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Full Length Research Paper

Assessment of fungi in soils of sugarcane crops and their potential for production of biomass-degrading enzymes

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Soil management practices are known to affect the biomass and enzyme activities of microbial soil communities. To assess whether burning of sugarcane prior to harvesting affects the community of soil-borne fungi, we collected soil samples in two sites: burned sugarcane culture prior harvesting (BS) and non-burned sugarcane culture (NBS). A total of 75 filamentous fungal isolates were recovered from soils in both sites. *Trichoderma* was the most prevalent genus in both sites, followed by *Fusarium*, *Cunninghamella* and *Aspergillus.* The Sorensen's index (0.62) suggested a slight difference in fungi associated with both areas, with high number of fungal isolates found on BS soil. The abundance of *Trichoderma* isolates in NBS soil was higher than BS soil; however, the abundance of *Fusarium*, *Aspergillus* and *Cunninghamella* was higher in the latter type of soil. In addition, fungi isolated from BS soil showed the highest production of xylanase and laccase in comparison with fungi derived from NBS soil. Our results indicate that the different types of sugarcane harvesting apparently did not interfere with the diversity of fungal communities as revealed by culture-dependent methods. In addition, our data indicates the potential of fungi from soils of sugarcane crops to produce relevant enzymes related to biomass conversion.

Key words: Culture-dependent analysis, harvest system, agriculture residues, biotechnological potential.

INTRODUCTION

Ethanol as an alternative energy source to petroleum-based fuels has been encouraged in Brazil since the 1970's. Due to suitable climate, available agricultural land and fertile soils, cultivation of sugarcane for ethanol production reached 600 million tons at 2014 (Union of Sugarcane Industry of São Paulo- UNICA). This dramatic increase in the sugar and ethanol sectors may entail significant changes in cultivated soils resulting from

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management practices and frequent use of pesticides (Viana and Perez, 2013).

The sustainability of soil ecosystems depends on the microorganisms which are responsible for the net fluxes of carbon soil through organic matter degradation. In general, the integrity of soil microbial communities can be used as indicator of changes in soil properties (Mataix-Solera et al., 2009). The abundance and activity of the soil microbiota may be directly affected by several environmental factors, thus highlighting the need for studies focused on genetic and functional diversity in such communities. Regarding the latter, different types of soil management disturbs functional microbial diversity (Yang et al., 2006; Capelle et al., 2012). The various soil management practices are also known to affect the microbial biomass and enzyme activities such as urease, phosphatase, deaminase and plant cell wall degrading enzymes like cellulase, xylanase and ligninase (Ajwa et al., 1999; Fioretto et al., 2002; Barreiro et al., 2010).

Although sugarcane burning is no longer a concern in Brazil, since all cane cutting has been mechanized (Viana and Perez, 2013), burning is still widely used in some countries such as South Africa and Nigeria (Are et al., 2009; Smithers, 2014). The practice of burning the sugarcane before harvesting facilitates manual cutting by removing leaves and insects (Thorburn et al., 2001); however, it results in modifications of the soil's physical, chemical, biological and hydrological properties (Cerri et al., 2007; Are et al., 2009).

In particular, fire may directly cause microbial mortality or indirectly induce changes in microbial community through modification of the physical/chemical properties and interactions with other soil organisms (Widden and Pakkinson, 1975; Certini, 2005). On the other hand, the mechanical sugarcane harvest causes soil compaction, resulting in a decrease in the dissolved oxygen, soil porosity and nutrient mobility (Blair, 2000; Braunack et al., 2006), ultimately reducing crop yield and fungal growth.

Information regarding the effects of periodic practices of burning on sugarcane crops has been generalized as microbial biomass determination (Graham et al., 2002; Galdos et al., 2009; Souza et al., 2012). Knowledge on the microbial communities and their activities can improve the understanding of the role played by microbes in soil communities. Here, we compared the effects of two types of sugarcane harvesting, burning and mechanical, on the functional role of the major fungal taxa found in soil. Fungi associated with oils under two types of sugarcane harvesting were isolated and the potential of these strains in the production of enzymes involved in biomass degradation was evaluated.

