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Influence of maize root mucilage on soil aggregate stability

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Abstract

This study was undertaken to determine the effects of root exudates on soil aggregate stability. Root mucilage was collected from two-month old maize plants (*Zea mays* L.). Mucilage and glucose solutions were added at a rate of 2.45 g C kg^{-1} dry soil to silty clay and silt loam soils. Amended soils, placed in serum flasks, were incubated for 42 d with a drying-wetting cycle after 21 d. Evolved CO_2 was measured periodically as well as the water–stable aggregates and soluble sugar and polysaccharide content of the soil. In mucilage-amended soils CO_2 evolution started with a lag phase of 2–3 days, which was not observed in glucose-amended soils. There was then a sharp increase in evolved CO_2 up to day 7. During the second incubation period there were only small differences in evolved C between treatments. Incorporation of mucilage in both soils resulted in a spectacular and immediate increase in soil aggregate stability. Thereafter, the percent of water-stable aggregates quickly decreased parallel to microbial degradation. On completion of the incubation, aggregate stability in the silty clay soil was still significantly higher in the presence of mucilage than in the control. This work supports the assumption that freshly released mucilage is able to stick very rapidly to soil particles and may protect the newly formed aggregates against water destruction. On the silty clay, microbial activity contributes to a stabilization of these established organo-mineral bounds.

Introduction

Plant roots are known to influence aggregation of soil particles in the rhizosphere. Various changes in aggregate stability around the root have been reported, and generally improvement was recorded (Habib, 1988; Helal and Sauerbeck, 1986; Monroe and Kladivko, 1987; Oades, 1978; Tisdall and Oades, 1979). In some cases, however, a reduction in stability was observed (Reid and Goss, 1981; Reid and Goss, 1982). As roots release amounts of organic compounds in the surrounding soil, these changes in aggregate stability in the rhizosphere have often been attributed to the presence of root exudates (Oades, 1984; Reid et al., 1982; Turcheneck and Oades, 1978).

Exudates are composed of molecules of different molecular weights (Cortez and Billes, 1982; Morel et al., 1986). Exudates of high molecular weight, i.e. mucilage, are dominant compounds released by root tips (Jones and Morre, 1967). Due to its polysaccharidic nature, root mucilage material could play a significant role in the 'cimentation' of soil particles, as has been suggested earlier (Turcheneck and Oades, 1978). In plant growth experiments it was shown to bind with the surface of minerals present in natural or artificial soils (Breisch et al., 1975; Dart and Mercer, 1964; Dorioz and Robert, 1987; Guckert

et al., 1975; Jenny and Grossenbacher, 1963; Tisdall and Oades, 1982). Recently, the use of intact root mucilage, collected from maize plants (Morel et al., 1986), produced evidence of the adsorption of exudates on clay minerals (Habib et al., 1990; Morel et al, 1987). SEM observations revealed the formation of micro-aggregates between Ca-montmorillonite and root mucilage, which may be the first step in soil aggregation in the vicinity of root tips (Habib et al., 1990).

Since many factors are involved in the development of soil structure, the specific effect of root exudates on aggregate stability can hardly be observed in experiments involving growing plants. For that reason, the role of exudates and especially root mucilage has never been clearly demonstrated and the debate on aggregation processes in the rhizosphere is still controversial. This work was undertaken with intact maize root exudates to shed some light on the specific influence of exudates on soil aggregate stability around root tips where mucilage is released.

Materials and methods

Root exudates

Root exudates were collected from two-month-old maize plants grown in the field according to the method of Morel et al. (1986). Fresh material was centrifuged at 4,000 g to discard root debris and the majority of sloughed cells. Supernatant, i.e. root mucilage (RM), was stored at −18°C without any further treatment. Elemental composition of RM was 38.2% C, 5.8% H, 44.1% O, and 0.5% N, on a freeze-dry weight basis. Mucilage was composed of polysaccharides (95.0%) and contained some proteins (4.0%). Two weak acidities, which had average pKa values of 4.4 and 10.1, and represented 2.4 ± 0.1 meq g^{-1} and 0.62 ± 0.05 meq g^{-1} of RM, respectively, were determined (Morel et al., 1987). The crude material contained mineral ash, mostly as K (0.5 meq g^{-1}), Ca (0.4 meq g^{-1}), Mg (0.2 meq g^{-1}) and Na (0.08 meq g^{-1}). Root exudates were concentrated under vacuum at 40°C and C content was measured using a Carmhograph (Wösthoff) analyzer.

