EDITORIAL



Editorial to the thematic issue: Ecological interactions in Central European forest under climate change

Dear readers,

Recent decades were characterized by dramatic changes in the environment, which challenged management of natural resources, including forestry. Many of these changes were driven by increasing air temperature, changing precipitation patterns, and increasing frequency of climate extremes. These dynamics affected forest ecosystems across Europe, triggering large pulses of tree mortality and species redistribution, and changing long-term disturbance regimes in many regions. Central Europe is a specific region where processes that are characteristic for low and high latitudes of Europe interact and generate unique ecosystem dynamics. Understanding these processes is central for informing forest management under climate change that needs to accommodate new concepts and address the emergent ecosystem dynamics.

Central European Forestry Journal is published jointly by National Forest Centre – Forest Research Institute Zvolen and Czech University of Life Sciences in Prague – Faculty of Forestry and Wood Sciences. We are pleased to bring to your attention the Special Issue entitled *Ecological Interactions in Central European forest under climate change* aiming at various ecological interplays in forest ecosystems facing the ongoing changes in climate. This issue presents a collection of six papers endeavouring to fill some gaps in our current understanding of the dynamics of Central European forests.

The article *Carbon sequestration in living biomass of Slovak forests: recent trends and future projection* (Barka et al.) informs about the carbon sequestration potential of Slovak forests during the next three decades. The authors used a model-based approach to quantify the amount of sequestered carbon under different management regimes, suggesting that changes in harvest timing and intensity are potent means for increasing the amount of carbon stored in the forest living biomass.

A study *Changes in diversity of protected scree and herb-rich beech forest ecosystems over 55 years* (Hájek et al.) is looking backward and explores changes in structure and herb layer composition of unique forest types, scree and herb-rich beech forests, over last 55 years, some of these changes reflecting on the ongoing changes in climate.

Roessiger et al. investigated the risk of stand-replacing disturbances to spruce stands, and options for alleviating this risk by admixing other trees species than spruce. The study *A high proportion of Norway spruce in mixed stands increases probability of stand failure* provided sound recommendations for forest management that should increasingly focus on comprehensive risk management rather than on the reductionist focus on productivity.

A paper *Physiological vitality of Norway spruce (Picea abies L.) stands along an altitudinal gradient in Tatra National Park* (Jamnická et al.) presents ecophysiological research of disturbed spruce stands in the mountain environment of the High Tatra Mts. The authors focused on tree-level processes such as tree growth, mineral nutrition of needles, and photosynthetic efficiency, informing about potential drivers of physiological performance of trees.

A paper Impact of the European bark beetle Ips typographus on biochemical and growth properties of wood and needles in Siberian spruce Picea obovata (Konôpková et al.) investigated how infestation by bark beetles affects biochemical properties of wood and needles of the Siberian spruce. The authors present interesting finding about the responses of photosynthesis-related pigments, extending our understanding of tree-pest interactions, and perhaps also informing efforts on early detection of infested trees via changes in their spectral reflectance.

The last paper *The response of Pinus species to ozone uptake in different climate regions of Europe* highlighted the fact that there are also other agents adversely affecting forest conditions in Central Europe than broadly studied drought, wind or bark beetles. Bičárová et al. investigated ozone injury to *Pinus mugo* and *Pinus cembra* in two mountain environments in Slovakia and France. The authors pointed out the differences in sensitivity of the two species and suggested interesting methodological improvements for mapping the effects of ozone on trees.

We believe that this diverse collection of studies will attract the readership and highlight some unique features of the Central European forests and forestry.

Zuzana Sitková & Tomáš Hlásny Editors of the issue

ORIGINAL PAPER



Carbon sequestration in living biomass of Slovak forests: recent trends and future projection

Ivan Barka*, Tibor Priwitzer, Pavel Pavlenda

National Forest Centre – Forest Research Institute Zvolen, T. G. Masaryka 2175/22, SK – 960 01 Zvolen, Slovak Republic

Abstract

The paper predicts development of forests in Slovakia from the perspective of carbon sequestration. Projection is based on actual trends of changes in forest area, age structure and tree species composition of forest stands managed according to forest management plans. Carbon balance in living biomass has been simulated until 2050 under two harvesting scenarios (based on planned and realized harvesting rates in reference period 2014–2019) with four possible combinations of fixed or changing tree species composition and size of forest area. Input data were stratified by tree species and 10-years wide age classes. A model simulating forest growth using yield tables and harvesting rates was developed and applied for prediction. Results indicate that the scenario based on realized harvesting would lead to a higher level of CO₂ removals from atmosphere by living biomass in Slovak forests for the whole simulated period, despite their decrease from the current level ~ -4,000 kt CO₂ to ~ -2,000 until 2040 and an increase to ~ -2,800 kt CO₂ in 2050. Conversely, scenario based on planned harvesting could result in a stronger decrease of CO₂ removals, with the culmination in 2040 at ~ -200 kt CO₂ followed by a slight increase to ~ -1,000 kt CO₂ in 2050. The influences of changes in tree species composition and increasing forest area were significantly lower than effect of different harvesting rates. Results achieved by this study suggest that adjusting harvested volume (e.g., by decreasing harvesting rates or modifying rotation periods) could be the most effective tool to intensify CO₂ removals by living biomass in Slovak forests in the upcoming decades.

Key words: carbon sequestration; carbon dioxide emissions/removals; living biomass; forest management; forest growth modelling

Editor: Erika Gömöryová

1. Introduction

Terrestrial ecosystems represent a major sink for atmospheric carbon (Schimel et al. 2001) and boreal and temperate forests play an important role in global carbon cycling and in carbon sequestration (Dixon et al. 1994; Peng et al. 2008). Estimation of the stock and accumulation of carbon in the forests is essential for assessing the role of forest ecosystems in global carbon budgets (Uri et al. 2012).

Carbon sequestration is very complex and varies greatly in consideration of age, composition, soil type and location of any forests. Terrestrial ecosystems and mostly trees store carbon due to their large storage volume and long–lived storage (Toochi 2018).

Sustainability of forest management and the potential of forests to mitigate climate change were discussed worldwide and many strategic documents related to these topics were elaborated (FAO 2016; Winkel 2017). There is international agreement that anthropogenic greenhouse gas (GHG) emissions need to be curbed to avoid dangerous changes in global climate (IPCC 2001). In response, Slovakia and more than 160 other countries have ratified the Kyoto Protocol to establish binding GHG emission reduction targets for industrialized nations. Forests are thought to be one of the most important solutions addressing the effects of climate change. For example, International Union for Conservation of Nature (IUCN) estimates that approximately 2.6 billion tonnes of carbon dioxide, one-third of the CO_2 released from burning fossil fuels, is absorbed by forests every year. Therefore, the functioning and management of forests are crucial for efforts to reduce climate change (climate change mitigation) and to reduce net GHG emissions into the atmosphere (emissions abatement).

Changing climate interacts with a wide range of factors. Higher atmospheric CO_2 concentrations are accelerating tree growth worldwide, while nitrogen emissions are fertilizing forest soils in Europe and so stimulating

^{*}Corresponding author. Ivan Barka, e-mail: ivan.barka@nlcsk.org

forest growth (Pretzsch et al. 2018). On the other hand, changing climate means risks to the ability of forests to sequester carbon from rising temperatures, changing seasonality of precipitation, and increases in the frequency, severity, or extent of natural disturbances such as drought, wildfire, and forest pests and pathogens (Bellassen & Luyssaert 2014). This may complicate or even disprove presumed best practice in carbon management, so that adaptation measures may be needed to maintain forest productivity and carbon stocks (Ontl et al. 2020). Uncertainty as to whether climate change will maintain the current forest sink or turn it into a carbon source is largely overlooked in the debate over the best forest management strategy to reduce the growth of atmospheric CO_2 concentrations (Valade et al. 2017).

Nevertheless, understanding how carbon dynamics are affected by stand age, density, management and rate of cuttings is fundamental to exploiting the capacity for sustainably managed forests to remove carbon dioxide from the atmosphere (Malsmheimer at al. 2011). These basic characteristics of the forests will play a crucial role in carbon sequestration in the upcoming decades.

As a member of the European Union, the Slovak Republic has a reporting obligation under the mechanism for monitoring and reporting GHG emissions and for reporting other information related to climate change at the national and Union level (Regulation (EU) No 525/2013, MMR). Every two years, Slovakia prepares a report containing projections of anthropogenic GHG emissions. These projections also include the Forestry sector with quantification of CO_2 emissions/removals in Slovak forests.

In the present study, we predicted development of carbon sequestration in the living biomass carbon pool in Slovak forests until 2050. The aim of this paper is to compare the development of CO_2 emissions/removals under planned and realized forest management in the near future, i.e., until 2050. This period (2020–2050) will be probably strongly affected by actual changes in tree species composition and an increase in the forest area. Therefore, the influence of these trends on future CO_2 emissions/removals was also simulated and analysed. A second objective of the paper is to introduce the FCarbon model, which was developed and used to predict future basic characteristics of forest stands (e.g., standing volume, current annual increment, harvested volumes), as well as to calculate CO_2 emissions/removals.

2. Material and methods

2.1. Study area and data sources

The study area covers a whole forest land in Slovakia managed according to the Act No 326/2005 Coll. on Forests. At the end of 2019, its total area was 1 943 156 ha. This forest land is subject to Forest Management Plans (FMPs) and quantitative and qualitative information on

forest characteristics and management is stored in central forestry databases. The official provider of data sets is the National Forest Centre - Institute of Forest Resources and Information (NFC–IFRI Zvolen) as the main administrator of Central forestry databases.

Management according to FMPs is obligatory for all forests in Slovakia. FMPs are updated on a regular basis with a 10-years period – i.e., one-tenth of the territory is surveyed each year and all forest stands are surveyed every decade. The elaboration process results in only one FMP proposal, which is considered to be optimal (Sarvašová et al. 2014). It is mandatory for forest managers that all management activities are reported to the state authority every year and recorded in the database.

As input data for the simulation of the future development of Slovak forests, summary reports from databases for one calendar year, stratified by tree species and 10-years wide age classes, were used. These reports included information on the area covered by each tree species and age class, growing stock (in m³ of merchantable wood, defined as tree stem and branch volume under the bark with a minimum diameter threshold of 7 cm), stocking (relative density of stands), yield class (site index - the mean height of the stand at the age of 100 years related to site conditions), current annual increment and harvested volume. The information on harvested volume, stratified in the same way, contained reported volumes of thinning, regeneration harvest and sanitary harvest (salvage logging).

The years 2014–2019 were chosen as a reference period for the simulation. This period was selected due to the consistency of input data processing (the data provider continuously updates the tools for database processing with an impact on the structure and consistency of data), as well as the representativeness of actual forest management in Slovakia. The length of the reference period is therefore 6 years and represents management practices and the country-specific age-related forestry dynamics (Grassi et al. 2018).

Within this period, the resulting CO_2 removalsby carbon stock changes in living biomass oscillated between -3500 and -4500 kt CO_2 (Szemesová et al. 2019), and the volume of total harvest varied between 9.2 and 9.9 million m³ of merchantable wood (Moravčík et al. 2019).

The stratification of the forest land in the present research follows tree species composition. The same approach is used in Slovak GHG Inventory (GHGI) under UNFCCC and Kyoto Protocol. In total, 20 groups of tree species are distinguished (Table 1, Fig. 1).

The age structure of forests in Slovakia at the end of 2014 and 2019 (used as an initial state for the simulation of the future development of forest stands) is presented in Fig. 2. The share of the youngest forest stands of the 1^{st} and 2^{nd} age classes has increased from 16% to 19% during the reference period. This is due to the high extent of forest damage caused by harmful agents and subsequent regeneration of damaged forests. Protective forests (for-

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| Name | Tree energies included | Percentage | of total area |
|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|---------------|
| INAILIC | nee species included | 2014 | 2019 |
| Spruce | Norway spruce (<i>Picea abies</i> L.), Silver spruce (<i>Picea pungens</i> Engelm.), Serbian spruce (<i>Picea omorika</i> Panc.) | 24.10 | 22.08 |
| Fir | Silver fir (Abies alba Mill.) | 4.02 | 4.04 |
| Pine | Scotch pine (Pinus sylvestris L.), Swiss stone pine (Pinus cembra L.), Black pine (Pinus nigra Arn.) | 6.86 | 6.63 |
| Larch | European larch (Larix decidua Mill.) | 2.49 | 2.63 |
| Other coniferous | Dwarf pine (<i>Pinus mugo</i> Turra), European yew (<i>Taxus baccata</i> L.), White pine (<i>Pinus strobus</i> L.), Doug- las fir (<i>Pseudotsuga menziesii</i> Mirb.) | 1.12 | 1.04 |
| Beech | European beech (Fagus sylvatica L.) | 32.73 | 34.22 |
| Oaks | Common oak (Quercus robur L.), Sessile oak (Quercus petraea Liebl.) | 10.64 | 10.47 |
| Turkey oak | Turkey oak (Quercus cerris L.) | 5.85 | 5.92 |
| Hornbeam | European hornbeam (Carpinus betulus L.) | 2.29 | 2.54 |
| Maple | Sycamore maple (<i>Acer pseudoplatanus</i> L.), Norway maple (<i>Acer platanoides</i> L.), Field maple (<i>Acer campestre</i> L.) | 1.58 | 1.57 |
| Ash | Common ash (Fraxinus excelsior L.), Narrow-leafed ash (Fraxinus angustifolia Vahl.), Manna ash | 0.03 | 0.03 |
| Elm | Wych elm (IIImus glabra Huds) Euronean white elm (IIImus leavis Pall) Field elm (IIImus minor Mill) | 2.54 | 2 57 |
| Linden | Small-leaved lime (<i>Tilia cordata</i> Mill.), Large-leaved lime (<i>Tilia nlatynhyllos</i> Scon.) | 1.71 | 1.79 |
| Locust | Common locust (Robinia nseudoacacia L.) | 1.51 | 1.67 |
| Birch | Silver birch (Betula pendula Roth). Downy birch (Betula pubescens Ehrh.) | 0.75 | 0.77 |
| Alder | Black alder (Alnus glutinosa L.), Grev alder (Alnus incana L.) | 0.41 | 0.42 |
| Poplar | Black poplar (<i>Populus nigra</i> L.), White poplar (<i>Populus alba</i> L.), Grey poplar (<i>Populus × casescens</i>), Aspen (<i>Populus tremula</i> L.) | 0.48 | 0.44 |
| Hybrid poplars | Hybrid poplars (<i>Populus</i> × euroamericana, <i>Populus</i> × hybr.) | 0.36 | 0.4 |
| Willows | Crack willow (Salix fragilis L.). White willow (Salix alba L.). Salix sp. | 0.11 | 0.10 |
| | Wild cherry (<i>Prunus avium</i> L.), Eastern black walnut (<i>Juglans nigra</i> L.), Planes (<i>Platanus</i> sp.). European | | |
| Other broadleaves | wild pear (Pyrus pyraster L.), Horse-chestnut (Aesculus hippocastanum L.), Sweet chestnut (Castanea | 0.42 | 0.64 |
| | sativa Mill.), Wild service tree (Sorbus torminalis L.), Rowan (Sorbus aucuparia L.) | | |
| Total area (ha) | | 1 933 217 | 1 943 156 |

Table 1. Area of the forest land in Slovakia by tree species with the percentage of the total area in the beginning and at the end of the reference period. In the case of more tree species in one stratum, the dominant tree species are shown in **bold**.



Fig. 1. Distribution of the main tree species of forest land in Slovakia. Information based on satellite data (Bucha 1999).

ests which protect soils, water resources and infrastructure) and forests in protected areas predominate in the 15th and older age classes due to the implementation of special management regimes and nature conservation restrictions (Moravčík et al. 2019).

2.2. Harvesting scenarios

For each tree species, two different harvesting scenarios (planned and realized) were identified according to the data on planned and realized harvesting in the reference period. "Planned harvest" scenario is based on the management envisaged in FMPs; it is a summary of the management activities planned for each forest compartment. It does not contain the influence of natural disturbances, because they are unpredictable for particular forest stand. "Realized harvest" scenario is based on real management, i.e., it summarizes results of activities that have actually been carried out in forest stands. Natural disturbances are the main factor that prevents full implementation or realisation of the planned activities in the planned period. Table 2 presents the total volumes (m³) of thinning and final harvesting (both planned and realized) in the reference period.

Input data on realized harvests were stratified into 3 basic types – thinning, final harvest and sanitary felling. However, no information was provided on the type of sanitary felling, i.e., which part had a character of sanitary thinning and which part was followed by the regeneration

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Fig. 2. The changes in the age structure of Slovak forests during the reference period.

Table 2. Total volumes (m³) of thinning and final harvesting (planned and realized) during the reference period in Slovak forests.

| | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 |
|---------------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Planned thinning | 1,600,101 | 1,661,263 | 1,724,948 | 1,746,986 | 1,796,827 | 1,841,392 |
| Realized thinning | 1,842,479 | 1,952,089 | 2,020,042 | 2,077,738 | 2,169,475 | 1,971,964 |
| Planned final harvesting | 7,749,342 | 7,862,276 | 7,966,011 | 8,000,780 | 8,089,135 | 8,100,647 |
| Realized final harvesting | 7,574,607 | 7,188,972 | 7,300,091 | 7,315,063 | 7,694,495 | 7,245,946 |
| Total planned harvests | 9,349,443 | 9,523,539 | 9,690,959 | 9,747,766 | 9,885,962 | 9,942,039 |
| Total realized harvests | 9,417,087 | 9,141,062 | 9,320,134 | 9,392,802 | 9,863,971 | 9,217,911 |

of the stand. Therefore, the volume of sanitary felling was split among the realized final harvesting and the realized thinning according to the ratio of the planned thinning or planned harvesting to the total planned harvests. This procedure was performed separately for each age class of tree species.

The rates of thinning and final harvests, as well as sanitary felling, were calculated using the merchantable wood volume available for thinning or final harvest and the recorded harvested volumes. Fig. 3 shows an example of planned and realized harvesting rates for beech. For each tree species and age class, the mean value and standard deviation of the rate were calculated in %.

2.3. The development of forest area and tree species composition

The analysis of data from the reference period identified a continuous increase in the total forest area – it increased from 1,933,217 ha at the beginning of 2014 to 1,943,156 ha at the end of 2019, i.e., the annual increase was 1,420 ha on average. However, there have been different trends in the area covered by particular tree species. While the area of beech increased by 5,366 ha every year, the area of spruce decreased simultaneously by 6,159 ha. Additional decreasing areas were found for pine, elm, oaks, hybrid poplars, and willow. All other tree species recorded an increase in area.



Fig. 3. An example of planned and realized harvesting rates for beech.

For both harvesting scenarios (based on planned and realized harvesting), the future development of forests was simulated with 2 scenarios of changes in the total forest area (static - fixed area from the end of the reference period, dynamic - constantly increasing total forest area). Similarly, 2 scenarios of changes in tree species compositions were implemented (static - fixed share of tree species from the end of the reference period, dynamic - the share of tree species constantly changing as in the reference period). Thus, 8 unique combinations of 2 harvesting scenarios, 2 scenarios of the development of the total forest area and 2 scenarios of the development of the tree species composition were simulated. In the combination of static tree species composition and dynamic total forest area, the increase of total forest area was divided among the tree species according to their share at the end of the reference period. In combination with the dynamic tree species composition and the static total forest area, the annual changes in the areas occupied by tree species from the reference period were adjusted proportionally so that the total forest area remained unchanged.

During simulations with the dynamic forest area or the tree species composition, all trends were linearly extrapolated for the whole simulation period until 2050 (i.e., no trend adjustments were applied to take into account future policies intended by different stakeholders like state forestry organizations, governmental bodies or owners). All the areas were changed every year with a constant average value calculated from the data for the reference period.

2.4. Forest growth modelling

A relatively simple model for estimation of future development of the forests in Slovakia was developed according to the methodology proposed by Grassi & Pilli (2017) and Forsell et al. (2018). The model (called FCarbon, https:// web.nlcsk.org/?page_id=17445) was developed for the needs of the Slovak Forest Reference Level calculation in connection with the application of the Regulation (EU) 2018/841. The main reason for model development was a specific requirement to include dynamic age-related forest characteristics. It was designed in consistency with Slovak GHG inventory (Szemesova et al. 2019) and with the national projections of GHG emissions reported under Regulation (EU) No 525/2013 as well (Szemesova & Zemko 2019).

The model is able to simulate the development of the age structure of forests, their growing stock, current annual increments and harvests of merchantable wood volume in each simulation step, which is 1 year. Shelterwood and clearcutting management systems can be modelled. The simulation of forest growth in the FCarbon model is based on yield tables (Halaj & Petráš 1998), which specify increments of merchantable wood volume in m³ for the main tree species in Slovakia (spruce, fir, pine, beech, oak, and hybrid poplar). The volumes of thinned and harvested wood are calculated by the application of the rates of harvests of the growing stock. The model is optionally able to simulate changes in the area occupied by tree species and changes in the total forest area.

The following information, stratified by tree species and age classes, must be provided as input to the model: growing stock (m³), area (ha), mean yield class and thinning and harvesting rates (in %, both average and standard deviation).

When initializing simulations, the model converts 10-years wide age classes into 1-year age classes. While the area of each 10-years wide age class is split equally (area of 1-year wide age class equals to 1/10 of the area of 10-years wide age class), the growing stock is split using a growing function taken from the yield tables (i.e., values of the growing stock per ha are calculated for each 1-year wide age class according to the growing function, then divided by the sum of the values within 10-years wide age class and the resulting ratios are applied to the growing stock of 10-years wide age class to calculate the growing stock of 1-year wide age classes; Fig. 4).

During each simulation step, the initial growing stock in the age class is increased by calculated increment (yield table value adjusted for 1-year wide age step and modified by stocking level - relative density) and reduced by harvested (or thinned) volume. Thinning and harvesting rates are applied to the growing stock of the corresponding age class to calculate thinned and harvested volumes, and the harvest rate is applied to the area of mature age classes as well to calculate the harvested area. The model allows stochastic simulation of thinning and harvesting activities by providing average thinning and harvesting rates and their standard deviation, assuming a normal distribution of possible values. A random value of the rate is generated for each age class in each simulation step (year). In the present study, the number of repeated simulations for each scenario was set to 150. The harvested area from all age classes within step is transferred to the youngest age classes. If the ratio of natural regeneration is specified for tree species, the naturally regenerated area is transferred to the 5-years old age class to simulate the shelterwood management system. The ratio of natural regeneration to artificial planting was summarized from the FMPs, where this ratio is estimated for each stand with planned regeneration cutting in the next decade. The rest of the harvested area (or the entire harvested area if this ratio is set to zero) is used to create a new age class, with age -1 simulating 2 years allowed for planting.

Simulation results for tree species and age classes are summarized within each step and CO_2 emissions/removals in living biomass carbon pool are calculated. The quantification is based on the methodology provided by the Intergovernmental Panel on Climate Change (IPCC) Guidelines (IPCC 2006 GL), using a Gain-Loss method. It is based on estimates of annual change in biomass from I. Barka et al. / Cent. Eur. For. J. 66 (2020) 191-201



Fig. 4. An example of growing stock conversion from 10-years wide age classes (left) to 1-year wide age classes (right). Growing stock of beech.

estimates of biomass gain and loss, where the gains represent an annual increase in carbon stocks due to biomass growth and losses represent an annual decrease in carbon stocks due to biomass losses (harvest).

Current annual increment (CAI) is converted to the annual biomass growth, using the biomass conversion expansion factor (BCEF₁) and root-to-shoot ratio (R). The annual increase in biomass carbon stock is calculated by multiplication of annual biomass growth by carbon fraction of dry matter (50% for coniferous and 49% for broadleaved wood; IPCC 2006 GL). The annual decrease in carbon stocks due to biomass loss is calculated using the harvested wood volume (m³), biomass conversion expansion factor (BCEF $_{R}$), root-to-shoot ratio (R) and the carbon fraction (IPCC 2006 GL). Changes in carbon stocks (gains-losses) are converted to units of CO₂ emissions by multiplying the change in carbon stock by -44/12. More detailed information on this methodology was published in the Slovak GHG National inventory report 2019 (Szemesova et al. 2019).

The whole simulation procedure of the FCarbon model and calculation of CO_2 emissions is coded in Python scripting language with data stored in the SQL-ite database.

The model was calibrated using data from Slovak GHG inventories in the period 2014–2019, because the first data on forest stands characteristics from the reference period were valid for the end of 2013. The aim of the calibration was to adjust the simulation process to reproduce GHG inventory as accurately as possible. In order to follow the real development of forest stands

during the reference period, variations of thinning and harvesting rates (both positive and negative, in %) were specified for each year of simulation so that the sum of the variations in the calibration period was zero. After the first run of the model, carbon stock changes in living biomass were calculated using the resulting values of harvested volumes and volume increments. It was found that the resulting average value of the carbon gains (5,141.1 kt C) was higher by 2.68% and the average value of simulated losses (-3,874.5 kt C) was lower by 0.05% when compared to average values from the NIR (5,006.9 kt C and -3,872.5 kt C). Carbon gains were compared according to tree species and the ratios of simulated values to inventory values were calculated. These ratios were applied as multipliers to the volume increments by tree species which were taken from the yield tables to adjust the simulated biomass gains. The calibrated model was used to simulate the development of the forest age structure, increments, and harvests in the period 2020–2050. Data on forest characteristics valid at the end of 2019 were used as inputs to the model.

3. Results

3.1. Age structure

Higher thinning and harvesting rates assumed by the scenario with planned harvesting resulted in a younger simulated age structure of Slovak forests compared to the results of simulations based on realized harvests from the reference period (Table 3, Fig. 5).

Table 3. Predicted age structure at the end of simulated period (2050), expressed as the share of age classes from the total forest area. The mean age at the end of the reference period was 70.8 years.

| How so ting cooperio | Onaging composition | Forest area | Tł | The share of age classes (%) | | | | | |
|----------------------|---------------------|-------------|------|------------------------------|-------|--------------------|--|--|--|
| narvesting scenario | Species composition | | 1-4 | 5-9 | 10-15 | - Mean age (years) | | | |
| | Statia | Static | 38.8 | 32.6 | 28.6 | 64.3 | | | |
| Dlannad | Static | Dynamic | 39.8 | 32.1 | 28.1 | 63.5 | | | |
| Fialilicu | Drmomia | Static | 41.2 | 31.4 | 27.4 | 62.4 | | | |
| | Dynamic | Dynamic | 41.9 | 31.0 | 27.1 | 61.9 | | | |
| | Statia | Static | 33.9 | 32.9 | 33.2 | 69.5 | | | |
| Doolizad | Static | Dynamic | 35.0 | 32.4 | 32.6 | 68.7 | | | |
| Realized | Drmomia | Static | 35.3 | 32.2 | 32.5 | 68.4 | | | |
| | Dynallic | Dynamic | 36.1 | 31.8 | 32.1 | 67.8 | | | |



Fig. 5. Predicted age structure of Slovak forests in 2050 according to different harvesting scenarios. Average from 150 simulations with combination of dynamic forest area and dynamic tree species composition.

3.2. Current annual increment

Due to changes in the age structure, the values of the current annual increment (merchantable volume) in the simulated period are lower compared to the reference period. For all simulated scenarios, minimal increments were recorded around 2040 (Table 4). The dynamic species composition and the planned harvesting scenario led to the lowest values, while the highest increments were achieved in the realized harvesting scenario and static tree species composition. The dynamic forest area led to higher increments until the end of the simulation period.

3.3. Harvested volumes

According to the simulations, the volumes of harvested wood under the realized harvesting scenario are constantly decreasing. However, simulations under the planned harvesting scenario assumes slightly increased and stabile harvested volumes until 2040, followed by a decrease until 2050 (Table 5, Fig. 6).



Fig. 6. Predicted total harvested volumes (m³) according to scenarios with planned and realized harvesting, dynamic tree species composition and dynamic forest area.

