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Assessment of the contribution of fungal biomolecules to soil carbon sequestration in organic and conservation agriculture

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Abstract
The assessment of the impact of agri-management on the microbial activities related to C sequestration was undertaken using two contrasting long term (2003-2020) agricultural managements in the rice-wheat rotation. The aim was to assess the role of microbial glycoproteins for carbon sequestration in agricultural soil, Soil microorganisms are key agents determining the fate of soil C and aid in its sequestration. The organic management included input of a combination of nutrient sources farm yard manure (FYM), vermicompost (VC) and biofertilizers (BF) in the rice - wheat rotation. The treatment VC+CR+BF had significantly higher ergosterol, peroxidase, phenol oxidase, FDA hydrolase activity, β-glucosidase activity & Xylanase activity implying higher fungal populations that are active in the mineralization and subsequent loss of the soil C. while FYM+CR+BF had significantly higher, water-soluble phenolic content, SMBC at 0-30 cm soil depth. a high degree of homology of these microbial metabolites with the SOM indicates superiority of this treatment with its potential to increase the soil labile C fraction. The long-term conservation agriculture (Rice-Wheat rotation) experiments included Zero tilled direct seeded rice (ZTDSR) and zero tilled wheat (ZTW) along with moong bean and input of respective crop residues. These were compared with the farmers practice conventionally tilled rice and wheat (CTR – CTW). (MR + ZTDSR) – (RR + ZTW) – (WR + mungbean) had significantly higher ergosterol content & β-glucosidase activity. ZTDSR – ZTW had significantly higher peroxidase activity, glomalin content & water-soluble phenolic content, (WR + ZTDSR + BM) – (RR + ZTW)) had significantly higher phenol oxidase activity, MBC & xylanase activity with respect to (CTR – CTW). The (CTR – CTW) were dominated by aerobic microbial populations that accounted for the higher activities of oxidative enzymes resulting in loss of C as carbon dioxide. Zero tilled soils were found to aid the development of microbial community and their metabolites that are known precursors of recalcitrant C.

Keywords: Conservation agriculture, soil carbon sequestration, soil microbial indices, microbial metabolites, precursors of recalcitrant C

Introduction
Soil carbon in Indian tropical soils is low and it is a key factor responsible for soil health and fertility. There is need to identify agricultural practices and the associated changes in soil microbial communities that may assist in buildup of soil organic carbon levels and minimize the C losses from soil. Therefore, in the present study, attempts have been made to identify agricultural practices and the associated changes in soil microbial communities that may assist in buildup of soil organic carbon levels and minimize the C losses from soil. Fungi are the major regulators of nutrient transformations among soil microbial communities. Temperature, moisture, texture, and structure, as well as tillage and cropping management, all have an interacting cumulative impact on carbon dynamics. Microbial degradation of external C sources applied to soil as agricultural waste results in the loss of 2/3 of the carbon. Although primary production plays a role in carbon sequestration in soils, it is the size and activity of the soil's microbial biomass that controls carbon accumulation through mineralization and immobilization of plant and microbially derived residues. Agricultural management practices influence AMF and thus account for soil carbon sequestration. Physical protection by aggregates related to the reactive characteristics of clays is directly and/or indirectly used by a soil to safeguard microbial biomass and microbially produced organic matter. The efficiency with which microorganisms utilize substrate C, as well as the chemical composition of the by-products they produce, are both important factors in soil C stabilization.
Microorganisms have the following benefits for CO_2 sequestration:

- Rapid generation,
- High photosynthetic conversion
- High capability for environmental bioremediation, such as CO_2 fixation from the atmosphere or fuel gas.

Materials and Methods

Soil sample collection

The soil samples were collected from the field at the flowering stage and transported to laboratory in ice box. The samples were divided into two parts, for biological studies the field moist samples were stored at–4 °C. A study on abundance and activities of fungi present in these two systems was undertaken, and fungal signature molecules and enzymes active in C mineralization were studied.

