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Wood-inhabiting macromycete communities in spruce stands on former agricultural land

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Abstract: Wood-inhabiting macromycete (WIM) communities in the ecosystem of uneven-aged spruce stands growing on former agricultural land were investigated in relation to the supply of wood substrate, degree of wood rot, and selected climatic and ecological conditions. Altogether, 58 WIM species were detected at research plots during 2016–2018. The abundance of fruiting bodies and WIM species richness increased from the youngest to the oldest forest stands. The highest numbers of fruiting body abundance were recorded for *Gymnopus perforans* (11 756), *Hypholoma fasciculare* (2 971), *Coprinellus disseminatus* (326), *Exidia pithya* (318) and *Panellus mitis* (147). The influence of stand age on WIM abundance was highly significant ($P < 0.001$), WIM abundance was not affected by precipitation ($P > 0.05$). The relationships between abundance and air temperature ($P < 0.001$), species richness and precipitation ($P < 0.001$), species richness and air temperature ($P < 0.001$) were highly significant. The most frequent damage to trees was caused by insects and forest animals (81%), which resulted in a high occurrence of resin secretion (70%). The total volume of coarse wood debris (CWD) and the decay rate were not statistically dependent. We confirmed the occurrence of *Heterobasidion annosum* s.s., *H. abietinum* s.s., *H. parviporum* s.s., *Armillaria ostoyae* s.s. and *A. cepistipes* s.s. by use of molecular genetic analyses.

Keywords: managed forests; *Picea abies*; phytopathology; rot; wood-decaying fungi; Western Carpathians

Spruce forest ecosystems in Slovakia provide habitats for many fungal species that grow only in such ecosystems or in an environment where spruce is present. Dynamics of species diversity, abundance of fruiting bodies, biomass production and successive relationships of wood-inhabiting macromycetes (WIM) associated with the occurrence of spruce vary depending on the supply of wood substrate, age of stands, woody species composition of stands, and climate conditions, such as precipitation, humidity, temperature and drought (e.g. Gáper, Mihál 2008; Soukup et al. 2008; Kałucka 2009; Štefančík, Kamenský 2009; Mihál, Luptáková 2017; Aghajani et al. 2018).

The ecology of WIM as a group of fungi separated from terrestrial saprotrophic or ectomycorrhizal macromycetes is generally known. WIM are typically subdivided into parasitic (obligatory and facultative) WIM and saprotrophic WIM. In forest management, parasitic WIM are particularly important due to the damage caused by their pathogenicity to forest stands (especially in monocultures). In Slovakia, spruce is mainly associated with the fungi *Armillaria* spp., *Fomitopsis pinicola* (Sw.) P. Karst., *Heterobasidion* spp., *Neonectria fuckeliana* (C. Booth) Castl. & Rossman, *Stereum sanguinolentum* (Alb. et Schwein.) Fr. (e.g. Mihál 1998, 2005; Uhlířová et al. 2004; Gáper, Mihál 2008;

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Mihál, Luptáková 2017). Perhaps the best known fungal parasites of spruce in Slovakia and in Europe in general are the species of the genus *Armillaria* spp. and *Heterobasidion* spp. (Figure 1). The ecology of these fungi in terms of bionomics, distribution, taxonomy, genetics, virulence, decomposition of wood, biology and other issues has been widely reported (Gáper, Mihál 2008; Garbelotto, Gonthier 2013; Sedlák, Tomšovský 2014; Oliva et al. 2015; Buza, Divos 2016; Trishkin et al. 2016).

Saprotrophic WIMs are important in forests because they decompose dead wood and other organic matter. Species richness of saprotrophic WIM mainly depends on the volume, availability, and type (hardwood or coniferous) of dead wood substrate. In commercial forests, the volume and diversity of dead wood are generally low as a result of plantation management; in such forests there are virtually no decaying thick branches or whole tree trunks (Pouska et al. 2010; Luptáková et al. 2018; Yakhyaev et al. 2019).

The most characteristic ecological role of WIM is the process of decomposition of wood mass followed by succession of WIM species on the decomposing substrate. This is the basis of the process of organic matter decomposition in forests that has been widely studied. Fukasawa et al. (2014) studied the process of rotting in coniferous forests in Japan. They reported the occurrence of both white and red-brown rot, and that biogenic elements (N, P, K, Na, Mg and Ca) were mineralized in the white rot phase, which was dominated by *Trichaptum abietinum* (Dicks.) Ryvarden and *T. fuscoviolaceum* (Ehrenb.) Ryvarden. In the red-brown rot phase, the dominant species were *Laetiporus sulphureus* (Bull.) Murrill and *Oligoporus caesius* (Schrad.) Gilb. & Ryvarden. Longuetaud et al. (2006) and Brüchert et al. (2017) studied the problem of wood damage during logging, e.g. resin secretion and the formation and spread of wounds on damaged spruce and fir trees. Kazartsev et al. (2018) documented the taxonomic composition of fungal communities on the bark of spruce branches during the progression of the rotting process. During the early stages of rot, the communities of yeasts, plant pathogens and cosmopolitan saprotrophic fungi emerged, while the transitional stage mainly involved saprotrophic fungi commonly found on decomposed wood.

Spruce stands often show interaction between the rotting process and trees damaged by forest animals. Månsson and Jarnemo (2013) studied spruce damage by deer herbivory in Sweden. They found that the susceptibility to damage was related to the morphological features of the trees (bark thickness, branching, and stem diameter) and the presence of old damage on the trunks. In the Czech Republic, Čermák and Strejček (2007) found that in 40-year-old spruce stands, bark wounds caused by deer were often infected with so-called "wound" fungal parasites such as *Stereum sanguinolentum*. The impacts of large animals (deer and elk) on 30-year-old spruce stands in Latvia in relation to the occurrence of wound fungal parasites was reported by Burneviča et al. (2016). Impacts of wounds on spruce caused by deer were reported by Finďo (2010) for the Low Tatras Mountains spruce stands of Slovakia.

WIM communities in uneven-aged spruce stands planted on former farmlands in the Vrchdobroč locality, central Slovakia, have been studied e.g. by Mihál (2005), Gáper and Mihál (2008), Mihál and Luptáková (2017). Mycological research was underway in the Vrchdobroč locality during 2016–2018 to monitor the process of mycoflora colonization and spread in spruce stands by the age of forest stand. The aim of our study was to characterize the status of WIM communities in uneven-aged spruce forests as a function of the supply of wood substrate, the degree of tree decay, and selected climatic variables.

