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REGULAR ARTICLE

Mucilage from fruits/seeds of chia (*Salvia hispanica L.*) improves soil aggregate stability

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Abstract
Background and aims Myxodiaspores have been shown to enhance soil-seed contact and improve soil stability. We aim to demonstrate the effect of myxodiaspory on the stability of soil aggregates and gain insight on the nature of bonds.
Methods Mucilage extracted from chia (*Salvia hispanica* L.) fruits after hydration was mixed with three soils (sandy-loam, loam, clay loam), incubated and tested at different times up to 30 days. We measured aggerate stability by wet sieving and the dynamics of soil CO_2 evolution. SEM imaging and ^{13}CPMAS spectroscopy of mucilage were performed in order to infer mechanisms of soil stabilization.
Results The incorporation of mucilage resulted in a dose- and soil-dependent rise in aggregate stability. The dose of 2% mucilage overcame textural effects on soil aggregate stability by providing a 2.3-fold stability increase in the loam and clay-loam and a 4.9-fold increase in the sandy-loam compared to control. The effect persisted after 30 days in spite of C losses due to soil respiration. Mechanisms of soil bonding analogous to xanthan can be inferred from SEM imaging and ^{13}C–CPMAS, since the mucilage was identified as a biopolymer containing 93.39% carbohydrates and 22.02% uronic acids.
Conclusions We demonstrate that mucilage extruded by hydrated diaspores strongly increases soil aggregate stability. This represents a potentially important ecosystem service provided by myxodiasporous crops during germination. Our findings confirm potential applications of mucilage from myxodiaspores as natural soil stabilizers.

Keywords Chia · *Salvia hispanica* L. · Myxodiaspory · Myxospermy · Soil stability · Aggregate stability

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Introduction

Myxodiaspory consists in the production of mucilage originating from epidermal and sub-epidermal cells or specialized tissues of seeds (myxospermy) and fruits (myxocarpy) (Makouate et al. 2012). Mucilage is exuded from plant dispersal structures when in contact with water. This occurs at the beginning of germination and causes a series of changes in the chemical and physical properties of the soil surrounding germinating myxodiaspores (Yang et al. 2012; Western 2012). The potential ecological role of mucilage in the spermosphere is analogous to that of root or microflora exudates in the soil but has been the object of less extensive research. Adhesive forces developed by mucilage cause reduction of seed scattering due to glueing to soil particles (van Rooyen et al. 1990; Engelbrecht et al. 2014) but also improved dispersion of seeds by animal vectors; hygroscopic behavior has been proposed as a mechanism in seed hydration, regulation of oxygen entry, and DNA repair, which affect seed viability, dormancy and germination (Western 2012). Rheological properties and attraction of microflora by mucilage have been also hypothesized as relevant in lubricating the radicle and enhancing seedling growth (Yang et al. 2012).

Besides their role in plant ecology, mucilage produced by plant diaspores have the potential of changing the physical behaviour of soils in analogy with other biological exudates. Root and bacterial mucilages may create a sheath around roots and affect soil structure by diffusing in the pore space upon hydration and binding soil particles (Watt et al. 1993, 1994; Czarnes et al. 2000). In the presence of biological exudates or their analogues soil structure stability often increases (Tisdall et al. 1978; Cheshire 1979; Amellal et al. 1998; Guckert et al. 1975; Tisdall and Oades 1982) and soil water holding capacity is higher (Chenu and Guèrif 1991; Carminati and Vetterlein 2013). The main compounds found in mucilages are polysaccharidic gums and their chemical composition plays an important role in soil binding properties; soil strength (Czarnes et al. 2000) or aggregate stability (Traorè et al. 2000), for instance, are improved by xanthan (Czarnes et al. 2000) and polygalacturonic acid (PGA) (Traorè et al. 2000; Czarnes et al. 2000) more than bydextran (Czarnes et al. 2000*)*.

Chia *(Salvia hispanica L.)* is an emerging oilseed crop belonging to the Lamiaceae family; its dry indehiscent fruit – commonly referred to as "seed" – is myxodiasporous: upon hydration it extrudes clear mucilage forming a capsule (Lin et al. 1994; Mu et al. 2012; Segura-Campos et al. 2014: Bochicchio et al. 2015*)* which is strongly connected to the fruit exocarp outer cell layers (de la Paz Salgado-Cruz et al. 2013) and is composed mainly by carbo-hydrate fibres of 18–45 nm width. According to Capitani et al. (2013) this phenomenon is possibly due to the association of mucilage with the columella and the cell wall, in analogy with findings on Arabidopsis. The mucilage of chia has been reported to be essentially composed of polysaccharides (de la Paz Salgado-Cruz et al. 2013). Lin et al. (1994) proposed a tentative structure, consisting of a tetrasaccharide with 4-O-metyl-α-D-glucoronopyranosyl residues occurring as branches at O-2 of some β-D-xylopyranosyl residues in the main chain of (1 → 4)-β-Dxylopyranosil-(1 → 4)-α-D-glucopyranosyl-(1 → 4)-β-D-xylopyranosil units. Through acid hydrolysis they obtained the monosaccharides β-D-xylose, α-D-glucose, and 4-O-metil-α-D-glucoronic acids in the proportion 2:1:1, respectively. The composition is therefore similar to that of maize *(Zea mays L.)* root exudates and has been used as an analogue of mucilages found in the rhizosphere to study plant-soil water relations (Carminati and Vetterlein 2013; Kroener et al. 2014; Ahmed et al. 2014).

