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SOIL MICROBIAL BIOMASS AND ACTIVITIES AS INFLUENCED BY GREEN MANURE LEGUMES AND N FERTILIZER IN RICE-WHEAT SYSTEM

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Abstract

The objectives of this study were to assess the influence of green manure legumes and N fertilizer on soil microbial biomass and activities in rice (*Oryza sativa*) -wheat (*Triticum aestivum*) system. Soil samples (0-15 cm) were collected from field experiment established in 2001 involving mungbean (*Vigna radiata*), cowpea (*Vigna unguiculata*), soybean (*Glycine max*), sesbania (*Sesbania rostrata*), pigeonpea (*Cajanus cajan*) and guar (*Cyamopsis tetragonoloba*) as green manure in rice-wheat system in Peshawar valley, Pakistan. The results showed that the green manure legumes and N fertilizer application significantly increased the microbial biomass and activities in rice-wheat system. The average improvement gained from the green manure legumes relative to (fallow-based-rice-wheat) FRW, was 1.79 times for microbial activities, 1.70 times for microbial biomass-C (MBC), 1.49 times for microbial biomass-N (MBN), 1.82 times for C mineralization, 1.92 times for N mineralization, 3.36 times for bacterial population and 1.46 times for fungal population. The average improvement gained from N fertilizer (+N) relative to no N unfertilizer (0N), was 1.40 times for microbial activities, 1.17 times for MBC, 1.29 times for MBN, 1.42 times for C mineralization, 1.45 times for N mineralization, 1.17 times for bacterial population and 1.42 times for fungal population. Our results thus suggest that the microbiological attributes proved to be highly responsive and sensitive to the beneficial influence of green manure legumes in rice-wheat system and can be used as indicator of soil quality.

Introduction

Green manure legumes may improve microbial biomass and soil organic fertility. Being N_2 fixers, legumes are believed to increase the N fertility of soil (Shah *et al*., 2003) and improve the soil quality when used as green (Biederbeck *et al*., 2005). Legumes are thus often recommended to include in crop rotation with cereals (Bakht *et al*., 2009). Among cereals, rice and wheat are the two major staple food crops for the people of developing countries. The rice-wheat cropping system is highly nutrient exhaustive and annually remove more than 650 kg ha^{-1} of N, P and K, and 0.5-1.0 kg ha^{-1} Zn, 2-3 kg ha^{-1} Fe and 3.0-3.5 kg ha^{-1} Mn. The rice-and/or wheat based cropping systems therefore cause a considerable depletion of soil nutrients. Consequently farmers have to use increased fertilizer doses each year to realize the same yield levels which were obtained with relatively lower amounts of fertilizers in the past This is however not possible by the farmers in developing countries because of low income. It is thus believed that the involvement of green manure legumes in rice-wheat system is a viable alternative for sustainable production.

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Although it is widely believed that the return of legume and other crop residues improve the soil fertility, it is not easy to measure improvement in soil fertility in the short-term. Microbial biomass which is a small but labile component of soil, can be used as an early indicator of changes occurring in soil due to management practices. Microbial biomass responds quickly to changes in soil management and is used as an indicator of soil quality. Biederbeck *et al*., (2005) reported that most of the soil biochemical and physical attributes were improved significantly by using legumes as green manure in fallow-wheat system. The labile attributes were more sensitive indicators of changes in soil quality than total C or N. Manna & Singh (2001) reported that guava enhanced the soil MBC approximately 2-fold after 38 years over 10 years of the same intercropped system.

Microbial biomass is not only used as an indicator of soil quality, it is the main agent that also controls the cycling of important nutrient elements such as C, N, P, S and other nutrients in terrestrial ecosystems. The soil microbial biomass serves as a sink (immobilization) and a source (mineralization) of important nutrient elements, controlling the supply of nutrients to crops through mineralization and immobilization processes (Abbasi *et al*., 2001; 2003; Fosu *et al*., 2007) depending on moisture, temperature and other environmental influences (Lodhi *et al*., 2009). Keeping in view the above facts that soil microbial biomass is sensitive to changes through management practices, environmental fluctuation and cropping system effects, the present study was undertaken to measure changes in microbial biomass, microbial activity, microbial population and C and N mineralization after green manure legumes (mungbean, cowpea, soybean, sesbania, guar and pigeonpea) in rice-wheat system.

