Nutrient input on rocket growth and soil microbial activity in alley cropping of pigeon pea¹

Aporte de nutrientes em rúcula e atividade microbiana do solo em cultivo com aléias de guandu

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Abstract - The effects of organic fertilization combining cattle manure and pigeon pea shoots on the culture of rocket, planted with one or two plants per hole, including soil microbial biomass carbon, soil respiration, the metabolic quotient, soil fumigation labile carbon, and the dry matter content and total N, K, P, Ca and Mg contents in the leaves and roots of rocket were investigated. The experimental design was randomized blocks in a 2 × 2 × 2 factorial experiment: 0 and 160 kg ha⁻¹ N from cattle manure, 0 and 160 kg ha⁻¹ N from pigeon pea shoots, and one or two plants per hole, with three replicates. The most significant and positive correlations were obtained between leaf K × soil respiration, microbial biomass × leaf N and root Ca × metabolic quotient. The use of 160 kg ha⁻¹ N from cattle manure along with 160 kg ha⁻¹ N from pigeon pea shoots with two plants per hole resulted in a lower relative loss of C-CO₂; the same result was found for the treatment of two plants per hole fertilized with 160 kg ha⁻¹ N from cattle manure. Increased leaf and root N contents were observed in the treatment that combined two plants in each plot, fertilized with 160 kg ha⁻¹ N from pigeon pea shoots, whereas the highest dry matter content was obtained by using one plant per hole, specifically: combining one plant per hole without fertilization; one plant per hole fertilized with 160 kg ha⁻¹ N from pigeon pea shoots; and one plant per hole fertilized with 160 kg ha⁻¹ N from cattle manure and pigeon pea shoots.

Key words - Eruca sativa L. Cajanus cajan L. Cattle manure. CO2 evolution. Green manuring.

Resumo - Investigou-se o efeito da adubação orgânica combinando esterco bovino, ramas de guandu e densidade populacional por cova na cultura da rúcula sobre carbono da biomassa microbiana do solo, respiração do solo, quociente metabólico, carbono lábil de solo fumigado, teor de matéria seca e conteúdo de N-total, K, P, Ca e Mg foliar e radicular de rúcula. O delineamento experimental adotado foi blocos ao acaso em ensaio fatorial 2 x 2 x 2 e três repetições: 0 e 160 kg ha⁻¹ de N de esterco bovino, 0 e 160 kg ha⁻¹ de N de ramas de guandu e uma e duas plantas por cova. As correlações mais significativas e positivas foram obtidas entre K foliar x respiração do solo, N foliar x biomassa microbiana e Ca radicular x quociente metabólico. A utilização de 160 kg ha⁻¹ de N de esterco bovino em conjunto com 160 kg ha⁻¹ de N de ramas de guandu com duas plantas por cova refletiu em menor perda relativa de C-CO₂, o mesmo ocorrendo com o tratamento de duas plantas por cova adubada apenas com 160 kg ha⁻¹ de N de esterco bovino. Maior conteúdo de N foliar e radicular foi observado no tratamento combinando duas plantas por cova adubada com 160 kg ha⁻¹ de N de ramas de guandu, enquanto maior teor de matéria seca foi obtido usando uma planta por cova, principalmente, nas combinações sem adubação; com 160 kg ha⁻¹ de N de ramas de guandu e com 160 kg ha⁻¹ de N de esterco bovino e ramas de guandu.

Palavras-chave - Eruca sativa L. Cajanus cajan L. Esterco bovino. Evolução de CO₂. Adubação verde.

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¹Recebido para publicação em 02/10/2010; aprovado em 06/06/2011

Pesquisa financiada pelo convênio entre TWAS (Third World Academy of Science), Unesco e CNPq

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Introduction

Concern over the rational use of environmental resources combined with the search for affordable alternatives to farmyard manure in line with the necessity of having indicators of soil quality that will assist in decision making is a current reality in the study of agroecosystems. Large quantities of animal manure, compost or other organic fertilizers are frequently used in intensive production systems for vegetables, often without the balance of nutrients for each situation being known (ALVES et al., 2004). This may contribute to unnecessary losses of nutrients in the planting of vegetable crops (PIMENTEL et al., 2009a).

