

# RESPIRATION AND SPECIFIC ACTIVITY OF SOIL MICROBIAL BIOMASS UNDER CONVENTIONAL AND REDUCED TILLAGE<sup>1</sup>

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**ABSTRACT** - The effects of two tillage systems, conventional (mold board plow) and reduced (harrow disk), on soil organic carbon and microbial biomass and activity were evaluated. Total organic carbon and microbial biomass were not affected by tillage, whereas carbon in the soil light fraction was higher in depth in plowed plots. Respiration of incubated samples and the specific activity of the microbial biomass were also higher in subsurface soil under conventional tillage. This was associated ( $r=0,80$ ) with carbon availability as indicated by the ratio light fraction-C/biomass-C. The  $CO_2$ -C produced by the soil was originated from both a labile and a resistant pool. Conventional tillage determined an increase of the labile one. The  $CO_2$ -C production in the field was positively correlated with soil temperature and rainfall ( $r^2=78-89$ ). During summer, plowed plots generated 44% more  $CO_2$ -C than unplowed ones. Plowing increased the available carbon pool size for microorganisms in depth but had no effect on  $CO_2$ -C emission to the atmosphere on a year basis.

**Index terms:** plow, harrow disk, organic carbon, incubated samples, organic matter.

## RESPIRAÇÃO E ATIVIDADE ESPECÍFICA DA BIOMASSA MICROBIANA DE UM SOLO SOB LAVRA CONVENCIONAL E REDUZIDA

**RESUMO** - Avaliaram-se os efeitos de um sistema de lavra convencional, (arado de relha e vertedoura) e um sistema de lavoura reduzida (grade de disco) sobre o carbono orgânico e sobre a biomassa e a respiração microbiana de um solo. O carbono orgânico total e o carbono da biomassa não foram afetados pela lavra, enquanto que o carbono na fração leve do solo foi maior durante o verão, nas glebas aradas. A respiração de amostras incubadas esteve correlacionada com o carbono na fração leve. A atividade específica foi superior em profundidade com lavra convencional, estando associada ( $r=0,80$ ) com a relação C-fração leve/C-biomassa. A produção cumulada de C- $CO_2$  foi originada a partir de dois componentes da matéria orgânica, um lábil e outro estável, determinando a lavra convencional um aumento da reserva de carbono do primeiro. A produção de C- $CO_2$  no campo esteve positivamente correlacionada com a temperatura do solo e com as precipitações ( $R^2=78-89$ ). Durante o verão as glebas aradas geraram 44% mais C- $CO_2$  que as que não o foram. Porém, as diferenças na quantidade total de C- $CO_2$  produzido anualmente não foram significativas.

**Termos para indexação:** arado, grade de disco, carbono orgânico, amostras incubadas, matéria orgânica.

## INTRODUCTION

The organic matter level of soils, especially in the upper 15 cm of the profile, decrease when they are destined to agricultural use (Smith & Young, 1975; Tiessen et al., 1982; Rasmussen & Collins, 1991). Tillage intensity affects the magnitude of organic carbon depletion. This is generally more dramatic when soils are tilled with the mold board plow (Doran, 1980; El-Harris et al., 1983; Sommerfeldt & Chang, 1985; Angers et al., 1993a; Cambardella & Elliot, 1993; Mahboubi et al., 1993). As a consequence of this

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process the reduction of total microbial biomass (Gupta & Germida, 1988; Carter & Mele, 1992; Angers et al., 1993a, 1993b) and active microbial biomass (Van de Werf & Verstraete, 1987) had been observed.

Variations in the microbial biomass content of soils, as influenced by cultural practices, can be taken as an early indication of changes in total organic matter level (Powlson et al., 1987; Saffigna et al., 1989; Ross et al., 1992). Tillage induces reductions in the bacterial population size (Doran, 1980) as well as in microbial biomass (Lynch & Panting, 1980a; Saffigna et al., 1989; Angers et al., 1993a) in superficial soil layers. Changes in microbial biomass content were detected by Lynch & Panting (1980a) during the first crop cycle after implementing different tillage systems. However other authors found no variation in the biomass level after one application of contrasting tillage methods (Lynch & Panting, 1980b; Holland & Coleman, 1987). From these results arose the question of which factors influence on the changes in biomass size due to tillage systems.

Our objectives were to determine under a wheat-soybean rotation: a) the effects of conventional and reduced tillage on the microbial biomass content of a typical soil from the Argentinean Rolling Pampa, during the establishing year of the tillage methods, b) to assess the influence of the tillage system on biological activity and specific respiration of microorganisms and c) to establish the relation between environmental factors and soil C-CO<sub>2</sub> emission in the field.

