DGfM

ORIGINAL ARTICLE

Fungi associated with stem collar necroses of *Fraxinus excelsior* affected by ash dieback

Sandra Peters[1] · Sebastian Fuchs[2] · Steffen Bien[1] · Johanna Bußkamp[1] · Gitta Jutta Langer[1] ·
Ewald Johannes Langer[3]

Abstract

In recent decades the vitality and physical stability of European ash trees in Germany have been reduced by European ash dieback, especially when associated with stem collar necroses and rots. This study was carried out to investigate the composition of the fungal communities associated with stem collar necroses. Filamentous fungi were isolated from 58 ash trees out of nine forest stands in northern, eastern, and central Germany. Obtained isolates were identified to a genus or species level by means of morphological and molecular analyses. In total 162 morphotypes including endophytic, saprotrophic, and pathogenic fungi were isolated. For 33 species found no prior reports from *Fraxinus excelsior* were recognised, including *Cryptostroma corticale* and *Diplodia sapinea*. None of the identified species were found at all studied sites, though *Diplodia fraxini* was the most common fungus with regard to frequency within all isolates, occurring at seven sample sites. This species is followed by *Hymenoscyphus fraxineus*, *Armillaria* spp., *Neonectria punicea*, *Diaporthe* cf. *eres*, *Fusarium* cf. *lateritium*, and *Paracucurbitaria* sp. in order of frequency within all isolates. The aforementioned species are characterised and analysed in respect to their occurrence in stem collar necroses and at sample sites. The influence of site conditions on the fungal composition was described for five intensively sampled sites with a minimum of five studied trees (Schwansee, Rhüden, Berggießhübel, Satrup, and Schlangen). The sampling site of Schlangen was further subdivided into four subplots with different positions in the terrain. In the remaining four extensive sample sites, either one or two trees, respectively, were sampled and analysed (Oranienbaumer Heide, Woltershausen, Wolfenbüttel, and Neuhege). Over all sample sites, fungal communities of symptomatic stem tissue are similar concerning the most frequent fungi, but vary greatly according to singularly isolated fungi.

Keywords *Fraxinus excelsior* · Endophytes · Fungal communities · Ash dieback · Stem collar necroses

Section Editor: Claus Baessler

✉ Sandra Peters
Sandra.Peters@nw-fva.de

1 Department of Forest Protection, Northwest German Forest Research Institute (NW-FVA), D-37079 Göttingen, Germany

2 Department of Environmental Control, Northwest German Forest Research Institute (NW-FVA), D-37079 Göttingen, Germany

3 Fachbereich 10 Naturwissenschaften, Institut für Biologie, Fachgebiet Ökologie, Universität Kassel, D-34127 Kassel, Germany

Introduction

Since the early 1990s, the European ash (*Fraxinus excelsior* L.) is threatened by European ash dieback, caused by *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz, & Hosoya (*Helotiaceae*, *Ascomycota*). First disease reports came from Poland and Lithuania (Przybyl 2002; Lygis et al. 2005). In Germany, the severe disease was observed since 2002 and the causal agent was first proven in the year 2006 (Heydeck et al. 2005; Schumacher et al. 2007). Meanwhile, this invasive fungal pathogen has become widespread in Europe. Affected European ash (hereafter referred to as ash) trees of all ages show a broad range of symptoms, such as leaf necrosis, wilting, shoot blight, inner bark discolorations, sunken cankers, epicormic shoots, as well as stem collar, and root necrosis (Gross et al. 2014; Langer 2017). Reduction of

tree stability and increase of mortality of ash is often connected to stem collar necrosis, which progresses towards xylem and heartwood. Stem collar necrosis can occur on trees with or without crown symptoms of ash dieback, but is often observed on diseased trees (Schumacher et al. 2009; Husson et al. 2012; Enderle et al. 2017; Langer 2017; Meyn et al. 2019). Stem collar necroses are defined as basal lesions with necrotic tissue on the outside and the inside of the stem mainly caused by fungi (Langer 2017). The actual shape of stem collar necroses depends on different factors, such as individual progress or associated fungi. Advanced necroses are often associated with wood rot caused by fungi colonising the stem following the initial infection by *H. fraxineus* (Langer 2017).

Even though many fungi are reported from *F. excelsior* (981 different species according to the USDA website, Farr and Rossman 2022, retrieved on 17.06.2022) fungi associated with necrotic stem tissue of ash, specifically in Germany, have rarely been described (Enderle et al. 2017; Langer 2017; Meyn et al. 2019). Most frequently isolated from the necroses at the collar base of diseased ash trees were *Armillaria* spp., *Diaporthe eres* Nitschke, *Diplodia* spp., *Fusarium avenaceum* (Fr.) Sacc., *F. lateritium* Nees, *Fusarium solani* (Mart.) Sacc. (syn. *Neocosmospora solani* (Mart.) L. Lombard & Crous), *H. fraxineus*, and *Neonectria punicea* (J. C. Schmidt) Castl. & Rossman (Lygis et al. 2005; Langer 2017; Meyn et al. 2019; Linaldeddu et al. 2020). A strong evidence for *H. fraxineus* being the causal agent of ash dieback was given by Chandelier et al. (2016), who proved occurrence of *H. fraxineus* in the majority of symptomatic tissue of ash stem collar. Langer (2017) confirmed frequent isolation from stem collar necroses and the assignment as primary agent. But not every type of stem collar necrosis must be primarily caused by *H. fraxineus*. Langer (2017) showed that basal necroses can be caused by *Phytophthora* under special site conditions, as found in floodplain forests or by *Armillaria* spp. on weakened ash trees. The path of infection by *H. fraxineus* still remains unknown and little is understood about the influence of environmental factors on stem collar necroses. Site characteristics, such as moisture content, are assumed to affect disease severity. Kenigsvalde et al. (2010) and Marçais et al. (2016) determined that disease severity correlates positively with soil humidity conditions. It has been suggested that stem collar necroses development and extent is also related to moist conditions or humid topographical positions (Marçais et al. 2016). Therefore the design of this study covers a wide range of water supply types at sampling sites. The composition of the forest stands combined with their nutrient and water availability could be a factor in assessing differences in fungal diversity per stand. Most trees moderately and severely damaged due to ash dieback were observed at forest sites with a high soil organic matter content and a neutral to slightly alkaline soil pH (Turczański et al. 2019). Hence, it can be suspected that fungal composition depends on soil and water availability just as well (Linaldeddu et al. 2011; Salamon et al. 2020).

This study is conducted as part of the demonstration project FraxForFuture and the sub-network FraxPath (Langer et al. 2022). The aims of this research are to fill knowledge gaps concerning the α-diversity of cultivable *Dikarya* Hibbett, T. Y. James & Vilgalys associated with stem collar necroses of trees affected by ash dieback and the composition of their fungal communities. Therefore, fungi associated with necrotic stem bases of ash were isolated and identified from 58 ash trees in order to determine the continuity and the frequency of *H. fraxineus* and secondary fungi. The role of the most frequent fungi in the process of stem collar necroses formation is discussed.

Materials and methods

Sampling sites

In total, six federal states of Germany (Lower Saxony, North Rhine-Westphalia, Saxony, Saxony-Anhalt, Schleswig-Holstein, and Thuringia) were investigated. Nine mixed broad-leaved forest stands with a substantial share of *F. excelsior* affected by ash dieback were selected in order to cover different sites with a wide range of soil water supply types (Table 1 and Fig. 1). The sample sites are located in northern, eastern, and central Germany with sub-oceanic to sub-continental temperate zones. All sites are eutrophic and cover the most common substrates of ash stands in Germany. Basic soil and geological data were acquired by using geological maps with a high resolution (scale 1:25000) of the respective federal geology departments and the forest inventory and forest site mapping data sets of the federal forestry authorities. Additional data from soil core sampling, soil profiles, and soil analyses were available for the sampling sites of Rhüden, Berggießhübel, Schwansee, and Schlangen because these sites are part of other studies associated with soil inventories: The site of Rhüden is part of the national forest soil inventory. Schwansee and Berggießhübel correspond to the intensive monitoring plots "TH_1 Schwansee" and "SN_2 Bienhof" of the research cluster FraxForFuture (Langer et al. 2022). The largest forest stand continuously including ash trees investigated in this study (Schlangen) has a pronounced relief and was divided into four subplots to investigate different positions in the terrain. In this case, intensive soil exploration was conducted to differentiate between the four subplots, including pedological assessments and soil sampling from soil profiles.

The shallow sites (Rhüden, Schlangen 2) in exposed terrain positions on limestone with high coarse soil fractions and low water storage capacities represent the driest

Table 1 Sampling site information sorted by sampling date; all forest sites are eutrophic

Forest site (coordinates UTM)	Sampling classification (sample number; sample date)	Metres above sea level	Exposition and inclination	Climate	Soil water supply	Soil and bedrock	Mixture of tree species in stand
Oranienbaumer Heide (33 U 318931 5730457)	Extensive sampling (1; 19.10.2020)	90	Flat	Sub-continental	Slightly moist, fluctuating ground water regime	Diluvial sands, boulder clay	*Quercus robur, Fagus sylvatica, Carpinus betulus, Betula pendula, Fraxinus excelsior*
Woltershausen (32 U 564973 5757894)	Extensive sampling (2; 20.10.2020 and 04.03.2021)	289	SSE, moderate inclination (9–18°)	Weakly sub-atlantic	Slightly moist	Clayey loam over cretaceous limestone	*Fagus sylvatica, Acer pseudoplatanus, Fraxinus excelsior*
Wolfenbüttel (32 U 632092 5778288)	Extensive sampling (1; 27.10.2020)	238	SE, slight inclination (0–9°)	Weakly sub-atlantic	Moist, high water holding capacity	Colluvial deposit over upper Muschelkalk (triassic limestone)	*Fagus sylvatica, Acer pseudoplatanus, Fraxinus excelsior*
Schwansee (32 U 646432 5660792)	Intensive sampling (8; 10.11.2020)	163	Flat	Weakly sub-continental	Gleysol, ground water influenced	Carbonatic lake gravel deposit, lake sediment	*Fraxinus excelsior, Quercus robur, Populus nigra, European alder*
Rhüden (32 U 579914 5757111)	Intensive sampling (10; 22.02.2021)	235	WSW, strong inclination (18–27°)	Weakly sub-atlantic	Slightly dry, well drained, low water holding capacity	Lower Muschelkalk, (triassic limestone) with shallow loess cover	*Fraxinus excelsior, Fagus sylvatica, Acer pseudoplatanus, Prunus avium*
Neuhege (32 U 593054 6010661)	Extensive sampling (1; 08.06.2021)	53	ESE, slight inclination (0–9°)	Moderately sub-atlantic	Stagnosol/gleysol, ground water influenced	Marly till, boulder clay	*Quercus robur, Alnus glutinosa, Fraxinus excelsior, Fagus sylvatica*
Berggießhübel (33 U 426320 5629803)	Intensive sampling (11; 01.07.2021)	475	NNW, slight inclination (0–9°)	Sub-continental, sub-montane	Slightly moist, slope water influenced, slightly stagnic	Basalt and gneiss solifluction soil with loamy loess cover	*Betula pendula, Acer pseudoplatanus, Fraxinus excelsior, Tilia cordata, Quercus robur, Prunus avium, Sorbus aucuparia*
Satrup (32 U 546373 6072699)	Intensive sampling (5; 06.07.2021)	49	ESE, slight inclination (0–9°)	Moderately sub-atlantic	Slightly moist, high water holding capacity, slightly stagnic	Marly till, boulder clay	*Fagus sylvatica, Fraxinus excelsior*
Schlangen 1 (32 U 492453 5740809)	Intensive sampling (3; 01.11.2021)	274	valley bottom, NNW, slight inclination (0–9°)	Moderately sub-atlantic	Moist, high water holding capacity, slightly stagnic	Colluvial deposit, silty loam	*Fagus sylvatica, Fraxinus excelsior, Acer pseudoplatanus, Prunus avium*
Schlangen 2 (32 U 492621 5741084)	Intensive sampling (6; 08.11.2021)	299	upper slope, S, strong inclination (18–27°)	Moderately sub-atlantic	Slightly dry, low water holding capacity	Cretaceous limestone, very shallow loess cover (clayey loam)	*Fagus sylvatica, Fraxinus excelsior, Acer pseudoplatanus, Prunus avium*

Table 1 (continued)

Forest site (coordinates UTM)	Sampling classification (sample number; sample date)	Metres above sea level	Exposition and inclination	Climate	Soil water supply	Soil and bedrock	Mixture of tree species in stand
Schlangen 3 (32 U 492515 5740880)	Intensive sampling (4; 01.11.2021)	305	upper slope, NNW, slight inclination (0–9°)	Moderately sub-atlantic	Slightly moist, moderate water holding capacity	Cretaceous limestone, moderate loess cover (clayey loam)	*Fraxinus excelsior, Fagus sylvatica, Acer pseudoplatanus, Prunus avium*
Schlangen 4 (32 U 492738 5741014)	intensive sampling (6; 01., 02., 08.11.2021)	316	hill top, W, slight inclination (0–9°)	Moderately sub-atlantic	Slightly moist, moderate water holding capacity	Cretaceous limestone, moderate loess cover (clayey loam)	*Fagus sylvatica, Acer pseudoplatanus, Fraxinus excelsior, Prunus avium*

end of the ecological niche of ash. Several other sites have moderate (Schlangen 3, Schlangen 4, Wolterhausen) or high water storage capacities (Wolfenbüttel, Schlangen 1) because of medium to deep loamy loess covers or colluvial deposits. The remaining sites are primarily characterised by either stagnic soil conditions (Satrup, Berggießhübel), slope water influence (Berggießhübel), or groundwater influence (Oranienbaumer Heide, Schwansee, Neuhege). Soil substrate, water retention capacity, terrain relief, climate, and presence/absence of groundwater or stagnic soil properties (Table 1) were combined to create a ranking of site water supplies of the sampling sites (Online Resource 1 and Online Resource 2).

Between one and nineteen trees were excavated and sampled per site. Hence, the sampling sites were divided into intensive and extensive sampling sites. The intensive sampling sites had a minimum of five and a maximum of 19 sample trees. Intensive sampling was conducted in Rhüden (10 sampled trees), Satrup (5), Berggießhübel (11), Schwansee (8), and Schlangen (19). Each intensive sampling site or subplot was 0.2–0.5 ha in size. The extensive sampling sites with only one or two examined ash trees were taken into account only for fungal occurrence: Woltershausen (2 sampled trees), Wolfenbüttel (1), Neuhege (1), and Oranienbaumer Heide (1). These individual trees were included to increase the sample set and the distribution of investigated stem collar necroses and their associated fungi.

Sampled trees

In total 58 ash trees were sampled, including six trees initially selected as control trees (two in Berggießhübel, one in Satrup, and three in Schlangen; Online Resource 3). The diameter at breast height of the sampled ash trees ranged from approximately 7–25 cm. The age of the sample trees ranged from 15 up to 80 years. The majority was approximately 40 years old. Classification of stem base and crown condition of the studied trees was carried out according to the guidelines of Peters et al. (2021a, b). Additionally, the neighbouring tree species occurring in the studied stands were noted (Table 1).

Ash trees were felled in the years 2020–2021 and cut at least 15 cm above the visible necrotic area. Subsequently, trunk bases and the uppermost parts of the main roots were dug out with picks and shovels. Depending on soil structure (rock content) final roots were cut by chainsaw with a .325-in. Rapid Duro 3 (RD3), 1.6-mm chainsaw chain (STIHL AG & Co. KG, Dieburg, Germany). The stem collars were transported to laboratory in clean and marked plastic bags.

