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Mechanisms of stabilization of earthworm casts and artificial casts*

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Summary. Fresh casts were collected from the earthworm species *Aporrectodea caliginosa*, and artificial casts were also made. The casts were subjected to ageing, drying–rewetting, and sterilization by hexanol vapour. Clay dispersion was determined, as a measure of the lack of stability of the casts. Two soils were used, the topsoil of a recently reclaimed polder soil in the Netherlands and the topsoil from a South Australian duplex soil. For both soils the fresh worm casts had higher dispersible clay than the artificial casts. During ageing, both types of casts became more stable. There are strong indications that this was mainly due to changes on the surface of the casts. Fungi developed on the surface of 6-day-old worm casts made of Australian soil. This gave a higher stability to the casts compared to artificial casts of the same age without fungal growth. With both types of casts, hexanol inhibited fungal growth on the surface of the casts, reducing the stabilizing effect of ageing. The fungus did not develop on Dutch soil casts until after 42 days, and the development of a higher stability with age was also less marked than in the Australian soil. When the casts were subjected to a drying and rewetting cycle before analysis, they became much more stable than the casts that were analyzed wet. The drying–rewetting cycle removed most of the differences between the worm casts and the artificial casts, and also removed any effects of ageing.

Key words: Aggregate stability – *Aporrectodea caliginosa* – Casts – Dispersion index – Age-hardening – Fungal hyphae – Earthworms

Earthworms are often regarded as highly beneficial to soils. This is because they construct channels, which facilitate the penetration of air, water and roots into the soil. Also, soils rich in earthworms often have a granular, porous, and stable soil structure. Much research has been aimed at measuring the stability of worm casts compared with control soil. Many authors have suggested that worm casts are more stable than natural aggregates of the same soil (Peele 1940; Swaby 1949; Brady 1984). However, more recently it has become apparent that casts are not always more stable, especially when they are fresh and wet (Shipitalo and Protz 1988). This apparent contradiction may be partly due to differences in the mechanisms of stabilization among soil types (Emerson 1959; Dexter 1988; Dexter et al. 1988). Also, methods used to measure stability may vary highly. In particular, introducing a drying or a drying-rewetting treatment before analysis may strongly increase the stability of worm casts in some experiments, as a result of irreversible changes in structure.

Several different processes may play a role in changing the stability of worm casts. If soil is ingested by worms, large amounts of watery mucus are added to it in the anterior part of the gut (Barois 1987), and the soil undergoes a thorough kneading. This moulding of the soil will break bonds between soil particles, thereby reducing stability (Griffith and Jones 1965; Blake and Gilman 1970; Utomo and Dexter 1981). This would explain the low stability of fresh, wet casts as found in the experiments of Shipitalo and Protz (1988). Further, fresh worm casts have a very low density because they have been moulded in the worm's gut at pressures as low as 260 Pa (McKenzie and Dexter 1987). If the casts are dried after egestion, the particles are pulled steadily closer together by tension produced by water menisci and the increasing matric potential of the water (Greacen 1960; Towner 1983). These closer arrangements of the primary particles cause stronger bonding, either between clay particles, or between organic and mineral particles.

However, ageing under continuously wet conditions may also increase stability (Lütjeharms 1952; Molope et al. 1987). During wet ageing both physical (thixotropic changes) and biological processes (microbial growth) can occur. Thixotropic- or age-hardening is caused by an internal rearrangement of clay and water films, causing stronger bonding between clay particles (Utomo and Dex-

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ter 1981). Maximum thixotropic hardening occurs at water contents around the lower plastic limit of the soil and becomes less significant when soil is drier or wetter than this. The particle rearrangements take place mainly during the first 10 days after moulding (Utomo and Dexter 1981; Molope et al. 1985, 1987), or even, according to Arya and Blake (1979), during the first 24 h.

