

Short Communication

Communities of Microscopic Fungi in Dead Spruce in Relation to the Season and Degree of Wood Decay in Poland's Karkonosze Mountains

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Abstract

Towards the end of the 20th century the Karkonosze Mountains in southwestern Poland were characterized by vast swathes of dead and dying spruce forests whose condition at the time was described as an ecological disaster. Wood-decaying fungi play an important role in global carbon and nitrogen circulation by promoting the bioconversion of organic matter. The aim of our study was to determine the composition of the fungal community in the 'post-disturbance' dead spruce wood in relation to the season and degree of wood decay in the Karkonosze Mountains. Mycological analyses were carried out in 2015 and 2016 in the submontane and mountain forest zone, as well as in the subalpine zone of Karkonoski National Park (KNP). The findings reveal that the prevalent fungi colonising the internal tissues of dead wood belonged to the genus *Trichoderma*. Their proportion in the material under study increased in advanced stages of wood decay. However, the mycobiota of dead spruce at lower levels of decay were characterized by the highest diversity. The analysis also showed seasonal variations in the composition of the fungal communities colonizing dead wood.

Keywords: fungi, dead spruce, stage of decay, Karkonosze Mountains

Introduction

By the end of the 20th century the Karkonosze Mountains in southwestern Poland were largely associated with vast swathes of land covered by dead or dying spruce forests [1]. Their condition was dire enough

to be regarded as an ecological disaster, and the blame was placed entirely on air pollution and acid rain. It is now a well-established fact that this was a multi-stage and multi-component process, and that these stages and components comprised, *inter alia*, herbivorous insect gradation, the presence of spruce monocultures and atmospheric conditions [2]. According to Manion [3], the phenomenon was also a consequence of the so-called 'decline disease spiral', which is a group of factors that

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contribute to the decline of a forest and that adversely affect one another, ultimately leading to the destruction of the ecosystem. Moreover, as demonstrated by Manion [3], tree diseases serve a purpose in nature, even if they occur on a large scale. More and more scientists believe that the process taking place in the Sudeten Mountains is neither isolated nor particularly extraordinary. It does, however, possess the characteristics of an ecological disturbance, although the extent to which human activity has contributed to it remains largely unknown. Moreover, it appears to be a recurring event. The research and experimentation revolving around remedial measures indicate that its effects might be reversible, and knowledge of the processes involved is likely to enable effective repair of the damage [4].

Dead wood is an essential component of the forest ecosystem [5]. It is crucial to the survival of saproxylic organisms that recycle nutrients and initiate the renewal of subsequent generations of trees and other organisms, including fungi [6]. Moreover, fungal endophytes are probably involved in triggering the early stages of wood decay [7]. This is one of the reasons why it has been suggested that dead wood provides indispensable nourishment for more than 1500 species of mostly microscopic fungi as well as 1300 insect species and ca. 100 species of vertebrates [8]. It should be noted that out of all fungi, the microscopic species are the least well-known, despite the important role they play in the decomposition of dead wood [9]. Wood-decaying fungi contribute to the global carbon and nitrogen cycle by promoting the bioconversion of organic matter. Therefore, identifying communities of these fungi, their colonization strategies and mechanisms of decomposition, as well as the factors influencing them, may help develop our understanding of the nutrients circulating in forest ecosystems. This

knowledge may help develop new vaccines, allow for the decomposition of the stumps and roots of felled trees, and facilitate composting, as well as the preliminary processing of timber intended for the production of bioethanol [10].

The aims of this study were: (i) identify the composition of fungal communities in dead spruce wood in the Karkonosze Mountains and (ii) assess to what extent the structure of these communities depends on the stage of wood decay, and whether or not it is subject to seasonal fluctuations.

