Forest Pathology

WILEY

Forest Pathology

ORIGINAL ARTICLE OPEN ACCESS

Assessing the Role of Mixed Tree Species in Mitigating Ash Dieback: Insights From Greenhouse Experiments

Falk Schrewe[1] | Kristin Morgenstern[2] | Sven Wagner[1]

[1]Institute of Silviculture and Forest Protection, Dresden University of Technology, Tharandt, Germany | [2]Institute of Forest Botany and Forest Zoology, Dresden University of Technology, Tharandt, Germany

Correspondence: Falk Schrewe (falk.schrewe@tu-dresden.de)

Received: 8 November 2024 | **Revised:** 18 October 2025 | **Accepted:** 29 May 2026

Keywords: ash dieback disease | disease control | *Hymenoscyphus fraxineus* | leaf litter decomposition | qPCR detection | spore trap | stand composition

ABSTRACT

Since its introduction, ash dieback has been a disease affecting ash (*Fraxinus excelsior*) stands across nearly their entire range. As the disease cannot be contained, various options for mitigating its severity have been explored in numerous studies. It has frequently been proposed that ash, particularly in dense pure stands, is more severely impacted by the disease. Additionally, it has been claimed that the presence of mixed tree species might have a species-specific effect in mitigating disease progression. To investigate the impact of admixed tree species, we conducted greenhouse experiments in which ash trees were grown under varying quantities of infected ash and mixed tree species leaf litter. The objective was to assess the effect of admixed tree species based on pathogen spore production and the assessment of disease symptoms. The mixed tree species used in the experiments were beech (*Fagus sylvatica*), sycamore (*Acer pseudoplatanus*) and winter lime (*Tilia cordata*). Spore production from the infected leaf litter in the greenhouse cabins followed patterns broadly consistent with previous field observations. We demonstrated that spore pressure in the cabins depended on the applied quantity of infected ash leaf litter and that the mixed tree species exerted no further influence. The number of infected shoots on an ash tree correlated with both its total number of shoots and the quantity of infected ash leaf litter. The number of shoots was considered an indicator for leaf area and thus the tree's potential to become infected. For stem necroses, only the quantity of infected ash leaf litter was found to be decisive. We conclude that the milder progression of ash dieback in mixed stands is primarily due to a dilution effect caused by the presence of mixed tree species. No additional effects of the three mixed tree species on spore production or disease progression were observed in our study.

1 | Introduction

Ash dieback disease, caused by the ascomycete fungus *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz, Hosoya, has emerged as a significant threat to European ash (*Fraxinus excelsior* L.; hereafter referred to as ash trees) populations across Europe and parts of Asia. Since its identification in the early 1990s in Poland and its subsequent spread across the continent, this devastating pathogen has led to widespread mortality of ash trees, with profound ecological, economic and cultural repercussions (Kowalski 2006). The rapid dissemination and severe impact of the disease have prompted extensive research efforts aimed at understanding its epidemiology, pathology and potential control measures, including large-scale projects such as FRAXBACK and FraxForFuture (Vasaitis and Enderle 2017; Langer et al. 2022).

The disease manifests through necrotic lesions on leaves and stems, wilting and dieback of shoots and branches, often resulting in the death of the affected trees within a few years of infection. The pathogen's life cycle involves a complex interplay between airborne spore dispersal, infection of host tissues and the formation of fruiting bodies on fallen leaf petioles, which facilitate further spore spread (Steinhart et al. 2024, 12). The high

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2026 The Author(s). *Forest Pathology* published by Wiley-VCH GmbH.

susceptibility of European ash and certain other *Fraxinus* species (Skovsgaard et al. 2017) to *H. fraxineus* has raised concerns over the long-term survival of ash-dominated woodlands and the ecosystems that depend on them. As infected leaf litter on the ground plays a crucial role in the pathogen's overwintering and re-emergence in the following season, many studies have focused on its treatment and management as a means of disrupting the disease cycle.

Containing the fungus in the leaf litter by using fungicides is an apparent solution, but numerous challenges make this approach impractical. Hauptman et al. (2015) found that various chemical fungicides, as well as urea, are effective in controlling the disease. Urea also stimulates soil organisms, increasing the decomposition rate of leaf residues (Hauptman et al. 2015, 170). However, the low selectivity of fungicides is the main argument against their use, as it could have far-reaching and unforeseeable consequences for ecosystems. Furthermore, the required large-scale and regular application of urea could lead to overfertilization of forests (Enderle 2018, 23) and the use of nursery-treated planting material in the forest carries the risk of introducing ash trees that are insufficiently resistant to the pathogen (NW-FVA 2016, 21).

More radical in its approach is the direct removal of infected leaf litter, as described by McCracken et al. (2017, 129, 132 sq., 137) as the Irish containment strategy. Unfortunately, this was not crowned by success (O'Brien and McDonagh 2023). In contrast to the expectations outlined by Volke et al. (2019, 170), the hypothesis that roadside trees are less affected due to reduced leaf accumulation and faster leaf drying could not be validated. According to Benoît Marçais (personal communication), an experiment in which the litter was removed from two plots of 774 m^2 each over 3 years indicated that sapling conditions can be positively influenced while spore load is reduced. However, he also noted that the method is highly labour-intensive, cannot be applied on a large scale, and is futile if the disease is established in neighbouring stands (Skovsgaard et al. 2017, 462).

It is commonly perceived that ash trees in dense, pure stands are particularly susceptible to damage from ash dieback. Grosdidier et al. (2020, 1797) demonstrated that an increased abundance of ash trees in the surrounding area leads to a higher density of infected rachises in the litter, which subsequently results in a higher apothecia density (Marçais et al. 2016, 1455). This, in turn, gives rise to a higher quantity of spores and thus the likelihood of successful infection (Tooley et al. 2013). Thus, a promising area of research is the investigation of mixed forests and their potential role in mitigating the impact of ash dieback.

In addition to the mitigating effect of a lower stand basal area of ash trees, there is the impression that ash dieback is less severe in mixed than in pure stands (Skovsgaard et al. 2017, 461). A species-specific effect is even attributed to admixed trees, although the results are sometimes contradictory (Havrdová et al. 2017; Pušpure et al. 2017).

The underlying mechanism responsible for such species-specific effects may be explained by the release of leaf compounds during decomposition, which could stimulate or inhibit the activity of both soil organisms and *H. fraxineus* (Chomel et al. 2016; Ehlers et al. 2020). This would then be reflected in changes in spore quantity and, subsequently, in disease progression. Concurrently, the presence of admixed tree species may exert a dilution effect on host trees, while also acting as a physical barrier between them (Keesing et al. 2006, 490).

The present study aimed to investigate the effects of different quantities of ash litter on the quantity of spores released and the progression of ash dieback, as well as the potential influence of admixed tree species on disease severity. We hypothesised that (1) an increase in the quantity of ash leaf litter leads to a proportional increase in the quantity of spores released, whereby (2) the leaf litter of mixed tree species has a species-specific effect on the quantity of spores released and (3) that this subsequently influences the severity of the disease in young ash trees. To test these hypotheses, we cultivated ash trees in greenhouses with varying quantities of litter from infected ash trees and other tree species: winter lime (*Tilia cordata* Mill.), whose foliage leads to a more extensive fragmentation of ash rachises (Bartha et al. 2017, 84), as well as European beech (*Fagus sylvatica* L.) and sycamore (*Acer pseudoplatanus* L.), due to their frequent occurrence in association with ash (Bartsch et al. 2020).

2 | Materials and Methods

2.1 | Layout of the Experiment

The present study sought to investigate the impact of litter quantity on the progression of ash dieback. For this purpose, the experimental treatments subjected ash trees to different weight-based proportions of ash litter, while keeping the total litter quantity constant at 0.4 kg/m^2—a value that reflects typical amounts of OL horizon (moist, freshly fallen leaves) on ash-dominated forest sites (own investigations). Four treatments were established, containing 0%, 10%, 90% and 100% ash litter by weight (see Figure 1).

To assess the potential influence of admixed tree species in addition to ash litter quantity, a replacement series was implemented following a substitutive design (Harper 1994, 255). In these treatments, the remaining portion of the 0.4 kg/m^2 total was supplemented with litter from other tree species. Ash litter always formed the bottom layer, as ash trees are capable of recognising a leaf infection by *H. fraxineus* and tend to shed infected leaves early, thereby preventing the pathogen from growing into branches (Kirisits and Freinschlag 2012, 189, Mayer and Lenz 2013, 39).

All treatments were repeated three times, and the ash trees were assessed biannually. The parameters recorded included tree height and length, total number of shoots, diseased or dead shoots, number of stem necroses and root collar diameter.

