Most bacteria in soil are found in biofilms, a complex aggregate of microorganisms attached to a solid surface and embedded within a matrix of EPS. The EPS matrix is a complex hydrated mixture of macromolecules, primarily composed of polysaccharides but also containing other substances such as proteins, lipids, and extracellular DNA (Sutherland, 2001; Or et al., 2007). Of these components, EPS polysaccharides hold a primary role in maintaining the biofilm mechanical and structural stability (Flemming and Wingender, 2010). The exact composition and properties of the polysaccharides is a unique feature of the microorganism as well as its physiological state and the environmental conditions (Sutherland, 2001). Typical molecular weights of EPS biopolymers ranges between $10^3$ to $10^8$ kDa (Allison, 1998), most of which are polyanionic, although neutral and positively charged polysaccharides have also been found in bacterial EPS (Sutherland, 2001).

In an unsaturated environment, one of the main stresses bacteria face is a severe water shortage. Very low water contents can lead to a cell's desiccation and death, while intermediate water contents may keep cells vital but limit the nutrient supply and thus reduce growth rates. It is believed that the hydrated EPS matrix can provide bacteria with some resistance against desiccation. Evidence supporting this hypothesis includes a higher survival rate found in EPS-coated cells that went through desiccation relative to cells not coated by EPS (Ophir and Gutnick, 1994; Tamaru et al., 2005; Chang et al., 2007) and an enhanced EPS production by Pseudomonas spp. as

**Water Retention Curves of Biofilm-Affected Soils using Xanthan as an Analogue**

This study investigated the effect of biofilms and, in particular, that of extracellular polymeric substances (EPS) on the hydraulic properties of porous media under unsaturated conditions. The quantitative understanding of the way biological activity alters hydraulic properties is a major key in understanding and engineering relevant systems such as soil aquifer treatment, bioremediation, and wastewater irrigation. Using an EPS analog (xanthan) we explored the effect of EPS on the water retention function of two sandy soils. The result was a significant increase in the water content at any given matric head that could reach 270% of its value within pure soil. For most of the water content range, we successfully modeled the effect of the EPS as a linear superposition of the original pure soil and the xanthan retention curves. Finally, we examined two mechanisms that can attribute to modification of the water retention curve: the EPS holding capacity and alteration of the soils' pore-size distribution; in our case, it appears that the first mechanism was dominant.

**Abbreviations:** EPS, extracellular polymeric substances; PEG, polyethylene glycolWRC, water retention curve.
a physiological response to desiccation (Roberson and Firestone, 1992; Roberson et al., 1993; Chang et al., 2007).

The presence of a bacterial biofilm in soil may alter its water holding capacity. In particular, EPS polysaccharides secreted by bacteria have a high water holding capacity, which can affect the soil environment even when present in small amounts. Chenu and Roberson (1996), for instance, found that the addition of only 1% (w/w) EPS to soil resulted in a significant increase in the water holding capacity. The EPS matrix can hold more than 15 to 20 kg water per kilogram EPS (Chenu and Roberson, 1996). When the matric potential decreases, the EPS remains saturated across a wide range of potential values, holding more than five and 10 times its weight in water at −0.5 and −1 MPa, respectively (Or et al., 2007). The EPS consists of polysaccharide strands. When dehydrated, the thickness of the double layer coating those strands decreases, reducing the distance between strands and causing the EPS to shrink. The distance between strands can vary between a few micrometers when fully saturated to a few nanometers in complete desiccation (Or et al., 2007). This mechanism of shrinking when dehydrated and swelling when wetted enables the EPS to remain water saturated on desiccation.

On top of the immense water holding capacity of the EPS itself, which increases the soil water content, Chenu and Roberson (1996) suggested that this increase can be partly attributed to the EPS separating soil particles, thus maintaining a more open pore structure. Although EPS content and properties can change from one microorganism to another as well as with environmental conditions and physiological state (Flemming and Wingender, 2010), the high water-binding capacity probably characterizes most bacterial EPS.

