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Sisällys — Contents

ILMARI SCHALIN: On the effect of nitrogen fertilization on the bacteria and microfungi in humus layer. 1
Seloste: Typpilannoituksen vaikutuksesta humuskerroksen bakteereihin ja homesieniin. 11

KOZLOWSKI, T. T., SASAKI, S. and TORRIE, J. H.: Effects of temperature on phytotoxicity of monuron, picloram, CDEC, EPTC, CDAA, and sesone to young pine seedlings. 13
Seloste: Lämpötilan vaikutuksesta monuronin, pikloramin, CDEC:n, EPTC:n, CDAA:n ja sesonen myrkyllisyyteen nuorissa männyntaimissa. 28

HELGE JOHNSSON: Olika vägar för rasmässig förbättring av vårt skogsodlingsmaterial. 29
Summary: Different ways of genetic improvement of forest trees in Scandinavia. 55

MATTI LEIKOLA: Havaintoja erään hoidetun männikön tuulisuhteista. 57
Summary: Observations on wind conditions in a managed Scots pine stand. 71

Uutisia — News. 72

Kirjallisuutta. 73

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ON THE EFFECT OF NITROGEN FERTILIZATION ON THE BACTERIA AND MICROFUNGI IN HUMUS LAYER

ILMARI SCHALIN

SELOSTE:

TYPPILANNOITUKSEN VAIKUTUKSESTA HUMUSKERROKSEN BAKTEEREIHIN JA HOMESIENIIN

Hyväksytty 1. 4. 1967

During the summer and fall of 1966 changes brought about by urea, calcium ammonium nitrate, nitrate of lime and ammonium sulphate in the bacterial and microfungal densities of a pine barren humus layer were observed. Application of the fertilizers corresponded to 100 kg/N per hectare.

The effect of urea was immediate. The pH rose and the bacterial density increased to 20—30 times more than that determined in the spring, while the microfungal density decreased to onethird of the spring density. In the ammonium sulphate plot corresponding, but completely opposite, changes occured almost as rapidly as in the previous case. A gradually increasing biological activity observed after application of calcium ammonium nitrate and nitrate of lime fertilizers seemed almost the same for bacteria and microfungi. Both microbe groups displayed consistent quantitative growth. pH 4.3 was the limit of acidity below which the bacteria showed a tendency to decline and the microfungi to increase, while the opposite was true above this limit.

Introduction

Several problems, difficult to solve, are related to the nitrogen fertilization of forest soils. The effect of fertilization on tree growth can be determined, with a certain accuracy, by size measurements and chemical analysis. The permanence of the effect and the significance of quality are more complicated to determine; perhaps the most difficult is the effect of nitrogen fertilization on the soil and its different phenomena.

Little is known about the influence of nitrogen fertilization on soil microbes and the role they play in the changes taking place in the fertilizers. These points have been demostrated, in part, by laboratory tests conducted singly with certain types of microbes, but thus far there is no information on the way that nitrogen fertilizers change in form, where, when subjected to predetermined microbial populations, they are affected by the entire mixed-population of the soil. Also there is little or no information on the changes that occur within the microbial population per se, resulting from the effect of the fertilization is wanting. This study deals with the latter condition where an attempt has been made to try to clarify it by means of a quantitative examination where minor details are by-passed to make the overall picture as clear as possible.

It also gives information relative to the ways bacterial and microfungal densities of the humus layer change as a result of nitrogen fertilization. This study is not so extensive, but deals with one dry heath forest stand, and the results are based on one summer's experiments and observations. The material should be considered adequate for a fairly thorough examination, because the organisms that were studied are generally more sensitive to natural environmental factors than other types of living organisms.

It should be noted that the results serve as only an orientation to the problem, because the information contained in them will be of practical value only to the extent that are followed by further research.

Material and methods

A VT* pine stand, the complete description is given in a previous study (SCHALIN 1967), chosen as the site of the investigation was located on a sandy barren of approximately ten hectares, where the character of both the soil and the tree stand was fairly homogenous and the mineral soil was covered with a less-than-2-cm-layer of rather poorly decomposed humus.

Five circular fertilization sample plots of ten ares were established in the stand and formed a row a distance of 25 meters between them to prevent any overlapping effects of the fertilizers. Each plot was divided into four sectors by cutting the circle with two diameters intersecting each other at right angles, thus providing four duplicate plots in each sample plot. With four replicate plots in each sample a considerable increase was represented in the realiability of the results obtained.

