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# Effect of long term fertilization on soil respiration and enzyme activities in floodplain soil

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#### Abstract

In agricultural farming system organic manuring and inorganic fertilizer application are the most common agricultural practices. Different fertilizer application lead to differences in soil nutrients, pH, and microbial species, which in turn affect the transformation and decomposition of organic carbon by soil microorganisms. Therefore, a laboratory incubation study was carried out to investigate the influence of long term manuring and fertilization on soil respiration by means of C mineralization and enzyme activities. A parallel first- and zero-order kinetic model was used to describe observed C mineralization in soil. The annual carbon mineralization was found to be significantly influenced by different fertilizer. This result indicates that more stable organic matter was formed in NP treated soil which is less prone to decomposition if present crop management has been changed. Other ward, NP has the highest potentiality to soil for the purpose of carbon sequestration in floodplain soil compared to other fertilizer. Urease activities varied from 4.7µg NH4-N/g soil/2h in NK treatment to 25.7µg NH4-N/g soil/2h in N+FYM treatment. N treatment had a significantly higher urease activity compared to the respective controls. When P, K, S and PK applied separately with N then the treatments show low enzyme activity to control and other treatments (N, N+FYM and NPKSZn). There were no significant differences for Arylamidase activities among the treatments. The arylamidase activities decreased when S applied in combination with N. On the other hand arylamidase activity increased with the application of all other treatment.

Keywords: Carbon mineralization, urease enzyme, arylamidase enzyme and floodplain soil

#### Introduction

Soil health is influenced by soil organic carbon (SOC) (Arias *et al.* 2005) <sup>[3]</sup>. It contributes to soil fertility, soil sustainability and crop yield in agricultural ecosystems (Tian *et al.*, 2015; Wang *et al.*, 2015) <sup>[21, 23]</sup>. Even a small change in SOC storage can greatly affect atmospheric carbon dioxide (CO<sub>2</sub>) concentrations (Cheng *et al.*, 2013) <sup>[6]</sup>. Soil organic carbon (SOC) is a basal resource for microbial communities and many of the soil meiofauna, and nutrients essential for plant growth are released as it decomposes and mineralizes. It also influences soil physiochemical properties (Colloff and Baldwin 2010) <sup>[8]</sup>. Fertilizer application has been widely used as a common agricultural management strategy to promote soil carbon (C) sequestration (Silveira *et al.*, 2013; He *et al.*, 2015) <sup>[19, 13]</sup>, which could directly or indirectly increase the SOC inputs and thereby influence nutrient availability and soil turnover (Schmidt *et al.*, 2015) <sup>[26]</sup>. Integrated application of organic and inorganic fertilizer improves soil fertility and crop yield long-term (Alizadeh *et al.*, 2012; Fallah *et al.*, 2013; Fereidooni *et al.*, 2013) <sup>[2, 10, 11]</sup>.

There have been many investigations the effects of longterm fertilization on the physicochemical properties and microbial characteristics of soil (Li *et al.* 2004; Diepeningen *et al.* 2006; Chu *et al.* 2007; Ma *et al.* 2010) <sup>[15, 9, 7, 17]</sup>. Many studies reported that inorganic fertilizer application resulted in significant increases in SOC and its fractions due to the positive effects of the fertilizer on crop growth and in turn crop C return (Gong *et. al.*, 2009) <sup>[12]</sup>. However, in other studies, inorganic fertilizer application, either balanced or unbalanced, showed no significant effects on SOC or its fractions. Organic manuring, either alone or in combination with inorganic fertilizer, has been widely shown more effective in increasing SOC and its factions than inorganic fertilizer alone mainly due to the significant increase in C input with the manure application. Mandal *et al.* (2007) <sup>[16]</sup> reported that microbial biomass can be controlled long-term

by application of fertilizer and manure. Soil microorganisms are the driving force for the transformation and cycling of soil organic matter and nutrients, and also provide a reserve of available nutrients for crops (Xu *et al.* 2016)<sup>[24]</sup>.

