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Research article

Soil type and integrated nitrogen nutrient-rice straw residue management techniques affect soil microbes, enzyme activities and yield of wheat crop

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A B S T R A C T

Sporadic burning of rice straw and the particulate air pollution caused consequently have created a pressing need for identification of practical environmentally sound *in situ* rice residue management methods. However, the agronomic interventions associated with the agri-inputs particularly the type of nitrogen fertilizer source must be worked out for these interventions. In a two-year field study performed at two different locations representing sandy loam and clay loam soil types, zero tillage with application of nitrophosphate (applied as basal dose through drilling) in combination with urea (applied at 1st irrigation + 3 foliar sprays of urea at weekly interval) significantly enhanced the grain and straw yield of wheat. The soil microbial viable cell counts and dehydrogenase and urease enzyme activities were also recorded to be highest in this treatment indicating the occurrence of higher living microbial population. The treatment × response variable Principle component analysis (PCA) biplot depicted relative variation among the residue management treatments/Nitrogen fertilizer sub-treatments and the enzyme activities as response variables. A variation in the soil organic content components was recognized through Fourier transform infra-red spectroscopy (FT-IRS) studies. Irrespective of the soil types under study, the FT-IR spectra exhibited presence of the aromatic carbon functional groups in residue incorporated treatments as compared to the no residue incorporation treatment.

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1. Introduction

In situ agri-residue management techniques are being encouraged as residue incorporation contributes to improve the labile and recalcitrant carbon pools besides helping to enhance the porosity, aggregate profile and fertility of the soil [1,2,3]. The zero and minimum tillage methods are considered to be the predominant approaches to prevent soil erosion and lead to stabilization of the soil carbon content in soil thereby improving the physical attributes of soil [1,4]. Therefore, these studies provide insights on changes in the soil physical and chemical properties in response to agri-residue incorporation or retention particularly regarding the soil organic carbon contents and the amount of soil available nutrient concentrations for uptake by the plants [4]. The changes in the soil physical and chemical properties get reciprocated as altered soil biotic components [5,6]. Organic matter amendments cause changes in the soil cation exchange capacity (CEC) leading to variation in the soil buffering capacity and thus the soil microbial communities are affected [6,7,8]. In a recent study involving identification of various soil physical and chemical properties on the soil microbial assemblages, the high soil calcium in cabbage cultivated fields led to abundance of three groups actino-, oxyphoto-, and γ-proteobacteria besides the fungal genera belonging to agarico- and eurotio-mycetes [9].

Soil represents a dynamic matrix comprised of inorganic non-living and soil dwelling biotic components. The soil microbiota is very diverse and is influenced in response to application of inorganic and organic agri-inputs or amendments particularly pesticides [10], fertilizers including nitrogen fertilizers [11,12] and organic amendments [13,14]. Incorporation of agri-residue and its amount and quality can lead to alteration in the viable cell counts and microbial community changes [15]. Published studies have indicated improvement in the microbial biomass, catabolic activity and functional diversity in response to organic amendments [16]. Biochemically, the agri-residues are rich sources of a variety of polysaccharides (cellulose, hemicellulose), and aromatic polyphenols (lignin and its derivatives) [17]. The lignin and its derivatives form the recalcitrant carbon compounds and get accumulated on continuous incorporation while the cellulose and hemicellulosic components get degraded within a time span of 6 months [17]. Therefore, the agri-residue components may further result in changes in the soil available nutrient pools specifically the soil mineral N levels and thus the uptake by the crop manifested as altered crop productivity levels. The agri-residues depending on their nature and origin bear variable carbon: nitrogen ratios mostly inappropriate for their proper microbial decomposition and mineralization in soil. To improve the C:N ratios (average range from 10:1 to 12:1), inorganic nitrogen compounds such as urea are applied which invariably improve the microbial decomposition rates of the added agri-residue [18]. Hence, the soil microbiota can be tweaked to achieve greater benefits from agri-residue incorporation approaches by identification of appropriate type and application rates of nitrogen fertilizer.

In a field study in an intensively cultivated cropland in China, researchers have obtained discrete interactions of applied nitrogen fertilizer with crop straw incorporation approach [19]. They have inferred that the N-supply to the crop plant was enhanced on incorporation of straw to the soil leading to improvement in the wheat grain productivity without substantial increase in the emission of potent GHG, the N_2O gas [19]. Furthermore, another study assessing the comparative impact of tillage and agri-residue incorporation revealed improved grain productivity and quality attributes including the enhanced N-content representing the increased grain protein content in paddy straw incorporated recommended N fertilizer treatment [20]. Thus, identification of the optimum agronomic interventions for the straw incorporation particularly the appropriate nitrogen fertilizer type and quantity will be pre-requisite to maximize the economic outputs from *in situ* management technique.

Table 1
Experiment details for the soil types, residue management treatments and Nitrogen fertilizer sub-treatments evaluated for the Rabi seasons 2020–21 and 2021–22 in wheat crop.

