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The effects of manure and NPK fertilizers on the soil microorganisms in a Danish long-term field experiment

Virkningerne af staldgødning og kunstgødning på jordens mikroorganismer i et dansk langvarigt markforsøg

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Summary

Microbial biomass, microbial activity and enzymatic activity were examined in soil samples originating from a field experiment, which was initiated in 1893 at the Askov Experimental Station. The unmanured, manured and fertilized plots, respectively, were since that time treated in the same way.

The results of the microbiological investigations varied with the time of the soil samplings. On an average of all soil samplings, plate counts of fungi, ATP content, oxygen uptake and dehydrogenase activity yielded the highest results for the fertilized soil, lower results for the manured soil and the lowest results for the unmanured soil (control). Plate counts of bacteria and the biomass determined by fumigation according to Jenkinson yielded the highest results for the manured soil.

In general, the differences between the microbiological results for unmanured, manured and fertilized soils were small.

Key words: Microbial biomass, microbial activity, enzymatic activity, manure, NPK fertilizers.

Resumé

Den mikrobielle biomasse, mikrobielle aktivitet og enzymatiske aktivitet er undersøgt i et fastliggende gødningsforsøg, anlagt i 1893 ved Askov forsøgsstation. Henholdsvis de ugødede, staldgødede og kunstgødede parceller blev gennem årene behandlet på samme måde.

Resultaterne af de mikrobiologiske undersøgelser ændredes med udtagningstidspunkterne for jordprøverne. Som et gennemsnit af alle jordprøveudtagninger gav svampepladespredninger, ATP indhold, iltforbrug og dehydrogenase aktivitet de største resultater for den kunstgødede jord, lavere resultater for den staldgødede jord og de laveste resultater for den ugødede jord (kontroljord). Bakteriepladespredninger og Jenkinsons biomasse index gav de største resultater for den staldgødede jord.

Forskellene mellem de mikrobiologiske resultater for ugødet, staldgødet og kunstgødet jord var normalt små.

Nøgleord: Mikrobiel biomasse, mikrobiel aktivitet, enzymatisk aktivitet, staldgødning, kunstgødning.

Introduction

The microorganisms in agricultural soils are affected by several factors, such as manuring, cultivation, crop-rotation and weather conditions. The factors all affect soil fertility and productivity (*Abd-El-Malek et al.*, 1976; *Alexander*, 1977). Soil microorganisms play an important role in the growth of plants and they are responsible for e.g. the decomposition of dead plant material and for nutrient transformations. The understanding of nutrient transformations in complex systems such as soil, requires information about microbial biomass, microbial activity and enzymatic activity (*Hersman & Temple*, 1979; *Nannipieri et al.*, 1978; *Nannipieri et al.*, 1979; *Poul & Johnson*, 1977).

The long-term effects of organic manure and NPK fertilizers on soil microorganisms have been examined to some extent (e.g. *Müller*, 1962; *Müller et al.*, 1972; *Pokorna-Kozová & Novák*, 1975; *Rübensam et al.*, 1962). In Denmark microbiological examinations of soils originating from different plots on the long-term fertilization experiments at the Askov Experimental Station have been carried out previously (*Christensen*, 1927; *Jensen*, 1951; *Eiland*, 1975).

The present study was undertaken to examine the effects of organic manure and fertilizers on the soil microorganisms under field conditions partly by use of new methods for determinations of microbial biomass, microbial activity and enzymatic activity.

Materials and methods

The field experiment

Field experiments were initiated in 1893 at the Askov Experimental Station in West Jutland with the purpose of elucidating the general problem whether soil fertility can be maintained by the use of fertilizers alone and to determine the value of fertilizers in comparison with farmyard manure. Solid as well as liquid manure were used (*Iversen & Dorph-Petersen*, 1951). From 1973 only liquid manure (slurry) was applied. The experimental plots were regularly cultivated in a four years rotation with wheat, sugarbeets, barley and clover-grass. During every rotation the same average amounts of plant nutrients have been applied. »1 manure« and »1 NPK fertilizers« contain the following amounts of plant nutrients per ha for barley: 75 kg N, 12 kg P and 75 kg K.

In 1978 slurry was applied on March 6th prior to the first sampling and fertilizers were applied on the 1st of May between the first and the second sampling. In 1979, slurry and fertilizers were not applied for clover-grass. In 1978, the crop was barley with clover-grass and in 1979 clover-grass.

