Fungi associated with Vitality loss of beech, with emphasis on the warmth-loving ascomycete *Biscogniauxia nummularia*

Pilze im Kontext der Buchenvitalitätsschwäche, mit besonderem Schwerpunkt auf dem wärmeliebenden Ascomyceten *Biscogniauxia nummularia*

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Contents

Summary

As a result of the dry and hot climate conditions since 2018, European beech has been exhibiting widespread signs of decline in Germany. The symptoms are related to the occurrence of Vitality loss of beech, a complex disease, triggered by abiotic factors such as drought and heat. Due to the weakening of the host tree, pathogens and insects appear as secondary agents, influencing the further progression of damage. Many fungi associated with Vitality loss of beech are already present as endophytes in healthy tissue. When the host is weakened, these fungi can transition to a parasitic phase. The documented decline of European beech in Germany is so severe that the debate about the suitability and adaptability of European beech in the context of climate change has been reignited.

While there are some studies on the fungal community of European beech, they focus almost exclusively on leaves and twigs. This is mainly due to the fact that sampling the trunk is more labour-intensive and inevitably results in more severe injuries to the tree compared to sampling leaves and twigs. Consequently, data on the fungal community in the wood of European beech trees affected by Vitality loss of beech are scarce. Therefore, the aim of this study was to systematically sample the woody tissue and cambium across the entire tree and to analyse the identified fungi in the context of Vitality loss of beech. In the studies conducted for this thesis, mature European beech trees were felled and sampled in three federal states in Germany. The sampled trees varied in their degree of damage related to Vitality loss of beech. Fungi were identified from both symptomatic and asymptomatic tissues, using a culture-based method. A total of 181 fungal morphotypes were identified, distributed across 15 orders of Ascomycota and four orders of Basidiomycota. Of the 181 morphotypes, 92 could be identified at species level. The isolated species varied greatly depending on the test tree, the sampled tree compartment, and the tissue type. The results suggest that, in the context of Vitality loss of beech, a large number of different fungal species occur as pathogens, depending on the affected tree. However, two ascomycetous fungal species, *Biscogniauxia nummularia* and *Neonectria coccinea*, were isolated in high numbers and throughout the entire study area, confirming their relevance in the current context. *Biscogniauxia nummularia* was isolated more frequently from the asymptomatic tissue of damaged and severely damaged trees than from the asymptomatic tissue of undamaged and slightly damaged trees. This led to the conclusion that *Biscogniauxia nummularia* may prove effective as a bioindicator

regarding the vitality of European beech. Genetic analyses revealed notable differences in the tubulin DNA region of tested *Biscogniauxia nummularia* strains. The growth characteristics of the strains in relation to temperature and nutrient medium, as well as their decay capacities on European beech wood, were examined *in vitro.* To model hyphal growth in culture, dose-response curves were fitted using the four-parameter Brain-Cousens hormesis model. The observed differences in growth characteristics may be related to the strains' geographic origin. Wood decay capacities of the various strains tested varied considerably. The host tree species from which the strains were originally isolated had no detectable effect on the wood decay capacities regarding European beech wood.

The conducted studies demonstrate that, under the dry and hot conditions of recent years, various fungal species can act as damaging agents regarding European beech. *Biscogniauxia nummularia*, however, represents a key pathogen that seems to have a marked degree of adaptability to high temperatures. Since climate change will lead to extended drought periods in Europe during the 21st century, further damage to European beech in the context of Vitality loss of beech is to be expected.

Zusammenfassung

Infolge der trockenen und heißen Witterungsbedingungen seit 2018 sind an Rotbuchen in Deutschland Absterbeerscheinungen auf großer Fläche zu beobachten. Die Symptome stehen im Zusammenhang mit dem Auftreten der Buchen-vitalitätsschwäche, einer komplexen Krankheit, die durch abiotische Faktoren wie Trockenheit und Hitze ausgelöst werden kann. Durch die Schwächung des Wirtsbaums treten Pathogene und Insekten als sekundäre Erreger auf, die das weitere Schadgeschehen beeinflussen. Viele Pilze, die mit der Buchenvitalitätsschwäche in Verbindung gebracht werden, sind bereits als Endophyten im gesunden Gewebe vorhanden. Gerät der Wirtsbaum unter Stress, können diese Pilze in eine parasitäre Phase übergehen. Die dokumentierten Absterbeerscheinungen der Rotbuche in Deutschland sind so gravierend, dass die Debatte über die Eignung und Anpassungsfähigkeit der Rotbuche im Kontext des Klimawandels neu entfacht wurde. Obwohl es einige Studien zur Pilzgemeinschaft der Rotbuche gibt, konzentrieren sich diese fast ausschließlich auf Blätter und Triebe. Das liegt vor allem daran, dass die Beprobung des Stammes mit einem erheblich höheren Aufwand verbunden ist und

zwangsläufig größere Verletzungen des Baumes verursacht als die Beprobung von Blättern und Zweigen. Entsprechend wenige Daten liegen zur Pilzgemeinschaft im Holz von Rotbuchen im Zusammenhang mit der Buchenvitalitätsschwäche vor. Ziel dieser Studie war es daher, das holzige Gewebe und das Kambium systematisch über den gesamten Baum hinweg zu beproben und die nachgewiesenen Pilze hinsichtlich ihrer Rolle im Kontext der Buchenvitalitätsschwäche zu analysieren.

Im Rahmen der vorliegenden Dissertation wurde dafür in drei Bundesländern in Deutschland Rotbuchenaltholz[1] gefällt und beprobt. Die beprobten Bäume wiesen, mit Bezug auf die Buchenvitalitätsschwäche, unterschiedliche Schädigungsgrade auf. Unter Verwendung eines kulturbasierten Verfahrens wurden Pilze aus symptomatischem und asymptomatischem Gewebe isoliert und identifiziert. Es wurden insgesamt 181 pilzliche Morphotypen identifiziert, die sich auf 15 Ordnungen der Ascomycota (Schlauchpilze) und vier Ordnungen der Basidiomycota (Ständerpilze) verteilten. Von den 181 Morphotypen konnten 92 bis auf Artebene bestimmt werden. Die isolierten Arten variierten je nach beprobtem Baum, dem Baumkompartiment und dem Gewebetyp stark. Die Ergebnisse legen nahe, dass im Kontext der Buchenvitalitätsschwäche je nach betroffenem Baum eine große Anzahl verschiedenster Pilzarten schädigend auftreten kann. Mit *Biscogniauxia nummularia* und *Neonectria coccinea* wurden allerdings zwei Schlauchpilzarten häufig und im gesamten Untersuchungsgebiet isoliert, was ihre hohe Relevanz im bestehenden Kontext bestätigt. *Biscogniauxia nummularia* wurde häufiger aus dem asymptomatischen Gewebe von geschädigten und stark geschädigten Bäumen isoliert als aus dem asymptomatischen Gewebe von ungeschädigten und leicht geschädigten Bäumen. Das führte zu der Schlussfolgerung, dass sich *Biscogniauxia nummularia* als potenzieller Bioindikator für die Vitalität der Rotbuche eignen könnte. Genetische Analysen zeigten deutliche Unterschiede im Tubulin-DNA-Bereich von getesteten *Biscogniauxia nummularia*-Stämmen. Die temperatur- und nährstoffmedien-abhängigen Wachstumscharakteristika der Stämme wurden *in vitro* untersucht, ebenso wie ihr Holzabbaupotential gegenüber Rotbuchenholz. Zur Modellierung des Hyphenwachstums in Kultur wurden Dosis-Wirkungs-Kurven unter Verwendung des

[1] Bestand dessen Bäume die Zielstärke erreicht haben und durch deren Nutzung die Verjüngung eingeleitet wird (Erlbeck et al. 1998).

vierparametrigen Brain-Cousens-Hormesis-Modells erstellt. Die beobachteten Unterschiede der Wachstumscharakteristika könnten im Zusammenhang mit der geographischen Herkunft der Stämme stehen. Das Holzabbaupotential der getesteten Stämme variierte erheblich. Die Wirtsbaumart, aus der die Stämme ursprünglich isoliert wurden, hatte keinen nachweisbaren Einfluss auf das Holzabbaupotential gegenüber Rotbuchenholz.

Die durchgeführten Studien zeigen, dass unter den trockenen und heißen Bedingungen der letzten Jahre verschiedene Pilzarten schädigend an der Rotbuche auftreten können. *Biscogniauxia nummularia* stellt jedoch ein Schlüsselpathogen dar, das in Bezug auf hohe Temperaturen ein hohes Anpassungsvermögen zu haben scheint. Da der Klimawandel im 21. Jahrhundert in Europa zu längeren Dürreperioden führen wird, sind weitere Schäden an der Rotbuche im Kontext der Buchenvitalitätsschwäche zu erwarten.

1. Introduction

1.1 Diseases of trees

According to Manion (1981), plant diseases are any deviation in the normal functioning of a plant caused by some type of persistent agent. Trees are affected by a wide range of pathogens, including bacteria, viruses, as well as fungi and Peronosporomycetes (Nienhaus and Castello 1989; Sieber 2007; Jung 2009; Denman et al. 2012). In addition, insects can act as pests, either directly damaging the tree through their feeding (Brück-Dyckhoff et al. 2019) or serving as vectors for pathogens (Carraro et al. 2004). Larvae and adults of the so-called ambrosia beetles (Schmidberger 1836) feed on cultivated fungi growing on woody tissues (Schedl 1956; Browne 1961; Wood 1982; cited in Kirkendall et al. 2015). While ambrosia beetles are primarily adapted to dead woody plants, many lineages have evolved to breed in living tissues, damaging or even killing their host tree in the process (Kirkendall et al. 2015). It can be assumed that plant diseases have troubled humans since the beginnings of plant cultivation (Manion 1981), and the management of plant diseases and pests has historically been associated with species grown for wood (Boyd et al. 2013). Most plant diseases are strongly influenced by environmental conditions. Climate change affects pathogens, hosts, and the interaction between the pathogens and their hosts, resulting in changes in disease impact (Sturrock et al. 2011). Additionally, through globalisation and climate change invasive pathogens and pests have increased in recent years, thereby heightening the threat to forests (Freer-Smith and Webber 2017). As a result outbreaks of forest diseases, driven by both native and introduced pathogens, are expected to increase in frequency and severity due to climate change, particularly through intensified drought and other abiotic stressors. However, predictions about their future impact remain uncertain, partly due to the complex effects of climate change on host–pathogen interactions (Sturrock et al. 2011). The proportion of timber harvested due to calamities has reached high values across various tree species in Germany in recent years (BMEL 2019, 2021, 2023, 2024a). This is not only reflected in higher economic harm, but tree diseases also threaten the additional benefits that trees provide to society, such as of air quality improvement, biodiversity, watershed services and climate regulation, to name just a few (Krieger 2001; Boyd et al. 2013; BMEL 2024a).

1.2 European beech (*Fagus sylvatica*)

According to Denk (2003), the genus *Fagus* Tourn. ex L. (Fagaceae) includes approximately ten monoecious, broad-leaved, deciduous tree species. European beech (*Fagus sylvatica* L.) is naturally widespread in Central Europe and is the only native species of the genus *Fagus* in this region (Schütt et al. 1992). Typical components of Germany's potential natural zonal vegetation include European beech forests, which represent the vegetation that would dominate under present environmental conditions without human influence (Tüxen 1956; Bohn et al. 2003; Ellenberg and Leuschner 2010). The European beech shapes a variety of natural forest communities in Central Europe, including *Luzulo-Fagetum*, *Asperulo-Fagetum*, *Carici-Fagetum*, *Cephalanthero-Fagion* and *Lonicero alpigenae-Fagenion* (Pott 1993; Fischer 2003; Meyer 2011).

According to the Fourth National Forest Inventory, European beech is the most common deciduous tree species in Germany, covering 16.6% of the forest area (BMEL 2024b). The climatic factors that primarily limit its distribution are cold winters, late frost, and severe droughts (Dittmar et al. 2006; Ellenberg and Leuschner 2010; Weigel et al. 2018). European beech is generally classified as a tree species with rather anisohydric behaviour (Leuschner 2020; Schumann et al. 2024). However, findings by Nguyen et al. (2017) suggest that the degree of anisohydry depends on the drought exposure of the provenance. European beech has a very wide soil chemical amplitude and is adapted to nearly all soil conditions. It can thrive in both acidic, nutrient-poor soils as well as calcareous sites. Only soils with long-standing groundwater and waterlogging are problematic for vitality of European beech (Leuschner et al. 1993; Leuschner 1998; Bartsch et al. 2020). Due to this broad ecological and climatic amplitude, its growth characteristics and its ability to tolerate shade, European beech is considered the most competitive tree species in Central Europe (Schütt et al. 1992; Bartsch et al. 2020). The growth of European beech in its youth is relatively slow compared to other tree species under full light conditions but continues well into old age. On a favourable site, a dominant European beech can reach a height of 30 metres by the age of 100 and up to 40 metres by 150 years (Schütt et al. 2004). European beech is an important tree species in German forests from both an ecological and economic perspective. Forests dominated by European beech offer distinct habitat conditions compared to other broadleaf forests, characterised by deep shade, persistent and accumulating leaf litter, and a relatively low diversity of shrubs and

mosses (Delagrange et al. 2006; Valladares and Niinemets 2008; cited in Brunet et al. 2010). The herbaceous layer of beech forests exhibits distinct seasonal variation. In spring, a carpet of flowering geophytes dominates, followed by shade-tolerant ferns, herbs, and grasses in summer. In canopy gaps, light-demanding herbs and grasses thrive periodically (Gálhidy et al. 2006; Hahn and Thomsen 2007; Naaf and Wulf 2007; cited in Brunet et al. 2010). Faunistic studies conducted by the Senckenberg Institute in the natural forest reserve „Niddahänge östlich Rudingshain“ (Hesse) estimated the presence of 4.500 animal species in 120 to 160 year-old European beech forests growing on basalt and red sandstone (Dorow and Flechtner 1999). Compared with the 45.000 animal species recorded in the Federal Republic of Germany according to Blab et al. (1984), Dorow and Flechtner (1999) concluded that the studied beech forests with an area of less than 0.75 square kilometres (0.000002% of the area of Germany) contain around 10% of the species of the native fauna. Data from the National Data and Information Centre for Fungi in Switzerland reveal that beech can serve as a partner or host for well over 2000 fungus species (Blaser and Gross 2022). Within the Kellerwald-Edersee National Park (Hesse, part of the UNESCO World Heritage site Ancient Beech Forests of Germany since 2011; UNESCO 2013), 1107 fungal species have been recorded across the 5724 hectares (Langer et al. 2015).

From an economic point of view, systematic beech management aims to produce high-value, large-diameter stem wood (Muck et al. 2009). In 2023, wood from the wood species group beech accounted for 15% of the total timber harvest in state forests in Germany (BMEL 2024a). The European beech has creamy white to reddish-white, diffuse-porous heartwood with fine pores and distinct growth rings. The largest rays are conspicuous on all surfaces. In contrast to the wood of American beech (*Fagus grandifolia* Ehrh.), crystals are absent in the longitudinal and ray parenchyma cells (Hoadley 1991). The use of European beech wood has undergone substantial changes since the last century. While European beech wood was mainly used as firewood at the beginning of the 20th century, today it is one of the woods with the greatest potential uses (Kucera and Pohler 1988). It is easy to process, easy to split and easy to impregnate. European beech wood is in high demand in the furniture industry and is a popular veneer wood. (Kucera and Pohler 1988; Erlbeck et al. 1998; Hapla and Militz 2008). In the form of "BauBuche" by the company Pollmeier, it is also used in structural applications (Hassan and Eisele 2015).

1.3 Fungal lifestyles with a focus on endophytes

Fungi are heterotrophic organisms, meaning they must obtain their energy and nutrients from external organic sources. According to Boddy (2021), fungi can be classified into three categories based on their lifestyle. However, these categories are not mutually exclusive. Fungi may shift between strategies or adopt multiple simultaneously. The three categories are saprotrophs, which feed on dead organic matter; necrotrophs, which kill host cells to extract nutrients; and biotrophs, which derive energy from living cells. Biotrophy can be either harmful, as in the case of biotrophic pathogens, or mutually beneficial, as observed in lichens, mycorrhizal associations, and certain endophytic relationships. The term endophyte is derived from the Greek words "éndon" (ἔνδον) = inside and "phytón" (φυτόν) = plant. However, various definitions of endophytes have been established (Schulz and Boyle 2006). According to the first definition by de Bary (1866), endophytes, in contrast to epiphytes, are all organisms that colonise internal plant tissue. In the present thesis, the term endophyte is used according to the definition by Boddy and Griffith (1989) and Sieber (2007), where endophytes are fungi that are present in the living tissue of an organism without causing symptoms. This condition persists either for the entire lifetime of the colonised plant tissue or for an extended period of time (Sieber 2007). The definition thus describes a momentary status (Schulz and Boyle 2006) and therefore does not exclude potentially pathogenic fungi that may switch from quiescence to pathogenicity when conditions become favourable for the endophyte and/or unfavourable for the host (Sieber 2007). Mutualism between endophytes and their host has been observed in grasses, where endophytes of the family Clavicipitaceae (C-endophytes) can produce alkaloids that enhance host defense and contribute to increased host fitness as well as metabolites that stimulate plant growth (Clay 1988, 1991). The growth of clavicipitaceous endophytes occurs systemically throughout the aboveground tissues of their hosts, and vertical transmission of these host specific microbes has been documented (Clay and Schardl 2002). In comparison to C-endophytes, non-clavicipitaceous endophytes (NC-endophytes) have been classified as an additional group. The latter inhabit asymptomatic tissues of non-vascular plants, and vascular plants such as ferns and their allies, conifers, and angiosperms. They are highly diverse fungi, forming a polyphyletic assemblage of primarily ascomycetous fungi with a broad host range. However, their ecological role remains poorly understood (Rodriguez et al. 2009).

1.4 The mycobiome of European beech

The mycobiome refers specifically to the fungal component of the microbiome (Ghannoum et al. 2010). It includes both fungi that can harm trees, as well as fungi that have beneficial relationships with trees. Beneficial relationships include mycorrhizal fungi, lichens, and endophytes (Boddy 2021). Both mutualistic and parasitic relationships between European beech and fungi have been extensively studied. Like many European temperate forest tree species, European beech is in natural condition obligatorily associated with ectomycorrhiza fungi (Meyer 1973; Piepenbring 2022). Studies on the fungal community of mycorrhizal fungi associated with European beech mostly focus on young plants. This is likely because root sampling is easier at this age, and drought stress can be more easily induced artificially, an important aspect, as the interaction between drought and mycorrhization is a frequently studied research field. Results from Hamp et al. (1999), Shi et al. (2002) and Pietras et al. (2013) on young plants indicate that the mycorrhizal fungi of European beech are highly diverse and that drought indeed affects the composition of the mycorrhizal fungal community. Regarding lichen diversity on European beech, research conducted by Hofmeister et al. (2016) in the Czech Republic revealed that none of the other tree species examined matched the lichen diversity recorded on European beech. Species rich lichen communities on European beech have also been reported in studies from Germany (Moning and Müller 2009), Sweden (Fritz and Brunet 2010), and Ukraine (Dymytrova et al. 2014). While the endophytic mycobiome of European beech has been extensively studied in leaves and to a slightly lesser extent in twigs by many authors, research on trunks and roots is comparatively rare. This is likely due to the fact that sampling of leaves, as well as twigs, is easier and involves fewer substantial injuries to the sampled tree. Sieber and Hugentobler (1987), Pehl and Butin (1994), Unterseher and Schnittler (2010) and Ceccarelli (2011) studied the endophytic community in European beech leaves using a culture-based approach. In contrast, Unterseher et al. (2013, 2016) and Siddique and Unterseher (2016) analysed leaves using NGS (Next-Generation Sequencing). Studies on the endophyte community in twigs or branches were conducted by Chapela and Boddy (1988), Petrini and Fisher (1988), Griffith and Boddy (1990), Danti et al. (2002), and Ceccarelli (2011), all using a culture-based approach. Gilmartin et al. (2022) investigated the endophyte community in various tree compartments including the trunks. Sapwood of mature European beech trees was sampled, using both culture-based and molecular

approaches. Langer and Bußkamp (2021) investigated the endophyte community in trunks and twigs of saplings (culture-based). Parfitt et al. (2010) used a culture-based approach and specific primers to attempt the detection of eleven fungal species endophytically in the sapwood of trunks and branches. Ahlich and Sieber (1996) examined the endophyte community in non-ectomycorrhizal fine roots of European beech (culture-based). Seeds were sampled for endophytes using a culture-based approach by Mańka et al. (2012) and Stolarek (2022). The studies presented demonstrate the diversity of the endophyte community, which not only varies greatly between the different tree compartments studied, but also according to the research approach the study was based on. All mentioned studies above showed that Ascomycota generally make up a larger portion of the detected endophytes. Across tree compartments, fungi of the genera *Alternaria*, *Diaporthe*, *Fusarium* and *Phoma* were frequently detected besides others in the before mentioned studies. Among the identified species were also known pathogens such as *Apiognomonia errabunda* (Roberge ex Desm.) Höhn. (Butin 2011), *Biscogniauxia nummularia* (Bull.) Kuntze (Granata and Whalley 1994), and *Neonectria coccinea* (Pers.) Rossman & Samuels (Langer and Bußkamp 2021).

Alongside to the above mentioned studies on fungi in asymptomatic tissue, there are also comprehensive studies on fungi in various types of symptomatic tissue (*e.g.* Hendry et al. 1998; Grüner and Metzler 2006; Hecht et al. 2015; Purahong et al. 2021; Kļaviņa et al. 2025), as well as studies on the mycobiome of dead wood of European beech (*e.g.* Ódor et al. 2006; Blaser et al. 2013; Hoppe 2015; Arnstadt et al. 2016; Baldrian et al. 2016).

1.5 Complex diseases of European beech

Manion (1981) postulated that in order to understand the dynamics between pathogens and hosts, interactions with the environment must always be considered. Complex diseases are characterised by the fact that they are not triggered by a single causal factor of abiotic or biotic origin, but by the interaction of a number of interchangeable specifically ordered abiotic and biotic factors. These produce a gradual general deterioration, often ending in the death of trees (Manion 1981). European beech has long been considered a tree species with rather few serious pathogens and pests (Hartig 1877; Klimetzek 1992; Schmidt 2005; Butin 2011). Nevertheless, with beech bark disease (BBD) (McIntosh 1849; Hartig 1878; Ehrlich 1934) and Vitality loss of

beech (VLB) (Bressem 2008), two complex diseases of European beech are known. Additionally, *Phytophthora* infections with complex damage progression have been documented on European beech, characterised by interactions between various pathogens (Bressem 2008).

1.5.1 Beech bark disease

In Germany BBD was first documented in 1849 (McIntosh 1849). The disease occurs on European beech following the interaction between the beech scale insect, *Cryptococcus fagisuga* Lind. and either *N. coccinea* or *Neonectria ditissima* (Tul. & C. Tul.) Samuels & Rossman (Thomsen et al. 1949; Niesar et al. 2007; Hirooka et al. 2013). Common symptoms are elongated bark necroses, extending several metres along the shady side of the trunks (Niesar et al. 2007; Bressem 2008). Wood boring insects and wood decay fungi characterise the advanced stages of damage (Arend et al. 2006; Niesar et al. 2007; Bressem 2008). The disease emerges periodically in Central Europe (Niesar et al. 2007). The last widespread outbreaks of the disease were documented in Germany, Luxembourg, France and Belgium between 1999 and the early 2000s (Eisenbarth 2001; Emschermann and Niesar 2001; Pankert 2001; Theisen 2001; Niesar et al. 2003, 2004). The outbreak mainly affected European beech trees over 120 years old (Arend et al. 2006; Niesar et al. 2007). The extent to which BBD is linked to a specific location especially altitude is debated (Schindler 1951; Arend et al. 2006; Niesar 2007). However, warm, rainy winters seem to favour the occurrence at high altitudes (Niesar 2007; Niesar et al. 2007).

In North America, American beech is, like the European beech in Europe, affected by BBD (Ehrlich 1934; Houston 1994). The non-native beech scale was first reported on American beech trees in 1911 (Hewitt 1914), more than 20 years after it was accidentally introduced to the Halifax Public Gardens on ornamental European beeches (Ehrlich 1934). In contrast to Europe, *Neonectria faginata* (M.L. Lohman, A.M.J. Watson & Ayers) Castl. & Rossman, and not *N. coccinea*, is associated with BBD outbreaks in North America, while *N. ditissima* is involved in the damage pattern in both Europe and North America (Ehrlich 1934; Spaulding et al. 1936; Thomsen et al. 1949; Houston 1994; Castlebury et al. 2006; Niesar et al. 2007; Hirooka et al. 2013). According to Hirooka et al. (2013), BBD is one of the greatest threats to beech forests on global scale.