Soils

Soils were sampled from the A horizon of two eluviated brown soils located in La Bouzule (Meurthe et Moselle) and Mons-en-Chaussée (Aisne). Wetted soils were sieved through a 2-mm screen and air-dried. The properties of the soils are reported in Table 1.

Incubation and measurement of evolved CO_2

Soils (25 g dry weight) were added to a series of 500 cm^3 serum flasks. They were amended at a rate of 2.45 g C kg^{-1} soil with root mucilage and glucose solutions plus KNO_3 to adjust solution C/N ratio to 10. Control soils were prepared by substituting distilled water for organic solutions. Amended soils were mixed thoroughly and their final water content was adjusted to 66% of the soil water holding capacity. The flasks were sealed with covers fitted with a serum stopper for gas sampling, and placed in an incubator at 25°C. Gas samples were collected periodically from the flasks by means of a syringe and needle, and analyzed for CO_2 with an Infra Red analyzer (RUBIS 3000). The stopper was then removed and compressed air was used for 20 seconds to

Table 1. Soil properties

Texture (Site)	pH_{H_2O}	Sand	Loam	Clay	$CaCO_3$	C_t	N_t	NH_4OAc-Exchangeable			
		(%)			(g kg^{-1})			Ca^{2+}	Mg^{2+}	K^+	Na^+
								(cmol$_c$ kg^{-1})			
Silty Clay (La Bouzule)	7.6	10	45	45	10	28	2.6	35	1.3	0.2	0.13
Silt Loam (Mons en Chaussée)	7.3	8	75	17	1	11	1.1	10	0.5	0.7	0.07

Samples were collected from two eluviated brown soils (Typic hapludalf).

replenish the O_2 supply. Control of flask weight revealed no loss of water during the whole experiment. Amended soils were incubated for a first 21 d period, then rapidly dried at 40°C under air flow, re-wetted at 66% of field capacity and incubated for another 21 d.

Aggregate stability

At 0, 7, 14, 21, 28, 35 and 42 d, two flasks per treatment were removed, and soil samples were dried at 40°C. Soils were gently passed through a 2-mm sieve and analyzed for water-stable aggregates using the test of Hénin et al. (1958). In a typical test, 2 cm^3 benzene are gently added to 5 g of soil placed in a 250 cm^3 beaker. Five min later, 20 cm^3 of distilled water are roughly poured on the soil. The suspension is left for 25 min, and placed in an Erlenmeyer flask with 60 mL H_2O. The flask is inverted 20 times during 20 sec, and the suspension is sieved in water on a 200 μm sieve with a Féodoroff apparatus (Féodoroff, 1960). Unsieved material, containing stable aggregates and coarse sand, is dried at 105°C. Sand is determined after destruction of aggregates with H_2O_2. Measurements are made in triplicate per soil sample.

Carbon and polysaccharide analysis

Polysaccharides were determined using the method of Guckert (1973). Samples (5 g) were suspended in 50 cm^3 H_2O and placed in an incubator at 80°C for 24 h. The suspension was centrifuged and the supernatant adjusted to 100 cm^3. A second extraction was performed under the same conditions replacing water by 3 *N* H_2SO_4. Both supernatants were assessed for sugars with the phenol method of Dubois et al. (1956).

Results

Mineralization of organic carbon

Cumulative CO_2 evolution during the first incubation period varied significantly between the different soils over time (Fig. 1). CO_2 evolution from the glucose-amended soils showed a sharp increase within a short period of time followed by a plateau. However, in presence of mucilage, C evolved from both soils presented a lag phase which lasted at least 48 hours, and was followed by a large increase in C evolution. The total organic C respired during incubation was significantly higher in presence of mucilage than in presence of glucose.

After the drying-wetting cycle, there were few differences between C evolved from control soils, glucose- and mucilage-amended soils, indicating an attenuation of the effects of the previous treatments on evolution of C from organic material. Nevertheless, respiration was still higher in presence of mucilage than in presence of glucose and no lag phase was recorded.

