



## Inoculating plant growth-promoting bacteria - effects on soil hydraulic properties and tomato root development under water stress conditions

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### Abstract

The plant growth promoting bacteria (PGPB) harbored in the rhizosphere develop specialized mechanisms that may have a key role to ameliorate soil properties and plant growth under prolonged dry conditions. Accordingly, this study aimed to assess the effects of bacterial growth on the soil hydraulic properties and the root response under water stress conditions induced by drip irrigated tomato. At pot scale, a silty soil was inoculated with two PGPB strains (*Micrococcus yunnanensis* M1 and *Pseudomonas stutzeri* SR7-77) to cultivate tomato plants under three different water regimes: full irrigation (100% of Pot Capacity- PC), moderate and severe water stress levels (75 and 50% of PC, respectively). Bacterized soil altered the pore size distribution of the rhizosphere compared to no-bacterized soil, increasing root zone plant-available water holding capacity. On the contrary, PGPB occupying the pores reduced the saturated hydraulic conductivity near-saturated soil conditions compared to the uninoculated trial. PGPB shown root surface density (RSD) equal to 0.540 % and 0.355 % to inoculated SR7-77 and M1 tests, respectively and under 50% PC, compared to 0.097% to the uninoculated soil test. Soil water potential values, retrieved through soil water retention parameters, were more negative to M1 and SR7-77 strains tests, corresponding to water hold in the pores with smaller radii conferring resistance to the plant following contrasting stresses. The results demonstrated that PGPB elongated continuous transmission pores and bridged with air-filled spaces in stressed periods.

**Keywords:** Irrigation water regimes, PGPB, Saturated hydraulic conductivity, Soil water potential, Soil water retention.

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### Contribution of this paper to the literature

The study delved into aspects of the soil hydraulic properties alteration processes induced by PGPB, not commonly assessed to address the drought. Highlighting that are soil water potential and pore size distribution changes to play a pivotal role on the performance improvement of prolonged water stress conditions ensuring agricultural productivity.

## 1. Introduction

Physical and hydraulic soil characteristics are greatly influenced by microbial community activity [1-4]. The microbe harbored in the rhizosphere assist water uptake under drought stress [5] ensuring crop yield, improving root development system, and reducing pathogen infection and maintaining a sustainable environment [6-9]. Among these, the plant growth-promoting bacteria (PGPB) has the role to aid the plants to withstand water stress [10-15]. Several studies have shown as the inoculation of PGPB influences plant growth and root development response. For instance, the bacterial inoculation with *Gluconacetobacter diazotrophicus* on red rice plants showed Induced a Systematic Tolerance (IST) response to the drought conditions with an increase of root area [16]. In addition, PGPB inoculation is a practice, especially in the arid and semi-arid irrigated areas, to ameliorate the soil physical and hydraulic properties [4, 17-19]. As a matter of fact, what occurs is the bacteria alter such soil physical and hydraulic properties in the rhizosphere, among them: pore system characteristics, soil water holding capacity and soil hydraulic conductivity. In detail, the bacteria occupying smaller soil pores space making available water at severe stress conditions, modifying its distribution [20] thus, is facilitated root water uptake even though prolonged drought conditions.

By assuming soil water potential as one of the soil variables that better may represent the root water uptake behavior, it can be taken into consideration to explore the root water uptake behavior to the inoculated bacteria soil. Under drought conditions, what was observed is that the bacteria community alter the range of water potential values (i.e. resulting more negatives) due to osmolytes which stimulated, boost the water holding capacity of dry soils [1]. What commonly happens to inoculated-bacteria soil, is the water content brings towards soil potential values nearly to those most negatives, which corresponds to the water adsorbed and hold in thinner and disconnected pores, making thus difficult the root water uptake under common conditions, since is water not promptly available to the plants. Therefore, PGPB might be an alternative strategy to reduce risk of drought, because of despite water held in the smallest pores is become available to the plants when experience water stress conditions. In point of fact, PGPB plays a relevant role in modifying water hold capacity and the soil porous structure, as consequence may ameliorate root water uptake to water scarcity contexts. Such results shown that bacteria growth increase air-entry value to coarse-grained soils, while first an increase then a decrease in the air-entry value was observed to fine-grained soils compared to uninoculated soil [21]. What was observed is bacterial-induced changes in the hydraulic properties and root growth inducing soil water potential and permeability reduction [22].

Moreover, at different levels of soil water storage, the soil and root hydraulic conductivities may increase or decrease accordingly to soil types and agricultural practices. In detail, both two conductivities require to be assessed when it reaches an intermediate soil water status condition [23] being the condition in which it observes a decrease of hydraulic conductivity and limited water root uptake under water stress conditions. Since the water root uptake not only depends on the hydraulic conductivity of the roots and of the soil, but also to that occurs at the root-soil interface; its efficient therefore is only occurred whereas mechanisms between soil and root fully satisfy water demand [24] which is not observed under water scarcity conditions. Furthermore, soil hydraulic properties and pore characteristics depend on spatio-temporal dynamics induced by differ among changes (i.e. long-term tillage systems, crop rotations) [25]. On the other side, PGPB may cause pores clogging which influence hydraulic conductivity under saturated or partially saturated soil conditions [26-30]. This effect arises from the fact that the bacteria regulate mechanisms between plant roots and soil by producing substances that alter soil structure [4]. In addition, knowing soil hydraulic properties plays a considerable role for estimation of available soil nutrients [31] using PGPB will also contribute to improve nutrient absorption capacity of plant, root biomass and area, as well [32]. In the light of this, PGPB can be considered a purposeful strategy to cope extreme events in agriculture.

Commonly, inoculation with PGPB adapts the plants to tolerate negative effects induced during drought periods, since they stimulate the root growth inducing the development of the lateral roots and root hair. In particular, the bacteria stimulate the roots to suck water even though water is only available at very high negative potential values, because of they allow to the root system to reach mesopores or micropores. It was observed that the inoculated plants develop lateral roots and root hairs [33, 34] which allow to enhance water stress tolerance [14]. Presumably, a good contact between roots and the soil surface was achieved under drought conditions because of bacteria stimulate the formation of extracellular polymeric substances become hydrophobic causing thus an alteration of the soil structure and of the soil hydraulic properties in the rhizosphere zone and influence the plant water use efficiency [19].

Knowing that the properties of rhizosphere around the root depend on the capability of a plant to extract water [35, 36] size, shape and continuity of pores [37] becomes therefore relevant to assess water interactions among bacteria-rhizosphere soil. For instance, investigating the pore structural properties of the soil adherent to the root shown the rhizosphere presents different behaviors [37]. In the rhizosphere of wheat, it was obtained higher water content values in comparison to the bulk density [20, 38] reasonably explicable to the effect of the mucilage properties at hygroscopic level. Increasing of water content in the rhizosphere was also observed for lupine and especially undergoing drying and wetting cycles [20]. Moreover, Raddadi, et al. [17] and Murgese, et al. [39] shown that bacteria have the ability to produce biosurfactants and bioemulsifiers substances contributes to improve soil hydraulic properties in arid soil. Among others, Murgese, et al. [39] shown as the bacterial consortium of PGPB improved *Barattiere* (*Cucumis melo* L.) physiological response at pot scale, as well as it reduced the use of mineral fertilizer doses.

Although these studies have demonstrated the effects of exudates in controlling the soil water dynamics in the rhizosphere, it not well-explored how some bio-hydrophysical properties change in the rhizospheric soil and alter pore-size distribution under different water regimes. To understand these behaviors, it becomes thus crucial to characterize soil hydraulic properties to inoculated soils, since they provide insights on the ability of the roots to

uptake water even under severe water conditions. So far, only few studies have been conducted thereon [19, 20, 25]. Thus, if the PGPB may or not improve the soil water holding capacity especially under water stress conditions this implies to estimate rhizosphere hydraulic parameters and pore size distribution under vary water irrigation regimes. The fact that the water roots dynamics and their growth strongly are influenced by adequate presence of air-filled pore space and by the stabilization of soil structure, to this study it was therefore characterized the soil properties at the end of tomato pot experiment, assuming that the bacteria would have accomplished their growth. And in doing so, soil water potential data was also retrieved along the irrigation season by using collected soil water content data throughout the experiment.

However, because of regulations are not yet well-defined on PGPB use at the open field in most jurisdictions, only trials at pot scale may be carried out since only allow to accurate monitor the effects of PGPB on soil characteristics and plant response, likewise allowing to keep under control the water dynamics reducing thus bacteria concentrations eventually lost by water drainage fluxes [40].

