Soil biochemistry and microbial activity in vineyards under conventional and organic management at Northeast Brazil

Nicácio de Oliveira Freitas[1]; Adriana Mayumi Yano-Melo[2]; Fábio Sérgio Barbosa da Silva[3]; Natoniel Franklin de Melo[4]; Leonor Costa Maia[1]*

[1]UFPE – Depto. de Micologia, Av. Nelson Chaves, s/n - 50670-420 – Recife, PE – Brasil.
[2]UNIVASF – Colegiado de Zootecnia, Av. José de Sá Maniçoba, s/n – 56304-917 – Petrolina, PE – Brasil.
[3]UPE – Faculdade de Formação de Professores de Petrolina, BR 203, km 2 – 56300-000 – Petrolina, PE – Brasil.
[4]Embrapa Semi-Árido – C.P. 23 - 56302-970 – Petrolina, PE – Brasil.
*Corresponding author <leonorcmaia@yahoo.com.br>

ABSTRACT: The São Francisco Submedium Valley is located at the Brazilian semiarid region and is an important center for irrigated fruit growing. This region is responsible for 97% of the national exportation of table grapes, including seedless grapes. Based on the fact that organic fertilization can improve soil quality, we compared the effects of conventional and organic soil management on microbial activity and mycorrhization of seedless grape crops. We measured glomerospores number, most probable number (MPN) of propagules, richness of arbuscular mycorrhizal fungi (AMF) species, AMF root colonization, EE-BRSP production, carbon microbial biomass (C-MB), microbial respiration, fluorescein diacetate hydrolytic activity (FDA) and metabolic coefficient (qCO$_2$). The organic management led to an increase in all variables with the exception of EE-BRSP and qCO$_2$. Mycorrhizal colonization increased from 4.7% in conventional crops to 15.9% in organic crops. Spore number ranged from 4.1 to 12.4 per 50 g^{-1} soil in both management systems. The most probable number of AMF propagules increased from 79 cm^{-3} soil in the conventional system to 110 cm^{-3} soil in the organic system. Microbial carbon, CO$_2$ emission, and FDA activity were increased by 100 to 200% in the organic crop. Thirteen species of AMF were identified, the majority in the organic cultivation system. *Acaulospora excavata*, *Entrophospora infrequens*, *Glomus* sp.3 and *Scutellospora* sp. were found only in the organically managed crop. *S. gregaria* was found only in the conventional crop. Organically managed vineyards increased mycorrhization and general soil microbial activity.
Key words: *Vitis vinifera* L., arbuscular mycorrhizal fungi, semiarid, soil microbial activity, sustainable agriculture

Atividade bioquímica e microbiológica do solo em videiras sob manejo orgânico e convencional no Nordeste do Brasil

RESUMO: O Vale do Submédio São Francisco é localizado na região do semi-árido brasileiro, sendo um importante centro da fruticultura irrigada, responsável por 97% da exportação nacional de uvas de mesa, incluindo as uvas sem sementes. Baseado no fato de que a fertilização orgânica pode melhorar a qualidade do solo, comparou-se o efeito do manejo orgânico e convencional sobre a atividade microbiana do solo e o estado micotrófico de videiras produtoras de uvas sem sementes. Foi avaliado o número de glomerosporos, número mais provável de propágulos (NMP), riqueza de espécies de fungos micorrízicos arbusculares (FMA), colonização de FMA, produção de PSRG-FE, carbono da biomassa microbiana (C-BM), respiração microbiana, atividade de hidrólise do diacetato de fluoresceína (FDA) e quociente metabólico (qCO$_2$). O manejo orgânico aumentou todas as variáveis, com exceção da PSRG-FE e do qCO$_2$. A colonização micorrízica foi 4.7% no cultivo convencional e 15.9% no orgânico. O número de esporos variou de 4.1 a 12.4 por 50 g^{-1} solo em ambos os sistemas de manejo. O NMP de propágulos de FMA foi de 79 cm^{-3} solo no sistema convencional e 110 cm^{-3} solo no sistema orgânico. O carbono microbiano, a emissão de CO$_2$ e a atividade do FDA apresentaram incrementos de 100 a 200% no cultivo orgânico. Treze espécies de FMA foram identificadas, a maioria no sistema orgânico. *Acaulospora excavata*, *Entrophospora infrequens*, *Glomus* sp.3 e *Scutellospora* sp. foram registradas apenas na cultura orgânica; *S. gregaria* foi exclusiva no cultivo convencional. O cultivo orgânico dos parreirais favorece a micorrização e a atividade microbiana do solo.
Palavras-chave: *Vitis vinifera* L., fungos micorrízicos arbusculares, atividade microbiana do solo, semi-árido, agricultura sustentável

