__Original Research Article__

Long term effects of integrated plant nutrition system on rice yield, nitrogen dynamics and biochemical properties in soil of rice-rice cropping system

ABSTRACT

Long term fertilization from manure and fertilizer influences crop yield as well as soil nutrient cycling. Therefore, a field experiment was conducted from 2010 to 2014 in order to observe the long term effects of integrated application of manure and fertilizers on rice yield, soil nitrogen dynamics and biochemical properties. The field experiment was carried out in a rice-fallow-rice cropping pattern under wetland condition with four fertilizer management practices: NPKS fertilizer as soil test based (STB), Cow dung (CD) @ 3 t ha^{-1} + NPKS as integrated plant nutrition system (IPNS) [CD+IPNS], Poultry manure (PM) @ 2 t ha^{-1} + NPKS as IPNS [PM+IPNS] and N control. The rice grain yield, soil N dynamics and other biochemical properties assessed in our study significantly varied with the organic sources used in IPNS system. The annual grain yield of double cropped rice with PM + IPNS was at per the STB treatment over the five cropping years while CD+IPNS responded from the third year. After five years of rice cropping PM+IPNS showed better impact on soil nitrogen dynamics and biochemical properties compared to STB fertilizer management. Considering the soil health, our study suggests that PM+IPNS could be a good practice for sustainable rice production in long run reducing the use of chemical fertilizer.

Keywords: rice yield, mineral nitrogen, nitrogen balance, urease enzyme activity, free living nitrogen fixing bacteria, soil depth

1. INTRODUCTION

Rice-fallow-rice is the dominant cropping system in Bangladesh which occupied about 27% of the net cropped area [1]. Among the major nutrient elements that limit rice crop growth and yield, nitrogen (N) considered as the most important. Farmers generally meet the crop N demand by applying chemical N fertilizer because of its effectiveness in improving yield [2]. However, the intensive application of chemical N fertilizer in the cropping system has raised the concerns of sustainable crop production. Moreover, the injudicious use of chemical N fertilizer coupled with modern high yielding varieties and little or no use of organic matter lead to soil organic matter deficiency, soil acidity and poor soil physical properties[3, 4, 5]. On the other hand, manure alone cannot substitute inorganic fertilizer to maintain the desired level of yield of the high yielding varieties [6].Therefore, the combined application of manure and fertilizer has emerged as an effective approach for sustainable crop production. Many studies have reported significant improvement in soil physical, chemical and biological properties in organic matter application system [7,8,9].

Inappropriate fertilizer management and excessive use of N fertilizer causes N losses through ammonia volatilization and leaching [10]. It is a major challenge in N management to minimize the use of N fertilizer in crop production avoiding N deficiency in soil. Combined application of fertilizer and manure is a recommended measure for reducing N loss and increasing N use efficiency [11]. The combined application of organic and inorganic fertilizers enhances N balance and minimizes losses by converting inorganic N to organic forms [12] resulting sustainable productivity [13, 14]. Crop cultivation with integrated approach of organic and inorganic fertilizers ensures plant nutrients in readily available form, good soil health and sustainable yield [15].

The term soil health refers to the chemical, physical and biological properties that enable soil to perform a wide range of function. The N fertilization from chemical and organic sources influences N cycling, soil C storage and mineralization rates [16], and also effects temporal and spatial distribution of inorganic soil N [17]. Soil biological properties respond quickly under different soil managements than chemical or physical properties [18, 19]. Soil enzyme activity is recognized as a good indicator of soil health as it reflects the effects of cultivation, soil properties and pedological amendments [18, 19]. Soil microbes also play an important role in nutrient cycling while the diversity of microbial population is largely affected by fertilizer management practices. The abundance of microbial community is directly or indirectly influenced by the fertilization practices [20].

