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**J.M. Moore · Susanne Klose · M.A. Tabatabai**

# Soil microbial biomass carbon and nitrogen as affected by cropping systems

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**Abstract** The impacts of crop rotations and N fertilization on microbial biomass C ($C_{mic}$) and N ($N_{mic}$) were studied in soils of two long-term field experiments initiated in 1978 at the Northeast Research Center (NERC) and in 1954 at the Clarion-Webster Research Center (CWRC), both in Iowa. Surface soil samples were taken in 1996 and 1997 from plots of corn (*Zea mays* L.), soybeans (*Glycine max* (L.) Merr.), oats (*Avena sativa* L.), or meadow (alfalfa) (*Medicago sativa* L.) that had received 0 or 180 kg N $ha^{-1}$ before corn and an annual application of 20 kg P and 56 kg K $ha^{-1}$. The $C_{mic}$ and $N_{mic}$ values were determined by the chloroform-fumigation-extraction method and the chloroform-fumigation-incubation method, respectively. The $C_{mic}$ and $N_{mic}$ values were significantly affected ($P<0.05$) by crop rotation and plant cover at time of sampling, but not by N fertilization. In general, the highest $C_{mic}$ and $N_{mic}$ contents were found in the multicropping systems (4-year rotations) taken in oats or meadow plots, and the lowest values were found in continuous corn and soybean systems. On average, $C_{mic}$ made up about 1.0% of the organic C ($C_{org}$), and $N_{mic}$ contributed about 2.4% of the total N ($N_{tot}$) in soils at both sites and years of sampling. The $C_{mic}$ values were significantly correlated with $C_{org}$ contents ($r \geq 0.41^{**}$), whereas the relationship between $C_{mic}$ and $N_{tot}$ was significant ($r \leq 0.53^{***}$) only for the samples taken in 1996 at the NERC site. The $C_{mic}:N_{mic}$ ratios were, on average, 4.3 and 6.4 in 1996, and 7.6 and 11.4 in 1997 at the NERC and CWRC sites, respectively. Crop rotation significantly ($P<0.05$) affected this ratio only at the NERC site, and N fertilization showed no effect at either site. In general, multicropping systems resulted in greater $C_{mic}:C_{org}$ (1.1%) and $N_{mic}:N_{tot}$ (2.6%) ratios than monocropping systems (0.8% and 2.1%, respectively).

**Key words** Crop rotation · Organic carbon · Organic nitrogen · Long-term cropping

J.M. Moore · S. Klose[1] · M.A. Tabatabai (✉)
Department of Agronomy, Iowa State University, Ames, IA 50011-1010, USA
e-mail: malit@iastate.edu
Fax: +1-515-2943163

[1] *Present address*: Institute of Soil Science, Technical University Dresden, D-01735 Tharandt, PO Box 10D, Germany

## Introduction

Recent interest in agroecology has been focused on studying soil and fertilizer management in agricultural systems to improve soil quality and minimize possible deleterious effects on the environment (e.g., soil erosion and eutrophication of natural ecosystems). Because crop residues are primary sources of organic matter, crop management and fertilizer regime can exert a significant influence on soil quality (Campbell et al. 1991). Compared with systems involving crop rotations, soils under monocultural systems, in general, contain significantly lower concentrations and qualities of soil organic matter, less soil structural stability, and reduced amounts of microbial biomass and activities. The positive effect of crop rotations on physical, chemical, and biological soil properties are related to higher C inputs and diversity of plant residues returned to soils (Biederbeck et al. 1984; Havlin et al. 1990; Varvel 1994; Miller and Dick 1995; Friedel et al. 1996; Robinson et al. 1996).

Studies on the effect of inorganic N fertilizer applications on soil biological properties have shown contradictory results. Some have reported increases in the size of microbial biomass (Fraser et al. 1994; Omay et al. 1997), whereas others have shown the opposite (Biederbeck et al. 1984; McAndrew and Malhi 1992; Ladd et al. 1994). The long-term application of $NH_4^+$ or $NH_4^+$-forming fertilizers may lead to changes in the structure of soil microbial communities in terms of promoting nitrifying populations, enhancing nitrification rates, and increasing potential risks of groundwater contamination with $NO_3$–N (Tabatabai et al. 1992).

Soil and crop management practices, including crop rotations and N fertilization, can influence soil biological activities through their effects on the quantity, structure, and distribution of soil organic matter. Systems with high organic matter inputs and easily available soil organic matter compounds tend to have higher microbial biomass contents and activities because they are preferred energy sources for microorganisms (Vaughan and Malcolm 1985).

The soil microbial biomass is involved in the decomposition of organic materials and, thus, the cycling of nutrients in soils. It is also frequently used as an early indicator of changes in soil chemical and physical properties resulting from soil management and environmental stresses in agricultural ecosystems (Brookes 1995; Jordan et al. 1995; Trasar-Cepeda et al. 1998). Although the soil microbial biomass C ($C_{mic}$) constitutes only 1–3% of total soil C and the biomass N ($N_{mic}$) up to 5% of total soil N, they are the most labile C and N pools in soils (Jenkinson and Ladd 1981). Therefore, nutrient availability and productivity of agroecosystems mainly depend on the size and activity of the microbial biomass (Friedel et al. 1996). The turnover time for N immobilized in the microbial biomass was found to be about ten times faster than that derived from plant material (Smith and Paul 1990). The determination of $N_{mic}$ is, therefore, important for the quantification of N dynamics in agricultural ecosystems because it controls soil inorganic N availability and loss, especially in high input systems.

Several methods have been proposed for the determination of $C_{mic}$ and $N_{mic}$ in soils (Horwath and Paul 1994; Alef and Nannipieri 1995; Joergensen 1995). In general, they can be grouped into direct and indirect methods. The direct methods involve microscopic procedures, including plate counting, estimation of biovolume, and culture methods. The indirect methods involve: (1) techniques, including fumigation-incubation (FI) and fumigation-extraction (FE), (2) estimation of cell components such as ATP, phospholipids, and catalase and dehydrogenase activities, and (3) substrate-induced activity of microorganisms. Among these, the FI and FE methods are widely used for estimation of $C_{mic}$ and $N_{mic}$. Results obtained by these methods are highly correlated (Brookes et al. 1985). Because of the availability of sensitive techniques for determination of organic C in soil extracts, including automated instruments, the FE method for estimating $C_{mic}$ is widely used. Similar equipment for determination of total N ($N_{tot}$) in soil extracts, however, is not yet widely available, and estimation of $N_{tot}$ in soil extracts by a semimicro-Kjeldahl method is difficult, time-consuming, and not accurate. A review of the methods available for estimation of $C_{mic}$ and $N_{mic}$ in soils led us to the selection of those used in this study, i.e., FE for estimation of $C_{mic}$ and FI for $N_{mic}$.

