A microbial index for esimation of the peaty forest soil fertility

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A simple method has been developed for qualitative and quantitative evaluation of the microflora of peaty soils as a fertility index. We have found that changes in the cellulose-decomposing microflora in Norway spruce stands are correlated to plant growth (height increment), when it is impossible to explain the difference in plant growth rate by chemical composition of the soil. The present method is useful not only for routine evaluation of soil microflora on sites to be reforested but also for assessment of the effect of different management practices on soil microflora.

Fertilizers containing K, when applied to soil, were found to reduce the number of fungal colonies and favour bacterial colonies. The strongest effect, as compared with the control, was seen from fertilizers containing P+K. This was true for a young stand of Norway spruce established on a clearcut, and a mature forest stand. N+P fertilizer, in its turn, reduced the number of fungal colonies without affecting the bacterial ones. The effect of fertilization could be observed only in the season following that of application. Soil treatment favoured the growth of bacterial colonies.

Thus, when evaluating the suitability of peaty soil for planting spruce, the numerical ratio of cellulose-decomposing fungi to bacteria may serve as an indicator. The value of this index has been related to the annual growth of Norway spruce plants.

Key words: forest productivity, soil fertility, cellulose-decomposing microflora, peaty soil, Norway spruce, microflora

Introduction

Nutrient availability dependent on organic matter decomposition is the principal factor determining forest productivity. Before planting a given site, the fertility of the soil may be evaluated using traditional indices, i.e. the total amount of humus, total concentration of N, P and K, as well as the availability of other nutrients. For peaty soils, these indices include the degree of peat decomposition, their botanical composition and the ash content.

The decomposition processes are of special importance in organic and peaty soils known to be rich in nutrients stored in the form of humus/peat. By management practices it is possible to control the decomposition of humus/peat and enhance the nutrient turnover. Drainage resulting in aerobic soil environment is one way of increasing the decomposition rate of peaty organic matter. Still, there are cases when this has no effect. Thus, in Latvia, ca 20,000 ha of drained, peaty forest land, predominantly former grass fens and flood-plain meadows, have been characterized as sites of low fertility, and the afforestation of such land has often failed (Zālītis, 1991).

So far, there are no reliable criteria for evaluating the suitability of drained peaty soils for cultivating forest. The traditional criteria guiding a forester in such a situation are insufficient for explaining poor growth of the planted seedlings. However, an important indicator of soil fertility is the decomposition of soil organic material, a process that is proportional to nutrient release (Stevenson, 1987; Staaf & Berg, 1982). In general, the factors limiting soil biological activity limit the mineralization rate and the release of nutrients (Vompersky, 1968). One of the main organic soils is cellulose and this compound could thus be expected to serve as a suitable energy source in tests of soil microbial activity.

The principal objective of the present study was to establish a relationship between the organic matter decomposition in forest soils and forest productivity. For this purpose we have for several years conducted research on the dynamics of cellulose decomposition in forest soils. The research was conducted on diverse

forest types with differing hydrothermal regimes and a number of meterological and ecological factors were evaluated (Gaitnieks, 1991).

Our idea was to apply this relationship to evaluate the effect of fertilization on the soil microflora and on stand productivity in mature and young forest and investigate its interaction further. We assumed that soil fertility should be related to the number of microorganisms active in decomposing soil organic matter and that the number of cellulose-decomposing microorganisms would serve here as a useful index. Moreover, such a method would allow an easy comparison of numerical data. To evaluate the composition of the soil microflora, a method was developed to obtain data on the composition of the cellulose-decomposing microflora in diverse forest site types.

Site description and experimental design

Plot for the first experiment

The experiments were made at the Forest Research Station Kalsnava, located in central Latvia at 56°44'N; 25°54'E and at an altitude of 100-150 m a.s.l. The forest, a Norway spruce (*Picea abies* Karsten) plantation, was situated on a drained peat bog. The experiment was carried out on 2 trial plots of different fertility and representing rich and poor growing conditions. A plot was characterized as poor (poor growth conditions) where the Norway spruce plants, 1-2m in height, had the annual increment less than 20 cm yr^{-1}, while an area with growth exceeding 50 cm yr^{-1} was judged to represent good growth conditions. In each of these plots, 6 subplots, each about 2,000 m^2 in size, were used.

