Czech University of Life Sciences Prague

Faculty of Forestry and Wood Science

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The effect of forest structure on the occurrence of tree-related microhabitats in primary mountain forests of Bulgaria

Master Thesis

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DIPLOMA THESIS ASSIGNMENT

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Forestry, Water and Landscape Management

Thesis title

The effect of forest structure on the occurrence of tree-related microhabitats in primary mountain forests of Bulgaria

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Objectives of thesis

1) Overview of current knowledge and literature about tree-related microhabitats, their function, and occurrence with regards to forest structure
2) Evaluation of the main factors influencing the occurrence of tree-related microhabitats in primary mountain forests of Bulgaria

Methodology

The first aim of the master thesis will be completed through a literature review of the current scientific literature. For the second goal, the student will participate in fieldwork which will be carried out in Bulgarian primary forests and will help with the data collection on permanent forest inventory plots, with the main focus on the survey of tree-related microhabitats. The student will be responsible for data preparation and subsequent statistical analysis and interpretation of results.

The proposed extent of the thesis

40-50 pages

Keywords

Biodiversity indicators, habitat trees, primary forests, tree-related microhabitats, forest structure

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Recommended information sources

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Declaration

I declare that I have written this bachelor's/master's thesis on the topic: The effect of forest structure on the occurrence of tree-related microhabitats in primary mountain forests of Bulgaria independently and quoted all information sources that I used in the work, and which I also listed at the end of the work in the list of references.

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With my signature, I also declare that the thesis electronic version is identical to the printed version and that the data provided in it was handled in connection with GDPR.

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The effect of forest structure on the occurrence of tree-related microhabitats in primary mountain forests of Bulgaria

Abstract

Tree-related microhabitats (TreMs) are becoming widely acknowledged as crucial substrates and structures for biodiversity. They serve as an indirect biodiversity indicator for species that use them as habitat or substrate for at least a part of their life cycle, highlighting their importance for ecological monitoring. Primary forests provide key ecosystem functions and serve as a baseline for studies of ecological processes. Even though they represent a small fraction of European forests, they still continue to disappear. This thesis focuses on the relationship between features of forest structure and their effects on the occurrence, abundance, and diversity of TreMs. Data collection was conducted in Bulgaria, in the primary mountain beech forests in the Central Balkan mountain range with the REMOTE Primary Forests project. A total of 42 permanent monitoring plots across three stands, dominated by European beech (*Fagus sylvatica*), were surveyed in September 2022, and TreMs were recorded using standardised hierarchical methodology. Generalised linear mixed models were used to test the effect of tree characteristics on the occurrence of TreMs on the tree level and the abundance and diversity of TreMs on the plot level. Diameter at breast height (DBH) and tree status significantly correlated with the occurrence of TreMs on the tree level. On the plot level, basal area, the density of trees, and altitude significantly increased the overall TreM abundance. In comparison, the density of trees and the density of large dead trees significantly increased the diversity of TreM types. An average abundance of 684.4 TreMs per ha is observed across stands, 532.2 TreMs per ha by live trees and 152.2 TreMs per ha by snags. While snags represent only 11% of all trees, they are not only more likely to host TreMs but also account for almost one third of all TreMs' total abundance. Our findings underline the importance of large trees and snags in contributing to the overall TreM abundance and diversity.

Key words: **Biodiversity indicators, habitat trees, primary forests, tree-related microhabitats, forest structure**

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1. Introduction

Human activities have significantly altered nature worldwide, leading to an unprecedented rate of species extinction. The main drivers of change in nature globally have been identified as changes in land use, direct exploitation of organisms, climate change, pollution, and invasion of alien species. The average abundance of native species has decreased by at least 20% since 1900 and may be accelerating. Around one million species are already facing extinction unless the intensity of biodiversity loss is slowed and reversed. The rate of forest loss has decreased globally since 2000. However, the loss is unevenly distributed worldwide, and net loss is often masked by regional gains in forest cover (IPBES, 2019).

Primary forests are defined as those relatively intact forests that follow natural dynamics. They are regenerated naturally and are composed of native species, showing no indication of human activities. They are characterised by standing and laying deadwood and old trees (Barredo et al., 2021). Although most are in the old-growth stage, natural disturbances contribute to their heterogeneity in structure, creating a mosaic of seral stages (Svoboda et al., 2014; Mikoláš et al., 2019). Primary forests are essential in the conservation of biodiversity. They support a higher genetic and functional diversity and act as an indispensable refuge for species (Watson et al., 2018). Primary forests are particularly scarce in Europe. While the forests cover about 35% of the continent (FOREST EUROPE, 2020), the primary forests are estimated to cover around 0.7% of Europe's forest area. Most of them are protected, however, only 46% strictly under the IUCN category I. They mainly occur in mountain and boreal regions, unevenly distributed across the continent (Sabatini et al., 2018).

Tree-related microhabitats (TreMs) can serve as an easy-to-implement biodiversity indicator. They can play an important complementary role in forest biodiversity monitoring alongside species diversity and deadwood profile (Larrieu et al., 2018). Integrating the monitoring methodology by Larrieu et al. (2018) alongside the retention of habitat trees (trees, bearing at least one TreM) is indispensable for organisms, depending on the habitat they provide. Standardised data can be used to evaluate measures taken to promote forest biodiversity (Bütler et al., 2021).

In this study, we evaluate the main factors influencing the occurrence, abundance, and diversity of TreMs. The fieldwork was conducted under the project REMOTE (REsearch on MOuntain TEmperate) Primary Forests (REMOTE Primary Forests, 2024) with the Department of Forest Ecology, Czech University of Life Sciences Prague. The study plots are in Bulgaria in the Central Balkan mountain range, specifically in the Boatin and Steneto forest reserves. The primary mountain beech forest plots were established there in 2017 and remeasured in September 2022. The dataset includes forest structural data from three study stands, Boatin, Steneto-1, and Steneto-2, each with seven pair plots, a total of 42 study plots. TreMs were recorded using the standardised methodology by Larrieu et al. (2018) on all live and standing dead trees with a diameter at breast height (DBH) ≥ 60 mm (N= 2251). We modelled the effect of selected factors on the richness of TreM types on the tree level, the abundance of TreMs on the plot level and the diversity of TreM types on the plot level. We fitted the models using generalised linear mixed models in R language version 4.3.2 (R Core Team, 2023).

2. Study Outline and Aims

The main aims of the thesis are:

1) Overview of current knowledge and literature about TreMs, their function and occurrence with regard to forest structure
2) Evaluation of the main factors influencing TreMs' occurrence in Bulgaria's primary mountain forests.

The first aim of the thesis is to provide an overview of the current knowledge on the forest structure, followed by a general overview of how it differs in intact primary forests compared to managed forests. TreMs serve as a surrogate biodiversity indicator, which is gaining importance due to the increasing need for effective and easily applicable biodiversity monitoring tools to quantify the current state of biodiversity better and measure its development through time. Various components of the forest structure impact the occurrence of TreMs, namely the diameter at breast height of the trees (DBH), their status (alive or dead standing), age, altitude, tree species, etc.

3. Literature Review

3.1 Primary Forests

Primary forests are defined as relatively intact forests that follow natural dynamics. They are regenerated naturally and are composed of native species while showing no indication of human activities. In comparison, old-growth forests are late-successional forests, containing structures and species that distinguish them from forests of younger age classes and early developmental stage. Old-growth forest attributes include deadwood and old trees, which reach higher ages than the rotation cycle for a given tree species (Barredo et al., 2021). Primary forests are characterised by natural disturbance cycles, contributing to a mosaic of different stand ages, including early succession stands (Swanson et al., 2011; Svoboda et al., 2014). Other related terms include “virgin forests” (Carpathian Convention, 2014), “primeval forests” and “ancient (beech) forests”, later synonymous with “old-growth (beech) forest” by UNESCO (Kirchmeir & Kovarovics, 2016). The term old-growth forest can include primary or secondary forests that have been undisturbed by humans for a long enough period. They are characterised by functional, structural, and compositional characteristics associated with old primary forests of the same type (Barredo et al., 2021).

Forests cover 4.06 billion hectares, accounting for 31% of the Earth’s land area. The tropical domain has the most significant proportion (45%), followed by the boreal (27%), temperate (16%) and subtropical (11%) domains. Over half (54%) of the world’s forests are in only five countries: The Russian Federation, Brazil, Canada, the United States of America, and China. The world’s forest area is decreasing; however, the rate of loss has slowed. Primary forests, defined by the FAO as “forests composed of native species in which there are no visible indications of human activities and the ecological processes have not significantly been disturbed”, account for 1.11 billion ha, approximately 27.3% of all forests (FAO, 2020).

Known primary forests in Europe cover an area of 1.4 million ha in 32 countries, accounting for 0.7% of Europe’s forest area (excluding Russia) (Sabatini et al., 2018a). The forests included in the study (Sabatini et al., 2018a) were previously indicated as “primeval”, “virgin”, “near-virgin”, “old-growth” and “long untouched” by the methodology by Buchwald et al. (2005). The forests included in the study were in reports and studies published between 2000 and 2017, in addition to the 32 existing regional datasets. Most forests are in Finland (0.9 million ha) and Eastern Europe (0.2 million ha), specifically in Ukraine, Bulgaria, and Romania. Switzerland, Lithuania, Slovenia, and Bulgaria each have about 0.5% of primary forests, while Madeira has a 1.5% primary forest coverage. However, the results might be skewed due to inadequate data availability. Primary forests are found mainly in the boreal and the alpine regions, with 1 million ha and 1% of the region and 0.4 million ha and 0.6% of the region, respectively. Most of them, 89.1%, are protected; however, only 46% of them are strictly by the IUCN category I and 24% are in the national parks as IUCN category II. They mainly occur in mountain and boreal areas, unevenly distributed across countries, biogeographical regions, and forest types. A general increase in the number of primary forest patches was found from the west to the east and from the south to the north direction. While the study found a higher share of primary forests than previously estimated, primarily due to the inclusion of early successional phases, Sweden had only a 0.03 million ha coverage, compared

to the previously estimated 2.4 million ha, representing a significant gap in the dataset. The explanatory variables behind the places where primary forest patches occur include historical political boundaries (e.g. Rhodope mountains between Bulgaria and Greece), lack of accessibility and infrastructure for transportation of timber, or forests that were historically protected as hunting areas (e.g. Białowieża in Poland) (Sabatini et al., 2018a). In 2021, a European-wide primary forest geodatabase was released, harmonising 48 datasets of primary forests spread across 33 countries, including Russia. The dataset accounts for 41.1 million ha of primary forests; a vast majority, 37.4 million ha, are in Russia, with the remaining 3.7 million ha across the other 32 European countries (Sabatini et al., 2021).

Field-based inventories of primary forests, although incomplete and with inconsistent methodologies, can help identify the unmapped primary forests in Europe. One of the first comprehensive studies on the primary forest distribution on a national scale in Europe was performed in Slovakia between 2009 and 2015. The study was carried out on the whole territory of Slovakia, based on the national forestry database, recent satellite images, national inventory of protected areas, and tips from local experts. A set of indicator properties was selected based on the primary forest criteria, including, for example, at least 30% of natural forest composition, the presence of veteran trees, at least ten pieces per ha of large standing trees, and at least ten pieces per ha of large lying deadwood, presence of stumps on less than 15% of the area and less than 15% of the area affected by cattle grazing. In addition to late succession, forest stands, characterised by the wide distribution of tree ages, diversity in vertical and horizontal structure, standing and downed dead trees of various decay stages, and a high percentage of old canopy trees, early seral stages and young forests were also considered (Mikoláš et al., 2019). These occur in primary forests after stand-replacing natural disturbances, followed by natural regeneration (Swanson et al., 2011; Svoboda et al., 2014). The results of the complete survey in Slovakia by Mikoláš et al. (2019) show that most forest stands, 93.5%, were in the old-growth stage, with the remaining stands in early-succession stages after disturbances. The early stages were more abundant in spruce forests, representing 17% of their total area. The distribution of primary forests was mostly in high elevations, steep slopes, and away from roads and settlements, as was previously found in other studies (Sabatini et al., 2018; Mackey et al., 2014). Most of the identified primary forests (70%) were strictly protected, while the remaining were protected to a lesser degree of protection and could potentially be logged (Mikoláš et al., 2019).

In the past, it has been commonly accepted that old-growth forests are carbon neutral, meaning that photosynthesis is balanced with respiration (Odum, 1969). However, more recent data indicates that carbon accumulation continues in forests that are centuries old (Zhou et al., 2006; Luyssaert et al., 2008; Ralhan et al., 2023). Primary mountain forests in the Carpathians reach the highest mean total biomass in mixed beech forests at 491 ± 81 megagrams per ha and 388 ± 106 megagrams per ha in spruce forests. Forests there sustain positive biomass accumulation rates, peaking at a mean plot age of 225 years (Ralhan et al., 2023). The large old trees in primary spruce forests in the Western Carpathians even exhibit continuous increase in growth throughout their lives, surpassing 400 years, indicating a strong carbon sink role (Begović et al., 2022). It is generally accepted and calculated that the mass of carbon is half that of the wood mass, although it may vary depending on the tree species (Russel et al., 2015). The carbon in coarse woody debris can have a long residence time, depending on the rate of microbial

decomposition, physical decay, or consumption by insects, depending on factors such as the tree species, stem diameter, environmental conditions, or occurrence of forest fires (Cornwell et al., 2009). Live trees enter the pool of standing dead trees or downed woody debris, depending on the nature of the disturbances and demographic processes. At the same time, its dynamics and turnover rates are affected by the decomposition rates (Russel et al., 2015). However, climate change could induce a higher severity and frequency of disturbance regimes, tipping the carbon balance of unmanaged mountain forests from a carbon sink to a carbon source (Albrich et al., 2022).

Primary forests are essential for the conservation of biodiversity. They sustain critical ecological processes while supporting higher functional diversity and higher intra-species genetic diversity. They are important for species dispersal and migration and serve as an important refuge (Watson et al., 2018). Biodiversity is affected by changes in forest structure, composition, and dynamics. Species dependent on the deadwood, large trees, and forest cover continuity, such as bryophytes, lichens, fungi, and saproxylic beetles, are negatively impacted by forest management (Thorn et al., 2017). Bird species, however, do not show a clear trend, as they are affected by broader landscape patterns. The forests that undergo the largest changes, such as clearcutting activities, are the most affected in terms of species richness. After the abandonment of management, some species, such as saproxylic beetles and fungi, will increase in abundance as the microhabitats that they require to survive to increase in abundance. However, other groups like bryophytes and lichens were not as much affected by the time since management, as the discontinuity of their life substrates is required in addition to dispersal limitations (Paillet et al., 2010). Increasing temperatures and drought risks due to climate change are expected to have different consequences for European forests. Their adaptive strategies may differ depending on local conditions and may include persistence of the current forest types, local adaptation by change in genotype frequencies, and gene flow among populations. Others may consist of migration or substitution of species or, finally, extinction of populations with low ecological plasticity or at the edge of their spatial distribution (Bussoti et al., 2015). An important characteristic of primary forests is the presence of large and veteran trees (Mikoláš et al., 2019). Large old trees provide key habitats for wildlife, some of which are irreplaceable by other structures. However, they are disproportionately affected by loss drivers, including fire and ungulate browsing, affecting their recruitment, accelerating mortality rates, or both (Lindenmayer et al., 2014). Protecting forests where large old trees are most likely to develop while managing trees and forests in longer rotation cycles than currently practised should, therefore, be a priority in biodiversity strategies (Lindenmayer et al., 2012a; Lindenmayer et al., 2014).

The retention of structural attributes to increase the old-growthness in managed forests includes increasing the number of habitat trees (trees, bearing one or more TreMs), standing dead trees, coarse woody debris on the ground, and patches with undisturbed vegetation, including advanced regeneration. Their densities and preferred spatial arrangement depend on the habitat requirements of species that use them, their stability and dynamics, and other risks that might affect them, such as the edge effect, spread of pathogens, fire risk, browsing ungulates, etc. (Bauhus et al., 2009). Some of the proposed silvicultural practices (Bauhus et al., 2009, expanded from Keeton, 2006) include selection cutting for vertical canopy stratification, group selection and variable density thinning for horizontal variation in stand density, crown thinning and long rotations

for large trees, allowing self-thinning, girdling, and no or limited salvage logging after disturbance for standing and fallen dead wood, etc (Bauhus et al., 2009). Other old-growth forest structures include the pit and mound structures that influence soil processes and habitat diversity. They are formed when the trees are uprooted and serve as preferential spots for tree regeneration due to the presence of exposed mineral soil. Mounds offer favourable conditions for early- and mid-succession tree species. However, the growth and survival of the regeneration varies depending on other factors (Barker Plotkin et al., 2017).

While managing for old-growth forest attributes is an oxymoron, as the old-growth forests have not experienced human intervention for a long time, certain silvicultural practices can improve the degree to which old-growth attributes are developed and maintained, the so-called "old-growthness" (Bauhus et al., 2009). Uneven-aged silvicultural practices can be modified to accelerate the rate at which late-successional forest development can be reached by implementing the so-called "structural complexity enhancement" approaches (Keeton, 2006). Two management options exist for managing forests for timber production. Forest stands can be managed on substantially longer production cycles well beyond the ages, considered optimal for tree growth, or alternatively, selected trees or other structural elements can be retained while the rest of the stand is managed on shorter production cycles. The knowledge about the quantity and distribution in time and space of the old-growth-related structural elements needed is limited. At the same time, their retention also depends on their stability and longevity within otherwise managed forest stands (Bauhus et al., 2009).

Forests interact with the climate system and absorb CO^2 (Luyssaert et al., 2010). They act as carbon pools in forest soils (Zhou et al., 2006; Russel et al., 2015) and tree biomass (Cornwell et al., 2009). Forests also impact the land-atmosphere exchange of energy and water vapour (Hudiburg et al., 2011). From 2003 to 2012, the change in forest cover generated mean biophysical warming on land, corresponding to about 18% of the global biogeochemical signal caused by the CO2 emissions from land-use change. Forest loss has been shown to amplify the diurnal temperature variation and increase the mean and maximum air temperature, the largest signal being in arid zones, followed by temperate, tropical, and boreal zones (Alkama & Cesca, 2016). The forest canopy mortality, including natural and human-induced, was, on average, 0.79% annually from 1984 to 2016. It increased by +2.4% annually, doubling the affected forest area since 1984. The increase was stronger in areas of low-severity mortality compared to stand-replacing mortality (Senf et al., 2018). The microclimate in forested mountain regions is influenced by elevation, microtopography, and vegetation. The elevation is a strong predictor of temperatures; however, vegetation and microtopography also have significant effects. Old-growth characteristics have shown an insulating effect and can potentially buffer and mitigate climate warming at local scales (Frey et al., 2016). Climate change has been demonstrated to impact changes in the species distribution at their altitudinal and latitudinal edges. A study of the distribution of 171 forest plant species between 1905 and 2005 along the elevational range of 0 to 2600 m a.s.l. has resulted in an upward shift in species optimal elevation on average by 29 m per decade. The effects of climate change are demonstrated to impact not only the distribution of plant species at the edge of their habitat but also at the core of their spatial distribution (Lenoir et al., 2008).

Climate change is already causing global declines in biodiversity (Butchart et al., 2010) and altering the distribution of forest tree species in Europe. Projections show that the species with the largest decline are the pioneer and coniferous species due to their current occupancy of northern latitudes and lack of areas to move further to (Dyderski et al., 2018). Similar is demonstrated for their alpine altitudinal distributions, as the environmental conditions are similar to those of northern latitudes (Lenoir et al., 2008). Meanwhile, mid- and late-successional species will be able to extend their habitat northwards. The study, however, did not consider the differences in the seed dispersal rates. In the most severe climate scenarios, even the mid- and late-successional species show signs of decline (Dyderski et al., 2018). While the pressures on biodiversity, such as the ecological footprint, nitrogen deposition, the number of alien species, and the climate impact, are all increasing significantly, the biodiversity indices, such as the living planet index, wild bird index, and forest extent, are all declining (Butchart et al., 2010).