Chia mucilage is highly adhesive (Svec et al. 2016), viscous (Capitani et al. 2015) and hygroscopic: it absorbs up to 27 times its own weight of water (Mu et al. 2012). Based on its rheological properties it has been proposed for food technology uses (e.g. Menga et al. 2017); however, no particular attention has been paid to the influence of mucilage of chia on the stabilization of soil aggregates, while this might open the way to potentially important applications of this additive as natural soil stabiliser. Furthermore, understanding the effect of hydrated chia seeds mucilage capsule on soil aggregate stability could help clarify the ecosystem services provided by myxosdiasporous crops during germination, a time when soils are bare at least on the sowing row, and virtually no other protection from erosion agents is provided by plant or soil features. In other species (i.e. Tamarind, Opuntia spp) the similarity of seed mucilage with commercial hydrocolloids used for food production lead to consider natural gels as a cheap environmental-friendly alternative to synthetic products with industrial perspectives (Alpizar-Reyes et al. 2017; Sáenz et al. 2004). Gardiner et al. (1999) investigated cactus mucilage ability to improve infiltration taking as reference commercial polyacrylamides; their results indicate a potential use of cactus extract as a soil conditioner.

Deng et al. (2015) have shown that mucilage from Shepherd's purse seeds increases soil complex shear modulus, viscosity and yield stress, and conclude that the soil seedbank from arable fields, and especially myxospermous species, may improve soil stability. Nevertheless, the effect of myxodiasporous plants on aggregate stability, one of the most important indices of soil quality related to management and environmental impact on erosion potential (Amézketa 1999), has not been investigated yet. This study tests the hypothesis that mucilage from a myxodiasporous crop improves the stability of aggregates in agricultural soils and investigates the persistence of this effect. To this end the stability of aggregates of three different agricultural soils was measured in response to different doses of mucilage extracted from chia *(Salvia hispanica L.)* and monitored up to 30 days. The chemical nature of chia mucilages was also investigated with 13 CPMAS and SEM imaging of soil + mucilage systems and of soil adhering to hydrated diaspores covered with mucilage was performed in order to infer mechanisms of soil stabilization.

Materials and methods

Soils

Soils were collected from the surface layer (0–30 cm) of three soils in the province of Potenza (Southern Italy): Piani del Mattino Lat. 40, 651,747 Lon. 15, 832,595 (Loam), Contrada Valle di Vitalba Lat. 40,860,595 Lon. 15,647,230 (Sandy Loam), and Costa della Gaveta Lat. 40,653,593 Lon. 15 851,022 (Clay loam); physical and chemical properties are reported in Table 1.

The soil was air-dried and sieved to obtain aggregates of size 1–2 mm.

Mucilage

Black chia seeds obtained from Eichenhain (www.eichenhain.com) were used to obtain mucilage with the procedure described by Mu et al. (2012), by extraction in distilled water at the temperature of 40 °C with seed/water ratio 1:20 *w*/w. The mixture was stirred and kept for 4 h at room temperature. The mixture was oven-dried at 50 °C for about 48 h and then pushed through a sieve with openings of 1 mm.

The dry matter of the mucilage was determined gravimetrically after oven drying at 70 °C and amounted to 90% of the sample weight. The ash content was determined by igniting the oven-dried sample from the moisture content determination in a muffle furnace at 440 °C. Organic matter was calculated by subtracting percent ash content from 100. Organic carbon was calculated by dividing the percent organic matter by 1.72 and amounted to 40.3% of the sample weight.

The mucilage was characterized by ^{13}C–CPMAS NMR in solid state. The spectrometer used was a Bruker AV-300 (Bruker Instrumental Inc., Billerica, MA, USA) equipped with a 4 mm wide-bore MAS (magic angle spinning) probe. NMR spectra were obtained with an MAS of 13,000 Hz of rotor spin, a recycle time of 1 s, a contact time of 1 ms, an acquisition time of 20 ms, and 2000 scans. Samples were packed in 4-mm zirconium rotors with Kel-F caps (Wilmad / Lab Glass, Buena, NJ, USA). The pulse sequence was applied with a 1H ramp to account for nonhomogeneity of the Hartmann–Hahn condition at high spin rotor rates. Each 13C–CPMASNMR spectrum was automatically integrated to calculate the area of the peaks which appeared in the chosen region. Spectral regions have been selected and C-types identified in previous reference studies (Bonanomi et al. 2011, 2015): 0–50 ppm = alkyl C; 51–60 ppm = methoxyl and N-alkyl C; 61–96 ppm = O-alkyl C; 97–108 ppm = di-O-alkyl C; 109–145 ppm = H- and C-substituted aromatic C; 146–162 ppm = O-substituted aromatic C (phenolic and O-aryl C); 163–195 ppm = carboxyl C; 196–220 ppm = carbonyl C.

Table 1 Physical and chemical characteristics of the soils. Sand silt and clay percentage and textural class were determined according to the USDA method

Texture (Site)	pH_{H2O}	Sand	Silt	Clay	E.C. $_{1:2,5}$	$CaCO_3$	Organic C Walkey-Black	N Kjeldahl
		(%)			(µS cm^{-1})	(g Kg^{-1})		
Sandy/Loam (Piani del Mattino)	7.93	76.5	16.8	6.7	210	17.86	5.46	0.5
Loam (Valle di Vitalba)	6.77	43.6	34.2	22.1	1149	8.19	17.74	1.9
Clay/Loam (Costa della Gaveta)	8.14	42.1	26.8	31.1	588	62.51	13.84	1.4

Soil aggregate stability

We tested the combination of concentration of mucilage with three levels: (0 = control, 1% *w*/w, 2% w/w), soil texture with three levels (sandy-loam, loam, clay loam) and time after mucilage incorporation (1,7 and 30 days).

