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1 **Vegetation growth promotion and overall strength improvement**  
2 **using biopolymers in vegetated soils**

3

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12

13 **Abstract**

14 Planting vegetation is a sustainable and eco-friendly method for shallow slope  
15 stabilization. However, in water-limited regions, this method is facing challenges like  
16 retarded vegetation growth, which leads to unprotected soils. Biopolymer, with potentials in  
17 both vegetation growth promotion and soil strength enhancement, is therefore tested in this  
18 paper with regard to its possibility in assisting soil reinforcement with vegetation through  
19 vegetation cultivation and direct shear tests. Both sugar-based and protein-based biopolymers  
20 improved water availability to growing plants and nutrient uptake. The most suitable  
21 polysaccharide xanthan gum was adopted to further explore the effects of treatment condition  
22 (i.e., blending content) and external environment (i.e., precipitation) on the vegetated soil  
23 performances. Under a variety of water supply, xanthan gum with a medium blending content  
24 of 0.5% (i.e., with respect to dry soil mass) led to the most substantial improvement in the  
25 ability to resist shear loading. This indicates that the appropriate dosage of biopolymer used at  
26 the initial stage of plant growth, should provide moderate bond strength between soil particles,  
27 whilst not impeding root penetration. Supported by the obtained results, biopolymer is  
28 suggested to be used in combination with plants for soil reinforcement for the best efficiency.

29

30 **Key words:** vegetation, biopolymer, clayey soil, shear strength, water retention.

31

## 32 1. Introduction

33 Plants are extensively involved in the stabilization of shallow slopes and the control of  
34 soil erosion (Veylon et al. 2015; Pollen-Bankhead and Simon 2010; Świtala et al. 2018). They  
35 provide mechanical reinforcement through frictional interlocking and interaction between  
36 plant roots and soil particles (Roering et al. 2003; Reubens et al. 2007; Stokes et al. 2009),  
37 and meanwhile increase soil effective stresses by elevating the level of matric suction via  
38 plant transpiration (Leung et al. 2015; Ng et al. 2016a; Ng et al. 2016b; Ni et al. 2019).  
39 However, soil stabilization using plants is always facing with the problem of scarce water  
40 resources. It has been reported that the lack of a stable equilibrium between water  
41 consumption (e.g., evaporation and transpiration) and water supply (e.g., local precipitation  
42 and soil water reserve) is responsible for unsatisfactory planting practices (Glenn et al. 1998;  
43 Jackson et al. 2002; Cao et al. 2007, 2008).

44 Biopolymer treatment is known to provide a better soil environment for vegetation  
45 growth (Chang et al. 2015a). Biopolymer hydrogels in soils allow great water retention under  
46 irrigation or precipitation, thus alleviating the impact of wetting-drying cycles on vegetation  
47 growth (Carminati et al. 2010; Zickenrott et al. 2016; Tran et al. 2019; Rajanna et al. 2022).  
48 Biopolymers have an abundance of sugar molecules and elements like nitrogen and  
49 phosphorus which can serve as soil nutrients to stimulate vegetation germination and growth  
50 (Schachtman et al. 1998; García-Ochoa et al. 2000). Soil particles glued by viscous hydrogels  
51 are less susceptible to wind erosion (Wu et al. 2020) and water erosion (Orts et al.  
52 2000). When hydrogels undergo dehydration and become stiffer, they may help to increase  
53 the soil resistance to the volumetric attraction, which is beneficial to soil aeration (Chang et al.  
54 2015a).

55 Meanwhile biopolymers exhibit considerable impacts on altering soil geotechnical  
56 properties. For sandy soils, viscous biopolymer hydrogels can remarkably improve soil  
57 cohesions immediately after a thorough mixing (Chang and Cho 2019). For clayey soils,  
58 biopolymers with high molecular weight can adsorb to several clay particles to cause  
59 flocculation and/or aggregation (Kang et al. 2019a; Kang et al. 2019b). The mechanism that

60 governs the adsorption process depends on biopolymer types. For non-ionic biopolymers (e.g.,  
61 guar gum), hydrogen bonding is the dominant method of adsorption, while for anionic  
62 biopolymers (e.g., xanthan gum) and cationic biopolymers (e.g., chitosan), electrostatic  
63 attraction dominates the interaction between functional groups of biopolymers and charged  
64 clay particles. Previous studies have shown that biopolymers at concentrations of 0.5%~1.5%  
65 by weight of dry soil mass have great potentials in improving the soil performances under  
66 unconfined compression (Chang and Cho 2012; Fatehi et al. 2018; Vydehi and Moghal 2022;  
67 Ni et al. 2022; Cheng and Geng 2023), triaxial compression (Khatami and O 'Kelly 2013),  
68 direct shear (Chang and Cho 2019; Chen et al. 2019a), interface shear (Lee et al. 2020),  
69 tension (Muguda et al. 2017), three-point bending (Aguilar et al. 2016; Nakamatsu et al.  
70 2017), and split (Aguilar et al. 2016; Nakamatsu et al. 2017). In addition, water resistance of  
71 soils can be enhanced by using thermo-gelation biopolymers, e.g., gellan gum, agar gum, and  
72 carrageenan (Chang et al. 2015b; Chang et al. 2017; Nakamatsu et al. 2017), as well as  
73 hydrophobic biopolymers, e.g., casein (Chang et al. 2018).

74 As biopolymers show possibilities of reinforcing soil mechanical performances and  
75 supporting vegetation growth, their usage is expected to increase the efficiency of soil  
76 reinforcement with vegetation, especially at the initial stage of vegetation growth when the  
77 plant root system is not well developed and is more vulnerable to the variation of water  
78 supply. Different from other existing researches (Chang et al. 2015a; Tran et al. 2019) in  
79 which the effects of biopolymers were largely evaluated with respect to reducing soil erosion  
80 and promoting plant growth, a preliminary work proposed by Ni et al. (2020a) also focused  
81 on the impact of biopolymer on the geotechnical behaviours of the vegetated soil. This current  
82 work is an extension of Ni et al. (2020a), to investigate the influencing factors including  
83 treatment conditions (i.e., biopolymer type and blending content) and external environment  
84 (i.e., precipitation) on the biopolymer performances in terms of stimulating vegetation growth  
85 and improving the overall strength of the vegetated soil.

## 86 **2. Materials and methods**

## 87 2.1. Clayey soil

88 The clayey soil obtained from a deltaic deposit (Shanghai, China) was used in this study.  
89 It contained both coarse-grained and fine-grained soils, consisting of sand (33.4%), silt  
90 (54.9%), and clay (11.7%) particles. The basic properties include liquid limit  $w_L = 38\%$ ,  
91 plastic limit  $w_P = 22\%$ , optimum moisture content  $w_{opt} = 23\%$ , maximum dry density  $\rho_{d,max} =$   
92  $1.59 \text{ kg/m}^3$ , and specific gravity  $G_s = 2.73$ . According to [ASTM D2487](#), the clayey soil was  
93 classified as sandy lean clay. Further detailed information of this clayey soil can be found in  
94 [Ni et al. \(2020b\)](#).

## 95 2.2. Plant

96 Oats (*Avena sativa* L.) were chosen as a representative of herbaceous species for soil  
97 reinforcement. They usually show a simple root structure ([Edmaier et al. 2014](#)), which is  
98 typical of many species at their early growth stages ([Mickovski et al. 2007](#)). They also  
99 possess a relatively fast germination process, e.g., germination of oat seeds can occur 30 h  
100 after sowing ([Edmaier et al. 2014](#)). In addition, oats have strong resistance to harsh  
101 environment, leading to high yields and popularity in most areas of China ([Yang 2010](#)). In  
102 order to better control the quality of commercially available oat seeds, seed screening was  
103 carried out manually before sowing by picking out the small grains, as well as seeds of other  
104 plants mixed in, such as barley, highland barley, grass seed and buckwheat.

## 105 2.3. Biopolymer

106 Both sugar-based biopolymers also called polysaccharides (i.e., xanthan gum, guar gum,  
107 agar gum, and beta-glucan) and protein-based biopolymer (i.e., casein) were used in this study.  
108 Their main chemical components, solubility in cold water, and performances in soils are  
109 summarized in [Table 1](#).

110 Xanthan gum is an anionic polysaccharide biopolymer produced by fermentation of  
111 sucrose or glucose by using *Xanthomonas campestris*. It has a leading chain consisting of a

112 linear  $\beta$ -1,4-linked D-glucose backbone with every two elements possessing a trisaccharide  
113 side chain (Jansson et al. 1975; Sutherland 1994). Xanthan gum in a small concentration can  
114 greatly increase the viscosity of aqueous systems (Hassler and Doherty 1990; Nugent et al.  
115 2009). Incorporation of xanthan gum into soils helps to increase soil strength (Latifi et al.  
116 2016; Chen et al. 2019; Ni et al. 2020b), reduce soil erosion (Chang et al. 2015a), and  
117 increase water resistance (Chen et al. 2020).

118 Guar gum is a non-ionic galactomannan polysaccharide extracted from guar seeds. It has  
119 a backbone consisting of  $\beta$ -1,4-linked D-mannose. Every second mannose possesses a short  
120 side branch made of  $\alpha$ -1,6-linked D-galactose residues (Mudgil et al. 2014; Mandal et al.  
121 2023). Guar gum is soluble in water and has a potential to improve soil strength when it is  
122 used in soils (Ayeldeen et al. 2016; Muguda et al. 2017).

123 Agar gum is a polysaccharide that is commonly extracted from marine red algae. It is  
124 composed of alternating sequences of  $\beta$ -1,3-linked D-galactose and  $\alpha$ -1,4-linked 3,6-  
125 anhydro-L-galactose residues. Agar gum has a low solubility in cold water, but dissolves in  
126 hot water ( $> 85\text{ }^{\circ}\text{C}$ ) and starts to form a gel when cooled to  $32\text{--}43\text{ }^{\circ}\text{C}$  (Normand et al. 2000;  
127 Ryou and Jung 2023). Other biopolymers that resemble this similar property (i.e., referred as  
128 thermo-gelation biopolymers) include carrageenan (Nakamatsu et al. 2017; Ni et al. 2022)  
129 and gellan gum (Chang et al. 2015b; Chang et al 2017; Chang and Cho 2019). The usage of  
130 agar gum in soils helps to raise soil strength (Khatami and O'Kelly 2013) and water resistance  
131 (Chang et al. 2015b).

