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# Soil porosity as a habitat for microorganisms

The soil pore system has to be characterized quantitatively in order to describe the soil as a habitat for microorganisms. Soil pore morphology as determined by soil structure may be as important as the size distribution for the transport of gases and nutrients. This study adresses the physical properties of differently textured soils in undisturbed and remoulded state and their effect on microbial activity.

Bulk soil was retrieved from 0-20 cm depth at six locations along a textural gradient in an arable field in Denmark. The samples ranged in clay content from 11 to 45%. The soils were crushed in air dry state, mixed, re-moistened, and exposed to a 9 month period of structure regeneration. Following application of $^{14}C$-labelled organic residues, the soils (labelled NA1 - NA6) were exposed to a further 8 month period of structure regeneration. Intact soil cores were then sampled and drained to four different matric potentials, analyzed for physical characteristics and subjected to a 15 weeks period of incubation with collection of evolved carbon dioxide. Cores of undisturbed but recently tilled topsoil from each sampling location in the field (labelled RE1 - RE6) were included as reference samples for the physical characteristics.

The previously disturbed (NA) soils regained some of their secondary structure during storage under field-like conditions, but they were still structurally different from undisturbed (RE) soils of similar texture. The habitable and the protective pore space defined as the volume of pores ranging in size from 0.8-30 $\mu$m and 0.8-3 $\mu$m, respectively, appeared to be nearly constant across soils with different clay content. NA soils had slightly smaller volume of protective pore space compared with RE soils. Although partly aggregated, disturbed clayey soils showed less continuous / more tortuous pore systems than the texturally corresponding and structurally intact soils. Generally, the disturbed NA soils had a pore system with relatively small pores 'enmeshed' in the soil matrix, whereas the undisturbed RE soils exhibited larger pores.

Measurements of oxygen diffusion confirmed that the relative water content - often quantified as the percentage of water filled pore space (WFPS) - may be a reasonable parameter in models simulating the activity of aerobic micro-organisms in remoulded soils. However, differences in the structure of undisturbed soils were shown to invalidate the use of WFPS as a universal parameter in simulation models applied to field conditions.

The results indicated that turnover of soil OM was mainly controlled by the soil volume occupied by water. Soil texture affected turnover indirectly through its effect on the soil water regime. The turnover of the native OM was regulated by the total volume of water, whereas water in micropores and cavities $<0.2$ $\mu$m in diameter was not involved in the decomposition process of recently added OM.

The studies encourage further investigations of the activity of soil microorganisms as related to the soil physical framework and the physical processes of importance for the function of the microbial community.

## INTRODUCTION

Quantitative measurements characterizing the soil pore system are needed in order to describe the soil as a habitat for microorganisms. The organisms have only access to locations in the soil where the physical dimensions of the pore space allow them to function. Referring to the surface area within the soil matrix, Van Veen & Kuikman (1) suggest that for some soils up to 90 % of the catalytic surface of organomineral complexes can be considered as sterile.
The characteristics of the pore system in the soil matrix is of great importance for the micro-environment of microorganisms. As soil structure is modified by tillage, soil management may significantly influence microbiological processes. Growth of decomposer organisms, migration of predators, and exchange of nutrients and gases take place along the tortuous pathways of the pore system. The size distribution and tortuosity of pores in the soil matrix is therefore of great importance to the transport of enzymes and partly decomposed substrates in water-filled pores, and to the diffusion of oxygen and carbon dioxide in the gas phase. Also the interaction between bacteria and their predators (protozoa and nematodes) is influenced by the relative volume of habitable and protective pore space (2).
Abiotic functions in models of soil OM turnover are typically based on experimental evidence from laboratory incubations of different soil types, most incubation studies employing homogenized and sieved soil samples in which aggregates are broken down before incubation. Recent results have indicated that such procedures may constrain the extrapolation of results to field conditions because soil structure is decisive for diffusion of gases to and from the microorganisms imbedded in the soil matrix (3). These findings may have importance also in considering the effects of different tillage strategies leading to significant differences in soil structure.
Clay is assumed to protect OM against decomposition, and the mechanisms proposed to explain OM stabilization are adsorption of organics onto surfaces of clays or organic complexes (4) and entrapment of particles in aggregates (1). Some investigations of turnover of organic matter in soil have shown problems in relating decomposition of recently applied litter to soil clay content, whereas the turnover of native organic matter from the soils followed the generally accepted theory of clay as a directly controlling factor in stabilization of OM in soil (5, 6). Recent results of decomposition studies for differently textured soils point out the effect of clay to be indirect through its influence on soil water characteristics (7).
This paper summarizes recent investigations on physical characteristics of structurally intact and disturbed soil samples from a textural gradient in the field. The studies are reported in detail elsewhere (3, 7).