One of the sample plots served as a control (0). The others (1—4) were treated with nitrogen fertilizers as follows:

(1) urea	46% N
(2) calcium ammoniun nitrate	26% N
(3) nitrate of lime	15% N
(4) ammonium sulphate $(NH_4)_2SO_4$	20% N

* *Vaccinium* forest type of Cajander.

The application of fertilizers corresponded to 100/N per hectare. Before fertilization, at the beginning of June, samples were taken from each sector of every one of the sample plots. After fertilization, four corresponding samples were taken in the same way, between June and September. These were homogenized and replicates prepared in eight plates containing soil agar for the bacteria, and Martin's medium for the microfungi. Prepared as they were in four replication, there were in all 32 plates for each of the two groups of cultures (bacteria and microfungi).

It is not mere coincidence that Poisson's distribution was shown to be valid for the bacteria when the dilution plate method was employed. This has also proven out in a number of other studies (JAMES and SUTHERLAND 1939, STEARMAN 1955, SCHALIN 1964), and was confirmed experimentally in the present study whenever the sample was diluted to 1: 20000 and was used for bacteria throughout this study. Reliability of results was tested with Fisher's dispersion index and it confirmed that standard deviations from the theoretical distribution were small. Although these were generally less than 5%, a number of instances fell below 1%.

A quantitative comparison of bacterial densities in the control plot and the fertilized sample plots was carried out using the *t*-test, which is based on direct comparison of the means, since they are the same size as the standard deviations in the Poisson series (SNEDECOR 1956).

The quantitative determination of microfungal population was made using a 1: 10000 dilution, inasmuch as this degree of dilution proves most useful for microfungi. It is to be noted here that the control results were the same as those given in a previous study of the VT pine stand (SCHALIN 1967).

The reliability of the results was not tested for microfungi because their probability distribution type could not be determined. Since a distribution very

Tabel 1. Course of pH in humus layer of the sample plots.
Taulukko 1. pH:n kehitys näytealojen humuskerroksessa.

Sample plot *Näyteala*	June 6 *Kesäk. 6*	June 12 *Kesäk. 12*	June 30 *Kesäk. 30*	July 29 *Heinäk. 29*	Aug. 18 *Elok. 18*	Sept. 28 *Syysk. 28*
(0) Control — *Kontrolli*	4.2	4.1	3.9	3.9	4.0	4.4
(1) Urea — *Urea* ..	4.2	4.5	4.8	4.6	4.4	5.1
(2) Calc. amm. nitr. — *Kalkkiammonsalp*	4.3	4.3	4.3	4.1	4.1	4.7
(3) Nitr. of lime — *Kalkkisalp.*	4.2	4.1	3.9	4.0	4.4	4.9
(4) Amm. sulph. — *Ammonium sulf.*	4.3	4.0	3.7	3.6	3.8	4.0

close to normal is quite often found in nature, a partial reliance has been constituted on this information. The results given are in simple figure symbols based on the means of the microfungi colonies in the replicate plates. Even though the results must be considered as highly unreliable from the standpoint of a detailed analysis, still they clearly show the general trend of development.

The pH of the humus layer was determined one week after fertilization, and each of the times that samples were taken. Determinations were made for each sector and from these values pH means were calculated for the different sample plots. They are given in table 1.

Every one of the fertilizers affected the pH on the humus layer. In comparison with the control plot, the greatest change was brought about by urea, which raised the pH by 0.7 and produced an immediate effect. The effect of calcium ammonium nitrate, and nitrate of lime, on the pH was similar to that of urea, but less and noticeable slower. But ammonium sulphate increased the acidity somewhat, as has been expected.

Results

The bacterial density

Figure 1 shows the differences in bacterial density in the humus layer of the control and the fertilized plots at various intervals.

Before fertilization, the humus layer of every sample plot contained almost the same size of bacteria. No significant differences existed from plot to plot or among the various plots as seen in table 2.

Tabel 2. Significance of differences (*t*-values) between means of bacterial densities in sample plots before fertilizing.

Taulukko 2. Näytealoissa havaittujen bakteeritiheyksien keskiarvojen erotusten merkitsevyys (t-arvot) ennen lannoitusta.

