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Forest Restoration in Croatia – Historical Overview

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ABSTRACT

In the era of climate change, increasing ecological disturbances, and declining biodiversity, forest ecosystems face growing challenges that require effective restoration approaches grounded in ecological principles, sustainability, and adaptive management. Forest restoration plays an important role in developing resilient, stable, healthy, and diverse forests capable of sustaining ecosystem services. As these challenges intensify, restoration efforts become more important for ensuring the long-term sustainability of forests. Understanding the history of restoration is therefore crucial for making informed choices and guiding both current and future decisions. This overview examines the development of forest restoration in Croatia through three significant historical periods: before 1940, from 1940 to 1990, and from 1990 to the present. Each period reflects the increasing significance and growing understanding of restoration, shaped by environmental needs, policy, knowledge, social awareness, and technological capabilities. Early efforts were based on demanding afforestation and reforestation practices, driven by economic and political events, environmental disasters, and conservation needs. In the mid-20th century, efforts were influenced by post-war forest degradation and industrial forestry. Since 1990, restoration has increasingly incorporated ecological principles, international frameworks, and adaptive strategies, supported by advances in science, rising public awareness, and improved technology and mechanisation. The growing role of forest restoration over time reflects a broader recognition of its complexity and importance in addressing both past degradation and future environmental uncertainties.

Keywords: afforestation; reforestation; silviculture; historical periods; contemporary restoration principles; lessons learned

INTRODUCTION

Forest ecosystems are increasingly threatened by climate change, biodiversity loss, invasive species, and ecological disturbances. These challenges call for restoration approaches based on ecological integrity and adaptive management. Forest restoration plays a key role in creating resilient, stable, and diverse ecosystems that can withstand environmental pressures (Menéndez-Miguélez et al. 2025). The European Nature Restoration Regulation (NRR) and the requirement for National Restoration Plans establish an important legal setting for restoration. With over 80% of habitats in poor condition in Europe, the NRR sets binding targets for forests, supports the EU's pledge to plant 3 billion trees by 2030, and promotes reconnecting rivers, restoring floodplains, and expanding urban green space. These measures aim to enhance biodiversity, secure essential forest services, mitigate climate change, and strengthen resilience to environmental risks. To plan and examine effectively under this framework, and other national restoration efforts, it is necessary to understand the historical trajectory of restoration—what has succeeded and what has failed—so that past lessons can inform present strategies and guide future initiatives.

Although often associated with recent environmental concerns, forest restoration has deep historical roots. The earliest known forest restoration practices date back to China during the Chou dynasty (1122–256 BCE). Restoration at that time focused on natural ecosystem management, resource use, forest protection, and reforestation (Lee, 1949, Erdozain et al. 2024). Ancient Chinese manuscripts described regulated timber harvesting, tree planting to preserve soil and water, and the importance of living in harmony with nature (Needham 1986). This is considered the first documented example of organised tree planting for restoration purposes. In Europe, early reforestation, as part of restoration, appeared during the medieval period. In the 13th century, large-scale tree planting was carried out near Nürnberg, Germany, on severely degraded land (Erdozain et al. 2024). The concept of sustainable forestry—Nachhaltigkeit—was first introduced in Germany in 1713. It promoted a balance between forest use and restoration (Agnoletti and Anderson 2000, Matić et al. 2015), a principle also adopted in Croatia and still applied today.

Croatia offers a uniquely valuable case for studying forest restoration history because it combines a long tradition of scientific forestry with a highly diverse forest landscape shaped by major political, economic, and social transitions. Croatia's forests have undergone cycles of degradation and recovery under different governance regimes from the Austro-Hungarian Empire to Yugoslavia and the modern EU. Restoration efforts in Croatia date back to 1514, when the Tripartitum—a legal code written in the Kingdom of Hungary, which at the time included the regions of Croatia and Slavonia— introduced regulations to limit tree felling and livestock grazing, aiming to protect and preserve forest ecosystems (Vac 1900). This reflects some of the earliest legal foundations for sustainable forest use in the Croatian historical context. This long and rich history makes Croatia an exceptional natural laboratory for understanding how historical legacies shape contemporary restoration challenges and opportunities.

Therefore, the goal of this article is to examine the historical development of forest restoration in Croatia and the conditions that have shaped it over time. It also considers current challenges and the growing need to expand restoration in response to climate change. An emerging restoration strategy known as prestoration emphasises selecting species based on their projected ecological suitability under future climate and hydrological conditions. Prestoration involves using species that are expected to persist both now and in the future (Butterfield et al. 2017).

This paper reviews forest restoration activities and examines their evolving role in Croatia over time by analysing available studies and data. Sources include academic databases such as Web of Science, Scopus, Google Scholar, and Hrčak, as well as historical manuscripts and documents from Croatian forestry institutions. Forest restoration in Croatia for the purposes of this research is understood in a broad sense, encompassing afforestation, reforestation, meliorative interventions, and ecological restoration. It also includes economic, political, and social dimensions such as timber production, erosion control, and rural development.

