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AN INCUBATION EXPERIMENT TO DETERMINE FACTORS INVOLVING AGGREGATION CHANGES IN AN ARID SOIL RECEIVING URBAN REFUSE

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Summary—The effect of the addition of urban refuse (UR) on the percentage of stable aggregates in a semi-arid structureless soil was studied in a 67-day incubation experiment. Twelve systems were established combining amendments with two doses of UR (2.4 and 4.8%) and treatments with cycloheximide, chloramphenicol or formaldehyde to evaluate the relative efficiency of carbohydrates and microbial (fungi and bacteria) communities in promoting changes in aggregate stability. The addition of UR to the soil increased the abundance of stable aggregates and this increase was proportional to the quantity of UR added. The degree of aggregate stability was related to all the factors considered, although this relationship was more evident for the fungal communities ($R = 0.81$, $P < 0.001$) and, to a lesser extent, for the bacterial communities and carbohydrate content. Carbohydrates were significantly correlated with stable aggregates during the first stages of incubation ($R = 0.83$, $P < 0.001$, at day 4), but this effect was short-lived and the maintenance and increase of the aggregate stability in the subsequent phases was attributable to the increases in microbial populations, and particularly to the proliferation of fungal mycelium.

INTRODUCTION

Many factors influence the structural stability of a soil. When the soil is neither saline nor sodic this stability is related to the texture, the organic matter content and iron and aluminium oxides (Goldberg *et al.*, 1990). Several correlations have been established between the improvement observed when organic matter is added to soil as an aggregate stabilizing agent, and the quantity of organic matter added (Christensen, 1986; Bernal *et al.*, 1987; Mbagwn, 1989). Most studies refer to particular organic matter fractions as the cause of this improvement. Dinel *et al.* (1991) found that long-chain aliphatic compounds play an important role in this respect, while Fortún *et al.* (1990) thought that the fulvic and humic fractions of the organic matter were most important. However, it is polysaccharides which have received most attention as possible stabilizers of soil structure (Cheshire, 1979; Theng, 1979). Some studies have concentrated on the polysaccharides produced in the soil during crop rotation (Roberson *et al.*, 1991) or during the addition of organic matter to a soil (Lax and García-Orenes, 1993).

The addition to the soil of organic residues rich in easily-decomposable carbohydrates results in an increase in the soil microflora (Lynch and Bragg, 1985) and in aggregate stability (Metzger *et al.*, 1987). The importance of microbial populations as a determining factor in soil aggregation has been stressed by Anderson (1991) and Jastrow and Miller (1991). The increase in stability produced by microorganisms can be of a physical nature (Tisdall and Oades, 1982) or through the formation and excretion of polysaccharides which act as binding agents (Cheshire *et al.*, 1983; Burns and Davies, 1986). Urban refuse (UR) can improve the quality of disturbed soils (Albaladejo and Díaz, 1990; Roldán and Albaladejo, 1993) by both promoting soil microbial populations and adding carbohydrates.

We studied the effect of UR application on the proportion of stable aggregates in a semi-arid structureless soil. Our aim was to evaluate the relative efficiency of bacteria, fungi and carbohydrates in promoting changes in aggregate stability.

MATERIALS AND METHODS

Materials

The soil used was a Xeric Torriorthent with a loamy clay texture, developed from marls. It was collected from Abanilla, located 30 km north of Murcia (Spain). Similar soils are found in many semi-arid zones along the Spanish Mediterranean coast and are characterized by low organic matter content and poorly developed structure. As a result they are degraded by erosion.

The refuse used in this experiment was a solid fresh material (without composting or grinding), which had undergone 10–15 days of natural maturation. The refuse came from the Murcia municipal treatment

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Table 1. Analytical characteristics of the soil used (% by wt)

Elect. conduct. (1:5 dS m^{-1})	1.66
Organic matter (%)	0.75
N total (%)	0.06
$CaCO_3$ (%)	54.00
P available (mmol kg^{-1})	0.16
K^+ available (cmol kg^{-1})	1.02
Na^+ (cmol kg^{-1})	0.91
Cl^- (cmol kg^{-1})	0.30
SO_4^{2-} (cmol kg^{-1})	5.44

Table 2. Analytical characteristics of the UR used (% by wt)

Water content (%)	23.1
pH (w. ext. 1:10)	7.9
Elect. conduct. (1:10 dS m^{-1})	3.8
Ashes (%)	40.7
Organic matter (%)	43.0
Carbohydrates (%)	13.0
N total (%)	1.2
P total (%)	0.6

plant. The analytical characteristics of both soil and UR, as determined by standard methods (Page *et al.*, 1982) are shown in Tables 1 and 2.

