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**A laboratory study to disentangle hydrological, mechanical and structural mechanisms of soil stabilisation by plant mucilage between eroding and depositional zones of a slope**

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**Running title:** Soil stabilisation by mucilage and slope position

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## Summary

Biological exudates, such as plant mucilage, can greatly stabilise soils but as the mechanical and hydrological drivers depend much on soil particle size composition, eroding and depositional areas of a slope may respond differently. Soils from an eroded midslope and a depositional footslope in an arable farm were amended with chia (*Salvia hispanica*) seed mucilage at concentrations of 0 g C kg<sup>-1</sup>, 0.46 g C kg<sup>-1</sup> and 2.3 g C kg<sup>-1</sup> mucilage, formed into cores, and then imparted with wetting and drying (WD) cycles. Mucilage increased the stability of these inherently stable soils from 80% to >98% water stable macroaggregates at 0WD cycles regardless of slope position. Aggregate stability was maintained after 5WD cycles by mucilage, whereas the stability of unamended soil dropped by 66.7% in the footslope and 30.1% in the midslope compared with 0 WD. Underlying physical stability properties were measured by tensile strength and penetration resistance for mechanical, water sorptivity and repellency for hydrological, and micro-, meso-, macro- and total porosity for structural properties. Almost every soil physical property measured changed less with WD cycles if mucilage was present. Compared to unamended soil, 2.3 g C kg<sup>-1</sup> mucilage amendment decreased water sorptivity from 0.289 mm s<sup>-1/2</sup> to 0.122 mm s<sup>-1/2</sup> in the midslope and 0.230 mm s<sup>-1/2</sup> to 0.182 mm s<sup>-1/2</sup> in the footslope after 5 WD cycles. Aggregate stability, total porosity and water sorptivity were correlated. In the midslope, hydrology and penetration resistance were affected most, likely driven by mucilage deposition in the macropores of this more coarsely textured soil. In the footslope, the greater impact of mucilage on tensile strength was likely driven by buffering of

macroporosity formation by WD cycles in this finer textured soil.

**Keywords:** hydrological, mechanical, plant mucilage, soil stability, slope, structural

**Highlights:**

- We explored how slope position interacts with plant mucilage to drive soil physical stability.
- Changes in soil physical stability by plant mucilage have rarely been considered with slope position.
- Interactions between mucilage and soil particles caused greater physical stability in midslope than footslope.
- Mucilage stabilised soil by easing changes in pore structure, DOC redistribution and water repellency, with particle bonding less important.

## Introduction

Soil erosion is a worldwide issue of both social and environmental concern. Plant exudates and mucilages could be very important to the stabilisation of soil against erosion (Wang *et al.*, 2017) as they have adhesive properties that bind soil particles through polymer bridges, they retain water providing physiological advantage to plant stress tolerance (Brax *et al.*, 2017), and they can also enhance soil hydrological stability by reducing the rapid wetting rate of dry soils (Zhang *et al.* 2004; Peng *et al.*, 2011). Plant exudates and mucilages interacting together with intense wetting and drying (WD) cycles due to evapotranspiration, stimulate changes in the formation and stability of soil structure (Morel *et al.*, 1991). On an eroding slope, the action of plant roots aggregating soil through a combination of particle bonding by plant exudates and mucilages, and physical enmeshment are major soil stabilisation mechanisms (Engelbrecht *et al.*, 2014; Deng *et al.*, 2012). Although it well known that vegetation restoration could greatly stabilize soil against erosion, the role or contribution of plant exudates and mucilages on soil physical stabilisation has received little attention (Traoré *et al.*, 2000). However, the effect of plant exudates and mucilages on soil stabilisation against water erosion may vary at different slope positions.

On a slope, selective transport induced by water erosion drives soil particles, soil carbon and soil microaggregates to be redistributed downslope (Papiernik, *et al.*, 2005; Shi, *et al.*, 2013; Chartier *et al.*, 2013). Therefore, in an eroded area more coarse soil particles (e.g. sands and gravels) and less carbon may remain *in situ*, restricting soil aggregation (Huang, *et al.*, 2014). This change in soil particle size composition along

the slope may cause great differences in pore size distribution between the midslope and the footslope. The greater proportion of fine material in footslope soils results in more micropores, which could preferentially absorb plant exudates, leading to enhanced aggregation (Benard *et al.*, 2018; Morel *et al.*, 1991). Coarser particle sizes in the midslope lead to macropores where organic substances with high molecular weight may be preferentially deposited through capillary action (Zhang *et al.*, 2008), potentially forming a gel film coating on macropore walls or bridges that block pore throats (Carminati *et al.*, 2016). Therefore, the difference in soil particle size distribution between midslope and footslope could cause a great discrepancy of binding capacity, interparticle cohesion and soil aggregate formation. However, the changes in soil physical stabilisation processes by the action of plant exudates have rarely been considered in relation to slope position.

Soil physical stability can be divided into soil mechanical and hydrological stability. Le Bissonais (1996) pointed out the main breakdown mechanisms of soil aggregates are slaking, breakdown by differential swelling, and mechanical breakdown by raindrop impact. The hydrological stability of soil aggregates depends on pore structure controlling the release of entrapped air (Le Bissonais, 1996) and the proportion of hydrophobic organic compounds that alter soil wetting properties (Hallett *et al.*, 2001). WD cycles have been indicated to be one of the non-biological factors driving soil water repellency development. Repeated WD cycles may redistribute soil hydrophobic dissolved organic compounds to the drying surfaces of aggregates (Zhang *et al.*, 2004), or depositing mucilage in smaller cavities among the particle surfaces during drying

(Benard *et al.*, 2018). These processes may create a hydrophobic surface that reduces the soil aggregate wetting rate, thereby enhancing slaking resistance.

Mechanical stabilisation is caused by interparticle bonding and the soil pore structure (Le Bissonais, 1996). It can be quantified from tensile strength or penetration resistance (Peng *et al.*, 2011). Tensile strength is the stress required to break a soil aggregate, and has been found to be a sensitive indicator of soil structural condition (Dexter & Watts, 2001). Soil penetration resistance is important for plant growth and soil biological activity (Bengough *et al.*, 1991; Sinnett *et al.*, 2008). Mechanical stabilisation of soil aggregates is governed by the properties of soil particle size composition, moisture and organic substances. Both slope position and plant mucilage should affect all these properties, driving different dominant stabilisation mechanisms of soil aggregates along an eroding slope.

We hypothesise that soil in the eroded midslope will be stabilised more by plant mucilages than the depositional footslope due to soil particle size composition. These differences will be exacerbated under WD cycles due to a greater redistribution of mucilage to aggregate/macropore surfaces in midslope soil compared to footslope soil. Dissolved organic carbon (DOC) and a range of processes underpinning soil structural stability were measured: macroaggregate water stability against fast wetting, sorptivity, water repellency (WR), tensile strength (TS), penetration resistance (PR), and porosity. The objectives of this study were: (i) to understand the interaction between plant mucilage and soil particles on soil pore structure evolution and DOC redistribution; and (ii) to explore the mechanisms of soil mechanical and hydrological and structural

stability by plant mucilage amendment and slope position.

## Materials and methods

### *Soil sampling*

Soils were sampled from 0-15 cm depth at the midslope and footslope of a commercial arable farm near Montrose in the northeast of the United Kingdom (56° 41' 1.0494"N, 2° 36' 0.126"W). The slope was north-facing, with about a 5° gradient, 1500 m distance and 20.83 m elevation difference between sampling locations. At the time of sampling, the field was under potatoes, with winter wheat planted the previous year, and other years with barley or oil-seed rape under a variable rotation. The soil at the midslope is classified as a Dystric Cambisol and at the footslope it is a Gleyic Cambisol. It formed from reddish brown till composed of andesitic lava, sandstone and conglomerate (Glenworth *et al.*, 2016). The area has a temperate maritime climate with an annual rainfall of approximately 683 mm. At the midslope and footslope, a mixed bulked soil sample was collected from 5 random spots in the area within a diameter of 10 m. Each bulked soil sample was then air-dried and passed through a 2 mm sieve with leaves and plant roots removed with tweezers, and then the soil was mixed by hand with a trowel to improve homogeneity.

