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 soil 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 the 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 similar to 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 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 impoverishment of the 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 elements [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 (OC) 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} OC, 1.1 g kg^{-1} total nitrogen (TN), 19 mg kg^{-1} available P, 0.14 cmol K 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}. The quantity of 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	10	15	0	
	Total	138	10	80	0	100	10	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 (OC) 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 (TOC) 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 N 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 Difference (LSD) test. 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. The annual grain yield in STB, CD+IPNS, PM+IPNS and N control treatments ranged between 9.83-11.23 t ha^{-1}, 7.53-10.47 t ha^{-1}, 9.37-10.44 t ha^{-1} and 5.52-7.81 t ha^{-1} respectively. During the five years cropping, the annual rice grain yield in PM+IPNS was identical to 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 similar to 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].

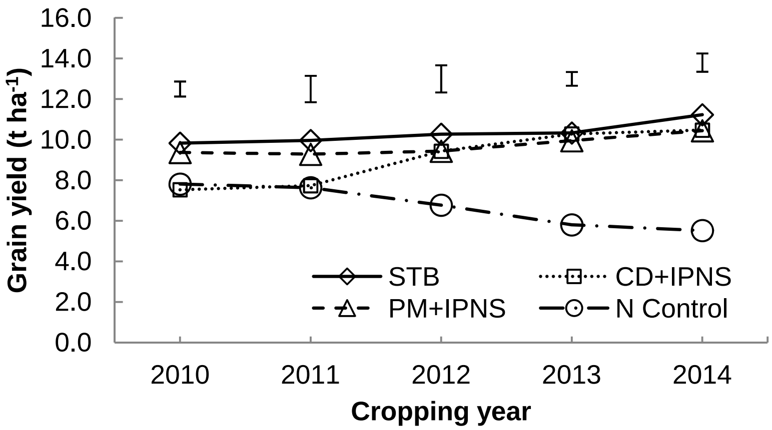

Figure 1: Fertilization effects on annual rice grain yield during five years of cropping.
Error bars represent LSD value

3.2 Net nitrogen balance

The N net 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 N 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 7). 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).

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].

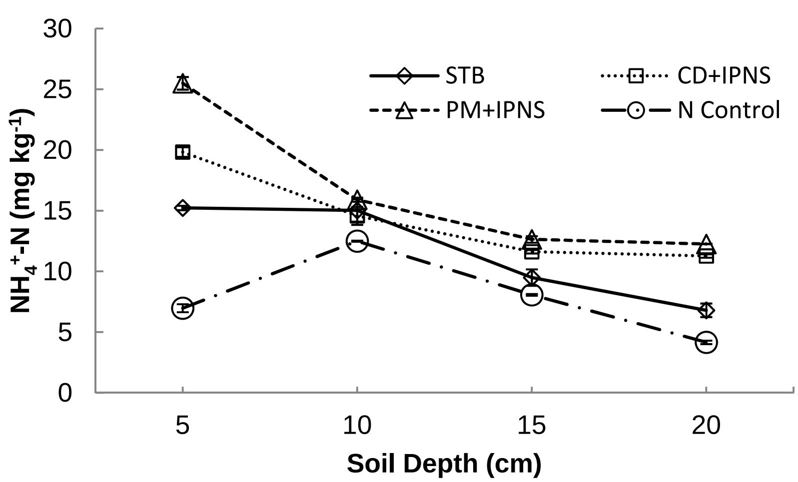

Figure 2: Fertilization effects on soil ammonium N after five years of rice cropping.

Error bars represent standard error (n=3) of the mean of ammonium N

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 3).
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.