Incubation procedures

Twelve systems were studied combining amendments with two UR doses (2.4 or 4.8%) and treatments with a fungicide (cycloheximide, Merck, 0.1%), a bactericide (chloramphenicol, Merck, 0.2%) or a general biocide (formaldehyde, Probus, 50 g l^{-1}) as shown in Table 3.

The mixtures were prepared by mixing 4.5 kg air-dried soil with their corresponding amounts of air-dried mechanically ground UR (<2mm) in 5-litre pots. The inhibitors were added to the mixtures with deionized water to 75% WHC. Pots were covered with a slightly perforated film and kept at 25°C and 75% r.h. To maintain soil moisture, the pots were weighed every 5 days and supplied with deionized water. Systems treated with biocides were supplied with their respective inhibitory solutions. Samples of the systems were taken for analysis after 0, 1, 4, 11, 25, 46 and 67 days of incubation.

Measurements

At each sampling, the contents of each system were mixed and 70 g of mixture per pot was removed and thoroughly mixed. Then 10-g aliquots of the samples were taken for fungal and bacteria counts and fixed with formaldehyde (4% v/v). The remainder was dried at 60°C and sieved (2 mm). The sieved samples were stored at room temperature until analysed for carbohydrate content and stable aggregate percentage. All the measurements were made in triplicate.

Total bacterial numbers were estimated by microscopic observation with epifluorescence after staining with acridine orange (Merck, 1%) and filtering through 0.2 μm Nucleopore polycarbonate membranes (Hobbie *et al.*, 1974). Two methods were used for homogenization of the samples: (I) moderate mechanical stirring after the addition of a non-ionic detergent (Triton X-100, 0.01%); and (II) cold sonication with a Dinatech sonicator (2 batches for 45 s, 0.7 output). Mycelium length was calculated by microscopic observation after staining with trypan blue (Merck, 0.05%) and filtering through 1.2 μm Millipore cellulose membranes (Hanssen *et al.*, 1974; Bardgett, 1991).

The carbohydrate content was determined by the anthrone colorimetric method of Brink *et al.* (1960) after hydrolysis with 1 N H_2SO_4 (120°C, 5 h) using glucose standards.

The percentage of water stable aggregates was determined by the method of Benito *et al.* (1986), modified as follows. A portion of sample was dry-sieved between 1 and 2 mm. Aliquots of 4 g of the sieved soil were placed on 7.5 cm dia 250 μm mesh size sieves and subjected to an artificial rainfall of 150 ml. This rainfall was performed by arranging a cylindric reservoir of 6 cm dia at 1 m height, with the bottom bored by 11 holes, in each one a pipette tip was assembled. The water drops fall on the soil sample distributed in a practically uniform way, with an energy of 270 J m^{-2}. The soil remaining on the sieve was dried (105°C, 5 h) and weighed (P_1). The air-dried soil was moistened and again sieved (250 μm) with the assistance of a small glass stick to disrupt the remaining aggregates. The particles on the sieve—coarse sand, small stones and some non-humified

Table 3. Description of the systems studied

System	Amendment (% UR)	Increase (in % org. C)	Treatment	Fungi	Bacteria
1	0.0	0.0	—	+	+
2	0.0	0.0	Chloramphenicol 0.2%	+	−
3	0.0	0.0	Cycloheximide 0.1%	−	+
4	0.0	0.0	Formaldehyde 50 g l^{-1}	−	−
5	2.4	0.6	—	+	+
6	2.4	0.6	Chloramphenicol 0.2%	+	−
7	2.4	0.6	Cycloheximide 0.1%	−	+
8	2.4	0.6	Formaldehyde 50 g l^{-1}	−	−
9	4.8	1.2	—	+	+
10	4.8	1.2	Chloramphenicol 0.2%	+	−
11	4.8	1.2	Cycloheximide 0.1%	−	+
12	4.8	1.2	Formaldehyde 50 g l^{-1}	−	−

+, Expected growth; −, expected decline.

organic matter—were dried (105°C, 5 h) and weighed (P_2). The percentage of water-stable aggregates with regard to the total aggregates was calculated by $(P_1 - P_2) \times 100/(4 - P_2)$.

