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Changes in soil organic carbon, labile carbon pools and microbial community as influenced by tillage systems, organic and synthetic fertilizers and straw alters in sub-tropical agro-ecosystem: A review

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Abstract

Soil microbial biomass plays a significant role in soils, and it is often used as an early indicator of change in soil quality. Soil microbial biomass is affected by different fertilization management practices. Therefore, the impact of different long-term fertilization management practices on the soil organic carbon (SOC) content, soil microbial biomass carbon (SMBC), and soil microbial biomass nitrogen (SMBN), as well as the soil microbial quotient (SMQ) in the tilled layer (0.00-0.20 m) were included in the review study. Organic Carbon content was highest in 0.25–1mm aggregate (6.9–9.6 g kg^{-1}) prior to incubation, followed by >2mm aggregates (2.2–5.8 g kg^{-1}), 1–2mm aggregates (2.4–4.6 g kg^{-1}) and <0.25 mm aggregates (3.3–4.5 g kg^{-1}). After 360-day incubation with straw incorporation, organic C content was 2.3–4.5 g kg^{-1},2.9–5.0 g kg^{-1},7.2–11 g kg^{-1} and 1.8–3.0 g kg^{-1} in >2, 1–2, 0.25–1, and < 0.25mm aggregates, respectively, with the highest in the IFMS treatment. Straw-derived C content was 0.02–0.05 g kg^{-1}, 0.03–0.04 g kg^{-1}, 0.11–0.13 g kg^{-1}, and 0.05–0.10 g kg^{-1} in >2, 1–2, 0.25–1, and < 0.25 mm aggregates, respectively. The relative distribution of straw-derived C was highest (40–49%) in 0.25–1 mm aggregate, followed by <0.25mm aggregates (21–31%), 1–2mm aggregates (13–15%), and > 2 mm aggregates (9.4–16%). Transition from conventional tillage to no-tillage may alter the depth distribution of soil organic carbon (SOC) and its chemical composition. Aggregate stability, as indicated with the mean weight diameter, was higher for NTS and CTS than that for CT. SOC occluded in the >1 mm fraction was higher under NTS and CTS than under CT. Both NTS and CTS had more aliphatic carbon than CT did, and CT contained more aromatic carbon in the 0–20 cm layer. Chemical fractionation showed long-term MNPK fertilization strongly increased the SOC storage in both soil layers (0-20 cm = 1492.4 g C m (2) and 20-40 cm = 1770.6 g C m (2)) because of enhanced recalcitrant C (RC) and labile C (LC). Overall, application of N and NPK fertilizers cannot significantly increase the SOC storage but enhanced C in mSOM of aggregates, whereas MNPK fertilizer resulted in the greatest amount of SOC storage (about 5221.5 g C m (2)) because of the enhanced SOC in LF, iPOM and mSOM of each aggregate. The SNPK fertilizer increased SOC storage in >250 µm aggregates but reduced SOC storage in <250 µm aggregates due to SOC changes in LF and iPOM.

Keywords: tillage practice, microbial community, labile carbon fractions, recalcitrant carbon fraction

Introduction

Soil organic matter, the largest terrestrial carbon (C) pool, plays an important role in improving soil fertility and sustaining soil productivity (Gong *et al*., 2009; He *et al*., 2015) [14, 18]. Many agricultural management practices, such as fertilizer application, straw return, and tillage, could affect the soil organic carbon (SOC) pool (Zhu *et al*., 2015; Zhao *et al*., 2016) [55, 52] especially the labile organic carbon fractions, such as dissolved organic carbon (DOC), microbial biomass carbon (MBC) and particulate organic carbon (POC). DOC and MBC, considered to be soil active organic C, have been reported as important factors in impacting soil fertility (Lei *et al*., 2010) [23]. Moreover, the formation, migration and transformation of DOC can affect soil microbial community structure and activity (Tian *et al*., 2012) [42] and the formation of mineral-associated organic carbon (MaOC) (Sokol *et al*., 2019) [39] which usually represents the largest fraction of SOC pool (Kogel-Knabner *et al*., 2008) [22]. POC has been considered as a transition state of stable organic C, which is predominantly of plant origin and has higher inherent biochemical recalcitrance than MaOC (Giannetta *et al*., 2018; Haddix *et al*., 2020) [12, 15]. Straw is rich in organic matter, and straw return can enhance SOC and crop yield (Kang *et al*., 2015; Wang *et al*., 2015a; Li *et al*., 2016) [21, 47, 25].

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Benbi *et al*. (2015) [3] showed marked increases in total organic carbon (TOC) and LOC contents in a 0-15 cm soil layer following the long-term application of farmyard manure and rice straw. In a subtropical paddy field in China, rice straw return could greatly enhance soil TOC, LFOC, DOC, and MBC contents in both early and late paddy seasons (Wang *et al*., 2015b) [46]. Hao *et al*. (2019) [16] showed that over the long-term, straw return significantly increased the SOC and MBC content in the 0-15 cm soil layer. In addition, Chen *et al*., 2017b showed that rice straw return markedly increased the soil TOC, DOC, and MBC contents but not that of LOC, with MBC and TOC being the major factors that influenced microbial communities under short-term straw return.

Microorganisms are the most sensitive part of the soil, influencing the ecological stability and biological productivity of cropland and grassland ecosystems (Andelkovic *et al*., 2019) [1] by participating in the biochemical transformation of mineral fertilizers and the synthesis of biologically active substances and nitrogen fixation (Zhao *et al*., 2014) [51]. Microbial communities such as fungi and bacteria may not necessarily be limited by the same elements that limit the plant community. Soil microorganisms can be limited by carbon or phosphorus, while net primary production in terrestrial ecosystems is generally limited to nitrogen availability. Excess N may inhibit soil microorganisms' activity, indicating that microbes are not always nitrogen restricted (Ramirez *et al*., 2012) [36]. Aboveground biomass production typically increases after nitrogen fertilization, while plant residues returning to the soil can increase the carbon source for soil microorganisms. On the other hand, the indirect effects of long-term N fertilization can cause significant changes in C availability and a dramatic loss of organic C (Raza *et al*., 2020) [37]. It is well known that indicators of microbial activity such as respiration, microbial biomass C and N (MBC and MBN), and metabolic quotient qCO_2, as well as light-fraction OM, potentially mineralizable C and N (PMC and PMN) and their C/N ratios are sensitive indicators to detect subtle changes in soil fertility parameters (Babur, 2019) [2] compared with soil organic C.

Soil microbial biomass is frequently used as an early indicator of change in soil quality. Compared with SOM, soil microbial biomass is more susceptible to changes in the soil environment and soil management practices (Liu *et al*., 2017) [29]. Although soil microbial biomass is a small portion of SOM, it plays a vital role in soil nutrient cycling because it can transform SOM and insoluble substances (Dou *et al.*, 2016) [7]. At the same time, soil microbial biomass is closely related to N, P, and S cycling, and can usually be considered a pool of soil nutrients that affects plant growth by some nutrient elements (Wu *et al.*, 1993). Some studies have shown that mineral fertilizers increase soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN) contents (Li *et al*., 2008) [24], but other studies have indicated that soil microbial biomass is not significantly affected by application of inorganic fertilizers and even that soil microbial populations and diversity were reduced by application of N fertilizer (Sarathchandra *et al.*, 2001) [38]. Meanwhile, some studies have reported that the soil microbial community was affected by application of inorganic fertilizers and organic matter in different crop rotation systems (Zhen *et al.*, 2014; Luo *et al.*, 2015) [53, 31]. The objective of review paper were (1) to examine the changes in SOC, labile organic carbon pools and microbial community composition, influenced by tillage with organic and synthetic fertilizers and rice straw return (2) to assess the effect of different tillage practices with organic and synthetic fertilizers on soil aggregate and labile organic carbon pools, and (3) to provide insights into the coupling relationships between soil organic carbon and labile organic carbon pools influenced by different tillage practices with organic and synthetic fertilizers and rice straw return.

SOC storage, sequestration, labile and recalcitrant C pools

Ding *et al*. (2012) [6] reported that different fertilization treatments had a significant influence on both SOC storage and sequestration. Topsoil (0–20 cm) C storage was significantly higher in the manure applied treatments (OM_1, OM_2, OM^3) compared with the CK and OM0 treatments (Fig. 1a). The plots under OM3 treatment stored 14.5% higher organic C than the plots under OM0 treatments in topsoil. No differences were found in organic C storage between OM_2 and either OM_1 or OM_3 treatments (Fig. 1a). SOC concentrations varied in the same way as organic C storage, with a range of 25.6–30.3 g kg^{-1}. The amount of sequestered organic C was highest under OM_3 treatment (10.5 Mg ha^{-1}), followed by OM_2 (8.4 Mg ha^{-1}) and OM_1 (7.2 Mg ha^{-1}) treatments, and lowest under OM_0 treatment (1.8 Mg ha^{-1}) at 0–20 cm soil depth (Fig. 1a). On average, organic fertilized treatments increased LPI-C, LPII-C, and RP-C by 7.8, 12.9, and 19.4%, respectively, as compared to CK. OM_3 soils contained the highest labile and recalcitrant C pools. OM_1 and OM_2 treatments resulted in different LPI-C and LPII-C pools but similar RP-C pool. RP-C pool accounted for a larger proportion in total SOC (54.0–59.3%) than both LPI-C (18.0–19.2%) and LPII-C (24.4–25.2%) pools. Moreover, increase in recalcitrant C (9.3– 29.5%) was significantly higher than labile C (2.8–10.2%) due to fertilization, as compared with CK. Significant correlations were found among SOC, LPI-C, LPII-C, and RP-C.

