

Soil microbial biomass nitrogen in relation to plant nitrogen uptake under paddy soils

EI-Sharkawi, H. M.

Central Laboratory for Agricultural Climate (CLAC), Agricultural Research Center (ARC), Egypt, Dokki 12411, Giza-Egypt

ABSTRACT

Soil microbial biomass is considered to be an important N pool in soil–plant N dynamics in terrestrial ecosystems. This study was carried out at the Rice Research and Training Center during 2007 and 2008 rice growing seasons to evaluate the potential use of these microorganisms as a natural bionitrogen source under two paddy soils. Three nitrogen sources i.e. rice straw compost, sludge and Urea were used under fresh and autoclaved soils with the rice variety Giza 177 in a pot experiment. Results obtained showed that the pot treated with organic matter recorded the maximum value of total N uptake and microbial biomass nitrogen (MBN) uptake, followed by Urea treated pots. While, pots amended with straw exhibited a lower microbial N forming ability than those amended with sludge under both soils. Nitrogen mineralization (NH_4) rate was significantly affected by nitrogen and microbial N sources. The ammonium concentration increased with straw compost application, which was delaying the utilization of microbial N sources in the rice culture. The results showed that MBN uptake and proportion of plant nitrogen derived from microbial nitrogen sources (P_{fix}) were under fresh soil treatment found to be higher than autoclaved soil treatment in both soils. A positive correlation was found between the P_{fix} and the total N in the shoot under both soils for the rice variety under study. The results also showed that microbial biomass N was governed not only by the soil type but also by the type of the nitrogen source. The addition of sludge to fresh soil increased total MBN uptake and consequently could be indirectly beneficial to rice production especially in poor soils. Thus, it can be concluded that soil microbes contribute to plant growth by serving as a N source during the rice growing season.

Keywords: nitrogen source, soil microbial biomass nitrogen, paddy soil

INTRODUCTION

Maximizing microbial biomass nitrogen (MBN) in the soil offers a potential approach to improving the production efficiency of soil. Recycling of microbial N in the soil occurs as a result of degradation of microbial biomass (Higa and Wididana, 1991 and Higa, 1995). Billions of organisms inhabit the upper layers of the soil, where they break down dead organic matter, releasing the nutrients necessary for plant growth. The micro-organisms include bacteria, actinomycetes, algae and fungi. Each group plays an important role in the soil ecosystem and can assist the organic farmer in producing a healthy crop. Microorganisms can be grouped according to their function: free-living decomposers convert organic matter into nutrients for plants and other microorganisms, rhizosphere organisms are symbiotically associated with the plant roots and free-living nitrogen fixers (Kennedy and Nelson, 1982; Saralov and Babanazarov, 1983; Yoneyama *et al.*, 2002; Hubbell and Gerald 2003 and El Sharkawi, *et al.*, 2006). Plant roots leak or

exude a large number of organic substances and continually slough off root caps into the soil. These materials are food for many micro-organisms living in a zone of intense microbial activity near the roots called the rhizosphere. Bacteria benefit most from the food supplied in the rhizosphere and may form a continuous film around the root. Other micro-organisms liberate nutrients from the clay and humus colloids (a colloid is a mass of fine particles) (Yoneyama *et al.*, 2002 and El Sharkawi *et al.*, 2006).