Introduction

Soil microorganisms have an important role in ecological processes such as the nutrient cycling (Marshall, 2000; Nannipieri et al., 2003). Organic fertilization is important for soil microbial activity (Fernandes et al., 2005; Truu et al., 2008) because it improves soil's quality due to increase on avail-ability of organic matter which benefits physical, chemical and microbiological soil properties. This includes soil aggregation, aeration, and fertility levels, providing energetic substrates that potentially can be degraded by the edaphic microbiota, increasing the oxidative metabolism (Bettiol et al., 2002; Fernandes et al., 2005; Sampaio et al., 2008). However, the chemical products used in the conventional systems

besides contaminating natural resources can suppress the soil microbial activity, what makes the system less sustainable and more dependent from agricultural inputs (Anaya, 1999; Bengtsson et al., 2005).

Arbuscular mycorrhizal fungi (AMF) live in a mutualistic association with roots of most plant species. This association improves the uptake of mineral nutrients by the plants, which also improves the nutritional status of the plant host and increases the crop productivity. AMF also interact with other rhizosphere organisms contributing for the equilibrium, quality and fertility of soils mainly through the stabilizing action of the mycelium network (Jeffries et al., 2003; Miyauchi et al., 2008; Srivastava et al., 2007). Higher AMF activity has been reported in agrosystems where mineral fertilizers were substituted by organic fertilizers (Mäder et al., 2000; Purin et al., 2006). Studies regarding the impact of organic *versus* conventional systems on AMF activity have the potential to increase the knowledge on biotechnological application of these fungi (Ryan et al., 2000; Ryan and Graham, 2002).

The São Francisco Submedium Valley is located at the Brazilian semiarid region and represents an important center of production of irrigated fruits. This area accounts for 97% of the produced table grapes (*Vitis vinifera* L.) that are exported. One of the main varieties of interest is the seedless grape whose production has increased in the past decade (Silva and Correia, 2000). Seedless grapes represent 25% of the fine table grapes exported by producers of the São Francisco Valley. They occupy approximately 2,500 ha of irrigated areas and this number tends to increase in the next years.

Although many studies regarding the effects of organic *versus* conventional systems on soil microbial dynamic have been conducted (Mäder et al., 2000; Purin et al., 2006), none has been performed on seedless grape crops under the semiarid conditions of Northeast Brazil. Beneficial effects of the mycorrhization in vineyards have been registered (Matsuoka et al., 2002; Schreiner, 2003; Cheng and Baumgartner, 2004) and are mainly related with improvement of the nutritional status of young plants during the nursery period (Matsuoka et al., 2002; Schreiner, 2003; Aguín et al., 2004; Cheng and Baumgartner, 2004) or during acclimatization of micropropagated plants. Among the factors that impact the AMF-plant association the plant genotype is one of the most important. This justifies studies to explore the importance of AMF for the seedless variety of grape (Miyauchi et al., 2008). We tested the hypothesis that areas of seedless grapes under treatment with organic manure have higher microbial population and higher AMF activity when compared to areas under conventional management. This study was conducted to evaluate the effect of organic *versus* conventional cultivation on soil microbial and AMF activity in seedless grape crops.

Material and Methods

The study was conducted on a commercial seedless grape farm in Petrolina (09°19' S, 40°21' W), Pernambuco State, Brazil. The climate of the region is semiarid (type Bswh – Köeppen). Average air temperature is 26°C, with 50% of relative air humidity, 450 mm annual precipitation and 3000 h/year of sunshine. Soil is classified as Typic Quartzipsamments (Soil Survey Staff, 1999) or Neossolo Quartzarênico (Empresa Brasileira de Pesquisa Agropecuária, 2006). Plots were planted with the seedless grape cultivar "Festival seedless/IAC 766 rootstock". Two areas with approximately ten years old vineyards were chosen: (i) one fertilized according to recommendations for the crop in the region, characterized as conventional (Pereira et al., 2000); (ii) one receiving a compost of plant debris (organic amendment) during the last three years, characterized as organic. Both, the organic and conventionally fertilized vineyards were daily irrigated through microsprinkling.