Samples of 10 g of soil were placed in airtight glass jars of 500 cm^3 volume. Mucilage was added and mixed with the dry soils. Distilled water was added to bring soil samples to 30% of field capacity (calculated as water retention at −0.033 MPa) plus 10 ml per gram of mucilage and incubated at 25 °C. At 1, 7 and 30 days of incubation three replicated jars per treatment were opened, samples were moistened under vacuum in distilled water, and then spread on the top mesh of a stack of sieves of the following mesh sizes; 1 mm, 0.5 mm, 0.2 mm and 0.1 mm. The stack was immersed in distilled water for 1 min, after which the soil was sieved for another minute under total immersion at 60 cycles min^{-1}. Aggregates remaining on each sieve were collected and each size fraction was oven-dried at 105 °C, weighed, and expressed as a percentage of the total sieved sample weight. We used this method to determine two soil structure characteristics: the size repartition of aggregates and the aggregate stability. The stability in water was calculated as the mass percentage of retained particles with effective diameter exceeding 1 mm (Traorè et al. 2000).

Carbon evolution

We tested the combination of concentration of mucilage with three levels: (0 = control, 1% *w*/w, 2% w/w), soil texture with three levels (sandy-loam, loam, clay loam) and time after mucilage incorporation (3, 10, 17, 24 and 30 days).

We tested the combination of:

A Concentration of mucilage with three levels: 0 (control), 1% w/w, 2% w/w.
B Soil texture with three levels: sandy-loam, loam, clay loam.
C Time after mucilage incorporation: 3,10,17, 24 and 30 days.

Samples of 20 g of soil were placed in airtight glass jars of 500 cm^3 volume. Mucilage was added and mixed with the dry soils. Distilled water was added to bring the soil samples to 30% of field capacity plus 10 ml per gram of mucilage. Carbon losses were measured through the titrimetric method (GU 13/03/2004 SG61), as CO_2 emission after incubation. On the bottom of the glass jars a small container containing 4 ml of NaOH 1 N was placed. Samples were incubated in the dark at 25 °C. At 3, 10, 17, 24 and 30 days of incubation three replicated jars per treatment were opened and the container was collected and treated with barium chloride, and afterwards titrated with hydrochloric acid for the determination of CO_2 production. A new becker with sodium hydroxide was introduced in the jar and incubation proceeded.

Scanning electron microscopy imaging

Images of mucilage and chia diaspores interactions with the three soils and quartz sand were taken with SEM (Scanning Electron Microscope) techniques using a Zeiss EVO LS15 and gold coating (Emitech K550X) in order to avoid electron scattering on the specimen surfaces (De-Paula et al. 2015).

Dry chia fruits were adhered to aluminum stubs and gold-coated (Robards 1978) for SEM imaging.

Chia fruits were hydrated through immersion in water at 40 °C for 4 h placed in contact with the three soils described above and quartz sand. Samples were dehydrated in oven at 25 °C for 12 h to verify the maintenance of the adhesive properties of the mucilage. For SEM images all samples were adhered to aluminum stubs and gold-coated.

Mucilage from chia fruits was mixed with the three soils used for the experiments in the proportion of 2% *w*/w and then hydrated with distilled water to 30% of field capacity and let stand for 72 h.

The samples were then dehydrated in an ethanol series, transferred to propilene oxyde, air-dried, mounted on aluminum stubs, coated with gold in a sputtering and examined in a Scanning Electron Microscope.

Statistical analysis

A factorial design was used to test the effect of mucilage addition, soil type, time of incubation and their interaction on stability index (aggregates >1 mm). Data were subjected to the Analysis of variance (3 –Way ANOVA with interaction) considering the three mentioned factors as categorical predictors. A Graphical residual analysis was carried out to check the absence of autocorrelation and residuals normality. The Least Significant Differences (LSD) test was used for post-hoc mean comparison.

Results

Mucilage composition

The composition of mucilage by ^{13}CPMAS NMR obtained in solid state is shown in Fig. 1, where signal intensity is reported as a function of the chemical shifts of different carbon atoms, while Table 2 features the different carbon atoms and percentages from peak areas corresponding to each spectral region. The main large peak of the spectrum in the interval from 61 to 96 ppm is due to the C2-C6 of carbohydrates. The anomeric carbons (C1), connected to two oxygens, resonate at lower field from 97 to 108 ppm. Finally, the interval from 163 to 195 ppm can be ascribed to the carboxyl-C of the uronic acids. Through integration of areas below peaks and their ratio with the total surface area of the spectrogram we calculate that the mucilage is composed of 93.39% carbohydrates and among them uronic acids represent 22.02%. Minor compounds are aliphatic amino acids (< 1%), aromatic aminoacids (<2%) and phenolic compounds (<1%).

Aggregate stability

Figure 2 represents the stability index as defined by Traorè et al. (2000). Effects of all tested factors and second and third degree interactions were significant ($p < 0.001$) at the analysis of variance for the stability index (Table 3) and the value of Least Significant Differences are reported as a segment on the graph. The percent weight of aggregates retained by sieves smaller than 1 mm on the stack is presented in Fig. 4 for the three soils treated with different doses of mucilage.

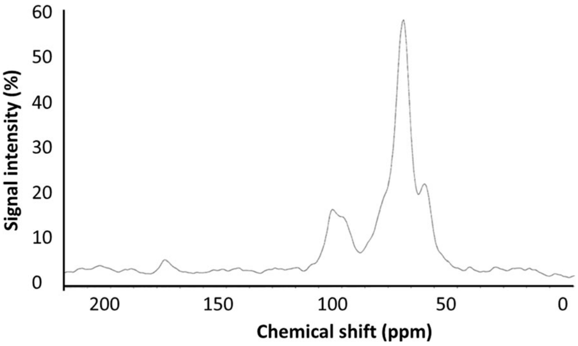

Fig. 1 Spectrogram of ^{13}CPMAS NMR of mucilage in solid state

For all soils and times of incubation the stability increased with mucilage concentration and values at 2% mucilage were always higher than the control (Fig. 2). At the dose of 2% mucilage differences in stability index were not significant between soil textures starting at day 7 (Fig. 2). In other terms, a dose of 2% mucilage overcame textural effects on soil aggregate stability by providing on average a 2.3-fold increase in the loam and clay-loam and a 4.9-fold increase in the sandy-loam compared to control soils' stability.

