

Article

Fungal Community Taxa Differ in Diversity and Number between Live and Dead *Prunus serotina* Ehrh. Wood in a Protected Forest within Its Secondary Range of Distribution

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Abstract: *Prunus serotina* is an important invasive plant species in Poland. It was introduced to European forests, even in places currently protected by law, such as national parks. One major factor contributing to the spread of this species is the lack of infecting pathogens and incomplete knowledge about the relationship between the plant and microorganisms. This study aimed to identify fungal communities collected from freshly cut stumps of live and dead black cherry tree specimens growing in a protected forest. The main working hypotheses were as follows: (i) fungal communities inhabiting the wood of dead and living trees will differ in diversity and the number of taxa; and (ii) saprotrophic fungi will dominate in the wood of dead tree stumps. This study applied Illumina sequencing based on the amplification of the fungal ribosomal *ITS1* region. The average number of sequences (OTU) obtained from the analysis of dead tree wood was 101,758, while that of living trees was 94,150. These sequences belonged to 312 taxa, among which 254 were isolated from the wood of dead trees and 171 from that of living trees. Among the saprotrophs on dead trees, the following species were identified: *Stereum rugosum*, *Ganoderma adspersum*, *G. applanatum*, *Peniophora cinerea*, and *Ascocoryne cylichnium*. On the other hand, in the wood of living trees, *Cytospora leucostoma* and *Botrytis cinerea* were the most abundant saprotrophic species. The fungal communities inhabiting the wood of dead and living trees differed in the diversity and abundance of taxa, thus confirming our hypothesis. The results of our research conducted in a protected area indicate that black cherry wood can be naturally colonized by many pathogen species that can further limit its expansion.

Keywords: biodiversity; black cherry; fungal communities; saprotrophs; Wielkopolski National Park; invasive species



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1. Introduction

Some species found outside the limits of their natural range spread intensively and spontaneously, thus gaining the status of invasive plants [1,2]. Together with non-native species [3], invasive species pose substantial threats to global and local biodiversity [4], and have a negative socio-economic and human health impact [5,6]. In forest ecosystems, such threats include hybridization, pathogen transmission, and species competition. Invasive species outperform native flora due to their faster growth, higher production capacity, more effective dispersal, higher tolerance to various environmental conditions, and fewer natural enemies, such as pathogens and herbivores [7]. Therefore, studying the role of plant–microbe/insect interactions—both antagonistic and synergistic—is essential for elucidating the mechanisms of the invasive plant species spread [8,9]. Plant invasion is also a major threat to forest biodiversity in protected areas [10,11].

More than 430 alien tree species worldwide are invasive species, and the list continues to grow as more tree species are moved around the world and thrive in new environments. According to Rejmánek and Richardson [12], 73 invasive alien tree species are found in Europe, 28 of which are used in forestry. The analysis of non-native species from the main European databases, DAISIE (www.europeliens.org, accessed on 1 April 2023) and EPPO [13], indicates that *Prunus serotina* Ehrh. (black cherry), *Acacia dealbata* L., *Ailanthus altissima* (Mill.) Swingle, and *Robinia pseudoacacia* L. are alien species particularly dangerous to native ecosystems. The dynamic development of the *P. serotina* population is most often observed in monocultures of *Pinus sylvestris* L. (Scots pine) or mixed stands with a large proportion of pine [14], in managed stands and stands under various forms of protection. Unfortunately, they are increasingly encroaching on the most fertile habitats of alder and riparian forests [15].

Reducing the occurrence of invasive species populations is essential to protecting native species [16]. However, regarding black cherry, no effective methods have been developed so far [17]. The current strategies used to reduce its occurrence include mechanical methods or their combination with a chemical (e.g., the Roztocze and Kampinoski National Parks in Poland) [18,19].

In Polish forests, restrictions are placed on chemical plant protection products [17], especially in protected areas (e.g., in national parks and nature reserves). An alternative could be the use of biological methods. Although wood pathogens can damage their hosts substantially, knowledge of the fungal pathogenic community and the mycobiome of *Prunus* wood is generally low, even regarding species native to Europe [20]. Black cherry wood is colonized by rich fungal communities, including taxa specializing in wood decay (saprotrophs) [21]. Interestingly, the use of the mycelium of *Chondrostereum purpureum* (Pers.) Pouzar was suggested to reduce the population of black cherry [22–24]. Few scientific reports refer to other fungi inhabiting the wood of black cherry. Black knot caused by *Apiosporina morbosa* (Schwein.) Arx, a widespread disease that can reduce timber value, can be identified on black cherry branches and stems [25]. Pathogens such as *Armillaria* spp. (Fr.) Staude, *Cytospora leucostoma* (Pers.) Sacc., *Coniophora cerebella* (Schumach.) P. Karst, *Fomitopsis spraguei* (Berk. & M.A. Curtis) Gilb. & Ryvarden, and *Polyporus berkeleyi* (Fr.) Bondartsev & Singer can also be found on black cherry trees and may be essential in limiting their spread [21]. Comprehensive studies of microscopic fungal communities inhabiting black cherry stumps have been conducted on the wood of *P. serotina* but only in managed pine stands, where this non-native plant had been previously controlled [26–28]. Macroscopic fungi associated with dead black cherry wood have been studied in the Kampinos National Park [18,29]. So far, no reports on the composition of fungal communities inhabiting black cherry stumps in stands not covered by standard forest management treatments are available.