## MATERIALS AND METHODS

Bulk soil was retrieved from 0-20 cm depth at six locations along a textural gradient in an arable field on Weichselian morainic deposits in Denmark. The samples (NA1 to NA6) ranged in clay from 11 to 45%. The soil was crushed in air dry state to $<2$ mm, mixed, re-moistened and exposed to freeze/thaw and dry/wet cycles as well as tillage to induce regeneration of soil structure. After nine months, $^{14}C$-labelled ryegrass was applied (1.2 mg C $g^{-1}$ soil) and allowed to decompose for 8 months before soil cores were sampled and equilibrated at four water matric potentials on ceramic plates (-30, -100, -500 and -1500 hPa). Volumetric water and air content, air diffusivity and permeability were measured before the soils were incubated for 15 weeks with determination of carbon dioxide evolved from $^{14}C$- and $^{12}C$-sources. Cores of undisturbed but recently tilled topsoil from each sampling location in the field (RE1 to RE6) were included as reference samples for the experimentally manipulated (disturbed) soils.

## RESULTS AND DISCUSSION

### Basic soil description

Some basic characteristics of the soils studied are given in Table 1. Analyses of clay, silt and sand fractions revealed that the soil from the six sampling locations had the same mineralogical composition (data not shown). Although the NA soils used for the incubation study had experienced a long period of structure regeneration including simulated tillage and rolling, they were less dense than the RE soils sampled in the seedbed in the field.

Table 1. Some basic characteristics of soils in investigation (See Schjønning et al. (3) for details). NA indicate samples for incubation and RE the undisturbed reference samples.

| Sample designation | Clay (<2 μm) | Carbon | Nitrogen | CEC | $pH_{CaCl_2}$ | Bulk density | |
|---|---|---|---|---|---|---|---|
| | | | | | | NA-soils | RE-soils |
| | | [g $kg^{-1}$] | | [$mmol_c$ $kg^{-1}$] | | [g $cm^{-3}$] | [g $cm^{-3}$] |
| NA1/RE1 | 109 | 13.3 | 1.37 | 114 | 6.6 | 1.22 | 1.25 |
| NA2/RE2 | 157 | 13.4 | 1.45 | 150 | 6.7 | 1.14 | 1.30 |
| NA3/RE3 | 208 | 12.8 | 1.39 | 181 | 7.2 | 1.10 | 1.25 |
| NA4/RE4 | 310 | 15.5 | 1.64 | 229 | 7.4 | 1.14 | 1.33 |
| NA5/RE5 | 374 | 13.0 | 1.54 | 264 | 7.5 | 1.02 | 1.22 |
| NA6/RE6 | 452 | 13.4 | 1.67 | 325 | 7.5 | 0.96 | 1.29 |