Fig. 1 Sampling sites in Germany divided in intensive (red) and extensive (yellow) sampling sites with a detailed view of the special study site Schlangen and its feature of splitting in four subplots with different terrain positions (resources: QGIS 3.24 © GeoBasis-DE/BKG (2022), DGM1 © GeoBasis NRW (2021))
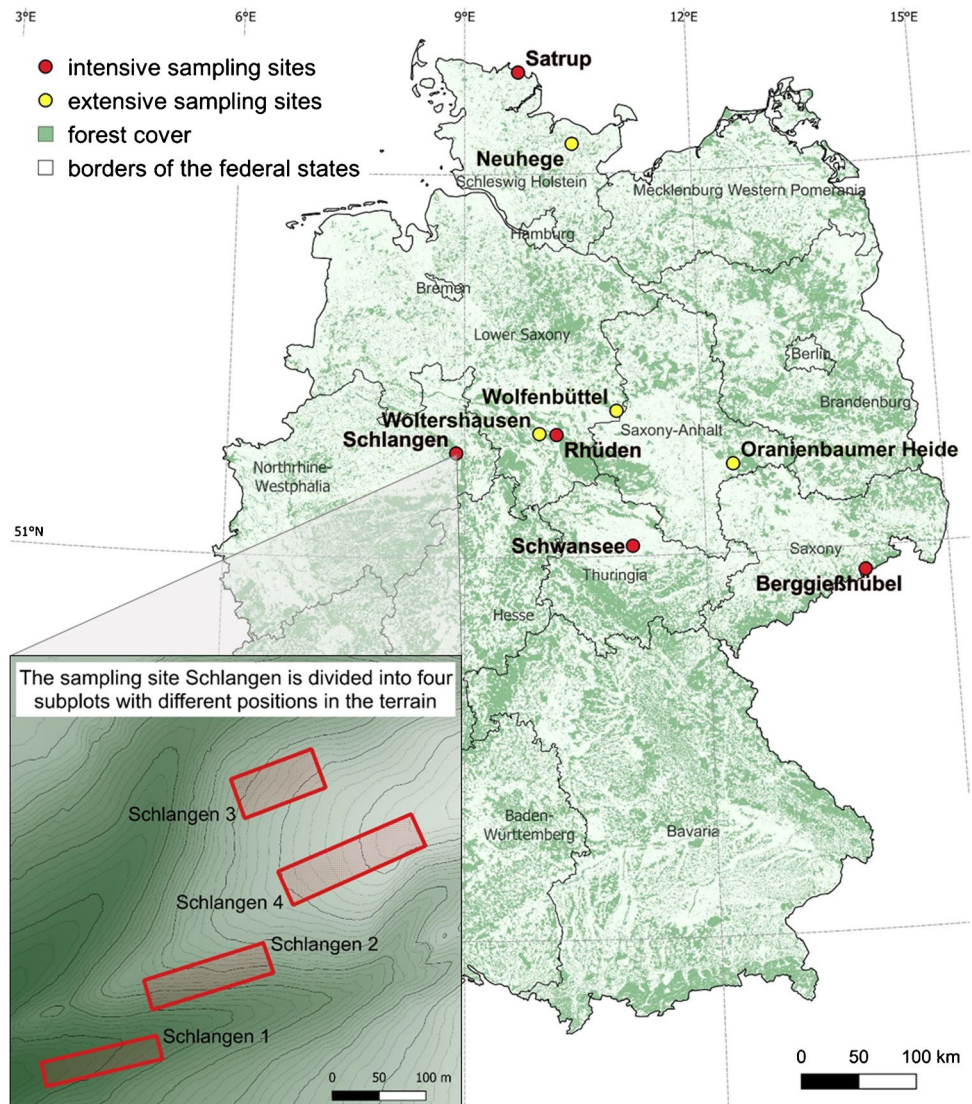
Isolation of fungi

In preparation for fungal isolation, tree stems were cleaned with a coarse brush under tap water. After air-drying overnight, the samples were processed in the laboratory. Each sample was photographed for documentation. Bark was removed from symptomatic stem areas at the transition zones of living and dead woody tissue. Depending on the thickness of the bark either a sterilised knife or a scalpel was used for removal of the bark. The exposed tissue was sprayed with 70% ethanol all over the necrosis. For chipping of wood tissue samples a chisel and hammer were used. All tools were sterilised by flame shortly before each use. The top tissue layer of the sample was discarded and only the sterile layer directly underneath was incubated. The number of wood chips taken from each tree varied depending on the size of the necrosis. 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). Once the surface of necroses was processed, stem collars were cut longitudinally with a band saw and carefully sanded for better visualisation of the discolorations. The longitudinal sections were treated according to the isolation method for the surface of necrosis described above. Wood chips were taken at the edge of the necroses and at the transition areas of different discolorations. The process was repeated with the cross section of the stem (Fig. 2).

The petri dishes containing wood chips were incubated at room temperature under ambient daylight for four weeks. The cultures were checked for isolates once a week. Emerging mycelia of filamentous fungi were sub-cultured into pure cultures. The pure cultures were grouped into morphotypes (MT) based on similarity of
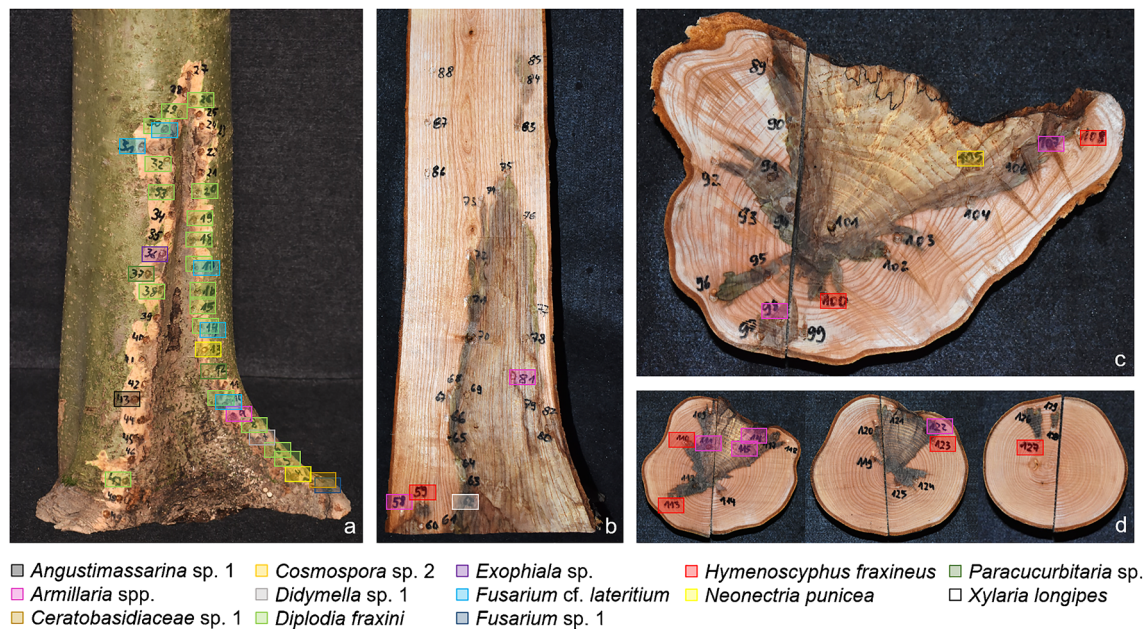
Fig. 2 Fungi associated with the stem collar necrosis of ash tree num-
ber 53 (Online resource 3) from the sampling site Schlangen 2. Iso-
lation loci are numbered. **a** Sampled stem base from the outside, **b**
longitudinal-section of stem base with visible wood discoloration, **c**
basal cross-section of stem base, and **d** cross-section of the stem base
above ground level every 10–15 cm

colony morphology. At least one representative culture
for each MT was stored in MYP slants at 4 °C at the fun-
gal culture collection of the Northwest German Forest
Research Institute (NW-FVA). Beside the MT assignment,
contaminated or overgrown fungi were summarised under
"Fungus sp.".

The frequency of each isolated fungal MT within all
isolates (f_{MT}) was specified as the percentage of this par-
ticular MT in all isolates. To measure the ratio of mor-
photype isolates to isolation attempts, the frequency of
isolated fungal MT in relation to the total amount of wood
chips (f_{WC}) is used. Continuity of isolated MT is defined as
the number of sampled trees where the MT was detected
in relation to the total number of sampled trees. Analyses
of the fungal diversity found in this study were conducted
using RStudio (v. 4.1.2, R Core Team 2021). The pack-
ages used were tidyverse (Wickham et al. 2019), ggplot 2
(Wickham 2016), and ggVennDiagram (Gao 2021).

The dependency of f_{WC} of the most commonly isolated
fungal MT on site water supply ranks of the intensive sam-
pling sites was tested by using Dirichlet regression (Maier
2014) and the R add-on package DirichletReg (Maier
2021). A non-parametric test is necessary because site
water supply is an ordinal variable. The ranks are based
on a combination of soil water retention, soil stagnic prop-
erties, relief, topography, and climate characteristics of
the study sites and correspond to the site descriptions in
Table 1 and Online Resource 1.

Molecular analysis

For molecular analysis, at least one representative strain
from each MT was chosen. Mycelium was placed in 1.5-ml
Eppendorf tubes with three glass beads (3 mm) and 150 µl
of TE buffer (10 ml 1 mmol Tris HCl (pH 0.8), 2 ml 0.5
mmol EDTA; Carl Roth, Karlsruhe, Germany), and crushed
in a Mixer Mill MM 200 (Retsch, Haan, Germany) with
25 vibrations per second for 90 s. Subsequently, genomic
DNA was extracted following the protocol of Izumitsu et al.
(2012).

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).
Additionally, for a selection of strains belonging to *Armil-
laria*, a partial sequence of the translation elongation factor
1α (*EF-1α*) was amplified using the primer pair EF595F +
EF1160R (Kauserud and Schumacher 2001). The PCR mix-
ture consisted of 1 µl of DNA and 19 µl mastermix which
contained 2.5 µl 10× PCR reaction buffer (with 20 mM
$MgCl_2$, Carl Roth, Karlsruhe, Germany), 1 µl of each primer
(10 mmol), 2.5 µl $MgCl_2$ (25 mmol), 0.1 µl Roti®-Pol Taq
HY Taq polymerase (Carl Roth, Karlsruhe, Germany) and
2.5 µl of 2 mmol dNTPs (Biozym Scientific GmbH, Hes-
sisch Oldendorf, Germany). Each reaction was topped up
to a volume of 20 µl by adding sterile water. A StepO-
nePlus™ PCR System (Applied Biosystems, Waltham,

Massachusetts, US) was used to carry out the DNA amplifications. The PCR conditions for the amplification of the ITS and *EF-1α* regions were set according to Bien et al. (2020) and Guo et al. (2016), respectively. A 1% agarose gel was used to visualise the PCR products. The products were sent to Eurofins Scientific Laboratory (Ebersberg, Germany) for sequencing. Initially, PCR samples of the ITS region were sequenced using the forward reaction (primer ITS-1F). In case of imprecise results, additionally, reverse reactions (primer ITS-4) were sequenced. PCR products of the *EF-1α* sequence region were sequenced by the respective forward and reverse reactions. All resulting sequences were visually checked and edited as follows using BioEdit Sequence Alignment Editor (v. 7.2.5; Hall 1999). Consensus sequences were generated, for all strains with forward and reverse sequences available. Defective sequence beginnings and ends were trimmed and erroneous nucleotide allocations corrected. Sequences were submitted to GenBank (Table 2).

Identification of fungi

Only cultivatable Dikarya fungi were investigated, however, yeasts were not taken into account. Only isolates which are clearly growing from the wood chips were determined. Obvious contamination with filamentous fungi was not considered. The genus *Trichoderma* was not included in the analyses, because it is difficult to assess whether these very fast-growing fungi were contaminations or real outgrowth from the wood.

MT were assigned to fungal taxa based on morphological observation and molecular analysis of representative strains following the method of Guo et al. (2000). For fungal taxon determination blastn searches based on ITS sequences were conducted on the GenBank database (http://www.ncbi.nlm. nih.gov/genbank, Altschul et al. 1997) excluding uncultured/ environmental sample sequences from the search. Results were critically interpreted with emphasis on well-curated culture collections, such as the Westerdijk Fungal Biodiversity Collection (CBS). In general, blastn results on a species level below a threshold of 98% identity were not trusted to be accurate enough for final determination. In case no definite affiliation to a specific taxonomic level was possible, for example because of more than one hit with a threshold of over 98% identity, the identification was marked by cf. (confer) to indicate uncertainties. The results were re-checked against literature and previously identified cultures from the institute's collection for confirmation. In addition to blastn searches, extended analyses for taxon determination on a species level were conducted for isolates belonging to *Diplodia* and *Armillaria* due to the considerable number of isolates from these genera. Phylogenetic analyses were conducted based on an ITS sequence-dataset and an ITS-*EF-1α* concatenated sequence-dataset for *Diplodia* and

Armillaria isolates, respectively, including appropriate reference sequences retrieved from GenBank. Both analyses 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 (Online Resource 4 and 5).

As a rule, current names were applied according to the nomenclatorial database MycoBank (Robert et al. 2005). Two exceptions from this generally applied rule have been made in the case of the MT designated here as *Fusarium solani* s. l. and *Armillaria gallica*. In the case of *F. solani* the authors are aware that there is a currently unsettled discussion about correct delimitation of this species, or rather species complex. Here we follow the "classic" nomenclature substantiated by Geiser et al. (2021) obverse that promoted by Lombard et al. (2015) and Sandoval-Denis et al. (2019) who place said species complex in the genus *Neocosmospora* (*N. solani* (Mart.) L. Lombard & Crous). The currently applied name for *A. gallica*, according to MycoBank is *A. lutea* Gillet. However, Marxmüller (1992) stated that *A. lutea* is a nomen ambiguum and the later introduced name *A. gallica* Marxm. & Romagn. is to be used (Burdsall and Volk 1993).

Results

Sampled trees

The crown health status regarding ash dieback of the sampled trees ranged from vital and nearly without dieback symptoms to almost dead. All sampled trees (Online Resource 3), except for the six planed control trees, had obvious stem collar necroses through discoloured, sunken, and in some cases ruptured bark. In cases of ruptured bark, subjacent stem collars were neither completely rotting nor dead. During processing of the sample trees in the laboratory two out of the six control trees (trees 32 and 42, Online Resource 3) showed necrotic woody tissue inside the stem base. Consequently, these two trees were transferred to and analysed as sample trees (symptomatic sample trees $n = 54$, asymptomatic control trees $n = 4$).

Isolated fungi

In total, 4401 wood chips of stem collar tissue originating from 58 trees were incubated. A total of 1511 isolates (from which 226 were not identifiable due to contaminations; marked as Fungus sp.) from 1413 wood chips (32%) were observed. 958 chips (22%) showed no outgrowth at all after four weeks of incubation, while 960 (22%) chips had been overgrown by fast-growing fungi from adjacent wood chips

Table 2 List of isolated fungi sorted alphabetically within orders; column "Species" is the name finally determined by the authors; fungi causing wood rot are marked with *; probable first reports of species isolated from *F. excelsior* are marked with [FR]; Phylum: A = *Ascomycota*, B = *Basidiomycota*. Datum Blast: 22./23.06.2022

Species	Phylum	Order	NW-FVA ID	ITS Accession no.	n isolates	Frequency (%)	Continuity (%)	Sites isolated from	ITS NCBI Blast results			Reference
									Basis of identification	ID	Identity (%)	
Acremonium sp.	A	*Hypocreales*	8031	OP023272	1	0.07%	2%	1	*Acremonium variecolor*	KF313108.1	99.63%	Min et al. 2014
Akanthomyces sp.[FR]	A	*Hypocreales*	6958	OP023220	1	0.07%	2%	1	*Akanthomyces muscarius*	MH858126.1	100.00%	Vu et al. 2019
Alternaria infectoria	A	*Pleosporales*	5940	OP023162	3	0.20%	5%	2	*Alternaria infectoria*	MT561399.1	100.00%	Langer and Bußkamp (unpublished)
Alternaria sp.	A	*Pleosporales*	6119	OP023191	5	0.33%	5%	2	*Alternaria angustiovoidea*	MH861939.1	100.00%	Vu et al. 2019
Angustimassarina sp. 1	A	*Pleosporales*	5951	OP023165	2	0.13%	3%	1	*Angustimassarina lonicerae*	KY496759.1	100.00%	Tibpromma et al. 2017
Angustimassarina sp. 2	A	*Pleosporales*	6207	OP023203	4	0.26%	3%	2	*Angustimassarina lonicerae*	KY496759.1	98.61%	Tibpromma et al. 2017
Armillaria spp.*	B	*Agaricales*	5952	OP023166	158	10.46%	50%	5	*Armillaria gallica*	KX618575.1	99.74%	Denman et al. 2017
									Armillaria cepistipes	MK966557.1	99.62%	Wei (unpublished)
Ascocoryne sp. 1*	A	*Helotiales*	7103	OP023244	3	0.20%	3%	2	*Ascocoryne* sp.	MH682237.1	100.00%	Johnston and Park (unpublished)
Ascocoryne sp. 2*	A	*Helotiales*	8030	OP023271	1	0.07%	2%	1	*Ascocoryne solitaria*	HM152545.1	100.00%	Griffin et al. 2010
Aureobasidium pullulans	A	*Dothideales*	6176	OP023196	5	0.33%	7%	3	*Aureobasidium pullulans*	KT693733.1	99.82%	van Nieuwenhuijzen et al (unpublished)
Beauveria bassiana	A	*Hypocreales*	6979	OP023247	2	0.13%	2%	1	*Beauveria bassiana*	MN122432.1	100.00%	Gasmi et al (unpublished)
Beauveria pseudobassiana	A	*Hypocreales*	6001	OP023182	1	0.07%	2%	1	*Beauveria pseudobassiana*	NR_111598.1	100.00%	Schoch et al. 2014
*Biscogniauxia nummularia*ature*	A	*Xylariales*	5926	OP023156	3	0.20%	5%	1	*Biscogniauxia nummularia*	NR_153649.1	100.00%	Wendt et al. 2018

Table 2 (continued)

