SEÇÃO III - BIOLOGIA DO SOLO

ENZYMATIC ACTIVITY AND MINERALIZATION OF CARBON AND NITROGEN IN SOIL CULTIVATED WITH COFFEE AND GREEN MANURES[1]

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SUMMARY

There are great concerns about degradation of agricultural soils. It has been suggested that cultivating different plant species intercropped with coffee plants can increase microbial diversity and enhance soil sustainability. The objective of this study was to evaluate enzyme activity (urease, arylsulfatase and phosphatase) and alterations in C and N mineralization rates as related to different legume cover crops planted between rows of coffee plants. Soil samples were collected in a field experiment conducted for 10 years in a sandy soil in the North of Paraná State, Brazil. Samples were collected from the 0–10 cm layer, both from under the tree canopy and in-between rows in the following treatments: control, *Leucaena leucocephala*, *Crotalaria spectabilis*, *Crotalaria breviflora*, *Mucuna pruriens*, *Mucuna deeringiana*, *Arachis hypogaea* and *Vigna unguiculata*. The soil was sampled in four stages of legume cover crops: pre-planting (September), after planting (November), flowering stage (February) and after plant residue incorporation (April), from 1997 to 1999. The green manure species influenced soil enzyme activity (urease, arylsulfatase and phosphatase) and C and N mineralization rates, both under the tree canopy and in-between rows. Cultivation of *Leucaena leucocephala* increased acid phosphatase and arilsulfatase activity and C and N mineralization both under the tree canopy and in-between rows. Intercropped *L. leucocephala* increased urease activity under the tree canopy while *C. breviflora* increased urease activity in-between rows.

Index terms: Soil enzymes, mineralization potential, green manure, mulching, nutrient cycling.

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RESUMO: *ATIVIDADE ENZIMÁTICA E MINERALIZAÇÃO DO CARBONO E NITROGÊNIO SOB SOLO CULTIVADO COM ADUBOS VERDES NA CULTURA DO CAFEEIRO*

*Existe grande preocupação sobre a degradação dos solos agrícolas. Tem sido sugerido que o cultivo de plantas intercalares no cafeeiro aumenta a diversidade microbiana e a sustentabilidade do solo. No presente trabalho foi avaliada a alteração na atividade de enzimas do solo (urease, arilsulfatase e fosfatase) e na mineralização do C e N devido ao cultivo intercalar de diferentes leguminosas de verão na cultura do cafeeiro. Foram feitas amostragens em um experimento de campo de longa duração instalado em Latossolo Vermelho distrófico em Miraselva, PR, na profundidade de 0–10 cm, na projeção da copa e na entrelinha, nos seguintes tratamentos: testemunha, leucena (*Leucaena leucocephala*),* Crotalaria spectabilis*,* Crotalaria breviflora*, amendoim-cavalo (*Arachis hypogaea *tipo virginia), mucuna-cinza (*Mucuna pruriens*), mucuna-anã (*Mucuna deeringiana*) e caupi (*Vigna unguiculata*). As amostragens de solo foram feitas em quatros estádios de desenvolvimento dos adubos verdes: pré-plantio (setembro), pós-plantio (novembro), florescimento (fevereiro) e pós-incorporação (abril), de 1997 a 1999. O cultivo de adubos verdes influenciou a atividade das enzimas do solo (urease, arilsulfatase e fosfatase) e a mineralização do C e N tanto na projeção da copa como na entrelinha. O cultivo da leucena aumentou a atividade da fosfatase ácida e da arilsulfatase e a mineralização de C e N na projeção da copa e na entrelinha do cafeeiro. O cultivo de leucena aumentou a atividade da urease na projeção da copa, enquanto* C. breviflora *incrementou a atividade da urease na entrelinha.*

Termos de indexação: enzimas do solo, potencial de mineralização, adubos verdes, cobertura morta, ciclagem de nutrientes.

INTRODUCTION

The degradation of agricultural soils has aroused great concern. Soil degradation in coffee (*Coffea arabica* L.) occurs due to soil acidification, e.g., by the use of fertilizer-N, due to soil erosion, nutrient depletion by leaching, runoff, organic matter mineralization, and unsubstituted crop removal (Pavan et al., 1999). Practices are being sought to reduce the accelerated soil degradation and increase soil quality.

It has been suggested that cultivating different plant species in the same field can increase microbial diversity and enhance soil sustainability. One possibility to increase microbial diversity in the agroecosystem is to plant legume cover crops, since legumes can be used as green manure. Legume species intercropped with coffee can help manage degraded soils by protecting the soil from erosion, inhibiting weed growth and promoting soil nutrient cycling by the addition of plant residues (Chaves et al., 1997). Legumes have been used as cover crops in view of the high biomass production per unit area, high nutrient content, ramified, robust and deep root system, high capacity to mobilize soil nutrients and biological N_2 fixation capacity. These characteristics ensure the surface protection of the soil, which is essential, aside from nutrient extraction and mobilization from deeper soil layers.

