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Integrated production systems revealing antagonistic fungi biodiversity in the tropical region

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Abstract. The antagonism and diversity of fungi have been studied in several environments, including agricultural soils. Nevertheless, information regarding fungi that are able to control *Fusarium* sp., *Rhizoctonia* sp. and *Sclerotium rolfsii* in integrated Crop-Livestock-Forest systems soils is unknown. Ten treatments were assessed, including monoculture, integration of Crop-Livestok-Forest, fallow and native forest. During the rainy and dry season was carried out fungi colony forming units (CFU), antagonistic potential and molecular identification. The results showed that CFU were higher in the rainy season and integrated systems of production. Fungal isolates as *Penicillium*, *Talaromyces*, *Eupenicillium*, *Trichoderma*, *Aspergillus*, *Chaetomium*, *Acremonium*, *Curvularia*, *Purpureocillium*, *Bionectria*, *Paecilomyces*, *Plectospharella*, *Clonostachy*, *Mucor*, *Fennellia* and *Metarhizium* were able to control *Rhizoctonia* sp., *Fusarium* sp. and *Sclerotium rolfsii.* This is the first report to describe culturable fungi species from the Amazon biome that are able to control pathogens. Furthermore, we suggest that integrated production systems can be a strategy for increasing fungal biomass and the rainy and dry season can modulate the density of soil fungi also, mainly in a tropical region.
Keywords: Microbial ecology, crop-livestock-forestry, Biocontrol, 18S rDNA, Plant soil

Introduction

Brazilian farms located in the transition areas between the Amazon and Cerrado biomes are characterised by the use of monocultures in areas that were previously occupied by native forests (FAO, 2012; Zilli et al., 2013). These transition areas make up a complex system, which is not yet well understood, which involves the cycling of nutrients between soil-plant-animal. Deforestation and lack of diversity of cultures may result in an unsustainable system, characterised by progressive soil degradation due to the emergence of pests and diseases in agricultural areas (Alves et al., 2011; Santos et al., 2012; Tonin et al., 2013), high production costs and resistance of pathogens to fungicides (Xiao et al., 2013).

Integrated systems of production may be a sustainable alternative to the recovery of degraded areas through the intensification of land use (Trecenti and Hassa, 2008). These production systems can be used in different productive configurations in the field, and the one that is the currently most adopted is integrated Crop-Livestock-Forest systems (iCLFs). iCLFs is a type of agrosilvopastoral system which can produce grains, fibres, wood, meat, milk and bioenergy in the same area, in consortium, in rotation or in succession, planting grain, pasture and crops associated with trees (Balbino et al., 2011). These systems optimise the use of soil and grain production in pastures, improving pasture productivity due to residual nutrient cycling and increasing soil organic matter (Trecenti and Hassa, 2008). Besides, agrosilvopastoral systems can be efficient for improving chemical and physical soil properties, the use of phosphorus and the dynamics of fungi (Ayarza et al., 1993; Sousa et al., 1997; Miranda et al., 2005; Balbino et al., 2011).

Rhizoctonia sp., *Fusarium* sp. and *Sclerotium rolfsii* infestations have resulted in large agricultural losses in monocultures, especially corn (Stumpf et al., 2013), soybean (Souza et al., 2013) and pastures (Cortinovis et al., 2013), but is still unknown on literature if iCLs may advantage the incidence of these pathogens. These diseases can be controlled by fungicides, but promote damage to human health and the environment, reducing the biodiversity of non-target organisms (Khot et al., 2012). Still, the incorrect use of these active

ingredients can result in the development of resistant pathogens (Souza et al., 2013).

In the other hand, biological control has been extensively studied in tropical agricultural crops in South America as a strategy for reducing phytopathogens (Souza et al., 2013; FAO, 2012; Silva et al., 2013), enabling sustainable and environmentally friendly agriculture. *Coniothyrium minitans*, *Ulocladium atrum*, *Trichoderma harzianum*, and *Trichoderma asperellum* have been reported as biological controllers of *Rhizoctonia* spp., *Fusarium* sp. and *Sclerotium rolfsii* by antagonistic actions (Jones et al., 2011; Ferraz et al., 2011; Fernando et al., 2007; Carvalho et al., 2011; Zeng et al., 2012), related enzymatic degradability of the cell wall (chitinase), inhibition of mycelial growth and sporulation (Santos et al., 2012), antibiosis, competition, parasitism and hypovirulence (Geraldine et al., 2013). These microorganisms can act involving several of these mechanisms, expressed simultaneously or synergistically (Alabouvette at al., 2009). In fact, studies of soil fungi that are able to control microorganisms are frequently carried out (FAO, 2012; Silva et al., 2013). However, the antagonist fungal diversity in iCLFs and how these systems could modulate soil fungi populations is still unknown (Lacombe et al., 2009; Vallejo et al., 2012).