2.2 | Research Site

Research was conducted at the Ecological Assessment Site in the Tharandt Forest, Saxony, Germany (N 50°57′50.3532″ E 13°33′55.3104″). Twenty-five greenhouse cabins were used, each containing a soil bed covered with different types of leaf litter,

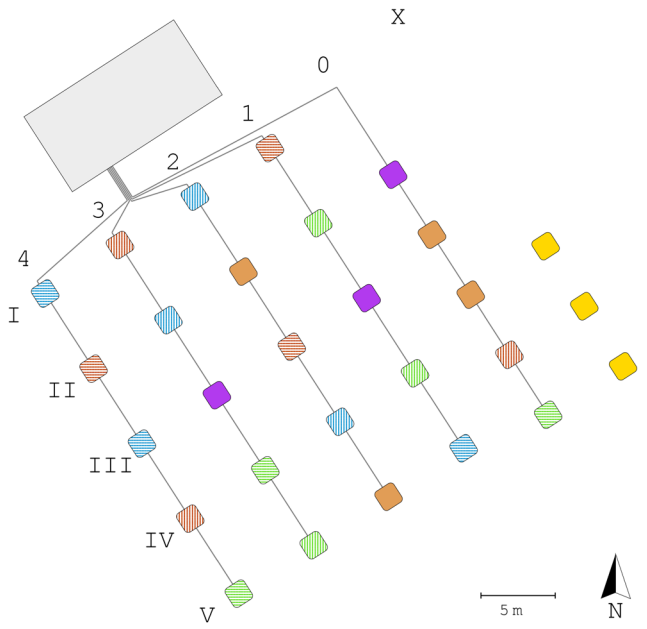


FIGURE 1 | Experimental design. Ash litter was distributed in the cabins with *Acer pseudoplatanus* (red), *Fagus sylvatica* (green) and *Tilia cordata* (blue), using two proportions: 900 g ash litter with 100 g admixed species litter (vertical lines) and 100 g ash litter with 900 g admixed tree species litter (horizontal lines). One treatment consisted of 1 kg pure ash litter (magenta), while another received no litter at all (orange). A final variant grew without added litter and without cabin coverage (yellow). All treatments were replicated three times. Rows X and 0 are compressed. Grey lines indicate underground pipes from the ventilation system connected to the main building.

in which ash saplings were planted. Each cabin covered a floor area of 1.5 by 1.5 m, with a height of 1.4 m at the rear and 1.85 m at the front ridge and was open at the bottom. Inside each cabin, the existing top 30 cm of soil was removed and replaced with standard soil (TS3, Recipe 404 of Klasmann-Deilmann GmbH). On the 6th May 2021, ten 1-year-old ash seedlings from the provenance '03 Central and eastern German lowlands and hills' (forest nursery 'Fürst Pückler' Zeischa GmbH) were planted in each cabin. On 18th October, whole ash leaves were collected from an infected ash seed orchard near Heinsen, Lower Saxony, Germany. The litter of the admixed tree species originated from nearly pure stands, either in urban green spaces or in nearby forests. On 25th October, the leaves were dispersed in the cabins. Since prolonged desiccation reduces the fructification (Gross and Holdenrieder 2013), the litter was regularly moistened with a hydraulic sprayer over the winter.

At the end of August 2021, a fully automated drip irrigation system was installed to ensure consistent daily watering. A magnetic valve opened every 4 h for 30 min, starting at 1:00 am. Each tree was equipped with a dripper that discharged 2 L of water per hour. This system watered seedlings precisely while leaving the rachises dry, thereby inhibiting fruiting body formation. Thus, a sprinkler with a discharge rate of 20 L per hour was installed in each cabin in May 2022. During the summer months, the theoretical discharge rate of 20 L per cabin and irrigation cycle was not achieved due to the low groundwater table. As a result, water was only available for 15–20 min per cycle; however, preliminary trials indicated that this duration was adequate for both *H. fraxineus* and the ash trees.

The automatic ventilation system was activated at 5:00 am, during the main sporulation period (cf. Timmermann et al. 2011) and ran at full capacity until 8:00 am. From then until 8:00 pm, it operated at 80% capacity. This served both to disperse the spores within the cabins and to create a slight overpressure in order to prevent contamination from other cabins. At the same time, a cooling effect was achieved by exchanging the air masses, which was further optimised in summer by the use of shading powder and nets. Parallel to the irrigation system, the ventilation system operated at 65% capacity for 1 h each at 9:00 pm and 1:00 am to prevent water from entering the ventilation pipes.

To validate the experimental setup, an additional control treatment was established adjacent to the main treatments, referred to here as 'row X'. This control used the same soil but excluded both the litter and the cabin enclosure. The aim was to determine whether the presence of the cabin or ventilation system influenced infection levels. If these factors had no effect, infection rates in this open, litter-free control should match those of the litter-free treatment within a cabin.

2.3 | Spore Trapping

To quantify spore density, passive spore traps were installed in each cabin above the sprinkler, as well as outdoors above each of the treatments without a cover (X) and in a garden situated at a considerable distance from ash stands (Z) (see Figures 1 and 2), during the period from 7th June to 1st November 2022. The traps were a modification of the protocol presented by Schweigkofler et al. (2004) and had previously been used by Grosdidier et al. (2018) and Aguayo et al. (2018), among others, in the context of *H. fraxineus*. Following a 7-day exposure period, the filters were individually stored in plastic bags at a temperature of −20°C until spore concentration was determined using qPCR.

2.4 | DNA Extraction

In order to detach fungal spores from the filter paper of the traps (150 mm diameter no. 1 Whatman filter) and prepare them for DNA extraction with subsequent TaqMan quantification, an optimised protocol was developed based on the method described by Grosdidier et al. (2017) to remove spores from the petroleum jelly used in the traps. Various solvents (TE, ddH_2O) and different incubation and centrifugation times were tested. For optimum sample preparation, the petroleum jelly was scraped from the filter with a sterile razor blade and transferred to a Falcon tube. 20 mL of prewarmed 4× TE buffer were added to the sample, which was then incubated for 15 min at 65°C in a water bath, mixed for 10 s using a test tube shaker and incubated again at 65°C for a further 10 min. The sample was then centrifuged for 10 min at 4°C, causing the petroleum jelly to settle on top of the suspension and the fungal spores to settle at the bottom of the Falcon tube. Using a cannula, approx. 18 mL of the aqueous phase were pipetted off and discarded. The petroleum jelly layer was carefully removed from the Falcon tube, the remaining spore solution was resuspended

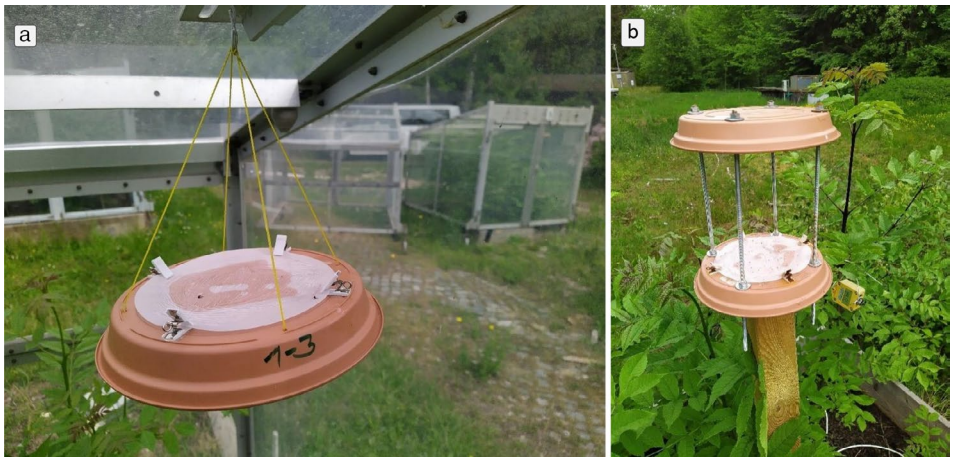


FIGURE 2 | Spore traps, as they have been installed in the cabins (a) hanging from the roof and (b) free-standing outside. Passive spore traps in the hanging indoor variant (a), as used in the greenhouse cabins and in the free-standing outdoor variant (b), as used in treatments X and Z.

and 1 mL of the solution was transferred to each of two new 2 mL reaction vessels. The reaction tubes were centrifuged at maximum speed for 10 min, the supernatant (approx. 950 µL) was discarded and the samples were stored at −25°C until further processing.

For DNA extraction, the samples were first homogenised with glass beads (2 and 3.5 mm) in a vibrating mill (3 min, 30 Hz). DNA extraction was carried out with the innuPREP Plant DNA kit (IST Innuscreen GmbH, Berlin) as far as possible according to the manufacturer's instructions. The two reaction vessels of a sample were combined in the first filter step. Deviating from the standard protocol, lysis was performed with 650 µL buffer and in the last step the DNA was eluted in 40 µL buffer. The extracted DNA was then stored at −25°C.

2.5 | qPCR Protocol

Based on the work of Ioos et al. (2009), a TaqMan real-time PCR assay was established to detect *H. fraxineus* and quantify the number of spores in the greenhouse cabins. As a positive control for the TaqMan protocol, a synthetic fragment was designed. The template for this fragment was derived from sequence data of the Internal Transcribed Spacer (ITS) region of ribosomal DNA (rDNA), available in the NCBI nucleotide database for *H. fraxineus*. These sequence data were aligned, and a representative consensus sequence of the target region was generated using the software Clustal Omega (Sievers and Higgins 2014). The consensus sequence was subsequently synthesised in an amount of 300 ng by Eurofins Genomics Germany GmbH (Ebersberg, Germany).