Different plant species can affect the soil environment in different ways due to variations in quantity and quality (C/N ratio and other variables), which may affect soil organic matter content, microbial activity and nutrient turnover. These changes alter the potential of soil to supply or sequester nutrients due to changes in mineralization and immobilization (Franzluebbers et al., 1995). Therefore, residue decomposition is a driving variable in nutrient cycling processes.

Soil microorganisms are responsible for nutrient mineralization via organic matter decomposition. Microbial biomass is a small but important nutrient reservoir (C, N, P, and S), and many transformations of these nutrients occur in the microbial biomass (Dick, 1992). The turnover of nutrients released from microbial cells is five times faster than of those from decomposition of vegetable residues (Paul & Clark, 1996).

Soil organic matter decomposition is mediated by microorganisms through enzymes that catalyze innumerable reactions necessary for the life processes of microorganisms in soils, decomposition of organic residues, nutrient cycling, and formation of organic matter and soil structure (Dick, 1994). Most soil enzymes are produced by microorganisms. These enzymes are constantly synthesized and can be accumulated, inactivated and/or decomposed in soil, with a great impact on nutrient recycling (Tabatabai, 1994; Dick, 1997). Soil enzyme activity can be used to indicate the intensity of certain biochemical processes. Soil enzyme activity can be used as a unique integrative biological indicator of the intensity of certain biochemical processes, underlying soil evaluations due to the close relationship of soil enzymes

with soil biology and the rapid response to changes in soil management (Dick, 1994, 1997; Bandick & Dick, 1999).

Therefore, soil management has great effects on soil chemical, physical and biological properties and on subsequent plant growth. Better knowledge of the dynamics of microbial activity and soil nutrient mineralization from plant residues is essential to quantify the potential benefits for soil quality and crop production due to changes introduced in the soil-plant system by green manure use.

The objective of this study was to evaluate soil enzyme activity (urease, arilsulfatase and phosphatase) and C and N mineralization as related to different legume species planted as green manures between coffee rows.

MATERIAL AND METHODS

Experimental conditions and soil sampling

The experiment was initiated in 1988 in Miraselva, in the North of Paraná State (22 ° 58 ' S, 51 ° 29 ' W), Brazil, in a sandy soil classified as Red distroferric Latossol. This soil was reported to contain 800 g kg^{-1} sand, 80 g kg^{-1} silt, and 120 g kg^{-1} clay with a pH of 3.9 ($CaCl_2$), 4.9 mg kg^{-1} P (*Mehlich-1*) and 6.7 g kg^{-1} organic carbon in the surface layer (0–10 cm). The experimental design was a randomized complete block with three replications. The treatments included different summer legumes used as green manure planted between coffee rows (cultivar Catuai): control, *Leucaena leucocephala, Crotalaria spectabilis, Crotalaria breviflora, Mucuna pruriens, Mucuna deeringiana, Arachis hypogaea,* and *Vigna unguiculata.*

Every year the legumes were sown in the beginning of October and cut at the flowering stage. The residues were left on the soil surface to cover the soil and to decompose. The control treatment was hand-weeded throughout the year, whenever necessary.

During the 10 years of the experiment, acidity was corrected twice by liming: the first time prior to the experiment (1988) and the second time in 1997. Both times, two tons of dolomitic limestone were applied per hectare. Annually, from September to March, mineral fertilizers (NPK) were applied under the tree canopy of the coffee plants. Each plant was treated with 400 g of ammonium sulfate, 60 g of triple superphosphate and 100 g of potassium chloride. The N was split in four applications per year, K in two and P applied in a single dose.

The soil samples were taken at four stages of the legume cover crop: pre-planting (September), after planting (November), at flowering (February), and after plant residue incorporation (April), from 1997 to 1999.

The soil samples were taken from a depth of 0–10 cm under the tree canopy and from the middle in-between the rows. Fresh soil samples were sieved (4 mm) to remove all major plant material and then stored at 4 °C until microbial, chemical and physical analysis.

Chemical analyses were conducted according to Pavan et al. (1992). Total organic C was determined by the Walkley-Black procedure, in which organic matter is oxidized by potassium dichromate in the presence of sulfuric acid. The remaining dichromate is determined by titration (Pavan et al., 1992).

Enzyme activity determination

All enzyme activities were measured by the method of Tabatabai (1994), during one hour of incubation. Urease activity (urea amidohydrolase, EC 3.5.1.5) was measured based on the determination of ammonia released by steam distillation when soil samples were incubated with a urea solution. Arylsulfatase activity (arylsulfate sulfohydrolase, EC 3.1.6.1) was determined by colorimetry of *p*-nitrophenol released when soil samples were incubated with *p*-nitrophenyl sulfate. Acid phosphatase activity (EC 3.1.3) was analyzed with a modified universal buffer (MUB) (pH 6.5) using colorimetric determination of *p*-nitrophenol released when soil samples were incubated with *p*-nitrophenyl phosphate. Urease activity results are expressed as μg g^{-1} h^{-1} $N\text{-}NH_4$. Activities of arylsulfatase and phosphatase are expressed as μg *p*-nitrophenol (PNP) g^{-1} h^{-1}.

