Agronomy Research **16**(1), 64–82, 2018
https://doi.org/10.15159/AR.18.020

Effects of fertilization on Picea abies stands situated on drained peat soils

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Abstract. Norway spruce used for afforestation of drained peat soils frequently has low productivity and decay in a long-term, which could be related to soil chemical composition and nutrient status. The research aim was to elucidate the effect of PSM on new Norway spruce plantings (1st experiment) and 20-year-old spruce stands (2nd experiment) on drained peat soils by evaluating: (1) nutrient accumulation in soil-plant system, (2) soil microbiological activity, (3) health status of spruce individuals, (4) growth intensity and productivity of spruce, (5) changes in composition of vascular plant and moss species. The study was conducted at two forest (*Myrtillosa turf.mel.*) sites in Latvia, each consisting of two plots: control and treated with PMS (100 g m^{-2} in September 2007, 50 g m^{-2} in April 2008). During 2008–2016, regular analysis of soil, spruce needles, soil microbiology, assessment of tree crown vitality, stand productivity, inventory of vascular plant and moss species were done. The results showed that the fertilization with PMS resulted in a significantly improved K, Ca, Zn, and N status of trees, crown vitality and up to three times increased tree growth parameters at both experiments during the study period. The average count of bacteria and fungi in soil of fertilized plots, accompanied by a remarkable variability in the study years, was significantly higher only for the 1st experiment. Fungi: bacteria ratio for the fertilized and control plots differed significantly only for the 1st experiment. Significant increase of cover with nitrophilic plant (*Urtica dioica, Antriscus sylvestris, Rubus idaeus*) and moss (*Plagiomnium cuspidatum*, *P. ellipticum*) species at both fertilized sites were stated.

Key words: Norway spruce, productivity, mineral nutrition, vegetation, microbiology.

INTRODUCTION

A common practice worldwide is to do afforestation in the soils unlikely suitable for agriculture like peatlands. In many northern countries, peatlands have been extensively drained for forestry (He et al., 2016), e.g., about 4.6 million hectares or 52% of peatlands and wet mineral soil sites have been drained for forestry in Finland (Finnish Statistical Year Book of Forestry, 2014). One of the most suitable commercial forest species used for afforestation of peatlands is Norway spruce (*Picea abies* (L.) Karst.). Norway spruce is also one of the dominant tree species in forests at the boreo-nemorale climate zone. Unfortunately, the afforestation is not always successful and sustainable. In Latvia, Norway spruce forests cover 18.2% of the total forest area, around 10% of them occurs on drained peat soils (VMD, 2016). Decay and low vitality have been observed mainly for 30–40-year-old pure spruce stands (Lībiete & Zālītis, 2007). During the last decades, spruce dieback has been observed also in various sites in Europe (Malek et al., 2012; Błońska et al., 2015). Low productivity and decay of spruce stands in a long-term frequently could be related to soil chemical composition (Zālītis, 1991; Berger et al., 2009; Moilanen et al., 2010; Błońska et al., 2015).

Important issue for forestry in boreal peatlands is sufficiency or deficiency of K (Finer, 1989; Tripler et al., 2006; Caisse et al., 2008; Moilanen et al., 2010; Sarkkola et al., 2016), B (Möttönen et al., 2005), and other nutrients. Sufficient supply with nutrients promotes not only intensity of tree growth, but also tolerance to diseases and stress conditions (Halmschlager & Katzensteiner, 2017). In this respect, the importance of potassium, copper, zinc, boron, calcium etc. has been specially emphasized (Mengel & Kirkby, 2001; Saarsalmi & Tamminen, 2005).

Soil fertilization is one of the effective forest management tools to increase forest vitality and tree growth (Saarsalmi & Mälkönen, 2001; Moilanen et al., 2015). It has been showed that the effects and success of fertilization varied depending on peat characteristics, nutrient status of stand, dose and kind of applied fertilizers and other factors (Finer, 1989; Zālītis, 1991; Saarsalmi & Tamminen, 2005; Moilanen et al., 2015; Klavina et al., 2016; Libiete et al., 2016; Okmanis et al., 2016; etc.). Nevertheless, there is a lack of information about complex impact of mineral K fertilizers like potassium magnesium sulfate (PMS) application on nutrient status of different age spruce stands, stand productivity, possible fertilization impact on soil microbiological activity and species composition in drained peat soil forests in the boreo-nemoral climate zone. PMS as K source is pH neutral, provides a readily available supply of K, Mg, and S to plants and is acceptable for use in organic crop production. The research aim was to elucidate the effect of PSM on different age Norway spruce stands on drained peat soils by evaluating: (1) nutrient accumulation in soil-plant system, (2) soil microbiological activity, (3) health status of spruce individuals, (4) growth intensity and productivity of spruce, (5) changes in composition of vascular plant and moss species. We hypothesized that fertilization with PMS might improve spruce supply not only with K, Mg and S, but also with other nutrients by significant changes in peat soil chemical composition, microbiological activity and species composition of herb layer plants, thereby facilitating tree growth.

MATERIALS AND METHODS

Site description and experimental design

The study, consisting of two experiments with fertilization, was conducted at two sites (Valka, 57°41´N, 26°09´E, and Kalsnava, 56°40´N, 25°50´E) in Latvia, situated in the boreo-nemoral climatic zone.

The first site 'Valka' is situated on Tālava Lowlands Seda Plain. The quarter sediment depth is 10–20 m. Glaciofluvial sand sediments are dominant, but peat deposits are dominant in relief depressions. The climate is moderately warm and humid: the average annual amount of precipitations is 610 mm, the average temperature in January is -6.0 °C, in July +16.9 °C, but annual – +5.2 °C (Kļaviņš et al., 2008). The aim of the 1st experiment was to assess the impact of treatment with PMS on new spruce planting developed on drained peat soil. The experiment was set up in a forest glade (*Myrtillosa turf.mel.,* alluvial land), where spruce trees were planted 25 years ago and > 95% decayed during the up-coming 10 years. The peat layer was > 1 m thick, the degree of humification according to Post scale (von Post & Granlund, 1926) varied between H3 and H8 in the 0–0.05 and 0.05–0.20 m layers and between H4 and H8 in the 0.20–0.30 m layer. Before fertilization, all shrubs and trees were sawed down and removed from the research area in August 2007. One experimental plot with a size of 100 m^2 was used as a control and another with the same size was fertilized with PMS (24.9% K, 6% Mg, 17% S): 100 g m^{-2} in September 2007 and 50 g m^{-2} during seedling planting in April 2008. 30 one-year-old container seedlings (ca. 20 cm in height) from the local tree nursery 'Strenci' were planted in each experimental plot, design of seedling sites: 1.5×2.0 m.