MATERIALS AND METHODS

Sampling

Soil samples were collected at two distinct sites from a sugarcane crop located in José Bonifácio, São Paulo State, Brazil (GPS: 21°03'S; 49°42'W). Each site was under different crop managements since 2005: (i) sugarcane was burned before harvesting (BS- dystrophic haplicgleysol soil) and (ii) green harvest (non-burned sugarcane, NBS- dystrophic red latosol soil). In each site, soil samples were collected four times across crop stages: (1) before soil preparation; (2) during soil preparation (tillage: when fertilizers and herbicides were applied); (3) mature culture and (4) after harvesting the sugarcane. In the case of stage #2, the herbicide mixture consisted of Velpar-k® (Diuron plus Hexazinone), Ametrine® (Triazine) and Msma® (Arsenical).

All soil samples were collected at three different points and pooled in order to obtain a composite sample. Thus, a total of four pooled samples were obtained from each area (representing the four crop stages). Approximately 1 g of surface soil (0 - 5 cm) was collected with a small shovel and transferred to vials containing sterilized culture medium. The samples were stored in boxes at room temperature and delivered directly to the incubator at 28 ± 2°C.

Isolation and identification of soil fungi

One gram of soil was inoculated in four types of liquid medium containing different carbon sources: glucose, wheat bran, sugarcane bagasse or filter paper as carbon sources. A variety of carbon substrates were employed due to the different responses of extracellular enzyme production by the fungi (Massadeh et al., 2010; Schneider et al., 2014). A modified version of the culture medium formulated by Kirk et al. (1978) was used (in g L^{-1} at pH 5.0): 5.0 of carbon source, 1.4 of $(NH_4)_2SO_4$, 2.0 of KH_2PO_4, 0.03 of $CaCl_2$, 0.02 of $MgSO_4.7H_2O$, 0.4 of peptone, 0.1 of Tween 80 and 0.5 mL of mineral solution (in g L^{-1}: 0.22 of $ZnSO_4.7H_2O$; 1.0 of H_3BO_3; 0.5 of $MnCl_2.4H_2O$, 0.5 of $FeSO_4.7H_2O$; 0,16 of $CoCl_2.5H_2O$; 0.16 of $CuSO_4.5H_2O$ and 0.11 of $(NH_4)Mo_7O_{24}.4H_2O$; 5.0 of EDTA). The antibiotics, ampicillin and chloramphenicol (200 µg L^{-1}) were supplemented to the media. All media were incubated at 28 ± 2°C for 72 h and a loop full of the homogenized culture was streaked on a Petri dish containing the solid version of the same medium and incubated at 28 ± 2°C for 24 to 72 h. Fungal isolates were subcultured until pure cultures were obtained. All strains were maintained on potato dextrose agar (PDA) slants and under mineral oil and were kept at room temperature.

Fungal isolates were first screened in order to group them into morphospecies. Fungal identification was carried out using morphological markers (colony macroscopic characters as well as micromorphology of asexual reproductive structures). Classical taxonomic treatments were used to carry out the identification (Domsch et al., 1980; Samson et al., 2000; Kirk et al., 2001).

Enzyme production in solid-state fermentation (SSF)

Five grams of mixtures of wheat bran and sugarcane bagasse (1:1, wt/wt) were placed in polypropylene bags and sterilized at 120°C for 20 min. This substrate was inoculated with 20 mL of a spore suspension, obtained from cultures previously grown for five days and was moistened with nutrient solution containing (g L^{-1}) 0.5 of $MgSO_4.7H_2O$, 3.5 of $(NH4)_2SO_4$, 3.0 of KH_2PO_4 and 0.5 of $CaCl_2$, pH 5,0. The solid state fermentation was carried out at 28°C for 10 days. Every 48 h, one bag was removed and the fermented material was mixed with 8 mL of distilled water per gram of fermented material, stirred for 30 min, filtered, and centrifuged at 8000 g and 5°C. The supernatant was used as crude enzyme solution.

Enzyme activity assays

Endo-glucanase and xylanase activities were assayed in a reaction

mixture containing 0.1 mL of crude enzyme solution and 0.9 mL of sodium acetate buffer solution at 0.1 M, pH 5.0 in addition to carboxy methyl cellulose (CMC) or xylan (Birchwood) at 5.0 g L^{-1}; and were incubated at 40°C for 10 min. The quantification of released reducing sugar (expressed as glucose or xylose) was carried out by the 3,5-dinitrosalicylic acid (DNS) method designed by Miller (1959) based on glucose and xylose standard curves.