Effect of root mucilage on aggregate stability

Soil aggregate stability was assessed from a test measuring water-stable aggregates after addition of benzene to soil samples. Benzene binds to organic matter, enhancing the non-wettability effect of soil organic matter. In general, the percentages of aggregates obtained with this test are lower than those determined with a test using only water. Data from both tests performed on different soil samples generally produce the same trend, but the benzene-water test appears to be more sensitive to discriminate between treatments with organic material than the water test.

Initial aggregate stability was almost nil in the silt loam soil (Fig. 2). In the silty clay soil, which was higher in clay and organic matter, the percentage of stable aggregates was about 5%. Incubation did not significantly change the aggregate stability in control soils. Additions of glucose produced a significant increase in aggregate stability in the silty clay soil during the first week of incubation. Later on, the percent of stable aggregates decreased steadily. It was almost equal to the value of the control at the end of the incubation. Additions of mucilage resulted in specific modifications in aggregate stability in both soils. A spectacular increase was observed immediately upon incorporating the mucilage. The percent of stable aggregates strongly decreased after a one-week incubation of the mucilage-amended soils. In the silt loam soil there was no significant difference between the

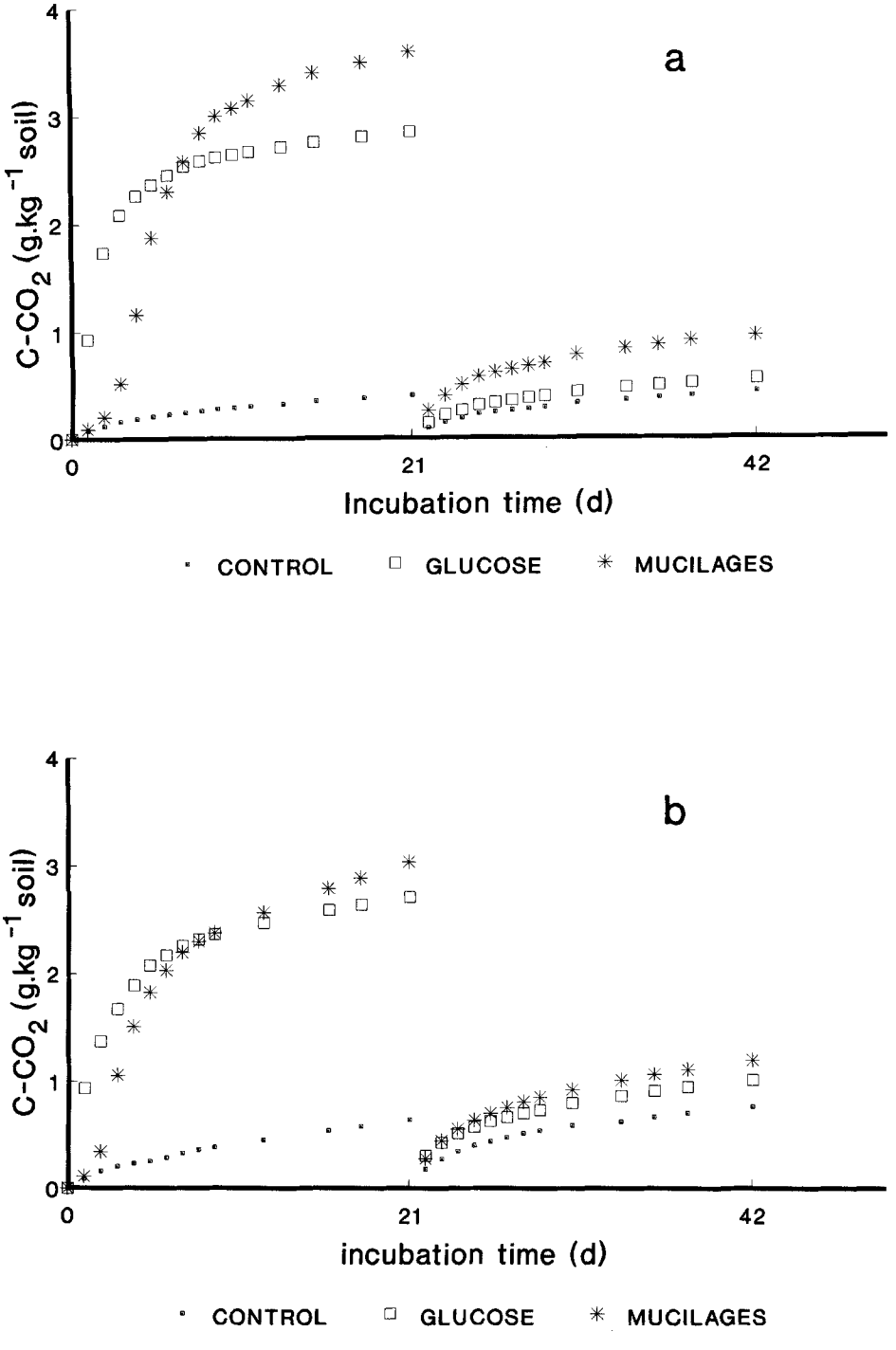

Fig. 1. Cumulative CO_2 evolution from unamended, glucose- and mucilage-amended silt loam (**a**) and silty clay (**b**). A dry wetting cycle was performed at day 21. Amendments were made at a rate of 2.45 g C kg^{-1} dry soil and incubation was conducted at 25°C. Confidence intervals calculated for daily $C–CO_2$ evolution were lower than 0.1 g kg^{-1} soil.

