

Article

Invertebrates in Fruitbodies of *Heterobasidion* spp., Infected *Picea abies* Logs and Adjacent Soil

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Abstract: *Heterobasidion* spp. pathogenic fungi produce conspicuous fruitbodies on infected wood, which may represent a habitat for a range of organisms, including invertebrates. The aim was to: (i) assess and compare invertebrate diversity in *Heterobasidion* spp. fruitbodies, infected *Picea abies* wood and adjacent soil; (ii) test methods for collecting invertebrates from *Heterobasidion* spp. fruitbodies. A total of 69 *Heterobasidion* fruitbody samples, 46 wood samples and 19 soil samples were collected at two forest sites in Latvia. In total, 7311 invertebrate individuals were collected representing 62 different taxa, among which 55 were in fruitbodies, 47 in wood and 36 in soil. The dominant invertebrates in fruitbodies were Acari (82.4%), Collembola (6.2%), Coleoptera (4.7%); in wood were Acari (78.6%), Collembola (9.6%), Diplopoda (3.1%); in soil were Acari (90.6%), Collembola (3.4%) and Coleoptera (1.4%). Among all taxa, 17.7% were found exclusively in fruitbodies, 3.2% in wood, 4.8% in soil, while 48.4% were shared among different substrates. Although many invertebrate taxa were shared, their relative abundance differed substantially among different substrates. The sampling methods used provided valuable information on invertebrate communities. Further development of these methods may be needed to obtain more detailed and specific information about invertebrates inhabiting different habitats.

Keywords: *Heterobasidion*; carpophores; fauna; Norway spruce; Tullgren funnels



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1. Introduction

Heterobasidion spp. pathogenic fungi cause considerable economic losses to forestry due to the infection of living trees and decay of wood. The impact of the pathogen is more severe in Norway spruce (*Picea abies*) stands as the fungal mycelia from the root system can expand several meters up in the stem, thereby causing rot in the most valuable part of the stem [1]. In Latvia, ca. 22% of *P. abies* stands are infected by *Heterobasidion* spp. and, on average, the economic loss at a final cutting exceeds EUR 4000/ha [2]. *Heterobasidion* spreads by basidiospores (primary infections) and by vegetative mycelia through root contacts (secondary infections) [3]. The abundance of fruitbodies of the pathogen in a particular stand has an impact on the primary infection by basidiospores. It is known that these fruitbodies mainly affect the rate of infection at the stand level but not in adjacent stands [4]. Both *Heterobasidion parviporum* Niemelä & Korhonen and *Heterobasidion annosum* (Fr.) Bref. are found in Latvia, and both of these species form fruitbodies on infected *P. abies* wood at a similar rate [5]. Studies conducted in Germany and Finland [6,7] have indicated that a large number of fruitbodies are formed on 3- to 4-year-old large dimension *P. abies* logs that are left in stands after forest operations. In Latvia, the area covered by fruitbodies on 3- to 4-year-old *P. abies* logs is on average 3759 cm²/m³, and can exceed 5000 cm²/m³ on logs in stands growing on drained peat soils [5].