The TaqMan reaction mixture (20 µL) contains 10.0 µL PerfeCTa qPCR ToughMix (Quantabio, distributed by VWR International GmbH, Darmstadt), 7.8 µL RNase-free water (Carl Roth GmbH + Co. KG), 0.4 µL each of forward and reverse primer (10 µM, Eurofins Genomics GmbH), 0.4 µL TaqMan probe (10 µM, FAM-Seq-BHQ1; Eurofins Genomics GmbH) and 1.0 µL template DNA. The PCR programme consists of an initial denaturation of 5 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 61.1°C. The sensitivity of the established TaqMan assay was tested using a decimal dilution series of the synthetic *H. fraxineus* fragment in the range of 0.1 ng/µL to 0.1 ag/µL. The reaction was repeated several times in double determination (syn. fragment) and with three No-Template-Control (NTC) samples as negative control. As a result, the *H. fraxineus* fragment could be reproducibly detected up to a concentration of 0.1 fg/µL. With a fragment length of 819 base pairs (bp) and 532.35 g/mol, 0.1 fg/µL corresponds to approx. 113 copies of the fragment in 1 µL sample volume.

The *H. fraxineus* concentration was determined using the established TaqMan assay. In each qPCR run, a dilution series of the synthetic *H. fraxineus* fragment in the range from 1.0 pg/µL to 0.1 fg/µL was included as a reference and three NTC samples as a negative control. The trap samples were tested in duplicate using 5 µL of DNA template each. If the duplicate determination of a sample showed different results or if the C_t values showed a standard deviation that was too high, the sample was repeated. The concentration was determined using relative quantification, in which the change in the C_t value of the sample compared to the reference is calculated.

2.6 | Statistics

The objective of this analysis was to determine whether the quantity of ash litter and admixed tree species exerted an effect on the release of *H. fraxineus* spores. To address this, the experimental treatments were broken down into their components, namely the quantity of ash litter and the species of admixed trees. These components were then additively implemented as fixed effects in a linear model, which was intended to explain the quantity of spores found in the traps.

Given that each experimental treatment exhibited a characteristic level of spore production over the study period, spore counts were aggregated across traps within treatments, thereby allowing the use of a linear model. A '0+' notation enabled the model to estimate distinct intercepts for each level of the categorical variable. The formula was as follows:

$$
\begin{aligned}
E[Y] \quad &= \beta_1 \times \text{Quantity. Ash} \\
&+ \beta_2 \times I_{A.\ pseudoplatanus} \\
&+ \beta_3 \times I_{F.\ sylvatica} \\
&+ \beta_4 \times I_{T.\ cordata} \\
&+ \beta_5 \times I_{\text{Empty}} \\
&+ \beta_6 \times I_{\text{Full}}
\end{aligned}
$$

Here, $E[Y]$ represents the predicted quantity of spores. The initial variable `Quantity.Ash` is numerical, with the coefficient β_1. The second predictor, `Species.admixed`, is categorical, comprising five levels: Empty (absence of any litter), Full (pure ash litter) and the identity of the three admixed tree species. To incorporate this categorical variable into the linear model, it was encoded using indicator variables, one for each level of the categorical factor. Each indicator variable takes the value of 1 if the corresponding category was present and 0 otherwise. Thus, β_2 to β_6 represent the distinct effects of each admixed species or litter condition on spore release. The model structure allowed for an additive interpretation, with each tree species or litter condition contributing independently to the release of *H. fraxineus* spores.

In a subsequent analysis, the quantity of ash litter and the total number of shoots per plant were examined for their influence on disease characteristics. The primary focus was on the number of infected shoots, as these were more reliable indicators than leaf spots and could be assessed in the leafless state. Both living and dead shoots, as well as stem necroses, were examined. The former were defined as shoots that exhibit evident discolouration or bark lesions in the living state; the latter were those that had died due to necrosis encompassing the entire branch or trunk. When examining stem necroses, the age or dimensions of the affected tissue were not considered.

The data obtained were of the count type, and showed a tendency to accumulate at low values (Cameron and Trivedi 2003, 332). This distribution was most appropriately modelled by a negative binomial distribution (Fahrmeir et al. 2021, 283 sqq., 308, Salinas Ruíz et al. 2023, 129). Accordingly, generalised linear mixed-effects models (GLMMs), as they allow the distribution of the response variables to be specified. GLMMs also permit inclusion of random effects within the linear predictor alongside the usual fixed effects.

GLMMs for the negative binomial family were fitted using the `glmer.nb()` function included in the R package `lme4` by Bates et al. (2015). The model was expressed as follows:

$$
E[Y] = \exp\left(\beta_0 + \beta_1 \times \text{Quantity. Ash} + \beta_2 \times \text{Shoots} + u_{\text{Cabin}}\right)
$$

In this equation, $E[Y]$ represents the expected count of the response variable, which in this case refers to the disease characteristics of infected shoots. The intercept β_0 represents the baseline log count when all predictors are zero. `Quantity.Ash` indicates the quantity of ash litter in a cabin, while `Shoots` corresponds to the number of shoots per tree; both are numerical variables with effects estimated by the parameters β_1 and β_2, respectively. The term u_{Cabin} denotes a random intercept for each cabin, accounting for potential variation between the groups of trees within each.

This model structure enabled the estimation of the fixed effects of ash litter and shoot quantity on disease characteristics while accounting for random variability between cabins.

3 | Results

3.1 | Seasonal Variation in Spore Spread

The qPCR analysis detected DNA of *H. fraxineus* in the majority of samples, although the quantities varied considerably. DNA was detected in samples collected between 7th June and 1st November 2022, demonstrating the pathogen's presence throughout the entire duration of the experiment. The largest quantities were recorded in the period between 28th June and 2nd August 2022, ranging from 0.01 to 237.67 fg (Figure 3). The peak concentration of 237.67 fg was found in a treatment with 100% ash litter (cabin 0-I) in the week preceding 5th July.

In all June samples, DNA quantities ranged from 0 to 6.68 fg, with no obvious trend across the three sampling dates. In July, spore production showed a marked increase, with the highest values of the study period. Already at the first July sampling, the concentration reached 237.67 fg, representing the overall peak, about 35 times higher than the maximum observed in the previous month.

In August, the maximum detected DNA quantity decreased to 100.07 fg and in September to 74.46 fg. However, this August peak occurred at the first sampling date. Since none of the four subsequent August samplings yielded similarly high values, the September measurements represent an increase compared to the preceding 4 weeks.

Due to limited laboratory capacity, part of the time series could not be evaluated, resulting in a data gap. In November, *H. fraxineus* DNA was still detectable in 22 of the 29 traps, but with a maximum of 2.13 fg—the lowest across all months.

3.2 | Effect of Admixture and Ash Litter Amount

The employed model indicated that the quantity of ash litter was a significant predictor of *H. fraxineus* spores on the traps (SE = 0.60, $t(2) = 8.53$, $p = 0.014$, cf. Table S1). This reflects a directly proportional relationship: for each additional unit of ash litter, the number of trapped spores increased by approximately 5.11 units on average. In contrast, the admixed tree species showed no statistically significant effect on spore counts. The estimated coefficients for the different species were associated with large standard errors and non-significant p values (all $p > 0.05$), as also illustrated by the broadly overlapping confidence intervals in Figure 4. Although the pure ash litter treatment exhibited a higher mean spore count (+218.65 units) compared to the other combinations, this difference was not statistically significant either (SE = 83.94, $t(2) = 2.61$, $p = 0.121$).

Overall, the regression model was statistically significant ($F(6, 2) = 56.55$; $p = 0.018$) and showed a high explanatory power (adjusted $R^2 = 0.98$). These results suggest that ash litter quantity was the main factor affecting spore abundance,

