Assessing the influence of rice roots and root exudates on nitrogen mineralization in soil using a novel protocol

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Abstract
Classical nitrogen (N) mineralization experiments are done using uncropped soil, thus completely neglecting the influence of roots and root exudates. Therefore, experiments were conducted at two field sites in Bangladesh during 'boro' season (winter rice), using two rice cultivars (BRRI Dhan 29 and BINA Dhan6) to investigate the influence of rice roots and root exudates on N mineralization. Rice cultivars were transplanted in three replicated plots maintaining 25 x 15cm spacing along with three replicated uncropped plots as control. A novel method was used to identify the most suitable location to assess N mineralization in soil having actively growing rice plants. For this purpose, soil samples were collected from three locations in soil namely; 1) rhizosphere (0cm, at the rhizosphere), 2) middle of the two plants (7.5cm apart from rhizosphere) and 3) middle of two rows (12.5cm apart from rhizosphere). There was significant stimulatory effect of rice roots and root exudates on N mineralization at both filed sites. Significant influences of rice varieties were also observed, with BINA Dhan 6 having greater influence on N mineralization than BRRI Dhan 29. Sampling location also had a significant effect on measured N mineralization. The highest stimulatory effects of rice roots and root exudates were recorded when soil was sampled from rhizosphere. Sampling between the plants and between the rows had similar effects. In conclusion, rice roots and root exudates had a large influence on N mineralization and the best sampling location to determine the effects of actively growing rice roots and root exudates on N mineralization, was the rice rhizosphere.

Key words
Nitrogen mineralization, paddy soil, rice rhizosphere, root and root exudates

Introduction
Plant roots influence the biological, chemical and physical properties of rhizosphere soil. These effects are a consequence of their growth, their activity and the exudation of organic compounds from them. In natural ecosystems, the linkages between inputs of carbon from plants and microbial activity driven by these inputs are central to our understanding of nutrient cycling in soil and the productivity of these systems. This coupling of plant and microbial productivity is also of increasing importance in agriculture, where a shift towards low input systems increases the dependence of plant production on nutrient cycling, as opposed to fertilizers. Rhizosphere processes play a key role in nutrient cycling in terrestrial ecosystems. The compounds released into the soil by roots are broadly referred to as root exudates. Through the exudation of the wide variety of compounds, root may regulate the soil microbial community in its immediate vicinity, cope with herbivores, encourage beneficial symbioses, change the chemical and physical properties of the soil and inhibit the growth of the competing plant species (Nardi et al., 2000). Rhizosphere microbial population densities are usually an order of magnitude higher than those found in bulk soil (Anderson et al., 1993).Microbial communities in soils are a key influence in regulating the dynamics of organic matter decomposition and the availability of plant nutrients such as nitrogen, phosphorus and sulphur (Paterson, 2003). The addition of representative amounts of three common low molecular weight organic substances (LMWOS) (organic acid, glucose and amino acid) into a simulated rhizosphere of a paddy soil showed that locally high concentrations of LMWOS (artificial root exudates) stimulate microbial biomass and soil organic matter decomposition (Begum *et. al.,* 2012).

Mineralization of native soil N is of special interest particularly in low-input agriculture that is practiced in large parts of South-east Asia, as it provides 20–80% of the N required by plants

(Broadbent, 1984). Lack of knowledge on N mineralization process from soil organic matter in South-east Asian paddy soils leads farmers to meet this N requirement exclusively by costly mineral fertilizers, which have typically an efficiency of less than 40% (Kader et al, 2013). The mineralization process and the availability of mineral N in soils are affected by various environmental factors and management practices, including the presence of growing plants. Root systems of growing plants are believed to exert considerable influence on soil microbial populations and activities through the release of various organic substrates, and thereby affect mineral N availability (Merckx et al., 1987). Gross rates of N mineralization in rhizosphere soil were found about 10 times higher than in bulk soil (Herman et al., 2006). Therefore, sophisticated management practices, based on better understanding of carbon (C) and N turnover processes in the soil, are required to maximise crop yields and quality, whilst simultaneously minimising negative environmental impacts of N fertilisation (Vance, 2001).To date, very little is known on the influence of root growth and rhizodeposition of root exudates on N turnover in paddy rice soils. Indeed, N mineralization is commonly studied in the laboratory using uncropped soil, thus completely neglecting the influence of roots and root exudates. In this research, we explicitly take into account the influence of root and root exudates of rice by using a novel sampling technique.