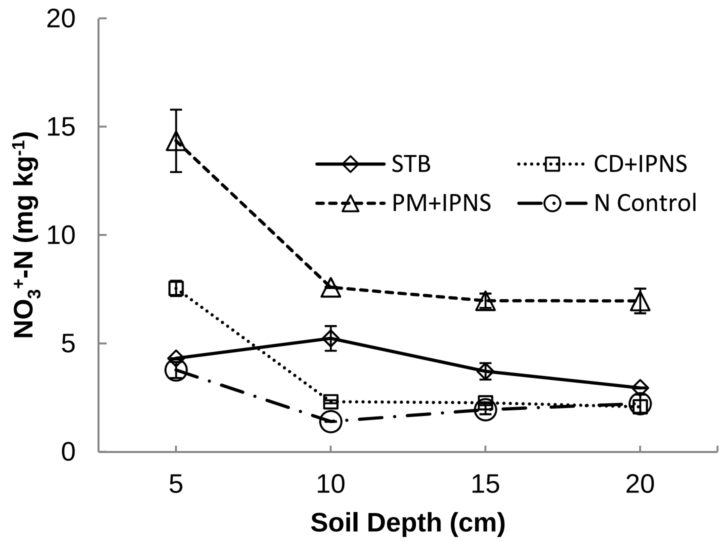

Figure 3: Fertilization effects on soil nitrate N after five years of rice cropping.
Error bars represent standard error (n=3) of the mean of nitrate N.

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 4).
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]. The higher N content in the top soil layer might be due to higher microbial activity which favors the availability of nutrient like N [43]. Moreover, the soil N is subjected to transformation of NH_4^+ and NO_3^- and in the submerged rice soil NH_4^+ is dominant [43]. The dynamics of NH_4^+ and NO_3^- are explained in section 3.3 and 3.4. The decreased N with the

increase in soil depth in our study coincides with the results of Lee and Choi, 2017 [5] and Rahman et al, 2016 [43].

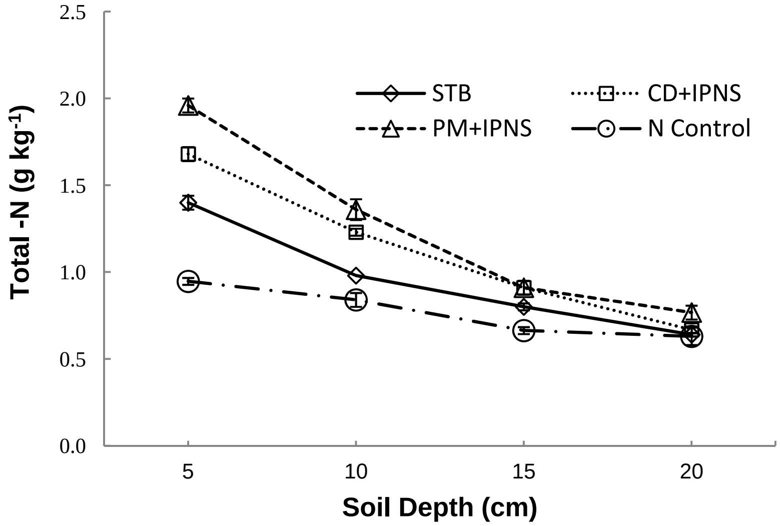

Figure 4: Fertilization effects on soil total nitrogen

Error bars represent standard error (n=3) of the mean of total nitrogen

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 5).

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 total N and OC (Figure 4 & 6) 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.

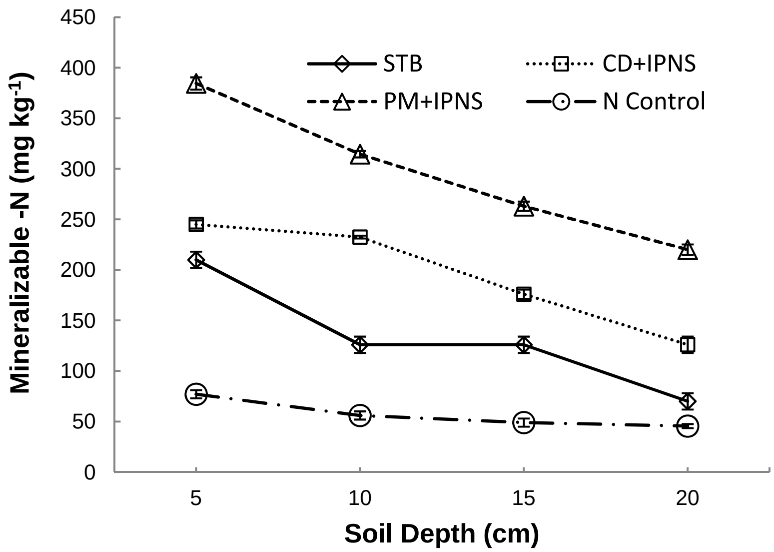

Figure 5: Fertilization effects on soil mineralizable N after five years of rice cropping.
Error bars represent standard error (n=3) of the mean of mineralizable N