Source	Description
Soil Type	
	Site-1: Sandy loam soil, Site-2: Clay loam soil
Residue Management Technologies	
T1	Wheat sown following conventional tillage after removing rice straw: **CT (-RS)**
T2	Zero tillage wheat sown with Happy Seeder by retaining rice straw: **ZT (+RS)**
T3	Wheat sown following conventional tillage after incorporation of rice straw using reversible Mould board plough: **CT (+RS)**
T4	Minimum tillage wheat sown with super seeder with rice straw incorporation: **MT (+RS)**
Nitrogen Levels	
NT1	No–N Control- No fertilizer N application **(N))**
NT2	Recommended practice: 23% N through DAP at sowing + 38.5% N as urea at 1st irrigation + 38.5% N at second irrigation **(23B + 38.5TD1+38.5TD2)**
NT3	23% N (as DAP) at sowing + 38.5% N at 1st irrigation + 28.5% N at second irrigation+ 10% N as urea foliar application (3 sprays at weekly intervals starting from 45 days after sowing) **(23B + 38.5TD1+28.5TD2+10F)**
NT4	23% N (as DAP) at sowing + 38.5% N at 1st irrigation + 38.5% N at second irrigation+ 10% N additional N through urea as foliar application (3 sprays at weekly intervals starting from 45 days after sowing) **(23B + 38.5TD1+38.5TD2+10F)**
NT5	50% N at the time of sowing (broadcast 27% N through urea before sowing + drill 23% N through DAP at sowing) + 50% N through urea at 1st irrigation **(50BD+50TD1)**
NT6	50% N through Nitrophosphate (24:24:0) at sowing through drilling + 50% N as urea at 1st irrigation **(50DN+50TD1)**
NT7	50% N through Nitrophosphate (24:24:0) at sowing through drilling+ 40% N as urea at 1st irrigation + 10% N through urea as foliar application (3 sprays at weekly intervals starting from 45 days after sowing) **(50DN+40TD1+10F)**

*Three to four irrigations each of 75 mm were provided according to irrigation schedule recommended for wheat crop in the region. First irrigation was applied on crown root initiation stage (21 days after sowing) whereas second irrigation to crop was applied on maximum tillering stage (45–50 days after sowing) remaining irrigations were applied according to requirement of wheat crop.

Apart from the grain yield benefits, in a long-term straw incorporation experiment the soil microbial biota and enzyme activities were also improved in the straw retention in zero tillage with happy seeder treatment [5]. Therefore, the alterations in the soil physical and chemical attributes are translated in variation in the soil microbial and associated soil enzymatic activities in both the short-as well as long-term agri-residue incorporation studies. In light of these aspects, this experiment was planned on following underlying hypotheses: 1) straw retention vs straw incorporation would have variable effect on the viable cell counts of fungi, actinobacteria, phosphorus and cellulose solubilizers in two types of soils *viz.,* sandy loam and clay loam; 2) type of nitrogen fertilizer source (highly water soluble (urea and diammonium phosphate) and sparingly/partially soluble (nitrophosphate) in various combinations) will bear an interactive effect with the straw retention/incorporation methods on the microbial counts and soil enzyme activities.

2. Materials and methods

2.1. Study site description, crop management, soil sampling, and basic properties

The field experiment was performed at two different sites representing the sandy loam (Fine loamy, calcareous, Typic Ustochrepts) and clay loam soil (Fine loamy, calcareous, Typic Ustochrepts) types i.e. at the Research Farm, Department of Soil Science and Department of Farm Power Machinery, Punjab Agricultural University, Ludhiana, Punjab during 2020-21 and 2021–22. Wheat variety PBW 725 was sown in the field (individual plot size of $7 \times 6.5\ m^2 = 45.5\ m^2$) during the first and second week of November in 2021 and 2022, respectively (Supplementary figure A1). The geographic coordinates of the two field sites were N30°54′24.811″, E75° 46′ 42.337″ and N30°.54′39.892″, E75° 48′43.189″ respectively. The experiment was laid down to compare the three methods of *in situ* incorporation of the rice residue i.e. zero tillage + sowing with happy seeder (T2), conventional tillage + mould board ploughing based incorporation of rice residue (T3) and minimum tillage with wheat sown with the super seeder (T4) vis-a-vis the conventional tillage with removal of rice residue i.e. control treatment (T1). The fertilizer sub-treatments included seven different doses of nitrogen sources (urea, nitrophosphate and diammonium phosphate as basal soil and foliar applications) were applied as detailed in Table 1. Wheat plots received equal basal dose of 26 kg P ha^{-1} as single super phosphate (in control plots) and diammonium phosphate and 25 kg K ha^{-1} as muriate of potash prior to sowing. The agrometeorological data revealed an average maximum temperature during crop season was 24.3 °C and 25.1 °C during 2020-21 and 2021–22, respectively at the two sites. While the average minimum temperature was 10.7 °C during first year and was 11.4 °C during second year of the study. Overall, 223.2 mm of rain was recorded throughout the crop season (Supplementary figure A2). The details of the soil physical and chemical properties of the two sites have been presented in Supplementary Table 1. Both the sandy loam and clay loam soils exhibited basic pH and were moderate in organic carbon content.

2.2. Grain and straw yield of the wheat

The wheat crop during both the Rabi 2020 and 2021 seasons was harvested at maturity after 180 days of sowing with a tractor mounted harvesting machine. The grains were obtained by manual threshing of the produce, weighed and expressed in tonnes ha^{-1}. The remaining straw was bailed in bundles and also weighed and expressed in tonnes ha^{-1}.

