Project Title: Beech DIV: Biodiversity in Deadwood - A Basis for Enhancing Sustainable Conservation of Beech Forests in Germany and the Caucasus

Final Progress Report

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Contents

1. Introduction

The Beech DIV project, titled "Biodiversity in Deadwood: A Basis for Enhancing Sustainable Conservation of Beech Forests in Germany and the Caucasus," was initiated in May 2021. This project aimed to evaluate and compare the biodiversity found in deadwood within both natural and managed forests of European and Oriental beech. Under the leadership of Prof. Dr. Jörg Müller, project-related activities were conducted by Ph.D. student Giorgi Mamadashvili. The primary project partners included several key figures and regions. In Germany, G. Mamadashvili supervised all project activities. In Georgia, research activities were carried out by Levan Tabunidze and Lars Drössler. Additionally, Mark Kalashyan supervised his research team in Armenia.

The project also involved collaboration with other countries. Laurent Larrieu supervised research in France, Thibault Lachat in Ukraine, Kostadin Georgiev in Bulgaria, Hazan Alkan Akinci in Turkey, and Razi Jahed in Iran. This international cooperation was crucial for the project's comprehensive evaluation of biodiversity in deadwood across different forest types and regions.

European beech (*Fagus sylvatica*) is widespread in Western and central Europe, replaced by *F. orientalis* in Bulgaria, Turkey, the Caucasus, and Iran. Beech forests are ecologically important in the western Palearctic region, but centuries of human intervention have fragmented and altered these habitats, with many now managed forests. The removal of damaged, deformed, diseased, and dead trees under "hygienic standards" of forest management has significantly impacted biodiversity. However, since the early 1980s, the importance of veteran and habitat trees, as well as deadwood, has been increasingly recognized for maintaining biodiversity. Many beech forests now hold protected status or are part of conservation networks such as Natura 2000 and the Emerald Network.

A multitude of studies have been conducted to ascertain the factors influencing biodiversity in beech forests across Europe, Asia, and in select other regions. The integrity of these forests has been assessed in relation to various management practices, elevation gradients, climate conditions, and comparisons with other tree species such as oak and conifers (e.g., pine, spruce, fir). Which significantly deepened our understanding of beech forest biodiversity over the past decades and influenced conservation strategies. However, there remains a lack of systematic comparative studies covering the entire range of Fagus species from western Europe to the

Caspian Sea using standardized methods to identify similarities and dissimilarities in biodiversity aspects across these forests. Therefore, to understand these ecological mechanisms operating in beech forests and to underscore their significance and potential future implications our project's aim was to systematically investigate beech forests of both Fagus species, *F. sylvatica* and *F. orientalis*, from west to east, spanning the Pyrenees, Central Europe, Balkan region, Carpathians, Great and Lesser Caucasus, and Hyrcanian Forest. This includes countries such as France, Switzerland, Germany, Bulgaria, Ukraine, Turkey, Georgia, Armenia, and Iran.

2. Overview of field work and data collection

2.1 Field Season Initiation and Data Collection in 2021

In April 2021, fieldwork commenced with the selection of study plots in production (10 plots) and old-growth (10 plots) forests dominated by *Fagus sylvatica* and *F. orientalis*. In Germany, 40 plots were selected across two regions: the Steigerwald forest and the Bavarian National Park (Mittelstaighütte\Scheuereck). Later, the same structure was established in other participating countries, and fieldworks were conducted (details in attached reports from participating countries).

Key activities:

1. Installation and monthly emptying of traps using copper sulphate.
2. Collection and sorting of insect samples for identification.
3. Collection of drill and sporocarp material from deadwood for future Meta-barcoding.
4. Assessment of tree-related microhabitats (TreMs) in each study site.

2.2. Continuation of TreMs fieldwork and initiation of further fieldwork in 2022

An educational video detailing the methods of deadwood fungi sampling and TreM assessment was prepared and shared with all project partners. This video facilitated standardized data collection across countries. Furthermore, we sampled deadwood fungi and beetles.

Key activities:

1. Continued assessment of TreMs in each plot.
2. Collection of deadwood fungi samples (drill and sporocarp) for Meta-barcoding.

3. Organization of a working trip to Georgia to discuss fieldwork progress and future plans with Georgian and Armenian colleagues.
4. Business trip of our Armenian project partner M. Kalashyan to Germany, meeting with J. Müller and G. Mamadashvili for sharing the experiences, discussions around the project implementation, short excursion in Steigerwald forest and Bavarian National Park, parallel to this - identification of some part of beetle samples.
5. Compilation and sharing of partner reports (Georgia, Armenia and Germany) for the 2021-2022 seasons, see the supplementary materials.

3. Further fieldworks, Data Analysis and Manuscript Preparation in 2023

Data analysis from the TreM assessments and meta-barcoding was completed, leading to the preparation of several manuscripts.

Key activities:

1. Two expeditions were carried out for collecting the extra materials for further lab. analyses in June and September in 2023 in Armenia.
2. Preparation and publication of the manuscript "Drivers of Tree-Related Microhabitat Profiles in European and Oriental Beech Forests" in the journal Biological Conservation (DOI: 10.1016/j.biocon.2023.110245).
3. Submission of the manuscript "Drivers of Wood-Inhabiting Fungal Diversity in European and Oriental Beech Forests" to Ecology and Evolution, currently under revision.
4. Publication of the manuscript “Comparison of tree species diversity, deadwood volume and regeneration of managed and old-growth Oriental beech (*Fagus orientalis* L.) forests in Eastern Georgia” by Lars Drössler in the journal of Caucasiana (Journal on the biodiversity of the Caucasus and adjacent regions) (DOI 10.3897/caucasiana.2.e106898).

4. Further Manuscripts and sharing with the data in 2024

The results of the beetles caught by flight interception and pitfall traps gave the opportunity to write next manuscript and, in the end, we prepared Thesis and submitted to the JMU University

Key activities:

1. Submission of the manuscript "Click Beetles (Elateridae) Identify Conservation Units in Oriental and European Beech Forests" to the Journal of Insect Conservation.
2. Compilation and sharing of partner final reports (Georgia, Armenia, and Germany) for 2024.
3. Workshop on tree-related microhabitats as indicators of forest integrity for the director and rangers of Lagodekhi National Park (Georgia), conducted by Giorgi Mamadashvili.

5. Results

Over the course of three years, all planned fieldwork and project-related activities were successfully completed. Our partners and collaborators dedicated themselves fully to achieving the project's goals. As a result of our collective efforts, we have prepared four manuscripts based on our research findings.

5.1 Paper N1 - "Drivers of Tree-Related Microhabitat Profiles in European and Oriental Beech Forests"

Published in Journal of "Biological Conservation"

Drivers of tree-related microhabitat profiles in European and Oriental beech forests

Giorgi Mamadashvili[a,1,*], Laurent Larrieu[b,1], Antoine Brin[c], Claus Bässler[d,s], Vasyl Chumak[e], Maksym Chumak[e], Valeriia Deidus[f], Lars Drössler[g], Céline Emberger[h], Kostadin B. Georgiev[i], Tigran Ghrejyan[j], Martyn M. Gossner[k,l], Ruslan Hleb[m], Razieh Rafiei-Jahed[n], Mark Kalashian[j], Ivan Kambarov[o], Gayane Karagyan[j], Joni Kevlishvili[p], Zviad Khutsishvili[p], Thibault Lachat[q,r], Ludwig Lettenmaier[a], Meri Mazmanyan[i], Oliver Mitesser[a], Peter I. Petrov[s], Nicolas Roth[q,r], Levan Tabunidze[o], Jörg Müller[a,t,1]

[*] Corresponding author at: Field Station Fabrikschleichach, Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Glashüttenstraße 5, 96181 Rauhenebrach, Germany

[1] these authors contributed equally to this work.

[a] *Field Station Fabrikschleichach, Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Glashüttenstraße 5, 96181 Rauhenebrach, Germany*

[b] *University of Toulouse, INRAE, UMR DYNAFOR, Castanet-Tolosan, France & CNPF, Toulouse, France*

[c] *University of Toulouse, Ecole d'Ingénieurs de PURPAN, UMR INRAE-INPT DYNAFOR, Toulouse, France*

[d] *Ecology of Fungi, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Germany*

[e] *Uzhhorod National University, Department of entomology and biodiversity preservation, Voloshyna str. 32, Uzhhorod, 88000, Ukraine*

[f] *State Museum of Natural History, National Academy of Sciences of Ukraine, Teatralna str., 18, Lviv, 79008, Ukraine*

[g] *Forestry Research and Competence Center ThüringenForst AöR, Gotha, Germany*

[h] *Conservatory of Natural Areas of Occitanie, Mercuès, France*

[i] *Hessian State Agency for Nature Conservation, Environment and Geology, Germany*

[j] *Laboratory of Entomology and Soil Zoology, Scientific Center of Zoology and Hydroecoly NAS RA, P. Sevak str., 7, Yerevan, 0014, Armenia*

[k] *Forest Entomology, Swiss Federal Research Institute WSL, CH-8903 Birmensdorf, Switzerland*

[l] *Institute of Terrestrial Ecosystems, Department of Environmental Systems Science, ETH Zürich, CH-8092 Zürich, Switzerland*

[m] *Carpathian biosphere reserve, Krasne Pleso St. 77, Transcarpathian Region Rakhiv 90600, Ukraine*

[n] *Research Institute of Forest and Rangeland (RIFR), Agriculture Research, Education and Extension Organization, Tehran, Iran*

[o] *Strandzha Nature Park Directorate, 1 Yanko Maslinkov St., 8162, Malko Tarnovo, Bulgaria*

[p] *Biodiversity Conservation Center "Nacres", B. jgenti str. 34, Tbilisi, Georgia*

[q] *Bern University of Applied Sciences University of Agricultural, Forest and Food Sciences HAFL, Department of Forest Sciences, Länggasse 85, 3052 Zollikofen, Switzerland*

[r]*Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland*

[s] *Forestry University Sofia, Field base Petrohan, 1 Petrohanska St., 3520 Barzia, Bulgaria*

[t] *Conservation and research department at the Bavarian Forest National Park, Freyunger str. 2. 94481 Grafenau, Germany*

Abstract

Tree-related microhabitats (TreMs) provide a quantitative indicator of habitat heterogeneity in forests, including beech (Fagus) forests. However, systematic analyses of the factors driving TreM diversity and composition in *Fagus sylvatica* and *F. orientalis* forests are lacking. In this study, the TreMs of beech forests on 203 plots of 22 forest sites (production and old-growth forest) across the full longitudinal range of both species were assessed following a standardized TreM protocol. A unified diversity and ordination framework based on Hill numbers was applied to account for unobserved TreM types and to extend the sensitivity of our findings focusing from rare to dominant TreMs. The composition of TreM assemblages was mostly determined by Fagus species and elevation, a surrogate for climate, and with focus on dominant TreMs by DBH, whereas old-growth versus production forest had no effect. The coverage of detected TreMs per plot increased with the number of trees assessed and DBH, but was lower in old-growth than in production forests. When standardized for sampling coverage, the diversity of rare and dominant TreM types was higher in old-growth than in production forests, but increased with elevation only focusing on dominant TreMs. These findings corroborate regional studies showing a higher TreM diversity in old-growth forests. Moreover, they demonstrate the importance of focusing conservation efforts on forests of both Fagus species and at different elevations, covering the full range of TreM assemblages. Future studies comparing TreM diversity in different forests should standardize diversity by sample coverage, as currently done in many biodiversity studies.

Keywords tree-related microhabitats (TreMs), forest management, *Fagus sylvatica*, *Fagus orientalis*, sample coverage, Hill numbers

1. Introduction

Some temperate forest regions are characterized by a few dominant species forming large-scale forests. For conservation strategies in these forests, it is important to understand the drivers of their habitat structures as vertical and horizontal structure, the dynamic of dead trees or the formation of microhabitats. Two of these species are European beech (*Fagus sylvatica* L.) and Oriental beech (*F. orientalis* Lipsky). European beech is widely distributed in the temperate forests of Western and Central Europe (von Wuehlisch, 2008) and replaced by Oriental beech from Eastern Bulgaria to Turkey, then to the Caucasus region and up to northeast Iran (Kavgaci et al., 2012). Both *F. sylvatica* and *F. orientalis* tend to dominate forests from lowlands to the upper montane level in areas with sufficient precipitation (Králíček et al., 2017) but they occur together with many other tree species (Dulamsuren et al., 2017). In colder regions, beech is mixed with other shade-tolerant and long-lived species, such as silver fir (*Abies alba*), Caucasian fir (*Abies nordmanniana*), Norway spruce (*Picea abies*), and Caucasian spruce (*Picea orientalis*). At lower elevations, as well as in the dry zone of the high elevations of Iran, beech is admixed with other broadleaf species (*Quercus* spp., *Carpinus* spp.) (Bardat et al., 2004; Sagheb Talebi et al., 2014). Beech forests comprise the most important temperate forest ecosystems in the western part of the Palearctic but most of the forests have been intensively managed for centuries (Brunet et al., 2010; Gossner et al., 2013; Ódor et al., 2006). The few remaining natural beech forests have thus been identified as important conservation targets. In Europe, *F. sylvatica* forests are well represented in the Natura 2000 network of protected areas (Bohn & Neuhäusl, 2000). In the Caucasus region, Georgia, and Armenia, arcto-tertiary relict beech forests, harboring many endemic plants and animals (Aliev, 2021; Fayvush & Aleksanyan, 2021; Goginashvili & Tvauri, 2013), belong to the Emerald Network of Areas of Special Conservation Interest (Artsivadze et al., 2018; Fayvush et al., 2016). Due to their high global importance for the conservation of temperate forest habitats in Europe and Western Asia, several remnants of natural beech forests have received the UNESCO World Heritage designation, including "The Ancient and Primeval Beech Forests of Carpathians and Other Regions of Europe," "Colchic Rainforests and Wetlands," and the "Hyrcanian Forest" (UNESCO, 2013, 2019, 2021).

Conservation strategies are applied in both production and old-growth forests as well as in forests with an enrichment of deadwood and the retention of veteran trees and tree-related microhabitats (hereafter TreMs, as used in Larrieu et al., 2018) (Brunet et al., 2010; Bütler et al., 2013; Winter & Möller, 2008). The importance of the old-growth attributes of beech forests for biodiversity has been shown for a number of the taxonomic and functional groups (Bauhus

et al., 2009) that develop in TreMs. Especially they are important for higher trophic levels that use TreMs as resources (water, food etc.), such as epiphytic and epixylic bryophytes (Ódor et al., 2006), lichens (Christensen et al., 2005; Moning & Müller, 2009), wood-inhabiting and soil related fungi (Abrego et al., 2017; Bässler et al., 2014), saproxylic beetles (Roth et al., 2019), true bugs (Gossner et al., 2007), cavity-nesting birds (Zahner et al., 2012), tree roosting bats (Russo et al., 2010), ground dwelling arthropods (Bátori et al., 2022), mollusks (Petermann & Gossner, 2022;Moning & Müller, 2009), vascular plants (Jiménez-Alfaro et al., 2018), and amphibians (Basile et al., 2017; Piraccini et al., 2017).

At the forest stand level, tree species composition, forest type, management approach (production vs. old-growth forest), diameter at breast height (DBH), tree status (standing/lying), and vitality (alive, dying, dead) are the main determinants of TreM abundance and diversity (Asbeck et al., 2019, 2021; Courbaud et al., 2021; Kozák et al., 2018; Larrieu et al., 2012; Larrieu, Cabanettes, Brin, et al., 2014; Martin et al., 2021; Paillet et al., 2019; Regnery et al., 2013; Sefidi & Copenheaver, 2020; Vuidot et al., 2011). The probability of TreM occurrence increases with tree age and DBH (Kozák et al., 2023; Winter et al., 2015; Winter & Möller, 2008) and thus increases in old-growth forests, where both large and old trees are more abundant. In addition, TreM density is consistently higher on broadleaf trees than on conifers (Asbeck et al., 2019; Larrieu & Cabanettes, 2012). Elevation has also been studied as an influencing factor for TreMs. Asbeck et al (2019) found an increase in TreM recordings with increasing elevation. They discuss this as possible effects of an increase in bearded lichens, in line with lichen studies (e.g., Bässler et al., 2016) and an increase in damage by rock fall, soil movement or other factors such as tree swaying.

In recent years, TreMs, including those in beech forests, have been recognized as an indicator of habitat heterogeneity (Asbeck et al., 2021; Basile et al., 2020; Khanalizadeh et al., 2020; Kozák et al., 2018; Larrieu et al., 2018). The cavity type and the presence of epiphytes, the fruiting bodies of fungi, and various types of perched deadwood are among the features that distinguish different TreMs (Larrieu et al., 2018) and contribute to their role as pivotal habitats, often for very small and difficult-to-record animal and fungal species (Möller, 2009). Accordingly, a higher diversity of TreMs provides a higher diversity of niches for a higher diversity of organisms, such that TreMs can serve as an indicator in biodiversity assessments and as a focus of efforts to promote integrative forest management (Asbeck et al., 2021; Larrieu et al., 2018; Martin et al., 2022; Paillet et al., 2018; Regnery et al., 2013; Winter & Möller, 2008).

While many TreM studies have been conducted in beech forests, most were at a local or regional scale and were restricted to either *F. sylvatica* or *F. orientalis*, a particular elevation, or a particular climate. Their results (Asbeck et al., 2019; Courbaud et al., 2017; Jahed et al., 2020; Larrieu & Cabanettes, 2012; Winter et al., 2015) are therefore strongly context dependent and difficult to transfer to other regions. Only one study based on extensive data collection investigated the role of DBH and management on the rate of TreM formation in European and Oriental beech (Courbaud et al., 2021). However, differences between production and old-growth forests were not assessed from a paired sampling design. So far no systematic study for old-growth and production forests of both *Fagus* species exist, hampering generalizations for their protection in terms of microhabitats. In this study, we sought to identify the general determinants of TreM diversity and composition in beech forests based on TreM data collected from 203 plots within 22 forest stands (old-growth and production forests) covering the entire longitudinal distribution of European and Oriental beech.

In biodiversity surveys, TreM sampling on a plot is inevitably incomplete, due to the limited sample size as well as to the difficulty in accounting for all TreMs within the complex structure of larger trees from the ground (Larrieu et al., 2018). To overcome these limitations, we applied the framework by Chao et al., (2014) considering unseen species in diversity and community analyses. Furthermore, and analogous to taxonomic assessments, we focused in our TreM diversity assessments on rare and on dominant TreM types using Hill numbers (Hill, 1973) in order to obtain insights into the habitat quality of a forest based on its TreMs.

Particularly, we made three hypotheses. H1: the diversity of TreMs would increase from production to old-growth forests (H1a), with a higher diversity in stands with larger trees (H1b), with an increasing number of admixed tree species (H1c) and, due to faster wood decomposition processes, towards warmer climates (H1d). H2: TreM assemblages at the stand scale are driven by (H2a) management, (H2b) elevation, and (H2c) the number of admixed tree species. H3: TreM diversity and composition differ generally between the two *Fagus* species as shown locally by Jahed et al. (2020).

2. Materials and methods

2.1 Study areas

The study included mature beech dominated forests with a Fagus proportion of >50% of basal area along the west-east gradient of European and Oriental beech, including France,

Switzerland, Germany, Bulgaria, Ukraine, Georgia, Armenia, and Iran. However, Bulgaria is the only country in our study that contains both *Fagus sylvatica* and *Fagus orientalis* (Figure 1). The forests can be characterized as following:

- Old-growth forests in all countries were set aside for at least 50 years. Therefore, they were all formally strictly protected with the exception of Bulgaria.
- The old-growth forests in Gorgan, Lagodekhi and Mala Uholka are described as primeval forests.
- The production forests in France, Switzerland and Germany are thinned at intervals of 5-15 years. The removal rate is around 20% of the basal area per logging operation.
- In Bulgaria, Lagodekhi (Georgia) and Iran, logging activities are carried out as irregular selective logging and removal of dead trees.
- In Borjomi (Georgia), the production forest is extensively managed as irregular single-tree-felling and removal of dead trees.

The 22 forest stands ranged from 65 to 1497 m above sea level (Table 1). In one of the plots, no TreMs were detected. Therefore, the community analyses comprised 202 plots. The stands were dominated by beech trees, ranging from 51-100% beech of basal area, admixed with oak (*Quercus* spp.), maple (*Acer* spp.), hornbeam (*Carpinus* spp.), lime (*Tilia* spp.) and sometimes with conifers such as pine (*Pinus* spp.), fir (*Abies* spp.), and spruce (*Picea* spp.).

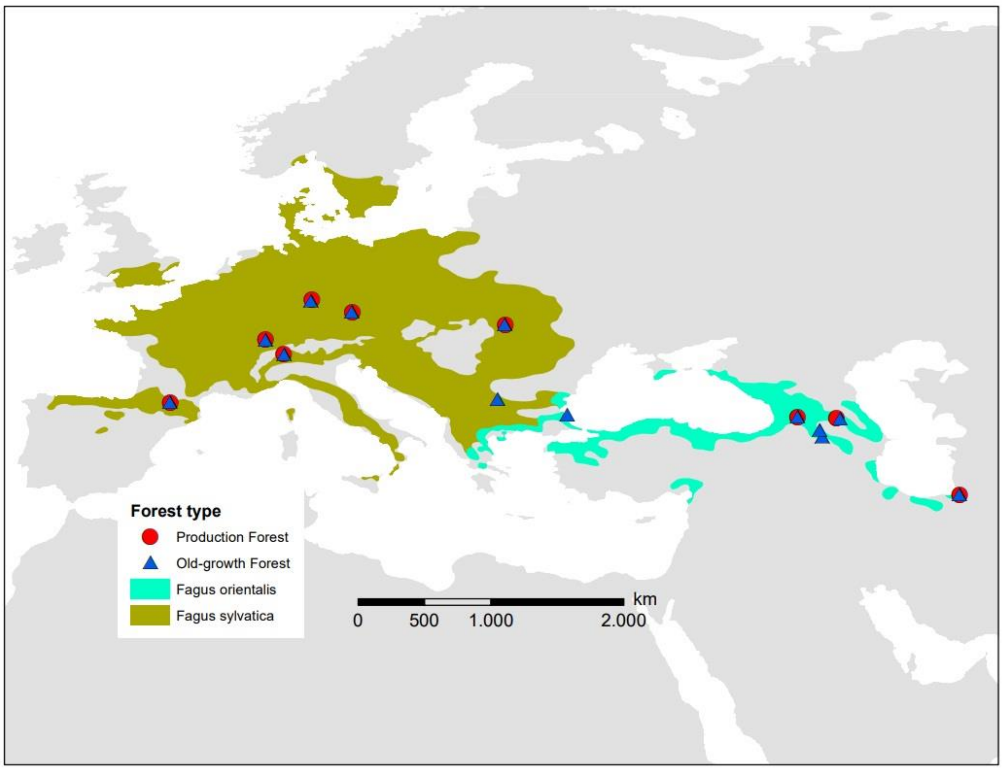

Figure 1. Geographical distribution of European (*Fagus sylvatica*) and Oriental (*F. orientalis*) beech (Caudullo et al., 2017) and the location of the 22 (old-growth and production forest) forests stands extending from the French Pyrenees to Iran, investigated for TreMs.