### Pore size distribution

The pore size distribution was calculated from the volume of water retained at the specific matric potentials as described by Schjønning (8). An estimate of water retained at a matric potential of -1.5 MPa (equivalent pore size of 0.2 μm) was calculated from the soil content of clay and organic carbon (9). The volume of pores in the size classes $<0.8$ μm, 0.8-3 μm, 3-30 μm and $>30$ μm was derived from the frequency curves obtained (Fig. 1). Considering 0.8 μm to be a lower limit for cell size of soil bacteria (2), the volume of soil not physically accessible to decomposer organisms was estimated to range from 10 to 21% for the NA soils and from 10 to 32% for the RE soils. As expected, the volume of small pores increased with increasing soil clay content. The smaller values obtained for the clay-holding, disturbed NA soils compared with the similar textured RE soils were ascribed to the lower bulk density for the NA soils (Table 1). The direct correlation between bulk density and relative volume of small pores was also reflected in the relatively high volume of pores registered for the RE4 (and NA4) soil having a higher bulk density (Table 1). For the undisturbed RE6 soil with a clay content of 45%, the pore volume inaccessible to the microorganisms accounted for more than 60% of the total soil pore volume (Fig. 1).

The *habitable pore space* defined as the volume of soil pores sizing from 0.8 to 30 μm (2) was similar for all soils across the textural gradient (Fig. 1). For both NA and RE soils, there was a tendency to a higher volume for the most sandy soils indicating that the volume of soil accessible to microorganisms at a water content of field capacity (pores drained to the equivalent diameter 30 μm) was about the same. Postma & van Veen (2) further considered the locations in soil where bacterial cells are not subject to grazing from higher organisms like protozoa. This *protective pore space*, defined as the volume of pores ranging in size from 0.8 to 3 μm (2), was found to be slightly higher for the undisturbed RE soils compared with

the NA soils prepared for incubation studies (Fig. 1). Also for this class of soil pores, the volume was rather constant across soil types although there was a tendency of increasing volume with increasing clay content for the NA soils. A significant decrease in large pores with diameters larger than 30 $\mu$m was observed with increasing clay content in the RE soils (Fig. 1).

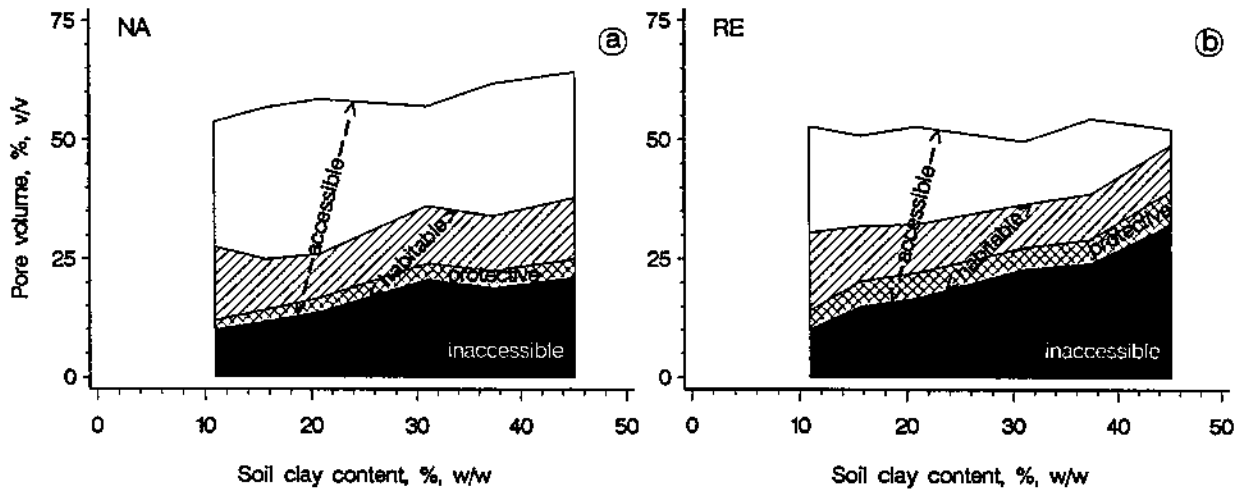


Fig. 1. Pore size fractions calculated from water retention characteristics determined for disturbed (NA) samples and intact reference (RE) cores. Inaccessible, protective, habitable, and accessible pore space relates to the pore fractions $<0.8$ $\mu$m, 0.8-3 $\mu$m, 0.8-30 $\mu$m and $>0.8$ $\mu$m, respectively.