Croatian forests are diverse, ranging from the Adriatic Sea along the coast, through mountainous regions, to floodplain and riparian areas in the east. To illustrate the geographical context relevant to forest restoration, Figure 1 presents a map of Croatia highlighting major regions, settlements, rivers, and the historical area of the Vojna Krajina.

The literature review covers the earliest available materials up to the present, using keywords such as "forest restoration in Croatia," "silviculture development in Croatia," "karst reforestation," "floodplain forest degradation," and "Croatian Forest Act." The results are organised into three historical periods—before 1940, from 1940 to 1990, and after 1990—chosen to reflect major socio-political changes, shifts in forestry policy, and

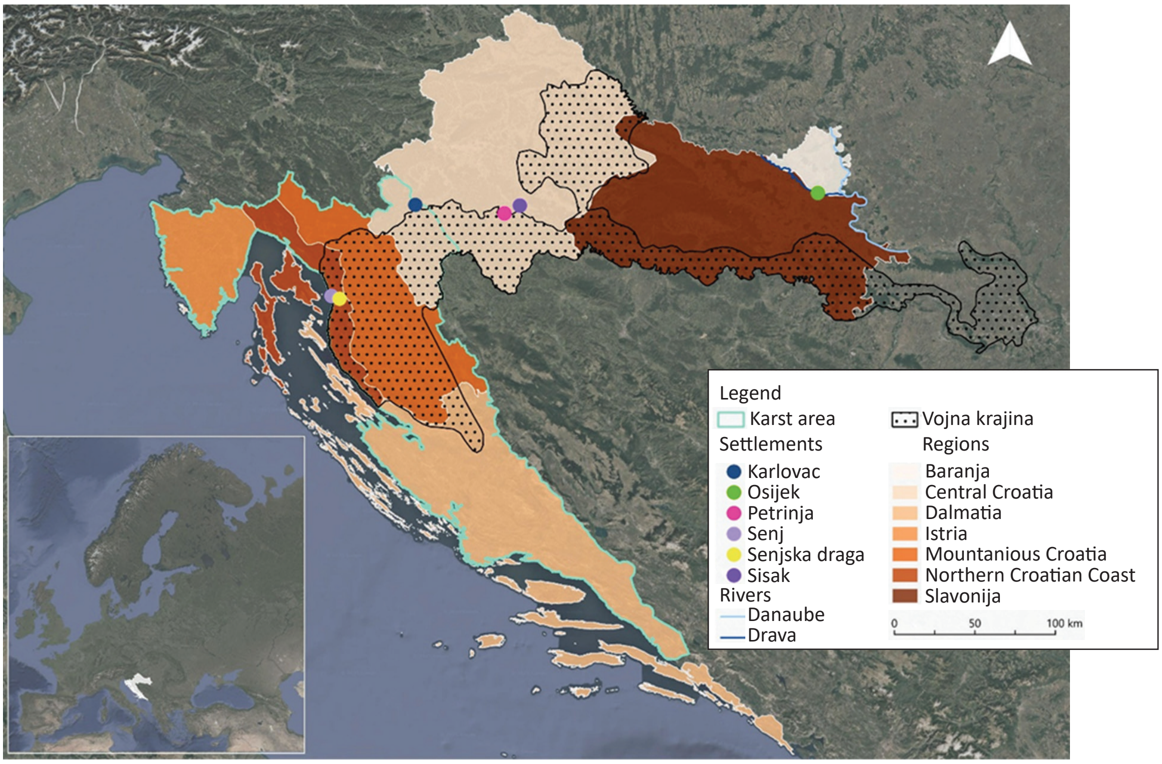


Figure 1. Map of Croatia with major regions, settlements, rivers, and the historical Vojna Krajina.

evolving environmental challenges and disturbances that influenced restoration practices.

