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Forest Biomass Dynamics of Temperate Mountain Forests in Central & Eastern Europe.

Doctoral thesis

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Ph.D. THESIS ASSIGNMENT

Dheeraj Ralhan, MRes., BSc (Hons)

Forestry Engineering
Forest Biology

Thesis title

Forest biomass dynamics of temperate mountain forests in Central and Eastern Europe.

Objectives of thesis

This thesis addresses how heterogeneous conditions, characterised by various tree sizes and uneven age distributions, contribute to the stable carbon-carrying capacity of primary forests. It explores how the diversity of tree genera, size distribution, natural disturbances, age, and abundance contribute to forest heterogeneity. Furthermore, the study investigates the relationship between biomass carbon and site-specific factors (both biotic and abiotic) that influence biomass accumulation across the Carpathian primary forests. The thesis emphasises the importance of primary forests as a stable carbon reservoir and the continued protection to safeguard them into the future.

1. To investigate the effects of natural disturbance regimes, tree age, and environmental conditions on forest biomass, this study focused on primary forests with minimal direct human impacts.

2. To investigate the roles of tree structure and genus-level diversity in determining biomass carbon storage in temperate forest ecosystems. Investigating the relationships that influence biomass and forest heterogeneity regarding the range of tree genus diversity and structural complexity across the primary mountain forests.

3. Investigating whether primary forests continue to accumulate carbon or whether the stocks reach equilibrium. Assessing the amount of survival, growth, mortality, and the transfer of carbon from the live to dead biomass pools across the temperate montane primary forests.

Methodology

For investigating the objectives of this thesis, data is used from a comprehensive network of permanent sample plots established in montane primary forests across the Carpathian Mountains, including Slovakia, Ukraine, and Romania. Individual tree diameters are used to calculate the amount of biomass present in each tree using a series of species-specific allometric equations to capture trends in forest biomass carbon. This is calculated by partitioning the mass of a tree into its stem, branches, and foliage and summing these to calculate individual tree mass (tree kg^-1). Individual tree biomass is then summed for each plot and extrapolated to represent the amount of biomass stock per hectare (Mg ha^-1). Site-specific biotic and abiotic factors, such as tree age, disturbance severity, temperature, climatic water deficit, and altitude, are used to assess how they influence the spatial and temporal trends in biomass stocks across the Carpathians.

The proposed extent of the thesis

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Keywords

Biomass carbon stock; Biomass distribution; Carbon carrying capacity; Ecosystem functioning; Forest composition; Primary Forest dynamics; Tree size distribution

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Declaration of Independence

I, Dheeraj Ralhan, hereby declare the originality and authenticity of this doctoral thesis titled "*Forest Biomass Dynamics of Temperate Mountain Forests in Central and Eastern Europe*." I confirm that this doctoral thesis was created independently and ethically, in accordance with the rules of the university and Czech law. All information sources, literature, and materials used have been appropriately documented and acknowledged. The thesis was produced under the direct supervision of my supervisor, prof. Ing. Miroslav Svoboda, Ph.D.

I agree with the disclosure of this doctoral thesis in accordance with Czech Law (Act No. 111/1998 Coll. Sb) and regardless of the outcome of the defence.

1st September 2024:

DKRalhan

Signature

Summary

Primary forests hold a multitude of unique traits that are not present in non-primary forests or terrestrial ecosystems. The presence of native vegetation that has remained in the ecosystem for centuries, as well as heterogeneity in species, structure, and age classes, are driven by natural disturbances. Moreover, primary forests, particularly in the Carpathian Mountains spanning Central and Eastern Europe, exist without any direct impact from human activity. In an ever-changing world continually being developed for human activity, from settlements to agriculture and forestry, it is rare for such natural forests to exist in Europe. Since primary forests are driven by natural processes that shape forest demography over centuries, this allows large-diameter trees (> 60 cm) to remain in the ecosystem and are valuable carbon stores. Such characteristics make primary forests an important ecosystem, not only for being driven by natural processes but its uneven age and tree size structure accommodates a stable carbon store and healthy biodiversity. Whilst forest biomass carbon is not a new approach for quantifying ecosystem functionality and services, most studies focus on either carbon sequestration, fluxes or biomass productivity.

The aim of this thesis is to investigate biomass carbon stocks across the temperate montane primary forests across Central and Eastern Europe. Specifically, to provide new knowledge into the spatial and temporal trends in biomass carbon stocks across the Carpathians Mountain forests, and their relationship with biotic and abiotic factors. Site-specific factors include mean plot-level tree age and disturbance severity which are derived from dendrochronological data, gridded climate data, tree size and genera diversity to topographical conditions across the region. The studies in this thesis represent data from two major forest types across the Carpathians: deciduous forests, dominated by beech (*Fagus sylvatica* L.) in the lower elevations and coniferous forests, dominated by Norway spruce (*Picea abies*) in higher elevation locations.

The first study focuses on the drivers of biomass accumulation across 726 permanent sample plots – ***see subchapters 3.1, 4.1 & 5.1***. Using forest inventory-based data and nonlinear regression models, I quantified aboveground biomass in mixed beech and spruce forests. The findings revealed that biomass stocks in these forests are comparable to other temperate primary forests, with significant carbon storage

capabilities. The highest mean total biomass was found in southern mixed beech forests and western spruce forests. The study highlights the importance of preserving unmanaged forests as a climate mitigation strategy, demonstrating their continued function as carbon sinks over centuries. Additionally, it underscores the resilience of these forests in maintaining positive biomass accumulation rates despite varying disturbance histories and tree ages.

The second study examined the interplay between tree structure, genus-level diversity, and biomass stocks from 726 permanent sample plots in the Carpathian Mountains – ***see subchapters 3.2, 4.2 & 5.2***. Focusing on how forest biomass, structure, and diversity vary spatially. The results indicate that both genus diversity and structural complexity are crucial for understanding biomass distribution. Local disturbances and varied tree ages enhance forest heterogeneity and biomass accumulation. Structural indices, supported by genus abundance, positively impact biomass stocks, with spruce forests exhibiting higher tree density and basal area compared to mixed beech forests. This study highlights the significance of primary forests as stable carbon reservoirs, maintained through their structural and biological diversity, and underscores the importance of protecting these ecosystems for future carbon storage and biodiversity conservation.

The third study investigates carbon dynamics in temperate montane primary forests, focusing on the carbon carrying capacity (CCC) and the factors influencing carbon stocks – ***see subchapters 3.3, 4.4 & 5.3***. Using data from two census periods across 454 permanent sample plots, analysing the fluctuations in live and dead biomass rates. Our findings reveal that primary forests continue to accumulate carbon over time, functioning as stable carbon reservoirs. The study highlights the importance of tree age, mortality, and local disturbances in driving carbon fluctuations. Mixed beech forests showed consistent increases in live carbon, while spruce forests exhibited more variability. This comprehensive analysis underscores the significance of primary forests in the global carbon cycle and the need for effective conservation strategies to mitigate climate change.

To synthesise the three specific research areas, findings from each are combined in a cumulative discussion subchapter (5.4) which includes a comprehensive discussion of biomass carbon stocks and their biogeographical relationships across the primary forests. The observed plateau in carbon accumulation at 200-225 years in the Carpathian primary forests likely represents a stability between growth and decomposition processes, within the interval between censuses. Natural disturbances, such as windstorms and bark beetle outbreaks, can disrupt this balance, altering carbon dynamics by removing biomass and promoting new growth. Until such events occur, forests are expected to maintain their carbon stock within a narrow range. Large-diameter trees enhance carbon storage and support forest heterogeneity, ensuring continued growth and stability of the forests' carbon-carrying capacity.

The findings from this thesis demonstrate the value of primary forests. They highlight how, despite of high maturity levels, continue to be an active and critical carbon sink. These forests are essential for preserving the ecological integrity of centuries-long diversity of tree size, age structures and species across the Carpathians. I believe such information is critical in understanding how natural forests exist without direct human intervention and how their unique traits in biodiversity and carbon stocks can be replicated in non-primary forests. Thus, supporting the protection and conservation of both primary and non-primary forests is essential for mitigating climate change and the long-term stability of the terrestrial ecosystem

Acknowledgement

This doctoral thesis represents the culmination of nearly half a decade of research, dedicated to understanding a small, yet vital aspect of our ever-changing environment. I hope that this work will somehow contribute towards informing and educating others about the importance of our natural forests. Completing the PhD has made me reflect on all of the work, challenges (both good and bad) and the countless experiences over the years. From numerous fieldworks in the European primary forests, to being able to complete an internship in Australia. I am truly grateful for all these experiences and to all the people who helped me on the way. Completing this PhD would not have been possible without the support of all the people I have met along the way. I thank you all, for your support and generosity over the years.

Firstly, I would like to thank and dedicate this thesis to my Mother, Father and Brother. Moving is never an easy task, especially to a new country. We have been through a lot over the years. Their unwavering support has been a major lifeline, and I owe them a great debt. Thank you for forever being by my side, for always being there, even when I was over 16,000 km away from home. To my good friend, Andrew Walles, thank you for your continued support from my Bachelors' dissertation to my Doctoral thesis.

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If you're still reading the acknowledgement section, I would like to thank you, the reader. Thank you, for your interest and patience in reading this thesis. And finally, I'd like to thank myself for not giving up, even if it would have been the easiest thing to do. From solving the endless errors in R to figuring out all things carbon stock and biodiversity related. I am now ready to rest, or at least for a little bit, before the next chapter in my life.

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List of Abbreviations

ΔAIC – Difference Akaike Information Criteria
AGB – Aboveground biomass
AGC – Aboveground carbon
AIC – Akaike Information Criteria
ACI – Aboveground carbon increment
BA – Basal area (m^2 ha^{-1})
BAI – Basal area index
C – carbon
CCC – Carbon-carrying capacity
CA - Percentage of disturbed canopy area removed
CWB – Climatic water balance (mm)
CWD – Climate water deficit (mm)
DBH – Diameter at breast height (cm, measured at 1.3 m aboveground)
D – Deviance
IVI – Importance Value Index
GAMM – Generalised additive mixed-effects model
PET – Potential evapotranspiration
ppt – precipitation (mm)
M.A.S.L – Metres above sea-level
R^2 – R-squared value
R^2 (C) – Conditional R^2 values accounting for both fixed and random effects
R^2 (M) – Marginal R^2 values accounting for fixed effects
REML – Restricted maximum likelihood
tmax - Temperature maximum
tmin - Temperature minimum
SD – Standard deviation

Note: All abbreviations are listed in alphabetical order.

List of Units

Length
mm - millimetre
cm – centimetre
m - metres

Area
m^2 – square metre
ha^{-1} – hectare, equal to 10,000 m^2

Volume
m^3 – cubic metre

Rate of change in an area
ha^{-1} – one hectare, equal to 10,000 m^2
ha^{-1} yr^{-1} – one hectare per year

Mass
kg – kilogramme
t – tonne, equal to 1,000 kg
Mg – Megagram, equal to one tonne
tC – tonne of carbon

Large masses
Mt – Megatonne, equal to one million or 1.0 x 10^6 tonnes
Gt – Gigatonne, equal to one billion or 1.0 x 10^9 tonnes

Note: All units are listed in order of ascending scale (i.e. smallest to largest).

Preface

There is a need to recognise the importance of naturally regenerative forests, and it is important to actively conserve and protect them to ensure their longevity into the future. Here is a passage from my favourite natural historian, biologist, and fellow Leicestrian, Sir David Attenborough:

> *"It might seem like an obvious thing to say but we need to keep saying it: our planet is precious.*
> *It provides the air we breathe, the food we eat, the water we drink. You have only to take a walk through a forest and look up at its canopy to see the outstanding beauty and complexity of ecosystems. Pause in the stillness among the trees and contemplate what is surrounding you: it's mind-blowing.*
>
> *But, rather than cherish this planet – our home – we have too often treated it with contempt. Today, as a consequence, we face disaster on a global scale"*
> *– Sir David Attenborough*

Photo of an ancient English Oak tree (Quercus Robur) from Bradgrate Park, Leicestershire. Source: Dheeraj Ralhan (2016)

In light of the current climate and environmental crises our world faces, it is more important than ever for scientists to provide evidence on the state of our environment.

Chapter 1 | Introduction

1.1 | Global Terrestrial Biosphere

Forest ecosystems cover 30% of the Earth's land surface and account for more than 80% of global terrestrial carbon storage (FAO, 2020). However, there are growing concerns over the capacity of future forest carbon storage due to climate change. Drastic changes in environmental conditions and disturbance regimes, as predicted by climate models (Price, 2013), are expected to strongly impact forest demography (tree species recruitment, growth and mortality) and the corresponding integrity of forest function, such as carbon storage (Allen et al., 2010; Jagodziński et al., 2020; McDowell et al., 2020).

Terrestrial ecosystems are in continuous flux, driven by interactions with biophysical conditions, such as atmospheric, hydrological and lithospheric processes, which vary from local to global scales (Pan et al., 2024; Reichstein and Carvalhais, 2019). If the trend in global temperature rise continues on its current trajectory, the integrity of terrestrial ecosystems, such as primary forests, may be compromised (Keeling and Phillips, 2007; Yuan et al., 2019). This can jeopardise the carbon-carrying capacity of some forests through changes in biodiversity and species composition, which could shift a forest into a novel successional phase with lesser recruitment, growth and biomass (Anderegg et al., 2020; Houghton, 2005; McDowell et al., 2020; Silva Pedro et al., 2015).

1.2 | Forest Biomass Carbon Stocks

Forests are critical for climate mitigation because they store large quantities of carbon (Mackey et al., 2020; Pan et al., 2024, 2011; Zhu et al., 2018). Climate change can impact the forest through long-term ‘slow’ changes, such as drier or wetter conditions over the course of several decades (Allen et al., 2010), or short-term ‘sudden’ natural disturbances, such as windstorms, drought-induced mortality, and pest and disease outbreaks. Although the effects can last for much longer than the event itself (Pugh et al., 2019; Zhu et al., 2018). To safeguard stable future carbon stocks, it is important to improve understanding of the current state of the forests and how they respond to variability in biotic and abiotic conditions. In this thesis, I focused on differences in biomass stocks due to natural disturbance and tree age, in addition to the effects of climate (temperature, climatic water deficit) and topography (altitude, slope position in a stand).

Understanding variation in forest biomass stocks can offer insights into the spatial variability in ecological traits (forest structure, age, competitive ability) and the conditions that influence carbon gains or losses (Jacobson et al., 2019; Keeton et al., 2011). Living biomass actively contributes to carbon sequestration and then accumulates as carbon stocks in both living and dead biomass (dead standing, downed trees, litter layer) and in soil organic matter (Jagodziński et al., 2020; Kueppers et al., 2004; Pugh et al., 2019). The partitioning among these biomass components, particularly above- and below-ground, varies with air temperature, water availability and soil nutrients (Álvarez-Dávila et al., 2017).

1.3 | Importance of Forest Monitoring

The development and application of forest inventories is a simple, yet efficient method for assessing the state of ecology in a terrestrial environment at the fine scale. While remotely sensed data enable assessments of large areas and conduct analysis at the global scale, information is aggregated at the macro-level, making it difficult to distinguish individual trees or niche conditions of a site, depending on the resolution of the data. Forest inventories, despite being time-consuming, difficultly accessing access remote locations and subject to human bias or error, offer a low-cost and fine-scale data collection. Moreover, forest inventories are important for capturing individual tree characteristics, as well as site conditions, that might not be possible through most remotely sensed approaches. Observing and measuring ecology at an individual, is crucial for understanding the dynamism within a forest ecosystem, including the variability in biomass stock which can be achieved for each individual tree. Such approaches can necessitate conservation and management strategies. Establishing permanent sample plots with regular monitoring can provide fine-scale data to assess the current state of the ecosystem and changes over time.

Establishing a baseline for sampling and methodologies to assess forested ecosystems is crucial for understanding current conditions and identifying potential obstacles to forest development (Elliott et al., 2016). It is essential to develop robust and efficient methods for estimating carbon stocks and their changes, covering a range of spatial scales suitable for various mitigation activities. Field data is vital to understanding ecosystem characteristics and calibrating estimates across all spatial scales. However, collecting field data involves many trade-offs due to constraints such as access to forest sites, labour-intensive work, required equipment, and the number of sites sampled, and time taken. This thesis utilises forest inventory data to analyse biomass carbon stocks and biodiversity in temperate montane primary forests. The aim is to provide new knowledge about the current state of the primary forests, including their variability in time and space from fine to coarse scale across Central and Eastern Europe.

1.4 | Primary Forest Dynamics and Natural Disturbances

Currently, we have an incomplete understanding of the effects of natural disturbances on the temporal and spatial stability of forest biomass (Seidl et al., 2014; Zhang et al., 2014). Given the important climate regulation function of forests, improved insights regarding relationships between factors that influence the successional development of forests, and their associated carbon storage are critical to predict the consequences of global change. Disturbance regimes are characterised by the extent, frequency and severity of reoccurring events. The nature of a disturbance regime influences the growth potential, canopy structure and development pathways of forest systems (Frelich and Lorimer, 1991). Low-frequency catastrophic disturbances that cause extensive tree mortality (for example, bark beetle outbreaks in monodominant spruce forests of the Carpathians) substantially impact the economies of wood production and biomass storage over short and long time frames (Mikoláš et al., 2021).

In contrast, more frequent gap-scale disturbances (which predominate in the mixed species forests of the Carpathian region) may lead to a shifting mosaic of forest conditions over large areas, whereby temporal changes in stand structure potentially oscillate around a mean level (Bormann and Likens, 1979). For example, Frelich and Lorimer (1991) suggested that the age structure of forest patches in primary temperate forests of Eastern North America, where gap-scale storm disturbances are prevalent, was temporally stable when considered at a broad regional scale (that is, substantially exceeding the extent of a typical gap-generating disturbance). However, whether and under what conditions the biomass accumulation and carbon storage of forests potentially reach steady-state levels is still under debate (Luyssaert et al., 2008; Zhu et al., 2018). An improved quantification of biomass levels at continental scales is critical in an era of anthropogenic climate change where disturbance events are becoming increasingly severe and frequent.

1.5 | Research Rationale

This thesis focuses on investigating trends in biomass carbon stocks in mountain temperate primary forests across Central and Eastern Europe. It aims to quantify the amount of biomass carbon stored in naturally regenerating ecosystems and understand how primary forests develop over time and space in the absence of direct human influence. The findings presented here provide a catalyst for understanding the current state and health of these primary forests, particularly in terms of their accumulated biomass. By exploring the variability in aboveground biomass of live trees, dead standing trees, and downed dead wood along the forest floor, this study provides deeper insight into the processes that drive and maintain a diverse ecosystem.

Moreover, this thesis is novel as it utilises forest inventory data in montane temperate forests from a large network of permanent sample plots, covering a large geographic area and gradient. This thesis can help to inform and improve our understanding of the natural processes that influence forest ecology at the fine scale. For assessing forest ecology in remote, steep, and difficult-to-access locations, remotely sensed data are often used to bridge knowledge gaps. Furthermore, this thesis offers new knowledge and novel insights into the importance of quantifying both live and dead biomass accumulation.

1.5.1 | Temperate primary forest biomass accumulates over centuries-long time frames

To investigate the effects of natural disturbance regimes, tree age, and environmental conditions on forest biomass, this chapter focused on primary forests where direct human impacts have been minimal. Compound disturbance events can lead to increased mortality of large-diameter trees and biomass losses if a considerable proportion of large trees are frequently killed by high-severity disturbances (Yuan et al. 2021). In this chapter, we assessed the extant levels and drivers of biomass stocks in the primary forests of the Carpathian Mountains in Central and Eastern Europe. The study area covers a broad a range of forest types from mixed conifer–deciduous stands at lower elevations, where beech is often prevalent, to monodominant spruce stands at upper elevations. We used a unique dataset from an extensive field-based inventory of remnant primary forest stands that are distributed across continental-scale geographic and environmental gradients.

The spatially explicit disturbance history and tree age of all sampled locations were reconstructed using dendrochronological techniques. We quantified variation in extant levels of biomass at plot, stand and landscape scales for aboveground woody live reservoirs, as well as standing and downed deadwood. We formulated nonlinear regression models to investigate the factors influencing biomass stocks, specifically to quantity (Q1) the relationships between observed plot-level biomass stocks (total, live and dead) and the corresponding tree species composition of mean plot-level tree age, past natural disturbance severity, temperature, climatic water deficit and altitude.

We posited that variation in the severity of natural disturbances regulates the biomass of a forest. We expected that high severity disturbances, which are more common in spruce forests due to bark beetle outbreaks, limit maximum biomass levels, while lower severity perturbations may be associated with an elevated carbon accumulation. The aim was to (Q2) identify any thresholds in tree age beyond which primary forests in Europe potentially cease functioning as carbon sinks. Based on prior literature evidence (e.g., Keeton et al. 2011), we hypothesised that (H1) temperate primary forests in this region have a capacity to maintain net positive biomass accumulation rates over long time frames.

1.5.2 | Tree structure and diversity shape the biomass of primary temperate mountain forests

To investigate the roles of tree structure and genus-level diversity in determining the biomass carbon storage in temperate forest ecosystems. We aimed to investigate (Q3) what are the relationships between forest biomass, tree genus, and structure across different spatial scales; and (Q4) what other factors influence the spatial distribution of forest biomass, with reference to tree age, disturbances, topography, and climate. Using these criteria, we assess the relationships that influence biomass and forest heterogeneity regarding the range of tree genus diversity and structural complexity across the primary mountain forests. We hypothesise that (H2) biomass stocks are strongly influenced by structural complexities, followed by tree genus diversity, which in turn supports age-size heterogeneity and increases biomass. Moreover, we expect that (H3) forest heterogeneity, mediated by age and disturbances, influences biomass positively in more heterogenous forests and negatively with less heterogeneity.

1.5.3 | Spatio-temporal variability in carbon dynamics across the primary forests

The focus of carbon accounting is commonly on the net changes in atmospheric carbon dioxide, with the amount of carbon stock viewed as the “bottom line” of many influx (gain) and efflux (loss) processes. However, our knowledge of current carbon accounting in temperate montane primary forests is limited due to the lack of consistent monitoring of naturally regenerative forests across a large gradient, both spatially and temporally. In this investigation, we use novel approaches to assess the current carbon carrying capacity (CCC) of primary forests.

Here, we provide a novel approach to quantifying the spatial and temporal changes in primary forest carbon stocks in both live and dead biomass accumulation across two census periods, with an average interval of 5 years between censuses, from 454 permanent sample plots across Central and Eastern Europe. We aimed to investigate (Q5) how carbon dynamics have changed across the primary forests over time and the rate of these changes, and (Q6) how site-specific factors (mortality, tree age, carbon stock, altitude) influence the spatial and temporal variations in carbon dynamics across the primary forests.

This chapter aims to assess whether primary forests continue to accumulate carbon or whether the stocks reach equilibrium. By addressing these questions, we seek to elucidate the factors influencing changes in carbon pools and ultimately evaluate the long-term carbon-carrying capacity of primary forests. This chapter further investigates trends in carbon dynamics across different forest types (mixed beech, spruce) and landscapes (Western, Eastern, Southern) within the Carpathian Mountains, assessing the amount of survival, growth, mortality, and the transfer of carbon from the live to dead biomass pools.

Moreover, we aim to assess the variation in both live and dead carbon stocks across the Carpathians to better understand their dynamics. We hypothesise (H4) that these primary forests function as stable carbon reservoirs, with a gradual increase in carbon stocks over time, suggesting the continued capacity of these forests to act as carbon sinks. This study is significant as it provides a comprehensive analysis of primary forest carbon dynamics, contributing to our understanding of how these ecosystems function and their role in the global carbon cycle. By integrating data from multiple regions and forest types, we offer a robust assessment of the factors influencing carbon stores and the stability of the primary forests, which is crucial for developing effective conservation and management strategies to mitigate climate change.

1.5.4 | Primary Forest Biomass: A Comprehensive Synthesis

The key findings and results in this thesis are synthesised in a dedicated subchapter, focusing on the carbon accounting and biodiversity in the temperate montane primary forests. The overall rationale is to provide a comprehensive insight into carbon and biodiversity trends, which can be used to inform the current status of the Carpathian primary forests, and how they relate to climate mitigation targets through enhanced carbon storage and biodiversity conservation. The final, cumulative subchapter investigates the role of carbon dynamics, the importance of large-diameter trees in maintaining carbon stores, and the effects of different tree age cohorts, size ranges and disturbance regimes. Additionally, to examine the relationship between biomass, tree diversity, and local (site-specific) factors such as tree size variability, tree density, topography and climatic water deficit.

Chapter 2 | Literature review

2.1 | Global Distribution of Biomass

Forest biomass refers to the biological mass of living and dead organic matter, accumulated by trees through growth and decomposition that exists in a terrestrial ecosystem (Bar-On et al., 2018; Houghton, 2005; Keeling and Phillips, 2007). Since biomass represents the amount of live and dead carbon stocks available in a forest, it can be useful for assessing the health of a forested ecosystem by quantifying the amount of carbon stock present and how site conditions, such as biodiversity to disturbance regimes influence biomass (Houghton, 2005; Keith et al., 2010; Mackey et al., 2013). Naturally regenerative forests benefit from continual accumulate of living biomass which transfer to the dead standing and forest floor carbon pool as coarse woody debris through decomposition (Mackey et al., 2013; Nord-Larsen et al., 2019).

FAO (2020) report that forest carbon stocks in Europe (excluding the Russian Federation) for the live, dead and soil carbon pool are, 68.4 tC ha^{-1}, 18.4 tC ha^{-1}, and 107 tC ha^{-1}, respectively. Moreover, (FAO, 2020) revealed a decrease in total forest carbon stocks between 1990-2020 by 6 Gt to 662 Gt, due to loss in forested areas, with a risk in most ecosystems shifting from a carbon sink to a source (FAO, 2020). However, FAO (2020) recognised that despite a global decline in total forest carbon, there have been significant increases in live and dead carbon stocks in some continents, including Europe which was attributed to an increase in forest area and improved protection (European Council, 2023).

Studies by Bar-On et al. (2018) distinguish biomass into three major biome types, including marine (~6 Gt C), deep subsurface oceanic (~70 Gt C) biome and terrestrial, which represented the biggest share of biomass carbon stocks (~ 470 Gt C). Accounting for the current amount of biomass carbon in a forest is essential for understanding its stability over time, whether that is seasonal, annual or a coarser time series to observe the spatial and temporal variability (Houghton, 2005; Reich et al., 2014). Assessing forest biomass and its ecology at the fine scale (i.e. < 1 ha) can provide a greater insight into the niche conditions and characteristics that influence biomass at the local level (Zhou et al., 2016). Local, endogenous processes such as tree species diversity, tree size, density, natural disturbances, topography, water availability, can have contrasting influence on forest demography which might not be detected at coarser scales (i.e. > 1 ha) (Houghton, 2005; Reich et al., 2014).

2.2 | Primary Forests

Terrestrial ecosystems, particularly forests, exist along a diverse gradient of conditions, including diversity of ecosystem functions (biomass), species, tree density, structural complexities (diameter and height distribution), age and the modality of disturbances (i.e., natural processes or human activity) (Houghton, 2005; Mackey et al., 2015; Reich et al., 2014; Sabatini et al., 2020; Zhang et al., 2018). These variations are largely influenced by their spatial distribution, both latitude and longitudinal position, driven by the type of climate and, in turn, forest type (i.e., broadleaved, conifer, mixed forest). Primary forests refer to naturally regenerated forests of native species, whose composition and structure are regulated by ecological processes, including natural disturbance regimes (Mackey et al., 2015). Primary forests can also be described a naturally regenerative, primaeval, virgin forests with varying degree of natural regeneration. FAO (2020) define primary forests as naturally regenerative forest that feature native tree species with no clear or direct impact from human activity, which attributes to these forests being globally rare and spatially fragmented. As a result, primary forests feature unique qualities compared to other forest systems, being populated by native tree species, whose composition, structure and dynamics are dominated by ecological and evolutionary processes including natural disturbance regimes (FAO, 2020; Mackey et al., 2015).

The legacy of ecological activity and development in a forest, along with extant conditions and land use, have a considerable influence on a forest with comprises of mostly early to late seral development (Mackey et al., 2015; Sabatini et al., 2020; Svoboda et al., 2010). Mackey et al. (2021) explain how the conditions of a given ecosystem depend on the ecological processes and the proportion or types of disturbances, either natural disturbances or a direct cause of human activity to the gradient of management or no management practises. These forests are an important store of carbon in the biosphere, but there is uncertainty about the persistence of this store under the increasing effects of climate change (Mackey et al., 2015; Case et al., 2021). Responses and adaptation of forest ecosystem dynamics, in terms of complexities in complexity from long history of development, structure and functioning, to changes in climate and disturbance regimes may affect the magnitude and longevity of the carbon stored (Pan et al., 2011; Houghton et al., 2009; Keeling and Phillips, 2007). Such changes in terrestrial carbon stocks could trigger feedbacks with the atmospheric carbon dioxide concentration and climate. Thus, primary forests can be considered important ecosystems that rely on the continual flow and occurrence of natural disturbance regimes and regeneration to support an uneven age and structured forest without human intervention (Mackey et al., 2021). Such diversity of conditions that are primarily driven by natural processes make primary forests an important refuge as a steady carbon reservoir that supports the accumulation of live and dead biomass (Duque et al., 2021; Keith et al., 2024; Sabatini et al., 2019).

2.2.1 | Protection of Primary Forests

The European Union has established the European Green Deal (European Council, 2019) as a roadmap to climate neutrality by 2050, in line with the Paris Agreement (UNFCCC, 2015). This ambitious goal involves reducing greenhouse gas emissions and restoring ecosystems. The EU Biodiversity Strategy aims to mitigate the direct and indirect impacts of environmental degradation on biodiversity, supporting sectors such as agriculture, fisheries, and forestry (European Council, 2023). The recent adoption of the natural restoration law targets 20% restoration by 2030 (European Council, 2023). There is a strong need for improved protection of primary as well as non-primary forests, since they represent a larger portion of forested areas globally. Enhanced protection leads to improved forest biodiversity, supporting native tree species, providing stable habitats for flora and fauna, and ensuring the stability of carbon store (European Council, 2023; Keith et al., 2024; Sabatini et al., 2020).

FAO (2020) report found that in Europe, recent decades have witnessed a positive trend in European primary forest area, driven by improved land use practises (Sabatini et al., 2020) and may continue to increase due to recent adoption of supportive legislation, notably the European Green Deal (European Council, 2019) with the goal of expanding forest area and their protection. The most substantial gains in primary forest area occurred between 2000 and 2010 (33,000 ha^{-1} yr^{-1}) with a subsequent decrease in the rate of increase coverage during the following decade (2010 – 2020; 9,000 ha^{-1} yr^{-1}) (FAO, 2020). The spatial scarcity of primary forests, both across Europe and globally, are valuable ecosystems that act as a carbon refuge in terms of their steadily in storing biomass carbon (Mackey et al., 2021). Such conditions are challenging, yet possible to replicate in non-primary forests where human intervention is present and had a larger impact in shaping the ecosystem. Therefore, primary forests can be considered as one of the last remaining terrestrial ecosystems where natural processes influence and drive forest productivity and shape its demography without human intervention.

2.2.2 | European Primary and Old-Growth Forests

Europe has a long history of intensive land use stemming from urban development, agriculture and silviculture and their expansion into formerly natural environments (Ciais et al., 2008). Moreover, Europe accounts for 27% of remaining primary forests globally, this figure drops to 3% when excluding the Russian Federation (FAO, 2020). The low proportion of primary forests in Europe is attributed to centuries of human land use and development, leading to a higher proportion of secondary and production forests across the continent (Sabatini et al., 2019). Despite the small land cover, European temperate and boreal regions are comparatively well-studied compared to those in Asia and Africa. While the latter continents feature more species-rich primary forests, data gaps limit a comprehensive global assessment of primary forests (Mackey et al., 2015; Sabatini et al., 2020).

Similarly, studies by Meyer et al. (2021) on deciduous forests in northwestern Germany revealed the nuanced trends in growth and mortality rates between pure beech, mixed oak and mixed beech forests. Pure beech forests had the highest biomass (478 Mg ha^{-1}), followed by mixed beech (434 Mg ha^{-1}) and mixed oak (334 Mg ha^{-1}), with biomass levels affected by species interactions (Meyer et al., 2021). Beech forests are particularly efficient at accumulating biomass in low-light conditions, while similar conditions in oak forests can reduce productivity. In old-growth forests, the diversity of species and age structures leads to higher deadwood accumulation, contributing to the forest's uneven age and structure (Meyer et al., 2021). The slow decay of wood allows deadwood to sustain biomass levels for decades, even as living biomass declines (Carmona et al., 2002; Meyer et al., 2021).

Martin-Benito et al. (2021) studies on old-growth *Quercus petraea* forest in the western Cantabrian Mountains, Spain, focusing on forest dynamics and carbon dynamics. The study found a decrease in tree abundance from smaller to larger diameters, with large trees (>70 cm, dbh) being less frequent but dominating the forest biomass, comprising 50% of the total aboveground biomass (AGB). Over the last 400 years, the forest has experienced frequent low-severity disturbances, with rare major events affecting less than 20% of the trees (Martin-Benito et al., 2021). These disturbances and recruitment periods in the 19th century have shaped the forest's current age distribution and structural diversity. Despite lower productivity in old-growth forests, the biomass of large tree cohorts significantly surpasses that of secondary forests. Forest demography was found to be driven by the disturbance of an individual tree, either by uprooting, partly breakage or gradual decay from the canopy which of large diameter trees, which can drastically shape the structure of a forest and its overall carbon carrying capacity (Janda et al., 2019; Keith et al., 2024; Martin-Benito et al., 2021; Synek et al., 2020). Martin-Benito et al. (2021) identified that the low tree slenderness and relatively open canopies in a forest reduce the likelihood of large-scale disturbances, such as wind-throw, compared to closed-canopy forests.

2.3 | Primary Forests – The Carpathians

The Carpathian primary forests consist of two main forest types, which are Norway spruce (*Picea abies* (L.) *Karst*) and mixed beech (*Fagus sylvatica* L.). In the mixed beech forests of the Carpathians, there is a diverse range of species composition, tree densities and low- to intermediate-severity natural disturbances (primarily wind driven), which in combination contribute to a multi-layered forest structure (Stillhard et al., 2022). Beech (*Fagus*) wood has a slow decay rate, and thus, dead wood is maintained in the forest carbon store for decades (Meyer et al., 2021). In the spruce forests of the Carpathians, windstorms and bark beetle disturbances of low to intermediate severity have a range of impacts. Localised windstorms may fell individual or groups of trees and generate small canopy gaps. Severe windstorms, which can occur in both forest types, or bark beetle outbreaks in spruce forests, may cause extensive tree mortality over large areas (Meigs et al., 2017). These processes have promoted both even- and uneven-aged forest stand structures that support a high variability in biomass levels (Janda et al., 2019). The effects of natural disturbances at stand or regional scales have been difficult to distinguish from the confounding effects of biophysical conditions that vary over gradients of topography and climate (Janda et al., 2019). However, stands can accumulate large carbon stocks after periods of high disturbance severity, while lower disturbance severity levels create less regeneration of carbon stores (Seedre et al., 2020).

Studies in Ukrainian Carpathian primary forests examine changes in forest attributes such as tree density, basal area, and the volume of living and dead trees (Stillhard et al., 2022). Stillhard et al. (2022) recognised the underrepresentation of key species, including *Acer pseudoplatanus*, *Carpinus betulus*, and *Abies alba*, and their contribution towards shaping forest demography in terms of tree size variation and diversity. Beech's shade tolerance and competitive dominance enable it to efficiently fill canopy gaps, contributing to the multi-layered structure characteristic of primary forests. The study supports the idea that these forests are in equilibrium regarding productivity and development (Stillhard et al., 2022). Since natural processes drive primary forests, any abrupt changes in disturbance regimes could disrupt this equilibrium, leading to a novel successional phase if recruitment is weaker and large-diameter trees are lost (Čada et al., 2013; Stillhard et al., 2022; Svoboda et al., 2014).

2.3.1 | The distribution of biomass across the Carpathians

In the Carpathians Mountains, Janda et al. (2019) investigated basal area variation in primary *Picea abies* forests to understand the influence of biophysical environments and natural disturbances. Stand-level total basal area was found to have a positive relationship between mean annual temperature and basal area of live biomass (Janda et al., 2019). Furthermore, Janda et al. (2019) identified forest age structures are synchronised, suggesting increased susceptibility to disturbances and a potential rise in disturbance frequency and severity (Mikoláš et al., 2019).

Fine-scale disturbances were identified as key drivers of basal area variation within stands, with the proportion of live to dead trees at finer scales indicating the impact of biophysical and natural disturbances (Janda et al., 2019; Mikoláš et al., 2019; Seedre et al., 2020). These findings were supported by Seedre et al. (2020) in assessing biomass carbon accumulation and their relationship with natural disturbances across the dominant spruce primary forests in the Carpathians. Primary forests store significantly more carbon than managed forests, but they face increasing threats of conversion to managed forests, leading to immediate carbon losses and reduced biodiversity (Keith et al., 2024; Mikoláš et al., 2019; Sabatini et al., 2020; Seedre et al., 2020). The long-term impact of disturbance severity on carbon stores highlights the need to account for these factors when predicting future carbon accumulation (both live and dead carbon pools) (Seedre et al., 2020). Mikoláš et al. (2021) investigated how long-term natural disturbances impact forest biodiversity and carbon dynamics over 250 years in the Carpathian primary forests. The study emphasises the importance of multi-scale analyses to understand how biodiversity and carbon stocks fluctuate across regenerating forests. By combining carbon stock with site specific data (i.e. tree species diversity, tree size, dendrochronology reconstruction) provides a detailed account into fine-scale carbon carrying capacity of the forest; capturing the historical influences of disturbances and maturity levels and how they shape present forest demography (Janda et al., 2019; Mikoláš et al., 2021; Seedre et al., 2020). The findings emphasise the need for forests to mature and have older trees, requiring the protection and expansion of primary forests to sustain biodiversity and carbon storage (Mikoláš et al., 2021), especially amid the current environmental crisis (Keith et al., 2024; Mina et al., 2017).

2.4 | Allometric Equations – Estimating Forest Biomass

Allometric equations are a crucial method for statistically estimating forest biomass carbon, calculating species-specific biomass while accounting for variables such as diameter, site conditions, wood density, and height introduces uncertainty and potential errors (Réjou-Méchain et al., 2021). Accurate measurement often requires destructive methods, such as tree felling, which disrupts ecosystems and reduces biomass. Pseudo-observation estimates, derived from species-specific wood density and structural parameters (diameter, height), are used to estimate biomass (Forrester et al., 2021, 2017; Pan et al., 2013; Pugh et al., 2019b; Vanderwel et al., 2013). The main challenge in biomass carbon-based studies is selecting suitable allometric equations, given the complexities of using species-specific parameters and forest inventory measurements (Forrester et al., 2017; Réjou-Méchain et al., 2021).

Numerous allometric equations exist for various terrestrial biomes, especially temperate and boreal regions. However, challenges arise in obtaining forest inventory data from remote sites and from the species diversity and density within tropical biomes (Bar-On et al., 2018; Houghton, 2005). Although temperate ecosystems are well-represented, limitations in allometric techniques and statistical estimations persist. Errors in estimation often stem from inaccurate forest inventories and small sample sizes (Forrester et al., 2021, 2017; Zianis and Mencuccini, 2004). Biomass predictions require precise diameter at breast height (dbh) measurements, usually obtained from the mean stem diameter used to fit the equation (Forrester et al., 2017; Zianis and Mencuccini, 2004). Applying these measurements to populations with different diameter distributions can reduce accuracy, highlighting the need for standardised methodological approaches. Forrester et al. (2021) emphasise the importance of including tree height alongside diameter to achieve accurate biomass estimates. Additional structural parameters offer greater insight into how competition and inter-tree characteristics influence biomass production, accounting for multiple factors affecting net primary production and stand development (Forrester et al., 2021).

Inaccuracies in allometric equations can misrepresent the true quantity of biomass in a location. Partitioning allometric equations allows for the subdivision of biomass analysis between long-lived components (stem, coarse root system) and short-lived components (fine-root system, foliage, branches) (Reich et al., 2014). Forrester et al. (2017) observed that a decline in foliage often coincides with increased basal area growth, reflecting a reduction in short-lived tissue productivity. This leads to reduced photosynthetic activity as the tree compensates for the suppression of stem growth. Variability in root and foliage biomass supports the hypothesis that mean annual temperature and biomass are influenced by temperature gradients, particularly in trees with limited or no access to the canopy (Reich et al., 2014). With less space for crown expansion or fine-root development, more biomass may be allocated to stems, enabling trees to reach the canopy layer (Senf et al., 2019). However, a recent study by Forrester et al. (2021) using a small sample area in a young Mediterranean plantation forest produced results partly incompatible with earlier findings (Forrester et al., 2017). Temperate biomes generally exhibit greater biomass carbon compared to

Mediterranean environments, due to more favourable conditions for net ecosystem production.

Réjou-Méchain et al. (2021) explore methods for upscaling and mitigating errors in forest biomass estimates, including the use of forest inventories and remote sensing techniques to scale from the landscape to the global level through satellite imagery. Forest biomass estimates are derived from allometric equations that factor in tree dimensions (diameter, height) and wood density to create an accurate representation of biomass carbon storage (Forrester et al., 2021; Lu et al., 2016; Réjou-Méchain et al., 2021). This non-invasive technique is preferable to destructive methods, which, although accurate, reduce tree density and can have detrimental effects on the ecosystem.

Allometry-based estimations are an ideal alternative for assessing forest ecosystem productivity. However, the potential for error depends on the suitability of the allometric equation and the parameters used (Forrester et al., 2017; Réjou-Méchain et al., 2021). Bias can weaken the robustness of the study by failing to accurately represent biomass distribution. Standardisation is essential to ensure forest inventory data are precise and consistent, thereby avoiding over- or underestimation in biomass estimates (Zianis and Mencuccini, 2004). Consistent data quality across study sites is crucial to minimising errors and ensuring reliable biomass estimates.

