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Microbial Effects on Hydraulic Properties of Sewage Sludge-Induced Water-Repellent Soil

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ABSTRACT

Soil water repellency (SWR) is a widespread natural phenomenon that results from a complex interplay between the hydrosphere, lithosphere, biosphere, atmosphere, and anthroposphere. Sewage sludge application can induce soil water repellency (SWR), impacting soil hydraulic properties. This research examined the effect of soil microbial manipulation (removal and addition) on SWR and water retention in a silty-clay-loam soil amended with varying sludge amounts. Three levels of water repellency (zero, weak and strong) were artificially created in a silty clay loam soil by adding urban sewage sludge. The results showed that the elimination of soil microorganisms such as fungi and bacteria and their interactions significantly ($P \leq 0.01$) affect the hydrophobicity, soil water retention curve (both wetting and drying) of the sludge-treated soils. Microbial exclusion significantly reduced SWR (21-49%), suggesting that microbial activity contributes to the formation of hydrophobic compounds. Conversely, microbial inoculation increased SWR (27.5-50%), indicating microbial production or transformation of hydrophobic substances. It is concluded that soil microorganisms can increase soil water repellency. Also, soil microorganisms can affect the soil water retention curve through their influence on soil water holding capacity, depending on microbial diversity. These findings highlight the critical influence of microbial activity on SWR and water holding capacity in sludge-treated soils.

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1. Introduction

Soil water repellency (SWR), characterized by a reduction in wetting and water retention. Soil water repellency (SWR) characterized by a decrease in wetting and water retention in soil (Jong *et al.*, 1999; King, 1981) arises from hydrophobic compounds coating soil particles. These compounds, including fatty acids, waxes, and tannins, create a water-repellent layer (Nourmahnad and Tabatabaei, 2012) that significantly changes soil properties (Burch *et al.*, 1987). The SWR can result in a water repellent layer on the soil surface, where it has many effects on soil properties (Debano, 1971). The consequences of the SWR include undesirable effects such as reduced water infiltration and increased surface runoff, leading to nutrient losses, agrochemicals leaching, and plant growth reductions and soil erosion (Wallis *et al.*, 1990). The formation and composition of these hydrophobic compounds are influenced by various biotic and abiotic factors, including wildfire (Debano, 1981), waste water (Arey *et al.*, 2011), sewage sludge (Ojeda, 2010), and notably soil microbial activity (Riling 2005; Veronica *et al.*, 2010).

Soil microorganisms, particularly fungi and bacteria, play a crucial role in shaping SWR through both direct and indirect mechanisms (Veronica *et al.*, 2010). Fungal and bacterial metabolism can release hydrophobic compounds or biosurfactants that mobilize existing hydrophobic substances (Veronica *et al.*, 2010). Fungi have long been assumed to be implicated in the development of soil water repellency (Feeney *et al.*, 2004, 2006). They are recognized to produce highly surface-active hydrophobics as a protection mechanism against drought stress (Hakanpaa *et al.*, 2004). As in the case of bacteria-induced water repellency, the effect of fungal hydrophobins that have on the porous medium will rely on the proportion of soil particles coated with the hydrophobic surfaces (Veronica *et al.*, 2010). Rillig *et al.* (2010) reported a causal relationship between the growth of arbuscular mycorrhiza fungal mycelia and soil water repellency. This relationship is due to the presence of a hydrophobin-related protein, glomalin. Rillig (2005) speculated that the hydrophobins and glomalin-related soil proteins (GRSP) on fungal surfaces might be the cause of the increased soil water repellency. Hydrophobias, a recently discovered class of small amino acids that are ubiquitous protein found in the filamentous fungi (Wessels, 1996) have received significant interest due to their impact on the SWR (Rillig, 2005). Linder (2009) found that the increase in hydrophobic wetting properties is related to the amount of hydrophobias produced on fungal surfaces. Iain *et al.* (2011) examined the impact of fungi, using the biomarkers glomalin and ergosterol, on the influence of water repellency on 15 land management treatments, sourced from century old managed arable and grassland sites. They observed strong and positive correlations between these biomarkers and water repellency.

Studies on the role of bacterial extracellular polymeric substances in soil water repellence development, such as the one by Schaumann *et al.* (2007) found that changes to soil wettability after being coated with specific biofilms depend on the bacterial strain producing it. Other studies have focused on the ability of exopolysaccharides to act as biosurfactants in order to increase the solubility of hydrophobic substances in the soil and make them available for the cells fixed in the EPS matrix (Ekschmitt *et al.*, 2005).

Both bacteria and fungi have the potential to greatly affect the porous media by altering soil water retention and its natural physical properties (Veronica *et al.*, 2010). Reduced effective porosity can arise from the high concentration of microbes in areas where basic survival requirements are met (such as in the vicinity of preferential flow paths), which can lead to: abundant production of extracellular polymers by highly active microbial cells; sloughing events of this polymeric material caused by overgrowth, starvation or shearing; and release of gaseous by-products of decomposition and endogenous decay (Veronica *et al.*, 2012).

Despite the recognized significance of microbial influence on SWR, the precise impact of specific microbial groups, especially fungi and bacteria, on SWR and related hydraulic properties, such as soil water retention, remains poorly understood. This study aims to address this gap by investigating the influence of soil microorganism manipulation (removal and addition) on SWR and the soil water retention curve in a water-repellent soil. We hypothesize that soil microorganisms will increase SWR, leading to subsequent changes in the hysteresis of the water retention curve.

