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Humidity conditions and the development of bacterial communities in soils of contrasting texture

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Abstract

The influence of different moisture conditions on the development of whole bacterial communities, as determined by the size of their biomass, was investigated in loamy sand and loose sandy soil. During the 4 months of the trial the soils were: (A) continuously stored at field-moist state (control); (B) constantly kept at about 59% of the maximum water holding capacity (WHC), corresponding to 100% of the capillary water capacity (CWC); (C) repeatedly air-dried and remoistened to 50% of the WHC at 2 week intervals ; (D) continuously stored at air-dry state. In untreated control loamy sand the mean bacterial biomass was found to be 0.19 mg dry mass g^{-1} dry soil, one third larger than the biomass found in loose sandy soil. This resulted both from the greater numbers as well as the larger mean size of the bacterial cells inhabiting the heavier soil. At persistent moistening of the samples to 100% of the CWC a doubling of the bacterial biomass was noted in both soils. The moisture level corresponding to the natural field-moist state thus limited the development potential of bacterial communities. After four drying-remoistening cycles the mean bacterial biomass was for air dry samples smaller than in the control: by one fourth and one third in the heavier and lighter soil, respectively. For remoistened samples the mean values were found to be higher than the control - by 16% in the heavier and by 18% in the lighter soil. The recurring increase in moisture level following a period of severe water deficit thus favoured bacterial biomass development, and that to a larger extent in the lighter soil than in the heavier one. In continuously air-dry samples the bacterial biomass in the soil decreased by one third and in the lighter soil by as much as four fifths in relation to the control. The diminishing of biomass size resulted primarily from the decrease in the mean size of bacterial cells. The reaction of bacterial communities to changes in moisture conditions was clearly dependent on soil type.

Keywords: Bacterial communities; Direct cell counts; Cell size; Biomass; Loamy sand; Loose sandy soil; Different moisture regimes

1. Introduction

Soil humidity is one of the major environmental factors that is decisive for the development of microorganisms. At prolonged water deficit the development of these organisms is limited (Zelles et al., 1991; Van Gestel et al., 1992) However, it has also been observed (Srvastava, 1992) that a strong remoistening of the soil such as during the rainy season following a severe drought (savannah) is linked with an increase in the numbers and activity of microorganisms. A sequence of alternate drying and remoistening cycles occurring within short peri-

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ods of time results in a disturbance of the equilibrium between the microorganisms, resulting not only in quantitative, but also in qualitative changes in their communities (Bottner, 1985). It was found, for example, that as a consequence of severe drying of soil a larger number of young cells are killed when compared with older cells (Soulides and Allison, 1961). During the recolonization of the soil following a prolonged drought period a particularly strong development of saccharomycetes has been observed (Sparling and Cheshire, 1979).

West et al. (1988) believe that the rate of decline of microbial biomass in air-dried soil is dependent upon it's texture. Other authors (Van Gestel et al., 1993a,b) attribute greater importance rather to the previous climatic history of soils than to their physico-chemical properties.

The present study investigates the influence of prolonged remoistening/drying as well as alternate drying and remoistening of two different soils on the development of bacterial communities as expressed by the size of their biomass.

2. Materials and methods

Two soils were tested - a heavier and a lighter one, differentiated by mechanical structure as well as the content of carbon and nitrogen (Table 1). The soils were collected in early spring from the 20 cm arable layer of cultivated fields belonging to one single farm and sieved (2 mm diameter mesh). After 14 days of storage in the dark at room temperature the soil samples were prepared according to the following experimental design:

A - control, field-moist state, i.e., 33% and 39% of the maximum water holding capacity (WHC) for the heavier and lighter soil, respectively,

Table 1
Soils studied

Soil	Contents (%)			
	Silt and clay	C	N	pH in H_2O
Loamy sand	32	0.95	0.087	7.0
Loose sandy soil	23	0.41	0.034	4.4

B - with 59% and 60% of the WHC for the heavier and lighter soil, respectively, corresponding to 100% of capillary water capacity (CWC),

C - alternately air-dried to 1% of the WHC and remoistened to 50% of the WHC, in 2 week intervals,

D - air dry soil (1.4% of the WHC).

In treatment A 3 kg soil samples as well as breakers with water were placed in containers and secured against drying by polyethylene film.