2.5 | Forest Biodiversity

Tree genus diversity and structure are critical factors in determining tree growth characteristics and, thus, how much biomass can be accumulated by an individual tree and a forest's overall carbon-carrying capacity. However, their effects can vary depending on local conditions, such as tree density, altitude, temperature and water availability (Fotis et al., 2018; Reichstein and Carvalhais, 2019). Combining tree genus diversity (abundance, richness, tree size distribution) with local, site-specific factors (i.e., climate, topography, tree age, natural disturbances) can enable a better understanding into the mechanisms that influence biomass stocks across a broad geographic gradient (Michaletz et al., 2018; Wang and Ali, 2022).

The structure of trees at different spatial scales can help identify how individual trees respond to stand-level conditions, such as age and size distribution. Ehbrecht et al. (2021) found that the range in uneven tree size distribution (small to large diameter range) strongly influences biodiversity (i.e., species diversity) and ecosystem functions (biomass). However, large-diameter trees (i.e., ≥ 60 cm dbh) may contribute ≥ 50% to the communities' total live biomass stocks (Keith et al., 2010; Lutz et al., 2018; Wang and Ali, 2022). Investigating the range in tree sizes can provides a deeper insight into the spatial range in biomass across communities.

Species distribution influences structural complexity and biomass stocks, but its effect can vary depending on local conditions and disturbance regimes which can influence forest ecosystem functioning (Fotis et al., 2018; Reichstein and Carvalhais, 2019). Szwagrzyk and Gazda (2007) found that beech-dominant stands across Central Europe contain high biomass, resulting from a low frequency of high disturbance events rather than the species being a particularly strong competitor. Furthermore, Mikoláš et al. (2021) demonstrated that local variability in disturbance regimes plays a crucial role in maintaining forest carbon stores and promoting biodiversity in the primary forests of the Carpathian Mountains.

Climate change induced by human activities may disrupt biomass accumulation, for example through exceptional disturbance events beyond that of the previous regime, with the potential of compromising the resilience of the forest ecosystem to future disturbances and affecting the structural integrity and biodiversity. Interestingly, Sabatini et al. (2019) found that maximising biodiversity and carbon storage at the stand level in temperate forests is challenging, and broader strategies, such as zoning, may offer better outcomes regarding their co-benefits. Explicitly considering all conservation-relevant taxa, rather than relying on biodiversity surrogates, is essential for quantifying how native species respond to changes in forest structure and carbon levels, which can influence overall biodiversity (Sabatini et al., 2019). Whilst Sabatini et al. (2019) may not have found a clear signal between carbon stock levels and biodiversity, safeguarding both to maximise their co-benefits can lead to improved ecosystem stability (Keith et al., 2024). This implies that such forests become stable and healthy carbon stores and overall carrying capacity. Therefore, understanding forest heterogeneity by assessing structural complexities and biomass is essential for revealing the state of the carbon store and its stability (Ehbrecht et al., 2021; Mackey et al., 2015; Wang and Ali, 2022).

2.6 | Natural Disturbance and Forest Maturity

Natural disturbances are an essential part of any terrestrial ecosystem, as a means of controlling and driving ecological processes and functionality across the ecosystem. Natural disturbances in primary forests create an uneven distribution in age and size structure of trees and their consequent biomass stocks, with mosaic effects at scales from small clusters of trees to landscape (macro) levels (Johnstone et al., 2016; Pugh et al., 2019b; Sprugel, 1991). The capacity of a forest to recover after a disturbance event depends on individual tree survival and regeneration traits, and their resulting post-disturbance legacies that produce an uneven-age forest structure (Janda et al., 2019; Schurman et al., 2019). The random distribution in disturbance occurrences and severities and their relationship with abiotic and biotic processes maintain a broad range in biomass stocks. Investigating the role of natural disturbances and tree age, in addition to forest biomass, climate, and topographical factors, provides a deeper understanding of the complex relationships between biotic and abiotic factors in maintaining a diverse ecosystem.

Disturbance regimes are characterised by the extent, frequency and severity of reoccurring events. The nature of a disturbance regime influences the growth potential, canopy structure and development pathways of forest systems (Frelich and Lorimer, 1991). Low-frequency catastrophic disturbances that cause extensive tree mortality (for example, bark beetle outbreaks in monodominant spruce forests of the Carpathians) substantially impact the economies of wood production and biomass storage over short and long time frames (Mikoláš et al., 2021). In contrast, more frequent gap-scale disturbances (which predominate in the mixed species forests of the Carpathian region) may lead to a shifting mosaic of forest conditions over large areas, whereby temporal changes in stand structure potentially oscillate around some mean level (Bormann and Likens, 1979). For example, Frelich and Lorimer (1991) suggested that the age structure of forest patches in primary temperate forests of Eastern North America, where gap-scale storm disturbances are prevalent, was temporally stable when considered at a broad regional scale (that is, substantially exceeding the extent of a typical gap-generating disturbance). However, whether and under what conditions the biomass accumulation and carbon storage of forests potentially reach steady-state levels is still under debate (Luyssaert et al., 2008; Zhu et al., 2018). An improved quantification of biomass levels at continental scales is critical in an era of anthropogenic climate change where disturbance events are becoming increasingly severe and frequent.

The capacity for a forest to recover after a disturbance event depends on individual tree survival and regeneration traits, and their resulting post-disturbance legacies that produce an uneven-age forest structure (Janda et al., 2019; Schurman et al., 2019). The random distribution in disturbance occurrences and severities and their effects with abiotic and biotic processes maintain a broad range in biomass stocks. Currently, we have an incomplete understanding into the effects of natural disturbances on the temporal and spatial stability of forest biomass. Given the important climate regulation function of forests, improved insights regarding relationships between factors that influence the successional development of forests, and their associated carbon storage are critical to predict the consequences of global change. Disturbance regimes are characterised by the extent, frequency and severity of reoccurring events. The nature of a disturbance regime influences the growth potential, canopy structure and development pathways of forest systems (Frelich and Lorimer, 1991). Low-frequency catastrophic disturbances that cause extensive tree mortality (for example, bark beetle outbreaks in monodominant spruce forests of the Carpathians) substantially impact the economies of wood production and biomass storage over short and long time frames (Mikoláš et al., 2021).

Chapter 3 | Methodology

3.1 | Introduction to the methodology

This chapter establishes the methodological framework of the research into primary forest biomass and carbon dynamics across Central and Eastern European temperate forests. This chapter aims to provide a full account of the methods for investigating montane temperate primary forest biomass carbon across Central and Eastern Europe. Featuring a detailed description of the protocols used throughout the thesis and the distinct approaches for each of the three main research aims. The first two subchapters (3.1 and 3.2) comprehensively describe the common methods used across all three research topics featured in this thesis. This includes details for determining the study area, forest inventories, sampling protocols and biomass calculations using species-specific allometric equations. A detailed description of methods for each specific research aim can be found in subchapters 3.3, 3.4, and 3.5.

3.1.1 | Study area

The study area for this research encompasses the Carpathian Mountains (hereafter referred to as the Carpathians'). The study area of the research for each research aim was conducted in the Carpathian which comprises of extensive tree-level data, collected across the temperate montane primary forests. The Carpathians is the one of the largest mountain ranges in Europe, being over ~1,500 km long (Kholiavchuk et al., 2023; Mráz and Ronikier, 2016). The Carpathian Mountain range stretches from Central to Eastern Europe, encompassing portions of Austria, the Czech Republic, Slovakia, Poland, western Ukraine, Romania, and parts of northern Serbia (Holeksa et al., 2009; Kholiavchuk et al., 2023; Mráz and Ronikier, 2016). In this thesis, the Carpathians hereby refers to portions of Slovakia, Ukraine and Romania, representing over ~80% cover of the mountain range (figure 1). Permanent sample plots were established across primary forests in the Carpathian Mountain Range, covering areas unaffected by direct human activity. The remoteness of most forest stands, combined with steep terrain and high altitude, naturally deters agricultural land use, thus attributing to the long periods of natural growth and development. For more detail on plots and sampling protocol, see subchapter 3.1.2.

The Carpathian forests experience natural disturbances at various scales. Localised events such as windstorms, snowstorms, and bark beetle outbreaks in spruce forests can cause physical damage to trees (crown breakage, stem snapping, or uprooting) (Frankovič et al., 2021; Mikoláš et al., 2021; Synek et al., 2020). Shifts in climate and the multifaceted threat of future climate change adds to the level of complexities in how natural disturbances influence the mountain region and the primary forests. Climate change adds another layer of complexity. Increasing temperatures due to drought have a widespread, disproportionate effect on forests at different elevations, may disrupt current disturbance regimes and elevate the impact by localised disturbance agents (Chivulescu et al., 2021; Schurman et al., 2019). Whereas outbreaks of European bark beetle (*Ips typographus* L.) are widespread but species-specific, only infesting spruce (Synek et al., 2020).

The unique geography of the Carpathians fosters extensive areas of naturally regenerating forests, unaffected by human intervention. These naturally regenerating ecosystems are globally rare and fragmented. Especially in Europe, considering the continent's long history of land use, which saw an increased loss in natural forests since the late 19th century (Holeksa et al., 2009; Sabatini et al., 2019). As such, areas such as the Carpathians, can be seen as the ecological refuge due to the existence of large, contiguous forest cover. Since the Carpathians features a complex and varied geography, this attributes towards a highly diverse topographic and climatic conditions, leading to the spatial variability in ecological traits (i.e., species distribution and development) across the range (Holeksa et al., 2009; Sabatini et al., 2019). The observed difference in species richness and the presence of endemic species between the Western and Southeastern Carpathians can be attributed, in part, to favourable palaeoecological conditions during the Pleistocene and Holocene epochs (Kholiavchuk et al., 2023; Mráz and Ronikier, 2016). Since the Last Glacial Maximum, the Carpathians have served as a crucial refuge for flora and fauna, thus making the region a valuable and critical biodiversity hotspot.

These primary forests represent ecosystems spanning early to late-seral stages, shaped primarily by natural processes due to the lack of human intervention. This allows for the accumulation of significant live and dead biomass (Holeksa et al., 2009; Sabatini et al., 2019). Disturbance agents vary from local to regional scales. Climatic extreme events, such as bark beetle outbreaks in spruce forests to wind/snowstorms, have a localised impact on the proportion of physical breakage of a tree (i.e., crown, stem or uprooting) (Frankovič et al., 2021; Mikoláš et al., 2021; Synek et al., 2020). Increasing temperatures due to drought have a widespread, disproportionate effect on forests at different elevations, may disrupt current disturbance regimes and elevate the impact by localised disturbance agents (Chivulescu et al., 2021; Schurman et al., 2019). Whereas outbreaks of European bark beetle (*Ips typographus* L.) are widespread but species-specific, only infesting spruce (Synek et al., 2020).

To investigate the variability in the accumulation of living and dead biomass and overall carbon carrying capacity of the primary forests, the study area is divided into two main forest types. This enables for a better understanding of the ecological functionality (i.e., biomass stocks) and site-specific conditions (tree size, age, disturbances, topography) vary in distinct forest types at different elevation ranges. Forest types include Norway spruce (*Picea abies* L. *Karst*) dominating the upper elevation sites, and European beech (*Fagus sylvatica* L.) at the lower elevations (Janda et al., 2019; Schurman et al., 2019). At lower elevations (600 - 1,200 metres above sea level – m.a.s.l), deciduous forests are characterised by varying proportions of beech (*Fagus sylvatica* L.) which is the dominant species, silver fir (*Abies alba* Mill.), and sycamore (*Acer pseudoplatanus* L), with less common small-leaved lime (*Tilia cordata* Mill.) and common hornbeam (*Carpinus betulus* L.). The western Carpathians (Slovakia) feature a cooler climate, whereas warmer, milder conditions occur in the Southern Carpathians (Ukraine and Romania) (Janda et al., 2019; Saulnier et al., 2020; Schurman et al., 2019). However, coniferous species such as Picea and Alba are not exclusive to the upper elevations (i.e., > 1,200 m a.s.l) and present in the deciduous, pre-dominantly beech forests. As such, identifying how genera respond in different parts of the Carpathians enables a deeper understanding of the ecological processes that influence biomass accumulation across the Carpathians.

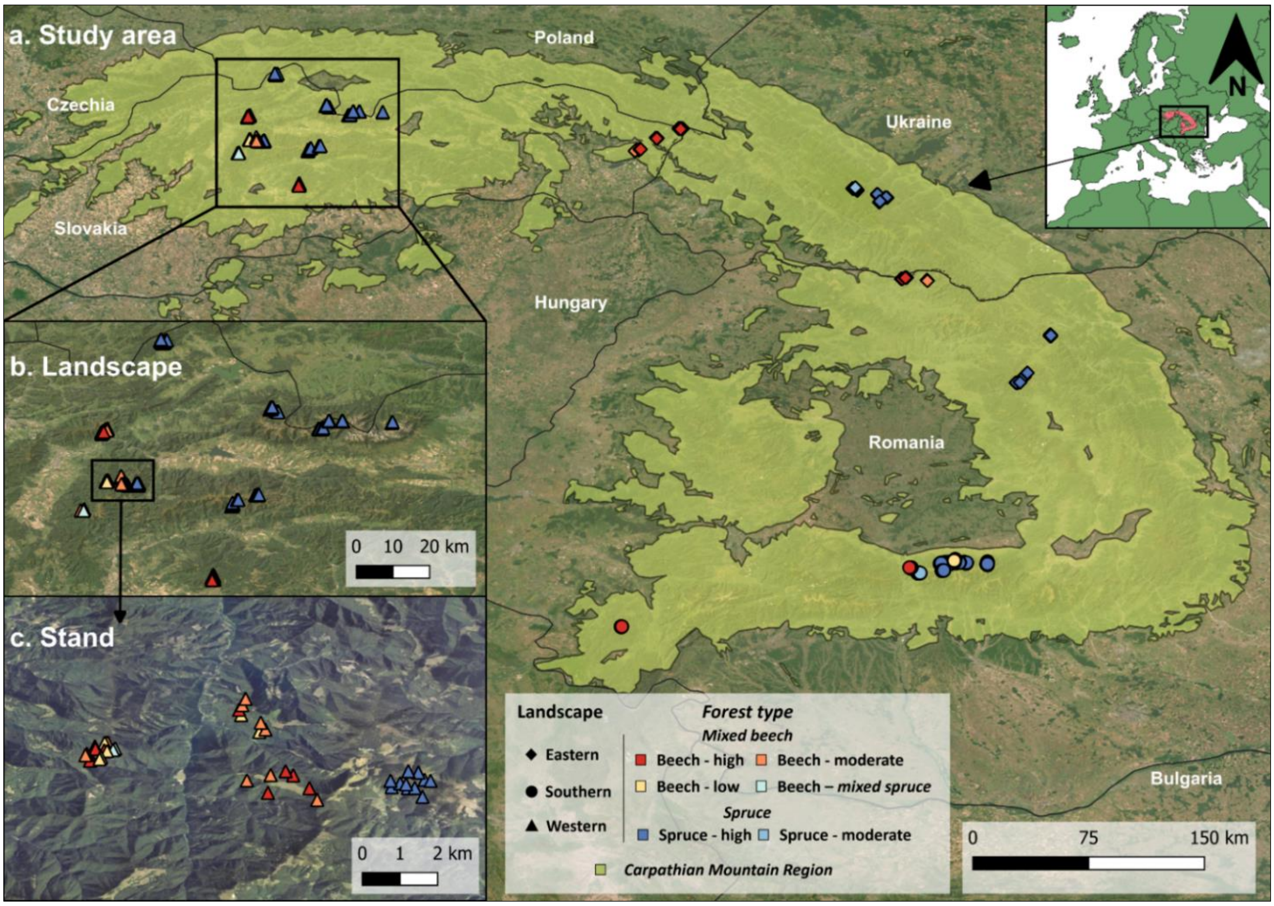


Figure 1: Study area - Map of the Carpathian Mountains showing the study area and the 726 plots with three spatial scales, representing the (a) study area, (b) landscape (Western, Eastern, and Southern Carpathians), and (c) stands situated within a given landscape containing each individual plot. Each plot was categorised by the prevalence of beech and spruce in their respective forest types, based on the Importance Value Index (IVI) score to identify genera presence by low (< 100), moderate (100 – 150) and high (> 150) IVI scores; Beech – mixed spruce (n plots = 4) being plots in the mixed beech forests characterised as being dominantly spruce in structure and genus coverage.

3.1.2 | Forest inventories: data collection and sampling protocols

We used data from the REMOTE Network (www.remoteforests.org; REMOTE Primary Forests, 2020) from permanent sample plots in the Carpathians across Slovakia, Ukraine and Romania. Plots were set up in areas identified as primary forests, following a stratified random design. We used a hierarchical scale comprising of three levels at different spatial scales: 1) macro-scale - landscape level (Western, Eastern, and Southern Carpathians); 2) intermediate-scale - stand, nested within a landscape; 3 - fine-scale - plot (or patch), nested within a given stand (figure 1). All plots followed a circular design with mixed beech forest at 0.1 – 0.15 ha and spruce forest plots at 0.05 – 0.1 ha plot area (Frankovič et al., 2021; Mikoláš et al., 2019; Svoboda et al., 2014) for mixed beech plots. In the spruce stands, single circular plots were established with an area of 0.05 - 0.1 ha. Mixed beech plots use a nested-circular design with size of 0.1 - 0.15 ha. To unify the methods across each sample year, we set the dbh threshold to ≥ 10 cm for both forest types (spruce and mixed beech).

Two classes of permanent sample plots were used to investigate the research aims across the Carpathian Mountains, based on the number of censuses conducted in each plot. The first class comprised 726 plots, each providing single-census data on biomass stocks collected during initial plot establishment, used for investigating research aims 1 and 2. The second class included 454 plots that have undergone re-measurement, allowing for analysis of changes over time to investigate research aim 3 - carbon dynamics. The first census in these plots represents the year of establishment, while the second signifies the first re-measurement event, with an average five-year interval between them. For more details on plots with multi-censuses and respective sampling protocols, see subchapter 3.4.1.

All individual live and dead standing trees ≥ 10 cm diameter at breast height (dbh) 1.3 m aboveground were identified in each plot. Tree position (x, y - metres) of each tree was also measured based on their distance from the plot centre using the spatial mapping software *FieldMap* using a *Getac* tablet. Field mapping of each tree using the tablet was placed in the plot centre, attached with a receiver and transponder on the outer bark of a tree to measure the precise distance from the plot centre. Tree cores were sampled from 5-20 non-suppressed living trees with a dbh ≥ 10 cm. Each tree was sampled with a single core extracted at a height of 1 m aboveground and perpendicular to the slope of the ground (Janda et al., 2019; Mikoláš et al., 2019). Dead standing trees were categorised by decay stage (table 3). Downed dead wood was measured using a line transect approach, where five 20 m transects, all starting at the centre of the plot, were set in cardinal directions (0°, 72°, 144°, 216°, and 288°; Mikoláš et al., 2019). We measured all downed dead wood debris ≥ 6 cm that intersected the transect line. Both standing and downed dead wood were identified to the species type where possible. Topographic data, including landform, denotes plot position along a slope gradient within a stand, slope and altitude were recorded onsite from the centre point of each plot during forest inventories (table 1).

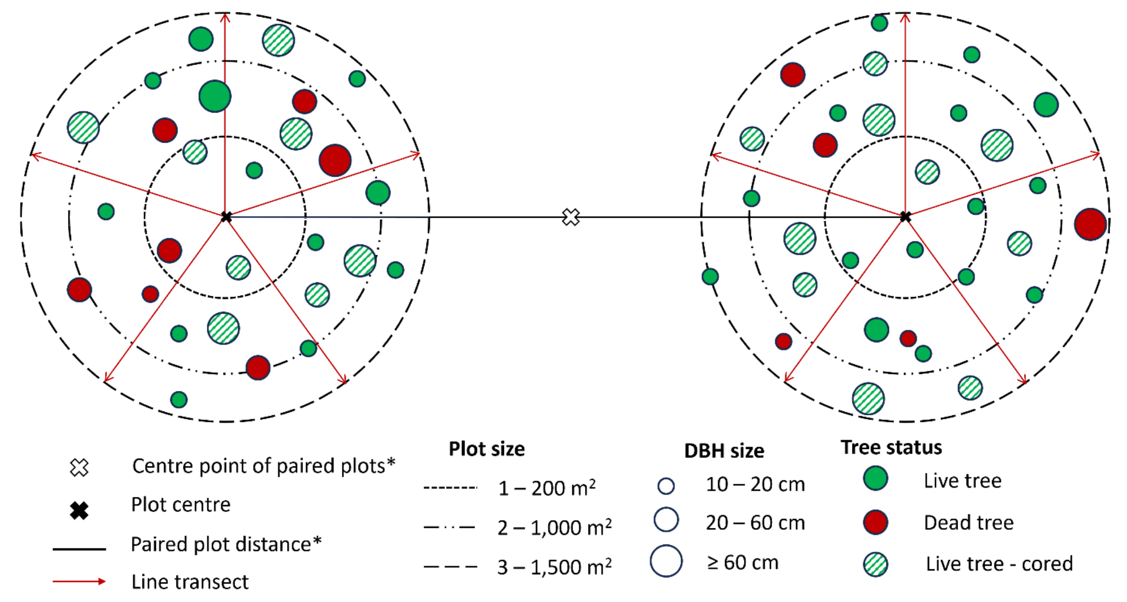


Figure 2: Circular plot design and sampling protocols – Illustration of plot design and sampling approach for identifying live and dead standing trees for diameter measurement and tree core sampling. Plots are split into inner (1 – 200 m²), middle (2 – 1,000 m²) and outer (3 – 1,500 m²) rings. For most of the spruce forest plots the plot size is 500 m², with only an inner and outer ring to divide a plot. Whilst mixed beech plots were established as a pair of plots, 40 m apart or 20 m equidistant from the centre point of the paired plots. Spruce plots were established singularly and most with a plot size of 500 m². Since plot size varies across stands and locations in the primary forests, the illustration includes three plot sizes, with the line transect approach, used for measuring the presence of downed deadwood ≥ 6 cm diameter at intersect along the transect line, measured from the plot centre to the perimeter. All individual trees were measured at diameter at breast height (dbh, cm). Tree status is denoted by colour: live trees (green), dead trees (red), and live trees that have been cored for samples (green with diagonal lines). Red lines indicate line transects within each plot.

Dead standing tree height was classified into predefined height classes approximated during field sampling (0 = 0.0 – 9.9 m; 1 = 10.0 – 19.9 m; 2 = 20.0 – 29.9 m; 3 = 30.0 – 39.9; 4 = 40.0 – 49.9; and 5 = 50.0 – 59.9 m) and median height was assigned for each class. Downed deadwood was measured using the line transect method (Van Wagner, 1968). To differentiate between dead tree characteristics, we categorise deadwood by decay class to assess the decay rate at the plot level and further estimate dead biomass: 1 – recently died tree bearing small twigs with leaves; 2 – recently died tree bearing twigs with no leaves; 3 – dead tree with large branches only; 4 – broken dead tree or a dead tree with few large branches present; and 5 – snag >1.3 m height and the highest decay class.

Status	Category	Description
Live	1	Full growth with no visual damage.
	2	Alive tree with only crown damage.
	3	Alive with stem breakage ≥1.3 m height.
	4	Alive, uprooted tree.
Dead	10	Dead tree stump ≤1.3 m height from a broken tree.
	11	Dead tree with no visual damage.
	12	Dead tree with crown damage.
	13	Snag, a dead tree with stem breakage ≥ 1.3 m height.
	14	Dead uprooted tree.
	15	Tree death caused by competition is typically a suppressed tree with no or minimal visible evidence from biotic agents (i.e. bark beetle outbreak).
	16	Dead tree uprooted after death.
	17	Dead tree with partial crown breakage, isolated to the upper canopy.
	21	Recent dieback caused by a large-scale disturbance, with no visual damage.
	22	Recent dieback caused by a large-scale disturbance, with crown breakage.
	23	Recent dieback caused by a large-scale disturbance, with stem breakage.

Table 1: Forest inventory level method for classifying live and dead standing tree status and types based on their characteristics.

Topographic data was captured onsite during forest inventories and taken from the centre point of each plot: mean altitude (metres above sea level – m.a.s.l), slope (°), denoting plot steepness and landform, which categorises the position of each plot relative to the stand it is nested within by the following levels: 1 – top of the slope; 2 – the peak of the slope; 3 – the midpoint between the valley and top of the slope; 4 – the lower part of the slope close to the base; and 5 – valley or a gradual slope at the base. See table 2 for mean landscape-scale characteristics in topographical conditions.

Landscape	Country	Forest type	Plots	Altitude (m)	Slope (°)
Eastern	(Northern) Romania	Mixed beech	40	874 - 1152	0 - 45
		Spruce	133	1266 - 1666	0 - 43
	(Eastern) Slovakia	Mixed beech	66	615 - 1068	0 - 38
	(Western) Ukraine	Spruce	96	1219 - 1501	8- 43
	Eastern (total)	Mixed beech	106	615 - 1152	0 - 45
		Spruce	229	1219 - 1666	0 - 43
Southern	(Southern) Romania	Mixed beech	84	923 - 1324	0 - 43
		Spruce	117	1267 - 1713	21 - 52
Western	(Western) Slovakia	Mixed beech	80	785 - 1285	0 - 44
		Spruce	110	1235 - 1535	0 - 39

Table 2: Landscape-scale characteristics of the total number of first-census plots (*n* = 726); altitude (m) and slope (°) (minimum and maximum range) across each geographical landscape, country and forest type in the Carpathians.

3.1.3 | Dendrochronological data

We used incremental tree cores to quantify two modes of interannual radial growth patterns using tree ring width analysis, being either (1) gap-recruitment or (2) growth release events. Gap-recruited events are those that initiated growth under a relatively open canopy soon after a canopy-opening disturbance event, as indicated by rapid initial growth. In contrast, growth released events signal radial growth was established in the shaded understorey and later recruited to the canopy through a gap formation, evidenced by slow initial growth followed by a sudden growth release (Lorimer and Frelich, 1989). An event was classified as gap-recruited if the mean ring width of the fifth to fifteenth ring from the pith exceeded the early growth rate threshold, based on comparisons between early growth rates in young trees sampled in gaps versus those under a forest canopy (i.e., suppressed growth). Released growth is a significant increase in the growth rate of a tree following the removal or death of neighbouring trees, observed in tree ring patterns as an abrupt growth increase (Čada et al., 2020). Gap-origin growth refers to the growth of trees that developed in the gaps created by the death or removal of canopy trees. We used the absolute increment method (Fraver and White, 2005) to identify releases estimating suppressed and gap-recruitment period in identifying signals in tree growth.

The difference between the 10-year mean before and after a peak disturbance event, with a threshold of 1.25 times the standard deviation based on growth differences throughout the chronology, was used to identify released growth. We allowed for multiple proxy evidence of disturbance events occurring within individual trees throughout the analysed tree ring width (Čada et al., 2020). To mitigate any effects from abrupt climatic inputs, we selected tree rings with releases that occurred > 20 years after potential rapid early growth rates and before the tree reached the diameter indicating canopy access. Since different species respond differently to growth trends and the period when a tree ascends to the canopy, we apply species- and region-specific diameter thresholds. Shade tolerant genera such as Fagus and Picea may have none to multiple releases during their chronologies. We identified gap trees based on statistically irregular variation in growth within the juvenile portion of a tree core that surpassed site-specific thresholds (Čada et al., 2020; Frelich and Lorimer, 1991).

Canopy area was determined by extant tree crown area, which was modelled using the coefficient between canopy area and dbh, divided by the sum of canopy area of all cored trees in a plot. To identify potential disturbance events, we employed a 30-year moving sum to capture long-term trends in canopy disturbance, calculated for each disturbance year from the full chronology. We calculated density using a 5-year smoothing bandwidth within a 30-year moving window. The year of a disturbance event was identified at peak years after rising for at least 5 years, with > 10 years gap between two peaks. The severity of a disturbance event was measured as the relative canopy area distributed, based on the sum of the relative current crown areas of trees that showed disturbance indicators within an 11-year window around the peak of a disturbance event (Čada et al., 2020). This method is based on the standard assumption that most trees exhibit responses to disturbances within a decade of the event, and that the aggregated relative current crown areas of these trees accurately reflect the proportion of the plot that was disturbed in the past (Lorimer and Frelich, 1989). We selected the maximum disturbance severity event, extracted from plot-level chronologies, as the main (historical) disturbance variable in this study, since we assume it to be the most influential parameter from the full chronology. Disturbance severity was categorised as being low (< 40%), moderate (0 – 60%) and high severity (>

60 %) (Janda et al., 2017). We omitted values with a disturbance severity < 10% to avoid any uncertainties in reconstructing such events due to the number of available cores.

We developed allometric models (Whittaker and Woodwell, 1968) to estimate extant canopy dimensions of gap trees and thus infer recent disturbances. Allometric models were based on dbh and crown measurements (canopy length and width) of > 5 randomly selected mature trees in each plot. We assessed recent disturbance severity by measuring canopy openness, which indicates recent changes to the canopy structure (Mikoláš et al., 2021). Canopy cover of individual living and dead-standing trees (for decay classes from 1 to 3 only) were calculate from their basal area, and then the proportion of live and dead trees occupying the canopy. This allowed canopy gaps to be identified and an inference to the severity of past disturbances that created the gaps. We aggregated plot-level maximum disturbance (dendrochronological data) and extant canopy openness (recent disturbance using structural data), selecting the extant canopy gap (recent disturbance) severity if it exceeded the historical, maximum disturbance severity. The data produced consisted of tree ring chronologies covering a 250-year period (details in Čada et al., 2020; Janda et al., 2019).

3.1.4 | Gridded climate data

To assess how regional climatic conditions influence biomass across the primary, unmanaged forests, we extracted monthly climate information from the TerraClimate database (Abatzoglou et al., 2018) with a 4 km spatial resolution. More specifically, we obtained temperature minimum (tmin), maximum (tmax), precipitation (ppt) and climatic water deficit (CWD [mm], defined as the difference between potential and actual evapotranspiration) values for the 1958–2019 period. Mean plot-level air temperature is calculated from the annual mean values for tmin and tmax. We computed monthly potential evapotranspiration (PET) values from tmin, tmax, ppt and latitude using the Hargreaves' equation (Hargreaves and Samani, 1985). Then, we calculated monthly climatic water balance (CWB [mm]) values as the difference between ppt and PET. Finally, we averaged yearly mean temperature, yearly CWB and yearly CWD for each plot and for the 1958–2019 period. To test our hypothesis regarding the spatial variability of biomass stocks, we use mean temperature (°C), CWB and CWD. CWD was selected *a priori* since it measures water deficit and identifies where demand for evapotranspiration exceeds available water and CWD performed better than CWB in the statistical modelling (see supplement S1.3 for model testing & S1.5 for mean climate data values). We used mean CWD in preference to other gridded climate data because moisture availability influences the amount of biomass accumulated in the forest (Houghton, 2007; Luyssaert et al., 2008; Pan et al., 2011).

3.1.4 | Statistical analysis: a common approach

We fit GAMMs using a Gaussian distribution and identity a link function with a restricted maximum likelihood, as implemented in the bam function from the R-package *mgcv* (Wood, 2011). Each model shares a common structure including biomass as the response, forest species composition as the fixed effects, pair of plots and stand per forest type set as the random effects (spline). To address spatial autocorrelation, we converted latitude and longitude into geographic distance as linear coordinates using the R-package *geodist* (Karney, 2013) and *vegan* (Oksanen et al., 2013).

GAMMs were calibrated with spatial autocorrelation using latitude and longitude as linear covariates, ensuring an accurate representation of geographic variations in the biological response variable. Auto-spatial correction is applied to each model with $longitude, latitude_{ts}$ using the thin smoothing penalty. We restricted the smooth basis to 3 knots to prevent biologically spurious responses, unless stated in each research study. We use the Akaike information criterion (AIC) (Akaike, 1987) to rank the models within each set. We use the ΔAIC to calculate the relative difference within each of the biomass models (total, live and dead biomass/carbon) (Anderson et al., 1994; Burnham et al., 2011), using the following approach: $\Delta AIC = AIC_i - AIC_{min}$, where AIC_i is the AIC model in series and AIC_{min} is the lowest AIC within the series. The candidate model selection approach (Burnham et al., 2011) limits the number of fixed effect variables to one per group, selecting the most parsimonious variable using the lowest ΔAIC score (Burnham et al., 2011). $\Delta AIC < 2$ supports the model (ideal candidate); $\Delta \geq 2$ and $\Delta < 7$ suggest partial support for the model and $\Delta \geq 7$ suggests no confidence in the model. All data preparation and statistical analyses were conducted using R 4.1.0 (R Core Team, 2022).

3.2 | Forest biomass

The main objective of this thesis is to investigate biomass accumulation across the primary forests of the Carpathians and their overall carbon-carrying capacity. To achieve this, biomass was calculated for individual live trees, dead-standing trees, and downed woody debris, measured in each plot. Live and dead-standing tree biomass was calculated using the dbh (cm) of each individual tree. Downed woody debris was measured using a diameter intersecting along a transect line. This comparative approach employs multiple methods that allow for a comprehensive assessment of forest biomass across the Carpathian region. The following subchapter details the foundational approach used for calculating forest biomass. This approach is the basis for both single-census and multi-census assessments of biomass carbon stores. All values upscaled to one hectare, with biomass values given as Mg ha^{-1}, whilst carbon is given as tC ha^{-1} which is approximate 0.5 of the biomass value. For further details on how carbon dynamics utilises multi-census data, see subchapter 3.5.

3.2.1 | Live biomass

Live standing biomass was calculated using a series of species and genus-specific allometric equations (Forrester et al., 2017, supplement S1.2). Allometric equations are suited for mature, uneven-aged temperate forest species – using equations 3 (eq 1) and 4 (eq 2) from Forrester et al. (2017):

$$\ln Y \;\; = \ln \beta_0 + \ln \beta_1 + d + \epsilon \tag{1}$$

$$\ln Y = \ln \beta_0 + \ln \beta_1 + d + \beta_{2\,BA} + \epsilon \tag{2}$$

where *Y* the natural log-transformed biomass estimation, β_0 and β_1 is the natural log species-specific weight with $\beta_{2\,BA}$ represent the β weight divided by the species-specific basal area, *d* represent diameter at breast height (dbh, cm) at 1.3 m aboveground, ∈ denotes the standard error. Individual live tree biomass was calculated by summing the mass of each tree component (stem, branches, foliage) using dbh (cm) and basal area (m^2 ha^{-1}) as predictors. The use of basal area as a secondary predictor was applied to *Abies alba, Fagus sylvatica, Picea abies, Pinus cembra, Pinus sylvestris* and *Taxus baccata*. Estimates were multiplied with a correction factor to address bias when back-transforming biomass from the natural log, upscaled from the raw values (kg tree^{-1}) to megagrams per hectare (Mg ha^{-1}). See supplement S1.2 – Forest biomass for a detail account of species-specific coefficients used in the allometric equations for calculating live tree biomass.

3.2.2 | Dead biomass

We include two types of deadwood in the study: standing dead trees and downed dead wood (coarse woody debris) found on the forest floor. In each plot, dbh and height of dead standing trees were measured, coarse woody debris was measured using the line intersect method, and the decay stage was classified in both cases (table 3).

Class	***Description***
1	Recent dead or cut trunk/ deadwood debris. The wood is hard and only able to penetrate a few mm into the wood using a spike. Completed covered with bark, phloem fresh remaining in some parts of the deadwood debris.
2	Wood mostly hard (spike can penetrate up to 1-2 cm into the wood), most of the bark remains (but not necessarily a bark beetle-infested trunk), no fresh phloem present.
3	Wood is partially decayed on the surface in the centre, spike penetrates 3-5cm into the wood, large piece(s) of bark is loose or detached.
4	Most of the wood is soft throughout, the entire spike (15-20cm) is able to penetrate the wood (depending on its diameter). However, the central parts of the wood remain hard, whilst the [outer] surface layers are missing.
5	The wood is very soft and disintegrates when lifted; the trunk is typically covered by field-layer vegetation.

Table 3: Forest inventory level method for classifying dead wood and deadwood [coarse woody] debris based on decay status when using a spike to measure the degree of firmness or softness of the deadwood debris.

The volume of deadwood (m^3) was calculated for dead standing trees based on the measured dbh and height using the *E_VOL_AB_HmDm_HT.f* function and the *SK.par.lme* model from *TapeR* package in R (Kublin et al., 2013a, 2013b; Zanne et al., 2009). For calculating downed deadwood, the volume was calculated based on the diameter at intersect using the line transect approach (Van Wagner, 1968) using the following approach (eq 3):

$$V = \frac{\pi^2 \sum d^2}{8L} \tag{3}$$

where V is the volume of wood per unit area, d is deadwood diameter, and L is the length of the sample line (100 m – the sum of five 20 m transect lines; Van Wagner, 1968). The line transect approach (Van Wagner, 1968) identifies the presence of downed deadwood on the forest floor. Subsequently, not all plots include downed deadwood since an individual coarse woody debris did not intersect along the transect line, with 644 plots from the first census plots. From the volumes of dead standing and downed trees, we estimated the biomass by multiplying the volume by species-specific wood density and a density reduction factor specific to the decay stage (Harmon et al., 2008).

3.3 | Temperate Primary Forest Biomass Accumulates over Centuries-Long Time Frames

3.3.1| Forest species composition

To address how tree species may influence biomass, we use two types of classifications. Forest type (mixed beech, spruce) and forest species composition nested within each forest type containing 12 groups. We use tree species to calculate a forest species composition index based on relative total biomass (percentage of total plot-level biomass) for each of the three top genera (*Fagus, Picea, Abies*) and other representing all other genera present in the forest, but with a lesser frequency and biomass. Each plot is divided into seven categories of forest species composition index across two main forest types (mixed beech, spruce) (table 4). Forest species composition is an intermediate scale between plot and stand levels, grouped within a landscape (subchapter 3.1.2). Landform measures the position of the plots along the slope within the scale of the stand (supplement—S1.1).

Forest type	*Forest species composition*	*Number of plots*	*Relative total biomass by category (%)*		
			Beech	*Spruce*	*Other*
Mixed beech	Pure beech	123	≥ 90		< 10
	Beech	57	≥ 75 to ≤ 90		10–25
	Mixed beech	63	≥ 50 to ≤ 75		25–50
	Mixed forest*	27		*Mixture*	
Spruce	Mixed spruce	19		≥ 50 to ≤ 75	25–50
	Spruce	38		≥ 75 to ≤ 90	10–25
	Pure spruce	399		≥ 90	< 10

Table 4: Forest Species Composition – Based on the Proportion of the Total Biomass Categorised by Three Major Species Groups: Beech, Spruce, Fir and Other. The 'other' category includes all remaining species identified during forest inventories that have a substantially lower frequency. *Mixed forest combines 27 plots from the categories due to low frequency of plots: mixed forest = (100% other [non-beech, spruce, fir], n = 1), spruce (spruce: ≥ 75 to ≤ 90%; other: 10–25%, n = 1), mixed spruce (spruce: ≥ 50 to ≤ 75%, n = 3) and fir–beech (fir: ≤ 50%; beech: ≤ 50%, n = 22).

3.3.2 | Statistical analysis: Investigating forest biomass stocks across the Carpathians

We built a series of generalised additive mixed models (GAMMs) to test our research questions. To investigate (Q1) the relationship between biomass type (total, live, dead) with biotic and abiotic factors, we use plot-level data for the following plot-level variables in the GAMMs: mean and standard deviation of tree age (year); disturbance severity (% CA); climate (mean temperature and CWD) and topographical variables (altitude and landform). Additionally, we use test for (Q2) any threshold in plot-level tree age across the primary forests potentially cease functioning as carbon sinks.

We built the GAMMs in two parts, to first identify how biomass type responds to each grouped variable and then select the most significant variable to build a hierarchical model to test (Q2) how biomass types are influenced by biotic and abiotic factors. We used the model selection approach and created three sets of models to describe the biomass types (total, live, dead) to identify individual trends with biomass types and factors. To investigate Q2, biotic and abiotic factors were grouped into four models: age, disturbance, topography and climate (supplement S1.3 —GAMMs). We use the candidate model selection method by selecting the most parsimonious model as the best fit model(s) influencing biomass. Moreover, for identifying (Q2) biotic and abiotic factors, we set a limit of one variable per group (topography and climate—Supplement S1.3 for details on the model selection and candidate model selection design) (eq 4):

$$\begin{aligned} log\ (biomass_n) \sim composition + s(x_1) + s(x_2) + \cdots s(x_n) + s(stand_{re}) + s(Paired\ plot_{re}) \\ + s(longitude, latitude_{ts}) \end{aligned} \tag{4}$$

where *log(biomass)* is the natural log-transformed biomass (total, live, dead) for each respective model series, composition is the forest species composition class and included in all models, *s* is the thin-plate regression spline applied to each variable with standre and paired plotre set as the random effects. Auto-spatial correction is applied to each model with *longitude, latitude*$_{ts}$ using the thin smoothing penalty. We restricted the smooth basis to 3 knots to prevent biologically spurious responses. We use the AIC (Akaike, 1987) to rank the models within each set. We use the ΔAIC to calculate the relative difference within each of the biomass models (total, live and dead biomass) (Anderson et al., 1994; Burnham et al., 2011).

