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Enhancing Soil Health Through Microbial Inoculation and Changing Cultivation Methods in Rice-Wheat Cropping System

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Abstract

India's rice-wheat cropping system (RWCS) is going through a paradigm shift to address resource degradation and sustainability issues. The increasing level of soil degradation has made soil biological health an important aspect of managing the problems of RWCS through understanding the role of microbes in enhancing soil health and by increasing the adoption of conservation agriculture-based practices. Our two-year study revealed that soil biological health, as measured by soil acetylene reductase activity (ARA), soil chlorophyll, microbial biomass carbon (MBC), soil dehydrogenase enzyme activity, and soil alkaline phosphatase activity (APA), was significantly impacted by the rice and wheat cultivation methods. The system of rice intensification (SRI) and zero tillage wheat (ZTW) system had significantly higher values for all the studied microbial parameters. The application of *Anabaena-Pseudomonas* biofilm formulation along with 75% recommended dose of nutrients (RDN) (90 kg N/ha and 19.35 kg phosphorus (P/ha) significantly improved all studied microbial parameters in both rice and wheat. The microbial properties such as dehydrogenase enzyme activity, alkaline phosphatase activity and microbial biomass carbon (MBC) had significantly higher values in treatment with RDN over 75% RDN indicating the role of optimal fertilization in soil biological health maintenance. A significant improvement in ARA and soil chlorophyll in inoculated treatment showed superior performance of inoculated microbes over inherent soil microbes in nitrogen fixation. The study of a combination of different rice and wheat cultivation methods and their influence in the long run on soil biological health in RWCS emphasizes the need for soil biological health maintenance considering its significant role in the system's sustainability.

Keywords: Cultivation methods, Nutrient management options, RWCS, Soil health

Introduction

The rice–wheat cropping system (RWCS), which is being followed on 10.2 million ha area, has played and is still playing a major role in food security of India. This cropping system is an outcome of the green revolution, which was initially based on three to four major pillars: crop improvement, use of chemical fertilizers, irrigation and agrochemicals for biotic stress management, including weeds. Due to the use of these purchased inputs in large areas for over 5-6 decades, RWCS has a higher contribution to total inputs and energy used in India. These technologies are associated with ill effects on soil health which arise due to the imbalanced use of chemical fertilizers, excessive dependence on agrochemicals and defective irrigation management strategies. Soil health is defined as an integrative property that reflects the capacity of soil to respond to agricultural intervention, so that it continues to support both agricultural production and the provision of other ecosystem services (Kibblewhite *et al*., 2008). Considering the three major groups of soil properties, soil health can also be defined in terms of soil physical, chemical and biological properties. The major problems related to soil chemical health in RWCS can be addressed through efficient nutrient management strategies like the use of secondary and micronutrients (sulphur zinc and iron), site-specific nutrient management and integrated nutrient management (SSNM and INM), accompanied by reduced use of agrochemicals and suggestions to use of biopesticides. In the case of soil physical health, alternative crop establishment methods, use of resource conservation technologies and following conservation agriculture principles can be useful.

The biological health is defined as the ability of soil to support large and diverse microbial communities, suppress pathogens and support healthy crop development (Brackin *et al*., 2017). The soil microbial population and diversity and conducive conditions of soil for the growth of microorganisms will decide the soil biological health. The soil microbial population and diversity are enhanced by periodic and need based inoculation with microorganisms having desirable characteristics such as nitrogen fixation, nutrient solubilisation, nutrient mobilization, and antagonism towards soil borne disease causing microorganisms. Considering this, an attempt was made to study the soil biological health as influenced by the addition of nitrogen-fixing and phosphorus solubilizing microbial consortia and by evaluating their performance under different cultivation methods. Soil acetylene reductase and alkaline phosphatase activity were measured to study the effect of inoculation on nitrogen fixation and phosphorus solubilisation, indicating the functional diversity of microbes. Dehydrogenase activity and microbial biomass carbon show changes in soil microbial population due to different crop establishment methods and microbial inoculation. Therefore, measuring these properties helps quantify soil biological health besides their role in crop growth improvement and nutrition.