The second site 'Kalsnava' is situated on East-Latvian Lowland, Arona Undulating Plain. The depth of quarter sediments is 10–50 m, consisting mainly of sand, gravel, as well as local clay deposits and peat deposits in relief depressions. The climate is also moderately warm and humid: the average annual amount of precipitations is 680 mm, the average temperature in January is -5.8 °C, in July +17.0 °C, but annual - +5.4 °C (Kļaviņš et al., 2008). The aim of the 2nd experiment was to assess the impact of treatment with PMS on 20-year-old spruce stand with low vitality (> 60% defoliation and discoloration of tree crown) growing on drained peat soil. Thus, in 2007, two experimental plots, each with a size of 100 m^2, were established in the spruce stand (*Myrtillosa turf.mel.*) planted at Kalsnava in 1989. The density of spruce trees was 5,000 trees ha^{-1} in 1989, and 1,500 – in 2007. One experimental plot was used as a control and another was fertilized with PMS: 100 g m^{-2} in September 2007 and 50 g m^{-2} in April 2008.

Soil samples and plant tissue collection

Soil and plant material sampling for laboratory analysis was done according to Table 1.

Table 1. Sample collection for laboratory analysis

Material	Time							
	08.2007	04.2008	09.2008	09.2010	09.2012	09.2014	09.2016	10.2016
Soil, chemistry	x		x	x	x	x	x	
Soil, microbiology					x		x	
Needles	x	x*	x	x	x	x	x	
Wood cores**								x

* only for the new seedlings for the 1st experiment; ** only for the 2nd experiment.

To detect the nutrient status and select the fertilizer dose, soil and needle samples from the current year shoots of spruces were collected for chemical analysis from all experimental plots before the experiment establishment in August 2007. Subsequent regular collection of soil samples was done until 2016. Soil samples were separately taken at 0 to 20 cm depth of the perimeter area of five randomly distributed spruce trees at each plot. Each soil sample (2 L) consisted of thoroughly mixed five subsamples collected by a soil probe was placed in a plastic bag and transported to the laboratory. Along with soil sampling, spruce needles from the current year shoots from previously selected five trees in each plot were collected for chemical analysis. For quantification of culturable microorganisms, four soil sub-samples from each plot were taken within 0–10 cm depth, placed in sterile plastic bags (*Nasco* WHIRL-PAK) and taken to the laboratory where stored at +4 °C until microbial analysis.

Tree measurements

At both experimental sites, trees were numbered. Measurements of total height were done for the trees of the 1st experiment in October 2008, 2012, 2016. The measurements of stem diameter at the stem basis and assessment of tree crown vitality according to the Forest monitoring guidelines (UN/ECE, 2006; Schomaker et al., 2007) to characterize the general physiological status of trees was done in autumn 2008 and 2016. This was based on the following bioindicators: visual evaluation of crown defoliation and needle discoloration (dechromation) in percent. Based on the results, tree condition was classified as following: healthy – 0–10%, slightly damaged – > 10–25%, medium damaged – > 25–60%, and seriously damaged – > 60% defoliation and discoloration. At the 2nd experimental site, the diameter of spruce trees at 1.3 m height and tree height were measured in October 2007, 2012, 2016. Tree-ring width samples (wood cores) were taken from all spruce trees with a Presler increment drill in 2016. Assessment of tree crown status was done similar to the 1st experiment.

Vegetation inventory

All tree (E_3), shrub (E_2), herb (E_1) and moss (E_0) layer species were recorded before the experiment establishment at both sites in August 2007, then in 2012, and 2016. The projective cover of each layer and the percentage of each plant and moss species were evaluated visually at each plot (Dierschke, 1994). To estimate the ecological growth conditions, Ellenberg indicator values (Ellenberg et al., 1992) were calculated for detected plant species.

Laboratory analysis

For chemical analyses, the collected soil samples were dried at room temperature and passed through a 2 mm sieve. The soil analyses were done using 1 M HCl extraction for all nutrients (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B), where soil/extractant (v/v) mixture was 1:5. This extractant is universal and quite aggressive thereby characterizes not only the amount of nutrient currently available for the plant uptake from the soil, but also indicates the amount of reserves of the element for the remaining vegetation season (Osvalde, 2011). For P, S and Mo determination, 1 M HCl extract of soil was oxidized with HNO_3, H_2O_2 and $HClO_4$ on a hot plate, the obtained salts were dissolved in HCl and diluted with distilled water (Rinkis et al., 1987). The concentrations of Ca, Mg, Fe, Cu, Zn and Mn in plant samples were determined by atomic absorption spectrophotometer (*Perkin Elmer AAnalyst 700*), acetylene-air flame (Page et al., 1982; Anonymous, 2000). The contents of P, Mo, N, S and B were determined by colorimetry: P by ammonium molybdate in an acid-reduced medium, Mo by thiocyanate in reduced acid medium, B by hinalizarine in sulfuric acid medium, S by turbidimetric method by adding $BaCl_2$, with a spectrophotometer JENWAY 6300 (Barloworld Scientific Ltd., Gransmore Green Felstad, Dunmow, Essex, UK). N in soil extraction (mineral (NH_4+NO_3) and total) – by

Nesler's reagent in an alkaline medium with a spectrophotometer JENWAY 6300. K – with the flame photometer *JENWAY PFPJ* (Jenway Ltd, Gransmore Green, Felsted Dunmow, Essex, UK). Soil pH was measured in 1 M KCl extraction (soil-extractant mixture 1:2.5) using the pH-meter *Sartorius PB-20* (Sartorius AG, Goettingen, Germany), but EC – in distilled water extraction (soil-water mixture 1:5) with the conductometer *Hanna EC 215* (Hanna instruments, USA). The obtained results of chemical elements were expressed in mg L^{-1}, because plant roots growth in a certain volume and in peat soil this volume significantly differ from the weight of this volume. The average volume weight for the peat soil of the 1st experiment control plot was 0.53 ± 0.04 g cm^{-1}, fertilized plot - 0.61 ± 0.02 g cm^{-1}; for the 2nd experiment control plot – 0.44 ± 0.03 g cm^{-1}, fertilized plot – 0.43 ± 0.03 g cm^{-1}.

Spruce needles were quickly washed with distilled water, dried at +60 °C and ground. Plant samples were dry-ashed in concentrated HNO_3 vapors and re-dissolved in 3% HCl for K, P, Ca, Mg, Fe, Cu, Zn, Mn and Mo detection. Wet digestion for N was done in conc. H_2SO_4, for S – in HNO_3, for B – plant samples were dry-ashed in concentrated HNO_3 vapors. N in plants was determined by modified Kjeldal method. P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, and B were analyzed similar to the procedures described for analysis of soil extracts.