3.4 | Tree structure and diversity shape the biomass of primary temperate mountain forests

3.4.1 | Structural and biodiversity indices

The selection of variables for our statistical analysis (see subchapter 2.7) was informed by the research hypotheses and information from the literature to support (supplement S2.2 – table S2.2). To test these hypotheses regarding the influence of biotic and abiotic factors on forest biomass, structure, and genus-level diversity, we selected variables capturing key aspects of both forest structure and genus-level diversity. For assessing structural complexity, we used dbh to calculate basal area (ba, m^2) for each individual tree. The sum of all individuals within a plot was upscaled to one hectare. Based on this approach for mean plot-level basal area (m^2 ha^{-1}), we calculated a basal area index (*bai*) using the standard deviation (σ) to address the variability in plot-level tree size distribution at the fine scale (eq 5):

$$bai = \sigma\left(plot\ level\ basal\ area\ m^2 ha^{-1} \frac{\sum individual\ tree\ basal\ area\ x\ 10000}{plot\ size\ [m^2]}\right) \tag{5}$$

Plot-level tree density was calculated as the number of trees per hectare. The basal area index, incorporating the full range of dbh classes, was preferred over small and large-diameter ranges, which showed no significant relationship with plot-level biomass in preliminary tests (supplement S2.2 – GAMMs). Since basal area was highly correlated with biomass, we used the basal area index to measure structural variance within each plot (Aponte et al., 2020). We also applied the Staudhammer and LeMay (2001) approach using the Shannon index to measure tree size distribution diversity. This method differs from the basal area index, which groups plots by mean basal area of all trees per dbh classes in 10 cm intervals (i.e., 10 – 100 cm, and ≥ 100 cm). The tree size distribution measures structural diversity, while the basal area index represents variability in mean basal area within a plot.

We quantified tree diversity across plots using the Shannon-Wiener diversity index (Shannon index; Hill, 1973). Genus diversity was calculated at the genus level and divided by the natural logarithm of the total number of genera in each plot. Genus abundance was measured by the total sum of individuals per genus in each plot (eq 6):

$$genus\ abundance = \sum_{i=1}^{n} number\ of\ individuals\ per\ genus \tag{6}$$

where *n* represents the number of genera within a plot (*i*). Tree genus diversity and tree size distribution indices were calculated using the R-package *vegan* (Oksanen et al., 2013). Shannon indices (tree genus diversity and tree size index) were standardised using a pairwise difference analysis (Ameztegui et al., 2022) (eq 7) to calculate the difference (Δ) between each Shannon index, calculated for live and dead standing trees:

$$\Delta Shannon = \frac{tree\ size\ index\ -\ tree\ diversity}{tree\ size\ index\ +\ tree\ diversity} \tag{7}$$

The pairwise analysis tests the similarity or dissimilarity between indices (*structure* - tree size index, *genus* - tree diversity) and their spatial distribution across the Carpathians. We measured the degree of beech and spruce prevalence in their respective forest types by calculating the Importance Value Index (IVI). We used the IVI to calculate the proportion of genera dominance and abundance in a plot, ranging from 0 (low presence) to 200 (high presence) based on the following approach: IVI = sum of relative dominance (eq 5) + relative abundance (eq 6) (Curits & McIntosh, 1950; Mueller-Dombois & Ellenberg, 1974).

3.4.2| Genera prevalence: Importance Value Index

We measured the degree of beech and spruce prevalence in their respective forest types by calculating the Importance Value Index (IVI). We used the IVI to calculate the proportion of genera dominance and abundance in a plot, ranging from 0 (low presence) to 200 (high presence) based on the following approach: IVI = sum of relative dominance (eq 8) + relative abundance (eq 9) (Curtis and McIntosh, 1950; Mueller-Dombois and Ellenberg, 1974).

$$relative\ dominance\ \frac{basal\ area\ per\ genus}{plot - level\ basal\ area} x\ 100 \tag{8}$$

$$relative\ abundance\ \frac{number\ of\ individual\ per\ genus}{number\ of\ genera\ in\ a\ plot} x\ 100 \tag{9}$$

Using the IVI score (eq 8, 9) we rank the prevalence of beech and spruce in their respective forest types, ranked by low (< 100), moderate (100 – 150) and high (> 150) IVI scores, with beech – mixed spruce (n plots = 8) being plots in the mixed beech forests characterised as being dominantly spruce in structure and genus coverage, ensuring the category contains a minimum of five levels associated with the random effects, when used for the statistical analysis (see section 2.7) (Zuur et al., 2009). Measuring beech and spruce prevalence using the IVI provides an insight into how genera influence a plot based on its degree of presence in a community (supplement S2.1 – Importance Value Index). We used these structural and genus-level variables to assess, at the fine-scale how tree structure varies across the Carpathians and their corresponding influence on biomass stocks in supporting an uneven age-size distribution and forest heterogeneity (H1, H2) – for detail on variable selection and rationale, see supplement S2.2 – GAMMs.

3.4.3 | Statistical analysis: Influence of tree genus and structure on forest biomass

We built a series of generalised additive mixed models (GAMMs) to test our research questions. To explore the relationships between forest biomass, genus-level diversity, and structural complexities, we developed a series of generalised additive mixed-effects models (GAMMs), divided into two sets: first (Q3) to test variations along three hierarchical scales (plot, stand, landscape) and second part (Q4) to examine their interactions with site-specific biotic and abiotic factors. The selection of key variables was guided by our hypotheses and information from the literature (supplement S2 – table S2.2). GAMMs were constructed using the following design (eq 10):

$$\begin{aligned} log(biomass) \sim \cdots + (plot_{re}) + (stand_{re}) + (landscape_{re}) \\ + \ s(longitude, latitude_{ts}) \end{aligned} \tag{10}$$

where the response variable *log*(*biomass*) is the mean plot-level biomass transformed using the natural logarithm. We used $plot_{re}$, $stand_{re}$, and $landscape_{re}$, as random effects, representing fine, intermediate, and macro scales, respectively, which were grouped by forest type (mixed beech, spruce). Random effects accounted for spatial variation in plot-level response and predictor variables across the Carpathians. Since not all plots contained dead standing trees > 10 cm dbh, we conducted separate approaches for live (*n* = 726 plots) and dead models (*n* = 607 plots) with plot-level total biomass (aboveground live, dead, and downed dead wood) as the consistent response variable throughout the analysis.

For assess how (Q3) biomass, genus-level diversity and structure vary along three hierarchical scales (plot, stand, landscape), we used the following models, testing different series of spatial scales as the random effects:

$$log(biomass) \sim s(x_n) + (plot_{re}) + (stand_{re}) + (landscape_{re}) \tag{11}$$

$$log(biomass) \sim s(x_n) + (landscape_{re}) \tag{12}$$

$$log(biomass) \sim s(x_n) + (stand_{re}) \quad (13)$$

$$log(biomass) \sim s(x_n) + (plot_{re}) \quad (14)$$

where, x_n represents predictors for live and dead standing trees, including structure (basal area index, tree size index), genus-level diversity (abundance, tree diversity), combined effects (basal area index + abundance), and *ΔShannon* (normalised pairwise difference between tree size index and tree diversity). To account for spatial scales, we used three classes (landscape, stand, and plot) as random effects in each model (equations 11 – 14, respectively). Plot-level random effect ($plot_{re}$) was represented by calculating the importance value index (IVI) at the genus level for each plot as a measure of beech and spruce prevalence. Variables were selected based on their performance during initial model testing to identify the most important variable per group. For details on model calibration and predictor selection, see supplement S2.2 – GAMMs, table S2.3.

To assess how forest biomass and composition (basal area index and genus abundance) interact with site-specific factors (Q4), we developed interactive GAMMs. These models included the following plot-level variables: *dendrochronology* (mean plot-level tree age × disturbance severity), *structure* (tree size index using Shannon index), *genus-level diversity* (tree diversity using Shannon index), *climate* (temperature), and *topography* (altitude). Interactive models tested the interaction between genus-level diversity (abundance) and structural complexity (basal area index) with site-dependent factors using the following approach:

$$log(biomass) \sim f(x_n\ x_{basal\ area\ index}) \cdots + (plot_{re}) + (stand_{re}) + (landscape_{re}) + s(longitude, latitude_{ts}) \quad (15)$$

$$log(biomass) \sim f(x_n\ x_{abundance}) \cdots + (plot_{re}) + (stand_{re}) + (landscape_{re}) + s(longitude, latitude_{ts}) \quad (16)$$

where *f* represents the tensor product smooths denoting the interaction between site-specific factor (x_n) with structure (eq 15 - *basal area index*) and genus (eq 16 - *genus abundance*) per live and dead standing trees. We used the candidate model selection approach (Anderson et al., 1994) to identify the most important variables from the most parsimonious model, limiting variables to one per category – see supplement S2.2 for detail on GAMMs and the variables selected using model selection.

3.5 | Temporal and spatial variability in carbon dynamics across the primary forests

3.5.1 | Forest inventories: two census data collection and sampling protocols

To investigate spatial and temporal changes in carbon dynamics, we adopted a standardised sampling protocol detailed in subchapter 3.1.2: Forest Inventories. This approach aligns with studies that rely solely on first-census data, for example, using the same plot size and tree diameter thresholds set in the first census. Notably, the protocol encompasses consistent methods for identifying and measuring live trees, dead-standing trees, and downed deadwood, ensuring comparability between the first and second censuses. A total of 454 plots were sampled twice to capture the spatial and temporal changes in the Carpathian montane primary forests. This includes the initial plot establishment (first census) and the first re-measurement event since establishment (second census). The interval between the first and second census varies across the network, ranging from 3 to 11 years, with an average of 5 years. See subchapter 3.1.2 for further detail on second-census data and use in this study, along with specific plot designs for mitigating changes in plot size between the first and second censuses.

To standardise the plot design, which was enlarged for most plots since the first census, we employ the original plot size defined during the first census (figure 3). This is achieved by identifying trees within the original size using their tree coordinates (x, y in metres) and proximity to the plot centre (designated as 0 m). The original plot size was maintained by converting the plot area (m^2) to radius (diameter – m) with the following distance from the plot centre: 1) 500m^2 = 12.6 m; 2) 1,000 m^2 = 17.8 m; and 3) 1,500 m^2 = 21.5 m, respectively. Individual tree positions were converted to distance from the plot centre (m) using the following approach (eq 17):

$$individual\ tree\ distance\ (m) = \sqrt{(x^2 + y^2)} \tag{17}$$

where x^2 and y^2 represent the gridded position of the tree within a plot, calibrated to the plot centre for a localised spatial coordinate of each tree.

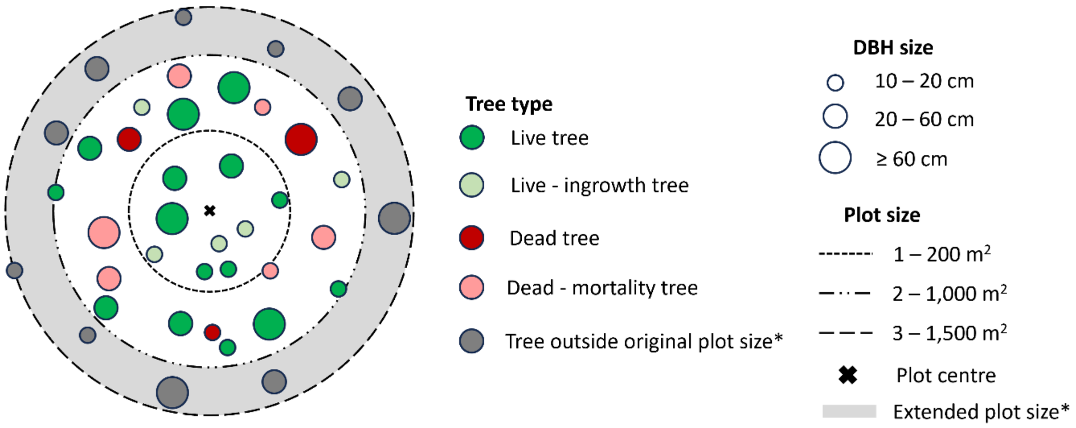


Figure 3: Changes in plot design and sampling across two censuses – illustration of the circular plot design and the types of standing trees after the second census. Since most plots were enlarged to 1,500 m^2 or 2,000 m^2 in the second census, the enlarged area was excluded from the analysis to ensure that only trees present within the original plot size are included. Trees located outside the original plot size (*) are therefore not used for quantifying carbon dynamics. The extended plot size area is represented in light grey, with trees within the enlarged plot area represented in dark grey shading. For categorising the changes in tree type between censuses, the following categories are used: live tree - recorded as alive in the first and second census (green); live ingrowth tree - a recruited tree that has surpassed the dbh threshold (> 10 cm) in the second census (light green); dead tree - dead standing tree that was not marked as alive in the previous census (light red); dead mortality tree - a tree that was alive in the first census but dead in the second (red).

We set a minimum dbh threshold (> 10 cm, measured 1.3 m aboveground) to ensure a consistent sampling protocol, and avoids bias in artificially inflating or underestimating the number of trees within a plot. This approach provides a precise measure of carbon dynamics based on the proportion of trees present. Furthermore, to address any uncertainties in dbh measurements of live standing trees between the first and second census, we prioritise the larger value from the first census if it differs from the second. This accounts for potential measurement errors or natural shrinkage that could lead to smaller dbh readings in the second census. Conversely, for dead standing trees, particularly mortality trees, the second-census measurements are retained due to potential size contraction through decomposition processes, influenced by the time since death and the interval between censuses.

3.5.2 | Forest carbon dynamics across multiple censuses

Prior to calculating carbon stocks for each plot, we grouped trees and downed deadwood into the following categories, to address the temporal changes across censuses: Live carbon: 1) alive in the first census. To account for changes in tree types, particularly those occurring after the second census, live and dead trees are categorised based on their status across both censuses (table 5). This categorisation allows for a more nuanced understanding of carbon dynamics: first census – live and dead; second census – live, remaining = denotes trees that were alive in both censuses; ingrowth – new, recruited growth where a tree has surpassed the dbh threshold (> 10 cm, dbh) in the second census (i.e., small diameter trees); dead – trees that remained dead in both censuses, and not previously identified as alive; mortality – previously alive in the first census, but dead in the second census. Categorising trees based on changes over time facilitates the identification of change in tree type, such as mortality, recruitment, changes in the remaining live trees and overall shifts in live and dead trees across the primary forest plots. All live and dead biomass values (Mg ha^{-1}) were multiplied by 0.5 to estimate the amount of carbon (tC ha^{-1}), as carbon constitutes approximately half of the biomass (Keith et al., 2009).

Forrester et al. (2017) approach uses plot-level basal area for most species as a secondary predictor for estimating biomass. Since live tree biomass is split into four different categories – live trees from the first census, ingrowth, live remaining trees in both census, mortality. Combining a single mean for plot-level basal area would not represent each of the standing tree types (live, mortality, ingrowth). Therefore, plot-level basal area is split into each of the groups, to better represent the changes across plots, for each census. By splitting the secondary predictor for estimating biomass provides a clear insight into the amount of biomass present for each carbon pool and types (table 5). Moreover, the calculation for live biomass follows the same approach outlined in subchapter 3.2 – Forest biomass, with the addition of ingrowth (new, recruited growth, surpassing the dbh threshold of > 10 cm), to calculate the change over time.

Census	*Carbon pool*	*Status*	*Description*
First	Live	Live - standing	Alive tree in the first census
	Dead	Dead - standing	Dead tree in the first census
		Downed dead wood	Presence of downed deadwood in the first census
Second	Live	Live - remaining	Trees that were alive in both the first and second censuses.
		Live - ingrowth	New, recruited growth where a tree has surpassed the diameter threshold (> 10 cm dbh) in the second census.
	Dead	Dead - standing	Trees that remained dead in both censuses and were not previously identified as alive.
		Mortality - standing	Trees that were alive in the first census but dead in the second census.
		Downed dead wood	Presence of downed deadwood in the second census.

Table 5: Summary of carbon pool statuses and their descriptions across two census periods. Trees and downed deadwood are categorised into live and dead standing and forest floor (i.e., deadwood) pools. Changes between the first and second censuses are detailed, including newly recruited growth and mortality trees.

Biomass of mortality trees, which were alive in the first and dead in the second census, was calculated using the dead-standing biomass approach, outlined in subchapter 3.2.2 - Dead biomass. To address a loss in biomass due to decay in dead-standing and downed deadwood, particularly mortality trees, we used the volumetric approach for establishing biomass, as detailed in subchapter 3.2.2 - Dead biomass. We will employ the TapeR package to estimate stem height for these trees (citation for the R package). Additionally, we will consider the decay stage of each dead-standing tree (table – decay stage). By incorporating the decay stage information, we can apply a decay reduction factor. This factor accounts for the loss of mass due to decomposition processes over time since the tree died.

Downed deadwood, which represents forest floor carbon stocks, was measured using the line transect approach (see subchapter 3.2.2). This approach relies on the original coordinates of the transect established during the first census. While the position of each individual deadwood piece intersecting the line transect was not recorded, this approach identifies the presence of downed deadwood debris on the forest floor. Since the decay stage of individual debris is highly varied, quantifying downed deadwood based on presence provides useful information into carbon dynamics, especially when combined with live and dead standing carbon stores. This information allows for the estimation of the amount of carbon stored within the forest floor over time.

3.5.3 | Statistical analysis: Spatio-temporal changes in carbon dynamics

Before developing the hierarchical models, we conducted a correlation analysis using the R package *corrplot* (Wei et al., 2017) to assess potential multicollinearity among predictor variables (age, mortality, altitude, and first-census carbon stock). This step aimed to ensure the feasibility of these variables in the models and prevent overfitting. The performance of each predictor was measured along a matrix, where the strength of a correlation from 1 to -1. The closer the correlation coefficient is to 1 (positive) or -1 (negative), the stronger the correlation, with 0 indicating no significant correlation between coefficients. The correlation analysis revealed no significant conflicting trends between the variables, supporting their inclusion in the subsequent generalised additive mixed-effect models (GAMMs) – see supplements S3.1 for correlation analysis outputs.

For developing the models, we created a series of GAMMs using the *bam* function from the R-package *mgcv* (Wood, 2011). We used the annual C increment between the first and second census as the response variable in all GAMMs (Q5, 6), as a representation of spatio-temporal changes in carbon stocks across the primary forest plots and quantify the overall carbon-carrying capacity of each site.

To calculate incremental changes for the total carbon stock, aboveground carbon increment (ACI) was calculated by calculating the difference in total carbon between the second and first census (eq 18) using the following approach:

$$ACI\ (tC\ ha^{-1}\ yr^{-1}) = \frac{c_{yr2} - c_{yr1}}{t_j} \tag{18}$$

where C_{cyr2} and C_{cyr1} represent the amount of carbon stock (total, live, dead) during the second and first census period respectively, t_j represents the interval between the first and second census (years) for each plot (*j*). To investigate how mortality rates influence carbon stock types (i.e., total, live, dead stand and dead downed) across the 454 plots, we used the following approach (eq 19):

$$mortality\ (tC\ ha^{-1}\ [\%]) = \frac{mort\ c_{yr2}}{total\ c_{yr2}} \times 100 \tag{19}$$

where *mort* c_{yr2} represents the amount of dead standing carbon that was alive in the first census but dead in the second, resulting in mortality stock, total c_{yr2} represents the total carbon present in the second census period (year 2). GAMMs utilise the uniform design in terms of the response variable, random effects and linear coordinates for autospatial corrections (eq 20):

$$log\ (C_{n\,yr2}) \sim \cdots + s(interval_{re}) + \ s(stand_{re}) + s(landscape_{re})\ + s(longitude, latitude_{ts}) \tag{20}$$

where the response variable $log(_n)$ represents the carbon stock in the second census, transformed using the natural logarithm. We include three levels of random effects ($_{re}$) including; $interval_{re}$ which denotes the time between the first and second census (years), with $stand_{re}$, *and* $landscape_{re}$ representing intermediate and macro scales, respectively, which were grouped by forest type (mixed beech, spruce). Auto-spatial correction was applied to each model with *longitude*, $latitude_{ts}$ using the thin smoothing penalty. Hierarchical GAMMs use the following approach in testing the relationship with key, site-specific factors (eq 21 – 24) (Q6):

$$log(C_{n\,yr2}) \sim s(\log\,(C_{n\,yr1})) + f(mort, age) + s(alt) \tag{21}$$

$$log(C_{n\,yr2}) \sim s(\log\,(C_{n\,yr1})) + f(mort, age) \tag{22}$$

$$log(C_{n\,yr2}) \sim s(\log\,(C_{n\,yr1})) \tag{23}$$

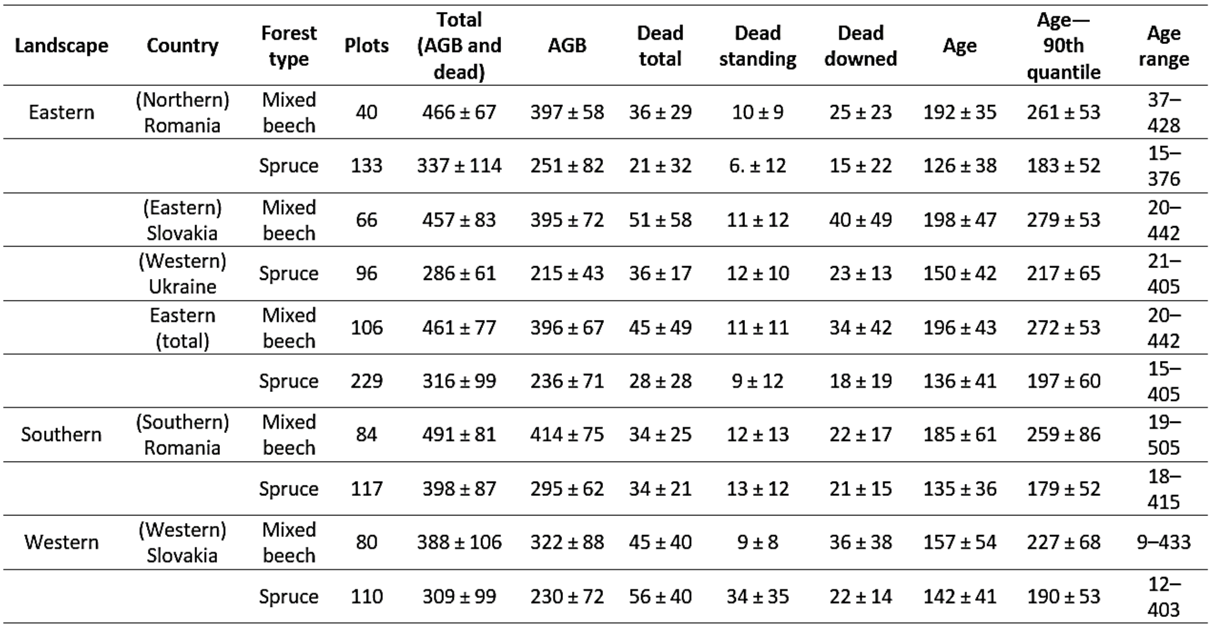
where $log(C_{n\,yr2})$ representing the amount of each carbon stock type (total, live, dead) present in the second census period, transformed using the natural logarithm. GAMMs also include *mort x age* which represent the interactions between mean plot-level mortality (%) and tree age (years), with *alt* denoting altitude (m). We used the candidate model selection approach (Anderson et al., 1994) to identify the most important variables from the most parsimonious model, based on the AIC and ΔAIC (Akaike, 1987).

Chapter 4 | Results

4.1 | Temperate Primary Forest Biomass Accumulates over Centuries-Long Time Frames

Forest species composition grouped by forest type (mixed beech and spruce) was the most influential factor in controlling the spatial variance in forest biomass stocks (total, live and dead biomass) across the landscapes (table 6). The influence of forest species composition on biomass is closely related to its altitudinal gradient in the occurrence of these species from plot to stand level. Biomass (total, live, dead) in the mixed beech forest plots is consistently higher than in the spruce forest plots, and this difference in forest type has a greater effect on biomass stocks than the geographical landscape in the Carpathians (table 6). Mixed beech forest plots in the southern landscapes contain the most diverse range in forest species composition (covering all four categories of pure beech, beech, mixed beech and mixed forest) and have the highest mean total biomass, being 491.74 ± 81.43 Mg ha^{-1} (supplement S1.4). Dead biomass has a small trend of higher stocks in the Western Carpathians (51.88 ± 40.49 Mg ha^{-1}) compared to the Eastern (33.74 ± 37.43 Mg ha^{-1}) and Southern (34.7 ± 22.99 Mg ha^{-1}) landscapes. The high abundance of dead biomass in the Western landscapes is attributed to a large amount of dead standing biomass in the spruce forests and dead downed biomass in the mixed beech forests (table 6).

Prior to the candidate modelling, we assessed the significance based on the ΔAIC amongst the biotic and abiotic variables. We found plot-level age is the most significant variable influencing the spatial variability upon all biomass types (total, live, dead) across different scales (supplement S1.3 – GAMMs). Disturbance severity and climate were the second and third most significant variable groups, while topography (landform and altitude) was the least significant variable group in total and live biomass. However, the effect of altitude is accounted for, at least partly, due to the covariance of altitude and forest type.



Landscape	Country	Forest type	Plots	Total (AGB and dead)	AGB	Dead total	Dead standing	Dead downed	Age	Age—90th quantile	Age range
Eastern	(Northern) Romania	Mixed beech	40	466 ± 67	397 ± 58	36 ± 29	10 ± 9	25 ± 23	192 ± 35	261 ± 53	37–428
		Spruce	133	337 ± 114	251 ± 82	21 ± 32	6. ± 12	15 ± 22	126 ± 38	183 ± 52	15–376
	(Eastern) Slovakia	Mixed beech	66	457 ± 83	395 ± 72	51 ± 58	11 ± 12	40 ± 49	198 ± 47	279 ± 53	20–442
	(Western) Ukraine	Spruce	96	286 ± 61	215 ± 43	36 ± 17	12 ± 10	23 ± 13	150 ± 42	217 ± 65	21–405
	Eastern (total)	Mixed beech	106	461 ± 77	396 ± 67	45 ± 49	11 ± 11	34 ± 42	196 ± 43	272 ± 53	20–442
		Spruce	229	316 ± 99	236 ± 71	28 ± 28	9 ± 12	18 ± 19	136 ± 41	197 ± 60	15–405
Southern	(Southern) Romania	Mixed beech	84	491 ± 81	414 ± 75	34 ± 25	12 ± 13	22 ± 17	185 ± 61	259 ± 86	19–505
		Spruce	117	398 ± 87	295 ± 62	34 ± 21	13 ± 12	21 ± 15	135 ± 36	179 ± 52	18–415
Western	(Western) Slovakia	Mixed beech	80	388 ± 106	322 ± 88	45 ± 40	9 ± 8	36 ± 38	157 ± 54	227 ± 68	9–433
		Spruce	110	309 ± 99	230 ± 72	56 ± 40	34 ± 35	22 ± 14	142 ± 41	190 ± 53	12–403

Table 6: Landscape-scale trends in biomass and age. Total = mean sum of live and dead biomass; AGB = live aboveground biomass; dead total = mean sum of dead standing and downed biomass; age = mean plot-level tree age (years); age range = minimum and maximum plot-level age (years) within each landscape per forest type; age—90th quantile: mean 90th quantile range based on mean plot-level tree age; total and live biomass contain 726 plots, dead biomass model contains 644 plots, respectively.

4.1.1 | Forest Biomass and Age

The most parsimonious models accounted for mean plot-level age and temperature for total biomass, mean age for live biomass and CWD for dead biomass (ΔAIC = 0 for the best models, table 7). Mean forest age therefore influenced total and live biomass, but did not affect dead biomass (table 7, figure 4). Live biomass exhibited a modest decline with increasing mean plot-level tree age beyond a threshold of ~ 225 years. The standard deviation of plot-level tree age was not an important predictor of any biomass type (by AIC). We found that over 68.3% of plots (496 of 726 plots) were 100–200 years old and had mean total biomass densities of 337.18 ± 99 Mg ha^{-1}.

Notably, 18% of all plots (mixed beech = 106, spruce = 25) with mean plot-level tree age > 200 years had a mean total biomass of 418.45 ± 97.62 Mg ha^{-1}. The mixed beech landscape in the Eastern Carpathians (196 ± 43 years) was found to be the oldest in our study region, with > 67% of the biomass being characterised as pure beech

(443.1 ± 50.58 Mg ha^{-1}). In contrast, the oldest spruce landscape was in the Western Carpathians (144 ± 43 years), with over 90% of the biomass characterised as pure spruce (291.71 ± 54.56 Mg ha^{-1}).

Model	AIC	R2 (C)	R2 (M)	ΔAIC	AIC rank
Total biomass					
M0: null (biomass)	109.75	0.4	NA	956.15	5
M1: age	− 829.5	0.87	0.5	16.9	2
M2: age + temp	− 846.4	0.87	0.5	0*	1
M3: age + temp + dist	− 801.87	0.86	0.5	44.53	3
M4: age + temp + dist + alt	− 780.58	0.86	0.51	65.82	4
Live biomass					
M0: null (biomass)	573.89	0.31	NA	719.4	5
M1: age	− 145.51	0.8	0.4	0*	1
M2: age + dist	− 124.6	0.8	0.41	20.91	4
M3: age + dist + temp	− 134.81	0.8	0.41	10.69	3
M4: age + dist + temp + alt	− 142.3	0.8	0.41	3.21	2
Dead biomass					
M0: null (biomass)	1544.15	0.14	NA	46.11	5
M1: CWD	1498.04	0.34	0.14	0*	1
M2: CWD + age sd	1498.4	0.33	0.15	0.35	2
M3: CWD + age sd + land	1501.32	0.34	0.15	3.28	3
M4: CWD + age sd + land + dist	1502.28	0.34	0.15	4.24	4

Table 7: Models of total and live biomass were fitted with 726 records, while the dead biomass models were fitted with 644 records. The strength of a model was quantified based on Akaike information criterion (AIC). Delta AIC (ΔAIC) is the difference between the AIC value of a given model and AIC of the best model. R^2 (M) and R^2 (C) are the marginal (fixed effects) and conditional (fixed and random) proportions of explained variance. Notes: age plot-level mean tree age (years), age sd standard deviation of plot-level tree age (years); dist disturbance severity (removed canopy area [CA] %); alt plot-level altitude (m); CWD climatic water deficit (mm); temp mean air temperature (°C); *Best model by AIC criteria.

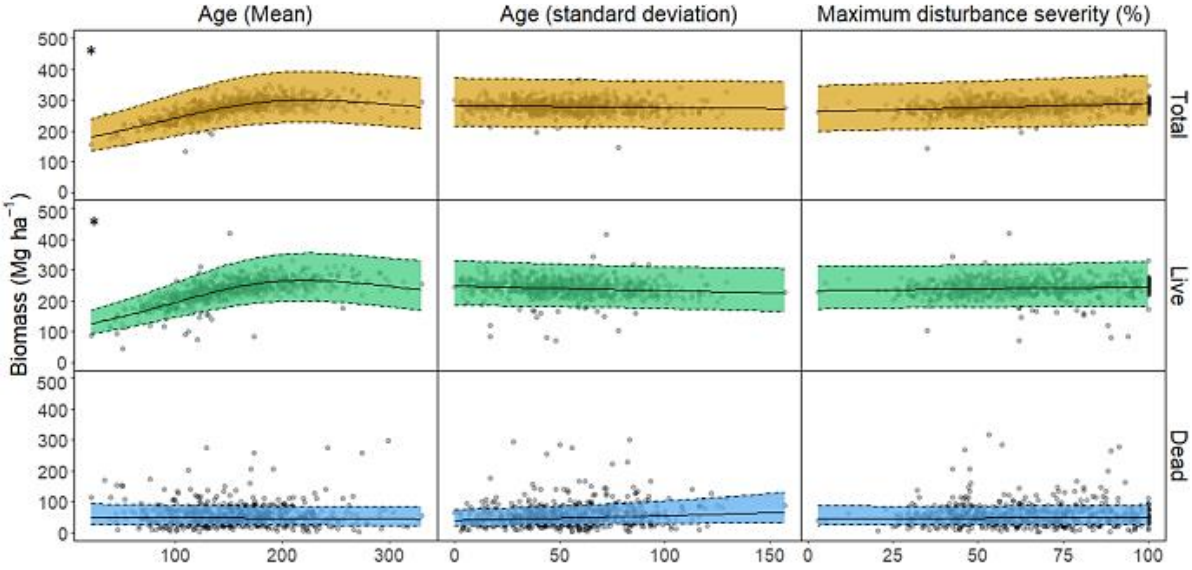


Figure 4: Conditional regression plots of plot-level biomass (total, live and dead) as a function of plot-level tree age and maximum disturbance severity from GAMMs. Shown are predicted total (upper panels), live (middle panels) and dead (lower) biomass levels (*y*-axis). Explanatory variables (*x*-axis) include mean plot-level tree age (left column), the standard deviation of plot age (middle column) and the maximum reconstructed disturbance severity for a site (right column). Each panel shows the effect of a given explanatory variable conditioned on or controlling for the effects of the other covariates in the model (that is, the other covariates were fixed at their observed median values). Forest species composition was also fixed at the most common level. The grey dots are the corresponding partial residuals for each conditional plot. Shaded areas delineate 95% confidence intervals (gold = total, green = live and blue = dead biomass). *Note*: * denotes an important relationship based on AIC. Conditional plots were generated using the R-package *visreg* (Breheny and Burchett, 2017).

4.1.2 | The Relationship Between Biomass and Disturbance

We found that mixed-severity disturbance regimes and plot-level tree age support a broad range in total, live and dead biomass across the Carpathians. The mean and variance of biomass were substantially different in each of the landscape areas (Western, Eastern, Southern) and forest types (mixed beech, spruce). Mean maximum disturbance severity was found to be broadly similar across mixed beech (63.47 ± 19.85%) and spruce (63.35 ± 20.25%) forest plots. GAMMs show that after controlling for forest species composition, tree age was critical for influencing biomass stocks. Disturbance severity had no noticeable effect on biomass types (total, live, dead), after accounting for age. We found 52.75% of plots (mixed beech = 142, spruce = 241) experienced high severity disturbance ≥ 60%. Dead biomass showed a narrow range of variance compared with total and live biomass because of the uneven distribution in disturbance regimes across the region (figure 4). However, we do not rule out its background effect in shaping forest composition and a driver of influencing an uneven tree age–size distribution at the fine scale.

4.1.3 | Forest Biomass and Environmental Conditions

Mean temperature displays a strong positive relationship with total and live biomass and was found to be the second most influential variable after age (figure 5). Interestingly, CWD was important for dead biomass with a pronounced increase after 50 mm, while tree age variance showed no clear trend. Mean CWD varied across each geographic landscape and forest type, with spruce forest plots showing a broad range in the Western, Eastern and Southern landscapes at 7.53 ± 3.98, 21.74 ± 11.8 and 42.13 ± 7.83 mm, respectively. CWD in the mixed beech forests across Western, Eastern and Southern landscapes was 25.13 ± 7.82, 26.6 ± 14.39, and 53.13 ± 13.2 mm, respectively.

Plot-level altitude had a moderate significance on live biomass with plot-level landform being significant for dead biomass (supplement S1.3). Total and live biomass stocks increased in a south-easterly direction along the Carpathians. Mixed beech forests increased in biomass stocks in a west-to-south direction. In contrast, no similar trend occurred in the spruce forests, with the highest mean total biomass in southern landscapes at 398.45 ± 87.32 Mg ha^{-1}. Landform was the most influential topographic variable for dead biomass; however, it shows no significant trend compared to altitude which depicts a reverse J-shaped distribution (figure 5).

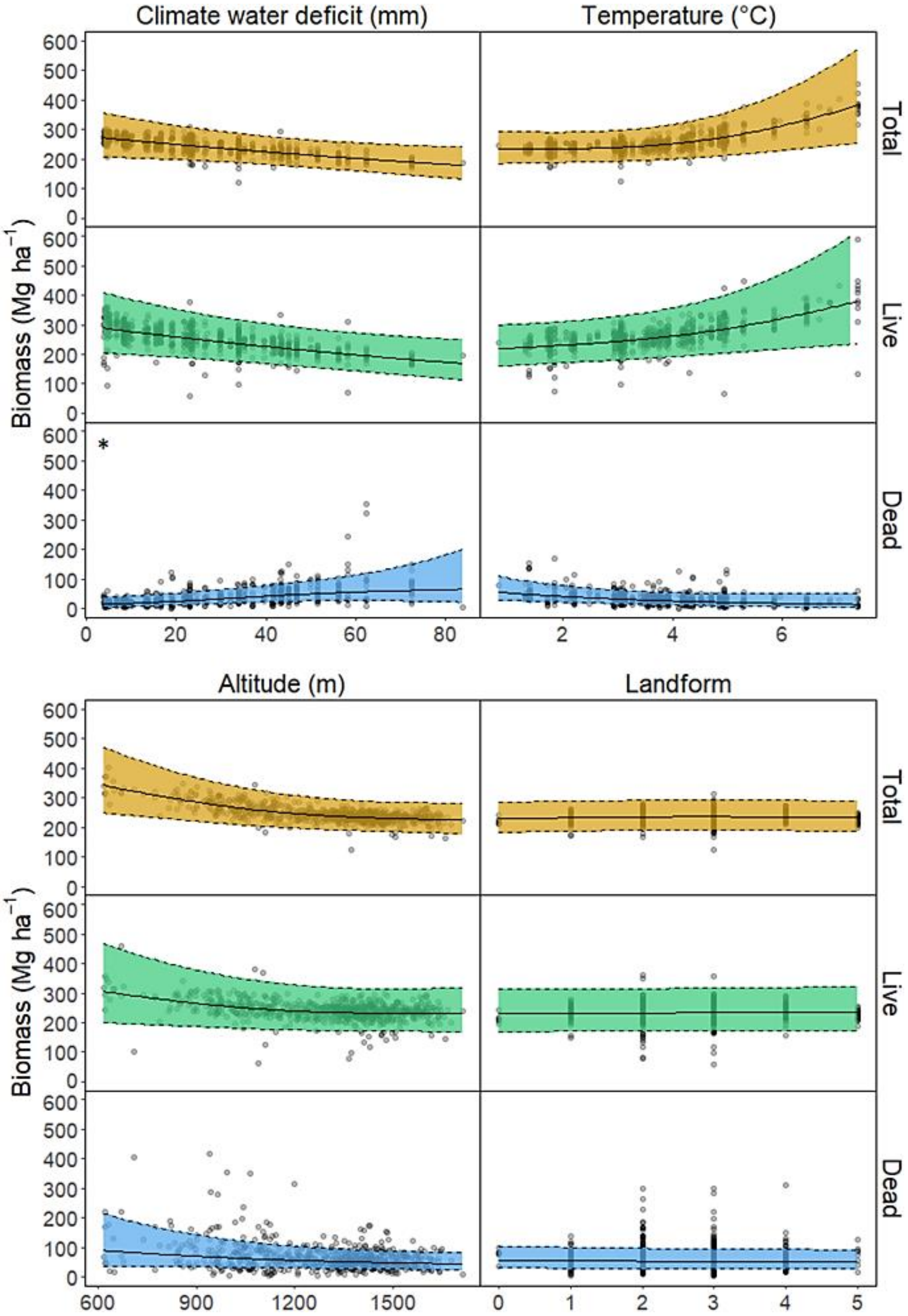


Figure 5: Conditional regression plots of plot-level biomass (total, live, dead) as a function of plot-level climatic water deficit, temperature, altitude and landform from GAMMs. Shown are predicted total (upper panels), live (middle panels) and dead (lower) biomass levels (y-axis). Explanatory variables (x-axis) include mean plot-level climatic water deficit (top left column), temperature (top right column), altitude (bottom left column) and landform (bottom right column). Each panel shows the effect of a given explanatory variable conditioned on or controlling for the effects of the other covariates in the model (that is, the other covariates were fixed at their observed

median values). Forest species composition was also fixed at the most common level. The grey dots are the corresponding partial residuals for each conditional plot. Shaded areas delineate 95% confidence intervals (gold = total, green = live and blue = dead biomass). Note: * denotes an important relationship-based AIC. Conditional plots were generated using the R-package visreg (Breheny and Burchett 2017).

4.2 | Tree structure and diversity shape the biomass of primary temperate forests

4.2.1 | Forest composition across spatial scales

We identified strong dissimilarities in forest composition (i.e., basal area index and genus abundance) and biodiversity indices (tree size index, tree diversity) between live and dead standing trees across different spatial scales (see subchapter 4.2.3). The proportion of live tree density and basal area was larger compared to dead trees, regardless of forest type (figure 6). Romanian spruce forests in the Southern landscapes had the highest mean live basal area at 56.2 ± 11.9 m^2 ha^{-1}. In contrast, Slovakian mixed beech forests in the Western landscape had a lower mean basal area of 39.2 ± 12 m^2 ha^{-1}, despite a wide range in basal area (1.2 ± 72.7 m^2 ha^{-1}). The presence of large dead trees varied considerably across landscapes, with Slovakian spruce forests in the Western landscapes having a notably high basal area of dead trees (11.7 ± 9.8 m^2 ha^{-1}), while live tree basal area was similar to other landscapes (42.3 ± 13.4 m^2 ha^{-1}) (supplement S2.2 – table S2.5).

Spruce forests generally had higher tree density and basal area than mixed beech forests, although mixed beech forests still represented a substantial proportion of biomass across landscapes (supplement S2.2 – figure S2.6). Genus abundance, indicating the sum of individuals per genus, varied across landscapes. Most spruce forests showed a lower range than mixed beech forest plots, except for Ukrainian spruce plots in the Eastern Carpathians (51.3 ± 17), which were comparable to mixed beech forest plots in the same landscape (supplement S5 – table S5, figure 6). Trends in basal area index per size class (i.e., full dbh range, smaller or larger than 60 cm dbh) revealed that all landscapes consistently contained a higher range of live standing basal area for trees with a dbh < 60 cm, with a pronounced difference in spruce forests. Romanian spruce forests in the Southern Carpathians had the highest live basal area for trees < 60 cm dbh (45.6 ± 12.8 m² ha⁻¹), compared to only 10.7 ± 6.8 m² ha⁻¹or trees > 60 cm dbh. Western Carpathian spruce forests showed the most notable range in basal area of dead trees < 60 cm dbh at 8.4 ± 7.6 m² ha⁻¹, while only 3.4 ± 4.6 m² ha⁻¹ for dead trees > 60 cm dbh.

