Core Ideas

- Transversal relaxation time spectra were a good proxy to determine pore size distribution.
- Chitosan provides more degradable C to the soil microbial community than Arabic gum.
- The water retention curve showed that 10 g Arabic gum kg\(^{-1}\) soil decreased plant-available water.
- The same amount of chitosan increased available water content compared with the reference soil.

Water Retention and Pore Size Distribution of a Biopolymeric-Amended Loam Soil

Mehdi Rahmati,* Andreas Pohlmeier, Sara Mola Ali Abasiyan, Lutz Weihermüller, and Harry Vereecken

The pore size distribution (PSD) of biopolymeric-amended soils is rarely investigated due to difficulties in its quantification using classical methods. In this study, we analyzed the impact of biopolymeric soil amendments on the PSD of a dryland loamy soil based on its physical and biological properties using a completely randomized design with four treatments consisting of two different dosages (10 and 5 g kg\(^{-1}\)) of two different biopolymers, (chitosan (CH) and Arabic gum (AG)) plus a reference soil. To determine the effects of CH and AG on the PSD, nuclear magnetic resonance relaxometry (NMRR) measurements were used to determine the longitudinal \(T_1\) and transversal \(T_2\) relaxation times. A set of soil structure–related characteristics was also determined in the laboratory. The results revealed that \(T_2\) spectra provided a good proxy to determine the PSD, showing good agreement between the PSD from \(T_2\) spectra and that calculated from the water retention curve (WRC) \((R^2 > 0.78; \text{RMSE} <1.38 \ \mu \text{m})\). The application of CH also increased the zeta potential of the soil to \(-18.5 \text{ mV}\), compared with \(-20 \text{ mV}\) obtained for the reference soil. The WRC measurements revealed that AG decreased the available water content for plant use compared with the reference soil, whereas CH increased the available water in comparison to the reference soil. Considering the parameters of the van Genuchten model, the application of AG and CH mainly affected the parameter \(\alpha\), confirming the dominant changes in macropores. This finding was confirmed by NMRR relaxation spectra. Furthermore, the application of CH and AG stimulated the microbial activity of the amended soil, leading to an increase in soil respiration.

Abbreviations: AG, Arabic gum; CPMG, Carr–Purcell–Meiboom–Gill; CH, chitosan; EC, electrical conductivity; HL, half-life; MRT, mean residence time; MWD, mean weight diameter of aggregates; NMRR, nuclear magnetic resonance relaxometry; OC, organic carbon; PSD, pore size distribution; SA, soil amendment; \(T_1\), longitudinal relaxation time; \(T_2\), transversal relaxation time; WRC, water retention curve; WAS, wet-aggregate stability.

The soil amendments (SAs), which are materials applied to the soil surface or incorporated into the surface layer to improve soil water retention characteristics, comprise a class of materials that can be organic (manure, compost, biopolymers, biochar, etc.), inorganic (lime, gypsum, etc.), synthesized (super-absorbents, polymers, and some kinds of biopolymers), and industrial byproducts (slag, mud, digestate, etc.) (Blanco-Canqui and Lal, 2008). Although the use of SAs has gained considerable interest in recent years (Blanco-Canqui and Lal, 2008), to our knowledge, few attempts have been made to investigate the changes in the pore size distribution (PSD) of amended soils.

The fraction of soil volume that is not occupied by solid material is considered as soil pore space, and the relative abundance of each pore size in a representative volume of soil is considered as PSD (Shi et al., 2017). Pore size distribution is a fundamental and key parameter in transmission and storage of soil water and solute (Mallants et al., 1997; Lipiec and Stepniowski, 1995), soil aeration, and root growth (Pagliai and Vignozzi, 2002; Valentine et al., 2012). Kutilek (2004) categorized pores into three groups according to the laws of hydrostatics and hydrodynamics (Kutilek and Nielsen, 1994): (i) submicroscopic pores, (ii) micropores or capillary pores, and (iii) macropores or noncapillary pores. In general, submicroscopic pores are considered too small to conduct water or to form continuous water flow paths (Kutilek, 2004). Micropores may occur in either within (intra-aggregate)
and between aggregates (interaggregate) or within blocks of soil particles if aggregates are not present (Kutilek, 2004). Macropores may occur in interaggregate spaces (Jullien et al., 2005) and/or in decayed roots, earthworm channels, fissures, and cracks (Kutilek, 2004). Macropores are defined as pores that do not form capillary menisci and therefore do not retain water against gravitation. Kutilek (2004) considered an equivalent pore radius of 15 to 30 μm (mean, 23 μm) as an approximate threshold between intra-aggregate and interaggregate pores and 1000 to 1500 μm (mean, 1250 μm) as an approximate threshold between micropores and macropores. For clay-rich materials, Jullien et al. (2005) also classified pores into interlayer, interparticle, and interaggregate (macro)pores. To merge these two classifications, we assume that submicroscopic pores introduced by Kutilek (2004) correspond to the interlayer pores defined by Jullien et al. (2005). In this class of pores (on nanoscale), the water molecules are retained in the interlayer spaces of clays or associated with clay particles. Therefore, we use the term interlayer pores for these types of pores. We use the term capillary pores for intra-aggregate and intraparticle pores (with \(d < 23 \mu m\), where \(d\) is pore radius) and interaggregate pores (with \(23 \mu m < d < 1250 \mu m\)) where capillary menisci are formed. The term macropores refers to larger interaggregate pores (with \(d > 1250 \mu m\)) where capillary menisci are not formed.