Although the effect of bacterial EPS on the soil water holding capacity has been demonstrated before, to the best of our knowledge no quantification of this phenomenon has yet been introduced. In this study, we quantitatively investigated the influence of the EPS polysaccharides on the soil water holding capacity by generating water retention curves (WRCs) of two different soils, xanthan as an EPS analog, and soil–xanthan mixtures. Based on these WRCs, we suggest a simple superposition model to assess the water holding capacity of a soil–EPS mixture given the EPS properties, soil properties, and EPS fraction.

**MATERIALS AND METHODS**

**Soils and Extracellular Polymeric Substances Analog**

Two soils were used in this study: Caesarea sand and Hamra soil (a Chromic Luvisol). Caesarea sand is a sandy soil taken from the Mediterranean shore. It is characterized by a narrow particle size distribution with >90% (w/w) of the particles (including clay aggregates) in the range of 210 and 350 μm and 0.27% organic matter. For the current study, the coarse and fine fractions (>590 μm and <105 μm) were removed. These fractions comprise <0.5% of the sand. A hydrometer test revealed that although the sand was sieved, it contained 0.5% silt and 1.5% clay. The Hamra soil was passed through a 590-μm sieve. The grain distribution of the Hamra soil consists of a higher percentage of fines (5% clay and 1.9% silt) and is characterized by a more graded distribution, where about 15% of the soil consists of aggregates >500 μm. The Hamra organic matter content was 0.58%.

Soils were soaked in 0.1 mol/L HCl overnight to remove carbonates and then rinsed with distilled water (Chenu, 1993). Both soils were sterilized before use by either wet autoclaving following by drying in 105°C (sand) or by dry heating at 200°C for 24 h (Hamra soil).

As an analog for the polysaccharide component of the EPS, we chose to use xanthan (Sigma Aldrich, St. Louis, MO), a polysaccharide naturally produced by the soil bacteria *Xanthomonas campesiris* and widely used as an EPS analog (Chenu, 1993; Chenu and Roberson, 1996; Or et al., 2007). The use of EPS analogs is a common practice that greatly simplifies experiments because it enables working under static conditions without dealing with undesired processes that arise from the presence of live bacteria while still using a substance that possesses the water holding characteristics of real EPS.

**Soil–Xanthan Mixtures**

Soil–xanthan mixtures were generated by mixing oven-dry soil and xanthan in a coffee grinder (SE-257, Selmor Int. Electrical Trading Co., Haifa, Israel). A preliminary experiment assured that this procedure generated a homogeneous mixture. The xanthan fractions (w/w) in the xanthan–soil mixtures were 0.25, 0.5, and 1%. These EPS fractions represent the high end of EPS content typically found in soils (0.1–5 g/kg soil, Or et al., 2007) and the expected EPS content in soils that are under heavier organic loads (such as at bioremediation sites).

**Water Retention Curves of Pure Soils and Soil–Xanthan Mixtures**

Water retention curves of the soil–xanthan mixtures were obtained for a range of matric heads *h* between 0 and −5000 cm. In all experiments, boiled distilled water spiked with 0.068% (0.005 mol/L) CaSO$_4$ was used to simulate the soil–water solution (Dane and Hopmans, 2002a). Boiling the water had the double purpose of sterilization and removal of dissolved air. As the system was open and therefore not sterile, 0.1% bronopol and 0.1% NaN$_3$ (Sigma Aldrich) were added to prevent bacterial contamination and degradation of the xanthan. Bacteria counts performed on soil samples taken at the end of the experiments ensured that no bacterial contamination existed. The xanthan content of each sample was checked at the end of the experiments to ensure that the xanthan wasn’t washed out of the system. The organic matter content was determined by burning the sample at 550°C for at least 4.5 h. The xanthan content was obtained by subtracting the organic matter content of the pure soil from that obtained for the sample. Typically, it was found that the xanthan content obtained was >90% of that added to the soil samples.

The water holding capacity between saturation and −150 cm (−180 cm in clean sand) was measured using the hanging column method (Dane and Hopmans, 2002a). Three to five replicates were made for each experiment, while two additional
samples were left at a constant water potential (0 and −100 cm) to estimate evaporation.