We established the following working hypotheses for the research of WIM communities in spruce stands of different ages in the Vrchdobroč local-

Figure 1. Syrrotium of *Armillaria* spp. (A) and sporocarp of *Heterobasidion* spp. (B) at the Vrchdobroč locality
photo: E. Luptáková

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ity: (*i*) the highest values of species diversity and abundance of fruiting bodies of WIM species will be recorded in the oldest stands, (*ii*) fruiting bodies of fungi of the genera *Armillaria* sp. and *Heterobasidion* sp. will occur only sporadically in all types of stands, (*iii*) the highest incidence of parasitic WIM will be recorded in the oldest stands, (*iv*) the highest value of the degree of rot will be recorded in the oldest stands.

MATERIAL AND METHODS

Study site. Our study sites were spruce stands planted on former farmland soils near the Vrchdobroč forest complex, central Slovakia, in the western part of the Slovenské Rudohorie Mountains, Veporské Vrchy Hills, in the spring area of the Ipeľ River. Elevations at the Vrchdobroč locality range from 740 to 917 m a.s.l., the bedrock is composed of crystalline granodiorite and the prevailing soil type is brown forest soil (Cambisol), sandy-loam. The average annual temperature in the growing seasons from 2016 to 2018 was 14.32 °C, the average precipitation during 2016 to 2018 was 492 mm (according to SHMÚ 2019 – Detvianska Huta Weather Station, 2 km away by air distance from the examined stands). In the area of individual permanent research plots (PRPs) we also determined the species composition of the herbal undergrowth that was represented by: *Athyrium filix-femina*, *Carex sylvatica*, *Fragaria vesca*, *Geranium robertianum*, *Rubus hirtus*, *R. idaeus*, *Senecio umbrosus*, *Urtica dioica*. Other recorded characteristics of each PRP are listed in Table 1.

Spruce culture stands at Vrchdobroč were established in the last decades of the last century on former farmland that was converted to forest by a governmental resolution enacted in 1960. The government reforestation program was adopted in response to unsustainable regional deforestation. The spring area of the Ipeľ River catchment, Lučenecký brook and Rimava River declined from about 60% to 29% over the last 50 years, the greatest decline in Czechoslovakia at that time. According to the reforestation plan, the forest area was to increase from 7 648 to 15 738 ha by 1980.

The structure of plantation stands in this area began to change rapidly after a number of winter storms (snow and wind storms) during winter 1993/1994. These storms largely destroyed spruce

Table 1. Basic characteristics of mycological permanent research plots (PRPs) in forest stands in the Vrchdobroč Hills

PRPs	Elevation (m a.s.l.)	Localisation	Dominant trees	Age	Number of trees	Growing phase	Area (m^2)	Exp.
A1	870	48°32'00.5"N 19°34'44.9"E	*Picea abies* 72.61%	21	157	pole stage stand	416.6	SW
A2	890	48°32'02.4"N 19°34'46.4"E	*Picea abies* 55.3%	21	85	pole stage stand	416.6	SW
A3	830	48°31'52.0"N 19°33'45.3"E	*Picea abies* 100%	21	60	pole stage stand	416.6	E
B1	850	48°31'58.3"N 19°34'31.9"E	*Picea abies* 100%	31	83	pole stage stand	416.6	E
B2	820	48°31'46.0"N 19°34'06.0"E	*Picea abies* 99.1%	31	111	pole stage stand	416.6	SW
B3	830	48°31'48.3"N 19°34'09.7"E	*Picea abies* 99.1%	31	111	pole stage stand	416.6	SW
C1	820	48°31'42.9"N 19°34'16.1"E	*Picea abies* 100%	51	54	mature stand	416.6	S
C2	800	48°31'33.6"N 19°34'05.8"E	*Picea abies* 96.72%	51	61	mature stand	416.6	E
C3	825	48°31'38.3"N 19°33'50.4"E	*Picea abies* 66%	51	50	mature stand	416.6	S

Age – age of stands (in 2016); exp. – exposition; other tree species on PRPs A1: *Larix decidua* 26.75%, *Populus tremula* 0.64%, A2: *Larix decidua* 44.7%, B2: *Populus tremula* 0.9%, B3: *Larix decidua* 0.9%, C2: *Abies alba* 3.28%, C3: *Abies alba* 32%, *Fagus sylvatica* 2%

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stands in the pole stage of growth, entire areas at the highest elevations, and patchy areas in the lower elevation stands. After these snow and wind storms, spruce forests in the Vrchdobroč locality were considerably fragmented. This weather-induced fragmentation was compounded by the planting of other woody species that were preferred to spruce, especially *Abies alba* Mill., *Fagus sylvatica* L. and *Acer pseudoplatanus* L. (by Štefančík, Kamenský 2009). Thus, at the time of our study, there were few extensive monoculture spruce stands in the Vrchdobroč locality. We sought to situate our research plots in these few spruce monocultures but in some cases we had no option but mixed species stands (Table 1).

Research methodology. We sampled WIM at nine mycological permanent research plots (PRPs) once a month from June to October in the years 2016 to 2018. PRPs were labelled A1–A3, B1–B3, and C1–C3 (Table 1). At each PRP, we recorded the species of WIM and the abundance of fruiting bodies on both spruce and deciduous trees. Some WIM were too small or too scattered for accurate counts. Others had fruticose or resupinate WIM fruiting bodies, which also complicated counting. We counted all fruiting bodies when they were detectable and when the accuracy of our counts was considered to be high. This was the case for the genera *Armillaria*, *Gymnopus*, *Hypholoma*, *Mycena*, *Rhodocollybia*, and *Tricholomopsis*. For some species, e.g. *Jackrogersella multiformis*, *Calocera viscosa*, *Chondrostereum purpureum*, *Dacrymyces stillatus*, *Diatrype disciformis*, genera *Stereum* and *Trametes, Xylodon radula*, due to the morphology of their fruiting bodies, exact values of fruit abundance could not be calculated.

The species that were unidentifiable in the field were brought to the laboratory for identification based on keys by Moser (1963, 1983), Červenka et al. (1972), Veselý et al. (1972), Jülich (1984), Breitenbach and Kränzlin (1986, 1991), Hansen and Knudsen (1992, 1997, 2000), Hagara et al. (1999), Papoušek (2004), Hagara (2014). We also used comparative material from the herbarium collection of the second author at the Institute of Forest Ecology SAS (Slovak Academy of Science) in Zvolen. The fungal species not previously recorded in the Vrchdobroč locality were collected and stored at the Institute of Forest Ecology SAS in Zvolen. The scientific nomenclature and the authors' abbreviations were adopted mainly from the database of Cooper, Kirk (2019). Data from 9 replications were obtained in each particular month. The age of the stand is a dependent variable between these samples.