RESULTS

Aggregate stability

Soils which were not treated with biocide (Fig. 1) showed an increase in stable aggregates, which was directly related to the amount of UR employed. Both the control and soils to which refuse had been added showed similar stable aggregate values of around 20% at time zero. These values had increased at 24 h, but after 11 days the increases were much larger in some cases, exceeding the original values 4-fold. These values then remained stable until the end of the experiment, except with the minimum UR addition, when the increase in stable aggregates at the end of the experiment was notable.

Soils treated with chloramphenicol to inhibit bacteria (Fig. 1) showed a similar behaviour to the above with significant increases in the proportion of stable aggregates. There were no significant differences between the two doses of UR used, although both were greater than the control. At the end of the experiment the percentage of stable aggregates in each treatment was similar to those in the previous system.

The soils treated with cycloheximide to inhibit fungi (Fig. 1) showed percentages of stable aggregates significantly less than those of the two previous systems. However, they generally followed the same pattern with appreciable differences between the soils receiving UR and the control. In this treatment, the percentage of stable aggregates increased slowly but constantly throughout the experiment with a pronounced growth being observed just before the final sampling.

The formaldehyde-treated soils showed erratic changes. The initial percentages of stable aggregates were slightly greater than in the other treatments, and varied erratically with time; no significant differences were observed between the doses of UR.

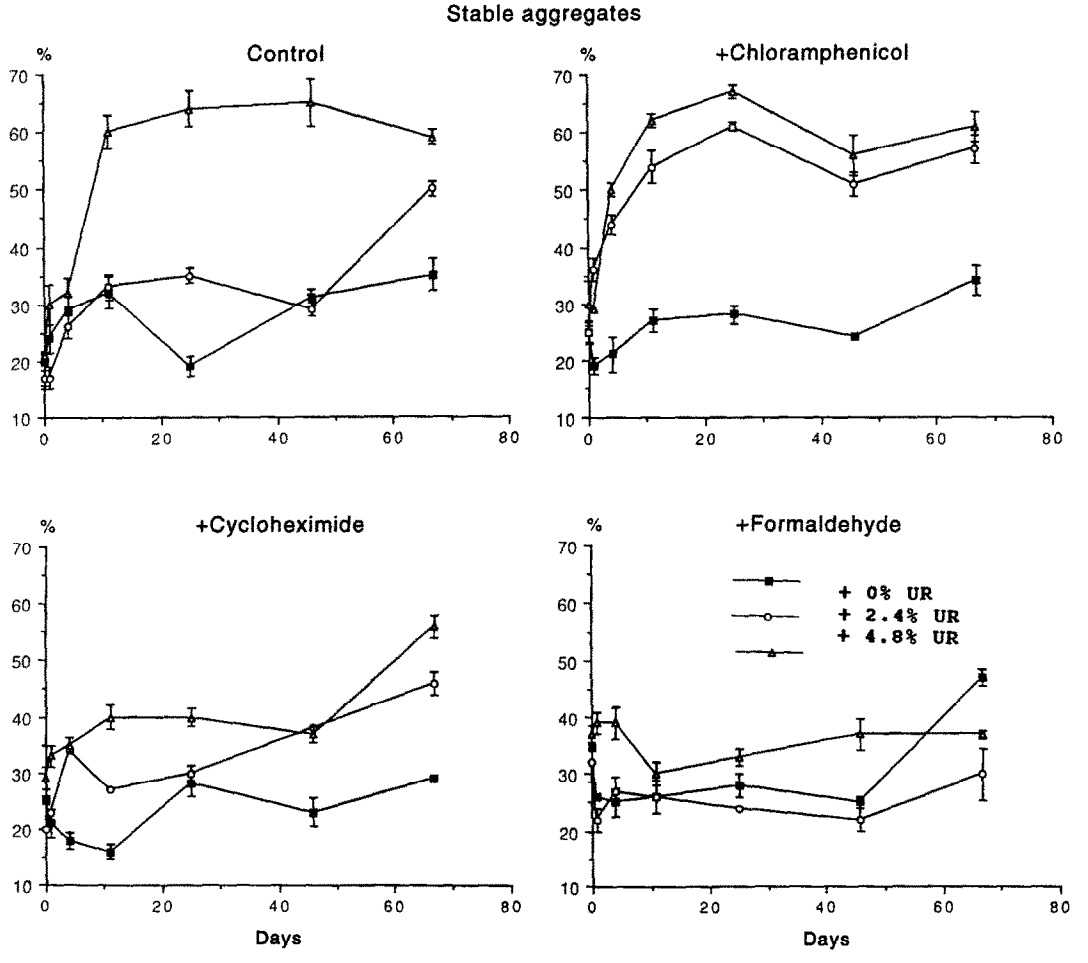

Fig. 1. Effect of UR addition and microbial inhibition treatments on the content of stable aggregates (1–2 mm) in an arid soil during 67 days of incubation.

