ISSN 1807-1929
Revista Brasileira de Engenharia Agrícola e Ambiental
v.21, n.2, p.116-121, 2017
Campina Grande, PB, UAEA/UFCG – http://www.agriambi.com.br

DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v21n2p116-121

Land use and vegetation cover on native symbionts and interactions with cowpea

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Key words:
arbuscular mycorrhiza
rhizobia
Legal Amazon
nodulation

A B S T R A C T

Arbuscular mycorrhizal fungi and rhizobia are important components of agroecosystems and they respond to human interference. The objective of this study was to investigate native communities of those microorganisms in soil collected under the native forest, four pastures (*Brachiaria brizantha*, *Panicum maximum*, *Arachis pintoi* and *Stylosanthes guianensis*) and a fallow soil after maize cultivation, in interaction with cowpea (*Vigna unguculata*). The cowpea grew in a greenhouse until flowering. They were randomly distributed depending on soil, in five replications. The lowest mycorrhizal fungi sporulation and mycorrhizal root colonization occurred under the *Panicum* and forest soil. In the soils under forest and *Stylosanthes*, the cowpea did not exhibit nodules and grew less. Among the anthropized areas, the effect was variable, with stimulus to the multiplication and symbiosis of these microorganisms, except in areas of *Panicum* and *Stylosanthes*. When the native vegetation is substituted by pasture or farming, the mycorrhizal fungi and rhizobia proliferation predominate. However, the effect and its magnitude depends on the grown plant species, with reflects on the plant species in succession, such as the cowpea.

Palavras-chave:
micorriza arbuscular
rizóbio
Amazônia Legal
nodulação

Uso do solo e coberturas vegetais sobre simbiontes nativos e interações com o feijão caupi

R E S U M O

Fungos micorrízicos arbusculares (FMA) e rizóbios são componentes importantes dos agroecossistemas e respondem à interferência antrópica. O objetivo deste trabalho foi estudar comunidades nativas desses microrganismos em solo coletado sob floresta nativa, quatro áreas de pasto (*Brachiaria brizantha*, *Panicum maximum*, *Arachis pintoi* e *Stylosanthes guianensis*) e uma área de pousio após lavoura de milho, em interação com o feijão caupi (*Vigna unguculata*). O caupi cresceu em casa de vegetação até o florescimento, distribuído ao acaso em função do solo, com cinco repetições. A esporulação e a colonização radicular por FMA foram mais baixas no solo das áreas de *Panicum* e floresta. No solo da floresta e com *Stylosanthes*, o caupi não nodulou e apresentou menor desenvolvimento. Dentre as áreas antropizadas o efeito foi variável, com estímulo à multiplicação e simbiose desses microrganismos, exceto nas áreas de *Panicum* e *Stylosanthes*. Ao substituir a vegetação nativa de floresta por pasto ou lavoura predominam a multiplicação dos FMA e os rizóbios nativos. Entretanto, o efeito e sua magnitude dependem da espécie vegetal cultivada, com reflexo sobre a espécie vegetal em sucessão, a exemplo do caupi.

Ref. 043-2016 – Received 26 Mar, 2016 • Accepted 11 Nov, 2016 • Published 5 Jan, 2017

Introduction

In Legal Amazon, most areas without original native vegetation were converted to pastures (Mendonça-Santos et al., 2006), altering the natural cycling of nutrients and organic matter in the superficial soil layers, previously in equilibrium (Boddey et al., 2003). These alterations also directly impact on soil microbiota, with reflex on the growth of the plants, making them more dependent on processes mediated by microorganisms (Moreira & Siqueira, 2006).

Among the various functional groups of soil microorganisms, arbuscular mycorrhizal fungi (AMF) and nodulating nitrogen-fixing bacteria (NNFB) stand out (Moreira & Siqueira, 2006). These organisms establish an intimate and evolved relationship with the hosts and contribute to the adequate maintenance of the agroecosystems. Additionally, they are very sensitive to changes in the environment and reflect the functioning of the ecosystem, directly interfering with the quality of soil physical and chemical attributes, necessary for a satisfactory plant growth (Ferreira et al., 2012), which has been evidenced by studies in anthropized areas, such as Stürmer & Siqueira (2011) for the natural occurrence of AMF, and Lima et al. (2009) and Melloni et al. (2006), for NNFB in symbiosis with 'siratro' (*Macroptilium atropurpureum*) and cowpea.

