



Population density of *Trichoderma* fungi in natural environments and agrosystems of a Cerrado area

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Abstract: Soil microorganisms present a great diversity, involving taxonomically distinct groups that play a role in the decomposition of organic matter, nutrient cycling, soil aggregation, among others. In this diversity, the fungi of the genus *Trichoderma* have been successful plant pathogen biocontrol agents, as plant growth promoters and as inducers of plant resistance to diseases. In addition, they are important in the sustainability of natural ecosystems. Aiming to verify the population density of *Trichoderma* fungi in natural environments and agroecosystems, in Cerrado area, samples of soils and roots from native vegetation and agroecological production system were collected in the Federal District, Brazil. The collection points were randomly selected, and each soil or root sample was individually wrapped. The soil adhered to the roots was removed for evaluations. Serial sample dilutions and number of Colony Forming Units (CFUs) of *Trichoderma* isolates were performed. The results showed that the number of CFU varied depending on the plant and location evaluated. The replacement of native vegetation by organic farming systems did not result in a significant reduction in this number.

Keywords: soil microflora; biocontrol agent; microbial population.

Densidade populacional de fungos do gênero *Trichoderma* em ambientes naturais e agrossistemas de uma área de Cerrado

Resumo: Os microrganismos de solo apresentam uma grande diversidade, envolvendo grupos taxonomicamente distintos que desempenham papel na decomposição da matéria orgânica, ciclagem de nutrientes, agregação dos solos, dentre outros. Nesta diversidade, os fungos do gênero *Trichoderma* tem apresentado sucesso como agentes de biocontrole de fitopatógenos, como promotores de crescimento de plantas e, ainda, como indutores de resistência de plantas a doenças. Além disso, são importantes na sustentabilidade dos ecossistemas naturais. Com o objetivo de verificar a densidade populacional de fungos do gênero *Trichoderma* em ambientes naturais e agroecossistemas, em área de Cerrado, amostras de solos e raízes oriundas de vegetação nativa e de sistema de produção agroecológica foram coletadas na região do Distrito Federal, Brasil. Os pontos de coleta foram selecionados aleatoriamente, e cada amostra de solo ou raiz foi acondicionada individualmente. O solo aderido às raízes foi removido para as avaliações. Foram realizadas diluições seriadas das amostras e contagem do número de Unidades Formadoras de Colônias (UFCs) de isolados de *Trichoderma*. Os resultados mostraram que o número de UFC variou dependendo da planta e da localidade avaliada. A substituição da vegetação nativa por sistemas de cultivo orgânicos não resultou em importante redução neste número.

Palavras-chave: microflora do solo; agente de biocontrole; população microbiana.

Introduction

Agroecology is an important instrument for the sustainability of small-scale agricultural activities or family farming, mainly due to the low dependence on external inputs from the recommended production systems (Aquino & Assis 2007). Several studies have shown the importance of this farm model in maintaining soil quality and biological activity, in contrast to conventional agriculture (Crowder et al. 2010). In this sense, agricultural practices adopted in agroecological systems are considered strategic to reduce the impact of agricultural expansion on biodiversity in the edaphic environment (Hole et al. 2005). By prioritizing the use of inputs produced on the property, this production model emphasizes the interrelation of the chemical, physical and biological components of the agroecosystem, promoting the conservation of biodiversity, which is important in soil formation (Vandermer 1995). A challenge for scaling up agroecology lies in translating agroecological principles into practical strategies for soil, water and biodiversity management to increase yield and resilience (Nicholls & Altieri 2018).

According to Altieri & Nicholls (2000), it is the interactions between the various biotic components of the agroecosystem that will contribute to biological pest control, nutrient recycling, water conservation, soil conservation and / or regeneration, and increased agricultural productivity in a sustainable way. In this regard, microorganisms have played a major role in the sustainability of agrosystems. Some of the beneficial microorganisms often used in agriculture worldwide include the genera *Bacillus*, *Azospirillum*, *Trichoderma*, *Rhizobium*, *Mycorrhizae*, *Pseudomonas*, *Streptomyces* and many other groups (Gupta 2012). As an example, *Trichoderma* strains have been successfully used as biological control agents of various plant pathogens, being one of the most studied and known microorganisms in the world (Verma et al. 2007). But initially, this biopesticide activity was considered as the only benefit to be considered. Subsequently, it was demonstrated that species of this genus could also be used as biofertilizers, biostimulants, among others (Lorito et al. 2010, Woo & Peppe 2018), being used as inoculant in several agricultural crops.

Therefore, any change that may cause a loss in environmental diversity, influenced by agricultural use or the absence / presence of rainfall, for example, may modify biological diversity in the edaphic environment (Lançon et al. 2013). Several methods have been used as indicators of changes in the soil microbial community. The isolation, cultivation and evaluation of microbial density in samples collected in this environment is the most widely used, due to its ease of execution (Antoniolli et al. 2010), although techniques based on the use of molecular markers may be more conclusive about the different groups of microorganisms, organisms and their ecology. These include the latest, based on extraction of microbial DNA directly from the soil (McPherson et al. 2018). However, there is little information on surveys and evaluation of the effects of environmental factors on the composition of beneficial fungal populations and plant pathogen antagonists in Brazilian soils.