The good growth site had a Norway spruce stand (forest type *Oxalidosa* turf.med.) aged 40 years, with a height of 17.0 m and a basal area of 26.1 m^2ha^{-1}. The dominant grass cover was *Urtica dioica* L., *Stellaria nemorum* L., *Pyrola rotundifolia* L., *Ramischia secunda* (L.) Opiz., *Mycelis muralis* (L.) Dum. Moss such as *Brachytecium curtum* (Lindb.) J. Lange et C. Jens, and *Pleurozium schreberi* (Brid.) Mitt, were found on the ground. The botanical composition of the peat at 5-10 cm – depth was: 45% wood , 40% *Sphagnum* moss and 15% *Carex* grass, and at 10-20 cm – depth: 15% wood and 90% *Carex* grass. The ash content was 9.8%, the degree of decomposition 26% and pH 3.8 (Table 2).

The poor site was a well-ditched low mire. The 70-year-old Norway spruce forest had an average height of 8.1 m with admixture of birches 13.0 m in height. The total basal area was 13.2 m^2 ha^{-1}. The dominant grass cover was *Calamagrostis canescens* (Web.) Roth, and *Festuca rubra* L. Moss such as *Brachythecium curtum* (Lindb.), J.Lange et C.Jens, and *Climacium dendroides* (Hedw.); Web et Mohr were found on the ground. The botanical composition of the peat was at 5-10 cm – depth: 75% wood and 15% *Carex*; at 10-20 cm – depth: 45% wood and 55% *Carex*. The ash content was 9.6%, degree of decomposition 46% and pH 3.8.

Plot for the fertilization experiment - a mature stand and a clearcut area

The experimental area was a former grass fen having intensively drained peaty soils. The soil may be characterized as potentially fertile: well-decomposed sedge (*Carex* - woody peat, the ash content 10%). Stand composition was the following: 50% spruce, 30% pine, and 20% birch with a total stock volume of 140m^3 ha^{-1}. Stand productivity, according to the forest typology corresponds to class IV.

On the plot evaluated as poor, according to the height increment (above), another experiment was carried out on a clearcut area in 1986, which was replanted with Norway spruce in the same year. Fertilizer was applied to the spots of 0.8 m^2 around planted Norway spruce seedlings. In the mature forest, the experimental design was identical. A total of 20 sample spots (24x17 m) were used, with 10 on each of the two of strips 70 m wide, one in the clearcut and one in the mature forest. In each such spot 96-98 plants were planted. The clearcut area was planted with the three-year-old bareroot Spruce plants one year prior to the start of the experiment. In both the clearcut and the forest, the fertilizers were applied in the following combinations: N+P, P+K, N+P+K. and K in the amounts of P (superphosphate) 171 gm^{-2}, K (as K_2SO_4) 25 gm^{-2}, and N (as NH_4NO_3) 44 gm^{-2}.

Materials and methods

Sampling for the microbial activity

The first experiment - comparison of plots with poor and rich conditions. In each of the 12 subplots (6 rich and 6 poor) peat was sampled from 3 spots in each subplot, taken from the depth of 10-12 cm and mixed together, resulting in 6 mixed samples from the rich and 6 samples from the poor plots, respectively. Samples were collected from all 12 plots, *viz.* 6 from each type of plot in the spring and autumn of 1987 and 1988.

Fertilization experiment. To determine the changes in the cellulose-decomposing microflora, soil samples were

collected from 4 control plots and from 16 plots of each type of fertilization (cf. experimental design) in the spring of 1987, prior to fertilization. After fertilization, sampling was made in the autumn of 1987, as well as in the spring and autumn of 1988.

Determination of cellulose-decomposing organisms

The essence of the method lies in estimating on a Petri dish the number of colonies with microscopic fungi and bacteria growing on cellulose agar on 25 small blobs of soil. To obtain the data, the method of Zakharov (1978) was used. As implied by this method, we attempted to evaluate the bacterial and fungal colonies, by using two different agar media (Kadota, 1956; Chastukhin, Nikolajevskaya, 1953). However, these media appeared to be less suitable for peat soils and were, therefore, combined into one, common for both bacteria and fungi with the following composition: to 1,000 ml water were added 1g NH_4SO_4, 1g K_2HPO_4, 1g NaCl, 1g $MgSO_4$ $7H_2O$, and 20 g agar. The pH was adjusted to 6.8-7.1.

The peat samples were wetted with sterile water to form a paste. Sterilized filter paper as a cellulose source was applied to the sterile agar plates. By using a glass tube, we transferred 25 small wetted blobs of soil to each Petri dish. From each mixed sample, 4 Petri dishes were inoculated. Incubation was done at 26-28°and the number of colonies was estimated after 6-7 days.

Soil analyses

Design for the first experiment. On each of the 12 plots, 2 soil samples were taken at depths of 10 and 30 cm, respectively, using a 100 cm^3 soil corer. The replicate samples were then mixed into one.

