unsuitable for rice-based cropping systems.

Discussion

LAI and CGR of rice-lentil as influenced by minimum tillage and integrated nutrient management

Direct-seeded plots did not perform well in the first year because of moisture stress owing to poor rainfall as there was poor germination of seeds, while it was quite a different scenario in the second year. Such a trend might be due to the overall effect of better climatic conditions (Supplementary Figure S1) during the second year and minimum tillage which was gradually getting pronounced with each passing year. Minimum soil disturbance results in the retention of soil organic matter and better soil chemical, physical and biological characteristics. More soil enzymatic activity, and microbial biomass, lead to higher nutrient availability and consequently higher uptake by the crop giving rise to higher leaf area per plant and better canopy cover hence improving the LAI. Higher LAI and better light interception have produced more dry matter causing higher CGR values in the case of

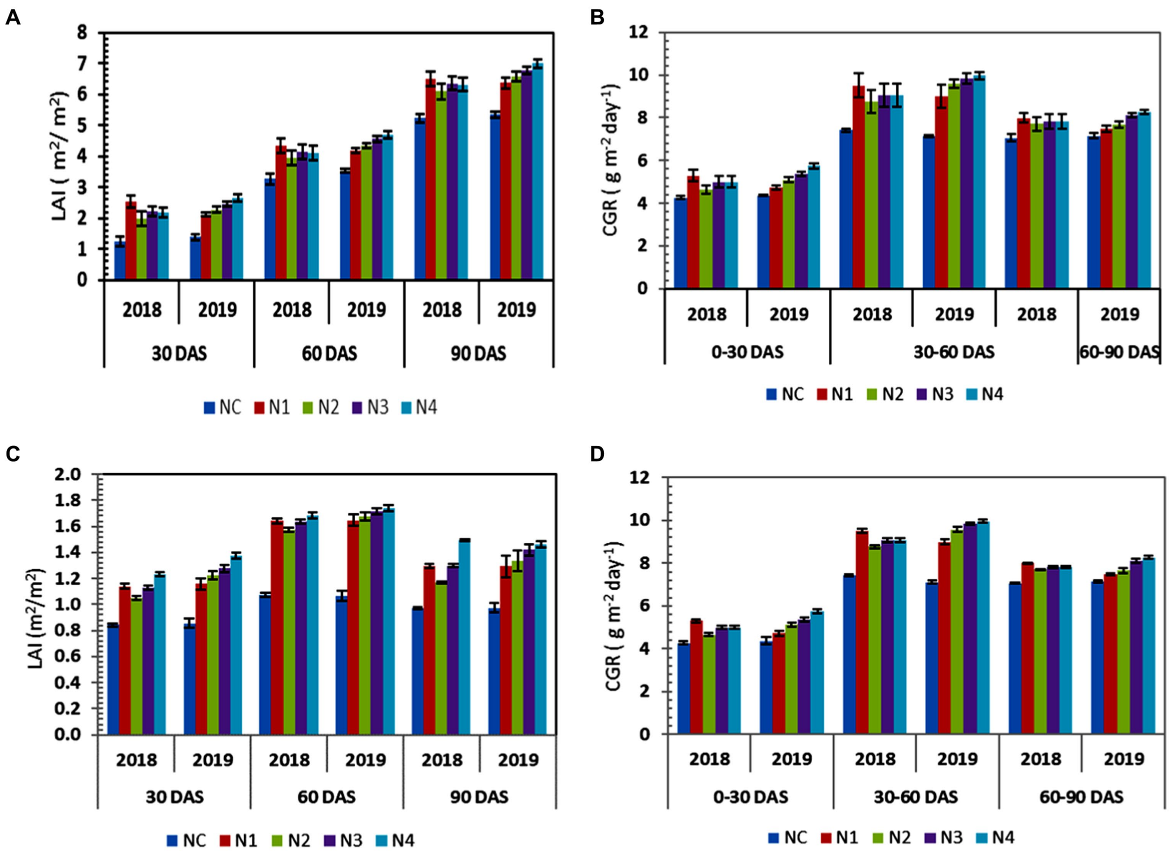

FIGURE 5
(A) LAI of rice under different nutrient treatments (2018 & 2019 crop seasons), **(B)** CGR of rice under different nutrient treatments (2018 & 2019 crop seasons), **(C)** LAI of lentil under different nutrient treatments (2018–2019 & 2019–2020 crop seasons), and **(D)** CGR of lentil under different nutrient treatments (2018–2019 & 2019–2020 crop seasons). The error bar represents the standard deviation.

TABLE 2 Data of 2 years for rice and lentil yield as influenced by different levels of tillage and nutrients.

Treatment	Rice grain yield (t ha^{-1})		Lentil grain yield (t ha^{-1})	
	2018	2019	2019	2020
CT_{DSR}-L	4.00^{c}	5.12^{a}	7.682^{b}	8.064^{c}
MT_{DSR}-L	4.38^{b}	5.17^{a}	8.084^{a}	8.817^{a}
CT_{TPR}-L	4.84^{a}	4.78^{b}	7.738^{b}	7.865^{d}
MT_{TPR}-L	4.67^{a}	4.82^{b}	8.051^{a}	8.579^{b}
N_C	3.3^{c}	3.51^{e}	6.43^{d}	6.444^{d}
N_1	5.17^{a}	4.93^{d}	8.236^{b}	8.25^{c}
N_2	4.53^{b}	5.3^{c}	7.412^{c}	8.349^{c}
N_3	4.71^{b}	5.47^{b}	8.2^{b}	8.797^{b}
N_4	4.65^{b}	5.65^{a}	9.165^{a}	9.816^{a}

The treatment description is given in Table 1.
Significant differences between means are represented by different letters.

TABLE 3 Data of 2 years for organic carbon (%) as influenced by different levels of tillage and nutrients.

Treatment	Organic carbon (%)	
	2019	2020
CT_{DSR}-L	$0.52^{a} \pm 0.007$	$0.52^{a} \pm 0.011$
MT_{DSR}-L	$0.52^{a} \pm 0.008$	$0.53^{a} \pm 0.014$
CT_{TPR}-L	$0.52^{a} \pm 0.007$	$0.52^{a} \pm 0.009$
MT_{TPR}-L	$0.52^{a} \pm 0.008$	$0.53^{a} \pm 0.013$
N_C	$0.51^{a} \pm 0.001$	$0.51^{a} \pm 0.004$
N_1	$0.51^{a} \pm 0.001$	$0.51^{a} \pm 0.005$
N_2	$0.52^{a} \pm 0.002$	$0.53^{a} \pm 0.008$
N_3	$0.52^{a} \pm 0.003$	$0.54^{a} \pm 0.008$
N_4	$0.52^{a} \pm 0.003$	$0.54^{a} \pm 0.008$

The treatment description is given in Table 1.
Significant differences between means are represented by different letters. Average means ± standard deviation ($n = 3$).

minimum tillage. Qamar et al. (2013) in his study found that zero tillage treatment had resulted in higher LAI and CGR values in the case of wheat, which was due to higher moisture and availability of nutrients near the crop root zone that accelerated the growth. Das et al. (2020) concluded that the availability of nutrients was more in conservation tillage treatments compared to conventional tillage treatments. Zero tillage outperformed conventional tillage in respect of various growth attributes of crops as observed by Parihar et al. (2016), which he assigned to better soil water regimes, aeration and better root growth resulting from lesser compaction of soil.

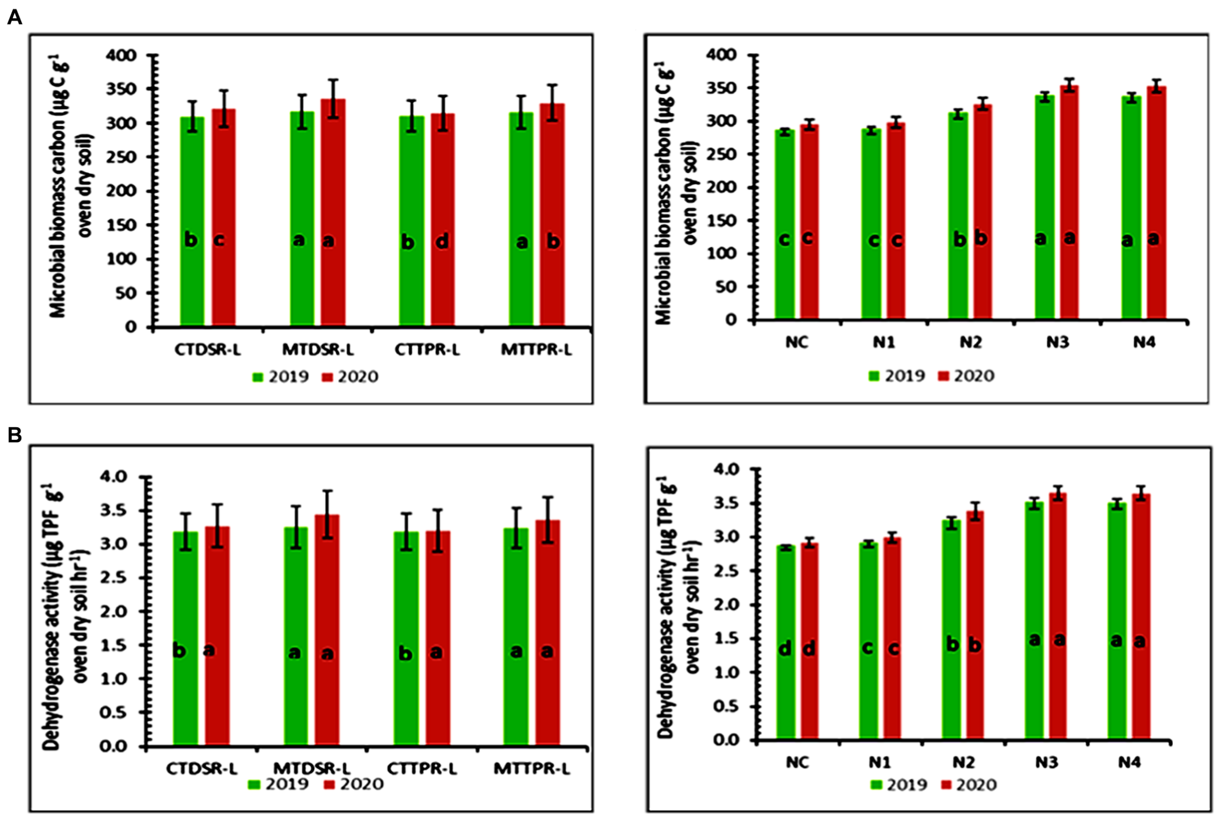

FIGURE 6
(A) Microbial biomass carbon as affected by different levels of tillage and nutrients in both years (Error bars represent standard deviation).
(B) Dehydrogenase activity as affected by different levels of tillage and nutrients in both years. Treatment details are given in Table 1.

Azospirillum sp. is said to promote the secretion of amino acids, cytokinins, gibberellins, indole acetic acid etc. which directly facilitates better root growth in plants, increasing nutrient as well as water uptake by plants (Zeffa et al., 2019). Hafeez et al. (2013) reported that zinc activates numerous plant enzymes for carbohydrate metabolism. This might be a valid reason for N_4 treatment to record the highest LAI and CGR values. Treatments receiving FYM showed better results in terms of LAI and CGR in the second year since it has a longer turnover period as it decomposes slowly due to its high C:N ratio to release nutrients. Imade et al. (2017) reported the highest LAI values in rice along with other growth attributes with the application of FYM. Singh et al. (2018) got similar results due to the application of FYM which improved growth attributes of rice due to lowering of nitrogen loss because of fixation of NH_4^+ ions in humus present in FYM and its slow release over time. Aerobic soil condition of the direct seeded rice establishment systems was responsible for better soil structure leading to better root growing conditions, which might result in higher nutrient uptake for lentil grown afterwards. Higher soil organic carbon content under minimum tillage plots due to the slow decomposition of organic residues on the soil surface as well as higher soil moisture conditions in minimally tilled plots might be a valid reason for residual lentil to have higher LAI and CGR values. The positive influence of minimum tillage practiced during the rice cultivation in improving growth attributes of the crop was quite visible in lentil, with minimally tilled plots recording comparatively more LAI and CGR than conventionally tilled plots. Bandyopadhyay et al. (2018) in his study observed higher LAI and CGR values in lentil in no-tillage and minimum tillage treatments which were due to better moisture content in the root zone which further led to higher relative water content and chlorophyll concentration. Higher relative water content is an important index for cell division whereas chlorophyll concentration determines the photosynthetic capacity. Both of these factors are important for the growth indices of any crop. Results tend to comply with the findings of Singh et al. (2016). This along with the application of farmyard manure might have resulted in better physiological characteristics of lentil which in turn improved LAI and CGR values.

The yield of rice-lentil as influenced by minimum tillage and integrated nutrient management

Various previous reports confirmed that during dry season or cases of low rainfall puddling has aided in enhancing the rice yield in general, owing to lowering of percolation loss of water and taming weeds which is due to less rainfall and deep water table (Yadav et al., 2017). This might be the reason behind the higher yield of transplanted rice in the first year, in both conventional and minimum tillage systems. While better climatic conditions favoring higher LAI and CGR values resulted in higher yield in the case of direct-seeded rice in the second year. Many researchers before have confirmed proper

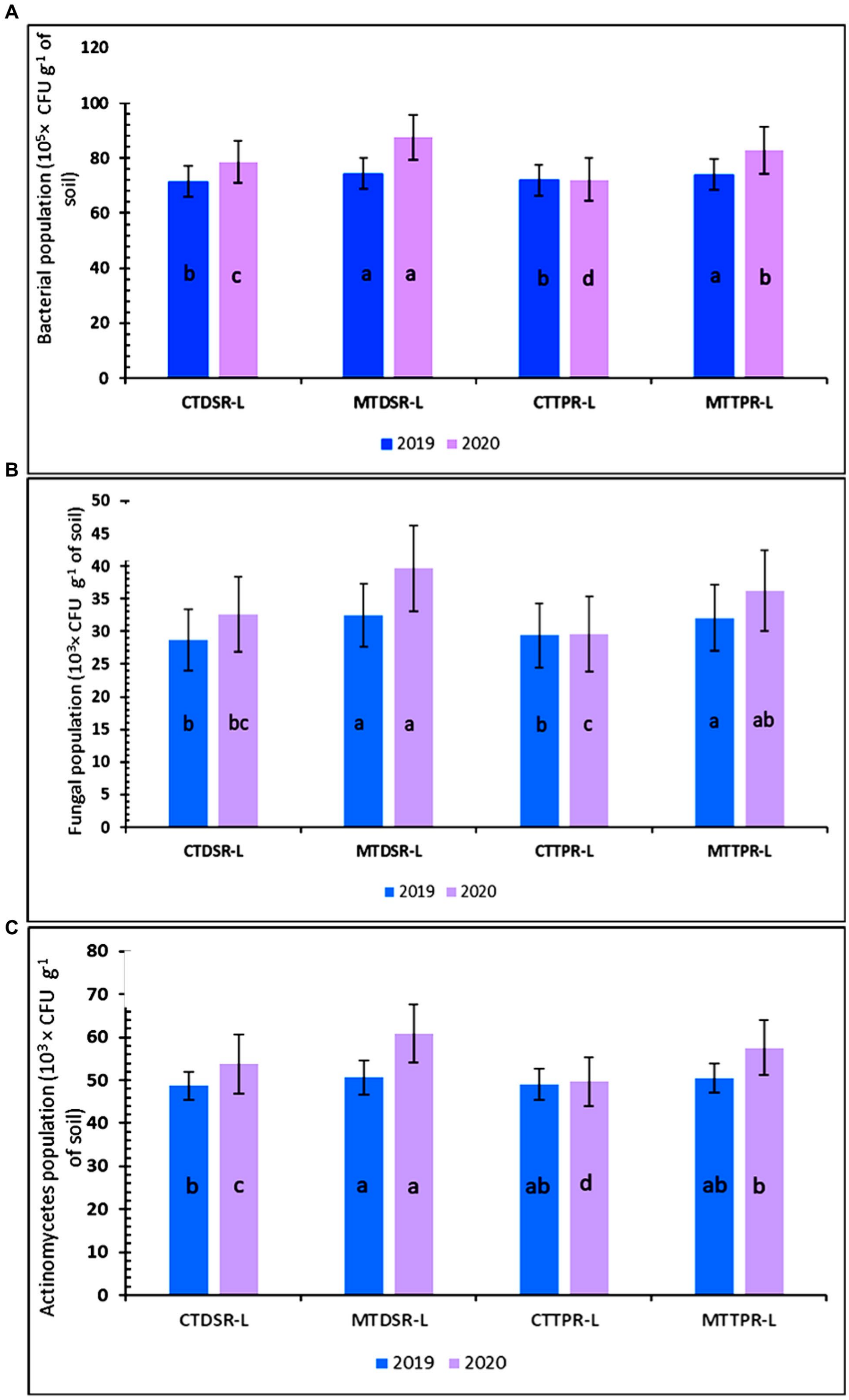

FIGURE 7
(A) The total bacterial, **(B)** fungal and **(C)** actinomycetes population as affected by different levels of tillage in both years (error bars represent standard deviation). Treatment details are given in Table 3.

agronomic management like weed and water management and favorable climatic conditions have resulted in direct-seeded rice performing better than transplanted rice (Tao et al., 2006). Liu et al. (2015) in his study pertained higher yield of direct seeded rice because of more number of panicles per m^2 than transplanted rice. Košutić et al. (2005) and Salahin et al. (2017) reported that minimum tillage with time improves the yield of crops. Less disturbance of soil in conservation practices like minimum tillage which leaves at least 30%

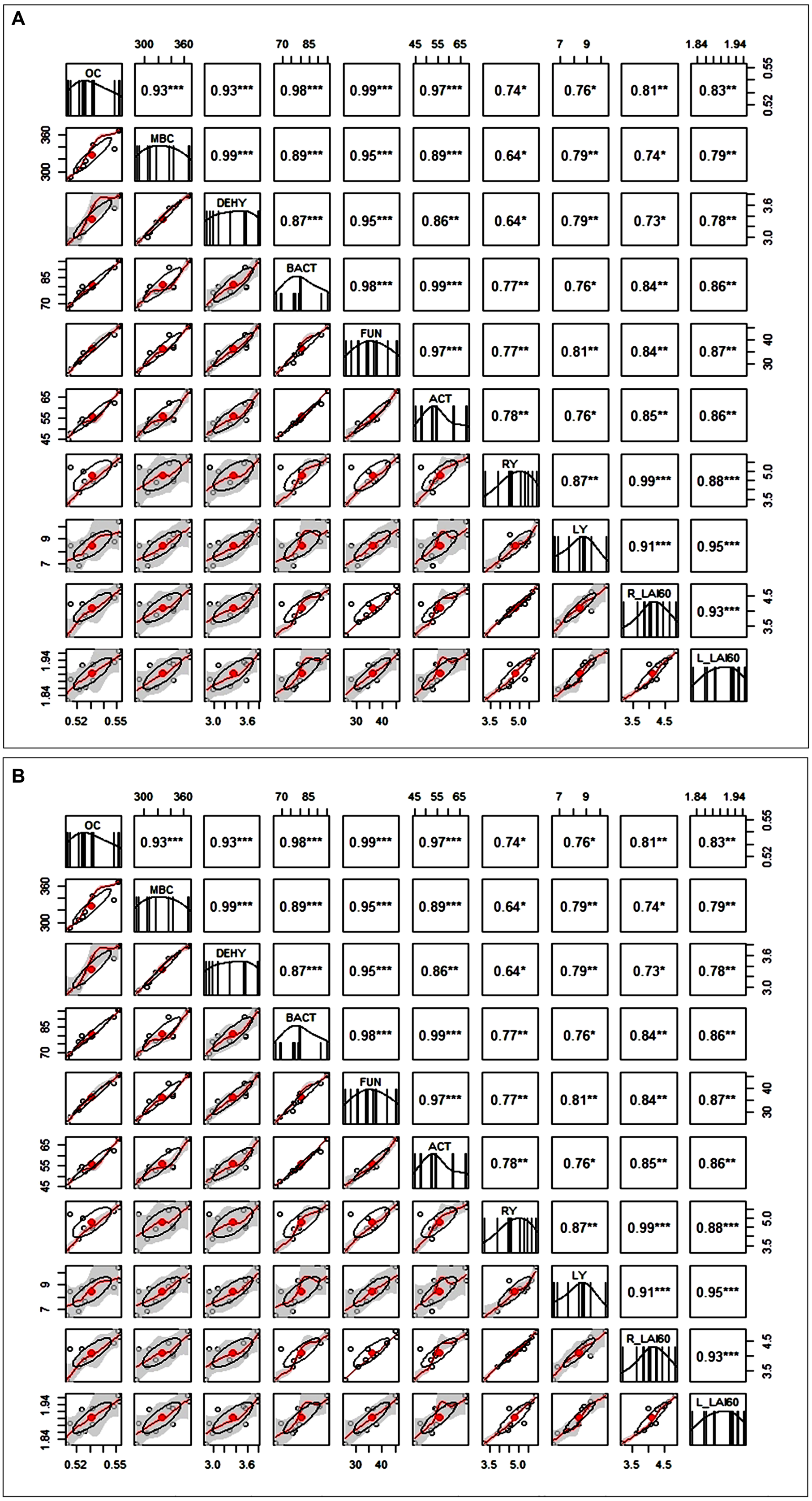

FIGURE 8
Correlogram showing the Pearson correlation coefficients between organic carbon (%OC), microbial biomass carbon (MBC), dehydrogenase (DEHY), bacterial population (BACT), fungal population (FUN), Actinomycetes population (ACT), rice (RY) & lentil (LY) yields, 60 DAS LAI of rice (R LAI60) and lentil (L LAI60) under **(A)** CT_{DSR}-L system and **(B)** MT_{DSR}-L system; *, Correlation coefficient significant at 0.05 level of probability, **, Correlation coefficient significant at 0.01 level of probability and ***, Correlation coefficient significant at 0.001 level of probability.

TABLE 4 Multiple linear regression with organic carbon (OC %) in soil with microbial population count under various tillage system.

Variables	Coefficients	*p* value	Significant level
CT Direct seeded rice-lentil			
(Intercept)	0.400	0.000	***
BAC2	0.000	0.815	
FUN2	0.000	0.217	
ACT2	0.001	0.013	*
BAC1	0.001	0.002	**
FUN1	0.000	0.699	
ACT1	0.000	0.692	
MT Direct seeded rice-lentil			
(Intercept)	0.422	4.03	***
BAC2	0.0015	0.146	.
FUN2	0.0001	0.769	
ACT2	−0.0005	0.436	
BAC1	−0.0006	0.676	
FUN1	0.0014	0.396	
ACT1	0.0002	0.779	
CT transplanted rice-lentil			
(Intercept)	0.407	0.000	***
BAC2	0.000	0.845	
FUN2	0.000	0.189	
ACT2	0.000	0.834	
BAC1	0.001	0.013	*
FUN1	0.001	0.203	
ACT1	0.000	0.560	
MT transplanted rice-lentil			
(Intercept)	0.386	0.000	***
BAC2	0.001	0.080	
FUN2	0.000	0.507	
ACT2	0.000	0.813	
BAC1	0.000	0.979	
FUN1	0.001	0.426	
ACT1	0.001	0.207	

Significant codes: "***" – 0.001, "**" – 0.01, "*" – 0.05, "." – 0.1.

residues on soil, stimulates the microbial biomass and increases its metabolic rate causing higher nutrient transformation of carbon, nitrogen, and phosphorus (Hungria et al., 2009). Nutrient availability is closely linked with robust dry matter accumulation, resulting in higher yield in the case of MT_{DSR}-L. N_4 recorded the highest yield due to release of phytohormones and nitrogen fixation by *Azospirillum* sp. along with the beneficial effect of zinc sulphate on nitrogen metabolism which might have led to maximum yield. Fukami et al. (2018) found out Azospirillum has a direct effect on root growth, resulting in higher nutrient and moisture uptake by plants. It also solubilizes phosphorus which further improves the yield attributes of crops. Integrated application of organic, and inorganic sources of nutrients along with biofertilizers improves soil organic carbon and soil structure (Das et al., 2015). Singh et al. (2009) reported similar instances of higher rice grain yield on the combined application of chemical fertilizers and Azospirillum owing to higher tillers m^{-2}, panicles m^{-2} and test weight. Integrated application of chemical fertilizers, organic manures and bioinoculants of *Azospirillum* was found to give 7.20% more rice grain yield than treatments not receiving *Azospirillum*, as it secreted growth-promoting substance which thereby gave rise to better root structure, enhancing mineral absorption by plants (Sravan and Singh, 2019). Minimum tillage and direct seeded rice establishment result in better seedbed than conventionally tilled transplanted systems because of a reduction in soil crusting and higher infiltration of water (Gangwar et al., 2006). Bandyopadhyay et al. (2018) pointed out that crop residue in minimum tillage plots lowered the solar radiation reaching the soil surface hence lowering energy for evaporation of residual soil moisture, after the rice harvest. Several researchers confirmed that no or minimum tillage lowers the change in soil moisture storage. Any kind of organic residue in soil improves the water-stable aggregate build-up because of polysaccharides and other organic compounds released after its decomposition, that act as a cementing material for soil particles. This enhanced aggregation of soil particles results in the development of low-density materials that are rich in mineral fractions (Bhanwaria et al., 2022). Mineralization of nutrients highly varies according to soil type, moisture, temperature and microbial activities of soil etc., (Moorhead et al., 1996). Higher moisture content, proper nutrient cycling and better biological parameters of minimum tillage system might have resulted in a greater number of pods per plant, seeds per pod and test weight of lentil which in turn led to its better seed yield as compared to conventional tillage system. Das et al. (2019) found similar results in a two-year experiment when lentil grown under no-tillage conditions in minimally tilled rice plots after its harvest recorded the maximum number of seeds per pod (1.29 & 1.37) and the number of pods per plant (22.7 & 23.1) respectively for both the years of study. Robust plant growth, in minimally tilled plots led to better biomass accumulation which was the reason for better stalk yield. Farmyard manure increases available nutrients such as nitrogen along with other micronutrients which further favors phosphorus and potassium use efficiency that results in better root structure, reduced leaching and higher nutrient uptake capacity of the crop (Zhang et al., 2016). The beneficial carryover effect of Azospirillum, FYM and zinc sulphate was clearly observed in the performance N_4 treatment, where Azospirillum acted as plant growth promoting rhizobacteria alongside slow nutrient-releasing bulky organic manure of FYM and zinc sulphate which perhaps facilitated nitrogen uptake by lentil, leading to better seed yield in the process. Application of organic manure improves the formation of stable metal–organic complexes, in the case of applied and natural micro-nutrient (Zn, Fe and Mn) in soil, which reduces their adsorption or precipitation in soil and enhances their uptake in crops (Swarup, 1984). This might be another reason for the N_4 treatment to perform better than the rest.

Organic carbon and microbial biomass carbon

Plant litter, root additions, and external application of organic manures improve the organic matter in soil, which transforms into

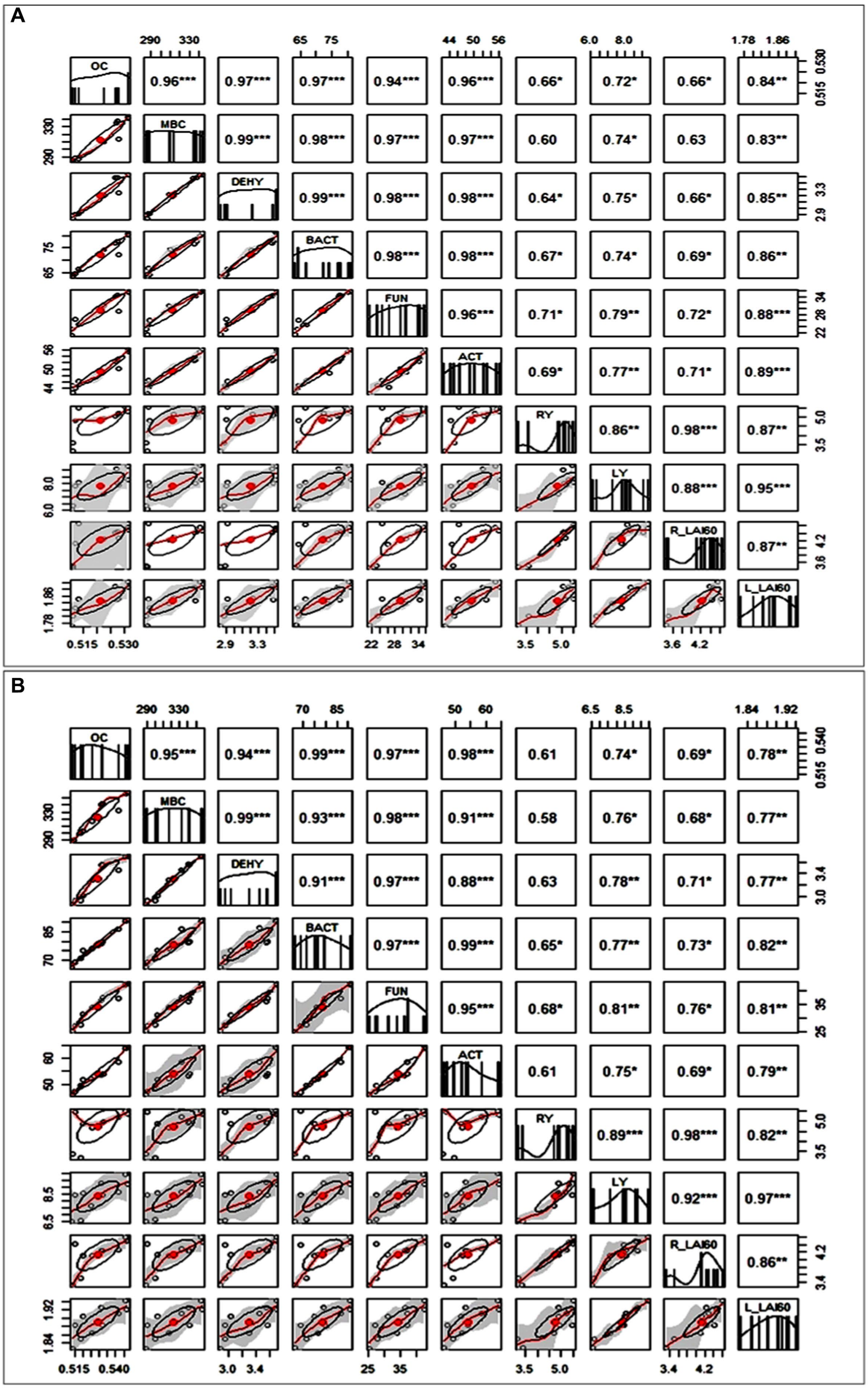

FIGURE 9
Different correlograms show the Pearson correlation coefficients between organic carbon (% OC), microbial biomass carbon (MBC), dehydrogenase (DEHY), bacterial population (BACT), fungal population (FUN), Actinomycetes population (ACT), rice (RY) & lentil (LY) yields, 60 DAS LAI of rice (R LAI60) and lentil (L LAI60) under **(A)** CT_{DSR}-L system and **(B)** MT_{TPR}-L system. *, Correlation coefficient significant at 0.05 level of probability; **, Correlation coefficient significant at 0.01 level of probability and ***, Correlation coefficient significant at 0.001 level of probability.

sources of humus carbon. It has been found that minimum tillage and zero tillage have considerably improved soil microbial biodiversity, their activity and biomass, since crop stubbles left on the surface act as their food source (Lupwayi et al., 2001) There was a circumspect improvement in carbon values from the first year to the second year. Minimally tilled plots had higher organic carbon content in soil than conventionally tilled plots throughout the entire experiment mainly because of limited manipulation of soil; oxidation of organic matter was reduced, hence conserving carbon within the soil. Various researchers (Beare et al., 1994; Six et al., 2000), stated that the presence of crop residues, in case of conservation tillage practices like minimum tillage, no-tillage etc., hinders the exposure of soil macroaggregates for microbial decomposition by increasing turnover rate of such soil macroaggregates by physically lowering the soil to residue contact, conserving soil organic carbon It takes several years for the organic carbon to increase to a substantial amount. Results from this experiment support this finding. After 11 years of experiment, Campbell et al. (1999), found that soil organic carbon improved to 0–3 Mg ha^{-1} under zero tillage within 0–15 cm. The introduction of legumes in the cropping system along with conservation tillage is another reason for the increase in the carbon values of soil (Yadav et al., 2017). Favorable crop rotations are associated with higher soil organic content as, root additions in conservation tillage systems, have a close relationship with the organic carbon stock of soil (dos Santos et al., 2011; Page et al., 2020). Khorami et al. (2018), from an experiment of 2 years, reported that soil organic carbon was highest in the case of reduced tillage (34%) followed by no-tillage (13%) as compared to conventional tillage Though some literatures suggest that leaving crop residues in soil or conservation tillage practices does not necessarily improve the soil organic content unless there is favorable soil temperature and humidity to act upon the decomposition rate of soil organic matter. Combined application of *Azospirillum* and farmyard manure accelerated robust root growth leading to higher root additions in soil, which might be a valid reason for N_3 treatment to record the highest organic carbon values with respect to other nutrient treatments by the end of second year of experiment. The results complied with findings of Zhao et al. (2020) where after 5 years of experiment organic carbon increased by 41.15% due to combined application of compost and inorganic sources of nutrients which was five times more than the increase because of the application of only inorganic fertilizers (Zhao et al., 2020). Again long-term experiments at various locations in India on the combined application of 50% NPK and 50% N through FYM showed similar results (Nayak et al., 2012). Different cereal-legume cropping systems were evaluated with respect to integrated nutrient management, Venkatesh et al. (2013) found that soil organic carbon content significantly increased by 26% in the treatment of farmyard manures + biofertilizers when compared to control treatments. Conventionally tilled treatments recorded lesser microbial biomass carbon as it is highly affected by soil management practices, which alters soil microbial habitat and microclimate. It is used as an important index of soil health and quality, which tends to increase in agricultural practices that maximize organic carbon content in soil (Malobane et al., 2020). Results from this experiment reveal that minimum tillage practices leave behind crop residues on the soil surface which acts as the energy source for various soil microorganisms, leading to greater soil microbial biomass carbon. Soil aggregate formation is always found to be higher in conservation tillage practices (Fuentes et al., 2009; Poonam et al., 2017; Upadhyay et al., 2018). The pore space within these aggregates acts as an essential habitat for soil microbial biomass, which is disrupted by conventional tillage practices. The results of this experiment are in accordance with Salinas-Garcıa et al. (2002) who concluded that in the upper 0–5 cm layer of soil, microbial biomass carbon was 25 to 50% higher in ZT and MT treatments than that CT treatments due to the presence of organic substrates in the case of both ZT and MT, which led to the assimilation of nutrients that further improved microbial biomass and their activity. Various researchers have found higher soil microbial biomass carbon in conservation tillage systems than in conventional systems (Hungria et al., 2009; Das et al., 2020). The addition of organic sources of nutrients, increased the carbon content of the soil for both the years of the experiment, while control plots and treatment receiving only chemical sources of nutrients did not have a higher carbon content in the soil. Various literature suggests (Vineela et al., 2008; Venkatesh et al., 2013; Francioli et al., 2016; Kumar et al., 2019) that integrated application of nutrients improves soil microbial biomass carbon. From a five-year experiment in the maize-mustard cropping system, continuous application of inorganic fertilizers along with FYM, biofertilizer and lime had the highest microbial biomass carbon (373.02 mg kg^{-1}) (Saha et al., 2010). Venkatesh et al. (2013) detected that the inclusion of pulses into any cropping system improves soil health through biological nitrogen fixation and their deep root system, hence increasing the below soil biomass. The results from this experiment confirm that the application of plant growth-promoting rhizobacteria like *Azospirillum* along with FYM resulted in higher root biomass and its exudates, providing carbon and energy for higher microbial proliferation in the soil, resulting in higher microbial biomass carbon.

Dehydrogenase activity

Minimally tilled systems recorded higher dehydrogenase values compared to conventionally tilled systems. Soil dehydrogenase enzymes are said to be one of the important enzymes taking part in biochemical routes to maintain balanced biogeochemical cycles. It's used as an important index for microbial activity in soil. Previous literature suggested that the more organic matter in the soil more will be more dehydrogenase activity which actually oxidizes the organic matter to release nutrients (Madejón et al., 2009; Das et al., 2020). Under permanent bed treatments dehydrogenase activity was 62% higher than conventionally tilled treatments in long-term maize-wheat cropping systems (Singh et al., 2009). Intensive tillage destroys soil aggregation and accelerates organic matter decomposition. Soil enzymatic activities are highly related to microbial biomass which is found higher in conservation tillage systems (Madejón et al., 2009). Minimally tilled plots had higher organic content that acted as an energy source for soil microbial multiplication which led to higher dehydrogenase activity. Higher dehydrogenase activity was confirmed under various conservation tillage systems like zero tillage, reduced tillage, etc., by other researchers (Nisha et al., 2016; Singh et al., 2016; Kumar et al., 2017; Hatti et al., 2018). Dehydrogenase activity (6.53 μg g^{-1}) at 0-15 cm soil layer was found to be more in CsT treatments than CT treatments as CsT methods improved the soil microbial activity and enzyme production under crop residue cover in the rice-rapeseed system (Das et al., 2020). Higher organic carbon content and microbial biomass carbon in FYM-applied treatments resulted in higher dehydrogenase activity Parewa et al. (2014) observed that in treatment where FYM along with bio inoculants were added accelerated the dehydrogenase activity due to the breakdown of organic substances present in the manure. The highest dehydrogenase activity was recorded by Brar et al. (2017) in their long-term experiment on maize when FYM, non-edible oilcake and biofertilizers were applied.

