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The effects of high doses of slurry and farmyard manure in microorganisms in soil

Virkningerne af store mængder gylle og fast staldgødning på mikroorganismer i jord

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Summary

The microbial biomass, microbial activity and enzymatic activity were examined in soil samples originating from a field experiment, which was initiated in 1972 at the Askov Experimental Station. The manured plots were added high doses of either slurry or farmyard manure, and in all other respects the plots have been treated in the same way since the start of the experiment.

The results of the microbiological examinations varied with the time of the sampling. On an average, ATP content, oxygen optake and dehydrogenase activity were higher in soils supplied with slurry and farmyard manure than in the corresponding control soils. Addition af manure every year and every two years gave almost identical results.

The same effects were seen for the number of bacteria determined by plate counts if relative values were used (control = 100). The number of fungal units, however, were higher when slurry and farmyard manure were supplied every two years than when added every year, whereas no significant differences with regard to amounts of fungi were seen between soils receiving manures every year and the corresponding control soils.

Key words: Microbial biomass, microbial activity, enzymatic activity, slurry, farmyard manure.

Resumé

Den mikrobielle biomasse, mikrobielle aktivitet og enzymatiske aktivitet er undersøgt i et fastliggende gødningsforsøg, anlagt i 1972 ved Askov forsøgsstation. De gødede parceller blev tilført store mængder enten af gylle eller af fast staldgødning, og alle parcellerne er i alle andre henseender behandlet på samme måde siden forsøgets start.

Resultaterne af de mikrobiologiske undersøgelser ændredes med udtagningstidspunkterne for jordprøverne. Som et gennemsnit af alle jordprøveudtagninger, gav ATP-indhold, iltforbrug og dehydrogenase aktivitet højere resultater i jord tilført gylle og fast staldgødning end i de tilsvarende kontroljorde. Tilførsel af gylle og fast staldgødning hvert år eller hvert andet år gav næsten ens resultater.

De samme virkninger blev fundet for bakterier bestemt på pladespredninger, når relative værdier blev anvendt. Antallet af svampe var derimod højere, når gylle og fast staldgødning blev tilført hvert andet år, end når det blev tilført hvert år, medens der ikke var signifikante forskelle i svampetallene mellem jorde, der fik tilført gødning hvert år og de tilsvarende kontroljorde.

Nøgleord: Mikrobiel biomasse, mikrobiel aktivitet, enzymatisk aktivitet, gylle, fast staldgødning.

Introduction

The effects on micororganisms in soil of addition of traditional amounts of semisolid farmyard manure and slurry to the soil have been studied quite extensively (*Eiland,* 1980; *Jensen,* 1951; *Müller,* 1962; *Novák et al.*, 1974; *Pokarná-Kozová & Novák,* 1975; *Rübensam et al.*, 1962; *Steinbrenner,* 1962), but little attention has been paid to the effects of addition of very large amounts of slurry or farmyard manure to the soil.

Changes in agricultural practice have led to concentration of the husbandry, resulting in production of large quantities of animal wastes with access to only restricted acreages for disposal of this waste. Therefore, it is important to know about the effects of addition of large quantities of such material on microbiological conditions in the soil.

The present investigation deals with the effects on the microorganisms and the microbiological processes in an arable soil caused by incorporation of high doses of either slurry or farmyard manure to the soil during several years.

Materials and methods

The field experiment

The field experiment was initiated in 1972 at the Askov Experimental Station in West Jutland with the purpose of elucidating the effects of addition of high amounts of slurry (SLU) and farmyard manure (FYM) to the soil. The following amounts of SLU and FYM were used in the examined plots since the start of the experiments: 100 tons per ha every year, 200 tons per ha every two years and 400 tons per ha every four years. The times when the manures were added are stated in Table 2 and Fig. 2. These amounts are equivalent to 500 kg N per ha, 1000 kg N per ha and 2000 kg N per ha, respectively (*Nemming,* 1976). Controls were included for both SLU and FYM amended soils, receiving 80 kg N per ha in NPK fertilizers.

The experimental plots were regularly cultivated in a four years rotation with sugarbeets, barley, Italian rye-grass and barley. In 1978, the crop was barley and in 1979, Italian rye-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; coarse sand (0.2–2.0 mm) 37.6 per cent, and humus 3.0 per cent (*Hansen,* 1976).