There is great diversity in the metabolic types of free-living microorganisms which are capable to extract the nitrogen from organic and soil substances and then release it (microbial nitrogen) to the soil pool as one of available nitrogen for plant nutrition in soil. Soil nitrogen pool is naturally maintained through the microbial activity (Roger and Ladha, 1992; Pilar, *et al.*, 2001). Sustainability of N cannot be considered in isolation; it must be considered holistically within the framework of system sustainability. With the current emphasis on the use of renewable resources for environmentally sustainable development, it is timely to assess the potential of microbial N to complement fertilizer inputs and to consider their contribution to rice plant under flooding condition. The capacity of soil micro-organisms under flooded rice conditions should be improved to maintain and enhance rice production on the long term in a way that is economically viable and socially acceptable on the short-term. Maintenance of adequate levels of soil organic matter is essential for a sustainable high production of crop (Bruulsema *et al.*, 2004). Organic amendments contain useful macronutrients such as nitrogen, phosphorus and potassium, as well as large amount of carbon which serve as a substrate for the production of microbial biomass (Materchera and Salagae, 2002). It appears that there are two ways in which organic matter is used for energy by soil micro-organisms, i.e. directly through the use of some hemicellulose components (Halsall and Gibson, 1985; Ladha *et al.*, 1986 and Yadvinder *et al.*, 2010), or indirectly through the use of products of organic decomposition (Lynch and Harper, 1983; Roper and Halsall, 1986 and Sylvain *et al.*, 2007). Use of organic matter such as sludge and rice straw compost are some of the major ways (along with crop rotation and green manure) throughout history to maintain soil fertility (McKenzie, 2003). Thus, intensive agriculture systems with high inorganic fertilizer inputs however, limit the return of crop residues to the soil (Roper and Ladha, 1995). Maximizing nitrogen content in plant not only entails maximizing the application of organic matter or chemical fertilizer, but also requires that recovery of nitrogen is optimized (Yadvinder *et al.*, 2010). Nitrogen transformations are occurring during breakdown of soil micro-organisms which influenced by residue management and soil physical and chemical properties (Peoples *et al.*, 1995). With the aim of evaluating the potential use of microbial biomass nitrogen under different soil types.

The objectives of this study were to;(1) to determine whether different nitrogen sources and soil types could increase the MBN which has been not evaluated and determined yet under paddy soils, (2) to evaluate the effect of different N sources on the relationship between the microbial N uptake by rice plant and the nitrogen mineralization determined by NH_4 concentration increase.

MATERIALS AND METHODS

Experimental Design and Treatments Structure

A pot experiments were conducted during 2007 and 2008 summer seasons using two paddy soils i.e. Clay and silt loam soils in a greenhouse. Both soils were collected from the paddy fields located near Rice Research & Training Center, Sakha, Kafre El sheikh governorate and New valley governorate, Egypt, respectively. Selected characteristics of the experimental soil and organic matters are listed in Table 1. Both locations have been cultivated rice several years ago. Nontransparent PVC cylinders (h=35 cm, d=25 cm) were filled with 4 kg of air-dried and sieved soil (2mm mesh). Three nitrogen sources were applied, (i) sludge manure, (ii) rice straw compost which is made from mixing of the rice straw and cow manure, (iii) basal dose of Urea as control and (iv) no-N application. Both of sludge and straw were sieved through a 2 mm sieve and were mixing with soil before seeding. There were a total of 16 treatments i.e. [three nitrogen sources and a control] X [two soil conditions (autoclaved and fresh soils)]X two soil types]. The 64 pots [counting 16 treatments replicated 4 times] were arranged into a randomized complete block design within 12 x 8 arrays of rows of pots 40 cm apart. Soil of 32 pots and both of sludge and straw compost were autoclaved separately. The other 32 pots were used as fresh soils which contained the natural microbial biomass. Soil microbes were killed by autoclaving in a Sterilmatic sterilizer (Market Forge Industries, Everett, MA) at 121°C for 30 min on two consecutive days. The rate of nitrogen sources was applied based on 146 Kg N ha⁻¹, then the amount of organic and inorganic sources were added to the soil according to their nitrogen concentrations. Half of the chemical nitrogen (Urea) fertilizer was applied at 15 days after seeding and the second half was applied at maximum tillering stage (60 days after sowing). Phosphorus fertilizer was applied at the time of seeding (36 Kg P₂O₅ ha⁻¹. Eight rice seeds of the variety Giza 177 were planted per pot on 22 May, 2007 and on 20 May, 2008 then thinned to four seedlings per pot 10 days after seeding. After 15 days the water level was raised to a height of about 2 cm above the soil and it was maintained at that level thereafter until maximum tillering stage where the time of sampling.