We evaluated the phytopathological manifestations on trunks (from the roots to the visible part of the crown) of 225 live trees on June 8, 2018. Twenty-five trees were sampled in the cross-sectional belt in the middle of each of our nine PRPs. We recorded the presence of 6 characteristics of damage with the presence or absence of damage being recorded for each tree. The method of phytopathological evaluation of trunks was later used by Barna et al. (2020) also in mature pine stands in Bulgaria.

We assessed the degree of rot of coarse wood debris (CWD) on 18th May 2018 according to the modified methodology of Heilmann-Clausen (2001), Heilmann-Clausen and Christensen (2003), and Jurina and Kunca (2014). We carried out the evaluation of rot at PRPs B and C, as there was hardly any CWD at PRP A. As a revision of the method of Jurina and Kunca (2014), we sampled trees of trunk diameter < 10 cm in the middle of the stem length in our evaluation. From the measured values, the volume of CWD and the degree of decomposition of wood mass were calculated for each PRP. We calculated the volume of CWD mass (m^3) using the formula for calculating the basal area, the calculated value was multiplied by the length of the stem (Šmelko 2015). We did not evaluate rot in the occasional old stumps left after earlier thinning operations because the stem diameter is the most important indicator for the calculation of wood volume and wood mass production.

***Armillaria* spp. and *Heterobasidion* spp.: preparation of isolate, extraction of DNA, Sanger sequencing.** A sample of a fruiting body of *Heterobasidion* spp. was cleaned from the soil, removed with tweezers and placed on the prepared solid Hagam culture medium (Oliva et al. 2015). DNA from sporocarps of *Heterobasidion* spp. (100 mg) was extracted using the glass milk method with the CTAB (cetyltrimethylammonium bromide) extraction buffer according to Gryndler et al. (2014, 2018).

Extracted DNA from *Heterobasidion* spp. was amplified by ITS1F and ITS4 primers that are specific to the internal transcribed spacer (ITS) RNA sequence in fungi (Adamčíková et al. 2015). For their use, a PCR reaction mixture was prepared in the required amount of 25 µL/1 sample, containing 12.5 µL Combi PPP Master Mix (hot start type,

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Top-Bio, Czech Republic), 0.5 µL forward primer ITS1F (10 µM), 0.5 µL reverse primer ITS4 (10 µM), 0.5 µL DNA template, 11.0 µL PCR water. PCR consisted of: denaturation phase at 94 °C for 4 min, denaturation time at 94 °C for 40 s undergoing 35 cycles, annealing phase at 54 °C for 40 s subjected to 35 cycles, annealing time at 72 °C for 80 s subjected to 35 cycles. The final elongation phase was run at 72 °C for 5 min. (Gryndler et al. 2018). The amplicon was quantified using PicoGreen Fluorometry, diluted to a concentration of 30 ng/µL, subjected to Sanger sequencing. The obtained sequence was evaluated by the Seed software (Větrovsky, Baldian 2013) and compared in the NCBI BLAST database (Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov/genbank) with the DNA sequences stored in GenBank.

DNA extraction from *Armillaria* spp. as well as some samples of *Heterobasidion* spp. sporocarps was done using the E.Z.N.A.® Fungal DNA Mini-Kit (Omega Bio-Tek Inc., Norcross, USA), according to the procedure specified by the manufacturer. The ITS regions (ITS 1 and ITS2) and 5.8S gene of ribosomal RNA operon were amplified using ITS1F and ITS4 primers (White et al. 1990; Gardes, Bruns 1993). Amplification of DNA was performed in 20 µL reaction volumes using Blend Master Mix (5× HOT FIREPol® DNA polymerase Blend Master Mix, Solis BioDyne, and Tartu, Estonia), 1 µM forward and reverse primers, ~5 ng of genomic DNA as template and molecular grade water. The PCRs were carried out on a T-professional Thermocycler (Biometra, Göttingen, Germany).

Prior to sequencing, target fragments were directly purified using a PCR Purification Kit (Qiagen, Hilden, Germany). Each amplified product was diluted with 30 µL H_2O. Sequence reactions were run at SEQme s.r.o. (Dobříš, Czech Republic). The retrieved sequences were compared by BLAST (Basic Local Alignment Search Tool, available at http://www.ncbi.nlm.nih.gov/genbank/) against DNA sequences deposited in GenBank for *Armillaria* spp.

Statistical analysis. Statistical analyses were performed using STATISTICA 12 (StatSoft, USA) and R-studio statistical programme. Every plot was sampled five times per annum, once per month for a total of 5 monthly visits. Three samples were collected from each plot for a total of 27 samples. Dependent variables such as abundance of sporocarps of WIM species showed deviations from the normal distribution – left-skewed asymmetrical (Scheer 2010), therefore abundances of individual fungi were transformed prior to statistical analysis using log ($x + c$, when is $c = 1$) (Lepš, Šmilauer 2016). The effects of area and seasonality in years on the WIM were tested using the permutation multivariate analysis of variance (perMANOVA, Anderson 2001) on Bray-Curtis distances (Bray, Curtis 1957). The metaMDS function in the vegan package (Oksanen et al. 2017) was used for analysis of WIM composition between areas. The test was done using unrestricted permutation of residuals with 9 999 random permutations. Results of two-way perMANOVA of WIM are displayed here by non-metric multidimensional scaling (NMDS, Kruskal 1964; Lepš, Šmilaur 2016; Luptáková et al. 2018). The NMDS final axis represents the largest deviation of the values. The stress method was used to assess interpretation reliability of the distance between individual points in final ordination. Association between individual sites and WIM in the research years was analysed using the IndVal function with 9 999 permutations (De Cáceres, Legarde 2009). Total results of the indicator species analysis were adjusted for multiple testing using Holm's method (Holm 1979). Variables such as abundance, species richness, rainfall and air temperature showed deviations from the normal distribution. We used the Wilcoxon pair test to test their dependence. The relationship between the decay level of dead wood and the volume of wood was quantified using Pearson's linear correlation coefficient (Lepš, Šmilauer 2016).

RESULTS

Altogether 58 species of WIM were identified, of which 27 species grew on PRP A, 34 on PRP B and 38 on PRP C. The greatest sporocarp abundance was recorded for *Gymnopus perforans* (11 756), *Hypholoma fasciculare* (2 971), *Coprinellus disseminatus* (326), *Exidia pithya* (318), *Hypholoma lateritium* (236), *Panellus mitis* (147), *Strobilurus esculentus* (134) (Table 2).