The objectives of this study were to estimate the sizes of the $C_{mic}$ and $N_{mic}$ in 19-year and 44-year-old field experiments, where different crop rotations and N fertilization levels have resulted in field plots with significant differences in pH, and in soil organic C ($C_{org}$) and $N_{tot}$ contents.

## Materials and methods

### Soils

Soils were collected from two long-term cropping systems at the Northeast Research Center (NERC) in Nashua, Iowa, and the Clarion-Webster Research Center (CWRC) in Kanawha, Iowa. Both sites were established to study the influence of crop rotation and N fertilization on crop yields. The NERC study was initiated in 1979 on Kenyon (fine-loamy, mixed, mesic Typic Hapludoll) and Readlyn (fine-loamy, mixed, mesic, Aquic Hapludoll) loams formed in reworked till sediment, with mean particle-size distributions ($\pm$SD) of $22 \pm 4.5\%$ clay, $47 \pm 5.3\%$ silt, and $31 \pm 3.2\%$ sand. The CWRC site was established in 1954 on a Webster clay loam (fine-loamy, mixed, mesic Typic Haplaquoll) formed in till-derived sediments, with mean particle-size distributions of $33 \pm 5.1\%$ clay, $43 \pm 5.9\%$ silt, and $24 \pm 6.2\%$ sand. The past 30-year average annual precipitation is 711 mm and 813 mm, and the mean annual temperature is 7.7 °C and 6.9 °C at NERC and CWRC, respectively. The experimental design at both sites was a split-plot design, replicated three times at the NERC site and twice at the CWRC site, with crop rotation as the main plot and N rate as the subplot (70 $m^2$ and 74 $m^2$ for the NERC and CWRC sites, respectively). The cropping systems at the NERC sites were continuous soybean (SbSbSbSb), continuous corn (CCCC), corn–soybean (CSbCSb), and corn–corn–oat–meadow (CCOM). The systems at the CWRC site were CCCC, CSbCSb, CCOM, and corn–oat–meadow–meadow (COMM). The N treatments chosen for each rotation at both sites were 0 and 180 kg N $ha^{-1}$, applied as urea ($NH_2CONH_2$) before corn only. All plots received an annual fertilization of 20 kg P and 56 kg K $ha^{-1}$, and the primary tillage system was chisel plowing in the fall (Robinson et al. 1996).

Surface soil samples (0–15 cm) were taken in corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), oats (*Avena sativa* L.), and meadow plots. The meadow was planted with alfalfa (*Medicago sativa* L.) alone or mixed with red clover (*Trifolium pratense* L.). The samples from the NERC site were collected in May 1996 and June 1997, and the samples from the CWRC site were collected in July 1996 and 1997 by pooling eight cores (diam 7.6 cm) taken from each plot at random. The field-moist soil samples were sieved through a 2-mm screen after removing any plant material and roots, and stored at 4 °C for several days until analysis for $C_{mic}$ and $N_{mic}$.

### Analytical methods

For the properties reported (Tables 1, 2), pH was measured following air-drying by using a combination glass electrode in both water and 0.01 m $CaCl_2$ solutions (soil:solution ratio = 1:2.5). Particle-size distribution was measured by pipette analysis (Kilmer and Alexander 1949). A portion of air-dried soil was ground to pass an 80-mesh (180μm) sieve. $C_{org}$ and $N_{tot}$ were determined by the Mebius method (1960) and by a semimicro-Kjeldahl method (Bremner and Mulvaney 1982), respectively.

### $C_{mic}$ and $N_{mic}$

Soil $C_{mic}$ was estimated on a 15-g oven-dry equivalent of field-moist soil sample by the chloroform-fumigation-extraction method (FE) described by Vance et al. (1987). The concentration of $C_{org}$ in the extractant was determined by a carbon analyzer (SHIMADZU Model TOC-5050) after acidification with one drop of

**Table 1** Properties of the soils at the Northeast Research Center (NERC) at Nashua, Iowa

| Crop rotation[a] | N treatment (kg ha$^{-1}$) | pH[b] | | $C_{org}$[b] | $N_{tot}$[b] |
|---|---|---|---|---|---|
| | | $H_2O$ | $CaCl_2$ | (g kg$^{-1}$) | |
| **C**-c-c-c | 0 | 6.7 (6.4) | 6.2 (5.9) | 25.3 (21.2) | 1.64 (1.67) |
| | 180 | 6.4 (6.5) | 6.1 (6.0) | 22.6 (22.6) | 1.49 (2.07) |
| **C**-sb-c-sb | 0 | 7.1 (7.1) | 6.6 (6.5) | 17.3 (18.7) | 1.21 (1.73) |
| | 180 | 6.6 (7.1) | 6.2 (6.3) | 17.7 (19.5) | 1.32 (1.87) |
| c-**Sb**-c-sb | 0 | 7.1 (7.1) | 6.6 (6.5) | 19.8 (21.4) | 1.37 (1.63) |
| | 180 | 7.1 (6.6) | 6.5 (6.3) | 19.2 (21.9) | 1.40 (1.67) |
| **C**-c-o-m | 0 | –[c] (6.9) | – (6.9) | – (22.7) | – (1.93) |
| | 180 | – (6.5) | – (6.5) | – (20.3) | – (1.90) |
| c-**C**-o-m | 0 | 7.0 (7.1) | 6.5 (7.1) | 21.4 (21.6) | 1.57 (1.70) |
| | 180 | 6.2 (6.8) | 6.0 (6.8) | 23.3 (22.4) | 1.68 (1.67) |
| c-c-**O**-m | 0 | 7.2 (6.8) | 6.6 (6.8) | 22.0 (22.7) | 1.40 (1.67) |
| | 180 | 7.0 (6.7) | 6.3 (6.7) | 21.4 (23.5) | 1.46 (1.90) |
| c-c-o-**M** | 0 | 7.0 – | 6.5 – | 25.5 – | 1.79 – |
| | 180 | 6.7 – | 6.1 – | 23.2 – | 1.63 – |
| **Sb**-sb-sb-sb | 0 | 7.1 (7.0) | 6.5 (7.0) | 17.4 (18.6) | 1.25 (1.77) |
| Mean | | 6.9 (6.8) | 6.4 (6.6) | 21.2 (20.1) | 1.48 (1.78) |
| LSD $P<0.05$[d] | | 0.5 (0.6) | 0.3 (0.5) | 1.9 (2.2) | 0.11 (0.17) |
| LSD $P<0.05$[e] | | 0.6 (0.4) | 0.2 (0.5) | 1.2 (2.5) | 0.10 (0.20) |

