

## SEASONAL CHANGES IN SOIL MICROBIAL BIOMASS CARBON UNDER A WHEAT-MAIZE CROPPING SYSTEM RECEIVING UREA AND FARMYARD MANURE IN DIFFERENT COMBINATIONS

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### Abstract

Seasonal changes in carbon (C) availability and soil microbial biomass carbon ( $C_{mic}$ ) were studied under an irrigated wheat-maize system receiving urea at 50 or 100 kg N ha<sup>-1</sup> year<sup>-1</sup> in combination with 8 or 16 t ha<sup>-1</sup> year<sup>-1</sup> of farmyard manure (FYM). Treatment effects on  $C_{mic}$  were not visible during the wheat growing season, whereas fertilizer application significantly increased  $C_{mic}$  during the maize growing season. In unfertilized soil,  $C_{mic}$  was similar under two crops, whereas fertilized soils showed 11–56% higher  $C_{mic}$  under maize than under wheat. Under both crops, C availability, as assessed by aerobically mineralizable C (AMC) and total organic C, was generally higher in fertilized soils than in the unfertilized. Fertilized soils showed much higher AMC under wheat than under maize; the stimulatory effect being much more pronounced due to increasing application rate of FYM than that of urea. In fertilized soils, but not in the unfertilized, specific respiratory activity (SRA) of soil microbial biomass was twice higher under wheat as compared to that under maize. Results suggested that the soil microflora under wheat was probably dominated by ‘*r*-strategists’, which respired more C as CO<sub>2</sub> than that incorporated into microbial biomass. In contrast, ‘*k*-strategists’ dominated under maize, incorporating relatively more C into their biomass than that respired as CO<sub>2</sub>. Fertilizer application significantly increased  $C_{mic}$  turnover rate; while increasing the FYM application rate further increased the turnover rate, the increasing urea level had no effect. The overall high  $C_{mic}$  turnover rate, particularly in fertilized soils (1.69–2.29 year<sup>-1</sup>) indicated that nutrient cycling through soil microbial biomass may be substantial under agro-climatic conditions prevailing in this region.

### Introduction

Soil microbial biomass, carbon on the average, constitutes only about 1% of the soil organic carbon (Moore *et al.*, 2000), yet it may comprise up to 44% of the active pool of soil nitrogen (Liang *et al.*, 1999). Therefore, the size of soil  $C_{mic}$  pool and its turnover have significant bearing on the overall productivity of soils. Several studies indeed show a close relationship between soil microbial biomass and nutrient availability to plants (Jenkinson & Lad, 1981; Houot & Chaussod, 1995).

Application of mineral N fertilizers and manures has been reported to significantly influence the build-up and dynamics of soil microbial biomass (Bolton *et al.*, 1985; Powlson *et al.*, 1987; Mahmood *et al.*, 1997). While organic amendments generally increase  $C_{mic}$  (Mazzarino *et al.*, 1993; Goshal and Singh, 1995), there are diverse reports

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regarding the effect of mineral N fertilizers on the size and activity of soil microbial biomass. Application of mineral N fertilizers either had no effect (Moore *et al.*, 2000), or caused an increase in the content of  $C_{mic}$  (Houot & Chaussod, 1995; McCarty *et al.*, 1995; Goshal & Singh, 1995). Cropping history, type of plant cover, plant age, and climatic changes are also well known to influence soil microbial biomass and its activity, thus leading to considerable seasonal fluctuations in  $C_{mic}$  (Franzluebbers *et al.*, 1994; Xu & Juma, 1993; Mendes *et al.*, 1999; Moore *et al.*, 2000; Piao *et al.*, 2000). However, only few long-term field studies exist to ascertain the practical significance of these fluctuations on crop growth and nutrient cycling (Kaiser & Heinmeyer, 1993; Joergensen *et al.*, 1994; Goshal & Singh, 1995).