Species	Phylum	Order	NW-FVA ID	ITS Accession no.	n isolates	Frequency (%)	Continuity (%)	Sites isolated from	ITS NCBI Blast results			Reference
									Basis of identification	ID	Identity (%)	
Bispora sp.*	A	Incertae sedis	8329	OP023300	1	0.07%	2%	1	Bispora antennata	KY462800.1	99.03%	Baral and Marson (unpublished)
Bjerkandera adusta*	B	Polyporales	5943	OP023163	5	0.33%	9%	3	Bjerkandera adusta	MK322310.1	100.00%	Chilean Collection of Microbial Genetic Resources (unpublished)
Botrytis cf. cinerea	A	Helotiales	6874	OP023212	6	0.40%	9%	3	Botrytis cinerea	MH860108.1	100.00%	Vu et al. 2019
Cadophora dextrinospora FR	A	Helotiales	7026	OP023226	11	0.73%	10%	3	Cadophora dextrinospora	NR_119489.1	100.00%	Schoch et al. 2014
Cadophora melinii	A	Helotiales	6985	OP023248	1	0.07%	2%	1	Cadophora melinii	MH791342.1	99.02%	Alborés 2018
Cadophora ramosa FR	A	Helotiales	6194	OP023202	8	0.53%	7%	4	Cadophora ramosa	MN232956.1	100.00%	Bien and Damm 2020a
Cadophora sp. 1	A	Helotiales	7100	OP023258	3	0.20%	3%	2	Cadophora malorum	MT561395.1	100.00%	Langer and Bußkamp (unpublished)
Cadophora sp. 2	A	Helotiales	8478	OP023311	1	0.07%	2%	1	Cadophora malorum	MT561395.1	100.00%	Langer and Bußkamp (unpublished)
Calycina herbarum FR	A	Helotiales	8287	OP023287	1	0.07%	2%	1	Calycina herbarum	MZ159660.1	99.42%	Gaya et. al (unpublished)
Camposporium sp.	A	Incertae sedis	8336	OP023307	1	0.07%	2%	1	Camposporium ramosum	MH866030.1	96.17%	Vu et al. 2019
Cephalotrichiella penicillata FR	A	Microascales	7042	OP023232	1	0.07%	2%	1	Cephalotrichiella penicillata	NR_153893.1	99.82%	Crous et al. 2014
Ceratobasidiaceae sp. 1	B	Cantharellales	8003	OP023265	2	0.13%	3%	1	Ceratobasidium sp.	KX786242.1	99.84%	Lakshman et al. 2017
Ceratobasidiaceae sp. 2	B	Cantharellales	8005	OP023267	1	0.07%	2%	1	Ceratobasidiaceae sp.	KX610454.1	98.26%	Yokoya et al. 2017
Chaetomiaceae sp.	A	Sordariales	8332	OP023303	1	0.07%	2%	1	Chaetomium sp.	MK182798.1	99.81%	Li and Xu (unpublished)
Chaetomium globosum	A	Sordariales	8326	OP023297	1	0.07%	2%	1	Chaetomium globosum	MH858130.1	99.81%	Vu et al. 2019

Table 2 (continued)

Species	Phylum	Order	NW-FVA ID	ITS Accession no.	n isolates	Frequency (%)	Continuity (%)	Sites isolated from	ITS NCBI Blast results			Reference
									Basis of identification	ID	Identity (%)	
Chaetomium sp.	A	Sordariales	8323	OP023295	1	0.07%	2%	1	Chaetomium subaffine	MH862288.1	100.00%	Vu et al. 2019
Chloridium virescens var. caudigerum	A	Chaetosphaeriales	8290	OP023290	6	0.40%	7%	1	Chloridium virescens var. caudigerum	MH857142.1	99.81%	Vu et al. 2019
Cladosporium sp.	A	Capnodiales	7029	OP023229	2	0.13%	3%	1	Cladosporium iridis	EU167591.1	100.00%	Simon et al. 2009
Clonostachys sp.	A	Hypocreales	5985	OP023174	1	0.07%	2%	1	Clonostachys rosea f. catenulata	NR_165993.1	99.25%	Vu et al. 2019
Coniochaeta velutina [FR]	A	Coniochaetales	8334	OP023305	1	0.07%	2%	1	Coniochaeta velutina	MK656234.1	99.81%	van der Merwe et. al (unpublished)
Coprinellus cf. domesticus*	B	Agaricales	6011	OP023184	1	0.07%	2%	1	Coprinellus domesticus	KP132301.1	100.00%	Irinyi et al. 2015
Coprinellus cf. radians 1*	B	Agaricales	6962	OP023221	1	0.07%	2%	1	Coprinellus radians	JN943117.1	100.00%	Nagy (unpublished)
Coprinellus cf. radians 2*	B	Agaricales	8590	OP023312	1	0.07%	2%	1	Coprinellus radians	JN943117.1	100.00%	Nagy (unpublished)
Coprinellus disseminates*	B	Agaricales	5991	OP023177	12	0.79%	10%	2	Coprinellus disseminatus	KY977599.1	99.85%	Kranjec (unpublished)
Coprinellus micaceus*	B	Agaricales	6048	OP023189	22	1.46%	19%	3	Coprinellus micaceus	KJ713992.1	100.00%	Jang et al. 2015
Cordycipitaceae sp.	A	Hypocreales	6965	OP023223	1	0.07%	2%	1	Samsoniella hepiali	NR_160318.1	100.00%	Wang et al. 2015
Cosmospora sp. 1	A	Hypocreales	7044	OP023234	1	0.07%	2%	1	Cosmospora sp.	MF495375.1	98.11%	Mejia et. al (unpublished)
Cosmospora sp. 2	A	Hypocreales	7095	OP023262	3	0.20%	5%	2	Cosmospora lavitskiae	KU563624.1	100.00%	Zeng and Zhuang 2016
Cryptostroma corticale* [FR]	A	Xylariales	5932	OP023158	3	0.20%	5%	2	Cryptostroma corticale	MH857008.1	100.00%	Vu et al. 2019
Cyclothyriella rubronotata [FR]	A	Pleosporales	8333	OP023304	1	0.07%	2%	1	Cyclothyriella rubronotata	NR_147651.1	100.00%	Jaklitsch and Voglmayr 2016
Diaporthe cf. eres	A	Diaporthales	5924	OP023155	58	3.84%	43%	7	Diaporthe eres	MK024685.1	100.00%	Hosseini et al. 2021

Table 2 (continued)

Species	Phylum	Order	NW-FVA ID	ITS Accession no.	n isolates	Frequency (%)	Continuity (%)	Sites isolated from	ITS NCBI Blast results			Reference
									Basis of identification	ID	Identity (%)	
Diaporthe cf. *rudis*	A	Diaporthales	6131	OP023194	4	0.26%	7%	1	*Diaporthe rudis*	KC343234.1	100.00%	Gomes et al. 2013
Didymella sp. 1	A	Pleosporales	5939	OP023161	1	0.07%	2%	1	*Didymella macrostoma*	MN588154.1	100.00%	Patejuk et. al (unpublished)
Didymella sp. 2	A	Pleosporales	5982	OP023173	1	0.07%	2%	1	*Didymella* sp.	MW582399.1	99.78%	Wang and Pecoraro 2021
Didymella sp. 3	A	Pleosporales	6147	OP023193	1	0.07%	2%	1	*Didymella pinodella*	MW945409.1	100.00%	Zhao et al. 2021
Didymella sp. 4	A	Pleosporales	6875	OP023213	1	0.07%	2%	1	*Didymella prosopidis*	NR_137836.1	100.00%	Crous et al. 2013
Didymellaceae sp.	A	Pleosporales	8289	OP023289	1	0.07%	2%	1	*Didymella* sp.	MN522472.1	100.00%	Rivedal et al. 2020
Diplodia fraxini	A	Botryosphaeriales	5921	OP023153	280	18.49%	71%	7	*Diplodia fraxini*	MT587349.1	100.00%	Zhang et al. 2021
Diplodia sapinea [FR]	A	Botryosphaeriales	5979	OP023170	1	0.07%	2%	1	*Diplodia sapinea*	NR_152452.1	100.00%	Alves et al. 2006
Geotrichum candidum [FR]	A	Saccharomycetales	6963	OP023222	4	0.26%	3%	1	*Geotrichum candidum*	MF044044.1	100.00%	Rongfeng et al. 2018
Dothiorella sp.	A	Botryosphaeriales	5944	OP023164	1	0.07%	2%	1	*Dothiorella* sp.	KF040058.1	100.00%	Zlatković et al. 2016
Entoleuca sp.	A	Xylariales	6018	OP023187	2	0.13%	3%	1	*Entoleuca* sp.	MN538292.1	100.00%	Pusz et. al (unpublished)
Eutypa cf. *petrakii* var. *hederae*	A	Xylariales	5994	OP023179	5	0.33%	3%	1	*Eutypa petrakii* var. *hederae*	MT153641.1	99.82%	Bien and Damm 2020b
*Eutypa lata**	A	Xylariales	5937	OP023160	20	1.32%	3%	2	*Eutypa lata*	MK547093.1	99.58%	Johnston and Park (unpublished)
Exophiala sp.	A	Chaetothyriales	8390	OP023310	4	0.26%	7%	1	*Exophiala* sp.	LC317596.1	92.77%	Takahashi and Yaguchi (unpublished)
*Flammulina velutipes**	B	Agaricales	8037	OP023274	4	0.26%	2%	1	*Flammulina velutipes*	MK934583.1	99.87%	Chilean Collection of Microbial Genetic Resources (unpublished)
*Fomitopsis betulina** [FR]	B	Polyporales	8324	OP023296	1	0.07%	2%	1	*Fomitopsis betulina*	MF967582.1	99.68%	Yang et al. 2017

Table 2 (continued)

Species	Phylum	Order	NW-FVA ID	ITS Accession no.	n isolates	Frequency (%)	Continuity (%)	Sites isolated from	ITS NCBI Blast results			Reference
									Basis of identification	ID	Identity (%)	
*Fomitopsis pinicola**	B	*Polyporales*	7048	OP023253	1	0.07%	2%	1	*Fomitopsis pinicola*	JQ341137.1	100.00%	Douanla-Meli and Langer 2012
Fusarium cf. lateritium	A	*Hypocreales*	5919	OP023151	53	3.50%	34%	6	*Fusarium lateritium*	MN588156.1	100.00%	Patejuk et. al (unpublished)
Fusarium sambucinum	A	*Hypocreales*	6043	OP023188	1	0.07%	2%	1	*Fusarium sambucinum*	KM231813.1	100.00%	Lombard et al. 2015
Fusarium solani	A	*Hypocreales*	6003	OP023183	15	0.99%	12%	2	*Fusarium solani*	FJ459973.1	100.00%	Zhang et. al (unpublished)
Fusarium sp. 1	A	*Hypocreales*	5920	OP023152	6	0.40%	9%	2	*Fusarium iranicum*	NR_175069.1	100.00%	Sandoval-Denis (unpublished)
Fusarium sp. 2	A	*Hypocreales*	5933	OP023159	1	0.07%	2%	1	*Fusarium sporotrichioides*	MT635298.1	100.00%	Cudowski (unpublished)
Fusarium stercicola	A	*Hypocreales*	7072	OP023275	1	0.07%	2%	1	*Fusarium stercicola*	MG250476.1	100.00%	Šišić et al. 2018
Geotrichum sp.	A	*Saccharomycetales*	6955	OP023237	2	0.13%	3%	2	*Geotrichum sp.*	AY787702.2	99.10%	Lygis et al. 2005
Gliomastix sp.	A	*Hypocreales*	6957	OP023219	2	0.13%	3%	1	*Gliomastix murorum var. felina*	MH864097.1	100.00%	Vu et al. 2019
Graphium sp.	A	*Microascales*	8007	OP023269	1	0.07%	2%	1	*Graphium sp.*	MF782695.1	98.49%	Jankowiak et al. 2019a
*Heterobasidion annosum**	B	*Russulales*	6111	OP023195	2	0.13%	3%	1	*Heterobasidion annosum*	MH859050.1	99.66%	Vu et al. 2019
Humicolopsis cephalosporioides [FR]	A	*Helotiales*	7045	OP023235	1	0.07%	2%	1	*Humicolopsis cephalosporioides*	NR_160150.1	99.24%	Vu et al. 2019
Hymenoscyphus fraxineus	A	*Helotiales*	5922	OP023154	179	11.85%	53%	8	*Hymenoscyphus fraxineus*	MT155386.1	100.00%	Panteleev et. al (unpublished)
*Hypholoma cf. acutum**	B	*Agaricales*	6345	OP023209	2	0.12%	3%	2	*Hypholoma fasciculare*	MT573401.1	100.00%	Alimadadi 2019
Hypocreales sp.	A	*Hypocreales*	6930	OP023217	1	0.07%	2%	1	*Albifimbria verrucaria*	KF887115.1	99.81%	Sun et al. 2014
*Hypoxylon fragiforme**	A	*Xylariales*	6113	OP023192	4	0.26%	7%	2	*Hypoxylon fragiforme*	MH855287.1	100.00%	Vu et al. 2019

Table 2 (continued)

Species	Phylum	Order	NW-FVA ID	ITS Accession no.	n isolates	Frequency (%)	Continuity (%)	Sites isolated from	ITS NCBI Blast results Basis of identification	ID	Identity (%)	Reference
*Hypoxylon howeanum**	A	Xylariales	7046	OP023251	1	0.07%	2%	1	*Hypoxylon howeanum*	MN588210.1	100.00%	Patejuk et. al (unpublished)
*Hypoxylon rubiginosum**	A	Xylariales	6051	OP023190	6	0.40%	7%	3	*Hypoxylon rubiginosum*	MZ045853.1	100.00%	Langer and Bußkamp 2021
Ilyonectria sp. 1	A	Hypocreales	7051	OP023252	4	0.26%	7%	2	*Ilyonectria lusitanica*	NR_156240.1	99.80%	Cabral et al. 2012
Ilyonectria sp. 2	A	Hypocreales	7068	OP023255	1	0.07%	2%	1	*Ilyonectria liliigena*	JF735297.1	99.80%	Cabral et al. 2012
Ilyonectria sp. 3	A	Hypocreales	8271	OP023281	1	0.07%	2%	1	*Ilyonectria protearum*	NR_152890.1	99.12%	Lombard et. al (unpublished)
*Jackrogersella cohaerens**	A	Xylariales	8292	OP023292	3	0.20%	5%	1	*Jackrogersella cohaerens*	MT561400.1	100.00%	Langer and Bußkamp (unpublished)
Jackrogersella sp.	A	Xylariales	6192	OP023200	4	0.26%	7%	3	*Jackrogersella* sp.	MT153658.1	99.80%	Bien and Damm 2020b
Juxtiphoma eupyrena	A	Pleosporales	8182	OP023280	2	0.13%	2%	1	*Juxtiphoma eupyrena*	MG098275.1	100.00%	Bußkamp et al. 2020
Kalmusia sp.	A	Pleosporales	7032	OP023231	1	0.07%	2%	1	*Kalmusia longispora*	JX496097.1	99.62%	Verkley et al. 2014
*Kuehneromyces mutabilis**	B	Agaricales	5988	OP023176	1	0.07%	2%	1	*Kuehneromyces mutabilis*	MH855190.1	99.55%	Vu et al. 2019
Lasionectria sp.	A	Hypocreales	6191	OP023199	1	0.07%	2%	1	*Lasionectria vulpina*	MZ159398.1	99.82%	Gaya et. al (unpublished)
Laxitextum bicolor	B	Russulales	8006	OP023268	1	0.07%	2%	1	*Laxitextum bicolor*	MW742677.1	99.15%	Ma and Zhao (unpublished)
Lepteutypa fuckelii	A	Amphisphaeriales	8284	OP023284	1	0.07%	2%	1	*Lepteutypa fuckelii*	MZ045855.1	100.00%	Langer and Bußkamp 2021
Leptodontidium sp.	A	Helotiales	8338	OP023309	1	0.07%	2%	1	*Leptodontidium* sp.	AB907597.1	96.72%	Fukasawa 2018
Leptosillia muelleri FR	A	Xylariales	6208	OP023204	2	0.13%	3%	2	*Leptosillia muelleri*	NR_164065.1	98.12%	Voglmayr et al. 2019
Lophiotrema rubi FR	A	Pleosporales	8327	OP023298	1	0.07%	2%	1	*Lophiotrema rubi*	AF383963.1	99.80%	Liew et al. 2002

Table 2 (continued)

