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SHORT COMMUNICATION

Effect of long-term fertilizers and manure application on microbial biomass and microbial activity of a tropical agricultural soil

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Abstract We investigated some aspects of soil quality and community-level physiological profiles (CLPP) of bacteria in soil under a long-term (37 years) trial with either exclusive inorganic fertilizers or fertilizers combined with farmyard manure cultivated with jute–rice–wheat system. The treatments consisted of 100% recommended dose (RD) of NPK, 150% RD of NPK, 100% RD of N, 100% RD of NPK+FYM (10 t ha^{-1} $year^{-1}$), and untreated control. Long-term application of 150% RD of NPK lowered the soil pH considerably while the soils in the other treatments remained near neutral. The 100% RD of NPK+FYM treated plot showed significantly highest accumulation of organic carbon, total nitrogen, microbial biomass carbon, basal soil respiration, and fluorescein diacetate hydrolyzing activity among the treatments. CLPP analysis in Biolog Ecoplates revealed that utilization of carbohydrates was enhanced in all input treated regimes, while the same for polymers, carboxylic acids, amino acids, and amines/amides were similar or less than the untreated control. However, within these groups of carbon sources, heterogeneity of individual substrate utilization between treatments was also noted. Taken together, addition of organic supplements showed significantly increased microbial biomass carbon and microbial activity, but input of nutrient supplements, both inorganic and organic, only marginally affected the overall substrate utilization pattern of soil microorganisms.

Keywords Long term · Fertilizer treatment · Manure application · Microbial biomass carbon · CLPP

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Introduction

In the quest for increasing food and fiber production to meet the ever-increasing demand, fertilizers have played a crucial role. In recent years, fertilizer cost and concern for sustainable soil productivity and ecological stability in relation to chemical fertilizer use have emerged as important issues (Aulakh et al. 2000). Chemical fertilizers which are sources of major plant nutrient elements, especially nitrogen (N), phosphorus (P), and potassium (K), serve the purpose of maintaining or enhancing crop production and increasing biomass carbon and nitrogen. On the other hand, their application to soil may also alter the environmental conditions and induce perturbations that ultimately affect soil properties (Øvreås and Torsvik 1998).

Development of soil quality changes is slow and it takes a reasonable period of time to achieve a long-term steady state after a change of management (Kennedy 1999). Therefore, long-term agricultural field experiments are invaluable to detect changes that are absent in short term (Powlson and Johnston 1994). In recent years, indicative components like soil microbial biomass carbon (MBC),

community structures, functions, and enzyme activities have been used to describe soil qualities under different agricultural practices (Mäder et al. 2002; Widmer et al. 2006; Badalucco et al. 2010; Vallejo et al. 2010). Farmyard manure and inorganic fertilizers (NPK) have been reported to have both positive and negative effects on the size of MBC and on microbial activities (Böhme et al. 2005).

Majority of the soil microorganisms are heterotrophs, and utilization of carbon sources by microbial communities is the driving force for soil functioning. Conventional determination of overall microbial activity by basal soil respiration (BSR) and fluorescein diacetate hydrolyzing activity (FDHA) does not reflect on the specific carbon source utilization profile. Determination of community-level substrate utilization patterns by aerobic heterotrophic bacteria with the Biolog technique has been used to differentiate bacterial community-level structure from different habitats (Garland and Mills 1991). Several workers have used this technique to study soil microbial community behavior (Fliessbach and Mäder 1997; Gomez et al. 2000; Widmer et al. 2001). However, this technique suffers from several limitations (Nannipieri et al. 2003) and substrate-induced respiration of soils treated with wide range of carbon substrates has been proposed as an alternative to quantify the catabolic diversity of microbial communities (Degens and Harris 1997). Studies in temperate and subtropical regions reveal that availability of C and N in soils is likely to change the substrate utilization of the soil microbial communities (Tate 2000; Zhang et al. 2008; Zhong et al. 2010). Limited work in the tropical soils show that Biolog analysis and enzyme activities had lower specificity with respect to soil type and land usage (Waldrop et al. 2000; Bossio et al. 2005). To our knowledge, no published information on long-term effect of fertilization is available from tropical regions using CLPP.

In the light of the above discussion, we evaluated detectable changes in the soil quality indicators under long-term integrated use of organic and inorganic supplements. Simultaneously, we undertook CLPP analysis of microbial communities under different soil management practices, which is lacking under tropical conditions. Microbial components consisted of the determination of MBC and determination of activity by BSR and FDHA. The CLPP analysis of the microbial communities under different treatments with Biolog Ecoplates is also reported.