2.3. Soil enzyme activity

Two critical soil enzyme activities for both types of soils i.e. sandy loam and clay loam in Rabi season 2022 were estimated to identify the effect of *in situ* rice straw management and nitrogen fertilizer sub treatments. The soil samples were collected at full maturity stage of the crop using a soil corer from three different soil depths i.e. 0-15, 15–30 and 30–60 cm. The soil samples were collected from four different sites from the treatment plot and three different soil depths were removed and pooled later for a particular depth. The pooled samples for a specific depth were placed in a container, hand-mixed mixed thoroughly and placed in a zip-lock bag and carried to the laboratory in an ice-cooled basket. The dehydrogenase enzyme assay included estimation of living microbial cells utilizing the respiration activity performed by them to convert 2, 3, 5-triphenyltetrazolium chloride to Triphenylformazan (TPF) recorded at $\lambda = 485$ nm against methanol as the blank. The values were calculated by preparing a TPF standard curve (10 to 100 μg) [21].

Further, as the type of nitrogen applied as fertilizer was anticipated to affect the available nitrate content, the urease enzyme activity was also determined [22]. The procedure involved measurement of red colour of the potassium chloride-PMA extracted soil solution by recording absorbance values at $\lambda = 527$ nm.

2.4. Enumeration of soil microbial counts

The soil samples were collected from two different soil depths (0–30 and 30 to 60 cm) at full maturity stage of the crop using a soil corer and were analyzed for the viable cell counts of the total fungi, actinobacteria, phosphate solubilizing and cellulose degrading microorganisms through the serial dilution plate assay on potato dextrose agar, actinomycetes agar, NBRIP agar and CMC agar, respectively. The soil suspensions were prepared by dispensing known quantity (10 g) of the soil sample in autoclaved normal saline (90 mL). The analysis was performed for three replicates per treatment. The contents were swirled and later kept on a rotary shaker for 5 min to disintegrate any bigger soil lumps or aggregates and allowed to decant for 2 h undisturbed. The supernatant aliquot (1 mL) was serially diluted and 100 μL aliquot from specific dilution blanks were spread plated on the surface of the solidified media (10^{-3} dilution was used). On incubation for a week at 27 ± 2 °C the microbial colonies appearing on the media were counted and depicted as log cfu g^{-1} dry soil [23].

2.5. Fourier transform infra-red spectroscopy of the soil samples

The vibrational IR spectroscopy was performed for functional group characterization of the dried whole soil samples. The FT-IR spectra were recorded from 4000 to 400 cm^{-1} wavenumbers on a FT-IR spectroscope (Cary 630, Agilent Technologies, Inc., Santa Clara, California, USA) equipped with a diamond attenuated total reflectance (ATR) assembly. The soil samples were analyzed by placing a known amount (5 mg) of the dried soil sample to obtain the peaks formed because of the interaction of the various soil components/compounds with the IR radiations in the % transmittance mode [24]. The % transmittance vs. wavenumbers (cm^{-1}) data was obtained in csv format and the FTIR graphs were prepared after normalization procedure using Origin software (Origin version 9.0, Origin Lab Corp., Northampton, MA, USA) [25].

2.6. Statistical analysis

The significant variations ($p < 0.05$) among the main residue incorporation methods, nitrogen fertilizer sub-treatments and the soil depths were obtained through the analysis of the data for analysis of variance (ANOVA) procedure using SAS software (version SAS 9.3, Cary, USA) [26]. The enzyme activities data set of the soil samples obtained from the two types of field sites (sandy loam and clay loam) from three different depths was subjected to principle component analysis (PCA) to identify the relatedness and variations among the main and sub-treatment factors with respect to the variables studied [5].

3. Results

3.1. Effect of rice residue management approaches and nitrogen fertilizer treatments on grain and straw yield in wheat crop

The rice straw incorporation as mulch/stubbles in zero till wheat sowing through happy seeder treatment (T2) exhibited significantly higher grain and straw yield in both the soil types for both the Rabi season 2020 and 2021 (Tables 2 and 3). Among the nitrogen level sub-treatments, NT7 nitrophosphate application in combination with urea exhibited consistently significant highest grain and straw yield for both seasons and soil types.

Therefore, these results indicated towards the zero-tillage approach as most useful for obtaining improved yields in wheat as compared to conventional tillage with mould board ploughing and minimum tillage with super seeder enabled rice straw incorporation methods. Further, significantly higher yield of wheat grains can be obtained by alternative application of a new nitrogen fertilizer source, 'nitrophosphate' in lieu of basal application of DAP followed by two splits of urea. This agronomic practice will thus be more efficient N-fertilizer application approach to reduce the time and cost of application of DAP followed by urea in two splits.

3.2. Dehydrogenase and urease activities of the soil samples

The ANOVA analysis of the data revealed the incorporation of the rice straw residue through super seeder (T4) exhibited significantly highest both the enzyme activities for both types of the sandy and clay loam soil types (Table 4) followed by zero tillage with happy seeder based sowing of the wheat crop (T2). Inspite of incorporation of rice residue through mould board ploughing method

Table 2
Effect of rice residue management technologies and different N application methods on grain yield of wheat crop cultivated at two different field sites.

