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Microbial biomass as an indicator of nutrient status of soil as influenced by parthenium and chromolaena as green manures and their compost under submerged condition

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ABSTRACT

Laboratory incubation was conducted to study the changes in the microbial biomass nitrogen and microbial biomass carbon under waterlogged condition of paddy soil with the use of parthenium and chromolaena as green manures and their compost. Based on the content of total C of plant material organic material was applied at the rate of 0.5, 1.0 and 2.0 per cent of organic carbon. The increase in extractable C in soil alone treatment was 24.53 mg/kg soil at 60th day of flooding. On 120th day of flooding it was 30.12 mg/kg. The E_N values also increased with time. Addition of organic C through different sources at 0.5, 1.0 and 2.0 per cent levels increased the E_C and E_N values. Biomass C content (B_C) across different treatments at 60th day of flooding ranged from 65 to 286 mg/kg in soil. Lowest microbial biomass content was noticed in T_5 (Parthenium green manure @ 0.5% C) among treatments which received organic carbon inputs.

Keywords: Microbial biomass; parthenium; chromolaena; compost; green manure

INTRODUCTION

Microbes are an integral component of a living soil. It is widely being recognized that the presence and abundance of microbial wealth makes soils healthy in terms of growth enhancement and protection against pests and diseases. Adding of organic manures significantly increases the microbial density and diversity in soils. Sensitive changes in microbial biomass, quantum and characters of biomass develop upon flooding, fertilization and residue management practices. Although microbial biomass only accounts for about 1 to 3 per cent of the soil organic matter it exhibits a rapid turnover and can be considered as a driving force of major nutrient cycles. Hence an attempt was made to assess the microbial biomass and changes in major nutrients of paddy soil upon addition of parthenium and chromolaena as green manures and their composts under flooded condition.

MATERIAL and METHODS

The soil used for the study was typic rhodustalf collected at Mandya Taluk, Mandya district, Karnataka. Soil samples were air-dried, sieved (<2 mm) and analyzed for physico-chemical properties (pH 6.47, EC 0.06 dS/m, total organic carbon 0.47%, NH_4^+-N 29.58 mg/kg, NO_3-N 6.08 mg/kg, Brays-P 6.57 mg/kg and NH_4OAc-K 62.37 mg/kg). There were 13 treatments and 3 replications. Chromolaena and parthenium were applied as green manures at the rate of 0.5, 1.0 and 2 per cent C respectively. Similarly chromolaena and parthenium composts were applied at the rate of 0.5, 1.0 and 2 per cent C. Control was maintained with treatments.

Appropriate amount of parthenium and chromolaena as green manures and their composts were added to soil (500 g) contained in polythene pots. Excess amount of water was added and mixed to create puddled conditions. At periodic intervals destruction sampling was done at 60 and 120 days after flooding for analyzing microbial biomass and nutrient status of soil. For all measurements wet soil samples were taken. Results were expressed on oven-dry weight basis after taking account of moisture per cent.

The NH_4^+-N of soil extracted with 2 M KCl was estimated by subsequent steam distillation (Bremner 1965). Phosphorus was extracted with 0.03 N NH_4 F in 0.025 N HCl and estimated according to Bray and Kurtz (1945). Potassium extractable with N, N NH_4OAc and estimated by flame photometry was analyzed as available K (Jackson 1973).

Microbial biomass carbon measurements

Chloroform fumigation–extraction technique standardized for paddy soils was employed for measuring biomass C content (Inubushi et al 1991). Moist soil samples were divided into two portions. One portion was extracted with 0.5 M K_2SO_4 by shaking for 30 minutes and filtered. Other portion was fumigated with addition of 1 ml ethanol-free chloroform in 250 ml conical flask with stopper for 24 hours at 25°C (Williamson et al 1995). Finally the fumigant was removed and the samples were extracted with 0.5 M K_2SO_4 for 30 minutes and filtered.

The contents of organic carbon in the fumigated and non-fumigated K_2SO_4 soil extracts were measured by dichromate digestion (Vance et al 1987) and E_C values were calculated. E_C value is the difference in C extracted from $CHCl_3$ fumigated sample and C extractable from non-fumigated sample. From E_C biomass C content was calculated using the relationship biomass C in mg/g oven-dry soil= 2.65 x E_C where E_C is also expressed in mg/g oven-dry soil (Shibahara Inubushi 1995).