These groups of symbiotrophic microorganisms are, therefore, important components of the ecosystems and can be studied as indicators of the impact of soil management and use on the environment and on the soil-plant-microorganism relationship. This is even more important considering fragile and marginal areas such as those of sandy soils in Northern Brazil. In this context, the objective of this study was to evaluate native communities of AMF and NNFB, subjected to various land uses and vegetation covers, and their interaction with the cowpea crop.

Material and Methods

The study was carried out in an experimental area of the Federal University of Tocantins (UFT), Campus of Araguaína (7° 12' 28" S; 48° 12' 26" W). The soil of the area is a typic orthic Quartzarenic Neosol (EMBRAPA, 2013). The soil was sampled in the layer of 0-0.20 m and five composite samples (three subsamples per point) were randomly collected per site of study.

The soils of the studied area were four pasture areas (two with forage grasses: *Brachiaria brizantha* cv. 'Marandu' – Brachiaria and *Panicum maximum* cv. 'Massai' – Guinea grass; two with forage leguminous species: *Arachis pintoi* – Pinto peanut and *Stylosanthes guianensis* – Brazilian stylo), a fallow area with spontaneous herbaceous vegetation (dominated by the species: *Neonotonia wightii* – Perennial soybean, *Senna obtusifolia* – Sicklepod, *Mimosa pudica* – Sleepy plant, *Bidens pilosa* – Black jack, *Anoda cristata* – Crested anoda and *Cyperus rotundus* – Coco-grass) and one area of forest without anthropic interference, representative of the Cerrado-Amazon ecotone. The pasture areas were subdivided into paddocks of approximately 1250.0 m² (25.0 x 50.0 m), under periodic grazing, implemented in 2005, after suppression of the native forest and soil tillage with plowing and harrowing. Soil fertility was initially corrected at planting with liming and establishment fertilization according to CFSEMG (1999). The statistical design was completely randomized and the treatments consisted of the soils of each area under different vegetation covers, with five replications.

Part of the collected soil was air-dried and sieved through a 2.0-mm mesh. From this soil, one sample was used for extraction and count of AMF spores, using the wet-sieving technique, according to Colozzi Filho & Balota (1994). The rest was subjected to determination of soil chemical attributes, using the methodology described in EMBRAPA (1997), presented in Table 1.

The soil samples collected in the various studied areas were placed in 2.0 dm³ pots, to which five pre-germinated seeds of cowpea (*Vigna unguculata*) were transplanted. At the end of the germination period (approximately five days), only two cowpea plantlets were left per pot, which in turn were conducted until flowering (approximately 45 days from germination). During the growth period, the pots were irrigated according to the needs of the plants, also using the completely randomized statistical design, in which the soil of each area under different vegetation corresponded to a treatment, with five replications.

At flowering, cowpea plants were collected and separated into shoots and roots. Roots were carefully washed in tap water to remove the soil; then, the nodules were collected, counted and dried in a forced-air oven at 60 °C, for approximately 72 h, when the dry weights of shoots, roots and nodules of the cowpea plant were determined.

While the roots were fresh, 0.5 g of thin roots were collected in each replication to determine root colonization. These roots were discolored with KOH and the fungal structures were colored with aniline blue, as described by Colozzi Filho & Balota (1994).