Considering the above, this study aimed to identify fungal communities collected from freshly cut stumps of live and dead black cherry tree specimens growing in a protected area. The working hypotheses were as follows: (i) fungal communities inhabiting the wood of dead and living trees will differ in diversity and the number of taxa; (ii) saprotrophic fungi will dominate in the wood of dead tree stumps; and, (iii) we will be able to identify fungi that could potentially be proposed for the biological control of *P. serotina*. This study is part of a broader research concept searching for species potentially helpful for the biological control of black cherry. Fungal communities are analyzed to identify species causing stump decomposition that may inhibit the regrowth of black cherry trees, thus contributing to the effectiveness of an alternative method to control their spread by intentionally inoculating their stumps.

2. Materials and Methods

2.1. Field Works

The research was conducted in the Wielkopolski National Park (Poland) in the Wiry Protection District, where five research plots (approx. 0.25 ha each) were located in four forest divisions. The taxation description of the stands from which the research material was collected is included in Appendix A (Table A1). Black cherry was likely introduced artificially in this location years ago, as was usually done in Central and Eastern Europe. Black cherry trees were cut in the first half of August 2022 using a chain saw, standardizing the height of the stumps to 2.5–3 cm. The diameter of black cherry stumps ranged from 4 to 7 cm. The biological material (chips) was collected from the stumps of freshly cut black cherry trees (live and dead) using a cordless drill with 6 mm wood drill bits. To capture a representative sample from the largest area possible, wood samples were randomly collected from stumps of live ($n = 25$, five from each experimental plot) and dead trees ($n = 25$, five from each experimental plot). The five samples of dead or live trees from each experimental plot were combined into one sample (one combined dead sample and one combined live sample per plot). The combined dead or live tree samples from all five experimental plots ($n = 5$ for dead tree samples, $n = 5$ for live tree samples) were subjected to further analyses.

2.2. Laboratory Works

Wood chips collected from the field were ground in a PEX™ SamplePrep™ Freezer/Mill™ cryogenic mill. A constant sample weight (1 g) was used for standardization. DNA was isolated using the Bead-Beat Micro AX Gravity kit (A&A Biotechnology, Gdynia, Poland) with an extended lysis step (up to 45 min). The metagenomic analysis of fungal communities was performed based on the *ITS1* region. Specific primer sequences were used (ITS1FI2, 5' GAA CCW GCGGAR TCA 3'; 5.8S, 5' CGC TGC GTT CTT CATCG 3') to amplify the selected region and prepare the library [30,31]. Each 25 μ L PCR mixture included 0.2 μ mol/L of each primer, 12.5 μ L 2 \times PCR MIX (A&A Biotechnology, Gdynia, Poland), and 2 μ L of total DNA extracted from the soil. The cycling conditions were as follows: 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s, and a final step at 72 °C for 7 min. The obtained DNA was purified using the Anti-Inhibitor Kit (A&A Biotechnology, Gdynia, Poland). The amplicons were sequenced using the Illumina system in the Genomed S.A (Warszawa, Poland). The PCR was performed using Q5 Hot Start High-Fidelity 2X Master Mix following the manufacturer's instructions.

The sequencing was performed with a MiSeq sequencer in paired-end (PE) runs. The automatic pre-analysis of the data was performed on the MiSeq instrument using MiSeq Reporter (MSR) v2.6 software. Negative control samples were prepared and sequenced for each variant to remove artifacts generated during the analysis. The analysis comprised two stages: (1) automatic demultiplexing of samples; and (2) generation of *fastq* files containing raw readings. The obtained sequences were subjected to further bioinformatics analysis to prepare the OTU (operational taxonomic unit) library. Bioinformatics analysis ensuring the classification of reads to the species level was conducted with the QIIME2 software package (<https://qiime2.org/>, accessed 3 April 2023) based on the NCBI reference sequence database [32]. The analysis consisted in removing the adapter sequences in the cutadapt software (<https://cutadapt.readthedocs.io/en/stable/>, accessed 1 April 2023) [33] and then in the qualitative analysis of the reads. In the next step, paired sequences were combined (fastq-join algorithm) [34], and chimeric sequences were removed (usearch61 algorithm). Clustering was performed based on the selected reference sequence database (uclust algorithm), and taxonomy was assigned based on the NCBI reference sequence database (blast algorithm). The sequencing depth was set at 10,000, and its quality was confirmed by determining rarefaction curves [35].

For the species identification, the percentage of similarity between the analyzed sequence and the reference was assumed to be 98%–100% at the species level, 94%–97% at the genus level, and 80%–94% at the order level, with at least 90% overlap with the

reference sequence. Communities were identified down to the lowest possible taxonomic level. OTUs that did not belong to Fungi or Oomycota were removed from further analysis. The function (trophic group) of individual taxa in the community was determined based on the FungalTraits database [36], and the applicable names were determined based on the Index Fungorum database (<http://indexfungorum.org/>, accessed on 2 April 2023).

2.3. Statistical Analysis

The obtained OTU library was compiled in an Excel spreadsheet and statistically analyzed using the R program [37]. Percentage data were transformed using a formula suggested by Bliss [38]. Before starting the statistical analysis of the results, the initial assumptions of the Shapiro–Wilk, Kolmogorov λ , and Bartlett’s tests were checked [39], and `adonis2` function from the `vegan` package was used [40]. The Shapiro–Wilk test indicated that the noted data in this experiment were not normally distributed; therefore, they were log-transformed. Then, the results were checked with the Kolmogorov λ concordance test, which confirmed that the data did not have a normal log distribution. No datasets met the assumptions of ANOVA; thus, the Kruskal–Wallis (one-way test, χ^2 approximation) was employed. The figures were generated using Microsoft Excel 2019 (Microsoft Corporation, 2019, Redmond, WA, USA).

