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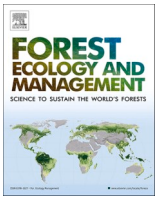

Tree vitality predicts plant-pathogenic fungal communities in beech forest canopies

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ABSTRACT

Forest canopies host diverse fungal communities that are crucial for tree vitality—defined as the physiological and structural traits influencing growth and resilience—and, consequently, for ecosystem functions. The canopy mycobiome has been shown to be closely associated with its tree hosts, especially in the case of pathogenic taxa. To better understand and predict how the canopy mycobiome will respond to changing environmental conditions, we used tree vitality-related variables to predict patterns in the beech canopy fungal plant-pathogens in two temperate forests— Bavarian Forest National Park, Germany and the Veluwe forest area, the Netherlands. Canopy water content, chlorophyll content, and crown diameter emerged as robust predictors of the canopy fungal plant-pathogen communities. We showed that these tree vitality-related variables predicted the unweighted relative abundance of plant-pathogenic fungi in the total fungal communities and the diversity of the fungal plant pathogen subgroup, but not the weighted relative abundance of plant-pathogenic fungi in the total fungal communities. Our model offers a powerful tool for monitoring this previously neglected biome in temperate beech forests in Europe.

1. Introduction

Tree vitality, often conceptualized as the capacity of trees to live, grow, and thrive under varying conditions, is influenced by a multitude of environmental and biotic stressors (Dobbertin, 2005; Cherubini, Battipaglia, and Innes, 2021). In temperate beech forests of Central Europe, factors like a warmer and drier climate (Einzmann et al., 2021; Martinez del Castillo et al., 2022), increased nitrogen input (Jantsch et al., 2013), and nutrient depletion in the soil (Pietsch and Hasenauer, 2002; Rennenberg et al., 2004; de Jong et al., 2022), can diminish a tree's physiological resilience, leading to symptoms like reduced canopy water content, defoliation, and crown dieback. These stressors compromise tree health, making them more vulnerable to pathogens and other environmental pressures. Declines in tree vitality are often associated with shifts in the microbiomes that, together with the tree, form the tree-microbes holobiont (Vandenkoornhuyse et al., 2015; Sanchez-Cañizares et al., 2017; Corinne et al., 2021). Tree-associated fungal communities are a diverse group of fungi that are critical in ecosystem functions, including mediating plant disease dynamics and nutrients cycling (Sun et al., 2021; Pajares-Murgó et al. 2024). The phyllosphere— the above-ground parts of plants (Leveau, 2019)— is an important habitat for these fungi. Recent studies have demonstrated that

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in response to environmental stressors, the plant-beneficial microbiota in the phyllosphere tend to decrease, while there is a notable increase in plant pathogen populations (Zhu et al., 2022; Perreault and Laforest-Lapointe, 2022).

Fungal plant pathogens are integral components of forest ecosystems. They can proliferate in response to tree stress, exploiting weakened defenses (Jactel et al., 2012; Rukh et al., 2023), or act as primary stressors themselves, directly impairing tree vitality (Langer and Busskamp 2021). They infect weakened trees, contributing to increased mortality (Hawkins and Henkel, 2011; Long et al., 2021; Jankowiak et al., 2022; Yin et al., 2023). This process also opens gaps in the forest, allowing for regeneration and succession, which supports forest diversity (Xiong, Yang, and Ni, 2023). Hence, fungal pathogens are an integral part of the tree-microbe holobiont and the forest ecosystem. Despite its importance, research into the forest canopy fungal plant pathogens has received less attention compared to their counterparts in the soil and rhizosphere.

Understanding the abundance and distribution of tree pathogens in the forest canopy is essential for effective forest management and conservation efforts. Traditional methods of pathogen detection, often based on visible symptoms or culturing techniques (Stewart, Kim, and Klopfenstein, 2018; Naderpour, 2020), have limitations in scope and sensitivity. In contrast, environmental DNA (eDNA) based approaches have emerged as a powerful tool for pathogen detection (Bulman et al., 2018; Barnes et al., 2020), offering a more comprehensive and sensitive means of surveying forest pathogens. These once cost-prohibitive methods are now more accessible and are being proposed as the foundation for next-generation forest monitoring programs (Lopes et al., 2017; Othman et al., 2023). eDNA surveys provide detailed insights into the presence and diversity of fungi in forests, enabling more proactive and informed management strategies.

European beech (*Fagus sylvatica*) is one of the dominant tree species in temperate European forests (Muffler et al., 2021; Welle et al., 2022), and it is facing a suite of stressors. These stressors influence tree vitality, and in beech trees, evident through physiological and structural changes such as loss of leaf water potential, defoliation and crown dieback (Ewald, 2000; Brück-Dyckhoff, Petercord, and Schopf, 2019; Ognjenovic et al., 2020; Walthert et al., 2021; Langer and Busskamp 2023; Heidenreich, Höwler, and Seidel, 2024). Given the complexity of tree vitality and the multiple ways it can be assessed, certain canopy-related variables stand out as particularly relevant in temperate European beech forests. These variables capture physiological processes and structural traits that are closely tied to how trees respond to environmental stressors. For example, leaf water content can reflect trees' hydration status, which supports transpiration and photosynthesis (Herbst et al., 2002; Brodrick, Anderegg, and Asner, 2019; Grulke et al., 2020). Additionally, reduced canopy water content has been shown to be associated with an increased relative abundance of certain plant-pathogenic fungal families, such as *Teratosphaeriaceae* and *Taphrinaceae* (Pérez et al., 2009; Zabalgogeazcoa, Pedro, and Canals, 2015; Prihatini et al., 2015). Canopy chlorophyll content, which serves as an indicator of photosynthetic capacity, provides insight into foliage health and productivity (Kmet and Ditmarová 2001; Ditmarová and Kmet, 2002; Wortman et al., 2012; West et al., 2022). Canopy nitrogen content is another vital metric, as nitrogen is a critical component of chlorophyll and proteins, directly influencing photosynthesis and overall metabolic activity (Witter et al., 2005; Wortman et al., 2012; Gottardini et al., 2020; Teglia et al., 2022; Ognjenovic et al., 2023). Stand crown diameter, meanwhile, reflects canopy structure, with crown reduction sometimes signaling physiological stress or competition (Jaworski and Paluch, 2002; Witter et al., 2005; Rötzer, Grote, and Pretzsch, 2005; Jacobs, Rais, and Pretzsch, 2021; Klesse et al., 2022). Lastly, Leaf Area Index (LAI), a widely used proxy for canopy density, captures the efficiency of light interception and overall stand productivity (Samalens et al., 2012; Pollastrini et al., 2016; Zolles et al., 2021; Fabolude et al., 2023). These studies underscore the potential of using tree vitality-related variables, such as canopy water content, chlorophyll content, and crown characteristics, to predict plant pathogen presence and abundance in the canopy, enhancing our understanding of the tree-microbe holobionts' response under conditions in the Anthropocene. While these variables do not represent an exhaustive assessment of tree vitality, they are particularly suited to understanding the physiological and structural dynamics in the European beech forests studied here. By examining these variables—hereafter referred to as tree vitality indicators—we can gain insights into the relationship between tree vitality and fungal plant pathogen communities in the canopy.