Heterobasidion fruitbodies can produce between 35,000 and 151,000 basidiospores per cm² of the fruitbody in one hour [8], and, in *P. abies* stands, the number of spores can exceed 1000 per m² per hour [9]. This spore density is a high risk to freshly cut *P. abies* stumps during tree felling as fresh stumps are the primary substrate for colonisation by *Heterobasidion* spores. Therefore, to reduce the risk of infection, several studies have recommended the removal of large dimension *P. abies* logs that are infected by *Heterobasidion* spp. from forest stands [5–7]. However, other studies have indicated that large dimension *P. abies* logs are important as a habitat to many fungal and invertebrate species [10,11]. Fungal fruitbodies are known to be inhabited by a number of invertebrate species [12,13]. Fruitbodies provide not only a food source for invertebrates but also shelter and a place for reproduction, thus influencing the density of invertebrates in a given substrate [11]. Invertebrate fauna have been investigated in the fruitbodies of *Fomitopsis pinicola* (Fr.) Karst, a fungal species commonly found in conifer forests [14–17]. However, in most studies on invertebrate fauna inhabiting fungal fruitbodies, multiple fungal species were analysed, without differentiation. The focus has often been on macro arthropods that feed on fungi, typically Coleoptera and Diptera [17–19]. However, fungal fruitbodies are also known to be commonly inhabited by Acari and Collembola [20,21]. Some studies have also investigated *Heterobasidion* spp. fruitbodies, however, the majority of these have examined a small number of fruitbodies, from which only adult Coleoptera were collected as these are relatively easy to identify. In a study that included several fungal species, adult Coleoptera were found in only two *H. parviporum* fruitbodies from a total of 44 collected [22], while in another similar study, only half of examined *H. parviporum* fruitbodies were inhabited by Coleoptera [23]. *Heterobasidion annosum* was shown to be associated with several Coleoptera species from the family Ciidae [24], and reproduction in fruitbodies was confirmed for *Cis lineatocribatus*, with both adults and larvae identified [18]. The possible role of insect vectors for dissemination of *Heterobasidion* spp. has previously been highlighted by Stenlid [25] and Piri et al. [26]. Indeed, spores of *Heterobasidion* spp. are known to be disseminated by the large pine weevil (*Hylobius abietis*) [27,28], but the role of other insects is largely unknown. To determine the effect of the removal of infected *P. abies* wood from forest stands on invertebrate diversity, quantitative and qualitative assessment of invertebrate fauna in *Heterobasidion* spp. fruitbodies is required, particularly focussing on rare and endangered species. The aim of this study was to: (i) assess and compare invertebrate diversity in *Heterobasidion* spp. fruitbodies, infected *P. abies* wood and adjacent soil; (ii) develop and test methods for collecting invertebrate samples from *Heterobasidion* spp. fruitbodies.

2. Materials and Methods

2.1. Sample Plots

Samples were collected from two sample plots in the autumn of 2016: (i) a 70-year-old forest stand consisting of 80% *P. abies* with an admixture of *Betula pendula* and *Pinus sylvestris* on peatland (forest type Oxalidosa turf.mel. [29]) near the Forest Research Station in the Kalsnava forest region (56°41'40.4" N, 25°54'21.8" E), further referred to as Kalsnava; (ii) a 50-year-old forest stand consisting of 90% *P. abies* with an admixture of *B. pendula* on mineral soil (forest type Oxalidosa [29]) in JSC Latvia's State Forests Central Daugava region, Ogre division (56°50'50.8" N, 24°48'17.8" E), further referred to as Ogre. Sample plots contained infected *P. abies* logs of different sizes and decomposition stages, where *Heterobasidion* spp. fruitbodies of varying sizes and ages were previously identified.

2.2. Field Methods

To sample fruitbodies, 46 infected *P. abies* logs with large *Heterobasidion* spp. fruitbodies (Figure 1) were randomly selected in each plot. For each log, the root end diameter and length were measured. The length of logs ranged between 107 and 487 cm (average 274 cm); diameters ranged between 15 and 42 cm (average 25 cm). Fruitbody samples (100 cm²) were collected by cutting a 10 × 10 cm piece from the fruitbody closest to the root end

of the log. During the collection of fruitbody samples, it was noted if the fruitbody was in contact with the soil and if any damage was visible. A similarly sized (100 cm²) wood sample was collected 20 cm from the fruitbody in the direction away from the root end of the log. One wood sample and at least one fruitbody sample was collected from each log. If multiple and well-separated fruitbodies were found on one log, two to three fruitbody samples were collected. Where the log was in contact with the soil, a 5 cm deep, 100 cm² soil sample was collected. If multiple logs were found less than 10 m from each other (in log piles), only one soil sample was collected. After collection, all samples were placed into plastic bags, and kept at +4 °C until processing. A total of 69 *Heterobasidion* spp. fruitbodies, 46 *P. abies* wood samples and 19 soil samples were collected. In Kalsnava, 46 fruitbodies, 30 *P. abies* wood samples and 15 soil samples were collected, while in Ogre, 23 fruitbodies, 16 *P. abies* wood samples and 4 soil samples were collected.