Materials and methods

Field site and sampling

A long-term continuous field trial was established in 1971 at the Central Research Institute for Jute and Allied Fibres, Barrackpore (88°26′E, 22°45′N), West Bengal, India. The soil classified as Eutrochrept was a sandy loam (18% clay, 28% silt, and 54% sand), neutral in reaction (pH 7.1) with a cation exchange capacity of 19 mol kg^{-1}, 6.6 gkg^{-1} carbon (C), and 0.86 gkg^{-1} total nitrogen (N). The experiment in jute–rice–wheat cropping sequence, the most common practice in the region, was laid in a randomized block design with five treatments replicated four times in plots of 200 m^2 each. Urea, single super phosphate, and muriate of potash were the sources of N, P, and K, respectively. The treatments consisted of 100% recommended dose (RD) of NPK, 150% RD of NPK, 100% RD of N, 100% RD of NPK+FYM, and untreated control. The recommended dose (RD) of N, P, and K were 60N:13P:50K for jute and 120N:26P:50K for rice and wheat. The farmyard manure (FYM) was applied once a year before jute (10 t FYM ha^{-1} $year^{-1}$). The study site is characterized as sub-humid tropical with 1,666 mm long-term average (37 years) of annual rainfall spread over 110 days per year. The average ambient maximum and minimum temperatures are 31.05°C and 20.84°C in summer and winter seasons, respectively.

Four soil samples from each replicated plot of each treatment were collected at a depth of 15 cm of the profile on April 2008 after the wheat crop harvest and were pooled to make a composite sample. Altogether, 20 soil samples (four samples for each treatment) were collected and brought to the laboratory in sealed polybags for physico-chemical, microbiological, and biochemical studies. The samples were then sieved (2.0 mm) and stored at 4°C. Physico-chemical properties of soils were determined with the sieved (2.0 mm) air-dried soils, and the microbiological and biochemical properties of soils were measured with the field moist soils. The results were expressed on a moisture-free basis.

Soil characteristics

The pH (1:2.5 H_2O), cation exchange capacity, organic C, and total N were determined by standard procedures (Jackson 1967). The MBC was determined by fumigation extraction method (Joergensen 1995) followed by determination of K_2SO_4 extractable C (Vance et al. 1987). Biomass carbon was estimated as biomass carbon=2.64 EC, where EC is the difference between K_2SO_4 extractable carbon from the fumigated and unfumigated soils. Fluorescein diacetate hydrolyzing activity (Alef 1995a) and basal soil respiration (Alef 1995b) of the soil samples were determined by the standard methods.

Community-level physiological profiles

The patterns of potential carbon source utilization by soil microbial communities under different fertilizer treatments were assessed by Biolog Ecoplate system containing

triplicates of 31 different environmentally relevant carbon sources and control well (Biolog Inc., Hayward, CA, USA) (Choi and Dobbs 1999). Soil suspension was prepared by vortexing 1 g of soil (on dry basis) in 10 ml of sterile phosphate buffered saline and was allowed to settle for 2 h. The supernatant was diluted (10^{-1} to 10^{-6}) and plated on Luria agar to obtain a colony forming unit (cfu) count roughly equivalent to the culturable bacteria present in each sample. The experiment was done in duplicate and cfu counts ranging from 30 to 300 at 48 h were used for enumeration. Aliquots of 150 μl corresponding to 10^3 cfu for each sample were inoculated to each well of Biolog Ecoplates and were incubated in the dark at 28°C.The absorbance values were read at 590 nm with a Biolog microplate reader at 12-h intervals until 72 h.

Ecoplate data analysis

A quantitative analysis was performed by plotting the ratio of absorbance of one well to the total sum absorbance of all the wells for that sample against time for the 31 response wells. Absorbance values used for such calculation were obtained after subtraction of each value with the absorbance of the control well containing water. All the positive absorbance values for the response wells were taken into account. The negative absorbance values were considered as zero. CLPP was expressed as the net area under curve (Guckert et al. 1996) for each well of the 31 response wells over a period of 72 h of incubation.

Statistical analysis

The differences in the variables attributed to treatments were analyzed with ANOVA using SPSS 10.0 software for Windows (SPSS Inc., USA). Duncan's multiple range test was used to determine significant differences in response to parameters. Statistical significance is indicated at 5% probability level.