Samples were drawn from three replications of similarly treated plots and from each of these plots, four soil cores (7 cm diam.) were taken from the 0–20 cm layer and mixed into one composite sample. The samples were stored at 5°C in polyethylene bags until use. Before the soils were analyzed 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.

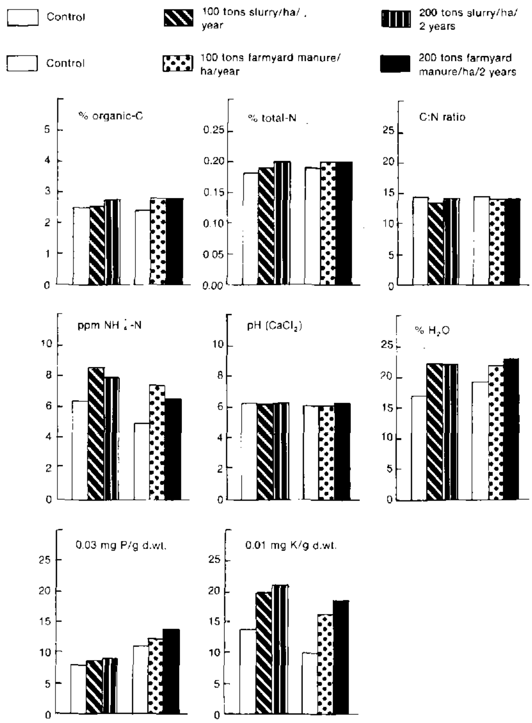

Fig. 1. The effects of high doses of slurry and farmyard manure on chemical properties of the soils from the L-field, Askov Experimental Station. The figures shown are the mean of the results for all soil analyses on each treatment.

Table 1. Some chemical charactetistics of the soils sampled at a different time of the year

	Organic-C		Total-N		C:N		H_2O		pH	NO_3^--N		NH_4^+-N		Ft*		Kt*	
	%	rel. val.	%	rel. val.	ratio	rel. val.	%	rel. val.	($CaCl_2$)	ppm	rel. val.	ppm	rel. val.	Ft	rel. val.	Kt	rel. val.
Date of soil sampling 25/4 1978																	
Control	2.41	100	0.17	100	13.9	100	9.7	100	6.2	1.9	100	3.9	100	7.8	100	12.8	100
SLU ev. year	2.46	102	0.19	112	13.2	95	22.5	232	6.1	28.6	1505	18.3	469	8.9	114	20.0	156
SLU ev. 2nd year	2.66	110	0,20	118	13.6	98	19.6	202	6.4	3.1	163	5.4	138	8.7	112	19.7	154
Control	2.33	100	0.17	100	14.0	100	17.8	100	5.9	2.9	100	3.0	100	10.1	100	9.3	100
FYM ev. year	2.71	116	0.20	118	13.3	95	21.8	122	6.0	14.3	493	8.0	267	12.3	122	17.3	186
FYM ev. 2nd year	2.71	116	0.20	118	13.5	96	23.0	129	6.2	5.9	203	5.9	197	13.5	134	18.8	202
Date of soil sampling 15/8-1978																	
Control	2.46	100	0.18	100	13.8	100	19.3	100	6.2	1.3	100	6.5	100	n.d.	-	n.d.	-
SLU ev. year	2.39	97	0.18	100	13.1	95	20.2	105	6.0	5.7	438	8.5	131	n.d.	-	n.d.	-
SLU ev. 2nd year	2.69	109	0.19	106	13.9	101	21.4	111	6.2	1.3	100	9.5	146	n.d.	-	n.d.	-
Control	2.39	100	0.17	100	14.5	100	18.2	100	5.9	1.3	100	5.9	100	n.d.	-	n.d.	-
FYM ev. year	2.77	116	0.20	118	13.7	94	19.4	107	6.1	7.9	608	9.7	164	n.d.	-	n.d.	-
FYM ev. 2nd year	2.77	116	0.20	118	13,9	96	21.2	116	6.2	2.2	169	9.9	168	n.d.	-	n.d.	-
Date of soil sampling 3/10-1978																	
Control	2.55	100	0.17	100	15.0	100	22.6	100	6.2	>1.3	>100	4.5	100	n.d.	-	n.d.	-
SLU ev. year	2.52	99	0.19	112	13.3	89	24.3	108	6.1	1.5	115	4.7	104	n.d.	-	n.d.	-
SLU ev. 2nd year	2.74	107	0.20	118	13.7	91	25.0	111	6.3	>1.3	>100	6.2	138	n.d.	-	n.d.	-
Control	2.41	100	0.17	100	14.2	100	22.2	100	6.0	>1.3	>100	2.9	100	n.d.	-	n.d.	-
FYN ev. year	2.82	117	0.20	118	14.1	99	25.6	115	6.1	>1.7	>131	7.3	252	n.d.	-	n.d.	-
FYM ev. 2nd year	2.84	118	0.21	124	13.5	95	26.0	117	6.4	>1.4	>108	5.9	203	n.d.	-	n.d.	-
Date of soil sampling 22/5-1979																	
Control	2.57	100	0.18	100	14.3	100	16.7	100	6.5	8.4	100	10.6	100	8.1	100	14.5	100
SLU ev. year	2.69	105	0.19	106	14.2	99	21.7	130	6.4	10.8	129	2.7	25	8.6	106	19.6	135
SLU ev. 2nd year	2.86	111	0.21	117	13.8	97	21.8	131	6.4	30.4	362	10.5	99	9.0	111	22.3	154
Control	2.51	100	0.17	100	15.3	100	18.7	100	6.4	10.2	100	7.6	100	12.1	100	10.9	100
FYM ev. year	2.93	117	0.20	118	14.7	96	21.2	113	6.3	9.5	93	4.5	59	12.1	100	15.1	139
FYM ev. 2nd year	2.91	116	0.20	118	14.4	94	21.5	115	6.2	20.4	200	4.4	58	13.8	114	18.1	166