1.5.2 Vitality loss of beech

The years between 2018 and 2022, with the exception of 2021, were characterised by drought and exceptional heat in Germany (Rakovec et al. 2022; Bose et al. 2022; Imbery et al. 2023). In central Germany, this has led to an increased incidence of VLB (Langer et al. 2020; Langer and Bußkamp 2021; Purahong et al. 2021), a complex disease affecting European beech (Bressem 2008). The disease is triggered by abiotic factors like heat, drought and exposure to intense direct sunlight (Bressem 2008; Asche 2016; Langer et al. 2020). Due to the reduced vitality of the host tree, fungi and insects appear as secondary pathogens and pests (Bressem 2008; Asche 2016; Brück-Dyckhoff et al. 2019; Langer et al. 2020). Some of the fungi associated with VLB like *B. nummularia* and *Hypoxylon fragiforme* (Pers.) J. Kickx f. are already present in the host tree as endophytes and switch to a pathogenic lifestyle once the host is weakened (Desprez-Loustau et al. 2006; Slippers and Wingfield 2007; Mehl et al. 2013; Langer and Bußkamp 2021, 2023; Langer et al. 2021). The presence of these latent pathogens can greatly influence the progression of damage (Bressem 2008; Langer 2019; Langer et al. 2020; Langer and Bußkamp 2021, 2023; Purahong et al. 2021). Symptoms of the disease are diverse, including declining growth (Scharnweber et al. 2020; Leuschner et al. 2023), premature leaf shedding, crown dieback, bark necrosis (Fig. 1) and the death of affected trees (Langer et al. 2020; Nussbaumer et al. 2020; Bigler and Vitasse 2021; Langer and Bußkamp 2021, 2023; Purahong et al. 2021; Arend et al. 2022). In 2022, 31% of all harvesting in the wood species group beech in Germany was carried out due to calamities (BMEL 2023). The drought related calamities since 2018 have thus led to various working groups and research projects increasingly focusing on this tree species (Hertel 2023). The debate about the future prospects of European beech in the context of climate change has been reignited, a debate that has already been ongoing for years (*e.g.* Rennenberg et al. 2004; Ammer et al. 2005; Geßler et al. 2006; Hanewinkel et al. 2013; Kasper et al. 2022; Wang et al. 2022; Klemmt et al. 2023; Rukh et al. 2023). The discourse is primarily shaped by questions regarding the adaptability of European beech to new climatic conditions characterised by prolonged droughts and the extent to which the tree species is declining in vitality and competitiveness under these circumstances.

1.5.3 *Phytophthora* infections with complex damage progression

The susceptibility of European beech to non-fungal, primary pathogenic *Phytophthora* de Bary (Peronosporomycetes, Stramenopile) species has long been known (Day

1938). Jung (2009) detected eleven different *Phytophthora* species in studies of damaged beech stands in Bavaria between 2003 and 2007. The most frequent detected species were *P. cambivora* (Petri) Buisman and *P. cactorum* (Lebert & Cohn) J. Schröt. Typical symptoms of *Phytophthora* infections include collar rot, characterised by orange-brown, tongue-shaped necroses that can extend up to seven metres up the trunk, as well as trunk cankers. These symptoms only occur when larger parts of the roots near the surface are already infected. The primary *Phytophthora* lesions are then colonised by a series of secondary fungal bark pathogens, including *N. coccinea*, and wood decay fungi, frequently *Ustulina deusta* (Hoffm.) Maire and *Armillaria* spp. In addition, infected trees are often attacked by various bark breeding and wood boring insects, in particular *Taphrorychus bicolor* (Herbst, 1793) [2] and *Trypodendron domesticum* L. (Bressem 2008; Jung 2009). Particularly endangered are European beeches on base-rich, waterlogged sites where infestation with *Phytophthora* spp. can lead to the extensive death of beech stands (Hartmann and Blank 1998; Hartmann et al. 2006; Bressem 2008).

[2] International Commission on Zoological Nomenclature, Article 51.3: “When a species-group name is combined with a generic name other than the original one, the name of the author of the species-group name, if cited, is to be enclosed in parentheses”. Available at https://www.iczn.org/the-code/the-code-online/, retrieved 10.04.2025.

Figure 1: Matured trees of *Fagus sylvatica* with typical symptoms of Vitality loss of beech. A) Bark necrosis on the sun-exposed side of the tree. B) Early leaf shedding and crown dieback. (Photos © Nordwestdeutsche Forstliche Versuchsanstalt).

1.6 *Biscogniauxia nummularia*

Species of the genus *Biscogniauxia* Kuntze (Ascomycota) are considered to be pathogens capable of causing damage at least when the host is severely weakened (Ju et al. 1998). Based on the data available at that time, Ju et al. (1998) also indicated that *Biscogniauxia* species are adapted to dry or at least seasonally dry habitats. Fungi of the genus *Biscogniauxia* belong to the family Graphostromataceae M.E. Barr, J.D. Rogers & Y.M. Ju (Wendt et al. 2018) which is part of the order Xylariales Nannf. It is known, that species of Xylariales are able to cause severe wood decay, which is generally exceptional among Ascomycota (Merrill et al. 1964; Duncan and Eslyn 1966; Worrall et al. 1997). While Ju et al. (1998) recognised 49 taxa of the genus *Biscogniauxia* worldwide, only two species are confirmed to occur on European beech: *B*. *nummularia* and *B. mediterranea* (De Not.) Kuntze (Ju et al. 1998; Ceccarelli 2011; Langer and Bußkamp 2021, 2023; Fig. 2).

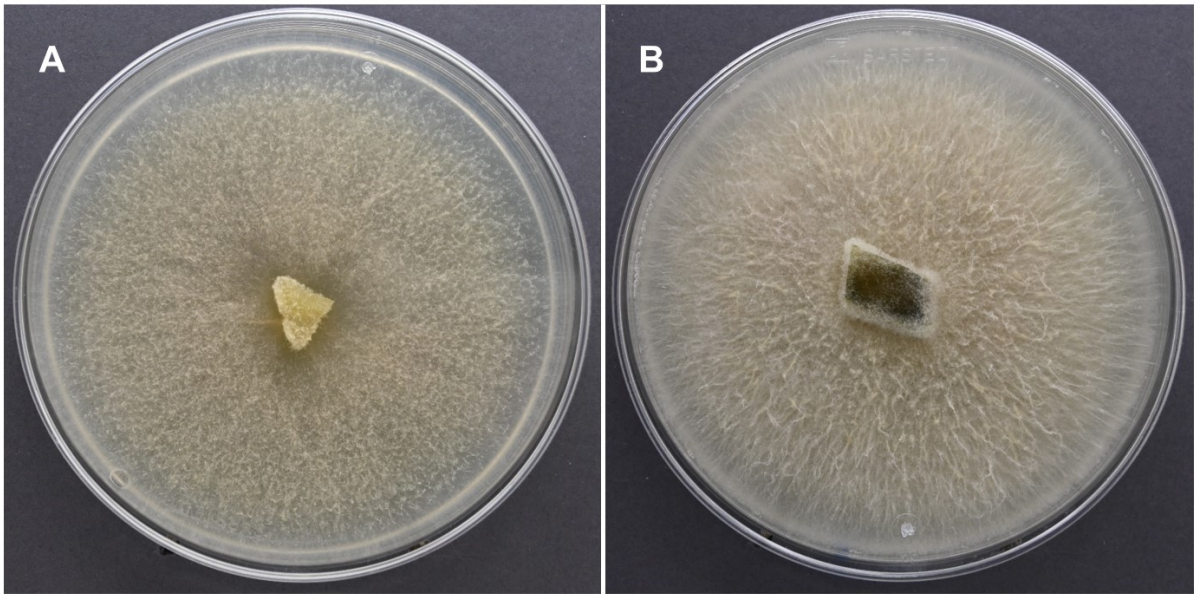

Figure 2: *Biscogniauxia* pure cultures. Seven-day-old fungal cultures grown on Malt Yeast Peptone Agar, modified according to Langer (1994). A) *Biscogniauxia nummularia*. B) *Biscogniauxia mediterranea*. (Photos © Nordwestdeutsche Forstliche Versuchsanstalt).

Biscogniauxia nummularia is considered to be one of the most frequently detected endophytes in European beech (Chapela and Boddy 1988; Nugent et al. 2005; Langer and Bußkamp 2021; Langer et al. 2021). The fungus is known to transition from an endophytic lifestyle to a pathogenic one when its host is stressed by drought and heat, causing various symptoms like bark necroses and strip-cankers, wood decay and beech decline (Granata and Whalley 1994; Hendry et al. 1998, 2002; Nugent et al. 2005; Luchi et al. 2015). However, while *B. nummularia* has been detected as an

endophyte on various host tree species (Bußkamp 2018; Schlößer et al. 2023; Peters et al. 2023), this transition, along with the associated symptoms and fruiting bodies, has, to date, only been documented on *Fagus orientalis* Lipsky and *F. sylvatica* (Nugent et al. 2005; Langer and Bußkamp 2023; Zamani et al. 2024). The ongoing VLB outbreak in Germany in 2018 is closely linked to the presence of *B. nummularia.* Both anamorphic and teleomorphic fructifications have been documented on affected trees (Langer and Bußkamp 2023; Fig. 3). However, reports of European beech decline associated with *B. nummularia* and drought are not confined to Germany. Cases of high mortality and beech decline linked to *B. nummularia* have been documented across various parts of Europe (Granata and Sidoti 2004; Lakatos and Molnár 2009; Mirabel and Gaertner 2023), highlighting the need for further research on *B. nummularia*.

Figure 3: *Biscogniauxia nummularia* on decayed thick branches of *Fagus sylvatica*. A) Anamorph, with a white conidia layer. B) Teleomorph, with black stromata. (Photos © Nordwestdeutsche Forstliche Versuchsanstalt).

Biscogniauxia nummularia and *B. mediterranea* exhibit similar behaviour from an ecological perspective, proliferating in asymptomatic host tissue during dry growing periods (Vannini et al. 2009; Luchi et al. 2015). However, *B. mediterranea* appears to be less associated with the recent beech decline in Germany than *B. nummularia* (Langer and Bußkamp 2023).

In 2020, Vujanovic et al. (2020) proposed the name *Biscogniauxia destructiva* Vujan for a presumed hybrid of *Biscogniauxia anceps* (Sacc.) J.D. Rogers, Y.M. Ju & Cand. and *B*. *nummularia*. The name *B*. *destructiva* was chosen due to its aggressiveness towards European beech. However, due to violation of the International Code of Nomenclature for algae, fungi, and plants the name is listed as invalid (Art. F.5.1 Shenzhen[3]).

1.7 Aim of the work

The presented studies were conducted at the Northwest German Forest Research Institute (Nordwestdeutsche Forstliche Versuchsanstalt) as part of the joint research project BucheAkut (funding reference number 2220WK10B1). The aim of the project was to analyse the causes of the occurring symptoms and to identify predisposing factors of VLB. While the presented studies investigated the role of fungi as predisposing and contributing factors, project partners examined the influence of forest management practices and site factors like water balance. Based on the results a risk analysis was carried out.

Although several studies have examined the fungal community of European beech, investigations into the fungal community in the context of VLB remain scarce. Consequently, it remains widely unclear which fungi contribute to the observed symptoms and to what extent the presence of endophytes may act as a predisposing factor for the onset of VLB. To address this question, the investigations focused on stands with varying degrees of damage. The aim was to determine whether severely damaged trees differ in their fungal community from undamaged trees. Since symptoms of this complex disease seem to occur independently in the crown and on the trunk, both symptomatic and asymptomatic tissue from different tree compartments were examined, and the isolated fungi were compared. To describe the lifestyle of the associated fungi more precisely, samples from the xylem and the cambium were distinguished. In the course of these investigations, key pathogens were identified. Since *B. nummularia* was identified as a key pathogen of VLB, further studies on *B. nummularia* were conducted. The aim was to examine whether *B. nummularia* strains exhibited genetic variability based on their geographic origin and host species. In the

[3] indexfungorum.org, retrieved 03.12.2024.

next step, it was tested whether these differences were reflected in their virulence and adaptation to specific temperature conditions.

The following hypotheses were formulated and investigated within the scope of this thesis: 1) The fungal community of symptomatic and asymptomatic woody tissues differs between vital and damaged matured European beech trees in central Germany. 2) Pathogens of European beech are also widely present in asymptomatic tissues of matured European beech trees in central Germany. 3) The endophyte community varies depending on the sampled tree compartment of matured European beech tress in central Germany. 4) *Biscogniauxia nummularia* strains exhibit genetic variability based on their geographic origin. 5) *Biscogniauxia nummularia* strains differ in their temperature-related growth characteristics. 6) Wood decay capacities of *B. nummularia* strains can vary considerably and 7) *B. nummularia* strains originating from host trees other than European beech cause considerably less mass loss on European beech wood compared to strains originating from European beech.

2. Manuscript I

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Fungi associated with Vitality loss of European beech in central Germany

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Abstract

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

Key words

Vitality loss of beech, Endophytes, European beech, *Biscogniauxia nummularia*, *Neonectria coccinea*

Introduction

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

2024) which leads to a „structural flipp“ through the loss of structural complexity in the upper stand layers (Höwler et al., 2024).

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

It can be reasonably assumed that drought events will become more prevalent in Europe during the 21st century due to climate change (Spinoni et al., 2018; Hari et al., 2020). As a result VLB will continue to affect large areas of Germany in the future. In order to identify areas at risk, it is essential to have an understanding of the damage process and to be aware of the pathogens involved. While there is some data about the endophyte community of European beech, many studies focus only on leaves, branches or twigs (*e.g.* Kowalski and Kehr, 1992; Unterseher and Schnittler, 2009; Ceccarelli, 2011; Unterseher et al., 2013; Guerreiro et al., 2018). In addition, the role of endophytes associated with VLB is largely unknown (Langer and Bußkamp, 2023). The initial studies on the fungi associated with European beech trees suffering from VLB were conducted by Langer and Bußkamp (2021, 2023) using a culture-based method and by Purahong et al. (2021) through Next Generation Sequencing.

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

Materials and methods

Forests plots

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

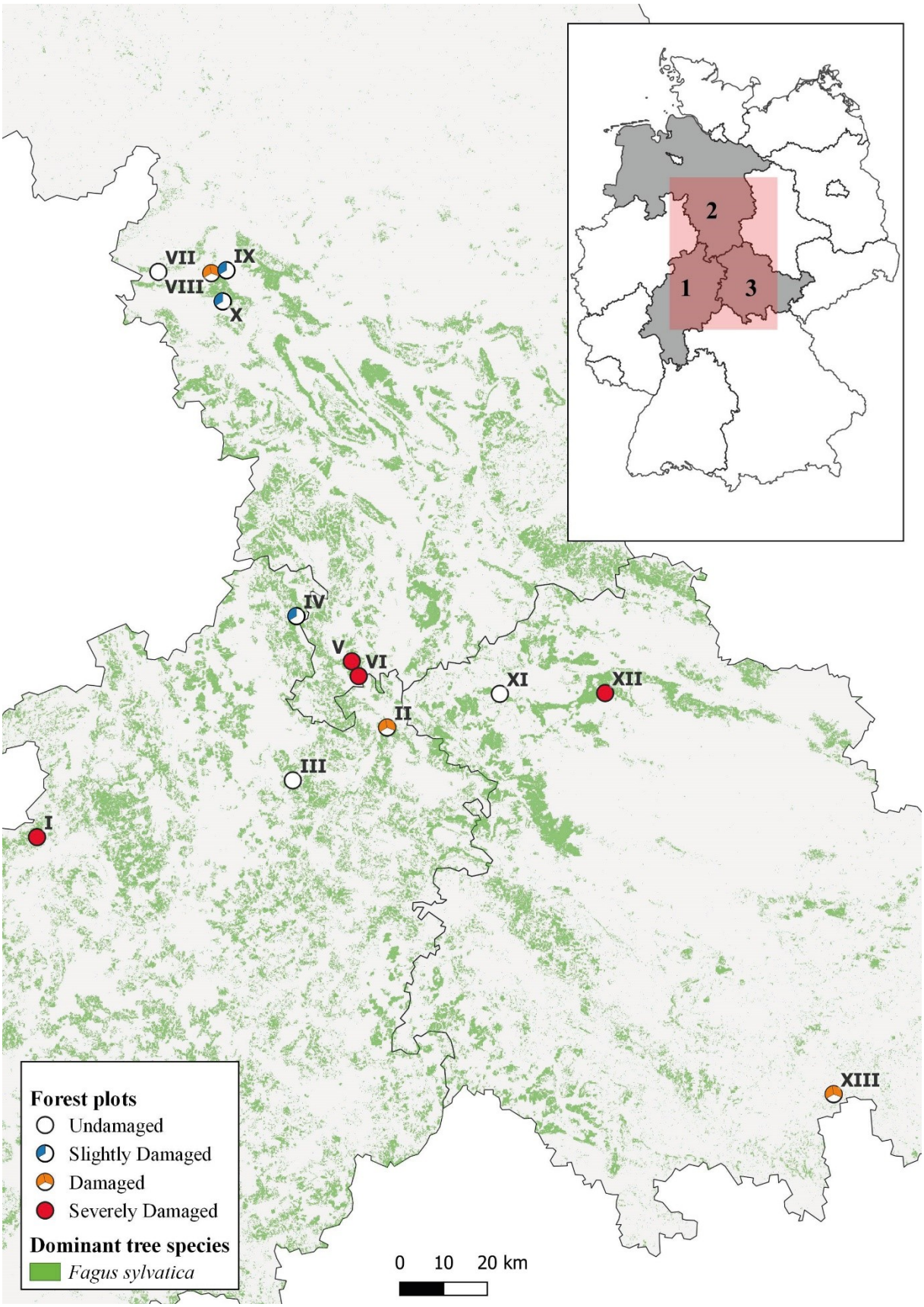

Figure 1: Detailed map showing the 13 locations of the forest plots (forest plots I-XIII) studied and areas where *Fagus sylvatica* is the dominant tree species (green) according to Blickensdörfer et al. (2024). Forest plots are differentiated according to their degree of damage due to Vitality loss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

Test trees

Since the plots were created as long-term observation plots, no tree was removed within the plot because the structure of the test area was not to be changed during the test period. One tree (*Fagus sylvatica*) per plot was cut down with a chainsaw in the immediate vicinity of each plot that was representative of the degree of damage to the plot. The diameter at breast height (1.3 m above ground), of the test trees was measured before harvesting and tree height after harvesting. Trees were assessed according to Kraft (1884) in their sociological position. After felling, the lying trees were examined intensively for signs of insect or fungal infestation. All test trees were harvested between January and March 2023.

Isolation of fungi

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

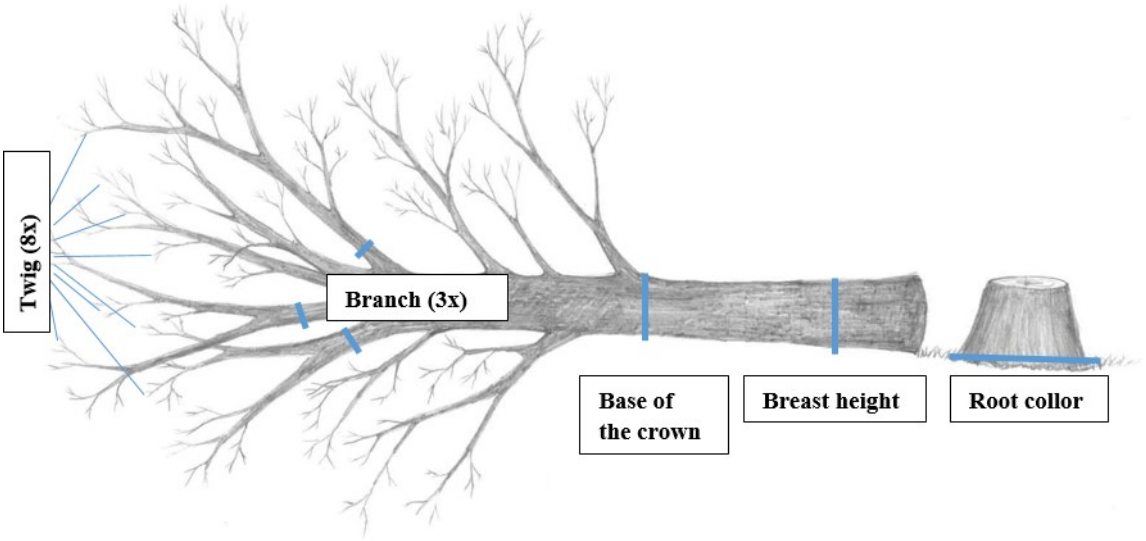

Figure 2: Schematic representation of a felled test tree and the selected areas (marked in blue) from which samples were taken from asymptomatic tissue of all test trees in a standardised manner. The graphic was designed by Victoria Tropf

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

of 2022 was smaller than 10 cm for twigs, the remaining intended pieces were taken from the previous year or the previous year's growth. Like the wood chips, the twig pieces were then transferred to Petri dishes containing MYP. The Petri dishes with the wood chips or twig pieces were incubated at room temperature and out of direct sunlight for four weeks. Isolates were checked once a week. Occurring mycelium of filamentous fungi was brought into pure culture. The pure cultures were grouped into morphotypes (MTs) based on similarity of culture morphology. At least one culture of each MT was stored in cryopreservation (-80 °C) at the fungal culture collection of the Northwest German Forest Research Institute (NW-FVA). In addition to fungi that could be assigned to a MT, fungi that were terminated or overgrown by other fungi and thus could not be brought into pure culture were summarised under “Fungus sp.”.

Molecular analysis

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

The 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers ITS-1 and ITS-2 (ITS region) was amplified for all strains using the primer pair ITS-1F (Gardes and Bruns, 1993) and ITS-4 (White et al., 1990). For a selection of fungal groups based on MT grouping (Table 3), a partial sequence of the 28S nrDNA (LSU) was amplified using the primer pair LROR (Rehner and Samuels, 1994) + LR5 (Vilgalys and Hester, 1990). Furthermore, additional DNA regions were amplified for a more precise taxonomic classification of selected taxa. The respective primer pairs and PCR conditions are given in suppl. mat. Table 1. The mixture for all PCR reactions consisted of 1 µl of DNA and 19 µl mastermix which contained 2.5 µl 10x PCR reaction buffer (with 20 mmol/l $MgCl_2$, Carl Roth, Karlsruhe, Germany), 1 µl of each primer (10 mmol/l), 2.5 µl $MgCl_2$ (25 mmol/l), 0.1 µl Roti®-Pol Taq HY Taq polymerase (Carl Roth, Karlsruhe, Germany) and 2.5 µl of 2 mmol/l dNTPs (Biozym Scientific GmbH, Hessisch Oldendorf, Germany). Each reaction was topped up to a volume of 20 µl by adding HPLC Water (Carl Roth, Karlsruhe, Germany). A StepOnePlus™ PCR System (Applied Biosystems, Waltham, Massachusetts, US) or a GeneExplorer 96 (Hangzhou BIOER Technology, Hangzhou, China) was used to carry out the DNA amplifications. The PCR conditions for the amplification of the ITS and LSU regions were set according to Bien et al. (2020) and Paulin and Harrington (2000), respectively. After visualisation in 1% agarose gel, PCR products were sent to Eurofins Scientific Laboratory (Ebersberg, Germany) for sequencing. From all resulting sequences consensus sequences were generated, and visually checked and edited if necessary using BioEdit Sequence Alignment Editor (v. 7.2.5; Hall, 1999). Sequences were submitted to GenBank (Table 3).

Identification of fungi

The analysis was restricted to fungi of the subkingdom *Dikarya*. Morphotype assignment based on morphology as stated above was supplemented by DNA information of representative strains and adjusted where necessary following Guo et al. (2000). For the taxonomic classification ITS sequences were used in blastn searches on the GenBank database (http://www.ncbi.nlm.nih.gov/genbank, Altschul et al., 1997). In case of an inconclusive blastn result (*e.g.* low percentage identity, different taxa with a similar degree of agreement), the identification was labelled cf. (confer) to imply a certain degree of

uncertainty. Cases of higher uncertainty were determined at the next higher conclusive taxonomic level. Results were confirmed based on literature and previously identified cultures from the institute's collection. Additional DNA loci were sequenced for a variety of MTs (see Table 3 and suppl. mat. Table 2) to improve the clarity of the respective taxonomic classification based on blastn searches and phylogenetic analyses. Phylogenetic analyses including appropriate reference sequences retrieved from GenBank were performed using RAxML v. 8.2.11 (Stamatakis, 2006, 2014) as implemented in Geneious R11 (Kearse et al., 2012) using the GTRGAMMA model with the rapid bootstrapping and search for best scoring ML tree algorithm including 1000 bootstrap replicates (data not shown).