Results and discussion

Continuous long-term (37 years) application of mineral fertilizers either alone or in combination with FYM resulted in large differences in pH, soil organic carbon, total N, MBC, BSR, and FDHA (Table 1). The soil pH values ranged from 6.42, the lowest in 150% RD of NPK treatment to 7.14, the highest in the untreated control. Although there was a tendency of lowering soil pH with application of the mineral fertilizers, the 100% RD of NPK +FYM, 100% RD of NPK, 100% RD of N, and control treatments remained statistically similar. Higher dose of 150% RD of NPK treatment showed significantly ($P<0.05$) lower pH value than the rest of the treatments. Such decrease in pH values in mineral fertilizer-applied soils may be due to the nitrification of NH_4^+ which produces H^+ ions, thus increasing the soil acidity (McAndrew and Malhi 1992). This variation in soil pH due to the treatments is consistent with the earlier works of Malhi et al. (1998) and Šimek et al. (1999). The total nitrogen (N) content of soil was significantly ($P<0.05$) highest in the 100% RD of NPK+FYM treatment (1.16 g kg^{-1}). The other mineral fertilizer treatments as well as the untreated control had statistically similar values of total N content of soil, although higher fertilizer doses correspond to higher values. The variation in organic C content of soil with the application of different treatments also followed a similar trend as that of the total N content of the soil. The highest accumulation of organic C occurred with 100% RD of NPK+FYM, while the other treatments had little difference between themselves. Manure application in combination with mineral fertilizer increased the total N content and enriched soil organic matter content than the exclusive mineral fertilizer treatments. This is consistent with the study conducted by Goyal et al. (1999).

Distinct differences in MBC content with values lying between 223 and 431 μg g^{-1} were observed in correspondence to the different treatments. The MBC was highest in the 100% RD of NPK+FYM treatment (431 μg g^{-1}) but was statistically similar to the 150% NPK treatment (392 μg g^{-1}). The MBC in the 100% RD of NPK treated soil (364 μg g^{-1}) was slightly lower than the 150% RD of NPK treatment. Among the exclusive mineral fertilizer treatments, the 100% N treatment recorded the lowest MBC (306 μg g^{-1}). Of all the treatments, the untreated control produced the lowest MBC (223 μg g^{-1}). Urea along with FYM increased MBC as FYM supplied readily available organic matter in addition to increasing root biomass and root exudates due to greater crop growth (Goyal et al. 1993). It is reported that MBC respond to a number of management practices, e.g., addition of animal manure, synthetic fertilizers, and residue incorporation (Schjønning et al. 2002). The MBC is regarded as one of the most sensitive indicators of the sustainability of a management system (Gregorich et al. 1997). It is apparent from Table 1 that MBC is highly correlated with the soil organic matter which is in accord with Anderson and Domsch (1980). Based on this long-term experiment, the MBC increased with the increase in soil organic carbon. However, inconsistent results on extensive studies related to the effects of nutrients on MBC are documented (Goyal et al. 1992; Sarathchandra et al. 2001). The BSR reflects the catabolic degradation of soil microbial communities under aerobic conditions. It decreased according to the order of 100% RD of NPK+FYM>150% RD of NPK>100% RD of NPK>100% RD of N>untreated control (Table 1).

Table 1 Some selected chemical, microbiological, and biochemical properties of soil after 37 years of continuous application of chemical fertilizers and organic manure

Treatment	pH (1:2.5 H_2O)	Organic carbon (g kg^{-1})	Total nitrogen (g kg^{-1})	Microbial biomass carbon (μg g^{-1})	Basal soil respiration (μg CO_2–C g^{-1} soil h^{-1} at 25°C)	Fluorescein diacetate hydrolyzing activity (μg fluorescein g^{-1} soil h^{-1} at 24°C)
100% RD of NPK	6.51 ab	7.47 b	0.84 b	364 b	1.08 c	72 c
150% RD of NPK	6.42 b	7.94 b	0.87 b	392 ab	1.65 ab	89 b
100% RD of N	6.56 ab	7.03 b	0.79 b	306 c	1.37 bc	69 c
100% RD of NPK +FYM	6.97 ab	9.93 a	1.16 a	431 a	1.96 a	104 a
Untreated control	7.14 a	6.39 b	0.78 b	223 d	0.67 d	52 d

The values produced in the table are the means of four replicates. Same letters in a column are not significantly different at $P<0.05$ by Duncan's multiple range test