C and N mineralization

Mineralized C was determined by incubation of 30 g moist soil in a 350 mL sealed jar with a NaOH trap, with water in a separate vessel to ensure high humidity, for 3, 6, 10, 17, and 24 days, at 30 °C in a dark chamber. In each sampling period, NaOH was collected and replaced. The CO_2 concentration released in each period was determined by Flow Injection Analysis (Kawazaki et al., 2000). Controls were run in six flasks without soil for CO_2 determination.

Nitrogen mineralization was determined based on a 24 day incubation of 5 g of moist soil at 30 °C. An initial 5 g of the soil sample, before and after the incubation, was extracted with 20 mL of K_2SO_4 solution-N (0.25 mol L^{-1}) by shaking for 50 min at 220 rpm, followed by centrifuging and filtering. Nitrate concentration was determined by the procedure described in Balota et al. (2004), which consisted of measuring NO_3^- in soil extracts without chemical reduction by ultraviolet spectrophotometry at 210 nm (NO_3^- + interfering ions) and at a longer wavelength (239 nm), where the ultraviolet light absorption due to NO_3^- is negligible and which, due to interfering ions, is similar to that measured at 210 nm (with chemical reduction). Nitrate concentrations were calculated as the difference between absorbance

values obtained at 239 nm (NO_3^- plus dissolved organics) and 210 nm (dissolved organics). Mineralized N was computed as the difference between nitrate concentration before and after incubation.

Microbial biomass C and N

The microbial biomass C (MBC) was determined by the fumigation-extraction method according to Vance et al. (1987), using a correction factor (k_c) of 0.33. The microbial biomass N (MBN) was determined by the method employed by Brookes et al. (1985), using a correction factor of 0.54.

All determinations were performed in triplicate and expressed on a dry matter basis. For each treatment, data were averaged over the four seasons and two years prior to statistical analysis by ANOVA, using the SAS statistical package.

RESULTS AND DISCUSSION

The soil chemical properties after 10 years under different legume green manures (Table 1) are discussed by Balota & Chaves (2010). The average values of enzyme activity, total C and C and N mineralization of eight samplings in two years indicated that intercropping of different summer legume species as green manure influenced microbial activity, both under the coffee canopy and in-between rows. In general, microbial activity was higher in-between rows than under the tree canopy (Tables 2 and 3).

Enzyme activities

Urease activity varied from 78 to 258 μg g^{-1} h^{-1} N-NH_4 in the soil under the tree canopy and from 63 to 154 μg g^{-1} h^{-1} N-NH_4 in-between rows (Table 2). Cultivation of *L. leucocephala* resulted in higher urease activity under the tree canopy than other legumes, with an increase of 166 % compared to the control and 230 % over *M. pruriens*. In-between rows, urease activity under *C. breviflora* increased up to 110 and 144 % compared to the control and *M. pruriens,* respectively.

The range of the urease activities was comparable to many regions of the world. In temperate climates conditions vary widely, from 23 to 270 μg g^{-1} N-NH_4 (Bandick & Dick, 1999; Klose & Tabatabai, 1999) and under Brazilian conditions from 4 to 169 μg g^{-1} N-NH_4 (Longo & Melo, 2005; Silveira, 2007). The results of this study were therefore consistent with other studies reporting that urease activity was significantly affected by different soil management systems.

Urease is the enzyme that catalyzes hydrolysis of urea to CO_2 and NH_3, which is a vital process in the regulation of N supply to plants after urea fertilization. Therefore, it is important to detect the factors that can reduce the efficiency of this enzyme in the ecosystem. On the other hand, reduction of urease activity in the *M. pruriens* treatment may suggest that potential N mineralization is being affected. Large reductions in urease activity may negatively affect plant growth and yield. Therefore, a better understanding of urease activity dynamics could indicate more effective ways of managing N fertilizers.

The increased urease activity under some green manures was not only due to the addition of larger

Table 1. Soil chemical properties (0–10 cm layer) after10 years of summer green manures in-between coffee rows

Green Manure	pH	Al saturation	CEC	Base saturation	P	C
		%	$cmol_c$ kg^{-1}	%	mg kg^{-1}	g kg^{-1}
			Tree Canopy			
Control	4.4	8.1	6.48	32.6	51.4 ab	7.6 b
L. leucocephala	4.5	10.4	6.94	36.7	42.4 abc	9.1 a
C. spectabilis	4.5	8.1	6.55	38.7	42.4 abc	8.0 b
C. breviflora	4.9	3.9	6.27	47.1	33.6 bc	7.7 b
M. pruriens	4.2	18.5	6.98	30.4	36.5 abc	8.1 ab
M. deeringiana	4.4	8.1	7.04	36.5	53.6 a	8.3 ab
A. hypogaea	4.0	20.0	6.89	26.8	44.2 abc	8.3 ab
V. unguiculata	4.4	14.9	6.83	34.4	30.1 c	8.1 ab
			In-Between Rows			
Control	6.0	0.0	6.74	62.2	13.8	8.6 b
L. leucocephala	5.6	0.0	7.81	60.6	9.7	11.6 a
C. spectabilis	5.8	0.0	6.28	58.0	8.2	7.5 b
C. breviflora	6.3	0.0	6.75	65.5	13.2	8.7 ab
M. pruriens	5.4	0.3	6.94	52.3	13.3	9.3 ab
M. deeringiana	5.5	0.0	7.02	54.7	13.8	9.2 ab
A. hypogaea	5.9	0.0	6.99	61.4	11.2	9.8 ab
V. unguiculata	5.9	0.0	6.86	60.8	12.9	8.9 b