Samples were collected during the harvest phase of the second production cycle. Ten compound samples were randomly collected from each area. Each compound sample consisted of four sub-samples taken from equidistant points around each plant. Soil and roots were collected from the 20 top centimeters in each sampling point. Roots samples were kept at 4°C until evaluations of mycorrhizal colonization were performed. Part of the soil samples was used for physical and chemical characterization (Table 1). Another fraction (200 mL per container) was used for preparing trap cultures. The remaining of the soil samples was kept at 4°C before being used for evaluation of mycorrhizal variables and soil biochemical and microbial activity.

AMF spores were extracted from soil by wet sieving and sucrose centrifugation (Gerdemann and Nicolson, 1963; Jenkins, 1964) and counted under a dissecting microscope. The quantity of AMF spores was expressed as number of spores per gram of soil. Seedless grape roots were treated with 10% KOH and stained with Chlorazol Black - E 0.03% (Brundrett et al., 1984).The mycorrhizal colonization was evaluated by the gridline intersect method (Giovanetti and Mosse, 1980).

Table 1 – Chemical and physical characterization of a typic Quartzipsamments or Neossolo Quartzarênico collected from the rhizosphere of apirenic grapeyards (IAC 766/Festival Seedless) cultivated under organic and conventional systems.

System	OM	pH*	EC	P	CEC	Sand	Silt	Clay
	g dm^{-3}		dS m^{-1}	mg dm^{-3}	mmol$_c$ dm^{-3}	——————— g kg^{-1} ———————		
Organic	25.0	6.9	0.31	543	7.62	920	50	30
Conventional	6.21	7.0	1.37	135	3.39	940	30	30

OM – organic matter; *H$_2$O (1:2.5); E.C. – electrical conductivity; CEC – cation exchange capacity.

The most probable number of AMF propagules was determined by the Feldmann and Idczak (1994) method. Corn seeds were sown in pots with soil taken from the field diluted in sand (0, 10^{-1}, 10^{-2}, 10^{-3}). Pots were daily watered and maintained in a greenhouse during 30 days. After that, presence of mycorrhizal colonization was assessed in roots stained as mentioned above and the MPN of propagules was estimated in cm^{-3} of soil using the Cochran (1950) table.

To quantify Easily Extractable Bradford-related soil protein (EE-BRSP), soil aggregates were mechanically separated in two fractions: < 1 mm and 1-2 mm were collected. EE-BRSP of each fraction was extracted from 0.25 g soil with 20 mM citrate pH 7.0 at 121°C for 30 min, as described by Wright and Upadhaya (1998). Protein in the supernatant was determined by the Bradford dye binding assay using bovine serum albumin as standard (Bradford, 1976).

Trap cultures were established with the objective of increasing detection of species that may not have been sporulating when field samples were collected (Stutz and Morton, 1996). Trap cultures were established in 250 mL containers using only soil samples from the field. Sorghum [*Sorghum bicolor* (L.) Moench, cv. IPA 1011] was the host plant. Pots were maintained under greenhouse conditions at 25 ± 3ºC; 75% relative humidity, 250 to 560 µmol m^{-2} s^{-1} luminosity. Soil samples were taken from the pots at the 45th, 90th, and 135th day after establishment of the cultures and the spores extracted for species identification. Spores were mounted onto microscope slides with PVLG (polyvynil alcohol lactoglycerol) and PVLG + Melzer (1:1). Species were identified based on Schenck and Pérez (1990), data from the International Culture Collection of Arbuscular Mycorrhizal Fungi (http://www.invam.caf.wvu.edu/taxonomy) and more recent publications.