3.4. CO₂ removals

A comparison of the resulting CO_2 emissions is given in Table 6 and Fig. 7. In all simulated scenarios, the values were negative, i.e., carbon stock changes in living biomass represented CO_2 removals. There were significant differences between the two scenarios based on realized

| Table 4. Predicted current annual increments, in m | /ear ⁻¹ . The average value in the reference p | period was 12,041,089 m ³ year ⁻¹ . |
|----------------------------------------------------|-----------------------------------------------------------|-----------------------------------------------------------|
|----------------------------------------------------|-----------------------------------------------------------|-----------------------------------------------------------|

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|---------------------|---------------------|-----------------|-----------------------------------------|------------|------------------|------------|
| Harvesting scenario | Species composition | Forest area | 2020 | 2030 | 2040 | 2050 |
| | Statia | Static | 12,127,652 | 11,233,697 | 10,284,235 | 10,561,362 |
| Dlannad | Static | Dynamic | 12,127,590 | 11,233,799 | 10,291,941 | 10,630,994 |
| Planned | Drmamia | Static | 12,138,922 | 11,346,983 | 10,273,218 | 10,421,628 |
| | Dynamic | Dynamic | 12,137,589 | 11,345,965 | 10,275,984 | 10,464,893 |
| | Statia | Static | 12,141,665 | 11,374,824 | 10,537,681 | 10,820,202 |
| Dealized | Static | Dynamic | 12,141,742 | 11,375,822 | 10,545,529 | 10,891,343 |
| Realized | Drmamia | Static | 12,145,289 | 11,450,324 | 10,487,516 | 10,503,830 |
| | Dynamic | Dynamic | 12,141,680 | 11,392,624 | 10,463,955 | 10,555,145 |

Table 5. Predicted average values of harvested volumes, in m^3 /year. The average value in the reference period was 9,392 988 m^3 /year.

| Harvesting scenario | Species composition | Forest area | 2020 | 2030 | 2040 | 2050 |
|---------------------|---------------------|-------------|-----------|-----------|-----------|-----------|
| | Statia | Static | 9,685,993 | 9,781,635 | 9,720,730 | 9,527,009 |
| Dlannad | Static | Dynamic | 9,689,569 | 9,783,372 | 9,721,469 | 9,533,076 |
| Flainicu | Drmamia | Static | 9,686,869 | 9,788,518 | 9,738,164 | 9,511,664 |
| | Dynamic | Dynamic | 9,690,780 | 9,794,849 | 9,744,225 | 9,520,856 |
| | Statia | Static | 9,160,538 | 9,061,451 | 8,947,151 | 8,738,873 |
| Doolized | Static | Dynamic | 9,150,555 | 9,050,701 | 8,957,716 | 8,756,694 |
| Realized | Drmamia | Static | 9,149,338 | 9,068,347 | 8,961,914 | 8,719,285 |
| | Dynamic | Dynamic | 9,146,890 | 9,058,471 | 8,950,370 | 8,694,860 |

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| Inon | C_2 into the constant of C_2 into the (C_2). The average value in the relation of period was C_2 . | | | | | | | | | | |
|------|--------------------------------------------------------------------------------------------------------------|---------------------|-------------|----------|----------|----------|----------|--|--|--|--|
| ID | Harvesting scenario | Species composition | Forest area | 2020 | 2030 | 2040 | 2050 | | | | |
| 1 | 1 2 Planned 4 | Statia | Static | -3,255.5 | -1,682.7 | -211.3 | -910.1 | | | | |
| 2 | | Static | Dynamic | -3,253.9 | -1,679.9 | -216.9 | -991.4 | | | | |
| 3 | | Dynamic | Static | -3,265.7 | -1,729.6 | -143.6 | -1,062.2 | | | | |
| 4 | | | Dynamic | -3,259.6 | -1,721.9 | -151.9 | -1,140.2 | | | | |
| 5 | | Statia | Static | -4,397.6 | -3,408.2 | -2,178.5 | -2,723.7 | | | | |
| 6 | Deskard | Static | Dynamic | -4,415.2 | -3,427.2 | -2,205.3 | -2,834.1 | | | | |
| 7 | Realized | Demenie | Static | -4,410.6 | -3,359.7 | -2,018.2 | -2,686.5 | | | | |
| 8 | | Dynamic | Dynamic | -4,412.5 | -3,329.8 | -2,021.3 | -2,810.4 | | | | |

Table 6. Predicted mean values of CO₂ removals (kt CO₂). The average value in the reference period was -4 159.3 kt CO₂



Fig. 7. Predicted CO₂ removals (kt CO₂). Numbers of simulated combinations of harvesting scenarios, tree species compositions and forest area (legend number) equals to ID in Table 6.

and planned harvesting. Both scenarios have led to a reduction of CO_2 removals by 2040, but this decrease is more intense in the planned harvesting scenario. After the culmination in 2040, CO_2 removals begin to increase. In both scenarios, the combination of the static forest area and the dynamic tree species composition led to the most intensive decrease of CO_2 removals in 2040, but the subsequent increase was higher than in the case of static tree species composition. The variability of the simulated CO_2 removals was slightly lower than in the reference period: the average standard deviation for the scenario based on the realized harvesting was 70 - 142 kt CO_2 . For the scenario based on the planned harvesting it was 25 - 43 kt CO_2 (Fig. 8).



Fig. 8. Predicted CO_2 removals according to scenarios with planned and realized harvesting, dynamic tree species composition and dynamic forest area.

4. Discussion

Different harvesting rates of scenarios based on planned and realized management led to the greatest contrasts in the resulting CO_2 removals. According to both scenarios, CO_2 removals levels decrease during the simulation period. Their harvesting rates affected the calculated current annual increments and harvested volumes, resulting in different levels of CO_2 removals. However, the general trends in all simulations were the same because they were driven by the initial (actual) age structure of Slovak forests.

The impact of changes in tree species composition on the resulting CO₂ removals was much lower in the present study compared to harvesting rates. The reason is that changes in tree species composition are slow, with the potential to modify CO₂ removals only after several decades of forest growth. However, in the long-term, the impact of these changes would be substantial. Carbon accumulation and storage vary according to species composition and site quality. Tree species differ in their wood density as well as growth potential and produce various current annual increments and wood stocks. For example, the carbon content in the biomass (carbon fraction of dry matter) is greater in conifers than in broadleaved trees (Bravo et al. 2017). In Mediterranean areas, Scots pine (Pinus sylvestris L.) stands store more CO₂ than pure oak (Quercus pyrenainca Willd.) stands, while mixed oak-pine stands store intermediate levels (Bogino et al. 2006). Forest species composition, structure, and tree age distribution can be altered by both natural and anthropogenic disturbances (Swanteson-Franz et al. 2018). Current forest management activities are aimed at increasing the quantity of fixed carbon in the forests (Bravo et al. 2017), despite the fact that the main goal of forest managers is to maximize productivity. Long-term experiments are necessary to gain knowledge about the effects of thinning on carbon storage and sequestration rates. A few studies have shown that unthinned stands provide higher carbon stocks in tree living biomass than thinned stands, as higher stocking from moderate or heavy thinning results in lower carbon stocks over the long-term time period (Skovsgaard et al. 2006; Powers et al. 2012). Along with thinning intensity, the type of thinning may also affect carbon stocks and sequestration rates (Bravo et al. 2017). In the present study, the effect of changes in tree species composition on the simulation results was expressed by slightly steeper curves of CO₂ removals and their more extreme culmination.

Despite the large afforestation potential of Slovakia, where the area of abandoned agricultural land is estimated at 400,000 ha (Nijnik & Bizikova 2007), the increase of forest area in the reference period was relatively small, averaging only 1,419.85 haper year, which is less than 1% of forest area. Such a small increase in the area had a very limited effect on the simulated CO, removals. During the implementation of previous rural development programs (RDP 2004-2006 and 2007-2013), only 332 ha of agricultural land were afforested in Slovakia. This area represents a very small share in the total forest area in Slovakia (1.938 million ha, specifically 0.017%). In the ongoing RDP (2014-2020), several stands of fastgrowing woody plants were established on 35 ha of agricultural land. Despite the fact that afforestation of agriculturally unused land has a high sequestration potential, there are currently no preconditions for accelerating the afforestation of such land in Slovakia, especially due to the lack of interest of landowners.

With respect to all the above-mentioned facts, the presented results suggest that adjusting harvesting rates could be the most effective tool to intensify CO_2 removals by living biomass in Slovak forests in the upcoming decades.

The selected modelling approach, in which thinning and harvesting rates were randomized by the mean and standard deviation separately for each 10-year wide age class of tree species, led to slightly lower variability in total harvested volume compared to the reference period. Some other approaches could be applied, such as first generating the total harvested volume and then dividing it between tree species and age classes. This will be the subject of future research and development of the FCarbon model. Higher variability of predicted harvested volumes may have an impact mainly on the planned harvesting scenario in a period with a very low annual sinks (around 2040). Higher variability of total harvests, as well as a different share of broadleaved wood (i.e., higher density wood) from the total harvested volume can produce net CO₂ emissions from living biomass, at least in some model runs.

Forest growth modelling in this study is based on yield tables. It therefore does not take into account expected future changes in growth due to climate change, in particular the impact of rising temperatures and modified precipitation regimes (Pretzsch et al. 2018). It also lacks a mortality model (as no information on natural mortality is available in the yield tables), which makes it virtually impossible to reasonably simulate a "no management" scenario and estimate changes of carbon stock in dead wood. These disadvantages are balanced by the simplicity of the model. However, given the most recent reference period used, the results reflect at least the impact of the actual frequency of natural disturbances, which are considered to be another important effect of climate change (e.g., Rötzer et al. 2013). The predicted development of the age structure demonstrates the evidence that both planned and realized harvesting scenarios lead to an increase of the area of the oldest age classes (older than 140 years). This is most likely due to the prolongation of regeneration periods in protective forests and the lack of management in natural reserves. Such an increase in the area of the oldest stands together with the preservation of CO_2 removals can support the sustainability of forest management in Slovakia and its compliance with the promotion of biodiversity.

5. Conclusions

Assuming that forests will be managed as in the last 6 years, a significant decrease of CO₂ removals can be expected in the period up to 2050. The results indicate that the scenario based on realized harvesting would lead to a higher level of CO₂ removals by living biomass in Slovak forests for the whole simulated period, despite their decrease from the current level $\sim -4,000$ kt CO₂ to \sim -2,000 until 2040 and an increase to \sim -2,800 kt CO₂ in 2050. Contradictory, scenario based on planned harvesting could result in a stronger decrease of CO₂ removals, with the culmination in 2040 at ~ -200 kt CO₂ followed by a slight increase to $\sim -1,000$ kt CO₂ in 2050. The influence of changes in tree species composition was stronger compared to the increasing forest area. The presented results suggest that adjusting harvested volume could be the most effective tool to intensify CO₂ removals by living biomass in Slovak forests in the upcoming decades.

Acknowledgments

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ORIGINAL PAPER



Changes in diversity of protected scree and herb-rich beech forest ecosystems over 55 years

Vojtěch Hájek¹, Zdeněk Vacek¹, *, Stanislav Vacek¹, Lukáš Bílek¹, Romana Prausová², Rostislav Linda¹, Daniel Bulušek¹, Ivo Králíček²

¹ Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, Department of Silviculture, Kamýcká 129, CZ – 165 21 Prague, Czech Republic

² University of Hradec Králové, Faculty of Science, Rokitanského 62, CZ – 500 03 Hradec Králové, Czech Republic

Abstract

Species composition along with spatial and age structure are the main attributes of forest ecosystems. The diversity of scree forests and herb-rich beech forests was analyzed in the Broumovsko Protected Landscape Area, the Czech Republic. The paper objective was to evaluate forest structure and dynamics of species diversity of tree layer, natural regeneration and herb layer in the period 1961–2016. Scree forests were structurally, and species very rich forest stands, in herb-rich beech forests the stands were of medium richness. Studied stands managed by small-scale methods, in the past especially by coppicing and later by shelterwood and selection felling, have maintained high species biodiversity during the 55 years of observation. Substantially higher biodiversity was found out in scree forests compared to herb-rich beech forests. Based on the comparison of predominantly coppiced forest stand and stand of generative origin at sites of scree forests, coppice have maintained higher biodiversity than high forests. Species richness increased during the observation period, but species evenness had mostly decreasing tendency. Species heterogeneity in coppiced scree forests increased in tree layer and natural regeneration, but it decreased in herb layer; inverse dynamics was observed in the high forests. Changes in biodiversity dynamics were remarkable for coppice, while high forests showed relatively high level of stability. During study period herb population of light-demanding species and species characteristic for broadleaved forests decreased, while an increase in shade tolerant, moisturedemanding and nutrient-demanding species, especially nitrophilous species was confirmed. Moreover, occurrence of thermophilic plants increased, respectively cold-tolerant plant population decreased in relation to climate change.

Key words: biodiversity; forest dynamics; stand structure; phytosociology; Natura 2000

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1. Introduction

Conservation of biological diversity is a crucial goal for sustainable management in forests (Piussi & Farrell 2000; Specker 2003; Machar et al. 2017). This is consistent with the present trend of forest management in Europe supporting higher biological diversity by the increased use of natural regeneration and creation of a higher proportion of mixed forests (Matuszkiewicz et al. 2013; Slanař et al. 2017). According to Lindenmayer (2000) the species composition of forest stand belongs among the most important elements of forest ecosystem sustainability and productivity. Species composition along with spatial and age structure are the main attributes of forest stand, influencing the ecosystem function and are mutually related with each other (Crow et al. 2002; Bulušek et al. 2016). The species composition of forest stands influences both the forest biotope by providing different cover and the microsite conditions and nutrient cycle in a forest ecosystem (Carvalho 2011; Vacek et al. 2015a, 2017b).

The tree species composition of forest stands is affected by many factors and requirements of the particular tree species. These mechanisms may have a direct influence on self-regulatory changes in the species composition and a mutual interaction in forest stands (Wilson 2011; Vacek 2014b). Among the most important factors are e.g. requirements for nutrients and light, growth or flowering phenology, long-term history of plant community and the type of forest management (Lindenmayer et al. 2000; Silvertown 2004; Kneewshaw & Prévost 2007; Paal et al. 2011; Lhotka 2013). Particularly silvicultural practices in the past often influenced the species

^{*}Corresponding author. Zdeněk Vacek, e-mail: vacekz@fld.czu.cz

composition of a given forest stand (Battles et al. 2001; Sitzia et al. 2012; Vacek et al. 2017a). Fluctuations in the natural environment belong to other important factors, mainly specific pressure of pests (Ammer 1996), air pollution load (Král et al. 2017) and extreme climate events (Serra-Diaz et al. 2012). In recent years, biodiversity and structure of forest ecosystems is strongly affected by ongoing climatic change (Čermák et al. 2018; Vacek et al. 2019a). Global warming, long-term droughts, frequent windstorms and fires and secondary fungal pathogens, insects (especially bark beetle) and other pests have caused distinct changes in species diversity and its distributions (Iverson & Prasad 2001; Morin et al. 2018). On the other site, forest biodiversity may mitigate climate change impacts (Hisano et al. 2018). In general, forest tree species have relatively low colonization capacity and therefore they have lower competitiveness compared to the species with wide ecological amplitude (Wulf 2003; Matuszkiewicz et al. 2013).

Tree species composition of near-natural forests can be considered as a certain expression of regeneration processes, which are highly stochastic phenomena, and so species abundance and representation may fluctuate substantially (Vacek et al. 2014a; Remeš et al. 2015; Bošela et al. 2016). Natural regeneration and subsequent interspecific interactions at this juvenile stage are influenced mainly by favourable microsite conditions in which seeds can survive, germinate and produce seedlings, and by seed availability (Snyder & Chesson 2004; Vacek et al. 2015a). According to Paluch & Jastrzębski (2013) an increase in the representation of one species in relation to another species is one of the factors increasing its reproduction chances. On the other hand, factors such as pathogens (Paluch & Stępniewska 2012), vegetation cover (Beckage et al. 2005), distance between parent trees (Packer & Clay 2003), light availability (Kunstler et al. 2005), soil moisture (Arrieta & Suarez 2005), herbivores (Vera 2000) or other animals feeding on seeds (Lucas-Borja et al. 2012) can strongly influence regeneration success of particular tree species. So especially food-attractive broadleaved tree species and silver fir (Abies alba Mill.) are often eliminated from forest stands while considerably lower food attractiveness is evident in Norway spruce (Picea abies [L.] Karst.) – (Motta 2003; Vacek et al. 2015b, Mattila & Kjellander 2017, Meier et al. 2017).

Studied herb-rich beech forests and scree forests in nature protection area (Natura 2000) Kozínek have maintained a high degree of forest stand naturalness and therefore a research on their diversity was launched in 1961. Long-term research plots are the most valuable objects for monitoring changes in forest ecosystems (Bakker et al. 1996). Currently the importance of these researches is confirmed by increasing number of publications dealing with long-term dynamics of forests (Hédl et al. 2017; Heinrichs & Schmidt 2017). The objective of the present paper was to determine changes in species diversity of tree layer, tree natural regeneration and herb layer of beech forests ecosystems from 1961 to 2016 in protected area with emphasis on structural and overall diversity of tree layer in 2016. Basic questions were: 1) How varied the biodiversity in coppice, coppice with standards (combined forest) and high forest? 2) What species and structural changes of forest stands occurred over the past 55 years in Kozínek? 3) Did distinct changes of species diversity appear in herb layer of scree forest and herb-rich beech forests? 4) Which factors caused changes in diversity of vegetation?

2. Material and methods

2.1. Study site

The research was conducted on five permanent research plots (PRP) situated in the locality Kozínek (Fig. 1, Table 1) – a protected locality in the Natura 2000 network. It was established on the area of 84.08 ha in 2004 as Special Area of Conservation (SAC) and Special Protection Area (SPA) – (AOPK 2004). The Kozínek SAC (CZ 0520507) is important for abundant occurrence of the priority biotope of scree forests (L4) along with biotopes of rock outcrops (S1.1) and also for its vast area of herb-rich beech forests (L5.1). By the representation of units L4 and S1.1 it is the most important locality in southern part of the Broumovsko Protected Landscape Area (PLA). Altitude of the locality above sea level is 378 – 504 m. The slopes with many rock outcrops of the valley are deeply cut into marlstone tablelands. The bedrock is mostly built of cretaceous sediments, of middle and lower Turonian or upper Cenomanian, while fluvial and fluvial-deluvial quaternary sediments are also represented (Vejlupek 1986, 1990). Prevailing soil types are Pararendzinas, Cambisols and Rankers (Mikeska et al. 2000). Climatically, according to the Hronov meteorological station (378 m a.s.l.) the Kozínek locality belongs to a moderately warm region (MT7 region; Quitt 1971) with average annual temperature of 7.2 °C and annual precipitation amount of 742 mm (Vesecký 1961; Tolasz et al. 2007). In relation to climatic change, temperature increased by 1.8 °C in period 1963-2017, while no changes were observed in precipitation regime.

Scree forests are composed of the plant associations *Aceri-Carpinetum*, *Mercuriali-Fraxinetum* and also *Lunario-Aceretum* to a lesser extent; herb-rich beech forests consist of the associations *Aceri-Fagetum*, *Asperulo-Fagetum* and *Dentarioenneaphylli-Fagetum*, and also of the association *Tiliocordatae-Fagetum*, which is a transition between beech forests and scree forests (Faltysová et al. 2002). Stand on PRP 2 and 3 was a high forest, coppice with standards (combined forest) were presented on PRP 1 and 4 (8% of trees) and coppice was characterized on PRP 5.



Fig. 1. Localization of permanent research plots 1 - 5 at Kozínek Special Area of Conservation.

3 – trees reaching a heights > 1/2 the height of the main level trees and < lower 1/3 of the length of their crown, 4 – tree individuals of heights > 130 cm and < 1/2 the height of the trees of the main level, 5_{1a} – individuals of heights in range 25 – 130 cm, 5_{1b} – individuals of heights < 25 cm, 5_2 – seedlings with uterine petals. In grades 1 – 4 average height, DBH and number of individuals were recorded for all tree species on the image area. For individual grades 5_{1a} , 5_{1b} and 5_2 , the average number for each species per 1 m² was stated. In 2016, this scale was modified for comparing both periods, where grades 5_{1b} , 5_{1a} and part of the grade 4 were indicated as natural regeneration (data recalculated from 2016 to 1961 for threshold value of DBH = 4 cm) and classes 1 – 3 and the rest of grade 4 as tree layer.

Table 1. Overview of the basic characteristics of permanent research plots (PRP) 1-5.

| | | | | | - | | | - ` | · · | | |
|-----|--------------------------------|-----|---------------|-------------|----------------------------------------------|-----------------|----------|-----------------|------------------------|-----------------|-------------------|
| PRP | GPS | Age | Height [m] | DBH [cm] | Volume [m ³ ha ⁻¹] | Altitude [m] | Exposure | Gradient [°] | Plant associations | Forest type | Soil type |
| 1 | 50°30'05.0" N 16°11'57.6" E | 146 | 22 | 37 | 593 | 425 | SE | 45 | Aceri-Carpinetum | Combined forest | Leptosol skeletic |
| 2 | 50°30'10.5" N 16°12'22.6" E | 162 | 25 | 44 | 591 | 435 | S | 29 | Asperulo-Fagetum | High forest | Cambisol skeletic |
| 3 | 50°29'57.1" N 16°12'17.4" E | 118 | 27 | 35 | 699 | 420 | NE | 36 | Tilio cordatae–Fagetum | High forest | Cambisol skeletic |
| 4 | 50°30'49.8" N 16°12'49.0" E | 148 | 17 | 35 | 612 | 440 | W | 40 | Tilio cordatae–Fagetum | Combined forest | Leptosol calcaric |
| 5 | 50°30'09.5"N 16°12'45.2"E | 73 | 18 | 26 | 340 | 430 | W | 28 | Mercuriali-Fraxinetum | Coppice | Leptosol calcaric |

2.2. Data collection

Midpoints of permanent research plots (PRP) at Kozínek SAC were always located near the central tree of typological plots dating back to 1961. In 2016 Field-Map technology (IFER-Monitoring and Mapping Solutions Ltd.) was used to determine the structure of the tree layer of tree species in forest ecosystems on five PRP of 50×50 m in size (2,500 m²). Using this system, the position of all tree layer individuals of breast height diameter (DBH) \geq 4 cm (to 1 mm accuracy) on five PRP and crown projections at a minimum of four directions perpendicular to each other were localized. In the tree layer tree heights and heights of live crown base were measured by a Vertex laser hypsometer (to 0.1 m accuracy).

In 2016 natural regeneration (seedlings older than 1 year, DBH < 4 cm) was measured on PRP in 10×50 m (500 m²) transects that were representative with regard to regeneration. These characteristics of natural regeneration were measured: position, heights, heights of green crown base and crown width.

Phytocoenological relevés on PRP 1–5 were recorded in 1961 by Dr. Gregor according to the combined Braun-Blanquet abundance/dominance scale as adapted and later published by Zlatník within the particular height layers (Zlatník 1978). The same modified methodology was used in 2016 by prof. Vacek. Scale used for the 1st monitoring according to Zlatník (1978) was fallowing: 1 – predominant trees (dominant), 2 – main level trees, In summary, herb layer on PRP was evaluated in circular plot around the central soil probe covering an area of 490 m² (r = 12.5 m) in both periods (1961, 2016). In 1961, natural regeneration was evaluated in the same circular plot (size 490 m²; 500 m² in 2016). In this year, tree layers were described and evaluated on a larger circular area with radius r = 15 m (size 707 m²; 2,500 m² in 2016).

2.3. Data processing

Tree species and structural diversity were evaluated by following indices: species richness D_i (Margalef 1958) and D_2 (Menhinick 1964), species heterogeneity $\hat{\lambda}$ (Simpson 1949) and H' (Shannon 1948) and species evenness E_i (Pielou 1975) and E_2 (Hill 1973). Structural and overall diversity was evaluated by these indices: Arten-profile index A (Pretzsch 2006), diameter TM_d and height differentiation index TM_h (Füldner 1995), index of non-randomness α (Pielou 1959, Mountford 1961), aggregation index R (Clark and Evans 1954) and total diversity index B (Jaehne and Dohrenbusch 1997). The criteria for these indices are given in Table 2.

The program PointPro (Zahradník, ČZU) was used to calculate the characteristics describing the spatial pattern of the overstorey. The test of significance of deviations from expected values for the random arrangement of points was done by Monte Carlo simulations. Basic stand characteristics and structural diversity of the tree V. Hájek et al. / Cent. Eur. For. J. 66 (2020) 202-217

| Criterion | Quantifiers | Label | Reference | Evaluation |
|---------------------------|--------------------------------|----------------------------|---------------------------------|-------------------------------------------------------------------------------------------------|
| | Species richness | D_1 (Mai) D_2 (Mei) | Margalef 1958 Menhinick 1964 | minimum $D=0$, higher $D=$ higher values |
| Spaciae divarcity | Species beterogeneity | λ(Sii) | Simpson 1949 | range 0 – 1; minimum $\hat{\chi} = 0$, maximum $\hat{\chi} = 1$ |
| Species urversity | Species neterogeneity | H' (Shi) | Shannon 1948 | minimum $H' = 0$, higher $H' =$ higher values |
| | Species evenness | E_1 (Pii) E_2 (Hi) | Pielou 1975 Hill 1973 | range $0-1$; minimum $E=0$, maximum $E=1$ |
| Vertical diversity | Arten-profile index | A (Pri) | Pretzsch 2006 | range $0-1$; balanced vertical structure $A < 0.3$; selection forest $A > 0.9$ |
| Structure differentiation | Diameter diff. Height diff. | TM_d (Fi) TM_h (Fi) | Füldner 1995 | range 0 – 1; low $TM < 0.3$; very high differentiation $TM > 0.7$ |
| Harizantal structure | Index of non-randomness | α (Pi&Mi) | Pielou 1959; Mountford 1961 | mean value $\alpha = 1$; aggregation $\alpha > 1$; regularity $\alpha < 1$ |
| 110112011tal Structure | Aggregation index | R (C&Ei) | Clark & Evans 1954 | mean value $R = 1$; aggregation $R < 1$; regularity $R > 1$ |
| Complex diversity | Stand diversity | B(J&Di) | Jaehne & Dohrenbusch 1997 | monotonous structure $B < 4$; uneven structure $B = 6 - 8$; very diverse structure $B > 9$ |

Table 2. Overview of indices describing the stand structure and their common interpretation.

layer were evaluated by the simulator SIBYLA (Fabrika & Ďurský 2005). Based on the measured dendrometric data stand characteristics including stand volume (Petráš & Pajtík 1991), stocking (Reinike 1933) and canopy density (crown closure and crown projection area) were computed. Localization figures were made in ARCGIS program (Esri). Ellenberg's indicator values (EIV) documenting ecological preferences of species were used for the interpretation of changes in environmental conditions. For each relevé unweighted means of EIV were computed for light, nutrients, moisture, soil reaction, temperature and continentality (Ellenberg et al. 1992). Analyses were processed in R software (R Core Team 2018). The differences in species diversity indices of tree layer, natural regeneration and vegetation cover between 1961 and 2016 and among research plots were separately tested by paired t-test. In addition, canopy of tree layer and herbal cover with species diversity were tested by the Pearson correlation coefficient. Unconstrained principal component analysis (PCA) in the CANOCO program (Šmilauer & Lepš 2014) was used to analyse relationships among species diversity of tree layer, herb layer and natural regeneration and stand characteristics, canopy parameters, soil type, forest type, plant associations and time in order to reveal similarity of five PRP in course of time (1961, 2016). Data were centred and standardized during the analysis.

3. Results

3.1. Tree layer structure

In 2016, the number of live trees ranged from 240 to 720 trees ha^{-1} with the stand density index (SDI) 0.70 \pm 0.09 SD (Table 3). The stand volume was 340 – 699 m³ ha⁻¹ in 2016, the highest stand volume of 699 m³ ha⁻¹ was on PRP 3 and the lowest volume of 340 m³ ha⁻¹ was on PRP 5. The tree layer was composed by three dominant species: European beech (Fagus syl*vatica* L.), Sycamore maple (*Acer pseudoplatanus* L.) and small-leaved lime (Tilia cordata Mill.; Table 3, Fig. 2). Beech was the dominant species on all research plots with the exception of PRP 5. On PRP 2 its volume share was nearly 99% almost without any admixture, on PRP4 than 83%. The maple was dominantly abundant on PRP 5 (42%), while on other plots without PRP 1 (18%) maple did not exceed 4%. Lime was relatively abundant on PRP 3 (35%), while on other plots it reached the maximum of 4%. The share of other tree species (Carpinus betulus L., Picea abies [L.] Karst., Quercus petraea [Matt.] Liebl.)