## MATERIALS AND METHODS

### Soil and experimental design

The Argentinean Rolling Pampa covers an area of about 10 Mha and includes the most productive soils of this country (Soriano, 1991; Hall et al., 1992). The experiment was conducted in the Estación Experimental Agropecuaria Pergamino (INTA), Province of Buenos Aires, on a Typic Argiudoll, Pergamino series. Main characteristics of the 0-15 cm layer were: sand 14%, silt 66%, clay 20%, organic carbon 2.11%, pH 6.0 and field capacity (w/w) 30%. The parcel had been under agricultural use for 10 years. The experiment started in July 1989 after a soybean crop. Plots were of 4 m width and 17 m long, with 4 replications by

treatment in a randomized block design. In conventional tillage, soil was plowed with mold board plow and refined with harrow disk. Under reduced tillage only the harrow disk was employed. Wheat (*Triticum aestivum* cv Pampa INTA) was sown in July 1989 and harvested in December 1989. The crop was protected with 10 g ha<sup>-1</sup> of sulfonyleurea and 130 ml ha<sup>-1</sup> of dicamba both post-emergence herbicides. The straw was incorporated to 15 cm depth in plots destined to conventional tillage, while it was partially buried to 5 cm depth in the reduced tillage treatment. In December 1989 soybean (*Glycine max* cv Agrrow 5308) was sown. Weeds were controlled with 650 g ha<sup>-1</sup> of metribuzin (preemergence) and 50 ml ha<sup>-1</sup> of cipermetrine was applied twice during flowering for pest control. The crop was harvested in June 1990. No fertilizer was used.

### Determination of field CO<sub>2</sub>-C production

*In situ* CO<sub>2</sub>-C production was evaluated 9 times in a year using the inverted box method. Three to six steel cylinders per plot (15 cm height and 8 cm diameter) were buried at 5 cm depth and covered with polyethylene and an aluminum foil. Cylinders were set up in the soil 3 days during each determination. The CO<sub>2</sub>-C generated by the soil was trapped using 10 ml N NaOH, allocated in vials disposed into the respirometers and titrated against HCl and phenolphthalein. Soil temperature was monitored with thermometers placed at 10 cm depth. Soil water content of the 0-30 cm layer was measured gravimetrically during the third day of each determination. Rainfall records were obtained from a local meteorological station.

### Determination of microbial biomass and activity

Three sampling moments were planned (July 1989, December 1989 and June 1990), and four samples per plot were taken in each of them and composited. In all cases samplings were performed after the crops had been harvested and before soil tillage took place. Samples were taken at 0-5, 5-10 and 10-15 cm depths and the soil was treated during the 48 h following extraction. Results for the 0-15 cm layer were calculated by averaging these data, after correcting them by the apparent density of each soil stratum. Dead roots and coarse plant debris were eliminated by hand. Microbial biomass was determined by the chloroform fumigation-incubation method (Jenkinson & Powlson, 1976) on 100 g fresh soil samples, incubated in 400 ml flasks at 25 °C and with a water content of 55% of the WHC. The CO<sub>2</sub>-C produced by unfumigated soil samples during the 10 to 20-day period of incubation was used as a control to avoid handling effects (Jenkinson &

Powelson, 1976). A  $k$  factor of 0.45 was considered (Oades & Jenkinson, 1979). Soil basal respiration was determined on unfumigated samples in the 0 to 10-day period. It was assumed that in this lapse the production of  $\text{CO}_2\text{-C}$  was regulated by the initial availability of readily metabolized substrate and that this substrate could be exhausted after some days of incubation. Soil was not dried, but it was grounded, so handling effects on soil biological activity may be small. Specific activity was calculated as the ratio between soil respiration and microbial biomass.

Total organic carbon was determined by wet digestion (Allison, 1965) on dried samples sieved by 500  $\mu\text{m}$  mesh. Carbon in the soil light fraction (density  $\leq 2 \text{ g ml}^{-1}$ ), a pool of carbon scarcely transformed and highly utilizable by microorganisms, was analyzed as described by Richter et al. (1975).

#### Evaluation of organic carbon mineralization intensity

Unfumigated soil samples were incubated for 5 months at 25 °C in order to evaluate carbon mineralization intensity.  $\text{CO}_2\text{-C}$  generated every 10-30 days was trapped in 5 ml N NaOH. Cumulative  $\text{CO}_2\text{-C}$  production fitted the simplified model used by Bonde et al. (1988) to describe soil nitrogen mineralization, adapted to carbon degradation:

$$C_m = C_o (1 - e^{-k_1 t}) + k_2 t$$

where

$C_m$  = cumulative mineralized carbon

$C_o$  = carbon in the labile pool

$k_1$  = rate constant of the labile pool

$k_2$  = rate of mineralization of resistant carbon

$t$  = time

The model describes the degradation of a two component substrate, a labile one mineralized by first order kinetic, and another resistant, with a constant decomposition intensity during the incubation.

