



Continuous application of inorganic and organic fertilizers over 47 years in paddy soil alters the bacterial community structure and its influence on rice production

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ARTICLE INFO

Keywords:

Bacterial community
Illumina-MiSeq[®]
Fertilization
Diversity indices
Rice

ABSTRACT

Soil bacterial communities are considered as an essential member of the microbial community, contributing to soil health. Continuous application of chemical fertilizers alters the bacterial community structure (BCS) thereby disturbing the soil biogeochemical cycling. The present study highlights the 16S rRNA amplicon sequencing-based variation of BCS through Illumina-MiSeq[®] in a 47 years old long-term fertilized paddy soil and its relation with grain yield (GY), straw biomass (SB) and various soil properties. The experiment comprising six treatments: control (no fertilizers), nitrogen (N), nitrogen + phosphorus (P) + potassium (K), farmyard manure (FYM), FYM + N and FYM + NPK. Data on rice crop performance indicated that GY and SB significantly ($p \leq 0.05$) enhanced by 45.1%–49.3% and 36.9–39.4% in FYM + NPK compared to control. Relative abundance of bacterial phyla varied across inorganic and organic fertilizer treatments. Dominant phyla across all treatments were Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, and Firmicutes, accounting for about 80–85% of total operational taxonomic units (OTUs). N application alone over 47 years encouraged certain bacterial phyla (Firmicutes, Actinobacteria, and Nitrospira) while major (Proteobacteria, Acidobacteria and Cyanobacteria) and minor (Fibrobacteres, Spirochaetes, TM7 and GNO4) bacterial phyla were found to be suppressed compared to other treatments. Moreover, continuous use of chemical N in paddy soil, considerably suppressed some diazotrophs taxa Burkholderiales, Enterobacteriaceae, and other taxa *Kaistobacter*, *Anaeromyxobacter*, *Bdellovibrio*, and *MND1*. Redundancy analysis coupled with principal component analysis revealed that BCS was significantly influenced by soil pH and presence of higher nitrogen content. Interestingly, the highest proportion of bacterial OTUs was recorded in balanced fertilizer (NPK) (without FYM) and therefore, this result suggested for the first time that continuous application of NPK encouraged the beneficial bacterial community without compromising of GY and SB. Overall, the present study indicated that continuous application of N and NPK with or without FYM for more than four decades in paddy soil, encouraged certain BCS whereas, N application alone suppressed certain beneficial bacterial phyla, resulting in the alteration of soil biodiversity and rice productivity.

1. Introduction

The soil is a dynamic living system which provides several ecosystem services including nutrient cycling, water regulation and controlling pests and diseases (Doran and Zeiss, 2000; Kumar and Dangar, 2013; Kumar et al., 2016a, 2016b, 2017c). Microorganisms are one of the originators of soil and play an important role to determine soil functions including decomposition of above and below-ground plant

residues (Biswas and Kole, 2017; Gupta et al., 2011). Shifts in the structure and composition of the distinct microbial community are strong indicators of soil biological activity and crop productivity of terrestrial agro-ecosystems (Edmeades, 2003; Torsvik and Ovreas, 2002).

Rice farming is the largest single use of land for producing food on the earth which makes a vital contribution to the reduction of hunger and poverty. Rice cultivation requires standing water which creates an

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anoxic condition in the soil, however, during the non-cropping season, fields are drained which give rise to aerobic condition (Mohanty et al., 2017). This situation creates a varied soil environment, both spatially and temporally, resulting in a diverse microbial community structure in paddy soils (Dong et al., 2014).

Despite the environmental importance of the microbial processes in soil, the knowledge of the microbial communities within long-term intensive rice mono-cropping is incomplete, primarily due to methodological limitations (Ansoorge, 2009; Kumar et al., 2017a). Culture-dependent methods only offer an incomplete picture of the complex microbial communities in soils (Jany and Barbier, 2008; Kumar et al., 2017b). Recently developed high-throughput sequencing technology such as “next-generation sequencing” has significantly enhanced researchers’ ability to look at the composition of abundances of microbial groups (Logares et al., 2014). This new technique has been extended to various environmental systems in recent years (Caporaso et al., 2012; Gibson et al., 2015; Wen et al., 2015). However, only a limited number of studies have employed Illumina-MiSeq® technology to investigate the microbial community structures in rice soil (de Souza et al., 2015). Thus, the present study provides the first-hand information about bacterial diversity based on 16S rRNA amplicon sequencing through Illumina-MiSeq® under sub-humid tropical Indian paddy soil cultivated under influence of inorganic and organic fertilizers for over four decades. This work also highlights the genetic diversity and functional ability of bacteria in relation to rice production since many studies may have looked one or the other.

Long-term experiments can be controlled in a particular way that can contribute significantly to understand the effects of fertilization, nutrient cycling in cropland, as well as soil biogeochemical cycles leading to the evolution of soil fertility. Thus, soil ecological research in recent years has focussed on measuring microbial communities and some key soil functions under long-term fertilizer management (He et al., 2008; Kumar et al., 2017a; Yamaguchi et al., 2009).

