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Nutrient characteristics and proline accumulation in relation to *Picea abies* status on drained peat soils

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Abstract: Monocultures of Norway spruce widely used for afforestation of drained peat soils often have low productivity and decline in vitality. The research aim was to elucidate: (1) imbalances in the nutrient status of soil and plants in relation to Norway spruce crown status and annual increment on drained peat soil in the sub-boreal zone; (2) the suitability of using proline accumulation as a biomarker for Norway spruce vitality and stress in nutrient imbalance conditions. The study was conducted at five forest (*Oxalidosa turf. mel.*) sites in Latvia, each containing trees with different crown condition status. Chemical analyses of soil and spruce needles, assessment of tree crown vitality and annual increment measurements were done. Our results revealed serious disturbances in the supply of nutrients, which is an important factor in the decline of Norway spruce in monoculture stands on drained peat soils. Deficiency of K, Fe, Cu, B, N and P was found in the current year needles for all trees, but in damaged trees (≥61% defoliation, ≥61% discoloration) Zn deficiency was also found. For 2-year-old needles, deficiency of K, P, S, Zn, and Cu was found in all trees, additionally, deficiency of Fe was found in healthy and medium damaged trees (26–60% defoliation, 21–60% discoloration), and N deficiency was found for medium damaged and damaged trees. Thus, K, Cu, B, Fe concentrations had significant negative correlations with crown status parameters – defoliation, discoloration, and density. K and B deficiency could play the most significant causal role in decreased stem increment. The results revealed that the stress amino-acid proline is a reliable biomarker, having a significant relationship with spruce crown condition parameters, annual increment and nutrient status in needles – low levels of K, Fe, S, Cu, B, N. The increase in proline content in spruce needles was pronounced in moderately damaged trees, indicating the potential for the use of proline accumulation as early stress indicator for spruce. Therefore, further studies on the identification of early stress and factors affecting nutrient uptake and accumulation in needles are particularly valuable for evaluation of stand management options before significant decline of spruce stands.

Key words: Norway spruce, crown status, soil and foliar analysis, proline, sub-boreal zone

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Introduction

In the boreal and temperate zones, peatlands or organic soils have been extensively drained and are widely used for forestry (Nieminen et al., 2018; Ojanen et al., 2019). One of the dominant and economically important tree species broadly used for afforestation of drained peat soils is Norway spruce (*Picea abies* (L.) Karst.) (Zālītis & Lībiete, 2005; Renou-Wilson et al., 2009). In Latvia, which is located in the sub-boreal zone, spruce as a target species is currently cultivated on drained meso-eutrophic and eutrophic peat soils (Hylocomiosa, Myrtillosa turf. mel., Oxalidosa turf. mel., etc.), comprising about 37 thousand hectares or 7% of the total spruce stands (State Forest Service, 2016). Norway spruce is also planted on drained shallow marshes and wetlands, which cover more than 20 thousand hectares (Zālītis & Lībiete, 2005; Lībiete & Zālītis, 2007). As Norway spruce is a fast-growing tree in juvenility, there is an increasing preference of many private forest owners prefer to plant Norway spruce compared to Scots pine (*Pinus sylvestris* L.) (Jansons, 2019).

Monocultures or even-aged stand management has been used as one of the forestry practices in Fennoscandia and other regions for several decades (Kuuluvainen et al., 2012; Lībiete et al., 2019). Unfortunately, spruce monocultures on organic soils outside typical forest lands often have low productivity and decline in vitality. Tree dieback is frequently observed in initially productive 30–40 year old spruce monocultures (Lībiete & Zālītis, 2007; Cekstere et al., 2018; Ruņģis et al., 2019). Moreover, spruce decline has also been observed in several countries in Europe (Cape et al., 1990; Małek et al., 2012; Błońska et al., 2015; Vacek et al., 2019; Vacek et al., 2020). Analysis of Norway spruce productivity (Zālītis & Lībiete, 2005) and dieback in peat soils in Latvia (Klavina et al., 2016) suggests that one of the factors influencing it could be related with ground water level fluctuations and formation of anaerobic conditions, negatively affecting tree root system development. However, other studies have reported that the destruction, low vitality and productivity of spruce stands could be related to soil chemical composition, nutrient status and air pollution effects on trees (Berger et al., 2009; Moilanen et al., 2010; Błońska et al., 2015; Vacek et al., 2019; Vacek et al., 2020). An additional issue is changes in soil chemical composition due to the effect of acidic deposition of airborne pollutants such as nitrogen oxides and sulfur dioxide and its impact on the forest vitality and productivity (Lomský et al., 2013). The complex effect of nutrient leaching due to acid deposition and increased nutrient demands by trees due to high N deposition has been a major discussion point for decades with regard to nutrient status in forest ecosystems in Europe and North America (Nilsen & Abrahamsen, 2003; Berger et al., 2009).

In boreal peatlands, several studies have emphasized K deficiency (Finer, 1989; Tripler et al., 2006; Caisse et al., 2008; Moilanen et al., 2010; Sarkkola et al., 2016), B deficiency (Möttönen et al., 2005; Nieminen et a., 2016), as well as imbalance of other nutrients. Moreover, K deposition has been reduced during the last decades (Ruoho-Airola et al., 2003) and depletion of K could be a significant issue in second rotation forests on drained peatlands (Nieminen et al., 2016). On one hand, sufficient supply with plant essential nutrients not only promotes tree growth, but also tolerance to diseases, insect damage and external stress (Halmschlager & Katzensteiner, 2017), such as frost, drought, heat, etc. Therefore, the importance of K, Cu, Zn, Ca, B, etc. has been emphasized (Mengel & Kirkby, 2001; Saarsalmi & Tamminen, 2005). On the other hand, the physiological mechanism of spruce response and tolerance to nutrient imbalance in the sub-boreal zone under global climate change conditions is not fully understood. When exposed to stressful conditions, plants accumulate large quantities of various types of compatible solutes, including the amino acid proline. The phenomenon of proline biosynthesis in cells as a plant defense mechanism against osmotic challenge and its specific role in cell adaptation to water loss is well documented (Meena et al., 2019). There are several other stress factors that also induce a massive accumulation of proline, e.g., increased salinity, photooxidative stress, heavy metal toxicity, etc. (Hare & Cress, 1997; Cekstere et al., 2015; Seneviratne et al., 2019). Only scant research on the potential role of proline in the conditions of nutrient imbalance is available (Hare & Cress, 1997; Ahmad et al., 2014). Although numerous studies have shown the close correlation between proline accumulation and the impact of environmental stressors (Ashraf & Foolad, 2007; Hayat et al., 2012), the relationship between proline production and stress tolerance in plants is always not conclusive (Seneviratne et al., 2019). As proline accumulation also occurs to a significant extent under mild and moderate stress (Sharma & Verslues, 2010), it has been suggested that the accumulation of proline may also act as a part of the stress signal affecting plant adaptive responses.

