FEDERAL UNIVERSITY OF MATO GROSSO DO SUL
CAMPUS OF CHAPADÃO DO SUL
GRADUATE PROGRAM IN AGRONOMY

VICTORIA ROMANCINI TOLEDO

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CHAPADÃO DO SUL – MS
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Supervisor: Prof. Dr Acacio Aparecido Navarrete
Co-Supervisor: Profa. Dra. Rita de Cassia Félix Alvarez

Master's thesis presented to the Federal University of Mato Grosso do Sul (UFMS), in partial fulfilment of the requirements for the degree of Master Science in Agronomy, area of concentration: Vegetable Production.

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VINASSE AND STRAW RETENTION DECREASE FUNGAL DIVERSITY AND POTENTIALLY PATHOGENIC FUNGI IN SUGARCANE SOIL

ABSTRACT

Soil management practices used in sugarcane agriculture in Brazil require synthetic mineral fertilizers and full recycling of waste products from ethanol production to sugarcane fields in the form of organic fertilizer. Vinasse (V) is a by-product of the sugar-ethanol industry, and it has been used as a liquid organic fertilizer in combination with mineral nitrogen (N) and straw retention. Despite numerous benefits to the physical and chemical characteristics of the soil, the effects of these organic residues combined with mineral N fertilizer on the soil fungal community are still largely unknown. This study focused on the effects of V combined with mineral N fertilizer and straw retention on the fungal microbial community diversity, richness, evenness, composition and structure in sugarcane-cultivated soils in a greenhouse mesocosm experiment. The experiment consisted of a combination of V, mineral N and sugarcane-straw blanket. Soil samples were collected at 7 (T7), 157 (T157) and 217 (T217) days after planting, corresponding to maximum carbon dioxide (CO_2) emissions from soil induced by the fertilizers after three repeated applications into the soil. Across 57 soil metagenomics datasets, it was revealed that the application the V in combination with mineral N and straw retention as a surface blanket decreased a diversity, evenness and richness of fungi at the community level in soil. Analysis of the soil fungal community composition based on the 20 genera most abundant in the soil revealed decrease in abundance for *Blastomyces*, *Melampsora* and *Penicillium* after the third application of V in combination with N fertilizer and straw blanket. An opposite response was revealed for *Amauroascus*, *Cantharellus*, *Chrysosporium*, *Clavaria*, *Morchella*, *Puccinia*, and *Tuber* in soils under this treatment. Shifts in fungal community composition were followed by increases in mycorrhizal and decomposers soil-borne fungi and decrease in potentially pathogenic fungi, but not by changes in community structure. Based on these results, it is possible to attest that repeated applications of V in combination with mineral N fertilizer and sugarcane-straw blankets affect ecological aspects of the soil fungal community composition and potentialfunctions played by fungi in sugarcane soil, which are essentials to ecosystem function and sustainable management of agricultural ecosystems.

Keywords: Decomposer fungi.Fungal community. Mycorrhizal fungi. Shotgun metagenome. Sustainability.

VINHAÇA E RETENÇÃO DE PALHA DIMINUEM A DIVERSIDADE FÚNGICA E POTENCIAIS FUNGOS PATÓGENOS EM SOLOS CANAVIEIROS

RESUMO

As práticas de manejo do solo utilizadas em campos de produção de cana-de-açúcar no Brasil requerem fertilizantes minerais sintéticos e a reciclagem completa de produtos resultantes da produção de etanol nas áreas de cultivo de cana-de-açúcar na forma de fertilizante orgânico. A vinhaça (V) é um subproduto da indústria sucroalcooleira e tem sido utilizada como fertilizante orgânico líquido em combinação com nitrogênio mineral (N) e retenção de palha. Apesar dos inúmeros benefícios para as características físicas e químicas do solo, os efeitos desses resíduos orgânicos combinados com o fertilizante N mineral na comunidade fúngica do solo ainda são amplamente desconhecidos. Este estudo teve como foco os efeitos de V combinado com fertilizante mineral N e retenção de palha na diversidade, riqueza, equitatividade, composição e estrutura da comunidade microbiana de fungos em solos cultivados com cana-de-açúcar em um experimento de mesocosmo em casa de vegetação. O experimento consistiu na combinação de V, N mineral e retenção de palha de cana-de-açúcar. Amostras de solo foram coletadas aos 7 (T7), 157 (T157) e 217 (T217) dias após o plantio, correspondendo às emissões máximas de dióxido de carbono (CO_2) do solo induzidas pelos fertilizantes após três aplicações repetidas no solo. Com base em 57 conjuntos de dados metagenômicos de solo foi revelado que a aplicação de V em combinação com N mineral e retenção de palha como uma cobertura de superfície diminuiu a diversidade, riqueza e equitatividade de fungos ao nível da comunidade no solo. A análise da composição da comunidade fúngica do solo com base nos 20 gêneros mais abundantes no solo revelou diminuição na abundância para *Blastomyces*, *Melampsora* e *Penicillium* após a terceira aplicação de V em combinação com fertilizante N e retenção de palha na superfície. Uma resposta oposta foi revelada para *Amauroascus*, *Cantharellus*, *Chrysosporium*, *Clavaria*, *Morchella*, *Puccinia* e *Tuber* em solos sob estes tratamentos. Mudanças na composição da comunidade fúngica foram seguidas por aumentos nos fungos micorrízicos e decompositores do solo ediminuição de potenciais fungos patogênicos, mas não por mudanças na estrutura da comunidade. Com base nesses resultados, é possível atestar que as aplicações repetidas de V em combinação com fertilizante de N mineral e a retenção de palha de cana-de-açúcar na superfície afetam os aspectos ecológicos da composição da comunidade fúngica do solo e as funções potenciais desempenhadas pelos fungos no solo da cana-de-açúcar que são essenciais para o funcionamento do ecossistema e gestão sustentável de ecossistemas agrícolas.

Palavras-chave: Fungos decompositores. Comunidade fúngica. Fungos micorrízicos. Metagenoma shotgun. Sustentabilidade.

SUMÁRIO

1. INTRODUCTION

Soil microbiological properties are early-warning indicators of agricultural soil management effects more than usually physical and chemical factors widely used in soil assessment (CHAER et al., 2009; KASCHUK et al., 2010; SOUZA et al., 2012; RACHID et al., 2012; NAVARRETE et al., 2013). While several recent studies have used deep sequencing approaches to assess the soil bacterial communities in sugarcane soils (NAVARRETE et al., 2015; RACHID et al., 2016; VAL-MORAES et al., 2016; DURRER et al., 2017; de CHAVES et al., 2019), the number of such studies addressing fungal communities is still limited (GUMIERE et al., 2016; LOURENÇO et al., 2020). This is true despite the fact that fungi comprise a large proportion of soil microbial biomass and have a dominant role in decomposition of organic material, nutrient cycling, mineral mobilization, formation of soil aggregates, elevated water holding capacity, plant growth promotion and suppression of phytopathogens (BUÉE et al., 2009). However, many fungi are also plant pathogens that reside either in soil (soil-borne) or persist on organic debris (FRAC et al., 2018). Compared to undisturbed natural ecosystems, fungal soil communities in agro-ecosystems are exposed to additional influencing factors related to soil and crop management practices, which regulate their diversity and activity (LÓPEZ-BUCIO et al., 2015; ROUPHAEL et al., 2015). The impact of different agricultural management regimes on fungal community composition gains rising interest, although, up to date, only few studies were dedicated to determine the effects of organic and inorganic fertilizers and retention of crop residues on soil fungal communities in sugarcane production systems.