This study aimed to establish a predictive model of the plant-pathogenic fungal communities in the beech canopies in two temperate European forests (Bavarian Forest National Park (BFNP), Germany and the Veluwe forest area, The Netherlands), using tree vitality indicators. We hypothesized that tree vitality indicators—canopy water content, stand crown diameter, canopy nitrogen content, canopy chlorophyll content, and Leaf Area Index (LAI) —are effective predictors of the relative abundance of fungal plant pathogens and their diversity in the beech canopies in both BFNP and Veluwe.

2. Methods

2.1. Site description and data collection

Two distinct temperate European forest ecosystems were sampled in this study— the Veluwe forest area in the Netherlands and Germany's Bavarian Forest National Park (Fig. 1). The Veluwe forest area comprises of different parks of which Het Nationale Park De Hoge Veluwe, Het Loo Royal Estate (in Dutch "Kroondomein Het Loo), and Nationaal Park Veluwezoom were sampled in this study. Featuring a diverse landscape of forests, heathlands, and sand dunes (Van Dijk, 2019), the Veluwe forest area encompasses approximately 900 km^2. Main tree species in the forested area include European beech (*Fagus sylvatica*), Scots pine (*Pinus sylvestris*), and English oak (*Quercus robur*) (Xie et al., 2024). Meanwhile, the mountainous Bavarian Forest National Park (BFNP), located along the border between Germany and the Czech Republic, forms part of the Bohemian Forest Ecosystem, the largest contiguous forested area in Central Europe, covering around 4000 km^2 when combined with the neighboring Sumava National Park and other protected areas (Heurich et al., 2010). BFNP is predominantly forested, with European beech being one of the dominant tree species (van der Knaap et al., 2020). These two forest areas also have different management strategies. Parks in the Veluwe forest area have a variety of management goals such as biomass production, recreation, and nature conservation (Kramer, Bruinderink, and Prins, 2006; Hein, 2011). BFNP, on the other hand, has implemented a non-intervention management strategy for large parts of the park to restore natural forest dynamics since 1983 (van der Knaap et al., 2020) and hosts intensive forestry and ecology research (Latifi et al., 2021). These two distinct forest woodlands, with their varying climatic and geographical characteristics, served as two case studies to examine how tree vitality indicators predict patterns in canopy fungal plant-pathogen communities.

We sampled 25 beech stands in BFNP and 21 beech stands in Veluwe in May-July, 2020 and 2021 (Supplementary Table 1) during the leaf growing season. The sampling design employed a stratified random approach, considering elevation gradients and forest stand age, with adjustments made for purposive sampling for areas where challenging terrain as well as road access limited accessibility. Each stand measured 30 m x 30 m and had a canopy dominated by beech trees, with at least 75 % canopy coverage. The central point of each stand was located using a Differential Global Positioning System Leica GPS 1200 (Leica Geosystems AG, Heerbrugg, Switzerland). In each stand, two to three beech trees were selected, representing the plot. The selection was based on visual assessments of tree characteristics, including diameter at breast height (DBH), canopy radius, and spatial distribution within the plot. This selection aimed to capture the natural variability in tree and canopy