[a] Capital bold letters indicate crop in which the sample was taken in 1996. In 1997, the samples were taken in the crop following the capital bold letter; *C* corn, *Sb* soybean, *O* oats, *M* meadow (alfalfa)
[b] Means of three field replicates from 1996. Means in parentheses are data for 1997
[c] Indicates that sample was not available in the specified crop in the rotation
[d] Least significant difference due to crop rotation at 0 N
[e] Least significant difference due to crop rotation at 180 N

**Table 2** Properties of the soils at the Clarion-Webster Research Center (CWRC) at Kanawha, Iowa

| Crop rotation[a] | N treatment (kg ha$^{-1}$) | pH[b] | | $C_{org}$[b] | $N_{tot}$[b] |
|---|---|---|---|---|---|
| | | $H_2O$ | $CaCl_2$ | (g kg$^{-1}$) | |
| **C**-c-c-c | 0 | 6.9 (7.0) | 6.2 (6.2) | 28.2 (29.8) | 1.79 (2.00) |
| | 180 | 6.0 (6.0) | 5.3 (5.4) | 35.2 (35.6) | 2.32 (2.20) |
| **C**-sb-c-sb | 0 | 7.1 (7.3) | 6.5 (6.7) | 31.2 (32.9) | 2.08 (2.20) |
| | 180 | 6.7 (6.8) | 6.2 (6.2) | 29.6 (32.1) | 1.96 (2.20) |
| c-**Sb**-c-sb | 0 | 6.7 (6.7) | 6.2 (6.1) | 30.6 (34.5) | 2.03 (2.25) |
| | 180 | 6.5 (6.3) | 5.9 (5.8) | 31.8 (33.6) | 2.15 (2.15) |
| **C**-c-o-m | 0 | 6.2 (6.5) | 5.6 (5.7) | 29.5 (31.2) | 2.05 (1.60) |
| | 180 | 5.8 (6.0) | 5.3 (5.3) | 34.9 (36.4) | 2.42 (1.80) |
| c-**C**-o-m | 0 | 6.4 (6.3) | 5.8 (5.4) | 32.1 (33.9) | 2.15 (1.95) |
| | 180 | 5.9 (6.2) | 5.4 (5.4) | 35.1 (37.7) | 2.40 (1.95) |
| c-c-**O**-m | 0 | 6.0 (6.0) | 5.3 (5.2) | 33.1 (35.7) | 2.18 (2.35) |
| | 180 | 5.9 (5.8) | 5.2 (5.1) | 34.2 (36.4) | 2.36 (2.30) |
| c-c-o-**M** | 0 | 5.9 (5.9) | 5.4 (5.2) | 38.4 (40.9) | 2.50 (2.40) |
| | 180 | 5.7 (5.6) | 5.2 (4.9) | 37.1 (39.5) | 2.45 (2.00) |
| **C**-o-m-m | 0 | 5.8 (6.2) | 5.2 (5.6) | 35.4 (36.1) | 2.43 (2.25) |
| | 180 | 5.5 (5.7) | 4.9 (4.9) | 34.8 (34.5) | 2.37 (2.30) |
| c-**O**-m-m | 0 | 6.0 (5.9) | 5.3 (5.3) | 34.5 (35.3) | 2.34 (2.50) |
| | 180 | 6.4 (6.4) | 5.8 (5.6) | 37.0 (44.3) | 2.52 (2.85) |
| c-o-**M**-m | 0 | 6.2 (6.2) | 5.6 (5.5) | 30.2 (30.5) | 2.12 (2.20) |
| | 180 | 6.1 (5.8) | 5.5 (5.1) | 32.6 (34.9) | 2.24 (2.35) |
| c-o-m-**M** | 0 | 5.9 (5.6) | 5.3 (5.1) | 37.7 (39.2) | 2.54 (2.50) |
| | 180 | 5.8 (5.4) | 5.2 (4.9) | 37.8 (39.1) | 2.67 (2.55) |
| Mean | | 6.2 (6.1) | 5.6 (5.4) | 33.7 (35.6) | 2.28 (2.22) |
| LSD $P<0.05$[c] | | 1.1 (1.1) | 1.1 (1.2) | 4.9 (6.0) | 0.33 (0.47) |
| LSD $P<0.05$[d] | | 1.3 (1.5) | 1.4 (1.5) | 5.5 (4.9) | 0.28 (0.28) |

[a] Capital bold letters indicate crop in which the sample was taken in 1996. In 1997, the samples were taken in the crop following the capital bold letter; *C* corn, *Sb* soybean, *O* oats, *M* meadow (alfalfa)
[b] Means of three field replicates from 1996. Means in parentheses are data for 1997
[c] Least significant difference due to crop rotation at 0 N
[d] Least significant difference due to crop rotation at 180 N

2 M HCl to remove any dissolved carbonate. Biomass C was calculated as follows:

$$C_{mic} = E_C/k_{EC}$$

where $E_C$ = (organic C extracted from fumigated soil) – (organic C extracted from non-fumigated soil) and $k_{EC} = 0.45$, which is the proportionality factor to convert $E_C$ to $C_{mic}$ (Wu et al. 1990).

Soil $N_{mic}$ was determined by the chloroform-fumigation-incubation method (FI) as described by Horwath and Paul (1994) using a 15-g oven-dry equivalent of field-moist soil sample, after adjusting the moisture content to 55% of the water-holding capacity (WHC), estimated by the method of Shaw (1958). Samples were conditioned for 7 days at 25 °C. The $NH_4^+$-N in the extracts was determined in a 20-ml aliquot by steam distillation (Keeney and Nelson 1982). Biomass N was calculated using the equation:

$$N_{mic} = E_N/k_{IN}$$

where $E_N$ = (flush of $NH_4^+$-N due to fumigation) – ($NH_4^+$-N produced in the non-fumigated soil during 10 days incubation) and $k_{IN} = 0.57$, which is the proportionality factor to convert $E_N$ to $N_{mic}$ (Jenkinson 1988). The $C_{mic}$ and $N_{mic}$ values were determined on the <2-mm mesh field-moist samples. All results reported are averages of duplicated analyses and are expressed on a moisture-free basis. Moisture was determined after drying at 105 °C for 48 h.

