Labile Soil Organic Matter Pools Are Influenced by 45 Years of Applied Farmyard Manure and Mineral Nitrogen in the Wheat—Pearl Millet Cropping System in the Sub-Tropical Condition

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Abstract: Labile soil organic matter pools (LSOMp) are believed to be the most sensitive indicator of soil quality when it is changed rapidly with varied management practices. In sub-tropical climates, the turnover period of labile pools is quicker than in temperate climates. Organic amendments are of importance in improve the LSOMp for a temperate climate and may be helpful in sub-tropical climates as well. Hence, the status of LSOMp was studied in long term farmyard manure (FYM) amended soils under wheat (Triticum aestivum L.) and pearl millet (Pennisetum glaucum L.) cropping systems in sub-tropical arid conditions. At the same time, we also attempt to determine the impact of mineral nitrogen (N) application in these pools. In this study, dissolved organic matter (DOM), microbial biomass (MB), and light fraction (LF) were isolated in the management practices involving different modes and rates of FYM applications along with the application of nitrogenous fertilizer. C and N contents of the labile pools were analyzed in the soil samples at different periods after FYM applications. Among the different pools, microbial biomass carbon (MBC) and dissolved organic carbon (DOC) were changed significantly with different rates and modes of FYM application and mineral N application. Application of FYM at 15 Mg ha$^{-1}$ in both the seasons +120 kg ha$^{-1}$ mineral N resulted in significantly higher MBC and DOC as compared to all of the other treatments. This treatment also resulted in 13.75% and 5.8% more MBC and DOC, respectively, as compared to the amount of MBC and DOC content in the control plot where FYM and mineral N were not applied. Comparing the labile organic matter pools of 45 years of FYM amendment with initial values, it was found that the dissolved organic carbon, microbial biomass carbon, and light fraction carbon were increased up to the maximum extent of about 600, 1200, and 700 times, respectively. The maximum amount of DOM (562 mg kg$^{-1}$ of DOC and 70.1 mg kg$^{-1}$ of DON), MB (999 mg kg$^{-1}$ of MBC and 158.4 mg kg$^{-1}$ of MBN), LF (2.61 g kg$^{-1}$ of LFC and 154.6 g kg$^{-1}$ of LFN) were found in case of both season applied FYM as compared to either summer or winter applied FYM. Concerning the different rates of FYM application, 15 Mg ha$^{-1}$ FYM also resulted in a significantly higher amount of DOM, MB, and LF as compared to other FYM rates (i.e., 5 Mg ha$^{-1}$ and 10 Mg ha$^{-1}$). Amongst different pools, MB was found to be the most sensitive to management practices in this study. From this study, it was found that the long-term FYM amendment in sub-tropical soil along with mineral N application can improve the LSOMp of the soil. Thus, it can be recommended that the application of FYM at 15 Mg ha$^{-1}$ in summer and winter with +120 kg ha$^{-1}$ mineral N can improve SOC and its labile pools in subtropical arid soils. Future studies on LSOMp can be carried out by considering different cropping systems of subtropical climate.
Keywords: dissolve organic matter; light fraction; microbial biomass; organic carbon; total nitrogen

1. Introduction

Soil organic matter (SOM) mainly consists of ‘labile’ pools with smaller sizes and rapid turnover and ‘recalcitrant’ pools of larger sizes with slow turnover [1]. Total soil organic matter content has an immense importance on soil structure, permeability, bulk density, and various chemical as well as biological properties. Hence, it is considered one of the most important attributes of soil quality [2]. However, there are no such rapid changes in organic carbon (C) content and total nitrogen (N) due to short term changes in management practices. Labile organic matter pools are highly sensitive to the changes in soil management practices and could be considered as an excellent soil quality indicator for controlling soil function in specific ways [3]. They include soil organic matter, light fraction, non-humic substances, soil micro, and macrofauna [4]. Different management practices, soil, and climate strongly influence the amount of these active fractions [5]. In temperate soils, these active fractions consist of almost 25–33% of the soil organic matter (SOM). However, they are possibly little in the case of tropical soils [6]. The soil nutrient dynamics is highly altered with active fractions. Precise and consistent measurement of turnover times, as well as the size of different SOM pools, must be present to comprehend the impact of varied soil management practices on the SOM dynamics [1]. Hence, it is important to understand SOM changes under tropical weather with the manipulation of management practices. Perhaps the most mobile form of SOC is dissolved organic matter (DOM) which is controlled and replenished by the addition of C through plant leaves, root exudates, and soil microbes [7]. It is reported that the majority of released DOM (up to 40%) is potentially biodegradable in solution within a period of days to a few months [8]. This form of DOM may be utilized by a crop during its growth period which can be a major pathway in element cycling and hence can be considered an important factor in plant nutrition point of view. The DOM is considered as an important substrate for microbial activity, a key resource of mineralizable N, S, and P, and its leaching greatly controls the SOM as well as nutrient content and the chemical state of ground-water [3]. In agricultural soils, fertilization with SOM has been shown to enhance the water-extractable organic carbon by a factor of 2.7 to 3.2 [9,10]. It is important to know the effect of mineral N-fertilizer on the concentration of DOM in cultivated soils. Most of the studies on DOM have been conducted in a temperate climate [11]. Thus, the research gap regarding the study of the status of soil DOM in tropical and sub-tropical climatic condition and cultivated soil per se is quite large. The light fraction of SOM is one of the most important indicators of soil quality as it is the primary fraction for soil C formation [12]. It is significant in controlling the activity of heterotrophic organisms as it provides energy to them, and it is also the reservoir of plant nutrients and relatively labile C. Hence, the determination of soil structure vis-à-vis ecosystem is largely dependent on the light fraction of SOM [13]. The labile character of the light fraction is significant owing to having a considerable impact on the plant-available N pool [14]. Balota and Auler [15] stated that soil microbial biomass mediates both immobilization and mineralization of nutrients and it is a storehouse of considerable amounts of readily mineralizable N than the N in most of the organic matter. Although accounting for only a little portion of soil organic matter, 1–3% of soil organic C [16], measurement of biomass may be helpful for the early assessment of changes in soil quality through varied soil managements long before such changes can be found in total soil organic N or C content. It is essential to study the changes in this fraction of soil organic matter due to the changes in soil management practices [17].