Xu *et al*. (2018) [50] indicated that SOC content in the paddy field was higher under the MF, RF, LOM, and HOM treatments than under the CK treatment (Fig.1b). At the main growth stages of late rice, the SOC content in all fertilization treatments was similar to the main growth stages of early rice. Soil organic carbon content in the paddy field at late growth stages was higher than at early growth stages of late rice. The highest SOC content in the paddy field under all fertilization treatments was at the heading stage of late rice, which decreased at the mature stage of late rice. The SOC content in the paddy field under treatments with fertilizer application (MF, RF, LOM, and HOM) was higher than under the treatment without fertilizer (CK) at the main growth stages of late rice.

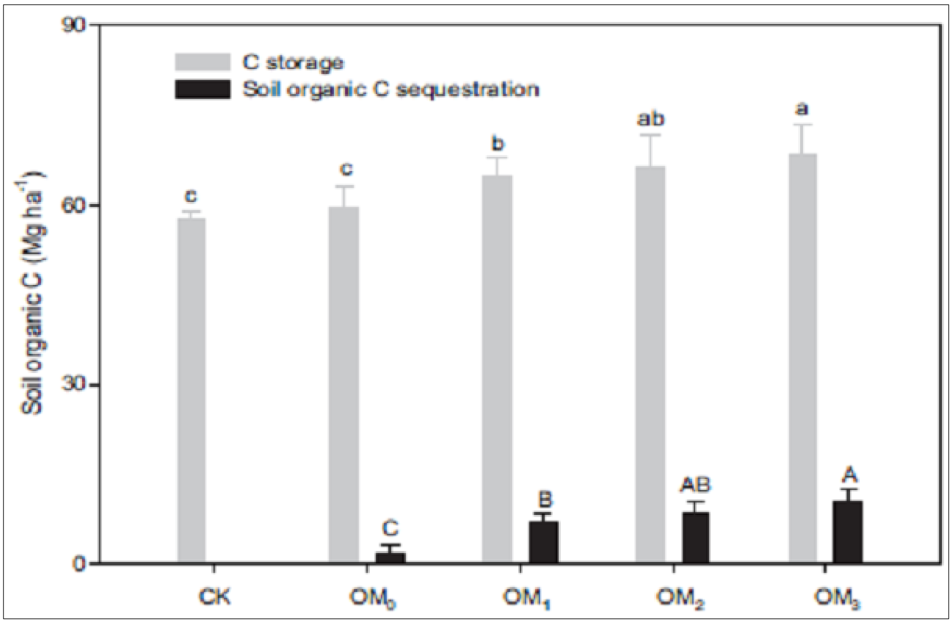

Fig 1a: Soil carbon storage and sequestration in soils after 10 years of different fertilization treatments. CK, unfertilized control; OM_0, only chemical fertilizer, no manure added; OM_1, organic manure added at 7.5 Mg ha^{-1} year^{-1} plus chemical fertilizer; OM_2, organic manure added at 15 Mg ha^{-1} year^{-1} plus chemical fertilizer; OM_3, organic manure added at 22.5 Mg ha^{-1} year^{-1} plus chemical fertilizer [Source: Ding *et al.*, 2012] [10]

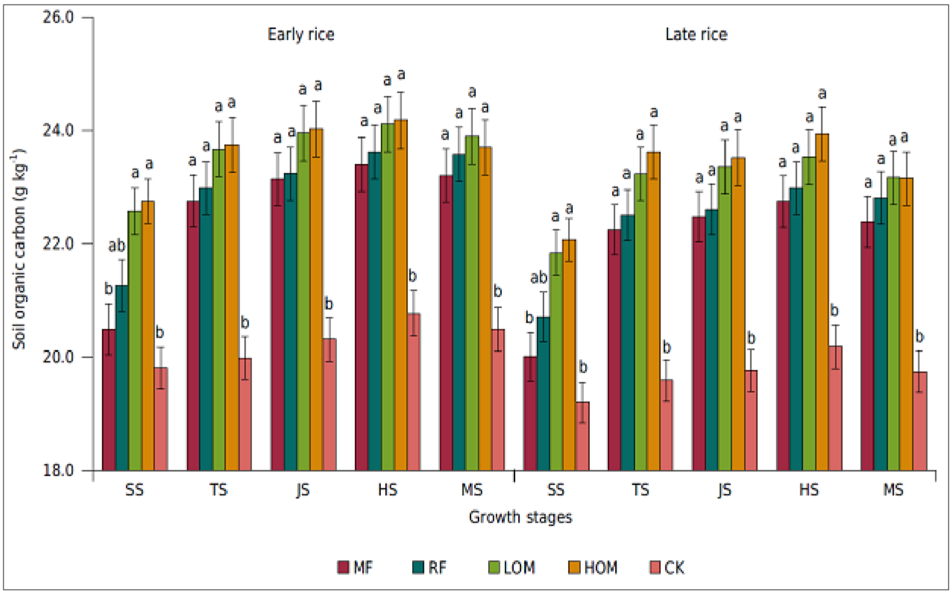

Fig 1b: Effects of different long-term fertilization treatments on soil organic carbon content in a paddy field at main rice growth stages. MF = mineral fertilizer alone; RF = rice straw residues and mineral fertilizer; LOM = 30% organic matter and 70% mineral fertilizer; HOM = 60% organic matter and 40% mineral fertilizer; CK = without fertilizer input; SS = seedling stage; TS = tillering stage; JS = jointing stage; HS = heading stage; MS = mature stage [Source: Xu *et al.*, 2018] [50]

Ge *et al*. (2021) [8] also found that Organic C content was highest in 0.25–1 mm aggregate (6.9– 9.6 g kg^{-1}) prior to incubation, followed by > 2 mm aggregates (2.2–5.8 g kg^{-1}), 1–2 mm aggregates (2.4–4.6 g kg^{-1}), and < 0.25 mm aggregates (3.3–4.5 g kg^{-1}) (Fig. 2a). Compared to CK and IF treatments, organic C content in IFM treatment was averagely increased by 159% (> 2 mm aggregates), 90% (1–2 mm aggregates), 38% (0.25–1 mm aggregates) and 33% (< 0.25 mm aggregates).

Organic C content was 2.3–4.5 g kg^{-1}, 2.9–5.0 g kg^{-1}, and 7.2–11 g kg^{-1} and1.8–3.0 g kg^{-1} in > 2, 1–2, 0.25–1, and < 0.25 mm aggregate, respectively (Fig. 2a). IFMS treatment has the highest organic C content during the whole incubation period. Organic C contents of all aggregate fractions in IFMS treatment were, on average, 79% and 84% larger than those in CKS and IFS treatments. Moreover, the relative distribution of straw-derived C to 0.25–1 mm aggregate was 54%, 40%, and 39% in CKS, IFS, and IFMS treatments on day 150, respectively (Fig. 2b). About one-half of straw-derived C was distributed to 0.25–1 mm aggregate in CKS and IFS treatments, and 40% of straw-derived C was incorporated into 0.25–1 mm aggregate in IFMS treatments on day 360. Besides, on day 150, the relative distribution of straw-derived C to > 2 mm aggregate and 1–2 mm aggregate was higher than that to < 0.25 mm aggregate in three treatments, while there was an opposite trend on day 360.

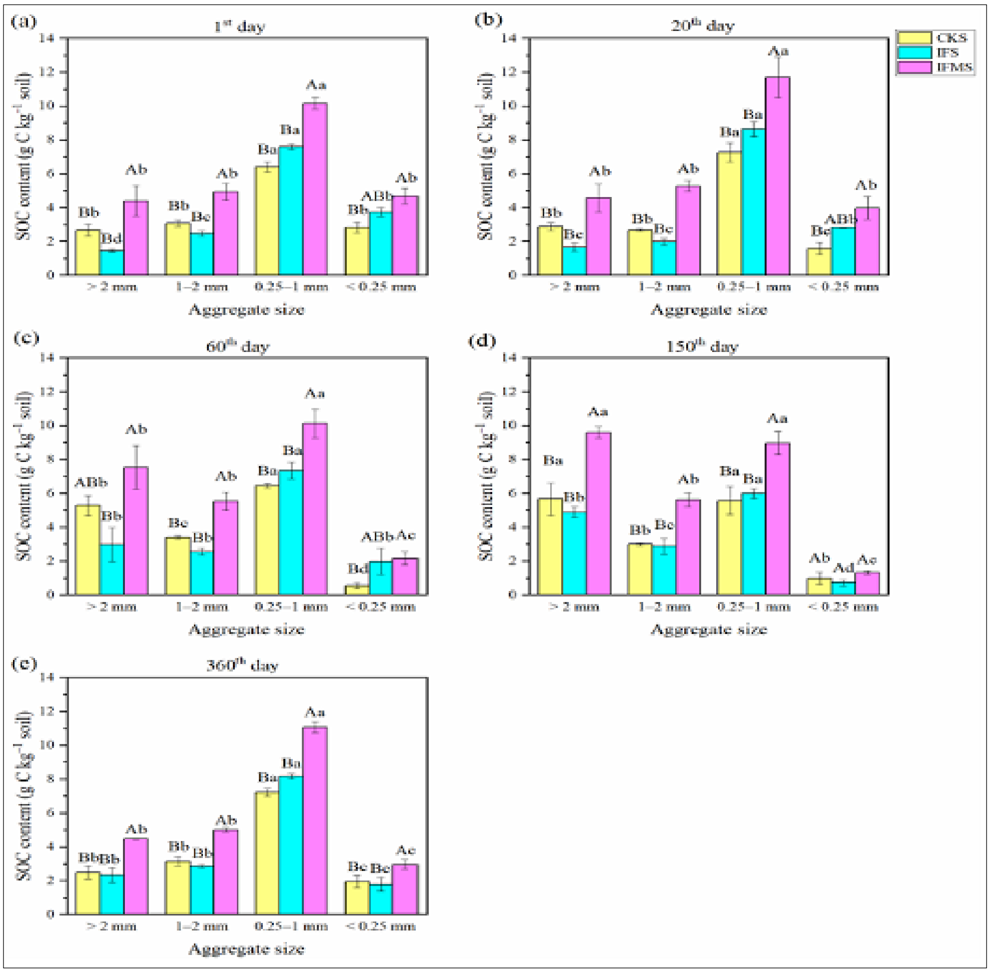

Fig. 2a: The SOC contents in aggregates under various fertilizer management strategies after adding straw. *Note*: Treatments including CKS (no fertilization control + straw), IFS (inorganic fertilizer + straw), IFMS (inorganic fertilizer plus manure + straw) [Source: Ge *et al*., 2021] [8]