Population is increasing day by day and agricultural land is gradually decreasing. So, the importance of fertilization and manuring of our land is increasing day by day and farmers are supposed to use these inputs intensively for sustained crop production. Intensive use of fertilization and manuring undoubtedly bring some changes in the physiochemical properties and biological properties of soil. Therefore an experiment was carry out to understand the long term fertilization effect on soil respiration and enzyme activities in floodplain soil.

#### **Materials and Methods**

The long term experimental soil used in this study was established in 1978 at the Department of Soil Science, Bangladesh Agricultural University (BAU), Mymensingh (24°43′ N, 90°25′ E), Bangladesh on loamy, mixed, nonacid Aeric Haplaquepts (Soil Taxonomy) developed from old Brahmaputra alluvium. The soil texture was silt loam (19, 63 and 18% sand, silt and clay, respectively). The treatments all have ayearly boro rice-fallow-aman rice cropping pattern and included an unfertilized control, six treatments with application of mineral fertilizer (control, N, NP, NS, NK, NPK, 50%N+FYM and NPKSZn) and one treatment with application of mineral N and farmyard manure (50%N+FYM). The application rates of N, P, K, S, and Zn per crop were 90, 20, 19, 30, and 5 kg ha<sup>-1</sup>, respectively applied as urea, triple superphosphate, muriate of potash, gypsum, and zinc oxide, respectively. Cowdung mixed with rice straw was applied as

farmyard manure at a rate of 5 Mg ha<sup>-1</sup> fresh material and once a year. Chemical fertilizers were applied to every crop and were incorporated to soil during final land preparation preceding rice transplantation, except for N which was applied as basal-dressing and two times as top-dressing in three equal doses. The experiment was conducted in a randomized block design with three replicate plots (12mx6m). The cropping pattern was rice-fallow-rice as high yielding Boro rice and high yielding T. Aman rice.

Soil samples were collected from long-term field experiments in May-July 2016. Surface soil samples (0-15 cm) were collected from 15 locations per replicate plot by means of an auger ( $\emptyset$  2.5cm). These samples were bulked into one composite sample and were thoroughly mixed. The field moist soil was gently broken apart by hand and was air-dried and ground to pass a 2-mm sieve prior to soil incubations, determination of soil properties and other studies.

#### Carbon and nitrogen analysis of the soils

The total carbon and nitrogen content of soil samples were analysed with a Variomax CNS-analyzer (Elementar Analysesysteme, Germany). Sub-samples of 0.8 g were used for analysis. The CNS analyser works according to the principle of catalytic tube combustion under excess oxygen supply and high temperatures (850 °C to 1150 °C).

#### **Carbon mineralization**

Carbon mineralization was studied in 2011 in an aerobic incubation experiment carried out in a closed system. For the  $CO_2$  determination, small vials containing 10 ml 1 M NaOH solution were placed in the jars to trap the evolved  $CO_2$ 



Fig 1: Scheme of an incubation jar

The jars were closed with air-tight seals and incubated at 25 °C temperature for104 days. The samples were incubated in three replications including two blank treatment (no soil added) in order to account for the  $CO_2$  present in the air. Decomposition was monitored during incubation period, as  $CO_2$  evolution. The excess alkali was back-titrated with standard 1M HCl after precipitating the carbonate with the presence of BaCl<sub>2</sub> (Anderson, 1982). The following reaction takes place when the

NaOH-solution is titrated: 2 NaOH + CO  $_2 \leftrightarrow$  Na  $_2$ CO  $_3 +$  H $_2$ O

#### Modeling of OC mineralization

Now a day's incubation studies are also widely used to measure the stable organic matter fraction of OM additives which is defined as the fraction of the organic matter that remains in soil one year after addition. Therefore, A parallel first- and zeroorder kinetic model (e.g. Saviozzi *et al.*, 1997; Van Kessel *et al.*, 2000)<sup>[22]</sup> was used to describe observed C mineralization in soil. The parallel first- and zero-order kinetic model assumes that the SOM consists of an easily mineralizable pool of C that is mineralized exponentially according to first-order kinetics, and a more resistant fraction that is mineralized according to zero-order kinetics (Van Kessel *et al.*, 2000)<sup>[22]</sup>, i.e. it is assumed that the resistant fraction is not depleted significantly during the incubation period considered:

$$C(t) = C_{A,f} \{ 1 - \exp(-k_f t) \} + k_s t$$

where C(t) is the cumulative amount of C mineralized at time t,  $k_f$  is the mineralization rate constant of the easily degradable carbon pool  $C_{A,f}$ , and  $k_s$  is the mineralization rate constant of the resistant pool.