Organic amendments have a huge role in the improvement of the labile SOM pools. Organics may contribute to the passive pools of the soil. Majumder et al. [18] reported that most of the pools were significantly correlated with each other under organic amendment for the rice-wheat cropping system. In this study, different rates and modes of FYM
amendments were considered believing that it might improve the labile SOM pools and that, by comparing the different rates and modes of application, the suitable FYM application schedule may come out from this study. Similarly, N application was considered to supplement the N nutrient to the crop and to find out whether there is any role of this fertilization in labile SOM pools or not. N is one of the vital factors that may influence SOC content by increasing inputs of plant residues to the soil [19]. The wheat–pearl millet cropping system is very much popular in the sub-tropical arid climatic condition of India, particularly in the western and north-western part of the country where there is a low amount of rainfall received in a year which does not allow the farmers to go for water-loving crops such as rice.

The major research gap lies in the limited information about the status of labile SOM pools in sub-tropical soils, especially in the cultivated soils where the rate of SOM decomposition is fast in comparison to temperate soil. Due to such a rapid turnover of labile SOM pools, it is very important to study the status of these pools in long term organic amendment of sub-tropical soils where SOM levels per unit of C input are lower. The research findings on the status of mineral N fertilization on these pools under organically amended soil of sub-tropical climate are also scarce. Considering all of his information, a long-term experiment having different rates of FYM applied at different times in a pearl millet–wheat cropping system was conducted with the hypothesis that long-term FYM and mineral N application would improve the labile SOM pools of the sub-tropical soils. The focal objectives of the study were to assess the status of labile soil organic matter pools and soil organic carbon as well as nitrogen build-up through the long-term application of FYM and mineral N in the sub-tropical climate. The novelty of the study is to generate the status of labile SOM pools of the sub-tropical soils with long-term different FYM application schedules along with mineral N.

2. Materials and Methods

2.1. Experimental Site

A long-term field experiment was initiated in October 1967 on a coarse loamy soil and classified as Typic Ustochrepts at CCS Haryana Agricultural University, Hisar, Haryana, India (29.16° N, 75.75° E, 215 m above mean sea level).

2.2. Weather and Experimental Soils

During the experimental period of 2012–2013, the site experienced rainfall of 770 mm annum⁻¹, of which 80 to 85% was received during August to October; the minimum and the maximum temperatures of 5 °C and 43 °C during December and May, respectively; variation in relative humidity from 81% in the morning hours to 45% in the evening hours; and total pan evaporation of 545 mm. Thus, the experimental site experienced a climate that was characterized by dryness, extremes of temperature, and scanty rainfall and thus the area was very hot in summers and cool in winters. The surface soil (0–15 cm) was sandy loam and well-drained. Initial surface soil (0–15 cm) before starting of the study was saline (pH 8.2); low in organic carbon (4.2 g kg⁻¹), medium in available P (0.5 M NaHCO₃-extractable, 13.0 kg ha⁻¹) and available K (N NH₄OAc extractable, 249 kg ha⁻¹); and low in available Zn (DTPA extractable, 0.68 mg kg⁻¹). The initial labile soil organic carbon and nitrogen pools are listed in Table 1.
Table 1. Initial soil organic carbon, labile soil organic carbon pools, total nitrogen and soil organic nitrogen pools during 1967.

| Attributes                        | Values |
|-----------------------------------|--------|
| Soil organic carbon (g kg⁻¹)       | 4.2    |
| Dissolved organic carbon (mg kg⁻¹) | 75.5   |
| Microbial biomass carbon (mg kg⁻¹) | 76.3   |
| Light fraction carbon (mg kg⁻¹)    | 328    |
| Total nitrogen (mg kg⁻¹)           | 359    |
| Dissolved organic nitrogen (mg kg⁻¹)| 6.23   |
| Microbial biomass nitrogen (mg kg⁻¹)| 10.9   |
| Light fraction nitrogen (mg kg⁻¹)  | 42.9   |

2.3. Treatments and Experimental Design

The treatments consisted of three rates of FYM (15, 30 and 45 Mg ha⁻¹ till 2007–2008 and 5, 10, and 15 Mg ha⁻¹ after 2008–2009) applied in three modes of application viz., in summer to pearl millet during May; in winter to wheat during November; and in both the summer and winter. The FYM applications were reduced to lower rates so as to make it realistic according to the availability to the farmers. The absolute control plot (no FYM) was applied in all of the seasons. The main plots were composed of nine combinations of FYM application (3 rates × 3 modes) and one absolute control. Each main plot was again divided into two sub-plots. Application of N at 0 and 120 kg ha⁻¹ for both the crops through urea was put into these two sub-plots. The statistical design used to analyze the data obtained from this experiment was a split-split plot design with four replications. The area of each sub-plot was 60 m² (10 m × 6 m). FYM was applied to the experimental field about 20–25 days before sowing of the crops and it was incorporated into the top-soil layer (above 15–20 cm). N was applied in two equal splits at sowing and 25–30 days after sowing for both the crops. The nutrient content of FYM applied in 2012 is represented in Table 2.

Table 2. Nutrient composition of farmyard manure applied during 2012.

| Nutrient | Content (%) | Nutrient | Content (mg kg⁻¹) |
|----------|-------------|----------|-------------------|
| C        | 39.53       | Zn       | 57                |
| N        | 1.21        | Mn       | 28                |
| P        | 0.58        | Cu       | 239               |
| K        | 0.426       | Fe       | 2214              |
| S        | 0.16        |          |                   |

No chemical fertilizers except urea were applied to the experimental site since the beginning of this experiment. The land was prepared conventionally for sowing the crops. Harvesting of both the crops was carried out at 2–5 cm above the ground level and the leftover stubble and roots of the crops were incorporated. No extra residues of any crops were added to the soil. Manual harvesting of the crops was carried out at their maturity stages.