## Morphology of soil pores

According to the model of Ball (10) the number of pores, $N_B$, in a soil transect can be estimated from the air-filled porosity, $\epsilon$, and the air diffusivity, $D_s/D_0$, and permeability, $K$, using the equation: $N_B = \epsilon^{1/2}(D_s/D_0)^{3/2} / 8\pi K$.

Estimates of $N_B$ were much higher for the previously disturbed NA soils than for structurally intact RE soils (Fig. 2a), which may be due to a higher volume and consequently larger number of large pores in the disturbed soils (Fig. 1). However, this is not the only explanation. The pore diameter $d_B = 2\ (8K/(D_s/D_0))^{1/2}$ (10), an estimate of the size of the 'effective' or 'average' pores active in the air transport, was shown to be much higher for the field-sampled RE soils compared with the disturbed NA samples (Fig. 2b). In other words, cores of disturbed soils exhibited a pore system of relatively small pores that were 'enmeshed' in the soil matrix, while the intact soils contained a smaller number of pores but larger in size.

## Air diffusivity and Water Filled Pore Space

The differences in soil morphology detected above will obviously influence the conditions for transport of gases in soil. In accordance with general models for air diffusivity in soils (11), the relative diffusivity for all NA soils followed a general relationship like $D_s/D_0 = k\epsilon^m$, where $k$ and $m$ are constants (Fig. 3a). However, for the undisturbed RE soils, no general model was able to describe data (Fig. 3b). This was obviously due to differences in pore tortuosity between the two groups of soils.

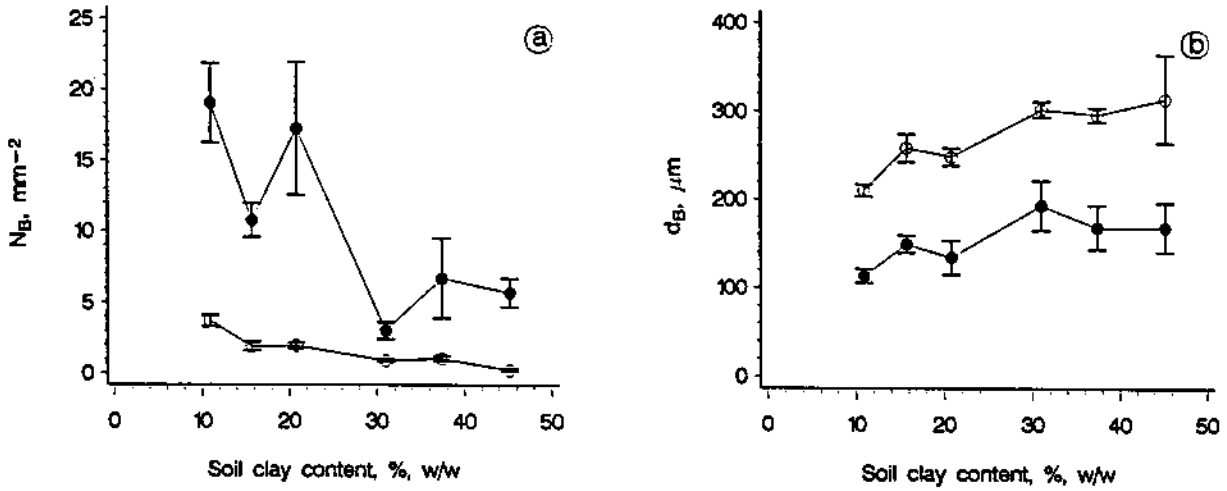


Fig. 2. Number of pores, $N_B$, in a soil transect (a) and effective diameter, $d_B$, of pores (b) for soil drained to -100 hPa matric potential as calculated by a model incorporating air diffusivity and permeability. Closed symbols refer to disturbed NA soils while open symbols refer to undisturbed RE soils. Bars indicate ±SE. See text for details.