The highest species diversity index (Figure 2A) was recorded at PRP B in 2018 and the lowest at PRP C in 2017. In 2016 and 2018 the same trend of the index was recorded at all PRPs. Simpson's community equitability (Figure 2B) was greatest at PRP A in 2018 and lowest at PRP B in 2016. Fruiting body abundance increased from the youngest

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Table 2. Abundance and species richness (in %) of wood-inhabiting macromycetes on permanent research plots (PRPs) in the Vrchdobroč Hills during 2016–2018

Species	PRPs		
	A/A	B/B	C/C
Ascomycota			
Ascocoryne sarcoides (Jacq.) J. W. Groves & D. E. Wilson			2/0.02
Chlorociboria aeruginascens (Nyl.) Kanouse ex C.S. Ramamurthi. Korf & L.R.Batra		3/0.05	
Diatrype disciformis (Hoffm.) Fr.		5/0.08	
Jackrogersella multiformis (Fr.) L. Wendt, Kuhnert & M. Stadler	3/0.15	9/0.14	
Neonectria fuckeliana (C. Booth) Castl. & Rossman		10/0.15	
Sarea resinae (Fr.) Kuntze	14/0.72	28/0.43	30/0.36
Scutellina sp.			6/0.07
Xylaria hypoxylon (L.) Grev			3/0.04
Basidiomycota			
Armillaria ostoyae (Romagn.) Herink		3/0.05	
Calocera viscosa (Pers.) Fr	25/1.28	21/0.32	9/0.11
Cerioporus varius (Pers.) Zmitr. & Kovalenko	2/0.1		
Chondrostereum purpureum (Pers.) Pouzar	4/0.2	9/0.14	
Coprinellus disseminatus (Pers.) J. E. Lange			326/3.88
Dacrymyces stillatus Nees	7/0.36	29/0.44	38/0.45
Daedaleopsis confragosa (Bolton) J. Schröt.	2/0.1	25/0.38	
Exidia glandulosa (Bull.) Fr	12/0.61	70/1.06	
E. pithya (Alb. & Schwein.) Fr	111/5.7	109/1.66	98/1.17
E. saccharina Fr	4/0.2		
Gloeophyllum odoratum (Wulfen) Imazeki		4/0.06	3/0.04
G. sepiarium (Wulfen) P. Karst.		2/0.03	
Gymnopilus junonius (Fr.) P. D. Orton			2/0.02
G. sapineus (Fr.) Murrill	10/0.51		20/0.24
Gymnopus perforans (Hoffm.) Antonín & Noordel.	1 561/80	5 976/90.8	4 219/50.2
Heterobasidion parviporum Niemelä & Korhonen	6/0.31		6/0.07
Hydropus subalpinus (Höhn.) Singer	9/0.46		
Hygrophoropsis aurantiaca (Wulfen) Maire		2/0.03	14/0.17
Hypholoma fasciculare (Huds.) P. Kumm.	6/0.31	90/1.37	2 875/34.2
H. lateritium (Schaeff.) P. Kumm			236/2.81
Megacollybia platyphylla (Pers.) Kotl. & Pouzar			20/0.24
Mycena epipterygia (Scop.) Gray	7/0.36	5/.08	
M. galericulata (Scop.) Gray	27/1.38	1/0.02	18/0.21
Panellus mitis (Pers.) Singer	66/3.38	1/0.02	80/0.95
Pholiota flammans (Batsch) P. Kumm.			34/0.4
P. lenta (Pers.) Singer			88/1.05
P. squarrosa (Vahl) P. Kumm.			1/0.01
Phyllotopsis nidulans (Pers.) Singer		36/0.55	16/0.19
Picipes melanopus (Pers.) Zmitr. & Kovalenko			14/0.17
Pleurocybella porrigens (Pers.) Singer	34/1.74		
Pluteus cervinus (Schaeff.) P. Kumm.		2/0.03	25/0.3
Postia caesia (Schrad.) P. Karst.		2/0.03	2/0.02

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Table 2 to be continued

Species	PRPs		
	A/A	B/B	C/C
Basidiomycota			
Postia fragilis (Fr.) Jülich	2/0.1	3/0.05	6/0.07
P. stiptica (Pers.) Jülich		6/0.09	6/0.07
P. subcaesia (A.David) Jülich		2/0.03	
Psathyrella laevissima (Romagn.) Singer	1/0.05		3/0.04
P. maculata (C. S. Parker) A. H. Sm.			5/0.06
Pseudohydnum gelatinosum (Scop.) P. Karst			36/0.43
Rhodocollybia maculata (Alb. et Schwein.) Singer	9/0.46	9/0.14	7/0.08
Stereum hirsutum (Willd.) Pers.	2/0.1	20/0.3	
S. rugosum Pers.	3/0.15		
S. sanguinolentum (Alb. et Schwein.) Fr.	3/0.15	25/0.38	5/0.06
Strobilurus esculentus (Wulfen) Singer		19/0.29	115/1.37
Stropharia aeruginosa (Curtis) Quél.			1/0.01
Trametes hirsuta (Wulfen) Lloyd		3/0.05	
T. versicolor (L.) Lloyd		26/0.39	
Trichaptum abietinum (Dicks.) Ryvarden	19/0.97	7/0.11	
Tricholomopsis decora (Fr.) Singer			2/0.02
T. rutilans (Schaeff) Singer	3/0.15	23/0.35	22/0.26
Xylodon radula (Fr.) Tura. Zmitr., Wasser & Spirin			11/0.13
Total abundance	1 952	6 585	8 404
Species richness	27	34	38
Simpson's evenness index (D)	0.64	0.82	0.37
Shannon index (H')	1.00	0.57	1.41
Simpson's equitability index (E)	0.06	0.04	0.07
Pielou index (J)	0.30	0.16	0.39

stands (PRP A) to the oldest stands (PRP C), with growth trends varying from one year to the next (Figure 3). The opposite trend was observed in the case of WIM species richness (Figure 4), which was highest at PRP A and decreased in the oldest stands (PRP C). These trends may be the result of influence of various factors and amount of dead wood substrate at the PRPs mentioned above. The influence of different age of stands on the WIM community ($P < 0.001$) as well as the influence of seasonality (occurrence of species during the growing season) on WIM proved to be statistically significant (Table 3, Figure 5). The fewest WIM indicator species were recorded at plot A and the most at plot C, possibly due to greater wood mass in the oldest stands (Table 4). WIM species which are typical of the spruce ecosystem were recorded among the indicator species.

