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## Effects of living mulches or residue amendments on soil microbial properties in direct seeded cropping systems of Madagascar

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### ABSTRACT

There is growing recognition for the need to study the impact of agricultural land uses on biological and biochemical properties of soils. In Madagascar, cropping systems based on direct seeding with permanent vegetation cover provide a new means for sustainable agriculture to protect the environment and make the most of natural resources. This study assessed the effects of different direct seeding mulch-based cropping systems on soil microbial biomass and activities. The soil was andic Dystrustept. Samples of the soil were taken from 0 to 5 cm soil layer of three direct seeding mulch-based cropping systems (DMC using crop residues and living mulches). The samples were compared with samples from conventionally tilled plots (CT) and natural fallows (NF). The field experiments were carried out over a 12-year-period and two types of amendment were applied once a year at sowing, farmyard manure (FYM) and farmyard manure combined with an NPK chemical fertilizer. The C and N content, microbial basal respiration and biomass and  $\beta$ -glucosidase, urease and acid phosphatase activities were determined. The results showed that there was no interaction between soil management strategies and the use of fertilizer. Furthermore, the fertilizer did not affect the soil C and N content or the acid phosphatase and urease activities. Farmyard manure with added NPK had a significantly greater effect than farmyard manure on its own, increasing the microbial biomass, soil respiration and  $\beta$ -glucosidase activity up to 26%, 52% and 20%, respectively but there was no significant difference between natural fallows and direct seeding mulch-based cropping systems. However, conventional tillage showed a significantly lower soil microbial biomass, C content, microbial respiration and urease activity than natural fallows. The results for direct seeding mulch-based systems varied according to the microbial activities measured. However, soil  $\beta$ -glucosidase and acid phosphatase activities were significantly higher for the direct seeding mulch-based systems using crop residues than for the direct seeding mulch-based systems using living mulches. Direct seeding mulch-based systems with *Desmodium unci-*

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*natum* living mulch had significantly lower microbial biomass and respiration than the other direct seeding mulch-based systems. There was less evidence of change in the soil between natural fallow and direct seeding mulch-based systems but a higher build-up of some microbial properties was obtained for direct seeding mulch-based systems soil than in conventionally tilled soils and natural fallow.

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## 1. Introduction

Soil organic matter (SOM) is considered to play a key role in determining the genetic and functional diversity of organisms (Dick, 1992). The abundance and composition of SOM are also referred to as indicators of soil quality (Carter et al., 1997; Haynes et al., 2003). The maintenance of SOM is, therefore, a key issue in the sustainability of agroecosystems (Conant et al., 2001) and plant productivity (Bending et al., 2000). Soil microbial biomass is an active component of the soil organic pool. Although soil microbial biomass comprises less than 5% soil organic matter, it performs critical functions in soil and the environment (Sparling, 1997; Dalal, 1998). Microorganisms are responsible for organic matter and residue decomposition (Sparling and Ross, 1993). Microbial biomass (Powlson et al., 1987; Rice et al., 1996) and activity (Tracy and Frank, 1998; Bending et al., 2000) can provide an early indication of changes in total SOM caused by soil management strategies (Uhlir et al., 2005). Aerobic microbial respiration ( $\text{CO}_2$ ) and enzymatic activities are appropriate indicators for highlighting the impact of land use management (e.g. addition of plant and animal residues) and pollution (Bååth, 1989; Anderson and Domsch, 1990; Dick, 1994; Brookes, 1995; Wardle and Ghani, 1995; Fernandes et al., 2005).

Intensified farming has a significant effect on the soil functions. In the Highlands of Madagascar where soils are intensively cropped and residues exported from the fields, organic matter is considerably depleted and erosion and decrease of soil fertility are common (Minten and Ralison, 2003; Ramanankasina and Rabeharisoa, 2003). Direct seeding mulch-based cropping systems have been suggested as an alternative for reducing soil erosion and regenerating soil quality. The principle of these systems is to keep the soil permanently covered by organic residue mulch and to sow crops without tillage (Séguy et al., 2003). Organic mulches are also commonly applied to the soil surface to suppress weeds, conserve soil moisture, moderate soil temperatures and suppress plant diseases (Robinson, 1998; Hoitink and Boehm, 1999). The potential of mulches to improve soil structure, increase organic matter and establish patterns of nutrient cycling resembling those observed in natural ecosystems have also been recognized (Scopel et al., 2002). It has also been reported that no-tillage agriculture stimulates soil C sequestration (Paustian et al., 1997; Wright et al., 2005).