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

Literature analysis

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

Data analyses

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

Results

Test trees

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

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

Isolated fungi

Of the 3999 incubated tissue samples, 1963 samples (48 %), showed outgrowths that could be assigned to filamentous *Dikarya*, 1436 (36 %) were sterile and 600 (15 %) were either not evaluable (terminated, overgrown) or the outgrowths did not belong to the filamentous *Dikarya*. From the 1963 samples with filamentous *Dikarya* outgrowths, 2156 fungi could be isolated, which were attributed to 181 MTs. In addition, 123 further outgrowths could not be brought into pure culture or determined („Fungus sp.”). Of the 181 MTs, 153 could be determined at least to the genus level and 92 of them even to the species level. A further 24 MTs were tentatively (cf.) identified to species level (table 3, marked with *). *Ascomycota* A to D are fungi that have been assigned to the group of black yeasts (Rosa and Péter, 2006) on the basis of their cultural characteristics. Based on a LSU-ITS phylogeny conducted, *Ascomycota* A1, A2, B1, and B2 are closely related to the type strain of *Lembosiniella eucalyptorum* Crous & Carnegie (CBS 144603) and a clade of unspecified strains of the genus (GenBank MT813964, MT813970, ON865956, OP467220). *Ascomycota* C also belongs to a clade of *Ascomycota* A-B, strains of *Lembosiniella* stated above and the type strain of the monotypic genus *Gobabebomyces* (*G. vachelliae*, CBS 146779). *Ascomycota* D can be assigned to the order *Myriangiales*, however, a more precise delimitation has not been possible. *Diaporthe eres* Nitschke and *Diaporthe rudis* (Fr.) Nitschke are regarded as species complexes (Gomes et al., 2013; Udayanga et al., 2014). When creating phylograms for fungal identification, reasonable reference sequences were consulted (*e.g.* Gomes et al., 2013, Dissanayake et al., 2017, Gao et al., 2017, Guarnaccia et al., 2018). It turned out that the different isolates belonging to *D. eres* and *D. rudis* were assigned to different clades within their group. Therefore, several MTs were assigned to the two groups. In the case of *Geoscypha tenacella* (Sacc.) Van Vooren, the cultures differed greatly from a macroscopic point of view. Comparison of the ITS DNA regions of the different strains also revealed minor differences. Therefore, two *G. tenacella* MTs were assigned.

Ascomycota were more frequently present and accounted for 167 of the MTs (92 %), whereas 14 MTs could be assigned to *Basidiomycota* (8 %). On the test trees, 15 orders of *Ascomycota* could be verified (Incertae sedis and "Not determinable" not included). The most common order among the MTs was *Pleosporales* (25 %) followed by *Diaporthales* (15 %) and *Xylariales* (11 %) (Figure 3). The majority of *Basidiomycota* MTs could be assigned to the order *Agaricales* (50 %), the others to *Polyporales* (29 %), *Auriculariales* (14 %) and *Trichosporales* (7 %) (Figure 3). The three most frequently isolated fungi overall were *B. nummularia* (*Xylariales*) with 515 isolates (*f* = *23*.9 %), followed by *Apiognomonia errabunda* (Roberge ex Desm.) Höhn. (*Diaporthales*) with 390 isolates (*f* =18 .1 %) and *Aureobasidium pullulans* (de Bary) G. Arnaud (*Dothideales*) with 174 isolates (*f* = 8.1 %). Only 15 MTs were isolated with a frequency ≥ 1 %, 79 MTs were isolated just once.

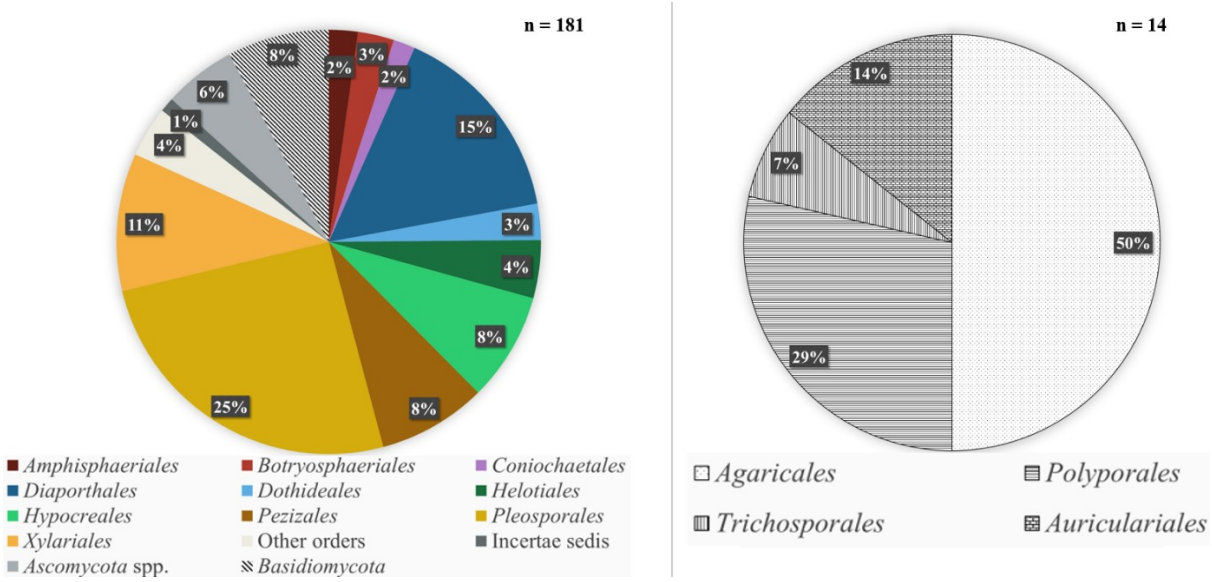

Figure 3: Proportion of orders by the morphotypes identified
Left: Proportion of *Ascomycota* orders by the morphotypes identified (with colour) and *Basidiomycota* (black and white with hatching, as one group). Orders of *Ascomycota* with two or fewer associated morphotypes are listed under "Other orders".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

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

Isolated fungi from asymptomatic tissue by test tree and damage class

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

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

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

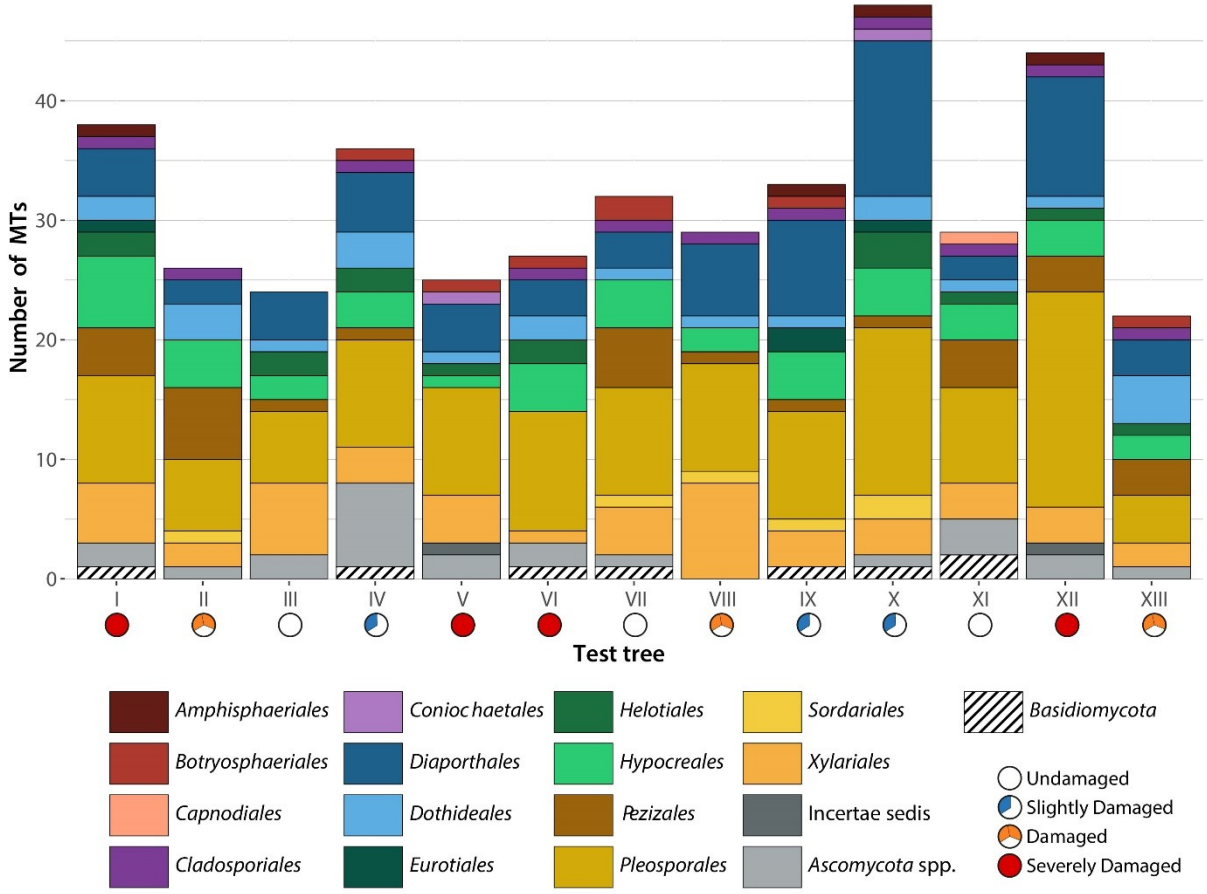

Figure 4: Number of endophytic morphotypes obtained from the various *Ascomycota* orders (with colour) and *Basidiomycota* (with hatching) differentiated according to the test tree from which they were isolated. "*Ascomycota* spp". summarises morphotypes of *Ascomycota* that could not be determined at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

Isolated Ascomycota by tree compartment

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

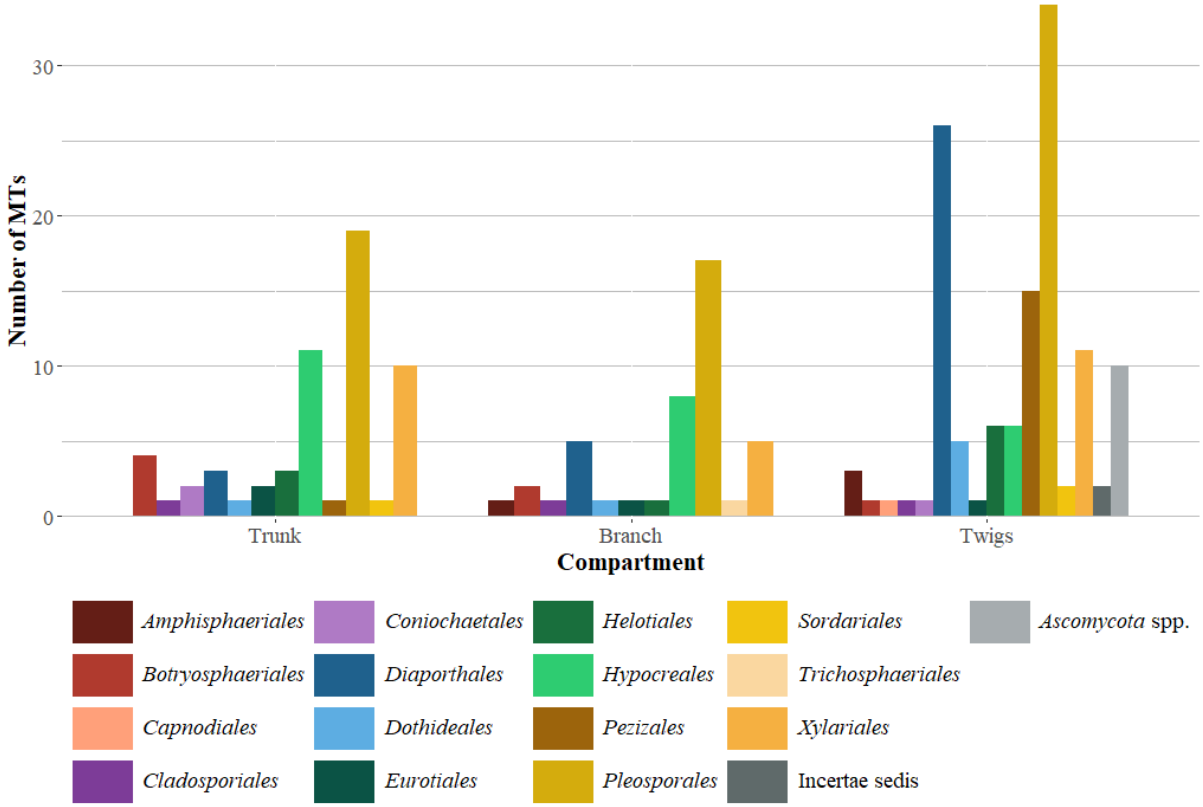

Figure 5: Number of ascomycetaceous morphotypes obtained from the various orders and differentiated according to the tree compartments (isolated from the trunk, the branches or the twigs). "*Ascomycota* spp." summarises morphotypes of *Ascomycota* that could not be determined at order level

Across all orders 58 different MTs were isolated from the trunk, 43 MTs from the branches and 125 different MTs were isolated from the twigs (Figure 6). Focusing on the trunk, 21 of the 58 MTs were not isolated from any other tree compartment. For the branches, eleven of the 43 MTs were isolated only from branches and for the twigs 92 of the 125 MTs were isolated only from the twigs. Of the 167 *Ascomycota* MTs detected across all test trees, 16 MTs were documented in all three compartments, namely *Alternaria infectoria* E.G. Simmons, *Alternaria* sp. 1, *Alternaria* sp. 2, *Asterosporium asterospermum* (Pers.) S. Hughes, *A. pullulans*, *B. nummularia*, *Cladosporium* spp., *Cytospora galegicola* Q.J. Shang, Camporesi & K.D. Hyde, *Didymella macrostoma* (Mont.) Qian Chen & L. Cai, *E. nigrum*, *Hypoxylon fragiforme*, *Neohendersonia kickxii* (Westend.) B. Sutton & Pollack, *N. coccinea*, *Penicillium* spp., *Pleosporales* sp. 7, and *Trichoderma* spp.

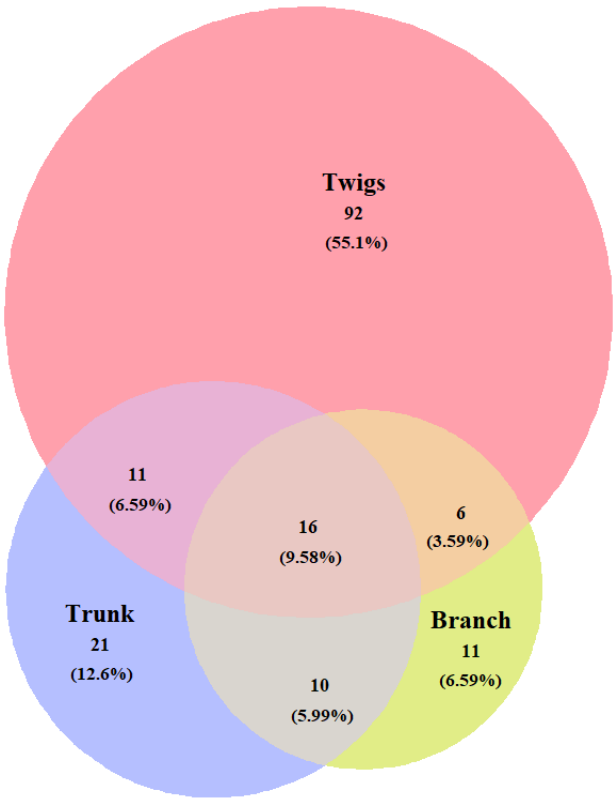

Figure 6: Number of ascomycetaceous morphotypes obtained differentiated according to the tree compartments into morphotypes isolated only from the trunk (blue), only from the branches (green) or only from the twigs (red), as well as all possible overlaps

Isolated Ascomycota by plant tissue

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

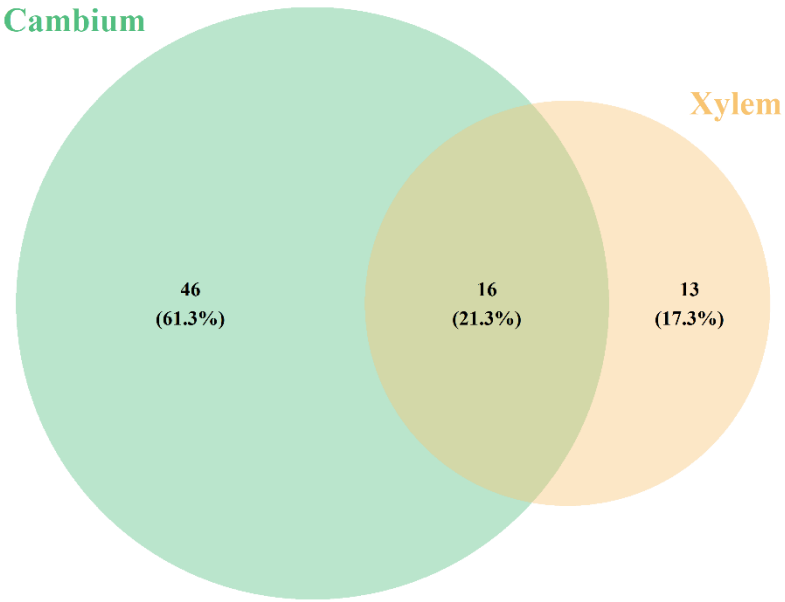

Figure 7: Number of ascomycetaceous morphotypes obtained from branches and trunks differentiated into morphotypes isolated from cambial tissue only (green), from xylem only (orange) or from both tissue types (overlap)

Isolated Ascomycota by tissue condition

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

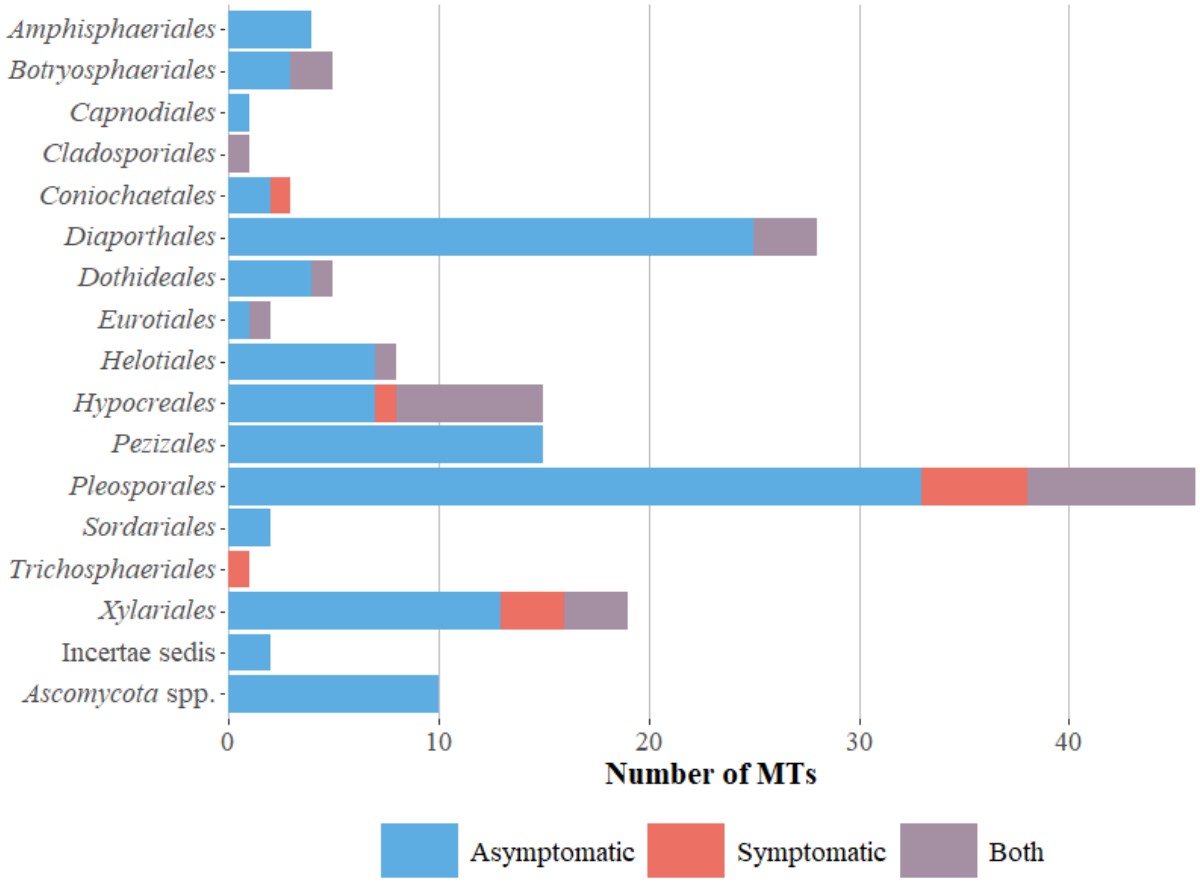

Figure 8: Number of ascomycetaceous morphotypes obtained within the orders and subdivided into morphotypes that were only isolated from asymptomatic tissue, only from symptomatic tissue or from both asymptomatic and symptomatic tissue. "*Ascomycota* spp". summarises morphotypes of *Ascomycota* that could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

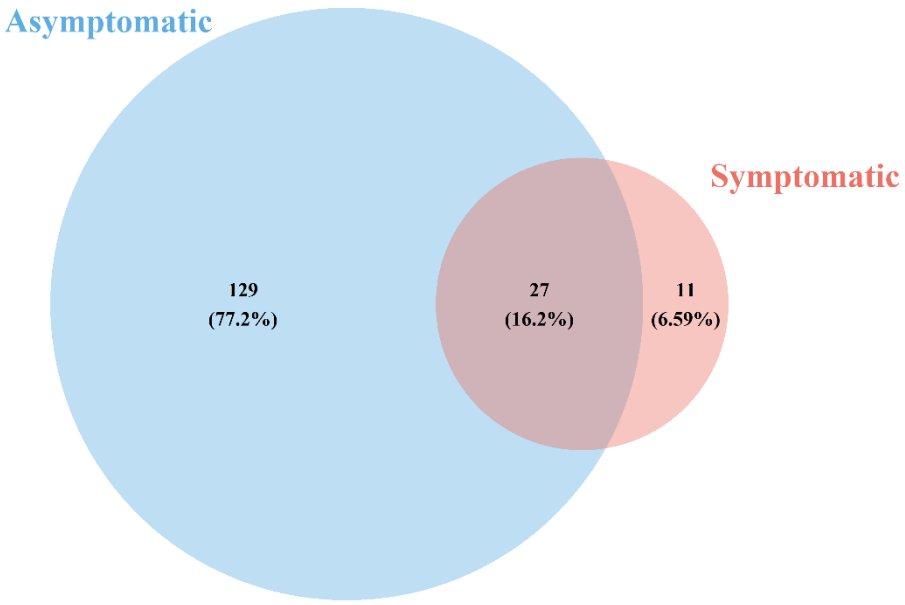

Figure 9: Number of ascomycetaceous morphotypes obtained that were isolated only from asymptomatic tissue (blue), only from symptomatic tissue (red) or from both asymptomatic and symptomatic tissue (overlap)

Apiognomonia errabunda

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

Biscogniauxia mediterranea

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

Biscogniauxia nummularia

With 515 isolates across all test trees, *B. nummalaria* was the most frequently detected fungus. *Biscogniauxia nummularia* was found 497 times in asymptomatic tissue and 18 times in symptomatic tissue (Figure 10). As an endophyte *B. nummularia* was detected on all 13 test trees. Of the twelve test trees on which symptomatic tissue could be sampled, the fungus was detected in symptomatic tissue on exactly half of the trees. *Biscogniauxia nummularia* was detected as an endophyte in damaged and severely damaged trees three times more frequently than in undamaged and slightly damaged trees (from 21 % to 7 %; proportion of asymptomatic tissue samples tested positive for *B. nummularia*). However, the damaged and severely damaged test trees XII and XIII are out of the ordinary, as the value here was only 2 % and 3 %, whereas it ranged between 12 % (II) and 43 % (VI) in the five other damaged or severely damaged trees. Twigs were the compartment from which *B. nummularia* was most frequently isolated as an endophyte with 467 isolates (detected in 19 % of all twig pieces). In asymptomatic tissue from branches and trunks, *B. nummularia* was detected 6 times and 24 times (3 % detection each of all tissue samples from the respective compartment). The symptomatic tissue from branches tested positive for *B. nummularia* with 14 isolates (5 %) and the tree trunks with 4 isolates (2 %). *Biscogniauxia. nummularia* was found both as endophyte and symptomatic more frequently in the cambium of branches (asymptomatic 5 % and symptomatic 8 %) and trunks (6 % and 5 %) than in the xylem of branches (0 %and 1 %) and trunks (1 %, and 1 %).

Neonectria coccinea

A total of 78 isolates were obtained from *N. coccinea*. The fungus was isolated from every test tree with the exception of test tree X (slightly damaged). The number of isolates from the positively tested trees ranged from two (IV and XI, slightly damaged and undamaged) to 21 (II, damaged). Of the twelve test trees from which *N. coccinea* was isolated, the fungus was isolated from the asymptomatic tissue of all all of them with the exception of tree VII. *Neonectria coccinea* was isolated from asymptomatic tissue in the damaged and severely damaged test trees about twice as often as in the undamaged and slightly damaged trees (3.1 isolates to 1.5 on average per group; outgrown from 1.2 % and 0.6 % of all asymptomatic samples accordingly). From symptomatic tissue, *N. coccinea* was detected on nine of the eleven trees that tested positive for *N. coccinea* and from which symptomatic tissue was sampled. With 47 isolates from symptomatic tissue, *N. coccinea* is the MT most frequently detected in symptomatic tissue. From symptomatic tissue of undamaged and slightly damaged trees, the fungus was isolated more frequently with an average of 12 % outgrowth than in the damaged and severely damaged trees, where this value was 9 %. Looking at the different tree compartments, *N. coccinea* was most frequently isolated

from the cambium of symptomatic branch and trunk tissue (22 % and 23 % of the described tissue samples) (Figure 10). In symptomatic xylem, *N. coccinea* was also detected in branches and trunks, but to a much lesser extent (2 % and 1 %). In asymptomatic tissue from branches and trunks *N. coccinea* was detected more frequently in the cambium (3 % and 5 %) than in the xylem (0 % and 1 %). The fungus was only isolated from 0.2 % of the incubated twig pieces.