The study of the impact on PSD is usually neglected in soil amendment experiments because it is difficult to quantify by classical field/laboratory methods, including direct or indirect measurement of PSD from water retention curve (WRC) measurements based on soil water content or geophysical measurements and hydrological inversion (as done by Jadoon et al., 2012; Busch et al., 2013; and Jonard et al., 2015) or WRC measured by pressure plate extractors (e.g., Bittelli and Flury, 2009); multistep outflow (e.g., Bayer et al., 2005; Hollebeck and Jensen, 1998; Neyshabouri et al., 2013; Weihermüller et al., 2009); or the evaporation (e.g., Schindler et al., 2010; Žydelis et al., 2018), mercury intrusion (e.g., Webb, 2001), and nitrogen sorption (e.g., Kowalczyk et al., 2003) methods. As an alternative, noninvasive measurement techniques can be used, such as MicroCT (e.g., Koestel, 2018; Pohlmeier et al., 2018; Smet et al., 2017), synchrotron radiation and/or microtomography (e.g., Peth et al., 2008), or nuclear magnetic resonance relaxometry (NMRR) (e.g., Jaeger et al., 2009; Stingaciu et al., 2010).

Nuclear magnetic resonance relaxometry has been successfully applied in studying the characteristics of the pore space in geological porous media like sandstone rocks (Kleinberg et al., 1994; Song, 2010) as well as natural soils (Brax et al., 2017; Hall et al., 1997; Hinedi et al., 1997; Pohlmeier et al., 2009). According to the empirical model of Brownstein and Tarr (1977, 1979), the observable relaxation rates are composed of the bulk rate plus a term describing enhanced surface relaxation. Because the average collision rate of water with the pore wall is higher in small pores than in large pores due to shorter translational diffusive pathways, the overall rate is faster, and one observes a linear relation between relaxation rate and reciprocal pore size. This relation allows the calculation of PSD from relaxation time distribution (Barrie, 2000; Hinedi et al., 1997; Ryu, 2009). In this regard, Morriss et al. (1997) compared the NMRR spectra with independently obtained PSD by mercury injection porosity measurements, leading to a classification of NMR-derived pore sizes for sandstones, where the transversal relaxation times \(T_2\) of 3 and 33 ms were considered as cutoff values to separate clay-bound water, capillary-bound water, and free water fractions. Later, Bird et al. (2005) compared WRCs with longitudinal relaxation time distribution \(T_1\) functions of model porous media and three natural soils. From bimodal distribution functions, they determined intra-aggregate and bulk water, which are characterized by short and long \(T_1\) values ranging from 3 to 100 ms. The direct scaling of NMR relaxation time to pore size was discussed by Jaeger et al. (2009), who found better correlation between PSD and \(T_2\) spectra by using a dual-relaxivity model, which defines separate surface relaxivity parameters for micropores and mesopores. Recently, Meyer et al. (2018) confirmed this approach by calibration and validation with 14 different soil materials. They found \(T_2\) surface relaxivities of 551.7 and 9.6 μm s\(^{-1}\) for macro- and medium-sized pores defined by matrix potentials of approximately ~32 kPa. The \(T_2\) spectra also correlate with wet-aggregate stability (WAS) and average particle size. Buchmann et al. (2015) differentiated between clay-associated water, micropores, and mesopores and macropores by defining \(T_2\) cutoff values of 3, 60, and 300 ms, respectively, and discussed the correlation of WAS, particle size, and \(T_2\) spectra. Recently, Shi et al. (2017) discussed the concept of separating pore size classes in swelling soil materials into clay interlayer, interparticle, and interaggregate pores, separated by \(T_2\) values of 10 and 100 ms.

In addition to the lack of the studies on the effects of different SAs on the PSD, there is a lack of studies on the use of natural biopolymers as SAs because most of the investigated biopolymers have a synthetic origin rather than being natural (Awad et al., 2013; Chang and Cho, 2012; Chang et al., 2015a, 2015b; Maghchiche et al., 2010; Orts et al., 2007). Natural biopolymers, such as Arabic gum (AG), agar, cellulose, alginate, psyllium gaur gum, bacterial exopolysaccharide, and chitosan (CH), have been investigated by few researchers (e.g., El-Jack, 2003; Hataf et al., 2018; Khatami and O’Kelly, 2013; Patil et al., 2011). To the best of our knowledge, there are few reports on the usages of AG and CH as SAs. For example, Mohamed (1999) reported that AG significantly increased the WAS compared with the reference soil. El-Jack (2003) also showed that AG increased the water retention (insignificantly) and WAS (significantly) and decreased the saturated hydraulic conductivity \(K_s\) (insignificantly) of three different soils with low, medium, and high clay contents compared with the reference soil. The increase in soil water retention may be due to the effect of gum on improvement of soil structure, leading to more water absorption, or due to the capacity of AG itself to store water. The increase in WAS might be caused by polysaccharides on cementation of soil particles. On the other hand, Hataf et al. (2018) used CH as soil stabilizer and showed that CH provided extra interparticle interaction under wet conditions soon after amendment and this effect reduced over time. The same
authors showed that aggregates of CH-amended soils were instable at dry condition.

The current work investigated the effect of AG and CH on PSD and the possible soil improvements with respect to water retention. To investigate the PSD of soils amended by CH and AG, NMRR was applied. The results from NMRR are underpinned by conventional soil physical and chemical measurements, including WRC, zeta potential ($\zeta$), and saturated water content ($\theta_s$), as well as soil electrical conductivity (EC), organic carbon (OC) content, and measurements of the soil respiration rate ($R_\text{p}$).

Materials and Methods

Soil Sampling

Soil samples were taken from Dryland Agricultural Research Institute (37°12’ N, 46°20’ E, 1730 m asl), 25 km east of Maragheh, East Azerbaijan Province, Iran. Soil samples were collected from depths of 0 to 25 cm. The reference soils were classified as loam texture (25% < 2 μm, 33% 2–50 μm; 42% 50–2000 μm), 0.54% OC, EC of 252 μS cm⁻¹, and pH of 6.72.

The soil (fine, mixed, mesic, Typic Calcixerepts based on the USDA system and Calci-sols based on the FAO system) (Hemmat and Eskandari, 2004) consists of 42% sand, 33% silt, and 25% clay. Therefore, the soil texture class can be characterized as loamy according to the USDA soil classification. The soil is poor in OC content with 0.5 mass %, and the EC value of 252 μS m⁻¹ indicates a normal, nonsaline soil. According to Iran Meteorological Organization report, the long-term average annual temperature and precipitation of the region are 13.2°C and 310 mm, respectively. Based on the thermochron and moisture regime maps of Iran’s soils, the study area has xeric and mesic regimes (Hemmat and Eskandari, 2004).