Samples of 100 g of the dry soil–xanthan mixtures were placed in a self-made Buchner funnel (7 cm in diameter, containing 0.025- or 0.05-MPa porous plates, Soilmoisture Equipment Corp., Santa Barbara, CA) and connected to a water reservoir. The height of the dry samples in the funnel was 1.5 to 2 and 1.2 to 1.7 cm in the Hamra–xanthan and sand–xanthan mixtures, respectively. The main drying curves were created by first saturating the samples and then measuring the water contents at a set of ascending prescribed matric heads. The samples were saturated by letting water flow from the bottom to the top of the samples, minimizing air entrapment. Matric heads were generated by raising the samples above the free water surface, while the water contents were calculated by measuring the volumes of the drained water when the system reached equilibrium and water drainage ceased (change of <0.1 mL of water per day). Water losses by evaporation were minimized by covering the soil samples and water reservoirs with aluminum foil and puncturing a small pinhole in it to sustain atmospheric pressure. Despite this, and because the experiments were very long, evaporative losses were still significant and taken into account when calculating water contents by adding them to the change in the water volume of the water reservoir.

At the last measurement point (at \( h = −150 \) cm), the gravimetric water content, \( \omega \), was determined by the gravimetric method. The soil sample volume was approximated at each of the stages by measuring the soil surface height, allowing calculation of the volumetric water content, \( \theta \), and monitoring the soil shrinkage. Water contents were determined every 10 to 30 cm, increasing the separation between measurements with decreasing matric head and obtaining a total of nine to 11 measurement points. Obtaining the WRC from saturation to −150 m (−180 m for clean sand) took between 3 wk (in pure sand) to 2 mo (in 1% xanthan mixtures).

At lower matric heads (−200 to −5000 cm), WRCs were determined by using the pressure plate method (Dane and Hopmans, 2002b). Porous plates having an air-entry pressure of 0.3 and 0.5 MPa (Soilmoisture Equipment Corp.) were used for most of the measurements, and 1.5-MPa porous plates (Soilmoisture Equipment Corp.) were used for the low end of the matric head range. Samples containing 10 to 30 g of dry soil each were placed in short open-ended cylinders (5 cm in diameter, 1 cm high) overlying the porous plates (typically 20 samples on each plate). The samples were saturated and left for 1 d to equilibrate before pressure was applied and drying began. At each pressure, samples were taken after equilibrium was achieved (3 d without drainage) and the water content was determined gravimetrically. Three to five replicates were taken at each pressure; for the pure sand three replicates were found to be sufficient, while for the soil–xanthan mixtures a higher number was needed. Altogether, 14 to 18 measurement points along the WRC were determined. Measurements were taken at 100-cm intervals at the lower end of the pressure range (up to −600 cm), increasing to 500-cm intervals at the higher end of the range. Because of the large number of samples and replicates, two separate pressure plate systems were used to cover the entire pressure range (typically one for −200 to −1500 cm and the other for −1500 to −5000 cm). The time required to achieve equilibrium (at each pressure) was between 3 wk and 3 d at the lower and higher pressures, respectively.

**Water Retention Curve of Pure Xanthan**

The pure xanthan WRC was determined by equilibrating xanthan samples stored within dialysis bags with different polyethylene glycol (PEG) solutions as described by Chenu (1993). Solutions of prescribed water potentials were prepared by varying PEG 8000 (Sigma Aldrich) concentrations according to the relation proposed by Michel (1983):

\[
P = 0.129 \left( C_{\text{PEG}} \right)^2 T - 14 \left( C_{\text{PEG}} \right)^2 - 0.4 \left( C_{\text{PEG}} \right) \quad [1]
\]

where \( P \) is the water potential (MPa), \( T \) is the temperature (°C), and \( C_{\text{PEG}} \) is the PEG concentration (kg PEG/kg water). To keep the same ionic strength as in the solutions used for the WRCs of the soil–xanthan mixtures and to prevent bacterial contamination, 0.068% CaSO₄, 0.1% bronopol, and 0.09% NaCl were added to the water solutions (NaCl was added instead of NaN₃ to keep the same ionic strength). All experiments were conducted in an air-conditioned room at a temperature of 23 ± 1°C.