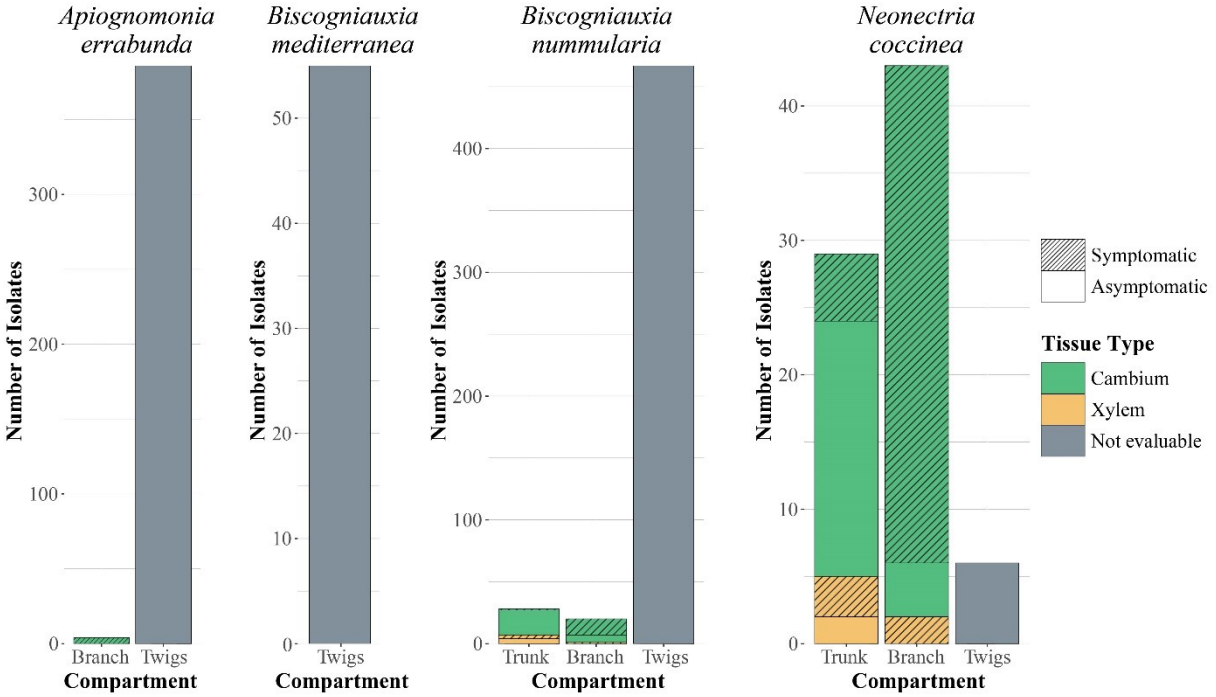

Figure 10: Number of isolates of *Apiognomonia errabunda*, *Biscogniauxia mediterranea*, *Biscogniauxia nummularia* and *Neonectria coccinea*, differentiated according to tree compartment and tissue type (with colour) as well as the health status of the tissue (with hatching) from which the species was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

Discussion

The role of beech-inhabiting fungi, especially endophytes, in the context of VLB was analysed in the present study using a culture-based approach. The present study aims to provide preliminary insights into the fungal species, particularly endophytes, that may be associated with VLB, and to illustrate the remarkable diversity of the fungal community inhabiting European beech. Nevertheless, care should be taken when attempting to generalise the findings, given the potential limitations of the study. A higher number of trees per plot would undoubtedly be beneficial for future studies in order to create statistical models. However, this is often precluded due to the considerable effort required for the study, as well as for ecological (nature conservation), economic (loss of value for the forest owner) and social reasons (critical reaction of citizens when trees are felled). As expected, the proportion of cultivated *Ascomycota* (92 %) was higher than *Basidiomycota* (8 %). Various studies on European beech (*e.g.* Sieber and Hugentobler, 1987; Petrini and Fisher, 1988; Kowalski and Kehr, 1992; Langer and Bußkamp, 2021, 2023), but also on other tree species (*e.g.* Singh et al., 2017; Bußkamp, 2018; Ghobad-Nejhad et al., 2018), have already shown that the number of isolated *Basidiomycota* occurring as endophytes is at a much lower level compared to *Ascomycota*. Even if only the MTs that were obtained from symptomatic tissue in the present study are considered, the *Ascomycota* account for a far greater proportion (81 %) than the *Basidiomycota* (19 %), though the gap is smaller. These results correspond to the findings by Langer and Bußkamp (2023) on European beeches affected by VLB in central Germany. Focusing on symptomatic beech tissue Langer and Bußkamp (2023) listed 31 associated fungal taxa whereby 87 % were *Ascomycota* and 13 % were *Basidiomycota*. The ratio of *Ascomycota* to *Basidiomycota* seems to shift slightly in favour of *Basidiomycota* in symptomatic tissue in trees affected by VLB, even though *Ascomycota* were still isolated much more frequently. Similar ratios between *Ascomycota* and

Basidiomycota as in the present study were found by Gilmartin et al., (2022) analysing the sapwood of young and old European beech trees with regard to the fungal endophyte community. They used a Next generation sequencing (NGS; ITS and LSU) as well as a culture-dependent approach in order to compare the results. The ITS and LSU sequencing datasets shared more OTUs than either of them did with the culture dataset. NGS using the ITS barcode showed a ratio of 67 % *Ascomycota* to 25 % *Basidiomycota* and LSU a ratio of 72 % to 22 %. With the culture-based method, the ratio was 84 % to 13%. The study of Purahong et al. (2021) on the bacterial and fungal community in symptomatic and asymptomatic tissue from European beech trees affected by VLB using a NGS approach, revealed a portion of 43 % *Basidiomycota*. Compared to the aforementioned comparable culture- and NGS-based studies on European beech, the portion of *Basidiomycota* in Purahong et al. (2021) is clearly higher in relation to *Ascomycota*. One possible explanation for the lower detection frequency of *Basidiomycota* using culture-based methods compared to NGS analyses is their requirement for lengthier incubation periods, particularly in the case of wood rot pathogens (Oses et al., 2008). Furthermore, the season has a proven influence on the species composition (*e.g.* Sieber and Hugentobler, 1987; Ceccarelli, 2011; Singh et al., 2017). Fewer *Basidiomycota* could have been present at the time of sampling, since the present study only sampled in winter. In addition, less filamentous basidiomycetes could also be present in the woody tissues of European beech during the investigated phases of the VLB damage process. Additionally, with the exception of one *Agaricales* and one *Hymenochaetales* amplicon sequence variant (ASV), Purahong et al. (2021) only detected taxa for which the majority of species shows yeast-like growth, a group that was not considered in the present study. Of the 14 *Basidiomycota* MTs identified in the present study, *Apiotrichum porosum* was the only *Basidiomycota* considered a yeast, however, the isolated culture clearly showed hyphal growth and was therefore not excluded from the analysis, limited to filamentous fungi. As reported from Fell et al. (2000) there appear to be strains that show hyphal growth and lack the yeast-like phase within the species *A. porosum*. The aforementioned factors may account for the relatively low prevalence of *Basidiomycota* observed in the present study and other culture-based investigations. Finally, the culture medium used has an influence on the detection of basidiomycetes. Bußkamp (2018) has discussed in detail the influence of the culture medium on the detection of basidiomycetes.

Across all MTs, the order *Pleosporales* accounts for the largest proportion (25 %) followed by *Diaporthales* (15 %) and *Xylariales* (11 %). Even in case of individual test trees, *Pleosporales* was either the most frequently represented order alone or together with another order (*Xylariales*, *Pezizales* and *Dothideales*). This contrasts with the results of Sieber (2007), who claimed that angiosperm endophyte communities are often dominated by *Diaporthales*. Although MTs of *Diaporthales* could be detected on every test tree, as with every other order (with the exception of *Pleosporales*), the number of associated MTs varied considerably between the different test trees. This could possibly be attributed to the differing site characteristics, namely climate, bedrock, water availability and trophy. The sites were affected to varying degrees by heat and drought, as illustrated by the deviation of temperature and precipitation from the reference period (Table 1). As shown by Wollan et al. (2008) and Tedersoo et al. (2014), climate and in particular temperature seems to be one of the main factors for the distribution of species of soil and macrofungi. In addition to the climate, the tree species assembly on the various sites differed in the present study. Hantsch et al. (2014) investigated fungal infestation on leaves of two particular host tree species and found a higher fungal species richness with increasing tree species diversity in the immediate vicinity. They concluded that different fungal species might be affected by tree diversity at different scales. It can therefore be assumed that the species communities observed in the present study were strongly influenced by the respective site characterics and probably also by the respective tree species assembly. In contrast, the vitality status of the test trees does not seem to have an effect on the orders detected or the effect is superimposed by other factors like the forest site. On the other hand, it seems to make a distinct difference which tree compartment is observed. Of the *Ascomycota* MTs, 55 % were isolated from the twigs alone, barely 10 % were detected in all three compartments. Fungi of the *Diaporthales* took the second largest share in the twigs after *Pleosporales*, but the proportion became gradually lower from the branches to the trunk. *Pezizales* was the third most

abundant order in the twigs, but was nearly absent in the branches and trunks. Morphotypes of the *Xylariales* were present in all three compartments, but were most common in the twigs, where they formed the fourth most common order. A comparison with other studies is hindered by the fact that for European beech often only the fungal community in leaves and twigs were analysed. However, the results on twigs in the present study were largely consistent with investigations on endophytes in beech leaves. In both Sieber and Hugentobler (1987) and Pehl and Butin (1994), the most common orders in European beech leaves were *Pleosporales*, *Diaporthales* and *Xylariales*, although the ranking was not the same. Consistent with this, Ceccarelli (2011) and Griffith and Boddy (1990) showed that species of the orders *Pleosporales* and *Diaporthales* are strongly represented in the twigs of European beech. However, contrasting to the present study, both Ceccarelli (2011) and Griffith and Boddy (1990) found that *Hypocreales* was one of the most represented orders. Here, *Hypocreales* was the second most dominant order on the trunk but becoming continuously less relevant across branches to the twigs. It is also noticeable that neither in the studies that analysed leaves nor in the studies that analysed twigs fungi of the order *Pezizales* were detected.

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

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

Considerably more taxa were detected in the present study than in other comparable culture-based research. There can be numerous reasons for this. One important factor is the investigation area. The studied sites in the present study covered three federal states and the linear distance between the two most distant trees investigated was almost 250 km. Since 48 % of the isolated *Ascomycota* MTs were limited to single trees, the site seems to have an important influence on the fungal community. Studies support the thesis that site factors have a measurable influence on the fungal community in European beech (Siddique and Unterseher, 2016; Unterseher et al., 2016). Thomas et al. (2019) analysed

spatial patterns of the endophyte community of different tree species in Taiwan. They hypothesised that even closely associated fungal species can be separated from their host trees when environmental conditions change greatly. In contrast, they found that there was a certain group of fungi species that were consistently present across the study area, while other fungi were only identified in single plots. The endophyte community of woody tissue is characterised by host-specific species (Sieber, 2007), as well as generalists, and species that are specific to the tissue type of the host (Bußkamp, 2018). Some fungal species have a broad host spectrum, such as generalists, but only sporulate on one or a few host species. The claims made in the studies mentioned above are consistent with the results of the present study. While almost half of the isolated MTs were limited to single trees and thus location, only four MTs were isolated from all test trees. *Aureobasidium pullulans* and *Epicoccum nigrum* are ubiquitous fungi with a large host species spectrum (Prasongsuk et al., 2018; Taguiam et al., 2021), while *B. nummularia* and *Apiognomonia errabunda* seem to be closely associated with the European beech despite their wide species host range (see the respective subchapters below).

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

Apiognomonia errabunda

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

Biscogniauxia nummularia and B. mediterranea

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

cases of VLB followed by *Neonectria coccinea* and *Eutypella quaternata* (Langer, 2019; Langer et al., 2020; Langer and Bußkamp, 2023).

It is striking that *B. nummularia* as an endophyte was isolated three times more frequently from trees of the damaged and severely damaged test trees than from the undamaged and slightly damaged test trees. Luchi et al. (2016) investigated the presence of *B. nummularia* in asymptomatic twigs of European beech using qPCR at two forest sites. They were able to detect *B. nummularia* more frequently in the twigs of those trees that stood on the site with longer dry periods. The authors of that study hypothesised that water stress increases the number of fungal inoculum, making the host more susceptible. They concluded that the increased presence of *B. nummularia* in asymptomatic tissue could have a negative impact on the vitality of the host and could lead to an outbreak of the pathogen if the climatic conditions are favourable for the pathogen. This type of behaviour has been reported in a number of studies examining various host-endophyte relationships (*e.g.* Bassett and Fenn, 1984; Vannini and Scarascia Mugnozza, 1991; Stanosz et al., 2001; Desprez-Loustau et al., 2006). Consequently, *B. nummularia* would be suitable as a bioindicator for vitality of European beech, *e.g.* regarding drought and heat induced damage. The results of the present study support the thesis of Luchi et al. (2016) and show that *B. nummularia* is more abundant in the asymptomatic tissue of trees that were more strongly affected by drought and heat induced damage. However, this did not apply to two damaged test trees (XII and XIII, sampled in Thuringia). Test tree XII was the most damaged tree analysed in the present study. The extent of the damage to the crown was such that it included very few twigs suitable for sampling, and even those were feeling quite dry. It is possible that the twigs had already dried out too much, as comparatively few fungi had grown from most of the sampled twigs of this test tree. Since fruiting bodies of *B. nummularia* were found on a dying thick branch of the test tree, the fungi must have been more present in the test tree at some time. The other test tree that stands out was tree XIII. One explanation for the low number of isolates of *B. nummularia* from test tree XIII could be that Plot XIII differs from the other analysed plots in terms of its site characteristics. On one hand, it is the highest plot above sea level, on the other hand, beech stands are very unusual for the area, instead spruce is dominant in the region (Blickensdörfer et al., 2024). *Biscogniauxia nummularia* is found to be the most abundant species and is found at all studies forest sites, which indicates its close connection to the European beech. The results of the present study show that this warm-loving species (Hendry et al., 2002) is most prevalent in twigs, rather than branches and trunks, and in xylem, rather than cambial tissue. *Hypoxylon fragiforme*, which along with *B. nummularia* is considered one of the most abundant fungi on European beech (Chapela and Boddy, 1988; Hendry et al., 2002), was only detected with low frequency (0.4 %) in the present study.

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

Neonectria coccinea

The findings of this study are consistent with the notion that *N. coccinea* is a highly abundant endophyte of European beech wood and bark (Chapela and Boddy, 1988; Hendry et al., 2002). It occurred at 92% of the forest sites analysed and was found in both symptomatic and asymptomatic tissue, however, less frequently in the latter. Although *N. coccinea* was not isolated most frequently across all samples, it was by far isolated most frequently in symptomatic tissue, and was most abundant in necrotic tissue from the cambium. This is in accordance with Purahong et al. (2021), who found that *N. coccinea* was among the most frequently detected fungal pathogens in discoloured wood of European beech affected by VLB. *Neonectria coccinea* along with woolly beech scale (*Cryptococcus fagisuga* Lindinger), is also a key pathogen of BBD (Ehrlich, 1934; Parker et al., 1980; Houston, 1994). However, *N. coccinea* is able to cause necrosis on European beech in the absence of *C. fagisuga*, *e.g.* when the host suffers from drought

stress (Lonsdale, 1980a, 1980b; Langer and Bußkamp, 2021). The growth of thalli is probably regulated by oxygen and/or nutrient availability (Sieber, 2007). The fact that the fungus was detected more frequently in the symptomatic tissue of undamaged and slightly damaged trees than in the more severely damaged classes suggests that *N. coccinea* plays a key role in the early stage of VLB. In the event of the host experiencing stress as a result of drought, as in VLB, *N. coccinea* can cause bark necrosis and may act as a precursor for wood decay fungi to gain access to the host tissue.

New records for European beech

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

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

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

Conclusion and outlook

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

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Tables

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

ID	Federal state	WGS84	Stand age	Metres above sea level	Water balance	Bedrock (simplified)	Mixture of tree species	Trophy	Deviation temperature [°C]	Deviation precipitation [mm]	Damageclass
I	Hesse	N 51° 4.764000 E 8° 43.732020	150	430	Moderately fresh	Greywacke	*Fagus sylvatica, Quercus* sp.	Mesotrophic	2,3	-205	Severely damaged
II	Hesse	N 51° 18.102000 E 9° 52.549980	150	359	Moderately fresh	Dolomite	*Fagus sylvatica, Fraxinus excelsior*	Eutrophic	2,2	-277	Damaged
III	Hesse	N 51° 11.703000 E 9° 33.909000	90	365	Fresh	Middle Old Red Sandstone	*Fagus sylvatica*	Mesotrophic	2,4	-281	Undamaged
IV	Hesse	N 51° 31.957020 E 9° 34.870980	140	297	Fresh	Middle Old Red Sandstone	*Fagus sylvatica*	Mesotrophic	2,4	-276	Slightly damaged
V	Lower Saxony	N 51° 24.894000 E 9° 46.752000	130	421	NA	Shell limestone	*Fagus sylvatica, Carpinus betulus, Acer pseudoplatanus, Acer platanoides, Tilia sp., Quercus sp., Sorbus torminalis, Ulmus sp.*	NA	2,2	-281	Severely damaged
VI	Lower Saxony	N 51° 24.493020 E 9° 47.053980	120	352	NA	Shell limestone	*Fagus sylvatica, Acer pseudoplatanus*	NA	2,2	-286	Severely damaged
VII	Lower Saxony	N 52° 14.499000 E 9° 7.681980	90	202	NA	Slate clay	*Fagus sylvatica, Quercus sp., Pinus sylvestris*	NA	2,2	-286	Undamaged
VIII	Lower Saxony	N 52° 14.203020 E 9° 19.038000	160	232	NA	Limestone	*Fagus sylvatica*	NA	2,2	-305	Damaged
IX	Lower Saxony	N 52° 14.536980 E 9° 19.626000	80	181	NA	Limestone	*Fagus sylvatica*	NA	2,3	-284	Slightly damaged
X	Lower Saxony	N 52° 10.834020 E 9° 20.604000	130	212	NA	Lime sandstone	*Fagus sylvatica*	NA	2,2	-303	Slightly damaged
XI	Thuringia	N 51° 22.057980 E 10° 14.835000	100	496	Moderately fresh	Limestone	*Fagus sylvatica, Acer pseudoplatanus, Fraxinus excelsior*	Eutrophic	2,4	-229	Undamaged
XII	Thuringia	N 51° 21.907980 E 10° 35.589000	190	424	Fresh	Limestone	*Fagus sylvatica, Carpinus betulus*	Meso-eutrophic	2,4	-217	Severely damaged
XIII	Thuringia	N 50° 31.696020 E 11° 18.112980	NA	543	Moderately fresh	Argillaceous schist	*Fagus sylvatica*	Mesotrophic	2,3	-228	Damaged

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

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

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

Morphotype	Order	NW-FVA-ID	NCBI-Accession Nr.	Isolated on n/ plots	Number of isolates										In total	Associated with *Fagus sylvatica*
					Healthy (n = 3432)					Symptomatic (n = 567)						
					Trunk (n = 702)		Branch (n = 234)		Twigs n = 2496	Trunk (n = 264)		Branch (n = 303)				
					X n = 351	C n = 351	X n = 117	C n = 117		X n = 242	C n = 22	X n = 127	C n = 170	F n = 6		
Ascomycota																
Alborbis galericulata	*Diaporthales*	10730	PP960607	2/13	0	0	0	0	2	0	0	0	0	0	2	Senanayake et al., 2018
Alternaria cf. *infectoria*	*Pleosporales*	10007	PP960608	10/13	0	5	0	1	24	0	0	0	1	0	31	**Langer and Bußkamp, 2023***
Alternaria sp. 1	*Pleosporales*	10012	PP960609	11/13	0	4	0	0	18	0	0	0	1	0	23	
Alternaria sp. 2	*Pleosporales*	10144	PP960610	3/13	0	0	0	0	2	0	1	0	1	0	4	
Amphisphaeria fuckelii	*Amphisphaeriales*	10659	PP960611	1/13	0	0	0	0	1	0	0	0	0	0	1	**Langer and Bußkamp, 2021**
Angustimassarina sp. 1	*Pleosporales*	10197	PP960612	2/13	0	0	0	0	7	0	0	0	0	0	7	
Angustimassarina sp. 2	*Pleosporales*	10243	PP960613	4/13	0	0	0	0	10	0	0	0	0	0	10	
Angustimassarina sp. 3	*Pleosporales*	10285	PP960614	1/13	0	0	0	0	3	0	0	0	0	0	3	
Apiognomonia errabunda	*Diaporthales*	9999	PP960615	13/13	0	0	0	0	386	0	0	0	4	0	390	**Langer and Bußkamp, 2023**
Apiognomonia hystrix	*Diaporthales*	10561	PP960616	2/13	0	0	0	0	2	0	0	0	0	0	2	Monod, 1983
Ascobolus cf. *crenulatus* ●	*Pezizales*	10570	PP960619	2/13	0	0	0	0	2	0	0	0	0	0	2	FRB*
Ascobolus sp.	*Pezizales*	10140	PP960620	1/13	0	0	0	0	2	0	0	0	0	0	2	
Ascomycota A1 ●		10543	PP960788	7/13	0	0	0	0	38	0	0	0	0	0	38	
Ascomycota A2		10550	PP960622	1/13	0	0	0	0	2	0	0	0	0	0	2	
Ascomycota B1 ●		10450	PP960790	2/13	0	0	0	0	24	0	0	0	0	0	24	
Ascomycota B2 ●		10703	PP960624	2/13	0	0	0	0	2	0	0	0	0	0	2	
Ascomycota C ●		10376	PP960625	2/13	0	0	0	0	15	0	0	0	0	0	15	
Ascomycota D ●		10545	PP960626	1/13	0	0	0	0	1	0	0	0	0	0	1	
Aspergillus inflatus	*Eurotiales*	10704	PP960627	1/13	1	0	0	0	0	0	0	0	0	0	1	FRB; FRG

Morphotype	Order	NW-FVA-ID	NCBI-Accession Nr.	Isolated on n/ plots	Number of isolates										In total	Associated with *Fagus sylvatica*
					Healthy (n = 3432)					Symptomatic (n = 567)						
					Trunk (n = 702)		Branch (n = 234)		Twigs n = 2496	Trunk (n = 264)		Branch (n = 303)				
					X n = 351	C n = 351	X n = 117	C n = 117		X n = 242	C n = 22	X n = 127	C n = 170	F n = 6		
Asterosporium asterospermum	*Diaporthales*	9983	PP960628	4/13	1	2	1	1	1	0	0	0	1	0	7	**Langer and Bußkamp, 2023**
Aureobasidium pullulans	*Dothideales*	10005	PP960629	13/13	0	5	0	2	164	2	0	0	1	0	174	Sieber and Hugentobler, 1987
Beauveria bassiana	*Hypocreales*	10206	PP960631	3/13	1	1	0	0	0	0	0	1	0	0	3	**Unterseher et al., 2013**
Bionectriaceae sp.	*Hypocreales*	10292	PP960632	1/13	0	1	0	0	0	0	0	0	0	0	1	
Biscogniauxia mediterranea	*Xylariales*	10112	PP960633	8/13	0	0	0	0	55	0	0	0	0	0	55	**Langer and Bußkamp, 2023**
Biscogniauxia nummularia	*Xylariales*	9991	PP960634	13/13	4	20	0	6	467	3	1	1	13	0	515	**Langer and Bußkamp, 2023**
Brunnipila cf. *fuscescens*	*Helotiales*	10452	PP960636	1/13	0	0	0	0	2	0	0	0	0	0	2	Suková, 2005*
Cadophora cf. *malorum*	*Helotiales*	10459	PP960637	1/13	1	1	0	0	0	0	0	1	0	0	3	**Langer and Bußkamp, 2023***
Calosporella innesii	*Diaporthales*	10656	PP960638	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB
Chaetomium sp.	*Sordariales*	10590	PP960639	1/13	0	0	0	0	1	0	0	0	0	0	1	
Cheirospora botryospora	*Helotiales*	10296	PP960640	2/13	0	0	0	0	2	0	0	0	0	0	2	**Crous et al., 2015**
Chromelosporiopsis carnea	*Pezizales*	10256	PP960641	2/13	0	0	0	0	3	0	0	0	0	0	3	Hennebert, 2020
Cladosporium spp.	*Cladosporiales*	10081	PP960642	12/13	0	6	1	2	45	1	0	1	4	0	60	
Coniochaeta cf. *hoffmannii*	*Coniochaetales*	10612	PP960643	1/13	0	0	0	0	1	0	0	0	0	0	1	Ceccarelli, 2011*
Coniochaeta cf. *velutina*	*Coniochaetales*	10223	PP960644	1/13	0	0	0	0	0	1	0	0	0	0	1	**Unterseher and Schnittler, 2010***
Coniochaeta sp.	*Coniochaetales*	10468	PP960645	1/13	0	1	0	0	0	0	0	0	0	0	1	
Coniothyrium ferrarisianum	*Pleosporales*	10093	PP960646	6/13	0	0	0	0	17	0	0	0	0	0	17	FRB
Cytospora cf. *cotini* ●	*Diaporthales*	10583	PP960650	1/13	0	1	0	0	0	0	0	0	0	0	1	FRB; FRG*
Cytospora cf. *galegicola* ●	*Diaporthales*	10249	PP980742	9/13	0	2	0	0	7	0	0	0	6	0	15	FRB; FRG*
Cytospora cf. *personata* ●	*Diaporthales*	10311	PP960652	1/13	0	0	0	1	1	0	0	0	0	0	2	FRB*
Diaporthe eres Group A	*Diaporthales*	10710	PP960653	1/13	0	0	0	0	3	0	0	0	0	0	3	**Langer and Bußkamp, 2023**
Diaporthe eres Group B	*Diaporthales*	10268	PP960654	2/13	0	0	0	0	2	0	0	0	0	0	2	
Diaporthe rudis Group A	*Diaporthales*	10332	PP960655	4/13	0	0	0	0	5	0	0	0	0	0	5	**Udayanga et al., 2014**