As conclusion, the study here proposed was undertaken to assess the effect of bacteria on soil hydraulic properties, root development and soil pore characteristics of tomato plants under three different water irrigation regimes with the project to consider PGPB as amendment to enhance the soil physical and hydraulic characteristics. Accordingly, silt-loam potting soil was inoculated with two PGP B strains: *Micrococcus yunnanensis* (M. yunnanensis M1) and *Pseudomonas stutzeri* (P. stutzeri SR7-77 PS) to determine soil hydraulic parameters at end of PGPB growth, and to estimate root surface density (RSD) in the rhizosphere.

## 2. Materials and Methods

### 2.1. Soil Hydraulic Properties Estimation

Simultaneous measurements of soil water content,  $\theta$ , and soil pressure head,  $h$ , were obtained using a suction table method [41, 42] to determine soil water retention curve; while hydraulic conductivity curve inferring from the evolution of  $\theta$  and  $h$ , and saturated hydraulic conductivity measurements,  $K_s$ , by Darcy's law [43].

$\theta(h)$  and  $K(h)$ - $K(\theta)$  functions were depicted by using van Genuchten Mualem (vG-M) semi-empirical model [44]. In detail,  $\theta(h)$  and  $K_s$  measurements obtained by laboratory methods [44, 45] allowed to solve vG-M model to the soil water retention curve  $\theta(h)$  and the hydraulic conductivity  $K(h)$ - $K(\theta)$ .

The shape of water retention curves is obtained as follows:

$$S_e(h) = \frac{\theta(h) - \theta_r}{\theta_s - \theta_r} = \frac{1}{(1 + |\alpha_{VG} h|^n)^m} \quad (1a)$$

$$h(\theta) = \frac{\left( \frac{1}{\left( \frac{\theta - \theta_r}{\theta_s - \theta_r} \right)^{\frac{1}{m}} - 1} \right)^{\frac{1}{n}}}{\alpha} \quad (1b)$$

Where  $S_e$  is the effective fluid saturation,  $\theta_r$  and  $\theta_s$  denote the residual and saturated water contents ( $L^3L^{-3}$ ), respectively; and  $\alpha_{VG}$ ,  $n$  and  $m$  ( $m = 1 - 1/n$ ) are empirical shape parameters.

A non-linear least-squares curve fitting procedure was used to determine  $\alpha_{VG}$ ,  $n$  and  $m$  parameters.

While the hydraulic conductivity function is deduced by applying the capillary bundle theory [46, 47] starting from water retention curve and here represented as:

$$k_r(S_e) = \frac{k(S_e)}{k_s} = S_e^\tau \left[ \int_0^{S_e} \frac{1}{h(S_e)} dS_e / \int_0^1 \frac{1}{h(S_e)} dS_e \right]^2 \quad (2)$$

In which  $k_s$  is the saturated hydraulic conductivity, and  $\tau$  is a coefficient which considers the reliance of the tortuosity and the parameters on the water content and being set an optimum average at about 0.5 by Mualem [47].

Assuming  $m = 1 - 1/n$  and applying Mualem's model, Van Genuchten [48] also found a closed-form analytical solution to eq. 2 to predict  $k_r$  at a specified volumetric water content:

$$k_r(S_e) = \frac{k(S_e)}{k_s} = S_e^\tau \left[ 1 - \left( 1 - S_e^{1/m} \right)^m \right]^2 \quad m = 1 - 1/n \quad (3)$$

Where  $k_s$  is the saturated hydraulic conductivity ( $LT^{-1}$ ) and  $\tau$  is the pore-connectivity parameter. The pore-connectivity parameter  $\tau$  in  $K(\theta)$  was estimated by Mualem [47] and set to 0.5 obtained as an average value from many different soil tests.

### 2.2. Soil Pore Size Distribution (PSD)

To define soil pore system, it is needed to determine the size distribution of pores because the bacteria function may also depend on their size. Several classifications are proposed to define the relation between size of pores and water storage. For instance, Greenland [49] classified the classes of pores as follows: i) bonding pores ( $< 0.005 \mu m$ ) which are fine pores able to aggregate primary particles; ii) residual pores ( $< 0.5 \mu m$ ) are those that establish chemical interactions at molecular scale; iii) storage pores ( $0.5$  to  $50 \mu m$ ) defined as the pores that retain water and make it available for plant and microbe community; iv) transmission pores ( $50$  to  $500 \mu m$ ) feed the root growth through the movement of water; v) the pores larger than  $50 \mu m$  and corresponding to the field capacity, and pores larger than approximately  $0.5 \mu m$  that could be emptied correspond to the wilting point; v) Pores larger than  $500 \mu m$  are interested to root excavation and water movement. However, the partition of pores between filled air and water and

root growth has not been adequately defined. As well known, to ensure root growth it is necessary to have adequate storage pores (0.5-50  $\mu\text{m}$ ) and adequate transmission pores (50-500  $\mu\text{m}$ ) in the soil [50].

Pore size distribution and water movement at specific water potentials are related through several physical equations and models [51, 52].

In fact, the soil pore size distribution (PSD) curve may be derived from soil water retention curve  $\theta = f(h)$  [53, 54]. This function converts into an equivalent pore size distribution curve based on the Young-Laplace law and assuming a parallel bundle of cylindrical pores [55, 56].

The shape of the distribution function of pore size can be described as follows:

$$d_e = \frac{(4\gamma\cos\omega)}{\rho gh} \approx \frac{2980}{h} ; h > 0(\text{cm})$$

$$f(h) = \frac{d(\theta)}{d(\ln h)}$$

Where  $f(h)$  is the distribution function of pore size versus  $\ln(h)$ , and relate to the equivalent pore diameter ( $\mu\text{m}$ ), on a log10 scale [57-59]  $h$  is the matrix head (cm) ( $h > 0$ ),  $\gamma$  is the water surface tension within the pores (72.8  $\text{g s}^{-2}$ ),  $\rho$  is the water density (0.998  $\text{g cm}^{-3}$ ),  $g$  is the gravitational acceleration (980  $\text{cm s}^{-2}$ ), and  $\omega$  is the water contact angle with the soil pores ( $\omega \approx 0$ ).

### 2.3. Laboratory Measurements

To estimate soil water retention and hydraulic conductivity properties, 27 undisturbed soil samples were collected at the end of the experiment by using stainless steel cylinders (inner diameter of 7.6 cm and height of 7 cm). Afterwards, soil samples were placed in a tank and slowly wetted from bottom with the purpose to reach a saturation condition. Water content,  $\theta$ , corresponding to negative pressure head,  $h$ , values were subsequently, measured using a sand-kaolin suction table, whereas saturated hydraulic conductivity,  $K_s$ , through the constant-head method [45]. In detail, water content,  $\theta$ , was monitored to the following pressure heads: -1.0, -3.0, -10.0, -15.0, -30.0, -50.0, -80.0, -110.0, -180.0 cm; while to more negative  $h$  values: -30 and -120 m was used the pressure plate. At end of the experimental measurements of water retention, the soil cores were oven-dried at 105  $^{\circ}\text{C}$  to determine also bulk density,  $\rho_b$ .

As mentioned above, a fitting procedure was used to optimize  $\alpha_{VG}$ ,  $n$  and  $m$  water retention parameters. These parameters were used to convert the trend of water contents recorded during the tomato season trial in soil potential by using the eq. 1b. These data were used to explain the bacteria growth and its interactions with rhizosphere soil.

### 2.4. Bacteria Inoculation Procedure

The chosen bacteria for this study were: *Micrococcus yunnanensis* (M1) and *Pseudomonas stutzeri* (SR7-77) strains within the five strains and selected from a previous pot experiment [60]. According to the results obtained from this first trial year, M1 and SR7-77 are the most promised strains by assessing tomato root development, yield, and soil quality.

*Micrococcus yunnanensis* Strain M1 originally described in 2009 [61] has been isolated from the endosphere of an extremophilic plant and some experimental validation on the strain M1 resistance to ampicillin, rifampicin, chloramphenicol and tetracycline was performed to demonstrate that strain M1 is sensitive to all the tested antibiotics.

*Pseudomonas stutzeri* was originally described as *Bacillus denitrificans* in 1895 [62]. The strain SR7-77 has been isolated from plants. The positive role of *P. stutzeri* on the plant growth is reported also by Bacilio, et al. [63]. They indicated that the use of humic acids and bacterial inoculation mitigated negative effects of salinity gradients in pepper. Moreover, different strains belonging to this species were isolated from polluted environment, sometimes described as pollutant degraders [64, 65].