Treatment	Grain Yield (t ha^{-1})			
	Sandy Loam (S1)		Clay Loam (S2)	
	2020–21	2021–22	2020–21	2021–22
Main Treatment				
T1	5.01c	4.67d	5.25d	4.86c
T2	5.17a	4.97a	5.37a	5.14a
T3	5.10b	4.73c	5.32b	4.92b
T4	5.13a	4.79b	5.29c	4.93b
Nitrogen Levels				
NT1	3.29e	3.01e	3.59e	3.24g
NT2	5.37d	5.07c	5.57c	5.26c
NT3	5.30d	4.98d	5.48d	5.15e
NT4	5.51b	5.17b	5.70b	5.34b
NT5	5.31d	4.99d	5.45d	5.12f
NT6	5.40c	5.08c	5.58c	5.22d
NT7	5.63a	5.24a	5.77a	5.41a

*Main treatments: T1- CT (-RS), T2-ZT (+RS), T3- CT (+RS), T4- MT (+RS).
**Sub Treatments: N1–0N (Control), N2-(23B + 38.5TD1+38.5TD2), N3-(23B + 38.5TD1+28.5TD2+10F), N4- (23B + 38.5TD1+38.5TD2+10F), N5-(50BD+50TD1), N6-(50DN+50TD1), N7-(50DN+40TD1+10F).

Table 3
Effect of rice residue management technologies and different N application methods on straw yield of wheat crop cultivated on two different soil types.

Treatment	Straw Yield (t ha^{-1})			
	Sandy Loam (S1)		Clay Loam (S2)	
	2020–21	2021–22	2020–21	2021–22
Main Treatment				
T1	5.01c	4.67c	5.25c	4.86c
T2	5.17a	4.97a	5.37a	5.14a
T3	5.10 ab	4.73b	5.32b	4.92b
T4	5.13a	4.79b	5.29b	4.93b
Nitrogen Levels				
NT1	3.29e	3.01e	3.59e	3.24g
NT2	5.37d	5.07c	5.57c	5.26c
NT3	5.30d	4.98d	5.48d	5.15e
NT4	5.51b	5.17b	5.70b	5.34b
NT5	5.31d	4.99d	5.45d	5.12f
NT6	5.40c	5.08c	5.58c	5.22d
NT7	5.63a	5.24a	5.77a	5.41a

The alphabetic scripting with different alphabets in a column represent values which are statistically significant.

Table 4
Effect of rice residue management technologies and different N application methods on soil dehydrogenase (DHA, μg TPF g^{-1} soil h^{-1}) and urease (UR, μg urea g^{-1} dry soil min^{-1}) enzyme activities in soil sampled from different depths of sandy loam (S1) and clay loam (S2) soils in wheat crop.

Sources of variation	Enzyme activities			
	DHA (S1)	DHA (S2)	UR (S1)	UR (S2)
Main Treatment				
T1	7.10d	9.30d	4.35d	1.62c
T2	14.99b	11.86b	6.27b	2.78b
T3	11.61c	10.06c	5.70c	2.69b
T4	15.04a	14.73a	6.88a	3.87a
Nitrogen Levels				
NT1	9.30g	5.89g	2.91f	1.56e
NT2	10.33f	8.35f	5.29e	2.20d
NT3	12.02d	10.66e	5.98d	2.22d
NT4	12.83c	13.95b	7.08b	2.67c
NT5	11.05e	11.32d	5.25e	2.89bc
NT6	13.33b	13.70c	6.25c	3.21b
NT7	16.45a	16.53a	7.86a	4.42a
Depth				
0–15	15.15a	14.85a	6.66a	3.30a
15–30	12.20b	11.28b	5.81b	2.77b
30–60	9.22c	8.33c	4.93c	2.15c

The alphabetic scripting with different alphabets in a column represents values which are statistically significant.

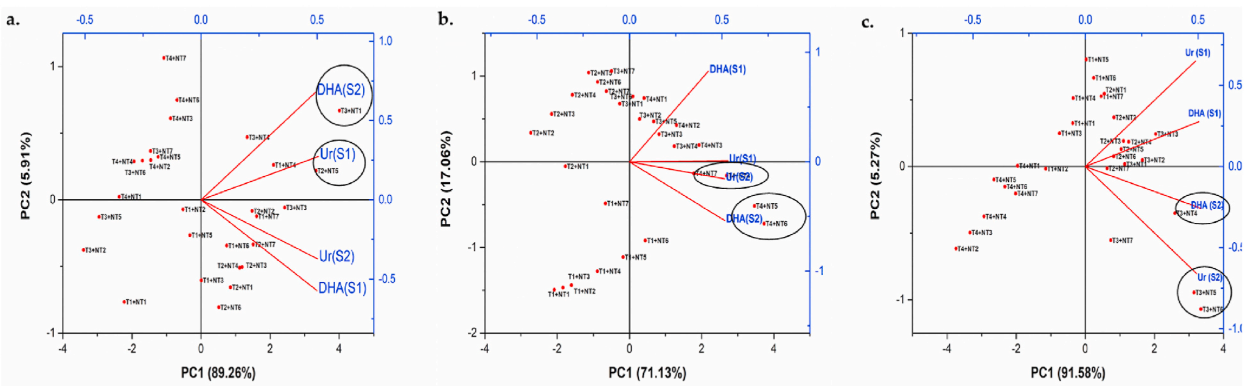

Fig. 1. Principal component analysis (PCA) for soil enzyme activities of the sandy and clay loam samples collected from different soil depths. (a) 0–15 cm, (b) 15–30 cm and (c) 30–60 cm. The encircled coordinates represent the treatment × response variable interactions.

resulted in least soil enzyme activities as compared to the other two residue management treatments. Among the two soil types, lower enzyme activities were recorded for clay loam soils as compared to the sandy loam soils probably due to higher gaseous and lower bulk density conditions in the former soil type. Among the nitrogen fertilizer sub-treatments, basal application of nitrophosphate (50% by drilling at sowing) followed by urea (40% at first irrigation) and foliar application resulted in significantly highest dehydrogenase and urease activities at both the field sites. The interactions among all the three factors were significant.

