Symbiosis (2017) 71:223–232
DOI 10.1007/s13199-016-0432-9

Incidence and diversity of arbuscular mycorrhizal fungi and successor herbaceous plants in an agro-system irrigated with produced water

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Received: 21 December 2015 / Accepted: 2 June 2016 / Published online: 11 June 2016

Abstract The purpose of this study was to evaluate the diversity of herbaceous plants and arbuscular mycorrhizal fungi following the cultivation of sunflower (*Helianthus annuus* L., cv. BRS 321) irrigated with produced water. The sunflower plants were irrigated during three successive cycles with different types of water: produced water obtained through simple filtration (PWSF), and secondly, produced water treated by reverse osmosis (PWRO), and the control with groundwater from the aquifer Açu (WCA). In June 2014, five months after the final harvest, the treatments were evaluated in terms of the diversity of successor plants and their roots colonized by arbuscular mycorrhiza (AM); and samples of soil, in which the following were measured: the spore abundance of AM fungi, the levels of glomalin in easily extracted glomalin and total glomalin. Of a total of eighteen species of herbaceous plants which were identified in the experimental field, *Dactyloctenium aegyptium* was related with the use of PWSF, *Panicum* sp. and *Diodella apiculata* with the use of PWRO, and *Trianthema portulacastrum* and *Eragrostis tenella* with the control WCA. The diversity of AM fungi was affected by irrigation with PWSF, in which two species of *Acaulospora*, one species of *Gigaspora* and species of *Paraglomus* were absent, compared to the treatment with PWRO. Acaulospora sp.1 was related with the WCA control as an indicator species. The use of produced water which had undergone reverse osmosis had a short-term effect on the content of glomalin which is easily extractable from the soil but did not change the mycorrhization rates of plants. These results enable us to infer that irrigation with produced water leads to a reduction in the diversity of herbaceous plants and of arbuscular mycorrhizal fungi in the soil, confirming the importance of monitoring agro-systems irrigated with residual water.

Keywords Wastewater · Plant succession · Mycorrhizal activity · Environmental microbiology

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1 Introduction

Agricultural production in semi-arid regions constitutes a challenge when there are problems with water distribution or a scarcity of drinking water, so it has been suggested that alternative sources, such as residual water, should be used for irrigation (Namazy et al. 2015). One of these alternative sources of water is produced water which is obtained when oil is extracted (Motta et al. 2013). In the Northeastern Brazil up to 90 % of the liquid extracted from oil wells is produced water (Melo et al. 2010). However, this water contains constituents (Melo et al. 2010) and residues of the chemical additives used in the process of separating out the oil (Figueredo et al. 2014), which makes the treatment of the produced water essential if it is to be re-used industrially.

Notwithstanding this, the treatments of produced water include the filtration and passing of water through ion-exchange

resin (Qian et al. 2012), and nanofiltration followed by reverse osmosis treatment (Alzahrani et al. 2013), among other methods of treating residual water (Igunnu and Chen 2012; Gregory et al. 2011; Das et al. 2014) which aim to remove toxic constituents.

Regardless of which water treatment is used in the petroleum industrial plant, the management of the wastewater must be monitored. The water residue from oil is brackish (Tabatabaei and Najafi 2009; Travis et al. 2012) and its use in the environment can lead to the salinization of the soil (Al-Haddabi and Ahmed 2007). In turn, high levels of salt interfere with the characteristics of the soil (Onojake and Abanum 2012) and with the biological activity of functional groups (Nwaugo et al. 2007), reducing the activity of arbuscular mycorrhizal fungi (Saint-Etienne et al. 2006; Bencherif et al. 2015), which is reflected in plant growth (Yang et al. 2015). Nevertheless, the concentration of salts determining this reduction varies according to the species of plant, and this fact could be related to the tolerance of salinity of each species (Bañuelos 2015).

The agricultural use of treated produced water has been suggested (Melo et al. 2010), but the irrigation of sunflower plants (*Helianthus annus* L.) and castor-oil plants (*Ricinus communis* L.) has led, in a short time, to changes in the proliferation and activity of fungi and cultivable bacteria (Lopes et al. 2014) and in the structure of mesofauna communities in the soil (Ferreira et al. 2015), reinforcing the need to monitor biotic and abiotic factors in soil which has been irrigated with the residual water from oil extraction.

Herbaceous plants are sensitive to changes in the environment and contribute to the formation of a microclimate, changing the soil's properties (Wang et al. 2014). They also establish associations with AM fungi in their roots. So too, these micro-symbionts are indicators of environmental impacts due to their use as a biotechnological input in degraded areas (Schreiner 2007), and can serve as a biological indicator of soil salinity (Flores 2010). An increase in the concentration of salts affects sporulation, selecting species of AM fungi which are saline resistant, (Sheng et al. 2008) and also the mycorrhization of plants (Saint-Etienne et al. 2006; Guo and Gong 2014; Bencherif et al. 2015).