Some chemical and physical characteristics of the soils are shown in Table 1. Sand content in the midslope was significantly greater than in the footslope ( $p < 0.01$ ), with a 3 times difference if only sand particles larger than 0.2 mm were considered. However, the silt and clay contents in the midslope were significantly less than in the footslope



( $p < 0.01$ ). The depletion of finer particles from the midslope and increase at the footslope suggests erosion.

Table 1 Selected soil properties in top soil layer from the midslope and footslope of the slope

#### *Plant mucilage extraction and soil cores preparation*

Mucilage was extracted from the seed coating of chia seeds. It has a chemical composition (primarily glucose, xylose and uronic acids) (Lin *et al.*, 1994) and physical properties similar to rhizodeposits produced by lupin and maize roots (Carminati, 2013). Mucilage was extracted by hydrating 50 g of dry chia seeds in 500 ml deionized water, followed by 2 minutes mixing with a magnetic stirrer and 2 hours resting at room temperature (20 °C). Seeds were separated from their hydrated mucilage coating by squeezing the mixture by hand through a nylon mesh (0.5 mm). The separated mucilage was stored in an airtight glass jar at 4 °C before amending into soil. The solid concentration of the collected mucilage was  $9.2 \pm 0.3 \text{ g kg}^{-1}$ . The dry mass of sieved mucilage consisted of  $407.8 \pm 1.4 \text{ g kg}^{-1}$  total organic carbon,  $10.9 \pm 0.1 \text{ g kg}^{-1}$  total nitrogen (TN) and  $1.55 \pm 0.02 \text{ g kg}^{-1}$  DOC.

Soil was wetted to about  $0.25 \text{ g g}^{-1}$  water content, which provided a consistency that allowed for packing into soil cores and was slightly drier than field capacity. This was achieved by wetting, 100 g sieved, air-dry soil with 25 g of either the original extracted mucilage, a 5 times dilution of the original extracted mucilage or deionized water. Mixing was done by hand with a spatula, with the solution applied gradually by dripping across the surface and constantly mixing. Agglomeration during mixing

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occurred, which was broken up to improve homogeneity. This mucilage amendment was equivalent to an amendment of 2.3 g C kg<sup>-1</sup> soil for the undiluted mucilage treatment and 0.46 g C kg<sup>-1</sup> soil for the 5 times dilution treatment. From herein we refer to the mucilage amendment treatments by this amount of C added to the soil.

The mixed soils were equilibrated for 24 hours at 4 °C and then packed into PVC cores (H=1.5 cm, D=3cm) to 1.55 g cm<sup>-3</sup> bulk density, which was similar to field conditions. Quantitative filter paper was secured to the bottom of the PVC cylinder with a rubber band. For each treatment, 39 soil cores were prepared, producing a total of 234 soil cores for the 2 soils and 3 mucilage treatments. The soil cores were stored in sealed plastic bags for 24 hours at 4 °C for further equilibration.

#### *Wetting and drying cycles*

The packed cores were treated with either 0, 1 or 5 WD cycles. Each WD cycle lasted 48 h, consisting of wetting at 0 kPa water potential with deionized water for 6 h at 4 °C followed by drying in an oven for 42 h at 40 °C. Through wetting of the base of the core by capillary action and drying from the top surface by heat, a ‘top’ surface was produced to explore whether DOC illuviation from the soil and added plant mucilage exacerbated observed physical impacts. WD cycles mimic processes that may occur towards the surface of soil or macropores that define interaggregate boundaries.

#### *Soil physical characteristics*

Soil aggregate stability was measured by the fast wetting method. Briefly, 10 g air-dried soil aggregates were immersed in 100 cm<sup>3</sup> of deionized water for 10 min. The

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slaked soils were then transferred to a 0.25 mm sieve that was moved up and down 20 times for 1 minute to a depth of 1 cm under the water. Soil remaining on the sieve was dried at 40 °C for 24 h and then weighed. The aggregate stability index was expressed by the weight percent of > 0.25 mm water stable aggregates. There were 3 replicates.

Soil pore size distribution and penetration resistance (PR) were measured on another 5 replicates on samples equilibrated to -5 kPa and then -30 kPa water potential. Cores were first saturated for 12 h and then equilibrated for 3 days for each water potential using a tension table (EcoTech, Bonn, Germany). PR was measured with a needle penetrometer (30° cone opening angle and 1.87 mm<sup>2</sup> base area) fitted to a loading frame (Zwick Z05, Germany) with a 5 kN load cell (accurate to 0.01 N). The crosshead speed was set to 2 mm min<sup>-1</sup>. The maximum force ( $F_{max}$ , N) was recorded from the surface to 6 mm depth of a core and used to calculate PR by dividing by the base area (Bengough *et al.*, 1997). The weight and volume of each soil core was measured after saturating, drying at -5 kPa and -30 kPa, and after drying at 105 °C. Macroporosity, mesoporosity and microporosity were inferred from the water content at each water potential, corresponding to pore diameter cut-offs of 60 µm at -5 kPa and 10 µm at -30 kPa (Kay, 1990).

On a further 5 replicates that had been dried at 40 °C, tensile strength (TS) was measured by the indirect tension test using the same Zwick loading frame and load cell used for PR measurements. The soil disc was removed from its plastic ring and placed vertically with opposite poles between two flat platens that compressed together during testing. The crosshead speed was set to 6 mm min<sup>-1</sup>, and the applied force was recorded

every 0.1 s. TS in kPa was calculated as

$$TS = 1000 \times \left( \frac{2F_{max}}{\pi DL} \right) \quad (1)$$

where  $F_{max}$  (N) is the applied load at failure,  $D$  (mm) is the diameter of soil core and  $L$ (mm) is the length of the soil core (Czarnes *et al.*, 2000).

Bulk density was measured with the same core used for TS, taking shrinkage during drying into account by measuring the height and diameter with calipers.

Sorptivity and water repellency were measured on the top and bottom surfaces of the soil core, using the approach developed by Hallett & Young (1999). After measuring TS, the soil sample was split in half, with each half used to measure either water sorptivity ( $S_w$ ) or ethanol sorptivity ( $S_E$ ). The soil cores were dried again at 40 °C for 12 h before testing. A 2 mm diameter infiltrometer tip, which was connected to a liquid reservoir on a logging balance (accurate to 0.1 mg), was brought into contact with the soil. The tip was covered with nylon mesh (4 µm in pore diameter) to allow for rapid liquid flow and the development of a hydraulic head of -10 mm to reduce macropore flow. Sorptivity was calculated based on the equations presented in Hallett & Young (1999), and used to determine the water repellency index ( $WR$ ) from the ratio between  $S_E$  and  $S_w$ , multiplied by 1.95 to account for surface tension and viscosity differences between the liquids.

#### *Soil chemical characteristics*

After measuring  $S_w$ , the same soil was used to measure soil DOC. Soils were first dried again for 12 hours at 40 °C and then divided into top (0-2 mm depth from the top surface), interior (2-13 mm from the top surface) and bottom (13-15 mm from the top

surface) layers for samples exposed to 0 and 5 WD cycles. Soil samples were weighed, ground and passed through a 2 mm sieve. DOC was extracted using the method of Ghani *et al.* (2003), and determined immediately by a Lab-TOC analyser (Pollution and Process Monitoring Ltd, Kent, UK). Briefly, DOC was extracted by adding 30 ml deionized water with a 1:10 (w/w) soil-to-water ratio into 3 g of soil sample. The suspension was stirred on a horizontal shaker at 120 rpm for an hour at room temperature. Next, a separation was done by centrifugation at 3500 rpm for 20 min. The supernatant was filtered to  $< 0.45 \mu\text{m}$  through a microfiltration membrane to obtain a solution for DOC determination.