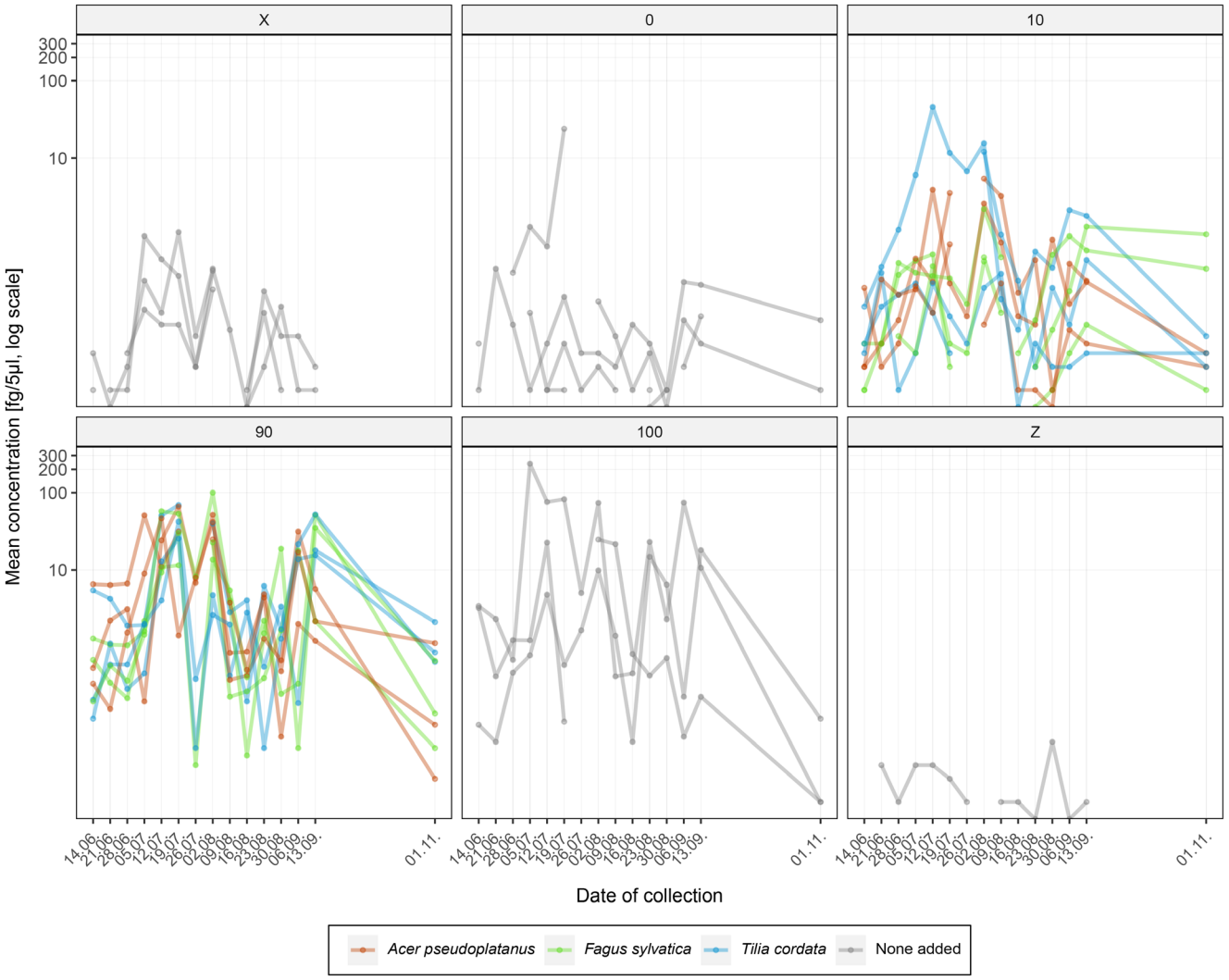


FIGURE 3 | Seasonal trend of the mean H. fraxineus DNA concentration (femtograms per 5 µL sample, logarithmic scale), detected by qPCR in spore traps on each collection date in 2022, grouped by experimental treatment. Facet labels indicate the experimental conditions: The number refers to the proportion of ash litter (as a percentage of 1 kg) distributed in the cabins, while the colour denotes whether—and if so, which—tree species was used to replenish the remaining litter to a total of 1 kg. 'X' represents spore traps placed in the corresponding row as the litter treatment; 'Z' refers to traps located at the maximum distance from ash trees.

whereas the identity of the admixed tree species had no measurable effect.

3.3 | Development of Ash Health Condition

The condition of the ash trees deteriorated continuously over the course of the experiment. In the 2022 winter inventory, 3.9% of the ashes exhibited some form of symptoms attributable to ash dieback. By the subsequent 2022 summer inventory, this figure had risen to 18.6%. At the final 2023 winter inventory, 32.5% of the trees (corresponding to 91 out of 280) were found to be infected, with only one tree dying.

3.4 | Analysing the Diseased and Dead Shoots

With regard to infected but still living shoots, the most appropriate model included the quantity of ash litter and the total number of shoots per tree (Table S2, Figure 5a). The likelihood of finding living infected shoots increased when a tree was in a cabin with more ash litter and when it had developed more branches, as confirmed by the GLMM analysis. Both the effect of ash litter (SE = 4.82e-03, $z = 2.03$, $p = 4.222e-02$) and the number of shoots (SE = 2.44e-02, $z = 3.35$, $p = 8.05e-04$) were statistically significant.

Using the same fixed effects as in the first model for living infected shoots, we also analysed dead shoots. As previously observed, both variables had a positive effect (cf. Table S2, Figure 5b). In accordance with the preceding model, the applied quantity of ash litter was found to be highly significant. The impact on dead shoots was stronger than on living shoots (estimate: 1.45e-02 compared to 9.8e-03), and with greater statistical significance (SE = 4.00e-03, $z = 3.62$, $p = 2.92e-04$). The number of shoots per tree was likewise significant (SE = 2.76e-02, $z = 3.48$, $p = 5.038e-04$), although its effect size on dead shoots remained similar to that on living infected shoots (9.60e-02 compared to 8.17e-02).

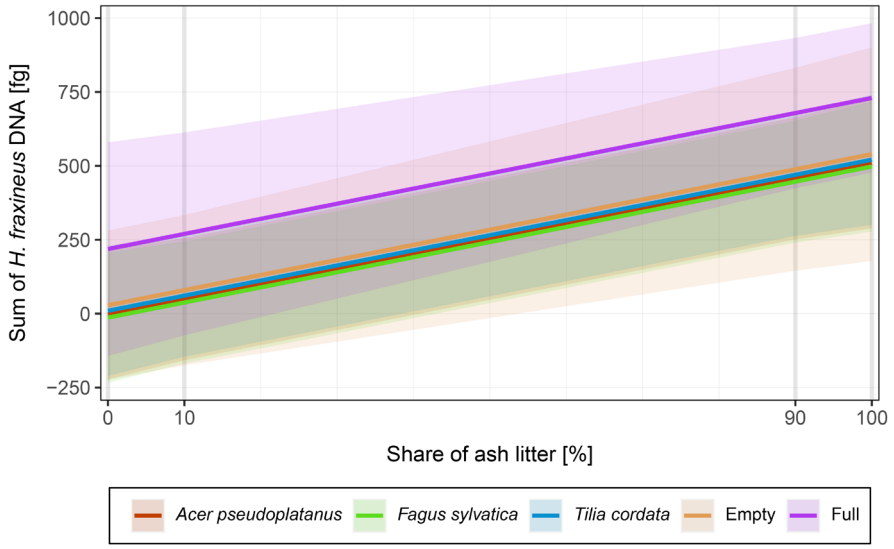


FIGURE 4 | Linear relationship between the proportion of ash litter (as a percentage of 1 kg) in the cabin treatments and the total amount of H. fraxineus DNA detected by qPCR in spore traps. The *x*-axis indicates the percentage of ash litter included in each 1 kg litter mixture; the *y*-axis shows the aggregated amount of H. fraxineus DNA detected in the corresponding treatment. Colours represent the tree species used to supplement the remaining litter in the 10% and 90% ash treatments. Shaded areas indicate confidence intervals.

A significant correlation was observed between the total number of infected shoots and the quantity of ash litter in a cabin ($_{\rho}=0.14$, $p=0.023$) as well as with a tree's number of shoots ($_{\rho}=0.20$, $p=0.001$). The proportion of trees showing symptoms of ash dieback increased with the amount of added ash litter: in cabins without any added litter, 7.5% of trees were symptomatic, compared to 21.1% with 10% added litter. Infection rates rose further to 38.8% with 90% litter and reached 50.0% with 100% litter. Across all treatments, on 19.0% of the trees with up to 10 shoots, ash dieback symptoms were found. More densely branched trees with 11 to 20 shoots were 36.5% infected and 50.0% of those with even more branches.

3.5 | Stem Necroses

The application of the same model to explain the total number of stem necroses demonstrated that the quantity of ash litter applied had a considerable positive effect on necrosis counts (Table S2). In contrast to the results for infected shoots, the total number of shoots per tree had no measurable effect, as indicated by the strongly overlapping confidence intervals in Figure 5c.

Alternative predictors, such as the use of root collar diameter, did not yield better results.

4 | Discussion

The protective effect of the cabins is evident from the results for the control row X. Although these trees were not treated with infected litter themselves, the absence of a cabin left them exposed to spores carried in from other treatments. This led to the highest infection rate of all (63.3%). In contrast, the lowest infection rate of only 7.5% occurred in the treatment without litter but with both a cabin and ventilation, confirming the protective benefit of these features. Nevertheless, the presence of some infections even in this treatment indicates that the protective measures were not entirely effective in preventing the entry of a small number of spores.

4.1 | Influence of Ash Litter

We found a significant positive correlation between the quantity of ash litter in a cabin, reflecting the share of ash in a forest stand and the severity of ash dieback. This was anticipated, given that a higher quantity of rachises in the litter leads to a higher density of apothecia (Marçais et al. 2016, 1455) and thus to a greater inoculum pressure. Close proximity of host trees creates optimal conditions for spore transmission, facilitating successful infection, while fallen leaves further contribute to the infection cycle, thereby increasing overall infection pressure (Grosdidier et al. 2020, 1797). Consequently, stands with a high ash proportion are more severely affected than solitary individuals (Meshkova and Borysova 2020, 160, Grosdidier et al. 2020, 1796).

It is well documented that particularly young and dense pure stands, as simulated in this study, tend to experience more severe disease progression (Schumacher 2011, 8, Enderle et al. 2017, 225). Disease severity is greater in dense, unthinned than in managed, thinned plots (Bakys et al. 2013, 122), while a low proportion of conspecifics among their nearest neighbours (Cracknell et al. 2023, 2129) or reduced stand basal area mitigates infection (Grosdidier et al. 2020, 1797). Notably, Pušpure et al. (2017, 213) found that mortality in natural ash regeneration reached 20% in pure stands, but only 1% in stands where ash occurred in admixture.