Methodology

Collection and measurement of rice root exudates

To quantify the rice root exudates, two rice cultivars BRRI Dhan 29 & BINA Dhan 6 were sown on sterile, organic matter-free cleaned sand and germinated at 37°C. Four days old rice seedlings were transplanted @ three seedlings per PVC plastic pot (15 cm internal diameter and 13 cm in length) containing 1.2 kg sterile washed sand. Sand was first washed with NaOH (5%) followed by distilled water and then again washed with HCl acid (0.5M) followed by several (about 10 times) washing with distilled water until the pH reached~pH 6.6. After that, the sand was sterilized in an oven at 550°C. PVC pots were filled with sand and a nylon filter was placed below the sand at the bottom of the pot covering the mouth of the outlet that was used for collecting root exudates. The experiment had four replicates with two treatments and was conducted in a rice growth room at the department of plant production, Ghent University, Belgium. The room was maintained at a day temperature of 28°C, 16/8 hour day/night duration, relative humidity of 72-80%, and 350 μmol m^{-2} s^{-1} light intensity. After transplanting, sand was kept well saturated with standard Hoagland's nutrient solution. After addition of nutrient solution, the top of the plastic pots were covered with aluminum foil to protect evaporation and algal growth. Root exudates were collected every week till 95 days through a leaching procedure as described by Kuzyakov and Siniakina (2001) with slight modification. After first leaching, the sands were subsequently leached 2-3 times with distilled water (around 100 ml each time). Immediately after collection, the leachates were filtered through a 0.45 μm membrane filter to remove root detritus and microbial cell debris and then frozen and subsequently freeze-dried. Rice plants were carefully removed from the pots at 95 days and the sand around the roots was washed off with gentle water spraying for several times and shoot and root samples were oven dried at 65°C.The dried solid residues, shoot and root samples were analyzed for the total C and N content using Variomax CNS-analyzer.

Nitrogen mineralization from soil organic matter from cropped soils

Field experiments were conducted at two sites in Bangladesh, namely the BAU farm (loamy, mixed, non-acid Aeric Haplaquepts with 2.14% organic carbon) and Sutiakhali (clayey, mixed, non-acid Aeric Haplaquepts having 1.62% organic carbon) during the 'boro' season (winter rice). Two rice cultivars, namely BRRI Dhan 29 and BINA Dhan 6 were used as test crop. Around 45 days old rice seedlings were transplanted at three seedlings $hill^{-1}$at both sites at the end of January using three replicated plots of $25m^2$ (5m x 5m) maintaining a row to row distance of 25cm and plant to plant distance of 15 cm. Prior to the transplantation, all fertilizers except N were applied as basal @ 20, 50, 10 and 1.5 kg P, K, S and Zn ha^{-1}respectively, during final land preparation in both sites. Three replicated uncropped plots of $25m^2$ were also included as control. Similar doses of fertilizers were also applied in uncropped plots. Weeds were removed by hand picking throughout the experiment at regular intervals both from cropped and uncropped plots to eliminate other effects of N mineralization except from rice roots and root exudates. Soil samples for measurement of mineral N were collected in each replicate using a small augur from both cropped and uncropped plots at two weeks interval throughout the rice growing season. In case of cropped plots, soil samples were collected from three locations namely;1)sampling from rhizosphere (0 cm, at the rhizosphere), 2) sampling the middle of the two plants (7.5cm apart from rhizosphere) and 3)sampling middle of two rows (12.5cm apart from rhizosphere) with three replications to determine the most suitable location for N mineralization under actively growing rice. The soil was mixed thoroughly and 15 g moist soil was extracted with 0.01N $CaCl_2$ and 1M KCl (1:50; soil weight (g): extractant volume (ml) solution as described by Kader et al (2013). The $CaCl_2$ extracts were analyzed for NO_3^--N concentration by spectrophotometer at a wavelength of 210 and 275 nm based on Goldman & Jacobs (1961) as modified by Kader *et al.* (2013). The KCl extracts were analysed for NH_4^+-N by Indophenol blue method using spectrophotometer at a wavelength of 636 nm.

Results

Shoot and root biomass, shoot to root ratio, shoot, root and root exuded carbon content of BINA Dhan 6 were found to be higher than for BRRI Dhan 29 (Table 1). Shoot biomass and shoot C content of BINA Dhan 6 were found significantly higher than the BRRI Dhan 29($P<0.05$). Between this two rice varieties, BINA Dhan 6 exuded 1.18 ± 0.07 mg C day^{-1} which was slightly higher than the BRRI Dhan 29 (1.12 ± 0.06 mg C day^{-1}). However, on a relative basis, higher C was exuded from BRRI Dhan 29 (0.99 ± 0.18 % of total biomass C) than BINA Dhan6 (0.76 ± 0.08 % of total biomass C).

