

Presence and diversity of arbuscular mycorrhizal fungi in soil regularly irrigated with vinasses

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Abstract

The application of organic waste products, such as vinasses to the soil is a common practice in different countries. Vinasses are a residual liquid generated during various processes such as ethanol production and beverages distillation. However, little is known about how the vinasses affect the content and species distribution of arbuscular mycorrhizal fungi (AMF) in soils irrigated with vinasses. The aim of this research was to determine the presence, spore density and taxonomic identification of AMF to assess in vinasses irrigated (VS) and unirrigated soils (CS). Sixteen species were identified in irrigated and unirrigated soils, with a predominance of *Acaulospora mellea*, *A. scrobiculata*, *Funneliformis geosporum*, *F. mosseae* and *Paraglomus occultum*. Only *Clareidoglomus etunicatum*, *Septoglomus constrictum*, and *Racocetra gregaria* were found in VS, while *A. morrowiae* and *Scutellospora reticulata* were only found in CS. The AMF abundance, root colonization, richness and biodiversity indices were not affected by vinasses application. It was found that irrigation of soil with vinasses seemed to have only a limited effect on AMF. To our knowledge, this is the first description of the species of AMF present in soil frequently irrigated with vinasse. This study could serve as a base for further investigations into the effect of vinasse on the soil microbial community and crop yields.

Keywords: Diversity, liquors/alcohols production, species richness, spore abundance, vinasses

1. Introduction

Irrigation of arable soil with wastewater is a common practice. It provides water to the growing crops and increases the soil organic matter content, plant nutrients, and microbial population. However, application of wastewater might reduce soil aeration and increase soil salinity, with an adverse effect on soil properties and fertility. Also, organic amendments applied to the soil can change the composition of the microbial community (Zaccardelli *et al.*, 2013). Despite this, the application of wastewater such as vinasses to arable soil is a widespread practice in many countries (Moran-Salazar *et al.*, 2016). Vinasses are a residual liquid generated during ethanol production from sugarcane (*Saccharum officinarum* L.), sugar beet (*Beta vulgaris* ssp.), or from the distillation of spirits, such as mezcal, tequila, and others.

The characteristics of vinasses depend mainly on the raw material used, but all of them share similar properties, such as a low pH (3.9 to 5.1), a high chemical oxygen demand (COD) (50,000 to 95,000 mg L⁻¹) and biological oxygen demand (BOD) content (18,900 to 78,300 mg L⁻¹) (Arimi *et al.*, 2014; Moran-Salazar *et al.*, 2016). They have a high concentration of suspended and dissolved solids, and large amounts of K⁺, Ca²⁺, and Mg²⁺ ions. The average proportion of vinasses generated during alcohol production (sugarcane and sugar beet) and distilled beverages (tequila or mezcal) ranges between 7 and 15 L by liter of final product (Moran-Salazar *et al.*, 2016). Vinasses share some characteristics and present many of the same issues for treatment, management and final disposal (Arimi *et al.*, 2014). Regularly, they are used without previous treatment as an organic amendment or

fertilizer for arable soil. Potential negative effects of vinasse on soil (e.g. acidification, increase in cations content and metals), water (contamination of rivers, lagoons and aquifers by nitrates or metals), air (increase of greenhouse gases emissions), microorganisms (decrease of biomass) and plants, have been reported when they are applied at high rates (Moran-Salazar *et al.*, 2016).

Little is known about how the vinasses affect arbuscular mycorrhizal fungi (AMF) population and diversity. The addition of sugarcane vinasses decreased the glomalin content of AMF (Velásquez Pomar and Sánchez de Prager, 2011). Meanwhile, Pereira Aleixo *et al.* (2014) reported no significant differences in AMF spore density between sugarcane crop with and without irrigation with alcohol vinasses. Montenegro-Gomez (2008) found an inhibition of AMF colonization in maize plants irrigated with sugarcane vinasses.

Thus, the aim of this research was to determine the presence and diversity of AMF in an unamended agricultural soil compared to a similar soil regularly amended with vinasses to elucidate how vinasses irrigation could change the AMF populations in the soil.