Table 1. Soil chemical attributes in the various studied areas

Chemical attributes	*A. pintoi*	*S. guaianensis*	*B. brizantha*	*P. maximum*	Fallow	Forest
pH ($CaCl_2$)	4.0	4.2	3.2	4.9	4.5	3.5
P - Mehlich-1 (mg dm^{-3})	3.0	3.0	3.1	3.1	3.1	3.0
K (mg dm^{-3})	3.0	2.0	3.0	8.0	2.0	3.0
Ca- EDTA (cmol$_c$ dm^{-3})	1.82	1.38	1.47	1.01	1.46	0.80
Mg- EDTA (cmol$_c$ dm^{-3})	0.87	0.66	0.66	2.25	0.63	0.83
H+Al (cmol$_c$ dm^{-3})	0.9	3.1	2.8	2.9	3.0	5.9
Al (cmol$_c$ dm^{-3})	0.19	0.19	0.19	0.19	0.19	0.19
CEC$_{pH=7.0}$ (cmol$_c$ dm^{-3})	15.2	13.4	14.3	19.4	14.0	14.9
Base saturation (%)	37.5	30.1	35.9	58.4	29.2	31.1
Organic matter (%)	2.22	0.35	0.71	1.24	0.69	1.09

CEC – Cation exchange capacity

The data were subjected to analysis of variance and test of means at 0.05 probability level. According to the variability and experimental design, the t-test (LSD) was the one that best fitted to the data.

Results and Discussion

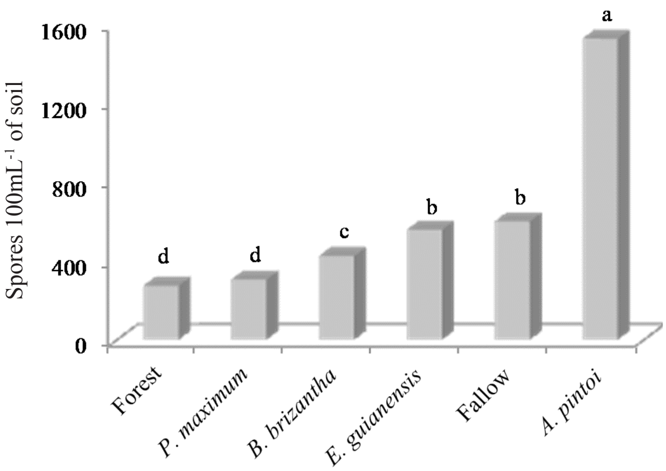
Studying native communities of soil organisms, especially those symbionts capable of influencing plant growth through nutrition, such as AMF and NNFB, has been an adequate strategy to verify the relationship between soil use and management and the anthropic interference suffered by the environment. It becomes more important in soils of low natural fertility and fragile, common in the humid tropic and confirmed in the present study by the data previously presented in Table 1, in which the plants are even more dependent on efficient biological network for the acquisition and natural cycling of nutrients (Paul, 2007).

Except for the soil under *P. maximum*, with base saturation above 50%, the other areas can be considered as of low fertility; all of them have acidic soil pH, presence of toxic aluminum and low contents of organic matter (CFSEMG, 1999). Regarding the chemical aspects, there was small difference between the areas anthropized by pasture and the area under native forest. Contrarily, Morais et al. (2015) observed expressive chemical alteration in the soil after converting areas of Caatinga into irrigated farming.

In the six studied areas, the native AMF communities were influenced by soil use and type of vegetation cover (Figure 1). AMF sporulation varied from 270 spores $100mL^{-1}$ soil, under native forest and *P. maximum*, to approximately 1500 spores $100mL^{-1}$ soil, in the area under *A. pintoi*, followed by fallow area and area under *S. guaianensis* with higher number of spores, around 600 spores $100mL^{-1}$ soil. Based on these results, the native AMF communities can be impacted by the interference in the environment. For most studied situations, the stimulus for sporulation was very dependent on the associated vegetation cover, which was also observed by Leal et al. (2013) in areas of the Amazon biome and Santos et al. (2013) in a semi-arid area in Bahia, who observed an increase in spore density in the soil when the native arboreal vegetation was substituted.

In quantitative terms, it was observed that certain soil uses and vegetation covers are situations that induce higher sporulation, while others cause slight alteration, such as the area under influence of *P. maximum*, in relation to the forest area (Figure 1).