Carbohydrates

In the systems which received no biocide treatment (Fig. 2), the initial concentration of carbohydrates increased in relation to the amount of UR applied. During the first stages of incubation, the carbohydrate content of the soil receiving the larger UR dose exceeded that of the control four-fold. However, it decreased with time and the final values were about 50% less than the initial value. The differences between UR treatments persisted throughout the experiment.

In the system containing chloramphenicol (Fig. 2), the initial carbohydrate concentrations increased, as in the previous system, and were related to the dose of UR applied. During the experiment the concentration of carbohydrate generally decreased, but there were some increases and final concentrations were similar to those of the no biocide treatment.

In the mixtures treated with cycloheximide the initial concentrations of carbohydrates were also related to UR dose. They remained almost constant during the first stages of incubation but were similar to those of the previous two systems by the end of the experiment.

For all four treatments there was an increase in the carbohydrate content, related to the dose of UR. With the exception of the soils treated with formaldehyde, there was a more or less pronounced decrease with the amount of carbohydrates in the soils, and at the end of the experiment all had similar amounts, which were increased by greater applications of UR.

Bacterial numbers

In the systems to which biocide agents had not been added (Fig. 3), there was a rapid growth of the bacterial numbers in both the sonicated and unsonicated samples, reaching maximum values near to 10^{11} g^{-1} for the larger UR dose before 20 days of incubation. After this time the bacterial numbers of the sonicated samples remained constant until the end of the experiment, but that of the unsonicated samples decreased gradually to values $< 10^{10}$ for the larger doses of UR. In all cases the addition of UR led to an increase in the total number of bacteria.