#### Statistical analysis

Data analysis was carried out using linear and multiple regression in order to find out an association between *in situ*  $\text{CO}_2\text{-C}$  production and different environmental factors. Comparisons between treatments and sampling dates means were carried out using ANOVA and Duncan multiple rank test. Mineralization model parameters were estimated using an iterative method.

## RESULTS AND DISCUSSION

### Production of $\text{CO}_2\text{-C}$ in the field

Mean soil temperature at 10 cm depth was 18.4 °C and the amount of rainfall during a one-year period (July 1989-June 1990) 1,076 mm. *In situ*  $\text{CO}_2\text{-C}$  production followed a seasonal pattern, being higher in the summer under both tillage systems (Fig. 1).  $\text{CO}_2\text{-C}$  production was significantly associated to temperature ( $r \geq 0.81$ ;  $P > 0.01$ ) but not to soil water content as measured the last day of the determination. No differences in soil temperature and hydric content were detected between tillage systems.

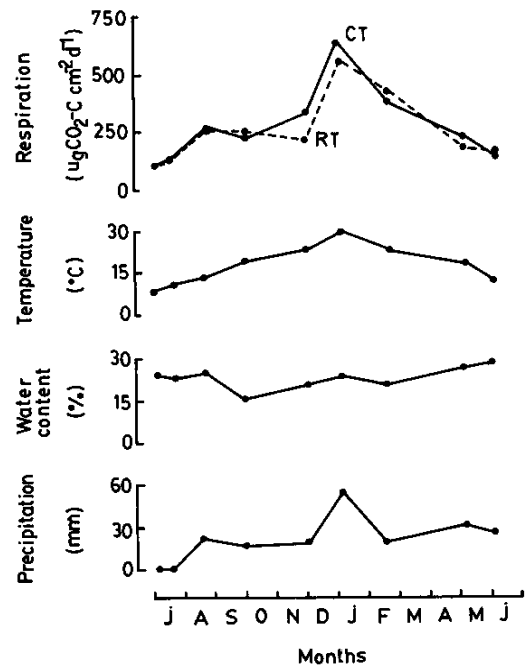


FIG. 1. Production of  $\text{CO}_2\text{-C}$  in the field, soil temperature and water content at the end of the respiration determinations, and rainfall in the period between 7 days before respirometers set up and the last day of the  $\text{CO}_2\text{-C}$  measurements. Data from July 89 to December 89 were obtained during wheat cropping and from January 90 to June 90 during soybean cropping. CT: conventional tillage, RT: reduced tillage.

Water available to microorganisms apparently did not restrict biological activity. Soil had in all sampling moments a water content of above 14% (Fig. 1) and was near field capacity in six out of nine determinations. Although temperature and water interrelationships are known to regulate soil respiration, it is considered that thermal conditions are more closely associated to CO<sub>2</sub>-C production in temperate and cold ecosystems (Edwards & Ross-Todd, 1983; Djellali et al., 1985; Reinke et al., 1981; Mathes & Schriefer, 1985; Koizumi et al., 1993). It should also be considered that, during the last two respiration determinations, it rained 23-25 mm while the respirometers were set up, so that soil water measurement after three days probably did not reflect real water availability along the whole determination period. Searching for a better index of the soil water status, rainfall data were employed. The amount of rainfall from 7 days before respirometers were set up to the end of the measurement periods was positively associated ( $r \geq 0.65$ ;  $P > 0.10$ ) with soil respiration. Temperature and rainfall together explained 78-89% ( $P > 0.05$ ) of the variability in soil CO<sub>2</sub>-C production depending on tillage system. These results suggest that the soil water condition before respiration measurements might have affected soil respiration. Peaks of CO<sub>2</sub>-C production had been detected in the field immediately after strong rains (Reinke et al., 1981; Mathes & Schriefer, 1985). An increase in the availability of readily assimilable carbon substrate to microbial biomass resulting from the drying and wetting effects that expose clay protected organic matter to biological attack could have been the cause of such peaks (Birch, 1958).

Tillage systems had no significant influences on soil respiration except in December, before wheat harvest, when the CO<sub>2</sub>-C generated under conventional tillage was 44% higher ( $P < 0.05$ ) than under reduced tillage (Table 1). Annual CO<sub>2</sub>-C production was ca. 9.8 t ha<sup>-1</sup> and no significant differences were found between treatments.