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					X n = 351	C n = 351	X n = 117	C n = 117		X n = 242	C n = 22	X n = 127	C n = 170	F n = 6		
Diaporthe rudis Group B	*Diaporthales*	10708	PP960656	1/13	0	0	0	0	1	0	0	0	0	0	1	
Diaporthe rudis Group C	*Diaporthales*	10310	PP960657	1/13	0	0	0	0	1	0	0	0	0	0	1	**Udayanga et al., 2014**
Diaporthe rudis Group D	*Diaporthales*	10275	PP960658	2/13	0	0	0	0	6	0	0	0	0	0	6	
Diaporthe sp. 1 ●	*Diaporthales*	10091	PP960659	6/13	0	0	0	0	64	0	0	0	0	0	64	
Diaporthe sp. 2	*Diaporthales*	10271	PP960660	1/13	0	0	0	0	8	0	0	0	0	0	8	
Diatrype stigma s.l.	*Xylariales*	10128	PP960661	1/13	0	0	0	0	0	0	0	0	1	0	1	**Langer and Bußkamp, 2023**
Didymella cf. *macrostoma*	*Pleosporales*	10013	PP960662	3/13	0	1	0	0	2	0	0	0	1	0	4	Griffith and Boddy, 1990*
Didymella cf. *pinodella*	*Pleosporales*	10559	PP960663	2/13	0	0	0	0	3	0	0	0	0	0	3	FRB*
Didymellaceae sp.	*Pleosporales*	10623	PP960664	1/13	0	1	0	0	0	0	0	0	0	0	1	
Didymosphaeria variabile	*Pleosporales*	10587	PP960665	1/13	0	0	0	0	5	0	0	0	0	0	5	FRB; FRG
Diplodia fraxini ●	*Botryosphaeriales*	10624	PP960666	1/13	0	1	0	0	0	0	0	0	0	0	1	FRB
Diplodia mutila	*Botryosphaeriales*	10154	PP960667	2/13	0	1	0	0	0	0	0	0	2	0	3	**Langer and Bußkamp, 2023**
Ditopella ditopa	*Diaporthales*	10288	PP960668	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB
Dothideales sp. 1	*Dothideales*	10344	PP960669	2/13	0	0	0	0	4	0	0	0	0	0	4	
Dothideales sp. 2	*Dothideales*	10407	PP960670	2/13	0	0	0	0	7	0	0	0	0	0	7	
Dothiorella iberica	*Botryosphaeriales*	10126	PP960671	2/13	1	1	0	0	0	0	0	0	2	0	4	FRB
Dothiorella sarmentorum	*Botryosphaeriales*	10461	PP960672	1/13	0	1	0	0	0	0	0	0	0	0	1	FRB
Epicoccum italicum	*Pleosporales*	10355	PP960673	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB; FRG
Epicoccum nigrum	*Pleosporales*	10099	PP960674	13/13	0	11	0	1	28	0	1	0	10	0	51	Ceccarelli, 2011
Eutypa maura	*Xylariales*	10216	PP960675	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB
Eutypa petrakii	*Xylariales*	10255	PP960676	1/13	0	0	0	0	0	2	1	0	0	0	3	FRB
Eutypa spinosa	*Xylariales*	10668	PP960677	1/13	1	0	0	0	0	0	0	0	0	0	1	**Langer and Bußkamp, 2023**
Eutypella quaternata	*Xylariales*	10121	PP960678	1/13	0	0	0	0	0	0	0	0	1	0	1	**Langer and Bußkamp, 2023**

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					Trunk (n = 702)		Branch (n = 234)		Twigs n = 2496	Trunk (n = 264)		Branch (n = 303)				
					X n = 351	C n = 351	X n = 117	C n = 117		X n = 242	C n = 22	X n = 127	C n = 170	F n = 6		
Fenestella sp.	*Pleosporales*	10260	PP960680	1/13	0	0	0	0	1	0	0	0	0	0	1	
Fusarium avenaceum	*Hypocreales*	10075	PP960682	4/13	0	3	0	0	4	0	0	0	0	0	7	Mańka et al., 2012
Fusarium cf. *acuminatum*	*Hypocreales*	10643	PP960683	1/13	0	0	0	0	1	0	0	0	0	0	1	Stępniewska et al., 2021*
Fusarium cf. *solani*	*Hypocreales*	10118	PP960684	2/13	0	1	0	0	0	0	0	0	1	0	2	Orlikowski et al., 2004*
Fusarium sp.	*Hypocreales*	10503	PP960685	3/13	0	0	0	0	5	0	0	0	2	0	7	
Geoscypha tenacella Group A ●	*Pezizales*	10193	PP960686	4/13	0	0	0	0	4	0	0	0	0	0	4	FRB
Geoscypha tenacella Group B ●	*Pezizales*	10439	PP960687	1/13	0	0	0	0	1	0	0	0	0	0	1	
Gibellulopsis nigrescens	*Trichosphaeriales*	10734	PP960688	1/13	0	0	0	0	0	0	0	0	1	0	1	FRB
Gnomoniaceae sp. 1	*Diaporthales*	9994	PP960689	1/13	0	0	0	0	1	0	0	0	0	0	1	
Gnomoniaceae sp. 2	*Diaporthales*	10554	PP960690	1/13	0	0	0	0	1	0	0	0	0	0	1	
Gnomoniopsis paraclavulata	*Diaporthales*	10074	PP960691	2/13	0	0	0	0	2	0	0	0	0	0	2	FRB; FRG
Hypoxylon fragiforme	*Xylariales*	9985	PP960693	4/13	0	3	0	1	4	0	1	0	0	0	9	**Langer and Bußkamp, 2023**
Hypoxylon rubiginosum	*Xylariales*	10469	PP960694	1/13	0	1	0	0	0	0	0	0	0	0	1	**Langer and Bußkamp, 2021**
Ilyonectria crassa	*Hypocreales*	9982	PP960695	1/13	1	0	0	0	0	0	0	0	0	0	1	Jankowiak et al., 2016
Iodophanus carneus ●	*Pezizales*	10096	PP960696	4/13	0	0	0	0	5	0	0	0	0	0	5	FRB
Jackrogersella cohaerens	*Xylariales*	9998	PP960698	2/13	0	2	0	1	0	0	0	0	0	0	3	**Langer and Bußkamp, 2023**
Lachnaceae sp.	*Helotiales*	10286	PP960699	1/13	0	0	0	0	1	0	0	0	0	0	1	
Lopadostoma turgidum	*Xylariales*	10715	PP960700	2/13	2	0	0	0	1	1	0	0	0	0	4	Vasilyeva and Scheuer, 1996
Melanconiella chrysodiscosporina	*Diaporthales*	10651	PP960701	1/13	0	0	0	0	5	0	0	0	0	0	5	Senanayake et al., 2017
Melanconiella chrysomelanconium	*Diaporthales*	10698	PP960702	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB
Melanconiella hyperopta	*Diaporthales*	10515	PP960703	1/13	0	0	0	0	8	0	0	0	0	0	8	FRB
Melanops fagicola	*Botryosphaeriales*	10732	PP960704	3/13	0	0	0	0	11	0	0	0	0	0	11	Li et al., 2020; FRG
Metapochonia suchlasporia	*Hypocreales*	10239	PP960705	1/13	0	0	0	1	0	0	0	0	0	0	1	FRB; FRG

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					X n = 351	C n = 351	X n = 117	C n = 117		X n = 242	C n = 22	X n = 127	C n = 170	F n = 6		
Microsphaeropsis olivacea	*Pleosporales*	10089	PP960706	6/13	0	0	0	1	10	0	0	0	0	0	11	**Langer and Bußkamp, 2023**
Nectria dematiosa ●	*Hypocreales*	10393	PP960707	2/13	0	1	0	0	1	0	0	0	0	0	2	FRB
Nectria nigrescens	*Hypocreales*	10252	PP960708	1/13	0	0	0	0	0	1	0	0	0	0	1	**Langer and Bußkamp, 2023**
Nectriaceae sp.	*Hypocreales*	10208	PP960709	1/13	0	1	0	0	0	0	0	0	0	0	1	
Nemania diffusa	*Xylariales*	10110	PP960710	2/13	0	0	0	0	4	0	0	0	0	0	4	**Unterseher and Peršoh, 2013**
Nemania serpens	*Xylariales*	10250	PP960711	6/13	0	1	0	0	9	0	0	0	0	0	10	**Unterseher et al., 2013**
Nemania sp.	*Xylariales*	10084	PP960712	1/13	0	0	0	0	1	0	0	0	0	0	1	
Neoascochyta sp.	*Pleosporales*	10440	PP960713	1/13	0	0	0	0	1	0	0	0	0	0	1	
Neocatenulostroma cf. *germanicum*	*Capnodiales*	10447	PP960714	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB*
Neocucurbitaria cava	*Pleosporales*	10514	PP960715	3/13	0	1	0	0	3	0	0	0	0	0	4	Ceccarelli, 2011
Neocucurbitaria cf. *vachelliae*	*Pleosporales*	10124	PP960716	4/13	1	4	0	0	0	0	0	1	1	0	7	FRB; FRG*
Neocucurbitaria sp.	*Pleosporales*	10284	PP960717	2/13	0	1	0	0	1	0	0	0	0	0	2	
Neohendersonia kickxii	*Pleosporales*	10219	PP960718	5/13	0	1	0	1	7	0	0	0	0	0	9	**Langer and Bußkamp, 2023**
Neonectria coccinea	*Hypocreales*	9988	PP960719	12/13	2	19	0	4	6	3	5	2	37	0	78	**Langer and Bußkamp, 2023**
Nigrograna sp.	*Pleosporales*	9990	PP960720	1/13	1	0	0	0	0	0	0	0	0	0	1	
Nigrospora sp.	*Xylariales*	10201	PP960721	1/13	0	0	0	0	1	0	0	0	0	0	1	
Nothophoma sp.	*Pleosporales*	10352	PP960722	2/13	0	0	0	0	3	0	0	0	0	0	3	
Paracamarosporium fagi	*Pleosporales*	10198	PP960723	1/13	0	0	0	0	0	0	0	1	0	0	1	**Crous et al., 2015**
Paraphaeosphaeria sp.	*Pleosporales*	10982	PP960724	1/13	0	0	0	0	3	0	0	0	0	0	3	
Paraphaeosphaeria sporulosa	*Pleosporales*	10397	PP960725	1/13	0	1	0	0	0	0	0	0	0	0	1	FRB; FRG
Penicillium spp.	*Eurotiales*	10082	PP960726	7/13	1	4	0	0	1	2	0	0	3	1	12	
Pestalotiopsis sp.	*Amphisphaeriales*	10262	PP960727	1/13	0	0	0	1	0	0	0	0	0	0	1	
Petrakia irregularis ●	*Pleosporales*	10657	PP960728	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB

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					X n = 351	C n = 351	X n = 117	C n = 117		X n = 242	C n = 22	X n = 127	C n = 170	F n = 6		
Pezicula cf. *neocinnamomea*	*Helotiales*	9987	PP960729	4/13	0	1	0	0	5	0	0	0	0	0	6	Chen et al., 2016*
Pezicula sporulosa ●	*Helotiales*	10616		3/13	0	0	0	0	18	0	0	0	0	0	18	FRB
Pezicula fagacearum ●	*Helotiales*	10525	PP960731	2/13	0	0	0	0	3	0	0	0	0	0	3	Chen et al., 2016; FRG
Peziza cf. *arvernensis* ●	*Pezizales*	10146	PP960732	4/13	0	0	0	0	11	0	0	0	0	0	11	Hansen et al., 2002*
Peziza cf. *pseudovesiculosa* ●	*Pezizales*	10138	PP960733	2/13	0	0	0	0	3	0	0	0	0	0	3	FRB*
Peziza sp. 3 ●	*Pezizales*	10437	PP960734	1/13	0	0	0	0	1	0	0	0	0	0	1	
Peziza subvesiculosa ●	*Pezizales*	10011	PP960735	2/13	0	2	0	0	4	0	0	0	0	0	6	FRB; FRG
Peziza varia ●	*Pezizales*	10123	PP960736	2/13	0	0	0	0	6	0	0	0	0	0	6	Hansen et al., 2002
Peziza vesiculosa	*Pezizales*	10636	PP960737	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB
Pezizomycetes sp. 2 ●		10316	PP980743	6/13	0	0	0	0	11	0	0	0	0	0	11	
Pezizomycetes sp. 4 ●		10109	PP960739	1/13	0	0	0	0	1	0	0	0	0	0	1	
Phoma cf. *herbarum*	*Pleosporales*	10158	PP960742	1/13	0	1	0	1	0	0	0	0	0	0	2	FRB*
Phoma sp. 1	*Pleosporales*	10098	PP960743	5/13	0	0	1	0	23	0	0	0	0	0	24	
Phoma sp. 3	*Pleosporales*	11068	PP960744	2/13	1	0	0	0	16	0	0	0	0	0	17	
Phoma sp. 4	*Pleosporales*	10595	PP960745	1/13	0	0	0	0	1	0	0	0	0	0	1	
Plagiostoma apiculatum	*Diaporthales*	9996	PP960746	3/13	0	0	0	0	4	0	0	0	0	0	4	FRB
Plagiostoma dilatatum	*Diaporthales*	10661	PP960747	2/13	0	0	0	0	2	0	0	0	0	0	2	FRB; FRG
Plagiostoma pulchellum	*Diaporthales*	10568	PP960748	2/13	0	0	0	0	2	0	0	0	0	0	2	FRB; FRG
Pleosporales sp. 1	*Pleosporales*	10194	PP960749	1/13	1	0	0	1	0	0	0	0	0	0	2	
Pleosporales sp. 2	*Pleosporales*	10281	PP960750	1/13	0	0	0	0	1	0	0	0	0	0	1	
Pleosporales sp. 3	*Pleosporales*	10090	PP960751	1/13	0	0	0	0	1	0	0	0	0	0	1	
Pleosporales sp. 4	*Pleosporales*	9993	PP960752	5/13	0	0	0	0	6	0	0	0	0	0	6	
Pleosporales sp. 5	*Pleosporales*	10638	PP960753	1/13	0	0	0	0	1	0	0	0	0	0	1	

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Pleosporales sp. 7	*Pleosporales*	9984	PP960754	8/13	1	4	0	2	1	0	0	0	1	0	9	
Pleosporales sp. 8	*Pleosporales*	10215	PP960755	1/13	0	0	0	0	1	0	0	0	0	0	1	
Preussia sp.	*Pleosporales*	10618	PP960757	1/13	0	0	0	0	1	0	0	0	0	0	1	
Prosthecium platanoidis	*Diaporthales*	10731	PP960758	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB
Pseudocamarosporium brabeji	*Pleosporales*	10261	PP960759	2/13	0	0	0	1	2	0	0	0	0	0	3	FRB
Pseudopithomyces chartarum	*Pleosporales*	10236	PP960760	1/13	0	0	0	0	0	0	0	0	1	0	1	FRB
Querciphoma minuta	*Pleosporales*	10147	PP960761	1/13	0	0	0	0	0	0	0	0	3	0	3	FRB
Rhizodermea veluwensis	*Helotiales*	10228	PP960762	1/13	0	1	0	0	0	0	0	0	0	0	1	FRB
Saccotheciaceae sp.	*Dothideales*	10149	PP960763	1/13	0	0	0	0	1	0	0	0	0	0	1	
Seimatosporium sp.	*Amphisphaeriales*	10088	PP960764	1/13	0	0	0	0	1	0	0	0	0	0	1	
Septoriella muriformis	*Pleosporales*	10130	PP960765	2/13	0	0	0	0	0	0	0	0	2	0	2	FRB; FRG
Sordaria sp.	*Sordariales*	10135	PP960766	5/13	0	1	0	0	10	0	0	0	0	0	11	
Sordariomycetes sp. 1		10526	PP960767	1/13	0	0	0	0	1	0	0	0	0	0	1	
Sordariomycetes sp. 2		10404	PP960768	1/13	0	0	0	0	1	0	0	0	0	0	1	
Sporormiella cf. *intermedia*	*Pleosporales*	10676	PP960769	1/13	0	0	0	0	1	0	0	0	0	0	1	Sieber and Hugentobler, 1987*
Sporormiella cf. *minima*	*Pleosporales*	10157	PP960770	9/13	0	0	0	0	35	1	0	0	0	0	36	Sieber and Hugentobler, 1987*
Stegonsporium pseudopyriforme ●	*Diaporthales*	10283	PP960771	1/13	0	0	1	0	0	0	0	0	0	0	1	FRB; FRG
Stemphylium vesicarium	*Pleosporales*	10209	PP960772	1/13	0	1	0	0	0	0	0	0	0	0	1	Ceccarelli, 2011
Sydowia polyspora	*Dothideales*	10297	PP960773	4/13	0	0	0	0	8	0	0	0	0	0	8	Mułenko et al., 2008
Thyridariaceae sp.	*Pleosporales*	10072	PP960774	1/13	0	0	0	0	0	1	0	0	0	0	1	
Thyridium sp.	Incertae sedis	10465	PP960775	1/13	0	0	0	0	1	0	0	0	0	0	1	
Tolypocladium sp. 1	*Hypocreales*	9995	PP960776	3/13	0	0	0	1	0	0	0	0	2	0	3	
Tolypocladium sp. 2	*Hypocreales*	10378	PP960777	6/13	1	6	0	0	0	0	0	0	1	0	8	

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Tricharina cf. *gilva* ●	*Pezizales*	10077	PP960778	2/13	0	0	0	0	2	0	0	0	0	0	2	FRB*
Tricharina cf. *tophiseda* ●	*Pezizales*	10101	PP960779	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB; FRG*
Tricharina sp.	*Pezizales*	10633	PP960780	1/13	0	0	0	0	1	0	0	0	0	0	1	
Trichoderma spp.	*Hypocreales*	10076	PP960781	9/13	1	11	0	1	5	25	0	0	5	5	53	
Ustulina deusta	*Xylariales*	10294	PP960782	2/13	1	1	0	0	0	0	0	0	0	0	2	**Langer and Bußkamp, 2023**
Xenocylindrosporium sp.	Incertae sedis	10517	PP960783	1/13	0	0	0	0	1	0	0	0	0	0	1	
Xenoseimatosporium cf. *quercinum*	*Amphisphaeriales*	10564	PP960784	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB*
Xylaria ellisii ●	*Xylariales*	10111	PP960785	1/13	0	0	0	0	1	0	0	0	0	0	1	FRB; FRG
Xylaria polymorpha	*Xylariales*	10229	PP960786	1/13	0	1	0	0	0	0	0	0	0	0	1	Arnolds et al., 1994
Xylariaceae sp.	*Xylariales*	10635	PP960787	1/13	0	0	0	0	1	0	0	0	0	0	1	
Basidiomycota																
Apiotrichum porosum	*Trichosporonales*	10203	PP960617	1/13	1	0	0	0	0	0	0	0	0	0	1	FRB
Armillaria gallica ●	*Agaricales*	10458	PP960618	1/13	0	0	0	0	0	3	0	0	0	0	3	**Gminder et al., 2001**
Auricularia auricula-judae	*Auriculariales*	10680	PP960630	1/13	0	0	0	0	0	1	0	0	0	0	1	**Krieglsteiner and Kaiser, 2000**
Bjerkandera adusta	*Polyporales*	10578	PP960635	2/13	0	2	0	0	0	0	0	0	0	0	2	**Krieglsteiner and Kaiser, 2000**
Coprinellus disseminatus	*Agaricales*	10572	PP960647	1/13	0	0	0	0	0	0	1	0	0	0	1	**Gminder, 2010**
Coprinellus micaceus	*Agaricales*	10078	PP960648	3/13	0	3	0	1	0	0	0	0	0	0	4	**Gminder, 2010**
Coprinellus sp.	*Agaricales*	10374	PP960649	1/13	0	0	0	0	0	0	0	0	1	0	1	
Exidia glandulosa	*Auriculariales*	10529	PP960679	1/13	0	0	0	0	0	0	0	1	0	0	1	**Krieglsteiner and Kaiser, 2000**
Fomes fomentarius	*Polyporales*	10523	PP960681	1/13	0	0	0	0	0	2	0	0	0	0	2	**Krieglsteiner and Kaiser, 2000**
Hypholoma fasciculare	*Agaricales*	10398	PP960692	1/13	0	1	0	0	0	0	0	0	0	0	1	**Gminder et al., 2003**
Ischnoderma resinosum	*Polyporales*	10527	PP960697	1/13	0	0	0	0	2	0	0	0	0	0	2	Bernicchia et al., 2007

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Phlebia sp. ●	*Polyporales*	10276	PP960740	1/13	0	0	0	0	0	1	0	0	0	0	1	
Pholiota aurivella	*Agaricales*	10524	PP960741	1/13	0	0	0	0	0	2	0	0	0	0	2	**Gminder et al., 2003**
Pleurotus ostreatus ●	*Agaricales*	10460	PP960756	1/13	0	0	0	0	0	1	0	0	0	0	1	**Langer and Bußkamp, 2023**

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

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

Statements and Declarations

Ethical Approval

Not applicable.

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Competing Interests

The authors declare no competing interests.

Author Contributions

Material preparation, data collection and analysis were performed primarily by Jan Tropf with support from G. Langer, S. Bien and J. Bußkamp. The first draft of the manuscript was written by Jan Tropf and revised by G. Langer, S. Bien, J. Bußkamp, and E. Langer. Funding acquisition by G. Langer, conceptualisation by G. Langer and J. Bußkamp.

Data Availability

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

3. Manuscript II

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Temperature-related growth limits and wood decay capacity of the warmth-loving fungus *Biscogniauxia nummularia in vitro*

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Temperature-related growth characteristics and wood decay capacities of *Biscogniauxia nummularia* strains were analysed *in vitro*, revealing variability between strains. To model the growth characteristics fitted dose-response curves were generated using the four-parameter Brain-Cousens hormesis model. The different strains showed distinct optimum growth temperatures, with some achieving maximum growth at 25°C, while others peaked at 28°C, depending on the tested culture media. Strains tested also exhibited variation in their temperature ranges for measurable growth, with some tolerating a broader range than others. The results of the study lead to the consideration that temperature tolerance as well as the optimal growth temperature might be influenced by the strains' geographic origin, with those from southern Germany possibly adapted to higher temperatures. In terms of wood decay, mass loss caused by the various strains differed clearly in many cases, suggesting potential strain-dependent differences in pathogenicity. Additionally, genetic analysis of the beta-tubulin DNA region of *B. nummularia* specimens examined revealed considerable variations between the strains.

KEYWORDS

***Biscogniauxia nummularia*, temperature-related growth, wood decay, Germany, *Fagus sylvatica*, Vitality Loss of Beech**

1 Introduction

Biscogniauxia nummularia (Bull.) Kuntze (*Graphostromataceae*, *Xylariales*, *Ascomycota*, Wendt et al., 2018), is considered one of the most abundant endophytes of European beech (*Fagus sylvatica* L.) (Chapela and Boddy, 1988; Langer and Bußkamp, 2021; Langer et al., 2021; Tropf et al., in press). Although *B. nummularia* has been detected as an endophyte on various coniferous and deciduous trees (*e.g.* Bußkamp, 2018; Peters et al., 2023; Schlößer et al., 2023), according to the current knowledge, it only fructifies and occurs as a pathogen on European beech and Oriental beech (*Fagus orientalis* Lipsky) (Petrini and Petrini, 1985; Nugent et al., 2005; Zamani et al., 2024). When the host (*Fagus*) comes under environmental stress, *e.g.* due to drought and heat, *B. nummularia* can switch from its endophytic lifestyle into a pathogenic phase and cause various symptoms ranging from bark necroses and strip-cankering to wood decay and beech decline (Granata and Whalley, 1994; Hendry et al., 1998, 2002; Granata and Sidoti, 2004; Nugent et al., 2005; Luchi et al., 2015).