Primary forests provide key ecosystem services and are important for the conservation of forest biodiversity. However, not all forest types are equally represented by remnants of primary forests. Out of 54 forest types, six have no existing primary forests, while the majority of types had less than 1% of the total forest area as primary forests. Although most are protected, not all are under strict protection, while many protected areas have non-primary forests, presenting an opportunity for restoration (Sabatini et al., 2020).

3.2 Forest Structure

Describing forest ecosystems includes the structural, functional, and compositional attributes. In these terms, forest structure refers to the spatial arrangement of ecosystem components, such as vertical and canopy structure, function refers to ecological processes such as the production of organic matter, and composition to the variety of ecosystem components, such as species richness and abundance (Franklin et al., 1981).

Forest structure can be defined as the degree of heterogeneity in biomass distribution in three-dimensional space and depends on the spatial patterns and efficiency of canopy space occupation. It is affected by climate variables, soil and topography, natural disturbances (Pavlin et al., 2023; Elbrecht et al., 2021), and forest management (Elbrecht et al., 2017). Those impact the tree species composition, functional diversity, tree size distribution and diversity, and complementarity in crown architecture, all of which influence the spatial patterns of the canopy space occupation (Ehbrecht et al., 2021; Fotis et al., 2018).

Several approaches have been made to quantify the forest stand structural complexity (Pommerening, 2002; McElhinny et al., 2006), such as the stand structural complexity index (SSCI). The SSCI is based on fractal dimension derived from single terrestrial laser scans and is positively correlated to conventional tree-based measures of stand structural complexity, tree size differentiation, diversity of tree diameters, and random tree spacing patterns. It increases with increasing tree species diversity (Elbrecht et al., 2017). Multiple stand structure attributes, representing vertical and horizontal heterogeneity, density, and diversity combined, allow for the characterisation of stand type, management effects, and stand productivity (Schall et al., 2018).

On the level of a forest stand, the stand structural complexity can be defined as a measure of several different attributes present and the relative abundance of each of these attributes. A summary of key attributes defined in a review by McElhinny et al. (2005) includes foliage arrangement, canopy cover, tree diameter, tree height, tree spacing, tree species, stand biomass, understory vegetation, and deadwood. Further, these attributes can be integrated into various indices of structural complexity.

Canopy structural complexity, describing the degree of heterogeneity in vegetation density, is correlated with several ecosystem functions (Gough et al., 2020). The vertical distribution of the canopy, appearing as continuous or multiple layers of foliage, serves as an important forest structure attribute (MacArthur & MacArthur, 1961; Franklin and Van Pelt, 2004), as it correlates with foliage height diversity, the foliage in different vertical layers (MacArthur and MacArthur, 1961). The vertical stratification maximises canopy packing through contrasting canopy architecture (Morin et al., 2015). The diversity of canopy structures also includes the irregular horizontal distribution of structures, such as canopy gaps and forest openings that correspond with dense patches of saplings and understory vegetation. Stand-replacing disturbances are usually followed by a predominantly even-aged forest with a single-layered, closed canopy. With time, the stand approaches maturity, and the upper canopy starts to thin due to competition and other mortality agents. The vertical heterogeneity develops as a response to increased light conditions within the stand, and shade-tolerant species gradually fill in the canopy gaps, creating vertical and horizontal heterogeneity - patchiness (Franklin and Van Pelt, 2004).

Crown morphology and aboveground biomass allocation patterns are affected by species richness and local structural variability (Kunz et al., 2019; Ishii et al., 2004). The tree species diversity positively affects structural complexity, as it can result in denser and structurally more complex canopies if trees with complementary physiological and morphological traits are mixed (Fotis et al., 2018; Juchheim et al., 2019; Jucker et al., 2015). Higher neighbourhood species diversity can induce wood volume allocation in favour of branches in morphologically flexible species, i.e. the overall potential of a tree to respond to the changing environment or competitive conditions with crown plasticity (Kunz et al., 2019). The growth and coexistence of different tree species depend on their tolerance to shade conditions (Pavlin et al., 2023; Coates et al., 2009), their mixture of evergreen and deciduous behaviours (Franklin et al., 2002) and their ability to acquire belowground resources under stress from competition. However, the relative magnitude of intra- versus interspecific competition differs among the tree species (Coates et al., 2009). Structurally complex canopies in species-rich forest stands enhance stand primary production by complementarity of resource utilisation through spatial, physiological, and temporal differentiation (Gough et al., 2020; Ishii et al., 2004). Maximum canopy height is correlated with a higher canopy rugosity, i.e. a measure of canopy structural complexity correlated with the primary production and light use efficiency (Gough et al., 2020).

Natural disturbance factors were found to be the main driver of canopy accession in primary temperate forests in Europe. Intensifying disturbance regimes could, therefore, cause shifts in species composition and their reached lifespan (Pavlin et al., 2021; Pavlin et al., 2023), specifically favouring the faster-growing, light-demanding tree species (Pavlin et al., 2023). Natural disturbances have various effects on forest ecosystems. An analysis of almost 500 disturbance studies published between 1981 and 2013 found that, on average, disturbance events

decreased the total ecosystem carbon by 38.5% (standardised for a stand-replacing disturbance in the year of its occurrence), while the biodiversity, measured in species richness, increased by 35.6%. The study, although geographically overrepresenting temperate forests in Europe and North America and focusing mainly on forest fire impacts, found that the intensification of disturbance regimes due to climate change will have positive impacts on biodiversity. However, other ecosystem services will mostly be negatively impacted. Managing for a low to medium severity and frequency disturbance regime could limit the negative impacts while continuing to be advantageous for biodiversity (Thom & Seidl, 2016). A recent study conducted in the primary mountain forests of the Carpathians has shown that past disturbances have a significant impact on the present-day communities of saproxylic beetles. The abundance, phylogenetic diversity, and functional diversity of these beetles are affected by the disturbance regime. The quantity and diameter of deadwood have a significant impact on the abundance and functional diversity of saproxylic beetles, while the phylogenetic diversity is positively correlated with the openness of the canopy (Kozák et al., 2021). Similarly, the local and regional richness of fungi in the primary spruce forests in the Western Carpathians are shaped by historical disturbances. While the diversity of local, red-listed species increases due to more frequent disturbance events, the species diversity at the regional scale decreased with more severe disturbances. While canopy openness impacts the deadwood-inhabiting fungi negatively, the species richness responds positively to deadwood volume (Ferenčík et al., 2022). Lichen species richness is lower in regeneration plots, compared to old-growth or recently disturbed forests. However, the legacies of natural forest dynamics influence the species composition and diversity of lichens. While the natural disturbances affect patches of forests, ensuring a larger protected area offers habitat variability at the landscape scale (Langbehn et al., 2021).

Changes in natural disturbance regimes due to human interference can have various consequences. Suppressing the low to medium severity of forest fires can lead to a higher likelihood of more intense disturbances. The changes in the forest structure, such as an increase in light-demanding species due to fire exclusion, coupled with restriction of understory thinning, result in decreasing numbers of large light-demanding species. Exacerbated by mortality from drought stress, insect outbreaks, disease, and competition further increase fuel loads and their spatial continuity, resulting in higher intensity wildfires and a decrease in large areas of late-successional habitat (Camp et al., 1999).

Large living and dead trees are characteristic of old-growth forests. In central Europe and south Scandinavia, the primary forests exhibit densities of 10 to 20 living trees per ha with a diameter at breast height (DBH) over 70 cm (Nilsson et al., 2002). Another study of beech primary and old-growth forests found the prevalence of very large trees with DBH of 80 cm or more, with the average density of very large trees typically ranging between 5 to 20 trees per ha with a median value of 13.1 trees per ha and a median diameter of 85 to 90 cm, rarely exceeding DBH of more than 100 to 130 cm. In specific conditions, the densities can reach even over 30 large trees per ha, as in the study in the Belgian lowland old-growth beech forest. An average annual DBH increment of 4.75 mm and a low annual mortality rate of 0.89% were found. However, above-average values are explained to occur due to the historical conditions of the establishment of the beech forest, with the forest stand being thinned and tended during its establishment, resulting in

continuous non-suppressed growth in ideal conditions, although the stand has been left unmanaged since 1986 (Vandekerkhove et al., 2018).

Large-diameter trees, specifically the size of the largest trees has been demonstrated to be correlated with the total forest biomass worldwide. Trees with DBH exceeding 60 cm comprise 41% of all aboveground live tree biomass and are an important consideration in global forest carbon cycling. However, large-diameter trees in forests with high biomass are represented by relatively few species compared to the overall forest species richness, but their individuals are abundant. The total biomass of the largest 1% of the trees and the forest density decline worldwide with the absolute latitude but generally represent a high proportion of biomass (Lutz et al., 2018).

On average, old-growth temperate and boreal forests exhibit a proportion of 10% of dead standing trunks including high stumps with the proportion increasing for the largest trees. The volume of deadwood is proposed to be directly correlated to the productivity of old-growth forests, as the share of dead standing trees is independent of the total basal areas. Standing dead trees, so-called snags, are generally larger than lying dead trees, while trees with DBH over 40 cm frequently dominate the basal area and volume of living and standing dead trees. About 20-40% of dead trees' volume and basal area is found in snags, independent of the total deadwood volume (Nilsson et al., 2002). Forest age positively correlates with most structural indicators, including basal area, large living trees, live aboveground biomass, and quadratic mean diameter. Compared to mature forests, old-growth forests differ by a higher amount of large living tree densities, higher quadratic mean diameter, greater live aboveground biomass, and coarse woody debris volume. Old-growth forests also show higher basal area and lower stem densities than mature stands, although these differences are not statistically significant and vary between forest types (Burrascano, 2013).

Deadwood volumes differ among different forest types. A study in Austrian natural forest reserves found volumes of 109 m^3 per ha in spruce-fir-beech forests, while only 23 m^3 per ha in larch forests. The amount of deadwood starts increasing in a relatively short time after the abandonment of management; hence, it is often used as an indicator of naturalness. Other factors affecting the compositional diversity of deadwood are tree diameter and volume of the living trees in the stand. Additionally, mean annual temperature influences the absolute deadwood volume, while precipitation influences its relative proportion (Oettel et al., 2020). A review of threshold data found that in mixed-montane forests, the ecological threshold for the amount of deadwood necessary for various species ranges from 30 to 40 m3 per ha, which is an amount that is difficult to reach in production forests. To satisfy the requirements of deadwood-dependant species, a network of connected forest landscapes should be the aim rather than a lower amount in all stands (Müller & Bütler, 2010). Deadwood is an important component of forest structure as many species depend on its presence in sufficient amounts and different compositions (Rondeaux & Sanchez, 2010; Lassauce et al., 2011; Burrascano et al., 2014). However, the correlation between the volume of deadwood and species richness is relative to other factors, such as the type of deadwood (snag, log, or stump), decay class (fresh or decayed), and biome. The deadwood volume and species richness of saproxylic beetles and fungi showed a higher correlation in boreal, compared to temperate forests (Lassauce et al., 2011). Natural small-scale disturbances produce an irregular pattern of ecological circumstances that lead to variations in the diversity and

composition of deadwood and, consequently, other deadwood-dependant species (Burrascano et al., 2014).

Tree regeneration is affected by climatic conditions, disturbance regimes, biotic factors, and their interactions, which create complex feedback. Generally, competition among trees is more prevalent in optimal conditions, while conditions under climatic stress decrease the competition pressure. Additionally, tree life history strategies add to the overall explanation of differences in regeneration of various tree species (Käber et al., 2022). Building on the established tree Life History Strategies (Bertness & Callaway, 1994; Callaway & Walker, 1997), Käber et al. (2022) expand the Stress Gradient Hypothesis, which states that abiotic factors, such as climate or soil, affect the species' performance at the edges of climatic gradients, that competition will be the determining factor under optimal climatic conditions and that stressful climatic conditions are more likely to result in positive interactions. Competition does not necessarily decrease under climatic stress; however, it is less prevalent under cold conditions than dry-range edges. Käber et al. (2021) found the main drivers of tree recruitment to be stand structure and shade tolerance, ranging across environmental gradient in temperate forests. The tree recruitment rates are positively affected by the higher temperatures; however, the drought tolerance and water relations need to be studied further.

Tree regeneration in forests occurs in forest gaps of varying sizes, e.g. after small disturbances, where different species can regenerate at different gap edges depending on the irradiance, microclimate, soil features, and ground vegetation (Vilhar et al., 2015). Stand-replacing disturbances lead to early-successional forest ecosystem establishment. These post-disturbance ecosystems are frequently rich in biological legacies, such as deadwood and surviving organisms. The early succession stage is not dominated by the tree canopies but by the herbaceous layer and shrubs with high spatial and structural complexity. It is characterised by high biodiversity, and many species depend on it for survival. Salvage logging reduces the key ecological processes and species richness, determined by the early succession stage, while tree planting decreases the duration of the stage (Swanson et al., 2011). While canopy openings have more light for the establishment of tree regeneration, the microclimatic buffering effect of the forest is decreased. Therefore, disturbances have both positive and negative impacts on the tree regeneration process, depending on light levels, air temperature, humidity, and the retention of deadwood. While tree regeneration is positively affected by the understory light level, the maximum vapour pressure deficit has a negative effect. The impact of deadwood and browsing on regeneration is inconclusive and varies in different conditions (Thom et al., 2023).

While the herbaceous layer represents less than 1% of the forest biomass, it can contain over 90% of the forest plant species and generate up to 20% of the foliar litter with high nutrient content. The early spring species can aid in nutrient retention and prevent leaching of nutrients, such as nitrogen. Competition in the understory vegetation determines the establishment of higher plants, including the tree species of the upper canopy layer (Gilliam, 2007). The herbaceous layer is largely affected by the tree species composition. Broadleaved forests generally provide a more diverse understory than coniferous forests, although no specific tree species have been found to have a strong effect. Mixed stands impact the understory diversity; however, pure stands have a more diverse herbaceous layer than mixed stands. Overstory structure and composition influence

the understory layer through their impact on light, water, and soil nutrient availability (Barbier et al., 2008).

Latitudinal changes in climate attributes translate into changing patterns of forest structure and affect the stand structural complexity in boreal, temperate, subtropical, and tropical primary forests. The hotspots of high structural complexity and hotspots of plant diversity are highly correlated. The climatic attributes, specifically the annual precipitation and precipitation seasonality, can largely explain the global variation of forest structural complexity (R2 = 0.89) (Ehbrecht et al., 2021). There is a correlation between precipitation amount and mean annual temperature and the attributes of the forest structure. Basal area and stem density are positively correlated with mean annual precipitation and negatively correlated with latitude. Coarse woody debris is negatively correlated with mean annual temperature, while large living trees correlate positively with precipitation (Burrascano et al., 2013).

Due to the increasing rate of global warming, forest ecosystems are anticipated to undergo significant changes. In a study conducted in Germany, Berchtesgaden National Park over a 28-year investigation period, the forests became denser, structurally more complex and showed an increase in species richness. The rate of change of all indicators accelerated over time, indicating intensifying forest dynamics. While the temperature rise accelerated the forest change, the old-growth forest characteristics had a dampening effect (Thom & Seidl, 2022). Individual trees and forest communities frequently have traits and ecological strategies, so-called legacies of exposure to past variable conditions, which make it possible for them to adapt to contemporary climate variability. Forests that have previously been exposed to large temperature variability are less sensitive to present temperature fluctuations. However, the precipitation and vapour pressure deficit do not show the same patterns, presumably due to the tree's response mechanisms to dry conditions (Hansen et al., 2022). In line with the hydraulic theory, the potential maximum tree height declines as the tree vapour pressure deficit rises. As the plants cannot reduce their height, the forests are showing trends of increasing mortality of tall trees, being replaced by the regeneration layer (McDowell & Allen, 2015). With the rising temperature and vapour pressure deficit, the conditions might benefit trees in cooler or wetter regions. However, most areas will experience negative impacts on plant demographic rates in addition to more severe and, in some regions, more frequent forest disturbances. The current knowledge of the net balance of tree mortality counteracted by tree regeneration is limited, limiting future predictability (McDowell et al., 2020). Studies of primary forests can give us the benchmark for comparisons of the natural processes. However, climate change affects young and even-aged forests more than old-growth forests as a result of their higher sensitivity to regional warming and drought. A study in Alberta, Canada, found that the predicted annual mortality increased significantly more in young forests compared to the old forests of quaking aspen (*Populus tremuloides*), balsam poplar (*Populus balsamifera*), black spruce (*Picea mariana*) and white spruce (*Picea glauca*). The differences are explained by the higher competition for space and nutrients in young trees occupying the same ecological niches, making them more vulnerable to stressors such as drought (Luo & Chen, 2013).

Mountain forests are particularly threatened by climate change and its impacts due to their temperature limitation and high exposure to warming. However, their complex topography can help them buffer the effects of climate change. For instance, the resilience of forest composition and size structure to climate change in the Eastern Alps was investigated using the iLand model,

testing for tipping points under different topography scenarios. When the warming reached +2°C, a threshold was crossed, and the system changed into an alternative state, in the specific case from conifer-dominated to a landscape dominated by smaller broadleaved trees. Even moderate warming, predicted under the best-case scenario of implicating current policy targets, could result in critical transitions of forest ecosystems, showing the importance of topographic complexity as a buffering agent (Albrich et al., 2020).

3.3 Biodiversity Indicators

The importance of ecologically sustainable forest management in preserving biological diversity is becoming more widely acknowledged (Secretariat of the Convention on Biological Diversity, 2009). Therefore, we need scientifically proven measures to assess the effectiveness and success of applied measures to restore and increase biodiversity. There are many different biodiversity indices that exist; however, some are difficult, expensive, or time-consuming to carry out, while others might be less effective in measuring the correlation (Burascano et al., 2023).

Taxon-based indicators of biodiversity include indicator (key) species (Lindenmayer et al., 2000), defined as "… an organism whose characteristics (e.g. presence or absence, population density, dispersion, reproductive success) are used as an index of attributes too difficult, inconvenient, or expensive to measure for other species or environmental conditions of interest" (Landres et al., 1988, p. 317). If used, they need to have explicitly defined criteria for the assessment aims, considering the natural variability in population attributes, specific species niches, etc. (Landres et al., 1988). Species lists act as a more comprehensive indicator; however, they are not practical apart from extreme conditions with only a few species present. Therefore, key structures can serve as biodiversity indicators, including stand complexity and plant species compositions, connectivity, and heterogeneity (Lindenmayer et al., 2000). Additionally, stand structural complexity necessarily involves the interaction between numerous variables. Pooling them into indices facilitates comparability between stands and their potential contribution to biodiversity. Stand-based assessments can be direct and easy to assess, for example, (non)removal of large dead trees (McElhinny et al., 2005).

Other strategies have been used to guide forest biodiversity conservation, such as a set of five guiding principles by Lindenmayer et al. (2006), proposing the maintenance of connectivity, landscape heterogeneity, stand structural complexity, aquatic ecosystem integrity and the use of natural disturbance regimes to guide human disturbance regimes. The measures are further specified at different scales, from regional to landscape and stand-level scales. At the regional scale, the establishment of large reserves should be ensured. The measures at the landscape level should include the creation of protected areas within production forests, aquatic ecosystem buffers, improved design of road networks and spatial and temporal design of harvest units. The stand-level measures include the retention of stand structural complexity and its key elements (e.g. large and old trees, deadwood), longer rotation cycles, alternative reduced impact silvicultural systems, etc.

Worldwide, 424 million hectares of forest are set aside for biodiversity conservation. Since 1990, 111 million hectares have been added, the majority between 2000 and 2010. Over the past ten years, the rate of expansion of forest areas, primarily for the conservation of biodiversity, has

slowed down (FAO, 2020). Globally, 7.7% of forests were protected in 1990, rising to 16.3% in 2015. In the tropical domain, protected areas have experienced a sharp increase from 12% in 1990 to 26.3% in 2015. However, between 1990 and 2015, the world's primary forest area decreased by 2.5% globally and by 10% in the tropics (based on data for countries that reported in all years) (Morales-Hildago et al., 2015).