No crop effects on soil respiration could be determined. Biological activity observed in August, after soybean straw incorporation, doubled June CO<sub>2</sub>-C production. However, higher soil temperature values (3°C) and rainfall produced before respirometer installation (31 mm) could be responsible for those results (Fig. 1). In the same way, respiration raised in January after wheat straw incorporation, but soil temperature also raised (6 °C) and rainfall was intense (58 mm).

### Microbial biomass and activity

On the basis of information from samples taken at different depths organic carbon, microbial biomass and biological activity were calculated for the 0-15 cm soil layer (Table 1). Organic carbon was not different between sampling days and tillage methods, and carbon in the light fraction was not affected by tillage. Only in June 1990, after the soybean crop had been harvested, higher carbon levels were detected in that fraction ( $P < 0.05$ ) in relation to preceding sampling times.

Microbial biomass carbon accounted for 1-2% of the total organic carbon and 18-36% of the carbon in the light fraction [(biomass-C/light fraction-C) \* 100], and was lower ( $P < 0.05$ ) in December 1989 than in July 1989 and June 1990. The higher spring and summer temperatures produced an increase of soil CO<sub>2</sub>-C production in the field, and might have induced a microbial metabolism activation thus determining the self consumption of microbial biomass carbon. Other authors have observed an *in situ* depletion of the biomass level and carbohydrate soil content during summer (Angers et al., 1993 a). An accumulation of nutrients in the microbial biomass and of extractable organic carbon in the soil occurred associated to low temperatures under controlled conditions (Sarathchandra et al., 1989). Soil disruption intensity did not affect biomass content.

In contrast, respiration of soil samples incubated 10 d was 23-26% higher ( $P < 0.05$ ) under conventional tillage. It is known that intense mechanical disruption induces an immediate flush of soil CO<sub>2</sub>-C production due to an increase in available carbon substrate; a consequence of dying of microorganisms and exposition of clay associated organic matter (Rovira & Greacen, 1957; Powlson, 1980). In our case, samples were taken about 6 months after soil tillage and no deleterial tillage effects were detected on microbial biomass, consequently, these results can not be attributed to differences in the soil disruption intensity. A larger carbon availability to microorganisms due to completely straw incorporation in plowed soil might be the reason for this phenomenon.

Specific respiration in July 1989 and June 1990, as determined on soil samples incubated 10 d in the

**TABLE 1. Soil organic carbon content, carbon in the soil light fraction, microbial biomass, and biological activity of microorganisms in the 0-15 cm layer.**

Date	Tillage system	Organic-C ( $\mu\text{g C g}^{-1}$ )	Light fraction ( $\mu\text{g C g}^{-1}$ )	Biomass-C ( $\mu\text{g C g}^{-1}$ )	Respiration		Specific activity <sup>2</sup> ( $\mu\text{g CO}_2\text{-C } \mu\text{g Biomass-C}^{-1} \text{ d}^{-1}$ )	
					Laboratory ( $\mu\text{g C g}^{-1}$ )	Field ( $\mu\text{g C cm}^{-2} \text{ d}^{-1}$ )	Laboratory	Field
July 89 <sup>1</sup>		21.100 a	1.066 a	381 a	132 a	92 a	0,0348 a	0,0127 a
	Reduced	21.900 a	1.109 ab	212 b	129 a	215 b	0,0616 b	0,0531 b
December 89								
	Conven.	21.300 a	1.224 ab	219 b	163 b	310 c	0,0783 b	0,0788 c
	Reduced	19.800 a	1.413 bc	281 c	176 b	146 ab	0,0626 b	0,0279 a
June 90								
	Conven.	20.400 a	1.464 c	277 c	216 c	126 a	0,0784 b	0,0255 a

<sup>1</sup> Data from July 89 correspond to the mean of all plots, before the installation of the experiment.

<sup>2</sup> The specific activity in the field was estimated by correcting the  $\text{CO}_2\text{-C}$  production *in situ* of each treatment by the correspondingly soil density. In the columns, numbers followed by the same letter are not different at the 5 % level.

laboratory at high temperature and high water content, was 2-3 times larger ( $P < 0.01$ ) than the estimated specific respiration in the field. This was computed assuming that the  $\text{CO}_2\text{-C}$  production of the soil arose from the microbial biomass placed in the 0-15 cm layer, as no living roots were present. The same parameter, recorded *in situ* in December 1989, was 49% larger under conventional tillage ( $P < 0.05$ ) than in non plowed soil. However, these differences had disappeared in June 1990. Specific activity of incubated samples was also higher under conventional tillage but differences were not significantly different. Soil temperature and water content are known to act together on biological activity (Wildung et al., 1975). Water content was high along the whole experimental period. Consequently, in our conditions, temperature must have been the only limiting factor for microbial metabolism. This could be the reason why differences between tillage systems were detected only during the summer. In contrasting agroecosystems, when biological activity is not restricted by temperature, it had been determined that the higher the amount in which carbon is supplied, the greater the respiration of soils (Koizumi et al., 1993). Respiration and specific activity of microbial biomass are indices of available energy in soils (Campbell et al., 1992) and greater specific activity has been observed in soils subjected to conventional tillage in relation to direct drilling, after 6 years implemented these two tillage methods, when total organic carbon and biomass had changed markedly (Saffigna et al., 1989).