Table 2. Urease, arylsulfatase and acid phosphatase activity under the tree canopy and in-between coffee rows as affected by different legume green manures

Green manure	Urease	Arylsulfatase	Phosphatase
	μg g^{-1} soil		
		Tree Canopy	
Control	97 cd	9.7 e	256 e
L. leucocephala	258 a	17.3 a	555 a
C. spectabilis	181 b	15.1 ab	549 a
C. breviflora	104 cd	12.6 bcde	489 ab
M. pruriens	78 d	13.0 bcd	364 cd
M. deeringiana	100 cd	13.6 bc	383 cd
A. hypogaea	153 bc	11.5 bcde	452 bc
V. unguiculata	118 cd	9.9 de	415 bc
		In-Between Rows	
Control	73 cd	10.7 b	333 e
L. leucocephala	145 ab	14.9 a	635 a
C. spectabilis	111 abcd	12.1 ab	378 de
C. breviflora	154 a	13.0 ab	520 bc
M. pruriens	63 d	10.6 b	320 e
M. deeringiana	123 abc	12.1 ab	460 cd
A. hypogaea	124 ab	12.8 ab	600 ab
V. unguiculata	101 bcd	11.6 b	477 c

Means within a column of the same sample position followed by a different lower-case letter are significantly different at $p \leq 0.05$.

Table 3. Carbon and N mineralization in 24 days and the C/N mineralization ratio under the tree canopy and in-between rows as affected by different legume green manures

Green manure	C_{MIN}	N_{MIN}	C_{MIN}/N_{MIN}
	μg g^{-1} C-CO_2	μg g^{-1} N-NO_3	
		Tree Canopy	
Control	21.4 bc	1.8 b	11.8 a
L. leucocephala	27.5 a	4.9 a	5.6 d
C. spectabilis	23.4 b	2.1 ab	11.1 a
C. breviflora	22.0 bc	2.0 b	10.9 ab
M. pruriens	18.0 d	1.9 b	9.5 abc
M. deeringiana	20.4 bc	1.9 b	10.7 ab
A. hypogaea	21.0 bc	2.8 ab	7.6 bc
V. unguiculata	19.2 c	2.6 ab	7.4 cd
		In-Between Rows	
Control	26.0 d	1.8 c	14.4 a
L. leucocephala	43.0 a	11.3 a	3.8 e
C. spectabilis	27.7 d	2.7 bc	10.3 b
C. breviflora	33.5 c	4.0 bc	8.4 bcd
M. pruriens	26.9 d	4.8 bc	5.6 de
M. deeringiana	33.0 c	5.5 b	6.0 cde
A. hypogaea	38.0 b	5.2 b	7.3 bcd
V. unguiculata	25.4 d	2.8 bc	9.1 bc

Means within a column of the same sample position followed by different lower case letters are significantly different at $p \leq 0.05$.

amounts of residue, which stimulated microbial activity, but also because these plants may have a higher level of substrates able to activate urease synthesis. Large additions of green manure residues increase soil organic matter. This alteration in the organic carbon pool can protect the soil enzymes by the association with organic and inorganic colloids, contributing to urease stabilization in soils (Nannipieri et al., 1996).

Acid phosphatase activity was lower under the tree canopy, varying from 256 to 555 μg g^{-1} h^{-1} PNP, than in-between rows, where activities ranged from 320 to 635 μg g^{-1} h^{-1} PNP (Table 2). Cultivation of *L. leucocephala* resulted in higher acid phosphatase activity both under the tree canopy and in-between rows. Under the tree canopy the increase was up to 116 % compared to the control, while in-between rows, *L. leucocephala* cultivation increased phosphatase activity up to 91 % of the control and 98 % of *M. pruriens*.

Our results for acid phosphatase activity were consistent with those reported in the literature, varying widely (14–1,165 μg g^{-1} h^{-1}) in many regions of the world, according to a variety of factors including soils, experimental conditions and climate. Studies include research in Indonesia (Salam et al., 1999), the USA (Dick et al., 1988), and Brazil (Fernandes et al., 1998; Baligar et al., 1999; Conte et al., 2002; Matsuoka et al., 2003; Carneiro et al., 2004; Balota et al., 2004). Under Brazilian conditions, acid phosphatase activity varies from 55 to 1165 (Baligar et al., 1999; Carneiro et al., 2004).