Microbial biomass carbon (C-MB): carbon was estimated by fumigation with chloroform free of ethanol in 20 g of soil, followed by extraction of carbon with 50 mL of 0.5 M K_2SO_4 and oxidation with 2 mL of 0.66 mM $K_2Cr_2O_7$ in a medium with 10 mL of concentrated H_2SO_4 and 5 mL of concentrated H_3PO_4. Carbon was quantified by titration with $FeSO_4$ $(NH_4)_2SO_4.6H_2O$ (0.033 eq g L^{-1}) using $(C_6H_5)_2NH$ (1%) as indicator. Calculations were performed in fumigated and non fumigated soil samples, using the correction factor K_c = 0.33. Values were expressed as µg C g^{-1} dry soil (De-Polli and Guerra, 1997).

To calculate microbial respiration (C-CO_2 evolution): 100 g of soil were incubated in 1000 mL recipients with 10 mL of KOH (0.5 eq g L^{-1}) for 15 days, in the dark. The CO_2 captured by KOH solution was quantified by titration with 0.1 eq g L^{-1} HCl, using phenolphthalein (0.1% in ethanol) and methyl orange (1%) as pH indicators. The CO_2 carbon liberated by microorganism respiration was expressed as µg CO_2 g^{-1} of dry soil day^{-1} (Grisi, 1978). The metabolic coefficient (qCO_2) was determined by the relation between the carbon of the evoluted CO_2 and the carbon of the soil microbial biomass.

To evaluate hydrolysis of fluorescein diacetate activity (FDA), 5 g of soil samples were incubated in an Erlenmeyer flask with 20 mL potassium phosphate buffer (66 mM; pH 7.6) and 200 µL of FDA solution (0.02 g 10^{-1} mL acetone) for 20 min. The reaction was interrupted by addition of 20 mL acetone and measurements were taken using a spectrophotometer (490 nm). Increasing concentrations of FDA that were previously hydrolyzed by heat (100°C) were used to construct a standard curve. The enzymatic activity was expressed in µg of hydrolyzed flourescein g^{-1} dry soil h^{-1} (Swisher and Carrol, 1980).

Data were submitted to one-way analysis of variance (ANOVA) and means were separated followed by the Tukey test (0.05%). Data transformation were applied to the percentage of colonization (Arc Sen $\sqrt{x}$ / 100) and to the number of AMF spores ($\sqrt{x}$ + 1) before statistical analysis.

Results and Discussion

The highest sporulation in field samples was observed in the organic system (Table 2). This may be due to the increase of organic matter in the soil under organic cultivation (Mohammad et al., 2003), considering that use of organic sources increases the abundance of AMF (Noyd et al., 1996). The organic input improves the physical and chemical soil properties, the nutritional status of the plant and, consequently, the amount of photosynthate for the fungus, which increase sporulation. Oehl et al. (2004) also observed higher number of spores in a soil from organic crops as compared to a soil with chemical fertilization. They attributed this difference to the excessive amount of applied mineral fertilizers. It is possible that fertilization had effects on sporulation in the areas chosen for the present experiment. However, we can only speculate on factors such as the quantity and nature of fertilizers since a more precise evaluation was not the objective of this study.

Mycorrhizal colonization was relatively low in both systems (Table 2) when compared to colonization rates higher than 80% which have been reported in vineyards (Schreiner, 2003). Root colonization was three times higher in the organic system (15.9%) when compared to the conventional system (4.7%). Increased AMF root colonization is one of the benefits of organic cultivation that has been also reported in other crops. Roots of *Secale cereale* L. (rye) under organic management presented 77% of mycorrhizal colonization, while in the conventional system this percentage was reduced to 11% (Sattelmacher et al., 1991). Levels of mycorrhizal colonization from 0 to 45% in vineyards (conventional) were observed by Matsuoka et al. (2002) who attributed these levels to the intensive use of chemical fertilizers and stage of plant development. Plants also have different colonization patterns and mycorrhizal dependency, and this variation is also observed in grapes. Mycorrhizal root colonization ranged between 46 to 76% according to the rootstock (Karagiannidis et al., 1997). Likewise, the low values of root colonization observed in this study may be typical of the variety used (IAC 766/Festival Seedless). We quantified 110 and 79 infective propagules of AMF cm^3 soil in the organic and conventional cultivation systems, respectively. Our data corroborates previous studies carried out with other economically important species such as *Spinacea oleraceae* and *Malus domestica* (Douds Jr. et al., 1997; Purin et al., 2006).

