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SOIL SCIENCE

Biological activity of soil cultivated with pigeon pea under different fertilization managements

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ABSTRACT: Fertilization management of pigeon peas can increase soil quality and the N utilization by plants. Therefore, we evaluated the biological activity of soil cultivated with pigeon peas (*Cajanus cajan* (L) Millsp.) under different fertilization treatments. A randomized block design was used with three replicates and a 3×5 factorial arrangement (genotype×fertilization and inoculation management). At full flowering stage, the plants were collected and shoot dry matter was evaluated. Soil was sampled at 0-20 cm for various analysis viz. nodulation assessment; soil organic carbon; total nitrogen; carbon and nitrogen from microbial biomass; C/N ratio; β-glucosidase and urease enzymatic activity. Data were subjected to analysis of variance and Tukey's test ($P \leq 0.05$). A Pearson correlation matrix was constructed, and the similarity between treatments was evaluated using the Mahalanobis distance and grouping was done using the unweighted pair group method with arithmetic average (UPGMA). Both the experimental genotypes (BRS03 and BRS04) showed similar nodulation and shoot dry matter pattern with respect to fertilizer treatments. Furthermore, the microbial inoculation promoted a higher shoot dry matter content in all the genotypes. The application of mineral N and inoculation increased the total N content in the soil, favoring the mineralization of this nutrient. During the testing phase, the genotypes exhibited an increase in microbial carbon and microbial quotient levels, indicating an improvement in soil quality. The combination of fertilization and inoculation increased the enzymatic activity of β-glucosidase and urease. The correlation matrix showed a strong association between N total and C/N ratio. The formation of groups by UPGMA was observed as a function of inoculation, demonstrating its effect on soil biological variables.

Key words: β-glucosidase, *Cajanus cajan* (L) Millsp., nitrogen fixing bacteria, soil quality, urease.

Atividade biológica do solo cultivado com feijão-guandu sob diferentes manejos de adubação

RESUMO: O manejo da adubação do feijão-guandu pode melhorar a qualidade do solo e o aproveitamento do N pelas plantas. Assim, objetivou-se avaliar a atividade biológica do solo cultivado com feijão guandu (*Cajanus cajan* (L) Millsp.) com diferentes manejos de adubação. Empregou-se o delineamento em blocos casualizados, com três repetições e arranjo fatorial 3x5 (genótipos x fertilização e manejo de inoculação). Na fase de florescimento pleno procedeu-se a coleta da planta, para determinação da massa seca da parte aérea e do solo em 0-20 cm, para avaliações da nodulação; carbono orgânico total; nitrogênio total; carbono e nitrogênio da biomassa microbiana; relação C/N; atividade enzimática de β-glucosidase e urease. Os dados foram submetidos à análise de variância e ao teste Tukey ($P \leq 0,05$). Foi construída uma matriz de correlações de Pearson, a similaridade entre os tratamentos foi avaliada pela distância de Mahalanobis e o agrupamento pelo método de pares não ponderados com média aritmética (UPGMA). Ambos genótipos experimentais (BRS03 e BRS04) apresentaram comportamento semelhante para nodulação e padrão de matéria seca da parte aérea semelhantes em relação aos tratamentos com adubação. A aplicação de N mineral e inoculação incrementou o N total do solo, favorecendo a mineralização deste nutriente. Durante as fases de testes, os genótipos obtiveram elevação nos teores de carbono microbiano e quociente microbiano, indicando melhoria da qualidade do solo. A combinação de adubação e inoculação aumentou a atividade das enzimas β-glicosidase e urease. A matiz de correlação demonstrou elevada associação entre N total e relação C/N. A formação dos grupos pelo UPGMA foi obtida em função da inoculação, evidenciando seu efeito sobre as variáveis biológicas do solo.

Palavras-chave: β-glicosidase, *Cajanus cajan* (L) Millsp., bactérias fixadoras de nitrogênio, qualidade do solo, urease.

INTRODUCTION

Pigeon pea (*Cajanus cajan* (L) Millsp.) stands out in the global agricultural scenario due to its rapid adaptability and stability in production under a variety of soil and climatic conditions. In addition, it presents interesting potential by providing essential dietary nutrients for human and animal consumption (BENÍTEZ et al., 2021; BUTHELEZI et al., 2019). The legume is well known for its medicinal use (WU et al., 2019), and capacity to conserve soil and water, which results from the process of biological nitrogen fixation, reducing the demand for nitrogen fertilizers (SINGH et al., 2020; FABINO NETO et al., 2021).

Therefore, the cultivation management of this legume through the application of fertilizers

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and inoculants can increase biological quality of soil and the use of nitrogen (N) by plants. The biological quality of soil is closely associated with attributes such as microbial biomass (carbon (C) and nitrogen (N)), enzymatic activity, soil respiration, metabolic and microbial quotients, and organic carbon. Among these attributes, microbial biomass, which is responsible for the decomposition and transformation of organic matter, is related to the use of N by plants, as they directly influence the C and N flow and nutrient cycling in the soil-plant system (PRIMO et al., 2011).