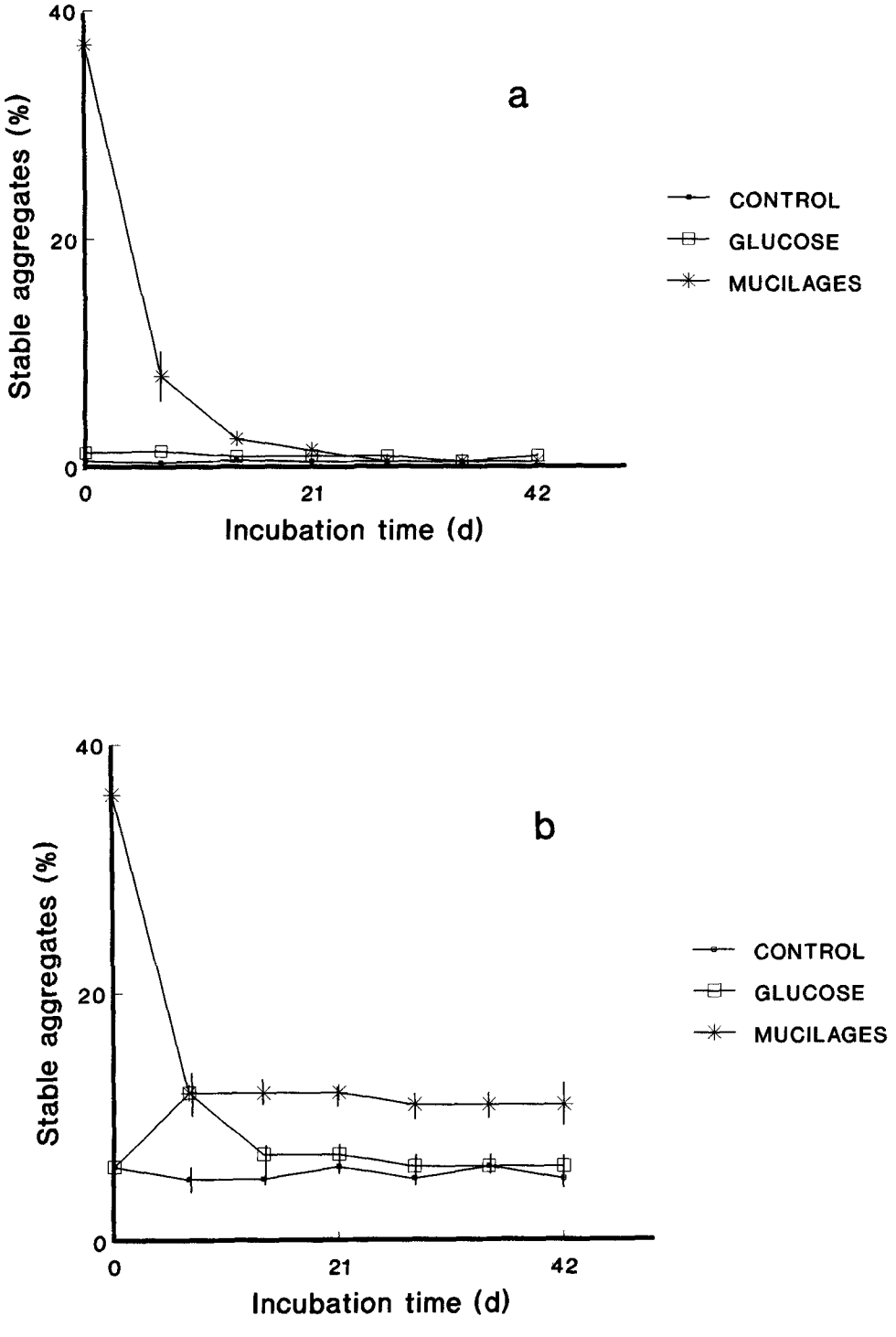

Fig. 2. Percentage of water stable aggregates during incubation of unamended, glucose- and mucilage-amended silt loam (**a**) and silty clay (**b**). Vertical *bars* indicate confidence intervals at the 5% probability level.