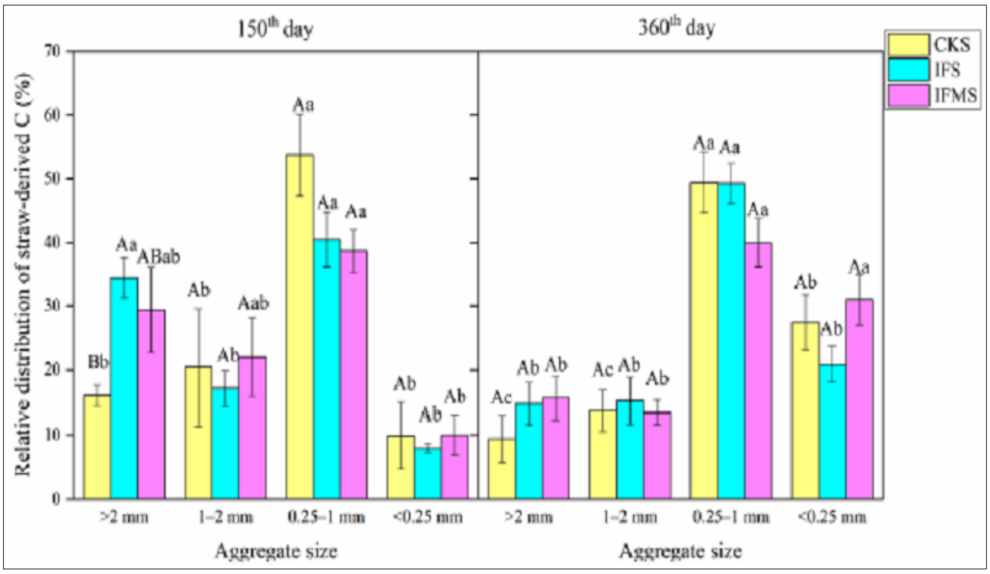

Fig 2b: The relative distribution of straw-derived C in soil aggregates (%) [Source: Ge *et al*., 2021] [8]

Jiao *et al*. (2020) [19] revealed that the concentration of SOC in forest land was significantly higher than arable land and grassland in the top 5 cm soil layer (Fig. 3a). The overall SOC stock showed similar patterns with SOC concentration among different land uses, except for the grassland in 10–30 cm soil profile, which was significant higher than arable land and forest land (Fig. 3a).

The concentration of soil labile carbon (LOC) in arable land was significant lower than both grassland and forest land in the top 50 cm soil profile (Fig. 3b). The CLOC to CORG ratio followed the order of forest land > grassland> arable land (Fig. 3b).

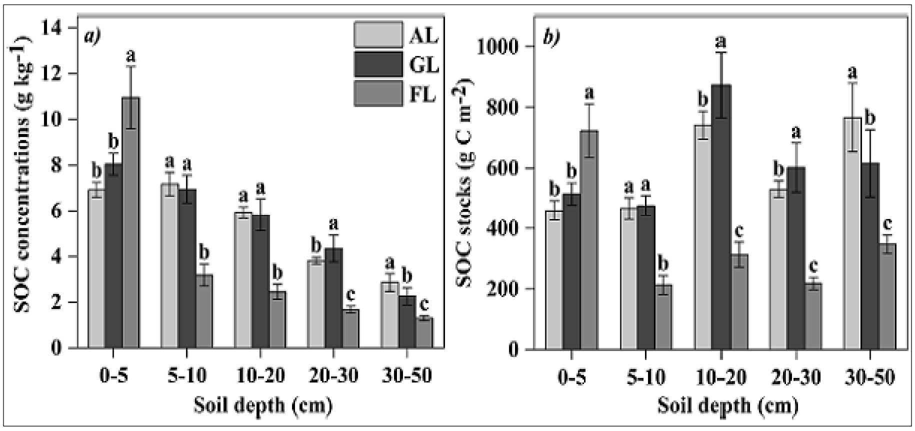

Fig 3a: (**a**) Soil organic carbon (SOC) concentration and (**b**) SOC stock with different land uses [Source: Jiao *et al*., 2020] [19]

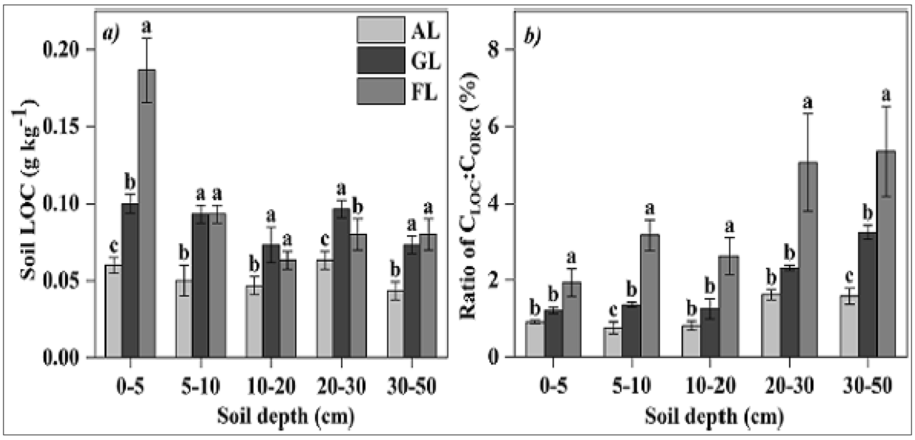

Fig 3b: (**a**) Soil labile carbon (LOC) concentration and (**b**) the CLOC to CORG ratios with different land use [Jiao *et al*., 2020] [19]

Lu *et al*. (2021) [32] observed that SOC and DOC contents varied with fertilization and aggregate size, both of which were higher under NP, NPS, and NPM than under CK in almost all aggregates (Fig. 4a).

In CK, SOC contents in macro-aggregates (>0.25 mm) were significant lower than in micro-aggregates (<0.25 mm). In NP, NPS, and NPM, there were no differences in SOC contents among all aggregates.

MBC and ROC contents were significantly affected by fertilization and aggregate size, respectively. The four treatments had no significant differences in ROC and MBC contents (Fig. 4a) in micro-aggregates (<0.25 mm), and a similar observation was also made concerning the ratio of MBC to SOC content (Fig. 4b).

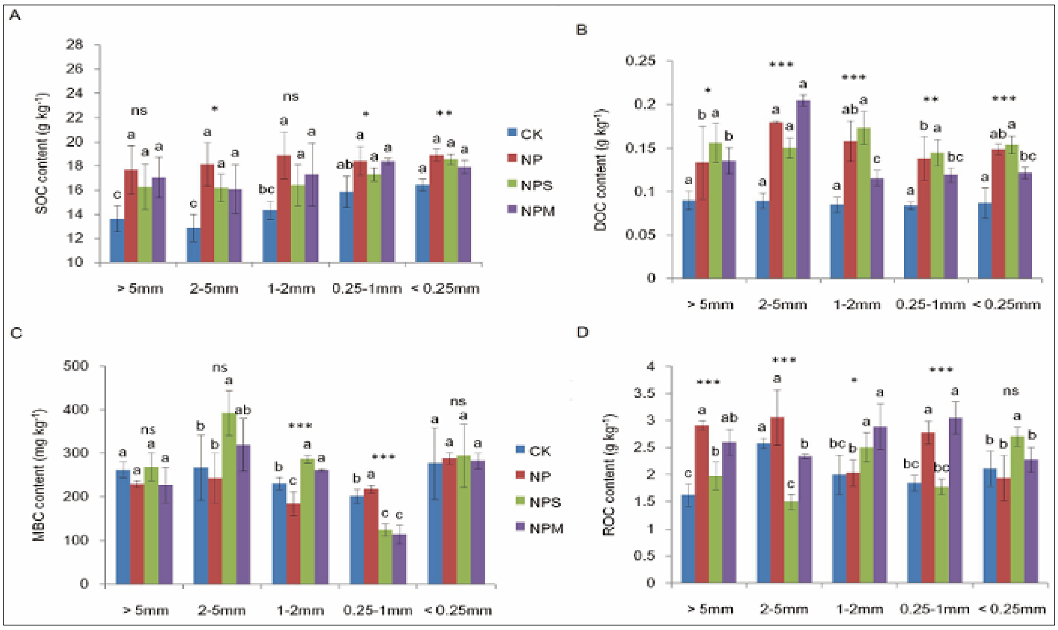

Fig 4a: The distribution of SOC (A), DOC (B), MBC (C), ROC contents (D) in different aggregates under 4 fertilization managements (CK, NP, NPS, and NPM). SOC: soil organic carbon; DOC: dissolved organic carbon; MBC: microbial biomass carbon; ROC: readily oxidizable organic carbon [Source: Lu *et al*., 2021] [32]