Sample plot *Näyteala*	June 6 — *Kesäk. 6* (0)	(1)	(2)	(3)
(1)	0.74			
(2)	0.34	0.95		
(3)	1.76	1.01	1.81	
(4)	0.80	1.27	0.49	1.96

An immediate fertilization effect was noted in only the urea plot (1). The continuous and relatively fast increase of bacterial density was clearly defined.

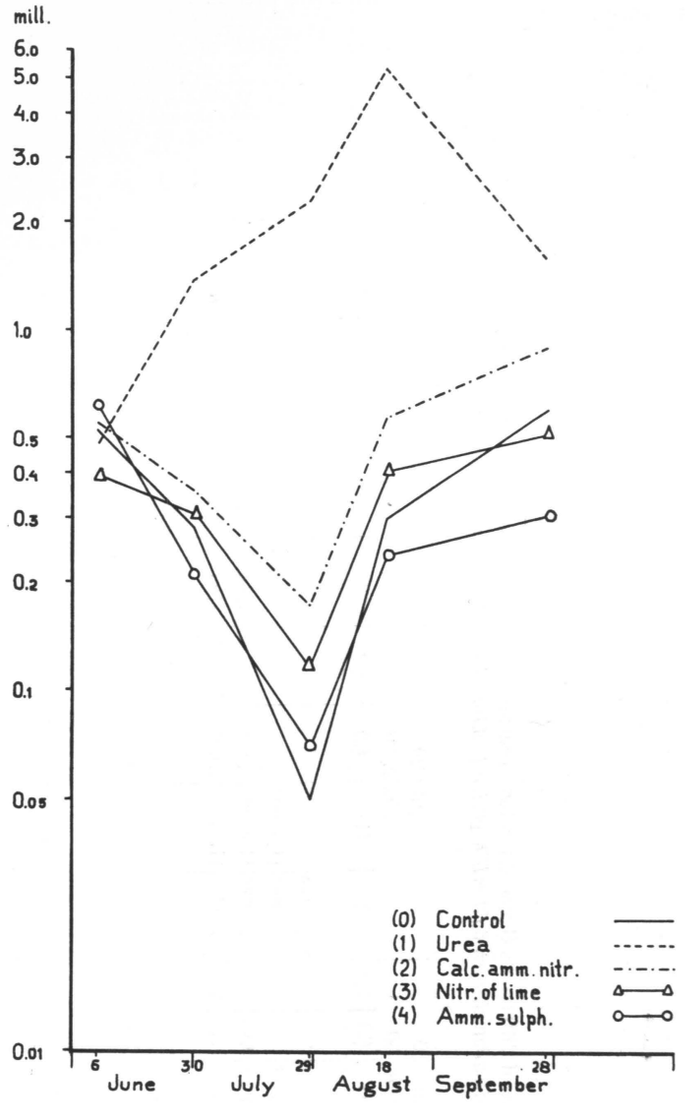

Figure 1. The bacterial density per cc in the fresh humus layer.
Kuva 1. Bakteeritiheys tuoreessa humuskerroksessa cm³ kohti.

In the remaining plots, including the control, bacterial densities with their defined minimums, by the end of July, showed a development that deviated completely from the urea plot, but conformed with the development generally observed in instances of the increase in bacterial density. The significance of the type of fertilizer was also indicated by the occurence of differences in bacterial density found in the various sample plots. A comparison shows that the differ-

Table 3. Significance of differences (t-values) between means of bacterial densities in sample plots after fertilization.
Taulukko 3. Näytealoissa havaittujen bakteeritiheyksien keskiarvojen erotusten merkitsevyys (t-arvot) lannoituksen jälkeen.