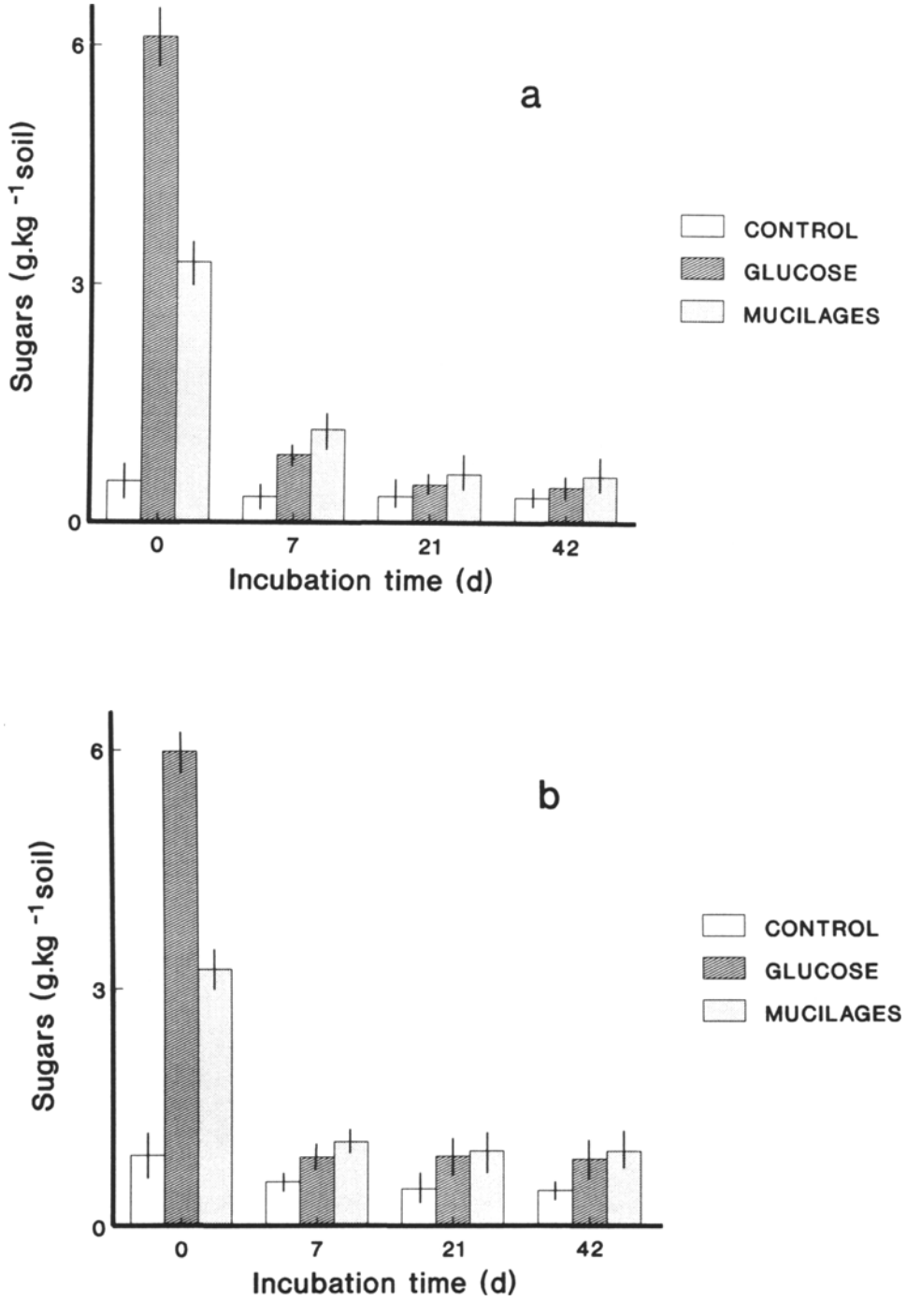

Fig. 3. Hydrosoluble sugar content during incubation of unamended, glucose- and mucilage-amended silt loam (**a**) and silty clay (**b**). Vertical *bars* indicate confidence intervals at the 5% probability level.

