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To determine possible relationship between the soil factors related to C sequestration and changes in the abundance, diversity of soil microorganisms under alternate tillage methods

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Abstract

An experiment on Influence of Conservation Agriculture on soil microbial indices contributing to soil carbon sequestration in rice–wheat cropping system was conducted during 2018– 20 at the research farm of the Indian Agricultural Research Institute (IARI), New Delhi, India. The treatments included: 1. Zero tillage (ZT) 100% N, 2. ZT 100%N + Brown manuring (BM), 3. ZT100%N +CR, 4. ZT100%N +CR+BM, 5. ZT100%N + Mungbean (MB), 6. ZT100%N +MB +CR, 7. Conventional transplanted rice (CTR) – zero tillage wheat (ZTW) 100%N, 8. CT 100%N. The 16:1w5c content (Arbuscular mycorrhizal fungi), 18:1w Hydroxy 9c content (Gram-negative bacteria) was maximum in ZT +CR+BM. CTR – ZTW had higher monounsaturated fatty acids, (MUFA) in rice crops. ZT had lower polyunsaturated fatty acids (PUFA) content (Saprotrophic fungi), 18:2w6,9c content (Saprotrophic fungi) and higher straight organism, branched organism, 18:1w9c content (Cyanobacteria; green algae), 10-methyl content (Actinomycetes, Actinobacteria), biomass. ZT+BM had higher straight organism, Cyclo organism (Gram-negative bacteria), 16:1w5c content (Arbuscular mycorrhizal fungi), biomass, and lower branched organism (Gram-positive bacteria), monounsaturated fatty acids, (MUFA), polyunsaturated fatty acids (PUFA) content (Saprotrophic fungi), DMA content, 18:1w9c content (Cyanobacteria; green algae), 18:2w6,9c content (Saprotrophic fungi), 10-methyl content (Actinomycetes, Actinobacteria) in rice crop. ZT+CR had higher straight organism, Cyclo organism (Gram-negative bacteria), DMA content, 10-methyl content (Actinomycetes, Actinobacteria), 16:1w5c content (Arbuscular mycorrhizal fungi), biomass, and lower branched organism, monounsaturated fatty acids, (MUFA), polyunsaturated fatty acids (PUFA) content (Saprotrophic fungi), 18:1w9c content (Cyanobacteria; green algae), 18:2w6,9c content (Saprotrophic fungi) in rice crop. ZT+MB had higher straight organism, 16:1w5c content (Arbuscular mycorrhizal fungi), and lower branched organism, monounsaturated fatty acids, (MUFA), polyunsaturated fatty acids (PUFA) content (Saprotrophic fungi), DMA content, 18:1w9c content (Cyanobacteria; green algae), 18:2w6,9c content (Saprotrophic fungi), 10-methyl content (Actinomycetes, Actinobacteria), biomass in rice crop.

Keywords: Determine, possible, microorganisms, alternate, methods

1. Introduction

Cereal–cereal systems, especially rice (*Oryza sativa* L.) –wheat (*Triticum aestivum* L.) (R-W), contribute to satisfy the bulk of the food demand in South Asia. Rice–wheat cropping systems possess important functions in food security in Asia by providing food grains for more than 20% of the population worldwide (Kumari M *et al.*, 2011) [17]. Thus, conservation tillage, along with some complimentary practices such as soil cover and crop diversity has emerged as a viable option to ensure sustainable food production and maintain environmental integrity. Soil organic carbon (SOC) plays an important role in improving soil fertility and sustaining soil productivity due to its effects on soil physical, chemical and biological properties (Gong *et al.*, 2009; He *et al.*, 2015) [8]. Changes in SOC are influenced by many management practices, such as fertilizer application, straw return and tillage (Zhu *et al.*, 2015) [27]. Microbial biomass C (MBC) are considered to be early and sensitive indicators of soil quality changes because of their rapid responses to changes in soil management practices (Cambardella and Elliott, 1992) [2]. AM fungi are root symbionts present in many terrestrial ecosystems and are known to form beneficial associations with nearly all agricultural crops (Douds and Millner, 1999) [5], though symbiotic associations, especially in high phosphorus or high nitrogen environments (Johnson *et al.*, 2015) [13]. AM fungi can provide benefits to crop plants through multiple mechanisms including pathogen resistance and nutrient acquisition (Wehner *et al.*, 2010) [26].