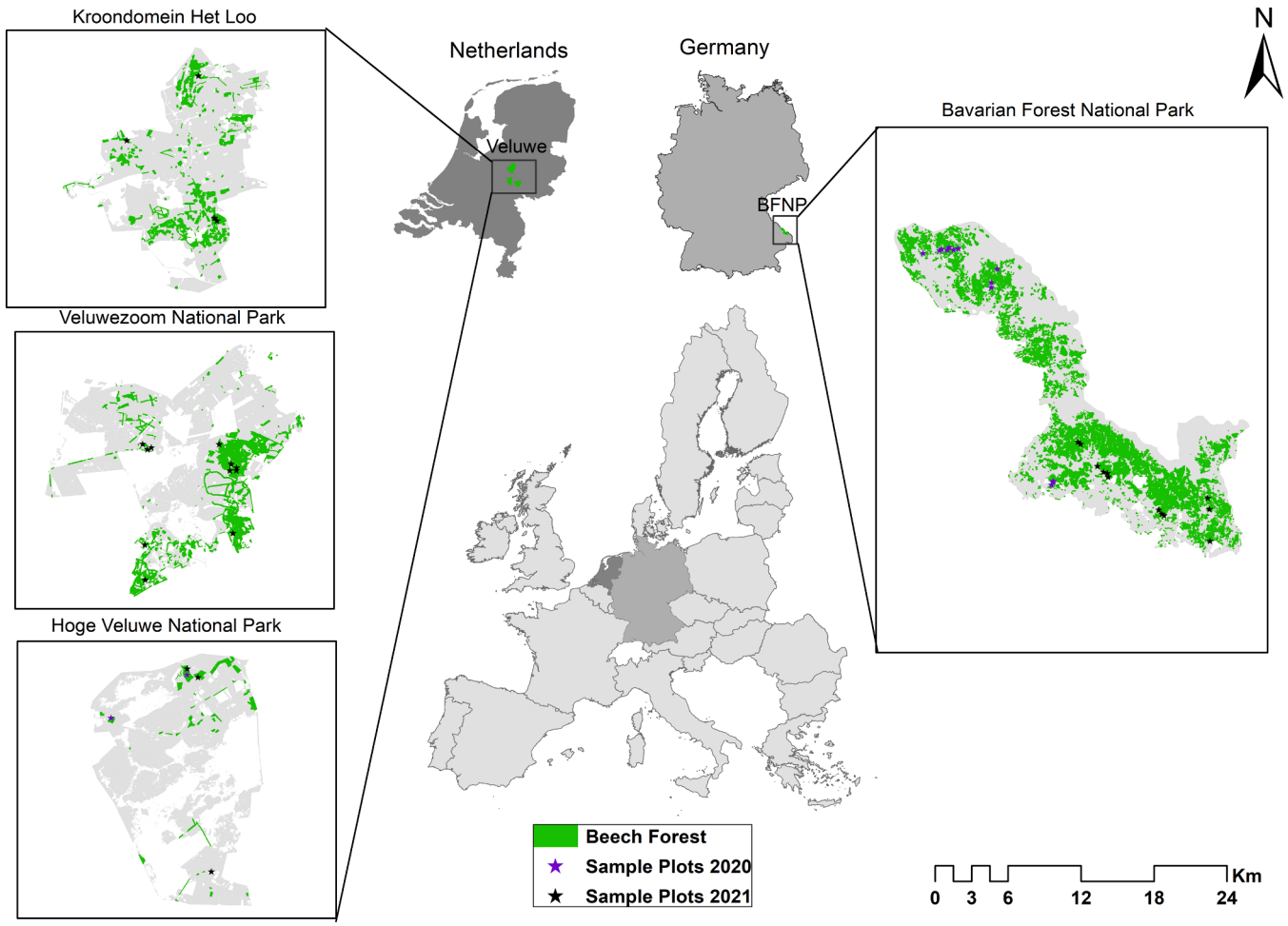

Fig. 1. Maps of study sites and sampling locations.

attributes within the stand while avoiding selection bias toward any specific tree condition. Leaf samples were obtained from the upper canopy using a crossbow and sling shot (Duan et al., 2024). A composite leaf sample was collected from each tree, from which leaves were randomly selected for DNA extraction and tree vitality measurements. Leaves for DNA extraction were collected and stored aseptically to prevent contamination. Leaves for chlorophyll measurements were protected from light. All samples were stored in a cooler during field collection and later frozen for subsequent analyses. Water content, nitrogen content, and chlorophyll content of the forest canopies were measured for each tree, also as described previously (Duan et al., 2024). Briefly, this includes measuring water content through oven-drying, measuring nitrogen content using a CHN analyzer, and measuring chlorophyll content using an UV–VIS Spectrophotometer. Leaf level measurements were then averaged between the sampled trees per stand and upscaled to the canopy level by multiplying the averaged values by the stand's LAI (Gara et al., 2019). The stand crown diameter was estimated as the average of the crown diameters of the representative beech trees sampled in each stand, with individual crown diameter visually estimated in the field (Bechtold, Mielke, and Zarnoch, 2002). Leaf area index (LAI) was obtained for each forest stand using a plant canopy analyzer (LI-COR LAI-2000) (Darvishzadeh et al., 2008).

2.2. DNA extraction and sequencing

For each tree, total genomic DNA was extracted from 0.1 g of composite leaf material as outlined in (Duan et al., 2024). The DNA extracts with concentrations higher than 5 ng/ml were normalized to 5 ng/ml. Fungal DNA was amplified using the ITS86/ITS4-ngs primer set, following the protocols by (Tedersoo et al., 2014) and (Turenne et al., 1999). Detailed polymerase chain reaction (PCR) procedures are provided in the supplementary materials (Supplementary Tables 2 and 3). DNA amplification and Illumina NovaSeq sequencing methodology are described in (Duan et al., 2024).

2.3. Bioinformatics

Bioinformatic analyses were performed using QIIME 2™ (Bolyen et al., 2019), while data analyses were performed in R version 4.2.3 (https://www.R-project.org/). Post-clustering curation was carried out using LULU (Froslev et al., 2017), and taxonomy was assigned utilizing the UNITE database (Nilsson et al., 2019). Details on the number of reads retained at each bioinformatic step are provided in Supplementary Table 4. Fungal ASVs were further processed by blank correction, filtered to retain only fungal reads, and corrected for tag-switching, following the methodology described by (Siegenthaler et al., 2024). Low-frequency noise, defined as ASVs with ≤ 5 reads, was excluded as per (Polling et al., 2022). The read depth was rarefied to 37,646 reads per sample, based on the sample with the lowest read depth (Duan et al., 2024), and the average of 999 iterations was used. To generate a stand canopy mycobiome profile, the number of reads per ASV was averaged across the two to three samples collected within each stand.

2.4. Functional prediction of fungal communities and data analysis

FungalTraits database (version 1.2) (Põlme et al., 2020; Tanunchai

et al., 2023), as implemented in the microeco R package (Liu et al., 2021) was used for the functional annotation of the fungal communities. Each ASV was assigned a binary classification (true or false) to indicate whether it was classified as a plant leaf, seed, or fruit pathogen (hereafter referred to as plant pathogens). For each sample, the relative abundance of plant pathogens was assessed using two metrics: (1) the unweighted relative abundance of plant-pathogenic ASVs, calculated as the percentage of plant-pathogenic ASVs in the total number of fungal ASVs, reflecting the occurrence of fungal plant-pathogenic taxa; and (2) the weighted relative abundance of plant-pathogenic reads, calculated as the percentage of fungal reads assigned to plant-pathogenic ASVs, capturing the dominance of fungal plant pathogens within the community. Phylogenetic trees for phylogenetic diversity were created in QIIME 2™ using the FastTree algorithm. Faith's phylogenetic diversity was then calculated for the subset of taxa classified as plant-pathogens using phyloseq (McMurdie and Holmes, 2013).