Some countries have implemented national strategies aiming to monitor and preserve forest biodiversity. Monitoring of fundamental compositional, structural, and functional ecosystem characteristics, from which indicators are derived, has been proposed in New Zealand. The indicators include forest area and spatial arrangement, tree recruitment and mortality, introduced plant species and herbivore species impacts, and deadwood (Allen et al., 2003). In the case of Austria, a single aggregated measure, the so-called Austrian Forest Biodiversity Index (AFBI), is proposed. AFBI is composed of nine state and four response indicators, with the final indicator value ranging from 1 to 100 representing the sum of all weighed and rescaled indicator measures. The selection and weighing of these indicators were done by an advisory board of a biodiversity monitoring project. The state indicators include naturalness of tree composition (exotic species, potential natural vegetation, game impact), forest structure (deadwood and large old trees), regeneration (amount, type, and seed sources) and forest fragmentation, while the response indicators include natural forest reserves, genetic reserve forests, seed stands, and seed orchards (Geburek et al., 2010). However, the comparability of such indicators across nations is difficult. Therefore, they need to be harmonised to ensure the comparability of international forest biodiversity monitoring.

The primary international process dealing with biodiversity is the Convention on Biological Diversity (CBD), under which the National Forest Inventories (NFIs) are the main source of information. The NFIs can be a source of comparable estimates of indicators for various biodiversity features, such as forest categories, deadwood, forest age, structure, and naturalness; however, the NFIs need to be harmonised internationally for reporting on ground vegetation and regeneration data (Chirici et al., 2012). Large-scale forest inventories have traditionally been used to assess forest stands for timber harvesting; however, with the increasing importance of multipurpose forests, the NFIs have expanded in scope to include assessments of forest carbon pools and sequestration estimates, forest health assessments and additional attributes for measuring biodiversity. However, the weakness of NFIs is the probabilistic sampling scheme typically used, which is inappropriate for quantifying changes in the abundance of rare, threatened, and endemic species. For the purposes of biodiversity monitoring, permanent inventory plots should be established to track changes with sufficient precision (Corona et al., 2011).

Compared to other taxon-based forest monitoring, forest structure is measured more easily during forest inventory and does not require specific taxonomic expertise, although it does not measure biodiversity directly. However, when such indices are linked and quantified in relationship to the species richness, they can act as an effective measure of biodiversity (Zeller et al., 2022). A review by Zeler et al. (2023) outlines evidence of 410 relationships between the forest structure and species richness based on 85 studies from the temperate forests in Europe, compiling positive, negative, and neutral correlations between them. The authors recognise structural attributes relating to old-growth forests, such as the presence and share of large old trees and

stand age, to be positively correlated with the species richness of various taxa. Additionally, they outline a positive correlation with canopy gaps.

Deadwood has been demonstrated to be correlated with the presence and diversity of saproxylic beetles and fungi (Bouget et al., 2011; Lassauce et al., 2011; Gao et al., 2015). The inventorization of deadwood requires properly described characteristics, such as snags, logs, stumps, branches, and other fine woody debris. The tree species, stage of decay, and volume calculations are additional characteristics that improve the strength of the indicators. Examples of proposed deadwood indicators include the forest surface containing at least 40 m^3 of deadwood per ha to conserve saproxylic organisms such as Coleoptera or a forest surface containing at least 20 m^3 of deadwood per ha with a diameter at breast height over 40 cm, which is important for multiple, red-listed invertebrate species, fungi, and birds (Rondeaux & Sanchez, 2010). A meta-analysis was performed to investigate the relationship between the volume of deadwood and species richness of saproxylic beetles and fungi, relating them to predictors at the forest stand level, including biome, deadwood type (stump, snag, and log) and decay class (fresh vs. decayed). A moderate but significant correlation is validated between deadwood volume and species richness of saproxylic organisms. Additionally, the biome has a strong effect, resulting in a higher correlation in boreal compared to temperate forests. However, it is emphasised that the total volume of deadwood is an insufficient indicator of saproxylic biodiversity, and the type of deadwood and decay class should be integrated into deadwood monitoring (Lassauce et al., 2011). Species composition and richness of saproxylic beetles depend not only on the presence of deadwood but have been demonstrated to prefer snags compared to laying deadwood in an oak forest, explained by the difference in ground contact, making the logs wetter, but also densely covered by bryophytes and ground vegetation, making them less accessible to beetles. Additionally, the dimensions of snags and logs are demonstrated to influence the occupation of deadwood by differing saproxylic beetles (Bouget et al., 2012).

More complex composite indices can provide a more reliable correlation, e.g. the Index of Biodiversity Potential (IBP) (Larrieu et al., 2019; Zeller et al., 2022). The IBP is designed to help practitioners with an efficient tool to estimate biodiversity at the local level. A study by Larrieu et al (2019) tested the IBP, used for rapid assessments of habitats in France, to test the power of relationships between the stand variables and data on species richness and composition for nine taxa, including lichens, beetles, birds, bats, and vascular plants. While the study found significant correlations with species composition, the correlation was weaker for species richness. Seven habitat variables and their threshold values are outlined, namely the number of tree species, the density of large logs (significant for bryophytes), and the number of aquatic macrohabitats (for polypores), all with positive relationships. In contrast, the number of vegetation strata had a negative relationship with ground beetles. The density of living polypore-bearing trees and the trees with missing bark likely mask other ecological components. Another study by Zeller et al. (2022) tested the IBP on 147 plots in three regions in Germany, quantifying the relationship between the values of IBP and changes in species richness for 13 taxonomic groups, such as bats, spiders, carabids, lichens, bugs, and moths. The IBP there considers factors such as the number of tree species and vertical layers, standing and lying deadwood, the number of large living trees, the optimal share of canopy gaps and the number of TreMs. Additionally, it includes aquatic and rocky habitats; however, those were absent in the study plots. The variables of vertical

layers, large living trees, share of gaps and TreMs were all positively correlated with the measured species richness. Birds, true bugs, moths, and lichens all positively correlated with the IBP, with a high correlation in most forest types. However, the results of the IBP application should be used with caution, as it does not differentiate species richness for rare, introduced, or red-listed species. The study was carried out in predominantly beech-dominated forests; therefore, the results are most representative of those. A study by Mikoláš et al. (2021) focusing on the primary spruce forests in the Carpathians used the so-called Biodiversity potential index (BPI). The BPI includes a different selection of predictors compared to the study by Zeller et al. (2022), including standing dead trees, downed logs, large old trees, diversity of understory vegetation and light availability at the ground floor, relating them to saproxylic beetles, lichens, mosses, wood-inhabiting fungi, light-demanding species of true bugs and understory vascular plants. These have previously been demonstrated in the study by Bače et al. (2023). The study by Mikoláš et al. (2021) also considers the presence or absence of data on a key umbrella bird species, the Capercaillie, based on the study by Suter et al. (2002). Although the study aimed to identify the trade-off relationship and co-benefits between forest biodiversity and carbon, it concludes that the BPI is a reliable predictor of species richness of wood-inhabiting fungi and lichens.

With an increasing number of forest biodiversity indicators, there is a growing need for their critical evaluation and assessment of the evidence supporting their correlation to the actual biodiversity. Each biodiversity indicator should have an established correlation with the species before being implemented in inventories, reporting, and policymaking regarding forest biodiversity. A review of 142 studies by Gao et al. (2015) reveals that 62 do not provide clear correlations between their indicator and direct biodiversity observations, while the remaining 80 studies have 412 correlations. Out of those, only six indicators are supported by solid evidence, having multiple studies with statistical correlations and no contradicting results. Mostly, these are related to deadwood, e.g. between deadwood volume and wood-inhabiting fungal species, deadwood diversity and saproxylic beetle species richness, the age of upper layer canopy trees and epiphytic lichen species richness and between gaps in canopy cover and the spider species richness.

Other indicators and new technologies for monitoring and assessment of biodiversity are emerging, such as environmental DNA barcoding. The biodiversity assessments are carried out via samples taken from water, air, soil, or sediments, from which DNA is extracted and analysed. This method enables the determination of species' presence while past species assemblages can be reconstructed. The technique is still advancing and needs standardisation of approaches; however, it is projected to become an essential tool for monitoring biodiversity (Yoccoz, 2012; Thomsen et al., 2015; Ruppert et al., 2019).

3.4 Tree-Related Microhabitats

3.4.1 Definition and Types of Tree-related Microhabitats

TreMs are defined as "… a distinct, well-delineated structure occurring on living or standing dead trees, that constitutes a particular and essential substrates or life site for species or species communities during at least a part of their life cycle to develop, feed, shelter or breed. TreMs are specific above-ground tree morphological singularities that are not to be found on every tree.

TreMs encompass both tree-originating modifications caused by biotic and abiotic impacts, such as intrusions, lesions, and breakages, which expose sap and heartwood and initialise outgrowth structures and wood decay (saproxylic TreM), as well as elements of external origin that are physically linked to the tree (epixylic TreM)" (Larrieu et al., 2018, p. 195). The definition does not include other morphological singularities, such as lying deadwood, wood decay structures, pits and mounds, root plates or species-specific traits (Larrieu et al., 2018).

TreMs can serve as an easy-to-implement biodiversity tool or, depending on the level of typology used, can play an important complementary role in combination with other structural indices, such as species diversity and deadwood profile. Integration of the preservation of TreMs in forest management could assist with slowing down the loss of forest biodiversity and help assess the biodiversity in European forests (Larrieu et al., 2018).

The definition and typology of TreMs by Larrieu et al. (2018), built on the definition by Kraus et al. (2016), established during an expert group led by the European Forestry Institute (Asbeck et al., 2021), has been harmonised, standardised, and ordered in a hierarchical typology. The typology aims to specify the features surveyed in the field and improve comparability between future studies performed in temperate and Mediterranean forests in Europe (Larrieu et al., 2018).

The methodology by Larrieu et al. (2018) includes TreM types occurring on living (Fig. 1) and dead standing (Fig. 2) trees. It consists of 47 specified TreM types, hierarchically grouped into 15 groups and further into seven basic forms based on their morphological characteristics. These forms include:

- **Cavities**: woodpecker breeding cavities, rot holes, insect galleries and bore holes, and concavities;
- **Tree injuries and exposed wood**: exposed sapwood, exposed sapwood and heartwood;
- **Crown deadwood**,
- **Excrescences**: twig tangles, burs and cankers;
- **Fruiting bodies of saproxylic fungi and slime moulds**: perennial fungal fruiting bodies, ephemeral fungal fruiting bodies and slime moulds;
- **Epiphytic, epixylic and parasitic structures**: epiphytic or parasitic crypto- and phanerogams, nests, microsoil;
- **Fresh exudates**.

Further division, morphological features and their dimensions are described in Appendix 1.

Figure 1: Live habitat tree with trunk base rot-hole.

Figure 2: Dead standing habitat tree with bryophytes and ephemeral fungal fruiting bodies.

The methodology is further described in a field guide by Bütler et al. (2021). The minimum inventory dimensions for each TreM are indicated, and the information about occurrence frequencies and replacement rates in the forest stands is given. It also includes information on whether certain types are more frequent in managed vs. unmanaged forests and provides a (non-exhaustive) list of associated species.

TreMs can be created by various biotic or abiotic events, such as a falling rock damaging the tree bark, lightning striking a tree, leaving a scar, splitting the wood, or woodpecker cavities created by the woodpeckers. For other types, such as vertebrate nests or witch brooms, the tree serves only as support. However, only features that are proven to have a direct link with associate species are classified as a TreM (Larrieu et al., 2018).

The literature concerning TreMs has grown in importance and abundance, especially in the last decade. A review by Martin et al. (2022) outlines that 60.3% of the articles included in the review were published in 2017 or after. The oldest study identified was published in 2000 (Lindenmayer et al., 2000), and the number of related publications stayed low (<4 articles/year) until 2013, after which it started rapidly increasing. Many publications on the topic existed before 2000; however, they focused on specific TreM forms (Martin et al., 2022) or wider habitat structures relating to particular species or species groups. Jonsson and Jonsell (1999) evaluated indicators in Sweeden's spruce forests to explore how the species groups co-vary in their occurrence and how much the species composition and richness can be predicted from habitat

structures, explicitly linking the occurrence of bryophytes, epiphytic lichens, and wood-inhabiting fungi, while additionally sampling beetles with window traps. Other indicators have also been used for accounting for species presence and abundance, such as in a study by Humphrey et al. (1995), where climatic conditions, (non)management, aspect, and vertical stand structure were observed to account for syrphid (hoverflies) and carabid (ground beetles) community composition and diversity.

The term "tree microhabitats" was coined in 2008, as the previous terminology lacked consistency. In a study by Winter and Möller (2008), 20 structural microhabitats on trees were defined and tested for comparisons of their occurrence in managed versus unmanaged stands. The study also explored the variety and frequency of microhabitats and their link to the occurrence of threatened saproxylic beetles. Since then, the majority (68.3%) of articles published on the topic have focused on forests in France, Germany, and Italy, representing 32.6%, 30.6%, and 11.8%, respectively (Martin et al., 2022). This is common in ecology-related research, as there is often a lack of global representativeness, with the Global North countries being overrepresented for various reasons (Martin et al., 2012; Thom & Seidl, 2016). The overrepresentation is particularly high in protected areas, temperate deciduous woodlands, and wealthy countries (Martin et al., 2012).

3.4.2 The Importance and Potential of Tree-Related Microhabitats as Biodiversity Indicators

In the post-2020 global biodiversity frameworks, an increasing need for establishing more specific and measurable targets and indicators is outlined. These are aiding in tracking the progress towards international targets across countries. Therefore, specific, measurable, ambitious, realistic, and time-bounding (SMART) targets should be implemented to monitor the progress of biodiversity-related targets, such as the Aichi Targets. The pressure-state-response model is a framework for identifying and structuring indicators ("Biodiversity: Finance and the Economic and Business Case for Action," 2019). TreMs can be used as structural indicators of the state of forest biodiversity at the scale of a forest stand. The implementation of TreMs as a structural surrogate indicator of biodiversity offers a quantitative measure for tracking the success of other measures aimed at the conservation of biodiversity, such as strict forest reserves and retention forestry approach to the management of forests (Asbeck et al., 2021). As most forests are managed and wood extracted from the forest, retaining selected structures and organisms at the time of harvest, such as large old trees and decayed logs, offers continuity and enrichment of forest structure, function, and composition. It promotes the maintenance of biodiversity at different spatial scales. The main difference between the retention approach from a conventional or uneven-aged selection (single selection, shelterwood, or seed-tree system) is the retention of some of the biomass, in contrast to its removal (Lindenmayer et al., 2012b; Gustafsson et al., 2020).

TreMs serve as an important indicator of biodiversity (Paillet et al., 2018; Basile et al., 2020a; Asbeck et al., 2021; Zeller et al., 2022; Martin et al., 2022). They can be used as a tool to assess forest management practices (Vuidot et al., 2011; Paillet et al., 2017; Paillet et al., 2018; Sever & Nagel, 2019; Martin & Raymond, 2020; Asbeck et al., 2022) or assess the factors at a

site-, stand- and tree-scales that favour their occurrence (Larrieu et al., 2022.). TreMs offer a variety of substrates and microclimatic conditions, offering important habitats. Some types are frequently occurring, including exposed sapwood and heartwood (e.g. from injuries during cutting operations in managed forests, falling items, etc.), while others, such as charred wood (fire or lightning strikes), epixylic features and supporting structures (e.g. vertebrate nests) are rarer (Larrieu et al., 2018).

A recent review by Asbeck et al. (2021) outlines the importance of TreMs in retention forestry, specifically addressing the criteria for selecting retained elements of forest structure, such as habitat trees. However, the main shortcoming of TreMs as surrogate indicators of biodiversity is the strength of the relationship between TreMs and the occurrence and abundance of forest-dwelling species from various taxonomic groups.

For example, a study by Basile et al. (2021) in the Black Forest in southwest Germany found that the bird species positively correlate to attributes of forest structure, namely the mean diameter of living trees, the mean diameter of dead trees, and the volume of laying deadwood. Additionally, the study found a positive relationship between bird species and the share of broadleaf and mixed forests in the landscape and a negative response to the distance between forest patches, resulting in a decrease in the richness and diversity of bird species. It outlines the importance of integrating structural characteristics in addition to the landscape context. Meanwhile, Betts et al. (2017) demonstrate the buffering effects of old-growth forests from warming, which affects climate-sensitive bird populations. Besides having a cooling effect, old-growth forests may also supply a higher availability of nest sites and food sources, benefiting the bird species at their upper edge of temperature limits. Another study by Kameniar et al. (2021) in primary spruce forests in the Carpathian Mountains in Slovakia focuses on the impact of natural disturbances on bird assemblage composition. Based on dendroecological data of forest disturbances between the years 1750 and 2000 and a dataset of bird counts from 2017 and 2018, different results were found. Early succession forest stands emerge following forest disturbances that create open habitats with more open canopy cover and larger amounts of deadwood structures. The main structural attributes found to be affecting changes in bird assemblages are basal area of deadwood, number of standing dead trees, total volume of deadwood, density of trees with missing bark, and mean canopy openness, all of which result from recent disturbances. The species significantly respond to the time since and the severity of the latest disturbance and their frequency. While some species respond positively to the open habitats, others are influenced negatively, keeping the net overall abundance, species richness, and Shannon diversity of the bird assemblage unchanged. A study by Basile et al. (2020b) in the Black Forest, Germany, focuses specifically on woodpecker cavities establishment in managed forests, in which case the TreMs (i.e. woodpecker cavities) result from direct activity of a bird species, while later serving as a habitat for breeding sites for other cavity-nesting species. The selection of trees by the woodpeckers is based not on the absolute diameter at breast height (DBH) but its relative deviation from the mean plot DBH. While the woodpeckers usually select larger trees, as they are likely older, potentially decayed, and consequently softer for excavation, the DBH class of 15 to 20 cm larger than the mean plot DBH was identified to be preferred, outlining the importance of relative, rather than absolute thresholds in the selection of trees to be retained. The authors emphasise the difference between managed and primary forests, where woodpecker cavities

serve a more important role for nesting species in managed forests, as the structures of natural cavities, created by decay, are less likely to occur than in primary forests.

The relationships between specific TreMs or TreM groups are frequently mentioned as a gap that needs further study. Basile et al. (2020a) address that in a study, identifying and quantifying the links between TreMs and the abundance and diversity of forest-dwelling bats, birds, and insects. The study is located in the Black Forest in Germany in a temperate mixed montane forest, dominated by Norway spruce, European beech, and silver fir at altitudes between 500 and 1400 m a.s.l. in a continuous cover forestry management system. TreM abundance was recorded at each plot on 15 trees with the largest canopy and, additionally, six smaller trees for representative results. Insects were continuously sampled with flight barrier window traps and collected monthly, bats were recorded for four nights with ultrasonic sound recorders, and birds were sampled by standardised point counts within a 50 m radius three times during the spring. The sampled dataset consists of 15 TreM groups (methodology by Larrieu et al., 2018), 10 insect groups, five bat groups and 29 bird species. The bird species correlated only weakly and were therefore excluded from the further analysis. The final model includes two TreM groups, concavities and rot holes, the insect orders of Heteroptera, Psocoptera, and Sternorrhyncha, and bats from Pipistrellus and Nyctaloid groups. Direct links are found between the abundance of rot holes and Psocoptera, Sternorrhyncha, and the Pipistrellus group, while Heteroptera and the Nyctaloid group correlate to concavities. While the TreMs contribute to the direct effect on organisms, other environmental predictors account for a larger share of the explanatory variation. While a strong relationship is demonstrated between invertebrates and bats, the birds show a lower correlation, explained by a wider set of attributes used by birds in addition to cavities. Some correlations, such as between the abundance of cavities and cavity-nesting birds, were not found but have been confirmed in other studies (e.g. Cockle et al., 2010; Paillet et al., 2018). The authors argue that this may be explained by the exclusion of snags, as the study only accounts for living habitat trees (trees bearing TreMs) while standing dead trees have been demonstrated to host higher densities of TreMs compared to live trees. Specifically comparing managed forests to strict forest reserves, the latter are proven to host significantly higher densities (+166%) of woodpecker cavities, mainly explained by the density of snags (Paillet et al., 2017). Basile et al. (2020a) conclude that several true biotic interactions can be disentangled from co-occurrence patterns. TreMs with the highest correlation to the tested organism groups are cavities and rot holes; however, apart from the Pipistrellus bat group, other forest structures, such as deadwood, explain a higher proportion of the variation in occurrence and abundance of organisms. TreMs can be used additionally to other structural indicators and used to objectively select habitat trees for retention in otherwise managed forests.