Unfortunately, visual symptoms of loss of tree vitality and productivity frequently appear too late to take measures for stress elimination. Therefore, there is an urgent need for biochemical parameters that respond to the early stages of environmental impact prior to the appearance of visual symptoms in the trees – i.e. early stress biomarkers. Only few studies (Kätzel et al., 2005) have reported on the possible use of proline as a stress biomarker for forest trees, especially in the conditions of some nutrient imbalances. There is a lack of information on the relationship between proline and macro- and micro element content essential for plants in spruce needles, as well as with stem annual increment.

The research aim was to elucidate: (1) imbalances in nutrient status of soil and plants in relation with Norway spruce crown status and annual increment on drained peatland in the sub-boreal zone; (2) suitability of the use of proline accumulation as a biomarker for Norway spruce vitality and stress in nutrient imbalance conditions. We hypothesized that (1) monoculture stands of Norway spruce have serious supply disturbances of various essential nutrients on drained peat soil; (2) as foliar stress metabolism can reflect the current health status of spruce, proline content in needles is applicable as a biomarker not only for crown status and annual stem increment but also as an indicator of nutrient imbalance.

Materials and Methods

Study area

The study was conducted at five different localities (Valka, Kalsnava, Birzi, Tireli and Olaine) in the central and eastern part of Latvia, which is situated in the Eastern part of the Baltic Sea region in the sub-boreal climatic zone. The climate in Latvia is moderately warm and humid: the average annual precipitation is 667 mm, the highest amount is in July and August – 78 mm per month, the lowest amount is in February and March – 33 mm per month, the average temperature in January is –4.6 °C, but in July +17 °C (Data of the State Ltd "Latvian Environment, Geology and Meteorology Centre"). Site "Valka" (57°41'N, 26°08'E) is situated on Tālava Lowlands Seda Plain, glaciofluvial sand sediments are dominant, but peat deposits are dominant in relief depressions. Site "Kalsnava" (56°40'N, 25°50'E) is situated on East-Latvian Lowland, Arona Undulating Plain. The quarter sediments consists mainly of sand, gravel, as well as local clay deposits and peat deposits in relief depressions. Site "Birzi" (56°25'N, 25°37'E) is located on East-Latvian Lowland, Aknikstes Tiled Plain. Unconsolidated quarter sediments are formed by moraine loam and sand with peat deposits in relief depressions. Sites "Olaine" (56°49'N, 24°06'E) and "Tireli" (56°50'N, 23°49'E) are located on Central Latvian Lowland, Tireli Plain. The quarter sediments consists of sand and aleirite deposits of the development stages of the Baltic Sea and younger bog sediments (Stivriņš, 2018).

Field work

The research was done at five sites (localities) with on average from 22.3±0.3 to 38.0±3.1 year old spruce stands on drained peat soil in August 2018. All stands were in commercial forests, typically managed in accordance with management practices in Latvia. In each stand, a research site with a size of 50 × 50 m was selected. The forest type for the selected spruce forest stands was Oxalidosa turf. mel. At each study site, all trees were numbered; measurements of total height and calculation of height increment per year for each tree were done using the Vertex IV ultrasound instrument system (Haglöf, Sweden). Measurements of stem diameter at a height of 1.3 m and assessment of tree crown health status was carried out according to the Forest monitoring guidelines (UN/ECE, 2006; Schomaker et al., 2007) to characterize the general physiological status of spruce trees. Visual evaluation was done for the following bioindicators: crown dieback, crown density, crown defoliation and needle discoloration, expressed as a percentage. Tree condition was classified based on, primarily, defoliation, then discoloration, as follows: healthy (0–10% defoliation, 0–5% discoloration), slightly damaged (11–25% defoliation, 6–20% discoloration), medium damaged (26–60% defoliation, 21–60% discoloration), and seriously damaged (61–100% defoliation, 61–100% discoloration). Tree-ring width samples or wood cores were collected from 25 spruce trees per site with a Presler increment drill. For tree-ring width (annual increment) measurements a *LINTAB IV* measuring table and *TSAP Win Scientific 0.55* software were used. The measurements were done at LSFRI "Silava".

10 samples of spruce needles for chemical analysis and 5 to 10 samples for biochemical analysis were separately collected from the current (1^{st}) year shoots and the same amount of samples from 2-year-old shoots from 10 randomly distributed trees with different crown status. Samples were collected at 2 to 3 m height around trees at each site at the beginning of August of 2018 (20 samples per site for chemical analysis and 10–20 – for biochemical analysis). Along with needle sampling, 10 soil samples were taken at each study site from a depth of 0 to 20 cm without organic (O) horizon for chemical analysis. Each soil sample consisted of thoroughly mixed five subsamples (volume of each subsample approximately 0.2 L) collected by a soil probe at the perimeter area of tree crown. The collected soil and needle samples were analysed at the Institute of Biology, University of Latvia.

Laboratory analysis

The soil samples were dried at room temperature and sieved <2 mm. The soil analyses were done using 1 M HCl extraction, where soil-extractant mixture was 1:5. This extractant is universal and quite aggressive, and therefore 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, the soil extract was oxidized with HNO_3, H_2O_2 and $HClO_4$, the obtained salts were dissolved in HCl and diluted with distilled water (Rinkis et al., 1987).

The levels of Ca, Mg, Fe, Cu, Zn and Mn in soil 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, 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, $N_{min.}$ (NH_4+NO_3) and $N_{tot.}$ (mineral + organic) by a modified Kjeldal method using Nesler's reagent in an alkaline medium, S by the turbidimetric method by adding $BaCl_2$, using a spectrophotometer JENWAY 6300 (Barloworld Scientific Ltd., UK). K was detected with the flame photometer *JENWAY PFPJ* (Jenway Ltd., UK). Soil pH was measured in 1 M KCl extraction (soil-extractant mixture 1:2.5) with the pH-meter *Sartorius PB-20* (Sartorius AG, Germany), EC – in distilled water extraction (soil-water mixture 1:5) with the conductometer *Hanna EC 215* (Hanna instruments, USA). The content of organic matter in soils was detected according to Tjurin (Rinkis et al., 1987; Carter & Gregorich, 2008). The obtained results of chemical elements in soil were expressed in mg L^{-1}, because the growth of plant roots take place in a certain volume and in peat soil this volume differs significantly from the weight of this volume. The average volume weight (g cm^{-3}) of peat soil in Kalsnava, Valka, Birzi, Tireli and Olaine was: 0.47±0.01, 0.62±0.02, 0.55±0.01, 0.58±0.03, and 0.46±0.03, respectively.