Studies on dynamics of soil microbial biomass have been particularly lacking under agro-climatic conditions prevailing in Pakistan. Previously, we reported seasonal changes in carbon availability and  $C_{mic}$  in a sandy-clay loam under a wheat-maize cropping system that had been under a long-term fertilizer trial and receiving for the past ten years 100-200 kg N ha<sup>-1</sup> year<sup>-1</sup>, either as urea or as FYM (Mahmood *et al.*, 1997). However, information on soil microbial properties has been lacking for cropping systems in which mineral N and FYM are applied together in different combinations; farmers in Pakistan possessing small land holdings often follow this practice. The present report describes the seasonal fluctuations in soil microbial biomass and its activity under a wheat-maize cropping system fertilized with two urea levels in combination with two levels of FYM.

### Materials and Methods

The experimental site at the Nuclear Institute for Agriculture & Biology, Faisalabad is located at 73.2° longitude, 31.4° latitude and 183m above the sea level. The area has a semiarid subtropical climate with a mean annual rainfall of 340 mm, most of which is received as heavy monsoon downpours during July and August. The hottest months are May and June, with mean maximum temperatures of 39.4° and 41.1°C, respectively, whereas January is the coldest month with a mean minimum temperature of 5°C. The soil belongs to Hafizabad series (Haplic Yermosol; Anon., 1966) and is a deep, well-drained sandy-clay loam developed in a mixed calcareous medium-textured alluvium derived from Himalayas. The soil had been under a long-term soil fertility experiment on irrigated wheat-maize cropping system receiving different urea and/or farmyard-manure (FYM) treatments for the past ten years.

Twenty experimental plots (7.5×8.5 m) were established for five treatments in a randomized complete block design, each with four replicates. The treatments included: an unfertilized control and urea applied at 50 and 100 kg N ha<sup>-1</sup> year<sup>-1</sup>, each combined with either 8 or 16 t ha<sup>-1</sup> year<sup>-1</sup> of FYM. Treatment details and physicochemical properties of the soil after ten years under different fertilizer treatments are given in Table 1. Wheat (*Triticum aestivum* L. cv. Pak-81) was sown on 6<sup>th</sup> December and harvested on 5<sup>th</sup> May, whereas maize (*Zea mays* L. cv. Akbar) was sown on 29<sup>th</sup> August and harvested as fodder on 30<sup>th</sup> October. A total of six irrigations were applied during the wheat season: all were equivalent to 75 mm except the first (presowing) and the fourth which were 100 mm and 50 mm, respectively. During the maize season, five irrigations were applied: all being equivalent to 75 mm except the first (presowing) and the last, which were 100 mm and 50 mm, respectively. The soil was sampled five and three times during the wheat and maize growing season, respectively. From each replicate plot, ten soil cores (3×15 cm, diameter×depth) were randomly collected, pooled, mixed, visible roots manually removed, and the soils sieved (<2 mm). The soil moisture content at the time of sampling

Table 1. Physico-chemical characteristics of the sandy-clay loam under wheat-maize cropping system receiving different fertilizer treatments for the past ten years.

Treatment <sup>a</sup>	Field capacity (%)	Bulk density (g cm <sup>-3</sup> )	Pore space (%)	Electrical conductivity (dS m <sup>-1</sup> )	pH	Total organic C (%)	Total nitrogen (%)	Mineralizable nitrogen (mg g <sup>-1</sup> )	CaCO <sub>3</sub> (%)
T <sub>1</sub> . U <sub>50</sub> +F <sub>8</sub>	16.95	1.40	47.95	0.76	7.70	1.32	0.078 (16.92) <sup>b</sup>	67.70	1.92
T <sub>2</sub> . U <sub>50</sub> +F <sub>16</sub>	16.78	1.43	46.65	0.95	7.65	1.33	0.084 (15.83)	67.95	1.98
T <sub>3</sub> . U <sub>100</sub> +F <sub>8</sub>	16.70	1.42	46.59	0.74	7.60	1.13	0.075 (15.07)	68.25	1.81
T <sub>4</sub> . U <sub>100</sub> +F <sub>16</sub>	17.03	1.42	46.90	0.88	7.65	1.34	0.084 (15.95)	69.40	1.79
T <sub>5</sub> . Unfertilized	16.38	1.43	46.32	0.63	7.70	1.11	0.069 (16.09)	62.40	1.97