132  $\beta$ -1,3/1,6 glucan (beta-glucan) is found in a variety of natural sources, e.g., fungi, yeast,  
133 mushrooms, seaweeds, and bacteria. It comprises glucose units mainly linked by  $\beta$ -1,3 bonds  
134 with a side chain joined by  $\beta$ -1,6 linkages (Chen and Seviour 2007; Wu et al. 2016; Uchiyama  
135 et al. 2020). Beta-glucan is water soluble and has possibilities to enhance soil strength (Chang  
136 and Cho 2012) and reduce soil erosion (Chang et al. 2015a).

137 Casein is used to refer a family of phosphoproteins typically found in mammalian milk.  
138 It is generally found as a suspension of casein micelles which are held together by calcium  
139 ions (Schmidt 1982; Holt 1992). Casein has a low solubility in neutral water (pH = 7). Due to

140 the hydrophobic bonds of the nonpolar side chains of amino acids in its protein structures,  
141 casein molecules have limited interactions with water molecules (Nemethy and Scheraga  
142 1962). Therefore, the usage of casein in soils can improve water resistance of soils (Chang et  
143 al. 2018; Fatehi et al. 2018) as well as soil strength (Ni et al. 2022).

#### 144 2.4. Experimental program

145 The experimental program consisted of Test series I and II which were conducted on the  
146 biopolymer-treated vegetated soils (Table 2). Test series I aimed to testify the feasibility of  
147 the method and assess the performances of various biopolymers in vegetated soils. The  
148 blending content, precipitation, and number of seeds in Test series I were the same as adopted  
149 by Ni et al. (2020a). Based on the results from Test series I, the most suitable biopolymer was  
150 selected for Test series II, which was designed to probe the effect of blending content on  
151 vegetated soils under the impact of precipitation.

152 For each test series, the behaviors of the biopolymer-treated vegetated soils were  
153 explored from the following three aspects: (1) pore fluid, i.e., volumetric water content and  
154 electrical conductivity; (2) plant growth, i.e., seed germination ratio, sprout height, and root  
155 content; and (3) mechanical properties in direct shear tests.

##### 156 2.4.1. Vegetation cultivation

157 Referring to the Standard for Geotechnical Test Method (GB/T 50123-2019 China), the  
158 procedures for sample preparation are described as follows. The clayey soil was oven dried at  
159 temperature  $105 \pm 5$  °C for 24 h, crushed down gently on a rubber plate with wood hammer,  
160 and sieved through a 2 mm sieve to become base soil. The base soil was thoroughly mixed  
161 with the biopolymer powder with certain blending content (defined as the mass of biopolymer  
162 powder with respect to the mass of base soil), before adding tap water. The optimum moisture  
163 content was employed as the initial water content for sample preparation according to Ng et al.  
164 (2014) and Ng et al. (2016b). In addition, as indicated by Ni et al. (2020b), biopolymer-  
165 induced soil strengthening effect hardly occurred for initial soil water contents smaller than



166 the optimum moisture content, probably due to the poor workability of highly viscous  
167 hydrogels (Chang et al. 2015c). To increase the solubility, the water was heated to 85 °C for  
168 dissolving agar gum. A metal trowel was used for mixing manually for about five minutes  
169 until a homogeneous biopolymer-soil mixture was obtained. The mixture was then placed into  
170 a plastic tray (length × width × height = 205 mm × 150 mm × 80 mm), covered with a piece  
171 of filter paper and statically compacted to a degree of compaction of 80% (defined as the ratio  
172 of compacted soil dry density to the maximum dry density) with a wood panel. After  
173 compaction, the filter paper and wood panel were removed, and 160 oat seeds were evenly  
174 sowed on the soil surface. A thin layer of the biopolymer-soil mixture (1 cm) was placed on  
175 top to cover the oat seeds. The volumetric moisture content (defined as the volume percentage  
176 of water in the soil per unit volume) of the soil was 25.5% after seeding.

177 Higher degrees of compaction (> 90%) were not adopted because of the following two  
178 reasons. On one hand, higher degrees of compaction might inhibit vegetation growth (Ng et al.  
179 2014), due to the increased mechanical resistance of root penetration (Bengough and Mullins  
180 1990; Lipiec and Hakansson 2000), as well as the reduced oxygen diffusion rate in soils  
181 (Granovsky and McCoy 1997) caused by the reduction of average soil pore size (Romero et al.  
182 1999). On the other hand, higher degrees of compaction tend to increase the water-holding  
183 capacity of soils (Romero et al. 1999; Ng et al. 2014; Ng and Peprah-Manu 2023), and then  
184 the effect of biopolymers on helping in retaining moistures would be less important, whilst  
185 the increased mechanical strength would also minimize the contribution from biopolymers.  
186 Biopolymers should therefore be more beneficial at lower degrees of compaction, when  
187 vegetation growth is not impeded so much.

188 The trays of the biopolymer-treated soils embedded with oat seeds were placed at a  
189 controlled environment (i.e., temperature of 20 °C and relative humidity of 75%) for a  
190 cultivation period of 14 d. Precipitation simulation was performed by providing designated  
191 amount of tap water on the 1st, 2nd, 3rd, 8th, 9th, and 10th days. It should be noted that only  
192 tap water without any additional nutrient was supplied during the vegetation growth. Multiple

193 holes, each with a diameter of 5 mm, were made at the bottom of the plastic tray to allow free  
194 drainage and to collect any water percolated from the base during watering.

#### 195 *2.4.2. Measurement of volumetric water content and electrical conductivity*

196 The volumetric water content and electrical conductivity of the biopolymer-treated  
197 vegetated soils were recorded every 24 h using a multi-functional soil sensor produced by  
198 Jingxun Changtong Electronic Technology Co., Ltd (Weihai, China).

#### 199 *2.4.3. Measurement of seed germination ratio and sprout height*

200 The number of germinated seeds and the height of sprouts above the soil surface were  
201 recorded every 24 h.

#### 202 *2.4.4. Direct shear test*

203 At the end of vegetation cultivation, a cutting ring (diameter  $\times$  height = 61.8 mm  $\times$  20  
204 mm) and a square hoe were used for sampling. The detailed sampling process is described as  
205 follows with a schematic diagram (see Fig. 1). Firstly, the upper seedlings above the surface  
206 (Fig. 1a) were cut off with scissors, after which a cutting ring was pushed into the soil where  
207 the sprouts were relatively uniformly distributed (Fig. 1b). The soil near the edge of the tray  
208 was excavated out so that the square hoe could be inserted below the cutting ring following  
209 the direction of arrows as shown in Fig. 1b. Then the sample was taken out (Fig. 1c). The  
210 excessive soil above and below the cutting ring was removed and then both surfaces of the  
211 sample were levelled off. It should be noted that all the samples were taken 1.5 cm below the  
212 soil surface, as shown in Fig. 1c, in order to ensure that there were no oat seeds in the cutting  
213 ring. In addition, the roots were pruned to extent 0.5 cm out of the cutting ring so as to assure  
214 the anchoring effect of the root system (Zhou et al. 2016).

215 The reinforcing effect of the combination of oat roots and biopolymer on the shear  
216 performances of the clayey soil was determined using direct shear tests following the

217 Standard for Geotechnical Test Method (GB/T 50123-2019 China). The shear stress was  
218 applied on the saturated sample at a rate of 0.8 mm/min under undrained condition, during  
219 which the overburden stress (i.e., 50, 100, and 150 kPa) was applied. For herbaceous species,  
220 the majority of roots are found in near surface soils (Jackson et al. 1996). When nutrients  
221 and/or water are limiting, roots tend to grow deeper (Jackson et al. 1996), e.g., up to several  
222 meters (Stokes et al. 2009). Therefore, the overburden stress up to 150 kPa which was also  
223 adopted by (Gonzalez-Ollauri and Mickovski 2017) was used here as it could cover the root  
224 depth of herbaceous species at extreme conditions. The root architecture and density near the  
225 soil surface will likely be very different from those at depth, as roots have a heterogeneous  
226 spatial distribution in soils (Jackson et al. 1996; Reubens et al. 2007; Stokes et al 2009).  
227 However, this effect of spatial root distribution on the shear resistance of the vegetated soil is  
228 not considered within the scope of this study. After the direct shear test, the root-containing  
229 damaged samples were dried in the oven at 80 °C for 48 h (Cornelissen et al. 2003). Then the  
230 dry mass of roots and dry mass of soil were weighed separately. The ratio of the dry mass of  
231 roots to the dry mass of soil in the specimen was determined as the corresponding root  
232 content.

### 233 3. Results and analysis

#### 234 3.1. Test series I

##### 235 3.1.1. Plant growth

236 The actual features of oat growth in biopolymer-treated soils examined every two days  
237 are shown in Fig. 2a. The germination of oat seeds was first found in the soil treated with  
238 xanthan gum followed by guar gum. It is seen in Fig. 2b that at the end of cultivation, xanthan  
239 gum was observed the most efficient biopolymer in promoting vegetation growth in terms of  
240 increasing the seed germination ratio (defined by the ratio of the number of germinated seeds  
241 to total number of seeds) by 300%, and raising the average sprout height by 31%, compared  
242 with the untreated soil. Guar gum, casein, agar gum, and beta-glucan were in a descending

243 order by effectiveness in stimulating seed germination and sprout growth. Even the least  
244 efficient biopolymer (beta-glucan) increased the seed germination ratio and average sprout  
245 height by 101% and 15%, respectively. Therefore, all the biopolymers were proved able to  
246 exert a favorable influence on the soil environment for plant growth to certain extent.

### 247 3.1.2. Water content and electrical conductivity

248 Although biopolymers may induce large pore spaces into soils (Chang et al. 2015a), and  
249 thus lead to more paths for moisture transport, they help to delay the moisture evaporation  
250 from soils, mainly attributed to the formation of hydrogen bonds between functional groups  
251 of biopolymers and water molecules (Narjary et al. 2012).