2. Materials and Methods

A calcareous soil from 0 to 20 cm layer was obtained from agricultural fields of Shahrekord University. The study soil was classified as mesic Fluventic Haplo xerepts with silty clay loam texture (13.20% sand, 53.90% silt and 32.90% clay). The soil was air-dried and passed through a 2-mm sieve for the experiment. Sewage sludge as the hydrophobic compounds was collected from Shahrekord Wastewater Treatment Plant (WTP). Sewage sludge was air-dried and ground to pass through a 1-mm sieve for a uniform mixture with the soil matrix. The chemical properties of both soil and sewage sludge analyzed were electrical conductivity, EC (Rhodes, 1996), pH (Thomas, 1996) organic carbon, OC (Nelson and Sommers 1996) and available copper (Cu), lead (Pb) and zinc (Zn) extracted with DTPA-TEA (Lindsay and Norvell 1978) using an atomic absorption spectrophotometer (AAS Model GBC 913 plus) (Table 1). The soil water holding capacity (WHC) was measured using pressure plate apparatuses. Total metal contents in soil and sewage sludge subsamples (extracted with 4 M HNO3 at 80 ^{0}C overnight) were determined according to the method described by Sposito *et al*. (1982). Sewage sludge was characterized by pH 6.7, 26.7% OC and 55.9 g N kg_1 based on oven-dry weight. The total concentration of metals in sewage sludge was 73.2 mg kg^{-1} for Cu, 46.1 mg kg^{-1} for Pb and 1163 mg kg^{-1} for Zn. The selected characteristics of the study soil and sewage sludge are listed in Table 1.

Table1. Basic physical and chemical properties of the soil and urban sewage sludge in the study

properties	Unit	Soil	Sewage Sludge
Sand	(%)	13.20	-
Silt	(%)	53.90	-
Clay	(%)	32.90	-
Texture	-	Silty clay loam	
K_s	(cmh^{-1})	2.19	-
pH (soil: water)	-	7.40 (1:10)	6.70
EC (soil: water)	(dSm^{-1})	0.30 (1:5)	3.90
OC	(%)	0.80	26.70
$CaCO_3$	(%)	30.00	3.30
Total metal [a]			
Cu	(mgkg^{-1})	73.21	11.70
Pb	(mgkg^{-1})	46.10	7.80
Zn	(mgkg^{-1})	1163.00	29.50
Available metal [b]			
Cu	(mgkg^{-1})	5.16	0.308
Pb	(mgkg^{-1})	1.36	0.128
Zn	(mgkg^{-1})	51.20	0.274

[a] Determined according to Sposito *et al*. (1982). [b]Extracted by DTPA-TEA according to Lindsay and Norvell (1978).

2.1. Experimental Design

Three levels of water repellency (zero, weak and strong) were artificially created in a silty clay loam soil adding urban sewage sludge (S0=0:100; S50=50:50 and S80=80:20 sludge weight: soil ratio). The rate of urbane sewage sludge application in the study was selected to achieve the desired degree of hydrophobicity (zero, weak and strong). Persistence and intensity of water repellency were determined for soil samples by the water drop penetration time (WDPT) test (Dekker and Ritsema, 1994). WDPT test measures how long repellency persists on a porous surface. It consists of placing a drop of distilled water on the soil surface and recording the time taken for the water drop to completely penetrate the soil. For every WDPT test, a small amount of soil was placed into a Petri dish and leveled. Four drops (0.5 μL in volume) of distilled water at 20 °C were applied with a syringe to the surface of soil samples. The penetration time for each drop was recorded and the average penetration time taken as representative of the WDPT for each sample. WDPT classes were classified according to Deker and Ritesma (1994), Hydrophilic (WDPT≤5 sec), slightly hydrophobic (WDPT: 5– 60 sec), strongly hydrophobic (WDPT: 60–600 sec), severely hydrophobic (WDPT: 600-3600 sec) and extremely hydrophobic (WDPT ≥ 3600 sec).

There were two different and independent experiments. The first experiment was carried out to eliminate the microbial population using biocides and to determine the influence of microbial exclusion (removal) on the water repellency. Treatments including S0, S50 and S80 were considered as main plots; and microbial removal treatments including fungi alone, -F; bacteria alone, -B; bacteria and fungi, +FB; and without bacteria and fungi, -FB were considered as subplots. After one month of laboratory incubation, soil water retention curve and water repellency were determined using sand box and pressure plate apparatus in matric potentials of 0, 10, 50, 100, 300, 1000, 3000, 50000, 15000 cm and water drop penetration time (WDPT) method, respectively. The second experiment was conducted to add the microbial population to steam-sterilized soils on order to ascertain the effect of soil microbial addition on those measured properties with similar experimental settings to the first experiment.

2.2. Analysis of chemical and microbiological properties

The soil chemical properties were analyzed as described above. The samples were incubated at 23-25 ºC for 30 days and their moisture was maintained at 70-80 % of the soil holding capacity. Microbial respiration rate (RR) or soil basal respiration was measured by analyzing the CO_2 accumulated in sealed plastic containers incubated at constant soil moisture (60% WHC) and temperature (25 ^{0}C) over 10 days (Anderson, 1982). A plastic vial containing 15 ml 0.5 M NaOH was placed inside the containers for CO_2 absorption. The amount of CO_2 evolved from the soil was determined by back-titrating the alkali with 0.25 N HCl after precipitating the carbonates with 10% BaCl2 solution. The CO_2 evolution was expressed as mg CO2-C kg^{-1}soil day^{-1} and the C mineralization quotient or C turnover rate was calculated by dividing C mineralization (i.e., microbial respiration) by OC content following Raiesi (2012). The microbial biomass carbon was calculated by the following equation (Anderson and Domsch, 1978).

$$MBC = SIR\ 40.04 + 0.37 \quad (1)$$

Where the unit for MBC is (μg C g^{-1} soil) and SIW is (μl CO2 g^{-1} soil h^{-1}).

