ARTICLES

The influence of nutritional conditions on forest-soil microflora

HEINO SEEMEN
Faculty of Forestry Estonian Agricultural University
Kreutzwaldi 5, EE 2400 Tartu, Estonia

HELGI LAITAMM
Estonian Institute of Agriculture
Teaduse 4, EE 3400 Saku, Estonia

JAAK PIKK
Faculty of Forestry Estonian Agricultural University
Kreutzwaldi 5, EE 2400 Tartu, Estonia

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Since 1970 in Estonian Forest Research Institute studies have been carried out to find opportunities to increase forest soil fertility and stand productivity by fertilization. Research work has shown after positive influence of the primary fertilization in some cases there will take place depression period of stands growth. Approximately half of pine stands on experimental areas of mineral soils are of such kind, where the stand fertilized 10 or more years ago grows worse than the unfertilized one. In order to find out the reasons for the depression of stand increment the volume increment and its soil microflora on four experimental areas were under research in *Rhodococcum* and *Myrtillus* site types. One area was with depression features from the both types and the other area without depression. The results do not confirm the opinion in certain conditions the influence of fertilization would be restraining on forest soil microflora. Microflora in fertilized forest soils is mostly numerous and for improving soil fertility more favourable than that in unfertilized forests.

Key words: site type, pine stand, volume increment, fertilization, depression of stand growth, soil microflora.

Introduction

Since 1970 in Estonian Forest Institute (closed in 1996) research has been carried out to find new ways for increasing forest soil fertility, stand productivity and its value. For that purpose forest fertilization experimental areas have been formed. As seen from the collected data, fertilization does help in improving productivity and value of forests, but not necessarily always. There is no use to fertilize if the soil is too humid or very fertile. After drainage potentially fertile peatsoils do not need any fertilization. Some good results can be seen if middle-aged and older pine stands (II and III quality class) of *Rhodococcum* and *Myrtillus* site types are repeatedly fertilized with nitrogen. The research work has shown that the primary fertilization has a good effect on stand growth and it will usually last for 5 to 8 years. After that period stand increment on fertilized and unfertilized soil is more or less equal or on fertilized soil it will be 10-20 % less. Thus the initial profit due to fertilization in stand increment becomes gradually less and in a long period may disappear at all. In Estonia on experimental areas with mineral soil approximately half of pine stands are of such kind, where the stand fertilized 10 or more years before grow worse than the unfertilized one. If a stand is fertilized again with nitrogen after 7 to 8 years the increment will become much more. Most probably the second fertilization with nitrogen will be positive over 5 to 6 years after it, but afterwards a depression period may start. Why a depression period, after nitrogen-fertilizer effect is over, does come up, it needs some explanation. It can be supposed in some conditions nitrogen fertilizer may brake the microflora of the soil. As a result, in fertilized soil microbiological activity lessens and, therefore, after fertilizer exhausts nutritional conditions of trees and the increment of a stand becomes poorer than that in unfertilized soil.

Materials and methods

In order to find out the reasons for the depression of stand increment the volume increment of stands and its soil microflora on four permanent experimental areas were under research. Two of the areas were situated in pine stands of *Rhodococcum*, the other two of *Myrtillus* site types. The objective of the research was one area with depression features from the both types (A and C) and the other area without depression (B and D).

Experimental areas in pine stands of Rhodococcum site type

Experimental area A (Kuuste II) was formed in medium podzol soil in a 46-year-old pine stand (II quality class) of *Rhodococcum* site type. The main fertilization was done by the norm $N_{100}P_{100}K_{100}$. In the fourth year after the main fertilization it was repeated with nitrogen (N_{100}). Experimental area B (Saare I) was formed in medium podzol soil in an 80-year-old pine stand (III quality class) of *Rhodococcum* site type. $N_{100}P_{100}K_{100}$ was given as the main fertilization, the repeated fertilization was done on the eighth (N_{100}) and the eighteenth (N_{150}) year after the main one.