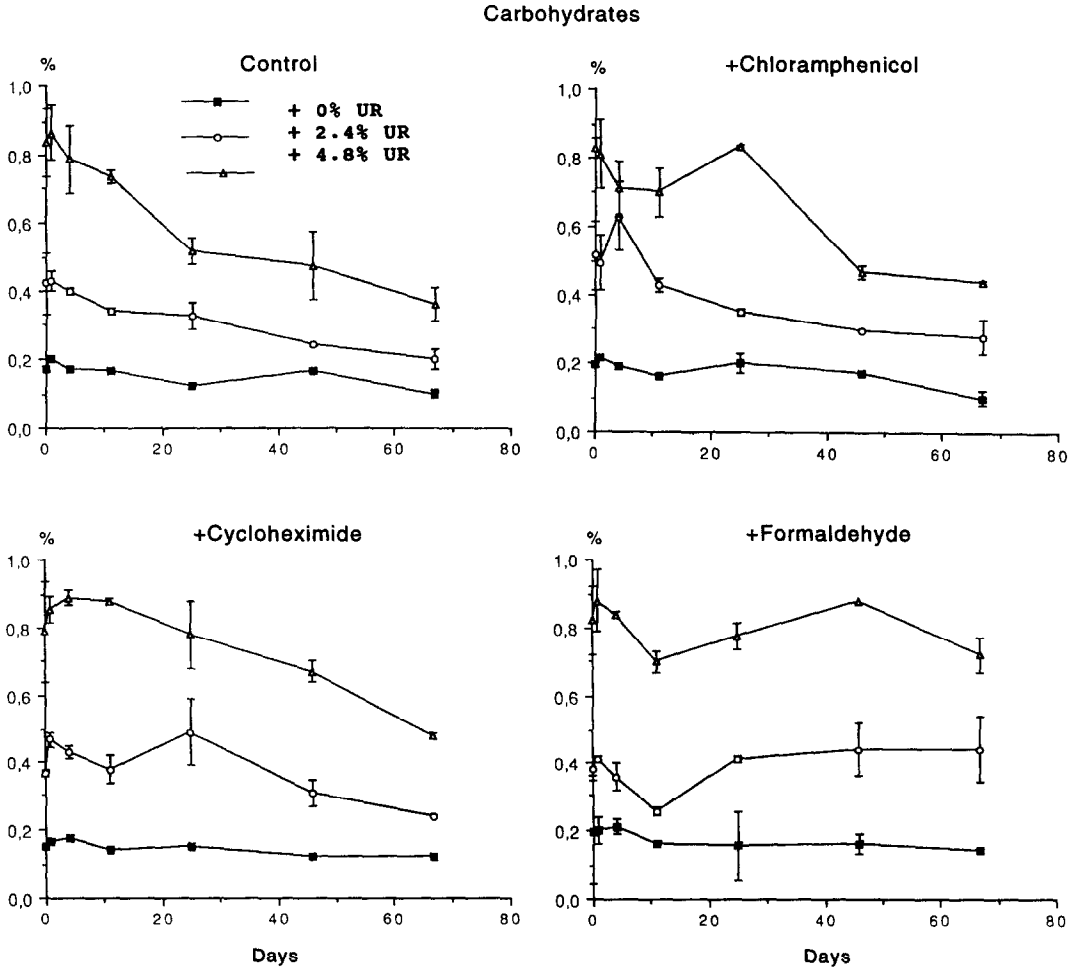

Fig. 2. Effect of UR addition and microbial inhibition treatments on the content of carbohydrates in an arid soil during 67 days of incubation.

The systems treated with cycloheximide (Fig. 3) showed similar results, the maximum being practically the same although these numbers fell more rapidly. Bacterial numbers in the unsonicated samples fell more sharply and final values were lower. As with the previous treatment, bacterial counts were higher with higher doses of UR.

Growth rates were much slower in the systems treated with the bactericide chloramphenicol (Fig. 3), reaching maximum numbers of $<10^{10}$ for the highest UR dose at the end of incubation. The unsonicated samples showed slightly higher growth rates during the early days of incubation, thereafter following the same evolution as the sonicated samples. The highest values were once again recorded with the highest UR doses.

As for the samples treated with formaldehyde (a non-selective biocide) (Fig. 3), bacterial numbers fell throughout the incubation, especially at the outset. Maximum values ($<10^8$) were recorded in the first sample, although at the end of the incubation they were $<10^4$. There was little difference between the sonicated and unsonicated samples. Nor was there any difference in bacterial numbers between the doses of UR used, indicating little or no bacterial growth.

Mycelium length

A count of total mycelium length in systems containing no biocide (Fig. 4) showed rapid growth during the early days of incubation. Maximum values of approximately 10^3 m g^{-1} were obtained in the soil receiving the maximum UR dose after about 25 days. These values remained stable before falling slightly at the end of the incubation period. The highest values corresponded to the highest UR doses.

The samples treated with chloramphenicol (Fig. 4) showed slightly higher values. The growth rate was faster and maximum values were observed at 20 days. There was practically no decrease in mycelium length during incubation of the samples receiving UR and very little difference between the different doses of UR.

There was a strong initial inhibitory effect of cycloheximide (a fungicide) on fungal growth, with maximum values $<10^2$ m g^{-1} (for the highest UR dose). These values remained practically constant until the end of incubation. Differences were observed between the different doses of UR, the highest values being obtained with the highest UR doses.

Lastly, treatment with formaldehyde (Fig. 4) led to maximum values of around 12 m mycelium g^{-1} of soil at time zero, decreasing sharply during the first 10 days until they reached almost zero. After this time, growth was only observed on one occasion. No significant differences were observed between the different doses of UR. All the values referring to mycelium length in this system were at the very limits of the quantification technique used.

DISCUSSION

This experiment shows that the addition of UR to a soil increased the abundance of stable aggregates and that this increase was proportional to the quantity of UR added. Pagliai *et al.* (1981), Christensen (1986), Mbagwn (1989) and Lax *et al.* (1990) have also shown that the addition of organic wastes has a positive effect on aggregate stability.

With the exception of formaldehyde, all the biocidal agents tested gave similar proportions of stable aggregates, the following order being established: chloramphenicol > none > cycloheximide > formaldehyde.