Figure 1. Large *Heterobasidion* spp. fruitbody.

2.3. Laboratory Methods

In the laboratory, each sample was weighed using a Kern EMB 200-2 balance (Balingen, Germany) before and after placing samples in Tullgren funnels (Figure 2), and macrofauna were collected (if present) using forceps. By weighing the samples before and after placing them in Tullgren funnels, the relative humidity of samples was calculated. The relative humidity of fruitbodies ranged from 40.6% to 85.6% with an average of 70.1%. In Ogre, the relative humidity of fruitbodies was on average 69.4%, but in Kalsnava it was on average 71.6%. The average weight of a fruitbody sample before drying was 68.7 g, maximal and minimal values being 151.0 g and 14.2 g, respectively. After drying, the weight varied between 5.1 g and 42.3 g with an average of 19.9 g.



Figure 2. Tullgren funnels utilized for the collection of invertebrates.

The thickness of fruitbodies was measured with a ruler. Most of the collected fruitbodies were between 0.4 and 2.5 cm thick, but some fruitbodies that were collected close to the root collar were up to 5.0 cm thick. The majority of fruitbodies collected were at least 3 years old with some being between 6 and 7 years old.

After measurements, samples were placed in Tullgren funnels for at least 72 h. Tullgren funnels (Figure 2) are commonly utilized to obtain quantitative data about arthropods in soil [30], but this method is also effective to obtain data on invertebrates in fruitbodies and

wood [14,31,32]. A 1:3 mixture of glycerine and 70% ethanol was placed in the collection vials to preserve the collected invertebrate individuals.

The collected invertebrates were examined using Leica NBO MSV266 (Wetzlar, Germany) and Optika B-383PH (Ponteranica, Italy) microscopes. Invertebrates were classified to the lowest possible taxonomic level, most to family level. Springtails (Collembola) were classified according to W. Dunger [33], mites (Acari) according to unpublished material by Dr U. Kagainis on the classification of oribatid mites (Oribatida) morphogroups in Latvia. Beetles (Coleoptera) were classified to the family level using the key by D. M. Unwin [34]. Taking into account the available information on Coleoptera of the Ciidae family inhabiting *Heterobasidion* spp. fruitbodies, these were classified to species level using the publicly available key by Lompes [35], which was developed from the results of studies by Reibnitz [24].

2.4. Statistical Analyses

To analyse invertebrate diversity in each substrate, the invertebrate data (actual observations) were used to construct accumulation curves [36], which was conducted in R [37] using the *vegan* package and *specaccum* function [38]. Differences in invertebrate taxa richness in different substrates (study sites as well as adults and larvae combined) were compared by nonparametric chi-square tests, taking into account the Bonferroni correction [39]. Shannon index values [40] were calculated for each substrate to determine the diversity in invertebrate communities. Values were calculated using invertebrate frequencies by taxa, i.e., the subclass or family taxonomic level. The invertebrate community structure in different substrates (fruitbodies, wood and soil) was analysed using principal component analysis in Canoco v.5.02 (Microcomputer Power, Ithaca, NY, USA).

3. Results

A total of 7311 invertebrate individuals were collected from all substrates and from both sample plots, including 7151 adults and 160 larvae. The number of invertebrates collected per individual fruitbody was between 1 and 396 (average 70). In wood and soil samples, invertebrate numbers ranged from 0 to 316 (average 35) and 0 to 192 (average 41), respectively. There were 55 different invertebrate taxa in 69 fruitbody samples, 47 taxa in 46 wood samples and 36 taxa in 19 soil samples. Consequently, the chi-square test showed that the absolute richness of invertebrate taxa differed significantly among all substrates, being highest in the soil, then in the wood and lowest in fungal fruitbodies ($p < 0.004$). A possible reason for the observed differences may be an uneven number of samples collected of different substrates. Indeed, when the same number of samples was taken from each substrate following the rarefaction analysis, the richness of invertebrate taxa did not differ significantly among different substrates ($p > 0.05$).

The accumulation curves for different substrates did not reach the asymptote (Figure 3).