<sup>a</sup>U<sub>50</sub> and U<sub>100</sub> indicate urea equivalent to 50 and 100 kg N ha<sup>-1</sup> year<sup>-1</sup>, respectively, whereas F<sub>8</sub> and F<sub>16</sub> indicate farmyard manure applied at 8 and 16 t<sup>-1</sup> year<sup>-1</sup>, respectively. The farmyard manure was stabilized for about six months in a pit and all was applied in November at land preparation for wheat. One half of the stated dose of urea was applied each to wheat and maize. For each crop, urea was again split into two, one half applied at sowing and the other with first irrigation. The farmyard contained 0.6% total N and 0.6% P<sub>2</sub>O<sub>5</sub>. The P<sub>2</sub>O<sub>5</sub> contained in the farmyard manure was balanced through the application of single superphosphate. Total N applied in T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> was equivalent to 98, 146, 148, and 196 kg ha<sup>-1</sup> year<sup>-1</sup>.

<sup>b</sup>Figures in parentheses represent the C:N ratio of soil.

was equivalent to field capacity. Soils were not stored and all analyses were carried out within six hours of collection.

Total organic carbon (TOC) of the soil was determined by an acid-dichromate method (Riehm & Ulrich, 1954). For the measurement of  $C_{mic}$ , the chloroform fumigation-incubation method of Jenkinson & Powlson (1976) was followed using 10g field moist soil. The fumigated-reinoculated and unfumigated (control) soils were incubated at 30°C for 10 days in air-tight 100-ml serum vials, and the headspace was analyzed for CO<sub>2</sub> on a gas chromatograph (Gasukuro Kogyo, Model 370) equipped with a thermal-conductivity detector. Microbial biomass C was calculated as:  $C_{mic}=F/k_C$ , where F is the flush of CO<sub>2</sub>-C (CO<sub>2</sub>-C evolved from the fumigated minus that from the unfumigated control) and  $k_C$  is the recovery factor, the value of which was taken as 0.41 (Voroney & Paul, 1984). Microbial biomass turnover rate was calculated by dividing the sum of losses of  $C_{mic}$  at different sampling intervals by the overall average  $C_{mic}$  (McGill *et al.*, 1986). Microbial biomass carrying capacity (MBCC) is defined as the amount of CO<sub>2</sub>-C evolved from the fumigated-inoculated soil during a 10-day incubation at 30°C divided by a  $k_C$  factor of 0.41. Aerobically mineralizable carbon was taken as the amount of CO<sub>2</sub>-C evolved from the unfumigated soil during a 10-day incubation at 30°C. Specific respiratory activity (SRA) of soil microbial biomass was calculated by dividing AMC by  $C_{mic}$  (Franzluebbers *et al.*, 1994). Relative mineralization rate of soil organic carbon was estimated by dividing AMC by TOC. Data were subjected to an analysis of variance, followed by Duncan's multiple range test (Steel & Torrie, 1980), and results are reported as means of four replicate plots.

## Results

**Seasonal changes in C availability and  $C_{mic}$ :** Carbon availability showed marked temporal variation during the growing seasons of wheat and maize (date means, Tables 2 & 3). Total organic C was relatively higher during the active growth period of both crops, when its values were comparable under wheat and maize. It declined toward the crop maturity in maize but even higher values recorded near the wheat maturity indicate a contribution from the decomposing root material. The mineralization of organic C pool (expressed as AMC) was strongly influenced by the growth stage of crops; in wheat, it was highest during the active growth period, whereas it continued to increase during the maize growing period. Relative C mineralization rate (expressed as mg CO<sub>2</sub>-C g<sup>-1</sup> TOC), was though quite low (2.9–10.2 mg CO<sub>2</sub>-C g<sup>-1</sup> TOC; equivalent to <1.0% of TOC), yet it showed marked seasonal trend, with maximum observed during the active growth period under both crops.