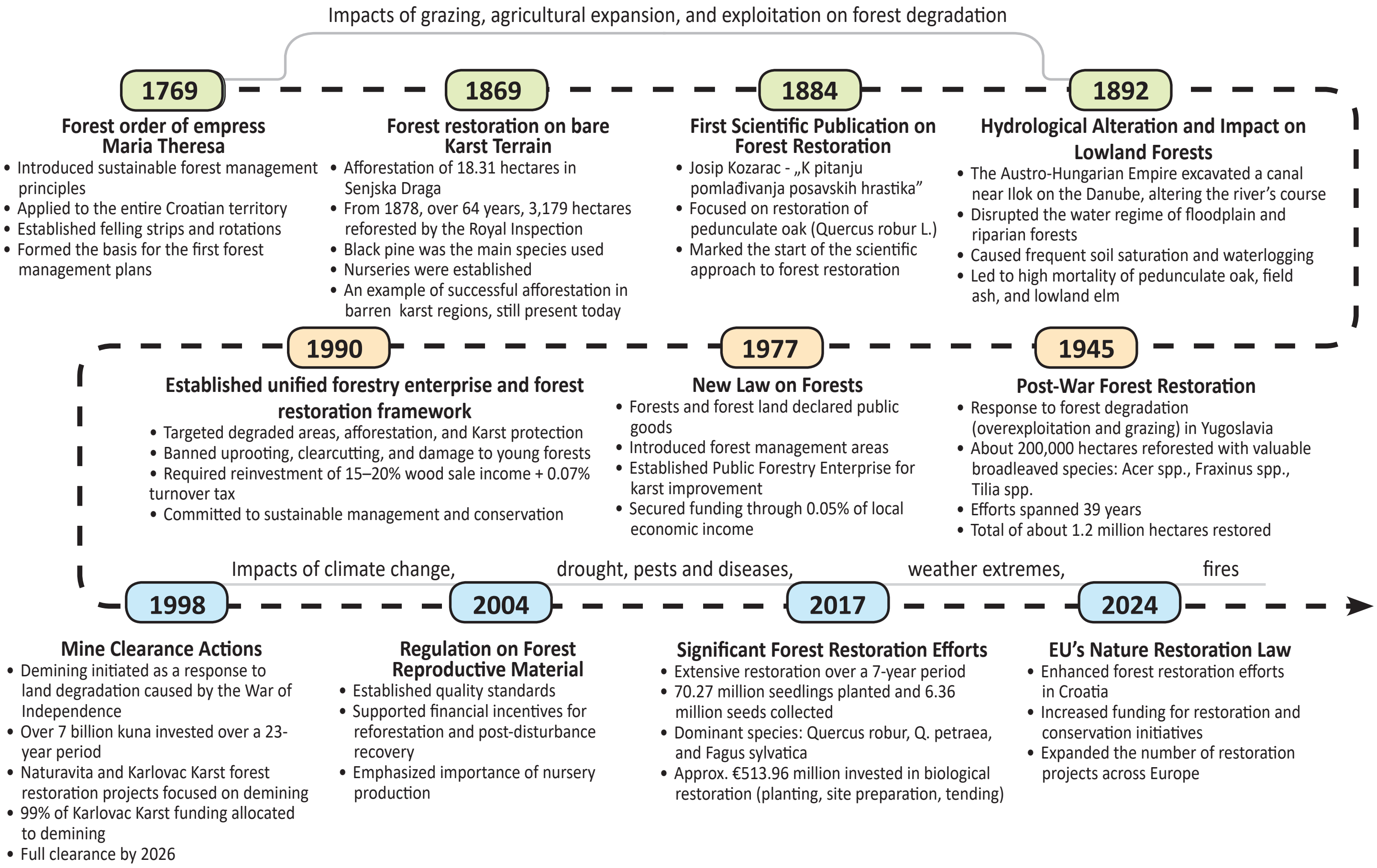
Changes in Restoration Perspectives and Efforts Throughout the Key Historical Periods

Forest restoration efforts in Croatia throughout history have been driven by different political regimes, wood exploitation, wars, mine contamination, population expansion, and other ecological and social challenges. From medieval forest regulations to today's more sustainable management, the Croatian approach reflects a mix of tradition, law, and scientific progress. This development can be divided into three main periods, each marked by the need for forest restoration and changes in restoration activities. Figure 2 presents a timeline highlighting key milestones and challenges in Croatia's forest restoration efforts.

Forest Restoration in Croatia Before 1940

The appearance of municipal statutes in Croatian towns during the 12th century marked the beginning of structured forest protection, introducing early regulations aimed at preserving forest areas and limiting excessive logging. Despite these initial steps, widespread deforestation before and during the First Industrial Revolution—combined with a lack of restoration—led to significant ecological and economic impacts. These effects were especially evident in lowland regions and floodplains, where industrial activity was most concentrated (Matić et al. 2015). In response to growing awareness of the long-term risks of unsustainable forest use and the need for balanced management, forestry began to develop, grounded in the principle of sustainability. In Croatia, forestry started to take shape as an established profession in the 18th century. The earliest formal regulation dates to 1755, when the Forest Order for Vojna Krajina introduced guidelines for managing and protecting forests in Slavonia, including instructions for foresters (Anić 2025). In 1765, the Habsburg military administration introduced forest-management guidelines for the Ogulin region and the Velebit area, formalised in the Instruction for the Karlovac Generalate (Pernar 2016). During this period, the military conducted extensive surveys that resulted in the mapping of the vegetation of eastern Croatia and Slavonia (Altić 2022).

