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EFFECT OF FERTILIZERS ON SOIL MICROBIAL BIOMASS UNDER LEGUMINOUS CULTIVATION

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Key words *: Microbial biomass carbon, population/fertilizer, groundnut/organic carbon*

Abstract - Soil microbial biomass carbon (C_{mic}) was measured under the cultivation of a high yielding variety of groundnut ICGS-76 *(Arachis hypogaea* L.) treated with inorganic and organic fertilizers at an upland experimental block of Agronomy division, Indian Council of Agricultural Research (ICAR) Barapani Shillong, Meghalaya, India. Soil samples were collected randomly at monthly intervals during two crop cycles from each different treatment at two-depths (0-10 cm and 10-20 cm). At the surface soil layer, the maximum C_{mic} (1061.2 |j,g C g^{-1} dry soil) was observed at NPK+FYM plot, whereas the minimum C_{mic} (42.5 \ag C g^{-1} dry soil) was observed at FYM plot. At the subsurface soil layer, peak microbial biomass carbon (817.7 |j.g C g^{-1} dry soil) was observed at NPK+FYM plot, whereas the minimum (16.2 |j,g C g^{-1} dry soil) was observed in FYM plot. Insignificant differences in C_{mic} were found among the treatments at both the surface and subsurface soil layers. The significant variation was obtained in microbial biomass carbon between surface and subsurface soil layers except only in NPK+FYM plot. Microbial population and total organic (TOC) carbon showed a significant correlation with C_{mic} at surface and subsurface soil layers. The investigation showed that C_{mic} increased shortly after the application of fertilizers and inconsistent distribution of C_{mic} within each treated plots. Fertilizers application has lowered the amount of C_{mic} as compared to the non-agricultural field soil. Surface soil layer showed higher accumulation microbial biomass carbon than the subsurface soil layer.

INTRODUCTION

Microbial biomass carbon (C_{mic}) can be used as sensitive indicator for the status and changes in the agro-ecosystem and soil fertility levels, and it can also predict the future soil quality and ecosystem functioning. Biomass measurement has also been used to give an early indication of changes in the organic matter content of soils due to variation in soil management (Insam and Haselwandter, 1989; Brookes, 1995). Soil microbial biomass, which represents about 1 -4% of total soil organic C, is more sensitive indicator of changing soil conditions than direct analysis of the organic C content. The quantity and composition of microbial biomass is sensitive to changes in the soil chemical and physical environments (Wolters and Joergensen, 1991; Wardle, 1992; Bauhus and Khana, 1994; Beck *etal.,* 1995).

Soil microorganisms play an important in the cycling of almost all the major plant nutrients (Smith and Paul, 1990), particularly so in natural and agriculture ecosystems with low inputs. A number of soil microbiological parameters, notably microbial biomass carbon and basal respiration (Doran and Parkin 1994; Sparling, 1997), have been employed in national and international monitoring programs. Soil microbial biomass can be further an important pool of plant nutrients and is often highly correlated with the organic matter content of soils (Pankhurst *et al.,* 1995). Consequently, a close relationship has also been reported between soil fertility and microbial biomass (Brookes, 1995; Insam *et al.,* 1991).

Carbon storage in agricultural and forest soils has attracted attention recently due to its potential as a substantial carbon sink (Hu *et al.,* 1997). The microbial biomass is a sensitive indicator of changes resulting from agronomic practices and other perturbations of the soil ecosystem (Doran, 1987; Smith and Paul, 1990). Management practices significantly affected organic carbon, carbohydrate contents, microbial biomass C and organic C turnover rates in agricultural soils (Hu *et al.,* 1997). Although small in

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mass, the microbial biomass is among the most labile pools of organic matter and thus serves as an important reservoir of plant nutrients, such as N and P (Jenkinson and Ladd, 1981; Marumoto *et al.*, 1982). The role of soil microbial biomass as a relatively labile nutrient pool in the cycling of C, N and P is well established (Van Veen *et al.*, 1987, Duxbury *et al.*, 1989; Jenkinson and Parry, 1989).