Phosphatase is the general name of a large group of enzymes that catalyze the hydrolysis of both esters and anhydrides of H_3PO_4. Acid phosphatases have been studied extensively because of their optimum activities under acid conditions and their importance in soil organic P mineralization and plant nutrition

(Tabatabai, 1994; Dick, 1997). Microorganisms would be the most productive phosphatase sources in soil, due to their high metabolic activity and short lifespan, with several generations a year, allowing the production of high amounts of enzymes.

It has been suggested that phosphatases are produced when the available P content reaches critical levels for plant and microorganism growth (Spiers & McGill, 1979). For example, natural systems such as forest sustain growth without phosphate fertilization, even at a low level of available P. In these systems, available P is controlled by organic P cycling, where microbial biomass is an essential component. On the other hand, in agricultural systems, P fertilizers may reduce phosphatase activity (Spiers & McGill, 1979).

Arylsulfatase activity varied from 9.7 to 17.3 μg g^{-1} h^{-1} PNP in the soil under the tree canopy and from 10.6 to 14.9 μg g^{-1} h^{-1} PNP in-between rows (Table 2). Under the tree canopy, cultivation of *L. leucocephala* increased arylsulfatase activity up to 78 % compared to the control and to *V. unguiculata*, while arylsulfatase activity in-between rows increased up to 39 % by *L. leucocephala* compared with the control.

Arylsulfatase activity varies widely in the literature (from 4 to 770 μg g^{-1} h^{-1}), depending on different factors such as soils, experimental conditions, and climates, in many regions of the world, including Indonesia (Salam et al., 1999), USA (Dick et al., 1988; Bandick & Dick, 1999); Canada (Gupta et al., 1993), and Brazil (Matsuoka et al., 2003; Baligar et al., 1999). The results of this study were consistent with studies in the literature reporting that arylsulfatase activity was significantly affected by different soil management systems.

Greater arylsulfatase activity obtained with *L. leucocephala* intercropping confirms a previous investigation showing that mulching can significantly increase arylsulfatase activity (Dick et al., 1988; Bandick & Dick, 1999). This result may be due to organic C inputs, which constitutes a principal reservoir of ester sulfate, the enzyme substrate.

Arylsulfatase is the enzyme involved in mineralization of ester sulfate in soils and has been detected in plants, animals, microorganisms, and soils (Tabatabai, 1994). Arylsulfatases are only one of the many types of sulfatases involved in mineralization of ester S compounds. The majority of arylsulfatases are not constitutive enzymes, and their synthesis by microorganisms may be controlled by the C and S contents of the system (Tabatabai, 1994; Germida et al., 1992; Dick, 1997). Consequently, arylsulfatase activity depends on the soil sulfate and nutrient contents. For example, since phosphate may displace or reduce sulfate adsorption to the soil colloids, a higher arylsulfatase activity may be associated with S deficiency due to a high P content in some areas.

This great variation in soil enzyme activities due to different green manures indicates the sensitivity of these enzymes to soil disturbance. The great increase of enzyme activity under *L. leucocephala* is likely due to high aboveground biomass production (almost 56 t ha^{-1} yr^{-1}), i.e., 3.2–6.2 times higher than under other green manures. As residue, this material works as a substrate for microbial growth and enzyme production. The increased mulch leads to an increase in the supply of readily available substrates, such as carbohydrates, for microorganisms that produce most soil enzymes.

Carbon and nitrogen mineralization

Carbon mineralization (C_{MIN}) in the 24 days of the incubation period varied from 18.0 to 27.5 μg g^{-1} CO_2-C under the tree canopy and from 25.4 to 43.0 μg g^{-1} CO_2-C in-between rows (Table 3). Cultivation of *L. leucocephala* resulted in higher C_{MIN} than other legumes. The increase under the tree canopy was up to 29 % compared to the control and 53 % compared to *M. pruriens*, while in-between rows C_{MIN} increased more than 55 % compared to the control, *C. spectabilis*, *M. pruriens* and *V.unguiculata*.

The C_{MIN} rates observed in this study are consistent and a little lower than other studies in many regions of the world. C_{MIN} varies widely from 10 μg g^{-1} d^{-1} C (Franzluebbers et al., 1995) to 222 μg g^{-1} d^{-1} C (Carter & Rennie, 1982) in temperate climates and from 1.5 to 7.1 μg g^{-1} d^{-1} C under subtropical conditions (Balota et al., 2004). This wide variation may be due to various factors, including soils, plants, climate, and experimental conditions. Plant factors include age, biomass, C/N ratio and other compounds in plant residues. Soil factors include soil water content, temperature, aeration, and available nutrients. The C_{MIN} observed in this study was two to four times lower than that observed by Balota et al. (2004). However, this experiment was conducted in a sandy soil with low natural fertility and about half the organic C content of the soils used by Balota et al. (2004), which were clay soils with high natural fertility.

Mineralization under laboratory condition is not truly representative of coffee field conditions, where soil disturbance is minimal. Most likely, the disturbance by sampling and processing exposed the protected soil organic matter pool, artificially stimulating C mineralization. Crop residues incorporated into the soil decompose several times faster than when they remain on the soil surface, with less immobilization and greater mineralization of nutrients. However, in this experiment the green manure residues remained on the soil surface, which may have slowed down decomposition rates.