In light of the paucity of research work related to the impact of produced water on the vegetation in agro-systems, this research was undertaken in order to determine the diversity of herbaceous plants and AM fungi in the soil after sunflowers had been grown using produced water for irrigation.

2 Material and methods

2.1 Study area and experimental approach used

This research was carried out on the Belém farm (FZB) belonging to Petrobras in the municipality of Aracati, Ceará, Brasil (4° 44′43.2″S, 37° 32′19.6″W). The soil is classified as Typic Quartzipsamment and the vegetation as semi-arid (seasonal), known as Caatinga (Sampaio 1995). In the years 2012 and 2013 the area was planted with sunflower (*Helianthus annuus* L., cv. BRS 321) and irrigated by different types of water (Sousa et al. 2016). The average air temperature registered in 2014 was between 26 °C and 28 °C and the annual rainfall was 564.9 mm with frequent periods of rain between March and May (Funceme 2015).

The sunflower plants were irrigated by means of a drip-irrigation system for three successive life-cycles, using adequate blades of water and two different types of produced water: produced water which was obtained through filtering and passing through columns of cation resin (PWSF), produced water which was filtered and treated by reverse osmosis (PWRO). Control plants were irrigated with groundwater collected (250 m depth) from the Açu aquifer (WCA). All types of water were supplied by the Operations Unit of the FZB belonging to Petrobras. All the irrigation treatments were repeated three times (in plots of land measuring 20 m x 20 m) which were randomly assigned within the experimental area. The amounts of water given to the plants during the three life-cycles (1124 mm of PWSF, 1060 mm of PWRO and 1033 mm of WCA) were calculated using the column mini-lysimeters (0.4 m diameter by 0.6 m depth) which had been installed in the different plots, taking into consideration the rates of evapotranspiration of the plant, and water loss through drainage in the soil profile (Sousa et al. 2016).

2.2 Collection and identification of successor herbaceous plants

In June 2014, five months after the last harvesting of sunflowers, rectangular sub-areas (1 m x 2 m) were marked out in the different experimental plots. In order to demarcate the sub-areas, a diagonal line was drawn through the plots, in order to obtain samples of plants which would be representative of the areas which had previously been cultivated. The period in which the successor plants were collected coincided with their flowering and seed production stage, making the separation into groups possible there in the field and the identification of species of vegetation easier.

The plants in the demarcated areas (2 m^2) were carefully collected, separated into groups and quantified. Representatives of the groups identified in the field were separated out and the parts containing reproductive structures were labelled, placed in presses and taken to the Laboratory of Angiospermae Taxonomy at the Universidade Federal do Ceará (UFC), in Fortaleza. After drying and screening they were exsiccated and donated for inclusion in the EAC Herbarium acronym according to (Thiers 2015) at UFC. In order to identify the herbaceous species, taxonomic keys were used together with the bibliography available at the EAC

Herbarium, and by making comparisons with the images provided by the Botanical Garden of Rio de Janeiro (JBRJ, Instituto de Pesquisas Jardim Botânico do Rio de Janeiro 2014) and the Brazilian Flora (http://floradobrasil.jbrj.gov.br/).

2.3 Evaluation of the mycorrhizal colonization of the herbaceous plants

The roots of the herbaceous plants prevalent in the plots of land which had undergone different irrigation treatments were kept in separate batches. The intermediate sections of fresh, fine roots (< 0.2 mm) were placed in small bottles containing an alcohol solution (5 % acetic acid and 90 % ethyl alcohol) in order to conserve them until their mycorrhizal colonization could be evaluated, in the Lab of Soils at the Embrapa Tropical Agroindustry, in Fortaleza.

To determine AM association in plants, 1 g pieces of fine roots were placed in a KOH 10 % basic solution in an autoclave at 121 °C for 12 min, as described by Philips and Hayman (1970). Following this procedure, the roots were washed with tap water and covered with a solution of hydrogen peroxide (H_2O_2 at 1.25 %) for 20 min. Immediately afterwards, the roots were washed in an acidic solution (HCl at 1 %), for 4 min, and soaked in aniline blue (875 mL of lactic acid, 63 mL of glycerine, 0.5 g of dye and 62 mL of distilled water), overnight. A total of 20 segments of dyed roots from each sample were mounted onto sheets of glass. The segments were covered with a lacto-glycerol solution and placed on glass slides so that observation through the microscope could be made (up to 400x) looking inside the roots for the presence of arbuscules, vesicles and other structures characteristic of AM fungi.