Attention has recently been focused on the impact of agricultural land use on biological and biochemical properties of soils (Nsabimana et al., 2004). Although the use of living mulches with legumes is increasing, little is known about their effect on soil organisms (Nakamoto and Tsukamoto, 2006). The objective of this study was to compare the effects of living

mulch versus crop residues amendments in direct seeding systems on soil properties. We hypothesized that soil microbial activities (respiration, enzyme activities) will effectively discriminate between these two management systems under direct seeded cropping.

## 2. Materials and methods

### 2.1. Field experiments

The study was conducted in the experimental station owned by Tany sy Fampanandrosoana (TAFSA), located in the district of Andranomanelatra ( $19^{\circ}46'45''\text{S}$ ,  $47^{\circ}06'25''\text{E}$ ), Antsirabe, Madagascar. The area has a cold tropical upland climate with 10–20 days of frost annually (Oldeman, 1990) and a mean temperature of  $16.9^{\circ}\text{C}$ . The site is 1600 m above sea level with an annual average rainfall of 1450 mm. The soil is andic Dystrustept (61.90% clay, bulk density  $0.76\text{ g cm}^{-3}$ ; pH ( $\text{H}_2\text{O}$ ) 5.72; CEC  $17.32\text{ cmol kg}^{-1}$  soil), figures given for soil at a depth of 0–10 cm (Razafimbelo et al., 2006). The experiment was set up in 1991 after clearing part of a 5-year-old fallow of *aristida* grassland, *Aristida rufescens* (Stend). The uncleared part was left intact and is referred to as natural fallow (NF). The direct seeding mulch-based cropping system (DMC) was a no-tillage system with the seed sown directly in a crop residue mulch or living mulch. The cropping systems studied were: (i) crop rotation of maize (*Zea mays* L.) and soybean (*Glycine max* L.) using conventional tillage without restitution of residue, (ii) crop rotation of maize (*Z. mays* L.) and soybean (*G. max* L.) using direct seeding through crop residue, (iii) crop rotation of common beans (*Phaseolus vulgaris* L.) and soybean using direct seeding through a living mulch of kikuyu grass (*Pennisetum clandestinum* Hochst. ex Chiov.) and (iv) continuous monoculture of maize using direct seeding through living mulch of silverleaf *Desmodium* (*D. uncinatum* (Jacq.) DC.). These cropping systems were compared with the natural fallow.

Two levels of amendment were applied once a year when the plots were sown: farmyard manure (FYM) at  $2\text{ Mg ha}^{-1}$  equivalent dry weight (C,  $18.8\text{ g kg}^{-1}$  equivalent dry weight; N,  $10.1\text{ g kg}^{-1}$  equivalent dry weight; total P,  $1.2\text{ g kg}^{-1}$  equivalent dry weight; K,  $12.9\text{ g kg}^{-1}$  equivalent dry weight) and farmyard manure combined with chemical fertilizer (FYM + NPK) at the recommended strength of  $30\text{ kg N}$ ,  $30\text{ kg P}$  and  $40\text{ kg K ha}^{-1}$  for soybean and beans, and  $70\text{ kg N}$ ,  $30\text{ kg P}$  and  $40\text{ kg K ha}^{-1}$  for maize. For maize, urea was added on the surface, 2/3 25 days after sowing and 1/3 at 60 days after sowing. The experiment was carried out using randomized sampling for the cropping system variable (five discrete values). There were three replicates for each treatment. For each cropping system,

except the NF, plots were split, FYM being applied to one half and FYM + NPK to the other. The whole experiment was conducted using split plots, with 6 m × 5 m individual plots.