Treatment details for conservation agriculture

T_1: ZTDSR–ZTW with 100% RDN (Rice & Wheat crops)
T_2: (WR + ZTDSR + BM) – (RR + ZTW) with 100% RDN (both crops)
T_3: ZTDSR – ZTW – ZT Mungbean with 100% RDN (both crops)
T_4: (MR + ZTDSR) – (RR + ZTW) – (WR + Mungbean) with 100% RDN (both crops)
T_5: CTR – ZTW with 100% RDN (both crops)
T_6: Farmers' practice (CTR – CTW) with 100% RDN (both crops)
CTW: Conventionally tilled wheat
CTR: Conventionally puddled transplanted rice
BM: Brown manuring of Dhaincha at 25 days after sowing by 2,4-D/ Bispyribac sodium spraying
RR: Rice residue (40%)
WR: Wheat residue (20%)
MR: Mungbean residue (100%)
RDN: Recommended dose of nitrogen (120 kg ha-1)

Crops

- Kharif season : Rice, variety PRH–10
- Rabi season : Wheat, variety HD CSW 18
- Summer season: Mungbean, variety Pusa Vishal

Experimental Design: Strip plot
Replication: 3
Variety: Pusa Basmati 1728 (Rice), HD 3086 (Wheat), Pusa Vishal (Mungbean)
Year of Start: 2003
Current Season: Kharif 2020 and Rabi 2020-2021

Treatment Details for Organic Cultivation

The experiment was laid out in a strip plot design with three replications which consisted of two rice-based cropping systems, i.e., Basmati Rice-Wheat-Sesbania (RWS) and Basmati Rice-Wheat-Mungbean (RWM) in strips and seven combinations of different organic materials and Biofertilizers (BF) preceding crop @ 3 t/ha for each rice, wheat and mungbean, VC+CR, FYM+CR+BF and VC+CR+BF, and control (no fertilizer applied) were applied in sub-plot. Pusa Basmati 1121, HD 2967 and Pusa Vishal varieties were used for rice, wheat and mungbean, respectively. Combinations of organic sources and biofertilizers were applied to both rice and wheat, whereas mungbean in rice-wheat-mungbean cropping system was grown on residual fertility. For biofertilizers, Blue green algae (BGA), Phosphate solubilizing bacteria (PSB) (*Pseudomonas striata*) and cellulolytic culture (*Aspergillus awamori*, *Trichoderma viride*, *Phanerochaete chrysosporium* and *Aspergillus wolulens*) used in rice, Azotobacter, PSB (*Pseudomonas striata*) and cellulolytic culture in wheat and Rhizobium + PSB in mungbean, and Soil samples were collected after harvest of rice crop.

Table 1: Standard protocols to analyze the proposed parameters

Contribution of fungi for mineralization of labile carbon pool	West and Sparling (1986)
Fluorescein diacetate (FDA) hydrolase activity	Green *et al*. (2006) [3]
Phenol oxidase and peroxidise activity	Bach *et al*. (2013) [1]
Ergosterol	Grant and West (1996) [5]
Soil microbial biomass carbon	Vance *et al*. (1987) [4]
Glomalin content	Wright and Upadhyaya (1999) [6]
β– glucosidase activity	Eivazi and Tabatabai (1988) [2]

Results and Discussion

An experiment on conservation agriculture treatments and organic cultivation followed by Rice-Wheat cropping system was conducted during 2020-21 at the research farm of the Indian Agricultural Research Institute (ICAR-IARI, New Delhi) India. Two sets of soil samples were collected after the wheat harvest from these long-term experiment field at 0-30 cm soil depth with a core sampler (7.5 cm diameter) using a soil auger according to treatments (6 in conservation agriculture treatments), (8 in organic cultivation) and cropping system (Rice-Wheat) at flowering stage. Each soil sample were divided into two parts: One part was kept in refrigerator for analysis of biological parameters and the other part was air-dried and processed for chemical analysis. Soil samples were examined for soil physical and/ or chemical parameters on rice & wheat crop. An experiment details given in material & methods were analysed for various parameters like Soil microbiological studies: Ergosterol estimation, FDA hydrolase activity, Soil respiration, Soil dehydrogenase activity, SMBC, β-glucosidase activity, Glomalin protein estimation, and PLFA analysis, etc.