Table 1. List of *Fagus* stands investigated for TreMs and recorded tree species in Production (PF) and Old-growth forests (OG): Aa: *Abies alba*, Ac: *Acer campestre*, Ag: *Alnus glutinosa*, Ao: *Acer opalus*, Apl: *Acer platanoides*, Ap: *Acer pseudoplatanus*, As: *Alnus subcordata*, Av: *Acer velutinum*, Bp: *Betula pendula*, B: *broadleaf*, C: *conifer*, Cb: *Carpinus betulus*, Cs: *Castanea sativa*, Cc: *Carpinus caucasica*, Dl: *Diospyros lotus*, Fe: *Fraxinus excelsior*, Fo: *Fagus orientalis*, For: *Fraxinus ornus*, Fs: *Fagus sylvatica*, Ld: *Larix decidua*, Pa: *Populus alba*, Pab: *Picea abies*, Pav: *Prunus avium*, Pd: *Prunus domestica*, Pp: *Parrotia persica*, Ps: *Pinus sylvestris*, Pt: *Populus tremula*, Qi: *Quercus iberica*, Qp: *Quercus petraea*, Qpo: *Quercus polycarpa* Qr: *Quercus robur*, Qs: *Quercus* sp, Sa: *Sorbus aria*, Sc: *Salix caprea*, St: *Sorbus torminalis*, Tc: *Tilia caucasica*, Tb: *Tilia begoniifolia*, Tp: *Tilia platyphyllos*, Ts: *Tilia* sp, Tba: *Taxus baccata*, Tt: *Tilia tomentosa*; Ug: *Ulmus glabra*.

Country/forest site	X	Y	Elevation (m)	Number of plots	Tree species	Management
France						
Montreich	42.986	0.973	811-984	3	**Fs**	PF
Montreich	42.990	0.967	939-958	3	Fe, **Fs**, Qp, Pa, Tp	OG
Switzerland						
Bettlachstock	47.221	7.413	611-631	11	Aa, Ao, Ap, B, C, Cb, Fe, **Fs**, Ia, Qp, Pab, Ps, Sa, Tba, Ts, Ug	PF
Bettlachstock	47.218	7.413	651-671	11	Aa, Ap, Fe, **Fs**, Pab, Ps, Tba, Sa, Ug	OG
Lodano	46.255	8.665	811-831	11	Bp, **Fs**, Ld, Pav	PF
Lodano	46.238	8.694	851-871	11	Bp, Cs, Fe, **Fs**, Pav, Qs, Sa	OG
Germany						
Steigerwald	49.930	10.562	438-475	10	Cb, **Fs**, Qr	PF
Steigerwald	49.860	10.499	355-404	10	**Fs**, Pd, Qr	OG
Scheuereck	49.064	13.306	765-831	10	Aa, Ac, **Fs**, Pd	PF
Mittelsteighuette	49.099	13.245	718-774	10	Aa, **Fs**	OG
Bulgaria						
Berkovitsa	43.176	23.121	600-1465	12	Cb, Bp, **Fs**, Pa, Qp	OG
Strandzha	42.110	27.852	65-456	12	Ac, Apl, **Fo**, For, Pt, Qpo, St, Tt	OG
Ukraine						
Velyka Uholka	48.222	23.651	492-553	11	Cb, **Fs**,	PF
Mala Uholka	48.269	23.621	731-779	11	Ap, Apl, Fe, **Fs**, Ug	OG
Georgia						

Lagodekhi	41.910	46.092	770-1128	10	Ap, Cc, Cs, **Fo**, Pt, Tb	PF
Lagodekhi	41.855	46.316	480-1497	10	Ac, Cs, **Fo**, Tc	OG
Borjomi	41.964	43.456	880-1113	10	Ag, Cb, **Fo**, Qi, Tc,	PF
Borjomi	41.975	43.454	994-1160	10	Ac, Cb, **Fo**, Pa, Qi, Sc, Ug, Tc	OG
Armenia						
Gegharkunik	40.651	45.121	1771	1	**Fo**	OG
Tavush	40.912	45.076	1298-1327	2	**Fo**	OG
Iran						
Gorgan	36.731	54.396	834-932	12	As, Av, Cb, Dl, **Fo**, Pp	PF
Gorgan	36.729	54.391	948-978	12	Av, Cb, **Fo**, Pp	OG

2.2 TreM assessment

All TreM data were collected during the summer in 2020 or 2022, depending on the country, since most of the sites are snow-covered in winter. Leaf-on detection of TreMs makes visibility difficult on the one hand, but on the other hand facilitates the detection of dead branches. In the Ukraine and Switzerland, the data were collected in fixed-radius circles with a horizontal area of 0.1 ha. For all other countries, angle-count sampling developed by Bitterlich (1984) was applied. The method focus on a balanced number of trees in all dimension classes. It assigns a higher probability of selection to larger trees. The sampling requires a defined opening angle, using a relascope or other device materializing the chosen angle, all trees that appear larger than opening angle when sweeping around 360° are counted. Just this counting allows an estimate of the basal area per ha.

On all plots, the number of trees inspected for sampling varied considerably due to the sampling method and variation in tree diameter. Both were accounted in all of the statistical analyses (see Sect. 2.3 Statistics). For each tree, the species was identified and its DBH was measured. TreMs were recorded as present or absent, without counting the number of TreMs of the same type at the tree level. TreMs were described following the typology of Larrieu et al. (2018), which classifies seven basic morphological forms: 1. cavities, 2. tree injuries and exposed wood, 3. crown deadwood, 4. excrescences, 5. fruiting bodies of saproxylic fungi and fungi like organisms, 6. epiphytic and epixylic structures and 7. exudates. Then they are differentiated into 15 groups and described in 47 different TreM types used in this study as diversity units.

2.3 Statistics

All analyses were conducted in R 4.2.3 (R Core Team, 2022) at the plot-level scale. Since observations of TreMs at the plot-level are inevitably incomplete, methods for the ordination of TreM type composition as well as for the diversity analyses were applied similar to those used in taxonomic measurements, taking into account unobserved TreMs (see Supplementary

material, R code). In order to better take this sampling effect into account in biodiversity analyses, Chao & Jost, (2012) suggested comparing diversity based on a standardized sample coverage rather than on a fixed sample size. This is required if sample coverage varies systematically along environmental gradients, which might be relevant in TreMs in production forests versus old-growth forests or in forest plots with different numbers of trees. Finally, among ecologists consensus has emerged, that Hill (1973) numbers (effective numbers of species) should be used to incorporate species abundance/evenness into species diversity measures (Ellison, 2010). Chao et al., (2014) demonstrated that this concept can be applied also to species composition. Here the Jaccard index (q=0), the Horn-index (q=1) and the Morisita-Horn-index (q=2) represent in the same way above the focus in the dissimilarity to rare, common, and dominant species, which can be applied to TreMs as well. We therefore estimated sample coverage for all plots and a diversity measure along the Hill numbers at a standardized completeness using the packages spadeR and iNEXT (Hsieh et al., 2016; Linderman et al., 2012). For details, see the R code in Supplementary material.

Different predictor sets for TreM community compositions at the plot level were tested using multiple regression on distance matrices (MRMs) (Lichstein, 2007) as follows: First distance matrices for the TreM composition for q=0, 1, and 2 were created considering unseen TreMs. In a second step, distance matrices were created for the number of inspected trees per plot (log transformed), management type (production\ old-growth forests), mean DBH of the inspected trees, number of admixed tree species, elevation, and *Fagus* species. Since the two species are distributed along a longitudinal gradient, the predictor distance beech species was replaced by a spatial distance that considers the nested structure of the plots in the stands.

In a first step, the number of TreM recordings and the number of observed TreM types per plot was counted. In the next step the sample coverage for each plot was extracted using iNEXT (Hsieh et al., 2016) and, before it was used in the model, was arc-sine square-root-transformed. Finally, TreM diversity was estimated for each plot for a fixed coverage of 75%. The latter values were log transformed for further analyses. The effect of the predictors on these response variables was tested using generalized linear mixed models (*lme4* package) with a Poisson distribution and an observation-specific random factor to control for overdispersion for the count data and a Gaussian distribution for log-transformed diversity values. In all models site served as a random factor to account for repeated measurements within forest stands (Bates et al., 2015). For Gaussian models, the p value was estimated using the function *cftest* in the package *multcomp* (Hothorn et al., 2008). To identify TreMs types characteristic for one of both beech species an indicator species analysis was applied, using *multipatt* in the package

indicspecies (Caceres, 2020). The identification is based on the relationship between occurrence and abundance of TreM types across the samples in both beech tree species.

3. Results

Of the 47 types of TreMs described by Larrieu et al. (2018), 45 were observed in our study. The two most abundant TreMs in both beech species were *dead branches* and *mosses and liverworts*. The next most abundant TreM types in *F. sylvatica* were *trunk base rot hole, buttress-root concavity* and *remnants of a broken limb*, and in *F. orientalis* the TreM types *foliose and fruticose lichens, remnants of a broken limb* and *ivy and lianas* (Figure 2). An indicator species analysis revealed the following nine TreM types as statistically typical ($p<0.001$) for *F. orientalis*: *ivy and lianas, foliose and fruticose lichens, epicormic shoots, invertebrate nest, hollow branch, canker, medium-sized woodpecker breeding cavity, bark-lined trunk concavity* and *fire scar*. Only *sap run* was identified as indicator TreM type ($p<0.001$) for *F. sylvatica*.

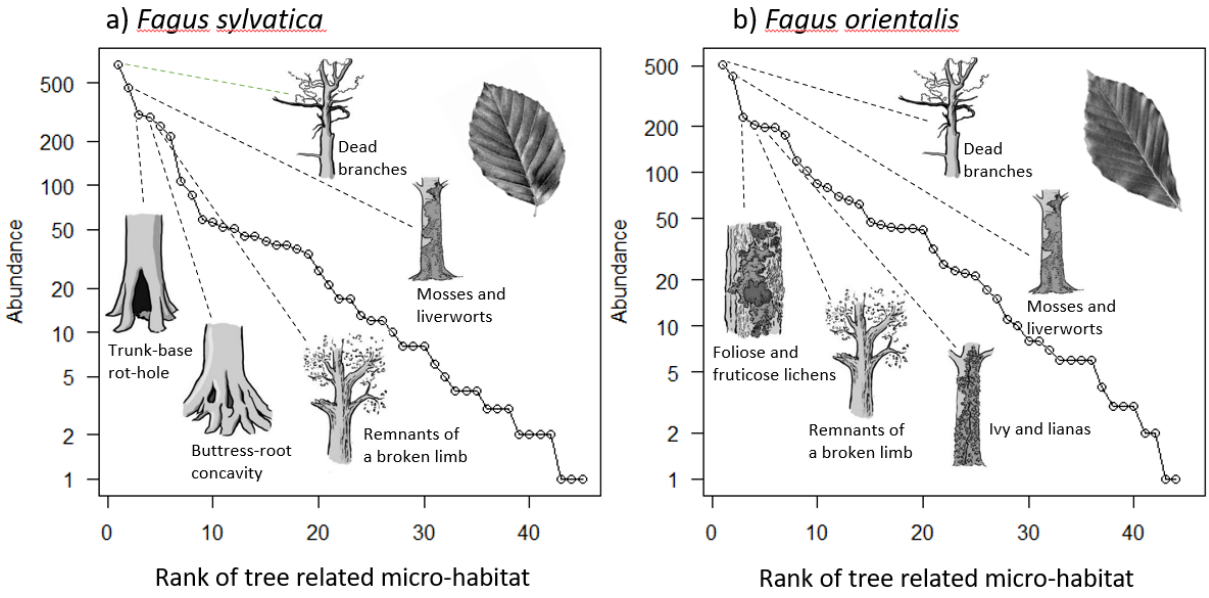

Figure 2. Rank abundance curve for TreM types in a) *Fagus sylvatica* and b) *Fagus orientalis* forests collected in 202 plots located in 22 beech forest stands from France to Iran.

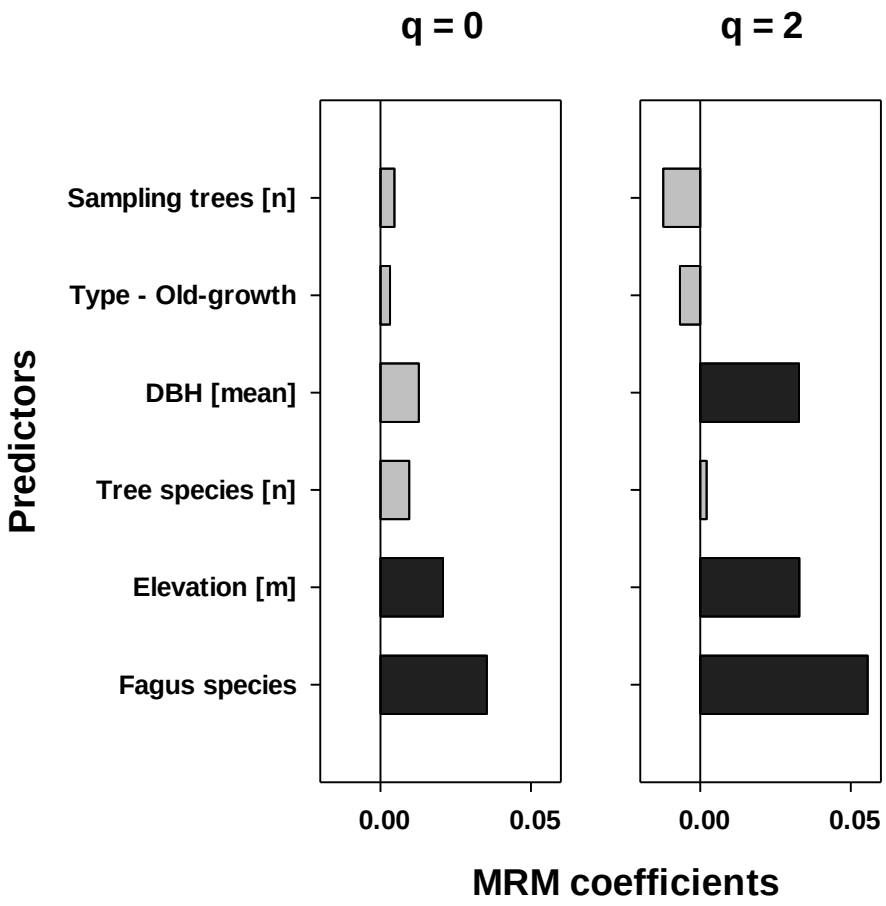

Figure 3. Multiple regression on distance matrices (MRM) coefficients of the predictors of the composition of tree-related microhabitats (TreMs) in 202 plots located in 22 beech forest stands. Black bars indicate significance in MRM $p < 0.05$. Results are shown only for rare (q=0) and dominant (q=2) TreM types. *Type* compared production with old-growth forest stands, and *Fagus species* compared *F. sylvatica* with *F. orientalis*. Note the results for q=1 are not shown because in terms of significance and value they were similar to q0 (see R code).

MRMs identified *Fagus* species and elevation as the most important drivers of TreM type composition, both for focus on rare and on dominant TreM types. On dominant TreM types the mean DBH of the inspected trees at a plot was another important driver (Figure 3). Management and the number of admixed trees did not affect the TreM type composition, regardless of the Hill number. An additional analysis using space instead of distance between *Fagus* species yielded similar results, supporting the correlation of the predictors beech species and space (Figure A1).

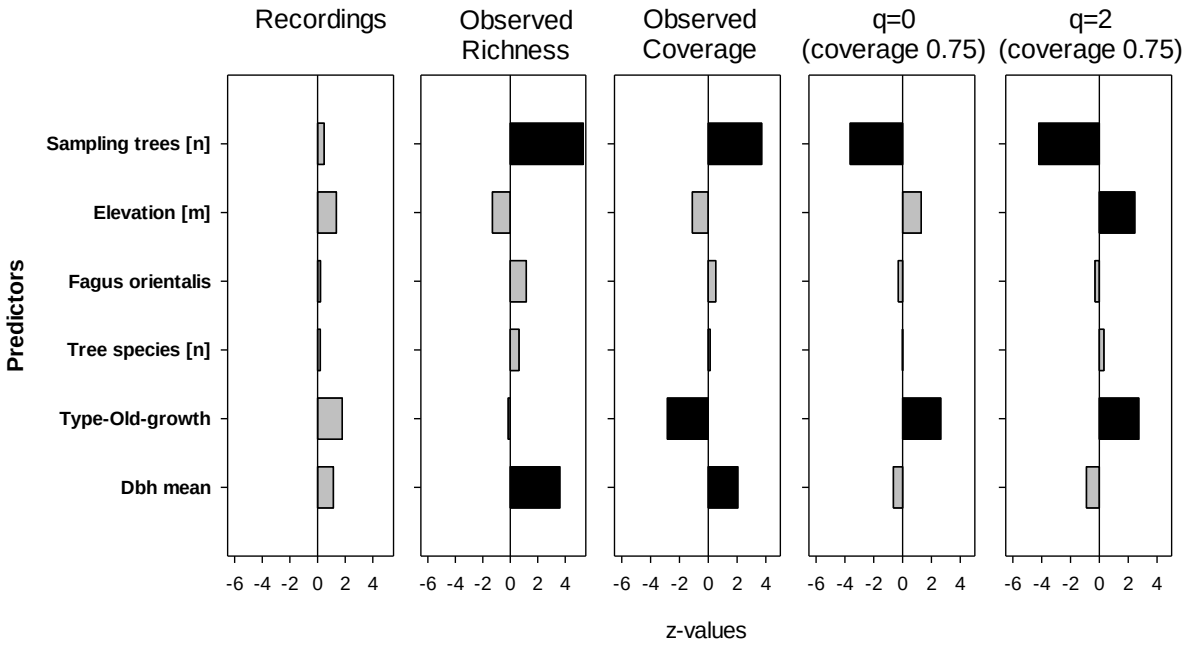

Figure 4. Results of generalized linear mixed models testing for the effects of different predictors of TreM metrics in 203 forest plots located in 22 beech forest stands. "Recordings variable" refers to the number of TreMs recorded per plot; "observed richness" refers to the number of different TreM types per plot; "observed coverage" is the plot-specific sample coverage; q=0 and q=2 are estimates of the effective number of TreMs for the Hill numbers 0 and 2 standardized by a sample coverage of 0.75. Forest stand served as a random effect in all models. Black bars indicate significance in glmms $p < 0.05$. Note the results for q=1 are not shown because in terms of significance and value they were similar to q0 (see R code).

The number of recorded TreMs could not be explained by any of our predictors (Figure 4). The observed TreM richness significantly increased with the number of inspected trees and in plots containing trees with a higher mean DBH (Figure 4). The sample coverage per plot increased with the number of inspected trees and was lower in old-growth sites than in production forest, which showed that coverage was not equally distributed among plots (Figure 4). This effect of tree sampling remained even if the areas from Switzerland, surveyed as fixed areas and not according to the Bitterlich method, were removed. However, the effect was weakened, indicating that Bitterlich sampling is less sensitive to sample coverage. After standardization for sample coverage TreM type richness (q=0) was significantly higher only in old-growth than in production forests, and decreased with the number of inspected trees. The diversity of dominant-type TreMs significantly decreased with the number of inspected trees per plot, but increased with elevation and was higher in old-growth sites than in production forests (Figure 4). The particular *Fagus* species had no effect on diversity.

4. Discussion

In forest dominated by both long-lived and shade-tolerant tree species such as beeches and silver fir, pioneer (e.g., birch, poplar) and post-pioneer (e.g., maple, rowan) species contribute significantly to TreM supply, mainly during regeneration and growing phases (Larrieu, Cabanettes, Gonin, et al., 2014). However, since they represent only a small part of the tree population, conservation issues focused on TreM-dwelling taxa need to focus on the dominant tree species. The previous studies comparing European and Oriental beech for TreM supply (Courbaud et al., 2021; Jahed et al., 2020) used unpaired sampling design and investigated only relatively few individual trees for Oriental beech (371 and 405 respectively). In addition, we took account of unobserved TreMs, a statistical method that has never been used before and that proved effective.

The results of our TreM study across beech forests from France to Iran provided support for a higher TreM diversity in old-growth forest (H1a) and in forests with trees of larger mean DBH (H1b) even when controlled for sampling effort, but not for positive effects of admixed species (H1c) or in lower elevations (H1d). Support was also obtained for our hypothesis on an effect of elevation (H2b), but not of management (H2a) or admixed species (2c) on TreM assemblages. An influence of *Fagus* species was demonstrated only for TreM composition, but not diversity, supporting H3 only partially, but in line with previous finding by Jahed et al (2018). In the following, a detailed discussion of these findings is preceded by a discussion of the limitation of the study in terms of uneven methodology between sites, potential observer effects or the wide spatial scale.