### 2.5. Pot Case Study

A pot experiment was carried out in a semi-controlled greenhouse of the Mediterranean Agronomic Institute of Bari (IAMB) located in Apulia region, south of Italy. The test was carried out between 13 February and 23 July 2018. The pots were filled by a silt-loam soil. Soil texture was determined by hydrometer and severs methods. According to the USDA textural classification (The United States Department of Agriculture) the percentage of each soil solid particle class was: 13.75, 16.50 and 69.75% for sand, clay and silt, respectively:

The experimental trial was consisted of three irrigation regimes that have been applied to potted tomato plant, which are: (T1) well-watered, with 100% irrigation based on pot capacity, (T2) and (T3) a controlled drought stress cycle with 50% and 75% of pot capacity, besides the 2 tests of bacteria: *Micrococcus Yunnanensis M1 (B1)*, *Pseudomonas stutzeri Sr7-77 (B2)*; that have been inoculated in the soil, and *control (C)* where there is no inoculation of the bacteria.

Pot capacity (PC) was measured after 48 hours once the pots were saturated and a fraction of delivered water was loosed through the soil gravity force. Overall, three water regimes were induced and 2 bacteria (B1 and B2) and 1 control (C) treatments were set up getting 9 experiment units.

The experimental design adopted was a randomized complete block design with 7 blocks; each block included 9 experimental units with 4 pots each one. The total number of pots used in the experiment was 252 pots.

The inoculation was applied once, at the beginning of the campaign, and two weeks after transplantation (13 April 2018).

The broth culture of the two inoculated bacteria tests, M1 and SR7-77, has been prepared. The concentration of the solution applied was equal to  $10^8$  cell/ml. Estimating that the plantlet roots occupy in the pot a soil volume of 200 ml. Hence  $10^8 * 200$  cells were calculated to be applied to each plant, to reach in the soil surrounding root a concentration of  $10^8$  cell/g. The 50 ml volume received contained a 10-time concentration of bacterial suspension, so it was diluted with a ratio of 1:9 (50mL of broth culture and 450mL of water). For each plant, 50 ml of the total

diluted bottle solution was poured to the top of the soil next to the tomato plant collar. This inoculation procedure was followed under optimal temperature conditions in order to allow to bacteria strain to colonize the root zone.

### 2.6. Root Density Measurements

126 root-soil samples were collected and scanned by ImageJ software. First, the samples were treated by a 5% sodium hexametaphosphate solution to disperse soil attached to the roots, then the roots were washed by hand. Afterward, each treated root sample was laid out on a white paper and scanned a 300 dpi resolution. Acquired images were analyzed by ImageJ [66]. To only distinguish live roots achieve, Red, Green and Blue (RGB) color threshold ranges were set between 0-70, 0-60 and 0-20, respectively.

Root Density Surface was then obtained with the “Analyze particles” command. Furthermore, it was selected a measurement range from 10 pixels to the infinity to not include small areas or single pixels that not corresponding to root areas. Assuming a cylinder shape, the area measured by the software was multiplied by  $\pi$  to gather the real surface of the roots. While root hair length was obtained with 10 root hair measurements per plant (U.S. National Institutes of Health, Bethesda, Maryland, USA). A photographic rank scale between 0 (no root hairs) and 5 (most dense) was chosen to determine root-hair density of plant.

### 2.7. Statistical Analysis

Statistical analyses are performed with Minitab 16 statistical software. Data are analyzed by two-way ANOVA-Analysis of Variance, using “bacteria” and “water regimes” as factors and block as random factor to assess differences among factors and the interactions between the factors using adjusted sum of squares for tests with an interval of confidence of 95% ( $p=0.05$ ).

## 3. Results and Discussion

The experiment units (water regime per treatment) are indicated hereafter with the abbreviations, as follows: *Micrococcus Yunnanensis M1 (B1)*, *Pseudomonas stutzeri SR7-77 (B2)*; and *control (C)* for treatments, while full (T1), moderate (T2) and severe (T3) for water irrigation regimes.

### 3.1. Soil Hydraulic Properties Characterization

The hydraulic parameters of water retention  $\theta(h)$  and hydraulic conductivity  $K(h)$  curves are averaged on 21 replications collected per each experiment unit (Exp. Unit): T1-B1, B2 and C; T2-B1, B2 and C, T3-B1, B2 and C.

The fitted hydraulic parameters  $n$ , and  $\alpha_{VG}$ ,  $\theta_r$  and  $\theta_s$  are listed in Table 1. According to the statistical analysis, there is a highly significant difference between the different bacteria inoculated, between the water regimes and between their interactions too.

Table 1. Soil hydraulic parameters for each experiment unit (Treatment X water regime).

Exp. unit	$\theta_r(-)$	$\theta_s(-)$	$\alpha(\text{cm}^{-1})$	$n(-)$	$m=1-1/n$	$K_s(\text{cmmin}^{-1})$	$\tau$
T1-B1	0.495 <sup>a</sup>	0.010 <sup>b</sup>	0.310 <sup>b</sup>	1.125 <sup>a</sup>	0.111	0.1789 <sup>b</sup>	0.5
T1-B2	0.511 <sup>a</sup>	0.015 <sup>b</sup>	0.243 <sup>b</sup>	1.140 <sup>a</sup>	0.123	0.1432 <sup>b</sup>	0.5
T1-C	0.497 <sup>b</sup>	0.000 <sup>a</sup>	0.173 <sup>a</sup>	1.146 <sup>b</sup>	0.127	0.2350 <sup>a</sup>	0.5
T2-B1	0.493 <sup>a</sup>	0.020 <sup>b</sup>	0.205 <sup>b</sup>	1.139 <sup>a</sup>	0.122	0.2434 <sup>b</sup>	0.5
T2-B2	0.482 <sup>a</sup>	0.035 <sup>b</sup>	0.212 <sup>b</sup>	1.143 <sup>a</sup>	0.125	0.2271 <sup>b</sup>	0.5
T2-C	0.510 <sup>b</sup>	0.000 <sup>a</sup>	0.193 <sup>a</sup>	1.158 <sup>b</sup>	0.137	0.1959 <sup>a</sup>	0.5
T3-B1	0.489 <sup>a</sup>	0.030 <sup>b</sup>	0.300 <sup>b</sup>	1.140 <sup>a</sup>	0.123	0.2194 <sup>b</sup>	0.5
T3-B2	0.494 <sup>a</sup>	0.040 <sup>b</sup>	0.177 <sup>b</sup>	1.170 <sup>b</sup>	0.146	0.2273 <sup>b</sup>	0.5
T3-C	0.501 <sup>b</sup>	0.000 <sup>a</sup>	0.213 <sup>a</sup>	1.161 <sup>b</sup>	0.138	0.4288 <sup>a</sup>	0.5