To first determine the differences in community compositions of beech canopy plant pathogenic fungi between BFNP and Veluwe, PERMANOVA (Anderson and Walsh, 2013) as implemented in the vegan R package (Oksanen et al., 2022) and LEfSe (Segata et al., 2011) as implemented in the microeco R package (Liu et al., 2021) were used. Based on these analyses, predictive models were constructed separately for the two sites. Partial least squares regression (PLSR) was used to predict the relative abundances and diversity of plant-pathogenic fungal taxa based on five tree vitality indicators: canopy water content, stand crown diameter, canopy nitrogen content, canopy chlorophyll content, and Leaf Area Index (Abdullah et al., 2024). The model employed leave-one-out cross-validation, and the optimal number of principal components was determined by adding components until R^2_{cv} stabilized (i.e., an increase of less than 2 % when adding additional component). Variable Importance in Projection (VIP) scores (with a threshold of VIP ≥ 1 indicating importance) and PLSR regression coefficients were used to assess the importance and effects of predictor variables. One BFNP sample was removed as an outlier in canopy water content measurement, identified using the Z-score method ($Z \geq 3$). Similarly, two Veluwe samples were excluded: one due to being an outlier in canopy water content and the other in canopy nitrogen content.

3. Results

3.1. Fungal DNA sequencing and functional annotations

Across the canopy mycobiome profiles of 46 beech stands, 1702 ASVs and 1588,728 fungal reads remained after bioinformatic processing (Supplementary Table 4). Each stand's canopy mycobiome was represented by an average of 123 ± 36 ASVs (mean ± SD) and 37646 ± 6 reads (mean ± SD). These fungal ASVs belonged to four phyla, 25 classes, 79 orders, 183 families, and 434 genera (Supplementary Figure 1). Taxonomic assignment at the genus level was successful for 79.26 % of the ASVs which accounted for 69.80 % of the total reads. Relying on genus level functional annotations, 56.28 % of all ASVs and 69.78 % of all reads were assigned to at least one function using FungalTraits. Leaf, fruit, and seed pathogens accounted for 20.74 % of all ASVs and 65.54 % of all reads. The composition of this plant-pathogenic sub-community is shown in Fig. 2. In both BFNP and Veluwe, *Naevala*, *Ramularia*, *Taphrina,* and *Aureobasidium* formed the four dominant plant-pathogenic genera that colonized the beech canopies. However, significant differences existed between BFNP and Veluwe, as suggested by PERMANOVA results using Bray–Curtis distance of Hellinger-transformed reads ($p < 0.01$, $R^2 = 28$ %, $F(1, 59) = 23$, Fig. 3a) and differential abundance analysis using LEfSe (Fig. 3b). In BFNP, genus *Naevala* made up 62.3 % of the pathogenic fungal reads, while in Veluwe, the most abundant group was the genus *Taphrina* (34.8 % of reads, Fig. 3). Beech canopies in BFNP also harbored more *Erysiphe* species and less *Aureobasidium* species compared to Veluwe (Fig. 2).

3.2. Predicting the relative abundance and diversity of plant-pathogenic fungi using tree vitality indicators

We first used PLSR models to assess the five tree vitality indicators—canopy water content, stand crown diameter, canopy nitrogen content, canopy chlorophyll content, and Leaf Area Index—for predicting the weighted and unweighted relative abundances of plant-pathogenic fungal taxa in beech canopies. Predicting the unweighted relative abundance, calculated as the percentage of pathogenic ASVs in the total fungal community, yielded robust results (Fig. 4). However, the model failed to accurately predict the weighted relative abundance, calculated as the percentage of plant-pathogenic reads in the total fungal community (Supplementary Figure 2). Then, using the same suite of tree vitality indicators, we also moderately predicted Faith's phylogenetic diversity of the plant-pathogenic fungi sub-community in both BFNP and Veluwe (Fig. 5).

3.3. Key predictors of plant-pathogenic fungi

Canopy water content, canopy chlorophyll content, and crown

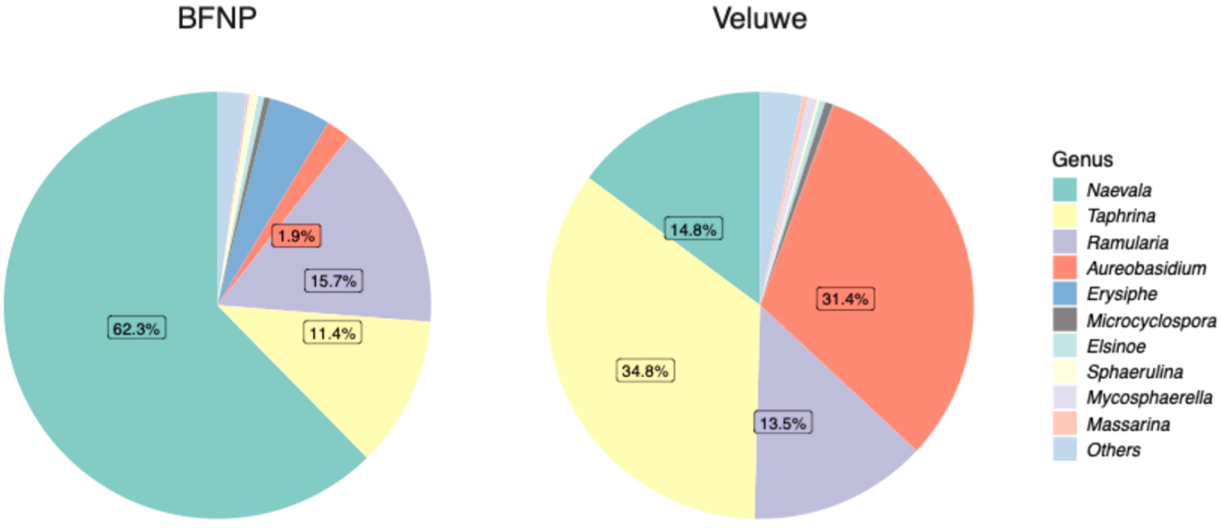

Fig. 2. Pie charts showing community composition of plant-pathogenic fungal genera in the beech forest canopies of Bavarian Forest National Park (BFNP) and the Veluwe forest area. To aid visualization, only the relative abundance percentages of the top four genera with the highest relative abundance are shown.