Half a gram of xanthan was placed in a dialysis bag (SpectraPor, Spectrum Labs Inc., Ranch Dominguez, CA) having a molecular weight cutoff of 3500 Da and was placed in 1 to 2 L of PEG solution. The volume of the PEG solution was at least 100 times the volume of the dialysis bag to assure that water flow into the bag wouldn’t significantly change the PEG concentration. The solutions were covered to prevent evaporation and left to equilibrate while being stirred continuously and weighed daily. The equilibrium time ranged from 6 to 13 d. When equilibrium was obtained, the xanthan samples were weighed and their water content was determined. Eight water potentials were measured, with each consisting of four replicates. Water potentials ranged from −23.2 to −11,345 cm, corresponding to PEG concentrations of 0.005 to 0.3 kg PEG/kg water. This range covered the range of water potentials measured for the soil–xanthan mixtures.

**RESULTS AND DISCUSSION**

**Water Retention Curve of Pure Xanthan**

The WRC of pure xanthan is presented in Fig. 1. The data points represent the average of four replicates, with a maximum standard deviation (not shown) of about 18% of the water content value. As evident, the water holding capacity of xanthan is very high, reaching >20 kg water/kg xanthan near saturation. The amount of water retained by the xanthan in our experiment was smaller than that reported by Chenu (1993), who found that near saturation, xanthan can hold >70 kg water/kg xanthan. This difference can be explained by the different ion composition of
the water solutions used by us and by Chenu (1993). Whereas we used distilled water spiked with 0.068% CaSO\textsubscript{4}, 0.1% bronopol, and 0.09% NaCl to match the water solution used for our soil WRC, Chenu (1993) used distilled water spiked with 5 mg/L NaN\textsubscript{3}. The increased ionic strength and the high concentration of Ca\textsuperscript{2+} ions in our water solution probably decreased the xanthan electrical double layer thickness, thus reducing the distance between xanthan strands and limiting its swelling. The importance of the water solution composition in determining the amount of water retained by xanthan was also demonstrated in an auxiliary experiment performed at a matric head of $h = -50$ cm with and without CaSO\textsubscript{4} addition (data not shown). We found that when CaSO\textsubscript{4} was added to the water solution, the amount of retained water was half the water retained without CaSO\textsubscript{4}.

The relation between the gravimetric water content, $\omega$, and the matric head, $h$, shown in Fig. 1 was expressed as a power law ($\omega = 105.76h^{-0.489}, R^2 \sim 0.98$). Such a power relation is often used to describe the equilibrium concentration of a polymer (polymer weight per water volume) under a given osmotic pressure (de Gennes, 1979).

**Soil and Xanthan Mixtures**

The WRCs of the pure soils and the soil–xanthan mixtures are presented in Fig. 2 in terms of the volumetric water content. Because the change in volume of the pressure plate soil samples could not be evaluated, the volumetric water content for these data points was calculated using the bulk density of the last point of the hanging water column experiments (at $h = -150$ cm). Although this assumption probably introduced some bias into the water content calculation, it was not expected to be too significant because it was found that most of the variation in the soil bulk density happened near saturation. The behavior of the soil bulk density is addressed below. For both soils (Hamra soil, Fig. 2a, and Caesarea sand, Fig. 2b) the effect of xanthan addition was pronounced. At saturation, the addition of 1% xanthan to the sand and Hamra soil increased the water contents by 45 and 24%, respectively, while at the lowest matric head measured ($h = -5,097$ cm), xanthan addition had an even greater relative effect, increasing the water content of the sand and Hamra soil by about 270 and 230%, respectively. Xanthan addition also changed the shape of the curves. The WRCs of the pure sandy soils are characterized by a nearly constant water content from saturation to the air-entry pressure (about $-20$ cm) followed by a sharp decrease in water content between $h = -20$ and $-50$ cm, while the WRCs of the soil–xanthan mixtures are characterized by a more gradual change in water content. As opposed to the sandy soils, which were characterized by a clear air-entry pressure above which the water content was approximately constant, xanthan changed its water holding capacity throughout the entire range of matric heads by shrinking and swelling without a distinct air-entry pressure. When xanthan is added to the soil,
it fills some of the pore space. This had a twofold effect on the shape of the WRC near saturation. At the range of matric heads between zero and the air-entry pressure, xanthan shrinkage caused a small gradual decrease in water content. In the range below the air-entry pressure, on the other hand, the xanthan moderated the water content decrease by keeping more water (that otherwise would have been drained) retained in the large pores by the xanthan matrix. As the matric head dropped, the xanthan continued to shrink and the water was slowly released, resulting in a more clayey-like WRC.