Vinasse is a by-product of ethanol from the sugarcane industry, generated during the distillation process (GLÓRIA,1992; DIAS et al., 2009), with a production rate of 10-18 L while preparing each liter of ethanol (GASPAROTTO et al., 2014). The chemical composition of vinasse is generally 93% water and 7% organic materials and minerals (HIDALGO et al., 2009; CHRISTOFOLETTI et al., 2013). Vinasse as a liquid fertilizer can be applied with sources of mineral nitrogen (N) in the culture of sugarcane to minimize the ecological problem of its disposal of residues in the environment (PENATTI et al., 1988; MORAES et al., 2014). However, even if this practice increases the productivity of sugarcane, it also causes physical, chemical and biochemical changes in the soil environment (MADEJÓN et al., 2001; TEJADA; GONZALEZ, 2006). Due to these changes that vinasse causes in the environment, its application to enrich agricultural soil must respect a dosage, regulated by technical standard P 4.231/2005 of the Environmental Company of the State of

São Paulo (CETESB), calculated considering the depth and fertility from the soil, the concentration of potassium in the vinasse and the average extraction of this element by the crop.

Although the use of vinasse combined with N fertilization is able to improve soil fertility and sugarcane productivity, there is a lack of information on the impacts of organic and inorganic amendments and straw retention on the fungal communities in tropical agricultural soils, and the changes in soil microbial communities are often correlated with the different chemical factors in this environment (FREY et al., 2004; NILSSON et al., 2007; LAUBER et al., 2009; JENKINS et al., 2009). Over the last five decades, culture-dependent and culture-independent approaches have been developed to assess microorganisms in soil. Conventional culture-dependent methods provide information on the active heterotrophic component and the functional role of the population. However, the evaluation of soil microbial diversity based on these methods has been limited only a tiny fraction of the total microbial diversity (PACE, 2009). Nowadays, two sequencing-based methods are generally used to study fungi in a mycobiome. The most common is PCR amplification of internal transcribed spacer regions (ITS) of rRNA operons, followed by sequencing (SCHOCH et al., 2012). The second approach identifies taxafrom shotgun metagenomes. Most tools use custom-built databases, together with search algorithms. These tools identify the database sequence most similar to a metagenome reading (DONOVAN et al., 2018).

Because of the substantial effects that organic and inorganic fertilizers and retention of crop residues may have on the microbial communities of agricultural soils, and the importance of fungal communities for the functioning of soil systems, we evaluated the fungal community in sugarcane-cultivated soil amended with vinasse and N fertilizer and with straw retention used as a surface ‘blanket’. We hypothesized that the incorporation of vinasse and N as fertilizer into thesoil and sugarcane straw-blanket increase the diversity, richness and evenness of soil fungal community. In a corollary hypothesis, we tested whether soil fungal community structure and composition change over time in repeated applications of vinasse and nitrogen as fertilizer. We also hypothesized that these fertilizers and straw-blanket favor the occurrence of beneficial soil-borne fungi. To address these hypotheses, we used shotgun metagenomic sequencing to analyze the ecological metrics, structure and composition of the fungal community from sugarcane-cultivated soil in a short-term greenhouse experiment. The results of this study are particularly important for the evaluation of management practices related to fertilizer use in sugarcane-cultivated soils.

2. MATERIALS AND METHODS

2.1. Experimental design and treatments

The sugarcane (*Saccharum* spp.) variety CTC-02 is characterized by medium-late maturation, high productivity and longevity, and it was grown from April until December 2013 (257 days) in a greenhouse mesocosm experiment. The influence of environmental parameters, such as moisture regime, soil type and fertilizer management, were normalized on the growth conditions for in vitro plants obtained via tissue culture techniques. Podzolic dark red soil (clay loam texture) was collected from the 0 to 20 cm topsoil layer in the experimental field of the Areão Farm at ESALQ/USP, Piracicaba, São Paulo, Brazil (22° 42' 30" S e 47° 38' 00" W). Eighteen mesocosms in plastic pots (100 L) were filled with 90 kg of soil, which was placed over a 15 cm layer of washed stones. Mineral fertilization that is common in all mesocosms and consisting of 150 kg ha^{-1} P_2O_5 (triple superphosphate) and 80 kg ha^{-1} KCl (potassium chloride) was used in this experiment. Six treatments and three replications were used in a completely randomized design. Mineral fertilizer was applied in the form of urea (450 g N kg^{-1}) to the 0 to 10 cm topsoil layer at a rate of 60 kg N ha^{-1} in treatments containing N fertilizer. A small shovel was used to mix the urea to the soil avoiding losses by volatilization. Vinasse is a liquid residue of ethanol distillation, and it was applied to the soil at a rate of 0.06 L kg^{-1} (120 m^3 ha^{-1}) as a source of K in addition to organic matter and other nutrients. An equivalent water volume was applied in treatments without vinasse. The experiment consisted of two conditions of soil-surface straw blanket as follows: surface blanket with sugarcane straw (10 t ha^{-1}) and uncovered surface. The straw blanket consisted of dry and chopped leaves from adult sugarcane plants. The KCl dosage was calculated minus the equivalent input of K in case of straw blanket and vinasse treatments according to previous measurements of K content in sugarcane straw and vinasse samples. Accordingly, the experiment included the following treatments: N, nitrogen fertilizer; N+S, N fertilizer and straw blanket; V+N, vinasse and N as fertilizers; V+N+S, V and N as fertilizers and straw blanket; C, excluding any N, V fertilizer and straw blanket (control); and C+S, excluding any N and V fertilizer and including straw blanket. In order to provide nutrients for the growth of the sugarcane plants until ripening phase, were made three applications of fertilizers (7, 157 and 217), defined based on plant deficiency symptoms and fertilizer-induced CO_2-C and N_2O-N emissions from the soil (NAVARRETE et al., 2015) (Figure 1).

The soil moisture was monitored daily in each mesocosm by using soil moisture sensor (Extech MO750, Nashua, NH, USA) in order to maintain the humidity at the 20%.

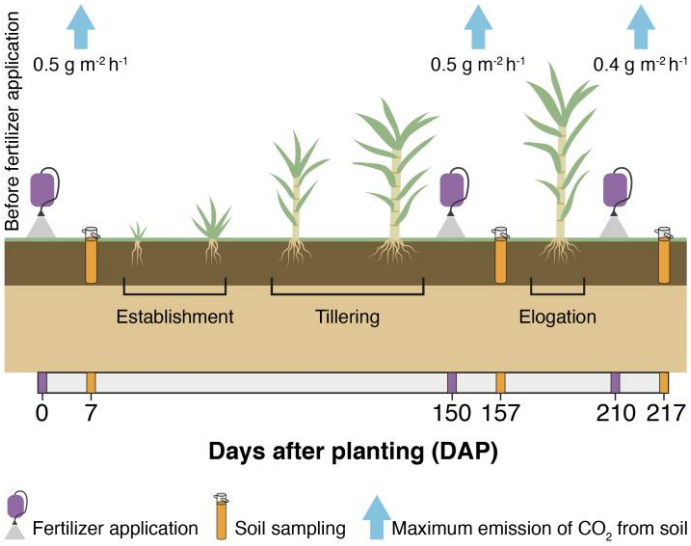

Figure 1. Repeated applications of fertilizers to the soil based on plant deficiency symptoms and fertilizer-induced CO_2-C emissions from the soil

Ten sugarcane plants were grown in each mesocosm, and only two sugarcane plants were left in each mesocosm until the end of the experiment. Sugarcane plants were removed in pairs from each mesocosm at 50, 90, 150 and 210 days after the first soil fertilization to maintain the root system under the limit capacity of the mesocosm.

2.2. Soil sampling

For each mesocosm, soil samples were collected before the first fertilization and on the maximum gas flux after the second fertilization for chemical factor analysis. Soil samples for DNA isolation were collected before the first fertilization and during the maximum CO_2-C and N_2O-N emissions from soil in each of the three applications of fertilizer. All of the soil samples were collected from the 0 to 10 cm topsoil layer using a cylindrical sampler (2 cm diameter) after removing the straw blanket when present. Soil samples for chemical analysis were immediately processed after sampling. Soil samples for DNA isolation were transported to the laboratory under ice and stored at -20°C until processing within 72 h after sampling.