Studies have indicated the presence of 10–50 thousand species of microorganisms in rhizosphere soil associated with grasses. However, only 1% of this microbial diversity could be isolated and cultivated under laboratory conditions. This demonstrates the need of studies for a better understanding of biological activity in the soil under different management systems (MATSUOKA et al., 2003). The evaluation of this biological component is crucial for creating a healthy and balanced environment, thus achieving success in agricultural activities.

Quantification of organic carbon and soil microbial biomass activity have been regarded as tools to indicate the soil quality in agriculture, as they are associated with the ecological functions of the environment and are capable of reflecting the changes caused by different soil management systems (ARAGÃO et al., 2012). Recent studies demonstrate the importance of biological techniques in detecting environmental changes (GONÇALVES et al., 2020; STIEVEN et al., 2020).

The biological activity of the soil can be represented almost entirely by microbial biomass, as this is the main source of enzymes that catalyze several reactions that represent the carbon source and sink relationship and nutrient exchange between the atmosphere and the soil-plant system. Any stress in this system affects the population, soil microbial diversity, and activity (REIS JUNIOR et al., 2007).

Research involving microbial analysis has become important for more detailed assessments of the agricultural environment as it allows quick evaluation of the necessary adjustments in relation to the systems of cultivation management. Therefore, this study analyzed the total carbon, nitrogen, and microbial and enzymatic activities of soil cultivated with pigeon peas (*Cajanus cajan* (L) Millsp.) under different fertilizer treatments.

MATERIALS AND METHODS

The study was conducted in a greenhouse, located in the municipality of Montes Claros - MG (16º40’58.5” S, 43º50’25.6” W, and 626 m altitude), in outline randomized complete block experiment, with three replications, in a 3×5 factorial scheme, the first factor being represented by the genotypes: BRS03 and BRS04 (experimental cultivars), and IAPAR 43 (commercial cultivar). The second factor corresponded to five treatments including combinations of fertilizer, nitrogen, and microbial inoculant (*Rhizobium tropici*): only base fertilizer (BF), base fertilizer with nitrogen and inoculant (BF+N+I), base fertilizer and nitrogen (BF+N), base fertilizer and inoculant (BF+I), and no fertilizer or inoculant application (WFI). The experimental plots consisted of one plant per 10 dm pot^3. The soil used to conduct the experiment was identified as Nitossolo (SANTOS et al., 2018), with the following chemical characterization before the experiment: pH (water) = 4.5; phosphorus (P) = 1.51 mg dm-3; potassium (K^+) = 20.69 mg dm-3; calcium (Ca+2) 2.50 $cmol_w$ dm-3; magnesium (Mg+2) = 1.00 $cmol_w$ dm-3; aluminium (Al) = 1.34 $cmol_w$ dm-3; H+Al = 9.62 $cmol_w$ dm-3; Effective CTC = 5.13 $cmol_w$ dm-3; saturation per base = 28%; and organic matter = 4.41%.

Soil correction was performed using the GEOX corrector with 60% calcium peroxide (CaO_2), 30% magnesium oxide (MgO), and 180% PRNT, with the aim of increasing the base saturation to 60%, following the recommendations proposed by FARIAS et al. (2013). The soil was incubated for 30 d to maintain the soil moisture at 60% of its field capacity. After the incubation, the base fertilizer with PA reagents was used for plants grown in pots in controlled environments according to CANTARUTTI et al. (2007), with the exception of nitrogen. In the treatments that received nitrogen fertilization, urea PA was used, twenty days after seedling emergence. Before sowing, the seeds were inoculated with a commercial inoculant @Nitro1000 composed of *Rhizobium tropici* (Semia 4077, Semia 4080, and Semia 4088), vitamins, minerals, carbon source, peat (powder)/water, thickener, preservative, and stabilizer PVP (aqueous) at a dosage of 100 ml g^{-1} for 25 kg of seeds.

The pigeon peas were sown with three seeds per pot. After germination, only one plant per pot was maintained, with soil moisture close to field capacity. Plants and ground soil were collected during full flowering. Soil was collected from a depth of 0-20 cm, close to the root system, for experimental evaluation. The soil samples were placed in plastic bags with ventilation, labeled, and stored in a refrigerator (4 ºC) until analysis. In the laboratory, the samples were sieved through a 2 mm mesh sieve, and all fragments of plants and animals were removed through manual scavenging.

After processing the samples, the following assessments were carried out: nodulation; soil organic carbon; total nitrogen; carbon and nitrogen from microbial biomass; C/N ratio; and β-glucosidase and urease enzymatic activities. Nodulation was assessed by counting the nodules per plant.