3.7 Total organic carbon

Soil 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 6).
Previous studies showed that combined application of manure and fertilizer increased soil TOC [61, 67, 68). Similar result was also found in our study where higher soil TOC content was found with PM and CD amended soil. However, the accumulation of TOC was higher in PM than CD. Rahman et al. 2016 [43] also reported increased TOC content in PM treated soil over CD after 2.5 years of rice cultivation. The increased soil TOC content in the top soil can be explained by the higher accumulation of manure and crop residues in the arable layer.

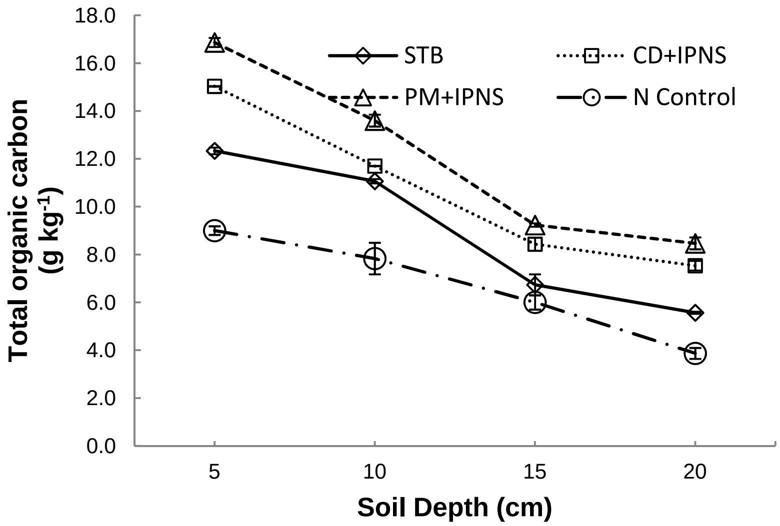

Figure 6: Fertilization effects on soil total organic carbon after five years of rice cropping.

Error bars represent standard error (n=3) of the mean of total 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 (Figure 7). 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 N fixation [70, 71] which might be the cause of lower NFB population in STB chemical fertilizer treatment.

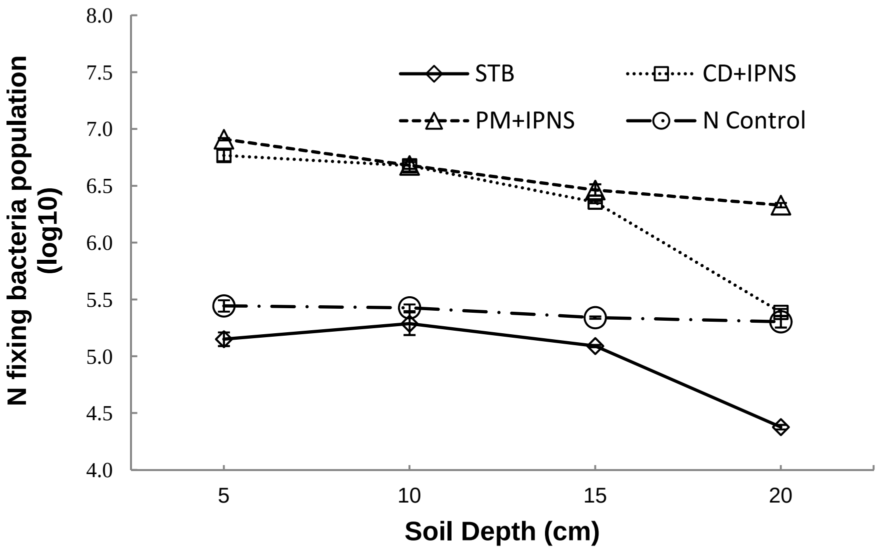

Figure 7: Fertilization effects on free living N fixing bacteria population at different soil depths after five years of rice cropping.

The data were subjected to log10 transformation. Error bars represent standard error (n=3) of the mean of free living N fixing bacteria.

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 8). 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.