2.3. Analysis of soil chemical factors

Soil samples were air dried and sieved through a 0.149 mm for total C and N determination by dry combustion on a LECO CN elemental analyzer at the Center for Nuclear Energy in Agriculture, University of São Paulo, Brazil. The fertility status of the soil from each soil sample was assessed as described in Navarrete et al. (2013), with organic matter (OM) determined according to Camargo et al. (2009) at the Soil Fertility Laboratory, Department of Soil Sciences, University of São Paulo. The evaluated soil fertility factors included pH, potential acidity (H+Al), Ca, Mg, P, K, S, available micronutrients (Fe, Mn, Zn and Cu), exchangeable bases (EB; the sum of Ca, Mg and K), cation exchange capacity (CEC), and base saturation (V%).

2.4. Isolation of DNA from soil and high-throughput sequencing of soil metagenome

DNA was extracted from 250 mg (wet weight) of 57 soil samples (3 samples taken before the first fertilization + 3 samples x 6 experimental treatments x 3 applications of fertilizer) using the Power Lyzer Power Soil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The DNA extracts were stored at −20°C until use.

Fifty seven DNA sequencing libraries were prepared using the Illumina Nextera sample preparation kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. The libraries were evaluated on 2100 Bioanalyzer using High Sensitivity DNA kit (Agilent, Santa Clara, CA, USA) to estimate the library size. Libraries were quantified using Qubit dsDNA HS kit on a Qubit 2.0 fluorometer (Life technologies, Carlsbad, CA, USA) and KAPA SYBR FAST qPCR Master mix and Illumina standards and primer premix (KAPA Biosystems, Wilmington, MA, USA) according to the Illumina suggested protocol. The resulting DNA libraries were denatured with NaOH, diluted to 8 pM in Illumina's HT1 buffer, and spiked with 1 % PhiX. Equal concentration of libraries was loaded on MiSeq Reagent v2 sequencing reagent kit (Illumina, San Diego, CA, USA). The equipment used for shotgun metagenomic sequencing was a MiSeq Personal Sequencing System by Illumina (Illumina, San Diego, CA, USA) operated in Rapid Run Mode to generate 2 x 250 pb paired-end reads. In summary, we captured an average of 105.5 MB of genomic sequences per sample.

2.5. Shotgun metagenomic data processing and taxonomic annotation of sequences

First, paired-end reads were merged using FLASH version 1.2.5 (MAGOC; SALZBERG, 2011) to produce consensus sequences and increase the annotation accuracy. Second, low-quality bases (quality score lower than 20) from merged and unmerged sequences were trimmed from both ends using the Phred algorithm with SeqClean script (http://www.bioinformatics.org/). Merged and unmerged trimmed sequences were concatenated into a single file for each metagenomic dataset, which are available through the Metagenomics Rapid Annotation (MG-RAST) server (http://www. metagenomics.anl.gov) under project accession 'Metagenomes of sugarcane soils–CENA USP' and accession numbers 4582104.3 to 4582153.3.

A taxonomic analysis of the DNA sequences was performed with FindFungi (SILVA et al., 2014), a method to identify fungal species in shotgun metagenomics datasets, without relying on rDNA amplicons. We combined read identification using Kraken (MUYZER et al., 1993) with an analysis of read distribution across the target genome, which greatly reduces false positives. The method has high sensitivity and specificity. We used FindFungi to identify fungal species in soil metagenomes. All code for FindFungi (version 0.23) is available on Github at https://github.com/GiantSpaceRobot/FindFungi-v0.23.

2.6. Ecological metrics and statistical analyses

Ecological metrics were calculated for the normalized number of sequences per library, as these metrics are correlated with the library size. Diversity index (Shannon), estimator of richness (Chao1), and evenness measure (Pielou) of the soil total fungal community were calculated to compare community-level diversity, richness and evenness, respectively, in the experimental treatments. The ecological metrics were calculated based on the detected members of the total fungal communities with the metangenomic sequence based analyses. A post hoc analysis using Tukey's HSD test was used to determine the significance of the differences between treatments and their respective controls within each of the three applications of fertilizer. Tukey's HSD test was carried out using the PAST software version 4.03.

A repeated measures analysis of variance (rANOVA) was performed using the R package "ExpDes.pt" version 3.5.2 (FERREIRA et al., 2014) to assess the effects of factors such as 'time' (repeated application of fertilizer) and experimental treatments on the relative

abundance of the 20 genera of fungi most abundant in the sugarcane soil in the different treatments over time in three applications of fertilizer. In order to analyze the similarity of the soil fungal community based on the 20 genera most abundant in the treatments within each of the three applications of fertilizers, principal component analysis (PCA) was carried out using the software CANOCO version 4.5 (ter BRAAK; ŠMILAUER, 2002).

3. RESULTS AND DISCUSSION

3.1. Soil chemical characteristics

Among the soil addition properties analyzed, 67% responded significantly (p <0.05) to the addition of vinasse 7 days after the second fertilizer aplication into the soil (Table S2). For this time, soil analysis revealed that pH, nutrients potassium, manganese and N, and carbon increased in the presence of vinasse. The boron micronutrient decreased by 33.33% in the N+V treatment, compared to the control treatment. The C/N ratio decreased by 17.03% with the addition of vinasse, and 19.15% with vinasse in combination with nitrogen fertilizer and straw blanket. This can be explained by the fact that the amount of N is greater than that of C, due to the application in fertilizer composed of N (V+N and V+N+S).

Use the vinasse result in modifications in different soil properties, and this effects of the application of this residue on the soil depend on various factors, such as the quantity applied in the soil, soil typeand chemical composition, relief, and crop type (CHRISTOFOLETTI et al., 2013). Studies conducted by Camargo et al. (1983), Glória and Orlando Filho (1983), Laime et al. (2011) and Jiang et al. (2012) in the disposal of sugarcane vinasse in the soil have reported beneficial effects on potassium. Neves et al. (1983) reported that the addition of vinasse combined with straw blanket can improve the physical characteristics of the soil and the mobilization of nutrients, as a result of higher solubility provided by the liquid residue. Canellas et al. (2003) reported improved in the physical conditions, on an increase in the level of organic matter. This is as a result of applications of vinasse throughout the years.

3.2. Diversity, richness and evenness of the soil fungal community

Shotgun sequencing of soil DNA from the 57 soil samples (DNA samples described in subsection 2.4) resulted in approximately 13.5 million merged sequence reads and 8.7 million non-merged sequence reads after the quality-based filtering procedure (Table S1). Sequence data were examined in soils to calculate ecological metrics at the community level for the total fungal community.

The diversity, richness and evenness results for the total soil fungal community are showed in the Figure 2. Statistical differences among the treatments were revealed only after the second and third fertilizer applications. Decrease in the total soil fungal community diversity was showed for the amended soil with the combination of vinasse and N after the third application with straw blanket and without straw retention as a surface blanket. The evenness also decreased in soils under these treatments after the second and third applications. The evenness in amended soil with vinasse and N and straw blanket on surface was 30.41% lower than in its respective control after the third fertilizer application. This result indicates increase in dominance at the community level for fungi in soil after vinasse fertilization use combined with N and straw retention.In turn, the richness decreased only after the third application of vinasse and N, and it did not revealed negative effect for amended soil with both fertilizers when straw was retented as a surface blanket.