Microbial population

Soil enzymes are positively correlated with soil health and microbial activity (Dick, 1994). Results complied with Li et al. (2020), who suggested that conservation tillage practices have impacts on the total bacterial population. Higher dehydrogenase activity recorded in MT_{DSR}-L can be an indicator of a higher bacterial population (Singh et al., 2009). Higher total and bioavailable carbon content enhance soil bacterial count (Navarro-Noya et al., 2013). Several other researchers have found a higher bacterial population under zero tillage and minimum tillage practices (Helgason et al., 2010). Diversified rotations of crops are another vital reason for the improvement in bacterial count in MT systems (Yang et al., 2013). During biological nitrogen fixation in legumes, hydrogen gas is produced in the soil rhizosphere which triggers the bacterial population of the soil (Gogoi et al., 2018). Fungal communities are found to similarly proliferate more in conservation tillage systems *viz.* minimum tillage, zero tillage etc. because of maintaining hyphal nets which otherwise get disturbed due to continuous tillage operations in CT (Hungria et al., 2009). Its abundance at the soil surface in conservation tillage systems has been reported by several researchers before (Zhang et al., 2012; Spurgeon et al., 2013). The fungal population increased in both soybean and wheat under zero tillage systems Singh et al. (2018). Nisha et al. (2016) found similar kind of results in his 3 years experiment where they reported 56.63% more fungal population in the rice-wheat-mungbean cropping system under zero tillage than that of CT. The results from this study conclude that the accumulation of organic residues in minimum tillage systems acted as carbon and nitrogen sources for higher fungal and bacterial proliferation and these were in support of the findings of Parewa et al. (2014), where they concluded treatment of 100% NPK + FYM+ plant growth promoting rhizobacteria + vesicular-arbuscular mycorrhiza increased fungal as well as bacterial population due to higher root exudates acting as a source of carbon and energy. The results were similar to Brar et al. (2017) who reported the highest actinomycetes population ($76.17 \times 104\ cfu\ g^{-1}$ soil) in treatment receiving 50% N as FYM + Biofertilizers. Conventionally tilled plots throughout the entire study recorded a lesser total actinomycetes population when compared to minimum tillage plots. From the results, it is evident that cropping intensification as well as minimum tillage creates crop residues and exudates causing micro-habitats for diversified microbial communities. In a paddy-maize-soybean cropping system grown under conservation tillage actinomycetes population increased by 8.51, 2.3 and 3.4% at the harvest stage of rice, maize, and soybean, respectively, when compared to conventional tillage systems (Dongre et al., 2017). The results were consistent with the findings of Hatti et al. (2018) and Nisha et al. (2016) who reported a higher actinomycetes population under zero and minimum tillage systems. Application of organic sources of nutrients along with biofertilizers and chemical sources of nutrients have been said to promote soil microbial communities, which significantly influences soil biological fertility ensuring better nutrient cycling and nutrient dynamics in the soil in the longer run (Suhaibani et al., 2020).

Relation between different soil microbial parameters with rice and lentil yield

It is quite evident from this study that a favorable soil environment provided by minimum tillage is responsible for organic matter accumulation and higher enzymatic activity indicating microbial multiplication, which clearly has a significant beneficial effect on the yield of successive crops. Higher correlation values between various soil biological parameters with a respective yield of rice and lentil were observed among minimum tillage systems after 2 years of experiment implying limited tillage operations resulted in higher microbial activity and higher decomposition of organic matter leading to more availability of nutrients which might be taken up by plants (Somenahally et al., 2018) giving rise to higher leaf area which is successively contributing to higher accumulation of photosynthates and better yield in case of both rice and lentil. Soil health and fertility improve over time under minimum tillage along with the inclusion of pulse crops which is a valid reason behind the yield increase (Kaye and Quemada, 2017).

Conclusion

The findings of the study show that though minimum tillage did not have any effect on the growth and yield of rice in the first year, it had a synergistic effect on rice in the following year. In the second year of the experiment, all FYM-treated plots outperformed treatments without FYM. The residual effects of tillage and nutrients on lentil planted after rice were highly significant. Minimum tillage with integrated nutrient combinations resulted in higher LAI and CGR values contributing to higher crop yield. Though minimum tillage did not have a substantial effect on organic carbon but had influenced the microbial biomass carbon, dehydrogenase activity and microbial populations which are indicators of soil health. Organic carbon was found to be closely related to all the soil biological parameters, especially in the case of minimum tillage systems. The improvement of soil health clearly increased crop yields due to higher nutrient availability compared to conventional tillage systems where the organic matter was oxidized. It is clear that to gain long-term stability and sustainability of agro-ecosystem this kind of experiment needs to be furthered. However, to reverse soil degradation caused by conventional tillage practices and mono-cropping farmers need to have locally adjusted conservation tillage practices along with the inclusion of pulse crops in the cropping system. Farmers can get profitable yield in the long run under the rice-lentil cropping system if the minimum tillage is practiced along with the addition of FYM, *Azospirillum* and zinc sulphate.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

UB, MN, VV, SS, and PB led the research work, planned, supervised, conducted field experiments, read, and edited the manuscript. UB, MN, and SS collected soil/plant samples and performed chemical analysis, also wrote the initial draft of the manuscript, prepared Figures, and Tables. PB, AG, AA, AH, and SM, project supervision, reviewed, read, and edited the manuscript with significant contributions. AG, AA, AH, and SM, performed the statistical analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1225986/full#supplementary-material

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Tillage-based nutrient management practices for sustaining productivity and soil health in the soybean-wheat cropping system in Vertisols of the Indian semi-arid tropics

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To achieve higher crop production in a soybean-wheat cropping system, comprehensive knowledge of soil fertility status and its variability is crucial. However, a significant gap exists between the potential and actual productivity of this system in the Vertisols of Indian semi-arid tropics. Therefore, 2 years of field research were conducted to investigate how different crop management practices affect soil fertility in this cropping system. The trial was conducted using a randomized complete block design (RCBD) with five crop management practices: CAO (conservation tillage + organic nutrient and weed management), CAC (conservation tillage + chemical nutrient and weed management), CTC (conventional tillage + chemical nutrient and weed management), OCT (conventional tillage + organic nutrient and weed management), and PoPs (package of practices). Results showed that CAO significantly ($p < 0.05$) increased soil organic C (6.8 g kg^{-1}), available N (129.5 mg kg^{-1}), P (11.0 mg kg^{-1}), K (232.6 mg kg^{-1}), Fe (9.17 mg kg^{-1}), and Mn (10.48 mg kg^{-1}) at topsoil (0–15 cm) and deeper layers (15–60 cm). In contrast, CAC had significantly ($p < 0.05$) higher soil availability of Ca (5,072 mg kg^{-1}) and Mg (901 mg kg^{-1}) and Cu (0.84 mg kg^{-1}). On the other side, PoPs resulted in the highest S (10.05 mg kg^{-1}) and Zn (0.85 mg kg^{-1}) availability in the topsoil. Our results evidently suggested S and Zn availability as key indicators of soil health sustenance in the present agroecosystem. Notably, CAC had significantly ($p < 0.05$) higher system productivity (4.62 t ha^{-1}) than the other treatments, showing a 14.0, 6.3, and 18.2% increase over CAO, CTC, and OCT, respectively. Based on the results, it is recommended that CAC is a better option for achieving higher system productivity, while CAO is the best option for ensuring long-term sustainability of soil fertility. The findings of this study could be useful for farmers and agricultural researchers in designing efficient crop management practices to improve the productivity and sustainability of soybean-wheat cropping system in arid to semiarid ecology.

KEYWORDS
conservation agriculture, nutrient availability, organic farming, soil depth, sustainability

1. Introduction

The soybean-wheat cropping system is one of the 30 prevalent cropping systems in India and is mostly practiced in the semi-arid tropics including the states of Madhya Pradesh, Maharashtra, and Rajasthan (NMOOP, 2014). This cropping system gained importance in the 1980s when soybean was introduced as a *kharif* (rainy season) crop in the wheat-based cropping systems of India with assured canal irrigation (Ramesh et al., 2017). Soybean and wheat have been vital in addressing food and nutrition security in India (NAAS, 2017). However, system productivity of this cropping system has remained low compared to the national average over the years (Choudhary et al., 2018), particularly in the semi-arid tropical zone of Rajasthan, India. Due to the irrigated ecology, this system productivity is not constrained by soil moisture stress, which is otherwise observed in many parcels of India (Dev et al., 2022; Garg et al., 2022). One of the major factors constraining the productivity of the soybean-wheat cropping system in India is depleted soil fertility and quality (Behera et al., 2007); while the other factors include diseases and pests (Ramesh et al., 2017) and heat stress and/or weather aberrations (Lenka et al., 2016). Soil fertility has deteriorated continually due to mismanagement practices, soil disturbances, and higher nutrient exhaustion caused by high-yielding, input-responsive cultivars of cereals and pulses that have been released since the 1960s (Debnath et al., 2020, 2022). The problem lies in the fact that farmers intensively practice farming in India without appropriate crop management practices, as they are more concerned with monetary returns than caring for soil health (Roos et al., 2018; Sharma et al., 2021). This lack of attention to soil health is adversely affecting crop production, productivity, and the sustainability of soil in the region.

One potential solution to this challenge is to adopt appropriate land management practices that include adequate nutrient management and minimum soil disturbance. The current over-reliance of farmers on inorganic fertilizers to boost crop production without incorporating organic amendments can cause irreparable damage to soil properties and negatively impact the soil environment (Devi et al., 2013). Using a combination of organic and inorganic sources of nutrients can improve soil physicochemical properties, promote the growth of beneficial soil microorganisms, and maintain soil nutrient balance in the cropping system (Zhao et al., 2023). On the other side, conventional tillage practices performed over an extended period can lead to physical degradation of the soil, reduce organic carbon and microbial biomass, and ultimately reduce desirable yield (Singh et al., 2009; Bhan and Behra, 2014). In constrast, adopting conservation tillage practices can efficiently manage the soil for resource efficiency and higher productivity (Carpenter-Boggs et al., 2003; Jayaraman et al., 2021a,b).

There is a growing agreement among experts that conservation tillage practices, such as minimum tillage and residue mulch, have significant benefits for soil health. For instance, minimum tillage leads to improved soil water and carbon storage, better aggregate stability, and increased saturated hydraulic conductivity while reducing bulk density compared to conventional tillage (Jalota et al., 2001; Hati et al., 2015; Dal Ferro et al., 2023). In addition, retaining crop residues in the field has a direct or indirect positive impact on soil quality by reducing soil erosion, conserving soil moisture, maintaining hydrothermal conditions, and increasing soil porosity and infiltration (Zhu et al., 2023). Crop residues also provide energy for the growth and activity of microbes, which increases soil microbial biomass and carbon substrate for microbial biomass (Govaerts et al., 2007; Kätterer and Bolinder, 2023). Therefore, combining conservation tillage with inorganic and organic plant nutrients has the potential to improve soil fertility and crop productivity, which could lead to sustainable development opportunities for a nation like India.

The current fertilization practices in India focus solely on the nutrient needs of the succeeding crops, disregarding the residual effect of applied nutrients on the previous crops. Achieving a balance in nutrient application is crucial for optimizing the output of any cropping system. Globally most of the studies under conservation agriculture were conducted in combination with inorganic nutrients application only. However, there is lack of clear understanding on how conservation agriculture behaves under organic nutrient managements in terms of soil quality improvement and crop yield sustainability. While there have been previous studies on nutrient and tillage management practices in rice-rice (Yadav et al., 2017), rice-wheat (Jha et al., 2023; Dhaliwal et al., 2023a) and maize-wheat (Pramanick et al., 2022; Rani et al., 2023) cropping systems in India, their integrative effect on soil properties and system productivity in soybean-wheat cropping systems remains unexplored. To address these gaps, a two-year field investigation was carried out to examine the impact of conservation, organic, and conventional crop management practices on (i) soil physical and chemical properties, (ii) yield and system productivity, and (iii) key drivers of system productivity in soybean-wheat cropping systems. This study is intended to design efficient crop management practices to improve the productivity and sustainability of soybean-wheat cropping system in the semi-arid to arid ecology of India and many other parcels of the world with similar agroecology.

2. Materials and methods

2.1. Study site characteristics

A two-year field study was conducted at the Agricultural Research Station, Agriculture University, Rajasthan, India (25°10' N, 75°50′ E, and 267 m above msl) from 2018–19 to 2019–20 (Figure 1). The site experiences a subtropical climate with extremely warm and dry summers (April to June) and wet monsoons (July to September) during the soybean season and cold and harsh winters (November to January) during the wheat growing period. The mean maximum temperature ranges between 40 to 48.4°C during May–June, and the mean minimum temperature ranges between 2.0 to 8.5°C during December–January. The study area receives an annual rainfall of 660.6 mm, with the majority falling between the months of June–September. The

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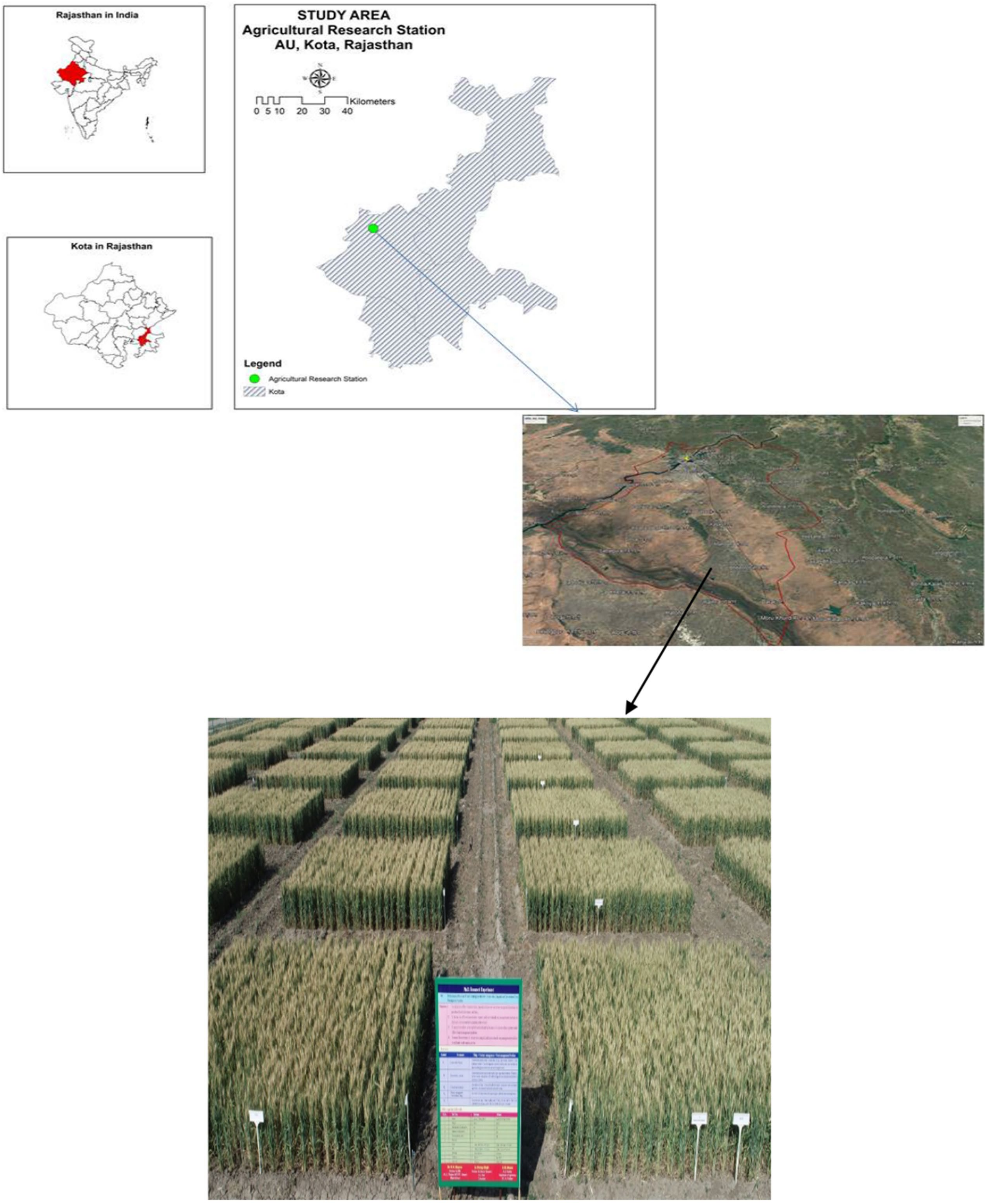

FIGURE 1
Location of the experimental site in the semi-arid tropics of India (Rajasthan).

experimental soil was moderately deep, well-drained, black Vertisols (United States Department of Agriculture classification), clay-loam in texture (sand 25.86%, silt 35.10%, and clay 38.94%), with neutral in soil reaction (7.41). The pre-crop topsoil (0–15 cm) contained organic C (5.1 g kg^{-1}), available N (104.5 mg kg^{-1}), P (9.4 mg kg^{-1}), K (196.0 mg kg^{-1}), S (7.5 mg kg^{-1}), and DTPA-extractable Zn (0.66 mg kg^{-1}), Fe (6.80 mg kg^{-1}), Cu (0.61 mg kg^{-1}), and Mn (7.21 mg kg^{-1}).

2.2. Treatments and experimental design

The study implemented various combinations of conservation tillage, organic, and chemical management practices, including nutrient and weed management, in a soybean-wheat cropping system. The treatments consisted of five scenarios, namely T1-conservation organic (CAO), T2-conservation chemical (CAC), T3-conventional chemical (CTC), T4-organic management + conventional tillage (OCT), and T5-package of practices (PoPs), which were randomly assigned in a randomized complete block design (RCBD) with four replications (Table 1). The plot size for each treatment was 389 m2 (48 m x 8.10 m). Before sowing the crops each year, well-decomposed farmyard manure (FYM) was incorporated into the soil based on the treatment requirements. The total N content in FYM was determined by Kjeldahl digestion following the distillation method (Jackson, 1973), and the FYM was also analyzed for total P, K, S, and Zn, Cu, Fe, and Mn contents through digestion with an HNO_3:$HClO_4$ (4:1) mixture and subsequent determination by vanadomolybdophosphoric acid, flame photometric method, and atomic absorption spectrophotometric method, respectively (Manna et al., 2012). The mean nutrient contents of FYM were analyzed as follows: N (0.50%), P (0.26%), K (0.50%), S (0.03%), Zn (24.80 ppm), Fe (173.90 ppm), Cu (5.15 ppm), and Mn (97.45 ppm). a slightly alkaline pH (7.41).

2.3. Crop management

Table 1 presents the agricultural techniques employed for cultivating the test crops, soybean and wheat, during the 2018–19 and 2019–20 seasons. In the rainy (*kharif*) season, soybean (cv. RKS 45) was sown using a seed drill in mid-July at a spacing of 30 cm (intra-row) x 10 cm (intra-plant) with a seed rate of 80 kg ha^{-1} and harvested in the last week of October. The experimental site was then irrigated for field preparation and sowing of the succeeding wheat crop in the winter (*rabi*) season. Wheat (cv. Raj 4,079) was sown at a spacing of 22.5 cm x 5 cm with a seed rate of 100 kg ha^{1}. Seeding was completed using a seed drill in the first week of December and harvested during the first week of April. Other crop management practices were carried out according to the assigned treatments (Table 1). During the 2018 *kharif* season, one life-saving irrigation was applied at the pod-filling stage [77 days after sowing (DAS)], while no irrigation was applied during the 2019 *kharif* season due to sufficient moisture availability at critical stages of soybean growth. However, during the *rabi* season, four irrigations were applied to the wheat crop at its critical growth stages during the experiment. For soybean, the total quantities of N, P, K, and S were applied as a basal dose, whereas for wheat, 100% of P, K, Zn, and 50% of N were applied at the time of sowing, and the remaining 50% of N was applied after the first irrigation (24 DAS) during the experiment.

2.4. Soil sampling and analysis

Composite soil sampling, involving four depth intervals (0–15, 15–30, 30–45, and 45–60 cm), was done form each plot using a soil auger with 6.0 cm internal diameter after harvest of each crop. The samples were dried in shade and then ground and passed through a 2.0 mm sieve for further laboratory analysis. The soil was analyzed for pH and electrical conductivity (EC; 1,2.5 w/v; Jackson, 1973), organic C (SOC; Walkley and Black, 1934), cation exchange capacity (CEC; Jackson, 1973), and exchangeable Ca and Mg (Jackson, 1973). The soil was also analyzed for also available nutrients like N (Subbiah and Asija, 1956), P (Olsen et al., 1954), K (Schollenberger and Simon, 1945), S (Chesnin and Yien, 1950), Zn, Fe, Cu, and Mn (Lindsay and Norvell, 1978). Bulk density (BD) of experimental site was measured using a core sampler (Blake and Hartge, 1986), and the soil porosity was calculated by the following equation:

$$\text{Porosity}\,(\%) = 1 - \frac{\text{Bulk Density}}{\text{Particle Density}} \times 100$$

2.5. Yield and system productivity of cropping system

At maturity, soybean and wheat crops were harvested manually for economic yield, and the equivalent yield and system productivity were calculated using the following equations:

$$\text{Soybean equivalent yield of wheat}\left(\text{tha}^{-1}\right) = \frac{\text{Yield of wheat}\left(\text{tha}^{-1}\right) \times \text{MSP of wheat}\left(₹\text{t}^{-1}\right)}{\text{MSP of soybean}\left(₹\text{t}^{-1}\right)}$$

System productivity was calculated by adding the soybean yield and soybean equivalent yield of the wheat for the respective years.

$$\text{System productivity}\,(\text{SEY}) = \frac{\text{Yield of soybean}\left(\text{tha}^{-1}\right) \times \text{MSP of soybean}\left(₹\text{t}^{-1}\right)}{\text{MSP of soybean}\left(₹\text{t}^{-1}\right)} + \frac{\text{Yield of wheat} \times \text{MSP of wheat}\left(₹\text{t}^{-1}\right)}{\text{MSP of soybean}\left(₹\text{t}^{-1}\right)}$$

The minimum support price (MSP) was for soybean (₹ 34,000 t^{-1} and ₹ 37,000 t^{-1}) and wheat (₹ 18,400 t^{-1} and ₹ 19,250 t^{-1}) during the year 2018–19 and 2019–20, respectively. 1 USD ($) = 82 INR (₹).

2.6. Statistical analysis

The data presented in this study were pooled from both years and analyzed using two-way analysis of variance (ANOVA) in Statistical Analysis Software v9.4 (SAS Institute, 2016; Supplementary Table S1) and R statistical software (R Core Team, 2016). The relationship between various treatment scenarios and variables was calculated, and the variables were classified based on stability and mean (Yan and Kang, 2002). The effect of different treatments on soil chemical properties was analyzed using a biplot analysis through principal component (PC) with the support of R Studio (RStudio Team, 2014). A Pearson's correlation matrix (correlogram) was constructed to determine the degree of correlation between the tested soil variables.

3. Results and discussion

3.1. Soil physical properties

It was observed that the effects of different management practices on soil bulk density (BD), particle density (PD), and porosity under

TABLE 1 Details of tillage, weed and nutrient management imposed in the present experiment.

	Season/crop	T_1: Conservation organic (CAO)	T_2: Conservation chemical (CAC)	T_3: Conventional chemical (CTC)	T_4: Organic management + conventional tillage (OCT)	T_5: Package of practices (PoPs)*
Tillage	*Kharif* / Soybean	One ploughing and direct sowing through seed drill, previous crop biomass retention (wheat @ 2.5 t ha^{-1})	Same as in T_1	One summer ploughing, two ploughing with planking and sowing through seed drill	Same as in T_3	Same as in T_3
	Rabi / Wheat	One ploughing and direct sowing through seed drill previous crop biomass retention (soybean 1.5 t ha^{-1})	Same as in T_1	three ploughing, sowing of wheat was completed through seed drill	Same as in T_3	Same as in T_3
Weed management	*Kharif* / Soybean	Non-chemical methods, i.e., dust mulch at 20 DAS under cultural method and hand weeding at 35 DAS under mechanical method.	Chemical method: a ready mixed herbicide *viz.* sodium acilfluorfen 16.5% + clodinafop propargyl 8% EC (165 + 80 g *a.i.* ha^{-1}) as sprayed at 25 DAS in soybean	Same as in T_2	Same as in T_1	Same as in T_2
	Rabi / Wheat	Non-chemical methods, i.e., dust mulch at 20 DAS under cultural method and hand weeding at 35 DAS under mechanical method.	Chemical method: a ready mixed herbicide *viz.* clodinafop-propargyl 15% WP + metsulfuron methyl 1% WP (48 + 4 g *a.i.* ha^{-1}) was sprayed as PE at 32 DAS in wheat crop	Same as in T_2	Same as in T_1	Same as in T_2
Nutrient management	*Kharif* / Soybean	Organic source N:P: K, 30:40:40 FYM 6 t ha^{-1} + PSB 600 g ha^{-1}	FYM + Fertilizer N:P: K, 30:40:40 FYM 5 t ha^{-1} + (Urea 11 + SSP 200 + MOP 25 kg ha^{-1})	FYM + Fertilizer N:P:K, 30:40:40 FYM 5 t ha^{-1} + (Urea 11 + SSP 200 + MOP 25 kg ha^{-1})	Organic source N:P:K:S, 30:40:40:30 FYM 6 t ha^{-1} + PSB 600 g ha^{-1}	FYM + N:P:K:S 20:40:40:30 FYM 10 t ha^{-1} + (Urea 65 + SSP 250 + MOP 67 + Elemental S 2.2 kg ha^{-1})
	Rabi / Wheat	Organic source N:P:K 180:40:30 FYM 36 t ha^{-1} + PSB 600 g ha^{-1}	FYM + Fertilizer N:P:K 180:40:30 FYM 5 t ha^{-1} + PSB 600 g ha^{-1} + (Urea 337 + SSP 250 + MOP 50 kg ha^{-1})	FYM+ Fertilizer N:P:K 180:40:30 FYM 5 t + PSB 600 g + (Urea 337 + SSP 250 + MOP 50 kg ha^{-1})	Organic source N:P:K 180:40:30 FYM 36 t ha^{-1} + PSB 600 g ha^{-1}	Fertilizer N:P:K:Zn 120:40:30:25 PSB 600 g ha^{-1} + (Urea 260.9 + SSP 250 + MOP 50 + $ZnSO_4$. $7H_2O$ 25 kg ha^{-1})

*T_5 – Incorporation of FYM@10 t ha^{-1} for package of practices once in a 3 year thus, applied only during experiment initiation *kharif* 2018.

the soybean-wheat cropping system were non-significant (p > 0.05; Table 2). However, the conservation organic crop management practice resulted in a reduction of BD (1.2 Mg m−3) and subsequently increased soil porosity (51.8%) at the topsoil (0–15 cm). Previous studies have shown that minimum tillage and the application of organic manures for many consecutive years can reduce soil bulk density and increase porosity (Govaerts et al., 2009; Gathala et al., 2011). Soil bulk density is an important indicator of changes in soil physical condition and water holding capacity due to different tillage practices (Jin et al., 2007). Alam et al. (2014) observed a significant reduction in soil bulk density under zero tillage, minimum tillage, conventional tillage, and deep tillage when compared to initial values, which supports the results of our study.

3.2. Soil physico-chemical properties

Unlike soil physical properties, tillage and crop management practices had significant influence ($p < 0.05$) on soil physico-chemical properties involving pH, EC, and SOC (Figures 2A–C). Conservation organic (CAO) and OCT resulted in lowering pH towards neutrality. Similarly organic management (CAO and OCT) significantly reduced EC as compared to CTC and PoPs. There was an increasing trend in pH and along the soil depths, irrespective of the treatments. Averaged across the depths, although CAO and OCT recorded higher CEC but remained at par with other treatments (Figure 2D). SOC showed a depleting trend along with increasing soil depth across the treatments (Figure 2). At 0–15 cm, the highest SOC was recorded under conservation organic (6.8 $g kg^{-1}$) followed by organic management + conventional tillage (6.6 $g kg^{-1}$), and the least was observed in the package of practices (5.6 $g kg^{-1}$). A similar trend was also noticed for other soil depths. Averaged over the soil depths, conservation organic (CAO) recorded 16.3% higher SOC as compared to PoPs over 2 years of experimentation.

Organic carbon in soil ecosystem is mostly controlled through a complex interaction between soil edaphic factors and land husbandry (Yadav et al., 2020). Residues and dead-decaying debris of crops are the main contributor to the organic carbon accumulation in the soil (Lal, 2004). Previous reports indicated that SOC can be increased by reduced tillage (Neugschwandtner et al., 2014) and by crop residues retention (Hati et al., 2015). Onward 60 cm depth, significant increase in SOC under CAO might be due to relatively high quantity of organic inputs through FYM and minimal soil disturbance resulting better permanency of soil organic matter (Hati et al., 2015; Mohanty et al., 2020; Yadav et al., 2020). A likewise increment in SOC under OCT again suggests that SOC accrual was chiefly governed by FYM addition rather than by tillage practices. Earlier, Meena et al. (2019) also demonstrated that FYM addition can significantly increase SOC content in the soil. Apart from increasing SOC, continuous addition of high quantity of FYM can also decrease soil pH (Hao and Chang, 2002; Rayne and Aula, 2020) by lowering the conductance, which corroborates our observation. On the other side, increase in CEC under CAO and OCT may be attributed to the presence of organic matter in manure that decomposes to increase the negatively charged sites on carboxyl and phenolic hydroxyl groups (Hao and Chang, 2002; Miller et al., 2016).

TABLE 2 Physical properties of soil as influenced by tillage, nutrient and weed management practices.

Treatments	Bulk density (g cm^3)	Particle density (g cm^3)	Porosity (%)
CAO	1.20 ^{a}	2.49 ^{a}	51.78 ^{a}
CAC	1.26 ^{a}	2.55 ^{a}	50.59 ^{a}
CTC	1.28 ^{a}	2.58 ^{a}	50.37 ^{a}
OCT	1.23 ^{a}	2.53 ^{a}	51.43 ^{a}
PoPs	1.30 ^{a}	2.60 ^{a}	49.98 ^{a}

Values followed by letter in common do not differ significantly ($p < 0.05$) as per Tukey's HSD test.

3.3. Availability of primary nutrients

Tillage and crop management had a significant ($p < 0.05$) impact on the availability of primary nutrients (N, P, and K) as shown in (Figures 2E–G). The CAO crop management treatment had the highest availability of primary nutrients across all soil depths and was significantly ($p < 0.05$) higher than the PoPs treatment. Compared to PoPs, the CAO and CAC treatments showed about a 10.0% increase in N availability. Nitrogen availability decreased across all treatments from 126.2 (0–15 cm) to 89.9 mg kg−1 (45–60 cm). Similarly, compared to CTC and PoPs, conservation tillage with organic and chemical management greatly increased the availability of P (7.9 to 14.7%) and K (5.5 to 7.4%). This is further illustrated in the biplot analysis (Figure 3). Additionally, the availability of P and K decreased with increasing soil depth across all treatments. On the other hand, a strong positive correlation between SOC and the availability of primary nutrients was observed (Figure 4).

The higher availability of N, P, and K in conservation tillage with organic and inorganic nutrient and weed management may be attributed to the increased supply of organic matter and improved soil ecosystem for nutrient cycling. Organic manures, in general, act as slow-release fertilizers, and their decomposition leads to an enhanced availability of nutrients (Mandal et al., 2000; Meena et al., 2019). Previous studies have shown that combining N fertilizer with quality crop residues can have a positive interactive effect on mineral N (Gentile et al., 2008). In contrast, straw incorporation in soil has been found to increase microbial biomass and N mineralization, leading to a higher nutrient supply in soil (Eagle et al., 2000; Choudhary et al., 2018; Dhaliwal et al., 2023b). These observations suggest that both organic and inorganic nutrient management can increase nutrient availability in soils, supporting our findings. Additionally, changes in SOC due to tillage practices can influence N content, with conventional tillage resulting in greater losses due to frequent tillage, higher leaching, and mineralization losses (Lal, 1997; Cui et al., 2023). This may explain the lower N availability observed under CTC.

Again, higher availability of N and P was observed in surface soil under zero tillage and minimum tillage. The build-up of available P in soil is attributed to its constrained downward movement, as reported in previous studies (NzeMemiaghe et al., 2022). The higher availability of K and P under conservation and organic management practices might be attributed to reduced fixation or solubilization of fixed forms due to the higher prevalence of organic acids as well as mineralization of added organic manure, as reported in previous investigations (Yadav and Kumar, 2002; Berner et al., 2008; Mahanta and Rai, 2008; Elayarajan et al., 2015; Meena et al., 2019). Moreover, mobilization of non-exchangeable K into the soil solution might have increased its

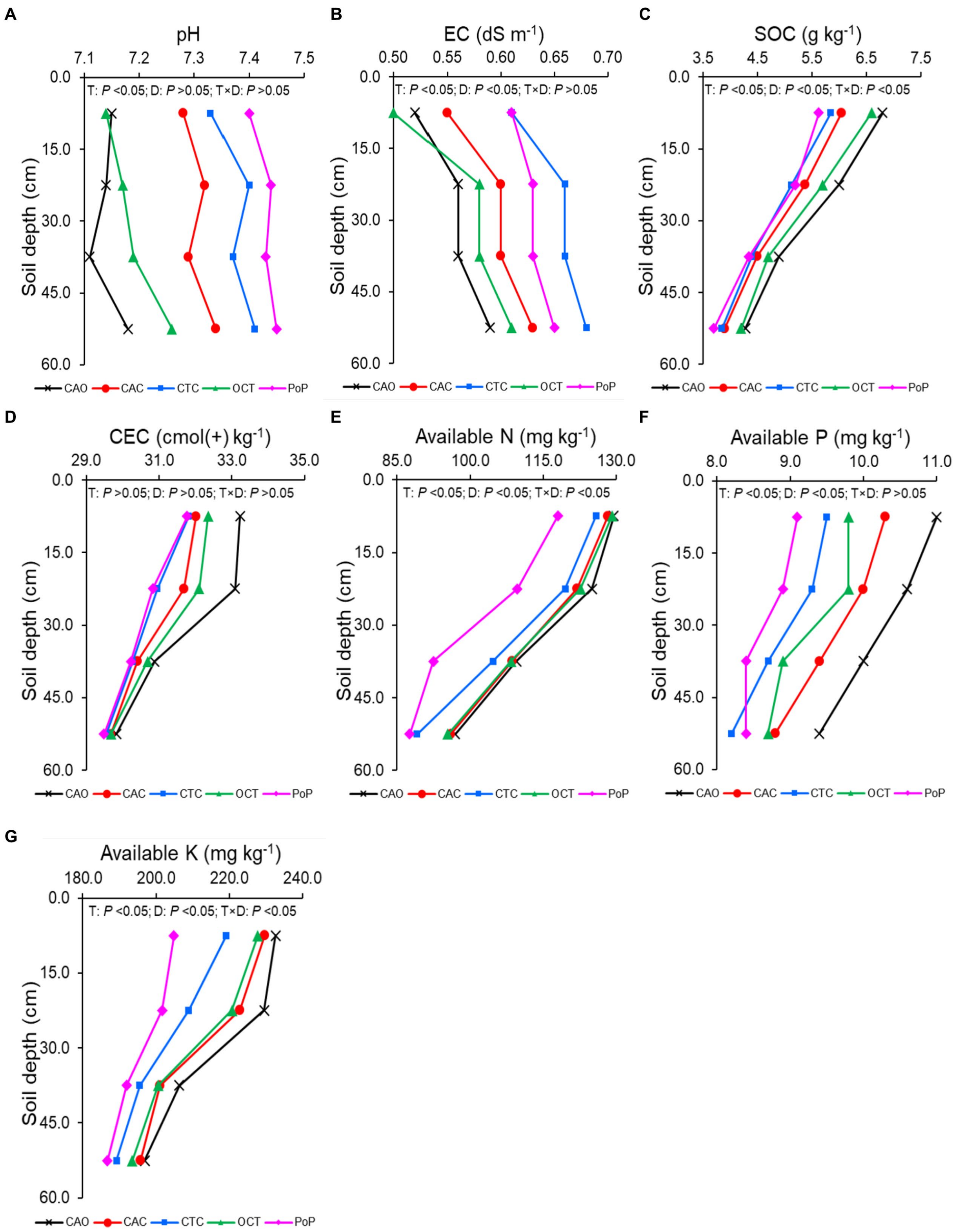

FIGURE 2
Depth-wise variation in the soil physico-chemical properties [**(A)**-pH; **(B)**-EC; **(C)**-SOC and **(D)**-CEC] and availability of primary nutrients [**(E)**-available N; **(F)**-available P and **(G)**- available K] as influenced by tillage, nutrient and weed management practices. Statistical significance ($p < 0.05$) was tested for treatment (T), soil depth (D) and treatment × soil depth (T × D). EC: electrical conductivity; SOC: soil organic C.

availability in the soil under conservation and organic management practices (Venkatesh et al., 2017). Simultaneous increase in soil organic carbon and availability of nutrients with crop residue amendments has also been reported in soybean (Singh and Rai, 2004) and is in line with our observation on positive correlation between SOC and primary nutrients.

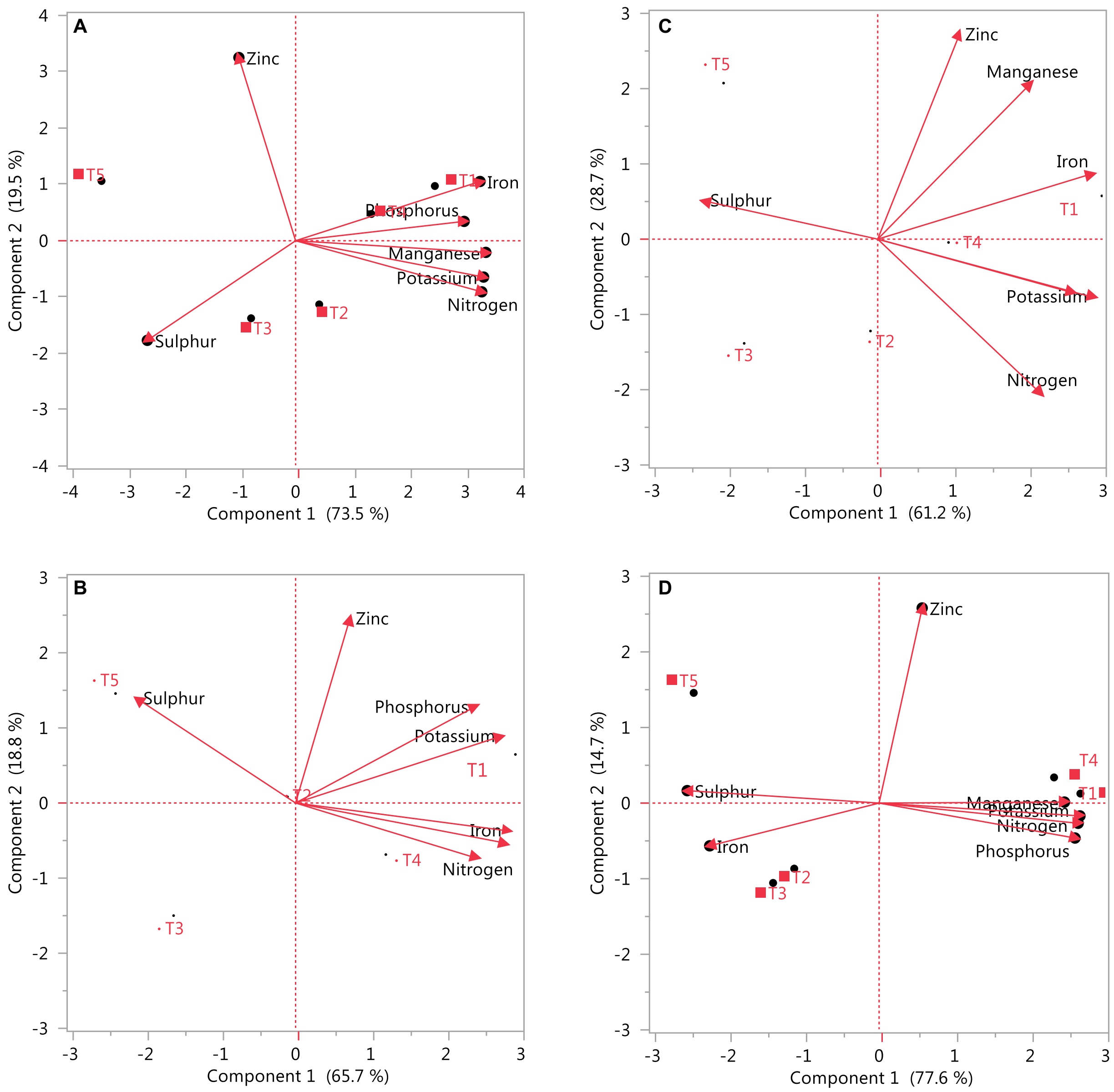

FIGURE 3
PCA biplot analysis of treatment effects (tillage, nutrient and weed management) on soil chemical properties. **(A)** 0–15 cm; **(B)** 15–30 cm; **(C)** 30–45 cm; **(D)** 45–60 cm. The abbreviation section includes key to the label of treatments.