Species	Phylum	Order	NW-FVA ID	ITS Accession no.	n isolates	Frequency (%)	Continuity (%)	Sites isolated from	ITS NCBI Blast results			
									Basis of identification	ID	Identity (%)	Reference
Lophiotrema sp.	A	*Pleosporales*	8337	OP023308	1	0.07%	2%	1	*Lophiotrema mucilaginosis*	NR_164039.1	100.00%	Phookamsak et al. 2019
Metapochonia bulbillosa FR	A	*Hypocreales*	6876	OP023214	2	0.13%	3%	2	*Metapochonia bulbillosa*	NR_154142.1	100.00%	Zare et al. 2000
Metapochonia suchlasporia FR	A	*Hypocreales*	8272	OP023282	1	0.07%	2%	1	*Metapochonia suchlasporia*	KU668952.1	100.00%	Palmer et al. 2018
Microcera cf. *larvarum*	A	*Hypocreales*	6195	OP023210	3	0.20%	5%	3	*Microcera larvarum*	EU860066.1	100.00%	Bills et al. 2009
Microcera rubra FR	A	*Hypocreales*	7028	OP023228	1	0.07%	2%	1	*Microcera rubra*	MH861019.1	100.00%	Vu et al. 2019
Microsphaeropsis olivacea	A	*Pleosporales*	7039	OP023250	1	0.07%	2%	1	*Microsphaeropsis olivacea*	MT561396.1	100.00%	Langer and Bußkamp (unpublished)
*Mycoacia nothofagi** FR	B	*Polyporales*	8043	OP023278	1	0.07%	2%	1	*Mycoacia nothofagi*	GU480000.1	99.83%	Moreno et al. 2011
Nectriaceae sp. 1	A	*Hypocreales*	5987	OP023175	3	0.20%	5%	2	*Cylindrocarpon* sp.	EF601608.1	99.14%	Belbahri et. al (unpublished)
Nectriaceae sp. 2	A	*Hypocreales*	8286	OP023286	2	0.13%	3%	1	*Dialonectria ullevolea*	MH855229.1	100.00%	Vu et al. 2019
*Nemania serpens**	A	*Xylariales*	6193	OP023201	6	0.40%	9%	3	*Nemania serpens*	MT790321.1	100.00%	Blumenstein et al. 2021
Neoascochyta sp.	A	*Pleosporales*	6968	OP023225	3	0.20%	5%	2	*Neoascochyta exitialis*	MT446179.1	100.00%	Liu (unpublished)
Neobulgaria sp.*	A	*Helotiales*	8331	OP023302	1	0.07%	2%	1	*Neobulgaria* sp.	MH191244.1	100.00%	Meyn et al. 2019
Neocucurbitaria acerina FR	A	*Pleosporales*	5981	OP023172	1	0.07%	2%	1	*Neocucurbitaria acerina*	MF795768.1	99.82%	Jaklitsch et al. 2018
Neocucurbitaria sp.	A	*Pleosporales*	7031	OP023230	1	0.07%	2%	1	*Neocucurbitaria cava*	KR909135.1	100.00%	Travadon et al. 2016
Neofabraea sp.	A	*Helotiales*	6125	OP023198	8	0.53%	9%	4	*Neofabraea kienholzii*	MH864120.1	100.00%	Vu et al. 2019
Neonectria punicea	A	*Hypocreales*	5980	OP023171	123	8.14%	33%	6	*Neonectria punicea*	MK955355.1	100.00%	Wu (unpublished)
Neopyrenochaeta acicola FR	A	*Pleosporales*	8328	OP023299	1	0.07%	2%	1	*Neopyrenochaeta acicola*	KJ395501.1	99.61%	Panno et al. 2013

Table 2 (continued)

Species	Phylum	Order	NW-FVA ID	ITS Accession no.	n isolates	Frequency (%)	Continuity (%)	Sites isolated from	ITS NCBI Blast results			Reference
									Basis of identification	ID	Identity (%)	
Neopyrenochaeta sp.	A	*Pleosporales*	8293	OP023293	2	0.13%	2%	1	*Neopyrenochaeta fragariae*	MT547823.1	100.00%	Bilanski et. al (unpublished)
Neosetophoma sp.	A	*Pleosporales*	5928	OP023157	1	0.07%	2%	1	*Neosetophoma rosigena*	NR_157525.1	99.64%	Wanasinghe et al. 2018
Nigrograna mycophila	A	*Pleosporales*	8330	OP023301	1	0.07%	2%	1	*Nigrograna mycophila*	NR_147654.1	99.39%	Jaklitsch and Voglmayr 2016
Obolarina dryophila[* FR]	A	*Xylariales*	6324	OP023207	1	0.07%	2%	1	*Obolarina dryophila*	GQ428313.1	100.00%	Pažoutová et al. 2010
Oliveonia sp.	B	*Cantharellales*	8004	OP023266	1	0.07%	2%	1	*Oliveonia* sp.	MT235640.1	99.07%	Spirin et. al (unpublished)
Paracucurbitaria sp.	A	*Pleosporales*	5999	OP023181	31	2.05%	34%	6	*Paracucurbitaria corni*	MT547826.1	99.80%	Bilanski et. al (unpublished)
Paraphaeosphaeria neglecta	A	*Pleosporales*	6014	OP023186	5	0.33%	7%	3	*Paraphaeosphaeria neglecta*	NR_155629.1	99.46%	Verkley et al. 2014
Penicillium daleae[FR]	A	*Eurotiales*	6931	OP023218	2	0.13%	3%	1	*Penicillium daleae*	MH862989.1	100.00%	Vu et al. 2019
Peniophora cf. *cinerea 1*[*]	B	*Russulales*	6180	OP023208	5	0.33%	7%	3	*Peniophora cinerea*	MK247860.1	100.00%	Zhang (unpublished)
Peniophora cf. *cinerea 2*[*]	B	*Russulales*	8039	OP023277	1	0.07%	2%	1	*Peniophora cinerea*	MZ018635.1	100.00%	Volobuev and Shakhova 2022
Peniophora cf. *incarnata*[*]	B	*Russulales*	7034	OP023249	1	0.07%	2%	1	*Peniophora incarnata*	MW740279.1	99.69%	Johnston and Park (unpublished)
Peniophora laeta[* FR]	B	*Russulales*	8036	OP023273	1	0.07%	2%	1	*Peniophora laeta*	MH857617.1	99.33%	Vu et al. 2019
Peniophora lycii[*]	B	*Russulales*	5995	OP023180	19	1.26%	10%	3	*Peniophora lycii*	MH857624.1	99.83%	Vu et al. 2019
Peniophora quercina[* FR]	B	*Russulales*	8044	OP023279	1	0.07%	2%	1	*Peniophora quercina*	MT156129.1	99.47%	Bien and Damm 2020b
Peniophora rufomarginata[* FR]	B	*Russulales*	7027	OP023227	1	0.07%	2%	1	*Peniophora rufomarginata*	MH857639.1	100.00%	Vu et al. 2019
Peniophora sp. 1[*]	B	*Russulales*	6927	OP023215	1	0.07%	2%	1	*Peniophora lycii*	MH857624.1	98.82%	Vu et al. 2019

Table 2 (continued)

Species	Phylum	Order	NW-FVA ID	ITS Accession no.	n isolates	Frequency (%)	Continuity (%)	Sites isolated from	ITS NCBI Blast results			Reference
									Basis of identification	ID	Identity (%)	
Peniophora sp. 2*	B	Russulales	7226	OP023263	1	0.07%	2%	1	Peniophora lycii	JX046435.1	99.83%	Fedorova et. al (unpublished)
Peniophora sp. 3*	B	Russulales	8038	OP023276	1	0.07%	2%	1	Peniophora lycii	MH857624.1	97.98%	Vu et al. 2019
Pezicula cf. sporulosa	A	Helotiales	7104	OP023168	5	0.33%	3%	1	Pezicula sporulosa	KR859257.1	100.00%	Chen et al. 2016
Pezicula cf. rostrupii	A	Helotiales	5976	OP023245	1	0.07%	2%	1	Pezicula rostrupii	MH665639.1	100.00%	Marson and Hermant (unpublished)
Pezicula sp. 1	A	Helotiales	6112	OP023205	1	0.07%	2%	1	Pezicula radicicola	MH862498.1	95.05%	Vu et al. 2019
Pezicula sp. 2	A	Helotiales	7154	OP023260	4	0.26%	3%	2	Pezicula sp.	KY977580.1	96.74%	Kranjec (unpublished)
Pezicula sp. 3	A	Helotiales	7060	OP023240	1	0.07%	2%	1	Pezicula ericae	NR_155653.1	97.85%	Chen et al. 2016
Phaeosphaeria sp.	A	Pleosporales	7321	OP023264	1	0.07%	2%	1	Phaeosphaeria glyceriae-plicatae	MH862724.1	100.00%	Vu et al. 2019
Phanerochaete sordida s. lat. Gruppe*	B	Polyporales	7052	OP023254	1	0.07%	2%	1	Phanerochaete sordida	KU761238.1	100.00%	Dufresne et al. 2017
Phialocephala fortinii [FR]	A	Helotiales	7056	OP023238	1	0.07%	2%	1	Phialocephala fortinii	MT276008.1	100.00%	Myrholm and Ramsfield (unpublished)
Phialocephala piceae [FR]	A	Helotiales	7038	OP023216	8	0.53%	9%	2	Phialocephala piceae	NR_111319.1	100.00%	Schoch et al. 2014
Phialocephala sp.	A	Helotiales	6952	OP023236	1	0.07%	2%	1	Phialocephala oblonga	MG553996.1	100.00%	Haelewaters et al. 2018
Phlebia radiata*	B	Polyporales	6013	OP023185	3	0.20%	5%	2	Phlebia radiata	MT551932.1	100.00%	Lodge et al (unpublished)
Pleosporales sp.	A	Pleosporales	8335	OP023306	1	0.07%	2%	1	Phoma herbarum	MG586981.1	99.81%	Kirtsideli (unpublished)
Pseudeurotiaceae sp. 1	A	Thelebolales	6966	OP023224	1	0.07%	2%	1	Geomyces asperulatus	MH861038.1	100.00%	Vu et al. 2019
Pseudeurotiaceae sp. 2	A	Thelebolales	7152	OP023246	1	0.07%	2%	1	Pseudogymnoascus pannorum	MH854615.1	100.00%	Vu et al. 2019

Table 2 (continued)

Species	Phylum	Order	NW-FVA ID	ITS Accession no.	n isolates	Frequency (%)	Continuity (%)	Sites isolated from	ITS NCBI Blast results			Reference
									Basis of identification	ID	Identity (%)	
Pseudogymnoascus sp.	A	*Thelebolales*	6432	OP023211	1	0.07%	2%	1	*Pseudogymnoascus appendiculatus*	NR_137875.1	100.00%	Malloch et al. 2016
Pseudopithomyces chartarum [FR]	A	*Pleosporales*	8294	OP023294	1	0.07%	2%	1	*Pseudopithomyces chartarum*	MH860227.1	99.82%	Vu et al. 2019
Ramularia sp.	A	*Mycosphaerellales*	7043	OP023233	1	0.07%	2%	1	*Ramulariacollo-cygni*	NR_154944.1	100.00%	Videira et al. 2016
Rhexocercosporidium sp.	A	*Helotiales*	7097	OP023257	1	0.07%	2%	1	*Rhexocercosporidium* sp.	MN124205.1	99.41%	Padamsee and Burgess (unpublished)
Rhizoctonia fusispora	B	*Cantharellales*	7057	OP023239	1	0.07%	2%	1	*Rhizoctonia fusispora*	MH857068.1	99.06%	Vu et al. 2019
Sarocladium cf. *dejongiae*	A	*Hypocreales*	7061	OP023241	1	0.07%	2%	1	*Sarocladium dejongiae*	NR_161153.1	99.62%	Lombard (unpublished)
*Schizopora paradoxa**	B	*Hymenochaetales*	7155	OP023261	1	0.07%	2%	1	*Schizopora paradoxa*	MH857218.1	100.00%	Vu et al. 2019
Sclerostagonospora cycadis	A	*Pleosporales*	8285	OP023285	1	0.07%	2%	1	*Sclerostagonospora cycadis*	KR611890.1	99.22%	Crous et al. 2015
*Scytalidium album** [FR]	A	*Helotiales*	6322	OP023206	1	0.07%	2%	1	*Scytalidium album*	NR_160102.1	99.81%	Vu et al. 2019
*Scytalidium lignicola**	A	*Helotiales*	7062	OP023242	1	0.07%	2%	1	*Scytalidium lignicola*	GU934579.1	100.00%	Bakys et. al (unpublished)
*Sistotrema oblongisporum** [FR]	B	*Cantharellales*	8008	OP023270	3	0.20%	3%	1	*Sistotrema oblongisporum*	KF218970.1	100.00%	Kotiranta and Larsson 2013
Sordariales sp.	A	*Sordariales*	7071	OP023256	1	0.07%	2%	1	*Podospora tetraspora*	MH859329.1	99.62%	Vu et al. 2019
Sporothrix sp.	A	*Ophiostomatales*	8291	OP023291	1	0.07%	2%	1	*Sporothrix* sp.	MH740965.1	99.62%	Jankowiak et al. 2019b
Steccherinum sp.*	B	*Polyporales*	8283	OP023283	1	0.07%	2%	1	*Steccherinum bourdotii*	MK795065.1	100.00%	Moiseenko et al. 2019
Stereum sp.*	B	*Russulales*	5970	OP023167	6	0.40%	5%	2	*Stereum armeniacum*	MH862626.1	100.00%	Vu et al. 2019
Thelonectria sp.	A	*Hypocreales*	5993	OP023178	1	0.07%	2%	1	*Thelonectria* sp.	OK161009.1	100.00%	Lutz et al. 2022

Table 2 (continued)

| Species | Phylum | Order | NW-FVA ID | ITS Accession no. | n isolates | Frequency (%) | Continuity (%) | Sites isolated from | ITS NCBI Blast results | | | Reference |
									Basis of identification	ID	Identity (%)	
Thysanophora penicillioides FR	A	*Eurotiales*	7063	OP023243	1	0.07%	2%	1	*Thysanophora penicillioides*	MH855455.1	100.00%	Vu et al. 2019
*Trametes versicolor**	B	*Polyporales*	6137	OP023197	5	0.33%	9%	2	*Trametes versicolor*	LC710148.1	100.00%	Mori and Hirai (unpublished)
Vexillomyces sp. FR	A	*Leotiales*	7102	ON809459	1	0.07%	2%	1	*Vexillomyces verruculosus*	NR_165533.1	91.83%	Bien et al. 2020
*Xylaria longipes**	A	*Xylariales*	8288	OP023288	1	0.07%	2%	1	*Xylaria longipes*	MN588219.1	100.00%	Patejuk et. al (unpublished)
*Xylaria polymorpha**	A	*Xylariales*	5978	OP023169	7	0.46%	12%	3	*Xylaria polymorpha*	MG098262.1	99.82%	Bußkamp et al. 2020

before outgrowth could be recognised. The remaining 1070 (24%) wood chips were colonised or contaminated by yeasts, mould, or fungi which do not belong to Dikarya. The resulting pure culture isolates were assigned to 162 MT (excluding *Trichoderma* spp.) and all but ten could at least be identified to a genus level. Eighty-nine isolates could be identified to a species level (Table 2).

The majority of the isolated filamentous fungi from all samples were *Ascomycota* (132 MT including *Trichoderma* spp., 77.8%), 36 MT (22.2%) belonged to the phylum of *Basidiomycota*. Within the *Ascomycota*, the most frequently observed orders (Fig. 3) were *Hypocreales* (23.0%) followed by *Pleosporales* (22.2%), and *Helotiales* (19.8%). The *Basidiomycota* fungi were mainly represented by *Russulales* (36.1%), *Agaricales* (25.0%), and *Polyporales* (22.2%).

Despite the diversity of 162 detected fungal MT, only a few fungi occurred with high f_{MT}. Sixty-seven MT (41%) were isolated more than once and from these only 13 fungi (8%) were obtained ten or more times. The remaining 95 fungi (59%) were only isolated once. Between one and 27 different fungi were found per stem collar. On average, nine MT were recorded on each tree. None of the identified fungi were found at all sites (including extensive sampling sites) and merely 46 MT (28%) were found at more than one site. In total, 116 fungi (72%) were found solely at one site (the four subplots of Schlangen are considered one site).