For variant B 200 g soil samples were placed in containers with a perforated bottom, secured with tight lids and placed in vessels with a thin layer of water to enable constant irrigation. The individual samples were liquidated at the subsequent dates of analyses.

In variant C 1.5 kg samples of soil were dried in darkness for 2 days at room temperature, by spreading the samples in a thin layer on an area of 50×35 cm. The air dried samples were stored in 2 l beakers. After conducting the analyses the soils were uniformly sprinkled with water up to approximately 50% of the WHC and stored in containers wrapped in polyethylene film.

In variant D 3 kg samples of air dried soil were stored in jars covered with paper.

All soil samples in two replicates of each treatment were kept at room temperature (21°C). During a period of 112 days analyses were carried out according to the following scheme: samples A were studied every 2 weeks of storage, samples B - every 4 weeks, samples C - in four 4 week - drying-remoistening cycles: after 2 weeks of storage of air dry soils (Cd) and after 2 weeks of storage of remoistened soils (Crm), and samples D - after 2 and 16 weeks of storage.

The biomass of bacteria was determined on the basis of their numbers and mean cell volumes, estimated in microscope preparations (Kaczmarek, 1984). Smears were prepared of soil solution (50-fold dilution) in sodium pyrophosphate with addition of Tween '80 (1 ml/l) and agar–agar (400 mg/l). Preparations were stained for 24 h with phenolic erythrosin. Bacterial cells were counted by means of the microscope (Jena-Med), without additional equipment, magnifying power $1000 \times$, in 24 fields of view for each trial (a minimum of 400 cells). Also the frequency of occurrence of cocci and rods was

determined in these preparations. Bacterial cell sizes were estimated in fuxin-stained stamp preparations from the surface of agarized soil samples (5-fold diluted soil solution + aqueous solution of agar, at a ratio of 1:1). The cells were measured in 48 fields of view with an ocular micrometer. Five size classes were distinguished. In the calculation of mean cell sizes the ratio of the numbers of cocci to rods was taken into consideration, as determined while counting the cells in erythrosin stained preparations. A specific cell gravity of 1.1 and a dry wt. of 20% were assumed, corresponding to 220×10^{-15} g μm^{-3}.

Statistical evaluation of the results consisted of analysis of variance to determine the effect of different humidity conditions, as well as of their duration on the dynamics of the bacterial number and biomass in soils studied.

3. Results and discussion

3.1. The cell numbers of entire bacterial communities

The total numbers of bacteria (Fig. 1) in untreated control soils were, as in earlier studies (Kaczmarek, 1984, 1985) differentiated according to soil type. The mean values were approximately 3×10^9 g^{-1} dry mass (dm) of the loamy sand and 2×10^9 g^{-1} dm of loose sandy soil. No significant changes in the bacterial numbers were found in the control samples during the 4 months of storage.

The increase in soil moisture from the field moist state to 100% of the capillary water capacity and its maintenance caused in both soils an increase of the mean bacteria counts by one half. The same phenomenon was observed by Schnürer et al. (1986) in the drip-irrigated experimental plot. This may be evidence of an increase in the nutrient pool available to the bacteria in conditions of a significant, prolonged increase in soil moisture.

During the first drying-remoistening cycle in air-dried soil stored during a period of 2 weeks the counts of bacteria decreased to 67% and 60% of those found in the control samples of the heavier and lighter soil, respectively. These interdependencies did not change significantly at subsequent dates of soil drying.

The mean bacteria counts from the four drying-remoistening cycles amounted for the remoistened samples of both soils to approximately 110% of the counts found in untreated soils. In repeatedly re-