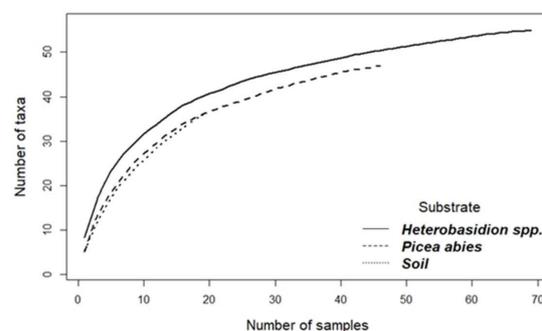


Figure 3. Accumulation curves showing the relationship between the cumulative number of invertebrate taxa and the number of collected samples.

The largest number of invertebrates was collected from *Heterobasidion* spp. fruitbodies—4886 adults and 101 larvae (Table 1). The dominant invertebrate taxa were Acari (82.4%),

with the other invertebrates being Collembola (6.2%), Coleoptera (4.7%), Diplopoda (2.5%) and Diptera (1.8%). In addition, Haplotaxida (0.8%), Thysanoptera (0.7%), Hymenoptera (0.3%), Aranea (0.2%), Gastropoda (0.1%) and Chilopoda (0.1%) were found in fruitbodies. Several invertebrate groups were represented by very few individuals in fruitbodies—Lepidoptera, Isopoda and Hemiptera (0.04% each) and Protura and Opiliones (0.02% each).

Table 1. Occurrence and relative abundance (%) of invertebrate taxa identified in *Heterobasidion* fruitbodies, *Picea abies* wood and soil samples. Absolute numbers for higher taxonomic groups are shown in parentheses.

Invertebrate Taxa	<i>Heterobasidion</i>		<i>Picea abies</i> Wood		Soil	
	Fruitbodies					
Subclass/Family	Adults	Larvae	Adults	Larvae	Adults	Larvae
Acari	(4111)		(1277)		(634)	
Hypochthoniidae	0.6	-	2.6	-	1.4	-
Pthiracaridae	0.9	-	4.8	-	2.2	-
Euphthiracaridae	0.2	-	5.9	-	0.3	-
Crotonioidae	0.7	-	0.1	-	1.4	-
Nanhermanniidae	0.1	-	0.3	-	0.4	-
Damaeidae	0.2	-	0.1	-	0.7	-
Eremaeidae	0.1	-	0.1	-	-	-
Tenuialidae	0.04	-	0.1	-	0.3	-
Xenillidae	0.02	-	0.2	-	-	-
Carabodidae	65.9	-	12.4	-	11.4	-
Oppiidae	7.3	-	39.2	-	32.7	-
Scheloribatidae	-	-	-	-	0.6	-
Ceratozetidae	0.8	-	5.3	-	34.9	-
Galumnidae	0.04	-	0.1	-	0.6	-
Other ¹	7.1	-	10.3	-	4.3	-
Collembola	(309)		(156)		(24)	
Hypogastruridae	0.2	-	0.2	-	0.1	-
Neanuridae	4.2	-	6.4	-	2.3	-
Onychiuridae	0.8	-	1.5	-	0.4	-
Entomobryidae	0.1	-	1.2	-	0.3	-
Isotomidae	0.7	-	0.1	-	0.3	-
Tomoceridae	0.2	-	0.4	-	-	-
Sminthuridae	0.02	-	0.1	-	-	-
Coleoptera	(170)	(62)	(12)	(29)	(7)	(3)
Anobiidae	0.02	-	-	-	-	-
Cantharidae	-	17.8	-	16.7	-	40.0
Carabidae	0.1	-	-	-	-	-
Ciidae	3.0	11.9	-	1.9	-	-
Cryptophagidae	-	-	-	-	0.1	-
Elateridae	0.02	23.8	-	33.3	-	-
Pselaphidae	-	-	0.1	-	0.1	-
Ptilidae	0.1	-	0.5	-	-	-
Silphidae	0.1	-	0.1	-	-	-
Staphylinidae	0.2	7.9	0.1	1.9	0.7	20.0
Diplopoda	(126)		(50)		(1)	
Blaniulidae	2.3	-	2.9	-	0.1	-
Julidae	0.02	-	-	-	-	-
Polydesmidae	0.3	-	0.3	-	-	-
Diptera	(50)	(39)	(10)	(22)	(3)	(1)
Bibionidae	0.02	0.9	-	-	-	-
Brachycera	0.1	15.8	-	7.4	-	20.0
Cecidomyiidae	0.04	2.9	-	-	-	-
Ceratopogonidae	0.1	-	-	3.7	-	-
Chironomidae	0.4	14.8	0.3	25.9	-	-

Table 1. Cont.