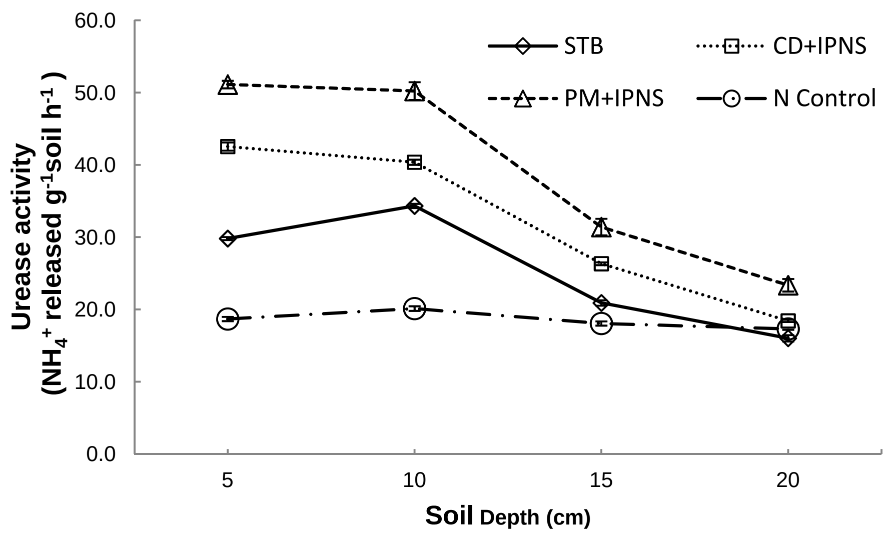

Figure 8: Fertilization effects on urease enzyme activity at different soil depths after five years of rice cropping.

Error bars represent standard error (n=3) of the mean of 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 similar to 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.

REFERENCES

1. Nasim M, Shahidullah SM, Saha A, Muttaleb MA, Aditya TL, Ali MA, Kabir MS. Distribution of crops and cropping patterns in Bangladesh. Bangladesh Rice Journal. 2017;21(2):1-55.
2. Zhu ZL, Chen DL. Nitrogen fertilizer use in China–Contributions to food production, impacts on the environment and best management strategies. Nutrient Cycling in Agroecosystems. 2002;63(2-3):117-27.
3. Celik I, Gunal H, Budak M, Akpinar C. Effects of long-term organic and mineral fertilizers on bulk density and penetration resistance in semi-arid Mediterranean soil conditions. Geoderma. 2010;160(2):236-43. doi:10.1016/j.geoderma.2010.09.028

