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# Response of hydrolytic enzyme activities and nitrogen mineralization to fertilizer and organic matter application in subtropical paddy soils

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ABSTRACT

Drivers of nitrogen (N) mineralization in paddy soils, especially under anaerobic soil conditions, are elusive. The influences of exogenous organic matter (OM) and fertilizer application on the activities of five relevant enzymes (β-glucosaminidase, β-glucosidase, L-glutaminase, urease and arylamidase) were measured in two long-term field experiments. Of the two field experiments, the 18-year field experiment was established in a weathered terrace soil with rice-wheat crop rotation at the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) farm with five OM treatments and two levels of mineral N fertilizer. The 30-year experiment was established in a young floodplain soil with rice-rice crop rotation at the Bangladesh Agricultural University (BAU) farm with five mineral fertilizer treatments including one with farm yard manure. At BSMRAU, N fertilizer and OM amendments significantly increased all enzyme activities, suggesting the availability of primarily substrate for microbial activity. Whereas at BAU, non-responsiveness of β-glucosidase activity, suggesting that fertilizer and OM amendments had little effect on overall soil microbial activity. Nevertheless the microbial demand for N, β-glucosaminidase and L-glutaminase activities differed among the treatments ($P < 0.05$) and showed opposite trends with soil N mineralization. Hence enzymatic pathways to acquire N differed with the treatment at BAU site, indicates differences in soil N quality and bio-availability. L-glutaminase activity was the sole investigated variable that positively correlated to both the aerobic and anaerobic N mineralization rates in both field experiments. Combined with a negative correlation between β-glucosaminidase activity and N mineralization rate, it appears that terminal amino acid $NH_2$ hydrolysis was a rate-limiting step for soil N mineralization at the BAU site. Future investigations with joint quantification of polyphenol accumulation and binding of N alongside an array of extracellular enzymes, including oxidases for phenols and hydrolases for N-compounds, would enable verification of the hypothesized binding and stabilization of N with accumulating polyphenols at BAU site under SOM storing management.



## 1. Introduction

A better understanding of the factors controlling nitrogen (N) mineralization in paddy soils and development of practical indicators of soil N supply is essential to improve N-use efficiency in South-east Asian paddy rice production, thereby reducing the application of relatively expensive N fertilizers. Several biological and chemical methods have been proposed as N mineralization indices [1,2]. However, limited progress has been made on reliable prediction of paddy soil N mineralization. In our previous work, basic soil properties as well as an array of physicochemical soil organic matter (SOM) fractions have been tested for the prediction of potential N mineralization from Bangladeshi paddy soils under laboratory incubations [3–5]. While some of these soil variables

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correlated with the potential aerobic N mineralization rate, relationships with anaerobic N mineralization were mostly insignificant or negative. Hence it was inferred that neither SOM quantity, nor SOM quality dominantly determine the anaerobic N mineralization process. A multitude of other biotic or abiotic constraining factors, which are not expressed in readily measurable soil variables or SOM fractions, could control the anaerobic N mineralization in paddy soils.

As organic N mineralization is mediated by microbial extracellular enzymes, assays of their activity should provide insight into key intermediate soil biochemical processes [6] and if successful, could be used as sensitive N mineralization indexes. Enzyme activities are the end result of the interaction of SOM biochemistry and physical soil conditions, both being shaped by management. The activities of urease and L-asparaginase and therefore the soil amidohydrolase activities in general have the potential to evaluate mineralizable N [7]. Tabatabai et al. [6] proposed N-acetyl-ß-D-glucosaminidase activity as an index of soil N mineralization among six amidohydrolases enzymes involved in N cycling and four glycosidase enzymes involved in carbon cycling in soils. However, such conclusions are not yet supported for flooded paddy soils by the lack of experimental data.

Specific practices, *i.e.*, wet cultivation, puddling, alternate wetting and drying make paddy soils distinct in physical, chemical and biological properties and role of enzymes to more frequently studied soil types. The present study considered five relevant eco-enzymes, covering initial and terminal steps in organic matter and N mineralization and urea hydrolysis. β-glucosaminidase and arylamidase are selected as representatives for extracellular enzymatic breakdown of complex organic N compounds into amides, amino sugars, and amino acids (aminization). This is assumed to be an initial rate-limiting step in soil N mineralization [8]. L-glutaminase was selected as representative for an array of enzymes involved in the production of $NH_4^+$ from amino acids through ammonification [9]. Microbial nutrient demand is determined by the elemental stoichiometry of microbial biomass in relation to environmental nutrient availability [10]. β-glucosidase activity, involved in cellobiose hydrolase and carbon (C) and energy supply, was therefore included as well, as this allowed further evaluation of the eco-enzymatic C:N-ratio as an indication of the tendency of microbial activity to be determined for nutrient or energy flow. Lastly, urease is an enzyme that catalyses the hydrolysis of urea, hydroxyurea, dihydroxyurea and semicarbazid into $CO_2$ and $NH_3$ [11]. The principal aim of this study was to evaluate the relative control of these selected enzymatic steps on aerobic and anaerobic N mineralization in young floodplain paddy soils. We interpret strong correlations between N mineralization rate and enzyme activity as likelihood that the mediated OM-transformation step would be limiting N mineralization. The secondary aim was to elucidate if enzyme activity was either determined by product demand or by substrate availability. It was hypothesized that: N fertilizer addition would reduce differences in enzyme activities between exogenous OM treatments due to a lifting of product demand, *in casu* mineral N; exogenous OM application would promote activity of all enzymes because of a generally enhanced substrate availability; a similar effect in mineral fertilizer treatments that would promote crop growth, and logically larger OM inputs from root exudation and incorporation of crop residues; and a higher demand for mineral N in treatments with exogenous OM with higher C:N ratio to result in a specific promotion of the activity of hydrolytic enzymes mediating N-transformations relative to β-glucosidase.