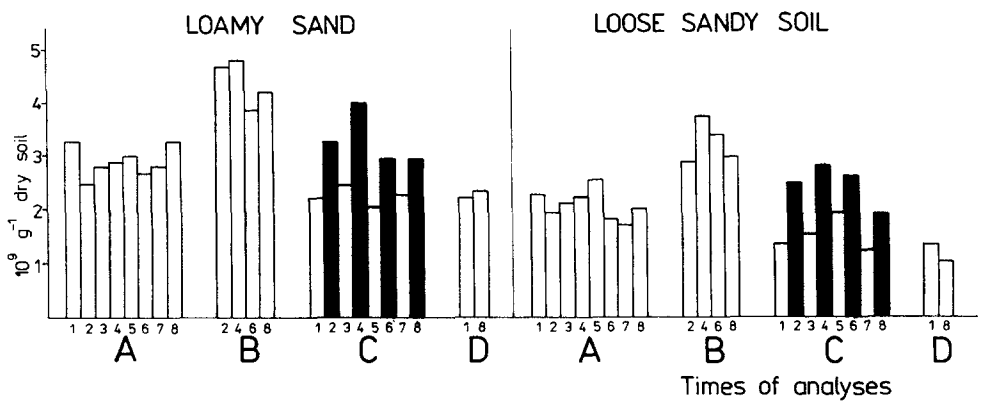

Fig. 1. Total numbers of bacteria. 1–8 - times of analyses, in 2 week intervals. (A) Control - field-moist samples, analysed every 2 weeks, after 2 to 16 weeks of storage. (B) Samples stored continuously at 59% (loamy sand) and 60% (loose sandy soil) of the WHC (e.g., 100% of the CWC), analysed after 4, 8, 12 and 16 weeks of storage. (C) Samples four times alternately air-dried and remoistened to 50% of the WHC: □ = analysed 2 weeks after drying (Cd), ■ = analysed 2 weeks after remoistening (Crm). (D) Continuously air-dry samples, analysed after 2 and 16 weeks of storage. Values for the treatments differ significantly at $P \leq 0.05$. Values for the time of analyses differ significantly at $P \leq 0.05$ for the treatments Crm. For other treatments values for the time of analyses do not differ significantly at $P \leq 0.05$.

moistened air-dried soil samples there was thus not only a recovery of the bacteria numbers that have decreased due to the water deficit, but even a certain increase was observed in relation to the continuously field-moist control.

The prolonged severe water deficit caused in the heavier soil a 20% decrease in bacteria counts in comparison with untreated samples. In the lighter soil the number of these organisms decreased by as much as one half. In an earlier study (Kaczmarek, 1979) a similar differentiation of the reaction to water deficit in bacteria inhabiting the heavier and lighter mineral soil was observed. However, it was also found that the drying of organic soil (muck) causes a much larger and a more lasting decrease in the bacterial community numbers than in mineral soils.

The mean volume of bacterial cells (Table 2) in the control samples was 0.30 μm^3 and 0.24 μm^3 for loamy sand and loose sandy soil, respectively. It was also found in earlier studies (Kaczmarek, 1979, 1984) that the size of the cells is positively correlated with the content of silt and clay particles as well as organic matter in the soil.

During the storage of soil samples with a moisture level brought up to 100% of the capillary water capacity the size of the cells increased systematically - from 0.32 μm^3 after 2 weeks of storage to 0.46 μm^3 after 4 months of storage and from 0.27 μm^3 to 0.29 μm^3 for the heavier and lighter soil, respectively. The mean increase of bacterial cell size for the period under study was in the heavier soil 30% and in the lighter soil 20% in relation to the controls. The growth in the size of bacterial cells may indicate that in a soil with prolonged high moisture level the carbon pool available for the bacteria was increasing. This element is the strongest growth limiting element for bacteria in soil (Stotzky and Norman, 1963).

In the samples of both soils intermittently dried and remoistened the repeated drying lowered the average size of bacterial cells in comparison to the control. This became more distinct in the lighter soil. However, after remoistening of the soils the mean cell size grew to the dimensions found in untreated samples.

At a severe prolonged water deficit in the heavier soil the mean bacterial size decreased by 30% and in the lighter soil even by 60%. This shows that in the heavier soil, with greater porosity, the hygroscopic moisture weakens more significantly the negative impact of the water deficit on the bacterial metabolism. The significant decrease in the bacterial cell size following a severe drying of soils, particularly of those that are poor in silt and clay particles,

Table 2
Volumes of bacterial cells (μm^3)

Time of analyses [a]	Loamy sand soil					Loose sandy soil				
	Treatment A	Treatment B	Treatment C		TreatmentD	Treatment A	Treatment B	Treatment C		Treatment D
			d	rm				d	rm	
1	0.31		0.29		0.29	0.23		0.24		0.24
2	0.30	0.32		0.30		0.24	0.27		0.25	
3	0.30		0.29			0.23		0.22		
4	0.30	0.39		0.31		0.24	0.29		0.24	
5	0.30		0.29			0.23		0.22		
6	0.30	0.39		0.31		0.24	0.29		0.24	
7	0.29		0.30			0.24		0.20		
8	0.30	0.46		0.32	0.20	0.25	0.31		0.24	0.10

Means for 400 measurements.