The use of green manure has shown promising results in terms of production in various crops such as cabbage (FONTANÉTTI et al., 2006), lettuce and carrots (PIMENTEL et al., 2009b; SALGADO et al., 2006). The inclusion of legumes is an excellent option for the diversification of intensive agricultural systems because legumes are able to fix substantial quantities of atmospheric nitrogen, which accumulate in the biomass (ALVES et al., 2004). The use of alleys greatly contributes to sustainable crop production. The beneficial effects of alleys in the soil include higher leaf N contents and grain yields of maize in the alley cropping system with pigeon pea and gliricidia (QUEIROZ et al., 2007) and the use of pigeon pea as an alley crop in maize cropping was shown to result in the best soil coverage and nutritional balance (MOURA et al., 2008).

In the case of rocket (roquette or arugula) (*Eruca sativa* L.), little is known about the nutrients in its leaves and roots, the effects of the use of alley cropping systems with legumes, and also on soil microbial activity. Investigations usually focus on phytotechnical indicators, and few studies have evaluated the nutrient contents in plant tissue (GRANJEIRO et al., 2003). Soil microbial indicators, such as microbial biomass, soil respiration and the metabolic quotient, have been increasingly used to measure soil quality. Soil respiration, for example, is able to detect changes in the levels of soil carbon associated with the type of soil management, enabling an evaluation of the effects of microbial activity on organic wastes added to the soil (FRANZLUEBBERS et al., 2000; FRIES; AITA, 1990).

According Matsuoka et al. (2003), the largest amount of readily mineralizable carbon in the soil at a depth of 0-5 cm was attributed to the accumulation of crop residues on the surface, which stimulated microbial activity through the release of C-CO₂. This was corroborated by Gama-Rodrigues (1999), who reported that the more efficient the microbial biomass, the lower the carbon loss as CO₂-C respiration, and a significant portion of the carbon is incorporated in microbial tissues, reflected in a lower metabolic quotient.

The specific objective of this study was to investigate the effect of organic fertilization combining cattle manure and pigeon pea shoots on the culture of rocket, planted with one or two plants per hole, including soil microbial biomass carbon, soil respiration, the metabolic quotient, soil fumigation labile carbon and the dry matter content and total N, K, P, Ca and Mg contents of the leaves and roots of rocket.

Material and methods

In the area of SIPA - Sistema Integrado de Produção Agroecológica (Integrated System of Agroecological Production) - a technical cooperation project between Embrapa Agrobiologia, Embrapa Solos, Pesagro-Rio and Universidade Federal Rural do Rio de Janeiro was developed in 2001 to assess the effect of direct fertilization using cattle manure and pigeon pea (Cajanus cajan L.) shoots as sources of nitrogen. This work was part of the cooperation project of the Third World countries, an agreement between TWAS (Third World Academy of Science), UNESCO and CNPq.

The region is located at a latitude of 22°46′ south and a longitude of 43°41′ west, at an altitude of 33 m. According to the Köppen climate classification, the climate is AW, characterized by heavy rainfall in the summer and dry in winter, with an average rainfall of 1275 mm and an average annual temperature of 23.5 °C. Soil tillage was carried out using a hoe in an Ultisol of sandy clay loam. Chemical analysis of the soil presented the following results: pH (water) 7.3; 4.5 cmol_c dm⁻³ Ca; 1.4 cmol_c dm⁻³ Mg; 165.7 mg kg⁻¹ of P; 195.0 mg kg⁻¹ K; 0.9 g kg⁻¹ total N (EMBRAPA, 1997).