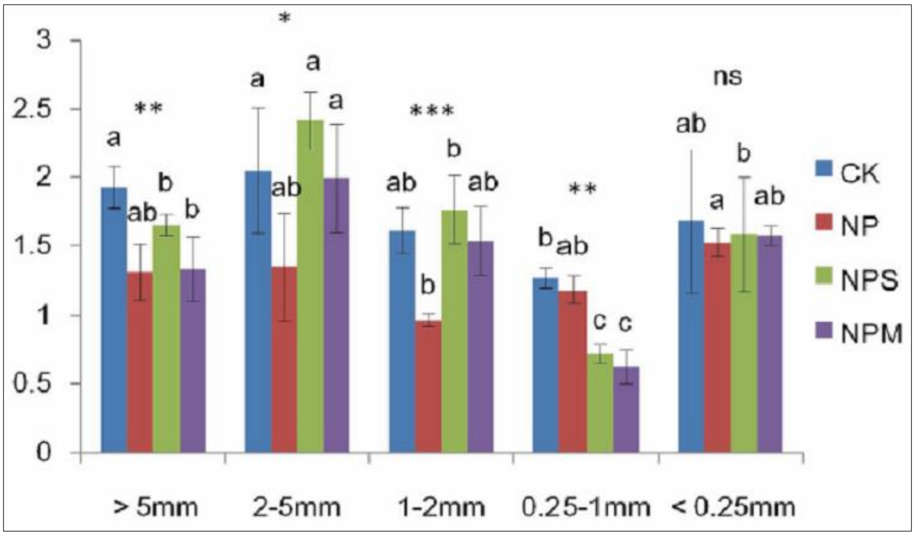

Fig 4b: The ratio of MBC to SOC in different aggregates under 4 fertilization managements (CK, NP, NPS, and NPM) [Source: Lu *et al.*, 2021] [32]

Ghosh *et al*. (2018) [9] reported that in soil surface, plots with NPK+FYM had significantly higher labile C within macro-aggregates compared with NPK and control plots (Fig. 5a). However, labile C concentrations within macro-aggregates of 150% NPK and NPK+FYM were similar. Labile: recalcitrant C in macro-aggregates of NPK+FYM was 1.38:1. There was a gradual decrease in labile C within macro-aggregates in NP, N and control plots. Similarly, recalcitrant C closely followed the trend of labile C within macro-aggregates, except under NP plots, which had significantly less labile C within macro-aggregates than all other plots. However, macro-aggregates of NPK+FYM had 19% and 46% higher recalcitrant C than NPK and NP plots. Like labile C within macro-aggregates, 150% NPK and NPK+FYM plots had similar recalcitrant C pools. Similar trend of C concentration was observed within micro-aggregates, as was observed within macro-aggregates. Moreover, in both 0–15 and 15–30 cm soil layers, plots with NPK+FYM had significantly higher labile and recalcitrant C pools than control and NP plots (Fig. 5a). Plots with NPK+FYM also had higher labile C than 150% NPK in the 15–30 cm soil layer, but both plots had similar recalcitrant C pools. While NPK+FYM had significantly higher non-labile C pool (Pool 4) than all other treatments in soil surface, all plots (except N and control plots) had similar non-labile C pools in 15–30 cm soil layer. On average, the relative preponderance of the fractions of SOC, extracted under a gradient of oxidizing conditions was: Pool 4 > Pool 1 > Pool 3 (less labile C) > Pool 2 (labile C) in surface soil layer constituting about 33.4%, 24.6%, 21.3% and 20.7%, respectively, of the total SOC.

However, labile: recalcitrant C in bulk soils and micro-aggregates of NPK, 150% NPK and NPK+FYM plots were similar and significantly higher than control, N and NP plots, signifying higher rate of C sequestration in the former treatments than the latter ones in sub-surface layer. Labile: recalcitrant C ratios within macro-aggregates were similar for all treatments.

When expressed on a total soil basis, macro-aggregates accounted larger part of total SOC in surface and subsurface soil layers. Interestingly, macro-aggregates of NP plots in soil surface had 1.36 and 1.38 times greater SOC enrichment than NPK+FYM and NPK treated plots. Whereas, macro-aggregates from N plots had 1.12 and 1.18 times greater C enrichment than NPK+FYM and NPK plots, respectively, in sub-surface soil. Carbon enrichment factor of soil micro-aggregates from all plots were <1, indicating net C depletion (Fig. 5b). Besides higher organics inputs, the greater amounts of recalcitrant C under NPK+FYM than NPK might be due to increased decomposition of labile compounds and accumulation of recalcitrant materials over time with NPK+FYM plots (Lopez-Capel *et al*., 2008) [30]. P addition could increase soil aggregation and physical and chemical C protection. This might be the cause of higher C distribution in recalcitrant pools under NP plots in both soil layers.

Distribution of recalcitrant C with respect to its total content was invariably higher in macro-aggregates than that in micro-aggregates. This could be due to SOC accumulation to form macro-aggregates, which might form a physical barrier between the substrates and microbes and in turn physically protect SOC from microbial decomposition (Tripathi *et al*., 2014) [44]. Increased recalcitrant C in micro-aggregates with fertilization could be due to: (i) Carboxyl-C that could accumulate within micro-aggregates than other C functional groups and (ii) increased humic substances and polysaccharides, the binding agents for micro-aggregate formation and decomposition protection.

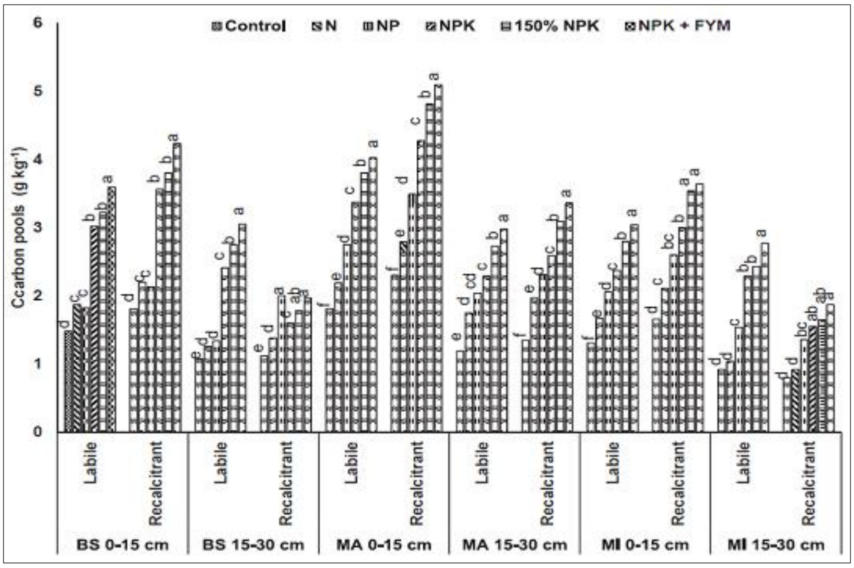

Fig 5a: Labile and recalcitrant carbon pools in bulk soils and aggregates as affected by long-term fertilization in the 0–15 and 15–30 cm soil layers under a wheat based cropping system in an Inceptisol [Source: Ghosh *et al*., 2018] [9]

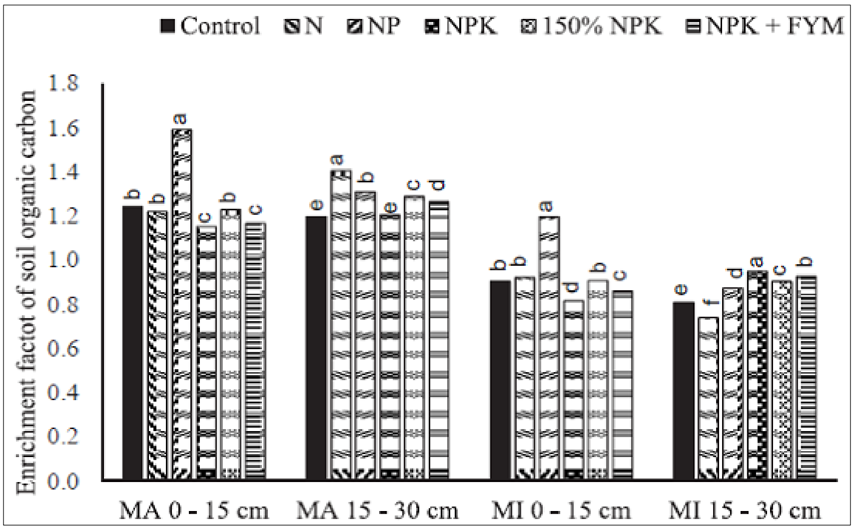

Fig 5b: Enrichment factor of soil organic C (SOC) in aggregates as affected by 44 years of fertilization under wheat based cropping system in an Inceptisol. MA: Macro-aggregates, MI: Micro-aggregates, MA 0–15 cm: Macro-aggregates of 0–15 cm soil depth [Source: Ghosh *et al*., 2018] [9]

Ghosh *et al*. (2018) [9] revealed that SOC accumulation rates in plots under NPK+FYM and NPK (over control plots) in the 0–90 cm soil profile was ~745 and 529 kg ha^{-1} yr^{-1}, respectively (Table 1). However, C sequestration (C accumulation in long-lived/recalcitrant pools) rates in the 0–90 cm soil profile for NPK and NPK+FYM treatments were only ~167 (31% of the accumulated SOC) and 224 (30% of the accumulated SOC) kg ha^{-1} yr^{-1}, respectively. Interestingly, NPK, 150% NPK and NPK+FYM treated plots had similar recalcitrant C contents in the said soil profile, but had significantly different C accumulation rates (with NPK+FYM treatment was the best). Nearly 54% of the accumulated SOC and 34% of the sequestered SOC under NPK+FYM plots (over control plots) were observed within deep soils (30–90 cm soil layer), implying role of INM on C sequestration in deep soils (Table 1).