Trichoderma fungi are often found in soil and organic matter in free-living form, adapt to different ecological conditions and colonize a multitude of substrates, as well as capable of more intimate associations with plant root systems. (Harman et al. 2004). As a constituent of the rhizospheric microbiota, *Trichoderma* acts on the translocation of minerals, solubilization and availability of nutrients to plants and the production of plant hormones. increase in productivity is related to the

ability to colonize roots, while its action as a biocontroller has been attributed to the mechanisms of antibiosis, hyperparasitism, induction of resistance, favoring the plant in tolerance to biotic and abiotic stresses, solubilization and nutrient sequestration. in addition to inactivation of pathogen-linked pathogen enzymes. However, the functional variability between isolates of the same species in relation to their biocontrol and plant growth promotion activities is a well-proven fact (Martínez et al. 2013, Munir et al. 2014). Given the agricultural importance of *Trichoderma*, this work was conducted to verify the population density of this fungus in natural ecosystems and agroecosystems (organic production) of the Cerrado biome.

Material and Methods

1. Collection of soil samples

Soil samples from native vegetation and agroecological production system were collected from healthy vegetables in the Cerrado area, in four localities of the Federal District, always in the morning. In each area five subsamples of non-rhizospheric soils (NRhZS) were collected, containing 200g each, at random points, with a distance of 5 cm from the cultivated species (in ridges between ridges) and 0-10 cm deep, making up a sample composed of 1 kg. Similar procedure was adopted in natural vegetation areas, except for the absence of furrows. Roots and root fragments of the plant species were also collected for each rhizospheric soil (RhZS) collection point. Soil and root samples were individually wrapped and, from these, the attached soil was removed (Ethur et al. 2008). The collection sites were: Rajadinha Rural Nucleus II, Planaltina region, in the cultivation of pumpkin (*Cucurbita* sp.), eggplant (*Solanum melongena* L.), kale (*Brassica oleracea* L. var. *acephala* DC.), cassava (*Manihot esculenta* Crantz), Mexican Sunflower (*Tithonia* sp.), maize (*Zea mays* L.), bell pepper (*Capsicum annum* L.), okra (*Abelmoschus esculentus* L. Moench), cabbage (*Brassica oleracea* var. *capitata* L) and tomato (*Lycopersicon esculentum* Mill.) and native plants *Cestrum* sp. (Solanaceae), *Cyathea* sp. (Cyatheaaceae), *Miconia elegans* (Melastomataceae), *Tibouchina* sp. (Melastomataceae); Taguatinga Rural Nucleus, Taguatinga region, for eggplant, coffee (*Coffea arabica* L.), persimmon (*Diospyrus kaki* L.), Mexican Sunflower, maize, mucuna (*Mucuna pruriens* L. DC), tomato, pod (*Phaseolus vulgaris* L) and native crops *Cyathea* sp., *Cordia* sp. (Rubiaceae) and *Trichilia pallida* (Meliaceae); Lamarão Rural Nucleus, Paranoá region, for eggplant, chives (*Allium schoenoprasum* L.), cabbage, spinach (*Spinacia oleracea* L.), cassava, Mexican Sunflower, maize, parsley (*Petroselinum crispum* Mill. Nym.) and native crops *Cordia macrophylla*, *Miconia elegans*, *Zanthoxylum rhoifolium* (Rutaceae); Boa Esperança Rural Center, Ceilândia region, in the cultivation of eggplant, coffee, kale, cassava, Mexican Sunflower, maize, mucuna, pepper (*Capsicum* sp.), cabbage and tomato. In this place there was no native ecosystem area.

2. Fungus isolation

For fungal isolation, 10 g of each soil sample was placed in Erlenmeyer, suspended in 90 mL of sterile water and stirred at 180 rpm at 25 ° C for 40 minutes. After the suspensions, the samples were diluted and 100 µL of each concentration were distributed in Petri dishes containing semi-selective Martin medium as described by Mello et al. (2007). For each sample four repetitions were performed.

3. Morphological identification of fungi

The plates were incubated at 25 ° C in B.O.D. (Biochemical Oxygen Demand) for two days in the dark and for 5-7 days with 12-hour photoperiod exposure. The cultures were evaluated daily until the appearance of a typical *Trichoderma* colony and considered as a colony forming unit (CFU). Slides were made for examination of morphological characteristics under the optical microscope and identification of the fungus at a generic level. The colonies confirmed as belonging to the genus were transferred to purified potato dextrose agar (PDA) medium, used for monospore cultivation and stored at 4°C.