The relationship between total biomass and IVI across different landscapes (Eastern, Southern, and Western) revealed contrasting results between mixed beech and spruce forests. Higher IVI values correlate with greater biomass, indicating that plots with a higher dominance of the respective forest types tend to have more biomass (figure 6). This correlation is stronger and more consistent in the Eastern and Southern landscapes, while the Western Carpathians exhibit more variability, particularly in mixed beech forests. The dbh class distribution shows a broad range of tree sizes, with larger trees significantly contributing to total biomass, thus highlighting the importance of forest composition and structure in controlling biomass across the Carpathians (S2.2 - GAMMs).

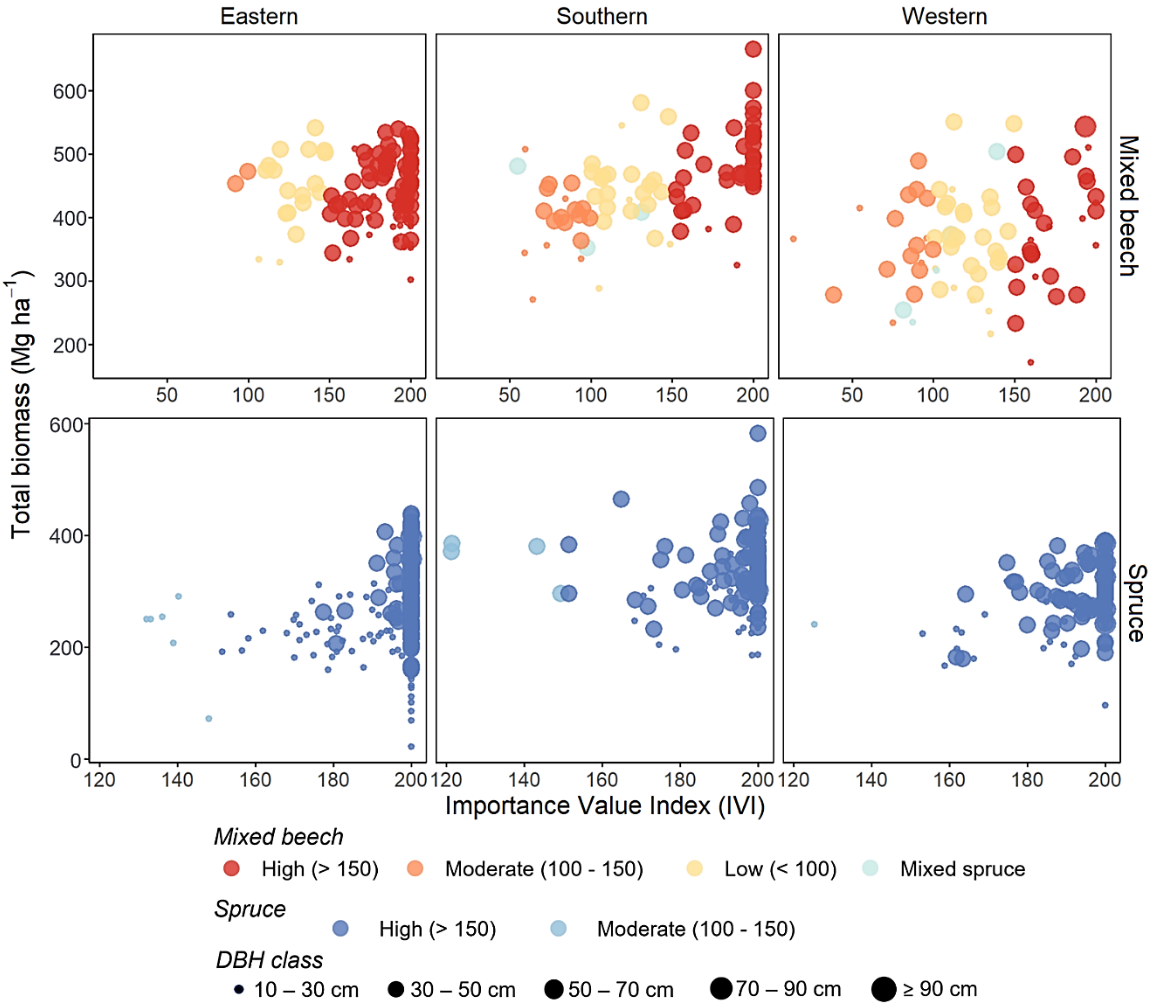


Figure 6: Spatial distribution in total biomass and the prevalence of beech and spruce genera in their respective forest types across each landscape (Eastern, Southern, Western Carpathians) using the Importance Value Index (IVI) score. *IVI score* denotes genera prevalence (beech, spruce) ranked by low (< 100), moderate (100 – 150) and high (> 150); *beech – mixed spruce* (*n plots = 4*) being plots in the mixed beech forests characterised as being dominantly spruce in structure and genus coverage. dbh class – plot-level mean diameter at breast height (cm, dbh) of live standing trees and total aboveground live and dead biomass (Mg ha^{-1}) across the 726 primary forest plots.

4.2.2 | The relationship between biomass and forest complexities

Assessing forest biomass, genus and structural variables at different spatial scales (Q1) revealed comparable results between live and dead standing trees. We found that the combined model, which included basal area index and genus abundance with all three spatial scales (landscape, stand, plot), was most influential in positively increasing biomass in both live and dead trees. Testing genus-level tree diversity at different scales revealed higher confidence when using all three spatial scales, supported by higher ΔAIC and R^2 values compared to using only stand and plot or plot-level (supplement S2.2 table S2.3). Combining structural (basal area index) and genus-level diversity (abundance) indices demonstrated a stronger relationship with total biomass variability, than the individual models with one variable. In contrast to the live models, dead tree models showed moderate support for basal area containing all three scales, indicating that structure has a greater influence on the distribution of dead trees and total biomass than genus-level diversity alone. The normalised difference test (Δtrees) between live and dead models showed a similar response when using both structural and genus-level indices at the three spatial scales, with stand-level models performing similarly (Δtrees -0.01), showing the least amount of variability between live and dead distribution, despite high ΔAIC (> 2) – see supplement S2.2 for further details on model testing and results.

4.2.3 | Interactions with biotic and abiotic factors

For testing how forest biomass, structural and genus-level indices interact with biotic and abiotic factors (Q4), we found tree age and disturbances, as a combined factor, positively influenced plot-level biomass and forest composition (i.e., basal area index and genus abundance), with the three spatial scales as the random effects helping maintain the positive effect. The second part of the analysis revealed that a mixed range in low-intermediate disturbance severity and a higher tree age range increased the structural complexities of live and dead trees, increasing total biomass. However, age and disturbance had a negative effect on dead tree abundance and total biomass when observed independently from other factors (figure 7).

Live tree models revealed that the full model, containing all five factors – dendrochronological (age x disturbance), structure (tree size index), genus (tree diversity), climate (temperature) and topography (altitude) – was most important for positively influencing total biomass (table 8). Altitude and temperature were moderately important for controlling live tree abundance but were less influential than the dendrochronological factors (age x disturbance). Both live and dead models showed a strong relationship with the dendrochronological factors, specifically plot-level mean tree age. The uneven age-size structure of the primary forest positively supports biomass stocks, demonstrated by the broad range of structural traits, such as tree size index and basal area index. The interactive models with biomass revealed contrasting relationships between structural and genus-level indices, with structural factors (basal area index) showing a stronger response to biomass than genus abundance. The relationship between biomass and genus-level factors (tree diversity, abundance) showed a slight positive increase, yet marginal compared to the interactive response with structural factors (table 8, figure 8).

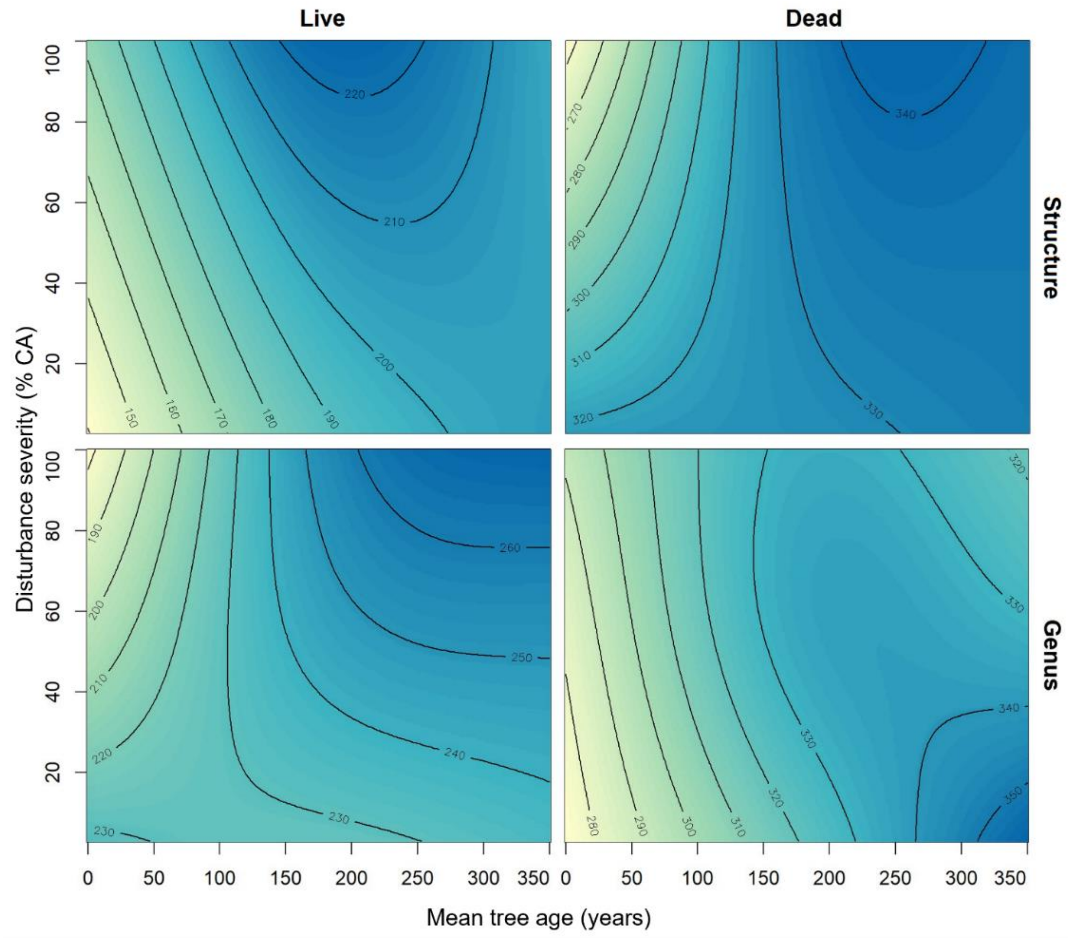
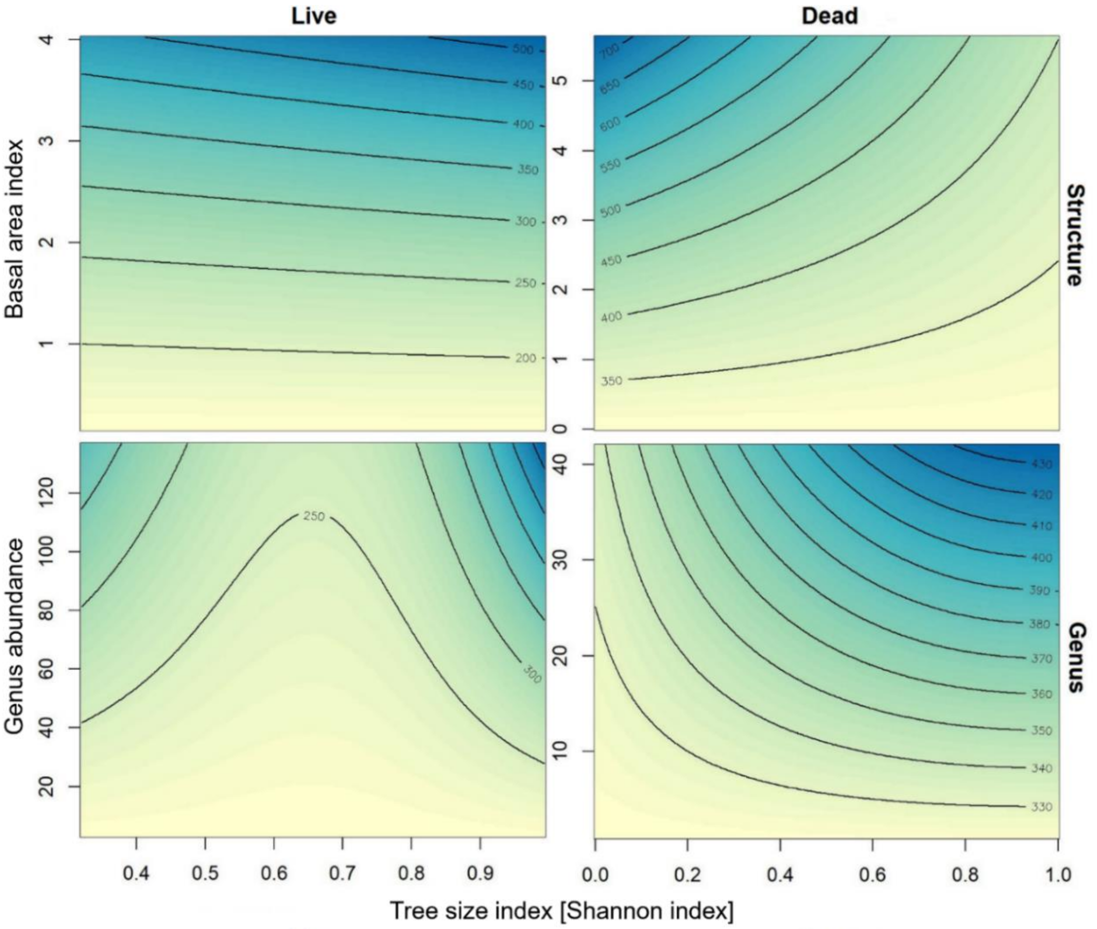


Figure 7: Generalised additive mixed-effects model (GAMM) – heatmaps showing the interactions between plot-level total biomass (Mg ha^{-1}), structure (basal area index), and genus (abundance) with age and disturbances. Basal area index – a measure of forest structural variability of plot-level basal area (m^2 ha^{-1}) standard deviation; genus abundance = plot-level genus count; Disturbance severity = mean plot-level disturbance severity represented as a proxy of disturbed canopy area (% CA); Mean tree age = mean plot-level tree age (years). Black contour lines represent the spatial distribution of total biomass values. The heatmap colour scheme shows light shading represents lower amounts of total biomass, while darker shading denotes higher amounts of total biomass. Narrow spacing between contour lines represent dense distribution in the interaction between variables, with wider spacing representing a sparse distribution.

		Live standing trees						***Dead standing trees***						
Model	Predictor	AIC	ΔAIC	R^2 (C)	R^2 (M)	D	Rank	AIC	ΔAIC	R2 (C)	R2 (M)	D	Rank	*Δtrees*
	Interaction with structure [basal area index]													
0	Null model	109.75	321.31	-	0.404	-	6	-197.04	140.57	-	0.472	-	6	3.51
1	Age x dist	-198.26	13.29	0.627	0.573	0.401	4	-337.61	0	0.603	0.53	0.284	1	-0.26
2	Age x dist + tree size index	-196.41	15.14	0.626	0.573	0.401	5	-337.22	0.39	0.603	0.529	0.284	2	-0.26
3	Age x dist + tree size index + tree diversity	-199.35	12.21	0.63	0.572	0.408	2	-337.12	0.48	0.601	0.53	0.281	3	-0.26
4	Age x dist + tree size index + tree diversity + temp	-198.93	12.63	0.63	0.571	0.409	3	-336.79	0.81	0.602	0.531	0.283	4	-0.26
5	Age x dist + tree size index + tree diversity + temp + alt	-211.56	0	0.63	0.57	0.41	1	-335.29	2.32	0.601	0.533	0.283	5	-0.23
	Interaction with genus [abundance]													
0	Null model	109.75	159.18	-	0.404	-	6	-197.04	134.363	-	0.472	-	6	3.51
1	Age x dist	-13.28	36.15	0.516	0.445	0.214	5	-331.4	0	0.598	0.503	0.276	1	0.92
2	Age x dist + tree size index	-39.43	9.99	0.535	0.46	0.249	4	-331.04	0.361	0.599	0.504	0.279	2	0.79
3	Age x dist + tree size index + tree diversity	-49.04	0.39	0.539	0.463	0.253	2	-324.77	6.63	0.599	0.504	0.28	5	0.74
4	Age x dist + tree size index + tree diversity + temp	-49.43	0	0.541	0.461	0.258	1	-328.02	3.374	0.599	0.505	0.281	3	0.74
5	Age x dist + tree size index + tree diversity + temp + alt	-47.71	1.72	0.544	0.478	0.265	3	-326.71	4.689	0.598	0.516	0.281	4	0.75

Table 8: Generalised additive mixed-effect models (GAMMs) analysing the relationship between plot-level biomass, structure (basal area index) and genus diversity (abundance) and interactions with the following site dependent factors: age x dist – mean plot-level tree age and disturbance severity; tree size index – the proportion of basal area per dbh class using the Shannon index; tree diversity – genus-level diversity using the Shannon index; temp – mean plot-level temperature; and alt – mean plot-level altitude. For testing the hierarchical models, we used the Akaike information criterion (AIC), and AIC difference (ΔAIC) represents measures of model comparison, R^2 (M) = marginal R^2 represents explained variance and account for the fixed effects (applicable to the model with no random effects and containing base R^2); R^2 (C) = conditional R^2 values accounting for both fixed and random effects; D = explained deviation to account of goodness-of-fit. *Δtrees* represents the normalised difference is a symmetrical, pairwise test which measures the degree of similarity or dissimilarity between models.


Live
Dead
Structure
Genus
Basal area index
Genus abundance
Tree size index [Shannon index]
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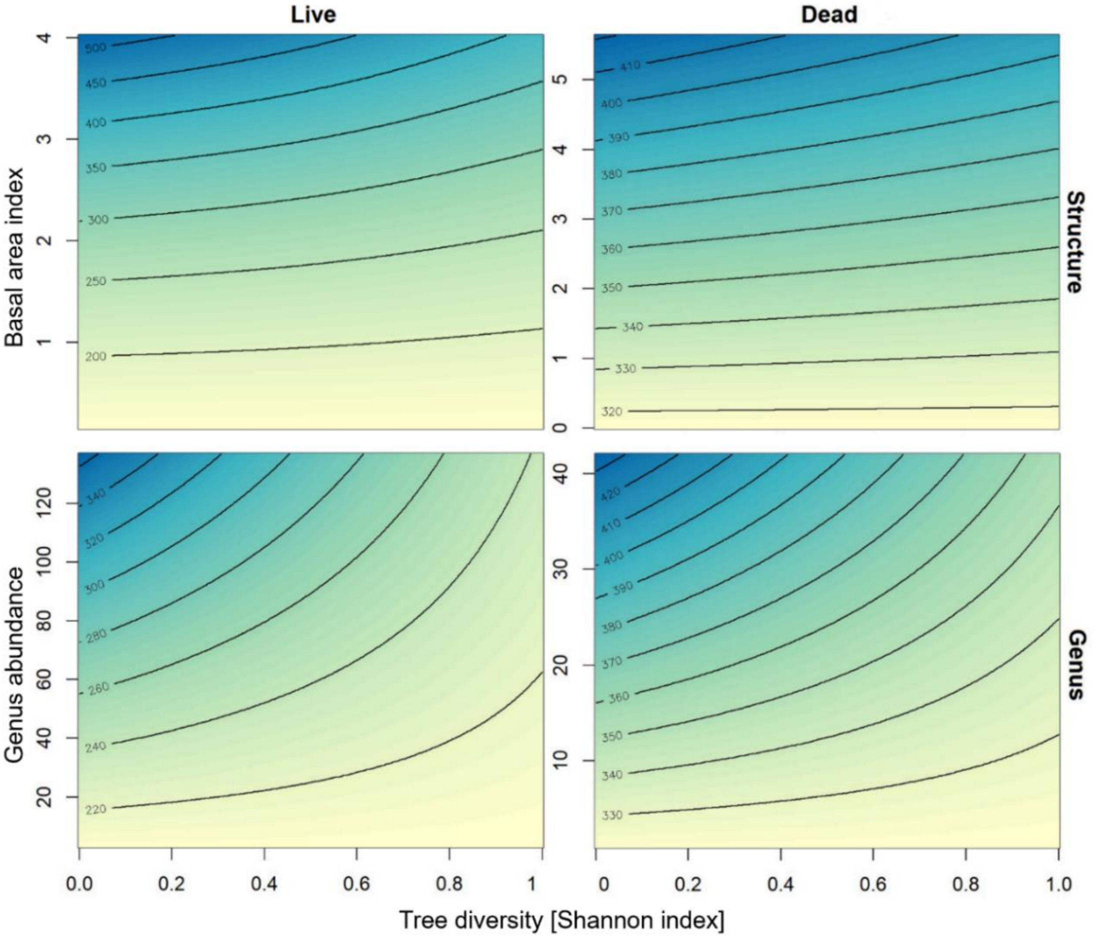
Live
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Basal area index
Genus abundance
Tree diversity [Shannon index]
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Figure 8: Generalised additive mixed-effects model (GAMM) – heatmaps showing the interactions between plot-level total biomass (Mg ha^{-1}), structure (basal area index), and genus (abundance) with tree size index, tree diversity, per tree type (live and dead standing). Main interactions: Basal area index – a measure of forest structural variability of plot-level basal area (m^{2} ha^{-1}) standard deviation; abundance = plot-level relative genus abundance. Predictor variables: Tree size index (top) – the proportion of basal area per dbh class; tree genus diversity (bottom panel) – genus-level tree diversity; each predictor variable was calculated using the Shannon Index. Black contour lines represent the spatial distribution of total biomass values. The heatmap colour scheme shows light shading represents lower amounts of total biomass, while darker shading denotes higher amounts of total biomass. Narrow spacing between contour lines represent dense distribution in the interaction between variables, with wider spacing representing a sparse distribution.

4.3 | Spatio-temporal variability in carbon dynamics across the primary forests

4.3.1 | Changes in carbon stocks over two censuses

The analysis of carbon dynamics across 454 forest plots in the Carpathian primary forests, reveals nuanced trends over two censuses (tables 9, 10). Mixed beech forests exhibited a slight increase in both total and live carbon stocks at all locations, with dead carbon pools showing varied changes. Specifically, Eastern Romania and Western Slovakia saw notable increases in live carbon, while Slovakia experienced minor increases. The maximum increase in live carbon was observed in the spruce forests of Eastern Romania, with a substantial gain (28 ± 43 tC ha^{-1}), whereas the minimum change in live carbon occurred in the Slovakian mixed beech forests (5 ± 8 tC ha^{-1}).

Spruce forests demonstrated more pronounced fluctuations, with Eastern Romania showing the highest gains in total carbon (30 ± 48 tC ha^{-1}) and a significant increase in live carbon, and Southern Romania with moderate increases in total carbon and a substantial rise in dead carbon stocks (table 10). Conversely, the maximum decrease in live carbon was noted in the spruce forests of the Western region, with a decline of 15 ± 40 tC ha^{-1}, while the mixed beech forests of Slovakia saw the least change in total carbon, with an increase of only 1 ± 17 tC ha^{-1}.

Trends in aboveground carbon increment (ACI) between censuses revealed a higher gain in total carbon than mortality across various landscapes and forest types. In the mixed beech forests, Eastern Romania showed an increase in total carbon stocks (1.55 ± 4.18 tC ha^{-1} yr^{-1}) and live carbon stocks (1.11 ± 4.17 tC ha^{-1} yr^{-1}). Dead carbon stocks also rose (0.44 ± 2.78 tC ha^{-1} yr^{-1}). Slovakian mixed beech forests exhibited a higher increase in total carbon stocks (4.26 ± 7.08 tC ha^{-1} yr^{-1}) and live carbon stocks (3.91 ± 6.03 tC ha^{-1} yr^{-1}), with minimal increase in dead carbon stocks (0.35 ± 2.96 tC ha^{-1} yr^{-1}).

In the spruce forests, Romania displayed a modest increase in total carbon stocks (0.27 ± 3.51 tC ha^{-1} yr^{-1}) and live carbon stocks (0.97 ± 1.78 tC ha^{-1} yr^{-1}), while dead carbon stocks decreased (-0.7 ± 3.13 tC ha^{-1} yr^{-1}). Ukraine’s spruce forests experienced a more substantial increase in total carbon stocks (1.03 ± 1.21 tC ha^{-1} yr^{-1}) and live carbon stocks (1.1 ± 0.85 tC ha^{-1} yr^{-1}), with a slight decrease in dead carbon stocks (-0.07 ± 1.27 tC ha^{-1} yr^{-1}). Southern Romanian spruce forests saw a more substantial increase in total carbon stocks (1.76 ± 3.6 tC ha^{-1} yr^{-1}) and live carbon stocks (0.6 ± 3.97 tC ha^{-1} yr^{-1}), with an increase in dead carbon stocks (1.15 ± 2.96 tC ha^{-1} yr^{-1}).

Overall, the data reflect the dynamic nature of carbon stocks across different forest types and regions, influenced by various ecological and environmental factors. These trends highlight the spatial variability in carbon stocks within a short period of time. Interestingly, dead carbon stocks exhibit more variability than live carbon stocks, with significant increases in some regions, suggesting changes in forest dynamics, potentially due to natural disturbances, and mortality affecting deadwood accumulation (supplement S3.2).

Forest type	Landscape	Country	*n plots*	Total – yr1	Total – yr2	Live – yr 1	Live – yr2	Dead yr 1	Dead yr 2	Dead standing - yr 1	Dead standing - yr 2	Dead CWD - yr 1	Dead CWD - yr 2	*ΔTotal*	*ΔLive*	*ΔDead*
Mixed beech	Eastern	Romania	33	213 ± 27	220 ± 36	195 ± 29	200 ± 35	18 ± 14	20 ± 16	5 ± 5	5 ± 8	12 ± 11	15 ± 11	8 ± 21	5 ± 21	2 ± 14
		Slovakia	70	223 ± 26	224 ± 29	198 ± 37	202 ± 39	26 ± 29	22 ± 22	6 ± 6	4 ± 6	20 ± 24	18 ± 21	1 ± 17	5 ± 8	-3 ± 15
Spruce		Romania	50	161 ± 35	191 ± 50	132 ± 36	160 ± 49	28 ± 14	30 ± 17	8 ± 7	12 ± 14	20 ± 10	18 ± 10	30 ± 48	28 ± 43	2 ± 16
		Ukraine	26	126 ± 24	131 ± 22	106 ± 22	112 ± 23	19 ± 8	19 ± 6	8 ± 6	9 ± 5	12 ± 5	10 ± 5	5 ± 6	6 ± 4	0 ± 6
	Southern	Romania	94	164 ± 32	176 ± 48	149 ± 32	153 ± 43	16 ± 9	23 ± 23	6 ± 5	14 ± 20	10 ± 7	9 ± 8	11 ± 26	4 ± 25	7 ± 19
Mixed beech	Western	Slovakia	20	190 ± 38	191 ± 36	159 ± 50	166 ± 47	31 ± 25	25 ± 24	4 ± 4	2 ± 3	27 ± 23	23 ± 23	1 ± 11	7 ± 9	-6 ± 12
Spruce			161	140 ± 30	147 ± 41	110 ± 39	95 ± 50	30 ± 23	52 ± 57	18 ± 21	39 ± 54	12 ± 9	13 ± 14	7 ± 23	-15 ± 40	22 ± 56

Table 9: Landscape-scale trends in carbon dynamics across censuses in the 454 forest plots, for total, live and dead carbon pools (tC ha^{-1}), dead carbon stocks include standing and coarse woody debris (CWD), for each census year (year 1, year 2) and their respective range in values between census years. The spatial distributions in carbon stocks are categorised by forest type (mixed beech, spruce), landscape (eastern, southern, western) and country (Romania, Slovakia, Ukraine) across the Carpathian primary forests. Difference (*Δ*) in carbon stocks between censuses represent the changes in total, live and dead pools, with all values containing mean and standard deviation in carbon stocks (±).

				tC ha^{-1} yr^{-1}					
Forest type	*Landscape*	*Country*	*n plots*	*Total*	*Live*	*Dead*	*Ingrowth*	*Mort*	*Downed*
Mixed beech	Eastern	Romania	33	1.55 ± 4.18	1.11 ± 4.17	0.44 ± 2.78	1.2 ± 2.76	0.29 ± 0.67	0.49 ± 1.99
		Slovakia	70	4.26 ± 7.08	3.91 ± 6.03	0.35 ± 2.96	4.03 ± 5.91	0.48 ± 1.12	-0.33 ± 1.51
Spruce		Romania	50	0.27 ± 3.51	0.97 ± 1.78	-0.7 ± 3.13	0.2 ± 0.37	0.21 ± 0.48	-0.49 ± 2.61
		Ukraine	26	1.03 ± 1.21	1.1 ± 0.85	-0.07 ± 1.27	0.4 ± 0.65	0.14 ± 0.28	-0.29 ± 0.9
	Southern	Romania	94	1.76 ± 3.6	0.6 ± 3.97	1.15 ± 2.96	0.63 ± 2.22	0.78 ± 1.33	-0.11 ± 0.9
Mixed beech	Western	Slovakia	20	0.24 ± 2.66	1.56 ± 1.85	-1.32 ± 2.74	0.36 ± 0.61	0.09 ± 0.25	-0.98 ± 2.55
Spruce			161	1.45 ± 5.19	-3.48 ± 9.64	4.92 ± 13.7	0.23 ± 0.69	3.03 ± 6.52	-0.12 ± 3.16

Table 10: Landscape-scale trends in incremental carbon stocks between censuses (tC ha^{-1} yr^{-1}) across the 454 forest plots, including the mean and standard deviation (±). For the following carbon stocks, values are based on the change between the second and first census: *Total* – net change in carbon stocks combining aboveground live, dead standing and downed dead stocks; *Live* – net change in aboveground live standing carbon stocks; *Dead* – net change in aboveground dead standing and downed carbon stocks; *Ingrowth* – the specific increase in aboveground live standing carbon, including new recruited trees; *Mort* – net change in mortality rates based on the amount of trees that were living in the first census and dead in the second census; *Downed* – the net change in downed dead carbon, representing the presence forest floor carbon pool.

4.3.2 | Spatio-temporal trends in carbon dynamics

Trends in the spatio-temporal distribution of carbon stocks in the Carpathians primary forests revealed a general pattern of stability across the region, particularly, in terms of total carbon stocks, encompassing both aboveground live and dead carbon reservoirs (tables 9, 10). We found that the spatio-temporal trends in carbon varied across each landscape (Western, Eastern, Southern) and forest type (mixed beech, spruce). For example, the one mixed beech stand in Western Slovakia, showed relatively stable amounts of total and live carbon accumulation between the first and second census, with less gain in dead carbon stores compared to the first census period. Trends in live and dead carbon accumulation across censuses revealed a high proportion of live carbon from the first census transferring into the dead carbon pool in the second census at specific sites (figure 9, 10, 11). Interestingly, trends in Eastern and Southern landscape remained stable, with considerably low amount of mortality and slightly higher rates of ingrowth compared to the Western landscapes (figure 9, 10, 11). This is emphasised by a pronounced increase in ingrowth, which was considerably greater in the Eastern and Southern spruce forests (figure 12). Notably, the spruce forests in Eastern Romania exhibit significant increases in total carbon stocks, indicating overall forest growth and increased carbon accumulation.

Analysing the trends in carbon dynamics revealed the contribution of live ingrowth carbon, which represents live tree carbon that surpassed the dbh threshold (> 10 cm) in the second census. This was a relatively minor component compared to the total amount of carbon from remaining trees that were present in both censuses, but indicated the processes of carbon accumulation. Mortality carbon, indicating trees that were previously alive in the first census but dead in the second, are prominent in some stands. Mortality was high at some sites in the Western Slovakian spruce forests, but lower in the Southern Romanian spruce forests (figure 12, 13).

Conversely, the presence of dead standing carbon, where trees were identified as dead in both censuses was generally low. Similarly, dead downed carbon, representing the presence of carbon on the forest floor from fallen trees and coarse woody debris, also revealed a low level across stands. Our results suggest that dead-standing trees remain standing for some time, with a minimal change to the forest floor carbon pool between censuses (figure 13). However, since downed dead carbon was measured using the line transect approach, we can only base these findings on the estimate of mean of coarse woody debris.

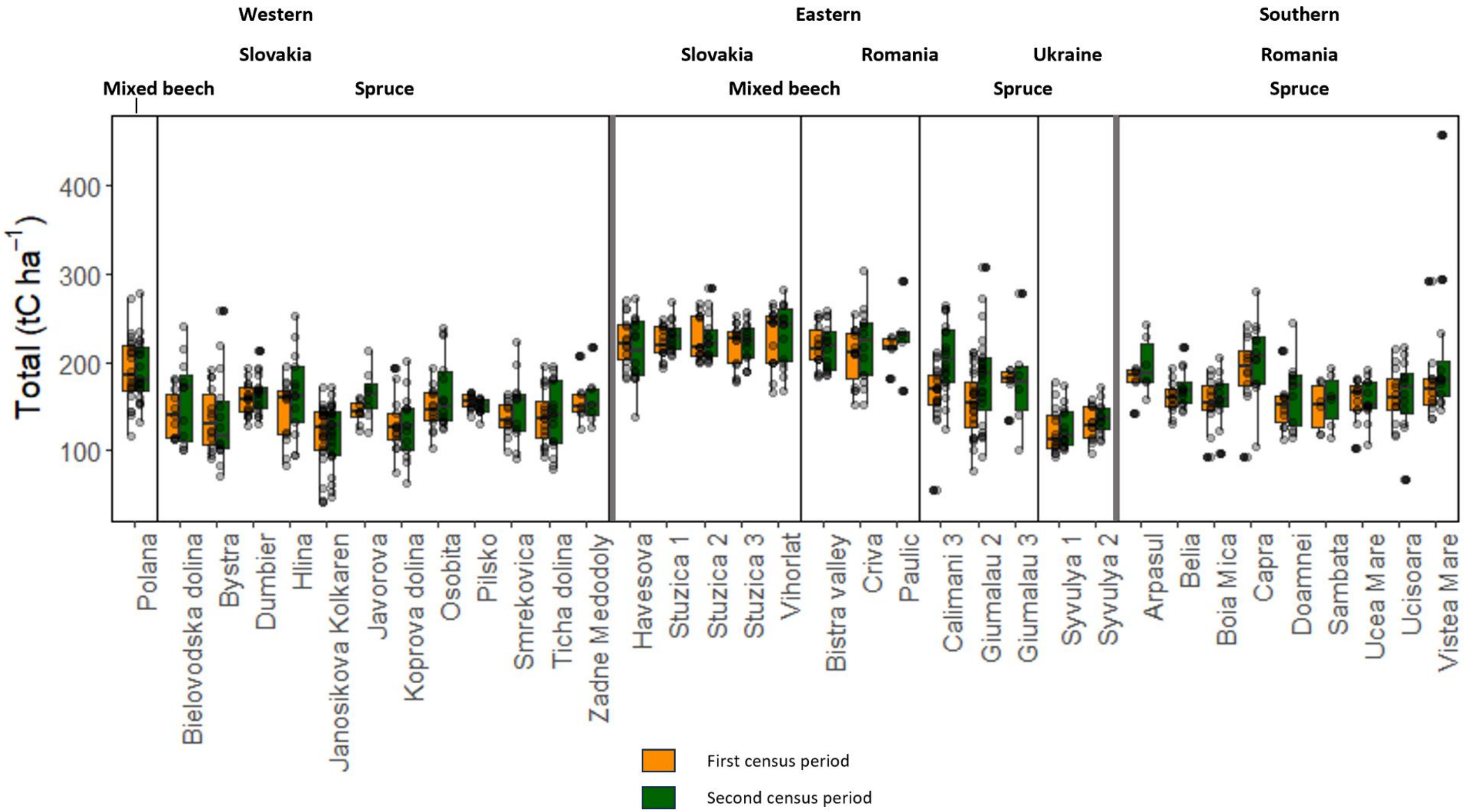

Figure 9: Trends in total carbon dynamics—a comparison of carbon stocks between the first (orange) and second (green) census periods across the Carpathian primary forests, including 454 plots. Trends depict total carbon, combining live and dead standing tree carbon and dead downed wood, representing the forest floor carbon pool stocks (tC ha^{-1}) across each landscape (Western, Eastern, Southern) and forest type (mixed beech, spruce).

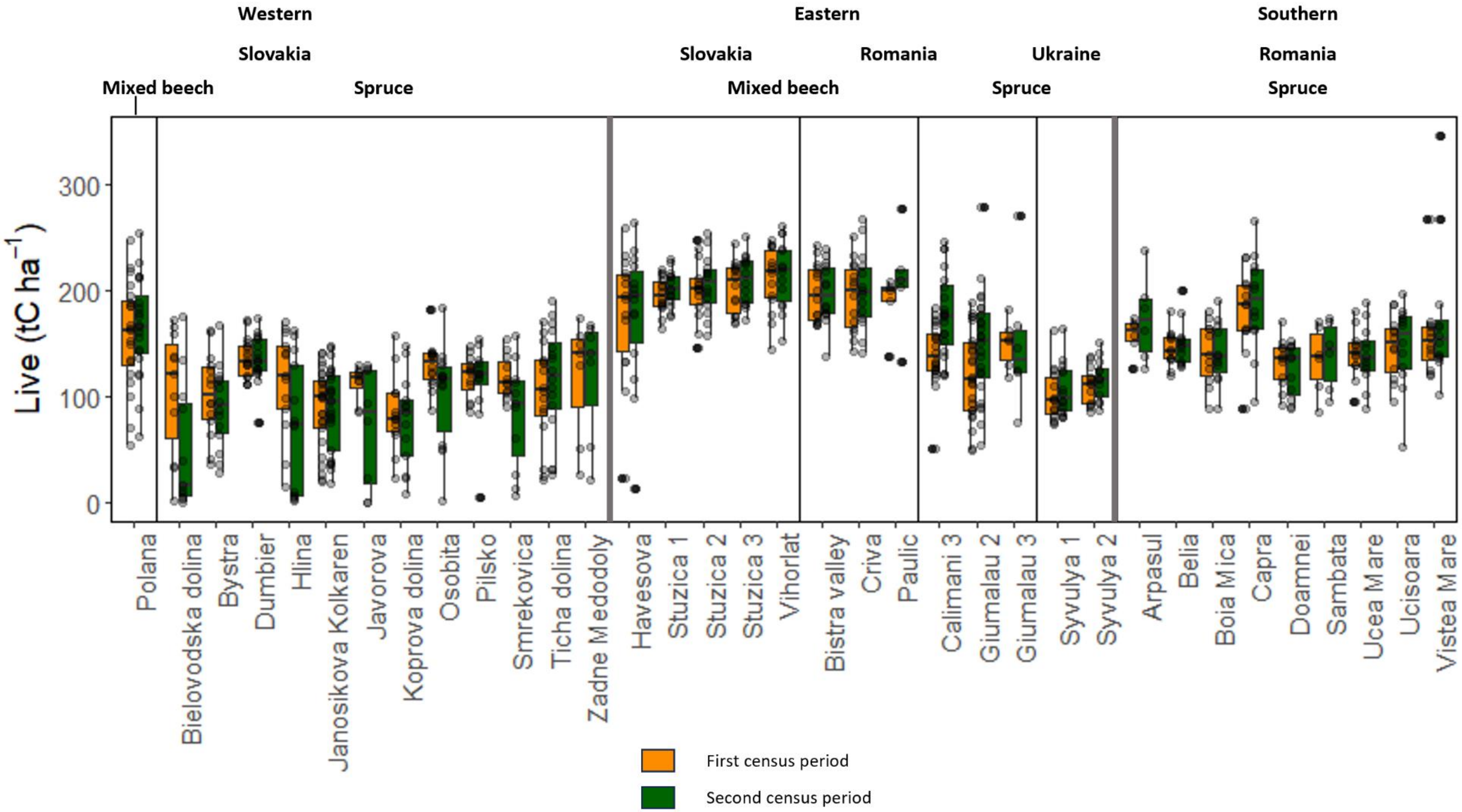

Figure 10: Trends in live carbon dynamics—a comparison of carbon stocks between the first (orange) and second (green) census periods across the Carpathian primary forests, including 454 plots. Trends depict live carbon (live standing tree) stocks (tC ha-^{1}) across each landscape (Western, Eastern, Southern) and forest type (mixed beech, spruce).