Microbial biomass N measurement

Microbial biomass N measurement was done after biocidal treatment with 0.5 M K_2SO_4. Total N in the K_2SO_4 extracts was measured after Kjeldahal digestion using 0.6 ml of 0.19 M $CuSO_4$ and 10 ml concentrated H_2SO_4. After digestion it was allowed to cool and distilled with 25 ml of 10 M NaOH and distillate was collected in 5 ml of two per cent boric acid with a mixed indicator. Finally distillate was titrated with 50 ml 1 M H_2SO_4 to faint pink colour (Brookes et al 1985). E_N was calculated by deducting quantity of N extractable with 0.5 M K_2SO_4 without $CHCl_3$ treatment from the quantity of N extractable with 0.5 M K_2SO_4 after $CHCl_3$ treatment. Biomass N in mg/kg oven-dry soil= E_N x 2.22 where E_N is mg/kg oven-dry soil (Shibahara and Inubushi 1995).

RESULTS and DISCUSSION

Microbial biomass properties

Data on chemical composition of organic materials used in the experiment are presented in Table 1. Highest carbon and nitrogen content was noticed in chromolaena followed by parthenium. Phosphorus content ranged from 0.63 to 1.93 per cent; lowest in parthenium and highest in chromolaena compost. Maximum K content was noticed in chromolaena compost (1.89%) followed by parthenium compost (1.46%). The C-N ratio showed wide variation among organic materials. It ranged from 15.50 (chromolaena compost) to 23.95 (parthenium).

Cellulose content was found to vary in the organic materials. Highest cellulose content was observed in chromolaena (18.76%) followed by parthenium (12.04%). Lignin content of the materials ranged from 5.20 per cent in chromolaena compost to 26.63 per cent in parthenium (Table 1). Lignin-N ratio was observed to be between 3.93 to 14.02.

In the present investigations E_C-E_N ratio ranged from 7.50 to 7.96 on 120th day of flooding in organic manure applied soils (Table 2). Generally E_C-E_N ratio of aerobically-treated soils was reported to be smaller (10-12) than those under anaerobic conditions (14-20). E_C-E_N ratio in the soils ranged from 4 to 6, values smaller than those recorded in well drained paddy soil (9-22) (Takai et al 1956, Jenkinson 1976, Inubushi et al 1991, Shibahara and Inubushi 1995). These findings suggest that during initial period up to 60th day of flooding microbes that are capable of thriving in oxygen depleted and ill-drained conditions decomposed added organic matter and immobilized soils nutrients depending on the quality of the organic manure.

Quantity of soil microbial biomass is another soil quality parameter that increases to a great extent due to incorporation of organic carbon sources at 2.0 per cent C level. The organic load given to soils modifies the properties of microbial biomass also. Organic matter-treated soils showed higher E_C-E_N ratio at 120th day than at 60th day of flooding. Very high build up of microbial biomass C was noticed in both parthenium and chromolaena compost treated soils and

Table 1. Biochemical composition of the organic manures

Parameter	Chromolaena	Chromolaena compost	Parthenium	Parthenium compost
Carbon (%)	39.30	20.47	38.32	19.32
Nitrogen (%)	1.90	1.32	1.60	0.99
Phosphorus (%)	0.69	1.93	0.63	1.03
Potassium (%)	1.08	1.89	0.98	1.46
C:N	20.68	15.50	23.95	19.15
Cellulose (%)	18.76	11.92	12.04	6.79
Lignin (%)	11.36	5.20	26.63	6.63
Lignin:N	7.04	3.93	14.02	6.69

B_C contents of these treatments were very high on 120th day of flooding. Biomass C content (B_C) across different treatments at 60th day of flooding ranged from 65 to 286 mg/kg. (Table 3). Lowest microbial biomass and microbial biomass nitrogen content was noticed in T_5 (Parthenium green manure @ 0.5% C) among treatments which received organic carbon inputs. Increasing the C level resulted in increasing B_N and B_C but to different degrees depending on the native of C sources. Increasing the C input from 0.5 to 2.0 per cent C narrowed B_C-B_N ratio.