The concept of TreMs is starting to be implemented in Central European temperate forests in various countries, although predominantly in retention practices, namely the selection of habitat trees, individually or in groups. Most frequently, older stands are considered to maintain about ten trees per ha. The main holdback is the evidence of a correlation between actual biodiversity and the occurrence and abundance of TreMs (Asbeck et al., 2021). Strong evidence exists for the link between TreMs and bats, birds, and, to some extent, saproxylic insects (Paillet et al., 2018; Basile et al., 2020a), while other species assemblages remain to have more evidence (Asbeck et al., 2021). TreMs are also only accounted for on living and dead standing trees (Larrieu et al., 2018),

while some species prefer lying deadwood (Kraus et al., 2013). TreMs do not catch the whole spectrum of site conditions affecting the occurrence and abundance of organisms, such as climate (Betts et al., 2017), distance to other populations (Ockinger et al., 2005), or management history (Paillet et al., 2010), while the observations may not detect rare or endangered species (Hedgren & Weslien, 2008). Another source of error may originate from the observer effect, having both true and false positive observations during an inventory (Paillet et al., 2015). An airborne laser scanning approach has been tested to detect TreMs with promising results (Frey et al., 2020; Santopuoli et al., 2020). The results are encouraging for some groups, such as epiphytic bryophytes, root buttresses, branch holes, and cavities. However, more abundant TreMs are generally detected more easily than rare ones, while TreMs at the top of canopies are more detectable than those at the bottom of the trunks (Santopuoli et al., 2020).

3.4.3 Factors Influencing Microhabitat Occurrence

The occurrence, abundance, and richness of TreMs are influenced by multiple factors. However, some have been demonstrated to have a stronger impact than others. The factors include tree-level structural attributes, such as DBH, age, tree health and vitality, tree status (alive or dead standing), tree species, etc. Other factors include management type (unmanaged and primary forests, compared to various degrees of management intensity), environmental conditions, such as climate and soil properties, disturbance regimes, biological interactions, and stand-level variables, such as slope, aspect, and altitude (Martin et al., 2022; Larrieu et al., 2022).

The tree dimension, generally expressed in terms of DBH, is a strong and significant driver of TreM occurrence, abundance and richness in all studies that consider its predictive power (Paillet et al., 2019; Paillet et al., 2017; Kozák et al., 2018; Kozák et al., 2023; Martin et al., 2022; Larrieu et al., 2022; Asbeck et al., 2021; Vuidot et al., 2011; etc.). In general, large trees support a higher richness and abundance of TreMs across all tree species (Asbeck et al., 2021; Martin et al., 2022; Kozák et al., 2023). For the same DBH, broadleaf tree species tend to host a higher abundance and richness of TreMs compared to coniferous tree species (Paillet et al., 2019; Martin et al., 2022). The tree dimension is related to the tree layer in the vertical forest structure, where different crown classes, from dominant to suppressed trees, undergo different developments depending on natural disturbance patterns and tree growth strategies (Pavlin et al., 2021; Pavlin et al., 2023). They are exposed to various processes relating to TreM formation, such as crown breakage by wind in the upper canopy layer (Hale et al., 2012). Dominant trees in the upper canopy layer generally provide more TreMs than suppressed trees (Großmann et al., 2018). Large trees exhibit a buffering effect on microclimate that attracts cavity-nesting species. Therefore, cavity builders preferentially excavate cavities on large trees (Paillet et al., 2017). The relation of mean TreM abundance to tree canopy layer differs across tree species. In a study in the mountain forests of southwest Germany, it decreased by half in beech trees from the first, predominant canopy class to the third, partly dominant canopy layer (classification by Kraft, 1884), while the mean TreM abundance in silver fir was reduced by 80% in the same classes. The correlation is not clear, as the abundance in beech trees increased again in the dominated fourth class, reaching the same level of abundance as the first (Großmann et al., 2018). The ontogenic stage describes the tree's developmental stage and is divided into young, adult, mature, and senescent stages (Drénou et al., as cited in Larrieu et al., 2022). While young trees have limited branching axes with thin and

ephemeral branches forming a temporary crown, the adult stage consists of the establishment of many forks which structure the crown. Reaching the mature stage, the branches continue to develop. However, the crown extent does not further increase. The senescent stage is characterised by the development of crown deadwood and other TreMs related to wounds, while the establishment of forks favours the presence of microsoils and dendrotelms (Larrieu et al., 2022). Larger and generally older trees have a longer life history, resulting in being exposed to more TreM-forming events, such as rock falls, storms, or frost (Paillet et al., 2017). The ontogenic stage is closely related to the tree DBH and age. However, it should be interpreted separately (Larrieu et al., 2022) except for its close correlation in even-aged forests (Meinzer et al., 2011). While the tree age often relates to the tree size and DBH in even-aged forest stands (Meinzer et al., 2011; Rozas, 2003; Sumida et al., 2013), the largest trees are not necessarily the oldest when the forests are uneven-aged (Meinzer et al., 2011; Pavlin et al., 2021). Using a tree-ring-based approach, the effect of DBH and tree age on the development of TreMs have been disentangled in the spruce and beech primary mountain forests in the Carpathians (Kozák et al., 2023). The tree age positively influences the overall TreM abundance and richness, significantly affecting the probability of occurrence of five TreM groups (typology by Larrieu et al., 2018), namely the burrs and cankers, insect galleries, exposed sapwood, concavities, and crown deadwood. In spruce forests, the tree age explained a larger proportion of the variation than DBH for the TreM groups of insect galleries, exposed sapwood, and burrs and cankers. At the same time, it was a stronger predictor compared to DBH in beech forests for TreM group burrs and cankers. Tree DBH was, however, the most important predictor for the overall TreM richness in both forest types. It positively influenced the probability of the occurrence of eight out of 13 TreM groups included in the study. Nevertheless, selecting habitat trees based on DBH alone is acceptable and presents a low risk of underrepresenting TreMs (Kozák et al., 2023).

The live status of trees is another major determinant of TreMs. Compared to living trees, snags have been demonstrated to provide more TreMs on trees of the same species and DBH (Asbeck et al., 2021; Larrieu & Cabanettes, 2012; Paillet et al., 2017; Kozák et al., 2018). Specifically, the TreM group of woodpecker feeding holes was higher by 166% when comparing managed forests to unmanaged forest reserves in French mountain and lowland forests, which was attributed to the higher density of snags (Paillet et al., 2017). The wood of snags is softer for excavation of cavities compared to living trees, while deadwood provides a food source, hosting a variety of insect species (Vuidot et al., 2011; Lassauce et al., 2011). Saproxylic fungi and cracks are more likely to occur on snags than on live trees (Vuidot et al., 2011; Larrieu & Cabanettes, 2012). A study in France found all tree genera but pine to have a higher abundance of TreMs on snags compared to living trees (Paillet et al., 2019). Not just snags, trees with decreased vitality host more TreMs than live and healthy trees (Kõrkjas et al., 2021; Vuidot et al., 2011; Larrieu & Cabanettes, 2012). A study in declining coniferous forests found no difference in the decline-induced local abundance of habitat trees. However, there was a significant increase in fungal TreMs, bark pockets, cracks, and an increased amount of injury related TreMs (Bouget et al., 2023).

Different TreM types are more likely to occur on different tree species (Vuidot et al., 2011; Courbaud et al., 2017; Kozák et al., 2018; Sever & Nagel, 2019; Larrieu & Cabanettes, 2012; Larrieu et al., 2014; Paillet et al., 2019). For example, sap runs are more likely to occur on

deciduous trees, while coniferous tree species are more susceptible to broken tops (Kozák et al., 2018). Outgrowths and bark characteristics occur more frequently on oaks (*Quercus spp.*), spruces, and firs compared to beech (Vuidot et al., 2011). Generally, higher tree species richness is positively related to TreM density and diversity (Kozák et al., 2018; Larrieu & Cabanettes, 2012; Paillet et al., 2019; Larrieu et al., 2022). Broadleaved species supply a higher abundance and richness of TreMs than conifers (Asbeck et al., 2017; Asbeck et al., 2019). However, the highest abundance of TreMs on the plot level occurs in mixed-species forest stands (Asbeck et al., 2019). Combining the data on the occurrence of habitat trees in fir and beech long unmanaged montane forests, the species explained around 16% and 10% of the variance. In comparison, a combination of species and DBH explained 26% of the variation (Larrieu et al., 2014). TreMs have been observed to exhibit co-occurrence patterns at the tree scale. The co-occurrences are more numerous on broadleaves than on conifers (Larrieu et al., 2021). Co-occurrences on European beech was confirmed between rot holes and root concavities, while the TreMs bark loss and crack and sap run and rot hole co-occurred on silver fir (Larrieu & Cabanettes, 2012; Larrieu et al., 2021). The dynamics of their evolvement explain some of the co-occurrences, for example, bark loss is prone to get infected by wood-decomposing fungi, leading to the development of a rot hole if the tree is unable to cover the wound (Larrieu et al., 2021).

Management practices have differing effects on TreM occurrence, richness, and abundance. The impact depends on the intensity of management, time since the abandonment of management, or if the forests are primary and have never been managed (Larrieu et al., 2022; Larrieu et al., 2018; Martin et al., 2021a; Martin et al., 2022; Paillet et al., 2017; Vuidot et al., 2011; Asbeck et al., 2022). Harvesting trees may, on one hand, remove habitat trees (Asbeck et al., 2022), while the harvesting operations themselves damage trees and trigger the development of new TreMs (Larrieu et al., 2012b; Vuidot et al., 2011; Asbeck et al., 2021). TreMs are generally more abundant and diverse in primary, compared to managed forests (Paillet et al., 2017; Winter et al., 2015; Asbeck et al., 2022). However, the main driver is a higher density of snags and large and old trees in primary, compared to managed forests, which drives the higher abundance and density of TreMs (Paillet et al., 2017; Vuidot et al., 2011; Larrieu & Cabanettes, 2012). When accounted for the DBH and live status, some studies found no significant difference between managed and unmanaged forest stands (Vuidot et al., 2011), although frequently the comparison was in forest stands left unmanaged for a period of time and not necessarily primary forests. However, studies have also been carried out in primary forests across Europe (e.g. Kozák et al., 2018; Kozák et al., 2023; Asbeck et al., 2022). When comparing managed and primary forests, the latter exhibited a higher abundance and diversity of TreMs on living trees. TreM abundance and richness are assumed to increase with tree senescence, leading to the development of more TreMs as the trees grow older compared to individuals of similar dimensions in managed forests (Asbeck et al., 2022). Certain TreM types are more likely to occur in managed, while others in unmanaged and primary forests. For example, large crown deadwood in lower parts of the canopy is significantly more likely to occur in unmanaged forests, rot holes, and concavities (Asbeck et al., 2022), while exposed heartwood, bark loss, dendrotelms, and broken limbs can be more frequently observed in managed forests (Larrieu et al., 2012b; Vuidot et al., 2011; Asbeck et al., 2021).

The abundance of TreMs generally increases with altitude, however, it does not significantly impact their diversity. It affects the occurrence of epiphytes, with more epiphytes

occurring in higher elevations (Asbeck et al., 2019; Asbeck et al., 2022; Kozák et al., 2023). The study by Asbeck et al. (2019) also found an increase in the abundance of root cavities with increasing elevation, driving an overall increase in TreM abundance with elevation. South-facing slopes may increase the occurrence of mistletoes, thin or compact soils, wind, and steep slopes increase the buttress formation, thin soils favour branch death, strong winds lead to stem and limb breakages and fork splits. The presence of cliffs upslope increases the probability of wounds and steam breakage due to impacts from falling rocks (Larrieu et al., 2022).

4. Materials and Methods

4.1 Study Site Characteristics

Bulgaria is a country located in southeast Europe on the Balkan Peninsula (Fig. 3). Lowlands and hills below 600 m cover 72% of the territory, while highlands of 600 to 1600 m cover 25% and mountains above 1600 m are 3% of the area. The Balkan Mountains act as an important divide, splitting Bulgaria into north and south and strongly impacting the temperature regime. The annual mean temperature in Bulgaria varies between -3.0 and 14.0 °C, depending on the elevation and location. It reaches -10.9 to 3.2 °C in January and 5.0 to 25.0 °C in July. The total mean annual precipitation is between 500 and 650 mm, ranging between 440 and 1020 mm, depending on circulation patterns, elevation, and the local orographic features. The driest months are February, March, and September (monthly average of 30 to 45 mm), while the most precipitation falls in May and June (monthly average of 55 to 85 mm). Mean precipitation between April and September is 333 mm with a standard deviation (std) of 72 mm and a maximum of 573 mm in the Balkan Mountains (Alexandrov et al., 2004).

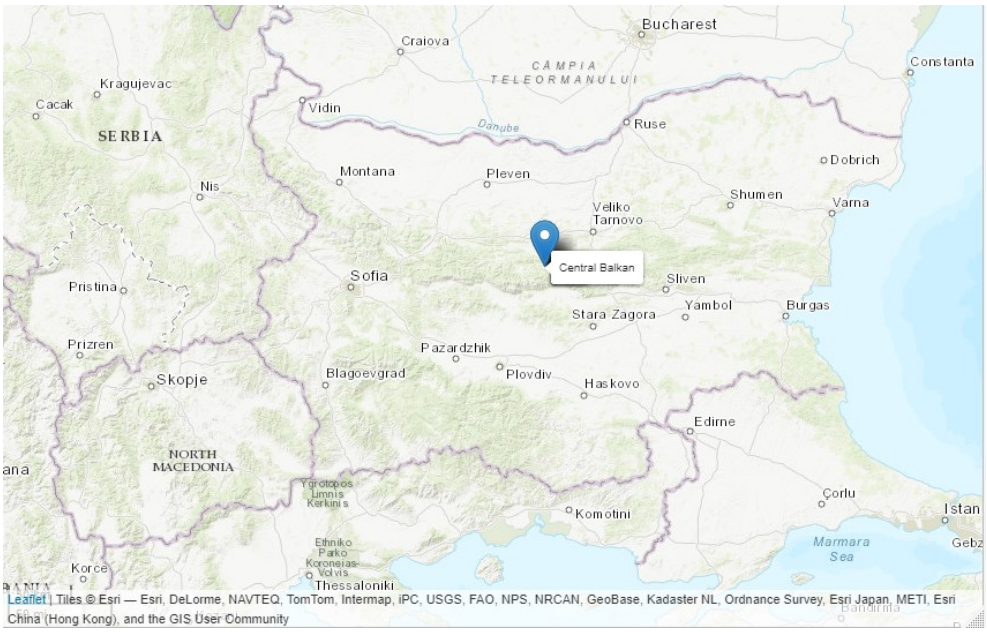

Figure 1: Map of study stands' location.

Forests cover an area of 3,551,500 ha in Bulgaria, covering 30% of the total area. Natural forest ecosystems represent 63.5% and plantation forests 36.5% (Raev et al., 2005). The majority of the forests in Bulgaria are owned by the state (73%), followed by municipal forests (13%), private forests (11%), and others (3%). Forest ownership shows an increasing trend in the share of non-state-owned forests compared to 2005 (Stoeva et al., 2020). Broadleaves represent 68.4 % (2,427,800 ha) and coniferous 31.6% (1,123,700 ha) of the forest area. The species composition is rich, resulting from the biogeographic position between the Mediterranean, Steppe, and European broadleaved forests, reaching from sea level up to 2927 m a.s.l. and forming altitudinal belts. The most abundant tree species are from the genus of oak (*Quercus spp.*)

(32.2%), found mainly in the plains up to 700 m and European beech (*Fagus sylvatica*) (16.9%), primarily growing between 600 to 1300 m a.s.l. Coniferous species are represented by Scots pine (*Pinus sylvestris*) with 16.5%, Austrian black pine (*Pinus nigra*) with 9.3 % and Norway spruce (*Picea abies*) with 4.6%. Other species include silver fir (*Abies alba*), Macedonian pine (*Pinus peuce*), Mountain pine (*Pinus mugo*), Pinus Heldreich (*Pinus heldreichii*) and others, adding up to 15 coniferous and 344 broadleaved tree and shrub species in Bulgarian forests (Raev et al., 2005). Protected areas in Bulgaria cover 45,503 km2, 41.04% of the land area (34% by the 5th National Report coverage). Out of all protected areas, 3.85% are under the IUCN category Ia strict nature reserves (Protected Planet, 2024).

Our study sites are located in the Central Balkan National Park (CBNP). CBNP is in central Bulgaria and includes the central and highest parts of Stara Planina mountain (The Balkan mountain range). The park lies in an East-West direction, expanding over 85 km in length and 10 km in width, ranging between 550 and 2376 m a.s.l., reaching the highest point at Botev peak. The forests cover an area of 44,000.8 ha (61%), while the mountain pastures and meadows cover an area of 27,668.7 ha (39%) (UNESCO World Heritage Centre [UNESCO], 2024).

The climate of the northern part of CBNP is moderately continental, while the southern part is transitional continental. The north part of the mountain range has cold winters with average January temperatures of -1.5 to 3.5 °C and cool summers of 16.0 to 22.0 °C. The southern part of the mountains has average January temperatures between 0.0 to 1.5 °C and summer temperatures of 17.0 to 21.0 °C (Mirchev, 2013, as cited in Nikolov & Dimitrov, 2023). The low-mountainous zone (1000-1500 m) of CBNP has, on average, 115 to 150 days with temperatures above 10 °C, and the duration of the vegetation season ranges between four to five months. The average annual temperature ranges between 4.5 to 7.0 °C and the average annual precipitation between 830 to 950 mm (Nikolov & Dimitrov, 2023). The geology of CBNP consists of granites, sandstones, diorites, South Bulgarian granites, schists, porphyres, dolomites, etc. The most common soil types are dystric and eutric cambisols, covering 97 % of the forested area, followed by chromic luvisols (2.0%) and umbric cambisols (0.8%) in the high mountain boreal zone. rendzinas cover some of the karst areas of the park, with a thickness of 10 to 30 cm (Petrova & Tzonev, 2013, as cited in Nikolov & Dimitrov, 2023).

CBNP was established in 1991 and, covering 720 km^2 (Protected Planet, 2024), is the third largest protected area in the country and one of the three national parks. It is classified under the second category (national park) according to the International Union for Conservation of Nature (IUCN) (Central Balkan National Park [CBNP], 2024), while the nine strict reserves; Boatin, Steneto, Tsarichina, Dzhendem, Severen Dzhendem, Kozya stena, Stara reka, Peeshti skali and Sokolna are classified under the first category as strict nature reserves (Protected Planet, 2024). CBNP is a protected zone under the European Union's NATURA 2000 Birds and Habitats directives and is certified as a member of the Federation of European Wildlife and National Parks (EUROPARC). Since 2017, the park and the area of five municipalities around it have been declared as Central Balkan Biosphere Reserve under the UNESCO's Man and Biosphere Programme, encompassing the four previously existing biosphere reserves Steneto, Boatin, Tsarichina, and Dzhendema, designated in 1977. Since 2017, some of the beech forests within the nine park reserves have been included in the serial site The Ancient and Primeval Beech

Forests of Carpathians and Other Regions of Europe to the UNESCO World Natural Heritage (CBNP, 2024).

Forests cover 61% of CBNP (UNESCO, 2024). Deciduous trees cover 83% of the forested area. Forests reach up to 1850 m a.s.l., and over 88% of the forests lie above 1000 m a.s.l. The relatively large area of CBNP, the border of transitional continental and moderately continental climate, and the large altitude range affect the variety of habitats. The European beech forests and the mesophilous beech forests cover the largest area. Austrian black pine forests grow under dry conditions on carbonate soils with poor nutrients. The riparian forests grow in the wettest and richest conditions at low altitudes in the moderately continental climate zone. The most thermophilic forests are silver linden (*Tillia tometosa*), while Macedonian pine and Norway spruce forests grow on acidic soils in lower temperatures (Nikolov & Dimitrov, 2023).