4. Statistical analysis

First, it was verified whether the plant types (explanatory variable), native and cultivated, differed statistically as to the number of isolates of *Trichoderma* (response variable). This analysis was developed under the focus of Generalized Linear Models (GLMs), assigning Poisson distribution to the response variable, a natural choice for variables of this type (discrete counting). When necessary, the heterogeneity factor present in the data (overdispersion) was corrected via the Quasiverossimilitude method.

Following this same logic, another model was adjusted to compare the locations within each culture. To obtain the variance analysis table in Poisson distribution GLMs, were used likelihood ratio (LR), which follows approximately Chi-Square distribution, this procedure is known as ANODEV. The analyzes were developed with the R free statistical language program. The adopted significance level was 5% (McCullagh & Nelder 1989).

Results

From the soil samples from the four properties, 530 isolates of *Trichoderma* were obtained, 361 from agroecosystems and 169 from natural ecosystems.

1. Analysis of results by location

1.1. Rajadinha Rural Center II - Property I

Vegetation type interfered with the number of *Trichoderma* isolates obtained (LR = 12.4790, df = 1, p-value = 0.0004116), regardless of soil type (NRhZS or RhZS). Native species presented, on average, a larger number of colonies than cultivated ones (Figure 1A).

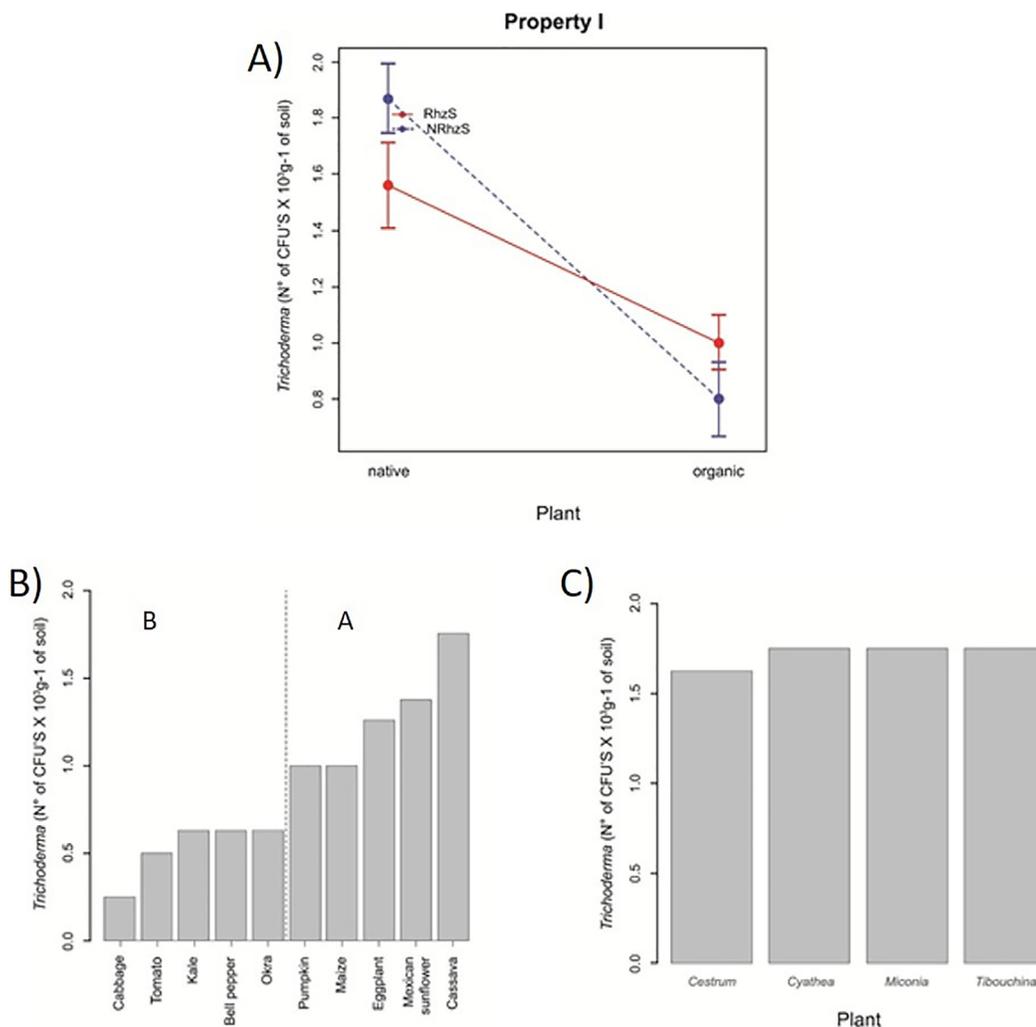


Figure 1. A) Average number of *Trichoderma* colony forming units (CFU) + standard deviation according to soil and plant type for property I. B) Average number of *Trichoderma* CFU obtained from rhizosphere of cultivated species. C) Average number of *Trichoderma* CFU obtained from rhizosphere of native species.