The soil microbial biomass is of importance in most ecosystems because it forms the base of the detritus food web and serves as a sink and source for most plant-available nutrients (Anderson and Domsch, 1980; Jenkinson and Ladd, 1981). This biomass thus has the potential to influence plant growth (Okano *et al.*, 1997). Studies have documented an increase in microbial biomass and activity (Mahmood *et al.*, 1997) as well as decrease (Burket and Dick, 1998) with increasing N fertilizer application. The main objectives of this investigation were to assess the response of soil C_{mic} to fertilizer treatments in agricultural field under surface and subsurface soil layers of leguminous cultivation and the relationship between C_{mic} and various soil properties.

MATERIAL AND METHODS

Study area

The study was carried out at an upland experimental block of Agronomy division, Indian Council of Agricultural Research (ICAR) for North Eastern Hill (NEH) region complex at Barapani Shillong; Meghalaya India on Groundnut *(Arachis hypogaea* L.) cultivation in northeast India. The geographical position of the study site is at 25° 38' N latitude and 91 52' E longitudes and is situated at an altitude of 850 msl. The soil of the study area has been broadly divided into four categories viz., (1) red loamy soil, (2) lateritic soil (3) red and yellow soil and (4) alluvial soil. The soil of the experimental site is sandy loam (54.50%) with moderate permeability, silt (30.80%) and clay (14.45%). The soil pH was slightly acidic and ranged from pH 4.9 to pH 6.0. The climate of the study area is humid and sub-tropical. The low clouds brought in by the south and west monsoon get interrupted in the southern face of Khasi hills and cause extremely heavy rainfall along the Cherapunjee range through long peninsular belt. The rain starts from middle of April and it continues till late October (Fig. 1).

Experimental Design

High yielding variety of groundnut ICGS-76 (International culture of groundnut selection-76) from International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Hyderabad India was sown for the investigation during the first year (2001) and the second year (2002). The experimental field was divided into four blocks with three replicates, each for different treatments and levels of fertilizer. The optimum fertilizers dosage for groundnut was applied into the field as recommended by ICAR (Table 1). According to the types of fertilizer treatment, each of the experimental plots viz., controls, inorganic, organic and combinations of inorganic and organic were designated as CTRL, NPK, FYM and NPK+FYM respectively. The experimental block was set up in a slope land terrace, with a good drainage system. Each triplicate plot had a size of 3 X 4 m . Before sowing and adding fertilizers the field was properly ploughed. After the application of fertilizers, groundnut was sown in a 10 X 30 cm spacing rows as recommended by ICAR.

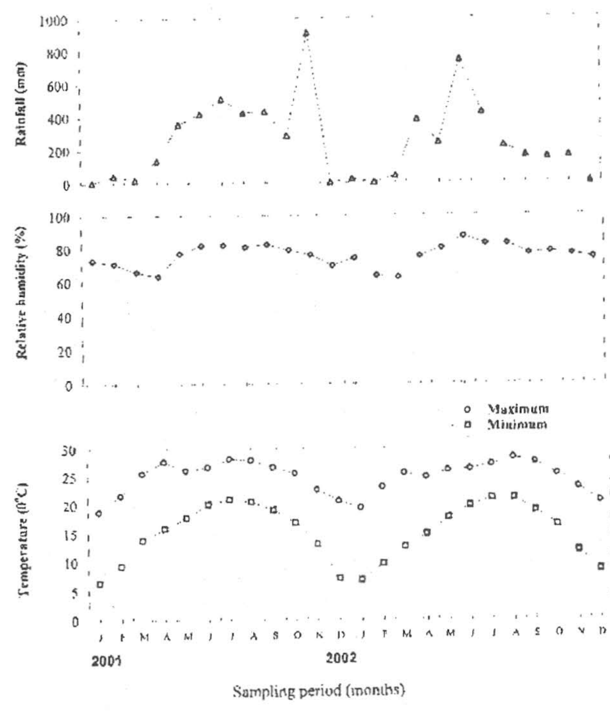

Fig. 1. Rainfall, relative humidity and temperature during the period of 2001-2002.