Species biodiversity was determined for each community using diversity indices. Species richness was defined as the total number of species in the community, and frequency was defined as the percentage of a given taxon in the community. The Shannon diversity index (H') was calculated. The dominance of a species in the fungal community was determined using the Berger–Parker dominance index in which the dominance of individual taxa in the community is deduced from the closeness of this value to unity. An NMDS analysis was conducted to illustrate differences between communities (NMDS, function `metaMDS`, `vegan` package; [40]). The results of the NMDS analysis, verified with a statistical ANOSIM test, were used to select indicator species of a given community.

3. Results

The OTU results indicated an average of 101,758 (SE = ± 3781 SE) and 94,150 (SE = $\pm 10,051$) sequences after analyzing the dead and live wood, respectively, corresponding to 312 taxa (254 from dead trees and 171 from living trees). Among these communities, 141 taxa were common for the dead and living tree samples, 113 taxa occurred only in the dead trees, and 58 only in the living trees.

In the wood of the living trees, Ascomycota (77.8%), Mucoromycota (11.5%), Basidiomycota (8.0%), and Entorrhizomycota (2.4%) were the most numerous. Regarding the dead wood, the highest frequency was found in Basidiomycota (70.5%), Ascomycota (19.2%), and Mucoromycota (9.6%) (Figure 1). Statistically significant differences in the proportion of individual fungal classes between the live and dead individuals of black cherry were observed only for Ascomycota ($p = 0.009$) and Basidiomycota ($p = 0.0122$).

In the wood of the living trees, *C. leucostoma*, *Diplodia sapinea* (Fr.) P. Karst. and *Penicillium canescens* Sopp displayed the highest frequency, while *Stereum rugosum* Pers., *Aspergillus parvulus* G. Sm., and *Ascocoryne cylichnium* (Tul.) Korf were most frequent in the wood of the dead trees (Figure 2 and Table A2). The trophic group with the highest frequency in the tested samples were saprotrophs and plant pathogens observed in the wood of the dead trees (Figure 3). Among the saprotrophs, the following species were identified: *S. rugosum*, *Ganoderma adspersum* (Schulzer) Donk, *G. applanatum* (Pers.) Pat., *Peniophora cinerea* (Pers.) Cooke, and *A. cylichnium*. On the other hand, in the wood of the living trees, *C. leucostoma* and *Botrytis cinerea* (Pers.) were the most numerous pathogen species (Table A2).

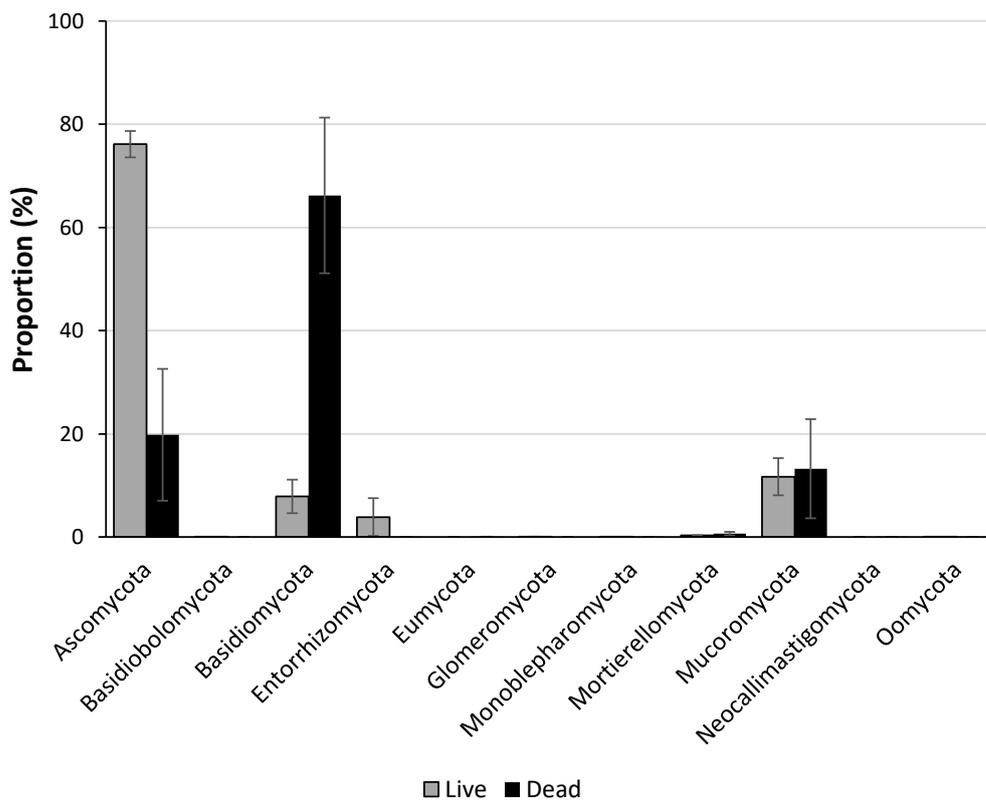


Figure 1. Proportion (mean and SE) of taxa assigned to classes in fungal communities from live and dead black cherry stumps (considered together) (%).

