

T. Mahmood · F. Azam · F. Hussain · K. A. Malik

Carbon availability and microbial biomass in soil under an irrigated wheat-maize cropping system receiving different fertilizer treatments

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Abstract Seasonal changes in carbon availability and microbial biomass were studied in soil under an irrigated wheat-maize cropping system receiving different fertilizer treatments over the past 10 years. Treatments included N-100 and N-200 (urea at 100 and 200 kg N ha⁻¹ year⁻¹, respectively), FYM-16 and FYM-32 (farmyard manure at 16 and 32 t ha⁻¹ year⁻¹, respectively) and a control (unfertilized). Aerobically mineralizable carbon (AMC; C mineralized after 10 days aerobic incubation at 30 °C) increased (13–16%) under wheat at both rates of urea whereas under maize it increased (22%) only with the lower rate of urea. Farmyard manure also increased the content of soil AMC under both crops, the effect being two- to threefold higher under wheat than under maize. Urea application caused an 32–78% increase in the specific respiratory activity (SRA) under wheat but caused an 11–50% decrease during the maize season. Farmyard manure also resulted in a higher SRA under both crops but only at the higher application rate. Under wheat, microbial biomass C (MBC) decreased in urea-treated plots but showed a slight increase at the higher rate of FYM. During the maize season, MBC was higher under both urea (42–46%) and FYM (36–47%) treatments as compared to the control. Microbial biomass turnover rate was highest for FYM-32 (2.08), followed by FYM-16 and urea treatments (1.35–1.49); control plots showed a turnover rate of 0.82. The higher AMC and SRA during the active growth period of wheat than that of maize indicated that root-derived C from wheat was higher in amount and more easily degradable.

Key words Microbial biomass · Carbon availability · Microbial biomass turnover · Wheat-maize rotation · Urea · Farmyard manure

T. Mahmood (✉) · F. Azam · F. Hussain
Soil Biology Division, Nuclear Institute for Agriculture and Biology,
PO Box 128, Faisalabad, Pakistan
Fax: 92-41-654213

K. A. Malik
National Institute for Biotechnology and Genetic Engineering,
PO Box 577, Faisalabad, Pakistan

Introduction

Microbial biomass represents a small percentage of soil organic matter, but because it is labile and dynamic in nature, it plays a significant role in nutrient cycling and ecosystem functioning. It interacts with ecosystem productivity by regulating nutrient availability, determining carbon and nitrogen storage and contributing to atmospheric CO₂ (Cheng and Virginia 1993). Several studies have shown a close relationship between microbial biomass and nutrient availability to plants (Jenkinson and Ladd 1981; Houot and Chaussod 1995). Microbial biomass has thus been suggested to serve as a reliable index of soil productivity that depends to a considerable extent on nutrient fluxes (Marumoto 1984; Hassink 1994). However, the estimates of microbial biomass vary considerably depending upon the method of estimation, choice of soil and its history (Lynch and Panting 1980; Jenkinson and Ladd 1981; Azam et al. 1986; Cheng and Virginia 1993; Hassink 1994; Joergensen et al. 1994; Goshal and Singh 1995; Houot and Chaussod 1995).

While reasonable agreement has generally been obtained between different methods of biomass estimation (Cheng and Virginia 1993), the variation in the estimates may almost be entirely attributed to the choice of soil and its treatment prior to biomass estimation. Of the different soil management practices, application of mineral fertilizers, plant residues and organic manures has been reported to have a significant bearing on the build-up, dynamics and activity of microbial biomass (Bolton et al. 1985; Schnurer et al. 1985; Powlson et al. 1987). Organic manures generally cause an increase in the microbial biomass (Mazzarino et al. 1993; Goshal and Singh 1995) and higher values have been reported for soils receiving mineral fertilizers (Houot and Chaussod 1995; McCarty et al. 1995; Goshal and Singh 1995).

Type of plant cover and cropping history of soils have been found to influence the microbial biomass and its activity mainly by regulating the supply of carbonaceous compounds and competing with soil microflora for nutrient acquisition (Franzluebbers et al. 1994; Campbell et al.