The average DOC ( $\text{DOC}_{\text{ave}}$ ) concentration of the whole soil core was calculated from the DOC concentrations by

$$\text{DOC}_{\text{ave}} = \frac{\text{DOC}_t \times W_t + \text{DOC}_i \times W_i + \text{DOC}_b \times W_b}{W_t + W_i + W_b} \quad (2)$$

where the subscripts  $t$ ,  $i$  and  $b$  refer to top, interior and bottom layers, respectively, and  $W$  is weight.

Soil pH and EC were measured with a pH meter (Hannah Instruments, UK) in 1:2.5 (w/w) and 1:5 soil-to-water suspensions, respectively. Soil texture was measured using the hydrometer method (Gee & Bauder, 1986). Soil organic carbon (SOC) and TN were measured by Carlo Erba Instruments, NA 2500 Elemental Micro Analysis, before detection, soil was treated with 0.1M HCl for removing inorganic C.

#### *Statistical analyses*

Three-way analysis of variances (ANOVA) were used to test the effects of plant mucilage amendment, WD cycles, and slope position on aggregate stability, PR, TS,  $S_w$

and  $S_E$ , WR, DOC concentrations, and porosity. Significant difference between means were evaluated by Fisher's least significant difference (LSD) at the 95% confidence level, and the LSD values were calculated following Webster (2007). Pearson correlation analysis was used to examine the relationships among soil parameters. All statistical analyses were carried out in SPSS version 19.0, and significance for all statistical analysis was accepted at  $p < 0.05$ . Graphs and linear regressions were made by Origin 8.5.

## Results

### *DOC redistribution in soil core*

In unamended soil samples, 5 WD cycles increased DOC by 46.5% in the midslope and 37.7% in the footslope, compared to 0 WD cycle (Fig. 1). DOC concentrations in different parts of the soil cores were affected significantly by mucilage amendment and WD cycles ( $p < 0.001$ ) (Fig. 1, Table\_1\_Supinfo). The top layers of the soil core had appreciably more DOC ( $p < 0.001$ ), containing 21.0%- 29.0% of the whole soil core DOC at 0 WD cycle, and 39.1%-56.8% of the whole soil core DOC after 5 WD cycles. However, the weight of DOC in the top layer was only 13.3% of the total DOC in the soil core. In the top layer, the midslope had greater DOC than the footslope ( $p < 0.001$ ), but DOC concentration in the interior and bottom layers did not vary between the midslope and footslope ( $p > 0.05$ ). DOC concentrations in the 2.3 g C kg<sup>-1</sup> added mucilage treatments were much greater than the water and 0.46 g C kg<sup>-1</sup> added mucilage treatments ( $p < 0.001$ ). Again, the top layer had the greatest DOC, with

2.3 g C kg<sup>-1</sup> added mucilage having 89.4% greater DOC in the midslope and 58.2% greater DOC in the footslope compared to unamended soil.

For the whole soil cores, DOC redistributed with mucilage amendment and WD cycles. Adding 2.3 g C kg<sup>-1</sup> mucilage resulted in more DOC movement to the top of the core surface than water or 0.46 g C kg<sup>-1</sup> added mucilage. This was exacerbated by 5 WD cycles. However, different parts of soil cores presented different changes. For both slope positions, frequent WD cycles resulted in about 80%-100% DOC being in the top layers, with only small changes of DOC measured in the interior and bottom layers (Table 2).

Table 2. The mass balance of dissolved organic carbon (mg) in different depths of soil cores (kg) with mucilage amendment and wetting and drying cycles.

#### *Soil aggregate stability*

Before imposing WD cycles, both the midslope and footslope soils were inherently stable and similar, with greater than 80% water stable aggregates >0.25 mm size (Fig. 2). Mucilage caused a significant increase in aggregate stability at 0 WD cycle ( $p<0.001$ ). After 5 WD cycles, aggregate stability dropped by 11.1%-66.7% in the footslope and 4.3%-30.1% in the midslope, but this was countered by mucilage. For 2.3 g C kg<sup>-1</sup> added mucilage treatment, aggregate stability was maintained close to 0 WD level and it was much greater than for unamended soils ( $p<0.001$ ). The impacts were greater for footslope soil ( $p<0.001$ ).

#### *Tensile strength (TS)*

Soil TS was affected most by slope position and WD cycles and to a lesser extent

by plant mucilage amendment ( $p < 0.001$ ) (Fig. 3, Table\_1\_Supinfo). TS in footslope soil was 36.1%~134.5% greater than in the midslope between the same WD cycles ( $p < 0.001$ ). TS had a large drop with WD cycles, decreasing by 50.5%-75.3% in midslope soil and 47.8%-75.3% in footslope soil between 0 and 5 WD cycles ( $p < 0.001$ ) (Fig. 3). This decrease was attenuated by 2.3 g C kg<sup>-1</sup> added mucilage, resulting in TS increases of 87.7% in midslope and 77.4% in footslope soils compared to unamended soil ( $p < 0.001$ ). At 0 and 1 WD cycles, however, plant mucilage caused gradual TS reductions in the footslope soil, but not in the midslope soil. Soil TS had significant positive correlation with the percentage of water stable macroaggregates for both slope positions ( $p < 0.001$ ), reaching coefficients up to 0.8 (Fig. 7).

#### *Soil porosity and pore size distribution*

Footslope and midslope soils were packed to the same initial porosity ( $p > 0.05$ ), but frequent WD cycles increased soil porosity (Fig. 4) ( $p < 0.001$ ). This porosity increase was in macroporosity and mesoporosity, at the expense of microporosity which decreased with WD cycles. For unamended soil at 0 WD cycles, there was 26.5% greater macroporosity in midslope soil compared to footslope soil, but 12.6% less mesoporosity and 7.2% less microporosity (Fig. 4). Macropore differences between the midslope and footslope soils reflect the textural differences between the slope locations.

The porosity changes with WD cycles, however, were attenuated by 2.3 g C kg<sup>-1</sup> added mucilage where changes were minimal ( $p < 0.05$ ), and far less than soils amended with water or 0.46 g C kg<sup>-1</sup> mucilage. After 5 WD cycles, soils with 2.3 g C kg<sup>-1</sup> added mucilage formed 8.5% and 50.1% less macropores and mesopores, respectively, in the



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midslope, and 24.0% and 43.6% less macropores and mesopores, respectively, in the footslope compared to unamended soils ( $p<0.05$ ). Moreover, 2.3 g C kg<sup>-1</sup> added mucilage had a small impact on micropore increases after 5 WD cycles compared to unamended soils, with the effect much greater for the footslope soil.

#### *Penetration resistance (PR)*

Midslope soils that were not amended with mucilage had greater PR than footslope soil, by 34.2% at -5 kPa water potential and 72.0% at -30 kPa water potential ( $p<0.001$ ) (Table 3). The F value indicates that slope position was the major factor affecting PR, with mucilage having the next greatest impact (Table\_1\_Supinfo). Adding 2.3 g C kg<sup>-1</sup> mucilage significantly increased soil PR in both midslope and footslope ( $p<0.001$ ). However, WD cycles showed a different trend between PR at -5 kPa and -30 kPa, with a 19.1% decrease at -5 kPa and a 6.2% increase at -30 kPa after 5 WD cycles compared to 0 WD cycles. Adding 2.3 g C kg<sup>-1</sup> mucilage had a greater impact on PR after 5 WD cycles for midslope than footslope soils (Table 3). Compared to unamended soils, PR values in 2.3 g C kg<sup>-1</sup> added mucilage were greater by 55.0% in the midslope and 38.9% in the footslope at -5 kPa water potential, and 22.6% in the midslope and 8.4% in the footslope at -30 kPa water potential.

Table 3. Soil penetration resistance as affected by slope position, mucilage amendment and wetting and drying (WD) cycles at different water potentials

#### *Water sorptivity ( $S_W$ ), ethanol sorptivity ( $S_E$ ) and water repellency (WR)*

These soils had small WR levels that were affected most by mucilage amendment

on the drying surface at the top of the core, and by slope position at the bottom of the core (Tables 4, Table 1- Suppinfo)). With WD cycles, WR generally increased at the top surface ( $p < 0.001$ ), with the impact exacerbated by increasing mucilage concentration ( $p < 0.001$ ). At the bottom of the core, however, WD cycles had no effect on the WR of the midslope soil ( $p > 0.05$ ) and caused a decrease in the footslope soil ( $p < 0.001$ ).