Distribution of organic carbon was homogeneous in depth. Since the parcel had been under cultivation for 10 years the soil had been plowed periodically and this determined the mixing of the 0-15 cm layer. No influences of tillage methods were detected on organic matter level but carbon in the light fraction decreased with depth ( $P < 0.05$ ) under both tillage systems (Fig. 2). This fraction contains coarse plant debris and decaying material which is partially associated to soil mineral components (Martel & Paul, 1974). The decrease in the content of carbon in the light fraction with depth was attributed to lower inputs of root materials from crops, as generally root masses are larger near the soil surface, and to the distribution of straw by tillage. A greater carbon content in the light fraction was detected in the 5-10 cm layer ( $P < 0.05$ ) during December 1989 in plowed soil. This is the result of soybean straw incorporation. Microbial biomass also decreased in subsuperficial soil layers. It was lower in winter (July 1989 and June 1990) at 5-10 cm depth ( $P < 0.05$ ) and during summer (December 1989) at the 10-15 cm layer ( $P < 0.05$ ) in relation to surface soil (Fig. 2). No differences were observed between tillage treatments.

$\text{CO}_2\text{-C}$  production of incubated samples in the 0-10 d period varied according to the fluctuation in the size of the light fraction (Fig. 3) and both were positively associated ( $r = 0.90$ ;  $P > 0.01$ ). Consequently the distribution of plant debris in

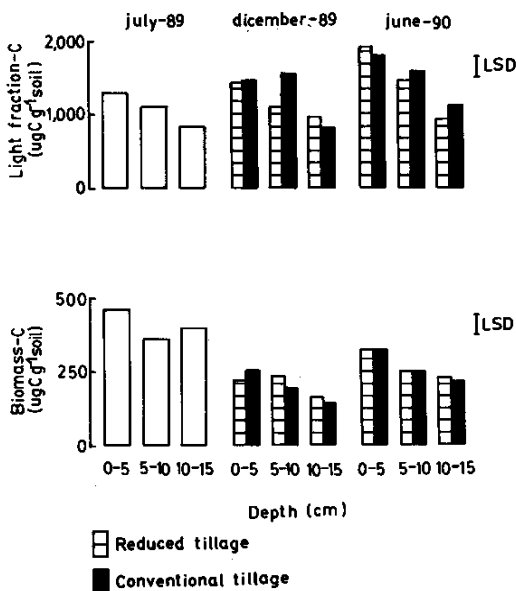


FIG. 2. Variation with depth of the carbon content in the soil light fraction and in the microbial biomass. White bars correspond to mean data from all the plots before the installation of the experiment.

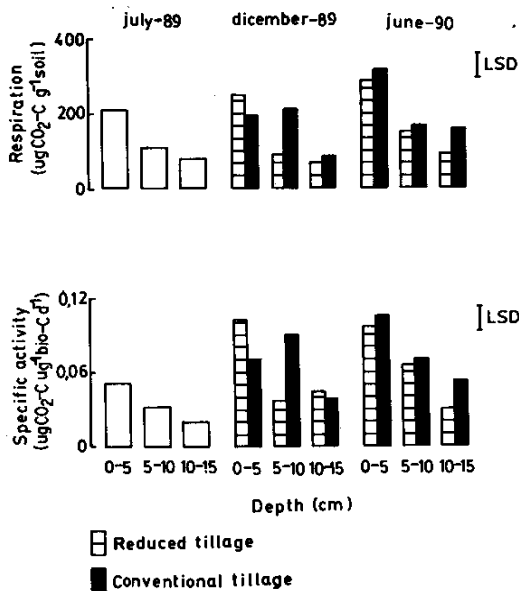


FIG. 3. Respiration and specific activity of microbial biomass on incubated soil samples.

depth showed a strong influence on biological activity. Under conventional tillage respiration was 140% greater ( $P < 0.05$ ) in December 1989 at the 5-10 cm layer and 78% higher ( $P < 0.05$ ) during June 1990 at the 10-15 cm depth than under reduced tillage, possibly as a consequence of deep straw incorporation.