Table 1: Total soil organic carbon (SOC) accumulation and sequestration rates (kg C ha^{-1} year^{-1}) over unfertilized control plots as affected by long-term fertilization [Source: Ghosh *et al*., 2018] [9]

Treatments	Total SOC content (Mg ha^{-1})	SOC accumulation rate (kg C ha^{-1} yr^{-1})	Recalcitrant SOC content (Mg ha^{-1})	SOC sequestration rate (kg C ha-^{I} yr^{-I})	Total SOC Content rate (Mg ha^{-1})	SOC accumulation rate (kg C ha^{-1} yr^{-1})	Recalcitrant SOC content (Mg ha^{-I})	SOC sequestration rate (kg C ha^{-I} yr^{-1})
	In the 0-90 cm soil layer				In the 30-90 cm soil layer			
Control	31.00d	-	21.89c	-	18.22d	-	14.98b	-
N	36.30d	120.48e	23.74bc	42.1	20.87d	60.16e	15.48b	11.5d
NP	42.15c	253.46d	25.32b	78.1c	25.61c	167.89d	15.91b	21.2c
NPK	54.271b	528.90c	29.17a	165.6c	30.55b	280.23c	17.67a	61.1b
150% NPK	60.77a	676.70b	30.55a	196.9b	35.28a	387.78b	18.22a	73.8a
NPK + FYM	63.80a	745.45a	31.73a	223.7a	36.11a	406.50a	18.37a	77.1a

Means with similar lower-case letters within a column are not significantly different at $P < 0.05$ according to Tukey's HSD test

Contribution of straw-C to SOC

Straw management is an essential way to increase the fertility of the soil and increase the SOC sequestration in soil, also protective and improving the soil quality in agriculture ecosystem. Van *et al*. (2011) [45] suggested that straw retaining with a no-tillage considerably enhanced the total SOC in the surface soil (0–30 cm) soil. Su *et al*. (2020) also found that during the straw C transformation and distribution process, the contents of soil DOC, MBC, POC and MaOC changed with time (Fig. 6). Within the first 7 days after straw inputs, soil DOC, MBC, POC and MaOC contents had the largest increase, which was 0.39, 4.69, 0.69 and 0.98 times higher than that of the control, respectively. The DOC content varied in the range of 430.7–447.0 mg kg^{-1} between days 14 and 28, and decreased to 280.6–303.6 mg kg^{-1} after 60 days, which was non-significantly different from the control (Fig. 6a). The content of soil MBC decreased dramatically after 7 days with straw inputs and maintained at 447.4–470.3 mg kg^{-1} from days 14 to 180, which was 0.3–1.6 times higher than that of the control (Fig. 6b). Straw-C contribution also peaked on day 7 after straw inputs for soil DOC (13.0%) and MBC (23.54%). The soil POC content after straw inputs increased with the time throughout the entire incubation period, while the soil MaOC content decreased during days 14 to 60, and increased slightly to about 3.71 g kg^{-1} on day 180 (Fig. 6c,d). The contribution rate of straw-C to soil POC increased from 33.94% on day 7 to 54.7% on day 60 after straw inputs, but decreased to 48.6% on day 180. For soil MaOC, straw-C contributed about 44.0% and 49.9% on average on day 7 and 14 after straw inputs, respectively, and then the straw-C contribution decreased with time, with only 20.0% of MaOC derived from straw on day 180 after straw inputs.

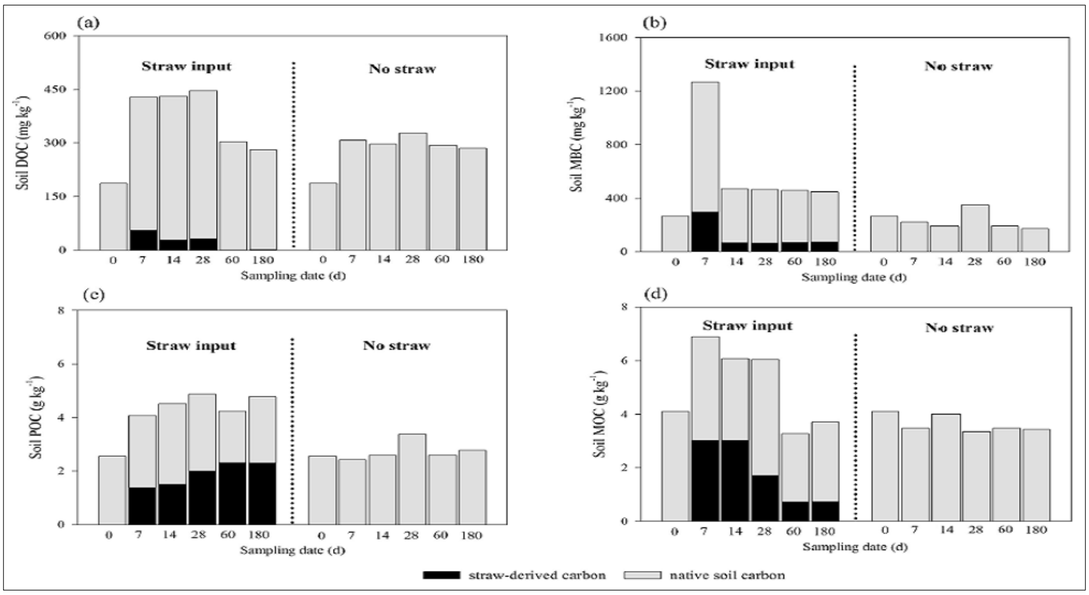

Fig 6: The effect of straw inputs on the contents of soil (a) DOC, (b) MBC, (c) POC and (d) MaOC, and the contribution of straw-C (black bar) and native soil carbon (gray bar) to these SOC fractions in the first 180 days of straw decomposition [Source: Su *et al*., 2020].

Singh and Benbi, (2018) also found that The change in WEOC (9.6 to 56.0%) in response to balanced and imbalanced application of fertilizer nutrients was lower compared with MBC (9.6 to 97.9%).

The corresponding change for Fract. 1, Fract. 2 and Fract. 3 ranged between 8.1 to 55.2%, 9.6 to 61.2%, and 4.7 to 47.5%, respectively. Among various C pools, $KMnO_4$-C had the highest change (2.0 to 306%) in response to balanced and imbalanced application of fertilizer nutrients with or without FYM (Fig. 7). Ghosh *et al*. (2012) reported magnitude of different organic C fractions in the order of Fract. 4>Fract. 1>Fract. 3>Fract. 2. Large amounts of C input through manure became gradually stabilized in the soil with a great proportion of recalcitrant C may be formed within first several years of experiment establishment (Ding *et al*. 2012) [10].

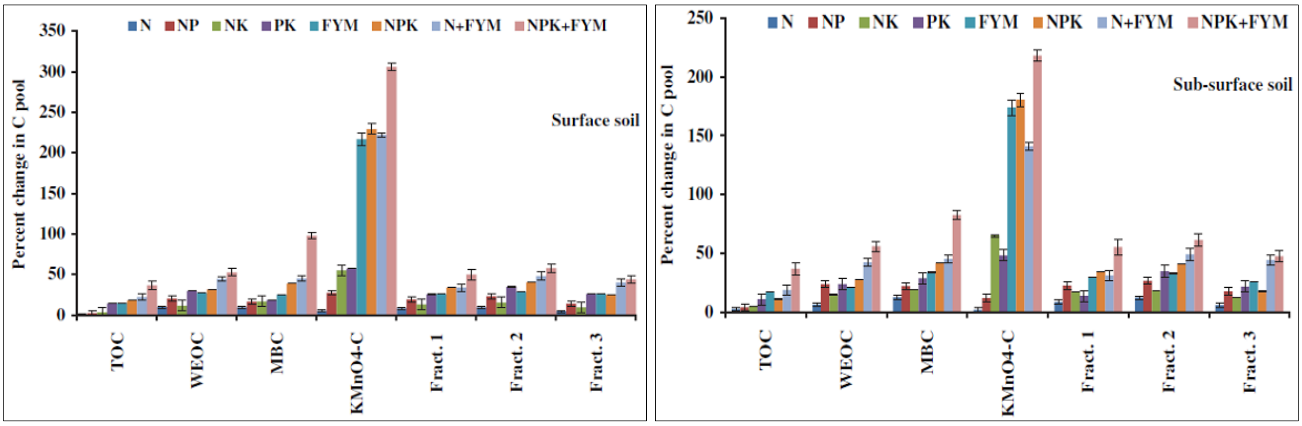

Fig 7: Percent change in various labile carbon pools and soil organic carbon fractions of different oxidizability in (a) surface and (b) sub-surface soils under rice-wheat cropping. [Fract. 1 = very labile C, Fract. 2 = labile-C and Fract. 3 = less labile-C]

Soil microbial community composition

The biodiversity of soil microbial communities is critical to maintaining soil quality, productivity, and ecological balance in agro-ecosystems (Li *et al*., 2014b) [27]. With the application of N fertilizer, the C: N ratio was decreased in the straw return treatment, which promoted the growth and increased the abundance of soil microbes. The higher C: N ratio *of T. angustifolia* has a lower decomposition rate and may lead to competition for N between soil microorganisms and the plant (Li *et al*., 2019) [28]. This competition inhibits the growth of soil microbial, resulting in the decreased soil microbial abundance. Wu *et al*. (2011) [49] observed that rice straw return did not influence bacterial community diversity, and rice straw return combined with chemical fertilizer application significantly increased bacterial abundance and altered the bacterial community composition. Tian *et al*. (2015) [43] observed that the application of compost significantly reduced the relative abundance of *Firmicutes*, which was negatively correlated with the organic carbon content. Ding *et al*. (2016) [11] showed that the relative abundance of *Betaproteobacteria* in the no-fertilizer treatment was higher than that in the manure and inorganic fertilizer treatments. Benbi *et al*. (2015) [3] in rice straw return enhanced the soil DOC, LOC, and MBC contents, with DOC content increasing the most and the LOC content increasing the least. The soil DOC, LOC, MBC, and TOC contents were highly significantly correlated.

Haynes (2000) [17] observed that compared with soil under long-term cultivation, the pastoral soil TOC, DOC, MBC, and LFOC contents were higher, and the active SOC fractions and TOC contents were significantly correlated. The contents of soil TOC, DOC, MBC, and LFOC in the AL treatment were higher than those in the S0 treatment, and the TOC content was significantly positively correlated with the active SOC fractions. Organic matter additions under AL treatment from senescing plant roots and tops and the exudation of organic compounds from roots increased the TOC and active SOC fractions in the soil. Under the S_0 treatment, the amount of organic material returned to the soil was much less than that in the AL treatment. In addition, the extent of soil cultivation affects oxygen availability and hence microbial activity (Johnston *et al*., 2009) [20], causing the soil TOC and active SOC fractions content in the S0 treatment to be lower than that in the AL treatment. Particulate organic matter is an active carbon pool of organic matter, and the recent history of organic matter addition is more likely to strongly influence POM (Gosling *et al*., 2013) [13]. Straw mulching significantly increased POC content compared to the straw removal.