Abundance was related to air temperature ($P < 0.001$), species richness and precipitation ($P < 0.001$), and species richness and air temperature ($P < 0.001$). We recorded no parasitic or saproparasitic WIM fruiting bodies directly on tree bark, and no manifestations of red-brown rot, which was indicated also by the zero value of the characteristic Fungus and Rot (Table 5). We recorded high levels of damage to trees by insects and forest animals (80.8%), resulting in a high incidence of resin secretion (70.2%). At all PRPs we frequently recorded *Sarea resinae* on the resin in a number of necrotic resin secreting wounds and other bark lesions which were also of anthropogenic origin – 12.8% (Table 5).

Total volume (m^3) of CWD mass and the degree of rot were significantly correlated. The degree of rot up to 41.8% depended on the volume of CWD (Fig-

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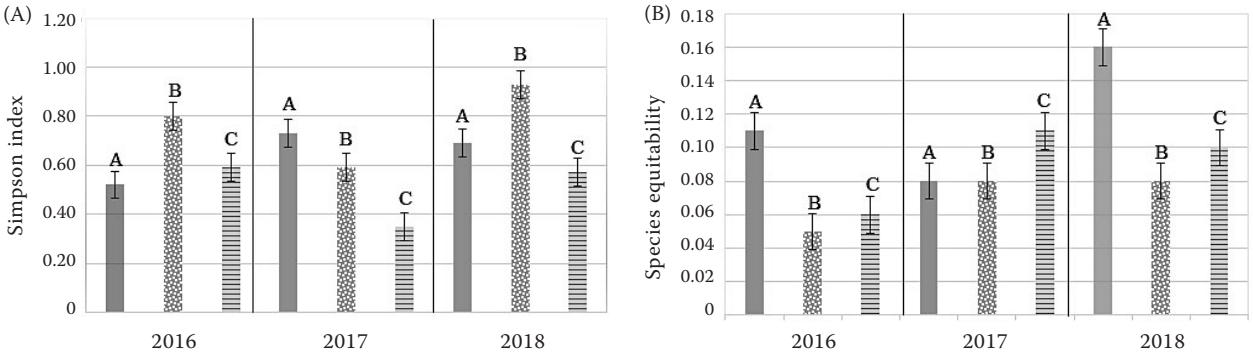

Figure 2. Degree of the species balance of wood-inhabiting macromycetes on research plots according to Simpson's evenness index (A) and Simpson's community equitability index (B)

Table 3. Results of preMANOVA on Bray-Curtis distances for assemblages of wood-inhabiting macromycetes on plots

Factors	Df	Sum Sq	Mean Sq	Pseudo-*F*	*P*-value
Area (A–C)	2	1.3288	0.66440	3.8398	0.0001***
Season (years)	2	0.5349	0.26743	1.5456	0.0529*
Area × season	4	0.6740	0.16849	0.9738	0.5201 NS
Residuals	18	3.1146	0.17303		

Df – degrees of freedom; sum Sq – sum of squares; mean Sq – mean square; ***$P \leq 0.001$; *$P \leq 0.05$, NS – not significant; *P* values are based on the specific resampling methods

ure 6). The greatest volume of CWD was recorded at PRP B3 (0.545 m^3 per whole area of PRP B3) and the lowest value at C1 (0.076 m^3 per whole area of PRP C1). The lowest degree of rot on fallen wood was at B3 (1.47) and the highest at B1 (3.0).

Molecular genetic analysis was carried out on samples of fruiting bodies of *Heterobasidion* spp. collected from PRP A3 and C1 as well as fruiting bodies of *Armillaria* spp. taken from stands outside the PRPs to identify the species of these two WIM genera. The sample sequences of *Armillaria* spp. were 99% identical to *Armillaria ostoyae* (KT822311), with one sample 99.99% identical to *Armillaria cepistipes* (KT822278) stored in Gen-

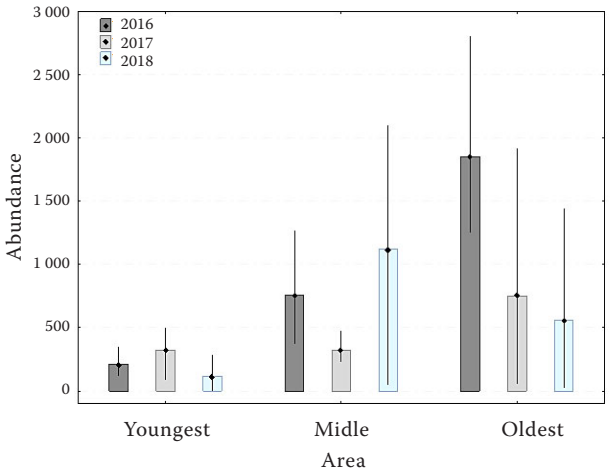

Figure 3. Mean abundance of wood-inhabiting macromycetes; vertical lines represent minimum and maximum values of abundance on the individual permanent research plots

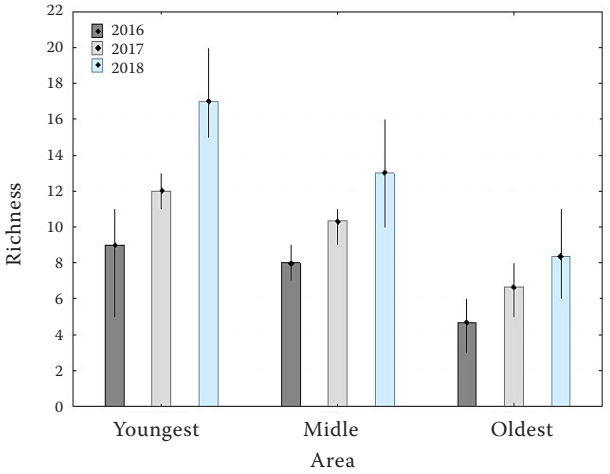

Figure 4. Mean species richness of wood-inhabiting macromycetes; vertical lines represent minimum and maximum values of richness on the individual permanent research plots

