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ORIGINAL ARTICLE

Fungi associated with Vitality loss of European beech in central Germany

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Abstract

Vitality loss of beech is a complex disease of European beech that has been occurring all across central Germany since the summer of 2018, leading to growth loss and causing damage to affected trees. Using a culture-based method, 13 matured European beech trees affected to varying degrees by Vitality loss of beech in central Germany were sampled. The isolation of fungi from each test tree was conducted in a standardised manner, with subsequent culturing, morphotyping and identification of filamentous species. All in all, 181 morphotypes were isolated from twigs, branches and trunks of symptomatic and asymptomatic tissue and identified. Fifteen different orders of *Ascomycota* and four orders of *Basidiomycota* were detected. Isolated species and corresponding orders differed greatly depending on the test tree, the sampled tree compartment and the tissue type. However, it could not be shown that the vitality status of the host tree had an effect on the fungal community in asymptomatic tissue, possibly because the effect was superimposed by the site or individual tree characteristics. While, depending on the individual tree, a large number of different fungal species probably contributed to the damage caused by Vitality loss of beech, *Biscogniauxia nummularia* and *Neonectria coccinea* were present throughout the whole study area, confirming their high relevance in Vitality loss of beech. *Biscogniauxia nummularia* was isolated more frequently from the asymptomatic tissue of damaged trees than from the asymptomatic tissue of undamaged trees and is therefore possibly suitable as a bioindicator for the beech vitality.

Keywords Vitality loss of beech · Endophytes · *Fagus sylvatica* · *Biscogniauxia nummularia* · *Neonectria coccinea*

Introduction

According to its relative area, European beech (*Fagus sylvatica* L., *Fagaceae*) is the most common deciduous tree species in German forests (BMEL 2018). An important factor limiting the spread of European beech is the availability of soil water (Ellenberg and Leuschner 2010). The years from 2018 to 2022 have been unusually dry and hot, with the exception of 2021 (Rakovec et al. 2022; Imbery et al. 2023). Due to the high precipitation deficits across the country, European beech suffered under drought stress in many places and showed losses in vitality. The consequences were declining growth (Scharnweber et al. 2020; Leuschner et al. 2023), early leaf shedding and discolouration as well as crown dieback on a large area (Langer et al. 2020; Nussbaumer et al. 2020; Bigler and Vitasse 2021; Langer and Bußkamp 2021, 2023; Purahong et al. 2021; Arend et al. 2022). In central Germany, older European beech trees on predisposed sites or trees with previous damage were initially affected in 2018. Due to the prolonged drought in the following years, the dieback spread, so that since 2019, European beech of all age classes and also trees on more favourable sites declined (Langer 2019; Langer et al. 2020). The observed symptoms have been assigned to the damage pattern Vitality loss of beech (VLB; Bressem 2008; Brück-Dyckhoff et al. 2019; Langer 2019). In 2022, 31% of all harvesting in the wood species group beech was carried out due to calamities (BMEL 2023). However, there are indications that the sub-canopy trees are less affected by the

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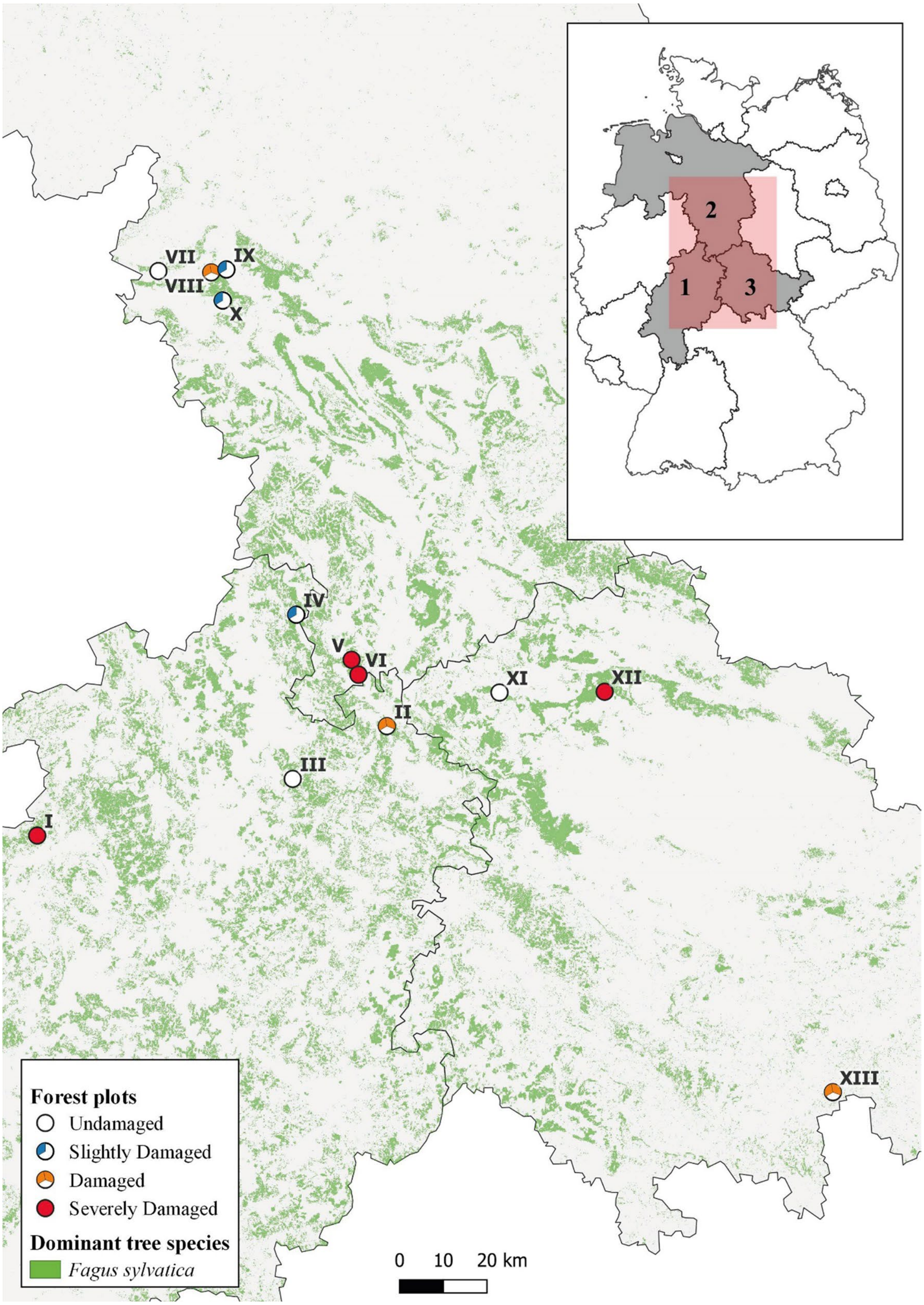
VII
IX
VIII
X
IV
V
VI
XI
XII
II
III
I
XIII
1
2
3
Forest plots
○ Undamaged
◔ Slightly Damaged
◔ Damaged
● Severely Damaged
Dominant tree species
🟩 Fagus sylvatica
0 10 20 km

◂**Fig. 1** Detailed map showing the 13 locations of the forest plots (forest plots I–XIII) studied and areas where *Fagus sylvatica* is the dominant tree species (green) according to Blickensdörfer et al. (2024). Forest plots are differentiated according to their degree of damage due to Vitality loss of beech. The inset shows the enlarged part within Germany (highlighted in red) and the federal states that were part of the study (grey: 1, Hesse; 2, Lower Saxony; 3, Thuringia). Resources: © GeoBasis-DE/BKG 2023 for boundaries of Germany and the federal states

drought (Mathes et al. 2024) which leads to a "structural flipp" through the loss of structural complexity in the upper stand layers (Höwler et al. 2024).

Along with the so-called beech bark disease (BBD; Ehrlich 1934) and the complex damage initially caused by infection with *Phytophthora* (Jung 2009; Langer 2019), VLB is the most important complex disease of European beech in Germany (Bressem 2008; Langer 2019). VLB is usually caused by precipitation deficits in combination with high temperatures and strong solar radiation (Asche 2016). Pathogenic bark fungi play a key role in the damage progression of this disease and various wood rot fungi reduce the stability of the affected trees and lead to wood degradation (Bressem 2008; Langer 2019; Langer et al. 2020; Langer and Bußkamp 2021, 2023; Purahong et al. 2021). Some of these fungi, such as *Biscogniauxia nummularia* (Bull.) Kuntze, *Hypoxylon fragiforme* (Pers.) J. Kickx f. or *Neonectria coccinea* (Pers.) Rossman & Samuels, are often already latently present in the host tissue (Langer et al. 2021). Once the host has been sufficiently weakened, these endophytes will then switch to their parasitic phase (Desprez-Loustau et al. 2006; Slippers and Wingfield 2007; Mehl et al. 2013). In the case of *B. nummularia* and *H. fragiforme*, this can result in the development of strip cankers as well as wood rot (Hendry et al. 1998; Nugent et al. 2005; Tropf et al. 2022).

It can be reasonably assumed that drought events will become more prevalent in Europe during the twenty-first century due to climate change (Spinoni et al. 2018; Hari et al. 2020). As a result, VLB will continue to affect large areas of Germany in the future. In order to identify areas at risk, it is essential to have an understanding of the damage process and to be aware of the pathogens involved. While there is some data about the endophyte community of European beech, many studies focus only on leaves, branches or twigs (e.g. Kowalski and Kehr 1992; Unterseher and Schnittler 2009; Ceccarelli 2011; Unterseher et al. 2013; Guerreiro et al. 2018). In addition, the role of endophytes associated with VLB is largely unknown (Langer and Bußkamp 2023). The initial studies on the fungi associated with European beech trees suffering from VLB were conducted by Langer and Bußkamp (2021, 2023) using a culture-based method and by Purahong et al. (2021) through Next-Generation Sequencing.

The aim of this research was to gain a deeper insight into the influence of the European beech fungal endophyte community in VLB. It was analysed how the endophyte community of woody tissues differs between vital and damaged European beech in central Germany and how the endophyte community differs between the different tree compartments. In addition, it was examined which fungal species occurring as pathogens are also isolated from asymptomatic tissue.

Materials and methods

Forest plots

The studied 13 forest plots (Fig. 1) are located in central Germany and distributed in three federal states: Hesse (plots I–IV), Lower Saxony (plots V–X) and Thuringia (plots XI–XIII). Although the aim was to analyse pure stands of European beech to improve comparability between plots, some plots are mixed with other tree species to a certain extent (Table 1), because the search for appropriate plots was more challenging than initially anticipated. Nevertheless, European beech is by far the dominant tree species on all plots. All plots are 0.25 ha in size and were categorised with regard to their damage progress of VLB as "undamaged" (3 plots), "slightly damaged" (3), "damaged" (3) and "severely damaged" (4), depending on the early leaf shedding, dead branches in the crown, wounds on the trunk and the detection of pathogenic fungi and insects in the stands. The plots were located between 180 and 540 m above sea level, and the stand age was between 80 and 190 years. The plots were created as long-term observation plots which means that the development of the crown structure of the trees on the plot and the occurrence of pathogens will be monitored in the long term. In 2022 and 2023, the crowns of the trees were assessed twice a year (summer and winter) using the method according to Eichhorn et al. (2016) and Wellbrock et al. (2020). Additionally, associated pathogens and disease symptoms on the trees were documented. Climatic and site-related data for the plots are shown in Table 1.

Test trees

Since the plots were created as long-term observation plots, no tree was removed from the plot because the structure of the test area was not to be changed during the test period. One tree (*Fagus sylvatica*) per plot was cut down with a chainsaw in the immediate vicinity of each plot which was representative of the degree of damage to the plot. The diameter at breast height (1.3 m above ground) of the test

Table 1 Spatial data and damage class of the 13 forest plots. For mixture of tree species, the tree species are sorted according to the frequency of belonging matured trees on the plot. Deviation temperature and deviation precipitation result from the annual mean values for temperature and precipitation in 2018 minus the corresponding mean value in the reference period from 1961 to 1990. Resources: Data of the forest owners for age; Garmin BaseCamp (v. 4.7.4.) for metres above sea level; Menge et al. (unpublished, suppl. mat. Table 4) for water balance and trophy; Geoportal-hessen.de for Bedrock in Hesse; NIBIS® Kartenserver for bedrock in Lower Saxony; Kartendienst des TLUBN for bedrock in Thuringia; DWD Climate Data Center (CDC) (n.d.a, b, c, d) for deviation temperature and deviation precipitation. *NA*, data not available

ID	Federal state	WGS84	Stand age	Metres above sea level	Water balance	Bedrock (simplified)	Mixture of tree species	Trophy	Deviation temperature (°C)	Deviation precipitation (mm)	Damage class
I	Hesse	N 51° 4.764000 E 8° 43.732020	150	430	Moderately fresh	Greywacke	*Fagus sylvatica, Quercus* sp.	Mesotrophic	2.3	−205	Severely damaged
II	Hesse	N 51° 18.102000 E 9° 52.549980	150	359	Moderately fresh	Dolomite	*Fagus sylvatica, Fraxinus excelsior*	Eutrophic	2.2	−277	Damaged
III	Hesse	N 51° 11.703000 E 9° 33.909000	90	365	Fresh	Middle Old Red Sandstone	*Fagus sylvatica*	Mesotrophic	2.4	−281	Undamaged
IV	Hesse	N 51° 31.957020 E 9° 34.870980	140	297	Fresh	Middle Old Red Sandstone	*Fagus sylvatica*	Mesotrophic	2.4	−276	Slightly damaged
V	Lower Saxony	N 51° 24.894000 E 9° 46.752000	130	421	NA	Shell limestone	*Fagus sylvatica, Carpinus betulus, Acer pseudoplatanus, Acer platanoides, Tilia sp., Quercus sp., Sorbus torminalis, Ulmus sp.*	NA	2.2	−281	Severely damaged
VI	Lower Saxony	N 51° 24.493020 E 9° 47.053980	120	352	NA	Shell limestone	*Fagus sylvatica, Acer pseudoplatanus*	NA	2.2	−286	Severely damaged
VII	Lower Saxony	N 52° 14.499000 E 9° 7.681980	90	202	NA	Slate clay	*Fagus sylvatica, Quercus sp., Pinus sylvestris*	NA	2.2	−286	Undamaged
VIII	Lower Saxony	N 52° 14.203020 E 9° 19.038000	160	232	NA	Limestone	*Fagus sylvatica*	NA	2.2	−305	Damaged
IX	Lower Saxony	N 52° 14.536980 E 9° 19.626000	80	181	NA	Limestone	*Fagus sylvatica*	NA	2.3	−284	Slightly damaged
X	Lower Saxony	N 52° 10.834020 E 9° 20.604000	130	212	NA	Lime sandstone	*Fagus sylvatica*	NA	2.2	−303	Slightly damaged
XI	Thuringia	N 51° 22.057980 E 10° 14.835000	100	496	Moderately fresh	Limestone	*Fagus sylvatica, Acer pseudoplatanus, Fraxinus excelsior*	Eutrophic	2.4	−229	Undamaged

Table 1 (continued)

ID	Federal state	WGS84	Stand age	Metres above sea level	Water balance	Bedrock (simplified)	Mixture of tree species	Trophy	Deviation temperature (°C)	Deviation precipitation (mm)	Damage class
XII	Thuringia	N 51° 21.907980 E 10° 35.589000	190	424	Fresh	Limestone	*Fagus sylvatica, Carpinus betulus*	Meso-eutrophic	2.4	− 217	Severely damaged
XIII	Thuringia	N 50° 31.696020 E 11° 18.112980	NA	543	Moderately fresh	Argillaceous schist	*Fagus sylvatica*	Mesotrophic	2.3	− 228	Damaged

trees was measured before harvesting and tree height after harvesting. Trees were assessed according to Kraft (1884) in their sociological position. After felling, the lying trees were examined intensively for signs of insect or fungal infestation. All test trees were harvested between January and March 2023.