Studies that state the opposite trend are less prevalent. Jönsson and Thor (2012) reported higher infection rates in traditionally managed than unmanaged forests. They attributed this to

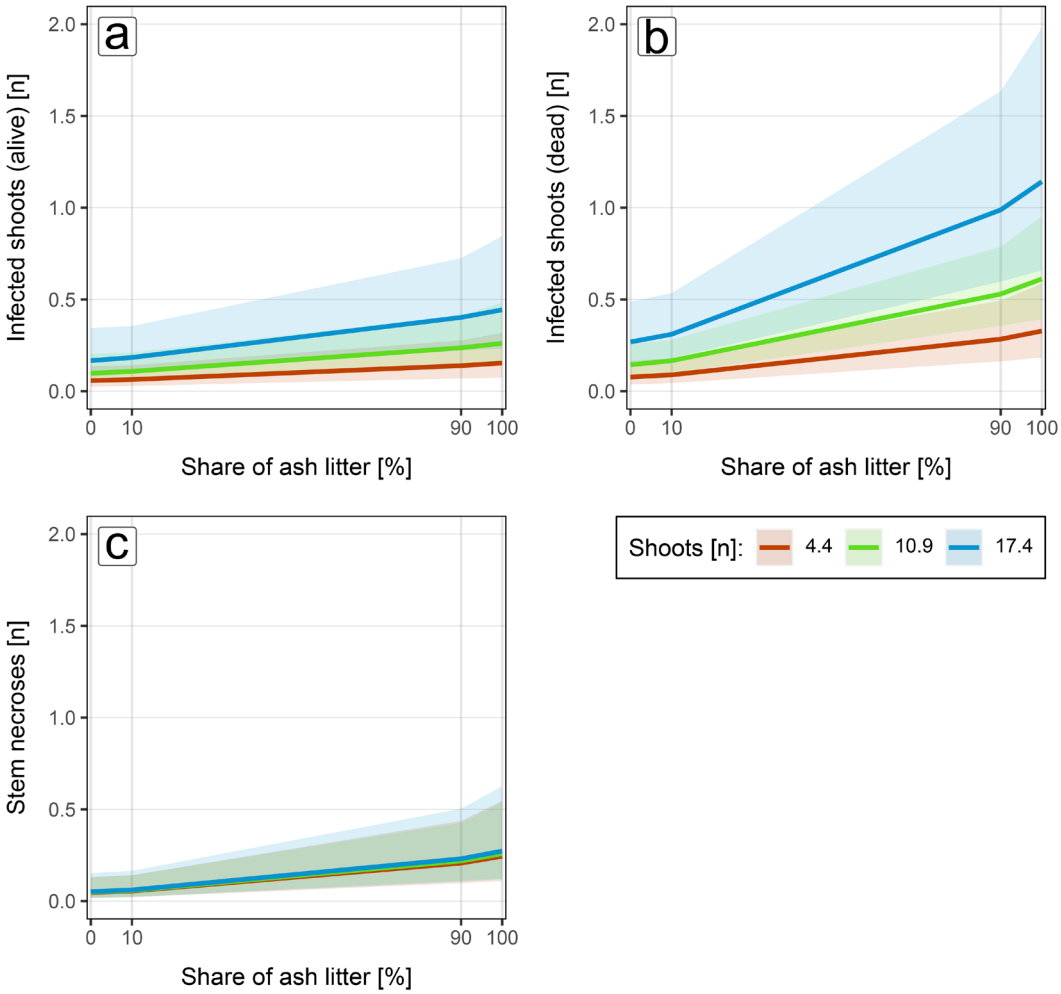


FIGURE 5 | Predicted values of (a) ill_alive (infected but living shoots), (b) ill_succumbed (shoots that became infected and succumbed to ash dieback disease) and (c) nekro_sum (number of stem necroses per plant), shown as a function of ash litter proportion (Quantity.Ash) and shoot number (Shoots). The *x*-axis represents the percentage of ash litter included in each 1 kg litter mixture; the *y*-axis shows the predicted number of infected shoots or stem necroses. Line colours indicate the mean value of Shoots and one standard deviation above and below it. Shaded areas represent confidence intervals.

frequent pollarding, which causes the trees to form new lower crowns that are potentially more susceptible. This interpretation is supported by studies showing a decline in ascospore concentration with increasing altitude (Dvořák et al. 2023, 239), regardless of site and sampling time (Chandelier et al. 2014, 1301).

Stand management generally benefits ash health. Thinning improves growing conditions, reduces the number of potential hosts and increases the vigour of remaining trees. Enhanced litter turnover (Bartsch et al. 2020, 400) reduces infection sources, leading to lower infestation rates and easier disease progression (Pušpure et al. 2017, 215, Cracknell et al. 2023, 2126).

The slightly disproportionate increase observed in our data may be attributed to *H. fraxineus* DNA accumulation in leaves until the fungus transitions from an endophytic to a pathogenic phase at a certain threshold. The occurrence of both symptomatic and asymptomatic ash trees with similarly high pathogen DNA levels suggests that this threshold is individual-specific, although higher DNA concentrations generally correlate with increased symptom expression (Cross et al. 2017, 1408, 1415).

Our findings corroborate earlier studies and confirm our initial hypothesis that an increase in the quantity of ash litter is proportionally associated with increased spore production.

4.2 | Limitations to an Effect of Admixed Tree Species

The findings of this study indicate that the tree species of the admixed litter had no discernible effect on spore load or disease progression. This contrasts with Havrdová et al. (2017, 178) and Pušpure et al. (2017, 212), who reported that the identity of neighbouring tree species could result in either more or less severe disease in ash via biogeochemical litter properties affecting *H. fraxineus* sporulation. As Skovsgaard et al. (2017, 461) noted, observations regarding the health benefits of mixed stands appear sometimes contradictory, warranting further investigation into the influence of individual tree species.

There is consensus that the species composition of a forest exerts a profound influence on the microbial communities in litter and soil (Harrison 1971; Prescott and Grayston 2013). In ash dieback research, several studies have reported reduced disease progression in stands with a higher proportion of admixed tree species (Grosdidier et al. 2020, 1797; Skovsgaard et al. 2017, 461; Havrdová et al. 2017, 176; Pušpure et al. 2017, 215; Schumacher 2011, 8; Enderle et al. 2018, 11), suggesting that such species confer positive effects extending beyond simple dilution (Hantsch et al. 2013, 8; Keesing et al. 2006).

A systematic investigation into the effect of individual tree species on ash litter decomposition rate has so far only been undertaken by Bartha et al. (2017, 84). After 1 year, they found that ash rachises covered by *T. cordata* leaf litter lost the most mass and were significantly more fragmented than rachises covered by *Carpinus betulus*, *Juglans nigra*, or left uncovered.

The absence of a detectable effect of admixed species on litter decomposition, as depicted by spore production and subsequently by disease severity, may have several explanations. It remains debatable whether rachis fragmentation is a major factor, as pseudosclerotial sections persist and produce fruiting bodies for longer (Kirisits 2015, 255; Gross and Holdenrieder 2013, 169). Moreover, ash litter is already among the fastest-decomposing litter types among tree species, leaving little scope for further acceleration in decay rate (Mitchell et al. 2014, 98). That said, since *H. fraxineus* mycelium exhibits different growth rates depending on the culture medium (Carrari et al. 2015, 542), interactions with compounds from other leaves cannot be ruled out. Further research is needed, however, as current information on the admixed species used here is limited. In general, the declining number of apothecia formed over time (Kirisits 2015, 255) suggests that their formation is influenced by litter decomposition.

Although the cabin environment may have temporarily increased spore pressure compared to a forest setting, spore production in each treatment corresponded with the amount of ash litter applied, as reflected in both trap counts and observed disease severity. The lack of differences among admixed species at equal ash litter quantities indicates that the species tested did not affect decomposition rate or spore output.

Thus, healthier ash in mixed stands is primarily the result of dilution rather than species-specific effects of the admixed trees. Accordingly, our second and third hypotheses are rejected and admixed tree species will not be considered in subsequent models.

4.3 | Determinants for a Successful Infection

Our findings suggest that trees with a greater number of branches are more susceptible to infection.

Two main patterns emerged regarding ash health. First, the proportion of healthy trees tends to decrease with age (Schumacher et al. 2009, 424; Timmermann et al. 2017, 153; Pušpure et al. 2017, 212; Giongo et al. 2017, 876). Dietrich (2016, 29) notes that recently lignified seedlings appear conspicuously healthy, which she attributes not to immunity but to their short lifespan and low leaf mass, reducing spore exposure and infection probability.

The significant correlation between shoot number and the number of infected living and dead shoots likely reflects the increased likelihood of infection as the number of shoots grows. More shoots also imply a larger leaf area, and since leaves are the primary route of infection, risk rises accordingly (Grote 2002, 783; Cleary et al. 2013).

Second, ash dieback severity is reduced in older trees. The disease progresses more slowly in these individuals, as both lower spore pressure and unfavourable microclimatic conditions in the crown hinder an infection (Dvořák et al. 2023, Gross et al. 2014, 14) and necroses also require more time to girdle the host (Combes 2022, 14). Older trees can also compensate for losses via epicormic shoots and may survive several years despite infection (Timmermann et al. 2017, 155).