Tang *et al*. (2011) [40] reported that *Betaproteobacteria* include *cellulolytic* bacteria and *Deltaproteobacteria* were primarily distributed in plant litter and areas of root exudation, playing key roles in carbon cycles. Straw return increased the relative abundances of *Betaproteobacteria*, *Deltaproteobacteria*, and *Bacteroidetes*. Changes in the relative abundance of *Sphingobacteriia* were relatively small, and the relative abundance of *Betaproteobacteria* in the AL treatment was the highest among the treatments. The soil organic matter content, and thus the relative abundance of *Betaproteobacteria* in the S_1, S_2, and AL treatments were higher than that observed in the S_0 treatment (Mi *et al*., 2019) [34]. Ding *et al*. (2016) [11] showed that the relative abundance of *Betaproteobacteria* in the no-fertilizer treatment was higher than that in the manure and inorganic fertilizer treatments.

Xu *et al*. (2018) [50] reported that soil microbial biomass carbon (SMBC) contents in the paddy field under the LOM and HOM treatments were higher than under the MF, RF, and CK treatments at the main growth stages of early and late rice. The SMBC contents in the paddy field under the MF and RF treatments were higher than under the CK treatment at the main growth stages of early and late rice (Fig. 8a). In early and late rice, the SMBC content in the paddy field at late growth stages was higher than at early growth stages. The highest SMBC content in the paddy field with five fertilization treatments was at the heading stage, and it then decreased at the mature stage of early and late rice. At the heading stage, in comparison with the CK treatment, the SMBC content in the paddy field increased by 144.34, 190.61, 330.84, and 365.90 g kg^{-1} under the MF, RF, LOM, and HOM treatments, respectively, in early rice. It increased by 145.36, 191.60, 331.86, and 359.93 g kg^{-1} under the MF, RF, LOM, and HOM treatments, respectively, in late rice.

The value of the SMBC: SMBN ratio under different fertilization treatments throughout the growth stages of early and late rice are shown in figure 8b. Throughout the growth stages of early rice, the value of the SMBC: SMBN ratio under the HOM treatment was higher than under the MF and CK treatments, and the value of the SMBC: SMBN ratio under the RF, LOM, and HOM treatments was also significantly higher than under the CK treatment (Fig. 8b). There is not a significant difference in the value of the SMBC: SMBN ratio between the MF and CK treatments at the main growth stages of early rice. Over the growth stages of early rice, the mean value of the SMBC: SMBN ratio was 13.58, 14.16, 15.15, 15.37, and 12.42 under the MF, RF, LOM, HOM, and CK treatments, respectively.

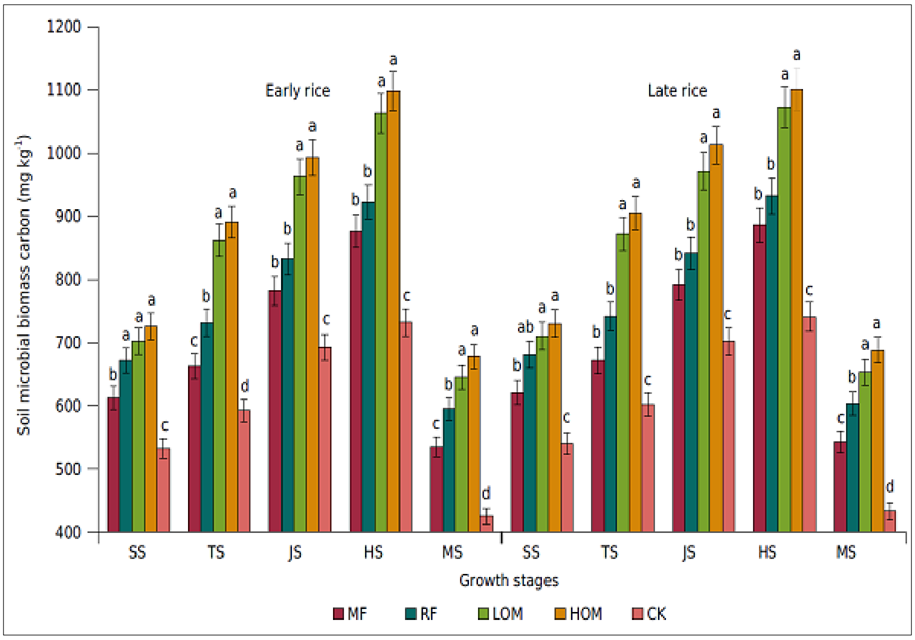

Fig 8a: Effects of different long-term fertilization treatments on soil microbial biomass carbon in a paddy field at main rice growth stages. MF = mineral fertilizer alone; RF = rice straw residues and mineral fertilizer; LOM = 30% organic matter and 70% mineral fertilizer; HOM = 60% organic matter and 40% mineral fertilizer; CK = without fertilizer input; SS = seedling stage; TS = tillering stage; JS = jointing stage; HS = heading stage; MS = mature stage [Source: Xu *et al*., 2018] [50].

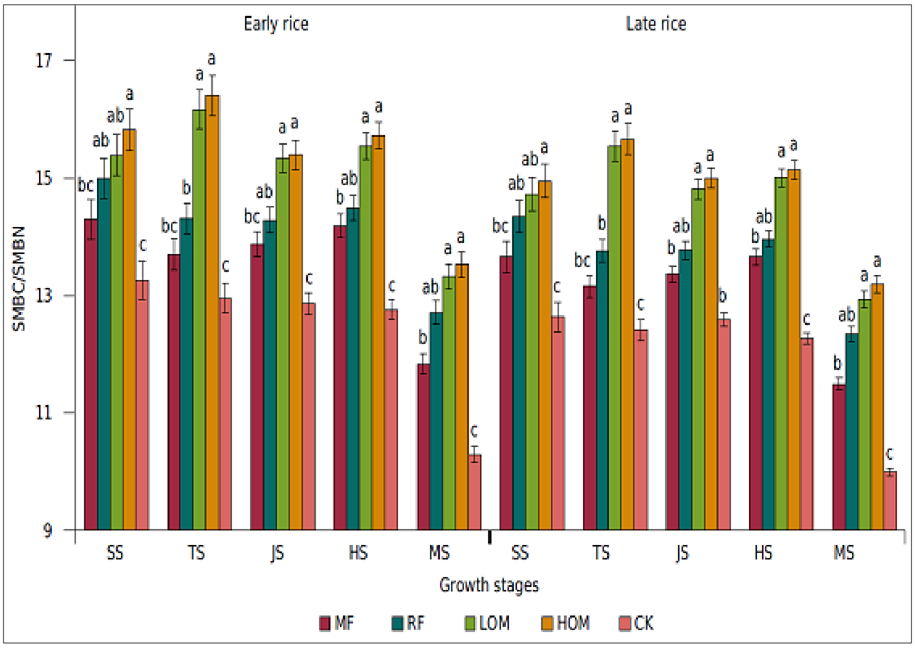

Fig 8b: Effects of different long-term fertilization treatments on soil microbial biomass carbon and nitrogen ratio in a paddy field at main rice growth stages (Source: Xu *et al*., 2018) [50].

Guo *et al*. (2016) [7] reported that as compared with CT treatments, NT treatments increased MBC by 11.2%, 11.5%, and 20%, and dissolved organic carbon (DOC) concentration by 15.5%, 29.5%, and 14.1% of bulk soil, >0.25 mm aggregate, and <0.25 mm aggregate in the 0−5 cm soil layer, respectively. Compared with NS treatments, S treatments significantly increased MBC by 29.8%, 30.2%, and 24.1%, and DOC concentration by 23.2%, 25.0%, and 37.5% of bulk soil, >0.25 mm aggregate, and <0.25 mm aggregate in the 0−5 cm soil layer, respectively. Conservation tillage (NT and S) increased microbial metabolic activities and Shannon index in >0.25 and <0.25 mm aggregates in the 0−5 cm soil layer. Naresh *et al*. (2018) revealed that the tillage intensity increased there was a redistribution of SOC in the profile, but it occurred only between ZT and PRB since under CT, SOC stock decreased even below the plow layer. However, higher SOC content of 8.14 g kg^{-1}of soil was found in reduced tilled residue retained plots followed by 10.34 g kg^{-1} in furrow irrigated raised beds with residue retained plots. Whereas, the lowest level of SOC content of 5.49 g kg^{-1}of soil were found in puddled transplanted rice followed by wheat planted under conventionally tilled plots. Average SOC concentration of the control treatment was 0.54%, which increased to 0.65% in the RDF treatment and 0.82% in the RDF+FYM treatment. Compared to F_1 control treatment the RDF+FYM treatment sequestered 0.33 Mg Cha^{-1} yr^{-1} whereas the NPK treatment sequestered 0.16 Mg C ha^{-1} yr^{-1}.

Singh and Benbi, (2018) reported that an applications of FYM alone or in combination with NPK significantly improved soil MBC. Soil MBC comprised about 4% of TOC, and between 150 and 297 mg kg^{-1}occurred as MBC in surface and between 128 and 234 mg kg^{-1}in sub-surface soil. It increased significantly with N application compared to UF, but did not differ significantly among other treatments involving imbalanced application of fertilizer nutrients (NP, NK and PK). Balanced fertilizer application significantly increased soil MBC. Farm yard manure application significantly increased MBC by 39% and 42% in the surface and sub-surface soils, respectively over the UF control. Compared with the application of NPK alone, NPK+FYM application increased MBC by 58.2% in the surface and 36.6% in the sub-surface soil. Averaged across treatments, surface soil had 15.3% higher MBC than sub-surface soil.