3.4. Availability of secondary- and micro-nutrients

The availability of Ca, Mg, S, (Figures 5A–C) and micronutrients (Figures 5D–G - available Zn, Fe, Cu, and Mn) at different soil depths was significantly ($p < 0.05$) affected by tillage and crop management practices (Figure 5). The CAC treatment showed the highest availability of exchangeable Ca and Mg, which was significantly ($p < 0.05$) higher than the CAO and OCT treatments. Conversely, S availability was comparable between CAC and PoPs but remained significantly ($p < 0.05$) higher than the other treatments. Notably, the least availability of secondary nutrients was observed in OCT. Zinc availability was significantly ($p < 0.05$) higher with PoPs, followed by CAO (Figure 5). Furthermore, Fe and Mn availability increased by 6.5 and 17.3%, respectively, with CAO compared to PoPs management. However, Cu availability was significantly ($p < 0.05$) higher with CAC than the other treatments. Similar to primary nutrients, the availability of secondary and micronutrients also decreased with increasing soil depth across all treatments. Apart from the availabilities, a close view of the biplot graphs revealed distinct position of S and Zn across the soil depths (Figure 3).

Overall, the availability of secondary and micronutrients in the soil was greatly improved by the addition of nutrients from organic and inorganic sources, as well as by tillage practices that affected both the quantity and mineralization of nutrients in the soil. Our results support the findings of Gadana et al. (2020), who also reported a positive impact of soil management practices on exchangeable cations and soil micronutrients. Crop residue retention and the addition of organic manure and mineral fertilizers provided an added advantage for better microbial growth, which accelerated nutrient mineralization and led to enhanced nutrient availability (Kiboi et al., 2021). It is worthy to note that the availability of secondary nutrients remained

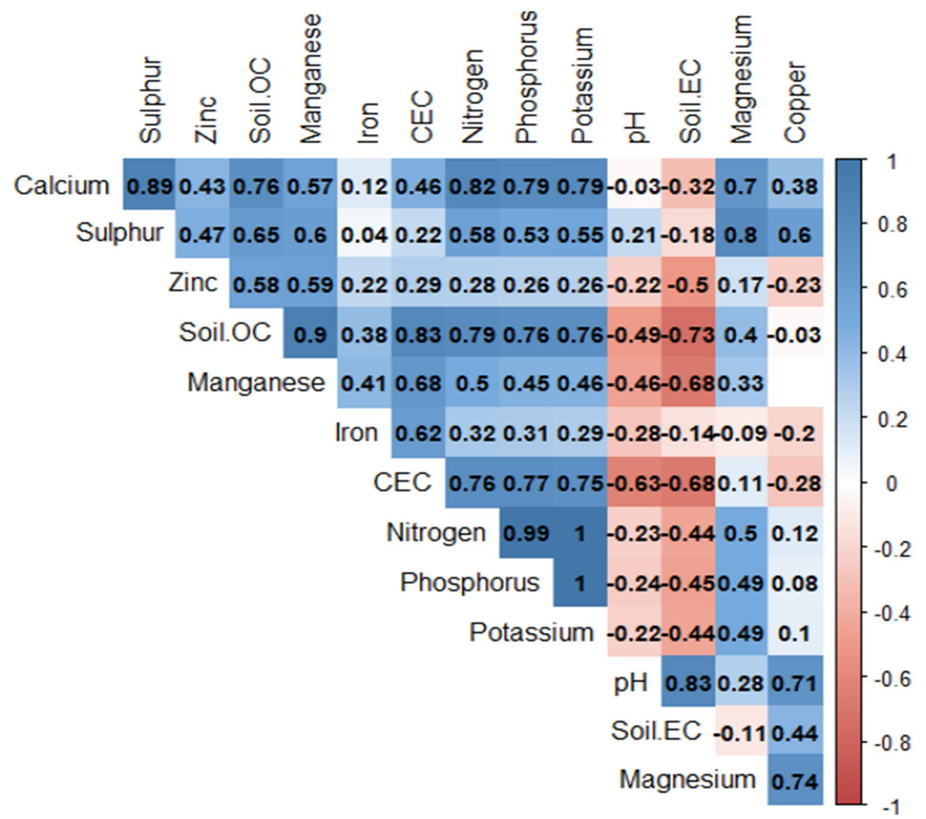

FIGURE 4
Correlogram (Pearson) of different soil fertility parameters as affected by tillage, nutrient and weed management practices.

high for the treatments receiving chemical fertilizers in conjunction with manure. In this study, we used single superphosphate as the P source, which contains a fairly high quantity of Ca and S (Barker, 2019). Therefore, the increased availability of these nutrients in chemically fertilized plots is not surprising.

Regardless of the management practices, the soil in this study showed high availability of micronutrients in comparison to the critical limits identified for Indian soils (Katyal and Sharma, 1991; Debnath et al., 2022), which contrasts with the marginal to medium status of available Fe and Zn and the medium to high status of available Mn and Cu concentration in farmed Vertisol parcels in India (Somasundaram et al., 2009). Therefore, conservation tillage coupled with organic nutrient management may be a sustainable practice to maintain the availability of micronutrients in the soils of this region. However, their removal from crop biomass with continuous cropping may decrease their phytoavailability in soils in the future. Periodical soil testing may thus help to decipher their depletion in soil so as to undertake appropriate remedial measures. Distinct position of S and Zn in biplots suggests their role as key indicators of sustaining soil health in the soybean–wheat systeam in arid ecology. Therefore, periodic monitoring of their availability remains indispensable to avoid yield trade-offs due to their short supply.

Compared to the 30–45 cm depth, a slightly higher availability of micronutrients was observed at the 45–60 cm depth, possibly due to leaching from the upper layer and accumulation at a later soil depth. However, this effect was absent for secondary nutrients, suggesting their leaching into deeper soil layers. This indicates that the leaching of micronutrients into deeper soil layers was possibly prevented by the formation of organo-mineral complexes or chelation due to organic inputs and increased soil organic matter (SOM). The higher availability of Zn with PoPs and CAO may be due to Zn application and organic matter addition, respectively. Our results also suggest that conservation tillage had a significant effect on Fe availability. Similarly, Jayaraman et al. (2021b) reported that available Fe concentration was relatively higher under no-till than conventional tillage in vertisols of Central India. The hierarchical clustering analysis of different soil properties suggests that organic matter addition has significantly improved the availability of Cu and Mn (Figure 6).

3.5. System productivity of cropping system

The soybean-wheat cropping system registered the maximum system productivity (SEY) in CAC with a yield of 4.62 t ha^{-1}, which was significantly higher by 13.96 and 18.17% compared to CAO and OCT, respectively (Table 3). However, SEY of CAC and PoPs was statistically similar. Although nutrient availability increased with organic nutrient management combined with conservation or conventional tillage practices, it did not enhance SEY. This suggests that other factors may have influenced the yields of different treatments. Kravchenko Alexandra et al. (2017) also reported yield penalties of 10–30% under organic management during the initial years. Effective weed management and timely availability of nutrients to the crop under CAC could be the prominent reasons for comparatively higher yields. Tillage

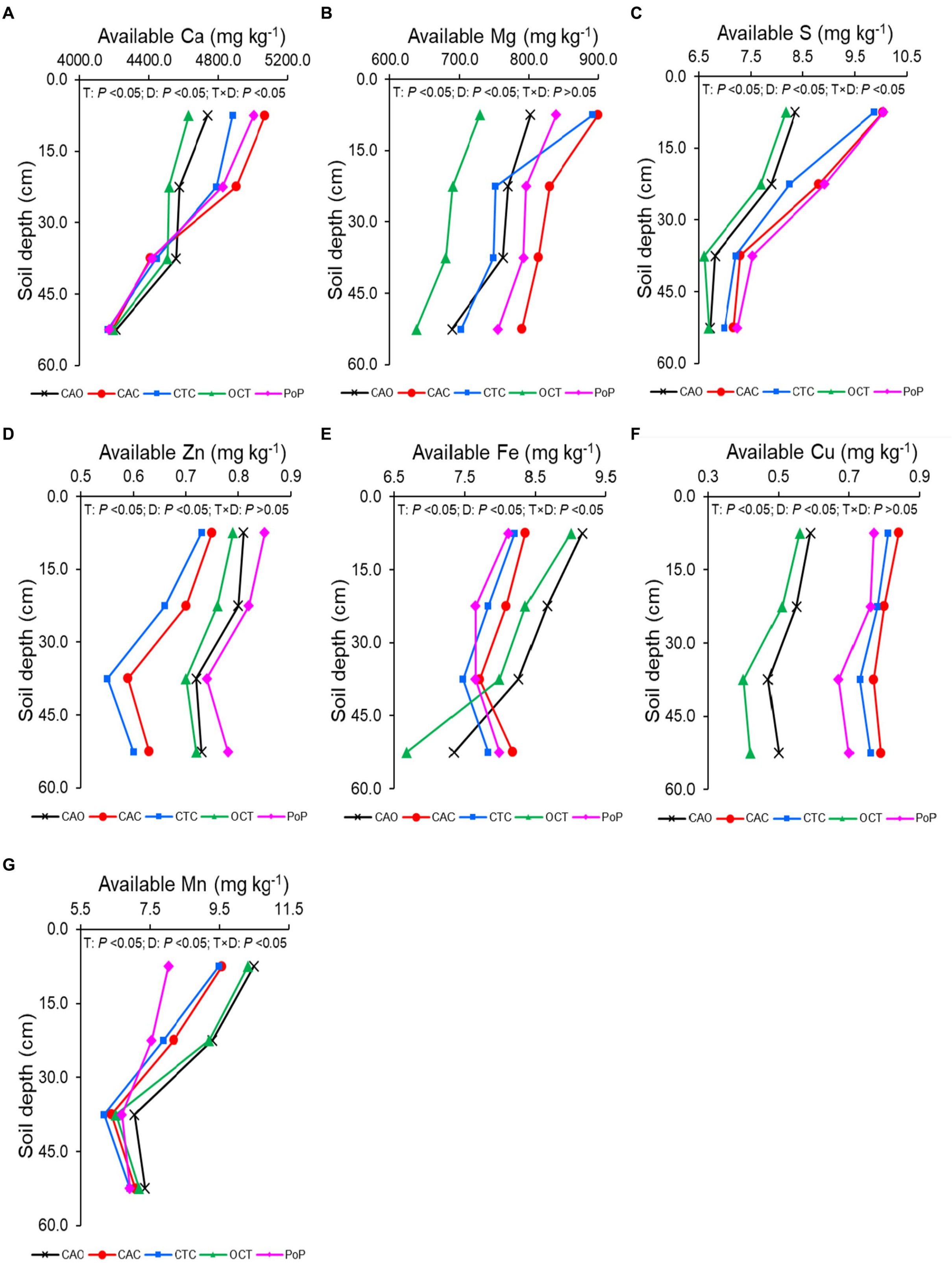

FIGURE 5
Depth-wise variation in the availability of secondary [**(A)**-available Ca; **(B)**-available Mg and **(C)**- available S] and micro-nutrients nutrients as [**(D)**- available Zn; **(E)**-available Fe; **(F)**- available Cu and **(G)**-available Mn] influenced by tillage, nutrient and weed management practices. Statistical significance ($p < 0.05$) was tested for treatment (T), soil depth (D) and treatment × soil depth (T × D).

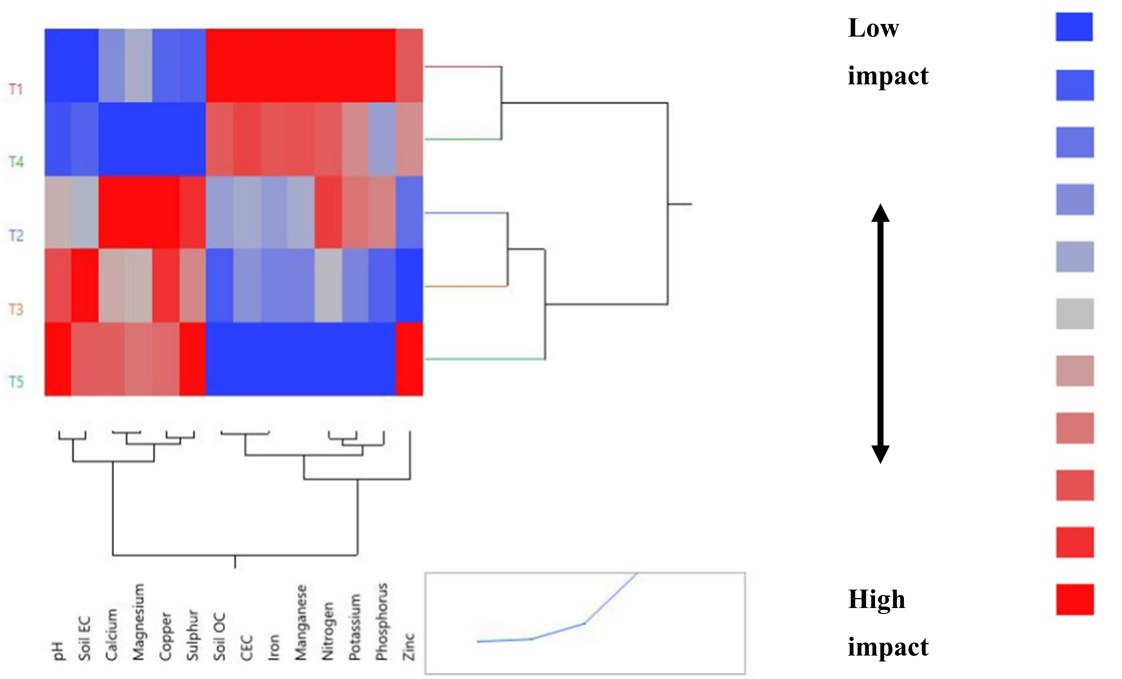

FIGURE 6
Hierarchical clustering of different soil chemical properties as affected by tillage, nutrient and weed management practices.

TABLE 3 Yield and system productivity of soybean-wheat cropping system as influenced by tillage, nutrient, and weed management practices.

Treatments	Soybean seed yield (t ha^{-1})	Wheat grain yield (t ha^{-1})	System productivity (t ha^{-1})
CAO	1.64	4.55	4.05
CAC	1.85	5.21	4.62
CTC	1.77	4.86	4.34
OCT	1.56	4.41	3.91
PoPs	1.85	5.09	4.55
SEm±	0.05	0.15	0.09
CD ($P < 0.05$)	0.15	0.43	0.27

SEm±: Standard error ($n = 4$). CD: Critical difference.

operation, nutrient management, and leguminous crops can significantly influence crop yields in a cropping system (Alam et al., 2020). Meena et al. (2022a, 2022b) also reported that CAC evolved as a better crop management practice under the soybean-wheat cropping system, producing the maximum economic yield of soybean over a regime of practice involving the recommended package of practices: conventional tillage + chemical, conservation tillage + organic, and conventional tillage + organic nutrient management.

4. Conclusion

Our study highlights the importance of the soybean-wheat cropping system for improving soil fertility and ensuring sustainable yields. Organic nutrient and weed management, combined with conservation or conventional tillage, improved soil fertility attributes such as SOC, N, P, K, Ca, Mg, Zn, and Fe but had no significant impact on soil physical characteristics. However, the effects of these management practices on soil fertility attributes were predominantly limited to topsoil (0–15 cm) alone. Our results clearly demonstrated that SOC accrual in the soil was primarily governed by organics addition rather than by tillage practices. Again, the results elucidated S and Zn as key indicators of sustaining soil health in the soybean–wheat systeam of arid ecology and therefore, warrants periodic monitoring of their availability to circumvent yield trade-offs, if there be any, due to their deficiency. The highest system productivity was achieved through the use of chemical herbicides and fertilizers in conjunction with conservation tillage (CAC), which is likely due to effective weed control and instantaneous nutrient availability. Nevertheless, adoption of conservation tillage with organic nutrients and weed management technology can be a long-term strategy for sustaining soil fertility under soybean-wheat cropping system in arid to semiarid ecology, which may help ensuring global food security in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

SN: implementation of field experiment, observation recording, monitoring, and other management practices related to study. SS:

designed the study and guidance. AR, BM, DJ, SD, and DS: manuscript writing, edition and statistical analysis and interpretation of data; DJ and SM: editing and interpretation of results. SY, PV, UD, JM, and SN: technical help, contributed in sample analysis, soil analysis, and drafting. PS: conceptualize the study and guidance during study. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1234344/full#supplementary-material

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Precise macronutrient application can improve cane yield and nutrient uptake in widely spaced plant-ratoon cycles in the Indo-Gangetic plains of India

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Introduction: Sugarcane is a long-duration and nutrient-exhaustive crop. To improve nutrient use efficiency, the 4R nutrient stewardship approach comprises applying nutrients at the right time and place with the right method and at the right proportion. Improper nutrient management in such a nutrient-exhaustive crop will result in various nutrient losses and environmental pollution.

Methods: Concerning this, a field study was performed on calcareous soils of the lower Indo-Gangetic plains of India during two subsequent years at the Sugarcane Research Institute, RPCAU, India, to explore the effect of precise application of macronutrients (N and K) in different methods of applications. The application methods (broadcasting and band application) were maintained in the main plot, and split N and K applications were put in the subplots.

Results and Discussion: A highly significant difference was observed in the numbers of millable cane, cane, and sugar yield under the split applications of fertilizer. The decline in millable cane numbers, cane, and sugar yield due to the broadcasting method was to the tune of 17.5 and 17.6%, 14.8 and 17.1%, and 14.7 and 15.8% in plant and ratoon crops, respectively as compared to band placement of the fertilizers. Yield increased by 16.0 and 15.1% under plant and ratoon crops, respectively, with seven split applications of N and K compared to the control (two split of N and no split application of K). Band placement of N and K fertilizers markedly improved the nitrogen uptake (284.1 and 287.3kgha^{-1}, in plant and ratoon, respectively) and phosphorus uptake (34.9 and 28.3kgha^{-1} in plant and ratoon, respectively) when compared to broadcasting. Application of N and K in seven splits resulted in better availability of nutrients in the soil, thereby facilitating the higher NPK uptake by the plants and ratoon both comparing two split applications of N and no splitting of K. From this study, it was observed that the band placement coupled with seven splitting of N and K is the best fertilizer application protocol, ensuring higher growth, yield, quality, and nutrient uptake of sugarcane in the calcareous soils of the Indo-Gangetic plains.

KEYWORDS

band placement, cane yield, crop quality, NPK uptake, sugarcane, split application of fertilizer

1. Introduction

Sugarcane is an important agro-industrial crop of tropical and subtropical climates and plays a significant role in the Indian economy. As a long-duration crop, sugarcane has a high demand for nutrients, especially nitrogen (N) and potassium (K) (Dotaniya et al., 2016). Among the macronutrients, N is more prone to leaching and volatilization losses. Fertilizer utilization is more prevalent in intensive sugarcane farming, which needs a lot of nitrogen since it generates an abundance of biomass (Otto et al., 2022). Sugarcane has a high water and nitrogen demand, which increases the risk of ground-or surface-water contamination (Dotaniya et al., 2016). N uptake by the crop has been affected by various factors such as the amount and quality of N fertilizer used, timing and frequency of its application, the kind and length of the crop, the ability of the crop to use N, the depth of the root system, rainfall, the hydraulic properties of the soil, and overall crop management techniques (Kumar et al., 2019b). A better fertilizer application strategy considering suitable method and time of application is crucial to maximizing the growth and yield of the crops with the least nutrient leaching (Pramanick et al., 2018; Lakshmi et al., 2021; Singh et al., 2021).

Strategies entail altering fertilizer application rates and frequencies to enhance the N efficiency and reduce the N leaching from the rooting zone (Pramanick et al., 2022). In most sugarcane growing areas, synthetic fertilizers are typically applied using the broadcasting method, which reduces nutrient use-efficiency to a considerable extent (Abdel Wahab, 2014). The health of the soil can be ensured by the judicious use of chemical fertilizers and by systematically implementing the 4R (correct rate, right time, right source, and proper technique) concept of nutrient management (Johnston and Bruulsema, 2014). This will help to develop a balanced relationship between soil quality and agronomic management of field soil. The fertilizer application method and rate must be changed concurrently for high crop yield and high nutrient use efficiency (Laik et al., 2021; Pramanick et al., 2023). The N, P, and K fertilizers, which are primarily imported, are significantly more expensive and are not utilized effectively by farmers in sufficient quantities, which causes stagnation or a fall in sugarcane productivity over time (Singh et al., 2019). All of these indicate a better potential for using more balanced fertilizers to increase cane output, improve quality, maintain system sustainability, and improve nutrient use efficiencies. Sugarcane seldom recovers more than 40% of its nitrogen fertilizer (Skocaj et al., 2013). Therefore, a well-balanced fertilizer application, which includes a significant amount of N and K, might be a first step toward attaining these critical objectives. However, phosphorus is also vital for cane production. Split applications describe adjusting fertilizer supply to a predetermined target yield and a specific soil moisture level (Pramanick et al., 2022). Fertilizers for sugarcane plants should have an appropriate quantity of nitrogen (N), as well as the proper ratios of phosphorus (P) and potassium (K).

The current focus should be on factors that limit sugarcane output and that are within farmers' control, as these appear to be more pressing and resolvable. Numerous studies have examined the effects of applying nitrogen split throughout two or three applications (Lakshmi et al., 2020). Sugarcane growers in India have been successfully persuaded to use balanced N and P fertilizing techniques. Potassium, a crucial component for cane productivity as well as for other quality indicators, is regrettably commonly ignored by farmers. Farmers regularly lose this costly nutrient, which is strongly linked to environmental degradation, climate change, and soil health, due to excessive amounts of nitrogen fertilizer. Due to the vulnerability of fertilizer N to leaching and de-nitrification losses, split N fertilizer applications seem advantageous. Nutrient loss is more significant in the calcareous soil in Bihar, India, and nitrogen and potassium in splits might result in less loss of nutrients and greater fertilizer use efficacy. A suitable fertilizer application method coupled with proper split applications of macronutrients in sugarcane is yet to be developed in the vast calcareous soil regions of the lower Indo-Gangetic plains. Considering the significance of splitting nitrogen and potassium fertilizers in sugarcane, the present study was conducted to determine the effective macronutrient application protocol for achieving better growth attributes, yield, juice quality, and nutrient uptake in sugarcane under plant-ratoon cycles. This study will provide important information on the macronutrient management strategy for sugarcane, ensuring better yield and lower nutrient loss in the calcareous soils of the Indo-Gangetic regions.

2. Materials and methods

2.1. Experimental site

The current study was carried out in the lower Indo-Gangetic plains of Bihar between 2019 and 2021 in a plant-ratoon system of sugarcane on calcareous soils. The available nitrogen, phosphorus, and potassium in the soil of the experimental field were 230.5, 20.3, and 130.1 kg ha^{-1}, respectively. The soil had a pH value of 8.2 and was a sandy loam texture. Rainfall was 1,590 and 1,881 mm during the growing seasons of the plant and ratoon crops, respectively. The detailed climatic scenario during the study period is included in Supplementary Figure S1.

2.2. Experimental design and sampling

The experiment was conducted using a split-plot design with two main plot factors (B1: Band placement and B2: Broadcasting) and four sub-plot factors with a split application of the recommended dose of nitrogen (RDN) and recommended dose of potassium (RDK) as follows: S1: Basal 10% remaining at 45, 75, 90, and 120 days after planting or DAP in equal splits (RDN + RDK in 5 splits); S2: Basal 10% remaining at 45, 75, 90, 120, and 150 DAP in equal splits (RDN + RDK in 6 splits); S3: Basal 10% remaining at 45, 75, 90, 120, 150, and 180

DAP in similar splits (RDN + RDK in 7 splits); and S4: Half of total N and a full dose of P and K at planting and rest of N at 45 and 120 DAP in equal quantity. There were three replications of the treatment combinations. In the case of band placement, the fertilizers were applied 20 cm below the surface, while in the broadcasting method, the fertilizers were broadcasted over the surface of the soil. Figure 1 represents the schematic depiction of the treatment details. The area of each plot was 56 m^2 (8 m × 7 m). Evaluation of plant growth, yield, and nutrient uptake was carried out on randomly selected plant samples. At harvest time, whole cane samples were collected, and cane juice was extracted using a power crusher. The juice quality was assessed using the Spencer and Meade method (Spencer and Meade, 1995).

2.3. Crop management

The recommended dose of fertilizer (RDF) was 150 kg ha^{-1} N, 85 kg ha^{-1} P, and 60 kg ha^{-1} K for the plant crop, and 170 kg ha^{-1} N, 50 kg ha^{-1} P, and 60 kg ha^{-1} K for the ratoon crop. The variety of sugarcane used in this study was Rajendra Ganna-1, which is a very popular variety of sugarcane with the farmers in the study area. The plant crop of sugarcane was planted on 20 March 2020 and the ratoon crop was initiated on 12 February 2021 in the same field. The planting was done in furrow using three budded setts in 120 cm row spacing. Four irrigations and one hand-weeding and hoeing were performed at appropriate stages.

2.4. Soil and plant analysis

In the current experiment, soil parameters such as electrical conductivity (EC), organic carbon (OC), available nitrogen (alkaline KMnO4 method), phosphorus (NaHCO3 extracted), and available potassium (NH4OAC extracted) were determined after harvesting of the ratoon crop. For determination of EC, the soil suspension (Soil: Water 10 g:50 mL) in the beaker was allowed to settle for a maximum of half an hour. Then, the standard KCl solution was prepared and the conductivity meter was calibrated with the standard KCl solution. Afterward, the EC was determined using an EC meter. The ground air-dried soil samples were passed through a 0.2 mm sieve to analyze soil organic carbon (SOC). SOC was determined through the modified Walkley and Black method (Jackson, 1973) using 1 N K2Cr2O7, conc. H2SO4, orthophosphoric acid, sodium fluoride, and ferrous ammonium sulphate solution. Available (mineralizable) nitrogen (N) in the soil was determined by using the alkaline permanganate (KMnO4-N) method (Subbiah and Asija, 1956). Phosphorus estimation was done by using sodium bicarbonate as an extractant (Olsen et al., 1954). Color development was measured using a spectrophotometer [Double Beam Spectrophotometer AU2703, Systronics (India) Ltd., India] at 720 nm wavelength with known standards. Available potassium (exchangeable + water-soluble K) in soil was determined by using a neutral normal ammonium acetate solution using a flame photometer [Systronics (India) Ltd., India] (Jackson, 1973). The total nitrogen content (%) by the crop was determined by a modified macro-Kjeldahl method (Jackson, 1973), while phosphorus content was determined by a vanado-molybdo-phosphoric acid yellow color method in an HNO_3 system with a colorimeter (Jackson, 1973). Potassium content was evaluated by a wet digestion method and a flame photometer (Jackson, 1973). Nutrient uptake was determined by multiplying the nutrient content in the crop with respective biomass production (Pramanick et al., 2017).

2.5. Statistical analysis

Analysis of variance method (ANOVA) method for split-plot design was followed to statistically analyze all the data. Fisher's LSD method (least significant difference) was adopted to differentiate the treatment means at 5% probability ($p \leq 0.05$), as stated in Gomez and Gomez (1976). All the statistical computations were done using SPSS software version 25 (IBM Corp. 2017).

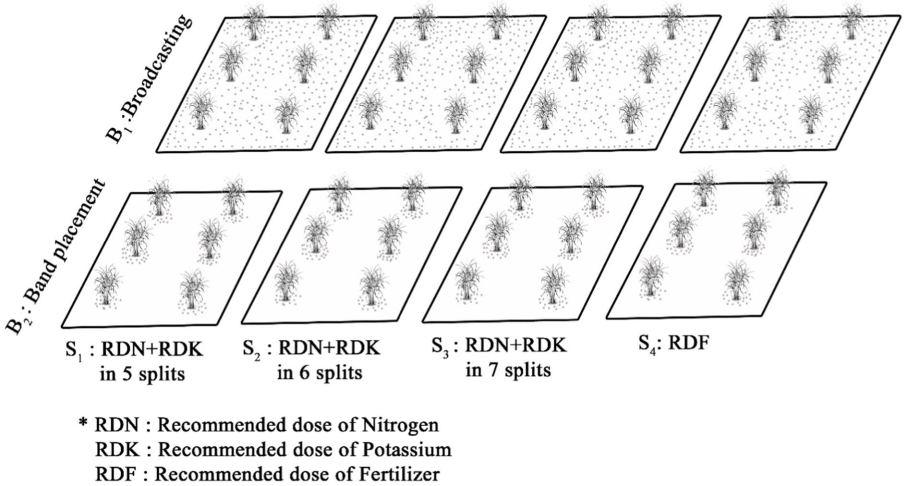

FIGURE 1
Schematic diagram of the treatment details.

3. Results

3.1. Growth

The tiller population was found to be increasing with advanced growth stages 2–4 months after planting. The study results showed a higher tiller population 4 months after planting (MAP) in the band placement method, accounting for an increase of 24.7% and 23.2% in plant and ratoon crops, respectively when compared to the broadcasting method (Figure 2). The tiller population of sugarcane was significantly ($p \leq 0.05$) influenced by the application of nitrogen and potassium in seven splits (S3), and it was found that S3 resulted in the increment of tiller number to the tune of 23.0% and 21.3% at 4 months after planting (MAP) as compared to the recommended dose of fertilizer (S4), in plant and ratoon cycles of sugarcane, respectively. Plant height under plant and ratoon systems was significantly ($p \leq 0.05$) influenced by splits application of macronutrients (nitrogen and potassium) and method of establishments. Splitting N and K seven times (RDN + RDK in 7 splits) resulted in the tallest plants, accounting for 7.7 and 6.2% increases in plant height for plant and ratoon crops, respectively, at 5 MAP, 8.3% and 7.9% increases in plant height for plant and ratoon crops, respectively, at 6 MAP, and 3.9% and 3.7% increases in plant height for plant and ratoon crops, respectively, at 7 MAP, as compared to the RDF application. Band

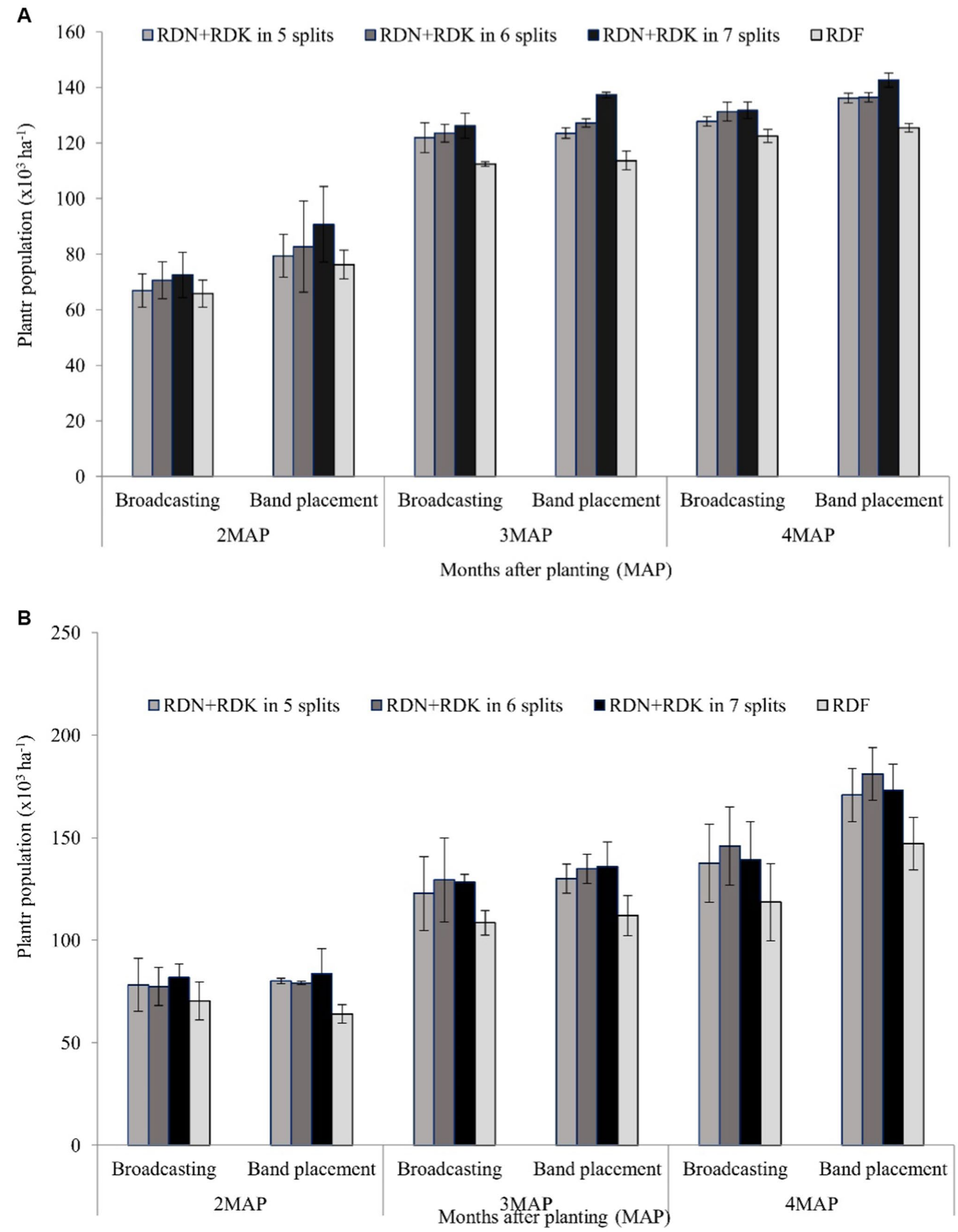

FIGURE 2
Effect of different treatments on plant populations in **(A)** sugarcane plant crop and **(B)** sugarcane ratoon crop. Lines above the bars denote the standard deviation ($n = 3$). RDN, RDK, and RDF denote recommended dose of N, recommended dose of K, and recommended dose of fertilizers.

placement also showed higher plant height as compared to broadcasting during the growth period of the crop. However, the application methods and split dose did not significantly affect plant height at harvest in plant and ratoon crops (Figure 3).

Dry matter accumulation (DMA) increased with the advancement of crop age. Moreover, the method of fertilizer application had a significant ($p \leq 0.05$) influence on the DMA of sugarcane (Figure 4). The maximum dry matter accumulation was recorded under the band placement method of fertilizer application compared to broadcasting at 6, 7 MAP, and harvest. The DMA was also found significantly ($p \leq 0.05$) higher in the plots receiving nitrogen in combination with potassium in seven splits as compared to the recommended dose of fertilizer, whereas DMA was found to be statistically at par with the treatment 6 and 5 split applications of N and K under plant and ratoon crops.

3.2. Yield parameters

Results showed that the millable canes were significantly ($p \leq 0.05$) affected by the method of planting and split application in plant and ratoon crops. The maximum number of millable canes (117.9 and 119.2×10^3 ha^{-1} in plant and ratoon crops, respectively) was recorded in band placement. This method of planting exhibited about 21.2% and 21.0% increments in millable cane numbers in plant and ratoon crops, respectively, as compared to the millable cane numbers under the broadcasting method. It was also revealed that seven times the splitting of nitrogen and potassium (RDN + RDK in 7 splits) in sugarcane produced more millable cane (114.9 and 116.3×10^3 ha^{-1} in plant and ratoon crops, respectively). However, this treatment was ($p \leq 0.05$) found at par with the six times splitting of N and K (RDN + RDK in 6 splits). The minimum number of millable cane (92.7

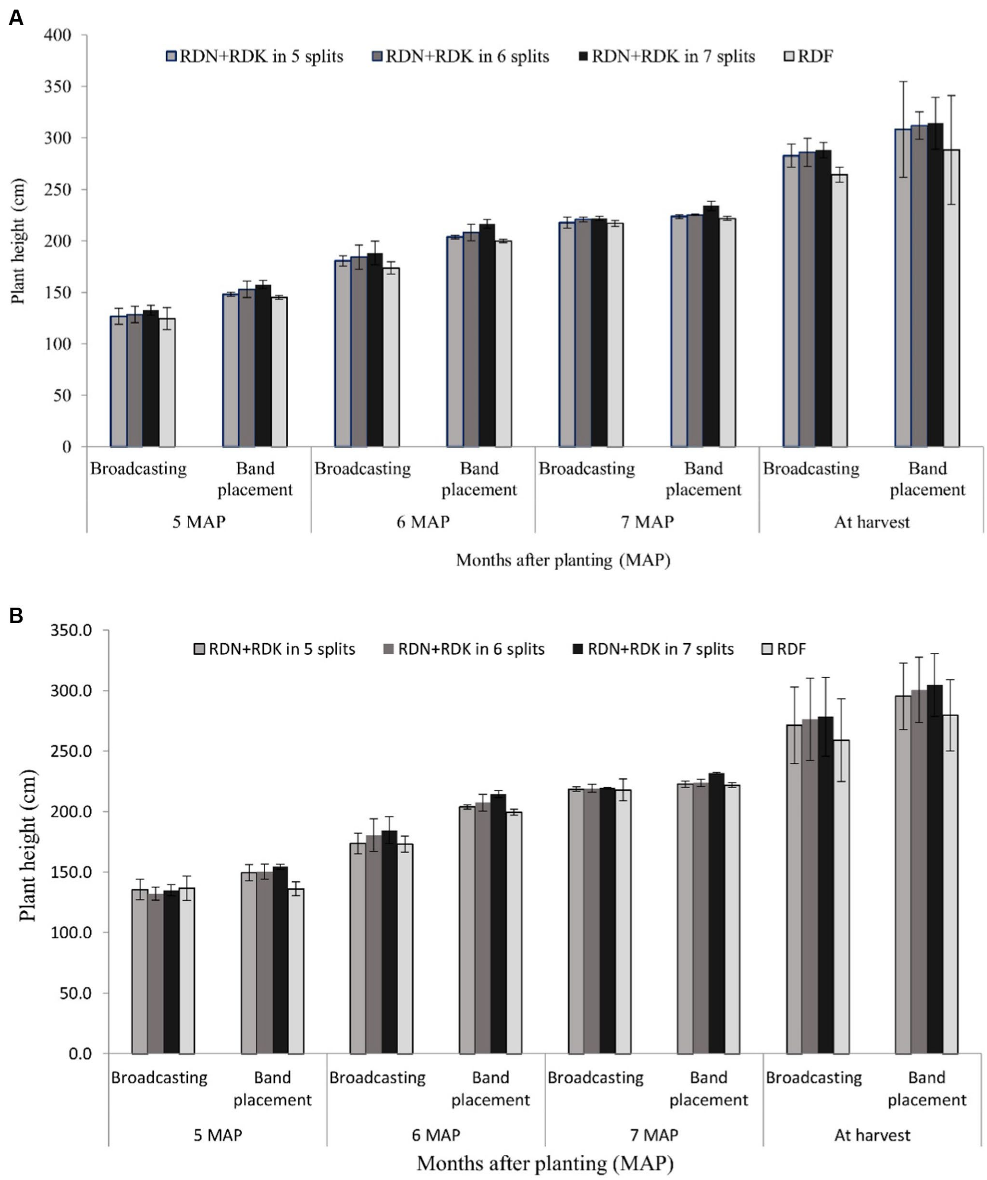

FIGURE 3
Effect of different treatments on plant height in **(A)** sugarcane plant crop and **(B)** sugarcane ratoon crop. Lines above the bars denote the standard deviation ($n = 3$). RDN, RDK, and RDF denote recommended dose of N, recommended dose of K, and recommended dose of fertilizers.