Microbial biomass carrying capacity and  $C_{mic}$  showed a distinct temporal pattern, which coincided with the temporal fluctuations in C availability, i.e. maximum during the active growth stage, and declining toward the crop maturity. The ratio of  $C_{mic}$  to TOC was small, ranging from 0.46–1.53%, the higher values corresponding to the active growth period of the two crops. Under both crops, SRA of soil microbial biomass was highest at the flowering stage ( $P<0.05$ ) indicating a fluctuating metabolic status of microbial communities during the crop growth. Averaged across treatments, the SRA values during the active growth period of wheat were significantly higher than those observed during the active growth stage of maize ( $P<0.05$ ). Compared to that of SRA, an opposite trend prevailed for  $C_{mic}$  ( $P<0.05$ ).

Table 2. Microbial biomass and carbon availability in soil during the wheat growing season<sup>a</sup>.

Treatment	Sampling date / Growth stage						Treatment mean	LSD	P<0.05	P<0.01
	13 Dec. Tillering	14 Jan. Stem elongation	24 Feb. Flowering	27 Mar. Grain filling	11 Apr. Dough					
	Microbial biomass ( $\mu\text{g C g}^{-1}$ )									
U <sub>50</sub> +F <sub>8</sub>	169	111	169	127	100	135 a	Treatment	15.17	20.22	
U <sub>50</sub> +F <sub>16</sub>	154	91	154	48	38	97 c	Date	8.76	11.68	
U <sub>100</sub> +F <sub>8</sub>	132	111	144	73	46	101 bc	Overall	7.89	10.52	
U <sub>100</sub> +F <sub>16</sub>	152	98	170	61	53	107 bc				
Unfertilized	111	142	151	86	77	114 b				
Date mean	144 b	111 c	158 a	79 d	63 e					
	Microbial biomass carrying capacity ( $\mu\text{g C g}^{-1}$ )									
U <sub>50</sub> +F <sub>8</sub>	291	398	464	318	201	335 a	Treatment	15.00	19.99	
U <sub>50</sub> +F <sub>16</sub>	282	410	464	260	149	313 b	Date	8.66	11.54	
U <sub>100</sub> +F <sub>8</sub>	254	369	434	259	145	292 c	Overall	19.36	25.81	
U <sub>100</sub> +F <sub>16</sub>	325	395	471	285	179	331 a				
Unfertilized	195	308	387	197	147	247 d				
Date mean	269 c	376 b	444 a	264 c	164 d					
	Total organic C (%)									
U <sub>50</sub> +F <sub>8</sub>	1.20	1.32	1.19	1.14	1.57	1.29 c	Treatment	0.066	0.087	
U <sub>50</sub> +F <sub>16</sub>	1.40	1.53	1.21	1.04	1.65	1.37 b	Date	0.066	0.087	
U <sub>100</sub> +F <sub>8</sub>	1.2	1.22	1.04	0.88	1.30	1.13 d	Overall	0.147	0.196	
U <sub>100</sub> +F <sub>16</sub>	1.85	1.62	1.17	1.01	1.58	1.44 a				
Unfertilized	1.08	1.28	1.20	0.98	1.06	1.12 d				
Date mean	1.36 b	1.39 ab	1.16 c	1.01 d	1.43 a					
	Ratio of microbial biomass C to total organic C ( $\text{mg C}_{\text{max}} \text{g}^{-1} \text{TOC}$ )									
U <sub>50</sub> +F <sub>8</sub>	14.1	8.4	14.3	11.1	6.3	10.8 a	Treatment	0.663	0.884	
U <sub>50</sub> +F <sub>16</sub>	11.0	6.2	12.8	4.7	2.3	7.4 d	Date	0.663	0.884	
U <sub>100</sub> +F <sub>8</sub>	11.0	9.1	13.8	8.3	3.6	9.2 c	Overall	1.483	1.977	
U <sub>100</sub> +F <sub>16</sub>	8.2	6.0	14.5	6.0	3.4	7.6 d				
Unfertilized	10.3	11.1	12.6	8.8	7.3	10.0 b				
Date mean	10.9 b	8.2 c	13.6 a	7.8 c	4.6 d					

Table 2 (Cont'd.)