The PCA of the enzyme activities estimated at three different depths is presented in Fig. 1. The biplot obtained from analysis of the data set of topsoil (0–15 cm) samples represented 95.17% of the total variance with PC1 and PC2 contributing to 89.26 and 5.91% variation for the two enzyme activities (Fig. 1a). Likewise, for 15 to 30 and 30 to 60 cm soil depths the total variance contributions were 88.19 and 96.85% respectively. The treatment × response variable biplot depicted relative variation among treatments/sub-treatments and the response variables. The minimum tillage wheat sown with super seeder treatment (T4) exhibited clear grouping with both dehydrogenase (NT5 and NT6) and urease (NT4 and NT7) activities for 15 to 30 cm soil depth clay loam samples (Fig. 1b). Similarly, the T3 sub-treatments (NT5 and NT6 for urease and NT4 for dehydrogenase activity) formed clear grouping for 30 to 60 cm soil depth samples (Fig. 1c).

3.3. Variations in microbial counts in response to soil depths, nitrogen fertilizer sub-treatments and different residue management treatments

Four different soil microbial viable cell counts were enumerated. The mean effects of the factors depicted in Table 5 relate to significantly higher microbial viable cell counts in zero tillage + sowing with happy seeder treatment (T2). This may have occurred probably due to the mulching effect of the rice straw residue and the standing stubbles which led to relatively optimum temperature conditions for the growth and proliferation of the four test microbial groups. The microbial counts decreased in a soil depth-dependent manner with significantly higher microbial counts for the 0–30 cm soil samples irrespective of the soil type or the two field sites. The two soil types did not show significant variations for the microbial counts. Among the nitrogen fertilizer sub-treatments, the application of nitrophosphate fertilizer in combination with urea (NT7) treatment showcased significantly highest viable cell counts of all the four test microbial groups except for fungal population in clay loam soil samples. Also, the other nitrophosphate treatment (NT6) also represented appreciably similar viable cell counts indicating this nitrogen fertilizer source to be a viable alternative to reduce dependence for the urea/diammonium phosphate fertilizer in the wheat crop. Irrespective of the soil type the rice residue incorporation improved the microbial viable cell counts as compared to the conventional tillage with removal of the rice residue treatment (T1). This indicated the stimulating and nutritional significance of the rice residue incorporation particularly for the soil bacterial and fungal communities.

3.4. FT-IR studies of the soil samples

The FT-IR spectra of the sandy soil upper layer (0–15 cm) soil samples (Fig. 2) indicated a broad absorption peak spanning over 3777 to 3000 cm^{-1} representing the hydroxyl functional group stretching vibrations of intramolecularly bonded water molecules as the common broad peak in all the soil samples. Further, the soil inorganic clay components exhibited broad peaks from 3000 to 2500 cm^{-1} representing the silicate bond vibrations. All the straw incorporation main treatments exhibited aromatic C=C stretching vibrations representing lignin at peaks 1630 to 1590 cm^{-1}. Multiple peaks in the 1580-1551 cm^{-1} region besides ~1400 cm^{-1} are attributable to organic acid carboxylate functional group vibrations.

Table 5
Effect of rice residue management technologies and different N application methods on viable cell counts of different groups of microbes in soil sampled from different depths of sandy loam (S1) and clay loam (S2) soils in wheat crop.

Sources of variation	Microbial Count (log cfu mL^{-1})							
	Phosphate Solubilizers		Actinobacteria		Cellulose degrading microbes		Fungi	
	S1	S2	S1	S2	S1	S2	S1	S2
Main Treatment								
T1	7.74b	7.44c	6.15d	5.56c	6.99d	6.58c	2.04d	1.96b
T2	8.05a	7.66a	6.29a	5.89a	7.21a	6.74a	2.17a	2.06a
T3	7.74b	7.51b	6.19c	5.71b	7.05c	6.66b	2.10c	2.02a
T4	7.83b	7.53b	6.24b	5.72b	7.13d	6.68b	2.12b	2.03a
Nitrogen Levels								
NT1	7.60e	7.35d	6.07f	5.58f	6.86f	6.58d	2.00d	1.96bc
NT2	7.75d	7.38d	6.17e	5.68d	7.07d	6.62c	2.11bc	1.93c
NT3	7.82cd	7.53c	6.18e	5.63e	7.05e	6.68b	2.13b	1.99b
NT4	7.88bc	7.59b	6.25c	5.76b	7.16c	6.69b	2.09c	2.00b
NT5	7.82cd	7.58b	6.21d	5.72c	7.04e	6.66b	2.10bc	2.13a
NT6	7.96 ab	7.61b	6.28b	5.78b	7.21b	6.67b	2.12bc	2.09a
NT7	8.03a	7.73a	6.36a	5.87a	7.26a	6.77a	2.21a	2.01b
Depth (cm)								
0–30	8.00a	7.76a	6.41a	6.08a	7.20a	6.82a	4.22a	0.03a
30–60	7.68b	7.32b	6.03b	5.35b	6.99b	6.51b	0.04b	4.04b

The alphabetic scripting with different alphabets in a column represents values which are statistically significant.