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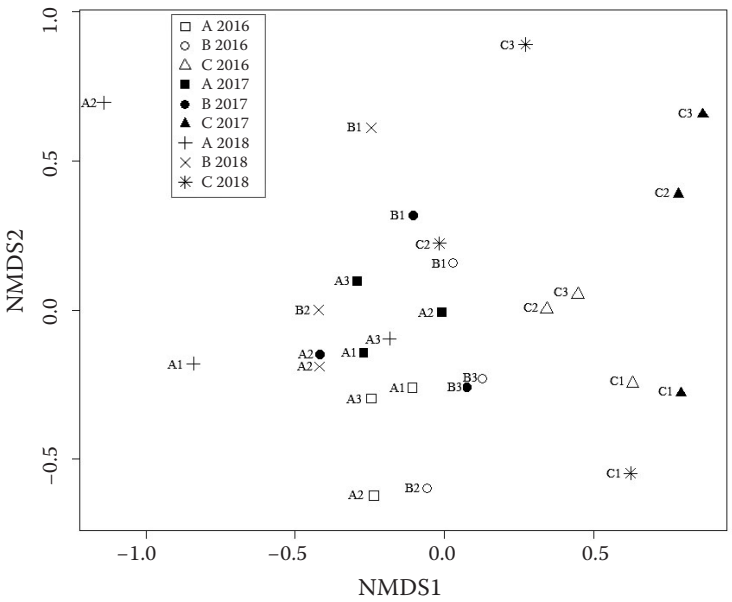

Figure 5. Non-metric multidimensional scaling by Bray-Curtis distances for wood-inhabiting fungi assemblages surveyed at permanent research plots A, B and C during 2016–2018; NMDS 1 and NMDS 2 axis represents the largest variance of the individual points; one unit in NMDS ordination space corresponds to the halving of community assemblage similarity, where stress = 0.187

NMDS – non-metric multidimensional scaling

Bank from China (Gou et al. 2016). *Heterobasidion* spp. from PRP A3 and C1 were 99.8% identical to the sequence of *Heterobasidion parviporum* (KT943925) stored in GenBank from Switzerland (Stroheker et al. 2018). The DNA sequence of one sample of *Heterobasidion* spp. collected outside the PRP stands was 99.97% identical to the sequence of *Heterobasidion annosum* (KC492910) stored in GenBank, Canada (Hamelin et al. 2013, unpublished). On one sample from the stands outside the PRPs, we confirmed 99% occurrence of *Heterobasidion abietinum* Niemelä & Korhonen (KT355028) stored in GenBank database from Russia (Kiyashko, Malysheva 2015, unpublished). In total, we confirmed the presence of up to three species of the genus *Heterobasidion* in spruce stands at Vrchdobroč. Using molecular genetic methods, we determined the species *Armillaria cepistipes*, *Heterobasidion abietinum* and *H. annosum* in stands outside our PRPs. In the PRP A and PRP C stands we determined the species *Heterobasidion parviporum* and in the PRP B stand the species *Armillaria ostoyae*

Table 4. The groups of indicator species (IndVal) on individual PRPs

Species	PRPs	IndVal	*P*-value	Species	PRPs	IndVal	*P*-value
TrichAbie	A	0.76	0.0015	HyphFasc	C	0.97	0.0001
ExidSacch	A	1.00	0.032	XyloRadu	C	1.00	0.0001
TramVers	B	1.00	0.0001	PlutCerv	C	0.93	0.0006
ExidGlan	B	0.84	0.0001	DacrStil	C	0.51	0.014
SterHirs	B	0.91	0.0001	PseuGela	C	1.00	0.0002
SterSang	B	0.76	0.0001	MegaPlat	C	1.00	0.004
DaedConf	B	0.93	0.0001	PholFlam	C	1.00	0.012
NeonFuck	B	1.00	0.0002	PiciMela	C	1.00	0.035
DiatDisc	B	1.00	0.013	HygrAura	C	0.87	0.055
JackMult	B	0.75	0.045				

TrichAbie – *Trichaptum abietinum*; ExidSacch – *Exidia saccharina*; TramVers – *Trametes versicolor*; ExidGlan – *Exidia glandulosa*; SterHirs – *Stereum hirsutum*; SterSang – *S. sanguinolentum*; DaedConf – *Daedaelopsis confragosa*; NeonFuck – *Neonectria fuckeliana*; DiatDisc – *Diatrype disciformis*; JackMult – *Jackrogersella multiformis*; HyphFasc – *Hypholoma fasciculare*; XyloRadu – *Xylodon radula*; PlutCerv – *Pluteus cervinus*; DacrStil – *Dacrymyces stillatus*; PseuGela – *Pseudohydhum gelatinosum*; MegaPlat – *Megacollybia platyphylla*; PholFlam – *Pholiota flammans*; PiciMela – *Picipes melanopus*; HygrAura – *Hygrophoropsis aurantiaca*

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Table 5. Evaluation of phytopathological factors (Σ/%) on spruce stems at the Vrchdobroč locality (n = 225)

Resin flow	Deformations	Insect and animals	Fungi and rot	Abiotic damages	Anthropogenic damages
158/70.2	93/41.3	182/80.8	0/0	10/4.4	29/12.8

Resin flow – especially fresh and old wounds after browsing by deer; deformations – 64 trees: necrosis, 29 trees: adhesions, tumours, coarsening stems; insects and animals – all trees: *Adelges* (*Sacchiphantes) abietis* (L.), 7 trees: fresh browsing by deer; abiotic damage – all trees: lightning; anthropogenic damage – 20 trees: blow with an axe, 9 trees: mechanical damage after cutting operation.

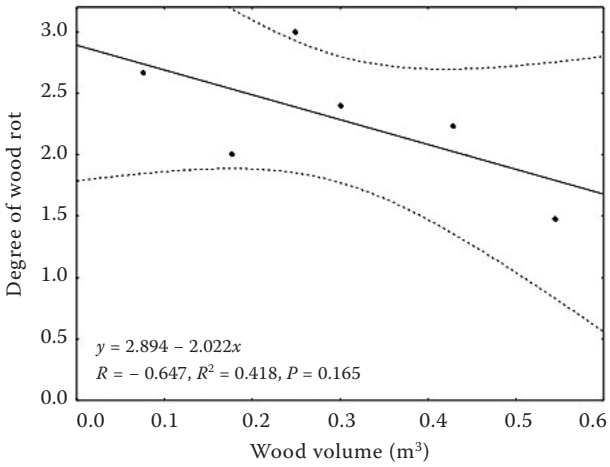

Figure 6. Pearson's correlation between wood volume and degree of wood rot

(see the subchapter *Armillaria* spp. and *Heterobasidion* spp. preparation of isolates, DNA extraction, Sanger sequencing).