Materials and Methods

The field experiment was conducted successively for two years at ICAR–Indian Agricultural Research Institute, New Delhi, India, located at a latitude of 28°38’ N, longitude of 77°10’ E and altitude of 228.6 m above mean sea level. The experiment was conducted in split plot design in rice–wheat cropping system involving two major factors: cultivation methods and rate and sources of crop nutrition as subplots. The three cultivation methods each of rice and wheat as the main plot, and nine combinations of nutrient sources and rates (chemical fertilizers, microbial inoculations and zinc fertilization) as subplots were studied in the present investigation (**Table 1**). For application of nutrient management treatments in rice for puddled transplanted rice (PTR) and SRI, phosphorus (P), potassium (K) and Zinc (Zn) were incorporated just before transplanting while broadcasting of N was

Table 1. Treatment details

Cultivation methods (Main plot treatments)
Puddled transplanted rice (PTR) followed by conventional drill-sown wheat (CDW)
System of rice intensification (SRI) followed by system of wheat intensification (SWI)
Aerobic rice system (ARS) followed by zero tillage wheat (ZTW)
Nutrient management options (Sub-plot treatments)
T1: Control (No nutrient application)
T2: Application of 120 kg N ha^{-1}, 25.8 kg P ha^{-1} (Recommended dose of nutrients (RDN)
T3: PDN + Zn (5 kg Zn ha^{-1} through ZnSO4.7H2O)
T4: 75% RDN
T5: 75% RDN + Zn
T6: *Anabaena sp.* (CR1) + *Providencia sp.* (PR3) + 75 % RDN
T7: *Anabaena sp.* (CR1) + *Providencia sp.* (PR3) + 75 % RDN + Zn
T8: *Anabaena-Pseudomonas* (An-Ps) biofilmed formulation+ 75 % RDN
T9: *Anabaena-Pseudomonas* (An-Ps) biofilmed formulation+ 75 % RDN + Zn

Zn^{**}: Soil applied 5 kg Zn ha^{-1} through Zinc sulphate heptahydrate; Potassium was applied uniformly @ 49.8 kg K/ha in all plots (including control).

done in three equal splits at 5, 25 and 45 days after transplanting (DAT). In aerobic rice system (ARS), the whole quantity of P, K and Zn were applied as per the treatments during sowing by drilling below the seed. For N, 1/3rd N was applied at the time of sowing by drilling below the seed, and the remaining 2/3rd N was applied as top dressing (broadcasting) equally at 30 and 60 days after sowing (DAS). For wheat, 1/3rd N, the complete dose of P, K, and Zn were applied by drilling below the seed at the time of sowing in all cultivation methods while the remaining 2/3rd nitrogen was applied equally at 30 and 60 DAS. Zn was soil applied at the time of sowing/transplanting @ 5 kg Zn/ha through zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) in each crop. Potassium was applied uniformly (49.8 kg K /ha/crop) in all plots (including control) before transplanting in rice and when sowing in wheat.

All treatments were replicated thrice, and rice variety '*Pusa Sugandh5*' and wheat variety '*HD2967*' were planted in the experiment. Two microbial cultures were used in the study *viz*., *Anabaena-Pseudomonas* (An-Ps) biofilmed formulation and *Anabaena sp.* (CR1) + *Providencia sp.* (PR3) consortia. For preparation of microbial inoculations, a mixture (1:1) of vermiculite (hydrous phyllosilicate mineral): compost (paddy straw compost with C/N 16.22 and humus 13.8% (pH 7.34) was used as carrier. The cyanobacterial, fungal, and bacterial colony forming units in the formulations were 10^4, 10^5, and 10^8 per gram of carrier, as reported by Prasanna *et al*. (2015) and Adak *et al.* (2016). A thick paste of inoculants was made in carboxyl methyl cellulose for inoculation. Rice seedlings were inoculated by dipping roots in a paste of respective culture for half an hour before transplanting in PTR and SRI. For ARS, pre-soaked seeds were treated with culture mixed in carboxyl methyl cellulose. In wheat, seeds were treated before sowing with thick paste of respective inoculations made in carboxyl methyl cellulose. The microbial inoculants were applied for their nitrogen fixation ability and phosphorus solubilization capacity.

Standard recommended management practices were followed for all cultivation methods. The data obtained were analyzed using F-test (Gomez and Gomez, 1984) and the least significant difference were used for comparing treatment means for their statistical difference. For the determination of soil microbial properties in rice, bulk soil samples were collected from each plot after 100 days in all three cultivation methods; while for wheat, bulk soil samples collected at 90 DAS were used for analysis. The soil microbial properties were analyzed following standard procedures (**Table 2**).