Laboratory Measurements

Biopolymer Application

In this experiment, biopolymers were dissolved in tap water at a concentration of 25 and 50 g L⁻¹. For CH, the solution was acidified by adding 1 mL acetic acid to obtain a pH of around 5. These solutions (200 mL kg⁻¹) were sprayed on disturbed soil material (<2 mm) and mixed simultaneously by a glass rod. The blank soil was treated similarly by spraying and mixing it with tap water only (200 mL kg⁻¹). After 3 wk of incubation time, we conducted the laboratory measurements. During incubation, soils were air dried and wetted by capillary rise several times by keeping soil water content near field capacity.

Soil Water Retention Curve

The soil WRC was measured using the HYPROP system (UM) as described by Schindler et al. (2010). Cylinders (250 cm³) were filled to known bulk density ($D_\text{b}$) of 1.2 ± 0.02 g cm⁻³ with dry soil/amendment mixtures. To do this, we transferred 300 g dry soil in small lots into the cylinder to fill it. For uniform packing, we tapped the cylinder gently with each addition of soil during filling process. Packed soils were incubated for 3 wk and then saturated over the course of 2 wk from below by setting the samples into a water bath. After full saturation, the samples were settled on the HYPROP system, and the tensiometer values were recorded permanently in predefined intervals (from 1 to 10 min). The gravimetric water content, $\theta_m$, of the samples was determined by settling the system on a balance at least five times a day. The mean soil suction ($h$ in cm) was calculated from the tensiometer readings for each water content measurement. Because the application of HYPROP system allowed a partial WRC measurement (0–100 kPa), we used the measured $\theta_m$–$h$ data pairs to fit the van Genuchten (1980) equation and then extrapolate the unmeasured ($h > 100$ kPa) part of the WRC:

$$\theta_s = \theta_r + \frac{\theta_s - \theta_r}{(1 + \alpha h^m)^n}$$

where $\theta_s$ (cm³ cm⁻³) is volumetric water content ($\theta_m \times D_\text{b}$) at each given $h$ (cm); $\theta_r$ and $\theta_s$ are the residual and saturated volumetric water contents (cm³ cm⁻³), respectively; $\alpha$ is the reciprocal of the air-entry value (cm⁻¹); and $n$ and $m$ (both dimensionless) are shape parameters, with $m = 1 - 1/n$. Fitting was performed with the data solver application in Microsoft Excel.

Nuclear Magnetic Resonance Relaxometry

The $T_1$ and $T_2$ relaxation times of water in the soil samples were measured in a custom-made Halbach magnet at a field strength of 0.15 T (Raich and Blümler, 2004) operated by a Kea² Spectrometer and Prospa software (Magritek). Applying the same procedure described in previous section, core samples were prepared by packing 16.3 g dry reference and biopolymer-amended soils into glass tubes (inner diameter, 24 mm; height, 30 mm) to reach a bulk density of 1.20 ± 0.02 g cm⁻³. Similar to other experiments, packed soils were incubated for 3 wk and then subjected to NMR measurements. The $T_2$ spectra were measured using a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence with up to 4096 echoes spaced at $t_2 = 0.12$ ms (Carr and Purcell, 1954; Meiboom and Gill, 1958). The repetition time was 2000 ms, and up to 128 echo trains were accumulated to improve the signal-to-noise ratio. To determine $T_1$, we used a saturation recovery pulse sequence ($T_1$-SR-Add) with CPMG pulse sequence detection with exponentially increasing encoding times between 0.4 and 1000 ms. The $T_1$ recovery curve was recorded with 16 logarithmically spaced saturation delays with a maximum delay of 1000 ms. All resulting relaxation curves were analyzed by inverse Laplace transformation embedded in the Prospa software, and the resulting relaxation time spectra are represented by the probability distribution $P[\log T_1, T_2]$.

The interpretation of the relaxation spectra proceeded on the empirical model of Brownstein and Tarr (1977), which relates the observed relaxation rates to the pore surface to volume ratio ($S/V$) pore:

$$\frac{1}{T_{1,2}} = \frac{1}{T_{1,2,\text{bulk}}} + p_{1,2} \left( \frac{S}{V} \right)_{\text{pore}} + \left( \frac{1}{T_{2,\text{diff}}} \right)$$

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where \( \rho \) is the surface relaxivity parameter, and the subscript indices 1 and 2 refer to longitudinal or transverse relaxation rates and surface relaxivities, respectively. The third term on the right hand side of Eq. [2] signifies the decay of the signal due to translational diffusion in magnetic field gradients, which is expressed in the short time regime as (Mitchell et al., 2010):

\[
\frac{1}{T_{2,\text{diff}}} = \frac{D}{12(\gamma G t_{E})^{2}} \quad [3]
\]

where \( D \) denotes the molecular translational diffusion coefficient, \( G \) is the magnetic field gradient strength, and \( t_{E} \) is the inter-echo spacing. This contribution can be minimized by the choice of a sufficient short value for \( t_{E} \).

**Determination of Pore Size Distribution from Relaxation Spectra**

According to Eq. [2], the average relaxation times are related to the average pore size parameter \((S/V)\) and to its distribution function. In the case that independent information about the pore diameter distribution is available (e.g., from WRC), the surface relaxivity parameters in Eq. [2] can be fitted by matching PSD functions, recalculated from NMRR relaxation time distributions to those obtained from the WRC curves (Stingaciu et al., 2010). Therefore, we translated the WRC into PSD using discrete retention points.

**Additional Soil Physical Measurements**

Additional soil physical characteristics were measured to support the effects of biopolymer amendment in soils and to help understand the findings from the NMRR experiments. The examined soil characteristics are soil texture, soil EC, pH, \( \theta_{v} \), \( \zeta \), OC content, and \( R \), measurements.