Material and Methods

Mycological monitoring of the 'post-disturbance' spruce wood (*Picea abies* (L.) H.Karst) was carried out three times in each growing season: in spring, summer and autumn, within the KNP (Poland), in 2015 and 2016. In the first year of the study, the sites included four areas of mountain forest and the subalpine zone of the western part of the KNP: Chojnik, Mumlawski Peak, Szrenica and Szrenicki Cirque. In the next year the sites were chosen in the submontane zone (under KNP management since 1 January 2016, i.e. Żar Mountain and Szerzawa), in mountain forests and the subalpine zone (Petrovka, Wilcza Poręba, Łomniczka Cirque, Łomniczka Valley, Skalny Stół), as well as in the vicinity of the Czech mountain shelter 'Jelenka'. In the course of the study the degree of wood decomposition was determined with the help of Pyle and Brown's scale [11] and the relevant classes were used in the description. The geographical coordinates and the altitude (above sea level) were measured using a Garmin 62s GPS device. The samples were taken within a 20-metre radius of the recorded coordinates (Table 1).

Table 1. Detailed information about sampling points.

Year	Location		Geographic coordinates	Height a.s.l. [m]
	Name	Abbreviation		
2015	Chojnik	C	N50° 50.369', E15° 38.465'	414
	Kocioł Szrenicki	KS	N50° 47.440', E15° 31.905'	1109
	Mumlawski Szczyt	M	N50° 47.820', E15° 29.539'	1134
	Szrenica	S	N50° 47.913', E15° 29.976'	1093
2016	Dolina Łomniczki	D	N50° 45.214', E15° 44.888'	971
	Góra Żar	G	N50° 49.547', E15° 38.010'	613
	Jelenka	J	N50° 44.553', E15° 46.450'	1319
	Kocioł Łomniczki	K	N50° 44.584', E15° 44.111'	1102
	Petrovka	P	N50° 47.909', E15° 37.099'	806
	Skalny Stół	Ss	N50° 45.097', E15° 47.393'	1221
	Szerzawa	Sz	N50° 48.968', E15° 37.979'	676
	Wilcza Poręba	W	N50° 45.835', E15° 45.334'	665

The samples were taken from three stumps of dead spruce trees or fallen spruce trunks, which were stripped of their bark using a sterile knife. Pieces of wood were then extracted with a sterile scalpel from a depth of approximately 1-2 cm and placed in sterile string bags. A laboratory analysis of the samples was carried out within three days of their collection. The wood fragments were divided into pieces of approximately 1 cm and underwent a 30-second-long process of superficial disinfection with a 1% solution of sodium hypochlorite. They were then placed in batches of six in Petri dishes with a PDA medium (Potato Dextrose Agar, Biocorp) in 10 replicates. In order to eliminate bacteria from the fungal samples, a 0.5% solution of acetic acid was added to the medium [12]. The biological material was incubated in Petri dishes for 4 to 14 days at $25\pm 0,2^{\circ}\text{C}$. The individual colonies of fungi grown from the wood samples were then counted and explanted onto the PDA medium.

Pure fungal cultures, obtained through the use of the single-spore method, were identified according to the methodology commonly employed in mycological laboratories, i.e., the methodology based on the macroscopic and microscopic appearance of the cultures and reference information in the monographic and diagnostic keys [13-34]. The majority of the fungi were identified on the PDA medium, but *Penicillium* and *Aspergillus* species were identified by growing them on the following additional media: malt extract agar (MEA, Biocorp), Czapek-Dox agar (1.2% agar, Biocorp) and Czapek yeast autolysate agar (CYA): $30.0\text{ g} \cdot \text{L}^{-1}$ sucrose, $15\text{ g} \cdot \text{L}^{-1}$ agar, $5.0\text{ g} \cdot \text{L}^{-1}$ yeast extract, $3.0\text{ g} \cdot \text{L}^{-1}$

NaNO_3 , $1.0\text{ g} \cdot \text{L}^{-1}$ K_2HPO_4 , $0.5\text{ g} \cdot \text{L}^{-1}$ KCl , $0.5\text{ g} \cdot \text{L}^{-1}$ $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $0.01\text{ g} \cdot \text{L}^{-1}$ $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$.