The plants were removed from the pot and aerial parts were separated from the root system at a height from the neck of the plant. The roots were washed under running water using sieves, and the nodules were counted. To identify viable nodules, the internal coloration of the nodule was observed. Active nodules possess an intense pink color. For determination of the dry mass of the aerial parts (DMAP), the plants were placed in a greenhouse with forced air circulation at a temperature of 65ºC until constant weight is reached. After obtaining the dry matter mass, the values were converted to g $plant^{-1}$.

Soil organic carbon (SOC) was determined using the method proposed by YEOMANS & BREMNER (1988). To determine total nitrogen, method given by MENDONÇA & SILVA (2017) was used. For the determination of carbon and nitrogen in the microbial biomass (Cmic and Nmic), the fumigation method was used for extraction (SILVA et al., 2007) as adapted from VANCE et al. (1987), in which the samples were fumigated in a desiccator using chloroform and kept for 48 h. Subsequently, the samples were extracted with a 0.5 M solution of potassium sulfate (K_2SO_4). Nmic was quantified by steam distillation (Kjeldahl method). The microbial quotient (qMic) was obtained from the relationship between Cmic and SOC.

Basal soil respiration (RBS) was determined according to the methodology described by MENDONÇA & SILVA (2017). The samples were incubated for seven days in flasks without light, with soil moisture adjusted to 60% of the field capacity for the stabilization of microorganisms. They were later transferred to air tight jars assembled with a bottle containing 0.5 mol L^{-1} NaOH, and the assessments were done at time intervals of 24, 48, 72, 96, and 120 h. To calculate RBS, we considered the last three values after stabilization at intervals of 72 and 96 h. O metabolic quotient (qCO_2) was calculated as the ratio between RBS and Cmic (SILVA et al., 2007).

β-glucosidase enzyme activity was determined based on the colorimetric measurement of p-nitrophenol released by soil β-glucosidases after incubation with a buffered solution of p-nitrophenyl-β-D-glucopyranoside. The absorbance was measured on a spectrophotometer at 420 nm. To evaluate the activity of the urease enzyme (AU), the soil sample was incubated with urea solution for 2 h at 37 ºC, followed by the determination of ammonium (NH+) released in the process (TABATABAI, 1994).

The data obtained were subjected to analysis of variance and the means were compared using the Tukey's test at 5% probability using R Software (R CORE TEAM, 2021).

For the variables, the total number of nodules (TNN) and number of viable nodules (NVN) did not present homogeneity of variances and/or normality of errors and the transformation of the data. Variables related to the biological attributes of the soil were subjected to multivariate analysis of variance. From the treatment averages, a matrix of Pearson correlations was used to establish relationships between the variables studied and better understand biological activity, along with results from the point of view univariate perspective.

The distance matrix between treatments was calculated using the Mahalanobis distance. From the distance matrix, grouping was performed using the "Unweighted Pair Group Method with Arithmetic Average" (UPGMA) to learn about the pattern of similarity of treatments and influencing factors, considering the variables together. The analyses were performed using RBio software (BHERING, 2017).

RESULTS AND DISCUSSION

The total number of nodules (TNN) and number of viable nodules (NVN) showed significant differences among the genotypes, whereas the nodulation did not show any difference among the fertilizer treatments within a genotype (Table 1).

The commercial cultivar IAPAR 43 was the one that showed the greatest nodulation among all the cultivars. In general, the fertilization with nitrogen supplementation and/or inoculation with *Rhizobium tropici* observed an increase in the dry mass of the aerial parts (Figure 1). Contrary to what was observed for TNN and NVN, cultivar IAPAR 43 presented the lowest values for the shoot dry mass than the two experimental cultivars (BRS03 and BRS04). Regarding fertilizer treatments, the treatment without base fertilizer, inoculant, or nitrogen (WFI) presented the lowest average value for the shoot dry mass. Pigeon pea is capable of nodulating natural soil bacteria; however, external inoculation can increase production, as the bacteria introduced are more competitive and efficient (XAVIER et al., 2008; FERREIRA et al., 2009). This characteristic corroborated the results which reported the highest dry mass of the value aerial part occurred for treatment with base fertilizer and inoculant.

Table 1 - Nodulation and dry mass of the aerial part (DMAP) of different pigeon pea genotypes under to five fertilization and inoculation treatments with *Rhizobium tropici*.

Genotypes	---Flowering---		
	---Number of nodules per plant---		DMAP (plant[1])
	Total nodules (TNN)	Viable nodules (NVN)	
BRS03	88.33 b	6.13 b	33.0747 a
BRS04	77.33 b	32.33 b	35.2933 a
IAPAR43	193.33 a	120.86 a	22.8093 b
CV (%)	28.18	66.86	26.98

[*]Means with the same letters in the columns do not differ from each other, using the Tukey test, at 5% probability. CV: Coefficient of variation. MSPA: Dry mass of the aerial part.