Among the organic sources that are available to farmers in Bangladesh, cow dung and poultry manure are good source of organic matter and can improve soil fertility providing essential plant nutrients [21, 22]. The application of fertilizers and manures of different properties differentially affects the physical, chemical and biological properties of soil [23]. N mineralization in wet land rice soil is significantly influenced by the quality and quantity of the organic matter applied [24]. However, many studies reported that the effects of organic matter application can be observed after 3 to 5 years [25, 26].Therefore, it is important to study the long-term effects of different organic fertilizers in combination with chemical N fertilizer on yield and soil properties. In Bangladesh several studies have been conducted on integrated application of manure and fertilizers on rice yield which are mainly short term study [27, 28] while there is lack of long term studies. Accordingly, this study was carried out to determine the long term effect of combined application of manure and fertilizer on soil N dynamics and biochemical properties from a five-year field experiment. Among the biochemical properties we assessed free living nitrogen fixing bacteria population, urease enzyme activity and soil organic carbon responses to applied treatment at different soil depths.

2. MATERIALS AND METHODS

2.1 Site description

The experiment was conducted at experimental farm of Bangladesh Rice Research Institute (23.59^{o} N, 90.24^{o} E, 8.4 m elevation) from 2010 to 2014. The climate of the experimental site is subtropical in nature and experiences periodic south western monsoon with an average annual rainfall of 2000 mm. The 80% of the rainfall occurs from mid-June to end of September. The lowest mean temperature (15^{o}C) prevails in January and highest (30^{o}C) in May. The soil of the experimental site belongs to the order Inceptisols in USDA soil classification having soil texture of silty clay loam. The initial surface (0-20 cm) soil sample had a bulk density of 1.40 g cm^{-3} and contained 12.21 g kg^{-1} organic carbon, 1.1 g kg^{-1} total nitrogen, 19 mg kg^{-1} available phosphorus, 0.14 cmol potassium in kg^{-1} soil and 28 mg kg^{-1} available sulphur.

2.2 Experimental design and crop management

The experiment was consisted of four treatments arranged in a randomized complete block design with three replications. The treatments were: Soil Test Based dose of NPKS fertilizer (STB), Cow dung (CD) @ 3 t ha^{-1} + NPKS as integrated plant nutrition system (IPNS) [CD+IPNS], Poultry manure (PM) @ 2 t ha^{-1} + NPKS as IPNS [PM+IPNS] and N control. The STB dose of NPKS in Boro (dry season) was 138-10-80-5 kg ha^{-1} and in T. Aman (wet season) it was 100-10-80-5 kg ha^{-1}. Nutrient supplied from per ton cow dung was 5 kg N, 1.5 kg P and 5 kg K and per ton poultry manure supplied 19 kg N, 5 kg P and 7.5 kg K. The N control treatment received the STB dose of chemical fertilizer except N. The STB dose was calculated using the BARC fertilizer recommendation guide [29]. In CD+IPNS and PM+IPNS treatments fertilizer dose was calculated by subtracting the nutrient supplied with manures from the STB dose [29]. The treatment details of the experiment are presented in Table 1. Urea, tripple super phosphate, muriate of potash and gypsum were used as fertilizer source for N, P, K and S nutrients. Manures and all the fertilizers except urea were applied at the time of final land preparation and incorporated into the soil. Urea was applied in three equal splits. The first split was applied at the time of final land preparation and the remaining two splits were applied at maximum tillering and 5-10 days before panicle initiation stage of the crop. The unit plot size was 6 m × 7 m. Rice crops were grown following Boro-Fallow-T. Aman cropping pattern. In Boro season rice variety (BRRI dhan28) was transplanted in the first week of January and was harvested in May. In T. Aman season rice variety (BRRI dhan49) was transplanted in the first week of August and harvested in last week of November.