Soil analyses

Soil ammonium (NH₄⁺) content was determined at maximum tillering stage in two fresh soil samples of each pot. Ammonium was determined after extraction with 2 M KCl using the indophenol-blue method (Keeney and Nelson 1982 and Rhine *et al.*, 1998). The water content of samples was determined in order to express the results of NH₄ on oven dry basis. Two samples were taken at the depth of 0-15 cm to determine the amount of total soil N at maximum tillering stage.

Table 1: Chemical properties of soil and organic matter.

Parameter	Clay* soil	Silt loam* soil	Organic matter	
			Sludge	Straw
pH (solid/water)	5.45	5.2	7.91	6.03
Total C (%)	3.01	3.2	33.30	22.00
Total N (%)	0.32	0.12	2.84	5.45
C/N ratio	9.38	26.6	11.9	4.00
Total P (mg Kg ⁻¹)	600	250	4800	2600
Organic matter (%)	5.17	0.25	38.72	40.55

*(According to Soil Survey Staff, 1996)

The soil samples were air-dried, ground and sieved using a 0.5-mm stainless sieve and stored under laboratory condition until been analyzed. The total content of nitrogen and carbon in the soil and plant were determined using the Sumi-Graph CHN-21 analyzer at Tottori Univ. Japan (Jimenez and Ladha, 1993 and El Sharkawi *et al.*, 2006).

Rice plant analyses

Two rice plants were harvested at maximum tillering (60 day from seeding) from each pot to determine the amount of N in rice shoot. The rice shoot washed with tap water followed by distilled water and then oven dried at 70°C. After determining the shoot dry weight (SDW), the plant samples were ground in a Vibrating Sample Mill (Heiko T1-100, Heiko Seisakusho Ltd, Tokyo, Japan). The ground samples were analyzed for total N content by a CHN-21 analyzer as described previously. Nitrogen uptake in rice shoot was determined by multiplying the N concentration by shoot dry weight. The total amount of microbial N absorbed by rice plant was determined by N difference method at maximum tillering stage (Bezdicsek *et al.* 1978 McNeill *et al.*, 1996; Kyuma, 2004 and El and harkawi *et al.*, 2006 and Sugihara *et al.*, 2010). Amount of microbial N were calculated from the difference between the extractable N from fresh soil and autoclaved soil samples under the same treatment as shown in the following equation. Microbial N flush was converted to MBN using a K_{EN} factor of 0.45 (Brookes *et al.*, 1985).

$$\text{MBN uptake} = (\text{TN under fresh soil} - \text{TN under autoclaved soil}) \times 0.45$$

Where; TN under autoclaved soil = Total N uptake in rice shoot grown in autoclaved soil

$$\text{TN under fresh soil} = \text{Total N uptake in rice shoot grown in fresh soil}$$

The MBN is controlled by two factors, (i) the amount of N accumulated during growth, and (ii) the proportion of plant nitrogen derived from microbial biomass (P_{fix}), which can be calculated as follows,

$$P_{\text{fix}} (\%) = \text{Amount of MBN uptake (mg pot}^{-1}\text{)} \times 100 / \text{TN uptake (mg pot}^{-1}\text{)}$$

Statistical analyses

Data were performed by analysis of variance (ANOVA) for a design type here i.e. randomized complete block design (Snedecor and Cochran, 1980). An Mstatc software program (citation) (MSTATC, 1990) was used to conduct the analysis of variance. A correlation procedure (SAS Inst., 1990) was used to determine the correlation coefficients between soil ammonium concentration, MBN uptake and P_{fix} for each treatment. Significant of treatments mean were determined at P < 0.05 with the LSD test.

RESULTS AND DISCUSSION

Effect of nitrogen sources and soil microbial biomass on nitrogen dynamics and shoot dry weight.