2. Materials and Methods

2.1. Sampling site, soil and vinasses characterization

The experimental site was located in El Arenal, Jalisco, Mexico (20° 46' 7" N and 103° 41' 18" W) at 1450 m a.s.l. The weather is dry with an average annual temperature of 20 °C and 1104 mm precipitation. The soil has been used for sugarcane cultivation

(*Saccharum officinarum* L.) for 30 years. The soil was collected in the last stage of sugarcane growth just before the plant was cut for sugar production. The experimental site has two different areas: 1) a field (2.4 ha) irrigated with tequila vinasses at $83 \text{ m}^3 \text{ ha}^{-1} \text{ month}^{-1}$ (considered the VS treatment) during 8 months each year, for at least 5 years, and 2) a field (1.8 ha) not applied with vinasses and considered the control soil (CS treatment). Each experimental area was divided into three plots to obtain three independent samples ($n = 3$). Twenty sub-samples were taken at random from each of plots ($n = 6$). The samples of each plot were pooled so that for each treatment three separate samples were obtained. The soil sampling procedure is given in Figure 1. The soil was transferred to the laboratory, sieved separately (5 mm mesh), and stored for characterization and AMF spore extraction. Additionally, samples of vinasses were collected immediately after distillation from a tequila factory that process agave traditionally. The vinasse from this factory has been used to irrigate the sampling site. The vinasse was then analyzed for physicochemical parameters.

Soil was characterized for pH, water holding capacity (WHC), cation exchange capacity (CEC), electrical conductivity (EC), total organic carbon, total nitrogen (N), total and available phosphorus (P) and particle size distribution as described by Dane and Topp (2002). Simultaneously, vinasses were characterized for pH, EC, biological oxygen demand (BOD), chemical oxygen demand (COD), arsenic, cadmium, copper, chrome, mercury, lead, nickel, zinc and iron, volatile suspended sol-

ids (VSS), settleable solids (SS), total and available P and total N (APHA, 1992). Vinasse applied to this field always came from the same distillery where the same cooking process has been followed since the beginning. It can thus be assumed that the composition of the vinasse was similar over the years.

2.2. Isolation and identification of AMF

The AMF spores were isolated three times from each plot of CS and VS soils ($n = 9$), using a wet sieving method with sucrose at 50% (w/v) and four sieves with 600, 150, 75 and 45 μm mesh (Brunnett *et al.*, 1996). Sieved spores were sorted by morphotypes under a stereomicroscope (NIKON SMZ745T, Beijing, China) and pooled. Slides were prepared with polyvinyl alcohol (PVLG) or Melzer reagent-PVLG (1:1, v/v). Taxonomic identification was carried out using an optical microscope with Nomarski interference contrast (AxioLab, A1 Carl Zeiss, Jena, Germany) and compared with descriptions from the international culture collection of vesicular arbuscular mycorrhizal fungi (INVAM, 2017) (<http://invam.wvu.edu/>). Spores were photographed with a digital camera (Axio Cam ERc 5s, Jena, Germany) by AxionVision® SE64, Release 4.9 software. The relative abundance (RA) of spores and number of species (richness S) was determined. Shannon-Wiener index (H'), Pielou's evenness index (J') and the Simpson index (expressed as 1-D) were calculated to determine species diversity (Magurran, 2004).

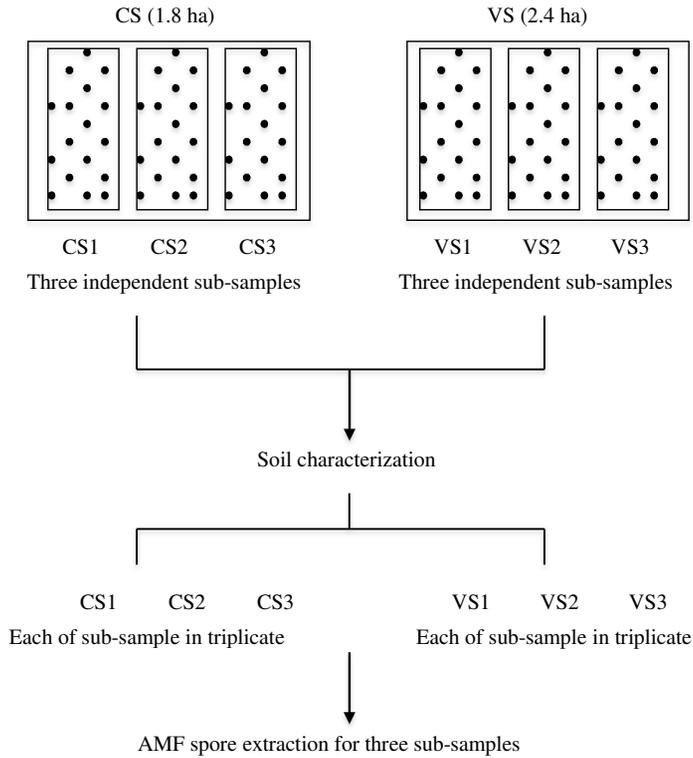


Figure 1. Scheme representation of sampling and management of replicates.