Soil texture was determined by hydrometer method (Gee and Or, 2002), soil EC, and pH in paste saturation extracts by EC meter and pH meter, and \( \theta_{v} \) was determined by weighing a defined volume of soil after full saturation by capillary rise from bottom and oven dried at 105°C.

The \( \zeta \), defined as the potential at the shear plane, was measured using the Zetasizer Nano ZS instrument (Malvern Instruments Ltd) (Zelazny et al., 1996). To do this, 100 g of soil plus 200 g \( \text{H}_{2}\text{O} \) was put into a 1-L glass bottle and shaken for 6 h upright on a horizontal shaker at 150 rpm. Then, another 600 g \( \text{H}_{2}\text{O} \) was added, and the bottle was shaken to homogenize the mixture. The suspension was left for 6 min, and all particles >20 \( \mu \text{m} \) settled to a depth below 12 cm. The supernatant was removed with a pipette and transferred into a new 1-L glass bottle, and a magnetic stick was added. The bottle was put onto a magnetic stirrer that was controlled by a timer. The suspension was slurred for 2 min. A sedimentation time of 12 h allowed the microaggregate phase (particle diameter, 2–20 \( \mu \text{m} \)) to separate. After 12 h, the supernatant (the colloidal phase with particle diameter <2 \( \mu \text{m} \)) was removed with a pipette and transferred to a new 0.5-L glass bottle. A diluted colloidal phase was then used for \( \zeta \) measurement by the Zetasizer. Because the ionic strength and pH value of the soil electrolyte solution affect the \( \zeta \) potential, a portion of the soil colloidal phase was centrifuged using a refrigerated centrifuge for 90 min at 8000 rpm and 20°C. The soil electrolyte section (i.e., the supernatant) was separated, and the ionic strength and pH value of the solution were determined.

The OC content of soil samples was measured according to Nelson and Sommers (1982) based on the wet combustion approach to determine the amount of \( \text{CO}_{2} \) liberated from organic C through the titrimetric technique.

Soil respiration rate was measured over the course of 31 d in a closed chamber using alkali traps to absorb \( \text{CO}_{2} \) (Bekku et al., 1997). To do this, 100 g of dry weight equivalent soil was put into the microcosms and wetted up to a water content of 0.2 g\( \text{H}_{2}\text{O} \) soil. The respiration experiment was performed at constant temperature of 20°C. The data from incubation experiments were fitted to single-pool model to predict the decomposition rate constant \( (k) \) (d\(^{-1}\)), half-life (HL) (d), and mean residence time (MRT) (d) of the organic C turnover for each amended soil as well as for the reference soil as recommended by Weihermüller et al. (2018):

\[
C_{t} = C_{0} \exp \left( -kt \right) \quad [4]
\]

\[
\text{HL} = \frac{\ln(2)}{k} \quad [5]
\]

\[
\text{MRT} = \frac{1}{k} \quad [6]
\]

where \( C_{t} \) is the cumulative \( \text{CO}_{2} \) evolved at time \( t \) (mg g\(^{-1}\)), and \( C_{0} \) is the total \( \text{CO}_{2} \) flux at \( t_{\text{end}} \) (mg g\(^{-1}\)).

**Statistical Analysis**

A completely randomized design was performed with five treatments and three replications. Treatments included soil amendment by 10 (high, indicated by H) and 5 (low, indicated by L) grams of CH and AG per kg soil (CH-H, CH-L, AG-H, and AG-L) as well as pure soil as reference (hereafter referred to as "reference soil"). The measured variables were subjected to ANOVA to evaluate the differences in measured soil parameters caused by soil amendments, and the Duncan (1955) method was used to conduct comparisons of means.

Regarding the relaxation times spectrums, both peak values (being related to mean value of the pore size) and their probability values (being related to their abundance) are important to interpret the effects of biopolymer amendment on examined soil. Therefore, both peak values and the integral relative area under curve (AUC) were determined. The latter parameter is defined as AUC(\( i \)) = \( i / A_{1} \), where \( A_{i} \) is area below the curve between two consecutive minimum values for each peak value, and \( A_{1} \) is area below the entire curve. More specifically, in the case of the \( T_{2} \) spectrum with three peaks, AUC(1), AUC(2), and AUC(3) are proportional to the water content in interlayer pores, capillary pores, and macropores, respectively. In the other words, AUC(1), AUC(2), and AUC(3)
are proportional to the fractional saturation of the examined soils, being equivalent to the total amount of water in the corresponding pores. The summation of AUC(1), AUC(2), and AUC(3) corresponds to the total porosity derived from NMRR measurements \(\pi_{\text{NMRR}}\). The peak values (i.e., the relaxation times) and their corresponding AUC values for each treatment were determined and subjected to ANOVA and comparisons of means.

Results and Discussion

Nuclear Magnetic Resonance Relaxometry Results

The averaged probability distribution functions of relaxation spectrum for reference soil and soils amended by biopolymers are plotted in Fig. 1. Probability distribution functions are bimodal for \(T_1\), having two peak values representing \(T_1(1)\) and \(T_1(2)\), and trimodal for \(T_2\), having three peak values representing \(T_2(1)\), \(T_2(2)\), and \(T_2(3)\) that correspond to different classes of pores (Fig. 1).

Water associated with clay particles or residing in the interlayer space in \(T_2\) in the range of few milliseconds for very different soil materials, whereas the association of modes with longer relaxation times is not straightforward and depends strongly on the type and texture of soil as well as on swelling and shrinking processes. Subject to a later clarification, we will base our work on the definition of Morriss et al. (1997) and identify \(T_2(1)\) with clay-associated water. This is approximately identical to the definition of Meyer et al. (2018) of water relaxing faster than 4.5 ms as micro- and medium-pore water. Water fractions relaxing slower are referred to in this scheme as capillary-pore and macropore water or, in the definition of Meyer et al. (2018), as macropore water. The differentiation between capillary pore and macropore water is arbitrary because the boundaries are not clearly defined. This range corresponds to the range of pore sizes that are easily measurable by WRC. Therefore, it is used for the derivation of surface relaxivities by rescaling NMR-derived PSD to WRC-derived PSD.