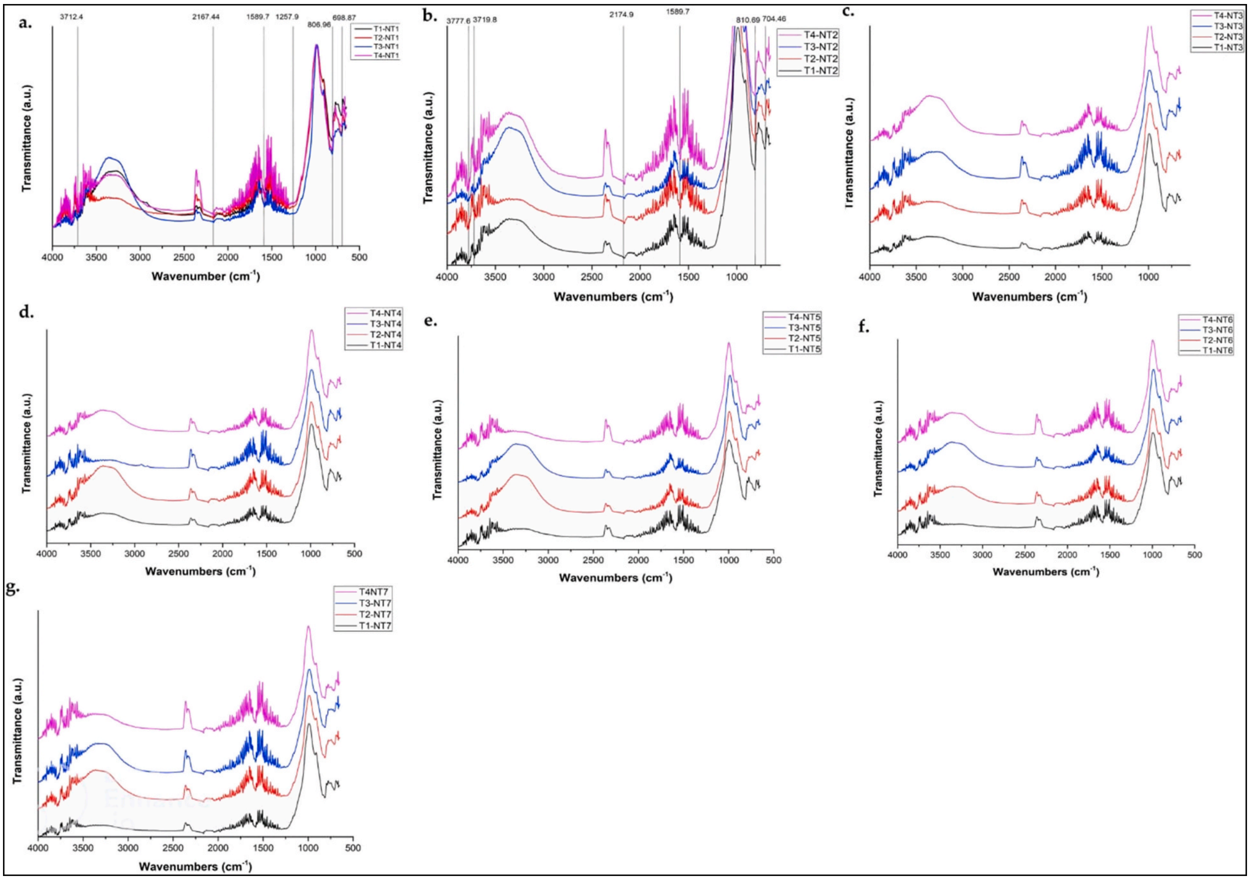

Fig. 2. FT-IR spectra depicting the alteration in the functional groups of soil components in response to main and sub-treatments for the sandy loam soil. The sub-treatments are represented as a) NT1, b) NT2, c) NT3, d) NT4, e) NT5, f) NT6 and g) NT7.

Also, the alkyl (methylene and methyl functional group) bending deformations appear as less distinct peaks in the 1500 to 1350 cm^{-1} region. The organic acid carboxyl functional group variations were more distinct in the rice straw incorporated treatments as compared to the conventional control treatment. Furthermore, the out-of-plane bending vibrations of the aromatic C–H functional group can be observed in the 909 cm^{-1} peak. The peaks at 806.96 and 698.87 cm^{-1} can be attributed to the silicate minerals including the quartz which is predominant in the sandy loam soil (Fig. 2). These peaks were less clear in the clay loam soil (Fig. 3).