#### **Enzyme Activity**

The enzyme activities of urease and arylamidase were measured from one week incubated (at 27 <sup>o</sup>C) soil with 60% water filled pore space and field bulk density. Urease activity was determined according to Tabatabi and Bremner (1972) <sup>[20]</sup>. Arylamidase activity was measured in soil according to Acosta-

Martinez and Tabatabai (2000)<sup>[1]</sup>.

#### **Statistical Analysis**

Means separation analysis was used to calculate parameters from cumulative C mineralization data in SPSS. One-way ANOVA with Duncan's multiple range post-hoc tests was used for statistical analysis. Correlation study was also performed between soil parameters, C mineralization parameters, enzyme activities and previously collected crop yields. All statistical analyses were carried out with SPSS 17.

#### **Results and Discussion** Carbon mineralization

Cumulative C-mineralization in soil showed an exponential course nearly one month at the beginning of the incubation followed by a linear course. Fig. 2 shows measured- C mineralization -as a function of incubation time and the fitted parallel first and zero order kinetic model for the different doses of fertilizer and manure application. In general the  $R^2$  values were all close to 1 and standard errors were very low, which shows that the selected model could describe the mineralization process satisfactorily.



Fig 2: Cumulative C-mineralization expressed as percentage of the total SOC for different fertilizer and manure application in soil.

The cumulative carbon mineralization was found to be influenced by different fertilizer application. The cumulative C mineralization expressed as percentage of the total SOC varies from 4.42 to 5.88 (g C/100g soil C) between different fertilizer and manure treatments. Our result closely match with the results of Zheng *et al* (2006) <sup>[27]</sup> who reported that that C mineralization varied between 3.8 and 5.6 g C/100g C in long term experimental soil in Jiangxi Province, China with rice-rice cropping pattern under different fertilizer and manorial treatments.

The highest C mineralization value was observed for NPKSZn (i.e.5.88 g/100g soil C) and the lowest in NP (4.42 g/100g soil C) (Fig. 3.1). There was a significant difference in annual C mineralization between NPKSZn, N and NP treatments. However, the cumulative C mineralization was statistically similar between control, NS and N+FYM treated soil. This result

indicates that more stable organic matter was formed in NP treated soil which is less prone to decomposition if present crop management has been changed. Other ward, NP has the highest potentiality to soil for the purpose of carbon sequestration in floodplain soil compared to other fertilizer. Our result is in contrast with Zheng *et al* (2006) <sup>[27]</sup> who reported a higher C mineralization in NP and NPKM treatments accounting 4.4 and 5.6 g C/100g C compared with control, N and NPK treatments accounting 3.8, 4.2 and 4.1 g C/100g C, respectively.

Parameters of a parallel first and zero order kinetic model fitted to these mineralization data are given in Table 1.Three parameter were estimated namely CAf (easily mineralizable C pool expressed in percentage), kf (mineralization rate constant of the easily degradable carbon pool) and ks (mineralization rate constant of the stable or resistant carbon pool). 

 Table 1: Estimated parameters of a fitted parallel first and zero order kinetic model. (Values in parentheses are standard deviation on parameter estimates).

Treatments	Parameters			
	CA.f (%)	Kf (%day-1)	Ks (%day <sup>-1</sup> )	<b>R</b> <sup>2</sup>
Control	1.919(0.06)	0.130(0.01)	0.018(0.00)ab	0.994(0.002)
Ν	1.827(0.08)	0.131(0.01)	0.017(0.00)bc	0.996(0.001)
NP	1.818(0.02)	0.116(0.01)	0.015(0.00)c	0.997(0.001)
NS	1.863(0.01)	0.104(0.00)	0.016(0.00)bc	0.998(0.001)
NK	1.867(0.11)	0.121(0.01)	0.019(0.00)ab	0.996(0.001)
NPK	1.824(0.15)	0.116(0.01)	0.019(0.00)a	0.997(0.000)
N+FYM	1.839(0.04)	0.120(0.02)	0.018(0.00)ab	0.995(0.002)
NPKSZn	1.819(0.12)	0.116(0.01)	0.020(0.00)a	0.996(0.001)
ANOVA	NS	NS	**	*
**, * Significant at P≤0.01 and P≤0.05, respectively; NS, not significant. Treatment indicated by different letters are statistically different				
(P < 0.05) from each other according to Duncan's multiple range post hoc test.				