A major milestone was reached in 1769 with the adoption of the first comprehensive forest law under the Forest Order of Empress Maria Theresa (Klepac 1965, Meštrović 1995). This legislation introduced the principle of sustainable forest management and, for the first time, applied to the entire territory of Croatia. It also outlined procedures for dividing forests into annual felling strips and establishing rotations, which later formed the basis of the first forest management plans (Meštrović 1995, Benko and Čavlović 1998). The first modern Forest Act was enacted by Emperor Franz Joseph I on December 3, 1852, applying to the entire Austro-Hungarian Monarchy. By special imperial decree, it came into force in Croatia and Slavonia on January 1st, 1858, and in Vojna Krajina on February 7th, 1860. The



1769
Forest order of empress Maria Theresa
- Introduced sustainable forest management principles
- Applied to the entire Croatian territory
- Established felling strips and rotations
- Formed the basis for the first forest management plans

1869
Forest restoration on bare Karst Terrain
- Afforestation of 18.31 hectares in Senjska Draga
- From 1878, over 64 years, 3,179 hectares reforested by the Royal Inspection
- Black pine was the main species used
- Nurseries were established
- An example of successful afforestation in barren karst regions, still present today

1884
First Scientific Publication on Forest Restoration
- Josip Kozarac - „K pitanju pomlađivanja posavskih hrastika"
- Focused on restoration of pedunculate oak (Quercus robur L.)
- Marked the start of the scientific approach to forest restoration

1892
Hydrological Alteration and Impact on Lowland Forests
- The Austro-Hungarian Empire excavated a canal near Ilok on the Danube, altering the river's course
- Disrupted the water regime of floodplain and riparian forests
- Caused frequent soil saturation and waterlogging
- Led to high mortality of pedunculate oak, field ash, and lowland elm

1945
Post-War Forest Restoration
- Response to forest degradation (overexploitation and grazing) in Yugoslavia
- About 200,000 hectares reforested with valuable broadleaved species: Acer spp., Fraxinus spp., Tilia spp.
- Efforts spanned 39 years
- Total of about 1.2 million hectares restored

1977
New Law on Forests
- Forests and forest land declared public goods
- Introduced forest management areas
- Established Public Forestry Enterprise for karst improvement
- Secured funding through 0.05% of local economic income

1990
Established unified forestry enterprise and forest restoration framework
- Targeted degraded areas, afforestation, and Karst protection
- Banned uprooting, clearcutting, and damage to young forests
- Required reinvestment of 15–20% wood sale income + 0.07% turnover tax
- Committed to sustainable management and conservation

1998
Mine Clearance Actions
- Demining initiated as a response to land degradation caused by the War of Independence
- Over 7 billion kuna invested over a 23-year period
- Naturavita and Karlovac Karst forest restoration projects focused on demining
- 99% of Karlovac Karst funding allocated to demining
- Full clearance by 2026

2004
Regulation on Forest Reproductive Material
- Established quality standards
- Supported financial incentives for reforestation and post-disturbance recovery
- Emphasized importance of nursery production

2017
Significant Forest Restoration Efforts
- Extensive restoration over a 7-year period
- 70.27 million seedlings planted and 6.36 million seeds collected
- Dominant species: Quercus robur, Q. petraea, and Fagus sylvatica
- Approx. €513.96 million invested in biological restoration (planting, site preparation, tending)

2024
EU's Nature Restoration Law
- Enhanced forest restoration efforts in Croatia
- Increased funding for restoration and conservation initiatives
- Expanded the number of restoration projects across Europe

Figure 2. Croatian forest restoration timeline.

law introduced a formal classification of forests into state-owned, municipal, and private categories. It emphasised sustainability, which was explicitly stated in Article 9, requiring that forests be maintained and improved through responsible management (Matić et al. 2015).

Scientific interest in forest restoration began with the work of forest engineer Josip Kozarac, who published an article in 1884 in a Croatian forestry journal on the restoration of *Quercus robur* L., one of the most important tree species in Croatia. The first legally grounded forest management plan for the Sisak and Petrinja area, which included the restoration of *Q. robur* forests, dates to 1853. These forests have been under continuous management for more than 170 years (Matić 1994). In 1894, a significant law was passed, establishing a formal system of forest governance and oversight by public authorities (Anić 2025). It regulated the appointment of professional forestry staff and the implementation of forest management practices in areas of special public interest, marking the beginning of organised restoration efforts in Croatian forestry. From then until the mid-20th century, Croatia began to see the first tangible results of restoration. Although data from this period are limited, records show that activities took place in various regions across the country, nearly all on public land and funded by the government (Čavlović 1994). Apart from royal and church-owned forests, most landscapes remained unmanaged and heavily exploited, leading to severe degradation, particularly in karst regions.

First Forest Restoration Activities in Croatia's Karst Regions

Throughout this period, forests in Croatia's karst regions have suffered from overexploitation and degradation. Excessive logging, which left forests degraded and exposed karst terrain, combined with uncontrolled grazing and limited public awareness, led to soil erosion, water loss, reduced agricultural productivity, and severe flooding (Matić et al. 2011). Historical manuscripts indicate that the first restoration efforts were mostly concentrated in Dalmatia, Istria, and other karst landscapes. Until the late 18th century, Dalmatia and Istria were under Venetian rule, with forest management beginning in the 13th century in Istria and the 15th century in Dalmatia. The Venetians established the first systematic forest surveys in Europe and the first forest cadastre (Morosini 1980), which included these regions. However, limited information is available on forest restoration from that time.