Isolation of fungi

Each tree was sampled equally for asymptomatic tissue. Wood discs (approx. 20–30 cm height) were taken at the root collar, at breast height, at the base of the crown and from three thick branches according to the scheme in Fig. 2. In addition, eight asymptomatic twigs were sampled from each test tree. From the different tree compartments (trunk, branch and twigs), wood chips were taken in equal proportions from the xylem and the cambium area with the exception of the twigs (Table 2). For the sampled twigs, it was not possible to distinguish between xylem and cambium. A total of 192 pieces from the eight twigs were taken from asymptomatic tissue for each test tree. If symptomatic tissue was observed on the test trees, these areas were additionally sampled as required. However, it was not possible to sample symptomatic twigs with the methodology used, as these broke during felling, and it was not possible to assign the twigs on the ground to the test trees. A total of 3999 wood chips and twig pieces from all tree compartments were incubated, with 3432 samples from asymptomatic tissue and 567 from symptomatic tissue (including six isolation attempts from highly decomposed tissue in the vicinity of a fruiting body).

For fungal isolation, the wood discs were surface sterilised with ethanol (70%). The top layer of tissue was then removed with a chisel and a hammer, and the layer below, which was free of contaminations, was incubated. The chisel was sterilised by flame before each use. Three of the 5–10-mm long wood chips were placed in a 90-mm Petri dish containing malt yeast peptone (MYP) agar, modified according to Langer (1994) containing 0.7% malt extract (Merck, Darmstadt, Germany), 0.05% yeast extract (Fluka, Seelze, Germany), 0.1% peptone (Merck) and 1.5% agar (Fluka). For the fungal isolation from twigs, starting from the twig tip, 10 cm of each twig was cut off and immersed in 70% ethanol for 1 min, then for 5 min in 1% sodium hypochlorite (NaOCl) and then again for 1 min in 70% ethanol. Each of the 10 cm long twig sections was then divided into 24 pieces (approx. 0.4 cm per piece) with a sterile scalpel. Thus, this year's growth (2022) was sampled for each twig. If the growth of 2022 was smaller than 10 cm for twigs, the remaining intended pieces were taken from the previous year or the previous year's growth. Like the wood chips, the twig pieces were then transferred to Petri dishes containing MYP. The Petri dishes with the wood chips or twig pieces were

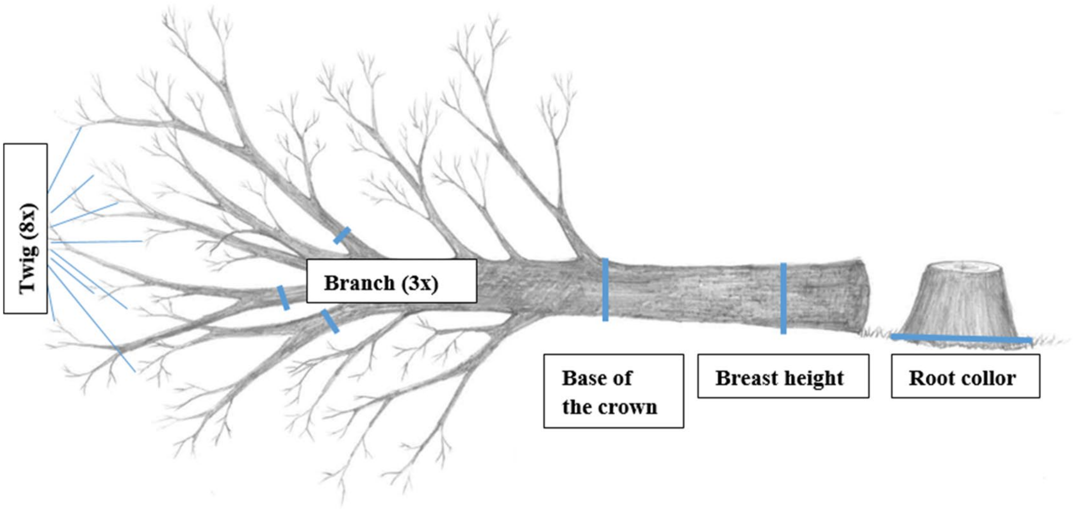

Fig. 2 Schematic representation of a felled test tree and the selected areas (marked in blue) from which samples were taken from asymptomatic tissue of all test trees in a standardised manner. The graphic was designed by Victoria Tropf

Table 2 The number samples taken from asymptomatic tissue of each test tree compartment differentiated according to the different tissue types (xylem and cambium). A categorisation regarding the tissue type was not possible for twigs. ND = not determinable

Compartment	Number of samples taken	Thereof xylem	Thereof cambium	Thereof ND
Root collor (trunk)	18	9	9	0
Breast height (trunk)	18	9	9	0
Base of the crown (trunk)	18	9	9	0
Branch	18	9	9	0
Twig	192	ND	ND	192
Total per individual tree	264	36	36	192
Total for all trees	3432	486	486	2496

incubated at room temperature and out of direct sunlight for 4 weeks. Isolates were checked once a week. Occurring mycelium of filamentous fungi was brought into pure culture. The pure cultures were grouped into morphotypes (MTs) based on the similarity of culture morphology. At least one culture of each MT was stored in cryopreservation (− 80 °C) at the fungal culture collection of the Northwest German Forest Research Institute (NW-FVA). In addition to fungi that could be assigned to a MT, fungi that were terminated or overgrown by other fungi and thus could not be brought into pure culture were summarised under “Fungus sp.”.

Molecular analysis

At least one representative strain from each MT was chosen for molecular analysis. For the extraction of genomic DNA, a cetyltrimethylammonium bromide (CTAB) protocol was applied as follows. Mycelium was taken from the pure cultures and placed in 1.5-ml Eppendorf tubes with eight glass beads (3 mm) and 100 µl of CTAB buffer (2% w/v CTAB, 1.5 mol/l NaCl, 100 mmol/l Tris HCl (pH 0.8), 50 mmol/l EDTA; Carl Roth, Karlsruhe, Germany) and crushed in a Mixer Mill Star-Beater (VWR, Darmstadt, Germany) with 30 Hz for 180 s. An additional 500 µl of CTAB buffer was added, and tubes were placed in a water bath at 65 °C for 30 min. Subsequently, 400 µl chloroform:isoamylalcohol (24:1) was added, and the fungal material was centrifuged down for 5 min at 15 800 × g. The aqueous supernatant was transferred to new tubes filled with 600 µl cold (− 20 °C) isopropanol. After incubation for 15 min and a further centrifugation step, the supernatant was discarded, and the pellets were washed twice with 300 µl 70% ethanol. DNA pellets were dried and resuspended in 100 µl deionised H_2O.

The 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers ITS-1 and ITS-2 (ITS region) was amplified for all strains using the primer pair ITS-1F (Gardes and Bruns 1993) and ITS-4 (White et al. 1990). For a selection of fungal groups based on MT grouping (Table 3), a partial sequence of the 28S nrDNA (LSU) was amplified using the primer pair LROR (Rehner and Samuels 1994) + LR5 (Vilgalys and Hester 1990). Furthermore, additional DNA

Table 3 Isolated morphotypes. Morphotypes marked with ● were determined with further primers in addition to ITS-1F and ITS-4. *X*, xylem; *C*, cambium; *F*, decomposed tissue in the vicinity of a fruiting body; *n* is the number of incubated tissue samples and is shown under the respective compartment/tissue type. For morphotypes that could be determined at species level, a reference where the fungus species was found previously on European beech is listed under Associated with *Fagus sylvatica*. *FRB*, first report on European beech; *FRG*, first report in Germany. References or first reports of morphotypes that could only be determined tentatively (cf.) are marked with *

Morphotype	Order	NW-FVA-ID	NCBI-accession Nr.	Isolated on *n*/ plots	Number of isolates										In total	Associated with *Fagus sylvatica*
					Healthy (*n* = 3432)					Symptomatic (*n* = 567)						
					Trunk (*n* = 702)		Branch (*n* = 234)		Twigs *n* = 2496	Trunk (*n* = 264)		Branch (*n* = 303)				
					X *n* = 351	C *n* = 351	X *n* = 117	C *n* = 117		X *n* = 242	C *n* = 22	X *n* = 127	C *n* = 170	F *n* = 6		
Ascomycota																
Alborbis galericulata	*Diaporthales*	10730	PP960607	2/13	0	0	0	0	2	0	0	0	0	0	2	Senanayake et al. 2018
Alternaria cf. *infectoria*	*Pleosporales*	10007	PP960608	10/13	0	5	0	1	24	0	0	0	1	0	31	Langer and Bußkamp 2023*
Alternaria sp. 1	*Pleosporales*	10012	PP960609	11/13	0	4	0	0	18	0	0	0	1	0	23	
Alternaria sp. 2	*Pleosporales*	10144	PP960610	3/13	0	0	0	0	2	0	1	0	1	0	4	
Amphisphaeria fuckelii	*Amphisphaeriales*	10659	PP960611	1/13	0	0	0	0	1	0	0	0	0	0	1	Langer and Bußkamp 2021
Angustimassarina sp. 1	*Pleosporales*	10197	PP960612	2/13	0	0	0	0	7	0	0	0	0	0	7	
Angustimassarina sp. 2	*Pleosporales*	10243	PP960613	4/13	0	0	0	0	10	0	0	0	0	0	10	
Angustimassarina sp. 3	*Pleosporales*	10285	PP960614	1/13	0	0	0	0	3	0	0	0	0	0	3	
Apiognomonia errabunda	*Diaporthales*	9999	PP960615	13/13	0	0	0	0	386	0	0	0	4	0	390	Langer and Bußkamp 2023
Apiognomonia hystrix	*Diaporthales*	10561	PP960616	2/13	0	0	0	0	2	0	0	0	0	0	2	Monod 1983
Ascobolus cf. *crenulatus*●	*Pezizales*	10570	PP960619	2/13	0	0	0	0	2	0	0	0	0	0	2	FRB*
Ascobolus sp.	*Pezizales*	10140	PP960620	1/13	0	0	0	0	2	0	0	0	0	0	2	
Ascomycota A1●		10543	PP960788	7/13	0	0	0	0	38	0	0	0	0	0	38	
Ascomycota A2		10550	PP960622	1/13	0	0	0	0	2	0	0	0	0	0	2	
Ascomycota B1●		10450	PP960790	2/13	0	0	0	0	24	0	0	0	0	0	24	
Ascomycota B2●		10703	PP960624	2/13	0	0	0	0	2	0	0	0	0	0	2	
Ascomycota C●		10376	PP960625	2/13	0	0	0	0	15	0	0	0	0	0	15	
Ascomycota D●		10545	PP960626	1/13	0	0	0	0	1	0	0	0	0	0	1	

Table 3 (continued)

Morphotype	Order	NW-FVA-ID	NCBI-accession Nr.	Isolated on n/plots	Number of isolates										In total	Associated with *Fagus sylvatica*
					Healthy ($n=3432$)					Symptomatic ($n=567$)						
					Trunk ($n=702$)		Branch ($n=234$)		Twigs $n=2496$	Trunk ($n=264$)		Branch ($n=303$)				
					X $n=351$	C $n=351$	X $n=117$	C $n=117$		X $n=242$	C $n=22$	X $n=127$	C $n=170$	F $n=6$		
Aspergillus inflatus	*Eurotiales*	10704	PP960627	1/13	1	0	0	0	0	0	0	0	0	0	1	FRB; FRG
Asterosporium asterospermum	*Diaporthales*	9983	PP960628	4/13	1	2	1	1	1	0	0	0	1	0	7	Langer and Bußkamp 2023
Aureobasidium pullulans	*Dothideales*	10005	PP960629	13/13	0	5	0	2	164	2	0	0	1	0	174	Sieber and Hugentobler 1987
Beauveria bassiana	*Hypocreales*	10206	PP960631	3/13	1	1	0	0	0	0	0	1	0	0	3	Unterseher et al. 2013
Bionectriaceae sp.	*Hypocreales*	10292	PP960632	1/13	0	1	0	0	0	0	0	0	0	0	1	
Biscogniauxia mediterranea	*Xylariales*	10112	PP960633	8/13	0	0	0	0	55	0	0	0	0	0	55	Langer and Bußkamp 2023
Biscogniauxia nummularia	*Xylariales*	9991	PP960634	13/13	4	20	0	6	467	3	1	1	13	0	515	Langer and Bußkamp 2023
Brunnipila cf. *fuscescens*	*Helotiales*	10452	PP960636	1/13	0	0	0	0	2	0	0	0	0	0	2	Suková, 2005*
Cadophora cf. *malorum*	*Helotiales*	10459	PP960637	1/13	1	1	0	0	0	0	0	1	0	0	3	Langer and Bußkamp 2023*
Calosporella innesii	*Diaporthales*	10656	PP960638	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB
Chaetomium sp.	*Sordariales*	10590	PP960639	1/13	0	0	0	0	1	0	0	0	0	0	1	
Cheirospora botryospora	*Helotiales*	10296	PP960640	2/13	0	0	0	0	2	0	0	0	0	0	2	Crous et al. 2015
Chromelosporiopsis carnea	*Pezizales*	10256	PP960641	2/13	0	0	0	0	3	0	0	0	0	0	3	Hennebert 2020
Cladosporium spp.	*Cladosporiales*	10081	PP960642	12/13	0	6	1	2	45	1	0	1	4	0	60	
Coniochaeta cf. *hoffmannii*	*Coniochaetales*	10612	PP960643	1/13	0	0	0	0	1	0	0	0	0	0	1	Ceccarelli 2011*
Coniochaeta cf. *velutina*	*Coniochaetales*	10223	PP960644	1/13	0	0	0	0	0	1	0	0	0	0	1	Unterseher and Schnittler 2010*

Table 3 (continued)