Values are the means of 3 replicate plots with 2 determinations from each of the plots.
n.d. = not determined.

* Ft = 3 mg P/100 g soil d.wt.; Kt = 1 mg K/100 g soil d.wt.
See caption to table 2 for the different amounts of manure.

Table 2. Results of microbiological analyses performed on soils sampled at a different time of the year

	Plate counts bacteria		Plate counts fungi		Jenkinson		ATP content		O_2 uptake		Dehydrogenase activity	
	x 10^6/ g d. wt.	rel. val.	x 10^4/ g d. wt.	rel. val.	mg biomass C/ 50 g d. wt.	rel. val.	ng ATP/ g d. wt.	rel. val.	µl O_2 per hour/ g d. wt.	rel. val.	m.eqv. [H]x10^{-4}/ g d. wt.	rel. val.
Date of soil sampling 25/4 1978												
Control[a]	67±16	100	13±2	100	n.d.	-	41± 4	100	4.3±1.7	100	5.0±0.7	100
SLU ev. year[b]	84±23	125	8±1	60	n.d.	-	55± 8	132	4.3±1.5	100	4.9±0.5	98
SLU ev. 2nd year[c]	97± 9	145	10±2	77	n.d.	-	108± 8	259	5.3±1.4	105	3.0±0.3	60
Control[a]	66±15	100	10±6	100	n.d.	-	59± 11	100	2.7±1.3	100	5.2±0.6	100
FYM ev. year[d]	76± 8	116	7±3	70	n.d.	-	95± 20	162	4.6±0.7	170	6.6±0.6	127
FYM ev. 2nd year[e]	75± 5	114	8±3	80	n.d.	-	54± 3	92	4.3±0.8	159	7.5±2.0	144
Date of soil sampling 15/8-1978												
Control[a]	96± 9	100	n.d.	-	22±0	100	698± 92	100	0.6±0.3	100	1.7±0.1	100
SLU ev. year[b]	85±13	88	n.d.	-	19±0	86	760± 61	106	1.0±0.3	167	2.3±0.3	136
SLU ev. 2nd year[c]	77± 1	80	n.d.	-	18±0	81	779± 94	109	1.5±0.3	251	3.3±0.3	195
Control[a]	71±17	100	n.d.	-	n.d.	-	673±133	100	1.2±0.1	100	1.4±0.0	100
FYM ev. year[d]	64± 3	90	n.d.	-	n.d.	-	880± 66	123	1.7±0.3	141	1.4±0.0	100
FYM ev. 2nd year[e]	50± 5	71	n.d.	-	n.d.	-	934± 73	131	2.2±0.3	183	1.4±0.0	100
Date of soil sampling 3/10-1978												
Control[a]	170± 0	100	n.d.	-	17±2	100	361± 42	100	1.8±0.5	100	1.7±0.7	100
SLU ev. year[b]	134±12	79	n.d.	-	21±2	124	588± 23	165	2.5±0.1	140	1.6±0.25	94
SLU ev. 2nd year[c]	89± 7	53	n.d.	-	21±2	124	555± 27	155	3.0±0.2	168	2.1±0.13	124
Control[a]	98±12	100	n.d.	-	n.d.	-	608±126	100	3.1±0.0	100	1.5±0.2	100
FYN ev. year[d]	107± 5	109	n.d.	-	n.d.	-	701±122	112	2.9±0.4	93	2.0±0.2	134
FYM ev. 2nd year[e]	115± 4	117	n.d.	-	n.d.	-	593± 94	95	3.6±0.6	115	2.3±0.1	154
Date of soil sampling 22/5-1979												
Control[a]	91± 8	100	7±1	100	12±1	100	321±124	100	1.2±0.3	100	2.7±0.2	100
SLU ev. year[b]	125±12	138	10±1	143	15±0	125	430± 96	133	1.7±0.1	141	3.2±0.2	118