## 2.2. Soil sampling

Soil samples obtained from six replicates (0–5 cm depth) were taken in each plot in March 2003 after 12 years continuous cultivation under the different systems. Samples were pooled, air-dried, sieved to <2 mm and stored at room temperature pending processing. Soil analyses had been performed within 2 months after sampling.

For biological activities, soil was rehumidified at 100% of the water-holding capacity (i.e. 30 g of water 100 g<sup>-1</sup> of soil).

## 2.3. Soil analysis

### 2.3.1. Organic carbon and total nitrogen

Total organic carbon (C) and total nitrogen (N) were determined by dry combustion using a CHN autoanalyzer (EA1112 Thermofinnigan Series, France) for dried (at 105 °C, 48 h) and ground soil samples (<200 μm). Results were expressed as mg(C)g<sup>-1</sup> soil.

### 2.3.2. Microbial basal respiration

Microbial basal respiration was measured on each pooled soil sample in triplicate using the method modified by Anderson and Domsch (1978). Each soil replicate (30 g equivalent dry mass) was incubated at 100% of the water-holding capacity (i.e. 30 g of water 100 g<sup>-1</sup> soil) in a 120 ml jar at 28 °C for 7 days.

Air samples were analyzed every day for CO<sub>2</sub> production using direct injection into a micro-GC Analytical Instruments SRA (MTI P200, Microsensor Technology Inc., Fremont, CA) with a TCD detector using Helium as carrier gas. After each CO<sub>2</sub> measurement, the headspaces were flushed with fresh air. Results were expressed as μg(CO<sub>2</sub>-C)g<sup>-1</sup> soil.

### 2.3.3. Microbial biomass

Microbial biomass C was estimated on soil after a 7-day incubation period using the chloroform fumigation-extraction procedure (Amato and Ladd, 1988). Ninhydrin-N reactive compounds were extracted from soils with 2 M KCl after 10 days fumigation. Fumigated and non-fumigated soil samples were suspended in KCl solution (1:3 dry soil/solution, w/v; 2 M final concentration) and shaken at 25 °C for 1 h. Extracts were filtered (0.45 μm) and stored frozen pending further analysis. The ninhydrin-reactive nitrogen content was determined using a continuous flow colorimeter (Evolution II, Alliance-Instrument, France) at 570 nm. Biomass C was calculated from the increase in ninhydrin-N between fumigated and unfumigated soils, multiplied by 21. Results were expressed as μg(C)g<sup>-1</sup> of dry soil.

### 2.3.4. β-Glucosidase activity

β-Glucosidase activity was determined using the method described by Hayano (1973). Hundred microliters of *p*-nitrophenyl-β-D-glucopyranoside as substrate and 400 μl of citrate-phosphate buffer (0.1 M, pH 5.8) were added to 100 mg of soil and incubated at 37 °C for 2 h. The reaction was stopped by adding 3 ml of sodium carbonate (0.2%, w/v). Released *p*-

nitrophenol was determined using a spectrophotometer (Spectronic 401, Spectronic Instruments, France) at 400 nm. Results were expressed as μg(glucose)g<sup>-1</sup>(soil)ha<sup>-1</sup>.

### 2.3.5. Urease activity

To measure urease activity, 100 mg of soil was incubated at 37 °C with 50 μl of 720 mm urea solution (SIGMA chemical, St. Louis, MO) and 400 μl of borate buffer (0.1 M, pH 10). Released ammonium was extracted with 3 ml of 2 M KCl solution and determined by modified Berthelot reaction (Kandeler and Gerber, 1988) using a spectrophotometer (Spectronic 401, Spectronic Instruments, France) at 400 nm. Results were expressed as μg(N-NH<sub>4</sub>)g<sup>-1</sup>(soil)ha<sup>-1</sup>.

### 2.3.6. Acid phosphatase activity

Soil (100 mg) was incubated with 400 μl of citrate-phosphate buffer (0.1 M, pH 5.8) and 100 μl of a 5 mm disodium paranitrophenyl phosphate solution (SIGMA) at 37 °C using the method described by Tabatabai and Bremner (1969). The reaction was stopped by adding 100 μl of 0.5 M CaCl<sub>2</sub> and 400 μl of 0.5 M NaOH. Released *p*-nitrophenol was determined using a spectrophotometer (Spectronic 401, Spectronic Instruments, France) at 400 nm. Results were expressed as μg PNP released g<sup>-1</sup>(soil)ha<sup>-1</sup>.