Table 3. Basic stand characteristics of permanent research plots 1-5 in 2016.

| PRP | Age | Crown closure | Crown projection [ha] | Stand density index | PAI [m ³ h | MAI a ⁻¹ v ⁻¹] | - Tree species ¹⁾ | Height [m] | DBH [cm] | Stand volume [m ³ ha ⁻¹] | Number of trees | Basal area [m² ha ⁻¹] |
|-----|-----|---------------|--------------------------|---------------------|--------------------------|------------------------------------------|------------------------------|---------------|-------------|----------------------------------------------------|--------------------|--------------------------------------|
| | | | | | L | 5 1 | E, beech | 26.2 | 47.9 | 274 | 104 | 18.6 |
| | | | | | | | S. maple | 27.8 | 55.0 | 108 | 32 | 7.5 |
| 1 | 140 | 0.04 | 2.40 | 0.7(| (0 | 4.1 | E. hornbeam | 20.6 | 33.3 | 80 | 88 | 7.6 |
| 1 | 140 | 0.94 | 3.48 | 0.76 | 6.8 | 4.1 | N. maple | 20.6 | 28.4 | 43 | 56 | 3.5 |
| | | | | | | | S. elm | 17.8 | 28.5 | 41 | 68 | 4.3 |
| | | | | | | | E. ash | 23.3 | 31.7 | 28 | 36 | 2.8 |
| 2 | 162 | 0.87 | 2.28 | 0.55 | 7.0 | 3.6 | E. beech | 28.9 | 49.5 | 582 | 184 | 35.4 |
| | | | | | | | E. beech | 28.7 | 38.1 | 417 | 240 | 27.3 |
| 3 | 118 | 0.94 | 3.21 | 0.79 | 8.2 | 5.9 | S-l. lime | 27.4 | 34.8 | 246 | 176 | 16.7 |
| | | | | | | | S. maple | 23.7 | 27.8 | 27 | 36 | 2.1 |
| | | | | | | | E. beech | 16.8 | 38.0 | 505 | 300 | 33.9 |
| 4 | 148 | 0.92 | 2.93 | 0.71 | 6.5 | 4.1 | N. spruce | 27.4 | 43.2 | 69 | 44 | 6.4 |
| | | | | | | | S. fir | 31.3 | 47.9 | 28 | 12 | 2.1 |
| | | | | | | | S. maple | 18.7 | 25.7 | 142 | 288 | 14.9 |
| | | | | | | | E. beech | 18.1 | 32.4 | 53 | 64 | 5.2 |
| 5 | 73 | 0.90 | 2.63 | 0.71 | 8.9 | 5.4 | S. oak | 19.2 | 32.0 | 46 | 64 | 5.1 |
| | | | | | | | S. birch | 17.5 | 23.2 | 27 | 88 | 3.7 |
| | | | | | | | S. fir | 22.1 | 31.7 | 17 | 20 | 1.5 |

Notes: ¹⁾ tree species reaching \geq 5% share.

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Fig. 2. Horizontal structure of forest stands on permanent research plots 1 – 5 in 2016.

ranged from 0 to13%. Average basal area in 2016 was $36.7 - 47.0 \text{ m}^2 \text{ ha}^{-1}$, periodic annual increment (PAI) ranged from 6.5 to $8.9 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ and mean annual increment (MAI) was $3.6 - 5.9 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$.

Spatial pattern of tree layer on PRP 1 was aggregated according to both indices (Table 4), however it was not statistically significant in the case of R. On PRP 2 the horizontal structure of tree layer was regular (not significant by α). On PRP 3 – 5 the horizontal structure of individuals of tree layer was random to regular. The vertical structure of tree layer was distinctly diversified on PRP 1 and 2 according to A, on PRP 3 and 4 it was moderately differentiated and on PRP 5 it was weakly diversified. According to TM_{d} diameter differentiation was medium on all PRP while TM_{μ} height differentiation was low on PRP 1, 3 and 5, and on PRP 2 and 4 it was medium. With respect to tree layer, PRP 1, 3 and 5 represented an extraordinarily diversified stand structure on, while the structural diversity on PRP4 and 2 was considerably lower.

Table 4. Structural diversity of tree layer on permanent research plots 1 - 5 in 2016.

| PRP | A(Pri) | TM_d (Fi) | TM_h (Fi) | α (Pi&Moi) | R(C&Ei) | B (J&Di) |
|-----|--------|-------------|-------------|-------------------|---------|----------|
| 1 | 0.718 | 0.371 | 0.296 | 1.620* | 0.865 | 10.339 |
| 2 | 0.252 | 0.364 | 0.305 | 0.806 | 1.341* | 7.088 |
| 3 | 0.497 | 0.369 | 0.266 | 0.980 | 1.083 | 9.272 |
| 4 | 0.546 | 0.496 | 0.429 | 1.085 | 1.057 | 8.648 |
| 5 | 0.674 | 0.374 | 0.289 | 1.173 | 1.072 | 11.277 |

Notes: A – Arten-profile index, TM_{d} – diameter differentiation index, TM_{b} – height differentiation index, α – index of non-randomness, R – aggregation index, B – total diversity index; *statistically significant (0.95 confidence interval) for spatial pattern – R and α indexes.

3.2. Species diversity of tree layer

The species richness according to tree layer D_1 was high on PRP 1, 3 and 5 and it was medium on PRP 2 and 4 in 1961 (Table 5). Until 2016 a moderate increase of species diversity was observed on all PRP. Tree layer D_2 was medium in 1961 on PRP 1, 3 and 5 and it was low on PRP 2 and 4. Until 2016 its slight to moderate increase to a medium level was found out on all PRP. In 1961 the species heterogeneity according tree layer λ was high on PRP 1, 3, 4 and 5 and medium on PRP 2. Over 55 years it moderately increased on PRP 5, on PRP 2–4 it decreased moderately. Tree layer entropy H' was high in 1961 on PRP 1 and 5, and it was medium on PRP 2–4. Until 2016 it moderately increased on PRP 5, and it decreased slightly to moderately on PRP 2 – 4. In 1961, the species evenness according to tree layer E_1 indicated high biodiversity on PRP 1, 4 and 5, on PRP 2 and 3 medium biodiversity; in 2016 the species eveness was the same on PRP 1 and it moderately decreased on PRP 2 – 5. Tree layer E_2 index indicated high biodiversity on PRP 1 and 3 in 1961 while medium diversity was recorded on PRP 2, 4 and 5. Until 2016 it moderately increased on PRP 3 whereas it moderately decreased on the other plots.

Comparing species diversity of tree layer in 1961 and 2016, forest stands showed several differences. In the course of 55 years, species richness in tree layer increased significantly (parameter D_2 , paired t–test, t = -7.22, df = 4, p = 0.002), but no significant changes were observed in species heterogeneity (p > 0.05 in all cases). In terms of species evenness, there was found marginal insignificance in terms of decrease (parameter E_1 , paired t–test, t = 2.41, df = 4, p = 0.07). Overall, the most substantial change occurred in coppice on PRP 5, where the species diversity increased by 17.3%, conversely high forest on PRP 3 showed its stability in diversity during study period (decrease –0.4%). The highest diversity was on PRP 5 and 1, while the lowest diversity was observed on PRP 2 representing high forest.

3.3. Species diversity of natural regeneration

The density of natural regeneration ranged on studied PRP from 13,880 recruits ha⁻¹ on PRP 4 to 186,462 recruits ha⁻¹ on PRP 2 with increasing share of maples and European ash compared to tree layer. Natural regeneration D_{t} (species richness) was high on PRP 1, 3 and 5 in 1961 whereas it was medium on PRP 2 and 4 (Table 6). Until 2016 it substantially increased on PRP 4, it was the same on PRP 2, on PRP 3 and 5 there was a moderate decrease and on PRP 1 a distinct decrease. Natural regeneration D_2 was medium on PRP 1 in 1961, low on PRP 2 - 4 and very low on PRP 5. Until 2016 it substantially decreased on PRP 1-3 while it decreased moderately on PRP 4 and 5. In 1961 natural regeneration $\hat{\lambda}$ (species heterogeneity) was high on PRP 1, 3, 4 and 5 and medium on PRP 2. Over 55 years it increased moderately on PRP 2 and 4 while vice versa on PRP 1, 3 and 5. Natural regeneration entropy H' was high in

Table 5. Change of species diversity of tree layer on permanent research plots 1-5 from 1961 to 2016.

| | • | - | | | - | | - | | | | | |
|----------------|------|---------|---------------|----------------------|----------------------|---|--------|---------|---|-------------|------------|---|
| PRP | Year | n (ind) | | D ₁ (Mai) | D ₂ (Mei) | | λ(Sii) | H'(Shi) | | E_1 (Pii) | E_2 (Hi) | |
| 1 1961 2016 | 1961 | 8 | | 1.105 | 0.337 | | 0.837 | 1.908 | | 0.917 | 0.892 | |
| | 2016 | 8 | \rightarrow | 1.155 | 0.387 | / | 0.836 | 1.912 | / | 0.920 | 0.885 | 7 |
| 2 | 1961 | 4 | | 0.525 | 0.229 | | 0.477 | 0.911 | | 0.657 | 0.613 | |
| 2 | 2016 | 5 | / | 0.730 | 0.323 | / | 0.394 | 0.823 | 7 | 0.511 | 0.509 | 7 |
| 2 | 1961 | 7 | , | 0.919 | 0.268 | | 0.662 | 1.342 | | 0.689 | 0.693 | |
| 3 | 2016 | 7 | \rightarrow | 0.969 | 0.317 | | 0.620 | 1.175 | 7 | 0.604 | 0.729 | 7 |
| 4 | 1961 | 6 | , | 0.762 | 0.225 | | 0.672 | 1.420 | | 0.793 | 0.653 | |
| 4 | 2016 | 6 | \rightarrow | 0.812 | 0.276 | / | 0.559 | 1.172 | 7 | 0.654 | 0.568 | 7 |
| 5 | 1961 | 7 | | 1.031 | 0.382 | | 0.723 | 1.566 | | 0.805 | 0.690 | |
| 5 | 2016 | 13 | | 1.672 | 0.447 | | 0.794 | 1.975 | | 0.795 | 0.620 | 7 |

Notes: $n - number of species, D_1 and D_2 - indexes of species richness, \lambda and H' - indexes of species heterogeneity, E_1 and E_2 - indexes of species evenness; code abbreviation of species diversity tendency over 55 years: \screw decreasing index value, \screw increasing index value, \screw no change index value.$

1961 on PRP 1, 3-5, and medium on PRP 2. Until 2016 there was a moderate increase on PRP 2 and 4, a substantial decrease on PRP 1 and 5, and a moderate decrease on PRP 3. Natural regeneration E_1 (species evenness) indicated in 1961 high biodiversity on PRP 1, 3-5 and medium biodiversity on PRP 2. Over 55 years it slightly increased on PRP 2 while it moderately decreased on PRP 1, 3-5. Natural regeneration E_2 indicates in 1961 high biodiversity on PRP 1, 3-5 and medium biodiversity on PRP 2. Until 2016 it moderately increased on PRP 2, it was the same on PRP 4, on PRP 1 and 3 it moderately decreased and on PRP 5 it substantially decreased.

Comparing species diversity of natural regeneration in 1961 and 2016, plots showed also several significant differences, similarly such as in tree layer. Comparing PRP at the beginning and the end of the observation period, there were significant decrease in species richness trough all PRP (parameter D_{2} , paired t-test, t=2.8, df = 4, p = 0.049). No significant changes were observed in species heterogeneity (p > 0.05), while marginal insignificance was found in species evenness (parameter E_{1} , paired t-test, t=2.17, df=4, p=0.10). Overall, the most substantial change occurred on PRP 1, where the species diversity decreased by 41.9%, conversely PRP 4 showed its stability in diversity for 55 years (increase 4.2%). The highest species diversity was in coppice with standards on PRP4, while the lowest diversity was observed in high forest on PRP 2.

3.4. Species diversity of herb layer

The species richness of herb layer was high in 1961 on PRP 1-3 and very high on PRP 4 and 5 (Table 7). Until 2016 it increased rather substantially on PRP 1-4, only

on PRP 5 it moderately decreased. Herb layer λ (species heterogeneity) was high in PRP 1 – 5 in 1961. Until 2016 there was a moderate increase on PRP 2 and 3, and it decreased moderately on PRP 1. Herb layer entropy H' was high in 1961 on PRP 4, and medium on PRP 1 – 3 and 5. Until 2016 it increased moderately on PRP 2 – 4, while opposite dynamics were on PRP 1 and 5. Herb layer E_1 (species evenness) indicated in 1961 high biodiversity on PRP 4 and medium biodiversity on PRP 1 – 3 and 5 while in 2016 it moderately decreased on all PRP. Herb layer E_2 indicated in 1961 high biodiversity on PRP 4 and medium biodiversity on PRP 4 and s. Until 2016 it moderately decreased on all PRP. Herb layer E_2 indicated in 1961 high biodiversity on PRP 4 and medium biodiversity on PRP 1 – 3 and 5. Until 2016 it moderately increased on PRP 3 and 5, and it moderately decreased on PRP 1 and 2.

Comparing species diversity of herb layer in 1961 and 2016, PRP showed no significant difference. Species richness showed marginal insignificance in term of increase (parameter D_1 , paired t–test, t = -2.6, df = 4, p = 0.06) and no significant changes were observed in species evenness and especially in species heterogeneity (parameter H', paired t–test, t = 0.03, df = 4, p = 0.98). Overall, the most substantial change in species diversity occurred in high forest on PRP 3 (increase 11.2%), respectively the minimum changes in coppice on PRP 5 (decrease –2.0%), both conversely than in tree layer and natural regeneration. The highest species diversity was on PRP 4, while the lowest diversity on PRP 3 in 1961, respectively on PRP 1 in 2016.

Retreating species were mainly light demanding ones like Veronica chamaedrys agg., Hypericum hirsutum, Melica nutans and Fragaria vesca. Partly disappeared also species typical for broadleaved forests like Lilium martagon, Viola mirabilis and Poa nemoralis and also cold-tolerant species (Actaea spicata, Campanula trache-

Table 6. Change of species diversity of natural regeneration on permanent research plots 1-5 from 1961 to 2016.

| PRP | Year | <i>n</i> (ind) | | D1 (Mai) | D_2 (Mei) | | λ (Sii) | H' (Shi) | | E_1 (Pii) | E_2 (Hi) | |
|-----|------|----------------|---------------|----------|-------------|-------|---------|----------|-------|-------------|------------|---|
| 1 | 1961 | 9 | | 1.227 | 0.345 | | 0.824 | 1.844 | | 0.839 | 0.880 | |
| 1 | 2016 | 8 | У | 0.657 | 0.039 | У | 0.608 | 1.219 | 7 | 0.586 | 0.651 | У |
| 2 | 1961 | 7 | , | 0.831 | 0.189 | | 0.476 | 1.015 | , | 0.522 | 0.517 | , |
| Z | 2016 | 13 | / | 0.824 | 0.025 | У | 0.691 | 1.397 | | 0.583 | 0.735 | |
| 2 | 1961 | 7 | , | 0.967 | 0.214 | | 0.715 | 1.505 | | 0.724 | 0.717 | |
| 3 | 2016 | 11 | / | 0.880 | 0.038 | У | 0.599 | 1.158 | 7 | 0.483 | 0.685 | У |
| 4 | 1961 | 7 | , | 0.767 | 0.140 | , | 0.687 | 1.372 | , | 0.705 | 0.745 | |
| 4 | 2016 | 11 | / | 1.048 | 0.093 | / | 0.750 | 1.612 | | 0.672 | 0.746 | 7 |
| 5 | 1961 | 13 | | 1.301 | 0.095 | | 0.821 | 2.001 | | 0.758 | 0.718 | |
| 5 | 2016 | 13 | \rightarrow | 1.159 | 0.074 | 0.550 | 1.339 | У | 0.522 | 0.433 | 7 | |

Notes: n – number of species, D_j and D_2 – indexes of species richness, λ and H' – indexes of species heterogeneity, E_j and E_2 – indexes of species evenness; code abbreviation of species diversity tendency over 55 years: \searrow decreasing index value, \nearrow increasing index value, \rightarrow no change index value.

| Table ' | Change of | fspecies | diversity o | of herb l | layer on | permanent researcl | h p | lots | 1-5 | from 1 | 1961 | to | 201 | 16 |
|---------|-----------------------------|----------|-------------|-----------|----------|--------------------|-----|------|-----|--------|------|----|-----|----|
|---------|-----------------------------|----------|-------------|-----------|----------|--------------------|-----|------|-----|--------|------|----|-----|----|

| PRP | Year | n (ind) | | D_1 (Mai) | D_2 (Mei) | | λ (Sii) | H'(Shi) | | E_1 (Pii) | E_2 (Hi) | |
|-----|------|---------|---|-------------|-------------|---|---------|---------|---|-------------|------------|---------|
| 1 | 1961 | 19 | | 4.801 | 2.915 | | 0.822 | 2.053 | | 0.697 | 0.680 | |
| 1 | 2016 | 26 | / | 6.759 | 4.091 | / | 0.688 | 1.746 | 7 | 0.536 | 0.633 | 7 |
| 2 | 1961 | 16 | | 3.744 | 1.859 | | 0.792 | 1.866 | | 0.646 | 0.699 | |
| Z | 2016 | 24 | / | 5.008 | 2.415 | / | 0.812 | 2.015 | / | 0.634 | 0.663 | 7 |
| 2 | 1961 | 14 | | 3.150 | 1.778 | | 0.779 | 1.922 | | 0.728 | 0.604 | |
| 3 | 2016 | 23 | / | 4.918 | 2.457 | / | 0.835 | 2.191 | / | 0.699 | 0.639 | <i></i> |
| 4 | 1961 | 33 | | 9.160 | 5.753 | | 0.939 | 3.008 | | 0.860 | 0.799 | |
| 4 | 2016 | 38 | / | 10.374 | 6.387 | | 0.942 | 3.073 | / | 0.845 | 0.794 | 7 |
| - | 1961 | 41 | | 9.033 | 4.479 | | 0.863 | 2.587 | | 0.697 | 0.513 | |
| 3 | 2016 | 40 | 7 | 8.514 | 4.049 | 7 | 0.858 | 2.396 | 7 | 0.649 | 0.605 | |

Notes: $n - number of species, D_1 and D_2 - indexes of species richness, <math>\lambda$ and $H' - indexes of species heterogeneity, <math>E_1$ and $E_2 - indexes of species evenness; code abbreviation of species diversity tendency over 55 years: <math>\searrow$ decreasing index value, \nearrow increasing index value, \rightarrow no change index value.

lium, Abies alba, Picea abies). On the contrary, increase was confirmed in the case of shade tolerant species like *Athyrium filix-femina, Geum urbanum, Viola reichenbachiana* and seedlings of tree species (*Fagus sylvatica, Acer pseudoplatanus, Fraxinus excelsior, Ulmus glabra*). Increase in case of thermophilic plants was also observed (*Heptica nobilis, Isopyrum thalictroides, Acer platanoides, Carpinus betulus*). From nitrophilous species increase was also recorded (*Urtica dioica, Galeobdolon montanum* and invasive *Impatiens parviflora*).

3.5. Relationships among species diversity and stand characteristics

Results of the PCA analysis are presented in the form of the ordination diagram in Fig. 3. The first ordination axis explained 32.8%, the first two axes together 57.3% and the first four axes together 85.1% variability data. The first axis x represented age, volume and canopy of tree layer, but also species richness of regeneration. Second axis y represented regeneration canopy with species evenness of regeneration. All indices of stand species diversity positively correlated to each other, while these parameters were negatively correlated with degree of naturalness (closer to autochthonous stands), regeneration canopy and species evenness of herb layer. Volume, age and canopy of tree layer were increasing in the course of time, while species diversity of regeneration and species richness of vegetation were decreasing in time. Canopy of tree layer were negatively correlated with regeneration D_t (r = -0.70, p < 0.05), λ (r = -0.63, p < 0.05) and H'(r = -0.75, p < 0.01) and regeneration canopy (r = -0.64, p < 0.05). Cover herb was negatively correlated with species diversity of natural regeneration and herb too, especially herb layer λ (r = -0.79, p < 0.01) and H'(r = -0.70, p < 0.05) and regeneration $E_2(r = -0.74, p < 0.05)$ p < 0.01). Species indices of individual layers (herb, tree, natural regeneration) were often very significantly correlated (p < 0.001), in the greatest extent in the tree layer. The dynamics of parameters in the course of 55 years was remarkable especially for coppice stands on the PRP 5, as marks of each record are relatively distant from one another, whereas marks for PRP 2 and 3 representing the high forests were relatively close together in the diagram. Slope and regeneration richness had relatively small importance for the explanation of the data variability. PRP were very different amongst one another; high forests on skeletic cambisol occupied the right part of the diagram typical for higher stand volume and age of tree layer, coppice or combined forest (coppice with standard) on leptosols were characterized by higher species diversity. According to the plant associations the greatest overall species diversity was observed at Mercuriali-Fraxinetum, the lowest at Asperulo-Fagetum.



Fig. 3. Ordination diagram showing results of PCA analysis of relationships among species diversity of tree layer, herb layer and natural regeneration (*E1*and *E2* species richness, *H* ' and λ species heterogenity, *D1* and *D2* species evenness, *Rege* natural regeneration); stand characteristics (*AgeTree* mean age of stand, *VolumeTree* stand volume, *Slope* of plot); canopy parameters (*CanopyRege, CanopyTree, CoverHerb*); soil type (*CambSkel* Cambisol Skeletic, *LeptSkel* Leptosol Skeletic, *LeptCalc* Leptosol Calcaric); forest type (*Coppice, High Forest, Combined* variant of the previous two type), plant associations (*Tilio cordatae-Fagetum, Aceri-Carpinetum, Asperulo-Fagetum, Mercuriali-Fraxinetum*) and time (*Year*). Code abbreviation: • identification of PRP and year of the record (1961, 2016), • plant associations, \blacksquare soil type and \blacksquare forest type.

4. Discussion

4.1. Structure and diversity of tree layer

Based on our results, the stand volume ranged from 340 m³ ha⁻¹ on PRP 5 (coppice forest) to 699 m³ ha⁻¹ on PRP 3 (high forest) in 2016. Similarly, the highest timber production was observed in high forest in Český kras PLA, respectively the lowest stand volume in coppice (Vacek et al. 2019b). In relation to spatial distribution, pronounced variability of the horizontal structure of tree layer has been revealed. On PRP 1 the structure was aggregated, on PRP 2 it was regular and on PRP 3-5 random to regular. Our results are consistent with those of Szwagrzyk & Czerwczak (1993), who studied tree distribution in old-growth forest stands in the Czech Republic and in Poland. They stated that the dominant spatial distribution of trees tends to be between random and regular arrangement. Similar results for trees with diameter at breast height larger than 35 cm from nearnatural forest were reported by Szwagrzyk et al. (1997). Also, von Oheim et al. (2007) reported random distribution of canopy-forming full-grown trees on 8 ha of near-natural forest in north-eastern Germany. Similar results from the beech forest of Sudeten range in the Czech Republic and Poland are reported by Vacek et al. (2014a, 2015c) and Bulušek et al. (2016). The total stand diversity was the highest in coppice (PRP 5), while the lowest was observed in high forest (PRP 2). The lowest biodiversity was also documented in lowland forest in middle Bohemia, although the highest total diversity was found in coppice with standards (Vacek et al. 2019b).

For the tree layer (such as herb layer), the highest number of species was recorded on PRP 5 in 2016. It is a plot in the scree forest that can be classified as overgrown coppice because 58% of the trees originated from shoots. In spite of its lowest age the forest stand on this plot has very high biodiversity. This trend, but less pronounced, was observed on the other plots in scree forests, especially on PRP 1 with 20% of the trees of vegetative origin and on PRP 4 with 8% of the trees of vegetative origin. There was an opposite trend in the herb-rich beech forest stand on PRP 2 (high forest).

On PRP 1 – 4 the number of species in the tree layer has not changed at all. Apronounced change has occurred only on PRP 5, where their number has increased almost to a double, which is caused by taking the relevés of forest stand shortly after coppicing when some species of trees have not grown up to the tree layer yet. Generally, species richness of tree layer significantly increased, while species evenness decreased during the observed 55 years. Comparable trend was observed from lowland stands during forest dynamics of 15 years (Vacek et al. 2019b). Overall, the most substantial change (diversity increase by 17%) occurred in coppice on PRP 5. Conversely, studies from Denmark (Strandberg et al. 2005) and in Italy (Ciancio et al. 2006) showed decreasing biodiversity trend in coppice forests.

4.2. Diversity of natural regeneration

On PRP 2 – 4 number of natural regeneration species increased by 57 - 86%, on PRP 5 it remained the same and on PRP1 it decreased by 11%. The highest increase in the number of natural regeneration species was observed in herb-rich beech forest. On all plots except PRP 5 the number of individuals of particular species of natural regeneration increased several times. The increase of the beech natural regeneration over the past two decades was also documented by many researches in the Sudeten range (Vacek et al. 2015a; Slanař et al. 2017). Expansion of beech can be explained by air pollution load (high SO₂ concentrations) fading from the 80's and 90's (Králíček et al. 2017; Vacek et al. 2017a; Šimůnek et al. 2019) and tree species distribution shift due to climatic change (Kramer et al. 2010; Dulamsuren et al. 2017). The highest species diversity was in coppice with standards (PRP4), while the lowest diversity was observed in high forest (PRP 2).

In the studied locality partial eutrophication of vegetation have taken place since 1961 (Hédl et al. 2010). Among the commonly appearing tree seedlings, newly occur Fraxinus excelsior, Ulmus glabra and Acer sp. that further contribute by their litterfall to eutrophication (Hofmeister et al. 2004). The trend of nutrient increase in the environment is evident from a comparison of EIV for nutrients and from soil analyses. The trend of eutrophication is very frequent in lowland forests (Van Calster et al. 2007; Keith et al. 2009). It is due to two factors that started to act approximately at the same time and that cannot be fully separated from each other. One factor is atmospheric deposition of nitrogen that has increased twice since the mid-20th century (Falkengren-Grerup 1995; Dentener et al. 2006; Hůnová et al. 2017), the other is a change in the management regime (Rackham 2008; Verheyen et al. 2012). Unlike the previous intensive removal of biomass, now organic matter remains in the environment and is accumulated there (Dzwonko & Gawroński 2002). Eutrophication is also fostered by the above-mentioned higher representation of tree species requiring more nutrients in the environment (Hofmeister et al. 2004).

Generally, newly appearing were shade-tolerant trees seedlings. On the other side in relation to climate change, increase occurrence of *Acer platanoides* and *Carpinus betulus* and decrease of *Abies alba* and *Picea abies* was observed in natural regeneration and may be closely related to rising temperature in study area. Decline of *Picea abies* in middle-elevations and increase of broadleaved lowland tree species due to climatic change was observed also in other studies (Falk & Hempelmann 2013; Sedmáková et al. 2019). Similarly, decline of *Abies alba* in changing environmental conditions was documented by Mrkva (1994) and Mikulenka et al. (2020).

The frequently reported impact of red deer on vegetation – in many localities causing severe browsing and bark stripping damage to regeneration (Corney et al. 2008; Rogers et al. 2008; Hédl et al. 2010; Vacek et al. 2015b; Cukor et al. 2019), was not confirmed in our study.

4.3. Diversity of herb layer

In the herb layer the relative level of species richness has increased on all plots except PRP 5. The species heterogeneity on PRP 2-4 has increased moderately, while it decreased slightly to moderately only on PRP 1 and 5. It was proved at the Kozínek SAC that the higher species richness of herb layer is usually characteristic of forest stands with higher tree species diversity. Similar conclusions were drawn by Vockenhuber et al. (2011). Some studies reported positive relations between the diversity of tree layer and herb layer (Ingerpuu et al. 2003; Mölder et al. 2008), others did not find any significant relation (Borchsenius et al. 2004; Houle 2007). In the study of Mölder et al. (2014) a positive relationship between the biodiversity of tree layer and herb layer was demonstrated in the Hainich National Park. In general, compared to coniferous forests higher biodiversity was observed in broadleaved forests (Berger & Puettman 2000; Hart & Chen 2008).

Currently, the herb layer on the studied plots (except PRP 5) is more shaded and trophic than in 1961 thanks to more abundant natural generative regeneration. On PRP 1-5 the number of species in the herb layer increased by 15-64% while on PRP 5 it decreased by 2%. This increase is caused mainly by a high number of seedlings of different tree species. But the total number of species in all relevés on PRP 1-5 has not basically changed. In similar studies a moderate increase in the number of species in a relevé is reported (Thimonier et al. 1994; Wild et al. 2004; Van Calster et al. 2007; Šamonil & Vrška 2008), but mostly a moderate to pronounced decrease was observed (Hédl 2004; Rogers et al. 2008; Naaf & Wulf 2010; Kopecký et al. 2013). There are also studies showing that the number of species did not change in time (Taverna et al. 2005; Keith et al. 2009), which is consistent also with our results.