2.4 Collection of soil, chemical evaluation and microbiological analysis

Soil samples composed of ten simple samples from the demarcated sub-areas were collected from the topsoil (up 0.1 m depth) and placed in properly labelled bags, for analyses to be carried out later at Embrapa Agroindústria Tropical, in Fortaleza. These samples were dried straightaway in the open air and then passed through a sieve with a 2 mm mesh for subsequent analysis. For the determination of pH in water (proportion 1:2.5) and the extraction of the elements P, K, Na, Fe, Cu, Mn, Zn (Merlich 1), Ca, Mg (solution KCL 1 mol) and the determination of organic carbon, the procedures described by Silva (2009) were followed. These analyses made it possible to describe the composition of the soil at the time it was collected in June 2014 (Table 1), after the irrigation and the cultivation of the sunflower plants.

Other parts of the soil samples were used for the extraction of the spores of AM fungi and the determination of the levels of glomalin. For the extraction of fungal spores, the humid-sieving technique was used, followed by centrifugation and sucrose gradient fluctuation, as described by Sieverding (1991). The different spore morphotypes of AM fungal communities were recognized under a stereomicroscope (up to 200x). Examples of typical spores from each group were mounted on glass slides in PVLG solutions (polyvinyl alcohol in lacto-glycerol) and PVLG/Melzer's reagent (1:1 *v*/v) (Sieverding 1991), for subsequent identification of the fungal species based on the color of the spores, the ornamentation of the surface and the structure of the wall as set out in the Identification Manual of Schenck and Pérez (1990). Descriptions on the site belonging to the INVAM (2015) were also consulted, together with original descriptions.

Other fractions of the soil samples were also analysed to determine the level of proteins related to glomalin in the soil in easily extractable glomalin (GRSP-EEG) and total glomalin (GRSP-TG), following the procedures adopted by Rilling (2004) and Wright and Upadhyaya (1998), with modifications in the extraction of proteins through the use of sodium citrate (20 mM in pH 7.4 GRSP-EEG and 50 mM pH 8.0 for GRSP-TG), and the quantification of both fractions following the Bradford die-binding assay with bovine serum albumin as the stander (Wright et al. 1996). The final concentrations of both protein fractions were adjusted to mg g-1 dry soil.

2.5 Analysis of the data

For the analysis of plant species and AM fungi, combinations of relative abundance and frequency relative to the species were used, as suggested by Dufrêne and Legendre (2007); and the calculation of the significance of indicator species in the different treatments was carried out through permutation tests (Dufrêne and Legendre 1997). In the evaluation, species which had an indicator value (*IndVal*) > 0.6; frequency > 3; $p \leq 0.05$ were considered valid for this study. It should be pointed out that this study of indicator species (*IndVal*) shows a tendency for a species to occur with greater frequency in one environment, and this should be considered in soils contaminated by salts or other chemical and organic constituents.

The effect of the different irrigation treatments on the abundance and diversity of species of vegetation and AM fungi, the levels of total and easily extractable glomalin and the intensity of mycorrhizal colonization of herbaceous plants were evaluated using the general Linear Model (GLM) based on the Poisson distribution. Before the evaluation, the data on the root colonization by AM fungi were arsine transformed $(x + 1/100)^{0.5}$ for normalization. The treatment averages were

Table 1 Values of pH, organic carbon (OC) and nutrients from the soil (up to 0.1 m depth), after the cultivation of sunflower irrigated with different types of water at the Belém farm, in Aracati, Ceará (Brazil)

Irrigation treatment	pH	OC g/kg	Ca mmol/cm^3	Mg	K	Na	P	Cu	Fe mg/cm^3	Mn	Zn
PWRO	7.20	5.30	25.10	13.80	2.20	2.00	240.50	4.20	6.80	25.50	3.20
PWSF	7.30	5.30	20.90	12.70	2.40	6.30	206.60	3.80	4.80	25.30	3.20
WCA	7.80	6.20	20.80	21.10	2.10	3.00	241.70	4.90	7.00	38.00	4.70

Produced water treated by reverse osmosis (PWRO), by simple filtration (PWSF), and groundwater from Açu Aquifer (WCA)

compared in terms of the different variables, using Tukey's test at 5 % probability and the SAS *software* (Release 9.2) (SAS INSTITUTE. SAS/STAT 2008).

3 Results

3.1 Floristic composition

A total of 2390 plants were identified in the experimental area evaluated, representing a dozen families and 18 species (Table 2). The most prevalent individuals belonged to the *Dactyloctenium aegyptium*, followed by *Eragrostis tenella*, *Mollugo verticillata*, *Croton glandulosus*, *Waltheria americana* and *Croton hirtus*. The diversity of plants was reduced to 14 species in the treatment previously irrigated with WCA (control); to 12 species treated with PWRO, and to 6 species in the plots irrigated with PWSF, where the levels of Na were higher (Table 1). It is worth pointing out that the PWSF passed through a sand filtering process and columns of ion-exchange resin before being used on the soil, demonstrating the impact that produced water has on spontaneous vegetation following sunflower cultivation.