## 2.4. Statistical analysis

Statistical analyses were performed using SAS software, release 9.1. Analyses of variance (ANOVA) were performed using the general linear model (GLM). Variables were first analyzed randomly to test for the effect of the cropping system and then according to the split plot layout to test the effect of adding fertilizer and interaction with the cropping system. Post hoc comparisons were done using the Student's *t*-test. A statistical probability of *P* < 0.05 was regarded as significant.

## 3. Results

### 3.1. Fertilization effects

The statistical analysis showed that there was no interaction between soil management and fertilization (Table 1). The effect of adding the fertilizer was, therefore, analyzed without distinguishing between DMC systems. Adding fertilizer was not found to have any effect on the soil C and N content, acid phosphatase or urease activities. However, it did have an effect on soil microbial biomass C, respiration and β-glucosidase activity. These were significantly higher in soils amended with FYM + NPK than in soils fertilized with FYM alone. The values recorded were 374.8 μg(C)g<sup>-1</sup> soil, and 297.3 μg(C)g<sup>-1</sup> soil for soil microbial biomass, 315.3 μg(CO<sub>2</sub>-C)g<sup>-1</sup>soil and 207.3 μg(CO<sub>2</sub>-C)g<sup>-1</sup> soil for soil respiration and 142.1 μg(glucose)g<sup>-1</sup>(soil)ha<sup>-1</sup> and 118.3 μg(glucose)g<sup>-1</sup>(soil)ha<sup>-1</sup> for β-glucosidase activity.

### 3.2. Carbon and nitrogen content

Soil organic carbon and nitrogen contents were higher in the NF than in DMC systems (Table 2). However, statistical

**Table 1 – Effects of fertilization and the interaction between soil management and fertilization on organic carbon (C) in  $\text{mg(C)g}^{-1}$  soil; total nitrogen (N) in  $\text{mg(N)g}^{-1}$  soil; microbial biomass (MB-C) in  $\mu\text{g(Cm)g}^{-1}$  soil; basal respiration ( $\text{CO}_2\text{-C}$ ) in  $\mu\text{g(CO}_2\text{-C)g}^{-1}$  soil;  $\beta$ -glucosidase ( $\beta\text{-glu}$ ) in  $\mu\text{g(glucose)g}^{-1}(\text{soil})\text{ha}^{-1}$ ; acid phosphatase (Ac. Pho.) in  $\mu\text{g(PNP)g}^{-1}(\text{soil})\text{ha}^{-1}$ ; urease (urea) in  $\mu\text{g NH}_4^+\text{-N g}^{-1}(\text{soil})\text{ha}^{-1}$**

Fertilization	C	N	MB-C	$\text{CO}_2\text{-C}$	$\beta\text{-gluc.}$	Ac. Pho.	Urea
FYM + NPK	46.4 a	3.8 a	374.8 a	315.3 a	142.1 a	467.0 a	58.4 a
FYM	45.3 a	3.7 a	297.2 b	207.3 b	118.3 b	439.3 a	52.8 a
Interaction SM $\times$ fert ( $P < 0.05$ )	0.095 ns	0.204 ns	0.583 ns	0.324 ns	0.356 ns	0.891 ns	0.626 ns

FYM: farmyard manure; NPK: fertilizer at recommended rate for each crop type; SM: soil management means followed by the same letter within each studied variable are not significantly different at  $P < 0.05$  ns: non-significant probability.

analysis did not show any significant difference. C and N content in soil in DMC systems using crop residues were similar to that in DMC systems using living mulch. The lowest soil organic matter content (C and N) was recorded for conventional tillage. The C/N ratio of soil organic matter was very similar for all soil treatments, ranging from 12.0 to 12.9.