In order to achieve our goal of covering as many regions of the east-west gradient of both beech species as possible, we were forced to combine data from two different sampling methods, fixed-radius circle, and angle-count. This of course affects the sampling effort per plot. However, as we show here, even within the same sampling technique the completeness of a plot sample varies along the environmental gradients. Under such conditions a focus only on the observed number of TreM types per tree or forest stand, ignores the variation in sample coverage among forests, analogous to a focus on the observed number of species in taxonomic studies on diversity. Collwell and Gotelli (2001) noted that such an approach is inappropriate in biodiversity studies if richness is not standardized according to the sampling effort, e.g., the number of specimens sampled in insect studies or the number of observations in bird studies. Our study of TreMs is the first showing that sample coverage did not vary randomly across

survey plots. Our finding of greater sample coverage in plots with a higher number of inspected trees demonstrates that TreM diversity in plots or forests with different numbers of trees, e.g., because of different DBH distributions, cannot be compared without statistical standardization, similar as the comparison of tree species in younger and older tropical forests (Collwell and Gotelli 2001). This effect was more pronounced when sampling TreMs within fixed areas, because of the larger proportion of smaller trees, which bear fewer TreMs, than when a Bitterlich approach was used. In addition, the significantly lower sample coverage in old-growth forests showed that they are regularly under-sampled for TreM diversity. Therefore, Chao & Jost (2012) recommended that diversity be compared at the same level of sample completeness, as was done in this study of TreMs. With our statistical approach, we solve two problems in one: firstly, the different recording methods in Ukraine and Switzerland on the one hand and the other countries on the other. Secondly, the problem of beech forests with different numbers of trees. Conceptually, the problems here are comparable to the recording of species diversity. A similar approach can be employed in future comparisons of TreM diversity in forests containing very different numbers of inspected trees or of forests with different types of management.

Another issue might be the role of observer effects (Paillet et al., 2015), although this might have a little impact in our study for three reasons. First, all pairs from old-growth and production forest were always sampled by the same observer. Secondly, all observations were carried out by persons who also had many years of experience with the TreM-typology of Larrieu et al., (2018). Thirdly, one observer (GM) sampled both oriental and European beech forests. A last topic affecting the results is tree height, as TreM detection is more difficult in taller trees (Santopuoli et al., 2020). This is an issue for all TreM studies and again can be considered only by methods accounting for the sample completeness, as we did with our approach. In summary, none of the issues is likely to have influenced our results. With the respect to our design one might ask the representativeness of our study. Here we have to admit that beech forests occur even wider as considered in our study particularly south - (e.g., Italy) and northwards (e.g., Sweden). However, our data cover the complete east-west gradient of both beech species for the first time covering a wide range of elevation where beech occurs. In Bulgaria, both beech species were even sampled in one country. This makes it the most comprehensive data set to date (Courbaud et al., 2021) and is likely to be rather representative for both beech species. Next studies should investigate similar pairs in Turkey and in Southern as well as Northern Europe to complete the picture of TreMs in beech forests.

A comparison of management type (production vs. old-growth) in the studied beech forests showed strong effects on the different TreM diversity metrics but not on TreM composition. An effect of management on TreM diversity is in line with a study in a montane beech-fir forest, which showed that management reduces tree species diversity and thereby the diversity and abundance of TreMs (Larrieu et al., 2012). Winter and Möller (2008) found both an increase in TreM abundance from managed to recently protected beech forests, with highest abundance found in long-protected beech forests. A higher abundance of TreMs in forest reserves than in production forests was also reported by Paillet et al. (2017). By contrast, a study of the beech forests of France found no difference in TreM diversity in production vs. old-growth forests (Vuidot et al., 2011). The latter finding might have been due to the short time since the old-growth forest stands were placed under protection (20 years on average) whereas the set-aside period required for TreM assemblages to return to their previous patterns after the last harvesting operation is 70–100 years (Bouget et al., 2014; Larrieu et al., 2012, 2017; Winter & Möller, 2008). In montane mixed forests, Larrieu et al. (2014) found a reduced richness of TreMs in production stands. For conifers, this decrease was mainly attributed to the reduction of the commercial DBH threshold, and for beech to the strong negative selection pressure resulting from the management decision to retain only those trees of high commercial value (Larrieu et al., 2014). In our model, we found an effect of old-growth despite controlling for the DBH, suggesting from DBH independent effects of protection on TreM diversity. The importance of controlling for indirect effects of management, beyond tree diameter, on TreM diversity is supported by Courbaud et al. (2021), who in a study of production forests showed a reduction in the rate of TreM formation, except polypores and buttress-root concavities, on European beech with increasing DBH. The slope, which is often steeper in old-growth forests, can also function as an indirect effect, by favoring the formation of reaction wood (Fournier et al., 2015) and therefore of buttress-root concavities (Larrieu et al., 2022). The formation of TreMs in trees with larger DBHs located on steep slopes in protected areas is promoted by rock falls (Stokes et al., 2007). Another indirect effect of management on TreM diversity is that of ownership (Johann & Schaich, 2016), with a higher TreM diversity in small-scale private forests, due to a lower logging intensity than in state forests. Logging as the cause of a reduced TreM diversity was also described in holm-oak-dominated Mediterranean forests (Regnery et al., 2013). Our production forest stands also represent a high diversity of management strategies. There might be a general gradient of decreasing systematic silviculture intensity in production forests from west to east, affecting our results. Such a view is supported by the findings of increasing functional diversity of saproxylic beetles by longitude within the range

of European beech forests (Gossner et al., 2013). However, to investigate this in more depth, more replicates of production beech forests have to be investigated along the longitude. In summary, these findings together with the results of our paired survey, extending from France to Iran, provide strong evidence that forest logging practices reduce TreM diversity beyond the mechanism of reducing the mean DBH of stands in forests dominated by *F. sylvatica* or *F. orientalis*.

Many TreM studies have focused on the relationship between TreM diversity and the DBH of single trees, with the results mostly showing a significant increase in TreM richness with increasing DBH (Augustynczik et al., 2019; Großmann et al., 2018; Johann & Schaich, 2016; Larrieu, Cabanettes, Brin, et al., 2014; Winter & Möller, 2008). However, only the largest trees are likely able to bear a high diversity of TreMs due to the time to accumulate them (Larrieu, Cabanettes, Brin, et al., 2014). Rather than single trees, the focus of our study was TreM diversity on the plot or stand scale of beech forests, and specifically on the mean DBH of trees per plot, with larger mean DBHs in plots containing fewer but larger trees, as is typical for the late succession phase of beech forests. Our systematic survey across production and old-growth beech forests of both *Fagus* species showed that, in assessments of rare TreMs, the mean DBH at the plot/stand scale can be used to predict TreM diversity throughout the distribution of beech, whereas the proportion of each DBH class and the number of very large trees can affect TreM diversity, depending on the phase of the silvigenetic cycle (Winter & Brambach, 2011).

Since beech is both shade-tolerant and long-lived, it often strongly dominates stands when environmental conditions are suitable (Rubio-Cuadrado et al., 2018). However, both pioneer (e.g. *Betula* spp.) and post-pioneer (e.g. *Fraxinus* spp. *Acer* spp.) tree species contribute to the diversity of TreMs at the stand scale (Larrieu et al., 2012; Vuidot et al., 2011), and likely play a key role throughout the silvigenetic cycle by providing TreMs when those from the dominant species beech are rare (Larrieu, Cabanettes, Gonin, et al., 2014). An increase in TreMs with increasing tree species richness was demonstrated in forests in the Carpathian and Dinaric mountains (Kozák et al., 2018). However, tree species richness in our study had no effect on TreM diversity in forests of either beech species, perhaps due to the higher TreM diversity of beech than of other species, as reported by Larrieu et al. (2012) and Vuidot et al. (2011) in the French Pyrenees and in French lowland forests, respectively. The very high diversity of TreMs across the range of both *Fagus* species implies a very high diversity of TreM-dwelling taxa. TreMs have been estimated to provide habitat for several thousand species, exemplified by the ~600 arthropod species present in the sporophores of tinder fungus (*Fomes fomentarius*) (Friess et al. 2019) and the ~380 species of beetles found in base rot-holes (Gouix & Brustel 2012).

Besides management, only elevation had a positive effect on TreM diversity, at least for rare and dominant TreM types. An increase in the diversity of TreMs with elevation was also observed in German broadleaf and conifer forests (Augustynczik et al., 2019) and in strictly protected forests of France (Paillet et al., 2017).

Our study provides new insights into the drivers of TreM assemblage composition, which thus far have hardly been examined. In addition to *Fagus* species, elevation was the most important determinant of TreM composition and its relevance increased for dominant TreM types (Figure 2). One reason might be that the reduced tree growth at higher elevations with harsher climates and slower growth leads to a greater accumulation of TreMs over time, as trees with a DBH range similar to that of trees at lower elevations tend to be older. This is in line with the results of Kozák et al. (2023), who found that common TreMs, such as rot-holes and concavities, are significantly influenced by tree age. It is also in line with the observation that critical thresholds for tree age with respect to the diversity of birds and lichens are higher in montane beech forests than in lowland beech forests (Moning & Müller, 2009). The increasing cloud cover at higher elevations, which favor specific TreM types such as epiphytes, may also play a role (Bässler et al., 2016; Moning & Müller, 2009).

Current evidence suggests that the *F. sylvatica* splitted from *F. orientalis* approximately eight million years ago (Renner et al., 2016), but so far only one study has compared their TreM composition (Jahed et al., 2020). In their survey of several primeval forest plots throughout the Hyrcanian Forest, one of the few primeval European beech forests in Ukraine, Jahed et al. (2020) found significant differences in the composition of TreMs on *F. sylvatica* vs. *F. orientalis*. A strong limitation of that study was that it only sampled one primeval *F. sylvatica* forest. However, our study, which covered a significant portion of *F. sylvatica* and *F. orientalis* and compared systematically both species with a number of replicates, also detected differences in the TreM composition of the two beech species. Interestingly, while a number of TreM types typical for *F. orientalis* were found, only one of these TreMs was identified as "indicators species" for *F. sylvatica*. This might have reflected the fact that *F. sylvatica* is the evolutionarily more recent species representing only a subset of phenotypes from the older *F. orientalis* and thus still lacks the full range of growth and microhabitat development. A difference in forest histories might also account for the difference.

5. Implications for research and management

Our comprehensive study of TreM diversity and composition in beech forests across eight countries covered the west-east range of *F. sylvatica* and *F. orientalis*. Our finding that TreM

diversity is sensitive to sample coverage, with Bitterlich sampling less sensitive than a fixed area count, should be taken into account in future TreM studies. The finding that TreM composition is mainly determined by *Fagus* species and elevation (Figure 4) suggests that management strategies to support TreM diversity at large spatial scales in beech-dominated forests should be applied to forests of both *Fagus* species and arranged along elevation gradients. Our results also showed a greater TreM diversity in long-term, old-growth beech forests and in forests at higher elevation, whereas the composition of TreM assemblages was less affected by management than by *Fagus* species, elevation, and, partly, the mean DBH at the stand scale. The effect of the particular beech species on TreM composition and assemblage should be studied by taking advantage of the presence of forests of both species in Bulgaria. In addition, studies following an historical ecology approach would allow a reconstruction of the long-term dynamics of European and Oriental beech forests. Since TreMs provide habitat for thousands of forest-dwelling species, managerial efforts to conserve biodiversity in forest ecosystems should include efforts to locally increase TreMs by increasing the area of long-term protected beech forests as well as the systematic retention of habitat trees in production forests. As TreM composition varies across the elevation gradient and between both species TreMs in beech forests at different elevations and in both *F. sylvatica* and *F. orientalis*, should be protected as well, similar to UNESCO's World Heritage declaration for forests of both species in lowlands and in the mountains.

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Data availability

Raw data for all analyses are publicly available from Figshare (https://doi.org/ 10.6084/m9.figshare.23912655).

Code availability

Annotated R code, including the data needed to reproduce the statistical analyses and figures, is publicly available from Figshare (https://doi.org/ 10.6084/m9.figshare.23912655). During review, code and data are available at: https://figshare.com/s/bdbe1a6b49d610287b30

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5.2 Paper N2 - "Drivers of Wood-Inhabiting Fungal Diversity in European and Oriental Beech Forests"

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Drivers of wood-inhabiting fungal diversity in European and Oriental beech forests

Giorgi Mamadashvili[1*], Antoine Brin[2], Maksym Chumak[4], Valeriia Diedus[3], Lars Drössler[5], Bernhard Förster[6], Kostadin B. Georgiev[7], Tigran Ghrejyan[8], Ruslan Hleb[9], Mark Kalashian[8], Ivan Kamburov[10], Gayane Karagyan[8], Joni Kevlishvili[11], Zviad Khutsishvili[11], Laurent Larrieu[12,13], Meri Mazmanyan[8], Peter I. Petrov[14], Levan Tabunidze[15], Claus Bässler[16,17,**], Jörg Müller[1,17, **]

[*]Corresponding author at: Field Station Fabrikschleichach, Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Glashüttenstraße 5, 96181 Rauhenebrach, Germany.

Email address: Giorgi.mamadashvili@uni-wuerzburg.de

[**]shared last author.

[1] *Field Station Fabrikschleichach, Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Glashüttenstraße 5, 96181 Rauhenebrach, Germany*

[2] *University of Toulouse, Ecole d'Ingénieurs de PURPAN, UMR INRAE-INPT DYNAFOR, Toulouse, France*

[3] *State Museum of Natural History, National Academy of Sciences of Ukraine, Teatralna str., 18, Lviv, 79008, Ukraine*

[4] *Uzhhorod National University, Department of entomology and biodiversity preservation, Voloshyna str. 32, Uzhhorod, 88000, Ukraine*

[5] *Forestry Research and Competence Center ThüringenForst AöR, Gotha, Germany*

[6] *Chair for Strategic Landscape Planning and Management, Technical University of Munich, Freising, Germany*

[7] *Hessian State Agency for Nature Conservation, Environment and Geology, Germany*

[8] *Laboratory of Entomology and Soil Zoology, Scientific Center of Zoology and Hydroecology NAS RA, P. Sevak str., 7, Yerevan, 0014, Armenia*

[9] *Carpathian biosphere reserve, Krasne Pleso St. 77, Transcarpathian Region Rakhiv 90600, Ukraine*

[10] *Strandzha Nature Park Directorate, 1 Yanko Maslinkov St., 8162, Malko Tarnovo, Bulgaria*

[11] *Biodiversity Conservation Center "Nacres", B. jgenti str. 34, Tbilisi, Georgia*

[12] *Université de Toulouse, INRAE, UMR DYNAFOR, Castanet-Tolosan, France*

[13] *CNPF-CRPF Occitanie, France*

[14] *University of Forestry Sofia, Field base Petrohan, 1 Petrohanska Str., 3520 Barzia, Bulgaria*

[15] *Caucasus Nature Fund – CNF, 2 Brose Str., 0108 Tbilisi, Georgia*

[16] *Ecology of Fungi, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Germany*

[17] *Conservation and Research Department at the Bavarian Forest National Park, Freyunger Str. 2. 94481 Grafenau, Germany*

Abstract

The hyperdiverse wood-inhabiting fungi play a crucial role in the global carbon cycle, but often are threatened by deadwood removal, particularly in temperate forests dominated by European beech (*Fagus sylvatica*) and Oriental beech (*Fagus orientalis*). To study the impact of abiotic drivers, deadwood factors, forest management and biogeographical patterns in forests of both beech species on fungal composition and diversity, we collected 215 deadwood-drilling samples in 18 forests from France to Armenia and identified fungi by meta-barcoding. In our analyses, we distinguished the patterns driven by rare, common, and dominant species using Hill numbers. Despite a broad overlap in species, the fungal composition with focus on rare species was determined by *Fagus* species, deadwood type, deadwood diameter, precipitation, temperature, and management status in decreasing order. Shifting the focus on common and dominant species, only *Fagus* species, both climate variables and deadwood type remained. The richness of species within the deadwood objects increased significantly only with decay stage. Gamma diversity in European beech forests was higher than in Oriental beech forests. We revealed the highest gamma diversity for old-growth forests of European beech when focusing on dominant species. Our results implicate that deadwood retention efforts, focusing on dominant fungi species, critical for the decay process, should be distributed across precipitation and temperature gradients and both *Fagus* species. Strategies focusing on rare species should additionally focus on different diameters and on the conservation of old-growth forests.

Key words: *deadwood fungi, Fagus sylvatica, Fagus orientalis, habitat heterogeneity, species diversity, Hill numbers*

Introduction

In the western part of the Palearctic, temperate forests are dominated by two species of *Fagus:* European beech *Fagus sylvatica* in the West and Oriental beech *Fagus orientalis* in the East, *ranging* from eastern Bulgaria to the Hyrcanian forest in Iran. The latter are often Arcto-tertiary relicts, harboring many endemic plants and animals (Aliev 2021; Fayvush & Aleksanyan 2021; Goginashvili & Tvauri 2013; Mathew *et al.* 2000). While for European beech the impact of forest management on several taxa has been well studied during the last decades (Brunet *et al.* 2010; Gossner *et al.* 2013; Hagge *et al.* 2019; Morales-Hidalgo *et al.* 2015; Ódor *et al.* 2006), drivers of biodiversity in Oriental beech are not well understood. Most of the studies in European beech forests have identified the loss of old growth trees and the reduction of deadwood amount by forest management as critical for biodiversity (Brunet *et al.* 2010;

Gossner *et al.* 2013). As one consequence, the amount of deadwood has been selected as one of nine pan European indicators for maintenance, conservation, and appropriate enhancement of the biological diversity in forest ecosystems (Schuck *et al.* 2015).

Aware of the global responsibility for beech forest ecosystems, conservation strategies have increasingly focused on these ecosystems. Today, European beech forests are well represented in the Natura 2000 network of protected areas (Bohn & Neuhäusl 2000), while Oriental beech forests of the Caucasus region belong to the Emerald Network of Areas of Special Conservation Interest (Artsivadze *et al.* 2018; Fayvush *et al.* 2016). Moreover, several remnants of natural beech forests have received the UNESCO World Heritage designation, including "The Ancient and Primeval Beech Forests of Carpathians and Other Regions of Europe," "Colchic Rainforests and Wetlands," and the "Hyrcanian Forest" (UNESCO 2013, 2019, 2021). Despite the unique fauna and flora of these Tertiary relicts, many managers of Oriental beech forests are often not aware of the key role of deadwood for biodiversity and ecosystem functioning. This became particularly clear when Iran decided to protect the whole Hyrcanian forest and restricted tree removal on deadwood and old growth trees, which would cause a major threat to deadwood organisms (Müller *et al.* 2016, 2017). This underlines the need to combine findings and research from regions of both beech species. Moreover, in the context of climate change, European forest managers are considering Oriental beech as a potential tree in the future (Mellert & Šeho 2022). This seems justified because a recent presence of this species in Italy is proved from 45.000 year old DNA pollen samples (Paffetti *et al.* 2007) and because many saproxylic species specialization to trees is more at the genus than the species level as shown recently for saproxylic beetles (Vogel *et al.* 2020).

Fungi form a functionally important and hyper-diverse group in beech forests, acting as mycorrhiza symbionts supporting forest productivity and as key decomposers of litter and deadwood. During deadwood decomposition, many coexisting fungi create habitat heterogeneity and new resources for many other wood inhabiting organisms, and play a crucial role in nutrient cycling processes (Boddy 2001; Friess *et al.* 2019; Gessner *et al.* 2010; Parisi *et al.* 2018; Valentín *et al.* 2014). The lignin barrier with the cellulose and hemicelluloses of deadwood which hinders the polysaccharides from microbial decomposition is only disintegrated and opened for other deadwood dwelling organisms by the help of fungi species and their various secretion of enzymes (Dix & Webster 1995; Fengel & Wegener 1983; Floudas *et al.* 2012; Hoppe *et al.* 2016; Liers *et al.* 2006; Stokland *et al.* 2012).

The drivers of wood-inhabiting fungi diversity in beech forest depends on spatial scale. At the local scale of a deadwood object, the tree species, decay stage, type of deadwood and microclimate are important drivers (Baber et al., 2016; Englmeier et al., 2023; Purahong et al., 2018; Krah et al., 2018; Müller et al., 2020; Rajala et al., 2012; Daniel & Nilsson, 1998; Rayner & Boddy, 1988), all affected by local forest management (Abrego *et al.* 2017a; Bässler *et al.* 2014; Müller *et al.* 2007). At the larger scale, connectivity and macroclimate became more important. For example, Abrego et al., (2015, 2017) and Heilmann-Clausen et al., (2014) identified forest connectivity, condition and decay stage of substrates and the climate across European beech forests as the most important factor for fungal species communities in protected areas. Furthermore, Ódor et al., (2006) investigated semi-natural beech forests in Europe and showed that diversity of saproxylic organisms are driven mostly by climate and forest management, deadwood volume and habitat fragmentation. Finally, Hagge et al., (2019) showed that functional diversity of wood-inhabiting fungi European wide are determined by latitude, elevation, forest cover and urbanization.

Despite an increasing interest in beech forests of both *Fagus* species, systematic studies among the entire beech forest belt from France to Caucasus and Iran are widely missing. However, only in such a synopsis it is possible to test the influencing factors of the two beech species in comparison to local factors such as deadwood type, decomposition, diameter, climate, and management for biodiversity. To unify biodiversity research of wood-inhabiting fungi in European and Oriental beech forests, and to guide future conservation strategies, we conducted a sampling campaign from France in the West to Armenia and Georgia in the East. Using drilling samples and metabarcoding, we aimed at identifying the drivers of fungal communities in *Fagus* forests of both species. In specific, we were interested in the impact of 1) large-scale factors, such as the *Fagus* species and the two climate variables temperature and precipitation; 2) stand scale factors such a forest management; 3) and small-scale factors like type of deadwood, decay stage, and diameter of the tree objects. For this, we used the concept of Hill numbers and focused on rare, common and dominant species (Hill 1973). The Hill numbers concept was applied for the tree-object scale (α-diversity) and the scale of overall production and old-growth beech forests of both *Fagus* species (γ-diversity).