Although morphologically similar to *Diplodia mutila* (Fr.) Mont., in the phylogenetic analysis based on ITS
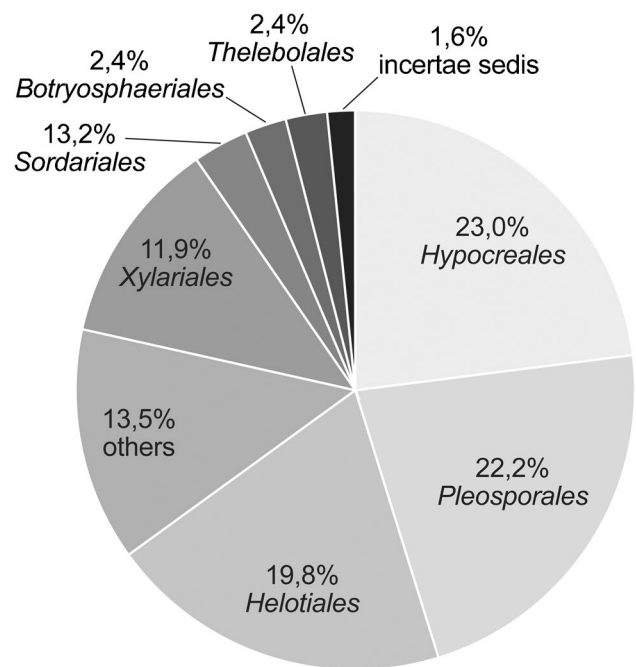
Fig. 3 Isolated orders of the *Ascomycota*, *n* = 132 of the isolated morphotypes belonging to the *Ascomycota* (Microsoft PowerPoint 2013)

sequences, the majority of *Diplodia* isolates from this study are placed clearly within a clade of strains of *Diplodia fraxini* (Fr.) Fr., including its ex-neotype (Online Resource 4 and Online Resource 6). One strain from this study (NW-FVA 5979) is placed within a clade formed by strains of *Diplodia sapinea* (Fr.) Fuckel including its ex-epitype strain and the ex-type strain of the synonymised *Diplodia intermedia*. The ITS sequences of this clade differ by one nucleotide, however, the ITS sequence of NW-FVA 5979 is identical to that of the ex-epitype strain of *D. sapinea*. Preliminary morphological observation of strains of *Armillaria* indicated affiliation to *A. gallica*. However, *A. gallica* cannot be clearly distinguished morphologically from *Armillaria cepistipes* Velen. (Tsopelas 1999). Of the 35 isolates included in the phylogenetic analysis of *Armillaria*, 33 isolates are placed within a clade containing reference strains of *A. gallica*. The two remaining isolates are placed within a clade of *A. cepistipes* (Online Resource 5 and Online Resource 7). Since only a selection of *Armillaria* isolates was included in the phylogenetic analysis due to limited lab resources, a clear distinction could not be made for all isolates of this genus. Hence, isolates of *Armillaria* are combined and referred to as *Armillaria* spp. in the final assessment of this study.

The most frequent MT isolated were *D. fraxini* (21.8%), *H. fraxineus* (13.9%), *Armillaria* spp. (12.3%), and *N. punicea* (9.6%), which account for nearly half of the isolated fungi. Perithecia of *N. punicea* were frequently observed on the ash bark above the necrotic lesions. All other isolated fungi were less frequent with <4% proportion. The most abundant MT in respect to continuity beside the aforementioned *D. fraxini* (71% continuity), *H. fraxineus* (53%), *Armillaria* spp. (50%), and *N. punicea* (33%), were *Diaporthe* cf. *eres* (43% continuity / 3.8% f_{MT}), *Fusarium* cf. *lateritium* (34% / 3.5%), and *Paracucurbitaria* sp. (34% / 2.1%). These MT were also most abundant in regard to occurrence at the nine sample sites (*H. fraxineus* occurs at eight sites, *Diaporthe* cf. *eres* and *D. fraxini* at seven sites, *Fusarium* cf. *lateritium*, *N. punicea*, and *Paracucurbitaria* sp. at six sites). When taking into account only the intensive sampling sites with at least five stem collars studied, there is an overlap of five occurring fungi: *Diaporthe* cf. *eres*, *D. fraxini*, *H. fraxineus*, *N. punicea*, and *Paracucurbitaria* sp.

The ash dieback pathogen *H. fraxineus* was isolated from 31 of the 54 stem collar necroses (57%). It was isolated at all studied sites except at Wolfenbüttel, where only a single tree with stem collar necrosis was sampled. The fungus was detected in both, young and advanced necroses, but not in the four control samples without symptomatic tissue. *Hymenoscyphus fraxineus* was isolated less frequently at the investigated sites with better water supply (soil water supply and climate combined; Online Resource 2).

147 MT (91%) were only isolated from necrotic stem tissue. Almost one-third of all MT according to their identification are able to decay wood (Table 2). A significant proportion of the isolated species were found here for the first time associated with *F. excelsior*, for example: three isolates from necrotic stem collar tissue were assigned to the MT identified as *Cryptostroma corticale* (Ellis & Everh.) P.H. Greg. & S. Waller and one isolate from stem collar tissue was identified as *D. sapinea*. The isolation of *Paracucurbitaria* sp. from the examined samples is the first proof of this genus from stem collar necroses of *F. excelsior*. Furthermore, one isolate from necrotic stem collar tissue was preliminarily assigned to the genus *Vexillomyces* S. Bien, C. Kraus & Damm based on ITS sequence comparison. Further morphologic as well as multi-locus phylogenetic investigations based on additional DNA regions (ribosomal large subunit, *EF-1α*, and a 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase) revealed this isolate to represent a novel yet undescribed species of *Vexillomyces* (Tan et al. 2022). The MT, which only occurred once in asymptomatic control samples were assigned to *Akanthomyces* sp., *Cephalotrichiella penicillata* Crous, and *Sclerostagonospora cycadis* Crous & G. Okada. The following fungi were isolated from stem collar necroses as well as from symptomless controls: *Alternaria infectoria* E.G. Simmons, *Alternaria* sp., *Diaporthe* cf. *eres*, *Exophiala* sp., *Hypoxylon fragiforme* E.G. Simmons, *Jackrogersella* sp., *Paracucurbitaria* sp., *Peniophora* sp. 1 and sp. 2, *Phaeosphaeria* sp., *Pseudeurotiaceae* sp., and *Sistotrema oblongisporum* M.P. Christ. & Hauerslev.

Fungal communities in stem collar necroses

The number of MT isolated from stem collar necroses at the different sites ranged from one (Wolfenbüttel, one sample tree) to ten (Woltershausen, two sample trees) at the extensive sites and from 29 (Schwansee, eight sample trees) to 87 (Schlangen, 19 sample trees) at the intensive sites. Hence, the fungal communities at the sites studied differed in their species composition and diversity (Fig. 4). In Schlangen 52 MT (32% of all 162 isolated MT of this study) out of 87 MT were found exclusively at this site. Nevertheless, the most common MT in Schlangen are identical with the most frequent MT over all sampling sites (*Armillaria* spp., *D. fraxini*, *H. fraxineus*, and *N. punicea*). *Diplodia fraxini* was isolated from almost 80% and *H. fraxineus* from almost 70% of the trees studied at this site. 54 MT were found in the samples from the site of Berggießhübel and 30 (19%) of those occurred exclusively at this site. Likewise, the most common MT at Berggießhübel were *D. fraxini*, *H. fraxineus*, and *Armillaria* spp. However, *N. punicea* was isolated only once there. At the Rhüden study site, 32 MT were found, 13 (8%) of which were found exclusively at this site. The most frequent MT were the same as the most abundant MT in respect to continuity from all samples - except *N. punicea*,
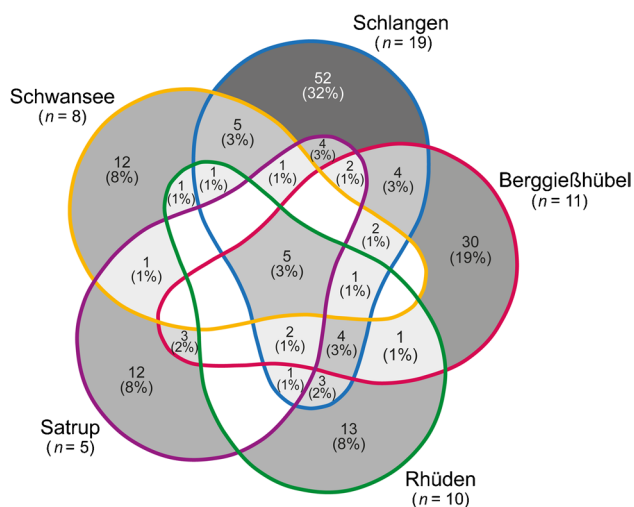
Fig. 4 Overlap between the fungi isolated from the main sites (with at least five samples) with the indication of how many of the isolated fungi were found at each site and between the different sites (absolute number as well as percentage), empty white areas indicating no overlap between the respective sites. Five fungi at the overlap of all sites are *Diaporthe* cf. *eres*, *Diplodia fraxini*, *Hymenoscyphus fraxineus*, *Neonectria punicea*, and *Paracucurbitaria* sp. (RStudio 4.1.2)

which was isolated only once in Rhüden. 31 MT were found in the Satrup samples, 12 of which (8%) exclusively at this site. The most common MT in Satrup were identical with the four most common MT in Schlangen. At Schwansee, 29 fungal MT were isolated in total, 12 of which (8%) were found exclusively at this site. At this location, the most common MT besides *D. fraxini* and *N. punicea* were *Coprinellus* species and *F. solani* s. l.

Discussion

Fungi associated with ash stem collars

In total, 162 fungi were isolated from ash stem collars and differentiated within this study. About half of these fungi were isolated from stem collar necroses in comparable studies as well (Lygis et al. 2005; Langer 2017; Meyn et al. 2019; Kranjec Orlović et al. 2020). Though 87 taxa (54%) isolated here were not reported by the aforementioned studies. It has to be taken into account that different sample sizes and sample site numbers lead to differing numbers of species. Considering the mentioned studies, Meyn et al. (2019) had the smallest sample size with four trees at one sample site and reported the smallest diversity with 16 fungal species. Langer (2017) isolated more fungal species (35) from 32 sample trees at seven sample sites. The correlation between sampling size and reported fungal diversity is also confirmed by Kranjec Orlović et al. (2020) with 68 fungal

species isolated from 90 sample trees examined at three sample sites. The non-negligible impact of the number of sample sites on the detectable fungal species diversity is shown in this study with 162 MT isolated from a smaller sample size (58 trees), but a higher number of sample sites (9) compared to the study of Kranjec Orlović et al. (2020). Other factors, such as number of the incubated wood chips or the isolation method may also have influence on the number of species isolated. But overall, there seems to be a positive correlation between sample size or number of sampled sites and species richness. Langer (2017) observed that, advanced stem collar necroses result in a higher number of isolated species. This could be confirmed in the present study when taking into account only the stem collar necroses with isolation of *H. fraxineus*.

Similar to studies focusing on endophytes of tree woody tissues (Bußkamp et al. 2020; Langer et al. 2021) in this study the majority of fungi isolated belong to *Ascomycota* (77.8%). A reason for the lower frequencies of *Basidiomycota* compared to *Ascomycota* might be that fungi belonging to the former often need longer incubation periods in order to grow out from incubated woody tissues (Oses et al. 2008) but since the incubated increment segments were kept for four weeks on nutrient media, it has to be assumed that enough time was given for fungi to grow out. However, the proportion of *Basidiomycota* (22.2%) in this study is higher than in the aforementioned studies. The reason for this discrepancy might be the focus on different woody tissue types in the mentioned studies and hence detection of differing fungal communities with divergent ecological functions. *Basidiomycota* isolated from woody tissues are often related to wood rot, because lignin is primarily decomposed by this fungal group and therefore they are more likely to be found in diseased or necrotic rather than asymptomatic woody tissue (Eriksson et al. 1990; Bugg et al. 2011). Hence, the occurrence of white and brown rot fungi in stem collar necroses is not unusual. Typical white rot fungi like *Armillaria* spp., *Coprinellus* spp., *Bjerkandera adusta* (Willd.) P. Karst., *Peniophora* spp., *Trametes versicolor* (L.) Lloyd, and few brown rot fungi like *Fomitopsis* spp. have been isolated from stem collar necroses in this study. The majority of soft rot fungi isolated here pertain to *Ascomycota* and the following representatives of this group were found: *Biscogniauxia nummularia* (Bull.) Kuntze, *Hypoxylon* spp., *Jackrogersella* sp.*, Nemania serpens* (Pers.) Gray, and *Xylaria* spp. (all *Xylariales*). Besides the occurrence of white and brown rot fungi, the frequent association of xylarialean wood decay fungi with stem collar necroses make it plausible that affected ash trees have a massive loss of stability and tend to topple over even without wind as a supporting factor.

Approximately one-third of the isolated MT detected in this study were listed for *F. excelsior* in the USDA fungal database (Farr and Rossman 2022). Only one of the most

frequent MT of this study, *N. punicea,* is not listed there, but other species from the genus *Neonectria* are mentioned. However, *N. punicea* was described as one of the most frequent MT associated with stem collar necroses in the context of ash dieback (Langer 2017; Meyn et al. 2019). The other most abundant MT isolated in our study *Armillaria* spp., *D. fraxini*, *H. fraxineus*, *Diaporthe* cf. *eres*, *Fusarium* cf. *lateritium,* and *Paracucurbitaria* sp. were already described to be associated with ash (Chandelier et al. 2016; Haňáčková et al. 2017; Langer 2017; Meyn et al. 2019; Linaldeddu et al. 2020; Kowalski and Bilański 2021; Barta et al. 2022). Langer (2017) investigated stem collar necroses of 32 ash trees and determined the aforementioned species as well—except for *Paracucurbitaria* sp. Meyn et al. (2019) isolated *D. fraxini* (labelled as *Botryosphaeria stevensii*), *H. fraxineus*, *N. punicea*, and *Diaporthe* cf. *eres* as well. The most commonly isolated species from stem collar necroses in the present study, except *Armillaria* sp. and *Paracucurbitaria* sp., were also isolated in high frequency by Linaldeddu et al. (2020), although they focussed on symptomatic branches of damaged ash trees in Italy. The absence of *Armillaria* sp. in branches was anticipated, because it is a soil-borne root and stem rot fungus, colonising its host through rhizomorph growth (Morrison 2004).

Within the genus *Armillaria, A. gallica* was the most frequently isolated species in the present study. Additionally to our isolations, mycelial fans and rhizomorphs of *Armillaria* spp. were observed at all sample sites of this study and the majority of further studied ash stands diseased by ash dieback. *Armillaria* species are common soil colonisers in Europe and therefore are probably existing in most forest sites even before ash dieback occurred (Morrison 2004; Lygis et al. 2005; Bakys et al. 2009b). They are considered secondary pathogens and wood-decaying fungi infecting stressed trees, which explains their occurrence in advanced stem necroses and root rot (Chandelier et al. 2016). On the one hand, *Armillaria* spp. can colonise stem collars after the necrosis has already formed by *H. fraxineus*. On the other hand, they are also able to independently attack a weakened ash tree without a stem collar necroses due to *H. fraxineus* (Langer 2017)*.* As in our study, the occurrence of *A. gallica* and *A. cepistipes* associated with stem collar necroses of trees affected by ash dieback has been shown by Chandelier et al. (2016) in Belgium. Enderle et al. (2017) also detected *A. gallica* in stem collar rots in south-western Germany. These results are in contrast to investigations by Lygis et al. (2005), who determined *A. cepistipes* as most frequent in Lithuania. Nevertheless, regardless of which of the two species caused infection, *Armillaria* spp. most likely accelerate the decline of ash dieback-affected ash trees (Chandelier et al. 2016) and reduce stem stability.

The most frequently isolated species in our study *D. fraxini* has been recognised as the dominant species in comparable studies as well. Linaldeddu et al. (2020) determined that many reports of *D. fraxini* on ash have earlier been assigned to *D. mutila* s. l. Phylogenetic analyses showed, that most of the *Diplodia* strains isolated in this study, although morphologically similar to *D. mutila*, certainly match with *D. fraxini*. It is an aggressive pathogen known to cause bark lesions and wood discoloration or to enlarge necroses, which are primarily caused by *H. fraxineus* (Alves et al. 2014; Linaldeddu et al. 2020, 2022). Kowalski et al. (2017) classified it as the second most pathogenic fungus after *H. fraxineus,* though it was not mentioned as a frequent coloniser of *F. excelsior* before ash dieback disease occurred (Kowalski et al. 2016). These facts might indicate that infections with *H. fraxineus* facilitate the colonisation of affected ash trees by *D. fraxini*. Another possible explanation for the more frequent occurrence of *D. fraxini* could be global warming because this species benefits from warm temperatures of around 25 °C (Alves et al. 2014). In our opinion, *D. fraxini* plays an important role in ash dieback disease and contributes undoubtedly to a greater damage extent, in particular at stem collar necroses. Besides the latter very frequent *Diplodia* species, to the knowledge of the authors, this is the first report of *D. sapinea* on ash. In contrast to the study by Linaldeddu et al. (2020), the species *D. subglobosa* could not be isolated in our analysis, maybe because they investigated branches and not stem collar necroses.

Neonectria punicea has a large host spectrum, including *F. excelsior* (Hirooka et al. 2013). However, this fungus has rarely been documented from this particular host species before (Langer 2017; Meyn et al. 2019; Karadžić et al. 2020). *N. punicea* was found to be associated with stem collar necroses and cankers of European ash in Germany (Langer 2017; Meyn et al. 2019) and it is able to cause necroses in juvenile ash trees (Karadžić et al. 2020). Its perithecia were observed frequently on the bark above the necrotic ash tissue (ibid. and Karadžić et al. 2020). *Neonectria punicea* is mainly known to be a secondary pathogen, but can also express an endophytic lifestyle (Langer 2017). Species of the genus *Neonectria* invade through natural entrances, like lenticels or artificial wounds, for infection (Flack and Swinburne 1977; Salgado-Salazar and Crouch 2019).