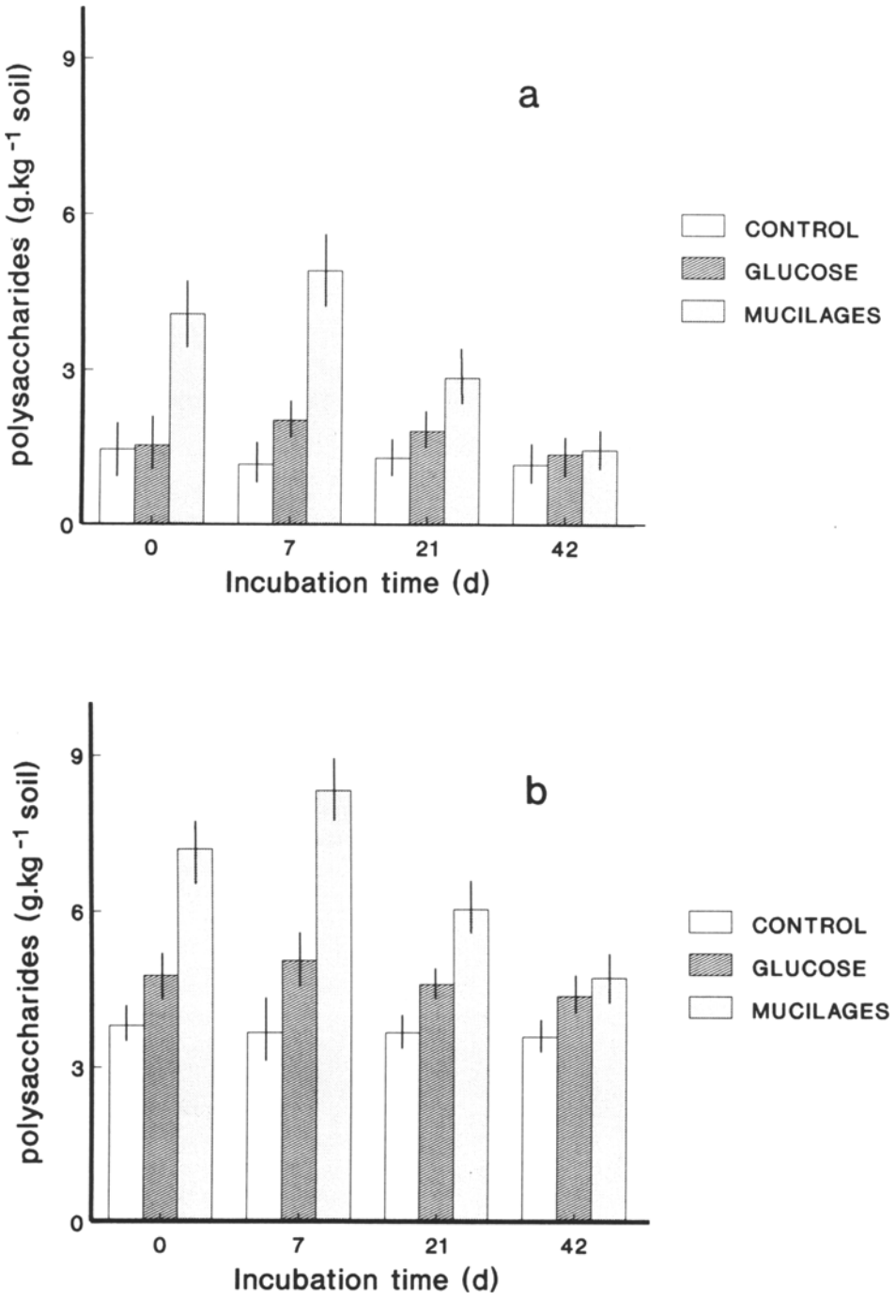

Fig. 4. Polysaccharide content during incubation of unamended, glucose- and mucilage-amended silt loam (**a**) and silty clay (**b**). Vertical *bars* indicate confidence intervals at the 5% probability level.