After evaluating the results, we can state that we confirmed the above formulated working hypotheses in only two cases: (*i*) species diversity and abundance of fruiting bodies of WIM species were highest in the oldest stands of PRP C (Table 2, H' = 1.41, abundance = 8.404). However, we confirmed also the second hypothesis that (*ii*) fruiting bodies of fungi of the genera *Armillaria* sp. and *Heterobasidion* spp. would occur only sporadically in all types of stands. Fruiting bodies of *Heterobasidion* spp. were sporadically collected from PRP A3 and C1 while most fruiting bodies of *Armillaria* spp. for genetic studies were taken only from stands outside the PRPs, because directly on PRPs the genus *Armillaria* sp. was sporadically recorded only on PRP B1. We did not confirm the hypothesis that (*iii*) the highest incidence of parasitic WIM would be recorded in the oldest stands – the occurrence of parasitic WIM was not significant in any PRPs. Similarly, we did not confirm the last hypothesis that (*iv*) the highest value of the degree of decay would be recorded in the oldest stands – the greatest volume of affected CWD was recorded at PRP B3 (0.545 m^3/whole area of PRP B3) and the lowest value at C1 (0.076 m^3/whole area of PRP C1). The lowest degree of rot on fallen wood was at B3 (1.47) and the highest at B1 (3.0).

DISCUSSION

WIM have been studied more or less continuously since 1989 in uneven-aged spruce monocultures at Vrchdobroč. Mihál (2005) and Mihál, Luptáková (2017) reported species diversity and WIM dominance at Vrchdobroč for 1993–2003. In total, 60 species of wood-decaying fungi were recorded in spruce stands at Vrchdobroč in 1993–2003, of which eight species were parasitic (*Armillaria ostoyae*, *Fomitopsis pinicola*, *Heterobasidion annosum*, *Neonectria fuckeliana*, *Schizophyllum commune* Fr., *Stereum sanguinolentum*, *Trichaptum abietinum*, *Verticillium* spp.) and 52 species were saprotrophic. In the years 2016–2018, the occurrence of 58 WIM species was recorded (Table 2). Of course, not all WIM species from 1993–2003 occurred in 2016–2018 and conversely. A total of 29 species and 3 genera occurred in both periods of research.

Aghajani et al. (2018) reported 25 fungal taxa of wood-decaying fungi in various cultivated oak-hornbeam Hyrcanian forests in Iran. Mean diversity was 2.52 for hornbeam and 1.94 for oak. Mean equitability was 0.84 for hornbeam and 0.73 for oak. Diversity and equitability were similar between the two tree species. However, both indices were statistically significant for the test sites, e.g. in unmanaged forests. Diversity in unmanaged forests ranged from 1.92 to 1.98, while in managed forests it ranged from 1.17 to 2.97. We recorded the highest WIM species richness in 51-year-old stands at PRP C (H' = 1.41, E = 0.07) and the lowest richness

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in 30-year-old stands at PRP B (H' = 0.57, E = 0.04, Table 2, Figure 2). Most often and more regularly, WIM species occurred in the oldest stands where there was enough CWD (Figure 4). A similar trend was observed by Luptáková et al. (2018) in the conditions of beech stands, where higher values of species richness and abundance of fruiting bodies of wood-inhabiting fungi were recorded in managed stand compared to the control unmanaged stand mainly due to a sufficient amount of CWD in managed stand. On the other hand, a higher proportion of WIM species was reported by Jankovský et al. (2004) in natural forests and virgin forests in the Železné hory Protected Landscape Area (Czech Republic), where they found the occurrence of 220 fungal species of which up to 50% were wood-destroying macromycetes.

The highest frequency throughout the study period was recorded for *Calocera viscosa*, *Dacrymyces stillatus*, *Trichaptum abietinum* and *Hypholoma fasciculare*. Of the typical spruce fungal parasites, *A. ostoyae*, *H. annosum*, *F. pinicola* and *Neonectria fuckeliana* occurred only occasionally. *Neonectria fuckeliana* is currently considered to be a threatening spruce parasite. Pettersson et al. (2018) reported the pathogenicity of *N. fuckeliana* to spruce. They researched the growth process and virulence of *N. fuckeliana* on inoculated young spruces and found that the development of symptoms in 8–11 months after inoculation was mild and lesions under the bark were usually small. The slow development of the infection is consistent with earlier studies (Huse 1981; Vasiliauskas et al. 1996) describing *N. fuckeliana* as a weak pathogen, but persisting in infected wood. We first recorded *N. fuckeliana* on spruce seedlings at Vrchdobroč in 1994. Twenty years later, we recorded it at the same place again (i.e. in the growth resulting from the seedling culture mentioned above) on spruce trunks. This suggests long latency and pathogenicity of *N. fuckeliana* (Mihál 1998, 2005; Mihál, Luptáková 2017).

Dangerous pathogens in spruce monocultures are the species of the *Heterobasidion* spp. complex. In the 1990s, only sporadic occurrences of *Heterobasidion annosum* were reported (cf. Gáper, Mihál 2008; Mihál, Luptáková 2017). In this study, we documented the presence of *Heterobasidion abietinum*, *H. parviporum* and *H. annosum* in several stands at Vrchdobroč. In localities unsuitable for spruce, such as monoculture stands on former farmlands at lower elevations, the frequency of parasitic *Heterobasidion* spp. is increasing (Uhlířová et al. 2004). *Fomitopsis pinicola* and *Heterobasidion annosum* were also recorded by Soukup et al. (2008) in 50- to 55-year-old spruce stands planted on former non-forest soils in the Czech Republic. They listed *Bjerkandera adusta*, *Calocera viscosa*, *Dacrymyces stillatus*, *Hypholoma capnoides*, *Postia caesia*, *P. stiptica*, *Stereum sanguinolentum* and *Tricholomopsis rutilans* as the most dominant WIM. These fungi were richly represented in our collections as well. Some of these fungi belong to the WIM indicator species (Table 4).

The most serious consequence of the parasitic infection of spruce stands by WIM is the rotting process that leads to tree death. Tree rot, especially in spruce monocultures, is well known to mycologists and forest managers. Rot often weakens stands that are then susceptible to damage by snow and wind. Vicena and Vokroj (1991) reported that rot greatly reduces the resistance to breakage of affected trees. Trees with surface rot were significantly more vulnerable to breakage than were trees with heartwood rot progressing mostly from the roots to the trunk. Gáper and Mihál (2008) quantified the extent of rot in 14- to 33-year-old stands at Vrchdobroč. They reported that the occurrence of rot in the first 14 years of stand development was negligible. In 23-year-old stands rot affected 28% of assessed trees with the extent of rot still quite small. In 33-year-old stands, they recorded a scarce occurrence of the most dangerous originators of rot – *Armillaria ostoyae* and *Heterobasidion annosum*.