Of the eighteen herbaceous species identified in the experimental area, *Dactyloctenium aegyptium* was related to the use of PWSF (*IndVal* = 0.62; p = 0.04), *Panicum* sp. (*IndVal* = 1.00; p = 0.04) and *Diodella apiculata* (*IndVal* = 1.00; p = 0.04) with the use of PWRO, and *Trianthema portulacastrum* (*IndVal* = 1.00; p = 0.04) and *Eragrostis tenella* (*IndVal* = 0.93; p = 0.04) with the treatment which received WCA. The greatest abundance of herbaceous plants (1132) occurred with the PWSF treatment (p = 0.031) and the smallest was observed in the plots irrigated with PWRO (p = 0.003), as can be seen in Fig. 1. Otherwise the diversity of herbaceous species was lower in the plots irrigated with PWSF (p = 0.005) compared to the other kinds of water used for irrigation (Fig. 1). The abundance of plants observed after treatment with PWSF could be associated with the proliferation of the species *Dactyloctenium aegyptium*. This species had the greatest dominance in the plots irrigated with PWSF. In the other treatments, the number of individuals belonging to different species varied a lot less.

3.2 Mycorrhization of the herbaceous plants prevalent in areas irrigated with produced water

All the individual plants belonging to *Dactyloctenium aegyptium*, *Eragrostis tenella* (both of these belong to the Poaceae), *Waltheria americana* (Malvaceae) and *Mollugo verticillata* (Moluginaceae) supported mycorrhizal association, although the colonization of their roots by AM fungi was not significantly affected by the type of irrigation treatment (Table 3). Other species, such as *Croton hirtus*, frequent in plots subjected to irrigation with PWRO and the *Borreria scabiosoides* present in both treatments with PWRO and WCA showed similar intensities of root colonization by AM fungi. In general, mycorrhization rates ranged from 19 % to 35 % for *Waltheria americana*, 22 % to 30 % for *Dactyloctenium aegyptium*, reaching up to 55 % for *Croton hirtus*, 10 % to 35 % for *Borreria scabiosoides*, 11 % to 20 % for *Eragrostis tenella*, and 13 % to 15 % for *Mollugo verticillata*.

3.3 Diversity of AM fungi in soil irrigated with produced water

Spores from ten species of AM fungi were identified in soil samples from the experimental area (Table 4), of which four belonged to the family Acaulosporaceae, two to the family Gigasporaceae and just one species of fungus belonging to the following families: Ambisporaceae, Diversispoareae, Glomeraceae and Paraglomeraceae. The diversity of spore morphotypes of AM fungi was significantly reduced when PWSF was used for irrigation, where there was a loss of four species of fungi compared to the PWRO treatment (*Acaulospora* sp1, *Acaulospora* sp2, *Gigaspora margarita* and *Paraglomus* spp.). It is worth pointing out, however, that small hyaline spores of *Paraglomus* were also undetectable in plots irrigated with WCA. A low frequency of spores (5.8 %) here identified as *Paraglomus* spp. was observed in the soil treated with PWRO, and this could be associated with the presence of plants

Table 2 Populations of communities of herbaceous plants after the cultivation of sunflower irrigated with different types of water at the Belém farm, in Aracati, Ceará

		Irrigation treatment					
Code EAC	Family/ Species	PWRO		PWSF		WCA	
		Individuals	F (%)	Individuals	F (%)	Individuals	F (%)
	Aizoaceae						
56,092	*Trianthema portulacastrum* L.	0.00	0.00	0.00	0.00	9.00	1.04
	Amaranthaceae						
56,103	*Froelichia humboldtiana* (Schult.) Seub.	0.00	0.00	0.00	0.00	1.00	0.12
	Convolvulaceae						
56,100	*Merremia aegyptia* (L.) Urb.	7.00	1.89	0.00	0.00	3.00	0.35
	Euphobiaceae						
56,094	*Cronton hirtus* (L.) Hér.	61.00	16.44	0.00	0.00	0.00	0.00
56,097	*Croton glandulosus* (L.) Hér.	10.00	2.70	1.00	0.09	16.00	1.84
56,086	*Jatropha ribifolia* (Pohl) Baill.	0.00	0.00	0.00	0.00	1.00	0.12
	Fabaceae						
56,095	*Mimosa candole* R.Grether	1.00	0.27	0.00	0.00	1.00	0.12
56,084	*Tephrosia purpurea* (L.) Pers. subesp. *Purpurea.*	0.00	0.00	0.00	0.00	1.00	0.12
	Malvaceae						
56,106	*Waltheria americana* L.	25.00	6.74	4.00	0.35	19.00	2.19
56,099	*Pavonia cancellata* (L.) Cav.	0.00	0.00	0.00	0.00	7.00	0.81
56,087	*Herissanthia tiubae* (R.Schum.) Brizicky.	1.00	0.27	0.00	0.00	0.00	0.00
	Moluginaceae						
56,107	*Mollugo verticillata* L.	20.00	5,39	12.00	1.04	48.00	5.52
	Nyctaginaceae						
56,098	*Boerhavia difusa* Cham. & Schltdl.	0.00	0.00	1.00	0.09	4.00	0.46
	Poaceae						
56,105	*Dactyloctenium aegyptium* (L.) Willd.	212.00	57.14	1130	98.26	495.00	56.96
56,108	*Eragrostis tenella* (L.) P.Beauv. ex Roem. & Schult.	16.00	4.31	2.00	0.17	258.00	29.69
56,102	*Panicum sp.*	6.0.00	1.62	0.00	0.0	0.00	0.00
	Rubiaceae						
56,109	*Borreria scabiosoides* Cham. & Schltdl.	4.00	1.08	0.00	0.00	6.00	0.69
56,090	*Diodella apiculata* (Willd ex. Roem. & Schuldt.) Delprete	8.00	2.16	0.00	0.00	0.00	0.00
	Total	371		1150		869	