Table 1: Nutrient added from different sources in each cropping season

Treatment	Source	Nutrient added								Annual N rate
		Boro				T. Aman				
		N	P	K	S	N	P	K	S	
STB	Fertilizer	138	10	80	5	100	10	80	5	238
CD+IPNS	Fertilizer	123	5.5	65	5	85	5.5	65	5	
	Cow dung	15	4.5	15	0	15	4.5	15	0	
	Total	138	10	80	5	100	10	80	5	238
PM+IPNS	Fertilizer	100	0	65	0	62	0	65	5	
	Poultry manure	38	10	15	0	38	11.2	15	0	
	Total	138	10	80	0	100	11.2	80	5	238
N control	Fertilizer	0	10	80	5	0	10	80	5	0

2.3 Plant and soil sample collection

Plant samples were collected at maturity stage of the crop growth for calculating grain yield and nutrient analysis. Grain yield was calculated from a harvesting area of 5 m^2 and adjusted to 14% moisture content. Total nitrogen content of plant tissue was estimated by Micro Kjeldhal method [30] and the crop N uptake was calculated from dry biomass (grain + straw) weight and N concentrations [28].

For this study soil samples were collected from four depths: 0 to 5, 5 to 10, 10 to 15 and 15 to 20 cm from each plot after the harvest of T. Aman crop in 2014. The soil samples were air-dried, grinded and passed through 2-mm sieve and stored in polythene bags at room temperature for total N, incubated ammonium and organic carbon determination. For determining NH_4^+-N, NO_3^--N, free living N fixing bacteria population and urease enzyme activity fresh soils were collected and refrigerated at 4^o C.

2.4 Net N balance

After ten cropping seasons the N balance was estimated as follows:

Net N balance = (Total N removal from ten cropping seasons + change in soil total N after 10 cropping seasons) – (N addition through manures and fertilizer)

In calculating net N balance, N inputs from other sources like rainfall, irrigation, crop residues, biological N fixation etc. and N outputs form various N loss mechanism were not considered in this study.

2.5 Soil ammonium N, nitrate N, mineralized N, total N and organic carbon determination

Soil NH_4^+-N and NO_3^--N was determined from moist soil extracting with 2 M KCl [31] followed by distillation with MgO and Dvarda alloy [32]. The mineralized N in soils received different fertilizers treatments was determined using a soil incubation study described by Sahrawat, 1983 [33]. Total N was determined by Kjeldhal digestion, distillation and titration [34]. Total organic carbon (OC) was determined following modified Walkley-Black method [35]. All the measurements were expressed on dry (105^{o}C) soil basis.

2.6 Free living nitrogen fixing bacteria (NFB) population and urease enzyme activity

The free living NFB population was counted as colony forming unit culturing on N free media [36] using serial dilution method [37].

Urease enzyme activity was determined by measuring the amount of ammonium released from the hydrolysis of urea and expressed as mg NH_4^+ released g^{-1} soil h^{-1} described by Tabatabai and Bermner, 1979 [38].

2.7 Statistical analysis

Data related to rice yield, soil ammonium N, nitrate N, total N, mineralizable N, organic carbon, free living nitrogen fixing bacteria population and urease enzyme activity in different treatments and soil depths were analyzed using ANOVA at 5% level of significance. Mean comparison was done using Least significance test (LSD). Results are presented as mean with standard error of three replicates. Statistical analysis was performed with STAR (Statistical Tool for Agricultural research) version 2.0.1.

3. RESULTS AND DISCUSSION

3.1 Grain yield

The different fertilizer management practices showed significant effects on rice grain yield and the yield scenario of five years rice cropping with different fertilization is shown in Figure 1. During the five years cropping, the annual rice grain yield in PM+IPNS was at per the STB treatment. The annual grain yield in CD+IPNS was significantly lower compared to STB treatment in the first two cropping years. However, from the third year it resulted in similar annual rice grain yield to STB. The omission of N fertilizer drastically reduced rice grain yield compared to N fertilized treatments from the third year (Figure 1). Our findings indicate that PM performed better than CD in obtaining the rice grain yield at par with STB treatment. The variation in crops yield with the types of organic matter used and their combination with inorganic fertilizers has been confirmed by previous studies [39, 40, 41, 42]. The better performance of PM on rice grain yield over CD might be due to higher nutrient content and low to optimum C:N ratio in PM than CD. Therefore, nutrient release from PM is faster resulting higher nutrient uptake and thereby higher yield. On the other hand manure like CD having high C:N ratio initially favors nutrient immobilization which eventually resulted in lower grain yield. These findings were supported by Rahman et al., 2016 [43]. Moreover, it is well documented that integrated application of manure and fertilizes in balanced doses increase crop yield by improving soil physical, chemical and biological properties [44, 45].