The solid lines in Fig. 2 are fitted curves of the experimental data points to the relation of van Genuchten (1980):

$$\theta_r - \theta_s = \left[1 + \left(\frac{\alpha}{\theta_s}\right)^{rn}\right]^{-1}$$

with $m = 1 - 1/n$ (dimensionless), where $n$ (dimensionless) and $\alpha$ [L$^{-1}$] are empirical fitting parameters related to the pore-size distribution, $\theta_s$ is the saturation water content, and $\theta_r$ is the irreducible water content. Fittings were performed by the RETC code (van Genuchten et al., 1991) designed to find soil hydraulic parameters. Table 1 lists the fitted parameters obtained for the eight measured cases and the fitting quality in terms of $R^2$. In almost all the curves, all four parameters, $n$, $\alpha$, $\theta_s$, and $\theta_r$ were fitted. The only exception was the sand + 1% xanthan curve, where the resulting fit strongly underestimated $\theta_s$ and yielded $\theta_r = 0$ (not shown). We therefore recomputed the fit with $\theta_s$ set to the measured value (water content measured at $h = 0$ cm, see Fig. 2b) and $\theta_r = 0.011$.

Increasing the xanthan fraction from 0 to 0.5% decreased the value of $n$, while for a xanthan fraction of 1%, $n$ slightly increased when compared with the soil + 0.5% xanthan. The parameter $n$ is related to the soil pore-size distribution and directly controls the slope of the WRC (van Genuchten, 1980). It tends to be high in coarse sandy soils, while lower values are usually obtained for soils with higher fine-particle fractions. When xanthan is added to the soil, a decrease in the value of $n$ is obtained. As discussed above, this is evidence that when xanthan is added, the shape of the WRC of sandy soils becomes less steep and shifts toward a more clay-like shape.

We expected that the value of $\theta_r$ would increase with the addition of xanthan, as was found by Al-Darby (1996) for sand amended with Jalma (a soil conditioner) and by Rockhold et al. (2002) for sand that was incubated with bacteria for a week. Our results, however, were inconclusive. In the Hamra soil, as expected, a general increase in $\theta_r$ was observed, with the exception of the 0.5% xanthan-amended Hamra soil having a $\theta_r$ value lower than that of the 0.25% xanthan-amended Hamra. This decrease may be attributed to the noisy data measured at the lower matric heads in the Hamra + 0.5% xanthan curve. This is reflected in a high value of the 95% confidence interval obtained for the fitted $\theta_r$ reaching 90% of its value, relative to values of 15 to 58% of $\theta_r$ in the other Hamra soil data sets (data not shown). In the sand, on the other hand, a decrease in $\theta_r$ was obtained with the increase in xanthan fraction. This decrease, however, may be an artifact because in all the data sets the 95% confidence intervals overlap and expand with the increase in xanthan fraction (confidence intervals are not shown). Because it is highly unlikely that the addition of xanthan decreased $\theta_r$ and given the unsound statistical basis, we conclude that fitting to the van Genuchten relation cannot accurately result in $\theta_r$ for several reasons. First, the lowest matric head measured was $-5000$ cm, relatively far from the irreducible saturation range (about $-15,000$ cm). Second, the soil bulk density wasn’t measured directly in the lower range of the WRC but was estimated as the density measured at the last step of the hanging column experiment. Nevertheless, it can be seen both visually and by the relatively high $R^2$ values (Table 1) that the van Genuchten model describes well the measured data within the measurement range, with seemingly reasonable model parameter values.