Regarding the cultivated species, significant differences were observed (LR = 17,119, df = 9, p-value = 0.04688), detecting two groups: cassava, Mexican Sunflower, eggplant, maize and pumpkin, with the highest number of CFU of *Trichoderma*; cabbage, tomato, kale, bell pepper and okra with the lowest number of CFU (Figure 1B). On the other hand, native species presented, on average, the same number of CFU (Figure 1C), not differing from each other.

1.2. Taguatinga Rural Center - Property II

Both soil sample type (NRhZS and RhzS) and vegetation type significantly influenced the average number of *Trichoderma* CFU (LR soil = 15.96, df = 1, p-value <0.0001; LR plant = 5.1549, df = 1, p-value = 0.02318). There was, on average, more CFU of this fungus in NRhZS than in RhzS, both for native and cultivated species (contrast = 0.39, standard error = 0.18, p-value = 0.0267), and the latter species had lower numbers. average of recovered isolates, in terms of CFU in relation to native vegetation (contrast = 0.74, standard error = 0.18, p-value <0.0001) - (Figure 2A).

Among the cultivated species there was significant difference (LR = 28.409, df = 9, p-value <0.0001). Two groups can be established: the first, presenting the highest average number of *Trichoderma* CFU composed of mucuna, pod and persimmon, coffee and Mexican Sunflower and the second group, with less CFU, containing kale, maize, eggplant, tomato and cassava (Figure 2B). Among native species, there were no significant differences in obtaining *Trichoderma* colonies (Figure 2C).

1.3. Lamarão Rural Center - Property III

The soil sample type (NRhZS and RhzS) had no effect on the mean number of CFU, which, however, suffered vegetation type interference (RV = 10.062, df = 1, p-value = 0.001514). Native species presented, on average, more CFU of *Trichoderma* than species of organic cultivation (Figure 3A).

There was a significant difference for at least two cultivated species regarding the average number of *Trichoderma* CFU (LR = 23,291, gl = 9, p-value = 0.005574). Mexican Sunflower and eggplant