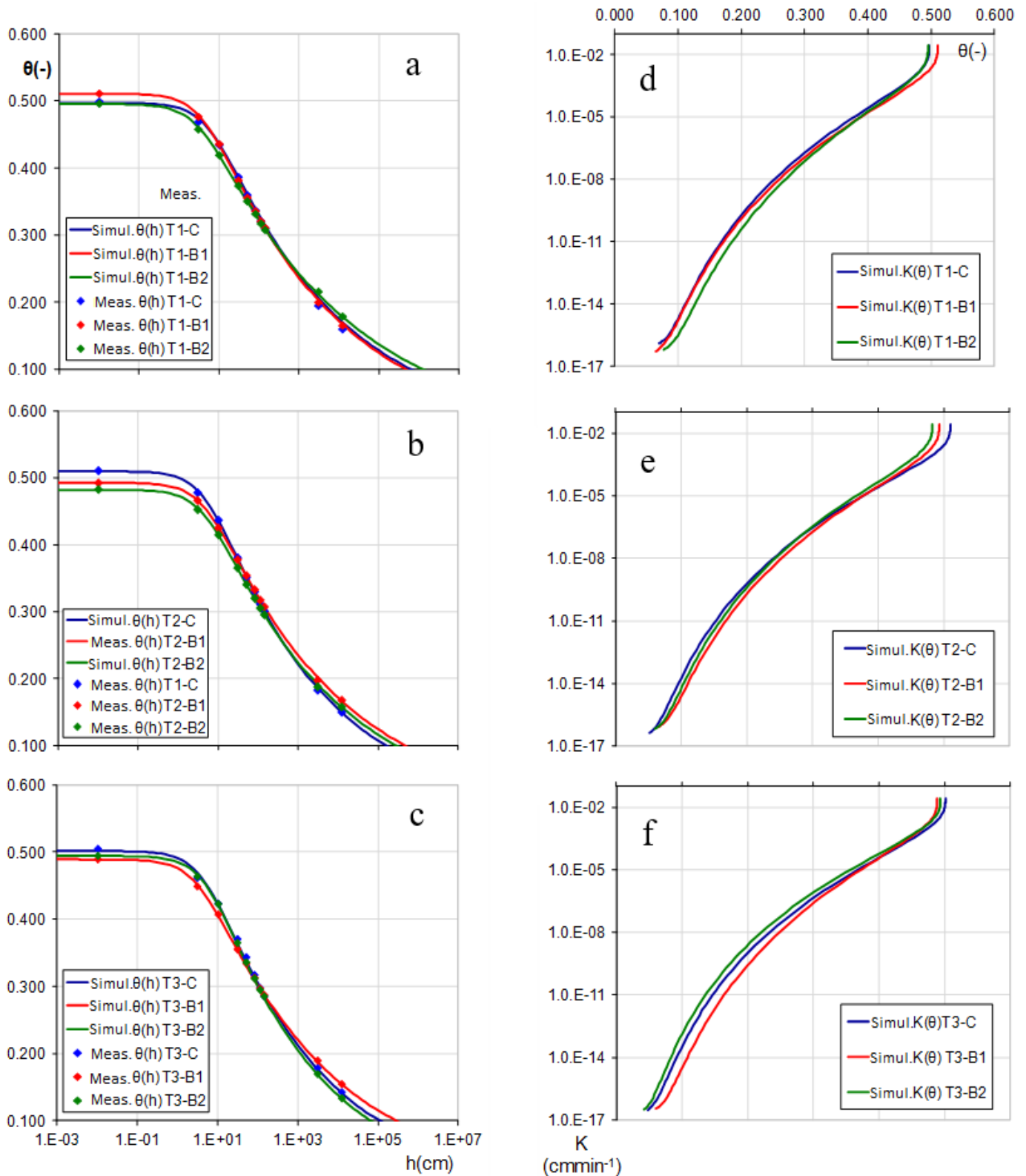
Note:  $p \leq 0.05$  were regarded as statistically significant. a, b letters indicate statistical significance.

In the Table 1, the shape curve parameter,  $\alpha_{VG}$ , observed for bacterized test was higher than the control for three water regimes (T1, T2, and T3); while T3-B2 was lower. In other hand, being  $\alpha_{VG}$  represents the inflection point of the soil water retention curve (WRC) indicates the amplitude of the water availability range. Thus, the greater value of  $\alpha_{VG}$  corresponds a lower potential head value which implies an enlargement of the water available range to the inoculated bacteria soil in comparison to the control, since the bacteria activity allowed to occupy smaller pores and increasing the held water.

On the contrary, the effect of bacteria growth has not increased saturated water content  $\theta_s$  (obtained as average on 21 replicates). It was observed that  $\theta_s$  is 49% for bacteria B1 and control C and 52 % for bacteria B2 under full water regime T1; 48% for bacteria T3-B1 and T2-B2. In the case of control test, the  $\theta_s$  is 51% and 50% for moderate T2 and severe T3 water stress, respectively. This means the bacteria do not show high significant difference when is reached soil porosity [67]. While to unsaturated soil conditions, bacteria are able to improve the soil water availability because of their capability to bridge air-filled gaps and consequently modify the hydraulic properties. Likely, fatty acid produced by PGP B in the silty-loam soils may have influenced the soil hydraulic properties improving the soil water retention. With this regard, it can observe as the soil residual water content  $\theta_r$  increased in both under full (T1) and moderate (T2) and severe (T3) water irrigation regimes for bacterized tests (B1 and B2).

As shown the Table 1, the soil saturated hydraulic conductivity values,  $K_s$ , highlight the effect of beneficial bacteria showing a main reduction to T1 (full irrigation) and to T3 (severe water stress conditions). To the contrary, by looking at the curve  $K(h)$  soil hydraulic conductivity,  $K$ , and soil water potential,  $h$ , the bacterial growth has caused a different behavior of the soil hydraulic conductivity trend, since to the variable saturated soil condition. Over the entire bacteria activity indeed, strong effects of the bacteria on hydraulic conductivity in saturated porous media were founded [30].

Based on the fitted soil hydraulic parameters from the measurements of soil water content,  $\theta$ , and potential,  $h$ , couple values, the hydraulic properties were determined to three tests (B1, B2 and C) and under three water regimes (T1, T2 and T3) as depicted in the Figure 1 a-c.



**Figure 1.** (a-c) averaged water retention  $\theta(h)$  and (d-f) soil hydraulic conductivity  $K(h)$  curves referred to *micrococcus yunnanensis* (B1 with red color line), *Pseudomonas stutzeri* (B2 with green color line), and control (C with blue color line) treatments: a) and d) under full water irrigation (T1); b) and e) moderate water irrigation c) and f) severe water irrigation regimes. The full symbols of water retention curves represent the measured couples data  $\theta$ - $h$ , while the lines refer to that simulated.

The Figure 1a-c shows as soil water retention  $\theta(h)$  is quite similar to bacterized and control soils, but with a slight difference. At given soil water content and relative soil water potential under optimal (T1) and water stress (T2 and T3) conditions, it is observed that at partially-saturation conditions changes the shape of curve near to the low potential values (more negative) for the bacterized soil compared to the control. This behavior is induced by a rearrangement of soil structure due to the bacteria growth occupying mesopores and micropores contributed to increase soil water available. In fact, soil water potential, which has been assumed as variable of the root uptake behavior, showed that there is more water but held in the smallest pores, which means yet water available, because the bacteria are able to regulate the mechanisms and overcome a water stress condition stimulating the root system to also search water among the smallest pores.

The Figure 1 d-f instead, depicts the soil hydraulic conductivity curves trend for all water regimes (T1, T2 and T3) and treatments (B1, B2 and C). Under full water regime, T1, the shape of the three water retention curves is quite similar, this is why the water held in the soil always allowed to satisfy the plant water demand in both trials: bacterized and non-bacterized, therefore the bacteria have not induced an evident variation under unsaturated soil conditions during the entire tomato season. PGPB have explicated a high significant under saturated soil conditions, in which the saturated hydraulic conductivity value,  $K_s$ , is equal to 0.1789 and 0.1432 cmday<sup>-1</sup> for B1 and B2, respectively and lower than the control test which provided a value equal to 0.2350 cmday<sup>-1</sup>. Under moderate water stress (T2), the soil hydraulic conductivity curve shows a different behavior. Over the entire soil water potential range, it may observe that to the same hydraulic conductivity value corresponds different and more negative potential

values compared to the unbacterized test. During the tomato growing season, water is also supplied according to the 75% of PC, which has shown a curve shape differs compared to the control test. And the explanation can be found in the fact that bacteria had the capability to develop within smallest pores.

In the case of severe drought stress (T3), the soil hydraulic conductivity vs. soil potential  $K(h)$  shows a decreasing, as shown in the [Figure 1d-f](#). By observing the last part of the curve, that is under a soil dry condition water storage is roughly kept to 50% of the PC treatment, B1 has pointed out a reduction of the soil hydraulic conductivity 23%; on the contrary to B2 shown an increasing equal to 23% in comparison to the control C. This can be due to the ability of the bacterized tests to uptake more water than non-bacterized due to the filamentous development induced by the bacteria. As explained [Wolf, et al. \[67\]](#) the capability of bacteria to form filamentous allowed to bridge air-filled pores in water shortage conditions, thus their mobility across a range of pore-size classes facilitated the water uptake under low hydraulic conductivity and lower water potential (i.e. to more negative values). Furthermore, bacteria are able to explore micro-habitats at lower water potential (negative values). This is the reason why soil water potential may explain water root uptake behavior. In practical, the root uptake activity depends on how much energy is spent by roots to suck water, and the bacteria stimulated the roots to explore larger range of potential values which means the plant could uptake water by smallest pores, allowing to reduce water stress experience under T3 (severe irrigation regime).

To understand the root uptake behavior induced by PGPB, the soil hydraulic properties were measured under three different water regimes (full, moderate and severe water regimes), and to two bacteria and control treatment at the end of tomato growth season.

Then, the soil hydraulic parameters were used to retrieve soil water potential data using measured soil water content data throughout the tomato season and the fitted soil hydraulic parameters. The inoculated soil (T1 and T2), shown that soil water potential values increase since bacteria growth stimulate bioclogging and biocementation processes especially in the smaller pores radii. As consequence, increase the number of smaller pores that could have contributed to the small amount of water content, in other words the soil water potential making available that water content that otherwise would be unavailable under control treatment (no-inoculated soil). In addition, bacteria altered pore-size distribution.