The extent of swelling in the soil–xanthan mixtures at matric heads higher than $-150$ cm is demonstrated in Fig. 3. The figure shows the change in the volume of the soil sample relative to the dry soil $\Delta V/V_{dry}$ at different matric heads. It can be seen that most of the change in the soil volume happened near saturation, while at lower matric heads the changes were small. For the Hamra soil curves and the sand + 0.25% xanthan curve, the swelling at the lowest point measured ($h = -100$ or $-150$ cm) was about 5% (compared with dry soil) at most. This indicates that the potential bias rising from calculating the volumetric water contents in $h < -150$ cm by using the bulk density of the lowest point of the hanging water column experiment is expected to be quite small. As for the sand with the highest xanthan fraction, the uncertainty is higher because the volume difference between the lowest measurement point and the dry soil is about 15%. Generally, can be seen that the sand was more susceptible to swelling than the Hamra soil. The reason for that may be the higher clay content of the Hamra soil, which results in an increased cohesion (14.7–47 kN/m$^2$, Arkin and Michaeli, 1985) relative to the

---

**Table 1. Fitted parameters of the van Genuchten model for soil–xanthan mixtures with 0 to 1% xanthan.**

<table>
<thead>
<tr>
<th>Parameter†</th>
<th>Sand 0%</th>
<th>0.25%</th>
<th>0.5%</th>
<th>1%‡</th>
<th>Hamra soil 0%</th>
<th>0.25%</th>
<th>0.5%</th>
<th>1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta_s$</td>
<td>0.012</td>
<td>0.011</td>
<td>0.008</td>
<td>0.011</td>
<td>0.026</td>
<td>0.048</td>
<td>0.034</td>
<td>0.054</td>
</tr>
<tr>
<td>$\theta_r$</td>
<td>0.385</td>
<td>0.469</td>
<td>0.506</td>
<td>0.556</td>
<td>0.452</td>
<td>0.468</td>
<td>0.475</td>
<td>0.534</td>
</tr>
<tr>
<td>$n$</td>
<td>7.26</td>
<td>2.43</td>
<td>1.94</td>
<td>2</td>
<td>2.32</td>
<td>2.11</td>
<td>1.83</td>
<td>1.89</td>
</tr>
<tr>
<td>$\alpha$, cm$^{-1}$</td>
<td>0.0279</td>
<td>0.0324</td>
<td>0.0284</td>
<td>0.0196</td>
<td>0.0424</td>
<td>0.0313</td>
<td>0.0315</td>
<td>0.0217</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.992</td>
<td>0.995</td>
<td>0.991</td>
<td>0.981</td>
<td>0.989</td>
<td>0.998</td>
<td>0.988</td>
<td>0.995</td>
</tr>
</tbody>
</table>

† $\theta_r$, irreducible volumetric water content; $\theta_s$, saturation volumetric water content; $n$ and $\alpha$, empirical fitting parameters related to the pore-size distribution.
‡ Saturation volumetric water content ($\theta_s$) was set to the measured value and the residual volumetric water content ($\theta_r$) was set to 0.011.
negligible cohesion in the sand. This yields larger aggregates with respect to the sand particles (as was also found in sieving tests, see above) that can better resist xanthan swelling. The change in soil volume due to xanthan swelling decreased the bulk density of the soil and increased its porosity. As seen in Fig. 2, the addition of 1% xanthan resulted in an almost 50% increase in the porosity of the sand and an approximately 25% increase in the Hamra soil. As the matric head decreased and water drained out of the soil, the soil–xanthan mixture shrank and the porosity decreased to its dry state value. Most of the samples regained their dry porosity at matric heads higher than −100 cm (data not shown).

Linear Superposition Model

We formulated a functional relation to describe the WRC of the soil–xanthan mixtures based on the xanthan fraction, the properties of the pure soils, and the properties of the pure xanthan. Because the volumetric water content is very sensitive to swelling, not insignificant in our case, and because the WRC of pure xanthan was measured in terms of the gravimetric water content (kg water/kg xanthan), we propose a superposition model to describe the WRCs in gravimetric terms. We suggest a simple linear superposition model by which

\[ \omega_{\text{mix}}(h) = \omega_{\text{soil}}(h)(1-f) + \omega_x(h)f \]  