252 Normally, precipitation, soil evaporation, and vegetation transpiration are the primary  
253 factors affecting the moisture level in soils. The volumetric water content  $w$  at any given time  
254 can be expressed by:

$$255 \quad (1) \quad w = w_0 + \Delta w_p - \Delta w_e - \Delta w_t$$

256 where  $w_0$  is the initial volumetric water content;  $\Delta w_p$ ,  $\Delta w_e$ , and  $\Delta w_t$  are the changes in  
257 volumetric water content due to precipitation, soil evaporation, and vegetation transpiration  
258 respectively. Since vegetation transpiration releases about 95% of the water absorbed by the  
259 roots (Nobel 2009; Mcelrone et al. 2013), the soil moisture absorbed by oat roots could be  
260 represented by  $\Delta w_t$  in eq. 1. Since  $w_0$  and  $\Delta w_p$  were the same for all the treatment conditions  
261 in Test series I,  $w$  was then only influenced by  $\Delta w_e$  and  $\Delta w_t$ .  $\Delta w_e$  is affected by the water-  
262 holding capacity of the biopolymer used for soil treatment (Chang et al 2015a; Muguda et al.  
263 2017).  $\Delta w_t$  is largely affected by the leaf area index (LAI) of vegetation (Jarvis and  
264 Mcnaughton 1986). When LAI is high, the total number of stomata on the leaves is also high,  
265 leading to a significant increase in water absorption from soils. In this sense, higher seed  
266 germination ratio and average sprout height can lead to higher  $\Delta w_t$ . It should be noted that  
267 there was hardly any water drained out from the drainage holes and therefore the impact of  
268 percolation on volumetric water content was not included in eq. 1. This might be partly  
269 attributed to the relatively low amounts of precipitation adopted in the current study. In

270 addition, upon precipitation, hydrophilic biopolymers help to adsorb the sudden water and  
271 swell, which leads to filling of soil pores and reduction in hydraulic conductivity via bio-  
272 clogging (Chang et al. 2015a).

273 The variations of volumetric water content during cultivation are presented in Fig. 3a.  
274 The vegetated soils treated with casein and agar gum respectively had the highest and the  
275 second highest values of  $w$ . This is because both casein and agar gum were less effective in  
276 promoting seed germination and sprout growth compared with xanthan gum or guar gum as  
277 shown in Fig. 2, which resulted in medium vegetation transpiration. In addition, although  
278 casein and agar gum have poor water solubility, they have strong water-absorbing and water-  
279 holding capacity, which helped to reduce soil evaporation. The vegetated soils treated with  
280 xanthan gum and guar gum respectively had medium values of  $w$  (Fig. 3a) due to an increased  
281 vegetation transpiration caused by highly promoted germination of oat seeds and sprout  
282 growth (Fig. 2). The untreated vegetated soil, although experienced the lowest level of plant  
283 transpiration corresponding to the worst plant growth (Fig. 2), had the smallest value of  $w$   
284 (Fig. 3a), indicating the highest level of soil evaporation due to lowest water-holding ability.

285 In the past, the electrical conductivity of biopolymer-treated soils has been rarely  
286 investigated, although the information could be potentially useful for soil scientists,  
287 geotechnical and environmental engineers. Figure 3b shows the variations of electrical  
288 conductivity of the vegetated soils during vegetation growth. The electrical conductivity is  
289 affected by a variety of soil properties, including the nature of solid grains, arrangement of  
290 voids, degree of water saturation, and electrical conductivity of the pore fluid (Samouëlian et  
291 al. 2005). Pore fluids containing different biopolymers could yield different electrical  
292 conductivities. In addition, the physicochemical interaction between functional groups of  
293 biopolymer molecules and charged clay particles could lead to different patterns of soil  
294 particle association (i.e., aggregation and/or flocculation), and in turn different arrangements  
295 of voids. Therefore, the values of initial electrical conductivity were not identical for the  
296 vegetated soils treated with various biopolymers (Fig. 3b). By comparison of Figs. 3a and 3b,  
297 it is seen that the electrical conductivity was positively correlated with the volumetric water

298 content, implying its strong dependence on degree of saturation. Generally, the electrical  
299 conductivity of tap water is between 125 to 1250  $\mu\text{s}/\text{cm}$ . The tap water used in the current  
300 study has an electrical conductivity of 483  $\mu\text{s}/\text{cm}$ , which is much higher than the electrical  
301 conductivity of air (i.e., air medium is an insulator). Thus, increasing the water-filled porosity  
302 raised the soil electrical conductivity and vice versa.

### 303 3.1.3. Direct shear test

304 [Figure 4](#) presents the relationships of shear stress-shear displacement of the vegetated  
305 soils with or without biopolymer treatment at overburden stresses of 50, 100, and 150 kPa.  
306 The enhanced shear behaviors due to the presence of biopolymers were noticeable. The  
307 vegetated soil with the addition of casein or agar gum had a greatly elevated shear strength  
308 (defined as the shear stress at 9 mm displacement) and exhibited a larger stiffness at the initial  
309 loading stage (i.e., < 3 mm displacement) compared with the control group. Xanthan gum and  
310 guar gum also effectively improved the shear strength and stiffness of the vegetated soil,  
311 albeit not as remarkable as casein or agar. Beta-glucan slightly increased the resistance of the  
312 vegetated soil to shear loading.

313 In the current study, the root-containing samples were sheared under the saturated  
314 condition and therefore soil suction as a component of soil strength was not considered. The  
315 question that then comes to mind is whether it could be possible that the untreated vegetated  
316 soil had a higher strength than the biopolymer-treated one if soil suction was included,  
317 because the untreated vegetated soil appeared to have the lowest water content ([Fig. 3a](#)) and  
318 therefore could have the greatest contribution from soil suction to mechanical performance.  
319 We think it could be largely unlikely because (1) there are evidences ([Zha 2014](#); [Muguda et al.](#)  
320 [2017](#); [Cao et al. 2018](#); [Tran et al. 2019](#)) indicating that biopolymer-treated soils exhibit higher  
321 suctions than untreated soils, due to the viscosity of biopolymer hydrogels and adhesive force  
322 between biopolymer hydrogels and solid surfaces; (2) under the unsaturated condition with  
323 lower water contents, the strengthening effect of biopolymers would be more remarkable due  
324 to the dehydration-induced strength gain and rigidity enhancement of biopolymer hydrogels,

325 and closer interaction between biopolymer molecules and soil particles (Chang and Cho 2012;  
326 Latifi et al. 2016). To sum up, the reinforcing effect of biopolymers was underestimated to  
327 some extent in the current study due to the adopted testing method.

#### 328 3.1.4. Evaluation of different biopolymers

329 As discussed above, all the selected biopolymers positively contributed to the soil  
330 reinforcement with plants. After comparing xanthan gum, casein, and agar gum that had  
331 better performances either in facilitating vegetation growth or increasing overall soil strength,  
332 it was determined to adopt xanthan gum for further study based on the following reasons, e.g.,  
333 reinforcing effect, implementation, and cost. (1) The strength of the vegetated soil treated  
334 with casein or agar gum was less dependent on the plant growth compared with the vegetated  
335 soil treated with xanthan gum (Figs. 2 and 4). Biopolymers are biodegradable from a long-  
336 term aspect, and hence the vegetated soil with a less developed root system could be more  
337 adversely affected when the direct mechanical reinforcement contribution from biopolymers  
338 wanes with time. By contrast, xanthan gum was the most effective polysaccharide in assisting  
339 plant growth (Fig. 2) and had also satisfactory performances in strengthening the vegetated  
340 soil (Fig. 4), and therefore the usage of xanthan gum is considered superior for improving the  
341 engineering behaviors of the vegetated soil. (2) Agar gum is a thermo-gelation biopolymer  
342 and hence hot water is needed for the preparation of agar gum-soil mixtures, which brings  
343 certain difficulties for implementation practices in field. Casein, on the other hand, has a low  
344 solubility in neutral water and exhibits cheesy state when mixed with water (Fatchi et al. 2018;  
345 Ni et al. 2022), which might lead to poor workability with soil. By comparison, xanthan gum  
346 has excellent solubility in cold water and other desirable functions like pH stability, storage  
347 stability, and ionic salt compatibility (Barrère et al 1986; Rosalam and England 2006). (3)  
348 Xanthan gum has been produced and used in large quantities with a relatively low price. Over  
349 the last three decades, the cost of soil treatment with 0.5% xanthan gum (i.e., 5 kg of xanthan  
350 gum per ton of soil) has decreased from approximately 70 USD to 10 USD (Mendonça et al.  
351 2021).

## 352 3.2. Test series II

### 353 3.2.1. *Plant growth, water content and electrical conductivity*

354 The results of vegetation cultivation performed on the xanthan gum-treated soils under  
355 the impacts of blending content and precipitation are presented in Fig. 5. The earliest oat seed  
356 germination was observed on the second day of cultivation. Within the first five days, the  
357 majority of oat seeds germinated except for the highest blending content (i.e., 1.00%) with  
358 low to medium precipitations (i.e., 25 and 50 mL). Due to the variation in both blending  
359 contents and precipitations, seed germination ratios changed broadly from 35% to 85% at the  
360 end of cultivation. Medium precipitations (i.e., 50 and 75 mL) allowed more seeds to  
361 germinate (Fig. 6a), while excessive water (i.e., 100 mL) lowered the seed germination ratio  
362 probably due to oxygen deficiency caused by the decreased air-filled porosity. On the other  
363 hand, low to medium blending contents (i.e., 0.25% and 0.50%) were more effective in  
364 stimulating seed germination (Fig. 6b). Medium to high blending contents (i.e., 0.75% and  
365 1.00%) were prone to form more viscous hydrogels that might increase the root penetration  
366 resistance. The high viscosity of the biopolymer hydrogels with 1.00% blending content may  
367 also be responsible for the delayed seed germination as shown in Fig. 5.

368 During the vegetation cultivation, the volumetric water content was M-shaped with two  
369 peaks (Fig. 7). For precipitations larger than 50 mL, the second peak value was higher than  
370 the first one, implying that precipitation exceeded the moisture loss due to soil evaporation  
371 and plant transpiration. For the minimum precipitation (i.e., 25 mL), the second peak value  
372 was lower than the first one. While increasing precipitations remarkably increased volumetric  
373 water contents (Fig. 8a), raising blending contents had a relatively small effect (Fig. 8b).  
374 Variations of electrical conductivity during vegetation cultivation is shown in Fig. 9. The  
375 impacts of precipitation and blending content on the electrical conductivity are shown in Figs.  
376 10a and 10b, respectively. The electrical conductivity showed a positive correlation with both  
377 influencing factors.