Morphotype	Order	NW-FVA-ID	NCBI-accession Nr.	Isolated on *n*/plots	Number of isolates										In total	Associated with *Fagus sylvatica*
					Healthy ($n=3432$)					Symptomatic ($n=567$)						
					Trunk ($n=702$)		Branch ($n=234$)		Twigs $n=2496$	Trunk ($n=264$)		Branch ($n=303$)				
					X $n=351$	C $n=351$	X $n=117$	C $n=117$		X $n=242$	C $n=22$	X $n=127$	C $n=170$	F $n=6$		
Coniochaeta sp.	*Coniochaetales*	10468	PP960645	1/13	0	1	0	0	0	0	0	0	0	0	1	
Coniothyrium ferrarisianum	*Pleosporales*	10093	PP960646	6/13	0	0	0	0	17	0	0	0	0	0	17	FRB
Cytospora cf. *cotini*●	*Diaporthales*	10583	PP960650	1/13	0	1	0	0	0	0	0	0	0	0	1	FRB; FRG*
Cytospora cf. *galegicola*●	*Diaporthales*	10249	PP980742	9/13	0	2	0	0	7	0	0	0	6	0	15	FRB; FRG*
Cytospora cf. *personata*●	*Diaporthales*	10311	PP960652	1/13	0	0	0	1	1	0	0	0	0	0	2	FRB*
Diaporthe eres Group A	*Diaporthales*	10710	PP960653	1/13	0	0	0	0	3	0	0	0	0	0	3	Langer and Bußkamp 2023
Diaporthe eres Group B	*Diaporthales*	10268	PP960654	2/13	0	0	0	0	2	0	0	0	0	0	2	
Diaporthe rudis Group A	*Diaporthales*	10332	PP960655	4/13	0	0	0	0	5	0	0	0	0	0	5	Udayanga et al. 2014
Diaporthe rudis Group B	*Diaporthales*	10708	PP960656	1/13	0	0	0	0	1	0	0	0	0	0	1	Udayanga et al. 2014
Diaporthe rudis Group C	*Diaporthales*	10310	PP960657	1/13	0	0	0	0	1	0	0	0	0	0	1	
Diaporthe rudis Group D	*Diaporthales*	10275	PP960658	2/13	0	0	0	0	6	0	0	0	0	0	6	
Diaporthe sp. 1●	*Diaporthales*	10091	PP960659	6/13	0	0	0	0	64	0	0	0	0	0	64	
Diaporthe sp. 2	*Diaporthales*	10271	PP960660	1/13	0	0	0	0	8	0	0	0	0	0	8	
Diatrype stigma s.l.	*Xylariales*	10128	PP960661	1/13	0	0	0	0	0	0	0	0	1	0	1	Langer and Bußkamp 2023
Didymella cf. *macrostoma*	*Pleosporales*	10013	PP960662	3/13	0	1	0	0	2	0	0	0	1	0	4	Griffith and Boddy 1990*
Didymella cf. *pinodella*	*Pleosporales*	10559	PP960663	2/13	0	0	0	0	3	0	0	0	0	0	3	FRB*
Didymellaceae sp.	*Pleosporales*	10623	PP960664	1/13	0	1	0	0	0	0	0	0	0	0	1	
Didymosphaeria variabile	*Pleosporales*	10587	PP960665	1/13	0	0	0	0	5	0	0	0	0	0	5	FRB; FRG

Table 3 (continued)

Morphotype	Order	NW-FVA-ID	NCBI-accession Nr.	Isolated on *n*/plots	Number of isolates										In total	Associated with *Fagus sylvatica*
					Healthy (*n* = 3432)					Symptomatic (*n* = 567)						
					Trunk (*n* = 702)		Branch (*n* = 234)		Twigs *n* = 2496	Trunk (*n* = 264)		Branch (*n* = 303)				
					X *n* = 351	C *n* = 351	X *n* = 117	C *n* = 117		X *n* = 242	C *n* = 22	X *n* = 127	C *n* = 170	F *n* = 6		
Diplodia fraxini●	*Botryosphaeriales*	10624	PP960666	1/13	0	1	0	0	0	0	0	0	0	0	1	FRB
Diplodia mutila	*Botryosphaeriales*	10154	PP960667	2/13	0	1	0	0	0	0	0	0	2	0	3	Langer and Bußkamp 2023
Ditopella ditopa	*Diaporthales*	10288	PP960668	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB
Dothideales sp. 1	*Dothideales*	10344	PP960669	2/13	0	0	0	0	4	0	0	0	0	0	4	
Dothideales sp. 2	*Dothideales*	10407	PP960670	2/13	0	0	0	0	7	0	0	0	0	0	7	
Dothiorella iberica	*Botryosphaeriales*	10126	PP960671	2/13	1	1	0	0	0	0	0	0	2	0	4	FRB
Dothiorella sarmentorum	*Botryosphaeriales*	10461	PP960672	1/13	0	1	0	0	0	0	0	0	0	0	1	FRB
Epicoccum italicum	*Pleosporales*	10355	PP960673	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB; FRG
Epicoccum nigrum	*Pleosporales*	10099	PP960674	13/13	0	11	0	1	28	0	1	0	10	0	51	Ceccarelli 2011
Eutypa maura	*Xylariales*	10216	PP960675	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB
Eutypa petrakii	*Xylariales*	10255	PP960676	1/13	0	0	0	0	0	2	1	0	0	0	3	FRB
Eutypa spinosa	*Xylariales*	10668	PP960677	1/13	1	0	0	0	0	0	0	0	0	0	1	Langer and Bußkamp 2023
Eutypella quaternata	*Xylariales*	10121	PP960678	1/13	0	0	0	0	0	0	0	0	1	0	1	Langer and Bußkamp 2023
Fenestella sp.	*Pleosporales*	10260	PP960680	1/13	0	0	0	0	1	0	0	0	0	0	1	
Fusarium avenaceum	*Hypocreales*	10075	PP960682	4/13	0	3	0	0	4	0	0	0	0	0	7	Mańka et al. 2012
Fusarium cf. *acuminatum*	*Hypocreales*	10643	PP960683	1/13	0	0	0	0	1	0	0	0	0	0	1	Stępniewska et al. 2021*
Fusarium cf. *solani*	*Hypocreales*	10118	PP960684	2/13	0	1	0	0	0	0	0	0	1	0	2	Orlikowski et al. 2004*
Fusarium sp.	*Hypocreales*	10503	PP960685	3/13	0	0	0	0	5	0	0	0	2	0	7	

Table 3 (continued)

Morphotype	Order	NW-FVA-ID	NCBI-accession Nr.	Isolated on *n*/plots	Number of isolates										In total	Associated with *Fagus sylvatica*
					Healthy (*n* = 3432)					Symptomatic (*n* = 567)						
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					X *n* = 351	C *n* = 351	X *n* = 117	C *n* = 117		X *n* = 242	C *n* = 22	X *n* = 127	C *n* = 170	F *n* = 6		
Geoscypha tenacella Group A●	*Pezizales*	10193	PP960686	4/13	0	0	0	0	4	0	0	0	0	0	4	FRB
Geoscypha tenacella Group B●	*Pezizales*	10439	PP960687	1/13	0	0	0	0	1	0	0	0	0	0	1	
Gibellulopsis nigrescens	*Trichosphaeriales*	10734	PP960688	1/13	0	0	0	0	0	0	0	0	1	0	1	FRB
Gnomoniaceae sp. 1	*Diaporthales*	9994	PP960689	1/13	0	0	0	0	1	0	0	0	0	0	1	
Gnomoniaceae sp. 2	*Diaporthales*	10554	PP960690	1/13	0	0	0	0	1	0	0	0	0	0	1	
Gnomoniopsis paraclavulata	*Diaporthales*	10074	PP960691	2/13	0	0	0	0	2	0	0	0	0	0	2	FRB; FRG
Hypoxylon fragiforme	*Xylariales*	9985	PP960693	4/13	0	3	0	1	4	0	1	0	0	0	9	Langer and Bußkamp 2023
Hypoxylon rubiginosum	*Xylariales*	10469	PP960694	1/13	0	1	0	0	0	0	0	0	0	0	1	Langer and Bußkamp 2021
Ilyonectria crassa	*Hypocreales*	9982	PP960695	1/13	1	0	0	0	0	0	0	0	0	0	1	Jankowiak et al. 2016
Iodophanus carneus●	*Pezizales*	10096	PP960696	4/13	0	0	0	0	5	0	0	0	0	0	5	FRB
Jackrogersella cohaerens	*Xylariales*	9998	PP960698	2/13	0	2	0	1	0	0	0	0	0	0	3	Langer and Bußkamp 2023
Lachnaceae sp.	*Helotiales*	10286	PP960699	1/13	0	0	0	0	1	0	0	0	0	0	1	
Lopadostoma turgidum	*Xylariales*	10715	PP960700	2/13	2	0	0	0	1	1	0	0	0	0	4	Vasilyeva and Scheuer 1996
Melanconiella chrysodiscosporina	*Diaporthales*	10651	PP960701	1/13	0	0	0	0	5	0	0	0	0	0	5	Senanayake et al. 2017
Melanconiella chrysomelanconium	*Diaporthales*	10698	PP960702	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB
Melanconiella hyperopta	*Diaporthales*	10515	PP960703	1/13	0	0	0	0	8	0	0	0	0	0	8	FRB

Table 3 (continued)

Morphotype	Order	NW-FVA-ID	NCBI-accession Nr.	Isolated on *n*/ plots	Number of isolates										In total	Associated with *Fagus sylvatica*
					Healthy (*n* = 3432)					Symptomatic (*n* = 567)						
					Trunk (*n* = 702)		Branch (*n* = 234)		Twigs *n* = 2496	Trunk (*n* = 264)		Branch (*n* = 303)				
					X *n* = 351	C *n* = 351	X *n* = 117	C *n* = 117		X *n* = 242	C *n* = 22	X *n* = 127	C *n* = 170	F *n* = 6		
Melanops fagicola	*Botryosphaeriales*	10732	PP960704	3/13	0	0	0	0	11	0	0	0	0	0	11	Li et al. 2020; FRG
Metapochonia suchlasporia	*Hypocreales*	10239	PP960705	1/13	0	0	0	1	0	0	0	0	0	0	1	FRB; FRG
Microsphaeropsis olivacea	*Pleosporales*	10089	PP960706	6/13	0	0	0	1	10	0	0	0	0	0	11	Langer and Bußkamp 2023
Nectria dematiosa●	*Hypocreales*	10393	PP960707	2/13	0	1	0	0	1	0	0	0	0	0	2	FRB
Nectria nigrescens	*Hypocreales*	10252	PP960708	1/13	0	0	0	0	0	1	0	0	0	0	1	Langer and Bußkamp 2023
Nectriaceae sp.	*Hypocreales*	10208	PP960709	1/13	0	1	0	0	0	0	0	0	0	0	1	
Nemania diffusa	*Xylariales*	10110	PP960710	2/13	0	0	0	0	4	0	0	0	0	0	4	Unterseher and Peršoh 2013
Nemania serpens	*Xylariales*	10250	PP960711	6/13	0	1	0	0	9	0	0	0	0	0	10	Unterseher et al. 2013
Nemania sp.	*Xylariales*	10084	PP960712	1/13	0	0	0	0	1	0	0	0	0	0	1	
Neoascochyta sp.	*Pleosporales*	10440	PP960713	1/13	0	0	0	0	1	0	0	0	0	0	1	
Neocatenulostroma cf. *germanicum*	*Capnodiales*	10447	PP960714	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB*
Neocucurbitaria cava	*Pleosporales*	10514	PP960715	3/13	0	1	0	0	3	0	0	0	0	0	4	Ceccarelli 2011
Neocucurbitaria cf. *vachelliae*	*Pleosporales*	10124	PP960716	4/13	1	4	0	0	0	0	0	1	1	0	7	FRB; FRG*
Neocucurbitaria sp.	*Pleosporales*	10284	PP960717	2/13	0	1	0	0	1	0	0	0	0	0	2	
Neohendersonia kickxii	*Pleosporales*	10219	PP960718	5/13	0	1	0	1	7	0	0	0	0	0	9	Langer and Bußkamp 2023
Neonectria coccinea	*Hypocreales*	9988	PP960719	12/13	2	19	0	4	6	3	5	2	37	0	78	Langer and Bußkamp 2023
Nigrograna sp.	*Pleosporales*	9990	PP960720	1/13	1	0	0	0	0	0	0	0	0	0	1	
Nigrospora sp.	*Xylariales*	10201	PP960721	1/13	0	0	0	0	1	0	0	0	0	0	1	
Nothophoma sp.	*Pleosporales*	10352	PP960722	2/13	0	0	0	0	3	0	0	0	0	0	3	

Table 3 (continued)

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					X *n* = 351	C *n* = 351	X *n* = 117	C *n* = 117		X *n* = 242	C *n* = 22	X *n* = 127	C *n* = 170	F *n* = 6		
Paracamarosporium fagi	*Pleosporales*	10198	PP960723	1/13	0	0	0	0	0	0	0	1	0	0	1	Crous et al. 2015
Paraphaeosphaeria sp.	*Pleosporales*	10982	PP960724	1/13	0	0	0	0	3	0	0	0	0	0	3	
Paraphaeosphaeria sporulosa	*Pleosporales*	10397	PP960725	1/13	0	1	0	0	0	0	0	0	0	0	1	FRB; FRG
Penicillium spp.	*Eurotiales*	10082	PP960726	7/13	1	4	0	0	1	2	0	0	3	1	12	
Pestalotiopsis sp.	*Amphisphaeriales*	10262	PP960727	1/13	0	0	0	1	0	0	0	0	0	0	1	
Petrakia irregularis●	*Pleosporales*	10657	PP960728	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB
Pezicula cf. *neocinnamomea*	*Helotiales*	9987	PP960729	4/13	0	1	0	0	5	0	0	0	0	0	6	Chen et al. 2016*
Pezicula sporulosa●	*Helotiales*	10616		3/13	0	0	0	0	18	0	0	0	0	0	18	FRB
Pezicula fagacearum●	*Helotiales*	10525	PP960731	2/13	0	0	0	0	3	0	0	0	0	0	3	Chen et al. 2016; FRG
Peziza cf. *arvernensis*●	*Pezizales*	10146	PP960732	4/13	0	0	0	0	11	0	0	0	0	0	11	Hansen et al. 2002*
Peziza cf. *pseudovesiculosa*●	*Pezizales*	10138	PP960733	2/13	0	0	0	0	3	0	0	0	0	0	3	FRB*
Peziza sp. 3●	*Pezizales*	10437	PP960734	1/13	0	0	0	0	1	0	0	0	0	0	1	
Peziza subvesiculosa●	*Pezizales*	10011	PP960735	2/13	0	2	0	0	4	0	0	0	0	0	6	FRB; FRG
Peziza varia●	*Pezizales*	10123	PP960736	2/13	0	0	0	0	6	0	0	0	0	0	6	Hansen et al. 2002
Peziza vesiculosa	*Pezizales*	10636	PP960737	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB
Pezizomycetes sp. 2●		10316	PP980743	6/13	0	0	0	0	11	0	0	0	0	0	11	
Pezizomycetes sp. 4●		10109	PP960739	1/13	0	0	0	0	1	0	0	0	0	0	1	

Table 3 (continued)

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					X *n* = 351	C *n* = 351	X *n* = 117	C *n* = 117		X *n* = 242	C *n* = 22	X *n* = 127	C *n* = 170	F *n* = 6		
Phoma cf. *herbarum*	*Pleosporales*	10158	PP960742	1/13	0	1	0	1	0	0	0	0	0	0	2	FRB*
Phoma sp. 1	*Pleosporales*	10098	PP960743	5/13	0	0	1	0	23	0	0	0	0	0	24	
Phoma sp. 3	*Pleosporales*	11068	PP960744	2/13	1	0	0	0	16	0	0	0	0	0	17	
Phoma sp. 4	*Pleosporales*	10595	PP960745	1/13	0	0	0	0	1	0	0	0	0	0	1	
Plagiostoma apiculatum	*Diaporthales*	9996	PP960746	3/13	0	0	0	0	4	0	0	0	0	0	4	FRB
Plagiostoma dilatatum	*Diaporthales*	10661	PP960747	2/13	0	0	0	0	2	0	0	0	0	0	2	FRB; FRG
Plagiostoma pulchellum	*Diaporthales*	10568	PP960748	2/13	0	0	0	0	2	0	0	0	0	0	2	FRB; FRG
Pleosporales sp. 1	*Pleosporales*	10194	PP960749	1/13	1	0	0	1	0	0	0	0	0	0	2	
Pleosporales sp. 2	*Pleosporales*	10281	PP960750	1/13	0	0	0	0	1	0	0	0	0	0	1	
Pleosporales sp. 3	*Pleosporales*	10090	PP960751	1/13	0	0	0	0	1	0	0	0	0	0	1	
Pleosporales sp. 4	*Pleosporales*	9993	PP960752	5/13	0	0	0	0	6	0	0	0	0	0	6	
Pleosporales sp. 5	*Pleosporales*	10638	PP960753	1/13	0	0	0	0	1	0	0	0	0	0	1	
Pleosporales sp. 7	*Pleosporales*	9984	PP960754	8/13	1	4	0	2	1	0	0	0	1	0	9	
Pleosporales sp. 8	*Pleosporales*	10215	PP960755	1/13	0	0	0	0	1	0	0	0	0	0	1	
Preussia sp.	*Pleosporales*	10618	PP960757	1/13	0	0	0	0	1	0	0	0	0	0	1	
Prosthecium platanoidis	*Diaporthales*	10731	PP960758	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB
Pseudocamarosporium brabeji	*Pleosporales*	10261	PP960759	2/13	0	0	0	1	2	0	0	0	0	0	3	FRB
Pseudopithomyces chartarum	*Pleosporales*	10236	PP960760	1/13	0	0	0	0	0	0	0	0	1	0	1	FRB