2.3. Root colonization

The AMF root colonization was determined based on the methods of Ishii and Loynachan (2004) which consisted in clearing the roots with KOH (10% w/v) at 90 °C for 90 min followed by staining with Trypan blue (0.05% w/v) in lactoglycerol:water (lactic acid-glycerol-water 1:1:1 v:v:v) at 90 °C for 30 min. The presence of mycorrhizal structures (hyphae, arbuscules, vesicles or coils) in one field of view were used for confirmation of root colonization. Average from several fields were examined using the previously described optical microscope at a 40 x magnification.

2.4. Statistical analysis

Spore abundance data were analyzed through a U-Mann-Whitney test to compare medians. Richness, diversity indices, root colonization and physicochemical parameters of soil were compared through a Student's test. Relative abundance (RA) data were $\log(x+1)$ transformed and subjected to a two-way variance analysis (ANOVA) to determine differences the two soils and the species. Centurion Statgraphics XVI.I. (Statpoint Technologies, Virginia, US) software was used to analyze the data.

3. Results

3.1. Soil and vinasse characterization

The sandy loam CS and VS soils were classified as phaeozem characterized by a high organic matter (9.4% in CS and 7.4% in VS) and nutrient content (nitrogen and phosphorus). The pH was neutral or near

neutral (6.5 in CS and 7.0 in VS), the electrical conductivity was similar with 83.8 and 80.4 $\mu\text{S cm}^{-1}$ in CS and VS respectively, and the cation exchange capacity was 27 and 25 Cmol kg^{-1} respectively. Additionally, heavy metals concentration was low (Table 1).

The vinasse was characterized by an acid pH, a low EC, total P content, total N content, COT, BOD, settleable solids and total volatile solids (Table 2).

Table 1. Control and vinasses irrigated soil characterization.

Parameter	CS	VS
Textural classification	Sandy loam	Sandy loam
Soil type	Phaeozem	Phaeozem
pH	6.5 \pm 0.2A	7.0 \pm 0.3B
Electrical conductivity (EC) ($\mu\text{S cm}^{-1}$)	83.8 \pm 31.2A	80.4 \pm 35.4A
Water holding capacity (%)	10 \pm 4.2A	17.9 \pm 5.3B
Moisture (%)	9.4 \pm 1.3A	12.4 \pm 2.0B
Cation exchange capacity (Cmol kg^{-1})	27.3 \pm 5.9A	25.5 \pm 6.8A
Ash (%)	92.6 \pm 1.4A	92.3 \pm 3.1A
Sand (%)	70.0 \pm 3.0A	68.7 \pm 1.8A
Clay (%)	16.9 \pm 4.1A	16.7 \pm 1.5A
Silt (%)	13.1 \pm 3.3A	14.7 \pm 2.4A
Phosphates (PO_4^{3-}) (mg kg^{-1})	9.9 \pm 2.6A	12.6 \pm 2.0B
Total phosphorus (TP) (mg kg^{-1})	83.3 \pm 3.9A	111.1 \pm 51.1A
Total organic carbon (TOC) (g C kg^{-1})	54.3 \pm 1.3A	43.2 \pm 1.9B
Organic matter (OM) (%)	9.37 \pm 0.15A	7.44 \pm 1.41A
Nitrites (NO_2^-) (mg kg^{-1})	0.01 \pm 0.0A	0.03 \pm 0.01B
Nitrates (NO_3^-) (mg kg^{-1})	0.45 \pm 0.07A	0.7 \pm 0.3B
Ammonium (NH_4^+) (mg kg^{-1})	0.04 \pm 0.03A	0.05 \pm 0.04A
Total nitrogen (TN) (mg kg^{-1})	971 \pm 147A	1259 \pm 284A
Lead (Pb) (mg kg^{-1})	12.0 \pm 0.2A	12.1 \pm 3.2A
Cadmium (Cd) (mg kg^{-1})	<0.2 \pm 0A	<0.2 \pm 0A
Nickel (Ni) (mg kg^{-1})	3.2 \pm 0.5A	2.9 \pm 0.2A
Iron (Fe) (g kg^{-1})	18.4 \pm 5.3A	14.4 \pm 7.0A
Manganese (Mn) (mg kg^{-1})	369 \pm 115A	309 \pm 2.7A
Zinc (Zn) (mg kg^{-1})	34.8 \pm 2.6A	34.0 \pm 5.3A
Copper (Cu) (mg kg^{-1})	10.1 \pm 10.6A	10.6 \pm 2.5A