Table 1. Types of fertilizer treatments and doses

Treatment	Source	Dose	
1. Control (CTRL)	--	--	
2. Inorganic fertilizers			
(N+P+K)	N= urea	20 kg/h	Recommentded dose
	P=single super phosphate	60 kg/h	
	K=muriate of potash	40 kg/h	
3. Organic fertilizer	FYM=Farmyard manure	10 t/h	
(FYM)	(Cow dung)		
4. Combination of inorganic	N	10 kg/h	50% of recommende d dose
and organic fertilizers	P	30 kg/h	
[(N+P+K) + (FYM)]	K	20 kg/h	
		5t/h	
	FYM	5 t/h	

Collection and Analyses of Soil Samples

Soil samples were collected randomly at monthly intervals during two crop cycles from each different treatment at two-depths (0-10 cm and 10-20 cm) from April to October in 200land 2002. Soil samples collected in the month of April was considered as pre-sowing and pretreatment of fertilizer, while soil samples collected in the month of October was considered as post harvest soil sample. All treatments were tested with three replicates. The samples were brought to the laboratory on the same day and kept in the refrigerator at 4°C (Fig. 2).

The soil microbial biomass carbon was determined by chloroform fumigation incubation (FI) method (Anderson and Ingram, 1993). The soil was sieved through 2 mm mesh sieve to remove stones, coarse roots and all visible litters. 10 g of each sample was taken in a beaker and was placed in a vacuum desiccator containing 30 mL of alcohol free chloroform in a shallow dish. The lid was closed and sealed and the vacuum was used till the last trace of chloroform evaporated and thereafter the desiccator was kept in the dark for 5 days at 25° C. Another 10 g of each sample was weighted for un-fumigated extraction (cti). The fumigated (cti) and un-fumigated soil (ct2) samples were then kept in a watertight extraction bottle (125 ml) and extracted with 50 ml of 0.5 M K.2SO4 and were shaken for 30 minutes. The extracted soil was then filtered through Whatman filter paper No.42. To a 4 ml filtrate, 1 ml of 0.0667 M potassium dichromate and 5 ml of concentrated sulphuric acid were added. The sample was then preheated at 150°C for 30 minutes. Two blanks were prepared i.e. one preheated at 150°C for 30 minutes and the other without heating. The digested sample was then transferred to a 100 ml conical flask and to it 0.3 ml of indicator solution (O-phenanthroline monohydrate) was added. The sample was then

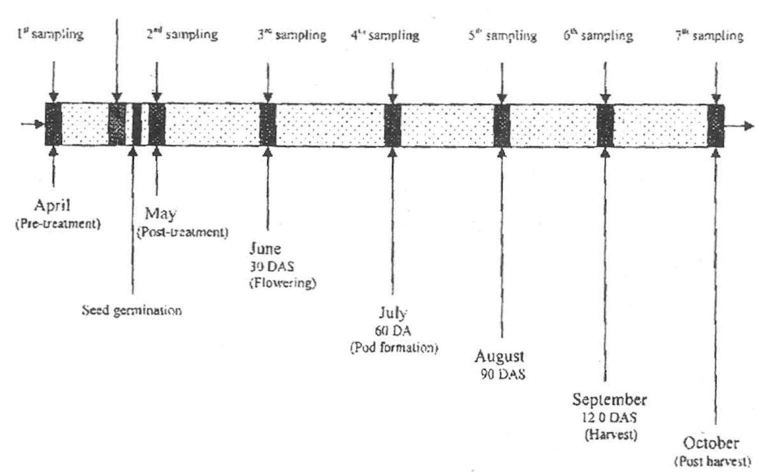

Fig. 2. Soil sampling period, stages of groundnut and period of soil treatment (DAS = day after sowing)

titrated with acidified ferrous ammonium sulphate solution. The end point was a colour change from green/violet to red. Three replicates were maintained in each case. For blank, 4 ml of 0.5 M KiSO4 solution was added in place of sample filtrate solution.