For chemical analysis, needle samples were 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 a modified Kjeldal method. P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, and B were analysed similarly to the procedures described for analysis of soil extracts.

The biochemical analyses included the determination of proline content according to Bates et al. (1973) using the ninhydrin method. Generally, 1 g of fresh plant material was homogenized in 3% sulfo-salicylic acid. Acid ninhydrin solution and glacial acetic acid (2 ml each) were added to the extract followed by heating at 100 °C for 1 h. After extraction with toluene, free proline was quantified spectrophotometrically at 528 nm. As a standard, L-proline was used.

Statistical analysis

Standard errors (SE) were calculated in order to reflect the mean results for each study site and average tree health status (healthy, medium damaged, and damaged). Differences between site averages of nutrient and proline concentrations, soil pH, and EC in the collected samples, bioindication parameters for tree crown status, and annual increment for the last year (2018, i1), 5 years (2014–2018, i5), and 10 years (2009–2018, i10) were tested by one-way ANOVA followed by Tukey's (HSD) post-hoc test, using the *Statistica 7.0* software (Statsoft Inc., Tulsa Ok). The relationships between needle age, tree status and nutrient and proline accumulation in needles, as well as proline and nutrient content relationship with tree crown parameters (defoliation, discoloration, density) were tested by general linear mixed models (GLMM) using *SPSS 14.0*. The individual tree formed the statistical unit whilst the site effect was treated as a random factor. Additionally, Pearson's correlation coefficients between nutrient and proline concentrations, crown status parameters and annual increment were determined. Based on the results, separate significant models, including the marginal R2, were calculated using the *Statistica 7.0* software. Soil characteristics for trees and multivariate responses of tree crown status parameters to nutrient and proline content in needles was assessed by principal component analysis (PCA), using *PC-ORD Version 6* (McCune & Grace, 2002).

Results

Peat soil

Generally, a wide variance of element concentrations in peat soil was found (Table 1, Fig. 1). The highest variability of concentration variance (v) was found for Fe, Ca, and P, the lowest for Cu and B. There were no significant ($p<0.05$) differences in the soil chemical composition between healthy, medium damaged and damaged spruce trees due to site or location particularities. The highest plant available concentrations of $N_{tot.}$, $N_{min.}$, P, K, S, Fe, Zn, and B were found at Valka, Ca and Mn at Birzi, Mg and Cu at Tireli. For most elements the lowest plant available content was found at Olaine (N_{tot}., N_{min}., P, K, S, Fe, Mn, and Cu); Ca and Mg – at Kalsnava, Zn and B – at Tireli. pH ranged from 4.01+0.07 at Kalsnava to 4.60+0.07 at Tireli, closely correlating with Ca and Mg in soil ($p<0.05$). Element ratio analysis revealed only a significant lower Mg:K ratio for medium damaged trees compared to trees with damaged crown status (healthy: 4.31±0.47; medium damaged: 3.87±0.43; damaged: 5.15±0.57). There were no

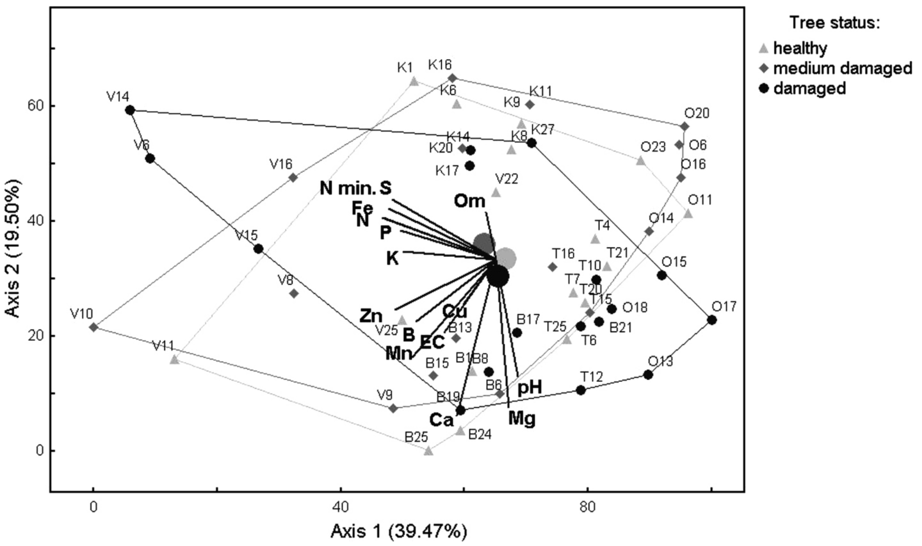

Fig. 1. Distribution of the analysed spruce trees on drained peat soil within the axes of principal component analysis of soil chemical dataset (n=50)

B – Birzi, K – Kalsnava, O – Olaine, T – Tireli, V – Valka, Om – organic matter.

Table 1. Chemical composition of drained peat soil at the Norway spruce study sites in Latvia (n=50]

Parameter	Mean	SE	Min.	Max.	Variance	Sufficiency range*
Organic matter [%]	72.18	2.3	14.08	88.95	259.03	–
pH/KCl	4.21	0.05	3.44	4.95	0.14	4.0–5.2
EC mS/cm	0.59	0.03	0.32	1.1	0.04	1.00–2.00
$N_{tot.}$ [mg L^{-1}]	292.88	20.9	76	685	21831.5	–
$N_{min.}$ [mg L^{-1}]	73.36	6.74	13	208	2271.26	60–140
P [mg L^{-1}]	318.08	79.44	68	2834	315496	100–200
K [mg L^{-1}]	82.7	2.96	43	134	438.79	60–150
Ca [mg L^{-1}]	5221	272.35	2100	10100	3708683	1000–2000
Mg [mg L^{-1}]	344.5	17.69	120	650	15638	200–450
S [mg L^{-1}]	37.74	2.84	6.9	95	402.76	20–60
Fe [mg L^{-1}]	8841	1127.7	750	33000	6.4E+07	600–2000
Mn [mg L^{-1}]	133.19	20.09	3.7	465	20179.9	15–30
Zn [mg L^{-1}]	8.76	1.39	1.15	49	96.02	5–15
Cu [mg L^{-1}]	1.34	0.04	0.6	2.15	0.09	2–5
B [mg L^{-1}]	0.4	0.05	0.1	1.4	0.14	0.4–1.2

*according to Nollendorfs V. (unpublished]

significant differences between various health status spruce trees for Ca:Mg and Fe:Mn ratio.