As shown in Tables 2 and 3, there was a significant difference among nitrogen sources for N in soil, NH₄, nitrogen uptake and SDW under both soils type. The highest ammonium content was recorded in organically treated pots with rice straw compost in clay and silt loam soils while the least content of NH₄ under Urea treated pots in both soils irrespective of the control. Nitrogen mineralization showed significant variation between inorganic and organic treatments in both soils. Inorganically treated pot amended with urea showed the highest N uptake and SDW in clay soil followed by sludge and rice straw treated pots and least in control. In contrast, organically treated pots amended with rice straw and sludge showed the highest N uptake and SDW in loamy soil followed by Urea treated pot.

Table 2: Ammonium concentration, nitrogen uptake and SDW of rice cv. Giza 177 as affected by nitrogen sources and microbial biomass in 2007 growing season.

Treatments	Clay soil				Silt loam soil			
	TN in soil (%)	NH ₄ (mg kg ⁻¹)	N uptake (mg pot ⁻¹)	SDW (g pot ⁻¹)	TN in soil (%)	NH ₄ (mg kg ⁻¹)	N uptake (mg pot ⁻¹)	SDW (g pot ⁻¹)
Nitrogen sources								
Zero N	0.30	15.71	168	11.77	0.12	12.37	63	4.88
Urea	0.49	23.58	451	23.79	0.23	16.99	115	11.78
Straw	0.50	26.32	609	23.05	0.25	21.45	168	13.87
Sludge	0.51	25.05	630	23.49	.26	20.59	231	16.17
F test	*	*	*	*	*	*	*	*
LSD at 5%	0.02	0.88	27.72	0.78	0.04	1.92	34.86	3.92
Microbial biomass								
Autoclaved soil	0.47	22.96	340	17.51	0.25	18.53	88.2	8.82
Fresh soil	0.54	22.27	593	21.27	0.51	19.85	150	9.52
F test	*	*	*	*	*	*	*	*

* = Significant at P ≤ 0.05, SDW = Shoot dry weight of rice.

The amount of mineralization (NH₄) of organic N was more than that from Urea fertilizer (Hasegawa, 1992). Organic matter with C/N ratio as seen in sludge produced a low NH₄ compared to straw compost with low C/N ratio. This may be attributed to the fact that materials with low C/N immobilize more N, and the release of the immobilized N is retarded (Ahmad et al., 1969 and Kyuma, 2004). This phenomenon plays a big role in microbial activity especially free living bacteria. Fresh soil treatment was affected the total N in the soil, nitrogen mineralization, N uptake and SDW in both soils. In the fresh soil inoculated pots, the organic matter was previously decomposed by general purpose decomposing bacteria before application. It has been shown that if organic matter applied to soil some of the liberated NH₄ is fixed in soil then, denitrification will be decreased (Kyuma, 2004). Although the total N in

the soil was higher under inoculated pots than un-inoculated one, also the ammonium in the soil was higher under un-inoculated treatment in both soils.

Table 3: Ammonium concentration, nitrogen uptake and SDW of rice cv. Giza 177 as affected by nitrogen sources and microbial biomass in 2008 growing season.

Treatments	Clay soil				Silt loam soil			
	TN in soil (%)	NH ₄ (mg kg ⁻¹)	N uptake (mg pot ⁻¹)	SDW (g pot ⁻¹)	TN in soil (%)	NH ₄ (mg kg ⁻¹)	N uptake (mg pot ⁻¹)	SDW (g pot ⁻¹)
Nitrogen sources								
Zero N	0.20	12.88	153	9.71	0.08	10.13	57	3.13
Urea	0.33	19.30	412	19.65	0.15	13.91	105	7.54
Straw	0.34	21.54	556	19.04	0.17	17.55	153	8.88
Sludge	0.34	20.49	576	19.40	0.18	16.85	211	10.35
F test	*	*	*	*	*	*	*	*
LSD at 5%	0.02	0.72	25	0.65	0.02	1.58	32	2.51
Microbial biomass								
Autoclaved soil	0.31	18.79	311	14.46	0.17	15.17	80	5.65
Fresh soil	0.36	18.23	542	17.57	0.34	16.25	137	6.10
F test	*	*	*	*	*	*	*	*