Table 2 – Number of AMF spores, most probable number of AMF propagules (MPN), easily extractable Bradford-related soil protein glomalin (EE-BRSP) and arbuscular mycorrhizal colonization (MC) in the rhizosphere of grape rootstocks (IAC 766/Festival Seedless), produced under organic and conventional systems.

System	Number of AMF spores	MPN of AMF propagules	EE-BRSP		MC
			(1-2 mm)	(< 1 mm)	
	50 g^{-1} soil	cm^{-3} soil	----- mg g soil^{-1} aggregates -----		%
Organic	12.00 a	110	2.52 a	1.33 a	15.9 a
Conventional	4.00 b	79	2.44 a	1.37 a	4.7 b

Means followed by the same letters, in the column, do not differ (Tukey test, $p < 0.05$).

EE-BRSP has been positively related to the increase of fertility (Lovelock et al., 2004a) and carbon levels (Wright and Upadhyaya, 1998; Lovelock et al., 2004a). However, the organic cultivation of grapes did not increase EE-BRSP production when compared with the conventional system (Table 2). It is possible that only three years of organic management are not enough to show differences in the quantities of EE-BRSP. All nutrient dynamics in soil are reflected first on components directly involved in mycorrhization, such as spores and root colonization, with changes in protein turnover being observed in long term studies. Also, there are differences on AMF composition between organic and conventional systems and the species probably differ regarding their capability to produce the EE-BRSP. This means that differences cannot be related only to soil management, but also to the microbial composition. Besides, the high levels of P commonly observed in soils under organic management can inhibit EE-BRSP production (Lovelock et al., 2004b). Differences of EE-BRSP quantities between organic and conventional management also depend on the stage of plant growth (Monokrousos et al., 2008).

Altogether, thirteen AMF taxa were identified in the two crop systems. Twelve species were detected in the organic systems, and nine were detected in the conventional (Table 3). Eight of the 13 species were found in both systems. Previous studies conducted in the Brazilian semiarid registered variable number of AMF species. Maia and Trufem (1990) identified only eight AMF species associated with cotton, bean, corn and cassava crops. Fifteen AMF species were identified in association with banana at the São Francisco Submedium Valley (Yano-Melo et al., 1997). Twenty four species of AMF have been registered in a semiarid area with native vegetation known as "caatinga" (Souza et al., 2003). These numbers suggest that in this region of Brazil the number of AMF species decreases when native vegetation is removed and crop species are introduced. The establishment of the vineyard also affected the AMF native population; successive monoculture usually reduces the number of AMF species (Douds and Millner, 1999). Because of this negative impact, it is important to adopt cultivation systems that minimize the loss of AMF species. The present study confirms the organic fertilization as a practice of this nature.

The species *Acaulospora excavata*, *Entrophospora infrequens*, *Glomus* sp.3 and *Scutellospora* sp. were found only in the organically managed area. *Scutellospora gregaria* was observed only in soil with conventional system. Some species such as *A. mellea, G. sinuosum* and *G. etunicatum* were always detected throughout evaluation of the trap cultures. Other species such as *A. scrobiculata* were observed only after 90 days of trap pot culturing (Table 3). *E. infrequens* and *G. albida* were identified only with the use of trap cultures in soil with organic fertilizer.

Higher microbial activity was observed in the organic system than in the conventional system (Table 4). This might have resulted from the higher amount of substrates with potential for microbial degradation, which were used as energy and carbon source by the soil microbiota (Fernandes et al., 2005). The soil from the organic system presented an evolution of 59.64 µg C-CO$_2$ g^{-1} dry soil day^{-1} (Table 4). This value is twofold higher than what was observed in the conventional system. Similar results were obtained by Sarangi et al. (2001) who registered an increase of 143% in CO$_2$ emission in a soil with organic amendment (17.5 t residue ha^{-1}) when compared to the treatment with chemical fertilizers (N:P:K 80:40:40 kg ha^{-1}). Possibly, the compost used in the organic cultivation of grapes increases soil microbial activity. In general, addition of organic materials enhances respiratory activity because the organic residues are energetic substrates consumed during oxidative metabolism of the soil heterotrophic microbiota (Bhattacharyya et al., 2001). The carbon of the microbial biomass (C-MB) is one of the most important variables that reflect differences between organic and conventional areas (Monokrousos et al., 2006). This was confirmed in the present study. The C-BM values were higher in the organic system than in the conventional area, reflecting differences on the microbial communities.