### 3.3. Microbial biomass C

Soil microbial biomass C under natural fallow ( $392.4 \mu\text{g(Cm)g}^{-1}$  soil) was lower than for DMC systems except for the living mulch treatment (e.g. *D. uncinatum* + maize monoculture) ( $235.5 \mu\text{g(Cm)g}^{-1}$  soil) although the difference was not statistically significant (Table 2). Soil microbial biomass under living mulch of *Desmodium* was significantly lower than for the other DMC systems (Table 2). The microbial biomass recorded in soils with DMC systems using organic residues (e.g. maize/soybean crop rotation) and living mulch of kikuyu grass was very similar. The lowest microbial biomass was recorded in soil with CT.

### 3.4. Soil respiration ( $\text{CO}_2$ )

Microbial respiration recorded in the soil in the NF ( $284.1 \mu\text{g(CO}_2\text{-C)g}^{-1}$  soil) was lower than in DMC except for that using *Desmodium* living mulch ( $192.8 \mu\text{g(CO}_2\text{-C)g}^{-1}$  soil), although the difference was not significant (Table 2).  $\text{CO}_2\text{-C}$  measured for *Desmodium* living mulch was significantly lower

than the other DMC systems, such as residue mulch ( $384.2 \mu\text{g(CO}_2\text{-C)g}^{-1}$  soil) and kikuyu grass living mulch ( $348.6 \mu\text{g(CO}_2\text{-C)g}^{-1}$  soil). Carbon respiration was lowest for soil under CT ( $119.7 \mu\text{g(CO}_2\text{-C)g}^{-1}$  soil).

No significant differences were observed in the calculated coefficient  $q\text{CO}_2$  for the various treatments (Table 2). It varied between  $0.059 \mu\text{g(CO}_2\text{-C)\mu g}^{-1}$  MB-C  $\text{j}^{-1}$  and  $0.073 \mu\text{g(CO}_2\text{-C)\mu g}^{-1}$  MB-C  $\text{j}^{-1}$ .

### 3.5. Enzyme activities

The lowest  $\beta$ -glucosidase activities were recorded in soils in the natural fallow ( $60.5 \mu\text{g(glucose)g}^{-1}(\text{soil})\text{ha}^{-1}$ ) and CT ( $68.2 \mu\text{g(glucose)g}^{-1}(\text{soil})\text{ha}^{-1}$ ) (Table 3). In the DMC systems, the *Desmodium* living mulch had the lowest  $\beta$ -glucosidase activity ( $131.4 \mu\text{g(glucose)g}^{-1}(\text{soil})\text{ha}^{-1}$ ) compared with the activity recorded for the residue mulch ( $172.0 \mu\text{g(glucose)g}^{-1}(\text{soil})\text{ha}^{-1}$ ) and kikuyu grass living mulch ( $149.3 \mu\text{g(glucose)g}^{-1}(\text{soil})\text{ha}^{-1}$ ).

Similarly, the lowest phosphatase activity was observed in the soil in the NF ( $154.8 \mu\text{g(PNP)g}^{-1}(\text{soil})\text{ha}^{-1}$ ) (Table 3). The conventionally tilled soil (CT) had a higher phosphatase level than the NF but less than that measured for DMC. The highest phosphatase activity was recorded for the DMC system,  $502.8 \mu\text{g(PNP)g}^{-1}(\text{soil})\text{ha}^{-1}$ ,  $451.1 \mu\text{g(PNP)g}^{-1}(\text{soil})\text{ha}^{-1}$ , and  $452.9 \mu\text{g(PNP)g}^{-1}(\text{soil})\text{ha}^{-1}$ , for crop residue, kikuyu grass and *Desmodium*. Unlike the other enzyme activities, the soil in the NF had significantly higher urease activity, except for *Desmodium*.