Treatment	Sampling date / Growth stage						Treatment mean	LSD	P<0.05	P<0.01
	13 Dec. Tillering	14 Jan. Stem elongation	24 Feb. Flowering	27 Mar. Grain filling	11 Apr. Dough					
Aerobically mineralizable C ( $\mu\text{g g}^{-1}$ )										
U <sub>50</sub> +F <sub>8</sub>	50	117	121	79	42	82 (6.6) b	Treatment	5.27	7.03	
U <sub>50</sub> +F <sub>16</sub>	53	131	127	87	46	89 (6.8) a	Date	3.04	4.06	
U <sub>100</sub> +F <sub>8</sub>	50	106	119	76	40	78 (7.2) b	Overall	6.81	9.08	
U <sub>100</sub> +F <sub>16</sub>	71	122	124	92	51	92 (6.9) a				
Unfertilized	34	68	97	46	29	55 (4.8) c				
Date mean	52 (3.8) <sup>b</sup> d	109 (7.8) b	118 (10.2) a	76 (7.8) c	42 (2.9) e					
Specific respiratory activity of microbial biomass ( $\text{mg CO}_2\text{-C d}^{-1} \text{g}^{-1} \text{C}_{\text{mic}}$ )										
U <sub>50</sub> +F <sub>8</sub>	30	105	71	62	42	62 d	Treatment	3.53	4.70	
U <sub>50</sub> +F <sub>16</sub>	34	145	86	179	122	113 a	Date	3.43	4.70	
U <sub>100</sub> +F <sub>8</sub>	38	96	83	105	87	82 c	Overall	7.89	10.52	
U <sub>100</sub> +F <sub>16</sub>	47	125	73	152	96	99 b				
Unfertilized	31	48	64	53	37	47 e				
Date mean	36 d	104 b	75 c	110 a	77 c					
Soil temperature, 5 cm depth ( $^{\circ}\text{C}$ )										
	9.8	11.3	17.5	26.0	23.0					

<sup>a</sup>Treatment or date means followed by different letter are significantly different ( $P<0.05$ ) by Duncan's multiple range test

<sup>b</sup>Figures in parentheses represent the relative C mineralization rate as  $\text{mg AMC g}^{-1} \text{TOC}$

Table 3. Microbial biomass and carbon availability in soil during the maize growing season<sup>a</sup>.

Treatment	Sampling date / Growth stage			Treatment mean	LSD	P<0.05	P<0.01
	17 Sep. Active growth	7 Oct. Flowering	19 Oct. Grain filling				
Microbial biomass ( $\mu\text{g C g}^{-1}$ )							
U <sub>50</sub> +F <sub>8</sub>	91	229	129	150 a	Treatment	13.08	17.62
U <sub>50</sub> +F <sub>16</sub>	130	208	118	152 a	Date	10.13	13.64
U <sub>100</sub> +F <sub>8</sub>	127	176	85	129 b	Overall	22.66	30.51
U <sub>100</sub> +F <sub>16</sub>	123	207	109	146 a			
Unfertilized	96	132	109	112 c			
Date mean	113 b	191 a	110 b				
Microbial biomass carrying capacity ( $\mu\text{g C g}^{-1}$ )							
U <sub>50</sub> +F <sub>8</sub>	217	358	281	285 b	Treatment	13.06	17.58
U <sub>50</sub> +F <sub>16</sub>	255	351	294	300 a	Date	10.12	13.62
U <sub>100</sub> +F <sub>8</sub>	240	308	271	273 b	Overall	22.62	30.46
U <sub>100</sub> +F <sub>16</sub>	262	347	282	306 a			
Unfertilized	210	232	270	237 c			
Date mean	236 c	324 a	280 b				
Total organic C (%)							
U <sub>50</sub> +F <sub>8</sub>	1.41	1.34	1.30	1.35 a	Treatment	0.075	0.100
U <sub>50</sub> +F <sub>16</sub>	1.36	1.22	1.29	1.29 ab	Date	0.058	0.078
U <sub>100</sub> +F <sub>8</sub>	1.36	1.23	1.23	1.27 ab	Overall	0.130	0.174
U <sub>100</sub> +F <sub>16</sub>	1.33	1.29	1.10	1.24 b			
Unfertilized	1.25	1.08	0.97	1.10 c			
Date mean	1.34 a	1.23 b	1.18 b				

Table 3 (Cont'd.)

