# Microbial Biomass Changes during Decomposition of Plant Residues in a Lixisol

S.K. Kachaka\*, R. Merckx\* & K. Vlassak\*

Keywords: Lixisol- Alley cropping- Microbial biomass- Incubation- Ninhydrin

### Summary

A lixisol was amended with four different alley cropping species: Senna siamea, Leucaena leucocephala, Dactyladenia barteri and Flemingia macrophylla. Soil samples were incubated for 140 days at 25 °C and the soil microbial biomass was determined by the ninhydrin extraction method along the incubation period. The soil microbial biomass values ranged between 80 and 600 mg.kg-1 and followed, in all cases, the decreasing order: Leucaena> Senna> Flemingia> Dactyladenia.

### Résumé

Variations de la biomasse microbienne au cours de la décomposition des résidus végétaux dans un lixisol

Un lixisol a été amendé avec les résidus de quatre plantes différentes utilisées en culture en couloirs: Senna siamea, Leucaena leucocephala, Dactyladenia barteri et Flemingia macrophylla. Les échantillons de sol ont été incubés à 25 °C pendant 140 jours et la biomasse microbienne du sol a été déterminée au cours de la période d'incubation selon la méthode d'extraction à la ninhydrine. Les valeurs de la biomasse microbienne du sol étaient comprises entre 80 et 600 mg.kg-1 et, dans tous les cas, on a observé l'ordre décroissant: Leucaena> Senna> Flemingia> Dactyladenia.

#### Introduction

According to Brookes *et al.* (3), the soil microbial biomass is the living part of soil organic matter, comprising all organisms less than about 5 x  $10^3 \mu m^3$  other than the living plant material. Soil microorganisms constitute a transformation matrix for all the organic materials in the soil and act as a labile reservoir for plant available N, P, and S (4). Soil microbial biomass can be estimated by different methods and each method has its own limitations.

Among the various methods proposed for soil microbial biomass measurement, the chloroform fumigation-incubation procedure is the most widely used (5). Problems mainly refer with the proportions of cell C and N converted to  $CO_2$  and  $NH_4^+$  respectively, the choice of suitable control soils, the denitrification or immobilization of N by the recolonizing soil population during incubation. Such problems may possibly be overcome by direct extraction of the released substances. Amato and Ladd (2) reported that about 16% of the total N released by the CHCl<sub>3</sub> fumigation after 24 hours and extracted by  $K_2SO_4$  are ninhydrin-reactive compounds and can be used to estimate the amount of biomass C in soil from the relationship: Biomass-C= 21 \* ninhydrin-N.

In our experiment, we used the ninhydrin-reactive N method (2) to estimate the dynamics of soil microbial biomass C in air-dried soil rewetted, amended with plant material and aerobically incubated at 25 °C. The aim was also to verify if the soil microbial biomass

contents can be considered as an effective index of sustainability in soils amended with plant residues when compared to control soils during the decomposition /mineralization process.

#### Material and methods

This study was carried out at the Laboratory of Soil Fertility and Soil Biology of the Catholic University of Leuven (Belgium) as a part of the collaborative project between the IITA (International Institute of Tropical Agriculture / Ibadan – Nigeria) and the KUL (Catholic University of Leuven / Belgium).

#### Soil

The soil was collected at the Main I.I.T.A. Station in Ibadan, southwestern Nigeria (Latitude 7° 26'N and Longitude 3° 4'E). As described by Moorman *et al.* (7), the soil is an Oxic Paleustalf (USDA) with cation exchange capacity values ranging from 6 to 13 meq per 100 g of clay and corresponds to a lixisol (FAO). The soil samples were taken from the top 10 cm, airdried, sieved to pass 2 mm and stored at room temperature. The characteristics of the soil used were: pH 5.4, 0.67% C, 0.06% N, 6.5% clay, 84% sand and 8.5% silt.

#### **Plant residues**

Four alley cropping species, Senna, Leucaena, Dactyladenia and Flemingia were collected at the

\*Laboratory of Soil Fertility and Soil Biology, Catholic University of Leuven, B- 3000 Leuven (Belgium). Received on 28.09.01. and accepted for publication on 30.09.02.

same location. All residues were fractionated into readily soluble, cellulose, hemicellulose and lignin parts using methods fully described by Van Soest (10) and Van Soest and Wine (11). Total C was determined by the method described by Amato (1) whereas total N was determined by the Kjeldahl method.

#### **Fumigation extraction method**

The determination of soil microbial biomass was carried out in the fumigated or unfumigated soil samples incubated aerobically for 10 days at 25 °C according to the fumigation extraction method described by Amato and Ladd (2). Briefly, after extraction by shaking 40 g of fumigated or unfumigated soil samples for 1 hour with 120 ml 2N KCl, the extracts were filtered and the optical density (OD) was determined spectrophotometrically at 570 nm after reacting aliquots with ninhydrin.