378 The volumetric water content was affected by precipitation, soil evaporation and plant  
379 transpiration. For a fixed xanthan gum blending content (Fig. 11), the moisture loss due to  
380 soil evaporation  $\Delta w_e$  could be deemed identical. Then the volumetric water content was  
381 mainly influenced by precipitation and vegetation transpiration. Taking Fig. 11a for an  
382 example, the volumetric water content had a small increment as the precipitation increased  
383 from 25 to 50 mL. This was a resultant value from a positive  $\Delta w_p$  in eq. 1 due to the increased  
384 precipitation and a positive  $\Delta w_t$  in eq. 1 due to the promoted plant growth. When precipitation  
385 increased from 50 to 100 mL, the volumetric water content had a more remarkable increment  
386 as the sign of  $\Delta w_t$  changed from positive to negative in eq. 1. Alternatively, for a fixed  
387 precipitation (Fig. 12), the moisture increment due to precipitation  $\Delta w_p$  was identical. Then  
388 the volumetric water content was mainly influenced by soil evaporation and vegetation  
389 transpiration. Taking Fig. 12b for example, the volumetric water content had a small  
390 decrement as the blending content increased from 0.25% to 0.50%. This is a resultant value  
391 from a negative  $\Delta w_e$  in eq. 1 due to the decreased soil evaporation and a positive  $\Delta w_t$  in eq. 1  
392 due to the promoted plant growth. When the blending content increased from 0.50% to 1.00%,  
393 the volumetric water content had a small increment as the sign of  $\Delta w_t$  changed from positive  
394 to negative in eq. 1.

### 395 3.2.2. Direct shear test

396 The effect of precipitation on the relationship between shear stress and shear  
397 displacement for different xanthan gum blending contents are presented in Fig. 13. It was  
398 observed that the precipitation corresponding to the highest curves at 50, 100, and 150 kPa  
399 overburden stresses increased as the blending content increased. For example, 50 mL  
400 precipitation led to the best improved shear resistance for the soils treated with low to  
401 medium blending contents (e.g., 0.25% and 0.50%, see Figs. 13a and 13b) and 75 ml  
402 precipitation for those treated with medium to high blending contents (e.g., 0.75% and 1.00%,  
403 see Figs. 13c and 13d), respectively. The dependence of shear performance on precipitation  
404 was in accordance with that of seed germination ratio on precipitation (Fig. 6a). For low to

405 medium blending contents (i.e., 0.25%, 0.50%, and 0.75%), the stiffness of the xanthan gum-  
406 treated vegetated soils decreased gradually with the increasing shear displacement. While for  
407 1.00% blending content, there was a more apparent change in the stiffness around 1 to 2 mm  
408 shear displacement, which might be due to the least satisfactory vegetation growth (Fig. 6b).  
409 The effect of blending content on the relationship between shear stress and displacement for  
410 different precipitations are shown in Fig. 14. The blending content leading to the highest  
411 strengths at 50, 100, and 150 kPa overburden stresses was observed to be a fixed value (i.e.,  
412 0.50%) regardless of the precipitation. Similarly, this result can be linked to the trend of seed  
413 germination ratio, i.e., 0.50% was the optimum dosage to facilitate plant growth (Fig. 6b).

414 The variations of internal friction angle and cohesion against root content (defined as the  
415 root mass with regard to the soil mass within the cutting ring) for different blending contents  
416 are provided in Figs. 15a and 15b, respectively. Fine oat roots actively increased the internal  
417 friction angle of the soil (Fig. 15a). When root content increased from 0.10% to 0.50%, the  
418 internal friction angle increased from 20° to 35°. By observing Fig. 15b, it was discovered that  
419 for each blending content, the dependence of cohesion on root content was not obvious.  
420 However, by comparing different soil groups, it is not difficult to find that the soil group  
421 containing more oat roots had higher soil cohesions, i.e., 0.50% xanthan gum-treated soil  
422 group had the highest cohesion followed by 0.25% XG-treated soil group, and the other two  
423 soil groups.

## 424 4. Discussion

### 425 4.1. The role of biopolymers in the vegetated soil

426 A schematic diagram is provided for elucidating the biopolymer-treated vegetated soil  
427 system in which many aspects are transient, e.g., plants grow and biopolymers degrade (Fig.  
428 16). At the initial stage after planting, there is primarily a biopolymer/soil composite.  
429 Biopolymer plays a dual role in the soil (i.e., promoting vegetation growth and improving soil  
430 strength). Once seeds germinate and roots grow, there exists a biopolymer/root/soil composite,

431 or more precisely a biopolymer/mucilage/root/soil composite. Mucilage is a range of organic  
432 materials secreted by plants and associated microorganisms (Naveed et al. 2017; Naveed et al.  
433 2019). Both mucilage and biopolymer have been shown to facilitate the adhesion of soil  
434 grains to plant roots and enhance the interparticle bonding of the soil grains in the rhizosphere,  
435 forming a root-associated soil region (Gregory 2006; Hinsinger et al. 2009; Chen et al. 2019b).  
436 Due to the presence of biopolymer in the periphery of the rhizosphere, the diameter of soil  
437 region associated with roots could be further enlarged. Under shearing, the movement of a  
438 root relative to the soil will therefore take directly or indirectly adhered grains with it,  
439 increasing the shear plane area between soil and roots and consequently raising the shear  
440 resistance of the biopolymer/mucilage/root/soil composite. Owing to the degradable nature of  
441 biopolymer, the biopolymer/mucilage/root/soil composite will be ultimately replaced with the  
442 mucilage/root/soil composite.

#### 443 4.2. Effect of biopolymer type

444 Polysaccharides and protein-based biopolymers have great water-holding abilities. It is  
445 seen from Fig. 3a that although biopolymers promoted vegetation growth, which in turn  
446 accelerated the water transportation from soil to plants through transpiration, they helped to  
447 increase the water use efficiency and had an overall beneficial effect on improving water  
448 availability to plants.

449 In addition, various biopolymers contain a variety of nutrients which are essential for  
450 plant growth. For example, polysaccharides chemically consist of repeated sugar units. These  
451 chemical components are not directly available to plants; polysaccharide degradation is  
452 normally required first to permit the uptake and usage of these components by plants.  
453 Products of enzymatic xanthan gum degradation caused by xanthan gum-degrading  
454 microorganisms include glucose, glucuronic acid, mannose, pyruvated mannose, acetylated  
455 mannose, and unidentified oligo- and polysaccharides (Hou et al. 1986), most of which can be  
456 utilized for plant photosynthesis and respiration (Meléndez-Hevia et al. 1996; Oexle et al.  
457 1999; Berg et al. 2002; Valpuesta and Botella 2004; Buchanan et al. 2009; Ceron-Garcia et al.

458 2011). Other polysaccharides (e.g., guar gum, agar gum, and beta-glucan) contain chemical  
459 species that are needed for plant metabolism as well; however, the diversity of nutrient  
460 components of these biopolymers is not comparable with xanthan gum (Table 1). This might  
461 be the reason that xanthan gum was observed the most efficient polysaccharide in promoting  
462 seed germination and sprout growth. A different result was obtained by (Chang et al. 2015a),  
463 saying that beta-glucan had a better performance than xanthan gum in promoting plant growth.

464 Casein is a protein-based biopolymer comprising phosphoproteins. Both nitrogen and  
465 phosphorus are the most frequently limiting macronutrients for the growth of terrestrial plants  
466 (Schachtman et al. 1998; Capek et al. 2018). In available forms (e.g., mediated by soil  
467 microbes) for plant uptake, nitrogen can be used to produce chlorophyll which is essential for  
468 plant photosynthesis, while phosphorus can raise biomass production (Moeneclaey et al.  
469 2022). Due to the strong interactions of nitrogen and phosphorus in biogeochemical processes,  
470 increasing the availability of either nitrogen or phosphorus promotes the uptake of the other  
471 one by plants (Xia et al. 2023). Therefore, casein also helps to stimulate plant growth by  
472 supplying nutrient elements. However, the contribution of casein might not be as great as  
473 xanthan gum or guar gum, as sugars are the most vital biomolecules and play the most  
474 important role in metabolism of plants (Ng 2017; Ahmad 2019).

#### 475 4.3. Effect of biopolymer blending content

476 Xanthan gum hydrogels can either lubricate the interface of roots and soil particles or  
477 increase mechanical impedance of plant roots depending on the blending content. Xanthan  
478 gum was used by Chen et al. (2019b) to mimic plant mucilage which provides root tip  
479 lubrication and helps to mobilize beneficial chemical components for plant uptake (Jones et al.  
480 2009). For low to medium blending contents (i.e., 0.25% and 0.50%), xanthan gum was  
481 observed effective in stimulating seed germination under varying water supplies (Fig. 6b),  
482 suggesting that the blending content up to 0.50% might not be restrictive for root penetration.  
483 By contrast, medium to high blending contents (i.e., 0.75% and 1.00%) adversely impacted  
484 seed germination ratios (Fig. 6b); the highest blending content of 1.00% combined with the

485 minimum precipitation of 25 mL retarded seed germination (Fig. 5). This is mainly because  
486 that the highly viscous hydrogels (especially upon drying) could result in the formation of a  
487 rigid soil crust at surface. It is deduced that there existed a relationship between vegetation  
488 growth and shear resistance of the vegetated soil. The inclusion of xanthan gum with 0.50%  
489 blending content which maximized the biomass production, was also corresponding to the  
490 highest soil shear strength (Fig. 14) due to both increased internal friction angle and cohesion  
491 (Fig. 15).