Results

In the two years of the study a total of 30 species of fungi were isolated, of which 25 were collected in 2016 and 20 in 2015 (Figs 1-2). The biodiversity of the fungi depended on the decay class of the wood and the time of sampling. Wood in the initial stages of decomposition was colonized by a greater variety of fungi. The correlation between the time of sampling and the number of isolated fungi was not confirmed until the second year of the study. Samples taken in spring exhibited the greatest biodiversity regardless of the degree of wood decomposition, with wood in the first decay class being the only exception (Table 2, Table 3).

The largest number (16) of fungi cultured in 2015 came from spruce samples taken in summer, while the lowest number (9) came from material sampled in spring (Fig. 1). Taking into account the degree of wood decomposition, the spruce wood sampled in summer and classified as decay class 3 contained the highest number of fungal species (12), whereas the samples taken in spring and classified as decay class 1 did not contain any fungi at all. However, during the spring and autumn sampling periods, material classified as decay class 2 was characterized by the highest fungal biodiversity (Table 2).

In 2016, the greatest number (21) of fungi was grown from spruce wood samples taken in spring, while

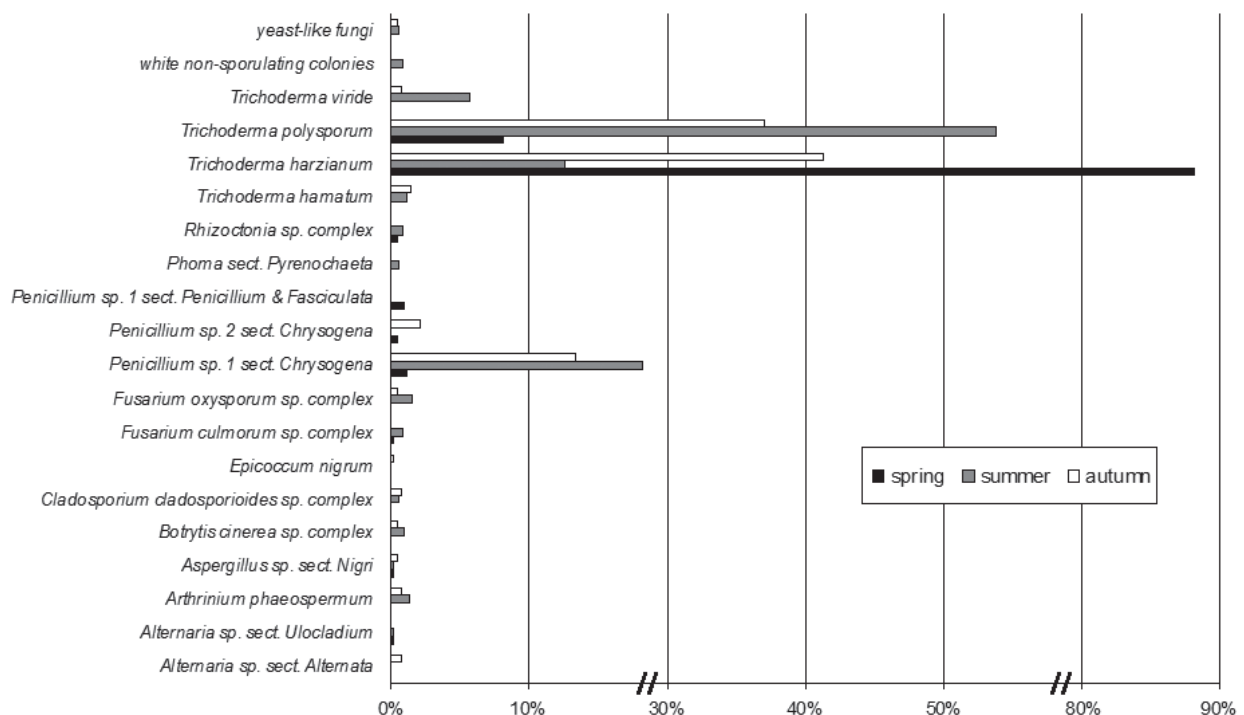


Fig. 1. Percentage of each fungus contributing to the totals for the dead spruce wood samples in 2015.