**Table 2 – Carbon and nitrogen content, soil microbial biomass and basal respiration within the DMC systems compared to the conventional tillage and the natural fallow**

Soil management	Carbon ( $\text{mg(C)g}^{-1}$ soil)	Nitrogen ( $\text{mg(N)g}^{-1}$ soil)	C/N	MB-C ( $\mu\text{g(Cm)g}^{-1}$ soil)	$\text{CO}_2$ ( $\mu\text{g(CO}_2\text{-C)g}^{-1}$ soil)	$q\text{CO}_2$ ( $\mu\text{g(CO}_2\text{-C)\mu g}^{-1}$ (Cm) $\text{j}^{-1}$ )
DMC Living mulch of kikuyu bean/soybean	47.9 a, b	4.00 a, b	12.0 a	458.2 a	348.6 a	0.073 a
DMC Living mulch of <i>Desmodium</i> maize/maize	48.8 a, b	4.02 a, b	12.2 a	235.5 b, c	192.8 b, c	0.073 a
DMC on crop Residue maize/soybean	48.1 a, b	3.81 a, b	12.6 a	439.3 a	384.2 a	0.069 a
CT maize/soybean	38.6 b	3.18 b	12.2 a	210.9 c	119.7 c	0.059 a
Natural fallow (NF)	64.4 a	5.01 a	12.9 a	392.4 a, b	284.1 a, b	0.062 a

CT: conventional tillage; DMC: direct seeding mulch-based cropping system. Means followed by the same letter within each studied variable are not significantly different at  $P < 0.05$ .

**Table 3 – Soil enzyme activities within the DMC systems compared to the conventional tillage and the natural fallow**

Soil management	$\beta$ -Glucosidase ( $\mu\text{g}(\text{glucose})\text{g}^{-1}$ (soil) $\text{ha}^{-1}$ )	Acid phosphatase ( $\mu\text{g}(\text{PNP})\text{g}^{-1}$ (soil) $\text{ha}^{-1}$ )	Urease ( $\mu\text{g NH}_4^+ \text{N g}^{-1}$ (soil) $\text{ha}^{-1}$ )	$\beta$ -Glucosidase /MB ( $\mu\text{g}(\text{glucose})\text{ha}^{-1}$ $\mu\text{g}^{-1}$ MB-C)	Acid phosphatase /MB ( $\mu\text{g}(\text{PNP})\text{ha}^{-1}$ $\mu\text{g}^{-1}$ MB-C)	Urease/MB ( $\mu\text{g}(\text{NH}_4^+ \text{N})\text{ha}^{-1}$ $\mu\text{g}^{-1}$ MB-C)
DMC living mulch of kikuyu bean/soybean	149.3 a, b	451.1 b	51.6 b, c	0.32 b	1.03 a, b	0.12 c
DMC living mulch of <i>Desmodium</i> maize/maize	131.4 b	452.9 b	71.7 a, b	0.63 a	2.32 a	0.27 a
DMC on crop residue maize/soybean	172.0 a	502.8 a	50.7 b, c	0.40 a, b	1.17 a, b	0.12 c
CT maize/soybean	68.2 c	405.8 c	48.3 c	0.36 b	2.25 a	0.19 b
Natural fallow (NF)	60.5 c	154.8 d	83.7 a	0.15 b	0.39 b	0.21 a, b

CT: conventional tillage; DMC: direct seeding mulch-based cropping system. Means followed by the same letter within each studied variable are not significantly different at  $P < 0.05$ .

### 3.6. Ratio of enzyme activity to microbial biomass

For all cultivation methods, the highest ratios were obtained for the acid phosphatase activity (Table 3), the lowest being recorded for urease activity. Except for urease, the ratios calculated for the soil in the NF were lower than those for the other methods. However, the soil microbial biomass under the living mulch of *Desmodium* had the highest ratio for all enzyme activity. No clear pattern emerged for these ratios when the various cultivation methods were compared.