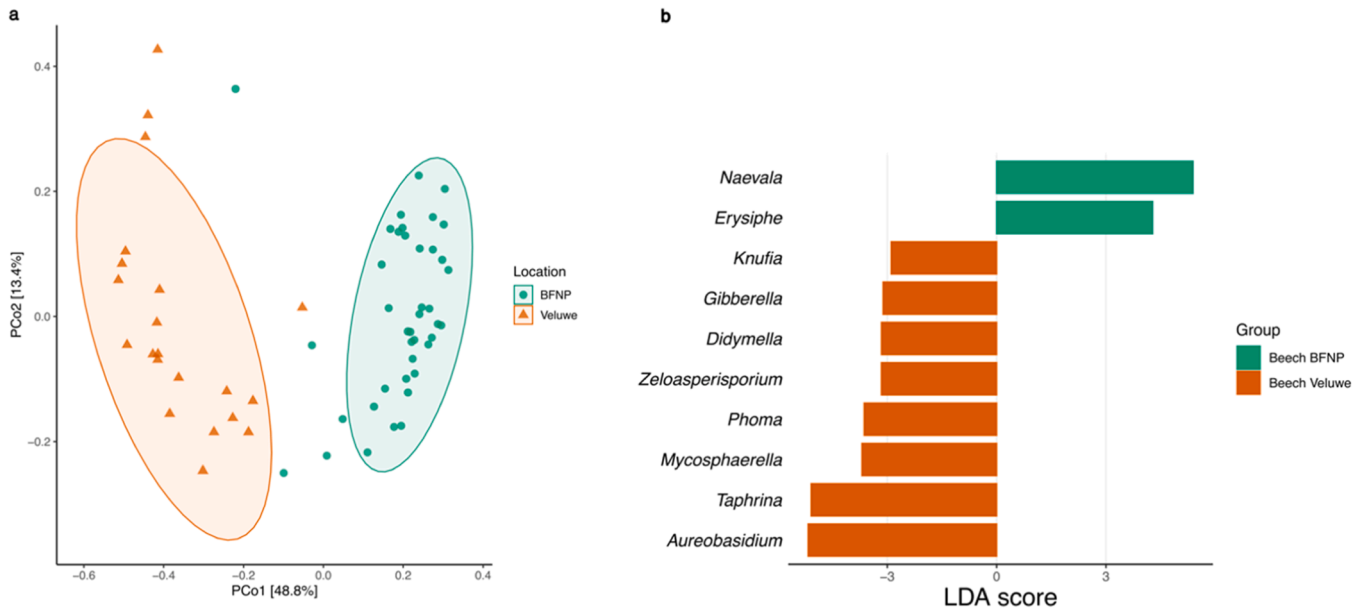

Fig. 3. PCoA plot showing variation in plant-pathogenic fungi community composition (Bray–Curtis distance of Hellinger-transformed reads) in Bavarian Forest National Park (BFNP) and Veluwe forest area (a) and LEfSe bar graph showing the differentially abundant genera in BFNP and Veluwe (b).

diameter emerged as key predictors (VIP $\geq$ 1) for canopy fungal plant pathogens in BFNP and Veluwe. Lower water content predicted 1) a higher unweighted relative abundance of plant-pathogenic ASVs in the overall fungal community in BFNP (Fig. 4), and 2) decreased diversity of plant-pathogenic taxa 6 In Veluwe, higher canopy chlorophyll level predicted a higher percentage of plant-pathogenic ASVs and a less phylogenetically diverse pathogen community (Figs. 4 and 5).

4. Discussion

4.1. Tree vitality indicators predicted patterns of fungal plant-pathogen community

Our study demonstrated that tree vitality-related variables can successfully predict patterns in the plant-pathogen fungal communities in temperate beech forest canopies, highlighting their critical role as a functional component of the canopy mycobiome. This finding underscores the holobiont theory (Vandenkoornhuyse et al., 2015) and the interconnection between forest conditions, tree health, and associated microbial communities. This robust interconnection, while supporting strong predictive relationships, reflect correlations and may involve bidirectional mechanisms. On one hand, tree vitality may influence fungal plant-pathogen communities by shaping microhabitats and resource availability, thereby modulating pathogen establishment and diversity (Beule et al., 2017; Busby et al., 2022). On the other hand, fungal pathogens could potentially impact measurable vitality variables by affecting water transport, photosynthetic capacity, or structural integrity, thus altering physiological responses (Oliva, Stenlid, and Martínez-Vilalta, 2014; Marçais and Desprez-Loustau, 2014). By shifting from descriptive observations to predictive modeling, our research represents a significant advancement in understanding these complex relationships. This predictive capacity offers valuable tools for improving the monitoring and management of forest ecosystems, especially in the face of changing environmental challenges.

We also found that the success of predicting the patterns of plant-pathogen communities varied among metrics of pathogenicity. Tree vitality indicators successfully predicted the unweighted relative abundance of pathogenic fungal ASVs in the total fungal community and their diversity but failed to predict their weighted relative abundance. This suggests that the mere presence of a diverse set of pathogens may be a more consistent indicator of tree health than their relative read abundance, which can be driven more by other factors or obscured by PCR bias (van der Loos Nijland, 2021; Stojan et al., 2023).