Table 1 Details of fertilizer treatments and some physicochemical characteristics of the (0–15 cm) soil

Treatment	Wheat	Maize	TOC (%)	Total N (%)	WHC (%)	pH ^a	Bulk density (g cm ⁻³)	Pore space (%)
N-100 ^b	50 kg N ha ⁻¹	50 kg N ha ⁻¹	1.14	0.07	37	7.3	1.44	46.9
N-200	100 kg N ha ⁻¹	100 kg N ha ⁻¹	1.05	0.08	36	7.3	1.42	47.4
FYM-16 ^c	16 t ha ⁻¹	None	1.17	0.08	36	7.4	1.42	47.5
FYM-32	32 t ha ⁻¹	None	1.18	0.09	37	7.4	1.41	47.6
Control	None	None	0.78	0.07	35	7.4	1.52	43.8

^a Saturation paste

^b To each crop, the stated dose of N-fertilizer was applied as urea in two equal splits, one at sowing and the other with second irrigation

^c Farmyard manure, all applied in November at land preparation for wheat; the total N applied as FYM-16 and FYM-32 treatments was equivalent to 96 and 192 kg ha⁻¹, respectively; the amount of P₂O₅ applied as FYM-16 and FYM-32 treatments was equivalent to 96 and 192 kg ha⁻¹, respectively, which was balanced in N-100 and N-200 treatments through application of single superphosphate

1991; Mazzarino et al. 1993; Xu and Juma 1993). Thus considerable seasonal variations in the microbial biomass and its activity have been reported (Kaiser and Heinemeyer 1993; Franzlubbers et al. 1994). However, only a few long-term field studies have involved different crop rotation patterns (Joergensen et al. 1994; Houot and Chaussod 1995; Goshal and Singh 1995; McCarty et al. 1995). Limited data are available on the magnitude of seasonal changes in soil microbial biomass and mineralizable carbon to ascertain the practical significance of these fluctuations on crop growth and nutrient cycling (Kaiser and Heinemeyer 1993; Patra et al. 1995).

Our objective was to follow seasonal changes in microbial biomass in relation to carbon availability in the field under irrigated wheat-maize cropping sequence receiving different fertilizer treatments.

Materials and methods

The study site located at the Nuclear Institute for Agriculture and Biology, Faisalabad, has a subtropic and semiarid climate with a mean annual rainfall of 340 mm, most of which occurs in the months of July and August. The hottest months are May and June, with mean maximum air temperatures of 39.3 and 41.1°C, respectively, whereas January is the coldest month with a mean minimum temperature of 5°C.

The experiment comprised 20 field plots (7.5×8.5 m) that received five fertilizer treatments in a completely randomized design, each with four replicates. Details of the treatments (which have been in effect since 1980) and some physicochemical properties of the plough layer are given in Table 1. Wheat (*Triticum aestivum* cv. Pak-81) was sown in November 1990 and harvested in April 1991 while maize (*Zea mays* cv. Akbar) was sown in August 1991 and harvested in October 1991. Wheat received six canal irrigations: all were 7.5 cm except the first (pre-planting) and the fourth, which were 10 and 5 cm, respectively. During the maize season, five irrigations were applied: all were 7.5 cm except the first (pre-planting) and the last, which were 10 and 5 cm, respectively. The soil was sampled 5 times during wheat and 3 times during maize growth. For each treatment, 16 soil cores (3×15 cm, diameter × depth) were randomly collected (four from each replicate plot), pooled, mixed and sieved (<2 mm). The soil moisture content at the time of sampling was equivalent to ca. 50% of water-holding capacity. All analyses were done in triplicate within 6 h of collection.

For measurement of microbial biomass carbon (MBC), the chloroform-fumigation method of Jenkinson and Powlson (1976b) was followed using 10-g soil samples. Fumigated-reinoculated and unfumigated soil samples were incubated at 30°C for 10 days in tightly stop-

pered 100-ml serum vials. The headspace was analysed for CO₂ on a gas chromatograph using a thermal conductivity detector. Microbial biomass C was calculated as: $MBC = F/k$, where MBC is the biomass C, F is the flush of CO₂-C (CO₂-C evolved from fumigated minus that from unfumigated soil) and k is the decomposition constant, the value of which was taken 0.45 (Jenkinson and Ladd 1981). Microbial biomass turnover rate was calculated by dividing the total losses in MBC at different sampling intervals by the average MBC (McGill et al. 1986). Microbial biomass-carrying capacity (MBCC) is defined as the amount of CO₂-C evolved from the fumigated-inoculated soil during 10 days of aerobic incubation at 30°C divided by a k factor of 0.45 (Groffman and Tiedje 1989). Aerobically mineralizable C (AMC) was taken as the amount of CO₂-C mineralized from unfumigated soil during 10 days of aerobic incubation at 30°C. Specific respiratory activity (SRA) of soil microbial biomass was estimated by dividing the AMC by MBC (Franzluebbers et al. 1994). Total organic carbon (TOC) in soil was analysed by an acid dichromate method (Riehm and Ulrich 1954). Analysis of variance was performed on all experimental data and means were compared using the least significant difference test (Steel and Torrie 1980).