Based on the above discussion, \(T_1(1)\) and \(T_2(1)\) in our experiments at 3 and 1 ms, respectively, correspond to clay-associated water molecules or the presence of interlayer pores, whereas \(T_1(2)\) and \(T_2(3)\) reflect the effects of the capillary pore–associated and macropore-associated water molecules on transversal relaxation times, respectively; \(T_1(2)\) also reflects the simultaneous effects of capillary pores and macropores associated water molecules on the longitudinal relaxation time (Shi et al., 2017).

The variation in the \(T_1\) spectrum under amended soils compared with the reference soil is small and insignificant due to the low resolution of the basic datasets using 16 inversion times (Fig. 1). Therefore, because \(T_2\) spectra had higher resolution, we focused on the variations in \(T_2\) spectrum because \(T_2\) gives more detailed information about the changes in pores related to capillary pores and macropores by differentiating their relaxation times.

Peak values and their corresponding relative abundances, which are reflected in the AUC values of \(T_2\), were determined and subjected to ANOVA. The results in Table 1 show that the application of AG-H and CH-H slightly but significantly shifted \(T_2(1)\) to smaller values (0.8 and 0.9 ms, respectively) compared with the reference soil \([T_2(1) = 1.2 \text{ ms}]\) (Fig. 2), indicating a reduction of average pore size because the relaxation rate is linearly related to the inverse pore size according to the Brownstein–Tarr model. The two other treatments (CH-L and AG-L) had no effect on \(T_2(1)\), showing a \(T_2(1)\) around 1.2 ms (Fig. 2). There is no clear reason why the AG-H and CH-H amendment resulted in faster relaxation times in clay-associated water molecules (interlayer pores).

Table 1. Analysis of variance of longitudinal relaxation time \(T_2\) and relative area under curve (AUC) values among different amendments.

<table>
<thead>
<tr>
<th>Variation</th>
<th>df</th>
<th>Mean squares</th>
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<tbody>
<tr>
<td>Amendment</td>
<td>4</td>
<td>0.002*</td>
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<tr>
<td></td>
<td></td>
<td>0.029**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0001 ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.091**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24.91 ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11278.6**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>0.0003</td>
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<td></td>
<td></td>
<td>0.003</td>
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<td>1250.88</td>
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</table>

* Significant at the 0.05 probability level; ns, not significant.
** Significant at the 0.01 probability level.
† Numbers in parentheses donate three peak values in the \(T_2\) relaxation time spectrum.
The results also revealed that CH-H insignificantly resulted in faster relaxation in $T_2^2$, showing a value of 14 ms compared with 16 ms under reference soil and AG-H. Both lower doses of applied biopolymers (AG-L and CH-L) resulted in insignificantly slower relaxation times for capillary-bound water molecules, showing a $T_2^2$ value of 21 ms for both compared with 16 ms for the reference soil. The CH-H treatment also resulted in a significantly faster relaxation time in macropore-related water molecules, showing a $T_2^3$ value of 194 ms compared with 281 ms for the reference soil. The AG-L, AG-H, and CH-L amendments insignificantly increased the relaxation times of $T_2^3$, showing $T_2^3$ values of 317 to 341 ms compared with the reference soil with $T_2^3$, 281 ms. Faster relaxation means that the CH-H has narrowed the pores diameters in both micropores (insignificantly) and macropores (significantly) and that AG-H insignificantly narrowed pore diameter in micropores and widened pore diameter in the macropore range.

The effect of CH-H on the pores system becomes more obvious by taking into account the AUC values. The comparison of mean values (Table 1) revealed that CH-H significantly increased the AUC value under $T_2^2$ (AUC = 0.35), illustrating an increase in the abundance of water bound by capillary forces compared with the reference soil and AG-L, AG-H, and CH-L, with AUC values of 0.14 ± 0.02. The total porosity derived from the $T_2$ measurement ($n_{NMRR}$) is reported in Fig. 3, which shows that CH-H had the highest $n_{NMRR}$ value of 0.65 (dimensionless) compared with AG-H and pure soil [$n_{NMRR}$, 0.52 (dimensionless)] and with AG-L and CH-L [$n_{NMRR}$, 0.49 (dimensionless)]. Figure 3 also shows the correlation between the $n_{NMRR}$ and $\theta_s$ with an $R^2$ value of 0.798. Excluding CH-H with an $n_{NMRR}$ value of 0.65 and a $\theta_s$ value of 0.54, the $n_{NMRR}$ and $\theta_s$ values for other treatments nearly match perfectly. The greater differences between the $n_{NMRR}$ and $\theta_s$ values of CH-H treatment are unclear and need to be investigated more in depth. Taking into account the results of the WAS and mean weight diameter (MWD) of the aggregate as published by Rahmati and Kousehlou (2019) for the same soil and treatments, CH-H resulted in more stable but smaller aggregates. One could expect higher abundance of the intermediate or capillary pores, which is in line with NMRR results. The slightly higher saturated water content, $\theta_s$, under CH-H treatment confirms this interpretation and is discussed in more detail below.

**Biopolymer Effects on Soil Physicochemical Properties**

The biopolymer-related changes in physicochemical properties of the amended soils were investigated to help interpret the NMR results. The changes in physicochemical properties were analyzed using ANOVA (Table 2). Significant differences were detectable in nearly all measured soil properties except soil pH. We expected lower pH in CH-H– and CH-L–amended soils because the raw biopolymer CH was acidified using acetic acid to solve chitosan in solution. However, this impact is negligible when the CH is added to the soil due to the buffering capacity of the soil.