In general, $N_{min.}$, K, Mg, S, Zn, and B content in soil, as well as soil pH and EC could be characterized as sufficient for spruce; P, Ca, Fe, and Mn as increased, and only Cu in deficient levels (Table 1). However, several sites were characterized by nutrient deficiency or low levels: K at Tireli and Olaine (71.80±2.61 mg L^{-1}), S – at Tireli, Olaine (22.40±2.24 mg L^{-1}), Mg – at Kalsnava (182.50±11.24 mg L^{-1}), Zn – at Olaine, Tireli, Kalsnava (5.98±0.20 mg L^{-1}), and Cu – at all sites. Thus, the best supply of most nutrients in soil was found at Valka and Kalsnava, which was demonstrated also by the PCA results (Fig. 1). Indeed, the PCA results confirmed grouping of the individual sampling points or trees in the ordination space according to the study site particularities. The 1st and 2nd axis explained 58.97% of the total variance ($p=0.001$). The highest correlations (tau values) for the 1st axis were found for N, S, Fe, Zn (−0.65 to −0.73), for the 2nd axis the highest values were found for Ca, Mg (−0.62 and −0.72).

Spruce needles

There were significant differences ($p<0.05$) in all nutrient contents, except N, S, between different age needles (Table 2): significant higher concentration of mean P, K, Zn, and Cu, but lower Ca, Mg, Fe, Mn, and B content was found in the needles of current year (1st) than in 2-year-old needles. Several differences ($p<0.05$) were found for the nutrient accumulation in the healthy, medium damaged and damaged spruce needles (Tables 3, 4). The most marked difference was found for K – up to two times lower concentration in damaged spruce needles compared with healthy ones, both in the current and 2-year-old needles. Significantly lower concentrations in medium damaged and damaged spruce needles compared to healthy trees were also found for Ca, Zn, B in the current year, and N in the 2-year-old needles. On the contrary, higher mean level of Fe in both year needles and Cu in the current year needles was characteristic of damaged spruce. GLMM results also confirmed significant higher Fe and Cu, as well as lower K and Ca content in needles of damaged trees compared with healthy ones (Table 2). No differences were found for N, P, Mg, S, and Mn in the 1st year and P, Ca, Mg, S, Mn, Zn, and Cu in 2-year-old needles for Norway spruce trees with different health status (Table 5).

Overall, deficiency of K, Fe, Cu, B, N and P in the current year needles was found for all trees, but for damaged trees – Zn as well. For 2-year-old needles, deficiency of K, P, S, Zn, and Cu was found for all trees, additionally, deficiency of Fe – for healthy and medium damaged, but N – for medium damaged and damaged trees. Generally, Mn content was high, it significantly exceeded Fe content by 9–15 times. However, it should be noted that a wide range of nutrient contents was measured in needles both at site level and in trees of different status, especially for Mn.

Analyzing the accumulation of elements in the soil-plant system, several significant ($p<0.05$) negative correlations were found between soil parameters and nutrient content in the current year needles: pH and Mn, pH-Zn, $N_{tot., min.}$-Ca, Mg; P-Ca, Mg; Ca-Fe; Fe-Ca, Mg; Fe-Fe, Mn-Fe ($-0.35<r<-0.69$), while positive relationships were found between K content in soil and needles, as well as Mn in soil and needles ($r=0.33$ and 0.45, respectively, $p<0.05$).

Table 2. Main effects of needle age and tree status on plant nutrients in Norway spruce needles on drained peat soils in Latvia. Within GLM models, the site was treated as a random factor. The significant models ($p\leq0.05$) are indicated in bold ($n=100$)

Source	Numerator df	Dependent variable			
		Macronutrient		Micronutrient	
		F	Sig.	F	Sig.
		N		Fe	
Intercept	1	6963.536	0.000	953.100	0.000
Needle age	1	2.213	0.140	10.566	**0.002**
Tree status	2	1.303	0.277	3.461	**0.035**
Needle age * Tree status	2	1.334	0.268	0.294	0.746
		P		Mn	
Intercept	1	1594.637	0.000	275.764	0.000
Needle age	1	14.964	**0.000**	16.447	**0.000**
Tree status	2	0.019	0.981	0.170	0.844
Needle age * Tree status	2	0.601	0.550	0.114	0.892
		K		Zn	
Intercept	1	902.487	0.000	1505.288	0.000
Needle age	1	32.100	**0.000**	17.598	**0.000**
Tree status	2	25.461	**0.000**	1.884	0.158
Needle age * Tree status	2	1.161	0.318	0.207	0.814
		Ca		Cu	
Intercept	1	617.860	0.000	2844.058	0.000
Needle age	1	75.418	**0.000**	12.748	**0.001**
Tree status	2	2.465	0.090	6.481	**0.002**
Needle age * Tree status	2	0.193	0.825	1.254	0.290
		Mg		B	
Intercept	1	2596.324	0.000	643.267	0.000
Needle age	1	11.427	**0.001**	18.955	**0.000**
Tree status	2	0.306	0.737	0.322	0.725
Needle age * Tree status	2	0.791	0.456	0.660	0.519
		S			
Intercept	1	1796.374	0.000		
Needle age	1	2.554	0.113		
Tree status	2	1.175	0.313		
Needle age * Tree status	2	0.843	0.434		

Table 3. Nutrient concentration in the current (1st) year needles of Norway spruce with different health status on drained peat soil. (Means annotated with different letters (a, b, c) were significantly different (Tukey's post–hoc test, $p<0.05$) between tree health status by one–way ANOVA, $p<0.05$)

Tree health status		K [%]	Ca [%]	Fe [mg kg^{-1}]	Zn [mg kg^{-1}]	Cu [mg kg^{-1}]	B [mg kg^{-1}]
Healthy (n=17)	Mean	0.28 c	0.38 b	39.50 ab	20.33 b	2.86 a	17.28 b
	SE	0.02	0.03	2.69	1.15	0.13	1.39
	Min	0.21	0.17	22.00	13.60	2.00	8.00
	Max	0.47	0.59	58.00	32.00	4.00	30.00
Medium damaged (n=16)	Mean	0.19 b	0.31 a	37.93 a	19.64 ab	2.92 a	15.87 ab
	SE	0.01	0.02	2.21	1.21	0.17	1.65
	Min	0.13	0.13	22.00	12.50	2.00	7.00
	Max	0.30	0.45	50.00	27.50	4.40	26.00
Damaged (n=17)	Mean	0.14 a	0.30 a	44.59 b	17.75 a	3.42 b	13.47 a
	SE	0.01	0.03	3.15	0.88	0.15	1.00
	Min	0.09	0.18	28.00	11.60	2.00	8.00
	Max	0.19	0.59	68.00	27.50	4.60	24.00
Sufficiency range*		0.4–1.6	0.2–0.8	60–300	20–150	4–15	20–100

*According to compilation by Nollendorfs V. (unpublished), Bergmann (1988), Mellert & Göttlein, 2012.