Different capital letters indicate significant difference to Student's T test ($P > 0.05$) ($n = 9$).

Table 2. Physicochemical characterization from tequila vinasses.

Parameter	Tequila vinasses
pH	3.5 ± 0.1
Electrical conductivity (EC) (mS cm ⁻¹)	3.3 ± 0.1
Phosphates (PO ₄ ³⁻) (mg L ⁻¹)	22.4 ± 1
Total Phosphorus (TP) (mg L ⁻¹)	26.4 ± 1
Total organic carbon (TOC) (g L ⁻¹)	16.8 ± 1
Organic matter (OM) (%)	4.6 ± 0.6
Nitrites (NO ₂ ⁻) (mg L ⁻¹)	2.2 ± 0.2
Nitrates (NO ₃ ⁻) (mg L ⁻¹)	580 ± 88
Ammonium (NH ₄ ⁺) (mg L ⁻¹)	1.4 ± 1.0
Total Nitrogen (TN) (g L ⁻¹)	4.2 ± 0.1
Total chemical oxygen demand (COD) (g L ⁻¹)	57.8 ± 1.9
Soluble chemical oxygen demand (COD) (g L ⁻¹)	53.7 ± 3.6
Total biological oxygen demand (BOD) (g L ⁻¹)	25.4 ± 1.7
Soluble biological oxygen demand (BOD) (g L ⁻¹)	12.7 ± 1.6
Total solids (mg L ⁻¹)	40.8 ± 3.0
Total suspended solids (mg L ⁻¹)	11.2 ± 2.0
Volatile suspended solids (mg L ⁻¹)	12.5 ± 3.0
Total volatile solids (mg L ⁻¹)	37.3 ± 3.0
Total dissolved solids (mg L ⁻¹)	29.6 ± 3.0
Settleable solids (mL L ⁻¹)	120 ± 5.0
Phenols (mg equiv. gallic acid L ⁻¹)	806.8 ± 36.9
Arsenic (As) (mg L ⁻¹)	<1.0
Cadmium (Cd) (mg L ⁻¹)	<0.2
Copper (Cu) (mg L ⁻¹)	<1.0
Chrome (Cr) (mg L ⁻¹)	<1.0
Mercury (Hg) (mg L ⁻¹)	<1.0
Lead (Pb) (mg L ⁻¹)	<1.0
Nickel (Ni) (mg L ⁻¹)	<1.0
Zinc (Zn) (mg L ⁻¹)	2.3 ± 0.0
Iron (Fe) (mg L ⁻¹)	24 ± 0.9

3.2. Identification of AMF species

In this study, sixteen AMF species were found in the CS and VS soil. Most species found were from the genus *Acaulospora*. They were identified as: *Acaulospora delicata*, *A. sp.*, *A. foveata*, *A. laevis*, *A. mellea*, *A. morrowiae*, *A. scrobiculata*, *A. spinosa*, *Claroideoglossum etunicatum*, *Funnelformis*

geosporum, *F. mosseae*, *G. margarita*, *Paraglossum occultum*, *Racocetra gregaria*, *Scutellospora reticulata* and *Septoglossum constrictum* (Figure 2). AMF such as *C. etunicatum*, *F. constrictum*, and *R. gregaria* were found in VS soil, but not in CS soil. *Acaulospora morrowiae*, *Acaulospora sp.* and *S. reticulata* were found in the CS soil, but not in VS soil.