Experimental areas in pine stands of Myrtillus site types

On experimental area C (Järvselja I) a 50-year-old pine stand (II quality class) of *Myrtillus* site type was growing on strongly podzol. Full fertilizer with the norm $N_{100}P_{100}K_{100}$ was used as the main fertilization. In the second (N_{100}), third (N_{100}) and sixteenth (N_{150}) year after the main fertilization, nitrogen was applied repeatedly

Experimental area D (Saare II) is situated on the lightly too-humid strong podzol with a thick humus layer in an 80-year-old pine stand (III quality class) of *Myrtillus* site type. The main fertilization was full fertilizer with the norm $N_{100}P_{100}K_{100}$, the repeated fertilization was done with full fertilizer ($N_{100}P_{100}K_{100}$) on the eighteenth year after the main one. The additional increment of stand volume was analysed by the data obtained in the 16th year after fertilization. Soil microflora research was conducted on the 19th and 20th years after the main fertilization. The data characterizing soil acidity and nutrient content are represented in Table 1. All the areas were on strongly acidic poor podzol.

Table 1. Agrochemical indices of the soil

Soil level	Thickness of soil level, cm	pH_{KCl}	General nitrogen content, %	Laktate soluble P_2O_5	Laktate soluble K_2O
		Experimental area A			
A_0	0- 7	2.3	1.03	18.6	57.2
$A_2(A_1)$	7-15	3.0	0.04	1.6	0.8
		Experimental area B			
A'_0	0- 3	3.1	1.31	27.0	64.8
A''_0	3- 6	2.6	1.02	1.8	10.4
$A_2(A_1)$	6-10	3.2	0.08	1.8	13.0
		Experimental area C			
A_0	0-10	2.55	0.76	22.6	67.5
A_2	10-27	3.20	0.04	0.50	1.3
		Experimental area D			
A'_0	0- 8	2.8	1.14	28.5	47.3
A''_0	8-20	2.7	1.09	12.5	40.8
A_2	20-39	3.4	0.04	0.0	1.3

Samples for soil microflora analysis were taken from 15-cm soil layer, including forest decay and podzol level. All the experimental treatments with fertilized and unfertilized soil were on three plots of 0.1 to 0.2 hectares. The mean soil sample, i.e. a mixed soil from three to five spots was taken from each plot. 6 samples – 3 from fertilized, 3 from unfertilized experimental treatments – were there from each area. Microbiological analysis of the soil sample was conducted at the Estonian Institute of Agriculture. By the dilution method the number of the main 12 microgroups were determined. It will be enable us to estimate microbiological activity and thus the soil fertility.

Results

The increment of pine stands of Rhodococcum site type

The results of the research on plot A (Fig.1.,Table 2) have shown the positive influence of fertilization on tree growth continues from the second to the ninth year after fertilization. The additional volume increment of stand was 24.3 m^3/ha (1.1 to 4.5 m^3/ha per year). From the twelfth year after fertilization the annual volume increment of stand on a fertilized area has been by far less than that on an unfertilized area. In the 10th up to the sixteenth year after fertilization in a fertilized stand volume supply per hectare in total was 10.1 m^3 less than the corresponding index in an unfertilized stand. By the end of the 16th year after fertilization the stand volume increment on fertilized soil per hectare was only 14.2 m^3 more than that on unfertilized one.

Table 2. Additional stand volume increment (m^3/ha) on fertilized soil in pine stands of *Rhodococcum* site type

Years after fertili-zation	Experimental area A			Experimental area B		
	ZT(M)	W(ZM)	ZT(M)*	ZT(M)	W(ZM)	ZT(M)*
1	-0.01	0.19	-0.01	0.31	0.11	0.31
2	2.93	0.23	2.92	1.58	0.15	1.89
3	3.23	0.26	6.15	2.14	0.15	4.03
4	4.53	0.33	10.68	1.95	0.16	5.98
5	4.10	0.36	14.79	1.83	0.20	7.81
6	3.07	0.35	17.85	1.22	0.17	9.03
7	2.46	0.35	20.31	0 78	0.16	9.81
8	2.95	0.41	23.26	0.44	0.19	10.25
9	1.06	0.63	24.32	1.95	0.26	12.20
10	-0.10	0.37	24.22	2.05	0.36	14.25
11	-0.21	0.41	24.01	1.58	0.33	15.83
12	-2.25	0.52	21.76	0.69	0.33	16.52
13	-2.21	0.58	19.55	0.44	0.43	16.96
14	-2.41	0.46	17.14	0.42	0.33	17.38
15	-1.35	0.43	15.79	0.47	0.29	17.85
16	-1.56	0.51	14.23	0.13	0.29	17.98