In the late 18th century, these areas briefly came under Austrian rule, during which public care for forests continued to decline. This led to increased deforestation and frequent flooding. In the early 19th century, French authority was established, and during the autonomous French administration in 1809, Croatia introduced forestry laws to address the gradual forest degradation. That same year, the Mediterranean region became part of the Illyrian Provinces. In Dalmatia, the main goal was to prevent goat grazing by building fences. About 30,826 families were required to maintain these forest areas. In total, 372 forest stands were established in Dalmatia, covering approximately 19,000 hectares (Piškorić 1976). These efforts also created jobs, giving the initiative economic value. The earliest professional afforestation plans for barren karst areas in Istria were developed by J. Ressel in 1842, marking the start of organised forest restoration in the region (Lipej 2005). Degradation continued until 1886, when a law mandated afforestation of bare karst areas.

Afforestation efforts began in Ogulin in 1865, in Gospić in 1867, in Otočac in 1868, and in Senj in 1869 (Severinski 1965, Ivančević et al. 1986). These early efforts focused on regeneration cuttings and reforestation of small clearings but had limited overall success. In 1869, afforestation began in Senjska Draga, restoring about 18.31 hectares. The project was paused until 1878, when the Royal Inspection for the Afforestation of Karst in the Croatian Military Border—also known as the Inspectorate for the Afforestation of Karst, Bare Rocky Grounds, and Flood Control—was established. This institution initiated a more systematic and large-scale restoration of degraded karst areas. The Inspection's activities covered 40,000 hectares of scrubland and barren terrain (Severinski 1965). The main goals were to reduce erosion, regulate wind and water impact, and protect infrastructure in torrent-prone areas. *Pinus nigra* JF Arnold was the dominant species used, and many of the region's largest forest cultures were established during this time. Regeneration cuts also improved stand vitality and structure, while grazing was prohibited on 2,000 hectares of degraded land (Ivančević and Piškorić 1986). From 1878 to 1942, the Royal Inspection managed 51,777 hectares. Within this, 95 crop areas and forest preserves were created on 10,097 hectares. During that time, 1,738 hectares were reforested, 1,441 hectares were additionally planted, and 7,435 hectares were regenerated naturally. To support these efforts, nurseries produced 82,724,368 seedlings and 3,612 kg of seeds for restoration (Severinski 1965, Ivančević 1999). Planting used a standard method involving 40 × 40 × 40 cm pits for bare-root seedlings. Both coniferous and broadleaf species were also sown. The quantity of seedlings and seeds used in these restoration efforts is presented in Table 1.

Table 1. Quantity of seedlings and seeds used in the early restoration of karst forests in Senj and the surrounding area (late 19th to early 20th century).

Species/Category	Quantity	Unit
Pinus nigra JF Arnold	67,023,543	seedlings
Fraxinus ornus L.	4,136,218	seedlings
Other species	11,564,103	seedlings
Coniferous species	57.79	kg (seeds)
Broadleaf species	3,554.21	kg (seeds)

Note: Percentages used to calculate the quantity of seedlings and seeds by species were originally reported by Ivančević (1999); the values presented are approximate calculations based on these data.

During its 64 years of operation, the Karst Afforestation Inspectorate functioned under four different empires. Over this period, the Inspectorate spent a total of 372,088 forints, 1,043,774 crowns, 15,534,211 dinars, and 404,996 kuna (Piškorić and Vukelić 1992). More than half of the expenditures covered personnel costs, one-third funded biological restoration activities, and the remaining amount was allocated to technical measures (Figure 3).

Due to intense torrent activity in karst areas, particularly around the city of Senj, a concrete barrier was built in 1892, along with 165 additional concrete structures. More than 21,358 m² of material were excavated, and over 8,457 m³ were used for torrent regulation in karst regions of Croatia (Ivančević 1999). These efforts successfully brought the torrents under control. Notably, Senj has not experienced severe flooding since 1856, demonstrating the effectiveness—as well as the complexity and cost—of these restoration measures. Between 1886 and 1913, 4,000 hectares were afforested, although the efforts were interrupted by World War I. Between the two world wars, another 2,000 hectares were afforested (Piškorić and Vukelić 1992).

First Restoration Efforts and Challenges in *Quercus robur* L. Forests of Slavonia

At the beginning of the 18th century, *Q. robur* forests were widespread across lowland and floodplain areas, with forest cover in Slavonia reaching 70% (Rauš 1973, Anić 2025). These stands resembled untouched primaeval forests (Anić 1971), and regeneration occurred naturally from seed. Natural regeneration was mostly achieved through individual tree cutting, while direct acorn sowing under the hoe was rarely used (Kozarac 1897). After 1699, when Slavonia came under Habsburg rule, the structure and natural regeneration ability of these forests began to decline. This was due to overexploitation, population growth, increased grazing, acorn shortages, and browsing of young saplings. As a result, restoration efforts focused on areas near villages, with acorn sowing under the hoe *for Q. robur* artificial regeneration (Kozarac 1886).