Laccase activity was determined via the oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonate (ABTS). The reaction mixture, containing 0.1 mL of 0.3 mM ABTS in 100 mM sodium acetate (pH 5.0) and 0.1 mL of crude enzyme solution, was incubated at 40°C for 1 min. The oxidation was followed by the increase in absorbance at 420 nm in spectrophotometer (Bechman) (ε = 36000 l mol^{-1} cm^{-1}). One enzyme unit (U) was defined as 1 µmol of ABTS oxidized per minute and activity was expressed in U L^{-1} (Buswell et al., 1995).

Data analysis

Composition of filamentous fungi assemblages from both areas was measured according to the Sorensen index, $S = 2(S_{12})/(S_1 + S_2)$; where S_1 is the number of genera found in area BS; S_2 is the number of genera found in area NBS and S_{12} is the number of genera common between the two areas.

Data regarding the enzymatic activity were log(x + 1) transformed in order to approach a normal distribution and homogeneity of variances. The Student-*t* test was used to determine significant differences in enzymatic activity (xylanase, CMCase and laccase) from each genus with a 5% significance level. The analysis was carried out in Statistica v. 7.

RESULTS

Filamentous fungi isolated from burned and non-burned sugarcane soils

To evaluate the possible effects of burning the sugarcane on the major fungal components of the soil mycota, we surveyed the fast-growing and dominant fungal taxa in two areas that differed in sugarcane harvesting treatments. A total of 75 filamentous fungal isolates were recovered from BS and NBS soils (Table 1). The Sorensen index of shared taxa resulted in the value 0.62, which indicates a slight difference in fungal composition between soil treatments. Overall, *Trichoderma* was the most prevalent genus in both types of soils followed by *Fusarium* and *Cunninghamella* (Table 2). The number of *Trichoderma* isolates was higher in NBS soil and *Aspergillus* sp. was isolated in a higher number in BS soil (Student-t; P = 0.25). The observed differences in the number of isolates belonging to *Fusarium* and *Cunninghamella* genera were not significantly different between soil types (t test; P = 0.006).

Although the experiment was performed in one sugarcane cultivation cycle, after four consecutive samplings, we recovered more fungal isolates during soil preparation (stage #2, tillage) in comparison with the other stages. The soil-borne fungi *Trichoderma* and *Fusarium* were found in all stages (Table 3). The intensity of rainfall and soil moisture in the sampling sites, although the great observed variation, did not affect the total number of fungal isolates. The temperature was fairly uniform in the days of sampling (Table 3).

Enzyme production by filamentous fungi

To investigate whether distinct soil conditions have impact on the functional role of fungi, the enzymatic production (cellulose, xylanase and laccase) was assessed. Overall, the observed values in the total enzyme activity by the isolated fungi from BS and NBS soils were not significantly different (*t* test; P = 0.002); although we found a higher production of laccases and xylanases by fungi recovered from BS soils (Table 4). Interesting to note, xylanase and CMCase production were higher for fungi from the genus *Aspergillus* and laccase production was higher for *Verticillium* sp. followed by fungi from genus *Fusarium* (Figure 1).

DISCUSSION

Understanding how management practices influence the diversity and functional roles of soil microbial communities is paramount to develop a comprehensive view of microbes interacting with plants that promote host productivity (Fioretto et al., 2002; Barreiro et al., 2010). Our survey revealed that all fungal genera recovered from soils of sugarcane crops were also found in samples from other types of soils as described by Bordjiba et al. (2001), Silva et al. (2009) and Kutorga et al. (2012). This reveals that soils under sugarcane crops harbor a fungal community composed of ubiquitous soil-borne fungi.

Our data suggest that the practice of burning the sugarcane prior harvesting did not significantly affect the composition of the most abundant taxa of soil-borne fungi, in comparison with the site where the sugarcane was harvested green (no burning procedures were applied). Several studies reported modifications on the chemical and physical properties of soil as a result of burning such as, nutrient lack due to loss of soil organic matter and the reduction of the soil moisture (Graham et al., 2002). Thus, it was expected that soil under green harvested sugarcane would present a higher richness of fungal genera, which was not observed in the present study.

The practice of burning the sugarcane may change the microbial communities but, due to its resilience, the communities can recover in short time span (Barcenas-Moreno and Baath, 2009). Fontúrbel et al. (2012) studying the effects of fire on soil microbiota showed that the biomass and activity of microorganisms in these soils were reduced by fire, however, the microbial diversity has been increased in relative proportion. In our study, we did not observe high differences in biomass composition between BS and NBS soils, but we observed that both types of soils had fungal genera that must have resisted environmental stress. One example is the genus

Table 1. Fungal strains isolated from sugarcane soils across crop stages, in different carbon source and types of harvesting.