mucilage-amended soil and other treatments at the end of the first incubation period. In the silty clay soil, however, the decrease in aggregate stability ended after 7 days of incubation, and the percent of stable aggregates remained significantly higher than in the control. In all soils, no change was observed after the drying-wetting cycle. After 42 days the percentage of stable aggregates was still significantly higher in the mucilage-amended silty clay soil than in the control.

Soil polysaccharides

Water-soluble sugar and polysaccharide contents in soils were significantly increased by mucilage and glucose additions (Figs. 3, 4). The concentrations of water-soluble sugars decreased in all treatments during the 42 d incubation period. In the presence of glucose, an increase in polysaccharides was observed, probably as a result of microbial activity, then the polysaccharide content decreased until the end of the experiment. Additions of mucilage increased the initial content in polysaccharides. After a further increase during the first week of incubation, the polysaccharide content decreased steadily until the end of the experiment. Nevertheless, values for glucose- and mucilage-amended soils were still higher than in control soils.

Discussion

Numerous factors, e.g. soil texture, organic matter content, climatic conditions and microbial activity, are involved in the development and stabilization of the soil structure. The presence of plants may also influence soil aggregation as has been observed in pasture soils (Monnier, 1965); this is generally attributed to the presence of root exudates. Previous work has shown that maize root mucilage is mainly composed of polysaccharides and is readily adsorbed on clay minerals (Habib et al., 1990; Morel et al., 1987). In this work, evidence is given that intact mucilage released by maize root tips exerts a significant increase in soil aggregate stability. Because it occurred immediately after the incorporation of exudates into the soil, the change was independent of any microbial activity. Since it was observed on dry as well as on wet soils (Habib, 1988), it can be assumed that the improvement in aggregate stability, analogous to a 'sticking effect', was due to a direct action of the root mucilage on soil aggregates and not only to drying during soil conditioning (Reid and Goss, 1982).

Mucilage represents a source of carbon and energy for the heterotrophic soil microflora. After a lag phase in C evolution observed also by Mary et al. (1991), microbial activity in mucilage-amended soils resulted in the biodegradation of root mucilage followed by a reduction in the soil aggregate stability. It may be assumed that the longer the lag phase, the longer the protective effect of mucilage against the destruction of aggregates by water. The decrease in aggregate stability was incomplete on the silty clay. In this case, the 'sticking effect' due to mucilage was possibly relayed by the establishment of firm bounds between minerals and newly synthesized microbial polysaccharides.

Assuming that incorporating freshly collected root exudates into soil samples is a means to simulate the rhizosphere conditions at root tips, the following scheme may be proposed to describe stabilization of aggregates around growing roots. In soils, root tips release amounts of mucilage which would strongly adhere to soil particles covering the pore wall, filling the interstices, and embedding soil aggregates. The root would 'slide' into this protecting sheath (Greenland, 1979). Later on, microbial colonization of the mucilage would partially destroy the previous organo-mineral associations to an extent which depends on the abundance of clay minerals. Polysaccharides released from microbial cells would relay the mucilage and result in a persistence of organo-mineral bounds allowing for a long lasting increased aggregate stability. Furthermore, when the soil dries because of water uptake, the mucigel (i.e. the complex of plant and microbial polysaccharides (Jenny and Grossenbacher, 1963) maintains bridges forming a buffer which keeps the root partially moist (Drew, 1979). Such a scheme requires, of course, a sufficient content in clay minerals allowing for the establishment of firm and durable mucilage-mineral bounds (Habib et al., 1990).

Such a close binding between soil and root can be easily observed from uprooted maize plants which exhibit a continuum between the soil aggregates and the root itself (Vermeer and McCully, 1982).

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