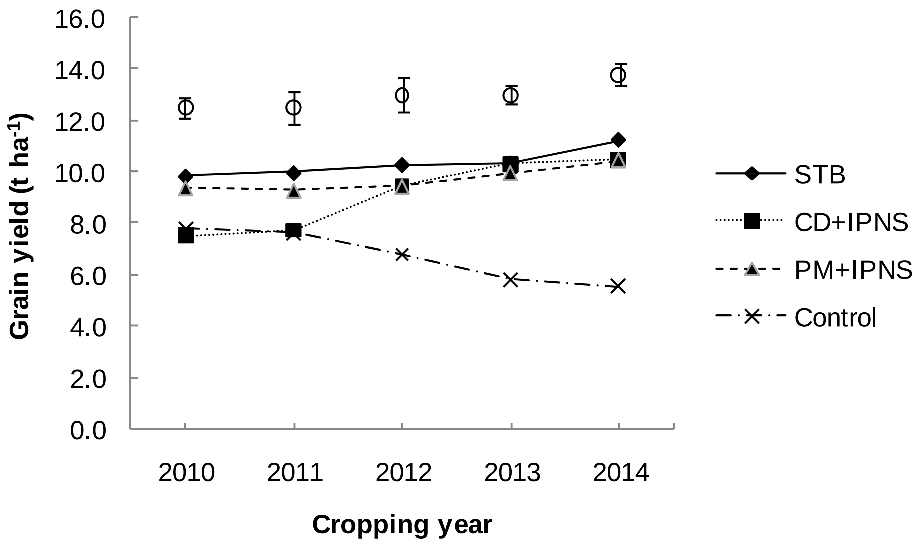

Figure 1: Fertilization effects on rice grain yield in Boro and T. Aman seasons during five years of cropping.

Error bars represent LSD value

3.2 Net nitrogen balance

The net N nitrogen balance in the 0-20 cm soil depth after five years of rice-rice cropping system as affected by different fertilizer management practices was shown in Table 2. All the fertilized treatments received an equal N input of 1380 kg ha^{-1} from fertilizer and manures. However, the highest (991 kg ha^{-1}) N removed by the rice crop was from STB treated soil resulting in a decrease (406 kg ha^{-1}) of soil N with a net N balance of -795 kg ha^{-1}. Among the manure treated soils, PM+IPNS showed an increase (910 kg ha^{-1}) in soil N with a net positive N balance of 435 kg ha^{-1} while CD+IPNS resulted in a negative net N balance of 148 kg ha^{-1}. Our study suggests that combined application of manure and fertilizer for long term can build up soil N while sole application of inorganic fertilizer or no nitrogen fertilizer resulted in a higher negative N balance. Kumar and Mukhopadhay, 2017 [46]; Tadesse et al., 2013 [47] reported a higher positive N balance with combined application of manure and fertilizer in rice based cropping system. Tiwari et al., 2010 [48] reported a negative nitrogen balance with inorganic fertilizers only and positive with manures. The positive balance in PM treated soil might be attributed due to the variation in N addition from different sources other than fertilizer and manure and variation in crop N removal. Because N inputs were equal in all fertilized treatments and N removal (except N losses) were considered, the positive N balance in the PM treated soil might be attributed to higher biological N fixation by free living N fixing bacteria which population was recorded higher in PM amended soil (Figure 4.a). Ladha et al., 2000 [49] also reported similar findings. The higher negative N balance in STB treatment where only chemical fertilizer was applied may be due to higher N loss through ammonia volatilization which was reported by several studies [50, 51, 52].