Organic Agriculture Soils

1) Quantitative estimation of the soil ergosterol as influenced by the organic agricultural practices in the Rice-Wheat cropping system

Ergosterol ($C_{28}H_{44}O$) is a lipid present in most fungus and some microalgae cell membranes. It is a sterol exclusively present in the fungi and used as a proxy for fungal biomass estimation. Commonly used as a biomarker to measure fungal biomass in soil. Under field conditions, fungal biomass in the soil can be a good sign of fungal activity and decomposition processes, and fungal biomass in the soil can be an essential indicator of soil health and internal biogeochemical processes. Fungi have both free and esterified forms of ergosterol, cell membranes contain the free form, whereas cytosolic lipid particles contain esters. The ergosterol activity under organic management was significantly influenced under different

cropping system. An application of VC+CR+BF in R-W-M resulted in significantly highest ergosterol activity while lowest due to VC+CR. The ergosterol activity ranged from 15.61±1.2 to 24.41±2.2 ug Ergosterol/g soil (Table-1) in the different treatments of organic cultivation R-W-M (Control), highest and lowest was found in VC+CR+BF and VC+CR respectively, the order of activity was VC+CR+BF>FYM+CR+BF>VC+CR. The ergosterol activity ranged from 17.62±1.5 to 28.46±2.5 ug Ergosterol/g soil (Table-1) in the different treatments of organic cultivation R-W-S (Control), highest and lowest was found in VC+CR and FYM+CR+BF respectively. The order of activity was VC+CR>VC+CR+BF>FYM+CR+BF.

Table 1: Quantitative Estimation of the Soil Ergosterol

Ergosterol (ug Ergosterol/g soil)	
Treatments	**MEAN±SD**
T1 : R-W-M (Control)	19.96±1.8 [d]
T3 : VC+CR	15.61±1.2 [f]
T5 : FYM+CR+BF	22.34±2 [c]
T7 : VC+CR+BF	24.41±2.2 [b]
R-W-S (Control)	
T2 : R-W-S (Control)	21.23±1.9 [cd]
T4 : VC+CR	28.46±2.5 [a]
T6 : FYM+CR+BF	17.62±1.5 [e]
T8 : VC+CR+BF	23.81±2.1 [b]
LSD (P=0.05)	1.746

2) Quantitative estimation of the Soil Peroxidase activity as influenced by the organic agricultural practices in the Rice - Wheat cropping system

Soil Peroxidase Activity

Primarily used for degradation or mineralization of relatively resistant C polymer, mainly of fungal origin. The Peroxidase activity ranged from 7.65±3.91 to 9.0±4.54 (µ mol/h/g dry soil) (Table-2) in the different treatments of organic cultivation R-W-M (Control), highest and lowest was found in VC+CR+BF and FYM+CR+BF respectively. The order of activity was VC+CR+BF> VC+CR>FYM+CR+BF. The Peroxidase activity ranged from 7.97±4.04 to 12.80±6.45 (µ mol/h/g dry soil) (Table-4. 2) in the different treatments of organic cultivation R-W-S (Control), highest and lowest was found in VC+CR and VC+CR+BF respectively. The order of activity was VC+CR > FYM+CR+BF > VC+CR+BF.

Table 2: Quantitative estimation of the Soil Peroxidase activity

Peroxidase activity (µ mol /h/g dry soil)	
Treatments	**MEAN±SD**
T1 : R-W-M (Control)	8.46±4.35 [b]
T3 : VC+CR	7.75±3.92 [b]
T5 : FYM+CR+BF	7.65±3.91 [b]
T7 : VC+CR+BF	9.0±4.54 [b]
R-W-S (Control)	
T2 : R-W-S (Control)	7.54±3.86 [b]
T4 : VC+CR	12.80±6.45 [a]
T6 : FYM+CR+BF	12.10±6.10 [a]
T8 : VC+CR+BF	7.97±4.04 [b]
LSD (P=0.05)	4.071

3) Quantitative estimation of the Soil Phenol oxidase activity as influenced by the organic agricultural practices in the Rice-Wheat cropping system.