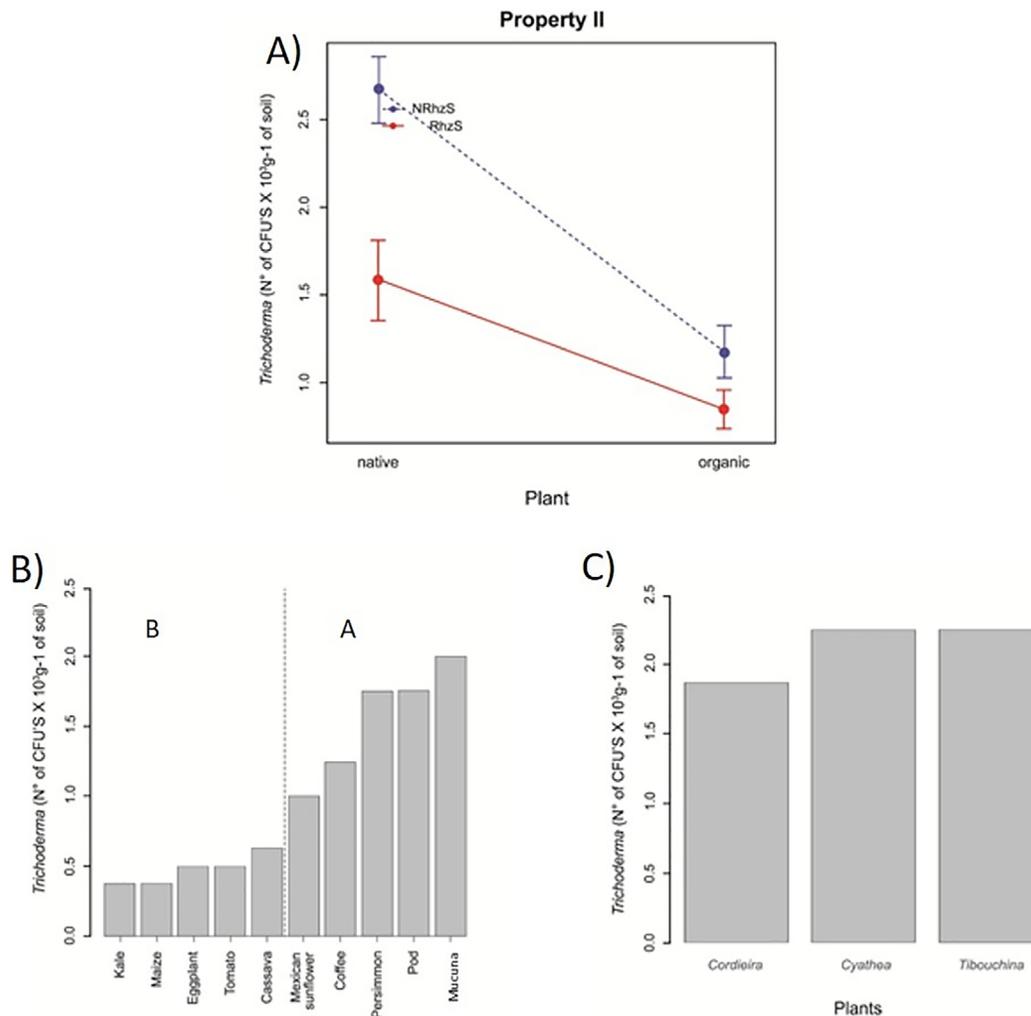


Figure 2. A) Average number of *Trichoderma* CFU + standard deviation according to soil and plant type for property II. B) Average number of *Trichoderma* CFU among cultivated species. C) Average number of *Trichoderma* CFU in native species.

Population density of *Trichoderma*

presented more CFU than spinach, pumpkin, parsley and tomato (Figure 3B). Regarding native species, there were also differences in the number of CFU (LR = 10,312, df = 2, p-value = 0.005765): *C. macrophylla* had, on average, more CFU than *Z. rhoifolium* and *M. elegans* - (Figure 3C).

1.4. Boa Esperança Rural Center - Property IV

For this property, only isolates from cultivated species were analyzed. As for the type of soil sample, there was no statistical difference regarding the number of CFU (LR = 0.13437, df = 1, p-value = 0.7139) - (Figure 4A).

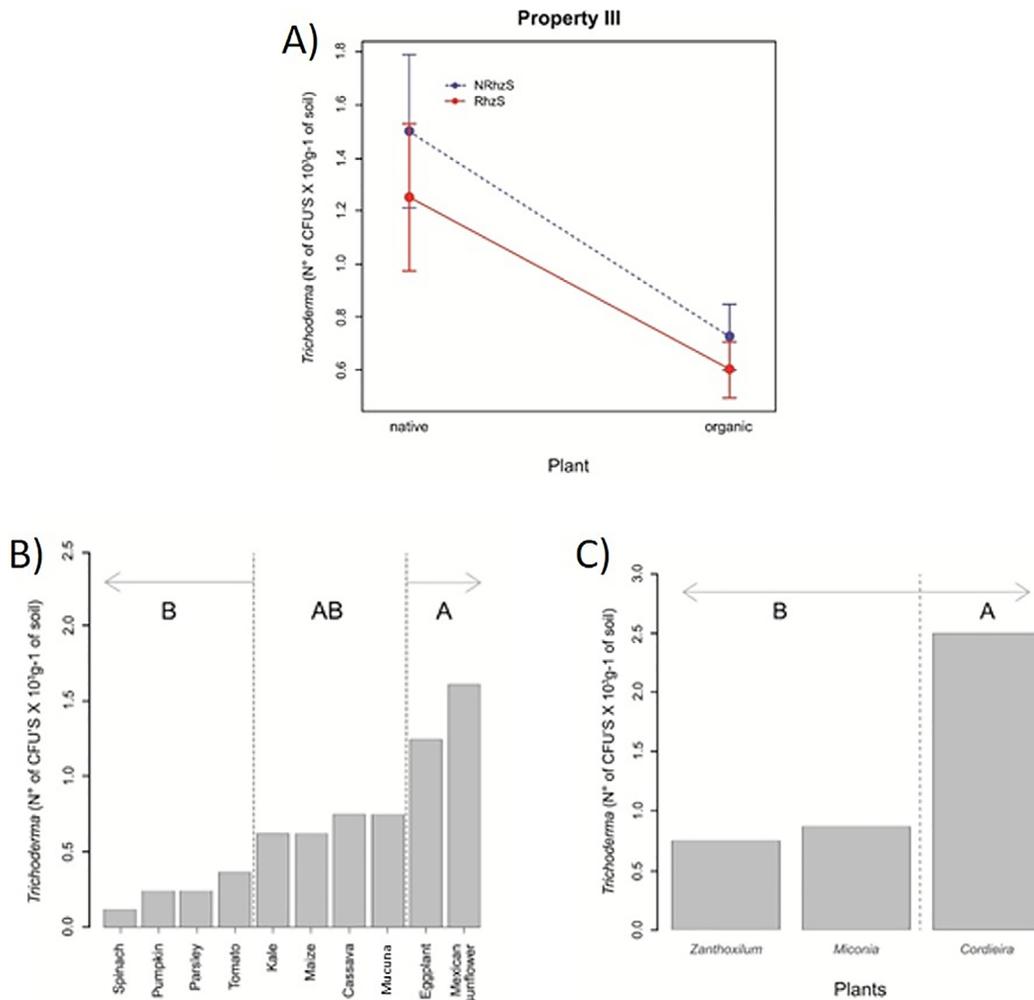


Figure 3. A) Average number of *Trichoderma* CFU + standard deviation according to soil and plant type for property III. B) Average number of *Trichoderma* CFU among cultivated species. C) Average number of *Trichoderma* CFU in native species