Material and methods

Study areas

We collected drilling samples from 215 deadwood objects in different type of deadwood in production and old-growth beech dominated forest stands in six countries during 2021-2022

summer and autumn seasons: France, Germany, Ukraine, Bulgaria, Georgia and Armenia. Bulgaria is the only country with both species of *Fagus*, where European beech from west to east is replaced by Oriental beech. The 18 forests investigated ranged from 65 to 1770 m above sea level (Table 1). For each plot we extracted the extracted and used the local climatic variables mean annual temperature BIO1 and annual precipitation (BIO12) from WorldClim (Hijmans *et al.* 2005) grid data in 30 s resolution and calculated mean values for a 1-km radius around sampled localities, following the methodology of (Gossner *et al.* 2013). Both variables were correlated with a Pearson's correlation coefficient of -0.54, allowing simulatanouse use in one model.

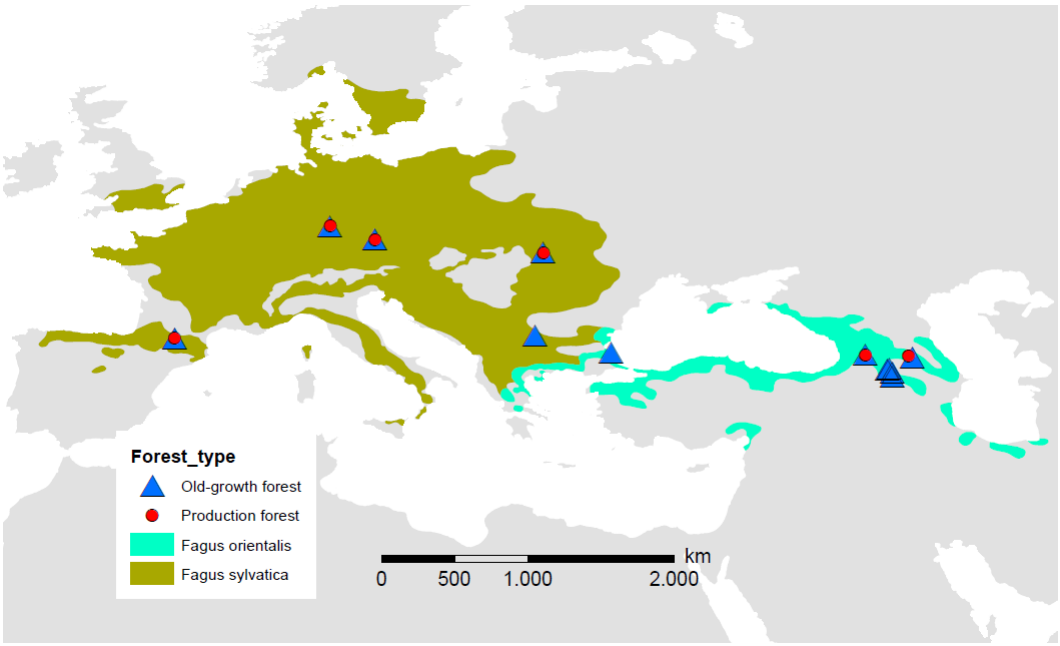

Fig. 1: Geographical distribution of European (*Fagus sylvatica*) and Oriental (*F. orientalis*) beech (Caudullo *et al.* 2017) and the location of the 18 sampling stands (old-growth and production forest stands) extending from the French Pyrenees to Armenia-Caucasus, investigated for saproxylic fungi communities in deadwood.

Table 1. Description of study sites and samples: FRA-France, GER-Germany, BUL-Bulgaria, UKR-Ukraine, GEO-Georgia, and ARM-Armenia; Fs-*Fagus sylvatica*, Fo-*Fagus orientalis*; Types: D = downed deadwood, S = standing deadwood, St = stump.

Country	Region	Region2	Forest type	Label	Tree species	Latitude	Longitude	Elevation	Samples	Diameter [cm]	Decay	Types
France	Montreich	FRA_Fs	Production	FrMPFs	Fs	42.98643	0.97321	811-984	11	30-45	2-4	D, S
France	Montreich	FRA_Fs	Old-growth	FrMOFs	Fs	42.99055	0.96659	951-958	11	30-75	2-4	D, S
Germany	Mittesteighuette	GER_Fs	Old-growth	GrMOFs	Fs	49.09767	13.24844	718-774	17	60-120	1-4	D, S

Germany	Scheuereck	GER_Fs	Production	GrSPFs	Fs	49.06692	13.30664	765-831	14	22-78	1-4	D, S
Germany	Steigerwald	GER_Fs	Production	GrTPFs	Fs	49.93008	10.55793	438-475	19	18-84	1-4	D, S, St
Germany	Steigerwald	GER_Fs	Old-growth	GrTOFs	Fs	49.86061	10.49864	355-404	20	60-120	2-4	D, S
Bulgaria	Berkovitsa	BUL_Fs	Old-growth	BuBOFs	Fs	43.15803	23.12848	600-1465	11	30-62	3-4	D
Bulgaria	Strandhza	BUL_Fo	Old-growth	BuSOFs	Fo	42.10796	27.78437	65-471	18	16-50	2-4	D, S
Ukraine	Mala Uholka	UKR_Fs	Old-growth	UkMOFs	Fs	48.26886	23.62126	742-799	22	18-72	1-4	D, S
Ukraine	Velyka Uholka	UKR_Fs	Production	UkVPFs	Fs	48.22146	23.65095	325-530	21	10-58	1-4	D, S
Georgia	Borjomi	GEO_Fo	Production	GeBPFo	Fo	41.96267	43.44907	880-1113	10	50-92	1-4	D, S
Georgia	Borjomi	GEO_Fo	Old-growth	GeBOFo	Fo	41.97949	43.45001	994-1160	9	26-46	1-4	D, S
Georgia	Lagodekhi	GEO_Fo	Old-growth	GeLOFo	Fo	41.84729	46.32148	480-1005	11	28-52	1-3	St
Georgia	Mtisdziri	GEO_Fo	Production	GeMPFo	Fo	41.91701	46.09561	770-1128	13	40-82	1-4	S
Armenia	Antaramej	ARM_Fo	Old-growth	ArAOFo	Fo	40.66068	45.07848	1768-1770	2	30-44	3-4	S, St
Armenia	Teghut	ARM_Fo	Old-growth	ArTOFo	Fo	41.09047	44.81056	1120-1042	2	51-75	1-3	D
Armenia	Yenokavan	ARM_Fo	Old-growth	ArYOFo	Fo	40.90897	45.06504	1388	1	22-22	3-3	St
Armenia	Zikatar	ARM_Fo	Old-growth	ArZOFo	Fo	41.12143	44.92292	1280	3	32-42	2-3	D, S

Field sampling

During the field sampling, we selected 10 deadwood beech items per site. Each item was a natural snag, natural log, or simply a stump. We chose only beech deadwood to exclude tree species effects beyond the genus *Fagus*. For each deadwood item, we recorded the type of deadwood (snag/standing tree, log, and stump), the diameter at breast height (DBH), the decay class (1-4; early, late early, middle, and late) (Müller-Using & Bartsch 2009), the coordinates (WGS84 world), the elevation and the *Fagus* species.

Fungal sampling

For the collection of the molecular fungal community, we followed Rieker et al., (2022) sample protocol using disposable laboratory gloves (fresh pair for each item and disinfection before sampling), a knife for removing the outer bark surface of drilling position (to avoid contamination by random attached fragments and spores), spray bottle with 75% ethanol do disinfect the gloves and the drill after each object, Bunsen burner for flaming the drill and knife, the cordless drill with auger bits (10mm diameter, 300 or 400 mm lengths), object with a diameter above 38 cm we drilled from both sides. We used Ziploc bags for collecting and storing the samples and labeling. And cool box with cold packs for transportation. Immediately placed into the freezer by -20°C.

Laboratory work

DNA extraction and isolation

The total community DNA was extracted from 0.150 g homogenized wood samples using NucleoSpin Soil, Mini kit for DNA (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) following the manufacturer's protocol. Bead beating was run on a FastPrep-24 instrument (MPBiomedicals; 2 cycles of 30s at speed 6.5). DNA concentrations were quantified using a NanoDrop UV-Vis spectrophotometer (Peqlab Biotechnologie GmbH, Erlangen, Germany). For sequencing the internal transcribed spacer (ITS2) regions of the fungal 18S rRNA gene, we applied for two-step, Nextera barcoded PCR libraries using the locus specific primer pair ITS3 (5′- GCA TCG ATG AAG AAC GCA GC -3′) and ITS4 (5′- TCC TCC GCT TAT TGA TAT GC -3′) with 20 PCR cycles for the first step and 15 PCR cycles for the second step were created. Subsequently the PCR libraries were sequenced on an Illumina MiSeq platform using a v2 500 cycles kit.

Amplicon-metagenomics data analysis

The produced paired end reads, which passed Illumina's chastity filter, were subject to de-multiplexing and trimming of Illumina adaptor residuals using Illumina's bcl2fastq software version v2.20.0.422. The quality of the reads was checked with the software FastQC version 0.11.8 and sequencing reads that fell below an average Q-score of 24 or had any uncalled bases (N) were removed from further analysis. The locus specific ITS2 primers were trimmed from the sequencing reads with the software cutadapt v3.2. Paired-end reads were discarded if the primer could not be trimmed. Trimmed forward and reverse reads of each paired-end read were merged to *in-silico* reform the sequenced molecule considering a minimum overlap of 15 bases using the software USEARCH version 11.0.667. Merged reads that contained ambiguous bases or were outliers regarding the expected amplicon size distribution were also discarded. Samples that resulted in less than 5000 merged reads were also discarded, to not distort the statistical analysis. From the remaining reads the fungal ITS2 subregions were extracted with help of the ITSx software suite v1.1.3 and its included database. The surviving reads were denoised using the UNOISE algorithm implemented in USEARCH to form zero-radius OTUs (zOTUs) also named amplicon sequence variants (ASVs) discarding singletons and chimeras in the process. The resulting OTU abundance table was then filtered for possible barcode bleed-in contaminations using the UNCROSS algorithm. OTU sequences were compared to the reference sequences of the UNITE database provided by https://www.drive5.com/usearch/manual/sintax_downloads.html, taxonomies were predicted and confidences were calculated using the SINTAX algorithm implemented in USEARCH. The identification revealed very similar proportions of unidentified OTUs with 43% in *Fagus orientalis* and 40% in *Fagus sylvatica* (Tab. S1). DNA extraction, library construction,

sequencing and data analysis described in this section were performed by Microsynth AG (Balgach, Switzerland). For a list of OUT identifications, see Tab. S1.

Statistics

All statistical analyses were performed using R 4.3.1 (R Core Team 2021). To determine fungal species richness and community composition, we followed two approaches. First, we used the observed species after excluding the records with only one read (Adamo *et al.* 2020). Removal of these records increase data quality for further analysis (Tedersoo *et al.* 2022). Second, we rarefied each community matrix (function *rrarefy*, package *vegan* by Oksanen et al., 2020). To determine a suitable rarefaction depth, we first calculated the read sums for each sample. Based on this exercise, we decided to use a minimum of 990 reads per samle as rarefaction depth. As diversity analyses of communities from both approaches revealed very similar results, we present the results only for the observed reads (first approach). We then calculated species richness for each object. Here, we are aware that OTUs are not equivalent to species but for reasons of readability, we chose the term species throughout the manuscript. Community matrices based on each log was calculated along the Hill numbers in 'ecodist' package (Goslee & Urban 2007) for dissimilarity indices representing a focus on rare (q=0, Jaccard Index), common (q=1, Horn Index) and dominant (q=2, Morisita Horn) species (Chao *et al.* 2014). This allowed giving increasing weights to species with high abundances.

Different predictor sets for fungi community compositions at the object level were tested using multiple regression on distance matrices (MRMs) (Lichstein 2007) as follows: First distance matrices for the fungi composition for q=0, 1, and 2 were created. In a second step, distance matrices were created for the management type (production\old-growth forests, gower distance), diameter of the deadwood object (Euclidian distance), temperature (Euclidian distance), precipitation (Euclidian distance), decay stage (Euclidian distance) and *Fagus* species (gower distance). Since the two species are distributed along a longitudinal gradient, the predictor distance beech species was replaced in a second approach by a spatial distance (Euclidian distance) that considers the nested structure of the plots in the stands (Mamadashvili *et al.* 2023).

To model the species richness per deadwood object, we used a multiple negative binomial model as species numbers are count data. We included sampling site as a random factor to account for replicated measurements of different objects in a forest. As predictors, we used *Fagus* species, temperature, precipitation, forest type, deadwood type, wood decay and

diameter (see Table S1). We finally repeated all analyses based on the rarefied communities (see above). As this did not change the results, we present these results only in the supplement.

To compare the gamma diversity of wood-inhabiting fungi in production and old-growth forests of both *Fagus* species, we fitted rarefaction-extrapolation curves across all objects of each category based on the incidences of fungi species per object using the function iNext in package iNext (Hsieh *et al.* 2016). To account for unequal sample coverage in the four categories due to variation in sampling size or even natural variation in sample completeness, we standardized by sample coverage as recommended by Chao & Jost (2012). Non-overlapping confidence intervals indicate significant differences.

Results

In total, we found 548 OTUs in 215 deadwood objects of beech. European beech revealed more unique OTUs than Oriental beech, but the majority (62%) could be found in both (Fig. 2). Moreover, it is important to note that twice as many deadwood items were sampled in European beech (n = 146) than in Oriental beech (n = 69) sampling sites (Fig. 2).

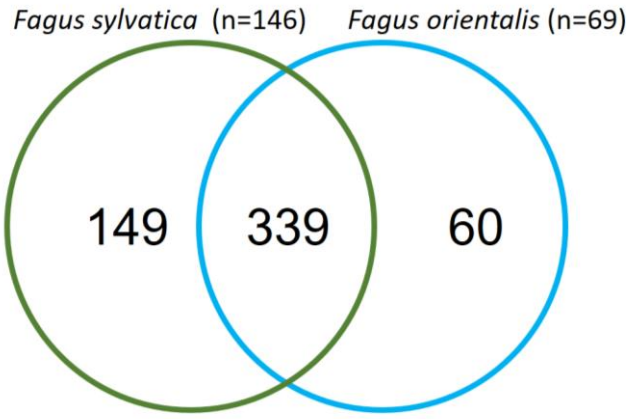

Figure 2: Venn Diagram of fungal OTUs in the two *Fagus* species collected in 18 beech dominated forests, n – deadwood items sampled.

Overall, the explained variance in fungal composition on the single object was low (0.22-0.35%), however, the multiple regression on distance matrices, identified significant environmental variables determining the community composition (Fig. 3). For q = 0, the *Fagus* species, the type of deadwood, the diameter, the precipitation, and management were identified as significant variables in descending order. With increasing Hill-numbers only *Fagus* species, temperature, precipitation, and type of deadwood remained significant determinants for

community composition (Fig. 3). However, as illustrated in Figure 4, the communities of both species showed a large overlap in wood-inhabiting fungi. Substituting the distance matrix *Fagus* species by a spatial distance matrix revealed similar results (Fig. S1), indicating that the effect of *Fagus* species and space cannot be distinguished due to the biogeographical distribution of the two *Fagus* species. These results were robust even when using the rarified species community (see R code in SM and Fig. S2).

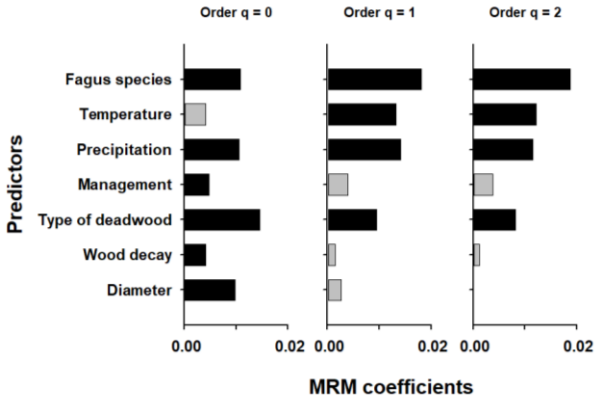

Figure 3: Multiple regression on distance matrices (MRM) coefficients of the predictors of the composition of fungi in 215 deadwood objects located in 18 beech dominated forests. Black bars indicate significance in MRM, with $p < 0.05$. Results are shown for rare (q=0), common (q=1) and dominant (q=2) fungi species, grey-nonsignificant. *Management* compared production with old-growth forest stands, and *Fagus species* compared *F. sylvatica* with *F. orientalis*.

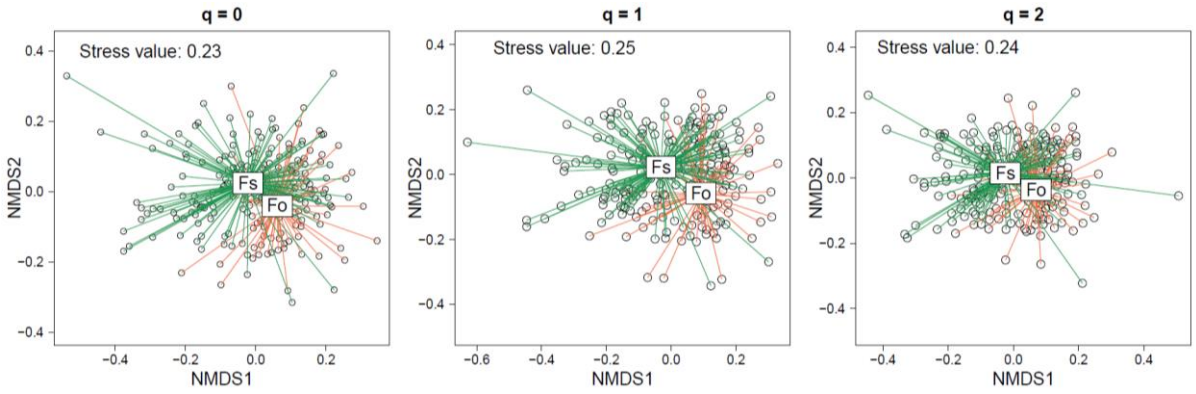

Figure 4: Community composition of fungi species identified by meta-barcoding from 215 beech deadwood objects collected from *Fagus sylvatica* (Fs) and *Fagus orientalis* (Fo). Distance matrix for q=0 is based on Jaccard Index, for q=1 on Horn, and for q=2 on Morisita-Horn as in Fig. 3.

The richness of the single deadwood object (alpha-diversity) was more or less independent from our predictors with one exception. With increasing decay stage, the richness per object increased significantly, which was again robust to the use of raw or rarified communities (Table S2).

Grouping all objects in four categories made by the combination of two *Fagus* species and the two management status (old-growth vs. production), the rarefaction-extrapolation curves showed higher beta-diversity for both groups from European beech than in Oriental beech with focus on rare species at the same sample coverage (Fig. 5, q=0). Focusing on dominant species, the highest diversity was found in old-growth European beech forests (Fig. 5, q=2).

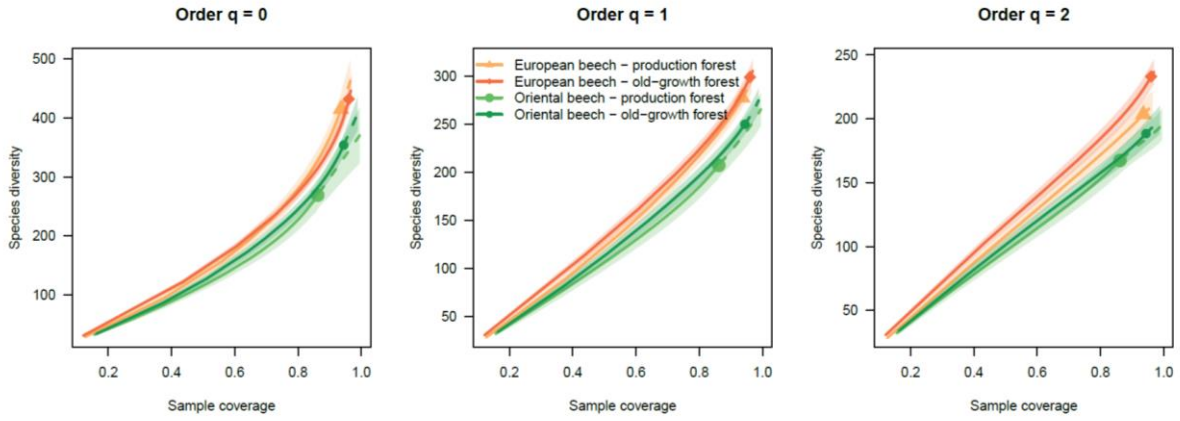

Figure 5: Gamma diversity of Beech forests of both *Fagus* species and in production as well as old-growth sites using rarefaction-extrapolation curves based on sample coverage. Solid lines indicate rarefaction, dashed lines extrapolation curves. Non-overlapping confidence bands indicate significant difference.

Discussion

Our systematic investigation of wood-inhabiting fungi in beech forests from France to Armenia revealed overall high similarity in species composition and a difference in □-diversity only by differences in decay stages. However, the species composition in European and Oriental beech revealed to be significantly different for all Hill numbers. Second, preciptation determined the species composition in all three Hill numbers. This underlines that large-scale drivers including the both *Fagus* species and climate drive the overall very similar community of wood-inhabiting fungi. Local factors as deadwood type, decay stage and diameter were only relevant for distinguishing composition of rare species.

Fagus species is important

Current research of fungal communities related to different host species compared mostly different host tree genera showing distinct fungal communities (Abrego *et al.* 2017b; Englmeier *et al.* 2023; Krah *et al.* 2018; Purahong *et al.* 2018). Therefore, our knowledge on differences in fungi within a host genus is rather scarce. Here our findings are a first confirmation that fungal species might differ even between very similar tree species within a genus. However, if looking at the distribution of OTUs between the two Fagus species and taking into account the OTUs that could be detected in at least 10 samples, there were hardly any OTUs that only occur in one of the two Fagus species (Tab. S1). If this was the case, it was mostly unidentified species and exclusively on *Fagus sylvatica* but not on *Fagus orientalis*. This emphasizes that there are hardly any more frequently occurring unique fungal species/OTUs in *Fagus orientalis* to be expected.