* = Significant at P ≤ 0.05, SDW = Shoot dry weight of rice

It has been shown that if organic matter applied to soil some of the liberated NH₄ is fixed in soil then, denitrification will be decreased (Kyuma, 2004). Although the total N in the soil was higher under inoculated fresh soil pots than un-inoculated one, the ammonium in the soil was higher under un-inoculated treatment in both soils. Jakobsen (1995) reported that in the first stage of plant life, the anaerobic and aerobic bacteria need, energy and nutrient especially nitrogen which are obtained from easily decomposable water-soluble organic compounds in soil solution or adsorbed to the soil itself. Later the microorganisms, soil biomass, are decomposed by other microbes and then the nitrogen will be mineralized to ammonium followed by nitrification to nitrate. It has observed that from the results in this experiment the markedly increase of the total N in soil under inoculated pot may be attributed not only to high population of microorganism and its activity but also some of bacteria able to fix the atmospheric nitrogen which add additional nitrogen to the soil (El Sharkawi et al., 2006). Clay soil had higher level of soil NH₄ than the Loamy soil. Schimel (1989) and Delgado et al. (1996) found that the lower N uptake associated with light soil is likely to be a function of greater volatilization losses as compared to clay soil. These results suggest that there may be subtle differences in N cycling between both soils. The relatively high NH₄ levels in clay suggest that plant available N may be higher in clay soil than loamy soil. Also, the total N uptake by rice shoot was lower under Urea application than in organic matter amended pots, although all pots had received equal dose of nitrogen. At low C/ N ratios as observed with straw manure, ammonia tends to accumulate and most of them loss through volatilization process and cause nitrogen loss (Fog, 1988). Organic materials with low C/ N are generally broken down more quickly than those materials with high C/ N (Ahmad et al., 1969 and Kyuma, 2004). These

results of literatures confirm why sludge amended pot generated NH_4 more than straw compost in both soils. Also, this phenomenon plays a big role in activity of free living bacteria under flooding condition. Microorganisms activity associated with coarse soil texture not grow well as compared to clay loam soil (Soil Quality Information Sheet, 2001).

Effect of different nitrogen sources on MBN uptake, P_{fix} and their relationship with nitrogen mineralization

Total microbial nitrogen is a natural system of microbial mobilization of atmospheric nitrogen in addition the nitrogen decayed from the bacteria itself which can be easily available and utilized by plants mediated by different microorganisms such as free living bacteria. The nitrogen sources appear to have the most relevance for microbial biomass in soil (Al-Kaisi *et al.*, 2008). Selection of the type of soil amendment especially under flooding conditions, played a very important role in enhancing microbial N inputs as will be explained below. Soil fertility was compensated by MBN under sludge amended soil while the ammonium was slow released, thereby, reducing the production cost (Peoples *et al.*, 1995). Leaching and loss of excess nitrogen derived from applying fertilizers are usually greater with flooded soil. From the data analyzed in this experiment, nitrogen sources and the microbial biomass had much greater effects on the microbial N uptake and P_{fix} in both soils under study (Tables 4 and 5). In spite of the differences found among nitrogen sources (Urea, rice straw and sludge) for the amount of microbial N absorbed by rice plant, there was no significant difference between rice straw and sludge manures under Clay soil compared with Loam soil.

Table 4: Total N uptake, MBN uptake and P_{fix} in rice cv. Giza 177as affected by nitrogen sources in 2007 growing season.

Treatments	Clay soil			Silt loam soil		
	TN in shoot (%)	MBN uptake (mg pot ⁻¹)	P_{fix} (%)	TN in shoot (%)	MBN uptake (mg pot ⁻¹)	P_{fix} (%)
Nitrogen source						
Zero N	1.75	28	15.4	1.11	3.7	6.6
Urea	2.50	75	19.3	1.39	15.8	15.0
Straw compost	3.59	137	21.0	1.56	31.6	20.6
Sludge	3.92	153	22.6	1.94	44.8	23.4
F test	*	*	*	*	*	*
LSD at 5%	0.27	17	1.26	0.23	13.1	2.2

*= Significant at $P < 0.05$, P_{fix} = the proportion of plant N derived from microbial biomass,

Table 5: Total N uptake, MBN uptake and P_{fix} in rice cv. Giza 177as affected by nitrogen sources in 2008 growing season.