2.4. Collection of Soil Samples and Analysis

Soil samples were taken in November 2013 before application of treatments to wheat from 0–15 cm depth using a 5 cm diameter augur. Each sample was a composite of three places in a plot. Each sample was mixed thoroughly, sieved through 2 mm sieve and stored in plastic bags below 4 °C until analysis for different soil properties. Thus, soil samples represent winter and summer applications of FYM which allowed 12- and 6-month mineralization periods, respectively, and also the combination of the two applications. Organic carbon in the surface soils was determined by Walkley and Black method [20] and total nitrogen as per the method suggested by Bremner [21].
2.5. **Dissolved Organic Matter**

50 mL of deionized water was added to 12 g of dry soil and this was subjected to shaking for 1 h on a horizontal shaker followed by centrifuging for 30 min at 8000 rpm. The solution was filtered through 0.45 μm polysulfone filter membrane and the filtrate was collected and stored in a freezer until laboratory analysis. Determination of dissolved organic carbon (DOC) was carried out with the dichromate acid oxidation method [22], while dissolved organic nitrogen (DON) was determined by Kjeldahl distillation after oxidizing the DOM by 1% potassium persulphate reagent [23].

2.6. **Microbial Biomass**

Moist soil samples of 25 g (oven-dry basis) were fumigated with ethanol-free CHCl₃ and extracted with 0.5 M K₂SO₄ [24]. Another set of unfumigated samples was similarly extracted. The extract was digested with K₂Cr₂O₇ followed by back titration with ferrous ammonium sulphate. Subsequently, biomass carbon was estimated using the following equation: Biomass C = 2.64 E_c, where E_c denotes the difference between organic carbon extracted by 0.5 M K₂SO₄ from fumigated and non-fumigated soils. The extra N released by CHCl₃ was calculated as the difference between N extracted by 0.5 M K₂SO₄ from the fumigated and non-fumigated soils [25].

2.7. **Light Fraction**

Light fraction SOM was isolated in the surface soil by dispersing the representative soil sample in NaI solution (specific gravity 1.70) and by removing the suspended material by vacuum suction. The light fraction was washed, dried, ground and analyzed for total C and N by CHN analyzer (Perkin Elmer Model 2400 series II).

2.8. **Statistical Analysis**

Effect of FYM application during both the seasons (summer and winter) with three doses in each mode of application and also with or without mineral N and their interactions on the three labile pools C and N were analyzed [26], where the F-test indicated a significant \((p = 0.05)\) effect. Means were separated according to LSD \((p = 0.05)\) using appropriate error mean squares.

3. **Results**

The effects of different treatment combinations on soil quality indicators viz., soil organic carbon (SOC), total nitrogen (TN), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), light fraction carbon (LFC) and nitrogen (LFN) were statistically studied with the help analysis of variance (ANOVA) technique. Table 3 represents the \(F\) values obtained for the main effects as well as interaction impacts of all treatments along with their statistical significance.

| Source of Variation   | df  | SOC     | TN     | DOC    | DON    | MBC    | MBN    | LFC    | LFN    |
|-----------------------|-----|---------|--------|--------|--------|--------|--------|--------|--------|
| Mode (M)              | 2   | 61.96 * | 137.3 *| 414.3 *| 482.8 *| 147.7 *| 241.4 *| 224.9 *| 36.12 *|
| Rate (R)              | 2   | 72.55 * | 141.6 *| 437.1 *| 465.2 *| 99.13 *| 99.45 *| 228.4 *| 44.25 *|
| M × R                 | 4   | 6.34 *  | 6.95 * | 87.17 *| 63.90 *| 7.42 * | 11.73 *| 28.60 *| 4.82 * |
| Inorganic N (N)       | 1   | 26.12 * | 34.27 *| 191.8 *| 156.7 *| 403.8 *| 210.8 *| 34.58 *| 60.49 *|
| M × N                 | 2   | 0.72    | 2.50   | 1.57   | 11.40 *| 22.81 *| 23.77 *| 0.65   | 1.78   |
| R × N                 | 2   | 0.25    | 0.02   | 7.68 * | 14.98 *| 9.28 * | 5.89 * | 0.20   | 2.68   |
| M × R × N             | 4   | 0.54    | 1.64 * | 3.70 * | 1.64   | 12.10 *| 2.27   | 1.08   | 0.83   |

* Significant at \(p \leq 0.05\).
3.1. FYM Modes and Rates Interaction

3.1.1. Organic Carbon and Total Nitrogen Build-Up

The ANOVA yielded a significant interaction between different modes and rates of FYM application concerning SOC and TN as depicted in Table 3. However, for mineral Nitrogen, the only main effects were significant. The interaction between modes and rates of FYM application suggested that the effect of different rates of FYM depended on its time of application (Figure 1a–h).