In that way, based on classical and molecular analyses such as isolation and antagonism analysis, followed by sequencing, this study aimed to: i) quantify fungi collected from soils used in different configurations of iCLFs and native forest from Amazon biome in Brazil; ii) assess the antagonistic potential of this fungi against *Fusarium* sp., *Rhizoctonia* sp. and *Sclerotium rolfsii*; and iii) use molecular tools to identify the fungi antagonists.

Methods

Soil characteristics, experimental design and pathogens used

The experiment was composed of 10 configurations of integrated Crop-Livestock-Forest systems (iCLFs) in Sinop, Mato Grosso, Brazil. The rainy and dry seasons were assessed during 2012. Details of different iCLFs configurations are described in Table 1.

Table 1. Description of treatments and reference areas assessed in this work.

Treatment	Description treatments in the year of sample collect
F - Forest	Eucalyptus cultivated in monoculture
C - Crop	Soybean crop cultivated in the rainy season followed by corn crop
G – Grass	*Brachiaria brizantha* cv. Marandu cultivated in monoculture
iGC – integration Grass/Crop	*Brachiaria brizantha* cv. Marandu cultivated in monoculture
iCG - integration Crop/ Grass	Soybean crop cultivated in the rainy season followed by corn crop.
iCF - Crop/Forest integration	Eucalyptus cultivated in triple ranks spaced 25 metres apart and soybean crop cultivated in that 25 metre space, followed by corn crop
iGF - integration Grass/Forest	Eucalyptus cultivated in triple ranks spaced 25 metres apart and *Brachiaria brizantha* cv. Marandu cultivated in that 25 metre space
iCFG - integration Crop/Forest followed by Grass/Forest	Eucalyptus cultivated in triple ranks spaced 25 metres apart and soybean crop cultivated in that 25 metre space, followed by corn crop. For 2 years
iGFC - integration Grass/Forest followed by Crop/Forest	Eucalyptus cultivated in triple ranks spaced 25 metres apart and *Brachiaria brizantha* cv. Marandu cultivated in that 25 metre space. For 2 years
iCLFs - integration Crop/Forest followed by Grass/Forest	Eucalyptus cultivated in triple ranks spaced 25 metres apart and soybean crop cultivated in that 25 metre space, followed by corn crop. Regular integrated Crop-Livestok-Forest system of production
W - Native Forest	Native forest area with vegetation characteristic from Amazon/Savana ecotone. Adjacent to the experimental area
R - Fallow	Reference area with no agricultural activities for 3 years

The experimental area belongs to Embrapa Agrosilvopastoral (Brazilian Agricultural Research Corporation), and is located in the transition region between the Cerrado and Amazon biomes. The climate is Am type with monsoon characteristics and average monthly rainfall less than 60 mm (Alvares et al., 2013).

The experiment was grown in four randomised blocks, each one consisting of ten plots (one plot per treatment); each plot was 2 hectares in size. The total area of the experiment was approximately 78 hectares. No-tillage and technical recommendations for each crop according to the species were prioritised.

The phytopathogens used in this work were provided by the Phytopathology Laboratory (Mato Grosso Federal University, Brazil). *Fusarium* sp. (obtained from corn crop), and *Sclerotium rolfsii* (obtained from soybean) were identified using molecular tools, as described below. *Rhizoctonia* sp. (obtained from soybean) were identified using morphological and *in vivo* assays. These isolates were grown and maintained in a culture of PDA medium.

Soil sampling and processing

During periods of the rainy (March) and dry season (September), soil was collected at a depth of 0-10 cm, using a Dutch auger. Twenty single samples were collected per plot to form a composite sample. All samples were packaged, identified and placed at 4°C and then transported to the laboratory of Microbiology and Molecular Biology Embrapa Agrosilvopastoral. In sequence, soils were homogenised, sieved at 4 mm mesh and stored at 4ºC until analysis.

Fungi isolation

Total soil fungi were isolated from samples diluted in serial steps when ten grams of each soil sample was diluted in 90 mL of sterile phosphate buffer solution (PBS, with [g L^{-1}] 1.44 Na_2HPO_4; 0.24 KH_2PO_4; 0.20 KCl; 8.00 NaCl; pH 7.4) and incubated under agitation (150 rpm) for one hour. Dilutions of 10^{-2}, 10^{-3} and 10^{-4} were applied in culture medium PDA (Potato-Dextrose-Agar), supplemented with 50 $\mu g\ mL^{-1}$ of tetracycline. Incubation was performed at 28°C and monitored for 15 days. To guarantee the sterilisation process, PBS was tested in culture medium using the protocol described above. The number of CFU (Colony Forming Units) was measured in CFU/g of soil. After purification and quantification, fungal isolates were stored in microtubes with autoclaved water at 4°C.

