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Variation of functional diversity of soil microbial community in sub-humid tropical rice-rice cropping system under long-term organic and inorganic fertilization

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ABSTRACT

Soil microbial communities play an essential role in maintaining soil fertility and considered as ecological indicators to evaluate soil health. In the present study, long-term effects of organic and inorganic fertilizers on functional diversity of soil microbial communities, and their correlation with soil organic carbon, microbial biomass and activities, were observed under sub-humid tropical rice-rice cropping system. This experiment comprises six treatments *viz.* control (plots without application of chemical fertilizers and farm yard manure), nitrogen (N at the rate of 60 & 80 kg ha^{-1} in wet and dry seasons, respectively), nitrogen + phosphorus + potassium (NPK), farm yard manure (FYM), FYM + N and FYM + NPK. The results indicated that pH of the soil under the treatments decreased from its initial value, whereas total organic carbon increased in FYM-treated plots. Microbial biomass carbon and nitrogen in FYM + NPK were increased by 50.0 and 46.4%, respectively as compared to control treatment. Carbon and nitrogen mineralization; and soil enzyme activities were significantly ($p \leq 0.05$) higher in FYM + NPK over control. The average well color development (AWCD) values derived from Biolog® eco-plates followed the order of FYM + N > FYM > FYM + NPK > NPK > N > Control. Shannon index was greater ($p \leq 0.05$) in FYM-treated soil as compared to control and chemical treated soil. Principal component analysis (PCA) indicates a clear separation of the cluster of treatments with FYM application (FYM and FYM + NPK) and treatments without FYM (N and NPK). Shannon index was significantly correlated with available phosphorus ($p \leq 0.002$) and carbon mineralization ($p \leq 0.015$). Biplot analysis suggested that polymer and amino acid utilizing microbes were dominant, irrespective of all treatments. These results revealed that continuous application of nitrogen fertilizer alone has responded a shift in soil microbial community in the long run and decreased the functional diversity of microbes. However, application of FYM either alone, or in combination with chemical fertilizers, could restore soil fertility. Moreover, the information generated from this experiment though Biolog® may be the first particularly with reference to the dominance of polymer and amino acid utilizing microbes, irrespective of treatments under long-term fertilized paddy soil.

1. Introduction

Soil microbiota is one of the essential components of soil. Their diversity and activity may be affected directly and indirectly by environmental (abiotic and biotic) factors and anthropogenic practices including application of fertilizers, pesticides and other agronomic practices (Simek et al., 1999; Sheik et al., 2011; Tamilselvi et al., 2015). The direct effects include accumulation of lethal metals and shift in soil pH which negatively impact soil microbiota (Chu et al., 2007; Enwall et al., 2007; Liu and Greaver, 2010), whereas, stimulation of plant growth, increase in rhizodepositions and plant residues input in the soil are major indirect effects which stimulates the growth and activity of soil microbiota

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in fertilized soil (Chu et al., 2007; Lugato et al., 2006; Salinas-Garcia et al., 1997).

Soil microbiota is also a vital component for various soil functions *viz.* breakdown of soil organic matter, degradation of xenobiotics and configuration of soil aggregates (Stenberg, 1999), and they can act as a source and sink for plant nutrients (Salinas-Garcia et al., 1997). Moreover, several environmental factors and management practices are responsible for mineralization of organic matter and accumulation of carbon in soil (Balkcom et al., 2009; Kandeler et al., 1999). Nutrient management is one of the most important agricultural practices which significantly affect soil microbial abundance, diversity and activity under long-term fertility management (Chinnadurai et al., 2014; Tamilselvi et al., 2015). However, long-term use of balanced chemical fertilizers of nitrogen (N), phosphorous (P) and potassium (K) may cause less negative impact on soil biological properties than unbalanced fertilization (NP, NK or PK) (Zhong and Cai, 2007).

Relatively lower quantities of organic manures which are also of low quality are being used by the Indian farming community to meet the plant nutrient needs (Roy et al., 2006). Moreover, overdose of nitrogen and low quantity of phosphorus and potassium application, to most of the crops, is a general practice (Tandon, 2004). These imbalances further deteriorate the soil health status, which in turn may possibly cause a risk for the productivity in future. Therefore, continuous monitoring of these soils is necessary for developing suitable indicators to sustain agricultural production.

Long-term fertilizer experiments (LTFEs) are valuable for evaluating the residual effects of fertilizer management on soil biological properties, including microbial abundance, activity and diversity (Liu et al., 2013; Balachandar et al., 2014; Tamilselvi et al., 2015). Changes of functional microbial communities are governed by long-term addition of organic or inorganic amendments under different ecosystems (Sarathchandra et al., 2001; Marschnera et al., 2003; Liu and Greaver, 2010; Moharana et al., 2012; Liu et al., 2013; Naher et al., 2013).