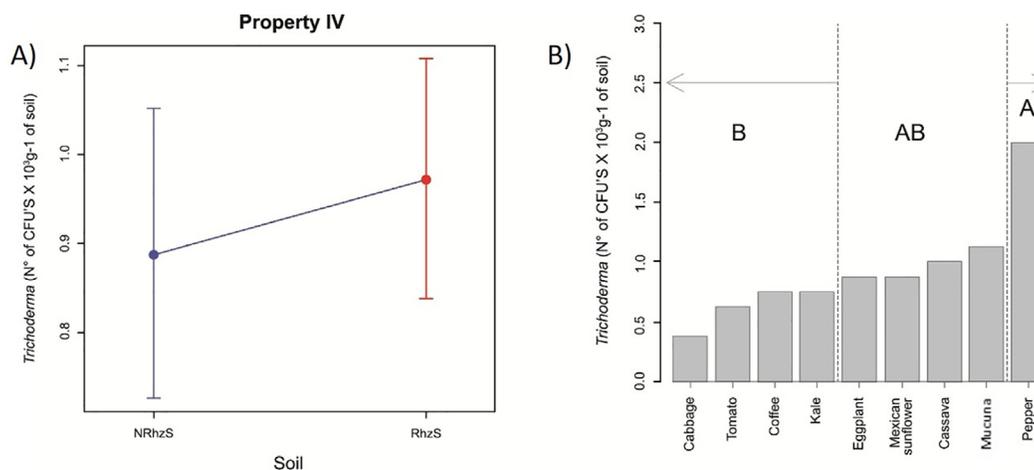


Figure 4. A) *Trichoderma* CFU mean number + standard deviation according to soil type for property IV. B) Average number of *Trichoderma* CFU among cultivated species.

In this case the average number of CFU in samples from ground cultivated with pepper was higher than in the cases of cabbage, tomato, coffee and kale (Figure 4B).

The locations for each crop were compared for the number of *Trichoderma* CFU obtained from eggplant, cassava, kale, Mexican Sunflower and tomato, species found concurrently in the four studied properties, since maize and mucuna were present in three of the four properties. With eggplant, the lowest average number of *Trichoderma* CFU was found for property II (LR = 10,059, df = 3, p-value = 0.01807). In the case of cassava, this number was higher in property I (LR =

9.5157, df = 3, p-value = 0.02317) and, with kale (LR = 1.2136, df = 3, p-value = 0.7498), Mexican Sunflower (LR = 3.1093, df = 3, p-value = 0.3751) and tomato (LR = 0.73695, df = 3, p-value = 0.8645) there was no significant difference in relation to the sample collection sites (Figure 5).

For some species (maize and mucuna), the fungus *Trichoderma* was recovered only at three sites (Figure 6). In this case, there was no significant difference between sites with corn crop (LR = 4.1822, df = 2, p-value = 0.1235) and, with mucuna, a higher number of CFUs were recovered in property II (LR = 12.552, df = 2, p-value = 0.001881).