Moreover, differences to the  $\alpha$ -parameter, which represent the inverse of the soil air-entry value, was observed among the treatments.

The two treatments (T1 and T2) shown slightly different trends to the value of  $\alpha$ , which is attributed to the competition occurred between pores bundle that contribute to both the capillary and adsorbed phase because bacteria allowed to involve smaller pores. Overall, the changes in the soil water retention trend are attributable to the precipitation-induced pore clogging.

To this regard, by considering the soil water retention  $\theta(h)$  and the hydraulic conductivity  $K(h)$  curves representative of root uptake, the pore space geometry is also derived to better understand the behavior of bacteria in the soil [\[58, 68\]](#).

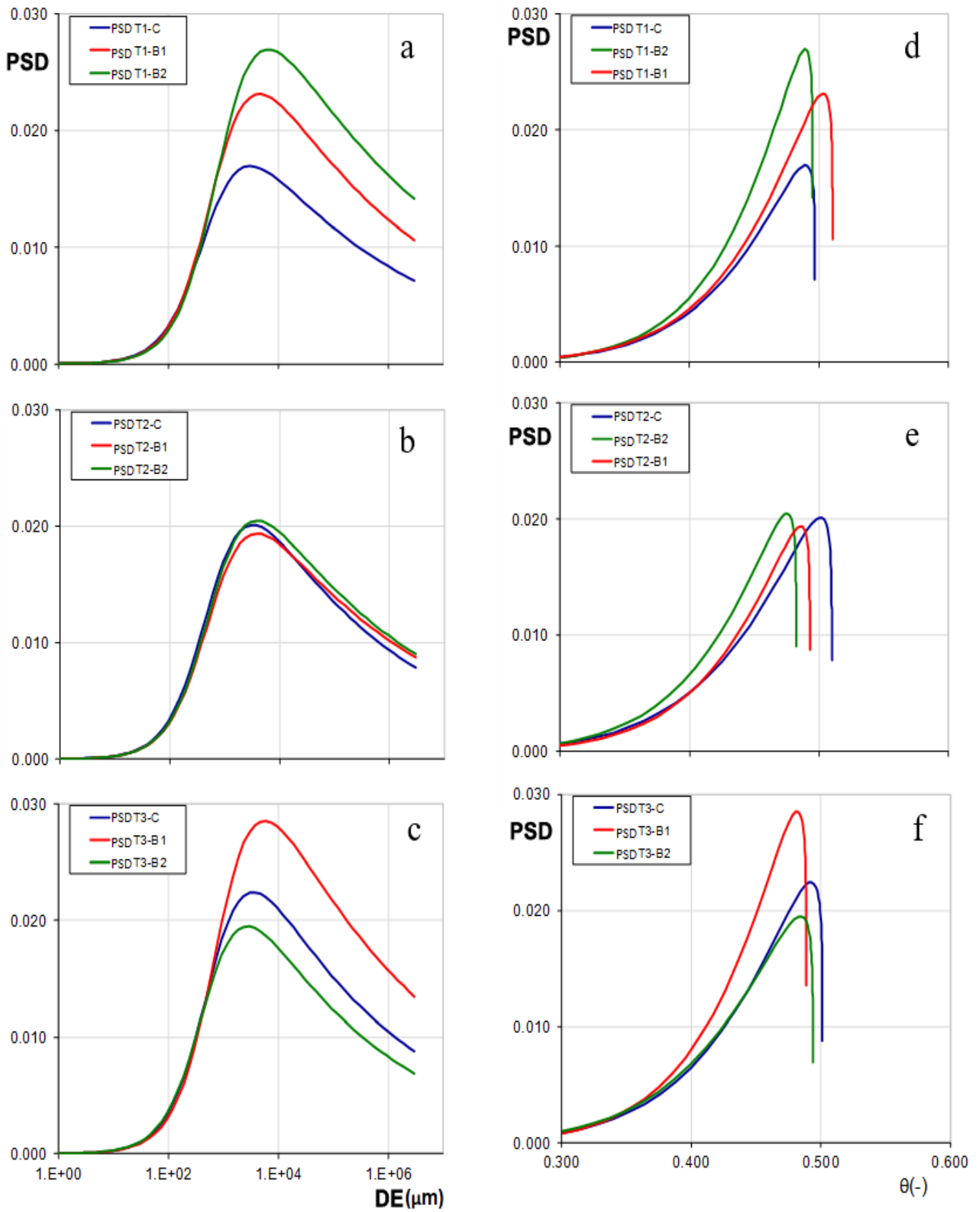
And here in attempts to relate soil hydraulic properties to soil structural under bacteria activity, the soil porous system is derived from soil water retention curve.

The Pore Size Distribution (PSD) representative of the physical structure of soils is obtained to describe the water flow movement and availability of water in the root zone.

In fact, the [Figure 2a-c](#) shows that at the same water content value, the aggregation of pores is different to the treatment (B1, B2 and C). In the [Figure 2a](#), it may observe a shift and increase of the peak of the PSD curve under full water regime (T1) showing for B1 and B2 that the peak moved from the 0.017 for control test (C) to 0.023 and 0.026 for B1 and B2; in other words, the peak is increased by 26% and 37% in comparison to C, respectively. Hence, the presence and mobility of bacteria in soil has permitted to bridge the air-pores, thus are progressively involved different classes of pores making available water to the root uptake even under long dry conditions, as occurred to T3 trial. To T2, the peak of the PSD curve is pretty similar to all treatments (B1, B2 and B3) and equal to 0.0200; and it is quite evident that the predominance of bacteria B1 induced to modify the soil pore characteristics. In detail it is observed a peak equal to 0.0286 and 0.019 to B1, which is lower than control (0.022).

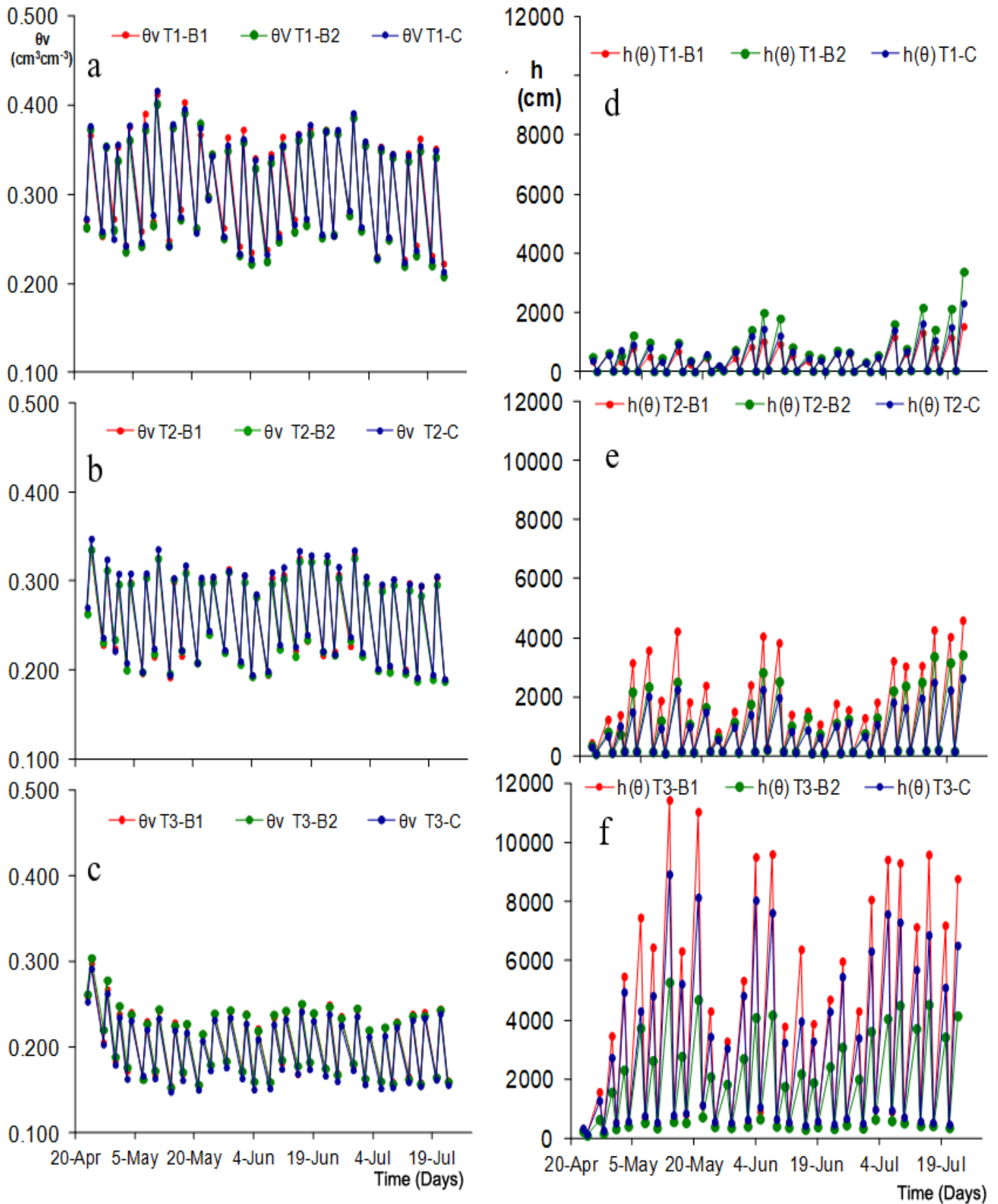
Now, explaining the trend of PSD curves in terms of soil water content available in the soil,  $\theta$ , it is observed that the pore size distribution changed under full water irrigation regime, as shown in the [Figure 2d](#). At maximum soil water content  $\theta$ , the pore size curves shown a different distribution between bacterized and un-bacterized tests. At highest soil water content values, the capability of bacteria to fill pores with water increase compared to C test and under full water regime.