Scree forests were richer in species number than herb-rich beech forests in the studied locality. However, they were highly variable proportionally to their developmental stage. In scree forests the number of species in the herb layer was a reaction mainly to light quantity when the species diversity was decreasing with increasing overshadowing. In herb-rich beech forests the effect of lower light intensities did not have any influence, but the effect of a change in moisture played its role.

Declining species were mainly light-demanding ones (e.g. Veronica chamaedrys agg.). On the contrary, among the newly appearing ones there were shade-tolerant species (e.g. Athyrium filix-femina, Geum urbanum, Viola reichenbachiana). The majority of the above-mentioned newly appearing species are also plants requiring higher soil moisture. Among the newly appearing species, increased the representation of the species of nutrientrich sites (e.g. Gallium odoratum) (Hofmeister et al. 2004). As a result of natural regeneration graminoids were also declining (mainly Melica nutans and Poa nemoralis). In relation to global warming, increase occurrence of Hepatica nobilis and Isopyrum thalictroides and decrease of Actaea spicata and Campanula trachelium was observed in study area.

In the forest vegetation due to an increase in the canopy closure (frequently caused by advanced natural regeneration) species were prospering that were adapted to overshadowing and specific conditions given by the leaf shedding of broadleaved species (e.g. *Hepatica nobilis*). The adaptation to restricted light access consists in a shift of phenological phases to earlier spring when the trees do not have any leaves yet, which allows the plants to receive sufficient solar radiation even in conditions of overshadowing (Dahlgren et al. 2006; Kopecký et al.

2013). An increase in the number of spring species was recorded by Brewer (1980) but von Oheim & Brunet (2007) reported a decrease.

Average Ellenberg's indicator values did not confirm any change in the moisture characteristics but other studies showed that the restriction of light and radiation into a forest stand decreases evapotranspiration from the undergrowth and the environment becomes more humid (Decocq et al. 2005; Hédl et al. 2010); on the contrary, moisture is decreasing with opening canopy (Hédl 2004).

There was no difference in EIV for the soil reaction, thus we conclude that the influence of acidification has not been evident in the area of Kozínek SAC in the last 55 years. Since the soil reaction has a crucial influence on diversity (Brunet et al. 1997; Hofmeister et al. 2009; Vacek et al. 2017a), an increase in soil acidity is considered as important factors changing the type of forest vegetation (Falkengren-Grerup 1995). The calcareous marlstone bedrock probably plays its role (Mikeska et al. 2000), acting as a buffer against acidification (Thimonier et al. 1994). However, in other areas changes caused by soil acidification were recorded (Hédl 2004; Van Calster et al. 2007). Important factors are atmospheric depositions of compounds, and forest succession and nitrogen accumulation in the environment that contributes to soil acidification (De Schrijver et al. 2006; Hůnová et al. 2017).

4.4. Effect of forest management

It is to state that studied forest stands which were managed by small-scale systems, in the past mainly by coppicing, and later by shelterwood and selection felling, have maintained high species biodiversity during the years of observation. Significantly higher biodiversity was observed in scree forests in comparison with herbrich beech forests. Based on a comparison of PRP 5 (forest with prevailing coppice shoots in the tree layer) and PRP 3 (forest of generative origin) it is to conclude that at sites of scree forests. Such a comparison is not available for herb-rich beech forests because they have always been regenerated generatively.

Similar results from forests where coppicing was abandoned were described by Corney et al. (2008), Rogers et al. (2008), Verheyen et al. (2012). Our results confirm the conclusions about vegetation changes presented by Mölder et al. (2008). Comparing different functional groups of forests Vockenhuber et al. (2011) found that higher tree diversity is consistent with an increased number of flowering herbs. It may be caused by plant functional groups that have different requirements for sources and therefore they have different reactions to gradients of ecological conditions (Tinya et al. 2009). Trees species often influence the herb layer by a change in the availability of resources and ecological conditions in the lower forest storeys (Barbier et al. 2008). It is especially the influence of light availability for the herb layer, which is a result of different light transmittance through the tree crowns, or the influence on the soil structure and its acidity through litterfall and its decomposability (Mölder et al. 2014).

Our study provided results that are many times consistent with similar published researches from other areas in the Czech Republic and in other countries (Hédl 2004; Van Calster et al. 2007; Rogers et al. 2008; Rooney 2008; Baeten et al. 2009; Keith et al. 2009; Bunn et al. 2010; Hédl et al. 2010; Verheyen et al. 2012; Kopecký et al. 2013). At the Kozínek SAC it is mainly a gradual change in forest management, i.e. conversion of coppice forest to high forest that modified the forest environment. This assumption is consistent with Durak (2012), who considered the forest management method as one of the most important factors influencing forest site and stand conditions.

5. Conclusion

The studied nature protected area Kozínek of the Natura 2000 belongs to the most valuable remnants of natural mixed beech forests in the Czech Republic. Biodiversity of scree forests in the past managed as a coppice or coppice with standards was greater than in herb-rich beech forests in both observation periods. There are several potential causes of changes in the biodiversity of scree forests and herb-rich beech forests at Kozínek SAC. The main cause is a change in forest management from coppice forest to high forest while small-scale management methods have been maintained. Climate change has also influenced the species diversity dynamics, but on a smaller scale compared to silviculture practises. In this connection, increase in the population of thermophilic plants was documented. During the 55 years, canopy of tree layer increased and thus a decrease was observed in light-demanding species of herb layer, while population of shade tolerant herb species and seedlings of trees increased. An important role played considerable expansion of natural regeneration. Eutrophisation was also an important factor, especially due to nitrogen deposition. As a result, new species of nitrophilous herbs appeared. The most substantial changes occurred in species richness, especially in its increase in the tree layer. However, limited number of research plots and slight differences of used methods in both periods in this case study must be considered when interpreting the present results.

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ORIGINAL PAPER



A high proportion of Norway spruce in mixed stands increases probability of stand failure

Joerg Roessiger*, Ladislav Kulla, Maroš Sedliak

National Forest Centre – Forest Research Institute Zvolen, T. G. Masaryka 2175/22, SK – 960 01 Zvolen, Slovak Republic

Abstract

The decline of pure spruce forests in the Beskydy Mountains in the Czech Republic and Slovakia is mainly driven by bark beetle attacks and storm events. Salvage-harvest records based on timber volume on the forest-stand level document the extent of stand failure processed by salvage logging. On the basis of these records, the stand failure proportion was expressed as the proportion of timber volume processed by salvage harvest divided by the standing timber volume over a period of 10 years (*sf*). Two null hypotheses to be tested are that *sf* is not influenced by (H1) the size of trees expressed by mean stand age (*age*) or alternatively by mean stand diameter (*dbh*); and (H2) the volume proportion of spruce within the stand (*spp*). The dataset was evaluated as a total and separately for the three site types, each for spruce, beech, fir, larch, pine, and other broadleaved species. The dataset was analysed using a binary logistic regression. The *sf* increased with *age*, *dbh*, and the *spp*. Pure spruce stands with high *age* and high *dbh* on mountain sites are associated with the highest *sf*. However, a slight admixture of 25% tree species other than spruce was shown to stabilise the stands significantly. Species other than spruce in a minor admixture to spruce stands were destabilised by the breakdown of spruce stands. For a realistic financial evaluation of forest-stand management, managers should consider the risk of stand failure, and reduce the risk by increasing species admixture in the spruce forest stands.

Key words: stand failure; risk; forest management planning; stabilisation; probability of survival

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1. Introduction

Old, pure even-aged spruce forests are rapidly declining in the Carpathian Mountains (Hlásny &Sitková 2010; Kulla & Sitková 2012; Hlásny et al. 2017). The initial event was a storm in 2004 that caused wind throws in old spruce stands. Storms in combination with hot dry summers are weakening spruce vitality and encouraging the spread of bark beetle and, thus, the further dying of whole stands on a large scale (Hlásny & Sitková 2010), which has mainly been seen since 2004. Forest management utilises a high amount of salvage cutting, which provokes debates regarding the different perspectives of spruce forest management.

Some general reasons discussed for the high intensity of spruce stand failure in the Carpathian Mountains are as follows:

- Climate change generating hot dry summers and warmer winters;
- Previous forest management strategies expanding spruce to unsuitable sites;
- Low thinning intensity leading to dense stands with short crowns, reducing single-tree stability and vitality;

- The prolonging of rotation periods to higher ages, which leads to exposure to a greater amount of risks;
- Pure, even-aged spruce forests rather than unevenaged forests being mixed with fir and beech.

Nevertheless, from the point of view of a forest manager, spruce is the most productive species due to its fast growth, short rotation cycles, and the cheap, schematic stand treatment. As a result of its high timber quality, spruce is in demand in forest industries. Additionally, because of natural spruce regeneration in the study region, often no planting is necessary. Contrarily, missing seed trees and insufficient or delayed natural regeneration from alternative species like fir and beech creates the need for expensive tree-planting programs. Moreover, adaptive management might even consider intermixing with non-native tree species in the face of shifts in the natural distribution areas caused by climate change (Bolte et al. 2009).

There are alternative solutions in the face of the high frequency of stand failure which will allow for continuous forest management in the future. As a possible solution, mixed rather than pure forests can reduce risks (Gayer

^{*}Corresponding author. Joerg Roessiger, e-mail: rossiger@nlcsk.org

1886; Knoke et al. 2005). "Near-natural forestry", "closeto-nature forestry", or "continuous-cover forestry" are terms which have no exact definition but typically mean the use of natural processes for management purposes and the avoidance of clearcuts.

Admixing of other tree species strongly reduces the risk of spruce stand failure and increases stability. Griess et al. (2012) and Roessiger et al. (2013) demonstrated that even a low admixture of 20% of broadleaved species to a pure spruce stand has a big effect on stabilisation while further admixture did not have an important impact on a stand with a low spruce area proportion. The reasons discussed for the stabilisation effect related to a lower failure risk (Knoke & Seifert 2008; Knoke et al. 2008; Griess & Knoke 2011; Griess et al. 2012) are as follows:

- The different types of roots using different soil horizons (the deeper roots of broadleaved species compared to spruce);
- The deeper and longer crown of broadleaved species, as compared to spruce, blocking the wind;
- The distinct or slower mechanical oscillation frequencies of broadleaved species reducing wind throws and breakage of spruce dominated stands during storms;
- Longer flight distances for bark beetles to reach the next spruce tree reducing beetle vitality;
- More ecological niches for antagonists against bark beetles or pathogens;
- Soil improvement by faster recycling of litter by broadleaved species.

While the mentioned ecological benefits are difficult to measure, the failure probabilities can be quantified with statistical regression models to estimate the countable financial long-term consequences when comparing the forests management options of pure spruce and mixed forests.

A regression model to estimate failure dependent on age based on Weibull function (Staupendahl & Zucchini 2011) was developed to also consider stabilisation of spruce forests by beech admixture for regional studies in Germany (Griess et al. 2012; Neuner et al. 2015) and on a pan-European dataset on crown condition on Level 1 and Level 2 plots (Paul et al. 2019; Brandl et al. 2020). The regional studies from Germany need to also be tested for other regions and conditions, and with a different type of dataset. While all the cited studies used plot-inventory data based on the individual tree level, the new application tests a salvage-harvest records based on timber volume. Existing statistical studies using mortality data in the Czech Republic and Slovakia (Pajtík et al. 2018) and salvage felling in Slovakia (Kunca et al. 2019) report average values but no regression models. Therefore, the new application in this study incorporates species mixture for spruce and other species into regression models for *sf* for the case of the Beskydy Mountains in Slovakia and the Czech Republic, which represent typical conditions in Central/Eastern European mountain forests.

Two null hypotheses to be tested are that stand failure *sf* (expressed by timber volume proportion processed by salvage logging over a period of 10 years) is not influenced by the following:

- H1: the size of trees expressed by mean stand age or alternatively by mean stand diameter;
- H2: the proportion of spruce within the stand.

2. Materials and method

Data from Forest management plans and Forest management records collected within a cross boundary project Interreg BESKYDY were used as the input for the analysis (Consortium Beskydy, 2019). The data cover 71,436 hectare of compact forest in the West Carpathian mountains, lying on both sides of the Czecho–Slovakian border. The project area is represented by the Protected landscape area of Beskydy in the Czech Republic, and the Protected landscape area of Kysuce in Slovakia (Fig. 1).

The territory is made up of flysh rocks with eutric, haplic, gleic, or dystrict cambisols with transitions to podsols in the upper parts, and luvisols in the lower parts. Tree species composition was significantly changed by the management in the past. Pure, even-aged spruce or mixed spruce stands predominate. Other spe-cies domi-



Fig. 1. The location of forest land covered by the data utilised for the analysis.

nating is less common, but there are cases in the foothills and in remote parts of the mountainous region, which were saved from intensive management in the past.

To also evaluate influence of site on stand failure, the territory dataset was split into three site types (Fig. 1):

- A: Mountain acid; including the mountain zone of beech-fir and beech-fir-spruce altitudinal vegetation zone (AVZ) on acid soils, 800 – 1 300 m a.s.l.;
- B: Mountain nutrient-rich; including the mountain zone of beech–fir and beech–fir–spruce AVZ on normal and rich soils, 800 – 1 300 m a.s.l.;
- C: Sub-mountain nutrient-rich; including the foothill zone of beech AVZ on normal and rich soils, 300 – 800 m a.s.l. (Zlatník, 1976).

Furthermore, the dataset was divided into six species groups (Table 1).

Table 1. Tree species composition calculated from standing volume per site type. Other broadleaved species mainly comprise hornbeam and oak.

| Site | Spruce | Beech | Fir | Pine | Larch | Other broadleaved species | Total |
|------|--------|-------|------|------|-------|------------------------------|--------|
| А | 88.9% | 6.5% | 3.7% | 0.3% | 0.2% | 0.3% | 100.0% |
| В | 75.2% | 17.0% | 5.8% | 0.2% | 0.6% | 1.2% | 100.0% |
| С | 56.8% | 24.3% | 8.5% | 5.8% | 2.6% | 2.1% | 100.0% |

A regression model was used to calculate the salvageharvest proportion of standing timber volume (Fig. 2) per species and per stand during the period 2009–2018.

Few errors were found in the original dataset. Illogical values with a *sf* higher than 120%, i.e. a salvage volume higher than the standing timber volume per species were removed from the analyses (652 cases were deleted). Cases with *sf* up to 20% higher than the standing volume were considered to be 100% *sf* regarding the increment between the inventory and the stand failure event, i.e., they were considered as possible errors in measurement (356 cases were corrected). Furthermore, cases with no volume per species, which counts for young stands, were not included (3,797 cases were deleted). Finally, 52,459

single cases were included into regression.

An evaluation using binary logistic regression was chosen, as it constrains the probability between 0% and 100%, it is flexible in terms of varying the steepness of the increase of the function, and it allows the scale to be shifted. The regression was carried out with the general linear model (GLM) function in the R statistical software (R Core Team, 2019). Two models were alternatively applied to test the hypotheses. The dependent variable was stand failure probability and the independent variables were as follows:

Model 1 (M1): age of a species and spp;

Model 2 (M2): *dbh* of a species and *spp*.

$$M1: sf = \frac{1}{1 + EXP(-(c_0 + c_1 * age + c_2 * spp))}$$

M2:
$$sf = \frac{1}{1 + EXP(-(c_0 + c_1 * dbh + c_2 * spp))}$$

Here, *sf* is decenal probability of stand failure (proportion calculated by the salvage-harvest volume divided by the standing timber volume over a period of 10 years) of a species in a stand; *age* is the mean age of a tree species in a forest stand in years; *dbh* is the mean diameter at breast height of a tree species in a forest stand in cm; and *spp* is the relative proportion of spruce volume divided by the total forest-stand volume on the stand level.

Dbh and *age* closely correlate. The reason for the alternative approach based once on *age* and once on *dbh* was to obtain potentially usable stand failure models for both: The common management based on age classes, as well as the close-to-nature or selective management based on diameter classes.

The regression model includes a weighting by total stand area in hectare to consider how representative the stand is in the total area.

Proportion of tree species other than spruce in the forest stand was not included as a further coefficient due





to its negative correlation to *spp* and due to the lower amount of data relate to the higher proportion of admixture of species other than spruce.

3. Results

Stand failure and subsequent salvage cutting increase with *age* and *spp* or *dbh* and *spp* (Fig. 3). This holds true for all the evaluated species groups and on all the sites evaluated. The only limited cases are the insignificant models for pine on sites A and B, other broadleaved species on site A both for M1, as well as pine on site A, and larch on site B both for M2 (Table 2). These cases occurred mainly on sites and for species where the number of cases was small and the increase in the functions was relatively small.

Spruce admixture had the biggest effect on sf for the interval between 100% and 75% spp, while lower spp mainly had a minor impact on sf. In the case of nearly no spp, only spruce on sites A and B both had a high sf of 10% and more for an age of 120 years or a dbh of 50 cm, while for all other cases sf was less than 8%. For nearly no spp and an an age of 120 years pine and other broadleaved species both on site C had a low sf around 2.5%. Nevertheless, beech, larch, and other broadleaved species on site C in a nearly pure spruce stand had a *sf* nearly as high as the sf of spruce. This clarifies that spruce management is always risky for spruce in the mountainous regions of Beskydy. For species other than spruce, management is risky when the species are a minority admixed in a nearly pure spruce stand. In the case of a young age in a nearly pure spruce stands only spruce on sites A and B had a high sf of 6% and 9%, respectively, while all other species had a lower sf.

Sf of spruce was higher on sites A and B (mountain) compared to C (sub-mountain). Contrarily, *sf* of beech, other broadleaved species, and larch was higher on site C compared to sites A and B.

Generally, the results from the two studies regarding *age* (M1) and *dbh* (M2) are strongly correlated. Exceptions occurred in the impact of the site on the *sf* of spruce: *Sf* related to *age* was higher on nutrient-rich site B (*age* 120; *spp* 100%: 34.2%) compared to acidic site A (*age*120; *spp* 100%: 30.0%). Contrarily, spruce *sf* related to *dbh* was higher on site A (*dbh* 50 cm; *spp* 100%: 39.6%) compared to site B (*dbh* 50 cm; *spp* 100%: 37.8%), but only for a high *dbh*.

4. Discussion

The results support the effect of species admixture in improving spruce survival, which had already been demonstrated in other studies (Griess et al. 2012; Neuner et al. 2015; Paul et al. 2019; Brandl et al. 2020). Studies such as those by Knoke et al. (2008), Griess et al. (2012), and Roessiger et al. (2013) interpreted the higher survival as a result of the effect of ecological stabilisation of admixed species on spruce.

The survival of spruce depends on beech admixture, but often studies assume the survival of species other than spruce to be constant and independent of the mixture (Griess & Knoke 2013, Roessiger et al. 2013). The study based in the Beskydy Mountains demonstrated the impact of species mixture, in the form of *spp*, on *sf* from beech, fir, pine, larch, and a mixed category of other broadleaved species. This is in line with various studies which showed the effects of admixture on survival, e.g.,

Table 2. Coefficients of the binary logistic regression analysis estimating proportion of stand failure over a 10 year period for Model M1 and Model M2.

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|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
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| Pine B 159 -7.52 *** 0.0110 4.397 ** 102 -9.01 *** 0.0637 ** 4.814 ** 96 | |
| C 1008 -7.50 *** 0.0319 *** 1.676 *** 555 -8.73 *** 0.1133 *** 1.581 *** 550 |) |
| All 2344 -7.86 *** 0.0279 *** 2.589 *** 870 -7.98 *** 0.0740 *** 2.331 *** 879 |) |
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| C 802 -10.58 *** 0.0530 *** 3.366 *** 296 -10.89 *** 0.1361 *** 3.214 *** 313 | 3 |
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| AIC: Akaike Information Criterion; Significance level: *** 0.001 ** 0.01 * 0.05 . 0.1 | |

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Fig. 3. Binary logistic regression analysis of the volume proportion processed by salvage cutting over a 10 year period for spruce, beech, fir, pine, larch, other broadleaved species dependent on mean age of the species, and dependent on the spruce volume proportion in the stand (Model M1) and dependent on mean diameter (Dbh) of the species, and dependent on the spruce volume proportion in the stand (Model M2), represented for the cases of 0%, 25%, 50%, 75%, and 100% spruce volume proportion in the stand.

for Douglas fir (Brandl et al. 2020) and for beech (Paul et al. 2019). Because currently the stabilising effect of a low spruce admixture on other species is not explained in the literature, an effect other than stabilisation might account for the impact of *spp* on *sf* in other species than spruce.

The region of interest is dominated by pure spruce stands, partly with little admixture, and intensive admixture is only present to a lesser extent in the lower parts and foothills. Consequently, in the case of a spruce stand failure, a small proportion of admixed species is also exposed to the failure and so is likely to be damaged by the breakdown of the nearby pure spruce forest stand. Additionally, salvage logging operations might damage or remove the remaining trees of other species. Even in cases where species other than spruce survive the failure event, they are exposed to further risks. Because spruce dominated stands are typically dense, trees develop short and narrow crowns and are not prepared to survive under free-land conditions. In the following years, the stems of remaining trees are suddenly exposed to the sun on the cleared area and can die through drying or being thrown by the wind.

While the continuous stabilisation effects of broadleaved species on spruce are related to the ecology of species mixture, the destabilisation effect of an increasing *spp* on all the species present is a coincidence caused by a single spruce stand failure event in the neighbourhood. Therefore, the results demonstrate how sensitive species react to destabilising by spruce. Generally, other species demonstrate a lower level of *sf* when growing in a stand with a high *spp*, as compared to spruce in a stand with a high *spp*. This lower *sf* demonstrates that other species better resist against stand failure compared to spruce; furthermore, they survive the harsh conditions during and after a stand failure event.

The study of the Beskydy Mountains used salvage records on the forest-stand level. This is a methodological difference to most other literature, which used inventory data on the single-tree level. Consequently, salvage records are better suited for calculating total stand failure and stand survival probability than continuous single-tree mortality rates in more intense mixtures. Further argument is the time frame of the salvage-harvest records of the Beskydy study region that covers one single, rapid, large-scale, and long-lasting event of spruce decline which affects whole stands. Contrarily, the previous literature used continuous data on larger, state-wide scales, e.g., between one (Griess et al. 2012) and three German states (Neuner et al. 2015), or on the European level (Paul et al. 2019; Brandl et al. 2020), in which such an event is not necessarily applicable.

Higher spruce *sf* in the mountainous region (A and B) compared to the sub-mountainous region (C) might be related to the increasing storm intensity with the altitude above sea level. The higher *sf* of beech and other broadleaved species at the lower altitudes of site C, as

compared to sites A and B, corresponds to the warmer climate, which is better biologically suited for the growth of broadleaved species, when compared with conifers. Consequently, broadleaved species on site C were more dominant, thicker, higher, and therefore more exposed to stand failure compared to broadleaved species at the higher altitudes on sites A and B. The *sf* of the broadleaved species and of spruce might correlate with a higher proportion of the same species on a site.

The inconsistency of spruce *sf* between site A and B, when comparing the evaluation dependent on age with the evaluation dependent on *dbh*, might be related to the fertility of the site: For a lower bonity (poor site A), the dbh and the height are lower compared to a higher bonity (richer site B), given the same age. The main exposure to sf is related to dbh (bark-beetle risk) and to height (storm risk), but only indirectly and secondarily to age. The higher spruce sf on site B compared to site A related to age corresponds to the much lower survival probability on good sites compared to bad sites found by Griess et al. (2012) and confirmed by Neuner et al. (2015) to a lesser extent. The reason for the different order might be that the nutrient-rich site B allowed for better growth compared to the acidic site A, and the higher trees were more exposed to stand failure.

The derived failure rates offer the option to implement them in single-tree models, forest-stand simulators, such as Silva or Sibyla, or in size-class structured (e.g., matrix) models. However, the interpretation of the (complete or partial) stand failure as a continuous single-tree mortality rate technically allows for remaining trees that are benefiting from a decreased stand density. Such a procedure might underestimate the extent of a single failure event in a stand which completely interrupts growth.

Regression models that consider the stabilising ecological effects of a proportion of mixed species on failure rates can be integrated into financial optimisation models that include risks (Griess & Knoke 2013; Roessiger et al. 2013; Neuner & Knoke 2017; Paul et al. 2019). The method is as follows: multiply the probability of stand failure for a given stand situation with the financial net result and weigh this stand part with a reduction factor, e.g., with 50% (Dieter 2001) to represent the damage of the timber, higher harvest costs, and reduced timber prices. Therefore, the failure rates can be used to find an optimal way to transform management, not only involving changing environmental conditions, such as changes in the proportion of species, the climate, or the site, but also to adapt to changing financial net incomes.

Regarding matrix modelling, Roessiger et al. (2019) demonstrated in a sensitivity study about Roessiger et al. (2018) how altered mortality rates and other characteristics can be implemented into forest simulation and optimisation. Liang et al. (2011) integrated the effect of continuous changing climatic temperatures and precipitation into their matrix model to demonstrate shifts in species composition. Furthermore, mortality rates change over time. Pretzsch et al. (2014) reported that beech mortality in Europe declined within the last century while spruce rate was stable. Griess et al. (2012) and Neuner et al. (2015) introduced climate and site factors into survival analyses by including factors such as water supply and nutrient supply into the regression analysis in addition to tree species ratio. The mortality rates sensitive to climate change the increased financial management risk (Neuner and Knoke 2017). Paul et al. (2019) evaluated the effect of beech admixture and climate variables related to temperature and precipitation on survival under expected climate change scenarios within a financial optimisation under risk.

Tree species mixture not only has a positive economic impact on survival but also on growth. The volume growth in mixed stands made up of single-tree or group-wise mixtures in Europe is 10% to 30% faster as compared to neighbouring pure stands (Pretzsch et al. 2010; 2015; Pretzsch & Biber 2016). Nowadays, spruce and beech need a shorter amount of time to reach the states documented in yield tables because of the higher temperatures, longer vegetation times, more nutrients, and higher CO₂ concentrations (Pretzsch et al. 2014). Pretzsch et al. (2020) artificially established summer drought conditions over five years in an experiment by excluding throughfall using a roof. They found that one or more beeches neighbouring a spruce reduced the decline in the growth rate of the spruce and the increase in mortality of the spruce during the drought and allowed for a faster recovery after the drought compared to a spruce in a pure spruce neighbourhood. However, a regional study demonstrated more diverse trends of growth in combination with other events. Bošela et al. (2019) evaluated growth over a hundred years using a tree-ring analysis in Smolícka Osada (Central Slovakia). They found a strong decline in spruce, a slight decline in pine, a slight increase in beech, and a strong recovery for fir, after the declining fir numbers in the 1980s caused by air pollution. The declining effect of light-demanding species such as spruce and pine might also be explained by a change from even-aged to uneven-aged management in the 1950s (Bošela et al. 2019).

5. Conclusion

This study clearly demonstrates an increase in stand failure expressed by salvage-harvest proportion with *age* (alternatively *dbh*) (H1) and spruce proportion (H2) in the stand. This paper indicates how a lower proportion of spruce and a simultaneously increased proportion of admixed species, to levels of 25% and higher, significantly reduces stand failure. Our results are based on data from forests managed using the common system of age classes on the stand level, hence they are best suited for modelling even-aged forest management. As a result of the close correlation between age and dbh on the stand level in even-aged forests, the results might be "carefully" utilised for modelling uneven-aged forest management based on diameter distributions or on the tree level. This might be the only way considering the critical lack of data from real uneven-aged forests within the region, especially during the first phase of transition from an even-aged to a stable uneven-aged management system. After a successful transition, matrix modelling may offer potential solutions regarding how to model and to subsequently develop forest management in the long term for cases like that in the Beskydy Mountains. Nevertheless, future research related to management risk and forest modelling should distinguish between the following two interpretations: (1) the mortality of single trees in the sense of a continuous reduction in the number of stems with a regularly scattered spatial distribution over the stand area; and (2) a large-scale failure event removing a complete stand or part of a stand. A realistic forest model should focus on the impact of large-scale failure in forestry characterised by long-lasting disruptions which disallow a continuous management, decreases and fluctuations in financial net income (including timber prices and costs for harvesting, planting, and silvicultural), and uncertainty regarding forest-management planning.