However, the BSR measured in the treatments 100% RD of NPK+FYM (1.96 μg CO_2–C g^{-1} soil h^{-1}) and 150% RD of NPK (1.65 μg CO_2–C g^{-1} soil h^{-1}) did not differ statistically. The 100% RD of N (1.37 μg CO_2–C g^{-1} soil h^{-1}) and 100% RD of NPK (1.08 μg CO_2–C g^{-1} soil h^{-1}) treatments had statistically similar values of BSR. A wide degree of variation in FDHA could be obtained between the treatments. The highest and the lowest FDHA were measured in 100% RD of NPK+FYM (104 μg fluorescein g^{-1} soil h^{-1}) and untreated plots (52 μg fluorescein g^{-1} soil h^{-1}), respectively, with intermediate values in the plots supplied with mineral fertilizers. Higher microbial activity, as determined by BSR and FDHA, in the 100% RD of NPK+FYM treatment shows that the soil microorganisms were rendered more active. Inorganic supplements exclusively may fulfill the demand for mineral nutrition but not the carbon for cell proliferation by the microorganisms. Integrated use of inorganic fertilizers and organic manure brings in more MBC in soil compared to exclusive inorganic fertilizer applications (Goyal et al. 1999).

The well color development in Biolog Ecoplates was recorded at regular 12-h intervals until 72 h. The color development in the wells was remarkably slow in the first 24 h and gradually increased with the progress of time recording maximum absorbance values at 72 h. A similar pattern was observed when the microorganisms were cultured in Luria agar plates by soil dilution plate technique (data not shown). Colony forming units developed after 48 h indicating the culturable population of microorganisms present in the soils being tested took about 48 h to adapt to the culture environment. The CLPPs tested for the utilization capacity of the 31 Eco-substrates are presented in Fig. 1. Ecoplate responses by the microbial communities of each treatment are expressed as net areas for sole carbon source tested. There were distinct differences in substrate utilization patterns by the microbial communities in the different treatments. The microbial communities in the soils were able to use all the 31 carbon sources. Among the carbohydrates, the microorganisms in the fertilizer treated as well as untreated control plots least utilized α-D-lactose, D-xylose, and L-erythritol. The microorganisms under 100% RD of N and 100% RD of NPK+FYM treatments preferred *N*-acetyl D-glucosamine the most, with D-cellobiose, β-methyl D-glucoside, and D-mannitol showing differential utilization by the soil microbial communities present under different treatments (Fig. 1a). Among the carboxylic acids (Fig. 1b), the pattern of utilization of D-galactonic acid-γ-lactone and D-galacturonic acid were dramatically different by the microbial communities in the input treated soils when compared to the untreated control soil. Microorganisms in the control treatment utilized D-galactonic acid-γ-lactone the most over other treatments, while for D-galacturonic acid it was least in the control treatment. However, the utilization of 2-hydroxy-butyric acid, itaconic acid, and α-keto butyric acid was minimal in all the treatments. Among the amino acids, the microbial communities in the untreated control plot maximally utilized L-asparagine while the latter could partially support microbial growth in 100% RD of NPK and 150% RD of NPK treated soils. Interestingly, the microbial communities present in 100% RD of NPK, 150% RD of NPK, and 100% RD of NPK+FYM treated plots could utilize L-serine as the sole carbon source while communities present in 100% N treated plots and untreated control could not (Fig. 1c). L-threonine, L-phenylalanine, and glycyl-L-glutamic acid were least utilized. Among the polymers (Fig. 1d), glycogen was the preferred substrate for the microbial communities in the 100% RD of NPK+FYM treatment, while Tween 40 and Tween 80 were preferred by communities present in 100% RD of N and 100% RD of NPK+FYM with α-cyclodextrin being least preferred by all. Figure 1e suggests that fertilizer treatments reduced the ability of the microbial communities to utilize amines/amides than the untreated control.