## 4. Discussion

### 4.1. Conventional tillage versus direct seeding mulch-based cropping system

The results of this study indicated that, 12 years after the clearance of the natural fallow and the setting up of the various DMC systems, the soil organic carbon and nitrogen content under CT was 60% of that of the soil from NF. Similarly, the microbial biomass under CT was 54% and 48%, respectively of that from NF, and that from DMC with crop residue. Soil microbial biomass is a sensitive indicator of early changes in total SOM caused by land use management (Powlson et al., 1987; Rice et al., 1996). Many previous studies had already shown that CT soils had less organic matter and MB-C, compared to no-tilled cropping, leading to a decline in other soil properties (e.g. aggregation, water-holding capacity, microbial activities, etc) (Saffigna et al., 1989; Dick, 1992; Alvarez et al., 1995). Al-Kaisi et al. (2005) found that soil organic C and N changes over 7 years are most likely to be caused by tilling rather than the level of annual organic C and N inputs from crop residues. Our study was unable to isolate the effect of residue from tilling, as it did not include treatment with residue restitution under CT, as this is not a common-farming practice. However, the results described by Saffigna et al. (1989), who compared CT plots with and without the restitution of organic residues, showed that the impact of residue restitution on total organic C was barely measurable. After 6 years, the authors measured an increase of 8% of SOM content in the CT amended treatment compared to that for the CT non-amended treatment. These findings of Saffigna et al. (1989) and Al-Kaisi et al. (2005) support the hypothesis that the determinant factor of the low performance of CT soil is the tillage. Tillage is well known for changing soil conditions, such as temperature, moisture and aeration, and increasing decomposition rates of the litter, resulting in a faster turnover of aggregates and higher losses in soil organic matter (Six et al., 1999). Despite the fact that MB-C and  $\text{CO}_2$  respiration were lowest under CT compared to that under NF,  $\beta$ -glucosidase activity was very similar to that obtained in the soil in the NF. Acid phosphatase activity under CT was higher than that in the soil under fallow. The higher activity of phosphatase enzyme in the soil might be related either to the increase of the decomposition of insoluble C substrates, or be an indication of P limitation in soils (Uhlir et al., 2005). On the other hand, urease activity using CT was similar to that recorded using DMC, except in the living mulch under a continuous maize crop. This uninhibited biomass activity under CT was

supported by the high enzyme activity-to-microbial biomass ratios which suggested small but active microbial biomass.

Unlike CT, DMC maintained about the same carbon and nitrogen content of soils as the NF. Interestingly, soil in DMC systems had a higher microbial biomass and microbial activities (e.g. CO<sub>2</sub> respiration,  $\beta$ -glucosidase, acid phosphatase) than those under CT and in the NF. The ability of the DMC systems to maintain their soil properties and functions may be attributed to the incorporation of crop residues and the suppression of tillage. We agreed with the suggestion of Villamil et al. (2006) that DMC stimulated soil biota (*sensu lato*) activities. Kladivko (1994) confirmed that even if the residues are not mechanically combined with the soil but left on the soil surface, the soil bulk density decreases as organic materials are slowly incorporated into the soil as a result of fauna burrowing activity. A significant decrease (e.g. 0.7–0.8 g.cm<sup>-3</sup> within DMC systems compared to 0.9 g.cm<sup>-3</sup> under CT) of the soil bulk density was recorded by Razafimbelo et al. (2006) who studied the same field plots as in this study. Furthermore, Dick (1997) found that enzyme activities were negatively correlated with soil bulk density.

No difference in the metabolic quotient ( $qCO_2$ ) was found between the various treatments (CT, DMC and NF), although this quotient was reported as a sensitive indicator of changes in soil quality (Giller et al., 1998). Nevertheless, the absence of differences between  $qCO_2$  in our results is consistent with the findings of Wardle and Ghani (1995) and Alvarez et al. (1998). These authors found that this metabolic quotient  $qCO_2$  was not a reliable or consistent indicator of different types of soil management or effect of soil disturbance. It was quite well related to stress (independent of disturbance), e.g. stress owing to the pH value (Wardle, 1993) or heavy metal-induced stress in soil (Brookes et al., 1986; Brookes, 1995; Landi et al., 2000). Bilgo et al. (2006), too, found that the  $qCO_2$  in a tropical sandy soil under different short-term fallows did not differ, even when perennial grasses were introduced. However, the absence of differences of  $qCO_2$  between CT and DMC systems confirms the findings of this study on the high enzyme activities-to-MB-C ratio of CT, showing small but active microbial biomass. Moreover, the CT had a low soil C/N ratio very similar to the soil C/N ratio of DMC systems and NF, suggesting easy decomposition of soil organic matter and active microbial biomass, probably owing to the effect of tillage. Enhanced decomposition tends to lower the soil C/N ratio to almost 12:1 (Brady and Weil, 2002).