Table 1. Shoot and root biomass, shoot to root ratio, shoot, root and root exuded carbon content of two rice varieties cultivated under control environment

Rice varieties	Biomass (g $hill^{-1}$)		Shoot : root	Carbon (g $hill^{-1}$)		Root exuded C (mg $hill^{-1}$)	Root exudation rate (mg C $hill^{-1} day^{-1}$)
	Shoot	Root		Shoot	Root		
BRRIdhan 29	26.12	4.15	6.9	9.68	1.40	106.4	1.12
BINAdhan 6	34.89	4.45	8.7	13.26	1.53	112.2	1.18
t-value	5.43	0.64	2.67	6.11	0.96	1.30	1.33
P-value	*0.01*	*0.56*	*0.07*	*0.01*	*0.41*	*0.28*	*0.28*

There was significant stimulatory effect of rice roots and root exudates on N mineralization over the two sampling sites (Fig 1). Higher N mineralization was measured under rice growing condition compared to control soil when sampled from the rhizosphere, particularly at the early growth stage of rice at both sites. Growing rice plants may also have influenced N content in soil through root uptake of mineralized N at the later growth stage, leaving less available N in the soil, particularly at the Sutikhali site. Indeed, this soil was less fertile having lower organic matter content and initial available N. There was also a significant effect of sampling location on measured N mineralization. The highest stimulatory effects of rice roots and root exudates were recorded when soil was sampled from rhizosphere. Sampling between the plants and between the rows had similar effects. There was also a significant influence of rice varieties on N mineralization, especially at the BAU site. Roots and root exudates of BINA Dhan 6 rice had greater influence on N mineralization than BRRI dhan 29 (Fig 1). This result closely matched with the higher root biomass and root exudation of BINA Dhan 6 (Table 1).Though the stimulatory effect varied between sites, however, they followed a similar trend.

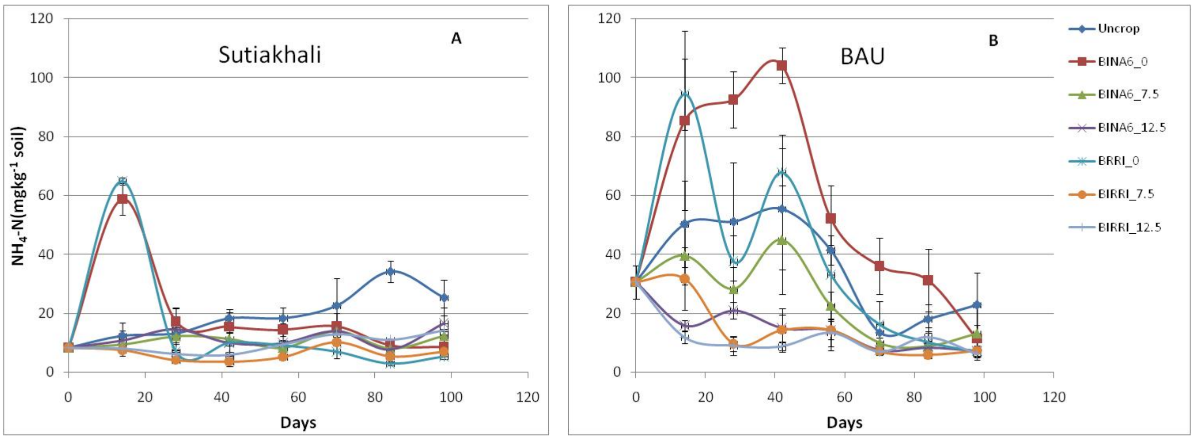

Figure 1. Presence of mineral N in soils incubated with and without rice crop under field condition at Sutiakhali (A) and BAU farm (B)

Conclusion

Actively growing rice root and root exudates has a stimulatory effect on N mineralization in paddy soil. The highest stimulatory effects of rice roots and root exudates were recorded when soil was sampled from rhizosphere compared to sampling between the plants and between the rows. Therefore, it could be recommended that the rice rhizosphereis the best location for sampling soil to determine the effects of actively growing rice roots and root exudates on N mineralization.

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