On the contrary, T2 regime is not depicted relevant difference between B1, B2 and C (see [Figure 2e](#)). Something quite different is observed for bacterized tests under severe water stress conditions T3, where PSD curve obtained to B1 was larger than the other two tests (B2 and C), as illustrated in the [Figure 2f](#).



**Figure 2.** (a-c) Pore size distribution (PSD) vs. pore diameter(de) and (d-f) pore size distribution (PSD) vs. soil water content ( $\theta$ ) curves referred to micrococcus yunnanensis (B1 with red color line), Pseudomonas stutzeri (B2 with green color line), and control (C with blue color line) treatments: a) and d) under full water irrigation (T1); b) and e) moderate water irrigation c) and f) severe water irrigation regimes.





**Figure 3.** Time evolution of (a-c) measured soil water content ( $\theta$ ) in whole tomato season and (d-f) the corresponding soil water matrix potential,  $h$ , obtained by convert the water content data based on optimized soil hydraulic parameters curves referred to *micrococcus yunnanensis* (B1 with red color line), *Pseudomonas stutzeri* (B2 with green color line), and control (C with blue color line) treatments: a) and d) under full water irrigation (T1); b) and e) moderate water irrigation c) and f) severe water irrigation.

The Figure 3a-c shows the soil water content evolution monitored during the whole tomato season. The trend is very similar between treatments and is observed a difference between water regimes. Once these data are converted in the corresponding soil water potential values, the behavior of bacteria is clearer. By looking to the trend of the soil water content overtime to control treatment, similar values are recorded to B1 and B2 too, and for all three water regimes. On the contrary, considering the soil water potential trend and compared to the other two treatments (B1 and B2), the capability of the soil to release water can be only attributed to such classes of pores, especially under severe water regime. This is due to the fact that the root tissues do not lose elasticity under drought periods but rather ensure root-water uptake, thanks to the bacteria activity.

As shown the Figure 3d-f, soil water potential trends are significantly affected by pore size distribution to all bacteria tests. This effect on soil water potential and pore size distribution (psd) explains how the bacteria improved the plant mechanisms, increasing the tolerance of the plant under water stress levels. To each irrigation event, it is evident as the trend of soil water potentials reached the same values in both of three treatments (B1, B2 and B3). On the contrary, between two irrigation events, that is when evapotranspiration fluxes are triggered due to less water available in the soil and showing water potential values twice higher than those observed to the un-bacterized test in whole the season.

Instead, the [Figure 3e](#) depicts the soil water potential values corresponding to water contents monitored in the soil along the irrigation season and to different bacteria tests under (T2) water regime. The graph shows that the soil water availability goes so far as at lower negative water potential to B1 and B2 compared to the control C.

Under moderate (T2) and severe (T3) water stress levels, a different shape of pore size distribution curve may be explained by the fact that the bacteria growing in the pores allowed to keep the connection between rhizosphere and root, which is pretty evident to water stress regimes.

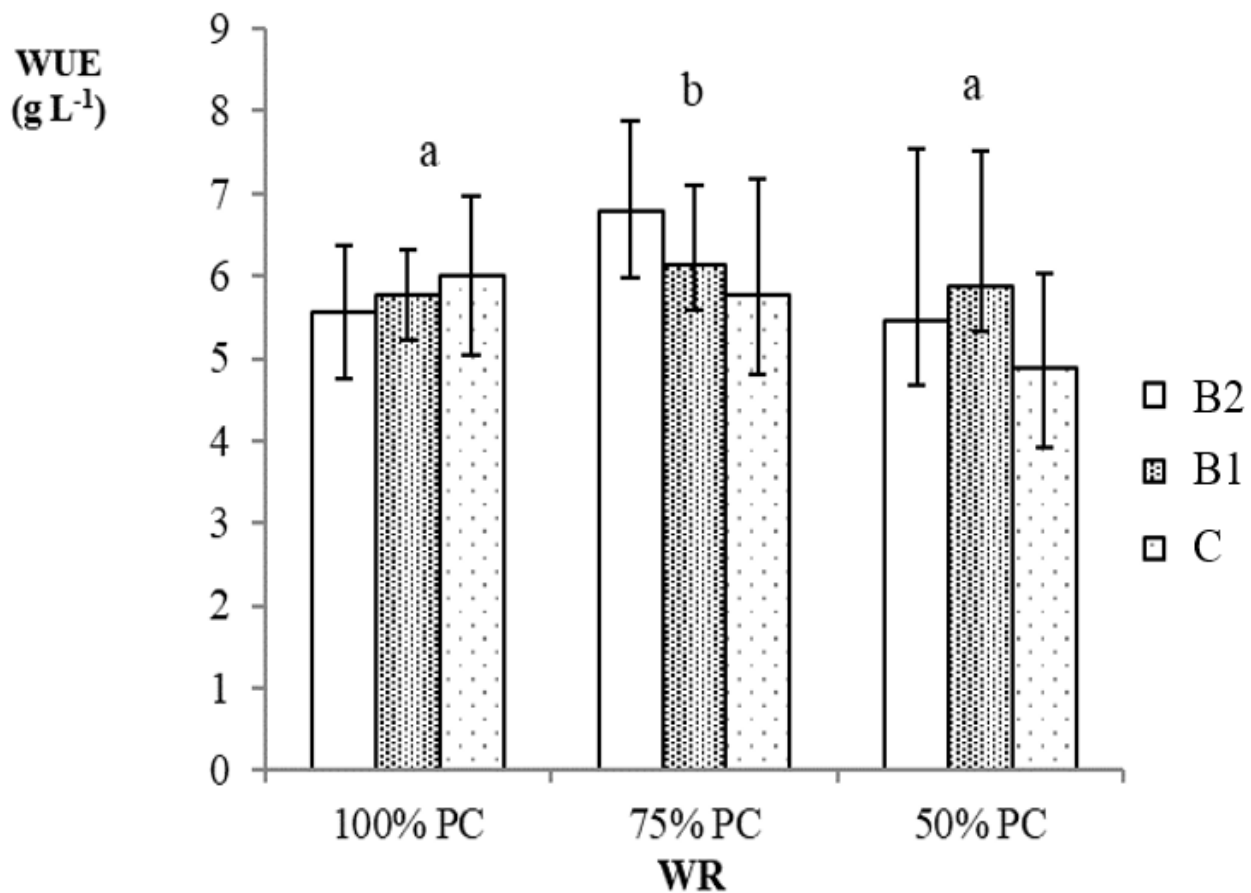
Traditionally, the response of the plant to water stress is to anticipate its vegetative phases in order to reduce the negative effects induced by drought stress conditions [69].

Overall, with the presence of bacteria, largely increased water fluxes under water drought conditions. In other words, the capillary force which allows to retain water in pores is altered by the presence of bacteria. In detail, the bacteria lead to a decrease surface tension and contact angle between water surface and particle surface allowing thus to a pore to easily release water [19].

This leads to conclude that the bacteria B2 and B1 may modify the pore system characteristics according to the water regime used. Therefore, the characterization of soil hydraulic properties is crucial to understand the bacteria growth behavior.