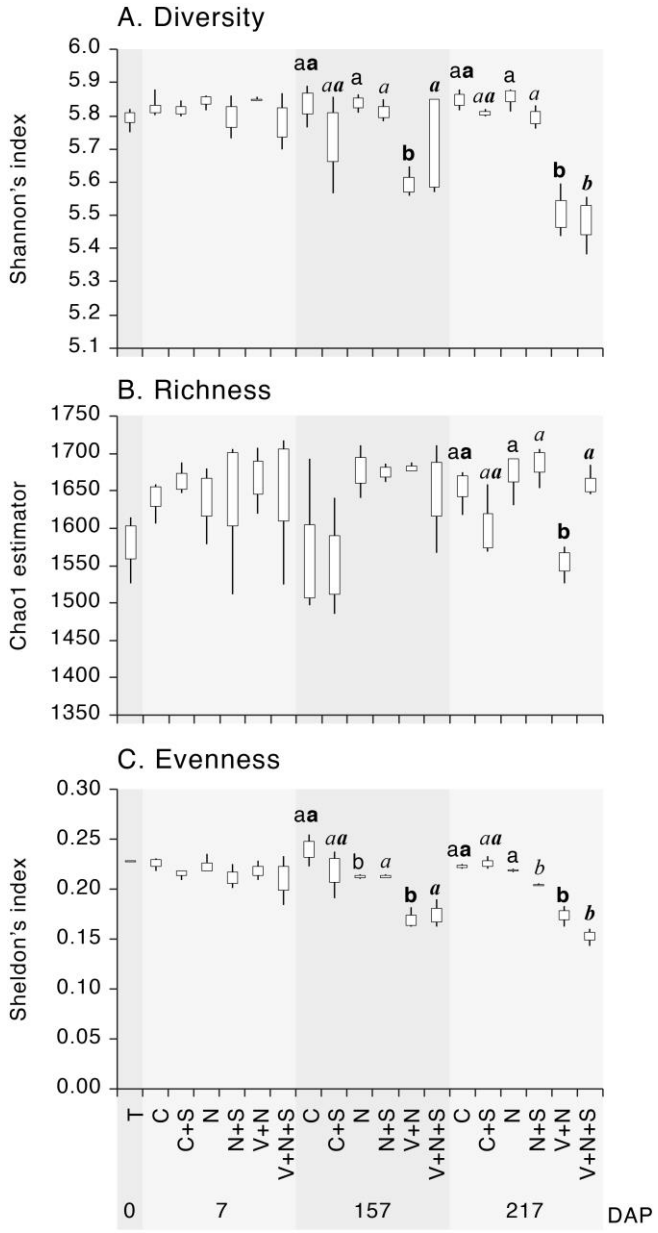

Figure 2. Diversity (A), richness (B) and evenness (C) of the total fungal community in soil cultivated with sugarcane under different experimental treatments over time in three applications of fertilizer. Tukey test (p <0.05) was conducted considering: nitrogen fertilization without straw vs. control without straw (standard small letters), nitrogen fertilization with straw vs. straw control (lowercase letters in italics), vinasse + nitrogen fertilization without straw vs. control without straw (bold small letters), vinasse + nitrogen fertilization with straw vs. straw control (lowercase letters in italics and bold). Mean values not accompanied by letters showed no statistical difference at the 5% level of significance.

Taken together, these results revealed negative effect of vinasse in combination with N fertilizer over time in three applications of these fertilizers on diversity, richness and evenness of the fungal community of sugarcane-cultivated soils with or without a straw blanket, excluding the richness in this amended soil with straw retention as a surface blanket. The soil microbial diversity has a positive correlation with the productivity and sustainability of a system (VAN DER HEIJDEN et al., 2008), and loss and simplification of soil community composition impair multiple ecosystem functions, including plant diversity, decomposition, nutrient retention, and nutrient cycling (WAGG et al., 2014). Soil microbial diversity, richness and evenness are sensitive to changes in land management practices, such as cropping systems, tillage and fertilization (HARTMANN et al., 2015; TANG et al., 2020).

These results concerning the ecological aspects of the fungal communities in our sugarcane soils corroborate results from previous studies that showed that soil fungal communities are susceptible to perturbations caused by nutrient amendment (HARTMANN et al., 2015; CASSMAN et al., 2016), with decreases in fungal biomass and diversity and alterations in fungal community composition (WALLENSTEIN et al., 2006; EDWARDS et al., 2011; PAUNGFOO-LONHIENNE et al., 2015). Thus, our hypothesis that the incorporation of vinasse and N as fertilizer into the soil and sugarcane straw-blanket increase the diversity, richness and evenness of soil fungal community was not supported based on the decreases in diversity and evenness of the fungal community of sugarcane-cultivated soils with or without a straw blanket, excluding the richness in this amended soil with straw blanket.

3.3. Soil fungal community structure and composition

To assess the effects of the repeated applications of fertilizer and experimental treatments on fungal community structure with their interactions, taxonomic profiles based on the 20 genera of fungi most abundantly detected in the soil samples were analyzed. It was not revealed effect for the repeated applications of fertilizer (time) neither for their interactions with the treatments on the soil fungal community structure (Table 1), with treatments nonclustered regarding the time (Figure 3). However,the abundance of *Amauroascus*, *Brettanomyces*, *Cantharellus*, *Chrysosporium* and *Puccinia* was affected by experimental treatments (Table 1).

Table 1. Repeated measures ANOVA (rANOVA) of the relative abundance of genera of fungi as a function of time (fertilizer applications), treatments and their interaction

Genera	Time		Treatment		Time x Treatment	
	F	*p*	F	*p*	F	*p*
Allomyces	0.75	0.478	1.26	0.301	0.74	0.684
Amauroascus	0.24	0.784	3.04	**0.021**	0.79	0.635
Aspergillus	1.13	0.335	1.25	0.306	0.88	0.563
Blastomyces	0.76	0.475	1.86	0.125	0.73	0.693
Brettanomyces	0.39	0.675	3.64	**0.009**	1.15	0.356
Cantharellus	0.05	0.954	2.65	**0.039**	0.85	0.581
Caulochytrium	1.36	0.268	1.28	0.291	0.77	0.648
Chrysosporium	0.48	0.621	2.81	**0.030**	0.73	0.689
Clavaria	0.44	0.647	1.92	0.115	0.80	0.626
Colletotrichum	1.31	0.283	1.37	0.258	0.72	0.701
Cutaneotrichosporon	0.81	0.452	1.79	0.139	0.66	0.754
Fusarium	0.67	0.519	2.20	0.075	1.58	0.152
Geotrichum	1.11	0.339	0.77	0.576	0.81	0.622
Grosmannia	1.13	0.334	1.46	0.227	0.63	0.775
Melampsora	0.52	0.598	1.82	0.133	0.69	0.727
Morchella	1.27	0.293	1.70	0.158	1.67	0.127
Penicillium	0.74	0.484	1.60	0.184	1.49	0.182
Puccinia	0.08	0.926	2.93	**0.025**	0.96	0.489
Rhodotorula	1.25	0.298	1.25	0.305	0.83	0.600
Tuber	0.35	0.704	2.47	0.050	0.75	0.673
Outros	0.75	0.481	1.34	0.270	0.75	0.669
Degrees of freedom (DF): Time: DF= 2; Treatment: DF = 5; e Time x Treatment : DF = 10						

Numbers in bold indicate statistical significance at 5% probability.