Tree vitality indicators can be closely associated with the unweighted relative abundance of pathogenic fungal ASVs and their diversity through several mechanisms. The occurrence of plant-pathogenic ASVs may reflect trees' ability to regulate fungal pathogens (Schuldt et al., 2017; Patkar and Naqvi, 2017; Tanunchai et al., 2022), and a high unweighted relative abundance of plant-pathogenic ASVs may indicate an imbalance in the plant's holobiont (Broberg et al., 2018; Ginnan et al., 2020) and a decline in tree vitality. The phylogenetic diversity of plant-pathogenic fungi, on the other hand, may serve as an indicator of both the level of competition among pathogens and the availability of niche space for pathogens in the canopy. It has been shown that trees with higher vitality can maintain a diverse pathogen community, fostering competition among species and preventing any single taxon from becoming dominant (Koskella et al., 2017). When a single taxon becomes dominant, it can be an indication of fungal infestation and is often accompanied by physiological symptoms indicative of reduced tree vitality, such as decreased canopy density and chlorophyll content (Rutten et al., 2020). By contrast, under stressed conditions, trees typically exhibit lower nutrient and energy content in the phyllosphere, providing a reduced niche space supporting fewer microbial taxa (Desprez-Loustau et al., 2006; Ghelardini et al., 2016), which can be reflected in the lower phylogenetic diversity of plant-pathogenic fungal community associated with reduced canopy water content and crown diameter. This illustrates a classic ecological scenario: the presence of a diverse group of taxa exploiting similar resources within the host plants (in this case, as plant pathogens) fosters competition that prevents any one taxon, or a few taxa from becoming dominant, thereby maintaining the health and vitality of the host plants.

In contrast, the weighted relative abundance of plant-pathogenic reads was not closely linked to tree vitality, likely due to the different ecological processes governing this metric. Biotic interactions, such as competition between pathogens, are not likely to eliminate the presence of a taxon, but can more strongly influence their relative abundance in terms of reads, making this metric more dynamic and less predictable (del Barrio-Duque et al., 2020). Additionally, variability in infestation progressions among different pathogens, where some may proliferate quickly while others more slowly (Belair et al., 2023; Huang et al.,

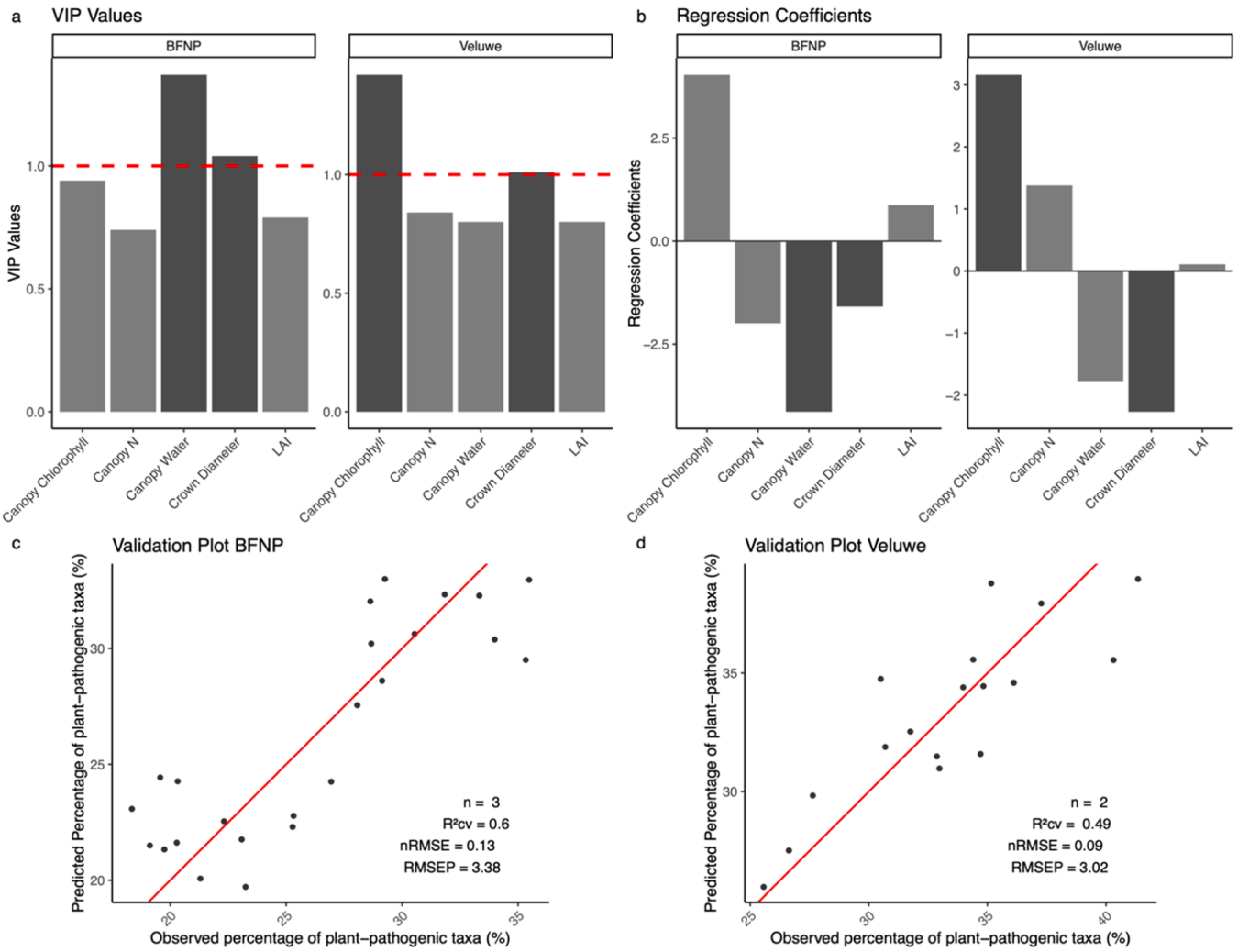

Fig. 4. (a) Variable Importance in Projection (VIP) values, (b) regression coefficients plot, and validation plots of PLSR predicted the unweighted relative abundance of plant-pathogenic fungal taxa (percentage of ASVs) in the total fungal communities in the beech forest canopies in (c) Bavarian Forest National Park (BFNP) and (d) the Veluwe forest area. Variables with VIP ≥ 1 are colored in darker grey. Number of components selected for the model is denoted as n.