Specific respiration was generally 1.5-3 fold larger in superficial soil than in depth (Fig. 3). During December 1989 at the 5-10 cm layer it was 165% greater ( $P < 0.05$ ) in plowed plots and in June-90 at the 10-15 cm layer, 89% higher ( $P < 0.05$ ) than in unplowed soil. Carbon availability in the light fraction, as a readily available substrate for microorganisms, seemed to determine the intensity of biomass metabolism. Straw incorporation to soil can produce an increase in energy consumption by microorganism (Ocio & Brookes, 1990). In this case, the correlation between the light fraction-C/biomass-C ratio and the specific respiration was 0.80 ( $P < 0.01$ ).

#### Organic carbon mineralization intensity

CO<sub>2</sub>-C production in samples incubated for five months was very intense initially but decreased after some weeks (Fig. 4). Respiration rate fell down 2-7 times between the 0-10 d period and the fifth month of incubation. Cumulative CO<sub>2</sub>-C production fitted in all cases a double exponential model ( $\geq 0.98$ ;  $P > 0.01$ ). The model allowed the estimation of the labile carbon pool (Table 2) which was closely associated to carbon in the light fraction ( $r = 0.80$ ;  $P > 0.01$ ). It also predicted that during December 1989 at 5-10 cm depth plowed soil contained 4 fold more labile carbon than the not plowed one and 2.5 times more at 10 to 15 cm depth during June 1990. These results indicate that under conventional tillage the deep incorporation of plant residues can increase carbon availability in the deep soil layers which would generate a larger CO<sub>2</sub>-C production. Indeed, the evolution of CO<sub>2</sub>-C was 88-92% greater ( $P < 0.05$ ) in this treatment.

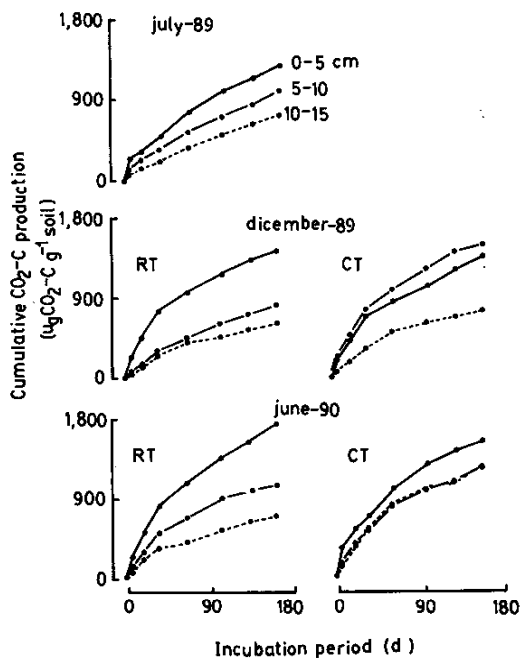


FIG. 4. Cumulative  $\text{CO}_2\text{-C}$  production from incubated samples during some months of incubation. 0-5 cm:  $\bullet\text{---}\bullet$ , 5-10 cm:  $\circ\text{---}\circ$ , 10-15 cm:  $\cdot\text{---}\cdot$ .

TABLE 2. Estimation of the size of the labile carbon pool and the kinetic parameters of the mineralization model:

$$C_m = C_o (1 - e^{-k_1 t}) + K_2 t$$

Depth (cm)	December 89		June 90		
	July 89	Reduced tillage	Convent. tillage	Reduced tillage	Convent. tillage
$C_o (\mu\text{g C g}^{-1})$					
0-5	552	733	612	936	871
5-10	371	230	926	556	698
10-15	303	282	376	326	825
$k_1 (\text{d}^{-1})$					
0-5	-0,0262	-0,0277	-0,0265	-0,0233	-0,0267
5-10	-0,0166	-0,0198	-0,0183	-0,0181	-0,0169
10-15	-0,0104	-0,0127	-0,0119	-0,0123	-0,0111
$k_2 (\mu\text{g C d}^{-1})$					
0-5	4,78	4,45	4,65	5,03	4,71
5-10	4,04	3,81	3,62	3,81	4,01
10-15	3,11	2,66	2,68	2,77	4,03

The labile carbon mineralization rate ( $k_1$ ) was affected neither by the sampling date nor by the tillage method, and it decreased with depth. The mineralization rate of resistant carbon ( $k_2$ ) was also lower in subsurface layers but was not affected by the sampling date. Under conventional tillage it was 45% higher at the 10-15 cm layer in June 1990 and this suggested a more intense microbial utilization of humified organic matter in that case.