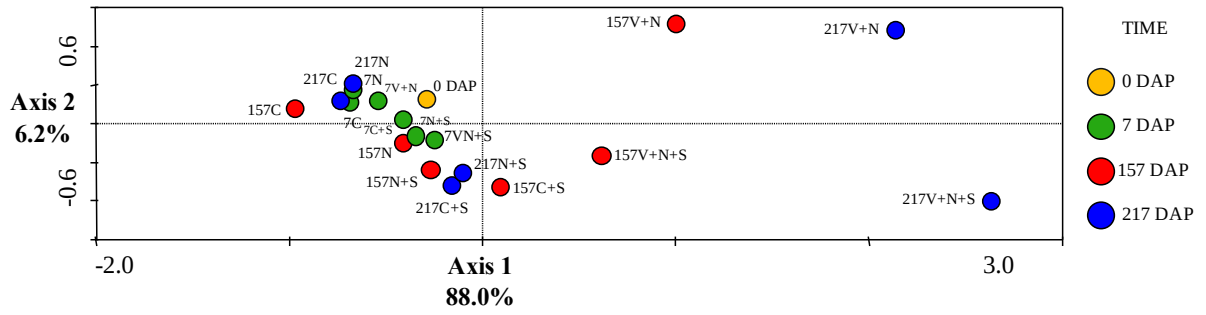

Figure 3. Principal component analysis (PCA) of the soil fungal community based on the 20 genera most abundant in the different treatments within each of the three applications of fertilizers

Analysis of the soil fungal community composition based on the 20 genera of fungi most abundant in the soil within each of the three applications of fertilizers revealed decrease in abundance for *Blastomyces*, *Melampsora* and *Penicillium* after the third application of vinasse in combination with N fertilizer and straw blanket (Table 2). An opposite response was revealed for *Amauroascus*, *Cantharellus*, *Chrysosporium*, *Clavaria*, *Morchella*, *Puccinia*, and *Tuber* in soils under this treatment (Table 2).

The application of sugarcane vinasse in the soil causes changes in abundance of soil microbial taxonomical groups (CHRISTOFOLETTI et al., 2013). Camargo (1954) observed an increase in the fungal abundance in soils amended with vinasse, with predominance of *Neurospora* spp, *Aspergillus* spp, *Penicillum* spp, and *Mucor* spp. Santos et al. (2009) also reported that the addition of vinasse into the soil can significantly alter the population of fungi in this environment. Leal et al. (1983) observed that the increase in soil pH after the application of vinasse may be associated with the development of the microbial populations and the transformation of N during the denitrification process of nitrate into nitrite.

Table 2. Relative abundance of the 20 genera of fungi most abundant in soil cultivated with sugarcane and of the other genera of fungi detected and collapsed as 'others' in the different treatments and sample time

Genera	Before fertilizer application	First application of fertilizer (7 DAP)				Control	
		N	N+S	V+N	V+N+S	C	C+S
Allomyces	0.42±0.13	0,46±0.14	0.47±0.32	0.45±1.12	0.49±0.34	0.48±0.17	0.49±0.27
Amauroascus	1.32±0.24	1.24±0.52	1.30±0.79	1.13±0.29	1.32±0.79	1.21±0.48	1.24±0.61
Aspergillus	2.88±0.59	2.90±0.99	2.88±1.78	3.00±1.19	2.84±1.88	2.88±0.98	3.06±1.63
Blastomyces	2.19±0.59	2.07±0.80	2.19±1.29	2.10±0.78	2.21±1.37	2.09±0.86	2.07±1.08
Brettanomyces	1.53±0.26	1.54±0.49	1.37±0.93	1.51±0.50	1.51±0.97	1.35±0.48	1.37±0.75
Cantharellus	9.03±1.73	8.98±3.36	8.98±5.70	8.73±3.51	8.99±5.69	8.90±2.97	8.98±4.59
Caulochytrium	0.41±0.09	0.39±0.15	0.40±0.26	0.38±0.16	0.40±0.26	0.43±0.19	0.40±0.18
Chrysosporium	1.10±0.20	1.07±0.46	0.97±0.65	1.03±0.35	1.01±0.70	1.03±0.38	1.02±0.40
Clavaria	4.30±0.89	4.40±1.79	4.35±2.79	4.41±1.68	4.53±2.85	4.38±1.55	4.51±2.09
Colletotrichum	2.40±0.68	1.87±0.73	2.26±1.47	2.16±1.16	1.86±1.22	2.10±0.69	1.97±1.00
Cutaneotrichosporon	2.98±0.44	2.96±0.81	3.00±1.98	2.69±0.84	3.02±2.04	2.70±1.15	2.65±1.44
Fusarium	0.83±0.17	0.83±0.22	1.09±0.64	1.16±0.40	0.88±0.53	0.80±0.27	0.90±0.55
Geotrichum	1.40±0.41	1.49±0.80	1.55±0.87	1.76±0.76	1.51±0.98	1.81±0.47	1.89±0.84
Grosmannia	0.45±0.16	0.48±0.18	0.43±0.30	0.41±0.16	0.44±0.29	0.44±0.17	0.43±0.21
Melampsora	1.97±0.56	1.87±0.75	2.02±1.26	1.82±0.77	1.94±1.30	1.86±0.57	1.96±1.10
Morchella	0.66±0.13	0.66±0.29	0.68±0.43	0.65±0.26	0.73±0.43	0.65±0.20	0.69±0.34
Penicillium	1.92±0.38	1.85±0.67	1.90±1.21	2.03±0.72	1.78±1.20	1.95±0.82	1.88±0.88
Puccinia	5.12±0.97	4.93±1.84	4.68±2.94	4.83±1.66	4.91±3.07	4.78±1.63	4.84±2.35
Rhodotorula	1.33±0.36	1.28±0.49	1.32±0.86	1.28±0.50	1.35±0.94	1.37±0.51	1.36±0.68
Tuber	4.84±0.90	4.67±1.97	5.23±3.07	5.00±2.01	5.49±3.43	4.80±1.53	5.10±2.59
Others	52.92±12.06	54.05±20.94	52.94±34.23	53.47±19.70	52.80±35.49	53.97±20.03	53.20±27.37

Tukey test ($P < 0.05$) was conducted considering: nitrogen fertilization without straw vs. control without straw (standard small letters), nitrogen fertilization with straw vs. straw control (lowercase letters in italics), vinasse + nitrogen fertilization without straw vs. control without straw (bold small letters), vinasse + nitrogen fertilization with straw vs. straw control (lowercase letters in italics and bold). Mean values not accompanied by letters showed no statistical difference at the 5% level of significance. Comparisons are interpretable on the lines.

Table 2. Continuation

Genera	Second application of fertilizer (157 DAP)				Control	
	N	N+S	V+N	V+N+S	C	C+S
Allomyces	0.48±0.22	0.50±0.16	0.39±0.04	0.40±0.29	0.52±0.45	0.43±0.29
Amauroascus	1.25±0.54	1.19±0.27	1.35±0.24	1.31±0.98	1.18±0.99	1.23±0.77
Aspergillus	2.78±1.19	2.76±0.66	2.71±0.53	2.50±2.06	2.81±2.36	2.88±1.65
Blastomyces	2.14±0.85	2.09±0.53	1.87±0.28	2.03±1.52	2.10±1.73	2.12±1.25
Brettanomyces	1.41a±0.46	1.26*a*±0.27	2.43**a**±0.92	1.99***a***±1.66	1.37a**b**±1.14	1.14***aa***±0.58
Cantharellus	9.28±3.96	9.40±1.83	10.17±2.49	10.24±7.74	8.69±7.59	9.65±5.08
Caulochytrium	0.38±0.14	0.39±0.12	0.32±0.04	0.31±0.23	0.42±0.41	0.34±0.25
Chrysosporium	0.94±0.30	0.99±0.30	0.96±0.12	1.16±0.88	0.87±0.83	0.93±0.37
Clavaria	4.41±1.87	4.43±0.88	4.42±0.95	4.65±3.71	4.19±3.47	4.47±2.54
Colletotrichum	1.86±0.78	2.02±0.58	1.56±0.32	1.57±1.17	1.97±1.68	1.97±1.17
Cutaneotrichosporon	2.78±1.30	3.03±0.80	2.49±0.16	2.40±1.63	3.09±2.55	2.85±1.71
Fusarium	0.78±0.35	0.89±0.24	1.45±0.59	0.98±0.88	0.88±0.80	0.82±0.44
Geotrichum	1.59±0.70	1.48±0.40	1.26±0.49	1.52±1.50	1.47±1.50	1.59±1.10
Grosmannia	0.39±0.15	0.44±0.08	0.36±0.05	0.39±0.30	0.44±0.41	0.38±0.31
Melampsora	2.02±0.89	2.16±0.57	1.59±0.17	1.82±1.25	1.94±1.62	2.11±1.28
Morchella	0.68±0.31	0.70±0.17	1.29±0.62	0.87±0.69	0.71±0.61	1.34±0.94
Penicillium	1.92±0.84	1.96±0.48	3.24±1.98	1.93±1.62	1.91±1.73	1.85±1.31
Puccinia	4.78±1.74	4.55±1.04	6.18±1.54	5.53±4.36	4.54±3.90	4.79±2.12
Rhodotorula	1.31±0.53	1.26±0.30	1.08±0.15	1.06±0.77	1.34±1.21	1.21±0.83
Tuber	5.43±2.51	5.58±1.10	4.81±0.32	6.21±4.48	4.85±4.13	5.87±3.43
Others	53.39±23.17	52.91±12.71	50.08±10.65	51.15±42.76	54.69±48.90	52.02±33.08