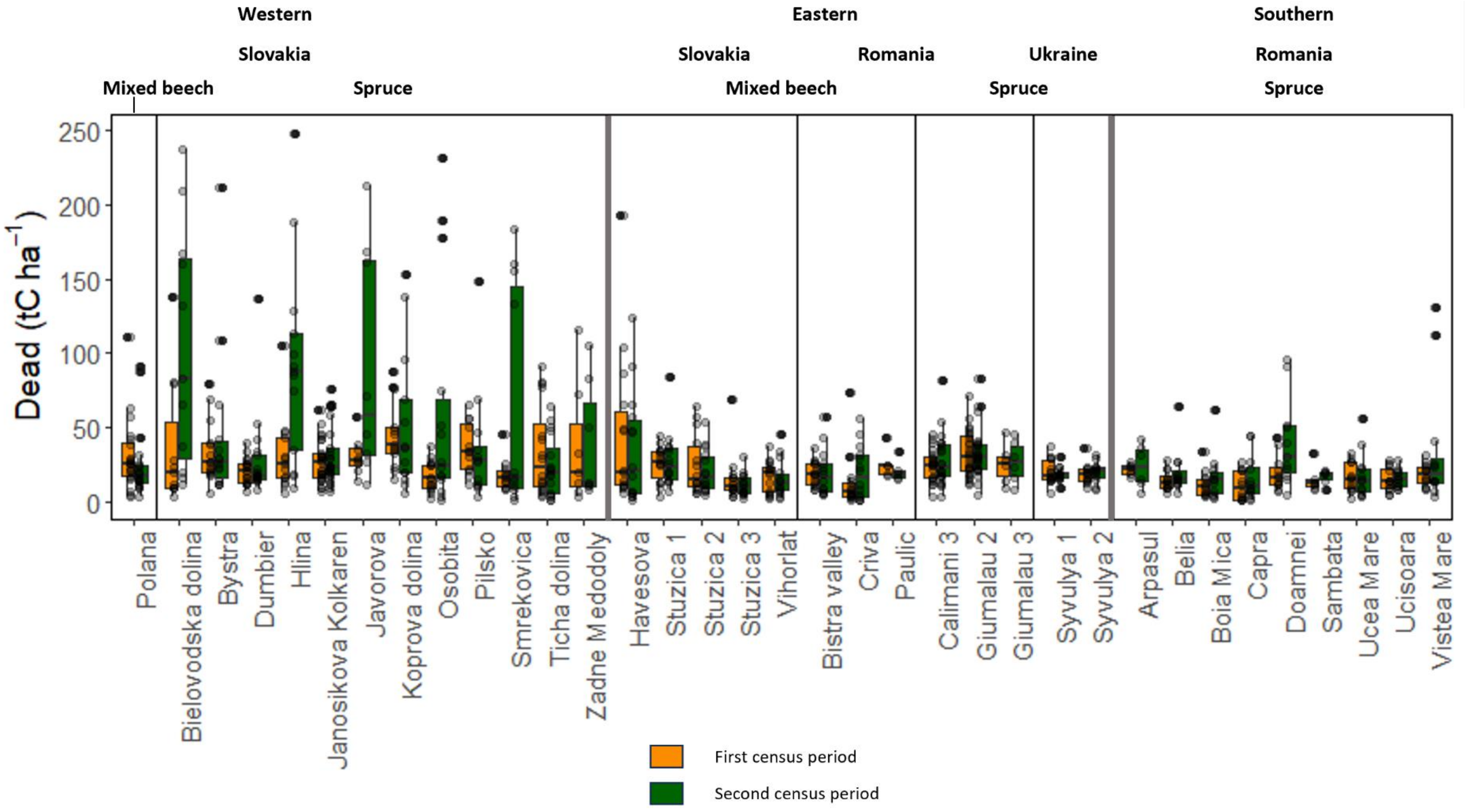

Figure 11: Trends in dead carbon dynamics—a comparison of carbon stocks between the first (orange) and second (green) census periods across the Carpathian primary forests, including 454 plots. Trends depict dead carbon, combining dead standing tree carbon and dead downed wood, representing the forest floor carbon pool stocks (tC ha^{-1}) across each landscape (Western, Eastern, Southern) and forest type (mixed beech, spruce)

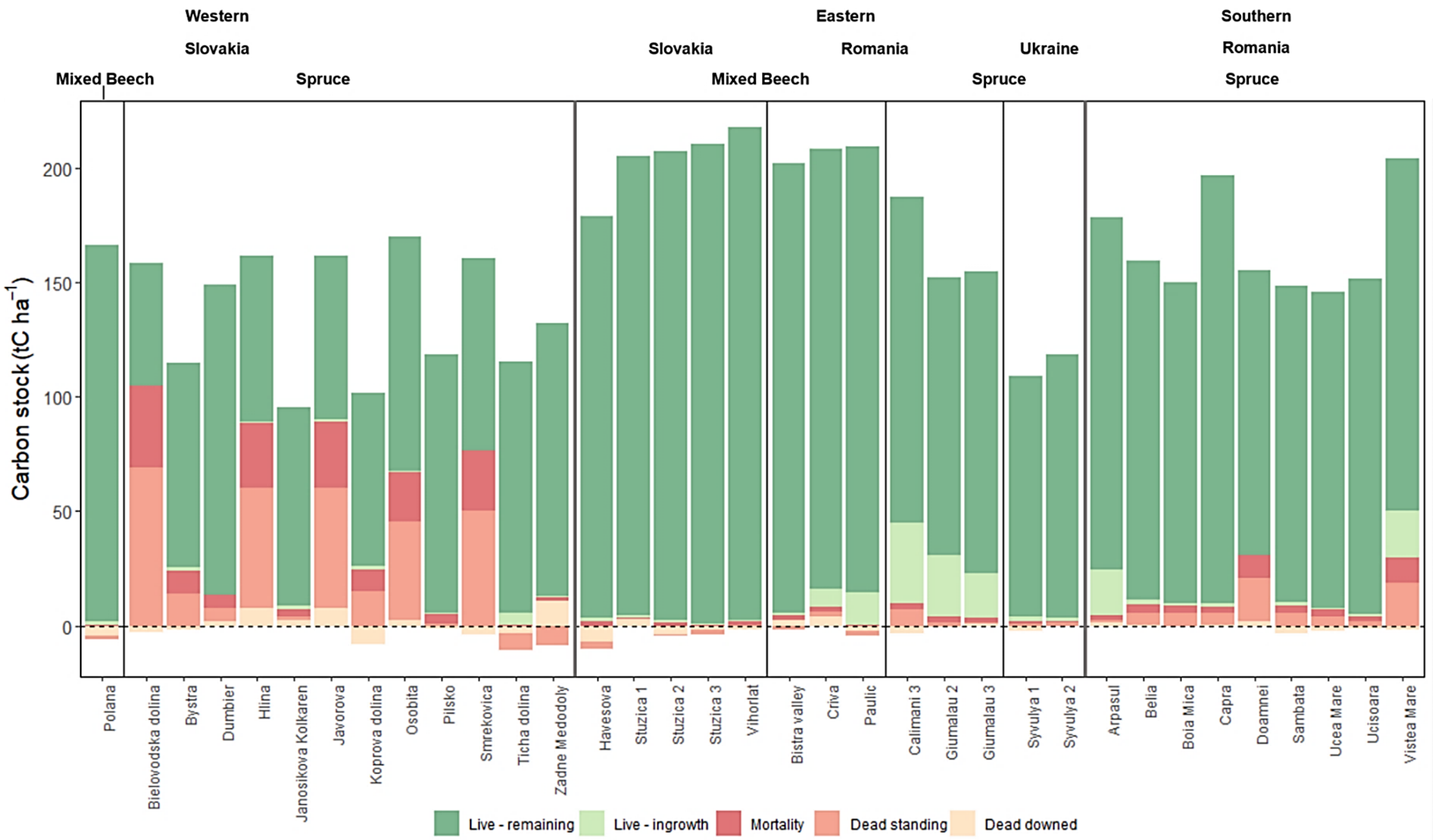

Figure 12: Total carbon stocks after the second census period for each landscape (Western, Eastern, Southern) and forest type (mixed beech, spruce). Carbon stock changes are categorised as follows: live carbon from trees present in both censuses (green); live ingrowth carbon from trees that grew past the diameter threshold by the second census (light green); mortality from trees alive in the first census but dead in the second (red); dead standing carbon from trees identified as dead in both censuses (light red); and the presence of dead downed carbon on the forest floor (yellow). Positive values represent carbon gains and negative values represent loss by decomposition

Figure 13: Net change in carbon stocks (Δ tC ha^{-1}) across the 454 primary forest plots, after the second census period for each landscape (Western, Eastern, Southern) and forest type (mixed beech, spruce). Change in carbon stocks (Δ) presenting the increase (+) and decrease (-) in the following carbon types: *live* – change in live carbon stocks (green); *mortality* – change in dead standing carbon stocks (red); and *dead downed* – changes in forest floor carbon stocks presence (yellow) denoting the difference in downed dead wood between the first and second census.

Net changes in carbon stocks, categorised by landscapes (Western, Eastern, Southern) and forest types (mixed beech, spruce), revealed that live carbon stocks generally increased across most locations (Figure 13). However, Slovakian spruce forests in the Western landscape showed a unique trend: after the second census, the majority of the carbon transferred from live to mortality, with most stands showing a loss of over 40 tC ha^{-1} in live carbon and an increase of more than 20 tC ha^{-1} in mortality. In contrast, the net change in carbon in the Eastern and Southern landscapes showed a moderate increase in live carbon, with live carbon gains ranging around 10 tC $ha\text{-}^{1}$ across most stands, and some stands in these landscapes showing gains > 20 tC $ha\text{-}^{1}$. This suggests higher levels of tree death in the Western landscape spruce forests, whilst mixed beech remained within its mean range (figure 13).

Spruce forests in Eastern Romania revealed the highest range in ACI (0 – 10 tC ha^{-1} yr^{-1}). Mixed beech forests generally exhibit moderate ACI, with some locations showing negative values, particularly in Western Slovakia (figure 14). These trends highlight the influence of site-specific conditions, such as maturity and topography, on the spatiotemporal distribution of carbon stocks across the Carpathians. Despite the substantial turnover in carbon type (live to mortality) in the Slovakian spruce forests, ACI revealed that most stands increased in carbon stocks. However, the Slovakian spruce stands in the Western landscape showed a consistently higher range of variability (-4 to 10 tC ha^{-1} yr^{-1}) compared to the Eastern and Southern landscapes, where the ACI was centred around 0 (figure 14).

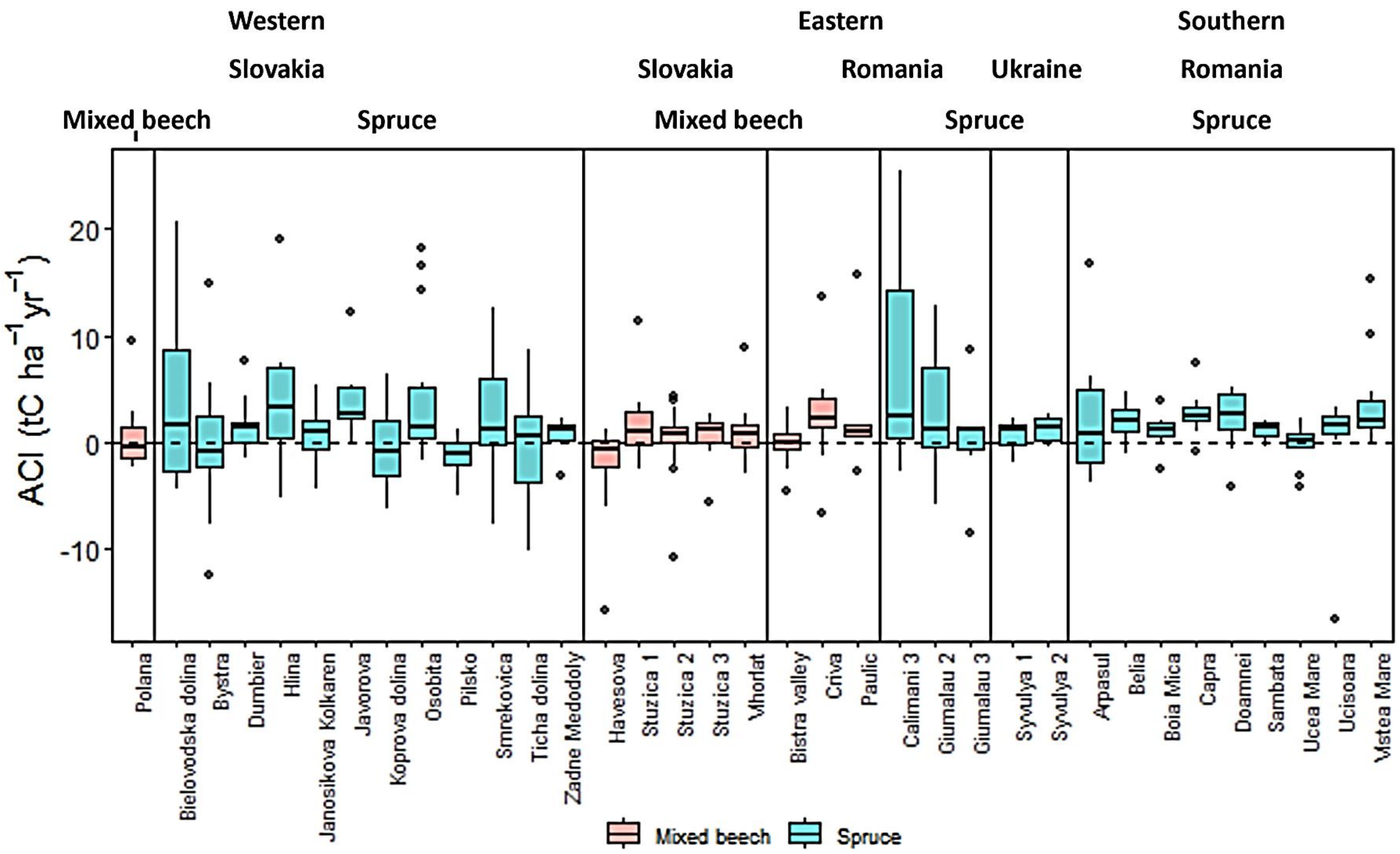

Figure 14: Aboveground carbon increment (tC ha^{-1} yr^{-1}) across the 454 primary forest plots, after the second census period for each landscape (Western, Eastern, Southern) and forest type (mixed beech). ACI represent the incremental change in total carbon stocks between censuses centred around 0, which represents no change in mean landscape-level incremental carbon stocks between censuses. Box represents the distribution and whiskers indicate the minimum and maximum range in values.

4.3.3 | Carbon dynamics and their relationship with biotic and abiotic factors

Testing carbon dynamics for each biomass component revealed that live and dead models performed similarly, identifying the full model (M1) containing carbon, mortality x age and altitude as the best fit and the only model within each series with an AIC < 2 (table 11). We identified that the changes in live and dead stocks separately were largely controlled by fluctuations in mortality since the first census and the uneven-age structure of the forest plots.

The trends were for live and dead carbon, were similar where the full model (M1), including all variables, was the best fit for each respective carbon type. Live carbon revealed the lowest AIC (-523.16), whilst dead carbon was considerably higher (-143.19), showing the best fit. Removing altitude (M2) resulted in a higher AIC for both live (-514.46) and dead (-127.82) models, respectively. This highlights the importance of altitude in predicting carbon stocks and their spatio-temporal distribution across the primary forests. The simplest model (M3: carbon) showed a considerable decrease in performance with a higher AIC for live (-442.97) and dead (-32.11) carbon. The difference between live and dead carbon models and their respective AIC may be attributed to the variability in carbon. Live carbon values show less variability compared to dead carbon, which is dependent on mortality and decomposition.

Additionally, AIC values within each model series were lower for dead carbon than total and live carbon, suggesting that the models fit better for dead carbon stocks. This could be due to the highly varied and uneven nature of the dead carbon dataset compared to total and live carbon datasets. However, the range in dead carbon stocks across each census period is considerably broader and more variable than total and live carbon stocks. In contrast, results for total carbon revealed that the model (M2) containing carbon from the first census with mortality and mean tree age provided the best fit with an ΔAIC < 2 (AIC = -586.31).

All models (total, live, dead) found that carbon stocks alone from the first census period had no strong effect on incremental carbon between censuses. The interactive effects of mortality x age improved the performance of all models, indicating a positive influence on incremental carbon stocks between the first and second census at the plot level. Indicating that incremental carbon between censuses was driven by mortality and age dynamics in support of all site-specific variables driving carbon stocks both spatially and temporally. The inclusion of altitude improved model performance for live and dead carbon; it was less critical for total carbon since it is the sum of total components of live and dead carbon, resulting in a stable trend with minimal variability compared to analysing each component separately (figure 15).

Model	***AIC***	***ΔAIC***	***R^2 (C)***	***R^2 (M)***	***D***	***Rank***
Total C						
M0: Null	17.23	603.54	-	0.38	0.4	4
M1: Carbon + mort x age + alt	-583.2	3.11	0.84	0.79	0.85	2
M2: Carbon + mort x age	-586.31	0	0.84	0.79	0.85	1
M3: Carbon	-511.69	74.62	0.81	0.79	0.82	3
Live C						
M0: Null	17.23	540.39	-	0.38	0.4	4
M1: Carbon + mort x age + alt	-523.16	0	0.82	0.78	0.83	1
M2: Carbon + mort x age	-514.46	8.7	0.81	0.77	0.83	2
M3: Carbon	-442.97	80.19	0.78	0.74	0.79	3
Dead C						
M0: Null	17.23	160.42	-	0.38	0.4	4
M1: Carbon + mort x age + alt	-143.19	0	0.59	0.5	0.62	1
M2: Carbon + mort x age	-127.82	15.37	0.57	0.49	0.6	2
M3: Carbon	-32.11	111.08	0.46	0.4	0.49	3

Table 11: Generalised additive mixed-effect models (GAMMs) analysing the relationship between plot-level carbon dynamics with site-specific variables, which includes: *mort* = mortality as a percentage of plot-level total carbon (%); *age* = mean plot-level tree age; *alt* = mean plot-level altitude (m); *carbon* = the amount of carbon (total, live, dead) present in the first census period for each model type (i.e., total, live, dead). These variables were tested with total incremental carbon stocks as the response variable, which is the average gain in total carbon stocks between the first and second census. For testing the hierarchical models, we used the Akaike information criterion (AIC), and AIC difference (ΔAIC) represents measures of model comparison, *R^2 (M)* = marginal R^2 represents explained variance and account for the fixed effects (applicable to the model with no random effects and containing base R^2); *R^2 (C)* = conditional R^2 values accounting for both fixed and random effects; *D* = explained deviation to account of goodness-of-fit. Rank denotes the order of importance based on the AIC score within each model series.

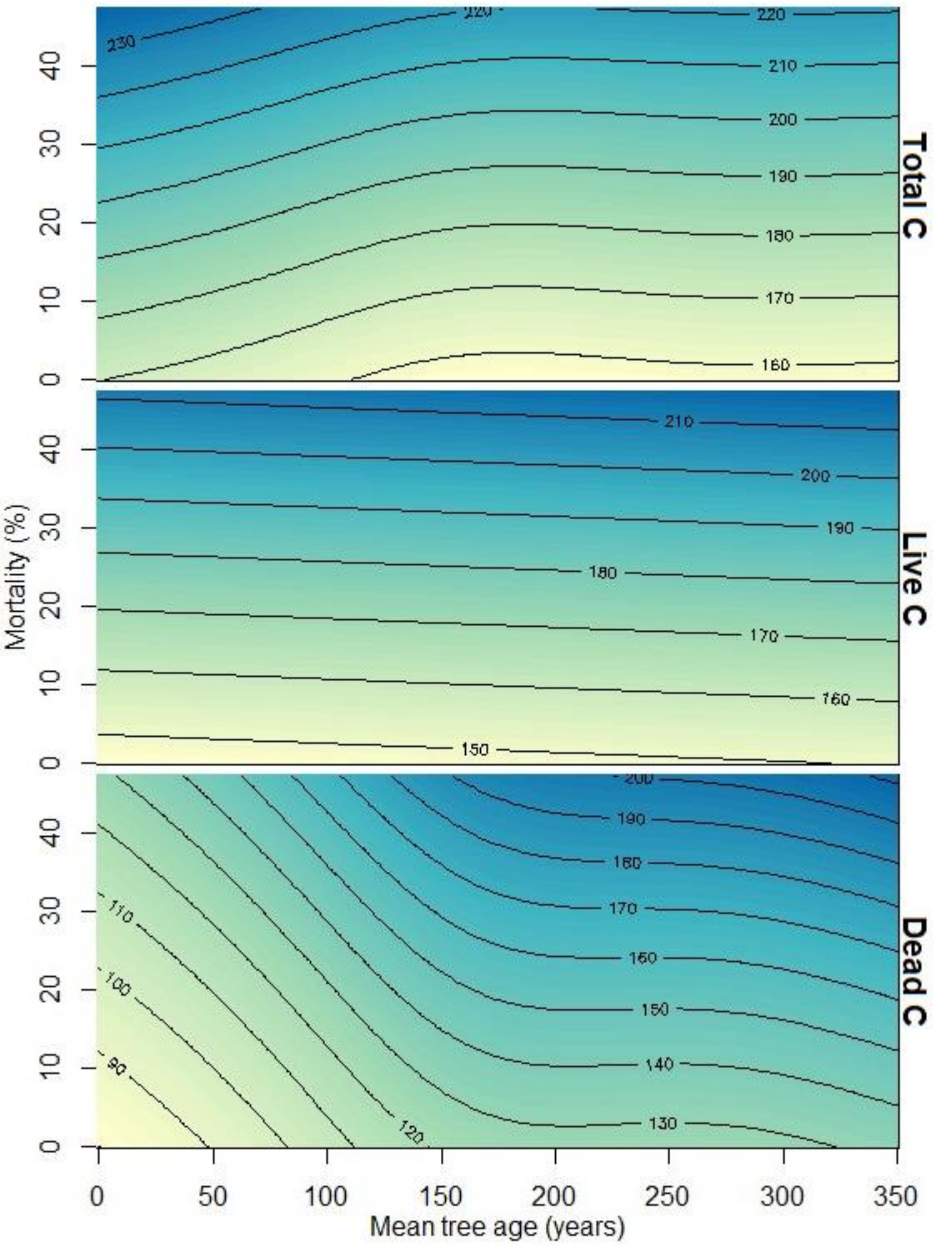

Figure 15: Generalised additive mixed-effects model (GAMM) – heatmaps showing the interactions between mean plot-level tree age (years) and mortality as a percentage of total carbon (%). Panels are split by carbon type, including total (top), live (middle) and dead (bottom) carbon stock (tC ha^{-1}) from the first census. Black contour lines represent the spatial distribution of total carbon after the second census. The heatmap colour scheme shows light shading represents lower amounts of carbon, while darker shading denotes higher amounts of carbon. Narrow spacing between contour lines represent dense distribution in the interaction between variables, with wider spacing representing a sparse distribution.

Chapter 5 | Discussion

5.1 | Temperate Primary Forest Biomass Accumulates over Centuries-Long Time Frames

Biomass stocks in the temperate forests of the Carpathian study region were asymmetrically distributed across broad classes of forest type, as well as spatially among subregions (termed landscapes in this study). In general terms, biomass amounts were greater by an average of ~ 106 Mg ha^{-1} in more diverse mixed beech forests versus spruce-dominated communities (Table 6). Geographically, the southern Carpathian landscapes support the largest stores of carbon in biomass in the region (~ 400 to 500 Mg ha^{-1}), while the Western landscape area maintains the lowest site biomass densities (~ 310 to 390 Mg ha^{-1}). According to a previous meta-analysis (Keith et al., 2009), our estimates of aboveground biomass carbon stocks for the Carpathian region correspond in magnitude with other temperate primary forests. Ecosystems with similar total biomass include the highly productive conifer forests of the Pacific Northwest region of North America. Based on a large dataset of widely distributed forest inventory plots, maximum biomass levels in that ecoregion were estimated to exceed 1,000 Mg ha^{-1} (Luyssaert et al., 2008; Smithwick et al., 2002), substantially greater than total biomass estimates in this study of primary forests in the Carpathians. Nevertheless, average live biomass in the Pacific Northwest ranges from 400 to 500 Mg ha^{-1} (varying by subregion) (Van Tuyl et al., 2005), like that in our study. The mixed species forests of the North-eastern United States support comparable but marginally lower, relative to the Carpathians, live biomass amounts, ranging from 250 to 350 Mg ha^{-1} in late successional old-growth forest conditions (Keeton et al., 2011). A few temperate forest types reported in Keith et al. (2009) do substantially exceed the maximum total biomass of the Carpathian region, including the *Eucalyptus regnans* (F. Muell.) forests of Southeastern Australia, where site-average biomass exceed 3,600 Mg ha^{-1}.

The biomass amounts quantified in this study are also consistent with previously documented global-scale patterns in the distribution of forest carbon, exceeding biomass densities reported for many tropical and boreal forest systems (Keith et al., 2009). At broad scales, variation in forest biomass density has been associated with gradients in and interactions between temperature and moisture supply, whereby total carbon storage is purportedly maximised under cool and mesic conditions (Fernández-Martínez et al., 2014; Keith et al., 2009; Larjavaara and Muller-Landau, 2012; Liu et al., 2014). Results from our study support a hypothesis for a temperature-dependent increase in biomass, although we did not detect evidence for a thermal threshold (figure 5). Presumably annual temperatures in the Carpathians (1–7 °C) are below the maximum levels, 10–20 °C according to Larjavaara and Muller-Landau (2012), that may lead to reductions in net carbon gain. Cool conditions have also been hypothesised to inhibit decomposition and thereby maintain dead biomass stocks (Keith et al., 2009). In the Carpathian region, our results show that drier conditions produced greater dead biomass (figure 5). Many interacting factors likely influence organic matter decomposition, including climate variables and the morphological and chemical properties of deadwood (Hu et al., 2018). In summary, a temperature threshold that limits the carbon economy of trees and potentially affects decomposition rates may partly explain the evidence in the literature for comparatively reduced total biomass pools in tropical versus temperate forest systems (Fernández-Martínez et al., 2014). In northern-latitude or high-elevation boreal forests, total biomass may be limited by the direct effects of low temperature on plant productivity, or by indirect effects on growing season length.

Controlling for broad categories of forest type, tree species composition within those types and climate factors, our analyses indicate that biomass stocks (total, live, dead) were strongly governed by tree age (table 7). Carbon stocks increased nonlinearly with the mean plot-level tree age, peaking when plot-level tree ages approached ~ 225 years (figure 4). A modest decline in total and live biomass was detected beyond this age threshold. A unimodal response to age is largely consistent with theoretical expectations and empirical evidence from prior studies (Fernández-Martínez et al., 2014; Liu et al., 2014; Luyssaert et al., 2008), though the underlying mechanisms are not fully resolved (Meyer et al., 2021). We discuss in more detail our modelling results and potential drivers in the following sections.

5.1.1 | Forest Biomass and Natural Disturbances

In this study, we hypothesised that disturbance history would strongly determine trajectories of forest development and the consequent range of biomass accumulation. However, our analyses did not detect an appreciable disturbance related signal in the biomass data. After accounting for plot-level tree age in our models, disturbance severity was not an important predictor of biomass stocks (total, live and dead). In contrast, previous research has found symptomatic relationships between the time interval of a prior extreme disturbance and contemporary levels of biomass. For example, carbon storage was found to reach maximum levels in primary Norway spruce forests circa 200 years following major historical disturbances (Mikoláš et al., 2021). Given that primary forests are often subject to re-occurring low-severity disturbances, we suggest that future studies investigate the effects of disturbance frequency or return interval on biomass stocks. Statistical uncertainties associated with disturbance reconstructions may also affect analysis outcomes. In our models, we used a measure of the most extreme disturbance event in a temporal chronology. However, multiple tree mortality events of low or moderate severity are likely to affect a given site over the course of centuries, leading to a progressive loss of information (data from tree cores) over time. A fading signal phenomenon, previously discussed in the context of retrospective analyses (for example, Swetnam et al., 1999), may lead to an underestimation of the importance of past event severity in biomass models.

5.1.2 | Forest Species Composition

Our modelling results suggest that biotic factors substantially regulate biomass stocks. Total, live and dead biomass stocks were higher in more diverse mixed-species forests (supplement figures S1.2, S1.4). Species-specific life-history strategies determine the demographic performance of trees (for example, survivorship and growth), hence their differential responses to climate and environmental variation, and thereby influencing the successional variability of forest communities (Grime, 1977; Kobe, 1997). We speculate that forest functions, such as carbon accumulation, may be buffered or stabilised in communities comprised of a diverse assemblage of species, consistent with a much-discussed theory linking diversity with ecosystem development (for example, Lasky et al., 2014). Additionally, a diversity of species that are characterised by substantial interspecific variation in life-history traits may contribute to greater long-term community-wide resistance to environmental perturbations (for example, climate extremes) (Zhang et al., 2018), thereby contributing to a conservation of forest biomass. For example, prior research has documented evidence for a greater drought tolerance in European beech, relative to Norway spruce, which may facilitate a greater potential for long-term biomass in mixed beech communities (Marchand et al., 2023).

5.1.3 | Forest Biomass and Maturity

Forest maturity, represented by mean plot-level tree age, was the main factor determining the accumulation of forest biomass (total, live and dead) across the Carpathian study region. We found forest maturity across the region was partly regulated by past disturbance severity (Keeton et al., 2011; Pavlin et al., 2021). Tree age effects have long been assumed to strongly influence rates of carbon gain in forests (for example, Whittaker et al., 1974). Recent continental and global scale analyses have indicated that forest age may account for as much as 92% of net ecosystem productivity (Magnani et al., 2007). Unresolved questions relate to the capacity of maturing and old-aged forests to capture and retain atmospheric carbon in biomass pools (Luyssaert et al., 2008). Theory indicates that biomass accumulation follows a predictable pathway as trees regenerate and increase in size following a stand disturbance. Then ecosystem productivity and net carbon gain are maximised, possibly within 100–200 years, and subsequently either decline (Gower et al., 1996; Ryan and Yoder, 1997). Bormann and Likens (1979) theory support that biomass stocks can vary substantial at different spatial scales. Results of plot-level total, live and dead biomass show a highly varied range. Meanwhile, at the coarse scale (that is, landscape level), biomass remains stable across the, respectively, forest types. More recent research indicates that uneven-aged forests may maintain positive carbon assimilation rates for several centuries (Keeton et al., 2011; Luyssaert et al., 2008).

Our results indicate that measured plot-level biomass of primary forests in our study region varied unimodally with mean plot-level tree age (figure 4). Specifically, controlling for other factors such as species composition, biomass stocks attained maximum levels as mean forest ages approached ~ 225 years. Previous studies based on analyses of large datasets have detected evidence for substantially older thresholds; for example, maximum forest biomass was associated with ages of 350–400 years in

Keeton et al. (2011) and 450–500 years in Liu et al. (2014). However, comparisons of age-dependent biomass thresholds among studies are confounded by inconsistent derivations of mean plot-level tree age. The two previously mentioned studies calculated stand age from a selected subset of canopy dominant trees (Keeton et al., 2011; Liu et al., 2014). In this study, tree age was derived from an unbiased and more integrative metric based on the average age of all mature trees in a plot (> 10 cm dbh). Therefore, our estimates of forest age and corresponding biomass thresholds are, by necessity, reduced in magnitude relative to values derived solely from the largest or oldest trees. Nevertheless, we demonstrate that primary forests in mesic temperate regions have a capacity to accumulate carbon for centuries, consistent with other studies and irrespective of uncertainties associated with age calculations. We did also consider an alternative measure of forest age founded on a supposition that biomass levels are determined by variation in the complexity of forest structure. We fit alternate competing models with the standard deviation of tree ages in a plot, assumed to reflect structural heterogeneity. However, according to model selection criteria, standard deviation was not an important predictor of biomass relative to mean tree age (table 6).

Biomass in the Carpathian was found to decline modestly beyond the 225-year age threshold. We did not detect evidence for an asymptotic biomass response to age, as in Keeton et al. (2011). Steady-state dynamics have been hypothesised to emerge in uneven-aged, multi-layered forests, where live biomass losses, associated with patch-scale disturbances or competition-driven tree mortality, may be relatively rapidly replaced by growth responses in extant canopy and sub-canopy trees (Luyssaert et al., 2008). We argue that our results are more consistent with previous findings that biomass growth in trees increases significantly with tree size, but declines with tree age when controlling for size (Foster et al., 2016). As no system can sustain unlimited growth, age-dependent biomass accumulation must ultimately be constrained by inherent physiological limits (Day et al., 2001). A range of mechanisms may lead to physiological declines in old trees. For example, hydraulic constraints in taller and presumably older trees (Ryan and Yoder, 1997) may negatively influence physiological function and demographic processes, including growth, defence and survival (Korolyova et al., 2022).

5.1.4 | Abiotic Conditions and Their Influence on Biomass Stocks: Climatic Effects

Our analyses identified that climatic water deficit plays a crucial role in determining the amount of dead biomass and influencing forest species composition in the Carpathians. Moisture availability also influences the decomposition rate of dead standing and downed wood and hence the time for biomass transfer from live to dead stock (Kueppers et al., 2004; Meigs and Keeton, 2018). This may explain why variance in tree age and CWD was significant for dead biomass, although the ΔAIC between temperature and CWD for total and live biomass was marginal (supplement S1.3—GAMMs). The amount of biomass accumulated in similar age cohorts may vary between different forest species composition types since wood density varies with species (Keeling and Phillips, 2007). Thus, the variability in tree age influences the range in dead biomass stocks across the Carpathians.

A possible explanation for why CWD is the most significant variable influencing dead biomass is that water availability varies along an altitudinal gradient and controls decay rate and forest type. CWD was higher in the Southern Carpathians landscapes compared to Western and Eastern (supplement S1.5—Climate data). This suggests that the Southern landscape has a partially elevated drought severity, regardless of species composition, yet biomass is consistently higher than in other landscapes (figure 3). CWD is positively related to the proportion of dead standing and downed biomass. Furthermore, cooler temperatures at higher elevations maintain a slower decomposition rate and delay the weakening of dead standing trees and their falling onto the forest floor. Kueppers et al. (2004) emphasise a lag in decay rates between the two forest types, for example, the difference in decay rate between mixed beech and spruce, and susceptibility to different disturbance agents. Spruce plots in Western Carpathians, Slovakia feature a significantly greater range in dead standing biomass compared with other landscapes (table 6) where cooler temperatures support a slower decay rate compared to mixed beech forest plots at lower elevations. Despite the lower range in dead biomass stocks compared to total and live biomass, our results demonstrate the amount of dead biomass present in the primary forests of the Carpathians is similar to other European temperate forests. Krueger et al. (2017) show in old-growth forests of Bavaria, Germany, the range of dead biomass was 23.2–30.4

Mg ha^{-1}, which is similar to levels in the Carpathians at coarse scales (Table 4). Deadwood is often omitted from studies on forest development due to difficulties of measurement. An inclusion of deadwood helps establish a deeper insight into the state and type of carbon storage in an ecosystem (Bormann and Likens, 1979).

5.1.5 | Abiotic Conditions and Their Influence on Biomass Stocks: Environmental Effects

Our findings are similar to Janda et al. (2019), whereby spruce forests exhibit a westward trend of decreasing basal area at stand and landscape scales. These general patterns may be influenced by higher disturbance severities in Western landscapes that generate larger stocks of dead biomass. High severity disturbance regimes are driven by intense windstorms and extensive bark beetle outbreaks, which are the main drivers of mortality in spruce forests (Janda et al., 2019). The combined effects of windstorms, bark beetle outbreaks and a higher portion of pure spruce stands with few mixtures may contribute to the higher disturbance severity in western landscapes. The most influential disturbance agents that cause mortality across the mixed beech forests are windstorms and senescence processes (Frankovič et al., 2021). In contrast, the spruce forest suffers from increased bark beetle outbreaks and windstorm events that occur at high elevations and steeper sites (Čada et al., 2020; Janda et al., 2019; Král et al., 2018). Mortality of these trees that produce the dead standing biomass cohort represents a stable carbon pool for many decades due to the low decomposition rates (Meigs and Keeton, 2018). Competition in the mixed forest may induce mortality since there is a higher demand for light availability, access to nutrients and space for growth, whereas growth in a pure forest displays less variability in biomass (Bartkowicz and Paluch, 2019; Keeling and Phillips, 2007).

5.1.6 | Conclusions of the first subchapter

A main goal in this study was to evaluate the capacity of primary forests to sustain net positive biomass accumulation rates. We demonstrate that primary forests in Central Europe reach maximum biomass over centuries-long time frames. Estimated biomass levels present in extant Carpathian primary forests are comparable in magnitude with biomass stocks that have been quantified for other temperate primary forest regions in mid latitudes. Mean plot-level tree age was identified as the most important driver of biomass in our analyses. We identified a mean age threshold (~ 225 years) at which biomass levels peaked at the plot level. We documented a reduction in biomass, albeit modest, in plots surpassing this age threshold. Unexpectantly, controlling for tree age, the effects of antecedent disturbance severity were not an important predictor of observed biomass amounts. We suggest that future studies evaluate the effects of alternate disturbance parameters on resulting biomass.

Our results support prior findings that primary forests serve as critical carbon sinks and store and thereby provide an important climate regulation function (Luyssaert et al., 2008). However, the functional integrity of primary forests is threatened by ongoing environmental changes. For example, global change factors have triggered a recent acceleration of tree mortality rates in divergent forest biomes across the globe (Hartmann et al., 2018). The effects of increasingly severe drought, warming temperatures, land clearing and wood harvest, among other factors, are multiplicatively impacting tree demography and forest productivity. Additionally, although disturbance effects were not important in our study, impacts related to the recent emergence of more extreme and frequent windstorms, fires and insect outbreaks are largely unknown. We suggest that results from this study demonstrate that safeguarding residual primary forests from land clearing and management impacts may serve as an effective climate mitigation strategy.

5.2 | Tree structure and diversity shape the biomass of primary temperate mountain forests

We found heterogeneity in tree size and genus distribution positively influenced biomass in supporting a diverse range across the three spatial scales (plot, stand, landscape) (Q3). Interactive models revealed that mean plot-level tree age, disturbances, and genus and structural complexities interact to create diverse forest compositions (Q4). These interactions support the accumulation of live and dead biomass through continual regeneration, growth and decay in a spatially diverse forest ecosystem. Thus, confirming both hypotheses regarding the interconnected role of structural complexities in basal area index, supported by genus-level abundance, positively influences total biomass stocks (H2). Forest composition complexity (tree genus diversity, structure) results from local processes such as disturbance regimes, uneven tree age and natural regeneration (H3).

We found the degree of heterogeneity in forest composition greatly controls the spatial distribution of biomass across the primary forests. Mono-dominant beech and spruce forest plots exhibit contrasting structures, with spruce-dominant plots featuring a similar size range, while beech-dominant plots show a broader range in tree sizes. Assessing the relationship in structure and genus with biomass shows that stocks are modulated by the effects of tree size index and tree diversity. Thus, the complexities in forest composition significantly influence the spatial variability and range in biomass stocks across forests at similar altitudes and levels of complexity.

5.2.1 | Influence of forest structure and genus diversity on biomass

Our findings show that complexities of genus-level tree diversity and structure have an interlinked effect on biomass, similar to the results reported by (Wang and Ali) 2022). This difference in biomass stocks varied between forest types, with mixed beech forest plots a greater range in the values of tree size and genus abundance compared to most spruce forest plots. We found that forest heterogeneity levels can lead to a decline in biomass stocks, due to the lack of diversity or minimal variability in structure and genus-level diversity, which is strongly influenced by the relative effects of forest composition (Figure 6). The spatial variability in forest structure was positively correlated with genus-level diversity, particularly in sites where there is a minimal difference between tree size index and tree diversity across most plots (supplement S2).

5.2.2 | Interactions between forest biomass and structure

We found the interactive effects of tree age and natural disturbances, Shannon indices (tree diversity and tree size index) to be the most important factors influencing the spatial variability in live and dead standing trees and biomass. Our results are similar to Ehbrecht et al. (2021), in finding the uneven distribution in plot-level biomass is influenced by a multi-age and tree size structure and interlinked with the variability in tree diversity (see subchapter 5.2.3). The distribution of dead trees showed a higher level of variance in tree structure, with a higher basal area index but lower genus abundance. This further demonstrates how the distribution of biomass is heterogeneous across the Carpathians and related to complexities in structure (basal area index, tree size index), and its interaction with tree diversity. Using a full range of tree sizes, rather than specifically analysing the influence of small or large trees, was more important for addressing the spatial variability in forest composition and biomass across forest plots (supplement S2.2 – GAMMs). This provided a better insight into the uneven structure of the primary forests at the plot scale and interlinked relationship between genus diversity and variability in tree structure with biomass (Fotis et al., 2018; Wang and Ali, 2022).

The variability in tree structure is modulated by the effects of age and natural disturbance in maintaining an uneven, heterogeneity ecosystem. Natural disturbances in the region typically leads to partial or total tree breakage, which facilitates an uneven tree size range through a mixture of canopy structures (Synek et al. 2020). The mechanisms of creation of canopy openings enable suppressed trees to ascend through the forest stratum, resulting in an uneven forest structure, which then contributes to overall biomass stock (Choi et al., 2023). Large-diameter trees are more susceptible to uprooting and breakage from windstorms, than smaller trees due to a lack of flexibility and often greater internal decay (Patacca et al., 2023). Small trees benefit from being sheltered by neighbouring trees (Lutz et al., 2018). Canopy openings formed by patterns of complex, moderate disturbance regimes facilitate younger individuals to develop into large-diameter trees (> 60 cm, dbh). In the context of the Carpathian primary forests, such processes maintain a structural complexity across forest strata. Whilst > 50% of the biomass is stored in larger-diameter trees, the uneven distribution in smaller trees helps maintain diversity in structure and genus (see supplement S5 – figure S5.4).

5.2.3 | Interactions between forest biomass and genus-level diversity

Our analysis showed that tree genus diversity is highly correlated with structure in supporting increased biomass stocks (figure 4). However, sites dominated by beech and spruce in the respective forest types, hence low diversity, showed that biomass stocks remained high due to heterogeneity in forest structure. We found tree diversity, observed at the genus level, to have a lower mean (as a dimensionless index) compared to tree size index (supplement S2.3 – table S2.5). This difference stems from tree size index containing a broader range in values due to the broad variability of basal area index, whilst number of genera is broadly similar within each forest type. Interestingly, we found dominant beech plots in Western Slovakia contained the largest range (0 - 0.99) across the Carpathian primary forest plots in our study (supplement S2.3 – table S2.5). In plots where dominant genus such as beech and spruce are mixed in their respective forest type have a higher amount of biomass if the ΔShannon between tree size index and genus diversity is minimal or closer to 0. Whilst most plots have a similar mean diameter range, higher IVI relating to a greater presence of dominant genus (beech, spruce) showed an increase in total biomass across all landscapes (figure 6). Interestingly, mono-dominant beech plots (IVI > 150) in the Southern Carpathians showed higher biomass with increasing dominance. However, the difference between other landscapes and forest types was minimal (figure 6). Mixture of sub-dominant genera, where beech and spruce have a lesser dominance in their respective forest plots, respond with a high biomass that is within a similar range to the mono-dominant plots (figure 6 & supplement S2.3 – figure S2.6).