Comparison of the mean values (Table 3) revealed that $\theta_s$ content under CH-H (0.54 cm$^3$ cm$^{-3}$) was slightly higher than that under AG-H (0.52 cm$^3$ cm$^{-3}$) and the reference soil...
AG-L and CH-L significantly decreased $q$ (0–100 kPa) performed for the reference and CH-H– and AG-H– (0.52 cm$^3$ cm$^{-3}$ Kousehlou (2019). They showed that both CH-H and AG-H treat-PSD, we discuss previously published results of WAS and MWD relaxa-ponsters, whereas the CH-H caused considerable changes in the tion curve (Fig. 4). The impacts of the amendments are discussed pore spectra, as reflected by the differences over the entire reten tion curve measured for the AG-H–amended soil is close to that tion curve. On the other hand, both AG-L and CH-L showed slower expected because they both have organic origins. It seems that aggregate size increase under AG-H amendment (MWD = 0.4 mm) and larger aggregate size under CH-H amendment (MWD = 4.1 mm). The reference soil showed a mean MWD value of 2.2 mm. The increase in WAS indirectly confirms the changes in interaggregate formation, which took place in both AG-H and CH-H treatments. On the other hand, smaller MWD under CH-H compared with the reference soil and AG-H confirms the higher frequency of narrower pores under CH-H, which is in line with NMRM results. The reason for the aggregate size increase under AG-H amendment seems to be clear because Sandford and Baird (1983) stated that polysaccharides can act as binding agents in soils. In fact, polysaccharides and polyacrylic amides bind to each other and act as a bridge for bonding clay minerals. Because AG has a gelatinous and adhesive state, it clogs up soil particles and facilitates aggregate formation and thereby improves soil structure (Whistler, 2012; Whistler and Hymowitz, 1979). Gardner (1972) reported that natural polymers, including AG, stabilize the soil structure, thereby improving aggregate formation and leading to better soil aeration, faster water flow, and improved germination of plants. However, reduced aggregate sizes accompanied by increased aggregate stability after CH-H seems to be more complex to describe. The main reason for reduced aggregate size can be explained by the reduction in the absolute value of soil $\zeta$. The absolute value of $\zeta$ under CH-H treatment is smaller (18.5 mV) compared with the soil, showing WAS values of 71 and 78%, respectively, vs. 23% for the reference soil, whereas an opposite effect was observed for the MWD (Fig. 5), showing smaller aggregate size under CH-H amendment (MWD = 0.4 mm) and larger aggregate size under AG-H amendment (MWD = 4.1 mm). The reference soil showed a mean MWD value of 2.2 mm. The increase in WAS indirectly confirms the changes in interaggregate formation, which took place in both AG-H and CH-H treatments. On the other hand, smaller MWD under CH-H compared with the reference soil and AG-H confirms the higher frequency of narrower pores under CH-H, which is in line with NMRM results. The reason for the aggregate size increase under AG-H amendment seems to be clear because Sandford and Baird (1983) stated that polysaccharides can act as binding agents in soils. In fact, polysaccharides and polyacrylic amides bind to each other and act as a bridge for bonding clay minerals. Because AG has a gelatinous and adhesive state, it clogs up soil particles and facilitates aggregate formation and thereby improves soil structure (Whistler, 2012; Whistler and Hymowitz, 1979). Gardner (1972) reported that natural polymers, including AG, stabilize the soil structure, thereby improving aggregate formation and leading to better soil aeration, faster water flow, and improved germination of plants. However, reduced aggregate sizes accompanied by increased aggregate stability after CH-H seems to be more complex to describe. The main reason for reduced aggregate size can be explained by the reduction in the absolute value of soil $\zeta$. The absolute value of $\zeta$ under CH-H treatment is smaller (18.5 mV) compared with the
AG-H and AG-L significantly increased OC contents, whereas AG amendments than in CH amendments. Previously published results showed that AG amendment caused more colloidal stability in AG-amended soil with respect to increased dosage can be related to a higher colloidal stability in AG amendments than in CH amendments. Previously published results showed that AG amendment caused more colloidal stability than CH amendment in comparison to a reference soil (Rahmati and Koushelou, 2019). The value of the $\zeta$ can be used as an estimate of the diffuse layer potential and is important in discussing the tendency for the soil colloids to disperse. In other words, by increasing ionic strength, the thickness of the electrical double layer is reduced, and thus soil colloids tend to flocculate. In general, there is a relationship between the effect of salt concentration on $\zeta$ and $K_s$: the higher the $\zeta$, the higher the $K_s$ (Aydin et al., 2004). Decreased $K_s$ can be related to the probable increase in thickness of the diffuse double layer (Aydin et al., 2004). The results indicated that CH-H caused the highest $K_s$ compared with all other amendments (Rahmati and Koushelou, 2019).

Since CH and AG have organic origins, we evaluated the changes in OC content of the examined soils and mixtures. Both AG-H and AG-L significantly increased OC contents, whereas CH-H and CH-L had no significant effects on OC content. However, this opposed our expectations because we expected a higher OC content under all treatments compared with reference soil. The likely reason that AG and CH amendments increase OC differently could be the different amounts of C atoms in the molecule structures. The different quality and the accessibility of the C atoms for biodegradation in AG- and CH-amended soils could be another reason for slower biodegradation of AG in the soil. To evaluate this hypothesis, we examined the soil $R_s$ as a measure of microbial activity, being sensitive to the quality and the accessibility of the C atoms in organic structures (Weihermüller et al., 2018). The cumulative CO2 flux rates over time under reference soil and soils amended by CH and AG (Fig. 6) showed that soil amended with CH-H and CH-L considerably increased the $R_s$ compared with the reference soil or with the soils amended by AG. In addition to visual inspection, we fitted the measured CO2 flux rates to a single-pool model (Eq. [4]) to estimate the $k$, HL, and MRT for each amendment and the reference soil. The results were subjected to ANOVA to explore the differences among amended soils and the reference soil. The measured incubation data could be accurately fitted to the single-pool model, with $R^2$ values of 0.84 to 0.99 and RMSE values of 0.15 to 0.89 mg g$^{-1}$. The ANOVA results (Table 4) show that the kinetic parameters $k$, HL, MRT, and $C_1$ significantly differ between reference soil and soil amended by CH and AG. Comparisons of means (Table 5) show that the decomposition rate constant of the reference soil ($k = 0.035 \text{ d}^{-1}$) is significantly higher than the amended soils; AG-H showed the