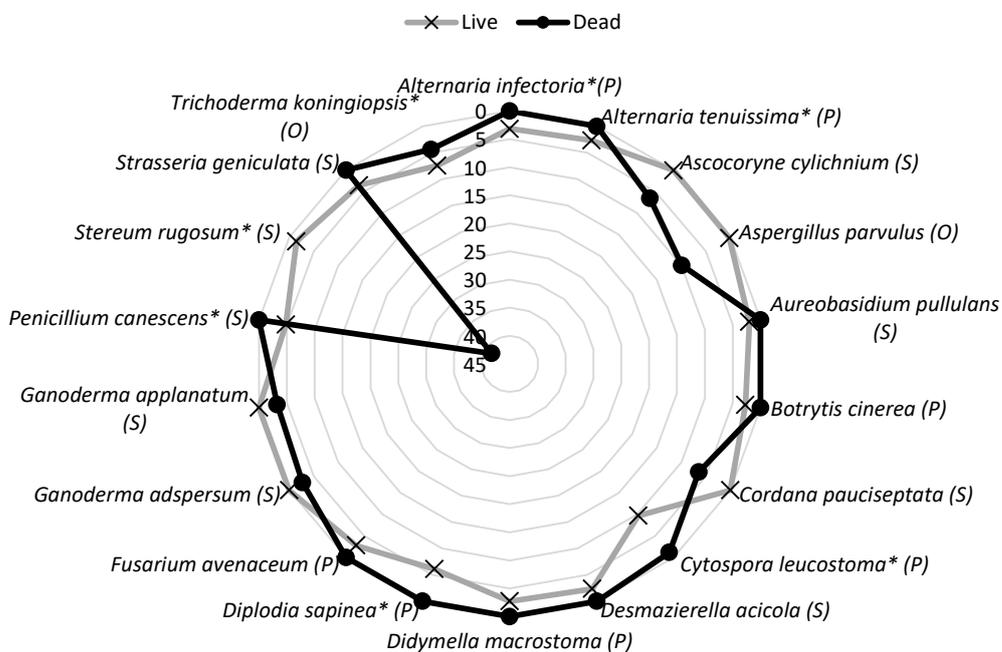


Figure 2. The most numerous taxa in fungal communities from live (x and grey line) and dead black cherry stumps (black dots and black line; considered together) [%]. An asterisk indicates a statistically significant difference, with $p < 0.05$. The letters in parentheses indicate the trophic groups of fungi: S—saprotroph, P—pathogen, and O—other.

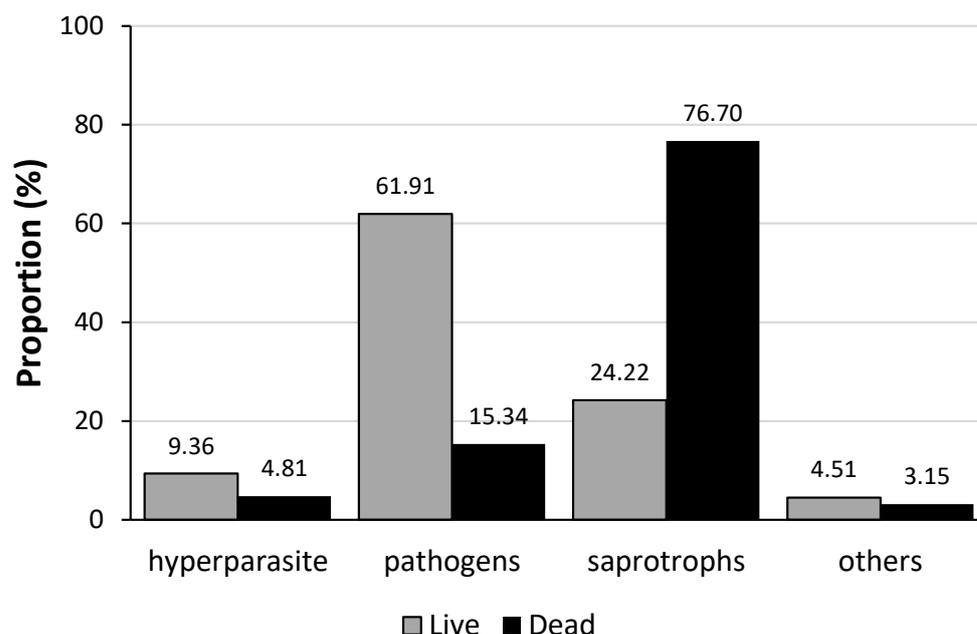


Figure 3. Proportion of identified taxa assigned to trophic groups in fungal communities from live and dead black cherry stumps (considered together) (%).

Statistically significant diversity was observed regarding the saprotrophs. This group was analyzed to identify indicator species, including 268 taxa; indicator taxa for the living and dead trees were selected based on their presence in a given sample. In the first group, forty-nine were identified, and in the second group, only six were. In the community isolated from the wood of the living trees, the following were indicator species ($p < 0.001$): *Absidia glauca* Hagem, *Allantophomopsis lycopodina* (Höhn.) Carris, *Alternaria axiaeriisporifera* E.G. Simmons & C.F. Hill, *A. hampshirensis* Wanas., E.B.G. Jones & K.D. Hyde, *A. infectoria* E.G. Simmons, *A. tenuissima* (Kunze) Wiltshire, *B. cinerea*, *Botrytis* sp., *Coniothyrium dispersellum* P. Karst., *C. pyrinum* (Sacc.) J. Sheld, *Didymella macropodii* Petr., *D. macrostoma* (Mont.) Qian Chen & L. Cai, *D. sapinea*, *D. seriata* De Not., *Dothiora prunorum* (C. Dennis & Buhagiar) Crous, *Epicoccum nigrum* Link, *Epicoccum* sp., *Fusarium avenaceum* (Fr.) Sacc., *Lewia infectoria* (Fuckel) M.E. Barr & E.G. Simmons, *Penicillium bialowiezense* K.W. Zaleski, *P. brevicompactum* Dierckx, *P. canescens*, *P. concentricum* Samson, Stolk & Hadlok, *P. scabrosum* Frisvad, Samson & Stolk, *P. soppii* K.W. Zaleski, *Pestalotiopsis* sp., *Phoma laundoniae* Boerema & Gruyter, *Phoma* sp., *Saitozyma podzolica* (Babeva & Reshetova) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, and *Stemphylium* sp. The taxa most strongly associated with the wood of dead trees ($p > 0.001$) were the following: *Talaromyces amestolkiae* Yilmaz, Houbraken, Frisvad and Samson, *S. rugosum*, *Collophora* sp., and *Phialocephala piceae* (T.N. Sieber & Grünig) Rossman.