The experimental design was randomized blocks in a $2 \times 2 \times 2$ factorial experiment with three replicates. The factors (treatments) were 0 and 160 kg ha⁻¹ N from cattle manure (E₁ and E₂), 0 and 160 kg ha⁻¹ N from pigeon pea shoots (G₁ and G₂) and planting densities of one or two plants per hole (P₁ and P₂), equivalent to 500,000 and 1,000,000 plants ha-1, respectively. Two rows of pigeon pea alleys were planted around the experimental area; when they were 1.8 m in height, the pigeon pea plants were pruned and their branches were placed on the 2 m² plots, whereas the cattle manure was applied by properly mixing it with the soil of the experimental site. The rocket seeds were sown in 128-cell trays and the plants were transplanted to the field with one or two plants per hole spaced at 0.20×0.10 m. The crops were harvested 27 days after transplanting.

The dry matter content was measured using plants from the central area of the plot, representing 10% of the area (0.2 m^2) . The plant material was then subdivided into leaves and roots and weighed to obtain the fresh weight

before being transferred to a ventilated oven at 65 °C for a period of 72 h (or until the dry weights became constant).

The evaluation of soil microbial biomass carbon was performed using the fumigation-extraction procedure modified by De-Polli and Guerra (2008), where soil fumigation labile carbon was used to estimate the microbial biomass carbon in the soil. Fumigation was carried out by the direct addition of 1 mL of chloroform-free ethanol for each 20 g soil sample in 100 mL bottles. The bottles were sealed and kept in the dark for 24 h before being left open in a fume cupboard for an hour to allow the chloroform to dissipate (BROOKES et al., 1982; WITT et al., 2000). The evaluation of soil respiration took place as stated by Stotzky (1965) and determination of the metabolic quotient was obtained from the ratio of the C respired per unit of microbial C at a time interval described by Anderson and Domsch (1990).

Statistical analysis was performed using the SISVAR program. First, the assumptions for the correct application of analysis of variance and the normality and/ or homogeneity of the data from the statistical model were tested. The treatment means were compared by the F test at 5% probability. Pearson's correlation coefficients (r) between the variables were obtained to verify the degree of the linear relationships between them. Pearson's r values can range between -1.00 to +1.00. A correlation

coefficient close to 1 (absolute value) indicates the presence of a strong relationship between variables. Positive values for r indicate that the values of the variables are directly proportional, whereas negative values correspond to an inverse proportional relationship. Coefficient values close to 0 indicate the absence of a linear correlation between the variables. Correlation coefficients that showed probabilities below p<0.05 and p<0.01 were considered significant and highly significant, respectively.

Results and discussion

In general, significant and positive correlations were obtained between the nutrient contents and microbial attributes of leaf K \times soil respiration (0.79), leaf N \times soil microbial biomass (0.73), root Ca \times soil respiration (0.64), and root Ca \times the metabolic quotient (0.74) (TAB. 1).

Among the combinations used, it was found that the use of higher doses of cattle manure and pigeon pea shoots (160 kg ha⁻¹ N) with two plants per hole resulted in a lower relative loss of C-CO₂, i.e., less detachment of the carbon by soil respiration and a lower metabolic quotient (TAB. 2).

In relation to the nutrient content in leaves and roots of rocket, higher leaf and root N contents were obtained from the combination of two plants per hole fertilized with

Table 1 - Pearson's correlation coefficients between soil microbial biomass (Smb), soil respiration (Sr), the metabolic quotient (qCO₂), soil fumigation labile carbon (Sflc) and the total root (¹) and leaf (¹) contents of Ca, Mg, P, K and N and of the dry matter contents of rocket grown with one or two plants per holes, with 0 and 160 kg ha⁻¹ of cattle manure N and 0 and 160 kg ha⁻¹ of pigeon pea shoot N