Common analytical methods. The total concentration of N was determined after Kjeldahl, and total Ca and Mg were analysed by titration with Trilon B (Anonymous, 1991). Ammonium and nitrate were extracted by shaking each sample in a 0.1 M Na Cl solution for 1h and analysed by the method of Nesler on a spectrophotometer. Soluble P was extracted by shaking each sample in a 0.2 M HCl solution for 1h and analysed by the method of Kirssanov on a colorimeter. Potassium was extracted by shaking each sample in a 1 M solution of ammonium acetate for 1 h and analysed on a flame photometer (Burril-Marti & Ramirez-Munos, 1957). The ash content was determined after heating to 400°C for fixed weight. The pH was measured in 1 M KCl solution.

Botanical composition of the peat was determined microscopically and the degree of decomposition was determined by using a centrifuge. Volume weight was also determined.

Results and discussion

Soil chemical analyses and botanical composition

Only the concentration of exchangeable K at the depth of 30 cm showed a significant difference between the plots with good and with poor growth, with 90.3 mg l^{-1} and 27.5 mg l^{-1} for the good-growth and poor plot, respectively (Table 1). The total concentrations of such nutrients as N, P_2O_5, Ca or Mg were not significantly different between the two types of plots nor were the concentrations of the extractable part of NH_4^+, NO_3^-, PO_4^{3-}, Ca^{2+} and Mg^{2+}. These findings corresponded with those of P.Zālītis (1990; 1991). He found that the above nutrients were not reliable indices of forest soil fertility for the growth conditions in Latvia. In our case, these nutrients thus could not be used to explain the differences in growth.

The botanical analysis did not exibit any difference between the plots. Thus, the degree of decomposition was similar (Table 2) at both depths, wood peat was found to be 55 and 50% for the good-growth and poor-growth plots, respectively, at the depth of 10 cm and 30 cm, and the values for wood peat, 38 and 21%, were not significantly different. *Carex* peat was found to be 27.5% for the good-growth and 44% for the poor-growth plot at 10 cm, and 59 and 74 at 30 cm – depth, respectively.

Investigation of cellulose-decomposing organisms on sites of good and poor growth

We compared the number of bacterial and fungal colonies growing on cellulose-agar plates and found that samples from the poor plots showed a significantly higher number of fungal colonies, whereas the number of bacterial colonies was high on the plates inoculated with samples from the rich plots. In fact, the number of bacterial colonies on plates representing plots with better growth conditions were 4 times higher than those of plates representing poor plots ($p<0.05$) Table 3). Further, the number of colonies of microscopic fungi on agar plates with inoculate from the poor plots was 5 times higher than those for the plates inoculated from the plots with better growth conditions ($p<0.05$).

The results were constant and each single pair of Petri dishes showed a significant difference (Table 3). When comparing the numbers of fungal and bacterial colonies for the plots with good growth conditions, we can see that the number of bacterial colonies was signi-

Table 1. Some chemical data on the experimental plots in a Norway spruce forest at the Kalsnava Research Station

Parameter	Depth (cm)	Fertility	Mean value	Standard deviation
pH_{KCl}	10	good	4.4	0.29
		poor	4.1	0.36
	30	good	4.9	0.44
		poor	4.6	0.26
Ash (%)	10	good	15.54	4.89
		poor	14.38	4.44
	30	good	14.25	2.41
		poor	23.06	12.98
N conc (%)·(total)	10	good	1.2	0.12
		poor	1.2	0.05
	30	good	1.2	0.07
		poor	1.2	0.12
NH_4^+ (mg l^{-1})	10	good	11.5	5.39
		poor	8.0	2.87
	30	good	16.0	5.26
		poor	16.0	7.86
NO_3^- (mg l^{-1})	10	good	14.0	8.02
		poor	19.0	11.32
	30	good	12.0	7.41
		poor	20.5	18.89
P_2O_5 (mg l^{-1})	10	good	10.0	3.88
		poor	17.0	9.82
	30	good	17.0	11.29
		poor	21.0	11.96
K (mg l^{-1})	10	good	36.5	19.31
		poor	27.5	11.79
	30	good	90.3*	19.97
		poor	27.5*	21.28
Ca (mg l^{-1})	10	good	1163	461.69
		poor	1026	501.21
	30	good	1446	731.4
		poor	1562	298.97
Mg (mg l^{-1}) (total)	10	good	138	72.13
		poor	76	67.3
	30	good	145	78.57
		poor	132	52.89

Significantly different values are indicated by an asterisk.