The results of the NMDS statistical analysis indicated differences between the fungal communities isolated from live and dead black cherry stump wood (Figure 4). Significant differences were found for the Shannon diversity and Berger-Parker dominance indexes between saprotrophic communities of dead and living trees (Figure 5). The value of the Shannon diversity index ($p = 0.009$) was higher for the community from living trees than that from dead trees. The Berger-Parker dominance index ($p = 0.0003$) was higher in dead trees than in live trees. These results confirmed the predominance of *S. rugosum* in the community.

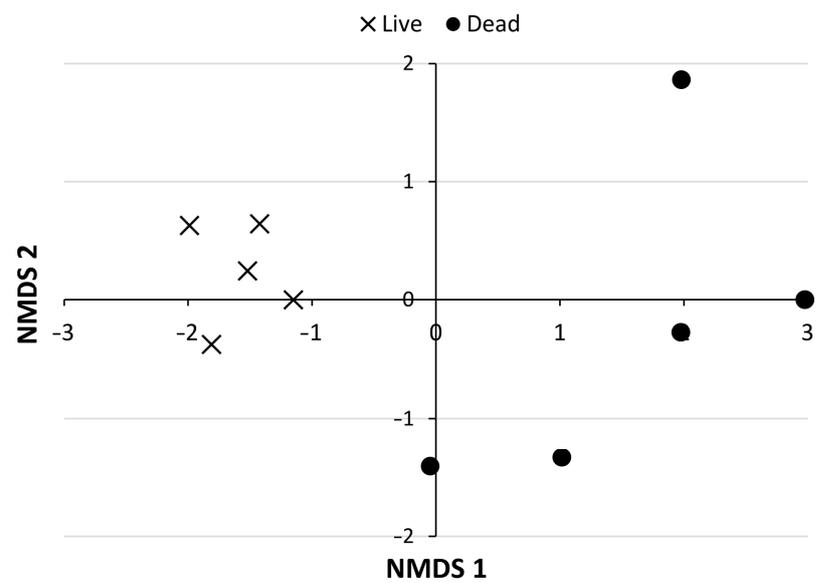


Figure 4. Results of the NMDS statistical analysis. × live trees, • dead trees.

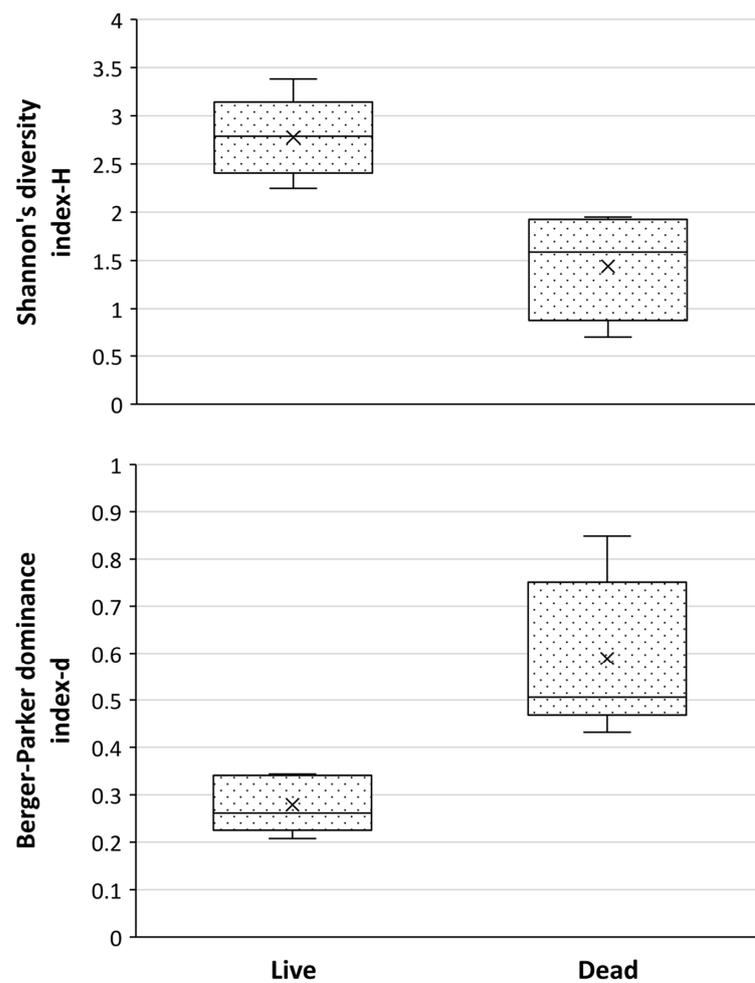


Figure 5. Boxplots for Shannon's index (H) (**upper graph**) and Berger–Parker dominance index (d) (**lower graph**) for living and dead trees. The mean value is marked with the symbol × in the figures.

4. Discussion

The results of this study indicated that the fungal communities of the living and dead trees differed, confirming our hypothesis. This difference was observed as early as the phylum level. Fungi from the Ascomycota phylum dominated the wood of the living black cherry individuals, while Basidiomycota dominated the wood of the dead trees. The dominance of Ascomycota in black cherry stumps has been demonstrated in previous studies [26–28]. The greater proportion of Basidiomycota in the wood of dead black cherry can be explained by its progressive decomposition. According to Kwaśna et al. [41], dead wood is a natural habitat for saprotrophic fungi, which explains the dominance of the latter in samples of dead wood.