Tree crown status and stand parameters

The average defoliation rate of spruce crowns at the selected study sites was 25.3±1.6%, discoloration – 40.9±2.7%, crown density – 57.3±1.4%, and dieback – 14.0±0.6%. Although all study sites had a similar number of trees with healthy, medium damaged and damaged crown status, on average the worst crown status was found at Olaine, where two parameters significantly ($p<0.05$) differed from results of other sites: 32.8±5.0% defoliation and 56.5±6.2% discoloration.

Significant negative correlations ($-0.38<r<-0.66$, $p<0.05$) were found between spruce defoliation, discoloration, dieback and K content in the 1st and 2-year-old needles. In turn, crown density and tree height had positive correlations with K content in both year needles ($0.30<r<0.45$). In addition, tree height had a positive significant correlation with Ca content in the 1st year needles, with S and Zn in 2-year-old year needles ($0.31<r<0.39$), but negatively correlated with Mn content in both year needles ($r_{1year}=-0.31$, $r_{2years}=-0.38$). B content in the current year needles had a significant positive relationship with crown density ($r=0.34$), but a negative correlation with defoliation ($r=-0.39$). The relationship of tree defoliation rate with K, Cu, and B, discoloration intensity with K, Fe, and Cu, as well as crown density with K and B content in needles was confirmed by the GLMM results (Table 6).

Comparing the average annual growth of spruce in different periods during the last ten years, significantly smaller annual increments were found for the coastal lowland stands (Tireli, Olaine) compared to

Table 4. Nutrient concentration in 2-year-old needles of Norway spruce with different health status on drained peat soil. (Means annotated with different letters (a, b) were significantly different (Tukey's post-hoc test, $p<0.05$) between tree health status by one-way ANOVA, $p<0.05$)

Tree health status		N [%]	K [%]	Fe [mg kg^{-1}]
Healthy (n=18)	Mean	1.19 b	0.19 b	46.06 a
	SE	0.03	0.01	3.32
	Min	0.99	0.15	23.00
	Max	1.45	0.33	68.00
Medium damaged (n=15)	Mean	1.08 a	0.12 a	47.93 a
	SE	0.04	0.01	4.30
	Min	0.83	0.09	25.00
	Max	1.48	0.19	90.00
Damaged (n=17)	Mean	1.12 ab	0.10 a	57.00 b
	SE	0.04	0.01	5.16
	Min	0.88	0.08	25.00
	Max	1.48	0.19	106.00
Sufficiency range*		1.2–2.5	0.4–1.6	60–300

*According to compilation by Nollendorfs V. (unpublished), Bergmann (1988), Mellert & Göttlein, 2012.

Table 6. Main effects of nutrient and proline content in Norway spruce needles on crown status parameters on drained peat soil. (Within GLM models, the site was treated as a random factor, for testing nutrients n=100, for proline - n=72. In the table, only significant models ($p<0.05$) have been included)

Dependent variable	Source	F	Sig.
Discoloration	Proline	15.073	0.000
Discoloration	K	39.607	0.000
Discoloration	Fe	4.331	0.040
Discoloration	Cu	5.599	0.020
Defoliation	Proline	17.248	0.000
Defoliation	K	20.064	0.000
Defoliation	Cu	4.278	0.041
Defoliation	B	4.353	0.040
Density	Proline	9.444	0.003
Density	K	12.524	0.001
Density	B	6.743	0.011

Table 5. Nutrient concentrations in the current (1st) and 2-year-old needles of the Norway spruce on drained peat soil (n=50)

Nutrient	Mean	SE	Min	Max	Variance	Sufficiency range*
			1st year needles			
N [%]	1.17	0.02	1.00	1.40	0.0118	1.2–2.5
P [%]	0.13	0.004	0.07	0.20	0.001	0.15–0.50
Mg [%]	0.11	0.003	0.07	0.14	0.0003	0.1–0.4
S [%]	0.05	0.002	0.04	0.08	0.0001	0.1–0.4
Mn [mg kg^{-1}]	375.28	32.49	80	1100	52790.16	30–250
			2-year-old needles			
P [%]	0.11	0.004	0.06	0.17	0.0007	0.15–0.50
Ca [%]	0.69	0.04	0.31	1.43	0.0717	0.2–0.8
Mg [%]	0.12	0.004	0.08	0.18	0.0006	0.1–0.4
S [%]	0.06	0.002	0.01	0.09	0.0002	0.1–0.4
Mn [mg kg^{-1}]	618.84	49.06	128	1480	120322.2	30–250
Zn [mg kg^{-1}]	15.49	0.63	9	30	19.97	20–150
Cu [mg kg^{-1}]	2.68	0.07	1.75	3.60	0.2242	4–15
B [mg kg^{-1}]	22.06	1.23	8.0	50.0	75.16	20–100

*According to compilation by Nollendorfs V. (unpublished), Bergmann (1988), Mellert & Göttlein, 2012.