Total soil moisture was determined for soil samples after drying in oven at +105 °C for 24 hours, analytical replication was three times. These results were further used to calculate coefficients to convert data of colony forming units (CFU) from 1 g soil (analysed) to 1 g dry soil. Number of culturable bacteria and fungi in soil was determined by plate count on nutrient agar ('Biolife', Italia) and bengal agar with chloramphenicol ('Laboratorios Conda', Spain), respectively. 10 g of analysed soil were added to 250 mL flask with 90 mL of sterile distilled water and shaken for 30 min. on automatic shaker (Alef, 1995). Decimal dilutions were prepared and inoculated on agar plates. Bacteria and fungi were incubated at temperature 20 ± 2 °C for 72 and 120 hours, respectively. After incubation, the numbers of CFU were counted and their abundance per one gram of soil was calculated.

Wood cores from the 2nd experimental site were processed using a LINTAB 4 measuring table and TSAPWIN software.

Statistical analysis

Statistical analysis (standard errors, *Student's t-test*, $p<0.05$, etc.) were done using R-Studio. Linear mixed models using *SPSS for Windows 14.0* were calculated to evaluate the fertilization effect on soil and needle chemical composition and tree growth parameters. Gradients in plant species composition, stand productivity and habitat factors were determined and analysed using detrended correspondence analysis (DCA) and PCord-5 software (McCune & Grace, 2002). To determine differences for fungal, bacterial CFU in soil between research plots, data were log-transformed to achieve normality and submitted to one-way ANOVA, followed by Tukey's test ($p < 0.05$) using program R 2.14.1. The spruce stock volume was calculated according to Liepa (1996) equation, because height of trees was > 1.5 m and diameter - > 1.5 cm:

$$V = 2.3106 \cdot 10^{-4} \cdot H_g^{0.78193} \cdot D_g^{0.34175 \cdot lgH_g + 1.18811} \cdot N \qquad (1)$$

where V – stand stock volume, m^3 ha^{-1}; H_g – average height of stand, m; D_g – average stem diameter for stand at 1.3 m height, cm; N – number of stand trees, ha^{-1}.

RESULTS AND DISCUSSION

Nutrient status of Norway spruce

The results did not reveal significant differences for the chemical composition of soil and needle samples between the selected plots before fertilization in August 2007 and were given in Table 2 as the average values for the site. The chemical composition of seedling needles before planting at the 1st experiment site in April 2008 are also given in Table 2.

Table 2. Mean nutrient concentrations at the Norway spruce experimental sites in Latvia before treatment with PMS (background status)

Element	Peat soil (August 2007)		Needles (August 2007)		Needles (seedlings before planting, April 2008)
	1st exp.	2nd exp.	1st exp.	2nd exp.	1st exp.
	$mg\ L^{-1}$, 1 M HCl extraction				
N min^{-1}	34.5 ± 1.5	31.0 ± 1.0	-	-	
Ntot	205.0 ± 5.0	190.50 ± 3.50	7.4 ± 0.4	8.1 ± 0.5	12.0
P	311.0 ± 38.0	105.5 ± 20.5	1.4 ± 0.2	1.2 ± 0.1	2.6
K	47.0 ± 3.0	47.5 ± 7.5	1.7 ± 0.2	1.6 ± 0.2	10.0
Ca	8,038.0 ± 125.0	3,225.5 ± 62.5	3.6 ± 0.3	5.2 ± 0.3	4.7
Mg	127.0 ± 5.0	188.5 ± 11.5	0.7 ± 0.1	0.9 ± 0.1	1.9
S	41.0 ± 10.0	28.0 ± 0.0	0.7 ± 0.1	0.5 ± 0.1	0.8
	$mg\ L^{-1}$		$mg\ kg^{-1}$		$mg\ kg^{-1}$
Fe	15,141.0 ± 710.0	6,350.0 ± 900.0	24.0 ± 1.5	60.0 ± 5.2	220.0
Mn	412.50± 17.5	25.00 ± 9.00	580.0 ±65.0	720.0 ± 134.5	134.0
Zn	16.25 ± 3.25	5.20 ± 0.30	24.00 ± 0.75	15.00 ± 1.05	36.00
Cu	1.33 ± 0.08	0.70 ± 0.15	2.60 ± 0.17	2.20 ± 0.12	5.00
Mo	0.05 ± 0.01	0.04 ± 0.00	0.10 ± 0.03	0.15 ± 0.01	1.20
B	0.50 ± 0.00	0.15 ± 0.05	11.00 ± 1.00	12.00 ± 0.09	23.00
pH_{KCl}	4.50 ± 0.04	4.39 ± 0.06	-	-	-
EC $mS\ cm^{-1}$	0.33 ± 0.03	0.47 ± 0.02	-	-	-

Both visual observation (yellowing or discoloration of the 2nd year and older spruce needles) and chemical analyses of peat soil and spruce needles before experiment establishment (Table 2) confirmed the deficiency of K at the experimental sites, as well as low level or deficiency of N, Mg, S, Fe, Cu, and B in spruce needles according to several authors: optimal for K – 0.5–1.2%, N – 1.35–1.70%; Mg – 0.10–0.25%; Cu – 4–10 $mg\ kg^{-1}$; B – 15–50 $mg\ kg^{-1}$ (Bergmann, 1988); satisfactory for N – > 1.5%; K – > 0.7%; S – > 0.15%; Fe – > 50 $mg\ kg^{-1}$; B – > 20 $mg\ kg^{-1}$; Cu – > 4 $mg\ kg^{-1}$ (compiled by Renou-Wilson & Farrell, 2007); critical concentrations (close to a general optimum range): N – 1.40–1.75 %; K – 0.52–0.82%; Mg – 0.08–0.13% (Mellert & Göttlein, 2012). Although the results of soil analysis in general showed sufficient concentration of Ca and Mg for tree growth, probably the unfavorable high Ca : Mg ratio (17–63 : 1) can be additional factor affecting spruce vitality for damaged stands. For plant nutrition the most optimal Ca : Mg ratio in soils using 1 M HCl extraction is 5–8 : 1 (Rinkis & Nollendorf, 1982; Cekstere & Osvalde, 2013). Additionally, the study indicated deficiency of N (in mineral form) and Cu in peat soil. In peat soils, N is mainly presented in organic form which is not directly available for plant uptake and can significantly limit tree growth (Moilanen et al., 2010). Our research showed that the N content in spruce needles was significantly below the optimal values, even in the range of extreme deficiency according to Mellert & Göttlein (2012). For high wood productivity sufficient N availability is one of the main prerequisite. Despite of overall foliar N deficiency, N : K ratio for damaged trees (5.43) were too high based on latest nutrient ratio thresholds for Norway spruce: N : K 1.7–3.3 (Mellert & Göttlein, 2012), pointing out severity of K deficiency. Therefore, N fertilization might be suggestable only after prevention of serious K starvation and should be based on the results of additional experimental studies. Thus, imbalanced conditions of mineral nutrition predisposed trees to discoloration and stunted growth frequently observed for middle-aged Norway spruce stands in Latvia.