[a] In 2 week intervals.

Treatment A: control - field-moist samples, analysed every 2 weeks, after 2–16 weeks of storage.

Treatment B: samples stored continuously at 59% (loamy sand) and 60% (loose sandy soil) of the WHC (e.g., 100% of the CWC), analysed after 4, 8, 12 and 16 weeks of storage.

Treatment C: samples four times alternately air-dried and remoistened to 50% of the WHC: d - analysed 2 weeks after drying; rm - analysed 2 weeks after remoistening.

Treatment D: continuously air-dry samples, analysed after 2 and 16 weeks of storage.

was also found in our earlier studies (Kaczmarek, 1979) as well as in the work of other authors (Jefremowa, 1975; West et al., 1988).

3.2. The biomass of bacteria

In untreated control loamy sand the mean biomass of bacteria (Fig. 2) was 0.19 mg and in loose sandy soil 0.11 mg dm g^{-1} of dry soil. The levels noted for both soils at the subsequent dates of analyses were not significantly differentiated ($P \leqslant 0.05$). This confirms the observations of Ross (1991) who found high stability of the microbial biomass size in soil with high humus content, stored for several months. The differentiation of the bacterial biomass size due to the type of soil, also found earlier in our studies (Kaczmarek, 1979; Kaczmarek, 1984) was greater than the differences in bacterial numbers. In the heavier soil not only the mean bacterial counts were higher by 30%, but also the mean cell size exceeded by 1/5 the values found for the lighter soil.

At a change in the moisture level from a field-moist state to 100% of the capillary water capacity there occurred in both soils almost a doubling of the bacterial biomass. This was due to an increase in their number in relation to the control as well as in the size of the cells in the trials with prolonged high moisture levels. In the heavier soil slightly lower increases in the numbers were noted and a stronger increase in cell size, whereas in the lighter soil these interdependencies were arranged in the opposite manner. Schnürer et al. (1986) observed a doubling of the bacterial biomass after 3 days of drip-irrigation of loam and a subsequent decrease. In our studies the biomass of bacteria remained at an elevated level over a 4 month period of intensive moistening of loamy sand and sandy soil. This indicates that the level of the field-moisture of these soils was much below the optimum level for bacterial development.

The first drying of the samples undergoing intermittent drying and remoistening resulted in an identical decrease of 40% in the bacterial biomass size of both the heavier and lighter soil. Also Van Gestel et al. (1991) have not found any differentiation in the reaction of the microbial biomass at short-term (3 days) drying of soils with different physical properties. After 14 days of storage of remoistened samples the bacterial biomass in the heavier soil reached a level observed in samples freshly collected from the field and was even 20% higher in the lighter soil. The mean bacterial biomass increases from the four drying-rewetting cycles were in the rewetted samples in relation to the dry samples 57% and 86% in the

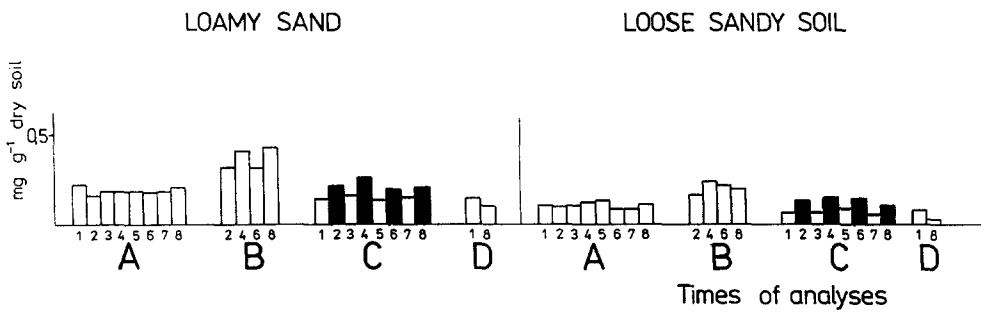

Fig. 2. The biomass of bacteria. 1–8 - times of analyses, in 2 week intervals. (A) Control - field-moist samples, analysed every 2 weeks, after 2 to 16 weeks of storage. (B) Samples stored continuously at 59% (loamy sand) and 60% (loose sandy soil) of the WHC (e.g., 100% of the CWC), analysed after 4, 8, 12 and 16 weeks of storage. (C) Samples four times alternately air-dried and remoistened to 50% of the WHC: □ = analysed 2 weeks after drying (Cd), ■ = analysed 2 weeks after remoistening (Crm). (D) Continuously air-dry samples, analysed after 2 and 16 weeks of storage. Values for the treatments differ significantly at $P \leqslant 0.05$. Values for the time of analyses differ significantly at $P \leqslant 0.05$ for the treatment Crm and D. For other treatments values for the time of analyses do not differ significantly at $P \leqslant 0.05$.