By contrast, younger trees show faster disease progression and higher mortality because of their limited capacity to compensate for damage and to lesions that quickly encompass their slender stems, causing all distal parts of the plant to die (Gross et al. 2014, 12; Marçais et al. 2022, 221). The mechanism also explains why additional ash litter had a stronger effect on dead shoots than on living ones: their small diameter makes them more vulnerable to rapid girdling by expanding lesions, cutting off resource flow and causing tissue death. Living infected shoots may therefore represent only a transient stage in the disease's progression before death (Marçais et al. 2022, 221).

Future research should examine how long individual branches persist in this transient stage and whether branch diameter affects their capacity for recovery.

4.4 | Occurrence of Stem Necroses

Our observations revealed that stem necrosis in ash trees was more common in cabins with greater quantities of ash litter.

The precise entry point of stem infections remains unclear (Peters et al. 2023, 2). However, two distinct types of stem necrosis were observed. In the first, a short branch lay at the centre of the lesion, suggesting an origin from a leaf-borne infection that subsequently spread into the stem. This type was relatively rare and occurred only on branches a few centimetres long at most. In the second, necrosis appeared more randomly along the stem. In these cases, infection was presumed to result from spores entering directly through lenticels (Husson et al. 2012, 894), with active lenticels and the thin bark of young trees increasing susceptibility (Chandelier et al. 2016, 295; Enderle et al. 2018, 11). Interestingly, neither shoot number nor the root collar diameter significantly influenced the occurrence of stem necroses, suggesting that infection is more closely linked to spore entry pathways than to structural characteristics of the tree. Since stem necroses are generally associated with high spore pressure and ash density (Grosdidier et al. 2020, 1795), our finding that higher litter quantities increased necrosis incidence reinforces this connection. Overall, the results underline the complex interplay

between environmental conditions and infection pathways in the development of stem necrosis.

4.5 | Functionality of Spore Traps

Passive spore traps have frequently been used to detect *H. fraxineus* spores in the past (Grosdidier et al. 2018; Aguayo et al. 2018), which supports the validity of our results.

The low number of spores trapped in treatments X and Z may be due to their installation in the open, and their roof design (see Figure 2b) not providing sufficient protection to prevent the spores from being washed away by rain or dew. A visible layer of dew often formed on the petroleum jelly of these traps, and similarly after rainfall, which led to the exclusion of both treatments.

In the retained treatments, spore concentration was in consistency with typical seasonal pattern of previous research, peaking between late June and early August (Cleary et al. 2013, 1296; Hietala et al. 2013, 305; Baral and Bemmann 2014, 248; Chandelier et al. 2014, 1299; Dvořák et al. 2016, 6; Grosdidier et al. 2018, 5; Cross et al. 2017, 1408), although local climate may shift this peak (Chandelier et al. 2014, 1303; Grosdidier et al. 2018, 8; Hietala et al. 2018, 6; Burns et al. 2022, 503). The increase in spore concentration at the beginning of September is somewhat curious, as it has not been reported previously. It may reflect particularly favourable growth conditions for the fungus in 2022, consistent with simultaneous observations of a renewed increase in apothecium density in forests after an earlier decline (Georgia Kahlenberg, personal communication). Given the pathogen's short incubation period, the possibility of a second sporulation cycle has already been proposed by Combes (2022, 7). Facilitated by the favourable conditions in the cabins, this suggests that *H. fraxineus* is capable of not only adjusting the onset of its sporulation phase to the local climate but also repeating the entire process. When this trend became apparent in the data, limited laboratory capacity only permitted us to include the samples from 1st November to confirm the expected low spore concentration at that time.

4.6 | Effect of Weather on *H. fraxineus* Sporulation

Understanding the effect of weather conditions is essential for interpreting spore production and disease progression in *H. fraxineus*, as both temperature and moisture are known to influence fungal growth and sporulation (Burns et al. 2022, 501; Dvořák et al. 2023, 240). In Saxony, 2022 was the fourth warmest year since 1881, with mean temperatures +2.1°C above the 1961–1990 reference period. Furthermore, annual precipitation had a deficit of −19%, primarily caused by monthly deficits of up to −63% from March to August (Franke et al. 2023, 9). In the cabins, despite the application of shading paint and nets, temperatures reached up to 45.6°C below the ridge, though data loggers showed that ground-level conditions were up to 20.1°C cooler. Regular irrigation further ensured sufficient moisture and maintained high humidity, likely mitigating some of the effects of high temperatures (Data not shown; Marçais et al. 2022, 228 sq.). Kowalski and Bartnik (2010) and Hauptman et al. (2013) found optimal growth at 20°C–22°C, with reduced growth still possible at 30°C, casting doubts on its ability to thrive under the higher temperatures observed. However, these studies measured mycelial growth on solid media at constant-temperatures and may not translate directly to in situ conditions (Blenis et al. 1984, 161 sq.).

The use of shading, regular irrigation and ventilation established conditions at ground level conducive to the growth of *H. fraxineus*, as confirmed by the observation of apothecia in every cabin containing ash litter. These findings suggest that *H. fraxineus* can tolerate higher temperatures than previously assumed and that its sporulation may be more plastic than earlier reported.

5 | Conclusion

This study provides a controlled evaluation of the impact of varying quantities of ash and mixed tree species litter under directly comparable greenhouse conditions. The three admixed tree species examined exerted no measurable influence on disease severity beyond a barrier or dilution effect. This finding makes a meaningful contribution to the field by providing empirical support for long-held assumptions. The results reinforce previous studies (e.g., Grosdidier et al. 2020) showing that stands with a higher proportion of ash trees experience greater spore pressure and more severe disease progression. Overall, the study underscores that silvicultural strategies such as establishing mixed stands can help mitigate the impact of ash dieback and enhance the resilience of ash populations.

Acknowledgements

This research was supported by the German Federal Ministry of Food and Agriculture (BMEL) through the Agency of Renewable Resources (FNR)—Forest Climate Fund (WKF) [grant number 2219WK23B4]. We would like to express our gratitude to Dirk Junkuhn, the forester of the Tharandt forest district, and his forestry workers for their assistance in the establishment and maintenance of the greenhouse facilities. Open Access funding enabled and organized by Projekt DEAL.

Funding

This work was supported by Fachagentur Nachwachsende Rohstoffe, 2219WK23B4.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

References

Aguayo, J., C. Fourrier-Jeandel, C. Husson, and R. Ioos. 2018. "Assessment of Passive Traps Combined With High-Throughput Sequencing to Study Airborne Fungal Communities Master." *Applied and Environmental Microbiology* 84: 1–17. https://doi.org/10.1128/AEM.02637-17.

Bakys, R., R. Vasaitis, and J. P. Skovsgaard. 2013. "Patterns and Severity of Crown Dieback in Young Even-Aged Stands of European Ash (*Fraxinus excelsior* L.) in Relation to Stand Density, Bud Flushing Phenotype, and Season." *Plant Protection Science* 49: 120–126. https://doi.org/10.17221/70/2012-PPS.

Baral, H.-O., and M. Bemmann. 2014. "*Hymenoscyphus fraxineus* vs. *Hymenoscyphus albidus*—A Comparative Light Microscopic Study on the Causal Agent of European Ash Dieback and Related Foliicolous, Stroma-Forming Species." *Mycology* 5: 228–290. https://doi.org/10.1080/21501203.2014.963720.

Bartha, B., A. Mayer, and H. D. Lenz. 2017. "Acceleration of Ash Petiole Decomposition to Reduce *Hymenoscyphus fraxineus* Apothecia Growth—A Feasible Method for the Deprivation of Fungal Substrate." *Baltic Forestry* 23: 82–88.

Bartsch, N., B. von Lüpke, and E. Röhrig. 2020. *Waldbau auf ökologischer Grundlage*. 8th ed. UTB GmbH. https://www.utb-studi-e-book.de/waldbau-auf-oekologischer-grundlage.html.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software* 67: 1–48. https://doi.org/10.18637/jss.v067.i01.

Blenis, P. V., R. F. Patton, and R. N. Spear. 1984. "Effect of Temperature on the Ability of *Gremmeniella abietina* to Survive and to Colonize Host Tissue." *European Journal of Forest Pathology* 14: 153–164. https://doi.org/10.1111/j.1439-0329.1984.tb00939.x.

Burns, P., V. Timmermann, and J. M. Yearsley. 2022. "Meteorological Factors Associated With the Timing and Abundance of *Hymenoscyphus fraxineus* Spore Release." *International Journal of Biometeorology* 66: 493–506. https://doi.org/10.1007/s00484-021-02211-z.

Cameron, A. C., and P. K. Trivedi. 2003. "Essentials of Count Data Regression." In *A Companion to Theoretical Econometrics*, edited by B. H. Baltagi, 331–348. Wiley. https://doi.org/10.1002/9780470996249.ch16.

Carrari, E., P. Capretti, A. Santini, and N. Luchi. 2015. "*Hymenoscyphus fraxineus* Mycelial Growth on Media Containing Leaf Extracts of Different Oleaceae Sieber." *Forest Pathology* 45: 540–543. https://doi.org/10.1111/efp.12238.