Soils and sampling methods

The soil at the Askov Experimental Station is a sandy-loam soil with the following characteristics: (0–23 cm depth), clay (<0.002 mm) 10.6 per cent; silt (0.002–0.02 mm) 11.8 per cent; fine sand (0.02–0.2 mm) 37.0 per cent; sand (0.2–2.0 mm) 37.6 per cent and humus 3.0 per cent (*Hansen*, 1976).

Samples were drawn from three replications (field B.2.) of similarly treated plots and from each of these plots, four soil cores (7 cm diam.) were drawn from the 0–20 cm layer and mixed into one composite sample. The samples were stored at 5°C in polyethylene bags until the next day. Before the soils were analysed they were thoroughly mixed, and stones and roots were removed. For each set of soil samples, all experiments were set up on the same day.

Chemical analyses

The following chemical analyses were made according to Danish standard procedures (*Anonymous*, 1972): Total organic-C, total-N, NO_3^--N, NH_4^+-N, Ft (P soluble in 0.2 N H_2SO_4), Kt (exchangeable K), soil pH ($CaCl_2$) and soil moisture content. Table 1 gives the analytical results of the individual samplings from the variously treated plots.

Microbiological analyses

Bacteria and fungi were enumerated by the standard dilution plate count method. The following plating medium were used for bacteria: Tryptone Soya Agar (TSA, Oxoid Ltd.), 10.0 g/l; Bacto

Table 1. Chemical characteristics of unmanured, manured and fertilized soils sampled at a different times of the year.

Plots B.2. field	Organic-C %	Total-N %	C:N ratio	H_2O %	pH ($CaCl_2$)	NO_3-N ppm	NH_4-N ppm	Ft*	Kt*
	Date of soil sampling 25/4-1978								
Unmanured (control)	1.13	o.1o	11.2	13.7	6.5	2.9	5.o	3.7	3.7
1½ slurry manure	1.48	o.13	11.o	18.5	6.4	22.4	4.7	6.2	9.8
1½ fertilizers	1.27	o.12	1o.6	16.3	6.6	3.2	3.4	7.3	1o.5
	Date of soil sampling 15/8-1978								
Unmanured (control)	1.22	o.o9	13.o	12.2	6.3	1.1	7.o	n.d.	n.d.
1½ slurry manure	1.49	o.13	11.5	15.6	6.1	1.2	8.1	n.d.	n.d.
1½ fertilizers	1.27	o.11	11.6	12.6	6.3	1.2	6.1	n.d.	n.d.
	Date of soil sampling 3/1o-1978								
Unmanured (control)	1.13	o.1o	11.3	14.8	6.5	<1.2	3.6	n.d.	n.d.
1½ slurry manure	1.49	o.13	11.5	18.9	6.5	<1.2	5.1	n.d.	n.d.
1½ fertilizers	1.29	o.11	11.7	15.5	6.4	<1.2	4.1	n.d.	n.d.
	Date of soil sampling 22/5-1979								
Unmanured (control)	1.25	o.1o	12.5	9.5	6.6	<1.2	o.5	3.8	3.2
1½ slurry manure	1.65	o.13	12.9	12.9	6.9	<1.2	2.6	6.1	6.2
1½ fertilizers	1.37	o.12	11.7	1o.6	6.9	<1.1	1.9	6.9	8.5

Results are the mean of 6 determinations (2 determinations from each of 3 similarly treated plots).
n.d. = not determined.
See caption to Table 2 for the amounts of manure and fertilizers.
* Estimated as described under "Materials and methods".

Agar (Difco Lab.), 9.0 g/l; Actidion (cycloheximide) 25.0 g/l; (Actidion was dissolved in a 95 per cent ethanol solution and 0.4 ml of this solution was added to 200 ml Agar medium). For fungi, Glucose Peptone Agar with addition of Rose Bengal were used. The medium had the following composition: Glucose, 10.0 g/l; Mycological Peptone, 5.0 g/l; KH_2PO_4, 1.0 g/l; $MgSO_4$, 0.5 g/l; Rose Bengal, 0.3 g/l; Bacto Agar (Difco Lab.), 20.0 g/l. Two subsamples were taken from each composite sample and seven plates were inoculated from each dilution. All plates were incubated for 8 days at 30°C.