SLU ev. 2nd year[c]	128±19	141	11±2	157	17±1	141	421± 48	131	1.9±0.1	155	4.7±0.7	174
Control[a]	91± 3	100	20±5	100	n.d.	-	218± 65	100	1.5±0.8	100	1.5±0.1	100
FYM ev. year[d]	102± 9	112	25±5	125	n.d.	-	267± 85	123	1.7±0.1	114	2.1±0.3	141
FYM ev. 2nd year[e]	118±23	130	35±2	175	n.d.	-	247± 93	114	1.9±0.7	127	2.5±0.1	168

Values are the means of 3 replicate plots followed by the standard deviation (two determinations from each of the plots).
a = 80 kg N/ha in fertilizers (supplied 27/4 1978 and 8/5 1979).
b = 100 tons slurry/ha every year (supplied 7/2 1978 and 13/3 1979).
c = 200 tons slurry/ha every 2 years (100 tons supplied 13/3 1979, 50 tons 3/7 1979 and 50 tons 6/8 1979).
d = 100 tons farmyard manure/ha every year (supplied 7/3 1978 and 13/3 1979).
e = 200 tons farmyard manure/ha every 2 years (100 tons supplied 13/3 1979, 50 tons 3/7 1979 and 50 tons 6/8 1979).

Chemical analyses

The following chemical analyses were made according to Danish standard procedures (*Anonymous*, 1972): Total content of organic-C, total-N,NO_3^--N, NH_4^+-N, Ft (P soluble in 0.2 N H_2SO_4 ; 0.03 mg P/g dry soil), Kt (exchangeable K; 0.01 mg K/g dry soil), soil pH ($CaCl_2$) and soil moisture content. Table 1 gives the chemical results of the individual samplings from the variously treated plots.

Microbiological analyses

Bacteria and fungi were enumerated by the standard dilution plate count method, as described by *Eiland* (1980). *Biomass C* was estimated by the chloroform fumigation technique (*Jenkinson & Powlson*, 1976).

ATP determinations were performed according to *Eiland* (1979) and *Eiland & Nielsen* (1979). The ATP content was extracted with 1 N sulfuric acid and measured the following day in a Lumac Cell-counter 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 & Sandberg* (1961).

All results are expressed on an oven dry basis (drying of soil samples at 105°C for 24 hours). The methods used are described by *Eiland et al.* (1979).

Results and discussion

Some chemical characteristics of the soils are shown in Fig. 1. The content of organic-C was slightly higher in FYM amended soil than in SLU amended soil whereas the content of total-N, C:N ratio and pH of the soils were nearly the same where soils were amended with FYM or SLU. The moisture content at the time of the samplings was lower in the unmanured soils than in soils supplied with FYM or SLU.