ZT(M) - additional stand volume increment per year in fertilized stand (m^3/ha)
W(ZM) - mistake of additional increment (± m^3/ha)
ZT(M)* - supplementary increment (m^3/ha)

The results on experimental area B concerning stand increment have shown the positive influence of fertilization on stand growth over 6 to 7 years, the second fertilization with nitrogen has shown a positive result over 4...5 years. Thus the positive result on tree growth has been continuous. The depression period. has been observed over the following 16 years after fertilization the stand volume has increased 18.0 m^3.

The increment of pine stands of Myrtillus site type

Positive influence of fertilization on stand increment on experimental area C has lasted from the second to the eighth year. In this period the additional volume increment was in total 15.0 m^3/ha (0.6...3.7 m^3/ha per year) (Fig. 1, Table 3). Since the ninth year after fertilization the annual increment of stand volume was 0.2...2.0 m^3/ha less than that on unfertilized soil. Thus, as a result of fertilization the achieved stand volume increment by the end of the experiment was 6.3 m^3/ha. The first favourable influence of fertilization on experimental area D lasted for 8 years. Over this period the volume increment in a fertilized stand was 15.7 m^3/ha more than that in an unfertilized stand. Since the ninth year after the fertilization

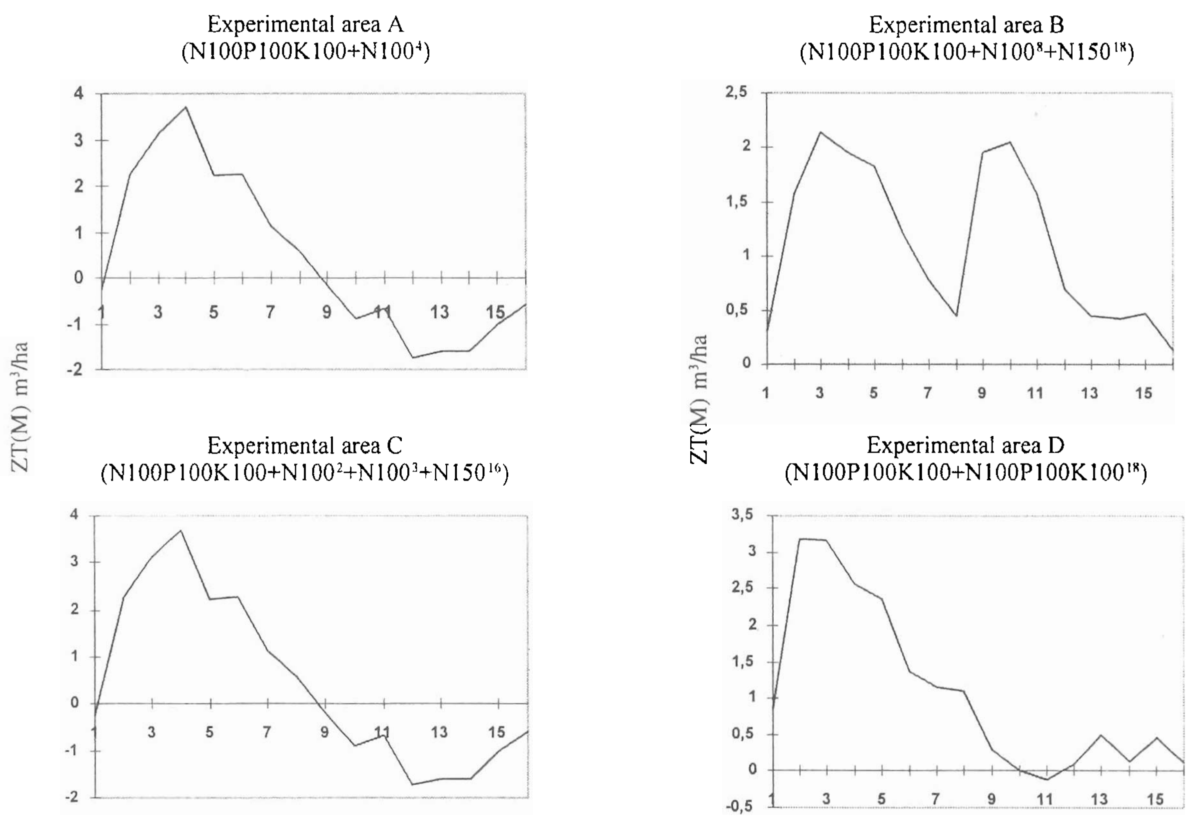

Fig. 1. The change of additional volume increment ZT(M) in stands fertilized 16 years ago. The increment of stand volume after the positive influence-period on experimental areas A and C is clearly smaller, but on experimental areas B and D more or less equal than on unfertilized soil

Table 3. Additional stand volume increment (m^3/ha) on fertilized soil in pine stands of *Myrtillus* site types

Years after fertilization	Experimental area A			Experimental area B		
	ZT(M)	W(ZM)	ZT(M)*	ZT(M)	W(ZM)	ZT(M)*
1	-0.25	0.13	-0.25	0.85	0.18	0.85
2	2.27	0.27	2.01	3.18	0.23	4.02
3	3.12	0.28	5.14	3.16	0.23	7.18
4	3.70	0.34	8.83	2.55	0.30	9.73
5	2.23	0.34	11.06	2.36	0.34	12.09