With the exception of 2021, the years between 2018 and 2022 were exceptionally dry and hot in Germany (Rakovec et al., 2022; Imbery et al., 2023), which led to the widespread occurrence of Vitality Loss of Beech (VLB) (Langer, 2019; John et al., 2019; Langer et al., 2020; Langer and Bußkamp, 2023). This complex disease of European beech is primarily caused by abiotic factors, but the damage progression is strongly influenced by accompanying fungi and insects (Bressem, 2008; Lakatos and Molnár, 2009; Brück-Dyckhoff et al., 2019; Langer and Bußkamp, 2021, 2023). Studies have shown that the current VLB outbreak, which started in 2018, is strongly associated with anamorphic and teleomorphic fructifications of *B. nummularia* (Langer and Bußkamp, 2021, 2023). Hendry et al. (1998) reported that *B. nummularia* continues to grow rapidly at 30°C. It is therefore not surprising that this fungus, which is sensitive to reductions in host vitality, is involved in a complex disease that is primarily triggered by high temperatures and drought. The average number of days (d) with daytime maximum of at least 30°C (hot days) was 20.4 d in 2018 and 17.0 d in 2019. For comparison, this value was 4.2 d for the reference period 1961 to 1990 (https://opendata.dwd.de/climate_environment/CDC/grids_germany/multi_annual/air_temperature_mean/DESCRIPTION_gridsgermany_multi_annual_air_temperature_mean_6190_en.pdf).

Reports of European beech decline associated with *B. nummularia* and drought are not confined to Germany. There are reports of high mortality and beech decline associated with *B. nummularia* from Hungary (Lakatos and Molnár, 2009), Italy (Granata and Sidoti, 2004), and France (Mirabel and Gaertner, 2023). Vujanovic et al. (2020) declared identification of a hybrid of *Biscogniauxia anceps* (Sacc.) J.D. Rogers, Y.M. Ju & Cand. and *B. nummularia* isolated in Montenegro, and proposed a new species based on an analysis of morphological and molecular data. The name *Biscogniauxia destructiva* Vujan was introduced due to its aggressiveness towards European beech, however, due to violation of the International Code of Nomenclature for algae, fungi, and plants the name is listed as invalid (Art. F.5.1 Shenzhen[1]). Further studies from Europe, for example from England and Wales (Nugent et al., 2005), Spain (Zabalgogeazcoa et al., 2015), the Czech Republic (Zíbarová and Kout, 2017), and Poland (Patejuk et al., 2022), confirm the widespread presence of *B. nummularia* in European beech. While the ability of Ascomycota to cause mass loss through wood decay is typically considered limited in comparison to Basidiomycota, it is well documented that species of the *Xylariales* are capable of causing severe mass loss (Merrill et al., 1964; Duncan and Eslyn, 1966; Worrall et al., 1997). Using microscopic cross-sections of heavily infected branches, Weber and Mattheck (2009) were able to verify that *B. nummularia* causes soft rot. The cells of the affected areas revealed cavities in the secondary cell walls, which speaks in favour of a type-I soft rot (Savory, 1954; Corbett, 1965). The convergence of these cavities led to an increasing degradation of the cellulose-rich S2 layer of the cell wall. In the final stage, only the primary wall and the middle lamella remained. As a result of the decay, branches with leaves still attached to them, are reported to break off. The fractures were brittle (Weber and Mattheck, 2009; Langer et al., 2020).

Fungal growth and the associated depolymerisation of lignocellulose in wood are primarily influenced by wood moisture and temperature, along with the interaction between both factors (Viitanen and Ritschkoff, 1991; Viitanen, 1997; Brischke and Rapp, 2008; Goodell, 2020). However, the optimum growth temperature for *B. nummularia* has never been determined in the past. Studies by Tropf et al. (2022) indicate that the wood decay capacities of various *B. nummularia* strains differ considerably *in vitro*. It is known that even under controlled laboratory conditions, different fungal strains of the same species can differ in growth rate, optimum growth temperature, and secondary metabolism production (Schwarze, 1992; Sørensen and Giese, 2013; Dresch et al., 2015). This led to the consideration that different strains of *B. nummularia* may have different optimum temperatures with regard to hyphal growth. The present study was conducted to verify the initial results of Tropf et al. (2022) concerning the wood decay capacities of various strains of *B. nummularia* from different parts of Germany. Temperature- and nutrient medium-related growth characteristics of the selected *Biscogniauxia* strains were examined *in vitro*. The optimum growth temperature and both the highest and lowest temperature at which the respective strain still showed measurable growth was determined (cardinal temperature). Furthermore, the temperature above the optimum temperature for each strain that resulted in the demise of the culture was identified.

The following hypotheses were formulated: 1) *B. nummularia* strains can differ in their temperature-related growth characteristics, 2) Wood decay capacities of *B. nummularia* strains can vary considerably, and 3) *B. nummularia* strains originating from host trees other than European beech cause considerably less mass loss on European beech wood compared to strains originating from European beech.

2 Materials and methods

2.1 Selection of fungal strains

All seven *B. nummularia* strains used were derived from the fungal culture collection of the Northwest German Forest Research

1 indexfungorum.org (retrieved 03.12.2024).

Institute growing on Malt Yeast Peptone Agar (MYP), modified according to Langer (1994) (0.7% malt extract (Merck, Darmstadt, Germany), 0.05% yeast extract (Merck), 0.1% peptone (Merck) and 1.5% agar (Merck)). The strains originated from European beech (NI2, HE2, BW1, BW2), Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco, HE1), and Scots pine (*Pinus sylvestris* L., NI1, ST1) across seven forest stands in Germany (Table 1; Figure 1). Strains derived from Douglas fir and Scots pine were included to test whether they differ from beech strains in their ability to cause mass loss in European beech wood. All strains were isolated either from tree compartments or ascocarps. Isolates were obtained during causal analyses of damaged forest trees in Germany, prior to this study. So all host trees exhibited declines in vitality during the sampling period. For the years 1991–2020, data was obtained from the nearest weather stations of every locality where the strains were isolated[2] (Supplementary Table 1).

2.2 Molecular analysis

Previous to molecular analysis, the strains were morphotyped according to Bußkamp et al. (2020). For species identification DNA extraction as well as amplification of the 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers ITS-1 and ITS-2 (ITS region) was carried out for all strains following Tropf et al. (acc.). In addition, various primer combinations were used to obtain as complete a picture as possible of the beta-tubulin gene region (*TUB*) of the strains used. Primers Bt1a, Bt1b, Bt2a, Bt2b (Glass and Donaldson, 1995), T1, T2, and T22 (O'Donnell and Cigelnik, 1997) were employed applying PCR conditions of (Paulin and Harrington, 2000). A StepOnePlus™ PCR System (Applied Biosystems, Waltham, Massachusetts, US) or a GeneExplorer 96 (Hangzhou BIOER Technology, Hangzhou, China) was used to carry out the DNA amplifications. After visualisation in 1% agarose gel, PCR products were sent to Eurofins Scientific Laboratory (Ebersberg, Germany) for sequencing. From all resulting sequences consensus sequences were generated, and visually checked and edited if necessary using BioEdit Sequence Alignment Editor (v. 7.2.5; (Hall, 1999)). Sequence regions generated by different *TUB* primer combinations were joined at the overlap points, using Geneious R11 (Kearse et al., 2012). Sequences were submitted to GenBank (Table 1). An ITS sequence dataset was compiled from sequences generated in this study and sequences analysed by Vujanovic et al. (2020). The ITS sequence dataset and a *TUB* dataset consisting of *TUB* sequences generated in this study were aligned automatically using MAFFT v. 1.5.0 (Katoh, 2002; Katoh and Standley, 2013) implemented in Geneious R11 and manually adjusted where necessary. Maximum Likelihood analyses were performed by RAxML v. 4.0 (Stamatakis, 2006, 2014) using the GTRGAMMA model with the rapid bootstrapping and search for best scoring ML tree algorithm including 1000 bootstrap replicates implemented in Geneious R11, respectively.

2.3 Determination of the temperature-related growth and mortality

The growth characteristics of the seven investigated *B. nummularia* strains were tested on four different culture media at temperature gradients from 0°C to 7°C and 25°C to 36°C at 1 degree intervals. For this purpose, Petri dishes (90 mm diameter) with the following media were inoculated with one of the strains each to be tested on the centre of each dish: MYP, Malt Extract Agar (MEA, 2% malt extract (Merck) and 1.6% agar (Merck)), Potato Dextrose Agar (PDA, 0.4% Potato extract (Sigma Aldrich, Steinheim, Germany), 2% Dextrose (Sigma Aldrich) and 1.5% Agar (Sigma Aldrich)) or Synthetic Nutrient-Poor Agar (SNA, 0.1% KH_2PO_4 (Merck), 0.1% KNO_3 (Sigma Aldrich), 0.05% $MgSO_4$*H_2O (Sigma Aldrich), 0.05% KCl (Merck), 0.02% Glucose (Sigma Aldrich), 0.02% Sucrose (Sigma Aldrich) and 2% Agar (Merck)). The inoculated Petri dishes were placed in climate chambers (KBW E6, Binder, Tuttlingen, Germany). To determine the growth optimum and the temperature at which the strains showed no measurable growth, the temperature gradient from 25°C to 36°C was tested. Temperatures between 25°C and 36°C were selected based on the findings of Hendry et al. (2002), who demonstrated that *B. nummularia* can still exhibit rapid growth at 30°C. Consequently, this temperature was used as the initial reference point in the experiment, with additional temperatures tested above and below this value. For each strain, two inoculated Petri dishes were tested per temperature and culture medium. Originally, an incubation period of one week was planned to test this temperature range. However, depending on the medium and temperature, the surface of the medium in the Petri dishes was already completely covered after one week. So after four days of incubation, the inoculated Petri dishes were already removed from the climate chambers and the extent of the hyphal growth was measured in mm with a ruler. The methodology for determining growth based on the diameter of the fungal colony was derived from the work of Brancato and Golding (1953). Two orthogonal straight lines were drawn on the undersides of the Petri dishes, which intersected at the inoculation point. Hyphal growth was measured along the four axes created and the values averaged (arithmetic mean). To determine the temperature, above the maximum growth temperature, at which the fungus dies, one petri dish per strain and culture medium was prepared in triple repetition, and incubated at temperatures 36°C and 37°C, respectively. After one week, two weeks, and three weeks each one culture for every medium was transferred to room temperature (approx. 22°C), without direct sunlight. If no growth of the fungal strain was observed after an additional 14 days incubation under these conditions, the culture was assumed to be dead.To assess the lowest temperature at which the *B. nummularia* strains still showed measurable growth, one inoculated Petri dish per strain, culture medium and temperature was incubated in climate chambers at temperatures between 0°C and 7°C for 14 days. The experiment was started at 0°C, and continued with increasing temperatures until all of the tested strains exhibited visible growth.

2 https://meteostat.net/de/ (retrieved 30.11.2024).

TABLE 1 Data of the *Biscogniauxia nummularia* strains.

Strain	ID NW-FVA	Year of isolation	Host	Origin	Federal state	WGS 84	NCBI-Accession No. ITS	NCBI-Accession No. *TUB*
ST1	4756	2018	*Pinus sylvestris*	Branch	Saxony-Anhalt	N: 52.3268 E: 11.1889	PQ722107	PQ757296
NI1	6186	2021	*Pinus sylvestris*	Branch	Lower Saxony	N: 52.4847 E: 10.4813	PQ722108	PQ757297
NI2	8204	2022	*Fagus sylvatica*	Fruiting body	Lower Saxony	N: 51.4199 E: 9.7605	PQ722109	PQ757298
HE1	8234	2022	*Pseudotsuga menziesii*	Branch	Hesse	N: 49.4451 E: 8.9174	PQ722110	PQ757299
HE2	8951	2022	*Fagus sylvatica*	Trunk	Hesse	N: 51.0800 E: 8.5861	PQ722111	PQ757300
BW1	9899	2019	*Fagus sylvatica*	Branch	Baden-Württemberg	N: 49.1964 E: 8.7363	PQ722112	PQ757301
BW2	9900	2020	*Fagus sylvatica*	Branch	Baden-Württemberg	N: 48.8276 E: 8.3659	PQ722113	PQ757302

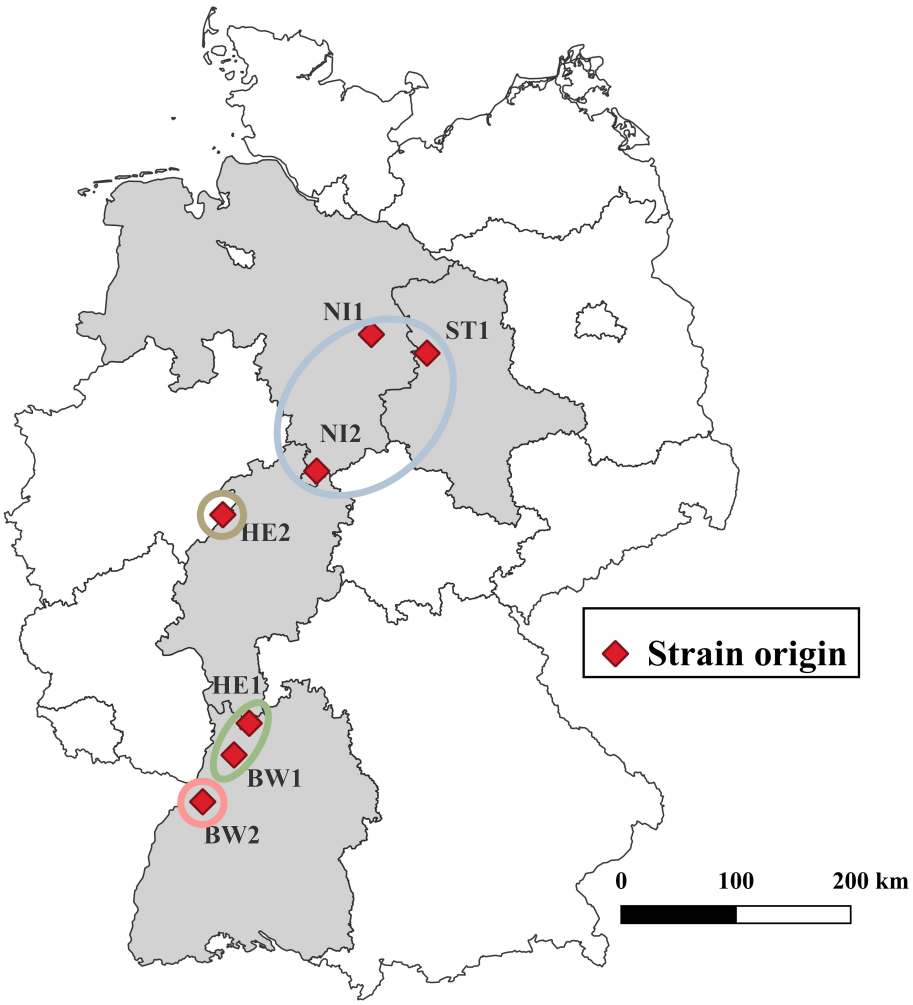

FIGURE 1
Origin of the various *Biscogniauxia nummularia* strains. The strains are grouped according to the illustration in Supplementary Figure 1. © GeoBasis-DE/BKG 2023 for boundaries of Germany and the federal states.

2.4 Determination of the wood decay capacities

The wood decay capacities of the *B. nummularia* strains tested were investigated in a test based on the DIN standard on the durability of wood and wood-based products – Wood preservatives – Method of test for determining the protective effectiveness against wood destroying basidiomycetes – Determination of the toxic values, German version (DIN EN 113, 1996). For the production of the wooden test objects (TOs), sapwood of the trunk from a freshly harvested beech was cut into cuboids measuring 5 cm · 2.5 cm · 1.5 cm (length · width · height). TOs with obvious wood defects were sorted out and excluded from the test. To determine the initial dry mass, the TOs were dried to constant weight in a drying oven (UM 500, Memmert, Schwabach, Germany), at 103°C for 24 hours. The weight of the TOs was determined to an accuracy of 0.001 g and defined as the initial dry mass m_0 before incubation. The TOs were then soaked in tap water for 24 hours and afterwards sterilised in an autoclave at 121°C for 20 minutes (VARIOKLAV® 400EP, HP Labortechnik GmbH, Oberschleißheim, Germany). Duran® square bottles (SCHOTT AG, Mainz, Germany) with membrane caps (test vessels) were filled with 80 ml MEA, autoclaved at 121°C for 20 minutes and then stored horizontally. Mycelium of strains tested, pre-cultivated on MEA, was transferred to the test vessels using an inoculation loop. Two inoculation points were chosen, one at the front of the bottle and one at the back. Control vessels also contained 80 ml MEA but were not inoculated. After the surface of the culture medium in the vessels was completely covered with a fungal strain, three sterile TOs were added to each test vessel, including control vessels. The incubation periods were set at six and nine weeks. For each incubation period, five test vessels were prepared for each strain plus five test vessels for control (corresponds to 15 TOs per treatment group). All test vessels were stored in the dark in a climate-controlled room where the temperature was set to 25°C. The air temperature was measured constantly over the incubation periods using a HOBO data logger (Onset, Bourne, USA). After each incubation period, the TOs were taken out of the test vessels and the mycelium on the surface of the TOs was removed with a razor blade. Thirteen TOs per treatment group were used to determine the wood decay capacities. The remaining two TOs per treatment group were excluded from the present study and used for the preparation of histological sections (not shown). The TOs were then dried at 103°C for 24 hours. The weight of the TOs was determined again to an accuracy of 0.001 g and defined as the final dry mass m_3 after incubation. The wood decay capacities was equated with relative mass loss of the TOs after the incubation period. The formula for calculating the relative mass loss was based on DIN EN 113 (1996):

$$RML = \frac{m_0 - m_3}{m_0} \cdot 100\ [\%]$$

RML: Relative mass loss of the TO after the incubation period [%].

m_0: Initial dry mass of the TO before incubation [g].

m_3: Final dry mass of the TO after incubation [g].

2.5 Data analyses

Statistical differences in relative mass loss caused by the different strains were investigated using beta regression models and Tukey multiple comparisons. We do not convert statistical inference statements based on p-values into binary test decisions, but rather use p-values as a continuous measure of evidence (Wasserstein, 2019). Where possible, the associated effect sizes are reported and evaluated in the applied context. To model the radial growth of the different strains at temperatures from 25°C to 36°C, dose-response curve models were fitted using the four-parameter Brain-Cousens hormesis model (95% credible intervals shown in Figure 2). Data was analysed using the statistical software environment R (R Core Team, 2024, version 4.4.1), with using R add-on packages betareg (Cribari-Neto and Zeileis, 2010), dplyr (Wickham et al., 2023), drc (Ritz et al., 2015), emmeans (Lenth et al., 2024), ggplot2 (Wickham, 2016), MASS (Venables and Ripley, 2002), and plyr (Wickham, 2011).

3 Results

3.1 Results of temperature-related growth and mortality

Temperature and the used culture medium had a strong influence on the growth of the *B. nummularia* strains. Comparing the culture medium, the lowest growth across all fungal strains between 25°C and 34°C, was observed for SNA (Figure 2). Strain HE2 did already show no measurable growth at 32°C within the four days of incubation on SNA. For the other six strains, the maximum temperature at which the strain still showed growth on SNA varied between 32°C and 33°C (Table 2). On the culture media other than SNA, there were strains that still grew out at 34°C and strain NI2 still showed growth on PDA even at 35°C. However, at 34°C, growth on all strains and independent of the culture media was at most very low. At 36°C, no strain showed growth within the incubation period regardless of the culture medium used. At 25°C, the measured growth across all fungal strains was almost equal between PDA and MYP, but with increasing temperatures up to 30°C, the growth measured for the fungal strains on PDA got increasingly higher in comparison to MYP. At 31°C, the average growth on the two culture media began to converge, and the difference between them decreased as the temperature increased further. For the MEA culture medium the growth values across all fungal strains were always located between MYP and SNA at temperatures between 25°C and 33°C.

If the modelled temperature-related growth depending on the media is considered individually for each strain, it is noticeable that the growth characteristics differ clearly (Figure 3). Strain ST1 already reached its maximum growth at 25°C on MEA, MYP and PDA (Table 2), but the growth curve declined slowly at rising temperatures compared to other strains like strain HE2. On SNA, the growth of strain ST1 peaked at 27°C. Strain NI1 showed low growth on all culture media compared to the other strains. On

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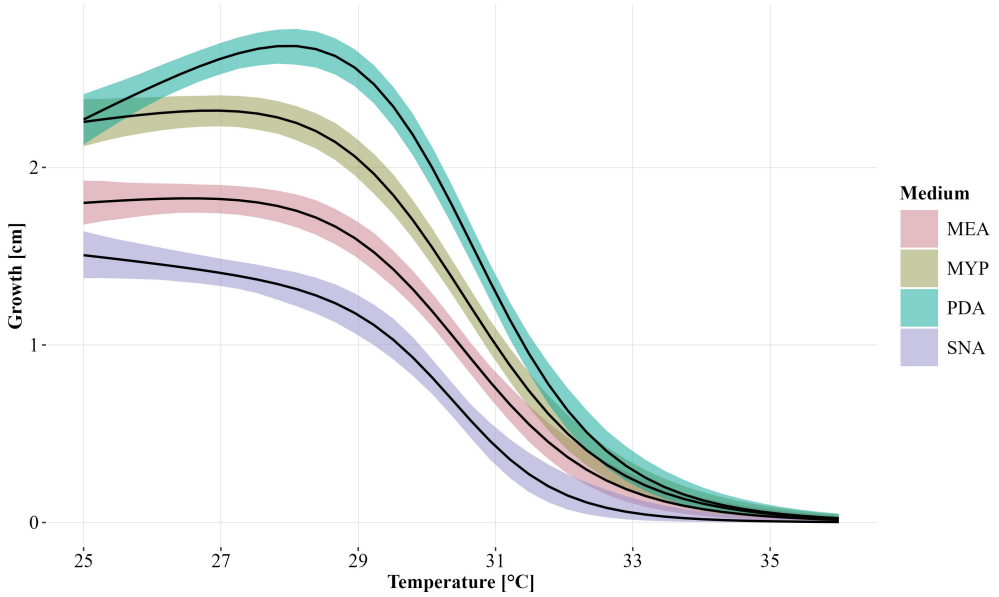

FIGURE 2
Modelled growth of *Biscogniauxia nummularia* as a function of temperature and grouped according to the culture media (MEA, Malt Extract Agar; MYP, Malt Yeast Peptone Agar; PDA, Potato Dextrose Agar; SNA, Synthetic Nutrient-Poor Agar) used without differentiation of the strains. The fitted dose-response curves were generated using the four-parameter Brain-Cousens hormesis model. The 95% credible interval is shown for each group. Growth was measured after four days of incubation.

MEA, growth of this strain culminated at 25°C and 26°C, after which growth declined rather evenly. On MYP, PDA and SNA, growth culminated at 27°C and 28°C, but growth dropped rapidly for MYP and PDA in particular at 30°C and 31°C. The growth of strain NI2 peaked rather late on all culture media (27°C or 28°C) except SNA and showed comparatively high growth even at temperatures above 30°C, until the growth curves converge at 33°C at the latest for all strains. At a temperature of 27°C on PDA, the two cultures of strain NI2 achieved the highest measured growth in the study at 3.6 cm and 3.7 cm. Strain HE1 showed similar growth characteristics to strain NI2 on MEA, MYP and PDA, but the measured growth between 26°C and 28°C was usually slightly lower than that of strain NI2. On MYP, the growth of strain HE1 already culminated at 25°C, and then declined slowly. On SNA, the measured growth was comparatively high up to 29°C and then dropped rapidly. Strain HE2 reached its maximum growth on MEA and MYP at 25°C. At this temperature, the measured growth was also considerably higher compared to the other strains. Above 25°C, the measured values for HE2 decreased more or less steadily. From 30°C onwards, the strain showed only low growth on the two culture media compared to many other strains. On PDA, HE2 culminated at 27°C and growth at 25°C was not as noticeable as on MEA or MYP. From 30°C, the strain also showed rather low growth on PDA in comparison. On SNA, strain HE2 peaked at 25°C, as on MEA and MYP, but growth was rather low in comparison across all tested temperatures. The growth of strains BW1 and BW2 peaked on MEA MYP and PDA at comparatively high temperatures of 27°C or 28°C. At all measured temperatures up to 34°C, however, the growth of strain BW2 remained at a much lower level than that of BW1. Above 35°C, both strains no longer grew. Between 28°C and 31°C on MEA MYP and PDA, strain BW1 achieved the highest measured growth of all strains at this high temperatures. On SNA, both strain BW1 and BW2 culminated at 25°C to 26°C, but strain BW2 showed almost constant growth up to 29°C.