C_{mic} = *(Extracted ct1,- Extracted* ct_2*) X 2.46*

Colony forming unit of fungi and bacteria were isolated by the serial dilution plate method (Parkinson *et* a/., 1971). For soil pH, 10 g of freshly collected soil was taken in a beaker containing 50 ml of distilled water and the soil water mixture was stirred and the solution was kept overnight and the pH was read by using electronic digital pH meter. Soil organic carbon was estimated by the method of Anderson and Ingram (1993). Total nitrogen was estimated by using the method of Jackson (1973). The available phosphorous was measured by the molybdenum blue method (Alien *et al.* 1974). Exchangeable potassium was measured by using the method of Alien *et al.* (1974).

Statistical Analysis

One-way analysis of variance (ANOVA) was used to test the effect of fertilizer treatment on soil C_{mic}. Inter relationship between C_{mic} at surface and subsurface soil layers were analyzed by computing coefficient of correlation tests (Pearson's correlation coefficient). A linear regression model was fitted to test which soil variable could be used as predictors of C_{mic}

RESULTS

The soil microbial biomass carbon increased shortly after the application of fertilizers and germination of groundnut throughout the investigation. At the surface soil layer, C_{mic} ranged between 42.5 and 1061.2 µg C g^1 dry soil in 2001 and 50.7 and 416.6 µg C g^{1}dry soil in 2002 (Table 2). At the subsurface soil layer, C_{mic} ranged between 16.2 to 817.7 µg C g^1 dry soil in 2001 and 31.7 and 414.4 µg C g^1 dry soil in 2002 (Table 3). The peak microbial biomass carbon was observed at NPK and NPK+FYM plots, whereas the minimum was observed at CTRL and FYM plots. The CTRL plot showed decreased in C_{mic} from September to October (Tables 2 and 3). The mean value of C_{mic} from 14 months also showed a closed C_{mic} within treatments and it was ranging from 217-270 µg C g^1 dry soil (Table 4). The analysis of variance

Table 2. Estimation of soil C_{mic} by chloroform fumigation incubation method in agricultural field at surface soil layer.

Soil treatment	Year of observation													
	2001							2002						
	Apr	May	Jun	Jul	Aug	Sep	Oct	Apr	May	Jun	Jul	Aug	Sep	Oct
	Soil microbial biomass carbon µg Cg^{-1} dry soil													
CTRL	92.69 ±27.93	328.74 ±48.12	222.24 ±23.82	144.7 ±50.11	303.98 ±58.41	349.18 ±89.53	122.7 ±10.0	176.63 ±0.58	206.62 ±41.49	218.78 ±47.35	414.36 ±35.0	386.24 ±23.90	90.0 ±11.63	50.73 ±1.62
NPK	80.62 ±0.12	692.28 ±31.49	260.94 ±61.55	471.6 ±47.62	82.8 ±34.77	166.97 ±83.47	246.71 ±41.12	269.92 ±46.75	327.33 ±48.10	187.89 ±46.48	416.58 ±30.0	227.26 ±11.65	137.19 ±21.15	132.96 ±2.37
FYM	129.44 ±59.05	478.67 ±89.26	326.97 ±22.77	349.2 ±57.74	42.46 ±8.49	140.76 ±24.36	171.84 ±4.88	192.68 ±50.03	201.83 ±9.0	145.31 ±21.35	396.73 ±24.54	205.65 ±0.0	132.20 ±43.38	129.56 ±0.70
NPK+ FYM	250.92 ±15.0	1061.73 ±22.45	309.69 ±65.81	450.81 ±45.07	334.92 ±65.61	311.81 ±22.3	157.39 ±12.39	111.52 ±44.36	258.71 ±47.15	79.98 ±9.0	124.47 ±17.0	69.74 ±24.06	132.64 ±14.32	130.30 ±0.34

Table 3. Estimation of soil C_{mic} by chloroform fumigation incubation method in agricultural field at sub surface soil layer.