In general, the pre-treatment nutrient status has been found to have a significant role as regards to the extent of the stand response after fertilization (Moilanen et al., 2015). Selection of PMS for our experiments underplayed on diagnosed nutritional disorders: overall K deficiency, Ca : Mg ratio unfavorable to Mg, as well as, in general, low level of S in current year needles. Regardless of spruce stand vitality the foliar S concentration found in the current year needles before the treatment were on the deficiency threshold according to Renou-Wilson & Farrell (2007). Additionally, PMS did not contribute to soil acidity or alkalinity. It is a relevant advantage of this fertilizer in the conditions of soil pH in general optimal for spruce growth.

A significant impact of fertilization on concentrations of several nutrients in the soil and spruce needles was stated according the results of LMM analysis (Tables 3, 4). Generally, a significantly higher K concentration in the soil showed a trend toward decrease from 2008 to 2016, while increase in N_{min} was found for both fertilized plots from 2008 to 2016 (Fig. 1, a, b). The pronounced increase in S concentrations were mainly characteristics for the fertilized plots during 2008 (Fig. 1, c). The fertilization significantly affected the concentrations of plant available Mg and N_{tot} in soil for the 1st experiment (Fig. 1, d, Table 5) without strong impact for the 2nd experiment.

Table 3. Results of LMM analyzing nutrient content in soil under fertilization with PMS (only significant results for nutrients, $p < 0.01$, were included in the table)

Dependent variable	Source	1st experiment			2nd experiment		
		Num. df	Den. df	F	Num. df	Den. df	F
K	Intercept	1	16.99	12,527	1	8.70	8,332
	Fertilization	1	16.99	4,523	1	8.70	1,444
	Year	4	9.09	143	4	7.32	120
	Fert.*Year	4	9.09	211	4	7.32	164
Nmin.	Intercept	1	15.90	20,275	1	18.81	12,758
	Fertilization	1	15.90	1,314	1	18.81	78
	Year	4	7.57	1,041	4	8.25	42
	Fert.*Year	4	7.57	59	4	8.25	90
Ntot.	Intercept	1	17.85	45,482	-	-	-
	Fertilization	1	17.85	586	-	-	-
	Year	4	6.90	169	-	-	-
	Fert.*Year	4	6.90	113	-	-	-

Table 3 (continued)

S	Intercept	1	8.80	8,136	1	13.79	9,775
	Fertilization	1	8.80	718	1	13.79	884
	Year	4	8.10	308	4	9.16	85
	Fert.*Year	4	8.10	136	4	9.16	124
Mg	Intercept	1	5.98	5,318	1	18.39	11,681
	Fertilization	1	5.98	1,730	1	18.39	46
	Year	4	6.72	231	4	7.26	45
	Fert.*Year	4	6.72	273	4	7.26	10

Table 4. Results of LMM analyzing nutrient content in spruce needles under fertilization with PMS (only significant results for nutrients, $p < 0.01$, were included in the table)

Dependent variable	Source	1st experiment			2nd experiment		
		Num. df	Den. df	F	Num. df	Den. df	F
K	Intercept	1	15.78	13,133	1	18.90	13,545
	Fertilization	1	15.78	4,886	1	18.90	4738
	Year	3	7.95	16	4	8.15	50
	Fert.*Year	3	7.95	12	4	8.15	55
N	Intercept	1	15.78	58,011	1	18.98	19,485
	Fertilization	1	15.78	1,363	1	18.98	57
	Year	3	7.76	76	4	7.83	133
	Fert.*Year	3	7.75	133	4	7.82	12
Ca	Intercept	1	15.80	26,124	1	19.17	18,468
	Fertilization	1	15.80	360	1	19.17	403
	Year	3	7.90	27	4	7.155	36
	Fert.*Year	3	7.90	39	4	7.155	17
Fe	Intercept	1	15.39	14,807	1	10.52	15,639
	Fertilization	1	15.39	133	1	10.52	25
	Year	3	7.99	78	4	6.56	429
	Fert.*Year	3	7.99	30	4	6.56	104
Zn	Intercept	1	13.64	23,406	1	18.17	18,069
	Fertilization	1	13.64	1,605	1	18.17	602
	Year	3	8.96	334	4	7.75	72
	Fert.*Year	3	8.96	212	4	7.75	84
B	Intercept	1	15.01	8,684	-	-	-
	Fertilization	1	15.01	735	-	-	-
	Year	3	7.05	51	-	-	-
	Fert.*Year	3	7.05	10	-	-	-

Significantly higher concentrations of K in needles were found at the both experimental plots treated with PMS compared to the control plots till 2016 (Fig. 1, e), as well as Ca and Zn, generally (Table 5). The increase in the content of N (Fig. 1, f), a significantly higher Fe, lower B level (Table 5) in needles was stated only for trees from the fertilized plot of the 1st experiment. There was no significant difference for other nutrients (Table 6). It is notable that the level of Cu, B and Mo in spruce needles was low for all experimental plots. It was not surprising for Mo which is the only micronutrient with low availability in acid soils (Mengel & Kirkby, 2001). Our study showed that mean Mo concentration in spruce needles was below 0.50 mg kg^{-1}; proposed as the sufficiency threshold for woody plants in acid growing medium (Karlsons & Osvalde, 2017).