Metabolic quotient (qCO2) or specific respiratory activity was calculated from MBC and respiration data measured to provide an indicator of substrate availability or stress within the microbial population (Anderson and Domsch, 2010), and was expressed as the CO2-C evolution per unit MBC and per unit time.

Using the fungicide Captan and the bactericide Streptomycin sulphate to inhibit fungal and bacterial activity, respectively. In a preliminary experiment, the optimal concentrations for inhibiting microbial respiration were determined separately for fungicide Captan (3mg g^{-1} soil) and this was found for bactericide Streptomycin sulphate (2mg g^{-1} soil). The evolution of CO2 was measured in the presence of inhibitors over 5–6 h. The inhibitors were used separately or combined to estimate inhibitor additivity ratio (IAR) expressed as the sum of reduction in SIR with separate addition of Captan and Streptomycin divided by reduction caused by the application of both inhibitors in combination (Lin and Brooks, 1999; Zhang *et al*., 2000; Bailey *et al*., 2003). The IAR is used to compute the extent to which the activities of the antibiotics overlap (Bailey *et al*., 2003). The IAR values observed in this study ranged from 0.96 to 1.04, indicating that there was no overlapping antibiotic effect on non-target microorganisms or antagonistic effect of one antibiotic on the other (i.e., lower effectiveness of Captan and Streptomycin at their combined introduction). The fungal to bacterial (F/B) activity ratio was calculated as the respiration inhibited by the fungicide divided by the respiration inhibited by bactericide (Bailey *et al*., 2003).

2.3. Statistical analysis

After testing the data for normal distribution and homogeneity of variance, two-way analysis of variance (ANOVA) was used to determine the effects of independent factors (soil water repellency, soil microorganism and their interaction) on the measured soil variables using SAS software version 9. In case of significant interaction effects, only the interaction terms were presented and discussed rather than the main effects. Mean values (n=4) were separated and compared using the post hoc analysis of Fisher LSD test. Differences in the measured soil properties as a result of treatments were considered significant only when p-values were lower than 5%, unless stated otherwise.

3. Results and Discussion

3.1. Soil water repellency

Based on the classification of Dekker and Ritsema (1994), the S0 treatment with WDPT less than 2 seconds was hydrophilic, the S50 treatment with WDPT less than 25 seconds was slightly hydrophobic, and the S80 treatment with WDPT 61 seconds was strongly hydrophobic. Results showed that the USS application had a significant effect ($P \leq 0.0001$) on WDPT. So that Water drop penetration time in soils with zero, weak and strong water repellency levels were less than 2, 25 and 61 sec, respectively. Sludge amendments reduce wettability due to hydrophobic compounds. The sewage sludge effects on soil wetting properties and biophysical parameters were dependent on sewage sludge origin and the type of post-treatment (Ojeda *et al*., 2010). Rahimkhani (2012) reported a significant relationship between USS and SWR and observed that sludge amendment causes water repellency reduces the hydrophobic of soil, via hydrophobic compounds.

3.2. Microbiological properties

Table 2 shows that the USS application affected the measured soil microbial properties (i.e. MBC, RR, qCO_2, FR, BR and F/B ratio). It can be concluded that the USS significantly increased the microbial biomass content and basal respiration during the first and second 10 days of the incubation period, but during the third period the basal respiration and microbial biomass increased with less speed. Soil microbial biomass carbon 15.2 and 26.5 times and basal respiration in soils with weak and strong water repellency levels was 16 and 27 times more than untreated soil, respectivelly (Table 3). Our results are consistent with those of many studies that the USS application increase the microbial biomass content and basal respiration due to a high content of organic carbon and nutrients in urban sewage sludge and decrease the labile organic matter and nutrients during incubation period (Banerjee *et al*., 1997; Fernandes *et al*., 2005; Jafari Vafa *et al*., 2016).

Table2. Results of ANOVA (mean square values) for the effect of urbane sewage sludge (USS) on microbial biomass carbon (MBC), basal respiration rate (RR), metabolic quotient (qCO2), fungal respiration (FR), bacterial respiration (BR) and fungal to bacterial respiration ratio (F/B).

Treatments	MBC ($mg\ CO_2 – C\ kg^{-1}$ soil)	R.R ($mg\ CO_2 – C\ kg^{-1}$ soil day^{-1})	qCO_2 ($\mu g\ CO_2 – C\ mg^{-1}$ MBC day^{-1})	F.R	B.R	F/B
				(%)		
			10day			
S0	195^c	13.06^c	66.70^b	55.20^a	47.50^c	1.16^a
S50	3079^b	208^b	67.90^a	46.09^b	57.14^b	0.81^b
S80	5265^a	359^a	68.20^a	35.70^c	60.30^a	0.60^c
			20day			
S0	189^c	12.19^c	64.50^b	55.45^a	46.10^c	1.20^a
S50	2854^b	185^b	65.00^a	47.90^b	53.30^b	0.90^b
S80	5099^a	333^a	65.30^a	38.20^c	60.40^a	0.63^c
			30day			
S0	176^c	11.30^c	64.20^b	56.08^a	42.20^c	1.33^a
S50	2613^b	169^b	64.50^a	48.70^b	52.50^b	0.93^b
S80	4632^a	300^a	64.70^a	39.72^c	58.20^a	0.67^c