Tukey test ($P < 0.05$) was conducted considering: nitrogen fertilization without straw vs. control without straw (standard small letters), nitrogen fertilization with straw vs. straw control (lowercase letters in italics), vinasse + nitrogen fertilization without straw vs. control without straw (bold small letters), vinasse + nitrogen fertilization with straw vs. straw control (lowercase letters in italics and bold). Mean values not accompanied by letters showed no statistical difference at the 5% level of significance. Comparisons are interpretable on the lines.

Table 2. Continuation

Genera	Third aplication of fertilizer (217 DAP)				Control	
	N	N+S	V+N	V+N+S	C	C+S
Allomyces	0.49±0.13	0.46±0.21	0.43±0.19	0.41±0.14	0.51±0.23	0.48±0.10
Amauroascus	1.17a±0.29	1.26*a*±0.54	1.99**a**±0.72	1.82***a***±0.49	1.10a**a**±0.33	1.30*a****b***±0.31
Aspergillus	3.01±0.87	2.77±1.22	2.53±0.84	2.51±1.00	2.84±1.20	2.72±0.57
Blastomyces	2.18a±0.63	2.12*a*±0.87	2.13**a**±0.69	2.10***b***±0.65	2.02a**a**±0.79	2.16*a****a***±0.34
Brettanomyces	1.52a±0.44	1.16*a*±0.47	2.55**a**±1.47	1.69***a***±0.42	1.31a**a**±0.40	1.13*a****b***±0.16
Cantharellus	8.78a±2.11	9.66*a*±3.51	12.16**a**±2.19	13.02***a***±2.89	9.00a**a**±3.43	10.13*a****b***±2.12
Caulochytrium	0.42±0.09	0.38±0.15	0.33±0.10	0.33±0.13	0.41±0.17	0.40±0.03
Chrysosporium	0.97a±0.15	1.02*a*±0.21	1.21**a**±0.52	1.36***a***±0.35	0.98a**a**±0.44	0.90*b****b***±0.15
Clavaria	4.25a±1.06	4.62*a*±1.63	4.52**a**±1.17	4.92***a***±1.53	4.36a**a**±1.56	4.60*a****b***±0.98
Colletotrichum	1.94±0.52	2.30±1.51	1.60±0.57	1.63±0.56	1.96±0.80	1.88±0.33
Cutaneotrichosporon	2.88±0.54	2.91±1.12	2.62±1.20	2.70±0.84	2.71±1.09	3.04±0.69
Fusarium	0.95±0.30	1.04±0.64	0.80±0.26	0.84±0.33	0.89±0.45	0.79±0.16
Geotrichum	1.57±0.65	1.61±0.78	1.64±0.44	1.38±0.60	1.85±0.84	1.32±0.16
Grosmannia	0.45±0.09	0.45±0.19	0.40±0.14	0.35±0.11	0.44±0.19	0.41±0.04
Melampsora	1.83a±0.39	2.01*a*±0.76	1.66**a**±0.43	2.09***b***±0.65	1.88a**a**±0.80	2.16*a****a***±0.41
Morchella	0.66a±0.17	0.74*a*±0.25	0.70**a**±0.09	0.78***a***±0.22	0.70a**a**±0.29	0.72*b****b***±0.11
Penicillium	1.89a±0.40	1.88*a*±0.80	1.80**a**±0.70	1.73***b***±0.58	1.94a**a**±0.72	1.78*a****a***±0.31
Puccinia	4.97a±1.27	4.50*a*±1.48	7.39**a**±2.96	6.51***a***±1.93	4.80a**a**±1.43	4.44*b****b***±0.76
Rhodotorula	1.42±0.37	1.24±0.50	1.19±0.47	1.07±0.35	1.34±0.52	1.33±0.24
Tuber	4.70a±1.35	5.41*a*±2.38	4.39**a**±0.89	6.13***a***±2.16	4.77a**a**±2.17	5.39*a****b***±0.86
Others	53.94±14.14	52.47±21.50	47.96±17.48	46.61±15.97	54.19±21.72	52.91±9.51

Tukey test (P <0.05) was conducted considering: nitrogen fertilization without straw vs. control without straw (standard small letters), nitrogen fertilization with straw vs. straw control (lowercase letters in italics), vinasse + nitrogen fertilization without straw vs. control without straw (bold small letters), vinasse + nitrogen fertilization with straw vs. straw control (lowercase letters in italics and bold). Mean values not accompanied by letters showed no statistical difference at the 5% level of significance. Comparisons are interpretable on the lines.

The organic matter from vinasse is an important source of soluble carbon, as glycerol, readily available to microorganisms (PRATA et al., 2001). The vinasse promotes immediate soil acidification, favoring the development of fungi, which are the microorganisms responsible for the early stages of the decomposition process (LAIME et al., 2011). The annual decomposition rate of straw typically ranges from 60% to 98% throughout the crop season (FORTES et al., 2012; CARVALHO et al., 2017), and the amount of sugarcane straw at different time points is expected to vary (OLIVEIRA et al., 1999; FORTES et al., 2012; CARVALHO et al., 2017; VARANDA et al., 2019), as are the different fungal functional groups, especially those decomposer of lignin, cellulose and hemicellulose (FORTES et al., 2012; RACHID et al., 2016). In addition, vinasse may act as a primer upon addition to soil by decreasing the C/N ratio (SILVA et al., 2013) and thus accelerating the changes in fungal community structure.

The use of N mineral fertilizer with the addition of vinasse provided an increase in the fungal genera that have beneficial functions for the soil and, consequently, for the crop (Table 3). Among the 20 most abundant genera in our sugarcane soils, ten of them differed statistically depending on the treatments. Regarding the genera that had an increase in abundance due to the application of vinasse and N as fertilizers, 85.71% are decomposers, mycorrhizal, oleaginous, or participate of fermentation processes. The *Puccinia* genus was the only pathogen that increased with the repeated application of vinasse and nitrogen as fertilizers. This fungal genus is responsible for transmitting sugarcane rust. However, our metagenomic data did not identify sequences belonging to the species *Puccinia melanocephala* and *Puccinia kuehnii*, that cause sugarcane rust.

The dominance of fungi genera indicates good species adaptation to the soils of sugarcane cultivation (RAMOS et al., 2018). According to Alves et al. (2011), the biological soil fraction is dynamic and affected by agricultural pratics. The main factors that have a direct influence on some populations are temperature, pH, and carbonand phosphorus levels (RAMOS et al., 2018). The release of nutrients from plant residues depends on their quality and on the activity of microorganisms in the soil (ASSIS et al., 2006). Microbial biomass controls N availability through mineralization and immobilization processes (BARRETO et al., 2008). Residues with a high C/N ratio show slower decomposition (SILVA; RESCK, 1997), and low amounts of N can generate competition between microbial biomass and plant roots, harming crop development (ASSIS et al., 2006). This fact is due to amounts of N present in these materials is noting enough for the decomposition of the straw, which leads to

the microbial immobilization of this nutrient in the soil. The application of N fertilizers reduces this effect during the decomposition of residues that have a high C/N ratio by microbial biomass (ASSIS et al., 2006).