4. Liu E, Yan C, Mei X, He W, Bing SH, Ding L, Liu Q, Liu S, Fan T. Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in Northwest China. Geoderma. 2010;158(3-4):173-180. doi:10.1016/j.geoderma.2010.04.029
5. Lee J, Choi HL. The dynamics of nitrogen derived from a chemical nitrogen fertilizer with treated swine slurry in paddy soil-plant systems. PloS one. 2017;12(3):e0174747. doi:10.1371/journal.pone.0174747
6. Efthimiadou A, Bilalis D, Karkanis A, Froud-Williams B. Combined organic/inorganic fertilization enhance soil quality and increased yield, photosynthesis and sustainability of sweet maize crop. Australian Journal of Crop Science. 2010;4(9):722.
7. Chang EH, Chung RS, Wang FN. Effect of different types of organic fertilizers on the chemical properties and enzymatic activities of an Oxisol under intensive cultivation of vegetables for 4 years. Soil Science and Plant Nutrition. 2008;54(4):587-99. doi:10.1111/j.17470765.2008.00264.x
8. Surekha K, Latha PC, Rao KV, Kumar RM. Grain yield, yield components, soil fertility, and biological activity under organic and conventional rice production systems. Communications in Soil Science and Plant Analysis. 2010;41(19):2279-92. doi:10.1080/00103624.2010.507834
9. Subehia SK, Sepehya S, Rana SS, Negi SC, Sharma SK. Long-term effect of organic and inorganic fertilizers on rice (Oryza sativa L.)–wheat (Triticum aestivum L.) yield, and chemical properties of an acidic soil in the western Himalayas. Experimental Agriculture. 2013;49(3):382-94. doi:10.1017/S0014479713000173
10. Moe K, Mg KW, Win KK, Yamakawa T. Effect of combined application of inorganic fertilizer and organic manures on nitrogen use and recovery efficiencies of hybrid rice (Palethwe-1). American Journal of Plant Science. 2017;8**:**1043-1064. doi:10.4236/ajps.2017.85069
11. Wen Z, Shen J, Blackwell M, Li H, Zhao B, Yuan H. Combined applications of nitrogen and phosphorus fertilizers with manure increase maize yield and nutrient uptake via stimulating root growth in a long-term experiment. Pedosphere. 2016;26(1):62-73. doi:10.1016/S1002-0160(15)60023-6
12. Kramer AW, Doane TA, Horwath WR, Van Kessel C. Combining fertilizer and organic inputs to synchronize N supply in alternative cropping systems in California. Agriculture, Ecosystems & Environment. 2002;91(1-3):233-43. doi: 10.1016/S0167-8809(01)00226-2
13. Satyanarayana V, Vara Prasad PV, Murthy VR, Boote KJ. Influence of integrated use of farmyard manure and inorganic fertilizers on yield and yield components of irrigated lowland rice. Journal of Plant Nutrition. 2002;25(10):2081-90. doi:10.1081/PLN-120014062
14. Jobe B. Integrated nutrient management for increased rice production in the inland valleys of the Gambia. In: Sanyang S, Ajayi A, Sy AA, editors. Proceedings of the Second Biennial Regional Rice Research Review; 2003.
15. Lakshmi CS, Rao PC, Sreelatha T, Madahvi M, Padmaja G, Rao PV, Sireesha A. Nitrogen use efficiency and production efficiency of rice under rice-pulse cropping system with integrated nutrient management. Journal of Rice Research. 2012;5(1):2.
16. Sardans J, Peñuelas J, Estiarte M. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. Applied Soil Ecology. 2008;39(2):223-35. doi: 10.1016/j.apsoil.2007.12.011
17. Shi Z, Li D, Jing Q, Cai J, Jiang D, Cao W, Dai T. Effects of nitrogen applications on soil nitrogen balance and nitrogen utilization of winter wheat in a rice–wheat rotation. Field Crops Research. 2012;127:241-7. doi: 10.1016/j.fcr.2011.11.025
18. Nannipieri P, Kandeler E, Ruggiero P. Enzyme activities and microbiological and biochemical processes in soil. In: Burns RG, Dick RP, editors. Enzymes in the Environment. New York: Marcel Dekker, 2002.