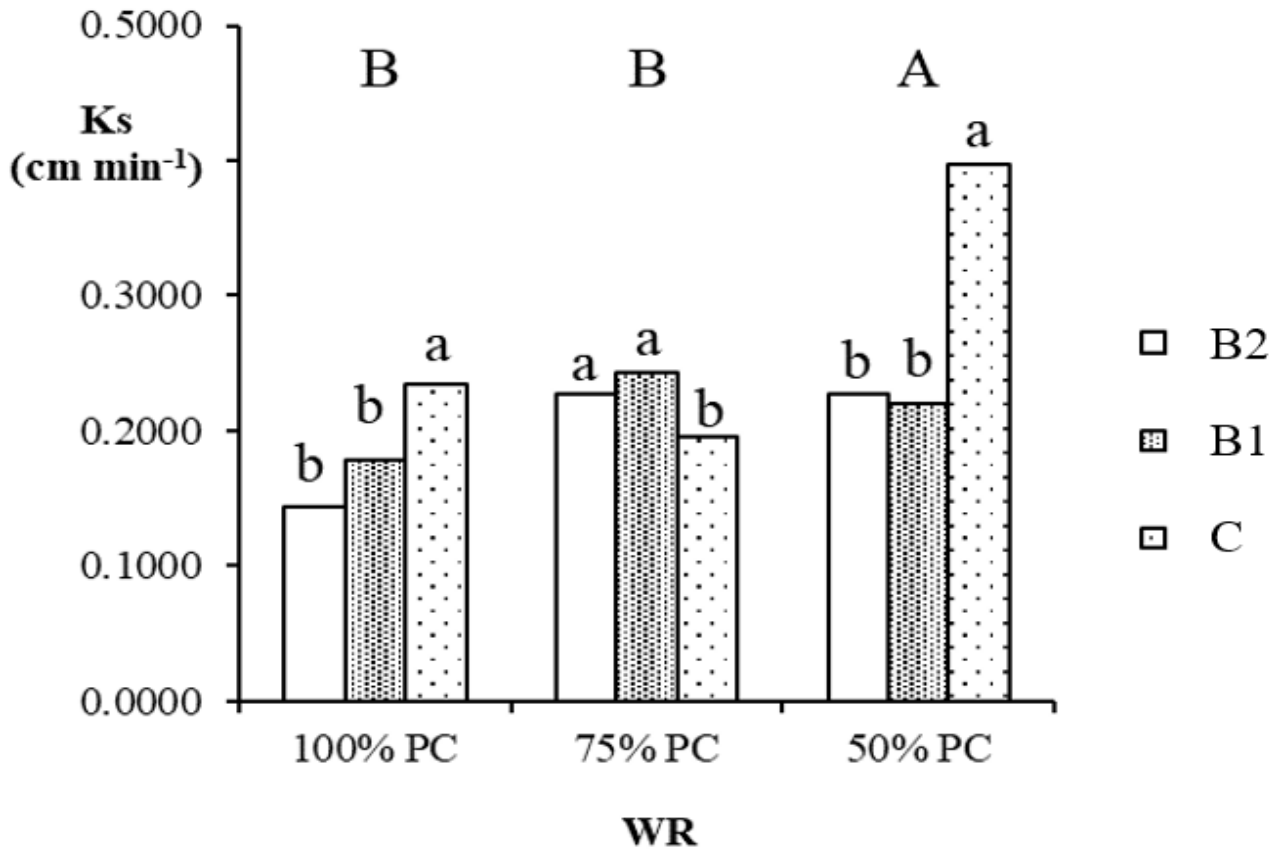
### 3.2. Water use Efficiency

According to the results observed, the bacteria inoculated surrounding the soil shown a significant difference to the soil parameters between the two treatments (bacteria and water regime). However, the bacteria influence the soil and in return the plant growth through specialized mechanisms, like the production of plant hormones. Moreover, uncontrollable conditions, which affect the plant parameters, such as non-controlled temperature and relative humidity, the nutrient deficit, have also a huge influence in the significance of results. This case is called type 2 error, where we accept the null hypothesis while it is wrong and that could be because of the sample size (in this case study is the number of pots per experimental unit that it's small), the effect of the treatments is really very low, or the significance threshold is high ( $p=0.05$ ).



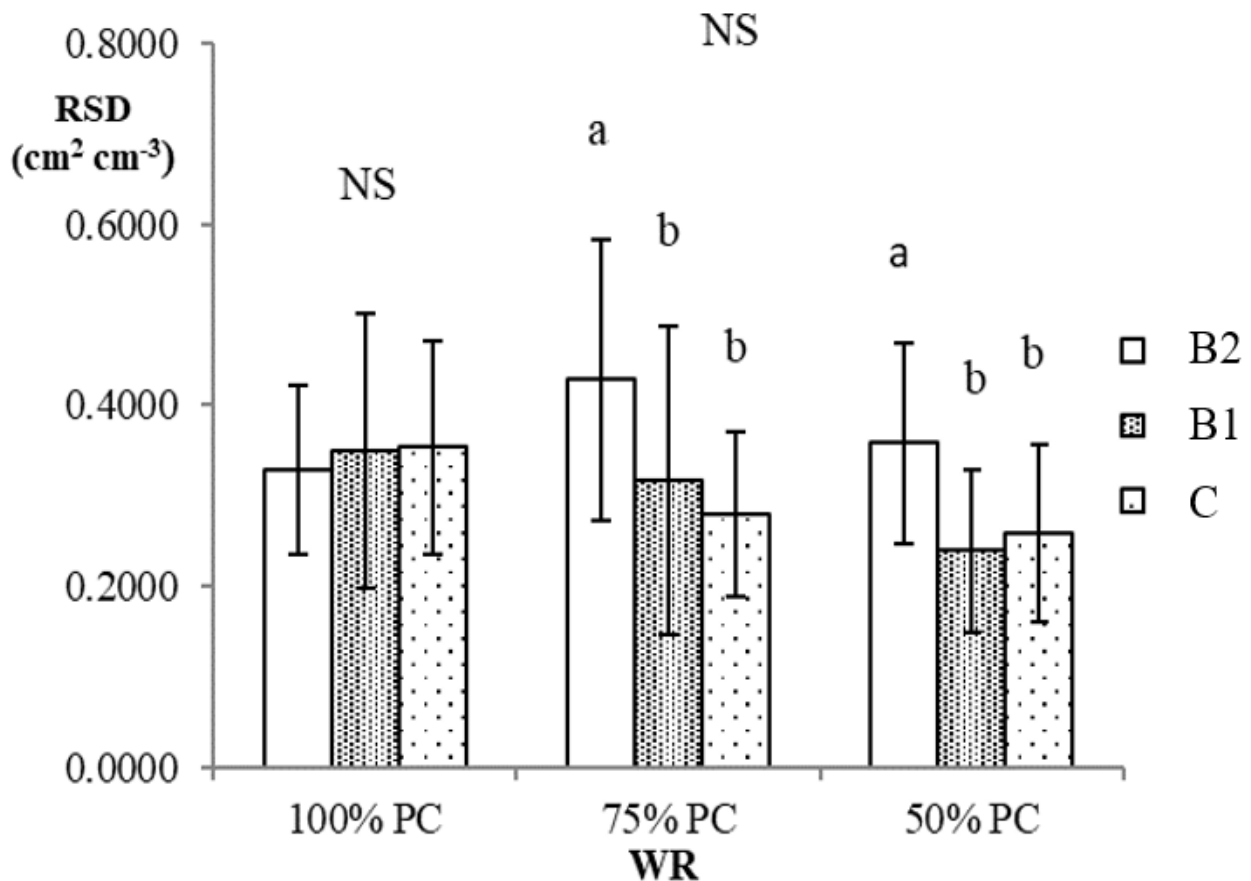
**Figure 4.** Effects of inoculating PGPB under three water regimes (100% PC, 75% PC and 50% PC) on observed data of WUE (Bars with the same letter(s) are not significantly different at  $p \leq 0.05$ ).

In order to explain non-significance difference between the bacteria effect on many parameters, the example of water use efficiency (WUE) is taken to show how the comparison between control and bacteria it becomes significant by changing the variability of each test in order to make them comparable. Observing the graph of WUE data ([Figure 4](#)); the highest variability was of (SR7-77 strain) bacteria tests represented by the bars of the standard deviation that represent how much the data differs from the mean.



**Figure 5.** Effects of inoculating PGPB under three water regimes (100% PC, 75% PC and 50% PC) on soil permeability (Bars with the same letter(s) are not significantly different at  $p \leq 0.05$ ).