Compared to un-amended soil, the dose of 1% mucilage caused a significant ($p < 0.001$) increase in the stability index from day 1 in the clay loam soil, from day 7 for the sandy loam and only at day 30 for the loam. In the sandy loam at day 30 and in the clay loam differences between the 1% and 2% doses were not significant (Fig. 2). For all soils and doses incubation time did not affect the stability index significantly ($p < 0.001$), except for the sandy loam at the dose of 1% where the index at 30 days of incubation was significantly higher than at day 1 and 7 (Fig. 2).

The percentage of aggregates collected on sieves with mesh smaller than 1 mm (Fig. 3) decreased with increasing mucilage dose in all soils and the reduction was most evident in the sandy loam soil (Fig. 4) where the amount of soil and aggregates passing the 1 mm sieve was higher than 80% of the initial sample in the control soil and was reduced to less than 8% at the highest mucilage rate. The percentage of soil passing the smallest sieve (< 0.1 mm) was reduced by 97.3% (from more than 27% to less than 1%) with the highest mucilage dose in this soil.

Mineralization of organic carbon

The cumulated amount of carbon lost by the samples during incubation (Fig. 4) showed a sharp increase within 10 days of incubation, followed by a slower increase especially in the amended treatments. Our experiment did not discriminate between C pools therefore losses are to be ascribed to original soil's organic or inorganic C or carbon added with the mucilage. The overall amount of carbon evolved from soils did not coincide with C added with mucilage: control treatments lost 0.06 (sandy loam soil) to 0.33 g C kg^{-1} (clay loam) during incubation; In all amended soils less carbon was

Table 2 Peak areas and spectral range intervals of ^{13}CPMAS NMR of mucilage in solid state with the corresponding different carbon atom types and sugar signals

Spectral range (ppm)	Peak areas	Carbon types	Sugar signals
0–50	0.07	Alkyl	–
51–60	3.23	Methoxy1/N-alkyl	–
61–96	75.94	O-Alkyl	C2-C6 sugars
97–108	14.30	di-O-Alkyl	Cl sugars
109–145	1.81	Aromatics	–
146–162	0.44	Penolics	–
163–195	3.15	Carboxyl	Uronic carboxyl C
196–220	0.06	Carbonyl	–

respired than the amount added at the doses of mucilage tested in this experiment, as shown by a ratio of carbon lost to carbon added (CL/CA) ratio always <1 in the labels corresponding to trend lines in Fig. 4. Absolute values of carbon loss increased with dose of mucilage (up to 2.09 g C kg^{-1} for the sandy loam at 2% mucilage) but the CL/CA ratio was lower at high mucilage amendment: values ranged between 25 and 38% of lost over added carbon at 1% mucilage dose, and between 24 and 28% at 2% mucilage dose (Fig. 4).

SEM imaging of the seed-soil and mucilage-soil complex

The nteraction of chia fruits with soil is shown in Fig. 5. The fruit surface shows hexagonal cells. After release, the mucilage exhibited a strong adhesive character, firmly fastening to quartz sand (Fig. 5b) and soils (Fig. 4c-g) that remained adherent even after dehydration. The mucilage not only caused adhesion to the diaspore as for quartz sand (Fig. 5b), but also inter-particle bonds resulting in aggregation (Fig. 4c-e) with larger aggregates forming in finer textured soils. In the clay-loam a large lump covering completely the surface of the seed was formed (Fig. 5e). Figure 5c and d and the details in Fig. 5f, g show that after hydration-dehydration the mucilage strands form thin films providing fruit-soil and soil-soil connections.

SEM images of the three agricultural soils amended with mucilage are shown in Fig. 6. In sandy/loam soil (Fig. 6a, b, c) mucilage strands alone or covered with adhering soil created connection

Fig. 2 Interaction of dose of mucilage x time of incubation x soil type for the stability index as defined by Traorè et al. (2000). Grey bars: control treatment; black bars: 1% mucilage; white bars: 2% mucilage. Values differing more than the LSD segment represent stability index significantly different for $p < 0.01$ at the post-hoc Least Significant Difference test

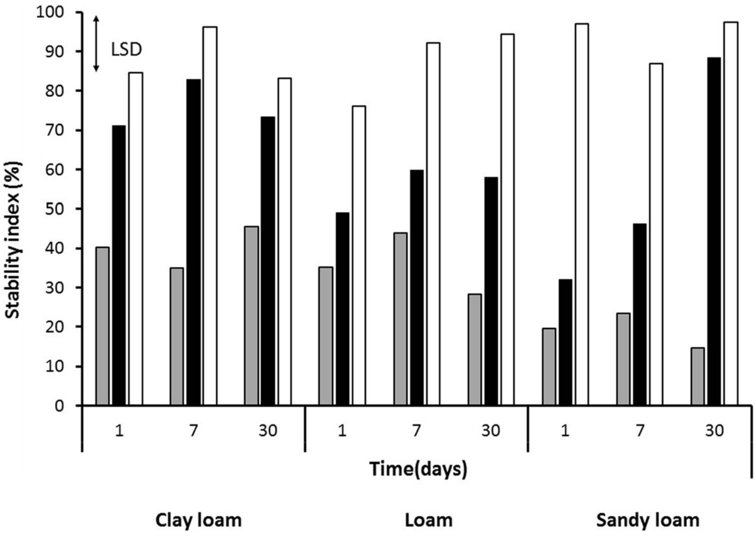

Table 3 Results of the analysis of variance for the stability index

	Df	Sum sq	Mean sq	F	Value	Pr(>F)
Time of incubation	2	0.114	0.057	11.05	0.0001	***
Soil	2	0.2	0.100	19.453	0.0000	***
Mucillage %	2	4.549	2.275	441.37	0.0000	***
Time x soil	4	0.124	0.031	6.022	0.0004	***
Time x mucillage %	4	0.144	0.036	6.989	0.0001	***
Soil x mucillage %	4	0.289	0.072	14.028	0.0000	***
Time x soil x mucillage %	8	0.352	0.044	8.534	0.0000	***
Residuals	54	0.278	0.005			

between soil particles and aggregates that are far apart. In finer textured soils (Fig. 6d-i) larger clumps are formed after mucilage treatment.