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AM fungi can also increase soil ecosystem function. Following colonization of the crop root, AM fungi typically create an extensive extra-radical hyphal (ERH) network to explore the soil and transfer nutrients. Conversion to less intensive row crop agricultural management systems such as biologically-based organic and no-till may reduce the negative effects of conventional management to AM fungi (Kabir, 2005) [14]. Similarly, adopting no-till management increased AM fungus abundance, root colonization. However, these less intensive agricultural systems may still affect soil microbial communities through more limited combinations of chemical (fertilizer) and mechanical (tillage) disturbance. The low AM fungus diversity in both organically managed and intensive conventional row crop systems, possibly due to residual high soil P levels from previous conventional management (Hijri *et al.*, 2006) [10]. Further research is needed to understand how the AM fungus community responds long-term to implementation of different row crop management systems for previously cultivated land.

2. Material and Methods

2.1 Phospholipid fatty acid (PLFA) analysis

PLFA in bulk soil was determined according to Vestal and White, 1989. Soil samples were obtained from two soil depths (0-15 and 15-30 cm) at flowering and harvest from rice and wheat crop soil. For the study of microbial communities, lipid analysis basically entails the extraction of lipids from a sample with organic solvents followed by analysis of certain fractions of the extracted material. The extraction and analysis are straightforward. In the field or laboratory, the sample is

exposed to a single-phase mixture of chloroform, methanol, and water in an initial ratio of 1:2:0.8. When these solvents are added, the lipids dissolve almost instantly and further lipid metabolism stops. PLFAs were extracted (from two replicate 30 g samples of inoculum for each treatment; these values were averaged before conducting statistical analyses), and analyzed according to Briefly, lipids were removed from samples into chloroform using an extraction procedure. Phospholipids were separated from other lipids by silica acid chromatography, and derivatized to their fatty acid methyl esters (FAMES) for analysis by gas chromatography. FAMES were identified by relative retention times, co-elution with purchased standards and comparison of samples between capillary columns of differing polarity [HP-5 (cross-linked 5% phenyl methyl silicone), 50 m 0.32 mm 0.52 mm film; HP-225 (50% cyanopropylphenyl methylpolysiloxane), 30 m 0.32 mm 0.25 mm film; Hewlett-Packard, Palo Alto, CA]. Final verification of peak identity was made by gas chromatography/mass spectroscopy on representative samples. FAMES were quantified on an HP-225 column using an HP 6890 series gas chromatography system (Hewlett-Packard) and a protocol according to. PLFAs were summed per sample to obtain the total amount (nmol PLFA per g inoculum) as an indication of total microbial biomass. Arbuscular mycorrhizal fungi themselves produce a variety of fatty acids. In order to avoid confounding effects, we analyzed PLFAs of predominantly fungal and bacterial origin separately.

3. Results and Discussion

Table 1: Phospholipid fatty acid analysis (PLFA) in different tillage treatments in rice soil

Treatments	PLFA (nmoles/gm)											Biomass content (nmoles/gm)
	Straight	Branched	Cyclo	MUFA	PUFA	DMA	18:1w9c	18:2w6,9c	10-methyl	16:1w5c	Hydroxy	
ZT100%N	19.62	44.63	_	5.41	9.81	5.16	8	3.82	3.55	_		627.1
ZT100%N+BM	36.34	6.35	0.36	4.01	37.04	4.35	4.15	2.05	2.14	3.21	_	2539.9
ZT100%N+CR	33.29	5.96	0.37	4.16	39.35	5.38	3.31	1.91	3.71	2.56	_	1515.02
ZT100%N+CR+BM	13.22	24.52	--	3.98	51.97	0.9	1.69	0.97	2.3	0.45	-	1922.45
ZT100%N+MB	43.74	7.94	--	2.65	40.42	0.62	1.59	0.72	0.99	1.32	-	1098.98
ZT100%N+MB+CR	30.71	5.46	_	6.98	34.58	5.24	5.52	3.47	_	6.99	1.06	319.2
CTR - ZTW 100%N	21.4	12.36	0.26	7.53	34.28	4.47	7.57	4.43	3.1	4.21	0.39	1814.6
CT100%N	15.51	6.86	0.11	_	56.97	5.25	5.41	7.89	2.9	1.11	-	300.19