During the 18th and 19th centuries, *Q. robur* forests in eastern Croatia continued to degrade, partly due to hydrotechnical interventions. These altered the course of the Danube River, drying out and destabilising forest ecosystems (Pilaš and Planinšek 2011). In 1892, the Austro-Hungarian Empire excavated a canal near Ilok, close to Šarengrad, to improve navigation. This significantly changed the river's course and disrupted the water regime of nearby forests (Mayer 1994, Mayer and Hećimović 2008). Similar interventions occurred along the Sava River, where numerous dredging operations were undertaken throughout the 18th century to facilitate navigation, including documented works on the Sava and Kupa in 1759. The rapid escalation in the economic and commercial

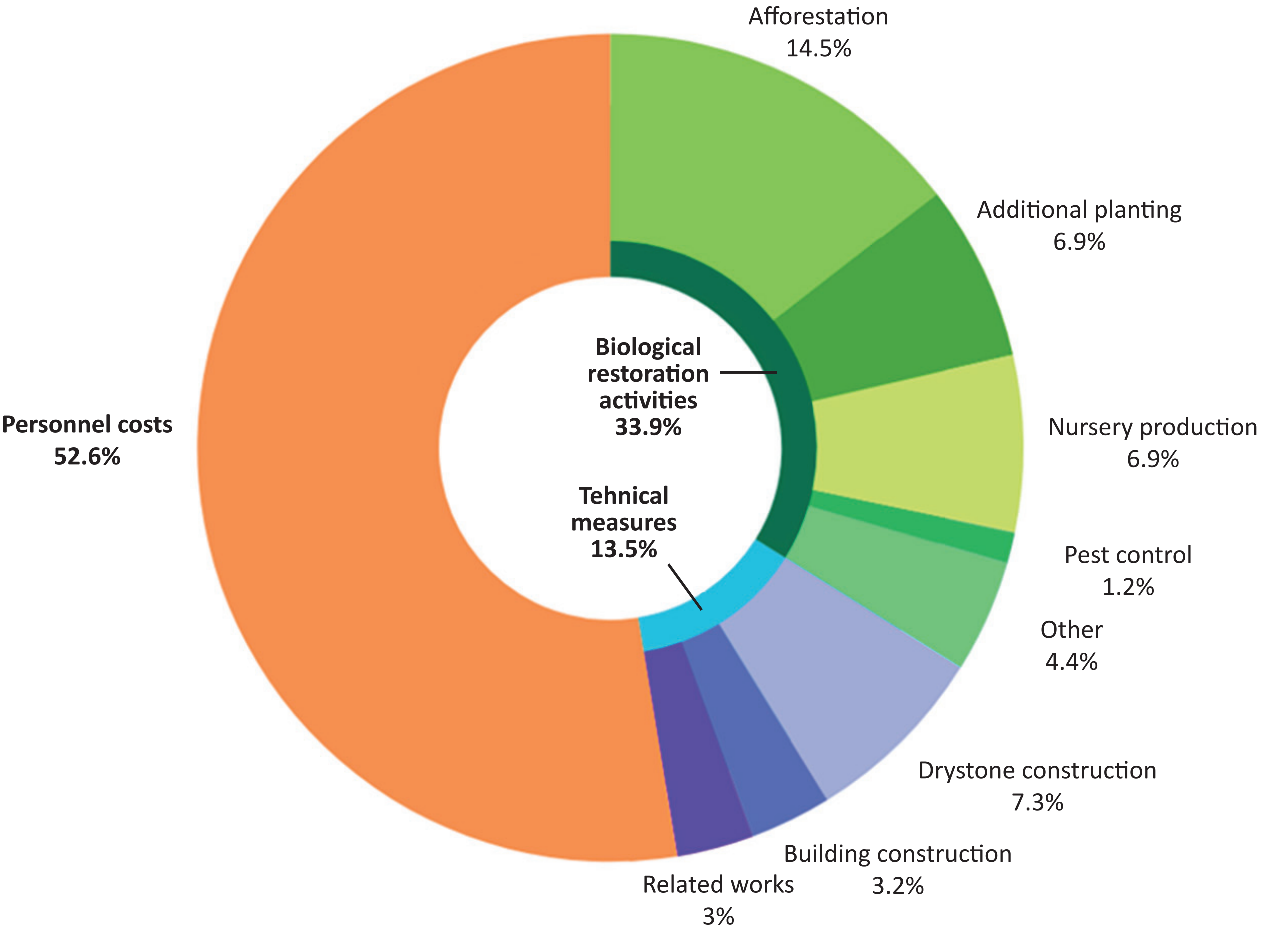


Figure 3. Distribution of Expenditures by the Karst Afforestation Inspectorate, 1878–1942 (Source: Piškorić and Vukelić 1992).

significance of the Sava during this period is closely linked to the liberation of large portions of the Sava–Drava interfluve from Ottoman rule. Following these conflicts, the entire Sava–Drava interfluve came under Habsburg administration, which stimulated the river's commercial use and further impacted its hydrology (Šimunjak 2022). By the 19th century, changes in the hydrological regime had severely affected the stability and ecosystem services of lowland forests dominated by *Q. robur* (Pilaš and Planinšek 2011). Frequent waterlogging led to tree dieback. High tree mortality rates were recorded in *Q. robur* and *Fraxinus angustifolia* Vahl, while *Ulmus minor* Mill. disappeared from the stand composition (Matić 1994). These extreme site conditions reduced the growing stock, although *Q. robur* remained the most economically important hardwood species in Croatia. Poor forest management—such as poorly planned infrastructure and heavy machinery use—worsened weed growth and increased soil water retention, further hindering natural restoration.