The underpinning driver of soil stabilisation by WR is its impact on  $S_w$ . Whereas WD cycles increased  $S_w$  by over 65% at the top surface for either midslope or footslope soil unamended with mucilage, when soils were amended with  $2.3 \text{ g C kg}^{-1}$  mucilage the  $S_w$  dropped by 13.5% in the midslope soil and increased by 29.1% in the footslope soil. By 5 WD cycles, there was a large increase in  $S_E$  for all treatments, but mucilage amendment decreased the amount of change.

Soil WR were significantly positively correlated with DOC concentration regardless of slope positions ( $p < 0.001$ ). Their coefficients reached up to 0.82 and 0.79 for midslope and footslope, respectively (Fig.6).

Table 4. Sorptivity and Repellency of soil cores formed from soil as affected by slope position, mucilage amendment and wetting and drying (WD) cycles

## Discussion

Plant mucilage had a positive impact on soil physical stability regardless of slope position (Fig. 2), but the dominance of different stabilisation mechanisms differed (Figs. 3-4, Tables 3-4). The most significant finding from this study was the attenuation of

physical weathering stresses by plant mucilage, simulated by imposed WD cycles. Almost every soil physical property measured changed less with WD cycles if mucilage was present, with impacts greatest for the larger mucilage concentration. This generally agrees with previous studies (Morel *et al.*, 1991; Traoré *et al.*, 2000; Peng *et al.*, 2011) that found plant mucilage increased soil physical stability by improving bond strength of soil particles, diminishing wetting rate, modifying soil porosity and reducing soil slaking stresses through increased water repellency. A schematic model of soil physical stability as affected by slope position, plant mucilage amendment and WD cycles is shown in Fig. 5.

#### *Impact of plant mucilage at different slope positions*

The new understanding from our study is to disentangle how slope position interacts with plant mucilage to drive soil physical stability. Compared to the midslope, the proportion of clay and silt in the footslope was greatly increased due to soil erosion, which redistributes soil particles, water and carbon in the landscape (Table 1) (Chartier *et al.*, 2013; Shi *et al.*, 2013). At the midslope, removal of finer material by colluviation (Table 1) would result in less interparticle bonding (Hallett *et al.*, 1995), as reflected in its smaller tensile strength compared to the footslope soil (Fig. 3). When the soils were wet, the finer material in the footslope soils eased penetration resistance (Oleghe *et al.*, 2017) compared to the midslope soils (Table 3). Mucilage amendment increased penetration resistance at either slope position, but by 5 WD cycles the increases were greater in midslope than footslope soils at either water potential. Hydrological behaviour was affected more in the midslope than the footslope, with water sorptivity

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driven by the combined effects of WD cycles, pore structure changes (as suggested by ethanol sorptivity) (Table 5) and water repellency (Table 4). These hydrological and mechanical processes interacted to affect soil aggregate stability, which was decreased far more with WD cycles in the footslope compared to the midslope, but was mitigated by mucilage at both slope positions.

Table 5. Correlations between soil hydrological and mechanical properties and soil pore structure

#### *Mechanical stabilisation by plant mucilage*

Plant mucilages are viscoelastic gels composed of large molecular weight organic compounds, low molecular weight organic acids and amino acids (Brax *et al.*, 2017; Naveed *et al.*, 2017). In soils, lower molecular weight organic substances add to DOC, which under WD cycles bind to soil particles and micropore surfaces through adsorption (Chenu & Guerif, 1991; Henao & Mazeau, 2009), surface tension and capillary forces of plant mucilage (Naveed *et al.*, 2019). Larger molecular weight substances in plant mucilage are more viscous, so are more likely to occupy larger void spaces (macropore) between larger soil particles (sands and aggregates). When soils dry, macropores are drained first, so these larger size organic substances may form a gel film coating on macropore walls or bridges that block pore throats (Carminati *et al.*, 2016). This has a mechanical effect by binding “loose” soil particles (Zhang *et al.*, 2008; Peng *et al.*, 2010), and a hydrological effect by clogging pores and inducing water repellency (Carminati *et al.*, 2016; Zhang *et al.*, 2004).

Amending soils with chia mucilage was to simulate rhizosphere soil in cores large enough to measure soil physical properties, as described by other studies (Peng *et al.*, 2011; Oleghe *et al.*, 2017). It allowed for mechanisms driving soil stability (Le Bissonnais, 1996) to be disentangled, such as tensile strength to quantify bond energy between soil particles (Czarnes *et al.*, 2000; Peng *et al.*, 2011). In the study, 2.3 g C kg<sup>-1</sup> added mucilage to either midslope or footslope soils increased aggregate stability, especially when soil was exposed to multiple WD cycles ( $p < 0.05$ ) (Fig. 2). Moreover, 2.3 g C kg<sup>-1</sup> added mucilage caused the smallest drop in tensile strength (Fig. 3) and the smallest increase in macroporosity (Fig. 4) caused by WD cycles. Given that tensile strength decreased with increasing mucilage concentration in the footslope soil and was hardly changed in the midslope soil at 0 WD cycles, the mechanical stabilisation by mucilage observed at 5 WD cycles was more likely due to its impact on pore structure than bond energy. Significant correlations between tensile strength and different pore size classes were found (Table 5) ( $p < 0.01$ ). The 0.46 g C kg<sup>-1</sup> added mucilage treatment had similar pore structure to unamended soils across the WD treatments (Fig. 4), but weaker tensile strength at 0 and 1 WD cycles for midslope and footslope soil. This suggests the mucilage decreased bond energy, but to disentangle directly would need fracture mechanics tests with imposed pore structures (Zhang *et al.*, 2008) or interparticle bond tests (Cole *et al.*, 2012). High molecular weight compounds found in plant mucilage and other exopolysaccharides have been found to increase dry soil strength in most studies (Chenu & Guerif, 1991; Cole *et al.*, 2012; Peng *et al.*, 2011), although Czarnes (2000) found dextran had no impact. The amount of clay along the

slope influenced the trends we observed, likely driven by differences in bonding between exopolysaccharides to sand (Cole *et al.*, 2012), silt and clay (Henao & Mazeau, 2009).

For both the footslope and midslope soils, tensile strength correlated with aggregate stability (Fig. 7), but given the arguments about particle bonding presented above, the drivers may be due to pore structure stabilisation and hydrological processes, rather than interparticle bonding by the plant mucilage. During drying, mucilage entraps water (Buchmann & Schaumann, 2017) and decreases the rate of water loss (Deng *et al.*, 2015), and during wetting mucilage imparts hydrophobicity that decreases the rate of water infiltration (Ahmed *et al.*, 2018). These hydrological drivers, coupled with enhanced microstructural stability (Brax *et al.*, 2017), could attenuate WD cycle induced pore structure changes. Microstructural stabilisation was possibly operating at a micrometer scale, that would be too small for the tensile strength measurement (larger than centimeter scale) to capture (Hallett *et al.*, 1995). As discussed earlier, penetration resistance, measured at -5 and -30 kPa water potential, suggest that mucilage increased microstructural stability of wet soil (Table 3).