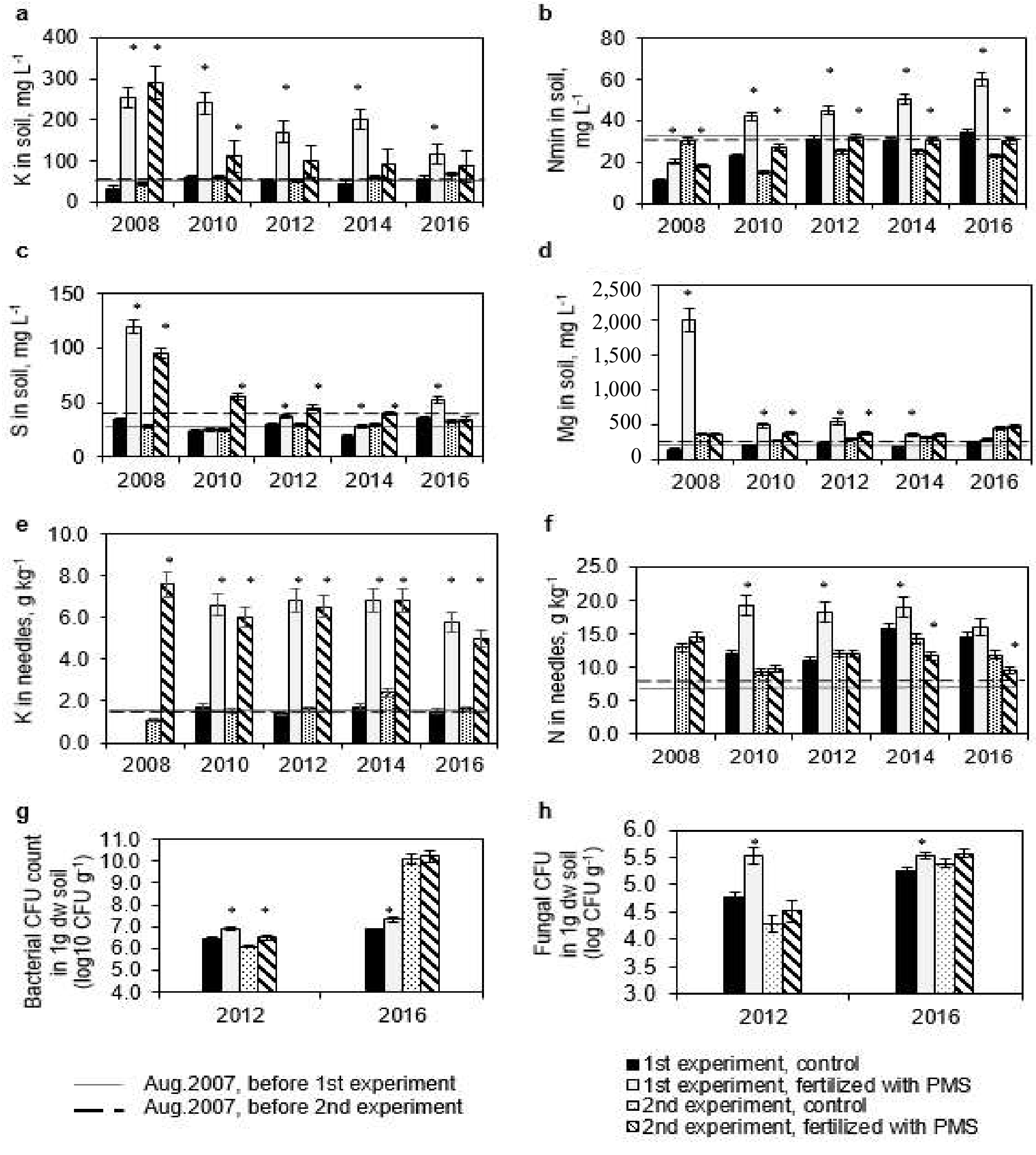


Figure 1. Changes in nutrient content in peat soil (1M HCl extraction) (a-d), needles (e, f) and bacterial (g) and fungal CFU count (h) in peat soils in the spruce experimental sites in Latvia. Date represent results of September 2008–2016. (Means ± SE, asterisk indicates significant difference (LMM, *p,*0.01) for the experimental site between the control and fertilized plot).

During the research at 2008–2016, almost permanent 3–4 times higher concentration of K in spruce needles, significantly above the deficiency limits, was characteristic for all experimental plots with K application. Moreover, the fertilization treatment completely balanced foliar N : K ratio. Based on the previous research (Silverberg & Moilanen, 2008; Moilanen et al., 2015) it is expectable that the effect of

K fertilization could last for the coming 10–15 years. It is surprising that soil K response to the PMS treatment also continued to be significant until the end of the study period. PMS is totally water soluble, but dissolves slower than most of other common K fertilizers. Unforeseen, the fertilization with PMS did not increase the content of S and Mg in spruce needles, which was still low or at deficiency level. Probably, the reasons could be the dilution effect caused by increased biomass production of trees and involvement of these elements in intensive growth of herb-layer vegetation, as well as element antagonism (Ca : Mg, K : Mg). For improvement of Mg and S status additional fertilization with appropriate fertilizers is suggestable.

Table 5. Average nutrient concentrations in peat soils and current year needles from the Norway spruce experimental sites in Latvia over 2008–2016 (fertilized in 2007–2008 with PMS)

	1st Experiment		2nd Experiment	
Element	Control	Fertilized	Control	Fertilized
Soils (mg L^{-1}, 1M HCl extraction)				
	2008–2016		2008–2016	
N_{tot}	218.6 ± 4.4 a*	272.8 ± 47.6 b ↑	201.3 ± 16.8 a	214.3 ± 20.2 a
Needles	2010–2016		2008–2016	
Ca, g kg^{-1}	3.5 ± 0.1 a	4.4 ± 0.3 b ↑	3.4 ± 0.3 a ↓	4.5 ± 0.2 b
Fe, mg kg^{-1}	42.50 ± 1.50 a ↑	51.50 ± 5.32 b ↑	53.50 ± 14.08 a	49.40 ± 13.85 a
Zn, mg kg^{-1}	17.55 ± 2.16 a ↓	30.00 ± 4.69 b	17.50 ± 0.29 a	25.12 ± 3.10 b ↑
B, mg kg^{-1}	17.75 ± 1.38 b ↑	9.75 ± 0.85 a	13.75 ± 2.66 a	13.00 ± 0.71 a

* – means with different letters in a row were significantly different for the experiment (*t-Test,* $p < 0.05$, b > a). ↑↓ – significantly lower or higher compared to the pre-treatment status in 2007.