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Querciphoma minuta	*Pleosporales*	10147	PP960761	1/13	0	0	0	0	0	0	0	0	3	0	3	FRB
Rhizodermea veluwensis	*Helotiales*	10228	PP960762	1/13	0	1	0	0	0	0	0	0	0	0	1	FRB
Saccotheciaceae sp.	*Dothideales*	10149	PP960763	1/13	0	0	0	0	1	0	0	0	0	0	1	
Seimatosporium sp.	*Amphisphaeriales*	10088	PP960764	1/13	0	0	0	0	1	0	0	0	0	0	1	
Septoriella muriformis	*Pleosporales*	10130	PP960765	2/13	0	0	0	0	0	0	0	0	2	0	2	FRB; FRG
Sordaria sp.	*Sordariales*	10135	PP960766	5/13	0	1	0	0	10	0	0	0	0	0	11	
Sordariomycetes sp. 1		10526	PP960767	1/13	0	0	0	0	1	0	0	0	0	0	1	
Sordariomycetes sp. 2		10404	PP960768	1/13	0	0	0	0	1	0	0	0	0	0	1	
Sporormiella cf. *intermedia*	*Pleosporales*	10676	PP960769	1/13	0	0	0	0	1	0	0	0	0	0	1	Sieber and Hugentobler 1987*
Sporormiella cf. *minima*	*Pleosporales*	10157	PP960770	9/13	0	0	0	0	35	1	0	0	0	0	36	Sieber and Hugentobler 1987*
Stegonsporium pseudopyriforme●	*Diaporthales*	10283	PP960771	1/13	0	0	1	0	0	0	0	0	0	0	1	FRB; FRG
Stemphylium vesicarium	*Pleosporales*	10209	PP960772	1/13	0	1	0	0	0	0	0	0	0	0	1	Ceccarelli 2011
Sydowia polyspora	*Dothideales*	10297	PP960773	4/13	0	0	0	0	8	0	0	0	0	0	8	Mułenko et al. 2008
Thyridariaceae sp.	*Pleosporales*	10072	PP960774	1/13	0	0	0	0	0	1	0	0	0	0	1	
Thyridium sp.	Incertae sedis	10465	PP960775	1/13	0	0	0	0	1	0	0	0	0	0	1	
Tolypocladium sp. 1	*Hypocreales*	9995	PP960776	3/13	0	0	0	1	0	0	0	0	2	0	3	

Table 3 (continued)

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Tolypocladium sp. 2	*Hypocreales*	10378	PP960777	6/13	1	6	0	0	0	0	0	0	1	0	8	
Tricharina cf. *gilva*●	*Pezizales*	10077	PP960778	2/13	0	0	0	0	2	0	0	0	0	0	2	FRB*
Tricharina cf. *tophiseda*●	*Pezizales*	10101	PP960779	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB; FRG*
Tricharina sp.	*Pezizales*	10633	PP960780	1/13	0	0	0	0	1	0	0	0	0	0	1	
Trichoderma spp.	*Hypocreales*	10076	PP960781	9/13	1	11	0	1	5	25	0	0	5	5	53	
Ustulina deusta	*Xylariales*	10294	PP960782	2/13	1	1	0	0	0	0	0	0	0	0	2	Langer and Bußkamp 2023
Xenocylindrosporium sp.	Incertae sedis	10517	PP960783	1/13	0	0	0	0	1	0	0	0	0	0	1	
Xenoseimatosporium cf. *quercinum*	*Amphisphaeriales*	10564	PP960784	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB*
Xylaria ellisii●	*Xylariales*	10111	PP960785	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB; FRG
Xylaria polymorpha	*Xylariales*	10229	PP960786	1/13	0	1	0	0	0	0	0	0	0	0	1	Arnolds et al. 1994
Xylariaceae sp.	*Xylariales*	10635	PP960787	1/13	0	0	0	0	1	0	0	0	0	0	1	
Basidiomycota																
Apiotrichum porosum	*Trichosporonales*	10203	PP960617	1/13	1	0	0	0	0	0	0	0	0	0	1	FRB
Armillaria gallica●	*Agaricales*	10458	PP960618	1/13	0	0	0	0	0	3	0	0	0	0	3	Gminder et al. 2001
Auricularia auricula-judae	*Auriculariales*	10680	PP960630	1/13	0	0	0	0	0	1	0	0	0	0	1	Krieglsteiner and Kaiser 2000
Bjerkandera adusta	*Polyporales*	10578	PP960635	2/13	0	2	0	0	0	0	0	0	0	0	2	Krieglsteiner and Kaiser 2000
Coprinellus disseminatus	*Agaricales*	10572	PP960647	1/13	0	0	0	0	0	0	1	0	0	0	1	Gminder 2010

Table 3 (continued)

Morphotype	Order	NW-FVA-ID	NCBI-accession Nr.	Isolated on *n*/plots	Number of isolates										In total	Associated with *Fagus sylvatica*
					Healthy ($n = 3432$)					Symptomatic ($n = 567$)						
					Trunk ($n = 702$)		Branch ($n = 234$)		Twigs $n = 2496$	Trunk ($n = 264$)		Branch ($n = 303$)				
					X $n = 351$	C $n = 351$	X $n = 117$	C $n = 117$		X $n = 242$	C $n = 22$	X $n = 127$	C $n = 170$	F $n = 6$		
Coprinellus micaceus	*Agaricales*	10078	PP960648	3/13	0	3	0	1	0	0	0	0	0	0	4	Gminder 2010
Coprinellus sp.	*Agaricales*	10374	PP960649	1/13	0	0	0	0	0	0	0	0	1	0	1	
Exidia glandulosa	*Auriculariales*	10529	PP960679	1/13	0	0	0	0	0	0	0	1	0	0	1	Krieglsteiner and Kaiser 2000
Fomes fomentarius	*Polyporales*	10523	PP960681	1/13	0	0	0	0	0	2	0	0	0	0	2	Krieglsteiner and Kaiser 2000
Hypholoma fasciculare	*Agaricales*	10398	PP960692	1/13	0	1	0	0	0	0	0	0	0	0	1	Gminder et al. 2003
Ischnoderma resinosum	*Polyporales*	10527	PP960697	1/13	0	0	0	0	2	0	0	0	0	0	2	Bernicchia et al. 2007
Phlebia sp.●	*Polyporales*	10276	PP960740	1/13	0	0	0	0	0	1	0	0	0	0	1	
Pholiota aurivella	*Agaricales*	10524	PP960741	1/13	0	0	0	0	0	2	0	0	0	0	2	Gminder et al. 2003
Pleurotus ostreatus●	*Agaricales*	10460	PP960756	1/13	0	0	0	0	0	1	0	0	0	0	1	Langer and Bußkamp 2023

regions were amplified for a more precise taxonomic classification of selected taxa. The respective primer pairs and PCR conditions are given in suppl. mat. Table 1. The mixture for all PCR reactions consisted of 1 µl of DNA and 19 µl mastermix which contained 2.5 µl 10 × PCR reaction buffer (with 20 mmol/l $MgCl_2$, Carl Roth, Karlsruhe, Germany), 1 µl of each primer (10 mmol/l), 2.5 µl $MgCl_2$ (25 mmol/l), 0.1 µl Roti®-Pol Taq HY Taq polymerase (Carl Roth, Karlsruhe, Germany) and 2.5 µl of 2 mmol/l dNTPs (Biozym Scientific GmbH, Hessisch Oldendorf, Germany). Each reaction was topped up to a volume of 20 µl by adding HPLC Water (Carl Roth, Karlsruhe, Germany). A StepOnePlus™ PCR System (Applied Biosystems, Waltham, MA, USA) or a GeneExplorer 96 (Hangzhou BIOER Technology, Hangzhou, China) was used to carry out the DNA amplifications. The PCR conditions for the amplification of the ITS and LSU regions were set according to Bien et al. (2020) and Paulin and Harrington (2000), respectively. After visualisation in 1% agarose gel, PCR products were sent to Eurofins Scientific Laboratory (Ebersberg, Germany) for sequencing. From all resulting sequences, consensus sequences were generated, visually checked and edited if necessary using BioEdit Sequence Alignment Editor (v. 7.2.5; Hall 1999). Sequences were submitted to GenBank (Table 3).

Identification of fungi

The analysis was restricted to fungi of the subkingdom *Dikarya*. Morphotype assignment based on morphology as stated above was supplemented by DNA information of representative strains and adjusted where necessary following Guo et al. (2000). For the taxonomic classification, ITS sequences were used in blastn searches on the GenBank database (http://www.ncbi.nlm.nih.gov/genbank, Altschul et al. 1997). In case of an inconclusive blastn result (e.g. low percentage identity, different taxa with a similar degree of agreement), the identification was labelled cf. (confer) to imply a certain degree of uncertainty. Cases of higher uncertainty were determined at the next higher conclusive taxonomic level. Results were confirmed based on literature and previously identified cultures from the institute's collection. Additional DNA loci were sequenced for a variety of MTs (see Table 3 and suppl. mat. Table 2) to improve the clarity of the respective taxonomic classification based on blastn searches and phylogenetic analyses. Phylogenetic analyses including appropriate reference sequences retrieved from GenBank were performed using RAxML v. 8.2.11 (Stamatakis 2006, 2014) as implemented in Geneious R11 (Kearse et al. 2012) using the GTRGAMMA model with the rapid bootstrapping and search for best scoring ML tree algorithm including 1000 bootstrap replicates (data not shown).

The current nomenclature of the isolated fungi was followed according to Mycobank (Robert et al. 2005), with one exception. Here, we refer to *Armillaria gallica* Marxm. & Romagn in contrast to *Armillaria lutea* Gillet which is listed in MycoBank as currently applied name. According to Marxmüller (1992), the latter is a nomen ambiguum, and the later introduced name *A. gallica* Marxm. & Romagn should be used instead (Burdsall and Volk 1993).

Literature analysis

In order to check whether MTs that could be determined at species level had already been described on European beech, 44 publications and books dealing with fungi found on European beech were automatically analysed using the Python CLI tool from Tropf and Tropf (2024) (output in suppl. mat. Table 3). Species that could only be tentatively identified (cf.) were also included. For species for which there were no matches using the current names from Mycobank, all synonyms and basionyms were also searched for. Fungal species that were not found in European beech in the 44 publications were checked in the USDA fungal database (https://fungi.ars.usda.gov/). Fungi that were neither documented on European beech in the 44 publications nor in the USDA database were searched additionally in publications focusing on the taxonomy and phylogeny of these fungi and less on the host European beech. The authors assume that a respective fungal species has not yet been described on European beech if there is no evidence linking it to this particular host species. Otherwise, one representative source was provided for each fungal species (Table 3), with current sources and sources with Germany as the study area being favoured. A previously published documentation of fruiting bodies of a particular species on European beech was recognised as confirmation of this species.

Data analyses

Data was analysed in RStudio (v. 4.4.1) using the packages "ggplot2" (Wickham 2016), "ggpattern" (FC et al. 2022), "eulerr" (Larsson 2024) and "Venndiagram" (Chen 2022), with the exception of Fig. 3 (Microsoft Excel 2013). The frequency (*f*) for each MT was calculated by dividing the number of isolates of one MT by the total number of isolates across all MTs and multiplying the result by 100%.

Results

Test trees

The diameter at breast height of the test trees ranged between 43.5 and 81.5 cm and the tree height was between 25 and 35.8 m. All test trees belonged to Kraft's classes 1 (predominant) or 2 (co-dominant). Test trees differed in their crown structure (Table 4). On the undamaged and slightly damaged plots, the crown structure of the harvested test trees varied between classes 2 and 3. On the damaged and severely damaged plots, the crown structure varied between class 3 and 7. In four trees, wood rot was already visible at the cut site after felling.

Infestation with insects (recognised by typical gallerie patterns, larvae, adult beetles) and/or infections with fungi (fruiting bodies) were detected on all test trees, although the test trees differed in terms of the organisms detected. Only on test tree IX were no signs of insect infestation found. *Agrilus viridis* L. (beech splendour beetle), *Taphrorychus bicolor* (Herbst, 1793) (beech bark beetle) and *Zeuzera pyrina* L. (wood leopard moth) were detected on four trees and were therefore the most common insect species. While *T. bicolor* and *Z. pyrina* were found on both damaged and undamaged trees, infestation by *A. viridis* was only found on damaged trees. Signs of *A. viridis* and *T. bicolor* were found in the canopy of all four trees and to a lesser extent on the trunk. As one would expect, signs of *Z. pyrina* were only found on thick branches. Fruiting bodies of *Ascomycota* or *Basidiomycota* were found on nine of the 13 test trees, with *Basidiomycota* being much rarer and only found on damaged trees. For example, *Neonectria coccinea* was discovered on six trees and *Biscogniauxia nummularia* on five trees. In contrast to *N. coccinea*, fruiting bodies of the latter could only be documented on severely damaged trees. Basidiocarps of *Auricularia auricula-judae* (Fr.) Quél., *Exidia* sp., *Fomes fomentarius* (L.) Fr., *Pleurotus ostreatus* (Jacq.) P. Kumm., *Schizophyllum commune* Fr. and one indeterminable *Basidiomycota* sp. were observed on four test trees. These trees were damaged or severely damaged. None of these fruiting bodies was observed on more than one test tree.