#### **Results and discussion**

#### Characterization of plant residues

The general characteristics of plant residues are given in table 1.

| Table 1   |
|---|
| Selected properties of plant residues.            |
| Results are expressed as mean of 6 replicates and |
| standard deviations are given beside mean values  |

| Plant material | % C        | % N             |
|----------------|------------|-----------------|
| Senna          | 42.7 ± 0.3 | 2.79 ± 0.02     |
| Leucaena       | 42.4 ± 0.4 | 4.33 ± 0.01     |
| Dactyladenia   | 39.5 ± 0.5 | $1.41 \pm 0.02$ |
| Flemingia      | 40.1 ± 0.3 | $3.26 \pm 0.02$ |

The four plant residues showed quite distinct chemical properties. Carbon percentages were quite similar but N content ranged between 1.41 and 4.33% for *Dacty-ladenia* and *Leucaena* respectively. As expected, the nitrogen fixing trees showed the largest concentrations of N when compared to *Dactyladenia* which is not a N<sub>2</sub>-fixing tree.

Table 2Fractionation of plant residues according<br/>to Van Soest method. Results are expressedas mean of 6 replicates and standard deviations (SD)<br/>are given below the mean values

| % dry weight  |       |          |              |           |
|---------------|-------|----------|--------------|-----------|
| Fraction      | Senna | Leucaena | Dactyladenia | Flemingia |
| Active        | 50.9  | 66.0     | 52.6         | 49.0      |
|               | 2.1   | 2.3      | 2.1          | 2.4       |
| Cellulose     | 14.7  | 5.6      | 16.3         | 24.6      |
|               | 2.3   | 2.2      | 2.1          | 2.2       |
| Hemicellulose | 17.0  | 12.5     | 5.2          | 2.5       |
|               | 1.8   | 2.4      | 0.6          | 0.5       |
| Lignin        | 10.4  | 8.1      | 14.9         | 17.2      |
|               | 1.3   | 1.2      | 2.0          | 1.1       |
| Ash           | 6.9   | 7.7      | 10.9         | 6.6       |
|               | 1.1   | 0.2      | 0.2          | 1.2       |

Fractionation of the plant materials into parts of varying decomposability showed that all the materials had a large active readily soluble fraction (Table 2).

This confirms that all materials consisted of fresh, growing leaves and were expected to contain significant amounts of metabolically active compounds, such as sugars, amino acids and proteins. Taking cellulose and hemicellulose together as the resistant fraction, the contents ranged between 18.1% for *Leucaena* and 31.7% for *Senna*. Taking lignin and ash on the other hand as the recalcitrant fraction, the contents increased from a low 15.8% for *Leucaena* to 25.8% for *Dactyladenia*.

# Dynamics of soil microbial biomass

# Rewetted soil

The microbial biomass C content of rewetted soils amended with the different residues is given in table 3.

| Table 3   |
|---|
| Microbial biomass C dynamics in rewetted soil amended |
| with plant residues and incubated at 25 °C            |

| Soil microbial biomass (mg.kg <sup>-1</sup> ) |         |       |          |              |           |
|---|---------|-------|----------|--------------|-----------|
| Time (days)                                   | Control | Senna | Leucaena | Dactyladenia | Flemingia |
| 2   | 131.3   | 212.5 | 595.4    | 162.5        | 237.5     |
| 7   | 137.5   | 250.0 | 475.0    | 221.9        | 242.8     |
| 14  | 100.0   | 505.4 | 164.0    | 125.0        | 237.5     |
| 28  | 93.8    | 375.0 | 325.0    | 175.0        | 212.5     |
| 56  | 125.0   | 287.5 | 300.0    | 112.5        | 200.0     |
| 84  | 87.5    | 175.0 | 125.0    | 100.0        | 100.0     |
| 112   | 112.5   | 225.0 | 150.0    | 112.5        | 125.0     |
| 140   | 75.0    | 112.5 | 100.0    | 100.0        | 137.5     |

In the control soil, the values remained fairly constant, at an average of 114 mg.kg<sup>-1</sup>, but pronounced increases due to their active fractions were recorded for all four amended soils, particularly when *Leucaena* and *Senna* were added.

The sequence of mineral N release and CO<sub>2</sub> production rates for the four residues was confirmed by the microbial biomass values, the increases following the same order: Leucaena> Senna> Flemingia> Dactvladenia (6). For Leucaena, the microbial biomass content increased rapidly and reached the maximum value (594.4 mg.kg<sup>-1</sup>) at the second day following plant residue application. Afterwards, it decreased considerably and reached the lowest value (164 mg.kg-1) after 14 days of incubation and finally increased and remained almost constant along the incubation period. For Senna, the microbial biomass increased considerably but at a slower rate when compared to Leucaena, and the highest value (504.4 mg.kg-1) of microbial biomass was observed after 2 weeks of aerobic incubation. Afterwards, it decreased slowly along the incubation period. For Dactyladenia and Flemingia, the maximum values of biomass C were observed after 7 days of incubation and were respectively 221.9 mg.kg  $^{\rm 1}$  and 242.8 mg.kg  $^{\rm 1}.$ 

# Comparison between predicted and measured microbial biomass after application of plant residues in the rewetted soil.

According to Payne (9), in view of the high amounts of active components of plant materials analyzed, a rapid and considerable increase in biomass can be predicted on the assumption that the amount of C present in this fraction is readily available and is assimilated by the microbial biomass with an efficiency of 60%.