#### *Hydrological stabilisation by plant mucilage*

Hydrological processes were quantified by water sorptivity, ethanol sorptivity and water repellency, which provide information about the rate of water infiltration and the disruptive effect of energy dissipation caused by rapid wetting (Czarnes *et al.*, 2000). In this study, increases in water and ethanol sorptivity in both midslope and footslope soils with increasing WD cycles were attenuated by plant mucilage (Table 4). Ethanol

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sorptivity depends on pore structure, as illustrated by the correlation we found (Table 5). Water sorptivity changed less with WD cycles in mucilage amended soils due to the development of water repellency. This was likely driven by the movement of mucilage to the top of the core when the soil dried, with the greatest impact observed for soils with 2.3 g C kg<sup>-1</sup> added mucilage. The impact on water repellency of 0.46 g C kg<sup>-1</sup> added mucilage was insignificant, possibly because the smaller volume of mucilage was deposited in contact regions between soil particles, forming thin filaments that were bypassed by infiltrating water (Benard *et al.*, 2018). DOC was also mobilised by WD cycles (Fig. 1), which was correlated to increased water repellency (Fig. 6). On closer examination, however, DOC in unamended soils changed markedly between 0 and 5 WD cycles (Fig.1) but did not result in increased water repellency (Table 4). This agrees with Zhang *et al.* (2004) who found no correlation between the concentration of native DOC in soil and water repellency. Mucilage was driving the development of water repellency in our study.

It was expected that mucilage would affect the physical behaviour of midslope soil more than footslope soil because of its coarser texture driven by soil erosion. For aggregate stability the reverse was found, with footslope soils destabilised far more by WD cycles, but stabilised more by mucilage amendment after 5 WD cycles. Bond energy is generally assumed to be the greatest driver of soil stabilisation by mucilage, but our results suggest that mucilage buffering pore structure dynamics and inducing water repellency have greater impact. With simulated physical weathering by WD

cycles, mucilage from plants has a large positive impact on stabilising soils on a slope, particularly in the footslope that has received unstable soil through colluviation.

Our experiment used idealised conditions of initially homogenised soils amended with a single mucilage input so that physical testing was possible. Using the same approach, exploring a broader range of soils and environmental stresses, particularly the impact of the severity of WD treatments, provides an opportunity for further study. There is scope to adopt new small-scale approaches to allow hydrological and mechanical processes driving soil stabilisation by roots and rhizodeposits to be measured along a slope in planted samples from controlled glasshouse experiments and in cores extracted from the field (Naveed *et al.*, 2018).

## Conclusion

This study has demonstrated that plant mucilage improved mechanical, hydrological and structural stabilisation mechanisms to a different extent depending on slope position. In the eroding midslope, more coarse particles, macroporosity and DOC accumulation on the surface of soil cores, may cause mucilage to impart stronger microstructural stabilisation and a greater drop soil wetting rate compared to footslope soil. This was reflected in the greater increase tensile strength, penetration resistance and water repellency for midslope compared to footslope soil after multiple WD cycles. However, there was less of an increase in soil macroaggregate stability caused by mucilage in the midslope because unstable aggregates were removed by erosion and deposited in the footslope. Soil hydrological and mechanical properties that drive soil



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stability in different slope positions were therefore mainly driven by the interaction of the plant mucilage with soil particles by attenuating changes in the soil pore structure, DOC redistribution and water repellency.

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### **Data Availability Statement**

The data used to support the findings of this study are available from the corresponding author upon request.

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## Tables

Table 1. Selected soil properties from the midslope and footslope of the slope.

Table 2. The mass balance of dissolved organic carbon (mg) in different depths of soil cores (kg) with mucilage amendment and wetting and drying cycles.

Table 3. Soil penetration resistance (MPa) as affected by slope position, mucilage amendment and wetting and drying (WD) cycles at different water potentials.

Table 4. Sorptivity and Repellency of soil cores formed from soil as affected by slope position, mucilage amendment and wetting and drying (WD) cycles.

Table 5. Correlations between soil hydrological and mechanical properties and soil pore structure.

Table 1. Selected soil properties from the midslope and footslope of the slope

Position	Soil texture / %				TN /g kg <sup>-1</sup>	SOC /g kg <sup>-1</sup>	pH ( H <sub>2</sub> O )	EC /μs cm <sup>-1</sup>
	Sand 2-0.02mm	Silt 0.02- 0.002mm	Clay <0.002 mm	Particle 2-0.2 mm				
Midslope	78.0±0.4	13.9±0.2	8.2±0.4	28.2±0.5	1.85±0.11	18.5±1.4	5.8±0.0	46.4±2.5
Footslope	64.6±0.4	21.2±0.3	14.2±0.4	8.5±0.2	1.57±0.02	19.5±2.0	6.4±0.0	67.9±0.3

Data are mean ± standard error of three replicates



Table 2. The mass balance of dissolved organic carbon (mg) in different depths of soil cores (kg) with mucilage amendment and wetting and drying cycles.

Slope position		Pre WD	0 WD			5 WD		
			water	0.46 g C kg <sup>-1</sup> mucilage	2.3 g C kg <sup>-1</sup> mucilage	water	0.46 g C kg <sup>-1</sup> mucilage	2.3 g C kg <sup>-1</sup> mucilage
Mid slope	Top	24	39 (+15)	38 (+14)	53 (+29)	99 (+75)	101 (+77)	168 (+138)
	Interior	100	87 (-13)	96 (-4)	120 (+20)	96 (-4)	103 (+3)	109 (+9)
	Bottom	23	21 (-2)	21 (-2)	29 (+6)	20 (-3)	20 (-3)	24 (+1)
	Whole	147	147	155 (+8)	203 (+55)	215 (+68)	224 (+77)	295 (+148)
	<sup>1</sup> DOC from mucilage		0	-8	-55	0	-23	-94
	DOC from SOC		0	0	0	-68	-54	-54
Foot slope	Top	22	33 (+11)	34 (+12)	45 (+23)	87 (+65)	88 (+66)	122 (+100)
	Interior	93	83 (-10)	94 (+1)	115 (+22)	95 (+2)	99 (+6)	115 (+22)
	Bottom	22	20 (-2)	23 (+1)	28 (+6)	20 (-2)	22	25 (+3)
	Whole	137	137	151 (+14)	188 (51)	202 (+65)	209 (+72)	262 (+125)
	DOC from mucilage		0	-14	-51	0	-23	-94
	DOC from SOC		0	0	0	-65	-49	-31

Note: The variation of DOC in different soil layer or whole soil core were listed in the bracket. “+” means that DOC is in the inflow state; “-” means that DOC is in the outflow state or decomposed from mucilage or soil organic carbon (SOC)

1. The increased mass of DOC in the whole soil core derived from amended mucilage or SOC, and we assumed that the mucilage was preferentially decomposed into DOC than SOC; The additive maximum OC mass from 0.46 g C kg<sup>-1</sup> mucilage and 2.3 g C kg<sup>-1</sup> mucilage for 1 kg soil were 23 mg and 94 mg, respectively.

Table 3. Soil penetration resistance (MPa) as affected by slope position, mucilage amendment and wetting and drying (WD) cycles at different water potentials.

Water Potential	Cycles	Midslope			Footslope		
		water	0.46 g C kg <sup>-1</sup> mucilage	2.3 g C kg <sup>-1</sup> mucilage	water	0.46 g C kg <sup>-1</sup> mucilage	2.3 g C kg <sup>-1</sup> mucilage
-5 KPa	0 WD	0.648±0.018	0.654±0.017	0.786±0.015	0.483±0.015	0.429±0.016	0.588±0.027
	1 WD	0.707±0.021	0.600±0.013	0.724±0.020	0.425±0.015	0.494±0.016	0.530±0.014
	5 WD	0.480±0.035	0.516±0.034	0.744±0.048	0.355±0.024	0.426±0.014	0.493±0.012
-30 KPa	0 WD	2.160±0.100	1.878±0.034	2.086±0.108	1.256±0.029	1.274±0.049	1.321±0.061
	1 WD	2.052±0.046	2.113±0.055	2.138±0.048	1.305±0.035	1.402±0.039	1.309±0.047
	5 WD	1.974±0.068	2.003±0.060	2.420±0.183	1.380±0.031	1.360±0.107	1.496±0.051

Data are mean ± standard error

Table 4. Sorptivity and Repellency of soil cores formed from soil as affected by slope position, mucilage amendment and wetting and drying (WD) cycles.