Statistical analyses, including analysis of variance, contrast comparisons, and separation of means by least significant differences were performed by using the general linear models (GLM) procedure in the SAS system (SAS Institute 1996). Non-orthogonal contrast comparisons were selected because we were interested in the following hypotheses: (1) a system under continuous corn will differ from that under continuous soybean (contrast 1, NERC only), (2) a system under continuous corn will differ from that under multicropping systems (contrast 2), (3) a system under continuous corn will differ from a 2-year rotation (contrast 3), (4) a system under a 2-year rotation will differ from that under a 4-year rotation (contrast 4), and (5) a system under continuous corn will differ from that under a 4-year rotation (contrast 5, NERC only). At all data points shown in the figures, the differences between duplicate laboratory values were smaller than the point size.

## Results and discussion

The differences in chemical properties of soils, such as pH, $C_{org}$, and $N_{tot}$, found between the two different sites and between the four different cropping systems studied at each of these sites (Tables 1, 2) may have led to differences in the microbial biomass in these soils. Crop rotation significantly affected soil pH ($H_2O$), $C_{org}$, $N_{tot}$, and C:N ratio in soils at the NERC site, while the effect of N fertilization was not clear because it varied between the two years (Table 3). Contrast comparisons revealed significantly lower soil pH ($H_2O$) in plots under continuous corn compared with the multicropping systems, probably resulting from the higher frequency of ammoniacal-N fertilizer additions before corn; as soybean, oats, and meadow did not receive any N fertilizer. The decrease of pH values in N-fertilized soils may be due to the nitrification of $NH_4^+$ and subsequent production of $H^+$ ions, thus increasing the soil acidity (McAndrew and Malhi 1992).

$C_{mic}$ and $N_{mic}$ showed, in general, higher values in the plots at the CWRC site compared with those at the NERC site. The microbial biomass values were, on average, 385 and 213 mg $C_{mic}$ $kg^{-1}$ soil, and 63 and 51 mg $N_{mic}$ $kg^{-1}$ soil, in the plots at the CWRC and NERC sites in 1996, respectively. A similar trend was found in 1997, although the values were generally lower. These findings may be attributed mainly to higher organic C contents in the soils at CWRC in comparison to those

**Table 3** Analysis of variance of effects of cropping systems on chemical and biological properties in soils from the Northeast Research Center (NERC)

| Source of variation | Chemical properties | | | | | Biological properties | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | pH ($H_2O$) | pH ($CaCl_2$) | $C_{org}$ | $N_{tot}$ | C:N | $C_{mic}$ | $N_{mic}$ | $(C:N)_{mic}$ | $C_{mic}:C_{org}$ | $N_{mic}:N_{tot}$ |
| 1996 | | | | | | | | | | |
| Crop rotation | ** | n.s. | ** | ** | ** | ** | ** | * | ** | ** |
| N fertilizer | ** | * | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Contrasts[a] | | | | | | | | | | |
| C1 | n.s. | n.s. | * | n.s. | n.s. | ** | ** | * | n.s. | n.s. |
| C2 | ** | ** | * | * | ** | * | ** | * | ** | ** |
| C3 | ** | ** | ** | ** | ** | n.s. | n.s. | * | * | ** |
| C4 | * | n.s. | ** | ** | n.s. | ** | ** | n.s. | * | n.s. |
| C5 | ** | ** | n.s. | n.s. | * | ** | ** | * | ** | ** |
| 1997 | | | | | | | | | | |
| Crop rotation | * | ** | * | * | ** | * | ** | ** | ** | ** |
| N fertilizer | n.s. | n.s. | n.s. | ** | * | n.s. | * | n.s. | n.s. | n.s. |
| Contrasts[a] | | | | | | | | | | |
| C1 | n.s. | n.s. | ** | ** | ** | n.s. | n.s. | n.s. | * | * |
| C2 | ** | ** | * | * | n.s. | n.s. | ** | n.s. | ** | ** |
| C3 | ** | ** | ** | ** | n.s. | n.s. | * | n.s. | n.s. | * |
| C4 | * | n.s. | ** | ** | n.s. | ** | * | n.s. | * | * |
| C5 | ** | ** | n.s. | n.s. | n.s. | * | ** | n.s. | ** | ** |

* and ** are significant at $P<0.05$ and 0.01 levels, respectively; *n.s.* not significant

[a] Contrasts: C1:CCCC vs. SbSbSbSb, C2:CCCC vs. multicropping systems, C3:CCCC vs. 2-year rotation, C4:2-year rotation vs. 4-year rotation, C5:CCCC vs. 4-year rotation

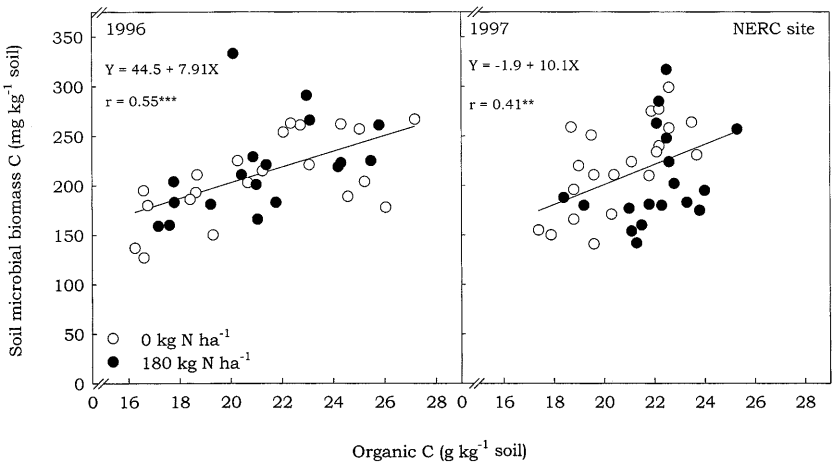


**Fig. 1** Relationship between organic C and soil microbial biomass C of soils at the NERC site sampled in 1996 and 1997

in the soils at NERC. The $C_{mic}$ values obtained in 1996 for the NERC site were significantly correlated with $C_{org}$ ($r = 0.55$***, Fig. 1). The corresponding correlation coefficient for the relationship at the CWRC site was 0.57***. The same relationship for the samples obtained in 1997 showed $r = 0.41$** for the NERC site (Fig. 1). No such relationship was found for the CWRC site in 1997. Similar significant positive linear correlations have been reported between $C_{mic}$ and $C_{org}$ for diverse soils (Anderson and Domsch 1989; Witter et al. 1993; Joergensen 1995; Weigand et al. 1995). Witter et al. (1993) reported $r = 0.905$*** for 14 soils from long-term field experiments under different treatments in Sweden, whereas Joergensen (1995) showed $r = 0.82$**** for this relationship in 82 German soils differing in management practices (arable, pasture, forest soils).