Sampling	Carbon source	Type of harvesting[1]	Strain	Code
1	Glucose	BS	*Cunninghamella* sp.1	G01
			Fusarium sp.4	G02
			Trichoderma sp.1	G03
			Trichoderma virens	G04
		NBS	*Trichoderma* sp.1	G06
	Wheat bran	BS	*Fusarium* sp.1	F01
		NBS	*Trichoderma* sp.1	F02
			Trichoderma harzianum species complex	F03
	Sugarcane bagasse	BS	*Trichoderma* sp.1	B01
			Fusarium sp.1	B02
		NBS	*Trichoderma* sp.1	B03
			Trichoderma sp.1	B04
	Filter paper	BS	*Trichoderma* sp.1	P01
		NBS	*Fusarium* sp.1	P02
			Mucor hiemalis	P03
2	Glucose	BS	*Aspergillus brasiliensis*	G08
			Cunninghamella elegans	G10
			Trichoderma sp.2	G13
		NBS	*Cunninghamella* sp.1	G14
			Trichoderma harzianum species complex	G15
			Trichoderma sp.2	G17
	Wheat bran	BS	*Verticillium* sp.	F04
			Trichoderma sp.1	F06
			Paecilomyces sp.	F07
			Penicillium sp.	F08
			Fusarium sp.6	F10
			Aspergillus sect *flavus*	F12
		NBS	*Fusarium* sp.5	F13
			Cunninghamella sp.2	F16
			Aspergillus sect *fumigati*	F18
	Sugarcane bagasse	BS	*Trichoderma* sp.1	B05
			Cunninghamella elegans	B06
			Aspergillus sect *flavus*	B07
		NBS	*Trichoderma* sp.1	B13
			Cunninghamella sp.2	B16
	Filter paper	BS	*Trichoderma* sp.1	P05
			Paecilomyces sp.	P07
			Cunninghamella sp.1	P08
		NBS	*Trichoderma* sp.1	P14
			Absídia sp.	P18
3	Glucose	BS	*Trichoderma* sp.1	G19
			FNI[1]	G20
		NBS	*Trichoderma* sp.1	G21
			Trichoderma sp.3	G22
	Wheat bran	BS	*Cunninghamella* sp.1	F19
			Trichoderma sp.1	F20
		NBS	*Trichoderma asperelloides*	F22
			Trichoderma sp.1	F24

Table 1. Contd.

	Sugarcane bagasse	BS	*Cunninghamella* sp.1	B18
			Fusarium sp.1	B19
			Aspergillus sect *flavus*	B20
			Aspergillus sect *nigri*	B21
		NBS	*Mucor hiemalis*	B22
			Trichoderma sp.1	B23
	Filter paper	BS	*Trichoderma* sp.1	P19
			Trichoderma sp.1	P20
		NBS	*Cunninghamella* sp.1	P21
			Aspergillus sect *flavus*	P22
4	Glucose	BS	*Mucor hiemalis*	G23
			Fusarium sp.1	G24
			Aspergillus sect *flavus*	G25
		NBS	*Fusarium* sp.1	G26
			Mucor hiemalis	G27
			Trichoderma sp.1	G28
	Wheat bran	BS	*Fusarium* sp.3	F25
			Trichoderma sp.1	F26
		NBS	*Absídia cylindrospora*	F27
			Trichoderma virens	F28
	Sugarcane bagasse	BS	*Aspergillus* sect *flavus*	B24
			Mucor hiemalis	B25
			Trichoderma sp.1	B27
		NBS	*Fusarium* sp.2	B26
	Filter paper	BS	*Mucor hiemalis*	P23
		NBS	*Fusarium verticillioides*	P24
			FNI[1]	P25

Crop stages: (1) before soil preparation; (2) during soil preparation (tillage); (3) mature culture and (4) after harvesting the sugarcane. [1]Type of harvesting - BS: soil where sugarcane was burned before harvesting; NBS: soil where green harvesting was applied (non-burned sugarcane). [2]FNI: not identified.

Table 2. Prevalence of fungi in soils of sugarcane crops under different harvesting systems. Figures indicate the number of fungal isolates obtained on burned (BS) and non-burned (NBS) soils.