**Figure 1.** Effect of FYM modes and rates interaction on (a) soil organic C (SOC); (b) Dissolved organic C (DOC); (c) microbial biomass C (MBC); (d) light fraction C (LFC); (e) total N (TN); (f) dissolved organic N (DON); (g) microbial biomass N (MBN); and (h) light fraction N (LFN). Bars with different letters in lowercase are significantly different at $p = 0.05$ according to LSD. Lines above the bars denote standard deviation ($n = 3$). S5, S10, and S15 denote summer applied FYM at 5, 10, and 15 Mg ha$^{-1}$, respectively; W5, W10, and W15 denote winter applied FYM at 5, 10, and 15 Mg ha$^{-1}$, respectively; S5W5, S10W10, and S15W15 denote both seasons applied FYM at 5, 10, and 15 Mg ha$^{-1}$ in each season, respectively.
A post hoc analysis using the least significant difference (LSD) further revealed that SOC were significantly higher when FYM was applied for both seasons. It was also found that FYM applied during summer only resulted in significant improvement of SOC over the winter applied FYM at all the rates of application. Almost 8 to 34% higher SOC build-up in the soil was observed in both season FYM applications at the three rates than the summer applications. Similar trends were also observed in TN build-up through the FYM application and about 16 to 25% higher build-up was recorded in both season FYM applications at the three rates than summer applications. Figure 2 depicts the increase in soil organic C (SOC) from the initiation of the study to 45 years of FYM application at different rates and modes. SOC was increased up to 19.1 g kg$^{-1}$ from the initial 4.2 g kg$^{-1}$ with the application of 15 Mg ha$^{-1}$ FYM both in summer and winter. The application of FYM at 5 Mg ha$^{-1}$ in the winter season only resulted in about 120% increment in SOC content from the initial soil status.

![Figure 2. Relative increase (%) in soil organic carbon (SOC) status from initial to 45 years of study. Initial SOC value was 4.2 g kg$^{-1}$; S5, S10, and S15 denote summer applied FYM at 5, 10, and 15 Mg ha$^{-1}$, respectively; W5, W10, and W15 denote winter applied FYM at 5, 10, and 15 Mg ha$^{-1}$, respectively; SSW5, S10W10, and S15W15 denote both seasons applied FYM at 5, 10, and 15 Mg ha$^{-1}$ in each season, respectively.](image)

3.1.2. Labile Soil Organic Matter Pools

Significant main and interaction effects of FYM modes and rates were observed on the carbon and nitrogen fractions of all the three LSOMp (Table 3). All of the carbon and nitrogen fractions had significantly higher concentrations when FYM was applied in summer as compared to winter in each FYM rate (Figure 1a–h). Higher concentrations were observed in summer of DOC and DON by 15–22% and 15–33%; MBC and MBN by 14–30% and 21–24%; and LFC and LFN by 14–23% and 12–14%, respectively, as compared to the winter application. Significantly higher concentrations were also observed in both season FYM applications as compared to summer and winter applications in each FYM rate due to the cumulative effect. Figure 3 represents the changes in the labile soil organic matter pools after 45 years of FYM amendments. Figure 3 clearly depicts that the dissolved organic carbon, microbial biomass carbon, and light fraction carbon increased to the tune of about 600, 1200, and 700 times, respectively, from their respective initial values.
organic carbon, microbial biomass carbon, and light fraction carbon increased to the tune of about 600, 1200, and 700 times, respectively, from their respective initial values.

3.2. Mineral N Interaction with FYM Modes and Rates

The application of mineral N at 120 kg ha⁻¹ significantly increased DON in all the three FYM modes (Table 4). The increase varied from 43 to 58% among the three modes. Interaction between mineral N and FYM modes was also significant with MBC and MBN. MBC and MBN increased due to mineral N application by 30 to 32% and 51 to 83%, respectively, among the three modes.

Nitrogen application also increased carbon and nitrogen contents of both dissolved organic matter and microbial biomass at the three rates of FYM (Table 5). The increase in DOC and DON concentrations were 12 to 21% and 33 to 61%, respectively, while MBC and MBN were 20 to 30% and 36 to 76%, respectively, at the three FYM rates. The mineral N did not have any significant effect on the light fraction of soil organic matter at the different rates or modes of FYM.

![Graph showing relative increase in soil organic matter pools from initial to 45 years of study.]

Table 4. Interaction effects of different FYM modes and rate of application on organic carbon, total nitrogen, and carbon and nitrogen contents of labile soil organic matter pools.

| FYM Modes       | Farmyard Manure Application Rate (Mg ha⁻¹) | 05  | 10  | 15  | 05  | 10  | 15  |
|-----------------|------------------------------------------|-----|-----|-----|-----|-----|-----|
|                 | OC a (g kg⁻¹)                            |     |     |     |     |     |     |
| Summer          |                                          | 10.8| 12.1| 14.2| 1052| 1188| 1309|
| Winter          |                                          | 9.3 | 10.8| 12.6| 967 | 1121| 1208|
| Both seasons    |                                          | 11.7| 14.6| 19.1| 1194| 1366| 1632|
| LSD (p ≤ 0.05)  |                                          | 1.4 |     |     |     | 66  |     |
Table 4. Cont.

| FYM Modes       | Farmyard Manure Application Rate (Mg ha\(^{-1}\)) | DOC \(^{c}\) (mg kg\(^{-1}\)) | DON \(^{d}\) (mg kg\(^{-1}\)) |
|-----------------|--------------------------------------------------|-------------------------------|-------------------------------|
|                 | 05 10 15 05 10 15 05 10 15                        |                               |                               |
| Summer          | 151 213 268 24.4 31.5 44.4                         |                               |                               |
| Winter          | 129 174 234 21.3 26.0 33.5                         |                               |                               |
| Both seasons    | 188 333 562 28.9 54.1 70.1                         |                               |                               |
| LSD (\(p \leq 0.05\)) | 24.14 2.9                                         |                               |                               |
| Summer          | 372 475 598 56.1 71.2 86.0                         |                               |                               |
| Winter          | 286 382 524 46.2 57.4 71.9                         |                               |                               |
| Both seasons    | 534 710 999 88.8 113.3 158.4                       |                               |                               |
| LSD (\(p \leq 0.05\)) | 77.29 10.6                                        |                               |                               |
| Summer          | 0.90 1.09 1.52 69.5 84.1 102.6                     |                               |                               |
| Winter          | 0.73 0.96 1.23 61.0 74.8 91.3                       |                               |                               |
| Both seasons    | 1.18 1.60 2.61 83.8 106.1 154.6                     |                               |                               |
| LSD (\(p \leq 0.05\)) | 0.15 16.8                                        |                               |                               |

\(^{a}\) SOC, soil organic carbon; \(^{b}\) TN, total N; \(^{c}\) Carbon contents of labile soil organic matter pools viz. DOC (Dissolved organic carbon), MBC (Microbial biomass carbon) and LFC (Light fraction carbon); \(^{d}\) Nitrogen contents of labile soil organic matter pools viz. DON (Dissolved organic nitrogen), MBN (Microbial biomass nitrogen) and LFN (Light fraction nitrogen).