ZT+MB had significantly higher straight organism (43.74) than ZT and CT (122.93% higher than ZT and 182.01% higher than CT). ZT had significantly higher value (26.49%) of straight organisms than CT. ZT +BM had significantly higher (85.21%) straight organism than without BM. ZT +CR had significantly higher (69.67%) straight organisms than without CR. ZT +MB had significantly higher (122.93%) straight organism than without MB in crop rotation.

ZT had significantly higher straight organisms (44.63) (550.58%) than CT. ZT +BM had significantly lower (602.83%) branched organisms (Gram-positive bacteria) than without BM. ZT +CR had significantly lower (648.82%) branched organism than without CR. ZT +MB had significantly lower (462.09%) branched organism than without MB in crop rotation.

ZT+CR had significantly higher Cyclo organisms (Gram-negative bacteria) (0.37) (236.36%) than CT. ZT +BM had significantly higher (227.27%) Cyclo organisms than CT.

CTR - ZTW had significantly higher monounsaturated fatty

acids, (MUFA) content (Gram-negative bacteria) (7.53) (39.18%) than ZT. ZT +BM had significantly lower (34.91%) MUFA content than without BM. ZT +CR had significantly lower (30.04%) MUFA content than without CR. ZT +MB had significantly lower (104.15%) MUFA content than without MB in crop rotation.

CT had significantly higher polyunsaturated fatty acids (PUFA) content (Saprotrophic fungi) content (56.97) (480.73%) than ZT. ZT +BM had significantly lower (53.80%) PUFA content than without BM. ZT +CR had significantly lower (44.73%) PUFA content than without CR. ZT +MB had significantly lower (40.94%) PUFA content than without MB in crop rotation.

ZT+CR had significantly higher DMA content (5.38) than ZT and CT (4.26% higher than ZT and 2.47% higher than CT). ZT +BM had significantly lower (18.62%) DMA content than without BM. ZT +CR had significantly lower (4.26%) DMA content than without CR. ZT +MB had significantly lower (732.25%) DMA content than without MB in crop rotation.

ZT had significantly higher 18:1w9c content (Cyanobacteria; green algae) (8.00) (47.87%) CT. ZT +BM had significantly lower (92.77%) 18:1w9c content than without BM. ZT +CR had significantly lower (141.69%) 18:1w9c content than without CR. ZT +MB had significantly lower (403.14%) 18:1w9c content than without MB in crop rotation.

CT had significantly higher 18:2w6,9c content (Saprotrophic fungi) (7.89) (122.25%) ZT. ZT +BM had significantly lower (86.34%) 18:2w6,9c content than without BM. ZT +CR had significantly lower (100%) 18:2w6,9c content than without CR. ZT +MB had significantly lower (430.55%) 18:2w6,9c content than without MB in crop rotation.

ZT +CR had significantly higher 10-methyl content (Actinomycetes, Actinobacteria) (3.71) ZT and CT (4.50% higher than ZT and 27.93% higher than CT). ZT had significantly higher value (22.41%) of 10-methyl content than CT. ZT +BM had significantly lower (65.88%) 10-methyl content than without BM. ZT +CR had significantly higher (4.50%) 10-methyl content than without CR. ZT +MB had significantly lower (258.58%) 10-methyl content than without MB in crop rotation.

ZT +MB +CR had significantly higher 16:1w5c content (Arbuscular mycorrhizal fungi) (6.99) (529.72%) than CT. ZT +BM had significantly higher (189.18%) 16:1w5c content than CT. ZT +CR had significantly higher (130.63%) 16:1w5c content (AMF biomarker whole cell fatty acid (WCFA) than CT. ZT +MB had significantly higher (18.91%) 16:1w5c content than CT.

ZT +MB +CR had significantly higher 18:1w Hydroxy 9c content (Gram-negative bacteria) (1.06) (171.09%) than CTR – ZTW.