Fungi	Soil type	
	BS[1]	NBS[1]
Absidia sp.	-	2
Aspergillus sp.	7	2
Cunninghamella sp.	6	4
Fusarium sp.	7	5
Mucor hiemalis	3	3
Paecilomyces sp.	2	-
Penicillium sp.	1	-
Trichoderma sp.	14	16
Verticillium sp.	1	-
FNI[2]	1	1
Total	42	33

[1]BS: Soil where sugarcane was burned before harvesting; NBS: soil where green harvesting was applied (non-burned sugarcane). [2]FNI: not identified.

Trichoderma found as the predominant genus in both soils. Using different isolation methods or high-throughput approaches such as next generation sequencing (454 pyrosequencing) will likely reveal the presence of rare species in these environments.

Although burning sugarcane apparently does not influence soil fungal community composition, the tillage practices modifies the mycota by altering the soil physical-chemical properties (Wanga et al., 2010). This step on culture management corresponds to the soil preparation where pH is corrected; fertilizers are added as well as herbicides. Our results show a higher number of fungi isolated from samples of soil in the preparation phase (stage #2), suggesting this management as an important step to restore fungal population in soil. However, this work was limited to only one crop cycle.

The greatest potential for production of xylanase and laccase presented by fungi isolated from BS samples may be related to modifications on the chemical and physical properties of the soil as a result of burning the sugarcane (Graham et al., 2002). The production of xylanase and CMCase were higher for *Aspergillus*

Table 3. Physical-chemical properties and filamentous fungi found in soils under different stages of sugarcane management. Figures correspond to the number of fungal isolates obtained in both areas (BS+NBS).

Fungi	Crop cycle[1]			
	1	2	3	4
Absidia sp.	-	1	-	1
Aspergillus sp.	-	4	3	2
Cunninghamella sp.	1	6	3	-
Fusarium sp.	4	2	1	5
Mucor hiemalis	1	-	1	4
Paecilomyces sp.	-	2	-	-
Penicillium sp.	-	1	-	-
Trichoderma sp.	9	8	9	4
Verticillium sp.	-	1	-	-
FNI[2]	-	-	1	1
Total	15	25	18	17
Precipitation (mm)	12.9	107.4	42.0	162.2
Humidity (%)	52.1	66.1	67.8	73.1
Temperature (°C)	29.5	31.4	29.4	29.2

[1]Soil sampling was carried out: (1) period after harvest and before soil preparation; (2) during soil preparation (see text for details); (3) mature culture and (4) just after harvest of sugar cane. [2]FNI: not identified.

Table 4. Production of xylanase, CMCase and laccase after 10 days of solid cultivation by the filamentous fungi isolated from sugarcane soil.

Fungi	Strain	Xylanase ($U.mL^{-1}$)	CMCase ($U.mL^{-1}$)	Laccase ($U.mL^{-1}$)
Abisidia sp.	P18	5.1	1.5	0.03
Absidia cylindrospora	F27	1.4	0.3	-
Aspergillus sect *flavus*	P22	64.0	3.3	0.04
Aspergillus sect *nigri*	B21	63.7	5.2	0.01
Aspergillus sect *flavus*	B24	60.2	2.6	-
Aspergillus sect *fumigati*	F18	60.0	3.8	0.01
Aspergillus sect *flavus*	G25	54.9	2.9	-
Aspergillus sect *flavus*	B07	52.4	2.6	0.01
Aspergillus sect *flavus*	B20	47.9	2.3	-
Aspergillus sect *flavus*	F12	47.6	3.4	-
Aspergillus brasiliensis	G08	43.7	4.2	0.01
Cunninghamella elegans	G10	8.7	0.9	-
Cunninghamella sp.1	G01	3.3	0.8	0.06
Cunninghamella sp.1	P21	2.9	0.4	0.01
Cunninghamella sp.2	B16	2.8	0.8	0.09
Cunninghamella sp.1	F19	2.6	0.4	0.01
Cunninghamella elegans	B06	2.5	1.2	0.13
Cunninghamella sp.1	P08	1.5	0.8	0.12
Cunninghamella sp.1	G14	0.7	0.4	0.01
Cunninghamella sp.1	B18	0.6	0.3	-
Cunninghamella sp.2	F16	0.6	0.4	0.10
FNI[1]	P25	1.6	0.5	0.01
FNI[1]	G20	5.8	1.4	0.01
Fusarium sp.1	B02	14.7	1.3	0.27

Table 4. Contd.