Table 5. Interaction effects of different farmyard manure modes of application and inorganic N on carbon and nitrogen contents of labile soil organic matter pools.

| FYM Modes       | Inorganic N (kg ha\(^{-1}\)) | DOC \(^{a}\) (mg kg\(^{-1}\)) | DON \(^{b}\) (mg kg\(^{-1}\)) |
|-----------------|------------------------------|-------------------------------|-------------------------------|
|                 | 0 120 0 120                  |                               |                               |
| Summer          | 192 229 27.0 39.8            |                               |                               |
| Winter          | 164 194 22.2 31.7            |                               |                               |
| Both seasons    | 340 381 39.5 62.6            |                               |                               |
| LSD (\(p \leq 0.05\)) | Ns 4.30                     |                               |                               |
| Summer          | 416 547 56.6 85.6            |                               |                               |
| Winter          | 345 449 44.8 72.1            |                               |                               |
| Both seasons    | 645 850 84.8 155.5           |                               |                               |
| LSD (\(p = 0.05\)) | 25.1 10.39                 |                               |                               |
| Summer          | 1033 1313 72.7 96.2          |                               |                               |
| Winter          | 887 1060 67.3 84.0           |                               |                               |
| Both seasons    | 1685 1911 108.5 121.1        |                               |                               |
| LSD (\(p \leq 0.05\)) | Ns Ns                       |                               |                               |

\(^{a}\) Labile soil organic matter pools viz. DOC (dissolved organic carbon), MBC (microbial biomass carbon) and LFC (light fraction carbon); \(^{b}\) Nitrogen contents of labile soil organic matter pools viz. DON (dissolved organic nitrogen), MBN (microbial biomass nitrogen) and LFN (light fraction nitrogen); Ns: not significant at \(p = 0.05\).

3.3. Modes and Rates of FYM and Mineral N Interaction

3.3.1. Soil Organic Carbon and Total Nitrogen

Tables 6 and 7 represent the treatment effects of mineral N and different modes and rates of FYM on SOC and TN build-up in soil, respectively. The variations in SOC and TN concentrations among the different treatments were between 4.6 to 19.5 g kg\(^{-1}\) and 402 to 1032 mg kg\(^{-1}\), respectively. TN increased significantly with increasing FYM rates.
at each mode and mineral N. TN was found to be higher in summer applications FYM as compared to winter applications at 5 and 10 Mg ha\(^{-1}\) in N control plots. At 120 kg ha\(^{-1}\) N, it was significantly higher only in summer FYM application of 5 Mg ha\(^{-1}\) as compared to winter application. It was interesting to understand that TN was significantly higher when FYM was applied at 5 Mg ha\(^{-1}\) during summer as well as winter than the one-time application of 10 Mg ha\(^{-1}\) during either summer or winter. The percent increase in SOC build-ups by mineral N at 120 kg ha\(^{-1}\) in the different FYM applications were between 2–16% in winter; 12–18% in summer and 4–13% and in both seasons. The corresponding increases in TN build-up were 4–15% in summer; 3–9% in winter; and 13–14% in both season FYM applications.

3.3.2. Dissolved Organic Matter

The variations in DOC and DON concentrations were from 85 to 578 mg kg\(^{-1}\) and from 6.9 to 87.7 mg kg\(^{-1}\), respectively. It was observed that DOC and DON were 1.38 to 2.96% and 1.71 to 5.06% of SOC and TN, respectively. There was a significant decrease in DOC in winter as compared to summer application at each FYM and mineral N rate. Mineral N was found to increase DOC concentrations in all the FYM treatments (Table 6).

| FYM Doses | Inorganic N (kg ha\(^{-1}\)) | DOC\(^a\) (mg kg\(^{-1}\)) | DON\(^b\) (mg kg\(^{-1}\)) |
|-----------|-----------------------------|-----------------------------|-----------------------------|
|           | 0  | 120 | 0  | 120 | 0  | 120 | 0  | 120 |
| 05        |    | 145 | 167 | 21.3 | 28.3 |
| 10        | 217 | 263 | 29.6 | 44.8 |
| 15        | 335 | 375 | 37.7 | 60.9 |
| LSD (\(p \leq 0.05\)) | 9.0 | 4.3 |
| MBC\(^a\) (mg kg\(^{-1}\)) | 377 | 452 | 46.1 | 81.2 |
| MBN\(^b\) (mg kg\(^{-1}\)) | 455 | 589 | 62.9 | 98.3 |
| 15        | 608 | 791 | 98.4 | 133.8 |
| LSD (\(p \leq 0.05\)) | 25 | 10.4 |
| LFC\(^a\) (g kg\(^{-1}\)) | 806 | 1067 | 59.9 | 83.0 |
| LFN\(^b\) (g kg\(^{-1}\)) | 1117 | 1325 | 79.4 | 95.2 |
| 15        | 1683 | 1893 | 109.3 | 123.0 |
| LSD (\(p \leq 0.05\)) | NS | NS |

\(^a\) Labile soil organic matter pools viz. DOC (dissolved organic carbon), MBC (microbial biomass carbon) and LFC (light fraction carbon); \(^b\) Nitrogen contents of labile soil organic matter pools viz. DON (dissolved organic nitrogen), MBN (microbial biomass nitrogen) and LFN (light fraction nitrogen); NS: not significant at \(p = 0.05\).