Table 2. Some botanical data on the experimental plots in a Norway spruce forest at the Kalsnava Research Station

Parameter	Depth (cm)	Fertility	Mean value	Standard deviation
Degree of decomposition	10	good	41.0	6.94
		poor	51.0	10.59
	30	good	34.0	10.38
		poor	42.0	16.31
Wood peat (%)	10	good	55.0	32.86
		poor	50.0	22.13
	30	good	38.0	33.11
		poor	21.0	13.34
Carex peat (%)	10	good	27.5	18.09
		poor	44.0	29.22
	30	good	59.0	32.77
		poor	74.0	18.28

Plot No.	No. of colonies									
	Fungi				Mean value	Bacteria				Mean value
Rich plot										
1	6	4	7	3	5.0	9	11	8	7	8.8
2	4	6	8	9	6.7	18	12	14	6	12.5
3	2	1	0	2	4.2	4	5	4	7	5.0
4	8	6	10	6	7.5	10	6	5	11	8.0
5	1	3	2	2	2.0	10	11	11	14	11.6
6	1	3	2	1	1.7	4	5	4	9	6.8
					4.4 (2.33)					8.78 (2.60)
Poor plot										
1	25	25	25	22	24.2	1	2	2	1	1.5
2	16	14	17	21	17.0	4	2	5	3	3.5
3	25	25	26	27	25.8	0	1	2	2	1.3
4	25	25	23	24	24.2	1	2	3	0	1.5
5	24	23	22	22	22.7	0	2	2	2	1.5
6	21	24	17	23	21.2	1	6	4	2	3.0
					22.5 (2.84)					2.05 (0.86)

Table 3. The number of colonies of fungi and bacteria growing on cellulose agar. Data from the count made in spring 1987. Standard deviation in parentheses.

ficantly higher ($p<0.05$) than that of fungal colonies and for the plot with poor growth conditions the number of fungal colonies was clearly higher ($p<0.05$). Further, on each of the three occasions (spring) the same tendency was visible, proving the stability of the test method.

We investigated the dynamics of the composition of bacterial and fungal colonies and found that over the year the relation between colonies of bacteria and fungi was constant for each type of plot. This was true for both types of plots. In plots with good growth conditions bacterial colonies dominated, and in poor plots with less optimal growth conditions fungal colonies prevailed. Thus, in the spring of 1987 the average value for bacteria in the rich plot was 8 with standard deviation (SD) of 2.60. The number of fungal colonies was 4.4 with standard deviation value of 2.33. In the poor plot, the corresponding values were 2.05 (SD 0.86) and 22.5 (SD 2.84), respectively. In the spring of 1987 and the autumn of 1988, the values were similar, showing relatively constant ratios.

As it follows from the experimental data, this numerical ratio of the numbers of colonies of microscopic fungi to bacteria can index the fertility of peaty soils under eutrophic conditions (Gaitnieks, 1988).

Fertilization experiment

Fertilization appears to be one of the principal ways of changing the soil microflora (Lettl&Langkramer, 1983; Lang&Beese, 1985; Mai&Fiedler, 1986). As we observed that the soil showing poor tree growth had a low concentration of K, we decided to investigate the bacteria-to-fungi ratio also in the soils with different fertilizers added. In this experiment, we used paired plots in a clearcut area and in a mature stand.

After planting, but prior to fertilization, we investigated the number of bacterial and fungal colonies in soils of both the forest and clearcut and found that they differed considerably. The number of bacterial colonies was significantly larger in the clearcut plot, where the soil had been disturbed by harvesting and site preparation.

After fertilization with N, P and K as described, the number of microscopic fungi decreased 11 times in the forested plot: the average value was 1.5 colonies per agar plate in the autumn of 1988, as compared with 16.5 colonies per agar plate for the control at the same time (Table 4). The number of bacteria had, in this case, increased to an average value of 13.3 colonies as compared with 1.0 for the control (autumn 1988). The controls showed constant values. The lowest effect shown by any fertilizer was that of N+P, both in the forest and clearcut area. In the latter case, the number of colonies with microscopic fungi decreased over the experimental period from 9.0 to 2.0 colonies after applying N+P+K fertilizer. The same tendency prevailed also for K alone and for P+K mix (11 to 3.3 and 11.8 to 3.5, respectively).

For bacteria, a significant and strong effect was achieved using the PK fertilizer, which resulted in a 14.6-fold increase in the number of bacteria: 1.3 for the control as compared with 19.0. The PK fertilizer showed the most pronounced effect on the number of bacteria over time (Table 4) with an increase from 1.3 in the spring of 1987 to 3.8 in the autumn, 7.5 in the spring of 1988 and 19 in the autumn. Also for N+P+K and K alone, increases were visible even if they were less pronounced.