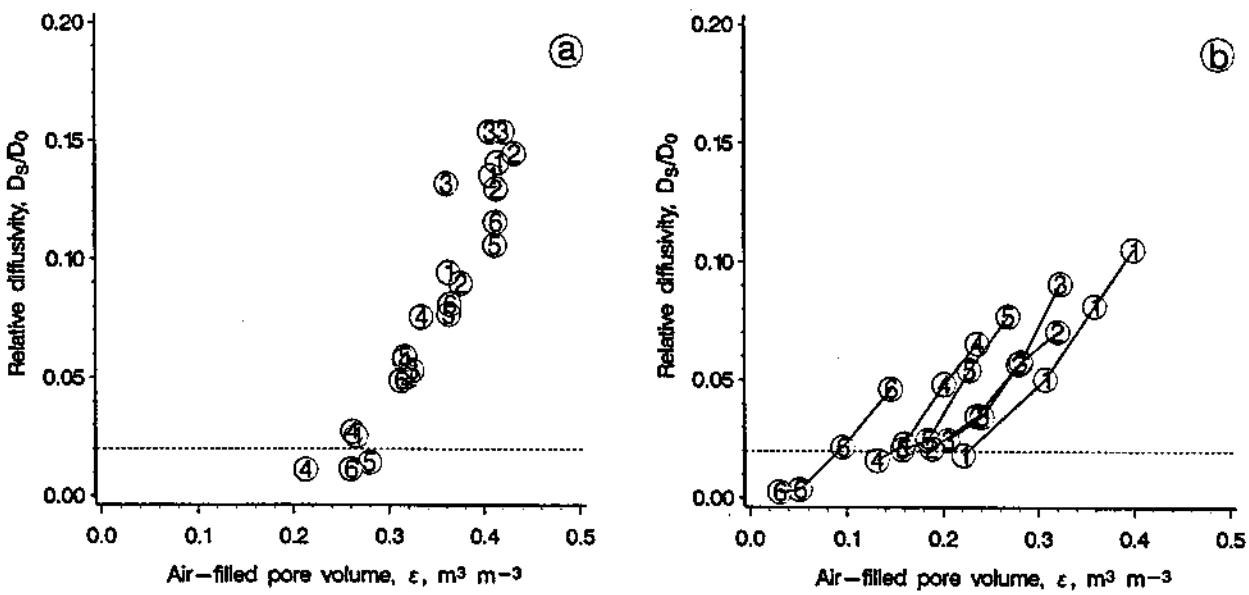


Fig. 3. Relative air diffusivity ($D_s/D_0$) for (a): cores of disturbed soils (NA) and (b): structurally intact soils (RE) as related to air-filled pore volume at each of the four water potentials. $D_s$ is the oxygen diffusion coefficient measured in soil, and $D_0$ is a tabulated value for oxygen diffusion in free atmosphere. The dotted line indicates a critical level of air diffusivity for aerobic biological processes in the soil (12, 13). Soil numbers are given inside the data markers (see Table 1).

A relative diffusivity of 0.005 - 0.020 was identified by Grable and Siemer (12) to limit plant growth. Furthermore, the oxygen diffusion rate (ODR) of a soil has been shown to decrease dramatically at a bulk soil relative diffusivity of about 0.02 (13). Taking this level of bulk soil diffusivity as limit of adequate aeration of the soil for microbial activity, the air-filled pore space that can be expected to be the lower limit for aerobic respiration for the soils investigated can be read from Fig. 3. Comparing these readings to total soil porosity, an estimate of the % WFPS at which soil respiration should be optimal can be calculated (Table 2).