Considering the importance of long-term fertilizer experiments particularly under intensive rice cultivation, we took up this study and hypothesized that 1) continuous application of nitrogen alone over four decades may alter some of the beneficial bacterial community which may be one of the factors for the decline in rice yields, and 2) combination of balanced chemical fertilizers alone and/or combination with organic manures may encourage the beneficial bacterial diversity thereby enhancing the rice yield. In view of the above hypothesis, the present study was initiated to assess the abundance and composition of bacterial communities based on 16S rRNA amplicon sequencing through Illumina-MiSeq® and their diversity in paddy soil as influenced by the long-term application of chemical fertilizers and organic manure treatments. In addition, a variation of rice yield and soil properties was also assessed to establish their relationship with bacterial community structure.

2. Materials and methods

2.1. Study site

The study site was situated at the experimental farm 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 annual temperature was 27.7 °C. During the study period, annual precipitation was 1500 mm of which 75–80% was received during June to 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 (52% sand, 17% silt and 31% clay). At the initiation of study (data received in the year 1969), the experimental site was homogeneous, flat and precisely leveled plot on which the ten treatments were replicated in a randomized design. Soil of experimental site was having bulk density (BD)

1.40 Mg m⁻³, cation-exchange capacity (CEC) 15.2 c mol (p+) kg⁻¹, pH 6.6 (using 1:2.5, soil/water suspension), total organic carbon (TOC) 0.66%, total-N (N_{tot}) 0.08%, exchangeable K 0.26 c mol (p+) kg⁻¹ and Bray's-P 13.0 kg ha⁻¹. Due to effect of treatments, there has been a change in physical and chemical properties and these have been previously reported by Kumar et al. (2017a); Shahid et al. (2017) and Tripathi et al. (2014).

2.2. Field experiment

The long-term fertilizer experiment initiated in 1969 with two rice crops per year as a mono-crop in wet (July–November) and dry (January–May) seasons. However, during dry seasons of 1984–1993, the field was maintained without a crop. The experiment was laid out in a randomized complete block design comprising absolute control (no fertilizers) and different combinations of chemical fertilizers and farmyard manure (FYM) viz., control, N, NP, NK, NPK, FYM, FYM + N, FYM + NP, FYM + NK and FYM + NPK with three replications. Out of these ten, the following six treatments viz., control, N, NPK, FYM, FYM + N, and FYM + NPK were selected for this study. The six selected treatments represented key combinations of fertilization strategies that are used and cover both chemical and organic manure applications. The field was ploughed thoroughly and flooded for puddling and levelling 2–3 days before transplanting. Twenty-five (25) days old seedlings of rice were transplanted at a spacing of 20 cm × 15 cm with two to three seedlings per hill. The rice varieties used for the present study was mentioned in our previous publications (Kumar et al., 2017a). The FYM (5 t ha⁻¹) was applied uniformly in all the FYM-treatments during the last week of May every year before puddling. FYM used in this study was prepared from the wastes of the Institute's dairy farm which contained 171–189 g kg⁻¹ total organic C, 4–16 g kg⁻¹ total-N and 3.7 g kg⁻¹ of P content. Fertilizers were applied as per the treatments requirement at the rate of 60-40-40 and 80-40-40 kg ha⁻¹ N-P₂O₅-K₂O 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 the top dressing. A full dose of P and K were 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

Soil samples were collected from five places randomly from the upper surface (0–20 cm) of each plot by using an auger. A composite sample representative of each treatment was prepared by mixing the sub-samples. Immediately after sample collection, part of the soil sample was air-dried and ground to pass through a 2 mm sieve and analyzed for total organic carbon (TOC) and total-N with an elemental analyzer (Thermo Scientific), available-N (Subbiah and Asija, 1956), Bray's-P (Bray and Kurtz, 1945), available-K (Piper, 1966), pH, electrical conductivity. Remaining soil samples were stored in the refrigerator at 4 °C for further biochemical and molecular analyses, which were completed within one month. Microbial biomass carbon (C_{mic}) and nitrogen (N_{min}) were determined using the chloroform fumigation-extraction methods (Vance et al., 1987; Ross, 1990). Carbon mineralization (C_{min}) was evaluated by using alkali traps (Anderson, 1982). To measure N mineralization (N_{min}), mineral N (NH₄ - N + NO₃ - N) of the incubated soil was determined by extraction with 2M KCl followed by alkali distillation in an automatic N analyzer (Keeney and Nelson, 1982). The activities of the dehydrogenase (DHA), urease, acid- and alkaline-phosphatase enzymes were determined by the methods as described by Tabatabai (1994) and Mahapatra et al. (2017). For enumeration of heterotrophic aerobic bacteria (HA), plates containing nutrient agar (NA) (HiMedia™) were incubated at 30 ± 0.1 °C for 72 h

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