Other indicators of microbial activity were also higher in the organic areas when compared to the conventional areas. There was a two-fold increase in carbon of microbial biomass and a three-fold increase in FDA activity (Table 4). These differences may be due to the higher levels of organic matter in the organic system (Haynes, 1999; Taylor et al., 2002). The organic amendment introduced in the vineyards probably presented more metabolically active microorganisms that contributed for higher enzymatic activity in this system (Debosz et al., 2002). The enzymes that adhere to the colloids of the organic compost can be another factor to increase the rate of FDA hydrolysis in the organic cultivation (Nannipieri et al., 2003). Organic fertilizers may also contribute to modify root exudates in the vineyards, by increasing the amount of organic compounds produced by the plant,

Table 3 – Taxa of AMF in soils with organic and conventional crops of vineyards (IAC 766/Festival Seedless) identified through direct exam of field material and after maintenance of soil samples in trap cultures, evaluated at the 45th, 90th and 135th day of established.

Species of AMF	Field		After trap culture					
	OC	CC	OC			CC		
			45	90	135	45	90	135
Acaulospora mellea Spain & Schenck	x	x	x	x		x	x	x
A. scrobiculata Trappe	x	x		x	x		x	x
A. excavata Ingleby & Walker	x			x	x			
Entrophospora infrequens (Hall) Ames & Schneid.				x				
Gigaspora albida Schenck & Sm.		x		x				x
Glomus sinuosum (Gerd. & Bakshi) Almeida & Schenck	x	x		x	x	x	x	x
G. etunicatum Becker & Gerd.	x	x	x	x	x	x	x	x
Glomus sp.1	x	x				x	x	x
Glomus sp.2	x	x	x	x				
Glomus sp.3	x							
Glomus sp.4	x	x					x	x
Scutellospora gregaria (Schenck & Nicolson) Walker & Sanders		x						
Scutellospora sp.1	x							
Total number of species	10	9	3	8	4	4	6	7

OC – organic crop; CC – conventional crop.

Table 4 – Carbon of microbial biomass (C-MB), microbial respiration (C-CO_2), FDA hydrolysis and metabolic coefficient ($q$$CO_2$) in the rhizosphere of vineyards rootstocks (IAC 766/Festival Seedless), under organic and conventional systems.

Crop system	C-MB	C-CO_2	FDA	$q$$CO_2$
	μg g dry soil^{-1}	μg g dry soil^{-1} d^{-1}	μg g dry soil $^{-1}$ h^{-1}	
Organic	163.41 a	59.64 a	24.63 a	0.365 a
Conventional	65.98 b	19.86 b	6.56 b	0.301 a

Means followed by the same letters, in the column, do not differ (Tukey test, $p < 0.05$).

maximizing soil microbial activity (Pascual et al., 1999). In addition, we hypothesize that higher AMF sporulation and MPN values observed in the organic system likely contributed to increase the microbial biomass carbon in this management system. It is possible that production of extraradical mycelial net was also increased by the organic system and contributed to the C stock in the soil and affecting soil aggregation (Rillig et al., 2001).

The metabolic coefficient ($q$$CO_2$) did not differ between the systems (Table 4). High values of $q$$CO_2$ usually may indicate a stressing condition in disturbed systems (Garcia et al., 2002) and, in general, conventional agrosystems present higher values in relation to the organic cultivation or the natural ecosystems (Dilly and Munch, 1998). However, not always the $q$$CO_2$ is sensible to measure the soil conditions (Wardle and Ghani, 1995). In semiarid soils, higher $q$$CO_2$ was registered in a native area compared with an irrigated area receiving saline waste (Pereira et al., 2004) and increase of $q$$CO_2$ in revegetated semiarid areas was also observed (Garcia et al., 2005). Other variables may be more sensible to indi-

cate the soil quality, as shown in this study. Thus, other relations based on microbiological variables, should be evaluated to elucidate the general microbial activity of a soil, as $q$$CO_2$ does not have universal values.

The organic cultivation of seedless grapes increased AMF and microbial activity as well as contribute to carbon immobilization by microorganisms in a short period of time. Evidence from this study supports organic management as a potential alternative to produce table grapes in the Brazilian semiarid region.

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