#### **Organic C mineralization**

The mineralization experiment was done for 104 days under laboratory condition at 25 °C. Therefore, the data were recalculated and extrapolated to one year at 25 °C, which is the mean annual temperature of Bangladesh. Cumulative annual C mineralization evolved from SOM (expressed as mg C 100 g<sup>-1</sup> soil) under field conditions were varied from 6.21 to 9.31% of total soil organic carbon. Among the different fertilizer treatments, NPKSZn treated soil had the highest annual C mineralization whereas NP fertilizer had the lowest annual C mineralization. The annual carbon mineralization was found to be significantly influenced by different fertilizer application (Fig.2). There was a significant difference in annual C mineralization between NPKSZn, N and NP. However, the annual C mineralization was statistically similar between control, NS and N+FYM treated soil. This result indicates that more stable organic matter was formed in NP and NS treated soils which are less prone to decomposition if present crop management has been changed. Other ward, NP and NS have the highest potentiality to soil for the purpose of carbon sequestration in floodplain soil compared to other fertilizer treatment.



Fig 2: Annual C mineralization rates (%) as influenced by repeated application of fertilizer and manure in floodplain soil.

# Enzyme Activities

## Urease enzyme Activities

Urease enzyme activities were determined from one week preincubated soils. There were significant differences in urease activities among the treatments. Urease activities varied from  $4.7\mu g$  NH4-N/g soil/2h in NK treatment to 25.7  $\mu g$  NH4-N/g soil/2h in N+FYM treatment (Fig. 3). The urease activity was significantly higher in N+FYM and NPKSZn treatments compared with other fertilizer treatments and control. The urease activities decreased when P, K, S and PK applied in combination with N. Manure amended soil recorded the highest urease activity compared to inorganic N in soil. Bhattacharyya *et al.* (2005)<sup>[4]</sup> reported that addition of organic manures increased the urease activity over mineral N and control to the significant extent. Low level of urease activity in fertilizer treated soil indicated that mineral N without sufficient amount of available organic substrate may not have impact on urease activity (Zaman *et al.*, 2008)<sup>[25]</sup>.



Fig 3: Urease enzyme activity as influenced by repeated application of fertilizer and manure in floodplain soil.

#### Arylamidase enzyme Activities

Arylamidase enzyme activities were also determined from one week pre-incubated soils. There were no significant differences for Arylamidase activities among the treatments (Fig. 4). The arylamidase activity was substantially high in control, N and NP treatments. The arylamidase activities decreased when S applied in combination with N. On the other hand arylamidase activity increased with the application of all other treatment. We know that when S fertilizer apply in soil, it increase acidic condition that's why in NS treated soil arylamidase activity decrease. Whereas the combination of N with P, K, Zn and FYM decrease acidic condition and enhanced microbial activity.



Fig 4: Arylamidase enzyme activity as influenced by repeated application of fertilizer and manure in floodplain soil.

#### Conclusion

The C mineralization rate constant for resistant carbon pool (ks) was significantly influenced by fertilizer application. For fertilization, on an average the highest ks value was estimated for NPKSZn application and the lowest in NP. The annual carbon mineralization was found to be significantly influenced by fertilizer and manure application. There was a significant difference in annual C mineralization between NPKSZn, N and NP. This result indicates that more stable organic matter was formed in NP treated soil which is less prone to decomposition if present crop management has been changed. Other ward, NP has the highest potentiality to soil for the purpose of carbon

sequestration in floodplain soil compared to other fertilizer. Long term fertilization in soil would result to variable properties of soil enhanced the microbial activity and properties in soils which may be favorable to sustained soil productivity and soil health.

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