Despite the cultivars in the test phase (BRS03 and BRS04) showing lower number of total and viable nodules, they obtained greater aerial part dry mass in relation to commercial cultivar IAPAR 43. Dry mass production is one of the aspects evaluated in relation to the efficiency of symbiosis with strains of *Rhizobium*, indicating the capacity of biological nitrogen fixation to meet part of the plant's demand and enable the development of culture (GUIMARÃES et al., 2016).

Furthermore, the soil cultivated with the BRS03 genotype in the presence of base fertilizer, nitrogen, and inoculant (BF+N+I) presented lower soil organic carbon (SOC) content when compared to the treatments that received only base fertilizer or base fertilizer with nitrogen or inoculant (Table 2). This plausible response may be associated with the effect of nitrogen fertilization. Some studies have already reported a negative effect of nitrogen fertilization on nodulation in cowpea and common bean roots as a result of N availability (BRITO et al., 2011; MARTINS et al., 2013). Similarly, the soil cultivated with cultivar IAPAR 43, in the treatment

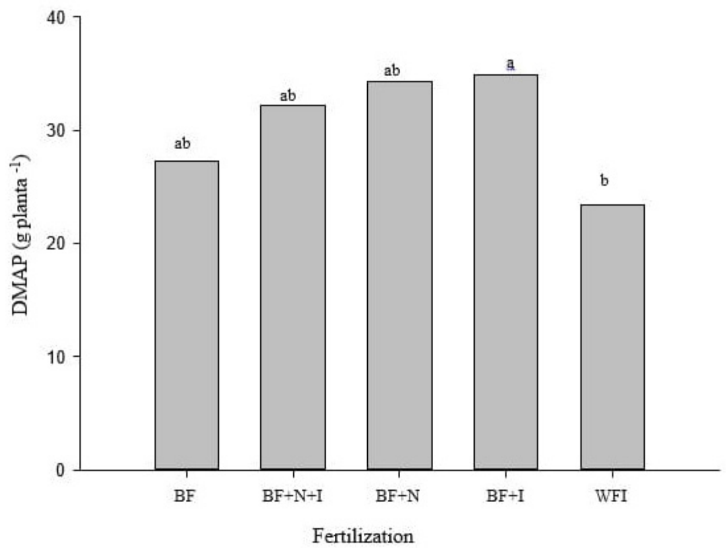

Figure 1 - Dry mass of the aerial part (g plant-1) of pigeon pea, subjected to five fertilization and inoculation management with *Rhizobium tropici*, in Nitossolo. Averages with letters equals do not differ from each other, using the Tukey test at 5% probability. CV: coefficient of variation. BF: fertilization base; BF+N+I: base fertilizer with nitrogen and inoculant; BF+N: base fertilizer and nitrogen; BF+I: base fertilizer and inoculant; WFI: only the amended soil, without application of fertilizer or inoculant.

Table 2 - Soil organic carbon (SOC, g kg-1) in the cultivation of pigeon peas under five fertilization and inoculation treatments with Rhizobium tropici, in Nitisols.

Genotypes	-----------------------------------Fertilization and inoculation management-------------------------------				
	BF	BF+N+I	BF+N	BF+I	WNI
BRS03	10.1 Aba	9.8 Bb	10.5 Aa	10.6 Aa	10.5 Aa
BRS04	10.6 Aa	10.6 Aa	10.6 Aa	10.4 Aa	10.5 Aab
IAPAR43	10.5 Aba	10.2 Abab	10.7 Aa	10.8 Aa	10.0 Bb
CV (%)	---2,71---				

*Averages with the same letters, uppercase in the row and lowercase in the columns, do not differ from each other, by the Tukey test, at 5% probability. CV: coefficient of variation. BF: fertilization base; BF+N+I: base fertilizer with nitrogen and inoculant; BF+N: base fertilizer and nitrogen; BF+I: base fertilizer and inoculant; WFI: only the amended soil, without application of fertilizer or inoculant.

with the absence of base fertilizer, inoculant, and nitrogen (WFI) showed lower values of SOC both in relation to other genotypes, as well as to the management they received only one source of variation (BF+N and BF+I). The soil cultivated with the BRS04 genotype did not show any statistically significant differences in the SOC levels among the various fertilizer treatments. Responses to the inoculation with N fixing bacteria in legumes can be different depending on the cultivars (BÁRBARO et al., 2009). SOC content has minor magnitude in case of cultivars that show inhibition in nodulation in presence of N as it is directly related to the largest population of microorganisms in the soil (TU et al., 2006). However, in some cultivars, the response of microbial resistance is higher when initially carrying out nitrogen fertilization, which is related to greater vigor of the plant before starting nodulation, improving this process and thus presenting larger microbial populations (OLIVEIRA et al., 2003). Therefore, these results demonstrated that soils cultivated with genotypes BRS03 and IAPAR 43 are more sensitive to contrasting conditions, regardless of the presence or absence of nitrogen sources. SOC content of soils cultivated with genotypes BRS03 and BRS04 (in the testing phase) exhibited potential equal to or greater than that grown with the commercial cultivar IAPAR 43, in addition to presenting less susceptibility to oscillation in relation to handling, with the exception of the BF+N+I treatment for soil with BRS03. According to MENDONÇA & MATOS (2017), the selection of materials that increase the organic matter content of soil contributes to improving its physical, chemical, and biological properties. For total N there was only the simple effect of management, and the values varied between 1.09 and 1.23 g kg^{-1}, with the lowest value being in BF and the highest in BF+N treatment (Table 3).