Treatment	Sampling date / Growth stage			Treatment mean	LSD	P<0.05	P<0.01
	17 Sep. Active growth	7 Oct. Flowering	19 Oct. Grain filling				
	Ratio of microbial biomass C to total organic C (mg C <sub>mic</sub> g <sup>-1</sup> TOC)						
U <sub>50</sub> +F <sub>8</sub>	6.5	17.1	10.0	11.2 ab	Treatment	1.038	1.379
U <sub>50</sub> +F <sub>16</sub>	9.6	17.1	9.2	12.0 a	Date	0.804	1.082
U <sub>100</sub> +F <sub>8</sub>	9.3	14.3	6.9	10.2 b	Overall	1.798	2.420
U <sub>100</sub> +F <sub>16</sub>	9.2	16.1	9.9	11.7 a			
Unfertilized	7.7	12.2	11.2	10.4 b			
Date mean	8.5 c	15.4 a	9.4 b				
	Aerobically mineralizable C (µg g <sup>-1</sup> )						
U <sub>50</sub> +F <sub>8</sub>	52	53	62	56 (4.1) c	Treatment	3.58	4.82
U <sub>50</sub> +F <sub>16</sub>	51	59	72	61 (4.7) b	Date	2.77	3.73
U <sub>100</sub> +F <sub>8</sub>	46	54	1	59 (4.7) bc	Overall	6.20	8.34
U <sub>100</sub> +F <sub>16</sub>	57	68	71	65 (5.3) a			
Unfertilized	47	41	66	51 (4.8) d			
Date mean	51 (3.7) <sup>b</sup> c	55 (4.5) b	70 (6.0) a				
	Specific respiratory activity (mg CO <sub>2</sub> -C d <sup>-1</sup> g <sup>-1</sup> C <sub>mic</sub> )						
U <sub>50</sub> +F <sub>8</sub>	57	23	48	43 c	Treatment	2.83	3.82
U <sub>50</sub> +F <sub>16</sub>	40	28	62	43 c	Date	2.20	2.95
U <sub>100</sub> +F <sub>8</sub>	37	31	90	52 a	Overall	4.91	6.61
U <sub>100</sub> +F <sub>16</sub>	46	33	62	48 b			
Unfertilized	49	31	61	47 b			
Date mean	46 b	29 c	65 a				
	Soil temperature, 5 cm depth (°C)						
	33.0	26.3	21.0				

<sup>a</sup>Treatment or date means followed by different letter are significantly different (P<0.05) by Duncan's multiple range test

<sup>b</sup>Figures in parentheses represent the relative C mineralization rate as mg AMC g<sup>-1</sup> TOC

**Table 4. Mineralization and turnover of soil microbial biomass carbon ( $C_{mic}$ ) under wheat-maize cropping system receiving different fertilizer treatments.**

Treatment	$\Sigma C_{mic}$ flux ( $kg\ ha^{-1}\ year^{-1}$ )	$C_{mic}$ turnover rate ( $year^{-1}$ ) <sup>a</sup>
U <sub>50</sub> +F <sub>8</sub>	317 a <sup>b</sup>	1.69 (216) <sup>b</sup> b
U <sub>50</sub> +F <sub>16</sub>	265 b	2.29 (159) a
U <sub>100</sub> +F <sub>8</sub>	252 b	1.88 (194) b
U <sub>100</sub> +F <sub>16</sub>	247 b	2.22 (164) a
Unfertilized	254 b	1.04 (351) c

<sup>a</sup>Figures in parentheses are the  $C_{mic}$  turnover time (days)

<sup>b</sup>Figures in a column followed by different letter are significantly different ( $P<0.05$ ) by Duncan's multiple range test