Acknowledgement

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ORIGINAL PAPER



Physiological vitality of Norway spruce (*Picea abies* L.) stands along an altitudinal gradient in Tatra National Park

Gabriela Jamnická¹, Alena Konôpková²*, Peter Fleischer jr.^{1,2}, Daniel Kurjak², Peter Petrík², Anja Petek², Hana Húdoková^{1,3}, Peter Fleischer², Zuzana Homolová⁴, Marek Ježík¹, Ľubica Ditmarová¹

¹ Institute of Forest Ecology, Slovak Academy of Sciences, Ľ. Štúra 2, SK – 960 01 Zvolen, Slovak Republic

² Technical University in Zvolen, Faculty of Forestry, T. G. Masaryka 24, SK – 960 01 Zvolen, Slovak Republic

³ Technical University in Zvolen, Faculty of Ecology and Environmental Sciences, T. G. Masaryka 24,

SK – 960 01 Zvolen, Slovak Republic ⁴ Research station of Tatra National Park, SK – 059 60 Tatranská Lomnica, Slovak Republic

Abstract

Nowadays, a large area of Norway spruce forest stands in Europe is disturbed by windstorm and, subsequently, bark beetle outbreaks. We investigated the state of three disturbed spruce stands along an altitudinal gradient in Tatra National Park (Slovakia) through various physiological processes. Tree-growth characteristics, the mineral nutrition in the needles, and photosynthetic efficiency were assessed. Two techniques of chlorophyll a fluorescence and analyses of assimilatory pigments were used to detect the changes in photosynthesis functioning. Also, the heat sensitivity of photosystem II was tested. Our results showed that these stands are located in nutrient-poor environments. We recorded similar contents of nitrogen, phosphorus, potassium, sodium, zinc, and iron in all stands. Down the vertical transect, the contents of calcium, magnesium, and manganese significantly decreased and the non-essential aluminium increased. Based on stem circumference measurements, water deficit occurred during the vegetation season in all stands, but with the smallest magnitude highest U-stand. We found some photosynthetic constraints: slightly lower chlorophyll contents in all stands were recorded; however, seasonal dynamics with increasing chlorophyll concentration in the highest U-stand were observed. Moreover, the photochemistry of the lowest D-stand was the most negatively influenced by simulated heat, as the photosynthetic performance index, and the density of the active reactions centres significantly decreased and the values of the K-step and basal fluorescence increased. Therefore, we can conclude the different levels of physiological vitality in these naturally damaged spruce stands, with the best physiological performance of the trees in the highest stand.

Keywords: spruce stand disturbances; heat stress; mineral nutrition; chlorophyll a fluorescence; stem circumference

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1. Introduction

In the context of climate and environmental change, the growth and vitality of Norway spruce, a dominant component of European mountains, may be called into question. Indeed, due to the increased scale of weather extremes and other damaging agents, the health status of spruce stands has considerably worsened in the whole Central European region (Uniyal & Uniyal 2009; Allen et al. 2010; Lindner et al. 2014). Climate-induced physiological stress and interactions with other processes, such as natural disturbances and insect pest gradations, can lead to the mortality of whole spruce populations and can create a major change in forest communities.

Windstorms play a crucial role as primary agents in the Atlantic and Continental temperate zone of Europe (Spiecker 2003; Schütz et al. 2006; Zúbrik et al. 2013). Windstorms and bark beetles (*Ips typographus* and *Pityogenes chalcographus*) have been the most damaging agents for spruce stands in Slovakia within the last two decades. The decline of spruce stands has increased, particularly after windthrows Alžbeta (on 19 November 2004) and Žofia (on 15 May 2014) (Koreň 2005; Fleischer & Homolová 2011; Kunca et al. 2019). The

^{*}Corresponding author. Alena Konôpková, e-mail: alena.konopkova@tuzvo.sk

co-occurrence of increasing drought periods additionally seriously weakens spruce trees, hence their shallow roots and relatively high sensitivity to water deficits (Jyske et al. 2014; Tužinský et al. 2017). Drought-weakened trees are not able to produce enough resin or protective substances to withstand the pressure of bark beetles (Hlásny et al. 2014; Vakula et al. 2015; Kunca et al. 2019). Moreover, there an increasing upward shift in altitudinal outbreaks of bark beetles has been observed (Schwarz & Moravčík 2009).

The physiological processes of such weakened trees are negatively impacted. The ability to conduct water effectively is reduced, and every change in the transpiration stream also influences the transport of nutrients to the foliage (Ditmarová et al. 2007a; Bonan & Van Cleve 1992). The disintegration of adult spruce stands is also promoted by serious nutrient disturbances (Šrámek et al. 2008) and low contents of basic cations (nutrition factors), primarily potassium and partially calcium. The low content of potassium indicates the insufficiency of the water regime in spruce trees (Jokela et al. 1998; Ditmarová et al. 2007a). The nutrient concentrations in conifer needles strongly influence their biochemical capacity for photosynthesis and growth, particularly the impact of N and P on changes in the structure and function of the photosynthetic apparatus (Fredeen et al. 1990; Chen et al. 2013). Also, the interaction of Al, Mn, and other risk elements may mirror the conditions in the air and soil. Needle biomass productivity may decrease, and if the defoliation is not temporary, it can bring about strong negative effects in photosynthetic efficiency and its related processes.

Chlorophyll a (*Chl a*) and b (*Chl b*) represent important pigments for the primary reaction of photosynthesis (Baker 2008). *Chl a* and *Chl b* absorb sunlight at different wavelengths, leading to the assumption that the total leaf chlorophyll content (*Chl a+b*) and the allocated ratio (*Chl a/b*) directly influence the photosynthetic capacity of plants. Consistently, the anti-oxidative mechanism of spruce needles also depends on the concentration of carotenoids. Low values of the *Chl a/b* and *Chl/Car* ratios manifest a weakened photo-protective function of the photosynthetic apparatus. However, it is still unsure how leaf *Chl* content varies among plant species, plant functional groups, and communities in natural forests, especially on a larger scale (Croft et al. 2017; Ying et al. 2018).

Recently, slow and fast chlorophyll *a* fluorescence (*Chl a* fluorescence) kinetics and the JIP test (Strasser et al. 2000) have become popular methods for the rapid screening of stress effects on the physiological processes of trees. In comparison to the basic fluorescence parameters, these measurements provide additional information on the photochemistry of photosystem II (PSII) and the photosynthetic electron transport chain. *Chl a* fluorescence techniques can also provide new insights into

the fundamental process of photosynthesis for forestry purposes (Kalaji et al. 2014; Bussotti et al. 2020).

In this study, we investigated the state of naturally disturbed spruce forest stands along the vertical transect of Lomnicky peak in Tatra Mountains, Slovakia, by analyzing their physiological traits. Using the data from three forest stands affected by a windstorm in 2004 and subsequently damaged by spruce bark beetles, we identified the tree-growth characteristics and the mineral nutrition in the needles, as well as explored variations in the photosynthetic efficiency through quantitative and qualitative analyses of photosynthetic pigments and chlorophyll *a* fluorescence. The main objectives of this study were: (1) To evaluate the changes in the stem circumference of spruce stands along the transect; (2) to compare the state of the mineral nutrition and contents and ratios of assimilatory pigments in the needles; (3) to test the PSII performance and thermostability using two fluorescence techniques, namely slow kinetics-rapid light curves (RLCs)-and fast kinetics of *Chl a* fluorescence; and (4) to investigate how the assimilatory pigments and Chl a fluorescence vary during the season in spruce forests of different vitality levels.

2. Material and methods

2.1. Transect description

The study sites are located along vertical transect on the south-east slope below the Lomnicky peak in Tatra Mts. (inner western part of the Carpathian Mountains), which are naturally covered by Norway sprucestands (Fig. 1). The lowest site (D) is located at 1,100 m a.s.l., the middle (M) at 1,300 m a.s.l., and the highest (U) at 1,500 m a.s.l.; the basic characteristics of individual sites are displayed in Table 1.

Due to occasional downslope winds, the age and structure of the forest differ in lower and higher altitudes. Mostly old growth pure spruce stands (over 120 years) dominate above 1,300 m and successional forests with European larch and Scots pine admixtures at lower elevations. Shallow stony podzols are typical at higher altitudes on steep slopes and deeper dystric cambisols in mountain foothills formed by moraines. According to the closest meteorological stations, the annual average air temperature ranges between 5.3 °C (Tatranska Lomnica, 830 m a.s.l.) and 1.6 °C (Skalnate Pleso, 1,360 m a.s.l.) and the average yearly precipitation from 800 to 1,200 mm.

The health status of the forests of the Tatra Mountains has been deteriorating for decades. In the past, the key factor was distance and ozone pollution. Recently, strong winds and especially bark beetle attacks stimulated by warmer and drier conditions have caused an unprecedented decline in health.

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Fig. 1. Forest spruce stands along the vertical transect below the Lomnicky peak in Tatra Mountains, Slovakia.

Table 1. Description of the studied sites – three forest spruce stands along the vertical transect: the D–stand (lowest), the M– stand (middle) and the U–stand (upper).

| Spruce | Altitude | Exp. | Slope | Surface/vegetation | Soil | Age | Stocking | Tree species composition | Defoliation |
|--------|------------------|------|--------|--------------------------------------------------------------------|------------------|---------|----------|-------------------------------|-------------|
| stand | stand [m a.s.l.] | | (deg.) | Sanaco regenation | 0011 | [years] | otooning | [%] | |
| D | 1,100 | SE | 10 | stony surface | Dystric Cambisol | 100 | 0.8 | spruce 65 pine 15 larch 25 | 43 |
| М | 1,300 | SE | 35 | boulder surface | Dystric Cambisol | 165 | 0.4 | spruce 70 larch 30 | 42 |
| U | 1,500 | SE | 35 | boulder surface, high occurrence of mosses and Vaccinium myrtillus | Podzol | 165 | 0.4 | spruce 70 larch 25 rowan 5 | 33 |

2.2. Meteorological data

Environmental variables (air temperature and humidity, solar radiation in 2 m above ground) were recorded every 20 minutes by automatic meteorological stations (Minikin, EMS Brno, CZ) on the D and U sites and precipitation by automatic ombrometer (EMS Brno, CZ) on the D site. Gypsum blocks (EMS Brno, CZ) were installed on all study plots in 15 cm depth to measure soil water potential (SWP) once an hour.

2.3. Stem circumference measurements

To describe tree water status as an indicator of tree vitality, we measured variations in stem circumference. Stem changes were recorded with high-resolution automatic band dendrometers (model DRL 26, EMS Brno, CZ, accuracy of $\pm 1 \mu$ m) that were installed on 30 sample trees (10 trees per plot). The dendrometers were installed in April 2017. On the U plot the tree no. 31 was attacked by bark beetle. Visible symptoms of dieback (strong discoloration and defoliation) occurred in mid-August 2017. Despite this fact dendrometer recording continued in order to identify dead wood response to environmental factors. To ensure the close contact of dendrometer bands with tree stems and to reduce the influence of bark swelling and shrinkage, the outermost part of the bark was carefully removed before the installation of the dendrometers. For the purpose of this study, only the period from the 12th of April to the 6th of September 2018 was taken into account. The circumference measurements were recorded in 20-min intervals, which allowed to extract water deficit (Δ W) as a proxy for tree water status. Water deficit was calculated from growth-detrended dendrometer records according to Ehrenberger et al. (2012).

2.4. Physiological data collection

Physiological measurements and sampling for pigment analysis were carried out on 10 individual trees per plot at the beginning of June and at the end of August 2018. The needles for the mineral nutrition analyses were collected at the end of August, according to the general recommendation that foliage should be sampled for mineral diagnostic purposes in the late summer, autumn, or winter, thus not in the period of intensive growth. Such sampling reduces the influence of stored carbohydrates and improves the nutrient status after growth has ceased (Linder 1995).

Sampling was performed with respect to minimizing the variability based on the age of the needles and their position within the crown. The needles of 1-year-old shoots were collected from sun-exposed branches from the upper part of the crown by professional climbers. The samples were immediately packed into plastic bags and put into a transportable refrigerator cooled to 15 °C to avoid dehydration or overheating during the transport to the laboratory. The measuring was done next day; hence the parameters of chl *a* fluorescence are relatively stable and can be reliably measured up to two days after sampling without significant changes in PSII photochemistry (Húdoková et al. 2017).

2.4.1 Analysis of the mineral nutrition in spruce needles

The contents of the individual mineral nutrients in the needles were determined from dry mass obtained by drying ground samples at 60–70 °C for 48–72 hours in an oven. Dried needle samples could be stored for longer period without deterioration occurring. Nitrogen (N) elemental analysis, which uses a thermal conductivity detector (the EA-TCD method), was used. The method of atomic emission spectrometry with inductively coupled plasma (AES–ICP) was used to determine the amounts of phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), manganese (Mn), aluminum (Al), boron (B), zinc (Zn), and copper (Cu). The analyses were performed in accredited the Central Forestry Laboratory of National Forest Centre, Zvolen, Slovakia.

2.4.2 Quantitative analysis of the pigments

A homogenized sample mixture (1 g) of spruce needles from each of the 10 individual trees per studied site were analyzed as 80% acetone extracts. The chlorophyll contents (*Chl a*, *Chl b*, and *Chl a+b*) and total carotenoids (*Car x+c*) were determined by spectrophotometry (*Cin*tra, GBS Australia) at 470, 646, and 663 nm and were calculated according to Lichtenthaler (1987). The pigment contents were related to the dry mass unit (mg.g⁻¹).

2.4.3 Fast kinetics of chlorophyll a fluorescence

The needles were dark-adapted for 30 min using the leaf clips, and then chlorophyll *a* fluorescence was excited by a saturation pulse with an intensity of 3,500 µmol m⁻².s⁻¹ for 1 s. A plant efficiency analyzer, namely, the Handy PEA (Hansatech Ltd., UK), was used for the OJIP transient measurements, which were analyzed based on the JIP test (Biolyzer 5 software, Laboratory of Bioenergetics, University of Geneva, Switzerland). We determined the basic fluorescence parameters: The basal fluorescence (F_0), measured 50 µs after illumination and the maximal quantum yield of PSII F_v/F_m , calculated as the ratio between the variable fluorescence ($F_v = F_m - F_0$) and

$$Wk = \frac{F0.3 - F0}{F2 - F0}$$
[1]

where $F_{0.3}$ and F_2 represent the fluorescence measured at 0.3 and 2 ms, respectively, and F_0 is the basal fluorescence (Strasser et al. 2000; Stirbet et al. 2018; Lazár et al. 1999).

2.4.4 Rapid Light Curves

RLCs were recorded using a fluorimeter Pam-2500 (Waltz, Germany). The measurements consisted of nine levels of actinic illumination with increasing intensities from 5 to 2,018 µmol m⁻².s⁻¹ and a duration of 30 s. The illumination periods were separated by a 1-s saturating flash with an intensity of 14,038 µmol m⁻².s⁻¹. RLCs for the electron transport rate (*ETR*), non-photochemical quenching (*NPQ*), and the effective quantum yield (ϕ_{PSII}) were measured. All curves were quantified as the sum of the individual points of the curve.

2.4.5 PSII sensitivity to heat stress simulation

The measurements were conducted at room temperature (25 °C) and stressing temperature (48 °C). The temperature of 48 °C was chosen as a threshold temperature in which the changes of PSII photochemistry certainly occur (Konôpková et al. 2018; Kurjak et al. 2019), and it is still lower than 50 °C, which can affect the results because of depigmentation and overall disorganization of PSII (Yamane et al. 1997).

Heat stress was simulated using a WNE22 water bath (Memmert, Germany). The shoot was enclosed in a glass Erlenmeyer flask and exposed to a temperature of 48 °C for 30 min by immersing the flask in a water bath. All measurements were repeated twice per individual and the values were averaged. The severity of heat stress was calculated for each determined parameter as the ratio between the value measured at 48 °C and the control value measured at room temperature ($R_{FV/Fm}$, R_{PI} , $R_{RC/ABS}$, R_{ETR} , R_{NPQ} , $R_{\phi PSIP}$, R_{FQ} , and R_{Wk}).

2.5. Data analysis

Mean of 10 trees on each plot was used for further dendrometric analysis. The raw dendrometer data were used to construct a "growth line," which represents the tree stem volume under fully hydrated conditions (Oberhuber et al. 2015). The growth line connects the maximum daily value with the next equal or bigger value. During the increment phases, the growth line followed the slope of the original dendrometer records. Tree water deficit $(\Delta W, \text{mm})$ was determined as a difference in stem size relative to the fully hydrated conditions $(\Delta W=0)$.

Identification of significant periodicities in the seasonal course of stem circumference variation, ranging from hours to weeks, was performed by wavelet analysis (Percival & Walden 2000; Torrence & Compo 1998). We used the WaveletComp R package (Rösch & Schmidbauer 2018) for the Morlet transformation, allowing a distinction between random and periodic fluctuations. The Morlet analysis in our study was based on average stem circumference variation per species. The output was a time scale plot, where the x- and y-axes represent the position along the time and periodicity scales, respectively, and the color contour at each x/y point represents the magnitude of the wavelet coefficient at that point. To detrend the stem circumference variation for growth, we used the Weibull function. Detrended data were used in the Morlet analysis where the lower period was set to 20-min intervals, while the upper to 28 days. To explore the influence of climatic variables, data were detrended according to Oberhuber et al. (2020) using fast Furrier transform low pass filter (further termed daily detrended stem circumference variation, DDSCV). We used Pearson correlation statistics to estimate relationship among DDSCV and environmental variables of living and dead trees.

Variability of the mineral nutrition data among the forest spruce stands was tested by one-way analysis of variance (ANOVA). The data of the photosynthetic pigments and Chl a fluorescence were tested by two-way ANOVA with the factors of the seasonal period and the forest spruce stand. Subsequently, the differences were tested by the post-hoc HSD Tukey's test. Prior to the test, the normality of the data distribution was tested by the Shapiro-Wilk test. The estimated parameters were separated into two groups, namely, mineral nutrition and parameters directly related to the photosynthesis performance. These were subjected to principal component analysis (PCA) to identify the general trends of the multi-dimensional data sets. The statistical analyses were performed using the Statistica 7 program (StatSoft, Tulsa) and the R 3.6.3 software (R Core Team, Austria).

3. Results

3.1. Variation in stem circumference

The peaks in dendrometer data occurred in mid-July on all study sites (Fig. 2A). Rapid decline of derived ΔW coincided with these peaks. No changes occurred in dead tree records (Fig. 2C). The observed increase in the negative values of ΔW due to dehydration of the storage pools indicates increasing tree water deficit. Water deficit derived from band dendrometer records was lowest in the U–stand during the study period, followed by the M–stand and then the D–stand. The results can be divided into two separate periods: The first lasting from the beginning of May until mid-July with only a slight water deficit, and the second from mid-July until the beginning of September with a more pronounced water deficit. In the second period, the water deficit in the D– and M–stands was roughly twice that measured in the U–stand.

SWP values on the M and U sites was close to zero during the entire study period indicating favourable soil moisture condition (Fig. 2D). The correlation between SWP and ΔW was very low 0.23 on the M and 0.02 on the U site. On the lowest D site, the correlation was notably higher (0.80). The course of water deficit was synchronous on all study sites with pronounced peak in mid-July. DDSCV data showed weak and non-significant correlations to climatic variables. The exception was RH on the plot D (r = 0.27) and GR on dead tree on the U plot (r =0.18). Correlation of DDSCV values among study plots was high (r > 0.9), contrary to negative correlations with dead tree (r < -0.45). When comparing the DDSTCVs of living and dead trees we observed remarkably lower amplitude in dead tree reaching less than 10% of living ones.

When inspecting the Morlet wavelet power spectra (Fig. 2B), we found a few significant periodicities in the region from 16 to 32 hours. These daily periodicities are of particular interest, reflecting the presence of increased diurnal variations, leading to the typical behavior of drought-exposed trees when the swelling phase was not able to compensate the daily shrinkage, which led to more long-term contractions.

3.2. Needle chemical composition

The concentrations of the individual elements are provided in Table 2, in which they were also evaluated and compared to the values arithmetical mean for spruce foliage under European conditions and the limit values of the optimal nutrition for spruce trees (Linder et al. 1995; Vrana et al. 1997; Stefan et al. 1997; Szaro et al. 2002; Maňkovská et al. 2002; Quesnel et al. 2006).

The contents of N (12,400–14,000 mg.kg⁻¹), P (1,100–1,200 mg.kg⁻¹), and K (4,000–4,600 mg.kg⁻¹) did not differ among the spruce stands along the transect (Table 2). Although the N/P concentration ratios (10.33–11.6) did not decrease below 10, indicating that phosphorus was the growth-restricting element, the contents of these elements (mainly potassium) are still under the limit of optimal nutrition for spruce stands (i.e., N: 14,000–18,000; P: 2,000; and K: 9,000). Also, the ratios of K/N (D–stand: 32.7%; M–stand: 32.3%; and U–stand: 36.2%) and P/N (D–stand: 8.7%; M–stand: 9.7%; and U–stand: 8.5%) are below the optimal values, i.e., 50% and 14%–15%, respectively.

Regarding the concentrations of Ca and Mg in the needles, these were found to be lower in the D–stand much (3,900 and 590 mg.kg⁻¹, respectively) than the



Fig. 2. Stem circumference variation with its relations for three spruce stands: A) growth-line, water deficit (ΔW) and stem circumference variation; B) Morlet wavelet power spectra; C) morning water deficit with confidence intervals for D–, M–, U– stands and dead tree; D) course of soil water potential on D–, M–, U–stands; E) precipitation on D–stand and air temperature on D– and U–stands.

Table 2. Concentration of the elements in 1-year-old needles of *Picea abies* L. Arithmetical means $(mg.kg^{-1})$ with their standard deviations. Different letters indicate statistically significant differences among spruce stands. Values in bold face are exceeded (+) or significantly lowered (-) than the arithmetical mean for spruce foliage under European conditions. *-limit value for risk elements.

| Element | D-stand (1,100 m a.s.l.) | | M-stand (1,300 m a.s.l.) | | U-stand (1,500 m a | a.s.l.) | European arithmetical mean | |
|---------|--------------------------|---|---------------------------|---|--------------------------|---------|----------------------------|--|
| N | 13,950±1.436ª | | 12,389±1,321ª | | 12,461±2,144ª | | 13,900 | |
| Р | 1,218±125.8ª | _ | 1,207±135.9ª | _ | 1,061±223.0ª | _ | 1,500 | |
| K | 4,558±599.7ª | _ | 4,001±334.3ª | _ | 4,505±904.6 ^a | _ | 6,700 | |
| Ca | 3,927±1,326ª | _ | 5,325±1,567 ^{ab} | | 5,952±2,003 ^b | | 5,000 | |
| Mg | 594±230.2ª | _ | 941±300.1b | _ | 714±158.8 ^b | - | 1,200 | |
| Zn | 15.1±5.9ª | _ | 21.6±10.9 ^a | _ | 22.7±6.4ª | _ | 45 | |
| Fe | 74.4±8.2ª | | 70.0±13.6 ^a | | 64.7±16.9ª | | 50-150 | |
| Mn | 312.5±108.9ª | | 641.7±173.0 ^b | + | 1,115±430.8° | + | 500 | |
| В | 23.0±6.1ª | | 29.3±5.8 ^b | | 20.7±3.9ª | | 22 | |
| Cu | 3.8±0.5 ^b | | 3.0±0.3ª | | 3.3±0.7 ^{ab} | | 3–4 | |
| Na | 28.6±2.7ª | | 27.8±2.6ª | | 24.7±4.8 ^a | | <100* | |
| Al | 198.4±31.5 ^b | + | 118.5±27.7ª | + | 112.0±23.5ª | + | <100* | |

values for the optimal nutrition of spruce, i.e., 6,000 and 1,200 mg.kg⁻¹, respectively. Similarly, the content of Mg in the M–stand (940 mg.kg⁻¹) and the U–stand (714 mg.kg⁻¹) was lower compared to the optimal values. However, the content of Ca was quite optimal, namely, 5,950 mg.kg⁻¹ in the U–stand and 5,330 mg.kg⁻¹ in the M–stand.

The Zn concentrations only reached about 15.1 mg.kg^{-1} in the D–stand and 22.7 mg.kg^{-1} in the U– stand (Table 2). The optimal concentrations for spruce needles are reported to be in the range of 40–60 mg.kg⁻¹. The concentrations of Fe (64.72–74.38 mg.kg⁻¹) were found to be similar to the average level for growing spruce in Europe (50–150 mg.kg⁻¹). Meanwhile, the Mn concentrations were found to be on par with the European spruce average (500 mg.kg⁻¹) only in the M–stand (642 mg.kg⁻¹). In the D–stand, the value was below average (313 mg.kg⁻¹), and on the contrary, it was above average in the U–stand (1,115 mg.kg⁻¹). The Fe/Mn ratios found in all of the spruce stands indicate rather disproportionate amounts of these two elements, since the ratio should be 1 : 2. The content of B can be assessed as satisfactory in all spruce stands (21–29 mg.kg⁻¹), with the highest concentration found in the M–stand. The value of Cu in all three spruce stands was found to be optimal within 3 mg.kg⁻¹. The sodium concentration (24.66–28.59 mg.kg⁻¹) in these forest stands was found to be lower than optimal of average value for growing spruce in Europe (30–100 mg.kg⁻¹) (Table 2).

As the allowable limit of Al in the foliage of forest tree species is 100 mg.kg⁻¹, it was very excessive in the D–stand (198.4 mg.kg⁻¹) (Table 2). Also, in the M– and U–stands, the concentrations were slightly higher (119 and 112 mg.kg⁻¹, respectively).

The state of the mineral nutrition in the needles of *P. abies* was also analyzed using PCA (Fig. 3). The main nutritional elements - N, P, and K - formed one group, which is not correlated with either the Zn, Mg, Mn, and Ca group or Al. The N, P, and K group is almost perpendicular to the clusters formed around the average values (i.e., the central point in the figure), hence there should be no statistically significant differences between these groups of variables in the studied stands. The same



Fig. 3. The principal component analyses showing relationships of nutritional elements to the studied spruce stands. The ellipse represents 95% confidence intervals around the centroid (bigger symbol) of each data cluster. The lowest D–stand of 1,100 m a.s.l. is presented by red cluster, the middle M–stand of 1,300 m a.s.l. by yellow cluster and the highest U–stand of 1,500 m a.s.l. by green cluster.

could be stated for Fe and Na. These independent relationships were confirmed by insignificant differences in the ANOVAs for above-mentioned elements (Table 2). Moreover, the M–stand especially follows the line of boron, and we can see a strong affinity of the D–stand to non-essential aluminum.

3.3. Photosynthetic pigment contents and ratios

The analysis of assimilatory pigments showed lower concentrations of chlorophylls and carotenoids, especially in the M–stand (Fig. 4). The highest spring concentration of photosynthetic pigments was found in D–stand from the lowest altitude of the transect; however, the overall highest amounts of chlorophylls and total carotenoids were recorded in late summer at the highest located U– stand (Fig. 4a,b). We recorded similar values of *Chl a/b* (3.5–3.8) and low values of *Chl/Car* in plot M (4.0–4.4) in late spring (Fig. 4c,d).

Interestingly, positive seasonal dynamics of chlorophyll and carotenoid contents were observed in the U– stand. Of note is also the fact that the seasonal dynamics of the photosynthetic pigments in the spruce needles in the D–stand were suppressed and the values were uniform during the vegetation season.

3.4. Photosystem II performance and thermostability

The parameters of the fast *Chl a* fluorescence kinetics were relatively stable. No differences in F_{v}/F_{m} among the plots over the whole measuring period were found; however, there were statistically significant differences between the D–stand with the lowest F_{v}/F_{m} , ratio (0.800) in August and the U–stand with the highest one (0.834) in June (Fig. 5). *RC/ABS* and *PI* were similar for all forest stands in June, as well as in August.

The measurements of the slow *Chl a* fluorescence kinetics were found to be more sensitive toward stress. The highest ϕ_{PSII} and, consequently, the highest *ETR* and the lowest *NPQ* were found in the U–stand in the spring (Fig. 6). Moreover, in the U–stand, seasonal dynamics were observed, with a significant decrease in the *ETR* and ϕ_{PSII} parameters during the late summer.

We simulated heat stress (up to 48 °C) in order to reveal differences in the response of PSII to increased temperature. The values of $R_{Fv/Fm}$ were slightly lower for samples measured in June compared to August, but no statistically significant differences between the months or stands were confirmed (Fig. 7). The ratios of R_{pp} , $R_{RC/}$ $_{ABS}$, R_{ETR} , and R_{NPQ} decreased to below 1, indicating a higher level of stress. These values were the lowest in the spring samples from the D–stand, and the highest in the late summer samples from the U–stand. On the contrary,



Fig. 4. Concentrations (mg. g^{-1}) of a) a+b chlorophylls, b) x+c carotenoids and their ratios: c) a/b, d) Chl (a+b)/Car (x+c) in spruce needles on the studied stands. The graph's bars with spruce stand's means and standard errors. Different letters indicate statistically significant differences among groups. Season is identified by different colors: the beginning of June (late spring) – dark grey color, the end of August (late summer) – light grey color.