The microorganisms which developed after incorporation of fresh residues initially had very high quantities of nitrogen with respect to carbon demand. Initially when abundant supply of labile C compounds was present microbes readily utilized them and built up biomass and incorporated large amounts of N in their skeletal structure. As labile compounds were used up gradually biomass C and N contents came down releasing nitrogen to soluble pool. Takai (1956) observed that biomass of waterlogged soils actually possessed high nitrogen concentrations. Certain anaerobic bacteria have narrow C-N ratio whereas fungi have wider C-N ratio of microbial biomass which confirm the dominance of bacterial population at the expense of fungi.

Addition of different levels of organic manures to soils showed highly significant changes in nutrient content of soils over control at 60 and 120 days after flooding. The observed increase in 2 M KCl extractable ammonical nitrogen content in control might be due to accumulation of ammonium as a consequence of N mineralization from native soil organic matter and due to lack of oxygen for nitrification (Table 4). Sahrawat (1983) observed a positive correlation between ammonia production and organic carbon content of soils.

In case of soils with standing water inactivation of aerobic bacteria causes an accumulation of NH_4^+-N (Murthy and Singh 1978). N release rate from N-rich organic material used in the experiment might be quick as explained by Weeraratna (1979) and Ladd et al (1981). Increasing the level of carbon application via these high N materials further increased KCl extractable N content.

N mineralization has shown that it is the form of N and C (Tenny and Waksman 1929) and C to N ratio present in the material is important. N mineralization from different organic materials also depends on certain biochemical compounds and their ratios (Palm and Sanchez 1991) in upland soils. Becker and Ladha (1997) showed that lignin-nitrogen ratio (L:N) is the best index, among C-N, polyphenols-N predict N release pattern in the soils.

Persual of the data presented in Table 4 indicates an increase in Brays-P content upon submergence. According to Ponnamperuma (1978) in general the increase in P availability under submerged conditions may be due to reduction of ferric phosphates present in soil, release of occluded phosphate and phosphate sorbed in amorphous iron and manganese oxides following soils reduction, increase in pH of acid soils, desorption following reduction of Fe (III) oxides, desorption by clays and aluminium oxides following pH increase and solvent action of inorganic and organic acids on soil phosphates.

Simultaneously some inorganic P may be released from mineralization of organic P pools of soils and applied organic manures during the incubation process. High available P content noticed in compost treatment compared to green manure treatments in both soils may be due to the release of organic P because composts are enriched with rock phosphate.

Table 2. 0.5 M K_2SO_4 extractable carbon (E_C) and nitrogen (E_N) (mg/kg oven-dry soil) after chloroform fumigation under flooded condition

Treatment	60 DAF			120 DAF		
	E_C	E_N	E_C:E_N	E_C	E_N	E_C:E_N
T_1: Control (soil alone)	24.53	3.36	7.28	30.12	4.05	7.43
T_2: Chromolaena as green manure @ 0.5% C	81.58	10.81	7.54	89.69	11.86	7.56
T_3: Chromolaena as green manure @ 1.0% C	85.31	11.10	7.68	91.70	11.83	7.75
T_4: Chromolaena as green manure @ 2.0% C	92.83	11.81	7.86	101.38	12.86	7.88
T_5: Parthenium as green manure @ 0.5% C	77.61	10.36	7.49	87.94	11.71	7.50
T_6: Parthenium as green manure @ 1.0% C	90.56	11.59	7.81	103.08	13.11	7.86
T_7: Parthenium as green manure @ 2.0% C	96.11	12.01	8.00	105.56	13.32	7.92
T_8: Chromolaena as compost @ 0.5% C	89.56	11.84	7.56	112.27	14.77	7.60
T_9: Chromolaena as compost @ 1.0% C	97.75	12.12	7.74	122.08	15.61	7.82
T_{10}: Chromolaena as compost @ 2.0% C	107.92	13.59	7.94	132.73	16.67	7.96
T_{11}: Parthenium as compost @ 0.5% C	81.13	10.81	7.50	84.99	11.24	7.56
T_{12}: Parthenium as compost @ 1.0% C	93.75	11.97	7.83	109.72	13.96	7.85
T_{13}: Parthenium as compost @ 2.0% C	101.64	12.64	8.04	114.55	14.56	7.90
SEm±	4.13	3.92	-	9.31	3.02	-
LSD (P= 0.05)	12.07	11.98	-	27.05	5.81	-

DAF= Days after flooding, E_C= Carbon extractable $CHCl_3$ minus C extractable without $CHCl_3$, E_N= Nitrogen extractable after $CHCl_3$ minus N extractable without $CHCl_3$