2024), makes the total percentage of plant-pathogenic reads in the fungal community less predictable. The percentage of reads can also be more sensitive to short-term environmental fluctuations, such as changes in resource availability, temperature, or UV radiation, which can cause rapid shifts in the composition of the fungal community (Hawkes et al., 2011). The multitude of factors driving read number-based relative abundance, therefore, further complicates its predictability using tree vitality alone.

4.2. *Key tree vitality indicators: canopy water, chlorophyll, and crown diameter*

Canopy water content was the most important tree vitality indicator that predicted patterns in pathogen communities – it both negatively predicted the percentage of plant-pathogenic taxa and positively predicted the diversity of the pathogen subgroup. With canopy water content being a common indicator of forest drought stress (Desprez-Loustau et al., 2006; Paz-Kagan and Asner, 2017; Lyons et al., 2021), our finding highlights the inter-connectivity of forest drought stress, tree vitality, and fungal plant pathogens. A decrease in canopy water content can lead to an increase in percentages of plant pathogens in the beech forest canopies through a suite of mechanisms. For example, water content can influence fungal taxa colonization and survival by directly affecting humidity, temperature, and substrate availability (Jacobson, 1997; Adejumo and Orole, 2010; Petronaitis et al., 2022), which are crucial for fungal spore germination and growth. Under conditions of reduced water content, commonly associated with drought stress, the dynamics of microbial communities can shift, leading to an increase in pathogenic taxa as plant defenses weaken (Yeoh et al., 2021; Ghanbary et al., 2021; Gomez-Gallego et al., 2022). Similarly, an increase in canopy water content can potentially support a more diverse fungal communities, leading to the increase of plant-pathogen diversity in the beech forest canopies (Castañeda et al., 2018; Chen et al., 2021). It is also possible that fungal pathogens can influence tree and canopy physiology by disrupting water transport. For example, species of *Biscogniauxia*—known to cause strip canker disease in beech—can damage vascular tissues, leading to localized dieback and reduced canopy water content (Zamani et al., 2024). Similarly, *Erysiphe alphitoides* (a common powdery mildew pathogen on beech) may alter leaf physiology, reducing water-use efficiency and contributing to canopy-level water stress (Marçais and Desprez-Loustau, 2014). This inter-connectivity between leaf water content, tree vitality, and pathogen dynamics underscores the vulnerability of these forest ecosystems to the anticipated warmer and drier conditions (Martinez del Castillo et al., 2022), which are expected to exacerbate drought stress and, in turn, influence canopy fungal plant pathogen communities. Understanding these mechanisms is essential for developing forest management strategies that mitigate the potential negative impacts on tree health and forest biodiversity.

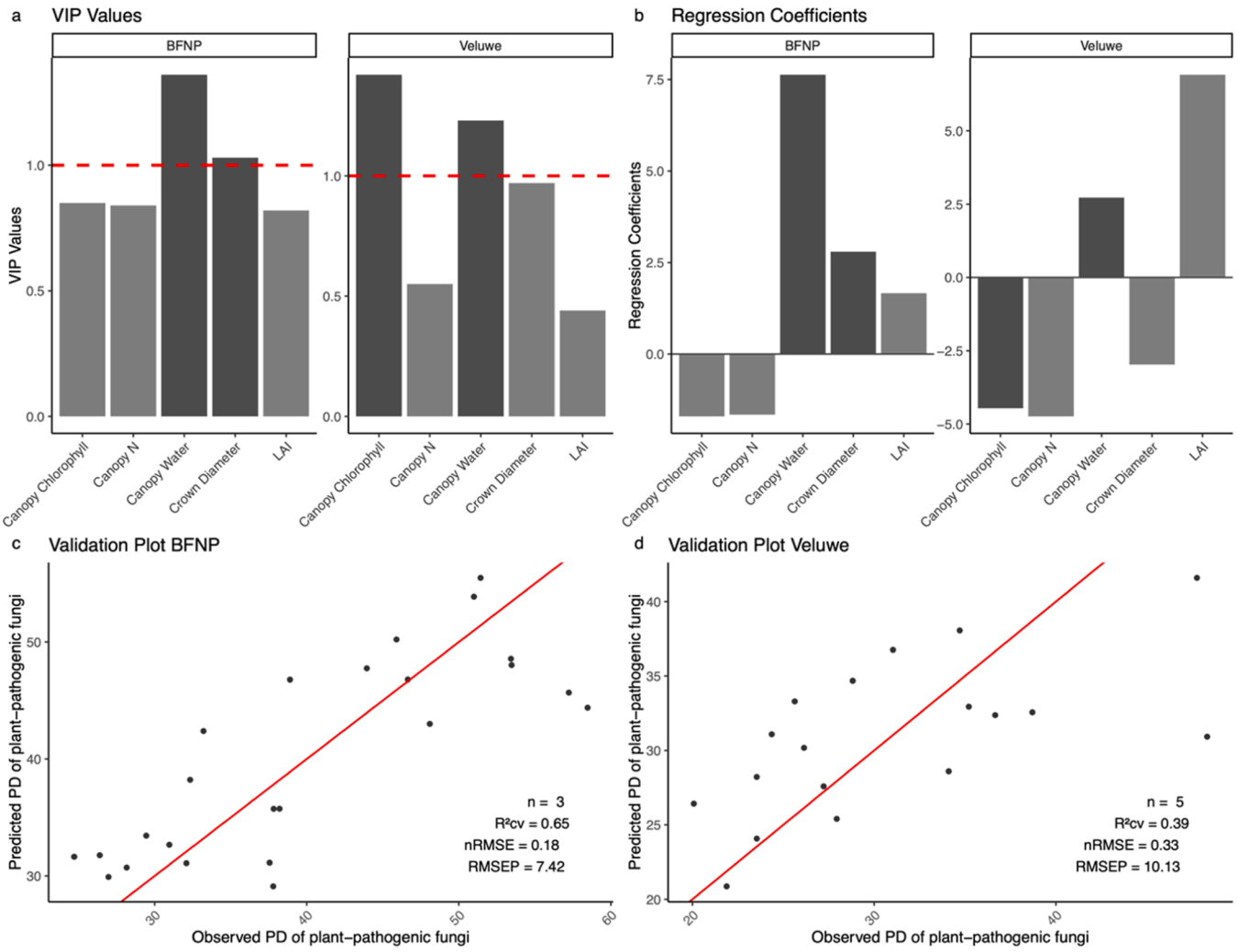

Fig. 5. (a) Variable Importance in Projection (VIP) values, (b) regression coefficients plot, and validation plots of PLSR predicted Faith's phylogenetic diversity (PD) of the plant-pathogenic fungal communities in the beech forest canopies in (c) Bavarian Forest National Park (BFNP) and (d) the Veluwe forest area. Variables with VIP ≥ 1 are colored in darker grey. Number of components selected for the model is denoted as n.