Table 2: Fertilization effects on net N balance after five years of rice cropping

Treatment	N Added		Initial soil N	N removed	Soil N after 10^{th}crop	Net N balance
	Fertilizer	Manure				
	(kg ha^{-1})					
STB	1380	0	3080	991.085	2674	-794.915
STB+CD	1230	150	3080	902.537	3409	-148.463
STB+PM	1000	380	3080	905.444	3990	435.444
N control	0	0	3080	456.3195	2156	-467.681

3.3 Soil ammonium N

After ten cropping season soil NH_4^+-N was significantly varied with fertilization practices and soil depths. The variation in NH_4^+-N concentration due to different fertilizer management practices was much higher at the top 0-5 cm soil depth where significantly highest NH_4^+ -N was found with PM+IPNS treatment (25.49 mg kg^{-1}) followed by CD+IPNS treatment (19.83 mg kg^{-1}). At 5-10 cm soil depth NH_4^+-N concentration was statistically similar with PM+IPNS (15.90 mg kg^{-1}) and STB (15.01 mg kg^{-1}) fertilizer management practices. In 10-15 and 15-20 cm soil depth the NH_4^+ -N concentration was identical between PM+IPNS and CD+IPNS treatments. In STB, CD+IPNS and PM+IPNS treatments NH_4^+-N concentration was higher at top soil layer and decreased with the increase of soil depths. However, in N control treatment the NH_4^+-N concentration was lower at 0-5 soil depth then it increased at 5-10 cm soil depth and then it gradually decreased up to 20 cm soil depth (Figure 2.a).

NH_4^+-N is the most reduced and preferred forms of N in flooded soil [53, 54]. NH_4^+-N builds up in wetland soils particularly in soils high in organic matter or when easily decomposable organic matter is added in high amounts [53; 55, 56]. This might be the reason of higher NH_4^+-N in manure treated soil especially in PM treated soils which is easily decomposable than CD. The lower concentration of NH_4^+-N in deeper soil layer might be attributed to its adsorbance on soil colloids which leads to lower percolation loss [57]. Lee and Choi, 2017 [5]; Mi et al., 2019 [58] also reported decreased NH_4^+-N with the increase of soil depth. As the mineralization of N from chemical N fertilizer is high the NH_4^+ released from STB treatment is subjected to instant crop uptake [5] and volatilization loss which resulted in lower concentration of NH_4^+ in the post harvested soil [52].

3.4 Soil nitrate N

Soil NO_3^--N was significantly affected by fertilizer management practices along the soil depths. At al the four soil depths the NO_3^--N concentration was significantly higher with PM+IPNS treatment and lowest with N control treatment. In CD+IPNS, PM+IPNS and N control treatments the NO_3^--N concentration was significantly higher at 0-5 cm soil depth and then it sharply decreased and remained static at 5-20 cm soil depths. However, in STB fertilizer treatment the NO_3^--N concentration was significantly higher at 5-10 cm soil depth and was identical at 0-5, 10-15 and 15-20 cm soil depths (Figure 2.b).

In this study the higher NO_3^- concentration in the PM+IPNS treated soil might be due to higher NH_4^+ concentration in this soil as the NH_4^+ adsorbed onto clay particles is gradually converted to NO_3^-via nitrification [5]. In an incubation study Murugan and Swarnam, 2013 [59] found higher NO_3^- in manure treated soils compared to inorganic N treated soils. The lower NO_3^--N concentration in the deeper soil may be due to less aerobic conditions in flooded soil [60]. Mi et al., 2019 [58] also reported lower NO_3-N with increasing soil depth.