Materials and Methods

A field experiment was established in 2001 at ARI Tarnab Peshawar (34°04′N, 71°40′E) with the objectives to involve green manure legumes in the rice-wheat system for sustainable production and study the effects of 6 green manure legumes (such as mungbean, cowpea, soybean, sesbania, guar and pigeonpea) and fertilizer N on rice and wheat production. A split-plot experiment was designed by assigning main plot to fertilizer N and sub-plots to legume treatments. The site was at an altitude of 360 m above see level. The climate is classified as semi-arid tropical. Annual precipitation average is 375 mm with summer dominance. The soil (surface 0-15 cm soil) of the experimental site was loam, alkaline in reaction, non-saline, calcareous in nature, and low in N, P and soil organic matter (Shah *et al*., 2010).

The experiment was commenced with legumes planted soon after wheat harvest in summer 2001. After 50 days of growth, legumes were incorporated into the soil in respective treatment plots by rotovator. Each treatment plot was planted to rice in July 2001 receiving nil (0N) or 120 kg N ha^{-1} fertilizer N (+N) alongwith basal dose of P and K. After rice, each treatment plot was planted to wheat, the 0N receiving no and the +N treatment receiving fertilizer N at 120 kg ha^{-1}. After wheat harvest, same legumes were planted in respective treatment plots in summer 2002 and incorporated in soil again after 50 days of growth. Rice in 2002 and 2003 and wheat in 2002/03 and 2003/04 were raised in similar passion as earlier.

Soil sampling and processing: For this study, soil samples (0-15 cm) were collected from each treatment plot immediately after wheat harvest in summer 2004 with the aim to assess the influence of green manure legumes on soil microbial biomass, microbial activity, microbial population and C and N mineralization, which were imposed in rice-wheat system for three years. The samples were broken down by hand and passed through 2 mm sieve while still moist. The samples were either run immediately for the required analysis or kept cool at 4^0C to avoid unnecessary microbiological changes in soil until ready to use.

Microbial activity: Microbial activities were measured from the rate of CO_2 evolution from soil during incubation (Neale *et al*., 1997). Soil samples were incubated in the presence of NaOH solution. The amount of CO_2 evolved was measured by titrating the NaOH solution against standard HCl solution.

Microbial biomass (C and N): Microbial biomass-C (MBC) and –N (MBN) were estimated by the methods of using chloroform fumigation incubation method of Brookes *et al*., (1985) and Vance *et al*., (1987) as described in Horwath & Paul (1994). Soil samples were fumigated with chloroform to the extent to kill all microbes present in the soil sample. The fumigated samples were inoculated with 1.0 g of unfumigated same soil sample. Both fumigated and unfumigated soil samples were incubated in the presence of NaOH solution. The amount of CO_2 evolved was measured by titrating the NaOH solution against standard HCl solution. The amount of mineral N was also measured both in fumigated and unfumigated samples. The amount of MBC and MBN were calculated as follows:

Calculation of biomass C: The amount of CO_2-C produced from fumigated and unfumigated samples were used to calculate soil microbial biomass C as follows:

$$\text{Biomass C} = (F_c - U_{fc})/k_c$$

where
F_c = CO_2 flush from fumigated soil
U_{fc} = CO_2 produced from unfumigated soil
K_c = 0.45 (Jenkinson & Ladd, 1981)

Calculation of biomass N: Microbial biomass N was calculated from the amount of mineral N produced in fumigated and un-fumigated samples using the following equation:

$N = (F_n - U_{fn})/k_n$
Fn = The flush of NH_4–N from fumigated soil
U_{fn} = The NH_4 –N mineralized during 10 days from unfumigated soil
K_n = 0.54 (Jenkinson, 1988).