The limitation for identifying the role of host tree identity versus biogeography remains in our study, because both beech species are spatially separated from West to East. Hence, finally we cannot distinguish between the impact of bioregion, history (Tertiary relicts in Oriental beech), and the host species and its traits itself. However, we can state that there are differences in fungi communities in deadwood of both species. Even earlier studies in European beech forests by Heilmann-Clausen et al., (2014) demonstrated a longitudinal effect on the composition of fungi in European beech forests and was discussed as an effect of differences in climate and land use history. Finally it is important to consider that some studies have shown that climate influence wood properties in *F. orientalis* (Topaloğlu *et al.* 2016) or in both *Fagus* species (Elzami 2018), which might affect the fungal composition.

We focused in this study on fungi species identified only via metabarcoding of wood samples. Studies on host effects with sporocarp surveys and metabarcoding revealed always a strong effect of the host identity on community composition in sporocarp datasets (Müller *et al.* 2020). Here sporocarp records represent probably more the dominant species in deadwood objects. This is further supported by the fact that the impact of host species for fungi by metabarcoding increased towards common and dominant species along the Hill numbers in (Müller *et al.* 2020). Similarly, in our data the role of the *Fagus* species increased towards dominant species (Fig. 3). In summary, we found either relatively similar communities or more diverse communities on the □- or on the □-level in much younger European beech forests than in the Tertiary relicts of Oriental beech forests.

Precipitation is always important, temperature towards dominant species

A second important variable for determining fungi community composition, for all Hill numbers, was precipitation (Fig. 3). In contrast, temperature proved to be important only for communities focusing on typical and dominant species (Fig. 3). Beech forests form very different temperate forest ecosystems from lowland to montane levels in both European and Oriental beech. With increasing elevation, the precipitation regularly increases and temperature decreases which is one of the fundamental drivers for fungal composition (Bässler *et al.* 2010a; Xu *et al.* 2023). Similarly, Heilmann-Clausen et al., (2014) identified elevation as an important driver for turnover in fungal species communities within European beech reserves in Europe as well. In contrast, along a local elevation gradient Bässler et al., (2010) found structural parameters more important than the elevation gradient, which could not be confirmed in our large-scale study. However, in most of these local studies (Bässler *et al.* 2010b) temperature and precipitation are to highly correlated to be distinguished. Here, our wide range of plots and only limited correlation of both, allowed to identify precipitation as more important in general and particularly for rare species communities, while the dominant communities were affected by both. This contrasts with studies from central Europe using wood-inhabiting fungi in specific (Heilmann-Clausen *et al.* 2014) and overall fungal diversity considering a broad range of guilds (Andrew *et al.* 2018). However, our spatial scale clearly exceeds the scale used in these studies which might explain the observed difference. Indeed, within increasing scale, both precipitation and temperature become important and have been suggested to drive global fungal diversity (Mikryukov *et al.* 2023; Tedersoo *et al.* 2014; Větrovský *et al.* 2019). As climate changes, temperature and precipitation are changing and many species shift their geographic range in these long-lasting ecosystems (Antão *et al.* 2022). From our findings, this is to be expected also for wood-inhabiting fungi (Bässler *et al.* 2010b, 2016).

Gamma diversity of managed and unmanaged beech forests of both species

Splitting the samples in both beech species and management types, we found the highest diversity of dominant species in old-growth European beech forests, but not in production forests. No difference could be found between both management types in Oriental beech (Fig. 5). This might indicate more impactful silviculture treatment in European beech than in Oriental beech forests, leading to reduction of functional diversity in the former in terms of deadwood (Gossner *et al.* 2013). However, the highest diversity in old-growth European beech might be surprising as Oriental beech forests are in generally much older and some of them untouched at least since many centuries (Aliev 2021; Kurz *et al.* 2023; UNESCO 2013, 2019). Moreover,

Oriental beech seems to be the older beech species harboring high levels of genetic diversity and should promote the diversity of its inhabitants (Azaryan *et al.* 2022; Cardoni *et al.* 2022; Kurz *et al.* 2023; Müller *et al.* 2016). On the other hand, the geographical range of European beech is much larger (see Fig. 1) and in general larger host ranges lead to higher diversity (Brändle & Brandl 2001). Additionally, the larger the range in European beech the broader the climate niche can be assumed, which then should promote diversity of fungi (Elzami 2018). Although, climate niche studies did not confirm a broader niche width in Oriental versus European beech (Mellert & Šeho 2022). Another reason might be larger phylogenetic tree diversity in European beech forests with oak in the lowlands and conifers as silver fir or spruce in higher altitudes, supporting more fungal species which might then jump over to beech deadwood (Krah *et al.* 2018). In contrast, most *Fagus orientalis* forests (with exception of Borjomi) lack conifers exhibiting in total a lower phylogenetic gamma diversity. On the other hand, wood-inhabiting fungi are highly mobile. Therefore, historically younger old-growth forests might be colonized successful by more dominant fungi species, which still coexist. In contrast, it might be that over the time since the Tertiary diversity of dominant fungi has shrunk as result of the high and long-lasting competition. However, this suggestion remains to be confirmed in future studies, e.g. by experiments as in Englmeier *et al.* (2023).

Management affected the species composition of rare species, but not of common or dominant species. Here we have to keep in mind that this effect was controlled for structural elements as deadwood type, diameter, or decay stage, which are respectively influenced by forest management as well. This means that silvicultural management has comprehensive impacts on fungal composition at least for rare species. Such subtle effects of management on fungal communities have been shown along regional logging intensity gradients in European beech forests in Germany (Bässler *et al.* 2014; Müller *et al.* 2007) or in Spain (Abrego & Salcedo 2011), where fungal communities in old reserves differed than communities in production forests. These studies identified species promoted or negatively affected by increasing forest management (Abrego *et al.* 2017b; Bässler *et al.* 2014).

Type of deadwood is important for rare species

Different types of deadwood offer very different substrates, e.g., from wet to dry (microclimate) conditions. This opens different environmental conditions for different species. Therefore, forests with high variation of deadwood types provide more different niches for more species (Uhl *et al.* 2022). In contrast, even large amount of deadwood dominated by similar types as after large scale disturbance, can lead to reduced fungal diversity (Beudert *et al.* 2015), because

wood-inhabiting fungi are highly competitive (Boddy 2021; Fukami *et al.* 2010). Regularly some dominant species occupy the major deadwood resources successfully and outcompete other species (Vogel *et al.* 2017). This has been shown on the scale of petri dishes in the lab (Fukami *et al.* 2010), to field experiments (Hagge et al., 2019) and indicated by global experiments (Seibold *et al.* 2021). As a consequence, fungi often show overdispersion in assembly patterns and have more species on more different objects as small twigs, when total resource volume is standardized (Bässler *et al.* 2014; Heilmann-clausen & Christensen 2004) and the decay is faster with a few dominant but efficient species than with a high diversity of species including many rare ones (Fukami *et al.* 2010). To escape the competition pressure, it seems therefore of advantage that deadwood is offered in many different types, which might have affected the composition in our data with focus on rare ones.

Diameter is important for rare species

Bader et al., (1995) studied the deadwood size as an indicator for fungal diversity and showed that some species prefer well decayed and large logs, which is why such species were well abundant in old growth forest sites but they became rare with increasing human activities-cuttings. In addition, according to Küffer & Senn-Irlet (2005) more species of fungi tend to be on deadwood with various diameters. However, Heilmann-Clausen & Christensen, (2004) confirmed that the dead small trees and branches host higher diversity then large trees and larger logs do. Also, in production forest stands the main driver of deadwood fungi (and other saproxylic organisms) diversity is fine woody debris, which creates a general deadwood volume (Brabcová *et al.* 2022).

Wood decay stage as driver for species richness

Deadwood decomposition is a succession with distinct species turnovers over time in all organisms, including fungi (Fukasawa 2018). Lindblad, (1998), Fukasawa et al., (2009) and Pouska et al., (2016) found a significant effect of deadwood decay stage on fungi species richness and community composition. In systematic surveys by fruiting bodies and metabarcoding the decay stage was critical for fungal species composition along the Hill numbers (Müller *et al.* 2020). This is in contrast with our study, as we could not confirmed these findings with our results. Here the main reason might be that our sampling did not cover a broader range of decay or the pattern on the large scale was overridden by other factors. However, we found decay stage as the only predictor to increase α-diversity per log (Table S1), which is in line with the findings from Bader et al., (1995) and Kubartová et al., (2012)

Conclusion for biodiversity conservation and forest management under climate change

Our study provides some important implications for conservation and forest management. First, the high diversity and similarity of wood-inhabiting fungi in beech forests across the 4k kilometer of temperate forest belt supports the view of a highly mobile organism group with a lot of functional insurance in rare and dominant species. From a mycological perspective the skepticism against the usage of Oriental beech in silviculture management in Western Europe seems not to be justified. Reason for that is the fact that fungi communities of both *Fagus* species are very similar. For conservation, our results show that effort should be put on establishment of protected areas in different climate conditions for both *Fagus* species, as currently mirrored in the Natural Heritages. For local managers interested in enhancing diversity of wood-inhabiting fungi the retention of different types of beech deadwood seems promising.

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Author's contribution:

GM, JM, and CB developed the underlying study design. Fieldwork management was done by GM, LD, KG, MK, LL and LT. Field work and drilling samples were collected by GM, AB, MC, VD, TG, RH, IK, GK, JK, ZK, MM and PP. BF, GM, JM and CB analyzed the data and built the manuscript's main draft. All co-uthors participated in the revision of the manuscript and approved the final version.

Conflict of interest

The authors have no conflict of interest.

Data availability

Annotated R code, including the data needed to reproduce the statistical analyses and figures, is publicly available from figshare (DOI: 10.6084/m9.figshare.24720003). During review, code and data are available at: https://figshare.com/s/5e64d74d00e18a27f402

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5.3 Paper N3 - "Click beetles (Elateridae) identify conservation units in Oriental and European beech forests"

submitted to "Journal of Insect Conservation"

Click beetles (Elateridae) identify conservation units in Oriental and European beech forests

Giorgi Mamadashvili[*,1], Andrea Jarzabek-Müller[2], Hazan Alkan Akinci[3], Antoine Brin[4], Claus Bässler[5,6], Vasyl Chumak[7], Maksym Chumak[7], Valeriia Diedus[8], Lars Drössler[9], Kostadin B. Georgiev[10], Tigran Ghrejyan[11], Martin M. Gossner[12,13], Ruslan Hleb[14], Razieh Rafiei-Jahed[15],

Mark Kalashian[11], Ivan Kambarov[16], Gayane Karagyan[11], Joni Kevlishvili[17], Zviad Khutsishvili[17], Thibault Lachat[18,19], Meri Mazmanyan[11], Peter I. Petrov[20], Nicolas Roth[18,19], Levan Tabunidze[21], Mariia Varyvoda[7], Jörg Müller[1,6]

[*] Corresponding author at: Field Station Fabrikschleichach, Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Glashüttenstraße 5, 96181 Rauhenebrach, Germany.

Email address: Giorgi.mamadashvili@uni-wuerzburg.de

[1] *Field Station Fabrikschleichach, Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Glashüttenstraße 5, 96181 Rauhenebrach, Germany*

[2] *Anton-Hilz-Straße 42, D-94566 Riedlhütte, Germany*

[3] Faculty of Forestry, Department of Forestry Engineering, Artvin Çoruh University, 08100 Artvin, Türkiye

[4] *University of Toulouse, Ecole d'Ingénieurs de PURPAN, UMR INRAE-INPT DYNAFOR, Toulouse, France*

[5] *Ecology of Fungi, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Germany*

[6] *Conservation and Research Department at the Bavarian Forest National Park, Freyunger Str. 2. 94481 Grafenau, Germany*

[7] *Uzhhorod National University, Department of entomology and biodiversity preservation, Voloshyna str. 32, Uzhhorod, 88000, Ukraine*

[8] *State Museum of Natural History, National Academy of Sciences of Ukraine, Teatralna str., 18, Lviv, 79008, Ukraine*

[9] *Forestry Research and Competence Center ThüringenForst AöR, Gotha, Germany*

[10] *Hessian State Agency for Nature Conservation, Environment and Geology, Germany*

[11] *Laboratory of Entomology and Soil Zoology, Scientific Center of Zoology and Hydroecology NAS RA, P. Sevak str., 7, Yerevan, 0014, Armenia*

[12] *Forest Entomology, Swiss Federal Research Institute WSL, CH-8903 Birmensdorf, Switzerland*

[13]*Institute of Terrestrial Ecosystems, Department of Environmental Systems Science, ETH Zürich, CH-8092 Zürich, Switzerland*

[14]*Carpathian biosphere reserve, Krasne Pleso St. 77, Transcarpathian Region Rakhiv 90600, Ukraine*

[15] *Research Institute of Forest and Rangeland (RIFR), Agriculture Research, Education and Extension Organization, Tehran, Iran*

[16] *Strandzha Nature Park Directorate, 1 Yanko Maslinkov St., 8162, Malko Tarnovo, Bulgaria*

[17] *Biodiversity Conservation Center "Nacres", B. jgenti str. 34, Tbilisi, Georgia*

[18] *Bern University of Applied Sciences University of Agricultural, Forest and Food Sciences HAFL, Department of Forest Sciences, Langgasse ¨ 85, 3052 Zollikofen, Switzerland*

[19] *Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland*

[20] *University of Forestry Sofia, Field base Petrohan, 1 Petrohanska Str., 3520 Barzia, Bulgaria*

[21] *Caucasus Nature Fund – CNF, 2 Brose Str., 0108 Tbilisi, Georgia*

[22]*Bern University of Applied Sciences University of Agricultural, Forest and Food Sciences HAFL, Department of Forest Sciences, Langgasse ¨ 85, 3052 Zollikofen, Switzerland*

Abstract

Beech trees form major parts of lowlands temperate forests in the west of the Palearctic. To conserve their biodiversity natural world heritages and networks of forest reserves have been established. However, the effects of these protected forests on insect's communities are still not well explored. To identify conservation clusters and hotspots of biodiversity we sampled elaterids (Elateridae, Coleoptera) in 26 beech forests from France in the west to Iran in the east using predominantly window traps. All specimens were identified to species level, comprising 118 species including one species new for science. Community composition analyses with focus on rare species identified five clusters with rather distinct communities, first the Hyrcanian

Forest in Iran, second the Lesser Caucasus in Türkiye, Georgia, and Armenia, third the Greater Caucasus in Georgia, fourth the Pyrenees and fifth a cluster with forests from Central Europe, the Balkan region, and the Carpathians. After controlling for sampling effort (individuals), highest richness was found in the Caucasus region. The proportion of endemics was highest in the Oriental beech forests of the Caucasus and the Hyrcanian Forest. Our findings suggest intensifying conservation actions in beech forests particularly in the Caucasian regions and the Hyrcanian forest, due to their unique fauna.

Key words: click beetles, endemic species, forest conservation, *Fagus sylvatica*, *Fagus orientalis*, Hill numbers

Introduction

The Eurasian beech forests form a belt from Western Europe to Iran, dominated by the two species, European beech (*Fagus sylvatica* L.) and Oriental beech (*F. orientalis* Lipsky). The classification of these beech forests into different habitats has challenged botanists for a century (Willner et al. 2017; Gholizadeh et al. 2020) and, in recent decades, also planners of continent-wide protected area systems such as Natura 2000 in Europe (Bohn et al. 2007). Advances in genetic methods over the last two decades have repeatedly yielded new insights into the origin of these West Eurasian beech forests (Renner et al. 2016). Today, the existence of both beech species is beyond question (Jiang et al. 2022). Progressive climate change has also reawakened interest in *Fagus orientalis* in European forestry. There is even some evidence of the existence of *Fagus orientalis* in Italy during the interglacial period (Paffetti et al. 2007). Overall, it could be shown that all beech species worldwide have a relatively similar niche width, independent of the distribution area (Cai et al. 2021). In a direct comparison, the oriental beech is currently considered to be better suited to higher temperatures and drought (Mellert et al. 2016). Therefore, there are considerations on assisted migration in European forest management, which is also fueling the debate about the effects of such tree species selection on biodiversity. Regarding the increase of awareness of the importance of near-natural beech forest ecosystems from both *Fagus* species has also targeted protected area designations and World Heritage Site awards have been made.

Despite the high physiognomic similarity of the forest ecosystems of both *Fagus* species and their overlap in Bulgaria, there have been hardly any comparative biodiversity studies across *Fagus* species to date. This complicates the argumentation for conservation efforts in a common West Eurasian context. Only in exceptional cases, such as deadwood and old trees, has it been possible to transfer findings from areas of European Beech to those of Oriental Beech. And one

study investigated Tree-related microhabitats (TreMs) on living trees across both Fagus species (Courbaud et al. 2022; Mamadashvili et al. 2023).

Among insects beetles are used in many ways to evaluate the impact of land use and/or biogeographical context in beech forests (Lachat et al. 2012; Gossner et al. 2013; Hagge et al. 2019). One diverse family are click beetles (Elateridae). They represent the largest family of beetles (Order Coleoptera) in the superfamily Elateroidea. They are one of the most ecological diverse families of beetles in terms of habitats and foraging strategies (Laibner 2000; Bouchard et al. 2009) and play an important role in many ecosystem, either as herbivores, predators or fungivores. Forests and grasslands harbor the greatest local richness of species (Scurlock et al. 2002).

Click beetles have been regularly used as model family in ecological studies on the impact of different management types. In rainforests of Indonesia different land-use types affected abundance, diversity and species composition of click beetles (Kasmiatun et al. 2020). Horák & Rébl (2013) showed that species richness of click beetles in ancient pasture woodland benefitted from a high level of sun exposure. Similarly, Mladenović et al. (2018) showed that click beetles mostly preferred sun-exposure, but also spruce trees in plantation forests. A study on mature oak forests and spruce plantations showed tree species as structuring the species composition and highest diversity of click beetles in oak forests (Loskotová and Horák 2016). In European beech forests the richness of click beetles increased with the amount of dead wood (Müller et al. 2008). Similarly in Silver fir relic forests of Italy click beetles responded to spatial patterns of forest structure (Parisi et al. 2016). Also, in US click beetle diversity responded to different management strategies in hardwood forests (Stewart 2013). Such a response to openings and deadwood for click beetles is also in line with a long-term experiment in hardwood forests in US (Thomas 1995). Another disturbance click beetles are sensitive to fires in boreal forests. Again, many species were favored by fire events. Finally, click beetles have been part of zoogeographical studies in some countries as Türkiye, regions as the North or North Western Caucasus (Lyubomir et al. 1996; Zamotajlov et al. 2010; Platia et al. 2018).

Here we compiled click beetle data from 26 old growth and production beech forests from France in the West to Iran in the East to address the following research question:

1. How similar are species communities in the different regions and under different management.
2. Which beech forests harbor (a) the highest richness, (b) proportion of endemics and (c) proportion of saproxylic species.

Material and Methods

Study sites

Study area covers a wide range of geographical distribution of both *Fagus* species*: F. sylvatica* and *F. orientalis*, starting from France, Germany, Bulgaria, Ukraine, Türkiye, Georgia, Armenia, and finally to Iran (Figure 2). Among these countries, Bulgaria is the only one with both Fagus species overlapping. For each country we sampled old growth and production forest stands with exception of Bulgaria, from which we got data only from old growth forests. Study plots varied from 65 to 1497 m above sea level. Stands were dominated by beech trees, admixed with hornbeam (*Carpinus* spp.), oak (*Quercus* spp.), maple (*Acer* spp.), lime (*Tilia* spp.) and in some areas with spruce (*Picea* spp.), pine (*Pinus* spp.) and fir (*Abies* spp.). Each site contained a particular type of deadwood, such as snag and log, stump and crown or dead standing (or lying) tree.

Sampling methods

Beetle sampling was implemented during warm seasons from April until October between 2018 and 2022. We arranged flight interception and pitfall traps per each plot, closed to the deadwood objects. Only in Switzerland and one forest in Iran only flight interception traps were used. However, as most elaterid specimen and species are collected by flight traps, this can be neglected. For both type of trap, we used a copper sulphate and Rocima solution as a collecting fluid. After every two to four weeks, we emptied the traps, placing the caught insects into tubes with 75% Ethanol and refilling the solution in the traps. The identification of the specimens was conducted by Antoine Brin in France, by Vasyl Chumak and Maksym Chumak in Ukraine and Switzerland, Alex Szallies in Switzerland, and Boris Büche in Germany. All other material was determined by Andrea Jarzabek-Müller. Two species from Türkiye were identified by Giuseppe Platia.

Statistical Analyses

All analyses were conducted in R 4.2.3 (R Core Team 2022) at the forest level, combining all specimen from one forest. Since the sampling effort was related to the number of traps, affected on the number of specimens and leaded to variation in sample completeness, we used methods for the ordination of elaterid communities and the diversity analyses, taking into account unobserved species (Chao et al. 2014a) (see Supplementary material, R code). Among ecologists consensus has emerged, that Hill (1973) numbers (effective numbers of species) should be used to incorporate species abundance/evenness into species diversity and also

composition measures (Ellison 2010). Moreover, Chao et al., (2014) demonstrated that the Jaccard index (q=0), the Horn-index (q=1) and the Morisita-Horn-index (q=2) can be used the same way as the Hill numbers q=0, 1, 2 for diversity measures, representing rare, common, and dominant species respectively. Therefore we estimated diversity for a standardized sample coverage for all plots and species composition considering unseen species using the packages spadeR and iNEXT (Linderman et al. 2012; Hsieh et al. 2016). For details, see the R code. We finally calculated a rank-abundance curve for all species based on their occurrence in the 26 forests.

Results

In total, we collected 6845 specimens of 118 different click beetles species. One species (*Athous* sp.) from Georgia is new for science and not yet described. All other specimens could be identified on species level. The most widespread species was *Melanotus villosus*, followed by *Athous vittatus*, *A. subfuscus*, *A. haemorrhoidalis*, *Denticollis linearis*, *Ampedus pomorum*.