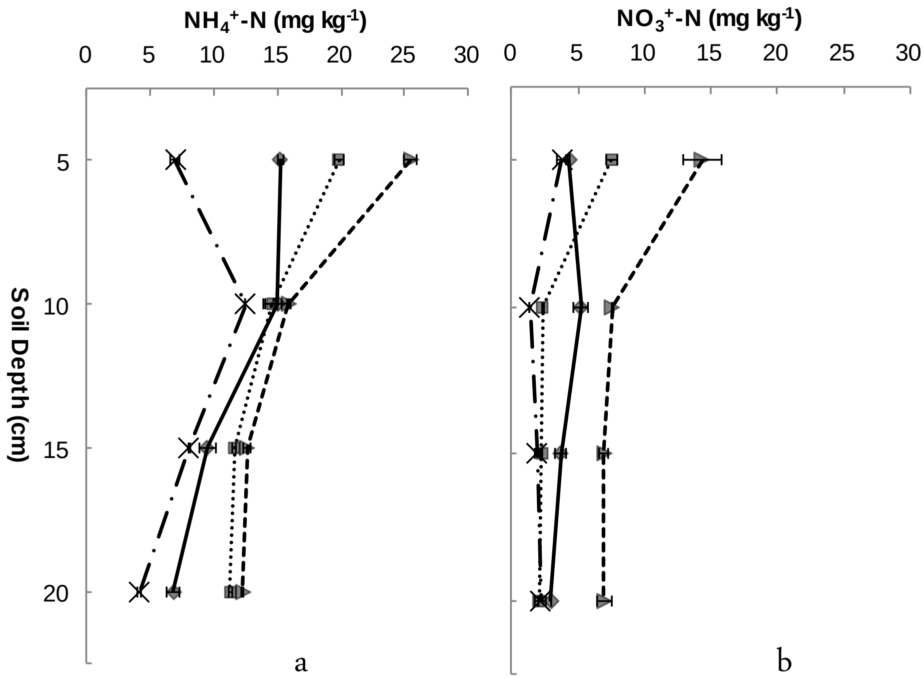

Figure 2: Fertilization effects on soil ammonium N and nitrate N after five years of rice cropping.

Error bars represent standard error (n=3) of the means of ammonium N and nitrate N.

3.5 Total N

The soil total N content was significantly affected with the different fertilization practices within a soil profile of 20 cm. At 0-5 cm soil depth the total N content was significantly higher with PM+IPNS followed by CD+IPNS while at 5-10 cm soil depth the trend was reverse. At 10-15 cm soil depth the effect of CD+IPNS and PM+IPNS treatments on soil total N was statistically similar. Significantly lowest soil total N was observed in N control treatment at all four soil depths. Regardless of the fertilizer treatments an overall decreasing trend of soil total N was observed with the increase of soil depths (Figure 3.a).
Previous studies reveled that combined application of different organic matter and chemical fertilizer for long term significantly increased soil total N compared to only chemical fertilizer [61, 62, 63]. Regardless of the fertilizer treatment total N decreased with the increase in soil depth which coincides with the results of Lee and Choi, 2017 [5].

3.6 Mineralizable N

Mineralizable N was determined from the anaerobic incubation of dried soils after harvesting of 10th rice crop. Different fertilization practices showed significant effect on soil mineralizable N at different soil depths after 14 days of incubation. PM+IPNS treated soils resulted significantly highest mineralizable N at all four soil depths (0-5, 5-10, 0-15 and 15-20 cm) compared to CD+IPNS and STB treated soils. In the PM+IPNS treated soil mineralizable N ranged from 384.50-220.00 mg kg^{-1}. The N controlled soils resulted in lowest mineralizable N at all soil depths. Irrespective of fertilizer treatments, minralizable N was highest at upper 0-5 cm soil layer and was lowest at 15-20 cm soil depth although in N control treatment after 0-5 cm the variation in mineralizable N was statistically similar (Figure 3.b).

The higher soil residual mineral N levels found in our study with the PM+IPNS treatment might be due to slow release of the N during the crop growth period. Mineralizable N is affected by the quantity and quality of organic matter applied in the soil [64] which might be cause of variation in mineralizable N in PM and CD treated soils. Sahrawat, 1983 [33] reported that mineralizable N in anaerobic incubation was significantly and positively correlated with soil OC and total N. This might be the reason of higher mineralizable N in manure treated soils in our study which contained higher OC and total N compared to chemical fertilizer treated soils. Pal et al., 2015 [65] found highest mineralizable N with the highest rate of chemical fertilizer combined with farm yard manure while Myint et al., 2010 [66] reported highest mineralized N with sole application of mineral N fertilizer than sole application of manure.