Produced water treated by reverse osmosis (PWRO), by simple filtration (PWSF), and groundwater from Açu Aquifer (WCA). Frequency (F) = 100 (Number of individuals of the species/ Total individuals of the plot). Code EAC = voucher specimen

belonging the genus *Panicum* sp., *Croton hirtus* or *Diodella apiculata* (Table 2).

With regard to the abundance of spore morphotypes of AM fungal communities, no difference was found between two of the treatments, that with WCA and that with PWRO, but there was a significant reduction in fungal spores in the PWSF treatment ($p = 0.015$) (Fig. 2), as well as a smaller number of species of AM fungi (Table 4). Just as floristic diversity was reduced in the herbaceous stratum (Table 2), there was also a reduction in the sporulation of AM fungi in the soil irrigated with PWSF, which may be attributed to the elevated level of Na present in this type of water (Table 1), and which, in the long-term, could affect the productive potential of the agro-system.

3.4 Level of glomalin in the soil

The levels of protein fractions related to glomalin (GRSP-EEG, GRSP-TG) were higher in the soil irrigated with WCA (Table 5); the lowest levels of GRSP-EEG derived mainly from the mycelium of AM fungi were detected in the soil irrigated with PWRO. During the treatment process, this water received glutaraldehyde, a biocide that prevents the formation of bacterial biofilms on the membrane filters used in the oil industry. On the other hand, the GRSP-TG fraction was more stable in the soil, regardless of the levels of the variables in the PWRO and PWSF treatments.

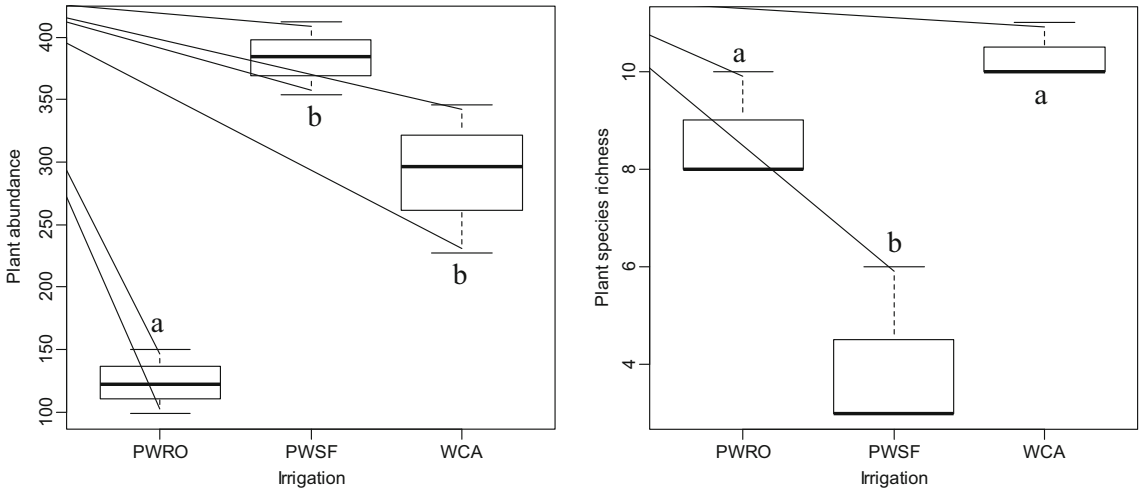

Fig. 1 Abundance and richness of herbaceous plants in succession to irrigated sunflower crop with produced water treated by reverse osmosis (PWRO), by simple filtration (PWSF), and groundwater from Açu Aquifer (WCA). Identical letters indicate that the treatments were not significantly different ($p < 5$ %)