Biomass C was estimated by the *chloroform fumigation technique (Jenkinson & Powlson,* 1976).

ATP determinations were performed according to *Eiland* (1979) and *Eiland* and *Nielsen* (1979). The ATP content was extracted with 1 N sulfuric acid and measured the following day in a Lumac Cellcounter 1030 (10 s integration period). The crude luciferin-luciferase enzyme (50 mg, Sigma FLE-50) was dissolved in 2.5 ml sterilized distilled water and stored for 2 hours at 4°C before use.

Oxygen uptake was measured in a Gilson differential respirometer using 10 g samples equilibrated overnight at 25°C. Readings were taken every 30 min. for 2 hours.

Dehydrogenase activity was determined using 2-p-iodophenyl-3-p-nitro-phenyl-5-phenyl-tetrazoliumviolet (INT) as an [H]acceptor. The method used is a modification of that of *Curl* and *Sandberg* (1961).

The results are expressed on an oven dry basis (drying of soil samples at 105°C for 24 hours).

All the methods used are described by *Eiland et al.*, (1979).

Results

The chemical characteristics of the soils from the differently treated plots are shown in fig. 1. Addition of manure to the soil has increased the content of organic carbon and total nitrogen compa-

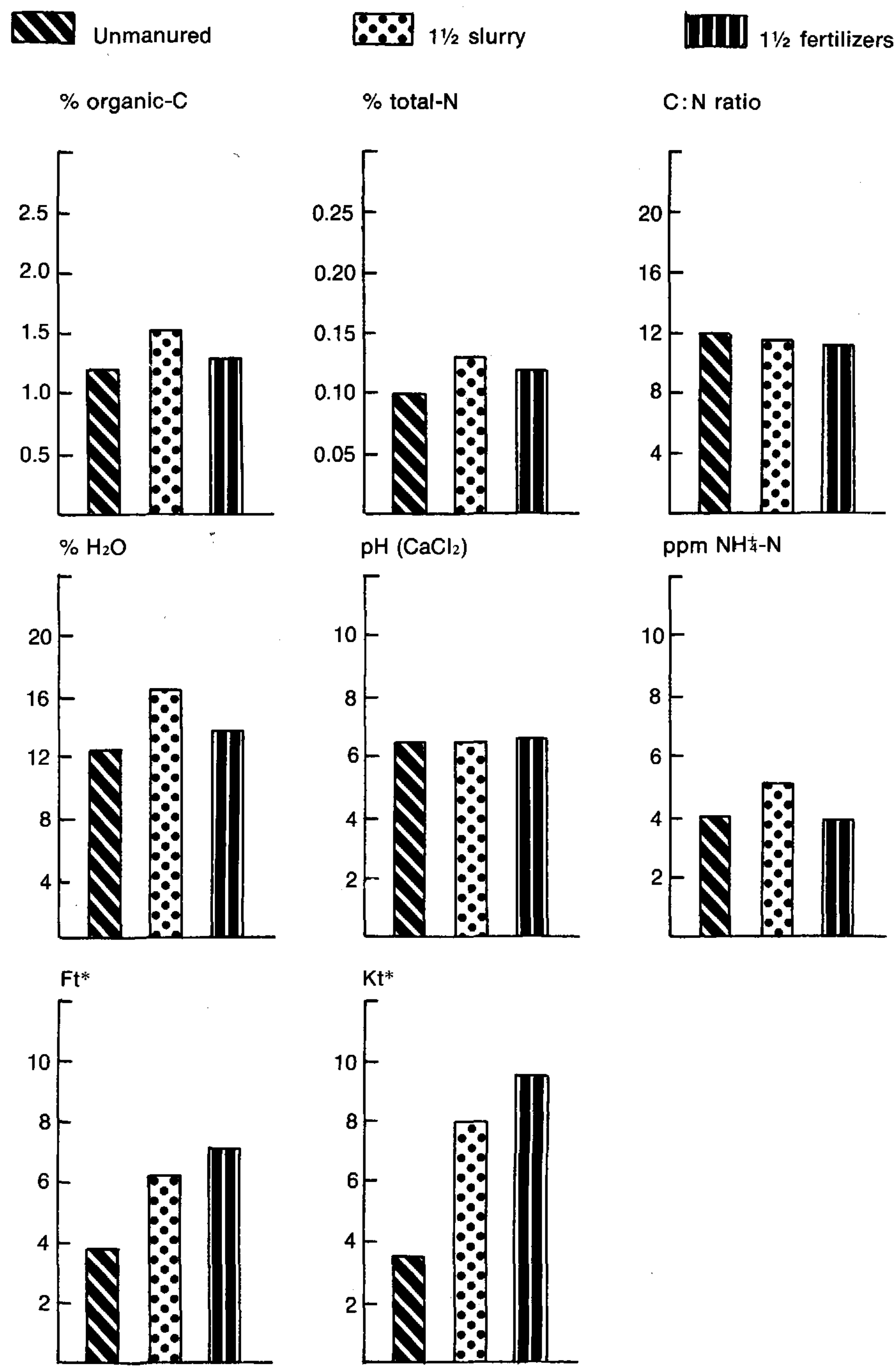

Fig. 1. The effects of slurry manure and fertilizers on chemical properties of soil from the B.2.-field, Askov Experimental Station. The figures shown are the average of the results of all soil analyses on each treatment.

* Estimated as described under »Materials and methods«.
Ft = 3 mg P/100 g soil dwt; Kt = 1 mg K/100 g soil dwt.

red with the unmanured soil. The same effect is seen after addition of mineral fertilizers to the soil but to a lesser extent. The same trends were observed during the years 1923–1969 (*Kofoed,* 1971). The C:N ratio varied between 10.6–13.0 in all soils. The variation in moisture content followed the variation in organic matter.

The number of bacteria determined by the plate count method was found to vary between 35×10^6 and 165×10^6 per g soil dwt with an average of 77×10^6 per g soil dwt (table 2). This amount corresponds to 3 per cent of the total amount of biomass determined by the fumigation technique, and to 11 per cent of the total biomass determined by the ATP analyses. These calculations were based on an average amount of 0.5×10^{-6} ng ATP per bacterial cell in a mixed population (*Anderson & Davies,* 1973) and 50 per cent carbon content in the cells with an average weight of 0.2×10^{-12} g biomass dwt per cell (*Alexander,* 1977). Jenkinson biomass index was calculated with the factor 0.5 (*Jenkinson & Powlson,* 1976).