Table 2. Air-filled porosity read from Fig. 3 at a limiting relative diffusivity of 0.02, and the corresponding percentage of water filled pore space (WFPS) as calculated from the air-filled pore space and the total porosity (calculated from bulk density, Table 1).

| Soil | Critical air-filled porosity read from Fig. 3 | Estimated WFPS for optimal soil respiration |
|---|---|---|
| | [$m^3$ $m^{-3}$] | [% v/v] |
| NA1 | 0.25[1)] | 54 |
| NA2 | 0.27[1)] | 52 |
| NA3 | 0.27[1)] | 54 |
| NA4 | 0.24 | 58 |
| NA5 | 0.28 | 55 |
| NA6 | 0.27 | 58 |
| RE1 | 0.22 | 58 |
| RE2 | 0.18[1)] | 65 |
| RE3 | 0.18[1)] | 66 |
| RE4 | 0.15 | 70 |
| RE5 | 0.16 | 71 |
| RE6 | 0.09 | 83 |

[1)] extrapolated from measured data.

The estimates of WFPS for cores of previously disturbed NA soils ranged from 52 to 58 %, in accordance with Linn and Doran (14). For the cores of structurally intact RE soils the WFPS ranged from 58 % in the most sandy soil (RE1) to more than 80 % for the most clayey soil (RE6). It appears that WFPS can not unambiguously be related to microbiological activity in undisturbed field soils.

## Microbial activity

The $^{14}C$ and $^{12}C$ evolved during the 15 weeks of incubation was tested for correlation to the abiotic parameters measured for the NA soils. It appeared that for native OM, a model incorporating only the volumetric water content gave a good description of data (Fig. 4a). Clay which often has been reported to have a direct controlling effect on OM stabilization in soil gave a poor fit (7). For the recently applied ryegrass material, water expressed as the total volumetric water content did not explain all variation in the observed data (Fig. 4b). Neither in this case gave clay or clay-related parameters as CEC a good description of data. Subtracting the water held in small pores ($<0.2$ $\mu$m diameter) considered not to be involved in the decomposition processes of recently applied OM, it appeared that the water held in $>2$ $\mu$m pores gave a good explanation of the accumulated evolution of $^{14}C$ from the soils (Fig. 5). It should be noted, that the choice of the pore size limit of 0.2 $\mu$m was taken solely because of the existence of a pedotransfer function for calculating the water held at the corresponding matric potential (9). However, as discussed by Hassink *et al.* (15), a limit close to 0.2 $\mu$m may be just as appropriate as the 0.8 $\mu$m value suggested by Postma & van Veen (2).

The results indicate that microbial turnover of organic matter in soil is controlled primarily

by the (water-filled) volume of soil where the substrate is located. The fact that organic matter found in cavities physically inaccessible to microorganisms are prone to decomposition may be interpreted as a significant role of diffusion of bacterial enzymes to the 'hidden' substrate.