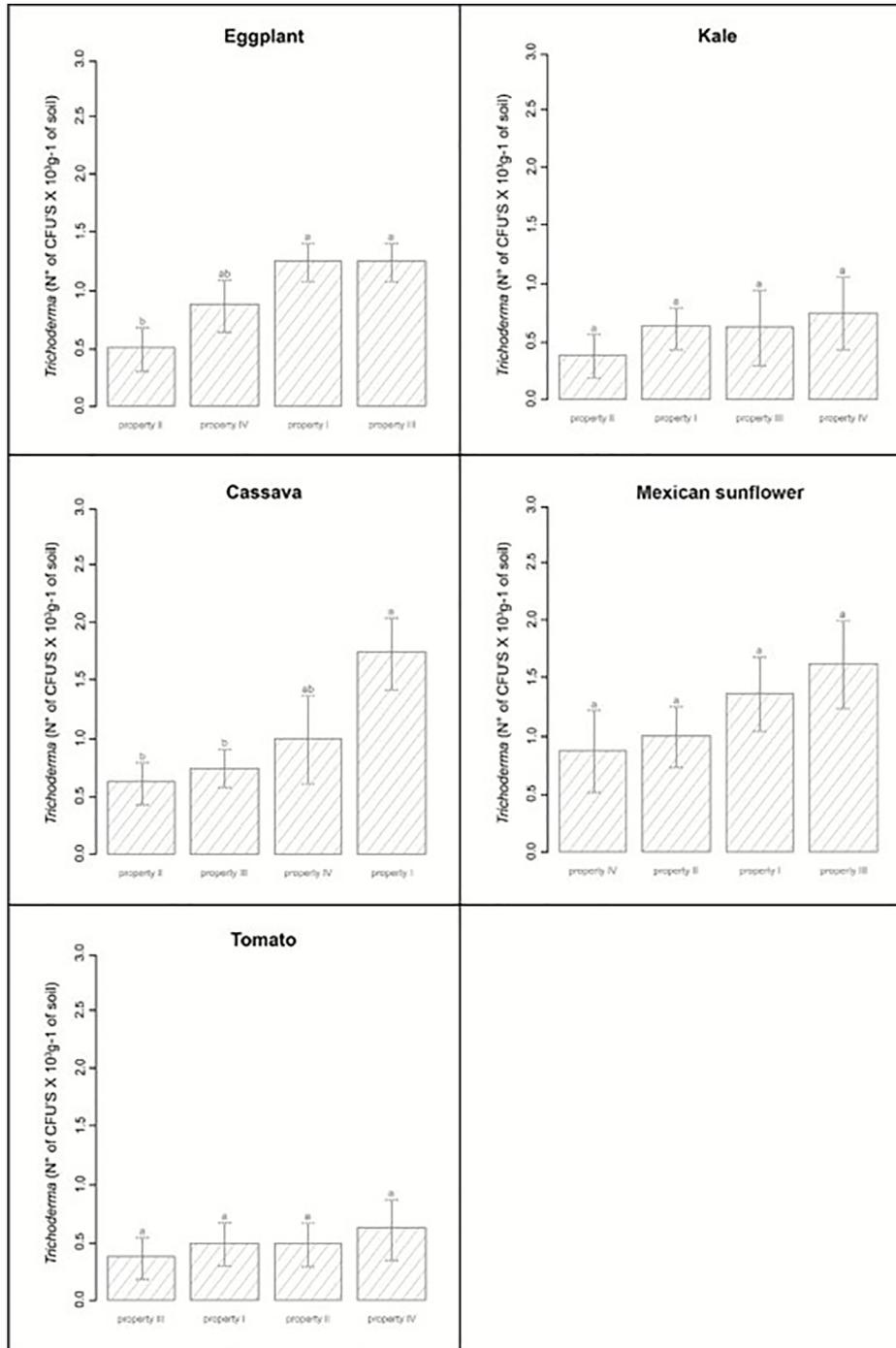


Figure 5. Average number of *Trichoderma* CFU + standard deviation observed at the four sites.

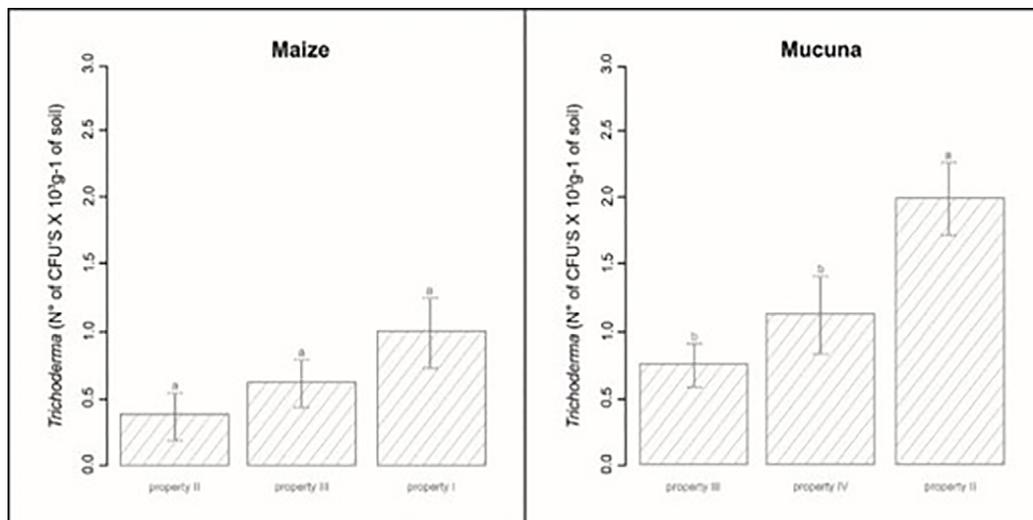
Population density of *Trichoderma*

Figure 6. Average number of *Trichoderma* CFU + standard deviation observed at the three sites.

Discussion

Only in property II the number of *Trichoderma* CFUs was higher in NRhZS than in RhZS (Figure 2A), probably due to the incorporation of crop residues or other organic matter in the soil, since in the other properties there were no significant differences in this number (Figures 1A, 3A and 4A). Moreover, according to Ethur et al. (2008), *Trichoderma* species settle better in soils when they contain vegetable remains and other forms of organic matter. Although in these soils there is greater interaction between microorganisms and plants (Mohammadi et al., 2011), it is also in them that the microbial flora suffers the most competition pressure (Dantas et al. 2009). Kredics et al. (2018) complement that various biotic and abiotic factors affect diversity populations of microbial communities in agroecosystems, including plant species and their growth stage, total microbial competition, pesticide or fertilizer application, as well as geographic region. However, only a few studies address the population, abundance and diversity of the genus *Trichoderma* in specific fields or agroecosystems. From the results obtained in this work, there is no need to remove parts of plant roots to obtain a representative number of *Trichoderma* isolates, since the number of *Trichoderma* isolates recovered from root-attached soil samples was similar to non-rhizospheric soil samples. In areas cultivated with tomato and cucumber in conventional system, Ethur et al. (2008) obtained similar results. It is worth mentioning that, in studies of antagonistic potential, Jash & Pan (2007) found no differences in antagonism against *Rhizoctonia solani* when testing *Trichoderma* isolates from RhZS and NRhZS.