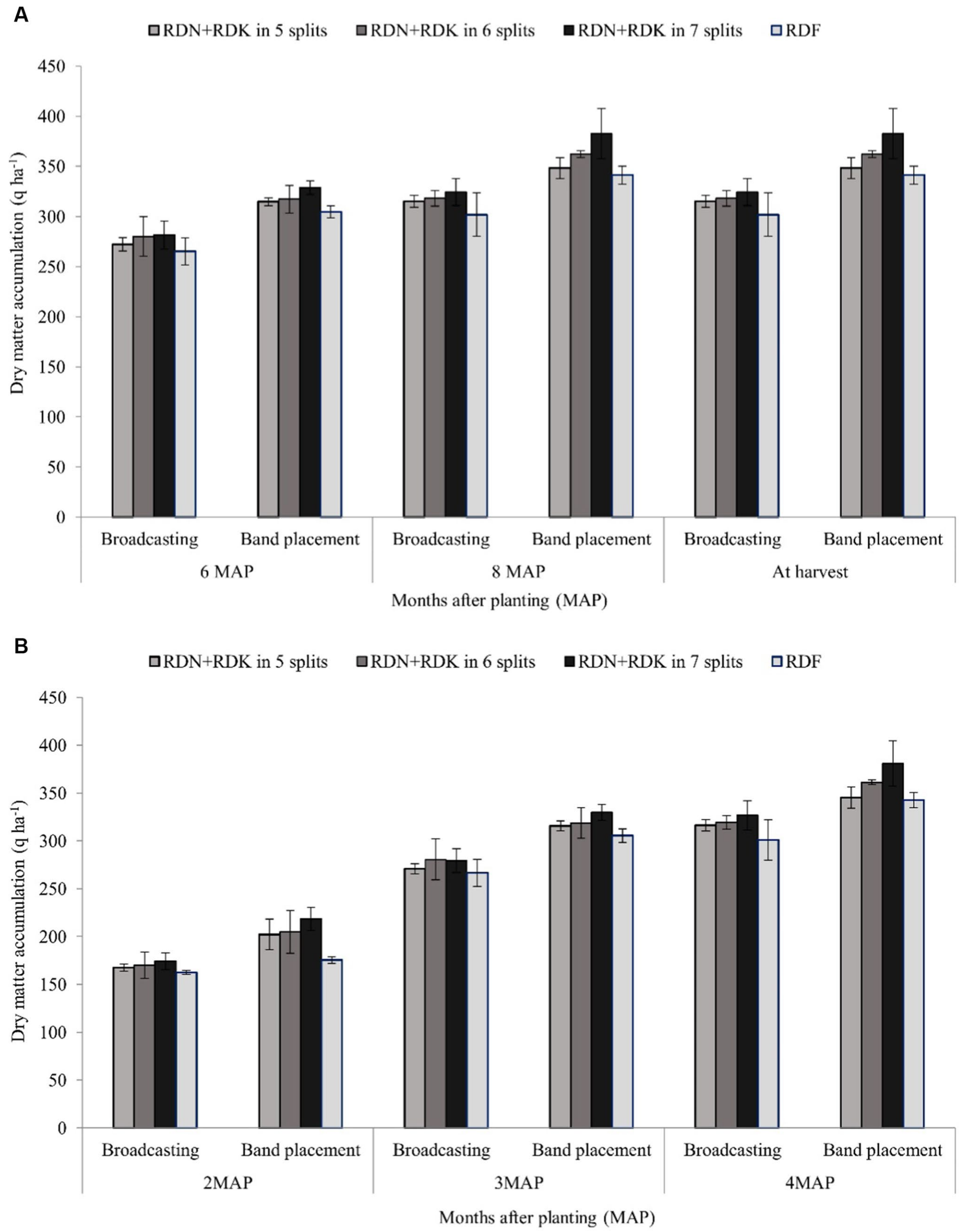

FIGURE 4
Effect of different treatments on dry matter accumulation in **(A)** sugarcane plant crop and **(B)** sugarcane ratoon crop. Lines above the bars denote the standard deviation ($n = 3$). RDN, RDK, and RDF denote recommended dose of N, recommended dose of K, and recommended dose of fertilizers.

and $92.4 \times 103\,ha^{-1}$) was observed in the RDF for plant and ratoon crops, respectively (Table 1).

Sugarcane growers must consider cane yield, which is majorly influenced by genotype, management techniques, and the environment. Harvesting at the right stage of crop maturity is critical for maximizing tonnage while minimizing losses in a growing environment. Harvesting of immature or over-mature canes results in significant losses in cane yield and sugar recovery. The present study indicated that the fertilizer application method, *viz.*, band placement and broadcasting, significantly ($p \leq 0.05$) influenced the cane yield in both the plant and ratoon crops, while different split applications of N and K did not influence the cane yield of ratoon crops. In the ratoon crop, cane yield was observed at the maximum ($78.3\,t\,ha^{-1}$) in 7 splits application and it was the minimum ($66.5\,t\,ha^{-1}$) in the recommended dose of fertilizer. The maximum cane yield (86.7 and $80.6\,t\,ha^{-1}$ in plant and ratoon crops, respectively) was observed in band placement, and the minimum cane yield (73.9 and $66.8\,t\,ha^{-1}$ in plant and ratoon crops, respectively) in the plant and ratoon crops was recorded in the broadcasting method. Cane yield was found to be increased by 19% with the split application of N and K for 7 times (RDN + RDK in 7 splits) as compared to the RDF (S4) where N was applied in 2 splits with no split application of K (Table 1). Sugar yield was not significantly ($p \geq 0.05$) influenced by the application methods of fertilizer (N and K) in both plant and ratoon crops. However, the splitting of nitrogen and

TABLE 1 Yield attributes, yield, and quality of plant-ratoon cycles of sugarcane (Rajendra Ganna-1) as influenced by method and split doses of nitrogen and potassium application.

Treatment	Millable canes ($\times 10^3$ ha^{-1})s		Cane yield (t ha^{-1})		Brix (%)		Pol (%)		Purity (%)		Juice recovery (%)		Commercial cane sugar (%)		Sugar yield (t ha^{-1})	
	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon
Fertilizer application methods																
B1	97.3	98.5	73.9	66.8	20.6	18.3	18.2	17.7	88.4	86.1	60.8	61.9	12.5	12.7	9.3	8.5
B2	117.9	119.2	86.7	80.6	20.8	18.2	18.3	17.7	88.7	87.2	62.7	63.8	12.6	12.5	10.9	10.1
SEm (±)	3.4	2.9	2.1	2.1	0.2	0.3	0.2	0.3	0.3	0.5	0.8	1.0	0.2	0.2	0.3	0.3
CD ($p \leq 0.05$)	20.5	17.9	12.6	12.7	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Splitting of N and K fertilizer																
S1	109.3	111.7	80.6	73.2	20.7	18.6	18.2	17.5	88.5	86.0	60.7	61.6	12.6	12.7	10.1	9.3
S2	113.7	115.2	83.8	76.7	20.8	18.3	18.3	17.6	88.6	86.3	61.9	63.2	12.6	12.6	10.6	9.7
S3	114.9	116.3	85.2	78.3	20.9	18.2	18.4	17.7	88.9	86.9	64.3	65.7	12.7	12.6	10.9	9.9
S4	92.7	92.4	71.6	66.5	20.4	18.1	18.0	17.8	88.2	87.4	59.9	61.2	12.5	12.5	8.9	8.3
SEm (±)	4.72	3.58	2.8	2.6	0.2	0.3	0.2	0.3	0.4	0.7	0.6	1.1	0.2	0.20	0.4	0.3
CD ($p \leq 0.05$)	14.5	11.1	8.71	NS	NS	NS	NS	NS	NS	NS	1.8	3.4	NS	NS	1.2	1.1

B1 and B2 denote broadcasting and band placement, respectively. S1, S2, S3, and S4 denote RDN + RDK in 5 splits, RDN + RDK in 6 splits, RDN + RDK in 7 splits, and RDF, respectively.

potassium exerts a significant effect on sugar yield under plant and ratoon crops, and plots receiving nitrogen and potassium in 7 splits (RDN + RDK in 7 splits) resulted in the highest sugar yield, accounting for an increase of approximately 23% and 19% over the recommended dose of fertilizer in plant and ratoon crops, respectively (Table 1).

3.3. Quality

Quality parameters such as brix, pol, and purity percentage were not significantly influenced by the methods and split application of nitrogen and potassium under plant and ratoon crops. It was also observed that juice recovery was not greatly affected by the method of fertilizer application. However, split fertilizer application (N and K) significantly affected the juice recovery in plant and ratoon crops. Higher juice recovery was achieved in the cane plant receiving nitrogen and potassium in 7 splits (64.3% and 65.7% in plant and ratoon crops, respectively) compared to the recommended dose of fertilizer (59.9% and 61.2% in plant and ratoon crops, respectively). Table 1 shows that commercial cane sugar percentage was not significantly influenced by the method and split application of nitrogen and potassium in both plant and ratoon crops.

3.4. Soil physicochemical parameters

Data presented in Table 2 show that the electrical conductivity of soil was not significantly ($p \geq 0.05$) influenced by the method of fertilizer application in plant and ratoon crops. The electrical conductivity (EC) of soil differed significantly ($p \leq 0.05$) among the split application of N and K.

The maximum value of EC was found in S4, i.e., RDF (0.28, 0.28 dS m^{-1} in plant and ratoon crops, respectively). The method of fertilizer application and split application of N and K fertilizer did not exert significant ($p \geq 0.05$) influence on soil organic carbon (OC) in plant and ratoon crops. Available soil N was found to be significantly ($p \leq 0.05$) affected by the method of fertilizer application under both plant and ratoon systems. Soil available N was found higher under the band placement method compared to that under the broadcasting method, accounting for a 4.6% and 3.4% increase in available N in the plant and ratoon-cropped soils, respectively. The split application of N and K significantly influenced the available N in plant crops. The higher values were observed in 7 splits application of N and K (RDN + RDK in 7 splits) over the RDF. Olson-P was not significantly influenced by the method and split applications of N and K fertilizer in plant and ratoon crops. The method of fertilizer applications (broadcasting and band placement) did not exert a significant effect on available K in both the years of study in plant-ratoon cycles. However, the splits application of N and K significantly influenced the available K in plant crops. Split application of N and K for 7 times (RDN + RDK in 7 splits) recorded the highest value of available K, which increased by 18.8% as compared to the available K under no splitting of K (S4).

3.5. Nutrient uptake

Band placement method of fertilizer application influenced the uptake of NPK in plant and ratoon crops, except for K uptake in

TABLE 2 Physicochemical properties of soil and uptake of NPK in plant-ratoon cycles of sugarcane (Rajendra Ganna-1) as influenced by method and split doses of nitrogen and potassium application.

Treatment	Electrical conductivity (dS/m)		Organic carbon (%)		Available N (kg ha^{-1})		Olson P (kg ha^{-1})		Available K (kg ha^{-1})		N uptake (kg ha^{-1})		P uptake (kg ha^{-1})		K uptake (kg ha^{-1})	
	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon	Plant	Ratoon
Fertilizer application methods																
B1	0.24	0.23	0.45	0.46	195.5	197.7	10.2	10.1	112.7	110.3	248.9	249.9	25.7	25.6	250.1	240.8
B2	0.25	0.27	0.44	0.47	204.4	204.4	11.1	11.2	120.5	118.6	284.1	287.3	34.9	28.3	292.9	252.4
SEm (±)	0.02	0.01	0.02	0.01	1.1	0.4	0.6	0.4	2.2	2.6	5.2	4.9	1.1	0.3	6.5	10.2
CD ($p \leq 0.05$)	NS	NS	NS	NS	6.56	2.3	NS	NS	NS	NS	31.9	24.6	6.2	1.6	39.7	NS
Splitting of N and K fertilizer																
S1	0.23	0.23	0.40	0.43	200.4	198.8	10.0	9.9	113.7	112.6	258.8	260.5	26.6	23.4	263.4	242.1
S2	0.23	0.23	0.43	0.48	202.4	201.2	10.8	10.8	123.7	114.6	272.5	273.8	31.1	26.6	286.5	242.9
S3	0.27	0.26	0.47	0.46	204.1	202.0	12.4	10.9	124.3	116.5	297.9	302.9	33.7	28.7	306.5	249.8
S4	0.28	0.28	0.47	0.49	192.9	202.2	9.4	11.0	104.6	118.9	236.6	237.3	29.7	29.0	229.8	251.5
SEm (±)	0.02	0.01	0.01	0.02	0.9	1.70	0.7	0.5	3.1	2.9	4.5	3.9	1.1	1.7	5.4	11.9
CD ($p \leq 0.05$)	0.06	0.02	NS	NS	2.9	NS	NS	NS	9.7	NS	13.7	12.1	3.3	NS	16.5	NS

B1 and B2 denote broadcasting and band placement, respectively. S1, S2, S3, and S4 denote RDN + RDK in 5 splits, RDN + RDK in 6 splits, RDN + RDK in 7 splits, and RDF, respectively.

ratoon crops. N, P, and K uptakes under band placement of fertilizers increased to the tune of 14.1%, 15.0%, and 35.8%, respectively, over the broadcasting method in the plant crop, and 10.5%, 17.1%, and 4.8%, respectively, over the broadcasting method in the ratoon crop. N uptake was significantly influenced by split application in plant and ratoon cycles. Higher N uptake was observed in plots that received 7 times splitting of N and K. Lower nitrogen uptake (236 and 237.3 kg ha^{-1} in plant and ratoon crops, respectively) was noticed with a recommended dose of fertilizer (S4). The treatments comprising S3 (RDN + RDK in 7 splits) and S2 (RDN + RDK in 6 splits) were the ones that most improved P and K uptakes, compared to other split application protocols in plant crops. Significant differences were not observed in P and K uptake in ratoon crops (Table 2).

4. Discussion

Growth of sugarcane was found to improve under 7 split applications of N and K in both plants and ratoon crops. This might be the result of optimal sugarcane metabolism (Cakmak, 2005; Wang et al., 2013), enzyme activation (Hawkesford et al., 2012), transport of carbohydrates, photosynthesis, hormone balance, auxin levels (Rubio et al., 2009), and cane root growth (Bhatt and Singh, 2021) upon the S3 treatment (split application of N and K for 7 times) compared to the other treatments. It is widely believed that factors such as the number of millable canes and the weight of a single cane can contribute to cane productivity (Singh et al., 2019). The findings of this study showed that the plant crop cane production was much higher than the ratoon crop one. The main factors that contribute to this low yield in the ratoon crop are cultivars with low and variable ratooning potential, inadequate crop management practices, the accumulation of toxic substances in the rhizosphere zone, inability to absorb nutrients by the ratoon, the loss of soil fertility, shallow ratooning, soil compaction, and an increase in the prevalence of pests and diseases (Xu et al., 2021). The cane yield and biomass could be greatly improved by applying nitrogen and potassium fertilizer using a band placement technique. This investigation found that adding bands to both plant and ratoon crops increased crop productivity. According to Bashagaluke et al. (2018), surface application without band placement of fertilizers resulted in significant plant nutrient loss due to fixation, erosion, and other losses, which may have led to an unbalanced soil nutrient environment and a decline in production. Higher cane yield (85.2 t ha^{-1}) was achieved with 7 times split nitrogen and potassium application in plant crops alone. Singh et al. (2008) and Lakshmi et al. (2020) also demonstrated the advantages of K split application regarding cane yield. In the research trial, split application of nitrogen and potassium in plant and ratoon crops significantly enhanced sugar yield, which might be due to the better availability of these macronutrients to the crop as split application facilitated the minimum loss of nutrients beyond the root zone.

Regarding sugar yield, the plant crop showed about 10% more yield compared to the ratoon crop. The lower cane yield and a smaller number of millable stalks in the ratoon crop might be responsible for the lesser increase in sugar yield. According to this research, split applications of nitrogen and potassium resulted in a higher number of millable canes than the standard approach. Early plant population has a significant impact on the millable stalks per hectare, whereas later nutrient competitiveness has an impact on individual growth (Kapur et al., 2011). Therefore, N application primarily influenced the millable stalks ha^{-1} by impacting the emergence and tillering rate and then jointly determining the cane yield by affecting the stalk length, diameter, and weight of sugarcane at a later stage. Potassium application primarily affects sugarcane juice quality (Kumar et al., 2019a). In this study, it was observed that split applications of N might be helpful to prevent the loss of nutrients such as nitrogen, which is vulnerable to leaching and volatilization losses. At the same time, K split application also might ensure better K availability soil and uptake by the plants. This was ultimately reflected in the better quality of the sugarcane. Sugarcane quality parameters, *viz.*, brix, pol, purity, and CCS percent, were all higher in the 7 splits application (S3) and band placement, although these differences were insignificant (Table 1). In this study, we have found that the higher juice recovery (65.7%) observed in ratoon crop over plant crop (64.3%) might be due to the early harvesting of the ratoon crop, resulting in better quality and better sugar recovery (Viator et al., 2010).

The findings of the current investigation showed that the NPK uptake by sugarcane under various split applications of nitrogen and potassium differed significantly. In increasing the number of splitting of macronutrients, N and K might have facilitated their even availability in soil throughout the development of cane, which ultimately led to a quicker rate of crop growth (Junejo et al., 2010). These results also confirm the findings recorded by Sandeep et al. (2013). The highest N, P, and K uptakes in sugarcane crops at harvest in plant and ratoon crops were found in 7 split-application of N and K. Many previous studies also reported similar results. The application of increasing N rates resulted in increased N accumulation in leaves, stalks, and crowns (Costa et al., 2016; Pramanick et al., 2022). Additionally, the results demonstrated that applying fertilizer with bands was the most effective compared with the broadcasting method. Soil available N, P, and K were significantly influenced by the band placement method. This might be because the band placement method reduced the loss of nutrients as compared to the broadcasting method (Nkebiwe et al., 2016), ultimately facilitating the better uptake of nutrients by both plants and ratoon crops.

5. Conclusion

In the present study, it was found that the band placement of fertilizers increases the availability of nutrients in both the sugarcane main crop and next-year ratoon crop, thereby, facilitating better nutrient uptake by the plant, which was ultimately reflected in better crop growth, yield, and quality of the crop. Concerning splitting of the macronutrients, it was found that more splitting of the soil mobile macronutrients such as N and K for long-duration crops such as sugarcane was always beneficial in terms of sugarcane growth, yield, and juice quality. From this two-year-long study on sugarcane to quantify the fertilizer application methods and number of split applications of N and K, it was found that the band placement of fertilizers was the most suitable method of fertilizer application ensuring yield and quality of the crop. Application of full dose of recommended P and 10% of recommended N and K at basal followed by splitting of remaining N and K fertilizers at equal rates at 45, 75, 90, 120, 150, and 180 days after planting was the best split application protocol for the macronutrients for sugarcane in the Indo-Gangetic plains.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

NK, LR, and AS led the research work, planned, supervised, and conducted field experiments, and read and edited the manuscript. NK, LR, and BP collected soil/plant samples, performed chemical analysis, wrote the initial draft of the manuscript, and prepared figures and tables. AS, NK, LR, AG, AA, MS, AH, and BP performed project supervision, reviewed, read, and edited the manuscript with significant contributions. NK, LR, AH, MS, AA, AG, and BP performed the statistical analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1223881/full#supplementary-material

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Unveiling the combined effect of nano fertilizers and conventional fertilizers on crop productivity, profitability, and soil well-being

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It is widely accepted that deficiency of macro (nitrogen) and micronutrients (zinc, copper etc.) affects the plant growth and development which cause a significant threat to crop production and food security. The Indian Farmers Fertilizer Cooperative (IFFCO) developed nano-urea (nano-N), nano-zinc (nano-Zn), and nano-copper (nano-Cu) liquid fertilizer formulations to enhance the crop yields, simultaneously addressing the nutrient deficiency, without causing toxicity. Therefore, this study was formulated to evaluate the effectiveness of nano-N (nano-urea), nano-Zn, and nano-Cu at varying N levels [0, 50, 75, and 100% of the recommended rates of nitrogen (RRN)] on maize-wheat and pearl millet-mustard systems during 2019–20 and 2020–21. The results exhibited that the application of nano-N + nano-Zn with 100% RRN exhibited significantly higher grain yields in maize (66.2–68.8%), wheat (62.6–61.9%), pearl millet (57.1–65.4%), and mustard (47.2–69.0%), respectively, over absolute control plots and combinations of three nano-fertilizers like nano-N + nano-Zn + nano-Cu applied plots. This was mainly attributed to the higher N and Zn uptake by the crops. However, 75% RRN with nano-N + nano-Zn also produced comparable yields. Thus, applying nano-N and nano-Zn via foliar applications, in conjunction with conventional urea, has the potential to reduce the required nitrogen fertilizer amount by up to 25%, while simultaneously maintaining equivalent yield levels. Similarly, 100% RRN and 75% RRN + nano-N + nano-Zn registered comparable profitability, soil mineral N, dehydrogenase activity (DHA), and soil microbial biomass carbon (SMBC), during both the study years. However, further research and field trials on nano fertilizers alone or in combination with conventional fertilizers are essential to fully unlock its benefits and ascertain its long-term effects which may offer a pathway to more efficient and eco-friendly crop nourishment.

KEYWORDS

energy efficiency, maize, mineral nitrogen, mustard, nano-urea, nano-Zn, pearl millet, wheat

1. Introduction

Macro and micronutrient deficiency in crop and soil have risen significantly over the past few years in India as well as globally (Shukla et al., 2021). The major reason behind the upsurge these deficiencies are growing of high-yielding crop varieties (Shukla et al., 2018), increased cropping intensity (Behera et al., 2021), and decreased or no usage of organic manures (Shukla et al., 2021). With the increasing demand of food for growing population, a disproportionate reliance on the excessive use of chemical fertilizers, specifically nitrogen (N) (Wen et al., 2017; Upadhyay et al., 2022) in agricultural practices has been evident in the recent past. Due to the injudicious use of conventional chemical fertilizers, the environment is polluted in terms of deteriorating soil quality (Oenema et al., 2014; Krasilnikov et al., 2022), eutrophication (Liu et al., 2021), groundwater pollution (Norton et al., 2015; Ye et al., 2020), and air pollution (Kumar et al., 2021) as well as diminished soil macro and micronutrient-supplying capacity (Babu et al., 2022). The most deficient among the micronutrients in Indian conditions is Zn (Shukla and Behera, 2020). The lack of micronutrients in the soil reduces grain nutritional quality in addition to crop yield (Fageria et al., 2002; Phattarakul et al., 2012; Dapkekar et al., 2018; Shukla et al., 2021). Micronutrient insufficiency occurs when animals and humans consume food (obtained from crops) with low micronutrient concentrations (Shukla et al., 2021). Insufficient dietary intake of zinc (Zn), which poses a significant health issue (Kihara et al., 2020), remains a pressing concern, especially in the underdeveloped countries, affecting both crop production and human nutrition (Manzeke et al., 2019). However, the foliar application of novel nano fertilizers (macro and micronutrients) in crops can reduce the nutrient deficiency in plants and animals.

Improved crop yields and grain quality can be achieved by the use of nano-fertilizers (Hu and Xianyu, 2021) which are built on nano-scale (1–100 nm) substrates (Peters et al., 2014; Babu et al., 2022). Many people believe that the use of these novel nano-fertilizers (Bartolucci et al., 2022) could lead to a shift in the agricultural practices (Verma et al., 2022). The adoption of nano-fertilizer (Jha et al., 2023) could be a big step toward the objective of sustainable agriculture (Mahapatra et al., 2022) in India and around the world, through curtailing of fertilizer dosages (Kumar et al., 2021; Upadhyay et al., 2023) and reducing runoff, leaching, and emission of gas in the atmosphere (Manjunatha et al., 2016). Indian Farmers Fertiliser Cooperative (IFFCO) has developed and patented three nano-fertilizer formulations *viz.* nano-urea/nano-N (Indian patent application number 201921044499), nano-Zn (Indian patent application number 201921044497) and nano-Cu (Indian patent application number 201921044498). Many researchers have found that spraying crops with nano-urea improves the crop yield under the field conditions (Das et al., 2016; Manikandan and Subramanian, 2016; Raliya et al., 2017; Du et al., 2019; Rathore et al., 2019; Kumar et al., 2021; Upadhyay et al., 2023).

Concurrently, nanoparticles, such as urea hydroxyapatite nanohybrid (Kottegoda et al., 2017), nano potassium (Al-Juthery et al., 2019), Zn nanoparticles (Drostkar et al., 2016), nano zinc oxide (ZnO) (Du et al., 2019), nano-micronutrients (Fe, Mn, Zn, Cu, Mo, and B) (Kanjana, 2020), silver nano particles (Mosa et al., 2021), nano copper oxide (CuO) (Dimkpa et al., 2019) etc. have been found to increase the plant growth in agricultural crops (Ahmed et al., 2021). However, most of this research has only been conducted in the lab or in pots. Although nanoparticles have been shown to be highly toxic to many plant species (Chen et al., 2015; Khan et al., 2019), they also play an important role in reducing heavy metal stress (Noman et al., 2020; Zhou et al., 2020) and promoting plant development (Salam et al., 2022). Plants can easily absorb excessive amount of Cu^{2+} and Zn^{2+} (Dong et al., 2022), leading to a wide range of structural and cellular abnormalities (Rizvi and Khan, 2018). Therefore, non-toxic nano-fertilizers are required to enhance the grain nutrient content as well as the crop yields.

Among micronutrients, zinc (Zn) plays a role in improving photosynthesis (Arough et al., 2016; Cabot et al., 2019), chlorophyll content (Sakya et al., 2018), grain yield (Ibrahim et al., 2017; Mahmood et al., 2019), relative water content (Pavia et al., 2019), the body's antioxidant defense system (Olechnowicz et al., 2018), and disease resilience etc. Therefore, for efficient utilization of N, Zn, Cu etc. their nano formulation is urgently needed (Ali et al., 2019). Nano fertilizers are gaining significant popularity and recognition as one of the most valuable nanomaterials (Salam et al., 2022) due to their small size, unique shape, and intriguing physicochemical properties (Selim et al., 2020). Increasing crop yield while using less conventional fertilizer on the environment is possible with nano-enabled agriculture (Milani et al., 2012; Sabir et al., 2020). A detailed study exploring the impact of the application of nano fertilizers or their judicious integration with traditional fertilizers on growth, yield and economics of crops under field condition is lacking (Kah et al., 2018; Mullen, 2019; Hu and Xianyu, 2021). Keeping these facts in view, the present study was planned to investigate the positive effect of nano-urea (nano-N), nano-Zn and nano-Cu on crop productivity, uptake, soil nutrient and biological health status under maize-wheat and pearl millet-mustard systems.

2. Materials and methods

2.1. Site description

The field trials were conducted at the experimental farm of ICAR-Indian Agricultural Research Institute, located in New Delhi. The specific coordinates for the trials were as follows: maize-wheat trials were conducted at N 28.38.0838 and E 077.09.1441, while pearl millet-mustard trials took place at N 28.38.1146 and E 077.09.1405. Table 1 provides detailed information about the soil properties of the location.

2.2. Experimental details

During *rabi* and *kharif* seasons of 2019–20 and 2020–21, field experiments on wheat, maize, mustard and pearl-millet under maize-wheat and pearl millet-mustard systems were established.

A total of 14 treatments were evaluated in a randomized complete block with three replications. The four rates of applied N as [0, 50, 75, and 100% of recommended rates of nitrogen (RRN)] were tested with different combinations of Nano-urea, Nano-Zn, and Nano-Cu application. The other major nutrients, *viz.* phosphorus and potassium were applied uniformly per the prescription. Table 2 shows the details of the treatments.

2.3. Nutrient management

The recommended fertilizer doses for the different crops were as follows: for maize, 150 kg N per ha, 75 kg P_2O_5 per ha, and 75 kg K_2O per ha; for pearl millet, 60 kg N per ha, 60 kg P_2O_5 per ha, and 30 kg K_2O per ha; for mustard, 80 kg N per ha, 40 kg P_2O_5 per ha, and 30 kg K_2O per ha; and for wheat, 120 kg N per ha, 60 kg P_2O_5 per ha, and 60 kg K_2O per ha. The recommended sources for nitrogen (N), phosphorus (P), and potassium (K) were prilled urea, single superphosphate, and muriate of potash, respectively. According to the treatment plan, mustard and pearl millet were provided with half of the nitrogen (N) requirement and the full doses of phosphorus (P) and potassium (K) at the time of sowing. The remaining half of the nitrogen (N) requirement was supplied as top-dressing later. Similarly, wheat and maize were supplied with half of their nitrogen (N) requirement and the full doses of phosphorus (P) and potassium (K) at the time of sowing, with the remaining half of the nitrogen (N) applied as top-dressing. Two sprays of Nano-urea were applied to the crops. The first spray occurred 30 days after sowing, followed by another spray one week before flowering. The rate of Nano-N spray was 4 mL/L, while Nano-Zn and Nano-Cu were sprayed at a rate of 2 mL/L. These sprays were applied using hand-operated knapsack sprayers with flat fan nozzles to ensure optimal foliage coverage. During harvesting, sickles were used to harvest the crops from the designated net plot area. Precautions were taken during spraying, including repeating the spray after rain and applying the spray in the afternoon when the dew had disappeared.

TABLE 1 Initial soil physico-chemical properties.

Soil properties	Value	Rating
Soil texture	Sandy loam	–
pH	8.22	Mildly alkaline
EC	0.24 dS m^{-1}	Non-saline
Organic carbon	0.58%	Medium
Available N	272 kg ha^{-1}	Low
Available P	22.3 kg ha^{-1}	Medium
Available K	311 kg ha^{-1}	High
DTPA-extractable Zn	0.84 mg kg^{-1}	Medium
DTPA-extractable Fe	4.72 mg kg^{-1}	Medium
DTPA-extractable Mn	19.9 mg kg^{-1}	High
DTPA-extractable Cu	1.91 mg kg^{-1}	High

2.4. Collection and processing of soil samples

Soil samples were collected at the flowering stage of each crop from the 0–15 cm depth using a core sampler with a diameter of 3.9 cm and a volume of 179.2 cm^3. Additionally, soil samples were obtained from the given plots for analysis of mineral nitrogen (N), microbial biomass carbon (MBC), and dehydrogenase activity (DHA). The collected soil samples from each plot were air dried, ground using a mortar and pestle, and passed through a 2-mm sieve. Subsequently, the samples were stored for further analysis. Similarly, another round of sampling was conducted after the harvest of each crop for nutrient estimation.

TABLE 2 Treatments details of experiments undertaken in maize-wheat and pearl millet-mustard systems.

S. No.	Treatment	Treatment details
T1	RRN_0PK	Recommended P and K (no-N)
T2	RRN $_{100}$PK	Recommended N, P and K
T3	RRN $_0$PK + Nano-Zn	Recommended P and K (no-N) and nano Zn sprays (2 times at the rate 2 mL/L)
T4	RRN $_{50}$PK+ Nano-Zn	50% of recommended N, recommended P and K, and nano Zn sprays (2 times at the rate 2 mL/L)
T5	RRN $_{75}$PK+ Nano-Zn	75% of recommended N, recommended P and K (no-N), and nano Zn sprays (2 times at the rate 2 mL/L)
T6	RRN $_{100}$PK+ Nano-Zn	Recommended N, P and K, and nano Zn sprays (2 times at the rate 2 mL/L)
T7	RRN $_0$PK + Nano-N+ Nano-Zn	Recommended P and K (no-N), and nano-N (2 times at the rate 4 mL/L) and nano Zn sprays (2 times at the rate 2 mL/L)
T8	RRN $_{50}$PK+ Nano-N+ Nano-Zn	50% of recommended N, recommended P and K, and nano-N (2 times at the rate 4 mL/L) and nano Zn sprays (2 times at the rate 2 mL/L)
T9	RRN $_{75}$PK+ Nano-N+ Nano-Zn	75% of recommended N, recommended P and K, and nano-N (2 times at the rate 4 mL/L) and nano Zn sprays (2 times at the rate 2 mL/L)
T10	RRN $_{100}$PK+ Nano-N+ Nano-Zn	Recommended N, P and K, and nano-N (2 times at the rate 4 mL/L) and nano Zn sprays (2 times at the rate 2 mL/L)
T11	RRN $_0$PK + Nano-N+ Nano-Zn + Nano-Cu	Recommended P and K (no-N), and nano-N (2 times at the rate 4 mL/L), nano Zn sprays (2 times at the rate 2 mL/L) and nano Cu sprays (2 times at the rate 2 mL/L)
T12	RRN $_{50}$PK+ Nano-N+ Nano-Zn + Nano-Cu	50% of recommended N, recommended P and K, and nano-N (2 times at the rate 4 mL/L), nano Zn sprays (2 times at the rate 2 mL/L) and nano Cu sprays (2 times at the rate 2 mL/L)
T13	RRN $_{75}$PK+ Nano-N+ Nano-Zn + Nano-Cu	75% of recommended N, recommended P and K, and nano-N (2 times at the rate 4 mL/L), nano Zn sprays (2 times at the rate 2 mL/L) and nano Cu sprays (2 times at the rate 2 mL/L)
T14	RRN $_{100}$PK+ Nano-N+ Nano-Zn + Nano-Cu	Recommended N, P and K, and nano-N (2 times at the rate 4 mL/L), nano Zn sprays (2 times at the rate 2 mL/L) and nano Cu sprays (2 times at the rate 2 mL/L)

*Recommended fertilizer doses were 150 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1}, 75 kg K_2O ha^{-1} for maize; 60 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, 30 kg K_2O ha^{-1} for pearl millet; 80 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1}, 30 kg K_2O ha^{-1} for mustard, and 120 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, 60 kg K_2O ha^{-1} for wheat crop.

2.5. Soil and plant analysis

The estimation of dehydrogenase activity (DHA) in the soil samples followed the standard protocol, which involved measuring the production rate of triphenyl formazan (TPF) from triphenyl tetrazolium chloride (TTC) under anaerobic conditions (Casida, 1977). For the extraction of mineral nitrogen (N), undisturbed soil samples collected at different growth stages were treated with 2 M KCl and estimated using the steam distillation method (Kjeldahl, 1883). Estimation of available zinc (Zn) and copper (Cu) were performed following the method described by Lindsay and Norvell (1978), and the analysis was conducted using an atomic absorption spectrophotometer. Similarly, the micro-Kjeldahl method described by Jackson (1973) was used to estimate the nitrogen (N) content in grain/seed and straw/stover samples. To ensure result accuracy, each plant and soil sample were analyzed thrice, and the mean values were utilized for the statistical analysis.

2.6. Nitrogen uptake

The estimation of nitrogen (N) uptake by the grain/seed and straw/stover of different crops was done based on the dry matter production per hectare using the equation provided by Rowell (1994).

$$\text{N uptake}\left(\text{kg ha}^{-1}\right) = \frac{\text{N content}\,(\%) \times \text{Grain yield}\left(\text{kg ha}^{-1}\right)}{100}.$$

2.7. Soil microbial biomass carbon

The method (fumigation-extraction) as described by Vance et al. (1987) was used for the estimation of soil microbial biomass carbon (SMBC)

$$\text{SMBC}\left(mg\ kg^{-1}\right) = 2.64 \times \left(\text{C1} - \text{C2}\right)$$

Where,

C1 = extractable C in fumigated soil.

C2 = extractable C in non xlix fumigated soil.

2.64 = Kc factor.

2.8. Profit analysis

The economic assessment encompassed an examination of cultivation expenses, net profits, and the benefit-to-cost ratio (B: C), across different experimental conditions. The cost of cultivating each treatment was determined using current market rates for inputs, factoring in all expenses associated with crop cultivation. This encompassed all costs incurred throughout the crop growth cycle, aggregated alongside shared expenses for various operations and inputs. The benefit–cost ratio (B: C) was derived by dividing gross profits by the cost of cultivation for each specific treatment combination.

$$\text{B} : \text{C} = \frac{\text{Gross return}}{\text{Cost of cultivation}}.$$

2.9. Statistical analysis

The standard analysis of variance (ANOVA) was conducted using SPSS 21.0 statistical software (IBM Corp, 2012) to compare the treatment means (Tables 3–11). The treatment means were compared at the 5% level of significance ($p \le 0.05$) using the critical difference method. For Figures 1, 2, the standard error (SE ±) of the treatment means was computed as

$$SE = SD\left(\sqrt{N}\right)^{-1}$$

Where, SD: standard deviation of the mean, and N: number of observations on which the mean is based. Contrast analysis (Supplementary Table 2) was done using SAS 9.4 (SAS Institute Inc, 2013) with generalized linear model procedure.