In Table 2 and Fig. 2 the microbiological results are shown for soil samples taken in the period April 1978 – May 1980, and Fig. 3 shows average results of the microbiological analyses.

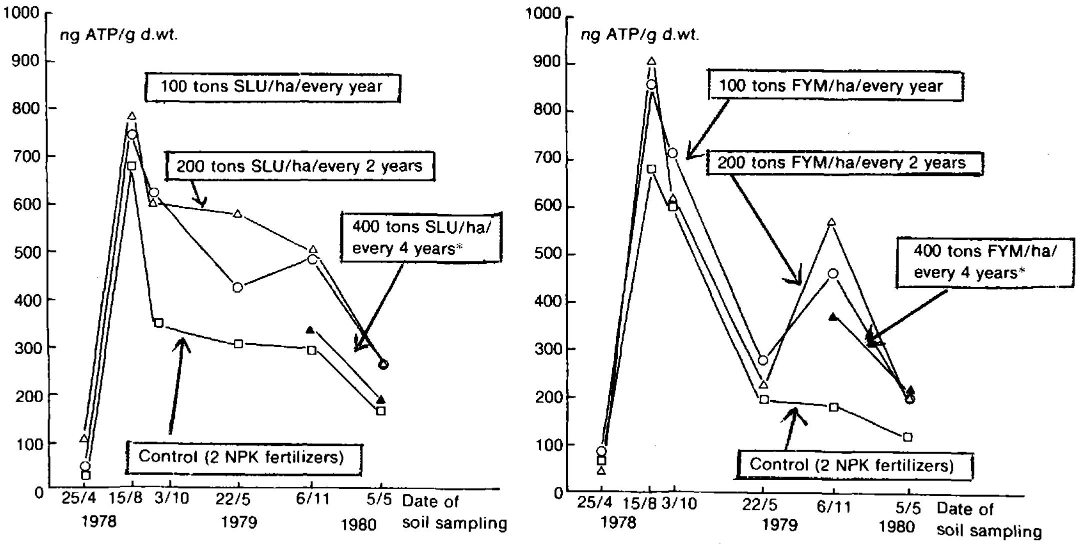

Fig. 2. The effects on the ATP content in soil caused by addition of high doses of slurry (SLU) or farmyard manure (FYM) to the soil. Values are the means of three replicated plots with two determinations from each of the plots.
* 400 tons SLU or FYM/ha/every 4 years were supplied 20/12–1976.
See caption to Table 2 for the time of supplying the other doses of manure.

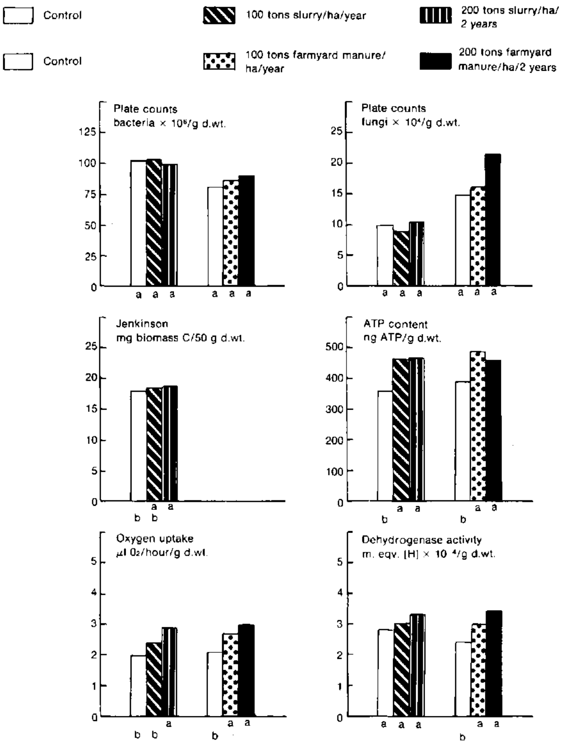

Fig. 3. The effects of high doses of slurry and farmyard manure on microbiological properties of the soils from the L-field, Askov Experimental Station. The results are the mean of the results for all soil analyses on each treatment.
Values with the same letter are not significantly different (level 0.05).