As shown in table 4, a reasonable agreement between theoretical and estimated efficiencies was observed with regard to soil microbial biomass dynamics after amendment of the rewetted soil with *Senn*a (64%) and *Leucaena* (61%) as opposed to a lack of agreement for *Flemingia* (17%) and *Dactyladenia* (22%).

Table 4 Comparison between predicted and measured biomass C after application of plant residues in the rewetted soil

|                | Biomass (mg.kg <sup>-1</sup> ) |          | Efficiency (%) |            |
|----------------|--------------------------------|----------|----------------|------------|
| Plant material | Predicted                      | Measured | Theoretical    | Calculated |
| Senna          | 358                            | 380      | 60             | 64         |
| Leucaena       | 461                            | 470      | 60             | 61         |
| Dactyladenia   | 342                            | 96       | 60             | 17         |
| Flemingia      | 324                            | 117      | 60             | 22         |
|                |                                |          |                |            |

A first possibility is that Senna and Leucaena had low values of Lignin:N and (Lignin+Polyphenol):N ratios

compared to *Flemingia* and *Dactyladenia* (6, 8) and these factors could restrict assimilation of the active components in the percentages of efficiency noted above. A second possibility is that, within the active fraction itself, a range of unknown compounds whose nature and proportions could impair its immediate assimilation have to be investigated. A third possibility is that the CHCl<sub>3</sub> fumigation can facilitate the extraction of unknown cell components of plant materials which in turn can vary with the plant species.

# Conclusion

The microbial biomass C content of rewetted soils followed the decreasing order: *Leucaena> Senna> Flemingia> Dactyladenia*, showing that the soil microbial biomass dynamics is more related to the plant residue characteristics.

We assumed that 60% of the amount of C present in the active fraction is readily available and is assimilated by the microbial biomass. Although, we are aware that such unsophisticated reasoning can be subject to some criticism, it is noteworthy that in the case of *Leucaena* and *Senna*, a reasonable agreement between predicted and observed values emerged, as opposed to the complete lack of agreement for *Flemingia* and *Dactyladenia*. In this respect, the high Lignin:N or (Lignin+Polyphenol):N ratios may be factors which restrict assimilation of the active components as quantified or estimated by efficiency percentages noted above.

#### Literature

- Amato M., 1983, Determination of <sup>12</sup>C and <sup>14</sup>C in plant and soil. Short communication. Soil Biol. Biochem. 15, pp. 611-612.
- Amato M. & Ladd J.N., 1988, An assay for microbial biomass based on ninhydrin-reactive nitrogen in extracts of fumigated soils. Soil Biol. Biochem. 20, pp. 107-114.
- Brookes P.C., Ocio J.A. & Wu J., 1990, The soil microbial biomass: its measurement, properties and role in soil nitrogen and C dynamics following substrate incorporation. Soil Microorganisms, 35, pp. 39-51.
- Jenkinson D.S. & Ladd J.N., 1981, Microbial biomass in soil: measurement and turnover. *In*: Paul E.A. & Ladd J.N., eds., Soil Biochemistry, Marcel Dekker, New York, pp. 415-471.
- Jenkinson D.S. & Powlson D.S., 1976, The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol. Biochem. 8, pp. 209-213.
- Kachaka S.K., Vanlauwe B. & Merckx R., 1992, Decomposition and mineralization of prunings of different quality. Proceedings, John Wiley and Sons, Sayce Publishing, pp. 199-208.

- Moorman F.R., Lal R. & Juo A.S.R., 1975, The soils of IITA. A description of eight soils near Ibadan, Nigeria with special reference to their agricultural use. IITA Technical bulletin 3, 48 p.
- Palm C.A. & Sanchez P.A., 1991, Nitrogen release from the leaves of some tropical legumes as affected by their lignin and polyphenolic contents. Soil Biol. Biochem. 23, pp. 83-88.
- 9. Payne W.J., 1970, Energy yields and growth of heterotrophs. Annual Review of Microbiology, 24, pp. 17-52.
- Van Soest P.J., 1963, Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. Journal of the AOAC, 46, pp. 828-835.
- Van Soest P.J. & Wine R.H., 1967, Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. Journal of the AOAC, 50, pp. 50-55.

S.K. Kachaka, Congolese (DRC), Doctor in Agricultural Sciences, Professor at the University of Kinshasa, D.R.C. Corresponding author.

R. Merckx, Belgian, Doctor in Agricultural Sciences, Professor at the Catholic University of Leuven, Belgium.

K. Vlassak, Belgian, Doctor in Agricultural Sciences, Professor at the Catholic University of Leuven, Belgium.