The isolation of strains assigned to *Diaporthe* cf. *eres* were made from diseased and also from healthy woody ash tissue in this study. This is in agreement with insights that *Diaporthe eres* can live as a plant pathogen, endophyte, or saprotroph and has a wide host range as well as a widespread distribution (Udayanga et al. 2014; Linaldeddu et al. 2020). This species often produces its tiny fruit bodies on dead woody tissues (Kowalski et al. 2016). In a study by Kowalski et al. (2017), *D. eres* showed the least virulence and caused significantly milder disease symptoms on *F. excelsior* plants

than the other tested fungal species. *Diaporthe eres* could be considered a weak pathogen in comparison to ash dieback on *F. excelsior*. In case of tree weakening by *H. fraxineus* the early endophytic presence of *D. eres* favours a fast pathogenic attack (Kowalski et al. 2016).

In this study *Fusarium* cf. *lateritium* Nees has been isolated frequently from symptomatic tissue and once from healthy wood tissue. The species is already known from *F. excelsior* in association with bacterial ash canker (Riggenbach 1956) but its virulence seems to be low in comparison with other fungal species (Bakys et al. 2009b). Kowalski et al. (2017) showed, that *F. lateritium* causes none or only small necroses on *F. excelsior*. In general, *Fusarium* spp. have a wide host range and are reported as the most common endophytes in ash bark and wood (Kowalski and Kehr 1992; Sieber 2007; Bakys et al. 2009b; Kowalski et al. 2016). The facts described above, indicate that *F. lateritium* is able to colonise the bark and woody tissue of ash independently of *H. fraxineus*. In association with ash dieback though it is more likely that the species contributes to the stem collar necroses as secondary pathogen. Thereby, it is non-essential, whether acceleration of ash dieback is established by shifting from endophytic to pathogenic lifestyle or colonising the tree as a secondary pathogen after tree weakening.

As far as it is known, the isolation of *Paracucurbitaria* sp. from the examined samples is the first proof of this genus in stem collar necroses. It was not isolated by Langer (2017) and Meyn et al. (2019) from rootstock. However, Kowalski and Bilański (2021) detected *Paracucurbitaria* sp. in previous year's ash leaf petioles in Poland, Barta et al. (2022) isolated it from ash twigs in Slovakia, and Haňáčková et al. (2017) reported *Paracucurbitaria corni* (Bat. & A. F. Vital) Valenz.-Lopez, Stchigel, Guarro & Cano as an endophyte of ash leaves and seeds. Therefore the occurrence of species from the genus *Paracucurbitaria* in plant material of *F. excelsior* is not striking, but its high frequency in stem collar necroses was unanticipated. It can be assumed that the high frequency of *Paracucurbitaria sp.* is no coincidence, because its detection in stem collar necroses of ash is increasing in ongoing research at the NW-FVA since sampling for this study.

Besides *D. sapinea*, there are a few species, which, to the knowledge of the authors, have not been previously reported from *F. excelsior* (Table 2). One of them is *C. corticale*, known as the causal agent of the sooty bark disease on maples. Its main host is *Acer pseudoplatanus* L., but it has been proven that *C. corticale* can colonise other maple species as well as *Aesculus hippocastanum* L. (Enderle et al. 2020). This species was found at sampling sites with sycamore. In addition to the first reports of ash as a host, one strain belonging to the genus *Vexillomyces* was isolated and recognised as undescribed species. The genus *Vexillomyces* was described in 2020 for two species

(*V. palatinus*, *V. verruculosus*) isolated from spore traps attached to vine shoots. No host organism is known for these species. Later several species of *Claussenomyces* and *Tympanis* were transferred to the genus (Baral and Quijada 2020). The respective species are known from dead or living angiosperm and gymnosperm wood, however, only for *V. atrovirens* (syn. *Claussenomyces atrovirens*) an affiliation to the host genus *Fraxinus* could be recognised (Dennis 1986).

Role of *Hymenoscyphus fraxineus* in stem collar necroses

The ash dieback pathogen *H. fraxineus* could not be isolated from all of the 54 symptomatic stem collars. Only in about half of the trees, the fungus could be determined. It has been already reported by several authors, that *H. fraxineus* could not be frequently isolated from symptomatic tissue of ash (Przybyl 2002; Bakys et al. 2009a; Enderle et al. 2017). A possible explanation for this could be its slow growth, unfavourable sampling conditions for the pathogen or too advanced necroses with antagonistic activity of other colonisers (Kowalski and Holdenrieder 2009; Hauptman et al. 2013; Gross et al. 2014; Langer 2017). Often, *H. fraxineus* could be solely isolated from recently discoloured woody tissues of the stem collar necroses (Fig. 2) and is probably supressed in the older parts of the necroses already colonised by secondary fungi. The aforementioned reasons might have contributed to the moderate isolation success of the ash dieback pathogen in this study. Perhaps, fungal community analysis by means of culture-independent methods, such as high throughput sequencing or qPCR could detect *H. fraxineus* more frequently than by culture based isolation, since these methods have the potential to detect inactively present fungi or even DNA residues if the initial fungus has been suppressed by secondary invaders (Lindahl et al. 2013). Our results on the f_{MT} and continuity of the association and localisation of *H. fraxineus* in basal stem necroses support the assumption, that this pathogen is very often the main or primary causal agent triggering stem collar necroses. Either way, *H. fraxineus* is confirmed as the main pathogenic agent of the ash dieback epidemic (Kowalski 2006; Bakys et al. 2009a; Kowalski and Holdenrieder 2009; Gross et al. 2014). The only lack of evidence for *H. fraxineus* at the study site Wolfenbüttel could be explained by the meagre sample size. It can be assumed that *H. fraxineus* may have been isolated if a larger wood chip number or sample tree size was examined. According to information from a co-researcher in FraxPath, *H. fraxineus* was present in branches of the sample trees at the study site Wolfenbüttel (Maia Ridley, personal communication).

Fungal communities in stem collar necroses

The observation of significant differences in the occurrence of fungal taxa between the investigated forest sites is consistent with the results of Bilański and Kowalski (2022). In the study of Meyn et al. (2019) only two species were found in all sample trees and many of the identified fungi were single isolates. Similarly, Kranjec Orlović et al. (2020) revealed just few predominating taxa representing half of all fungal isolates from stem bases of *Fraxinus angustifolia* Vahl. In addition, species represented only by a single isolate make up one-third of all isolates in the study by the latter authors. The fact, that the composition of fungi isolated in this study differs with only a little overlap between the sampling sites, leads to the assumption that adding further sampling sites would reveal new sets of fungi not recorded in this study.

Relation of the most common fungi to the site characteristics

Independent of age class and site conditions, European ash trees can be vulnerable to an infection by *H. fraxineus* (Pautasso et al. 2013). However, the extent of ash dieback in the crown and stem collar necroses and tree mortality, most likely depend on many different factors. Susceptibility of ash trees to the pathogen, the range of subsequent colonising fungal species (Langer et al. 2022), tree vitality, or the environmental context of the forest site and stand (Havrdová et al. 2017) are some examples. Ash tree vitality is encouraged at fertile and (moderately) wet soils, conditions which are preferred by ash (Walentowski et al. 2017). It has to be taken into account that for this study only a selection of forest sites from a rather narrow area out of the wide range of European ash was investigated. An optimal soil and water supply with a sufficient percentage of ash trees was fundamental. Furthermore, the selection of sample trees was subjected to different restrictions. For example the condition of a diameter at breast height less than 25 cm because of logistics and processing abilities in the lab. Besides that, trees with very advanced necroses like completely necrotic or rotten stem base or dead trees were not suitable for investigation.

Our preliminary results indicate that *H. fraxineus* was isolated less frequent at sites with higher water availability (Online Resource 2). This is in accordance with the guess that the fungal composition of stem collar necroses depends on soil and water availability of the forest stand (Linaldeddu et al. 2011; Salamon et al. 2020). As mentioned before, this assumption refers only to the selection of the investigated forest sites. One explanation could be, that secondary fungi have more favourable conditions at sites with higher water availability and thus are able to overgrow *H. fraxineus* faster than at drier sites.

For the other most common fungi *Armillaria* spp., *Diaporthe* cf. *eres*, *D. fraxini*, *Fusarium* cf. *lateritium*, *N. punicea*, and *Paracucurbitaria* sp., Dirichlet regression indicated no correlation between f_{WC} and water supply rank of the site (Online Resource 2). Assuming that *H. fraxineus* as the sole pathogen influences the extent of damage caused by stem collar necrosis, this this would be in contrast to the suggestion of several authors that stands with wet soil conditions show a higher probability that the individual trees affected by *H. fraxineus* exhibit greater damage (Gross et al. 2014; Erfmeier et al. 2019). At Schwansee, the wettest sampling site, *H. fraxineus* was only isolated twice. However, the stem collar necroses were most advanced at these sampling trees, where a lower isolation rate of *H. fraxineus* was generally expected, as mentioned previously.

It was noticeable, that *D. fraxini* and *N. punicea* had a significantly different f_{WC} at the various sampling sites (Online Resource 2), but there was no indication for a correlation with the site characteristics water supply, soil and bedrock, climate, or mixture of trees. However, it was observed that ash trees with a low f_{WC} of *D. fraxini* had a thinner bark. Compared with *D. fraxini* and *N. punicea*, the MT *Diaporthe* cf. *eres*, *Fusarium* cf. *lateritium*, and *Paracucurbitaria* sp. had a consistent f_{WC} over all sites. But *Fusarium* cf. *lateritium* was not isolated at Satrup and at the valley bottom in Schlangen. Due to the lower amount of isolations in this study, the authors assume there is also a lower probability of occurrence in stem collar necroses.

Armillaria species were not present at all studied sites and could not be isolated from the trees in Schwansee. This result is contradictory to those of Enderle et al. (2017), who found older necroses to be more often colonised by *Armillaria* spp. The progress of the necroses formation was clearly visible by their partially ruptured wood surface and presence of fruiting bodies on the necrotic stem areas of wood decay fungi, such as *Coprinellus* sp. and *Xylaria polymorpha* (Pers.) Grev. (Liers et al. 2011). Furthermore, the absence of *Armillaria* spp. isolates in Schwansee, the most moist of all sampling sites which is influenced by its ground water, do not correspond to preference of *Armillaria* species for continuously moist soil conditions (Whiting and Rizzo 1999). A possible explanation for the lack of this species in Schwansee, could be the specific forest site background as a former lake. The area was earlier used as fishpond until the eighteenth century. Thus, the soil was subjected to special formation conditions (Welk 2017) and perhaps it was not possible for *Armillaria* spp. to colonise the soil like in other forest sites.

Many of the other MT detected in this study were isolated just once, which may indicate no direct correlation with the investigated forest sites, thus site characteristics like soil and water supply relatedness cannot be assumed. However, it cannot be ruled out that the one-time isolated fungi occur

in other forest sites, than the investigated ones, too. As well as a higher abundancy is theoretical possible. Additionally, it is to be expected that the composition of fungi might differ according to tree age, tree species composition, forest management type, season, and the like (Scholtysik et al. 2013; Tomao et al. 2020). For example, a more diverse tree species composition at a forest site could contribute to the occurrence of a wider spectrum of fungi colonising a tree (Cavard et al. 2011; Kowalski et al. 2016; Krah et al. 2018; Tomao et al. 2020). This is confirmed by the isolation of sycamore-typical fungi like *C. corticale* und *Cy. rubronotata* from *F. excelsior* in stands with maple trees. It is furthermore supported by the fact that the most mixed intensive sampling site of Berggießhübel has one of the highest fungal diversity in relation to its sample tree amount. In addition to its diverse tree species composition, Berggießhübel is the most eastern sampling site. Satrup is the most northern sampling site and shows high fungal diversity despite its smallest sample tree amount. This observation suggests that widely varying sites in Germany lead to differing fungal communities. Furthermore, a possible underestimation of fungal diversity in the studied trees may occur since not all fungi are detectable through standardised culture based methods or in general (Guo et al. 2001; Allen et al. 2003; Unterseher 2011; Muggia et al. 2017).

Conclusion and outlook

This study provided new insights into the fungal diversity and communities of endophytes, primary and secondary pathogens, wood-decaying fungi, and saprotrophic fungi associated with stem collar necroses of European ash trees. A rich fungal composition inhabiting symptomatic stem tissue has been revealed with four frequent species occurring at most of the studied forest sites, but with little overlap between the sites. The fungal species richness detected in this study (162) is considerably higher compared to previous investigations in which 16–75 different species were detected (Lygis et al. 2005; Enderle et al. 2017; Langer 2017; Meyn et al. 2019). This difference in diversity can be explained by the larger sampling size (not only tree number, but also amount of wood chips taken) and the partially greater number of sites studied. Single trees with only about 20 studied chips of stem collar tissue each (Oranienbaumer Heide, Wolfenbüttel) had the fewest amount of isolated MT. Further studies on stem collar necroses can increase the knowledge of fungal biodiversity on *F. excelsior*, as clearly demonstrated by the newly described species, *Vexillomyces fraxinicola* (Tan et al. 2022), which was collected in this study.

The ash dieback pathogen was isolated from only about half of the trees sampled. Different reasons like its slow growth can cause a low isolation rate of the primary pathogen. Nevertheless, it is possible that stem collar necroses are commonly initiated by this fungus. The occurrence of several pathogenic fungi from necrotic stem tissue of ash beside *H. fraxineus* is striking, because of their high f_{MT}. It was shown that the different fungal communities of the sample trees are largely dominated by three MT (*D. fraxini*, *Armillaria* spp. and *N. punicea*) next to *H. fraxineus* representing almost 50% of all isolates. They are considered to play a major role in the progression of stem collar necroses and rot and therefore also contribute to a loss of tree stability. The remaining fungi which were isolated from the stem collars necroses turned out to be very diverse with much lower f_{MT}, in the majority of cases were represented with only one isolate. Overall, the synergistic interaction of different pathogens in the context of ash dieback, for example *H. fraxineus* and *D. fraxini* or *N. punicea,* can lead to a larger damage in contrast to infection by only one pathogen (Marçais et al. 2010). In this context, *N. punicea* poses a serious threat to planted ash forests and natural regenerations of *F. excelsior*, especially if another host tree species, such as European beech (*Fagus sylvatica* L.) is in mixture. European beech is potentially an inoculum reservoir of *N. punicea* for future infections of ash stem collars (Karadžić et al. 2020). Therefore, in the future, the susceptibility of ash to form stem collar necroses and to be diseased by *D. fraxini* and *N. punicea* should be considered in breeding programmes to develop more resistant ash trees in relation to ash dieback.

However, stem collar necrosis types caused by other fungi than *H. fraxineus* or *Phytophthora* spp. (Langer 2017), should not be disregarded. The results of this study show, that at least one fungal pathogen can be found in the necrosis without evidence of *H. fraxineus*. For example, one of the control samples, which turned out to have necrotic tissue inside the wooden body, was colonised by *Armillaria* sp. In this case, it is likely that the fungus attacked the weakened tree independently of a pre-colonisation of the stem collar by *H. fraxineus*.

Since in this study no correlation between the site factors and fungal occurrence could be calculated because most of the isolated fungi were only detected once, further studies should be carried out at additional comparable forest sites. Inventories of stem collar necroses at a higher number of locations may reveal dependence of MT to forest side conditions and their individual role in the fungal communities in detail. Future studies need to be conducted in order to estimate potentially high-risk characteristics of forest sites for pronounced and fast-advancing stem collar necroses and rot. Additionally, the investigation of genotypes of *H. fraxineus* associated with single-stem collar necroses could help to better understand the path of infection with *H. fraxineus* and the secondary colonisation by other fungi.