Fusarium verticillioides	P24	3.6	0.8	0.03
Fusarium sp.1	P02	8.5	0.6	0.01
Fusarium sp.2	B26	7.9	1.7	0.15
Fusarium sp.1	G24	6.9	5.5	0.24
Fusarium sp.4	G02	5.0	0.8	0.07
Fusarium sp.1	F01	3.8	2.2	0.10
Fusarium sp.5	F13	3.5	2.2	0.17
Fusarium sp.6	F10	3.2	0.6	0.03
Fusarium sp.3	F25	2.7	1.5	0.02
Fusarium sp.1	B19	2.1	1.8	0.01
Fusarium sp.1	G26	2.0	1.8	0.07
Mucor hiemalis	B25	16.8	1.8	0.01
Mucor hiemalis	G27	12.2	1.5	-
Mucor hiemalis	P03	6.2	1.9	0.02
Mucor hiemalis	P23	2.4	0.9	0.03
Mucor hiemalis	G23	2.2	0.5	0.24
Mucor hiemalis	B22	0.5	0.4	-
Paecilomyces sp.	F07	1.9	0.8	0.04
Paecilomyces sp.	P07	1.0	0.6	0.12
Penicillium sp.	F08	13.7	1.9	0.02
Trichoderma sp.1	P01	18.3	1.8	0.01
Trichoderma virens	F28	16.8	4.3	-
Trichoderma sp.1	P20	15.0	3.6	0.01
Trichoderma sp.1	B03	13.7	1.4	0.01
Trichoderma virens	G04	13.5	2.9	0.02
Trichoderma sp.1	G28	13.4	3.1	0.01
Trichoderma sp.1	P05	12.7	2.2	0.01
Trichoderma harzianum species complex	F03	12.4	1.5	0.02
Trichoderma sp.2	G17	12.3	2.2	0.01
Trichoderma sp.1	G19	11.6	3.9	0.05
Trichoderma sp.2	G13	9.5	3.2	0.01
Trichoderma sp.1	P19	9.4	4.4	0.01
Trichoderma sp.1	F02	9.0	2.5	-
Trichoderma sp.1	P14	8.9	3.2	0.03
Trichoderma sp.1	G06	8.8	2.1	0.03
Trichoderma harzianum species complex	G15	8.0	4.7	0.05
Trichoderma sp.1	G03	7.4	3.4	0.02
Trichoderma sp.1	F26	6.9	2.2	-
Trichoderma sp.1	B23	6.0	1.6	0.02
Trichoderma sp.1	B27	5.3	2.0	-
Trichoderma sp.1	B05	4.9	2.1	0.01
Trichoderma sp.1	F24	4.6	2.4	0.06
Trichoderma sp.1	B13	4.6	1.2	0.01
Trichoderma sp.1	B04	4.5	1.3	0.01
Trichoderma sp.1	G21	4.4	2.1	0.01
Trichoderma sp.1	F06	4.2	1.5	-
Trichoderma sp.3	G22	4.0	1.0	0.01
Trichoderma sp.1	B01	3.6	0.8	0.13
Trichoderma asperelloides	F22	3.5	2.1	0.02

Table 4. Contd

Trichoderma sp.1	F20	3.5	1.9	0.03
Verticillium sp.	F04	1.3	0.3	0.50

[1]FNI: not identified.