The soils managed with BF+N (1.23 g kg^{-1}), BF+N+I (1.21 g kg^{-1}), and BF+I (1.21 g kg^{-1})

Table 3 - Total Nitrogen (total N, g kg^{-1}) and C/N ratio of soil cultivated with beans guandu, subjected to five fertilization and inoculation treatments with *Rhizobium tropici*, in Nitosols.

Fertilization management	Total N	C/N Ratio
BF	1.09 b	9.63 a
BF+N+I	1.21 a	8.46 b
BF+N	1.23 a	8.67 ab
BF+I	1.21 a	9.03 ab
WFI	1.16 ab	8.95 ab
CV (%)	4.99	7.86

*Averages followed by the same letter in the column do not differ from each other, using the Tukey test, at 5% probability. CV: coefficient of variation. BF: fertilization base; BF+N+I: base fertilizer with nitrogen and inoculant; BF+N: base fertilizer and nitrogen; BF+I: base fertilizer and inoculant; WFI: only the amended soil, without application of fertilizer or inoculant.

were observed to possess the highest total N content, possibly due to the application of nitrogen fertilizer and the biological N-fixing activity of the inoculant. N supply to the soil is of great importance for crop yield as well as for further plantations and maintenance of fertility in the soil (PEREIRA et al., 2015). In the present study, high productivity with increased N supply is also evidenced by the higher DMAP values observed for the treatments with nitrogen or/and inoculant application.

Total N content in the soil may explain the C/N ratios observed in the soil. The BF treatment compared to BF+N+I corresponded to the C/N ratio of 9.63 and 8.46, respectively (Table 3). The higher nitrogen content in the BF+N+I treated soils contributed to a reduction in the C/N ratio, demonstrating that greater mineralization and availability of N may have occurred for plants (DELBEM et al., 2011). It is also worth highlighting that the C/N ratios obtained in the soils in this study demonstrated a predominance of the process of nutrient availability for plants.

The carbon content of microbial biomass (Cmic) was not influenced by fertilization or inoculation with *Rhizobium* ($P > 0.05$). However, cultivation with the genotypes BRS03 and BRS04 (114.0 and 120.1 mg C kg^{-1} soil, respectively) provided higher Cmic levels in the soil compared to IAPAR 43 (59.80 mg C kg^{-1} soil), reaffirming the potential of these experimental genotypes in increasing the biological activity of the soil. According to SOUZA (2015), these results can be associated with greater volumes of roots and root exudates, increasing carbon input, thus providing more energy to the microbial community and stimulating the proliferation of microbial biomass in the soil. Higher Cmic values result in lower nutrient losses in the soil–plant system via temporary immobilization; hence, it is important to implement fertilizer treatments and genotypes that favor the development of microbial biomass (ROSCOE et al., 2006).

The Cmic value in IAPAR 43 represented less than 1% of the SOC value, while the acceptable range of Cmic is 1%–5% of the SOC (JENKINSON & LADD, 1981), indicating that the adoption of this cultivar may have provided unfavorable conditions for the development of soil microorganisms under the evaluated conditions.

The application of fertilizers, nitrogen, and *Rhizobium* (BF+N+I) treatment observed significant differences among cultivars for soil microbial biomass nitrogen content (Nmic), and in cultivar BRS03 an average of 38.89 mg N kg^{-1} soil was obtained, while in BRS04 Nmic content corresponded to 10.98 mg N kg^{-1} soil. This result demonstrated that the cultivation of BRS03 may have provided a more favorable environment for the development of microorganisms responsible for N immobilization.

The value of microbial quotient (qMic) was seen to vary between the experimental and commercial genotypes, with BRS03 and BRS04 presenting higher values of 1.10 and 1.13%, respectively, compared to IAPAR 43 (0.57%). According to JAKELAITIS et al. (2008), the qMic varies from 1 to 4% under normal conditions. Some studies also suggested that certain genotypes present better conditions for the development of microorganisms and are more efficient in using organic compounds (GONÇALVES et al., 2020).