Soil treatment	Year of observation													
	2001							2002						
	Apr	May	Jun	Jul	Aug	Sep	Oct	Apr	May	Jun	Jul	Aug	Sep	Oct
	Soil microbial biomass carbon µg Cg^{-1} dry soil													
CTRL	56.83 ±21.86	155.6 ±18.09	164.39 ±8.21	50.05 ±12.52	182.38 ±98.69	41.32 ±11.0	64.03 ±21.18	98.44 ±19.65	101.16 ±19.15	174.12 ±23.21	401.46 ±37.0	160.79 ±2.34	39.95 ±7.0	37.07 ±1.34
NPK	66.81 ±23.14	420.82 ±23.61	217.35 ±40.21	85.43 ±42.74	40.77 ±38.17	45.95 ±14.07	199.25 ±39.85	121.60 ±68.37	150.15 ±23.63	184.75 ±22.83	414.35 ±95.73	188.24 ±18.74	33.56 ±11.62	31.69 ±1.70
FYM	22.71 ±16.17	91.06 ±48.51	235.36 ±25.37	339.2 ±12.51	33.26 ±8.3	121.63 ±2.37	142.17 ±20.64	155.86 ±5.05	167.22 ±72.11	108.83 ±17.56	320.73 ±48.30	165.95 ±13.91	41.14 ±7.0	39.31 ±0.73
NPK+ FYM	235.39 ±24.01	817.65 ±95.44	253.77 ±110.27	146.73 ±67.24	213.92 ±42.77	160.82 ±45.04	69.29 ±24.0	65.71 ±45.54	205.55 ±41.10	79.56 ±13.0	107.81 ±21.89	54.00 ±23.21	52.03 ±26.19	49.27 ±0.52

Table 4. Estimation of various biological and physico-chemical properties of soil (mean for 14 months observation).

Soil treatment	Soil depth (cm)	PH	MC (%)	TOC (%)	TN (%)	AP (µgg⁻¹ dry soil)	K (%)	C (µg C g⁻¹ dry soil)	FP (CFUX 10^{-3})	BP (CFUX 10^{-5})
CTRL	0-10	5.48	23.94	2.07	0.15	28.87	0.019	223.39	44.44	6.08
	10-20	5.26	22.88	1.63	0.10	16.33	0.013	123.40	15.06	3.10
NPK	0-10	5.39	23.68	2.06	0.16	32.70	0.017	264.36	44.57	6.23
	10-20	5.23	22.92	1.65	0.12	20.25	0.012	157.19	17.82	3.67
FYM	0-10	5.61	24.92	2.23	0.17	28.37	0.017	217.38	35.68	7.17
	10-20	5.43	23.91	1.78	0.12	19.49	0.013	141.74	15.29	2.97
NPK+FYM	0-10	5.52	25.01	2.32	0.15	25.31	0.017	270.33	37.24	5.99
	10-20	5.51	24.12	1.88	0.11	18.30	0.014	179.39	18.26	3.20

MC=soil moisture content, TOC=total organic carbon, TN=total nitrogen, AP=available phosphorus, K=exchangeable potassium, C_{mic}=microbial biomass carbon, FP=fungal population, BP=bacterial population)

revealed that fertilizers treatment (inorganic or organic) showed insignificant variation (P< 0.05) on microbial biomass carbon as compared to control plot (Table 5) and the soil microbial carbon showed significant declined (P< 0.01) of C_{mic} from surface to sub-surface layers (Table 6).