[3]

where \( f \) is the xanthan fraction (by dry weight) and \( \omega_{\text{mix}}, \omega_{\text{soil}}, \) and \( \omega_x \) are the gravimetric water contents of the mixtures, pure soil, and xanthan, respectively. Equation [3] is adapted from double-porosity (Gerke and van Genuchten, 1993) and multiporosity (Durner, 1992, 1994) models of WRCs, but with the difference that those models are based on volumetric properties while ours is weight based. Zhang and Chen (2005) showed that such a representation (based on volumes) can accurately describe the WRCs of soils consisting of a bimodal pore distribution by using mixtures of sand and diatomaceous earth. Because these soils do not swell, it was possible to use volumetric water contents. For our swelling soil–xanthan mixtures, we prefer to use a weight-based relation.

Figure 4 presents the WRCs of the pure soils and the soil–xanthan mixtures in terms of the gravimetric water content. The
We conclude that as the pure xanthan curve was generated in an unconstrained environment, different from real pore conditions, water when free relative to when it is confined inside the soil. This greatly improved the predictions at the higher matric heads (10% for kaolin and 14% for montmorillonite). Figure 5 presents the predicted water contents calculated from Eq. [3] against the measured values. For three of the points, the difference between the measured and the predicted water contents is <5%, whereas the fourth point shows a 21% difference. Although we have analyzed only a few data points measured at a single matric

### Table 2. Root mean square errors computed for the superposition model for soil–xanthan mixtures with 0.25 to 1% xanthan.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sand</th>
<th>Hamra soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using pure xanthan curve</td>
<td>0.022 (0.01)†</td>
<td>0.018 (0.012)</td>
</tr>
<tr>
<td>Using soil + 1% xanthan curve</td>
<td>0.011</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>0.041 (0.025)</td>
<td>0.028 (0.019)</td>
</tr>
<tr>
<td></td>
<td>0.055 (0.033)</td>
<td>0.05 (0.02)</td>
</tr>
</tbody>
</table>

† Values in parentheses were computed for the $h < −100$ cm range of the water retention curve.

The change in soil water holding capacity due to xanthan addition can stem from two mechanisms: (i) the increased water holding capacity of the xanthan increases the water holding capacity of the soil–xanthan mixture (relative to pure soil); and (ii) the xanthan addition changes the pore-size distribution of the soil (whether by occupying some of the pore space or by swelling or expanding the pore space) and by that changes the soil WRC. In our case where the xanthan was homogenously distributed, it was expected that the change in pore-size distribution would affect all pores in the same way and therefore might be less pronounced relative to a case where xanthan would potentially reside in small or large pores. The superposition model only takes into consideration the change in the WRC due to the water holding capacity of the added xanthan and doesn’t account for the change in the soil WRC due to the modified soil pore-size distribution. The good fit obtained between the superposition model and the measurements indicates that the dominant mechanism affecting the WRCs of the soil xanthan mixtures was the intrinsic water holding capacity of the xanthan and not the change in the soil pore-size distribution. Nevertheless, it is possible that some of the deviations between the model and the measurements stem from the change in soil pore-size distribution that was not captured by the model.

Furthermore, we tried to apply the linear superposition approach to the data on water retention of kaolin and montmorillonite amended with different fractions of scleroglucan (a polysaccharide secreted by the fungi *Sclerotium glucanicum*) measured by Chenu (1993) at a water potential of $-0.0032$ MPa. As with our data, the curves of intermediate concentrations were calculated using the water retention of the pure soils and that of the soil + the maximum fraction of the polysaccharide measured (10% for kaolin and 14% for montmorillonite). Figure 5 presents the predicted water contents calculated from Eq. [3] against the measured values. For three of the points, the difference between the measured and the predicted water contents is <5%, whereas the fourth point shows a 21% difference. Although we have analyzed only a few data points measured at a single matric
head, these are encouraging results that may suggest that the mixing model may apply also for clayey soils.