Table 3. Functions of the 20 genera of fungi most abundant in sugarcane-cultivated soil

Genera	Principal Genera Function	Reference
Amauroascus *A. niger* *A. mutatus*	Decomposer	(MUÑOZ, J. F. et al., 2018)
Blastomyces *B. dermatitidis* *B. gilchristii* *B. parvus* *B. percursus* *B. sp. MA-2018*	**Pathogen (human)**	(MCBRIDE, J. A. et al., 2019)
Cantharellus *C. appalachiensis* *C. cibarius* *C. cinnabarinus* *C. lutescens*	Mycorrhizal	(SOKOVIĆ, M. et al., 2018); (BRUNDETT, M. et al., 1996);
Chrysosporium *C. queenslandicum*	Decomposer	(KANALY, R. A.; HUR, H.-G., 2005)
Clavaria *C. fumosa*	Decomposer	(FURTADO, A. N. M. et al., 2016)
Melampsora *M. abietis-canadensis* *M. aecidioides* *M. allii-populina* *M. larici-populina* *M. medusae* *M. occidentalis* *M. pinitorqua*	**Pathogen (plant)**	(DEAN, R. et al., 2012); (DUPLESSIS, S. et al., 2011)
Morchella *M. septimelata*	Mycorrhizal	(SOKOVIĆ, M. et al., 2018)

Functions in underline and bold indicate fungal genera that increased (underline) and decreased (bold) the abundance after the third application of vinasse in combination with nitrogen fertilizer and straw blank.

Table 3. Continuation

Genera	Principal Genera Function	Reference
Penicillium		
P. antarcticum		
P. arizonense		
P. brasilianum		
P. capsulatum		
P. carneum		
P. citrinum		
P. coprophilum		
P. decumbens		
P. digitatum		
P. expansum		
P. flavigenum		
P. freii		
P. fuscoglaucum		
P. griseofulvum		
P. italicum		
P. janthinellum	**Pathogen (human and plant), decomposer**	(FORTES, C. et al., 2012); (OLIVEIRA, I. S. et al., 2006)
P. nordicum		
P. oxalicum		
P. paneum		
P. paxilli		
P. polonicum		
P. roqueforti		
P. rubens		
P. sclerotiorum		
P. sp. HKF2		
P. sp. MA 6036		
P. sp. MA 6040		
P. sp. MT2 MMC-2018		
P. sp. 'occitanis'		
P. sp. SPG-F1		
P. sp. SPG-F15		
P. steckii		
P. subrubescens		
P. vulpinum		
Puccinia		
P. horiana		
P. sorghi	<u>Pathogen (plant)</u>	(AIME, M. C. et al., 2017); (DEAN, R. et al., 2012)
P. striiformis		
P. triticina		
Tuber		
T. melanosporum	<u>Mycorrhizal</u>	(SOKOVIĆ, M. et al., 2018); (BRUNDETT, M. et al., 1996);
T. microsphaerosporum		
T. umbilicatum		

Functions in underline and bold indicate fungal genera that increased (underline) and decreased (bold) the abundance after the third application of vinasse in combination with nitrogen fertilizer and straw blank.

Among the genera that had an increase with the treatments that contained vinasse, we have *Cantharellus*, *Morchella* and *Tuber* as being mycorrhizal. Azevedo (2008) reported the existence of a symbiosis between mycorrhizal fungi and sugarcane. Kelly et al. (2001) concluded that, when there is a sufficient number of mycorrhizal fungi propagules in the soil, fertilization with phosphatic fertilizer, for the conditions evaluated, is only necessary when the availability of phosphorus is less than 30 mg kg^{-1} (H_2SO_4 0.005 M), while in the absence of mycorrhizal fungi propagules, this level rises to 47 mg kg^{-1} of phosphorus.

In sugarcane, the straw decomposition process is slow, due to the high C/N ratio of this residue (COTRUFO et al., 2009), and accelerating it would bring benefits to the current production system, making nutrients available in periods use of the crop in the same harvest. For this, the action of decomposing microorganisms needs to be potentiated. In this work, we have as decomposers the genera *Amauroascus*, *Chrysosporium*, *Clavaria*. This response to these genera mentioned occurred 250 days after the straw retention on the surface soil. Those genera of decomposers fungi that have had this rapid increase in a short period of time certainly benefit from most readily available forms of carbon. These genera of soil fungi had an increase with the application of vinasse combined with N fertilizer. The enhancement of these fungal community member simply a better breakdown of both cellulose, which constitutes about half to a third of plant tissues, and lignin, a polymer that makes cells and tissues rigid and difficult to decompose (RAES et al., 2003; CABANÉ et al., 2004; TAIZ & ZEIGER, 2004).

According to the literature, organic vinasse amendment mainly increases the abundance of saprotrophs, species capable of fungal denitrification (LOURENÇO et al., 2020), and fungi capable of producing extracellular enzymes (CAESAR-TONTHAT, 2002; DAYNES et al., 2012). Some members utilize labile carbon resulting from organic matter decomposition as a nutrient (BÖDEKER et al., 2016; NGUYEN et al., 2016). Previous results indicate that the nutrient-rich content of vinasse (organic carbon, organic N, and potassium) favors oligotrophic fungi (PÖGGELER, 2011; ENTWISTLE et al., 2013; HO et al., 2017). It alters trophic guilds related to saprotrophic, fungal and copiotrophic denitrification and oligotrophic fungi (LOURENÇO et al., 2020).

Considering the genera of fungi that showed a decrease after vinasse and N-fertilizer application to the soil in this study, *Blastomyces*, *Melampsora* and *Penicillium* are potentially pathogenic fungi. Among the known fungal species, two-thirds of them establish parasitic,

commensalistic or mutualistic relationships with other living organisms (BARBIERI; CARVALHO, 2001). Some fungi are well known due to the diseases that they cause in plants, devastating large crops (JAROSZ; DAVELOS, 1995; THORN, 1997). Among the most studied are *Colletotrichum falcatum*, which causes red rot; *Ustilago scitaminea*, which causes coal; *Leptosphaeria sacchari*, which causes ring spot; *Bipolaris sacchari*, which causes eye spot; *Puccinia melanocephala*and *Puccinia kuehnii*, which causesrust; *Fusarium moniliforme*, causing "*Fusarium*rot" and "pokkah-boeng" and *Thielaviopsis paradoxa*, causing pineapple rot.

The genus *Penicillium* showed the greatest diversity in species among all the top 20 most abundant fungal genera in the soil in this study. Romão (2010) found a predominance of 33% of this genus in experiments on root and rhizosphere isolation of sugarcane plants. However, in addition to its pathogenic action, this genus may have other non-disease related functions, such as phosphate solubilization (KUCEY, 1987; REYES et al., 2002). Whitelaw (2000) reported this potential for several species belonging to this genus. This potential was also reported by Pradhan and Sukla (2005), with the genus *Penicillium* solubilizing phosphate in a culture medium in the presence of C and N sources. Phosphorus in the soil is available, in its majority (95 to 99%), in its insoluble form, which cannot be used directly by the plants (PRADHAN; SUKLA, 2005). Hence, it is importante develop studies focusing on the potentiation of the phosphorus availability to the plants, considering the higher capacity of the Fungi to solubilize inorganic phosphorus than bacteria (NAHAS, 1996).