Discussion

Aggregate stability

Our study demonstrates a strong dose-dependent effect of the mucilage extruded by chia fruits upon hydration on the stability of soil aggregates, and the persistence of this effect after 30 days of incubation. Interactions of doses with soil type and time were significant. The clay loam soil showed a significant response to mucilage already at the dose of 1% since day 1 of incubation whereas in the other soils the response to low doses was slower. The dose of 2% mucilage provided a more than double increase in the sandy-loam compared to other soils. Most studies on the relations of biological mucilages with soil stability refer to root exudates or bacterial/fungal exopolysaccharides. Their effect on aggregate stability has been shown to vary according to their nature, and gums such as xanthan and PGA (polygalacturonic acids) - containing uronic groups like our mucilage - appear to increase the stability of aggregates or other rheological parameters linked to structure stability (Czarnes et al. 2000; Traorè et al. 2000; Ayeldeen et al. 2016). More recently mucilage from myxodiaspores have been studied in relation to soil physical properties as a model of other exudates (e.g. Carminati and Vetterlein 2013; Kroener et al. 2014), but mainly in relation to the hydrology of the soil-root interface. Mechanical properties relevant for stability have been investigated by Deng et al. (2015) on the wild myxodiasporous *Capsella busrsa pastoris* L. (Sheperd's purse). Besides hydrological effects, they demonstrated that mucilage from seeds of this plant improve the soil's complex shear modulus, viscosity and especially yield stress and discuss this data suggesting that the soil wild seedbank may enhance soil stability. Our data report the effect of myxodiaspores on soil aggregate stability, an index which is widely used in agricultural and environmental research to quantify the behavior of soils under management and often considered a proxy of soil erosion, crusting potential and hydrological properties (Amézketa 1999). The effect is difficult to compare with reports of other types of natural organic matter on aggregate stability (as reviewed in Amézketa 1999) or synthetic soil conditioners (as reviewed in Sojka et al. 2006), due to different methodology. However commonly used synthetic hydrogel such as anionic polyacrylamide (PAM) efficacy is influenced by texture and SOM content (Lee et al. 2010) while in our case chia mucilage at 2% inclusion sharply increases aggregate stability to the point of overcoming any difference in texture. Results from Traorè et al. (2000) with the same sieving method and time-frame as the one used in our experiment show that amendment with root exudates, PFA, glucose or model exudate containing 74.6% sugars, organic acids and aminoacids at the dose of 2 g Kg^{-1} of C induces changes of 2–4 fold values of the stability index which is the same order of what shown in our data. They also report that time-dynamics of aggregate stability was extremely variable between soils amendments and that most changes between experimental variables were observed in the class of aggregates >1 mm and <0.1 mm as in our data.

Morel et al. (1991) added maize root exudates and glucose at the rate of little over 0.2% and performed a

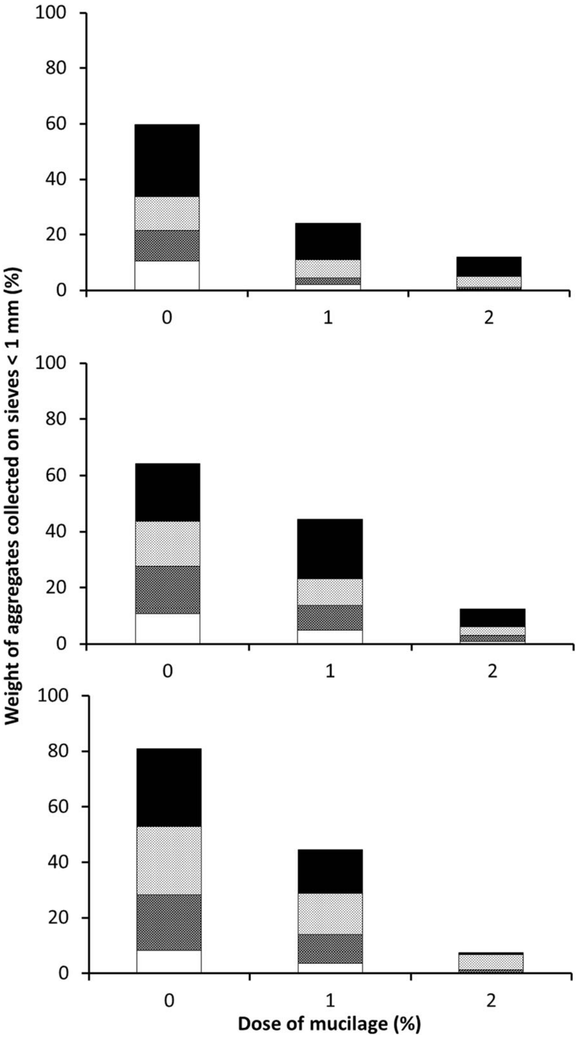

Fig. 3 Percent weight of soil collected in the wet-sieving experiment at mesh sizes <1 mm. White bars: soil collected on the 0.5 mm sieve; grilled bars: soil collected on the 0.2 mm sieve; dotted bars: soil collected on the 0.1 mm sieve; black bars: soil passing the 0.1 mm sieve. Top: clay-loam, mid: loam, bottom: sandy-loam