The treatment with formaldehyde was responsible for the least amounts of stable aggregates suggesting that microorganisms are important in aggregate stabilization. Another important factor is the presence of polysaccharides, either those produced microbially or those introduced with the UR. To investigate the relative importance of the microbiological factors involved and those relating to carbohydrates, correlation coefficients between these indices and the percentages of stable aggregates were calculated (Table 4). This shows that aggregate stability is to a greater or lesser degree related with all the factors considered, although this relation is most evident for the fungi component ($R=0.81$, $P<0.001$). Many studies have suggested that fungal communities are mainly responsible for aggregation (Metzger *et al.*, 1987). The correlation coefficients between bacterial numbers and stable aggregates vary considerably and depend on the quantification technique used. Homogenization by ultrasound not only led to higher total bacteria numbers but also to a highly significant correlation coefficient ($R=0.42$, $P<0.001$) with the percentage of stable aggregates.

Some authors have suggested that bacterial communities are equally or more important than fungal communities in deciding final aggregate stability (Lynch, 1981). Hattori (1988) and others have found differences in the levels of correlation with stable aggregates according to whether only those bacteria which remain in suspension are considered or those released by ultrasound. Our results fully support Hattori's observation. Looking at the overall results, the correlation between the difference in bacteria released by ultrasound and those not and the percentage of stable aggregates shows a correlation coefficient even greater than that observed with bacteria released by ultrasound ($R=0.49$, $P<0.001$). Consideration of some specific treatments confirms this observation. For example, in the system to which no biocide was added, the correlation coefficient between the bacteria released by ultrasound and stable aggregates is 0.74 ($P<0.001$), but in the system with cycloheximide the coefficient is 0.32 ($P<0.05$). In both the coefficient is greater than that found with sonicated bacteria and much greater than that found with unsonicated bacteria.

The duration of the incubation (67 days) allowed stabilization of the effects considered by the end of the experiment. At the same time, regular sampling