6	2.27	0.29	13.33	1.37	0.36	13.46
7	1.14	0.29	14.47	1.14	0.33	14.60
8	0.57	0.30	15.04	1.10	0.37	15.70
9	-0.18	0.27	14.86	0.29	0.37	15.99
10	-0.90	0.30	13.96	0.01	0.37	16.00
11	-0.68	0.29	13.28	-0.11	0.27	15.89
12	-1.73	0.37	11.55	0.10	0.37	15.99
13	-2.03	0.46	9.52	0.49	0.49	16.48
14	-1.60	0.35	7.92	0.13	0.44	16.61
15	-1.01	0.31	6.91	0.47	0.36	17.08
16	-0.58	0.45	6.34	0.12	0.41	17.20

ZT(M) - additional stand volume increment per year in fertilized stand (m^3/ha)
W(ZM) - mistake of additional increment (± m^3/ha)
ZT(M)* - supplementary increment (m^3/ha)

the stand volume increment in a fertilized and unfertilized stand continued to be more or less equal. There was no depression period.

Soil microflora

The data in Figure 2 and Table 4 imply that the fertilization has not decreased microbiological activity of the soil, just the opposite. The number of ammonification bacteria grown on meat-peptone agar is greater in fertilized soil: in pine stands of *Rhodococcum* 1.3...1.5 and in these of *Myrtillus* site types 1.8...2.3 times. Sporogenous bacteria in both site types in fertilized soil are 1.4 to 3.0 times more, but in spite of that their number remains small even in fertilized soil. More numerous sporogenous bacteria were *Bacillus cereus. Bac. mycoides* and *Bac. cyanogenes. Actinomycetes* have been 1.5 to 2.5 times more in fertilized pine stands of *Rhodococcum* and 2.1 to 4.6 times more of *Myrtillus* site type than that in unfertilized stands. Sporogenous bacteria as well as *Actinomycetes* take part in final mineralizing of organic matter and thus they improve soil quality. Microfungi characteristic of forest soil which are from the genus *Trichoderma, Mortierella, Penecillium, Acremo-*

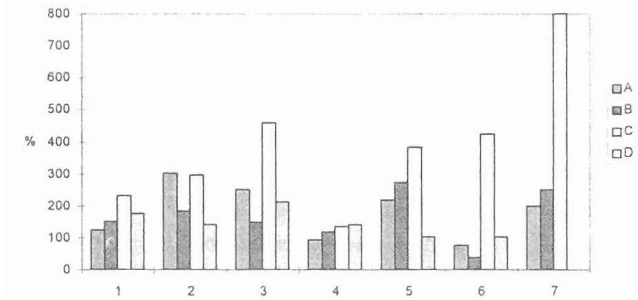

Fig. 2. The number of microorganisms in fertilized soil (percentage from the number of microorganisms of unfertilized soil) of *Rhodococcum* (A, B) and *Myrtillus* (C, D) pinewoods. 1- bacteria growing on meat-peptone agar, 2- sporogenous bacteria, 3- actinomycetes, 4- microfungi on wort agar, 5- clostridium pasteurianum, 6- butyric acid bacteria, 7- cellulose decomposing bacteria