Old forests in Bulgaria have been described in the 19th century, mentioning beech and oak forests in the Central Balkan range, oak forests in Strandzha mountain, pine, fir, spruce, and beech forests in Rila mountain and in the Rhodopes. The extent of forests gradually decreased between the 16th and 19th centuries due to increased demand for energy sources, such as wood and charcoal, for iron ore processing by the Turkish army and heating of Istanbul. Large-scale exploitation started during the Crimean War (1853-1856) and continued with the construction of the telegraph, railroad network, and for export. Following the Liberation in 1878, the demand for timber and firewood increased, and many forests were converted into arable land due to increased demand by the developing industry, small-scale farming, transport, and trade. Forest fires and illegal exploitation increased due to the multiple wars: Balkan (1912-13), Inter-Alliance (1913) and WWII (1914-18). The forests were nationalised after WWII (1941-45), leading to intensive cutting, large-scale afforestation, and the development of forest road networks in previously inaccessible areas (Raev et al., 2005).

The mapping, inventory, and creation of a strategy for sustainable management and protection of primary forests in Bulgaria was carried out by the Royal Dutch Society for Nature Conservation (KNNV) and the Forest Research Institute of the Bulgarian Academy of Sciences (FRI-BAS) between 2002 and 2006. The degree of naturalness (considered synonymous with primarity) was used as a basis for selecting and assessing virgin forests (considered synonymous with primary forests), carried out between 2003 and 2005. The considered selected indicators include the presence of naturally occurring vegetation, the absence (or near absence) of human activity, the age structure of stands, the presence of old trees and various ages, the presence of endemic species (non-obligatory), the presence of typical mammals and birds and good health status of vegetation (presence of dry and fallen trees considered natural). Another important consideration was the minimal area so that natural processes can flow normally. It was determined as 30 ha for deciduous forests, 20 ha for coniferous forests and 25 ha for mixed forests. Smaller patches were also described, as they must be preserved and can serve as stepping stones (green corridors) and a genetic pool. Buffer zones around the reserves should be present and protected, ranging from at least 50 to 100 m (Raev et al., 2005).

All the virgin forests in Bulgaria are in the mountainous areas. They cover 103,356.1 ha of forest areas, accounting for 2.9% of the forested area of Bulgaria. The largest share of the virgin forests is found in the Balkan range (33.82%), followed by Pirin Mountain (26.03%) and Rila

Mountain (19.73%). Smaller proportions are in the Rhodope Mountains (8.54%), Strandzha Mountain (5.99%) and the remaining 5.89% in other mountains. They are characterised by steep slopes, while the areas with the largest shares are additionally characterised by the so-called closed basins with no road infrastructure present. Their location can largely be explained by the altitude and steepness of the terrain, ranging above 1000 m a.s.l. and in 88% on slopes steeper than 10° (Raev et al., 2005).

The protected virgin forests in the Balkan range are predominantly Fagus sylvatica forests. They grow continuously between 800 and 1600 m a.s.l., sometimes also in lower elevations. They mostly grow in pure stands, although they occur also mixed with European hornbeam (*Carpinus betulus*), silver fir, Austrian black pine, and other tree species. They are followed by Norway spruce forests (6.7%), silver fir forests (4.9%), sessile oak (*Quercus petraea*) forests (3.1%), European hornbeam forests (2.6%), Oriental hornbeam (*Carpinus orientalis*) forests (2.0%) and others (Raev et al., 2005).

Figure 2: Primary mountain forest in Central Balkan mountain range, Boatin forest reserve.

A total of 60,494.2 ha of forests were investigated in the Balkan range, out of which 34,954.3 ha, representing 57.8% of the total forest area, were identified as virgin forests. Most, 85.4%, were found in the Central Balkan, followed by 9.9% in Western and 4.7% in Eastern Balkan. The largest areas, 22,548.6 ha, representing 64.5%, were already protected as reserves. The size of the Steneto locality is 3593.8 ha and Boatin 1598.0 ha, all of which are classified as virgin forests. While Steneto is classified as pure beech forest, Boatin has an admixture of Norway spruce. The protected virgin forests in the Balkan range are predominantly Fagus sylvatica forests (Fig. 4). They grow continuously between 800 and 1600 m a.s.l., sometimes also in lower elevations. They mostly grow in pure stands, although they occur also mixed with European

hornbeam, silver fir, Austrian black pine and other tree species. They are followed by Norway spruce forests (6.7%), silver fir forests (4.9%), sessile oak forests (3.1%), European hornbeam forests (2.6%), Oriental hornbeam forests (2.0%) and others. Most virgin forests, 68.9%, were found on steep terrain of slopes above 21°, probably the main reason for their continuity until the present day (Raev et al., 2005).

4.2 Sampling Design

The plots in Bulgaria were established and measured for the first time in 2017, following the methodology of the Department of Forest Ecology, Czech University of Life Sciences Prague (REMOTE Primary Forests, 2024). They were later remeasured in September 2022, when the data used in this study was collected. Inside the selected area, chosen with the help of local experts, the first points were generated randomly in a 10 ha grid area. The plots were created as nested plots, from the point centre in two directions along the contour with a distance of 40 m from the point, 80 m distance between the two pair plot centres (Fig. 5). On each end, a subplot was established. If the point centre was unsuitable due to, e.g. stone field, water, or steepness, the second randomly selected point was generated or, in rare cases, the third.

The data was sampled on composite three-level sampling concentric circles. The inventory plot consists of 3 circular nested plots:

- 1 – inner circle, radius 7.98 m, area 200 m^2,
- 2 – middle circle, radius 17.84 m, area 1000 m^2,
- 3 – outer circle, radius 21.85 m, area 1500 m^2.

Each plot was given a unique plot identification number, which includes the country, stand (location), plot number and subplot (pair plots 1 and 2), e.g., BUL_BOA_412_1. The data on precise plot location is available in latitude and longitude. The altitude, slope, and aspect were measured and the plot's landform and hillform were assessed.

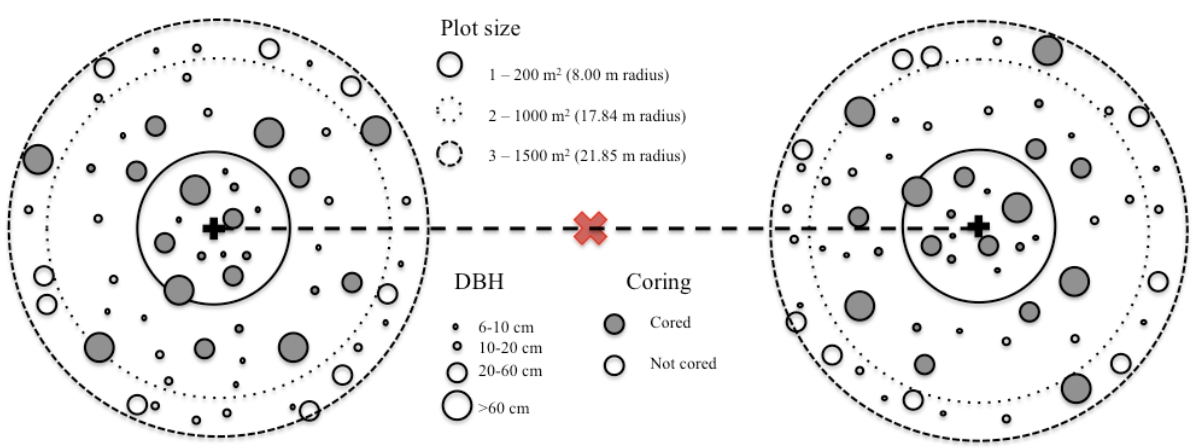

Figure 3: Example of the nested plot location. The red cross indicates the randomly generated centre point from which the two plots are established (source: KEL methodology).

The measured data includes all live and dead standing trees with a diameter at breast height (further DBH, measured in 1.3 m of height) larger or equal to 60 mm, stumps that have at least 200 mm diameter at 0.3 m height above ground, and freshly uprooted trees with a diameter at 1.3 m of tree height of 200 mm or more. The trees are mapped within all three circular plots (1500 m^2 plots). In total, tree height was measured on 15 trees, including 5 biggest trees based on the DBH measurements. From the most abundant tree species, in total 10 trees were selected (e.g., if there were 60% beech, 30% fir, and 10% spruce, 6 beech trees, 3 fir and 1 spruce were selected), covering the diameter range from small, medium, and large, preferably the trees where the tree core was obtained. The crown projection area was recorded for five to 15 trees per plot. Further, the DBH, species, tree status, tree layer, and social status (suppressed or non-suppressed with at least one half of the crown projection under the canopy condition) were recorded for each tree with a DBH ≥ 60 mm. For the dead standing trees, the decay class, decay height (in 10 m classes), and wood decay class were recorded for each tree with a DBH ≥ 60 mm or a diameter of at least 200 mm at 0.3 m of the tree height in case of stumps under 1.3 m height. TreMs were recorded for all live and dead standing trees with a DBH ≥ 60 mm following the methodology by Larrieu et al. (2018) at the 47 TreM types level (Appendix 1).

The tree age for the plot level was calculated based on the tree chronological data. All live trees within the first circle with the DBH ≥ 60 mm were cored. In the second circle, 25% of the released trees in the DBH class 100-200 mm were cored with an additional random selection of every 4th tree. In the third circle, all living trees with DBH ≥ 600 mm were cored. The cores were extracted at 1 m tree height, perpendicular to the slope, to avoid reaction wood. The tree cores were processed using standard chronological procedures. Ring-widths were measured with LintabTM sliding stage measuring system (Rinntech, Heidelberg, Germany; http://www.rinntech.de). The cores were cross-dated using marker years (Yamaguchi, 1991) and verified with COFECHA (Holmes, 1983) and CDendro (Larsson, 2003). For the core samples that did not intersect the pith, the number of missing tree rings was extrapolated from the curvature and average growth rates of the innermost rings (Duncan, 1989). For a more in-depth methodology, see Pavlin et al. (2021).

4.3 Data Analysis

The dataset includes 42 plots (21 pairs of plots), 14 of them each in Boatin, Steneto-1, and in Steneto-2 stands. It consists of 2251 trees, 1211 of which (53.8%) bear at least one TreM (habitat trees). The altitude ranges between 1236 and 1452 m a.s.l. (Tab. 1), without much difference between the stands. The slope ranges between 6 and 45° and is, on average, higher in Boatin than in both stands in Steneto. The mean tree age is 275 years, reaching a maximum of 528 years. The DBH averages at 345 mm, ranging from 316 mm in Boatin to 361 mm in Steneto-1. The largest DBH measured was in Steneto-1, measuring 1290 mm.

Table 1: Study stand characteristics. Age data was acquired in 2017, to which we added 5 years for consistency with other data from 2022. Diameter at breast height data is based on all trees ≥ 60 mm.

	Mean altitude (min, max) (m a.s.l.)	**Mean slope (min, max) (°)**	**Mean age (max age)**	**Mean DBH (max DBH) (mm)**	**Mean DBH live trees (max DBH) (mm)**	**Mean DBH snags (max DBH) (mm)**
Boatin	1327 (1236, 1449)	32 (20, 45)	267 (528)	316 (1088)	310 (1088)	375 (1026)
Steneto-1	1334 (1246, 1452)	20 (15, 31)	321 (504)	361 (1290)	352 (1290)	436 (1290)
Steneto-2	1335 (1255, 1482)	23 (6, 32)	238 (449)	357 (1149)	364 (1149)	307 (1055)
total	1332 (1236, 1482)	25 (6, 45)	275 (528)	345 (1290)	342 (1290)	372 (1290)

The species composition in our study predominantly consists of European beech (Tab. 2). While there is an admixture of silver fir, sycamore maple, and wych elm in Steneto-1 and Steneto-2, there is a total predominance of European beech in Boatin.

Table 2: Tree species composition on study stands.

	European beech	**Wych elm**	**Silver fir**	**Sycamore maple**	**European beech (%)**
Boatin	821	0	0	0	100
Steneto-1	637	0	18	1	97.1
Steneto-2	659	2	104	9	85.14
total	2117	2	122	10	94.08

We excluded all TreM types that had no occurrences from our analysis, namely the TreM types of small woodpecker breeding cavity, woodpecker “flute”, fire scar, mistletoe, and vertebrate nest. Heavy resinosis was excluded from the tree level analysis as it was not present on beech trees but was later included again on the plot level analysis, having occurred on silver fir trees. Some other TreM types had very low occurrences (e.g. large woodpecker breeding cavity, witch broom, ivy and lianas, and others), which we decided to retain, as they contributed to the overall abundance and diversity of TreMs. We presumed that these are not the result of the observer effect (Paillet et al., 2015). The final model, therefore, consists of the remaining 41 TreM types, described in Appendix 1.

Following Zuur et al. protocol for data exploration (2010) we inspected the dataset. All analyses were performed in R language version 4.3.2 (R Core Team, 2023) using libraries “tidyverse” (Wickham et al., 2019) for data analysis and packages “ggplot2” (Wickham, 2016) and “ggpubr” (Kassambara, 2023) for plotting the graphs. We fitted models using “glmmTMB” (Brooks et al., 2017) and plotted model residuals using “DHARMa” (Hartig, 2022).

We visualised the outliers on boxplots, separately for each of the stands (Fig. 6). We plotted the TreM type richness against DBH and tree status to visually inspect the data (Fig. 7).

Based on the visual inspection we excluded two outliers, the tree with the TreM richness of 12 TreM types and a specimen of a snag with 1 TreM, but a DBH of 1290 mm, to enhance the model performance.

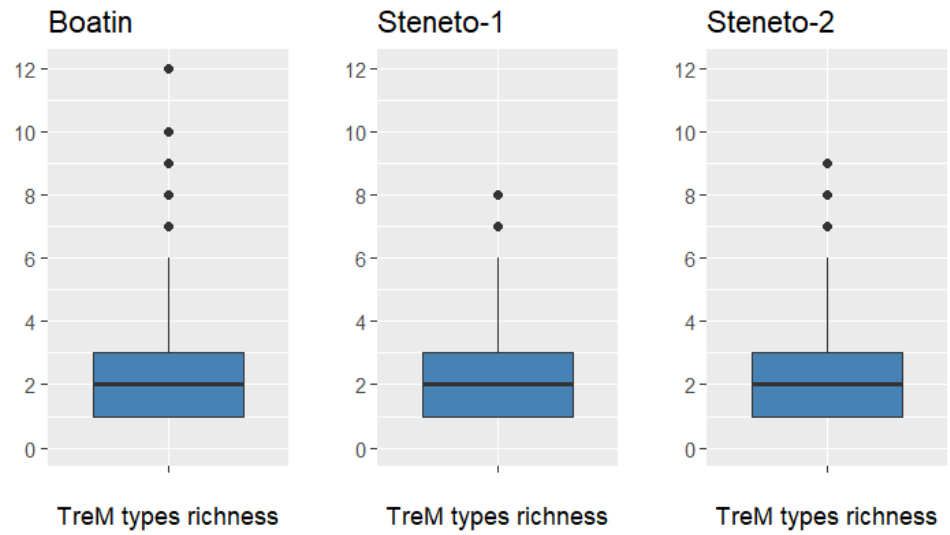

Figure 4: Boxplot of tree-related microhabitat types richness per all habitat trees across the study stands.

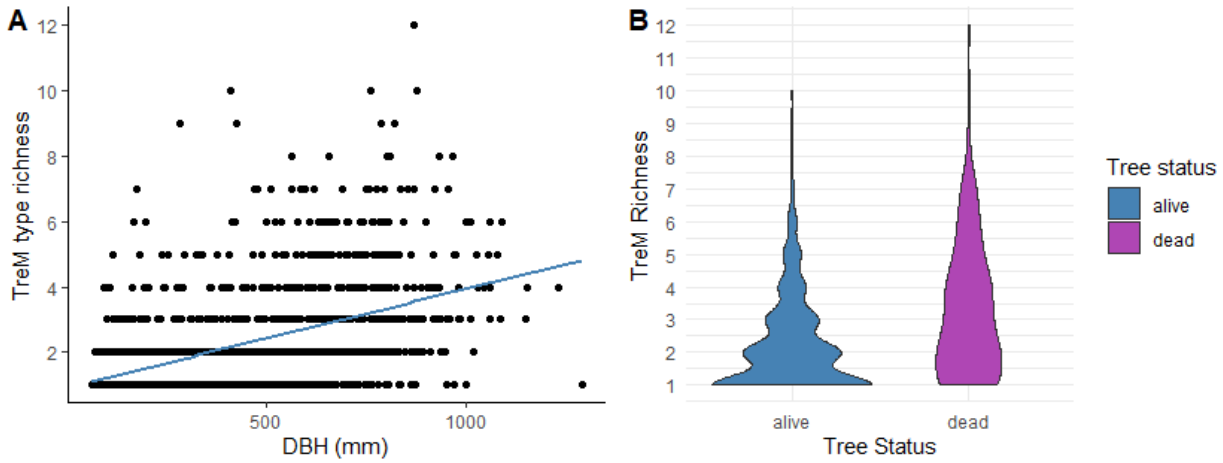

Figure 5: A) Scatter plot with linear trendline with diameter at breast height plotted against tree-related microhabitat type richness, B) violin plot with tree-related microhabitat type richness distribution by tree status.

We tested the correlation between selected predictors to the TreM richness per habitat tree. We define TreM richness as the sum of TreM types (Larrieu et al., 2018); each type is counted as a zero or one depending on the occurrence. Based on the literature review (e.g. Paillet et al., 2019; Larrieu et al., 2022; Asbeck et al., 2022), we evaluated the effect of tree DBH (continuous variable), altitude (positioned at the centre of the plot), tree status (categorical variable, expressed as alive or dead standing), and tree species, separately. Since the species composition of habitat trees was predominantly European beech (N= 1156), while other species, namely silver fir (N= 46), sycamore maple (N= 10), and wych elm (N= 2), accounted for a small fraction of total population, we decided to exclude the predictor from further analysis. Based on a low number of

occurrences and different TreM profiles (Paillet et al., 2019), we decided to exclude the trees from further analysis on the tree level. We additionally excluded three trees based on their missing values of DBH and the two aforementioned outliers. The final tree-level analysis dataset, therefore, consists of 1151 habitat trees (DBH ≥ 60 mm). Due to the non-significant effect of altitude on TreM richness, we omitted it from the model to avoid unnecessary complexity based on the improved AIC (Akaike Information Criterion). The AIC is used as a measure to compare the relative goodness of fit based on the trade-off between the model complexity and model fit (Bolker et al., 2009). We included only the habitat trees to avoid the issue of zero inflation (Asbeck et al., 2022). We tested the Pearson and Spearman indices of correlation between the DBH and tree status. The Pearson index of correlation is 0.113, and the Spearman index of correlation is 0.121, both of which indicate no significant correlation.

To model the richness of the TreM types per habitat tree, we built a generalised linear mixed model (GLMM) with the “glmmTMB” function from “glmmTMB” library package (Brooks et al., 2017) with a Poisson error distribution as our data is in counts of TreM types per tree. We set the predictor values DBH (mm) and tree status (dead, alive) as fixed effects. To prevent the pseudoreplication of trees within the same subplot, plot, or stand, where the trees in the same group might have similar characteristics compared to other individuals (Bolker et al., 2010), we set the random effect in a hierarchical nested sample plot design, as previously demonstrated in other studies (Paillet et al., 2019; Asbeck et al., 2022; Kozák et al., 2023). We additionally tested a GLMM with a negative binomial distribution, “glmer.nb” function, from “lme4” package due to the overdispersion of Pearson residuals. The glmmTMB model with Poisson distribution had an AIC value of 3757.3, while the “glmer.nb” had an AIC value of 3781.6. Hence, we continued with the glmmTMB model with Poisson distribution.

The final formula is designed as follows:

TreM richness ~ DBH + tree status + (1 | stand/plot/subplot).

To test for potential plot-level effects of the forest structure, we modelled the total TreM abundance, expressed in total TreM count per plot, and the diversity of TreM types, expressed in the count of all TreM types present on the plot. Due to missing values from the DBH measurements from one of the plots in Boatin, we excluded it and performed the analysis for the remaining 41 plots.