#### 4.2. Comparison between direct mulch-based systems: residue mulch versus living mulch

This study revealed other differences in microbial biomass and activities between the DMC systems. These suggested that the performance of the DMC systems was not determined by the absence of tilling or the presence of mulch alone or by a combination of the two. Furthermore, the cropping system pattern and the type of mulch were also found to be important. The MB-C and CO<sub>2</sub> respiration in DMC on crop residue mulch was similar to that using kikuyu grass living mulch. However, for the DMC with *Desmodium* living mulch, these soil properties were as low as for CT. Several studies have reported the positive relationship between organic carbon content and soil

microbial biomass (Chaussod et al., 1992; Alvarez et al., 1998; Wright et al., 2005; Sall et al., 2006). This relationship was confirmed for the two DMC systems on crop residues and kikuyu grass living mulch but not for DMC with *Desmodium* living mulch. The quality of organic inputs is known to be one of the major determinants affecting the size, composition, and activity of the soil microflora (Nannipieri et al., 1983; Franzluebbers et al., 1995; Badiane et al., 2001; Nsabimana et al., 2004). The inhibitory effect of *Desmodium* might be attributed to its biochemical composition. *D. uncinatum* is known to contain high levels of tannins (Skerman et al., 1988) and isoflavones exudates from the roots (Tsanuo et al., 2003). The inhibitory effect of phenolic compounds on microbial growth and activity has been reported by many authors (see Hättenschwiler and Vitousek, 2000; Nsabimana et al., 2004).

Differences in enzyme activities between DMC systems were also found, depending on the type of enzyme. The DMC with crop residue had higher soil  $\beta$ -glucosidase and acid phosphatase activities compared to DMC with living mulch. Roldán et al. (2003) found that  $\beta$ -glucosidase is stimulated where crop residues are left intact on the soil surface. Dick (1992) indicated that phosphatase activity is enhanced in the presence of fresh organic matter. These results emphasized the importance of the plants (and their residues) cropped in association in direct seeding systems. Our results were consistent with those of Villamil et al. (2006) studying a no-tillage system combined with corn and soybean crop rotation. The authors stressed the benefit of the differences in rooting patterns of the two crops and the quality of residue left in the field.

A comparison of the two living mulch systems (kikuyu grass and *Desmodium*) did not show any significant difference in  $\beta$ -glucosidase and acid phosphatase activities. Therefore, the inhibitory effect of phenolic compounds of the *Desmodium* on microbial biomass may not explain this result. Although our study did not allow us to draw a clear conclusion on the impact of phenolic compounds, it did reveal the selection of a specific community affected by these compounds. As for  $\beta$ -glucosidase and acid phosphatase activities, urease activity differed among the various DMC systems, showing higher activity in the presence of *Desmodium* living mulch than of kikuyu grass. Roldán et al. (2003) observed that urease activity increases in the presence of legumes.

## 5. Conclusion

This study shows that the organic matter (C and N content) of the soil in a 12-year-old direct seeding experiment was very similar to that observed in the nearby NF. Moreover; microbial activities were greatly stimulated by DMC systems compared to NF. No-tillage, permanent soil cover, plant composition (crops and mulch) and rotation effects are likely to play a key role in the improvement of these soil properties.

When comparing the different direct seeding systems, the choice of crop sequences and combinations seemed to affect the soil microbial properties. Residue-based direct seeding mulch-based systems can be recommended as they stimulate these properties to a greater extent than living mulch systems. However, the use of residue mulch for crops may

possibly be limited on farms by the competitive use of these residues as fodder or firewood. Living mulch has a high potential to fulfill soil biological functions and alleviation fodder problems by selecting appropriate plant species (fodder species) and crop rotation. Our study confirms that microbial biomass, soil respiration and enzyme activities are sensitive indicators in impact studies relative to land use management and cropping systems. Crop rotation within DMC on living mulch requires further study for optimizing their potential as a means of responding to agricultural production and environmental concerns.

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