Analysis of variance showed that $C_{mic}$ and $N_{mic}$ values were significantly ($P < 0.05$) affected by crop rotation and plant cover at sampling time, but not by N fertilizer treatment (Tables 3, 4). At the NERC site, the highest $C_{mic}$ values were found in plots of the 4-year rotation (CCOM) taken in oats or meadow in both years (Fig. 2). The lowest $C_{mic}$ values were found in plots under continuous soybean followed by continuous corn and corn–soybean rotation (2-year rotation). At the CWRC site, the $C_{mic}$ contents followed a similar trend, with the highest values found in plots of the 4-year rotations (CCOM, COMM) and the lowest under the continuous and the 2-year cropping systems (CCCC, CSbCSb). The highest $N_{mic}$ values at the NERC site were found in the 4-year rotation, taken in meadow and in first year corn, for soils sampled in 1996 and 1997, respectively (Fig. 3), and the lowest values under the continuous cropping system. At the CWRC site, no effect of crop rotations on $N_{mic}$ was found in either sampling year. Differences in $N_{mic}$ values between the years are evident for both sites (Figs. 3, 4). The reasons for the dramatic variations are unclear. Temporal fluctuations in $C_{mic}$ and $N_{mic}$ values have

**Table 4** Analysis of variance of effects of cropping systems on chemical and biological properties in soils from the Clarion-Webster Research Center (CWRC)

| Source of variation | Chemical properties | | | | | Biological properties |
|---|---|---|---|---|---|---|
| | pH ($H_2O$) | pH ($CaCl_2$) | $C_{org}$ | $N_{tot}$ | C:N | $C_{mic}$ |
| 1996 | | | | | | |
| Crop rotation | n.s. | n.s. | * | ** | * | * |
| N fertilizer | n.s. | n.s. | * | ** | * | n.s. |
| Contrasts[a] | | | | | | |
| C2 | n.s. | n.s. | n.s. | ** | * | n.s. |
| C3 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| C4 | ** | ** | ** | ** | ** | * |
| 1997 | | | | | | |
| Crop rotation | n.s. | n.s. | * | ** | ** | * |
| N fertilizer | n.s. | n.s. | ** | * | n.s. | n.s. |
| Contrasts[a] | | | | | | |
| C2 | n.s. | n.s. | * | n.s. | n.s. | ** |
| C3 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| C4 | * | * | * | * | ** | n.s. |

* and ** are significant at $P < 0.05$ and 0.01 levels, respectively, *n.s.* not significant
[a] Contrasts: C2:CCCC vs. multicropping systems, C3:CCCC vs. 2-year rotation, C4:2-year rotation vs. 4-year rotation

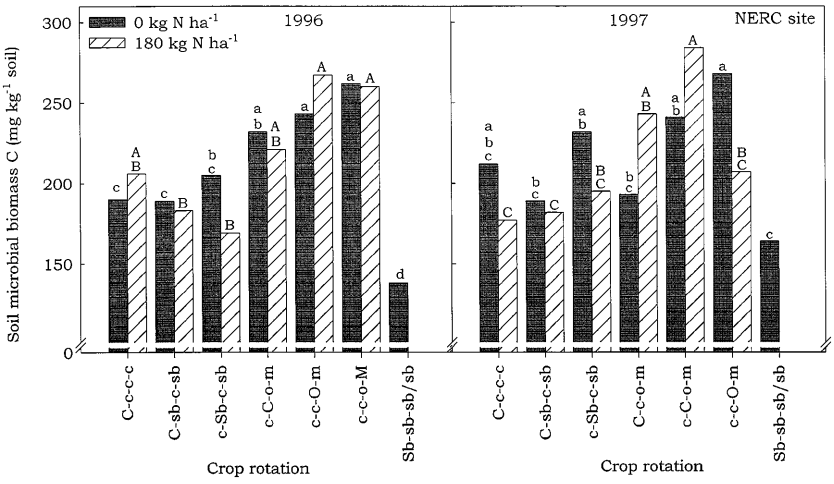


**Fig. 2** Effect of cropping systems on the soil microbial biomass C at the NERC site. *Capital letter* indicates crop at which samples were taken in 1996. In 1997, the samples were taken in the crop following the capital letter. All results are the averages of three field replicates. Within each N treatment, *bars* with the same letter are not significantly different at $P<0.05$ using the LSD test (0 N treatment *lower-case letters*; 180 N treatment *upper-case letters*)

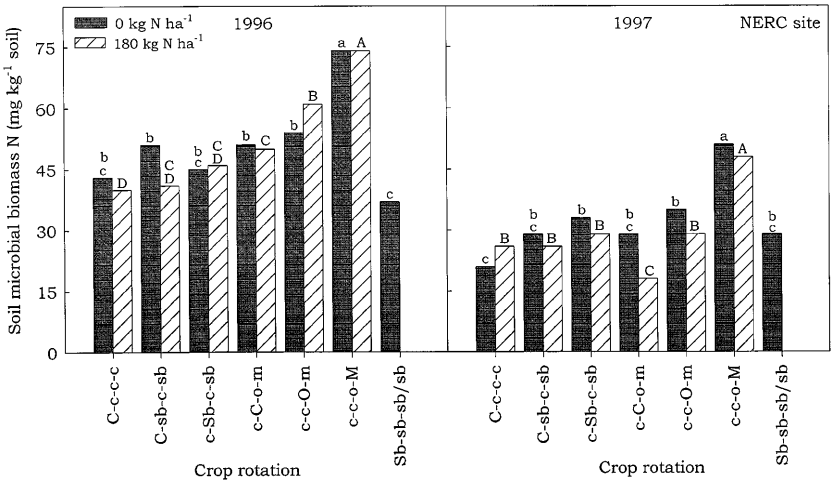


**Fig. 3** Effect of cropping systems on the soil microbial biomass N at the NERC site. *Capital letter* indicates crop for which samples were taken in 1996. In 1997, the samples were taken in the crop following the capital letter. All results are the averages of three field replicates. Within each N treatment, *bars* with the same letter are not significantly different at $P<0.05$ using the LSD test (0 N treatment *lower-case letters*; 180 N treatment *upper-case letters*)