Ma *et al*. (2016) [33] reported that the differences in SMBC were limited to the surface layers (0–5 and 5–10 cm) in the PRB treatment. There was a significant reduction in SMBC content with depth in all treatments. SMBC in the PRB treatment increased by 19.8%, 26.2%, 10.3%, 27.7%, 10% and 9% at 0–5, 5–10, 10–20, 20–40, 40–60 and 60–90 cm depths, respectively, when compared with the TT treatment. The mean SMBC of the PRB treatment was 14% higher than that in the TT treatment.

Li *et al*. (2017) reported that an *Acidobacteria* (16–21%), *Bacteroidetes* (11–20%), and *Proteobacteria* (23–30%) were the dominant phyla across treatments. Among the classes of *Proteobacteria*, *Betaproteobacteria* (8–14%) had the highest relative abundance (Figure 9A). MNPK had higher relative abundance of *Betaproteobacteria*, and SNPK had higher Bacteroidetes than other treatments. Acidobacteria in HNPK and MNPK were more abundant (Figure 9A). Top 15 families with average relative abundance of >3.5% were analyzed (Figure 9B). MNPK led to a remarkable increase (4.4-fold increase) in the relative abundance of *Xanthomonadaceae*, but significant decrease in the relative abundances of *Planctomycetaceae*, *Gaiellaceae*, and *Nitrospiraceae*. Similarly, the relative abundances of *Planctomycetaceae*, *Gaiellaceae*, and *Nitrospiraceae* (especially *Gaiellaceae* were largely decreased by SNPK. The significantly increased abundances of *Chitinophagaceae* and *Sphingomonadaceae* occurred in HNPK (Figure 9B).

However, network edges were predominantly composed of strong positive associations, and the dominant identifiable OTUs belonged to *Acidobacteria*, *Bacteroidetes*, and *Gammaproteobacteria* (Figure 9A). SOC showed a strong positive association with one *Acidobacteria* subgroup 6 (Gp6) member (Figure 9B; Dataset S2). TN showed strong positive associations with *Gemmatimonas*, one *Acidobacteria* Gp6 member, one *Myxococcales* member and two members within *Betaproteobacteria* and *Bacteroidetes* (Figure 9C; Dataset S2). Based on betweenness centrality scores, the OTUs identified as keystone taxa were *Gemmatimonas*, *Flavobacterium* and one Subdivision3 member within Verrucomicrobia.

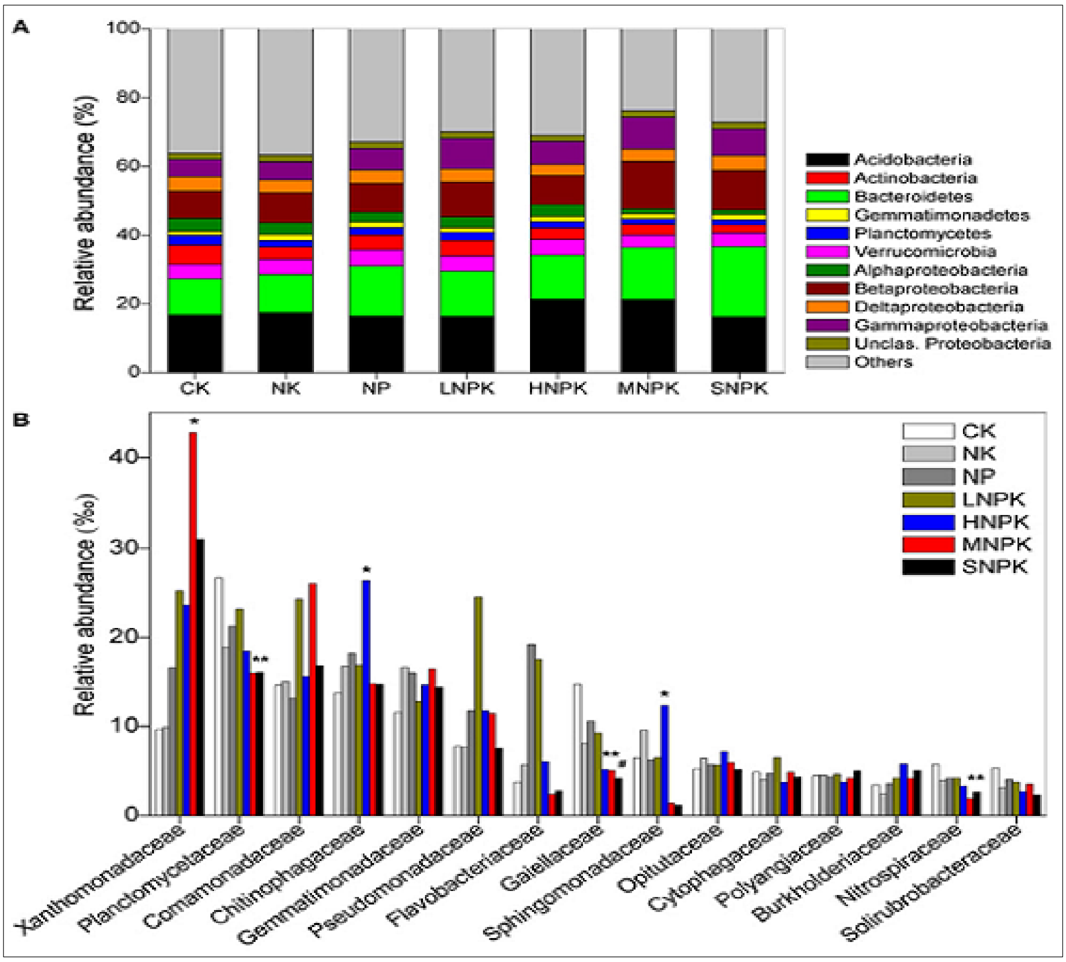

Fig 9a: Stacked and unstacked histograms showing the relative abundance of (A) major bacterial phyla and dominant classes of *Proteobacteria* and (B) 15 most abundant bacterial families, respectively, in treatments CK (unfertilized control), NK, NP, LNPK (low rate of N, regular PK), HNPK (high rate of N, regular PK), MNPK (manure plus NPK) and SNPK (straw plus NPK) [source: Li *et al*., 2017]

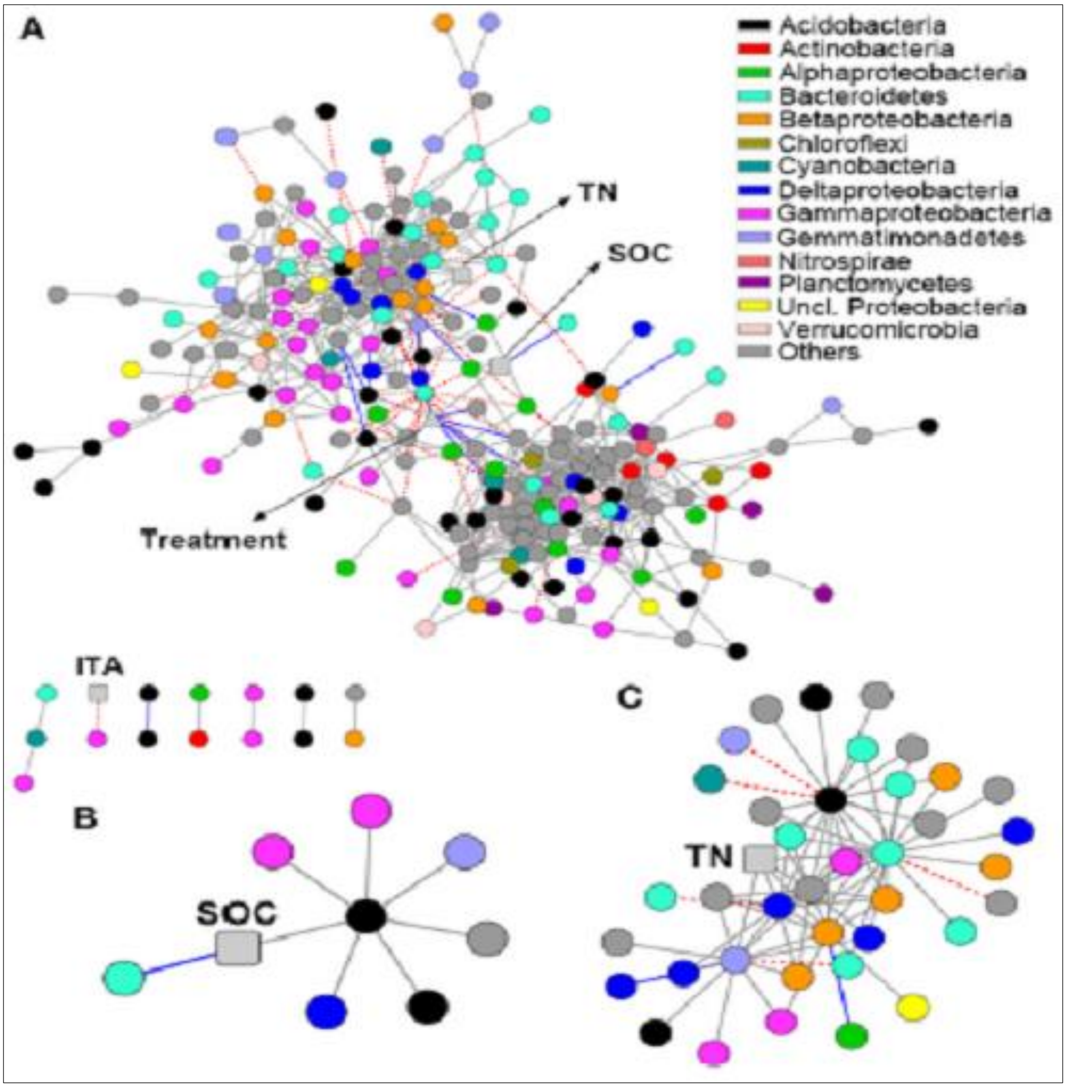

Fig 9b: Network analysis revealing the associations among bacterial OTUs and soil properties. Gray solid line, blue solid line and red dash line represent strong positive linear ($r > 0.8$), strong negative linear ($r <-0.8$) and strong nonlinear (MIC-$r^2 > 0.8$) relationships, respectively. Colored nodes signify corresponding OTUs assigned to major phyla and classes. Soil properties are indicated with round rectangle, and treatment with triangle. SOC, soil organic C; TN, total N; ITA, invertase activity. (A) Network co-occurrences of OTUs substantially enriched by long-term fertilization; (B, C), Subnetworks for the associations of SOC (B) and TN (C) [Li *et al*., 2017]