Isolated fungi

Of the 3999 incubated tissue samples, 1963 samples (48%) showed outgrowths that could be assigned to filamentous *Dikarya*, 1436 (36%) were sterile and 600 (15%) were either not evaluable (terminated, overgrown) or the outgrowths did not belong to the filamentous *Dikarya*. From the 1963 samples with filamentous *Dikarya* outgrowths, 2156 fungi could be isolated, which were attributed to 181 MTs. In addition, 123 further outgrowths could not be brought into pure culture or determined ("Fungus sp."). Of the 181 MTs, 153 could be determined at least to the genus level, and 92 of them even to the species level. A further 24 MTs were tentatively (cf.) identified to species level (Table 3, marked with *). *Ascomycota* A to D are fungi that have been assigned to the group of black yeasts (Rosa and Péter 2006) on the basis of their cultural characteristics. Based on a LSU-ITS phylogeny conducted, *Ascomycota* A1, A2, B1 and B2 are closely related to the type strain of *Lembosiniella eucalyptorum* Crous & Carnegie (CBS 144603) and a clade of unspecified strains of the genus (GenBank MT813964, MT813970, ON865956, OP467220). *Ascomycota* C also belongs to a clade of *Ascomycota* A-B, strains of *Lembosiniella* stated above and the type strain of the monotypic genus *Gobabebomyces* (*G. vachelliae*, CBS 146779). *Ascomycota* D can be

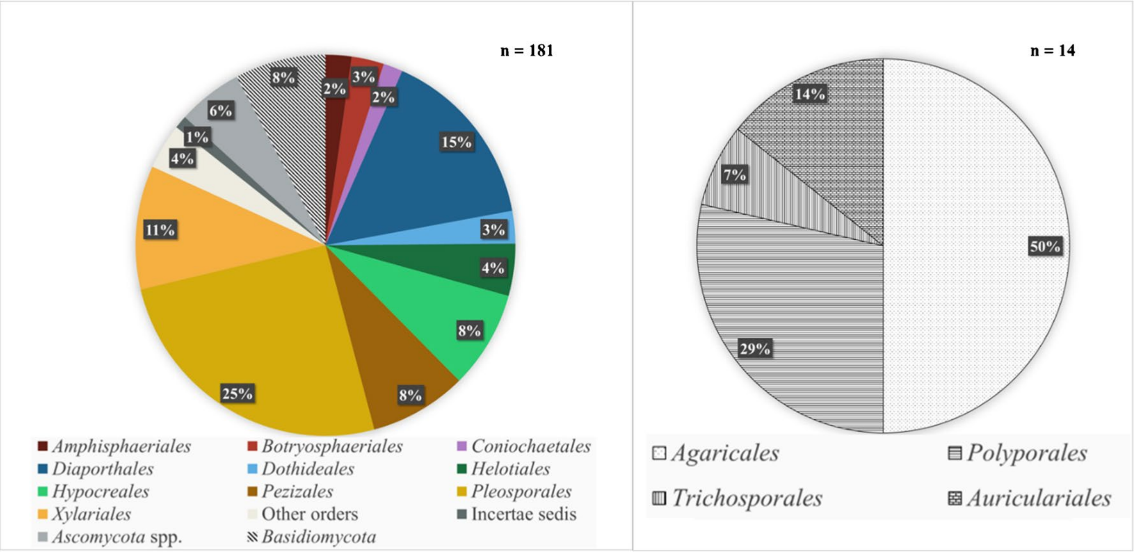


Fig. 3 Proportion of orders by the morphotypes identified. Left: Proportion of *Ascomycota* orders by the morphotypes identified (with colour) and *Basidiomycota* (black and white with hatching, as one group). Orders of *Ascomycota* with two or fewer associated morphotypes are listed under "Other orders". "*Ascomycota* spp." summarises morphotypes of *Ascomycota* that could not be determined at order level. Right: Proportion of *Basidiomycota* orders by the morphotypes identified (with hatching)

Table 4 Sampled test trees. Height of trees marked with > could not be measured accurately because the crown broke during felling. Kraft's class was determined according to Kraft (1884), Crown structure was determined according to the methodology of Eichhorn et al. (2016) and Wellbrock et al. (2020). Discolouration and Wood decay were recorded in binary form with 1 present on the tree and 0 absent

ID	Diameter at breast height (cm)	Height (m)	Kraft 's class	Crown structure	Discolouration	Wood decay	Observed insects	Observed fruiting bodies	Damageclass (plot)
I	49	> 28	2	5	1	1	*Cerambycidae* sp. *Taphrorychus bicolor*	*Biscogniauxia nummularia* *Neonectria coccinea*	Severely damaged
II	61	35.8	1	3	1	0	*Agrilus viridis*	*Neonectria coccinea*	Damaged
III	59	34.8	2	2	0	0	*Zeuzera pyrina*	*Asterosporium asterospermum*	Undamaged
IV	51	31	2	3	0	0	*Taphrorychus bicolor*		Slightly damaged
V	48.5	> 26	1	7	0	1	*Agrilus viridis* *Zeuzera pyrina*	*Biscogniauxia nummularia* *Exidia* sp. *Neonectria coccinea*	Severely damaged
VI	43.5	> 26	2	6	1	1	*Agrilus viridis* *Taphrorychus bicolor*	*Biscogniauxia nummularia* *Hypoxylon fragiforme* *Neonectria coccinea* *Pleurotus ostreatus*	Severely damaged
VII	54	32.5	2	3	0	0	*Zeuzera pyrina*		Undamaged
VIII	50.5	> 33	2	6	0	0	*Agrilus viridis*	*Auricularia auricula-judae* *Biscogniauxia nummularia* *Hypoxylon fragiforme* *Schizophyllum commune*	Damaged
IX	56	32.8	1	2	0	0		*Neonectria coccinea*	Slightly damaged
X	46	26.5	1	3	0	0	*Sesiidae* sp.		Slightly damaged
XI	50	32	2	3	1	0	*Taphrorychus bicolor*		Undamaged
XII	81.5	> 28	1	7	1	1	*Cerambycidae* sp.	*Biscogniauxia nummularia* *Fomes fomentarius* *Indeterminable Basidiomycota sp.*	Severely damaged
XIII	53.5	> 25	2	4	0	0	*Zeuzera pyrina*	*Neonectria coccinea*	Damaged

assigned to the order *Myriangiales*; however, a more precise delimitation has not been possible. *Diaporthe eres* Nitschke and *Diaporthe rudis* (Fr.) Nitschke are regarded as species complexes (Gomes et al. 2013; Udayanga et al. 2014). When creating phylograms for fungal identification, reasonable reference sequences were consulted (e.g., Gomes et al. 2013, Dissanayake et al. 2017, Gao et al. 2017, Guarnaccia et al. 2018). It turned out that the different isolates belonging to

D. eres and *D. rudis* were assigned to different clades within their group. Therefore, several MTs were assigned to the two groups. In the case of *Geoscypha tenacella* (Sacc.) Van Vooren, the cultures differed greatly from a macroscopic point of view. Comparison of the ITS DNA regions of the different strains also revealed minor differences. Therefore, two *G. tenacella* MTs were assigned.

Ascomycota were more frequently present and accounted for 167 of the MTs (92%), whereas 14 MTs could be assigned to *Basidiomycota* (8%). On the test trees, 15 orders of *Ascomycota* could be verified (Incertae sedis and "Not determinable" not included). The most common order among the MTs was *Pleosporales* (25%) followed by *Diaporthales* (15%) and *Xylariales* (11%) (Fig. 3). The majority of *Basidiomycota* MTs could be assigned to the order *Agaricales* (50%), the others to *Polyporales* (29%), *Auriculariales* (14%) and *Trichosporales* (7%) (Fig. 3). The three most frequently isolated fungi overall were *B. nummularia* (*Xylariales*) with 515 isolates (*f* = *23.9%*), followed by *Apiognomonia errabunda* (Roberge ex Desm.) Höhn. (*Diaporthales*) with 390 isolates (*f* = 18.1%) and *Aureobasidium pullulans* (de Bary) G. Arnaud (*Dothideales*) with 174 isolates (*f* = 8.1%). Only 15 MTs were isolated with a frequency ≥ 1%, 79 MTs were isolated just once.

Of the 87 MTs identified at species level counting the different MTs of *D. eres*, *D. rudis* and *G. tenacella* as only one MT each, 36 MTs or species had already been reported in the tissue of European beech in Germany. The authors were able to find reports of a further 16 species of European beech with the study area outside of Germany. A total of 35 species (corresponds to 41% of all species that could be identified at species level) have not previously been reported to be associated with European beech (Table 3). These were primarily species belonging to the Ascomycota, for example, *Botryosphaeriales* such as *Diplodia fraxini* (Fr.) Fr., *Dothiorella iberica* A.J.L. Phillips, Luque & Alves (≡ *Botryosphaeria iberica* A.J.L. Phillips, Luque & Alves) and D. *sarmentorum* (Fr.) A.J.L. Phillips, Alves & Luque (≡ *Botryosphaeria sarmentorum* A.J.L. Phillips, Alves & Luque = *Diplodia pruni* Fuckel = *Diplodia sarmentorum* (Fr.) Fr.) fide Phillips et al. (2005), and *Xylariales* like *Eutypa petrakii* Rappaz and *E. maura* (Fr.) Fuckel. *Apiotrichum porosum* Stautz was the only *Basidiomycota* for which the authors found no reports on European beech. The obtained culture of *A. porosum* clearly showed hyphal growth. The other species of the *Basidiomycota* detected in this study had already been reported on European beech. For 14 species, the first record in Germany was provided in the present study (Table 3), e.g. *Didymosphaeria variabile* (Riccioni, Damm, Verkley & Crous) Ariyaw. & K.D. Hyde, *Melanops fagicola* W.J. Li, Camporesi & K.D. Hyde and *Xylaria ellisii* J.B. Tanney, Seifert & Y.M. Ju.

Isolated fungi from asymptomatic tissue by test tree and damage class

Only isolates from asymptomatic tissue (endophytes) are listed in this section since not every tree had symptomatic tissue that could be examined. Five of the 14 *Ascomycota* orders identified in asymptomatic tissue were found in all test trees, i.e. *Pleosporales*, *Diaporthales*, *Xylariales*, *Hypocreales* and *Dothideales* (Fig. 4). *Capnodiales*, with a single isolation of *Neocatenulostroma germanicum* (Crous & U. Braun) Quaedvl. & Crous, was only detected at a single plot (XI) (Table 3. The other orders could be detected in two to eleven trees. For each of the 13 test trees, *Pleosporales* was the order to which most MTs could be attributed. Three trees each had a further order with the same number of MTs (test tree II, *Pezizales*; test tree III, *Xylariales*; test tree XIII, *Dothideales*). The absolute number of *Ascomycota* MTs detected per test tree differed clearly in some cases; for instance, more than twice as many MTs were detected in tree X (47 MTs) than in tree XIII (22 MTs). Four MTs were detected in all 13 felled beech trees, namely *B. nummularia*, *A. errabunda*, *A. pullulans* and *Epicoccum nigrum* Link. Eighty-seven *Ascomycota* MTs (including MTs only obtained from symptomatic tissues) were isolated from single-test trees, respectively, which corresponds to 52% of the total number of *Ascomycota* MTs detected.

Basidiomycota were detected in asymptomatic tissue in seven of the 13 test trees. Only five MTs could be isolated from this group, namely *A. porosum* (*Trichosporonales*), *Bjerkandera adusta* (Willd.) P. Karst. (*Polyporales*), *Coprinellus micaceus* (Bull.) Vilgalys, Hopple & Jacq. Johnson (*Agaricales*), *Hypholoma fasciculare* (Huds.) P. Kumm (*Agaricales*) and *Ischnoderma resinosum* (Schrad.) P. Karst. (*Polyporales*). *Coprinellus micaceus* was isolated from the asymptomatic tissue of three test trees (two trees severely damaged, one slightly damaged), *B. adusta* from two (undamaged and slightly damaged) and the remaining three MTs only from a single tree. With the exception of tree XI, from which two *Basidiomycota* MTs were isolated, only one MT was detected in each of the other six test trees from which *Basidiomycota* species were isolated from asymptomatic tissue. Since MTs of the *Basidiomycota* section accounted for such a small amount of the MT detected compared to the *Ascomycota*, the *Basidiomycota* were excluded from the following sections.

No trend was observed for the number of MTs isolated from asymptomatic tissue in relation to the damage class of the test trees (suppl. mat. Figure 1). Most MTs were isolated from the asymptomatic tissue of severely damaged trees (85 MTs). However, four test trees were analysed in this damage class and only three in the other damage classes, respectively. The second highest number of MTs was isolated from the tissue of slightly damaged trees (80 MTs), followed by undamaged trees (69 MTs), and the lowest number of MTs was isolated from the asymptomatic tissue of damaged trees (52 MTs). No fungal order that was frequently represented was found exclusively or clearly more frequently in only one damage class. Although the number of MTs isolated solely from trees within one damage class differed between the four damage classes (suppl. mat. Figure 2).

Isolated *Ascomycota* by tree compartment

The number of recorded MTs and the corresponding orders differed between the three tree compartments, trunk, branch and twigs (Fig. 5). In the trunk, twelve of the 15 detected orders could be identified and eleven different orders could be detected on the branches. With the exception of *Trichosphaeriales*, all of the detected orders were found in the twigs. In all three compartments, most of the isolated MTs belonged to the order *Pleosporales* (33% of the isolated MTs from the trunk, 40% from the branches and 27% from the twigs). In the trunk, MTs of the order *Hypocreales* were second most frequently isolated (19% of the MTs isolated from the trunk) and MTs of the order *Xylariales* were the third most frequently isolated (17%). In the branches, *Hypocreales* ranked second (19% of the isolated MTs from the branches), with *Xylariales* and *Diaporthales* sharing third place (12%). In the twigs, the second most frequent order was *Diaporthales* (21% of the isolated MTs from the twigs), followed by *Pezizales* (12%).

Across all orders, 58 different MTs were isolated from the trunk, 43 MTs from the branches and 125 different MTs were isolated from the twigs (Fig. 6). Focusing on the trunk, 21 of the 58 MTs were not isolated from any other tree compartment. For the branches, eleven of the 43 MTs were isolated only from branches, and for the twigs, 92 of the 125 MTs were isolated only from the twigs. Of the 167 *Ascomycota* MTs detected across all test trees, 16 MTs were documented in all three compartments, namely *Alternaria infectoria* E.G. Simmons, *Alternaria* sp. 1, *Alternaria* sp. 2, *Asterosporium asterospermum* (Pers.) S. Hughes, *A. pullulans*, *B. nummularia*, *Cladosporium* spp., *Cytospora galegicola* Q.J. Shang, Camporesi & K.D. Hyde, *Didymella macrostoma* (Mont.) Qian Chen & L. Cai, *E. nigrum*, *Hypoxylon fragiforme*, *Neohendersonia*

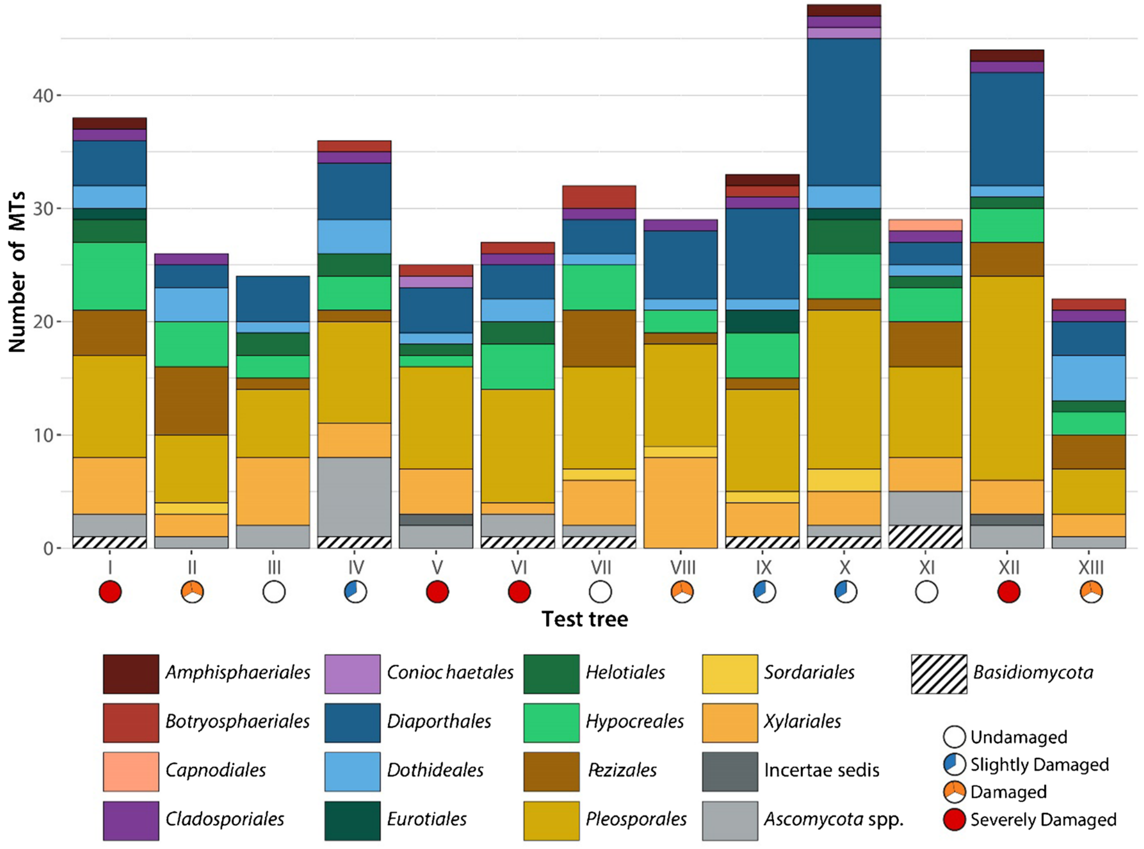


Fig. 4 Number of endophytic morphotypes obtained from the various *Ascomycota* orders (with colour) and *Basidiomycota* (with hatching) differentiated according to the test tree from which they were isolated. "*Ascomycota* spp." summarises morphotypes of *Ascomycota* that could not be determined at order level. The corresponding damage classes (symbols) are listed under each test tree

kickxii (Westend.) B. Sutton & Pollack, *N. coccinea*, *Penicillium* spp., *Pleosporales* sp. 7 and *Trichoderma* spp.