ZT +BM had significantly higher biomass (2539.9) than ZT and CT (305.02% higher than ZT and 746.09% higher than CT). ZT had a significantly higher value (108.90%) of biomass than CT. ZT +BM had significantly higher (305.02%) biomass than without BM. ZT +CR had significantly higher (141.59%) biomass than without CR. ZT +MB had significantly lower (75.24%) biomass than without MB in crop rotation.

3.1 Microbial abundance and diversity in rice-wheat cropping system: Phospholipid fatty acid analysis (PLFA)

The microbial densities are affected by tillage in agricultural soils (Wang *et al.*, 2016). Our study represents a comprehensive synthesis and strives to reveal the effect of zero tillage (ZT) on microbial abundance and diversity in the rice-wheat cropping system. In our study, ZT show significantly increased abundance and diversity of straight organism and branched organism (Gram-positive bacteria), 18:2w6,9c content (Saprotrophic fungi), 18:1w9c (Cyanobacteria; green algae), 10-methyl content (Actinomycetes, Actinobacteria), higher total biomass than conventional tillage (CT). The effect of management practices on AM fungal diversity and community structure are more nuanced. No-till increased AM fungus diversity than organic management, removed the mechanical disturbance to the hyphal network (De la Providencia *et al.*, 2007) ^[4], while conventional tillage is intermediate. The AM fungus diversity decreased in conventional horticultural and agricultural cropping systems (Jansa *et al.*, 2002) ^[12]. The no-till than conventional-till showed increased bacterial diversity (Cejanavarró *et al.*, 2010) ^[3]. The fungal growth and activity are favored by zero tillage due to enhanced establishment and

maintenance of extensive hyphal network for organic matter decomposition and nutrient cycling processes to natural ecosystems (Bailey *et al.*, 2002) +. Tillage reduced densities of total and metabolically active hyphae. Soils under NT and reduced tillage (RT) practices were richer in fungal hyphae than CT soils. There were negative effects of soil disturbance on AM hyphal growth and sporulation (Douds *et al.*, 1993) ^[6]. Arbuscular mycorrhizal fungi having biotrophic nature hurted by tillage much more severely than other soil fungi. There were reduction in growth and development due to disruption of their extraradical hyphae and detachment from their host root system due to tillage practices. A low proportion of viable hyphae was associated with CT, particularly harmful to AMF. The AMF proliferation is less repressed by tillage than saprophytic hyphae having the lower proportion of carbon (C). Arbuscular mycorrhizal hyphae survived in winter in soil relatively well, when hyphae overwinter attached to dead roots (77% of initial metabolically active hyphae survive). However, their survival is reduced (34% survive) when they are detached from their host roots and submitted to soil disturbance in fall (Kabir *et al.*, 1997). The zero tillage (ZT) and permanent bed (PB) showed increased microbial count than conventional tillage (CT). The minimal soil disturbance and retention of crop residue increased soil microflora populations in zero tillage (ZT) and permanent bed (PB). Bacterial population depends on management of crop residues and tillage operation. Higher total bacterial count in zero tillage (ZT) (10.63%) followed by permanent bed (PB) (7.6%) than conventional tillage (CT) irrespective of all the cropping system was reported (Sharma *et al.*, 2011) ^[24].

NT showed an increased contribution of Glomeraceae than CT. The reason being the ability of Glomeraceae to propagate mainly through mycelia and not spores. However, NT and CT had similar proportions of Acaulosporaceae, Gigasporaceae and Glomeraceae. Fungal species exhibit associations with different plant species and thereby promote species richness in non disturbed soils (Menendez *et al.*, 2001) ^[18]. The contribution of species of genera *Glomus* was favored by the presence of mycelia and the absence of spores. The soils and extramatrical hyphae remain intact in NT, showing an increased percentage of Glomeraceae. There is confirmation that AMF families use different approach for colonizing new roots (e.g. Hart and Reader, 2002) ^[9], and in addition based on life history strategy studies suggested that, in contrast to *Glomus* species, members of Gigasporaceae are 'K' strategists. There is evidence that AMF families use different strategies for colonizing. The Gigasporaceae is less sensitive to soil disturbance than Glomeraceae because after disturbance some hyphal fragments lose viability due to cytoplasmic leakage, Where as spore are not greatly affected, and that Gigasporaceae colonize roots primarily from spores (Schalamuk *et al.*, 2006) ^[23].