Table 4. The number of microorganisms (thousands per 1 g of absolutely dry soil) in fertilized (F.) and unfertilized (Unf.) pine stands soils of *Rhodococcum* and *Myrtillus* site types

Group of microbes	*Rhodococcum* site type				*Myrtillus* site type			
	Experimental area							
	A		B		C		D	
	Unf.	F.	Unf.	F.	Unf.	F.	Unf.	F.
Bacteria growing on meat-peptone agar	450	567	521	779	400	920	630	1110
Sporogenous bacteria	1.7	5.1	3.2	5.9	0.5	1.6	1.2	1.7
Actinomycetes	4.2	10.4	3.8	5.7	3.5	16.3	3.9	8.2
Microfungi on wort agar	54.5	51.1	45.6	54.7	28.6	38.3	72.2	102
Fusarium on Nash and Snyder culture medium (CM)	0.06	0.16	0	0	0.04	0.41	0	0.08
Lipomyces on Ashby culture medium	0	0.05	0.02	0	0.06	0.17	0	0.01
Nitrification bacteria	0	0	0	0.01	0	0.02	0.06	1.86
Denitrification bacteria on Hiltay culture medium	0.01	0.03	0	0	+	0	0.03	0.1
Clostridium pasteurianum on Vinogradski culture medium	0.4	0.8	0.1	0.3	0.2	0.8	10.1	10.3
Butyric acid bacteria on potato culture medium	80.7	62.3	28.8	11.2	38.6	163.3	248	252
Desulfating bacreria on Starkey culture medium	0.10	0.09	0.03	0.03	0.05	0.07	0.06	0.06
Cellulose decomposing bacteria on Hutchinson culture medium	0.02	0.04	0.02	0.05	0.01	0.08	0	0.05
Bacteria growing on starch-ammonium agar	-	-	792	779	-	-	2140	1730

nium have been most numerous. The number of cellulose-decomposing microfungi and sulfate-decomposing desulfating bacteria has not considerably changed due to fertilization. The number of *Fusarium* has considerably increased on one experimental area (2.7 times) of *Rhodococcum* site type and in one of *Myrtillus* site type (10.3 times). In most cases the number of air-nitrogen connective *Clostridium* has increased, in fertilized *Rhodococcum* site type soil by 2.2 to 2.7 times and in *Myrtillus* site type soil (experimental area C) by 3.8 times. The number of cellulose-decomposing bacteria in fertilized soils *Rhodococcum* site type has grown 2...2.5 times and that of *Myrtillus* site type 8 times. Nitrification and denitrification bacteria could be found less, but in pine stands of *Myrtillus* type in fertilized soil they were by far more numerous than in unfertilized soil. In pine stands of *Rhodococcum* site type the above-mentioned bacteria were practically absent in fertilized as well as in unfertilized soils. Carbohydrates-decomposing unaerobic butyric acid bacteria were relatively rare in *Rhodoccum* site type soils, while after fertilizing the number of butyric acid bacteria in *Rhodococcum* site type pine stands decreased from 23 to 61 %. In fertilized soils of pine stand of *Myrtillus* type on one experimental area the number of bacteria remained the same, but on the other one became 4 times greater. The number of sulfate decomposing desulfating bacteria did not considerably change in fertilized pine stands of *Rhodococcum* site type, but in *Myrtillus* site type pine stands after fertilization the above index increased 1.4 times in one case, in another case it remained unchanged.