The number of bacteria seems to be affected by the different treatments as well as the time of the soil samplings but the results are however not consistent. The average of the results for all determinations (fig. 2), indicate higher numbers in manured than in fertilized soil with the control in between.

Plate counts of fungi were made only in April 1978 and May 1979. The numbers of fungal units were found to vary between 9×10^4 and 18×10^4 per g soil dwt with the lowest numbers in the unmanured soil and the highest numbers in the fertilized soil.

Estimates of total biomass (bacteria, fungi and protozoa) by the fumigation method, indicate an average amount of 13 mg biomass C varying from 8–23 mg per 50 g soil dwt (table 2). Average results for the whole period (fig. 2) show the largest amount of biomass in the manured soil with only a small difference between fertilized and unmanured soils.

The ATP content varied within the range of

Table 2. Results of microbiological analyses performed on unmanured, manured and fertilized soils sampled at a different time of the year.

Plots B.2. field	Plate counts bacteria x 10^6 /g soil dwt	Plate counts fungi x 10^4 /g soil dwt	Jenkinson mg C biomass /50 g soil dwt	ATP ng ATP /g soil dwt	O_2-uptake µl O_2/hour /g soil dwt	Dehydrogenase m.eqv.[H] x 10^{-4} /g soil dwt
	Date of soil sampling 25/4-1978					
Unmanured (control)	135±28	18±6	8±1	155± 5	4.1±0.0	4.1±1.2
1½ slurry manure	164±26	26±6	23±3	235± 22	3.6±0.4	5.1±0.8
1½ fertilizers	92± 8	36±5	12±2	225± 5	5.1±1.1	5.6±0.1
	Date of soil sampling 15/8-1978					
Unmanured (control)	53± 5	n.d.	11±1	443± 14	0.5±0.1	1.2±0.2
1½ slurry manure	48± 5	n.d.	15±1	360± 41	1.3±0.3	1.7±0.1
1½ fertilizers	50± 4	n.d.	12±3	562± 31	0.7±0.3	3.7±0.4
	Date of soil sampling 3/10-1978					
Unmanured (control)	35± 8	n.d.	15±0	436± 69	1.3±0.2	0.7±0.1
1½ slurry manure	55± 1	n.d.	18±2	567± 49	2.2±0.8	1.0±0.2
1½ fertilizers	59± 5	n.d.	13±1	479± 61	2.4±1.0	1.8±0.8
	Date of soil sampling 22/5-1979					
Unmanured (control)	100±10	8±4	10±4	293± 61	1.5±0.5	2.2±1.6
1½ slurry manure	88±11	9±2	8±3	318± 13	1.5±0.4	2.6±1.6
1½ fertilizers	49± 6	13±3	11±3	333±114	1.6±0.5	3.2±0.3