DISCUSSION

The increased in C_{mic} after application of fertilizers could be due to the increased in colony forming unit of fungi and bacteria, as they are reported to be the main component of soil microbial biomass (Jordan *et al.*, 1995). The microbial counts were higher at post treatment as compared to the pre-treatment which is in agreement with the observation of Polyanskaya *et al.*, (1997), that C_{mic} measures the total biomass C (fungi + bacteria) and the specific studies have indicated that fungi are dominant component of the total soil microbial biomass accounting for up to 90% of total. In agricultural soils, ploughing, tillage, application of fertilizers and types of cultivation affect the microorganisms (Domsch, 1986). A linear relationship was obtained between C_{mic} and microbial population at both the soil layers (Fig. 3 and 4). Though the soil microbial population was increased after the application of fertilizers, the major controlling factors of microbial population could be the leguminous plant (root exudates) and soil processing (ploughing) rather than the application of fertilizers. Reductions in C_{mic} in fertilized soils have been discussed extensively by Lovell *et al.*, (1995) and attributed to changes in substrate quality and root growth (Hassink, 1992; Lovell *et al.*, 1995), changes in microbial competition and community structure (Fog, 1988). It is well known that plant root exudates can influence soil microbial growth. Thus, leguminous plant was expected to support soil microbial population especially those of symbiotic nitrogen fixing bacteria i.e. *Rhizobium* sp. Further, higher C_{mic} values are commonly found in crops with intensive root growth and root density (Perfect *et al.*, 1990). The variation in the concentrations and types of organic compounds released by the roots of different plants can also effect the microbial population in the rhizosphere (Lynch and Bragg, 1985).

The linear relationship between C_{mic} and total organic carbon was also in conformity with the result of Wardle (1992) which indicated that soil microbial biomass are usually resource limited and thus microbial C concentration is generally related to amount of soil C (Fig.5). The effects of inorganic fertilizer and organic amendments on organic matter-microbial biomass relationships in field experiments under tropical conditions have shown that soil microbial biomass C and N increased with balanced

Table 5. One-way ANOVA of soil microbial biomass carbon (P<0.05)

Source of Variation	Surface layer (0-10cm)		Subsurface Layer (10-20 cm)	
	F-ratio	P-level	F-ratio	P-level
CTRL X NPK X FYM X NPK+FYM	1.040	NS	1.259	NS
CTRLXNPK	1.645	NS	1.845	NS
CTRL X FYM	0.050	NS	0.712	NS
CTRL X NPK+FYM	1.203	NS	2.710	NS
NPK X FYM	2.118	NS	0.37	NS
NPK X NPK+FYM	0.016	NS	0.374	NS
FYM X NPK+FYM	1.512	NS	1.210	NS

NS=not significant

Table 6. One-way ANOVA of soil microbial biomass carbon (C_{mic}) between surface and subsurface soil layers (p/<0.001)

Treatments	F-ratio	P-level
Canned tuna	76	19
CTRL	17.3848	7.5×10^{-5}
NPK	10.8026	1.4x 10'3
FYM	9.3436	3xlO'3\
NPK + FYM	3.4275	NS

NS=not significant

fertilization and the also the addition of organic amendments increased microbial biomass even when the organic C content of the soil did not increase (Goyal *et al.*, 1992). The soil management practice i.e. manuring increased carbon input to the soil (Ritz *et al.*, 1997) and therefore the ratio of microbial C to soil organic carbon has thus been used as an indicator for C availability (Insam and Domsch, 1988).