Mineralizable C and N: Mineralizable C was estimated from the total amount of CO_2 produced from an unfumigated soil sample during 10 days of incubation. The amount of mineralizable C was calculated as 44 g of CO_2 equals 12 g of C. Mineralizable N was determined by analyzing soil samples for mineral N before (day 0) and after incubation (10 days) at 25°C (Abbasi *et al*., 2003). The amount of mineralizable N was calculated by difference. The soil mineral N (NH_4-N + NO_3-N) was determined by the steam distillation method described by Mulvaney (1996).

Bacterial and fungal population: Bacterial and fungal population was determined by the dilution plate technique as described by Wollum (1982). In this method, 10 g of soil sample was shaken with 95 ml of sterilized diluents in the presence of few glass beads in a 200 ml bottle for 10 minutes. Further dilutions were prepared by transferring 10 ml of the suspension to another bottle containing 90 ml diluents keeping on diluting the suspension to the required dilution. Bacterial population was assessed on nutrient agar medium and fungi on peptone glucose acid agar medium (Wollum, 1982).

Statistical analysis: The data were analyzed statistically using randomized complete block design with split-plot arrangement as described by Steel & Torrie (1980).

Results and Discussion

Rate of CO_2 evolution: Averaged across legume-based rice-wheat (LRW) plots, the rate of CO_2 evolution was consistently greater in the LRW than in the fallow-based rice-wheat (FRW) plot during all incubation periods (Fig. 1a). The rate of CO_2 evolution in the LRW plots was 1.71 times greater at day 3, 1.96 times at day 6 and 1.79 time at day 10 relative to FRW plot. Differences in CO_2 evolution between the LRW and FRW plots were statistically significant. The effect of fertilizer N on rate of CO_2 evolution was also significant (Fig. 1b). Averaged across cropping systems, the rate of CO_2 in the N fertilized (+N) treatment was 1.37 time greater at day 3, 1.46 times at day 6 and 1.40 times at day 10 relative to the unfertilized (0N) treatment.

The rate of CO_2 evolution, however, varied with the type of legume used for green manuring. Among legumes, the rate of CO_2 evolution was greatest in the sesbania and lowest in the guar based plot (Table 1).

Cumulative CO_2 production: The cumulative CO_2 produced was consistently greater in the LRW than in the FRW plot (Fig. 2a) and such differences between the two systems were statistically significant. We observed that the amount of CO_2 produced in the LRW plots was1.71 times greater at day 3, 1.86 times at day 6 and 1.82 times at day 10 compared with FRW plots. Our results further showed that N fertilizer (+N) treatment significantly increased the amount of CO_2 produced during 10 days of incubation period (Fig. 2b).

The amount of CO_2 produced varied with the type of green manure legumes. Among legumes, the greatest amount of CO_2 was produced in the sesbania and lowest in the guar-based RW plot during 10 days of incubation period (Table 2). These results suggested that green manure legumes and application of N fertilizer in rice-wheat system increased microbial activity in soil. The high microbial activities could be due to increased inputs of organic matter from green manure legumes (Shah *et al*., 2009). Chirinda *et al*., (2008) observed that high C and N inputs enhanced microbial activity in the 10-year old cropping system experiment in Denmark. Similar to our results with respect to responses of microbial activities to green manuring legumes were also observed in other studies (Biederbeck *et al*., 2005) where microbial activities in terms of dehydrogenase (202%), phosphatase (171%) and arylsulfatase (287%) were obtained in cropping involved green manure legumes compared with fallow-wheat.

Table 1. Rate of CO_2 evolution (ug g^{-1} soil d^{-1}) as influenced by green manure legumes and N fertilizer in rice-wheat system.