Discussion and conclusions

In interpreting the presented data one should bear in mind microbes activity will favour soil fertility. As a result of it, acids separate from organic matter, which, in their turn, react with other compounds forming fast water-soluble salts. Microbes secrete vitamins, growing substances and other organic compounds useful to forest. The more microorganisms in forest soil, the better conditions for tree growth. If after fertilization the number of microflora decreases, it will effect mineralization of organic matter and it will be unfavourable for tree growth which is noticeable after fertilizer stock has been exhausted. From the results we can conclude that on the fertilized experimental areas in pine stands of *Rhodococcum* and *Myrtillus* site type the soil is poor in microbes in fertilized and unfertilized stands. It seems the low forest soil microbiological activity is caused by high acidity of the soil. The soil of experimental areas has pH KCl 2.3...3.4. The depression period is apparently connected with nitrogén deficiency to trees. If nitrogen fertilizers have been applied in time, there will be no depression period and stand volume will increase as expected. In accordance with the research results more microorganisms are in the soil, if it has more organic matter. Thus the number of microbe-groups is bigger in pine stands of *Myrtillus* site type than that in *Rhodococcum* site type. Microflora in a fertilized forest is mostly numerous and for improving soil fertility more favourable than that in an unfertilized forest. The results do not confirm the opinion in certain conditions the influence of fertilization on soil microflora would be restraining. When the nitrogen supply by fertilization has been exhausted, the number of microorganisms may fall lower than that in unfertilized soil and thus a depression period in stand growth would take place. However, the stand increment may drop for some other circumstances, caused by the natural changes as well as by human activity. Stand fertilization does change a fixed balance of the forest ecosystem. Fertilization adds nutrients necessary to plants. As a result tree growth will improve, but at the same time fungipests, in their own turn, restrain stand increment.

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ВЛИЯНИЕ УСЛОВИЙ ПИТАНИЯ НА МИКРОФЛОРУ ЛЕСНОЙ ПОЧВЫ

Х. Сеемен, Х. Лайтамм, Я. Пикк

Резюме

Начиная с 1970 года в Эстонском институте леса (закрыт в 1996 г.) проводились исследования с целью установления новых научно обоснованных возможностей повышения плодородия лесных почв и производительности насаждений с помощью минеральных удобрений. Результаты исследовательской работы показывают, что удобрение может оказаться эффективным при повышении производительности и ценности лесов, однако не всегда.

Благоприятное влияние первичного удобрения на рост насаждений продолжается обычно 5-8 лет. После этого прирост насаждения на удобренной и неудобренной земле более или менее уравновешивается или остается в удобренном насаждении на 10-20% меньше, в результате чего полученная от удобрения первичная прибыль в виде дополнительного прироста начинает постепенно уменьшаться и в течение более длительного времени может исчезнуть. Приблизительно половина опытов, заложенных в Эстонии в сосняках на минеральных почвах, являются такими, где насаждение растет на почве, удобренной 10 и более лет назад, хуже, чем на неудобренной почве.

Для выяснения причин периодов депрессии в росте насаждений изучали прирост запаса насаждений и микрофлору почвы тех же насаждений на четырех постоянных опытных участках по лесоудобрению. Два опытных участка располагались в брусничниковом и два опытных участка в черничниковом типе местопроизрастания в 45-80-летних сосняках II и III бонитета. Сосняки-брусничники росли на типично-среднеподзолистой почве и сосняки-брусничники на типично-сильноподзолистой почве. В обоих типах местопроизрастания объектом исследования выбрали по одному опытному участку с признаками депрессии (опытные участки А и С) и по одному опытному участку без депрессии (опытные участки В и D).

Из-за периода депрессии сосняк-брусничник (опытный участок А) потерял к концу опытного периода 42% и сосняк-черничник 58% от дополнительного прироста запаса (который в сосняке-брусничнике составлял 24,3 $м^3$/га и в сосняке-черничнике 15,0 $м^3$/га), полученного в период положительного влияния удобрений

Приступая к изучению микрофлоры удобренных насаждений полагали, что азотное удобрение может при некоторых условиях повлиять на микрофлору почвы отрицательно, в результате чего микробиологическая активность в удобренной почве уменьшится, в результате чего после исчерпания удобрения также и условия питания деревьев и прирост насаждения станут хуже, чем в неудобренной почве. Однако исследовательская работа показала, что удобрение не уменьшило микробиологическую активность почвы, а скорее наоборот (рис. 2, табл. 4). Микрофлора почвы в удобренном лесу в большинстве случаев многочисленнее и благоприятнее для улучшения плодородия почвы, чем в неудобренном лесу.

Ключевые слова: тип местопроизрастания, сосновое насаждение, прирост запаса, удобрение, депрессия прироста насаждения, микрофлора почвы.