The uneven species distribution across the Carpathian reflects the characteristics of primary forest and how varying local conditions can modulate genus diversity, tree size and the overall biomass stocks in an ecosystem (Wang and Ali, 2022; Zeller and Pretzsch, 2019). Our results showed that the structural complexities in tree size (basal area index and tree size index), rather than tree diversity, may be a better indicator; with complexities in forest structure supporting better resilience and resistance to disturbance events that allows trees to continue growing to larger sizes (Choi et al., 2023; Mikoláš et al., 2021; Silva Pedro et al., 2015). However, both structural and species indicators have a crucial role in assessing the degree of biodiversity in a forest and their interlinked relationships with biomass stocks.

5.2.4 | The relationship between forest biomass, tree age and size distribution

We found that plots containing a mixture of tree age and size range positively influenced the amount of biomass. Primary forests with large old trees together with cohorts of trees with a range of ages facilitate a stable carbon store for ~225 years (Ralhan et al., 2023) which was found to be supported by complexities in basal area index. Moreover, the variability in age cohorts, ranging from plots or stands containing a large number of old trees to less mature trees through regeneration, maintains a diverse age and size structure (Janda et al., 2019; Keith et al., 2009; Yuan et al., 2019). Our findings are similar to other regional studies, such as Zeller & Pretzsch (2019), in assessing forest structure and species diversity from tree to stand level across Central Europe. Additionally, we found that complexities in the full tree size and species diversity range were positively linked with an increase in total biomass stocks (supplement S2.2 – GAMMs). Biomass can remain stable, if the range in tree age and size within an extant community are uneven and forest development is not heavily compromised by disturbances (Piponiot et al., 2022).

5.2.5 | Effects of natural disturbances and structural complexities on biomass

We found a positive relationship between biomass stocks, age and disturbances for both live and dead tree models (table 8). Dead tree models revealed a stronger relationship due to a wider size range compared to live trees (supplement S2.3). Natural disturbances can have variable effect on forest development, based on the severity, intensity, frequency, and impact on different tree sizes (Patacca et al., 2023; Sabatini et al., 2020; Seedre et al., 2020; Szwagrzyk and Gazda, 2007). Natural disturbances are key components in a forest, in supporting forest heterogeneity by maintaining diversity in tree size and structural complexities.

Whilst moderate disturbance severity may not lead to stand-replacement impacts in most forests, their effects vary due to local processes and site conditions. In the spruce dominant primary forests in the Carpathians, moderate disturbances produced high structural complexities, which led to an optimal balance in carbon storage and biodiversity (Mikoláš et al., 2021). In the eastern United States, Choi et al. (2023) found that moderate disturbances enhanced canopy structure complexity, supporting resistance and resilience to a disturbance. Higher severity disturbances (i.e., > 60% CA) produce larger openings, and topography influences the degree of exposure to solar radiation, thus affecting canopy recruitment (Pavlin et al., 2024). Disturbance events can greatly influence tree size ranges and spatial scales and are a key characteristic of a forest shaped by natural disturbances (Choi et al., 2023; Lutz et al., 2018; Szwagrzyk and Gazda, 2007).

Most plots in our study experienced disturbance events that affected forest composition at scales of less than 1 ha, with isolated occurrences of high-severity events, typically in Slovakian spruce forest plots at the stand-scale of greater than 1 ha (Čada et al., 2020; Frankovič et al., 2021; Janda et al., 2019; Seedre et al., 2020; Synek et al., 2020). Patacca et al. (2023) found a dramatic increase in frequency of natural disturbance events across the European continent between 1950 and 2019, with bark beetle outbreaks, windstorms and fire being the primary disturbance agents. Increasing trends in disturbance events, and changes to both frequency and severity, could amplify the effects of sequential disturbance agents such as bark beetle outbreaks in the dominant spruce forests, adding to the highly stochastic pattern in damage (Holeksa et al., 2009; Patacca et al., 2023).

5.2.6 | Effects of climate and topography on forest composition and biomass

We found temperature and altitude strongly interacted with live genus-level abundance in influencing biomass. Interestingly, no other model found a substantial effect on forest composition (basal area index and abundance), which had a lesser degree of importance than interactions with age, disturbances, and Shannon indices (tree size index, tree diversity). This may be due to the high correlation between plot-level genus indices (abundance, tree diversity) and structural data (basal area index, tree size index) indices with total biomass. Changes in species and structural complexities across different altitudes may be sensitive to global environmental change, specifically, increases in thermal sensitivity in controlling species occurrence, growth and biomass stocks (Anderegg et al., 2020; Wang and Ali, 2022), moisture limitations can also affect forest growth and biomass, which can be more pronounced along an altitudinal gradient (Case et al., 2021; Wang and Ali, 2022). Whilst we did not find an important trend with climate water deficit (CWD), we do not rule out the importance of CWD, in addition to temperature, in controlling species growth and biomass.

Swetnam et al. (2017) found comparable results with topography being highly correlated to carbon store, in monitoring forest carbon and water constraints in a sub-alpine coniferous forest with fir, spruce and pine in the US. The variability in local topographical conditions, such as slope position and aspect, were found to have a stronger influence on biomass carbon accretion than climatic conditions (Swetnam et al., 2017). Duduman et al. (2021) found a positive correlation between maximum biomass of three main genera (*Abies, Picea, Fagus*) strongly varied with altitude, with species diversity declined with increasing altitude with stands above an intermediate altitude gradient (i.e., > 1,000 m), which contained maximum carbon stocks, showed a decline in stand-level species to > 2. The temperature-altitudinal gradient directly influences the amount of biomass a forest can accumulate by affecting tree height, exposure to solar radiation, growth rate, age, and wood density (Anderegg et al., 2020; Dolezal et al., 2020; Duduman et al., 2021; McDowell et al., 2020; Ullah et al., 2021).

5.2.7 | Conclusions of the second subchapter

Our study aimed to assess the spatial variability in aboveground living and dead biomass stocks, its relationship with structural and species diversity, and environmental conditions across the Carpathian primary forests. We found that (Q3) the combined effects of basal area index and abundance were important for explaining the variability in live and dead biomass across three spatial scales (plot, stand, landscape) (H2). Total biomass stocks showed a strong interactive effect with mean age and disturbance when testing their relationship with the spatial variability in forest composition (i.e., basal area index, genus abundance) (Q4). Our results showed how the interaction between tree age and natural disturbances supports a complex forest composition. These interactions enable the forest ecosystem to provide a stable carbon store through current and ongoing structural complexities and species diversity (H3). Thus, confirming our hypotheses that (H2) structural complexities and tree genus diversity influence biomass stocks and (H2) forest heterogeneity, mediated by age and disturbances, positively influences biomass.

The interactions of biotic and abiotic factors that create heterogeneity in forest composition enable accumulation of large stable carbon stocks in the ecosystem. We emphasise the importance of assessing carbon stock, which is often understudied, in favour of carbon flows and ecosystem productivity. Climate change presents multi-faceted uncertainties upon ecosystems globally, which could threaten the stability of primary mountain forests. Understanding the current state of these forests offers a deeper insight into the processes that facilitate forest development, benefiting carbon storage and biodiversity. Quantifying biomass carbon stocks in primary forest ecosystems is crucial to better evaluate the ecological and functional processes without direct impacts from human activities. Therefore, protecting primary forests is paramount to ensure their future security and ability to maintain large carbon stores that contribute to climate mitigation.

5.3 | Spatio-temporal variability in carbon dynamics across the primary forests

5.3.1 | Trends in carbon dynamics across the Carpathians

Across the majority of the primary forest stand, (Q5) carbon dynamics showed a steady trend across the Carpathians, with a gradual increase in carbon stocks across most sites, over time with an average five-year interval between censuses. Notably, total carbon stocks remained within range of the first census. However, spruce forest plots showed a wider range in carbon, particularly in the Western landscapes, which showed a higher mortality rate than the Eastern and Southern landscapes. Trends in dead carbon stocks were highly varied between sites, due to variations in disturbance events causing mortality which supports a heterogenous forest. Landscape-scale analysis of carbon stocks across censuses revealed stable total carbon across the Carpathians, where at the coarser scale increases in live carbon stocks was greater than mortality (H4). Mixed beech forests showed a consistent increase in live carbon, averaging between 5 and 7 tC ha^{-1}. In contrast, spruce forests displayed a wider range of live carbon changes across landscapes, from -15 tC ha^{-1} in Western Slovakia to 28 tC ha^{-1} in Eastern Romania. The variability in mortality across the Carpathians stems from spatial and temporal differences in specific disturbance events (e.g., bark beetle outbreaks) at some sites, which converted live biomass to dead biomass.

Assessing the drivers of carbon dynamics (Q6) revealed support for age and mortality influencing carbon fluctuations over time and space. Changes in carbon stores, whether total, live, or dead, was found to be driven by the interaction of tree age and recent mortality between censuses. The continued presence of large diameter trees (> 60 cm, dbh) in both censuses allows for live carbon stocks to remain stable and steady (figure

S3.4). The interplay of local factors, from climate, tree species traits, natural disturbances, and historical land management practices, drives the accumulation of large carbon stocks in from large-diameter trees (Keith et al., 2024, 2009; Lutz et al., 2018; Mildrexler et al., 2020). This further highlights the important quality of primary forests, where the absence of direct human activity allows large carbon stocks to accumulate and be maintained in the forest for centuries. While most spruce plots revealed an increase in live carbon, they suffered higher mortality rates than mixed beech forests. Notably, a significant portion of the dead carbon in the spruce plots remained standing during the second census, while forest floor carbon levels (i.e., coarse woody debris) remained relatively constant. The variability in dead carbon type (standing and downed) may be due to the difference in disturbance agents influencing each forest type. Wind and snowstorm events occur in both forest types (Čada et al., 2020; Janda et al., 2019; Seedre et al., 2020; Synek et al., 2020).

The frequency and intensity of these disturbance agents are more pronounced in the spruce forests compared to the deciduous forest plots (Čada et al., 2020; Mikoláš et al., 2021; Synek et al., 2020). This is due to the characteristics of the forest itself where spruce trees at higher altitudes are exposed to more storms, in addition to being affected by bark beetle outbreaks in some locations (Janda et al., 2019; Seedre et al., 2020; Synek et al., 2020). The combined impact of multiple disturbance agents increases the threat of bark beetle outbreaks, specifically affecting spruce trees. Such disturbance regimes can substantially threaten the survival of spruce and are a factor for elevated mortality rates in the spruce-dominated forests in the Western Carpathians (Patacca et al., 2023; Synek et al., 2020). This difference in mortality and transfer to dead biomass highlights the difference in disturbance regimes and the responses of each forest type, with mixed beech forests showing higher resilience and stability than spruce forests.

5.3.2 | Variability in live, dead, and total carbon stocks over time and space

The accumulation of live and dead biomass is a critical part of the biosphere carbon cycle in maintaining a healthy and biodiverse ecosystem and a heterogeneous forest (Mikoláš et al., 2021; Ralhan et al., 2024; Thom and Seidl, 2016). Our findings indicate a complex dynamic in forest development across forest types, driven by the uneven age structure and recent local (plot-level) changes by mortality. These characteristics in ecosystem processes demonstrate that the Carpathian primary forests are a stable carbon stock, with the accumulation of live biomass and decomposition acting as the main change in forest demography before a natural disturbance event. Abrupt changes in forest structure facilitate the natural regeneration of the ecosystem through a mixture of low-to-intermediate recent disturbances (Janda et al., 2019; Ralhan et al., 2024).

Trends in carbon accumulation and their dynamics revealed a distinct difference between the mixed beech and spruce forests. Carbon accumulation between the census periods differed between forest types, with mixed beech forests always positive and ranging from 0.6 to 3.91 tC ha^{-1} yr^{-1}, whereas spruce forests were negative or positive at different sites with a range from -3.48 to 1.76 tC ha^{-1} yr^{-1}. While spruce forests displayed a significantly higher carbon accumulation rate in live biomass, there was also higher mortality at many sites. Recruitment rates (i.e., ingrowth rates) also differed between the two forest types, with mixed beech plots ranging from 0.36 to 4.03 tC ha^{-1} yr^{-1} and spruce forests from 0.2 to 0.63 tC ha^{-1} yr^{-1}.

Trends in carbon accumulation and their dynamics revealed a distinct difference between the mixed beech and spruce forests. Both forest types showed signs of carbon accumulation, with mixed beech forests ranging from 0.6 to 3.91 tC ha^{-1} yr^{-1} and spruce forests from -3.48 to 1.76 tC ha^{-1} yr^{-1} (figure 14, table 9). While spruce forests displayed a significantly higher carbon accumulation, the distinct characteristics of beech and spruce species dominance significantly influence carbon accumulation patterns. Recruitment rates (i.e., ingrowth rates) also differed between the two types, with mixed beech plots ranging from 0.36 to 4.03 tC ha^{-1} yr^{-1} and spruce forests from 0.2 to 0.63 tC ha^{-1} yr^{-1}.

5.3.3 | Dynamics of carbon stocks

We found the rates of mortality and ingrowth were consistent with other forest ecosystems, with live carbon stock accumulating at a slightly higher rate than mortality rates (Duque et al., 2021; Dyderski et al., 2023; Gonzalez-Akre et al., 2016; Yuan et al., 2019). Studies in tropical and subtropical Andean montane forests (Duque et al., 2021) revealed higher amounts of carbon gains than losses in old growth forests, driven by size-dependent mortality and influence from disturbance regimes. Duque et al. (2021) found that increased stem density, rather than changes in average wood density, were the main drivers influencing carbon gains. Moreover, Duque et al. (2021) observed significant aboveground carbon gains in Andean forests despite changes in tree species composition due to warming. Interestingly, trends in the Andean montane forests showed a high mortality rate between 1,000 and 1,800 m.a.s.l., the zone where species have varying thermal tolerances (Duque et al., 2021). They attributed this mortality to changes in species composition (supplement S3, table S3.1).

Beech wood has a slow decay rate and thus dead wood is maintained in the forest carbon store for decades (Meyer et al., 2021). Downed dead carbon showed a decline in both forest types, with mixed beech showing a slightly larger decline (mixed beech: -0.31 ± 2.48 tC ha^{-1} yr^{-1}; spruce: -0.16 ± 2.34 tC ha^{-1} yr^{-1}). The decrease in amount of dead downed carbon between censuses represents loss of carbon from the total biomass pool, even though the absolute amounts are small. This decline in dead carbon accumulation on the forest floor represents the balance between inputs to the pool from standing dead trees falling and decomposition of the dead biomass. However, the mean interval of 5 years between censuses may be a short period of time for any substantial decomposition to occur (supplement S3 – figures S3.2, S3.3).

A substantial portion of live carbon was converted to dead carbon (mortality) after the second census, highlighting the need to monitor all biomass pools. This was particularly noticeable in the spruce forests in the Western landscapes, where mean annual increment rate of mortality was 3.03 ± 6.52 tC ha^{-1} yr^{-1}, whilst the range for the Eastern and Southern landscapes spruce forests was considerably lower, being 0.37 ± 0.94 tC ha^{-1} yr^{-1} and 0.78 ± 1.33 tC ha^{-1} yr^{-1}, respectively. In the mixed beech forests, mortality rates were considerably lower, in the Western (0.09 ± 0.25 tC ha^{-1} yr^{-1}) and Eastern (0.24 ± 0.55 tC ha^{-1} yr^{-1}) landscapes, respectively. Our findings are within range of Dyderski et al. (2023) in quantifying biomass dynamics across Gorce National Park, Polish Carpathians; revealing an average mortality increment of 2.9 ± 0.01 tC ha^{-1} 5 $years^{-1}$ in spruce forests. Dominant beech forests in the Polish Carpathians revealed an average increment of live carbon at 6.65 ± 0.01 tC ha^{-1} 5 $years^{-1}$, however, these values are based on an increment of five years interval between measurements than annual increment per year (Dyderski et al., 2023). The altitudinal range in our study of the Carpathian primary forests (600 – 1,735 m.a.s.l) was comparable to Dyderski et al. (2023) (640 – 1,310 m.a.s.l) and observed a similar trend with increasing mortality rates with altitude (supplement S3, figure S3.2). Dyderski et al. (2023) found that the variability in local to regional disturbance regimes to altitude and topographic wetness index, as a measure of potential soil moisture accumulation and availability, were the main drivers of biomass in the Polish Carpathians. Mortality rates over a 25-year period revealed a retreat of spruce and increased growth rate of beech and fir. Old growth forests have larger trees that grow more slowly and die less frequently. Findings by Dyderski et al. (2023) revealed that forests with a mixture of tree sizes have lower mortality rates for older trees due to higher resilience to disturbance regimes.

Mortality rates also differed between the two types, with mixed beech ranging from 0.09 to 0.48 tC ha^{-1} yr^{-1} and spruce forests from 0.14 to 3.03 tC ha^{-1} yr^{-1}. This aligns with studies by Szewczyk et al. (2011), supporting the more pronounced and variable growth responses of beech and spruce in the Western Carpathian, when analysing changes in stand density and growth chronologies. The difference in growth rates between spruce and beech due to greater climatic variability, as well as local climatic conditions. Spruce are more thermally sensitive, with changes in temperature and precipitation significantly reducing growth. Beech is less sensitive but still impacted by such fluctuations in climatic conditions (Szewczyk et al., 2011). This explains why the range in mortality was greater in the spruce forest than in the mixed beech, because the spruce dominant forests become more stressed, due to adverse climate conditions which makes them susceptible to bark beetle outbreaks as a secondary disturbance agent (Marchand et al., 2023). Hence, these distinct characteristics of beech and spruce forest types significantly influence carbon accumulation patterns across the landscape.

The higher range in live carbon increment and rate of mortality in the spruce dominant forests demonstrates these plots exhibit a higher degree of variability in their total carbon stocks. Live carbon displayed no clear pattern with increasing mortality and age. In contrast, total carbon stocks peaked at approximately ~225 years, aligning with previous research (Ralhan et al., 2023). Dead carbon stocks exhibited an inverse trend, decreasing significantly after 200 years. In a pan-European study by Di Filippo et al.

(2015), the maximum lifespan limit of 25 temperate deciduous tree species was 300 – 400 years, whilst the maximum mean-plot level tree age in the Carpathian primary forests plots was 442 years. Our findings suggest that trees can attain a maximum lifespan of approximately 300-400 years in these temperate deciduous forests. Beyond this threshold, growth rates may continue at a substantially reduced rate or potential plateau in carbon accumulation.

5.3.4 | Drivers of Carbon Dynamics: Disturbances

Historical disturbances shape the growth trajectories of mature trees, particularly those with long lifespans that remain for centuries and continue to be an important carbon sink (Keith et al., 2024; Kueppers et al., 2004; Mikoláš et al., 2021; Pavlin et al., 2024; Seedre et al., 2020; Yuan et al., 2019). However, uncertainties concerning future climate change may introduce a novel phase in disturbance regimes (McDowell et al., 2020), impacting the accumulation of live and dead carbon. In the Norway spruce (*Picea abies*) forests of the Carpathians, the effects of natural disturbances at the coarse scale have been difficult to distinguish when confounded by biophysical conditions such as topography and climatic conditions (Janda et al., 2019). However, stands can reach high carbon stocks after periods of high disturbance severity, whilst lower disturbance severity levels lead to a stagnating carbon store (Keeton et al., 2011; Seedre et al., 2020). The mixed beech (*Fagus sylvatica*) forests of the Carpathians have a diverse range in species and tree density and experience intermediate severity natural disturbances that result in a multi-layered forest structure (Choi et al., 2023; Meigs and Keeton, 2018; Stillhard et al., 2022).

The amount of live and dead carbon remained within the mean range from the first census for most landscapes. Western Slovakian spruce forests showed the highest mortality, with a high proportion of trees in most stands identified as dead in the second census. Despite the high mortality, which was limited to the spruce forests in the Western landscapes and a few stands in the Southern landscape, overall total carbon stores remained stable across the Carpathians. This stability is due to the carbon transferring from the living to the dead pool, as the majority, if not all, of the mortality trees identified in the second census remained standing, allowing their presence to be accounted for during forest inventories. However, such dynamics between censuses may be difficult to capture for downed dead wood since it requires the use of the line transect approach, which identifies per census the average presence of coarse woody debris not the individual tree or logs. Considering these caveats in sampling downed dead wood, this approach remains suitable in quantifying the amount of carbon on the forest floor at the fine scale that can be remeasured along the same spatial dimensions set by the plot size. The position of the line transects, using the same approach for individual trees position (i.e., x,y coordinates), across all plots provides an account of the change in coarse woody debris present per census.

Studies on temperate forests in northeast China (Yuan et al., 2021) demonstrate the importance of large-diameter trees (> 60 cm, dbh), which were found to represent over 50% of a plot's total carbon store. We identify similar patterns in carbon stock distribution with varying tree size, with > 50% represented in large-diameter trees, which is also supported by Keith et al. (2024) in a European-focused study on the carbon-carrying capacity of naturally regenerating forests. Compound disturbance events have the potential, depending on their intensity, frequency, and duration, to compromise the stability of large trees, drastically reducing the amount of live carbon in a plot (Keith et al., 2024; Ralhan et al., 2024; Yuan et al., 2021). It may take centuries to restore carbon stores foregone to pre-disturbance levels of live carbon.

The mortality of a large, mature tree in a plot can significantly impact carbon dynamics, with mortality rates exceeding the growth rate of younger trees as ingrowth and incremental growth of remaining trees. Understanding how primary forests, even those containing similar dominant genera (beech, spruce), respond to these different disturbance regimes is crucial. In contrast to the Western Carpathians, mortality rates in the Eastern and Southern Carpathian landscapes were relatively similar across forest types. Interestingly, these eastern landscapes exhibited substantial carbon gains (0.01-25 tC ha^{-1} yr^{-1}) in both spruce and mixed beech forests.

Despite substantial fluctuations in carbon stocks between live, dead, and ingrowth components, particularly evident in Western spruce forests, the overall maximum carbon of the primary forests remained relatively stable. This emphasises the importance of disturbance regimes in maintaining forest structure and function.

Notably, the increased level of mortality was particularly evident in spruce forests (> 1200 m.a.s.l), especially in the Western Carpathians. Beech forests plots showed a This aligns with the increasing threats of bark beetle outbreaks and windstorms in these spruce forests, leading to higher mortality rates in this specific landscape (Synek et al., 2020). Although total annual carbon increment remained high in Western spruce landscapes, separating live ingrowth and mortality components revealed a contrasting trend.

5.3.5 | Drivers of carbon dynamics: age distribution

We established one of the first studies on the Carpathian primary forests to investigate the spatio-temporal changes in carbon dynamics, but also establish protocols for assessing primary forests natural carbon carrying capacity. Similar to trends discussed in subchapter 5.1.3 – Forest Biomass and Maturity, changes in carbon stores over time and space supports a peak in accumulation at ~225 years (Ralhan et al., 2023), which is much higher than previous studies (Keeton et al., 2011). After an average interval of 5 years between census, the primary forest plots show a steady increase in live carbon that is greater than mortality rates across the majority of plots. However, the short interval between censuses showed minimal deviation from the mean carbon stock and so there remains uncertainties about the longevity of accumulation and maximum carbon stocks. Similar to single census studies on biomass (see subchapter 5.1), we did not detect evidence for asymptotic carbon stocks over time in response to age, as in Keeton et al. (2011) and Zhu et al. (2018), in terms of saturated carbon stocks limited by age.

Trends in carbon dynamics with age revealed that whilst net change in live and dead carbon strongly varied between plots within a stand (i.e., at the fine-scale), stand-level trends identified the overall stability of the carbon reservoir across the landscape. Our findings suggest that physiological tree maturity is not the primary driver of a loss in the rate of carbon gain in living trees. Instead, individual tree characteristics, such as reduced hydraulic conductivity, seem to be more critical factors which limit a tree's resilience to disease and pathogens, ultimately leading to mortality. This aligns with Pavlin et al. (2024) and Synek et al. (2020), where intermediate disturbances play a critical role in shaping canopy tree structure and in turn driving tree mortality. In addition to shaping tree structure, the physiological characteristics of a tree, such as reduced hydraulic conductivity, can impact tree growth by restricting root system development (Reich et al., 2014; Ryan and Yoder, 1997; Yuan et al., 2019). This restriction can increase susceptibility to windstorm damage and tree uprooting, These characteristics are common in *Picea* trees, which trend to have shallower root systems compared to *Fagus*, especially at higher altitudes and in soils with a higher proportion of rocks which prevent *Picea* trees from developing deep roots for stability (Noreika et al., 2019; Yuan et al., 2019). Additionally, Korolyova et al. (2022) highlighted that other physiological characteristics, such as the reduced plasticity with maturity, can also impact tree structure, particularly with older trees compared to younger trees. This diminished ability to adapt to changing climate conditions makes older trees more vulnerable to partial windstorm damage, potentially exposing them to disease and compromising their defence mechanisms and survival. Therefore, individual tree physiology and local factors like disturbance regimes emerge as crucial determinants of forest structure

5.3.6 | Conclusions of the third subchapter

Our findings on carbon dynamics in the Carpathians reveal that the current state of the primary forests, calibrated using field inventories and local species information such as tree diameter and basal area, remains stable and continues to accumulate live and dead carbon stores (H4). However, the stability of these forest carbon stores may be threatened by future climate change. Uncharacteristic disturbance events, such as prolonged or abrupt droughts or windstorms, which are the primary disturbance agents across the Carpathians, may increase the risk of a novel forest development phase (Falk et al., 2022; Janda et al., 2019; McDowell et al., 2020; Schurman et al., 2019). Such conditions may occur in the spruce forest plots, which exhibited a higher degree of variability in terms of live carbon loss and increased mortality after the second census.

We acknowledge the limitations of this study, particularly in covering both spatial and temporal changes in carbon stock, excluding temperature and climatic water deficit. Uncertainties in data coverage influenced this decision. Despite this omission, we recognise the critical role of climatic conditions, particularly temperature and water availability, in regulating carbon dynamics and tree age distribution across primary forests. Our findings indicate that, over a short period, the primary forests have remained stable with sustained growth across most stands. Notably, an exception exists in the Western Slovakian spruce forests, which displayed significant mortality.

While our research provides high spatial resolution of carbon stores across the temperate mountain primary forests, further studies are needed to capture changes over longer periods, thus providing greater temporal resolution into carbon dynamics. The absence of management and human land use can significantly benefit a forest’s resilience to future climate change. Unmanaged forests promote a diversity of traits within the ecosystem, such as a range of ages and structural sizes and a variety of genera. This heterogeneity ensures the long-term stability of its carbon stocks and the health of the forest as a carbon reservoir. Trends in carbon dynamics show the potential for mature primary forests in mitigating climate change.

5.4 | Primary Forest Biomass: A Comprehensive Synthesis

5.4.1 | Primary Forest Biomass Carbon

Based on our findings, the observed plateau in carbon accumulation at approximately 200-225 years in Carpathian primary forests may represent an equivalence of processes of growth and decomposition until the next natural disturbance event at the site (subchapter 5.3). It is crucial to note that natural disturbance events, such as windstorms, bark beetle outbreaks in the spruce forests, and water stress due to warmer drought conditions, may likely disrupt this equilibrium if they disturb the forests at a larger scale (i.e., > 1 ha). Such disturbances can significantly alter carbon dynamics by removing biomass and creating opportunities for new growth, potentially resetting the carbon accumulation process. Until the occurrence of such a disturbance, the forest is expected to maintain its carbon stock within a relatively narrow range with continued accumulation of live and dead biomass.

The presence of one or two large-diameter trees greatly increase a plot's total carbon store since larger trees store more carbon than smaller trees. Larger trees can shelter them from intermediate disturbance. Such processes help maintain an uneven age and size structure that ensures the continual growth and development of the forest. Thus, it supports the primary forests' overall carbon-carrying capacity, which can remain stable due to the presence of larger trees, facilitating heterogeneity in structure and species. Luyssaert et al. (2008) highlight the role of disturbance in creating uneven-aged forests, essential for long-term carbon storage and stability of old-growth forests.

Moreover, Urrutia-Jalabert et al. (2015) investigated old-growth temperate rainforests in Southern Chile and found that differences in disturbance regimes significantly influence carbon accumulation. Forests with a history of fire disturbances tend to have lower carbon storage (114.1 Mg C ha^{-1}) and lack large, old-growth trees. This is likely due to the fires eliminating these long-lived carbon stores (Urrutia-Jalabert et al., 2015). Conversely, forests that escaped major historical fires, as evidenced by pre-fire estimates of large-diameter trees, exhibit a higher proportion of old-growth trees and, consequently, greater carbon storage (447.5 Mg C ha^{-1}). This highlights the importance of large trees in maintaining an uneven age distribution in a forest and accumulating biomass over centuries.

Interestingly, studies by Ondei et al. (2023) on the relationship between tree diversity and biomass across different biogeographic regions revealed that localised variability in tree species and basal area were key drivers of biomass. Whilst Ondei et al. (2023) did not include tree age in their study, our study revealed the importance of tree age, specifically the variability in age ranges within an ecosystem, in mediating tree size distribution. The combination of large-diameter trees (> 60 cm, dbh), uneven age cohorts in a stand and varying rates of mortality, helps to mediate carbon dynamics and a positive increase in biomass stocks across the Carpathians (see subchapter 5.3).

Noreika et al. (2019) explored the relationship between tree species, forest biomass, and biogeographic regions. Their work highlights how local-scale variations in factors, such as stem density, tree size, topography, and latitude, significantly influence forest dynamics and carbon accumulation. While Noreika et al. (2019) focused on a global scale and did not consider tree age, our findings from the Carpathians support the importance of site-specific factors. Here, topography, tree density, and diversity of genera play vital roles in controlling carbon stocks and the ability of mature trees to actively accumulate biomass (see subchapter 5.2).

5.4.2 | International Agreements and Carbon Accounting

International agreements, such as the Paris Agreement (UNFCCC, 2015) and the Glasgow Climate Pact (UNFCCC, 2021), aim to limit global warming, but concerns regarding carbon accounting methods remain, as addressed in Keith et al. (2024). To achieve climate goals, Europe needs to prioritise protecting existing stocks, increasing forest carbon stocks, and improving carbon account protocols that are fit for the purpose of quantifying the carbon-carrying capacity of primary forests and the potential for natural regeneration of secondary forests (Keith et al., 2024). Protecting existing primary forests is vital for maintaining carbon storage and the safeguard biodiversity that ensures the integrity and stability of ecosystems. While large-scale afforestation is also important, a tailored approach prioritising native species and biodiversity is essential and must take into consideration local conditions to facilitate the restoration of native species and support a heterogenous forest. Therefore, a balanced approach that replicates the composition and structure of native ecosystems, such as primary forests, combined with strict protection, would enhance biodiversity and carbon storage capacity (Keith et al., 2024; Mackey et al., 2015).

The strict protection and restoration of primary forests, alongside responsible management of other forests, offer rapid mitigation benefits and contribute to biodiversity conservation (Keith et al., 2024; Luyssaert et al., 2008; Mackey et al., 2021, 2015; Sabatini et al., 2020). This aligns with the European Green Deal and focuses on protecting and restoring these vital ecosystems by supporting the long-term security of the naturally regenerative forests and their biodiversity. Therefore, supporting an uneven-aged, multi-tiered forest structure with a mixture of tree species that occupy different strata will benefit the long-term stability and security of the primary forests.

5.4.3 | Safeguarding Primary Forests: Future Management and Challenges

In recognition of the future threats to the biosphere, there are increasing uncertainties as to how terrestrial ecosystems – particularly the longevity of primary forests, will respond and adapt to future climate changes. Effective management protocols necessitate the continued existence of primary forests to ensure their protection into the future. This requires comprehensive information on the state of these forests to better understand how the ecosystem may evolve. This thesis demonstrates the importance of forest inventories in assessing the state of the primary forests.

Mina et al. (2017) investigated the impact of various management strategies on projected carbon stocks between 2080 and 2100. Their findings suggest that unmanaged (natural) forests will experience the greatest increase in carbon storage, followed by alternative management strategies. In contrast, business-as-usual scenarios, where forest protection is limited, and land-use change persists, resulted in the lowest projected carbon stocks. Notably, the scenario with increased unmanaged forests consistently showed higher carbon stores and offered additional benefits such as greater biodiversity, improved water-holding capacity to prevent drought-induced dieback, enhanced erosion control, and protection against rockfall and avalanches in specific forest areas (Keith et al., 2017; Mina et al., 2017).

Predicted warmer temperatures until 2150 in the Dinaric Mountains indicated a marginal increase in projected carbon stocks along an altitudinal gradient, comparable to the Carpathian primary forests (Mina et al., 2017). While Mina et al. (2017) found that warmer temperatures would briefly benefit forests at different altitudes, they do not stipulate the range in temperature increase but rather the probability that such locations may warm under different climate scenarios. Such projected changes in carbon stores may enhance the capacity for carbon storage, but only if there is improved resource availability in hydrologic activity and soil nutrients, resulting in minimal water stress due to drought conditions (Yuan et al., 2021). Therefore, if carbon uptake is going to increase, then there has to be a comparable increase in other limiting resources, mainly water and nutrients.

If future climates continue to warm, this may lead to significant shifts in forest development and natural disturbance regimes (McDowell et al., 2020; Meigs and Keeton, 2018). Native vegetation, such as *Picea*, may be outcompeted by genera that typically thrive at lower altitudes, such as *Fagus* and *Acer*. However, post-disturbance successional development could introduce a forest composition that is less diverse in structure, age, and species, with a lower proportion of large-diameter trees (McDowell et al., 2020; Meigs and Keeton, 2018).

Our findings reveal that primary forests, which are prominently old-growth forests, are a stable carbon reservoir. This underlines the need to safeguard all primary forests, which hold a dynamic range of carbon stocks, biodiversity and age structure which is characterised by their centuries of development (Ralhan et al., 2023). Sabatini et al. (2020) found that while many European primary forests are within protected areas, non-primary forests encompass a larger proportion of land cover. If these non-primary forests are given similar or the same protection status as primary forests, this could potentially restore degraded forests within these same boundaries.

In the context of European forests, safeguarding primary forests, which only represent 0.7% of forested areas (Sabatini et al., 2019) necessitates substantially expanding protected areas (1,132 km^2) (Sabatini et al., 2020). Studies by Sabatini et al (2019, 2020) underline not only the importance of primary forests, but their vulnerability to human activity such as land use. To guarantee the long-term survival of these primary forests into the future, the ecological conditions need to be protected, such as diversity of age cohorts, structure, and species, and these conditions need to be replicated in non-primary forests to support their restoration (Sabatini et al., 2020). This further addresses the urgent need for integrated policy reforms, as seen by the ambitious directive of the European Green Deal (European Council, 2023, 2019), that acknowledge primary forests' irreplaceable value and prioritise enhanced protection and restoration efforts (Keith et al., 2009; Sabatini et al., 2020).

Chapter 6 | Conclusions

Primary forests serve as invaluable carbon stocks and sinks and thus helping mitigate climate change. These forests, characterised by their centuries-old trees, maintain diverse traits in tree size, age structure, and species diversity, contributing to their ability to accumulate biomass and store carbon effectively. These centuries-old forests contain a long legacy of growth histories, preserved through the presence of old-growth trees, and they continue to accumulate biomass. Therefore, maintaining the presence of primary forests is critical to ensuring the long-term security of the ecosystem. Primary forests are spatially fragmented and rare, particularly in temperate Europe. Inadequate security and protocols for quantifying forest ecology and health may expose these native forests to future climate change. This could compromise the overall carbon-carrying capacity of primary forests, potentially shifting them from carbon sinks to carbon sources. Effective forest monitoring is needed to quantify and understand that changes occurring in the dynamics of their carbon stores and biodiversity, and thus contribute to mitigating climate change through active conservation and protection.

This thesis emphasises that primary forests reach their maximum biomass over long time frames, with tree age being the most significant driver of biomass accumulation (subchapter 5.1). Despite the modest reduction in biomass in older plots, primary forests continue to serve as stable carbon stores. The interaction between tree age and natural disturbances supports a complex forest composition, enhancing the ecosystem’s stability and carbon storage capacity (subchapter 5.3).

However, the functional integrity of these forests is threatened by ongoing environmental changes, such as severe droughts, warming temperatures, and increased frequency of windstorms, fires, and bark beetle outbreaks. The absence of human management and land use in primary forests promotes resilience to climate change by fostering a diversity of traits within the ecosystem (subchapter 5.2). Supporting a heterogeneous forest ensures the long-term stability of carbon stocks and the health of the forest as a carbon reservoir. To ensure the future security of these ecosystems, it is crucial to protect primary forests from land clearing, harvesting and management impacts. Effective forest monitoring and conservation efforts are needed to maintain their role as carbon sinks and mitigate climate change. Given their spatial fragmentation and rarity, particularly in temperate Europe, safeguarding these forests is paramount to prevent them from shifting from carbon sinks to carbon sources.

This thesis provides new knowledge and information on the state of ecology in temperate montane primary forests, focusing on a detailed account of their carbon stocks and their variability over space and time. While temperate forests are well-researched in terms of their dynamics, more information must be provided covering a large geographic area for temperate European primary forests. This thesis is one of the few studies utilising a large network of permanent sample plots across Central and Eastern Europe, combining forest inventories, dendrochronological, and gridded climate data to assess the relationship between biomass and biotic and abiotic factors across the Carpathians.

Moreover, the research conducted in this thesis addresses the importance of primary forest biomass and demonstrates that mature, old-growth forests are capable of accumulating biomass for approximately 200 years and maintaining their stores beyond 225 years with minimal decline at a fine scale. While highlighting the importance of primary forest as a stable, long-term carbon reservoir, it is equally crucial to address how susceptible these native forests are to future climate change and human activities such as land use. Despite the forest plots being in remote locations, far from human settlements, it is important to protect these ecosystems to avoid any losses due to poor or inadequate protection, which poses a high risk to the forests in the face of climate change.

Chapter 7 | References

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Supplement | S1

S1.1 | Forest Inventories

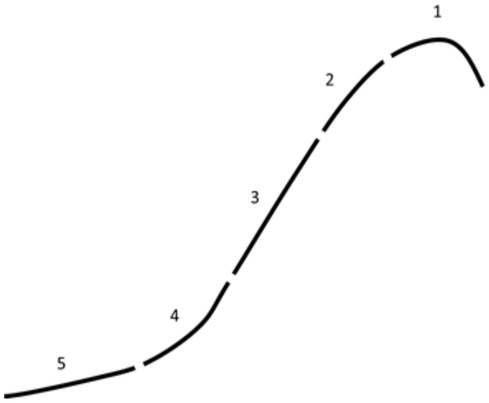


Figure S1.1: Landform features the slope position of a plot within the scale of the stand and identified during forest inventories and categorised by 1 – top of the slope; 2 – the peak of the slope; 3 – middle position of the slope between the valley and top; 4 – lower position of the slope close to the base; and 5 – valley or a gradual slope

S1.2 | Forest Biomass

Biomass: Part I - Branch mass

Species	Equation
Abies alba, Picea abies	exp(-3.3163 + 2.1983 x log(dbh cm)) x 1.00803763592252
Acer, Acer platanoides, Acer pseudoplatanus, Betula, Betula pendula, Broadleaves, Carpinus betulus, Corylus, Corylus avellana, Fraxinus, Fraxinus excelsior, Salix, Salix caprea, Sambucus, Sambucus nigra, Sambucus racemosa, Sorbus, Sorbus aucuparia, Tilia, Tilia cordata, Ulmus, Ulmus glabra	exp(-3.7241 + 2.4069 x log(dbh cm)) x 1.38607162595035
Taxus baccata	exp(-3.248 + 2.3695 x log(dbh cm) + (-0.0254 x basal area)) x 1.00258646540519
Fagus sylvatica	exp(-3.7694 + 2.8003 x log(dbh cm) + (-0.0247 x basal area)) x 1.46653457042711
Larix decidua	exp(-3.2409 + 2.1412 x log(dbh cm)) x 0.967330408815134
Pinus cembra, Pinus sylvestris	exp(-3.6641 + 2.1601 x log(dbh cm)) x 1.04508984648314

Table S1.1: Species-specific allometric equations for calculating branch mass devised from Forrester et al. (2017).

Biomass: Part II – Foliage mass

Species	Equation
Abies alba, Picea abies	exp(-2.1305 + 2.0087 x log(dbh cm) + (-0.0324 x basal area)) x 1.04450283517212
Acer, Acer platanoides, Betula, Betula pendula, Broadleaves, Carpinus betulus, Corylus, Corylus avellana, Fraxinus, Fraxinus excelsior, Salix, Salix caprea, Sambucus, Sambucus nigra, Sambucus racemosa, Sorbus, Sorbus aucuparia, Tilia, Tilia cordata, Ulmus, Ulmus glabra.	exp(-4.2286 + 1.8625 x log(dbh cm)) x 1.0636530778921
Acer pseudoplatanus	exp(-4.0625 + 2.0662 x log(dbh cm)) x 1.00318132717147
Taxus baccata	exp(-2.6019 + 2.1097 x log(dbh cm) + (-0.0404 x basal area)) x 1.01325784347909
Fagus sylvatica	exp(-4.4813 + 1.9073 x log(dbh cm)) x 1.08751755461533
Larix decidua	exp(-3.8849 + 1.7502 x log(dbh cm)) x 0.956852217638524
Pinus cembra, Pinus sylvestris	exp(-2.4122 + 1.8683 x log(dbh cm) + (-0.0537 x basal area)) x 1.03396760040159

Table S1.2: Species-specific allometric equations for calculating foliage mass devised from Forrester et al. (2017).