The Phenol oxidase activity ranged from 4.28±2.23 to 10.09±5.07 (µ mol/h/g dry soil) (Table-3) in the different treatments of organic cultivation R-W-M (Control), highest and lowest was found in FYM+CR+BF and VC+CR+BF respectively. The order of activity was FYM+CR+BF> VC+CR > VC+CR+BF. The Phenol oxidase activity ranged from 5.15±2.67 to 14.48±7.30 (µ mol/h/g dry soil) (Table-3) in the different treatments of organic cultivation R-W-S (Control), highest and lowest was found in VC+CR+BF and VC+CR respectively. The order of activity was VC+CR+BF> FYM+CR+BF > VC+CR.

Table 3: Quantitative estimation of the Soil Phenol oxidase activity

Phenol oxidase (µ mol/h/g dry soil)	
Treatments	**MEAN±SD**
T1 : R-W-M (Control)	7.27±3.73 [bc]
T3 : VC+CR	6.40±3.322 [c]
T5 : FYM+CR+BF	10.09±5.07 [b]
T7 : VC+CR+BF	4.28±2.23 [c]
R-W-S (Control)	
T2 : R-W-S (Control)	5.15±2.67 [c]
T4 : VC+CR	5.15±2.67 [c]
T6 : FYM+CR+BF	5.48±2.82 [c]
T8 : VC+CR+BF	14.48±7.30 [a]
LSD (P=0.05)	4.57

4) FDA Hydrolase Activity

This stain is used specifically for measuring enzyme activity of actively growing fungal hyphae, amount of fluorescein produced by the hydrolysis of fluorescein diacetate (FDA) was exactly proportional to the microbial population. The ability to hydrolyze FDA appears to be widespread among decomposers, bacteria, and fungi, because microbial decomposers account for more than 90% of the energy flow in a soil system, an assay that measures microbial decomposer activity and overall microbial activity.

The FDA Hydrolase activity ranged from 2.56±0.21 to 3.38±0.29 (µg/g oven dry soil) (Table-4) in the different treatments of organic cultivation R-W-M (Control), highest and lowest was found in VC+CR+BF and VC+CR respectively. The order of activity was VC+CR+BF> FYM+CR+BF > VC+CR.

The FDA Hydrolase activity ranged from 2.25±0.2to 3.08±0.30 (µg/g oven dry soil) (Table-4) in the different treatments of organic cultivation W-S (Control), highest and lowest was found in FYM+CR+BF and VC+CR+BF respectively. The order of activity was FYM+CR+BF> VC+CR> VC+CR+BF.

Quantitative estimation of the FDA Hydrolase activity as influenced by the organic agricultural practices in the Rice-Wheat cropping system.

Table 4: Quantitative estimation of the FDA Hydrolase activity

FDA Activity (µg/g oven dry soil)	
Treatments	**MEAN±SD**
T1 : R-W-M (Control)	1.49±0.12 [g]
T3 : VC+CR	2.56±0.21 [e]
T5 : FYM+CR+BF	3.07±0.31 [c]
T7 : VC+CR+BF	3.38±0.29 [b]
R-W-S (Control)	
T2 : R-W-S (Control)	2.70±0.23 [d]
T4 : VC+CR	2.98±0.24 [c]
T6 : FYM+CR+BF	3.08±0.30 [c]
T8 : VC+CR+BF	2.25±0.2 [f]
LSD (P=0.05)	0.173

5) Soil microbial biomass carbon (SMBC)

The Soil microbial biomass carbon (SMBC) ranged from 60.22±5.08 to 473.21±46.5 (mg/kg soil) (Table-5) in the different treatments of organic cultivation R-W-M (Control), highest and lowest was found in FYM+CR+BF and VC+CR+BF respectively, the order of activity was FYM+CR+BF> VC+CR> VC+CR+BF.

The Soil microbial biomass carbon (SMBC) ranged from 154.87±14 to 1058.27±103**(**mg/kg soil) (Table-5) in the different treatments of organic cultivation R-W-S (Control), highest and lowest was found in FYM+CR+BF and VC+CR+BF respectively, the order of activity was FYM+CR+BF > VC+CR > VC+CR+BF.