To address this, artificial regeneration of *Q. robur* stands was implemented, along with habitat restoration measures such as drainage system construction to regulate soil water levels (Posarić 2008). The goal was to initiate regeneration before weeds established, and further hydrological stress occurred. Restoration included direct sowing of acorns and planting of seedlings, based on regeneration methods. This involved seed and final cut, use of forest reproductive material, monitoring, and tending. Clear-cutting was considered unsuitable for restoring *Q. robur* stands (Starčević 1990). Under optimal conditions, regeneration cuts could restore stands within 10–12 years (Matić 1994). These efforts reflected a strong commitment to sustainable forest management. This principle was formally adopted in the Kingdom of Yugoslavia with the Forest Act of December 21st, 1929 (Matić et al. 2015). The law required sustainable practices in both state-owned and private forests (Meštrović 1978).

Forest Restoration in Croatia from 1940 to 1990

During this period, up to the 1990s, Croatia was part of Yugoslavia, and most available data on forest restoration and management refer to the entire Yugoslavian territory. Forests were transferred to state ownership when Yugoslavia took control of previously privately-owned land, resulting in 76% of forests becoming state-owned and 24% remaining private. Forestry efforts focused on afforestation, reforestation, and implementing sustainable forest management. By the mid-20th century, sustained forest management aimed to preserve and enhance biodiversity, productivity, vitality, ecosystem protection, and the social role of forests (Klepac 1998, Matić et al. 2015).

Between 1945 and 1990, the Forest Law was amended six times, with an emphasis on conservation, preventing degradation, and promoting afforestation. Despite these efforts, forests experienced intensive logging after both world wars, increasing the need for restoration. In 1948, timber harvesting in Croatia reached 7.24 million m^3, exceeding the estimated increment of 5.5–6.0 million m^3 by roughly 20–30%. It wasn't until the late 1950s that harvested volume fell below the estimated increment—a trend that has continued (Krpan 1992, Piškorić and Vukelić 1992).

To address overexploitation, about one million hectares were afforested across Yugoslavia after World War II, of which around 200,000 hectares were planted with valuable species such as *Acer* spp., *Fraxinus* spp., and *Tilia* spp. to enhance timber quality and ecological resilience. The remaining 800,000 hectares were predominantly afforested with conifers (*Pinus nigra, Pinus sylvestris* L., *Picea abies* (L.) Karst.) on karst and degraded soils, *Robinia pseudoacacia* L. on poor sites, and *Populus* spp. and *Salix* spp along rivers. Between 1945 and 1956, site preparation led to the restoration of 220,000 hectares. From 1957 to 1965, 177,300 hectares were reforested, along with 369,400 hectares of meliorative interventions. Reforestation continued with 91,140 hectares (1966–1970), 77,170 hectares (1971–1975), 168,075 hectares (1976–1980), and 181,523 hectares (1981–1984) (Stilinović 1986).

From the 1960s, the use of mechanisation significantly improved restoration efficiency. Chainsaws increased labour productivity by 75%, improved cost-effectiveness by 16%, and doubled overall economic efficiency. Forwarders were introduced for more efficient timber extraction (Sever and Slabak 1988). Most restoration efforts targeted karst forests and were financed through taxes and grazing bans. Support also came from the United Nations, beginning in 1954—the same year the Institute for Practical Forestry Research was founded in Zagreb. This later evolved into the Croatian Forest Research Institute (since 2009), after merging with the Institute for Forest Seed Control in Rijeka (1959) and the Institute for Conifers (1961). These developments led to the establishment of the Forest Research Institute in Jastrebarsko in 1974 (Gračan 2015).

In 1947, the Law on Declaring Forests Public Property and the formation of forest offices consolidated forest management in Croatia. The 1977 Forest Law declared forests and forest land as public goods, introduced the concept of the forest management area, and established a Public Forestry Enterprise for karst forest improvement, funded by a 0.05% levy on the gross income of local economic entities.

Restoration Activities in Karst Regions of Croatia from 1940 to 1990

The karst regions of Croatia have undergone significant environmental degradation, including deforestation, soil erosion, and forest land loss. During this period, these forests were heavily exploited, often without any management, and their condition further deteriorated due to frequent fires, making restoration increasingly urgent (Biškup and Vondra 1996). Many of these forests had important protective roles, such as preventing erosion, landslides, and floods.