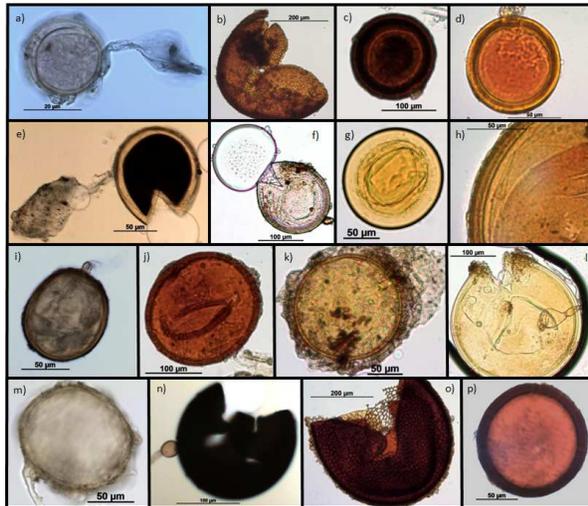


Figure 2. AMF isolated and identified in CS and VS soil. **a)** *Acaulospora delicata* (10x objective); **b)** *A. foveata* (10x); **c)** *A. laevis* (10x); **d)** *A. mellea* (40x); **e)** *A. morrowiae* (10x); **f)** *A. scrobiculata* (10x); **g)** *A. sp.* (10x); **h)** *A. spinosa* (40x); **i)** *Claroideoglomus etunicatum* (10x); **j)** *Funneliformis geosporum* (10x); **k)** *F. mosseae* (10x); **l)** *G. margarita* (10x); **m)** *Paraglomus occultum* (10x); **n)** *Racocetra gregaria* (10x); **o)** *Scutelospora reticulata* (10x) and **p)** *Septoglomus constrictum* (10x)

3.3. Spore abundance, richness and root colonization of AMF

The abundance of AMF spores in the CS (248 ± 37 spores per 100 g soil) and VS soil (209 ± 12 spores per 100 g soil) did not show a significant difference between soils ($P = 0.1528$) (Figure 3). Likewise, the CS and VS soil had similar AMF species rich-

ness (S) and no statistically significant differences were found between CS and VS in this parameter ($P = 0.8797$) (Figure 3). Root fragments collected in both soils showed AMF colonization. There was no significant difference ($P > 0.05$) between CS ($45.0\% \pm 8.7\%$) and VS ($60.0\% \pm 6.0\%$) in root colonization percentage (Figure 4).

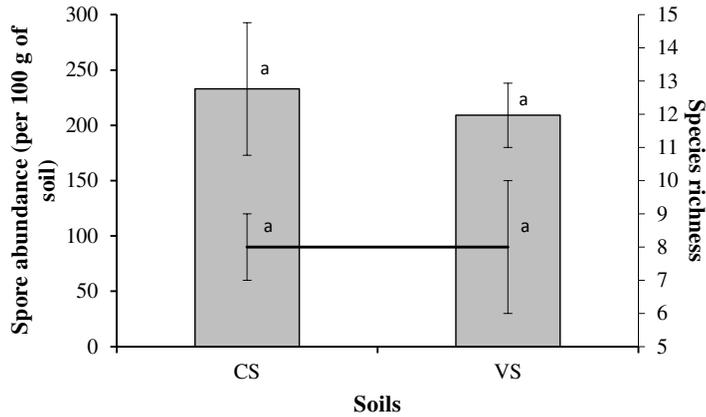


Figure 3. Spore abundance (bars) and species richness (line) of arbuscular mycorrhizal fungi in control (CS) and vinasses irrigated (VS) soil. Bars with the same letter were not significantly different ($P > 0.05$) ($n = 9$).

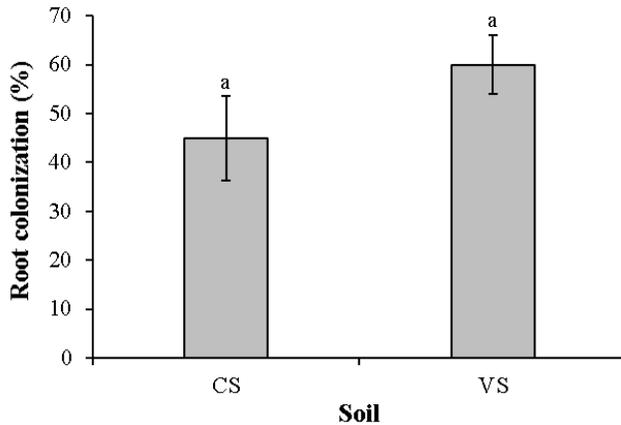


Figure 4. Root colonization (%) of AMF present in CS and VS soils. Bars with the same letter were not significantly different ($P > 0.05$) ($n = 9$).