The number of bacteria determined by plate counts was higher in SLU amended soil than in FYM amended soil. However, a comparison of the relative values (control = 100), showed very similar results for the two different manure treatments. The observed differences, therefore, must be due to other factors than the treatments. There were no significant differences between soils amended with FYM or SLU every year, every two years, and the controls. Furthermore, the variations with the time of the soil samplings were relatively small.

The number of fungal units was higher when SLU and FYM were supplied every two years than when added every year, but the difference was not statistically significant. The control soils gave sometimes higher and sometimes lower values than the manured soils.

Davis et al. (1980) examined microbial populations by plate counts in clay loam receiving large applications of cattle feetlot waste. They found only transitory effects on the soil microbial populations as also indicated by plate counts in the present work.

Determination of the biomass by chloroform fumigation was higher in the SLU treated soils than in the control, except for soils sampled 15th August 1978. Addition of the same amount of SLU every year or the double amount every two years gave very similar results.

The ATP content varied with the time of the soil samplings (Fig. 2). The seasonal fluctuations for SLU and FYM treated soils were nearly the same. During April 1978 rather low ATP concentrations were recorded. In late May 1979 higher values were obtained for SLU treated soils than for FYM treated soils and the opposite situation was observed in November 1979. The overall highest values were obtained in the middle of August 1978, where 800-900 ng ATP/g dry soil were recorded. In the beginning of October 1978, slight decreases were obtained and in November 1979 and May 1980 even lower values were found.

The effects of addition of 400 tons SLU or FYM/ha/every four years (supplied 20th December 1976) to the plots were examined in November 1979 and May 1980. The SLU treated soils gave only slightly higher ATP values than did the fertilized soil (control), and the FYM treated soils gave the double amounts of ATP, compared with the fertilized soil.

The results of the ATP analyses clearly indicate seasonal fluctations. *Söderström* (1979) examined the amount of FDA-active hyphae in a Swedish pine-forest for three successive years. He reported a fairly high fungal biomass during the autumn. After a depression during the winter, fungal activity again increased in the spring resulting in high biomasses. *Lynch* and *Panting* (1979) examined the size of the biomass in an English arable soil during one year by a modified fumigation method. They reported that the biomass evidently remains at a fairly constant amount until the spring, where it increased to a maximum around June and slightly lower values were observed in August. Fluctuations in the FDA-active fungal biomass and the total biomass determined by fumigation are in good agreement with the present results of the ATP determinations.

On the whole, the ATP content was significantly higher in the soils supplied with SLU or FYM, than in the corresponding controls. Addition of manure every year or the double amount every two years gave very similar results (Fig. 3).

Oxygen uptake and dehydrogenase activity showed the highest activities in soils supplied with SLU and FYM every two years. Lower activities were found for addition af manure every year (SLU and FYM), and the lowest activities were seen in the corresponding control soils.

The total number of earthworms at the same levels of slurry and farmyard manure expressed as the mean of data from October 1976 to October 1978 *(Andersen,* in press), showed the same effects of soil treatments as observed in the ATP determinations. But he found that farmyard manure favoured more balanced proportions of the individual species than did slurry.

Nemming (1978) examined the yields of crops during a four years period (1972–1976) in the same field experiment. He found as an average that slurry gave higher yields of crops than did farmyard manure, but an addition of the same amount of NH_4^+-N in the two types of manure gave nearly the same yields. Normally, addition of manure every year gave higher yields than higher amounts of manure every two years or every four years. The yields of crops were not in good agreement with the microbial determinations.

Conclusions

High doses of slurry and farmyard manure both when supplied every year and every two years increased ATP content, oxygen uptake and dehydrogenase activity, compared with a fertilized control soil. However, the metabolically active biomass determined by the ATP assay and the microbial activities (oxygen uptake and dehydrogenase activity) were nearly the same for soils receiving SLU and FYM treatments. A comparison between normal SLU treatment (112.5 kg N in SLU/ha/every year) in the B.2.-field *(Eiland,* 1980), and addition of high doses of SLU or FYM (500 kg N/ha/every year or 1000 kg N/ha/every two years), showed that the ATP concentration, the oxygen uptake and the dehydrogenase activity were increased by the higher doses.

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