3. Results

3.1. Productivity

Nano-fertilizers like N, Zn and Cu exerted a strong influence on both the grain and straw yield of maize-wheat and pearl millet-mustard systems during 2019–20 and 2020–21 crop seasons (Table 3). Nano-N + nano-Zn with 100% RRN applied plots recorded significantly higher grain yields of 6.55 and 6.43 t ha^{-1}, 5.48 and 5.39 t ha^{-1}, 3.52 and 3.59 t ha^{-1}, and 2.40 and 2.45 t ha^{-1} in maize, wheat, pearl millet and mustard crops during first and second years, respectively over control (N_0PK or N_0PK+ nano-N or N_0PK+ nano-N+ nano-Zn or N_0PK+ nano-N+ nano-Zn + nano-Cu). The percentage increase in yield under N_{100}PK+ Nano-N+ Nano-Zn treatment was 72.1–84.1% in maize, 73.8–77.1% in wheat, 55.5–62.8% in pearl millet, and 50.3–73.3% in mustard over control plots (N_0PK+ nano-N+ nano-Zn). Likewise, there was 66.2–68.8%, 62.6–61.9%, 57.1–65.4%, and 47.2–69.0% yield enhancement was noted in maize, wheat, pearl millet and mustard crops, respectively under N_{100}PK+ Nano-Zn treatment over N_0PK + nano-Zn. Similarly, combination of all the three nano fertilizers like, nano-N + Zn + Cu with 100% RRN enhanced maize grain yield by 64.4–73.7%, wheat yield by 58.2–63.7%, pearl millet yield by 61.3–66.2%, and mustard yield by 50.0–72.9% over control plots (N0PK + Nano-N+ Nano-Zn + Nano-Cu). Therefore, sole application of nano-Zn or in combination with nano-N had higher yield advantage in all the crops compared to combination of all the three nano-fertilizers. However, in all the crops during both the study years, treatment with100% RRN with sole application of nano-Zn or a combination of nano-N+ Nano-Zn was found to be at par with 75% RRN + Nano-N + Nano-Zn. Likewise, the percentage yield enhancement with 75% RRN + nano-N + nano-Zn was 51.0–56.2% in maize, 53.7–54.7% in wheat, 50.0–51.6% in pearl millet, and 41.1–57.2% in mustard crops over control (N_0PK + Nano-N+ Nano-Zn) during both the study years. The application of RRN_{100}PK+ nano-N+ nano-Zn + nano-Cu led to slightly lower yields in all crops compared

TABLE 3 Effect of nano-fertilizers on productivity (t ha^{-1}) of maize, wheat, pearl millet, and mustard systems.

Treatment	Maize		Wheat		Pearl millet		Mustard	
	2020	2021	2019–20	2020–21	2020	2021	2019–20	2020–21
RRN$_0$PK	3.35	3.24	2.81	2.75	1.90	1.78	1.28	1.23
RRN $_{100}$PK	5.98	6.01	5.02	5.14	3.33	3.35	2.28	2.31
RRN $_0$PK + Nano-Zn	3.69	3.46	3.05	2.97	2.18	2.07	1.55	1.35
RRN $_{50}$PK+ Nano-Zn	5.03	4.94	4.20	4.17	2.85	2.65	2.00	1.81
RRN $_{75}$PK+ Nano-Zn	5.35	5.30	4.85	4.61	2.91	2.83	2.25	1.93
RRN $_{100}$PK+ Nano-Zn	6.35	6.37	5.30	5.26	3.39	3.37	2.33	2.34
RRN $_0$PK + Nano-N+ Nano-Zn	3.94	3.81	3.37	3.33	2.24	2.17	1.63	1.45
RRN $_{50}$PK+ Nano-N+ Nano-Zn	5.30	5.19	4.66	4.59	2.90	2.88	2.15	1.93
RRN $_{75}$PK+ Nano-N+ Nano-Zn	5.95	5.95	5.18	5.15	3.36	3.29	2.30	2.28
RRN $_{100}$PK+ Nano-N+ Nano-Zn	6.55	6.43	5.48	5.39	3.52	3.59	2.40	2.45
RRN $_0$PK + Nano-N+ Nano-Zn + Nano-Cu	3.79	3.69	3.42	3.25	2.17	2.13	1.60	1.40
RRN $_{50}$PK+ Nano-N+ Nano-Zn + Nano-Cu	5.23	5.15	4.60	4.53	2.86	2.85	2.20	1.91
RRN $_{75}$PK+ Nano-N+ Nano-Zn + Nano-Cu	5.77	5.74	5.09	5.05	3.27	3.21	2.25	2.21
RRN $_{100}$PK+ Nano-N+ Nano-Zn + Nano-Cu	6.23	6.41	5.41	5.32	3.50	3.54	2.40	2.42
Sem±	0.20	0.21	0.22	0.19	0.16	0.12	0.13	0.13
CD ($p \leq 0.05$)	0.58	0.61	0.64	0.55	0.46	0.36	0.39	0.39

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to RRN_{100}PK+ nano-N and nano-Zn, although these results were statistically comparable (Table 3). Furthermore, a contrast analysis (between RRN_0PK + Nano-N+ Nano-Zn Vs. RRN_0PK + Nano-N+ Nano-Zn + Nano-Cu; RRN_{75}PK+ Nano-N+ Nano-Zn Vs. RRN_{75}PK+ Nano-N+ Nano-Zn + Nano-Cu; RRN_{100}PK+ Nano-N+ Nano-Zn Vs. RRN_{10}PK+ Nano-N+ Nano-Zn + Nano-Cu) was performed to elucidate the individual effect of nano-Cu from that of nano-N and nano-Zn, aiming to understand any potential antagonistic interactions (Supplementary Table 2). It was observed that the effect on nano-Cu in all the treatment combinations was non-significant in all the crops.

3.2. Profitability

Across various crop types, the highest cultivation costs were recorded in plots treated with 100% recommended rate of nitrogen (RRN) along with nano-N + nano-Zn + nano-Cu application, with values of 570, 507, 379, and 397 US\$ ha^{-1} for maize, wheat, pearl millet, and mustard crops, respectively (Supplementary Table 1). Furthermore, the maximum net returns were observed in plots treated with 100% RRN along with nano-N + nano-Zn application for all crops, amounting to 996, 866, 898, and 1,209 US\$ ha^{-1} for maize, wheat, pearl millet, and mustard crops, respectively. Notably ($p \leq 0.05$), maize exhibited significantly higher net returns (996 US\$ ha^{-1}) and a Benefit–Cost ratio (B: C) of 2.77 under the 100% RRN along with nano-N + nano-Zn treatment, compared to the control (net return of 314 US\$ ha^{-1} and B: C of 1.65). This performance remained comparable to the RRN_{75}PK + Nano-N + Nano-Zn, RRN_{100}PK, RRN_{100}PK + Nano-Zn, and RRN_{100}PK + Nano-N + Nano-Zn + Nano-Cu treatments (Supplementary Table 1).

Wheat demonstrated a notably elevated net return of 866 US\$ ha^{-1} under the RRN_{100}PK + Nano-N + Nano-Zn treatments, in stark contrast to the control group's net return of 272 US\$ ha^{-1}. This performance remained on par with other treatments: RRN_{75}PK + Nano-N + Nano-Zn (804 US\$ ha^{-1}), RRN_{100}PK (816 US\$ ha^{-1}), RRN_{100}PK + Nano-Zn (843 US\$ ha^{-1}), and RRN_{100}PK + Nano-N + Nano-Zn + Nano-Cu (841 US\$ ha^{-1}) (Supplementary Table 1). Additionally, the statistical analysis unveiled a higher B: C of 2.76 under the RRN_{100}PK treatment, surpassing the control's B: C of 1.63. This performance was consistent with the B: C observed under RRN_{75}PK + Nano-N + Nano-Zn (2.63), RRN_{100}PK + Nano-N + Nano-Zn (2.73), and RRN_{100}PK + Nano-N + Nano-Zn + Nano-Cu (2.66) treatments.

The treatment involving RRN_{100}PK + Nano-N + Nano-Zn exhibited significantly elevated net returns in pearl millet, reaching 898 US\$ ha^{-1}, in contrast to the control's net return of 374 US\$ ha^{-1}. This performance remained consistent with the net returns observed under RRN_{75}PK + Nano-N + Nano-Zn (816 US\$ ha^{-1}), RRN_{100}PK (863 US\$ ha^{-1}), RRN_{100}PK + Nano-Zn (841 US\$ ha^{-1}), and RRN_{100}PK + Nano-N + Nano-Zn + Nano-Cu (877 US\$ ha^{-1}) treatments (Supplementary Table 1). Furthermore, the analysis revealed a statistically higher B: C of 3.41 under the RRN_{100}PK treatment, surpassing the control's B: C of 2.18. This B: C performance remained consistent with the ratios observed under RRN_{100}PK + Nano-Zn (3.36), RRN_{75}PK + Nano-N + Nano-Zn (3.21), and RRN_{100}PK + Nano-N + Nano-Zn + Nano-Cu (3.31) treatments.

Net return in mustard was registered higher under RRN_{100}PK+ Nano-N+ Nano-Zn treatments (1,209 US \$ ha^{-1}) over control (509 US \$ ha^{-1}) and it was remained at par with RRN_{75}PK+ Nano-N+ Nano-Zn (1,120 US \$ ha^{-1}), RRN_{100}PK (1,156 US \$ ha^{-1}), RRN_{100}PK+ Nano-Zn (1,157 US \$ ha^{-1}) and RRN_{100}PK+ Nano-N+ Nano-Zn + Nano-Cu (1,183 US \$ ha^{-1}) (Supplementary Table 1). Significantly higher B: C was noticed under RRN_{100}PK treatment (4.28) over control (2.54) and it was remained at par with RRN_{75}PK+ Nano-N+ Nano-Zn (3.90), RRN_{100}PK+ Nano-Zn (4.09), RRN_{100}PK+ Nano-N+ Nano-Zn (4.10), and RRN_{100}PK+ Nano-N+ Nano-Zn + Nano-Cu (3.98).

3.3. Nitrogen uptake

In all crop seasons, maize, wheat, pearl millet and mustard grains exhibited significantly higher N uptake during the study years. In general, nano-N + nano-Zn with 100% RRN had higher N uptake [(87.8 and 86.8 kg ha^{-1} in maize during first and second year, respectively), (68.3 and 66.8 kg ha^{-1} in wheat during first and second year, respectively), (59.7 and 62.0 kg ha^{-1} in pearl millet during first and second year, respectively) and (67.6 and 70.2 kg ha^{-1} in mustard during first and second year, respectively)] over control (N_0PK). However, sole or combination of nano-fertilizers had similar grain N-uptake in mustard crop during both the study years (Table 4). In maize and wheat crops, maximum N uptake of 86.8–87.8 kg ha^{-1}, and 68.3–66.8 kg ha^{-1}, respectively was recorded with 100% RRN + nano-N + nano-Zn plots over other combinations. However, superior treatment was at par with other treatments as compared to N_0PK + Nano-Zn, N_0PK + Nano-N + Nano-Zn, and N_0PK + Nano-N + Nano-Zn + Nano-Cu during both the cropping seasons. Likewise, application of 100% RRN + Nano-Zn recorded significantly higher grain N uptake of 60.5 kg ha^{-1} in pearl millet during 2019–20, while it was comparatively higher in 100% RRN + Nano-N + Nano-Zn (62.0 kg ha^{-1}) during 2020–21 than other nano-fertilizer applied plots. Furthermore, the treatments with 75% RRN + Nano-N+ Nano-Zn, and 75% RRN + Nano-N + Zn + Cu with 100% RRN + nano fertilizers applied plots registered the slightly lesser but similar grain N uptake in all the crops during all the study years. In mustard, the treatment 100% RRN + nano-N + nano-Zn + nano-Cu registered the higher grain N uptake by 67.9–70.2 kg ha^{-1} over other treatments during both the study years.

Total N uptake (grain + stover/straw) in maize, wheat, pearl millet and mustard crops were significantly influenced by nano fertilizer application. Application of nano-N + nano-Zn along with 100% RRN had higher total N uptake by 174–181 kg ha^{-1} in maize, ~123 kg ha^{-1} in wheat, 114–172 kg ha^{-1} in pearl millet, and 195–204 kg ha^{-1} in mustard over other combinations of nano-fertilizers with 100% RRN plots as well as in lower levels of fertilizer application, but it was at par with 75% RRN levels (Table 5).

3.4. Zn uptake

In comparison to the other combinations of nano-fertilizers with 100% RRN plots, the Zn uptake with nano-N + nano-Zn was greater than 1,100 mg ha^{-1} in maize, wheat and pearl millet crops, while it was >900 mg ha^{-1} in mustard (Table 6). Zinc uptake was significantly higher with 100% RRN + Nano-N + Nano-Zn plots, and the Zn uptake was higher by 1,435–1,508 mg ha^{-1} in maize, 1,626–1,195 kg ha^{-1} in wheat, 1,144–1,195 mg ha^{-1} in pearl millet, and 962–972 mg ha^{-1} in mustard over other treatments. However, application of 75% RRN

TABLE 4 Effect of nano-fertilizers on grain N uptake (kg ha^{-1}) under maize-wheat and pearl millet-mustard systems.

Treatment	Maize		Wheat		Pearl millet		Mustard	
	2020	2021	2019–20	2020–21	2020	2021	2019–20	2020–21
RRN$_0$PK	47.9	45.7	38.2	37.3	34.6	32.3	37.8	36.4
RRN $_{100}$PK	80.5	81.0	63.0	64.5	59.3	62.0	64.7	66.5
RRN $_0$PK + Nano-Zn	52.8	49.3	40.7	38.0	39.7	38.0	46.9	40.4
RRN $_{50}$PK+ Nano-Zn	72.0	70.7	56.2	54.1	52.7	49.2	58.6	53.6
RRN $_{75}$PK+ Nano-Zn	74.1	73.0	63.1	59.1	52.7	52.6	65.1	56.3
RRN $_{100}$PK+ Nano-Zn	86.7	84.7	67.7	67.4	60.5	60.6	67.5	67.4
RRN $_0$PK + Nano-N+ Nano-Zn	56.7	54.8	45.5	43.2	41.2	40.6	48.9	43.2
RRN $_{50}$PK+ Nano-N+ Nano-Zn	74.0	70.2	61.1	59.3	51.3	51.7	62.1	56.8
RRN $_{75}$PK+ Nano-N+ Nano-Zn	79.0	78.5	63.4	63.2	57.9	58.1	66.6	65.0
RRN $_{100}$PK+ Nano-N+ Nano-Zn	87.8	86.8	68.3	66.8	59.7	62.0	67.6	70.2
RRN $_0$PK + Nano-N+ Nano-Zn + Nano-Cu	55.6	53.2	47.2	44.2	40.0	39.5	49.0	42.3
RRN $_{50}$PK+ Nano-N+ Nano-Zn + Nano-Cu	77.6	74.2	63.3	61.3	52.2	52.9	64.7	57.4
RRN $_{75}$PK+ Nano-N+ Nano-Zn + Nano-Cu	78.2	76.3	65.0	63.7	58.6	58.1	64.3	63.9
RRN $_{100}$PK+ Nano-N+ Nano-Zn + Nano-Cu	80.4	82.8	65.4	64.5	59.7	61.8	67.9	68.2
Sem±	5.3	4.6	4.3	4.6	3.6	3.5	2.8	4.1
CD ($p \leq 0.05$)	15.7	13.5	12.5	13.3	10.5	10.1	8.2	12.0

TABLE 5 Effect of nano-fertilizers on total (grain + straw/stover) N uptake (kg ha^{-1}) under maize-wheat and pearl millet-mustard systems.

Treatment	Maize		Wheat		Pearl millet		Mustard	
	2020	2021	2019–20	2020–21	2020	2021	2019–20	2020–21
RRN$_0$PK	102	97	71	66	102	95	104	104
RRN $_{100}$PK	163	163	115	120	164	172	178	186
RRN $_0$PK + Nano-Zn	106	101	75	71	69	106	129	114
RRN $_{50}$PK+ Nano-Zn	139	134	101	97	77	134	154	141
RRN $_{75}$PK+ Nano-Zn	150	144	117	107	84	140	178	159
RRN $_{100}$PK+ Nano-Zn	172	172	124	123	100	168	185	186
RRN $_0$PK + Nano-N+ Nano-Zn	115	112	82	79	70	114	132	122
RRN $_{50}$PK+ Nano-N+ Nano-Zn	151	141	113	109	87	137	171	159
RRN $_{75}$PK+ Nano-N+ Nano-Zn	164	158	121	116	100	154	186	183
RRN $_{100}$PK+ Nano-N+ Nano-Zn	181	174	123	123	114	172	195	204
RRN $_0$PK + Nano-N+ Nano-Zn + Nano-Cu	116	108	84	79	70	109	130	116
RRN $_{50}$PK+ Nano-N+ Nano-Zn + Nano-Cu	158	151	111	108	94	144	182	169
RRN $_{75}$PK+ Nano-N+ Nano-Zn + Nano-Cu	165	160	117	115	103	163	179	181
RRN $_{100}$PK+ Nano-N+ Nano-Zn + Nano-Cu	172	167	119	119	99	158	180	183
Sem±	10	7	75	5	7	6	12	10
CD ($p \leq 0.05$)	28	21	101	16	21	18	37	30

along with nano-N + nano-Zn was at par with Zn uptake of 100% RRN + nano-N + nano-Zn applied plots. Interestingly, all the three combinations of nano-fertilizers like nano-N + nano-Zn + nano-Cu exhibited lower Zn uptake in grains of all the crops as compared to combination of nano-N + nano-Zn during the study years.

When applied to maize, wheat, pearl millet and mustard, nano fertilizers dramatically increased total Zn uptake (grain + straw/stover) and irrespective of crops, >52–62% of that Zn was retained in the straw over grain/seed (Table 7). Likewise, Zn uptake was significantly higher with 100% RRN+ Nano-N+ Nano-Zn plots in all the tested crops like maize (5636–5,670 mg ha^{-1}), wheat (2753–2,843 kg ha^{-1}), pearl millet (4386–4,603 mg ha^{-1}), and in mustard (4520–4,635 mg ha^{-1}; Table 7). However, it was at par with 75% RRN+ Nano-N+ Nano-Zn applied plots in all the crops.

3.5. Cu uptake

Over the years, harvests of maize, wheat, pearl millet, and mustard have all seen considerable increases in grain Cu consumption with 100% RRN applied plots (Table 8). However, application of nano-N + Zn + Cu either in alone or in combination had little effect in Cu uptake in maize, wheat and mustard crops, while slight variation in Cu uptake was observed in pearl millet crop. The variation of only about 2–4 mg ha^{-1} was observed in all the crops with respect to nano-fertilizer application. However, the uptake of Cu in pearl millet plant was 3–4 times higher than maize, wheat and mustard crops. Similarly, total Cu uptake by all the crops also followed the same trend as that of grain Cu uptake during the study years (Table 9). However, total plant Cu uptake was significantly higher in 100% RRN + nano-N + nano-Zn

TABLE 6 Effect of nano-fertilizers on grain Zn uptake (mg ha^{-1}) under maize-wheat and pearl millet-mustard systems.

Treatment	Maize		Wheat		Pearl millet		Mustard	
	2020	2021	2019–20	2020–21	2020	2021	2019–20	2020–21
RRN$_0$PK	713	695	792	775	685	670	543	520
RRN $_{100}$PK	1,310	1,348	1,588	1,656	1,165	1,180	963	916
RRN $_0$PK + Nano-Zn	839	776	941	971	754	734	617	515
RRN $_{50}$PK+ Nano-Zn	1,135	1,151	1,280	1,257	944	868	793	718
RRN $_{75}$PK+ Nano-Zn	1,331	1,238	1,571	1,420	955	935	935	792
RRN $_{100}$PK+ Nano-Zn	1,245	1,373	1,482	1,443	1,100	1,134	957	980
RRN $_0$PK + Nano-N+ Nano-Zn	787	804	953	920	747	721	650	587
RRN $_{50}$PK+ Nano-N+ Nano-Zn	1,234	1,262	1,450	1,450	1,029	992	898	847
RRN $_{75}$PK+ Nano-N+ Nano-Zn	1,399	1,303	1,605	1,662	1,099	1,180	893	874
RRN $_{100}$PK+ Nano-N+ Nano-Zn	1,508	1,435	1,663	1,626	1,195	1,144	962	972
RRN $_0$PK + Nano-N+ Nano-Zn + Nano-Cu	882	838	1,051	992	686	710	635	552
RRN $_{50}$PK+ Nano-N+ Nano-Zn + Nano-Cu	1,200	1,089	1,396	1,413	993	953	855	724
RRN $_{75}$PK+ Nano-N+ Nano-Zn + Nano-Cu	1,198	1,228	1,405	1,430	1,006	1,097	864	820
RRN $_{100}$PK+ Nano-N+ Nano-Zn + Nano-Cu	1,306	1,386	1,599	1,591	1,192	1,147	963	901
Sem±	97	118	95	93	99	63	68	76
CD ($p \leq 0.05$)	283	345	280	272	290	184	201	222

TABLE 7 Effect of nano-fertilizers on total (grain + straw) Zn uptake (mg ha^{-1}) under maize-wheat and pearl millet-mustard systems.

Treatment	Maize		Wheat		Pearl millet		Mustard	
	2020	2021	2019–20	2020–21	2020	2021	2019–20	2020–21
RRN$_0$PK	2,973	2,855	1,411	1,358	2,875	2,840	2,883	2,820
RRN $_{100}$PK	5,040	5,068	2,731	2,923	4,395	4,550	4,483	4,536
RRN $_0$PK + Nano-Zn	3,245	3,148	1,652	1,650	2,890	2,850	3,040	2,614
RRN $_{50}$PK+ Nano-Zn	4,179	4,199	2,244	2,206	3,566	3,459	3,904	3,510
RRN $_{75}$PK+ Nano-Zn	5,099	4,747	2,706	2,520	3,666	3,647	4,531	4,020
RRN $_{100}$PK+ Nano-Zn	4,549	5,036	2,593	2,544	3,979	4,339	4,540	4,585
RRN $_0$PK + Nano-N+ Nano-Zn	3,189	3,215	1711	1,646	3,071	2,990	3,271	3,168
RRN $_{50}$PK+ Nano-N+ Nano-Zn	4,708	4,687	2,481	2,446	4,027	3,916	4,435	4,124
RRN $_{75}$PK+ Nano-N+ Nano-Zn	5,385	5,134	2,781	2,802	4,221	4,481	4,204	4,480
RRN $_{100}$PK+ Nano-N+ Nano-Zn	5,670	5,636	2,843	2,753	4,603	4,386	4,520	4,635
RRN $_0$PK + Nano-N+ Nano-Zn + Nano-Cu	3,722	3,402	1823	1728	2,787	2,839	3,059	2,759
RRN $_{50}$PK+ Nano-N+ Nano-Zn + Nano-Cu	4,850	4,607	2,430	2,391	3,941	3,737	4,155	3,733
RRN $_{75}$PK+ Nano-N+ Nano-Zn + Nano-Cu	4,837	5,076	2,499	2,509	3,930	4,163	4,123	4,050
RRN $_{100}$PK+ Nano-N+ Nano-Zn + Nano-Cu	5,217	5,305	2,816	2,871	4,564	4,273	4,478	4,522
Sem±	334	308	153	133	403	228	379	204
CD ($p \leq 0.05$)	981	904	449	391	1,182	669	1,110	598

applied plots in maize, wheat and mustard crops as compared to other combinations. Whereas, 100% RRN + nano-N + nano-Zn + nano-Cu applied plots had significantly higher total Cu uptake in pearl millet than other combination of fertilizers.

3.6. Soil mineral nitrogen

The data presented in Table 10 indicated that the soil mineral nitrogen in maize, wheat, pearl millet and mustard crops was significantly influenced by nano-fertilizer application at different sampling times during both the study years. Soil mineral nitrogen ranged from 16.4–30.1 μg g^{-1} of soil during flowering and post-harvest stages in maize crop. Application of 100% RRN + Nano-Zn, and 100% RRN + Nano-N + Nano-Zn exhibited significantly higher values for soil mineral N uptake at flowering (30.8–31.0 μg/g of soil) and post-harvest soils (30.0–31.1 μg/g of soil) than other combinations, and it was at par with 100% RRN+ Nano-N+ Nano-Zn + Nano-Cu. While the variation in soil mineral N was slightly higher in wheat than maize. Mineral N in soil varied significantly from 17.1 to 31.6 μg/g of soil in wheat (Table 10). Among growth stages of wheat, application of 100% RRN+ Nano-N+ Nano-Zn recorded significantly higher mineral N at flowering (31.0 μg/g) and at post-harvest soils (30.3 μg/g) over other plots during 2019–20. During 2020–21, application of 100% RRN+ Nano-N+ Nano-Zn + Nano-Cu (31.6 and 28.6 μg/g of soil at flowering and post-harvest stages, respectively) noted maximum values for mineral nitrogen and remained at par with almost all the other treatments except treatments N_0PK + Nano-Zn and N_0PK + Nano-N+ Nano-Zn + Nano-Cu.

TABLE 8 Effect of nano-fertilizers on grain Cu uptake (mg ha^{-1}) under maize-wheat and pearl millet-mustard systems.

Treatment	Maize		Wheat		Pearl millet		Mustard	
	2020	2021	2019–20	2020–21	2020	2021	2019–20	2020–21
RRN$_0$PK	65	61	62	56	312	294	87	82
RRN $_{100}$PK	119	117	113	106	552	588	160	157
RRN $_0$PK + Nano-Zn	67	61	62	63	389	378	107	94
RRN $_{50}$PK+ Nano-Zn	95	92	90	92	499	456	140	126
RRN $_{75}$PK+ Nano-Zn	102	98	115	107	519	509	153	133
RRN $_{100}$PK+ Nano-Zn	120	125	121	125	588	612	162	163
RRN $_0$PK + Nano-N+ Nano-Zn	75	68	68	71	440	411	113	102
RRN $_{50}$PK+ Nano-N+ Nano-Zn	99	99	99	95	517	495	153	135
RRN $_{75}$PK+ Nano-N+ Nano-Zn	111	109	123	119	552	596	160	156
RRN $_{100}$PK+ Nano-N+ Nano-Zn	126	120	124	121	624	686	168	166
RRN $_0$PK + Nano-N+ Nano-Zn + Nano-Cu	71	68	76	74	393	388	114	99
RRN $_{50}$PK+ Nano-N+ Nano-Zn + Nano-Cu	97	95	107	99	526	551	151	129
RRN $_{75}$PK+ Nano-N+ Nano-Zn + Nano-Cu	105	106	118	114	598	615	152	146
RRN $_{100}$PK+ Nano-N+ Nano-Zn + Nano-Cu	115	117	123	122	644	662	165	165
Sem±	7	5	13	9	49	36	10	10
CD ($p \leq 0.05$)	20	16	37	26	145	104	29	30

TABLE 9 Effect of nano-fertilizers on total Cu (grain + straw/stover) uptake (mg ha^{-1}) under maize-wheat and pearl millet-mustard systems.

Treatment	Maize		Wheat		Pearl millet		Mustard	
	2020	2021	2019–20	2020–21	2020	2021	2019–20	2020–21
RRN$_0$PK	564	504	321	300	1,632	1,524	1,327	1,372
RRN $_{100}$PK	948	950	559	549	2,662	2,698	2,230	2087
RRN $_0$PK + Nano-Zn	544	547	318	310	1870	1760	1,646	1,417
RRN $_{50}$PK+ Nano-Zn	717	719	453	447	2,245	2,138	2,328	1817
RRN $_{75}$PK+ Nano-Zn	808	792	578	546	2,604	2,379	2,375	2040
RRN $_{100}$PK+ Nano-Zn	874	911	591	599	2,651	2,901	2086	2052
RRN $_0$PK + Nano-N+ Nano-Zn	616	618	355	349	1900	1912	1884	1766
RRN $_{50}$PK+ Nano-N+ Nano-Zn	792	757	500	490	2,159	2,321	2,441	2006
RRN $_{75}$PK+ Nano-N+ Nano-Zn	891	892	613	589	2,611	2,562	2,518	2,267
RRN $_{100}$PK+ Nano-N+ Nano-Zn	974	955	625	616	2,893	2,939	2,418	2,569
RRN $_0$PK + Nano-N+ Nano-Zn + Nano-Cu	632	592	378	365	1879	1819	1,686	1,568
RRN $_{50}$PK+ Nano-N+ Nano-Zn + Nano-Cu	819	779	511	496	2,385	2,263	2,253	2,136
RRN $_{75}$PK+ Nano-N+ Nano-Zn + Nano-Cu	877	861	575	560	2,725	2,735	2,305	2086
RRN $_{100}$PK+ Nano-N+ Nano-Zn + Nano-Cu	951	917	603	570	2,920	2,915	2,134	2,209
Sem±	61	46	52	32	118	104	231	169
CD ($p \leq 0.05$)	180	136	152	94	346	304	678	496

Mineral nitrogen in pearl millet during 2019–20 and 2020–21, at flowering and post-harvest stages ranged from 17.7 to 32.3 μg/g of soil (Table 11). Application of 100% RRN + Nano-Zn and 100% RRN+ Nano-N+ Nano-Zn + Nano-Cu noted the highest values for soil mineral N of 32.0 and 31.0 μg/g, 32.3 and 30.7 μg/g soil at flowering and post-harvest stages, respectively during the studied seasons and recorded comparable values of mineral nitrogen with treatments 75% RRN + Nano-Zn, 75% RRN+ Nano-N + Nano-Zn, 100% RRN + Nano-N + Nano-Zn and 75% RRN + Nano-N + Nano-Zn + Nano-Cu in both the years, respectively. Significant variation in soil mineral nitrogen in mustard crop was recorded and ranged from 20.1 to 33.3 μg/g of soil (Table 11). Adoption of 100% RRN + Nano-Zn registered highest value for soil mineral N at flowering (~33.1 μg/g soil) and 100% RRN+ Nano-N + Nano-Zn + Nano-Cu at post-harvest stages (~32.3 μg/g soil), respectively during both the study years and it was at par with 75% RRN + Nano-N + Nano-Zn, 100% RRN + Nano-N + Nano-Zn and 75% RRN + Nano-N + Nano-Zn + Nano-Cu.

3.7. Dehydrogenase activity

Dehydrogenase activity (DHA) of soil under different treatments was measured in maize-wheat and pearl millet-mustard systems (Figure 1). In maize, maximum DHA activity was recorded under treatment of 100% RRN + nano-N + nano-Zn (35.5 μg TPF g^{-1} 24 h^{-1}, average of 2 years) which remained at par with 100% RRN+ Nano-N + Nano-Zn + Nano-Cu and 75% RRN + Nano-N + Nano-Zn. The treatment 100% RRN + Nano-Zn registered similar dehydrogenase activity (34.7 μg TPF g^{-1} 24 h^{-1}, average of 2 years) and also remained at par with treatment 100% RRN+

TABLE 10 Effect of nano-fertilizers on soil mineral nitrogen (μg/g of soil) at flowering and post-harvest stages of maize and wheat crops.

Treatment	Maize				Wheat			
	2020		2021		2019–20		2020–21	
	Flowering	Post-harvest	Flowering	Post-harvest	Flowering	Post-harvest	Flowering	Post-harvest
RRN_0PK	19.5	16.5	19.1	15.5	20.7	17.6	20.8	17.8
RRN $_{100}$PK	30.7	28.5	31.9	28.0	30.8	28.6	30.9	27.8
RRN $_0$PK + Nano-Zn	20.2	17.4	19.5	16.4	21.3	18.0	23.3	18.1
RRN $_{50}$PK+ Nano-Zn	22.7	22.5	21.8	21.4	23.4	20.6	25.0	24.6
RRN $_{75}$PK+ Nano-Zn	25.1	24.2	25.9	24.0	25.9	24.7	27.6	25.6
RRN $_{100}$PK+ Nano-Zn	30.8	29.6	29.9	29.7	30.8	30.2	31.8	28.4
RRN $_0$PK + Nano-N+ Nano-Zn	19.7	19.3	19.0	18.1	21.9	18.9	21.1	17.1
RRN $_{50}$PK+ Nano-N+ Nano-Zn	21.4	19.9	22.5	21.4	22.2	19.2	24.5	18.9
RRN $_{75}$PK+ Nano-N+ Nano-Zn	26.2	25.3	26.0	25.5	27.0	26.2	26.6	24.9
RRN $_{100}$PK+ Nano-N+ Nano-Zn	30.3	30.0	31.0	30.1	31.0	30.3	29.4	26.7
RRN $_0$PK + Nano-N+ Nano-Zn + Nano-Cu	20.7	19.4	19.9	18.3	23.5	20.7	23.2	19.1
RRN $_{50}$PK+ Nano-N+ Nano-Zn + Nano-Cu	23.4	21.7	23.1	21.6	24.1	21.5	24.3	23.9
RRN $_{75}$PK+ Nano-N+ Nano-Zn + Nano-Cu	24.7	24.3	27.0	25.8	25.4	24.3	26.1	26.0
RRN $_{100}$PK+ Nano-N+ Nano-Zn + Nano-Cu	30.3	29.6	30.7	29.2	30.9	30.4	31.6	28.6
Sem±	1.46	1.43	1.75	1.73	1.36	2.15	2.70	2.01
CD ($p \leq 0.05$)	4.28	4.18	5.13	5.09	4.00	6.30	7.93	5.90

TABLE 11 Effect of nano-fertilizers on soil mineral nitrogen (μg/g of soil) at flowering and post-harvest stages of pearl millet and mustard crops.

Treatment	Pearl millet				Mustard			
	2020		2021		2019–20		2020–21	
	Flowering	Post-harvest	Flowering	Post-harvest	Flowering	Post-harvest	Flowering	Post-harvest
RRN_0PK	20.8	19.0	20.2	17.9	22.3	19.6	22.3	19.3
RRN $_{100}$PK	32.4	29.7	32.0	29.4	33.3	30.7	33.9	31.1
RRN $_0$PK + Nano-Zn	21.3	20.9	20.0	19.4	22.8	21.6	22.0	20.8
RRN $_{50}$PK+ Nano-Zn	26.2	24.7	25.4	24.6	26.8	25.3	27.1	24.9
RRN $_{75}$PK+ Nano-Zn	28.8	27.0	28.0	26.2	29.6	28.0	29.7	27.6
RRN $_{100}$PK+ Nano-Zn	32.0	29.5	31.7	29.9	33.1	32.0	33.3	33.1
RRN $_0$PK + Nano-N+ Nano-Zn	20.5	19.9	19.7	18.6	22.7	20.6	20.9	20.1
RRN $_{50}$PK+ Nano-N+ Nano-Zn	25.7	24.8	25.1	23.8	26.5	25.6	26.1	24.9
RRN $_{75}$PK+ Nano-N+ Nano-Zn	29.1	28.2	28.7	27.0	30.1	29.3	29.6	28.7
RRN $_{100}$PK+ Nano-N+ Nano-Zn	30.7	30.0	31.0	30.0	32.1	31.1	32.6	32.0
RRN $_0$PK + Nano-N+ Nano-Zn + Nano-Cu	21.2	18.9	19.7	17.7	22.1	19.9	21.4	20.3
RRN $_{50}$PK+ Nano-N+ Nano-Zn + Nano-Cu	27.0	23.9	25.9	22.7	29.0	24.7	28.1	24.4
RRN $_{75}$PK+ Nano-N+ Nano-Zn + Nano-Cu	31.0	28.9	30.5	27.4	31.4	30.3	30.8	29.4
RRN $_{100}$PK+ Nano-N+ Nano-Zn + Nano-Cu	31.9	31.0	32.3	30.7	32.8	32.3	33.0	32.8
Sem±	2.34	1.85	2.43	2.63	2.03	1.62	1.94	1.78
CD ($p \leq 0.05$)	6.87	5.43	7.14	7.71	5.95	4.76	5.69	5.23

Nano-N + Nano-Zn. The maximum dehydrogenase activity was recorded under 100% RRN + nano-Zn treatment (39.3 μg TPF g^{-1} 24 h^{-1} for wheat, 42.2 μg TPF g^{-1} 24 h^{-1} for pearl millet, 46.1 μg TPF g^{-1} 24 h^{-1} for mustard, average of 2 years) and it remained at par with 100% RRN + nano-N + nano-Zn (38.2 μg TPF g^{-1} 24 h^{-1} for wheat, 40.2 μg TPF g^{-1} 24 h^{-1} for pearl millet, 41.3 μg TPF g^{-1} 24 h^{-1} for mustard, average of 2 years) and 100% RRN + nano-N + nano-Zn + nano-Cu (36.7 μg TPF g^{-1} 24 h^{-1} for wheat, 42.4 μg TPF g^{-1} 24 h^{-1} for pearl millet, 40.3 μg TPF g^{-1} 24 h^{-1} for mustard, average of 2 years).

Furthermore, the treatments 75% RRN + Nano-Zn (32.1 μg TPF g^{-1} 24 $h^{-1}$for wheat, average of 2 years), 75% RRN + Nano-N + Nano-Zn (34.9 μg TPF g^{-1} 24 $h^{-1}$for wheat, 36.3 μg TPF g^{-1} 24 $h^{-1}$for pearl-millet, average of 2 years) and 75% RRN + Nano-N + Nano-Zn + Nano-Cu (36.0 μg TPF g^{-1} 24 h^{-1} for wheat, 36.1 μg TPF g^{-1} 24 h^{-1} for pearl-millet, average of 2 years) also remained at par with 100% RRN + Nano-N + Nano-Zn during the 1st and 2nd years, respectively.

3.8. Soil microbial biomass carbon

A significant effect on soil microbial biomass carbon was recorded under various treatments of maize-wheat and pearl millet-mustard

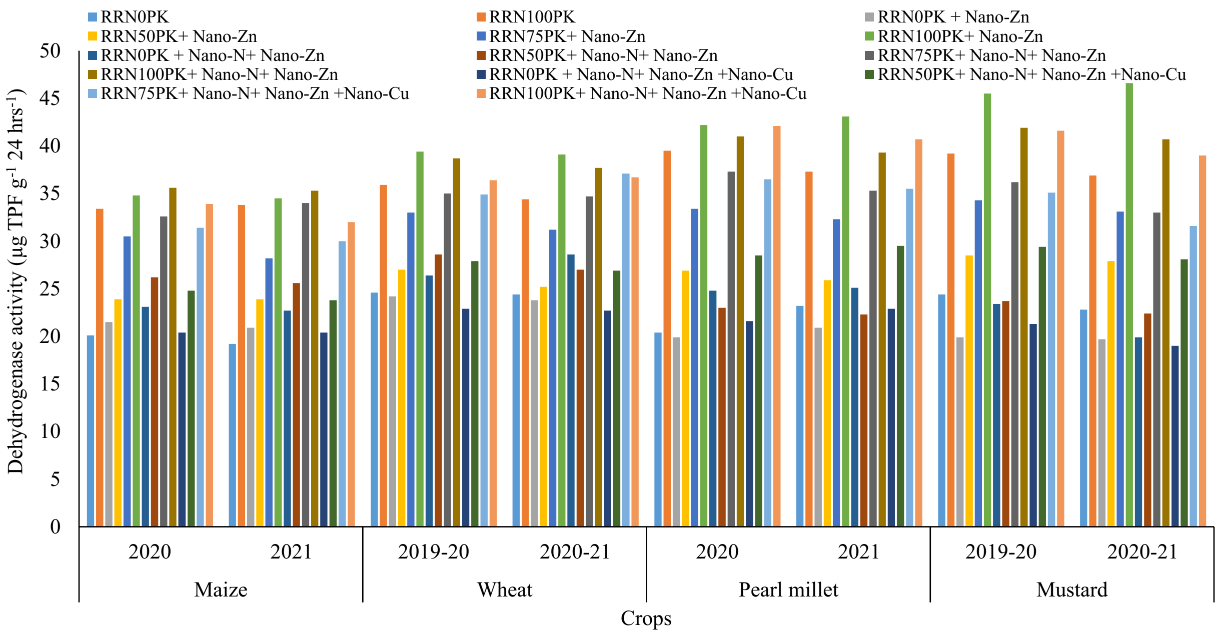

FIGURE 1
Effect of nano-fertilizers on dehydrogenase activity (µg TPF g^{-1} 24 h^{-1}) under maize-wheat and pearl millet-mustard systems.

system (Figure 1). Application of 100% RRN + Nano-N + Nano-Zn recorded maximum value for microbial biomass carbon for maize (282–316 µg g^{-1} of soil), wheat (291–296 µg g^{-1} of soil), pearl-millet and Mustard (289–349 µg g^{-1} of soil) during first as well as second year, while application of 100% RRN + Nano-Zn recorded maximum value for microbial biomass carbon during first year in wheat crop (293 µg g^{-1} of soil) (Figure 2). The treatments 100% RRN + Nano-Zn and 100% RRN + Nano-N + Nano-Zn + Nano-Cu recorded comparable values for microbial biomass carbon for maize, wheat and pearl millet during 2019–2020 and 2020–2021. In mustard, during 2019–2020 and 2020–2021, application of 100% RRN + Nano-N + Nano-Zn and 100% RRN + Nano-N + Nano-Zn + Nano-Cu recorded the highest and same values (300 µg g^{-1} of soil) for soil microbial biomass but did not show any significant difference (Figure 1). Both the treatments remained at par among themselves and with treatments 100% RRN + Nano-Zn (298 µg g^{-1} of soil), 75% RRN + Nano-N+ Nano-Zn (252 µg g^{-1} of soil), and 75% RRN+ Nano-N+ Nano-Zn + Nano-Cu (259 µg g^{-1} of soil) during both the years.