The fungal communities inhabiting the wood of the dead and live black cherry trees differed in taxon diversity and abundance. Wood fungal communities associated with the live black cherry trees were more diverse, and more indicator species were selected for the living trees than for dead trees. The taxa most strongly associated with the wood of the dead trees while having a significant proportion in the fungal community were *S. rugosum* and *P. piceae*. *S. rugosum* (Basidiomycota) is a cosmopolitan pathogen, weakness parasite, and saprotroph, inhabiting mainly dead trunks and branches of deciduous trees [42]. Its fruiting bodies were observed on black cherry trunks during our research in the Wielkopolski National Park. In Poland, this species has already been isolated from black cherry wood [26], and the presence of its fruit bodies on *P. serotina* has been identified [29]. However, in the abovementioned studies, the proportion of this taxon in the studied community was lower. A significant proportion of *S. rugosum* in the analyzed fungal communities and the visible presence of its fruiting bodies in the study area (unpublished data) indicate that it can significantly affect the black cherry trees' condition and health status. In addition to *S. rugosum*, other saprophyte species isolated from dead black cherry wood deserve more attention. The wood sample from plot I was dominated by *Cordana pauciseptata* Preuss (>50% proportion) and the sample from plot IV by *A. cylindrium*. *Phialocephala piceae* (Ascomycota) also had a significant proportion in the community of the dead wood fungi (Table A2). Until now, this species was considered an endophyte of coniferous needles [43], although other species of this genus have been isolated from black alder [44]. Its presence in the analyzed samples may result from the structure of the stand from which the samples were taken; the black cherry trees grew under the canopy of Scots pines. A similar situation may apply to *D. sapinea* (*Sphaeropsis sapinea*), which has been identified only in the wood of live black cherry trees. It is an endophyte, a pathogen causing the decline of coniferous species, in particular pines [45,46]. Further research should clarify the presence of these fungi in the tissues of deciduous trees.

On the other hand, the taxa most strongly associated with the wood of the living trees while having a significant proportion in the fungal community were *B. cinerea*, *D. macrostoma*, *D. sapinea*, *D. prunorum*, *Epicoccum* sp., *F. avenaceum*, *P. bialowiezense*, *P. brevicompactum*, *P. canescens*, *P. laundoniae*, and *Stemphylium* sp. *Botrytis cinerea* (Ascomycota) is a cosmopolitan and dangerous plant pathogen causing gray mold [47]. It can colonize plants internally (endophyte) without causing disease or stress symptoms [48]. The relationship between the presence of this fungus in generative organs and its presence in wood is unknown. Van Kan et al. [48] provided data on the occurrence of *B. cinerea* only in the generative organs of the woody plants *Sorbus acuparia* and *Rubus fruticosus* (Rosaceae) (B. A. P. Rajaguru, University of Reading, unpublished results). The presence of this fungus as an endophyte in black cherry tissues may indicate increased inbreeding and reduced genetic diversity in this area [48]. *Prunus serotina* trees in the Wielkopolski National Park displayed various signs of weakness. The symptoms of stone tree leucostoma caused by *C. leucostoma*, isolated mainly from the wood of live black cherry individuals, were visible, especially in plot III (Table A2). Species from the genus *Cytospora* are described as endophytes, saprotrophs, or phytopathogens. These pathogens cause cankers on the branches of various trees, such as peaches, nectarines, plums, ashes, maples, birches, and willows, and may lead to tree dieback [49]. The development of the pathogen is favored by stress factors, such as insect

feeding or damage caused by hail, snow, or drought. Field observations (unpublished data) of leaves have indicated significant damage caused by the feeding of the monophagous moth *Yponomeuta evonymellus* L. (Lepidoptera) and polyphagous beetles, such as *Gonioctena quinquepunctata* F. and *Phyllobius* sp. (Coleoptera), which prefer *Prunus padus* L. and *P. serotina* leaves [50–53]. Wounds are the gateway to infection by pathogens of this type [54]. Both of the abovementioned fungus species can naturally support the methods used to reduce the occurrence of black cherry.

The presence of the following fungi in black cherry wood may positively limit its expansion: *D. macrostoma* (*Phoma macrostoma*), *P. laundoniae*, *F. avenaceum*, and *Stemphylium* sp. These are endophytes but can also transform from opportunists into pathogens after contact under appropriate conditions with a suitable host [55–58]. The fungi of the genus *Stemphylium*, which also infect plants of the genus *Prunus* [59], and *Fusarium* sp., whose presence in black cherry wood has also been confirmed [26], are interesting. On the research plots, numerous weakened *P. serotina* sprouts were observed with powdery mildew on their leaves (unpublished data), a symptom similar to those caused by *Stemphylium* pathogens. The natural presence of these species in black cherry wood is interesting, and their infectious potential could help limit the expansion and regrowth of *P. serotina*, especially since the lack of natural enemies is a major reason for the success of invasive species [60].

Dothiora prunorum (*Aureobasidium prunorum*) is a fungus classified as yeast, which was isolated from the fruit of *Prunus domestica* var. Belle de Louvain [61]. It has been recognized as an endophyte of crops [62]. Its role, similar to that of *Epicoccum* sp., is not clear. The genus *Epicoccum* includes endophytes, pathogens, saprotrophs, and antagonists to other pathogens [63]. These taxa should be further analyzed to determine their role in black cherry tissues. Further research should also be conducted on *Aspergillus parvulus*, which dominated dead wood in plot V (>50% proportion; Table A2) because its function in the dead wood fungal community has not been recognized so far [64].

Species of the genus *Penicillium* were previously isolated from stumps of *P. serotina* [26] and roots of black cherry [65]. The high frequency of *Penicillium* is due to its preferences and ecological specialization, abundant sporulation, and the nature of its spores and their effective dispersal [66]. The abovementioned researchers suggest that *Penicillium* spp. fungi participate in the decomposition of wood components due to their cosmopolitan nature combined with their cellulolytic properties. The scale of this process is probably limited, and their role as promoters of black cherry wood decomposition should not be overestimated [27].