492 Therefore, higher blending contents do not necessarily lead to a higher reinforcing  
493 efficiency when vegetation is involved. As illustrated in Fig. 16, when biopolymers were  
494 initially mixed with soils, they dominated the role of soil reinforcement to a large extent,  
495 thereby 1.00% blending content was expected to create the maximum soil reinforcing effect  
496 owing to the increased soil cohesion and bonded soil particles. However, upon dehydration,  
497 the soil treated with 1.00% biopolymer might become excessively stiff which was restrictive  
498 for root elongation, resulting in a less protected soil from the long-term perspective. By  
499 comparison, an appropriate blending content (i.e., 0.25% and 0.50%) will provide a moderate  
500 reinforcing effect while ensuring a long-term plant-reinforced soil system.

501 In a biopolymer/mucilage/root/soil composite, both biopolymer and mucilage can offer  
502 beneficial effect on soil stabilization. The mucilage secreted by plants and associated  
503 microbes, although is biodegradable in nature, is produced continuously and there exists a  
504 consistent level of total mucilage estimated 0.005% to 5% with regard to the dry soil mass  
505 (Zickenrott et al. 2016). Therefore, the higher mucilage contents associated with the better  
506 vegetated soils (i.e., treated with 0.25% and 0.50% blending contents) should be taken into  
507 account when the effect of blending content of xanthan gum on shear strength indices is  
508 analyzed (Fig. 15).

#### 509 4.4. Effect of precipitation

510 Precipitation had a greater effect on the soil water content than the biopolymer blending  
511 content, by comparing Figs. 8a and 8b. Precipitation of 25 mL led to the least amount of

512 available water to the growing plants (Fig. 8a) and the worst vegetation growth (Fig. 6a),  
513 which could be attributed to the water stress under which plant metabolism was restricted.  
514 The highest precipitation of 100 mL resulted in the highest volumetric water content (Fig. 8a),  
515 but not the best plant growth (Fig. 6a). This might be attributed to the fact that upon  
516 precipitation, biopolymer hydrogels absorbed water, swelled, and clogged the pore spaces,  
517 which decreased the air-filled porosity and in turn led to an unbalanced air-water circulation.  
518 Precipitations of 50 and 75 mL allowed the highest seed germination ratios to occur for low to  
519 medium blending contents (i.e., 0.25% and 0.5%) and medium to high blending contents (i.e.,  
520 0.75% and 1.00%), respectively (Fig. 6a), and led to efficiently improved strength (Fig. 13).  
521 This suggests that moderate soil moisture contents (Fig. 8a) may satisfy the requirements of  
522 both water demand for plant metabolisms and soil aeration.

#### 523 4.5. Economic feasibility of biopolymer usage in vegetated soils

524 The economic feasibility of biopolymer usage in geotechnical engineering has been  
525 reviewed by Chang et al. (2016), Chang et al. (2020), and Mendonça et al. (2021). Over the  
526 last thirties years, the cost of xanthan gum has dropped approximately from 14,000 USD/ton  
527 to 2,500 USD/ton. The expanded applications of biopolymers in geotechnical engineering as  
528 well as in other fields (e.g., food, medicine, cosmetics, farmland irrigation, and construction)  
529 and the resultant mass product intend to further reduce the cost of biopolymers. In addition,  
530 the price of biopolymers can reasonably be expected to decrease when they are produced  
531 specifically for the purpose of geotechnical engineering, e.g., by reducing the high levels of  
532 food-grade purity of biopolymers as they are unnecessary for geotechnical applications.

### 533 5. Conclusion

534 The application of biopolymers in vegetated soils to promote vegetation growth and  
535 improve overall strength is evaluated in this paper. Both polysaccharide and protein-based  
536 biopolymers improved water availability to the growing plants. In addition, they contain  
537 sugars and elements like nitrogen and phosphorus which in available forms (e.g.,

538 monosaccharides and oligosaccharides) can be utilized by plants for photosynthesis and  
539 respiration. It has been revealed that polysaccharide xanthan gum with 0.5% blending content  
540 greatly facilitated plant growth and improved overall vegetated soil strength under a variety of  
541 water supplies. Higher blending contents, although provide a greater soil reinforcing effect at  
542 the initial stage, may increase the root penetration resistance and hinder the formation of root-  
543 soil composite from a long-term perspective. From the obtained outcomes, biopolymers are  
544 suggested to assist in the plant-reinforced soil system especially at the initial stage of  
545 vegetation growth when the plant roots do not actively reinforce the soil and are more  
546 vulnerable to water stress.

#### 547 **Acknowledgment**

548 The work described in this paper was supported by the National Natural Science  
549 Foundation of China (51608323, 51978533). In addition, this project has received funding  
550 from the European Union's Horizon 2020 Framework programme Marie Skłodowska-Curie  
551 Individual Fellowships under grant agreement No. 897701.

#### 552 **Data availability**

553 Data generated or analyzed during this study are available from the corresponding author  
554 upon reasonable request.

#### 555 **Competing interests**

556 The authors declare that there are no competing interests.

#### 557 **Reference**

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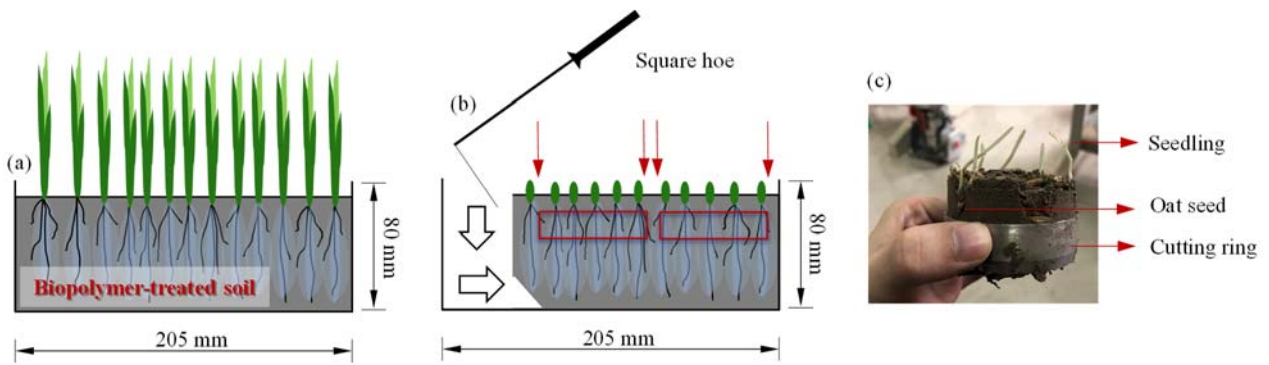
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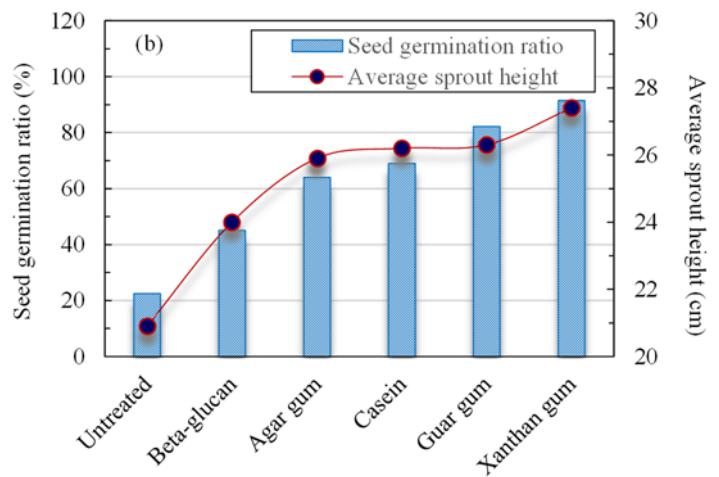
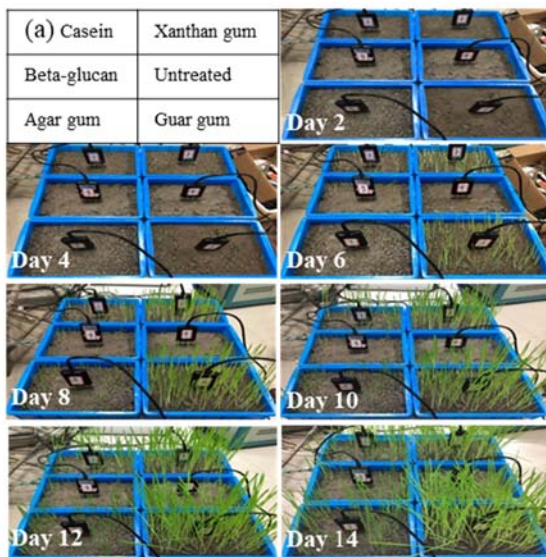
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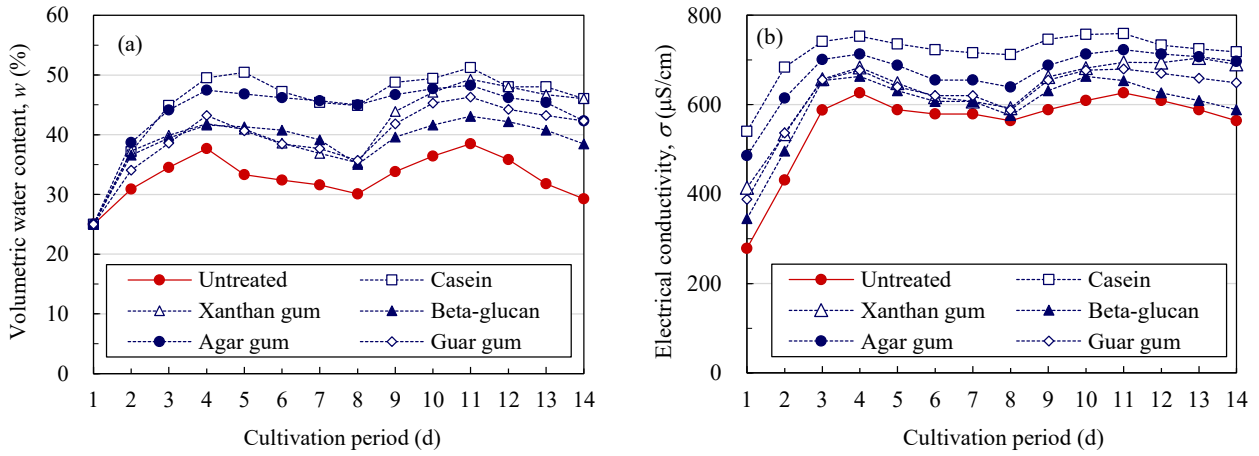