As there was no difference in the total N content between the cultivars, it was observed that in the BF+N+I treatment, the greater values of Nmic in BRS03 was responsible for the higher Nmic/N ratio (%) compared to BRS04 (3.17 and 0.90%, respectively) (Table 4). In general, nitrogen immobilization in microbial biomass was inefficient, regardless of the genotype or treatment, remaining below 4% (GONÇALVES et al., 2020). One of the factors that may have contributed to this low efficiency

Table 4 - Nmic/Ntotal ratio (%) of soil cultivated with pigeon pea, subjected to five fertilization and inoculation management with *Rhizobium tropici*.

Genotypes	-----------------------------------Fertilization and inoculation management--------------------------------				
	BF	BF+N+I	BF+N	BF+I	WNI
BRS03	2.78 Aa	3.17 Aa	1.88 Aa	1.55 Aa	1.1 Aa
BRS04	1.86 Aa	0.90 Ab	1.87 Aa	1.37 Aa	2.3 Aa
IAPAR43	1.69 Aa	1.32 Aab	2.46 Aa	3.03 Aa	3.0 Aa
CV (%)	--48.04--				

*Averages with the same letters, uppercase in the row and lowercase in the columns, do not differ from each other, by the Tukey test, at 5% probability. CV: coefficient of variation. BF: fertilization base; BF+N+I: base fertilizer with nitrogen and inoculant; BF+N: base fertilizer and nitrogen; BF+I: base fertilizer and inoculant; WFI: only the amended soil, without application of fertilizer or inoculant.

was the intense soil disturbance for correction and implementation of the experiment, which may have reduced the Cmic and Nmic values, given the sensitivity of these microbiological attributes to soil management (ASSIS et al., 2019).

For soil basal respiration (SBR), the soil cultivated with IAPAR 43 (0.194 mg C-CO2 kg^{-1} solo h^{-1}), even with a lower Cmic content, showed results similar to those of BRS03 and BRS04 (0.196 and 0.197 mg C-CO_2 kg^{-1} solo h^{-1}, respectively). REIS JUNIOR & MENDES (2007) stated that high rates must be analyzed according to context, which may represent stressful situations or systems with high production; in this study, it refers to production, as this cultivar had a shorter time (days) for flowering (IAPAR 43: 73.07; BRS03: 169.20; and BRS04: 170.53). Furthermore, according to DELBEM et al. (2011), high SBR values may result in the long-term loss of SOC.

The qCO_2 differed depending on the interaction between fertilization management, inoculation, and genotype (Table 5). In the soil cultivated with BRS03 cultivar, the metabolic quotient was lower in BF+I management (1.19 mg C-CO2 g^{-1} Cmic-C h^{-1}) compared to BF (5.90 mg C-CO_2 g^{-1} Cmic-C h^{-1}). The inoculation that occurred in BF+I in this genotype favored the development of more efficient microorganisms for the use and storage of organic compounds, considering that there was no significant difference in the SOC content between the treatments. Thus, there was greater incorporation of Cmic and less loss of C via CO_2 during respiration, characterizing a more efficient biomass for the use of these compounds (REIS JUNIOR & MENDES, 2007; ASSIS et al., 2019).

In the soil that received only BF, cultivation of BRS03 provided greater qCO_2 values than BRS04 and IAPAR 43. In BF+I, this behavior was modified, with IAPAR 43 obtaining a higher value than BRS03 and BRS04 (Table 5). DELBEM et al. (2011) pointed out that there is an increase in qCO_2 due to disturbances in the agroecosystem, and that this behavior is characterized by a reaction of the microbial community. This result can also be explained by the fact that the high levels of qCO_2 indicate microbial communities in the early stages of development; that is, a higher proportion of active microorganisms (ROSCOE et al., 2006). The biological activity of the soil was also evaluated by the activity of β-glucosidase and urease enzymes (Table 6). We reported that the activity of the β-glucosidase enzyme in the WFI treatment was inferior to that in other fertilizer treatments in the three genotypes evaluated, indicating that the lack of fertilization and inoculation negatively affects the activity of this enzyme in the soil. As β-glucosidase is associated with the carbon cycle, the absence of mineral fertilization and inoculation (WFI) may have contributed to lower plant growth and microbial activity (MATSUOKA et al., 2003). Although, it does not differ from other treatments, a reduction in Cmic was observed in the WFI, which may be related to the result presented by β-glucosidase.

BF, BF+N, and WFI treatments in the BRS03 genotype contributed to greater urease enzymatic activity than in the BRS04 and IAPAR 43 genotypes (Table 6). In WFI treatment, the activity of this enzyme in IAPAR 43 cultivar was lower than that in the other fertilizer treatments. Soil cultivated with this cultivar and subjected to certain management practices may have less potential to convert organic N into minerals, thereby damaging the N mineralization process (LANNA et al., 2010). There are factors that influence the production of enzymes by microorganisms, mainly those which interfere with the development of microbes, such as water availability and temperature in an adequate range, and presence of other nutrients that favored the metabolic processes of microorganisms (STIEVEN

Table 5 - Metabolic quotient (qCO_2, mg C-CO_2 g^{-1} Cmic-C h^{-1}) of soil cultivated with pigeon pea under five fertilization and inoculation treatments with *Rhizobium tropici*.