4 Discussion

Irrigating sunflower plantations with produced water had an impact on the diversity of successor herbaceous plants and functional groups of AM fungi in the soil. The reduction in the diversity of plants and AM fungi could be linked to the levels of salts, which were found to be as high as 6.3 mmol Na for each cm^3 of soil in the PWSF treatment. According to Yang et al. (2015), an excess of Na can lead to smaller plant growth. However, the level of concentration of salts in the soil affects different plant species in different ways (Bañuelos 2015). According to Heinze et al. (2015) biotic factors, such as competition, pathogenic micro-organisms, parasites and mutualistic interactions, along with abiotic factors, such as temperature, humidity, and the incidence of light, as mentioned by Sproull et al. (2015) can also affect the plant community structure. In the PWSF treatment, there was a high level of *Dactyloctenium aegyptium* (Poaceae), a species which may possibly be more tolerant of saline stress in the soil. This grass is not halophytic, but it does have a good capacity to adapt to different environments (Adu et al. 1994). Some species of the Poaceae family produce large quantities of seeds and this significantly increases dissemination in different environments and conditions (Holm et al. 1991).

Other herbaceous species, such as *Panicum* sp., *Diodella apiculata* were indicators of PWRO treatment, while *Trianthema portulacastrum* and *Eragrostis tenella* were associated with the control treatment which received WCA. It should be noted that *Panicum* sp. was not found in the plots irrigated with PWSF, an outcome which corroborates the observations made by Koyro et al. (2013). These researchers demonstrated that representatives of the *Panicum* genus have low levels of tolerance to soil salinity.

The species *Trianthema portulacastrum* was an indicator of the WCA control and its frequency may be linked to the potential to produce substances which inhibit the germination of other plants, as mentioned by Mubarik et al. (2015). The species *Diodella apiculata* and *Eragrostis tenella*, (which belong, respectively, to the families Rubiaceae and Poaceae), are common herbaceous components of the semiarid regions of Brazil (Machado Filho et al. 2015).

The arbuscular mycorrhizal colonization of the most common herbaceous plants was not affected by the types of irrigation. However, the rates of root colonization by AM fungi decreased in the following order: *Waltheria americana* > *Dactyloctenium aegyptium* > *Croton hirtus* > *Borreria scabiosoides* > *Eragrostis tenella* > *Mollugo verticillata*. Even with low levels of mycorrhization, the plants possibly benefit from mycorrhizal symbiosis. It was observed that there was a high level of phosphorus in the soil, 206.6 mg P per dm^3 of soil irrigated with PWSF and more than 240 mg P per dm^3 of soil irrigated with PWRO and WCA, and this might have

Table 3 Arbuscular mycorrhizal colonization rate (%) of frequent herbaceous plants in the experimental plots previously cultivated with sunflower irrigated with different types of water at the Belém farm, in Aracati, Ceará

Irrigation treatment	Plants			
	D. aegyptium	*W. americana*	*M. verticillata*	*E. tenella*
PWRO	21.7	35.0	5.0	8.3
PWSF	26.7	11.7	6.7	5.0
WCA	13.3	35.0	5.7	13.3

Produced water treated by reverse osmosis (PWRO), by simple filtration (PWSF), and groundwater from Açu Aquifer (WCA). Root colonization rates were transformed before analysis of variance into arc sine $(x + 1/100)^{0.5}$. The means of the treatments did not differ in the 5 % Tukey test for plant species

Table 4 Populations of spore morphotypes of AM fungal communities in the soil (n° per 100 g dry soil) and frequency (F, %) of species and families following cultivation of sunflower irrigated with different types of water at the Belém farm, in Aracati, Ceará

	Irrigation treatment					
Family/ Species	PWRO		PWSF		WCA	
	Fungal spores	F (%)	Fungal spores	F (%)	Fungal spores	F (%)
Acaulosporaceae						
Acaulospora aff. *Bireticulata* F.M. Rothwell & Trappe	46.00	8.30	19.00	7.20	43.00	7.72
Acaulospora aff. *Excavata* Ingleby & Walker	239.00	43.30	151.00	57.00	0.00	0.00
Acaulospora sp.1.	32.00	5.80	0.00	0.00	227.00	40.75
Acaulospora sp.2	0.00	0.00	0.00	0.00	98.00	17.59
Ambisporaceae						
Ambispora fennica C. Walker, Vestberg & A. Schüßler	22.00	4.00	12.00	4.50	34.00	6.10
Diversisporaceae						
Diversispora aurentia (Błaszk., Blanke, Renker & Buscot) C. Walker & Schüβler	53.00	9.60	56.00	21.10	48.00	8.62
Gigasporaceae						
Gigaspora margarita Becker & Hall	68.00	12.30	0.00	0.00	35.00	6.28
Racocetra castanea (C. Walker) Oehl, F. A. Souza & Sieverd	28.00	5.10	22.00	8.30	35.00	6.28
Glomeraceae						
Funneliformis geosporum (Nicol. & Gerd.) Walker & A. Schüβler	32.00	5.80	5.00	1.90	37.00	6.64
Paraglomeraceae						
Paraglomus spp.	32.00	5.80	0.00	0.00	0.00	0.00
Total	552.00		265.00		557.00	