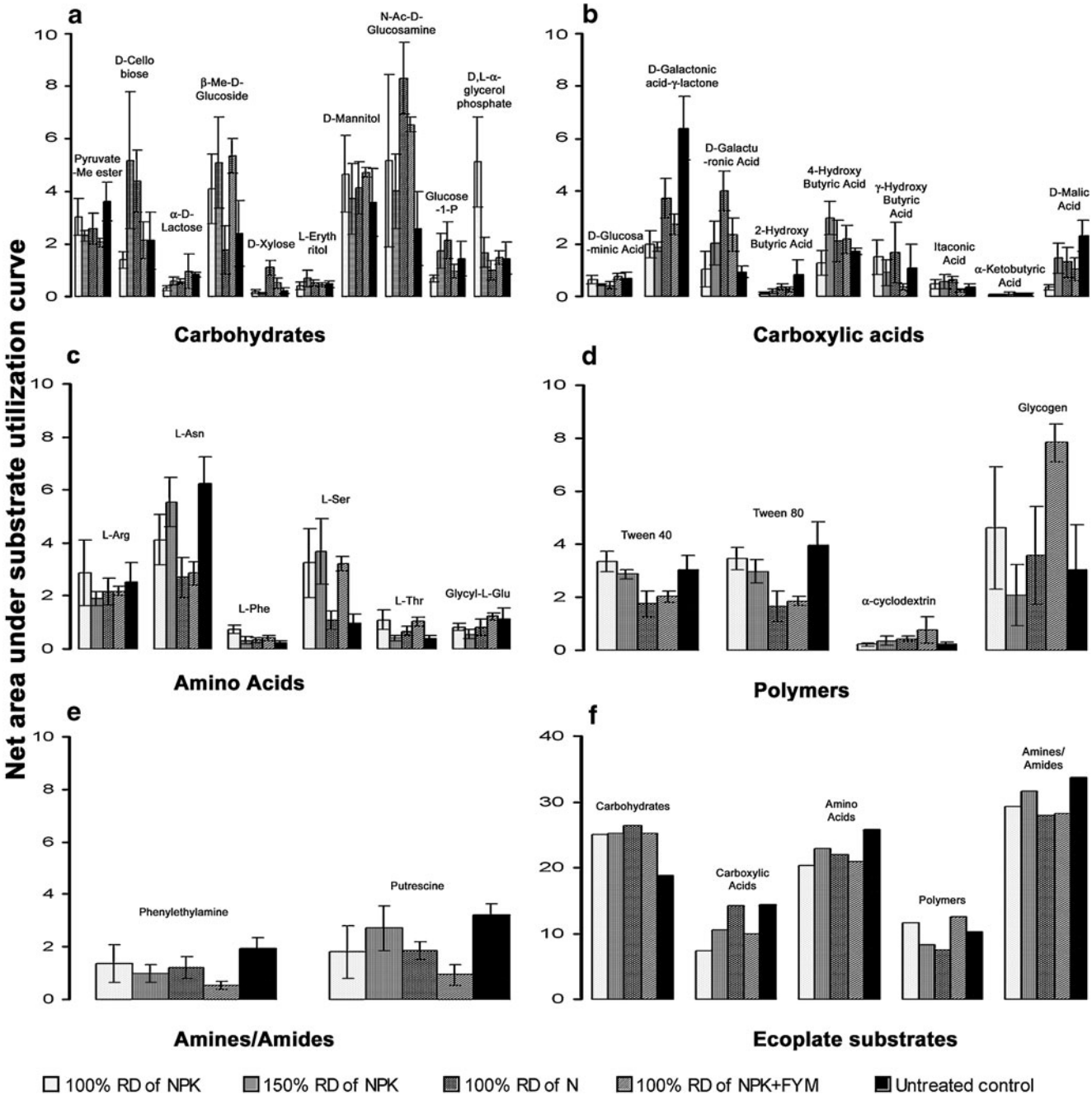

Fig. 1 Community-level physiological profiles (CLPP) of different soil samples. Utilization of each substrate is expressed as net area under the utilization curve for five different treatments. The *error bars* indicate the standard error of mean. Substrates were classified under **a** carbohydrates, **b** carboxylic acids, **c** amino acids, **d** polymers, **e** amines/amides, and **f** overall utilization of the above five categories of substrates. Utilization of different C sources varied across treatments, but utilization of carbohydrate sources was higher in all fertilizer treated soils than control

To simplify direct comparison, five main categories of substrates were classified as carbohydrates, carboxylic acids, amino acids, polymers, and amines/amides. The overall utilization of carbohydrates was more or less similar in the input treated plots and was higher in magnitude than the untreated control soil (Fig. 1f). However, the microbial communities in the untreated plots utilized more amino acids and amines/amides than the input treated plots. Difference in utilization of substrates in the different treatments shows diverse metabolic capabilities of the soil microbial communities. It is interesting to note that application of nitrogen fertilizer had compromised the ability of the soil microbial communities to catabolize the amino acids and amines/amides (Fig. 1f).

In conclusion, long-term application of integrated use of organic and inorganic supplements helped in accumulation of organic matter, which in turn had substantial incremental effect on the soil microbial biomass and its activities. The CLPP data showed that soil microbial communities in all the input treated soils could better utilize only the carbohydrates. For other carbon sources, no enhanced spectrum of utilization was seen over untreated soil. Microbial biomass represents total microbial communities while CLPP is confined to only a subpopulation of culturable aerobic microorganisms. This possibly explains the inability of CLPP to distinguish between entire gamuts of catabolic functions of the microbial communities.

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