Declarations

References

Alborés S (2018) Biodiversity and antimicrobial activity of Antarctic fungi from the Fildes Peninsula, King George Island. Sydowia Int J Mycol 185–192. https://doi.org/10.12905/0380.sydowia70-2018-0185

Alimadadi N (2019) Molecular identification of some wild medicinal macrofungi from Northern Iran. Stud Fungi 4:26–36. https://doi.org/10.5943/sif/4/1/4

Allen TR, Millar T, Berch SM, Berbee ML (2003) Culturing and direct DNA extraction find different fungi from the same ericoid mycorrhizal roots. New Phytol 160:255–272. https://doi.org/10.1046/j.1469-8137.2003.00885.x

Altschul SF, Madden TL, Schäffer AA et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein databases search programs. Nucleic Acids Res 25:3389–3402. https://doi.org/10.1093/nar/25.17.3389

Alves A, Correia A, Phillips A (2006) Multi-gene genealogies and morphological data support *Diplodia cupressi* sp. nov., previously recognized as *D. pinea* f. sp. *cupressi*, as a distinct species. Fungal Divers 23:1–15

Alves A, Linaldeddu BT, Deidda A et al (2014) The complex of *Diplodia* species associated with *Fraxinus* and some other woody hosts in Italy and Portugal. Fungal Divers 67:143–156. https://doi.org/10.1007/s13225-014-0282-9

Bakys R, Vasaitis R, Barklund P et al (2009a) Investigations concerning the role of *Chalara fraxinea* in declining *Fraxinus excelsior*. Plant Pathol 58:284–292. https://doi.org/10.1111/j.1365-3059.2008.01977.x

Bakys R, Vasaitis R, Barklund P et al (2009b) Occurrence and pathogenicity of fungi in necrotic and non-symptomatic shoots of declining common ash (*Fraxinus excelsior*) in Sweden. Eur J For Res 128:51–60

Baral HO, Quijada L (2020) Nomenclatural novelties. Index Fungorum 454:1–3

Barta M, Pastirčáková K, Ostrovský R et al (2022) Culturable endophytic fungi in *Fraxinus excelsior* and their interactions with *Hymenoscyphus fraxineus*. Forests 13:1098. https://doi.org/10.3390/f13071098

Bien S, Damm U (2020a) *Arboricolonus simplex* gen. et sp. nov. and novelties in *Cadophora*, *Minutiella* and *Proliferodiscus* from *Prunus* wood in Germany. MycoKeys 63:119–161. https://doi.org/10.3897/mycokeys.63.46836

Bien S, Damm U (2020b) *Prunus* trees in Germany - a hideout of unknown fungi? Mycol Prog 19:667–690. https://doi.org/10.1007/s11557-020-01586-4

Bien S, Kraus C, Damm U (2020) Novel collophorina-like genera and species from *Prunus* trees and vineyards in Germany. Persoonia 45:46–67. https://doi.org/10.3767/persoonia.2020.45.02

Bilański P, Kowalski T (2022) Fungal endophytes in *Fraxinus excelsior* petioles and their in vitro antagonistic potential against the ash dieback pathogen *Hymenoscyphus fraxineus*. Microbiol Res 257:126961. https://doi.org/10.1016/j.micres.2022.126961

Bills GF, Platas G, Overy DP et al (2009) Discovery of the parnafungins, antifungal metabolites that inhibit mRNA polyadenylation, from the *Fusarium larvarum* complex and other Hypocrealean fungi. Mycologia 101:449–472. https://doi.org/10.3852/08-163

Blumenstein K, Bußkamp J, Langer GJ et al (2021) The Diplodia tip blight pathogen *Sphaeropsis sapinea* is the most common fungus in Scots pines' mycobiome, irrespective of health status - a case study from Germany. J Fungi 7:607. https://doi.org/10.3390/jof7080607

Bugg TDH, Ahmad M, Hardiman EM, Rahmanpour R (2011) Pathways for degradation of lignin in bacteria and fungi. Nat Prod Rep 28:1883–1896. https://doi.org/10.1039/C1NP00042J

Burdsall HH, Volk TJ (1993) The state of taxonomy of the genus *Armillaria*. McIlvainea 11:4–12

Bußkamp J, Langer GJ, Langer EJ (2020) *Sphaeropsis sapinea* and fungal endophyte diversity in twigs of Scots pine (*Pinus sylvestris*) in Germany. Mycol Prog 19:985–999. https://doi.org/10.1007/s11557-020-01617-0

Cabral A, Groenewald JZ, Rego C et al (2012) *Cylindrocarpon* root rot: multi-gene analysis reveals novel species within the *Ilyonectria radicicola* species complex. Mycol Prog 11:655–688. https://doi.org/10.1007/s11557-011-0777-7

Cavard X, Macdonald SE, Bergeron Y, Chen HYH (2011) Importance of mixedwoods for biodiversity conservation: evidence for understory plants, songbirds, soil fauna, and ectomycorrhizae in northern forests. Environ Rev 19:142–161. https://doi.org/10.1139/a11-004

Chandelier A, Gerarts F, Martin GS et al (2016) Temporal evolution of collar lesions associated with ash dieback and the occurrence of *Armillaria* in Belgian forests. For Pathol 46:289–297. https://doi.org/10.1111/efp.12258

Chen C, Verkley GJM, Sun G et al (2016) Redefining common endophytes and plant pathogens in *Neofabraea, Pezicula,* and related genera. Fungal Biol 120:1291–1322. https://doi.org/10.1016/j.funbio.2015.09.013

Crous PW, Schumacher RK, Wingfield MJ (2015) Fungal systematics and evolution: FUSE 1. Sydowia Int J Mycol 81–118. https://doi.org/10.12905/0380.sydowia67-2015-0081

Crous PW, Shivas RG, Quaedvlieg W et al (2014) Fungal Planet description sheets: 214-280. Persoonia 32:184–306. https://doi.org/10.3767/003158514X682395

Crous PW, Wingfield MJ, Guarro J et al (2013) Fungal Planet description sheets: 154–213. Persoonia 31:188–296. https://doi.org/10.3767/003158513X675925

Denman S, Barrett G, Kirk SA et al (2017) Identification of *Armillaria* species on declined oak in Britain: implications for oak health. Forestry 90:148–161. https://doi.org/10.1093/forestry/cpw054

Dennis RWG (1986) Fungi of the Hebrides. Royal Botanic Gardens, Kew

Douanla-Meli C, Langer E (2012) Diversity and molecular phylogeny of fungal endophytes associated with *Diospyros crassiflora*. Mycology 3:175–187. https://doi.org/10.1080/21501203.2012.705348

Dufresne PJ, Moonjely SS, Ozaki K et al (2017) High frequency of pathogenic *Aspergillus* species among nonsporulating moulds from respiratory tract samples. Med Mycol 55:233–236. https://doi.org/10.1093/mmy/myw064

Enderle R, Riebesehl J, Becker P, Kehr R (2020) Rußrindenkrankheit an Ahorn - Biologie, Pathologie und Entsorgung von Schadholz. In: Dujesiefken D (ed) Jahrbuch der Baumpflege 2020, 24th edn. Haymarket Media, Braunschweig, pp 85–100

Enderle R, Sander F, Metzler B (2017) Temporal development of collar necroses and butt rot in association with ash dieback. IForest - Biogeosci For 10:529–536. https://doi.org/10.3832/ifor2407-010

Erfmeier A, Haldan KL, Beckmann L-M et al (2019) Ash dieback and its impact in near-natural forest remnants – a plant community-based inventory. Front Plant Sci 10:658. https://doi.org/10.3389/fpls.2019.00658

Eriksson K-E, Blanchette RA, Ander P (1990) Biodegradation of lignin. In: Timell TE (ed) Microbial and enzymatic degradation of wood and wood components, 1st edn. Springer, Heidelberg, pp 225–333

Farr DF, Rossman AY (2022) Fungal databases - fungus-host distributions. In: US Natl. Fungus Collect. Fungal Database. https://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm. Accessed 4 May 2022

Flack NJ, Swinburne TR (1977) Host range of *Nectria galligena* Bres. and the pathogenicity of some Northern Ireland isolates. Trans Br Mycol Soc 68:185–192. https://doi.org/10.1016/S0007-1536(77)80007-7

Fukasawa Y (2018) Fungal succession and decomposition of *Pinus densiflora* snags. Ecol Res 33:435–444. https://doi.org/10.1007/s11284-017-1557-x

Gao C-H (2021) ggVennDiagram: a "ggplot2" implement of Venn diagram. In: GgVennDiagram Ggplot2 Implement Venn Diagr. https://CRAN.R-project.org/package=ggVennDiagram

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Geiser DM, Al-Hatmi AMS, Aoki T, et al (2021) Phylogenomic analysis of a 55.1-kb 19-gene dataset resolves a monophyletic *Fusarium* that includes the *Fusarium solani* species complex. Phytopathology® 111:1064–1079. https://doi.org/10.1094/PHYTO-08-20-0330-LE

Gomes RR, Glienke C, Videira SIR et al (2013) *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia 31:1–41. https://doi.org/10.3767/003158513X666844

Griffin MA, Spakowicz DJ, Gianoulis TA, Strobel SA (2010) Volatile organic compound production by organisms in the genus *Ascocoryne* and a re-evaluation of myco-diesel production by NRRL 50072. Microbiol 156:3814–3829. https://doi.org/10.1099/mic.0.041327-0

Gross A, Holdenrieder O, Pautasso M et al (2014) *Hymenoscyphus pseudoalbidus*, the causal agent of European ash dieback. Mol Plant Pathol 15:5–21. https://doi.org/10.1111/mpp.12073

Guo LD, Hyde KD, Liew ECY (2000) Identification of endophytic fungi from *Livistona chinensis* based on morphology and rDNA sequences. New Phytol 147:617–630

Guo LD, Hyde KD, Liew ECY (2001) Detection and taxonomic placement of endophytic fungi within frond tissues of *Livistona chinensis* based on rDNA sequences. Mol Phylogenet Evol 20:1–13. https://doi.org/10.1006/mpev.2001.0942

Guo T, Wang HC, Xue WQ et al (2016) Phylogenetic analyses of *Armillaria* reveal at least 15 phylogenetic lineages in China, seven of which are associated with cultivated *Gastrodia elata*. PLoS One 11:e0154794. https://doi.org/10.1371/journal.pone.0154794

Haelewaters D, Dirks AC, Kappler LA et al (2018) A preliminary checklist of fungi at the Boston Harbor Islands. Northeast Nat 25:45–76. https://doi.org/10.1656/045.025.s904

Hall T (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98. https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29

Haňáčková Z, Havrdová L, Černý K et al (2017) Fungal endophytes in ash shoots - diversity and inhibition of *Hymenoscyphus fraxineus*. Balt For 23:89–106

Hauptman T, Piškur B, de Groot M et al (2013) Temperature effect on *Chalara fraxinea*: heat treatment of saplings as a possible disease control method. For Pathol 43:360–370. https://doi.org/10.1111/efp.12038

Havrdová L, Zahradník D, Romportl D et al (2017) Environmental and silvicultural characteristics influencing the extent of ash dieback in forest stands. Balt For 23:168–182

Heydeck P, Bemmann M, Kontzog H-G (2005) Triebsterben an Gemeiner Esche (*Fraxinus excelsior*) im nordostdeutschen Tiefland. Forst und Holz 60:505–506

Hirooka Y, Rossman AY, Zhuang W-Y et al (2013) Species delimitation for *Neonectria coccinea* group including the causal agents of beech bark disease in Asia, Europe, and North America. Mycosystema 32:485–517

Hosseini B, Voegele RT, Link TI (2021) Establishment of a quadruplex real-time PCR assay to distinguish the fungal pathogens *Diaporthe longicolla, D. caulivora, D. eres,* and *D. novem* on soybean. PLoS One 16:e0257225. https://doi.org/10.1371/journal.pone.0257225

Husson C, Caël O, Grandjean JP et al (2012) Occurrence of *Hymenoscyphus pseudoalbidus* on infected ash logs. Plant Pathol 61:889–895. https://doi.org/10.1111/j.1365-3059.2011.02578.x

Irinyi L, Serena C, Garcia-Hermoso D et al (2015) International Society of Human and Animal Mycology (ISHAM)-ITS reference DNA barcoding database - the quality controlled standard tool for routine identification of human and animal pathogenic fungi. Med Mycol 53:313–337. https://doi.org/10.1093/mmy/myv008

Izumitsu K, Hatoh K, Sumita T et al (2012) Rapid and simple preparation of mushroom DNA directly from colonies and fruiting bodies for PCR. Mycosci 53:396–401

Jaklitsch WM, Checa J, Blanco MN et al (2018) A preliminary account of the *Cucurbitariaceae*. Stud Mycol 90:71–118. https://doi.org/10.1016/j.simyco.2017.11.002

Jaklitsch WM, Voglmayr H (2016) Hidden diversity in *Thyridaria* and a new circumscription of the *Thyridariaceae*. Stud Mycol 85:35–64. https://doi.org/10.1016/j.simyco.2016.09.002

Jang Y, Jang S, Min M et al (2015) Comparison of the diversity of basidiomycetes from dead wood of the Manchurian fir (*Abies holophylla*) as evaluated by fruiting body collection, mycelial isolation, and 454 sequencing. Microb Ecol 70:634–645. https://doi.org/10.1007/s00248-015-0616-5

Jankowiak R, Ciach M, Bilański P, Linnakoski R (2019a) Diversity of wood-inhabiting fungi in woodpecker nest cavities in southern Poland. Acta Mycol 54:1126. https://doi.org/10.5586/am.1126

Jankowiak R, Strzałka B, Bilański P et al (2019b) Ophiostomatoid fungi associated with hardwood-infesting bark and ambrosia beetles in Poland: taxonomic diversity and vector specificity. Fungal Ecol 39:152–167. https://doi.org/10.1016/j.funeco.2019.02.001

Karadžić D, Stanivuković Z, Milanović S et al (2020) Development of *Neonectria punicea* pathogenic symptoms in juvenile *Fraxinus excelsior* trees. Front Plant Sci 11:592260. https://doi.org/10.3389/fpls.2020.592260

Kauserud H, Schumacher T (2001) Outcrossing or inbreeding: DNA markers provide evidence for type of reproductive mode in *Phellinus nigrolimitatus* (*Basidiomycota*). Mycol Res 105:676–683. https://doi.org/10.1017/S0953756201004191

Kearse M, Moir R, Wilson A et al (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kenigsvalde K, Arhipova N, Laiviņš M, Gaitnieks T (2010) Fungus *Chalara fraxinea* as a causal agent for ash decline in Latvia. For Sci 21:110–120

Kotiranta H, Larsson K-H (2013) *Sistotrema luteoviride* sp. nov. (*Cantharellales, Basidiomycota*) from Finland. Acta Mycol 48:219–225. https://doi.org/10.5586/am.2013.023

Kowalski T (2006) *Chalara fraxinea* sp. nov. associated with dieback of ash in Poland. For Pathol 36:264–270. https://doi.org/10.1111/j.1439-0329.2006.00453.x

Kowalski T, Bilański P (2021) Fungi detected in the previous year's leaf petioles of *Fraxinus excelsior* and their antagonistic potential against *Hymenoscyphus fraxineus*. Forests 12:1412. https://doi.org/10.3390/f12101412

Kowalski T, Bilański P, Kraj W (2017) Pathogenicity of fungi associated with ash dieback towards *Fraxinus excelsior*. Plant Pathol 66:1228–1238. https://doi.org/10.1111/ppa.12667

Kowalski T, Holdenrieder O (2009) Pathogenicity of *Chalara fraxinea* For Pathol 39:1–7. https://doi.org/10.1111/j.1439-0329.2008.00565.x

Kowalski T, Kehr R (1992) Endophytic fungal colonization of branch bases in several forest tree species. Sydowia 44:137–168

Kowalski T, Kraj W, Bednarz B (2016) Fungi on stems and twigs in initial and advanced stages of dieback of European ash (*Fraxinus excelsior*) in Poland. Eur J For Res 135:565–579. https://doi.org/10.1007/s10342-016-0955-x

Krah F-S, Seibold S, Brandl R et al (2018) Independent effects of host and environment on the diversity of wood-inhabiting fungi. J Ecol 106:1428–1442. https://doi.org/10.1111/1365-2745.12939

Kranjec Orlović J, Moro M, Diminić D (2020) Role of root and stem base fungi in *Fraxinus angustifolia* (Vahl) dieback in Croatian floodplain forests. Forests 11:1–15. https://doi.org/10.3390/f11060607

Lakshman D, Vieira P, Pandey R et al (2017) Symptom development in response to combined infection of in vitro-grown *Lilium longiflorum* with *Pratylenchus penetrans* and soilborne fungi collected from diseased roots of field-grown lilies. Plant Dis 101:882–889. https://doi.org/10.1094/PDIS-09-16-1336-RE

Langer GJ (1994) Die Gattung *Botryobasidium* DONK (*Corticiaceae, Basidiomycetes*). J. Cramer, Berlin, Stuttgart

Langer GJ (2017) Collar rots in forests of Northwest Germany affected by ash dieback. Balt For 23:4–19

Langer GJ, Bußkamp J (2021) Fungi associated with woody tissues of European beech and their impact on tree health. Front Microbiol 12:702476. https://doi.org/10.3389/fmicb.2021.702467

Langer GJ, Bußkamp J, Terhonen E, Blumenstein K (2021) Fungi inhabiting woody tree tissues - stems, branches, and twigs. In: Asiegbu FO, Kovalchuk A (eds) Forest Microbiome Forest Microbiology. Forest Microbiology, Tree Microbiome: Phyllosphere, Endosphere, vol 1, pp 175–205

Langer GJ, Fuchs S, Osewold J, et al (2022) FraxForFuture - research on European ash dieback in Germany. J Plant Dis Prot https://doi.org/10.1007/s41348-022-00670-z

Liers C, Arnstadt T, Ullrich R, Hofrichter M (2011) Patterns of lignin degradation and oxidative enzyme secretion by different wood- and litter-colonizing basidiomycetes and ascomycetes grown on beech-wood. FEMS Microbiol Ecol 78:91–102. https://doi.org/10.1111/j.1574-6941.2011.01144.x