Cropping system with	Day 3			Day 6			Day 10		
	0N	+N	Mean	0N	+N	Mean	0N	+N	Mean
Fallow	7.4	10.2	8.8c*	7.8	20.5	14.2c	13.2	25.8	19.5c
Mungbean	13.3	18.8	16.0a	22.4	35.5	28.9a	27.7	38.9	33.3b
Cowpea	14.8	21.4	18.1a	24.7	35.2	30.0a	30.6	44.3	37.4ab
Soybean	12.3	16.9	14.6ab	24.5	28.9	26.7a	26.5	34.5	30.5b
Sesbania	13.8	19.8	16.8a	26.9	38.4	32.7a	35.5	49.6	42.5a
Guar	9.5	11.7	10.6bc	16.2	25.1	20.6b	23.6	29.6	26.6b
Pigeonpea	12.8	15.8	14.3ab	23.9	30.5	27.2a	33.3	44.3	38.8ab
Mean legumes	12.7	17.4	15.1	23.1	32.3	27.7	29.5	40.2	34.9
% Increase by legumes	173	170	171	296	157	196	224	156	179

*Means followed by different letter(s) within column differ significantly ($p<0.05$)

Table 2.Cumulative CO_2 production (ug g^{-1} soil) in 10 days as influenced by green manure egumes and N fertilizer in rice-wheat system.

Cropping system with	Day 3			Day 6			Day 10		
	0N	+N	Mean	0N	+N	Mean	0N	+N	Mean
Fallow	22	31	26b*	46	92	69b	98	196	147b
Mungbean	40	56	48a	107	163	135a	218	318	268a
Cowpea	45	64	54a	119	170	144a	241	347	294a
Soybean	37	51	44a	110	138	124a	216	276	246a
Sesbania	41	59	50a	122	175	148a	264	373	318a
Guar	28	35	32b	77	110	94b	172	229	200b
Pigeonpea	38	47	43a	110	139	125a	243	316	280a
Mean legumes	38	52	45	108	149	128	226	310	268
% Increase by legumes	173	170	171	236	162	186	230	158	182

*Means followed by different letter(s) within column differ significantly ($p<0.05$)

Microbial biomass-C (MB-C) and -N (MB-N): Averaged across legumes-based cropping system, significantly greatest MB-C was obtained in the LRW than in the FRW plot (Table 3). Overall, MBC was 1.70 times greater in the LRW than in the FRW plots. Significantly greatest MBC was obtained in the +N than in the 0N treatment. On average, +N produced 1.17 times greater MBC than the 0N treatment. The effect however varied with the type of green manure legume. Sesbania produced the greatest and guar the lowest MBC in soil under RW system.

Like MBC, MBN was significantly increased in the LRW compared with the FRW plots (Table 3). On average, MBN was 1.49 times greater in the LRW than in the FRW soil. However, the effect varied with the type of legume. The greatest MBN was observed in the sesbania and lowest in the guar plot. Similarly, significantly greatest MBN was obtained for +N than for 0N treatment. On average, +N produced 1.29 times greater MBN than the 0N treatment.

These results suggested that MBC and MBN were significantly increased bt the green manure legumes and the N fertilizer application in soil under rice-wheat system. Similar results were also reported by Biederbeck *et al*., (2005) where the average improvement gained from green manure legumes was 107% for MBC and 191% for MBN compared with fallow-wheat cropping. Chirinda *et al*., (2008) also obtained higher MBN and nitrification rate in cropping systems involving green manure legumes compared with those reliant on inputs from manure and mineral fertilizer. Patro *et al*., (2009) however observed that soil MBN values gradually declined with time and reached minimum at rice harvest in two consecutive years the treatment in which rice was grown after incorporation of Crotalaria green manure and wheat straw where SMB values increased initially.