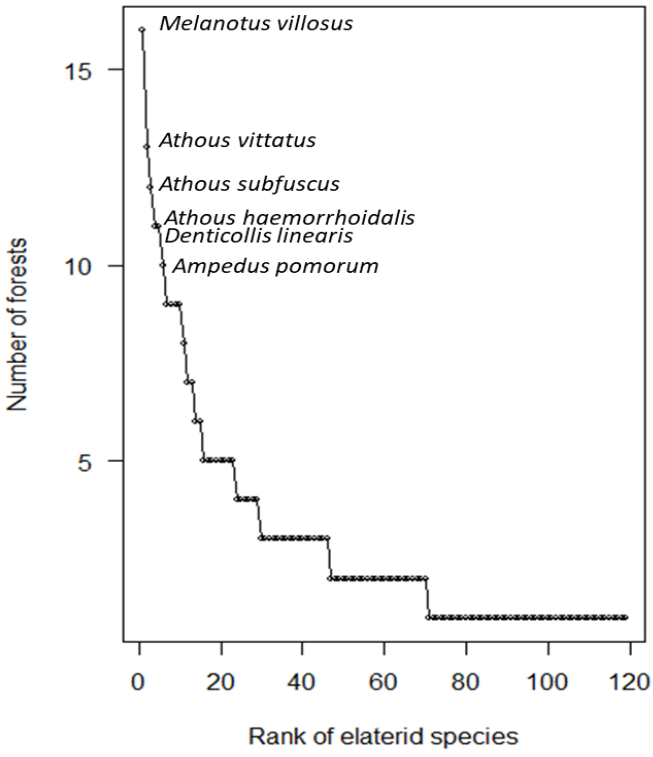

Figure 1: Rank frequency curve for all 118 species identified. Note that about 50 species were recorded only in one of 26 forest.

Our non-metric multidimensional scaling (NMDS) ordination with focus on rare species (Fig. 3a) showed a clear separation of 5 clusters: The first cluster was the west the Pyrenees, followed by a cluster including all Central European sites up to the first *Fagus orientalis* in the east of

Bulgaria, the third cluster contained the species of all sites in the Lesser Caucasus, fourth cluster was in the Greater Caucasus and fifth cluster was in the Hyrcanian forest of Iran. This pattern was rather robust when shifting the focus to dominant species in the ordination distance matrix (Fig 3b), but with some outliers for the dominant species.

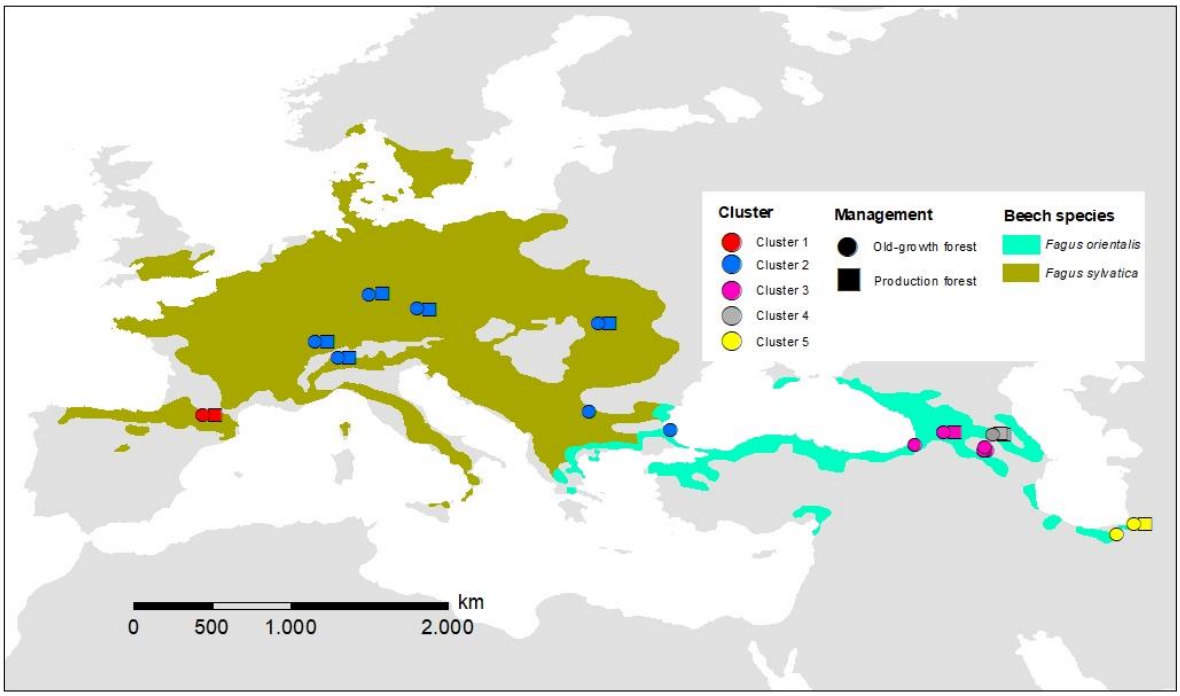

Figure 2: Global distribution of *Fagus sylvatica* and *F. orientalis* and the location of 26 forests investigated for click beetle communities. Note that some locations were shifted to increase visibility. Colors indicate the clusters identified by ordination on distance matrix with focus on rare species (q=0, see Figure 3).

Table 1: List of 26 beech forests investigated for elaterid species.

Forest	Latitude	Longitude	Elevation	Country	Region	Forest_type
AR_DDU	40.947	45.208	1232	Armenia	Ditaven	Unmanaged
AR_TTU	40.912	45.077	1327	Armenia	Teghut	Unmanaged
AR_TZU	41.122	44.923	1298	Armenia	Tavush	Unmanaged
BU_BBU	43.176	23.122	1067	Bulgaria	Berkovitsa	Unmanaged
BU_TSU	42.107	27.788	298	Bulgaria	Tsarevo	Unmanaged
FR_OMM	42.987	0.973	917	France	Occitania	Managed
FR_OMU	42.990	0.966	950	France	Occitania	Unmanaged
GE_BMU	49.099	13.246	748	Germany	Bavaria	Unmanaged
GE_BSM	49.066	13.306	786	Germany	Bavaria	Managed
GE_FSM	49.930	10.562	452	Germany	Franconia	Managed
GE_FSU	49.861	10.500	389	Germany	Franconia	Unmanaged

GG_KLM	41.843	46.272	800	Georgia	Kakheti	Managed
GG_KLU	41.841	46.325	837	Georgia	Kakheti	Unmanaged
GG_KMM	41.918	46.096	931	Georgia	Kakheti	Managed
GG_SBM	41.963	43.449	1006	Georgia	Samtskhe Javakheti	Managed
GG_SBU	41.980	43.450	1101	Georgia	Samtskhe Javakheti	Unmanaged
IR_GGM	36.733	54.392	881	Iran	Golestan	Managed
IR_GGU	36.728	54.393	959	Iran	Golestan	Unmanaged
IR_MSU	36.150	53.405	1573	Iran	Mazanderan	Unmanaged
SW_BBM	47.221	7.415	932	Switzerland	Bettlachstock	Managed
SW_BBU	47.219	7.410	833	Switzerland	Bettlachstock	Unmanaged
SW_LLM	46.255	8.665	910	Switzerland	Lodano	Managed
SW_LLU	46.239	8.695	691	Switzerland	Lodano	Unmanaged
TU_AVU	41.237	41.854	800	Turkey	Artvin	Unmanaged
UK_CMU	48.221	23.651	521	Ukraine	Carpathian Ruthenia	Unmanaged
UK_CVM	48.269	23.621	755	Ukraine	Carpathian Ruthenia	Managed

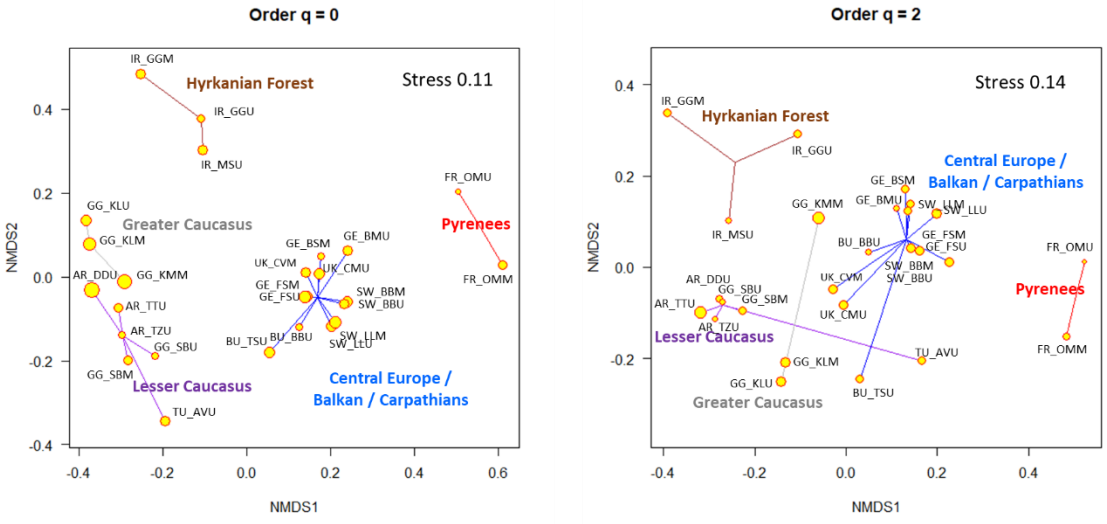

Figure 3: Non-metric ordination on distance matrix with focus on rare species (Jacard Similarity) and dominant species (Morisita Horn similarity). Stress values indicate the robustness of the findings (for abbreviation of forests see Tab. 1).

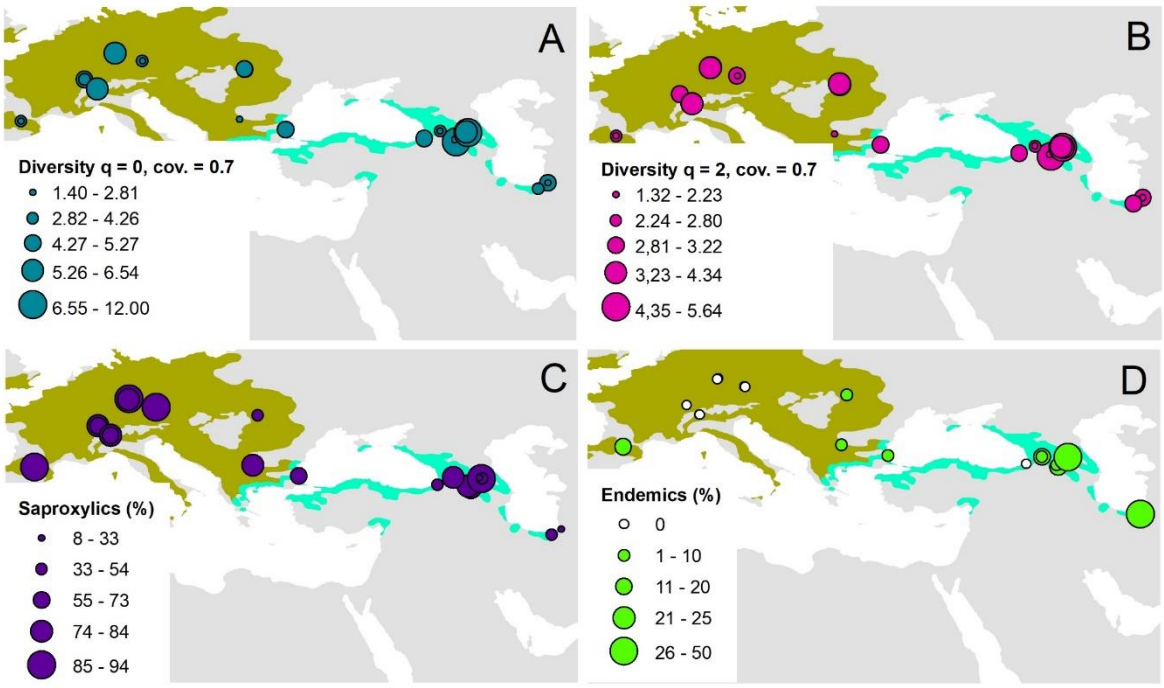

Figure 4: Display of the site-specific richness (A) of click beetles calculated on the same sample coverage, the (B) diversity with focus on dominant species (q=2), the proportion of (C) saproxylic beetle individuals and (D) endemics per forest site.

The coverage standardized diversity was high in forests in Switzerland and Southern Germany, but particularly in the Caucasus region. This was similar for focus on dominant species, but Ukraine forests also revealed high diversity here. The proportion of saproxylics were high in the Caucasus and Central European part. High proportion of endemics were found in descending order in the Hyrcanian and Caucasus forests, the Pyrenees and the eastern part of Europe. Finally, conservation status of the forest had no important effect on the metrics investigated

Discussion

Our comparison of elaterid species in beech forests across both *Fagus* species revealed many similarities among communities, with a number of species occurring in both type of forests. This is one more hint towards the view that for the fauna in Central Europe it might be less problematic if foresters use *Fagus orientalis* in silviculture (Mellert et al. 2016). From a conservation perspective however, using different aspects of biodiversity, as total species richness, number of endemics or some functional groups revealed different hotspots at the continental scale. This is common and well known even from global comparisons of biodiversity, e.g. for birds or fishes (Orme et al. 2005). The comparison of elaterid assemblages

in beech forests showed a number of clusters, differences in biodiversity and different proportions of endemics. These patterns seems to be connected to biogeography, isolation, climate history and evolution, which leads species to historical expansion and colonization in specific geographical ranges (Bruchmann and Hobohm 2014; Gutiérrez-Rodríguez et al. 2017; Manes et al. 2021).

In Europe, the Pyrenees mountains formed one of a few glacial refuges for *Fagus sylvatica* (Magri et al. 2006). The high variation in topography, some near natural forests, combined with the forests excluded from glaciation might explain why Pyrenees are one of the richest European mountains hosting e.g. 3652 plant species, of which 119 are endemics (Gómez et al. 2022). Researching *Saxifraga longifolia* – endemic species of Pyrenees, Pomeda-Gutiérrez et al. (2023) named this place as a "cradle" of plant diversity. Similar species forming evolutionary processes have been shown for mammal (Gillet et al. 2017), amphibian (Valbuena-Ureña et al. 2018) or spider species in the Pyrenees. These findings are in line with the largest proportion of endemics in the elaterid species in our study in European beech forests.

The majority of European beech forests are concentrated in Central Europe covering a wider area (see Fig. 1). However, these forests are rather young, and when beech trees recolonized the area after last glaciation (Magri et al. 2017), around 6-5k years ago, humans were intensively using forests, actively decreasing the natural ecosystems, ending up in habitat loss and habitat degradation, a decline of rare species in many regions (Gossner et al. 2013; Eckelt et al. 2018). This long-term negative trend has promoted many conservation activities in form of designation of protected areas as a refuge for species who survived after such harsh human interventions (Berg et al. 1994; Peterken 1996; Bengtsson et al. 2000). However, in these protected beech forests old-growth stands still are almost absent (Glatzel 1991; Schulze et al. 2016; Ammer et al. 2018).

Despite a similar or even more harsh land-use history in the Balkan region, forests are still known as European hotspot for biodiversity, with high proportion of endemism. Here the Balkan region represents a refugium from Pleistocene, lying on crossroads of Europe and Asia (Griffiths et al. 2004) sheltering almost 8000 plants, from which around 2700 taxa are endemics to the region (Stevanović et al. 2007). Guéorguiev et al. (1997) demonstrated this for endemic Balkan ground beetles (Carabidae) in Bulgaria. According to Geological history, many biogeographical territories collide here and as a result the fauna is extremely heterogenous, as well as including number of endemic and relict species (Makarov and Dimitrijević 2008). This is line with our endemic proportion shown in Figure 4D.

For European beech, the Carpathian mountains form an important backbone for biodiversity including some large primeval forests as represented in our data, which also show a relative high diversity in our data (Fig. 4B). Even in the Carpathians a considerable part of species (12% of flora) is endemic (Voloscuk 1999). However, this is less supported for beech forest inhabiting elaterids. Here a similar fauna could be shown in the primeval Uholka forests as in more degraded forest in Germany or Switzerland.

In contrast, the Caucasus Ecoregion is an area of outstanding importance with regard to a very unique region characterized by many endemic species: high mountains of Great (North) and Lesser Caucasus (South), also Black sea (West) and Caspian sea (East) create biogeographical islands (Zazanashvili et al. 2020), separated from surrounding regions during millennia. Isolated for long time formed a specific diversity of vegetation (trees, shrubs, herbs) dating back to the Tertiary period, especially in the Colchic Region of the Black Sea basin, which was shaped mainly from the end of Middle Sarmatian, i.e., 11-12 million years ago (Shatilova et al. 2011; Nakhutsrishvili et al. 2015). Western Caucasus represents one of those regions of Europe which has not experienced significant human impact and it is a refugium of a unique flora and fauna diversity with high endemism, which is why this region was designated as World Heritage site under Natural Criteria ix and x (UNESCO 1999). In addition, because ecosystems of the Caucasus survived the last Ice age, today, the Colchic Refugium (Georgia, Russia, and Türkiye) is an important relict for many endemic species. Together with Armenia, Azerbaijan and Georgia, the North Caucasus portion of the Russian Federation and north-eastern Türkiye and part of north-western Iran this region is one of the 34 biodiversity hotspots in the world (Mittermeier et al. 2004). The temperate forests here form one of the most diverse and unique temperate forest ecosystems globally, with more than twice the plant and animal diversity found in adjacent regions of Europe or Asia (Williams et al. 2006; Zazanashvili and Mallon 2009). This assessment is also supported by studies on elaterids. Orlov (1994) described several species, unique for the Caucasus region. Similarly, Chantladze (1988) reported new records of click beetles of Caucasus region in western part of Georgia, including endemic species. Magomedova (2017) compared species composition of the elaterid genus *Agriotes* in the Caucasus region, demonstrating highest species diversity in the Northern part of the Caucasus similar to our results. The importance of the Greater Caucasus mountains for diversity and endemics, as shown in our study by elaterids, was demonstrated by a study on Tenebrionids and explained by geographical or climatological isolations (Abdurkhmanov and Nabozhenko 2015).

The last important region, identified in our study as important for beech forest insect diversity is the Hyrcanian region, south of the Caspian Sea. These forests host similar high numbers of endemics or species highly threatened in Europe (see Fig. 4D, (Müller et al. 2016)). But in terms of species composition they form a very distinct community (see Fig. 3), which seems to be caused by geographical history and isolation as well as climate (Safarov 1979; Nakhutsrishvili et al. 2015). The beech forests in the Hyrcanian belt date back 25 to 50 million years conserving natural forest ecosystems and promoting speciation with many endemics in all species groups (Mathew et al. 2000; Javidkar et al. 2007). To this day, species from even well-studied species groups such as beetles are still being described new for science from the beech forests of the Hyrcanian Forest (Löbl and Ogawa 2016; Bussler 2017; Müller and Schuh 2021). This is true also for elaterids (Jarzabek-Müller et al. 2017; Kundrata et al. 2019).

As mentioned above, this high importance of the larger Caucasus Ecoregion for rare and endemic species, as demonstrated even in our elaterid data from beech forests, was the reason for designation as UNESCO Worlds Heritage Sites (Tohidifar et al. 2016; UNESCO 2019). It seems that the isolation by mountains and deserts of the Oriental beech forests (see Fig. 1) in this region together with the fact that these forests are the oldest temperate forests of Western Eurasia explains well the specific role of beech forests for biodiversity.

If we summarize our results on elaterids in beech forests from France to Iran and compare them with findings from other species groups, it becomes clear that the biogeographically old beech forests are of outstanding importance for a unique beech forest fauna. These are found predominantly in the Caucasus Ecoregion with several very distinct species communities. Unfortunately, in these regions there is not always a public awareness of the importance of old-growth features such as deadwood and decaying trees (Müller et al. 2017). For this reason, community efforts for more protection should be intensified, especially in these beech forests of Georgia, Türkiye, Armenia, Azerbaijan, and Iran, which are so important for biodiversity of beech forests.

Table 2: Distribution of 118 Elaterid species in beech forests. In the brackets the number of forests investigated.