Microbial growth, also, can cause increases in stability. Bacterial cells or colonies can form gel coats, to which clay particles can adhere, thus forming micro-aggregates (Foster 1978; Emerson et al. 1986). Fungi tend to bind particles directly with their hyphae (Tisdall and Oades 1982), and grow on the outside of aggregates, thus stabilizing macro-aggregates. The influence of fungi reaches a peak, according to most studies, after 10–15 days (Aspiras et al. 1971; Metzger et al. 1987; Molope et al. 1987), but Griffith and Jones (1965) found a measurable influence on stability for up to 36 weeks.

From the foregoing, the following hypotheses about stability changes in earthworm casts were formulated. Fresh, wet casts are unstable after moulding in the worm gut. During ageing they will become more stable by thixotropic processes and binding through microbial activity. Microbial growth will be greater in worm casts compared to artificially moulded casts because of a higher organic matter content. Bacterial stimulation will lead to micro-aggregate formation, while fungal growth will stabilize the cast as a whole. Drying and rewetting casts will increase their stability, independently of other treatments. An experiment was designed to test these hypotheses.

Materials and methods

Soils

Two soils with a similar texture, but highly different in the degree of development, were used in the experiment. The older soil was sampled from a site near the Mount Bold Reservoir in South Australia (35°04′30″S, 138°42′50″E). The young soil came from the experimental farm "Lovinkhoeve" in the Noord Oost Polder (Land Parcel S38) in the Netherlands. Data on texture and chemical composition are given in Table 1. The Mount Bold soil was sampled and slightly dried before sieving with a 2-mm sieve. The polder soil was air-dried, sealed into plastic bags, packed into sealed containers, and sterilized by γ-radiation before being surface-mailed to Australia, where it was sieved through a 2-mm sieve. The fractions < 2 mm of both soils were wetted to 50 cm suction on porous ceramic plates.

Table 1. General characteristics of the soils used

	Soil	
	Lovinkhoeve (The Netherlands)	Mount Bold (South Australia)
Land use	Arable	Grassland
C (%)[a]	2.8	4.8
$CaCO_3$ (%)[a]	9.3	0.2
pH	7.3 (KCl)	4.8 (1 : 5 soil : H_2O)
Texture (%)[b]		
< 2 µm	20	13
2 – 50 µm	68	43
50 – 2000 µm	12	44

[a] Mass fractions of oven-dried soil
[b] Mass fractions of mineral material

Earthworms

Worms (*Aporrectodea caliginosa*) were collected by hand-sorting from the botanic garden of the Waite Institute and from the Mount Bold site. The worms were kept for a week in moist soil of the same type that they would be put into during the experiment. Fifteen worms were kept per 5-litre plastic container, filled with 2 kg wet soil (50 cm suction). The lids of the containers were lined with moist filter paper to keep the air humidity high in the containers. The containers were stored in a climate room with a day/night regimen of 12/12 h, with temperatures of 16 °C in the daytime and 14 °C at night. No food was added to the containers. There were two containers with Lovinkhoeve soil and three with Mount Bold soil.

Collection of worm casts

The worms were hand-sorted from the soil in the containers, rinsed clean of adhering soil, and placed overnight on moist filter paper in individual Petri dishes. The filter paper had been wetted with de-ionized water and brought to 50 cm suction on suction plates. The next morning, the worms were returned to the soil. The Petri dishes with the casts were kept cool with ice until analysis or further treatment.

Production of artificial casts

Artificial casts were made by moulding the wet soil for 2 min at a gravimetric water content of 100%, which was the water content of the freshly deposited worm casts. The slurry was then shaped into small casts by pushing it through a syringe with a mouth opening of 1.5 mm. The artificial casts were slightly bigger than the worm casts. The water content of both types of casts was well above the lower plastic limits of these soils, which would have been at gravimetric water contents of around 20%.

Further treatment

Both the worm casts and the artificial casts were analysed either fresh or after ageing in a pressure cell (12.5 cm diameter, 11.5 cm high) at 50 cm water pressure for either 6 or 42 days. Half the samples were kept in a pressure cell containing a vial with 5 ml of the sterilant hexanol. Before analysis, half the casts were air-dried, rewetted the next day over a water vaporizer for 20 min, and then left on a porous ceramic plate at a suction of 50 cm for 4 h. Ten casts were analysed for each combination of treatments.