The large differences in C_{MIN} among treatments demonstrate that green manure cultivation changes the pool of soil labile organic C. Our results agree with previous studies (Carter & Rennie, 1982; Franzluebbers et al., 1995; Chander et al., 1997; Balota et al., 2004) that demonstrate the responsiveness of soil organic matter quality (potential C mineralization) to changes due to soil management practices.

The ratio of mineralized C to organic C (C_{MIN}/C_{org}) ranged from 0.22 to 0.30 % under the tree canopy and from 0.29 to 0.39 % in-between rows (Table 4). In the literature, the C_{MIN}/C_{org} ratio varies widely, from 0.2 % (Franzluebbers et al., 1995) to 3.6 % (Carter & Rennie, 1982). The ratio C mineralized/microbial biomass C (C_{MIN}/MBC) was from 9.0 to 18.8 % under the tree canopy and from 9.6 to 12.7 % in-between rows, while the C_{MIN}/MBC ratio in the literature ranges from 48 % (Franzluebbers et al., 1995) to 221 % (Chander et al., 1997). The ratio N mineralized/microbial biomass N (N_{MIN}/MBN) was 12.6–62.4 % under the tree canopy and 15.5–52.7 % in-between rows, while in the literature the N_{MIN}/MBN ratio ranges from 6.9 % (Balota et al., 2004) to 36.0 % (Singh & Singh, 1995).

In general, potential C_{MIN} in the 0–10 cm layer appeared to be controlled by the soil organic C concentration since it is a substrate for heterotrophic activity. The correlation between C_{MIN} and soil organic C ($r^2 = 0.63$ and $r^2 = 0.33$) was lower than C_{MIN} and MBC ($r^2 = 0.96$ and $r^2 = 0.84$) (Figure 1) and C_{MIN} and the C_{mic}/C_{org} ratio ($r^2 = 0.65$ and $r^2 = 0.84$). These higher correlations demonstrate that microbial biomass and the C_{mic}:C_{org} ratio play an important role in C_{MIN}.

Nitrogen mineralization (N_{MIN}) in 24 days varied from 1.8 to 4.9 μg g^{-1} NO_3^--N in the soil under the tree canopy and from 1.8 to 11.3 μg g^{-1} NO_3^--N in-between rows (Table 3). Cultivation of *L. leucocephala* resulted in higher N_{MIN} than of the other legumes. The increase under the tree canopy was up to 172 % of the control and more than 145 % compared to *C. breviflora*, *M. pruriens* and *M. deeringiana*, while in-between rows N_{MIN} increased up to 528 % compared to the control.

Table 4. The ratio of C mineralization (C_{MIN}) to organic C (C_{org}) or microbial biomass C (MBC) and the ratio of N mineralization (N_{MIN}) to microbial biomass N (MBN) after 24 days under the tree canopy and in-between rows as affected by different legume green manures

Green manure	C_{MIN}/C_{org}	C_{MIN}/MBC	N_{MIN}/MBN
	%		
	Tree Canopy		
Control	0.28 ab	18.1 ab	20.2 cd
L. leucocephala	0.30 a	9.0 e	29.1 b
C. spectabilis	0.29 ab	11.7 cde	12.6 e
C. breviflora	0.28 ab	13.0 cd	18.1 d
M. pruriens	0.22 c	15.3 b	17.7 d
M. deeringiana	0.25 abc	14.6 c	16.6 de
A. hypogaea	0.26 abc	10.5 de	23.9 c
V. unguiculata	0.24 c	18.8 a	62.4 a
	In-Between Rows		
Control	0.30 bc	12.7 a	15.5 d
L. leucocephala	0.37 a	10.1 a	52.7 a
C. spectabilis	0.37 a	10.8 a	19.6 d
C. breviflora	0.39 a	10.3 a	19.6 d
M. pruriens	0.29 c	10.1a	33.5 b
M. deeringiana	0.36 ab	10.2 a	28.1 c
A. hypogaea	0.39 a	9.6 a	27.9 c
V. unguiculata	0.29 c	10.0 a	17.4 d

Means within a column of the same sample position followed by different lower-case letters are significantly different at $p \leq 0.05$.