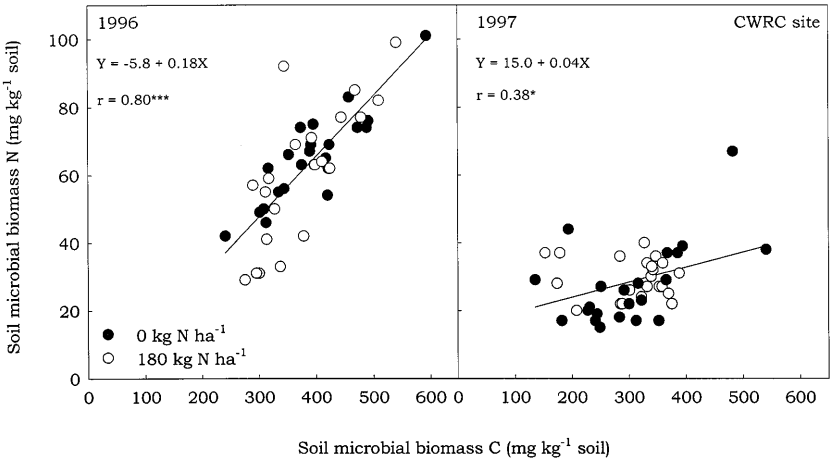


**Fig. 4** Relationship between microbial biomass C and microbial biomass N in soils at the CWRC site, sampled in 1996 and 1997

been reported as a result of variations in soil moisture and temperature, stage of plant growth, and available substrate (Insam 1990; Witter et al. 1993; Kaiser et al. 1995; Lovell et al. 1995; Chang and Juma 1996). The $N_{mic}$ values show more pronounced temporal fluctuations than those of $C_{mic}$ (Brookes et al. 1985; Joergensen 1995), because microorganisms differ much more in their N content than in their C content, depending on their stage of growth (Anderson and Domsch 1980; Jenkinson and Ladd 1981). This is one reason for the larger variations in $k_N$ values compared with $k_C$ values found in the literature (Joergensen 1995). Therefore, small shifts in the structure of the microbial community can result in large changes in $N_{mic}$ (Campbell et al. 1991). Close relationships between total N, $N_{mic}$ and active N in soils have been reported by McCarty et al.

(1995), with correlation coefficients of $r>0.91$*. Although these N pools are highly correlated, the slopes of the regression lines indicated that $N_{mic}$ and active N pools are distinct from the total N pool. This could explain the lack of influence of N fertilization on the $N_{mic}$ values found in this study. The relationship between $N_{tot}$ and $N_{mic}$ was significant, with non-zero intercepts, for the samples from the NERC site in 1996 only ($r=0.53$***), and the slope of the regression line decreased substantially between the two years of sampling (not shown). The non-zero intercepts may indicate a pool of largely inert soil-N that obviously did not have a positive influence on the $N_{mic}$ values.

The positive effect of the 4-year rotations on microbial biomass compared with the continuous corn and soybean systems and the 2-year rotation may be due to several reasons, such as enhanced soil structure, greater amounts and diversity of residues produced, the nearly year-around rhizosphere and plant cover, a stabilized microclimate, and higher root density under diverse crop rotations. Contrast comparisons revealed that $C_{org}$ and $N_{tot}$ were, generally, lowest in plots under continuous soybean, followed by corn–soybean (2-year) rotation, and highest in CCCC and CCOM (4-year rotation) plots, with no significant difference between the latter two systems (Tables 3, 4). Varvel (1994) and Havlin et al. (1990) reported lower $C_{org}$ and N contents in soils from crop rotations that involved soybean and related this to the lower amounts of crop residues left after soybean as compared with those left after the corn harvest. Higher $C_{mic}$ values are commonly found in rotations that include high residue-producing crops (Omay et al. 1997), crops with intensive root growth and root density (Stone and Buttery 1989; Perfect et al. 1990), and under reduced or no-till systems (Granatstein et al. 1987; Campbell et al. 1992). These results are strongly related to the positive influence of the rhizosphere on soil microbial communities. In addition, it is known that there are differences in rhizosphere populations because of the variations in the concentrations and types of organic compounds released by the roots of different plants (Lynch and Bragg 1985). Besides the effects of rhizosphere and the amounts and diversity of plant material returned to the soil, the decomposition rate of these residues is considered to be important for the accessibility of the organic material to soil microorganisms. A comparison of various plant materials showed that the amounts of easily decomposable $C_{org}$ fractions decreased from alfalfa residues to corn and soybean residues and, thus, their decomposition rates varied (Ajwa and Tabatabai 1994).

Linear regression analyses showed that $C_{mic}$ was significantly correlated with $N_{mic}$ for the soils sampled at the CWRC site in both years (Fig. 4). A similar correlation was found for the samples from the NERC site in 1996 ($r=0.71$***, not shown), but not in 1997. A high correlation coefficient ($r=0.80$***) has been reported between $C_{mic}$ and $N_{mic}$ for 82 soils under different management systems (Joergensen 1995). The inconsistency in the relationship between $C_{mic}$ and $N_{mic}$ at both sites may be a result of the large temporal variation in $N_{mic}$ values between the 1996 and 1997 data.

The influence of moisture content of soils on biomass measurements has been a subject of controversy. A study involving the FI method with soils at 18, 23, and 33% of the WHC revealed no significant differences in $CO_2$–C or mineralizable N flush even when these values were increased to 60% (Ross 1989). Nevertheless, the adjustment of soils at 50–60% WHC is considered as necessary requirement for a higher accuracy of the FI method (Jenkinson 1988). The FE method was shown to be unaffected by soil water content (Kaiser et al. 1995; Joergensen 1995). However, the water content in the soils studied at sampling time did not show significant variations between the two years and, thus, could be excluded as a possible reason for the changes in $N_{mic}$ values. It may be inferred that $N_{mic}$ was more sensitive to factors that could have influenced the size and structure of microbial biomass in this study. These include microclimate, crop rotation, fertilizer practices, soil pH and structure, and amount and distribution of soil organic matter. Therefore, $N_{mic}$ seems to be more labile in soils than $C_{mic}$.

The ratio of $C_{mic}$:$N_{mic}$ is often used to describe the structure and the state of the microbial community. A high $C_{mic}$:$N_{mic}$ ratio indicates that the microbial biomass contains a higher proportion of fungi, whereas a low value suggests that bacteria predominate in the microbial population (Campbell et al. 1991). The $C_{mic}$:$N_{mic}$ ratios of selected microbial isolates, extracted from soils or obtained from culture collections, and cultivated under optimal conditions, range from 7 to 12 in fungi and from 3 to 6 in bacteria (Jenkinson 1976; Anderson and Domsch 1980). Differences in these ratios are expected between microbial populations cultivated under laboratory conditions and those grown under natural conditions (Joergensen 1995). Joergensen (1995) reported C:N ratios of the microbial biomass varying from 5.2 in an arable soil to 20.8 in a forest soil, with an average of 6.8 for 82 soils. Shen et al. (1984) and Anderson and Domsch (1980) summarized data suggesting that the average microbial biomass C:N ratio is 6.7. The $C_{mic}$:$N_{mic}$ ratios of the soils from the two different experimental sites were, on average, 4.3 and 6.4 in 1996, and 7.6 and 11.4 in 1997 at the NERC and CWRC sites, respectively (Table 5).