We based our decision for the selection of predictors to model the TreM abundance partially on the literature review (Larrieu et al., 2014; Larrieu et al., 2022; Kozák et al., 2018). However, we wanted to test possible interactions with other forest structure variables that might not have been proven significant yet. We tested the predictor variables in separate GLMMs with already present random effect set to account for spatial correlation of pair plots and stands. Specifically, the variables identified include the basal area of all trees with DBH ≥ 60 mm, the density of all trees with DBH ≥ 60 mm, the density of dead trees with DBH ≥ 100 mm, the density of live trees with DBH ≥ 100 mm, the density of live trees with DBH ≥ 500 mm, altitude (m a.s.l.), and patchiness. Based on the AIC value of the model with different combinations of density predictors, we selected the predictor density of all trees with DBH ≥ 60 mm, as it improved our model the most. We tested for the Pearson and Spearman index of correlation and plotted the

variables to visually inspect the correlations of fixed variables versus the response variable (Fig. 8).

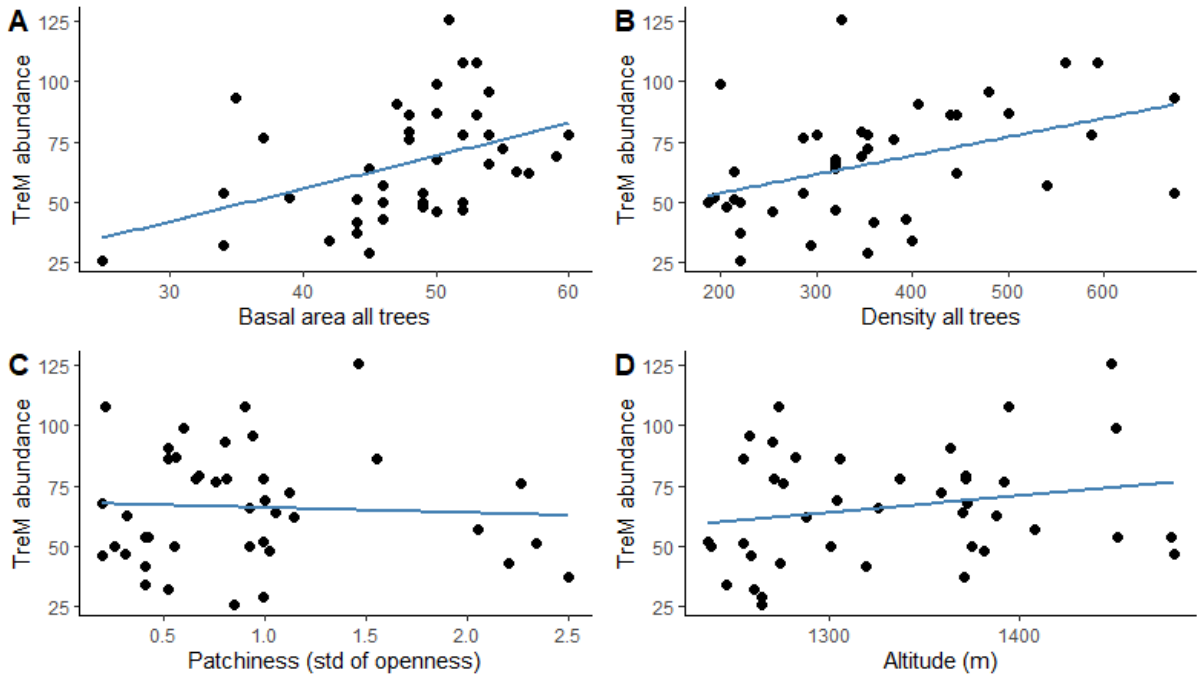

Figure 6: Scatter plots with linear trendline with A) basal area of all trees (DBH ≥ 100 mm) plotted against TreM abundance, B) density of all trees (DBH ≥ 60 mm) plotted against TreM abundance, C) patchiness (std of openness) plotted against TreM abundance, and D) altitude (m) plotted against TreM abundance.

After fitting the full model, we excluded variables that no longer exhibited significance and increased the AIC value until we got a fitting model. We fitted it with a "glmmTMB" function and Poisson distribution, which showed significant effect of basal area of all trees with DBH ≥ 100 mm), density of all trees (DBH ≥ 60 mm), patchiness (std of openness), and altitude (m). However, when we fitted the model with a negative binomial distribution with log link, the AIC value decreased significantly, from 376.6 to 363.3. The model with negative binomial distribution no longer exhibited significance for the predictor patchiness. Comparing the reduced models (Tab. 3), we fitted the final model with negative binomial distribution and included the basal area of all trees with DBH ≥ 100 mm, density of all trees with DBH ≥ 60 mm, and altitude (m) as the model performed the best out of compared. The inclusion of random effects improved the model fit. We scaled out variables using function scale(). The final model formula is designed as follows:

TreMs abundance per plot ~ scale(basal area of all trees with DBH ≥ 100 mm) +

scale(density of all trees with DBH ≥ 60 mm) +

scale (altitude (m)) +

(1 | stand/plot).

Table 3: Results of fitted GLMMs with Poisson and negative binomial distribution including different combinations of fixed and random effects, explaining TreM abundance on plot level.

	AIC	BIC	logLik	deviance	df.resid
Poisson distribution model (full)	376.6	388.6	-181.3	362.6	34
Negative binomial distribution (full)	363.3	377.0	-172.6	347.3	33
Negative binomial distribution (reduced, no patchiness)	**361.7**	**373.7**	**-173.8**	**347.7**	**34**
Negative binomial distribution (reduced, no basal area, no patchiness)	371.9	381.2	-179.5	359.0	35
Negative binomial distribution (reduced, no density of all trees, no patchiness)	365.8	376.1	-176.9	353.8	35
Negative binomial distribution (reduced, no altitude, no patchiness)	363.4	370.3	-177.7	355.4	37
Negative binomial distribution (no random effects, no patchiness)	362.4	371.0	-176.2	352.4	36

We followed the same approach for the selection of variables to model the TreM type diversity richness. The variables that exhibit significant predictive power are the density of all trees (DBH ≥ 60 mm) and density of dead trees (DBH ≥ 500 mm). We compared the model to a reduced model without accounting for the random effect of the stand and plot (Tab. 3), which significantly improved the model, so we decided to exclude it from the final model. We tested the negative binomial distribution, but, based on the AIC values, 223.0 for negative binomial distribution and 221.0 for Poisson distribution, the latter exhibits a better fit (Tab. 4). We tested for the Pearson and Spearman index of correlation and plotted the variables to visually inspect the correlations of fixed variables versus the response variable (Fig. 9)

Table 4: Results of fitted generalised linear mixed models with Poisson and negative binomial distribution including different combinations of fixed and reduced effects, explaining the diversity of tree-related microhabitats on the plot level.

	AIC	BIC	logLik	deviance	df.resid
Poisson distribution model (full)	225	233.6	-107.5	215	36
Poisson distribution model (reduced, no random effects)	**221**	**226.2**	**-107.5**	**215**	**38**
Negative binomial distribution (reduced, no random effects)	223	229.9	-107.5	215	37

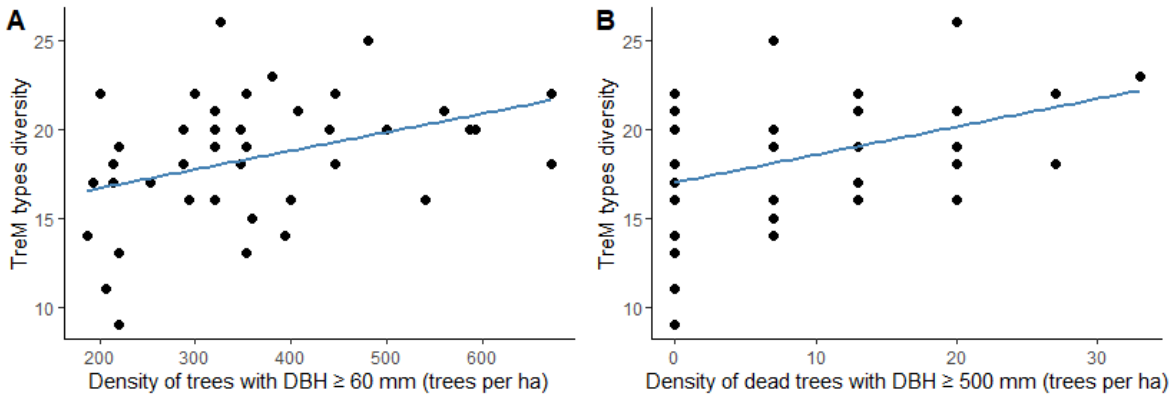

Figure 7: Scatter plots with linear trendline with A) density of all trees DBH ≥ 60 mm (trees per ha) plotted against TreM types diversity, B) density of dead trees with DBH ≥ 500 mm (trees per ha) plotted against TreM types diversity.

The final model formula is designed as follows:

TreMs types diversity richness per plot ~ scale (density all trees DBH ≥ 60 mm) +

scale (density of dead trees with DBH ≥ 500 mm).

In the final part of our study, we analyse the stand level characteristics and TreM profile with data aggregation to study stands. We compare the tree densities, densities of live trees and snags, and the density and percentage of habitat trees. Our aim here is to present stand-level data from mountain primary beech forests in Bulgaria, that can serve as benchmark data for other studies, while interpreting the »random effect« of the stand level, previously used in the models.

5. Results

We've identified 41 TreM types out of 47 described in the methodology by Larrieu et al. (2018), across 42 surveyed plots. The response variable, expressed as the richness of TreM types present on habitat trees, varies between 1 and 12 TreMs per tree. However, many trees only bear a single TreM (N= 459) with the mean TreM richness at 2.38. The model was fitted with the selected fixed and random effects with a GLMM with Poisson distribution. The fitted model (Fig. 10) exhibits a level of overdispersion (Appendix 1), identified with the DHARMa package (Hartig, 2022). However, it performs comparatively better (lower AIC) than other models (e.g. glmmTMB with negative binomial dispersion) while the model assumptions meet the criteria. Due to the overdispersion, the results (Tab. 5) should be interpreted with caution. The random effects, namely the stand, plot and subplot nested hierarchical spatial structure, significantly contribute to the improvement of the model fit. The calculated standardised coefficients (stdcoef) indicate that for each std in DBH (mm) increase, TreM richness corresponds to an increase of 0.2 std while holding all other predictors constant, with a standard error (stdse) of 0.013. An increase of one std in tree status (meaning the change from category live to dead standing) corresponds to an increase of 0.106 std in TreM richness and a stdse of 0.01.

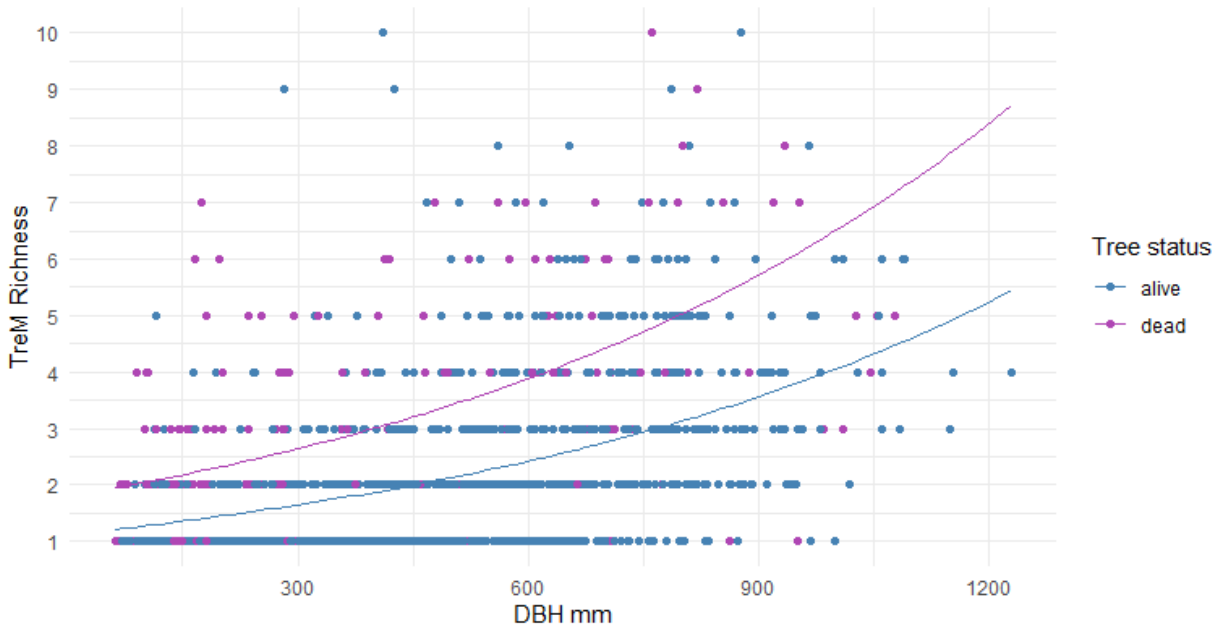

Figure 8: Fitted model with fixed effects of diameter at breast height, the tree status, and the predicted value of tree-related microhabitat richness, expressed in tree-related microhabitat types present on habitat trees on the tree level.

Table 5: Model output summary of the fitted glmmTMB with Poisson distribution, analysing the effect of DBH (mm) and tree status as fixed effects and the hierarchically nested random effects stand, plot, and subplot, on TreM types richness on tree level. Additionally, the standardized coefficients and standardized errors are calculated.

AIC	**BIC**	**logLik**	**deviance**	**df. resid**
3757.3	3787.6	-1872.6	3745.3	1145

Random effects:	**Variance**	**Std. Dev.**
subplot:plot:stand	0.016004	0.12651
plot:stand	0.006563	0.08101
stand	0.003458	0.05880

Fixed effects:	**Estimate Std.**	**Error**	**Z value**	**Pr (>|z|)**
(Intercept)	3.974e-02	7.016e-02	0.566	0.571
DBH (mm)	1.366e-03	8.674e-05	15.753	<2e-16 ***
Tree status	4.833e-01	4.777e-02	10.118	<2e-16 ***
Signif. Codes:	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'

Fixed effects:	**stdcoe**	**stdse**
(Intercept)	0.0000000	0.00000000
DBH (mm)	0.1996008	0.01267060
Tree status	0.1062862	0.01050494

TreMs abundance varies from 26 to 126 per plot, with an average of 66 TreMs per plot. For the analysis of the factors affecting the absolute TreM abundance on the plot level we fitted a GLMM with a negative binomial distribution. The results of the model summary (Tab. 6) indicate a good fit compared to more and less complex combinations of variables tested (Tab. 3, p. 47), based on the AIC value of 361.7 and plotted DHARMa residuals (Appendix 2). The estimated variance of TreM abundance attributed to the nested combination of stand and plot effects improves the model fit. The calculated stdcoef indicate that for each increase of one std in the basal area of trees with DBH ≥ 100 mm, TreM abundance increases by 0.007 std with a stdse of 0.002. For each increase of one std in density of all trees (DBH ≥ 60 mm), TreM abundance increases by 0.005 std with a stdse of 0.002. For each increase of one std in altitude, TreM abundance increases by 0.003 std with a stdse of 0.002.

Table 6: Model output summary of the fitted glmmTMB with negative binomial distribution, analysing the effect of basal area of all trees with DBH ≥ 100 mm, density of all trees with DBH ≥ 60 mm, and altitude (m) as fixed effects and the hierarchically nested random effects stand and plot on TreM abundance on plot level. Additionally, the standardized coefficients and standardized errors are calculated.

AIC	BIC	logLik	deviance	df. resid
361.7	373.7	-173.8	347.7	34

Random effects:	Variance	Std. Dev
plot:stand	0.016	0.125
stand	1.015	0.123

Fixed effects:	Estimate Std	Error	Z value	Pr (>|z|)
(Intercept)	4.15326	0.08469	49.04	<2e-16 ***
Basal area of all trees with DBH ≥ 100 mm	0.16552	0.04565	3.63	0.000288 ***
Density of all trees with DBH ≥ 60 mm	0.11516	0.04489	2.57	0.010309 *
Altitude (m)	0.07853	0.04705	1.67	0.095099 .
Signif. Codes:	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'

	stdcoef	stdse
(Intercept)	0	0
Basal area of all trees with DBH ≥ 100 mm	0.007002330	0.001931453
Density of all trees with DBH ≥ 60 mm	0.004871804	0.001899131
Altitude (m)	0.003322418	0.001990556

TreM types diversity on the plot level varies between 9 and 26 TreM types with a mean at 18.34. The model to account for TreM type diversity on the plot level was fitted with a GLMM with Poisson distribution. Due to higher value of AIC when including the random effects stand and plot, we excluded them from our final model. The results of the final model summary (Tab. 7) indicate a good fit compared to a model with negative binomial distribution (Tab. 4, p. 47), based on the AIC value of 221.0 for Poisson distribution (no random effects), compared to a value of 223.0 for a negative binomial distribution (no random effects). We plotted the DHARMa residuals (Appendix 3). The calculated stdcoef indicate that for each increase of one std in density of all trees (DBH ≥ 60 mm), we predict an increase of 0.022 std in diversity of TreM types with a stdse 0f 0.01. For an increase of one std in density of dead trees (DBH ≥ 500 mm), we predict an increase of 0.022 std in diversity of TreM types with a stdse 0.01.

Table 7: Model output summary of the fitted glmmTMB with Poisson distribution, analyzing the effect of density of all trees (DBH ≥ 60 mm) and density of dead trees (DBH ≥ 500 mm) as fixed effect on TreM abundance on plot level. Additionally, the standardized coefficients and standardized errors are calculated.

AIC	BIC	logLik	deviance	df. resid
221.0	226.2	-107.5	215.0	38

Fixed effects:	Estimate Std	Error	Z value	Pr (>|z|)
(Intercept)	2.90305	0.03669	79.13	<2e-16 ***
Density all trees DBH ≥ 60 mm	0.08007	0.07657	2.19	0.0286 *
Density of dead trees with DBH ≥ 500 mm	0.08181	0.03610	2.27	9.0234 *
Signif. Codes:	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'

	stdcoef	stdse
(Intercept)	0	0
Density all trees DBH ≥ 60 mm	0.0220558	0.010072814
Density of dead trees with DBH ≥ 500 mm	0.02253491	0.009942389

We aggregated the data on the forest stand level. The density of trees varies considerably, ranging from 312 trees per ha in Steneto-1 to 391 trees per ha in Boatin, with an average of 357 trees per ha across stands (Tab. 8). There is an average of 39 snags per ha, reaching a maximum of 48 snags per ha in Steneto-2. The average share of snags, compared to all standing trees, is 11%.

Table 8: Study stand, density of live and dead standing trees.

	Trees per ha	Live trees per ha	Snags per ha	Percent of snags compared to all trees (%)
Boatin	391	356	35	9.1
Steneto-1	312	279	33	11.7
Steneto-2	369	321	48	12.2
total	357	319	39	11

The total mean density of habitat trees varies between different stands, reaching 202 habitat trees per ha in Boatin, while Steneto 1 and Steneto 2 have total mean densities of 161 and 186 habitat trees per ha, respectively (Tab. 9). The average density across all stands is 183 habitat trees per ha. While the densities of habitat trees varies widely, the percentage of all living trees, carrying at least one TreM is relatively constant across stands and averages at 51.9% for living trees. Compared to living trees, dead standing trees are on average more likely to be habitat trees. The share varies between stands, from 65% in Steneto-2 to 87.9% in Boatin, averaging at 78.6% (Fig. 11).

Table 9: The density and share of habitat trees on study stands.