Table 3. Changes in the properties of microbial biomass (mg/kg oven-dry soil) in the soil incorporated with different organic manures under flooded condition

Treatment	60 DAF			120 DAF		
	B_C	B_N	B_C:B_N	B_C	B_N	B_C:B_N
T_1: Control (soil alone)	65	7	9.28	79	9	8.77
T_2: Chromolaena as green manure @ 0.5% C	213	24	8.88	226	26	8.69
T_3: Chromolaena as green manure @ 1.0% C	226	26	8.69	243	28	8.67
T_4: Chromolaena as green manure @ 2.0% C	243	28	8.82	266	31	8.58
T_5: Parthenium as green manure @ 0.5% C	203	23	8.74	226	26	8.69
T_6: Parthenium as green manure @ 1.0% C	236	27	8.56	256	30	8.53
T_7: Parthenium as green manure @ 2.0% C	257	30	8.74	278	32	8.68
T_8: Chromolaena as compost @ 0.5% C	236	27	8.63	256	32	8.00
T_9: Chromolaena as compost @ 1.0% C	259	30	8.66	276	32	8.62
T_{10}: Chromolaena as compost @ 2.0% C	286	33	8.60	307	35	8.77
T_{11}: Parthenium as compost @ 0.5% C	215	25	8.60	223	26	8.58
T_{12}: Parthenium as compost @ 1.0% C	247	28	8.82	265	31	8.54
T_{13}: Parthenium as compost @ 2.0% C	265	33	8.03	281	32	8.78
SEm±	15.52	11.01	-	18.04	14.52	-
LSD (P= 0.05)	45.56	32.08	-	53.63	42.08	-

DAF= Days after flooding, B_C= Biomass carbon, B_N= Biomass nitrogen

Data on available potassium content of flooded soils indicated a slight increase with time (Table 4). NH_4OAc extractable potassium showed highly significant variation over control upon addition of different levels of organic manure at 60 and 120 days after flooding. These findings are in line with the results of Ponnamperuma (1978) and Katyal (1977). Among all the organic treatments major increase in available K content was noticed. It is believed that K present in plant residues could be extracted with water as K will not be a structural constituent in residues unlike C, N, P and S. Therefore major portion of K is liberated to soil solution immediately after flooding (Chaminade 1955, Nagarajah et al 1989). Highest and lowest

Table 4. Changes in the ammonical N, P_2O_5 and K_2O content of soil (mg/kg) during decomposition of added organic manures under flooded condition

Treatment	NH_4^+– N		P_2O_5		K_2O	
	60 DAF	120 DAF	60 DAF	120 DAF	60 DAF	120 DAF
T_1: Control (soil alone)	49.63	54.84	7.52	5.26	74.29	79.81
T_2: Chromolaena as green manure @ 0.5% C	51.61	54.84	17.96	16.50	78.63	85.60
T_3: Chromolaena as green manure @ 1.0% C	53.73	59.09	18.56	18.75	82.16	88.34
T_4: Chromolaena as green manure @ 2.0% C	55.10	63.60	19.63	24.34	91.00	98.34
T_5: Parthenium as green manure @ 0.5% C	49.74	55.78	19.02	17.36	76.53	82.58
T_6: Parthenium as green manure @ 1.0% C	51.00	57.38	18.03	18.72	78.00	84.51
T_7: Parthenium as green manure @ 2.0% C	52.23	59.38	19.22	19.63	81.39	86.36
T_8: Chromolaena as compost @ 0.5% C	58.75	66.74	18.53	19.65	96.74	108.56
T_9: Chromolaena as compost @ 1.0% C	61.34	71.84	19.63	21.62	98.63	110.57
T_{10}: Chromolaena as compost @ 2.0% C	71.03	83.71	20.84	21.00	99.99	112.74
T_{11}: Parthenium as compost @ 0.5% C	56.34	62.71	20.96	22.60	92.72	100.53
T_{12}: Parthenium as compost @ 1.0% C	59.09	66.07	21.63	22.64	93.38	105.99
T_{13}: Parthenium as compost @ 2.0% C	62.74	70.46	21.39	21.50	95.34	109.74
SEm±	12.58	16.87	3.90	3.00	12.04	15.34
LSD (P= 0.05)	35.87	46.35	10.13	9.07	34.57	42.65

DAF= Days after flooding

available K contents were observed among various treatments depended mainly on K content of the organic manures and rate of incorporation of the material.