Table3. Effects of urban sewage sludge (USS) application on soil microbial properties

	df	MBC	R.R	qCO_2	MBC/OC	F.R	B.R	F/B
USS	2	3630	249	0.0330	0.0590	2.1300	0.8300	0.0900
Error	9	0.0040	0.0020	0.0010	0.0050	0.0020	0.0020	0.0001
CV(%)	-	1.1400	0.1300	0.4400	0.9000	0.6700	0.6100	0.4000
Pr>F	-	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$
USS	2	3507	227	0.0150	0.0470	1.6100	0.9700	0.0900
Error	9	0.0020	0.0010	0.0020	0.0020	0.0002	0.0007	0.0001
CV(%)	-	1.1100	0.3000	0.5900	1.6100	0.2100	0.3500	0.3800
Pr>F	-	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$
USS	2	3160	203	0.0060	0.0140	1.4200	1.3900	0.1100
Error	9	0.0200	0.0030	0.0007	0.0002	0.0006	0.0003	0.0002
CV(%)	-	1.1300	0.2000	1.2900	0.5400	0.3700	0.2600	0.3900
Pr>F	-	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$

Mean values (n = 4) are displayed. Significant differences between means are marked by different letters (Fisher LSD test) at a = 0.05). MBC microbial biomass carbon, RR respiration rate, qCO2 metabolic quotient, FR fungal respiration,
BR bacterial respiration, F/B fungal to bacterial respiration ratio.

Adding USS had a positive effect on soil MBC/OC ratio. The more MBC/OC ratio is closely linked with increase in MBC (15.20-16.50) than OC (-14.60- 24.80 times) contents in soil hydrophobicity (Table 3). It can be due to an increase in providing organic matter for microbial biomass per unit of organic carbon (Banerjee *et al*., 1997). Other studies of USS effects on MBC/OC ratio have reported similar results (Fernandes *et al*., 2005).

The metabolic quotient (qCO2) was significantly affected by USS and was higher in the presence of USS than the control soil without USS addition (Table 2). However, amplifications in qCO2 were much in USS-treated (1.02–1.43%) than untreated soils (Table 3). The increased qCO2 shows a rejuvenation of the microbial community with more catabolic activity, and has been found in other studies (Anderson 2003; Fernandes *et al*., 2005; Jafari Vafa *et al*., 2016).

USS factors ($p < 0.0001$) significantly affected both fungal (FR) and bacterial (BR) respiration and subsequently F/B ratio (Table 2). The presence of USS decreased the FR by 14.34-31.80% and increased the BR 16.73-32.14% with a corresponding decrease of F/B ratio (28.45-48.70%) in in soil hydrophobicity (Table 3). The similar result has also been reported by Jafari Vafa *et al*., (2016).

3.3. The first experiment

Addition of the antibiotics (Captan and Streptomycin) was effective and these antibiotics were able to inhibit the respiration when added to the soil. The inhibitor additive ratio (IAR) was calculated for Streptomycin and Captan and the result confirmed that streptomycin and Captan show neither an additive nor antagonistic effect (Table 4).

Table4. The effect of Captain and streptomycin and the simultaneous use of both antibiotics on basal respiration (mg CO2 - C kg-1 soil day-1)

	Microbial Treatment				
USS	-B	-F	-BF	+BF	IAR
S0	7.20	6.90	5.80	12.20	1.04
S50	112.04	109.65	93.78	187.30	1.01
S80	212.54	202.20	187.87	330.60	0.97

Fungi alone, -F; bacteria alone, -B; bacteria and fungi, +FB; and without bacteria and fungi, -FB

3.3.1. The influence of soil microorganism on WDPT

Table 5 shows that soil microorganisms and USS and their interactions had significant effect on WDPT of the treatments ($P \leq 0.01$). The elimination of fungal and bacterial populations led to a decrease in soil water repellency. In S50 treatment, -F (33%), -B (21%), -FB (30%) and in S80 treatment, -F (49%), -B (34.4%) and -FB (60.42%) decreased the WDPT when compared with the control treatment (Table 6). It could be due to the production of Hydrophobins in fungal surface (Linder 2009). Rillig (2010) reported increasing in water WDPT can because of the presence of a hydrophobin-related protein; glomalin. Soil water repellence may be caused by exuded compounds from fungi and bacteria that are either intrinsically hydrophobic, change their surface properties to become hydrophobic when desiccated, or liberate with biosurfactants existing hydrophobic compounds in the soil (Veronica *et al*., 2010).

3.3.2. The influence of soil microorganism on soil retention curve (wetting and drying)

Both soil microorganisms such as fungi and bacteria and USS and their interactions effects on soil water retention curve (wetting and drying) in the treatments of S50 and S80 were significant (Table 7). The elimination of fungal and bacterial populations significantly decreases soil water content (Figure 1 and 2). By increasing the suction effect of soil microorganisms, soil hydrophobicity and their interactions on soil water content decreased, So that in the suction of 3000, 5000 and 15000 cm there were no significant effect on water content (Table 7). Soil microorganisms by producing organic compounds (Rilling, 2005) and polymeric materials outside the bacterial cells (EPS) (Veronika *et al*., 2010), fungal hyphens (Scotch 2010) causing water holding. The existence of different organic compounds including fatty acids, waxes and tannins can affect the soil water content and soil water retention curve through their influence on soil water holding, depending on microbial diversity (Franco *et al*., 2000). Inhibiting fungal and bacterial activity resulted in a decreased in soil water content, with the subsequent changes in the hysteresis of water retention curve (figure1, 2).