Antagonist isolates assessing

Antagonism assays were carried out with twenty fungal isolates obtained from each treatment, including the area of Native Forest (W) and Fallow (R), against *Fusarium* sp., *Rhizoctonia* sp. and *Sclerotium rolfsii*. The evaluations followed the method of paired culture, plating the two microorganisms directly onto solid medium (Mariano, 1993). Fungal isolates were inoculated on PDA culture medium and incubated at 28°C for 2 days. Subsequently, the phytopathogenic isolates were inoculated on the opposite side of the Petri dish followed by incubation for 10 days. The control plates were prepared with pathogens only. The presence of inhibition zones between paired cultures and radial growth of the pathogen compared to the control indicated positive isolates. All antagonism tests were performed in duplicate.

DNA extraction from antagonistic isolates

DNA was extracted from fungal isolates using the Wizard® Genomic DNA purification Kit (Promega, EUA), according to technical recommendations and with an extra step using phenol and chloroform (1:1). The isolates were incubated in 100 ml of PD (200 g of potato broth, 20 g of dextrose in 1 L of water, [pH 6.0]), for 10 days at 28ºC. The mycelium was filtered and triturated using liquid nitrogen. The DNA extraction process used 200 mg samples of mycelium. The integrity and quantification of DNA were checked using agarose gel electrophoresis (0.8% v/v), followed by staining with Gel Red® (Biotium) and visualisation under ultraviolet light.

Molecular identification of antagonistic isolates

Primers ITS-1 (5`-TCCGTAGGTGAACCTGCGG-3`) and ITS-4 (5`-TCCTCCGCTTATTGATATGC-3`), were used to amplify the region ITS1-5.8S-ITS2 from rDNA (White et al., 1990). The amplified region was approximately 700 base pairs (bp) in length. Reactions were performed in a final volume of 25 µL containing 5 ng of DNA, 1x buffer (50 mM of KCl, 20 mM of Tris-HCl (pH 8.4)), 3.75 mM of $MgCl_2$, 0.2 mM of each triphosphate deoxyribonucleotide, 0.2 µM of each primer and 0.05 $U.\mu L^{-1}$ of Taq DNA polymerase (Sigma). A negative control (PCR reaction without DNA) was included in all amplification reactions. Amplifications were performed in a thermocycler (Bio Rad - T100) programmed for initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 30 seconds, 55ºC for 30 seconds and 72°C for 30 seconds. A final extension at 72°C for 10 minutes was performed to complete the reaction. Amplification was checked using agarose gel electrophoresis (1.5% v/v), followed by staining with Gel Red® (Biotium) and observation of the 700 bp fragment under ultraviolet light.

The products amplified by PCR were purified with isopropanol as follows: precipitation with 100% isopropanol alcohol; centrifugation for 15 minutes at 9000Xg; two washes with 70% ethanol and resuspension of DNA in 10 µL of ultrapure water. DNA was measured using a Nanodrop (Thermo Scientific) and sequenced at the Instituto do Genoma Humano (USP, São Paulo, Brazil). The sequences were evaluated in BLASTn (National Center for Biotechnology Information website) against the GenBank database and were deposited in GenBank in the process. After comparison, alignment and phylogenetic analysis were performed using version 4.0 of the MEGA program (Tamura, 2007).

Statistical Analysis

Data normality and homogeneity of variances were checked by the tests of Lilliefors ($P<0.05$) and Bartlett ($P<0.05$), respectively. Subsequently, the results were analysed by the comparison of means. The tests were performed using the Statistica package and Excel software.

Results and discussion

The amount of fungi present in different configurations of iCLFs soil and reference areas,

native forest and fallow, was assessed. The method allows fungi isolates from different morphochromatic groups to be obtained. In total, 1440 fungi were isolated and purified, while the control plates had no fungal colonies.

The number of fungi colony forming units observed in the treatments during the dry season (10.1 x 10^5 CFU.g^{-1} of soil) was lower than that observed in the rainy season (23.0 10^5 CFU.g^{-1} of soil; F = 77.23 and p = 0.01) (Table 2).

The results of the treatments were analysed by average contrast (Table 3). The average CFU of fungi in monoculture treatments (F, G and C; 14.4x10^5) was lower than the average CFU observed in the integrated production treatments (iGC, iCG, iCF, iGF, iCGF, iGCF, iCGF; 17.5 x10^5) (C1, F=5.14 and p=0.032). The lowest CFU value was maintained, even in comparison with monoculture treatments, for double integrated treatments (C2, F=4.32 and p=0.047). The CFU number of fungi on Grass integrated with Forest (19.5 x10^5) was higher than in the Crop integrated with Forest (15.5 x10^5) (C5, F=4.11 and p=0.053). The monoculture of Forest had a lower CFU count for fungi (11.8 x10^5) than treatments with Forest as an integrated component (17.5 x10^5) (C8, F=6.87 and p=0.014).

Table 2. Average of colony forming units (10^5 UCF.g^{-1} of soil) obtained in a different treatments and reference areas during the rainy and dry season.