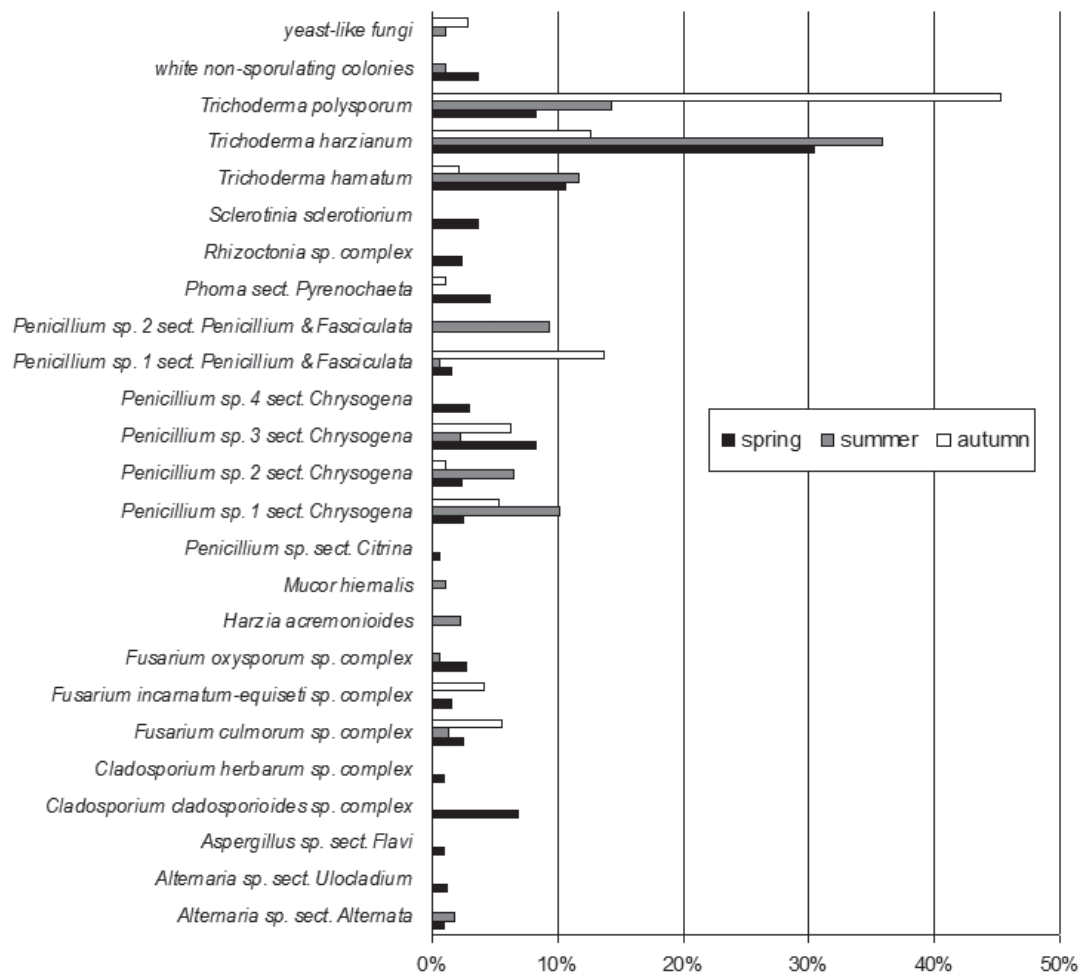


Fig. 2. Percentage of each fungus contributing to the totals for the dead spruce wood samples in 2016.

the fewest (11) were cultured from the material sampled in autumn (Figs 1-2). In terms of wood decay classes, spring samples in decay class 4 were characterized by the greatest fungal biodiversity. Autumn samples assigned to decay classes 1 and 3 contained the lowest number of fungal species (3 per sample). In the summer, the samples with the highest fungal biodiversity were those in decay class 1, whereas during the autumn sampling those were classified as decay class 5 (Table 3).