	Smb	Sr	qCO ₂	Sflc	Car	Mg ^r	Pr	Kr	Nr	Cal	Mgl	P¹	K ¹	N^{l}	Dmc
Smb	1.00														
Sr	0.45	1.00													
qCO_2	0.16	0.89**	1.00												
Sflc	0.63*	0.78**	0.77*	1.00											
Car	-0.11	0.64*	0.74*	0.56	1.00										
$Mg^{r} \\$	-0.05	0.42	0.41	0.29	0.72	1.00									
P^{r}	-0.66*	0.03	0.31	-0.01	0.65	0.17	1.00								
\mathbf{K}^{r}	-0.36	0.08	0.20	-0.00	0.55	0.69	0.37	1.00							
N^{r}	0.27	0.11	-0.26	-0.22	-0.07	0.36	-0.43	0.21	1.00						
Cal	0.24	-0.55	-0.65*	-0.23	-0.44	-0.06	-0.47	0.23	0.29	1.00					
$Mg^l \\$	0.58	0.06	0.07	0.47	-0.01	0.04	-0.35	0.19	-0.09	0.63	1.00				
P^{l}	0.20	-0.02	-0.08	-0.15	-0.23	0.06	-0.39	0.24	0.34	0.42	0.51	1.00			
K^{l}	0.56	0.79**	0.55	0.60	0.31	0.16	-0.16	-0.41	0.23	-0.57	-0.22	-0.30	1.00		
N^{l}	0.73*	0.38	0.08	0.35	-0.04	-0.24	-0.26	-0.54	0.27	-0.07	0.17	0.14	0.62*	1.00	
Dmc	-0.03	0.08	0.27	0.04	-0.32	-0.41	-0.20	-0.18	-0.47	-0.18	0.17	0.35	-0.15	-0.20	1.00

^{*(}p < 0.05); **(p < 0.01)

Table 2 - Interactions effect of one (P_1) or two (P_2) plants per hole, (E_0) and (E_0) and (E_{160}) kg ha⁻¹ N from cattle manure and (E_0) and (E_0) kg ha⁻¹ N from pigeon pea shoots on soil respiration (mgC-CO₂ kg⁻¹ solo h⁻¹) and the metabolic quotient (mg C-CO₂ g⁻¹ C mic h⁻¹) in rocket cultivation

Factor		Soil res	piration		Metabolic quotient				
P	E_0G_0	$E_{160}G_{0}$	$E_{0}G_{160}$	$E_{160}G_{160}$	E_0G_0	$E_{160}G_{0}$	$E_{0}G_{160}$	$E_{160}G_{160}$	
1	1.32a	1.64a	0.95a	0.78a	5.60a	6.93a	3.60a	3.27a	
2	1.75a	2.72a	1.00a	0.66a	9.17a	8.61a	3.87a	2.99a	
Е	P_1G_0	$P_{1}G_{160}$	P_2G_0	$P_{2}G_{160}$	P_1G_0	$P_{1}G_{160}$	P_2G_0	$P_{2}G_{160}$	
0	1.32a	1.64a	1.75a	2.72a	5.60a	6.93a	9.17a	8.61a	
160	0.95a	0.78a	1.00a	0.66b	3.60a	3.27a	3.87b	2.99b	
G	P_1E_0	$P_{1}E_{160}$	P_2E_0	$P_{2}E_{160}$	P_1E_0	$P_{1}E_{160}$	P_2E_0	$P_{2}E_{160}$	
0	1.32a	0.95a	1.75a	1.00a	5.60a	3.60a	9.17a	3.87a	
160	1.64a	0.78a	2.72a	0.66a	6.93a	3.27a	8.61a	2.99a	

Means followed by same letters in the column, between the levels of plants or manure or pigeon pea shoots, did not significantly differ in the F test (p < 0.05)

160 kg ha⁻¹ N from pigeon pea shoots without cattle manure (TAB. 3). The highest dry matter contents were obtained from the combination of one plant per hole fertilized with 160 kg ha⁻¹ N from pigeon pea shoots and one plant per hole fertilized with 160 kg ha⁻¹ N from cattle manure plus pigeon pea shoots; however, one or two plants per hole without cattle manure but with pigeon pea shoots also showed high dry matter contents (TAB. 4).