Treatments*	Rainy season	Dry season
F	15.9	7.6
C	19.0	7.2
G	27.4	9.1
iGC	28.6	14.0
iCG	18.4	8.9
iCF	19.9	14.8
iGF	24.7	10.9
iCFG	28.8	8.0
iGFC	25.6	10.1
iCLFs	21.5	10.6
Mean ± SE	23.0 ± 4.6	10.1 ± 2.2
W	13.1	10.4
R	19.9	14.3

*F-Forest; C - Crop; G - Grass; iGC - integration Grass/Crop; iCG - integration Crop/Grass; iCF - integration Crop/Forest; iGF - integration Grass/Forest; iCFG - integration Crop/Forest followed by Grass/Forest; iGFC - integration Grass/Forest followed by Crop/Forest; iCLFs - integrated Crop-Livestok-Forest system. W – Native Forest; R - Fallow. Means obtained from four repetitions with three replicates.

Assays of antagonism to pathogens *Fusarium* sp., *Rhizoctonia* sp. and *Sclerotium rolfsii* were performed by selecting twenty isolates per treatment and reference areas from each season. In total, 480 fungi isolates for each soil pathogen were tested; of these, 173 had an antagonistic effect (Figure 1). Integrated systems showed a highest amount of isolates able to control the pathogens in the dry season (Figure 2). In the other hand, the monocultures showed a highest amount of antagonists in rainy season (Figure 2). Ten isolates showed antagonism to three pathogens tested and 37 showed antagonistic potential to at least 2 pathogens (Figure 3 and 4). Besides, the inhibition zones of each fungus varied from 0.001 to 1 cm diameter in size, according to the pathogen assessed.

Molecular identifications were performed from isolates that presented antagonism that was positive to one or more pathogens. Eighty-five isolates were identified by partial sequencing of the ITS rDNA. This identification promoted access to genus and species of fungi in soil samples from all of the treatments and reference areas studied (Tables 4 and 5). In the rainy season was obtained the biggest number of genera able to control *Fusarium* sp., *Rhizoctonia* sp. and *Sclerotium rolfsii*, with 13 genera: *Acremonium, Aspergillus, Bionectria, Chaetomium, Clonostachys, Curvularia, Eupenicillium, Purpureocillium, Penicillium, Paecilomyces, Plectosphaerella, Talaromyces, Trichoderma.* (Table 4). Already, in the dry season was obatined 9 genera: *Aspergillus, Bionectria, Chaetomium, Fennellia, Metarhizium, Mucor, Penicillium, Paecilomyces, Talaromyces.* (Table 5).

For decades, the Cerrado/Amazon ecotone was occupied for low technologies planting (Silva et al., 2012) and/or without environmental sustainability, gradually impacting on biological diversity in the soil (Teixeira et al., 2012). Apparently, the intensification of land use can promote a reversal of this characteristic, as the crop diversification can influence the proliferation of microorganisms in soil.

The data showed that the intensification of land use, e.g. the use of integrated production systems, increased the amount of fungi present in the soil compared to soils used for monoculture production, mainly with regard to the treatments containing Forest in the system. The proliferation of fungi in the soil may be reflected in greater diversity, ecosystem stability and increased nutrient cycling (Tótola and Chaer, 2002; Mendes et al., 2009).

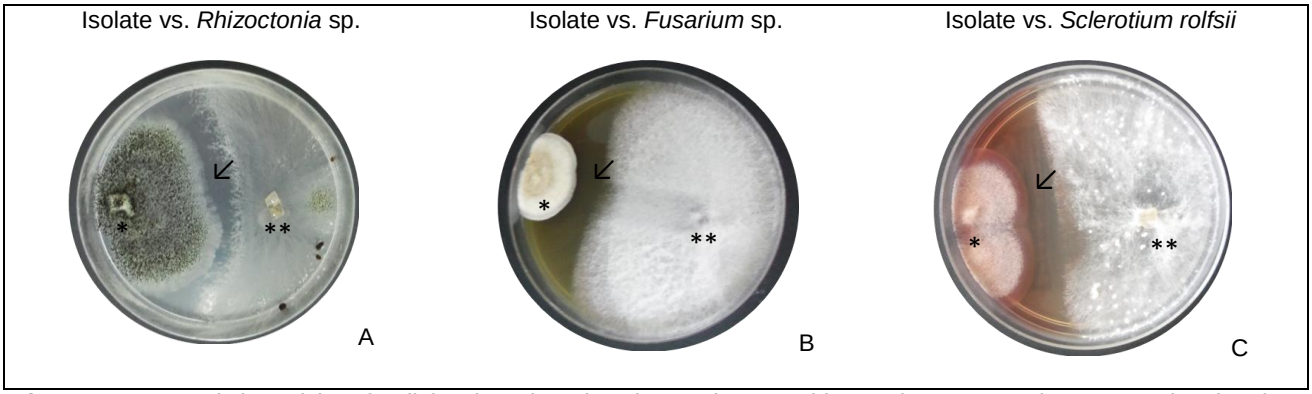

Figure 1. Antagonistic activity of soil fungi against the phytopathogen *Rhizoctonia* sp., *Fusarium* sp., and *Sclerotium rolfsii* strains. A) Fungi isolate against *Rhizoctonia* sp; B) Fungi isolate against *Fusarium* sp.; and C) Fungi isolate against *Sclerotium rolfsii*. *Antagonist fungi isolate able to control the pathogen; ** Pathogen. Results obtained from three replicates.