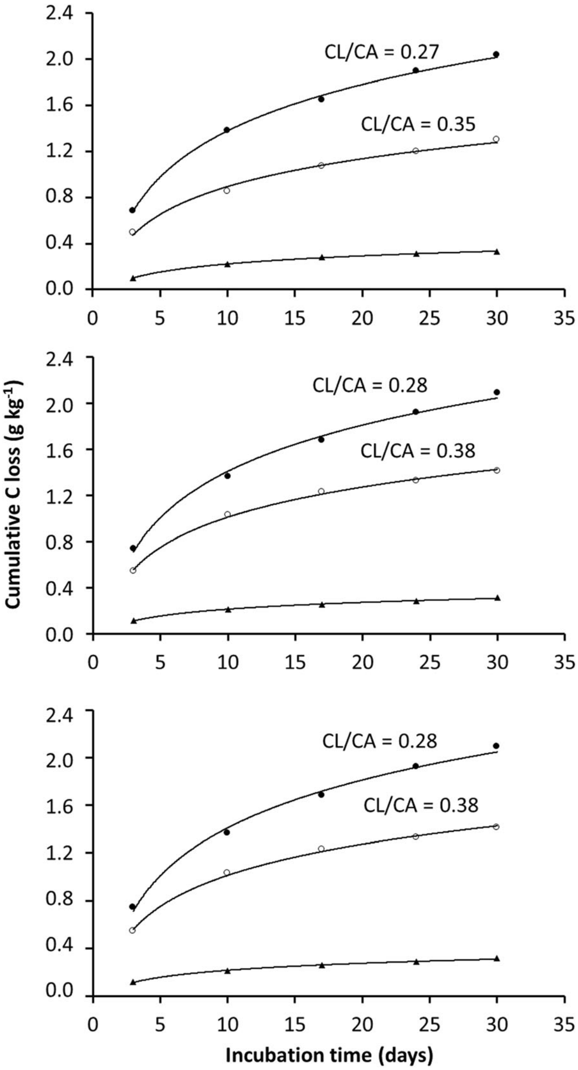

Fig. 4 Cumulative carbon loss from un-amended and mucilage-amended soils during incubation. Top: clay-loam; mid: loam; bottom: sandy-loam. Triangles: control; empty circles: 1% mucilage; full circles: 2% mucilage. Where not visible, error bars are smaller than the symbol's size. Labels on trend lines of amended soils indicate the ratio of carbon respired to carbon added (CL/CA)

wetting-drying cycle during incubation. They found a strong stabilization of aggregates depending on soil texture and decreasing after a few days of incubation, but they report that after 42 days the addition of mucilage still resulted in a higher stability in silty clay but not in silty loam.

The amount of carbon provided to soils by myxodiaspores cannot be directly compared with that of other soil amendments or extracellular polysaccharides. Research on biological exudates often refers to the work of Chenu (1995), who estimated that 0.26–1.34 g kg − 1 soil of extracellular polysaccharides are associated with microorganisms in deciduous forests or permanent grassland. Amounts of 2 g kg^{-1} of carbon are commonly used (e.g. in Traorè et al. 2000). The mucigel capsule extruded by myxodiaspores is a different type of input, quite localized around the diaspore with high concentrations establishing in a few seconds after hydration (Deng et al. 2012). Research on the effects of this mucilage on soil physical properties is only recently emerging but available work is conducted using amounts varying between 0.5 and 1.25% of mucilage

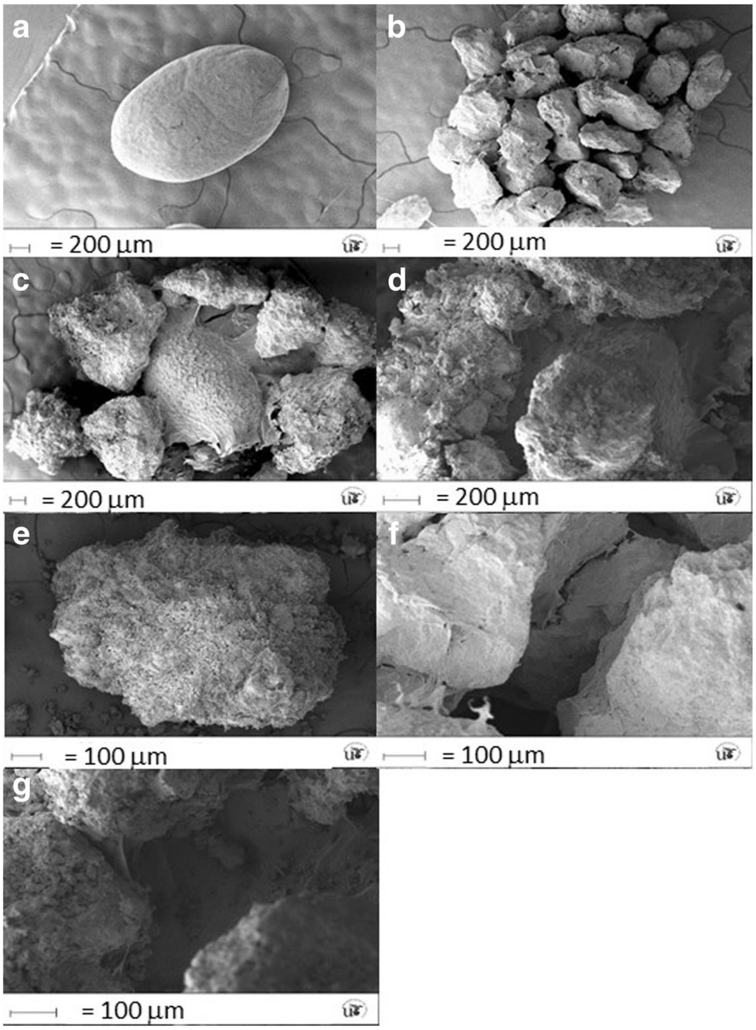

Fig. 5 Interaction of Chia (*Salvia hispanica L.*) fruits with soil. **a** dry fruit (**b**-**g**) hydrated fruits put in contact with soil and then dehydrated. **b** quartz sand **c** sandy-loam soil (**d**) loam soil (**e**) clay-loam soil: (**f**-**g**) details of mucilage membranes and filaments connected to soil

or corresponding carbon (Deng et al. 2015; Chang et al. 2015; Kroener et al. 2014). In order to provide results within the same order we therefore choose values of 1 and 2% of mucilage, corresponding to 0.37 and 0.74% (3.7 and 7.4 g kg^{-1} respectively) of C based on our ^{13}CPMAS NMR data on carbohydrate percent and a ratio of 0.4 C in carbohydrates. Our work shows that even at such high C input values the response of aggregate stability was dose-dependent for the lighter textures, and stability with 2% mucilage was still significantly higher than with 1% at day 30 for the loam soil (Fig. 2).