3.7 Total organic carbon

Soil total organic carbon (TOC) significantly varied with fertilizer management practices and soil depth. Application of PM @ 2 t ha^{-1} for five consecutive cropping seasons resulted in significantly highest accumulation of TOC at all the four studied soil depths followed by application of CD @ 3 t ha^{-1}. In PM+IPNS treated soil TOC varied from 16.87-8.45 g kg^{-1}. The lowest TOC was found in N controlled soil. Regardless of the fertilizer treatments, soil TOC decreased with the increase of soil depth (Figure 3.c).

Previous studies showed that combined application of manure and fertilizer increased soil OC [67, 68, 61]. Similar result was also found in our study where higher soil OC content was found with PM and CD amended soil. However, the accumulation of OC was higher in PM than CD. Rahman et al. 2016 [43] also reported increased OC content in PM treated soil over CD after 2.5 years of rice cultivation. The increased soil OC content in the top soil can be explained by the higher accumulation of manure and crop residues in the top soil.

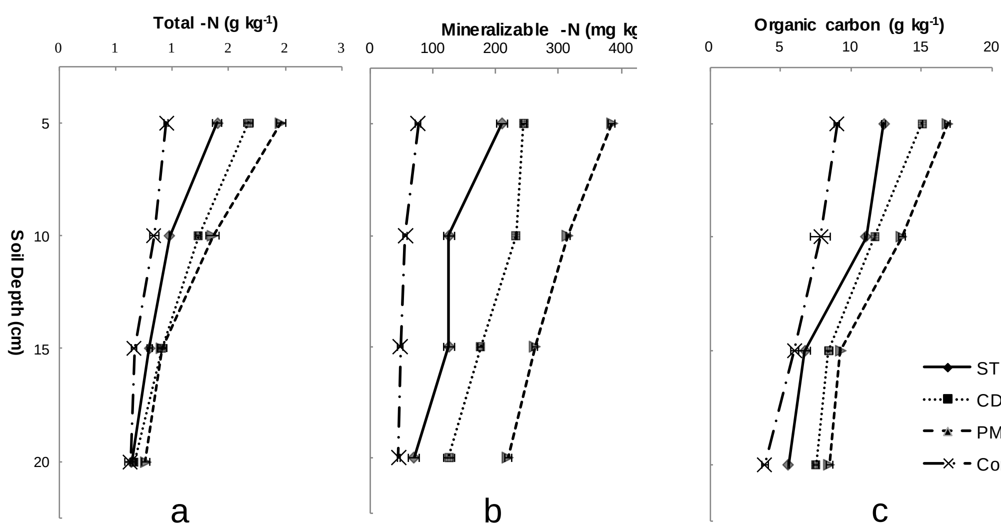

Figure 3: Fertilization effects on soil total N, mineralizable N and organic carbon after five years of rice cropping.

Error bars represent standard error (n=3) of the means of total N, mineralizable N and organic carbon.

3.8 Free living nitrogen fixing bacteria population

Free living nitrogen fixing bacteria (NFB) population was significantly affected by fertilizer treatments and soil depths. The NFB population was counted as colony forming unit (cfu) which have been presented transforming into log10 value (Figure 4.a). Application of CD and PM following IPNS approach significantly increased NFB population irrespective of soil depths compared to STB fertilizer management and N control. At 0-5 and 15-20 cm soil depths the NFB population was significantly higher with PM+IPNS than rest of the treatments while at 5-10 and 10-15 cm soil depths the NFB population was found statistically similar with PM+IPNS and CD+IPNS. The STB and N control treatment exerted similar effect on NFB population at different soil depths. The NFB population was higher in upper soil layer and decreased with the increase of soil depth irrespective of treatments. However, in STB and N control treatments the variation in NFB population along the soil depths was statistically similar. Our study results clearly indicate that repeated application of organic manure in combination with chemical fertilizer resulted in the abundance of NFB population which supports previous findings. Mujiyati and Supriyadi, 2009 [69] reported increased population of *Aztobacter* and *Azospirillium* with manure and fertilizer application. On the other hand, application of high N fertilizer inhibits biological nitrogen fixation [70, 71] which might be the cause of lower NFB population in STB chemical fertilizer treatment.