The differences found for these ratios between the two years are mainly attributed to the variations in $N_{mic}$ values already discussed. The $C_{mic}$:$N_{mic}$ ratio is affected by soil properties such as moisture content, texture, pH, $C_{mic}$:$C_{org}$ and $N_{mic}$:$N_{tot}$ ratios (i.e., the substrate availability), N incorporation in fungi and the ratio of active to dormant microorganisms (Jenkinson 1976; Anderson and Domsch 1980; Campbell et al. 1991). However, crop rotations significantly ($P\leq0.05$) affected the $C_{mic}$:$N_{mic}$ ratio at the NERC site only, whereas rotation and N fertilization had no effect on this ratio at the CWRC site (Tables 3, 4). Results indi-

**Table 5** Values of the microbial biomass C:N ratios in soil samples of the different cropping systems in 1996 and 1997

| Crop rotation[a] | N treatment (kg ha$^{-1}$) | Microbial biomass C:N ratio[b] | | | |
|---|---|---|---|---|---|
| | | NERC site | | CWRC site | |
| | | 1996 | 1997 | 1996 | 1997 |
| **Sb**-sb-sb-sb | 0 | 4.0 | 5.7 | – | – |
| **C**-c-c-c | 0 | 4.4 | 11.1 | 5.5 | 7.6 |
| | 180 | 5.2 | 6.9 | 6.0 | 7.7 |
| **C**-sb-c-sb | 0 | 3.8 | 6.5 | 5.9 | 11.4 |
| | 180 | 4.4 | 7.6 | 4.7 | 11.5 |
| c-**Sb**-c-sb | 0 | 4.6 | 7.1 | 5.6 | 5.2 |
| | 180 | 3.8 | 7.2 | 6.0 | 12.6 |
| **C**-c-o-m | 0 | –[c] | 7.0 | 5.9 | 11.4 |
| | 180 | – | 13.8 | 7.5 | 12.3 |
| c-**C**-o-m | 0 | 4.6 | 6.8 | 5.7 | 10.5 |
| | 180 | 4.4 | 9.9 | 8.0 | 16.3 |
| c-c-**O**-m | 0 | 4.5 | 5.3 | 7.0 | 14.1 |
| | 180 | 4.4 | 4.5 | 7.0 | 13.3 |
| c-c-o-**M** | 0 | 3.6 | – | 6.0 | 12.2 |
| | 180 | 3.5 | – | 5.9 | 13.9 |
| **C**-o-m-m | 0 | – | – | 6.4 | 13.0 |
| | 180 | – | – | 9.9 | 11.2 |
| c-**O**-m-m | 0 | – | – | 6.6 | 9.9 |
| | 180 | – | – | 7.3 | 8.3 |
| c-o-**M**-m | 0 | – | – | 6.1 | 9.3 |
| | 180 | – | – | 5.8 | 10.6 |
| c-o-m-**M** | 0 | – | – | 6.0 | 14.7 |
| | 180 | – | – | 6.0 | 14.7 |
| Mean | | 4.3 | 7.6 | 6.4 | 11.4 |
| LSD $P<0.05$[d] | | 0.8 | 3.6 | 1.4 | 5.1 |
| LSD $P<0.05$[e] | | 1.3 | 2.8 | 3.4 | 9.0 |

[a] Capital bold letters indicate crop in which the sample was taken in 1996. In 1997, the samples were taken in the crop following the capital bold letter; *C* corn, *Sb* soybean, *O* oats, *M* meadow (alfalfa)
[b] Means of three (NERC) and two (CWRC) field replicates
[c] Indicates that sample was not available in the specified crop in the rotation

cated that plant cover in the year of sampling had a particular impact on this ratio, because regardless of the cropping systems, the values were lowest in plots under soybean. Contrast comparisons of the 1996 values showed that plots under continuous corn differed significantly from those under continuous soybean and multicropping systems. In 1997 the $C_{mic}$:$N_{mic}$ ratios for both sites were, in general, about twice as great as those in 1996. Contradictory results have been reported concerning the response of the $C_{mic}$:$N_{mic}$ ratio to changes in environmental conditions or soil management practices. Some researchers found that this ratio is relatively constant in arable soils and independent of soil moisture and temperature, N fertilization, development of the root system (Joergensen 1995), incorporation of straw (Ocio et al. 1991) and application of pesticides (Harden et al. 1993). Others reported significant temporal changes in $C_{mic}$:$N_{mic}$ ratios, depending on the stage of plant growth (Collins et al. 1992) and plant cover (Drury et al. 1991). Drury et al. (1991) found that grass species were associated with a greater $C_{mic}$:$N_{mic}$ ratio (4.3) than red clover (3.6), alfalfa (2.8), and corn (2.5). These results are associated with differences in root density and depth as well as in types of root exudates and, thus, result in differences in soil structure and rhizosphere populations.

The $C_{mic}$ and $N_{mic}$ values, expressed as percentages of $C_{org}$ and $N_{tot}$, respectively, give an estimation of the quantities of nutrients in the microbial biomass, substrate availability, and organic matter dynamics in soils (Sparling 1992). The higher the $C_{mic}$:$C_{org}$ ratio in systems with similar properties and conditions, the higher the portion of easily decomposable organic C compared to stable humus compounds (Anderson and Domsch 1989). On average, $C_{mic}$ made up about 1.0% of the $C_{org}$, and $N_{mic}$ contributed about 2.4% to the $N_{tot}$ in soils at both sites and years of sampling (Table 6). Crop rotation significantly ($P \leq 0.01$) influenced both ratios in plots at the NERC site in both years, but showed no effect at the other site (Tables 3, 4). Contrast comparisons revealed consistent differences in $C_{mic}$:$C_{org}$ and $N_{mic}$:$N_{tot}$ ratios between monocropping and multicropping systems with 4-year rotations, especially when samples were taken in meadow. Generally, higher values ($C_{mic}$:$C_{org}$ = 1.1% and $N_{mic}$:$N_{tot}$ = 2.6%) were found for multicropping than for monocropping systems (0.8% and 2.1%, respectively). The N fertilizer treatment did not affect these ratios (Tables 3, 4). A wide range of $C_{mic}$:$C_{org}$ ratios, varying from 0.27% to >4.0% with an average of 2–3%, are reported in the literature (Jenkinson and Ladd 1981; Anderson and Domsch 1989). The ratios of $N_{mic}$:$N_{tot}$ are generally much greater than those of $C_{mic}$:$C_{org}$; they can range from 1% to 7% (Jenkinson 1988; Fauci and Dick 1994). Average values of 1.7% for the $C_{mic}$:$C_{org}$ ratio and 2.8% for the $N_{mic}$:$N_{tot}$ ratio for 82 soils under different management systems have been reported, indicating the varying importance of microbial biomass as a sink for these elements (Joergensen 1995). A survey of the $C_{mic}$:$C_{org}$ ratio of 143 soils from long-term field experiments in Central Europe revealed lower ratios in monoculture (2.3%) than in multicropping systems (2.9%) (Anderson and Domsch 1989). These results suggest that microbial communities in the latter systems have evolved a more complex system of substrate-use efficiency with the heterogeneous input of organic matter, enabling them to fix a greater proportion of the C in their biomass than those in monocropping systems.