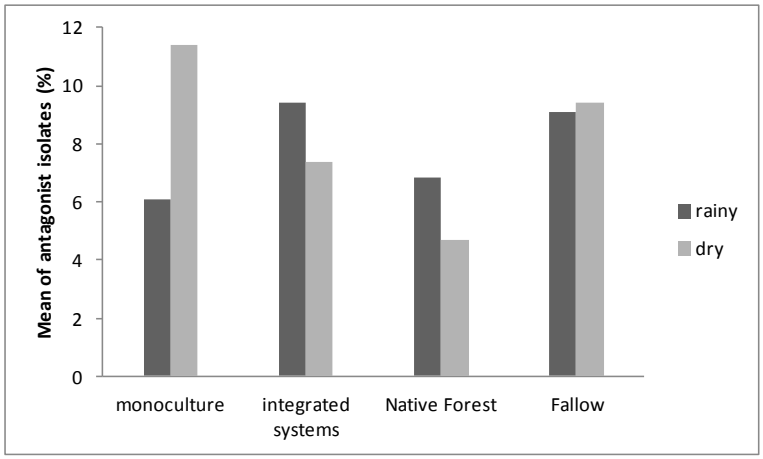

Figure 2. Antagonist isolates able to control at least 1 pathogen assessed (*Rhizoctonia* sp., *Fusarium* sp., and *Sclerotium rolfsii*), in rainy and dry season. Monoculture – mean (%) obtained from F-Forest; C - Crop; G – Grass. Integrated systems - mean (%) obtained from iGC - integration Grass/Crop; iCG - integration Crop/Grass; iCF - integration Crop/Forest; iGF - integration Grass/Forest; iCFG - integration Crop/Forest followed by Grass/Forest; iGFC - integration Grass/Forest followed by Crop/Forest; iCLFs - integrated Crop-Livestok-Forest system. Results obtained from three replicates.

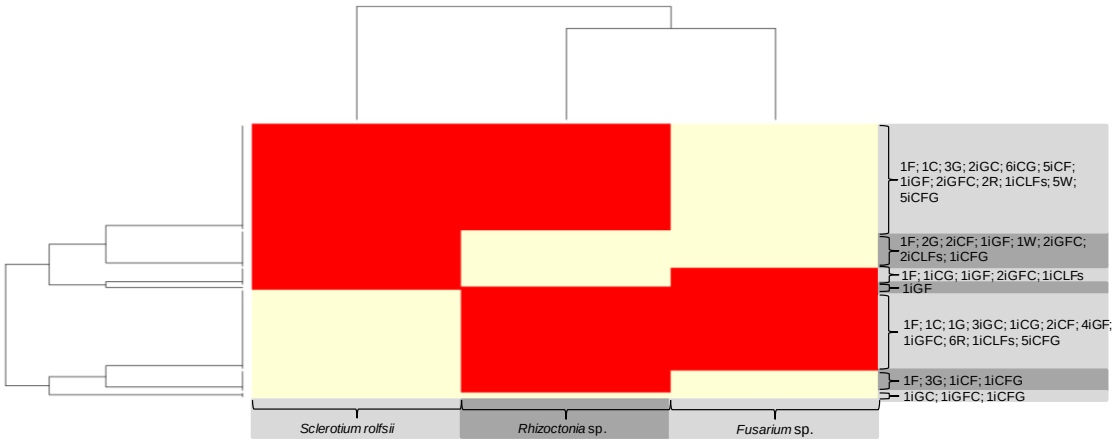

Figure 3. Similarity dendrogram showing the antagonistic effect of eighty-eight fungi isolates able to control *Fusarium* sp., *Rhizoctonia* sp. and *Sclerotium rolfsii*, in each treatment in the rainy season. F-Forest; C - Crop; G - Grass; iGC - integration Grass/Crop; iCG - integration Crop/Grass; iCF - integration Crop/Forest; iGF - integration Grass/Forest; iCFG - integration Crop/Forest followed by Grass/Forest; iGFC - integration Grass/Forest followed by Crop/Forest; iCLFs - integrated Crop-Livestok-Forest system. W – Native Forest; R - Fallow. Results obtained from three replicates.