3.9 Urease enzyme activity

Urease activity is a good indicator of changes in soil quality for soil management [72]. In this study soil urease activity was significantly affected by fertilizer management practices and soil depths. Regardless of the soil depth urease activity was significantly higher in PM+IPNS followed by CD+IPNS treated soils compared to STB fertilized soil. In PM+IPNS treated soil the urease activity ranged between 51.12-23.36 µg NH_4^+ $g^{-1}h^{-1}$ and in CD+IPNS treated soil it was 42.54-18.43 µg NH_4^+ $g^{-1}h^{-1}$. The lowest urease activity was found in N control treated soil. Urease activity was higher at upper soil layer and decreased with the increase of soil depth in all applied treatments (Figure 4.b). The higher urease activity in the organic matter amended soil and upper soil layer is due to high OC and microbial activity which was reported by several studies [73, 74, 75]. The OC provides energy for microbial activity resulting in higher enzyme activity. The constituents of the organic matter also influence the soil urease activity and it is positively correlated with OC and total N [76]. Application of PM performed better than CD which might be due to high N content in PM.

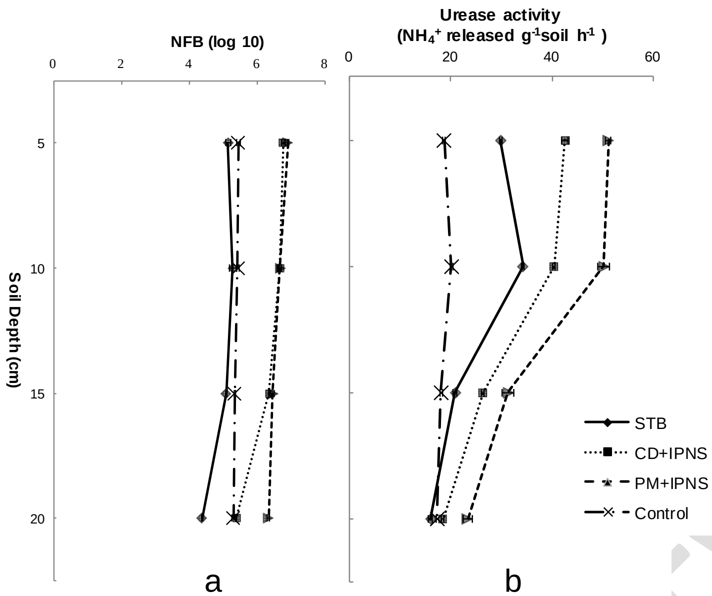

Figure 4: Fertilization effects on free living N fixing bacteria (NFB) and urease enzyme activity at different soil depths after five years of rice cropping.

Error bars represent standard error (n=3) of the means of free living N fixing bacteria and urease enzyme activity.

4. CONCLUSION

From this study it can be concluded that long term (5 years) application of organic and inorganic fertilizers following IPNS approach in the rice-fallow-rice cropping system has profound influence on rice yields, N forms and biochemical properties. Application of PM @ 2 t ha^{-1} in each cropping season resulted in annual grain yield at per the STB treatment during the five years of rice cropping. After five years of rice cropping PM+IPNS resulted in a positive soil N balance while it was negative in CD+IPNS and STB treatments. The consecutive use of PM+IPNS also showed significant effects on N forms (ammonium N, nitrate N, mineralizable N and total N), organic carbon and biochemical properties (free living N fixing bacteria population and urease enzyme activity). Considering the soil health, our study suggests that PM+IPNS could be an effective fertilization practice for sustainable rice production in long run reducing the use of chemical fertilizer.

COMPETING INTEREST

The authors have declared that no competing interests exist.

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