Sample plot *Näyteala*	June 30 *Kesäk. 30*				July 29 *Heinäk. 29*				August 18 *Elok. 18*				September 28 *Syysk. 28*			
	(0)	(1)	(2)	(3)	(0)	(1)	(2)	(3)	(0)	(1)	(2)	(3)	(0)	(1)	(2)	(3)
(1)	7.61***				12.10***				24.31***				6.20***			
(2)	1.35	7.04***			5.41***	11.13***			2.64*	21.39***			1.89	7.04***		
(3)	0.51	7.41***	0.85		6.83***	11.64***	2.45*		1.16	24.07***	2.08		0.36	9.54***	2.29*	
(4)	0.33	7.78***	1.72	0.87	2.19*	11.97***	4.62***	3.96***	1.09	24.32***	3.19**	2.25*	4.03***	12.22***	5.51***	5.29***

ences in bacterial density in the plots, fertilized with calcium ammonium nitrate (2), nitrate of lime (3), and ammonium sulphate (4), follow the same orderly trend, although differences increase over period of time. At the close of the investigation period the first named plot contained more bacteria, and last one less bacteria than in the spring, before fertilization. In the nitrate of lime fertilized plot the bacterial size remained almost the same, but in the urea plot the bacteria had increased from 10 to 30 times compared to the rest of the plots from the first determination on.

The significance of differences in bacterial densities brought about by fertilization is shown in the comparison of the sample plots in table 3.

When *t* shows a highly significant deviation in the comparison of two samples it can be taken as proof that they represent two different populations. The immediate quick growth in bacterial density in the humus layer resulting from the application of urea fertlizer must be considered a real, rather than a random effect. A coincident increase in bacterial density caused by calcium ammonium nitrate, and nitrate of lime, is also an indiputable phenomen, even though the effects in these instances are less and a lot slower. Ammonium sulphate affected

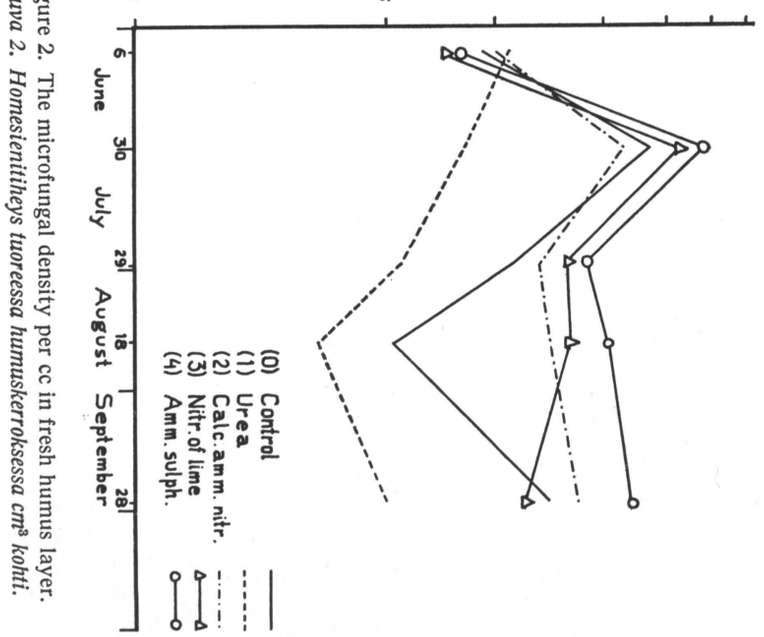

Figure 2. The microfungal density per cc in fresh humus layer.
Kuva 2. Homesienitiheys tuoreessa humuskerroksessa cm³ kohti.

bacterial density slowly and in a manner contrary to that of the other fertilizers. The presence of this difference was under question until it was fully establish in the fall.

The microfungal density

Figure 2 shows the differences in microfungal density in the control plot and fertilized sample plots, determined at the same time as the bacterial densities.

According to determinations made before the application of fertilizers, microfungal occurence was approximately the same in the sample plots. This indicates that the chance of error can be expected to be relatively small in the light of final results.

One of the immidiate effects of fertilization on microfungi was noticed in the instance of urea (1) wich had also brought about an immediate increase in bacterial density. In the instance of the microfungi, the density decreased continuosly, as shown by successive measurements. There was a minor increase in the fall.

An almost similar microfungal density development was detected in all other fertilized plots. Here the control (0) differed a little from the others. There was a considerable deviation, a clear minimum in August that was not apparent in the sample plots of calcium ammonium nitrate (2), nitrate of lime (3), and ammonium sulphate (4). Here microfungal densities followed the regular trend right up to fall, when a slight irregularuty appeared. This, in addition to the ammonnium sulphate plot which contained the highest microfungal density, at the same time that the urea one contained the lowest, is, in all probability related to the different acidity of the humus layer of the sample plots. The discussion that follows substantiates this evidence.