Table 5. Results of ANOVA (mean square values) for the effect of main effects and interactions of soil microorganisms (MT) and soil water repellency (WRT) on WDPT

	df	WDPT
WRT	2	119^{***}
r(WRT)	9	0.014
MT	3	3.310^{***}
WRT × MT	6	0.670^{***}
r(MT)	27	0.021
CV (%)	-	3.700

Fungi alone, -F; bacteria alone, -B; bacteria and fungi, +FB; and without bacteria and fungi, -FB. In each row all values with different letters are significant at p≤0.01 (***)

Table6. The effect of soil microorganisms (fungi and bacteria) on WDPT of repellent soils

	Microbial Treatment			
USS	-B	-F	-BF	+BF
S0	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}
S50	19.70^{b}	16.70^{d}	17.50^{c}	25.00^{a}
S80	40.00^{b}	31.00^{d}	35.00^{c}	61.00^{a}

***were significant at P ≤0.01

Table 7. Results of ANOVA (mean square values) for the effect of main effects and interactions of soil microorganisms (MT) and soil water repellency (WRT) on soil water content in matric potentials of 0, 10, 50, 100, 300, 1000, 3000, 50000, 15000 cm

	df	0	10	50	100	300	1000	3000	5000	15000
					drying					
WRT	2	0.6370^{*}	0.6580^{*}	0.5420^{*}	0.5080^{*}	0.3560^{*}	0.7820^{*}	0.4830^{*}	0.4620^{*}	0.4900^{*}
r(WRT)	9	0.0008	0.0005	0.0002	0.00006	0.00003	0.00003	0.0060	0.0040	0.0060
MT	3	0.0050^{*}	0.0040^{*}	0.0030^{*}	0.0030^{*}	0.0020^{*}	0.0030^{*}	0.00007^{ns}	0.0001^{ns}	0.0002^{ns}
WRT × MT	6	0.0020^{*}	0.0007^{*}	0.0007^{*}	0.0060^{*}	0.0040^{*}	0.0002^{*}	0.0004^{ns}	0.0004^{ns}	0.00001^{ns}
r(MT)	27	0.0030	0.0030	0.0040	0.0002	0.0003	0.0003	0.0480	0.4620	0.00006
CV (%)	-	1.6200	0.5500	0.6400	0.6300	0.7400	0.9500	1.1300	1.2800	1.5400

Table 7. Continued

	df	0	10	50	100	300	1000	3000	5000	15000
					wetting					
WRT	2	0.2810*	0.3470*	0.4390*	0.3560*	0.3560*	0.7820*	0.4830*	0.4620*	0.4900*
r(WRT)	9	0.0006	0.0002	0.0002	0.0001	0.00003	0.00003	0.0060	0.0040	0.0060
MT	3	0.0250*	0.0520*	0.0090*	0.0010*	0.0020*	0.0030*	0.00007^{ns}	0.0001^{ns}	0.0002^{ns}
WRT × MT	6	0.0060*	0.0260*	0.0018*	0.0007*	0.0040*	0.0002*	0.0004^{ns}	0.0004^{ns}	0.00001^{ns}
r(MT)	27	0.0003	0.0380	0.0030	0.00002	0.0003	0.0003	0.0480	0.4620	0.00006
CV (%)	-	1.6700	0.9900	0.6200	0.6800	0.7400	0.9500	1.1300	1.2800	1.5400