Invertebrate Taxa	<i>Heterobasidion</i>		<i>Picea abies</i> Wood		Soil	
	Fruitbodies					
Subclass/Family	Adults	Larvae	Adults	Larvae	Adults	Larvae
Drosophilidae	-	-	0.1	-	-	-
Limoniidae	-	-	-	3.7	-	-
Psychodidae	0.02	-	-	-	-	-
Rhagionidae	-	-	-	-	0.1	-
Sciaridae	0.3	0.9	0.3	-	0.3	-
Stratiomyidae	0.02	2.9	-	-	-	-
Haplotaaxida	(41)		(18)		(8)	
Lumbricidae	0.4	-	0.6	-	0.7	-
Enchytraeidae	0.4	-	0.5	-	0.4	-
Thysanoptera	0.7 (35)	-	0.6 (10)	-	0.1 (1)	-
Hymenoptera	(15)		(7)		(8)	
Parasitica	0.3	-	0.4	-	1.2	-
Formicidae	0.02	-	-	-	-	-
Aranea	0.2 (10)	-	0.5 (8)	-	0.4 (3)	-
Gastropoda	(6)		(6)		(1)	
Arionidae	0.02	-	-	-	-	-
Clausiliidae	0.02	-	-	-	0.1	-
Zonitiidae	0.1	-	0.4	-	-	-
Chilopoda	0.1 (5)	-	0.2 (3)	-	0.1 (1)	-
Hemiptera	0.04 (2)	-	0.1 (1)	-	0.3 (2)	-
Lepidoptera	(2)			(3)		(1)
Tineidae	0.04	-	-	5.5	-	20.0
Isopoda	(2)		(2)			
Trichoniscidae	0.04	-	0.1	-	-	-
Opiliones	0.02 (1)	-	-	-	-	-
Protura	0.02 (1)	-	-	-	-	-
Pseudoscorpionida	-	-	0.6 (10)	-	0.3 (2)	-

¹ Acari individuals that were not identified to family level.

Coleoptera of the Ciidae family were most prevalent in fruitbodies (86.8% of all beetles collected): *Cis nitidus* (27.6% of all beetles collected), *C. castaneus* (19.4%), *C. lineatocribatus* (18.8%), *C. jacquemartii* (14.7%) and *C. glabratus* (5.8%). Adult *Phosphuga atrata*, *Daliopius marginatus* and *Oxypselaphus obscurus* were observed, as well as larvae of several beetle families, i.e., Elateridae, Cantharidae, Ciidae and Staphylinidae. Larvae of these taxa were also found in wood samples; however, they were in a smaller number.

Three Diplopoda species were found in fruitbodies. The most prevalent species was *Proteroiulus fuscus*, which was twice as prevalent than in wood samples. One *Cylindroiulus caeruleocinctus* individual was found only in fruitbodies, as well as thirteen *Polydesmus denticulatus* individuals. Three earthworm species *Lumbricus castaneus*, *L. rubellus* and *L. terrestris* were found in fruitbodies. These species were also found in wood samples; however, they were in a smaller number.

The second largest number of invertebrates was collected from wood samples, i.e., 1570 adults and 54 larvae (Table 1). The dominant invertebrate taxa were Acari (78.6%), with the other invertebrates being Collembola (9.6%), Diplopoda (3.1%), Coleoptera (2.5%), Diptera (1.9%) and Haplotaaxida (1.1%). Several invertebrate groups were represented by a few individuals in wood samples, which were Pseudoscorpionida and Thysanoptera (0.6% each), Aranea (0.5%), Hymenoptera (0.4%), Gastropoda (0.4%) Lepidoptera larvae (0.2%), Chilopoda (0.2%), Isopoda (0.1%) and Hemiptera (0.1%).