Chandelier, A., F. Gerarts, G. San Martin, M. Herman, and L. Delahaye. 2016. "Temporal Evolution of Collar Lesions Associated With Ash Dieback and the Occurrence of *Armillaria* in Belgian Forests." *Forest Pathology* 46: 289–297. https://doi.org/10.1111/efp.12258.

Chandelier, A., M. Helson, M. Dvořák, and F. Gischer. 2014. "Detection and Quantification of Airborne Inoculum of *Hymenoscyphus pseudoalbidus* Using Real-Time PCR Assays." *Plant Pathology* 63: 1296–1305. https://doi.org/10.1111/ppa.12218.

Chomel, M., M. Guittonny-Larchevêque, C. Fernandez, et al. 2016. "Plant Secondary Metabolites: A Key Driver of Litter Decomposition and Soil Nutrient Cycling." *Journal of Ecology* 104: 1527–1541. https://doi.org/10.1111/1365-2745.12644.

Cleary, M. R., G. Daniel, and J. Stenlid. 2013. "Light and Scanning Electron Microscopy Studies of the Early Infection Stages of *Hymenoscyphus pseudoalbidus* on *Fraxinus excelsior*." *Plant Pathology* 62: 1294–1301. https://doi.org/10.1111/ppa.12048.

Combes, M. J. 2022. *The Ecology and Pathology of Ash Dieback Disease*. PhD Thesis, 182. Cardiff University. https://orca.cardiff.ac.uk/id/eprint/151199/2/2022combesphd.pdf.

Cracknell, D. J., G. F. Peterken, A. Pommerening, P. J. Lawrence, and J. R. Healey. 2023. "Neighbours Matter and the Weak Succumb: Ash Dieback Infection Is More Severe in Ash Trees With Fewer Conspecific Neighbours and Lower Prior Growth Rate." *Journal of Ecology* 111: 2118–2133. https://doi.org/10.1111/1365-2745.14191.

Cross, H., J. H. Sønstebø, N. E. Nagy, et al. 2017. "Fungal Diversity and Seasonal Succession in Ash Leaves Infected by the Invasive Ascomycete *Hymenoscyphus fraxineus*." *New Phytologist* 213: 1405–1417. https://doi.org/10.1111/nph.14204.

Dietrich, M. 2016. *The Impact of Ash Dieback on Ash Regeneration in the Forest Reserve Dalby Söderskog. Mathesis*, 46. Swedish University of Agricultural Sciences. http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-5146.

Dvořák, M., G. Rotkova, and L. Botella. 2016. "Detection of Airborne Inoculum of *Hymenoscyphus fraxineus* and *H. albidus* During Seasonal Fluctuations Associated With Absence of Apothecia." *Forests* 7: 1–13. https://doi.org/10.3390/f7010001.

Dvořák, M., P. Štoidl, and M. Rost. 2023. "Vertical Spread of *Hymenoscyphus fraxineus* Propagules." *NeoBiota* 84: 231–246. https://doi.org/10.3897/neobiota.84.90981.

Ehlers, B. K., M. P. Berg, M. Staudt, et al. 2020. "Plant Secondary Compounds in Soil and Their Role in Belowground Species Interactions." *Trends in Ecology & Evolution* 35: 716–730. https://doi.org/10.1016/j.tree.2020.04.001.

Enderle, R. 2018. "Warum ist die Bekämpfung des Eschentriebsterbens so schwierig?" *AFZ - Der Wald* 73: 22–24.

Enderle, R., J. Bußkamp, and B. Metzler. 2017. "Growth Performance of Dense Natural Regeneration of *Fraxinus excelsior* Under Attack of the Ash Dieback Agent *Hymenoscyphus fraxineus*." *Baltic Forestry* 23: 218–228.

Enderle, R., B. Metzler, U. Riemer, and G. Kändler. 2018. "Ash Dieback on Sample Points of the National Forest Inventory in South-Western Germany." *Forests* 9: 25. https://doi.org/10.3390/f9010025.

Fahrmeir, L., T. Kneib, S. Lang, and B. D. Marx. 2021. *Regression: Models, Methods and Applications*. 2nd ed, 744. Springer. https://doi.org/10.1007/978-3-662-63882-8.

Franke, J., U. Schreiber, and D. Rumpf. 2023. "Klimatologische Einordnungen in Sachsen." In *Wetter Trifft auf Klima—Jahresrückblick 2022*, 1–59. Landesamt für Umwelt, Landwirtschaft und Geologie Sachsen. https://www.klima.sachsen.de/download/klima/Jahresruecckblick2022_Fachbeitrag_2023.pdf.

Giongo, S., C. M. Oliveira Longa, E. Dal Maso, and L. Montecchio. 2017. "Evaluating the Impact of *Hymenoscyphus fraxineus* in Trentino (Alps, Northern Italy): First Investigations." *iForest - Biogeosciences and Forestry* 10: 871–878. https://doi.org/10.3832/ifor2486-010.

Grosdidier, M., J. Aguayo, B. Marçais, and R. Ioos. 2017. "Detection of Plant Pathogens Using Real-Time PCR: How Reliable Are Late C_t Values." *Plant Pathology* 66: 359–367. https://doi.org/10.1111/ppa.12591.

Grosdidier, M., R. Ioos, C. Husson, O. Cael, T. Scordia, and B. Marçais. 2018. "Tracking the Invasion: Dispersal of *Hymenoscyphus fraxineus* Airborne Inoculum at Different Scales." *FEMS Microbiology Ecology* 94: fiy049. https://doi.org/10.1093/femsec/fiy049.

Grosdidier, M., T. Scordia, R. Ioos, and B. Marçais. 2020. "Landscape Epidemiology of Ash Dieback Thrall." *Journal of Ecology* 108: 1789–1799. https://doi.org/10.1111/1365-2745.13383.

Gross, A., and O. Holdenrieder. 2013. "On the Longevity of *Hymenoscyphus pseudoalbidus* in Petioles of *Fraxinus excelsior* Hantula." *Forest Pathology* 43: 168–170. https://doi.org/10.1111/efp.12022.

Gross, A., O. Holdenrieder, M. Pautasso, V. Queloz, and T. N. Sieber. 2014. "*Hymenoscyphus pseudoalbidus*, the Causal Agent of European Ash Dieback." *Molecular Plant Pathology* 15: 5–21. https://doi.org/10.1111/mpp.12073.

Grote, R. 2002. "Foliage and Branch Biomass Estimation of Coniferous and Deciduous Tree Species." *Silva Fennica* 36: 779–788. https://doi.org/10.14214/sf.520.

Hantsch, L., U. Braun, M. Scherer-Lorenzen, and H. Bruelheide. 2013. "Species Richness and Species Identity Effects on Occurrence of Foliar

Fungal Pathogens in a Tree Diversity Experiment." *Ecosphere* 4: art81. https://doi.org/10.1890/ES13-00103.1.

Harper, J. L. 1994. *Population Biology of Plants*. 9 imprint ed, 892. Acad. Press.

Harrison, A. 1971. "The Inhibitory Effect of Oak Leaf Litter Tannins on the Growth of Fungi, in Relation to Litter Decomposition." *Soil Biology and Biochemistry* 3: 167–172. https://doi.org/10.1016/0038-0717(71)90011-3.

Hauptman, T., F. A. Celar, and M. de Groot. 2015. "Application of Fungicides and Urea for Control of Ash Dieback." *iForest - Biogeosciences and Forestry* 8: 165–171. https://doi.org/10.3832/ifor1272-008.

Hauptman, T., B. Piškur, M. de Groot, N. Ogris, M. Ferlan, and D. Jurc. 2013. "Temperature Effect on *Chalara fraxinea*: Heat Treatment of Saplings as a Possible Disease Control Method Holdenrieder." *Forest Pathology* 43: 360–370. https://doi.org/10.1111/efp.12038.

Havrdová, L., D. Zahradník, D. Romportl, V. Pešková, and K. Černý. 2017. "Environmental and Silvicultural Characteristics Influencing the Extent of Ash Dieback in Forest Stands." *Baltic Forestry* 23: 168–182.

Hietala, A. M., I. Børja, H. Solheim, N. E. Nagy, and V. Timmermann. 2018. "Propagule Pressure Build-Up by the Invasive *Hymenoscyphus fraxineus* Following Its Introduction to an Ash Forest Inhabited by the Native *Hymenoscyphus albidus*." *Frontiers in Plant Science* 9: 1087. https://doi.org/10.3389/fpls.2018.01087.

Hietala, A. M., V. Timmermann, I. Børja, and H. Solheim. 2013. "The Invasive Ash Dieback Pathogen *Hymenoscyphus pseudoalbidus* Exerts Maximal Infection Pressure Prior to the Onset of Host Leaf Senescence." *Fungal Ecology* 6: 302–308. https://doi.org/10.1016/j.funeco.2013.03.008.

Husson, C., O. Caël, J. P. Grandjean, L. M. Nageleisen, and B. Marçais. 2012. "Occurrence of *Hymenoscyphus pseudoalbidus* on Infected Ash Logs." *Plant Pathology* 61: 889–895. https://doi.org/10.1111/j.1365-3059.2011.02578.x.