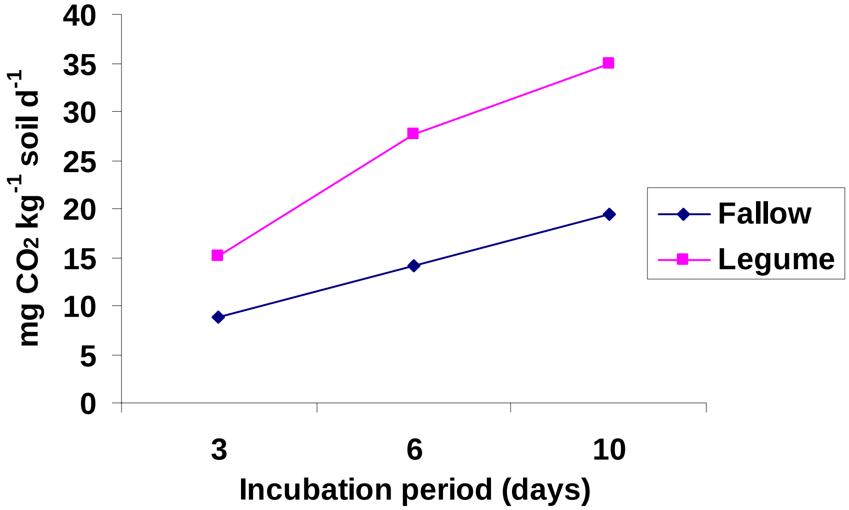

Fig. 1a. Rate of CO_2 evolution as influenced by green manure legumes in rice-wheat system

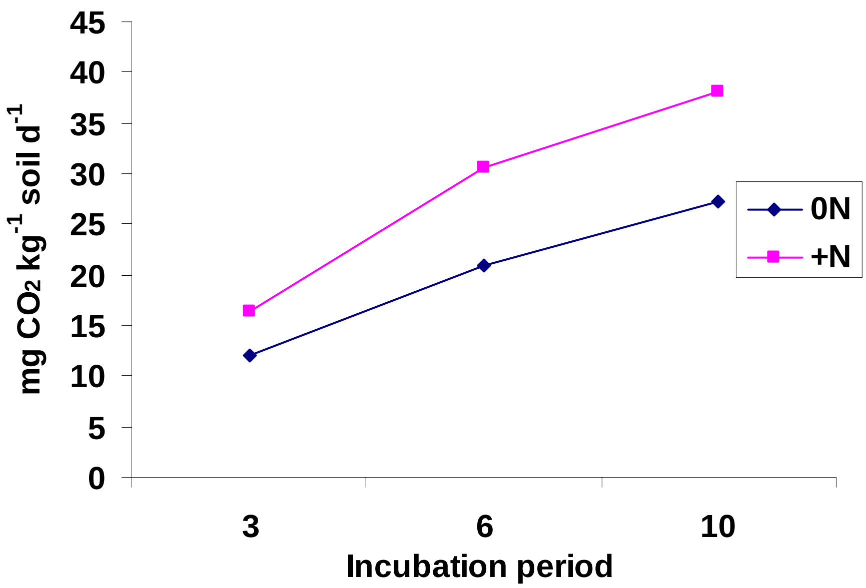

Fig. 1b. Rate of CO_2 evolution as influenced by N fertilizer in rice-wheat system

Carbon (C) and nitrogen (N) mineralization: Carbon mineralization was significantly greater in the LRW relative to FRW plot (Table 4). On average, legumes increased C mineralization by 1.82 times compared with FRW plot. The effect, however, varied with the type of green manure legume. The greatest C mineralization occurred in sesbania and lowest in guar plot. The application of N fertilizer to rice and wheat also had significant effect on C mineralization. On average, C mineralization was 1.42 times greater in the +N than in the 0N treatment.

Nitrogen mineralization followed similar pattern of response to green manure legumes and N fertilizer as of C mineralization. Like C mineralization, N mineralization was significantly affected by green manure legumes and N fertilizer application in soil under rice-wheat system (Table 4). Averaged across legumes, N mineralization was 1.92 times greater in the legume than in fallow plots. Similarly, +N treatment increased N mineralization by 1.45 times compared with 0N treatment.