			Midslope			Footslope		
			water	0.46 g C kg <sup>-1</sup> mucilage	2.3 g C kg <sup>-1</sup> mucilage	water	0.46 g C kg <sup>-1</sup> mucilage	2.3 g C kg <sup>-1</sup> mucilage
Top surface	S <sub>w</sub>	0 WD	0.173±0.003	0.180±0.002	0.141±0.004	0.136±0.004	0.140±0.002	0.141±0.004
		1 WD	0.163±0.002	0.214±0.004	0.138±0.001	0.126±0.004	0.135±0.002	0.139±0.005
		5 WD	0.289±0.006	0.274±0.014	0.122±0.008	0.230±0.006	0.216±0.029	0.182±0.018
	S <sub>E</sub>	0 WD	0.252±0.006	0.248±0.005	0.231±0.004	0.171±0.003	0.174±0.002	0.203±0.004
		1 WD	0.241±0.002	0.261±0.004	0.231±0.004	0.162±0.003	0.172±0.002	0.196±0.004
		5 WD	0.449±0.067	0.430±0.025	0.278±0.018	0.317±0.038	0.366±0.016	0.340±0.050
	WR	0 WD	2.763±0.052	2.689±0.069	3.210±0.130	2.455±0.074	2.422±0.028	2.819±0.101
		1 WD	2.887±0.051	2.379±0.065	3.252±0.051	2.515±0.050	2.494±0.051	2.769±0.112
		5 WD	2.634±0.266	3.122±0.109	4.500±0.202	2.885±0.264	3.301±0.353	3.869±0.447
Bottom surface	S <sub>w</sub>	0 WD	0.214±0.009	0.186±0.003	0.139±0.005	0.134±0.002	0.144±0.002	0.128±0.003
		1 WD	0.191±0.005	0.203±0.006	0.149±0.006	0.140±0.002	0.143±0.004	0.136±0.005
		5 WD	0.648±0.075	0.422±0.040	0.202±0.005	0.801±0.046	0.754±0.059	0.467±0.047
	S <sub>E</sub>	0 WD	0.252±0.003	0.255±0.003	0.243±0.004	0.172±0.002	0.183±0.002	0.194±0.002
		1 WD	0.249±0.006	0.268±0.004	0.235±0.004	0.170±0.006	0.184±0.003	0.206±0.009
		5 WD	0.867±0.094	0.615±0.094	0.334±0.003	0.661±0.090	0.623±0.081	0.390±0.029
	WR	0 WD	2.317±0.099	2.664±0.065	3.419±0.119	2.497±0.036	2.470±0.036	2.959±0.074
		1 WD	2.540±0.037	2.590±0.082	3.100±0.130	2.365±0.079	2.501±0.087	2.957±0.085
		5 WD	2.639±0.400	2.671±0.309	3.220±0.108	1.420±0.256	1.537±0.190	1.650±0.196

S<sub>w</sub>: water sorptivity (mm s<sup>-1/2</sup>); S<sub>E</sub>: ethanol sorptivity (mm s<sup>-1/2</sup>); WR: water repellency;

Data are mean ± standard error

Table 5. Correlations between soil hydrological and mechanical properties and soil pore structure.

Slope Position	Soil porosity	Water Sorptivity	Ethanol Sorptivity	Water Repellency	DOC in top surface soil	DOC in bottom surface soil	Aggregate stability	Tensile strength
midslope	macro-	0.57**	0.80**	0.36*	0.67**	-0.53*	-0.65**	-0.87**
	meso-	0.86**	0.93**	-0.04	0.44*	-0.73**	-0.90**	-0.94**
	micro-	-0.57**	-0.73**	-0.24	-0.68**	0.77**	0.76**	0.86**
	Total	0.70**	0.88**	0.18	0.53*	-0.53*	-0.72**	-0.89*
footslope	macro-	0.79**	0.74**	0.25	0.52*	-0.67**	-0.90**	-0.94**
	meso-	0.80**	0.75**	0.23	0.55*	-0.67**	-0.92**	-0.91**
	micro-	-0.45**	-0.31*	0.11	-0.29	0.88**	0.80**	0.49**
	Total	0.77**	0.77**	0.34*	0.59*	-0.51*	-0.79**	-0.94**

\*,\*\* indicate the significance of  $p < 0.05$  and  $p < 0.01$ , respectively.

## Figures lists

Fig.1. Dissolved organic carbon (DOC) concentration at different depths in the soil cores in the top soil layer (0-2 mm depth from the top surface), bottom soil layer (13-15 mm from the top surface), interior soil layer (2-13 mm from the top surface) and the whole soil core (0-15 mm) as affected by slope position, plant mucilage amendment and wetting/drying (WD) cycles.

Fig. 2. Percentage of water stable aggregates ( $>0.25$  mm) of soil cores as affected by slope position, plant mucilage amendment and wetting/drying (WD) cycles. The dashed lines show the proportion of primary sand particles  $>0.25$  mm, with 18.23% at the midslope and 6.12% in the footslope.

Fig.3 Tensile strength of soil cores as affected by slope position, plant mucilage amendment and wetting/drying (WD) cycles.

Fig.4 Soil porosity expressed as macroporosity ( $>60$   $\mu\text{m}$ ), mesoporosity (10-60  $\mu\text{m}$ ) and microporosity ( $<10$   $\mu\text{m}$ ) of the soil cores as affected by slope position, plant mucilage amendment and wetting/drying (WD) cycles.

Fig. 5 Schematic model of soil physical stability as affected by slope position, plant mucilage amendment and wetting/drying (WD) cycles.

Fig. 6 Relationship between water repellency and dissolved organic carbon (DOC) concentration in top surface soil layer (0-2 cm). The fitted line and analysis are for linear regression.

Fig. 7 Relationship between tensile strength and percentage of water stable aggregates (>0.25 mm) after wet sieving. The fitted line and analysis are for linear regression.

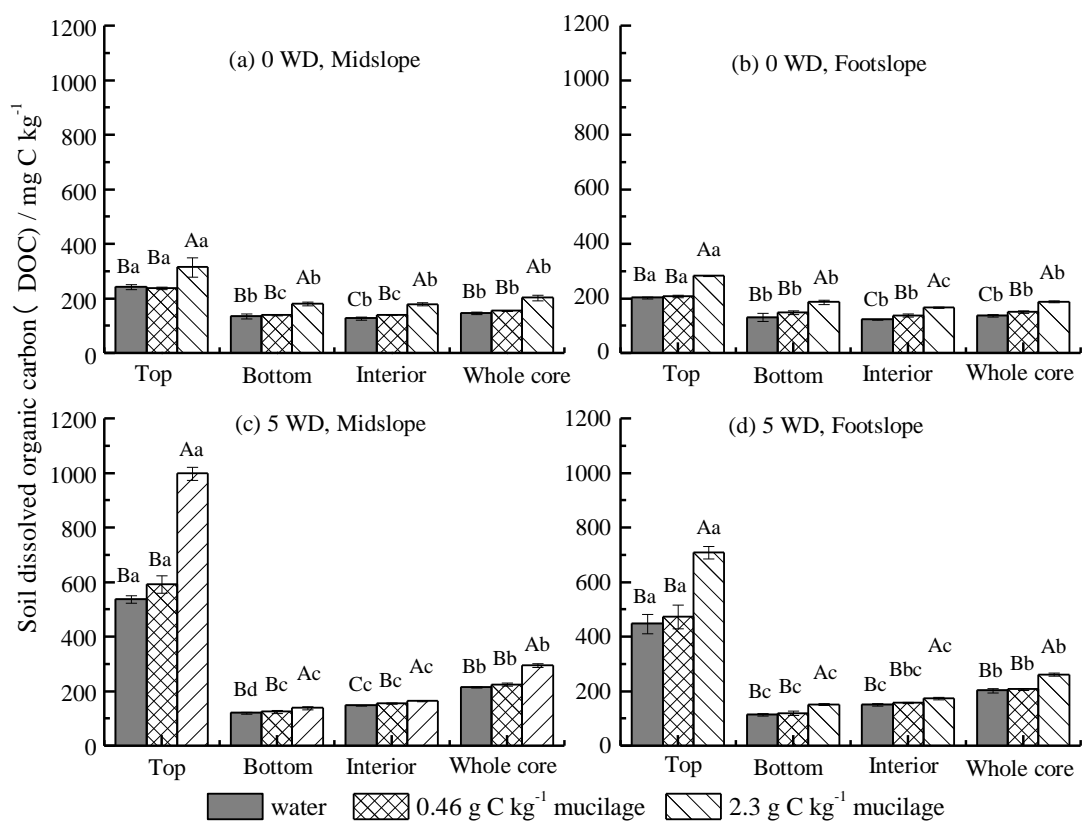


Fig.1. Dissolved organic carbon (DOC) concentration at different depths in the soil cores in the top soil layer (0-2 mm depth from the top surface), bottom soil layer (13-15 mm from the top surface), interior soil layer (2-13 mm from the top surface) and the whole soil core (0-15 mm) as affected by slope position, plant mucilage amendment and wetting/drying (WD) cycles

The uppercase letters mean the differences among mucilage treatments ( $p < 0.05$ ), and the lowercase letters represent the differences among soil layers and whole soil core ( $p < 0.05$ ).