The agricultural soil has lower C_{mic} as compared to non-agricultural soil and as reported inorganic fertilizer reduced soil microbial biomass carbon (Biederbeck *et al.*, 1984; McAndrew and Malhi, 1992; Ladd *et al.*, 1994). In our experiment, control and FYM plots were expected to show higher amount of C_{mic} rather than inorganic (NPK) treated plot (Sakamoto and Oba, 1994; Lovell *et al.*, 1995; Hopkins and Shiel, 1996). It was also noted that manure applications typically result in increased soluble organic C in soil (Bhogal and Shepard, 1997; Gregorich *et al.*, 1998; Liang *et al.*, 1998). But our investigation showed inconsistent distribution of C_{mic} (higher C_{mic} in NPK or NPK+FYM plots in some month). Therefore, it can be hypothesized that this inconsistent distribution of C_{mic} could be due to the retention of inorganic fertilizers, which were already applied earlier in this field for other experimental purposes. This hypothesis is in agreement with the experimental observation of Bardgett and Shine (1999) that neither the cessation of fertilizer application nor changes in cutting and grazing management significantly affected soil microbial biomass or the fungal: bacterial biomass ratio and the lack of effects on the soil microbial community may be related to high residual fertility caused by retention of fertilizer N in the soil. The analysis of variance revealed that fertilizers treatment (inorganic or organic) showed insignificant variation ($P < 0.05$) on microbial biomass carbon as compared to control plot.

Studies on the effect of inorganic fertilizer application on soil microbial biomass have increase in the size of microbial biomass (Omay *et. al.* 1997; Srivastava and Lal, 1994), whereas others have shown the opposite which is in conformity with our finding (Biederbeck *et al.*, 1984; McAndrew and Malhi, 1992; Wardle, 1995; Srivastava, 1992; Singh and Singh, 1993; Henrot and Robertson, 1994; Ladd *et al.*, 1994). The reduction in Cmic under fertilizers treatment might be due to the high level of mineral N availability and have been attributed to changes in substrate quality, root growth (Hassink, 1992; Lovell *et. al.*, 1995) and changes in microbial competition and community structure, repression of enzyme activity and the build-up of recalcitrant and toxic compounds (Fog, 1988). Reductions in microbial biomass in fertilized soil could be due to the acidifying

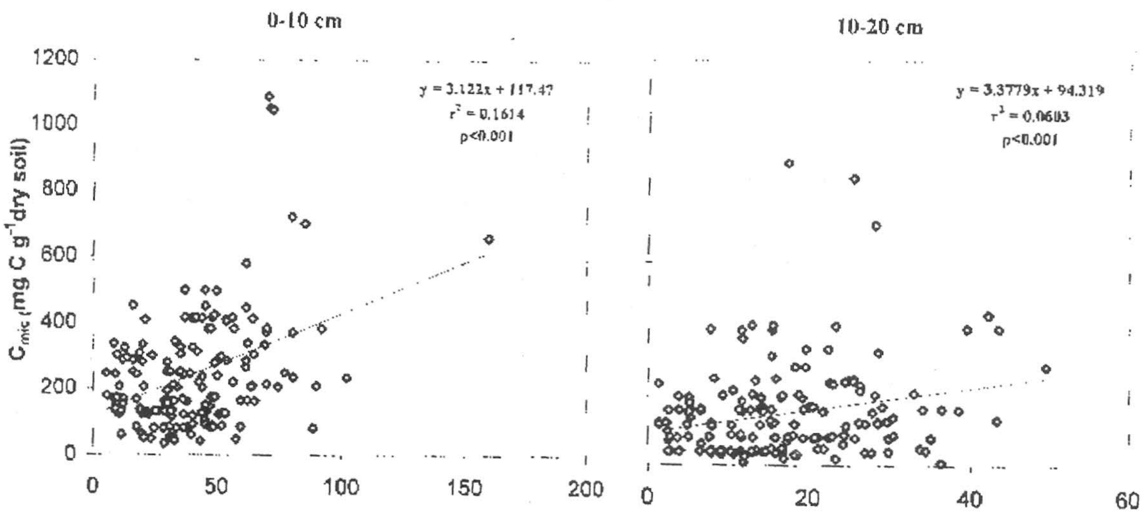

Fig. 3. Relationships of C_{mic} with fungal population at surface and sub surface soil layers.