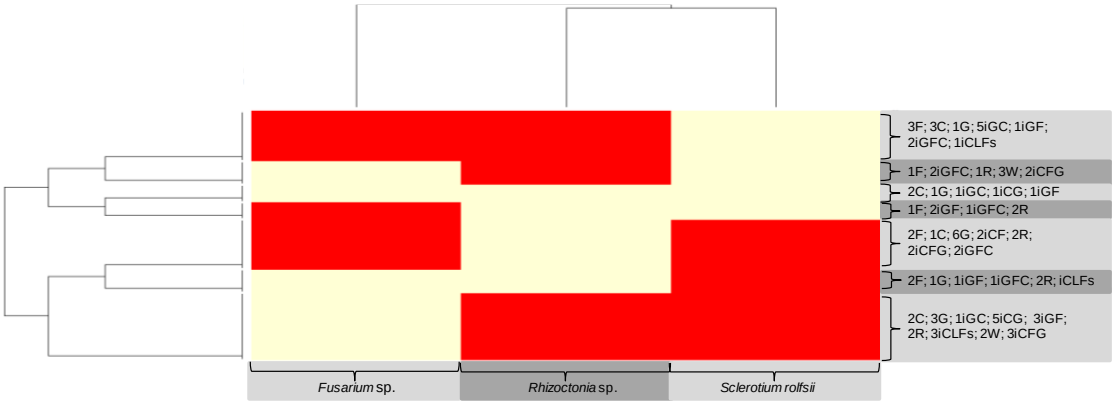

Figure 4. Similarity dendrogram showing the antagonistic effect of eighty five fungi isolates able to control *Fusarium* sp., *Rhizoctonia* sp. and *Sclerotium rolfsii*, in each treatment in the dry season. F-Forest; C - Crop; G - Grass; iGC - integration Grass/Crop; iCG - integration Crop/Grass; iCF - integration Crop/Forest; iGF - integration Grass/Forest; iCFG - integration Crop/Forest followed by Grass/Forest; iGFC - integration Grass/Forest followed by Crop/Forest; iCLFs - integrated Crop-Livestok-Forest system. W – Native Forest; R - Fallow. Results obtained from three replicates.

The greatest number of fungi found in integrated systems over monocultures may be the biggest ecological complexity in these treatments. Moreira and Siqueira (2006) reported that the exudates of plants may interfere directly in the composition of soil microbial communities. The complexity of these exudates is related to the complexity of the vegetation cover in the area. Integrated systems are effective in increasing the amount of many of the fungi. However, the quantity of antagonistic fungi able to control *Rhizoctonia* sp. *Fusarium* sp. and *Sclerotium rolfsii* was similar in all treatments. Antagonism is the way in which fungi compete with other microorganisms in the soil (Ownley and Windham, 2008). In this study, the antagonism was considered when the fungi had antibiosis to pathogens, which may have been inhibited by the production of antibiotic and volatile or non-volatile substances (Alabouvette at al., 2009). Those metabolites with fungicidal effect diffused into the culture medium are responsible for the inhibition of mycelial growth, and promote the disruption of cells and the lysis of pathogen hyphae (Ahmed et al., 2003).

The fungal diversity obtained from the different soil treatments included *Eupenicillium shearii, Eupenicillium ochrosalmoneum, Talaromyces pupurogenus, Talaromyces tracyspermus, Trichoderma viride, Aspergillus terreus, Aspergilllus flavipe, Aspergillus foetidus, Aspergillus brasiliensis, Aspergillus niger, Chaetomium aureum, Chaetomium cupreum, Acremonium cellulolyticus, Clonostachys rosea, Penicillium citrium, Penicillum verruculosus, Penicillium pinophilum, Curvularia affinis, Bionectria ochroleuca, Purpureocillium lilacinum, Plectosphaerella cucumerina, Fennellia nívea, Paecilomyces formosus,* and *Metarhizium anisopliae. Aspergillus flavipes*, *Talaromyces trachyspermus*, and *Clonostachys rosea*, with seven other non-affiliated isolates showing antagonism to the three pathogens assessed. Besides, the fungus *C. rosea* was considered the controller of other pathogens such as *Pythium aphanidermatum* (Corrêa et al., 2010).

There was the presence of other fungi usually described as phytopathogen antagonists, such as *Trichoderma* sp., *Clonostachys* sp., *Curvularia* sp. and *Metarhizium* sp., which were able to produce different compounds and have been used in the biological control of agricultural pests (Pérez et al., 2010; Teixeira et al., 2012; Vinale et al., 2013; Gao et al., 2014; Wang et al., 2013).

Nevertheless, it is important to obtain those species occurring in the Cerrado/Amazon ecotone and Amazon biome, because those are adapted to the soil type, temperature and humidity of the region, increasing the future success of biological control strategies.