Finally, it should ne noted that no statistical test was carried out in the case of microfungi.

Discussion

When examining the changes brought about by nitrogen fertilizers in microbial densities of dry heath forest land, the moist effective procedure might be that of starting with the facts already established.

Clear-cut differences exist between solubility rates of the nitrogen fertilizers. One essential reason for this is the form of the nitrogen present in the fertilizer. It is known that the ammonium-N dissolves faster than the nitrate-N (i.a. HILTBOLD, BARTHOLOMEW and WERKMAN 1951, BROADBENT 1964), even though it is not actually known how great the differences are. Solubility in nature is dependent on so many factors that the establishment of a close correlation between nitrogen quality and the solubility rate remains difficult to prove.

Nitrogen fertilizers bring about considerable change in the humus layer. They free part of the organic nitrogen of the soil (CHU and KNOWLES 1966), but the

mineralization of the same is dependent on the acidity of the environment; and, that pH is dependent on the fertilizers.

The effect of pH in mineralization is most clearly defined in the formation of nitrates, which is common in acid soil, although low acidity steps up the production of ammonium (HARMSEN and VAN SCHREVEN 1955, ROBERGE and KNOWLES 1966).

Soil microbes are, to a limited extent, particular in regard to the quality of nitrogen nutrients. Because organic matter is their most important source of nitrogen, this does not rule out the change of their nitrogen needs by taking them from inorganic compounds. Observation shows some bacteria use ammonium-N almost entirely (GIBSON 1935), and it is apparently preferred by microfungi (JANSSON, HALLMAN and BARTHOLOMEW 1955).

A low acid environment is better for bacteria, and microfungi adjust well to considerably acid environments. Small pH fluctuations common under normal conditions during the growing season are of little importance in either of these microbe groups, expect when the environmental conditions are otherwise acceptable.

In accordance with statements based on observations as above, some cautious conclusions can be drawn pertaining to the effects of nitrogen fertilizers on bacterial and microfungal densities of the humus layer.

A deviation in results was noted in urea fertilization of the two microbe groups involved. This was no doubt because of its organic nitrogen which appeared in its orginal form and as readily soluble ammonium-N (ERNST and MASEY 1960, WARREN 1962) is a particularly suitable nutrient for bacteria and produces fast growth (GIBSON 1935). Urea reduced the acidity and created an environmental condition in the humus layer such that nitrogen could be readily assimilated, thus increasing the bacterial density.

The factors that brought about bacterial density increased it in the first three months (June—August) followed by a decrease later on and a coincident, but reverse, development in the pH are not contradictory despite the observations above. The development of bacterial activity increases environmental acidity. When this restricts efficient bacterial function, the factors that increase the acidity lessen. This does not provide a sufficient reason for the rise in pH, observed in September, and it must be assumed that the phenomenon is related to the decrease of bacteria that had started at the same time that it was apperent that the temperatur of the humus layer was beginning to drop, although the moisture increased (VEZINA 1965).

A phenomenon brought about by ammonium sulphate in the humus layer, namely, the creation of the kind of environmental conditions there were few bacteria but a considerable large size of microfungi, represent an indirect effect of acidity. Ammonium sulphate brought about an immediate and rather unusual sink in the pH that proved permanent. Increasing acidity is known to lead to neutralization of ammonium-N which forms irreversibly complex compounds

with organic debris (i.a. ROBERGE and KNOWLES 1966). Such a considerable increase in density in microfungal population under these conditions is related to the ability of this microbe to adjust to a low reaction plus its capacity to use several kinds of nitrogen and complex nitrogen compounds effectively.

The increased biological activity brought about by calcium ammonium nitrate, and nitrate of lime, was almost the same for both the bacteria and the microfungi. Among the fertilizers, the most active factor was seemingly represented by gradually soluble nitrate-N that increases its useability along a gradual rise in the pH of the humus layer. A balance between bacteria and microfungi can be maintained rather well at pH 4.3, but below this value the bacteria showed a tendency to decline, while the microfungi increased. Both tendencies were reversed at a higher pH.