![Fig. 5. Aggregate sizes formed by the application of different amounts of chitosan (CH) and Arabic gum (AG). The photo is taken by Nikon D80FLR digital camera with an 18- to 135-mm lens and with a magnification ratio of 1:5.](image_url)

![Fig. 6. Cumulative CO2 flux rates over time for the reference soil and for soil amended with chitosan (CH) and Arabic gum (AG). For amendments, L refers to low (5 g kg$^{-1}$ soil) and H refers to high (10 g kg$^{-1}$ soil).](image_url)
lowest decomposition rate ($k = 0.007 \text{ d}^{-1}$). As a consequence, the HL and MRT (20 and 29 d) of the reference soil was significantly lower than the amended soils. Treatment with AG-H showed an HL of 101 d and an MRT of 145 d, indicating the most stabilized process. We conclude that AG seems to need much more time to affect soil properties.

Application of AG caused $R_2$ rates lower than those of the reference soil after 2 wk (Fig. 6). This could be caused by the existence of antibacterial compounds in AG or by its higher gelling characteristic (i.e., AG sticks more strongly to soil particles and stays out of the reach of the soil microbial community). The last case could be confirmed by large soil clods (aggregates) created under AG amendment, but this effect needs to be investigated in more depth. The exact mechanisms affecting microbial degradation with AG and CH are not well understood, and more studies are needed to explore whether any toxic effects are detectable or if the amendments cause shifts in the soil microbial community.

### Table 4. Comparison of means of physicochemical properties of the reference soils and the soils amended by low and high dosages of chitosan (CH-L and CH-H) and Arabic gum (AG-L and AG-H).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameters†</th>
<th>EC$_{colloid}$</th>
<th>EC$_{electrolyte}$</th>
<th>OC</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference soil</td>
<td>$\zeta$</td>
<td>mV</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>–20.0 (b)§</td>
<td>118 (b)</td>
<td>104 (b)</td>
<td>0.54 (c)</td>
<td>0.520 (ab)</td>
<td></td>
</tr>
<tr>
<td>CH-H</td>
<td>–18.5 (a)</td>
<td>241 (a)</td>
<td>232 (a)</td>
<td>0.63 (c)</td>
<td>0.544 (a)</td>
</tr>
<tr>
<td>CH-L</td>
<td>–20.8 (bc)</td>
<td>116 (b)</td>
<td>96 (b)</td>
<td>0.55 (c)</td>
<td>0.492 (b)</td>
</tr>
<tr>
<td>AG-H</td>
<td>–21.6 (c)</td>
<td>103 (b)</td>
<td>86 (b)</td>
<td>1.11 (a)</td>
<td>0.524 (ab)</td>
</tr>
<tr>
<td>AG-L</td>
<td>–21.1 (bc)</td>
<td>108 (b)</td>
<td>91 (b)</td>
<td>0.95 (b)</td>
<td>0.492 (b)</td>
</tr>
</tbody>
</table>

† For amendments, L refers to low (5 g kg$^{-1}$ soil) and H refers to high (10 g kg$^{-1}$ soil).‡ EC$_{colloid}$: electrical conductivity of colloid section of soil; EC$_{electrolyte}$: electrical conductivity of electrolyte section of soil; OC, organic carbon; $\theta_1$, saturated water content; $\zeta$, zeta potential. § The letters a, b, and c represent different groups with mean values from greater to less. Combined letters indicate that there is no difference between the two related groups.

### Table 5. Comparison of means of respiration data the reference soils and the soils amended by low and high dosages of chitosan (CH-L and CH-H) and Arabic gum (AG-L and AG-H).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameters†</th>
<th>$k_1$</th>
<th>C$_1$</th>
<th>Half-life</th>
<th>MRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference soil</td>
<td>$T_1$</td>
<td>d$^{-1}$</td>
<td>mg g$^{-1}$</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>0.035 (a)§</td>
<td>5.36 (c)</td>
<td>20 (d)</td>
<td>29 (d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH-H</td>
<td>0.012 (c)</td>
<td>14.48 (b)</td>
<td>57 (b)</td>
<td>82 (b)</td>
<td></td>
</tr>
<tr>
<td>CH-L</td>
<td>0.022 (b)</td>
<td>9.60 (d)</td>
<td>33 (c)</td>
<td>47 (c)</td>
<td></td>
</tr>
<tr>
<td>AG-H</td>
<td>0.007 (d)</td>
<td>15.13 (a)</td>
<td>101 (a)</td>
<td>145 (a)</td>
<td></td>
</tr>
<tr>
<td>AG-L</td>
<td>0.012 (c)</td>
<td>10.07 (c)</td>
<td>60 (b)</td>
<td>86 (b)</td>
<td></td>
</tr>
</tbody>
</table>

† For amendments, L refers to low (5 g kg$^{-1}$ soil) and H refers to high (10 g kg$^{-1}$ soil).‡ $C_1$, organic carbon content at time zero; $H$, half-life; $k_1$, degradation rate constant; MRT, mean residence time. § The letters a, b, c, and d represent different groups with mean values from greater to less.