Functional diversity of soil microbial community can be measured by using Biolog® eco-microplates, which provide comprehensive information on various soil microbial functions. The Biolog plates were originally designed to identify bacterial isolates. Later, the plates were found useful in microbial community studies and also used widely to characterize bacterial communities from various environments, including soil (Garland and Mills, 1991; Garland, 1996). Biolog data are well-suited for multivariate statistical analyses such as principal component analysis and cluster analysis, tools which can distinguish among bacterial communities from various environments and can be used to describe temporal changes in physiological characteristics (Choi and Dobbs, 1999). Profiling of carbon source utilization by microbial communities present in different fertilization treatments in paddy soil can be readily generated from Eco- plates (Zak et al., 1994; Li et al., 2012). Some of the workers have mentioned that the Biolog® method is not a valid method to measure diversity of the soil microbial community alone (Ros et al., 2006, 2008), however it gives a comprehensive indication of the functional diversity of the soil microbiota.

Rice-rice cropping system is one of the most intensively cultivated cropping systems and consumes most of the nitrogenous fertilizer used in agriculture (Dawe et al., 2000). However, many researchers reported that decline in productivity in a number of long-term intensive rice-rice systems (Cassman et al., 1997; Dawe et al., 2000; Shahid et al., 2015) due to reduction in indigenous N supply over time. Moreover, information on long-term effect of use of chemical fertilizers with or without farm yard manure (FYM) in an intensive rice-rice cropping system on soil fertility and temporal shift in functional diversity of the microbial community is limited. Thus, the present study was undertaken in a long-term fertilization experiment which was started in the year 1969 with rice-rice system. Aims of the present work were (1) to find out the effects of chemical fertilizers, either alone or in combination with FYM, on soil chemical and biological properties, at the most active and critical stage of crop growth; and (2) to explore potential feedback mechanisms and changes of functional diversity of soil microbial communities due to fertilization after 41 years of intensive rice-rice cultivation. We further hypothesized that whether long-term application of carbon and nitrogen through organic and inorganic fertilizers will have distinct effects on soil organic carbon pools, nutrient availability and functional diversity of soil microbial community.

2. Materials and methods

2.1. Study site

The study was conducted at long-term fertilization experimental field of ICAR-National Rice Research Institute, Cuttack, India (20°25′N, 85°55′E; 24 m above mean sea level). Mean annual maximum and minimum temperatures were 39.2 and 22.5 °C, respectively, and mean temperature was 27.7 °C. During the study period, annual precipitation was 1500 mm of which 75–80% were received during June–September. The soil on the farm has developed in recent times from deltaic sediments of the Mahanadi River and classified as Aeric Endoaquept (Soil Survey Staff, 2010) with a sandy clay loam texture (31% clay, 17% silt and 52% sand). At the initiation of the study, soil at the experimental site had a bulk density (BD) of 1.40 Mg m^{-3}, cation-exchange capacity (CEC) 15.2 cmol (p+) kg^{-1}, pH 6.6 (using 1:2.5, soil/water suspension), total organic carbon 6.6 g kg^{-1}, total-N 0.8 g kg^{-1}, exchangeable K 0.26 cmol (p+) kg^{-1} and available-P 13.0 kg ha^{-1}.

2.2. Field experiment

The long term fertilization experiment was laid out in a randomized complete block design comprising of absolute control (no fertilizer) and different combinations of chemical fertilizers and farm yard manure *viz.* N, NP, NK, NPK, FYM, FYM + N, FYM + NP, FYM + NK and FYM + NPK with three replications. Out of these ten, six treatments *viz.* control, N, NPK, FYM, FYM + N and FYM + NPK were selected for present study. Twenty five (25) days old seedlings of rice varieties (Gayatri during wet season and Lalat during dry season) were transplanted at a spacing of 20 cm × 15 cm with two seedlings per hill. FYM (5 t ha^{-1}) was applied uniformly in all the treatments, during the last week of May in every year before puddling. The wastes from the institute's dairy farm were used to prepare the FYM which contained 171–189 g kg^{-1} total organic C and 4–16 g kg^{-1} total-N. Fertilizers were applied as per the requirement at the rate of 60–40–40 and 80–40–40 kg ha^{-1} N-P_2O_5-K_2O for wet and dry seasons, respectively. Nitrogen was applied in the form of urea, 50% of this as basal and the rest in two equal split after transplanting as top dressing. Full dose of P and K was applied as basal in the form of single super phosphate and muriate of potash (KCl). All the plots remained continuously flooded to a water depth of 7 ± 3 cm during the entire period of crop growth and water was drained out from plots 10 days before the harvest. The crop was raised as per local recommended agronomic practices.

2.3. Soil sampling and analyses

Rhizospheric soil (soil that adheres to roots) samples were collected at panicle initiation (PI) stage of rice. In each treatment, five soil samples were collected and mixed to form a composite sample. Immediately after sample collection, a part of the soil sample was air-dried and ground, then passed through a 2 mm sieve and analyzed for total organic carbon (TOC) and total-N (N_{tot}) with an