4. Discussion

The effect of the agri-residue returns on the yield, and soil microbiological and enzyme characteristics has been documented with no effect, sparingly to substantial positive impacts on the studied yield and soil biological traits [27,28,29]. The present field study illustrated wheat grain and straw yield enhancing potential of the residue incorporation/retention treatment with application of nitrophosphate fertilizer in combination with the urea (applied at 1st irrigation and three sprays at one week interval) in comparison to the no straw incorporation (unamended control) treatment. These results are in line with the reports of other researchers [5,20,30]. The improved grain and straw yields may be attributed to improved soil biological traits in reciprocation to the residue return based organic matter enhancement in the soil [31]. Furthermore, the reinstatement or restoration of the nutrients mined by the previous rice crop in form of rice straw residue (straw and stubbles) furnishes back the lost nutrients for uptake by the crop plants [32]. The nitrogen nutrition is crucial for the growth of the crop plants. The combined application of nitrogen fertilizer at the recommended rate along with rice straw incorporation treatment exhibited improved grain and straw yields in rice [33,34] and wheat [2] crops as studied by other researchers. However, certain research reports project a contrary positive impact on the crop yield on residue incorporation with application of N-fertilizer at a reduced rate vis-à-vis unamended control treatment though the effect is evident after a longer time-span [35]. This positive effect is unlikely to be observed for short-term incorporation studies. As the rice straw exhibits wide C:N ratio unfavourable for its microbial decomposition, application of nitrogenous fertilizers particularly urea at over and above the recommended rates have been identified to be beneficial for acceleration of the *in-situ* microbial decomposition of the incorporated residue [18]. The enhanced decomposition though may result in greater N-immobilization in form of the microbial biomass and can have a negative impact on the N-nutrient availability for the wheat crop while it can be useful for the subsequent rice crop [30]. The use of a

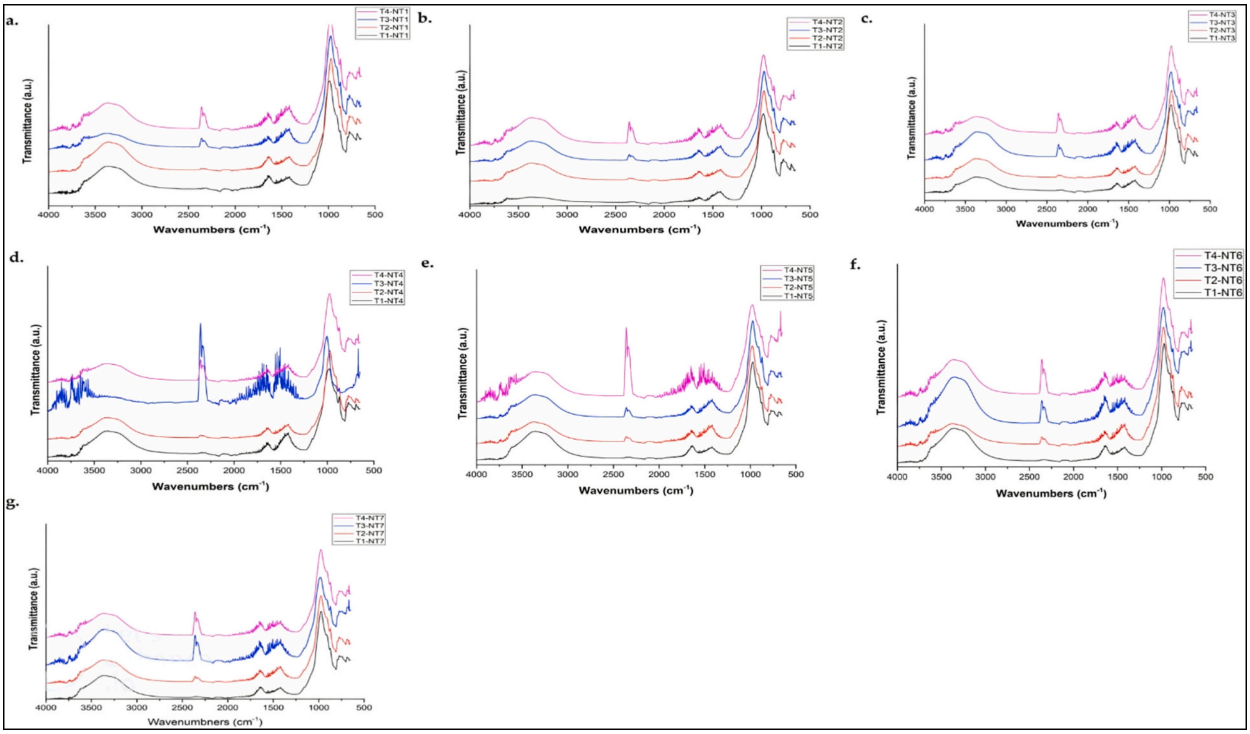

Fig. 3. FT-IR spectra depicting the alteration in the functional groups of soil components in response to main and sub-treatments for the clay loam soil. The sub-treatments are represented as a) NT1, b) NT2, c) NT3, d) NT4, e) NT5, f) NT6 and g) NT7.

sparingly soluble nitrophosphate in lieu of the basal DAP application can be useful strategy to decrease the N-immobilization by the straw decomposing microbial populations. In this study, basal application of nitrophosphate (50% RDN) followed by urea split application (40% at first irrigation)+three sprays urea (10%) at one week interval had the most stimulating effect on the plant growth which resulted in improved straw and grain returns. The soil texture had no impact on the grain and straw yield as the zero tillage-main treatment (T2) and nitrophosphate (NT7) sub-treatment exhibited significantly highest values for both the soil types.