Treatments	Clay soil			Silt loam soil		
	TN in shoot (%)	MBN uptake (mg pot ⁻¹)	P_{fix} (%)	TN in shoot (%)	MBN uptake (mg pot ⁻¹)	P_{fix} (%)
Nitrogen source						
Zero N	0.78	18	13.38	0.52	2.9	4.40
Urea	1.12	48	16.74	0.65	12.4	10.00
Straw compost	1.60	89	18.29	0.73	24.7	13.76
Sludge	1.75	100.	19.57	0.91	35.1	15.60
F test	*	*	*	*	*	*
LSD at 5%	0.12	11	1.09	0.11	10.2	1.48

*= Significant at $P < 0.05$, P_{fix} = the proportion of plant N derived from microbial biomass,

On the other hand, there was a difference among nitrogen sources for P_{fix} , which was the highest under sludge treatment in both soils and both years followed by rice straw. Increasing microbial N uptake rates under sludge treatment leads to the possibility of improving nitrogen use efficiency in both soils. These results indicated that organic amendments with high C content as shown in sludge (Table 1) may increase the N immobilization by soil microbes, resulting in a lower rate and longer period of N uptake by plants (Sugihara *et al.*, 2010). Positive correlations were found between N uptake and MBN uptake in both clay and silt loam soils ($R^2=0.94$ and $R^2=0.99$, respectively) as affected by nitrogen sources regardless of the control (Figs.1 and 2). Soil microbial biomass in paddy soil grows well without the addition of nitrogen fertilizer or slow N mineralization (Ladha, 1986). Our results suggested that not only the nitrogen source affecting the amount of MBN uptake in rice plant but also the amount of ammonium concentration under flooding conditions. The relationship between MBN uptake and NH_4 in both clay and silt loam soils ($R^2=0.78$ and $R^2=0.80$), confirmed the reduction in MBN uptake under straw treatment as shown in tables 4 and 5. Optimum level of the available nitrogen (NH_4^+) in soil has been shown to enhance the capacity of total microbial biomass (Hogh Jensen and Schjoerring, 1994). But the presence of more NH_4^+ greatly inhibits microbial biomass activity (Roper and Halsall, 1986). It was shown that NH_4^+ was more severely inhibiting than NO_3^- or NO_2^- to free living and endophytic bacteria in paddy soils (Kyuma, 2004). Also, ammonium is well known to inhibit the synthesis of nitrogenase but not its activity. In a paddy soil, growing rice plants with high concentration of NH_4^+ lowered the microbial N production. Similar result has reported by Yoshida *et al.* (1973). However, application of organic nitrogen sources such as sludge to paddy soil not only stabilize soil pH and nutrients availability (El Sharkawi *et al.*, 2006) but also enhance the soil microbial activity due to the organic phosphorus (4800 mg Kg^{-1} , Table 1) and carbon which are contained in these materials (Materechera and Salagae, 2002).

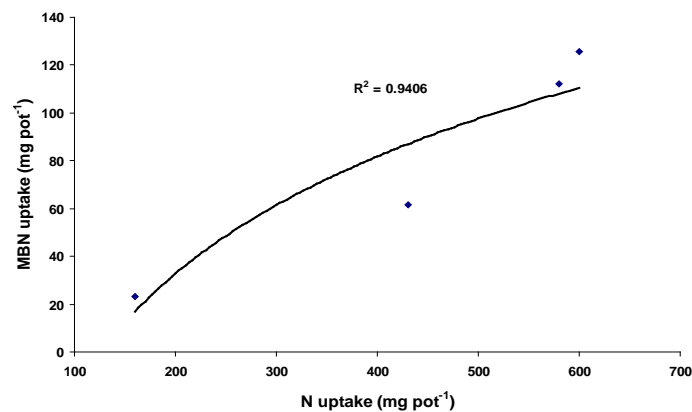


Fig. 1: Relationship between total N uptake and MBN uptake as affected by nitrogen sources in rice cv. Giza 177 under Clay soil.