* and ns, respectively, not significant and is significant at $p \leq 0.05$

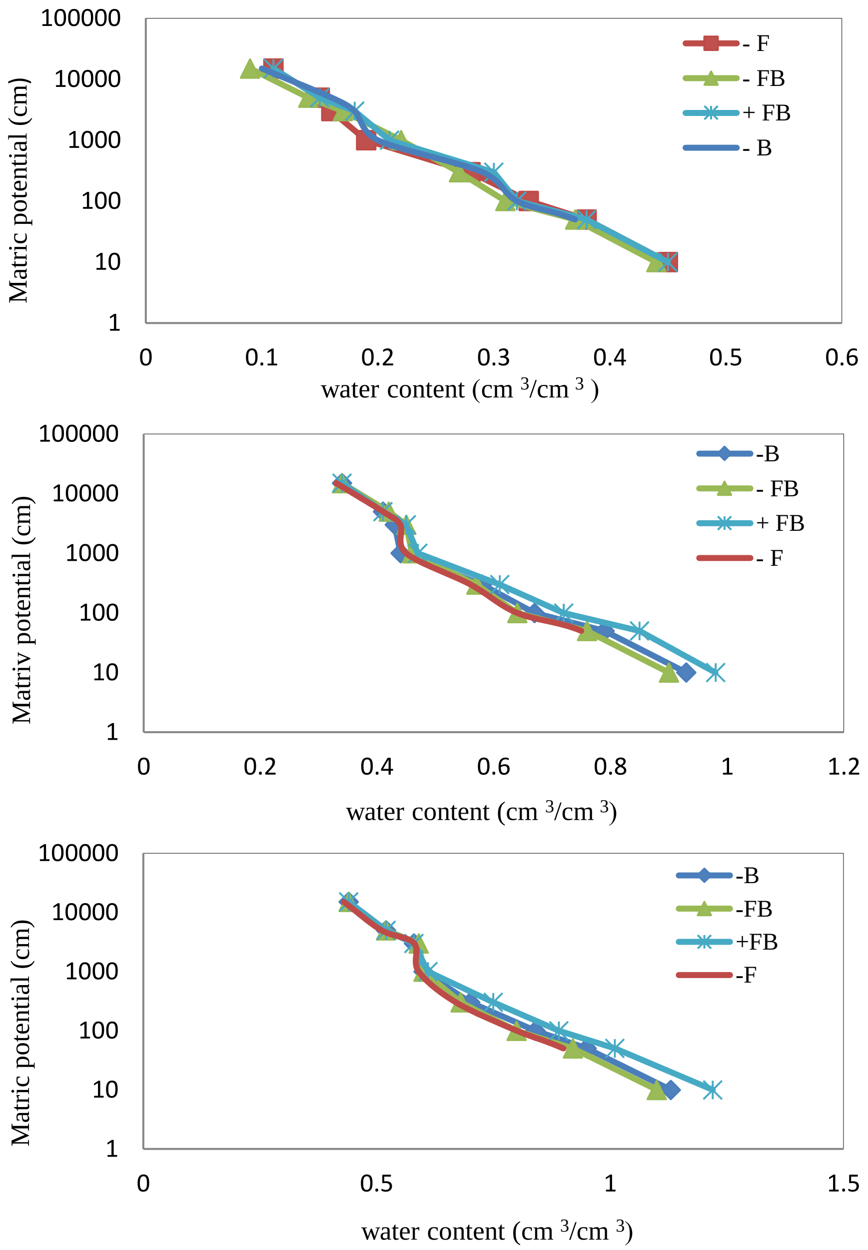

Figure1. Soil water retention curve (drying) in S0 (a), S50 (b), S80 (c) affected by soil microorganisms (fungi and bacteria

Fungi alone, -F; bacteria alone, -B; bacteria and fungi, +FB; and without bacteria and fungi, -FB.

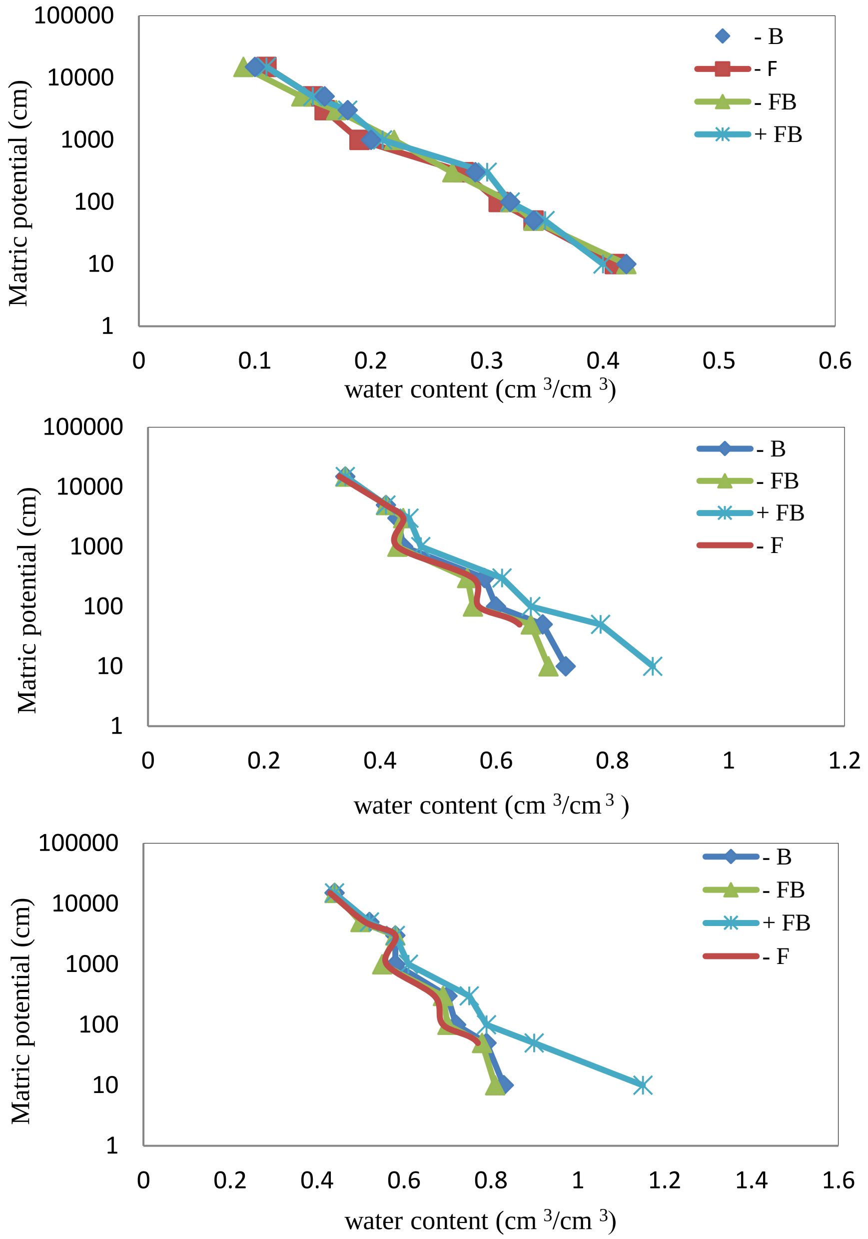

Figure2. Soil water retention curve (wetting) in S0 (a), S50 (b), S80 (c) affected by soil microorganisms (fungi and bacteria)
Fungi alone, -F; bacteria alone, -B; bacteria and fungi, +FB; and without bacteria and fungi, -FB

3.4. The second experiment
3.4.1. The influence of soil microorganisms on WDPT
Soil microorganisms such USS and their interactions on WDPT in repellent soils were significant (table 8). Addition of fungi alone, bacteria alone and bacteria and fungi to sterile soils led to an increased 37.5, 25, 47.5% in S50 and 40, 27.5, 50 % in S80, respectively (Table 9). The results of the first experiment confirm that soil microorganisms such as fungi and bacteria could increase soil water repellency. The results of the first experiment confirm that soil microorganisms such as fungi and bacteria can affect soil water content with the subsequent changes in the hysteresis of water retention curve (Figures 3 & 4).