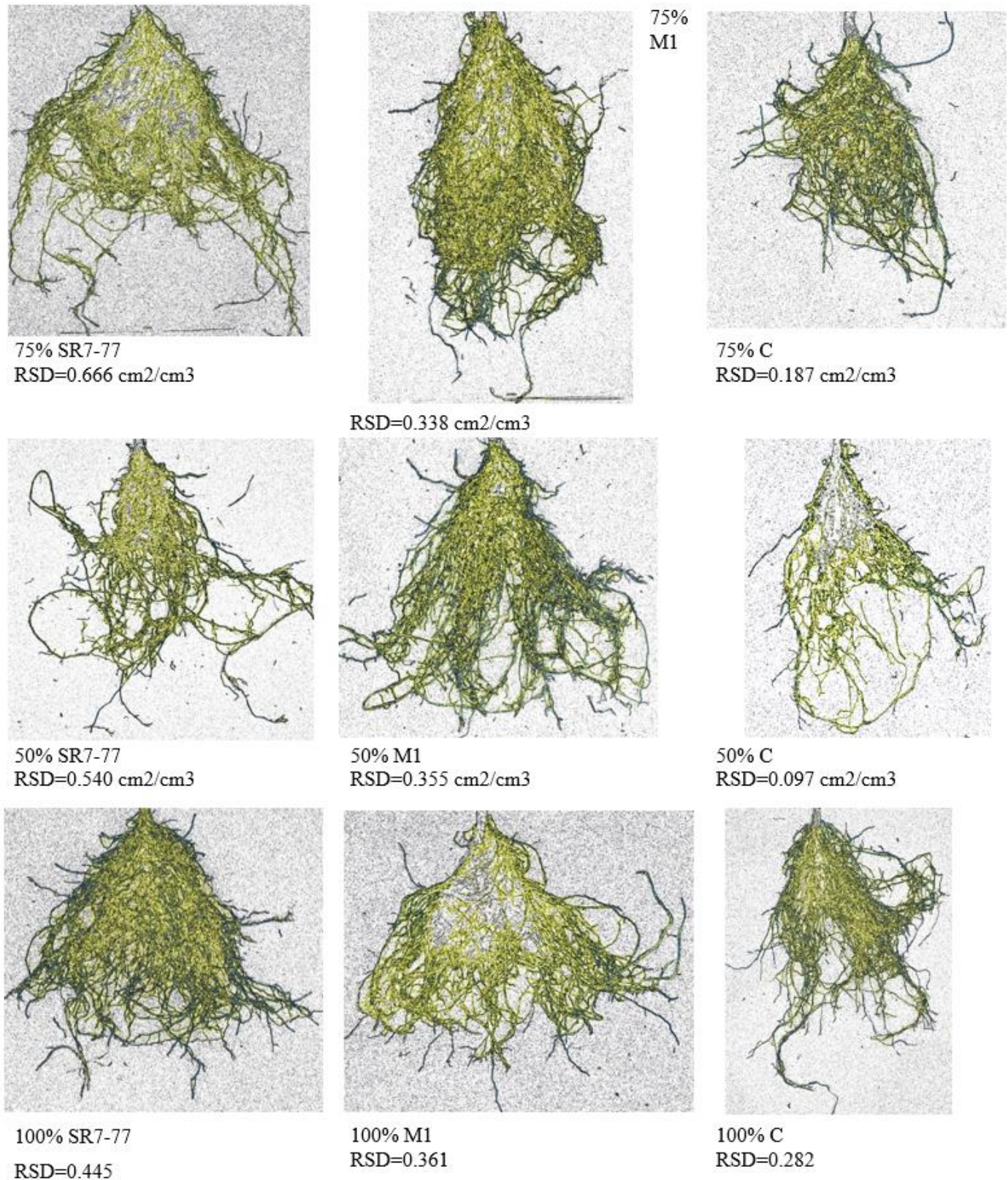
According to the statistical analysis, there is a highly significant difference between the different bacteria inoculated ( $p=0.000$ ) and between the water regimes ( $p=0.000$ ) and also between their interactions ( $p=0.000$ ). Observing the Figure 5, the presence of the beneficial bacteria reduces the soil permeability mainly at full irrigation regime and at severe water stress condition (50% PC). It is known that in rhizosphere, the moisture holding capacity of the soil is improved by the exopolysaccharides (EPS) produced by the PGP Bacteria. Also, it has been proved that the EPS production has the ability to improve permeability by increasing soil aggregation and maintains higher water potential around the roots [70]. In addition to the shrink–swell behaviour of EPS for different water potentials that affects mean pore size and passage of solutes and colloids of different size is the main cause of the decrease of soil permeability [4].



**Figure 6.** Averaged root surface density data under three water regimes (100% PC, 75% PC and 50% PC) (NS: Non-significant differences between treatments, bars with the same letter(s) are not significantly different at  $p \leq 0.05$ ).

### 3.3. Tomato Root Development

The results shown in the **Figure 6** confirm a significant difference in Root Surface Density between bacteria, but not between water regimes or their interaction. Therefore, according to the graph, it is obvious that under the two induced deficits irrigation 75% PC and 50% PC levels, the impact of the bacteria (SR7-77 strain) on the root surface density is higher than (M1) bacteria and control (C), confirming what is approved before by **Patten and Glick [71]** that the inoculation of various plant species with such bacteria has resulted in increased root growth and formation of lateral roots and root hairs, which affect positively the water acquisition and nutrients uptake, helping plants to cope with water deficit [72] as well as the results of **Sharafzadeh [73]** revealed that *P. fluorescens* (92rk) increased total root surface area and volume.



**Figure 7.** Images of scanned roots by imageJ software for the three treatments: Non-bacterized and two bacterized (M1 and SR7-77) under three water regimes: 100%, 75% and 50% of pot capacity (PC).

While to full regime irrigation, there is no difference between the effect of two bacteria and control, which can be explained by the efficiency of (B2 - SR7-77 strain) under stress conditions. The bars also show standard deviation (SD) values added to show how the data spread around the mean value and how accurately the mean value represents the data. The observed standard deviation of each treatment is quite large, but this doesn't mean that the data are not reliable because biological measurements are notoriously variable.

The **Figure 7** shows instead, images of scanned roots obtained by imageJ software for each treatment. The root images are representative of 126 collected roots samples. The images highlight as M1(B1) and SR7-77 strain (B2) treatment improved the root surface density (RSD) and higher than those obtained to control test and full irrigation regime (100% PC). In detail, RSD was equal to 0.540 % and 0.355 % to M1(B1) and SR7-77 strain (B2), respectively

and under 50% PC compared to 0.097% observed to uninoculated bacteria test, and 0.445 and 0.361 to PS and MY, respectively and 0.282% to C, under 100% PC to control test.

#### 4. Conclusions

Prolonged dry conditions reduce plant growth and its development, but an alternative strategy as the PGP B would allow to alter soil water hydraulic properties and enhance the water stress tolerance of plants. Therefore, PGPB may be considered as an amendment able to improve the soil hydraulic characteristics with a double benefit: 1) PGPB inoculation extend water stress conditions conferring tolerance to plant; 2) PGPB modify root growth and pores distribution.

In term of rhizosphere soil hydraulic properties, *Micrococcus yunnanensis* (M1-B1) and *Pseudomonas stutzeri* (SR7-77 strain-B2) have altered both pore size distribution and water potential in the rhizosphere. Results demonstrated that inoculating bacteria test altered the soil water retention curves in different ways. To M1 and SS7, van Genuchten  $\alpha$ -parameter (air entry point value of the SWRCs) increased. While, the saturated hydraulic conductivity,  $K_s$ , decreased due to the capability of PGP B to grow within pores. Bacteria tended to keep all pores filled by water at high soil water potentials (i.e. less negative values), which is a condition already ensured with a full irrigation regime and where bacteria do not have an evident benefit to enhance the water root efficiency. At partially unsaturated soil conditions, PGP B stimulated the roots development in a way that water may be available even at low water potential values (higher negatives-toward low soil water content values). Also, the two PGPB strains shown a change in the soil pore distribution which allowed to connect full and empty pores, an essential condition to reduce the negative effects induced by prolonged water stress conditions.

The results indicated these selected PGP strains have the potential to significantly improve pores size distribution (psd) under water stress conditions. The pore size distribution curve shown different trend of pores classes for bacterized and non-bacterized treatments. Instead, the soil hydraulic conductivity at soil saturation conditions has been reduced by the bacteria growth inner the pores. Moreover, soil water potential variable assumed as representative of the root water uptake efficiency showing a better root growth and uptake because of bacteria allowed a greater stock of water at severe stress conditions in comparison to the control; in other words, the water stress condition was alarmed by the bacteria and overcame because of the rapid releasing of water compared to the control treatment.

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