Isolated *Ascomycota* by plant tissue

A total of 75 MTs were found in the two tissue types (Fig. 7). With 46 MTs (61% of the MTs detected in branches and on the trunk), the majority were only found in the cambial tissue. Thirteen MTs were exclusively detected in the xylem (17%). In both tissue types, 16 MTs were isolated (21%). The 16 MTs were as follows: *Asterosporium asterospermum*, *A. pullulans*, *Beauveria bassiana* (Bals.-Criv.) Vuill., *B. nummularia*, *Cadophora malorum* (Kidd & Beaumont) W. Gams, *Cladosporium* spp., *D. iberica*, J. Luque & A. Alves, *E. petrakii*, *Neocucurbitaria vachelliae* Jaklitsch & Voglmayr, *N. coccinea*, *Penicillium* spp., *Pleosporales* sp. 1, *Pleosporales* sp. 7, *Tolypocladium* sp. 2, *Trichoderma* spp. and *Ustulina deusta* (Hoffm.) Maire.

Isolated *Ascomycota* by tissue condition

Fewer samples were taken from symptomatic than from asymptomatic tissue (3432 asymptomatic to 567 symptomatic) because not every tree contained symptomatic tissue, so symptomatic tissue could not be systematically sampled. From the four orders *Amphisphaeriales* (4 MTs), *Capnodiales* (1 MT), *Pezizales* (15 MTs) and *Sordariales* (2 MTs), the corresponding MTs could only be obtained from asymptomatic tissue (Fig. 8). *Trichosphaeriales*, represented by just one isolated MT, *Gibellulopsis nigrescens* (Pethybr.) Zare, W. Gams & Summerb., was the single order that occurred only in symptomatic tissue. Across all orders, 27 MTs were both isolated from asymptomatic and from symptomatic tissue (16% of all *Ascomycota* MTs, Fig. 9). Most MTs (129 MTs, 77%) were isolated exclusively from asymptomatic tissue. Eleven MTs (7%) were isolated exclusively from symptomatic tissue, i.e., *Coniochaeta velutina* (Fuckel) Cooke, *Diatrype stigma* s.l. (Hoffm.) Fr., *E. petrakii*, *Eutypella quaternata* (Pers.) Rappaz, *G. nigrescens*, *Nectria nigrescens* Cooke, *Paracamarosporium fagi* Crous & R.K. Schumach., *Pseudopithomyces chartarum* (Berk. & M.A. Curtis) Jun F. Li, Ariyaw. & K.D. Hyde, *Querciphoma minuta* (J.C. Carter) Crous & P.M. Kirk, *Septoriella muriformis* (Ariyaw., Camporesi & K.D. Hyde) Y. Marín & Crous and *Thyridariaceae* sp. These MTs were only isolated once, with the exception of *E. petrakii* (3 isolates), *Q. minuta* (3 isolates) and *S. muriformis* (2 isolates). *Septoriella muriformis* was the only one of these MTs that was found on more than one test tree.

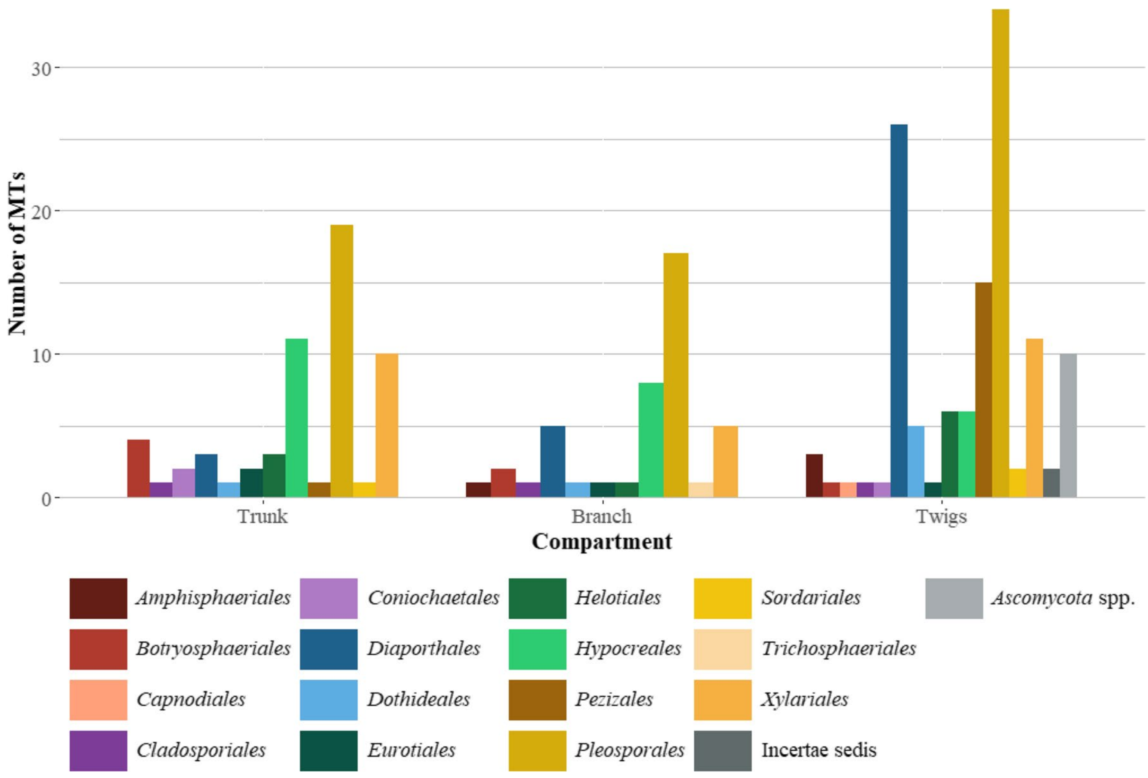


Fig. 5 Number of ascomycetaceous morphotypes obtained from the various orders and differentiated according to the tree compartments (isolated from the trunk, the branches or the twigs). "*Ascomycota* spp." summarises morphotypes of *Ascomycota* that could not be determined at order level

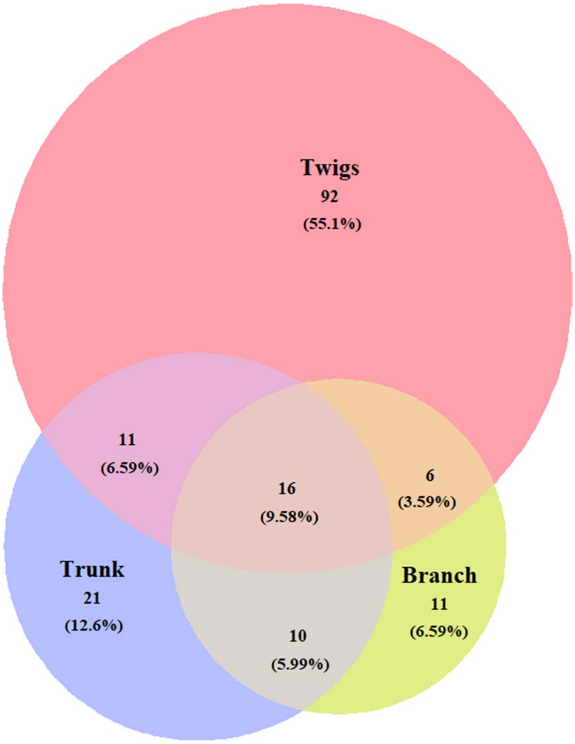


Fig. 6 Number of ascomycetaceous morphotypes obtained differentiated according to the tree compartments into morphotypes isolated only from the trunk (blue), only from the branches (green) or only from the twigs (red), as well as all possible overlaps

Apiognomonia errabunda

For this study, 390 isolates of *A. errabunda* were obtained. Most isolates (99%) came from twigs (detected in 15% of all twig samples) (Fig. 10). *Apiognomonia errabunda* was found as an endophyte in the twigs of every test tree. Although the test trees differed in the frequency with which *A. errabunda* could be isolated from the twigs (values ranged from 2% for test tree III to 41% for test tree XIII), the values hardly differed for undamaged to slightly damaged and damaged to severely damaged trees (17 to 16%). The fungus was isolated four times from symptomatic tissue, distributed over three test trees (either damaged or severely damaged). All four of these isolates were obtained from the cambial tissue of branches.

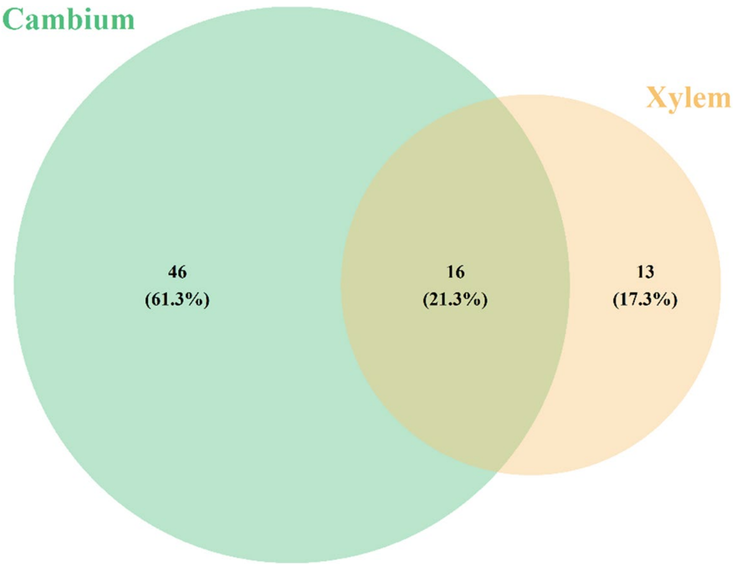
Biscogniauxia mediterranea

Biscogniauxia mediterranea (De Not.) Kuntze was isolated a total of 55 times. All isolates were restricted to the twigs (Fig. 10), which, as already mentioned, were only obtained from asymptomatic tissue for this study. Eight of the 13 trees tested positive for the fungus. The number of isolates from the positively tested trees ranged from two (II and XII) to 22 (I). For each test tree where we were able to detect *B. mediterranea*, there were either oaks on the plot (e.g. tree I) or the information received from the forest owners indicated that at least some oaks were in the vicinity of the respective plot.

Biscogniauxia nummularia

With 515 isolates across all test trees, *B. nummalaria* was the most frequently detected fungus. *Biscogniauxia nummularia* was found 497 times in asymptomatic tissue and 18 times in symptomatic tissue (Fig. 10). As an endophyte, *B. nummularia* was detected on all 13 test trees. Of the twelve test trees on which symptomatic tissue could be sampled, the fungus was detected in symptomatic tissue on exactly half of the trees. *Biscogniauxia nummularia* was detected

Fig. 7 Number of ascomycetaceous morphotypes obtained from branches and trunks differentiated into morphotypes isolated from cambial tissue only (green), from xylem only (orange) or from both tissue types (overlap)



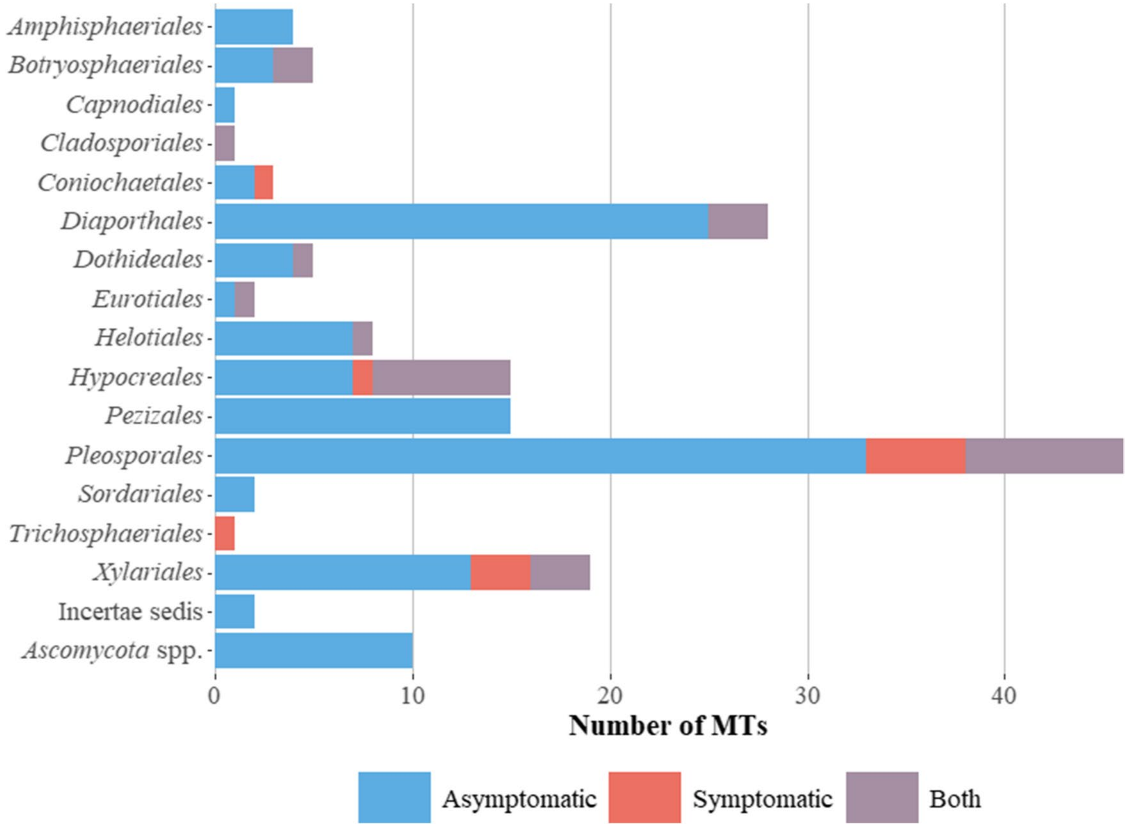


Fig. 8 Number of ascomycetaceous morphotypes obtained within the orders and subdivided into morphotypes that were only isolated from asymptomatic tissue, only from symptomatic tissue or from both asymptomatic and symptomatic tissue. "*Ascomycota* spp." summarises morphotypes of *Ascomycota* that could not be determined at order level