In terms of the correlations between leaf $K \times soil$ respiration and leaf $N \times soil$ microbial biomass, some studies indicated a direct relationship between these factors. Silva et al. (2007) evaluated soil respiration after the application of biofertilizers in the organic cultivation of maize and observed positive and significant correlations

between the amount of carbon that was mineralized with foliar K. Paim (2007) evaluated the effect of the use of lime mud and potassium chloride in an Oxisol soil and found no significant difference between the basal respiration rates depending on the application of industrial waste and potassium chloride.

Leaf K is directly involved in the process of photosynthesis and a shortage of K results in a reduction in the photosynthetic rate per unit leaf area, and also higher rates of plant respiration. In the case of soil microbial biomass, their dry matter stocks may reach the range of 1 to 6 tons ha⁻¹ (LOOMIS; CONNOR, 1998), since a large amount of N is stored (67 to 420 kg ha⁻¹ N) in soil microbial biomass that has a C/N ratio close to 6.7 (FERREIRA, 2005). There were also

Table 3 - Interactions effect of one (P_1) or two (P_2) plants per hole, (P_0) and (P_0) kg ha⁻¹ N from pigeon pea shoots on the total nitrogen contents in roots (P_0) and leaves (P_0) of rocket

Factor			Nr		N ^I				
P	E_0G_0	$E_{160}G_{0}$	$E_{0}G_{160}$	$E_{160}G_{160}$	E_0G_0	$E_{160}G_{0}$	$E_{0}G_{160}$	$E_{160}G_{160}$	
1	1.51a	1.35a	1.42a	1.36a	2.88a	3.17a	2.95a	3.15a	
2	1.22b	1.56a	1.51a	1.45a	2.89a	3.26a	3.06a	3.03a	
Е	P_1G_0	$P_{1}G_{160}$	P_2G_0	P ₂ G ₁₆₀	P_1G_0	$P_{1}G_{160}$	P_2G_0	$P_{2}G_{160}$	
0	1.51a	1.35a	1.22b	1.56a	2.88a	3.17a	2.89a	3.26a	
160	1.42a	1.36a	1.51a	1.45a	2.95a	3.15a	3.06a	3.03a	
G	P_1E_0	$P_{1}E_{160}$	P_2E_0	P ₂ E ₁₆₀	P_1E_0	$P_{1}E_{160}$	P_2E_0	P ₂ E ₁₆₀	
0	1.51a	1.42a	1.22b	1.51a	2.88a	2.95a	2.89b	3.06a	
160	1.35a	1.36a	1.56a	1.45a	3.17a	3.15a	3.26a	3.03a	

Means followed by same letters in the column, between the levels of plants or manure or pigeon pea shoots, did not significantly differ in the F test (p < 0.05)

Factor	Dry matter content							
P	E_0G_0	$E_{160}G_{0}$	$E_{0}G_{160}$	E ₁₆₀ G ₁₆₀				
1	25.31a	21.73a	24.59a	24.67a				
2	23.50a	17.79a	19.76b	17.72b				
Е	P_1G_0	$P_{1}G_{160}$	P_2G_0	$P_{2}G_{160}$				
0	25.31a	24.59a	23.50a	19.76a				
160	21.73a	24.67a	17.79b	17.72a				
G	P_1E_0	$P_{1}E_{160}$	P_2E_0	P ₂ E ₁₆₀				
0	25.31a	21.73a	23.50a	17.79a				
160	24.59a	24.67a	19.76a	17.72a				

Table 4 - Interactions effect of one (P_1) or two (P_2) plants per hole, (P_2) and (P_3) and (P_4) kg ha⁻¹ N from cattle manure and (P_4) and (P_5) and (P_5) and (P_5) have (P_5) and (P_5) and (160 (G₁₆₀) kg ha⁻¹ N from pigeon pea shoots on the dry matter contents of rocket

Means followed by same letters in the column, between the levels of plants or manure or pigeon pea shoots, did not differ in the F test (p<0.05)

significant and negative correlations between root $P \times soil$ microbial biomass (-0.66) and leaf Ca × the metabolic quotient (-0.65) (TAB. 1).