The lower incidence of AMF spores in the area of native forest can be a consequence of the seasonality (Carneiro et al., 2009) or the reduced mycorrhizal dependence of the climax species, of low growth rate (Sturmer & Siqueira, 2011; Bonfim et al., 2013), a reflex of the stability of the system. Carneiro et al. (2009) also found lower sporulation under native vegetation of Cerrado, for both sandy soil, similar to that of the present study, and clayey soil. A similar pattern was also observed by Sturmer & Siqueira (2011) in the Amazon biome, by Lima et al. (2007) in areas of Caatinga and Zandavalli et al. (2008) in Araucaria forests in Southern Brazil. According to Lima et al. (2007), the negative anthropic interference has different effects in the study on variations in each system; while the increase in degradation level stimulated AMF multiplication in areas of native vegetation, the opposite effect occurred in crop areas.

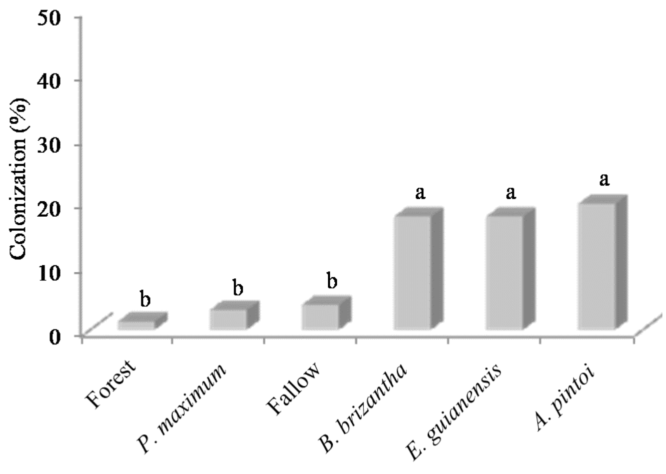


Means with the same letter do not differ by t-test at 0.05 probability level

Figure 1. Number of spores of native arbuscular mycorrhizal fungi (in 100 mL of soil), in areas under various vegetation covers

The native AMF of all studied areas could colonize the cowpea crop, but the anthropic interference and type of vegetation cover were able to influence the plant-microorganism relationship (Figure 2). Root colonization was low when the cowpea crop grew in soil under forest, *P. maximum* and fallow, whose values were below 5%. However, under the effects of the other soil covers, the AMF were more efficient in colonizing the cowpea (about 18% of colonization).

The lower spore density in the soil under forest clearly led to lower colonization of the cowpea crop, which was also observed in the area with *P. maximum* (Figures 1 and 2). These results suggest more factors controlling the colonization of the host, besides the inoculum potential of the soil in the form of spores, such as that observed for the fallow area. Similar results were reported by Gomide et al. (2009), who found that the vegetation



Means with the same letter do not differ by t-test at 0.05 probability level

Figure 2. Mycorrhizal colonization of cowpea by native fungi, after growth and soil under the effect of various vegetation covers

cover modified an artificial community of mycorrhizal fungi, strongly influencing the symbiotic relationship of these organisms with the host species in succession, a consequence of the host specificity and mycorrhizal dependence of the previous crops.

Biological nitrogen fixation is one of the most important natural biological processes, responsible for the maintenance of life, and it is speculated that only photosynthesis is more important (Moreira & Siqueira, 2006). Studying the occurrence of these organisms in the soil, especially those that nodulate leguminous crops, is relevant for the succession of crops and as a strategy to recover degraded areas, particularly in soils of low natural fertility, such as that of the present study.

The native NNFB communities responded to the anthropic interference and type of vegetation present in the area, altering the nodulation of the host plant cultivated in succession, for both number of nodules per plant and weight of these nodules (Figure 3A and 3B). The cowpea did not nodulate in the soil under forest, but there was nodulation in the soil under the spontaneous herbaceous plants and the various forage crops studied, except for *S. guaianensis*.

The highest nodulation was observed in the soil of the fallow area (Figure 3A), which was composed of various native species and dominated by two leguminous crops, perennial soybean (*N. wightii*) and sicklepod (*S. obtusifolia*). However, these were small nodules, because the highest weight of nodules was found in plants grown in soils under *B. brizantha* and *P. maximum*, i.e., two grass crops (Figure 3B).