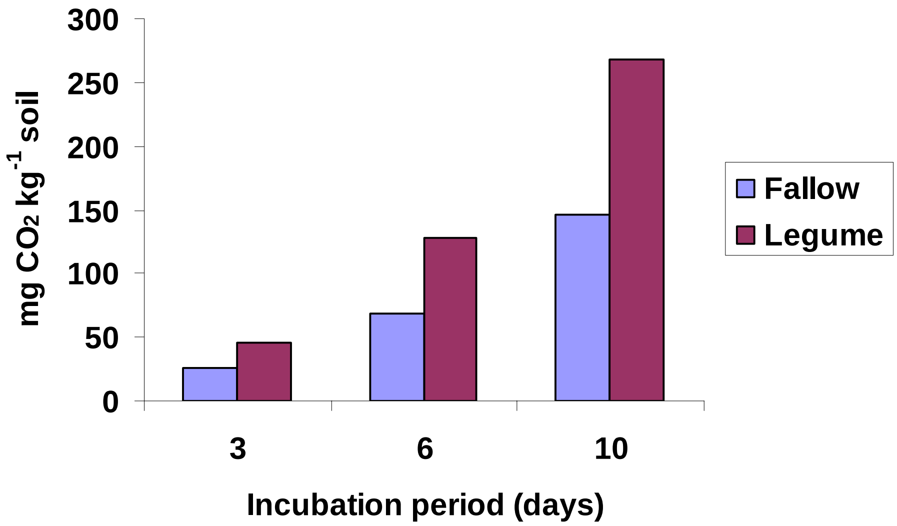

Fig. 2a. Cumulative CO_2 production as influenced by green manure legumes in rice-wheat system.

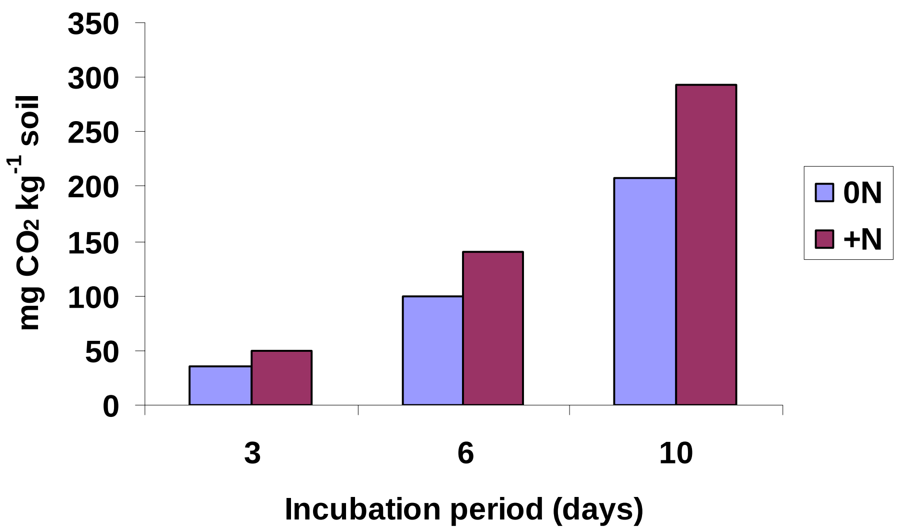

Fig. 2b. Cumulative CO_2 production as influenced by N fertilizer in rice-wheat system.

The enhanced rate of C and N mineralization after green manuring legumes could be due to increased inputs of organic matter particularly organic N (Abbasi *et al*., 2009; Shah *et al*., 2010). The soil organic fertility built up has consequences on soil biological properties and subsequently influences N and C availability in the soil (Robertson *et al*., 2000; Mader *et al*., 2002). Biederbeck *et al*., (2005) observed 205% increase in cumulative C mineralization after the green manuring legumes compared with the fallow-wheat cropping.

Microbial population: Green manure legumes and N fertilizer application significantly increased the bacterial and fungal population in soil. The average increase from the legumes relative to FRW, was 3.36 times for bacteria and 1.46 times for fungi (Table 5). However, the effect varied with the type of green manure legume. The greatest increase in bacterial and fungal population occurred in the sesbania and lowest in the guar plots. Similarly, the average increase from the +N treatment relative to 0N treatment, was 1.17 times for bacteria and 1.42 times for fungi. The interaction between legumes and N fertilizer was non-significant.

Table 3. Microbial biomass-C and -N as influenced by green manure legumes and N fertilizer in rice-wheat system.