Results

Treatment effects on carbon availability

Carbon availability and soil microbial biomass under wheat and maize are presented in Tables 2 and 3, respectively. Fertilizer treatments had a pronounced effect on available carbon as revealed by the results from AMC, TOC and SRA. The two urea treatments had 13–16% higher AMC than the control when averaged across sampling dates for wheat ($P < 0.01$). Under maize, 22% more AMC was recorded with the N-100 treatment while at a higher rate of urea, AMC slightly decreased ($P < 0.01$). Application of FYM also increased the AMC under both wheat and maize, the stimulatory effect being two- to threefold higher for wheat. During the wheat season, AMC was similar at both urea levels, but under maize N-100 showed a 32% increase ($P < 0.01$). Doubling the rate of FYM caused a 28% and 24% increase in AMC of soil under wheat and maize, respectively ($P < 0.01$). Under both crops, TOC was slightly (9–14%) higher with the N-100 treatment than the control ($P < 0.01$), but there was little effect of the higher rate of urea application. Under both crops, FYM treatments had 15–31% higher TOC than control ($P < 0.01$). Urea application produced a 32–78% increase in SRA un-

Table 2 Carbon availability and soil microbial biomass under wheat receiving different fertilizer treatments

Treatment	Sampling date (1990-1991) growth stage					Treatment mean	LSD	<i>P</i> <0.05	<i>P</i> <0.01
	13 Dec Tillering	14 Jan Tillering	24 Feb Flowering	27 Mar Grain	11 Apr Dough				
Aerobically mineralizable C ($\mu\text{g C g}^{-1}$)									
N-100	39	77	125	45	34	64	Treatment Date Overall	3.96	5.28
N-200	41	77	109	45	40	62			
FYM-16	56	129	125	83	46	88			
FYM-32	108	164	136	95	64	113			
Control	34	68	97	46	29	55			
Date mean	56	103	119	63	43				
Total organic C (%)									
N-100	1.19	1.52	1.14	0.97	1.57	1.28	Treatments Date Overall	0.066	0.087
N-200	1.95	1.41	1.12	1.03	1.30	1.18			
FYM-16	1.18	1.49	1.27	1.02	1.50	1.21			
FYM-32	1.29	1.61	1.34	1.18	1.94	1.47			
Control	1.08	1.28	1.20	0.98	1.06	1.12			
Date mean	1.16	1.46	1.21	1.04	1.47				
Specific respiration activity ($\text{mg C g}^{-1} \text{MBC day}^{-1}$)									
N-100	30	66	107	49	76	66	Treatment Date Overall	7.54	10.05
N-200	34	69	92	66	184	89			
FYM-16	38	139	82	80	53	79			
FYM-32	106	285	83	127	72	135			
Control	34	53	70	58	38	50			
Date mean	48	122	87	76	85				
Microbial biomass-carrying capacity ($\mu\text{g C g}^{-1}$)									
N-100	217	289	398	191	122	243	Treatment Date Overall	6.76	9.01
N-200	213	282	362	167	112	227			
FYM-16	270	379	431	288	188	311			
FYM-32	344	422	465	287	230	350			
Control	177	281	352	180	134	225			
Date mean	244	330	402	222	157				
Microbial biomass ($\mu\text{g C g}^{-1}$)									
N-100	130	117	118	92	45	100	Treatment Date Overall	4.65	6.20
N-200	121	111	120	68	22	88			
FYM-16	147	93	152	103	87	116			
FYM-32	103	58	164	75	88	98			
Control	101	129	137	79	75	104			
Date mean	120	101	138	83	63				

der wheat, and a decrease (11–50%) under maize ($P<0.01$). Of the two urea treatments, N-200 exhibited a higher SRA than N-100 in wheat; the trend was reversed under maize ($P<0.01$). Application of FYM also caused an increase in SRA that was significantly higher with the FYM-32 than with the FYM-16 treatment under both crops ($P<0.01$). Microbial biomass-carrying capacity was fairly dependent on AMC as suggested by the highly significant correlation between the two ($r=0.89$; $P<0.001$). Although a small increase in MBCC was recorded in N-100-treated wheat, the stimulatory effect was much greater under maize ($P<0.01$). In N-200, no effect was observed on MBCC during the wheat season, but an increase of 16% occurred under maize ($P<0.01$). Application of FYM caused an increase in MBCC under both crops ($P<0.01$) that was higher at the higher rate of application. Increasing urea application rates from 100 to 200 kg N ha⁻¹ produced 7% and 14% increases, whereas increasing the FYM application rate from 16 to 32 t ha⁻¹ produced 8% and 13% in-

creases, in MBCC of the soil under wheat and maize crops, respectively ($P<0.01$).