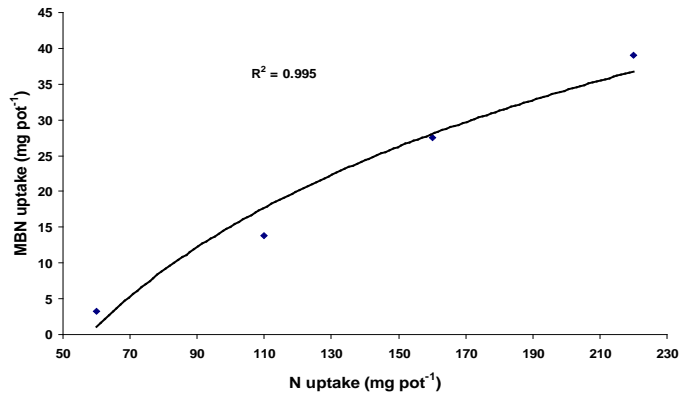


Fig. 2: Relationship between total N uptake and MBN uptake as affected by nitrogen sources in rice cv. Giza 177 under silt loam soil

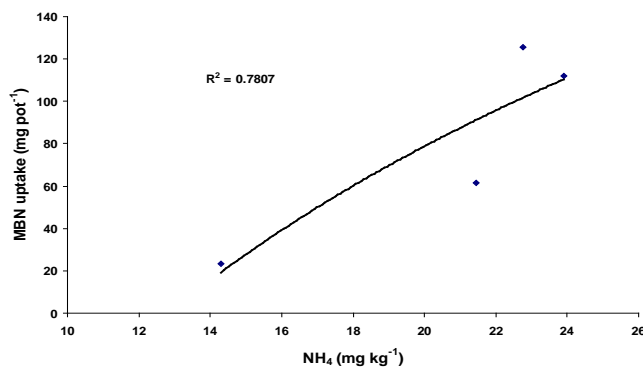


Fig. 3: Relationship between ammonium concentration and MBN uptake as affected by nitrogen sources in rice cv. Giza 177 under Clay soil.

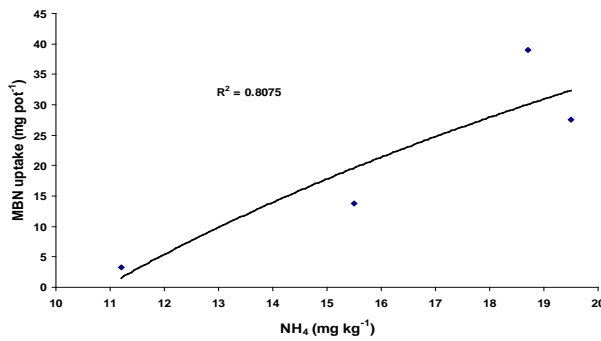


Fig. 4: Relationship between ammonium concentration and MBN uptake as affected by nitrogen sources in rice cv. Giza 177 under silt loam soil.

This could also demonstrate that why the amount of soil microbial N absorbed by rice plant was higher under sludge amended soil compared with straw compost or chemical fertilizer. However, further study is necessary to support this hypothesis.

Many studies have indicated that reducing NO_3^- leaching is the greatest challenge to improve the efficiency of N use in soil (Hartermink *et al.*, 2000 and Chikowo *et al.* 2006). If we can use MBN as a temporal N sink during the flooding condition by applying organic amendment as observed by Hirai *et al.* (2006) we should be able to decrease the service N leaching and improve the efficiency of N use in the soil (Mtambanengwe and Mapfumo 2006). Because the microbial N uptake by rice plant in the sludge and straw amended pots were greater than that in the Urea amended pot, organic matter application appeared to improve the efficiency of N use in the soil as compared with chemical fertilizer alone. As we could not observe a clear effect on organic matter application on the dynamic of microbial biomass nitrogen, future studies should focus on the effect of plant residues and organic matter and /or fertilizer application on temporal of MBN variations in relation to N loss from flooded soil and plant N uptake.