Bässler et al. (2012) investigated WIM in distinct spruce forests under varied management regimes. They reported that the volume of CWD and crown canopy were the most important variables affecting the abundance of fungal species. They reported low numbers of species in logged forests and a positive relationship between numbers of species and the reduced canopy cover resulting from logging. The low crown canopy (25.7% ± 10.4) resulted in higher volumes of rotten CWD mass (2.3 m^3 ± 1.8) as well as rot in more advanced stage (0.74 ± 0.28). Similarly, Yakhyaev et al. (2019) reported the distribution of stem decay in beech stands in Azerbaijan. They found that as the age of the stand increased, the extent, diameter, and volume of decay increased significantly: the extent of decay from 1.47 to 6.43 m; the diameter of decay from 8.15 to 32.7 cm; and the volume of decay from 2.5 to 13.2%. From these

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studies and from our own study we conclude that the older stands were characterized by greater volumes of CWD, higher diversity of WIM, and larger volumes of rot.

The occurrence and pathogenicity of parasitic WIMs are closely connected to the issue of rot in spruce stands, particularly in cultivated forest stands. The species that most threaten spruce stands are *Armillaria ostoyae*, *Fomitopsis pinicola*, *Heterobasidion* spp., *Neonectria fuckeliana*, *Stereum sanquinolentum*, *Trichaptum abietinum*. A second important driver of the incidence of rot is the size of deer populations that browse trees, causing bark damage and resin secretion through which parasitic fungi infect the trees. Månsson and Jarnemo (2013) studied spruce damage by deer browsing in Sweden. Finďo (2010) reported damage to mountain spruce stands by deer in the Low Tatras in Slovakia at 34.5% in young stands and 55.7% in older stands. Similarly, Čermák and Strejček (2007) reported high rates of damage in 40-year-old spruce stands, overall 7 348 trees (44%) of the total of 16 700 evaluated trees were damaged by deer browsing. Wounds on the bark of spruce caused by deer damage are often infected with so-called "wound" fungal parasites such as the species *Stereum sanguinolentum* (Uhlířová et al. 2004). The impact of large animals (deer and moose) on 30-year-old spruce stands in Latvia was described by Burneviča et al. (2016). The fungus *Heterobasidion parviporum* usually infects trees through root contacts, but it can also infect open wounds, especially at roots or tree trunk bases (Redfern, Stenlid 1998).

Significant, even epiphytic occurrence on spruce trees at Vrchdobroč was reported in the past for the species *Adelges* (*Sacciphantes) abietis* (L.) (aphids of the family Adelgidae), which forms galls on the shoots of spruces. We recorded the occurrence of old and young galls of *A. abietis* on as many as 182 spruces (80.8%) out of 225 evaluated trees (Table 5). Uhlířová et al. (2004) reported the general occurrence of this species especially on young spruces in cultivated plantations. The mass occurrence of *A. abietis* significantly deforms the spruce branches, reduces the assimilation apparatus of the tree and the formation of galls requires a lot of nutrients from the tree, which can reduce its resistance and vitality.

All browsing damage and resulting fungal infections of spruce trees planted in unsuitable habitats on former agricultural land have a negative impact on health, ecological stability and vitality of the stands. Míchal et al. (1992) ranked the stability of the even-aged spruce stands at levels lower than those of the uneven-aged natural forest. They concluded that wind and snow were the main negative abiotic factors in less stable spruce stands. They assessed the stability of spruce stands in nutrient-rich ecotopes as lower than the stability of stands in ecotopes with medium or weak supplies of minerals. Spruce monocultures at Vrchdobroč grew on former agricultural soils with abundant supply of nutrients. In light of Míchal et al. (1992), we suggest that the ecological stability of spruce stands at Vrchdobroč in the past was not as high as that of natural spruce stands at higher elevations. This is supported by the records of extensive snow and wind damage during winter 1993/1994 that affected all older spruce stands in the Vrchdobroč locality (cf. Mihál, Luptáková 2017). Decreased ecological stability and increased sensitivity of spruce to wood-decaying fungal infections may be due to a lack of mycorrhizae associated with spruce roots. Pešková et al. (2015) studied the dynamics of ectomycorrhizae on spruce roots in dependence on drought stress in an 80-year-old spruce monoculture in central Bohemia. They reported more active mycorrhizae in control (not stressed by drought) stands than in the stands stressed by drought. The drought-induced stress and exposure of trees at the edge of the stand, where they are increasingly exposed to soil desiccation, bark damage by sunlight, and subsequent increased secretion, are ideal conditions for attack by parasitic fungi. Old spruce stands at Vrchdobroč are considerably fragmented and thinned by wind and snowfall, which exacerbate drought stress and lead to parasitic fungal infections.

CONCLUSION

The abundance of fruiting bodies and species richness of WIM increased from the youngest to the oldest stands. The highest values of fruiting body abundance were recorded for *Gymnopus perforans* (11 756), *Hypholoma fasciculare* (2 971), *Coprinellus disseminatus* (326), *Exidia pithya* (318), and *Panellus mitis* (147). WIM species diversity and abundance increased with increasing age of stand ($P < 0.001$). Fruiting body abundance values increased from the youngest stands to the oldest stands, with growth trends varying from one year

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to the next. The fewest WIM indicator species were at PRP A (*Exidia saccharina*, *Trichaptum abietinum*) and the most were at PRP C (*Megacollybia platyphylla*, *Pseudohydnum gelatinosus*, *Xylodon radula* and others). The amount of precipitation did not affect WIM abundance. WIM abundance was related to air temperature ($P < 0.001$), species richness and precipitation ($P < 0.001$), and species richness and air temperature ($P < 0.001$). The most frequent damage to trees was caused by insects and forest animals (80.8%), which resulted in a high incidence of resin secretion (70.2%). *Sarea resinae* was frequently recorded on the resin in many necrotic resin secreting wounds and other bark lesions at all PRPs. Total volume (m^3) of coarse woody debris (CWD) mass and the degree of rot were significantly negatively correlated. The degree of rot up to 41.8% depended on the volume of CWD. The greatest volume of CWD was recorded at PRP B3 (0.545 m^3/whole area of PRP B3) and the lowest value at C1 (0.076 m^3). The lowest degree of rot on fallen wood was at B3 (1.47) and the highest at B1 (3.0). The occurrence of *Heterobasidion annosum*, *H. abietinum* and *H. parviporum*, *Armillaria ostoyae*, and *A. cepistipes,* was confirmed using molecular genetic analyses.

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