Liew ECY, Aptroot A, Hyde KD (2002) An evaluation of the monophyly of *Massarina* based on ribosomal DNA sequences. Mycologia 94:803–813. https://doi.org/10.1080/15572536.2003.11833174

Linaldeddu BT, Bottecchia F, Bregant C et al (2020) *Diplodia fraxini* and *Diplodia subglobosa*: the main species associated with cankers and dieback of *Fraxinus excelsior* in north-eastern Italy. Forests 11:883. https://doi.org/10.3390/f11080883

Linaldeddu BT, Bregant C, Montecchio L et al (2022) First report of *Diplodia fraxini* and *Diplodia subglobosa* causing canker and dieback of *Fraxinus excelsior* in Slovenia. Plant Dis 106:26–29. https://doi.org/10.1094/PDIS-06-21-1204-SC

Linaldeddu BT, Sirca C, Spano D, Franceschini A (2011) Variation of endophytic cork oak-associated fungal communities in relation to plant health and water stress. For Pathol 41:193–201. https://doi.org/10.1111/j.1439-0329.2010.00652.x

Lindahl BD, Nilsson RH, Tedersoo L et al (2013) Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. New Phytol 199:288–299. https://doi.org/10.1111/nph.12243

Lombard L, van der Merwe NA, Groenewald JZ, Crous PW (2015) Generic concepts in *Nectriaceae*. Stud Mycol 80:189–245. https://doi.org/10.1016/j.simyco.2014.12.002

Lutz T, Langer G, Heinze C (2022) Complete genome sequence of a new quadrivirus infecting a member of the genus *Thelonectria*. Arch Virol 167:691–694. https://doi.org/10.1007/s00705-021-05353-y

Lygis V, Vasiliauskas R, Larsson K-H, Stenlid J (2005) Wood-inhabiting fungi in stems of *Fraxinus excelsior* in declining ash stands of northern Lithuania, with particular reference to *Armillaria cepistipes*. Scand J For Res 20:337–346. https://doi.org/10.1080/02827580510036238

Maier M (2014) DirichletReg: dirichlet regression for compositional data in R

Maier MJ (2021) DirichletReg: dirichlet regression. R package version 0.7-1. https://github.com/maiermarco/DirichletReg

Malloch D, Sigler L, Hambleton S et al (2016) Fungi associated with hibernating bats in New Brunswick caves: the genus *Leuconeurospora*. Botany 94:1171–1181. https://doi.org/10.1139/cjb-2016-0086

Marçais B, Caël O, Delatour C (2010) Interaction between root rot basidiomycetes and *Phytophthora* species on pedunculate oak. Plant Pathol 60:296–303. https://doi.org/10.1111/j.1365-3059.2010.02378.x

Marçais B, Husson C, Godart L, Caël O (2016) Influence of site and stand factors on *Hymenoscyphus fraxineus*-induced basal lesion. Plant Pathol 65:1452–1461. https://doi.org/10.1111/ppa.12542

Marxmüller H (1992) Some notes on the taxonomy and nomenclature of five European *Armillaria* species. Mycotaxon 44:267–274

Meyn R, Langer GJ, Gross A, Langer EJ (2019) Fungal colonization patterns in necrotic rootstocks and stem bases of dieback-affected *Fraxinus excelsior* L. For Pathol 49:e12520. https://doi.org/10.1111/efp.12520

Min YJ, Park MS, Fong JJ et al (2014) Diversity and saline resistance of endophytic fungi associated with *Pinus thunbergii* in coastal shelterbelts of Korea. J Microbiol Biotechnol 24:324–333. https://doi.org/10.4014/jmb.1310.10041

Moiseenko KV, Glazunova OA, Shakhova NV et al (2019) Fungal adaptation to the advanced stages of wood decomposition: insights from the *Steccherinum ochraceum*. Microorganisms 7:527. https://doi.org/10.3390/microorganisms7110527

Moreno G, Blanco M-N, Checa J et al (2011) Taxonomic and phylogenetic revision of three rare irpicoid species within the Meruliaceae. Mycol Prog 10:481–491. https://doi.org/10.1007/s11557-010-0717-y

Morrison DJ (2004) Rhizomorph growth habit, saprophytic ability and virulence of 15 *Armillaria* species. For Pathol 34:15–26. https://doi.org/10.1046/j.1439-0329.2003.00345.x

Muggia L, Kopun T, Grube M (2017) Effects of growth media on the diversity of culturable fungi from lichens. Molecules 22:824. https://doi.org/10.3390/molecules22050824

Oses R, Valenzuela S, Freer J et al (2008) Fungal endophytes in xylem of healthy Chilean trees and their possible role in early wood decay. Fungal Divers 33:77–86

Palmer JM, Jusino MA, Banik MT, Lindner DL (2018) Non-biological synthetic spike-in controls and the AMPtk software pipeline improve mycobiome data. PeerJ 6:e4925. https://doi.org/10.7717/peerj.4925

Panno L, Bruno M, Voyron S et al (2013) Diversity, ecological role and potential biotechnological applications of marine fungi associated to the seagrass *Posidonia oceanica*. New Biotechnol 30:685–694. https://doi.org/10.1016/j.nbt.2013.01.010

Pautasso M, Aas G, Queloz V, Holdenrieder O (2013) European ash (*Fraxinus excelsior*) dieback – a conservation biology challenge. Biol Conserv 158:37–49. https://doi.org/10.1016/j.biocon.2012.08.026

Pažoutová S, Šrůtka P, Holuša J et al (2010) The phylogenetic position of *Obolarina dryophila* (*Xylariales*). Mycol Prog 9:501–507. https://doi.org/10.1007/s11557-010-0658-5

Peters S, Langer G, Kätzel R (2021a) Eschentriebsterben - Kriterien zur Schadensbonitur an Eschen, 1st edn. Fachagentur für nachwachsende Rohstoffe e.V. (FNR), Gülzow-Prüzen

Peters S, Langer G, Kätzel R (2021b) Bonitur geschädigter Eschen im Kontext des Eschentriebsterbens. AFZ-DerWald 76:28–31

Phookamsak R, Hyde KD, Jeewon R et al (2019) Fungal diversity notes 929–1035: taxonomic and phylogenetic contributions on genera and species of fungi. Fungal Divers 95:1–273. https://doi.org/10.1007/s13225-019-00421-w

Przybyl K (2002) Fungi associated with necrotic apical parts of *Fraxinus excelsior* shoots. For Pathol 32:387–394. https://doi.org/10.1046/j.1439-0329.2002.00301.x

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Version 4.1.2. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Riggenbach A (1956) Untersuchung über den Eschenkrebs. Doctoral Thesis, ETH Zürich. https://doi.org/10.3929/ethz-a-000294364

Rivedal HM, Stone AG, Severns PM, Johnson KB (2020) Characterization of the fungal community associated with root, crown, and vascular symptoms in an undiagnosed yield decline of winter squash. Phytobiomes J 4:178–192. https://doi.org/10.1094/PBIOMES-11-18-0056-R

Robert V, Stegehuis G, Stalpers J (2005) The MycoBank engine and related databases. In: MycoBank Database. https://www.mycobank.org/. Accessed 15 Sep 2022

Rongfeng X, Bo L, YuJing Z et al (2018) Spatial distribution characteristics of fungal population in microbial fermentation bed for pig rearing. Chin J Eco-Agric 26:493–504. https://doi.org/10.13930/j.cnki.cjea.170904

Salamon S, Mikołajczak K, Błaszczyk L et al (2020) Changes in root-associated fungal communities in *Triticum aestivum* ssp. *spelta* L. and *Triticum aestivum* ssp. *vulgare* L. under drought stress and in various soil processing. Plos One 15:e0240037. https://doi.org/10.1371/journal.pone.0240037

Salgado-Salazar C, Crouch JA (2019) Genome resources for the stem and bark canker pathogens *Corinectria fuckeliana, Neonectria hederae* and *N. punicea*. Plant Dis 103:389–391. https://doi.org/10.1094/PDIS-05-18-0904-A

Sandoval-Denis M, Lombard L, Crous PW (2019) Back to the roots: a reappraisal of *Neocosmospora*. Persoonia 43:90–185. https://doi.org/10.3767/persoonia.2019.43.04

Schoch CL, Robbertse B, Robert V et al (2014) Finding needles in haystacks: linking scientific names, reference specimens and molecular data for Fungi. Database 2014:bau061. https://doi.org/10.1093/database/bau061

Scholtysik A, Unterseher M, Otto P, Wirth C (2013) Spatio-temporal dynamics of endophyte diversity in the canopy of European ash (*Fraxinus excelsior*). Mycol Prog 12:291–304. https://doi.org/10.1007/s11557-012-0835-9

Schumacher J, Kehr R, Leonhard S (2009) Mycological and histological investigations of *Fraxinus excelsior* nursery saplings naturally infected by *Chalara fraxinea*. For Pathol 40:419–429. https://doi.org/10.1111/j.1439-0329.2009.00615.x

Schumacher J, Wulf A, Leonhard S (2007) Erster Nachweis von *Chalara fraxinea* T. KOWALSKI sp. nov. in Deutschland – ein Verursacher neuartiger Schäden an Eschen. Nachrichtenblatt Dtsch Pflanzenschutzdienstes 59:121–123

Sieber TN (2007) Endophytic fungi in forest trees: are they mutualists? Fungal Biol Rev 21:75–89. https://doi.org/10.1016/j.fbr.2007.05.004

Simon UK, Groenewald JZ, Crous PW (2009) *Cymadothea trifolii*, an obligate biotrophic leaf parasite of *Trifolium*, belongs to *Mycosphaerellaceae* as shown by nuclear ribosomal DNA analyses. Persoonia 22:49–55. https://doi.org/10.3767/003158509X425350

Šišić A, Baćanović-Šišić J, Al-Hatmi AMS et al (2018) The "forma specialis" issue in *Fusarium*: a case study in *Fusarium solani* f. sp. *pisi*. Sci Rep 8:1252. https://doi.org/10.1038/s41598-018-19779-z

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Sun L, Zhu J, Li X et al (2014) Diversity of endophytic fungi associated with *Ferula sinkiangensis*. Acta Microbiol Sin 54:936–942

Tan YP, Bishop-Hurley SL, Shivas RG et al (2022) Fungal Planet description sheets: 1436-1477. Persoonia 49:261–350. https://doi.org/10.3767/persoonia.2022.49.08

Tibpromma S, Hyde KD, Jeewon R et al (2017) Fungal diversity notes 491–602: taxonomic and phylogenetic contributions to fungal taxa. Fungal Divers 83:1–261. https://doi.org/10.1007/s13225-017-0378-0

Tomao A, Antonio Bonet J, Castaño C, de-Miguel S (2020) How does forest management affect fungal diversity and community composition? Current knowledge and future perspectives for the conservation of forest fungi. For Ecol Manag 457:117678. https://doi.org/10.1016/j.foreco.2019.117678

Travadon R, Lecomte P, Diarra B et al (2016) Grapevine pruning systems and cultivars influence the diversity of wood-colonizing fungi. Fungal Ecol 24:82–93. https://doi.org/10.1016/j.funeco.2016.09.003

Tsopelas P (1999) Distribution and ecology of *Armillaria* species in Greece. Eur J For Pathol 29:103–116. https://doi.org/10.1046/j.1439-0329.1999.00139.x

Turczański K, Rutkowski P, Dyderski M et al (2019) Soil pH and organic matter content affects European ash (*Fraxinus excelsior* L.) crown defoliation and its impact on understory vegetation. Forests 11:22. https://doi.org/10.3390/f11010022

Udayanga D, Castlebury LA, Rossman AY et al (2014) Insights into the genus *Diaporthe*: phylogenetic species delimitation in the *D. eres* species complex. Fungal Divers 67:203–229. https://doi.org/10.1007/s13225-014-0297-2

Unterseher M (2011) Fungal Biodiversity Research – from cultivation of foliar tree endophytes to the combination of ecological theory and next generation sequencing. Doctoral Thesis, ErnstMoritz Arndt Universität Greifswald. https://doi.org/10.13140/RG.2.1.2045.3526

Verkley GJM, Dukik K, Renfurm R et al (2014) Novel genera and species of coniothyrium-like fungi in *Montagnulaceae* (*Ascomycota*). Persoonia 32:25–51. https://doi.org/10.3767/003158514X679191

Videira SIR, Groenewald JZ, Braun U et al (2016) All that glitters is not *Ramularia*. Stud Mycol 83:49–163. https://doi.org/10.1016/j.simyco.2016.06.001

Voglmayr H, Aguirre-Hudson MB, Wagner HG et al (2019) Lichens or endophytes? The enigmatic genus *Leptosillia* in the *Leptosilliaceae* fam. nov. (*Xylariales*), and *Furfurella* gen. nov. (*Delonicicolaceae*). Persoonia 42:228–260. https://doi.org/10.3767/persoonia.2019.42.09

Volobuev S, Shakhova N (2022) Towards the discovery of active lignocellulolytic enzyme producers: a screening study of xylotrophic macrofungi from the Central Russian Upland. Iran J Sci Technol Trans Sci 46:91–100. https://doi.org/10.1007/s40995-021-01245-7

Vu D, Groenewald M, de Vries M et al (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Stud Mycol 92:135–154. https://doi.org/10.1016/j.simyco.2018.05.001

Walentowski H, Falk W, Mette T et al (2017) Assessing future suitability of tree species under climate change by multiple methods: a case study in southern Germany. Ann For Res 60:101–126. https://doi.org/10.15287/afr.2016.789

Wanasinghe DN, Phukhamsakda C, Hyde KD et al (2018) Fungal diversity notes 709–839: taxonomic and phylogenetic contributions to fungal taxa with an emphasis on fungi on *Rosaceae*. Fungal Divers 89:1–236. https://doi.org/10.1007/s13225-018-0395-7

Wang W-J, Li Y, Wang X-L et al (2015) Neotypification of *Paecilomyces hepiali* (*Hypocreales*). TAXON 64:147–150. https://doi.org/10.12705/641.28

Wang X, Pecoraro L (2021) Analysis of soil fungal and bacterial communities in tianchi volcano crater, northeast China. Life 11:280. https://doi.org/10.3390/life11040280

Welk A (2017) Managementplan (Fachbeitrag Offenland) für das FFH-Gebiet 165 "Schwansee." Management plan final report, RANA - Büro für Ökologie und Naturschutz on behalf of the Thüringer Landesanstalt für Umwelt und Geologie, Halle (Saale)

Wendt L, Sir EB, Kuhnert E et al (2018) Resurrection and emendation of the *Hypoxylaceae*, recognised from a multigene phylogeny of the *Xylariales*. Mycol Prog 17:115–154. https://doi.org/10.1007/s11557-017-1311-3

White TJ, Bruns T, Lee SJWT, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc Guide Methods Appl 18:315–322

Whiting EC, Rizzo DM (1999) Effect of water potential on radial colony growth of *Armillaria mellea* and *A. gallica* isolates in culture. Mycologia 91:627–635. https://doi.org/10.1080/00275514.1999.12061061

Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer, New York

Wickham H, Averick M, Bryan J et al (2019) Welcome to the Tidyverse. J Open Source Softw 4:1686. https://doi.org/10.21105/joss.01686

Yang L, Peng M, Shah SS, Wang Q (2017) Transcriptome sequencing and comparative analysis of *Piptoporus betulinus* in response to birch sawdust induction. Forests 8:374. https://doi.org/10.3390/f8100374

Yokoya K, Postel S, Fang R, Sarasan V (2017) Endophytic fungal diversity of *Fragaria vesca*, a crop wild relative of strawberry, along environmental gradients within a small geographical area. PeerJ 5:e2860. https://doi.org/10.7717/peerj.2860

Zare R, Gams W, Culham A (2000) A revision of *Verticillium* sect. *Prostrata* I. Phylogenetic studies using ITS sequences. Nova Hedwigia 71:465–480. https://doi.org/10.1127/nova/71/2000/465

Zeng Z-Q, Zhuang W-Y (2016) A new species of *Cosmospora* and the first record of sexual state of *C. lavitskiae*. Mycol Prog 15:59. https://doi.org/10.1007/s11557-016-1201-0

Zhang W, Groenewald JZ, Lombard L et al (2021) Evaluating species in *Botryosphaeriales*. Persoonia 46:63–115. https://doi.org/10.3767/persoonia.2021.46.03

Zhao P, Crous PW, Hou LW et al (2021) Fungi of quarantine concern for China I: *Dothideomycetes*. Persoonia 47:45–105. https://doi.org/10.3767/persoonia.2021.47.02

Zlatković M, Keča N, Wingfield MJ et al (2016) *Botryosphaeriaceae* associated with the die-back of ornamental trees in the Western Balkans. Antonie Van Leeuwenhoek 109:543–564. https://doi.org/10.1007/s10482-016-0659-8