Results are the mean of 6 determinations ± Standard Deviation (2 determinations from each of 3 similarly treated plots).

n.d. = not determined.

1½ slurry manure and 1½ fertilizers equivalent to 112.5 kg N per ha.

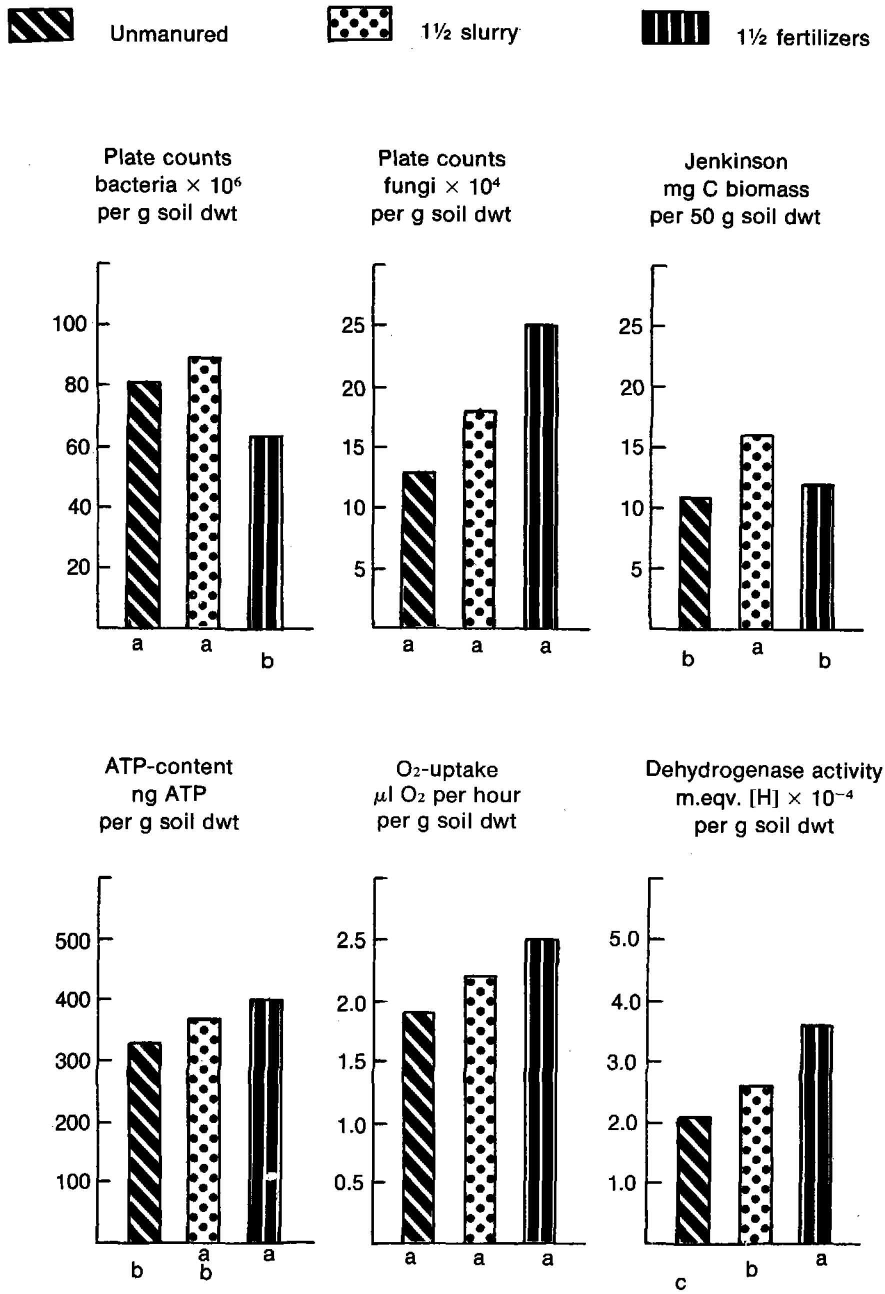

Fig. 2. The effects of slurry manure and fertilizers on microbiological properties of soil from the B.2.-field, Askov Experimental Station. The figures shown are the average of the results of all soil analyses on each treatment.