Quantitative estimation of the Soil microbial biomass carbon (SMBC) as influenced by the organic agricultural practices in the Rice-Wheat cropping system

Table 5: Quantitative estimation of the Soil microbial biomass carbon (SMBC)

MBC (mg /kg soil)	
Treatments	**MEAN±SD**
T1 : R-W-M (Control)	714.122±70 ^{c}
T3 : VC+CR	94.64±9 ^{fg}
T5 : FYM+CR+BF	473.21±46.5 ^{d}
T7 : VC+CR+BF	60.22±5.08 ^{gh}
R-W-S (Control)	
T2 : R-W-S (Control)	782.95±76 ^{c}
T4 : VC+CR	301.13±29 ^{e}
T6 : FYM+CR+BF	1058.27±103 ^{b}
T8 : VC+CR+BF	154.87±14 ^{f}
LSD (P=0.05)	105.677

6) PLFA analysis

The PLFA analysis ranged from 1501.11 to 1901.22 Biomass content (n moles/gm) (Table-6) in the different treatments of organic cultivation, highest and lowest was found in VC+CR and VC+CR (R-W-S field) respectively, the order of activity was VC+CR> FYM+CR+BF > VC+CR+BF >VC+CR (R-W-S field).

- Straight organism-Eukaryotes
- Branched organism–Gram-positive bacteria
- Cyclo organism–Gram-negative bacteria
- MUFA Conent–Gram-negative bacteria
- PUFA Content–Saprotrophic fungi
- 18:1w9c Conent–Cyanobacteria; green algae
- 18:2w6,9c Conent-Saprotrophic fungi
- 10-methyl Content–Actinomycetes, Actinobacteria
- 16:1w5c Content–Arbuscular mycorrhizal fungi
- 18:1w Hydroxy 9c Conent–Gram-negative bacteria

Table 6: Quantitative estimation of the PLFA Analysis as influenced by the organic agricultural practices in the Rice-Wheat cropping system

Organic soil–PLFA	
Treatments	**Biomass Content (n moles/gm)**
T1 : R-W-M (Control)	983.3
T3 : VC+CR	1901.22
T4 : VC+CR (R-W-S field)	1501.11
T5 : FYM+CR+BF	1735.9
T7 : VC+CR+BF	1589.98

Conservation Agriculture Soils

7) Ergosterol Activity

The ergosterol activity ranged from 2.30±0.2 to 6.15±0.5 ug Ergosterol/g soil (Table-7) in the different treatments of conservation agriculture soils, highest and lowest was found in (MR + ZTDSR) – (RR + ZTW) – (WR + mungbean) with 100% RDN and (WR + ZTDSR + BM) – (RR + ZTW) with 100% RDN (both crops) respectively(Plate 4.5),The order of activity was (MR + ZTDSR) – (RR + ZTW) – (WR + mungbean) with 100% RDN > ZTDSR – ZTW – ZT mungbean with 100% RDN (both crops) >ZTDSR – ZTW with 100% RDN (Rice & Wheat crops)> CTR – ZTW with 100% RDN (both crops) >(WR + ZTDSR + BM) – (RR + ZTW) with 100% RDN (both crops).

Table 7: Quantitative estimation of the soil ergosterolas influenced by the conservation agricultural practices in the Rice-Wheat cropping system

Conservation Agriculture Soils	
Ergosterol Estimation (ug Ergosterol/g soil)	
Treatments	**MEAN±SD**
T1: ZTDSR – ZTW with 100% RDN (Rice & Wheat crops)	3.71±0.23 ^{c}
T2: (WR + ZTDSR + BM) – (RR + ZTW) with 100% RDN (both crops)	2.30±0.2 ^{e}
T3: ZTDSR – ZTW – ZT mungbean with 100% RDN (both crops)	5.58±0.51 ^{b}
T4: (MR + ZTDSR) – (RR + ZTW) – (WR + mungbean) with 100% RDN	6.15±0.5 ^{a}
T5: CTR – ZTW with 100% RDN (both crops)	3.21±0.29 ^{d}
T6: Farmers' practice (CTR – CTW) with 100% RDN (both crops)	3.04±0.31 ^{d}
LSD (P=0.05)	0.343

8) FDA Hydrolase Activity

The FDA hydrolase activity ranged from 2.26±0.24 to 5.03±0.51 (µg/g oven dry soil) (Table-8) in the different treatments of conservation agriculture soils, highest and lowest was found in CTR–ZTW with 100% RDN (both crops) and (WR + ZTDSR + BM) – (RR + ZTW) with 100% RDN (both crops) respectively(Plate 4.3), The order of activity was CTR – ZTW with 100% RDN (both crops) > (MR + ZTDSR) – (RR + ZTW) – (WR + mungbean) with 100% RDN >ZTDSR – ZTW with 100% RDN (Rice & Wheat crops) >ZTDSR – ZTW – ZT mungbean with 100% RDN (both crops) >(WR + ZTDSR + BM) – (RR + ZTW) with 100% RDN (both crops).