According to Tokarska-Guzik et al. [4], black cherry is an established species in Poland, and two main reasons for its invasiveness are its ability to escape from natural enemies [67] and the lower pathogenicity of new enemies due to the lack of coevolution [68]. Our research indicates that some pathogens can weaken its population, such as *B. cinerea*, *C. leucostoma*, *D. macrostoma*, *P. laundoniae*, *F. avenaceum*, and *Stemphylium* sp. Additionally, the presence of *B. cinerea* in its tissues indicates a different research direction: the need to determine the genetic diversity of the population of this invasive plant species. The hitherto unsatisfactory effects of introducing *P. serotina*, manifested in the low quality of the wood material (with small dimensions, such as a shrub or a small tree), may result from inbreeding, a result of low genetic diversity in its population.

5. Conclusions

The differences between the fungal communities inhabiting the wood of dead and living trees were confirmed regarding taxon diversity and abundance. The results of the research conducted in a protected area indicate that black cherry wood can be naturally colonized by many pathogen species that can limit its expansion. Such pathogens include *B. cinerea*, *C. leucostoma*, *D. macrostoma*, *P. laundoniae*, *F. avenaceum*, and *Stemphylium* sp. A natural promoter of black cherry stump decomposition, the potential of which should be tested in further research, is *S. rugosum*, whose proportion in the dead wood communities was significant. *C. leucostoma* is an interesting species causing the leucostoma of stone

fruit trees. The infection of black cherry by this pathogen indicates its establishment and gives us hope that naturally occurring antagonists can slow down the rate of black cherry expansion. In addition, we propose that identifying fungal communities based on molecular biology techniques should be supplemented by an inventory of macroscopic fungi and other symptoms for a more accurate recognition of the condition of examined trees and to facilitate inferences.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Taxation description of the selected forest divisions on the basis of the forest inventory (data current for end of 2013; access to data (30 November 2022): Data Bank of Forests, Poland). Data for forests in Poland is refreshed once every 10 years during detailed inventory.

Forest Division (GPS Coordinates)	Research Plot (no.)	Area (ha)	Forest Habitat Types	Forest Layer	Tree Species	Share of Species in Layer (%)	Age (Year)	Mean DBH (cm)	Mean height (m)
46c (16.831801; 52.301139)	1. and 2.	3.96	fresh broadleaved forest	Canopy	<i>Pinus sylvestris</i>	80	48	21	22
					<i>Betula pendula</i>	20	48	22	23
					<i>Prunus serotina</i>	60	20		5
				Understory	<i>Fagus sylvatica</i>	20	20		4
					<i>Quercus petraea</i>	20	20		3
34b (16.827777; 52.312627)	3.	0.97	fresh broadleaved forest	Canopy	<i>Pinus sylvestris</i>	90	51	22	22
					<i>Betula pendula</i>	10	51	23	22
				Understory	<i>Prunus serotina</i>	100	35	8	10
37g (16.824575; 52.307312)	4.	6.11	fresh mixed coniferous forest	Canopy	<i>Pinus sylvestris</i>	100	79	30	24
					<i>Betula pendula</i>	50	25	12	13
					<i>Quercus petraea</i>	20	25	9	11
				Understory	<i>Prunus serotina</i>	20	25	10	10
					<i>Fagus sylvatica</i>	10	25	10	9
29h (16.815404; 52.316147)	5.	4.89	fresh broadleaved forest	Canopy (first layer)	<i>Pinus sylvestris</i>	90	79	28	24
					<i>Quercus petraea</i>	10	79	28	23
				Canopy (second layer)	<i>Quercus petraea</i>	100	60	19	17
				Understory (first layer)	<i>Prunus serotina</i>	100	40	9	11
				Understory (second layer)	<i>Prunus serotina</i>	100	20		
	Shrub layer	<i>Prunus serotina</i>							

Appendix B

Table A2. Frequency of individual taxa, the share of which was greater than 0.05%. L and D symbols with numbers indicate samples collected from live and dead trees from plots 1 to 5 (see Section 2).