heavier and lighter soil, respectively. The improvement in the moisture regime thus favoured to a larger extent the recovery of bacterial communities in the lighter than in the heavier soil. In an earlier study (Kaczmarek, 1979) it was also found that severe drying limits to a much greater extent the potential of bacterial biomass recovery in soil with a high level of organic matter in comparison with light soil. Independently of the differentiation linked with soil type there is reason to believe that the actual reaction of bacterial communities to the remoistening of dry soil was stronger than noted in our study. Our data are derived from Day 14 after rewetting of the dry samples. Schnürer et al. (1986) found the largest bacterial biomass increases after 3 days of storage of the remoistened soil.

The mean sizes of bacterial biomass from the four drying-remoistening cycles were in dried samples of heavier soil lower by 1/4 and in lighter soil lower by 1/3 when compared with continuously field-wet control samples. The limitation of bacterial development in conditions of repeated water deficit was thus stronger in the lighter than the heavier soil. However, in remoistened samples the mean bacterial biomass reached values that were 16% higher than in the control in the heavier soil, and 18% higher in the lighter soil. Thus, at an improvement of moisture conditions the bacterial biomass is reconstructed to a greater extent in the lighter than in the heavier soil. This may demonstrate a pre-adaptation of bacterial communities inhabiting light soils to a development within conditions of severe moisture changes. Also the findings of other authors lead to similar conclusions (West et al., 1988). The intensified production of bacterial biomass occurring at the remoistening of severely dried soil manifests an increase in the nutrient level in the soil. It is believed (Van Gestel et al., 1991) that the nutrient pool expands by incorporating the compounds of microbial cells killed by the desiccation of soil. Most of all, however, it is growing due to the accessibility to the microorganisms of substrates contained in native soil organic matter. Drying and remoistening causes a physical disruption of soil structure and as a consequence substrate desorption from soil surface.

In continuously air-dry soil the bacterial biomass found after 4 months was smaller by 1/3 and 4/5 than in the control for loamy sand and loose sandy soil, respectively. The decrease in the biomass size in the heavier soil was linked solely with the lower mean volume of bacterial cells while their number, after an initial decrease, stabilized. In the lighter soil also smaller bacterial numbers were noted. However, here the decrease in the size of the biomass of these organisms resulted mainly from the smaller size of their cells. The negative impact of severe drying of soil on the size of bacterial cells has also been reported by other authors (Jefremowa, 1975).

Van Gestel et al. (1993a,b) conclude that the differentiation in the reaction of microorganisms to moisture stress results rather from the previous climatic history of the soils than from their properties such as texture and organic matter content. Our observations indicate that in a relatively brief desiccation period of some 2 weeks the factors linked with soil type do not determine the degree of the decline of bacterial biomass, whereas in conditions of lasting or frequently repeated severe water deficit the development of bacterial communities is limited to a much greater extent in the lighter than the heavier soil. The survival potential of a greater number of bacteria with a larger cell volume in the heavier soil certainly results from the greater abundance of organic matter having a protective influence. Of great importance is furthermore the higher porosity of the heavier soil (West et al., 1988) which, among other factors, is decisive for water retention.

The differentiation of the reaction of the bacterial biomass to drought in heavier and lighter soils may also be linked with a qualitatively different composition of these microorganism communities. For example arthrobacters, believed to be the most abundant bacterial group inhabiting the soil (Hagedorn and Holt, 1975) are found to be more numerous in heavier soils (Mulder and Antheunisse, 1963; Hagedorn and Holt, 1975; Kaczmarek, 1984, unpublished data). These organisms demonstrate at the same time a greater resistance to drought than other soil bacteria incapable of spore production (Chen and Alexander, 1973).

Our results show that all the factors linked with soil type distinctly differentiate the potential of bacterial biomass reconstruction after degradation caused

by drought. The biomass of bacterial communities is recovered much more readily in remoistened lighter soil than in a heavier one.

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