A second relation that was considered to describe the WRCs of the soil–xanthan mixtures is the composite media model that was proposed by Rockhold et al. (2002) to describe the water holding capacity of biofilm-amended soils. In this relation, the EPS-affected soil is treated as a composite porous medium having primary and secondary porosities of the soil and EPS, respectively. In this approach it is assumed that the EPS occupies some of the soil's pore volume, keeping the total soil volume constant and equal to the total volume of the EPS-free soil. Because this model doesn't account for swelling, it results in a reduction of the total porosity of the soil–EPS system with respect to the porosity of the pure soil. In their study, Rockhold et al. (2002) didn't validate their model experimentally but analyzed the biofilm effect on the WRC by considering hypothetical curves for cases in which the biofilm occupies 25, 50, and 75% of the pore space (Rockhold et al., 2002, Fig. 11). These curves exhibit a decrease in water content with an increase in the biofilm fraction for high matric heads, while at low matric heads the opposite trend appears. From our results (Fig. 2), it is clearly seen that as the soil–xanthan mixtures swelled, the total porosity and the water content (across the entire matric head range) increased with the xanthan fraction. Thus, we concluded that the composite media model is not suited to describe the WRC of the soil–xanthan mixtures when they are allowed to swell. This conclusion, however, may change for deep soil layers, in which the pressure of the overlying soil will prevent the soil from swelling freely. It is worth noting that when the xanthan fraction is small and the matric head is low, the extent of xanthan swelling is expected to be small and mostly confined within the soil pores. In this case, the total soil volume is expected to stay approximately constant and therefore the composite media model should apply.

In this work, we used xanthan as an EPS analog to describe natural EPS. As stated above, the use of an EPS analog is the method of choice because it greatly simplifies the experiments while maintaining most of the traits of natural EPS (Chenu, 1993; Chenu and Roberson, 1996; Or et al., 2007). Other studies (for instance, Chenu and Roberson, 1996) have used EPS that were extracted from biofilms and then mixed with soil. It was found that in both cases, the same qualitative behavior of the EPS was obtained. A question that remains open, regardless of whether an analog or an extracted EPS is being used, is the possible effect of the homogeneous distribution of the analog within the soil vs. that of the EPS distribution under natural conditions. This pore-scale change in the EPS spatial distribution may significantly affect the pore-size distribution and thus change the soil hydraulic properties, i.e., the soil hydraulic conductivity and water holding capacity. We postulate that the EPS spatial distribution has a larger effect on the soil hydraulic conductivity and a lesser effect on the water holding capacity because the latter is not dependent on pore connectivity and can be viewed primarily as an additive property. To the best of our knowledge, the spatial distribution of biofilms at the pore scale and under unsaturated conditions hasn't been studied. In the absence of more information, the use of a homogeneous xanthan distribution seems reasonable when studying the water holding capacity.

**SUMMARY AND CONCLUSIONS**

We measured the WRCs of two sandy soils amended with different xanthan fractions under free swelling conditions. The results showed that an addition of <1% xanthan dramatically increased the water holding capacity of the soil. The increase in porosity and water content at high matric heads was attributed to xanthan swelling. This behavior contradicts the composite media model proposed by Rockhold et al. (2002). Instead, we propose a linear superposition model based on the WRCs of the pure soil and that of the pure xanthan to describe the WRCs of the soil–xanthan mixtures. While a reasonable fit was obtained for low matric heads, at high matric heads the use of the pure xanthan curve resulted in an overprediction of the water content because the swelling of the xanthan confined within the soil pores cannot be described using the pure xanthan. To solve this, we used the curves of the pure soil and that of the soil + 1% xanthan to reconstruct the WRC of the pure xanthan in its confined state. We found that the prediction of the intermediate curves was greatly improved. The proposed relation was also verified against the water retention measurements of clays amended with scleroglucan in the study by Chenu (1993). A good fit was obtained for three of the four data points, showing that the linear superposition approach may also be applied to clayey soils.

The proposed relation is a simple model that allows evaluation of the water holding capacity of soils amended with EPS throughout the matric head range. Such an evaluation is useful for microbial ecology research, where the amount of water available to soil bacteria is of interest, and for the analysis and design of bioremediation processes performed in the unsaturated zone, where high bacteria and EPS concentrations exist. Although in this study we used xanthan, our verification of the results obtained with scleroglucan indicates that qualitatively similar results may be expected for actual EPS–soil mixtures.

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