3.4. AMF species relative abundance and diversity indices

In the CS soil, *Acaulospora mellea* (27%) and *F. mosseae* (25%) presented a significantly greater

relative abundance (RA) than the other species ($P < 0.05$), followed by *A. scrobiculata* (18%), *Paraglomus occultum* (14%), *Funneliformis geosporum* (7%) and *Gigaspora margarita* (4%) (Table 3). In the VS soil, the species that presented a significantly

greater RA ($P < 0.05$) than the other species were *F. mosseae* (32%) and *A. mellea* (27%) followed by *A. scrobiculata* (13%), *F. geosporum* (10%), *P. occultum* (4%) and *Gigaspora margarita* (5%) (Figure 5). The relative abundance of AMF was not significantly different between the CS and VS soil ($F = 0.29, P = 0.2733$).

The AMF Shannon-Wiener index (H') in the CS soil was 1.98 ± 0.28 and not significantly different from that in the VS soil (1.88 ± 0.40) ($P=0.8502$), the Simpson index (1-D) was 0.78 in CS and 0.76 in VS ($P=0.3748$) and the evenness (J') was 0.83 for CS and 0.82 for VS ($P=0.8086$). The Simpson index and evenness were not significantly different between soils (Table 3).

Table 3. Diversity indices of AMF species present in CS and VS soils.

Soil	Shannon index (H')	Simpson index (1-D)	Evenness (J')
CS	$1.67 \pm 0.14a$	$0.78 \pm 0.04a$	$0.83 \pm 0.06a$
VS	$1.66 \pm 0.13a$	$0.76 \pm 0.03a$	$0.82 \pm 0.08a$

Different letters indicate significant differences between soils ($P < 0.05$) ($n = 9$).

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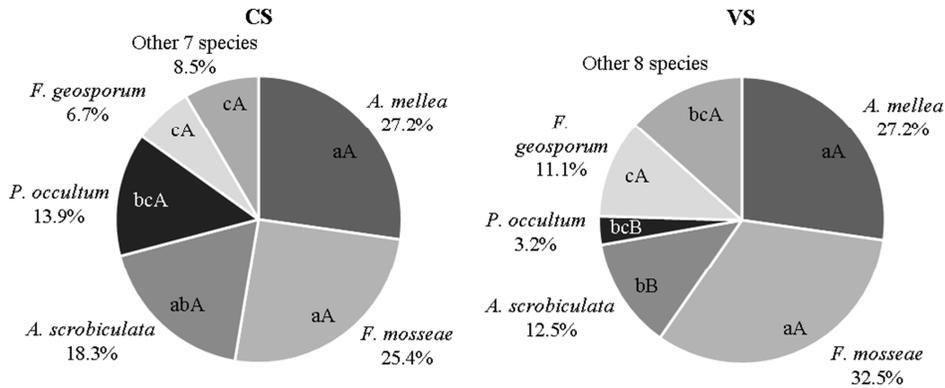


Figure 5. Relative abundance (% of total spores) of AMF present in CS and VS soils. Different lowercase letters indicate significant differences between species and capital letters between soils ($P < 0.05$) ($n = 9$).

4. Discussion

4.1. Soil and vinasse characterization

Physicochemical parameters in the soils were classified according to Mexican and American standard regulations (NOM-021-SEMARNAT, 2000; USDA, 2014). The organic matter content was high in the soils as is reported for Phaeozem soils (FAO, 2014). They were non-saline with a high CEC probably because of the high clay content in both soils. A soil with a high organic matter content and CEC as found in CS and VS has been related to a large pH buffering capacity (Xu *et al.*, 2012). This could explain why the application of highly acidic vinasses (as described later) did not acidify the soil. The mineral N content was low although the total N content was medium. This suggested that mineralized N was immobilized immediately, and the amount of heavy metals was low, which did not exceed Mexican and American standards regulations (NOM-021-SEMARNAT, 2000; USDA, 2014).