In this sense, our hypothesis concerning changes in soil fungal community structure and composition over time in repeated applications of vinasse and N as fertilizer was partially accept based on changes in soil fungal community composition but not on its structure. Increases in fungal genera that have beneficial functions for the soil achieved after repeated applications of these fertilizers and straw retention as a surface blanket lead us to accept the third hypothesis concerning to the favoring the occurrence of beneficial soil-borne fungi.

4. CONCLUSION

In conclusion, our results support decreases in fungal diversity, evenness and richness at the community level in soil due to repeated application of vinasse and incorporation of N as fertilizer into the soil, with this negative effect on both diversity and evenness when straw

was retained as a surface blanket as well. Shifts in fungal community composition were followed by increases in mycorrhizal and decomposers soil-borne fungi,and decreases in potentially pathogenic fungi, but not by changes in community structure after repeated combined applications of vinasse and mineral N fertilizer and straw retention as a surface blanket. Our findings based on a short-term greenhouse experiment provide the initial attempt to understand how ecological aspects of soil fungal community are affected by repeated applications of organic and inorganic fertilizers and retention of crop residues into sugarcane-cultivated soils.

5. REFERENCES

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Supplementary material

Table S1. File size in megabytes and number of sequencing reads obtained for each treatment over time in the greenhouse experiment

Fertilizing	Treatments	Size megabytes*	N. of merged sequence reads*‡	N. of not merged sequence reads*
Before fertilizer amendment				
		51.98 ± 8.68	77,278 ± 12,057	65,147 ± 10,801
First fertilizer amendment (0 DAP)				
	N	105.21 ± 33.43	170,571 ± 36,449	115,515 ± 40,043
	N+S	146.58 ± 77.49	307,954 ±145,974	137,797 ± 78,930
	N+V	129.67 ± 39.40	285,914 ± 98,865	119,885 ± 33,686
	N+V+S	145.81 ± 78.09	149,788 ±115,593	148,739 ± 79,535
	C	93.07 ± 26.39	90,930 ± 56,480	140,029 ± 55,345
	C+S	122.12 ± 50.39	212,817 ±116,025	130,531 ± 39,513
Second fertilizer amendment (150 DAP)				
	N	143.83 ± 52.41	161,646 ± 45,267	191,277 ± 74,958
	N+S	103.24 ± 19.73	145,699 ± 51,650	123,819 ± 33,647
	N+V	115.95 ± 13.70	315,227 ± 24,330	99,072 ± 14,172
	N+V+S	112.50 ± 66.47	243,234 ± 21,739	112,128 ± 49,951
	C	77.94 ± 55.83	83,454 ± 47,730	94,744 ± 68,511
	C+S	56.89 ± 28.75	77,271 ± 20,182	71,516 ± 44,002
Third fertilizer amendment (210 DAP)				
	N	131.68±28.87	268,238 ± 87,468	119,388 ± 15,553
	N+S	136.25±45.29	280,168 ±142,636	121,574 ± 25,975
	N+V	55.16±14.78	76,737 ± 7,995	65,121 ± 23,398
	N+V+S	110.95±31.71	216,580 ± 78,245	101,667± 26,214
	C	108.56±36.09	140,470 ± 88,178	112,154 ± 32,263
	C+S	60.47±8.97	71,796 ± 20,310	78,546 ± 8,660

DAP = days after planting

* Post quality control on SeqClean script

‡ After merge paired reads using FLASH

Table S2. Chemical factors of the soil in the 0-20 cm surface layer in different experimental treatments 150 days after planting of sugarcane in the mesocosms

Factors from soil	Before fertilizer application	Treatments				Control	
		N	N+S	V+N	V+N+S	C	C+S
pH	5.23±0.06	5.23a ± 0.15	5.13*a* ± 0.06	5.60**a** ± 0.00	5.43***a*** ± 0.06	5.23a**b** ± 0.06	5.17***ab*** ± 0.06
P	61.00±17.69	99.67a ± 12.66	51.00*b* ± 16.46	90.00**a** ± 12.12	84.67***a*** ± 15.57	61.00b**a** ± 17.69	105.33***aa*** ± 27.65
S	6.33±1.53	11.67a ± 0.58	7.33*b* ± 1.15	183.67**a** ± 16.50	66.00***a*** ± 40.85	6.33b**b** ± 1.53	12.00***aa*** ± 2.00
K	1.23±0.06	1.13a ± 0.06	1.23*a* ± 0.06	11.43**a**± 0.92	4.90***a*** ±1.08	1.23a**b** ± 0.06	1.57***ab*** ± 0.21
Ca	54.00±2.65	55.00a ± 1.00	51.67*a* ± 3.06	48.67**a** ± 3.51	49.00***b*** ± 2.00	54.00a**a** ± 2.65	56.67***aa*** ± 2.08
Mg	16.67±1.53	17.33 ± 0.58	17.00 ± 1.00	18.67 ± 0.58	17.67 ± 1.15	16.67 ± 1.53	17.00 ± 0.00
H+Al	43.67±2.89	44.00 ± 5.20	50.33 ± 2.89	34.00 ± 0.00	40.67 ± 2.31	43.67 ± 2.89	47.00 ± 0.00
SB	71.53±3.94	73.03 ± 1.92	69.47 ± 2.85	79.17 ± 4.85	71.57 ± 3.10	71.53 ± 3.94	74.87 ± 2.21
CTC	115.30±6.22	116.97a ± 3.42	119.83*a* ± 4.76	113.33**a** ± 4.90	112.37***b*** ± 4.56	115.30a**a** ± 6.22	121.77***aa*** ± 2.21
V%	61.67±1.15	62.33a ± 3.21	57.67*b* ± 1.53	70.00**a** ± 1.00	63.67***a*** ± 1.53	61.67a**b** ± 1.15	61.33***aa*** ± 0.58
B	0.22±0.04	0.22a ± 0.01	0.26*a* ± 0.05	0.16**b** ± 0.04	0.26***a*** ± 0.03	0.24a**a** ± 0.04	0.26***aa*** ± 0.04
Cu	0.93±0.06	0.93 ± 0.06	0.83 ± 0.06	2.23 ± 2.31	0.83 ± 0.06	0.93 ± 0.06	0.93 ± 0.06
Fe	37.00±3.61	37.00 ± 4.00	33.33 ± 2.31	48.33 ± 19.60	35.00 ± 1.00	37.00 ± 3.61	38.33 ± 3.51
Mn	7.40±0.50	7.47a ± 1.12	8.10*a* ± 1.90	21.03**a** ± 3.78	25.50***a*** ± 1.21	7.40a**b** ± 0.50	7.70***ab*** ± 0.89
Zn	1.97±0.50	1.70 ± 0.10	1.60 ± 0.35	2.27 ± 1.48	2.23 ± 1.01	1.97 ± 0.50	1.67 ± 0.15
C	2.04±0.07	2.29a ± 0.03	2.21*a* ± 0.07	2.59**a** ± 0.07	2.48***a*** ± 0.07	2.24a**b** ± 0.05	2.33***ab*** ± 0.04
N	0.18±0.01	0.19a ± 0.01	0.17*a* ± 0.01	0.26**a** ± 0.02	0.24***a*** ± 0.01	0.19a**b** ± 0.01	0.18***ab*** ± 0.01
C/N	11.63±0.22	11.96a ± 0.55	12.73*a* ± 0.29	9.94**b** ± 0.53	10.47***b*** ± 0.21	11.98a**a** ± 0.37	12.95***aa*** ± 0.16

Tukey test (P <0.05) was conducted considering: nitrogen fertilization without straw vs. control without straw (standard small letters), nitrogen fertilization with straw vs. straw control (lowercase letters in italics), vinasse + nitrogen fertilization without straw vs. control without straw (bold small letters), vinasse + nitrogen fertilization with straw vs. straw control (lowercase letters in italics and bold). Mean values not accompanied by letters showed no statistical difference at the 5% level of significance. Comparisons are interpretable on the lines.