Fig. 5. Parameters of fast kinetics of chlorophyll *a* fluorescence: a) F_v/F_m , b) *RC/ABS*, c) *PI*. The graph's bars present spruce stand's mean (relative unit) with standard errors. Different letters indicate statistically significant differences among groups. Season is identified by different colors: the beginning of June (late spring) – dark grey color, the end of August (late summer) – light grey color.



Fig. 6. Fluorescence parameters of Rapid Light Curves (RLCs): a) ϕ_{PSIP} b) *ETR*, c) *NPQ*. The graph's bars present spruce stand's mean (relative unit) with standard errors. Different letters indicate statistically significant differences among groups. Season is identified by different colors: the beginning of June (late spring) – dark grey color, the end of August (late summer) – light grey color.

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Fig. 7. The ratios of fluorescence parameters after heat stress simulation (the value measured at 48 °C) and before stress simulation (control value measured at room temperature 24 °C): a) $R_{FV/Fm}$, b) R_{Pl} , c) $R_{RC/ABS}$, d) R_{ETR} , e) R_{NPQ} , f) $R_{\phi PSI/P}$ g) R_{F0} , h) R_{Wk} . The graph's bars present spruce stand's mean (relative unit) with standard errors. Different letters indicate statistically significant differences among groups. Season is identified by different colors: the beginning of June (late spring) – dark grey color, the end of August (late summer) – light grey color.

the values R_{Wk} and R_{F0} describe opposite behaviour compared to previously mentioned parameters. Therefore, as expected, the lowest values of R_{Wk} were detected in the U-stand and the highest in the D-stand. Noteworthy, the highest R_{F0} was found in the D-stand in June, and also the lowest R_{F0} with the highest ϕ_{PSII} was found in the same stand in August.

Based on the PCA, we captured the seasonal variability of PSII performance. In June, there were remarkable differences among the forest stands along the Lomnicky peak transect (Fig. 8). The D–stand (lowest) followed mainly chlorophylls, carotenoids, R_{ETR} , and $R_{\phi PSII}$ after heat simulation, but also NPQ before and after heat simulation. Meanwhile, the M–stand (middle) followed mainly $R_{RC/ABS}$ and R_{PI} after heat stress, and the U–stand (highest) showed significant *ETR* and ϕ_{PSII} . The parameters of *Fv/Fm*, *RC/ABS*, *PI*, and R_{Wk} stayed uniform, without clear inclination toward a certain forest stand. In the late summer, the forest stands were more overlapped and showed more uniform functioning (Fig. 9). The significance of the assimilatory pigment content for the D–stand weakened, while the performance of the photosystems under normal conditions stayed, more or less, constant among stands, with only post-heat stress differences being slightly noticeable. The significance of R_{F0} and R_{Wk} increased for the D– and M–stands; hence, the significance of R_{PI} and $R_{RC/ABS}$ increased for the U– stand.



Fig. 8. The principal component analyses showing relationships of chlorophyll pigments and chlorophyll *a* fluorescence parameters to the studied spruce stands at the beginning of June (late spring). The ellipse represents 95% confidence intervals around the centroid (bigger symbol) of each data cluster. The lowest D–stand of 1,100 m a.s.l. is presented by red cluster, the middle M–stand of 1,300 m a.s.l. by yellow cluster and the highest U–stand of 1,500 m a.s.l. by green cluster.



Fig. 9. The principal component analyses showing relationships of chlorophyll pigments and chlorophyll *a* fluorescence parameters to the studied spruce stands at the end of August (late summer). The ellipse represents 95% confidence intervals around the centroid (bigger symbol) of each data cluster. The lowest D–stand of 1,100 m a.s.l. is presented by red cluster, the middle M–stand of 1,300 m a.s.l. by yellow cluster and the highest U–stand of 1,500 m a.s.l. by green cluster.

4. Discussion

The course of the stem circumference variations and the derived growth lines at 1,100 and 1300 ma.s.lwere almost identical. In both cases, water deficit was relatively low, indicating favorable hydric conditions, despite the occurrence of short drought periods ($\Delta W < 0$) (Fig. 2). Complete rehydration occurred until mid-July, when stem circumference size culminated. Since then, pronounced drought conditions occurred (ΔW ranged from -1.5 up to -2.0 mm.m^{-1}). The hydric conditions in the upper site differed notably as the rehydration remained continuous. However, water deficit occurred at the same time as in the lower sites, but to a smaller magnitude (-0.5 up to) -1.0 mm.m^{-1}). Based on the results of Ježík et al. (2015), we may estimate the presence of increased drought stress when ΔW falls below -1.0 mm.m^{-1} in young spruce stands, while based on that of Nalevanková et al. (2018), it seems that the values signalizing increased and more pronounced drought were approximately three times lower (absolute deviation from zero values) for mature beech stands.

We interpret the peaks in dendrometer records as representation of stem water status rather than stem reaction to surrounding atmosphere conditions. This is in contrast with Oberhuber et al. (2020) who found highly synchronous course (amplitude and frequency) of DDSTCV on both living and dead trees. He attributed it to hygroscopic swelling and shrinkage of the outer bark tissues. In our study the DDSTCV of dead tree showed only partial amplitude of living trees (less than 10%). We understand this fact because of continuously water saturated bark tissue under moist mountain conditions. Low correlations among DSTCV and environmental variables on different plots and on dead tree supports the statement on water saturated bark tissue conditions. Thus, we interpret the peaks in dendrometer data as phenophysiological process which needs to be further investigated. Despite favourable SWP and weather condition even on the most humid U site water deficit was present. We attribute this reaction to sensitivity of spruce to the irregularities in precipitation (Fig. 2E) which might not influence SWP.

The Morlet spectra revealed almost identical patterns of periodical change in stem circumference on a diurnal scale (58–80 20-min intervals), confirming the same water status and response in the D– and M–stands. Similar periodic events also occurred in lower frequencies (i.e., several days). The Morlet analysis of the U–stand showed less pronounced daily periodicity, indicating less effort to balance the tree and environment water requirements. Low-frequency periodicity was also less evident than in previous stands, especially from mid-July.

The majority of the detected elements in the spruce foliage showed values below the average concentrations for first-year needles, as documented for Carpathian spruce forests (Linder et al. 1995; Bublinec 1994; Maňkovská et al. 2002) and also Canadian boreal spruce forests (Quesnell et al. 2006). Our results show that these spruce stands (growing mainly on podzols and rankers), located in high mountainous regions, do have not optimal mineral nutrition. The geographical extension of Norway spruce is primarily located in nutrient-poor, infertile environments, and spruce typically grows under acidic soil conditions with a thick organic layer (Schmidt-Vogt 1991; Baier et al. 2006). Boreal and temperate spruce forest stands have a naturally low availability of nitrogen (N), which is often the primary growth-limiting nutrient in these soils (Tamm 1991; Binkley & Fisher 2013). Nowadays, there are increased polemics if the fertilization enhances the vitality and growth of poor spruce stands (Bergh et al. 2014), and also if it is suitable as a measure to sequester more carbon (C) (Gundale et al. 2014). The increased biomass growth, after supplying a growth-limiting nutrient, tends to dilute other nutrients (Koricheva et al. 1998; George & Seith 1998), and decreases the tolerance of trees against soil-borne (e.g., deficiency of other mineral nutrients) stress factors. Also, nitrogen deposition has been shown to have a positive effect on herbivorous insect populations (Throop & Lerdau 2004; Strengbom et al. 2005). Another meta-analysis showed that species adapted to resource-rich habitats grow inherently faster and invest less in defences than species adapted to less-productive habitats (Endara & Coley 2011; Nybakken 2018).

Regarding Ca and Mg, which are common enzymatic activators in metabolic physiological processes, optimal concentrations were found only in case of Ca in the upper M– and U–stands. Especially a shortage of magnesium, as a core component of chlorophylls, may lead to damage of the processes in chlorophyll synthesis, which is then reflected in a reduction of chlorophyll content and premature needle yellowing. Since the equilibrium of individual elements in trees is a precondition of their vitality and normal growth (Markert 2003), the ratios of Ca/Mg were limited (3.7–8; Maňkovská et al. 2002) in all three stands, and the Ca/N ratios were optimal only in the M– and U–stands.

Synergic and antagonistic relationships between individual elements can be disturbed by different stress factors (Markert 2003). We recorded relatively high amounts of Mn in the upper U-stand. Although Mn mobilization may indicate the disturbance of the equilibrium in spruce physiology, leading to disproportionate levels of iron, which was also confirmed in our case, this phenomenon is quite common, since a few studies have confirmed that the highest mobilization of manganese appeared in higher altitudes (Kaupenjohan et al. 1989; Maňkovská et al. 2002). On the other hand, an enormously high aluminum content recorded in the lower D-stand can have a much greater negative effect on the balance of the physiological processes. Based on the decreases in exchangeable base cations (Ca²⁺ and Mg^{2+}) and the increases in exchangeable Al in the spruce needles of this stand, we can presume soil acidification occurred. For the entire Carpathian Mountains range, the highest concentrations of Al were determined for spruce in Poland: 140 mg.kg⁻¹ (Maňkovská et al. 2002). The Al concentrations detected in the D–stand were even higher than this value. We expect that in this case, aluminum is deposited mainly in the form of precipitation from more distant polluted areas, and may be leached from higher to lower stands.

Moreover, stress factors negatively affected the state of the photosynthetic pigments of the spruce forests along the Lomnicky peak transect, mainly in the M-stand. Ditmarová et al. (2007b) found that the values of total chlorophyll pigments during the vegetation season in the healthy trees of natural spruce stands in the Zadná Polana National Nature Reserve (Slovakia) were in the range of 3.26–4.81 mg.g⁻¹. The average concentrations in our study were 2.53–4.37 mg.g⁻¹. The values of the Chl/ Car ratio (3-5) revealed the weakened photo-protective function of the photosynthetic apparatus of all evaluated stands. Although the percentage of defoliation was quite high in the D- and M-stands, the assimilatory organs were not seriously damaged with regard to the photosynthetic pigment ratios, because the values did not decrease to below 3 (Lichtenthaler 1987). Additionally, the findings of the maximal pigment concentration during late summer in the U-stand may provide useful insight into the seasonal dynamics of pigment concentrations. Similar results were found by Kmet et al. (2010) with a natural seasonal course for undamaged spruce trees and without such dynamics for damaged trees with yellowing symptoms. Based on our results, we can presume that forest stands with a seasonal increase in chlorophyll pigment concentration are more vital than stands without seasonal improvement.

The parameters of the fast and slow Chl a fluorescence measured in all spruce stands did not indicate reduced capacity of the primary photosynthetic processes during the vegetation season. The values of one of the basic parameters, namely, F_v/F_m , for the whole sample set in June and August were recorded as favorable, not dropping close to the disturbance limit (0.725) (Critchley 2000). Measurements before and after the occurrence of high temperatures allowed us to evaluate changes in the performance of PSII; indeed, the Chl a fluorescence parameters have been recognized as excellent indicators of high temperature stress in PSII (Brestic & Zivcak 2013; Mathur et al. 2014; Kalaji et al. 2017). The photosynthetic performance of PSII evaluated by fluorescence traits was higher at the beginning of June. Nevertheless, the thermostability of PSII during June was lower in comparison to that in August. Simulation of heat stress led to a decrease in the efficiency of the primary photochemistry in PSII. The most negatively influenced were the fluorescence parameters in the D-stand. The photosynthetic performance index (R_{pl}) , as well as the density of active reactions centers ($R_{RC/ABS}$), significantly

reduced and the values of K-step (*Wk*) and the basal fluorescence (F_0) increased. This was expected, as increasing of the variable fluorescence in the K-step has previously been observed, especially under thermal stress and high levels of F_0 and *Wk*, indicating serious damages to the PSII (Strasser et al. 2000).

After heat simulation, we also observed a high stimulation of non-photochemical quenching (NPQ) in all spruce stands, due to the increase in energy dissipation as heat. Leaf temperatures above 35 °C cause an initial decrease in the photochemical efficiency of most plants, which is accompanied by an increase in NPQ in order to reduce the energy available for photochemistry and as a mechanism of plant defense against thermal stress (Müller et al. 2001; Mathur et al. 2014).

Our results also indicate that the differences in the performance of PSII among the forest stands were well detected at the beginning of June (late spring), as we can see from the PCA results. Since the fluorescence parameters are closely linked to photosynthesis, we suppose that these processes are more active during the rapid growth (i.e., in spring and at the start of summer) of spruce trees, and are also more influenced by heat stress in this period.

5. Conclusion

We analysed vitality status of three spruce stands affected by windstorms in 2004 and 2014, drought stress (annually from 2015), and consequently, disturbed by bark beetles. Based on our research, we can conclude that there are certain differences in the vitality of these spruce stands, which were recorded by using an eco-physiological assessment/approach. The lowest spruce stand, i.e., the D-stand at 1,100 m a.s.l., displayed the lowest concentrations of the basic nutrition cations and high Al concentrations in the needles, the most affected performance of the PSII after heat stress simulation, and no seasonal dynamics of photosynthetic pigments. The best physiological vitality was detected in the highest spruce stand at 1,500 m a.s.l. However, as shown in this study, using heat stress simulation, future increasing temperatures and drought periods may lead to the rapid disintegration of these spruce forest stands, which are already seriously weakened by Ips typographus. Photosynthesis, as the most sensitive physiological process, might be negatively influenced by high temperature during periods of intensive tree growth, when the photosystems are the most active, and concurrently, the highest nutrient consumption occurs.

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ORIGINAL PAPER



Impact of the European bark beetle *Ips typographus* on biochemical and growth properties of wood and needles in Siberian spruce *Picea obovata*

Alena Konôpková^{1*}, Konstantin E. Vedernikov², Egor A. Zagrebin², Nadezhda A. Islamova², Roman A. Grigoriev², Hana Húdoková^{3,4}, Anja Petek¹, Jaroslav Kmet¹, Peter Petrík¹, Anna S. Pashkova², Anastasia N. Zhuravleva², Irina L. Bukharina²

¹ Technical University in Zvolen, Faculty of Forestry, T. G. Masaryka 24, SK – 960 01 Zvolen, Slovak Republic

- ² Institute of Civil Defense, Udmurt State University, Universitetskaya Ulitsa 6, RU 426 034 Izhevsk, Udmurt Republic,
- Russian Federation

³ Institute of Forest Ecology, Slovak Academy of Science, L. Štúra 2, SK – 960 01 Zvolen Slovak Republic

⁴ Technical University in Zvolen, Faculty of Ecology and Environmental Science, T. G. Masaryka 24,

SK – 960 01 Zvolen, Slovak Republic

Abstract

European spruce bark beetle *Ips typographus* is an important driver of ecological processes in spruce stands, with severe effects on forestry economies. To prevent bark beetle outbreaks, early detection of infestations is a crucial step in forest management. It is expected that bark beetle infestation modifies biochemical composition of wood and needles, alters physiological responses in the early stage of infestation, which results in the reduction of tree growth and ultimately a tree death. Therefore, we studied the differences in biochemical composition of wood, content of photosynthesis-related pigments, shoot morphology, and growth between the healthy Siberian spruce trees (*Picea obovata*) and trees which were infested by *I. typographus*. The study was performed in five experimental plots established in the south of the Udmurt Republic in the European part of the Russian Federation. Three infested and three non-infested trees were chosen on each plot. Our results showed no significant effect of bark beetle infestation on the content of main structural components of wood – holocellulose (cellulose and hemicellulose) and lignin. On the other hand, we found differences in the content of photosynthesis-related pigments differed between the non-infested and infested trees as well. Unexpectedly, bark beetle infestation caused the overproduction of both pigment types rather than their degradation. Moreover, we observed that a higher amount of total extractive substances positively affected the incremental growth, whereas tannins supported the growth of shoots and needles.

Key words: wood extractive substances; photosynthesis-related pigments; shoot morphology; tree-ring growth; spruce stand decline

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1. Introduction

Bark beetle outbreaks mediated by climate change (Pureswaran et al. 2018), are major disturbance agent causing large-scale mortality of spruce stands and altering ecosystem function worldwide (Grodzki 2010; Hlásny & Sitková 2010; Hicke et al. 2012; Økland et al. 2016). As bark beetle is natural in conifer forests, their negative impact on *Picea abies* (L.) H. Karst, coniferous tree species with high economic and ecological value, is evoking the interest of foresters over the last two decades. In Europe, distribution of spruce is partitioned into three major domains: a northern area extending from Norway to central Russia, where the species is introgressed and gradually replaced by Siberian spruce (*Picea obovata* Ledeb.), and two smaller areas, an Alpine territory and a Carpathian one (Chen et al. 2019). It is still debatable, but most of the scientist agree that Siberian spruce is closely related to northern populations of Norway spruce and they can be considered as subspecies or geographical races of the same species undergoing significant gene exchange (Krutovskii & Bergmann 1995; Lockwood et al. 2013). As the increased decline of Norway spruce

*Corresponding author. Alena Konôpková, e-mail: alena.konopkova@tuzvo.sk

stands in Western and Central Europe was documented, there had been also reported the high mortality of both, Norway spruce and its subspecies Siberian spuce, across Eastern Europe. In the European part of Russia, over 2 million hectares of spruce stands were declined (Kharuk et al. 2015). The spruce dieback was also detected in Latvia (Arhipova 2013), Lithuania (Vasilyauskas 2013), Belarus (Kharuk et al. 2015), and Ukraine (Parpan et al. 2014). Ongoing climate changes in synergy with overgrowing biotic agents and overmaturity of stands have been reported as a potential cause of these events in Eastern Europe (Chuprov 2008; Zamolodchikov 2011). For example, Grinenko & Hegay (2018) reported dieback of over 60 thousand hectares of spruce stands in the European part of Russia triggered by the bark-beetle outbreak during the period of 2010-2012. As bark beetle completes most of its life cycle under bark, its detection is often observed too late, after reaching a damage threshold. Hence, to detect bark beetle in the early stage, could be a possible way to regulate the spreading of the bark beetle outbreak. The first visual sign of weakened vitality of trees is the crown defoliation and/or decolourization. Based on changed colour of the crown, there are three foliage degradation levels caused by infestation (Wermelinger 2004). The green level with no visible damage of the tree or decolouration of needles. The second level of the infestation, named as red, represented by the degradation of needles continues in changing foliage colour to reddishbrown (Wermelinger 2004; Abdullah et al. 2018). If these changes are permanent, the substantial impairment of physiological processes and subsequently the biomass production, is occurred. The last level of foliage degradation terminates as defoliation of tree crowns (Wermelinger 2004; Abdullah et al. 2018). Along with biomass production decrease, the differences in biochemical composition of wood between trees with different infestation level were reported. Several studies (Matyssek et al. 2012; Lee et al. 2019) have shown that lignified cells in the outer bark may represent the physical barrier against pathogens. Sclerenchyma and sclereids are formed by lignin and thus, protect the tree mechanically. Moreover, sclereid cells are able to accumulate flavonoids and stilbenes which can be involved in chemical and physical defence. These compounds maintain the resistance of the bark and protection of the cambium against biotic agents. Furthermore, spruce trees may stir and exudate the terpenoid resinous substances, which may be crucial resistance mechanism (Zeneli et al. 2006; Zhao et al. 2011; Hlásny et al. 2014), or may upregulate synthesis of the other protective substances e.g. tannins. Tannins are secondary metabolites with antioxidant activity and play a major role during tree-environment interactions, both local and systemic responses against pathogenic microbes and pest infestations (Felicijan et al. 2016). Therefore, linking spruce performance (growth parameters) with morphological and biochemical parameters of the needles and stems could provide a wider view of spruce respond to bark beetle infestation.

The aim of the present study was to evaluate the impact of bark-beetle infestation on Siberian spruce trees and to find out how they affect the biochemical and growth properties of wood and needles. We hypothesize: (i) that infested trees will show the different biochemical composition of wood. We expect that bark-beetle infestation will cause an overproduction of the extractive substances, as the result of excited defence system. However, higher costs of such synthesis will be at the expense of holocellulose and lignin production. (ii) For the photosynthesisrelated pigments, we expect that infestation will result in degradation and thus lower content of both pigment types – chlorophylls and carotenoids. (iii) Consequently, we hypothesize that above-mentioned changes will limit the growth of shoots, needles, and trunk as well.

2. Material and methods

2.1. Study sites

The research was conducted on the territory of the Udmurt Republic. The Udmurt Republic is located at the European part of the Russian Federation in the west of the Ural Mountains (Fig. 1). The territory of the Udmurt Republic is bordered by the Kama and Vyatka rivers. It stretches broadly north to south for about 320 km, and west to east for 200 km. Significant elongation from north to south and hilly-rugged terrain cause major differences in temperature, humidity, atmospheric pressure, and rainfall between the northern and southern parts of the Republic. In this regard, the territory of the Udmurt Republic is located within two landscape zones: taiga (boreal/south-taiga zone) and sub-taiga (boreal-sub-boreal/coniferous-deciduous forest zone) (Fedorova et al. 2016).

For this study, we established five experimental plots with a size of 100×100 m in the south of the Udmurt Republic. The experimental plots were located in the sub-taiga zone in the two forest districts; two plots in the Malaya Purga and three plots in the Mozhga district (Fig. 1). The plots lay in stands predominantly consist of spruce, whereas the spruce has been actively drying out in these regions. A more detailed description of experimental plots is displayed in Table 1. All plots are comparable from the perspective of tree age, height, and diameter at the breast height. The dominant woody species is spruce which creates at least 90% of stand composition. The proportion of dead trees is in the range of 50-58% for all experimental plots, except Mozhga 3, where it was just 20%. The soils of the experimental plots were sodpodzolic, and loamy in granulometric composition.



Fig. 1. The locations of established experimental plots in the Udmurt Republic (Russian Federation).

Table 1. Basic characteristics of experimental plots. Mean \pm standard deviation of stand age (A), height (H), diameter at breast height (DBH), the number of trees (pcs/ha), the proportion of the dead trees, and the species composition of the stands are presented.

| District | Plot No. | A [age] | H [m] | DBH [cm] | Number of trees [pcs/ha] | Proprotion of dead trees [%] | Stand composition |
|--------------|----------|------------|----------|-------------|-----------------------------|------------------------------|-------------------------------------------|
| Malaya Purga | 1 | 60±1.7 | 18.3±0.5 | 25.9±0.8 | 252 | 50.8 | 10S (100% spruce) |
| | 3 | 60±1.1 | 18.7±0.7 | 20.3±0.2 | 155 | 57.4 | 10S (100% spruce) |
| Mozhga | 1 | 60±1.6 | 23.1±0.6 | 25.7±0.5 | 383 | 56.7 | 9S 1F+L (90% spruce, 10% fir + linden) |
| | 2 | 50±1.1 | 20.3±0.9 | 22.6±0.3 | 408 | 50.2 | 9S 1L (90% spruce, 10% linden) |
| | 3 | 60±1.5 | 19.1±0.5 | 19.1±0.2 | 456 | 20.1 | 9S 1P+B (90% spruce, 10% pine + birch) |

2.2. Experimental design

The sampling of Siberian spruce trees was performed at the end of August 2019. On each plot, we selected six spruce individuals, whereas three of them showed good health status (hereinafter represent as "non-infested trees") and three individuals showed bark beetle infestation (hereinafter represent as "infested trees"). As "infested trees", the trees with noticeable crown defoliation, and/or needles depigmentation, especially at the top of the crown and expanding to the bottom, were marked. Moreover, the visible boreholes on the bark, and resin lumps melted with sawdust on the tree trunks had to be present as indicators of successful bark beetle infestation. On all surrounding dead trees, we observed the presence of galleries under the bark, which correspond by their shape and size to the European spruce bark beetle (Ips typographus L.). Therefore, there is also a high probability of the presence of this species on the studied infested trees.

The biochemical properties of the wood, the content of photosynthesis-related pigments in needles, the shoots and needles morphology and the growth traits derived from tree-ring analysis were assessed.

2.3. The biochemical composition of wood

Wood samples were taken using the Pressler drill at a height of 0.3 m from the root neck of the tree. The analyses were carried out in the laboratory of Ecological and

Fire Safety of the Federally Funded Educational Institution of Higher Education Udmurt State University (Izhevsk). Firstly, the wood moisture was established by the gravimetric method as a difference in weight of the fresh and air-dried samples. Then, the biochemical substances were fractionated in accordance with their chemical nature by sequential extraction with solvents of increasing polarity. The biochemical compounds were estimated using hot distillation in a Soxhlet apparatus by the hot water in the case of water-soluble holocellulose (cellulose and hemicellulose) and lignin and by the alcohol-toluene mixture for resinous substances. The tannin content was determined using the permanganometric method (Babkin 2017). Moreover, the content of total extractives containing the tannins, resins and group of other extractive substances was assessed. Yields of individual compounds were estimated as a percentage of the dry mass.

2.4. The content of photosynthesis-related pigments

The content of photosynthesis-related pigments was assessed spectrophotometrically according to Gavrilenko & Zhigalova (2003). The 0.2 g of needles from the newly grown shoots was firstly extracted in 80% ethanol and then the absorbances of extract at 600, 642.5 and 440.5 nm were measured by using the spectrophotometer Экрос ПЭ-5400ВИ. The content of chlorophyll *a*

(chl *a*), chlorophyll *b* (chl *b*), the total concentration of chlorophylls (chl a+b) and the carotenoids (car)concentration in fresh weight of needles were assessed for each individual in 2–3 replications as follow:

$$chl a = (13.7 * A600) - (5.76 * A642.5)$$

$$chl b = (25.8 * A642.5) - (7.6 * A600)$$

$$chl a + b = (6.1 * A600) - (20.04 * A642.5)$$

$$car = (4.695 * A440.5) - (0.268 * chl a + b)$$

2.5. Tree-ring analysis

Increment cores were sampled the same way as described above, using the Pressler drill at the height of 0.3 m from the root neck of the tree. The cores were air-dried and then glued at the wooden core mounts and they were shaped using the 400-grit sandpapers. For measurements of tree-ring widths, the digital positiometer equipped by binocular with accuracy $\pm 0,01$ mm was used. The width of current year tree-ring (Z_R^{-1}) and the mean periodic (over 10 years) tree-ring width (\overline{Z}_R^{-10}) was assessed.

 Z_{R}^{1} is an indicator of the increment width of the tree trunk during the last year of its growth (before sampling) calculated as:

$$Z_R^{\ 1} = R_a - R_{a-1}$$

where R_a – the current radius of the trunk (at the time of sampling); $R_{a_{-1}}$ – trunk radius 1 year ago; a – the age of the tree; \overline{Z}_R^{10} is an average indicator of the annual increment width of a tree trunk over the past 10 years (before sampling), calculated as:

$$\overline{Z}_{R}^{10} = \frac{R_{a} - R_{a} - 10}{10}$$

where R_a – the current radius of the trunk (at the time of sampling); R_{a-10} – the trunk radius from 10 years ago; a – the age of the tree.

2.6. Shoots and needles morphology

The length and width of the newly grown central shoots of 15 branches were sampled. The shoots were photographed at the millimetre paper and then measured using the ImageJ 1.51k software (National Institute of Health, USA). The width was measured on the widest part of the shoot. Moreover, approx. 60 needles from the central part of the same shoots were sampled to measure the mean needle area (needle area, cm²) and specific leaf area (*SLA*, cm²g⁻¹). Needles were firstly scanned to obtain the area of needles. Scans were processed using the Image J 1.51k software. Then, the needles were dried to constant weight and SLA was calculated as the ratio between the area of needles and their dry weight.

2.7. Statistical analysis

The obtained data were processed using software R 3.6.3 (R Core Team, Austria). Prior to the statistical tests, the normality of data distribution was tested using the Shapiro-Wilk test. If needed, the data were transformed. Then, the differences between the non-infested and infested trees were tested by the mixed model ANOVA with "health status" as a fixed factor and "plot" as random factor. The differences were assessed using Tukey's honestly significant difference (HSD) test with $p \le 0.05$. Moreover, all the estimated parameters were processed by principal component analysis (PCA) to identify the general trends of the multi-dimensional data sets. Finally, to find out the relationships between the biochemical properties and growth traits, the simple linear regression was used.