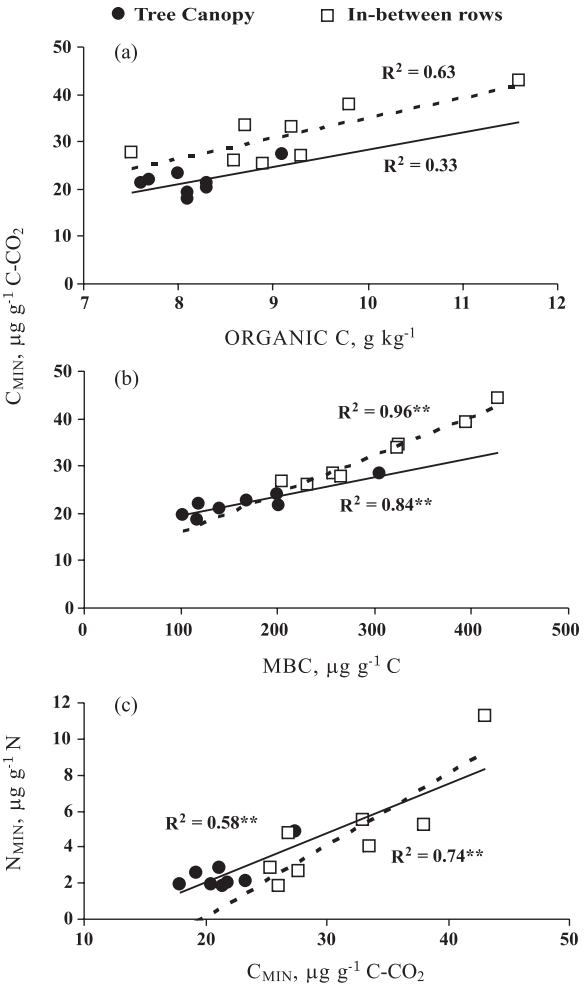

Figure 1. Relationship of C mineralization (C_{MIN}) to soil organic carbon (a), to microbial biomass C (MBC) (b) and to N mineralization (N_{MIN}) (c) under the tree canopy and in-between rows. * and ** Significant at 5 and 1 %, respectively.

Greater N_{MIN} variation due to soil management was observed by several authors. Franzluebbers et al. (1995) observed values ranging from 4.8 to 24.0 μg g^{-1} N in temperate climates, while Balota et al. (2004) observed values from 1.2 to 5.2 μg g^{-1} N under subtropical conditions, both in 24 day incubations.

Soil C and N cycles are intimately related by the processes of mineralization and immobilization. This suggests a strong relationship between soil N transformation and soil CO_2 flux. Therefore, the increased potential N_{MIN} under *L. leucocephala* was probably due to the larger organic N pool. However,

green manure residues were left on the soil surface. Under this kind of residue management, nitrification can be inhibited due to the organic matter, and nutrients such as N that accumulate at or near the soil surface may restrict N-mineralization. In undisturbed soil, nitrification may be inhibited due to acidification of the surface soil or due to substrate limitation for nitrifiers due to lower mineralization or unfavorable ammonium spatial distributions (Blevins & Frye, 1993).

According to Smith & Paul (1990), a considerable portion of N available for plant uptake comes directly from the turnover of soil microbial biomass. This source can act as a nutrient source for plants during the critical period of plant growth in tropical soils. N flow through soil microbial biomass can be sufficient to supply crop demands (Lethbridge & Davidson, 1982). However, nutrient release from decomposed green manure residues to coffee depends on the synchrony between nutrient release and coffee requirements.

N-mineralization from soil organic matter can provide useful, integrated information on chemical, physical and biological aspects of soil health because it involves N accumulation through previous biological activity, the present soil organic matter status and current N mineralization activity of soil microorganisms (Sparling, 1997).

These varied results of C and N mineralization show clearly that variations in soil management cause different decomposition rates of organic matter and affect microbial biomass and its activity, with consequent differences in substrate availability. Therefore, the determination of the dynamics of C and N mineralization of different green manure residues is important to adopt appropriate strategies for soil sustainability.

C/N mineralization ratio

The C/N mineralization ratio varied from 5.6 to 11.8 under the tree canopy and from 3.8 to 14.4 in-between rows (Table 3). Changes in the C/N mineralization ratio demonstrate effects due to soil management. Nonetheless, the ratio was higher in the control than in the other treatments, both under the tree canopy and in-between rows. The greatest decreases of C_{MIN}/N_{MIN} ratio in relation to the control were to 53 and 74 % for *L. leucocephala*, respectively, under the tree canopy and in-between rows.

Higher C/N mineralization ratios may suggest that microbial populations use organic matter with wide C/N ratios, while lower C/N mineralization ratios can suggest that the C and N flow through the mineralizable fraction became less stable, possibly reducing C and N conservation in the long term (Franzluebbers & Arshad, 1996). However, our results showed that the cultivation of *L. leucocephala* resulted in a lower C/N mineralization ratio than in other treatments, while organic C increased up to 35 % under the tree canopy and 73 % in-between rows, compared to the initial content (6.7 g kg^{-1}). Under other treatments, organic C increased by about 24 % under the tree canopy and 46 % in-between rows.

The C/N mineralization ratio can be used as an index of labile substrate availability. C/N mineralization ratios greater than 25 may indicate that the crop residues and resulting soil organic matter fractions are low in N concentrations (Franzluebbers & Arshad, 1996). Higher C/N mineralization ratios may indicate N limitation for heterotrophic microorganisms, with greater potential for N immobilization. The variation of C/N mineralization ratio in this study was narrow (3.8 - 14.4), reflecting the low variation of the C/N content of the green manures, from 14.8 in *L. leucocephala* to 23.4 in *C. spectabilis* (Calegari, 1995). In general, C/N ratios below 30 do not affect microbial activity (Trinsoutrot et al., 2000). The mineralization ratio is also strongly influenced by other residue properties, such as polyphenolic, lignin and cellulose content as well their ratios (Aulakh et al., 1991; Tian et al., 1992). Polyphenols may form a complex with proteins and can reduce N availability to microorganisms (Hattenschwiller & Vitousek, 2000).