Thus, the role of fertilizer nitrogen may be seen as a catalyst in increasing the concentration of DOM in FYM amended soils. In summer, the maximum increase in DOC concentration was about 30% observed in 10 Mg ha\(^{-1}\) FYM applied plot, while in winter it was about 25% in the 15 Mg ha\(^{-1}\) FYM applied plot. Thus, an increase in the concentration of DOC due to mineral N application depends on both the rate as well as the time of FYM application. When FYM was applied during both seasons the percent increase in DOC with the application of mineral N application depends on both the rate as well as the time of FYM application. When FYM was applied during both seasons the percent increase in DOC with the application of mineral N application depends on both the rate as well as the time of FYM application. When FYM was applied during both seasons the percent increase in DOC with the application of mineral N application depends on both the rate as well as the time of FYM application. When FYM was applied during both seasons the percent increase in DOC with the application of mineral N application depends on both the rate as well as the time of FYM application. When FYM was applied during both seasons the percent increase in DOC with the application of mineral N application depends on both the rate as well as the time of FYM application.
3.3.3. Microbial Biomass

MBC varied from 85 mg kg\(^{-1}\) in the control plot to 1169 mg kg\(^{-1}\) in the plot at the highest FYM application rate per annum (30 Mg ha\(^{-1}\) year\(^{-1}\)) and mineral N at 120 kg ha\(^{-1}\) (Table 6). MBN varied between 12.14 and 208.75 mg kg\(^{-1}\) in the corresponding treatments (Table 7). Thus, MBC and MBN increased up to 13.75 and 17.21 times due to the various management practices of FYM and mineral N. MBC varied between 1.85 and 5.72% of soil organic carbon while MBN between 3.01 and 12.05% of total nitrogen. A significant decrease in MBC was observed in winter as compared to summer application at each FYM rate and mineral N. Thus, mineral N increased microbial activity at each rate of FYM in each mode.

**Table 7.** Soil organic carbon and labile soil organic carbon pools as influenced by varied FYM modes, rates of application, and inorganic nitrogen after 42 years of experimentation.

| FYM Application | OC (g kg\(^{-1}\)) | DOC (mg kg\(^{-1}\)) | MBC (mg kg\(^{-1}\)) | LFC (mg kg\(^{-1}\)) |
|-----------------|-------------------|-------------------|-----------------|------------------|
| Modes Rates (Mg ha\(^{-1}\)) | N\(_0\) | N\(_{120}\) | N\(_0\) | N\(_{120}\) | N\(_0\) | N\(_{120}\) | N\(_0\) | N\(_{120}\) |
| S 05 | 9.9 \(\text{Aa}\) | 11.7 \(\text{Aa}\) | 140 \(\text{Gb}\) | 162 \(\text{Ha}\) | 300 \(\text{Fb}\) | 442 \(\text{Fa}\) | 714 \(\text{Aa}\) | 1082 \(\text{Aa}\) |
| 10 | 11.4 \(\text{Aa}\) | 12.8 \(\text{Aa}\) | 185.0 \(\text{Eb}\) | 240 \(\text{Ea}\) | 411 \(\text{Eb}\) | 538 \(\text{Ea}\) | 1003 \(\text{Aa}\) | 1195 \(\text{Aa}\) |
| 15 | 13.2 \(\text{Aa}\) | 15.1 \(\text{Aa}\) | 250.0 \(\text{Cb}\) | 285 \(\text{Ca}\) | 534 \(\text{Cb}\) | 662 \(\text{Ca}\) | 1382 \(\text{Aa}\) | 1664 \(\text{Aa}\) |
| W 05 | 9.2 \(\text{Aa}\) | 9.4 \(\text{Aa}\) | 124 \(\text{Bb}\) | 133 \(\text{Ia}\) | 243 \(\text{Gb}\) | 328 \(\text{Ga}\) | 584 \(\text{Aa}\) | 872 \(\text{Aa}\) |
| 10 | 10.2 \(\text{Aa}\) | 11.3 \(\text{Aa}\) | 160.0 \(\text{Fb}\) | 188 \(\text{Ga}\) | 330 \(\text{Fb}\) | 433 \(\text{Fa}\) | 875 \(\text{Aa}\) | 1055 \(\text{Aa}\) |
| 15 | 11.6 \(\text{Aa}\) | 13.5 \(\text{Aa}\) | 208.0 \(\text{Db}\) | 260 \(\text{Da}\) | 462 \(\text{Db}\) | 542 \(\text{Da}\) | 1202 \(\text{Aa}\) | 1253 \(\text{Aa}\) |
| B 05 | 11.0 \(\text{Aa}\) | 12.4 \(\text{Aa}\) | 170.0 \(\text{Fb}\) | 205 \(\text{Fa}\) | 482 \(\text{Db}\) | 585 \(\text{Da}\) | 1120 \(\text{Aa}\) | 1246 \(\text{Aa}\) |
| 10 | 14.1 \(\text{Aa}\) | 15.1 \(\text{Aa}\) | 305.0 \(\text{Bb}\) | 360 \(\text{Ba}\) | 624 \(\text{Bb}\) | 795 \(\text{Ba}\) | 1473 \(\text{Aa}\) | 1724 \(\text{Aa}\) |
| 15 | 18.7 \(\text{Aa}\) | 19.5 \(\text{Aa}\) | 546.0 \(\text{Ab}\) | 578 \(\text{Aa}\) | 829 \(\text{Ab}\) | 1116 \(\text{Aa}\) | 2464 \(\text{Aa}\) | 2763 \(\text{Aa}\) |
| Control | 4.6 | 6.1 | 85 | 105 | 85 | 134 | 370 | 370 |

S, summer; W, winter; B, both seasons; N\(_0\), no inorganic N; N\(_{120}\), 120 kg N ha\(^{-1}\); OC, organic carbon; DOC (dissolved organic carbon), MBC (microbial biomass carbon), LFC (light fraction carbon); means in columns within each N rate with the same upper case letters are not significantly different according to least-square means for FYM rates and modes and inorganic N interactions adjusted for multiple comparisons at \(p = 0.05\); means in rows with each FYM modes and rates with the same lower case letters are not significantly different according to least-square means for FYM doses and modes and inorganic N interactions adjusted for multiple comparisons at \(p = 0.05\).