19. Paz-Ferreiro J, Gascó G, Gutiérrez B, Méndez A. Soil biochemical activities and the geometric mean of enzyme activities after application of sewage sludge and sewage sludge biochar to soil. Biology and Fertility of Soils. 2012;48(5):511-7. doi: 10.1007/s00374-011-0644-3
20. Gu Y, Wang Y, Lu SE, Xiang Q, Yu X, Zhao K, Zou L, Chen Q, Tu S, Zhang X. Long-term fertilization structures bacterial and archaeal communities along soil depth gradient in a paddy soil. Frontiers in Microbiology. 2017;8:1516.doi:10.3389/fmicb.2017.01516
21. Khanam M, Rahman MM, Islam MR, Islam MR. Effect of manures and fertilizers on the growth and yield of BRRI Dhan30. Pakistan Journal of Biological Sciences. 2001; 4: 172-174. doi: 10.3923/pjbs.2001.172.174
22. Mitchell CC, Tu S. Nutrient accumulation and movement from poultry litter. Soil Science Society of America Journal. 2006; 70(6): 2146-2153. doi:10.2136/sssaj2004.0234
23. Hossaen MA, Shamsuddoha AT, Paul AK, Bhuiyan MS, Zobaer AS. Efficacy of different organic manures and inorganic fertilizer on the yield and yield attributes of Boro rice. The Agriculturists. 2011;9(1&2):117-25.
24. Sahrawat KL. Organic matter and mineralizable nitrogen relationships in wetland rice soils. Communications in Soil Science and Plant Analysis. 2006;37(05-06):787-96. doi:10.1080/00103620600564034
25. Ohyama N, Katano M, Hasegawa T. Effects of long term application of organic materials to the paddy field originated from Aso volcanic ash [Japan] on the soil fertility and rice [Oryza sativa] growth, 1: Effects on the rice growth and nutrient uptake for the initial three years. Proceedings of Faculty of Agriculture-Kyushu Tokai University (Japan). 1998.
26. Uenosono S, Nagatomo M. Effect of application of only manure on yield and quality of paddy rice. Kyushu Agricultural Research (Japan). 1998.
27. Ali ME, Islam MR, Jahiruddin M. Effect of integrated use of organic manures with chemical fertilizers in the rice-rice cropping system and its impact on soil health. Bangladesh Journal of Agricultural Research. 2009;34(1):81-90.
28. Sarkar MU, Rahman MM, Rahman GK, Naher UA, Ahmed MN. Soil test based inorganic fertilizer and integrated plant nutrition system for rice (*Oryza sativa* L.) cultivation in inceptisols of Bangladesh. The Agriculturists. 2016;14(1):33-42. doi: 10.3329/agric.v14i1.29098
29. BARC (Bangladesh Agricultural Research Council). Fertilizer Recommendation Guide 2012. Dhaka, Bangladesh: BARC; 2012.
30. Yoshida S, Forno DA, Cock JH. Laboratory manual for physiological studies of rice. Philippines: Los Banos IRRI; 1971.
31. Maynard DG, Kalra YP. Nitrate and exchangeable ammonium nitrogen. In: Carter MR, editor. Soil sampling and methods of analysis. Boca Raton: Lewis Publishers; 1993.
32. Bremner JM, Keeney DR. Steam distillation methods for determination of ammonium, nitrate and nitrite. Analytica Chimica Acta. 1965; 32: 485-495.
33. Sahrawat KL. Mineralization of soil organic nitrogen under waterlogged conditions in relation to other properties of tropical rice soils. Soil Research. 1983; 21:133-138.
34. Bremner JM. Determination of nitrogen in soil by the Kjeldahl method. The Journal of Agricultural Science.1966; 55:11-33.
35. Page AL, Miller RH, Keency DR. Methods of soil analysis- Part 2. Madison: American Society of Agronomy; 1982.
36. Dobereiner J, Day JM. Associative symbioses in tropical grasses: characterization of microorganisms and dinitrogen-fixing sites. In: Newton WE, Nyman CJ, editors. Proceedings of the 1st international symposium on nitrogen fixation. Pullman: Washington State University Press; 1976
37. Dhingra OD, Sinclair JB. Basic plant pathology methods. London: CEC press; 2000.
38. Tabatabai MA, Bermner JM. Use of P-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biology and Biochemistry. 1969; 1:301-307.