Trichoderma sp. and *Gliocladium* sp. were described as mycoparasitic on plant pathogens as they produce enzymes such as chitinase, endochitinase, glucanase, cellulase and hemicellulose, which are able to lyse cell walls (Lahlali and Hijri, 2010). Indeed, some products based on *Trichoderma* spp. have been sold and recommended for use in biological control, such as Trichodel® (ECCB, 2013) and Trichodermil® (Itaforte, 2013). *Penicillium* sp., *Talaromyces* sp. and *Eupenicillium* sp. were effective in antagonism against *Rhizoctonia* sp., *Fusarium* sp. and *S. rolfsii*; also, there was antibacterial activity with the production of penicillin (Veiga et al., 2013), thus increasing the range of control of these fungi and exemplifying the complexity of the interactions between microorganisms in the soil. Thereby, *Metarhizium* sp. and *Paecilomyces* sp. have been described as biocontrollers of other organisms, such as insects and nematodes (Alves and Bateman, 2013).

Table 4. Identification of fungal isolates from soil collected in all treatments and reference areas obtained in a rainy season. The molecular identification was performed by sequencing of ITS regions and compared by BLASTn (National Center for Biotechnology Information Website).

Treatment[+]	Isolate	Genus	Species(Blast –NCBI)	%[++]	GenBank reference
F	49	*Eupenicillium*	-	96	GQ924907.1
F	57	*Talaromyces*	Sp	99	GU973739.1
C	54	*Talaromyces*	*T. purpurogenus*	97	AB872825.1
G	4	*Trichoderma*	-	93	GU048860.1
G	5	*Aspergillus*	*A. terreus*	98	KC762934.1
G	17	*Chaetomium*	*C.aureum*	98	JX186515.1
G	22	*Trichoderma*	-	89	GU048860.1
G	31	*Aspergillus*	*A. flavipes*	99	FR733808.1
G	37	*Aspergillus*	*A.flavipes*	98	GU566238.1
G	43	Uncultured soil fungus	Sp	98	DQ421253.1
iGC	50	*Acremonium*	*A. cellulolyticus*	98	AB474749.2
iGC	59	*Clonostachys*	*C. rósea*	98	KC878702.1
iCG	14	*Aspergillus*	-	91	EF669591
iCG	17	*Talaromyces*	*T.purpurogenus*	97	AB872825.1
iCG	31	*Eupenicillium*	*E. chrosalmoneum*	100	EF626960.1
iCG	38	*Penicillium*	*P. citrinum*	91	HQ245157.1
iCG	48	*Curvularia*	*C. affinis*	98	J467361.1
iCG	52	*Purpureocillium*	*P. lilacium*	98	KC478538.1
iCF	27	*Aspergilllus*	sp	98	FR733808.1
iCF	32	*Aspergillus*	*A. flavipe*s	98	FR733808.1
iCF	35	*Bionectria*	Sp	99	HM849058.1
iCF	42	*Aspergillus*	-	93	FJ545246.1
iCF	46	*Aspergillus*	-	95	JX556221.1
iCF	48	*Talaromyces*	Sp	99	GU973739
iCF	51	*Aspergillus*	-	93	HD219673.1
iCF	57	*Acremonium*	*A. cellulolyticus*	97	AB474749.2
iGF	19	*Aspergillus*	*A. Brasiliensis*	98	JQ316521
iGF	54	*Purpureocillium*	*P. lilacinum*	98	KC157756.1
iGF	56	*Talaromyces*	*T. trachyspermus*	97	EU076917.1
iGF	60	*Talaromyces*	Sp	99	JF714646.1
iCFG	14	*Purpureocillium*	-	91	KC478538.1
iCFG	24	*Aspergillus*	*A. Flavipes*	99	FR733808.1
iCFG	28	*Paecilomyces*	Sp	97	HQ607808.1
iGFC	25	*Talaromyces*	*T. purpurogenus*	97	AB872825.1
iGFC	55	*Plectosphaerella*	*P. cucumerina*	99	KC93994.1
iCLFs	4	*Talaromyces*	-	87	HQ60823.1
iCLFs	27	*Talaromyces*	Sp	99	GU973739.1
iCLFs	36	*Aspergillus*	*A. Flavipes*	99	GU566238.1
iCLFs	48	*Penicillium*	*P. verruculosus*	97	JN565299.1
R	4	*Talaromyces*	Sp	98	GU973739.1
R	5	*Clonostachys*	*B. Rósea*	97	KC878702.1
R	10	*Aspergillus*	*A. flavipes*	98	GU566238.1
R	25	*Aspergillus*	sp	98	KC007332
R	39	*Chaetomium*	*B. cupreum*	98	AB509372.1
W	6	*Penicillium*	*P. citrinum*	97	HQ245157.1
W	15	*Talaromyces*	-	87	HD608123.1
W	28	*Trichoderma*	Sp	88	JX416583.1
W	40	Fungal endophyte	-	89	EU977237.1
W	44	*Penicillium*	*P. pinophilum*	99	GU595046.1

[+]F-Forest; C - Crop; G - Grass; iGC - integration Grass/Crop; iCG - integration Crop/Grass; iCF - integration Crop/Forest; iGF - integration Grass/Forest; iCFG - integration Crop/Forest followed by Grass/Forest; iGFC - integration Grass/Forest followed by Crop/Forest; iCLFs - integrated Crop-Livestok-Forest system. W – Native Forest; R - Fallow. [++]Most similar GenBank sequence.