It has been reported that soils cultivated with sugarcane crop have an organic matter high content (59 g kg⁻¹ to 6.9 g kg⁻¹) and high total nitrogen content (1.8 g kg⁻¹ to 2.4 g kg⁻¹) (Mthimkhulu *et al.*, 2016), which are concordant with values and the classification found in this study. Also a phaeozem soil is characterized by a pH ranging from 5 to 7 (FAO, 2014) and the pH of sugarcane soils is reported on a wide range from 4.5 to 6.0 depending on fertilizer application rates and soil type (Azevedo *et al.*, 2014; Mthimkhulu *et al.*, 2016). The USEPA (2004) regulations do not establish maximum limits for EC, BOD, COD or nitrates for wastewater discharge on agricultural soils, only for pH (9), total BOD (4.5 g L⁻¹) and TSS (45 mg L⁻¹). In Brazil, irrigating soil with sugarcane vinasses is permitted by standard regulation (CETESB, 2006). Only limits for the amount of K₂O that can be applied to soil are

given (185 kg K₂O ha year⁻¹), but not for other parameters, such as BOD, COD, and N or P. The heavy metal content in vinasses was below limits established by standard regulation for the discharge of wastewater in agricultural soils (USEPA, 2004).

4.2. Identification of AMF species

Some of the genus found in CS and VS soil have been reported in soil from sugarcane crops in Brazil without vinasses irrigation such as *Acaulospora*, *Glomus*, *Funneliformis*, *Paraglomus*, *Gigaspora*, *Scutellospora*, and *Racocetra* (Azevedo *et al.*, 2014). This could suggest certain preference of AMF by sugarcane plants regardless of environment conditions and resistance of AMF species to vinasses irrigation found in this study.

The genera *Claroideoglomus* and *Funneliformis* (present in VS soil) are normally described as generalists species (Castillo *et al.*, 2016) because they are found in a wide range of ecosystems, which is probably why they resisted the disturbance caused by vinasses. Other species, which are considered specialists, are only found in specific conditions, and they could probably not resist the change in pH or the high amount of phenolic compounds as a result of vinasse application.

4.3. Spore abundance, richness and root colonization of AMF

The abundance of AMF in both soils fell into the interval reported by Datta and Kulkarni (2012) (135-395 spores per 100 g soil) in a sugarcane soil without vinasses irrigation. However, in sugarcane crops with different management systems (burning and unburning) the AMF spore abundance was reported from 31 to 40 spores per 50 g of soil (Azevedo *et al.*, 2014). These values were lower than found in this research in

sugarcane soils irrigated with vinasses. The colonization percentage agreed with that reported by Azevedo *et al.* (2014) for AMF colonization in sugarcane roots (31-36%) in perturbed (burning) and not perturbed soil (not burned) (49-51%).

On the other hand, the tequila vinasse had high phenolic compounds content ($806.8 \pm 36.9 \text{ mg L}^{-1}$) (Table 2). This was higher than reported by Ferral-Pérez *et al.* (2016) in tequila vinasses ($44\text{-}81 \text{ mg L}^{-1}$) and (603 mg L^{-1}), or sugarcane (34 mg L^{-1}), beet (450 mg L^{-1}) and mezcal ($478\text{-}542 \text{ mg L}^{-1}$) vinasses (Moran-Salazar *et al.*, 2016). Some phenolic compounds reported for sugarcane vinasses are protocatechuic acid, *p*-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, benzoic acid, vanillin, *p*-coumaric acid, ferulic acid and syringic acid (Arimi *et al.*, 2014), the last three are also reported for sugar beet vinasses (Bostyn *et al.*, 2009), whereas for tequila vinasses there are no reports about the specific phenolic compounds.

Johnson *et al.* (1992) reported that phenolic compounds could reduce the AMF colonization, other reports showed that it depends on the concentration and kind of phenolic compounds, which could affect positive or negatively to AMF (Becard *et al.*, 1992), i.e. ferulic acid ($>50 \text{ mg L}^{-1}$) inhibited AMF colonization (Wacker *et al.*, 1990); hesperetin, apigenin, chrysin and biochanin-A in $>10 \text{ }\mu\text{M}$ concentration (≈ 0.302 , 0.270 , 0.254 , 0.284 mg L^{-1} respectively) presented hyphal growth inhibition in AMF spores *in vitro* (Becard *et al.*, 1992). Compounds such as *p*-coumaric acid, *p*-hydroxybenzoic acid, or quercetin at $> 1 \text{ mM}$ (≈ 164 , 138 and 302 mg L^{-1} respectively) were inhibitory for growth and AMF colonization in clover and sorghum plants (Fries *et al.*, 1997). Piotrowsky *et al.* (2008) reported decrease in AMF colonization in roots from *Sorghum sudanese* plants with coumarin, ferulic, caffeic and vanillic acid at 250 mg kg^{-1} soil. However, other phenolic compounds such as quercetin, myricetin and kaempferol stimulated spore germination and