39. Gupta V, Sharma RS, Vishwakarma SK. Long-term effect of integrated nutrient management on yield sustainability and soil fertility of rice (*Oryza sativa*)–wheat (*Triticum aestivum*) cropping system. Indian Journal of Agronomy. 2006;51(3):160-4.
40. Saha PK, Ishaque M, Saleque MA, Miah MA, Panaullah GM, Bhuiyan NI. Long-term integrated nutrient management for rice-based cropping pattern: effect on growth, yield, nutrient uptake, nutrient balance sheet, and soil fertility. Communications in Soil Science and Plant Analysis. 2007;38(5-6):579-610. doi: 10.1080/00103620701215718
41. Baishya LK, Rathore, SS, Singh D, Sarkar D, Deka BC. Effect of integrated nutrient management on rice productivity, profitability and soil fertility. Annals of Plant and Soil Research. 2015;17, 86-90.
42. Gawde N. Long term effect of integrated nutrient management on soil nutrient status under rice-wheat cropping system in Inceptisols. International Journal of Chemical Studies. 2017; 5:1050-1057.
43. Rahman F, Rahman MM, Rahman GM, Saleque MA, Hossain AS, Miah MG. Effect of organic and inorganic fertilizers and rice straw on carbon sequestration and soil fertility under a rice–rice cropping pattern. Carbon Management. 2016;7(1-2):41-53. doi: 10.1080/17583004.2016.1166425
44. Rahman MM. Potential supplies and use efficiencies of nutrients from different organic wastes under tomato cultivation. Annals Bangladesh Agriculture. 2012;16: 25-39.
45. Rahman MM. Nutrient-use and carbon-sequestration efficiencies in soils from different organic wastes in rice and tomato cultivation. Communications in Soil Science and Plant Analysis. 2013;44(9):1457-1471. doi: 10.1080/00103624.2012.760575
46. Kumar B, Mukhopadhyay SK. Effect of integrated nutrient management on system productivity, nutrient uptake, nitrogen balance, soil structural properties and nitrogen use efficiency under wheat-rice cropping system. Journal of Pharmacognosy and Phytochemistry. 2017;SP1:1030-1033.
47. Tadesse T, Dechassa N, Bayu W, Gebeyehu S. Effects of farmyard manure and inorganic fertilizer application on soil physico-chemical properties and nutrient balance in rain-fed lowland rice ecosystem. American Journal of Plant Sciences. 2013;4(02):309. doi: 10.4236/ajps.2013.42041
48. Tiwari KR, Sitaula BK, Bajracharya RM, Børresen T. Effects of soil and crop management practices on yields, income and nutrients losses from upland farming systems in the Middle Mountains region of Nepal. Nutrient Cycling in Agroecosystems. 2010;86(2):241-53. doi:10.1007/s10705-009-9289-0.
49. Ladha JK, Dawe D, Ventura TS, Singh U, Ventura W, Watanabe I. Long-term effects of urea and green manure on rice yields and nitrogen balance. Soil Science Society of America Journal. 2000; 64: 1993–2001. doi:10.2136/sssaj2000.6461993x
50. Huang S, Lv W, Bloszies S, Shi Q, Pan X, Zeng Y. Effects of fertilizer management practices on yield-scaled ammonia emissions from croplands in China: a meta-analysis. *Field Crops Research*. 2016;192:118–125. doi: 10.1016/ j.fcr.2016.04.023
51. Ke J, Xing X, Li G, Ding Y, Dou F, Wang S, Liu Z, Tang S, Ding C, Chen L. Effects of different controlled-release nitrogen fertilisers on ammonia volatilisation, nitrogen use efficiency and yield of blanket-seedling machine-transplanted rice. Field Crops Research. 2017;205,147–156. doi: 10.1016/j.fcr.2016.12.027
52. Liu T, Huang J, Chai K, Cao C, Li C. Effects of N fertilizer sources and tillage practices on NH_3 volatilization, grain yield, and N use efficiency of rice fields in Central China. Frontiers in Plant Science . 2018;9:385. doi: 10.3389/fpls.2018.00385
53. Ponnamperuma FN. Effects of flooding on soils. In: Kozlowski T, edotor. Flooding and plant growth. New York: Academic Press; 1984.
54. George T, Ladha JK, Buresh RJ, GarrityDP. Managing native and legume fixed nitrogen in lowland rice-based cropping systems. Plant and Soil. 1992;141: 69–91.
55. Narteh LT, Sahrawat KL. Influence of flooding on electrochemical and chemical properties of West African soils. Geoderma. 1999;87(3-4): 179–207.