Species	France (2)	Germany (4)	Switzerland	Ukraine (2)	Bulgaria (2)	Türkiye (1)	Armenia (3)	Georgia (5)	Iran (3)
Adrastus dolini								1	
Agriotes acuminatus		2			1				
Agriotes bogatschevi								3	
Agriotes tauricus								1	
Agriotes danieli									2
Agriotes infuscatus					1	1	2	3	1
Agriotes integricollis							2		
Agriotes lineatus								1	
Agriotes litigiosus				1	1				
Agriotes obscurus		1							
Agriotes pallidulus		2		1					
Agriotes pilosellus		2	3		2				
Agriotes sputator		1							
Agriotes starcki								1	
Agriotes ustulatus				1					
Agrypnus murinus					1				
Ampedus aethiops		1	1						
Ampedus balteatus			2						
Ampedus biformis									2
Ampedus brunnicornis			1						
Ampedus cinnabarinus							1	1	
Ampedus elegantulus				2					1
Ampedus elongatulus	1		2						
Ampedus erythrogonus		1	4	1					
Ampedus hjorti									1
Ampedus lenkoranus							3		2
Ampedus melanurus			1	1					
Ampedus nigerrimus		1	1	1					
Ampedus nigrinus		2	2						
Ampedus pomonae				2					
Ampedus pomorum		2	4	2	1			1	
Ampedus quercicola			4		1				
Ampedus rubellus								1	
Ampedus rufipennis					2			2	1
Ampedus sanguineus			3	1					
Ampedus sinuatus					1	1	2	1	1
Anostirus castaneus		2	1	1					
Anostirus purpureus		2	2	1					
Anostirus sulphuripennis			1						
Athous (Alcimathous) mokrzecki					1				

Athous (Athous) haemorrhoidalis		3	4	2	2			
Athous (Athous) vittatus		4	4	2	2	1		
Athous (Euplathous) proximus					1			
Athous (Haplathous) austriacus					2			
Athous (Haplathous) circassiensis							1	
Athous (Haplathous) circumductus							3	
Athous (Haplathous) turcicus						1		
Athous (Haplathous) subfuscus		4	4	2	1		1	
Athous (Orthathous) abkhazianus							1	
Athous (Orthathous) dasycerus					1			
Athous (Orthathous) artvinensis						1		
Athous (Pleurathous) circassicus							3	2
Athous (Pleurathous) hyrcanicus								2
Athous astrabadensis								1
Athous bicolor				1				
Athous emaciatus			1					
Athous laevistriatus	1							
Athous longicornis	1							
Athous mandibularis	2							
Athous mollis				1				
Athous rosinae								3
Athous zebei		3	4					
Brachygonus megerlei			3		1			
Calambus bipustulatus			1		1			
Cardiophorus gramineus							1	
Cardiophorus kryzhanovskyi							2	
Cardiophorus nigerrimus			2					
Crepidophorus mutilatus				1				
Ctenicera cuprea			1					
Dalopius marginatus		4	4	1				
Denticollis linearis	2	4	2	2	1			
Denticollis parallelicollis							2	3
Denticollis rubens		3	3	2			1	
Diacanthous undulatus				2				
Dicronychus cinereus			1				1	
Dicronychus decorus							1	
Dicronychus marani					2			
Drapetes talyschensis								2
Drasterius bimaculatus							1	
Drilus concolor			2		1		1	
Drilus fulvitarsis							1	
Drilus novoathonius							1	
Elater ferrugineus								1
Elathous brucki								1
Hemicrepidius hirtus		1	1					1
Hemicrepidius niger			3					
Hemicrepidius nigritulus						1	1	3
Hypoganus inunctus			2		1			
Hypoganus stepanovi								1

Idolus adrastoides							1	2	
Idolus picipennis	2		1	1					
Idolus sp. nov.							2		
Ischnodes sanguinicollis				2					
Lacon mertliki									1
Lacon punctatus								1	
Limoniscus wittmeri									1
Limonius minutus				1					
Limonius poneli							1		
Megathous menentriesi									3
Melanotus castanipes	1	2	4	1					1
Melanotus crassicollis					1				
Melanotus dichrous					1				
Melanotus villosus		3		2	2		3	5	1
Nothodes parvulus	1	2	4		2				
Paraphotistus impressus			2						
Pheletes aeneoniger			1						
Pheletes quercus			1						
Procraerus carinifrons								1	
Procraerus stepanovi									2
Procraerus tibialis	1			2					
Prosternon admirabilis									1
Prosternon tessellatum			1	1	1				
Pseudocrepidophorus flavescens					2		1		
Sericus subaeneus			1						
Stenagostus probosus							1		
Stenagostus rhombeus			2	1	2				
Stenagostus rosti								3	
Synaptus filiformis				2					

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5.4 Paper N4 - "Comparison of tree species diversity and deadwood of managed and old-growth Oriental beech forests in Eastern Georgia"

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Comparison of tree species diversity and deadwood of managed and old-growth Oriental beech forests in Eastern Georgia

Lars Drössler, Tobias Wolff, Jonas Glatthorn

Abstract: Main stand characteristics and tree species composition are studied. Oriental beech dominates both regeneration layer and upper story in old-growth forest. In managed forest, Caucasian hornbeam and Oriental beech co-dominate the tree layer as well as the regeneration layer. Tree species diversity and deadwood proportion are higher in managed forest than in old-growth forest. However, absolute deadwood proportion are more similar in both studied forest types. Hence, deadwood proportion is not a suitable indicator to assess the naturalness or management intensity of forests in the Caucasus region.

Keywords: Coarse woody debris, natural regeneration, stand characteristics, *Fagus orientalis*, *Carpinus caucasica*

Introduction

Fagus sylvatica is the prevailing tree species in the forests of Georgia, about 50% of the forest are dominated by this tree species. In eastern Georgia, it occurs mostly at 1000 to 2000 m elevation above sea level (Nakhutsrishvili, 2013). Optimum growth conditions are 1000 to 1500 m a.s.l. (Dolukhanov 2010) where also most cuttings occur nowadays. While *Rhododendron* forms a dense understory in western beech forests of the country where precipitation is very high (Nakhutsrishvili 2013), eastern beech forests are characterized by *Rubus* and grass species and 1000 mm precipitation in the study area.

Study goal is the comparison of stand structure (stem number, basal area, standing volume, deadwood proportions) and regeneration (density and tree species proportions) in old-growth and managed beech forests. Based on western European literature, we hypothesize that i) deadwood proportion is higher in old-growth forest than in managed stands. We also assume that ii) regeneration is more abundant and diverse in old-growth forest.

Methods

The plots are located in the east of Georgia, between 870 and 1182 m elevation a.s.l. at a northwestern slope where optimum growth conditions prevail for Oriental beech (Dolukhanov 2010). Old growth forest is located in the core zone of the protected area of Lagodekhi, near the border to Azerbaijan. The managed stands are located at the same exposition towards NW and elevation 20 km east on the same soil type and similar site conditions. The two managed areas were unmanaged before logging which took place 15 and 5 years ago.

There, 10 study plots with 37.5 m x 37.5 m area (1406 m^2) were randomly distributed and established, four plots in the cut area 15 years after cutting, six plots in the area which was cut 5 years ago. The cuttings were conducted during two subsequent years (hence 5-6 and 15-16 years ago). 10 other study plots of the same size were distributed in two unmanaged areas of the protected area Lagodekhi. The size of the study plots is a result of nine smaller squares with 12.5 m x 12.5 m area comparable to the size of crown cover by a canopy tree to analyze stand structure and natural forest dynamics according to Tabaku (2000) and Droessler and Meyer (2006). On these small squares, forest developmental stages were determined according to the method by Tabaku (2000) with corrections according to Zenner et al. (2016). On the large plot, coordinates, tree species and diameter at breast height (dbh ≥ 7 cm) was recorded. Standing and laying deadwood was recorded with minimum diameter of 20 cm, following the method of Kucbel et al. (2012). To assess tree species composition and diversity of both forest areas in Lagodekhi and Mtisdziri, the abundance of tree species per plot was calculated (based on tree

numbers). The vegan package (Oksanen et al., 2020) was then used to calculate tree species richness.

In the center of each small square natural regeneration was recorded on a circular plot with 1.78 cm radius (10 m^2 area). Wooden plants were measured from 10 cm to 200 cm plant height. The regeneration survey follows the survey method by Staupendahl (1997) where all seedlings are counted, but only the nearest individual to the regeneration plot center is measured and represents the tree species. In addition, the canopy density was estimated with a forest canopy densiometer (Lemmon 1956).

Results

Stand characteristics

The old-growth forest was characterized by a large proportion of *Fagus orientalis* (82%, std. dev. 11%). The two other most frequent species *Carpinus caucasica* and *Tilia begoniifolia* comprised 7% of the tree species each. In managed forest where mostly beech was harvested, the proportion of *Fagus orientalis* was only 41% (std. dev. 18%) while *Carpinus caucasica* was 39% (std. dev. 17%) and Tilia begoniifolia 8% (std. dev. 7%). *Castanea sativa* and *Acer* species were found in both managed and unmanaged stands, but the proportion was less than 5% like for all other following tree species. Contrary to the old-growth forest, single *Populus tremula* and *Salix caprea* occurred in the managed forest, as well as *Prunus avium* and *Ulmus glabra*. Anyhow, statistically the tree species richness did not vary significantly between the two studied areas (p = 0.09).

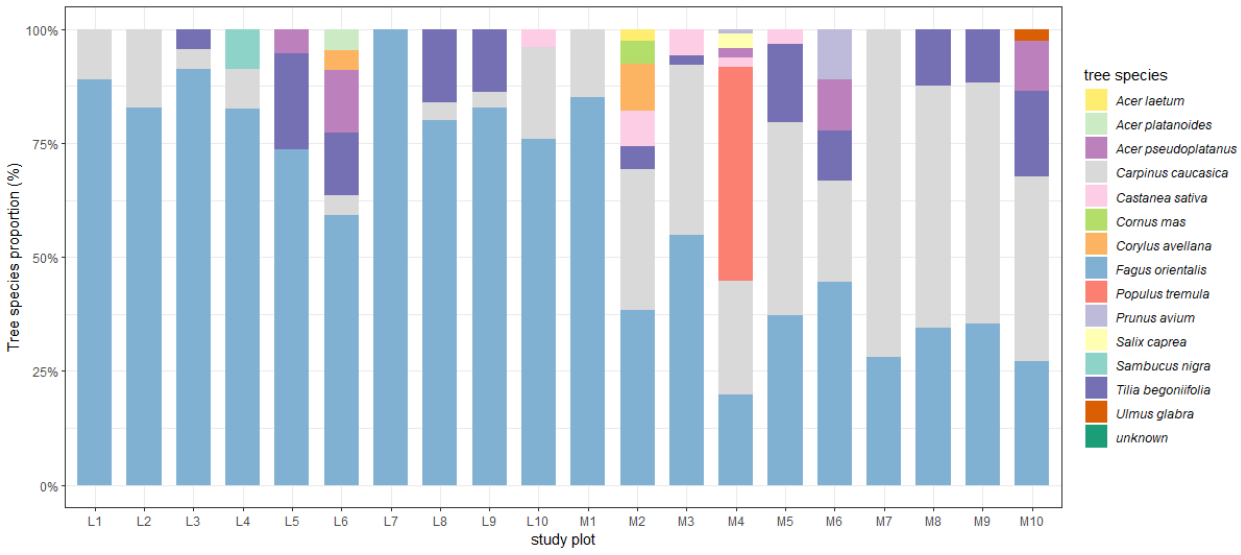

Fig. 1. Tree species proportion of the number of trees for each study plot.

In the old-growth forest, standing volume of living trees was 640 m^3 ha^{-1}. There, the deadwood proportion was only 5%. In managed stands the relative proportion was 14% five and fifteen years after cutting. Standing volume was 271 m^3 ha^{-1}. Tree density was 197 trees per ha in the unmanaged stands and 274 trees per ha in the managed forest. The latter was considerably smaller with 34 cm dbh of the mean basal area tree (table 1). Basal area in Mtisdziri was also much lower than in Lagodekhi.

*Tab. 1. Main stand characteristics per study area for all tree species combined; statistical significant differences for the mean between Lagodekhi and Mtisdziri are indicated by bold text and asterisk: *= $p \leq 0.05$, **= $p \leq 0.01$, ***= $p \leq 0.001$, Wilcoxon rank-sum test/t-test.*

Study area		Tree number (N ha^{-1})	BA (m^2 ha^{-1})	d_g (cm])	Stand volume (m^3 ha^{-1})	Deadwood volume (m^3 ha^{-1})
Lagodekhi	Mean	197	**33.2*****	**47.5****	**640*****	34
	95% CI	[153, 241]	[29.3, 37.1]	[41.9,53.1]	[521,759]	[12,56]
	SD	61.6	5.5	7.9	166.9	31.0
	CV	31.5 %	16.5 %	16.6 %	26.1 %	91.9 %
Mtisdziri	Mean	274	**19.0*****	**34.0****	**271*****	43
	95% CI	[145,403]	[14.6,23.4]	[25.2,42.8]	[193,349]	[20,66]
	SD	180.3	6.2	12.3	109.4	31.5
	CV	65.7 %	32.4 %	36.0 %	40.5 %	72.8 %

BA = Basal area of living trees DBH ≥ 7 cm, d_g = DBH of the mean basal area stem, 95% CI = 95 % Confidence-Interval, SD = standard deviation, CV = Coefficient of variation; Tree number, BA, d_g, stand volume was measured of living trees DBH ≥ 7 cm, deadwood diameter ≥ 20 cm

Regeneration

9588 seedlings per ha were counted in the old-growth forest Lagodekhi, and 14200 seedlings per ha in the managed forest Mtisdziri. In Lagodekhi, 50% was beech, 28% was lime, 12% was *Acer* species, and 9% was hornbeam. 40% of the plots were without forest regeneration. In Mtisdziri, hornbeam was much more frequent: 57% was hornbeam, 42% was beech, and single seedlings of lime and *Acer* species also ocurred. 25% of the regeneration plots were without regeneration.

In Lagodekhi, 86% of seedlings were 10-50 cm high, dominated by 47% beech and 34% lime. Only beech seedlings occurred in the second height class from 51-100 cm. 12% were 1-2 m high, mostly sycamore trees growing in natural canopy gaps. In Mtisdziri, 55% of seedlings were 10-50 cm tall. 32% was 51-100 cm tall, and 13% were 1-2 m high. Here, in the lowest height class, hornbeam was 54% and beech was 45%. Single *Acer* species occurred too. In the

second smallest height class from 51-100 cm, beech was 54% and hornbeam was 45%. Single lime and *Acer* species occurred. In the highest recorded height class from 1-2 m, 92 % (1800 individuals) was hornbeam in the managed forest.

In Lagodekhi, 5000 seedlings were found where the canopy cover was 96-100% (45% beech and 33% lime as well as single hornbeam and Acer species). Similar proportions were found in the canopy class 91-95% covered. The lowest canopy coverage was 71% in the old-growth forest. From 71 to 90% occurred 1388 seedlings with 81% beech. In the managed forest Mtisdziri, hornbeam dominated the lowest canopy class by 93% (2250 individuals). While beech prevailed with 58% (525 individuals) in the second lowest canopy class with 91-95% canopy coverage, hornbeam dominated when canopy coverage was 81-90% (69%, equal to 4262 individuals). When the canopy was open (10-80%), then beech was most frequent with 74%. Hornbeam proportion was 25%. Single lime and *Acer* species also occurred.

Discussion

While Oriental beech is the prevailing tree species in the old-growth forest Lagodekhi and the proportion of hornbeam is small, the latter is much more frequent in the managed forest with the same soil type, elevation and exposition. Moreover, in unmanaged parts near the plots of Mtisdziri, beech also prevailed. Dolukhanov (2010) already mentioned that Carpinus caucasica can replace beech in managed areas. Considering natural regeneration in both study areas, beech dominates in Lagodekhi while hornbeam and beech compete with each other in the managed forest Mtisdziri. In addition, Rubus species were more competitive in Mtisdziri when the canopy coverage was low. However, diversity of the ground floor vegetation was also higher in Mtisdziri. To maintain the beech forest ecosystem, less intense cuttings can be recommended.

Deadwood proportions are an important indicator to assess sustainable forest management in Central Europe today. However, in Georgia with many old-growth forest and managed stands left with habitat trees and deadwood after cutting, this indicator can be misleading: the absolute amount of deadwood can be the same in both types of forest, unmanaged and managed – while only the standing volume of living trees is reduced by the logging. In general, there is no lack of habitat trees and coarse woody debris in Georgian native beech forests.

Conclusion

Hornbeam can replace beech if local logging intensity is to much. After heavy removals, even competitive *Rubus* species can prevent forest regeneration for 1-2 decades. In conclusion, local

harvest intensity should be reduced to maintain the same ecosystem. On the other hand, the logging area could be expanded by using wires and winches for logging on slopes.

Maximum diameter or a special old growth indicator (Meyer et al. 2021) may be more suitable to assess the natural conditions of a forest than the proportion of deadwood. Deadwood proportion is not suitable as indicator for management intensity in the Caucasus region, because it is the same absolute amount and even higher in relation to the standing volume of living trees after the cutting.

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6. Dissemination of the results

Additional activities: During this project some additional activities were carried out too, that expended our knowledge and experience:

1. Biodiversity excursions for German students in Georgia in 2022 and in 2024.
2. Presentation for Lagodekhi National Park (Georgia) rangers about the project and the achievement of our research in 2024.
3. Fauna excursions in Fabrikschleichach\Steigerwald forest in 2022 and in 2024.
4. Oral presentation in GFÖ International Conference in Leipzig, in 2023.
5. 20 long-term forest study plots were established by Lars Droessler in eastern Georgian beech forests for future forester student's researches.
6. Permanent marking of trees and plots, marking plot center and borders by Lars Droessler in 2022.
7. Training of the University students by Lars Droessler in the differences between managed and natural forests, beech forest dynamics and biodiversity monitoring in 2021, 2022 and 2023.
8. Annual excursions by Lars Droessler with master students and CaBoL BioBlitz with students to Lagodekhi, Georgia in 2021 and 2022.
9. Training of the rangers in Lagodekhi and teaching PA staff by Lars Droessler about the importance of deadwood and natural forest dynamics in 2022.
10. Excursion to Germany with Georgian master students and teaching the exploration of managed and protected beech forests in Germany (DAAD funded) in 2022 and 2023.
11. Soil sampling on the study plots by Lars Droessler in 2022.
12. Regeneration survey with light/canopy assessment by Lars Droessler in 2022.
13. Wood quality assessment by a bachelor student from FH Erfurt, under the supervision of Lars Droessler in 2022.
14. Knowledge exchange with the forest inventory and taxation company in Lagodekhi by Lars Droessler in 2022.
15. Woodpecker survey in Lagodekhi by Lars Droessler in 2022.
16. Collection of genetic samples of beech in Lagodekhi by Lars Droessler in 2022.
17. Terrestrial laser scans of beech forest structure along an elevation gradient from 500-2000 m in Lagodekhi under the collaboration between Nika Gobronidze and the University of Göttingen, Germany in 2022.

18. Meeting BeechDIV project partners: Lars Droessler and Levan Tabunidze in Borjomi, for sharing the experience and idea of establishing the long-term study plots in Borjomi, Georgia in 2022.
19. Establishment of a small exercise stand (2 ha) by Lars Droessler to introduce foresters and students to first thinning in 20-30 years old, overexploited mixed forest near Mtisdziri\Lagodekhi, Georgia (the forest district with the well-managed beech forest study area) in 2022.
20. Establishment of a Marteloscope site by Lars Droessler in the flat area of the management zone of the protected area Lagodekhi to teach silviculture and forest biodiversity with easy forest access in Lagodekhi, Georgia in 2022.

7. Outlook

During the project, everything proceeded smoothly. The comprehensive investigation of tree-related microhabitats (TreMs), deadwood fungi, and elaterid beetles across beech forests from France to Iran provided valuable insights into biodiversity drivers, such as the presence of Fagus species, elevation, and forest type. Our research revealed that TreM diversity is significantly influenced by tree species, elevation, tree diameter, and forest age (old-growth vs. production). The study underscores the need for conservation efforts across different elevations and Fagus species to capture the full range of TreM diversity. Meta-barcoding of deadwood samples from France to Armenia showed that fungal composition is primarily influenced by tree species, elevation, and deadwood type, with decay stage significantly increasing species richness. This highlights the importance of deadwood retention across various elevations and tree species, especially in old-growth forests to protect rare species. The analysis of elaterid beetles across 26 beech forests identified five distinct biodiversity clusters, with the highest species richness and endemic proportions in the Caucasus and Hyrcanian forests. This suggests prioritizing conservation efforts in these regions due to their unique insect communities.

Overall, these findings advocate for targeted conservation strategies that consider tree species, elevation, forest management, and specific regional biodiversity hotspots. And finally, the identification of the principal predictors of biodiversity provided an opportunity to elucidate the principal patterns of biodiversity in beech forests and to propose a functional perspective for future biodiversity conservation efforts in beech forests. This perspective may be employed by foresters, scientists, and all those engaged in forest management activities.

8. Future Plans

We are also planning a future manuscript involving the analysis and identification of the remaining beetle samples collected during the study. This upcoming work will further contribute to understanding beetle diversity and its role in the conservation of beech forest ecosystems. We will share with this results to our partners and collaborating countries. Additionally, we will expand our knowledge and findings to the specialists professionally connected to this topic such as foresters, biologists, ecologists and for future collaboration and for next scientific researches.

9. Conclusion

The Beech DIV project successfully established a comprehensive dataset on biodiversity in deadwood within beech forests. Collaboration across multiple countries enhanced the robustness of the study, providing valuable insights into the conservation of these critical forest ecosystems. The outcomes, including several scientific publications, underscore the importance of biodiversity in deadwood for sustainable forest management and conservation strategies.

Supplementary materials

Supplement 1: Factual report from Armenia – 2021

Annex to the Proof of Use of Funds (12.2021)

Factual report for 2021

In the framework of BeechDiv Project the following activities were carried out by working group of Scientific Center of Zoology and Hydroecology, National Academy of Sciences of Armenia (Secondary recipient):

Initial expeditions in April-May, 2021 towards three provinces of Northern Armenia where beech forest are presented were carried out to select areas for further sampling. Four plots were selected, namely:

Gegharkunik prov., env. Antaramej, N40.66193 E45.08014 1730 m

Lori prov., env. Teghut, Teghut mining area, N41.09063 E44.81085 1016 m

Tavush prov., env. Yenokavan, N40.90897 E45.06504 700 m

Tavush prov., env. Koghb, "Zikatar" Environmental Center, E44.92292 1134 m

In each plot 5 window traps together with 5 pitfall (Barbier) traps were put in July, 2021. Samples were taken each month until the end of October; during the last expedition at the end of November-beginning of December traps only were collected which didn't contain any material due to worsening of weather conditions. All the material was preliminarily elaborated and stored in freezer in our lab.

Besides, in each plot were collected samples of tinder fungus *Fomes fomentarius*. These were put into cages in the lab for breeding of associated insects. Emerged insects were stored in 70% alcohol.

Fruit bodies of other fungi associated with beach were collected and stored in silica gel.

In each plot drilling samples were collected as well.

All the material concerning fungi was passed to Mr. G. Mamadashvili and transported to Germany.

Responsible Investigator

Mark Kalashyan

Note: The work started before the signing of the Contract and thus, the corresponding bureaucratic procedures could not be implemented in our Center, so the activities were carried out without appropriate documentary evidence. Accordingly, the corresponding expenses are not reflected in our documents. This part of work for period April-July was carried out at our own expenses.

Supplement 2: Factual report from Armenia – 2021-2024

Annex to the Proof of Use of Funds (03.2024)

Factual report for period June-September, 2022

In the framework of BeechDiv Project the following activities were carried out by working group of Scientific Center of Zoology and Hydroecology, National Academy of Sciences of Armenia (Secondary recipient).