Smaller crown diameter also predicted higher percentages of plant-pathogenic taxa in the fungal communities in both sites. Crown diameter can be affected by factors such as growth conditions, past disturbances, and tree age (Fichtner et al., 2013). This potentially suggests that trees with a broader canopy have better-developed structural integrity and energy reserves, allowing them to allocate more resources to defense mechanisms (Chauvin et al., 2018), thereby keeping the relative abundance of pathogens low. In addition, crown size could reflect the competitive status of a tree within a stand, where larger crowns may indicate trees that have experienced less competition for light and resources. Conversely, smaller crowns might result from higher competition or environmental stress, potentially weakening the tree's ability to resist pathogenic colonization. Moreover, fungal pathogens such as *Biscogniauxia nummularia* can directly affect crown size by damaging vascular tissues and reducing water transport, which impairs crown vitality (Luchi et al., 2016). Similarly, the onset of beech decline has been associated with crown thinning and reduced growth, likely due to physiological stress induced by both biotic and abiotic factors (Woodcock et al., 1995).

Interestingly in Veluwe, a higher canopy chlorophyll content predicted a higher percentage of fungal taxa in the canopy to be made up by a less diverse group of plant pathogens. This finding is intriguing, as higher chlorophyll levels typically indicate healthier trees with more efficient nutrient uptake and photosynthesis (Sanchez-Cañizares et al., 2017). However, our results suggest that elevated leaf nutrient levels may not equate to more effective defenses against pathogens. In fact, these nutrient-rich conditions might create a more favorable environment for specific clades of plant pathogens, which can outcompete non-pathogenic fungal taxa. These pathogens, often fast-growing generalists (Veresoglou et al., 2013; Fatima and Senthil-Kumar, 2015), may exploit the enriched nutrient conditions, leading to their increased prevalence within the fungal community. This points to a potential trade-off between nutrient uptake and pathogen defense in forest trees, highlighting that higher vitality in terms of chlorophyll content does not necessarily correspond to a more resilient canopy microbial community against plant pathogens.

Our eDNA surveys recorded a diverse range of plant-pathogenic fungal taxa in both study sites. Many of these fungal genera are sparsely documented in the literature, exposing significant gaps in our knowledge. The identification of prevalent and unexpected fungal pathogens also enables more proactive forest management. For instance, we detected *Erysiphe*, a common fungal pathogen found on beech leaves (Burke et al., 2024). The comprehensive detections of both known and underexplored taxa highlights the utility of eDNA for expanding our understanding of fungal pathogens in forest ecosystems. It is emphasized that DNA metabarcoding allows for the analysis of patterns in relative abundances and diversity but cannot quantify absolute pathogen load (Lamb et al., 2019), which is more directly associated with visible

disease symptoms and reduced tree vitality (Zhang et al., 2018; Chen et al., 2021). As our sampling was conducted during the growing season (May–July), the observed fungal community composition represents a snapshot of this period. Seasonal variation in fungal communities is well-documented, and future studies could benefit from sampling across multiple seasons to capture these temporal dynamics and further elucidate their relationship with tree vitality. The success of these predictions based on annotated fungal community functions also demonstrated the usefulness of functional annotation tools like the recently developed FungalTraits database (Põlme et al., 2020) in extrapolating microbial community functions from amplicon sequencing results. Despite instances where these annotation tools may produce uncertain results (Tas et al., 2021), our study supported their utility. Our study opens possibilities for modeling more functional aspects of this microbiome in the future, using environmental and host-related variables. For example, future studies can model other phyllosphere aspects such as saprotrophic fungi, fungal plant or animal parasites. The list of available microbial functions can be further expanded using metagenomics. The ability to predict changes in trees' microbiome that serve important ecosystem functions (e.g., regulation of plant populations by pathogenicity, thereby influencing nutrient cycling and carbon sequestration) can be very valuable. Our study demonstrated that predictive models of microbial communities based on eDNA can be used for improved ecosystem monitoring in the forest canopies.

5. Conclusion

We predicted patterns of the forest canopy mycobiome using tree vitality indicators, with canopy water content, chlorophyll content, and crown diameter emerging as effective predictors. Given the anticipated warmer and drier climate in temperate European forests, further declines in tree vitality in beech stands are expected (Ognjenovic et al., 2020; Langer and Busskamp 2023). Our model predicts that changes in beech vitality can have corresponding changes in the relative abundance of plant pathogens and/or reduced pathogen diversity, further threatening forest's resilience against fungal diseases. Our study presents a powerful approach for the prediction and enhanced monitoring of this previously overlooked biome in forest canopies, facilitating more informed forest management.

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CRediT authorship contribution statement

de Groot Arjen: Writing – review & editing, Methodology, Conceptualization. **Rousseau Mélody:** Methodology. **Laros Ivo:** Methodology. **Chariton Anthony:** Writing – review & editing, Methodology, Conceptualization. **Abdullah Haidi:** Writing – review & editing, Data curation. **Skidmore Andrew:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Siegenthaler Andjin:** Writing – review & editing, Software, Methodology, Data curation, Conceptualization. **Duan Yiwei:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2025.122588.

Data availability

Data will be made available on request.

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