Tang *et al*. (2020) [41] also found that phyla *Actinobacteria* (8–17%), *Acidobacteria* (11–14%), *Alphaproteobacteria* (8–11%), *Chloroflexi* (7–10%), *Fimicutes* (9–14%), *Proteobacteria* (8–13%) and *Planctomycetes* (9–13%) were the most abundant in the soil samples. And the phyla *Proteobacteria*, *Actinobacteria* with M30, M50 and M100 treatments were significantly higher ($p < 0.05$) than that of with M0 and CK treatments (Fig. 10a). The phyla *Alphaproteobacteria*, *Acidobacteriales*, *Chloroflexi*, and *Fimicutes* with M30, M50 and M100 treatments were significantly lower ($p < 0.05$) than that of with CK treatment. The results also indicated that relative abundance of *Gammaproteobacteria* were richer higher with M30 (5%), M50 (6%) and M100 (4%) treatments soil, and the relative abundance of *Acidobacteriales* and *Chlorobi* were higher with CK (14%, 10%) treatment soil. Compared with CK treatment, the relative abundance of *Proteobacteria* and *Actinobacteria* with M100 treatment was increased 62.50% and 112.50%, respectively.

Tang *et al*. (2020) [441] showed that phyla *Ascomycota* (50.3–72.5%), *Basidiomycota* (17.4–31.2%) and *Zygomycota* (7.5–16.6%) were the most abundant in the soil samples. And the order of phyla with different fertilizer treatments was showed *Ascomycota* > *Basidiomycota* > *Zygomycota*. And the phyla *Ascomycota* with CK (72.5%) treatment were significantly higher ($p < 0.05$) than that of with M30 (59.4%), M50 (55.6%) and M100 (50.3%) treatments (Fig. 10b). The phyla *Basidiomycota* and *Zygomycota* with M30, M50 and M100 treatments were significantly higher ($p < 0.05$) than that of with M0 and CK treatments. The results indicated that relative abundance of *Basidiomycota* and *Zygomycota* were richer higher ($p < 0.05$) with M30 (26.6%, 11.3%), M50 (28.6%, 13.5%) and M100 (31.2%, 16.6%) treatments soil, and the relative abundance of *Ascomycota* were higher ($p < 0.05$) with CK (72.5%) treatment soil. Compared with CK treatment, the relative abundance of *Basidiomycota* and *Zygomycota* with M100 treatment was increased 79.31% and 121.33%, respectively.

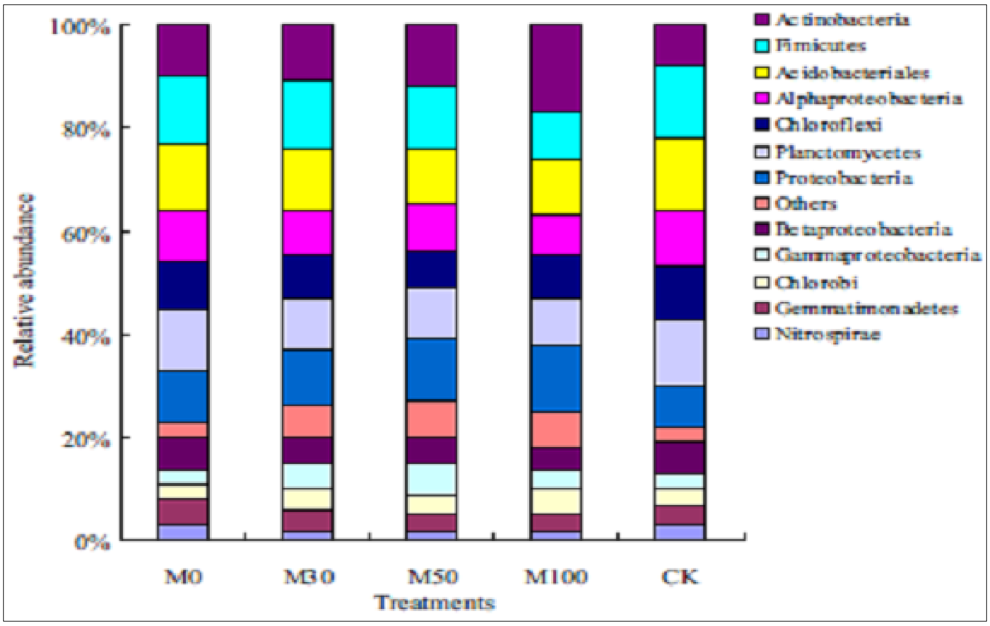

Fig 10a: Relative abundance of the dominant bacterial phyla in all soil samples combined and in each fertilizer treatments. M0: 100% N of chemical fertilizer, M30: 30% N of organic manure and 70% N of chemical fertilizer, M50: 50% N of organic manure and 50% N of chemical fertilizer, M100: 100% N of organic manure, CK: without N fertilizer input as control [Tang *et al*., 2020] [41]

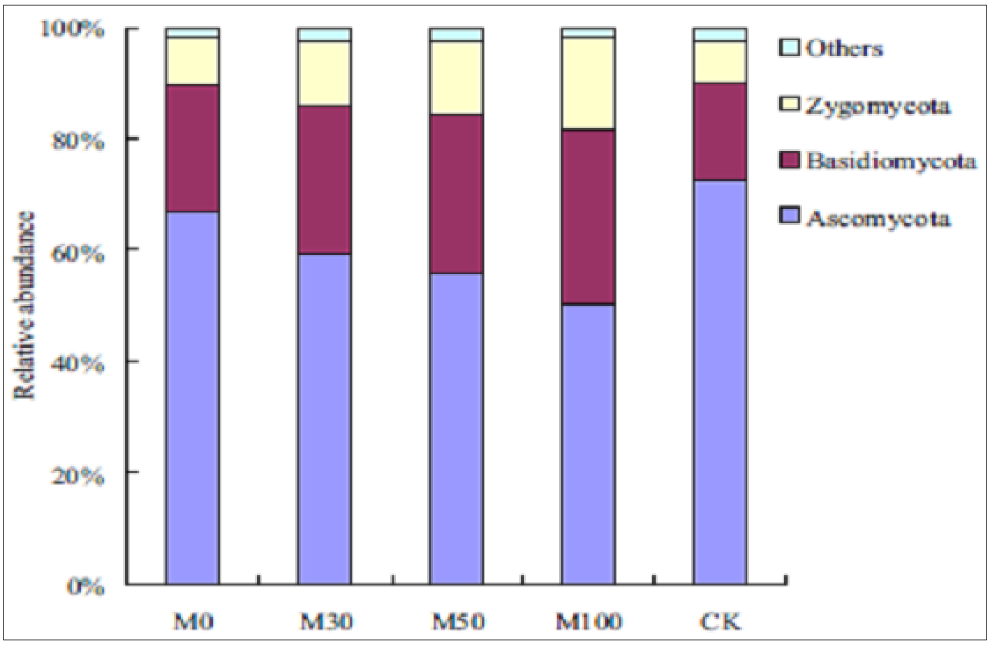

Fig 10b: Relative abundance of the dominant fungal phyla in all soil samples combined and in each fertilizer treatments [Tang *et al*., 2020] [41]

Conclusions

Use of organic manure with chemical fertilizer had positive effects on sequestering organic C in 0–20 cm layer of an Inceptisol, cultivated intensively in a sub-tropical agro ecosystem in North West IGP. Moreover, higher addition rates of organic manure benefited more prominent increase in the total SOC and its various C fractions. Manure application had beneficial effects on SOC stocks in the labile pool and to a greater extent in the recalcitrant pool, suggesting a pronounced change in SOM quality caused by long-term different fertilization treatments. Straw return and abandoned farmland significantly increased the contents of soil TOC and active SOC fractions. The soil TOC and active SOC fractions contents increased with the amount of straw returned, and straw return could slow the reduction in soil TOC content.

The INM increased total SOC concentrations due to substantial increments not only in the macro-aggregates and labile C pools, but also in the micro-aggregates and recalcitrant C pools. Increased labile and recalcitrant C in aggregates under INM is of specific importance, as it would reduce CO_2 emission from soils. INM also had a positive effect on the redistribution of SOC among the particle-size fractions, with obvious depletion of SOC in fine particles and pronounced enrichment in macro-aggregates. The C associated with the macro-aggregates could be used to assess the effects of long-term fertilization on SOC sequestration in an Inceptisol, as macro-aggregate- associated C was the largest C fraction of the total SOC pool across all treatments. Long-term abandoned farmland reduced soil microbial abundance, whereas straw return treatment enhanced soil microbial richness but did not influence soil microbial diversity. Straw return enhanced the relative abundances of microbes involved in the carbon cycle; including *Acidobacteria*, *Betaproteobacteria*, and *Deltaproteobacteria* Microbial community composition was closely related to the active SOC contents, with soil MBC and DOC being the primary factors influencing the soil microbial community at the phylum level. Balanced fertilizer application conjointly with FYM increased TOC pool and favorably impacted soil organic matter composition under rice-wheat system. Therefore, there is a need for balanced fertilizer application conjointly with FYM on long-term basis to sequester C in different pools in soil under rice-wheat cropping.

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