Biomass: Part III – Stem mass

Species	Equation
Abies alba	exp(-3.2683 + 2.5768 x log(dbh cm)) x 0.987286775425715
Acer, Acer platanoides, Acer pseudoplatanus, Betula, Betula pendula, Broadleaves, Carpinus betulus, Corylus avellana, Fraxinus, Fraxinus excelsior, Salix, Salix caprea, Sambucus, Sambucus nigra, Sambucus racemosa, Sorbus, Sorbus aucuparia, Tilia, Tilia cordata, Ulmus, Ulmus glabra	exp(-2.4521 + 2.4115 x log(dbh cm)) x 0.936149672763302
Taxus baccata	exp(-2.7693 + 2.3761 x log(dbh cm) + (0.0072 x basal area)) x 1.03850427882888
Fagus sylvatica	exp(-1.4487 + 2.1661 x log(dbh cm)) x 0.997918893742347
Larix decidua	exp(-2.4105 + 2.424 x log(dbh cm)) x 1.01854983592874

Picea abies	exp(-2.5027 + 2.3404 x log(dbh cm)) x 1.05988395278679
Pinus cembra, Pinus sylvestris	exp(-2.3583 + 2.308 x log(dbh cm)) x 1.03342764129309

Table S1.3: Species-specific allometric equations for calculating stem mass devised from Forrester et al. (2017).

S1.3 | Generalised Additive Mixed-Effects Models [GAMMs]

The generalised additive mixed model (GAMM) is built in two stages. The first stage involves testing (Q1) each grouped parameter with biomass type (total, live, and dead) and forest composition. The second stage selects the most significant variable from each grouped parameter to identify the trends between (Q2) biotic and abiotic factors and biomass type.

Generalised additive mixed-effect model: Part I – model selection:

Group	*Variable*	*Equation*
-	-	$(0)\ log(biomass_n) \sim NULL + (Paired\ plot_{re}) + (stand_{re}) + s(lng, lat_{ts})$
Age	Plot level tree age	$(1)\ log(biomass_n) \sim composition + s(age) + (Paired\ plot_{re}) + (stand_{re}) + s(lng, lat_{ts})$
	Plot level tree age variance	$(2)\ log(biomass_n) \sim composition + s(age\ sd) + (Paired\ plot_{re}) + (stand_{re}) + s(lng, lat_{ts})$
Disturbance severity	Plot level disturbance	$(3)\ log(biomass_n) \sim composition + s(dist) + (Paired\ plot_{re}) + (stand_{re}) + s(lng, lat_{ts})$
Topography	Plot level altitude	$(4)\ log(biomass_n) \sim composition + s(alt) + (Paired\ plot_{re}) + (stand_{re}) + s(lng, lat_{ts})$
	Plot level landform	$(5)\ log(biomass_n) \sim composition + s(landform) + (Paired\ plot_{re}) + (stand_{re}) + s(lng, lat_{ts})$
Climate	Plot level mean temperature	$(6)\ log(biomass_n) \sim composition + s(temp) + (Paired\ plot_{re}) + (stand_{re}) + s(lng, lat_{ts})$
	Plot level climatic water deficit	$(7)\ log(biomass_n) \sim composition + s(CWD) + (Paired\ plot_{re}) + (stand_{re}) + s(lng, lat_{ts})$

Table S1.4: Generalised additive mixed-effect models expressed as a series of equations for testing the variability in biotic and abiotic factors with biomass type (total, live, age). Age = mean plot-level tree age (years); Age sd = variance in plot-level tree age (years) represented by the standard deviation; dist = mean plot-level disturbance severity (removed canopy area [CA%]); alt = mean plot-level altitude (m); landform = plot-level slope position; temp = mean plot-level temperature (°C); CWD = mean plot-level climatic water deficit (mm).

Where log(biomass) is the natural log-transformed biomass (total, live and dead) for each respective model series, s is the thin-plate regression spline applied to each variable with standre and paired plotre set as the random effects.

Generalised additive model: Part II – Candidate model selection:

Total model	***Live model***	***Dead model***
M0: -	*M0: -*	*M0: -*
M1: age	*M1: age*	*M1: age sd*
M2: age + temp	*M2: age + dist*	*M2: age sd + CWD*
M3: age + temp + alt	*M3: age + dist + alt*	*M3: age sd + CWD + land*
M4: age + temp + alt+ dist	*M4: age + dist + alt + temp*	*M4: age sd + CWD + land + dist*

Table S1.5: Structure of the candidate model selection approach for testing the significance or non-significance of the explanatory variables with total, live and dead biomass for each respective model series. Intercept (-); Age = mean plot-level tree age (years); Age sd = variance in plot-level tree age (years) represented by the standard deviation; dist = mean plot-level disturbance severity (removed canopy area [CA%]); alt = mean plot-level altitude (m); land = plot-level slope position; temp = mean plot-level temperature (°C); CWD = mean plot-level climatic water deficit (mm). Based on the ΔAIC, the models follow a hierarchical structure by testing the 1st, 2nd,…, nth most significant variables.

		Group								
Model		**Intercept**	**Age**		**Disturbance severity**	**Topography**		**Climate**		
			Age	*Age sd*	*Dist*	*Alt*	*Landform*	*Temp*	*CWB*	*CWD*
Total model	*AIC*	109.75	-829.5	-693.06	-699.82	-693.77	-687.32	-721.21	-697.03	-703.71
	R^2 (C)	0.4	0.87	0.84	0.84	0.84	0.84	0.85	0.84	0.84
	R^2 (M)		0.5	0.45	0.45	0.45	0.45	0.45	0.45	0.45
	ΔAIC	939.25	0	136.44	129.68	135.72	142.18	108.29	132.46	125.79
	AIC rank	9	1	7	4	6	8	2	5	3
Live model	*AIC*	573.89	-145.51	67.67	41.37	58.94	74.74	58.75	84.22	67.02
	R^2 (C)	0.31	0.8	0.74	0.75	0.74	0.74	0.74	0.74	0.74
	R^2 (M)		0.4	0.32	0.32	0.32	0.32	0.32	0.39	0.32
	ΔAIC	719.4	0	213.18	186.88	204.45	220.25	204.26	229.73	212.53
	AIC rank	9	1	6	2	4	7	3	8	5
Dead model	*AIC*	1544.15	1499.67	1498.31	1501.86	1503.76	1498.85	1504.04	1504.17	1498.04
	R^2 (C)	0.14	0.33	0.33	0.33	0.33	0.32	0.32	0.32	0.34
	R^2 (M)		0.14	0.15	0.14	0.14	0.15	0.15	0.09	0.34
	ΔAIC	44.77	46.11	1.63	0.27	3.82	5.72	0.81	6	0.14
	AIC rank	9	4	2	5	6	3	7	8	1

Table S1.6: Results from the generalised additive mixed models (GAMMs) with initial model selection approach for total (left); live (middle); and dead (right) biomass for each of the four groups: Age, Disturbance severity, Topography and Climate. For comparing the performance of each model, the Akaike information criterion (AIC); the difference AIC (ΔAIC) for standardising the model for each biomass type (total, live, and dead); R2 (M) = Marginal R2 values accounting for the fixed effects; R2 (C) = Conditional R2 values accounting for both fixed and random effects; Age = plot-level mean tree age (years); Age sd = plot-level tree age (years) represented by the standard deviation; dist = disturbance severity (removed canopy area [CA] %); alt = plot-level altitude (m); landform = plot position along a scale gradient; CWB = climatic water balance (mm); CWD = climatic water deficit (mm); temp = plot-level mean air temperature (°C).

S1.4 | Trends in Forest Biomass

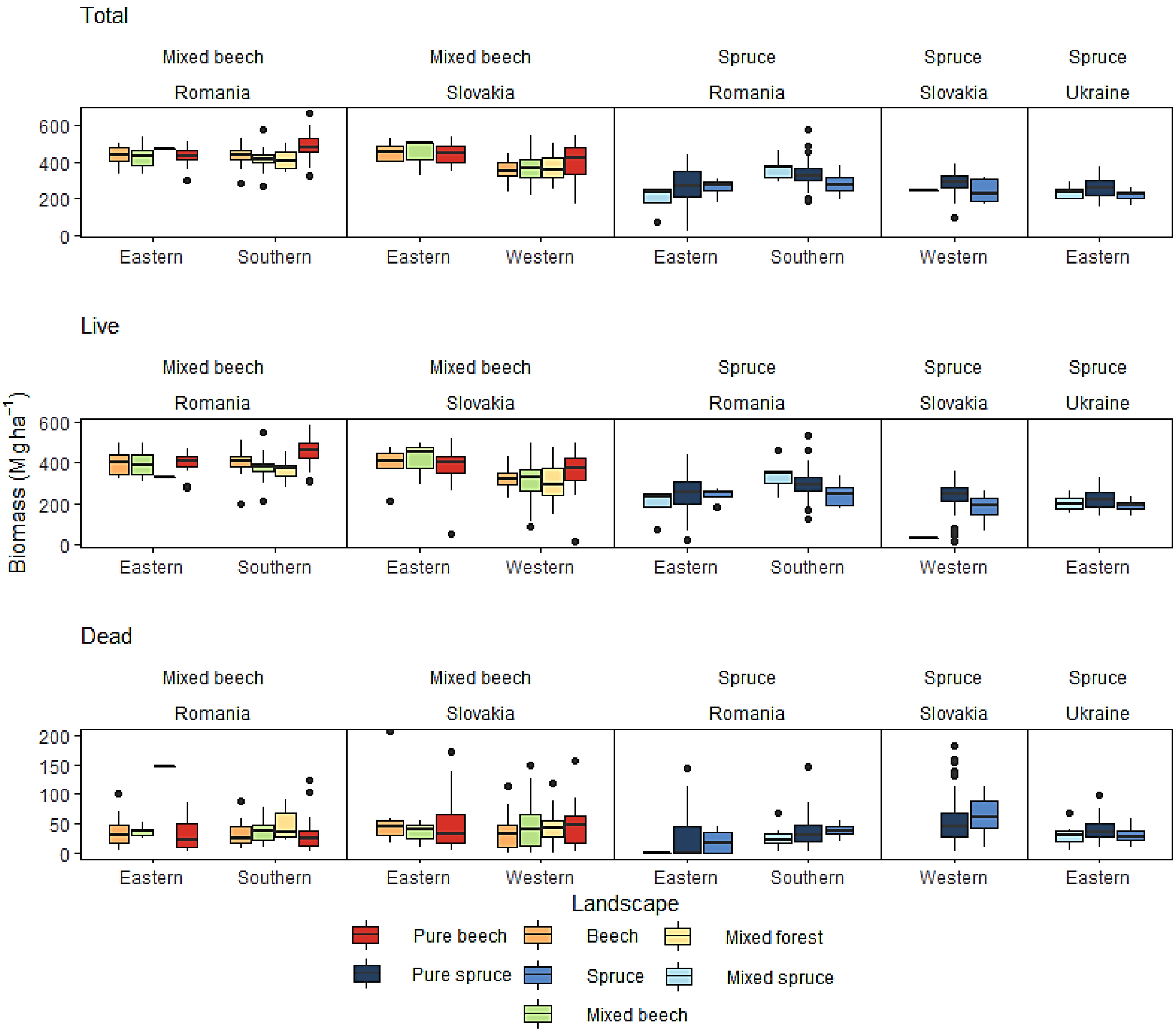


Figure S1.2: Landscape-scale spatial variability in forest biomass categorised by forest species composition (pure beech, beech, mixed beech, fir-beech, pure spruce, spruce, mixed spruce, and mixed) and forest type (mixed beech and spruce). Forest species composition: Pure beech = > 90%; beech = 75 - 90%; mixed beech = 50 - 75%; Fir-beech = fir < 50%, beech < 50%; mixed spruce = 50 - 75%; spruce = 75 - 90%; pure spruce = > 90%; *Mixed forest combines 27 plots from the categories due to low frequency of plots: Mixed forest (100% other [non-Beech, Spruce, Fir], n = 1), Spruce (Spruce: ≥ 75 - ≤ 90%; other: 10 – 25%, n = 1), Mixed spruce (Spruce: ≥ 50 - ≤ 75%; other: 25 – 50%, n = 1) and Fir-beech (Fir: ≤ 50%; Beech: ≤ 50%, n = 22). Total and live biomass plots = 726; dead biomass plots = 644. Box presents the distribution and whiskers indicate the minimum and maximum range in values.

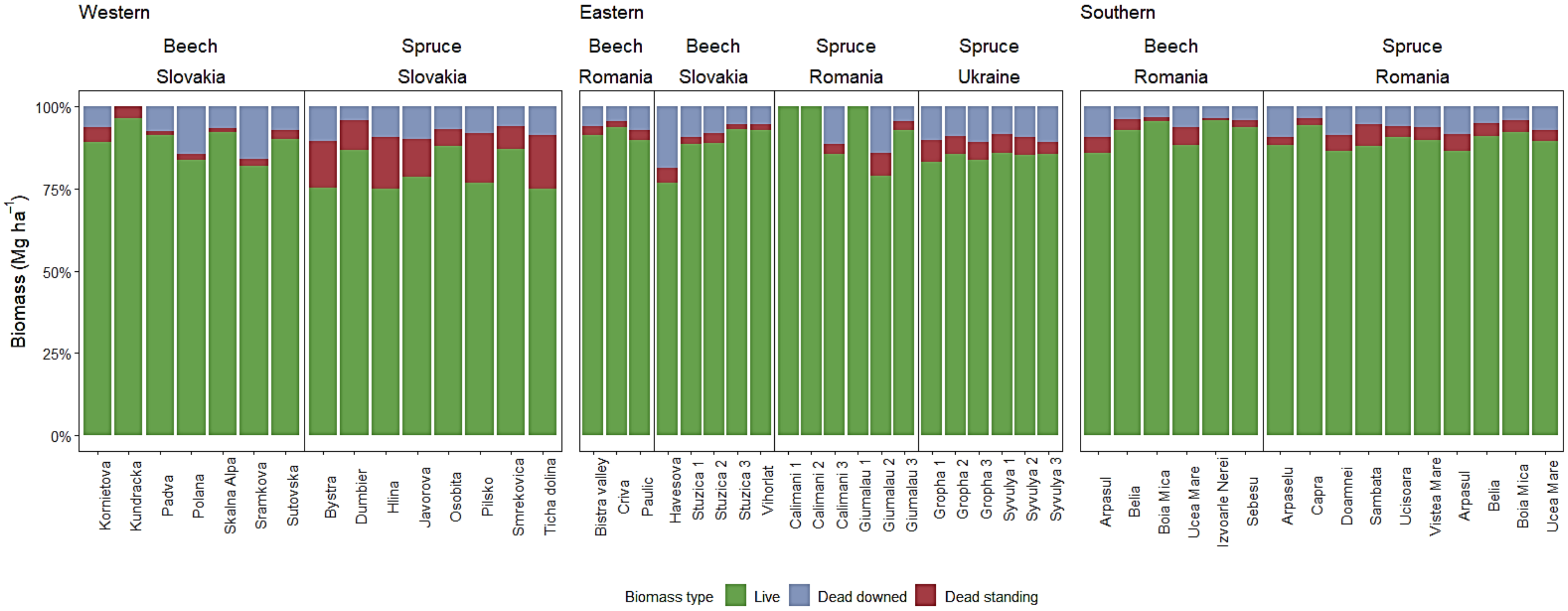


Figure S1.3: Stand-level aboveground live, dead standing and downed biomass (Mg ha^{-1}) across the Carpathians. (Eastern, Southern, Western) and two major forest types (mixed beech and spruce), represented as relative coverage of biomass types (%) per stand: aboveground live (green), dead standing (light blue) and dead downed biomass (red). Spruce forests, Eastern Carpathians = Dead biomass was not measured during forest inventories in Calimani 1, 2, and Giumalau 1 when using the transect line approach for indicating dead downed trees and coarse woody debris and the presence of dead standing trees in these stands at the time measurements was taken.

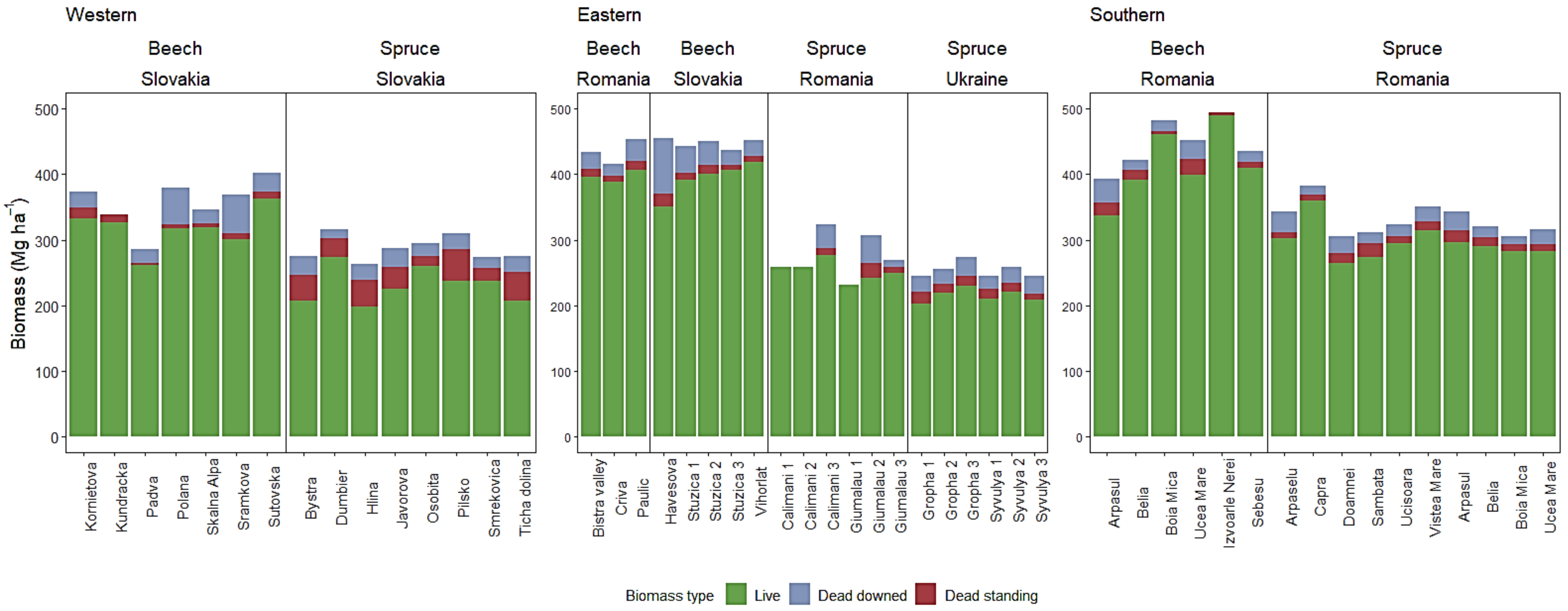


Figure S1.4: Stand-level aboveground live, dead standing and downed biomass (Mg ha^{-1}) across the Carpathians. (Eastern, Southern, Western) and two major forest types (mixed beech and spruce): aboveground live (green), dead standing (light blue) and dead downed biomass (red). Spruce forests, Eastern Carpathians = Dead biomass was not measured during forest inventories in Calimani 1, 2, and Giumalau 1 when using the transect line approach for indicating dead downed trees and coarse woody debris and the presence of dead standing trees in these stands at the time measurements was taken.

S1.5 | Climate data

Landscape	***Forest type***	***Forest species composition***	N plots	***Temp (°C)***	***CWB (mm)***	***CWD (mm)***
Eastern	Mixed beech	Pure beech	72	5.35 ± 1.33	474.21 ± 163.26	27.9 ± 15.98
		Beech	24	4.88 ± 0.99	525.74 ± 116.89	24.26 ± 10.55
		Mixed beech	9	4.81 ± 1.27	518.49 ± 153.23	25.16 ± 10.87
		Mixed forest	1	4.38	594.25	19.11
	Spruce	Pure spruce	197	2.89 ± 0.57	437.23 ± 298.84	23.08 ± 11.71
		Spruce	20	3.07 ± 0.55	702.59 ± 258.2	13.51 ± 10.63
		Mixed spruce	12	2.61 ± 0.62	649.56 ± 285.66	13.35 ± 7.44
Southern	Mixed beech	Pure beech	36	4.53 ± 1.33	245.31 ± 137.2	56.95 ± 16.06
		Beech	14	4.32 ± 1.21	172.49 ± 115.43	53.59 ± 14.67
		Mixed beech	25	3.98 ± 0.55	178.16 ± 59.28	49.2 ± 7.56
		Mixed forest	9	3.97 ± 0.22	164.66 ± 34.98	50.15 ± 5.76
	Spruce	Pure spruce	103	3.45 ± 0.8	225.25 ± 74.06	42.75 ± 7.8
		Spruce	8	3.6 ± 0.57	219.4 ± 72.91	43.35 ± 5.65
		Mixed spruce	6	2.62 ± 0.7	346.68 ± 88.49	33.8 ± 6.95
Western	Mixed beech	Pure beech	15	4.79 ± 0.56	501.4 ± 129.56	26.24 ± 9.77
		Beech	19	4.66 ± 0.53	499.05 ± 115.26	25.64 ± 8.82
		Mixed beech	29	4.7 ± 0.51	507.67 ± 96.39	24.76 ± 6.95
		Mixed forest	17	4.63 ± 0.36	488.43 ± 87.48	25.86 ± 6.86
	Spruce	Pure spruce	99	2.57 ± 0.98	845.14 ± 161.5	7.54 ± 3.91
		Spruce	10	2.74 ± 0.98	779.91 ± 202.67	9.18 ± 5.39
		Mixed spruce	1	1.85	982.64	4.51

Table S1.7: Trends in climate forest species composition group, forest types (Mixed beech, spruce) and landscape (Eastern, Southern, Western) across the Carpathians, using mean (±) standard deviation. Mean stand-level trends in temperature and CWD per forest type across the Carpathian primary forest plots. Mean annual climate data is obtained from calculating mean trends from 1958-2020 at the plot level. Temp = mean annual temperature (°C); CWB = mean annual climatic water balance (mm); CWD = mean annual climatic water deficit (mm)

Supplement | S2

S2.1 Importance Value Index

Forest type	***Genus prevalence***	***IVI range***	***Number of plots***
Mixed beech	Beech – high	>150	152
	Beech – medium	100–150	72
	Beech – low	<100	38
	Spruce*	–	8
Spruce	Spruce – medium	100–150	11
	Spruce – high	>150	445

Table S2.1: *Plot-level genus category* – Plot-level category in assigning the most common genus based on the Important value index (IVI) score (on a scale of 0–200) within each plot. *IVI rank* prevalence of beech and spruce in their respective forest types, ranked by low (<100), moderate (100–150) and high (>150) IVI scores, with *beech – mixed spruce* (plots *n* = 4) being plots in the mixed beech forests characterised as being dominantly spruce in structure and genus coverage.

S2.2 Generalised Additive Mixed-Effects Models [GAMMs]

GAMMs were calibrated using spatial autocorrelation (ts) with latitude and longitude, with geographic coordinates converted into linear covariates using the R-packages geodist and vegan. Thin-plate regression splines were applied to fixed and random effects, and spatial autocorrelation variables, with each predictor containing a smooth function restricted to three knots to reduce artificial biological responses. By calibrating each GAMM with spatial autocorrelation using latitude and longitude converted into linear covariates, we account for spatial dependence in the models by factoring in plot location. This allows the model to capture how the biological response variable changes geographically, providing more accurate results that consider the spatial context of the data.

Prior to building the main models, we tested each variable with tree indices (live and dead) independently, to investigate their performance, before creating the models using the model selection approach. Exploratory testing was conducted to identify how each variable per group response to mean plot-level biomass (Mg ha^{-1}) across the Carpathians. Preliminary model testing with no assumptions and interactions to identify the performance of each predictor with biomass, per live and dead standing trees. We calculated a filtered basal area and basal area index by excluding large-diameter trees within each plot ≥ 60 cm dbh to disseminate the effects small-sized trees may have on the structural diversity in a given community. Plot-level tree density is calculated to quantify the number of trees per hectare. Moreover, we tested the relationship smaller (≤ 60 cm) and large (≥ 60 cm) diameter trees and their respective variance using the standard deviation. However, we opted to use the full dataset since we found no important relationship during preliminary testing (see supplement S1 for method and S4 for statistical analysis).

In building the GAMMs, we tested the variables in Table S4.1 to identify the ideal biotic and abiotic factors to assess how they interact with forest biomass, structure (basal area index), and genus-level diversity (abundance). We used the basal area index as the main interaction variable based on the assumption that tree size strongly influences the distribution of total biomass and contributes to genus-level diversity. Since basal area at the plot level is highly correlated with forest biomass, we opted to use the coefficient of variance of basal area at the plot level as an index of tree size variability, providing insight into the variation of tree size range in different site conditions (i.e., genus-level tree diversity, abundance).

Diameter at breast height (dbh) is measured for all live and dead standing individuals across each of the 726 plots, providing a deeper understanding of tree size variability at the fine scale. Furthermore, using genus abundance as the main interactive variable to represent genus-level diversity provides insight into how genus distribution and the number of individuals belonging to each genus are correlated with biomass and structure (basal area index).

Prior to developing the main models, we first conducted a series of tests to identity the performance of each variable independently. Structural variables: basal area index - coefficient of variance of plot-level basal area which includes the full range of trees, with the latter variables representing the coefficient of variance based on trees < 60 and > 60 cm, dbh, respectively; Tree density = plot-level tree density (ha^{-1}); Tree size index – the proportion of basal area per dbh class using the Shannon index. Genus variables: Genus abundance: the total sum of genus count present in each plot; Tree diversity - spatial variation in genus based on the Shannon Index; genus abundance - plot-level genus count. Combined - ΔShannon – a pairwise comparison of tree size index (structure) and tree diversity (genus). Topography: Altitude – mean plot-level altitude (m); landform – plot steepness and landform, which categorises the position of each plot relative to the stand it is nested. Climate: Temperature – mean plot-level temperature (°C); CWD – mean plot-level climate water deficit (mm). Age variables: Age – mean plot-level tree age; Age variance – represent the coefficient of variance in mean plot-level tree age. Disturbance variables – Disturbance severity – mean plot-level disturbance severity represented as a proxy of disturbed canopy area (%CA); Combined disturbance variables; age × dist – mean plot-level tree age and disturbance severity.

Category	***Variable***	***Description***	***Supporting Literature***
Structure	Basal Area Index - Coefficient of variance of plot-level basal area including full range of trees.	Indicates tree size variability, which influences biomass distribution and genus-level diversity.	Since basal area was highly correlated with biomass, we used the basal area index to measure structural variance within each plot (Aponte et al., 2019).
	Basal Area Index (< 60 cm) - Coefficient of variance based on trees < 60 cm dbh.	Highlights variability in smaller tree sizes.	Uneven tree size distribution has a significant influence on forest biodiversity and biomass stocks (Ehbrecht et al, 2021, Wang and Ali, 2022).
	Basal Area Index (> 60 cm) - Coefficient of variance based on trees > 60 cm dbh.	Highlights variability in larger tree sizes.	
	Tree Density - Plot-level tree density (·ha^{-1}).	Reflects stand density, which can influence competition and resource availability.	
	Tree Size Index - Proportion of basal area per dbh class using the Shannon index.	Provides a measure of structural complexity and diversity within a plot.	Structural complexity in tree size classes influences forest processes (Staudhammer and LeMay, 2001).
Genus-level diversity	Genus Abundance - Total sum of genus count present in each plot.	Represents overall genus richness, important for understanding biodiversity.	Spatial variability in species/genus diversity can have a strong impact in controlling biomass accumulation (Wang and Ali, 2022).
	Tree Diversity - Spatial variation in genus based on the Shannon index.	Measures genus diversity, which can affect ecosystem resilience and productivity.	
	Genus Abundance (Plot-level) - Plot-level genus count.	Reflects local genus richness, useful for fine-scale biodiversity assessments.	
Combined	ΔShannon - Pairwise comparison of tree size index (structure) and tree diversity (genus).	Integrates structural and genus diversity to assess overall forest heterogeneity.	Quantifying the difference between biodiversity indices to see how the degree of similarity or dissimilarity influence biomass stocks.
Topography	Altitude - Mean plot-level altitude (m).	Influences climate and species composition, affecting forest structure and biomass.	Altitude affects temperature and precipitation, influencing biomass (Wang and Ali, 2022).
	Landform - Plot steepness and landform, categorising the position of each plot, nested within a stand.	Determines microhabitat conditions, influencing tree growth and forest dynamics.	Plot position along a slope profile can influence the accumulation of biomass.

Climate	Temperature - Mean plot-level temperature (°C).	Affects metabolic rates and growth patterns, impacting biomass accumulation.	Climatic conditions control the spatial distribution of forest biomass, its structural complexity and diversity levels (Ehbrecht et al., 2021).
	CWD - Mean plot-level climate water deficit (mm).	Indicates water availability, crucial for understanding drought stress and its effects on forest biomass.	
Dendro	Age - Mean plot-level tree age.	Provides insight into forest development stages and their impact on biomass, structure, and genus-level diversity.	The uneven-age structure support a heterogenous forest with higher levels of biomass which are maintained through natural disturbances (Ralhan et al., 2023).
	Age Variance - Coefficient of variance in mean plot-level tree age.	Reflects age heterogeneity, important for understanding the variance in maturity levels and how it influences forest processes.	
	Disturbance Severity - mean plot-level disturbance severity represented as a proxy of disturbed canopy area (% CA).	Measures the impact of disturbances, influencing forest structure and a plot's overall carbon carrying capacity.	
	Age × dis - Combined dendrochronological variables; mean plot-level tree age and disturbance severity.	Examines the interaction between age and disturbance, providing insights into how chronological development influences biomass accumulation and the distribution of live and dead standing trees.	

Table S2.2 Description of site-specific variables used for developing the models: Descriptive summarise of the biotic and abiotic variables tested prior to developing the main generalised additive mixed-effects models (GAMMs). Each variable is categorised into *structural*, *genus-level diversity*, *combined*, *topography*, *climate*, *and dendrochronological (dendro) variables*. The importance of each variable is provided to highlight their relevance in assessing interactions with forest biomass, structure, and genus-level diversity.

Model selection: no interactions		**Live**					**Dead**					**Normalised difference**
Group	*Predictor*	AIC	ΔAIC	R^2 (C)	R^2 (M)	Rank	AIC	ΔAIC	R^2 (C)	R^2 (M)	Rank	
-	*Null model*	109.75	296.71		0.4	17	–197.04	179.41		0.47	17	3.51
Structure	Basal area index	–186.95	0	0.61	0.56	1	–295.21	81.23	0.56	0.49	3	0.22
	Basal area index [< 60 cm, dbh]	–97.53	89.42	0.56	0.49	2	–285.73	90.71	0.55	0.47	10	0.49
	Basal area index [> 60 cm, dbh]	–80.03	106.93	0.55	0.51	3	–285.32	91.12	0.55	0.47	11	0.56
	Tree density	32.04	218.99	0.47	0.4	12	–285.77	90.68	0.55	0.48	9	1.25
	Tree size index [Shannon index]	–6.99	179.96	0.5	0.43	5	–289.93	86.52	0.56	0.48	4	0.95
Genus	Genus abundance [count]	15.11	202.06	0.49	0.42	6	–285.05	91.39	0.55	0.48	12	1.11
	Tree diversity [Shannon index]	15.8	202.75	0.48	0.41	8	–284.72	91.72	0.56	0.47	13	1.12
	ΔShannon [combined: species, structural]	15.46	202.41	0.49	0.4	7	–282.01	94.44	0.55	0.48	16	1.12
Topography	Altitude [m]	31.42	218.37	0.47	0.43	11	–285.99	90.45	0.55	0.51	7	1.25
	Landform	34.7	221.66	0.47	0.4	15	–285.95	90.49	0.55	0.47	8	1.28
Climate	Temperature	36.14	223.09	0.47	0.4	16	–287.92	88.53	0.56	0.47	5	1.29
	CWD	33.35	220.3	0.47	0.41	14	–283.69	92.75	0.55	0.47	14	1.27
Age	Age	–51.08	135.87	0.53	0.47	4	–376.44	0	0.62	0.55	1	0.76
	Age variance [standard deviation]	33.17	220.12	0.47	0.41	13	–283.43	93.01	0.55	0.48	15	1.27
Disturbance	Disturbance severity	30.82	217.78	0.48	0.41	10	–287.64	88.8	0.56	0.48	6	1.24
	age × dist [combined, interactive effects]	17.19	204.14	0.49	0.33	9	–311.55	64.9	0.57	0.49	2	1.12

Table S2.3: Generalised additive mixed-effect models (GAMMs) - Model testing with no interactions: Full list of predictor variables used to test their effect on total biomass (Mg ha^{-1}) per tree type (live and dead standing) across the Carpathians. Structural variables: *basal area index* - coefficient of variance of plot-level basal area which includes the full range of trees, with the latter variables representing the coefficient of variance based on trees < 60 and > 60 cm, dbh, respectively; *Tree density* = plot-level tree density (ha^{-1}); *Tree size index* – the proportion of basal area per dbh class using the Shannon index. Genus variables: *Genus abundance:* the total sum of genus count present in each plot; *Tree diversity* - spatial variation in genus based on the Shannon Index; *genus abundance* - plot-level genus count. Combined - *ΔShannon* – a pairwise comparison of tree size index (structure) and tree diversity (genus). Topography: *Altitude* – mean plot-level altitude (m); *landform* – plot steepness and landform, which categorises the position of each plot relative to the stand it is nested. Climate: *Temperature* – mean plot-level temperature (°C); *CWD* – mean plot-level climate water deficit (mm). Age variables: *Age* – mean plot-level tree age; *Age variance* – represents the coefficient of variance in mean plot-level tree age. Disturbance variables – *Disturbance severity* – mean plot-level disturbance severity represented as a proxy of disturbed canopy area (%CA); Combined disturbance variables; *age × dist* – mean plot-level tree age and disturbance severity. For testing the hierarchical models, we used the Akaike information criterion (AIC), and AIC difference (ΔAIC) represents measures of model comparison, R^2 (M) = marginal R^2 represents explained variance and account for the fixed effects (applicable to the model with no random effects and containing base R^2); R^2 (C) = conditional R^2 values accounting for both fixed and random effects; *D* = explained deviation to account of goodness-of-fit. *Normalised difference* is a symmetrical, pairwise test which measures the degree of similarity or dissimilarity between models.

S2.2.1 | GAMMs: Forest biomass and complexities at different scales (Q1)

To analyse the relationship between forest biomass, genus-level diversity, and structural complexity across three hierarchical spatial scales to quantify spatial variability. The following variables were selected using these three hierarchical spatial scales based on their performance in the model selection: Structural variables: basal area index - coefficient of variance of plot-level basal area; tree size index - spatial variation in basal area per dbh class using the Shannon index. Genus variables: Tree diversity - spatial variation in genus based on the Shannon Index; genus abundance - plot-level genus count. Combined variables: ΔShannon – a pairwise comparison of tree size index (structure) and tree diversity (genus); basal area index + genus abundance – structural and genus-level diversity variables combined into a single model. These structural and genus-level diversity variables were selected due to their performance in the initial testing (see Table S2.4) and model selection (see Results 3.3). The spatial scales include the landscape scale (i.e., macro-scale), which divides the Carpathians into three regions at the coarser scale, based on ordination (western, eastern, southern); stands are nested within each landscape and denote an intermediate scale; plots are nested within each stand and represent individual sites at the fine scale.

		Live					*Dead*					
Predictor	*Spatial scale*	AIC	ΔAIC	R^2 (C)	R^2 (M)	*D*	AIC	ΔAIC	R^2 (C)	R^2 (M)	*D*	*Δtrees*
Null model	-	109.75	417.16	NA	0.4	NA	-197.04	99.76	NA	0.47	NA	3.51
Structure												
Basal area index	Landscape + Stand + Plot	109.75	417.16	0.404	0.564	0	–295.21	1.58	0.561		–295.21	3.51
	Landscape	–183.72	123.69	0.616		0.38	–284.08	12.71	0.538		–284.08	0.23
	Stand	–126.25	181.16	0.565		0.26	–287.9	8.89	0.561		–287.9	0.38
	Plot	–182.11	125.3	0.614		0.38	–281.2	15.6	0.54		–281.2	0.23
Tree size index (Shannon)	Landscape + Stand + Plot	–129.64	177.77	0.574	0.43	0.29	–289.92	6.87	0.557	0.475	–289.92	0.37
	Landscape	–6.99	300.42	0.504		0.18	–280.94	15.86	0.536		–280.94	0.95
	Stand	11.44	318.85	0.474		0.11	–282.61	14.19	0.558		–282.61	1.08
	Plot	5.92	313.33	0.503		0.2	–278.64	18.16	0.538		–278.64	1.04
Genus												
Tree diversity (Shannon)	Landscape + Stand + Plot	19.43	326.83	0.483	0.406	0.14	–285.05	11.74	0.553	0.476	–285.05	1.15
	Landscape	31.1	338.51	0.457		0.08	–276.6	20.19	0.532		–276.6	1.25
	Stand	44.52	351.93	0.477		0.15	–276.38	20.41	0.553		–276.38	1.38
	Plot	44.43	351.84	0.455		0.08	–273.41	23.38	0.534		–273.41	1.39
Genus abundance	Landscape + Stand + Plot	21.68	329.08	0.487	0.42	0.15	–284.71	12.08	0.556	0.471	–284.71	1.16
	Landscape	31.66	339.06	0.459		0.08	–278.15	18.64	0.535		–278.15	1.26
	Stand	29.15	336.56	0.484		0.17	–280.3	16.5	0.556		–280.3	1.23
	Plot	38.17	345.57	0.461		0.1	–276.27	20.52	0.538		–276.27	1.32
Combined												
ΔShannon	Landscape + Stand + Plot	20.52	327.93	0.487	0.404	0.15	–282.01	14.78	0.555	0.481	–282.01	1.16
	Landscape	27	334.41	0.461		0.08	–278.87	17.93	0.537		–278.87	1.21
	Stand	34.98	342.38	0.481		0.16	–272.52	24.27	0.554		–272.52	1.29
	Plot	39.74	347.15	0.46		0.09	–274.39	22.4	0.538		–274.39	1.34
Basal area index + genus abundance	Landscape + Stand + Plot	**-307.41**	**0**	**0.676**	0.63	**0.47**	**-296.79**	**0**	**0.562**	0.489	**-296.79**	–0.02
	Landscape	–242.71	64.7	0.63		0.37	–284.21	12.58	0.54		–284.21	0.08
	Stand	–293.99	13.42	0.67		0.47	–288.69	8.1	0.562		–288.69	–0.01
	Plot	–254.77	52.64	0.642		0.4	–281.29	15.51	0.543		–281.29	0.05

Table S2.4: Diversity and structural indices at different spatial scales (plot, stand, landscape) and forest type (mixed beech, spruce) using (GAMMs). Structural variables: basal area index - coefficient of variance of plot-level basal area; tree size index – the proportion of basal area per dbh class using the Shannon index. Genus variables: Tree diversity - spatial variation in genus based on the Shannon Index; genus abundance - plot-level genus count. Combined variables: ΔShannon – a pairwise comparison of tree size index (structure) and tree diversity (genus); basal area index + genus abundance – structural and genus-level diversity variables combined into a single model. Akaike information criterion (AIC) and AIC difference (ΔAIC) represents measures of model comparison, R^2 (M) = marginal R^2 represents explained variance and account for the fixed effects (applicable to the model with no random effects and containing base R^2); R^2 (C) = conditional R^2 values accounting for both fixed and random effects; D = explained deviation to account of goodness-of-fit. Δtrees is a symmetrical, pairwise test (i.e., the normalised difference) to measure the degree of similarity or dissimilarity between live and dead models.

S2.2.2 | GAMMs: Heatmaps (Q2)

Generalised additive mixed-effects model (GAMM) – heatmaps from the optimum GAMM featuring an interactive effect between plot-level total biomass (Mg ha^{-1}) and structure (basal area index) and species (abundance). Heatmaps show the combined interactive effects of plot-level mean tree age and disturbance severity, per structure and species component. Featuring the spatial variability in biomass per live and dead standing tree indices in the temperate primary forests of the Carpathians.