Cropping system	Microbial biomass C			Microbial biomass N		
	(µg g-1 soil)					
	0N	+N	Mean	0N	+N	Mean
Fallow	152	288	220c*	13.0	20.7	16.9b
Mungbean	379	419	399a	24.5	28.9	26.7a
Cowpea	384	407	396a	23.2	30.5	26.9a
Soybean	376	402	389a	22.4	26.8	24.6a
Sesbania	411	424	418a	25.3	31.9	28.6a
Guar	266	350	308b	16.6	22.6	19.6ab
Pigeonpea	306	375	341b	21.5	27.0	24.3a
Mean legumes	354	396	375	22.3	28.0	25.1
% Increase by legumes	233	138	170	171	135	149

*Means followed by different letter(s) within column differ significantly ($p<0.05$)

Table 4.Carbon and nitrogen mineralization in 10 days as influenced by green manure legumes and N fertilizer in rice-wheat system.

Cropping system	C mineralization			Net N mineralization		
	(µg g-1 soil)					
	0N	+N	Mean	0N	+N	Mean
Fallow	26.8	53.4	40.1b*	5.3	8.7	7.0b
Mungbean	59.4	86.9	73.2a	9.9	12.6	11.2ab
Cowpea	65.7	94.8	80.2a	10.2	18.8	14.5a
Soybean	59.0	75.2	67.1a	12.4	12.9	12.6ab
Sesbania	72.0	101.8	86.9a	13.5	22.3	17.9a
Guar	46.8	62.4	54.6b	9.4	13.7	11.5ab
Pigeonpea	66.4	86.2	76.3a	11.0	14.7	12.8ab
Mean legumes	61.6	84.6	73.1	11.0	15.8	13.4
% Increase by legumes	230	158	182	208	183	192

*Means followed by different letter(s) within column differ significantly ($p<0.05$)

Table 5.Soil microbial population as influenced by green manure legumes and N fertilizer in rice-wheat system.

Cropping system	Fungi ($x10^5$)			Bacteria ($x10^8$)		
	Colony forming units g^{-1} soil					
	0N	+N	Mean	0N	+N	Mean
Fallow	9.6	14.0	11.8b*	1.9	2.8	2.4c
Mungbean	13.6	20.8	17.2ab	7.6	9.6	8.6a
Cowpea	17.1	22.8	20.0a	10.0	10.2	10.1a
Soybean	12.6	25.8	19.2a	5.9	5.1	5.5bc
Sesbania	18.4	26.2	22.3a	10.0	11.3	10.7a
Guar	9.1	12.9	11.0b	3.8	5.3	4.6bc
Pigeonpea	14.6	13.1	13.9b	6.4	9.5	8.0ab
Mean legumes	14.4	20.2	17.3	7.3	8.5	7.9
% Increase by legumes	150	144	146	384	304	336

*Means followed by different letter(s) within column differ significantly ($p<0.05$)

Increased microbial population in soil could be due to enhanced organic matter inputs from the green manure legumes and cropping intensity (Stromberger *et al*., 2007; Shah *et al*., 2009). The fertility building in organic cropping system has consequences for soil biological properties including microbial population (Robertson *et al*., 2000; Mader *et al*., 2002). Similar improvement in bacterial and fungal population by the green manure legumes was observed in other studies. For example, Biederbeck *et al*., (2005) observed 385% increase in bacterial and 210% in fungal population after the green manuring relative to fallow-wheat cropping.

Conclusions

This experiment has shown that the green manure legumes and N fertilizer application increased the microbial biomass and activities in rice-wheat system. The average improvement gained from the six green manure legumes relative to FRW, was 1.79 times for microbial activities, 1.70 times for MBC, 1.49 times for MBN, 1.82 times for C mineralization, 1.92 times for N mineralization, 3.36 times for bacterial population and 1.46 times for fungal population. The average improvement gained from +N relative to 0N, was 1.40 times for microbial activities, 1.17 times for MBC, 1.29 times for MBN, 1.42 times for C mineralization, 1.45 times for N mineralization, 1.17 times for bacterial population and 1.42 times for fungal population. Our results thus suggest that the microbiological attributes proved to be highly responsive and sensitive to the beneficial influence of green manure legumes in rice-wheat system.

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