**Treatment effects on carbon availability and  $C_{mic}$ :** Total organic carbon of the soil increased as a result of fertilization (15–29% increase under wheat, 15–23% increase under maize,  $P<0.05$ ), except that U<sub>100</sub>+F<sub>8</sub> treatment had no effect during the wheat growing season (treatment means, Table 2 & 3). Treatment effects on the relative mineralization rate of TOC were inconsistent under two crops. However, averaged across all sampling dates under wheat and maize, only U<sub>50</sub>+F<sub>8</sub> showed slightly higher rate (9% increase over control,  $P<0.05$ ), while other treatments slightly decreased the rate (6–12% decrease over control,  $P<0.05$ ). Treatment effects on AMC were much more pronounced compared to other parameters. At a given FYM application rate, doubling the urea application rate either did not affect the level of AMC, or caused a little (7%) increase but only during the maize season under higher FYM application rate. However, with both urea application rates, doubling the FYM application caused an 8–18% increase in AMC ( $P<0.05$ ). The overall carbon availability to microbes expressed as MBCC increased due to fertilization (18–36% increase under wheat, 15–29% increase under maize,  $P<0.05$ ). Treatment effects on  $C_{mic}$  however, followed a pattern that was not consistent with that observed for C availability parameters. During the wheat growing season, the effect of fertilization on  $C_{mic}$  was observed only in U<sub>50</sub>+F<sub>8</sub> treatment, which showed an increase of 18% over the unfertilized control ( $P<0.05$ ). A pronounced effect of fertilization on  $C_{mic}$  was recorded during the maize growing season, showing a 15–36% increase over the unfertilized ( $P<0.05$ ). On the other hand, treatment effects on SRA were more obvious under wheat than under maize. Averaged across sampling dates, SRA in the unfertilized soil was similar under both crops. However, in fertilized treatments it was 45–162% higher under wheat than under maize. The stimulatory effect on SRA was higher due to increasing FYM than urea application rate.

**Turnover of  $C_{mic}$ :** Fertilizer application substantially increased the turnover of  $C_{mic}$  (63–120% increase over unfertilized,  $P<0.05$ ) (Table 4). Increasing urea application from 50–100 kg N ha<sup>-1</sup> had no effect, but doubling the FYM application rate produced an 18–35% increase in the  $C_{mic}$  turnover rate ( $P<0.05$ ). Except that U<sub>50</sub>+F<sub>8</sub> treatment showed higher values, fertilizer application had no effect on the flux of  $C_{mic}$  (Table 4). However, the flux was significantly but negatively correlated with the concurrently determined soil mineral N ( $r=-0.611$ ,  $P<0.01$ ; point data for soil mineral N not shown).

#### Discussion:

Seasonal fluctuations in different carbon availability indices and microbial properties were not related to soil temperature. This is consistent with findings of Raubuch and Joergensen (2002) but contrary to several earlier reports describing seasonal variations in

soil C as a function mainly of soil temperature (Tate *et al.*, 1993; Zak *et al.*, 1993; Carnol & Ineson, 1999). An inverse relationship has also been reported between soil temperature and  $C_{mic}$  (Piao *et al.*, 2000).

Increase in soil organic C due to organic amendments is well documented (Marschner *et al.*, 2003) and the higher TOC values observed in the present study during the active crop growth periods indicate contribution by the wheat and maize crops in the form rhizodeposits. These rhizodeposits not only served as C source for the soil microflora (as indicated by increased AMC during the active plant growth), but might also have accelerated mineralization of the native pool of soil organic C. Our contention is supported by the data on relative mineralization rate of soil organic C ( $\text{mg AMC g}^{-1}$  TOC), which followed a strong seasonal trend i.e. being higher during the active crop growth, particularly during the wheat growing season. Soil microbial biomass is known to be triggered into activity by trace amounts of substrates like root exudates, resulting in 'priming effect' on decomposition of the native pool of soil organic matter (Nobili *et al.*, 2001).