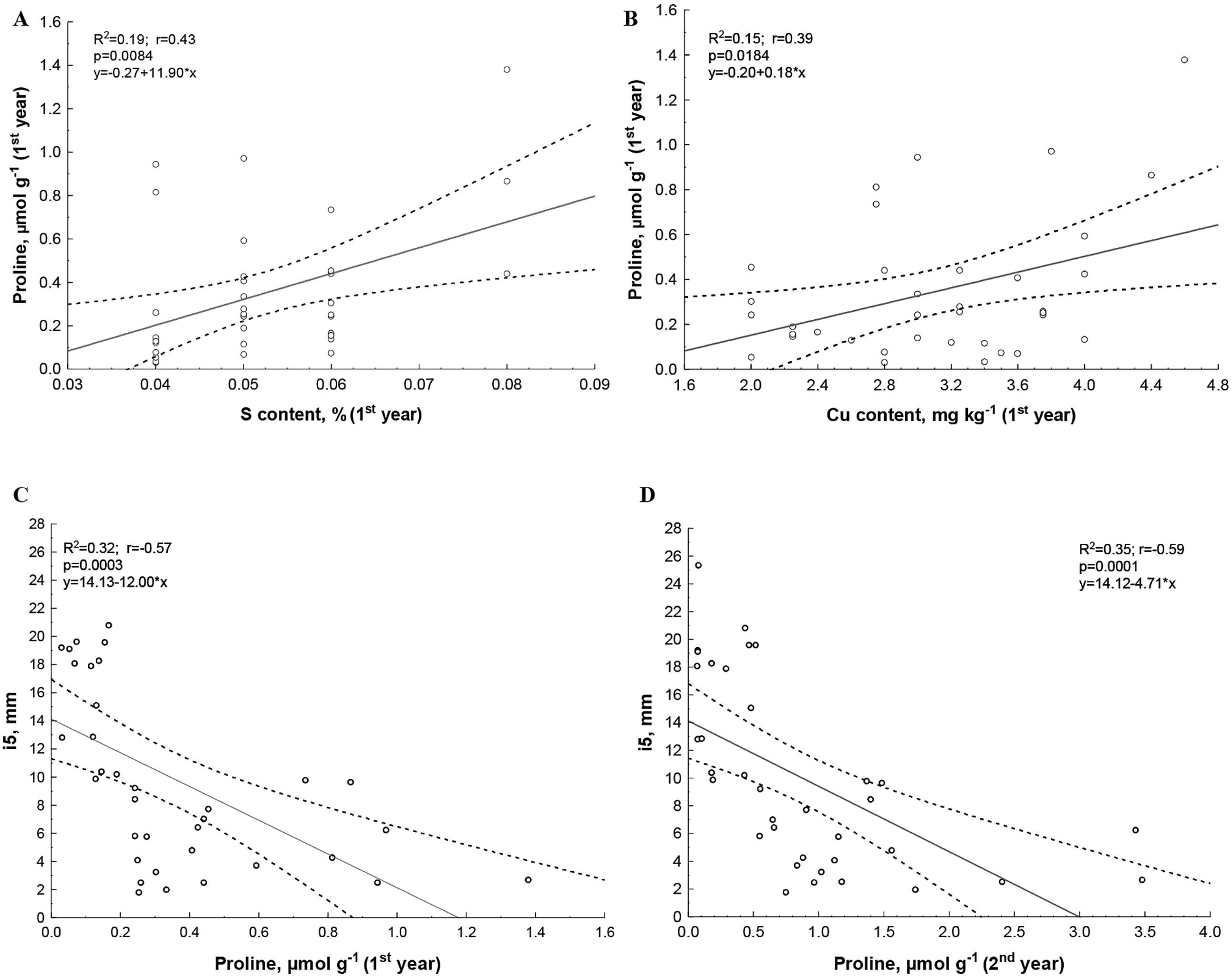

Fig. 2. Changes in proline content, as a function of nutrient accumulation in Norway spruce needles on drained peat soil (A, B), and changes in annual increment, as a function of proline accumulation within different age Norway spruce needles (C, D). Each data point represents one tree (n=36). Solid and dashed lines indicate significant ($p \leq 0.05$) linear regressions and 95% confidence intervals

the inland stands (Valka, Kalsnava, Birzi), with the most significant differences for i5: 147.55±17.95 mm < 242.37±24.47 mm, $p<0.05$. A significant ($p<0.05$) negative relationship was found between i1; i5 and i10 and needle discoloration, defoliation, dieback rate ($-0.49<r<-0.67$), the closest correlation was with i5, while the weakest was with i1. There were significant positive correlations between i1, i5, i10 and K content in both year needles ($0.47<r<0.70$), as well as between i5 and i10 and B content in the 1st year needles ($r_{i5}=0.29$, $r_{i10}=0.29$).

Proline

Overall, the analysis of proline content in spruce needles revealed significant close correlation with the nutrient content in the needles, as well as tree status and annual increment. GLMM results showed a significant higher proline content in needles of damaged trees compared with healthy ones (F=7.464, p=0.008), a significant relationship between proline content and N, K, Fe and B content in spruce needles (Table 7), while regression and correlation analysis revealed a significant relationship with content of S and Cu in the current year needles (Fig. 2). Moreover, increase of proline content in both year needles had significant positive relationships with crown discoloration, defoliation and density intensity, but a negative correlation with tree annual increment, the strongest correlation being with i5 (Fig. 2, Table 6).

To determine the most important parameters characterizing Norway spruce status, content of several essential nutrients and proline in the current and 2-year-old needles, tree crown status parameters and i5 were selected for PCA analysis. Results revealed a relatively good structure of the individual study sites in the ordination space and grouping according to the crown status (Fig. 3). For the PCA using chemical results of the current year needles, the 1st and 2nd axis explained 52.76% of the total variance (Fig. 3A).

Table 7. Main effects of needle age and nutrient concentration on proline content in Norway spruce needles on drained peat soil (within GLM models, the site was treated as a random factor. The significant models ($p \leq 0.05$) are indicated in bold (n=72))

Source	F	Sig.
Needle age	2.750	0.103
N	7.176	**0.010**
P	1.079	0.303
K	6.208	**0.016**
Ca	1.584	0.213
Mg	0.246	0.622
S	0.004	0.949
Fe	6.447	**0.014**
Mn	1.577	0.214
Zn	0.117	0.734
Cu	0.000	0.998
B	3.912	**0.050**

The highest negative correlations (tau values) for the 1st axis were found for the crown status parameters as discoloration, defoliation, dieback and proline (−0.76, −0.75, −0.63, −0.68), but positive tau value for K, i5 and crown density (0.57, 0.67, 0.62), whereas for the 2nd axis the highest negative tau values were found for Mn, N and Cu (−0,55, −0.49, −0.48). For the PCA using chemical results of 2-year-old needles, the 1st and 2nd axis explained 50.99% of the total variance (Fig. 3B). The highest negative tau values for the 1st axis were found for defoliation, discoloration, dieback, and proline (−0.78, −0.77, −0.66, −0.65), but positive tau values were found for K, i5 and crown density (0.61, 0.69, 0.63), whereas for the 2nd axis the highest negative tau values were found for S, Zn and tree height (−0.63, −0.63, −0.49). Both PCA results revealed that of all nutrients, K had the most