Produced water treated by reverse osmosis (PWRO), by simple filtration (PWSF), and groundwater from Açu Aquifer (WCA). Fungal spores represent the sum of three composite soil samples

restricted the mycorrhizal activity in the herbaceous plants. Lower levels of plant mycorrhization have been observed in soil which has high levels of available nutrients, such as phosphorus (Propster and Johnson 2015). In addition, a high level of exchangeable Na was detected in the soil belonging to the plots irrigated with PWSF, and according to various authors (Saint-Etienne et al. 2006; Guo and Gong 2014; Krishnamoorthy et al. 2014; Bencherif et al. 2015) the presence of Na reduces mycorrhizal colonization and can affect plant growth and seed production.

The impact of irrigation with PWSF was shown in the spore morphotypes of AM fungal communities in the soil

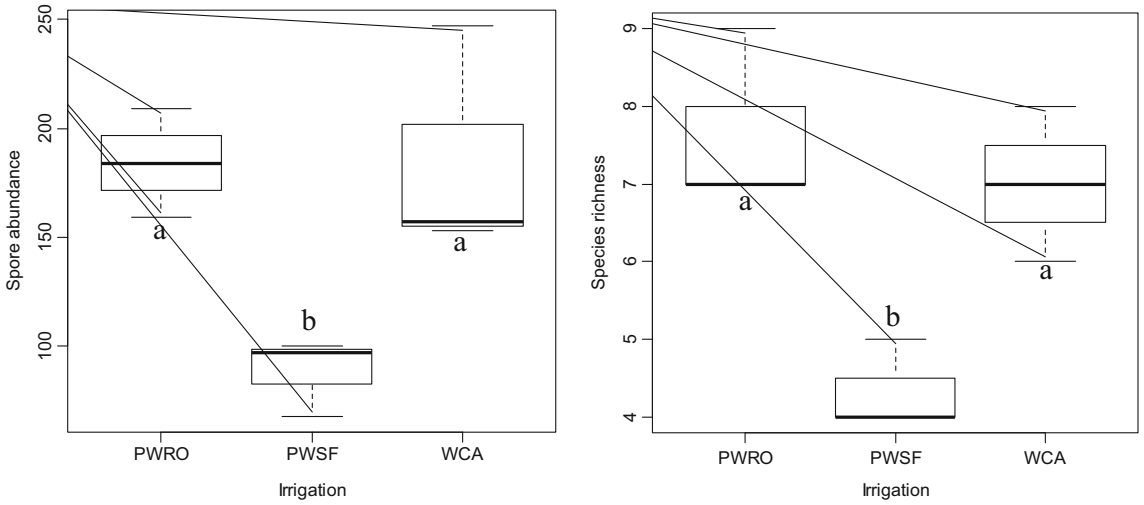

Fig. 2 Abundance and richness of spore morphotypes of AM fungal communities in succession to irrigated sunflower crop with produced water treated by reverse osmosis (PWRO), by simple filtration (PWSF), and groundwater from Açu Aquifer (WCA). Identical letters indicate that the treatments were not significantly different ($p < 5$ %)

Table 5 Levels of proteins related to total glomalin (GRSP-TG) and easily-extractable glomalin (GRSP-EEG) in the soil following the cultivation of sunflower irrigated with different types of water at the Belém farm, in Aracati, Ceará

Variables	Unit	Irrigation treatment		
		PWRO	PWSF	WCA
PCRG-T	mg g^{-1} dry soil	1.07 b	0.94 b	1.40 a
PCRG-F	mg g^{-1} dry soil	0.28 c	0.45 b	0.63 a

Produced water treated by reverse osmosis (PWRO), by simple filtration (PWSF), and groundwater from Açu Aquifer (WCA). Means followed by the same letter on the line do not differ by 5 % Tukey test

(Table 4). However, mycelium growth and the consequent AM fungi sporulation are also affected by environmental conditions. In this research, the samples were collected at the end of the rainy season, and the systems with humid soil could support only small populations of AM fungi (Pagano et al. 2013; Sousa et al. 2014). The production of glomerospores is characteristic of AM species of fungi (Stürmer and Siqueira 2011), and can be affected by plants and other environmental factors, given that some types of activity, such as systems which integrate crop cultivation and livestock-raising or which use direct planting can lead to a reduction in AM fungi sporulation in the soil (Cordeiro et al. 2007).

The stage of herbaceous succession and the low levels of diversity of the plants observed in the plots irrigated with PWSF could have been unfavourable for the proliferation of AM fungi, where grasses of the Poaceae family dominated the vegetation. In a more advanced plant succession, a richer presence of AM fungi has been observed (Sousa et al. 2014), and in the present research the diversity of plants and AM fungi was greater in plots irrigated with PWRO and WCA. It seems that the diversity of successor herbaceous plants in sunflower cultivation can interfere directly with the diversity of AM fungi.