Although *Leucaena* resulted in higher microbial activity, probably due to the high biomass and biological N_2-fixation capacity, compared to other legumes, some studies have indicated that *Leucaena* have a high polyphenol content (7.1 %), which can negatively influence residue mineralization, probably due to protein complexation by polyphenols (Monteiro et al., 2002). Monteiro et al. (2002) also observed that the polyphenol contents of other legumes (*Arachis*, *Mucuna*, *Cajanus*, *Centrosema*) were lower (1.8–4.0 %) than of *Leucaena*.

Our results demonstrate that legumes as green manures can contribute to enhance soil quality. This contribution can be explained by the high amount of biomass produced and the capacity to form symbioses with N_2-fixing bacteria, which contribute with expressive amount of N to the soil-plant system. Green manure usually has a low C/N ratio and is cut during the flowering period, when the nutrient content of legumes is higher and the lignin content lower. N content in *Crotalaria* (sunnhemp) plants can decrease from around 3 % at flowering to 1 % in the mature plant, while lignin increases from 6 to 18 % (Giller, 2001).

The improvement of soil N through biological N_2-fixation by legume green manures is particularly important in tropical soils, which have low natural fertility and high organic matter decomposition rates. When the soil-plant system is considered, legumes between coffee rows introduce a large amount of N derived from biological N_2-fixation, which would be enough to balance the N exports through coffee fruits. According to Calegari (1995) *Leucaena* have the

highest biomass N content (4.3 %), followed by *C. breviflora* and *M. deeringiana* (3.2 %), *Vigna unguiculata*, *Arachis hypogaea* and *M. pruriens* (2.5 %) and *C. spectabilis* (2.2 %). The total amount of N in the plant biomass of Leucaena was about 764 kg ha^{-1} $year^{-1}$ (Calegari, 1995), while *C. breviflora* contained about 68 kg ha^{-1} $year^{-1}$, *Vigna* 75 kg ha^{-1} $year^{-1}$, *M. deeringiana* 91 kg ha^{-1} $year^{-1}$, and *M. pruriens* 100 kg ha^{-1} $year^{-1}$ of N. Around 60–70 % of this amount of N incorporated into the soil was derived from biological N_2-fixation (Giller, 2001). If about 60 % of the total plant N is derived from biological N_2-fixation, *Leucaena* would have incorporated about 453 kg ha^{-1} $year^{-1}$ of N through biological N_2-fixation, which constitutes an excellent strategy of N supply to the soil. Perin et al. (2004) previously observed that 57 % of total N accumulated by *Crotalaria* (about 305 kg ha^{-1}), was derived from biological N_2-fixation.

This practice can contribute to increase N in soil, allowing a reduction of mineral fertilization. The usefulness of legume green manures in maintaining or building up soil fertility has been recognized by several authors. In this experiment, the legumes were not inoculated with *Rhizobium*, so infection and biological N_2-fixation occurred through natural *Rhizobium* populations.

The use of legume green manure between coffee rows makes it possible to release available N to coffee at legume harvest. Legume intercropping can be beneficial to coffee due to N_2 fixation, excretion of N compounds, nodule and root decomposition and, more intensely by residue decomposition, which can release a high amount of nutrients.

Several studies showed that green manure influences the soil chemical properties. For example, in legumes intercropped with grape (*Vitis vinifera*), Faria et al. (2004) observed that sunnhemp and jack bean increased organic C by 102 and 81 %, respectively, after six years and 11 legume cycles. Other studies demonstrated no effect of green manure on soil chemical properties. According to Matos et al. (2008), green manure improvement in soil quality and nutrient cycling depend on the climate, soil characteristics, soil management and quantity and quality of plant biomass. Most studies on the effect of green manure on soil properties were short-term experiments, several of which used only one plant cycle of about six months. Matos et al. (2008), studying the effects of different green manure on coffee, suggested the use of more than one green manure species in the system, due to the different characteristics of biomass production and nutrient content of the species.

Our results agree with previous studies (Carter & Rennie, 1982; Franzluebbers et al., 1995; Franzluebbers & Arshad, 1996; Chander et al., 1997; Balota et al., 2004) that demonstrated the responsiveness of soil organic quality (potential mineralization) to changes in soil management practice. This observation agrees with Smith & Paul (1990), who suggested that a major N source for plant growth can be supplied by soil organic matter through microbial mineralization processes. These results demonstrate that microbial biomass represents a substantial nutrient reserve for coffee. However, the values of biomass nutrient flux do not represent the nutrient amount available for crop growth per year, as a portion of the available nutrients may be used by the following generation of soil organisms or may be adsorbed onto soil colloids. Nevertheless, soil microbial biomass remains an important source of plant nutrients.

CONCLUSION

Legume crops influenced microbial activity, both under the coffee canopy and in-between rows. Cultivation of *Leucaena leucocephala* increased soil enzyme activity and C and N mineralization rates.

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