Conclusion

It can be safely said that not all nitrogen sources can serve well the anaerobic free living bacteria and make balance of soil nitrogen in paddy soil regardless of the type of soil. Consequently, free living bacteria in sludge-amended soil enhanced the total nitrogen uptake by rice plant in both Clay and Silt loam soil, and then increased the dry matter production. Fresh soil amended by sludge increased plant nitrogen consistently. Positive microbial N uptake by rice plant to the native free living microbes was enhanced rather than suppressed at high nitrogen mineralization rate. Based on the results obtained and on the information available, we suggest ways for considering soil microbial biomass for sustainable agriculture in paddy field condition.

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نيتروجين الكتلة الميكروبية وعلاقتها بامتصاص النيتروجين في النبات و أراضى الأرز
هيثم محمد الشرقاوى
المعمل المركزى للمناخ الزراعى – مركز البحوث الزراعية – الجيزة

تعتبر الكائنات التي تعيش حرة في التربة لها دور في ديناميكية النيتروجين في التربة و النبات ، أجرى هذا البحث لدراسة وتقييم الكائنات التي تعيش حرة في اراضى الأرز كمصدر للنيتروجين الطبيعي تحت نوعين من اراضى الأرز، حيث تمت دراسة ثلاثة مصادر للنيتروجين (السماد العضوى من قش الأرز، الحماة واليوربا) تحت ظروف التربة الغير معقمه (الطبيعية) و ظروف التربة المعقمه (autoclaved soil) في تجربة أصص خلال موسمي 2007,2008 . وقد اظهرت النتائج أنه في الأصص المُعاملة بالمادة العضوي سُجلت أعلى القيم للامتصاص النيتروجين الكلي و نيتروجين الكتلة الميكروبية ، تليها الأصص المُعاملة باليوربا، ولكن يقل امتصاص النيتروجين الميكروبي عند استخدام السماد العضوى من قش الارز بالمقارنة بتلك المُعاملة بسماد الحماة في كل من نوعى التربة. وبالنسبة الى عملية معدنة النيتروجين (NH_4) فأنها قد تأثرة بشكل ملحوظ بمصادر النيتروجين و الكائنات التي تعيش حرة في اراضى الأرز ، حيث ان زيادة تركيز الأمونيوم كان ملحوظاً مع السماد العضوى من قش الأرز ، والذي كان يؤخر استفادة الكائنات التي تعيش حرة في اراضى الأرز من الاستفادة من المادة العضوية مقارنة بالمعاملات الأخرى. وقد أظهرت النتائج ايضا ان MBN ونسبة النيتروجين الممتص بالنبات المستمد من مصدر ميكروبي (Pfix) تحت ظروف التربة الغير معقمة ارتفعت ارتفاعا ملحوظا بالمقارنة بالتربة المعقمة في كل من نوعى التربة، أيضا كانت هناك علاقة إيجابية بين Pfix وكمية النيتروجين الكلى الممتص بالنبات . وأظهرت النتائج أيضا أن نيتروجين الكتلة الميكروبية كان متأثراً ليس فقط من خلال نوع التربة ولكن أيضا من خلال نوع مصدر النيتروجين المضاف، بالإضافة إلى ذلك ، إضافة سماد الحماة في التربة الغير معقمة ادت الى زيادة نيتروجين الكتلة الميكروبية . من النتائج المتحصل عليها يمكن القول بأن اضافة سماد الحماة مفيدا بشكل غير مباشر في إنتاج الأرز وخاصة في التربة الفقيرة. وهكذا ، يمكن ان نستنتج ايضا أن الكائنات التي تعيش حرة في اراضى الأرز تسهم في نمو النباتات كمصدر للنيتروجين الحيوى خلال الموسم.

قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة
مركز البحوث الزراعية

أ.د / محمد حسين غنيمه
أ.د / عبد الله عبد النبي عبد الله