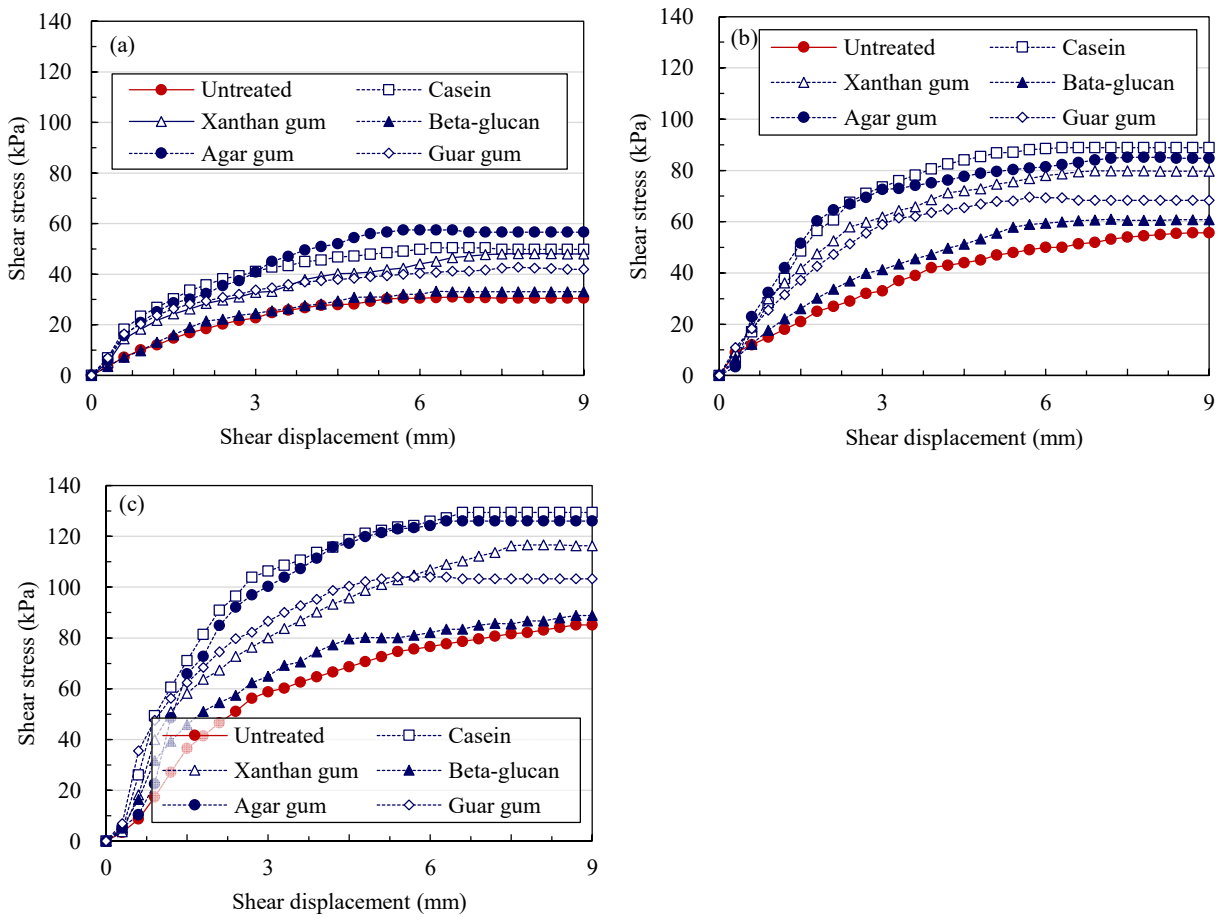
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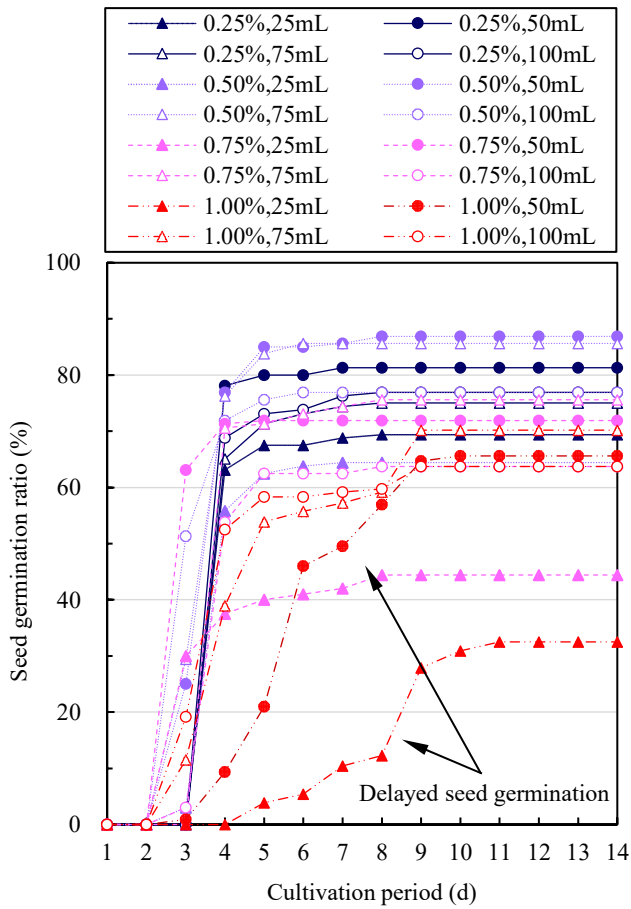


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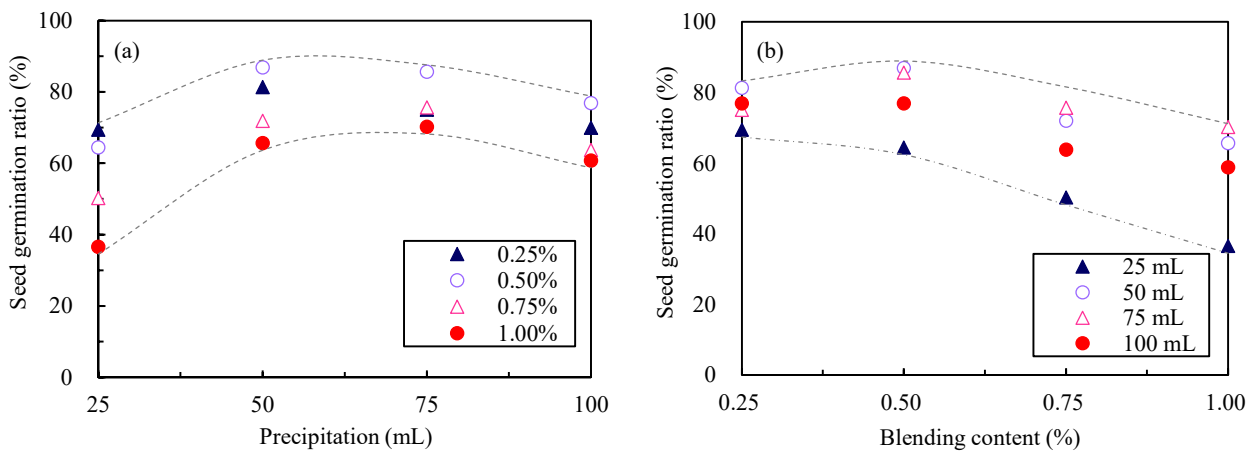
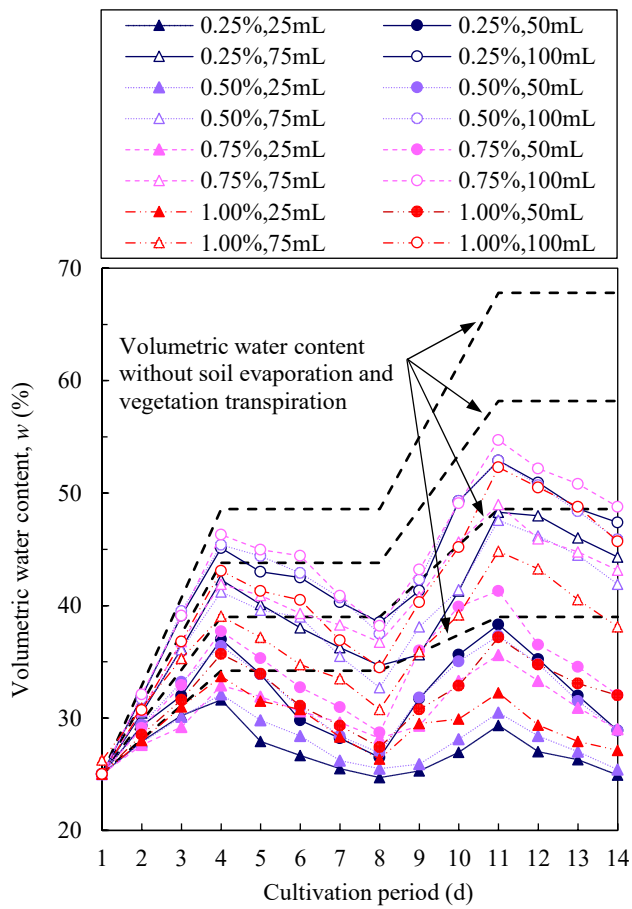
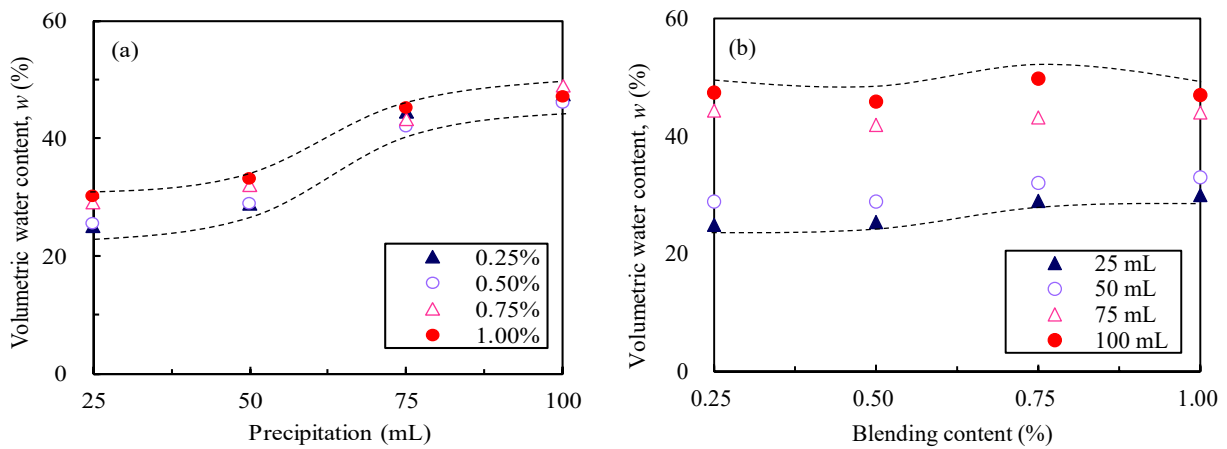