Table 2. Procedures for measurements of soil microbial properties

Soil microbial properties	Procedure	References
Nitrogenase enzyme activity	Acetylene reductase activity	Prasanna *et al.* (2003)
Soil chlorophyll	Chlorophyll extraction using organic solvents	Nayak *et al.* (2004)
Dehydrogenase activity	Triphenyl tetrazolium chloride incubation	Casida *et al.* (1964)
Microbial biomass carbon	Fumigation method	Nunan *et al.* (1998)
Alkaline phosphatase activity	p-nitrophenyl phosphate hydrolysis method	Tabatabai and Bremner (1969)

Results and Discussion

Acetylene reductase activity (ARA) and soil chlorophyll

The nitrogenase enzyme is responsible for biological nitrogen fixation and reduces nitrogen (N_2) from the atmosphere to ammonia (NH_3). This enzyme also reduces the acetylene to ethylene and this principle is used to determine the biological nitrogen fixation potential of cyanobacteria using gas chromatography and expressed as acetylene reductase activity (ARA) in unit of nmole ethylene/g soil/h. The ARA in rice and wheat was significantly affected due to cultivation methods as well as due to application of microbial inoculation (**Figure 1** and **Table 3**, respectively). In rice, the highest ARA activity was observed in SRI (7.99 nmole ethylene/g soil/h), which was significantly higher than PTR (7.71 nmole ethylene/g soil/h) and ARS (6.88 nmole ethylene/g soil/h). Among the nutrient management treatments, the highest ARA was recorded in 75% RDN + MI2 (11.20 nmole ethylene/g soil/h) which was significantly higher than 75% RDN + MI1 (10.18 nmole ethylene/g soil/h). This indicates the positive effect of inoculation on biological nitrogen fixation by improving population of inoculated microbes. In wheat, ZTW had the highest ARA, which was significantly higher than other methods. The ARA was significantly higher in all treatments with application of microbial inoculation, with the highest value in 75% RDN + MI2 + Zn in both years. The increase in ARA with microbial inoculation was 4.63 to 5.77 n mole ethylene/g soil/h in rice at 100 DAS and 2.60 to 2.91 n mole ethylene/g soil/h in wheat at 90 DAS.

The soil chlorophyll extracted with the help of organic solvent (Dimethyl sulphoxide and acetone) was measured as an indicator of the growth of applied cyanobacteria. The soil chlorophyll in rice (0.47 – 2.57 µg/g) was higher than wheat. The soil chlorophyll in SRI and PTR was significantly higher than ARS which might be due to saturated condition of soil in both methods. In wheat, ZTW recorded the highest soil chlorophyll content, which was significantly superior over CDW and SWI. Among the nutrient management treatments, the trend remained the same as that of ARA, with the highest value in 75% RDN + MI2. This variation in soil ARA and soil chlorophyll across the crop establishment methods and nutrient rates indicate the impact of change in soil microclimate on soil microbial properties and suggest their role as indicators of soil biological health (Prasanna *et al*., 2015, and Adak *et al*., 2016); while improvement in both properties with application of inoculations (treatment T6 to T9) indicates their role in enhancing soil biological health (Shivay *et al*., 2022).

Dehydrogenase activity, microbial biomass carbon (BMC) and alkaline phosphatase activity

The dehydrogenase activity was significantly increased due to microbial inoculation and applying chemical fertilizers (**Table 3** and **Figure 1**). The increase in

Table 3. Effect of microbial inoculations in combination with chemical fertilizer on soil microbial parameters in different cultivation methods of wheat at 90 days after sowing (data pooled over two years)

Treatment	Acetylene reductase activity (ARA) (n mole ethylene g/soil/h)	Soil Chlorophyll (µg/g)	Dehydrogenase activity [µg triphenyl formazan (TPF)/g soil/h)	Microbial biomass carbon (mg/kg)	Alkaline phosphatase activity (APA) (µg PNP/g soil/h)
Cultivation methods					
CDW	4.04	1.56	43.2	337.8	76.9
SWI	4.06	1.59	43.4	338.8	79.1
ZTW	4.36	1.76	46.2	342.7	82.1
SEm±	0.03	0.02	0.40	0.54	0.37
CD (*P* = 0.05)	0.14	0.10	1.57	2.11	1.44
Nutrient management options					
Control (no fertilizer application)	1.20	0.24	20.2	183.7	25.9
RDN*	3.15	1.38	44.4	349.3	81.9
RDN + Zn**	3.20	1.39	45.1	350.2	82.6
75% RDN	3.13	1.35	44.1	347.8	80.8
75% RDN + Zn	3.15	1.37	44.2	348.5	81.0
75% RDN + MI1	5.73	2.16	48.9	367.1	88.0
75% RDN + MI1 + Zn	5.77	2.18	49.0	367.8	89.0
75% RDN + MI2	6.00	2.31	50.8	371.2	92.2
75% RDN + MI2 + Zn	6.06	2.31	51.4	372.0	92.6
SEm±	**0.08**	**0.06**	**0.67**	**1.12**	**0.87**
CD (*P* = 0.05)	**0.21**	**0.17**	**1.90**	**3.18**	**2.47**