Ioos, R., T. Kowalski, C. Husson, and O. Holdenrieder. 2009. "Rapid In Planta Detection of *Chalara fraxinea* by a Real-Time PCR Assay Using a Dual-Labelled Probe." *European Journal of Plant Pathology* 125: 329–335. https://doi.org/10.1007/s10658-009-9471-x.

Jönsson, M. T., and G. Thor. 2012. "Estimating Coextinction Risks From Epidemic Tree Death: Affiliate Lichen Communities Among Diseased Host Tree Populations of *Fraxinus excelsior* Roberts." *PLoS One* 7: 1–10. https://doi.org/10.1371/journal.pone.0045701.

Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. "Effects of Species Diversity on Disease Risk." *Ecology Letters* 9: 485–498. https://doi.org/10.1111/j.1461-0248.2006.00885.x.

Kirisits, T. 2015. "Ascocarp Formation of *Hymenoscyphus fraxineus* on Several-Year-Old Pseudosclerotial Leaf Rachises of *Fraxinus excelsior* Woodward." *Forest Pathology* 45: 254–257. https://doi.org/10.1111/efp.12183.

Kirisits, T., and C. Freinschlag. 2012. "Ash Dieback Caused by *Hymenoscyphus pseudoalbidus* in a Seed Plantation of *Fraxinus excelsior* in Austria." *Journal of Agricultural Extension and Rural Development* 4: 184–191. https://doi.org/10.5897/JAERD12.046.

Kowalski, T. 2006. "*Chalara fraxinea* sp. nov. Associated With Dieback of Ash (*Fraxinus excelsior*) in Poland." *Forest Pathology* 36: 264–270. https://doi.org/10.1111/j.1439-0329.2006.00453.x.

Kowalski, T., and C. Bartnik. 2010. "Morphologial Variation in Colonies of *Chalara fraxinea* Isolated From Ash (*Fraxinus excelsior* L.) Stems With Symptoms of Dieback and Effects of Temperature on Colony Growth and Structure." *Acta Agrobotanica* 63: 99–106. https://doi.org/10.5586/aa.2010.012.

Langer, G. J., S. Fuchs, J. Osewold, et al. 2022. "FraxForFuture—Research on European Ash Dieback in Germany." *Journal of Plant Diseases and Protection* 129: 1285–1295. https://doi.org/10.1007/s41348-022-00670-z.

Marçais, B., C. Husson, L. Godart, and O. Caël. 2016. "Influence of Site and Stand Factors on *Hymenoscyphus fraxineus*-Induced Basal Lesions." *Plant Pathology* 65: 1452–1461. https://doi.org/10.1111/ppa.12542.

Marçais, B., C. Kosawang, S. Laubray, E. Kjær, and T. Kirisits. 2022. "Ash Dieback." In *Forest Microbiology*, 215–237. Elsevier. https://doi.org/10.1016/B978-0-323-85042-1.00022-7.

Mayer, A., and H. D. Lenz. 2013. "Infektionsversuche an Eschen." *LWF Aktuell* 97: 39–41.

McCracken, A. R., G. C. Douglas, C. Ryan, M. Destefanis, and L. R. Cooke. 2017. "Ash Dieback on the Island of Ireland. 15."

Meshkova, V. L., and V. L. Borysova. 2020. "Incidence of Ash Dieback in the Left-Bank Forest-Steppe Depending on Stand Characteristics." *Forestry and Forest Melioration* 136: 157–164. https://doi.org/10.33220/1026-3365.136.2020.157.

Mitchell, R. J., J. K. Beaton, P. E. Bellamy, et al. 2014. "Ash Dieback in the UK: A Review of the Ecological and Conservation Implications and Potential Management Options." *Biological Conservation* 175: 95–109. https://doi.org/10.1016/j.biocon.2014.04.019.

NW-FVA. 2016. *Eschentriebsterben*. Vol. 4. Nordwestdeutsche Forstliche Versuchsanstalt (NW-FVA), Abteilung Waldschutz. https://www.nw-fva.de/fileadmin/user_upload/Abteilung/Waldschutz/Praxis-Informationen/Praxis-Informationen_Eschentiebsterben_update_15.08.2016_NW-FVA.pdf.

O'Brien, T., and M. McDonagh. 2023. *Ash Dieback Continues to Devastate Irish Tree Population*. Irish Times. https://www.irishtimes.com/ireland/2023/08/21/ash-dieback-continues-to-devastate-irish-tree-population/.

Peters, S., S. Fuchs, S. Bien, J. Bußkamp, G. J. Langer, and E. J. Langer. 2023. "Fungi Associated With Stem Collar Necroses of *Fraxinus excelsior* Affected by Ash Dieback." *Mycological Progress* 22: 52. https://doi.org/10.1007/s11557-023-01897-2.

Prescott, C. E., and S. J. Grayston. 2013. "Tree Species Influence on Microbial Communities in Litter and Soil: Current Knowledge and Research Needs." *Forest Ecology and Management* 309: 19–27. https://doi.org/10.1016/j.foreco.2013.02.034.

Pušpure, I., R. Matisons, M. Laiviņš, T. Gaitnieks, and J. Jansons. 2017. "Natural Regeneration of Common Ash in Young Stands in Latvia." *Baltic Forestry* 23: 209–217.

Salinas Ruíz, J., O. A. Montesinos López, G. Hernández Ramírez, and J. L. Crossa-Hiriart. 2023. *Generalized Linear Mixed Models With Applications in Agriculture and Biology*. Springer.

Schumacher, J. 2011. "The General Situation Regarding Ash Dieback in Germany and Investigations Concerning the Invasion and Distribution Strategies of *Chalara fraxinea* in Woody Tissue." *EPPO Bulletin* 41: 7–10. https://doi.org/10.1111/j.1365-2338.2010.02427.x.

Schumacher, J., R. Kehr, and S. Leonhard. 2009. "Mycological and Histological Investigations of *Fraxinus excelsior* Nursery Saplings Naturally Infected by *Chalara fraxinea*." *Forest Pathology* 40: 419–429. https://doi.org/10.1111/j.1439-0329.2009.00615.x.

Schweigkofler, W., K. O'Donnell, and M. Garbelotto. 2004. "Detection and Quantification of Airborne Conidia of *Fusarium circinatum*, the Causal Agent of Pine Pitch Canker, From Two California Sites by Using a Real-Time PCR Approach Combined With a Simple Spore Trapping Method." *Applied and Environmental Microbiology* 70: 3512–3520. https://doi.org/10.1128/AEM.70.6.3512-3520.2004.

Sievers, F., and D. G. Higgins. 2014. "Clustal Omega." *Current Protocols in Bioinformatics* 48: 3.13.1-3.13.16. https://doi.org/10.1002/0471250953.bi0313s48.

Skovsgaard, J. P., G. J. Wilhelm, I. M. Thomsen, et al. 2017. "Silvicultural Strategies for *Fraxinus excelsior* in Response to Dieback Caused by *Hymenoscyphus fraxineus*." *Forestry: An International Journal of Forest Research* 90: 455–472. https://doi.org/10.1093/forestry/cpx012.

Steinhart, F., A. Westhauser, K. Mausolf, et al. 2024. *Zukunft der Esche—Empfehlungen zum forstbetrieblichen Umgang mit dem Eschentriebsterben*, 72. Fachagentur Nachwachsende Rohstoffe e.V. https://mediathek.fnr.de/zukunft-der-esche.html.

Timmermann, V., I. Børja, A. M. Hietala, T. Kirisits, and H. Solheim. 2011. "Ash Dieback: Pathogen Spread and Diurnal Patterns of Ascospore Dispersal, With Special Emphasis on Norway." *EPPO Bulletin* 41: 14–20. https://doi.org/10.1111/j.1365-2338.2010.02429.x.

Timmermann, V., N. E. Nagy, A. M. Hietala, I. Børja, and H. Solheim. 2017. "Progression of Ash Dieback in Norway Related to Tree Age, Disease History and Regional Aspects." *Baltic Forestry* 23: 150–158.

Tooley, P. W., M. Browning, and R. M. Leighty. 2013. "Inoculum Density Relationships for Infection of Some Eastern US Forest Species by *Phytophthora ramorum*." *Journal of Phytopathology* 161: 595–603. https://doi.org/10.1111/jph.12107.

Vasaitis, R., and R. Enderle, eds. 2017. *Dieback of European Ash (Fraxinus spp.)—Consequences and Guidelines for Sustainable Management*, 322. SLU Service/Repro. https://www.cost.eu/publication/european-ash-consequences-and-guidelines-for-sustainable-management/.

Volke, V., S. Knapp, and A. Roloff. 2019. "Survey of *Hymenoscyphus fraxineus* in a Central European Urban Area and Exploration of Its Possible Environmental Drivers." *Urban Forestry & Urban Greening* 40: 165–173. https://doi.org/10.1016/j.ufug.2018.05.013.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Regression results for *H. fraxineus* DNA from filter traps across the different experimental variants. The table presents the estimated value for each fixed effect with standard error in parentheses below. **Table S2:** Regression Results Test for (1) ill_alive (infected, but living shoots), (2) ill_succumbed (shoots that have contracted and succumbed to ETS) and (3) nekro_sum (number of stem necroses per plant). The table presents the estimated value for each fixed effect with standard error in parentheses below.