Table 6. Average chemical composition of peat soil and current year needles from the Norway spruce experimental plots over 2008–2016 (control+fertilized*) in Latvia

	1st Experiment	2nd Experiment
Peat Soils (mg L^{-1}, 1M HCl extraction)	2008–2016	2008–2016
P	234.31 ± 35.02	151.57 ± 24.36
Ca	5,316.05 ± 401.50 ↓	3,556.07 ± 362.04
Fe	14,739.25 ± 1,321.45	6,640.00 ± 630.08
Mn	233.33 ± 23.75 ↓	58.00 ± 19.92
Zn	11.32 ± 1.01	5.99 ± 1.81
Cu	0.89 ± 0.11	0.89 ± 0.13
Mo	0.04 ± 0.01	0.06 ± 0.01
B	0.76 ± 0.21	0.37 ± 0.06
pH_{KCl}	4.35 ± 0.10	4.24 ± 0.07
EC, mS cm^{-1}	0.43 ± 0.08	0.47 ± 0.06
Needles	2010–2016	2008–2016
P, g kg^{-1}	2.0 ± 0.1 ↑	1.7 ± 0.2 ↑
Mg, g kg^{-1}	1.1 ± 0.2	0.9 ± 0.1
S, g kg^{-1}	0.8 ± 0.1	0.7 ± 0.1
Mn, mg kg^{-1}	614.00 ± 26.02	721.00 ± 157.18
Cu, mg kg^{-1}	2.65 ± 0.57	2.50 ± 0.24
Mo, mg kg^{-1}	0.20 ± 0.06	0.24 ± 0.08

* – no statistically significant ($p < 0.05$) differences were found, on average, between the control and fertilized plot of the experiment during 2008–2016. ↑↓ – significantly lower or higher compared to the pre-treatment status in 2007.

Assessment of soil microbiological activity

The average count of bacteria and fungi in soil of fertilized plot, accompanied by a remarkable variability in the study years, was significantly higher than in the control plot only for the 1st experiment (Fig. 1, g, h). As high abundance of bacteria was found for all soil samples analyzed, fungi : bacteria (F : B) ratio did not exceed 0.044. This highest value was obtained for the fertilized plot of the 1st experiment in 2012. The lowest values were characteristics for the 2nd experiment in 2016 (F : B 0.000019-0.000022). In general, F : B ratio in the fertilized and control plots differed significantly only for the 1st experiment. Different studies demonstrate that soil microbes and fungi are highly sensitive to environmental conditions; fungi often dominate in acid soil while bacteria in nutrient-rich soils, often with high pH (Schimel & Bennett, 2004; Shen et al., 2014). Our results revealed a high abundance of soil bacteria in both low pH study sites regardless of the fertilization. Although there was a tendency of the promotive impact of fertilization on the count of bacteria and fungi in soil, this increase was statistically significant only for the 1st experiment with young trees. Our results were also in a good agreement with findings by Klavina et al. (2016) on significantly higher microbial abundance in fertilized (wood ash, K_2SO_4) peat soil of young spruce. It is approved that F : B ratio depends on forest ecosystems of different vegetation types, forest fertility and N availability (Högberg et al., 2007). Our research revealed that F : B ratios could vary in response to changes in herb and moss layer vegetation caused by PMS fertilization (1st experiment, 2012), as well as due to local soil conditions, for example increased soil moisture for the 2nd experiment in 2016.

Spruce crown status and stand productivity

A significant impact of fertilization on spruce stand productivity of both experiments was stated (Table 7). Fertilization with PMS resulted in up to 3 times higher spruce height (4.20 ± 1.50 m > 1.71 ± 0.11 m, $p < 0.05$) and 2 times larger stem diameter (7.23 ± 0.24 cm > 3.05 ± 0.19 cm, $p < 0.05$) compared to the control at the end of 1st experiment in 2016. Simultaneously, significant differences in crown status were also stated between plots: the crown status was healthy for fertilized trees (defoliation: 14.8 ± 0.9%, discoloration: 5.3 ± 0.4%), but seriously damaged for the control (defoliation: 27.3 ± 2.8%, discoloration: 85.4 ± 0.7%).

Results of the 2nd experiment revealed a significant continuous improvement of tree crown vitality at the fertilized plot compared to the control already from 2008. Thereby in 2008 and 2016, the defoliation and dechromation level of tree crown was significant lower ($p < 0.05$) at the fertilized plot compared to the control (2008: defoliation $21.7 \pm 2.2\% < 30.7 \pm 2.5\%$; discoloration $5.2 \pm 0.6\% < 82.7 \pm 0.5\%$; 2016: defoliation $14.7 \pm 7.0\% < 29. \pm 3.1\%$, discoloration $5.2 \pm 0.4\% < 90.7 \pm 0.7\%$, respectively).

Table 7. Results of LMM analyzing responses of tree growth parameters under fertilization with PMS (p < 0.01)

Dependent Variable	Source	1st Experiment			2nd Experiment		
		Num. df	Den. df	F	Num. df	Den. df	F
Mean tree height	Intercept	1	66.01	2,304	1	70.49	5,374
	Fertilization	1	66.01	334	1	70.49	47
	Year	2	54.66	790	2	46.84	97
	Fert.*Year	2	54.66	189	2	46.84	18
Mean tree diameter	Intercept	1	50	1,150	1	71.760	2,503
	Fertilization	1	50	190	1	71.760	27
	Year	0	-	-	2	49.15	60
	Fert.*Year	0	-	-	2	49.15	9
Mean tree volume	Intercept	-	-	-	1	50.49	622
	Fertilization	-	-	-	1	50.49	57
	Year	-	-	-	2	40.25	100
	Fert.*Year	-	-	-	2	40.25	26

Abbreviations: Num. df – numerator degree of freedom; Den. df – denominator degree of freedom; Fert. – fertilization.

The rapid increase in the increment of tree rings (planting 1989, 2nd experiment) started on the 2nd year after fertilization (Fig. 2, a). Generally, the positive effect of fertilization significantly facilitated spruce growth: annual tree ring increment was up to 3.5 times higher compared to the control, tree height – 1.5 times, stem diameter 1.4 times larger at the end of 2016 (Table 8). As the result (Fig. 2, b), the stock volume for the fertilized plot was 3.5 times higher compared with the calculated prognostic (theoretical) stock volume without application of PMS and 2.8 times higher compared to the control.

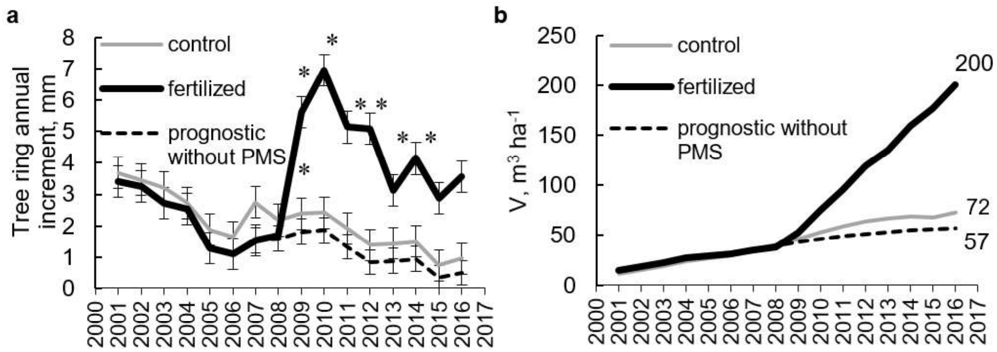


Figure 2. Annual increment of tree rings (a) and stock volume (b) for control and fertilized with PMS (2007, 2008) Norway spruce at the 2nd experimental site (planting 1989); a: ± standard error, *n*=15; b: V – stand stock volume; prognostic without PMS – theoretical stock volume for the fertilized plot without fertilization. Asterisk indicates significant difference (*t-Test, p* < 0.05) for the experimental site between the control and fertilized plot.