The microbiological attributes that correlated positively and significantly with each other were: the metabolic quotient × soil respiration (0.89), soil microbial biomass × soil fumigation labile carbon (0.63), soil respiration × soil fumigation labile carbon (0.78) and soil fumigation labile carbon \times the metabolic quotient (0.77). For the metabolic quotient × soil respiration, similar results were observed by Fernandes et al. (2005), who reported that basal respiration rates and the metabolic quotient were positively correlated with increasing doses of sewage sludge. Concerning the relationship between soil fumigation labile carbon × soil microbial biomass (0.63), De-Polli et al. (2007) also found a positive correlation between these attributes. Both cases reinforce the results found in this work.

The nutrient contents of leaf N × leaf K also showed positive and significant correlations, a result corroborated by Cogo et al. (2006), who investigated the relationship of N/K and observed a linear relationship between the K and N in whole plants throughout the crop cycle. This further highlighted the existence of a simple proportionality between these two nutrients.

In terms of the interaction between pigeon pea shoots × plant density per hole, it was observed that the use of higher doses of cattle manure and pigeon pea shoots (160 kg ha⁻¹ N) with two plants per hole resulted in a lower relative loss of C-CO₂, i.e., less detachment of carbon by soil respiration and a lower metabolic quotient; the same result was found with the treatment of two plants per hole fertilized with 160 kg ha⁻¹ N from cattle manure. In this sense, as soil microorganisms

become more efficient in the use of ecosystem resources, a smaller amount of carbon is lost as CO, through respiration and a greater proportion is incorporated into microbial cells (ANDERSON; DOMSCH, 1985). Moreover, when considering conventional planting, the opposite results to this work were observed by Cabezas (2008), who found no significant increase in soil respiration after the application of nitrogen in the form of ammonium sulfate fertilizer on maize crops. Furthermore, Jakelaitis et al. (2007) observed a higher metabolic quotient after the application of herbicides.

The nutrient contents in the leaves and roots of rocket revealed higher leaf and root N contents from the combination of two plants per hole fertilized with 160 kg ha⁻¹ N from pigeon pea shoots, while dry matter content of rocket followed the same pattern from the combination of two plants per hole with 160 kg ha⁻¹ N from cattle manure and pigeon pea shoots. This result may be linked to the balance of nutrients between the supplied and exported by the crops, since Alves et al. (2004) evaluated the balance of nitrogen and phosphorus in growing beets, carrots and green beans after the incorporation of pigeon pea biomass, and found that there was a positive balance after this incorporation, accompanied by an increase in the absorption of P. These results were corroborated by Sampaio et al. (2003), who stated the importance of maintaining soil cover for retaining nutrients in the system.

Finally, the use of pigeon pea shoots as fertilizer has been proven to be a soil management practice with quantitative results of interest to the producer. This practice resulted in the greatest leaf N contents, besides positively contributing to the incorporation of carbon in the soil microbial biomass during the experimental period, reducing the relative emissions of C-CO, into the atmosphere.

Conclusions

- 1. Higher and positive correlations were obtained between leaf $K \times soil$ respiration, leaf $N \times soil$ microbial biomass and root $Ca \times the$ metabolic quotient;
- 2. The use of higher doses of cattle manure and pigeon peas shoots with two plants per hole resulted in a lower relative loss of C-CO₂;
- 3. Higher N contents in leaves and roots were observed from the combination of two plants per hole fertilized with 160 kg ha⁻¹ N from pigeon pea shoots, while higher dry matter contents were obtained by using one plant per hole, specifically: the combination of one plant per hole without fertilization; fertilized with 160 kg ha⁻¹ N from pigeon pea shoots; and fertilized with 160 kg ha⁻¹ N from cattle manure and pigeon pea shoots.

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