The smallest number of samples were collected from soil samples, i.e., 695 adults and five larvae (Table 1). As in the fruitbody and wood samples, Acari were the dominant invertebrate taxon (90.6%). Other invertebrates were present in small numbers: Collembola (3.4%), Coleoptera (1.4%), Haplotaaxida (1.1%) and Hymenoptera (1.1%). Several

invertebrate groups were represented by very few individuals in soil samples: Diptera (0.6%), Aranea (0.4%), Pseudoscorpionida (0.3%) and Hemiptera (0.3%), and Chilopoda, Diplopoda, Thysanoptera, Lepidoptera larvae and Gastropoda (0.14% each).

Shannon index values were 2.37 in wood samples, 1.63 in fruitbodies and 1.95 in soil samples.

Dominance of one invertebrate taxonomic group was pronounced with Acari dominating in all substrates, and Collembola was the second most common group (Table 1). The third most prevalent taxonomic group varied between substrates, i.e., in *Heterobasidion* spp. samples it was Coleoptera, in wood it was Diplopoda and in soil it was Coleoptera and Haplotaaxida. Among the 62 invertebrate taxa found in the present study, eleven (17.7%) were exclusively found in fruitbodies, two (3.2%) in the wood and three (4.8%) in the soil, while thirty (48.4%) were common to all substrates (Figure 4). There were 43 shared invertebrate taxa between fruitbodies and wood, 31 between fruitbodies and soil and 32 between wood and soil (Figure 4).

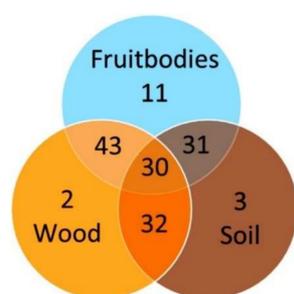


Figure 4. Venn diagram showing the diversity and overlap of invertebrate taxa in different substrates (sites as well as adults and larvae are combined).

In principal component analysis (PCA) of invertebrate communities, axis 1 explained 81.7% of variation and axis 2 explained 18.3%. PCA showed that invertebrate communities from different substrates were separated from each other on axis 1, i.e., on the axis, which explained most of the variation (Figure 5).

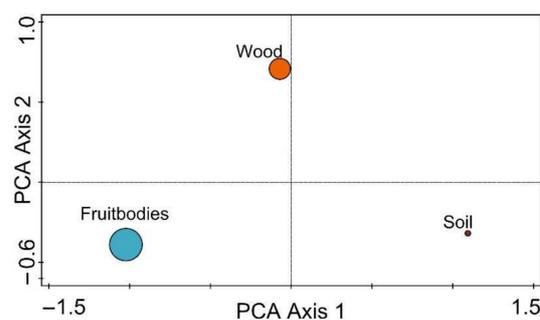


Figure 5. Ordination diagram based on principal component analysis of invertebrate communities from *Heterobasidion* spp. fruitbodies, *Picea abies* wood and soil samples. The size of each point reflects the relative richness of invertebrate taxa.

4. Discussion

The taxonomic composition of invertebrates found in *Heterobasidion* spp. samples reflects the biology of fruitbodies. Fruitbodies develop on the part of the infected substrate that are in contact with the soil [7]. If the wood substrate is embedded in the soil, the fruitbody develops in a narrow line at the point of contact between the soil and the wood [5]. Therefore, the dominant invertebrate taxa in fruitbodies were also present in wood and soil samples (Table 1, Figure 4). Although the majority of invertebrate taxa were shared among different substrates, the PCA showed that invertebrate communities in different substrates were separated from each other, which was likely due to differences in the abundance data

of different taxa (Table 1), thereby demonstrating the specificity and substrate preferences of different invertebrates. Our data show that Acari were the predominant invertebrate taxa found in *Heterobasidion* spp. fruitbodies (Table 1). This is consistent with other reports about fauna of invertebrates found in *Heterobasidion* spp. [32] and *F. pinicola* [14] fruitbodies. Some of the Acari that were not identified to family level belong to the order Mesostigmata, which was previously identified in fruitbodies from other species, such as *F. pinicola* [41]. Acari of the genus *Carabodes* were the most prevalent in fruitbodies. The presence of this genus in fruitbodies has been studied extensively in coniferous forests in Norway [14,42], and many taxa inhabiting soil also occupy fruitbodies as an additional food resource. This was also observed for Acari collected from *Heterobasidion* spp. [32].