Fungi of the genus *Trichoderma* were isolated from dead spruce wood in all decay classes, at all sampling sites and in both years of the study (Tables 2 and 3). Moreover, *Trichoderma* spp. was the dominant species in the fungal communities colonizing the samples, with the proportion of other taxa in the same samples reaching <10% – especially in the case of wood in the final stages of decomposition. The species most frequently isolated in both spring samplings, as well as in the autumn of 2015 and in the summer of 2016, was *Trichoderma harzianum*. However, in the summer of 2015 and the autumn of 2016, the dominant species turned out to be *T. polysporum* (Figs 1 and 2).

Discussion

Because fungi are so diverse – more than five million species have been described to date [35] – their identification to species level is, in many cases, difficult. This is particularly true when only one identification method is employed, such as the classical diagnostic one based on the phenotype of the fungus and an assessment of its biochemical capacity, or in a situation where only a single genetic marker is used [17, 32, 36, 37]. In the case of the classical identification method, the composition of the culture medium and the conditions of incubation also play an important role. Only in fungi do the aforementioned factors determine the appearance of the colony and, in many cases, facilitate the production of distinctive morphological forms (such as fungal spores) that can prove useful in the identification process [36]. This is why the majority of the fungi analyzed using the classical method can be identified down to a specific complex, genus or section [37, 38]. This is one of the reasons why most of the fungi sampled in this study were identified as belonging to a complex (e.g., the *C. cladosporioides* sp. complex) or a section (e.g., *A. sp. sect. Flavi*) in accordance with the most recent suggestions and recommendations.

Table 2. Microscopic fungi isolated from dead spruce in 2015: I – spring, II – summer, III – autumn; A (+) indicates the presence of fungi.

Fungi	Wood on decomposition scale															Sampling location		
	1			2			3			4			5			I	II	III
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III			
<i>Alternaria</i> sp. sect. <i>Alternata</i>						+												C, KS, M*
<i>Alternaria</i> sp. sect. <i>Ulocladium</i>				+				+								M, S	M	KS
<i>Arthriniium phaeospermum</i>			+					+									M	KS
<i>Aspergillus</i> sp. sect. <i>Nigri</i>			+	+								+				M, C	C, S	
<i>Botrytis cinerea</i> sp. complex			+					+									KS	KS
<i>Cladosporium cladosporioides</i> sp. complex		+	+														KS	KS
<i>Epicoccum nigrum</i>												+						S
<i>Fusarium culmorum</i> sp. complex		+		+				+								KS, M	KS	
<i>Fusarium oxysporum</i> sp. complex		+			+			+									KS	C
<i>Penicillium</i> sp. 1 sect. <i>Chrysogena</i>		+		+	+	+	+	+	+	+	+	+				KS, M, S	KS, M, S	M, S
<i>Penicillium</i> sp. 2 sect. <i>Chrysogena</i>				+		+	+		+							M, S		C, M
<i>Penicillium</i> sp. 1 sect. <i>Penicillium & Fasciculata</i>				+			+									M, S		
<i>Phoma</i> sect. <i>Pyrenochaeta</i>		+															KS	
<i>Rhizoctonia</i> sp. complex				+				+								M	KS	
<i>Trichoderma hamatum</i>					+	+		+			+	+					C, M, S	C, KS, M, S
<i>Trichoderma harzianum</i>			+	+	+	+	+	+	+	+	+	+	+	+	+	C, KS, M, S	C, KS, M	C, KS, M, S
<i>Trichoderma polysporum</i>				+	+	+	+	+	+		+	+		+	+	KS, M, S	KS, M, S	C, KS, M, S
<i>Trichoderma viride</i>								+	+								M	M
White non-sporulating colonies					+			+			+						KS, M	
<i>Yeast-like fungi</i>											+	+					KS	KS
Total number of fungal taxa	0	5	5	9	5	7	5	12	5	2	7	6	1	2	2	—		
	9			13			14			8			2					