56. Narteh LT, Sahrawat KL (2000) Ammonium in solution of flooded West African soils. Geoderma**.** 2000;95(3-4): 205–214.
57. Cassman KG, Peng S, Olk DC, Ladha JK, Reichardt W, Dobermann A, Singh U. Opportunities for increased nitrogen-use efficiency from improved resource management in irrigated rice systems. Field crops research. 1998;56(1-2):7-39. doi: 10.1016/S0378-4290(97)00140-8
58. Mi W, Gao Q, Xia S, Zhao H, Wu L, Mao W, Hu Z, Liu Y. Medium-term effects of different types of N fertilizer on yield, apparent N recovery, and soil chemical properties of a double rice cropping system. Field Crops Research. 2019;234: 87-94. doi.org/10.1016/j.fcr.2019.02.012
59. Murugan AV, Swarnam TP. Nitrogen release pattern from organic manures applied to an acid soil. Journal of Agricultural Science. 2013;5(6):174..doi:10.5539/jas.v5n6p174
60. Roldán A, Salinas-Garcia JR, Alguacil MM, Diaz E and Caravaca F. Soil enzyme activities suggest advantages of conservation tillage practices in sorghum cultivation under subtropical conditions*.* Geoderma. 2005;129(3-4):178–185. doi: 10.1016/j.geoderma.2004.12.042
61. Li W, Chen H, Cao C, Zhao Z, Qiao Y, Du S. Effects of long-term fertilization on organic carbon and nitrogen dynamics in a Vertisol in Eastern China. Open Journal of Soil Science. 2018; 8:99-117. doi: 10.4236/ojss.2018.83008
62. Chang EH, Wang CH, Chen CL, Chung RS (2014) Effects of long-term treatments of different organic fertilizers complemented with chemical N fertilizer on the chemical and biological properties of soils. Soil Science and Plant Nutrition. 2014;60(4):499-511. doi: 10.1080/00380768.2014.917333
63. Gao X, Han X, Zhan X, Sun Z, Jiang L, Chen H. Effect of long-term fertilization on total nitrogen storage in a brown soil. Plant Nutrition and Fertilizer Science. 2009;15(3):567-72.
64. Sahrawat KL. Nitrogen mineralization in lowland rice soils: the role of organic matter quantity and quality. Archives of Agronomy and Soil Science. 2010;56(3):337-53.
65. Pal B, Pati S, Badole S, MalothuV, Patra PK. Effect of integrated nutrient management on nitrogen dynamics in soil of rice-potato based cropping sequence. Journal of Applied and Natural Science. 2015;7(2): 652-655. doi: 10.31018/jans.v7i2.661
66. Myint AK, Yamakawa T, Kajihara Y, Zenmyo T. Application of different organic and mineral fertilizers on the growth, yield and nutrient accumulation of rice in a Japanese ordinary paddy field. Science World Journal. 2010; 5(2): 47-54. doi:10.4314/swj.v5i2.61513
67. Stark C, Condron LM, Stewart A, Di HJ, O'Callaghan M. Influence of organic and mineral amendments on microbial soil properties and processes. Applied Soil Ecology. 2007;35(1):79-93. doi: 10.1016/j.apsoil.2006.05.001
68. Kuntal MH, Anand S, Mishra B,Manna MC, WanjariRH,Mandal KG, Misra AK. Impact of long-term application of fertilizer, manure and lime under intensive cropping on physical properties and organic carbon content of an Alfisol. Geoderma. 2008;148:173–179. doi: 10.1016/j.geoderma.2008.09.015
69. Mujiyati M, Supriyadi S. Effect of manure and NPK to increase soil bacterial population of *Azotobacter*and*Azosprillus*in chili (*Capsicum annum*) cultivation. BioScience. 2009;1(2):59-64. doi: 10.13057/nusbiosci/n010202
70. Shrestha RK, Ladha JK (1996) Genotypic variation in promotion of rice dinitrogen fixation as determined by nitrogen-15 dilution. Soil Science Society of America Journal. 1996;60(6):1815-1821. doi: 10.2136/sssaj1996.03615995006000060029x
71. Ayuni N, Radziah O, Naher UAA, Panhwar QA, Halimi MS. Effect of nitrogen on nitrogenase activity of diazotrophs and total bacterial population in rice soil. Journal of Animal and Plant Sciences. 2015; 25(5):1358-1364.
72. Diaz-Marcote I, Polo A (1995) MSW compost for the restoration of degraded soil. In World Congress on Waste Management. Proc. of the 25th Anniversary of the

International Solid Waste Association. 15th-20th October, 1995 ISWA, Vienna (CD Rom).

73. Chakrabarti K, Sarkar B, Chakraborty A, Banik P, Bagchi DK. Organic recycling for soil quality conservation in a sub-tropical plateau region. Journal of Agronomy and crop Science. 2000 Apr;184(2):137-42. doi: 10.1046/j.1439-037x.2000.00352.x
74. Chang EH, Chung RS, Tsai YH. Effect of different application rates of organic fertilizer on soil enzyme activity and microbial population. Soil Science and Plant Nutrition. 2007;53(2):132-40. doi: 10.1111/j.1747-0765.2007.00122.x
75. Goutami N, Rani PP, Pathy RL, Babu PR. Soil properties and biological activity as influenced by nutrient management in rice-fallow-sorghum. International Journal of Agricultural Research, Innovation and Technology. 2015; 5:10-14. doi: 10.3329/ijarit.v5i1.24581
76. Dharmakeerthi RS, Thenabadu MW. Urease acitivity in soils: a review. Journal of the National Science Foundation of Sri Lanka. 1996;24:159-195. doi: 10.4038/jnsfsr.v24i3.5548