Our data suggest that the seeds of *S. hispanica* as a model of myxodiapores provide ecosystem services related to increasing soil aggregate stability around seeds when they are surrounded by a mucigel capsule. Engelbrecht et al. (2014) report that germinating myxodiaspores of different species resist the force of run-off at flow shear stress values corresponding to the range of values that produces detachment and transport of soil particles on hillslopes. This suggests that mucigel emission in soils can protect seeds from dispersal and soil surrounding seeds from detachment. Although this mechanism is quite localized it can contribute to soil stability locally. Deng et al. (2015) comment their findings on rheological effects of the myxodiaspore of Capsella bursa pastoris in terms of the potential of this weed for soil stabilization. In the case of *S. hispanica*, coming from an agricultural crop our findings are relevant to a management condition (early stages of germination after sowing) where the soil is generally bare and seedbed preparation has left it without protection from

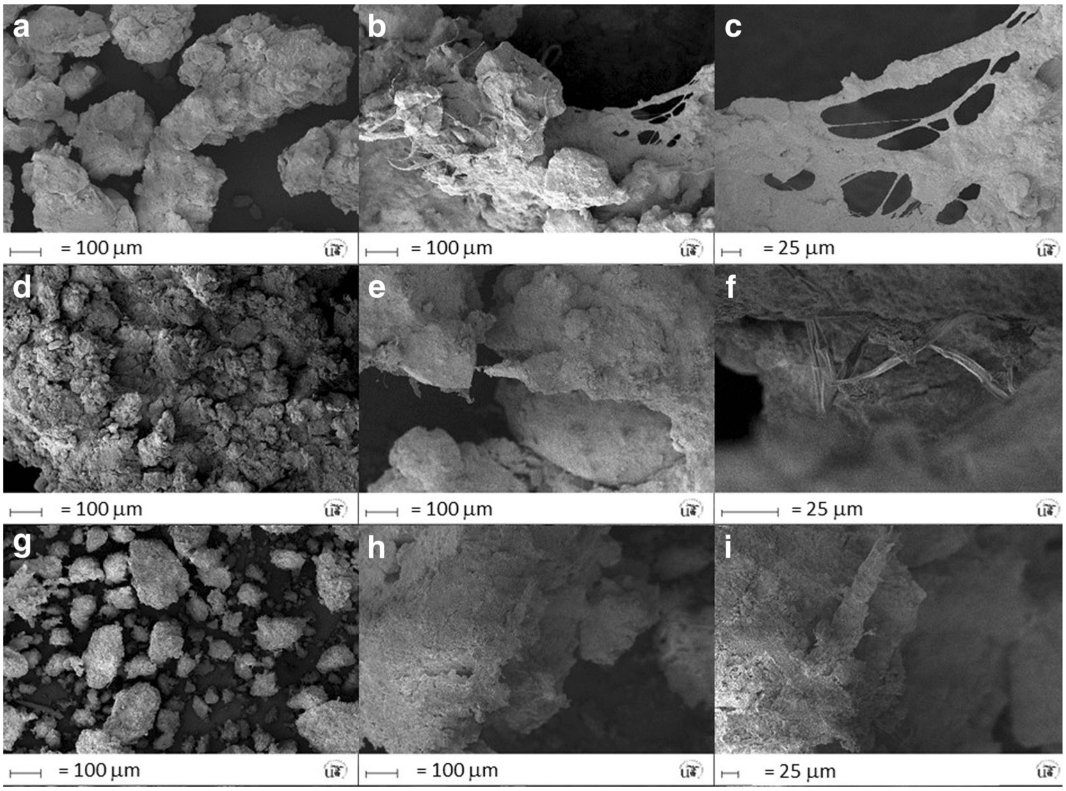

Fig. 6 SEM images of soils before and after amendment with the chia (*Salvia hispanica L.*) mucilage. Sandy-loam - (**a**) control; (**b**, **c**) mixed with mucilage. Loam - (**d**) control; (**e**, **f**) mixed with mucilage. Clay-loam - (**g**) control; (**h**, **i**) mixed with mucilage

water erosion, at least on the row where the local effect of mucigel capsule is relevant. Moreover Deng et al. (2015) showed that the effect of myxospermy on soil rheology increases at low water content thus suggesting an even greater agronomic potential of chia gel in rainfed cropping systems. The persistence of high soil stability for 30 days after mixing with mucilage indicates that the effect may cover the most crucial time for row soil erosion potential, when other mechanisms related to canopy development and reduction of water splash have not developed yet.

Our work is primarily aimed at the ecological roles of mucilage from *S. hispanica* and the methods and amounts used in this paper refer to the high concentration of mucilage around germinating seeds. Nevertheless our findings add evidence to research on natural polysaccharides as soil stabilizers (Gardiner et al. 1999). The technical and economic feasibility remain to be explored and possible alleys for future research on this issue include the development of high-mucilage yielding varieties, mucilage extraction techniques (Capitani et al. 2013) and methods of application to soils. The latter include highly efficient delivery with irrigation water in order to deposit very little amounts of conditioners on the irrigated soil surface only, rather than treating the soil to some depth (Sojka et al. 2006).