Temperature-related mortality differed only slightly between the different strains. After an incubation period of one week at 36°C and subsequent storage of the cultures at room temperature for a further two weeks, all strains grew out (Table 3). After the incubation period at 36°C was increased to two weeks, only twelve of the 28 cultures tested grew out after the two-week storage period. Strains NI1 and BW2 did not grow out on any culture medium during this treatment, while strains NI2 and HE2 grew on all tested culture media. None of the strains grew out after a three-week incubation period at a temperature of 36°C. Increasing the temperature to 37°C resulted in only 19 of the 28 cultures growing out after a one-week incubation period and two weeks of storage. Strains ST1 and HE2 grew out on all tested culture media during this treatment. An increase in the incubation period to two weeks and three weeks at 37°C resulted in none of the cultures showing outgrowth. After an incubation period of two weeks at a temperature of 0°C up to 2°C, none of the 28 cultures showed measurable growth (Table 2), however all of the cultures grew out after two weeks of storage. Increasing the temperature to 3°C resulted in 7 of the 28 cultures showing measurable growth. Strains ST1 and HE2 showed measurable growth on all culture media used except PDA. Increasing the temperature to 5°C resulted in 20 of the 28 cultures showing measurable growth. All strains showed measurable growth on all culture media used, with the

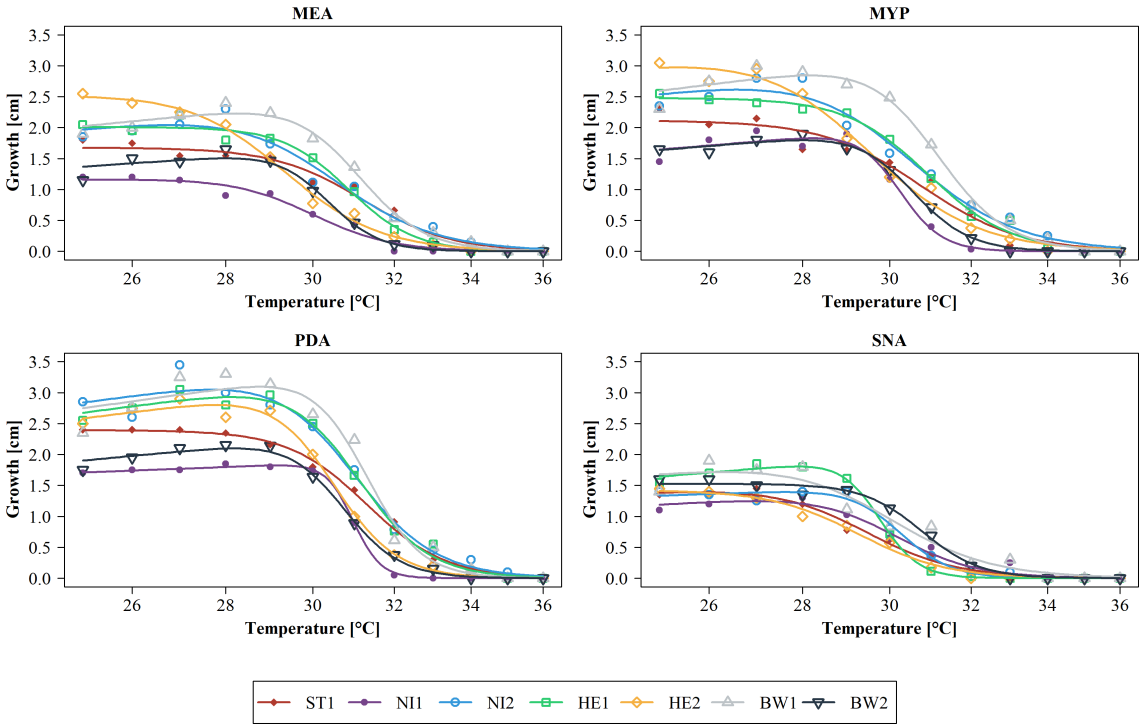

FIGURE 3
Modelled growth per *Biscogniauxia nummularia* strain (BW1-2, HE1-2, NI1-2 and ST1) as a function of temperature and subdivided according to the culture media used (MEA, Malt Extract Agar; MYP, Malt Yeast Peptone Agar; PDA, Potato Dextrose Agar; SNA, Synthetic Nutrient-Poor Agar). Dose-response curves were fitted using the four-parameter Brain-Cousens hormesis model. Actual measured growth values (n = 2 per strain, temperature, and culture medium) were averaged and are presented as dots. Growth was measured after four days of incubation.

exception of strains NI1 and BW2, which did not show measurable growth on any culture medium. Both strains grew out at 6 and 7 degrees, but the measured growth was at a very low level.

3.2 Results of the wood decay capacities

Measured temperatures ranged between 25.6°C and 25.9°C. Mass loss was observed for all TOs incubated with *B. nummularia* strains, with the exception of a single TO, which was incubated with strain NI2 for an incubation period of nine weeks. Therefore, the latter TO was not included in the evaluation. Additionally, three TOs from the control group with an incubation period of nine weeks could not be evaluated and were therefore also excluded. The average mass loss of the TOs across all strains was 4.5% after an incubation period of six weeks and 6.5% for the TOs that were incubated for nine weeks. An average mass loss of 0.17% was observed in the controls after both six and nine weeks. After a six-week incubation period, the mass loss of the TOs caused by the different *B. nummularia* strains exhibited some variation. The p-values ranged from 1 to values < 0.0001. (Figure 4, beta regression model and Tukey multiple comparison). On average, the greatest mass loss was observed in TOs incubated with strain HE2 (5.69%). The mass loss differed clearly – p-values between 0.0388 and < 0.0001 – from all other strains with the exception of strain NI1 (5.5%, p-value = 0.9985). Strain BW2 (2.7%) caused the lowest mass loss. It was clearly lower (p-values < 0.0001) than the mass loss caused by any other strain. In addition, the mass loss by strain NI1 was noticeably higher than that by strain ST1 (4.32%, p-value = 0.0016), NI2 (4.15%, p-value = 0.0002) and BW1 (4.16%, p-value = 0.0002). Differences in mass loss were also observed after an incubation period of nine weeks. The p-values were between 0.9801 and < 0.0001. Test objects incubated with strain HE2 showed the highest average mass loss (8.89%). The mass loss was clearly higher – p-values between 0.0031 and < 0.0001 – than the mass loss of all other strains except BW1 (7.83%, p-value = 0.4499). As with the six-week incubation period, TOs incubated with strain BW2 also showed the lowest average mass loss after nine weeks (3.56%, p-values < 0.0001). The mass loss caused by strain BW1 was considerably higher than the mass loss caused by strains NI1 (5.36%, p-value < 0.0001), NI2 (4.15%, p-value = 0.001) and HE1 (6.58%, p-value = 0.0183). Test objects incubated with strain NI1 showed a clearly lower relative mass loss than TOs incubated with strain ST1 (6.96%, p-value = 0.0069). Strain NI1 was the only strain that did not cause a higher average relative mass loss after nine weeks of incubation than after six weeks.

3.3 Results of the molecular analysis

Based on the ITS analysis all tested strains could be assigned to *B. nummularia*. The strains of the present study exhibit no or up to

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TABLE 2 Temperature-related growth characteristics of *Biscogniauxia nummularia* strains.

Strain	MEA	MYP	PDA	SNA
Temperature with maximal growth [°C]				
ST1	25	25	25-27	27
NI1	25-26	27	28	27-28
NI2	28	27-28	27	25; 28-29
HE1	27	25	27	27
HE2	25	25	27	25
BW1	28	27	28	26
BW2	28	28	28	25-26
Highest temperature with measurable growth [°C]				
ST1	34	33	34	32
NI1	31	32	32	33
NI2	34	34	35	33
HE1	33	34	34	32
HE2	33	33	34	31
BW1	34	34	34	33
BW2	33	32	33	32
Lowest temperature with measurable growth [°C]				
ST1	3	3	4	3
NI1	6	6	6	6
NI2	4	4	3	4
HE1	5	5	5	5
HE2	3	3	4	3
BW1	5	5	5	5
BW2	6	6	6	6

Growth characteristics of the strains BW1-2, HE1-2, NI1-2 and ST1 were determined in culture on different artificial media (MEA, Malt Extract Agar; MYP, Malt Yeast Peptone Agar; PDA, Potato Dextrose Agar; SNA, Synthetic Nutrient-Poor Agar). Maximal growth and highest temperature with measurable growth was determined after four days, lowest temperature with measurable growth was determined after two weeks.

two nucleotides differences to *B. nummularia* strains MUCL 51395 (GenBank acc. NR_153649), H07 (LN714525), and BI21 (EF155488, data not shown). However, the ITS sequences of the strains analysed in this study differ in two to three nucleotides from the strain designated as *B. destructiva* (nom. inval.; GenBank acc. MT804371; Vujanovic et al., 2020).

For all strains tested, DNA sequences of the *TUB* DNA region of approximately 1800 bp were retrieved. The strains show considerable nucleotide differences between 2 and 29 nucleotides (Supplementary Table 2). Based on *TUB* sequence similarity four groups can be distinguished (Figure 1; Supplementary Figure 1). Group one consists of NI1, NI2, and ST1 with 3 to 6 nucleotide differences between them. Group two consists of BW1 and HE1 with 2 nucleotide differences between them. Both BW2 and HE2 have at least 17 nucleotide variations from every other strain that was examined.

4 Discussion

The results of the present study show that the temperature-related growth of *B. nummularia* can differ considerably between strains *in vitro*. However, it must be taken into account that the density of the colony cannot be inferred from the diameter growth of the colony (Wells and Uota, 1970). The growth extent of some of the tested strains was observed to reach its maximum at 25°C on the tested culture media. In contrast, the optimum growth temperature for other strains was found to be 28°C. Since temperatures between 8 and 24°C were not tested in the present study, it is possible that a few of the strains tested would have reached their maximum measured growth on certain media at even lower temperatures. Overall, the growth of the strains for temperatures between 25 and 32 degrees differed considerably less on SNA than on the other culture media tested. This is probably due to the fact that SNA is a nutrient-deficient medium compared to the other culture media used. There were also differences between the strains with regard to the lowest and highest temperature at which growth was measurable. Depending on culture medium, some strains appear to have a considerably wider temperature range in which measurable growth is possible than others. Strain ST1, for example, showed measurable growth on MEA at 3°C up to 34°C, while strain NI1 only showed measurable growth between 6°C and 31°C. Strains that did not show measurable growth at temperatures under 5°C or 6°C tended to reach their maximum growth at temperatures above 26°C, at least on most of the tested culture media. Examples of this are strains BW1 and BW2 from Baden-Württemberg and, to a lesser extent, strain HE1 from southern Hesse. For all localities from which strains were isolated the average summer temperature (June, July, and August) for the reference period 1991 to 2020 was compared (Supplementary Table 2). According to this comparison the latter strains originated from localities with higher average summer temperatures. The values were 19.2°C for strain BW1 and 18.6°C for strain BW2. At 17.8°C, the value of the closest weather station for strain HE1 is at least slightly higher than the rest of the strains (17.0°C to 17.6°C). So it is possible that there is an influence of the site and that *B. nummularia* strains that have adapted to the higher temperatures are becoming established in southern Germany. Patejuk et al. (2022) report indications for a northern population of the fungus, which might be adapted better to the Central European climate. In contrast to the strains BW1 and BW2 strain NI1 (Lower Saxony) did not show measurable growth for temperatures under 6°C and yet achieved its greatest growth already at 25°C at least on MEA. Overall, strain NI1 appears to be a strain with a very small temperature-related growth amplitude.

There were slight differences at temperatures (above the optimal temperature), which led to the death of the various strains. After an

TABLE 3 Mortality of *Biscogniauxia nummularia* strains in culture.

	Strain	MEA	MYP	PDA	SNA		Strain	MEA	MYP	PDA	SNA
36°C	One week of incubation					37°C	One week of incubation				
	ST1	1	1	1	1		ST1	1	1	1	1
	NI1	1	1	1	1		NI1	0	1	0	0
	NI2	1	1	1	1		NI2	1	1	0	1
	HE1	1	1	1	1		HE1	1	1	1	0
	HE2	1	1	1	1		HE2	1	1	1	1
	BW1	1	1	1	1		BW1	1	1	1	0
	BW2	1	1	1	1		BW2	0	0	1	0
	Two weeks of incubation						Two weeks of incubation				
	ST1	0	1	0	0		ST1	0	0	0	0
	NI1	0	0	0	0		NI1	0	0	0	0
	NI2	1	1	1	1		NI2	0	0	0	0
	HE1	0	0	1	0		HE1	0	0	0	0
	HE2	1	1	1	1		HE2	0	0	0	0
	BW1	1	0	1	0		BW1	0	0	0	0
	BW2	0	0	0	0		BW2	0	0	0	0
	Three weeks of incubation						Three weeks of incubation				
	ST1	0	0	0	0		ST1	0	0	0	0
	NI1	0	0	0	0		NI1	0	0	0	0
	NI2	0	0	0	0		NI2	0	0	0	0
	HE1	0	0	0	0		HE1	0	0	0	0
	HE2	0	0	0	0		HE2	0	0	0	0
	BW1	0	0	0	0		BW1	0	0	0	0
	BW2	0	0	0	0		BW2	0	0	0	0

Mortality of strains (BW1-2, HE1-2, NI1-2 and ST1) is differentiated regarding to culture media (MEA, Malt Extract Agar; MYP, Malt Yeast Peptone Agar; PDA, Potato Dextrose Agar; SNA, Synthetic Nutrient-Poor Agar), temperature and incubation period. 1 = Outgrowth of the culture after incubation period and two weeks of storage at room temperature, 0 = No Outgrowth after incubation period and two weeks of storage.

incubation period of one week at 36°C, all strains still grew out. Increasing the temperature to 37°C resulted in some strains no longer surviving on all tested media after a one-week incubation period. Here too, differences between strain ST1 and strain NI1 became apparent. Strain NI1 died on all tested media except MYP, while strain ST1 did not die on any medium. An increase in the incubation period to two weeks at 36°C resulted in strain ST1 dying on all media except MYP. Strains NI2 and HE2 survived under these conditions. Strain HE2 in particular reached its maximum growth on most media at comparatively low temperatures.

Differences were observed between the wood decay capacities of the various strains tested. After both, six and nine weeks of incubation, the TOs incubated with strain HE2 exhibited the greatest average relative mass loss. For both incubation periods, this relative mass loss differed clearly from most of the other strains tested. TOs incubated with strain BW2 exhibited the lowest average relative mass loss after both, six and nine weeks of incubation. This mass loss was clearly different from all other strains for both incubation periods. The reasons why the strains differ so much in their wood decay capacities can only be surmised at this point. Numerous authors have shown that strains of one fungal species can differ in their pathogenicity towards their host (*e.g.* Elgersma and Heybroek, 1979; Jarosz and Davelos, 1995; Lee et al., 2015; Ghelardini et al., 2016). It is possible that the HE2 strain is more virulent than the BW2 strain. However, as the study was carried out *in vitro*, the results cannot simply be used to draw conclusions about the pathogenicity of the fungal strains in living tissue. Host-pathogen relationships are highly complex. Due to a variety of influencing factors, it is impossible to predict the exact growth rate of a fungus in a living tree (Schwarze et al., 1999). It is noteworthy that the strain HE2, which caused the highest degree of wood degradation, also showed the greatest growth on almost all culture

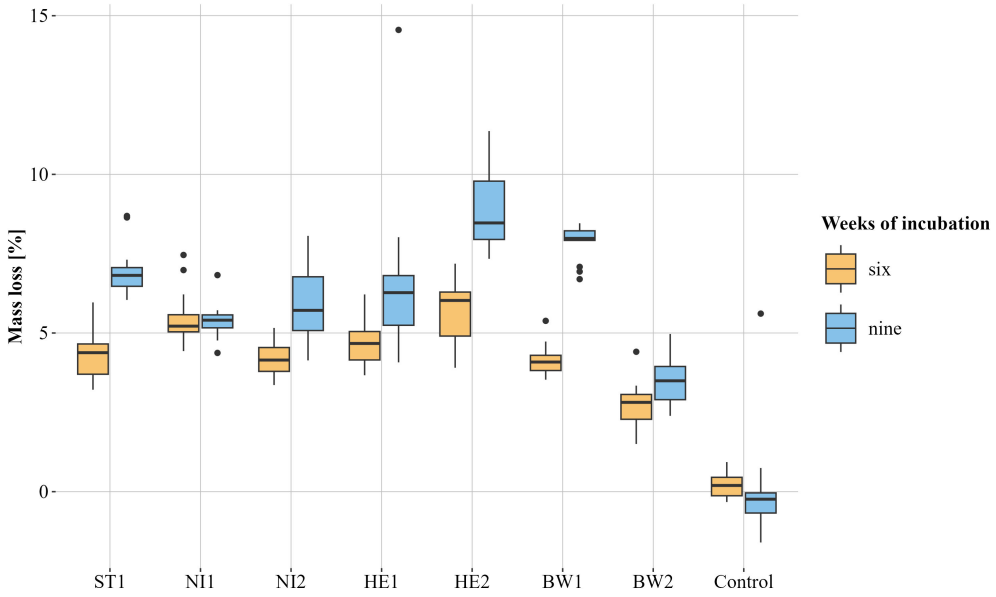

FIGURE 4
Visualisation of the relative mass loss caused by the different *Biscogniauxia nummularia* strains (BW1-2, HE1-2, NI1-2 and ST1) as box plots. The respective median is shown. Test objects that were incubated for six weeks are orange and those that were incubated for nine weeks are blue. Controls were incubated without fungus. n = 13 for each group, except for the group control, nine weeks (n = 10) and NI2, nine weeks (n = 12).

media at 25°C. The optimum growth temperature for strain BW2 was 28°C on almost all media. It is possible that if the incubation temperature had been set at 28°C, strain BW2 would have caused a greater relative mass loss. Strain BW1, which had similarly high optimum growth temperatures as strain BW2 depending on the medium, also caused a comparatively low relative mean mass loss after six weeks. However, the average relative mass loss after nine weeks was almost twice as high. This contrasts with strain NI1, for which the relative mass loss caused did not increase on average between six and nine weeks of incubation. In conjunction with the results of the growth experiment, it can be assumed that the wood decay capacity of each strain is temperature-dependent since the optimal temperature for hyphal growth varies between strains. Nevertheless, this is merely a single factor, and it can be assumed that a multitude of factors contribute to the wood decay capacities of *B. nummularia*. However, the data presented in this study demonstrate that the host species from which the strain was isolated has no obvious impact on its capacity to decay European beech wood. Strain NI2 (*F. sylvatica*) does not differ noticeably from strains ST1 (*P. sylvestris*) and HE1 (*P. menziesii*) after either six or nine weeks of incubation. Both, the strain causing the highest relative mass loss (HE2) and the strain causing the lowest relative mass loss (BW2) were isolated from European beech.

To our surprise, notable differences were found in the tubulin DNA region of the tested strains, with high similarities between strains isolated from plots located within distinct circular radii, implying a geographic component. These groups are not necessarily reflected in the results of the growth or wood decay capacities. The findings are at odds with those of Patejuk et al. (2022), who discovered no noteworthy variations in the beta tubulin region with a considerably larger number of strains but a much smaller number of compared base pairs (using primer pair Bt2a + Bt2b, representing a approx. 450 bp region). The tubulin region is usually one of the more conserved gene coding regions (Sullivan and Cleveland, 1986). The observed dissimilarities can be primarily explained by differences in intron regions. Additionally, for a number of fungal species, the presence of paralogue or pseudogene regions is known in association with the tubulin region (May et al., 1987; Ayliffe et al., 2001; Hubka and Kolarik, 2012). Unfortunately, since there is currently little data available on the tubulin DNA region of *B. nummularia* from other strains and localities, and only seven strains were tested in the study, this apparent phenomenon at present cannot be investigated in more detail. However, the observations presented in this study justify reassessing the species concept of *B. nummularia* while considering intraspecific variability. Ideally, examination of the phylogenetic relationships between different populations should be done over a larger geographic region, using additional markers and an adequate number of isolates.

In conclusion, the present study is the first to show that *Biscogniauxia nummularia* strains can differ in their temperature-related growth characteristics. There are indications that the temperature-related differences could be related to the origin of the respective strain and therefore the site. For future studies, to gain a more comprehensive understanding of the subject matter, it would be beneficial to analyse a greater number of strains across an even larger geographical area. Furthermore, including multiple strains isolated

from the same site would facilitate a more in-depth investigation into the influence of the site. The results of the present study demonstrate the variability in the temperature-related growth characteristics and the wood decay capacities of *B. nummularia* strains, thereby contributing to a more comprehensive understanding of VLB.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

JT: Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization. SB: Writing – review & editing, Conceptualization, Investigation, Methodology. JBu: Writing – review & editing, Funding acquisition. HS-R: Writing – review & editing. JBe: Writing – review & editing. JG: Writing – review & editing. GL: Writing – review & editing, Funding acquisition, Resources, Supervision. EL: Writing – review & editing, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffunb.2025.1548128/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Unrooted tree retrieved from Maximum likelihood phylogenetic analysis of *Biscogniauxia nummularia* TUB sequence alignment. ML bootstrap support values above 60% are shown at the branches.

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4. General discussion

4.1 Disease concept of Vitality loss of beech

The decline of European beech observed and studied in central Germany since 2018 is consistent with the characteristics of VLB, including inciting factors, damage progressions and occurring symptoms (Bressem 2008; Langer et al. 2020). Building on the “Decline Disease Spiral” concept proposed by Manion (1981), the results of the present thesis provide a basis for the systematic illustration of VLB (Fig. 4).

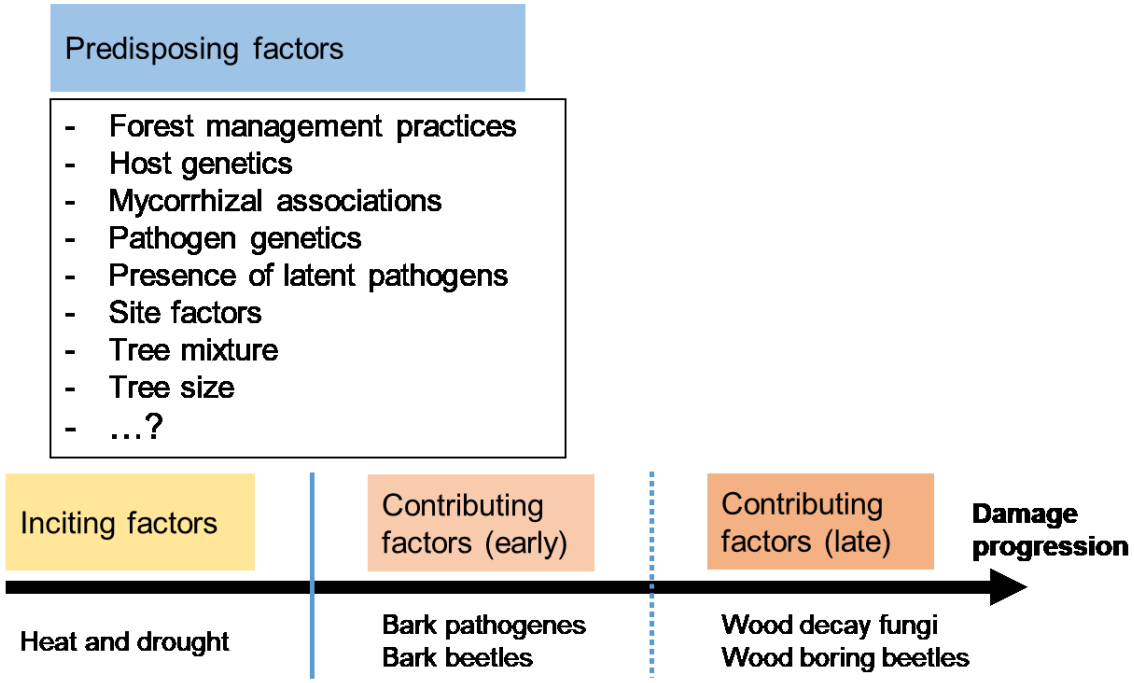

Figure 4: Model of Vitality loss of beech, based on the “Decline Disease Spiral” concept by Manion (1981). Possible predisposing factors are listed in alphabetical order.

Various predisposing factors for the occurrence of VLB have already been discussed by other authors, including forest management practices, host genetics, mycorrhizal associations, site factors, tree height, and tree diversity (Manzanedo et al. 2018; Cuervo-Alarcon et al. 2021; Pfenninger et al. 2021; Klesse et al. 2022; Meyer et al. 2022; Höwler et al. 2024; Sachsenmaier et al. 2024). The findings of the present thesis regarding *B. nummularia* in asymptomatic tissue suggest that a high presence of this fungus in the host may act as a predisposing factor (Manuscript I). This hypothesis is supported by the conclusion of Luchi et al. (2015) who stated that a higher presence of *B. nummularia* in asymptomatic tissue may compromise host vitality and potentially lead to disease outbreaks under climatic conditions favourable to the pathogen. Accordingly, the presence of latent pathogens should be taken into account in the

discussion of predisposing factors. Subsequent investigations suggest that *B. nummularia* strains may exhibit genetic differences (Manuscript II). Furthermore, the conducted laboratory studies in the present thesis demonstrated that temperature-related growth characteristics and wood decay capacities varied among the tested *B. nummularia* strains. These results support the assumption that virulence may differ between strains, which is consistent with the results of Granata and Sidoti (2004), who found that some *B. nummularia* isolates led to a more rapid formation of cankers. So alongside host genetics, pathogen genetics should also be taken into consideration as a possible predisposing factor.