Genotypes	--Fertilization and inoculation management--				
	BF	BF+N+I	BFN	BF+I	WFI
BRS03	5.90 Aa	1.52 ABa	1.62 ABa	1.19 Bb	2.55 Aba
BRS04	1.48 Ab	1.70 Aa	2.15 Aa	1.61 Ab	2.65 Aa
IAPAR 43	2.54 Aab	3.97 Aa	3.69 Aa	6.81 Aa	5.72 Aa
CV (%)	---29.63---				

*Averages with the same letters, uppercase in the row and lowercase in the columns, do not differ from each other, by the Tukey test, at 5% probability. CV: coefficient of variation. BF: fertilization base; BF+N+I: base fertilizer with nitrogen and inoculant; BF+N: base fertilizer and nitrogen; BF+I: base fertilizer and inoculant; WFI: only the amended soil, without application of fertilizer or inoculant.

Table 6 - β-glucosidase (μg p-nitrophenol h^{-1} g^{-1} soil) and urease (μg NH_4^+ - N g^{-1} solo h^{-1}) activity of soil cultivated with pigeon pea under five fertilization treatments and inoculation with *Rhizobium tropici*.

	---Fertilization and inoculation management---					
Enzyme	Genotype	BF	BF+N+I	BF+N	BF+I	WFI
B-glucosidase	BRS03	34.2 Ba	38.4 ABab	39.5 Aa	35.5 ABb	25.3 Cb
	BRS04	37.9 Bca	34.4 CDb	41.5 ABa	42.9 Aa	31.2 Da
	IAPAR43	36.1 Aba	39.2 Aa	33.2 Bb	37.7 ABb	23.0 Cb
CV (%)	---5.71---					
Urease	BRS03	166.3 Aa	138.6 Ba	143.6 ABa	148.7 ABa	148.4 Aba
	BRS04	142.6 Ab	147.0 Aa	114.0 Bb	150.8 Aa	114.8 Bb
	IAPAR43	117.1 Bc	134.5 ABa	131.2 ABb	143.4 Aa	92.6 Cc
CV(%)	---6.21---					

*Averages with the same letters in the same session, capital letters on the same line and lower case letters on the same line columns, do not differ from each other, using the Tukey test, at 5% probability. CV: coefficient of variation. BF: fertilization base; BF+N+I: base fertilizer with nitrogen and inoculant; BF+N: base fertilizer and nitrogen; BF+I: base fertilizer and inoculant; WFI: only the amended soil, without application of fertilizer or inoculant.

et al., 2020). Under natural soil conditions (WFI), a reduction in enzymatic activity was observed, indicating the importance of fertilization and inoculation for microbial activity. Fertilization and inoculation promote several improvements in soil quality with microbial populations, as they are more sensitive to treatment and can increase rapidly; consequently, increasing the release and activity of enzymes (SICZEK et al., 2016).

The Pearson's correlation matrix obtained using biological soil variables demonstrated few associations between the variables studied (Figure 2).

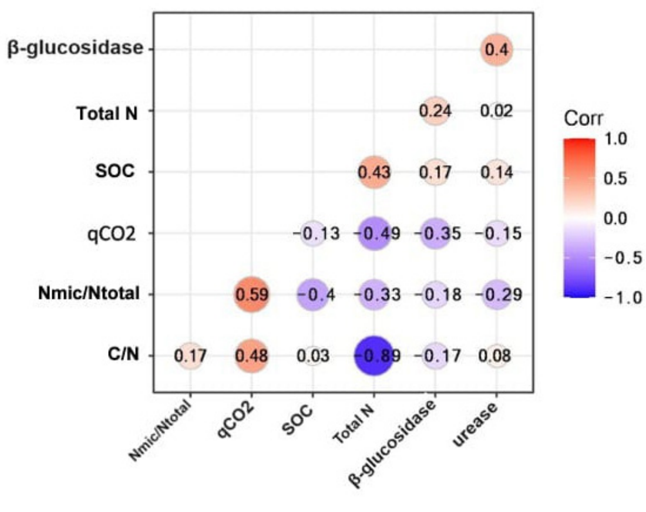

Figure 2 - Pearson correlation matrix of the means of the evaluated variables. Circles larger, darker circles indicate greater correlation magnitudes and smaller, darker circles light colors indicate lower correlation magnitudes. Positive correlations are red and negatives in blue. β-glucosidase (μg p-nitrophenol h^{-1} g^{-1} ground); Ntotal (total nitrogen, g kg^{-1}); SOC: soil organic carbon (g kg^{-1}); urease (μg NH_4^+ - N g^{-1} solo h^{-1}); qCO_2: quotient metabolic (mg C-CO_2 g^{-1} Cmic-C h^{-1}); Nmic.Ntotal: Microbial N/Ntotal (%); C:N C/N ratio.