Among the spore morphotypes of AM fungal communities of the ten identified fungal species, four belonged to the genus *Acaulospora* (Table 4). The diversity of this genus can be linked to the diversity of herbaceous plants and characteristics of the soil. Mafaziya and Madawala (2015) also observed a greater frequency of spores from the *Acaulospora* genus and a larger number of species from the Poaceae family in degraded areas. The frequency of some species of the *Acaulospora* genus may be related to the pH of the soil (Stürmer et al. 2006; Sheng et al. 2008), given that in the present study there was a variation between pH 7.2 in soil irrigated with PWRO and pH 7.8 in the plots which were given WCA (Table 1). The conditions of the soil could have favored the proliferation of some species, since the *Acaulospora* genus is usually observed in periods of greater rainfall in both North and Southeastern Brazil (Caproni et al. 2003; Aidar et al. 2004). The saline stress of the soil can also be a limiting factor for the proliferation of AM fungi. Factors such as the rhizosphere and floristic diversity affected the proliferation of AM fungi in the soil, but this outcome may not be enough to consider the specificity of herbaceous plants with *Paraglomus*. Mycotrophic plants belonging to the genus *Croton*, *Panicum*, *Herissanthia* or *Diodella*, which we observed in the treatment with PWRO, may have been able to produce and exude flavonoids in their rhizosphere, thus favoring the sporulation of some fungal species. According to a review of Ellouze et al. (2014) flavonoids and other phytochemicals act positively in mycorrhizal symbiosis. The *Paraglomus* association with herbaceous plants should be further explored, using molecular analysis.

Some species, such as *Acaulospora* sp.1 and *Gigaspora margarita* were absent when the soil was irrigated with PWSF, and the *Gigaspora* genus has frequently been observed in soils with low levels of salinity (Bencherif et al. 2015). Other species of AM fungi, such as *Acaulospora bireticulata*, *Ambispora fenica*, *Racocetra castanea* and *Funneliformis geosporum* were present in all the different plots, indicating a possible adaptation to soil and local climate conditions. The diversity of AM fungi and their symbiosis with plants may have affected the communities of dominant plants in the area. Püschel et al. (2007) have shown that AM fungi interfere with the community structure and vegetation succession. In this context, the most frequent fungal species could be tested in future research involving the management of sunflower cultivation with residual water.

With regard to glomalin, the protein fraction easily extractable from the soil decreased in the following order: WCA > PWSF > PWRO. This protein fraction was more sensitive to the irrigation treatment that that of total glomalin content in the soil. The reduction in both protein fractions is evidence of the negative impact of produced water (PWSF and PWRO) on the mycelium of AM fungi deposited in the soil. The low levels of the easily extractable protein fraction, which was recently deposited in the soil and had not yet undergone biochemical transformations (Wright et al. 1996), could be due to the presence of glutaraldehyde, a biocide added during the industrial process when purifying produced water. This biocide can be detected even after it has been treated with PWRO (0.198 mg/L) (Melo et al. 2010), and may possibly affect the synthesis of glomalin and the spread of AM fungal mycelium in the soil. This biocide acts on the metabolism of microbial cells (Leung 2001) and is used to prevent the formation of bacterial film on the membranes used in water purification. During research carried out in the same area as the present study, although with reference to the first cultivation cycle of sunflowers, Lopes et al. (2014) also observed a reduction in the proliferation of culturable bacteria and fungi in soil irrigated with PWRO. In addition, glutaraldehyde alters the groups of mesofauna (Ferreira et al. 2015) which naturally feed on the fungal mycelium and other microbes. This could have led to disequilibrium in the biota and

food web in soil irrigated with PWRO. The negative effect of PWSF irrigation on glomalin could be due to the exchangeable Na in the soil. Na-salts have a negative impact on the production of proteins related to glomalin (Krishnamoorthy et al. 2014).

5 Conclusions

The diversity of herbaceous plants and arbuscular mycorrhizal fungi which succeed sunflower cultivation is reduced to a greater degree when irrigation has been carried out using produced water obtained by simple filtration than when irrigated with water from an aquifer.

The use of produced water which has been treated by reverse osmosis significantly reduces, in the short term, the fraction of glomalin which is easily extractable from the soil.

Plants with an herbaceous component and AM fungi in the soil both react in different ways to the stresses induced by irrigation with produced water. This leads to changes in the structure of plant communities and arbuscular mycorrhizal fungi in agro-systems irrigated with residual water.

Acknowledgments The authors thank the PETROBRAS and Embrapa for providing research funding. Thank the Ceará State Foundation for the Support of Scientific and Technological Development the award of a master's scholarship.

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