	Density of habitat trees per ha	Density of live habitat trees per ha	Density of habitat snags	Percent of live trees carrying at least 1 TreM	Percent of snags carrying at least 1 TreM
Boatin	202	172	29	51.7	87.9
Steneto-1	161	133	28	51.1	82.9
Steneto-2	186	158	28	52.9	65
total	183	155	28	51.9	78.6

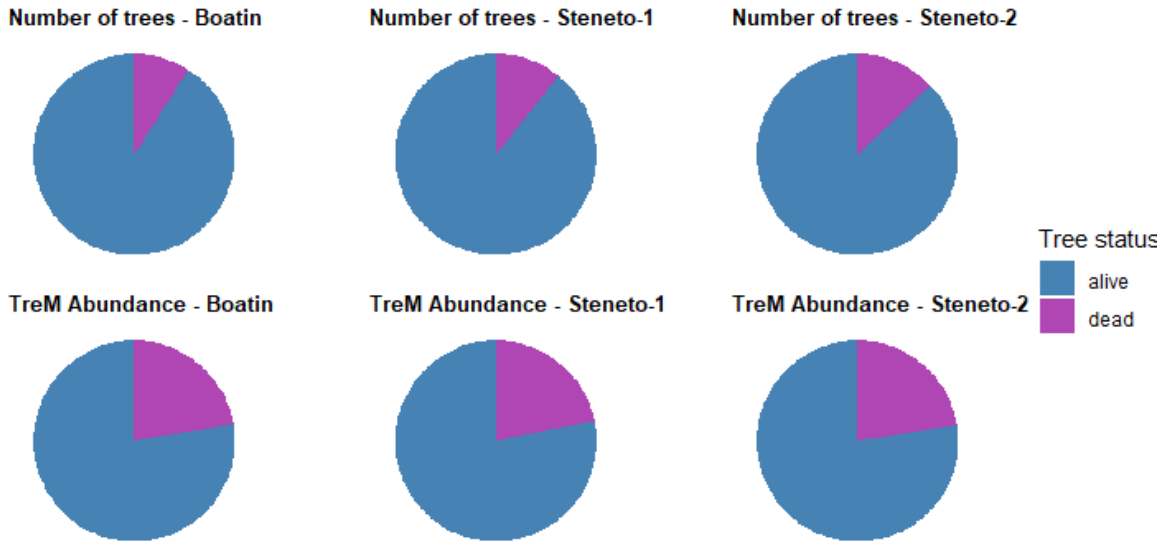

Figure 9: Pie charts, representing the share of live and dead trees on study stands (first row) and their relative contribution of tree-related microhabitats (bottom).

Additionally, total TreM abundances, presented as densities on stand level, were calculated. They are expressed as TreM densities per ha (Tab. 10), TreM densities per ha on all living trees (Tab. 11), and TreM densities per ha on all snags (Tab. 12). We calculated the densities for 42 types of TreMs present, as we included all trees of all tree species. Therefore, the tables additionally include TreM type heavy resinosis, present on silver fir trees.

The most abundant TreMs across all stands are root-buttress concavities (countable, N= 1386, average density of 220.0 per ha across stands), burrs (countable, N= 377, average density of 59.8 per ha across stands), bark loss (countable, N= 296, average density of 47.0 per ha across stands), bryophytes (presence only, N= 272, average density of 43.2 per ha across stands), bark shelter (countable, N= 200, average density of 31.7 per ha across stands), and foliose and fruticose lichens (presence only, N= 195, average density of 31.0 per ha across stands). An overall TreM abundance of 684.4 TreMs per ha is observed across stands, 532.2 TreMs per ha by live trees and 152.2 by snags. While snags represent only 11% of trees (Tab. 9), they are not only more likely to be habitat trees but also account for, on average, 28.6% of all TreMs' total

abundance, varying between different TreM types. The relative contribution of live and dead trees is displayed in Fig. 12.

Table 10: Total tree-related microhabitat abundance densities on all habitat trees for the 42 tree-related microhabitat types calculated separately by stands and averaged across all stands. They are expressed in the number of tree-related microhabitats per ha. Some tree-related microhabitat types are countable on the tree level, while others are only marked as present. We marked all with only presence with a star (*).

	Boatin	Steneto-1	Steneto-2	Total
Medium-sized woodpecker breeding cavity	0.0	0.0	3.3	1.1
Large woodpecker breeding cavity	0.0	0.5	0.0	0.2
Trunk base rot hole	12.4	12.4	10.5	11.7
Trunk rot hole	25.2	13.8	21.9	20.3
Semi-open trunk rot hole	9.0	4.3	3.8	5.7
Chimney trunk base rot hole	2.9	1.0	0.5	1.4
Chimney trunk rot hole	2.4	1.0	0.0	1.1
Hollow branch	7.1	4.3	13.3	8.3
Insect galleries and bore holes *	26.7	9.5	27.6	21.3
Dendrotelm (phytotelmata, water-filled hole)	15.7	14.8	11.4	14.0
Woodpecker foraging excavation	32.9	9.5	17.6	20.0
Trunk bark-lined concavity	8.1	2.4	16.7	9.0
Buttress-root concavity	249.0	129.5	281.4	220.0
Bark loss	42.4	47.6	51.0	47.0
Bark shelter	25.7	19.0	50.5	31.7
Bark pocket	17.6	9.0	43.8	23.5
Stem breakage *	9.0	1.9	3.8	4.9
Limb breakage (heartwood exposed)	1.0	2.9	4.8	2.9
Crack	2.4	2.4	1.0	1.9
Lightning scar *	0.0	1.4	0.5	0.6
Fork split at the intersection	0.0	1.4	1.4	1.0
Dead branches *	20.0	20.5	28.6	23.0
Dead top *	6.2	2.9	5.7	4.9
Remaining broken limb	9.0	12.4	20.0	13.8
Witch broom	0.5	0.0	0.0	0.2
Epicormic shoots *	9.5	14.3	17.1	13.7
Burr	70.0	52.9	56.7	59.8
Canker	15.7	9.0	7.1	10.6
Perennial polypore *	21.0	11.0	9.5	13.8
Annual polypore *	5.7	7.1	2.4	5.1
Pulpy agaric *	8.1	3.3	3.8	5.1
Large pyrenomycete *	5.2	2.4	2.9	3.5
Myxomycetes *	4.8	0.0	0.0	1.6
Bryophytes *	55.7	37.6	36.2	43.2

Foliose and fruticose lichens *	27.1	32.9	32.9	31.0
Ivy and lianas *	0.0	0.0	0.5	0.2
Ferns *	1.0	0.0	1.0	0.6
Invertebrate nest	0.5	0.0	1.4	0.6
Bark microsoil *	3.3	1.0	2.9	2.4
Crown microsoil *	1.4	0.5	0.5	0.8
Sap run *	2.4	1.0	0.5	1.3
Heavy resinosis *	0.0	0.5	4.8	1.7
Total	**756.7**	**497.6**	**799.0**	**684.4**

Table 11: Total tree-related microhabitat abundance densities on all live habitat trees for the 42 tree-related microhabitat types calculated separately by stands and averaged across all stands. They are expressed in the number of tree-related microhabitats per ha. Some tree-related microhabitat types are countable on the tree level, while others are only marked as present. We marked all with only presence with a star (*).

	Boatin	**Steneto-1**	**Steneto-2**	**Total**
Medium-sized woodpecker breeding cavity	0.0	0.0	2.9	1.0
Large woodpecker breeding cavity	0.0	0.5	0.0	0.2
Trunk base rot hole	11.4	10.5	9.5	10.5
Trunk rot hole	24.8	11.4	21.0	19.0
Semi-open trunk rot hole	6.7	3.8	3.3	4.6
Chimney trunk base rot hole	0.5	0.5	0.0	0.3
Chimney trunk rot hole	0.5	0.5	0.0	0.3
Hollow branch	7.1	3.8	13.3	8.1
Insect galleries and bore holes *	9.5	1.0	5.7	5.4
Dendrotelm (phytotelmata, water-filled hole)	15.7	14.3	11.4	13.8
Woodpecker foraging excavation	11.4	3.8	4.3	6.5
Trunk bark-lined concavity	8.1	2.4	14.8	8.4
Buttress-root concavity	232.4	117.1	260.0	203.2
Bark loss	22.4	28.6	27.6	26.2
Bark shelter	4.3	4.8	16.2	8.4
Bark pocket	3.8	3.3	11.0	6.0
Stem breakage *	3.8	1.4	3.8	3.0
Limb breakage (heartwood exposed)	1.0	2.9	4.8	2.9
Crack	1.0	1.4	1.0	1.1
Lightning scar *	0.0	1.0	0.5	0.5
Fork split at the intersection	0.0	1.4	1.4	1.0
Dead branches *	19.0	20.0	28.6	22.5
Dead top *	5.2	1.9	4.3	3.8
Remaining broken limb	9.0	11.9	19.5	13.5
Witch broom	0.5	0.0	0.0	0.2
Epicormic shoots *	9.5	14.3	17.1	13.7

Burr	66.2	52.4	56.2	58.3
Canker	15.2	9.0	7.1	10.5
Perennial polypore *	5.2	1.4	0.0	2.2
Annual polypore *	2.4	1.9	1.0	1.7
Pulpy agaric *	4.3	1.0	1.4	2.2
Large pyrenomycete *	2.9	0.0	1.9	1.6
Myxomycetes *	4.3	0.0	0.0	1.4
Bryophytes *	47.6	26.7	28.6	34.3
Foliose and fruticose lichens *	25.2	31.9	31.9	29.7
Ivy and lianas *	0.0	0.0	0.5	0.2
Ferns *	0.5	0.0	0.5	0.3
Invertebrate nest	0.5	0.0	1.4	0.6
Bark microsoil *	1.9	1.0	1.9	1.6
Crown microsoil *	1.0	0.5	0.5	0.6
Sap run *	2.4	1.0	0.5	1.3
Heavy resinosis *	0.0	0.5	4.8	1.7
Total	**587.1**	**389.5**	**620.0**	**532.2**

Table 12: Total tree-related microhabitat abundance densities on all dead standing habitat trees for the 42 tree-related microhabitat types calculated separately by stands and averaged across all stands. They are expressed in the number of tree-related microhabitats per ha. Some tree-related microhabitat types are countable on the tree level, while others are only marked as present. We marked all with only presence with a star (*).

	Boatin	**Steneto-1**	**Steneto-2**	**Total**
Medium-sized woodpecker breeding cavity	0.0	0.0	0.5	0.2
Large woodpecker breeding cavity	0.0	0.0	0.0	0.0
Trunk base rot hole	1.0	1.9	1.0	1.3
Trunk rot hole	0.5	2.4	1.0	1.3
Semi-open trunk rot hole	2.4	0.5	0.5	1.1
Chimney trunk base rot hole	2.4	0.5	0.5	1.1
Chimney trunk rot hole	1.9	0.5	0.0	0.8
Hollow branch	0.0	0.5	0.0	0.2
Insect galleries and bore holes *	17.1	8.6	21.9	15.9
Dendrotelm (phytotelmata, water-filled hole)	0.0	0.5	0.0	0.2
Woodpecker foraging excavation	21.4	5.7	13.3	13.5
Trunk bark-lined concavity	0.0	0.0	1.9	0.6
Buttress-root concavity	16.7	12.4	21.4	16.8
Bark loss	20.0	19.0	23.3	20.8
Bark shelter	21.4	14.3	34.3	23.3
Bark pocket	13.8	5.7	32.9	17.5
Stem breakage *	5.2	0.5	0.0	1.9
Limb breakage (heartwood exposed)	0.0	0.0	0.0	0.0
Crack	1.4	1.0	0.0	0.8

Lightning scar *	0.0	0.5	0.0	0.2
Fork split at the intersection	0.0	0.0	0.0	0.0
Dead branches *	1.0	0.5	0.0	0.5
Dead top *	1.0	1.0	1.4	1.1
Remaining broken limb	0.0	0.5	0.5	0.3
Witch broom	0.0	0.0	0.0	0.0
Epicormic shoots *	0.0	0.0	0.0	0.0
Burr	3.8	0.5	0.5	1.6
Canker	0.5	0.0	0.0	0.2
Perennial polypore *	15.7	9.5	9.5	11.6
Annual polypore *	3.3	5.2	1.4	3.3
Pulpy agaric *	3.8	2.4	2.4	2.9
Large pyrenomycete *	2.4	2.4	1.0	1.9
Myxomycetes *	0.5	0.0	0.0	0.2
Bryophytes *	8.1	11.0	7.6	8.9
Foliose and fruticose lichens *	1.9	1.0	1.0	1.3
Ivy and lianas *	0.0	0.0	0.0	0.0
Ferns *	0.5	0.0	0.5	0.3
Invertebrate nest	0.0	0.0	0.0	0.0
Bark microsoil *	1.4	0.0	1.0	0.8
Crown microsoil *	0.5	0.0	0.0	0.2
Sap run *	0.0	0.0	0.0	0.0
Heavy resinosis *	0.0	0.0	0.0	0.0
Total	**169.5**	**108.1**	**179.0**	**152.2**

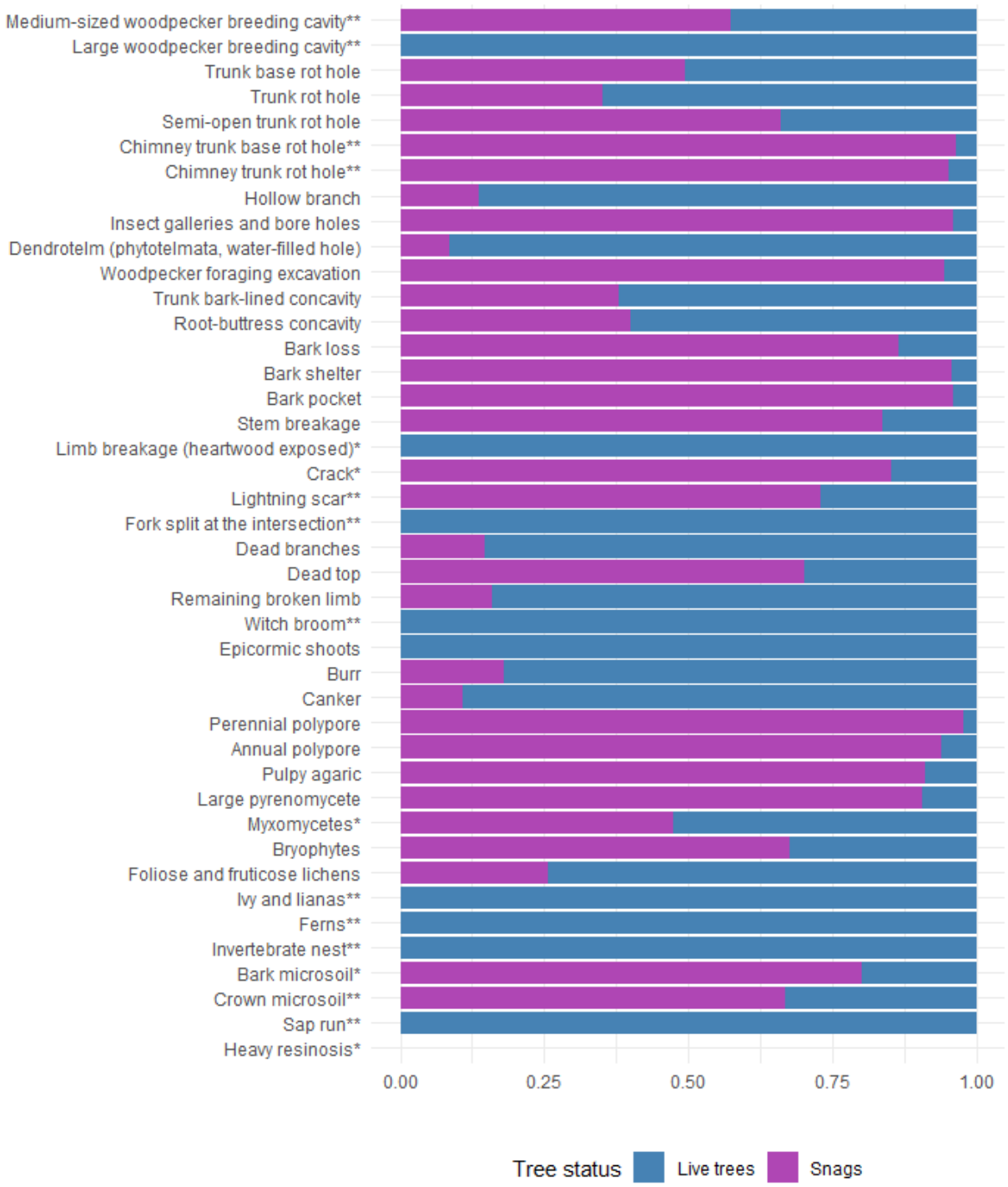

*Figure 10: Relative contribution of live trees and snags to tree-related microhabitat abundance. The tree-related microhabitats that occur <10 times are marked with **, those with <20 occurrences are marked with *.*

6. Discussion

To better understand forest ecosystems and evaluate their biodiversity, many biodiversity indicators have been developed (Burascano et al., 2023). In this study, we analysed the effect of structural characteristics of primary mountain beech forests in Bulgaria and their contribution to the occurrence, abundance, and diversity of TreMs on the tree, plot, and stand level. TreMs are used as an indirect surrogate indicator of forest biodiversity at the scale of a forest stand. The definition and typology of TreMs consists of 47 specified TreM types, grouped into 15 groups and 7 basic forms, namely the cavities, tree injuries and exposed wood, crown deadwood, excrescences, fruiting bodies of saproxylic fungi and slime moulds, epiphytic, epixylic and parasitic structures, and fresh exudates (Larrieu et al., 2018). TreMs occur in higher abundance in primary, compared to managed forests (Asbeck et al., 2022).

The factors that we found to be significantly positively correlated with the richness of TreM types on the tree level are the tree DBH and tree status. Both factors have been found to be significantly correlated to the occurrence and abundance of TreMs on the tree level (e.g. Paillet et al., 2019; Larrieu et al., 2022; Martin et al., 2022). Snags, compared to living trees, have been demonstrated to host more TreMs on trees of the same species and DBH (Larrieu & Cabanettes, 2012; Paillet et al., 2017; Asbeck et al., 2021). Due to the wood decomposition processes, they have softer wood for excavation while offering habitat to a variety of insects that, in turn, also serve as food sources (Vuidot et al., 2011; Lassauce et al., 2011). Large trees, reaching the mature stage, are characterised by the development of crown deadwood and other TreMs related to wounds, while the establishment of forks by the branches favours the formation of microsoil and dendrotelms (Larrieu et al., 2011). Larger and generally older trees have, in general, a longer life history, which results in the trees being exposed to more TreM-forming events, such as storms, rockfalls, or frost events (Paillet et al., 2017). Due to the forest stands being dominated by the European beech trees and other tree species only occurring in small numbers, we could not test for the effect of tree species. However, European beech and other broadleaf species have been observed to host a higher abundance and diversity of TreMs compared to trees of coniferous species (Asbeck et al., 2021; Kozák et al., 2023). Since not all the trees have the age data available, we decided to test its effect on the plot level instead. We hypothesise that the overdispersion of residuals in the fitted model occurs partially due to other predictor variables that could potentially explain a portion of the variance but were not yet identified, such as the history of suppression and release events. The overall TreM richness has been observed to increase with higher elevation. It significantly increases the abundance of epiphytes, although the higher abundance may be a result of higher precipitation, increasing with the higher elevation (Asbeck et al., 2019; Asbeck et al., 2022; Kozák et al., 2023). An increase in the abundance of root cavities was also found with higher elevation (Asbeck et al., 2019); however, the method used in the study was different and the dimensions, required to fit the assignment of the countable TreM were smaller, compared to the standardised methodology by Larrieu et al. (2018) used in this thesis. The south facing slopes have been observed to impact the occurrence of mistletoes (Larrieu et al., 2022), however, we didn’t observe any of them on our study plots. The inclusion of hierarchically nested study sample design, added to the model as a random effect to avoid pseudoreplication, improved the model fit.

In the second part of the analysis, we tested the correlation between the selected predictors and the overall abundance of TreMs on the plot level. While some TreM types are countable, we only mark presence for others (Appendix 1), which can potentially lead to an inequality between TreM types and overrepresentation of the most frequent countable types. The most observed TreM type across all plots are root-buttress concavities, with the average density across plots of 220 TreMs per ha, although ranging from 129.5 in Steneto-1 to 281.4 root-buttress cavities per ha in Steneto-2. Other frequently observed TreMs include burrs, bark loss, bryophytes, bark shelter, and foliose and fruticose lichens. The basal area, representing the cross-sectional area of trees, is affected mainly by the DBH. Individual trees with high DBH in the primary forests account for a high share of the total basal area (Seiwa et al., 2023). However, we did not detect the maximum DBH or RMS DBH of trees on the plot to significantly improve the model, most likely due to the fact that large trees might be present also on disturbed plots, where the overall number of trees is decreased. The second predictor identified is the density of trees all trees (DBH ≥ 60 mm). The more trees there are, the more likely it is that more of them, in the absolute sense, might host TreMs. This can also be addressed in another way, implementing rarefaction curves to account for the difference in sample size on a given plot (i.e. number of trees present) (Chao et al., 2014). There is uncertainty with both approaches, though, as a recently severely disturbed plot might have a high density of regeneration with DBH ≥ 60 mm while not yet hosting many TreMs (Paillet et al., 2019). Therefore, we decided to add it as a predictor variable to the model. The inclusion of altitude as a predictor improved our model fit and lowered the AIC by 1.7 (Tab. 3). As already discussed, altitude mostly influences the higher abundance of root-buttress concavities and epiphytic TreMs (e.g. bryophytes and lichens) (Asbeck et al., 2019; Asbeck et al., 2022; Kozák et al., 2023), all of which were highly abundant on our plots (Tab. 10).