The areas of native vegetation, located around the crop, suffer a reduced anthropic effect, so the soil supposedly represents the ecological conditions of environmental stability because it is not influenced by disturbances of preparation and application of inputs, unlike cultivated areas, even treating it itself from organic production. Probably, this fact explains the higher number of CFUs found in native vegetation areas in the three evaluated sites (Figures 1C, 2C and 3C), compared to those of cultivated area (Figures 1B, 2B and 3B). Brouwer & Riezebos (1998) has mentioned that in forest soils, nutrient losses from the ecosystem are lower.

This provides better soil cover, higher organic matter content and greater floristic diversity, determining factors for larger soil settlement in number and microbial diversity (Ramos et al. 2012). According to Lourente et al. (2011), the diversity of native vegetation species (quantity and quality) implies the continuous deposition of organic substrates with varied composition, favoring the microbial mass content, but the substitution of native vegetation by cultivation systems may cause important changes in the attributes. soil chemicals in the first year of implementation. There are several reports that disturbances caused by land use and crops may also result in decreased microbial biodiversity (Bending et al. 2004, Mendes et al. 2012). Louzada et al. (2009), who studied the antagonistic action of *Trichoderma* isolates from various regions of Brazil against plant pathogens *Sclerotinia sclerotiorum* and *Fusarium solani*, mention studies by other authors showing that isolates from native areas would result in a higher percentage of potentially active *Trichoderma* isolates against plant pathogens in *in vitro* tests, with a mycelial growth inhibition of around 80%. However, in this work, the substitution of native vegetation by organic cultivation systems did not cause a significant reduction in the number of *Trichoderma* CFUs, confirming postulations made by different authors about the advantages of the organic cultivation system regarding the preservation of soil microflora and stabilization of agroecosystems.

Therefore, the results presented show the effect on the number of CFU of *Trichoderma*, depending on the culture and location evaluated (Figures 5 and 6). These properties, although located in Cerrado areas, have soils probably subjected to different treatments, which would have interfered with the number of recovered *Trichoderma* colonies. According to Frazão et al. (2010), soil microbial community is generally influenced by variations in soil temperature, water content and aeration, aggregate disruption, decreased soil cover, nutrient availability and organic substrates. Studies by Saravanakumar et al. (2016) from samples of coastal regions showed that the biodiversity of *Trichoderma* spp. was influenced by temperature, redox potential and pH. In the set of results, it was found a variation between *Trichoderma* population levels in the various organic crops evaluated, due to the different factors mentioned, although no correlation was studied or made regarding soil types and characteristics, only with the cropping systems.

Regarding diversity, molecular characterization work of *Trichoderma* isolates from target properties should be performed to verify the prevalent species in the soil of organic crops and native vegetation, because the methodology used for sample dilution and calculation of forming units of colonies (CFUs) do not allow the distinction of an introduced strain of *Trichoderma* populations residing in the investigated environment (Kredics et al. 2018). According to Louzada et al. (2009), there are no data in the literature reporting the loss of diversity of *Trichoderma* spp. with the continuous agricultural use of soils or even the possible relationship of such interferences with the reduction of the frequency of antagonism to pathogens. *Trichoderma* spp. are highly successful settlers in their habitats and are able to overcome adversity related to environmental variations around the world (Schuster & Schmoll 2010). Studies of this nature coupled with the knowledge of the real distribution and population dynamics of this fungal genus and its associations with different plant species and soils are crucial to ensure the efficiency and safety of the use of these microorganisms, especially in the biocontrol and promotion of plant growth.

Given the above and based on the results observed in this work, it is concluded that the types of crop and native vegetation influenced the distribution of the population of *Trichoderma* fungi in soils of organic farming system. On the other hand, the substitution of native vegetation by organic cultivation systems did not result in a significant reduction in the number of *Trichoderma* CFUs.

Author Contributions

João Batista Tavares da Silva: substantial contribution in the concept and design of the study; contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation, contribution to critical revision and adding intellectual content.

José Eustáquio Menezes: contribution to data collection, contribution to data analysis and interpretation and contribution to manuscript preparation.

Eder Marques: contribution to data analysis and interpretation and contribution to manuscript preparation.

Joseane Padilha da Silva: contribution to data analysis and interpretation.

Sueli Corrêa Marques de Mello: substantial contribution in the concept and design of the study; contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation, contribution to critical revision and adding intellectual content.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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