## CONCLUSIONS

1. The incorporation of crop residues by means of the mold board plow produced an increase in the readily assimilable substrate content for microbial biomass in depth in relation to reduced tillage.
2. This phenomenon caused a rise of microbial metabolism and a greater generation of  $\text{CO}_2\text{-C}$  in incubated samples.
3. In the field, soil respiration and specific activity were more intense in plowed plots during summer, when temperature and soil water content were not limiting biological processes.
4. Microbial biomass was not significantly affected by tillage system nor was the annual  $\text{CO}_2\text{-C}$  production in the field.

## REFERENCES

- ANGERS, D.A.; BISSONNETTE, N.; LÉGÈRE, A.; SAMSON, N. Microbial and biochemical changes induced by rotation and tillage in a soil under barley production. *Canadian Journal Soil Science*, v.73, p.39-50, 1993a.
- ANGERS, D.A.; N'DAYEGAMIYE, A.; CÔTÉ, D. Tillage induced differences in organic matter of particle-size fractions and microbial biomass. *Soil Science Society of America Journal*, v.57, p.512-516, 1993b.
- ALLISON, L.E. Organic carbon. In: BLACK, C.A. (Ed.). *Methods of soil analysis*. Part 2. Chemical and microbiological properties. Madison: American Society of Agronomy, 1965. p.1367-1378. (Agronomy, 9).

- BIRCH, H.F. The effects of soil drying on humus decomposition and nitrogen availability. *Plant and Soil*, v.10, p.9-31, 1958.
- BONDE, T.A.; SCHNÜRER, J.; ROSSWALL, T. Microbial biomass as a fraction of potentially mineralizable nitrogen in soils from long-term field experiments. *Soil Biology and Biochemistry*, v.20, p.447-452, 1988.
- CAMBARDELLA, C.A.; ELLIOT, E.T. Methods for physical separation and characterization of soil organic matter fractions. *Geoderma*, v.56, p.449-457, 1993.
- CAMPBELL, C.A.; BRANDT, S.A.; BIEDERBECK, V.O.; ZENTNER, R.P.; SCHINTZER, M. Effect of crop rotations and rotation phase on characteristics of soil organic matter in a Dark Brown Chernozemic soil. *Canadian Journal of Soil Science*, v.72, p.403-416, 1992.
- CARTER, M.R.; MELE, P.M. Changes in microbial biomass and structural stability at the surface of a duplex soil under direct drilling and stubble retention in North-Eastern Victoria. *Australian Journal Soil Research*, v.30, p.493-503, 1992.
- DJELLALI, N.; BILLÉS, G.; BOUNAGA, N.; LOSSAINT, P. Étude de l'activité biologique des sols de la steppe à alfa d'Algérie. Minéralisation du carbone et de l'azote. *Acta OEcologica*, v.6, p.289-307, 1985.
- DORAN, J.W. Soil microbial and biochemical changes associated with reduced tillage. *Soil Science Society of America Journal*, v.44, p.765-771, 1980.
- EDWARDS, N.T.; ROSS-TODD, B.M. Soil carbon dynamics in a mixed deciduous forest following clear-cutting with and without residue removal. *Soil Science Society of America Journal*, v.47, p.1014-1021, 1983.
- EL-HARRIS, M.K.; COCHRAN, V.L.; ELLIOT, L.F.; BEZDICEK, D.F. Effect of tillage, cropping and fertilizer management on soil nitrogen mineralization potential. *Soil Science Society of America Proceedings*, v.47, p.1157-1161, 1983.
- GUPTA, V.V.S.R.; GERMIDA, J.J. Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. *Soil Biology and Biochemistry*, v.20, p.777-786, 1988.
- HALL, A.J.; REBELA, C.M.; GHERSA, C.M.; CULOT, J.P. Field-crop systems of the Pampas. In: PEARSON, C.J. (Ed.). *Field crop ecosystems*. Cap. 19, New York: Elsevier, 1992. p.413-450.
- HOLLAND, E.A.; COLEMAN, D.C. Litter placement effects on microbial and organic matter dynamics in an agroecosystem. *Ecology*, v.68: p.425-433, 1987.
- JENKINSON, D.S.; POWLSON, D.S. The effects of biocidal treatments on metabolism in soil -V. A method for measuring soil biomass. *Soil Biology Biochemistry*, v.8, p.209-213, 1976.
- KOIZUMI, H.; USAMI, Y.; SATOH, M. Carbon dynamics and budgets in three upland double-cropping agro-ecosystems in Japan. *Agriculture Ecosystems and Environment*, v.43, p.235-244, 1993.
- LYNCH, J.M.; PANTING, L.M. Cultivation and the soil biomass. *Soil Biology and Biochemistry*, v.12, p.29-33, 1980a.
- LYNCH, J.M.; PANTING, L.M. Variations in the size of the soil biomass. *Soil Biology and Biochemistry*, v.12, p.547-550, 1980b.
- MAHBOUBI, A.A.; LAL, R.; FAUSSEY, N.R. Twenty-eight years of tillage effects on two soils in Ohio. *Soil Science Society of America Journal*, v.57, p.506-512, 1993.
- MARTEL, Y.A.; PAUL, E.A. The use of radiocarbon dating of organic matter in the study of soil genesis. *Soil Science Society of America Proceedings*, v.38, p.501-506, 1974.
- MATHES, K.; SCHRIEFER, T. Soil respiration during secondary succession: influence of temperature and moisture. *Soil Biology and Biochemistry*, v.17, p.205-211, 1985.
- OADES, J.M.; JENKINSON, D.S. Adenosine triphosphate content of the soil microbial biomass. *Soil Biology and Biochemistry*, v.11, p.201-204, 1979.
- OCIO, J.A.; BROOKES, P.C. An evaluation of methods for measuring the microbial biomass in soils following recent additions of wheat straw and the characterization of the biomass that develops. *Soil Biology and Biochemistry*, v.22, p.685-694, 1990.
- POWLSON, D.S. The effects of grinding on microbial and non-microbial organic matter in soil. *Journal of Soil Science*, v.31, p.77-85, 1980.
- POWLSON, D.S.; BROOKES, P.C.; CHRISTENSEN, B.T. Measurement of soil microbial biomass provides an early indication of change in total soil organic matter due to straw incorporation. *Soil Biology and Biochemistry*, v.19, p.159-164, 1987.