Analysis

Individual casts were weighed in pre-weighed beakers, and 75 ml deionized water was added. The casts were soaked for 20 h. Next, the soil–water mixture was stirred, and after 1.5 h the dispersed clay was determined by pipetting a 5-ml aliquot at a 2-cm depth. The concentration of clay in the sample was assessed by measuring the turbidimetry in a Hach Ratio Turbidimeter and comparing this with a standard curve. The remaining soil–water mixture was then treated with an ultrasonic probe for 1 min, while being cooled and stirred. This was expected to bring all the clay into suspension (Wace and Hignett 1988). The clay released was measured again by pipetting after 1.5 h. The beaker with the remaining soil was then dried in an oven at 105 °C for 24 h, and the soil dry mass was determined after cooling in a desiccator.

Two different methods were used to stir the soil–water mixture after the 20-h soaking. One method used a paddle-stirrer, which only brought back into suspension the clay that had dispersed (seeped) out of the cast during the soaking, and the second method used a magnetic stirrer, which broke down the cast. With the paddle-stirrer, the capacity of the surface structure to prevent clay dispersion can be tested. The magnetic stirrer, in contrast, provides information about the dispersion potential of the clay inside the cast. If the clay is bound into micro-aggregates, the internal stability may be lower than if the micro-aggregates are not

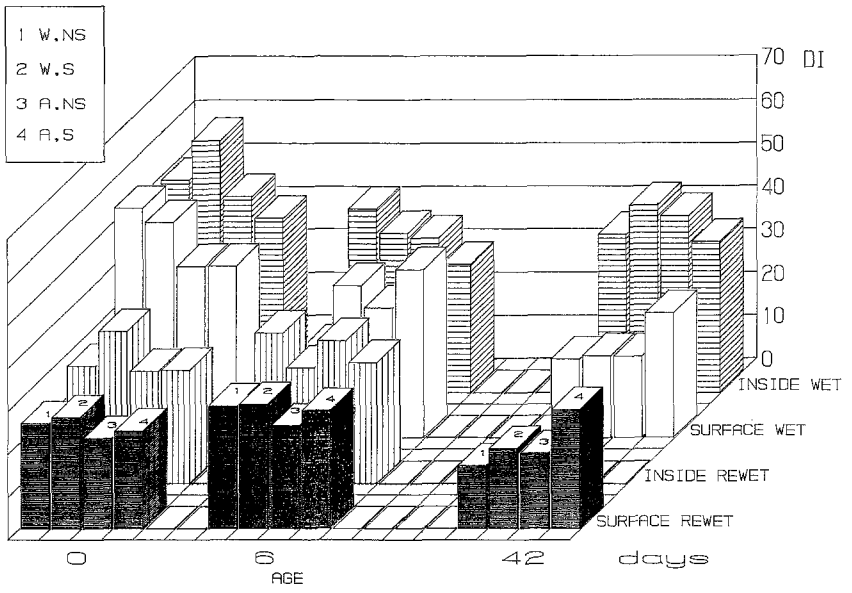

Fig. 1. Dispersion index (in %) of casts made of Mount Bold soil. *W,NS*, worm casts, non-sterile; *W,S*, worm casts, sterile; *A,NS*, artificial casts, non-sterile; *A,S*, artificial casts, sterile

present. Thus the internal stability may be an indirect measure of the degree of micro-aggregation.

Worm casts and artificial casts differ in their clay contents (Shipitalo and Protz 1988). Therefore, the amount of clay dispersed was related to the total amount of clay in the cast by obtaining the mass ratio between dispersed clay and total clay. This was called the dispersion index.

Statistical testing

The measurements of the dispersion index were replicated 10 times. However, because casts of different ages were not kept in the same pressure cell, the measurements cannot be seen as complete replications of the treatments. Therefore a $2\times2\times2\times2\times3$ analysis of variance was carried out on the means of the dispersion index per combination of treatments, using the PC version of the Statistical Package for the Social Sciences (SPSS). To make the design more complete, 5 of the 10 replicates from age 0 were randomly assigned to be either sterile or non-sterile. Because some internal stability values for the age of 42 days were missing, only the main effects and two-way interactions could be tested.