3. Results

3.1. The biochemical composition of wood

We observed the different wood composition in the noninfested and infested trees of Siberian spruce (Table 2, Fig. 2). The fraction of total cellulose and hemicellulose of the wood (holocellulose) and lignin showed no significant differences between the non-infested and infested trees. Just on one plot, the slightly higher content of holocellulose was recorded in the non-infested individuals. The lignin shows no distinguishable response pattern as we observed higher content of lignin in the wood of infested trees on two plots and on one plot the higher content of lignin in the wood of non-infested spruces. The total amount of extractive substances did not significantly differ between the infested and non-infested spruces as well. However, we recorded differences in the content of tannins. On all established plots, we observed the significantly lower fraction of tannins in infested individuals compared to the non-infested spruces. The content of tannins was, in average, 1.5 times lower in these trees. On the other hand, the fraction of resins showed inconsistent response through different trial plots: On one plot, the fraction of resins was substantially lower in the non-infested individuals. On two plots, the same fraction of resins for spruce with different health status were recorded. The higher fraction of resins in the noninfested individuals were observed in two plots.

3.2. The content of photosynthesis-related pigments

The composition of photosynthesis-related pigments differed between the trees with different health status. Overall, we recorded higher content of all pigment types in infested trees. However, the significantly higher values in infested individuals were recorded for chlorophyll *a*,

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Fig. 2. The extractive substances of wood in non-infested and infested Siberian spruce trees. The mean percentage \pm standard errors in the content of holocellulose (A), lignin (B), total amount of extractive substances (C), resins (D) and tannins (E) are presented. The percentages of individual groups of extractive substances (F) are displayed. "N" represents non-infested trees, and "I" infested trees.

chlorophyll *b* and, therefore content of total chlorophylls (Table 2, Fig. 3A). Moreover, as results shown in Fig. 3B, the infested individuals exhibited higher content of carotenoids as well. However, no significant difference between the non-infested and infested trees of Siberian spruce was recorded.

3.3. Growth traits

The results of shoot, needles and trunk growth traits are displayed Fig. 4 and Table 2. As expected, the infested trees had significantly reduced shoot growth. The noninfested spruces showed longer and wider newly grown shoots compared to the infested trees. This behaviour was observed on all five and four plots, respectively. Similarly, the needles of the non-infested trees had overall higher area compared to those with weak health status. However, no significant difference was observed for specific leaf area. This reflects the same thickness of needles regardless of the health status of individual trees. From the point of view of tree-ring analysis, it showed no recognizable growth pattern. For most trial plots, the width of current and periodic increments was comparable for the non-infested and infested Siberian spruces or

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|---------------------------------------------|----------------|
|---------------------------------------------|----------------|

| Parameter | Source of variance | numDF | denDF | F-value | p-value |
|---------------------------------------|--------------------|-------|-------|----------|---------|
| Holocolluloco | (Intercept) | 1 | 24 | 905.48 | <.0001 |
| Holocellulose | Health status | 1 | 24 | 0.04 | 0.8517 |
| Lignin | (Intercept) | 1 | 24 | 317.63 | <.0001 |
| Liginii | Health status | 1 | 24 | 0.74 | 0.3982 |
| Total amount of autroative autorage | (Intercept) | 1 | 24 | 50.93 | <.0001 |
| Total amount of extractive substances | Health status | 1 | 24 | 1.41 | 0.2472 |
| Tonning | (Intercept) | 1 | 24 | 70.38 | <.0001 |
| Tammis | Health status | 1 | 24 | 67.99 | <.0001 |
| Desina | (Intercept) | 1 | 24 | 81.52 | <.0001 |
| NC51115 | Health status | 1 | 24 | 0.54 | 0.4681 |
| Chla | (Intercept) | 1 | 55 | 4999.44 | <.0001 |
| Unia | Health status | 1 | 55 | 5.19 | 0.0267 |
| Chik | (Intercept) | 1 | 55 | 280.61 | <.0001 |
| Chi D | Health status | 1 | 55 | 4.24 | 0.0442 |
| Chlath | (Intercept) | 1 | 55 | 4872.44 | <.0001 |
| CIII a+D | Health status | 1 | 55 | 5.26 | 0.0257 |
| Con | (Intercept) | 1 | 55 | 47668.75 | <.0001 |
| Car | Health status | 1 | 55 | 1.91 | 0.1721 |
| Shootlangth | (Intercept) | 1 | 406 | 448.51 | <.0001 |
| Shoot length | Health status | 1 | 406 | 325.61 | <.0001 |
| Shootwidth | (Intercept) | 1 | 406 | 523.90 | <.0001 |
| | Health status | 1 | 406 | 102.88 | <.0001 |
| Maan noodla araa | (Intercept) | 1 | 1598 | 350.44 | <.0001 |
| אוכמוז ווככעוב מוכמ | Health status | 1 | 1598 | 32.42 | <.0001 |
| SI V | (Intercept) | 1 | 24 | 954.37 | <.0001 |
| JLA | Health status | 1 | 24 | 0.02 | 0.8901 |
| 7 1 | (Intercept) | 1 | 24 | 93.90 | <.0001 |
| ^L _R | Health status | 1 | 24 | 1.76 | 0.1973 |
| 710 | (Intercept) | 1 | 24 | 141.72 | <.0001 |
| Z _R | Health status | 1 | 24 | 0.05 | 0.8187 |

Table 2. The results of mixed model ANOVA for the estimated parameters.



Fig. 3. The content of photosynthesis-related pigments of the non-infested and infested Siberian spruce trees. The mean ± standard errors of the total content of chlorophyll (Chl a+b, A) and total content of carotenoids (Car, B) are presented.

we observed the contradictory results across the established experimental plots.

3.4. Overall assessment of the biochemical and growth traits

Fig. 5 shows the principal component analysis of multifactorial data. The first two axes of the presented biplot describe almost 50% of the variability. However, there exists a noticeable separation of the non-infested and infested spruce trees, which shows the uneven biochemical and growth response of these two groups. According to this along with the above-mentioned, we can assess the content of photosynthesis-related pigments in the needles and content of tannins in the wood as the main biochemical substances distinguishing non-infested and infested Siberian spruce trees. From the point of view of the growth traits, shoot width seems to be the most contributed to the differentiation of these two groups, but the mean area of needles and shoot length could be also considered.

Moreover, we observed strong correlations between the total content of extractive substances in wood and the



Fig. 4. The shoot and needles morphology and growth traits of the non-infested and infested Siberian spruce trees. The mean \pm standard errors of the shoot length (A), shoot width (B), the specific leaf area (C), the needle area (D), the mean periodic treering width (E) and the width of current year tree-ring are presented (F).

mean width of tree-ring during the last 10 years and the width of tree-ring of the current year, which suggests that the amount of extractive substances positively impact on incremental growth. On the other hand, the specific extractive substances of wood, tannins, support the growth of newly grown shoots of spruce. The individual with the higher content of tannins showed greater shoot growth in the length as well as in the width (Fig. 6).

4. Discussion

The crown defoliation, and decolouration are relatively well-described symptoms of bark beetle infestation (Wermelinger 2004; Abdullah et al. 2018), however, the studies focused on the biochemical nature of such changes are still quite scarce. Therefore, the presented study demonstrates the impact of bark beetle infestation on both, the biochemical properties of wood and needles and growth rate of tree-rings and shoots, in Siberian spruce.

There is expected that the changes in the biochemical composition of wood and needles could occur as a response of bark beetle infestation (Thoss & Byers 2006). We predicted that the infested trees would synthesize more extractive substances in wood, such as resins and tannins, at the expense of the structural components (holocellulose and lignin) production. Such results were observed e.g. by Watson (2006), who confirmed the sig-



Fig. 5. Principal component analysis for the estimated parameters. The ellipses represent 95% confidence intervals around the centroid (bigger symbol) for the non-infested (green colour) and infested Siberian spruce trees (red colour). The percentage on the axes indicates the proportion of the variance that is explained by the given principal component.

nificant reduction of lignin content and soluble sugars in the sapwood of lodgepole pine after infestation by mountain pine beetle *Dendroctonus ponderosae* (Hopkins). Similarly, Lieutier (2002) supposed the downregulated production of carbohydrates in favor of protein production, which is needed for defense, as a result of defense systemic change in whole tree metabolism.

However, we observed no significant differences in content of holocellulose and lignin regardless of the bark beetle infestation. We recorded just slightly higher content of holocellulose in non-infested trees on one of the five experimental plots. This discrepancy probably results from the parallel colonization by fungi in the mentioned study, not from the bark-beetle itself. The fungal colonization of trees infested by Ips typographus is relatively well described. Most often, the fungal colonization follows the successional pattern with the most pathogenic blue-stain fungi of Ophiostoma and Ceratocystis species as the primary colonizers, followed by Graphium species, but the fungi composition of trees from different geographic areas or in different stages of infestation may vary (Solheim 1992; Lieutier 2002; Viiri & Lieutier 2004; Kirisits 2010). However, Krokene & Solheim

(1996) reported that the Ips typographus carry more pathogenic fungi comprared to other bark beetle species. The relationship of pathogenic fungi and bark beetle is symbiotic; the fungi may provide nutrients to bark beetle, and/or impairment tree defenses by the degradation of its defense phenolic compounds (Zhao et al. 2019). On the other hand, most of the fungi may be beetle-transmitted, which supports their expansion and accelerating tree death (Furniss et al. 1990; Solheim 1992). As most of the bark beetle associated fungi are ascomycetes, which are not typically capable to degrade the cellulose (Hofstetter et al. 2015), the reduction of hollocelulose content in studied infested trees could be attributed to co-occurring wood-decay fungi, which during incipient growth on lignocellulosic material, firstly consume soluble sugars (Zabel & Morrell 1992; Goeffrey 2016). In the case of lignin, we observed no recognizable pattern between the infested and noninfested treed. However, the several earlier studies (Lieu et al. 1979; Larsson 2002; Goeffrey 2016) documented that the above-mentioned blue-stain fungi could cause the lignin degradation. Therefore, the different response patterns, from the point of lignin and hollocelulose contents, across our five experimental plots



Fig. 6. Correlation between the shoot and tree-ring growth traits and content of extractive substances in wood.

could be explained by the different levels of fungi colonization.

Although extractives make up only a small percentage of the total chemical composition of wood, they play several significant roles in plant-insect interactions, so we expected higher content of total extractives in infested trees. We confirmed no significant differences between the infested and non-infested trees in the content of total extractives substances, however, we observed differences in the proportion of the content of tannins, resins, and the other unspecified extractive substances. To improve survival ability against the bark beetle attack, the trees often produce the resins. This was proved by Lombardero et al. (2000), who recorded, that the bark beetle induced damage leads to increase of resin production in the xylem. However, in our case, the differences in resin content were inconsistent across different experimental plots. Only on two plots, the higher content of resins in infested trees was observed. Otherwise, on other plots, non-infested trees showed significantly higher content of resins. Stoffel & Sitz (2008) and Hood et al. (2015) emphasized that the increase of resin production can be also caused by the other environmental factors and specific disturbances such as windthrows, fires, avalanches, etc. Therefore, the impact of such factors can lead to increase in resin production in the xylem of non-infested trees as well. From the point of view of tannin production, Felicijan et al. (2016) have shown that bark beetle attacks strongly stimulate tannin production. Tannins can play an important role in defence against bark beetles by inhibiting the feeding of the insect as they reduce the digestibility of proteins by binding to them (Salminen & Karonen 2011; Dowd 2018; Hammerbacher et al. 2018). Moreover, they are known to possess high in vitro antioxidant capacity and as such have an important role in protection against oxidative stress (Gourlay & Constabel 2019). In our results, the significantly lower tannin content was observed in the infested trees compared with the non-infested ones. The lower content of tannins does not explicitly indicate the reduced production of tannins due to the impair defence system in infested trees. However, it could be caused by the immediate oxidation of synthesized tannins and hence its utilization during the defence response. Therefore, in such comparisons, there would be more suitable to consider oxidized tannins as well, not just the content of reduced tannins. However, it could be difficult, as the tannin oxidation may create new bonds, make tannins water-insoluble, and thus resistant to the most conventionally used quantification methods (Poncet-Legrand et al. 2010).

The content of photosynthesis-related pigments showed higher values in the infested trees on all experimental plots, but those differences were significant just for the chlorophyll content and not for carotenoids. As mentioned above, pigment bleaching is one of the major visible symptoms of needle damage (Young et al. 1990), therefore we expected higher content of chlorophylls in non-infested trees. Such results were also observed by Abdullah et al. (2018), who confirmed the higher values of chlorophylls in healthy individuals of Norway spruce. However, those results were obtained in infested trees in the "green attack stage" and trees studied in our experiment were at the second "red attack stage". Therefore, we supposed that the increased demand for assimilates, which are essential for maintaining metabolic processes, causes excitation of synthesis of chlorophylls as well as carotenoids as one of the main protective substances (Abdullah et al. 2018). Moreover, this demand could be much higher in the trees with a reduced leaf area. This assumption corresponds also with our results as we observed an upregulated synthesis of photosynthesisrelated pigments and simultaneously the significant reduction of growth of new shoots and lower needle area after bark beetle infestation. On the other hand, tree-ring analysis showed not so evident reduction of incremental growth. We observed just slightly reduced incremental growth during the last year before the sampling in infested individuals on the one plot. However, as the bark beetle infestation may have relatively rapid progress and the time of occurrence of the bark beetle attack was not determined, the reduction of incremental growth cannot be reliably attributed to the bark beetle attack itself and could be also the result of a previous tree weakening. Even more, the infested spruce trees on another plot had noticeably, but not significantly, higher increments during the year of the sampling compared to non-infested ones. The higher radial incremental growth after bark beetle attack was also recorded for Picea engelmanii (Parry ex Engelm.) (De Rose & Long 2007). As Stoffel & Hitz (2008) emphasized, this could be connected to the production of traumatic resin ducts due to the mechanical injury. Such behaviour was observed after wounding for Picea abies and Larix decidua (Mill.) as well (Gärtner & Heinrich 2009). Moreover, we supposed the radial growth is positively impacted by the content of total extractive substances, whereas better growth of shoot can be attributed to a specified group of extractives, to tannins.

5. Conclusion

This study demonstrates that the infestation by European bark beetle causes several differences in biochemical properties of wood and needles in Siberian spruce. Although the content of the main structural components of wood, holocellulose and lignin, did not significantly differ between the non-infested and infested trees, the variation in the composition of wood extractive substances was observed. The non-infested trees showed significantly higher content of reduced tannins compared to infested ones, but no visible pattern regarding the bark beetle infestation was recorded for resin production. On the other hand, the infested trees of Siberian spruce had upregulated synthesis of photosynthesis-related pigments in the needles. This is probably a result of higher demand for the assimilates production for maintaining the essential metabolic processes.

As was expected, the better growth of new shoots was observed in non-infested trees as well as the mean needle area was significantly higher compared to infested trees, whereas we suppose shoot growth was affected by the tannin production in a positive way. On the other hand, the tree-ring analysis showed no significant difference in increment between the non-infested and infested trees. However, we recorded that the trees with a higher content of wood extractive substances showed higher increments.

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The response of *Pinus* species to ozone uptake in different climate regions of Europe

Svetlana Bičárová^{1*}, Anumol Shashikumar², Laurence Dalstein- Richier², Veronika Lukasová¹, Katarína Adamčíková³, Hana Pavlendová⁴, Zuzana Sitková⁴, Anna Buchholcerová¹, Dušan Bilčík¹

³ Institute of Forest Ecology SAS Zvolen, Department of Plant Pathology and Mycology, Akademická 2, SK – 949 01 Nitra,

⁴ National Forest Centre - Forest Research Institute Zvolen, T. G. Masaryka 2175/22, SK – 960 01 Zvolen, Slovak Republic

Abstract

This study is focused on the research of selected *Pinus* species exposed to high ozone concentrations in the mountain environment. We noticed different values of modelled ozone doses (MOD) up-taken by Mountain pine (*Pinus mugo* Turra) in the High Tatra Mts (SK–HTMts) and Swiss stone pine (*Pinus cembra* L.) in the Alpes-Mercantour (FR–AlpMar) during the growing season 2019. The MOD values were obtained by multiplicative DO3SE model, while we also tested a new approach based on modification of input ozone data. The MOD values were obtained by multiplicative DO3SE model, while we also tested a new approach based on modification of input ozone data. Testing has shown that ozone input based on passive sampling may be used in MOD modelling for sites situated in the subalpine zone where the operation of active monitors is limited. . Presented results confirmed the assumption regarding stomatal ozone flux reduction due to the occurrence of soil drought in hot and dry summer weather typical for the Mediterranean climate region. Despite the limitation of stomatal flux, foliar ozone specific injury on two years needles of *P. cembra* was substantially higher in comparison to the incidence of ozone injury symptoms observed on two years needles of *P. mugo* in SK–HTMts. It may suggest low phytotoxicity of given MOD or efficient resistance of *P. mugo* against oxidative stress. In addition, the visible injury index (VINX) covering the broad effect of biotic and abiotic harmful agents was appraised on *P. mugo*. Percentage of affected surface indicated moderate deterioration of needle injury at the end of the growing season, particularly due to traces of mechanical damage.

Key words: Modelled Ozone Dose (MOD); Visible Injury Index (VINX); passive O₃ sampling; soil humidity; mountain environment

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1. Introduction

Air quality is still a serious problem in contemporary society. Recent geographical models that provide transparent information about the world's (WAQI 2019) or European (EAQI 2019) air pollution highlights the Air Quality Index (AQI) based on the measurement of key pollutants in the ambient air including ozone concentration (O_3). The abundant O_3 formation manifesting by AQI increase may lead to an adverse effect on human health (Analitis et al. 2018; Orru et al. 2019) and vegetation (Bendáková & Hůnová 2015; Feng et al. 2017; Gong et al. 2020). The risk of exposure of forest area to ozone is estimated by the AOT40 index (Directive 2008/50/EC) derived from the O_3 concentration measured in the ground level of the atmosphere. However, scientific evidence (Ashmore et al. 2004; Matyssek et al. 2007; Sicard et al. 2016) has suggested that AOT40-based critical levels for vegetation should be replaced by stomatal flux-based critical levels that reflect the amount of ozone transported into the leaves (Mills et al. 2011). The accumulated stomatal flux over a specified time interval is represented by the modelled parameter of PODY (the Phytotoxic Ozone Dose over a threshold flux of Y (nmol m⁻² PLA s⁻¹) where Y means a detoxification threshold and PLA is the projected leaf area. Expert judgement was used to set Y=1 nmol m⁻² PLA s⁻¹ based

*Corresponding author. Svetlana Bičárová, e-mail: bicarova@ta3.sk, phone: +421 527 879 165

¹ Earth Science Institute of the Slovak Academy of Sciences, Stará Lesná, SK – 059 60 Tatranská Lomnica, Slovak Republic

² GIEFS (Groupe International d'Études des Forêts Sud-Européennes), 69 avenue des Hespérides, FR – 06300, Nice, France

Slovak Republic

on observation of O₃ sensitivity under controlled conditions (Dizengremel et al. 2013). The threshold Y below which it is assumed that any O₃ molecule absorbed by the plant will be detoxified may be due to species specificity and the difference in the real environment. The high sensitive conifers are Pinus species (Dalstein & Vas 2005), however different visible O₂ injury response may be expected under natural conditions due to differences in O₂ sensitivity controlled by genotype and micro site conditions of growth, exposure, and O₃ flux (Coulston et al. 2003; Nunn et al. 2007; Braun et al. 2014). Considering that, the goal of this work is to specify the phytotoxic O₃ effect on mountain timberline tree species using the modelled value of ozone dose (MOD), regardless of "Y", against visible injury index (VINX) that traces biotic and abiotic damage including visible O₂ injury.

Mountain timberline is exposed to relatively high O₂ concentration (Bytnerowicz et al. 2004; Hůnová et al. 2010; Bičárová et al. 2019) produced by photochemical transformation of precursors from both anthropogenic and biogenic sources with the addition of large-transmission transport of polluted air masses. Monitoring of O₃ pollution in complex terrain is commonly performed by passive sensors, while modelling of PODY requires continuous measurement of O₂ concentration in hourly step. Therefore we consider it beneficial to introduce a new approach consists of the modification of MOD calculation incorporating O₃ data from passive samplers. Along with ozone, environmental conditions play a key role in stomatal O₃ uptake and climate affects the mountain ecosystems in a complex way (Zapletal et al. 2012; Kopáček et al. 2017; Fleischer et al. 2017; Mezei et al. 2017). In this context, we investigated the effect of environmental factors on stomatal O₃ flux covering two different mountain bioclimatic regions of Europe such as (1) the High Tatra Mts (SK-HTMts) in the Western Carpathians with a temperate climate and (2) the Alpes-Mercantour (FR-AlpMar) in the Alpes-Maritimes with a Mediterranean climate.

The objectives of this study were: (i) to model ozone dose (MOD) for sensitive mountain conifers Swiss stone pine (*Pinus cembra* L.) and Mountain pine (*Pinus mugo* Turra) during the growing season 2019; (ii) to appraise the role of environmental factors in O_3 uptakes under contrasting climate conditions; (iii) to test the use of passive O_3 sampler measurements in MOD modelling; and (iv) to analyse the relationship between MOD results and field observation of visual injury for *P. mugo* in SK–HTMts and *P. cembra* in FR–AlpMar.

2. Material and methods

2.1. Study area

The study area (Fig. 1) includes a subalpine zone of two different climate regions situated in the Tatra National park (SK–HTMts) and in the Mercantour National

Park (FR-AlpMar). The territory of the High Tatra Mts. belongs to the continental climate zone characterized by cold winters, mildly warm, and wet summers. Based on climate data obtained at Skalnaté Pleso Observatory (SPO: 49°11'21" N; 20°14'02" E; 1,778 m a.s.l.) since 1943, the mean temperature of the coldest month (February) is -5.6 °C and mean temperature in two summer months (July, August) is close above 10 °C. Long-term (1943–2019) mean annual air temperature is 2.2 °C. The annual mean of 2.8 °C confirms climate warming during the last decades (1991–2019). Precipitation is concentrated mostly in the warmer months from May to October, the monthly maximum is in July and achieves 221 mm on average (1943–2019). The most vulnerable vegetation in the subalpine zone is P. mugo (Bičárová et al. 2019) that reaches the highest coverage at 1,450 m a.s.l. and decreases with increasing altitude, where forests become sparser. Positions around 2,100 m a.s.l. is occupied by individual shrubs with very low density. We selected 10 monitoring sites in the vicinity of SPO (Fig. 2) for inspection of visible injury on *P. mugo* during vegetation season 2019. In order to model O₃ fluxes, the O₃ concentration, and environmental parameters were considered for the site SPO where both, meteorological and O₂ measurements are carried out.

FR-AlpMar within Alpes-Maritimes County is renowned for its hot temperatures and pleasant climate throughout the year. Although the weather is sunny and dry during the summer months, it can be quite cool and fresh in the mountains. In the Mercantour National Park, less than 70 km from the Mediterranean Riviera, the summits of Mercantour culminate up to more than 3,000 m of altitude leading to a multitude of bioclimatic floors. The climate of subalpine zone FR-AlpMar represented by sites situated along Route du Col de Salèse (RCS: 44°07'42"N, 7°15'14"E, 1,790 m a.s.l.) is hot with dry summers and mild, wet winters. Climate patterns based on 30 years of hourly weather model simulations (Meteoblue 2019) available for RCS site show that a period of mean temperature above 10 °C lasts from June to September with a peak of 15 °C in July and August. The mean temperature of the coldest month (January) is around -2.5 °C. Precipitation amount increases in the spring months (April and May) when the monthly total is about 300 mm. In summer months (June, July, August) precipitation amount decreases deeply under 200 mm. Studies have shown (Dalstein et al. 2005; Sicard et al. 2011) that many areas in the south of France, and more particularly the rural alpine Mediterranean area of the Mercantour National Park, may be affected by considerable quantities of ozone that originates from regional road traffic combined with the strong hot season of the Mediterranean climate, along the French Riviera, and on the Pô River plain in Italy. The regional forests have thus become the primary victims of the photochemical pollution given off by metropolitan Nice areas and their associated automobile traffic.



Fig. 1. The geographical position of the Tatra National Park (SK–HTMts) in the Carpathian mountain range and the Mercantour National Park in the Alpes-Maritimes region (FR–AlpMar) including selected sites: SPO – Skalnaté Pleso Observatory; RCS – Route du Col de Salèse.

2.2. Ozone dose modelling

Model simulation of MOD up-taken by *Pinus* species was performed by the multiplicative deposition model DO₃SE (Büker et al. 2012). In this work, the model output of phytotoxic ozone dose without threshold limitation (Y=0) i.e. POD0 was considered identical to MOD. As demonstrates Eq. 1, MOD (mmol O₃ m⁻² PLA) represents the amount of O₃ taken up by the vegetation via open stomata as stomatal ozone flux (F_{st} in nmol m⁻² s⁻¹) aggregated over the period between the start (SGS) and end (EGS) of the growing season [Eq. 1]. We considered the length of the growing season from 1st June to 31st August 2019 (JJA 2019) that cover the summer season when the intensity of the physiological process of mountain vegetation is the highest.

$$MOD = \sum_{SGS}^{EGS} \left[F_{st} * (3,600 / 10^6) \right]$$
 [1]

 $\rm F_{st}$ stands for the rate of passage of $\rm O_3$ entering through the stomata of a leaf and is defined (Eq. 2) by stomatal conductance (G_{sto} in mmol O₃ m⁻² s⁻¹) and concentration of O₃ c(z₁) in nmol m⁻³) at the top of the canopy measured in the tree height (z₁)

$$F_{st} = F_{sto} * c(z_{t}) * R_{st} = G_{max} * f_{ENVI} * c(z_{t}) * R_{st}$$
[2]

where R_{st} is a resistance factor reflecting the quasi-laminar resistance and leaf surface resistance (s m⁻¹) on F_{st} .

Key parameter for G_{sto} calculation is maximal stomatal conductance (G_{max} in mmol $O_3 m^{-2} s^{-1}$) that is species specific parameter optional in model assignment or derived from experimental measurements. Model values of G_{sto} correspond to G_{max} limited by environmental factors (f_{ENVI}). The effects of f_{ENVI} on G_{max} [Eq. 3] including meteorological and site conditions such as air temperature (f_{temp}), vapour pressure deficit (f_{VPD}), solar radiation or light (f_{light}), soil water potential (f_{SWP}), plant phenology (f_{nhen}), and O_3 concentration (f_{O3}).

$$f_{ENVI} = [min(f_{phen}, f_{O_3})] * f_{light} *$$

$$max\{f_{min}, (f_{temp} * f_{VPD} * f_{SWP})\}$$
[3]

Generally, there is no limitation of stomatal conductance associated with the leaf development stage of conifer species (i.e. $f_{phen} = 1$). We also considered $f_{03}=1$ because stomatal O_3 flux is driven particularly by air temperature defined according to the f_{temp} function (ICP, 2017).

The preset, built in version (3.0.5) of the DO₃SE model (SEI, 2014) with the collection of parameters for coniferous forests and parameter G_{max} (110 mmol O₃ m⁻² s⁻¹) for *P. mugo* obtained from field experiments (Bičárová et al. 2019) were used. Selected meteorological data allowed for specification of the environmental functions associated with air temperature [Eq. 4], vapour pressure deficit [Eq. 5], and irradiance radiation and light [Eq. 6]:

$$f_{temp} = max \left\{ f_{min}, \left[\left(\frac{AT - T_{min}}{T_{opt} - T_{min}} \right) + \left(\frac{T_{max} - T_{opt}}{T_{opt} - T_{min}} \right) \right] \left(\frac{T_{max} - T_{opt}}{T_{opt} - T_{min}} \right) \right\}$$

$$(4)$$

$$f_{VPD} = \min\left\{1, \max\left[f_{min}\left((1 - f_{min})\right) + \left(\frac{VPD_{min} - VPD}{VPD_{min} - VPD_{max}}\right)\right] + f_{min}\right\}$$

$$(5)$$

$$f_{light} = 1 - EXP((-light_a) * PFD)$$
[6]

where AT is measured air temperature (°C); VPD is vapour pressure deficit calculated on base of measurement of air temperature and relative air humidity (kPa); PFD represents the photosynthetic photon flux density in units of µmol m⁻² s⁻¹ i.e., photosynthetically-active radiation (PAR) derived from measurement of global solar radiation R (W m⁻²). These variables are completed with the species-specific parameters f_{min} (0.1), T_{min} (1°C), T_{opt} (18 °C), T_{max} (36 °C), VPD_{min} (-3.3 kPa), VPD_{max} (0.6 kPa), and light_a (0.008). The functions f_{temp} , f_{VPD} , and f_{hight} are expressed in relative terms (i.e., they accept values between 0 and 1 as a proportion of G_{max}).