4. Discussion

4.1. Productivity of crops

Overuse of conventional fertilizers is a globally followed practice to meet plant nutrient needs. However, the efficiency of fertilizer use in crops rarely exceeds 30–35%, which is due to the loss of nutrient through leaching, evaporation and fixation (Mahmud et al., 2021). Therefore, nano-fertilizers have gained momentum over the decade to make fertilizer use more efficient and facilitate fertilizer application. However, research has evolved over a decade from laboratory studies and concentric pot experiments. Few systematic studies have been conducted so far to demonstrate the effects of nano-fertilizers or the combination of nano-fertilizers with conventional fertilizers on crop yield and economics under the field conditions (Kah et al., 2018; Hu and Xianyu, 2021; Upadhyay et al., 2023).

The application of 100% RRN in conjunction with nano-N + nano-Zn increased grain yields by 66.2–68.8% in maize, 62.6–61.9% in wheat, 57.1–65.4% in pearl millet, and 47.2–69.0% in mustard compared to control plots. However, for maize, wheat, pearl millet, and mustard, 75% RRN combined with two sprays of Nano-urea + nano-Zn produced statistically equivalent yields to 100% RRN + nano-N + nano-Zn (Table 3). This increase in crop yield with the application of nano-N + nano-Zn could be attributed to increased uptake of applied nano-fertilizers in addition to the basal application of traditional fertilizers. Foliar use of nano-fertilizers at important crop growth stages in various crops, either alone or in conjunction with fertilizers, boosts the crop yield (Kumar et al., 2021). According to Al-Juthery et al. (2019), foliar spraying of nano-fertilizers considerably increased plant growth parameters and yield of maize and wheat crops. Nano-urea, nano-Zn, and nano-Cu were sprayed on leaves in the current investigation, resulting in direct penetration through stomatal holes, and transfer through plasmodesmata (Kumar et al., 2021). Similarly, 75% RRN alone or in conjunction with nano-N + Nano-Zn was determined to be equivalent to 100% RRN+ Nano-N + Nano-Zn. Although maize, wheat, pearl millet, and mustard yields were statistically equal during the first year, yield was significantly lower under 75% RRN than 100% RRN during the second study year. This could be related to a deterioration in the soil's intrinsic fertility state, which contributed N nutrition to both crops during the first year (the year the experiment began). These nano-fertilizers release N and Zn in a regulated manner after entering plant systems. The absorption efficacy of nano-urea by plants is 80% greater than that of regular urea (Kumar et al., 2021). However, the efficiency of these nano-fertilizers is dependent on their concentration, application method, and also on the weather conditions. According to

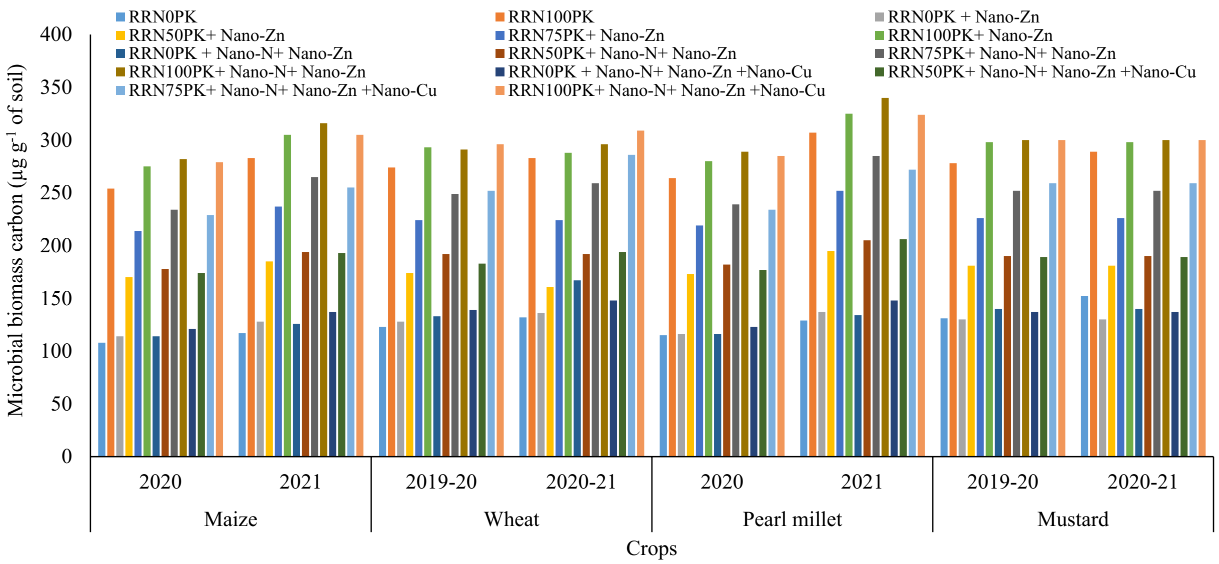

FIGURE 2
Effect of nano-fertilizers on Microbial biomass carbon (μg g^{-1} of soil) under maize-wheat and pearl millet-mustard systems.

Babu et al. (2022), in warm weather better acquisition and translocation of nano-urea results in achieving higher efficiency of nano-urea by plants. Interestingly, the use of ZnO nano particles (NPs) enhanced gas exchange parameters and chlorophyll concentration, leading to a better photosynthetic rate (Srivastav et al., 2021). As a result, either alone or in combination with nano-N, nano-Zn delivered a higher yield advantage. Zn has already been shown to improve chlorophyll synthesis by stimulating chlorophyll pigment formation and protochlorophyllide development, which ultimately improve photosynthesis (Sadak and Bakry, 2020; Del-Buono et al., 2021; Salam et al., 2022).

4.2. Uptake of nutrients

Regardless of crop, the application of nano-fertilizers with 100% RRN + Nano-N + Nano-Zn plots increased N and Zn uptake (Tables 4–7). However, the use of 75% RRN in conjunction with nano-N + nano-Zn produced comparable N and Zn uptake to that of 100% RRN + nano-N + nano-Zn plots. This was mostly attributable to the statistically same level of productivity noticed in all crops under mentioned treatments compared to statistically at par N and Zn levels. It implies that the application of nano-urea as a foliar spray additionally stimulates the uptake mechanism. Nano-N absorption is dependent on the leaf surface area (Babu et al., 2022), plant nutritional needs (Tarafdar et al., 2012), applied N (Grillo et al., 2021), and usage efficiency of native soil N (Tarafdar et al., 2014). In this work, Zn nano fertilizers and nano-N dramatically increased Zn uptake in all crops. As a result, our research enables us to decipher the Zn nano-fertilizer, allowing it to be used as an effective growth regulator to boost crop output under stress situations. Salam et al. (2022) discovered that adding ZnO NPs to maize plants decreased Co stress by lowering its uptake and bioaccumulation, boosting critical nutrient intake, and improving photosynthetic efficiency. Interestingly all the three combinations of nano-fertilizers like nano-N + nano-Zn + nano-Cu had similar Cu uptake with no nano-Cu applied plots (Tables 8, 9).

4.3. Mineral nitrogen and biological activities

Soil mineral nitrogen (Tables 10, 11), dehydrogenase activity (DHA) (Figure 1), and soil microbial biomass carbon (SMBC) (Figure 2) in maize, wheat, pearl millet, and mustard crops were significantly higher with the application of 100% RRN + Nano-N + Nano-Zn at flowering and post-harvest soils than other combinations. As a result, using Zn nano-fertilizers in conjunction with nano-N in addition to traditional fertilizers provided a greater advantage in terms of increasing DHA and SBMC. Zinc (Zn) is an essential element which involved in photosynthesis, the antioxidant defense system, and disease resistance (Olechnowicz et al., 2018; Cabot et al., 2019). Post-flowering applications of ZnO NPs had a larger effect on grain Zn content and a relatively lesser impact on grain yield was reported by Dapkekar et al. (2018) and Srivastav et al. (2021). In the current study, the applications of 100% RRN and 75% RRN + Nano-N + nano-Zn yielded statistically similar mineral N values throughout the seasons, implying that nutrient mining did not occur. The superior plots' increased root biomass frequently serves as a substrate for microbial development and metabolism. The addition of nano-urea increased root development and activity, which favored soil enzymatic activity. Nevertheless, the recommended N applications, along with Nano-N and Nano-Zn spray, produced the highest soil mineral N levels. However, lesser or no application of conventional fertilizers resulted in significantly lower mineral N, DHA, and SMBC levels across the seasons. Therefore, to avoid nutrient mining, at least 75% of the recommended nitrogen along with 2 sprays of nano-urea or nano-urea and nano-Zn should be applied. Further, it is observed from the study that maintaining ecological balance between aboveground (in terms of plant growth and yield) and underground (soil mineral N, DHA, SMBC etc.) the conjoint use of conventional fertilizers and nano-fertilizer (nano-N and nano-Zn) could be one of the best option.

5. Conclusion

The use of nano-N and nano-Zn in combination with traditional nitrogen fertilizers has immense scope to improve crop yields, nutrient uptake, soil mineral N, dehydrogenase activity, and soil microbial biomass carbon in wheat-maize and mustard-pearl millet cropping systems. Maximum grain yield of maize, wheat, pearl millet and mustard crops was observed under RRN $_{100}$PK+ Nano-N+ Nano-Zn treatments. The alone application of nano-Zn or nano-N+ nano-Zn or nano-N+ nano-Zn + nano-Cu could not suffice the requirement of the crops. Basal N application (75% of recommended) through prilled urea with full dose of P_2O_5 and K_2O along with nano-urea (2,500 mL ha^{-1} $spray^{-1}$) + nano-Zn (1,250 mL ha^{-1}) sprays recorded on par grain yield (wheat, mustard, maize and pearl millet) over 100% N + full dose of P_2O_5 and K_2O (recommended dose of fertilizer). Furthermore, the application of nano-Cu did not produced any significant results concerning crops yield. Overall, continued exploration demands a rigorous pursuit of additional research and expansive field trials concerning nano fertilizers, both in isolation and in tandem with conventional counterparts. It is imperative to carry out these endeavors across diverse crops and varied locations, a vital undertaking aimed at unraveling the true scope and potential of this innovative approach.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

PU: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. VS: Conceptualization, Methodology, Writing – original draft. GR: Data curation, Formal analysis, Methodology, Writing – review & editing. BD: Investigation, Methodology, Project administration, Writing – review & editing. AD: Data curation, Formal analysis, Methodology, Supervision, Writing – original draft. RS: Data curation, Validation, Writing – review & editing. SR: Data curation, Formal analysis, Writing – review & editing. KS: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. SB: Data curation, Formal analysis, Validation, Visualization, Writing – review & editing. TS: Funding acquisition, Resources, Writing – review & editing. YK: Funding acquisition, Resources, Writing – review & editing. CS: Data curation, Resources, Writing – review & editing. MR: Data curation, Methodology, Writing – review & editing. AK: Resources, Writing – review & editing. SS: Formal analysis, Writing – review & editing. SD: Formal analysis of data. SR: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1260178/full#supplementary-material

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Monitoring the effect of integrated nutrient management practices on soil health in maize-based cropping system

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Introduction: Soil organic matter (SOM) plays a vital role in enhancing soil characteristics and promoting sustainable crop production. The active and passive components of SOM tend to be more effective indicators of soil changes than total SOM content.

Methods: This study aimed to examine the impact of integrated nutrient management (INM) on the active and passive segments of SOM in maize during the kharif seasons of 2019 and 2020 at the Instructional Farm of Rajasthan College of Agriculture, Udaipur. A total of 11 treatments comprising of control (no application of manures/fertilizers/biofertilizers), different INM combinations, and application of inorganic fertilizers were laid in a randomized block design (RBD) with three replications tested in this study.

Results: The use of INM through enriched phosphorus compost (EPC), biofertilizers, and chemical fertilizers significantly boosted both the active and passive constituents of the organic matter of the soil. In this study, a combination of 75% NPK fertilizers via soil test response (STR), EPC @ 5 t ha^{-1}, an *Azotobacter* consortium, phosphorus solubilizing bacteria (PSB), and a foliar spray of 0.5% Zn considerably increased the active fraction of SOM than other treatments as indicated by microbial biomass carbon (251 mg kg^{-1}), microbial biomass nitrogen (36.8 mg kg^{-1}), microbial biomass phosphorus (6.82 mg kg^{-1}), water-soluble organic carbon (73.9 mg kg^{-1}), water-soluble carbohydrates (43.8 mg kg^{-1}), presence of dehydrogenase in soil (6.82 µg TPF g^{-1} soil 24 h^{-1}), and carbon mineralization (43.8 mg CO_2C kg^{-1} soil 24 h^{-1}). This treatment was also found to increase the passive fraction as shown by the presence of humic acid (0.332%), fulvic acid (0.210%), hymatomelanic acid (0.052%), brown humic acid (0.252%), and humin (0.604%).

Discussion: From this study, it can be concluded that the application of 75% NPK fertilizers as per STR + EPC + *Azotobacter* + PSB + 0.5% foliar Zn spray can improve soil health in maize-based cropping systems.

KEYWORDS

integrated nutrient management, active fractions, passive fractions, soil organic matter, humic acid

1. Introduction

Soil organic matter (SOM) encompasses all organic materials present in the soil, excluding living roots and animals. Constituting 58% of total SOM, carbon is a prominent indicator of soil health and fertility. High levels of soil organic carbon (SOC) are critical for soil biogeochemical processes (Doetterl et al., 2015; Laik et al., 2021a; Pramanick et al., 2023). The rates of decomposition of stable SOC and newly added carbon are regulated by immobilization and mineralization processes. The soil carbon storage capacity defines the rate of increase in SOC stock after the adoption of improved management practices such as the addition of organic matter in the soil. The amount of mineralization and equilibrium levels of carbon are crucial indicators of potential soil productivity (Riffaldi et al., 1996).

The primary constituents of SOM are humic substances (Stevenson, 1994a). These substances, particularly humic and fulvic compounds, directly boost plant growth through physiological and nutritional means (Vaughan and Malcolm, 1985; Chen and Aviad, 1990; Nardi et al., 2002). Some can act as natural plant hormones, accelerating seed germination, root formation and development (Piccolo et al., 1993), and uptake of nutrients (Dell'Agnola and Nardi, 1987; Piccolo et al., 1993), and serve as sources of nitrogen (N), phosphorus (P), and sulfur (S) (Arslan and Pehlivan, 2008). These substances indirectly impact plant growth by modifying soil's physical (Johnston, 2007), chemical, and biological properties, enhancing soil water holding capacity and cation exchange capacity and improving tilth and aeration (Stevenson, 1994b; Johnston, 2007; Murphy, 2015). SOM comprises active and passive fractions. The passive fraction, encompassing of durable stock of materials that can remain for a longer period of hundred decades in soil, is a decomposition product. It accounts for 58–82% of most SOM, and its quality changes slowly. Passive fractions enhance the soil's cation interchanging ability, water retention potential, and colloidal properties (Smith and Paul, 1990).

In sustainable agriculture, organic matter not only contributes to soil fertility but also stimulates and moderates soil formation, biology, physio-chemical properties, and hydro-thermal characteristics (Katyal, 2000; Pramanick et al., 2018, 2022; Naik et al., 2022). The nature, content, composition, and transformations of SOM play a crucial role in crop productivity in varying climate conditions. However, the costs associated with the procurement, transportation, and application of organic manures in large quantities are significant for farmers in developing countries such as India. Thus, a combination of inorganic fertilizers and organic manure could offer a solution to maintain crop yield (Sher et al., 2022) and soil health (Huang et al., 2010; Song et al., 2015; Lakshmi et al., 2021).

Maize-based cropping system in India is known as a chemical fertilizer-intensive cropping system as the nutrient requirement of maize is higher than other cereals (Singh et al., 2017; Pramanick et al., 2022). Continuous maize cultivation with the application of chemical fertilizers may deplete soil health in the long run. Hence, organic amendment as well as the application of biofertilizers are important to make the soil sustainable for production. In this study, the soil of a maize-based cropping system was selected to know whether the INM practices can improve the SOM pools or not.

Given these factors, the present experimental trial was directed to assess the operation of impacts of integrated utilization of biological, organic, and inorganic nutrient cause of sources on the SOM pools of maize soils and to identify the optimal nutrient sources and combinations for maintaining soil health.

2. Materials and methods

2.1. Experimental location

The study was carried out at the College Farm of the Rajasthan Agricultural College in Udaipur, Rajasthan, across two crop seasons: Kharif 2019 and Kharif 2020. The experimental plot range is positioned at 24°34′ N latitude and longitudinal reference of 73°42′ E, at an elevation of 579.5 m more than the average sea level (Figure 1). The site falls under the CA'w Climatic classification as per Thornthwaite's system, signifying a humid subtropical climate with warm, wet summers and dry winters that receive sparse rainfall. The location typically receives an average rainfall of 637 mm, with over 70% of this precipitation occurring during the southwest monsoon season (July to September). The relative humidity during these monsoon months is relatively high, often exceeding 65%.

The soil at the experimental site is classified as clay loam, falling under the *Vertisols* category—typified by fine texture, iso-hyperthermic temperature regime, montmorillonitic mineralogical class, and Typic *Haplustept* taxonomic class. The soil has a slightly saline pH level of 8.29 and contains 0.62% organic carbon.

The experiment consisted of 11 treatments replicated thrice in a randomized block design (RBD). The treatment imposition details are given in Table 1.

FIGURE 1
Experimental location of the study area on the map.

TABLE 1 Treatment imposed in the current research study plan.

Treatments	Treatment details
T_1	Control
T_2	125% NPK via STR
T_3	100% NPK via STR+ 0.5% Zn foliar spray at 35 DAS
T_4	100% NPK via STR + *Azotobacter* consortium + Phosphorus Solubilizing Bacteria (PSB)
T_5	100% NPK via STR + *Azotobacter* consortium + PSB + 0.5% Zn foliar spray at 35 DAS
T_6	75% NPK via STR + Enriched phosphorus compost (EPC) @ 5 t ha^{-1}
T_7	75% NPK via STR + EPC @ 5 t ha^{-1} + *Azotobacter* + PSB
T_8	75% NPK via STR + EPC @ 5 t ha^{-1} + *Azotobacter* + PSB+ 0.5% Zn foliar spray at 35 DAS
T_9	50% NPK via STR + EPC @ 5 t ha^{-1}
T_{10}	50% NPK via STR + EPC @ 5 t ha^{-1} + *Azotobacter* + PSB
T_{11}	50% NPK via STR + EPC @ 5 t ha^{-1} + *Azotobacter* + PSB + 0.5% Zn @ foliar spray at 35 DAS

NPK, STR, and DAS denote nitrogen, phosphorus, and potassium fertilizers, soil test-based recommendation, and days after sowing, respectively.

2.2. Treatment imposition and management

The control treatment (T1) was conducted without the application of any fertilizers. The soil test-based recommendation (STR) was used to calculate the appropriate amounts of urea, diammonium phosphate, and muriate of potash for each treatment. These were subsequently applied at the recommended stages of crop growth. Enriched phosphorus compost (EPC) was locally prepared using maize stover, rock phosphate, and waste mica. To decrease the C:N ratio of the maize stover, we applied a urea solution at a rate of 0.25 kg N per 100 kg of stover and fresh cow dung at 10 kg per 100 kg of stover. These were used as natural inoculants along with phosphate solubilizing microorganisms (*Aspergillus awamori, Pseudomonas striata,* and *Bacillus polymixa*) at 50 g per 100 kg for treatments T_6 to T_{11}. The fully decomposed EPC contained 0.85% N, 1.21% P, 1.15% K, and 115 ppm of Zinc (Table 2). The EPC was applied to the field as dictated by the treatments and was thoroughly mixed into the soil a week before sowing. To administer treatments T_4 and T_5, we treated the seeds with a consortium of *Azotobacter* and phosphorus-solubilizing bacteria, i.e., PSB (*Bacillus megatherium* var. *phosphaticum*). For seed treatment, 200 g of each inoculant of *Azotobacter* and PSB was mixed in 500 ml water and a slurry was prepared with 50 g of jaggery. Afterward, the slurry was mixed evenly with the seeds before sowing. Nutrient management was applied as per the treatment details. For maize cultivation, two irrigations were given at silking and tasseling stage. Weed management was performed using the preemergence application of Atrazine at 1,000 g ha^{-1} applied 2 days after sowing (DAS) followed by one manual weeding at 30 DAS. The cost of cultivation is included in Supplementary Table S1.

2.3. Soil analyses

The soil microbial biomass carbon content was determined using the chloroform fumigation-extraction method as described by Vance et al. (1987). The soil microbial biomass nitrogen was analyzed using a similar method by Brookes et al. (1985). Soil microbial biomass phosphorus was assessed using the standard procedure proposed by Brookes et al. (1982). Water-soluble carbon was determined using the acid extraction method outlined by Meloon and Sommers (1996), and water-soluble carbohydrates were quantified using the hydrolytic extraction with the H_2SO_4 method by Cheshire and Mundie (1966). The Anthrone extraction procedure, as explained by Casida et al. (1964), was utilized for analyzing soil dehydrogenase activity. Carbon mineralization was calculated using the rubber cork method (Kukreja et al.,

TABLE 2 Characteristics of enriched phosphorus compost (EPC) and in-organic fertilizers used in the experimental field study.

Nutrient source	OC (%)	C:N	*N* (%)	P_2O_5 (%)	K_2O (%)	Zn (ppm)
EPC	7.90	<20:1	0.85 ± 0.02	1.22 ± 0.02	1.16 ± 0.02	115 ± 1
Urea	-	-	46	0	0	-
DAP	-	-	18	46	0	-
MOP	-	-	0	0	60	-

OC, organic carbon; EPC, enriched phosphorus compost; DAP, di-ammonium phosphate; MOP, muriate of potash.

1991). For the analysis of humic substances, various methods were employed. Humic acid (HA) and fulvic acid were determined using a 0.5 N NaOH solution method. Hymatomelanic acid (HyMA) was analyzed using Soxhlet's apparatus method. Brown humic acid (BHA) was measured using a 5% NaOH solution method. Gray humic acid (GHA) was quantified using the formula GHA = (weight of HA before extracting HyMA—weight of HA after extracting HyMA)—BHA. Humin was determined using a 1-N HCl method. All these methods were based on the procedures provided by Stevenson (1965).

2.4. Statistical analysis

Data were analyzed using the standard techniques of analysis of variance (ANOVA) for randomized block design (RBD) with the help of statistical software, SAS (version 9.2). Bartlett's $\chi 2$ test was performed to analyze the homogeneity of the error variance. Then, pooled analysis was performed as per Cocharan and Cox (1957) as the error variance was homogeneous. Differences were tested for Fisher's *F*-test at a 5% level of significance. When the *F*-test was found significant at a 5% level of significance, the least significant difference (LSD) was used to test the significance of differences between the two treatment means (Gomez and Gomez, 1976).

3. Results and discussion

3.1. Active pools of soil organic matter

The dynamics of SOM are crucial for enhancing soil quality and maintaining crop productivity under intensive farming. In the following sections, we will discuss the results and interpretations of the active pools of SOM.

3.1.1. Soil microbial biomass carbon

The integration of organic manure with fertilizer and biofertilizers got a positive impact on soil microbial biomass carbon (Table 3). Compared to non-compost treatments (T_1-T_5), the microbial biomass carbon was higher in compost-based INM treatments (T_5-T_{11}). The lowest microbial carbon (180 mg kg^{-1}) was found in control (T_1), whereas treatment with 75% NPK via STR + EPC+ *Azotobacter* + PSB + Zn (T_8) recorded the highest value (251 mg kg^{-1}). Compared to the unfertilized plot, treatment T_8 had 1.4 folds of higher soil microbial biomass carbon. Biofertilizers application also poses a significant and pragmatic influence on soil microbial biomass carbon. The application of Zn as foliar spraying did not induce significant changes as evident from comparisons made between T_7 and T_8 and T_{10} and T_{11}. In addition to EPC and biofertilizers (BF), i.e., *Azotobacter* and PSB, increasing levels of NPK bring significant improvement in microbial biomass carbon to control.

Microbial biomass is closely tied to SOC, and its levels rise with the addition of a carbon substrate and fall when the carbon content is consumed (Manna and Ganguly, 2000; Zhao et al., 2009). The increase in soil microbial biomass carbon in treatments with EPC could also be due to a decrease in soil pH and electrical conductivity resulting from the addition of organic matter. This improvement in soil microbial biomass carbon with compost application aligns with findings from Kallenbach and Grandy (2011) and Meena and Biswas (2015). Similarly, numerous studies have found that organic amendments significantly impact microbial biomass carbon levels due to the range of macro- and micronutrients added via these amendments (Hu et al., 2011; Chinnadurai et al., 2014; Tamilselvi et al., 2014; Obalum et al., 2017; Rajput et al., 2019; Ogumba et al., 2020; Padbhushan et al., 2021).

3.1.2. Soil microbial biomass nitrogen

Significant differences in the microbial biomass of soil nitrogen were recorded in response to various nutrient sources and their combinations (Table 3). Like microbial biomass of soil carbon, the highest (36.8 mg kg^{-1}) and lowest values (26.4 mg kg^{-1}) of microbial biomass of soil nitrogen were observed in 75% NPK via STR + EPC+ *Azotobacter* + PSB + Zn (T_8) and control (T_1), respectively. The biomass nitrogen content of the soil microbial population is significantly influenced by the levels of NPK. Compared to the control, 17.25% and 6.5% higher biomass nitrogen were obtained in 125% NPK and 100% NPK + Zn, respectively. In addition to EPC and inorganic fertilizers, the co-application of Zn and biofertilizers also brought significant improvement in microbial biomass nitrogen as evident from comparing T_9 and T_{11} and T_6 and T_8.

The enhancement in the microbial biomass of soil carbon is perhaps ascribed to the balanced nutrient provision to soil microbes through an INM approach (Rajput et al., 2019). The higher levels of SOC and improved nitrogen availability mediated by EPC can be an additional factor contributing to the increased soil microbial biomass nitrogen observed in EPC-based plots. Significantly higher soil microbial biomass nitrogen levels were observed in 100% and 125% NPK treatments compared to the control. This aligns with findings by Verma and Mathur (2009) and Padbhushan et al. (2021), who noted that microbial biomass nitrogen increased with rising nitrogen levels.

TABLE 3 Effect of integrated nutrient management practices on soil microbial biomass.

Treatments	Soil microbial biomass (mg kg^{-1} soil)								
	Soil microbial biomass carbon			Soil microbial biomass nitrogen			Soil microbial biomass phosphorus		
	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled
T_1	179 ± 5	181 ± 7	180 ± 6	26.2 ± 1.0	26.5 ± 0.5	26.4 ± 0.8	4.45 ± 0.5	4.53 ± 0.2	4.49 ± 0.4
T_2	209 ± 9	211 ± 2	210 ± 5	30.7 ± 2.1	31.1 ± 0.9	30.9 ± 2.0	5.95 ± 0.6	6.05 ± 0.1	6.00 ± 0.3
T_3	189 ± 4	191 ± 4	190 ± 4	27.9 ± 1.2	28.3 ± 2.1	28.1 ± 1.6	5.71 ± 0.8	5.80 ± 0.5	5.76 ± 0.6
T_4	197 ± 5	199 ± 3	198 ± 4	29.1 ± 1.5	29.5 ± 2.2	29.3 ± 1.8	5.80 ± 0.9	5.89 ± 0.2	5.85 ± 0.5
T_5	200 ± 3	202 ± 5	201 ± 4	29.6 ± 1.1	30.0 ± 2.5	29.8 ± 1.8	5.86 ± 0.5	5.96 ± 0.4	5.91 ± 0.4
T_6	235 ± 6	237 ± 4	236 ± 5	34.6 ± 1.6	35.0 ± 1.5	34.8 ± 1.5	6.64 ± 0.9	6.74 ± 1.0	6.69 ± 0.9
T_7	246 ± 7	249 ± 11	247 ± 9	35.8 ± 1.7	36.2 ± 1.6	36.0 ± 1.6	6.72 ± 0.6	6.83 ± 1.0	6.77 ± 0.8
T_8	249 ± 9	252 ± 12	251 ± 10	36.6 ± 1.6	37.0 ± 1.8	36.8 ± 1.7	6.77 ± 0.4	6.88 ± 0.6	6.82 ± 0.5
T_9	209 ± 11	212 ± 10	211 ± 10	31.9 ± 1.9	32.3 ± 1.5	32.1 ± 1.7	6.11 ± 0.5	6.21 ± 0.8	6.16 ± 0.6
T_{10}	219 ± 10	221 ± 5	220 ± 8	33.0 ± 2.2	33.5 ± 1.0	33.2 ± 1.6	6.33 ± 0.4	6.43 ± 0.5	6.38 ± 0.5
T_{11}	224 ± 8	226 ± 7	225 ± 8	33.4 ± 1.8	33.8 ± 2.0	33.6 ± 1.9	6.39 ± 0.3	6.49 ± 0.8	6.44 ± 0.6
LSD at 5 %	9	9	6	1.8	1.9	1.3	0.31	0.35	0.23

Treatment details are mentioned in Table 1.

3.1.3. Soil microbial biomass phosphorus

Differences in phosphorus content of microbial biomass between the non-compost treatments (T_1-T_5) and the compost-based treatments (T_6-T_{11}) are significant except for T_2 and T_9 which were non-significantly different (Table 3). The maximum microbial biomass phosphorus values were found in T_8, followed by T_7, T_6, T_{11}, T_{10}, and T_9 treatments, respectively. The treatments T_1 to T_4 recorded the least value of microbial biomass phosphorus. The addition of biofertilizers and Zn did not improve the microbial biomass of soil phosphorus significantly. The microbial biomass of soil phosphorus was higher under integrated nutrient application than sole use of NPK fertilizers. However, compared to the control (T_1) and 100% NPK + Zn (T_3), significantly higher soil microbial biomass phosphorus was observed in 125% NPK (T_2). Balanced NPK application in conjunction with EPC enhanced the soil microbial biomass phosphorus more than the rest of all treatments. Integrated nutrient management provides substrates needed for microbes' growth and activity, thus enhancing microbial biomass phosphorus (Verma and Mathur, 2009; Kumar et al., 2017). Lack of nutrient addition in the soil might have resulted in low microbial biomass phosphorus in control plots. Additionally, the optimal level of P fertilizer might have promoted microbial metabolism processes that in turn raised the level of microbial biomass phosphorus (Verma and Mathur, 2009).

3.1.4. Water-soluble organic carbon

Integration of organic manure with fertilizer and biofertilizers recorded a positive impact on the water-soluble organic carbon (WSC). Its value in soil varied from 46.2 to 73.9 mg kg^{-1} under different treatments (Table 4). The maximum value of WSC (73.86 mg kg^{-1}) in soil was recorded under T_8 (75% NPK via STR + EPC+ *Azotobacter* + PSB + Zn), and this treatment was found at par with T_7 (75% NPK via STR + EPC+ *Azotobacter* + PSB). The lowest value of WSC (46.2 mg kg^{-1}) in soil was recorded in the control treatment (T_1). The WSC is the most mobile as well as most labile C pool, and it serves as an immediate C source for soil microbes (Verma and Mathur, 2009). Verma and Mathur (2009) also reported that the highest WSC was recorded in INM treatments. In the INM, organic manures, inorganic fertilizers, and biofertilizers all are applied in combination to the soil and this kind of management practice increases the SOC as well as the activities of the soil beneficial microbes which ultimately attributed to higher WSC in soil (Laik et al., 2021b). In contrast to the above, the beneficial effects of organic manures on WSC content were reported by Dutta et al. (2018). The addition of organic manures increased WSC irrespective of inorganic fertilizers usage.

3.1.5. Water soluble carbohydrate

The integrated nutrient management practices significantly enhanced the water-soluble organic carbohydrates of soil (Table 4). The water-soluble organic carbohydrates in soil varied from 26.45 to 43.80 mg kg^{-1} under various treatments. The minimum value (26.45 mg kg^{-1}) of water-soluble organic carbohydrates was recorded in control (T_1) whereas the maximum value (43.80 mg kg^{-1}) was recorded in T_8 (75% NPK via STR + EPC+ *Azotobacter* + PSB + Zn) which was statistically at par with T_7 (75% NPK via STR + EPC+ *Azotobacter* + PSB). Water-soluble carbohydrates function as both sources and sink for mineral nutrients and organic substrates in the short term. Over a longer period, they act as catalysts for plant nutrient conversion, therefore, influencing crop productivity and nutrient cycling (Kumari et al., 2011). Water-soluble carbohydrates are the most readily accessible energy source for microbes, and they play a substantial role in stabilizing soil structure (Verma and Mathur, 2009). Studies conducted by

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TABLE 4 Effect of integrated nutrient management practices on water-soluble organic carbon and water-soluble carbohydrates, dehydrogenase activity, and carbon mineralization in the soil after harvest of maize.

Treatments	Water soluble organic carbon (mg kg^{-1} soil)			Water soluble carbohydrates (mg kg^{-1} soil)			Dehydrogenase activity (μg TPF g^{-1}soil 24 hr^{-1})			Carbon mineralization (mg CO_2-C kg^{-1} soil 24 hr^{-1})		
	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled
T_1	45.9 ± 0.6	46.5 ± 0.5	46.2 ± 0.5	26.3 ± 0.1	26.6 ± 0.2	26.5 ± 0.1	4.83 ± 0.05	4.83 ± 0.01	4.83 ± 0.03	26.3 ± 0.2	26.6 ± 0.3	26.5 ± 0.3
T_2	58.1 ± 0.4	58.8 ± 0.5	58.5 ± 0.4	34.3 ± 0.2	34.7 ± 0.1	34.5 ± 0.2	5.64 ± 0.04	5.64 ± 0.01	5.64 ± 0.02	34.3 ± 0.3	34.7 ± 0.4	34.5 ± 0.4
T_3	49.9 ± 0.2	50.5 ± 0.3	50.2 ± 0.2	29.8 ± 0.1	30.2 ± 0.2	30.0 ± 0.2	5.39 ± 0.01	5.39 ± 0.01	5.39 ± 0.01	29.8 ± 0.2	30.2 ± 0.2	30.0 ± 0.2
T_4	54.0 ± 0.1	54.6 ± 0.2	54.3 ± 0.2	31.8 ± 0.1	32.3 ± 0.3	32.0 ± 0.2	5.48 ± 0.01	5.48 ± 0.01	5.48 ± 0.01	31.8 ± 0.5	32.3 ± 0.5	32.0 ± 0.5
T_5	55.5 ± 0.6	56.2 ± 0.8	55.8 ± 0.7	32.4 ± 0.2	32.8 ± 0.1	32.6 ± 0.1	5.52 ± 0.01	5.52 ± 0.02	5.52 ± 0.01	32.4 ± 0.3	32.8 ± 0.4	32.6 ± 0.4
T_6	70.8 ± 0.7	71.7 ± 0.8	71.3 ± 0.7	40.9 ± 0.1	41.5 ± 0.3	41.2 ± 0.2	6.28 ± 0.01	6.28 ± 0.01	6.28 ± 0.01	40.9 ± 0.4	41.5 ± 0.2	41.2 ± 0.3
T_7	72.2 ± 0.8	73.1 ± 0.7	72.6 ± 0.7	42.8 ± 0.1	43.4 ± 0.1	43.1 ± 0.1	6.78 ± 0.02	6.78 ± 0.02	6.78 ± 0.02	42.8 ± 0.5	43.4 ± 0.3	43.1 ± 0.4
T_8	73.4 ± 0.5	74.3 ± 0.4	73.9 ± 0.4	43.5 ± 0.2	44.1 ± 0.1	43.8 ± 0.1	6.82 ± 0.01	6.82 ± 0.01	6.82 ± 0.01	43.5 ± 0.1	44.1 ± 0.4	43.8 ± 0.3
T_9	63.1 ± 0.2	63.9 ± 0.3	63.5 ± 0.3	36.2 ± 0.3	36.6 ± 0.2	36.4 ± 0.3	5.71 ± 0.02	5.71 ± 0.02	5.71 ± 0.02	36.2 ± 0.5	36.6 ± 0.6	36.4 ± 0.6
T_{10}	67.1 ± 0.1	67.9 ± 0.5	67.5 ± 0.4	38.1 ± 0.2	38.6 ± 0.5	38.3 ± 0.3	5.78 ± 0.01	5.78 ± 0.01	5.78 ± 0.01	38.1 ± 0.2	38.6 ± 0.5	38.3 ± 0.3
T_{11}	68.3 ± 0.3	69.1 ± 0.1	68.7 ± 0.2	39.0 ± 0.1	39.5 ± 0.2	39.3 ± 0.2	5.89 ± 0.01	5.89 ± 0.02	5.89 ± 0.02	39.0 ± 0.3	39.5 ± 0.1	39.3 ± 0.4
LSD at 5 %	3.3	3.3	2.3	1.7	1.7	1.2	0.25	0.25	0.25	1.7	1.7	1.2

Treatment details are mentioned in Table 1.

Padbhushan et al. (2014) and Verma and Mathur (2009) have also reported that the addition of organic manure to soil increases the total SOM which ultimately increases the carbohydrate content in the soil.

3.1.6. Soil dehydrogenase activity

The activity of soil dehydrogenase is known as the index of microbial activities happening inside the soil (Dutta et al., 2018); therefore, it is a good indicator of soil biological activities. The effect of different treatments on dehydrogenase activity in soils was found significant (Table 4). The highest value of dehydrogenase activity in soil (6.82 μg TPF g^{-1} soil 24 hr^{-1}) was estimated under T_8 (75% NPK via STR + EPC+ *Azotobacter* + PSB + Zn) and this treatment was found statistically at par with T_7 (75% NPK via STR + EPC+ *Azotobacter* + PSB). Soil dehydrogenase activity was greatly stimulated by organic, inorganic, and integrated management practices (Gao et al., 2020). Application of EPC augmented dehydrogenase activity in the soil. The extended activity of dehydrogenase in EPC-amended plots might be due to increased microbial activity under a higher organic manure supply. Earlier, Dutta et al. (2018) also reported similar results while working on various nutrient management practices.