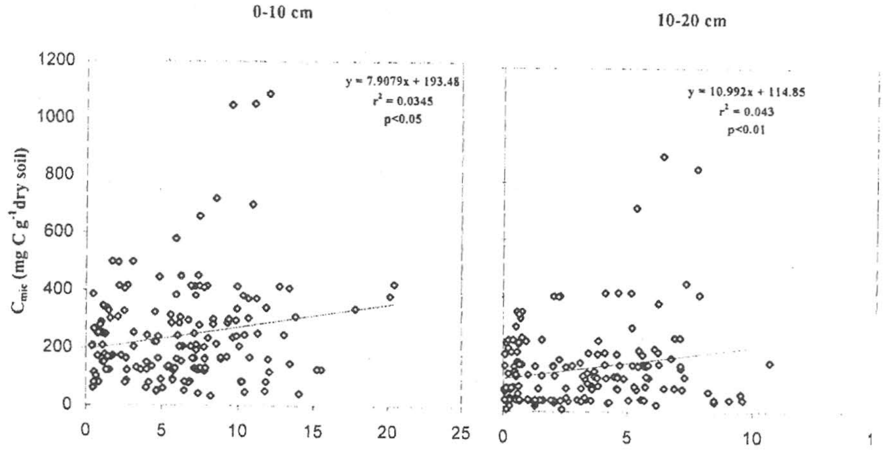

Fig. 4. Relationships of C_{mic} with bacterial population at surface and sub surface soil layers.

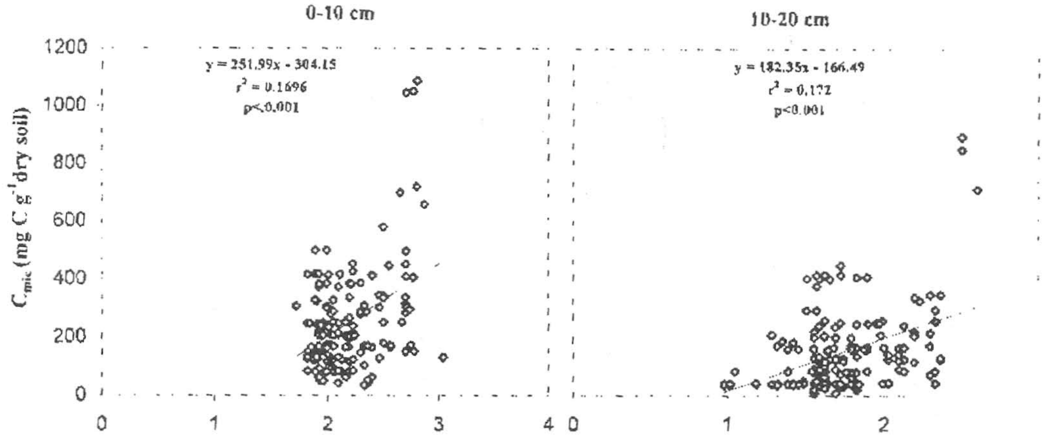

Fig. 5. Relationships of C_{mic} with total organic carbon at surface and sub surface soil layers.

effect of nitrogenous fertilizers (microbial production of nitric oxide) in soil (Christie andBeattie, 1989).

Higher accumulation of microbial biomass carbon was observed at the surface soil layer and has been attributed to higher microbial population and more accumulation of organic carbon than that at the subsurface soil layer (Lavahun *et al.*, 1996). Thus, it can be concluded that C_{mic} increased shortly after the application of fertilizers and inconsistent distribution of C_{mic} within each treatment. Fertilizers application has lowered the amount of C_{mic} as compared to the non-agricultural field soil. Surface soil layer showed higher accumulation of microbial biomass carbon than the subsurface soil layer. The insignificant variation of soil microbial biomass carbon and its inconsistent distribution within treatments may be due to the effects of host legumes root exudation, retention of fertilizers, environmental factors and microbial efficiency (Wardle and Ghani 1995).

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