Initial expeditions in April-May, 2021 towards three provinces of Northern Armenia where beech forest are presented were carried out to select areas for further sampling. Four plots were selected, namely:

Gegharkunik prov., env. Antaramej, N40.66193 E45.08014 1730 m

Lori prov., env. Teghut, Teghut mining area, N41.09063 E44.81085 1016 m

Tavush prov., env. Yenokavan, N40.90897 E45.06504 700 m

Tavush prov., env. Koghb, "Zikatar" Environmental Center, E44.92292 1134 m

The work started before the signing of the Contract and thus, the corresponding bureaucratic procedures could not be implemented in our Center, so the activities were carried out without appropriate documentary evidence. Accordingly, the corresponding expenses are not reflected in our documents.

Further expeditions towards the plots above mentioned were carried out in July-beginning of December, 2021. In each plot 5 window traps together with 5 pitfall (Barbier) traps were put in July, 2021. Samples were taken each month until the beginning of December. All the material was preliminarily elaborated and stored in freezer in our lab.

Besides, in each plot were collected samples of tinder fungus *Fomes fomentarius*. These were put into cages in the lab for breeding of associated insects. Emerged insects were stored in 70% alcohol.

Fruit bodies of other fungi associated with beech were collected and stored in silica gel.

In each plot drilling samples were collected as well.

In 2022 expeditions in March- April, 2022 were carried out towards two plots previously selected, namely:

Tavush prov., env. Yenokavan, N40.90897 E45.06504 700 m

Tavush prov., env. Koghb, "Zikatar" Environmental Center, E44.92292 1134 m

Due to adverse weather conditions (the presence of snow cover in the forest) and the impassability of field roads two other plots – in Teghut and Antaramej – were unavailable and putting traps is planned for the beginning of June. Later it was found that plots in Teghut remained unavailable due to stopping of work of Teghut mining enterprise and destruction of field-roads to the plot. The

same unavailability of field roads was found near Antaramej plot. Thus, additional plot was selected during initial visit in May, 2022, namely:

Gegharkunik prov., env. Kalavan, N40.65124 E45.12188, 1774 m.

Like in 2021, in each plot 5 window traps together with 5 pitfall (Barbier) traps were put in March, 2022 for first two plots and in May for the last one. During further expeditions in June-November, 2022 samples were taken each month. Material from pitfall (Barbier) traps was elaborated to select invertebrates collected. This material as well as window trap samples were stored in freezer in our lab.

Samples of fungi (*Fomes fomentarius* and other fungi associated with beach) and drilling samples were collected as well. These were put into cages in the lab for breeding of associated insects. Emerged insects were stored in 70% alcohol. In each plot were collected drilling samples as well.

Business trip of M. Kalashyan to Germany was carried out in September-October, 2022. During the visit some consultations and discussions concerning the Project implementations with colleagues were carried out, some materials, particularly on beetles collected, were elaborated and identified together with G, Mamadashvili. Short-time excursions to beech forests of Bavarian National Park were carried out as well.

All the material, including window trap samples, preliminarily selected soil traps materials, drilling samples and materials concerning fungi was transported to Germany for further processing partly via J. Müller and Mr. G. Mamadashvili and partly by myself during my visit.

Two expeditions in 2023 (June and September) were carried out to the Zikatar locality to collect some additional materials. These materials were elaborated in our lab and identified for further analysis.

Some equipment and consumables were purchased to re-equipment of lab and carry out the works properly.

Was published one article in which among other materials Armenian data were used:

Mamadashvili G., Brin A., Bässler C., Chumak V., Chumak M., Deidus V., Drösslerf L., Embergerg C., Georgiev K. B., Ghrejyan T., Gossner M.M., Hleb R., Rafiei-Jahed R., Kalashian M., Kambarov I., Karagyan G., Kevlishvili G., Khutsishvili Z., Kraus D., Lachat Th., Lettenmaier L., Mazmanyan M., Mitesser O., Petrov P.I., Roth N., Tabunidze L., Larrieu L., Müller J. Drivers of tree-related microhabitat profiles in European and Oriental beech forests Biological Conservation 285 (2023) 110245: [1-10] https://doi.org/10.1016/j.biocon.2023.110245 0006-3207

Two other papers concerning beech-associated click-beetles and fungi are currently submitted for publication.

Responsible Investigator

Mark Kalashyan

Supplement 3: Factual report from Georgia, NACRES – 2021

Biodiversity Conservation Center "Nacres"

Biodiversity in deadwood - basis for improving the sustainable protection of beech forests in the Caucasus and in Germany

Factual Report

2021

Collecting standardized data in Natural and Managed forests in Borjomi-Kharagauli National Park, Georgia

Prepared by: Biodiversity Conservation Center "Nacres"

Author: Levan Tabunidze

Director: Irakli Shavgulidze

28.02.2022

Georgia

Two locations in oriental beach forest of Borjomi-Kharagauli National Park were identified. One location covers the natural forests, while another one covers the managed forest. Each Sample area covers 0.5 ha.

Sample areas were visited and defined in May 2021. In both managed and natural forest 10 sample plots of 0.5 ha were established. Each sample plot consists of 20 traps, out of 20 traps 10 were window traps. Altogether, there were established 40 traps.

There were carried out a rapid assessment of the plots. Field officers collected data on habitat quality and quantity (amount of deadwood, age, crown closure), according to prior shared methods and data was sent to Germany.

Collecting the data from the established traps started after a month period from setting up the traps. Data collection and sorting the beetles collected from the traps were carried out monthly till October 2021.

NACRES

Biodiversity Conservation Center "Nacres"

Number of sample plots according to months data was collected

Months	Natural Forest		Managed Forest		Fungi Sampling	Drilling wood samples
	Ground Trap	Window Trap	Ground Trap	Window Trap		
June	10	9	10	10		
July	6	10	7	8		
August	5	10	5	6		
September	10	10	5	6	20	20

As the figures show some traps were damaged and data collection was impossible. Each months the field officers repaired the demaged traps.

The data (beetles and insects) from window traps and ground traps were sorted and placed in the special containers and sent to Germany for further studies.

Fungi sampling was carried out in Septmeber 2021, according to Fungi sampling field protocol. According to protocol, the snags and logs were identified in the sample plots. The information about the snags – Tree species, height, DBH, cause of death (wind broken etc) and logs - Decay class, length, diameter, Bark cover, moss cover, soil contact was collected. Altogether there were collected 20 fungi samples and sent to Germany for further studies.

To study molecular fungal community in Borjomi-Kharagauli National Park, data was collected from dead wood (logs and snags) in the sample areas. Drilling method was used to get the data from the dead wood according to protocol shared with us. There were collected 20 samples from dead wood and sent to Germany.

Supplement 4: Factual report from Georgia, NACRES – 2021-2024

NACRES

Biodiversity Conservation Center "Nacres"

Biodiversity in deadwood - basis for improving the sustainable protection of beech forests in the Caucasus and in Germany

Factual Report

2021-2024

Collecting standardized data in Natural and Managed forests in Borjomi-Kharagauli National Park, Georgia

Prepared by: Biodiversity Conservation Center "Nacres"

Author: Natia Muladze

Director: Irakli Shavgulidze

28.02.2024

Georgia

NACRES

Biodiversity Conservation Center "Nacres"

Project Report: Biodiversity in Deadwood for Sustainable Protection of Beech Forests

The project "Biodiversity in Deadwood - Basis for Improving the Sustainable Protection of Beech Forests in the Caucasus and in Germany" aimed to assess and compare biodiversity in deadwood between natural and managed forests in Borjomi-Kharagauli National Park, Georgia and to collect a standardized data.

The primary goal of the project was to showcase the critical role that deadwood and unmanaged forests play in fostering biodiversity within beech forests. By comparing biodiversity patterns between managed and natural forest areas, the project aimed to highlight the significance of preserving deadwood habitats for the conservation of diverse species communities.

Project Scope: Two locations within the oriental beech forests of Borjomi-Kharagauli National Park /Kvishkheti Section were selected for the study. One location represented natural forests, while the other represented managed forests. Each sample area was above 1000 m. and covered 0.5 hectares, with 10 sample plots established in both managed and natural forests. Each sample plot consisted of 20 traps, including 10 window traps, totaling 40 traps across all plots.

Methodology: The project began in May 2021 with the identification and delineation of sample areas. Field officers established sample plots and traps according to predetermined methods. A rapid assessment of the plots was conducted to collect data on habitat quality and quantity, focusing on parameters such as deadwood amount, age, and crown closure.

Data Collection and Analysis: Following the establishment of traps in the sample plots, data collection commenced after a one-month period to allow for sufficient time for beetles and other organisms to populate the traps. During the project period field officers conducted monthly visits to the sample plots to collect data from the traps and sort the beetles collected.

During each visit, field officers carefully examined the traps to record the presence and abundance of beetles and other insects. Data collected included species identification, abundance counts, and morphological characteristics when necessary. Additionally, environmental factors such as temperature, humidity, and weather conditions were documented to assess their potential impact on beetle activity and diversity.

The collected beetles were sorted, categorized, and preserved for further analysis. Special attention was paid to identifying indicator species associated with deadwood habitats, as their presence can provide valuable insights into the biodiversity and ecological health of the forest ecosystem.

During the course of the project, it was observed that some traps were damaged, rendering data collection impossible. Factors such as adverse weather conditions, wildlife interference, and natural decay may have contributed to trap damage. In response, field officers repaired damaged traps each month to ensure the continuity of data collection efforts. This proactive approach aimed to minimize disruptions to the study and maintain the integrity of the data gathered.

Following sorting and containerization, the collected specimens up to 260 in total, were sent to collaborating partners in Germany for further studies.

As part of the project's research objectives fungi sampling activities were conducted in Borjomi-Kharagauli National Park. The sampling protocol involved the identification and characterization of snags (standing dead trees) and logs within the sample plots. Field officers collected detailed information about each snag, including tree species, height, diameter at breast height (DBH), and the cause of death (e.g., windbreak). Similarly, logs were assessed for decay class, length, diameter, bark cover, moss cover, and soil contact.

A total of 20 fungi samples were collected from the identified snags and logs following the prescribed sampling protocol. These samples were carefully selected to represent the diversity of fungal species inhabiting deadwood substrates within the study area. Each sample was labeled, packaged, and prepared for transportation to Germany for further molecular analysis.

The project represented a significant step towards understanding the role of deadwood in supporting biodiversity in beech forests. By comparing natural and managed forests, the study aimed to inform forest management practices that promote biodiversity conservation and sustainable forest protection. The methodology used during the project can serve as a tool for future biodiversity assessments and conservation efforts in similar forest ecosystems.

Supplement 5: Final report from Lagodekhi, Georgia by Lars Drössler

Final report about the BeechDIV field work in Lagodekhi

by Lars Droessler, a Georgian professor in forestry and forest sciences from 2017-2022, responsible for the field work of the BMBF-project **BeechDIV: Biodiversity in deadwood - base of an improvement of sustainable conservation of beech Forests in Germany and the Caucasus (reference no 01DK21002)** in Lagodekhi, eastern Georgia

General remark and thanks

I was surprised that the project proposal was granted so early. Lagodekhi is still no National park and there is no Institute of Ecology in Lagodekhi, as I corrected in the draft version of the project proposal. We started the field work already in March 2021 (despite official papers were not finalized yet as the state university has to wait for the approval by the Georgian ministry of finances and follows not only German rules in the project). I am grateful to the Georgian university administration and the Ministry of Finances for their fast processing. I did not like the attitude from German side to expect official papers with stamp and signature from authorities to be sent to Germany within a week. We are not so fast with paper copies and our whole system is based on digital signatures and copies. But I thank the German university staff and research station for the reminders and patience. In general, I am proud that Ilia State university managed all these small bureaucratic and logistic hurdles of the project in time.

Selection of students and study plots

From the beginning, the two forest students Tinatin Lobjanidze and Nika Gobronidze were designated to work in the project after defending their master thesis. But both were too busy in our DendroLab to analyze bore cores of the National forest inventory for GIZ. We organized a field trip to the old-growth beech forest in Lagodekhi for other students of the master program "Nature protection and forestry" which I was coordinating (my main task in Georgia), but no other master student or graduate was available at that time, so we focused on the recruitment of BSc ecology students at the university. Two potential forest students, namely Shota Tsiklauri and Revaz Kvaratskhelia joined me in the field during scouting of suitable study areas in the protected area and in managed forests. In addition, I employed the forester Giorgi Gigauri for one month to introduce us to the regional and local forest administration of the National forest agency and the Agency of Protected Areas to access their maps and old management plans. Unfortunately, after the establishment of a few study plots, the two potential forest students

found work for better money than I was ready to pay. Shota Japarashvili, another BSc ecology student with a special interest in beetles, was eager to use and establish the insect traps on the plots, so I employed him for this task and expected him to collect the insect samples as well. However, he was not able to collect insects every month, because of writing his BSc thesis (that's what he said) and due to his new duty as country coordinator of another, larger BMBF infrastructure project named CaBoL at the Institute of Ecology in Tbilisi.

Basically, I ended up to train one new student every month in the woods when we went 40 km through the mountains to empty the traps. Luckily, in the Rachisubani district, I found reliable help by the local ranger, during his spare time. Without him we would not have managed to collect all insects in 2021 due to other duties and forest projects. The rangers are busy at work with their daily tasks, the director Dr. Giorgi Sulamanidze explained to me one day when I complained and pointed on the following sentence in the BeechDIV project proposal: "Die Schutzgebietsverwaltung unterstützt das Projekt mit Ortskenntnis, Vermittlung von Unterkünften und die lokale Betreuung von Fallen." The director and me then tried two times with two locals who were unemployed to collect samples, but they already told me at the end of the day that walking on steep slopes was too much.

Typical Oriental beech forest in eastern Georgia is located 1000-1500 m above sea level. That is were the study plots are located, remote from villages and at least two hours hiking uphill from the parking lot. The plots were randomly selected in two distinct areas of typically managed forest, and in two distinct areas of the natural forest on similar site (same soil, elevation and exposition, and climate).

While insect and fungi samples were taken in 2021 and the sampling could be finished during the first year, most of the tree marking and measurements were conducted in 2022. Fungi sampling was fast and could be done easily. In 2022, I taught seven foreign students how establish long-term study plots. Also, I introduced them to the TReM manual and taught them how to record micro-habitats accordingly. Four German students and their FH professor decided to limit the TReM assessment to the lower 6 m of the trunk for their bachelor thesis, because it is so difficult to see micro-habitats in the crown (that is what they said). The only good thing for me with that decision was that the Georgian PhD student Giorgi Mamadashvili finally had to visit these 20 study plots by himself. That made me very happy, but I became grumpy again after his statement or joke that nobody will remeasure these long-term study plots again after 5 or 10 years (in terms of growth and forest development). To establish modern forest sciences in

the Caucasus, that would be really important, I still think. Well, let's see how things develop after BeechDIV...

In any case, BeechDIV substantially contributed to the establishment of 20 long-term forest study plots in eastern Georgian beech forests.

Beside the insect and fungi sampling in 2021, additional activities could be organized by "AG Drößler":

- Teaching the differences between managed and natural forests, beech forest dynamics and biodiversity monitoring to university students (2021, 2022 and 2023)

- Annual excursions with master students and CaBoL BioBlitz with students to Lagodekhi (2021 and 2022)

- Beetle species pre-identification in Tbilisi (2021)

- Ranger training in Lagodekhi, teaching PA staff about the importance of deadwood and natural forest dynamics (2022)

- excursion to Germany with Georgian master students and teaching staff to explore managed and protected beech forests in Germany (DAAD funded, 2022 and 2023)

- Permanent marking of trees and plots, marking plot center and borders (2022)

- Soil sampling on the study plots (2022)

- Regeneration survey with light/canopy assessment (2022)

- Wood quality assessment (by a bachelor student from FH Erfurt, 2022)

- Knowledge exchange with the forest inventory and taxation company in Lagodekhi (2022)

- Wood pecker survey in Lagodekhi (2022)

- Collection of genetic samples of beech in Lagodekhi (2022)

- removing the window traps and other plastics from the plots and giving them to Shota Japarashvili (2022)

- Terrestrial laser scans of beech forest structure along an elevation gradient from 500-2000 m in Lagodekhi (collaboration between Nika Gobronidze and University of Göttingen, 2022)

- Meeting with the second BeechDIV project partner Levan Tabunidze (NACRES) in Borjomi, telling him briefly about our method to establish long-term study plots in the forest, and offer students help in case they also would like to establish long-term plots in Borjomi (2022)

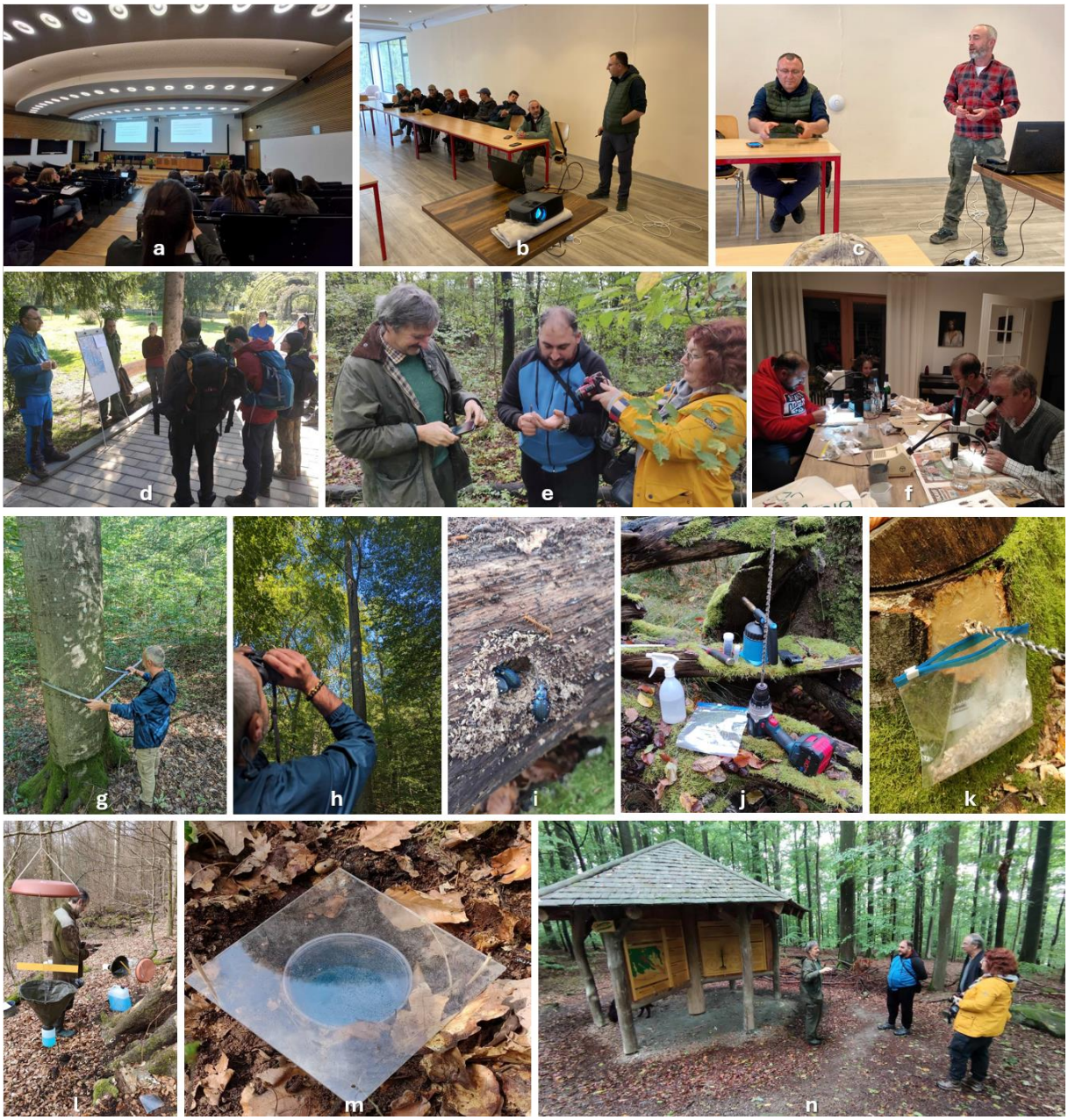
- Establishment of a Marteloscope site in the flat area of the management zone of the protected area Lagodekhi to teach silviculture and forest biodiversity with easy forest access in Lagodekhi (2022)

- Establishment of a small exercise stand (2 ha so students don't get lost) to introduce foresters and students to first thinning in 20-30 years old, overexploited mixed forest near Mtisdziri (the forest district with the well-managed beech forest study area) (2022)

The main project partner Lars Droessler left Georgia in November 2022.

Finally, in year 2023, a scientific article about managed and unmanaged beech forest in Lagodekhi and Mtisdziri, based on the 20 long-term study plots was published: https://caucasiana.pensoft.net/article/106898/

Supplement 6: Photos captured during fieldworks, meetings, and conferences



a - Oral presentation by G. Mamadashvili in GFÖ Conference in Leipzig, Germany. b - Presentation by G. Sulamanidze (the director of Lagodekhi National Park) in Lagodekhi, Georgia. c – Presentation of G. Mamadashvili in Lagodekhi National Park, Georgia. d – Meeting the director of National park and German students during their "Biodiversity excursion in Georgia". e – Fieldwork meeting of Armenian specialists Gayane Karagyan, Tigran Ghrejyan with German forester Ulrich Mergner in Steigerwald forest, Germany. f – Meeting of specialists and beetle identification: Mark Kalashian, Tigran Ghrejyan, Andrea Jarzabek-Müller, and Jörg Müller. g – Measuring the beech tree DBH. h – Looking for tree-related microhabitats in the canopy. i – Saproxylic beetles (Carabidae). j – Field equipment's for drill sampling from the

deadwood, for future DNA sequence. k – Drilling the stump by the driller. l – Flight interception trap. m – Pitfall trap. n – meeting the specialists in the field.