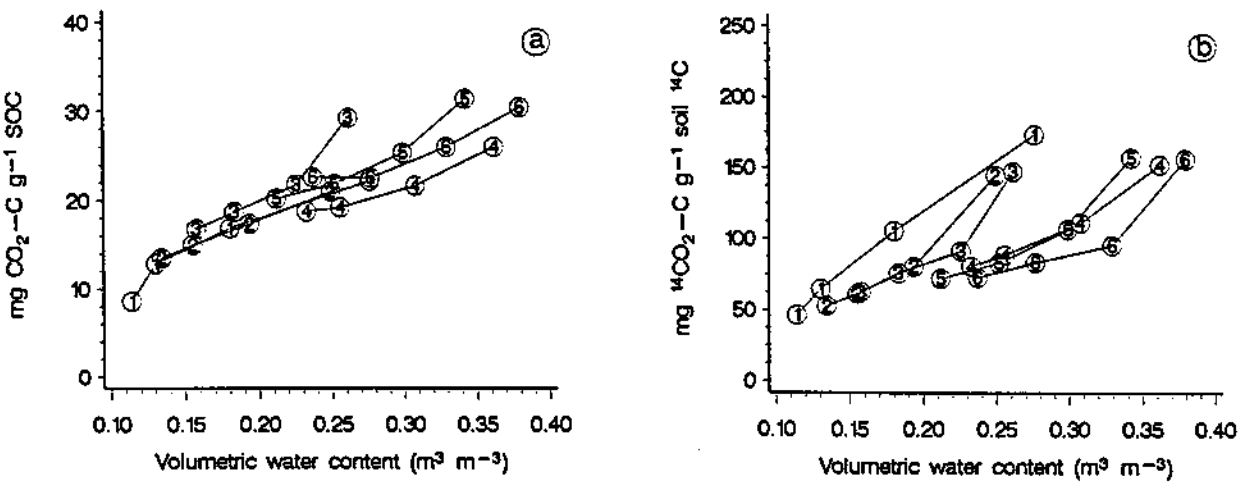


Fig. 4. Volumetric water content as related to (a) accumulated $CO_2$-evolution relative to native soil OM and (b) $^{14}CO_2$-evolution relative to residual $^{14}C$ at day 0 of incubation.

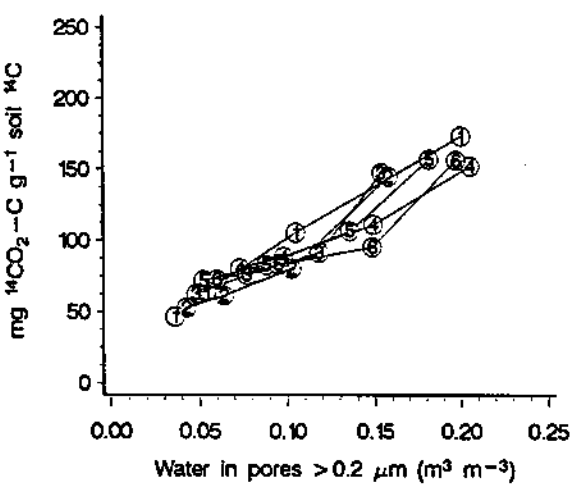


Fig 5. Accumulated $^{14}CO_2$-evolution relative to residual $^{14}C$ at day 0 of incubation as related to water contents in pores $>0.2$ $\mu$m at the specific water potential.

## CONCLUSIONS

Previously disturbed soils regained some of their secondary structure during a 17 month period of storage under field-like conditions, but they were still structurally different from intact soils of similar texture. The habitable and the protective pore space as defined by Postma & van Veen (2) appeared to be nearly constant across soils with different clay content. Disturbance of soil produced a slightly smaller volume of protective pore space compared with undisturbed seedbed samples. Although partly aggregated, disturbed clayey soils showed less continuous / more tortuous pore systems than the texturally corresponding and structurally intact soils. Generally, the disturbed soil had a pore system with relatively small pores 'enmeshed' in the soil matrix, whereas the intact soils exhibited larger pores.

Measurements of oxygen diffusion confirmed that the relative water content - often quantified as the percentage of water filled pore space (WFPS) - may be a reasonable parameter in models simulating the activity of aerobic micro-organisms in remoulded soils. However, differences in the structure of intact soils were shown to invalidate the use of WFPS as a universal parameter in simulation models.

The results indicated that turnover of soil OM is mainly controlled by the soil volume occupied by water. Soil texture affected turnover indirectly through its effect on the soil water. The turnover of the native OM was regulated by the total volume of water, whereas water in micropores and cavities $<0.2$ $\mu$m in diameter was not involved in the decomposition process of recently added OM.

The studies encourage further investigations concerning the activity of soil microorganisms as related to the soil physical framework and the physical processes of importance for the function of the microbial community.

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