a, b, c: Values with the same letter are not significantly different ($P = 0.05$).

155–567 ng ATP per g soil dwt with a total average of 367 ng ATP per g soil dwt (table 2). The average ATP content in the unmanured soil was 332 ng per g soil dwt, in the manured soil 370 ng, and in the fertilized soil 400 ng.

The oxygen uptake was used as a measure of the overall activity of the aerobic soil microflora. The results varied between 0.5–5.1 μl O_2 per hour per g soil dwt (table 2) with an average of 2.2 μl (fig. 2). The differences between the treatments correspond to the results of the ATP analyses.

The dehydrogenase activity in the soils varied between 0.7–5.6 m.eqv. [H] $\times 10^{-4}$ per g soil dwt (table 2) with a total average of 2.7 m.eqv. (fig. 2). This was the only method showing the same order between treatments throughout the experimental period. The differences between treatments corresponded to the order between average results of the ATP analyses and the oxygen uptake measurements.

Discussion

It was found that manure and NPK fertilizers affected microbial biomass, microbial activity and enzymatic activity but the effects were not so great as expected. In general, fertilizers have given higher average crop yields than manure for the same amounts of available nitrogen, phosphorus and potassium (*Iversen & Dorph-Petersen,* 1951). The beneficial effect of fertilization on the number of microorganisms, is probably due to the increased growth of the plants leaving an increased amount of roots, root exudates and post-harvest residues.

Christensen (1927) made plate counts of bacteria and fungi and found that usually both types of organisms were most numerous in the manured plots, and the counts were consistently low in the unmanured plots. He also measured CO_2-production and found only insignificant differences between differently treated plots.

Jensen (1951) made both direct counts and plate counts of bacteria but not of fungi, and he also measured CO_2-production. His conclusion was that it was possible to detect a somewhat higher microbiological activity including both higher numbers and higher respiration activity in the manured than in the unmanured plots but the differences were small, and there were no significant differences between NPK fertilized plots and control plots.

Müller (1962) examined a similar long-term fertilization experiment in Germany. The experiment was initiated in 1878 on sandy soil and the plots were regularly cropped with rye (»ewigen Roggenbau«). The microbiological studies were carried out in 1959, and numbers of both bacteria, actinomycetes and fungi were found to be significantly higher in manured than in unmanured plots. The NPK fertilized plots also contained relatively high numbers of microorganisms but the difference between these and the control plots was less consistent.

In another German field trial on sandy soil *Rübensam, Steinbrenner* and *Naglitsch* (1962) found the highest number of microorganisms in plots receiving either manure or artificial fertilizers and the lowest numbers in unfertilized control plots and in plots, lacking calcium and therefore showing a low pH-value (pH = 4.0).

Steinbrenner (1962) also found that addition of manure caused significant increases in the numbers of soil bacteria but he did not observe any effect on soil fungi.

Both the original results reported here and the previously published reports reviewed above, indicate that addition of farmyard manure to agricultural soils may have a more or less stimulative effect on the soil organisms, resulting in increased numbers, increased activity or both. The most surprising thing in this connection is perhaps that the effects of manuring often are quite modest and even may be difficult to prove.

The explanation may be that the stimulating effect of manure is not permanent but restricted to a period of variable length after the addition to soil. The organic matter originating from farmyard manure represent many years accumulation of the most inavailable (biostable) part of the organic matter in the manure. The higher number of microorganisms in the manured soils may partly be the results of better physical conditions that improve the survival. The manure only forms a rather small part of the total amount of organic matter available to soil microorganisms, the remaining part being supplied by the growing crop. Better growth of the crop caused by supply of artificial fertilizers, may therefore, under certain circumstances more than equivalize the effect of the manure.

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