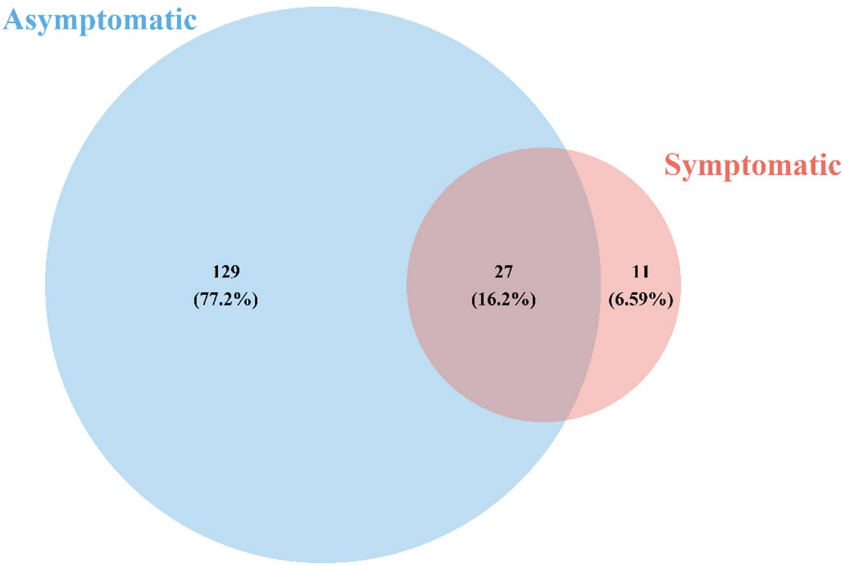


Fig. 9 Number of ascomycetaceous morphotypes obtained that were isolated only from asymptomatic tissue (blue), only from symptomatic tissue (red) or from both asymptomatic and symptomatic tissue (overlap)

as an endophyte in damaged and severely damaged trees three times more frequently than in undamaged and slightly damaged trees (from 21 to 7%; proportion of asymptomatic tissue samples tested positive for *B. nummularia*). However, the damaged and severely damaged test trees XII and XIII are out of the ordinary, as the value here was only 2% and 3%, whereas it ranged between 12% (II) and 43% (VI) in the five other damaged or severely damaged trees. Twigs were the compartment from which *B. nummularia* was most frequently isolated as an endophyte with 467 isolates (detected in 19% of all twig pieces). In asymptomatic tissue from branches and trunks, *B. nummularia* was detected 6 times and 24 times (3% detection each of all tissue samples from the respective compartment). The symptomatic tissue from branches tested positive for *B. nummularia* with 14 isolates (5%) and the tree trunks with 4 isolates (2%). *Biscogniauxia nummularia* was found both as endophyte and symptomatic more frequently in the cambium of branches (asymptomatic 5% and symptomatic 8%) and trunks (6% and 5%) than in the xylem of branches (0%and 1%) and trunks (1%, and 1%).

Neonectria coccinea

A total of 78 isolates were obtained from *N. coccinea*. The fungus was isolated from every test tree with the exception of test tree X (slightly damaged). The number of isolates from the positively tested trees ranged from two (IV and XI, slightly damaged and undamaged) to 21 (II, damaged). Of the twelve test trees from which *N. coccinea* was isolated, the fungus was isolated from the asymptomatic tissue of all of them with the exception of tree VII. *Neonectria coccinea* was isolated from asymptomatic tissue in the damaged and severely damaged test trees about twice as often as in the undamaged and slightly damaged trees (3.1 isolates to 1.5 on average per group; outgrown from 1.2% and 0.6% of all asymptomatic samples accordingly). From symptomatic tissue, *N. coccinea* was detected on nine of the eleven trees that tested positive for *N. coccinea* and from which symptomatic tissue was sampled. With 47 isolates from symptomatic tissue, *N. coccinea* is the MT most frequently detected in symptomatic tissue. From symptomatic tissue of undamaged and slightly damaged trees, the fungus was isolated more frequently with an average of 12% outgrowth than in the damaged and severely damaged trees, where this value was 9%. Looking at different tree compartments, *N. coccinea* was most frequently isolated from the cambium of symptomatic branch and trunk tissue (22% and 23% of the described tissue samples) (Fig. 10). In symptomatic xylem, *N. coccinea* was also detected in branches and trunks, but to a much lesser extent (2% and 1%). In asymptomatic tissue from branches and trunks *N. coccinea* was detected more frequently in the cambium (3% and 5%) than in the xylem (0% and 1%). The fungus was only isolated from 0.2% of the incubated twig pieces.

Discussion

The role of beech-inhabiting fungi, especially endophytes, in the context of VLB was analysed in the present study using a culture-based approach. The present study aims to provide preliminary insights into the fungal species, particularly endophytes, that may be associated with VLB, and to illustrate the remarkable diversity of the fungal community

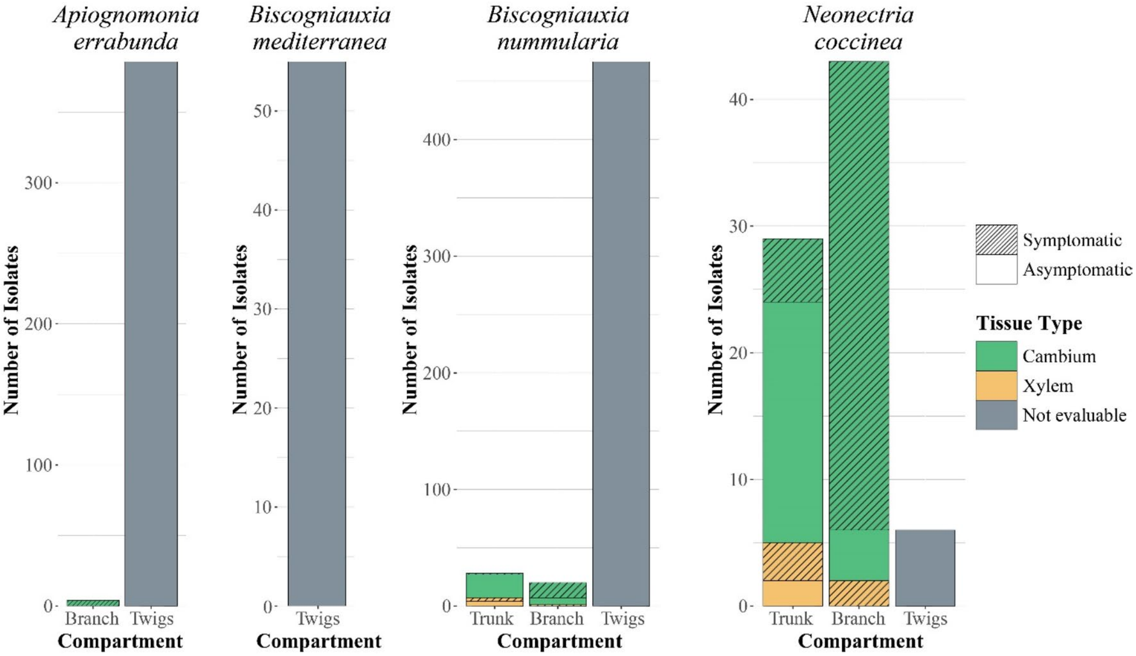


Fig. 10 Number of isolates of *Apiognomonia errabunda*, *Biscogniauxia mediterranea*, *Biscogniauxia nummularia* and *Neonectria coccinea*, differentiated according to tree compartment and tissue type (with colour) as well as the health status of the tissue (with hatching) from which the species was isolated. For twigs, no differentiation was possible with regard to tissue type

inhabiting European beech. Nevertheless, care should be taken when attempting to generalise the findings, given the potential limitations of the study. A higher number of trees per plot would undoubtedly be beneficial for future studies in order to create statistical models. However, this is often precluded due to the considerable effort required for the study, as well as for ecological (nature conservation), economic (loss of value for the forest owner) and social reasons (critical reaction of citizens when trees are felled). As expected, the proportion of cultivated *Ascomycota* (92%) was higher than *Basidiomycota* (8%). Various studies on European beech (e.g. Sieber and Hugentobler 1987; Petrini and Fisher 1988; Kowalski and Kehr 1992; Langer and Bußkamp 2021, 2023), but also on other tree species (e.g. Singh et al. 2017; Bußkamp 2018; Ghobad-Nejhad et al. 2018), have already shown that the number of isolated *Basidiomycota* occurring as endophytes is at a much lower level compared to *Ascomycota*. Even if only the MTs that were obtained from symptomatic tissue in the present study are considered, the *Ascomycota* account for a far greater proportion (81%) than the *Basidiomycota* (19%), though the gap is smaller. These results correspond to the findings by Langer and Bußkamp (2023) on European beeches affected by VLB in central Germany. Focusing on symptomatic beech tissue Langer and Bußkamp (2023) listed 31 associated fungal taxa whereby 87% were *Ascomycota* and 13% were *Basidiomycota*. The ratio of *Ascomycota* to *Basidiomycota* seems to shift slightly in favour of *Basidiomycota* in symptomatic tissue in trees affected by VLB, even though *Ascomycota* were still isolated much more frequently. Similar ratios between *Ascomycota* and *Basidiomycota* as in the present study were found by Gilmartin et al., (2022) analysing the sapwood of young and old European beech trees with regard to the fungal endophyte community. They used a Next-Generation Sequencing (NGS; ITS and LSU) as well as a culture-dependent approach in order to compare the results. The ITS and LSU sequencing datasets shared more OTUs than either of them did with the culture dataset. NGS using the ITS barcode showed a ratio of 67% *Ascomycota* to 25% *Basidiomycota* and LSU a ratio of 72 to 22%. With the culture-based method, the ratio was 84 to 13%. The study of Purahong et al. (2021) on the bacterial and fungal community in symptomatic and asymptomatic tissue from European beech trees affected by VLB using a NGS approach revealed a portion of 43% *Basidiomycota*. Compared to the aforementioned comparable culture- and NGS-based studies on European beech, the portion of *Basidiomycota* in Purahong et al. (2021) is clearly higher in relation to *Ascomycota*. One possible explanation for the lower detection frequency of *Basidiomycota* using culture-based methods compared to NGS analyses is their requirement for lengthier incubation periods, particularly in the case of wood rot pathogens (Oses et al. 2008). Furthermore, the season has a proven influence on the species composition (e.g. Sieber and Hugentobler 1987; Ceccarelli 2011; Singh et al. 2017). Fewer *Basidiomycota* could have been present at the time of sampling, since the present study only sampled in winter. In addition, less filamentous basidiomycetes could also be present in the woody tissues of European beech during the investigated phases of the VLB damage process. Additionally, with the exception of one *Agaricales* and one *Hymenochaetales* amplicon sequence variant (ASV), Purahong et al. (2021) only detected taxa for which the majority of species shows yeast-like growth, a group that was not considered in the present study. Of the 14 *Basidiomycota* MTs identified in the present study, *Apiotrichum porosum* was the only *Basidiomycota* considered a yeast; however, the isolated culture clearly showed hyphal growth and was therefore not excluded from the analysis, limited to filamentous fungi. As reported from Fell et al. (2000), there appear to be strains that show hyphal growth and lack the yeast-like phase within the species *A. porosum*. The aforementioned factors may account for the relatively low prevalence of *Basidiomycota* observed in the present study and other culture-based investigations. Finally, the culture medium used has an influence on the detection of basidiomycetes. Bußkamp (2018) has discussed in detail the influence of the culture medium on the detection of basidiomycetes.

Across all MTs, the order *Pleosporales* accounts for the largest proportion (25%) followed by *Diaporthales* (15%) and *Xylariales* (11%). Even in the case of individual test trees, *Pleosporales* was either the most frequently represented order alone or together with another order (*Xylariales*, *Pezizales* and *Dothideales*). This contrasts with the results of Sieber (2007), who claimed that angiosperm endophyte communities are often dominated by *Diaporthales*. Although MTs of *Diaporthales* could be detected on every test tree, as with every other order (with the exception of *Pleosporales*), the number of associated MTs varied considerably between the different test trees. This could possibly be attributed to the differing site characteristics, namely climate, bedrock, water availability and trophy. The sites were affected to varying degrees by heat and drought, as illustrated by the deviation of temperature and precipitation from the reference period (Table 1). As shown by Wollan et al. (2008) and Tedersoo et al. (2014), climate and in particular temperature seems to be one of the main factors for the distribution of species of soil and macrofungi. In addition to the climate, the tree species assembly on the various sites differed in the present study. Hantsch et al. (2014) investigated fungal infestation on leaves of two particular host tree species and found a higher fungal species richness with increasing tree species diversity in the immediate vicinity. They concluded that different fungal species might be affected by tree diversity at different scales. It can therefore be assumed that the species communities observed in the present study were strongly influenced by the respective site charactericstics and probably also by the respective tree species assembly. In contrast, the vitality status of the test trees does not seem to have an effect on the orders detected, or the effect is superimposed by other factors like the forest site. On the other hand, it seems to make a distinct difference which tree compartment is observed. Of the *Ascomycota* MTs, 55% were isolated from the twigs alone, barely 10% were detected in all three compartments. Fungi of the *Diaporthales* took the second largest share in the twigs after *Pleosporales*, but the proportion became gradually lower from the branches to the trunk. *Pezizales* was the third most abundant order in the twigs but was nearly absent in the branches and trunks. Morphotypes of the *Xylariales* were present in all three compartments but were most common in the twigs, where they formed the fourth most common order. A comparison with other studies is hindered by the fact that for European beech, often, only the fungal community in leaves and twigs was analysed. However, the results on twigs in the present study were largely consistent with investigations on endophytes in beech leaves. In both Sieber and Hugentobler (1987) and Pehl and Butin (1994), the most common orders in European beech leaves were *Pleosporales*, *Diaporthales* and *Xylariales*, although the ranking was not the same. Consistent with this, Ceccarelli (2011) and Griffith and Boddy (1990) showed that species of the orders *Pleosporales* and *Diaporthales* are strongly represented in the twigs of European beech. However, contrasting to the present study, both Ceccarelli (2011) and Griffith and Boddy (1990) found that *Hypocreales* was one of the most represented orders. Here, *Hypocreales* was the second most dominant order on the trunk but becoming continuously less relevant across branches to the twigs. It is also noticeable that neither in the studies that analysed leaves nor in the studies that analysed twigs fungi of the order *Pezizales* were detected.

Focussing on the branches and tree trunks in the present study, considerably more MTs were detected in the cambial tissue than in the xylem. Consistently, Petrini and Fisher (1988) showed that fewer taxa were isolated from twigs of European beech where the bark was removed than from twigs where the bark was left on. Various studies have shown that the asymptomatic xylem of other tree species is also less species-rich than asymptomatic cambial tissue and bark (Fisher and Petrini 1990; Wang and Guo 2007; Bußkamp 2018). According to Singh et al. (2017) and Juybari et al. (2019), the tissue type has the greatest influence on the fungal species community within a host, even before site locality and season.