*Abbreviation: C – Chojnik; KS – Kocioł Szrenicki; M – Mumlawski Szczyt; S – Szrenica

Our observations have shown that dead spruce wood, and especially wood in the highest decay classes, was colonized to the greatest degree by *Trichoderma* spp. Fungi of this genus commonly occur in the natural environment. They are present in soil and are responsible for the decomposition of dead organic tissue [39]. *Trichoderma* spp. are ubiquitous and colonize dead wood very quickly, although they are unable to colonize living trees. They are frequently isolated from dead spruce wood [39-41]. Interestingly, fungi belonging to this genus (e.g., *T. harzianum*) may be antagonistic to insects colonizing spruce trees, an example of such an insect being the bark beetle [9]. The insect's defence mechanism against the fungi involves the use of substances produced by the bacterium *Micrococcus luteus*, which is present in their oral secretions and prevents fungi from growing in the insect-bored corridors [41].

The correlation between wood decay class and composition of wood-decay fungi as well as the frequency of their occurrence was observed in both years of the study. Wood in lower decay classes was colonized by a higher number of fungal taxa compared to wood in decay class 4 or 5. Furthermore, wood in advanced stages of decomposition was mostly colonized by taxa of *Trichoderma*, particularly in the first year of the study. Our results confirm the observations made by Oszako [42], who isolated more species of fungi from young alder trees (*Alnus* spp., Betulaceae) than from older specimens, which were mostly infested by *Trichoderma* spp. Similar results were also obtained by Czekaj and Kowalski [43], as well as Linder [44]. On the other hand, Wrzosek [45] claims that *Fusarium* spp. is the genus most frequently isolated from dead spruce wood. Fungi belonging to this genus were indeed found during the course of our study. According to the existing

Table 3. Microscopic fungi isolated from dead spruce wood in 2016: I – spring, II – summer, III – autumn; A (+) indicates the presence of fungi.

Fungi	Wood on decomposition scale															Sampling location			
	1			2			3			4			5			I	II	III	
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III				
<i>Alternaria</i> sp. sect. <i>Alternata</i>	+	+		+	+							+					K, P, Ss, Sz*	K, Ss	
<i>Alternaria</i> sp. sect. <i>Ulocladium</i>	+																D, G, K, W		
<i>Aspergillus</i> sp. sect. <i>Flavi</i>														+			G		
<i>Cladosporium cladosporioides</i> sp. complex	+			+				+				+					G, J, K, Ss, Sz		
<i>Cladosporium herbarum</i> sp. complex				+													S		
<i>Fusarium culmorum</i> sp. complex		+			+	+					+				+	+	P	P, Ss, W	D, Ss
<i>Fusarium incarnatum-equiseti</i> sp. complex	+			+				+									G, Ss, W		
<i>Fusarium oxysporum</i> sp. complex	+	+		+	+	+	+										G, K, P, Ss	P	G, Sz
<i>Harzia acremonioides</i>		+																J	
<i>Mucor hiemalis</i>		+			+													D, P, W	
<i>Penicillium</i> sp. sect. <i>Citrina</i>														+			P		
<i>Penicillium</i> sp. 1 sect. <i>Chrysogena</i>		+		+	+			+	+			+	+			+	K	Ss	Ss
<i>Penicillium</i> sp. 2 sect. <i>Chrysogena</i>		+		+	+			+	+			+			+	+	J, K	D, K, P, Ss	G
<i>Penicillium</i> sp. 3 sect. <i>Chrysogena</i>	+			+				+	+			+	+			+	D, G, J, P, Ss, Sz, W	Sz	S
<i>Penicillium</i> sp. 4 sect. <i>Chrysogena</i>															+		D, G, K, P, Sz	J	
<i>Penicillium</i> sp. 1 sect. <i>Penicillium & Fasciculata</i>		+		+		+			+	+			+			+	G, J, P, W	J	D, J, K
<i>Penicillium</i> sp. 2 sect. <i>Penicillium & Fasciculata</i>									+				+			+			
<i>Phoma</i> sect. <i>Pyrenochaeta</i>	+		+	+				+				+					G, K, W		G
<i>Rhizoctonia</i> sp. complex				+				+									K, P, Ss, W		
<i>Sclerotinia sclerotiorum</i>				+				+							+		K, P, Ss, Sz		
<i>Trichoderma hamatum</i>		+		+	+			+	+			+	+	+	+	+	D, G, J, P, Sz	P, Ss, W	G, K, P
<i>Trichoderma harzianum</i>	+	+		+	+			+	+	+	+	+	+	+	+	+	J, G, K, P, Ss, Sz, W	D, G, J, K, P, Ss, Sz, W	D, G, K, P, W
<i>Trichoderma polysporum</i>		+	+		+	+		+		+	+	+	+	+	+	+	G, J, K, P, Sz, W	D, G, P, Ss, Sz, W	D, J, K, P, Ss, Sz, W
White non-sporulating colonies	+	+		+											+		K, P, Sz	J, P	
Yeast-like fungi		+	+															P	G
Total number of fungal taxa	9	13	3	15	9	4	12	6	3	19	7	4	12	4	7				
	18			18			14			12			15						