Lastly, we fit a GLMM to account for diversity of TreM types on the plot level. The diversity of TreM types varies between 9 and 26 TreM types, with a mean of 18.34. The variables that improved the model fit are the density of all trees with DBH ≥ 60 mm and the density of dead trees with DBH ≥ 500 mm. As discussed earlier, a higher density of trees translates into more trees potentially bearing TreMs, increasing the likelihood of them hosting different types of TreMs. The second variable, the density of dead trees with DBH ≥ 500 mm, is in line with other study findings (e.g. Paillet et al., 2017; Paillet et al., 2019; Kozák et al., 2018). Snags host significantly more TreMs, linked with wood decay processes, such as woodpecker feeding holes (Paillet et al., 2019). Snags and large trees are also highlighted as the main driver of TreM abundance and diversity when compared to managed forests (Paillet et al., 2017; Vuidot et al., 2011; Larrieu & Cabanettes, 2012). TreM abundance and diversity are assumed to increase with tree senescence, leading to the development of more TreMs as the trees grow older compared to individuals of similar dimensions in managed forests (Asbeck et al., 2022). We expected to see an increased diversity of TreM types on plots with more tree species present. Sap runs are, for example, more likely to occur on deciduous trees, while coniferous trees are more susceptible to broken tops (Kozák et al., 2018). Outgrowths and bark characteristics are more frequently observed on oaks, spruces, and firs compared to beech (Vuidot et al., 2011). However, we had a largely beech-dominant forest. Only one plot had four different tree species present, and while several had an admixture of fir, the whole study stand in Boatin was composed of 100% beech.

We expected to see the influence of plot age on the abundance and diversity of TreMs; however, as demonstrated by earlier studies, due to long periods of suppression, the correlation between DBH and age is not necessarily strong. TreM types on beech trees, which were previously demonstrated to be influenced by the tree age, can result from longer exposure to damage and injuries (e.g., bark loss, bark pockets, and bark shelters) or need a longer time to develop, such as rot holes (Kozák et al., 2023). Generally, tree age and DBH are closely related. However, the largest trees are not necessarily the oldest in primary forests. Beech trees are shade tolerant species, that survive long periods of initial suppression, slower growth rate, and can exhibit more release events (Pavlin et al., 2021). The result of divergence in DBH and age correlation may result in different pathways of TreM formation and development among large and old trees (Kozák et al., 2023). The effect of tree age might be found significant if we fitted the model to TreM types or groups separately. However, it did not impact the overall TreM abundance and diversity on the plot level. We hypothesise, that other predictors might only affect some TreM types or groups as well, while they are not significant across all TreM types combined, but such analyses were out of scope in this thesis.

Finally, we want to highlight the observed contribution of snags to the overall abundance and diversity of TreMs. While snags present, on average, 11% of all trees across our study plots (Tab. 8), they contribute a total of 28.6% of total TreM abundance across the study plots (Fig. 11). They are much more likely to host TreMs. On average, 78.6% of all snags hosted at least one TreM, while 51.9% of live trees hosted at least one TreM (Tab. 9). In relative terms, they contribute a higher abundance of rot holes, insect galleries, woodpecker excavations, exposed wood, perennial polypores and microsoils (Fig. 12).

7. Conclusions

This study aimed to identify the elements of forest structure influencing the occurrence, abundance, and diversity of TreMs in the primary mountain beech forests of Bulgaria. We conducted a review of the current state of knowledge and literature on TreMs and their function with regards to forest structure, primary forests, and biodiversity. The selected set of predictors was tested and fitted with GLMMs, disentangling the impact of the various elements of the forest structure. GLMMS are an effective statistical tool that take into account the effect of spatial correlation between different study stands and plots. DBH and tree status have significant predictive power for the occurrence of TreMs on the tree level. Tree density, basal area and altitude significantly increase the abundance of TreMs on the plot level while the tree density and density of large snags significantly increase the diversity of TreM types. Snags are significantly more likely to host TreMs signifying their importance in preserving biodiversity. This study serves as the first assessment of TreMs in the primary mountain forests in Bulgaria, in the Central Balkan mountain range. The reference values may provide a baseline to guide forest management practices focused on biodiversity enhancement. Our study outlines the importance of snags and old trees for TreMs, which are found in higher abundances in primary, compared to managed forests. Retention forestry practices, such as retention of snags and allowing trees to get old and die, are therefore recommended in order to support forest biodiversity in managed stands. Remaining primary forests represent only a small fraction of European forests and their protection should be a priority, while setting aside new areas to connect the patches of protected areas should be implemented. TreMs' ability to serve as an indirect biodiversity indicator is still understudied, therefore more research is needed to link the occurrence of specific taxa based on their abundance and diversity. New methods of ecosystem monitoring are emerging, aiding the field surveys with remote techniques able to cover larger areas. Therefore, it is important to understand the relationships between forest structure, TreMs, and its impact on biodiversity to utilise them to the advancement of biodiversity monitoring, leading to more informed and targeted management actions.

8. References

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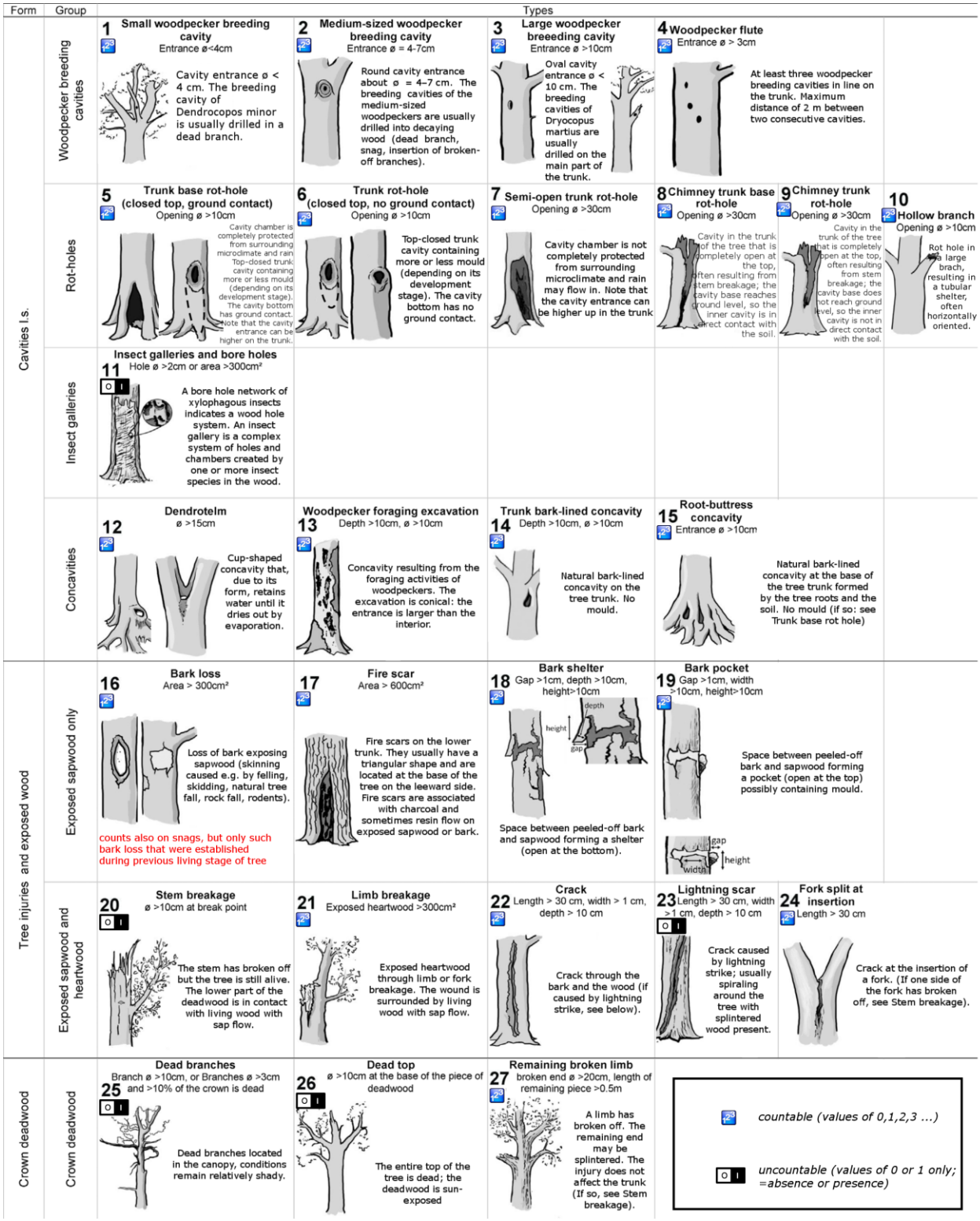
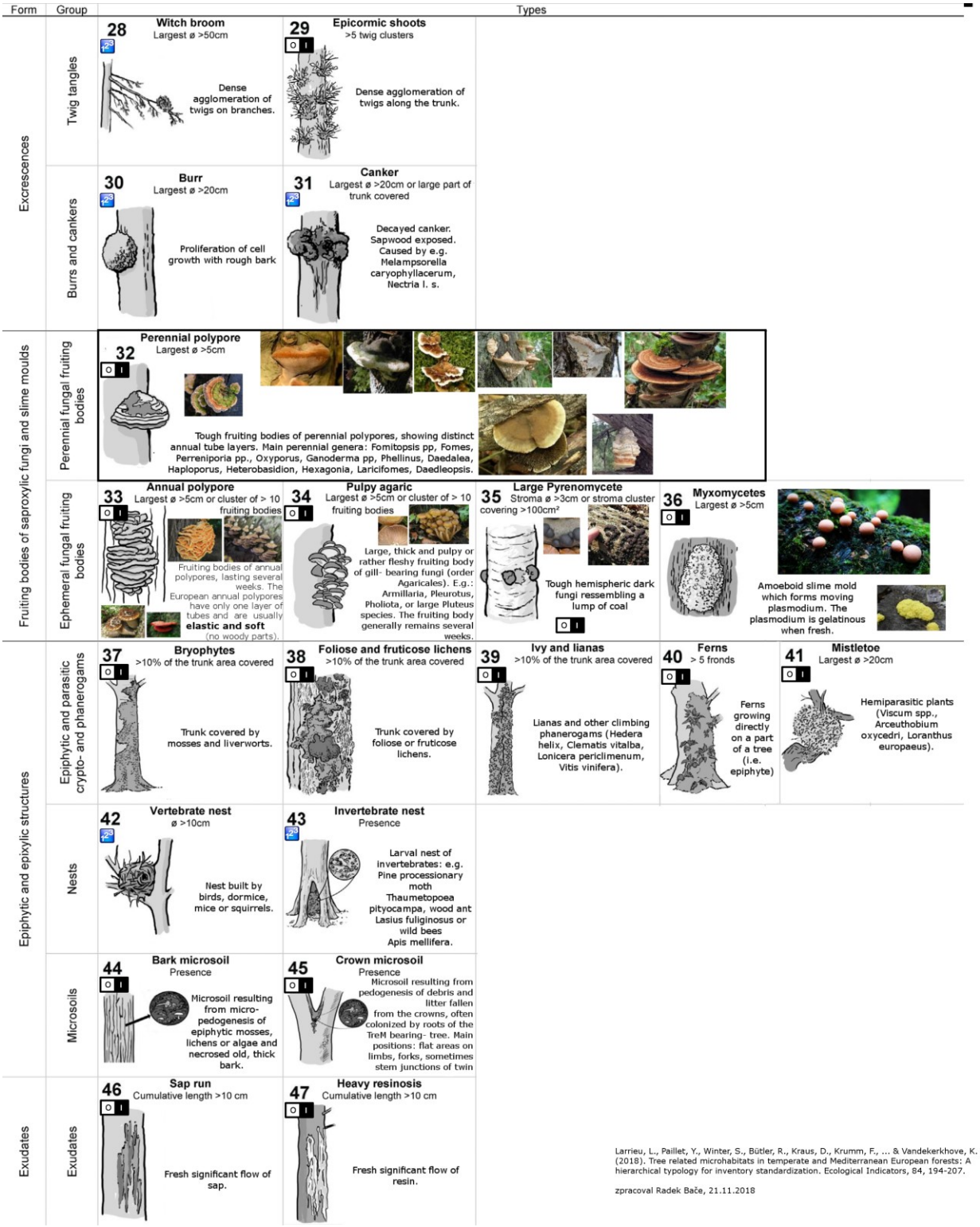
9. Appendices

Appendix 1: TreM methodology used in REMOTE forests project. Prepared by Bače, 2019, following the methodology by Larrieu et al., 2018.





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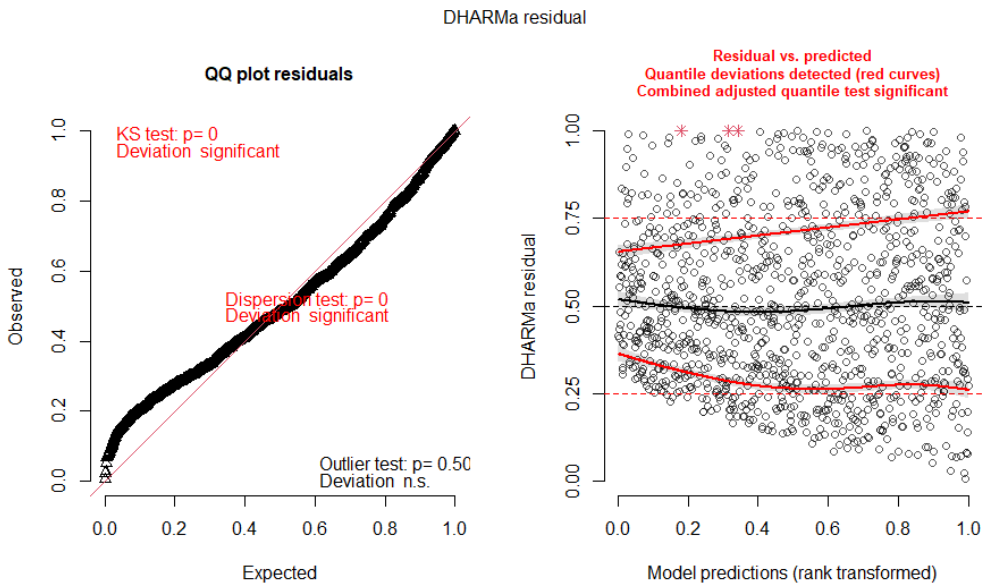
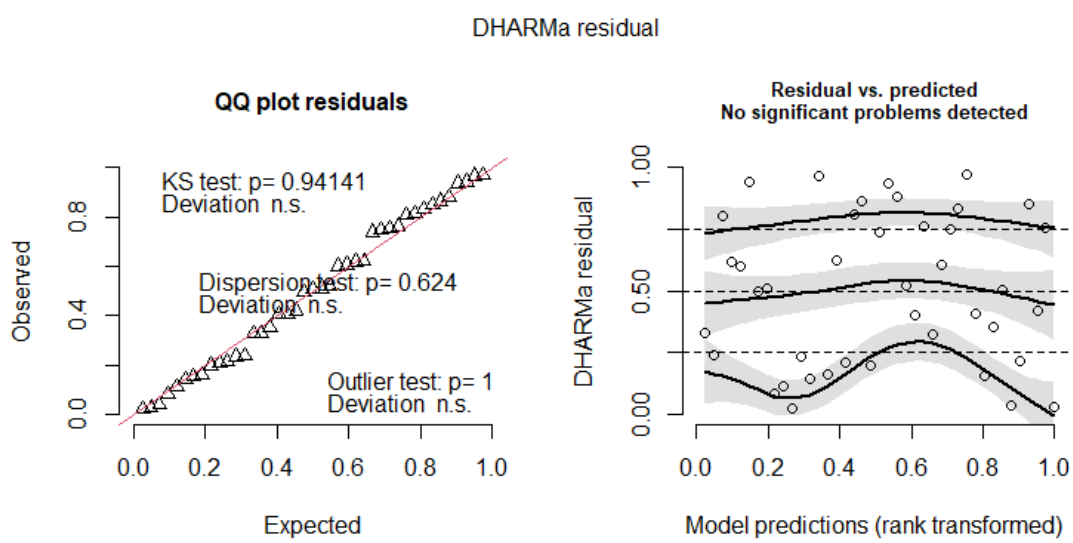
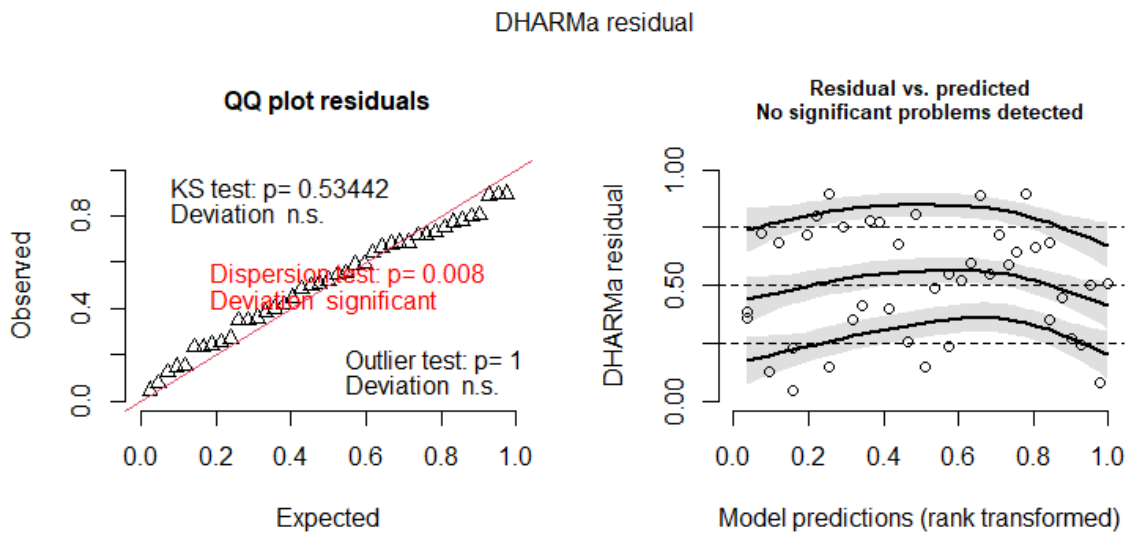
zpracoval Radek Bače, 21.11.2018

Appendix 2: DHARMa plotted residuals of the fitted generalised linear mixed model with Poisson distribution for tree-related habitat richness on the tree level. Quantile-quantile plots (left) and scatter plots (right) with plotted residuals against tree-related microhabitat richness are shown along with the results of the Kolmogorov-Smirnov tests (KS test), dispersion tests and tests for outliers.



Appendix 3: DHARMa plotted residuals of the fitted generalised linear mixed model with negative binomial distribution for tree-related habitat abundance on the plot level. Quantile-quantile plots (left) and scatter plots (right) with plotted residuals against tree-related microhabitat abundance are shown along with the results of the Kolmogorov-Smirnov tests (KS test), dispersion tests and tests for outliers.



Appendix 4: DHARMa plotted residuals of the fitted generalised linear mixed model with Poisson distribution for tree-related habitat abundance on the plot level. Quantile-quantile plots (left) and scatter plots (right) with plotted residuals against the diversity of tree-related microhabitats are shown along with the results of the Kolmogorov-Smirnov tests (KS test), dispersion tests and tests for outliers.