Table 8. Results of estimated marginal means for spruce growth parameters at the 2nd experiment plots in Kalsnava (planting 1989)

Dependent Variable*	Plot	Year	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
Mean tree diameter, cm	Fertilized	2007	8.67	0.41	7.82	9.51
		2012	13.79	0.57	12.63	14.95
		2016	16.66	0.67	15.27	18.04
	Control	2007	8.66	0.41	7.82	9.51
		2012	10.86	0.57	9.69	12.02
		2016	12.23	0.75	10.70	13.77
Mean tree height, m	Fertilized	2007	5.86	0.16	5.54	6.19
		2012	9.54	0.19	9.16	9.92
		2016	11.58	0.20	11.17	12.00
	Control	2007	5.94	0.16	5.61	6.26
		2012	7.28	0.19	6.90	7.67
		2016	8.25	0.23	7.78	8.71
Stand stock volume, m^3 ha^{-1}	Fertilized	2007	34.89	3.46	27.83	41.95
		2012	119.17	7.93	102.97	135.36
		2016	200.22	12.08	175.43	225.01
	Control	2007	36.24	3.46	29.18	43.29
		2012	64.21	7.93	48.01	80.40
		2016	72.50	10.89	50.15	94.85

* LMM showed a significant effect of 'fertilization', 'time' and 'fertilization*time' on the dependent variable (Table 6).

Thereby our study convincingly approved significant effect of fertilization with PMS on additional increment of stock volume by up to 3 times during the study period, as well as a higher growth response compared to the previous studies with other fertilizers (wood ash, KCl, K_2SO_4) for Norway spruce at drained peat soil in the boreo-nemoral climate zone (Zālītis, 1991; Okmanis et al., 2016). It was found that four years after fertilization with K in dose 62–65 kg ha^{-1}, applied by wood ash or potassium sulphate, the additional volume increment reached 9–19 m^3 ha^{-1} (Okmanis et al., 2016). Our results revealed 55 m^3 ha^{-1} of additional increment for 2009–2012, and 128 m^3 ha^{-1} in 2016. We suggested that higher growth response could be attributed to approximately five times higher K dose, complexity of PMS for simultaneously ameliorating nutrient (K, Mg, S) deficiency in growing medium, as well as possibility to use higher amount of fertilizer without detrimental increase in soil reaction and electrical conductivity. Several studies have demonstrated a distinct positive correlation between K, Ca and Zn nutrition and wood production of trees (Barrelet et al., 2006; From, 2010; Guerriero et al., 2014). Our study also revealed a higher Ca and Zn content in spruce needles from the fertilized plots of both experiments. It is notable that these foliar concentrations of Ca and Zn in needles completely met the standards for Norway spruce reported by Renou-Wilson & Farrell (2007).

Dynamics of stand vegetation

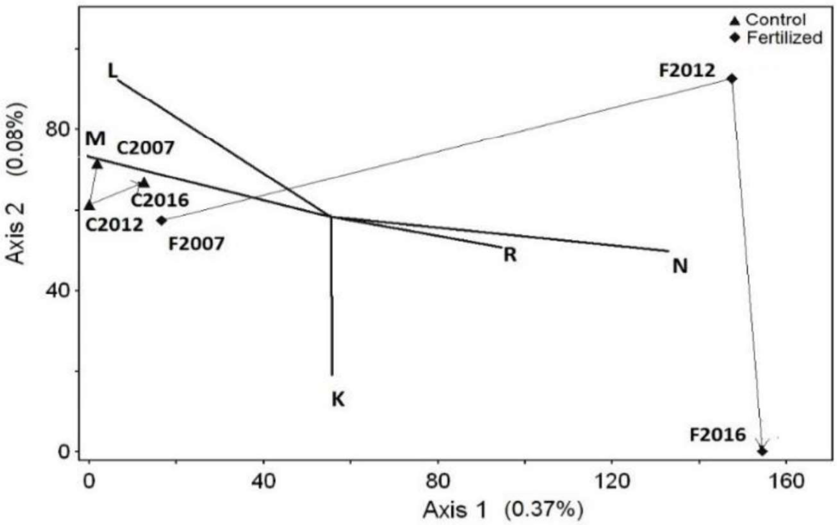
The vegetation observations revealed no significant differences for species cover and composition between the plots of the 1st experiment before the treatment with PMS in 2007. Generally, herb layer covered 72% of the total plot area, while moss layer – 52%. 18 vascular plant species and 8 moss species were recorded. The most dominant plant species were *Festuca rubra* with projective cover of 30%*, Deschampsia cespitosa* – 11%*, Geum rivale* and *Galium album* – each 8%, and the dominant moss species were *Rhytidiadelphus squarrosus* – 21%, *Climacium dendroides* – 12%*,* and *Plagiomnium cuspidatum* – 5%. Although the amount of vascular plant (24) and moss (10) species had increased in 2016, the same dominant species were prevalent for the control also after 10 years. During the first years after treatment, the amount of nitrophilic tall herbaceous species, e. g., *Antriscus sylvestris* (40%)*, Urtica dioica* (10%), *Rubus idaeus* (8%), and *Chamerion angustifolium* (8%) has rapidly increased at the fertilized plot. With the increase of spruce role as edificator, the amount of previously mentioned nitrophilic species has decreased forward to 2016, nevertheless they were dominant at the herb layer also during 2016. The amount of *Plagiomnium* sp. (*P. cuspidatum* – 30%, *P. ellipticum* – 16%, *P. undulatum* – 10%) has rapidly increased at the moss layer after fertilization, reaching 56% of the total plot area in 2016.