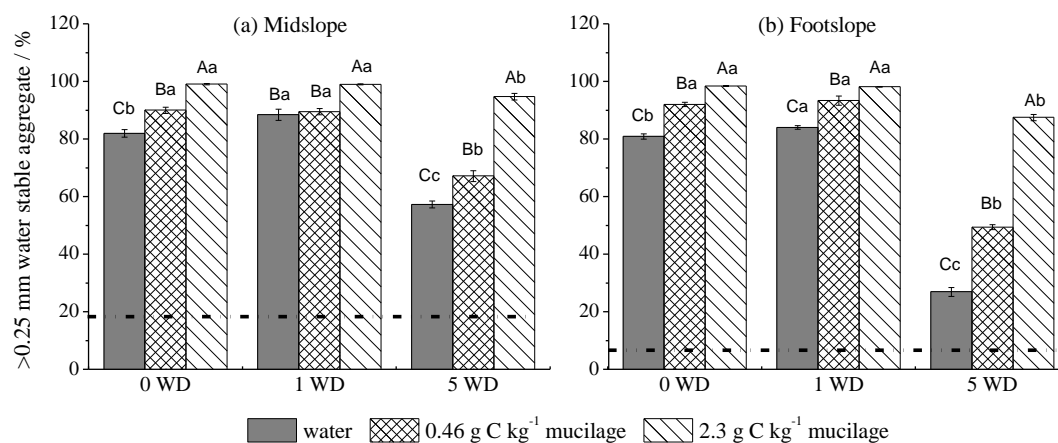


Fig. 2. Percentage of water stable aggregates (>0.25 mm) for soil cores as affected by slope position, plant mucilage amendment and wetting/drying (WD) cycles. The dashed lines show the proportion of primary sand particles >0.25 mm, with 18.23% at the midslope and 6.12% in the footslope

The uppercase letters mean the differences among mucilage treatments ( $p < 0.05$ ), and the lowercase letters represent the differences among WD cycles ( $p < 0.05$ ).



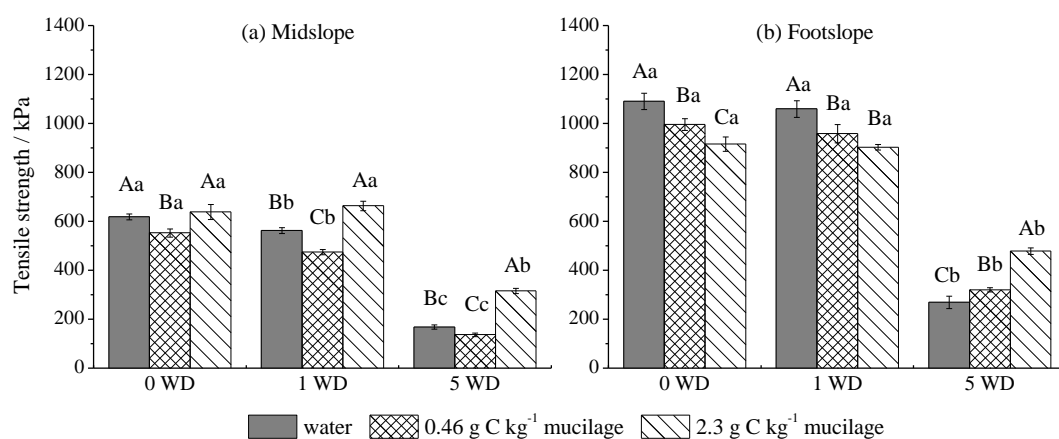


Fig.3 Tensile strength of soil cores as affected by slope position, plant mucilage amendment and wetting/drying (WD) cycles

The uppercase letters mean the differences among mucilage treatments ( $p < 0.05$ ), and the lowercase letters represent the differences among WD cycles ( $p < 0.05$ ).

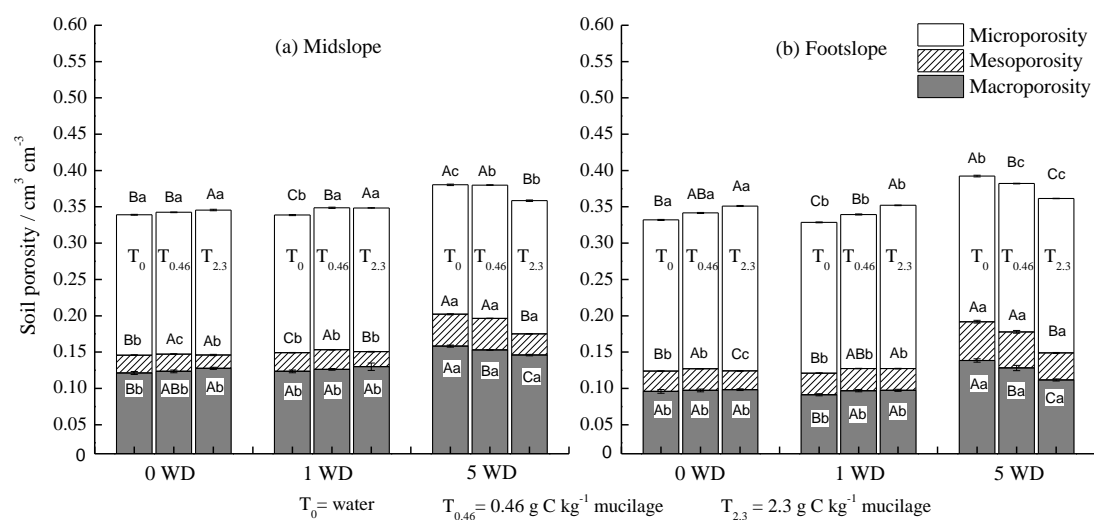


Fig.4 Soil porosity expressed as macroporosity (>60  $\mu\text{m}$ ), mesoporosity(10-60  $\mu\text{m}$ ) and microporosity (<10  $\mu\text{m}$ ) of the soil cores as affected by slope position, plant mucilage amendment and wetting/drying (WD) cycles.

The uppercase letters mean the differences among mucilage treatments ( $p < 0.05$ ), and the lowercase letters represent the differences among WD cycles ( $p < 0.05$ ).