3.1.7. Carbon mineralization

Carbon mineralization plays a critical role in the dynamics of SOM as it determines the amount of carbon that accumulates in the soil (Zhao et al., 2008). The combined use of organic manures, fertilizers, and biofertilizers significantly influenced carbon mineralization in soil (Table 4). The value of carbon mineralization in soil ranges from 26.45 to 43.80 mg CO_2C kg^{-1} soil 24 hr^{-1} under various treatments. The maximum value of carbon mineralization (43.80 mg CO_2-C kg^{-1} soil 24 hr^{-1}) in soil observed in T_8 (75% NPK via STR + EPC+ *Azotobacter* + PSB + Zn), and this treatment was found statistically at par with T_7 (75% NPK via STR + EPC+ *Azotobacter* + PSB), whereas the lowest value (26.45 mg CO_2-C kg^{-1} soil 24 hr^{-1}) was observed in control treatment (T_1) (Table 4). Balanced nutrient supply and adequate carbonaceous substrate availability and a favorable environment under INM may enhance microbial growth that leads to an increased soil carbon mineralization (Mandal et al., 2020). Depletion of nutrients in unfertilized control plots can be the reason for the least carbon mineralization. This study echoes the findings of Mandal et al. (2020), who observed similar effects of INM on carbon mineralization. The increase in carbon mineralization observed in plots treated with both NPK fertilizer and organic amendments can be attributed to the ample supply of labile carbon substrates, as pointed out by Majumder et al. (2008). This finding is corroborated by studies from Kaur et al. (2008) and Mohanty et al. (2013). Zhao et al. (2009) further proclaimed that long-duration employed usage of both intensive fertilizers and organic manures not only provides a supportive domain for microbial growth and activity but also supplies the necessary substrates for the course of carbon mineralization.

3.2. Passive pools of soil organic matter

3.2.1. Humic acid

Humic acid, as the second most significant component of SOM, plays a crucial role in enhancing nutrient recovery and transformation in soil, thereby contributing to higher yield production. This study discovered that INM significantly increased the humic acid levels in the soil post-cutting of the maize (as shown in Table 5). The content of humic acid in the soil ranged from 0.230% to 0.332% under different treatment conditions. The lowest value of humic acid (0.230%) in soil was observed under control. The treatment T_8 (75% NPK via STR + EPC+ Azotobacter + PSB + Zn) showed a significantly higher amount of humic acid (0.332%) status in soil and this treatment was closely followed by T_7 (75% NPK via STR + EPC+ *Azotobacter* + PSB) which can be due to enhanced soil physical parameters and directed favorable conditions for the evolution of humic acid (Santhy et al., 2001). The findings are in line with those reported by Deshmukh et al. (2015). Similarly, studies by Prasad et al. (1991), Banik and Sanyal (2006), Gathala et al. (2007), Bhoye et al. (2011), Meshram et al. (2016), and Meshram et al. (2018) all found that the greatest amount of humic acid was present when organic manure/residue was integrated with inorganic fertilizers. This might be attributed to the enhancement of SOM through the application of organic manures coupled with the biofertilizers that created a suitable condition for humus evolution (Kurbah and Dixit, 2020).

3.2.2. Fulvic acid

It is the uttermost dominant pool of SOM which was also significantly affected by various nutrient management practices (Table 5).

Fulvic acid, a component of the passive pool of SOM, remains soluble in alkaline solutions and stays in the solution even after humic acid has precipitated with acid. Fulvic acid components assist in binding soil particles into skeletal portions known as aggregates, which are crucial in mobilizing and transporting sesquioxides in the soil complex. The amalgamation of diverse nutrient sources noticeably elevated the levels of fulvic acid in the soil following the maize harvest. It ranges from 0.136 to 0.210% under various treatments. Similar to humic acid, fulvic acid (0.210%) also recorded the highest in T_8 (75% NPK via STR + EPC+ *Azotobacter* + PSB + Zn), which was found statistically at par with T_7 (75% NPK via STR + EPC+ *Azotobacter* + PSB). Kumari et al. (2011) noted that the repeated usage of organic manures, either alone or in tandem with intensive fertilizers, had a significant impact on the fulvic acid fraction in the soil. This might be due to the increase in SOM and beneficial microorganisms in the soil. These results align with the findings of Gathala et al. (2007) and Deshmukh et al. (2015), who documented increased fulvic acid levels following the combined application of NPK and farmyard manure.

3.2.3. Hymatomelanic acid

Integrated nutrient management significantly enhanced hymatomelanic acid (HyMA) in the soil after cutting the maize crop in both years and in the combined analysis (Table 5). The

TABLE 5 Effect of integrated nutrient management practices on humic, fulvic, and hymatomelanic acid in soil.

Treatments	Humic acid (%)			Fulvic acid (%)			Hymatomelanic acid (%)		
	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled
T_1	0.227	0.233	0.230	0.133	0.138	0.135	0.031	0.035	0.033
T_2	0.268	0.274	0.271	0.177	0.182	0.180	0.039	0.043	0.041
T_3	0.243	0.249	0.246	0.159	0.164	0.162	0.034	0.038	0.036
T_4	0.253	0.259	0.256	0.167	0.172	0.170	0.036	0.040	0.038
T_5	0.258	0.264	0.261	0.170	0.175	0.173	0.037	0.041	0.039
T_6	0.304	0.311	0.307	0.198	0.204	0.201	0.046	0.050	0.048
T_7	0.319	0.326	0.323	0.204	0.210	0.207	0.049	0.053	0.051
T_8	0.328	0.335	0.332	0.207	0.213	0.210	0.050	0.054	0.052
T_9	0.279	0.286	0.282	0.184	0.189	0.187	0.040	0.044	0.042
T_{10}	0.289	0.296	0.292	0.190	0.196	0.193	0.042	0.046	0.044
T_{11}	0.294	0.301	0.297	0.192	0.198	0.195	0.043	0.047	0.045
LSD at 5 %	0.014	0.015	0.010	0.008	0.008	0.006	0.003	0.004	0.002

Treatment details are mentioned in Table 1.

maximum value of HyMA (0.052%) in soil was obtained in T_8 (75% NPK via STR + EPC+ *Azotobacter* + PSB + Zn), and this treatment was found statistically at par with T_7 (75% NPK via STR + EPC+ *Azotobacter* + PSB). The lowest value of HyMA (0.033%) in soil was obtained under control (T_1). These outcomes are in tune with the salient findings of Kumari et al. (2011). The content of HyMA was low because it consists of more than 90% aliphatic structure and is poorer in amino acid fragments and twice as rich in hydrocarbons as humic acid (Ranjana et al., 1984).

3.2.4. Brown humic acid

Application of compost with NPK fertilizer and biofertilizers enhanced the brown humic acid (BHA). The brown humic acid in the soil varied from 0.175 to 0.252% under varied treatments (Table 6). The maximum value of BHA (0.252%) in soil was obtained in T_8 (75% NPK via STR + EPC+ *Azotobacter* + PSB + Zn), and this treatment was statistically at par with treatment T_7 (75% NPK via STR + EPC+ *Azotobacter* + PSB). It might be due to added N through inorganic N and EPC and these fractions of SOM, especially BHA, showed stronger signals for N compounds (Saizet et al., 1979). These outcomes are perfectly synced with the findings of Kumari et al. (2011).

3.2.5. Gray humic acid

This is the smallest passive SOM pool in the study region. Despite the various nutrient management strategies applied, no significant improvement was noted in the content of gray humic acid. The values varied between 0.023 and 0.034% under different treatment conditions (Table 6). The lowest value of gray humic acid (0.023%) in soil was recorded in the control (T_1). Combined use of EPC, 50% NPK fertilizers, foliar application of Zn, and biofertilizer (T_{11}) exhibited the maximum value of gray humic acid (0.034%) in soil followed by the treatment T_8 (75% NPK via STR + EPC+ *Azotobacter* + PSB + Zn). It might be due to the added N through inorganic nitrogen and EPC and a gray humic acid fraction of SOM showed stronger signals for N compounds (Saizet et al., 1979). These outcomes are fine-tuned with the evident findings of Kumari et al. (2011). Moreover, the potential explanation for the lack of significant improvement in gray humic acid content may be due to already optimized soil physical parameters and a conducive environment for this fraction.

3.2.6. Humin

The extremely impervious and resistant fraction of SOM is humin and its distribution or extent of share is the largest among the passive fraction of SOM. Among various passive pools of SOM, humin is the biggest soil pool followed by humic acid and fulvic acid fractions. The INM practices in maize significantly increased humin content in the soil after the harvest of the crop (Table 6). The humin in soil varied from 0.383% to 0.604% under various treatments. Unfertilized plots had the lowest humin (0.383%) whereas integrated use of 75% NPK via STR + EPC @ 5 t ha^{-1} + *Azotobacter* + PSB + Foliar application of Zn @ 0.5% (T_8) exhibited the largest humin pool, and this treatment was closely followed by T_7 (75% NPK via STR + EPC+ *Azotobacter* + PSB). The increase in humin fraction could be related to increased mineralization activity due to higher surface soil temperatures in tropical regimes by Santhy et al. (2001). When organic manure is added, it can serve as a source of humus. Compounds, such as cellulosic, xylose, mono and polysaccharides, glucosides, fatty acids, and amino acids, that are utilized by microorganisms can be indirectly converted into humic substances within microbial cells. These transformed substances can then be involved in the setup of humic substances evolution (Kononova, 1966). Previous studies by Prasad et al. (1991), Banik and Sanyal (2006), Gathala et al. (2007),

TABLE 6 Effect of integrated nutrient management practices on brown humic acid, gray humic acid, and humin in soil.

Treatments	Brown humic acid			Gray humic acid			Humin		
	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled
T_1	0.172	0.177	0.175	0.024	0.021	0.023	0.367	0.396	0.382
T_2	0.204	0.209	0.206	0.025	0.023	0.024	0.499	0.530	0.514
T_3	0.185	0.190	0.187	0.024	0.022	0.023	0.434	0.464	0.449
T_4	0.192	0.197	0.194	0.025	0.023	0.024	0.453	0.483	0.468
T_5	0.196	0.201	0.198	0.025	0.023	0.024	0.464	0.495	0.479
T_6	0.231	0.236	0.234	0.027	0.025	0.026	0.574	0.606	0.590
T_7	0.242	0.247	0.245	0.028	0.026	0.027	0.583	0.615	0.599
T_8	0.249	0.254	0.252	0.029	0.027	0.028	0.588	0.620	0.604
T_9	0.212	0.217	0.215	0.027	0.025	0.026	0.544	0.576	0.560
T_{10}	0.220	0.225	0.223	0.027	0.025	0.026	0.560	0.591	0.576
T_{11}	0.225	0.231	0.228	0.031	0.036	0.034	0.563	0.595	0.579
CD at 5%	0.016	0.016	0.011	NS	NS	NS	0.032	0.033	0.022

Treatment details are mentioned in Table 1.

Bhoye et al. (2011), Meshram et al. (2016), and Meshram et al. (2018) have all shown that applying 100% NPK via STR together with organic manures outstandingly enriched humus values in soil. This could be attributed to the stabilized employment of intensive fertilizers in conjunction with organic manure or matter, which led to more humus contributions in the soil, eventually resulting in greater nutrient accessibility for plants. Meena (2017) also reported that the highest humin fraction was obtained under the application of organic manure, compared to other treatments comprising chemical fertilizers.

4. Conclusion

Based on the findings of this research study, it was found that the combined application of organic and inorganic nutrient sources coupled with the application of biofertilizers led to a significant improvement in the active and passive fractions of SOM in the maize-based cropping system. From this study, it was observed that the application of 75% NPK via soil test response (STR) + enriched phosphorus compost (EPC) at 5 t ha^{-1} + *Azotobacter* + PSB + Zn foliar spray at 0.5% during 35 DAS resulted in the maximum attainments of microbial biomass carbon (251 mg kg^{-1}), microbial biomass nitrogen (36.8 mg kg^{-1}), microbial biomass phosphorus (6.82 mg kg^{-1}), water-soluble organic carbon (73.9 mg kg^{-1}), water-soluble carbohydrates (43.8 mg kg^{-1}), presence of dehydrogenase in soil (6.82 μg TPF g^{-1} soil 24 hr^{-1}), and carbon mineralization (43.8 mg CO_2C kg^{-1} soil 24 hr^{-1}). This treatment was also found to increase the passive fraction of SOM in the maize-based cropping system. This INM practice was also found to be closely followed by the application of 75% NPK via STR + EPC + *Azotobacter* + PSB. Hence, from this 2-year-long study, it can be concluded that the active and passive pools of SOM in a maize-based cropping system can be increased through the application of 75% NPK via STR + EPC @ 5 t ha^{-1} + *Azatobacter* + PSB + 0.5% Zn foliar spray at 35 DAS.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

JB, HP, BN, AH, AA, AG, and BP performed the statistical analysis, project supervision, reviewed, read, and edited the manuscript with significant contributions. JB, HP, BN, BP, ShB, SuB, KD, SS, and SM collected soil/plant samples and performed chemical analysis, also wrote the initial draft of the manuscript, prepared figures and tables, led the research work, planned, supervised, and conducted field experiments, and read and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Organic management increases beneficial microorganisms and promotes the stability of microecological networks in tea plantation soil

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Introduction: Organic agriculture is highly regarded by people for its commitment to health, ecology, care, and fairness. The soil microbial community responds quickly to environmental changes and is a good indicator for evaluating soil microecology. Therefore, from the perspective of soil microbial communities, elucidating the impact of organic management on soil microecology in tea plantations has great significance for improving local tea plantation systems.

Methods: The study collected bulk soil from organic management (OM) and conventional management (CM) tea plantations in Pu'er City, a major tea-producing area in China, and analyzed their species diversity, structural composition, and co-occurrence networks using metagenomics technology.

Results: Compared with CM, the diversity index (Shannon) and evenness index (Heip) of soil fungi increased by 7.38% and 84.2% in OM tea plantations, respectively. The relative abundance of microorganisms related to the nitrogen cycle increased. Specifically, there was a significant increase in *Rhodobiales*, a 2-fold increase in *Nitrospirae*, and approximately 1.95 and 2.03 times increases in unclassified genera within *Betaproteobacteria* and *Deltaproteobacteria*, respectively. The relative abundance of plant residue degradation species, *Gemmatimonadetes, Ascomycota, and Basidiomycota*, increased by 2.8, 1, and 1.4 times, respectively. The OM was conducive to the establishment of collaborative relationships among bacterial species and increased the diversity and complexity of species relationships in fungal communities. The network stability of soil ecosystems was promoted. The organic tea plantations' keystone taxa contained mycorrhizal fungi (*Pezoloma_ericae, Rhizophagus_irregularis, Rhizophagus_clarus*), as well as species involved in soil nitrogen metabolism (*Acidobacteria_bacterium, Acidobacteriia_bacterium_AA117, Sphingomonas_sp._URHD0007, Enhydrobacter_aerosaccus*), pathogen (*Erysiphe_pulchra*), and parasites (*Paramycosporidium saccamoeba*). The partial least squares method (PLS-SEM) indicated that OM affected N-NH_4^+ negatively, increasing the abundance of fungi, and thereby positively affecting the Shannon index.

Conclusion: In brief, reasonable organic management can improve the diversity of soil microorganisms, increase the relative abundance of beneficial bacteria in tea plantation soil, and promote the stability of the soil microbial ecological network.

KEYWORDS

tea, organic planting, bacterial community, fungal community, co-occurrence networks

1. Introduction

As an important cash crop, tea is widely cultivated in developing countries. In 2020, the global tea plantation area reached 5.098 million ha. China is a big tea-producing country. The tea plantation area reached 3.165 million ha, accounting for 62.1% of the total global tea area in 2020. The total annual production was 2.986 million tons, accounting for nearly 50% of the total global tea production (ITC, 2022). The tea plantation ecosystem provides humans with not only tea but also by-products such as fruits, vegetables, medicinal materials, and poultry. It also maintains the Earth's life system by conserving water resources, improving soil, preventing water and soil loss, mitigating natural disasters, regulating climate, purifying the environment, breeding and preserving biodiversity, having social functions such as ornamental field value, and displaying traditional culture, tourism, and recreation (Xue et al., 2013). Therefore, the sustainability of tea plantation ecosystems is of great significance for the global ecological environment and human wellbeing.

Soil is the foundation of agricultural production. Without healthy and fertile soil, food security cannot be guaranteed. As an important part of the soil environment, soil microorganisms play the roles of decomposers, pathogens, and symbionts in the ecological environment and participate in the decomposition of organic matter, the activation of nutrients, the prevention and control of plant diseases, and the maintenance of soil biodiversity. It drives the biogeochemical cycle on Earth (Stott and Taylor, 2016; Essel et al., 2019; Ney et al., 2020). Microorganisms are small in size, abundant in quantity, and highly sensitive to environmental changes (Karimi et al., 2017; Wagg et al., 2019; Bei et al., 2021; Wang C. et al., 2021; Wang et al., 2022). Microorganisms can serve as biological indicators for evaluating soil quality (Karimi et al., 2017).

Tea trees are perennial crops. The soil acidification caused by the accumulation of leaf residue and rhizosphere exudates has a severe impact on the growth of tea trees (Rothenberg et al., 2022). Previous studies have shown that organic management can prevent soil acidification (Yan et al., 2020). Additionally, organic agriculture based on the principles of health, ecology, care, and fairness has gradually become a highly recommended agricultural production mode by protecting the health and welfare of humans and future generations (FiBL and IFOAM, 2022). Therefore, organic management can provide the possibility for the sustainable development of tea plantation ecosystems.

The characteristics of the soil microbial community, as a sensitive indicator of soil quality, have been extensively studied by researchers. However, these research conclusions were inconsistent. For example, the abundance of bacteria and fungi under organic management was higher than that under conventional management (Kundel et al., 2020), and beneficial microorganisms increased (Suyal et al., 2021). Many studies have shown that, compared with conventional management, the microbial diversity of organic management has not changed or decreased (Karanja et al., 2020). The difference in the microbial community and abundance between organic and conventional farming systems changes with the change in land use (arable land, orchards, and grassland), climate, and other factors (Lori et al., 2017), and there is no unified conclusion yet. Therefore, it is necessary to study the impact of organic management on the microbial community of farmland based on specific climates, crop types, and cultivated land types and to elucidate soil microecological status under organic management.

To date, many studies have analyzed the soil microecology of organic tea plantations from the perspective of soil microbial communities, but relatively little research has been conducted in Pu'er City, the world's tea source. Therefore, the investigation of soil microorganisms in organic tea plantations in this region has reference value for the study of soil microecology in global organic tea plantations and also provides a theoretical basis for the improvement of local organic management systems and tea plantation management systems.

Bulk soil samples from organic and conventional tea plantations in Pu'er City were collected to continue exploring soil microecology changes. The soil bacterial and fungal alpha diversity, composition structure, and co-occurrence network of tea plantations under organic and conventional management were studied through metagenomics. Combining previous studies and tea plantation conditions, we hypothesized that (1) organic management will change the microbial community structure and increase the beneficial microorganisms in the tea plantation soil and (2) organic management will improve the complexity of the microbial symbiosis network and help to build a stable micro-ecological network. The research objectives are (1) to study the impact of organic management on the diversity and community structure of bacteria and fungi in tea plantation soil; (2) to reveal soil biomarkers that distinguished between organic and conventional management, microbial symbiotic patterns, and keystones taxa that exerted considerable influence on microbiome structure and function, and (3) to reveal the relationship between organic management and soil environmental factors, soil microbial species, and diversity index. This study comprehensively analyzes soil microecology from the perspectives of microbial community structure, network analysis, biomarkers, and keystone taxa and provides a theoretical reference for improving the farmland management system of organic tea plantations in Pu'er City, the world's tea source, and similar climate regions.

2. Materials and methods

2.1. Study area

The study area is located in Pu'er City, Yunnan Province, China. Yunnan Province is a typical region for tea production. The tea plantation area of Yunnan Province accounts for more than 15% of the Chinese tea production area. By 2021, the certified area of organic tea plantations and the certified number of organic products in Yunnan Province were 70,500 ha and 1,406, respectively. The certified area of organic tea plantations and the certified number of organic products in Yunnan Province were the

Abbreviations: OM, organic management; CM, conventional management; SWC, soil water content; SOC, soil organic carbon; N-NH_4^+, ammonia nitrogen content; N-NO_3^-, nitrate nitrogen content; AP, available phosphorus content; AK, available potassium content; TN, total nitrogen content; TP, total phosphorus content.

TABLE 1 Introduction of treatments.

Treatment name	Longitude and latitude	Management type	Planting years	OM years
OM-11	N22°44′, E100°53′	OM	11 years	11 years
CM-11		CM		
OM-16	N22°48′, E100°55′	OM	16 years	14 years
CM-16		CM		
OM-24	N22°45′, E100°52′	OM	24 years	14 years
CM-24		CM		

OM, organic management; CM, conventional management.

first in China for many consecutive years (Fu et al., 2022). Pu'er is a subtropical region known as the World Tea Source.

The information on the three sampling sites in this study is shown in Table 1. The average altitude of the three sampling sites is 1,326 m. The farmland type is a field with a ladder section (terrace). The soil was classified as *Laterite* based on the United States Department of Agriculture's (USDA) textural classification system. The local area has a low-latitude plateau subtropical monsoon climate, with an annual average temperature of 17.8°C and an annual average precipitation of 1,500–1,600 mm. The frost-free period is 325 days, and the annual sunshine hours are 2133.6 h. Before the implementation of organic management (OM), the local soil had the following physical and chemical properties: a pH of 5.13, organic matter content of 24.2 g kg^{-1}, total nitrogen of 1.07 g kg^{-1}, alkali-hydro nitrogen of 117 mg kg^{-1}, available phosphorus of 14.6 mg kg^{-1}, available potassium of 64 mg kg^{-1}, and slowly available potassium of 321.5mg kg^{-1}.

2.2. Field management and soil sampling

The three tea plantations selected in the study have the same agronomic measures and are relatively close to each other (within 10 km). The distance between the OM and CM in each plantation is ∼1 km. In this way, sampling sites were kept under the same climate and soil type, and the differences stemmed from agronomic measures.

The variety of tea trees was *Xueya 100*. The tea plantation was cultivated artificially, and the height of the tea tree was 60–70 cm. The three sampling sites both contained organic management (OM) and conventional management (CM) models (six treatments in total). The management method of OM or CM was consistent between all sampling sites. The organic management methods were as follows: the base fertilization period was from November to December every year, and topdressing was in May. Base fertilizer was 9,000 kg ha^{-1}, and topdressing was 3,000 kg ha^{-1}. After fertilization, 20–30-cm deep ditches were dug and covered with soil. Organic fertilizer had passed the national organic management standard certification. The organic fertilizer contained 42% organic matter, with a total nutrient content of 6% (N 2.1%, P_2O_5 2.71%, and K_2O 1.54%) and a pH value of 7.6. The tea plantations used physical methods such as yellow boards and pest control lamps to control pests. The conventional management treatment adopted the production habits of local farmers: the base fertilizer (N-P-K: 25-6-9) was applied in December, and the topdressing (urea) was applied in the middle of May. The base fertilizer was 1,200 kg ha^{-1}, and the top dressing was 600 kg ha^{-1}. Glyphosate was used for weeding, and acetamiprid was used for pest extermination.

Soil samples were collected in July 2022, and the period was the peak period for tea tree growth. The selected sampling point should avoid the shade of other trees and the side of the road and be close to the middle of the tea plantation to avoid marginal interference. In each treatment, three plots (10 m × 10 m) were selected at the same altitude. The distance between plots should be at least 10 m or more. Five sampling points were set as "plum blossoms" in each plot. The position for soil sampling was selected 10 cm outside the fertilization ditch, and then plant residues were removed on the surface. Bulk soil was collected using a drill with a diameter of 3 cm at a depth of 0–20 cm. On each plot, five soil samples were mixed and homogenized through a 2-mm sieve. In this way, three samples were obtained from every treatment, and a total of 18 samples were obtained from the six tea plantations. Afterward, the soil sample was divided into two parts. One part was stored in a refrigerator at −80°C for the sequencing of microbial metagenomics, and the other part was taken back to the laboratory to measure soil water content (SWC) and for physicochemical analyses.

2.3. Measurement index and method

2.3.1. Physicochemical analyses

The SWC was determined using the drying method: weigh 10 g of fresh soil, dry it at 105°C in the aluminum box weighed in advance, and calculate the soil moisture content (Bao, 2000). The organic carbon (SOC) content was determined via a multi-N/C 3100 TOC/TC analyzer (Analytik Jena, Germany). The total nitrogen (TN) content was determined using the Kjeldahl method (Kirk, 1950), and nitrate nitrogen content (N-NO_3^-) and ammonia nitrogen content (N-NH_4^+) were determined by Auto Analyzer 3 (Seal company). The content of available phosphorus (AP), total phosphorus (TP), available potassium (AK), and soil pH were measured through standard soil testing procedures (Bao, 2000).

2.3.2. DNA extraction, construction of a PE library, and genome sequencing

According to the E.Z.N.A® Soil DNA Kit (Omega Bio-tek, USA) instructions, soil genomic DNA was extracted. The quality of the DNA extraction was assessed using 1% agarose gel electrophoresis. The concentration and purity of DNA were determined using a micro fluorometer (TBS 380) and a nucleic acid protein meter (NanoDrop ND 2000). DNA fragments (~400 bp in length) were fragmented using an ultrasonic instrument (Covaris M220). The sequencing library was constructed using the NEXTFLEX^{TM} Rapid DNA-Seq Kit (BioScientific USA). Paired-end (PE) sequencing was performed on the Illumina NovaSeq platform (Shanghai Majorbio Technology Co., Ltd.).

2.3.3. Analysis of metagenomics sequencing data

Metagenomics sequencing data used fastp (Version 0.20.0) (Chen et al., 2018) to control the quality of the sequence. Megahit (Version 1.1.2) software (Li et al., 2015) was used to conduct the multiple mixed splicing of the optimized sequence, and more than 300 bp overlap groups (contigs) were screened as the final assembly result. Then, Prodigal (Hyatt et al., 2010) was used to predict the open reading frame (ORF), and genes with nucleic acid lengths of more than 100 bp were screened and translated into amino acid sequences. CD-HIT (Version 4.6.1) software (Fu et al., 2012) was used to cluster the predicted gene sequence. The cluster similarity and coverage of the gene sequence were 90%. The longest gene was taken as the representative sequence of each category to construct a non-redundant gene set. SOAPaligner (Version 2.21) software (Li et al., 2009) was used to compare high-quality reads with non-redundant gene sets and then calculate the gene abundance information with a similarity of over 95% (Lawson et al., 2017).

2.3.4. Statistical analysis and data visualization

Diamond (Version 0.8.35) software (Buchfink et al., 2015, 2021) was used to compare the amino acid sequence of the non-redundant gene set with the NR database. The species annotation results were obtained through the corresponding taxonomic information database, and the species abundance table was constructed at each taxonomic level. According to the results of taxonomic analysis, the structural composition of species or genes in different groups (or samples) would be understood. The relative abundance of bacteria (or fungi) at different classification levels is the ratio of the abundance of each bacteria (or fungi) to the total abundance of bacteria (or fungi).

Sample and microbial species relationship diagrams were visualized using Circos-0.67-7 (http://circos.ca/). The alpha diversity indices (Shannon, Chao, and Heip) based on read numbers at the species level were calculated in QIIME2 (Caporaso et al., 2010). The PCoA analysis based on the read number at the species level used the Binary Jaccard distance algorithm in the R "vegan" package and visualized the results using the "ggplot2" package. The intergroup differences in microorganisms were determined via ANOSIM analysis. ANOSIM analysis was implemented through the R "vegan" package.

This study discovered multi-level biomarkers through linear discriminant analysis effect size (LEfSe). Specifically, the non-parametric Kruskal–Wallis sum-rank test was used to test the species abundance differences between groups, and species with significant differences were obtained. Then, LDA linear discriminant analysis (LDA > 3.0) was used to estimate the degree of impact of these different species on the intergroup differences. The analysis process was performed using LEfSe software (http://huttenhower.sph.harvard.edu/lefse/) (Segata et al., 2011). The correlation coefficient between environmental factors and the alpha diversity index (Spearman) was calculated using the R "psych" package, and the results were calculated using the "ggplot2" package.

The microorganism co-occurrence network was constructed using the Python "Networkx" package. The top 50 species (species level) with abundances were selected in this analysis. Spearman's rank correlation coefficient was adopted in this study. A *p*-value of <0.05 was considered a valid co-occurrence. The correlation threshold was set to 0.5. The visualization of networks and calculation of topology parameters were completed through Gephi 0.9.7. Topology parameters included degree, network diameter, density, modularity, number of communities, average clustering coefficient, average path length, and positive and negative links (Bastian et al., 2009). In this study, the top five species with the highest clustering coefficients were identified as keystone taxa.

Analysis of variance (ANOVA) and least significant difference (LSD) multiple comparison tests ($p < 0.05$) were used for one-way or multivariate analysis of variance. The statistical analysis of the data was conducted in Excel 2016 and SPSS 26 (SPSS Inc., Chicago, USA). The structural equation model was constructed using the partial least squares method. The model construction platform was SmartPLS 3.

3. Results

3.1. Soil microbial diversity

The community coverage showed that the current sequencing depth covered all the bacteria and fungi in the soil, which can comprehensively reflect the situation of soil bacteria and fungi communities (Table 2). Compared with the CM, the bacterial Chao (richness index) significantly decreased by 5.5% in the OM ($p < 0.01$), while Shannon (diversity index) and Heip (evenness index) had no significant change. The fungal community Chao index reduced (29%) significantly in OM tea plantations, but the Heip and Shannon indices increased (84 and 7%, respectively). The fungi Alpha diversity indexes significantly differed between different management methods ($p < 0.01$).

The physical and chemical properties of OM and CM plantation soils are shown in Table 3. The correlation analysis between the alpha diversity index and soil environmental factors showed that most of the environmental factors examined in the study were significantly associated with the alpha diversity index ($p < 0.05$). SOC was significantly negatively correlated with Shannon-B and Chao (Figure 1). There was a significant positive correlation between AP and Shannon, Heip-B, and Chao-F. AK was significantly positively correlated with Shannon-F and Heip-F indices. N-NH_4^+ influenced Shannon-F and Chao-F positively. TP and N-NO_3^- showed a significant positive correlation with the diversity index other than Heip-F. TN influenced Chao negatively. pH was negatively correlated with Chao-F and positively correlated with Heip-F.

TABLE 2 Alpha diversity of bacteria and fungi in tea plantation soil.

Sample	Bacteria				Fungi			
	Shannon	Chao	Heip	Coverage	Shannon	Chao	Heip	Coverage
OM-24	5.63 ± 0.16b	20832 ± 303c	0.013 ± 0.0020b	1	4.14 ± 0.27b	386 ± 2.12d	0.17 ± 0.03a	1
CM-24	5.74 ± 0.41ab	21809 ± 1712bc	0.015 ± 0.0047ab	1	3.81 ± 0.08c	595 ± 9.90c	0.09 ± 0.01b	1
OM-16	6.20 ± 0.08a	23848 ± 446a	0.021 ± 0.0013a	1	4.66 ± 0.09a	662 ± 72.8b	0.15 ± 0.01a	1
CM-16	6.21 ± 0.08a	23868 ± 165a	0.021 ± 0.0017a	1	4.49 ± 0.17a	778 ± 11.3a	0.11 ± 0.02b	1
OM-11	5.44 ± 0.36b	20731 ± 764c	0.012 ± 0.0049b	1	3.61 ± 0.18c	381 ± 29.0d	0.10 ± 0.00b	1
CM-11	5.79 ± 0.16ab	23537 ± 834ab	0.014 ± 0.0020b	1	3.30 ± 0.06d	601 ± 17.7c	0.05 ± 0.00c	1
Locale (L)	**	**	**	—	**	**	**	—
Management (M)	NS	**	NS	—	**	**	**	—
L*M	NS	**	NS	—	NS	**	NS	—

OM, organic management; CM, conventional management. ** represents $p < 0.01$. NS represents no significant difference. The same below. Values are means of three replicates ± SD followed by different letters that differ significantly (Turkey's test, $p < 0.05$).

TABLE 3 Soil physical and chemical properties of OM and CM.

Treatment	SWC	$N\text{-}NH_4^+$ (mg kg^{-1})	$N\text{-}NO_3^-$ (mg kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	SOC (g kg^{-1})	pH
OM-11	0.19 ± 0.02	1.97 ± 0.23	3.66 ± 0.37	57.4 ± 2.91	342 ± 11.2	2.55 ± 0.11	0.38 ± 0.04	59.11 ± 0.92	5.46 ± 0.03
CM-11	0.23 ± 0.00	0.71 ± 0.20	5.68 ± 1.39	17.5 ± 2.24	247 ± 8.06	1.66 ± 0.23	0.21 ± 0.01	30.55 ± 1.57	4.51 ± 0.04
ANOVA	*	**	*	**	**	**	**	**	**
OM-16	0.25 ± 0.01	1.83 ± 0.01	9.44 ± 0.06	119 ± 19.9	640 ± 8.69	2.01 ± 0.14	0.43 ± 0.04	33.49 ± 1.95	5.55 ± 0.22
CM-16	0.23 ± 0.01	8.9 ± 1.26	11.2 ± 1.61	106 ± 27.8	232 ± 9.93	1.53 ± 0.08	0.35 ± 0.03	27.18 ± 1.23	4.31 ± 0.07
ANOVA	NS	**	NS	NS	**	**	NS	**	**
OM-24	0.24 ± 0.00	3.19 ± 0.08	3.10 ± 0.37	41.6 ± 10.0	460 ± 13.9	2.07 ± 0.02	0.32 ± 0.05	36.93 ± 1.34	5.98 ± 0.10
CM-24	0.25 ± 0.01	4.32 ± 0.33	10.32 ± 0.78	34.5 ± 3.92	315 ± 9.46	2.08 ± 0.39	0.31 ± 0.01	28.39 ± 1.90	4.19 ± 0.01
ANOVA	NS	**	**	NS	**	NS	NS	**	**

SWC, soil water content; $N\text{-}NH_4^+$, ammonia nitrogen content; $N\text{-}NO_3^-$, nitrate nitrogen content; AP, available phosphorus content; AK, available potassium content; TN, total nitrogen content; TP, total phosphorus content. Values are means of three replicates ± SD. ** represents $p < 0.01$. * represents $0.01 \le p < 0.05$. NS represents no significant difference.

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The study was based on PCoA analysis (Binary-Jaccard) at the species level to compare the differences in the structure of bacterial or fungal communities under two management methods (Figure 2). The first and second coordinate axes represented the contribution values of the first and second principal components to the treatment difference. In the 24-year plantation, the first and second principal components accounted for 58.53 and 52.96% of the variance in bacterial and fungal communities, respectively. In the 16-year plantation, these figures were 63.91 and 70.79%, while in the 11-year plantation, they were 60.69 and 62.01%. Bacterial and fungal communities were clearly separated between organic and conventional management.

3.2. Bacterial community structure

In this study, the non-redundant gene set annotated by the NR database was used to compare the relative abundance of bacteria species in tea plantation soil under different management modes. An analysis of variance between OM and CM was performed on bacterial phyla with a relative abundance of >1%. Among the dominant bacterial phyla (relative abundance ≥ 10%) (Supplementary Figure S1A), compared with CM-16, the relative abundance of *Proteobacteria* in OM-16 significantly increased by 24.3% ($p < 0.05$) (Figure 3A). In the non-dominant bacterial phylum (relative abundance < 10%), *Gemmanimonadetes* showed 1.1 times ($p < 0.05$), 5.89 times ($p < 0.001$), and 1.37 times ($p < 0.01$) higher levels in OM-11, OM-16, and OM-24 compared to the CM treatment, respectively. *Planctomycotes* significantly decreased by 29% ($p < 0.001$) and 48% ($p < 0.01$) under OM-16 and OM-11, respectively. *Verrucomimicrobia* significantly increased by 2.26 times under OM-16 ($p < 0.001$). *Bacteroidetes* significantly increased by 56% ($p < 0.05$) in OM-16 compared to CM-16 but decreased by 44% ($p < 0.001$) in OM-11 compared to CM-11. OM could improve the relative abundance of the *Nitrospirae* community, especially when the OM-24 treatment was three times higher than that of CM-24.

The dominant bacterial order (relative abundance ≥ 10%) included *Rhizobiale*s and the unclassified bacterial orders of *Acidobacteria, Chloroflexi,* and *Actinobacteria* (Supplementary Figure S1C). An analysis of variance between OM and CM of bacteria orders with relative abundance in the top 15 was conducted between OM and CM. The relative abundance of unclassified bacterial orders of *Acidobacteria* was significantly higher in OM-24 than in CM-24 (43%, $p < 0.05$) (Figure 3B). The relative abundance of the unclassified bacterial orders of *Chloroflexi, Actinobacteria, and Rhizobiales* in OM-11 was significantly higher than that in CM-11. The growth rates were respectively 69% ($p < 0.05$), 30% ($p < 0.05$), and 49% ($p < 0.05$). The non-dominant bacteria orders (relative abundance < 10%) in the three tea plantation locations were the same, mainly including *Corynebacteriales, Rhodospirillales, Acidobacteriales, Ktedonobacteria,* and so on. The *Acidobacteriale* levels of the OM were significantly lower than those of the CM. Compared with CM-24 and CM-16, OM-24 and OM-16's *Corynebacteriales* and *Rhodospirillales* were significantly lower.

The dominant bacteria genus (relative abundance ≥ 10%) in the three sampling sites were unclassified bacteria genera under *Acidobacterium, Actinobacia*, and *Chloroflex* (Supplementary Figure S1E). An analysis of the variance of bacteria genera with relative abundance in the top 15 between OM and CM was conducted. The dominant bacterial genus had no significant difference between the OM and CM (Figure 3C). The non-dominant bacteria genus (relative abundance < 10%) included unclassified bacteria genera of *Acidobacterium, Chloroflexi, Actinobacia, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Streptomyces*, *Bradyrhizobium*, *Mycobacterium,* and so on (Supplementary Figure S1E). Organic management changed the relative abundance of non-dominant bacteria in tea plantations. As shown in Figure 3C, the relative abundance of *unclassified_c__Betaproteobacteria* significantly increased by 1.3 times ($p < 0.01$), 3.3 times ($p < 0.05$), and 1.2 times ($p < 0.01$) in OM-11, OM-16, and OM-24, respectively. The *unclassified_c__Deltaproteobacteria* in OM-16 and OM-24 also showed an increasing trend, with 2.6 times ($p < 0.05$) and 1.5 times ($p < 0.01$) compared to CM, respectively. Compared with the CM, *Mycobacterium* significantly decreased by 70 and 63% ($p < 0.05$) in OM-16 and OM-24, respectively. *Streptomyces* significantly decreased by 61% ($p < 0.01$) in OM-24 (Figure 3C).