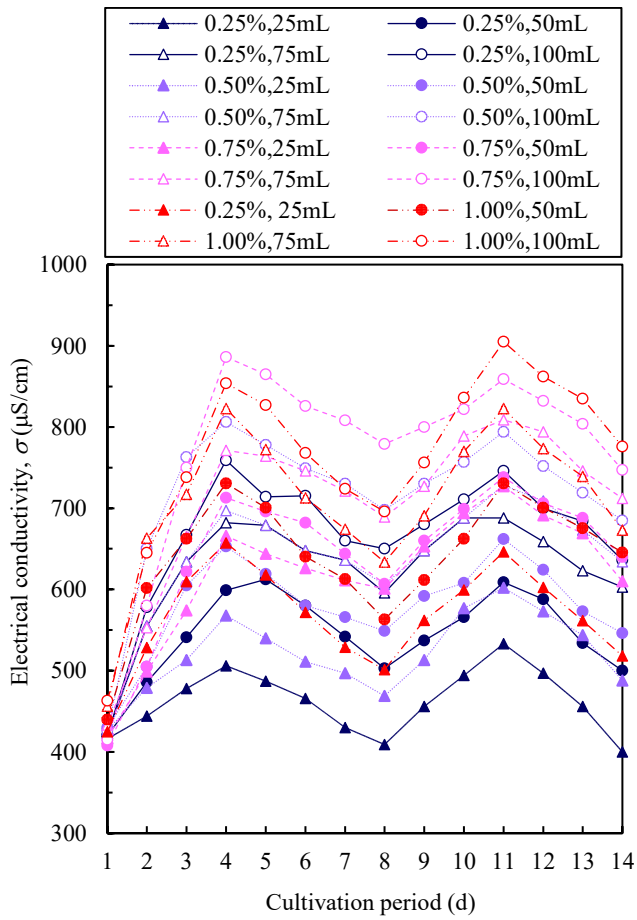
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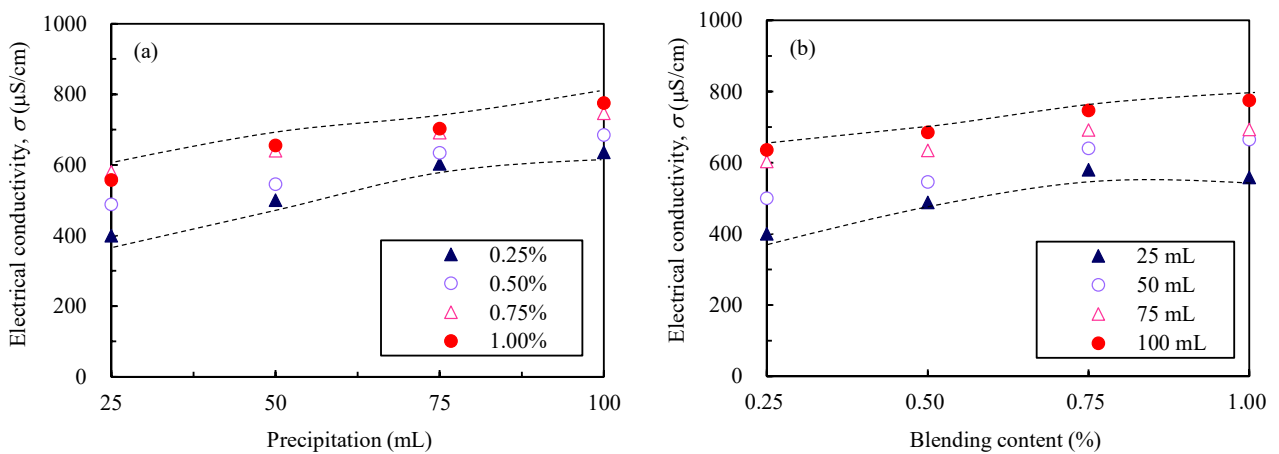
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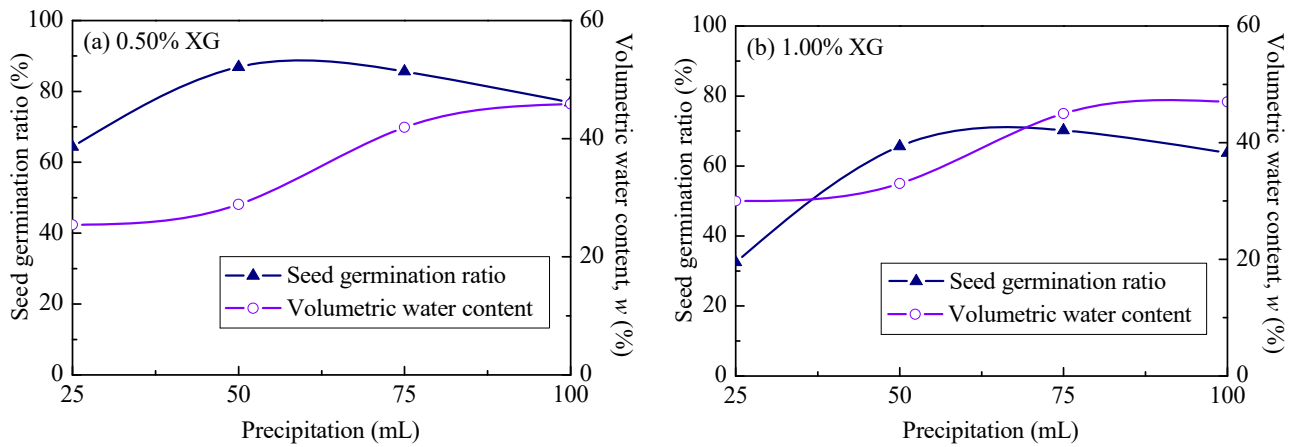
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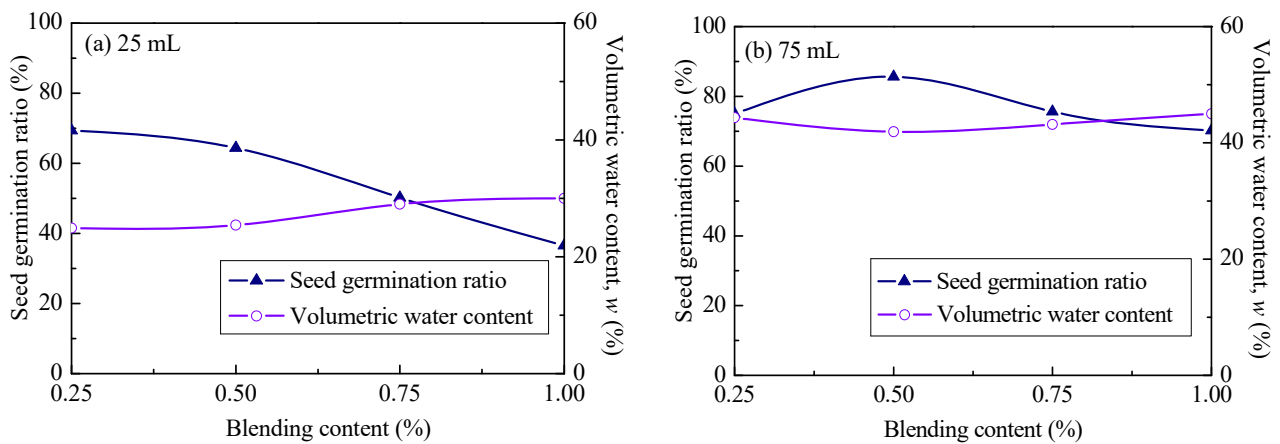
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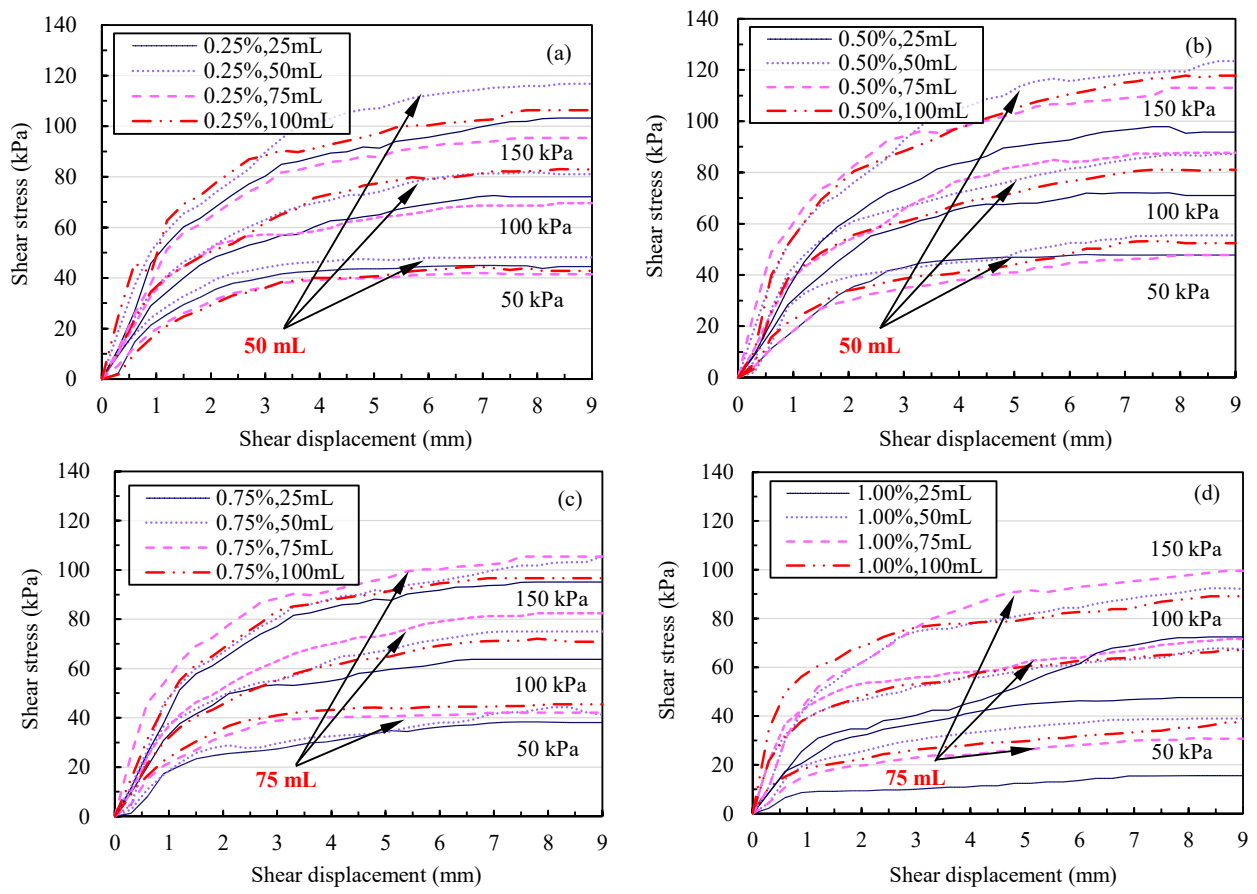
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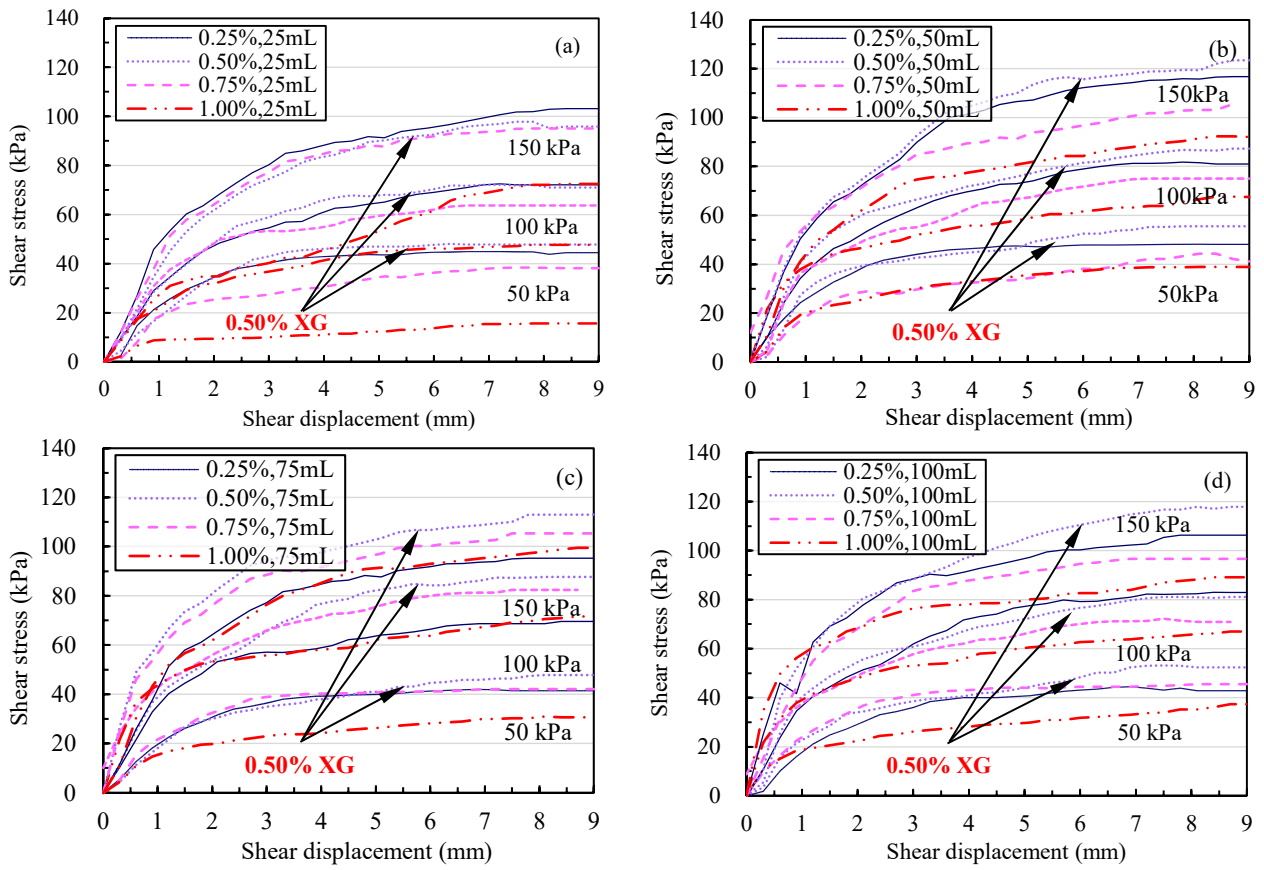
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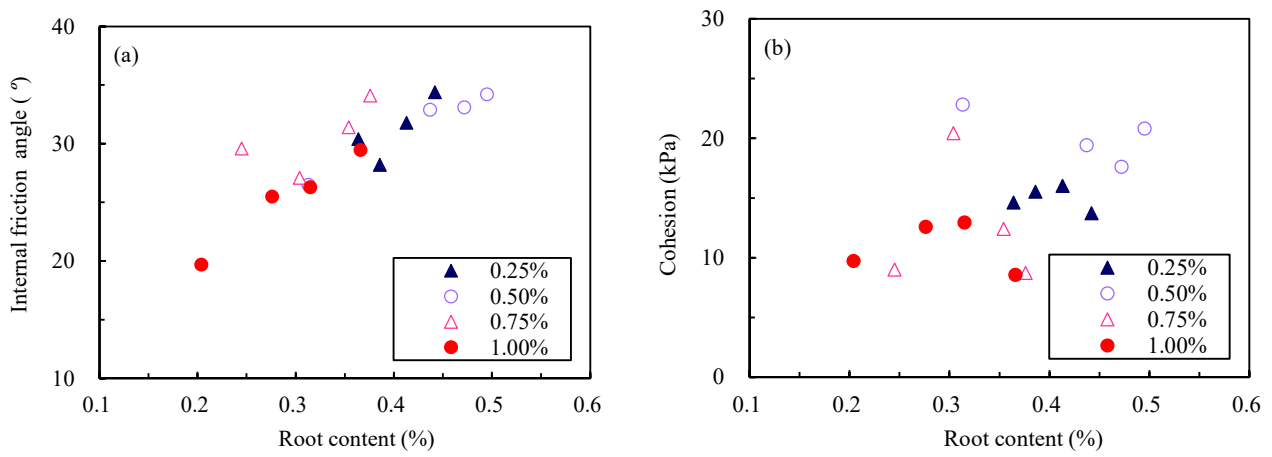