3.3.4. Light Fraction

LFN and LFN varied from 370 to 2763 mg kg\(^{-1}\) and 49 to 160 mg kg\(^{-1}\), respectively, in the plots with different management practices (Tables 6–8). LFC constituted 7.21 to 14.17% of organic carbon while LFN constituted 5.52 to 9.23% of total N. It was observed that the different FYM and mineral N treatments did not have any significant influence on these contents of the light fraction.

**Table 8.** Soil organic nitrogen and soil organic nitrogen pools as influenced by FYM modes, doses, and inorganic nitrogen after 35 years of application.

| FYM Application | TN (mg kg\(^{-1}\)) | DON (mg kg\(^{-1}\)) | MBN (mg kg\(^{-1}\)) | LFN (mg kg\(^{-1}\)) |
|-----------------|-------------------|-------------------|-----------------|------------------|
| Modes Rates (Mg ha\(^{-1}\)) | N\(_0\) | N\(_{120}\) | N\(_0\) | N\(_{120}\) | N\(_0\) | N\(_{120}\) | N\(_0\) | N\(_{120}\) |
| S 05 | 979 \(\text{Eb}\) | 1124 \(\text{Fa}\) | 22.0 \(\text{Aa}\) | 26.7 \(\text{Aa}\) | 43.1 \(\text{Aa}\) | 69.1 \(\text{Aa}\) | 54.0 \(\text{Aa}\) | 84.8 \(\text{Aa}\) |
| 10 | 1143 \(\text{Da}\) | 1233 \(\text{Da}\) | 25.1 \(\text{Aa}\) | 37.9 \(\text{Aa}\) | 59.6 \(\text{Aa}\) | 82.8 \(\text{Aa}\) | 71.5 \(\text{Aa}\) | 90.7 \(\text{Aa}\) |
| 15 | 1283 \(\text{Ba}\) | 1334 \(\text{Ca}\) | 33.8 \(\text{Aa}\) | 54.9 \(\text{Aa}\) | 67.0 \(\text{Aa}\) | 105.1 \(\text{Aa}\) | 92.5 \(\text{Aa}\) | 113.0 \(\text{Aa}\) |
| W 05 | 923 \(\text{Fa}\) | 1010 \(\text{Fa}\) | 18.1 \(\text{Aa}\) | 24.5 \(\text{Aa}\) | 31.0 \(\text{Aa}\) | 61.3 \(\text{Aa}\) | 48.1 \(\text{Aa}\) | 73.9 \(\text{Aa}\) |
| 10 | 1102 \(\text{Eb}\) | 1140 \(\text{Da}\) | 21.6 \(\text{Aa}\) | 30.3 \(\text{Aa}\) | 47.1 \(\text{Aa}\) | 67.7 \(\text{Aa}\) | 67.3 \(\text{Aa}\) | 82.2 \(\text{Aa}\) |
| 15 | 1192 \(\text{Ba}\) | 1224 \(\text{Ca}\) | 26.8 \(\text{Aa}\) | 40.2 \(\text{Aa}\) | 56.3 \(\text{Aa}\) | 87.4 \(\text{Aa}\) | 86.6 \(\text{Aa}\) | 96.0 \(\text{Aa}\) |
### Table 8. Cont.

| FYM Application | TN (mg kg<sup>−1</sup>) | DON (mg kg<sup>−1</sup>) | MBN (mg kg<sup>−1</sup>) | LFN (mg kg<sup>−1</sup>) |
|-----------------|-------------------------|------------------------|------------------------|------------------------|
| Modes Rates (Mg ha<sup>−1</sup>) | N₀ | N₁₂₀ | N₀ | N₁₂₀ | N₀ | N₁₂₀ | N₀ | N₁₂₀ |
| B 05            | 1162<sup>Ca</sup> | 1194<sup>Ba</sup> | 23.9<sup>Aa</sup> | 33.8<sup>Aa</sup> | 64.3<sup>Aa</sup> | 113.3<sup>Aa</sup> | 77.2<sup>Aa</sup> | 90.4<sup>Aa</sup> |
| 10              | 1279<sup>Bb</sup> | 1453<sup>Ba</sup> | 42.0<sup>Aa</sup> | 66.2<sup>Aa</sup> | 82.1<sup>Aa</sup> | 144.6<sup>Aa</sup> | 99.3<sup>Aa</sup> | 112.8<sup>Aa</sup> |
| 15              | 1532<sup>Ab</sup> | 1732<sup>Aa</sup> | 52.5<sup>Aa</sup> | 87.7<sup>Aa</sup> | 108.1<sup>Aa</sup> | 208.8<sup>Aa</sup> | 149.1<sup>Aa</sup> | 160.0<sup>Aa</sup> |
| Control         | 402 | 685 | 6.9 | 14.6 | 12.1 | 25.3 | 49 | 68 |

S, summer; W, winter; B, both seasons; N₀, no inorganic N; N₁₂₀, 120 kg N ha<sup>−1</sup>; TN, total N; DON (dissolved organic nitrogen); MBN (microbial biomass nitrogen); LFN (light fraction nitrogen); means in columns within each N rate with the same uppercase letters are not significantly different according to least-square means for FYM rates and modes and inorganic N interactions adjusted for multiple comparisons at p ≤ 0.05; means in rows with each FYM modes and rates with the same lowercase letters are not significantly different according to least-square means for FYM doses and modes and inorganic N interactions adjusted for multiple comparisons at p ≤ 0.05.