Taxon	Live					Dead				
	L1	L2	L3	L4	L5	D1	D2	D3	D4	D5
<i>Alternaria infectoria</i> E.G. Simmons	2.41	4.28	0.82	5.22	0.14	---	---	---	---	0.01
<i>Alternaria tenuissima</i> (Kunze) Wiltshire	1.72	2.88	2.61	4.21	0.43	---	---	0.01	---	---
<i>Ascocoryne cylichnium</i> (Tul.) Korf	---	0.24	0.02	---	---	0.06	---	---	24.87	0.02
<i>Aspergillus parvulus</i> G. Smith	0.01	1.31	0.04	---	---	---	---	---	0.01	50.60
<i>Aureobasidium pullulans</i> (de Bary & Löwenthal) G. Arnaud	2.93	6.65	1.14	0.16	0.18	0.01	---	0.03	---	---
<i>Botrytis cinerea</i> Pers.	3.86	1.58	0.31	0.08	11.23	---	---	---	---	---
<i>Chaetosphaeria</i> sp. Tul. & C.Tul.	---	0.03	0.01	---	0.08	0.04	0.02	---	1.81	---
<i>Cladosporium</i> sp. Link	0.60	3.31	1.60	0.46	0.10	0.01	---	0.01	---	---
<i>Coniophora arida</i> (Fr.) P. Karst.	---	---	---	---	---	4.88	---	0.01	---	---
<i>Coniophora</i> sp. DC.	---	---	---	---	---	---	---	1.16	---	---
<i>Cordana pauciseptata</i> Preuss	---	---	---	---	---	50.43	---	0.01	---	---
<i>Cylindrobasidium evolvens</i> (Fr.) Jülich	---	---	---	---	---	---	8.22	---	---	---
<i>Cytospora leucostoma</i> (Pers.) Sacc.	9.23	2.07	4.55	10.63	23.01	0.01	0.02	5.60	---	0.03
<i>Desmazierella acicola</i> Lib.	---	0.95	18.71	---	---	---	---	---	---	---
<i>Diaporthe eres</i> Nitschke	---	---	0.13	---	---	---	1.81	0.06	---	---
<i>Didymella macrostoma</i> (Mont.) Qian Chen & L. Cai	3.34	1.77	3.23	3.25	0.31	---	---	---	---	---
<i>Diplodia sapinea</i> (Fr.) Fuckel	2.01	4.53	5.46	12.31	1.25	0.02	---	---	---	---
<i>Dothiora prunorum</i> (C. Dennis & Buhagiar) Crous	1.83	1.96	0.19	3.35	0.03	---	---	---	---	---
<i>Epicoccum</i> sp. Link	24.28	20.80	26.18	33.80	3.34	---	---	0.02	---	---
<i>Exophiala</i> sp. J.W.Carmich.	---	0.03	---	0.01	---	1.63	0.03	---	0.01	0.02
<i>Fusarium avenaceum</i> (Fr.) Sacc.	1.64	1.63	1.76	5.59	0.10	---	---	---	---	---
<i>Ganoderma adspersum</i> (Schulzer) Donk	---	---	---	---	---	---	---	13.22	---	---
<i>Ganoderma applanatum</i> (Pers.) Pat.	---	---	---	---	---	---	---	15.88	---	---
<i>Mucor moelleri</i> (Vuill.) Lendn.	---	0.08	---	---	1.62	---	---	---	---	---
<i>Mucor plumbeus</i> Bonord.	0.43	1.73	0.36	0.98	---	---	---	---	---	---
<i>Myxarium varium</i> Hauerslev	---	---	---	---	---	---	---	---	1.70	---
<i>Penicillium bialowiezense</i> K.W. Zaleski	0.97	6.07	1.89	1.43	0.56	0.01	---	0.03	---	---
<i>Penicillium brevicompactum</i> Dierckx	0.18	1.12	0.24	0.21	0.13	---	---	---	---	---
<i>Penicillium camemberti</i> Thom	0.07	1.64	2.16	0.56	0.51	---	---	0.04	---	---
<i>Penicillium canescens</i> Sopp	2.73	1.25	0.22	0.15	32.79	---	---	0.01	---	---
<i>Penicillium lilacinoechinulatum</i> S. Abe	0.09	0.20	0.04	---	7.21	---	---	0.01	---	---
<i>Penicillium</i> sp. Link	0.56	2.01	0.91	0.24	0.39	0.06	0.02	0.04	0.01	0.04
<i>Penicillium waksmanii</i> K.W. Zaleski	2.16	2.47	0.87	0.43	2.55	0.70	0.01	0.08	---	0.01
<i>Peniophora cinerea</i> (Pers.) Cooke	---	0.01	0.02	0.05	0.01	---	---	43.22	---	---
<i>Peniophora</i> sp. Cooke	---	---	---	---	---	---	---	1.05	---	---
<i>Pezicula cinnamomea</i> (DC.) Sacc.	0.04	0.21	0.27	0.05	0.06	0.94	0.11	2.60	3.23	2.38
<i>Pezicula sporulosa</i> Verkley	0.01	0.21	0.13	0.02	0.01	1.12	0.30	0.43	0.03	0.08
<i>Phialocephala piceae</i> (T.N. Sieber & Grünig) Rossman	0.04	1.02	0.65	0.09	0.05	12.45	0.97	1.67	0.21	0.45
<i>Phoma laundoniae</i> Boerema & Gruyter	0.27	0.60	1.44	0.27	1.23	---	---	---	---	---
<i>Pluteus cervinus</i> (Schaeff.) P. Kumm.	---	---	---	---	---	2.21	---	---	---	---
<i>Pluteus rangifer</i> Justo, E.F. Malysheva & Bulyonk.	---	---	---	---	---	1.73	---	---	---	---
<i>Pyrenochaeta</i> sp. De Not.	---	0.05	0.01	3.36	---	0.01	---	---	---	---
<i>Stemphylium</i> sp. Wallr	0.14	0.27	2.83	0.19	0.01	---	---	---	---	---
<i>Stereum rugosum</i> Pers.	0.02	1---	0.11	---	---	12.33	84.86	5.62	65.39	18.28
<i>Strasseria geniculata</i> (Berk. & Broome) Höhn.	12.03	0.01	0.01	---	---	---	---	---	---	---
<i>Trichoderma atroviride</i> P.Karst.	1.20	0.04	0.04	0.01	0.01	0.01	---	0.01	---	0.02
<i>Trichoderma koningiopsis</i> Samuels, C. Suárez & H.C. Evans	12.66	3.14	12.13	3.15	7.11	0.24	0.01	6.99	0.20	14.63
<i>Trichoderma reesei</i> E.G. Simmons	1.75	0.02	0.25	---	0.13	---	---	---	---	---
<i>Trichoderma</i> sp. Pers.	0.98	0.10	0.02	0.01	0.10	2.20	---	0.01	---	0.11
<i>Umbelopsis isabellina</i> (Oudem.) W. Gams	0.03	0.16	0.38	1.47	0.02	0.02	---	0.01	0.01	6.55
<i>Vararia</i> sp. P. Karst.	---	---	---	---	---	1.89	---	---	---	---

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