2.3. Measured input data

Meteorological variables at SPO site were continuously monitored in hourly step using the measurement system based on a PROlog ultra-low power datalogger (Physicus, SK) connected with the following sensors: (AT) temperature probe with platinum resistance thermometers Pt100 for air temperature (at 2 m above the surface); (RH) Prove-HumiAir 9 for relative air humidity; (R) Pyranometer CMP6 (Kipp and Zonen) for global solar radiation; (WS) Wind Transmitter Compact (Thies Clima) for wind speed, (P) PressAir sensor for air pressure, and (Ppt) Rain Gauge (MR3H – Meteoservice CZ) for precipitation. Meteorological input data at RCS site were derived from measurements of Meteo-France in region Provence-Alpes-Côte d'Azur by approximation method. Air temperature (AT, °C) and relative humidity (RH, %) values were derived by linear interpolation with respect to altitudinal gradient. VPD (kPa) for a given AT and RH was calculated by a specific formula for saturated and actual vapour pressure. Precipitation (Ppt, mm) and wind speed (WS, m s⁻¹) data were derived from the nearest weather stations with respect to altitude zones. Air pressure (P, kPa) calculation used the barometric formula for given AT and altitude. Solar global radiation (R, W m⁻²) corresponded to measurement at the nearest weather stations.

Soil moisture data were obtained by modelling soil water potential (SWP in MPa), useful forn specifying the f_{SWP} function [Eq. 7].

$$f_{SWP} = \min\left\{1, \left\{f_{min}\left((1 - f_{min}) * (SWP_{min} - SWP)\right) / (SWP_{min} - SWP_{max})\right\} + f_{min}\right\}$$
[7]

The f_{SWP} function defines the effect of soil moisture on G_{max} [Eq. 2] in relative terms, similar to the aforementioned functions [Eq. 4-6] using additional threshold parameters, such as SWPmin (-1.20 MPa) and SWPmax (-0.76 MPa). In addition, field measurement of SWP was conducted at three soil depths (-0.1, -0.2, -0.4 m) only at SPO site. SWP values were measured using gypsum blocks at a range up to -1.5 MPa (GB2, Delmhorst Instrument, U.S.A.). SWP data were stored in integrated data loggers (MicroLog SP3, EMS Brno, CZ) at 1-hour intervals. Differences between f_{swp} based on measured and modelled SWP allow for verification of reliability for the soil moisture module included in the DO₂SE model. This modelling approach incorporated hydraulic resistance (steady state, SS) to water flow through the plant system (Büker et al. 2012).

Measurement of O_3 concentration at SPO was employed by calibrated active monitors (Thermo Electron Environmental 49C) based on the well established technique of absorption of UV light at 254 nm. Hourly mean data were recorded in a continuous regime without major gaps throughout the year 2019. For purposes of this study, the hourly meteorological and O_3 concentration data for summer months (June, July, and August) were analysed.

In FR–AlpMar, passive samplers developed in Sweden by IVL (Svenska Miljöinstitutet) were used for measurement of O_3 concentration. These passive O_3 sensors have the advantage of allowing large-scale monitoring of concentrations of gaseous pollutants in remote rural areas (Krupa & Legge 2000). ThePpassive sampler was placed in the open air, not under forest cover, and was protected by a metal sheet approximately 1.8 m above the ground. Ionic chromatography was used to analyse the ozone concentration (IVL Laboratories Sweden). This technic was validated in 2000, accuracy was verified by

comparing the results from the passive tube samplers with the results from the UV absorption analysers (Dalstein et al. 2001). Passive O₃ sampling provides input O₃ data in a monthly step that is not sufficient for standard MOD calculation. For this reason model processing was divided into two parts. In the first part, measured hourly meteorological data and theoretical O3 concentration 1 ppb were processed by the DO₃SE model to obtain the theoretical MOD. In the second part, the final MOD was calculated by multiplying MOD, and average O₃ concentration measured over the period. This procedure was applied individually for *P. mugo* (STO) and *P. cembra* (RCS). Model calculation of MOD based on continuously measured hourly values of O₃ concentration and meteorological variables at SPO site were used to verify this modified approach.

2.4. Visible injury

Inspection of visible injury on *Pinus* species was primarily focused on the identification of visible ozone and visible ozone like symptoms. Chlorotic mottling is the most common symptom that can be described as yellow or light green areas of similar size without sharp borders between green and yellow zones. It frequently appears only in second-year needles, and older (ICP, 2016). Observation of visible O_3 injury on *P. cembra* in FR–AlpMar at RCS plot was carried in accordance with the proven techniques for evaluation of the ozone-specific symptoms, which are described in the ICP Forests protocol (Schaub et al. 2010; Michel et al. 2014).

The thick leaves of *P.cembra* intercept more light at low angles of incidence than at a high angle of incidence, this has an affect on the ozone symptoms appearance (Jordan & Smith 1993) and thus at each plots O₂ injury was assessed on five adult trees well exposed to sunlight. From these trees five branches with at least 30 needles per branch were removed from the upper third part of the crown and were assessed for foliar injury based on needle age classes, the percentage of needle surface affected was scored for current year needles (C), 1 year old (C+1), and two year old (C+2) needles. The observations were made by experts from the GIEFS (Groupe International d'Études des Forêts Sud-Européennes). For P. cembra spots or mottling were recorded during late summer from mid-August to early September a period during which the concentration of ozone is highest of the year. The ozone specific foliar injury was calculated for its mean percentage by considering needles/leaf surface affected per plot. The ambition of research on P.mugo in SK-HTMts was to provide a comprehensive evaluation of surface visible injury where we considered also other harmful agents such as fungal and viral diseases, leaf biting insects, red spider mites mentioned in ICP Vegetation Ozone Injury Recording Application (ICP, 2018). Inspection of visible injury on *P. mugo* in SK-HTMts was realised only on two years-old (C+2) needles, as these needles show pronounced injury (Bičárová et al. 2019). We selected a total of 10 monitoring sites near SPO (Fig. 2 left) including 6 sites with high tree coverage (S1-S6) and 4 sites situated at the highest position (S7-S10) of P. mugo belt, above 2,000 m a.s.l. We marked three trees at each S1-S6 and one tree at each S7-S10 site, respectively. Sample of ten twin needles from each marked tree was collected at the beginning of summer (early June) and autumn (early October) season 2019 with an aim to notice changes of visible injury influenced by ozone uptake during the growing season. In addition, we performed control observation on young seedlings located indoors, protected from external influences. Two years old P. mugo seedlings were obtained from Gene pool center managed by specialist forest workers of the State Forests of Tatra National Park situated at the area near SPO called Rakúskelúky (49°12'37" N; 20°19'32" E; 800 m a.s.l.).

The visible injury both for field and control samples were identified by specialists in visible ozone injury, fungal disease, and other forest tree damage. For this study we define the visible injury index (VINX, Eq. 8) covering different harmful agents (ICP, 2018): a) fungi diseases b) stinging insects c) spider mites d) ozone, and e) abioticmechanical effects (Fig. 2 right).

$$VINX(\%) = \frac{\Sigma X_{score}}{X_{max}}$$
[8]

where $\Sigma X_{score} = X_a + X_b + X_c + X_d + X_e$ is the sum of the scores for each harmful agent (a–e). The score corresponds to the extent of injury observed on needle surface in scale from 0 (without visible injury symptoms) to 5 (damage to the entire surface of the needles) that was evaluated individually for each harmful agent. X_{max} is the sum of the maximal score for each harmful agent. According to VINX value (%), the degree of damage was classified according to the classes mentioned in Schaub et al. 2016 (Table 1) in scale from 0 (no injury) to 3 (large damage).

Table 1. Visible injury index (VINX) classification.

| Legend | VINX (%) | Class |
|-----------|------------------------------------|-------|
| No injury | 0% | 0 |
| Low | 1-5% of the surface is affected | 1 |
| Moderate | 6-50% of the surface is affected | 2 |
| Large | 51-100% of the surface is affected | 3 |

3. Results

3.1. Environmental factors and stomatal conductance

Effect of air temperature (AT, Eq. 4), vapour pressure deficit (VPD, Eq. 5), photosynthetically-active radiation (PFD, Eq. 6), and soil water pressure (SWP, Eq. 7) on environmental factors (f_{temp} , f_{VPD} , and f_{light} , and f_{sWP}) covering JJA 2019 period illustrates Fig. 3. As expected, f_{temp} , f_{light} , reflect more appropriate air temperature and solar

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Fig. 2. The scheme of sites (S1–10) selected for collecting *P. mugo* needle samples in SK–HTMts (upper and lower left rectangle), triangle marks the position of Skalnaté Pleso Observatory (SPO); examples of needle damage differentiated according to the harmfull agent: a) fungi diseases b) stinging insects c) spider mites d) ozone e) abiotic-mechanical effects (lower right rectangle).

radiation conditions in Mediterranean FR–AlpMar than colder climate in SK–HTMts. Contrary, in FR–AlpMar water insufficiency in both, air and soil was more pronounced in July and August.

It is evident that f_{SWP} (Fig. 3) is the crucial environmental factor with a relevant influence on G_{sto} in FR-AlpMar (Fig. 4 right). Effect of f_{swp} contributed to G_{sto} limitation to minimal level up to 20 mmol m⁻² s⁻¹ during a relative long-lasting time window started at the end of June (number of the day (NOTD) \approx 180) and continuing with short interruptions until the end of summer. On the contrary, the period of G_{sta} limitation (close above 40 mmol $m^{-2} s^{-1}$) in SK-HTMts (Fig. 4 left) lasted only a few days in July (NOTD around 190) and was influenced primarily by cold wave episode (AT, f_{temp} in Fig. 3). Differences between measured and modelled SWP values for SPO site in SK-HTMts (Fig. 5) show relatively good agreement when we take into account threshold limits (SWP max - SWP min) as well as f_{SWP} . Due to the absence of field SWP measurement, the comparison with the modelled data is not presented for RCS in FR-AlpMar. For future work, it should be beneficial to include SWP data from field measurement into MOD modelling, especially for areas with soil water deficit such as FR-AlpMar. The relevance of f_{SWP} in relation to model outputs of F_{st} and MOD [Eq. 1–2] demonstrate Fig. 6 with nearly two times higher MOD₁ at SPO in SK–HTMts than RCS in FR–AlpMar, considering theoretical $c(z_1) = 1$ ppb.

3.2. Ozone concentration and ozone dose

The measurement of O₂ concentration at SPO using the active ozone monitor equipment suggests relatively high O₂ abundance in the subalpine zone SK-HTMts with a mean annual value of 45.9 ppb (2000-2019). It can be assumed that O₃ formation at this site depends on the complex actions of O₃ precursors originated from various sources (local, regional, from long-distance transport, anthropogenic, biogenic). Measured O₃ concentration recalculated to reference height of 20 m (ICP, 2016) refers to nearly two times higher average O₃ values for SPO (>50 ppb) than RCS site (>25 ppb) during the summer season from June to August 2019 (Fig. 7 left). Standard model processing input O₂ data in the hourly step resulted in the value of MOD close below 15 mmol m⁻² PLA (Fig. 7 right) at SPO in SK-HTMts. Modified calculation linking to MOD_m value shows good agreement with MOD (Table 2, Fig. 8). Modification approach asso-



Fig. 3. Seasonal development of hourly measured meteorological data (left part) and corresponding environmental factors (right part) processed by DO₃SE model for SK–HTMts and FR–AlpMar sites.


Fig. 4. Model results of G_{sto} processed by DO₃SE model for SK–HTMts and FR–AlpMar sites.

ciated with the value of MOD_m consists of multiplying of model MOD_1 output for theoretical O_3 concentration $c(z_1) = 1$ ppb and O_3 concentration averaged over considering period i.e. $c(z1) = O_3$ avg (ppb). This approach was used to estimate MOD at RCS in FR–AlpMar where only monthly O_3 data obtained by passive samplers are avail-



Fig. 5. Measured hourly values of SWP at SPO site in SK–HT-Mts in comparison with model results of SWP and f_{swp} .

able. For this site, the average value $c(z_1)$ was 32.3 ppb (Table 2) in the period JJA 2019. According to achieved MOD outputs, we can assume that *P. mugo* in SK–HTMts absorbed substantially higher O₃ amount (MOD = 14.1 mmol m⁻² PLA \approx MOD_m) than *P. cembra* in FR–AlpMar (MOD_m = 4.0 mmol m⁻² PLA) during the same time period JJA 2019.

3.3. Visible injury

Foliar ozone specific injury observed on the surface of *P. cembra* needles at RCS plot in FR–AlpMar in 2019 (Fig. 9), presented a percentage of deterioration of 3% (C), 16% (C+1), and 25% (C+2) on an average i.e. in a range from low to moderate damage (class 1–2). As expected, the symptom of mottling was most commonly observed on older needles. Mottling occurred as a small spot of yellow/light green or mottling with a diffuse outline, especially on the upper surface and at the tip of the needles. Despite the low MOD of 4.0 mmol $O_3 m^{-2}$ PLA (Table 2), the frequency of mottling identified on *P. cembra* (C+2) was markedly higher when comparing



Fig. 6. Model results of stomatal ozone flux (F_{st} , left) and modelled ozone dose (MOD₁, right) processed theoretical input O₃ concentration c(z_1)=1ppb for SK–HTMts and FR–AlpMar sites.



Fig. 7. Measured O_3 concentration (left): long straight line depicts the average of hourly data obtained from active analyzer at SPO in SK–HTMts and short straight lines illustrate monthly averages derived from passive samples at RCS in FR–AlpMar; on right: standard model outputs F_{st} and MOD using real hourly O_3 data from measurement at SPO in SK–HTMts; to estimate MOD value for RCS in FR–AlpMar requires modification approach.

Table 2. The MOD value based on hourly O_3 data (O_3 _h) and MODm results linking to averaged O_3 concentration (O_3 _avg) for JJA 2019 period.

| Study site | MOD mmol m ⁻² PLA | | MOD _m mmol m ⁻² PLA | MOD vs MOD _m | |
|------------|------------------------------------|---------|----------------------------------------------|---------------------------------|----------------|
| Study site | $c(z_1)$ O ₃ h (ppb) | MOD_1 | $c(z_1)$ O ₃ -avg (ppb) | MOD_1 xO ₃ -avg | Difference (%) |
| SK-HTMts | 14.0 | 0.25 | 56.2 | 14.1 | 0.7% |
| FR–AlpMar | : | 0.12 | 32.3 | 4.0 | : |

ozone symptoms evidenced on *P. mugo* (C+2) in Table 3. The rare incidence of visible O_3 injury (VINX= 3% on an average, Class =1) suggests the tolerance of *P. mugo* in SK–HTMts towards MOD value of 14.1 mmol O_3 m⁻² PLA. On the other hand, low MOD associated with frequently mottling occurrence could denote a high sensitivity of *P. cembra* on ozone and environmental stress in FR–AlpMar.

Visible injury on *P. mugo* (C+2) needles in SK–HTMts represented by VINX (%) confirm the increase of field sample damage from 14 % at the beginning (June 2019) to 21% at the end of growing season (October 2019) on an average (VINX (a–e) in Table 3), which corresponds



to class 2 (i.e. Moderate injury) based on Table 1. corresponds VINX (a, b, c, d, e) differentiated according to the type of harmful agent accents similarity to the incidence of O_3 symptoms and injury due to fungi diseases or biting



Fig. 8. Correlation between MOD corresponding to the input of O_3 concentration measured in hourly step and MOD_m based on O_3 concentration averaged over JJA 2019 period at site SPO in SK–HTMts.

Fig. 9. Box-whiskers statistic plot shows foliar ozone specific injury on *P. cembra* at RCS site in FR–AlpMar for different-aged needles: current year (*C*), one year old (*C*+1), and two years old (*C*+2) inspected in 2019. For each box-whisker plot, the center dot represents the average of the foliar injury during the study period.

| June/October 2019 | | Score: 0 (without injury) 5 (extensive damage) | | | | | (a-e) | (a, a) | (a, a) |
|----------------------|--------|------------------------------------------------|-----------------------|---------------------|----------------------|-----------------------|--------------|-----------------|----------------|
| | | (a) Fungi diseases | (b) Biting insects | (c) Spider mites | (d) Ozone mottles | (e) Abiotic traces | Score sum | (a-e) VINX % | (a-e) Class |
| | | | | | | | | | |
| S1 | 0/0 | 0/0 | 0/1 | 1/0 | 0/1 | 4/9 | 5/12 | 1/2 | |
| | 0/2 | 1/1 | 1/1 | 0/0 | 0/1 | | | | |
| | 0/1 | 0/0 | 1/1 | 0/0 | 0/1 | | | | |
| S2 | 1/1 | 2/2 | 1/1 | 1/0 | 0/3 | 11/14 | 15/19 | 2/2 | |
| | 0/1 | 1/1 | 2/1 | 2/0 | 0/1 | | | | |
| | 1/1 | 1/1 | 1/1 | 1/1 | 0/2 | | | | |
| S3 | 0/0 | 1/1 | 1/1 | 0/1 | 0/1 | 10/17 | 13/23 | 2/2 | |
| | 0/1 | 1/2 | 2/1 | 1/1 | 0/2 | | | | |
| | 2/1 | 0/0 | 1/2 | 1/1 | 0/2 | | | | |
| S4 | 1/1 | 0/1 | 2/2 | 0/1 | 0/3 | 14/23 | 19/31 | 2/2 | |
| | 1/0 | 1/0 | 3/3 | 2/2 | 0/4 | | | | |
| | 1/1 | 1/2 | 1/2 | 1/1 | 0/1 | | | | |
| S5 | 1/1 | 0/1 | 2/2 | 0/2 | 0/1 | 12/22 | 16/29 | 2/2 | |
| | 1/1 | 2/2 | 1/2 | 1/2 | 0/1 | | | | |
| | 0/1 | 0/0 | 1/2 | 0/1 | 0/1 | | | | |
| S6 | 4/0 | 1/0 | 2/1 | 0/0 | 0/1 | 10/9 | 13/12 | 2/2 | |
| | 0/0 | 0/0 | 1/1 | 1/0 | 0/1 | | | | |
| S7 | 1/1 | 0/0 | 2/1 | 1/1 | 0/1 | | | | |
| S8 | 1/1 | 0/0 | 1/1 | 1/1 | 3/1 | 19/23 | 19/23 | 2/2 | |
| S9 | 1/1 | 1/0 | 1/2 | 1/1 | 0/1 | | | | |
| S10 | 2/2 | 0/1 | 2/2 | 1/2 | 0/3 | | | | |
| Sum | 18/18 | 13/15 | 30/32 | 16/18 | 3/34 | 80/117 | : | : | |
| (a,b,c,d,e) VINX (%) | 3/3 | 2/3 | 5/6 | 3/3 | 1/6 | : | 14/21 | : | |
| | Class | 1/1 | 1/1 | 1/2 | 1/1 | 1/2 | : | : | 2/2 |
| Control site | | 1/1 | 0/0 | 1/1 | 0/0 | 0/0 | | | |
| | K0 | 0/0 | 0/0 | 1/0 | 0/0 | 0/1 | 4/3 | 5/4 | 1/1 |
| | | 0/0 | 0/0 | 1/0 | 0/0 | 0/0 | | | |
| | Sum | 1/1 | 0/0 | 3/1 | 0/0 | 0/1 | 4/3 | : | : |
| | VINX % | 1/1 | 0/0 | 4/1 | 0/0 | 0/1 | : | 5/4 | : |
| | Class | 1/1 | 0/0 | 1/1 | 0/0 | 0/1 | : | : | 1/1 |

Table 3. Inspection of visible injury on *P. mugo* (C+2) needles in SK–HTMts at the beginning (June) and the end (October) of the growing season 2019.

insects. Although spider mites symptoms dominated in the biotic agent group in June as well as in October 2019, deterioration during the growing season was particularly due to abiotic/mechanical type of damage. The inspection of the control plants situated in a protected indoor environment revealed low injury up to 5% i.e. class 1 (Table 1), primarily due to the occurrence of spider mites. We did not notice deterioration, contrary; improving concerning the occurrence of spider mites suggests high regeneration ability of *P. mugo* seedlings.

4. Discussion

Commonly used methods for assessing the impact of ozone on forests are based on the measurement of O₃ concentration (AOT40) and modelling of accumulated stomatal O, flux (POD) that provide AQI generally related to the abiotic element of the environment. On the other hand, the core of the O₃ phytotoxicity problem lies in the disruption of the biological integrity of plant cells due to oxidative stress. The major challenge in the development of O₃ standards is their validation against biologicallybased field data (Paoletti & Manning 2007). Specific ozone visible symptoms are still the best indicator of ozone induced injury (Sicard & Dalstein-Richier 2015; Paoletti et al. 2019). In this work, we present results of visible O₂ injury inspection undertaken on *P. cembra* in FR-AlpMar and P. mugo in SK-HTMts. The inspection of visible injury on P. mugo highlights the importance of complex evaluation of all biotic and abiotic agents operating in mountainous zones beside the O_3 symptoms (Table 3). Ozone may have an impact on discoloration and defoliation and should be considered together with the influence of other factors (Badea et al. 2004).

Although substantially different MOD values (Table 2), relatively low incidence of O₂ symptoms suggests milder O₂ effect on *P. mugo* in SK-HTMts than on *P.* cembra in FR-AlpMar (Table 2). The presence of foliar visible symptoms can be interpreted as a strategy of Pinus species adapted to limiting environmental conditions and does not mean necessarily damage resulting in growing reduction (Marzuoli et al. 2019). The discrepancy between a low value of VINX (Table 3) and a high level of MOD (Table 2) related to P. mugo in SK-HTMts could be associated e.g. with the activation of antioxidant enzymes in the needles acclimating to increased levels of oxidative stress. Superoxide dismutase enzymes (SODs) concentrations typically increase with the degree of stress conditions. SODs act as antioxidants and protect cellular components from being oxidized when catalyzing the production of O2 and H2O2 from superoxide (O2-) (Alscher et al. 2002). High levels of SOD activity could protect the plant from visible injury caused by ozone when the overproduction of SOD in the chloroplasts may result in a 3-4 fold reduction of visible O₂ injury (Van Camp et al. 1994). Kormuťák et al. (2019) found the increasing content of SOD in the P. mugo needles in High Tatra Mts. from April, with the peak in August, followed by a slow decrease until November.

The phytotoxic effect of ozone on Pinus species we investigated by tracing biological symptoms such as visible O₃ injury in relationship to MOD. The amount of ozone absorbed by forest trees can be estimated by modelling that requires precise and continual field measured input data supplemented by species-specific model parameters. To achieve more accurate results of MOD in FR-AlpMar, measurements of input data should be realized within selected forest plots in hourly step using automatic types of equipment both for meteorological variables and O₃ concentration. On the other hand, to obtain uninterrupted hourly O₃ data based on active O₃ monitor measurement in mountain field conditions is difficult. Passive sensors providing O₃ concentration averaged over a month period seem operationally more friendly than the active monitors. In that context, it would be appropriate to arrange the O₃ measurement that consists of at least one point equipped by both the active monitor and passive sensor with additional points at remote plots using passive sensors. Substitution of measured hourly O₂ concentrations by average O₂ concentration over the growing season period for calculation MOD (Fig. 8) is possible for plots situated in a subalpine zone with the typical flattened daily course due to the nondestruction of ozone at night. It follows that average O₂ values derived from passive samplers could be used in MOD modelling although there is a study referring to the differences between active and passive O₂ sampling that can range for e.g. from -14% to 77% when comparing seasonal mean O₂ concentrations (Pitar et al. 2018). Model results of SWP, as well as f_{SWP} , revealed a relevant decrease of G_{sto} as a response to soil moisture deficit in summer season in FR-AlpMar (Fig. 4). Soil moisture conditions can have a significant effect on stomatal conductance (De Marco et al. 2016). Field measurement of SWP is important to take into account when modelling the stomatal O₂ flux especially in areas where soil drought events in association with lower precipitation occurs.

For the future, the system of air quality with respect to the biological response of mountain tree species to O_3 could be innovated by introducing modern methods providing the opportunity to analyze large areas e.g. employing the remotely sensed satellite data and spectral indices.

5. Conclusion

Respecting the objectives of this study, research of *Pinus* species response to ozone pollution suggests that *P. mugo* in SK–HTMts received substantially higher ozone dose than *P. cembra* in FR–AlpMar during considering period JJA 2019. Relatively low MOD uptaken by P. cembra was particularly due to soil drought linking to fSWP factor. This confirms our assumption that hot, sunny, and dry summer weather typical for the Mediterranean climate plays the principal role in ozone uptake. Despite the low MOD, a high degree of surface damage on two years old needles of *P. cembra* was identified. This may indicate

high sensitivity *P. cembra* to ozone and environmental stress. On the other hand, although high MOD, an inspection of visible O_3 injury on *P. mugo* showed a low incidence of O_3 symptoms on *P. mugo* needles surface in SK–HTMts. This may be associated with the activation of antioxidant enzymes under oxidative stress conditions. In this work, we also tested the use of passive O_3 sampler measurements in the model simulation of MOD. Our results present that the average O_3 concentration for the considered period can replace O_3 concentration in an hourly step in the model input file, primarily for field sites situated in the subalpine and alpine zone where nearly flat daily O_3 course is observed.

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List of abbreviation

Study area

SK FR SPO RCS

Ozone dose modelling DO.SE

PODY (nmol m⁻² PLA s⁻¹) $PLA(m^{-2})$ POD0 (nmol m⁻² PLA s⁻¹) MOD (mmol O₃ m⁻² PLA) $\begin{array}{l} \text{MOD}_{\text{m}} (\text{nmmol } \text{O}_3 \text{ m}^{-2} \text{ PLA}) \\ \text{MOD}_{\text{m}} (\text{nmmol } \text{O}_3 \text{ m}^{-2} \text{ PLA}) \end{array}$ $C(Z_1)$ F_{st} (nmol O₃ m⁻² s⁻¹) G_{sto}^{*} (mmol $O_{3} m^{-2} s^{-1}$) G_{max} (mmol O_{3} m⁻² s⁻¹) R_{st} – Resistance factor f_{ENVI} ĂT (⁰C) T_{min} (1 °C), T_{opt} (18 °C), T_{max} (36 °C) RH (%) VPD (kPa) VPD_{min} (-3.3 kPa), VPD_{max} (0.6 kPa) f_{VPD} PFD (μmol m⁻² s⁻¹) PAR (μ mol m⁻² s⁻¹) R (W m⁻²) light_a (0.008) f_{light} SWP (MPa) SWPmin (-1.20 MPa) SWPmax (-0.76 MPa) f_{SWP} $f_{min}^{03}(0.1)$ SGS EGS P (kPa) Ppt (mm) WS ($m s^{-1}$)

Visible injury VINX (%) – HTMts: Slovakia, the High Tatra Mts.

- AlpMar: France, the Alpes-Maritimes region
- Skalnaté Pleso Observatory
- Route du Col de Salèse
- Multiplicative Deposition Model
- Phytotoxic Ozone Dose over a detoxification threshold flux of Y
- Projected Leaf Area
- Phytotoxic Ozone Dose without threshold limitation (Y=0)
- Modelled Ozone Dose
- Modelled Ozone Dose Modified
- Modelled Ozone Dose for theoretical O_3 concentr. $c(z_1) = 1$ ppb
- $-O_{2}$ concentration at the top of the canopy measured in the tree height (z,)
- Stomatal Ozone Flux
- Stomatal Conductance
- Maximal Stomatal Conductance
- -Environmental factors
- Air Temperature
- Species-specific parameters of Air Temperature
- Factor of Air Temperature
- Relative Humidity
- Vapour Pressure Deficit
- Species-specific parameters of Vapour Pressure Deficit
- Factor of Vapour Pressure Deficit
- Photosynthetic photon Flux Density
- Photosynthetically Active Radiation
- Global Solar Radiation
- Species-specific parameters of Solar light
- Factor of Solar radiation or light
- Soil Water Potential
- Species-specific parameters of Soil Water Potential
- Species-specific parameters of Soil Water Potential
- Factor of Soil Water Potential
- Factor of Plant Phenology
- Species-specific parameter
- Start of Growing Season
- End of Growing Season
- Air Pressure
- Precipitation Amount
- Wind Speed

- Visible Injury Index