### 4. Discussion

#### 4.1. SOC and TN Build-Up

Long term application of organic amendment such as FYM, root biomass, and crop residues to the soil were ultimately reflected through the increase in SOC [27]. Improved soil physicochemical properties and the biological environment through the addition of root biomass C was observed, as they are beneficial for crop growth and these resulted in higher C-sequestration [28]. Significant loss of SOC and TN was found in the winter FYM applied plot as compared to summer applied plots. The winter application was about five months earlier than the summer application. Application of organic manures increases C-sequestration as they contain most of the C in recalcitrant form and these organic manures have already gone through some decomposition before their application in the agricultural fields [29]. Still, under subtropical climate, SOC and TN of winter applied FYM plots were lower by 11% to 16% and 6% to 8%, respectively, as compared to summer applied plots. Recycling of soil C into the atmosphere might be controlled by FYM application as its application to the soil helped in the formation of chemically resistant humic substances [30]. These humic substances are accumulated in the soil, and so they are supposed not to be decomposed by the soil microbes. However, their relative inherent recalcitrance may depend upon the climatic condition and microbial mineralization rates of humic and fulvic acid fractions [30]. Such recalcitrance refers to molecular level characteristics such as functional groups, elemental composition, and molecular conformation that control the natural biodegradability [31]. Similarly, the N status of soil is altered significantly due to changes in organic matter sowing to their composition, varying stages of decomposition and ecological factors [32]. Thus, the increase in SOC and TN depends upon the addition of organic matter in soil and their recalcitrance.

#### 4.2. Dissolve Organic Matter

In this study, DOC content was increased with the FYM application. This might be discussed as the application of FYM in the soil helps in the direct addition of water-soluble C. Antil and Singh [33] also reported that there is a linear relationship between organic manure application and the buildup of water-soluble C in the soil. Soil DOC content has increased to the tune of almost 2.7–3.2 times through the application of both organic manures and chemical fertilizers into the soil [34]. There was a significant increase in DOC and DON concentrations in soil by N application. The increase in DOC might be due to the application of fertilizer N which improved crop biomass and returned more crop remains into the soil. DOM, being highly mobile in soils can have an influence on acidity, microbial activity, nutrient availability and mobility [5].

#### 4.3. Microbial Biomass Carbon

The abundance of microbial activity owing to the application of mineral N was also previously reported by Francioli [35]. SOC buildup was higher with the application of FYM and this might be the reason behind such enhancement of MBC in the present study.
Metabolic activity of the soil microbes might be increased by utilizing root exudation under FYM treated plots where soil C was higher [36]. Singh et al. [37] also reported that the increase in MBC through organic manure amendment was much higher than that of mineral fertilizer application. Moreover, binding agents such as exocellular mucilaginous polysaccharides, major food and energy for soil fauna, is formed due to the abundance of the varied microbial community associated with the decomposing particulate organic matter which is considered as one of the key attributes of soil quality [38]. Hence, soil microbial biomass measurements are considered as one of the fundamental tools to assess the changes in SOM under varied soil management in different climatic conditions.

4.4. Light Fraction of SOM

LFC and LFN varied from 370 to 2763 mg kg\(^{-1}\) and from 49 to 160 mg kg\(^{-1}\), respectively, in the plots with different management practices which constituted 6.30–14.15% of organic carbon, and 5.72–9.73% total N. The light fraction comprises a small portion of soil mass. However, owing to having high C and N content, it could explain a substantial portion of total carbon and nitrogen pools in the soil [39]. Janzen et al. [40] reported that the light fraction accounts for 2–18% of the soil carbon and 1–12% of the total N. LFC is highly labile and mainly composed of partially decomposed plant residues [41]. In this study, a higher amount of LFC was found through the FYM application. This finding confirms that the light fractions can be changed rapidly in responses to management. The gap of six months between the two modes of FYM application might have led to more decomposition of soil organic matter, and reduced the LFC in winter applied plots.

5. Conclusions

Thus, in cultivated soils under wheat–pearl millet cropping sequence, proper management of FYM and mineral N will have to be carried out for improvement in labile SOM pools, SOC, and TN. The application of 15 Mg ha\(^{-1}\) FYM in both seasons significantly improved all the labile soil organic matter pools. Application of N at 120 kg ha\(^{-1}\) also showed significant improvement in the microbial biomass and dissolve organic N in the soil. It was also comprehended that the microbial biomass was the most sensitive among all the different LSOMp to the changes in FYM amendment as it had the most variation in its carbon and nitrogen content. On the other hand, light fraction showed persistence in the soil during the year, as there was no significant change in its concentration due to the different modes or rates of FYM application along with mineral N. Thus, from this study it can be recommended that the FYM application at 15 Mg ha\(^{-1}\) in both summer and winter + 120 kg ha\(^{-1}\) mineral N can improve the labile soil organic matter pools and build-up soil organic carbon under wheat–pearl millet system in sub-tropical soil. This finding will help the researchers those who are working on the different soil organic matter properties as influenced by varied management practices in the sub-tropical climatic condition. The fate of the LSOMp should further be explored in different cropping systems under sub-tropical and tropical climates where the turnover is rapid.

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Abbreviations

| Abbreviation | Description                       |
|--------------|-----------------------------------|
| DOC          | Dissolved organic carbon          |
| DOM          | Dissolved organic matter          |
| DON          | Dissolved organic nitrogen        |
| FYM          | Farmyard manure                   |
| LF           | Light fraction                    |
| LFC          | Light fraction carbon             |
| LFN          | Light fraction nitrogen           |
| LSD          | Least significant difference      |
| LSOMp        | Labile soil organic matter pool   |
| MB           | Microbial biomass                 |
| MBC          | Microbial biomass carbon          |
| MBN          | Microbial biomass nitrogen        |
| SOC          | Soil organic carbon               |
| SOM          | Soil organic matter               |
| TN           | Total nitrogen                    |

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