Table 8: Quantitative estimation of the FDA hydrolase activity as influenced by the conservation agricultural practices in the Rice-Wheat cropping system

FDA Activity (µg/g oven dry soil)	
Conservation Agriculture Soils	
Treatments	**MEAN±SD**
T1: ZTDSR – ZTW with 100% RDN (Rice & Wheat crops)	2.65±0.21 [d]
T2: (WR + ZTDSR + BM) – (RR + ZTW) with 100% RDN (both crops)	2.26±0.24 [e]
T3: ZTDSR – ZTW – ZT mungbean with 100% RDN (both crops)	2.61±0.28 [d]
T4: (MR + ZTDSR) – (RR + ZTW) – (WR + mungbean) with 100% RDN	4.01±0.41 [b]
T5: CTR – ZTW with 100% RDN (both crops)	5.03±0.51 [a]
T6: Farmers' practice (CTR – CTW) with 100% RDN (both crops)	3.19±0.34 [c]
LSD (P=0.05)	0.245

9) Soil microbial biomass carbon (SMBC)

The soil microbial biomass carbon (SMBC) ranged from 576.46±55 to 1626.13±158(mg/kg soil) (Table-9) in the different treatments of conservation agriculture soils, highest and lowest was found in (WR + ZTDSR + BM) – (RR + ZTW) with 100% RDN (both crops) and ZTDSR – ZTW with 100% RDN (Rice & Wheat crops) respectively, The order of activity was (WR + ZTDSR + BM) – (RR + ZTW) with 100% RDN (both crops) > (MR + ZTDSR) – (RR + ZTW) – (WR + mungbean) with 100% RDN > ZTDSR – ZTW – ZT mungbean with 100% RDN (both crops) >CTR – ZTW with 100% RDN (both crops) > ZTDSR – ZTW with 100% RDN (Rice & Wheat crops).

Table 9: Quantitative estimation of the soil microbial biomass carbon (SMBC) as influenced by the conservation agricultural practices in the Rice-Wheat cropping system

MBC (mg /kg soil)	
Conservation Agriculture Soils	
Treatments	**MEAN±SD**
T1: ZTDSR – ZTW with 100% RDN (Rice & Wheat crops)	576.46±55 [d]
T2: (WR + ZTDSR + BM) – (RR + ZTW) with 100% RDN (both crops)	1626.13±158 [a]
T3: ZTDSR – ZTW – ZT mungbean with 100% RDN (both crops)	636.68±65 [d]
T4: (MR + ZTDSR) – (RR + ZTW) – (WR + mungbean) with 100% RDN	886.20±86 [b]
T5: CTR – ZTW with 100% RDN (both crops)	619.48±59 [d]
T6: Farmers' practice (CTR – CTW) with 100% RDN (both crops)	739.93±71 [c]
LSD (P=0.05)	99.945

Conclusion

Soil carbon stocks of agricultural land are experiencing a continuance declining trend. In order to meet the eve growing demand for food and simultaneously reduce carbon losses/increase soil carbon storage alternative agricultural methods are being adopted. In this direction, the organic agriculture and the conservation agriculture practices are reported to improve natural resource use efficiency and gaining popularity globally. The key practices of organic agriculture, are focused on closed nutrient cycles by recycling plant residues and manures from livestock back to soil thereby significantly reducing the soil carbon losses or even to higher soil carbon concentrations and net carbon sequestration over time. another approach, the conservation agriculture also relies on the input of crop residues as surface mulch, rotation of crops and minimum or no disturbance of soil by avoiding tillage, agricultural management play a critical role in plant-microbe interaction.

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