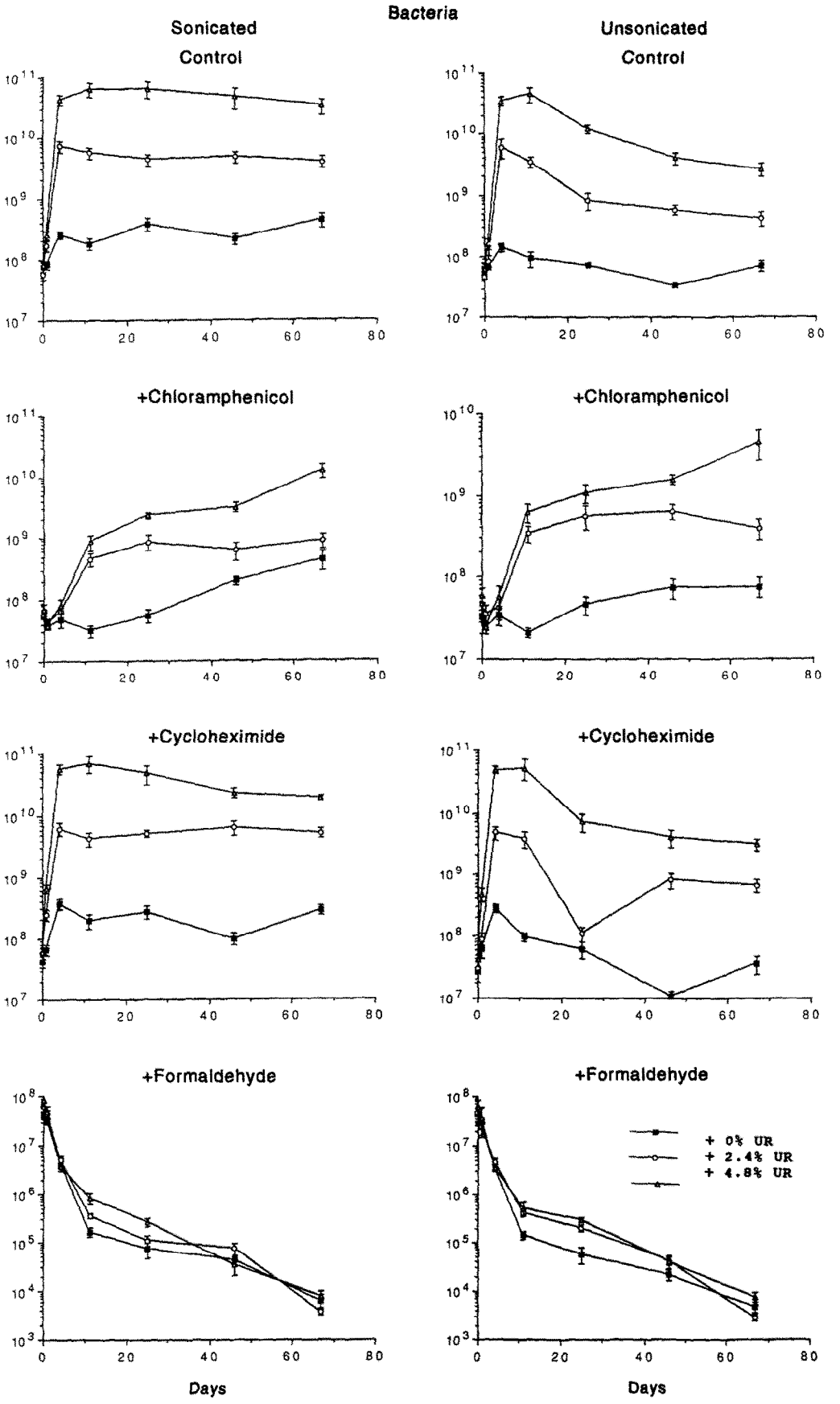

Fig. 3. Effect of UR addition and microbial inhibition treatments on total bacteria populations in an arid soil during 67 days of incubation.

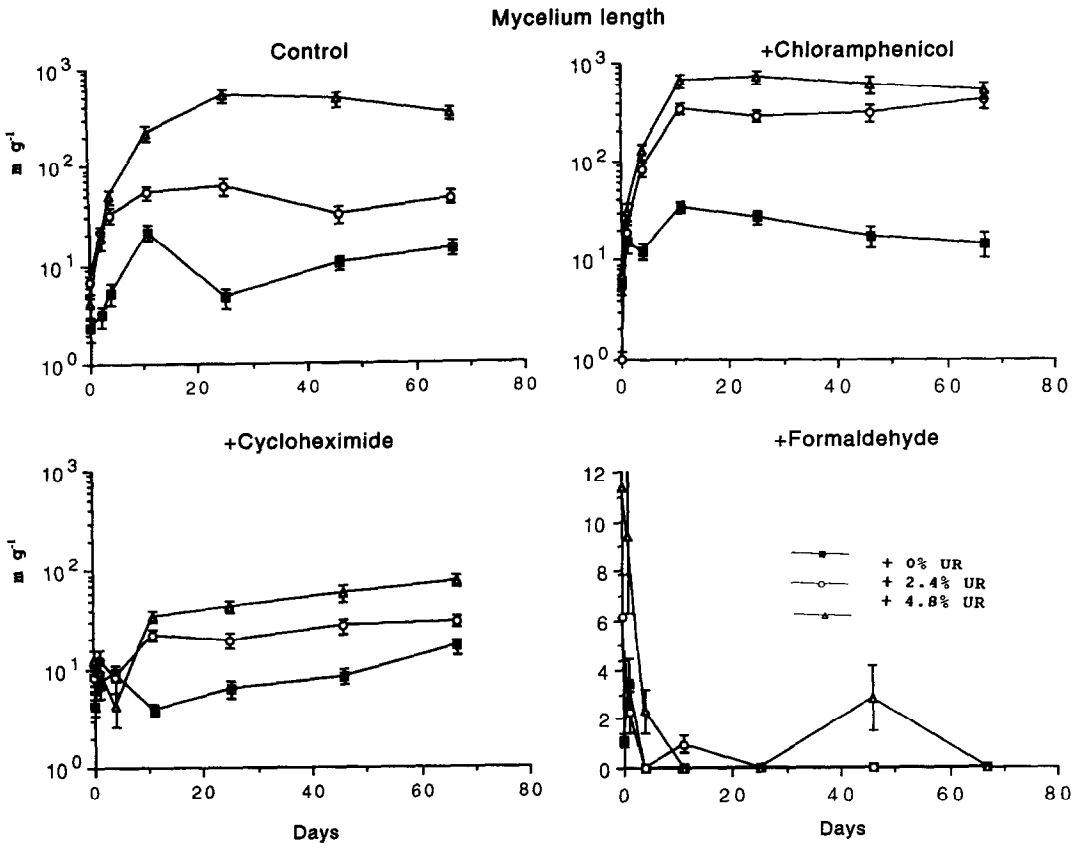

Fig. 4. Effect of UR addition and microbial inhibition treatments on fungal populations (expressed as mycelium length) in an arid soil during 67 days of incubation.