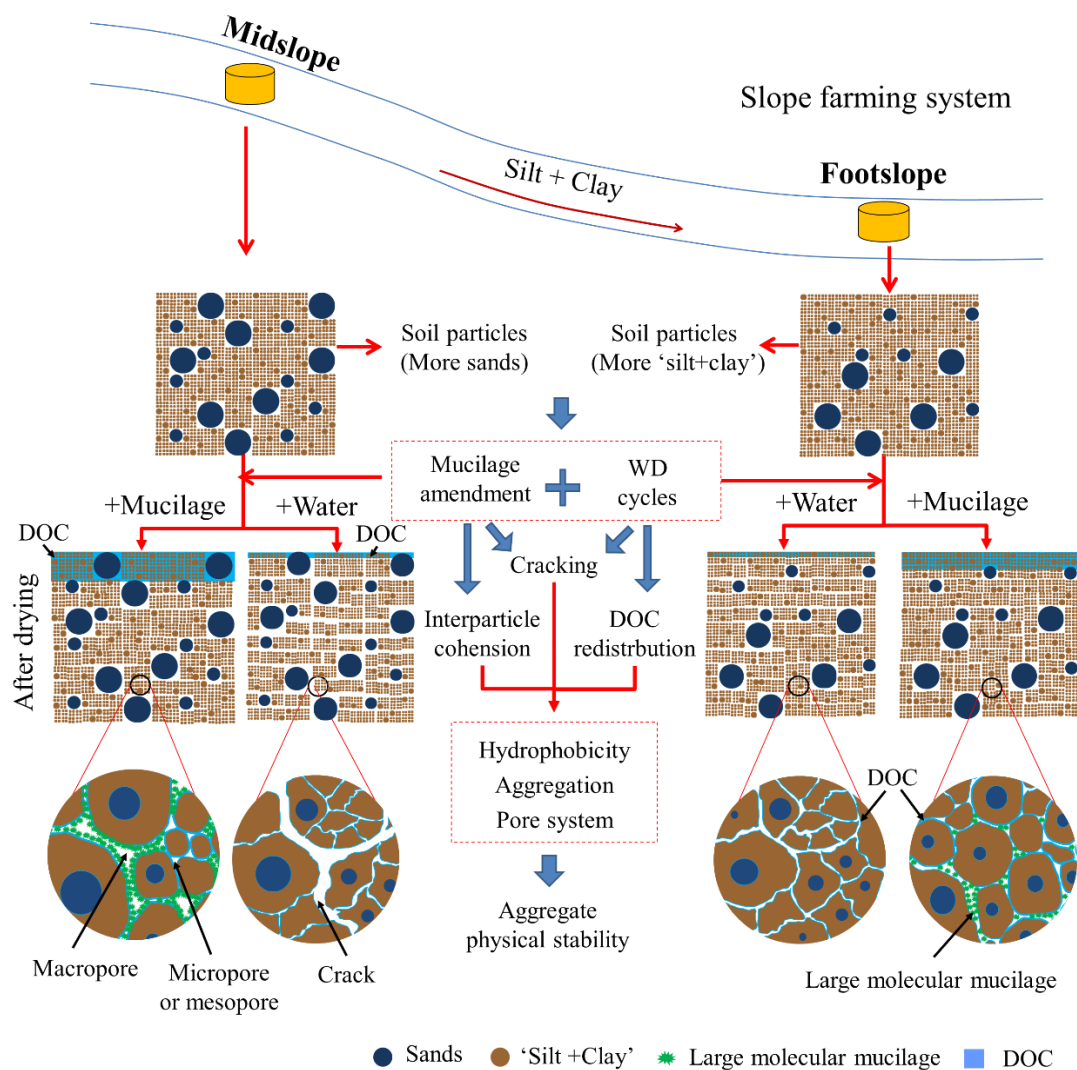


Fig. 5 Schematic model of soil physical stability as affected by slope position, plant mucilage amendment and wetting/drying (WD) cycles

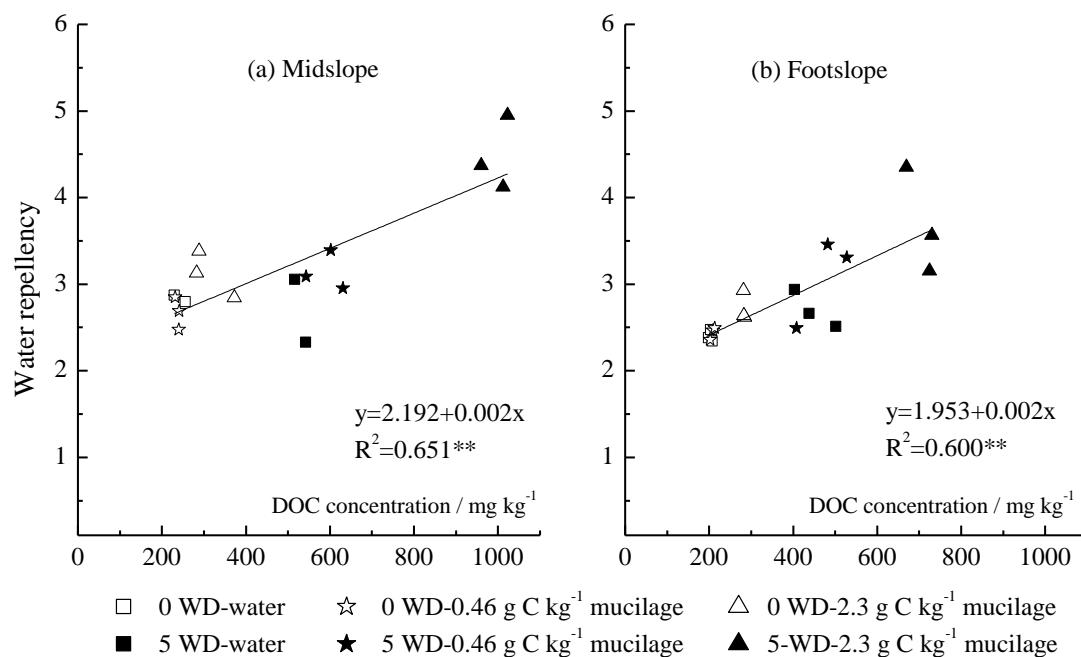


Fig. 6 Relationship between water repellency and dissolved organic carbon (DOC) concentration in top surface soil layer (0-2 cm). The fitted line and analysis are for linear regression

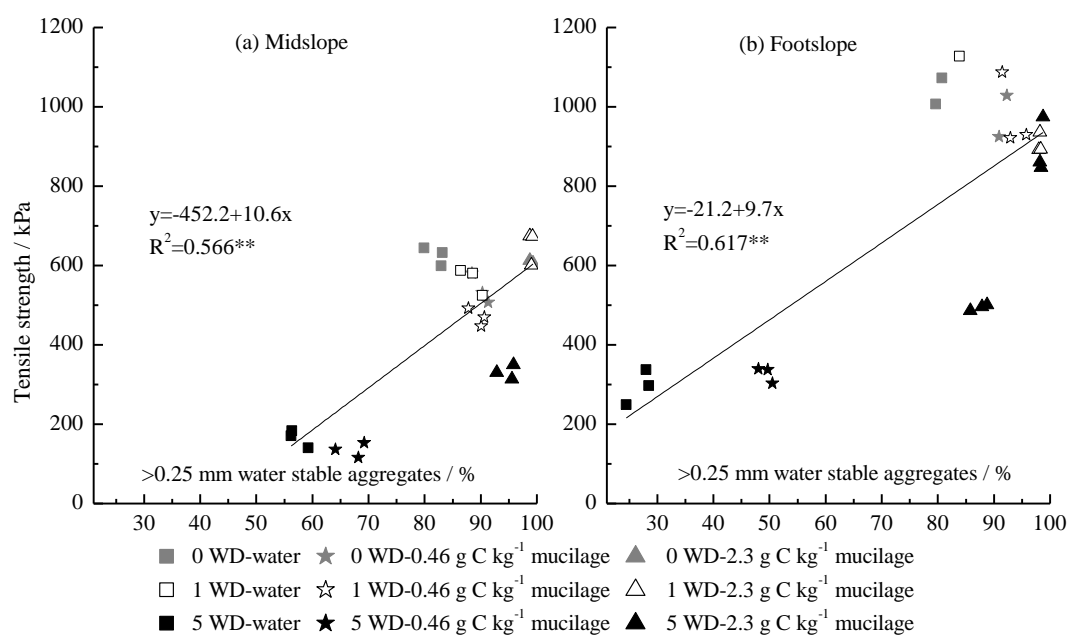


Fig. 7 Relationship between tensile strength and percentage of water stable aggregates (>0.25 mm) after wet sieving. The fitted line and analysis are for linear regression.