3.3. Fungal community structure

At the dominant phylum level (relative abundance ≥ 10%) (Supplementary Figure S1B), analysis of variance between OM and CM was performed on fungal phyla with a relative abundance of >1%. In the OM, the relative abundance of *Mucoromycota* significantly decreased by 56% ($p < 0.05$) and that of *Ascomycota* significantly increased by a fold ($p < 0.01$) (Figure 3D). The relative abundance of *Basidiomycota* in OM-24 and OM-16 significantly increased by 1.48 and 1.23 ($p < 0.001$), respectively, compared to the CM. Among non-dominant fungal phyla (relative abundance < 10%), *Cryptomycota, Chytidiomycota,* and *Zoopagomycota* showed a certain degree of decrease in OM. At the fungi orders, *Glomerales, Diversisporales,* and *Tremella* (relative abundance ≥ 10%) were the dominant orders (Supplementary Figure S1D). An analysis of variance between OM and CM of fungi orders with relative abundance in the top 15 was conducted. Compared to CM, the relative abundance of *Glomerales* and *Diversesporales* in OM decreased, while the relative abundance of *Tremella* increased (Figure 3E). Non-dominant orders (relative abundance < 10%) included *Chaetothyriales, Helotiales, Eurotiales, unclassified_p__Cryptomycota, Mucorales, Hypocreales, Saccharomycetales, Pleosporales,* and so on. The relative abundance of *Chaetothyriales* in OM-16 significantly increased by 93% ($p < 0.05$), while the relative abundance of *Helotiales* doubled ($p < 0.05$). The relative abundance of *Mucorales, Hypocreales, Saccharomycetales, and Pleosporales* significantly increased in OM-11 and OM-24 ($p < 0.05$).

At the genus level of fungi, the dominant genera (relative abundance ≥ 10%) include *Rhizophagus, Saitozyma,* and *Diversispora* (Supplementary Figure S1F). An analysis of variance between OM and CM of fungi genera with relative abundance in the top 15 was conducted. Compared with CM, the relative abundance of *Rhizophagus* in OM-11 and OM-24 decreased

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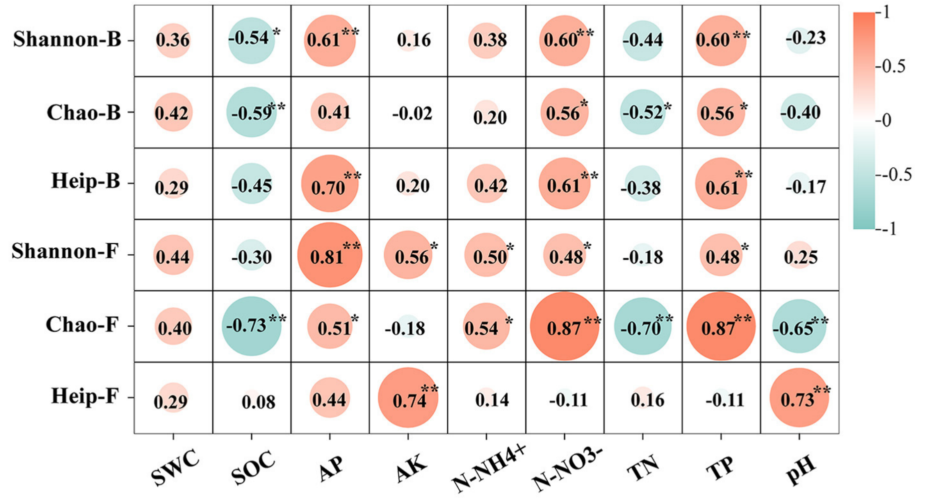

FIGURE 1
Correlation of environmental factors and bacteria or fungi Alpha diversity index. Shannon-B represents the bacteria's Shannon index. Chao-B represents the bacteria's Chao index. Heip-B represents bacteria's Heip index. Shannon-F represents the Shannon index of fungi. Chao-F represents the Chao index of fungi. Heip-F represents the Heip index of fungi. **Represents $0.001 \leq p < 0.01$. *Represents $0.01 \leq p < 0.05$. No * representes no relevance. The same below. The values are the correlation coefficient r^2 between the alpha diversity index and environmental factors.

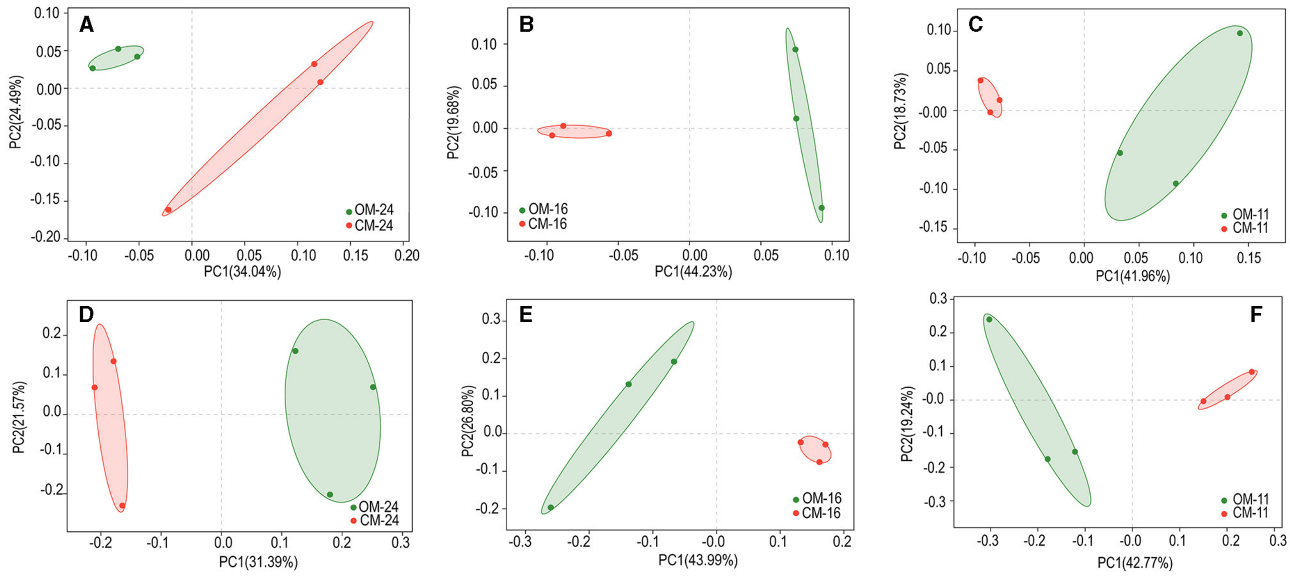

FIGURE 2
Bacterial **(A–C)** and fungal **(D–F)** Beta diversity of tea plantations soil in different management modes. OM, organic management; CM, conventional management.

significantly by 70% ($p < 0.01$) and 62% ($p < 0.05$) (Figure 3F). Diversispora of OM-11, OM-16, OM-24 significantly reduced by 86% ($p < 0.001$), 65% and 66% ($p < 0.01$), respectively. The relative abundance of *Saitozyma* significantly increased by 3-fold in OM-24 ($p < 0.01$). The non-dominant genera (relative abundance < 10%) mainly included *Gigaspora, Glomus, Exophiala, Aspergillus, Paramicrosporidium, Mucor, Lipomyces,* and so on. The relative abundance of *Gigaspora, Glomus,* and *Paramicrosporium* significantly decreased ($p < 0.05$) or did not show significant changes in OM. However, the relative abundance of *Exophiala, Aspergillus, Mucor, and Lipomyces* significantly increased ($p < 0.05$) or remained unchanged in OM.

3.4. The biomarkers of the organic tea plantation

In this study, LEfSe was used to further clarify the soil biomarker groups under different management modes in order to

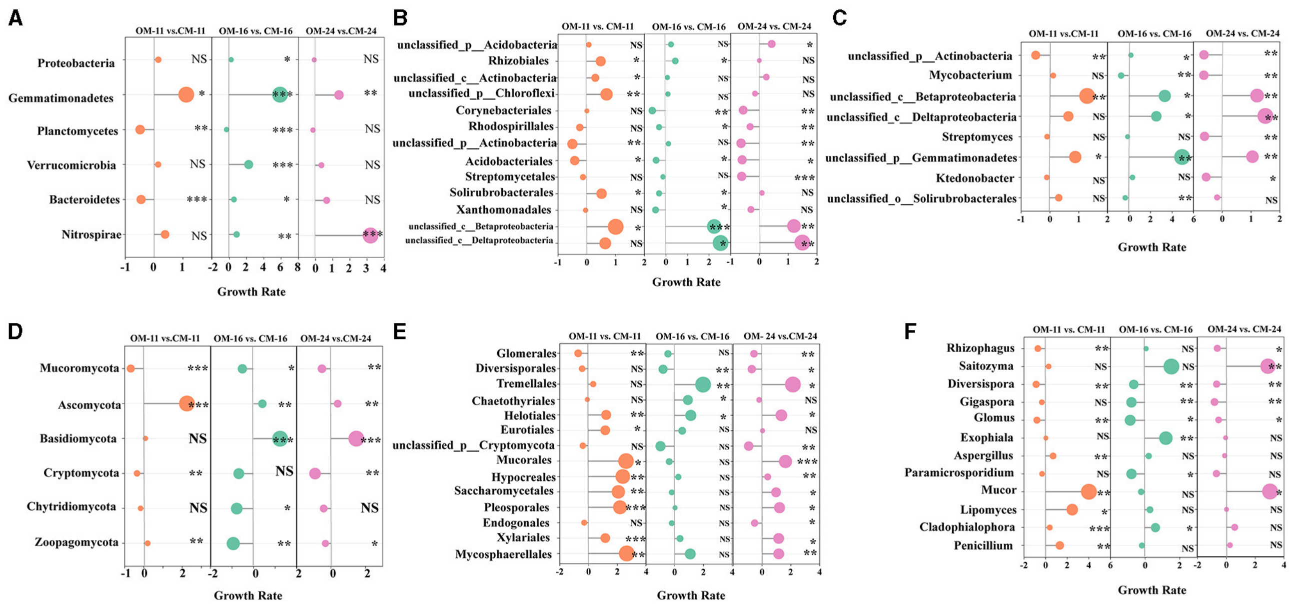

FIGURE 3
The growth rate of species relative abundance of OM relative to CM. **(A)** Bacterial phylum level **(B)** Bacterial order level **(C)** Bacterial genus level **(D)** Fungal phylum level **(E)** Fungal order level **(F)** Fungal genus level. The size of points represents the numerical value. NS represents no significant difference. ***Represents $p < 0.001$. **Represents $0.001 \leq p < 0.01$. *Represents $0.01 \leq p < 0.05$. OM, organic management; CM, conventional management.

evaluate the species causing the difference between OM and CM by setting a linear discriminant analysis (LDA) value. When the LDA score was >3.0, there were significant differences between OM and CM in 12 bacterial species and 22 fungal species ($p < 0.05$) (Figure 4). In this study, these different species were considered the biomarker group. "OM" contained eight bacteria and 12 fungal biomarkers, while CM contained four bacteria and 10 fungal biomarkers.

Among the bacteria biomarker group in OM, *Betaproteobacteria_ bacterium* was the species with the highest LDA score (LDA score = 3.655, *Proteobacteria*) (Figure 4A). More than 50% of the members of the bacteria biomarker group belonged to the *Proteobacteria*. Other members came from the *Germatimonadetes, Verrucomicrobia*, and *Actinobacteria*. The four members of the biomarker group of CM were derived from *Actinobacteria, Proteobacteria, Planctomycotes,* and *Acidobacteria*. *Mycobacterium_ rhizamasilianse* (*Actinobacteria*) had the highest LDA score (3.159).

Saitozyma_ Podzolica had the highest LDA score among the soil fungi biomarker group in OM (LDA score = 4.608, *Basidiomycota*). Other biomarkers were derived from *Ascomycota and Mucormycota* (Figure 4B). Most (67%) of the biomarkers belonged to *Ascomycota*. The highest LDA score for the biomarker group of soil fungi in CM was *Rhizophagus_ Iregularis* (LDA score = 4.66, *Mucormycota*), and the rest of the biomarkers were derived from *Cryptomycota* and *Basidiomycota*. A total of 70% of the biomarkers of soil fungi in CM belonged to *Mucormycota*.

3.5. Taxonomic co-occurrence networks

To explore the health condition of soil bacterial and fungal ecosystems under the OM and CM, the study used taxonomic co-occurrence networks to analyze the symbiotic patterns of bacteria and fungi in the top 50 soil species abundances (Figure 5).

From the perspective of bacterial network topology parameters, compared to the CM, OM reduced the total number of connections (edges) and the connectance (degree) of bacteria's co-occurrence network (Table 4). The average degree of bacterial co-occurrence networks in the CM was 17.4, which was 1.3 times higher than the OM, indicating that the bacterial co-occurrence networks in the CM were more complex than the CM. The diameter and density of the soil bacterial co-occurrence network were higher in CM than in OM. Although the number of nodes in the bacterial network was the same between the OM and CM, there were more diverse connections between nodes in the CM.

From the perspective of fungal network topology parameters, compared to the CM, the OM increased the total number of connections (edges) and the connectance (degree) (Table 4). The average degree of fungal co-occurrence network in the OM was 11.59, which was 1.5 times higher than that in the CM. It indicated that the fungal networks were more complex in the OM. The diameter of the soil fungal co-occurrence network under the CM was higher than that under the OM, but the number of edges under the OM was higher than that under the CM, which led to a higher density of the OM's fungal network structure.

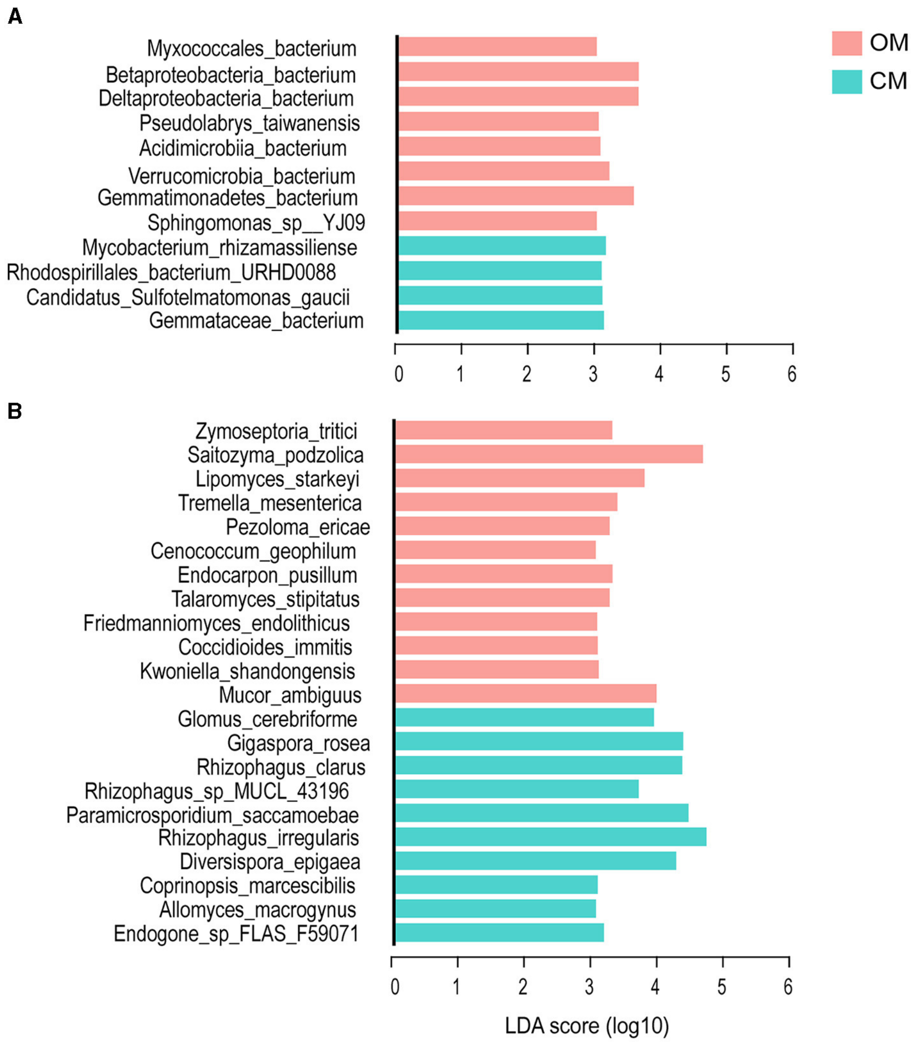

FIGURE 4
The biomarkers of the tea plantation soil under OM and CM. **(A)** Bacteria and **(B)** fungi. OM, organic management; CM, conventional management.

The modularity of the bacterial network in OM was higher than that in CM, while the opposite was true for fungi. The average clustering coefficient of the soil bacterial and fungal co-occurrence network nodes in CM was higher than that in OM, indicating that the species in the co-occurrence network of CM had closer connections with their neighboring species compared to those in OM (Table 4). The edge number of the bacterial co-occurrence network in CM was higher than that in OM. However, the proportion of positive correlation between bacterial species in OM was 11% higher than that in CM, indicating that OM was more conducive to the establishment of a positive correlation between bacterial species. The edge number of the fungal co-occurrence network in OM was higher than that in CM, and the relationships between fungal species in CM were all positive. There was also a nearly 4% negative correlation between OM species. It indicated that OM led to a certain degree of negative correlation between fungal community species.

The top five species with multiple correlations with other microorganisms were classified as keystone taxa based on the clustering coefficient. The contribution of these keystone taxa to the community network structure was due to their influence, not abundance (Figure 5, Supplementary Table S1).

3.6. Correlation between management mode, environmental factors, and soil microorganisms

The relationship between management mode, environmental factors, and soil microorganisms was further analyzed by constructing a PLS-SEM model (Figure 6). First, the autocorrelative environmental factors were removed (VIF > 10), which preserved the less interacting SWC, AK, AP, $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, and TN. Then, the above environmental factors, phylum-level species with significant differences in relative abundance between OM and CM, and the Shannon index were included in PLS-SEM. The indicators with weights of <0.2 and VIF > 10 were removed

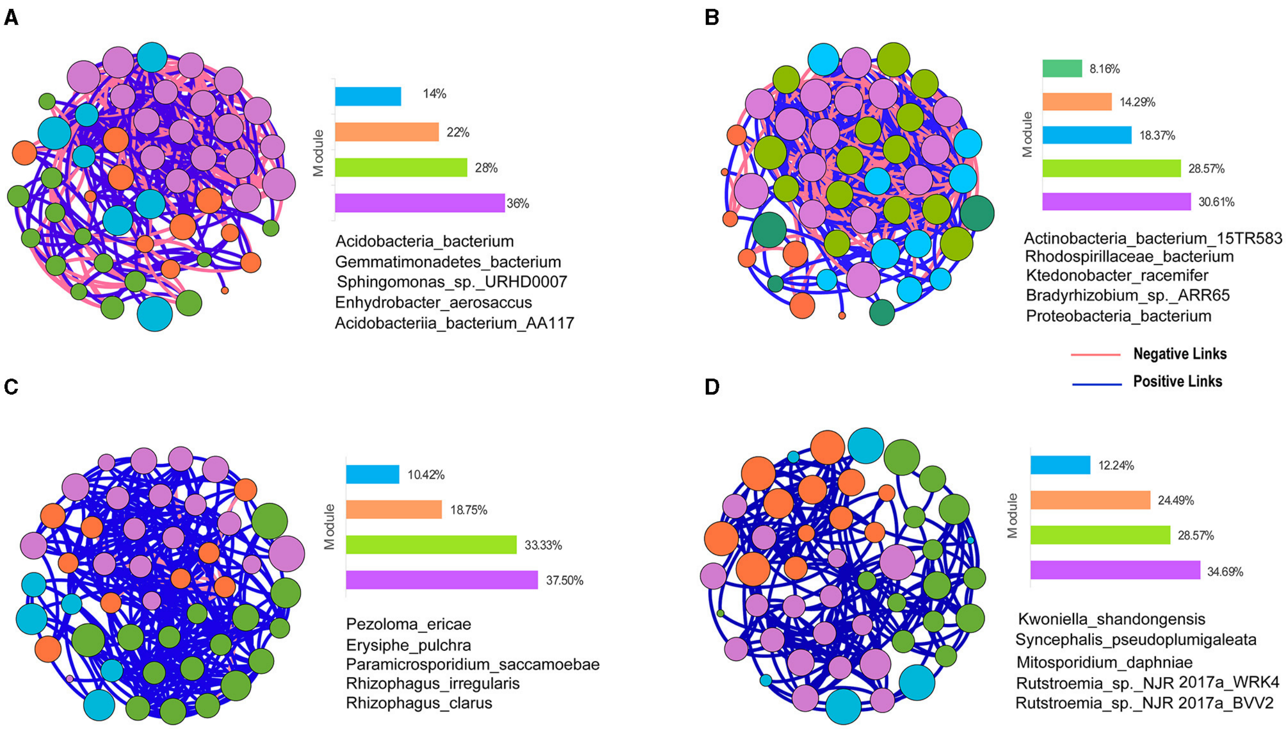

FIGURE 5
Taxonomic co-occurrence networks of bacterial and fungal species in the OM and CM. **(A)** Bacterial taxonomic co-occurrence network in the OM. **(B)** Bacterial taxonomic co-occurrence network in the CM. **(C)** Fungal taxonomic co-occurrence network in the OM. **(D)** Fungal taxonomic co-occurrence network in the CM. OM, organic management; CM, conventional management. Nodes in the figure represent species. The size of nodes represents the size of the clustering coefficient. Nodes of the same color form a module. The bar chart represents the proportion of each module in the whole symbiotic network. The red lines between nodes indicate a negative correlation between the two species, and the blue lines indicate a positive correlation. The species shown in the figure are keystone taxa in the co-occurrence network.

from the PLS-SEM model. SWC AP had no correlation with the management mode, so they were removed. The results of PLS-SEM showed that all latent variables had discriminant validity. The organic management had a positive correlation with AK and TN and a significant negative correlation with N-NO_3^- and N-NH_4^+. However, only N-NH_4^+ provided a significant impact on soil microorganisms, that is, N-NH_4^+ affected the relative abundance of *Chytidiomycota* and *Zoopagomycota* in the soil and then further positively affected the Shannon index of soil microorganisms.

4. Discussion

Microorganisms are omnipresent and abundant, and their genetic and metabolic diversities are the main participants in the biogeochemistry process (Stott and Taylor, 2016; Essel et al., 2019; Ney et al., 2020). Additionally, their body size is small, and they respond quickly to environmental changes. Based on these characteristics, microbes can be used as excellent biological indicators to characterize environmental disturbance and soil quality (Karimi et al., 2017). The present study used metagenomics sequencing technology to evaluate the microbial community composition and co-occurrence network of three tea plantations under organic and conventional management to understand the soil quality of tea plantations from the perspective of soil microorganisms. In this study, there were significant differences in soil microorganisms in tea plantations between organic and conventional management.

4.1. Organic management induced positive changes in microbial alpha diversity in tea plantation soil

The alpha diversity of microorganisms can quantify the diversity characteristic of microorganisms in the soil, which plays a key role in assessing soil quality and the sustainable development of farmland (Lemanceau et al., 2015; Delgado-Baquerizo et al., 2016). There was controversy in previous studies regarding alpha diversity. Previous studies have shown that organic management increased the diversity of soil microorganisms (Kundel et al., 2020; Suyal et al., 2021), remained unchanged (Sugiyama et al., 2010), or even decreased it (Bonanomi et al., 2016; Karanja et al., 2020). This study indicated that OM improved the Shannon index of fungi, while there was no significant change in bacteria. The richness (Chao) of bacteria and fungi was reduced in the OM compared to the CM (Table 2). The result was similar to most research (Bonanomi et al., 2016; Kundel et al., 2020; Suyal et al., 2021). However, some scholars believed that community composition was more important than diversity in soil ecological function due to functional equivalence or redundancy between microorganisms.

TABLE 4 Topological network parameters.

Features	OM		CM	
	Bacteria	Fungus	Bacteria	Fungus
Nodes	50	48	49	49
Edges	330	278	426	194
Degree	13.2	11.6	17.4	8.0
Network diameter	5	4	7	7
Density	0.269	0.246	0.362	0.165
Modularity	0.259	0.339	0.151	0.552
Number of communities	4	4	5	4
Average clustering coefficient	0.607	0.597	0.737	0.622
Average path length	2.08	2.067	2.182	2.737
Positive links	64.24%	96.04%	53.76%	100%
Negative links	35.76%	3.96%	46.24%	0.00%

OM, organic management; CM, conventional management.

Therefore, the diversity evaluation of microbial communities needs to be combined with community composition (Bonanomi et al., 2016).

The Heip index reflects the evenness of soil microbial communities. The higher the Heip index, the more uniform the distribution of microbial communities and the more stable soil microecology is. The higher evenness of microbial communities in organic management indirectly characterizes the high stability of soil microecology (Sugiyama et al., 2010). The stability of soil microecology is an important indicator of soil quality (Seybold et al., 1999). The present results were similar to them. The organic management improved the fungus evenness in the soil of tea plantations (Table 2). The lower evenness of soil microorganisms in CM may be due to the increased interference of soil microorganisms caused by the application of insecticides, fertilizers, and herbicides (Sugiyama et al., 2010).

4.2. Organic management increased the abundance of beneficial microorganisms in tea plantation soil

The composition of soil microbial communities and the role of species in soil are of great significance for soil quality evaluation (Sugiyama et al., 2010). *Gemmatimonadetes* can degrade soil pollutants and organic matter, producing alkaline phosphatase in the soil (Ding et al., 2016; Durrer et al., 2021). *Gemmatimonadetes* is a eutrophic bacteria that needs enough nutrients (Ghosh et al., 2016). Therefore, the increase in the relative abundance of *Gemmatimonadetes* was regarded as a sign of sufficient nutrients. In this research, *Gemmatimonadetes* in OM was significantly increased, which indirectly suggested that organic management might increase soil nutrient content in tea plantations (Figure 3). To verify this hypothesis, soil nutrients were tested and showed that organic management promoted an increase in soil total nitrogen (TN), organic carbon (SOC), and available potassium content (AK) (Table 3).

Proteobacteria was the dominant phylum in organic tea plantations (with a relative abundance of over 30%), with a higher relative abundance compared to conventional management (Figure 3; Supplementary Figure S1A). Biomarkers are often used in microbial research to identify species that cause significant differences between groups. These biomarkers are evaluated based on species abundance, using LEfSe as the method of analysis. In the study, more than half of the bacterial biomarkers originated from the *Proteobacteria* (Figure 4), indicating that the species in the *Proteobacteria* were key species that caused soil microecology differences between OM and CM. *Proteobacteria* mainly include *Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria,* and *Gammaproteobacteria* (Dedysh et al., 2004; Ghosh et al., 2016). *Pseudolabs_ Taiwanensis* was a bacterial biomarker belonging to *Rhizobiales (Alphaproteobacteria). Rhizobiales* have biological control and nitrogen fixation functions, often coexisting with leguminous plants, and can persist in soil (Bastida et al., 2016; Degrune et al., 2017).

In addition, in this study, the relative abundance of *Rhizobiales* in OM tea plantations was significantly increased compared to CM, possibly due to the leguminous weeds included in organic tea plantations that promote the symbiosis of nitrogen-fixing bacteria. *Rhodospirillales* belonging to *AlphaProteobacteria*, including *Azospirillum* and *Rhodospirillum*, can promote plant growth through nitrogen fixation and hormone synthesis (Souza et al., 2013). Our results suggested the relative abundance of *Rhodospirillales* in OM was significantly reduced (Figure 3). *Betaproteobacteria_Bacterium* and *Deltaproteobacteria_Bacterium* are bacterial biomarkers that belong to *Betaproteobacteria* and *Deltaproteobacteria,* respectively. Aerobic ammonia oxidation mediated by *Betaproteobacteria* is a rate-limiting step in the nitrification process related to plant nitrogen availability, nitrate leaching into groundwater, or N_2O emissions (Di and Cameron, 2016). *Deltaproteobacteria* promote nitrogen and organic matter cycling in soil (Zhao et al., 2019). In addition, this research also indicated that the relative abundance of unclassified genera in *Betaproteobacteria* and *Deltaproteobacteria* significantly increased in the OM compared to the CM (Figure 3). *Nitrospirae* is an aerobic bacterium with high nitrification activity. It is an important bacterium involved in the oxidation of nitrite in the soil nitrogen cycle (Daims et al., 2015; Durrer et al., 2021). Our result suggested that the relative abundance of *Nitrospirae* was significantly increased, especially in the OM-24, which increased by three times compared to conventional management (Figure 3). The result is that the longer the organic management period, the higher the nitrogen availability, which induces an increase in *Nitrospirae* (Wang et al., 2020).

In summary, most of the biomarkers in OM were related to soil nitrogen metabolism. It indicated that there may be significant differences in soil nitrogen metabolism between OM and CM and that nitrogen metabolism performs better in organic tea

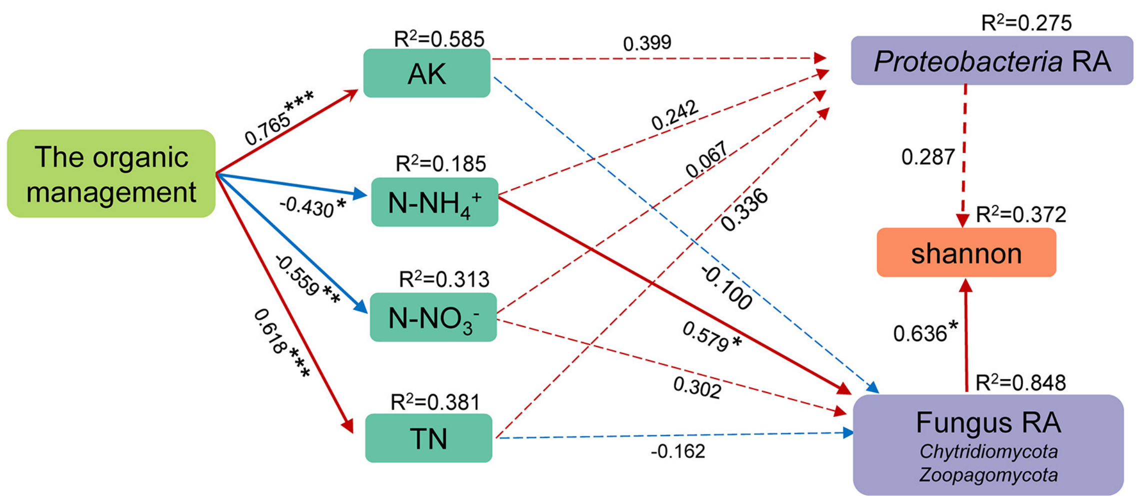

FIGURE 6
The structural equation model (SEM) for the management mode, soil environmental factors, the relative abundance of bacteria and fungi at the phylum level, and the Shannon index. The value above the SEM line represents the path coefficient. RA represents relative abundance. The red line represents the positive path coefficient, and the blue line represents the negative path coefficient. ***Represents $p < 0.001$. **Represents $0.001 \leq p < 0.01$. *Represents $0.01 \leq p < 0.05$. The solid line represents a significant correlation between two factors, while the dashed line represents a non-significant correlation.

plantations. The results of PLS-SEM can also precisely explain this phenomenon. OM reduced $N\text{-}NH_4^+$ in the soil, increasing the relative abundance of *Proteobacteria* (Figure 6).

Streptomyces can produce antibiotics, plant growth hormones, and extracellular enzymes, enhancing the plant's resistance to biotic and abiotic stresses (Ponmurugan and Saravanan, 2013; Zhu et al., 2019). Previous studies have shown that *Streptomyces* preparations can maintain a good level of yield and quality in tea plants infected with bird's eye spot or red rust disease (Gnanamangai and Ponmurugan, 2012; Ponmurugan and Saravanan, 2013). Currently, the relative abundance of *Streptomyces* in the OM-24 is lower than that in the CM-24 (Figure 3). It was due to the acidophily of *Streptomyces*, which was an important factor in its better biological control effect in tea plantations (Gnanamangai and Ponmurugan, 2012). Soil acidification is a universal problem in perennial tea plantations (Rothenberg et al., 2022). In our study, the OM alleviated the problem of soil acidification in conventional tea plantations (Table 3), which explained the significant decrease in the abundance of *Streptomyces* in organically managed tea plantations.

Ascomycota and *Basidiomycota* are mainly saprophytic nutrition types. They are related to the degradation of crop residues, and play an important role in soil carbon, nitrogen cycle, and other nutrient cycles (Bastida et al., 2016; Li et al., 2017; Zheng et al., 2018). In the present study, fungi biomarkers in the OM come from *Ascomycota*. The relative abundance of *Ascomycota* and *Basidiomycota* increased significantly in the OM ($p < 0.05$), which was similar to the previous studies (Ding et al., 2017). It was the large input of organic matter, such as weeds and crop residues, in organic tea plantations that accelerated the reproduction of *Ascomycota* and *Basidiomycota*.

Eurotiales, Mucorales, Hypocreales, Saccharomycetes, and *Tremelales* are free-living saprotrophic fungi (Vargas-Gastelum et al., 2015). The majority of species in the *Eurotiales* and *Hypoceras* can produce N_2O (Mothapo et al., 2015). In our study, *Hypoceras* and *Eurotiales* significantly increased in OM, compared to CM. Further research is needed on their contribution to N_2O emissions. *Rhizophagus* is one of the AMFs, consuming less carbon but helping host plants absorb more phosphorus (Stefani et al., 2020). Our results suggested that *Rhizophagus* was found as a dominant fungal genus in OM tea plantations, and its relative abundance was significantly lower than that in CM tea plantations. This result may be because less organic carbon in conventional soil management attracts more low-carbon-consuming species. In summary, organic management increased the soil fungi that degrade plant residues and turnover nutrients in tea plantations.

4.3. Organic management promoted the stability of soil microecology in tea plantations

Microorganisms do not exist in isolation in soil ecosystems. A microbial species often interacts with other species in multiple ways. These interactions can occur simultaneously and in space, forming a network of interactions (Karimi et al., 2017). Microbial symbiotic networks provide an overall perspective for ecosystems because they integrate the direct and indirect effects of environmental changes on species diversity, taxonomy composition, and relationships between species within communities. A growing number of studies have taken microbial symbiotic networks as indicators of soil quality (Berry and Widder, 2014; Wang et al., 2020; Guseva et al., 2022; Xue et al., 2022; Yang et al., 2023).

The co-occurrence network was constructed by calculating the correlation coefficients between species. When species were significantly correlated, two species were connected by a line. The edge number of bacterial co-occurrence networks was reduced in the CM compared to the OM, but the positive correlation ratio between bacterial species in the OM was 11% higher than that in the CM. It indicated that the OM was more conducive to the establishment of collaborative relationships between bacterial species (Table 4). Modules are considered ecological niches or functional units that constitute different ecological processes in a community (Zhang et al., 2018). In this study, organic management led to a higher modularity of co-occurrence networks (Table 4). The increase in the modularity of microbial networks can indicate that microbial communities have formed more refined ecological niches and functional units (Wang L. X. et al., 2021). The lower modularity of bacterial co-occurrence networks in the CM showed that microbial functional diversity was inhibited, which may be due to the application of pesticides and fertilizers in the CM. Compared with the CM, the fungal co-occurrence networks in the OM had a higher density, which indicated that the OM increased the diversity of species relationships. The OM has increased the number of connections (edges) and the degree of connectivity between fungal network nodes. It proved that the OM increased the complexity of fungal co-occurrence networks and promoted the exchange of metabolites and information between species (Table 4). The result was consistent with that of the study by Chen et al. (2022). In addition, there was also a partial negative correlation between fungal species in the OM, which was not found in the CM (Table 4). This negative correlation means that there is competition, exclusion, or adaptation to ecological niches among species (Cuartero et al., 2022). However, these negative correlations promote the stability of the microbial network of the soil ecosystem because they compensate for the overexpression of some members. Overexpression may lead to the instability of the network (Coyte et al., 2015). Therefore, organic management can protect soil microbial functional diversity and promote the stability of soil ecological networks compared to conventional management.

In the co-occurrence network of soil microorganisms, the clustering coefficient represents the connection between a node and its neighboring nodes. The larger the clustering coefficient, the more important the node is. The more important nodes are regarded as keystone taxa. The top five species ranked by clustering coefficient in our study are called keystone taxa (Figure 5). Banerjee et al. (2018) defined keystone taxa as "highly connected taxa that individually or in a guild exert considerable influence on microbiome structure and functioning irrespective of their abundance across space and time." All the bacterial keystone taxa in the organic tea plantations belong to *Acidobacteria, Gemmatimonadetes, and Alphaproteobacteria*. According to previous research, *Acidobacteria* participate in the nitrogen cycle and promote the conversion of nitrate and nitrite (Liu et al., 2017). *Alphaproteobacteria* are related to the nitrogen fixation process in soil (Basit et al., 2014).

The fungal keystone taxa in the OM included three types of mycorrhizal fungi (*Pezoloma_ericae, Rhizophagus irregularis,* and *Rhizophagus_clarus*). To our knowledge, mycorrhizal fungi play an important role in improving soil quality, nutrient cycling, and alleviating stress (Fei et al., 2022). *Erysiphe_ Pulchra* originates from *Erysiphe. Erysiphe* is a pathogen of powdery mildew, infecting tea trees (*Litsea Coreana Levl.Var.Lanuginose*) (Zhu et al., 2007). *Paramicrosporidium saccamoebae* was described as an intranuclear parasite of *Saccamoeba* (Quandt et al., 2017). It is necessary to further study their pathogenicity through field observation and pathogenicity verification in the future. In summary, we inferred that organic management may promote soil nitrogen metabolism and nutrient uptake by tea plants. This conjecture still needs our later research to verify it.

5. Conclusions

Organic management increased the relative abundance of microorganisms related to nutrient metabolism and plant residue degradation and improved the stability of the microbial ecological network in tea plantation soil. Organic management induced positive changes in microbial alpha diversity in tea plantation soil. Organic management impacted N-NH_4^+ negatively, which increased the relative abundance of fungi, thereby positively affecting the Shannon index. Overall, organic management can increase beneficial microorganisms and promote the stability of microecological networks. The study can provide a theoretical reference for the implementation of organic planting systems in Yunnan Province, China. The existence of pathogens and parasites also reminds us that there is a need to further improve the supporting agricultural management measures for organic tea plantations in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

XH, WG, YC, JC, and PS: conceptualization. XH, YZ, and PL: methodology and investigation. XH: formal analysis, visualization, and writing original draft preparation. WG, YC, and JC: writing review and editing. WG and YC: funding acquisition and resources. WG, YC, JC, and PS: supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1237842/full#supplementary-material

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