Several studies have noted that *Heterobasidion* spp. fruitbody development is promoted by shade and moisture [5,43,44]. In this study, invertebrate groups that prefer moist environments, such as Haplotaxida, Diplopoda and Chilopoda, were also frequently detected in fruitbodies. However, these invertebrates are probably incidental and temporary inhabitants of fruitbodies even though their numbers in the analysed samples was relatively high (Table 1). Diplopoda and Chilopoda are prone to dehydration, and therefore seek moist places to forage [45]. The relative moisture content of the fruitbodies analysed in this study was 70%, and water drained from them when compressed. A portion of the Haplotaxida was collected from the oldest peripheries of the fruitbodies, where other invertebrates had already formed tunnels.

Heterobasidion spp. form perennial fruitbodies with a layered, cork-like structure [3]. Several Diptera adults and larvae were identified in fruitbodies, however the number of individuals collected was relatively small in comparison with the total number of invertebrates collected, which could be due the structure of fruitbodies. Diptera most often inhabit softer, fruitbodies of annual fungi that decay faster [46]. However, despite the structural differences of various fungal fruitbodies, our study is in agreement with the results from other studies [47,48], i.e., showing the low level of specialization between Diptera. Therefore, fungal fruitbodies can be colonized by various groups of Diptera. The number of amassed Diptera could be affected by the time of sampling as these insects are mostly inactive during the dormancy period.

One of the aims of this study was to compare invertebrate fauna inhabiting fruitbodies, rotting wood and soil. While these substrates are in close contact with each other in nature, differences in the invertebrate fauna inhabiting them were observed (Table 1, Figures 4 and 5). These differences may be due to the season when samples were collected (October). Assessment of invertebrate fauna during the vegetation period may be more representative. Each substrate had a characteristic invertebrate fauna profile (Figure 5), and the number of individuals and taxa collected varied between different substrates. For example, Acari of the genus *Carabodes* were most prevalent in fruitbodies but were less common in wood and soil samples (Table 1). By contrast, some other Acari were most often found in wood and soil samples (Oppiidae and Ceratozetidae, respectively) but were less commonly found in fruitbodies. In total, 90% of invertebrate individuals collected from soil samples were Acari, while in fruitbody and wood samples the proportion of Acari was lower (82% and 78%, respectively). Although the majority of invertebrate taxa were found in all substrates, some characteristic taxa were only found in fruitbodies (e.g., Ciidae), but not in wood or soil samples (Figure 4).

In studies of polypore inhabitants, usually the entire fruitbody is collected [17,32]. Studies in Latvia on the development of *Heterobasidion* spp. fruitbodies on decaying *P. abies* wood indicate that the size of fruitbodies can vary widely, and older fruitbodies coalesce with each other [5]. Therefore, in this study, only partial fragments of fruitbodies were collected. Although *Heterobasidion* spp. form large fruitbodies on windthrown trees and large dimension logging debris in favourable conditions [3,5], collection of a large number of 10 cm × 10 cm fragments of *Heterobasidion* spp. fruitbodies is difficult. Although the area of collected fruitbodies was uniform (100 cm²), the volume of fruitbodies varied. This was due to differing thicknesses of the collected fruitbodies, which is influenced by the age

of fruitbodies. In addition, the dry weight of collected fruitbodies varied between 5.1 g and 47.3 g, which has also been reported to influence invertebrate numbers and diversity in *H. annosum* fruitbodies [32]. The obtained data indicate that in further research, a larger number of samples of smaller size could be collected.