Tillage, residue incorporation and nitrogen fertilizer application have impact on soil microflora. Soil microorganisms are a sensitive indicator of any perturbations caused due to application of different amendments whether inorganic or organic to the soil [10]. An increase in soil microflora abundance/richness can be considered as an integral component representing the occurrence of various forms of organic carbon compounds besides their stability in the soil [36,37]. A recent soil microbiome study illustrated the potential role of microbe-mediated C and N-mineralization processes in response to residue incorporation to exhibit an impact of residue N on soil C-sequestration [37]. The soil microbial populations were recorded to be maximum in the zero tillage (T2) main treatment and nitrophosphate (NT7) sub-treatment except significantly lower count of fungi in NT7 as compared to other N-fertilizer combinations. It can be argued that similar to previously published reports, the zero tillage treatment provided the conducive optimum temperatures and retained more moisture helping in the proper growth and proliferation of the soil microbes as a result of the mulching effect of straw retained on the soil surface [38,39]. The application of nitrophosphate was beneficial as it released both the N and P nutrients in a slow-release manner that helped in sustained growth of the heterotrophic bacterial communities. However, because of probability of a lower nitrate-N leading to high (basic) pH conditions did not allow for significantly highest counts of soil fungi in the NT7 sub-treatment. Among the differential groups of microbes, the increased cellulose degrader viable cell counts were anticipated as predominant components of rice straw include the cellulose and hemicellulose fractions which were utilized by these group of microbes as their preferred carbon substrate. A likewise enhanced cellulose degrading populations have been reported by Refs. [5,27]. The phosphate solubilizing microbes (PSMs) were improved in zero tillage + rice residue main treatment in this study. Likewise enhanced PSM counts have also been recorded in response to rice residue incorporation [40,41,42]. The increased P-solubilizers will lead to improved available P content of the soil due to production of organic acids particularly the citric and oxalic acid on decomposition of the incorporated rice straw [43]. Further, the phosphate solubilizing microbial population exhibited as shift from bacterial to more fungal counts on residue incorporation treatments [41,44]. The actinobacterial populations were also improved in the agri-residue incorporated treatments signifying the improved organic carbon content, mulching effect and alkaline pH of the incorporated soil [45,46].

Soil enzyme activities have a response similar to the conjugate physiological activities of the microbes, plants and other biotic components of the soil [47]. Among the soil enzymatic activities, the dehydrogenase activity is an indicator of the living and respiring soil microbial population [48,49]. Therefore, it corresponds to the viable cell counts of the various soil microbial groups [50]. The higher dehydrogenase activity recorded in T2 (zero tillage + rice residue retention) could be attributed to improvement in the organic carbon content in both types of soils [30]v. The urease activity was also recorded to be highest in the same treatment representing development of conditions conducive for proliferation of these group of microbes [5].

The soil organic and clay component variations in response to various agri-residue and nitrogen fertilizer sub-treatments were identified through the vibrational spectroscopy studies. The FT-IR spectroscopy analysis predominantly provide information on the chemical functional groups representing different compounds/components of the soil sample. As the soil sample inherently contains the inorganic clay as the major component, the FTIR spectra from both the soil types exhibited clay lattice adsorbed inner hydroxyl functional groups (3712 to 3200 cm^{-1}) [51,52]. The predominance for the less intensity of these peaks in both the soil samples represented the occurrence of quartz in sandy loam and kaolinite as the prominent clay component [53,54]. Similar FT-IR peaks have been reported by other researchers for the soil samples [53,54]. Furthermore, these peaks also exhibited the presence of polysaccharide (-OH group) i.e. cellulose and hemicellulose content in the residue amended soils [55]. As the straw is considered to be a rich in cellulose and hemicellulose, the addition/incorporation of the straw is expected to improve the specific IR peaks for the functional groups for these compounds. The T2 main treatment exhibited presence of specific peaks representing the aromatic C=C aromatic carbon bending vibrations [56] and hence the lignin and its derivatives obtained on straw incorporation [17].

5. Conclusions

This research intervention revealed the agronomic and soil microbiological aspects associated with the three *in situ* rice residue incorporation treatments in combination with the seven nitrogen sub-treatments. Among the main treatments, zero tillage with happy seeder based sowing treatment was identified as the most appropriate method of rice residue management as it exhibited significantly improved grain and straw yield besides the viable cell counts of the microbes and second best for the soil enzyme activities. The application of nitrophosphate in combination with urea (NT7) was responsible to significantly improve the yield, soil microbial counts and enzyme activities as compared to the other six sub-treatments. This study specifically identified that among the mould board, super seeder and happy seeder enabled rice straw incorporation, zero tillage + happy seeder treatment had the most significant economic and environmental benefit. Furthermore, nitrophosphate + urea can be a useful N-fertilizer as it can supply both 50% N as well as required P to the crop at the CRI (crown root initiation) growth stage. However, the basal application of DAP followed by urea (two-split) application in agri-residue incorporated conditions could not provide the required N-nutrition at the CRI stage of the wheat crop due to no irrigation.

Author contribution statement

Vicky Singh: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Rajeev Kumar Gupta: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Anu Kalia: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nadhir Al-Ansari: Conceived and designed the experiments; Analyzed and interpreted the data; Funding acquisition; Wrote the paper.

Abed Alataway: Analyzed and interpreted the data; Funding acquisition; Wrote the paper.

Ahmed Z. Dewidar: Contributed reagents, materials, analysis tools or data; Funding acquisition; Wrote the paper.

Mohamed A. Mattar: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Funding acquisition; Wrote the paper.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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