Inciting factors in VLB are heat and drought (Bressem 2008; Rukh et al. 2023), two extreme weather events which are expected to increase strongly in a warming climate (Coumou and Rahmstorf 2012). Two main hypotheses have been proposed to explain the causes of drought-induced damage in trees, one being hydraulic failure (Engelbrecht 2012; Arend et al. 2022) and one being carbon starvation (McDowell and Sevanto 2010). Hydraulic failure results from the disruption of water transport caused by a large number of embolised vessels (cavitation), ultimately leading to tissue desiccation. While stomatal closure limits water loss, it also reduces photosynthetic carbon uptake, potentially causing carbon starvation due to insufficient carbohydrate supply (Urli et al. 2013). Various studies on drought-related damage in European beech since 2018 indicate that hydraulic failure is the primary explanatory factor (Schuldt et al. 2020; Braun et al. 2021; Walthert et al. 2021). It can be assumed that such cavitations facilitate the symptomatic activity of bark pathogens as well as bark beetles (Fig. 4). Among the fungal species, *N. coccinea* seems to be particularly relevant at this early stage, as it was more frequently isolated from symptomatic tissues of slightly damaged trees than from those of moderately and severely damaged ones in the present thesis (Manuscript I). *Biscogniauxia nummularia* was isolated not only from the symptomatic tissue of moderately and severely damaged test trees, but also from two slightly damaged test trees. So the fungus might also cause wood decay in rather early stages of decline. Typical white rot fungi of European beech, such as *Fomes fomentarius* (L.) Fr. (Schwarze 1994) and *Pleurotus ostreatus* (Jacq.) P. Kumm. (Bezalel et al. 1996), were exclusively isolated from symptomatic tissue of severely damaged test trees, indicating an association with advanced stages of decline. Nevertheless, *F. fomentarius* has also been detected as an endophyte in healthy European beech trees, suggesting a potential latent presence prior to symptom

development (Baum et al. 2003). Primarily Basidiomycota, specifically aerobic white rot fungi, are able to completely degrade lignin (Dashtban et al. 2010). The enzymatic cleavage of the aromatic ring depends on oxygen and thus cannot occur under anaerobic conditions (Berg and McClaugherty 2014). According to Kazemi et al. (2001), reducing the available oxygen concentration results in very low weight loss of wood caused by Basidiomycota. Oxygen supply is therefore essential for the wood-decaying activity of these fungi, for instance through insufficient compartmentalization following pruning (Metzler 2012) or, in the context of VLB, due to preceding damage caused by bark pathogens or bark beetles.

With regard to insects, *T*. *bicolor* seem to be closely associated with VLB. The bark breeding beetle appears to be a key species in this context, as it was frequently detected even in rather undamaged test trees, though restricted to the tree crown (Manuscript I). As vitality of the test trees declined, larvae of *Agrilus viridis* L. were found more frequently beneath the bark of damaged trees. The appearance of both mentioned species is not surprising, as both have been known as pests of weakened European beech trees, especially under conditions of heat and drought (Heering 1956; Schönherr and Krautwurst 1979). Larvae of the family Cerambycidae (longhorn beetles) were exclusively found in the xylem of severely damaged trees. The fact that certain species of Cerambycidae exclusively infest severely damaged trees, such as in the context of drought and prior bark beetle infestation, is confirmed by Hanks (1999). Manuscript I supports the interpretation of VLB as a complex disease in the sense of Manion's "Decline Disease Spiral", characterised by the interaction of several abiotic (drought, heat) and biotic (fungi, beetles) factors. Depending on the damage progression, different fungi and beetles are involved.

4.2 Recent drought induced calamities affecting European beech: An evaluation in the context of Vitality loss of beech

According to the Intergovernmental Panel on Climate Change Report (Pachauri et al. 2014) impacts from recent climate-related extremes, such as heat waves and droughts, reveal significant vulnerability and exposure of some ecosystems. The consequences of these climate-related extremes are reflected in reported damage to European beech stands across large areas of Central and central-western Europe. As a result of the weather extremes from 2018 to 2020 (Rakovec et al. 2022) premature leaf shedding and decline of European beech was reported in the DACH countries

(Germany, Switzerland and Austria) (Langer et al. 2020; Schuldt et al. 2020; Bigler and Vitasse 2021; Braun et al. 2021). Similar to this, beech decline as a consequence of these climate conditions has also been reported from France (Mirabel and Gaertner 2023). In 2022, central-western Europe was hit by prolonged drought and heatwaves once again (Faranda et al. 2023; Imbery et al. 2023; Knutzen et al. 2025).

Extended drought periods associated with decline of European beech are well-documented in the past. Reports in Hesse date back to 1850, 1860, and 1880 (Anonymous 1858, 1860; Hess 1900; cited in Hoppmann et al. 2022). One of the earliest and most extreme droughts in historical records was the megadrought of 1540, which affected large parts of Europe (Pfister 2018). In more recent times, prolonged drought periods were observed throughout the 20th century, such as the one from 1947 to 1952 (Rathgeb et al. 2020). Additionally, there was the extremely dry and hot year 1976 (Green 1977) and at the beginning of the 21st century, the extremely dry and hot summer of 2003 (Hamberger and Menzel 2004; Raspe et al. 2004). Both, 1976 and 2003, led to a sharp decline in annual growth of European beech stands (Borchert 2004; Utschig et al. 2004). The mentioned time periods have also been associated with an increased occurrence of *A. viridis* and *T. bicolor* (Kamp 1952; Schönherr and Krautwurst 1979; Delb 2004), which, as already mentioned, were also frequently identified in the conducted studies of the present thesis. It is noteworthy, however, that no increased occurrence of fungi as pathogens on the weakened trees was reported, in contrast to the mentioned insects, following 1947, 1976, or 2003 in Germany. This either means that the pattern of damage in the 20th century and in 2003 differed from the one observed since 2018, or that the presence of fungi was not detected or considered relevant back then. However, since strip-cankers on European beech trees were documented in England following the drought in 1976 (Hendry et al. 1998)[4], the latter hypothesis is the more likely explanation. In conclusion, the calamities affecting European beech reported since 2018 are not a one-time event but have been observed recurrently in Germany, as evidenced by historical records. However, this should in no way lead to any form of mitigation. Even though the damage pattern was likely similar during the presented drought periods, the extent of the damage might differ considerably. Due to limited data availability, however, comparisons with calamities

[4] At the time, the strip-cankers were attributed to fungi of the genera *Hypoxylon* and *Diatrype*.

from the 19th and early 20th centuries are challenging. Climatically the drought of 2018 was more extreme and had a greater impact on forest ecosystems of Central Europe than the 2003 drought (Schuldt et al. 2020; Rukh et al. 2023). Climate change will most likely lead to more extended periods of drought stress in Europe during the 21st century (Lindner et al. 2010). Additionally, the occurrence of heatwaves is very likely to increase (Pachauri et al. 2014). As a result, it is to be expected that VLB will occur more frequently and over larger areas in Central Europe in the future. In this context, the adaptations of *B. nummularia* strains to different temperature conditions, as shown in the present thesis (Manuscript II), should be taken into account.

4.3 Evaluation of Vitality Loss of beech and other calamities in different *Fagus* species

The potential replacement of European beech by Oriental beech at critical sites in the context of climate warming is debated in Germany (Mellert and Šeho 2022). The following chapter explores whether other beech species are also affected by VLB or if they face other complex diseases involving fungal participation.

4.3.1 Oriental beech (*Fagus orientalis*)

The natural range of Oriental beech borders the southeastern distribution area of European beech (Schütt et al. 1992). It is one of the forest-forming broadleaved tree species of Asia Minor and adjacent regions (Liesebach et al. 2021). Due to its suggested climatic tolerance, Oriental beech is being discussed for cultivation on sites critical for European beech in German forests (de Avila et al. 2021; Liesebach et al. 2021; Mellert and Šeho 2022), although its suitability as an alternative tree species could not always be confirmed in field trials (von Wuehlisch et al. 2008). To date, there are no known diseases that cause widespread mortality in Oriental beech comparable to the dieback observed in European or American beech. However, in 2024, damage caused by *B. nummularia* on Oriental beech was reported for the first time in Iran (Zamani et al. 2024). The authors point out that *B. nummularia* is known to cause damage in association with water and temperature stress. However, the report does not provide information on whether the affected trees were subjected to environmental stress. Even though Oriental beech reaches warmer and drier climates than European beech (Mellert and Šeho 2022) observations by Zamani et al. (2024) suggest that Oriental beech will also be affected by VLB if water stress and heat become too severe. Additionally, BBD was detected on Oriental beech in the USA (Ewing et al. 2019).

4.3.2 American beech (*Fagus grandifolia*)

The American beech is the only native beech species in North America and its natural range is limited to the eastern third of the North American continent (Schütt et al. 1992). At this point in time, no cases of VLB are known, and *B*. *nummularia* does not appear to have been detected on American beech. However, as already mentioned in the introduction of this thesis, the American beech is affected by BBD (Ehrlich 1934; Houston 1994). In contrast to central-western Europe, where the last large-scale infection wave was recorded around the turn of the millennium (Eisenbarth 2001; Emschermann and Niesar 2001; Pankert 2001; Theisen 2001; Niesar et al. 2003, 2004), North America has reported widespread occurrences of this complex disease even in more recent times (McLaughlin and Greifenhagen 2012; Kasson and Livingston 2012; Reed et al. 2022). The disease has spread rapidly in the eastern USA since the introduction of the beech scale around 1890 (Ehrlich 1934) and has invaded most of the regions where American beech is a dominant component of forest stands (Morin et al. 2007). The different damage dynamics observed in North America and Europe are attributed by Houston (1994) to the higher tolerance of European beech to infection and a greater resistance to the beech scale compared to American beech.

With the beech leaf disease (BLD), an additional threat to American beech has emerged in 2012. Symptoms were first observed in Ohio and have since been documented in multiple U.S. states as well as in Canada (Ewing et al. 2019). Initial symptoms of the disease are dark green, inter veinal banding patterns on lower canopy foliage. Later symptoms manifest in solidly darkened leaves that are shrunken and crinkled (Ewing et al. 2019). According to Shepherd et al. (2025) 29.6% of beech trees died within the American study plots between 2011 and 2023. The vast majority of this mortality occurred since the initial documentation of BLD in Cuyahoga County in 2014. Saplings were most severely affected, while the lowest mortality was observed in large trees. The presence of the non-native nematode *Litylenchus crenatae* ssp. *mccannii* (Carta et al. 2020), was found in infected leaves throughout the range of BLD (Reed et al. 2020). However, it remains unclear whether the nematode itself causes the disease or merely acts as a vector for a so far unknown pathogen (Carta et al. 2020). Cases of BLD have also been documented on European beech in the USA (Ewing et al. 2019). In Europe, the disease has not been reported so far, but poses an imminent threat of disease to European beech.

4.3.3 East Asian *Fagus* species

The genus *Fagus* shows its greatest diversity in East Asia. Two species display notable disjunctions: *Fagus hayatae* Palib. between mainland China and Taiwan, and *Fagus engleriana* Seemen between China and the South Korean island of Ullung-do. The other East Asian species are native to China and Japan (Denk 2003). However, no reports of large-scale calamities affecting East Asian beech species have been found in the English-language literature. *Phytophthora* species have been confirmed on *F. hayatae*, but symptoms are largely absent (Jung et al. 2017). In Japan Hirooka et al. (2013) were able to identify *N. coccinea* on *Fagus crenata* Blume, sister species of *F. sylvatica* (Denk 2003). This confirms the presence of at least one of the two key pathogens of VLB in Japan. In contrast, the beech scale, the inciting factor of the BBD has not yet been introduced to Japan (Hirooka et al. 2013). Regarding BLD, *Litylenchus crenatae* (Kanzaki et al. 2019) was originally described on *F. crenata* in Japan, causing only minor symptoms on its host (Kanzaki et al. 2019). Due to morphological and host range differences, the North American populations have been nomenclaturally distinguished from the Japanese population *Litylenchus crenatae* s. str. as *Litylenchus crenatae* ssp. *mccannii*. Contrary to *F. sylvatica* and *F. grandifolia*, *F. crenata* may be resistant to the *Litylenchus crenatae* ssp. *mccannii* population from North America (Ewing et al. 2019; Carta et al. 2020). *Litylenchus crenatae* s. str. has not been reported on American beech in Japan (Carta et al. 2020).

4.4 Endophytes of European beech as pathogens of other tree species

For some of the fungi identified as endophytes in the present thesis, it is still unclear to what extent they act as latent pathogens of European beech. However, for several of these fungi, their pathogenic effects on other tree species have been documented.

4.4.1 *Biscogniauxia mediterranea*

With 55 isolates, *B. mediterranea* was among the most frequently detected fungal species (Manuscript I). *Biscogniauxia mediterranea* is a well-known endophyte of European beech (Ju et al. 1998; Ceccarelli 2011; Langer and Bußkamp 2021, 2023), however, it is still not conclusively determined to what extent *B. mediterranea* is able to damage European beech. *Biscogniauxia mediterranea* has been frequently identified as an endophyte in both conifers and other deciduous tree species (Bußkamp et al. 2020, 2024). In terms of pathogenic characteristics, *B. mediterranea* has primarily been associated with damage in water-stressed oaks (*Quercus* spp.)

(Vannini and Scarascia Mugnozza 1991; Vannini et al. 1996; Capretti and Battisti 2007), causing charcoal disease (Mirabolfathy et al. 2011; Diminić et al. 2019; BakhshiGanje et al. 2024). Yet, results from Tropf et al. (2022) indicate that the fungus is capable of causing mass loss to dead European beech wood, comparable to some of the tested *B. nummularia* strains under laboratory conditions. On young European beech plants inoculated with *B. mediterranea*, Langer and Bußkamp (2021) observed necroses of a similar extent to those infected with *B. nummularia*. Nevertheless, as reported in the introduction, *B. mediterranea* is clearly less associated with damage to European beech in the context of VLB (Langer and Bußkamp 2023), and in the present thesis, it was exclusively isolated from asymptomatic tissue (Manuscript I).

4.4.2 *Diplodia* spp.

According to the results of the study presented here (Manuscript I) and previously published by other authors, *Diplodia* species (Botryosphaeriaceae) appear to be particularly closely associated with VLB. While *Diplodia mutila* (Fr.) Fr. had already been reported on European beech prior to the disease outbreak in 2018 (LFE 2016), the number of repots has increased considerably since then (Langer and Bußkamp 2023) and the fungus was also detected in asymptomatic tissue in the course of the present thesis. Following the dry period in 2018, *Diplodia corticola* A.J.L. Phillips, A. Alves & J. Luque was isolated for the first time from diseased European beech trees and simultaneously reported for the first time in Germany (Langer et al. 2020; Langer and Bußkamp 2021). However, the presence of *D. corticola* was not confirmed in any of the samples examined in the present thesis and previous detections have only been made in symptomatic tissue (Langer and Bußkamp 2021; Bregant et al. 2024). *Diplodia corticola* and *D. mutila* are associated with oak decline, especially in the Mediterranean region (Luque et al. 2000; Sánchez et al. 2003; Linaldeddu et al. 2009, 2014). Both of the species have repeatedly proven to be virulent on European beech saplings in infection tests (Langer and Bußkamp 2021; Tropf et al. 2022). In the context of the present thesis, the first record of *Diplodia fraxini* (Fr.) Fr. on European beech was reported (Manuscript I). Since *D. fraxini* was found solely in asymptomatic tissue and, to date, no pathogenicity tests have been conducted, it remains unclear to what extent *D. fraxini* acts as a pathogen of European beech. However, *D. fraxini* is a known pathogen of European ash (*Fraxinus excelsior* L.) and manna ash (*Fraxinus ornus* L.), causing cankers and is also associated with stem collar necroses in the context of ash dieback (Linaldeddu et al. 2020; Peters et al. 2023; Benigno et al. 2024).

4.4.3 *Dothiorella* spp.

In addition to *Diplodia* spp., two further fungal species belonging to the family Botryosphaeriaceae, *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves and *D. sarmentorum* (Fr.) A.J.L. Phillips, Alves & Luque, were asymptomatically detected in the course of the present thesis. This marks the first record of both fungal species on European beech. *Dothiorella iberica* is associated with a wide range of tree hosts in Europe, Africa, and the USA (Phillips et al. 2005; Azouaoui-Idjer et al. 2012; Adesemoye et al. 2014; Dissanayake et al. 2016). In Algeria, *D. iberica* has been identified as a pathogen on Monterey cypress (*Cupressus macrocarpa* Hartw.), causing bark discoloration, cankers on branches, dieback and tree mortality (Azouaoui-Idjer et al. 2012). In the USA, this fungus has similarly been reported as a pathogen, inducing trunk and branch cankers on almond trees (*Prunus dulcis (*Mill.) D.A.Webb) in California (Doll et al. 2015). Subsequently, the fungus was also linked to symptoms of disease (Almond Decline Syndrome) in almond trees in Spain (Antón-Domínguez et al. 2023). Symptomatic occurrences on typical forest trees of Central Europe have not been described in the literature for *D. iberica*. Similar to *D. iberica*, *D. sarmentorum* is associated with a variety of tree hosts across continents and is widely associated with tree decline (Inderbitzin et al. 2010; Dissanayake et al. 2016; Kazemzadeh Chakusary et al. 2019; Iqbal et al. 2023). The fungi has also been linked to symptoms on European ash trees (Ivanová 2018) a common forest tree in Europe (Dobrowolska et al. 2011). In contrast to *D. sarmentorum, D. iberica* was in the present thesis not exclusively isolated from asymptomatic tissue but also from symptomatic tissue. So, *D. iberica* may act as a latent pathogen of European beech.

4.4.4 *Nectria dematiosa*

Species of the genus *Nectria* (Fr.) Fr. are typically weak parasites of woody plants (Samuels et al. 2009). *Nectria dematiosa* (Schwein.) Berk. and *N. nigrescens* Cooke, both isolated from European beech during the course of this thesis, are members of the *Nectria cinnabarina* (Tode) Fr. species complex (Hirooka et al. 2011). In contrast to *N. nigrescens*, *N. dematiosa* was identified as an endophyte in the present thesis. This is the first record of *N. dematiosa* on European beech, confirmed through DNA analyses. Although the pathogenic role of *N. dematiosa* in European beech, particularly in the context of VLB, remains unclear, the fungus is known from various continents and affects twigs and branches of various broadleaf and coniferous trees (Hirooka et al. 2011; Yang et al. 2018). Based on phylogenetic analyses of DNA

sequence data from six DNA loci, Hirooka et al. (2011) assigned *N. dematiosa* strains to three subclades: Subclade A, known from Europe and North America; Subclade B, represented by two isolates from Canada; and Subclade C, restricted to Asia. Sequence analysis of the ITS (internal transcribed spacer) and *TEF* (translation elongation factor 1α) DNA data generated from the *N. dematiosa* strain isolated in the present thesis (Manuscript I) suggests its affiliation with Subclade A, thus supporting the findings of Hirooka et al. (2011).

4.4.5 *Stegonsporium pyriforme* s. l.

In the context of the present thesis, *Stegonsporium pseudopyriforme* Voglmayr & Jaklitsch was recorded for the first time on European beech. Based on phylogenetic analyses, Voglmayr and Jaklitsch (2014) separated *S. pseudopyriforme* and *S. protopyriforme* Voglmayr & Jaklitsch from *S. pyriforme* (Hoffm.) Corda. According to Voglmayr and Jaklitsch (2014), there are no clear morphological distinctions among the three species and ITS sequences alone do not allow a further distinction. Therefore, identifications of *S. pyriforme* based on morphological features or ITS sequences should be considered with caution. In the presented study (Manuscript I), species identification was conducted using *TEF* sequence data. *Stegonsporium pyriforme* s. l. is documented on different maple species (*Acer* spp.) (Voglmayr and Jaklitsch 2008, 2014; Moročko-Bičevska et al. 2019). It is associated with necroses on sycamore maple (*Acer pseudoplatanus* L.) (Cech 1995), on which *S. pyriforme* s. l. is also known to occur as an endophyte (Bußkamp et al. 2024). Thereby, *S. pyriforme* s.l. may act as a latent pathogen of sycamore maple. However, results of Kowalski and Materniak (2007) and Bußkamp et al. (2024) indicate that the *Stegonsporium*-complex has only low pathogenic potential with respect to sycamore maple. To what extent the three fungal species differ in their lifestyle and ecological niche remains unknown. To date, only *S. pseudopyriforme* has been detected on European beech and there is no indication that *S. pseudopyriforme* or either of the two closely related species acts as a pathogen of European beech.

4.5 Conclusion and outlook

The present thesis has provided valuable new insights into the mycological aspects of VLB, particularly regarding the role of endophytic fungi. There are no comparable culture based studies on fungi associated with VLB in which differently damaged trees have been systematically examined across such a large geographical area — from the

trunk to the twigs — including both symptomatic and asymptomatic tissue. The results also demonstrate the remarkable diversity of the mycobiome in European beech. Numerous first records of fungal species of European beech have been reported. In addition, some of the isolated species have not yet been fully identified. There are indications that new species may be among them. The precise identification and, if necessary, first description of these species will follow.

In the context of VLB, numerous fungi appear to be present during different stages of this complex disease, and their presence or absence likely has a substantial impact on the disease progression. Certain wood decay fungi, such as *F. fomentarius* and *P. ostreatus*, were exclusively detected in symptomatic tissues of severely damaged trees, whereas the bark pathogen *N. coccinea* was more frequently isolated from symptomatic tissues of slightly damaged trees. So, these findings support hypothesis 1, suggesting that the fungal communities at least in symptomatic woody tissues differs depending on the vitality status of mature European beech trees in central Germany. Although it could not be clearly demonstrated that the fungal community in asymptomatic tissue differs in relation to the vitality of the host tree, the high presence of *B. nummularia* in asymptomatic tissues of moderately and severely damaged test trees is particularly notable. Hypothesis 2 could be confirmed by demonstrating that the key pathogens *B. nummularia* and *N. coccinea* are indeed widespread and frequently present in asymptomatic woody tissue of European beech. Only a small proportion of the isolated fungi was detected in all three investigated tree compartments, thereby confirming hypothesis 3. Consequently, different tree compartments should be considered in future monitoring efforts, particularly with regard to risk assessments concerning host-associated pathogens. The studies on *B. nummularia* (Manuscript II) suggest that this fungal species may not only influence disease progression, but also act as a predisposing factor and could serve as bioindicator for Eurpean beech vitality, as previously discussed by Luchi et al. (2015). Evidence for genetic variability among the strains, seemingly linked to their geographic origin, supports hypothesis 4. The results of the hyphal growth experiment conducted as part of the present thesis confirm hypothesis 5 and indicate that *B. nummularia* exhibits a high degree of adaptability to varying temperature conditions. In conjunction with Tropf et al. (2022) this study was the first to provide evidence of varying wood decay capacities among different *B. nummularia* strains confirming hypothesis 6. However, the host species from which the strain was originally isolated does not

appear to considerably influence its ability to decay European beech wood, thereby refuting hypothesis 7. The confirmed hypotheses 4, 5, and 6 suggest that for a reliable risk assessment regarding host-associated pathogens, not only the fungal species but also the specific fungal strain present is of importance.

The presented studies should be regarded as preliminary research, and further investigations are necessary to build upon these findings. To further investigate the mycobiome of European beech in this context, the study area should be expanded, and a larger number of trees per area should be sampled to allow for inferential statistical analyses. Pathogenicity tests *in planta* are needed to verify the pathogenic potential of newly identified fungi associated with European beech, such as *D. fraxini* and *N. dematiosa*. Inducing drought stress in host plants during these pathogenicity tests could provide insights into the relevance of these potential pathogens in the context of VLB. With regard to *B. nummularia*, more strains should be included in genetic analyses and studies of growth behaviour. Ideally, strains should be analysed across the entire natural distribution range of European beech. If the conclusions drawn are correct, the observed differences are likely to be even greater.

Climate change creates regional but, to an even greater extent, cross-border challenges. Therefore, a strong network with regular exchange between researchers working on the beech microbiome, plant diseases and forestry is essential.

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Anlage

Zusätzliche Veröffentlichungen

im Zusammenhang mit dem Forschungsprojekt dieser Dissertation in chronologischer Reihenfolge

Tropf J, Bien S, Eurich L, Grüner J, Langer GJ (2022) Pilzliche Schäden an der Rotbuche. AFZ Der Wald 177:32-35

Tropf J, Gawehn P, Langer GJ (2022) Buchenkalamitäten im Klimawandel - Ursachen, Folgen, Maßnahmen. ImDialog 3:34-35

Tropf J, Langer GJ (2022) Verbundprojekt untersucht Vitalitätsschwächen länderübergreifend. Waldi 8:4

Tropf J, Menge J, Langer GJ (2023) Buchenkalamitäten im Klimawandel –Erste Ergebnisse zur Pathologie aus dem Forschungsprojekt Buche-Akut. Das Blatt: Mitarbeitermagazin Thüringenforst 3:29

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