Conventional tillage is detrimental to AMF, reducing the abundance of soil mycelium in spring, and delaying and limiting the mycorrhizal development of the following crop. Reduced tillage had much less severe negative impact on the abundance of soil hyphae and mycorrhizal colonization by indigenous AMF. We observed that conventional transplanted rice-zero tillage wheat (CTR – ZTW) had significantly increased content of monounsaturated fatty acids (MUFA) (Gram-negative bacteria) than ZT. CT shows significantly higher polyunsaturated fatty acids (PUFA) content (Saprotrophic fungi) than ZT.

The crop residues on the soil surface present continuously throughout the year showed increased microbial diversity in conservation agriculture (CA). Under CA, not only zero tillage but also crop residues determined fungal dominance, because residues served as a continuous energy source for microorganisms, and increased their dominance by providing enhanced conditions for reproduction. It was observed that crop residue affects the abundance of general bacterial markers due to the decomposition process carried out by microorganisms. ZT with residue of rice, wheat, and mungbean show increased amount of straight organism, cyclo organism (Gram-negative bacteria), DMA content, 10-methyl content (Actinomycetes, Actinobacteria), 16:1w5c content, higher biomass and decreased amount of Gram-positive bacteria, monounsaturated fatty acids, (MUFA) PUFA content, DMA content, 18:1w9c (Cyanobacteria; green algae) content, 18:2w6,9c content. Zero tillage with crop residue and mungbean in rotation (ZT +MB +CR) had significantly increased 16:1w5c content (Arbuscular mycorrhizal fungi), 18:1w 9c Hydroxy content (Gram-negative bacteria) than CT. Although, the incorporation of crop residues by fall plowing combined with spring disking in CT leads to warmer spring temperatures in plowed soils and to conditions favorable to proliferation for the fungal saprophytes. The impact of CT on fungal saprophytes has the negative impact of disking on total soil hyphae. The initial low abundance of soil hyphae after spring disking in RT followed by a rapid increase in AM hyphal density, and at silking, the effect of disking had disappeared suggesting that spring disking had less effect than CT (Salinas-Garcia *et al.*, 2002) ^[21, 22].

We observed that the straight organism are the general bacterial marker, which were higher in zero tillage (ZT) with inclusion of mungbean in rotation with rice-wheat cropping system, while crop rotation increased the cyclo organism (Gram-negative bacteria), 10-methyl content, 16:1w5c content, and decreased the amount of branched organism (Gram-positive bacteria) and monounsaturated fatty acids (MUFA) content (Gram-negative bacteria), 18:1w9c (Cyanobacteria; green algae) content, 18:2w6,9c content, reduced total biomass. Our study represents a comprehensive synthesis and strives to reveal the effect of crop rotation on AMF and microbial diversity. Similarly, no-tillage showed more dominance of Ascomycota, followed by Basidiomycota (Miura *et al.*, 2015). The maximum (74%) dominance of Ascomycota for residue degradation is due the zero tillage maize-wheat-mungbean (ZT-M-W-Mb) system with maximum crop residue biomass. The quality of the substrate influenced the abundance of Ascomycota Classes of Ascomycota *viz.* Sordariomycetes, Dothideomycetes, and Eurotiomycetes are the dominating classes (Wang *et al.*, 2016). The cellulolytic enzymes in residue rich soils are produced by the members of Sordariomycetes (Phosri *et al.*, 2012) ^[20]. The degradation of cellulose and other complex carbohydrates in dead or partially digested plant organic matter (Hyde *et al.*, 2013) ^[11] carried out by Dothideomycetes lives as endophytes or epiphytes on plants and are saprobes. The dominating Ascomycetous orders are Sordariales followed by Hypocreales (Klaubauf *et al.*, 2010) ^[16]. ZT-M-W-Mb showed increased diversity which indicates more stability of that management system (Giller *et al.*, 1997) ^[7]. The more stability of that management are indicated by increased diversity of The high species richness in ZT-M-W-Mb correlated with the presence of maize in crop rotation

system which provide greater amounts of plant residue with more decomposability that support greater microbial populations and diversity (Moore *et al.*, 2000) ^[19].

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