Mineralization of organic carbon

The evolution of C showed a higher emission rate in the 0–10 days incubation period and subsequently a lower rate, The total amounts of carbon lost at the end of incubation correspond to a range of 0.06 g C kg^{-1} of soil in the sandy loam without amendment to 2.09 g C kg^{-1} of soil in the amended loam and are of the same order as those found by Traorè et al. (2000) for soil with different biopolymer amendments. Absolute carbon loss was more pronounced with higher percentage of mucilage but still more than 60% of the added C was left in soils amended with 1% mucilage and more than 70% in in soils with 2% mucilage after 30 days of incubation. The persistence of a considerable amount of the added C in soils may be invoked to discuss the general duration of effects of mucilage on aggregate stability.

Nevertheless the time-dynamics of the stability index (unaffected or increasing in time after 30 days of incubation) don't match the time-course of mucilage losses from soils, and effects go beyond the simple amount of mucilage probably due to the complex development of mucilage-soil bonds in time (Deng et al. 2015). Also, effects of microflora developed during incubation may be responsible for newly developed bonds as also hypothesized by Morel et al. (1991) who found that aggregate stability did not covariate with the dynamics of mineralization of root exudate added to soils, and commented that this may be due to bonds established between soil minerals and microbial polysaccharides of new synthesis which relay the mineralized exudates. Abiven et al. (2007) explain the same phenomenon in terms of emerging fungal biomass.

SEM imaging of the seed-soil and mucilage-soil complex

The chia fruit surface shows hexagonal cells which are also shown by other authors (De la Paz Salgado-Cruz et al. 2013) as the source of mucilage strands developing throughout the fruit upon hydration and of thin films after dehydration. In our images both strands and films are shown to provide fruit-soil and soil-soil connections which create larger aggregates in finer textures. Soil-type was also relevant in modulating the extent and timing of the increase in aggregate stability. Differences in the effect of mucilage on soil aggregation (Figs. 5 and 6) and aggregate stability (Fig. 2) linked to texture and time dynamics are probably to be explained by the nature of within soil and soil-mucilage bonds. In general polysaccharides are known to interact more closely with clays (Chenu 1993) due to surface properties. Our data show that the chia mucilage used in this experiment has 22.02% of uronic acids out of 93.39% of carbohydrates in agreement with findings by Lin et al. (1994) after hydrolysis. Due to the presence of uronic groups interactions with soil may be commented in analogy to the behavior of xanthan gums in soil reinforcement (Chang et al. 2015): the strengthening mechanisms can be explained as the formation of a mucilage strands matrix or a soil-mucilage complex according to the type of bonds between mucilage and soil particles. In sands Chang et al. (2015) describe the effect as the formation of a mucilage matrix coating particles and thereby increasing their contact area and bridging particles that are not in contact. The strength of the soil-mucilage complex therefore depends on the characteristics of the mucilage matrix in the pore-space. Their measurements of soil rheology show that the dose of 1% of xanthan was enough to increase particle-particle contact and provide cementation of sand and no improvement was recorded at higher doses. In the presence of clays, in addition to coating and bridging, the electrical surface properties of fine particles allows the creation of strong interactions (hydrogen or electrostatic bonding, cation bridging or others) with this kind of mucilages. Strong bonds of biopolymers with clay minerals and cations have been known to establish for a long time (e.g. Morel et al. 1987; Gessa and Deiana 1992). In our images we see this soil-mucilage matrix in the form of large aggregates (Figs. 5d-e and 6d-i) for the loam and clay-loam. In our sandy-loam soil we see strands of mucilage partly covered with the soil's fine components (Fig. 6a-c). These strands create contact between coarser particles and aggregates which are apart and don't lump as much as the fine soils do. Bond strength is reported to increase with time ("soil curing") especially during soil drying or wetting-drying cycles due to deposition of biopolymer materials along soil surfaces, (Albalasmeh and Ghezzehei 2014) the increase in water repellency of polysaccharides upon drying (Rillig et al. 2015) and the gelling of polysaccharides at a given water content (Albalasmeh and Ghezzehei 2014).

Conclusions

The mucilage extracted from chia seeds, exerts a dose-dependent significant increase of soil aggregate stability, which persists in time after 30 days from application. Mechanisms of soil bonding analogous to xanthan and linked to the presence of charged groups can be inferred from ^{13}C–CPMAS and SEM imaging of the soil-diaspore surface and of soil amended with mucilage.

The extent and time-dynamics of aggregate stability increase together with SEM images suggest different mechanisms of soil-mucilage interactions according to soil texture. In coarser-textured soils particle coating and bridging seems to prevail while in finer textures the creation of larger aggregates is most likely the result of widespread mucilage interactions (i.e., hydrogen or electrostatic bonding) with the charged surfaces of clays.

All soils including un-amended controls lost C during the experiments. More than 60% of the carbon added with mucilage was left at the lowest dose (1% mucilage) and more than 70% remained at the highest dose (2%) after 30 days of incubation.

We report of the effect of mucilage from a diaspore on soil aggregate stability, and results are of the same order as what reported for root exudates. Our data therefore show that the germinating fruit of *S. hispanica* provides ecosystem services related to reducing the soil erosion potential. Besides the general implications of this finding, our research is relevant to a critical plant stage in agricultural management since data come from an agricultural species. This suggests that crop myxospermy is important for ensuring soil protection from erosive effects of irrigation water and rainfall during the first stages of plant growth, when the soil is generally bare and in a non-optimal state of aggregation after seedbed preparation. The persistence of high soil aggregate stability after mixing needs to be further inquired within a longer timeframe, but since we found no reduction in the aggregate stability index after 30 days we suggest that the stabilizing effect may cover the most crucial time for row soil erosion potential, when the plant has not yet developed other mechanisms related to reduction of water splash and transport linked to leaf and stem size and root anchorage to deep soil layers.

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