- RASMUSSEN, P.E.; COLLINS, H.P. Long-term impacts of tillage, fertilizer, and crop residue on soil organic matter in temperate semiarid regions. *Advances in Agronomy*, v.45, p.93-134, 1991.
- REINKE, J.J.; ADRIANO, D.C.; McLEOD, K.W. Effects of litter alteration on carbon dioxide evolution from a South Carolina pine forest floor. *Soil Science Society of America Journal*, v.45, p.620-623, 1981.
- RICHTER, M.; MIZUNO, I.; ARANGUEZ, S.; URIARTE, S. Densimetric fractionation of soil organo-mineral complexes. *Journal of Soil Science*, v.26, p.112-113, 1975.
- ROSS, D.J.; SPEIR, T.W.; COWLING, J.C.; FELTHAM, C.W. Soil restoration under pasture after lignite mining: management effects on soil biochemical properties and their relationship with herbage yields. *Plant and Soil*, v.140, p.85-92, 1992.
- ROVIRA, A.D.; GREACEN, E.L. The effects of aggregate disruption on the activity of microorganisms in the soil. *Australian Journal Agricultural Research*, v.8, p.659-673, 1957.
- SAFFIGNA, P.G.; POWLSON, D.S.; BROOKES, P.C.; THOMAS, G.A. Influence of sorghum residue and tillage on soil organic matter and soil microbial biomass in an Australian Vertisol. *Soil Biology and Biochemistry*, v.21, p.759-765, 1989.
- SARATHCHANDRA, S.J.; PERROT, K.W.; LITTLER, L.A. Soil microbial biomass: influence of simulated temperature changes on size, activity and nutrient content. *Soil Biology and Biochemistry*, v.21, p.987-993, 1989.
- SMITH, S.J.; YOUNG, L.B. Distribution of nitrogen forms in virgin and cultivated soils. *Soil Science*, v.120, p.354-360, 1975.
- SOMMERFELDT, T.G.; CHANG, C. Changes in soil properties under annual applications of feedlot manure and different tillage practices. *Soil Science Society of America Journal*, v.49, p.983-987, 1985.
- SORIANO, A. Natural grasslands. In: COUPLAND, R.T. (Ed.). *Ecosystems of the world*. New York: Elsevier, 1991. p.367-407.
- TIESSEN, H.; STEWART, J.W.B.; BETTANY, J.R. Cultivation effects on the amount and concentration of carbon, nitrogen, and phosphorus in grassland soils. *Agronomy Journal*, v.74, p.831-835, 1982.
- VAN DE WERF, H.; VERSTRAETE, W. Estimation of active soil microbial biomass by mathematical analysis of respiration curves: calibration of test procedure. *Soil Biology and Biochemistry*, p.19, p.261-265, 1987.
- WILDUNG, R.E.; GARLAND, T.R.; BUSCHBOM, R.L. The interdependent effects of soil temperature and water content on soil respiration rate and plant root decomposition in arid grassland soils. *Soil Biology and Biochemistry*, v.7, p.373-378, 1975.