CDW, Conventional drill-sown wheat; SWI, System of wheat intensification; ZTW, Zero tillage wheat; RDN*, Recommended dose of nutrients (120 kg N/ha and 25.8 kg P/ha); Zn**, Soil applied 5 kg Zn/ha through zinc sulphate heptahydrate; MI1: *Anabaena sp*. (CR1) + *Providencia sp* (PR3); MI2: *Anabaena-Pseudomonas* biofilmed formulation).

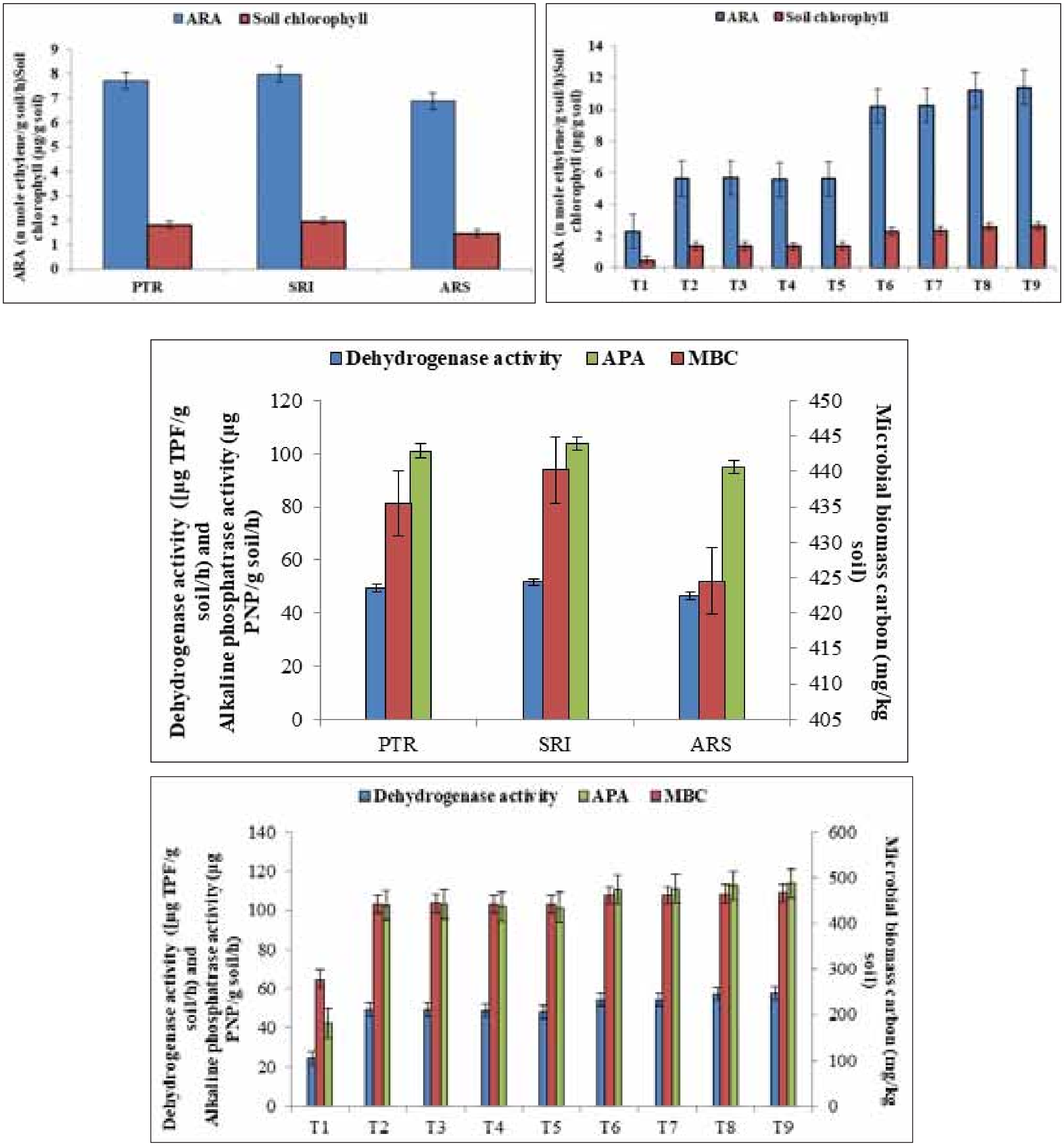

Figure 1: Effect of crop cultivation methods and nutrient management on soil microbial properties in rice field (soil) at 100 days after sowing (Data pooled over two years)

T1: Control (no fertilizer application), T2: RDN*, T3: RDN + Zn**, T4: 75% RDN, T5: 75% RDN + Zn, T6: 75% RDN + MI1, T7: 75% RDN + MI1 + Zn, T8: 75% RDN + MI2 and T9: 75% RDN + MI2 + Zn; PTR: Puddled transplanted rice; SRI: system of rice intensification; ARS: Aerobic rice system; RDN*, Recommended dose of nutrients (120 kg N/ha and 25.8 kg P/ha); Zn**, Soil applied 5 kg Zn/ha through zinc sulphate heptahydrate; MI1: *Anabaena sp*. (CR1) + *Providencia sp* (PR3); MI2: *Anabaena-Pseudomonas* biofilmed formulation enzyme activity over control due to the application of RDN was 24.6 µg triphenylformazan (TPF)/g soil/h in rice at 100 DAS, and in wheat, the increase was 24.2 µg TPF/g/soil/h at 90 DAS over control. Microbial inoculation increased the dehydrogenase activity over RDN and control by 5.1–8.7 and 29.8–33.3µg TPF/g soil/h respectively in rice, while in wheat, the increase was 4.5–7 and 28.7–31.2µg TPF/g/h, respectively. Among cultivation methods, SRI was found significantly superior to both PTR and ARS; while in wheat, ZTW was significant. This signifies that microbial inoculations and optimal dose of chemical fertilizers had a significant and positive effect on enhancing soil microbial activities (Mader *et al*., 2011; Nath *et al*., 2011). At the same time, increased dehydrogenase activity in non-inoculated treatment indicates the role of inherent soil microbial population in maintaining biologically active soil.