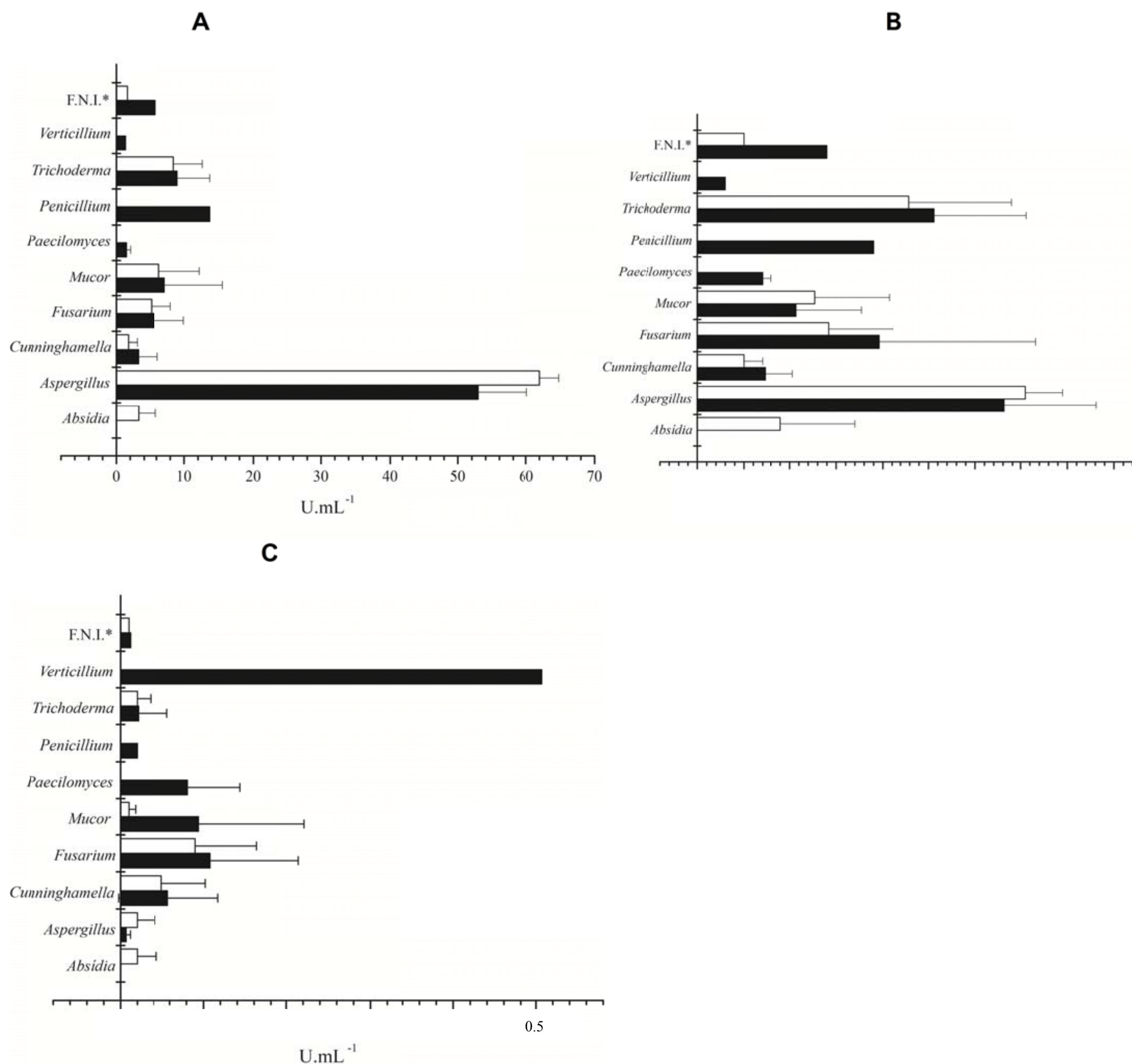

Figure 1. Enzyme activities (mean and standard deviations) of filamentous fungi isolated from burned (dark bars) and non-burned (open bar) sugarcane soils. A. Xylanase activity; B. CMCase activity and C. Laccase activity. * FNI: fungi not identified.

isolates, followed by fungi of the genus *Trichoderma*, *Fusarium* and *Mucor*. For laccase, the largest amount was produced by *Verticillium* sp., followed by fungi of the genera *Fusarium* and *Mucor*. This result shows the

important role of several fungi in the degradation of lignocellulosic biomass in the soil environment. However there was wide variation in the production of these enzymes within the same genus, which can be explained by the difference in the type of soil, period of sampling and carbon source used to isolate the fungi.

This result raises an interesting hypothesis regarding the physiological effects of burning on soil fungal communities. It is likely that burning acts as a selective pressure on the soil fungal communities and that heat resistance fungi with high enzymatic profile may help the fungal community recover during the first stages after burning.

Conclusions

In conclusion, the culture-dependent methods applied in the present study showed the major fungal taxa in sugarcane soils under different stages. Our data indicates that burning the sugarcane may not affect the composition of the major fungal taxa, but the tillage practices could interfere in the soil fungal community and its functional role. Future studies using high-throughput sequencing technologies should be carried out in order to investigate in depth the modifications of burning and sugarcane managements in soil fungal communities.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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