hyphal growth *in vitro* conditions at $10 \text{ }\mu\text{M}$ concentration (Becard *et al.*, 1992).

These highlights in the literature suggest that phenolic compounds could be one of constituents from vinasses that could have positive or negative effect on AMF and then influence changes in the AMF populations. However, more information is necessary about AMF association and colonization of plants, populations and diversity, with different sources and rate applications of vinasses to improve our understanding on changes induced by vinasses irrigation in agricultural systems. Few studies have reported on AMF in soil irrigated with vinasses. So, to our knowledge, this is one of the first description of AMF species in soil frequently irrigated with vinasse.

4.4. AMF species relative abundance and diversity indices

Among the species reported by Azevedo *et al.* (2014) as the most frequent species ($> 50\%$) in soil from sugarcane crops without vinasses irrigation, *Acaulospora scrobiculata* coincides with the present work, while other species are different (*A. morrowiae*, *Glomus macrocarpum*, and *Scutellospora pellucida*) to found in our study.

Diversity indices showed that although there were no significant differences, H' is higher in CS soil than in the disturbed soil from VS, which has been reported previously. Azevedo *et al.* (2014) reported a similar H' for AMF (1.50 ± 0.28) in unburned and burned soils (1.57 ± 0.22) cultivated with sugarcane. Marín *et al.* (2016) however, reported a significantly higher H' (2.24), (1-D) (0.88) and J' (0.89) ($P < 0.05$) for AMF in forests soil compared to disturbed forests ($H' = 1.586$, (1-D) = 0.747 and $J' = 0.815$).

The high 1-D and J' value found in this study indicate that there was no dominant species and the species were distributed more or less uniformly. This could

mean that all species in the AMF consortium were equally adapted to vinasse application, since all of them have been subjected by years of the same abiotic stress induced by the application of vinasses.

The pH of the soil is other factor that could determine the distribution or amount of AMF and regulates their life cycle, i.e. *G. margarita* is found in soil with pH < 5.5 and *A. scrobiculata*, *A. spinosa* and *G. etunicatum* are found in tropical soils with pH 3.8-8.0 and *Funneliformis* is common in neutral and slightly alkaline soils (Mukerji *et al.*, 2002), while *Acaulospora* genus has been reported in acid soils (Abbott and Robson, 1991), or *Glomus* genus with highest spore abundances in sugarcane soil (pH 5.9) followed by *Scutellospora* and *Acaulospora* species (Azevedo *et al.*, 2014). In this work, the predominant genus was *Acaulospora* although the soils were neutral (VS) and lightly acid (pH 6.5) for CS.

Although the vinasse has a very acid pH, after application to soil, it was not acidic. This indicated a buffer effect of soil due to the organic matter decomposition, which contributed the buffer effect in soils (Quiroz and Rojas, 2009). So, the AMF species associated to soil could have an acid-neutral interval of pH in this habitat induced by vinasses irrigation.

5. Conclusions

Sixteen AMF were found in two agricultural soils: irrigated with vinasses or left unirrigated. This irrigation is not detrimental to arbuscular mycorrhizal fungi. The predominant arbuscular mycorrhizal fungi were *Funneliformis mosseae* and *Acaulospora mellea*. No significant differences were found in the abundance, colonization, richness, and diversity of arbuscular mycorrhizal fungi as a result of vinasses irrigation. The species found were more or less evenly distributed and there was no increase in dominance in vinasse amended soil, which could mean that the

AMF consortium was adapted to the application of vinasses and not only a single species.

However, although no significant differences were found in the abundance, colonization, richness and diversity of the arbuscular mycorrhizal fungi, their metabolic activity might have been altered which might affect their beneficial effect on crop growth. Further studies are thus required to investigate possible changes in the metabolic activity of the arbuscular mycorrhizal fungi.

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