At the 2nd experiment, no significant differences for species cover and composition between the plots of the 2nd experiment before the treatment were stated. Thus, in 2007, 33 vascular plant species and 12 moss species were recorded in total, covering 80% and 70% of each plot area, respectively. The dominant plant species were *Ranunculus repens* (12%), *Viola palustris* (12%), *Deschampsia cespitosa* (8%), but for the moss species – *Climacium dendroides* (25%), *Rhytidiadelphus squarrosus* (16%) and *Plagiomnium cuspidatum* (12%). These species with no significant changes were dominant at the control plot during all the study period. Whereas at the fertilized plot, a rapid increase of cover with *Urtica dioica* (12%) and *Antriscus sylvestris* (21%) was stated in 2012 compared to 2007. In 2016, a significant increase of moss species *Plagiomnium cuspidatum* (30%) and *P. ellipticum* (10%) cover*,* as well as decrease of plant species cover (35%) and diversity (25 species) was recorded due to high level of shading formed by spruce crown cover (95%) at the fertilized plot. Thereby, the cover of *Ranunculus repens* was only 5%, *Urtica dioica* – 2%.

The DCA of the 1st experiment vegetation results revealed the highest positive values ('tau coefficient') for *Urtica dioica* (0.966), *Plagiomnium elatum* (0.828) and *Anthriscus sylvestris* (0.690), as well as soil reaction (0.467) and nitrogen (0.759) in ordination with the 1st axis. Similar results were stated also for the 2nd experiment: the highest positive correlations with the 1st axis were determined for plant species typical for neutral and nitrogen abundant sites (*Plagiomnium cuspidatum* (0.900), *Anthriscus sylvestris* (0.602) and soil reaction (0.501). Whereas the highest negative correlations with the 1st axis were stated for the background species of the control plot: *Veronica chamaedrys* (-0.828) and *Rhytidiadelphus squarrosus* (-0.867) in the 1st experiment, and *Climacium dendroides* (-0.966) and *Dechampsia cespitosa* (-0.867) at the 2nd experiment.

The content of N, P, and K in soils may affect not only plant growth but also plant community composition (Knecht & Göransson, 2004; Tripler et al., 2006). The results emphasized the fact that fertilization with PMS undeniable caused changes in drained peat soil chemical composition, microbiological activity and plant species composition. Significant increase of nitrophilic plant species indicated increase of biologically active N in soil surface and thus on activation of nutrient cycling, also reflected by environment gradient analysis by Ellenberg values, specially four years after fertilization (Fig. 3). During the study period, the increase in plant available N_{min} was stated for the fertilized plots of both experimental sites established on peat soils. The contributing impact of fertilization on total N was found only for the new planting where, according to Ellenberg's values for N, the treated plot could be characterized as more fertile habitat. In peat soils, N is mainly presented in organic form which is not directly available for plant uptake and can significantly limited tree growth (Moilanen et al., 2010). However, several studies have shown that a range of plant species, including conifers, can take up N also from organic N sources (Öhlund & Näsholm, 2001). Consequently, foliar N status of trees from fertilized plot of 1st experiment was from latent deficiency to normal range, while for older trees (2nd experiment) – at the deficiency level.

a 1st experiment (planting 2008)



b 2nd experiment (planting 1989)

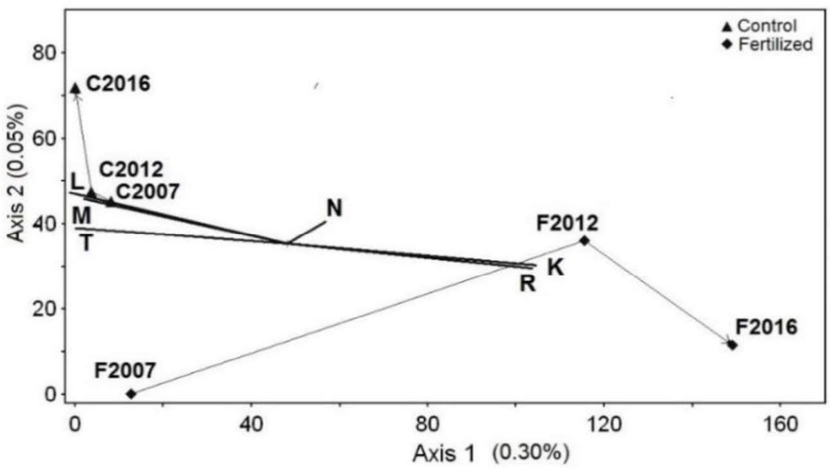


Figure 3. Detrended correspondence analysis of distribution of plant and moss species composition at the Norway spruce experimental plots and gradients of ecological factors. (Vectors: Ellenberg values for detected plant species: L – light; T – temperature; K – continentality; M – humidity; R – reaction; N – nitrogen).

Undeniable, the fertilization results might depend on various factors as stand age, initial nutrient status, kind and dose of fertilizer, tree density, microclimate, etc. Besides a significant aspect is also the fertilization impact on vegetation, changes in soil/peat chemical composition, nutrient cycling, mycorrhiza, etc. Thus further research and additional experiments with various PMS doses and study sites should be carried out in the future. In addition, our study revealed significant deficiency of N, Cu, Mo and B for spruce needles on drained peat soils. Thereby, studies on optimization of fertilization regimes in order to provide tree demands, to increase wood production, and to find a balance between economy and ecological aspects is very important task. Correct application of appropriate fertilizers in adequate doses is critical to avoid soil and groundwater pollution as well as imbalance with other nutrients.

CONCLUSIONS

The results revealed that fertilization with 150 g m^{-2} of PMS resulted in a significantly better nutrient (K, Ca, Zn, and N) status of Norway spruce stands, improved crown vitality and tree growth at both experiments on drained peat soil. This effect lasted all the study period of 2008–2016. Nine years after the fertilization, up to three times higher spruce height, two times larger stem diameter for a new planting, and up to 3.5 times higher annual tree ring increment, 1.4 times – tree height, 1.3 times – stem diameter, and the additional stock volume – 128 m ha^{-1} was found for older spruce stand (2nd experiment). The average count of bacteria and fungi in soil of fertilized plots, accompanied by a remarkable variability in the study years, was significantly higher only for the 1st experiment (new planting). F : B ratio did not exceed 0.044 for both experiments, with significant differences between fertilized and control plots only for the 1st experiment. Significant increase of cover with nitrophilic plant (*Urtica dioica, Antriscus sylvestris, Rubus idaeus*) and moss (*Plagiomnium cuspidatum, P. ellipticum*) species at both fertilized experimental sites indicated increase of biologically active N and activation on nutrient cycling.

ACKNOWLEDGEMENTS. The financial support partly provided by the Forest Development Fund in Latvia for project '*Degradation of Norway spruce stands in Latvia: reasons and measures for possible solutions*' during 2007-2008, as well as the Basic Research Funding, University of Latvia, for project 'Biological diversity – impacts, functions and protection', N° AAP2016/B034, ZD2015/AZ81, during 2016-2017, are gratefully acknowledged. The authors thank two anonymous reviewers for their valuable scientific advises and comments.

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