permitted us to follow the evolution of these effects. In order to relate the amounts of stable aggregates with microbial communities and carbohydrates, a correlation matrix according to sampling date was made (Table 5). Within 24 h of the addition of the UR, the amounts of stable aggregates were correlated with amounts of carbohydrates. This correlation was especially significant after 4 days ($R=0.83$, $P<0.001$) and remained significant, although less so, until day 11. Among the microbial communities, only the fungi were related to aggregate stability during these first stages of incubation, although with a degree of significance less than that with the carbohydrates, and with a slower evolution (between days 4 and 11). This suggests that the polysaccharides introduced with the refuse are effective in increasing aggregation although, in this experiment, the effect is short-lived. This agrees with the observations of Martens and Frankenberger (1992), who demonstrated that the adherence of soil particles increased with the addition of polysaccharides to the soil, although the effect was only evident during the first week ($R=0.78$, $P<0.001$) and less so during the second week ($R=0.61$, $P<0.05$).

As the amounts of stable aggregates in our experiment did not decrease during subsequent phases of incubation, the beneficial effect on aggregation shown by carbohydrates in the refuse must have been replaced by other factors. In our experiments the microbial communities were probably responsible for

Table 4. Spearman rank correlations between the factors studied for the whole experiment

	Stable aggregates	Carbohydrates	Bacterial numbers (sonicated:Bs)	Bacterial numbers (unsonicated:B ns)	Bacterial numbers (Bs – Bns)	Mycelium length
Stable aggregates	1	0.2822**	0.4201****	NS	0.4983****	0.8072****
Carbohydrates		1	0.3417***	0.3675***	NS	NS
Bacterial numbers (sonicated:Bs)			1	0.8338****	NS	0.8267****
Bacterial numbers (unsonicated:Bns)					0.8267****	0.2739**
Bacterial numbers (Bs – Bns)					1	NS
Mycelium length						1

Significance level: **$P<0.05$; ***$P<0.01$; ****$P<0.001$.

Table 5. Spearman rank correlations between the percentages of stable aggregates and the factors studied for each sampling date

Incubation (days)	Carbohydrates	Bacterial numbers (sonicated)	Bacterial numbers (unsonicated)	Mycelium length
0	NS	NS	NS	NS
1	0.7022**	NS	NS	NS
4	0.8314****	NS	NS	0.7316***
11	0.6630**	NS	NS	0.8481****
25	NS	NS	NS	0.9179****
46	NS	0.6332**	0.6479**	0.8953****
67	NS	0.6649**	0.7418***	0.7626***

Significance level: $^{**}P<0.05$; $^{***}P<0.01$; $^{****}P<0.001$.

maintaining or increasing the amounts of stable aggregates after 11 days of incubation. Among the microbial communities, fungi gave strong correlations with stable aggregates from day 11 until the end of the experiment (day 67). The correlations between bacterial populations and aggregate stability began to be significant much later (from day 46) but never showed such high levels of significance as did the fungi.

The importance of microbial communities (particularly the fungi) in aggregate stabilization seems clear in our experiment. However, the mechanisms involved in this process remain to be clarified, although it is known that fungal mycelia can form mechanical associations between soil particles, which are very resistant to disaggregating factors (Allen, 1989). The same effect can be produced by encapsulated bacteria or those with special adherence mechanisms, which adhere to one another when they occupy recesses between the soil particles (Lynch, 1981). However, this bacterial mechanism is limited by the scant biomass of the bacterial populations when compared with the quantity of fungal hyphae which can exist in a soil. For this reason it is considered that bacteria are responsible for forming aggregates <500 μm (Tisdall and Oades, 1982), and fungi are responsible for larger aggregates (Metzger *et al.*, 1987). Only the fraction of stable aggregates between 1 and 2 mm was considered in our experiment. This may have made the bacterial communities appear to be less important in aggregate stabilization than fungal communities. Also, our experiments were in "closed" systems in which there was no primary production of carbohydrates by plants or autotrophic microorganisms. In an "open" system, as in a field soil, the continous supply of new carbohydrates through root and cell exudates or through decomposition of plant tissues would lead to a dynamic equilibrium, in which the role of polysaccharides in the formation of aggregates would not be confined to the first stages of incubation.

It can be concluded that the addition of UR to a weakly-structured soil increased the proportion of stable aggregates. Carbohydrates in the UR improved soil aggregation inmediately after addition. The maintenance and increase of the proportion of the stable aggregates in the subsequent phases was attributable to increases in microbial numbers, and particularly to the proliferation of fungal mycelia.

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