The annual increments for the trees were compared (Table 5) and we may conclude that, fertilization with N and P exibited no significant difference in this experiment, while P+K, N+P+K and K alone created considerably higher growth as compared with the control.

We may, thus, form a simple ratio from the number of bacterial and fungal colonies and compare it with the

Table 4. Variation in the number of cellulose-decomposing microorganisms in the soil of mature forest and a clear-cut after fertilization (number of colonies/Petri dish) (average values from 4 counts)

Kind of fertilization	Forest			Planted clearcut			
	Spring 1987 (before fertiliz.)	Autumn 1987	Autumn 1988	Spring 1987 (before fertiliz.)	Autumn 1987	Spring 1988	Autumn 1988
Number of microfungi							
Control	16.0	16.8	16.5	12.0	14.8	20.3	20.0
NP	14.5	21.3	4.5	11.3	9.5	12.8	8.0
PK	16.5	24.3	3.0	11.8	5.3	6.0	3.5
NPK	12.8	20.8	1.5	9.0	9.3	7.3	2.0
K	17.3	21.8	3.8	11.0	8.3	3.8	3.3
Number of bacteria							
Control	1.0	1.3	1.0	2.3	2.5	1.8	1.3
NP	0.5	1.8	4.5	3.0	2.8	3.8	1.3
PK	1.0	3.3	7.5	1.3	3.8	7.5	19.0
NPK	1.5	5.5	13.3	2.3	3.3	5.0	6.0
K	1.8	9.5	8.3	1.0	6.0	12.0	9.5

Table 5. Growth of Norway spruce plants on the clear-cut one year after application of fertilizer. (Significant difference in the growth on the control plot is given. In all cases n=30)

Fertilizer	Height (cm)			Annual increment, (cm)		
	Mean value	Standard deviation	sign diff	Mean value	Standard deviation	sign diff
Control	76.5	16.3		21.13	7.05	
NP	78.0	17.25	n.s.	24.53	7.52	n.s.
PK	88.0	25.70	$p<0.05$	31.87	12.34	$p<0.05$
NPK	87.0	17.59	$p<0.05$	29.80	10.32	$p<0.05$
K	85.0	18.49	$p<0.05$	31.87	9.56	$p<0.05$

increment, whereby we can see a very clear relationship between this ratio and shoot length of the spruce plants. Thus, K-containing fertilizers have produced, in terms of the overall and annual increment, the most significant effect on the young spruce.

Concluding remarks

1. When evaluating peaty soils for planting spruce, the numerical ratio of the number of microscopic fungi to bacteria growing on cellulose may serve as an indicator of soil suitability for it.

2. We may conclude that fertilization affects the cellulose-decomposing microflora in forest soils. In this case, K fertilizers may be regarded to act as a catalyst rather than an additional nutrient.

3. Fertilization with K may reduce the number of microscopic fungi, but at the same time the number of bacteria increases.

4. Although this observation was made for peaty soils, it may be developed as a tool for other soil types as well. Thus, purposeful control of the described kind of index may be a useful tool in evaluating soil suitability for one or another type of forest crops and, consequently, in improving forest productivity.

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ПОЧВЕННАЯ МИКРОФЛОРА КАК ПОКАЗАТЕЛЬ ПЛОДОРОДИЯ ТОРФЯНЫХ ЛЕСНЫХ ПОЧВ

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Резюме

В работе отмечено преимущество микробиологических показателей при определении фактического плодородия торфяных почв. Установлено, что объективным показателем плодородия субстрата являются количественные соотношения целлюлозоразрушающей микрофлоры. Улучшение условий произрастания ели на осушенных торфяных кисличниках связано с уменьшением микроскопических грибов и сувеличением количества бактерий.

Разработан метод, с помощью которого можно оперативно получить информацию о структуре микрофлоры в предусмотренных для облесения площадях. Предложенный метод проверен на практике лесного хозяйства в Латвии, оценивая влияние минерального удобрения на микрофлору. Установлено, что на осушенных низинных болотах на втором году после удобрения с K, PK и NPK уиеличивается количество бактерий и уменьшается количество микроскопических грибов. Следовательно, механизм положительного влияния минеральных удобрений связан с изменением количественных соотношений целлюлозоразрушающей микрофлоры в почве.

Ключевые слова: продуктивность леса, плодородие почвы, целлюлозоразрушающая микрофлора, целлюлоза, торфяные почвы, уль, микрофлора.