Table 8. Results of ANOVA (mean square values) for the effect of main effects and interactions of soil microorganisms (MT) and soil water repellency (WRT) on WDPT

	df	WDPT
WRT	2	152***
r(WRT)	9	0.026
MT	3	0.780***
WRT × MT	6	0.270***
r(MT)	27	0.015
CV (%)	-	2.850

***were significant at (P ≤0.01)

Table 9. The effect of soil microorganisms (fungi and bacteria) on WDPT of repellent soils

	Microbial Treatment			
USS	+B	+F	-FB	+FB
S0	1[a]	1[a]	1[a]	1[a]
S50	20[C]	22[b]	16[d]	24[a]
S80	51[C]	56[b]	40[d]	59[a]

Fungi alone, +F; bacteria alone, +B; bacteria and fungi, +FB; and without bacteria and fungi, -FB. In each row all values with different letters are significant at p≤0.0001 (***)

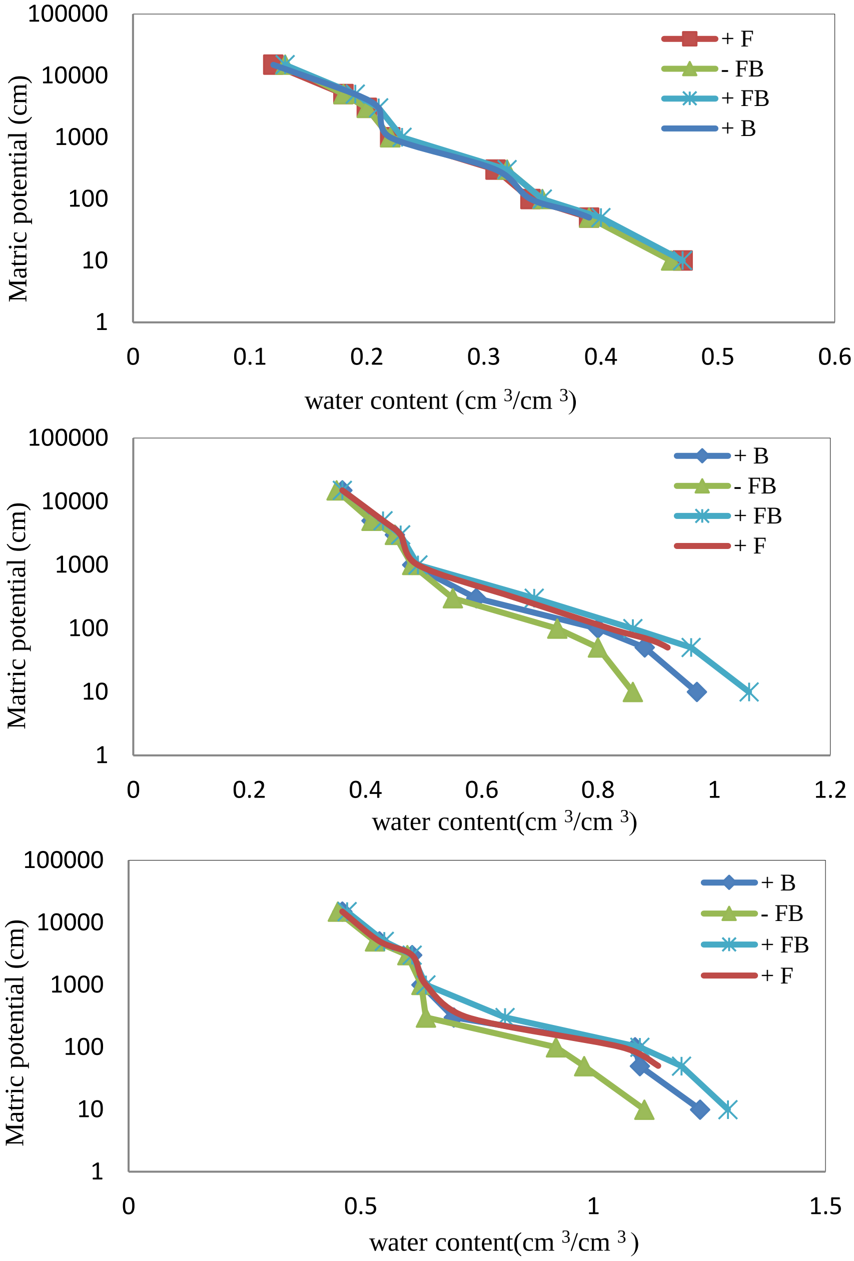

Figure3. Soil water retention curve (drying) in S0 (a), S50 (b), S80 (c) as affected by soil microorganisms (fungi and bacteria)
Fungi alone, -F; bacteria alone, -B; bacteria and fungi, +FB; and without bacteria and fungi, -FB