### Pore Size Distribution from Nuclear Magnetic Resonance Relaxometry Experiments

The PSD functions of the soils were examined by rescaling $T_1$ and $T_2$ distribution functions using Eq. [2]. For this purpose, we matched the NMRR-PSD to the WRC-PSD measured with HYPROP in the range of 0 and 100 kPa by adjusting the surface relaxivity parameter. Because the soil amended with AG-L and CH-L showed a negligible effect on NMRR results compared with the reference, we only discuss the results obtained for CH-H and AG-H amendments. Additionally, the measured water retention data were translated to the approximate PSD curves using discrete retention points. Figure 7 illustrates the PSD curves derived from WRC and from $T_1$ and $T_2$ distributions for the reference soil and the soil amended by CH-H and AG-H. We did not use the fast relaxation modes for the calculation because these modes were associated with clay-bound water only (organic bound and bound to the biopolymer structures is also possible), and therefore do not provide information of the PSD for water molecules associated with capillary pores and macropores. The effect of the fraction on mineral (clay)-bound water is also not reflected in the WRCs because the measurements only span $b < 100$ kPa. The fitted van Genuchten parameters, bulk water relaxation times, and surface relaxivity parameters are listed in Table 6. Finally, the weighted mean pore diameter of the reference and amended soils calculated from knowledge of the measured discrete points along the WRC and those obtained by NMRR ($T_1$ and $T_2$) are listed in Table 4.

There is satisfactory agreement between predicted rescaled pore diameters from WRC and NMRR measurements showing $R^2$ values of 0.79 to 0.92 and RMSE of 0.61 to 1.45 $\mu$m for $T_1$ and $R^2$ values of 0.78 to 0.99 and RMSE values of 0.57 to 1.38 $\mu$m for $T_2$ (Fig. 7). However, for CH-H, the CPMG-derived PSD curve is slightly shifted vs. the WRC-derived curve. The $T_2$ distribution apparently provides less information about the PSD than $T_1$. On the other hand, $T_2$ measurements show more detailed
information about the PSD compared with those obtained from the WRC because $T_2$ shows a bimodal distribution, which is not shown in the WRC. Therefore, NMRR $T_2$ measurements contain more information about capillary pores and macropores compared with classical soil water retention curves.

Comparing the PSD for the AG-H– and CH-H–amended soils with the reference soil (Fig. 7) shows that CH-H had more effect on pores where the water was bound by capillary forces, whereas AG-H had more effect on macropores. Although the reference soil and the soils amended by CH-H and AG-H show a mean pores diameter value of 1 μm (the peak values in NMR-derived PSD curve), which is the pore region where the water is held by capillarity, it seems that CH-H had increased the pore spectrum in this capillary bound region compared with the reference soils, showing wider probability for pore related to capillary-bound water molecules. The weighed mean macropore diameter value was estimated to be 31, 44, and 24 μm for the reference and CH-H– and AG-H–amended soils, respectively. By taking into account the peak values, it seems that CH-H resulted in larger macropores and AG-H in narrower macropores. However, the increased pore sizes under CH-H seems to be compensated by a decrease in the number or the availability of the pores. On the other hand, the
decreased pore sizes under AG-H could also be compensated for by the increase in the number of the availability of the pores.

In general, CH-H decreased the weighted mean value of entire pore sizes compared with the reference soil ($d = 13 \mu m$ vs. $16 \mu m$), and AG-H increased the weighted mean value of entire pore sizes ($d = 18 \mu m$) (Table 4).

Taking into account the water contents at field capacity (FC, in $b = 10$ kPa from measured data) and permanent wilting point (PWP, from extrapolated data) shows that AG-H decreased the available water (AW = FC – PWP) for plant use, showing FC and PWP values of 0.40 and 0.27 cm$^3$ cm$^{-3}$, respectively, compared with those of 0.39 and 0.24 cm$^3$ cm$^{-3}$ for the reference soil, whereas CH-H increased the available water calculated from FC and PWP of 0.38 and 0.23 cm$^3$ cm$^{-3}$.

### Conclusion and Outlook

In this study, we evaluated the effects of two different bio-polymers (CH and AG) on the physical and biological properties of a dryland loamy soil. Results from NMRR experiments revealed that AG caused faster NMRR relaxation times in amended soils, indicating an increase of pores with larger diameters compared with the reference soils, whereas CH mostly increased the number and size of pores representing capillary-bound water. These results were confirmed in previous studies where application of both AG and CH significantly increased aggregate stability. However, the size of stable aggregates decreased in CH-amended soils and increased in AG-amended soils compared with reference soils. Application of AG and CH also significantly affected the soil water content, where the water content at FC and PWP from measured data showed that AG-H decreased the AW by changing the FC and PWP to 0.40 and 0.27 cm$^3$ cm$^{-3}$, respectively, compared with those of the reference soil with 0.39 and 0.24 cm$^3$ cm$^{-3}$. On the other hand, CH-H increased the AW due to changes in FC and PWP to 0.38 and 0.23 cm$^3$ cm$^{-3}$, respectively.

The NMRR results revealed that the PSD can be predicted from both $T_1$ and $T_2$ measurements in a satisfactory manner, showing reasonably high agreement between PSD obtained from WRC and NMRR measurements, with $R^2$ values of 0.79 to 0.92 and RMSE of 0.61 to 1.45 $\mu m$ for $T_1$ and $R^2$ values of 0.78 to 0.99 and RMSE values of 0.57 to 1.38 $\mu m$ for $T_2$. In general, the $T_2$ distribution provided more information about the PSD and the WRC compared with $T_1$ because $T_2$ showed a bimodal distribution for capillary pores and macropores associated with water molecules, which is not seen in the $T_1$ and/or WRC distribution.

The results from incubation experiments showed that CH-amended soils had considerably increased respiration rates compared with the reference soil or AG-amended the soils. In addition to respiration rate, we analyzed the effect of biopolymers on soil biological conditions by determining the decomposition rate constant ($\delta$), HL, and MRT of all examined soils. In our study, AG-H with an HL of 101 d and MRT of 145 d proved to be more biologically stable in soil compared with the reference soil and the CH-amended soil. Based on these results, CH was more likely to provide more degradable carbon to the soil microbial community than AG.

Our results show that AG and CH significantly affected the hydraulic and structural properties as well as the moisture conditions of the examined soils. Therefore, it seems that these biopolymers can be used to modify the structural properties of loamy soils.

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### References