In non-anthropized arboreal native vegetation, NNFB density tends to be very low, because these are plants with low growth rate and the cycling of N from soil organic matter is able to supply most of the demand of these plants (Lima et al., 2009; Moreira & Siqueira, 2006). Still according to these latter authors, the weight and activity of nodules are the most relevant factors for the response of the host plant in growth, compared with the number of nodules.

Areas cultivated for a long period with grass crops can still maintain a NNFB community capable of establishing symbiosis with the host leguminous plant in succession, i.e., an effect of resilience, also observed by Lima et al. (2009), studying bacteria from this group under the effect of land use, in area of Amazon forest. However, the prolonged monoculture of certain leguminous plant may induce a selection pressure on the native NNFB and negatively affect the nodulation of the other leguminous plant in succession (Moreira & Siqueira, 2006), such as the effect of *A. pintoi* and especially *S. guaianensis*, which showed wide nodulation in their roots (176.6 and 50.6 nodules per portion of sampled roots, respectively).

The modification of vegetation cover interferes with both the capacity of native bacteria to colonize the host and their efficiency for plant growth (Melloni et al., 2006; Prévost et al., 2012), because, besides changes in soil chemical conditions, there are genetic and functional alterations of the bacterial community (Pereira et al., 2007; Lima et al., 2009).

The cowpea crop can grow in the soils collected in all studied areas. This growth was determined by soil use and type of pre-existing vegetation cover (Figures 4A and 4B). The lowest dry weights of shoots and roots were observed in cowpea plants grown in soils of areas of *S. guaianensis* and native forest, while the highest growth was observed in soil of the area under *P. maximum* and *B. brizantha*. This lower growth of cowpea under the effect of *S. guaianensis* and forest coincides with the absence of nodulation of the leguminous plant in these areas.

For the areas of *P. maximum* and *B. brizantha*, nodulation seems to be the variable that best explains cowpea growth, especially the dry weight of nodules, which was higher under the influence of these two grass crops, in relation to the other treatments. In addition, the better fertility of the soil under the influence of *P. maximum* may have been combined with the effect of nodulation to stimulate cowpea growth, since this area showed the highest contents of organic matter, magnesium, potassium and cation exchange capacity (Table 1).

These results suggest an interrelationship between soil use and vegetation cover on the native communities of symbiont microorganisms of plants, with reflex on growth of the host, especially regarding NNFB.