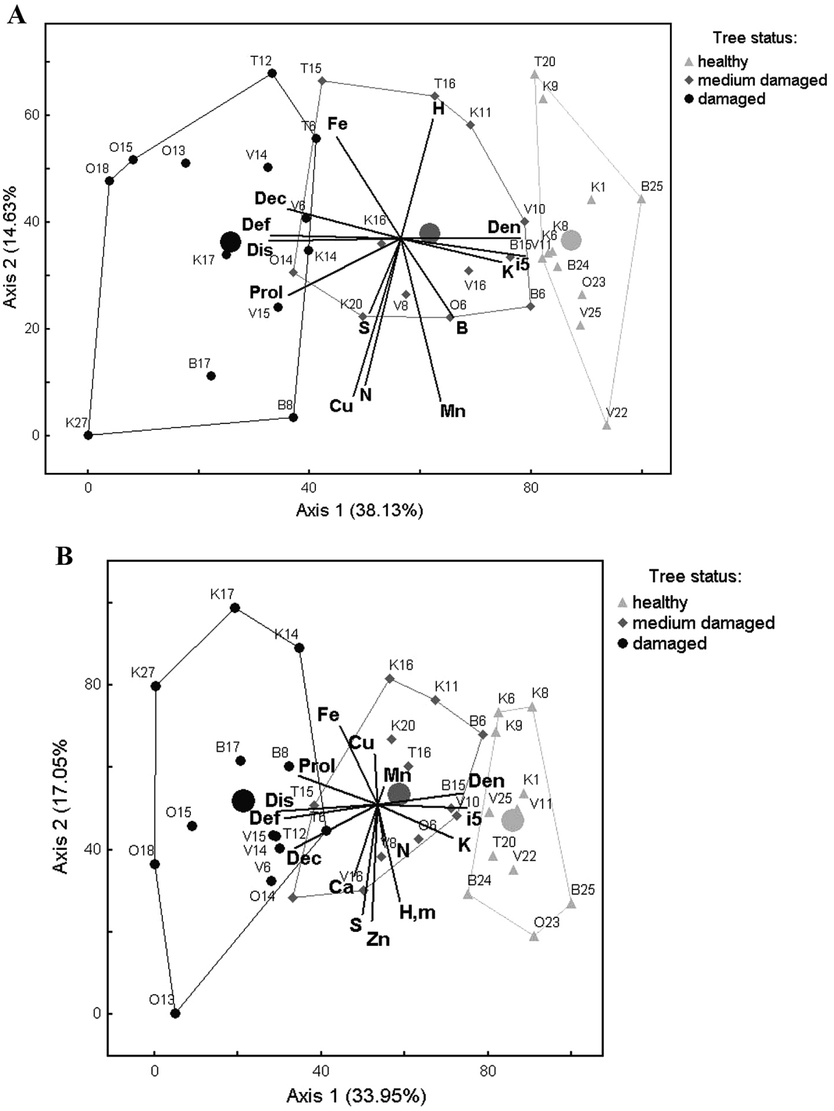

Fig. 3. Distribution of spruce trees within the axes of principal component analysis (PCA) of dataset of crown status and chemical results for current (A) and 2-year-old (B) needles (n=36)

B – Birzi, K – Kalsnava, O – Olaine, T – Tireli, V – Valka, Prol – proline, Dis – discoloration, Def – defoliation, Dec – decay, Den – density, H – height [m], i5 – annual increment 2014–2018.

important positive impact on the annual increment and tree crown status, but proline was a significant biomarker having an important relationship with spruce crown status parameters.

Discussion

Studies on the relationship of genetic diversity with growth potential of even-aged Norway spruce stands in Latvia have revealed low genetic differentiation between stands and groups with various growth potentials. This suggested that growth potential was more subject to the impact of environmental factors and management regime than genetic factors (Ruņģis et al., 2019). Indeed, soil nutrient content, their availability and uptake might play an important role in ensuring and regulating plant growth, development and biomass accumulation. Relevant factors for maintenance of favorable tree growth condition are adequate soil reaction and balanced nutrient status. Whereas no significant deviation from the optimum soil pH, provided by sufficient or high concentrations of Ca and Mg, for Norway spruce was found, the main reason for spruce decline in peat soils was likely associated with nutrient imbalances, particularly with deficiency of K, B, S, Cu, as well as low levels of N and Zn in spruce needles.

Various studies have revealed that the low availability of K and B, as well as P and N are the main limiting factors for tree growth on drained peatlands. The importance of K is especially notable because K bound in stand biomass can exceed the amount of K in the rooting zone in thick peat soils (Finér, 1989; Westman & Laiho, 2003; Sarkkola et al., 2016). Our results did not reveal K deficiency in the top-soil (sampling depth 0–20 cm) of spruce stands in Latvia using 1 M HCl extract. However, they showed K deficiency in spruce needles, significantly correlating with K content in soil. According to Laiho et al. (1999), in forests growing on thick peat soils, K has a vertical distribution – strong enrichment in the top 10 cm, sharply decreasing to very low content already at a depth of 30 cm. In the present study, insufficient foliar K concentration was found for almost all spruce trees, while severe K deficiency, even to the extent of visible signs of deficiency was recorded in medium and damaged trees, especially in 2-year-old needles. A significant close relationship with tree crown parameters and annual increment indicates that K deficiency is one of the main reasons for growth disturbances in Norway spruce stands on drained peat soil in Latvia, which is located in the sub-boreal zone. Unfortunately, K deposition has been reducing during the last decades. Data by Sarkkola et al. (unpublished) have showed that on average 2 kg of K ha^{-1} annually was lost by leaching from minerotrophic, Norway spruce-dominated peatland forests (Nieminen et al., 2016) in the boreal zone. Given that K deposition might not add as much K to drained peatlands as is lost by annual leaching, and as trees grow and their demand for K increases, the problem of K deficiency is expected to increase in the future, which will increasingly negatively affect the status and productivity of spruce stands. Such a trend would be comparable with results from studies on K content in spruce needles in the Czech Republic during 1980–2015 (Vacek et al., 2020).

In forest trees, B deficiency impairs the primary cell wall, affects apical meristems, and interrupts the structural development of organs and whole plants, resulting in dieback of leading shoots, adverse impacts on tree formation, wood quality, cold and drought tolerance (Lehto et al., 2010; Wang et al., 2015). Since B is taken up through mass flow, the availability of B is largely determined by water status (Thelin, 2000). Although the annual water balance is positive in Latvia, generally, the precipitation distribution has been becoming more heterogeneous in summer (Avotniece et al., 2010). Therefore water status, as well as increased Ca content in soil and formation of insoluble B compounds, could cause B deficiency in spruce needles.

One of the main preconditions for high wood productivity is sufficient supply of N. Improved N nutrition due to increased atmospheric N deposition in recent decades could be one of the most important factor for growth acceleration of Norway spruce in central Europe (Mellert et al., 2008). However, the situation for spruce in Scandinavia may be different. In Northern Europe, spruce stands are usually adequately provided with N and the trends in foliar N content are mainly negative (Mellert et al., 2004). Although our results showed sufficient or even an increased amount of N in soil, the content of N in needles could be characterized as low or decreased, especially in 2-year-old needles of medium damaged and damaged trees. In peat soils, N is mainly in organic form, which is not directly available for plant uptake (Moilanen et al., 2010), and this can significantly limit tree growth. The rate of N mineralization is dependent on peat temperature, moisture, and microbial activity, leading to severe deficiency, especially during cold growing seasons (Pietiläinen & Kaunisto, 2003). Therefore, our findings are in good agreement with results by Vacek et al. (2020) on N content in spruce needles growing on peat soil.