Results and discussion

The results for the Mount Bold soil are given in Fig. 1, and for the Lovinkhoeve soil in Fig. 2. The results of the statistical analysis are given in Table 2. Dispersion index values ranged from 50–60% for fresh worm casts, to between 5 and 20% in dried and rewetted, aged casts. The Lovinkhoeve soil worm casts were generally less stable than artificial casts made of the same material. This was reflected in a significant main effect. Both worm casts and artificial casts became more stable with age, but most strongly in the worm casts, as evidenced by a significant interaction between the factors of age and type. After ageing for 42 days, the worm casts were still slightly less stable than the artificial casts. In the Mount Bold soil there was no significant main effect of the type factor, but there was an interaction with the age factor; the fresh worm casts were less stable than the fresh artificial casts

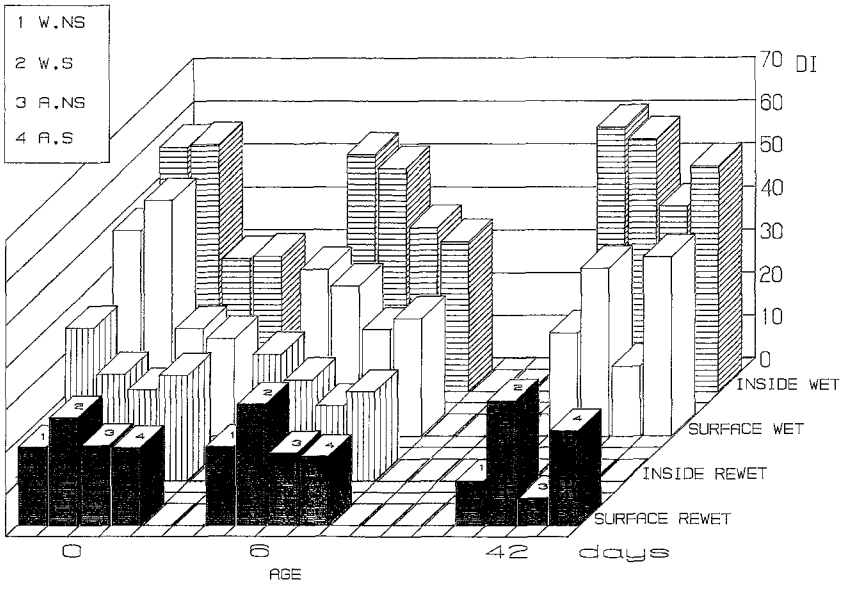

Fig. 2. Dispersion index (in %) of cast made of Lovinkhoeve soil. For abbreviations see Fig. 1

when analysed wet, but in older casts this pattern was less clear. Differences in properties of the Mount Bold and Lovinkhoeve soils may explain the dissimilarities in behaviour of the two soils as reflected by changes in the dispersion index in the worm casts and the artificial casts. The Mount Bold soil is very old, and most of its clay is probably aggregated into micro-aggregates. The Lovinkhoeve soil, however, has undergone structural formation for only 45 years. Therefore the Lovinkhoeve soil may be more susceptible to the relatively thorough kneading inside the worm gut, than to the less intensive disruption caused by artificial moulding. This may explain why the ratio between the dispersion index of the artificial casts and that of the worm casts was often much lower for the Lovinkhoeve soil than for the Mount Bold soil.

As expected, there were also significant differences between the stability of the aggregate surface and the internal stability. In both soils the differences between these two measures of stability were smaller in fresh casts than in older ones. Throughout the experiment, the changes in internal stability in the two soils were negligible. However, the surface-related stability in both soils increased somewhat with ageing. This interaction between the factors of method and age was significant in both soils. In the Mount Bold soil there was a big difference between the wet, surface-related stability at the age of 6 days and all the other wet treatments. In the Lovinkhoeve soil the pattern was different; here the main differences were between the surface-related stability of the non-sterile treatments at the age of 42 days and all others, which was reflected in a significant interaction between the factors of age and hexanol. If it is true that the internal stability depends on the percentage of micro-aggregates, the absence of change in the internal stability during this experiment implies that practically no new micro-aggregates were formed in any of the treatments.