Most MTs detected in the present study were exclusively found in asymptomatic tissue. A smaller proportion was detected both, in asymptomatic and symptomatic tissue, and the smallest proportion was found exclusively in symptomatic tissue. In contrast to the tissue type, there was no trend recognisable regarding the vitality status of the test tree and the number of MTs detected. However, *Biscogniauxia nummularia* was detected much more frequently in damaged trees than in undamaged ones. In contrast, *B. nummularia*, was not detected by Danti et al. (2002) on either undamaged or damaged European beech trees. Danti et al. (2002) compared the fungal community associated with damaged and undamaged trees. While the most frequent taxa could be detected in both groups, the number of isolates per frequent taxa differed to a certain degree. Nevertheless, one *Apiosphaeria* species was the only species that could be detected significantly more frequently in damaged than undamaged trees. In the present study, no *Apiosphaeria* species was detected. Although Danti et al. (2002) were able to detect *Asterosporium asterospermum* five times more frequently in twigs of damaged European beech trees (high crown transparency) than from undamaged ones (low crown transparency), *A. asterospermum* was only isolated from 2% of the tissue samples from damaged trees. In the present study, *A. asterospermum* was one of the few MTs isolated in all three tree compartments, in both tissue types (xylem and cambium) and from asymptomatic and symptomatic tissue. Thus, the results also contradict Butin (2011) who described *A. asterosporium* as a frequent first coloniser of bark of dead branches and trunks of European beech. In the present study, *A. asterospermum* was isolated with a low frequency ($f < 1\%$), and only one of the isolates came from a damaged tree. Thus, according to the data collected in the present study, *A. asterospermum* is not suitable as a bioindicator for the vitality of European beech.

Considerably more taxa were detected in the present study than in other comparable culture-based research. There can be numerous reasons for this. One important factor is the investigation area. The studied sites in the present study covered three federal states, and the linear distance between the two most distant trees investigated was almost 250 km. Since 48% of the isolated *Ascomycota* MTs were limited to single trees, the site seems to have an important influence on the fungal community. Studies support the thesis that site factors have a measurable influence on the fungal community in European beech (Siddique and Unterseher 2016; Unterseher et al. 2016). Thomas et al. (2019) analysed spatial patterns of the endophyte community of different tree species in Taiwan. They hypothesised that even closely associated fungal species can be separated from their host trees when environmental conditions change greatly. In contrast, they found that there was a certain group of fungi species that were consistently present across the study area, while other fungi were only identified in single plots. The endophyte community of woody tissue is characterised by host-specific species (Sieber 2007), as well as generalists, and species that are specific to the tissue type of the host (Bußkamp 2018).

Some fungal species have a broad host spectrum, such as generalists, but only sporulate on one or a few host species. The claims made in the studies mentioned above are consistent with the results of the present study. While almost half of the isolated MTs were limited to single trees and thus location, only four MTs were isolated from all test trees. *Aureobasidium pullulans* and *Epicoccum nigrum* are ubiquitous fungi with a large host species spectrum (Prasongsuk et al. 2018; Taguiam et al. 2021), while *B. nummularia* and *Apiognomonia errabunda* seem to be closely associated with the European beech despite their wide species host range (see the respective subchapters below).

Due to the high number of MTs detected in the present study, it is not possible to discuss each individual MT. In the following, selected species are discussed with regard to their role in VLB.

Apiognomonia errabunda

Apiognomonia errabunda was the second most frequently detected fungus in this study (390 isolates), which confirms that *A. errabunda* is a very abundant endophyte in leaves, buds, twigs and branches of European beech (Sieber and Hugentobler 1987; Kowalski and Kehr 1992; Danti et al. 2002). However, the species has also been detected on various other tree species (Monod 1983). *Apiognomonia errabunda* is known to be the causal agent of leaf anthracnose on various deciduous trees including European beech. Leaf anthracnose on European beech is characterised by erratic brown necrosis on the leaves (Butin 2011). Although the number of isolates obtained per test tree differed clearly in some cases, the vitality status of the tree does not appear to be the explanatory factor in the present study. *Apiognomonia errabunda* was isolated from each of the 13 test trees and was mainly detected in the asymptomatic twigs and less often in symptomatic tissue of the branches. While Butin (2011) reported that young shoots can be affected by *A. errabunda* the authors of the present study found no report that *A. errabunda* is able to damage perennial, strong branches. The high frequency of *A. errabunda* within the endophyte community of European beech in leaves and twigs (Sieber and Hugentobler 1987; ibid.) leads us to the assumption that *A. errabunda* is commonly associated with *Fagus sylvatica* and coexists closely with the tree species. Morphological, physiological and biochemical studies of *A. errabunda* isolates from different hosts indicate host-specific differences (Haemmerli et al. 1992; Toti et al. 1992). As per Butin (2011), even in cases when this highly abundant species manifests symptomatically in European beech, no severe damage to the host occurs, suggesting that *A. errabunda* either plays no role in the damage produced by VLB or only a minor one. The latter is supported by the fact that Langer and Bußkamp (2023) associated only 4% of the investigated cases of VLB with *A. errabunda*.

Biscogniauxia nummularia and *B. mediterranea*

The fact that *B. nummularia* is one of the most abundant endophytes of European beech (Chapela and Boddy 1988) can be confirmed by the results of this study. The species has also been recorded as an endophyte on various other deciduous and coniferous tree species (e.g., Bußkamp 2018; Peters et al. 2023; Schlößer et al. 2023). However, to the knowledge of the authors of the present study *B. nummularia* only fructifies and occurs as a pathogen on European and Oriental beech (Zamani et al. 2024). If a host tree is weakened, especially by drought, the endophytic *B. nummularia* can switch to a parasitic life phase causing necrosis as well as strip-cankers on branches and trunks, which leads to beech decline (Chapela and Boddy 1988; Granata and Whalley 1994; Hendry et al. 1998; Granata and Sidoti 2004; Nugent et al. 2005; Luchi et al. 2006). The year 2018 and the subsequent drought years made this irrefutably clear, when *B. nummularia* was substantially involved in the damage caused by VLB in Germany. *Biscogniauxia nummularia* was the most frequently fungal species associated with cases of VLB followed by *Neonectria coccinea* and *Eutypella quaternata* (Langer 2019; Langer et al. 2020; Langer and Bußkamp 2023).

It is striking that *B. nummularia* as an endophyte was isolated three times more frequently from trees of the damaged and severely damaged test trees than from the undamaged and slightly damaged test trees. Luchi et al. (2016) investigated the presence of *B. nummularia* in asymptomatic twigs of European beech using qPCR at two forest sites. They were able to detect *B. nummularia* more frequently in the twigs of those trees that stood on the site with longer dry periods. The authors of that study hypothesised that water stress increases the number of fungal inoculum, making the host more susceptible. They concluded that the increased presence of *B. nummularia* in asymptomatic tissue could have a negative impact on the vitality of the host and could lead to an outbreak of the pathogen if the climatic conditions are favourable for the pathogen. This type of behaviour has been reported in a number of studies examining various host-endophyte relationships (e.g. Bassett and Fenn 1984; Vannini and Scarascia Mugnozza 1991; Stanosz et al. 2001; Desprez-Loustau et al. 2006). Consequently, *B. nummularia* would be suitable as a bioindicator for the vitality of European beech, e.g. regarding drought and heat-induced damage. The results of the present study support the thesis of Luchi et al. (2016) and show that *B. nummularia* is more abundant in the asymptomatic tissue of trees that were more strongly affected by drought and heat-induced damage.

However, this did not apply to two damaged test trees (XII and XIII, sampled in Thuringia). Test tree XII was the most damaged tree analysed in the present study. The extent of the damage to the crown was such that it included very few twigs suitable for sampling, and even those were feeling quite dry. It is possible that the twigs had already dried out too much, as comparatively few fungi had grown from most of the sampled twigs of this test tree. Since fruiting bodies of *B. nummularia* were found on a dying thick branch of the test tree, the fungi must have been more present in the test tree at some time. The other test tree that stands out was tree XIII. One explanation for the low number of isolates of *B. nummularia* from test tree XIII could be that Plot XIII differs from the other analysed plots in terms of its site characteristics. On one hand, it is the highest plot above sea level; on the other hand, beech stands are very unusual for the area, instead spruce is dominant in the region (Blickensdörfer et al. 2024). *Biscogniauxia nummularia* is found to be the most abundant species and is found at all studies forest sites, which indicates its close connection to the European beech. The results of the present study show that this warm-loving species (Hendry et al. 2002) is most prevalent in twigs, rather than branches and trunks, and in xylem, rather than cambial tissue. *Hypoxylon fragiforme*, which along with *B. nummularia* is considered one of the most abundant fungi on European beech (Chapela and Boddy 1988; Hendry et al. 2002), was only detected with low frequency (0.4%) in the present study.

In contrast to *B. nummularia*, the closely related species *Biscogniauxia mediterranea*, which is known to be a pathogen on oaks (Desprez-Loustau et al. 2006; Henriques et al. 2015), was isolated less frequently in the present study. It was detected exclusively in the asymptomatic twigs and only in test trees that were in the vicinity of oak trees. Bußkamp (2018) suspected the same for pines in the vicinity of oaks.

Neonectria coccinea

The findings of this study are consistent with the notion that *N. coccinea* is a highly abundant endophyte of European beech wood and bark (Chapela and Boddy 1988; Hendry et al. 2002). It occurred at 92% of the forest sites analysed and was found in both symptomatic and asymptomatic tissue, however, less frequently in the latter. Although *N. coccinea* was not isolated most frequently across all samples, it was by far isolated most frequently in symptomatic tissue and was most abundant in necrotic tissue from the cambium. This is in accordance with Purahong et al. (2021), who found that *N. coccinea* was among the most frequently detected fungal pathogens in discoloured wood of European beech affected by VLB. *Neonectria coccinea* along with woolly beech scale (*Cryptococcus fagisuga* Lindinger) is also a key pathogen of BBD (Ehrlich 1934; Parker et al. 1980; Houston 1994). However, *N. coccinea* is able to cause necrosis on European beech in the absence of *C. fagisuga*, e.g. when the host suffers from drought stress (Lonsdale 1980a, 1980b; Langer and Bußkamp 2021). The growth of thalli is probably regulated by oxygen and/or nutrient availability (Sieber 2007). The fact that the fungus was detected more frequently in the symptomatic tissue of undamaged and slightly damaged trees than in the more severely damaged classes suggests that *N. coccinea* plays a key role in the early stage of VLB. In the event of the host experiencing stress as a result of drought, as in VLB, *N. coccinea* can cause bark necrosis and may act as a precursor for wood decay fungi to gain access to the host tissue.

New records for European beech

In this study, 35 species were detected for the first time on European beech. Among them was *Diplodia fraxini*, a species associated with stem collar necrosis, cancers and dieback on various ash species (Alves et al. 2014; Peters et al. 2023). *Diplodia fraxini* was previously classified as part of the *Diplodia mutila*-complex. However, recent taxonomic revisions have led to its revaluation and reinstatement as a distinct species (Linaldeddu et al. 2020). The MT obtained in the present study could be clearly assigned to *D. fraxini* based on multilocus phylogenetic analysis (ITS-*EF1α*-*TUB*). Whether *D. fraxini* is pathogenic on European beech in a similar way to *Diplodia mutila* (Fr.) Fr. and *Diplodia corticola* A.J.L. Phillips, A. Alves & J. Luque (Langer and Bußkamp 2021; Tropf et al. 2022) remains to be tested. In contrast to the investigation by Langer and Bußkamp (2023), *D. mutila* was only detected with a low frequency and *D. corticola* was not detected at all in the present study.

In the present study, three different *Cytospora* species were isolated. The authors assume that they are the following species: *Cytospora cotini* Norph., Bulgakov & K.D. Hyde, *Cytospora galegicola* and *Cytospora personata* (Fr.) Sacc. If the assumption is confirmed, this would be the first detection of all three species on European beech, and *C. galegicola* und *C. cotini* would have been detected for the first time in Germany. Of the three species, *C. galegicola* was the most common species that was isolated in this study and was dectected on nine test trees and in both, symptomatic and asymptomatic tissue. *Cytospora galegicola* was previously detected in Italy in a dead stem of *Galega officinalis* L. (Goat's-rue) and introduced as a new taxon in 2020 (Shang et al. 2020). Since the six isolates of *C. galegicola* from symptomatic tissue originated exclusively from cambial tissue of branches, the species seems to be a bark pathogen, similar to *N. coccinea*. In contrast to *N. coccinea*, *C. galegicola* was only found in the symptomatic tissue of the crown but not in the trunk and could neither be isolated asymptomatically nor symptomatically from the xylem. Various other species of the genus *Cytospora* are known as broad-spectrum pathogens, which, in combination with drought, can cause cankers and tree dieback (Desprez-Loustau et al. 2006; Shang et al. 2020). According to Purahong et al. (2021), species of the genus *Cytospora* were among the most frequently detected pathogens on bark necroses of European beech affected by VLB. They concluded that species of the genus *Cytospora* can contribute to the damage progression of VLB in Thuringia. The latter thesis can be confirmed for Thuringia in the present study and extended for the federal states of Lower Saxony and Hesse.

Three species of the genus *Eutypa* were identified in the present study, including the first detection of *E. maura* and *E. petrakii* on European beech. None of the three species was detected on more than one test tree, and *E. petrakii* was the only species from which more than one isolate was obtained. *Eutypa petrakii* was isolated exclusively from symptomatic tissue (trunk, xylem and cambial tissue), *E. maura* and *Eutypa spinosa* (Pers.) Tul. & C. Tul. only from asymptomatic tissue. *Eutypa petrakii* has been detected on various tree and shrub species (Rolshausen et al. 2006). The extent to which *E. petrakii* might occur as a serious pathogen on European beech could not be conclusively clarified. *Eutypa maura* is a common inhabitant of living and dead tissue of sycamore (e.g. Trouillas and Gubler 2004; Unterseher et al. 2005; Brglez et al. 2020). As far as the authors know, there is no evidence that *E. maura* occurs as a pathogen. *Eutypa spinosa* is known to cause strip-canker on European beech, similar to the symptoms of *B. nummularia* (Hendry et al. 1998) and has been previously associated with VLB by Langer and Bußkamp (2023).

Conclusion and outlook

The present study has created a unique data set on fungi in the context of VLB. The damage progression of VLB appears to be heavily influenced by the two species *B. nummularia* and *N. coccinea*. However, there seems to be a large number of species that are to a different extent involved in the damage process, depending on the site, maybe even the characteristics of the individual tree or stage of disease progression. The vast majority of these fungi are already endophytic in the host tissue and react sensitively to a reduction in the vigour of the host, particularly through drought (Desprez-Loustau et al. 2006). As the site has a major effect on the fungi that are present as endophytes in European beech trees, the large study area in the present study is certainly a reason for the high number of taxa detected in comparison to some of the studies mentioned. It is likely that even more taxa would have been detected in the present study if additional sites were sampled. Similar to the results of Petrini and Fisher (1988) and Ceccarelli (2011), the influence of the sampled compartment and tissue type on the isolated fungal community of European beech can be confirmed. It has been shown that some species were specific to a single tree compartment or tissue type. In some cases, representatives of a particular order were found exclusively or at least in high abundance in one individual tree compartment, e.g. MTs of *Pezizales* and *Diaporthales* in the twigs. While the fungal community in asymptomatic and symptomatic tissue also seems to differ, it could not be shown that the fungal community in asymptomatic tissue differs with regard to the vitality of the host tree. The results of the present study give further reason to test that *B. nummularia* may be suitable as a vitality indicator species for European beech, especially with regard to heat and drought stress. It would be of considerable interest to undertake comparable studies in northern Germany, where VLB is currently not common (Langer et al. 2020), with the prospect of comparing the endophyte community with that of central Germany. In addition, it must be investigated whether pathogens that have already been detected as an endophyte in the tissue of adult European beeches are also present in the regeneration in the understorey of the infected matured trees. These pathogens may cause damage when the regeneration reaches a certain height and is therefore more susceptible to damage from drought (Bennett et al. 2015; Stovall et al. 2019). Finally, to fulfil Koch's postulates, further pathogenicity and wood decay tests are recommended for potential pathogens associated with VLB which have not yet been addressed by Langer and Bußkamp (2021) and Tropf et al. (2022).

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Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval Not applicable.

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