Table 5 Identification of fungal isolates from soil collected in all treatments and reference areas in a dry season. The molecular identification was performed by sequencing of ITS regions and compared by BLASTn (National Center for Biotechnology Information Website).

Treatment[+]	Isolate	Genus	Species (Blast –NCBI)	%[++]	GenBank reference
F	3	*Aspergillus*	Sp	96	KC007332.1
F	8	*Penicillium*	-	89	HQ245157.1
F	22	*Penicillium*	*P. verruculosim*	96	JN565299.1
F	27	*Penicillium*	Sp	80	GU973810.1
F	59	*Chaetomium*	*A. Aureum*	99	KF245432.1
C	9	*Aspergillus*	*A. Flavipes*	97	GU5662238.1
C	14	*Aspergillus*	*A. Flavipes*	97	FR733808.1
C	28	*Aspergillus*	-	88	FR733808.1
C	53	Fungal endophyte	-	99	KF673666.1
G	11	*Aspergillus*	-	96	JF817254.1
G	19	*Mucor*	Sp	96	KF158220.1
G	31	*Talaromyces*	*T. trachyspermus*	99	EU076917.1
G	45	*Aspergillus*	*A. brasiliensis*	97	JQ316521.1
iCG	33	*Fennellia*	-	95	FJ155814.1
iCG	44	*Penicillium*	*P. citrinum*	97	KF414682.1
iCG	59	*Aspergillus*	-	94	HQ219673.1
iCF	12	*Penicillium*	-	89	HQ245157.1
iGF	3	*Aspergillus*	*A. flavipes*	98	GU566238.1
iGF	13	*Aspergillus*	*A. candidus*	99	HQ607958.1
iGF	24	*Talaromyces*	-	96	AB872825.1
GFTi	19	Uncultured *Aspergillus*	-	85	KC143754.1
iGFC	21	*Aspergillus*	-	96	KC007332.1
iGFC	26	*Bionectria*	*B. ochroleuca*	99	JQ794833.1
iGFC	30	*Aspergillus*	-	95	JX556221.1
iGFC	32	*Paecilomyces*	*P. formosus*	99	FJ389927.1
iCLFs	25	*Aspergillus*	Sp	98	KC007332
iCLFs	40	*Aspergillus*	*A. flavipes*	98	GU5662238.1
iCLFs	48	*Aspergillus*	*A. brasililensis*	98	JQ316521.1
iCLFs	55	*Aspergillus*	*A. Flavipes*	98	GU5662238.1
R	32	*Aspergillus*	*A. brasililensis*	98	JQ316521.1
R	41	*Aspergillus*	Sp	99	KC007332.1
R	58	*Aspergillus*	*A. brasiliensis*	98	JQ316521.1
R	60	*Metarhizium*	*M. anisopliae*	99	AJ608970.1
W	28	*Penicillium*	Sp	98	JN565301.1

[+]F-Forest; C - Crop; G - Grass; iGC - integration Grass/Crop; iCG - integration Crop/Grass; iCF - integration Crop/Forest; iGF - integration Grass/Forest; iCFG - integration Crop/Forest followed by Grass/Forest; iGFC - integration Grass/Forest followed by Crop/Forest; iCLFs - integrated Crop-Livestok-Forest system. W – Native Forest; R - Fallow. [++]Most similar GenBank sequence.

Thus, it is possible that production systems to support these fungi are less vulnerable to diseases, insects and nematode attacks. The intensification of land use with integrated systems can be a strategy for increasing fungi in the soil and stimulating natural equilibrium, which are among the benefits for fungi and phytopathogens. Furthermore, this can contribute to decreasing the use of chemical molecules like fungicides, which may promote degradation and have residual effects on the environment (Santos et al., 2012).

Also, in this study, human and animal opportunistic fungi were identified, such as the genera *Purpureocillium* sp., *Chaetomium* sp., *Acremonium* sp., *Aspergillus* sp. and *Fennellia* sp. (Laung et al., 2011; Soleiro et al., 2013), which should be studied carefully. A good study strategy for these cases is the search for non-pathogenic isolates, which are either plant pathogen controllers or suppliers of genes related to biological control or compound production.

This is the first report to describe culturable fungi species from the Amazon biome able to control *Fusarium* sp., *Rhizoctonia* sp. and *Sclerotium rolfsii*. Furthermore, the results of this study suggested that iCLFs can be a strategy to increase the fungal biomass and the rainy and dry season can modulate the density of the soil fungi too. After greenhouse and field assays, the biological control using isolates from this study could be a strategy to suppress pathogen populations in integrated systems in the Amazon biome.

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