The microbial biomass carbon (MBC) is the most sensitive fraction of the total soil organic carbon to change in management practices and input addition and has a high turnover rate. Considering its sensitivity, MBC is one of the important soil property used in soil quality analysis (Onwosi *et al*., 2020). Hence its measurement as affected by a change in crop cultivation methods and input addition in different crops is important for evaluating the impact of crop cultivation on soil health. The MBC varies between 278–466.3 mg/kg soil at 100 DAS in rice, while in wheat, it varied between 183.7–372.0 mg/kg soil (**Table 3** and **Figure 1**). In our study, the order of significance of applied treatment in affecting MBC was nutrient application (RDN)>microbial inoculations>cultivation methods >Zn fertilization in both rice and wheat. This order indicates that both inherent soil microbial populations triggered by fertilization and inoculated microbes have a major role in increasing MBC. Therefore fertilization and application of microbial inoculation improve soil biological health besides their impacts on crop growth and yield (Zhang *et al*., 2022). The alkaline phosphatase activity (APA), an indicator of P solubilization capacity of microbes, was found to be significantly higher in SRI in rice and ZTW in wheat, indicating their superiority in enhancing soil microbial health (Swarnalakshmi *et al*., 2013). Microbial inoculation increased the APA by 8.6–11.0 µg PNP/g soil/h in rice and 7.2–11.4 µg PNP/g soil/h in wheat. The interaction effect was found significant for all the five soil microbial properties studied, which indicates the sensitivity of selected parameters to changes in soil, water and plant management (Prasanna *et al*., 2015; Adak *et al*., 2016).

Our study concluded that SRI system of cultivation in rice and ZTW in wheat had a positive and significantly better impact on soil biological health. The use of *Anabaena-Pseudomonas* biofilmed formulation showed promise in improving all the studied soil biological parameters thereby contributing to soil biological health improvement.

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