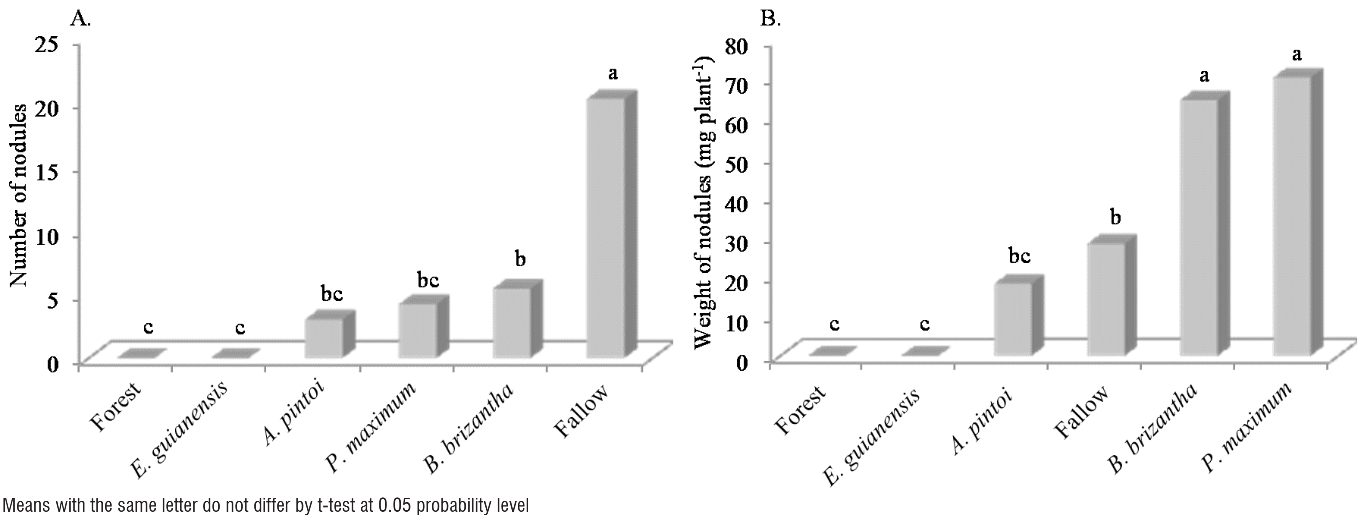


Figure 3. Number of nodules (A) and dry weight of nodules (B) per cowpea plant, after growth in soil under the effect of various vegetation covers

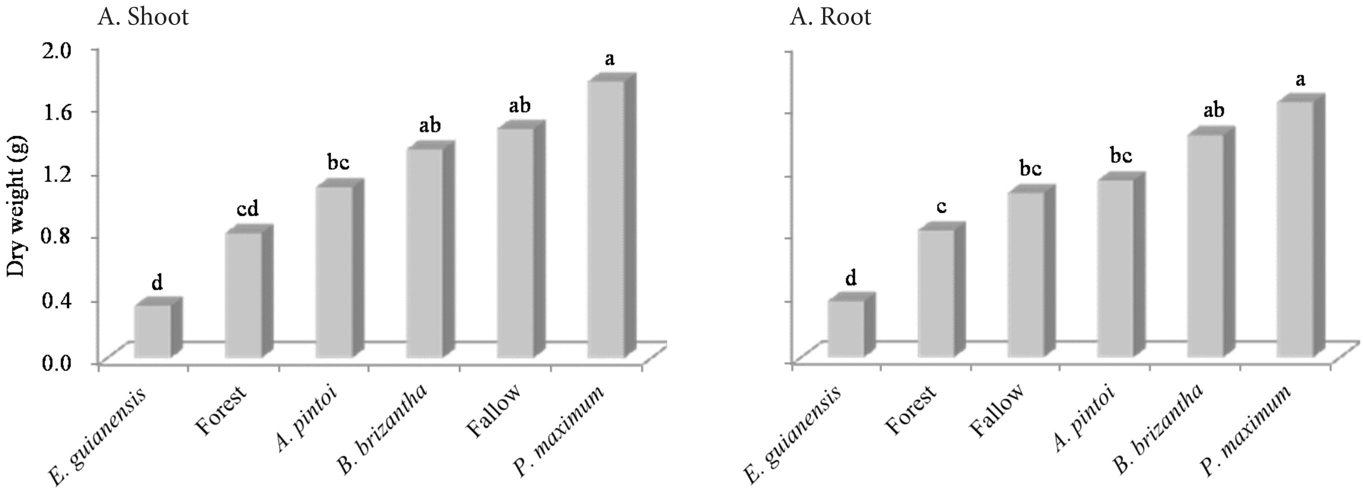


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Figure 4. Dry weight of shoots (A) and roots (B) of cowpea after growth in soil under the influence of various vegetation covers

Conclusions

1. The native communities of arbuscular mycorrhizal fungi and nodulating nitrogen-fixing bacteria are impacted by soil use and type of vegetation cover.

2. In most situations, the conversion of native forest area to pasture or cultivation, followed by fallow, stimulates the multiplication of AMF and NNFB.

3. The magnitude of the stimulating effect for the multiplication of these microorganisms depends on the type of vegetation, and there are also situations without quantitative alteration in relation to the native area, such as the soil under *P. maximum* for AMF and *S. guaianensis* for NNFB.

4. Soil use and management and the previously cultivated vegetation cover are able to regulate the growth of cowpea in succession.

5. Cowpea plants benefit in growth, especially in soil previously cultivated with grass crops. However, there may be incompatibility when another leguminous plant is previously maintained in monoculture.

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