*Abbreviation: D – Dolina Łomniczki; G – Góra Żar; J – Jelenka; K – Kocioł Łomniczki; P – Petrovka; Ss – Skalny Stół; Sz – Szerzawa; W – Wilcza Poręba

literature, most *Fusarium* species are plant pathogens, yet their pathogenicity is highly variable even at the intra-species level. As such, in some circumstances saprotrophic forms may develop and use dead organic matter as a substrate [46]. These fungi may also constitute an element of the mycoflora that acts as an inhibitor of natural cycles of renewal, since they pose a threat to seedlings and young trees [9], as well as herbaceous plants [38]. Nevertheless, in environments that experience low human disturbance, *Fusarium* fungi are a regular component of plant communities and, unlike in agricultural crops or nurseries, their incidence is not of epidemic proportion [47].

The existing scholarly papers [42, 43, 45] seem to conclude that the mycobiota of dead 'post-disturbance' spruce wood found in the Karkonosze Mountains is not significantly different from that of other tree species. Furthermore, they demonstrate a similar positive correlation between the level of *Trichoderma* spp. colonization and wood decay class. This validates the hypothesis [48] that, despite the ecological disaster that occurred within the area more than 25 years prior to the study, the ecosystem has achieved a state of equilibrium, and the mass occurrence of dead wood together with living organisms capable of decomposing dead organic matter, including fungi, is much the same as in woodland areas experiencing less anthropic disturbance. The conclusion can therefore be drawn that the natural renewal of spruce stands is possible and can be observed in the Karkonosze Mountains. New trees are continually overgrowing the dead stumps as well as the accumulation of spruce trees that died off more than 25 years ago.

Conclusions

Trichoderma spp. predominated in the mycobiota of dead spruce wood and their proportion increased along with the degree of decomposition. The mycobiota of dead spruce in lower decay classes were characterized by greater biodiversity. The effect of the seasons on the composition of the fungal communities colonizing dead wood was observed, too. The biodiversity of the fungi colonizing dead post-disturbance wood accumulated in the Karkonosze Mountains is not markedly different from that found in other areas or tree species. This lends validity to the claim that the ecosystem of the Karkonosze Mountains is slowly approaching equilibrium after the ecological disaster it suffered 25 years ago.

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Author Contributions

W.P. designed the experiment, collected all the samples (fieldwork), and performed the analysis. W.P. and R.O. wrote the paper. All the Authors revised the paper and approved the final version.

Conflict of Interest

The authors declare no conflict of interest.

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