Microbial biomass carbon was a minor fraction of soil organic matter, constituting on the average 0.91% and 1.11% of TOC during the wheat and maize growing seasons, respectively. These figures are close to those (about 1.0%) reported by Moore *et al.*, (2000). However, this minor component of the soil organic matter showed marked seasonal fluctuations, and was also strongly influenced by the application of urea and FYM. An increase in  $C_{mic}$  due to application of mineral N fertilizer, as observed during the maize growing season, agrees to some earlier reports (Mahmood *et al.*, 1997; Raiesi, 2004). The  $C_{mic}$  was not correlated with carbon availability indices such as TOC and AMC, whether utilizing point data ( $n=40$ ), date means ( $n=8$ ) or treatment means ( $n=10$ ). These results are contrary to the reports of Ross *et al.*, (1995) which related temporal fluctuations in  $C_{mic}$  to fluctuations in TOC.

The temporal trend of C availability, soil microbial biomass and its activity was much more pronounced as compared to treatment effects. It seems that treatment effects on  $C_{mic}$  were ramified due to temporal variations in the microbial community structure. As evidenced from the AMC data, carbon availability to microbes was consistently higher under wheat than under maize. However, proportionally less C was incorporated into microbial biomass than that respired under wheat than under maize. This is also evident from SRA data, which showed a highly significant but negative correlation with  $C_{mic}$  (under wheat:  $r=-0.579$ ,  $n=25$ ,  $p<0.001$ ; under maize:  $r=-0.780$ ,  $n=15$ ,  $p<0.001$ ; under both crops:  $r=-0.628$ ,  $n=40$ ,  $p<0.001$ ). Specific respiratory activity of soil microbial biomass was also negatively correlated with  $C_{mic}$ :TOC ratio (under wheat:  $r=-0.532$ ,  $n=25$ ,  $p<0.01$ ; under maize:  $r=-0.736$ ,  $n=15$ ,  $p<0.001$ ; under both crops:  $r=-0.591$ ,  $n=40$ ,  $p<0.001$ ). A strong seasonal pattern of SRA, which was opposite to that observed for  $C_{mic}$ , has also been reported under continuous corn (Salinas-Garcia *et al.*, 1997). The higher SRA and lower  $C_{mic}$  values under wheat than under maize indicate that the rhizodeposits under wheat compared to maize crop probably favoured the microbial community that was dominated by 'r-strategists' which respire more  $\text{CO}_2$  per unit degradable C, incorporating relatively less C into biomass (Lynch, 1984; Insam, 1990). In contrast, 'k-strategists' probably dominated during the maize growing season, respiring relatively less C as  $\text{CO}_2$  and incorporating more into biomass. Plant age, fertilizer application, rhizosphere development and substrate loading rates are well known to cause shifts in the microbial community structure (Griffiths *et al.*, 1999; Steer & Harris, 2000;

O'Donnell *et al.*, 2001). A strong but negative correlation between SRA and  $C_{mic}$  indicates that SRA of the soil microflora was a decisive factor in determining the size of soil microbial biomass pool under the cropping system investigated.

The increased  $C_{mic}$  turnover rate due to fertilizer application rate is consistent with the results of our earlier study (Mahmood *et al.*, 1997). In addition, lack of the effect due to increasing urea application rate and a pronounced stimulatory effect of increasing FYM application rate on the  $C_{mic}$  turnover are also in agreement with our previous findings (Mahmood *et al.*, 1997). The relatively high  $C_{mic}$  turnover rates observed during the present study also concur to some earlier reports (Mahmood *et al.*, 1997; Raiesi, 2004) indicating a considerable nutrient cycling through soil microbial biomass under irrigated cropping systems in this region.

A negative relationship between  $C_{mic}$  flux and soil mineral N indicates that seasonal fluctuations in soil mineral N also contributed significantly to the buildup and decomposition of soil microbial biomass; the lower soil mineral N levels favored the decomposition of microbial biomass and *vice versa*. Considering the annual fluxes of  $C_{mic}$  (Table 4), and assuming a  $C_{mic}: N_{mic}$  ratio of 6, about 40 kg N ha<sup>-1</sup> year<sup>-1</sup> (in unfertilized) to 53 kg N ha<sup>-1</sup> year<sup>-1</sup> (in U<sub>50</sub>+F<sub>8</sub> treatment) might be released due to the death and decay of microbial biomass under the cropping system investigated. This further suggests that nutrient cycling through soil microbial biomass may be substantial under agroclimatic conditions prevailing in this region.

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