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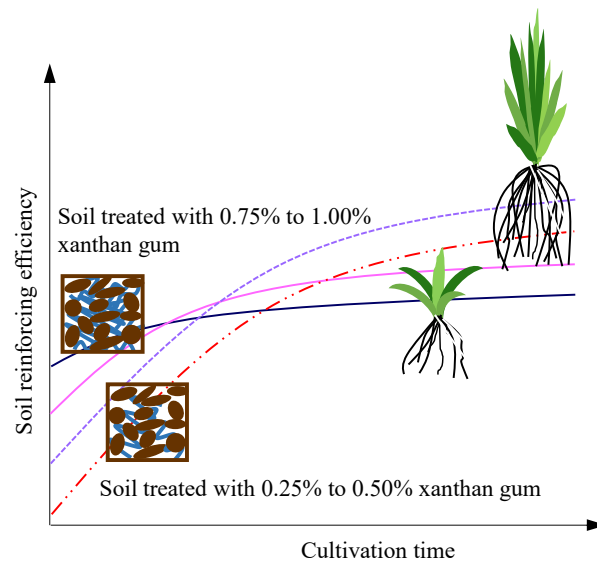


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**Table 2.** Experimental program for two test series.

**Table 1.** Properties of various biopolymers used in this study.

Biopolymers	Chemical components	Solubility (g/100 mL water)	Performances with soils
Xanthan gum	Glucose, mannose, and glucuronic acid	0.5~1.5	Strengthening (Latifi et al. 2016; Chen et al. 2019; Ni et al. 2020b) Erosion reduction (Chang et al. 2015a) Water resistance (Chen et al. 2020)
Guar gum	Mannose and galactose	0.5~1.5	Strengthening (Ayeldeen et al. 2016; Muguda et al. 2017)
Agar gum	Galactose	0.01~0.5	Strengthening (Khatami and O'Kelly 2013) Water resistance (Chang et al. 2015b)
Beta-glucan	Glucose	0.5~1.5	Strengthening (Chang and Cho 2012) Erosion reduction (Chang et al. 2015a)
Casein	Aggregates of micelle ( $\alpha$ -, $\beta$ -, and $\kappa$ -casein) and phosphate calcium	0.01~0.5	Strengthening (Chang et al. 2018; Fatehi et al. 2018; Ni et al. 2022) Water resistance (Chang et al. 2018; Fatehi et al. 2018)

**Table 2.** Experimental program for two test series.

Variables	Test series I	Test series II
Plant	Oat	Oat
Biopolymer type	Xanthan gum, guar gum, agar gum, beta-glucan, casein	Xanthan gum
Blending content, %	0.50	0.25, 0.50, 0.75, 1.00
Precipitation, mL	70	25, 50, 75, 100
Number of seeds	160	160