The present investigations showed that addition of organic C significantly increased microbial biomass C and N contents in soils under flooded condition. The increased microbial biomass also increased the availability of ammonical nitrogen, phosphorus and potassium in soils. The increase in biomass contents varied with the nature of organic manure added, rate of C application and the duration of decomposition process.

REFERENCES

Becker M and Ladha JK 1997. Synchronizing residue N mineralization with rice N demand derived in flooded conditions. In: Driven by nature: plant litter quality and decomposition (Cadisch and KE Gille eds), CAB International, Wallingford, UK, pp 231-238.

Bray RH and Kurtz LT 1945. Determination of total, organic and available forms of phosphorus in soils. Soil Science **59:** 39-46.

Bremner JM 1965. Inorganic forms of nitrogen. In: Methods of soil analysis (CA Black, DD Evans, JL. White, LE Ensminger, and FE Clark eds), Part 2, 2nd edn, Agronomy Monograph # 9, American Society of Agronomy and Crop Science Society of America, Madison, pp 1179-1237.

Brookes PC, Landman A, Pruden G and Jemkinson DS 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method for measuring microbial biomass nitrogen in soil. Soil Biology and Biochemistry **17(6):** 837-842.

Chaminade R 1955. Le potassium et le matiere organique. Symposium, International Potash Institute, Rome, pp 203-214.

Inubushi K, Brookes PC and Jenkinson DS 1991. Soil microbial biomass C, N and ninhydrin – N in aerobic and anaerobic soils measured by the fumigation-extraction method. Soil Biology and Biochemistry **23:** 737-741.

Jackson ML 1973. Soil chemical analysis. Prentice Hall of India Pvt Ltd, New Delhi, India, 498p.

Jenkinson DS 1976. The effects of biocidal treatments on metabolism in soil. IV. The decomposition of fumigated organisms in soil. Soil Biology and Biochemistry **8:** 203-208.

Katyal JC 1977. Influence of organic matter on the chemical and electro-chemical properties of some flooded soils. Soil Biology and Biochemistry **9:** 259-266.

Ladd JN, Oades JM and Amato M 1981. Distribution and recovery of nitrogen from legume residues decomposing in soils sown to wheat in the field. Soil Biology and Biochemistry **13:** 251-256.

Murthy GK and Singh TA 1978. Kinetics of ammonification and denitrification in submerged calcareous soils. Oryza **15:** 52-57.

Nagarajah S, Neue HV and Alberto MCR 1989. Effect of sesbania, azolla and rice straw incorporation on the kinetics of NH_4, Fe, Mn, Zn and P in some flooded soils. Plant and Soil **116(1):** 37-48.

Palm CA and Sanchez PA 1991. Nitrogen release from the leaves of some tropical legumes as affected by their lignin and polyphenolic contents. Soil Biology and Biochemistry **23:** 83-88.

Ponnamperuma FN 1978. Electro-chemical changes in submerged soils and the growth of rice (under Philippine conditions). In: Soils and rice, International Rice Research Institute, Los Banos, Philippines, pp 421-443.

Sahrawat KL 1983. Mineralization of soil organic nitrogen under waterlogged conditions in relation to other properties of tropical rice soils. Australian Journal of Soil Research **21(2):** 133-138.

Shibahara F and Inubushi K 1995. Measurements of microbial biomass C and N in paddy soils by the fumigation-extraction method. Soil Science and Plant Nutrition **41:** 681-689.

Takai Y, Koyama T and Kamura T 1956. Microbial metabolism in reduction process of paddy soils. Part I, Soil and Plant Food **2:** 63.

Tenny FG and Waksman SA 1929. Comparison of natural organic materials and their decomposition in the soil. IV. The nature and rapidity of decomposition of the various organic complexes in different plant materials under aerobic conditions. Soil Science **28:** 55-84.

Vance ED, Brookes PC and Jenkinson DS 1987. An extraction method for measuring soil microbial biomass. Soil Biology and Biochemistry **19:** 703-707.

Weeraratna CS 1979. Pattern of nitrogen release during decomposition of some green manures in a tropical alluvial soil. Plant and Soil **53(3):** 287-294.

Williamson WM, Blunt JW and Greenfield LG 1995. Method for rapid removal of ethanol from chloroform in soil microbial biomass determinations. Communications in Soil Science and Plant Analysis **26(3-4):** 407-410.