The Shannon diversity index values indicated that invertebrate diversity in fruitbodies was moderate. As mentioned before, the results of the study were influenced by the season when samples were collected. *Heterobasidion* spp. sporulate throughout the year, however, the maximum rate is between June and August [49,50]. Collection of fruitbodies at this time may identify invertebrate taxa that feed on spores at some developmental stage or taxa that are inactive during colder periods of the year. Analysis of invertebrates present in fruitbodies during peak sporulation times may identify potential vectors that can influence the primary infection pathways of this pathogen [51]. Previous studies on the role of different invertebrates as vectors of *Heterobasidion* have mainly focused on fungal conidia [3,52], but further studies are required to better understand the nature of such associations.

In this study, samples were placed in Tullgren funnels to collect invertebrates for three days. In total, 95% of the individuals collected were collected within the first 24 h. Some reports [45,53] indicate that in order to identify certain invertebrates, samples need to be held in Tullgren funnels for a longer period, for example, to allow development of larvae. Some Diptera and Coleoptera larvae were collected in this study, however, the proportion of larvae of the total number of individuals collected was low (2% in fruitbodies). To obtain a more comprehensive assessment of invertebrate fauna inhabiting *Heterobasidion* spp., methods that allow the development of larvae should be considered, by not preserving collected individuals in glycerine and placing collected larvae in rearing chambers, or by placing fruitbodies in vials and collecting emerged insects. In addition, Tullgren funnels are only partially effective for the collection of mobile invertebrates such as Collembola and should be used in conjunction with other collection methods [54], for example, by covering funnels. In this study, Collembola were observed on the surface of the samples during both sample sorting and collection in Tullgren funnels.

The stage of wood decay also influences invertebrate numbers and diversity, similarly to the decay stage of fruitbodies. The majority of organisms associated with polypores utilise fruitbodies in the later succession stages [11]. During the sterile phase, when the fungal hymenium layer is developing, perennial polypore species produce chemicals that protect them against invertebrate damage, which ceases when the fruitbody starts to decay [14]. Invertebrates may only start to inhabit fruitbodies after several years. In addition, fruitbodies on a single log do not start to decay simultaneously, ensuring suitable conditions for invertebrates that colonise decaying fruitbodies, even over several decades. Studies in Latvia have identified *Heterobasidion* spp. fruitbodies in various stages of decay on more than ten-year-old rotting spruce wood [5].

Ciidae was the most prevalent Coleoptera family collected from fruitbodies. This is a functionally important group that inhabits fruitbodies during both larval and adult stages, and several successive generations are often found in a single fruitbody. The diversity of Ciidae species is significantly influenced by host organism incidence within a territory, as well as fruitbody developmental stages [17,55]. *Cis bidentatus* was the only Coleoptera species collected from *H. parviporum* fruitbodies in Finland, and that colonised decaying fruitbodies or decaying parts of still living fruitbodies [23]. However, this species was not identified in this study.

While a comparatively small number of *Cis glabratus* individuals were identified in this study, this species has been reported to be prevalent in *H. annosum* [24] and *F. pinicola* fruitbodies [15,17]. In addition to species collected in this study (*C. lineatocribatus*, *C. nitidus*, *C. glabratus* and *C. castaneus*), another study also reported the presence of *C. bidentatus* and *Ennearthron cornutum* on *Heterobasidion* spp. [24].

According to the European Union Council directive 92/43/EEC (Protecting Europe's biodiversity (Natura 2000)), no rare or protected species of invertebrates were identified in this study.

Further research is needed to reveal the effect of the decay stage of wood and fruitbodies, as well as the effect of wood dimensions on the invertebrate fauna found in *Heterobasidion* spp. fruitbodies. Differences in the invertebrate fauna inhabiting decaying wood in areas of mineral and peat soils could be assessed by collecting samples from forest stands of different forest types.

5. Conclusions

The results demonstrated the presence of a taxa-rich community of invertebrates inhabiting *Heterobasidion* fruitbodies, decayed *P. abies* logs and adjacent soil. Although a large number of invertebrates were shared among different substrates, their relative abundance varied substantially showing certain specificity and substrate preferences of particular taxa.

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