Seasonal changes in carbon availability

In both urea-treated and the control plots sown to wheat, AMC doubled during tillering, reached a maximum at flowering and then declined ($P<0.01$). With FYM treatments, maximum AMC was observed at tillering (14 January) followed by a gradual decline towards crop maturity ($P<0.01$). For maize, AMC increased very slightly during the active growth period with a peak in all treatments at the dough stage when the fodder was harvested ($P<0.01$). Total organic carbon in soil during the wheat season increased during tillering followed by a decline until maturity, when it increased again. During the maize season average TOC recorded at the active growth stage slightly decreased near crop maturity ($P<0.05$). For wheat, ma-

Table 3 Carbon availability and soil microbial biomass under maize receiving different fertilizer treatments

Treatment	Sampling date (1991) growth stage			Treatment mean	LSD	<i>P</i> <0.05	<i>P</i> <0.01
	17 Sep Active growth	7 Oct Flowering	29 Oct Dough				
Aerobically mineralizable C ($\mu\text{g C g}^{-1}$)							
N-100	38	63	84	62	Treatment	2.65	3.57
N-200	31	47	63	47	Date	2.05	2.76
FYM-16	53	59	79	63	Overall	4.59	6.18
FYM-32	78	70	87	78			
Control	47	41	66	51			
Date mean	49	56	76				
Total organic C (%)							
N-100	1.34	1.11	1.16	1.20	Treatment	0.075	0.100
N-200	1.09	1.14	1.22	1.15	Date	0.058	0.078
FYM-16	1.26	1.34	1.33	1.31	Overall	0.129	0.174
FYM-32	1.47	1.52	1.35	1.45			
Control	1.25	1.08	0.97	1.10			
Date mean	1.28	1.24	1.21				
Specific respiration activity ($\text{mg C g}^{-1} \text{MBC day}^{-1}$)							
N-100	23	37	77	46	Treatment	2.83	3.82
N-200	22	29	50	34	Date	2.20	2.96
FYM-16	31	34	77	47	Overall	4.91	6.61
FYM-32	56	37	99	65			
Control	54	34	67	51			
Date mean	37	34	74				
Microbial biomass-carrying capacity ($\mu\text{g C g}^{-1}$)							
N-100	250	310	297	286	Treatment	11.03	14.85
N-200	215	269	267	250	Date	8.54	11.50
FYM-16	289	302	278	290	Overall	19.10	25.72
FYM-32	310	345	281	312			
Control	191	212	246	216			
Date mean	251	288	274				
Microbial biomass ($\mu\text{g C g}^{-1}$)							
N-100	166	170	110	149	Treatment	7.22	9.72
N-200	145	165	127	145	Date	5.59	7.53
FYM-16	171	172	103	149	Overall	12.50	16.10
FYM-32	138	189	88	138			
Control	87	121	99	102			
Date mean	141	163	105				

imum SRA under FYM treatments was recorded during tillering while peaks for urea treatments and the control occurred at flowering stage ($P<0.01$). The SRA declined gradually in all treatments except FYM-32 and N-200, which showed a second peak at grain formation and the dough stages, respectively ($P<0.01$). During the maize season, the SRA remained almost unchanged between active growth and flowering but increased to a maximum in all treatments at the dough stage ($P<0.01$). Average SRA in all treatments except the control was higher in wheat than in maize whereas for the control it was similar in both crops.

Microbial biomass carbon

Under wheat, the high rate of urea caused a significant reduction in the MBC. During the maize season, however, the urea treatments produced 42–46% higher MBC than

the control ($P<0.01$). Application of FYM before wheat sowing caused a slight increase in MBC at the lower and a slight decrease at the higher application rate ($P<0.01$). For maize, both the FYM treatments caused an increase (36–47%) in MBC ($P<0.01$), the stimulatory effect being higher with the lower rate of application.

The MBC also showed marked seasonal changes. During tillering in wheat, a sharp decline was observed in MBC in all treatments, the decline being more pronounced (37–44%) in FYM treatments. The control showed a 28% increase in MBC during this period. Between tillering and flowering, a significant increase in MBC was observed in FYM-treated plots, whereas negligible changes occurred in control and urea-treated plots. After flowering, the MBC continued to decline until maturity. At the start of the maize-growing season, the MBC in all treatments was significantly higher than that recorded at wheat maturity ($P<0.05$). During the active growth phase of maize, an increase in MBC was observed in N-200, FYM-32 and con-

Table 4 Microbial biomass carbon (MBC) turnover rate under a wheat-maize cropping system receiving different fertilizer treatments