Narrow spacing between contour lines represents dense distribution in the interaction between variables, with wider spacing representing a sparse distribution. The heatmap colour scheme shows light shading represents lower amounts of total biomass, while darker shading denotes higher amounts of total biomass. Narrow spacing between contour lines represents dense distribution in the interaction between variables, with wider spacing representing a sparse distribution

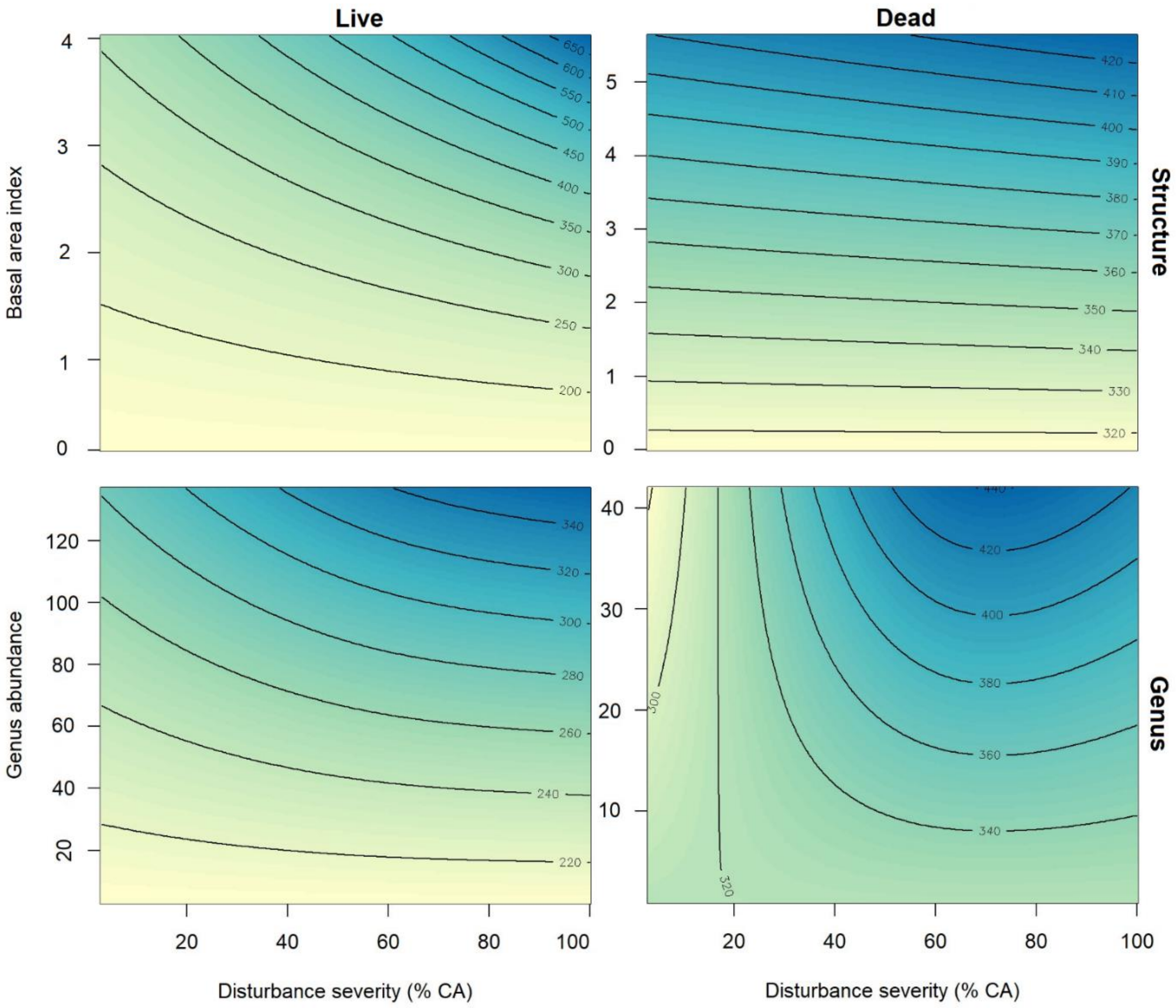


Figure S2.1. *GAMMs – Disturbance: Basal area index* – a measure of forest structural variability of plot-level basal area ($m^2 \cdot ha^{-1}$) standard deviation (σ); *abundance* = plot-level relative species abundance; *Disturbance severity* = mean plot-level disturbance severity represented as a proxy of disturbed canopy area (% CA).

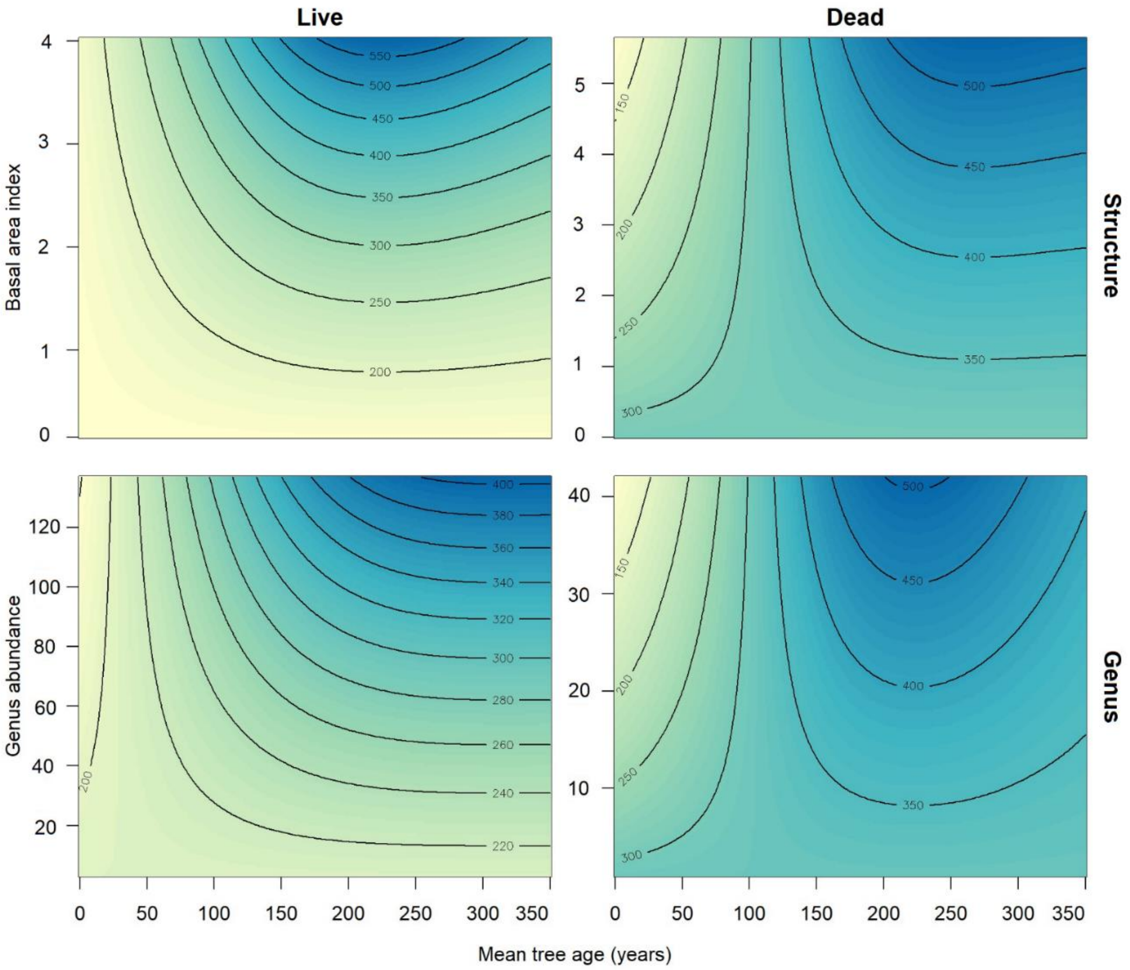


Figure S2.2. *GAMMs – Age: Basal area index* – a measure of forest structural variability of plot-level basal area ($m^2 \cdot ha^{-1}$) standard deviation (σ); *abundance* = plot-level relative species abundance; *Age* = mean plot-level tree age (years).

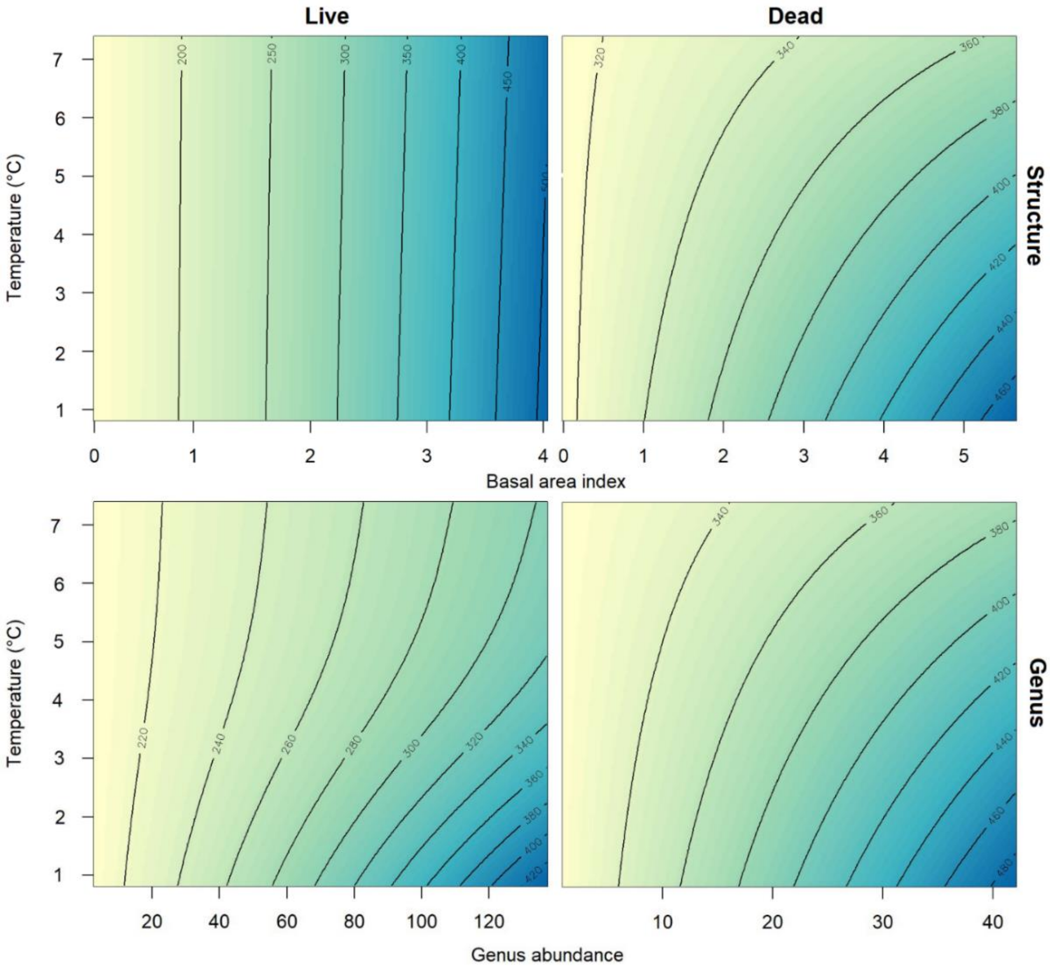


Figure S2.3. *GAMMs – Temperature: Basal area index* – a measure of forest structural variability of plot-level basal area (m²·ha⁻¹) standard deviation (σ); *abundance* = plot-level relative species abundance; *Temperature* = mean plot-level temperature (°C).

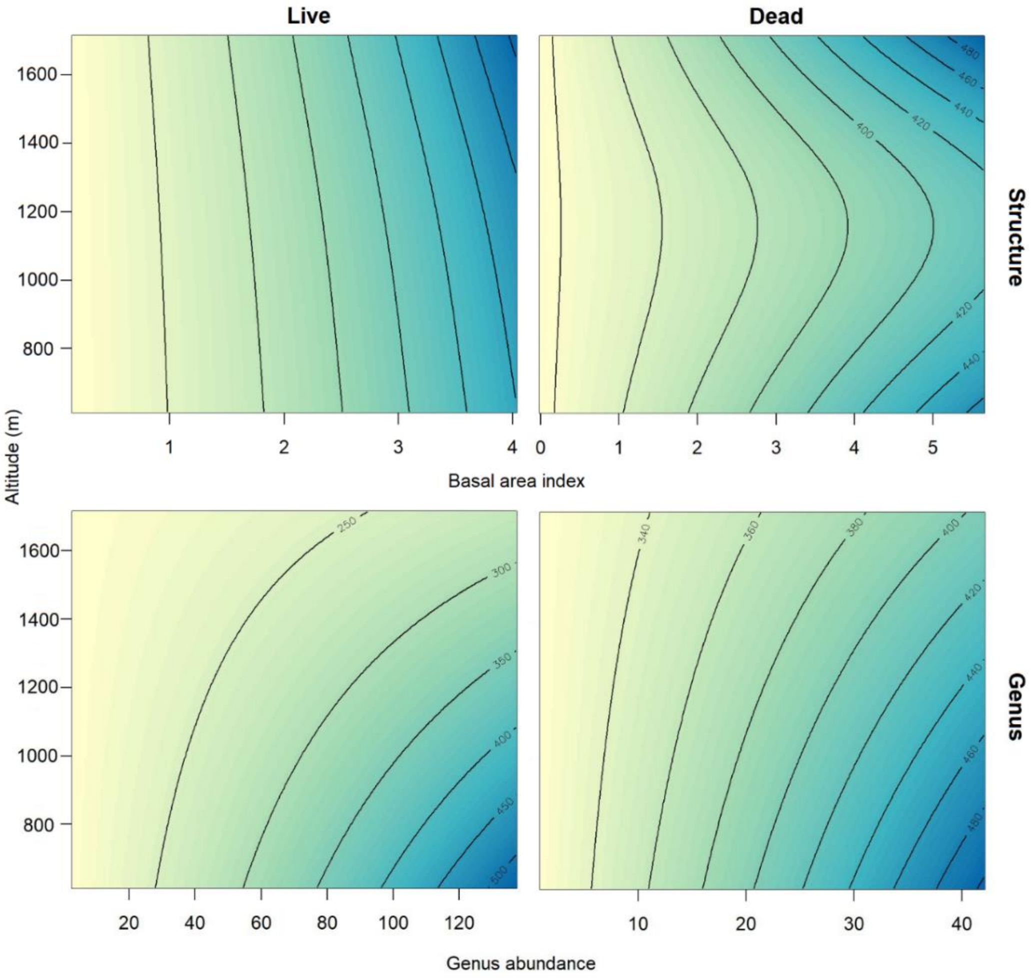


Figure. S2.4 *GAMMs – Altitude: Basal area index* – a measure of forest structural variability of plot-level basal area ($m^2 \cdot ha^{-1}$) standard deviation (σ); *abundance* = plot-level relative species abundance; *Altitude* = mean plot-level altitude (m).

S2.3 Forest Composition

Landscape	Forest type	Country	Plots	Basal area index			Tree density	Genus abundance	Tree size index		Tree genus diversity	
				Full range	< 60, dbh	> 60, dbh			Mean	Range	Mean	Range
Live standing tree												
Eastern	Mixed beech	Romania	40	46.3 ± 9.5	26.8 ± 9.2	19.5 ± 8.3	376.6 ± 96.9	56.5 ± 14.5	0.85 ± 0.08	0.52–0.96	0.59 ± 0.21	0–0.95
		Slovakia	66	41.4 ± 9.4	20.9 ± 9.3	20.6 ± 9.5	372.7 ± 140.1	55.9 ± 21	0.79 ± 0.09	0.5–0.97	0.19 ± 0.2	0–0.73
	Spruce	Romania	133	47.4 ± 15.8	38.4 ± 15.2	9.3 ± 10.8	516.8 ± 237	34 ± 13.8	0.83 ± 0.12	0.39–0.98	0.05 ± 0.13	0–0.69
		Ukraine	96	41.6 ± 8.4	36.3 ± 9.5	5.4 ± 6.3	569.6 ± 250	51.3 ± 17.4	0.82 ± 0.11	0.41–0.98	0.17 ± 0.2	0–0.79
Southern	Mixed beech	Romania	84	49.5 ± 9.3	25.4 ± 10	24.3 ± 10.8	390.4 ± 135.9	42.9 ± 13.9	0.84 ± 0.08	0.55–0.96	0.56 ± 0.35	0–1
	Spruce		117	56.2 ± 11.9	45.6 ± 12.8	10.7 ± 9.2	566.9 ± 222.6	53.1 ± 18.9	0.87 ± 0.06	0.67–0.98	0.16 ± 0.21	0–0.98
Western	Mixed beech	Slovakia	80	39.2 ± 12	22.1 ± 9	17.2 ± 11.6	354.6 ± 147	42.9 ± 16.7	0.82 ± 0.13	0.32–0.98	0.61 ± 0.25	0–0.99
	Spruce		110	42.3 ± 13.4	33.4 ± 12.7	8.9 ± 9.1	377.3 ± 181	36.8 ± 16	0.86 ± 0.08	0.48–0.99	0.23 ± 0.27	0–0.95
Dead standing tree												
Eastern	Mixed beech	Romania	38	5.8 ± 4	2 ± 1.8	3.9 ± 3.5	36.6 ± 19.7	5.3 ± 2.9	0.77 ± 0.37	0–1	0.61 ± 0.42	0–1
		Slovakia	63	6.5 ± 5.3	1.8 ± 1.3	4.7 ± 5.1	39.9 ± 22.7	5.7 ± 3.4	0.8 ± 0.31	0–1	0.28 ± 0.38	0–1
	Spruce	Romania	48	7.7 ± 5	6.1 ± 4.2	1.7 ± 3.1	120.4 ± 111.9	8 ± 6.7	0.8 ± 0.29	0–1	0.03 ± 0.11	0–0.54
		Ukraine	92	4.8 ± 3.3	4.2 ± 2.9	0.6 ± 1.5	100.1 ± 56.1	5.4 ± 3.7	0.77 ± 0.35	0–1	0.06 ± 0.22	0–0.97
Southern	Mixed beech	Romania	77	6.1 ± 4.5	2.5 ± 2.2	3.5 ± 4.2	57.7 ± 46.2	5.9 ± 4.7	0.75 ± 0.36	0–1	0.46 ± 0.43	0–1
	Spruce		110	5 ± 3.8	4.2 ± 3.4	0.8 ± 1.8	149.4 ± 104.3	8.7 ± 7	0.71 ± 0.33	0–1	0.1 ± 0.24	0–1
Western	Mixed beech	Slovakia	71	6.3 ± 4.9	3 ± 3	3.3 ± 4	47.9 ± 35.9	4.6 ± 3.5	0.73 ± 0.4	0–1	0.64 ± 0.41	0–1
	Spruce		108	11.7 ± 9.8	8.4 ± 7.6	3.4 ± 4.6	132 ± 93	10.6 ± 7.8	0.83 ± 0.25	0–1	0.08 ± 0.22	0–0.92

Table S2.5 Landscape-scale characteristics of forest composition, comprising structural and species diversity indices for live and dead standing trees at the macro-scale, each landscape and forest type. Number of plots – live tree = 726; dead tree = 607. *Structural indices* – basal area index: including mean plot-level values with no filtering (full range), mean plot-level values of smaller trees < 60 dbh (cm) and larger trees > 60 dbh (cm); tree size index denotes the variability in basal area per dbh classes (0 – 100, ≥ 100 cm) using the Shannon Index approach; basal area ($m^2 \cdot ha^{-1}$) values at the landscape scale and tree density (n ha^{-1}). *Genus indices* – tree genus diversity is based on the Shannon index for measuring genus-level diversity; genus abundance is the total sum of genus count present in each plot. Values include mean, with standard deviation denoted by ±, with minimum and maximum range (-) for tree size index and tree diversity values.

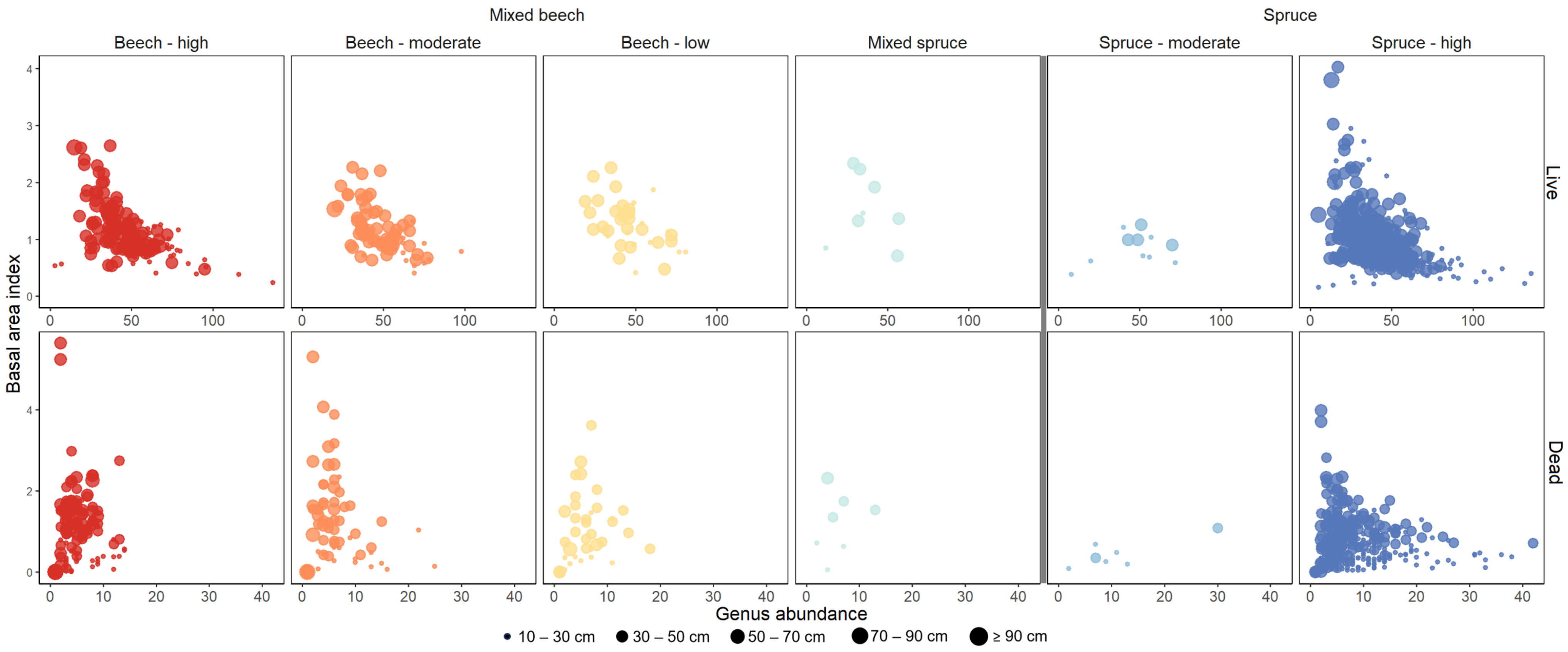


Figure S2.5: Variability in forest structure and genus, defined by the distribution of genus-level abundance (species) and basal area index (structure), by the most common genera contained within each forest type (Mixed beech, spruce) across the Carpathian primary forests. IVI score – prevalence of beech and spruce in their respective forest types, ranked by low (<100), moderate (100–150) and high (>150) IVI scores, with beech – mixed spruce (n plots =4) being plots in the mixed beech forests characterised as being dominantly spruce in structure and genus coverage. Plot-level species and structural indices are shown for live (top panel; n = 726) and dead (bottom panel; n = 607) standing trees.

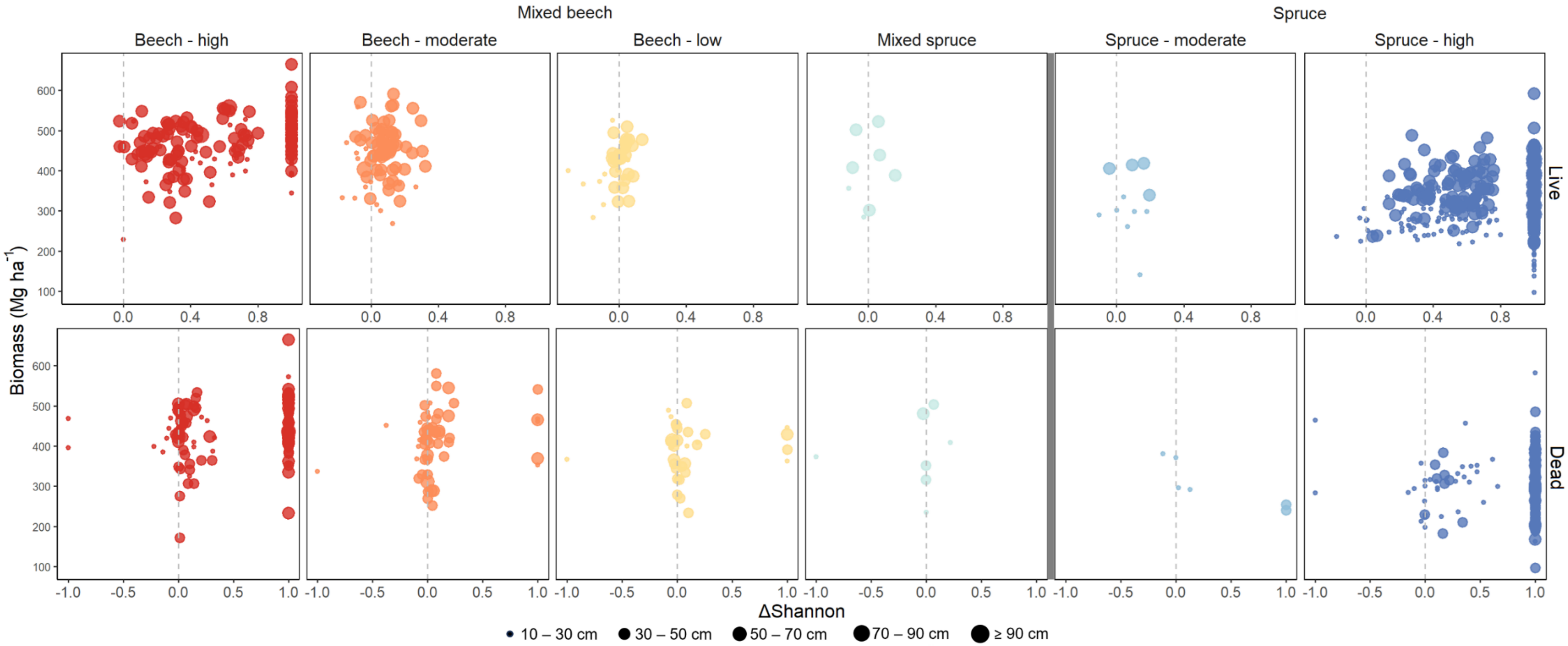
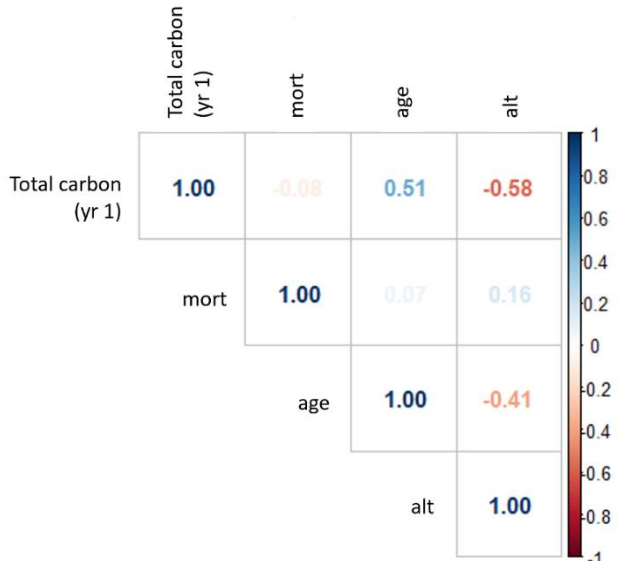


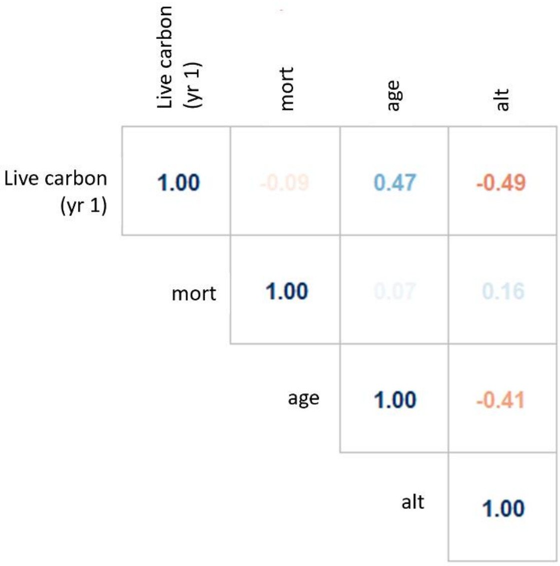
Figure. S2.6: Spatial distribution in mean plot-level ΔShannon index values and total (live and dead) biomass (Mg ha^{-1}) by the most common genera contained within each forest type (Mixed beech, spruce) across the Carpathian primary forests. ΔShannon – Normalised difference (Δ) between the structural (tree size index) and species (tree diversity) Shannon indices per tree type (live, dead), denoting the degree of similarity or dissimilarity between each plot-level mean values. IVI rank prevalence of beech and spruce in their respective forest types, ranked by low (<100), moderate (100–150) and high (>150) IVI scores, with beech – mixed spruce (*n plots* = 4) being plots in the mixed beech forests characterised as being dominantly spruce in structure and genus coverage. Plot-level species and structural indices are shown for live (top panel; *n* = 726) and dead (bottom panel; *n* = 607) standing trees.

Supplement | S3

S3.1 | Statistical analysis: correlation analysis

Before developing the hierarchical models, we conducted a correlation analysis using the R package *corrplot* (citation: R-package) to assess potential multicollinearity among predictor variables (age, mortality, altitude, and first-census carbon stock). This step aimed to ensure the feasibility of these variables in the models and prevent overfitting. The correlation analysis revealed no significant conflicting trends between the variables, supporting their inclusion in the subsequent Generalised Additive Mixed Models (GAMMs).





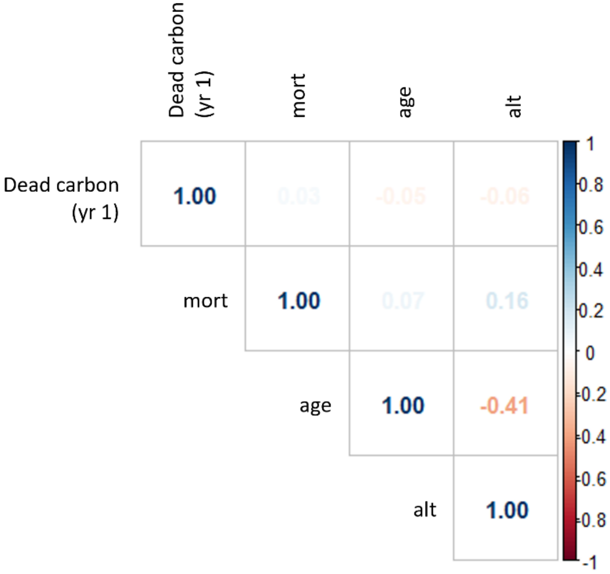


Figure S3.1: Correlation matrix of predictors for each carbon type (total, live, dead). . The performance of each predictor was measured along a matrix, where the strength of a correlation from 1 to -1. The closer the correlation coefficient is to 1 (positive) or -1 (negative), the stronger the correlation, with 0 indicating no significant correlation between coefficients.

S3.2 | Trends in carbon dynamics

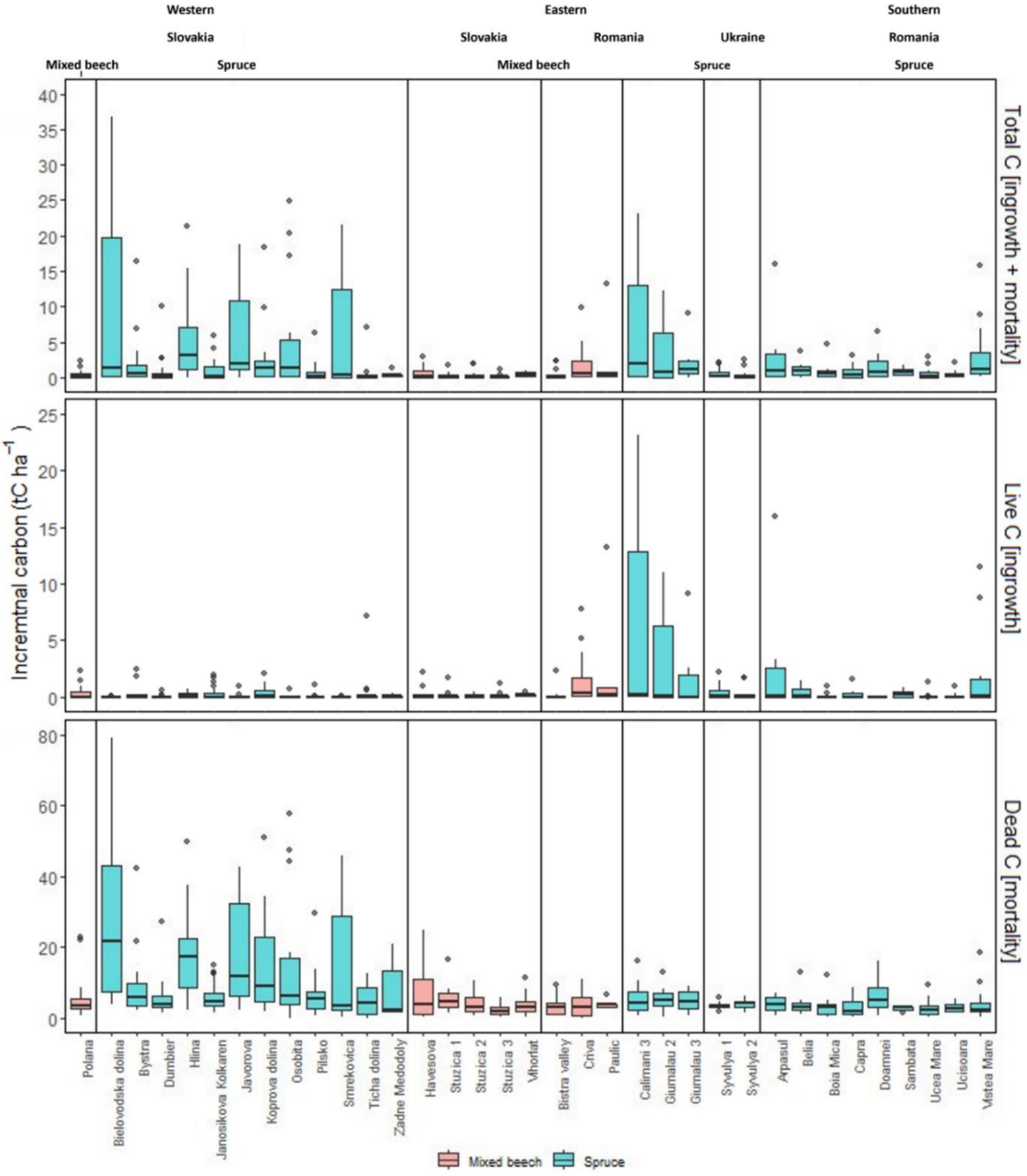


Figure S3.2: Trends in incremental carbon (tC ha^{-1}) for each carbon type (total, live, dead), across each landscape (Western, Eastern, Southern) and forest type (mixed beech, spruce). Total incremental carbon pools together the gain in live stock, representing ingrowth, and increase in dead standing carbon, mortality. Incremental carbon represents the net change in carbon stocks.

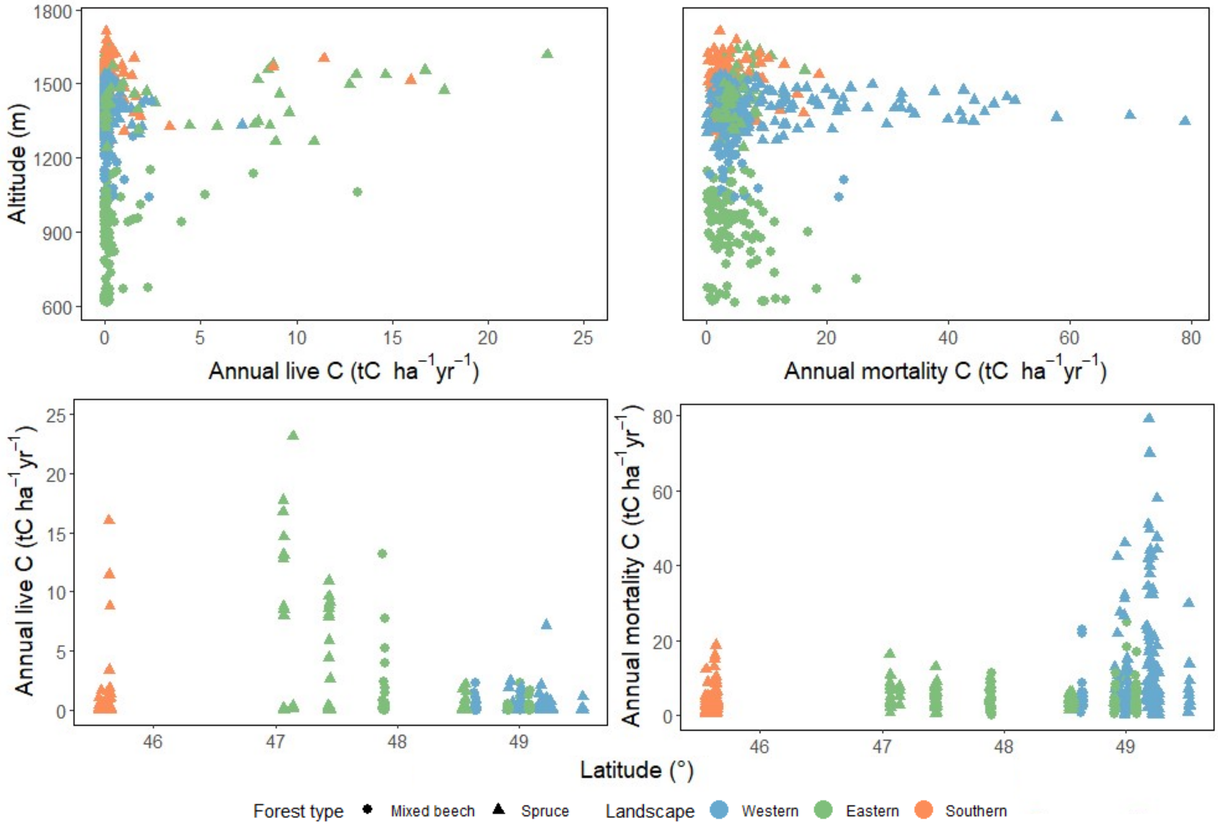


Figure S3.3: Annual net changes in carbon stocks (tC ha^{-1} yr^{-1}) between the first and second census for each of the 454 primary forest plots, per landscape (Western, Eastern, Southern) and forest type (mixed beech, spruce). Top panel show the trends in plot-level altitude (m) and mean changes in carbon between censuses, with the bottom panel representing the change across latitude (°), respectively.

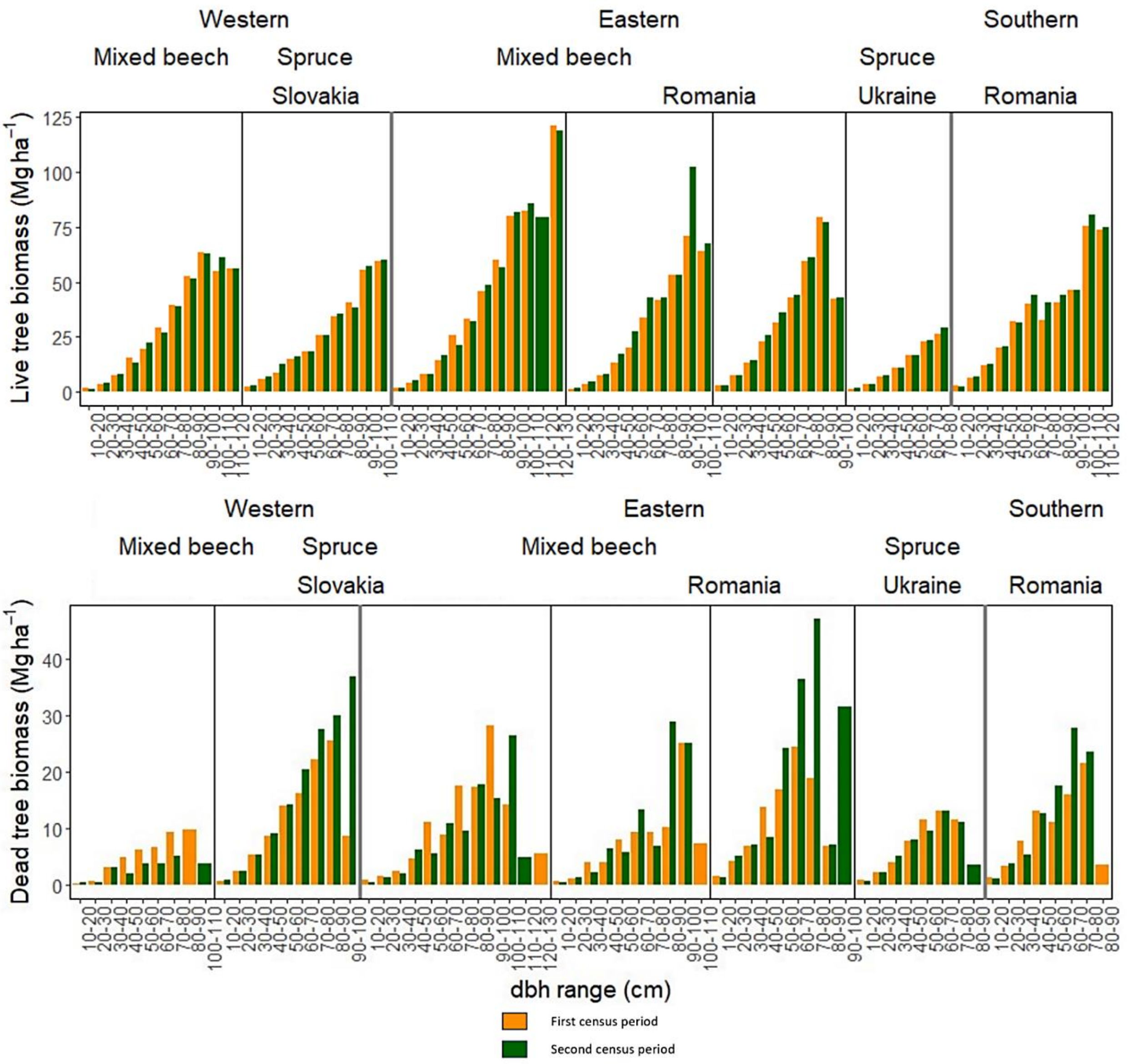


Figure S3.4: Landscape-scale trends in aboveground standing biomass and tree—a comparison of tree biomass stocks between the first (orange) and second (green) census periods across the Carpathian primary forests, including 454 plots, per dbh class (cm). Trends depict trends in live (top panel) and dead (bottom panel) biomass stocks (Mg ha^{-1}) across each landscape (Western, Eastern, Southern) and forest type (mixed beech, spruce). Dead biomass combines standing trees identified as dead and mortality tree.