Since the 1990s, atmospheric sulfur dioxide emissions have fallen dramatically worldwide, and as a result, S input to forest ecosystems has also decreased significantly (Jonard et al., 2012). Under these conditions, foliar S status is highly dependent on plant available S in soil. Similarly to N, there was no S deficiency found in peat soils from the study sites in

Latvia, except at Olaine. However, the timing and rate of mineralization of organic matter as an S reservoir are likely inconsistent with periods of active nutrient accumulation and spruce growth, resulting in S deficiency in needles for spruce trees of all health statuses. Several studies have reported low S content in needles of conifers as a symptom of reduced atmospheric S deposition in Europe (Pietrzykowski et al., 2013; Jonard et al., 2015; Talkner et al., 2019). Therefore, forestry practices aimed at nutrient sustainability must increasingly take into account sulfur deficiency also in the sub-boreal zone.

Additionally, this study identified deficiency of Cu and high levels of Mn as a common problem for peat soils in almost all study sites. Although soil acidity stimulates micronutrient uptake (Mengel & Kirkby, 2001), low levels of Cu in peat soil caused significant deficiency of Cu in needles regardless of spruce vitality. In addition to impaired apical dominance, Cu deficiency may also cause permanently bent and twisted stems and branches of conifers, thus significantly affecting the quality of the stand (Thelin, 2000; South et al., 2004). Despite the fact that Cu is generally closely bound to soil organic matter, thus limiting the reliability of soil diagnostics, the soil testing method used in the present study (1M HCl extraction) provides a good indication of low Cu availability also to the trees. Foliar Mn concentrations found in the study largely exceeded the optimal level for spruce (up to 500 mg kg^{-1}, Bergmann, 1988), negatively affecting Fe:Mn ratio in needles and probably decreasing Fe uptake. In conditions of high Mn supply and availability in acid soils, Mn could be taken up by spruce with no limitation in concentration (Kazda & Zvacek, 1989). Although the concentration of Zn in peat soils was generally optimal and there were no significant differences between healthy and damaged spruce trees, Zn deficiency in the current year needles was found only for damaged trees. Zn deficiency impairs various plant metabolic functions; especially enzyme system functions, seed production, auxin synthesis etc. and could result in stunted branches and needles, as well as significant foliar loss.

Comparing the average annual increment widths at the study sites at different time intervals over ten years (2009–2018), differences were identified between the growth of spruce stands in the coastal lowland (Tireli, Olaine) and inland (Valka, Kalsnava, Birzi): significantly shorter annuals and smaller increments were found for the coastal lowland stands compared with the inland stands. This is highly consistent with our soil chemical and PCA results indicating a better supply of most nutrients at Valka and Kalsnava, while generally lower of P, K, S, Zn and B content was found in Olaine and Tireli. Therefore, imbalanced mineral nutrition conditions predisposed trees to vitality losses (discoloration, defoliation, stunted growth) and reduced annual increment which was frequently observed for middle-aged Norway spruce stands on drained peat soil. This is consistent with results of studies in Finland (boreal zone), that the geographical variation in the nutrient status of trees on drained mires could be caused by climatic and weather conditions, as well as by the site's geological history (Moilanen et al., 2010).

Although crown and foliage condition in terms of crown dieback, density, defoliation and needle discoloration as well as foliage chemical composition are well known indicators of tree stand vitality, a number of other tree condition variables also being examined. Preventive measures to reduce the effects of environmental stress require special markers that can assess the current level of stress in almost healthy trees. Different biochemical compounds, as arginine, malondialdehyde, phenolic compounds, superoxide dismutase, photosynthetic pigments, proline, etc. can be considered as parameters of physiological tree stress, including nutrient imbalances. Proline accumulation in plants is known to occur under various stresses such as drought, salinity, heavy metal exposure, etc. (Hayat et al., 2012; Cekstere et al., 2015; Jamnická et al., 2019). However, there is currently little knowledge about the relationship between the foliar level of proline and the vitality and nutrient status of Norway spruce. According to Song et al. (2016), nutrient deficiency could inhibit proline synthesis and disturb metabolism in tree leaves. Whereas Simmleit et al. (1991) reported that proline accumulation in needles could be used as a stress indicator to describe and monitor the vitality of spruce trees. The results of our study revealed that proline is a reliable biomarker having a significant relationship with spruce crown condition parameters (crown discoloration and defoliation intensity), annual increment, as well as status of several nutrients in needles – deficiency or low level of K, Fe, S, Cu, B and N. An increase in proline content in spruce needles was already pronounced in moderately damaged trees. Some evidence of the impact of soil nutrient status on proline content in needles was found in studies on wood ash fertilization in a Scots pine stand growing on nutrient poor soil in Lithuania. The foliar concentration of proline decreased with addition of wood ash as a source of such nutrients as K, P, Ca and Mg (Ozolinèius et al., 2007), thus indirectly indicating the potential role of proline as an indicator of nutrient deficiency.

As a non-specific marker, proline could be particularly promising in field studies, where trees are often exposed to several stressors. Future studies should focus on proline as a potential early stress indicator for spruce in the sub-boreal zone. There is some evidence on detectable changes in metabolism, including proline accumulation, in response to environmental stress before visual symptoms appear in pine and oak (Minocha et al., 2015). While these data are undeniably not a direct predictor of tree stand productivity and sustainability, they could be useful for both early detection of stress and long-term monitoring of forest tree health, especially in the context of nutrient imbalances, which in most cases are considered as stress conditions that develop slowly/gradually.

Conclusions

Serious disturbances in supply of various essential nutrients was identified as an important reason for the decline of Norway spruce in monoculture stands on drained peat soils in the sub-boreal zone. Generally, a deficiency of K, Fe, Cu, B, N and P in the current year needles was found for all trees, but for damaged trees – Zn as well. For 2-year-old needles, deficiency of K, P, S, Zn, and Cu was found for all trees, additionally, deficiency of Fe – for healthy and medium damaged, but N – for medium damaged and damaged trees. Thus, K, Cu, B, and Fe had significant negative correlations with crown status parameters – defoliation, discoloration, and density. This research showed that from the analyzed complex of essential nutrients, the deficiency of K and B could play the most significant causal role in decreased annual stem increment. The results revealed that proline is a reliable biomarker having a significant relationship ($p<0.05$) with spruce crown condition parameters (discoloration and defoliation intensity), annual increment, as well as the status of several nutrients in needles – deficiency or low level of K, Fe, S, Cu, B, and N. The increase in proline content in spruce needles of the current and 2-year-old needles was pronounced in moderately damaged trees, and indicated the potential of proline as an early stress indicator for spruce. Therefore, further studies on the identification of early stress indicators and factors affecting nutrient uptake/ accumulation in needles is particularly valuable for the implementation of stand management options before the significant decline of spruce stands on drained peat soils in Latvia.

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