Treatment	MBC turnover rate (year ⁻¹)
N-100	1.35 b ^a
N-200	1.35 b
FYM-16	1.49 b
FYM-32	2.08 a
Control	0.82 c

^a Means followed by a different letter are significantly different at $P < 0.01$

At the dough stage, however, MBC declined in all treatments, the decline being more in FYM (40–55%) and urea (23–35%) treatments than the control (18%). Microbial biomass turnover rate was highest in the FYM-32 treatment followed by FYM-16 and urea treatments ($P < 0.01$). The MBC turnover rate recorded for the control was 2.5-fold less than FYM-32 and almost 1.6-fold less than FYM-16 or urea treatments (Table 4).

Discussion

The average values of MBC under wheat (98–116 $\mu\text{g C g}^{-1}$) and maize (102–149 $\mu\text{g C g}^{-1}$) crops observed in the present study are lower than those reported for different Pakistani soils (Azam et al. 1986). One reason may be that soils used by Azam et al. (1986) were air-dried, stored and conditioned before MBC measurement whereas field-moist soils were used in the present study. An increase in the extractable organic carbon due to air-drying and its availability to soil microbes has been reported (Powlson and Jenkinson 1976). Moreover, a 0- to 10-day control was employed in the present study, which may have also led to underestimation of the MBC due to the large initial respiration rates from unfumigated samples caused by sampling and handling procedures (Jenkinson and Powlson 1976b). Nevertheless, the observed values of MBC are within the range reported by others (Jenkinson and Powlson 1976a; Lynch and Panting 1980; Insam et al. 1991). The increase in MBC due to application of N fertilizer conforms with other reports (Shen et al. 1989; Insam et al. 1991). A decrease in MBC with increasing rate of N fertilizer has also been reported (Ritz and Robinson 1988; Smolander et al. 1994). However, the reasons for decreased biomass and activity after N addition are not well understood. The stimulatory effect of FYM on MBC is in agreement with the findings of Bolton et al. (1985), Goyal et al. (1993) and Houot and Chaussod (1995). However, a smaller increase in MBC with the higher FYM level is not consistent with the results of Goyal et al. (1993), who found an increase in the stimulatory effect with increasing FYM application rates between 15 and 90 t ha^{-1} . The decline in MBC in the initial stages of the wheat crop, particularly in FYM treatments, is consistent with the findings of Joergensen et al. (1994). Although a significant increase in AMC was recorded in all treatments at the event of decline

in MBC, most of the available carbon was probably respired with little incorporation into microbial cells. This is evident from the exceptionally high SRA and lower MBC in both FYM treatments at wheat tillering (14 January). The overall seasonal trends in MBC during the rest of the wheat season were similar to those reported by Lynch and Panting (1980) and Franzluebbers et al. (1994).

The seasonal changes in AMC observed in this study are similar to those reported by Franzluebbers et al. (1994). The higher AMC recorded at the tillering and flowering stages was probably due to the availability of rhizodeposits (Xu and Juma 1993). The higher AMC recorded in FYM treatments reflects the availability of degradable carbon. Fluctuations in the SRA also indicate the availability of plant-derived carbon in the active growth stages of wheat. An increase in SRA at flowering and a decline at harvest was reported by Franzluebbers et al. (1994). However, the increase in SRA observed for both crops near maturity was perhaps due to the availability of carbon from decomposing roots. An increase in SRA due to FYM application is well documented in the literature (Schnurer et al. 1985; Goyal et al. 1993).

The higher AMC and SRA during the active growth period of wheat than that of maize indicates that root exudates from wheat were of greater quantity and more easily degradable than those of maize. On the other hand, higher MBC values obtained during the maize season reflect the fact that the microflora prevailing under maize was endowed with a more efficient metabolism. The differences in SRA during the active growth phases of wheat and maize reflect the seasonal changes in microbial diversity, particularly in response to freshly added carbonaceous substrate in the form of root exudates. According to Insam (1990), a high SRA is associated with a recent input of easily degradable substrate which induces a kind of microflora that respire more CO_2 per unit of degradable carbon; Lynch (1984) defined these as r-strategists. In contrast, low SRA is associated with K-strategists, which develop on the relatively complex food web and respire less CO_2 per unit of degradable carbon.

The results of the present study confirm that fertilizer application significantly influences the carbon availability as well as the size and activity of microbial biomass in cultivated land. The type of plant cover has a significant bearing on the quality and quantity of the available carbon and thus on the dynamics of microbial communities. Moreover, as supported by the very high turnover rate, nutrient cycling through microbial biomass may be substantial under semiarid subtropical conditions.

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