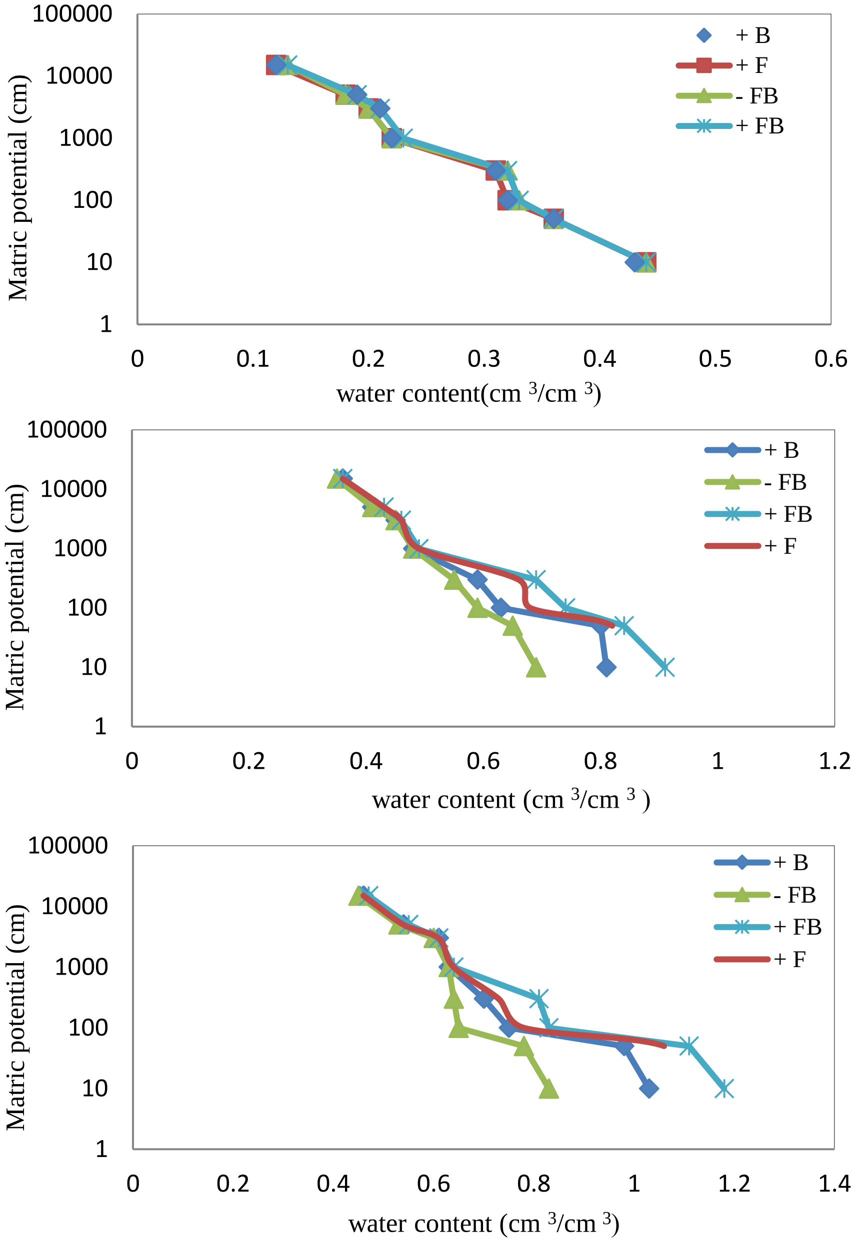

Figure4. Soil water retention curve (wetting) in S0 (a), S50 (b), S80 (c) affected by soil microorganisms (fungi and bacteria)
Fungi alone, -F; bacteria alone, -B; bacteria and fungi, +FB; and without bacteria and fungi, -FB

4. Conclusion

Our study provided evidence that except the influence USS on MBC, R.R, qCO_2, FR, BR and F/B. It was shown that USS application could increase MBC, R.R, qco_2, BR but decreased FR and F/B rate. It can due to a high content of organic carbon and nutrients in urban sewage sludge. Our results showed both bacteria and fungi have the potential to greatly affect the (WDPT), increasing in water WDPT can due to the presence of a hydrophobic-related protein; glomalin and it may be caused by exuded compounds from fungi and bacteria that are intrinsically hydrophobic and both bacteria and fungi could change soil water contents and consequently changed soil water retention curve by altering soil water holding.

Author Contributions

For this research Nasrin Karimian, Shoja Ghorbani-Dashtaki, Fayez Raiesi, Sayyed-Hassan Tabatabaei and Bijan Khalilimoghadam; methodology, Nasrin Karimian; software, Nasrin Karimian and Shoja Ghorbani-Dashtaki and Fayez Raiesi; validation, Nasrin Karimian, Shoja Ghorbani-Dashtaki and Fayez Raiesi; formal analysis, Nasrin Karimian; investigation, Nasrin Karimian and Shoja Ghorbani-Dashtaki ; resources, Nasrin Karimian and Shoja Ghorbani-Dashtaki and Fayez Raiesi; data curation, Nasrin Karimian; writing—original draft preparation, Nasrin Karimian, Shoja Ghorbani-Dashtaki, Fayez Raiesi, Sayyed-Hassan Tabatabaei and Bijan Khalilimoghadam; writing—review and editing, Shoja Ghorbani-Dashtaki; visualization, Shoja Ghorbani-Dashtaki and Fayez Raiesi; supervision, Nasrin Karimian, Shoja Ghorbani-Dashtaki and Bijan Khalilimoghadam ; project administration.

Data Availability Statement

Not applicable

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Ethical considerations

The study was approved by the Ethics Committee of Shahrekord University. The authors avoided from data fabrication and falsification.

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