## 2. Materials and methods

### 2.1. Site description and soil sampling

Soil samples were collected from two long-term field experiments. One experiment was established in 1989 at the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) farm at Salna (24°05′ N, 90°16′ E), Bangladesh on a clayey, kaolinitic, Ultic Ustocrept soil [12] developed from Madhupur clay. The soil texture was silty clay loam (15:46:39) [13]. A yearly Wheat (variety *Akbar*)-Fallow-Transplanted rice (variety *T. Aman*) cropping pattern was practised. The BSMRAU field trial involved five OM application treatments (1° no-application, 2° rice straw (air dry) at 2 Mg ha$^{-1}$, 3° green manure at 7.5 Mg ha$^{-1}$ fresh biomass of *Sesbania rostrata*, 4° compost at 25 Mg ha$^{-1}$ made from cow dung and rice straw, and 5° cow dung at 25 Mg ha$^{-1}$ as fresh manure). These were combined with 2 levels of inorganic N fertilizer amendments (0 and 100 kg N ha$^{-1}$ for rice and 0 and 120 kg N ha$^{-1}$ for wheat). Triple super phosphate (TSP) was applied at 44 kg P ha$^{-1}$ for both crops and muriate of potash (KCl) was applied at 55 and 45 kg K ha$^{-1}$ for rice and wheat, respectively, during the final land preparation. The experiment was laid out in a 5 × 3 factorial design with replications. The dimension of each plot was 12 m × 7 m with a plot-to-plot distance of 1.5 m. Details on the agronomic management can be found in Ref. [13].

The second field experiment was established in 1978 at Bangladesh Agricultural University (BAU) farm at Mymensingh (24°43′ N, 90°25′ E), Bangladesh on a loamy, mixed, non-acidic Aeric Haplaquept [12], developed from old Brahmaputra alluvium. The soil texture was silt loam (19:63:18). The treatments all had a yearly Boro rice (irrigated winter rice transplanted on mid-January and harvested mid-May)-Fallow-T. Aman rotation and included treatments with application of mineral fertilizer (control, N, NP, NPK) and one with application of mineral N and farmyard manure (N+FYM). F The application rates of N, P, K, S, and Zn per crop were 90, 20, 19, 30, and 5 kg ha$^{-1}$, respectively applied as urea, triple super phosphate, potassium chloride, gypsum, and zinc oxide. Cow dung was mixed with rice straw applied once a year 10–15 days prior transplantation of Boro rice at a rate of 5 Mg ha$^{-1}$ fresh material. The experiment was conducted in a randomized block design with three replications (12 m × 6 m). Details on the agronomic management can be found in Ref. [13].

Surface soil samples (0–15 cm) were collected from 15 locations per replicate plot by means of a 2.5 cm inner diameter auger in May 2008 at BSMRAU, and in July 2008 at BAU. These samples were bulked into one composite sample and thoroughly mixed. The field moist soil was gently broken apart by hand and air-dried and ground to pass a 2-mm sieve prior to the assessment of N mineralization, and enzyme activities.

### 2.2. Nitrogen mineralization

Fourteen-week laboratory incubations were carried out to determine both aerobic and anaerobic N mineralization rates, as described in detail by Kader et al. [4]. Three replicate plot soils per treatment at BAU and two replicate plot soils per treatment at BSMRAU were used to quantify potential N mineralization. In total 42 tubes were filled for each BAU treatment such as 21 tubes (3 replicates × 7 dates) for aerobic and 21 tubes for anaerobic incubation; and 28 tubes per BSMRAU treatment such as 14 tubes (2 replicates × 7 dates) for aerobic and 14 for anaerobic incubation. Removal of mineral N from soil solution by denitrification or immobilization was not considered and only net N mineralization was measured in the incubation experiments. The net aerobic N mineralization data were best described by a zero-order kinetic