Assimilation of various kinds of nitrogen nutrients by the microbes creates new cellular tissue; that dies and during the decomposition that follows, the nitrogen is biologically activated and liberated (HILTBOLD, BARTHOLOMEW and WERKMAN 1951, SWABY and LADD 1962, TIURIN and KONONOVA 1962). The reason for this, and just how much of the nitrogen fertilizers are activated by microbe cells, is not dealt with in this study. Inasmuch as further information is not presently available on the real significance of these two microbe groups in the humus layer, it would be assuming too much, on the basis of their greater or lesser abundance, to draw any far-reaching conclusions relative to their actual significance as agents in the circulating process of nitrogen fertlizers.

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SELOSTE:

TYPPILANNOITUKSEN VAIKUTUKSESTA HUMUSKERROKSEN BAKTEEREIHIN JA HOMESIENIIN

Tutkimuksessa on yritetty selvittää urean, kalkkiammonsalpietarin (calcium ammonium nitrate), kalkkisalpietarin (nitrate of lime) ja ammoniumsulfaatin aikaansaamaa vaikutusta kuivan mäntykankaan bakteeri- ja homesienipopulaatioiden kvantitatiivisten erojen muodostumiseen ja pH:n merkitykseen tässä yhteydessä.

Lannoitteiden annostus vastasi 100 kg N/ha. Bakteerien kohdalla tulosten luotettavuus koeteltiin Poissonin jakautuman ja Fisherin dispersioindeksin avulla. Kontrollin ja lannoitettujen kohteiden erojen vertailu suoritettiin *t*-testillä. Homesienien kohdalla voitiin suorittaa vertailu vain rinnakkaismaljojen pesäkkeiden keskiarvojen perusteella.

Lannoituksen jälkeen muuttui mikrobitiheys kaikissa kohteissa (kuvat 1 ja 2). Kalkkiammonsalpietari ja kalkkisalpietari kasvattivat tasaisesti bakteerien ja homesienien määrää. Urealannoituksen jälkeen bakteerit lisääntyivät välittömästi, mutta homesienet sen sijaan vähenivät. Ammoniumsulfaattilannoitus vaikutti täysin päinvastoin, homesienet lisääntyivät ja bakteerit vähenivät.

Ensisijaisina tekijöinä lannoitteiden aiheuttamiin bakteerien ja homesienien tiheyden muutoksiin oli ko. aineista johtunut pH:n muuttuminen ja ilmeisesti myös niiden typen laatu.

Urea nosti välittömästi pH:a. Kalkkiammonsalpietari ja kalkkisalpietari pysyttivät sen koko kesän suunnilleen kontrollin tasolla, mutta syksyllä tapahtui siinä huomattavaa nousua. Ammoniumsulfaatti edellisistä poiketen aiheutti pH:ssa merkittävää laskua, joka lisäksi osoittautui melko pysyväksi.

Tasapaino bakteerien ja homesienien välisissä tiheyssuhteissa säilyi melko hyvin pH 4.3:n kohdalla; tämän rajan yläpuolella tendenssi oli ensiksi mainittujen kohdalla ylenevä ja jälkimmäisten kohdalla aleneva ja sen alapuolella päinvastoin.

Urean vaikuttavana aineosana oli ilmeisesti sen orgaaninen typpi joko sellaisenaan tai helppoliukoisena ammoniumtyppenä, jotka yhdessä suhteellisen lievän happamuuden kanssa loivat humuskerrokseen olosuhteet, joissa bakteerien mahdollisuudet typen assimilaatioon olivat niille varsin edulliset ja niiden määrä näistä syistä kasvoi.

Ammoniumsulfaatin aiheuttama happamuuden lisäys johtaa yleensä typen sitoutumiseen kompleksiyhdisteiksi humuskollodeihin. Näissä olosuhteissa tapahtunut homesienien huomattava tiheytyminen liittyy niiden ominaisuuteen bakteereja paremmin sopeutua alhaiseen reaktioon sekä niiden kykyyn käyttää useampaa erilaatuista typpeä ja sen yhdisteitä.

Kalkkiammonsalpietarin ja kalkkisalpietarin vaikuttavana tekijänä on pääasiassa pidettävä niiden vähitellen liukenevaa nitrattityppeä, jonka käyttökelpoisuus lisääntyy pH-tason suksessiiviseen nousuun liittyen. Näistä syistä lisääntynyt biologinen aktiviteetti ilmeni lähes samansuuruisena sekä bakteerien että homesienien kohdalla.