Table 2. Results of analysis of variance: P values

Factor	Mount Bold	Lovinkhoeve
Main effects	0.000*	0.000*
Method, surface vs inside	0.000*	0.000*
Dry-rewetting (DRew)	0.000*	0.000*
Type, worm vs artificial	0.076	0.000*
Age	0.000*	0.311
Hexanol, sterile vs non-sterile	0.185	0.018*
2-Way interactions	0.001*	0.001*
Method/DRew	0.851	0.177
Method/Type	0.157	0.450
Method/Age	0.006*	0.016*
Method/Hexanol	0.068	0.036*
DRew/Type	0.364	0.002*
DRew/Age	0.000*	0.945
DRew/Hexanol	0.850	0.696
Type/Age	0.015*	0.040*
Type/Hexanol	0.548	0.521
Age/Hexanol	0.684	0.006*

*$P<0.05$

In both soils, a single drying–rewetting cycle led to a sharp reduction in the dispersion index and resulted in fairly constant values during the ageing. This is in accordance with the findings of Shipitalo and Protz (1988). The highest reduction in the dispersion index caused by a drying–rewetting cycle in Mount Bold soil occurred in fresh casts, and was reflected in a significant interaction between the factors of age and rewetting. In the Lovinkhoeve soil the dispersion index did not decrease significantly with age, and only a main significant effect of the rewetting factor was observed. Regardless of soil type, the worm casts and the artificial casts reacted similarly upon drying and rewetting. This suggests that organic binding materials, which are expected to differ in worm casts from those in artificial casts, do not play a major role in the enhancement of stability upon drying.

In the non-sterile samples after 6 days a net of fungal hyphae developed on the worm casts of Mount Bold soil. The appearance of the hyphae was associated with a large difference between the internal and the surface-related stability. The hyphae were still present in a melanized form on the casts after 42 days. By this time, a few hyphae were visible on the artificial casts. In the Lovinkhoeve soil, however, there was only a very slight development of fungus on some worm casts after 6 days, with more growth on both worm casts and artificial casts after 42 days. This slow rate of fungal growth compared to the Mount Bold soil may be related to the sterilization with γ-radiation, or to other soil properties, e.g., the Lovinkhoeve soil is poor in fungal growth in field situations (J. A. Van Veen, personal communication, 1989). In both soils, hyphae were invariably absent in all hexanol treatments, but inside the casts bacterial growth occurred in all cases (Foster and Marinissen, unpublished results, 1989). The pattern of fungus growth was closely related to the pattern of surface-related stability in both soils. Because the fungal hyphae formed a net on the surface of the casts, and are known to bind particles with their hyphae, the stabilization of the older casts from both soils was probably caused by the growth of fungus on the surface.

Conclusions

This study confirmed some of the hypotheses formulated in the Introduction. The first is that, because of moulding in the gut, earthworm casts are susceptible to dispersion when wet and fresh. This effect seems to be stronger in soils with a low structural organization. Bacterial growth occurred in both artificial casts and worm casts, but as far as could be measured by the methodology used, this did not lead to a higher percentage of micro-aggregates. Direct estimation of the amount of micro-aggregates will be necessary in the future. However, it is likely that repeated stimulation of bacterial growth, probably in combination with drying–rewetting cycles, causes the formation of micro-aggregates over a number of cycles of cast formation. Worm casts can become colonized by fungi very quickly, and this will increase the surface-related stability. The stability of surface-sterilized casts of

both types, when subjected to ageing, did not increase as much as that of casts of the non-sterile treatments. This implies that the biological processes were more important than the age-hardening. This is probably because earthworm casts are too wet for thixotropic processes to occur to a significant effect. One drying–rewetting cycle produced an instant increase in stability, but the same levels of stability came about by fungus grown on casts of Mount Bold soil after 6 days. However, it can be assumed that the effects of drying–rewetting will be more persistent with time than the effect of the hyphae.

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