High correlations between these variables can explain the fundamental relationships that maintain balance in the soil microbiota (SANTOS et al., 2011). It was possible to verify that the variables N total and C/N obtained higher magnitudes of correlation (-0.89) and median relationships between qCO_2 and Nmic/N total. A low C/N ratio indicated a high rate of organic matter decomposition.

As shown in table 3, the highest nitrogen content in the soil of BF+N+I contributed to reducing the C/N ratio, which was proven by the high magnitude and negative correlation between total N and the C/N ratio. That is, the increase in total nitrogen contributed to reducing the C/N ratio, which is an extremely important factor that indicates that greater nutrient availability stops the plants. It can also be observed that the ratio of SOC and total N was only 0.43. SANTOS et al. (2011) observed a correlation of 0.98 for the same variables. The positive correlations among SOC, total N, and qCO_2, indicate higher concentrations of these elements in the soil, supporting a larger population of microorganisms and, consequently, a greater rate of respiration in the soil. This provides better conditions for plant establishment (TU et al., 2006).

Multivariate analysis of variance was performed using Wilks' test with 5% probability for the cultivar × management interaction. The dendrogram obtained by the method UPGMA (average connection between groups) and based on the Mahalanobis distance obtained a cophenetic correlation of 0.77 (Figure 3), this indicated that there was a good representation of the matrix of the distance between treatments in the graphic scatter. Three groups were formed based on the variables analyzed and considering the MOJENA Method (1977) for the establishment of groups, with a value of 26.19 for k = 1.25. According to the analysis, the closest treatments were BRS03: BF+I and BRS04: BF+I, and the most distant were BRS03: BF and IAPAR 43: WFI.

Groups G1 and G2 drew attention because they were managed with no application of fertilizer or inoculant (WFI) or only base fertilizer (BF); that is, the absence of the inoculant has an effect on the biological responses of the soil. These results complement the lower enzymatic rates observed in the WFI treatment (Table 6), indicating lower microbial activity. It is worth highlighting that the dendrogram concerns all biological variables evaluated together, and it can be inferred that biological activity in general was affected by the absence of the inoculant. The G3 group is formed by the majority of the treatments that received nitrogen fertilization or inoculants. Inoculation can have an effect similar to nitrogen fertilizer (BÁRBARO, 2009). Because the multivariate analysis results were significant for the interaction among cultivars, the results were also influenced by them. This strengthens the importance of nitrogen in general, for microbial activity in the soil.

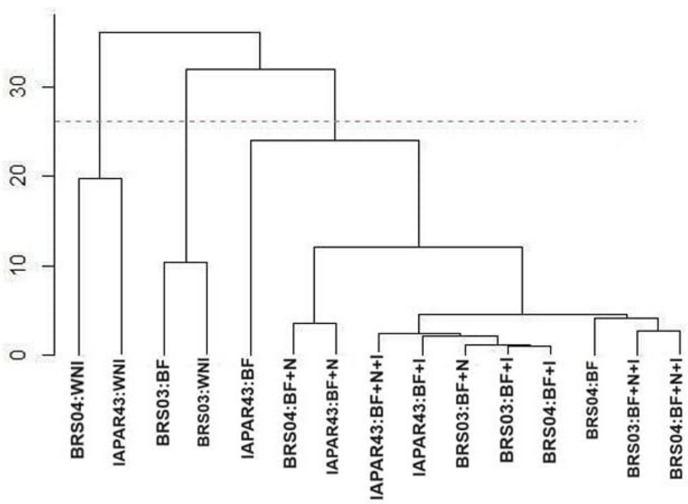

Figure 3 - Dendrogram obtained by the UPGMA method from Mahalanobis distances of the variables studied. Genotypes: BRS03, BRS04 and IAPAR 43. BF: fertilization base; BF+N+I: base fertilizer with nitrogen and inoculant; BF+N: base fertilizer and nitrogen; BF+I: base fertilizer and inoculant; WFI: only the amended soil, without application of fertilizer or inoculant.

CONCLUSION

The two pigeon pea genotypes in the test phase showed similar nodulation and dry mass of the aerial parts an increase in Cmic and qMic levels in the soil, indicating the potential of these genotypes to improve soil quality.

The application of mineral N and microbial inoculation of pigeon peas increased the total N in the soil, so that the association of these sources favored the mineralization of this nutrient in the ground in addition increased the activity of β-glycosidase and urease in the soil.Microbial inoculation increased the shoot dry mass in all the genotypes.

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DECLARATION OF CONFLICT OF INTEREST

The authors have no competing interests to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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