## Conclusions

Microbial biomass C and N showed significant responses to crop rotations and plant cover at sampling time, mainly attributed to differences in chemical properties between the four cropping systems studied. Soil $C_{mic}$ and $N_{mic}$ contents were generally greater in 4-year rotations than in 2-year rotations or with corn or soybean monocultures. Changes in $C_{mic}$ and $N_{mic}$ contents in response to the cropping systems seem to be related

**Table 6** Effect of different cropping systems on $C_{mic}$ and $N_{mic}$ values, expressed as percentages of $C_{org}$ and $N_{tot}$, respectively, in 1996 and 1997

| Crop rotation[a] | N treatment (kg ha$^{-1}$) | $C_{mic}$ as percentage of $C_{org}$[b] | | $N_{mic}$ as percentage of $N_{tot}$[b] | |
|---|---|---|---|---|---|
| | | NERC site | CWRC site | NERC site | CWRC site |
| **Sb**-sb-sb-sb | 0 | 0.8 (0.8) | – – | 2.9 (1.6) | – – |
| **C**-c-c-c | 0 | 0.8 (1.0) | 1.0 (0.7) | 2.7 (1.2) | 3.0 (1.5) |
| | 180 | 0.9 (0.8) | 1.0 (0.5) | 2.7 (1.3) | 2.6 (1.1) |
| **C**-sb-c-sb | 0 | 1.1 (1.0) | 1.2 (1.0) | 4.3 (1.8) | 3.2 (1.3) |
| | 180 | 1.0 (0.9) | 1.1 (1.0) | 3.1 (1.3) | 3.8 (1.3) |
| c-**Sb**-c-sb | 0 | 1.0 (1.1) | 1.0 (0.5) | 3.3 (1.9) | 2.8 (1.5) |
| | 180 | 0.9 (0.9) | 1.0 (0.8) | 3.3 (1.6) | 2.5 (1.5) |
| **C**-c-o-m | 0 | –[c] (0.8) | 1.2 (1.0) | –[c] (1.5) | 3.0 (1.9) |
| | 180 | – (1.2) | 1.1 (0.9) | – (0.9) | 2.4 (1.6) |
| c-**C**-o-m | 0 | 1.1 (1.1) | 1.1 (1.0) | 3.2 (2.1) | 3.1 (1.8) |
| | 180 | 0.9 (1.3) | 1.0 (1.1) | 3.0 (1.7) | 2.0 (1.6) |
| c-c-**O**-m | 0 | 1.1 (1.2) | 1.2 (1.0) | 3.9 (3.1) | 2.5 (1.1) |
| | 180 | 1.3 (0.9) | 1.0 (0.9) | 4.2 (2.5) | 2.2 (1.2) |
| c-c-o-**M** | 0 | 1.0 – | 1.2 (0.8) | 4.1 – | 3.2 (1.2) |
| | 180 | 1.1 – | 1.1 (0.7) | 4.5 – | 2.8 (1.1) |
| **C**-o-m-m | 0 | – – | 1.1 (1.0) | – – | 2.5 (1.3) |
| | 180 | – – | 0.9 (0.7) | - – | 1.4 (0.9) |
| c-**O**-m-m | 0 | – – | 1.2 (0.9) | – – | 2.8 (1.3) |
| | 180 | – – | 1.3 (0.8) | – – | 2.9 (1.6) |
| c-o-**M**-m | 0 | – – | 1.4 (1.1) | – – | 3.4 (1.6) |
| | 180 | – – | 1.3 (1.0) | – – | 3.3 (1.4) |
| c-o-m-**M** | 0 | – – | 1.3 (0.9) | – – | 3.3 (1.0) |
| | 180 | – – | 1.3 (0.8) | – – | 3.0 (0.8) |
| Mean | | 1.0 (1.0) | 1.1 (0.9) | 3.5 (1.7) | 2.8 (1.3) |
| LSD $P<0.05$[d] | | 0.3 (0.1) | 0.4 (0.3) | 0.9 (0.6) | 1.2 (1.0) |
| LSD $P<0.05$[e] | | 0.3 (0.3) | 0.4 (0.6) | 0.6 (0.4) | 2.0 (1.4) |

[a] Capital bold letters indicate crop in which the sample was taken in 1996. In 1997, the samples were taken in the crop following the capital bold letter; *C* corn, *Sb* soybean, *O* oats, *M* meadow (alfalfa)

[b] Means of three (NERC) and two (CWRC) field replicates from 1996. Figures in parentheses are data for 1997

[c] Indicates that sample was not available in the specified crop in the rotation

[d] Least significant difference due to crop rotation at 0 N

[e] Least significant difference due to crop rotation at 180 N

to the amount and diversity of crop residues, the proportion of easily decomposable organic compounds returned to the soil, root density, microclimate, and soil structure. N fertilization had no effect on $C_{mic}$ and $N_{mic}$ concentrations. There is evidence that the $N_{mic}$ pool is a distinct fraction, although not necessarily related to the size of the $N_{tot}$ in soils. Variations between the two years were less pronounced for $C_{mic}$ than for $N_{mic}$ values. The ratio of $C_{mic}$:$N_{mic}$ seems to be more related to the plant cover at sampling time than to the cropping system; the lowest ratios were found in plots under soybean. These findings may indicate a close relationship between the amount and composition of root exudates and the structure of the microbial communities in the rhizosphere. Generally, multicropping systems resulted in greater $C_{mic}$:$C_{org}$ and $N_{mic}$:$N_{tot}$ ratios than monocropping systems.

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