Restoration efforts focused on planting various tree species, along with technical measures to control torrents and floods. In karst areas, species such as *R. pseudoacacia*, *P. nigra, P. sylvestris*, and *Pinus halepensis* Mill. were commonly used (Stilinović 1986). Between 1945 and 1975, around 25,000 hectares were afforested in these regions (Matić et al. 2011). Despite an average afforestation success rate of 67%, these efforts are considered notable given the extreme conditions and the extent of land degradation. The restoration of forests in Senjska Draga is a prominent example of successful afforestation in barren karst areas. The planting of *P. nigra* proved especially effective in stabilising soil and controlling torrents and floods (Severinski 1965). Both biological and technical restoration measures have been crucial for soil protection, native species vitality, improved water retention, and the emergence of new springs. Today, *P. nigra* forest cultures are seen as one of the most successful forest restoration efforts in the Mediterranean, offering economic, ecological, protective, recreational, and health benefits (Severinski 1965). After 1948, afforestation intensified, but as *P. nigra* began to spread naturally through self-seeding, active planting gradually declined, and the landscape transitioned toward native deciduous shrubs and tree forest species (Lipej 2005).

After 1956, afforestation and melioration in karst areas gradually declined due to the introduction of a self-financing system, which shifted funds to other sectors such as tourism and industry (Matić et al. 2011).

Restoration Actions in *Quercus robur* L. Forests from 1940 to 1990

Q. robur forests have historically been restored naturally through self-seeding and regeneration cuts, which were applied in two stages—seed cut and final cut—to promote seed dispersal and seedling growth. However, the lack of suitable shrub species to provide vertical stand structure, which is essential for shading and creating optimal conditions, often resulted in weed overgrowth, preventing the successful establishment of oak seedlings. This, combined with poor seed production, agricultural expansion, and habitat degradation from hydrological changes, caused a significant decline in oak forests by the mid-20th century. As a result, artificial regeneration using planting and seeding became increasingly necessary (Starčević 1990, Matić 1994).

The natural restoration of *Q. robur* was further hindered by the aggressive spread of *Carpinus betulus* L., which easily overgrew oak seedlings, highlighting the need for timely tending activities. Successful natural restoration requires approximately 800 kg/ha^{-1} of acorns (Anić 2025). During this period, artificial regeneration in oak forests was mainly carried out through regeneration cuts, followed by planting nursery-grown seedlings or manual/broadcast seeding. Since the 1970s, tree dieback has increased, largely due to drought and changes in water regimes, with *Q. robur* especially affected. Research from the late 20th century proposed that, in severely degraded stands, *Q. robur* should be partially or fully replaced with pioneer species such as *Fraxinus angustifolia, Alnus glutinosa* L., *Salix alba,* and *Populus* spp., in line with the natural course of forest succession (Oršanić et al. 1996, Anić 2025). Although clear-cutting was previously used, it has not been part of restoration practices for decades. For the amelioration of degraded *Q. robur* and *Fagus sylvatica* L. stands, species like *P. abies, Abies alba*, and other broadleaf species were used.

Between 1969 and 1989, 392 wagonloads of acorns were collected from the Vinkovci forests, averaging 18.5 wagonloads per year. Major obstacle during this period was vole overpopulation, which peaked from 1987 to 1989 and caused severe damage to restored areas. Despite protection measures, some sites were almost destroyed (Rubić 1997). In restoration projects by the Vinkovci Forest Administration, site preparation involved removing shrub-layer vegetation. After seed cutting, sunlight was allowed to reach the forest floor, promoting seedling growth. Fungicide treatments were applied to protect young plants from powdery mildew. Mechanised removal of felled trees increased efficiency. From 1960 to 1970, transport infrastructure and forest roads were constructed, but they did not reach the lowland *Q. robur* forests, which remain crucial for effective restoration (Sever 1988). From 1987 onward, sustainability became a key principle of global environmental policy (Glavač 1999), which set the foundation for subsequent national frameworks.

Forest Restoration in Croatia After 1990

Since 1990, forest restoration in Croatia has been regulated by the Forest Act (NN 52/90), with a major revision in 2018 (NN 68/18) and amendments through 2023. These laws emphasise sustainable forest management and reforestation. The Regulation on Forest Management (NN 97/18, 101/18, 31/20, 99/21, 38/24) defines procedures for updating forest management plans and applying silvicultural measures, especially in degraded or mine-suspected areas. Additional regulations—such as the Regulation on Forest Reproductive Material (2004, amended in 2010 and 2013) and rural development measures (from 2015)—support the use of high-quality seedlings and offer financial incentives for reforestation and post-disturbance recovery.

The 1990 amendments to the Forest Act created a unified state forestry enterprise and introduced a legal framework for restoring degraded forests, expanding afforestation, and strengthening protection, particularly in the Karst region. The Act prohibits clear-cutting and any activity that damages forests or individual trees. In the 1990s, forest owners and companies were legally required to reinvest 15–20% of wood sales income and pay a 0.07% turnover tax to fund reforestation, afforestation, Karst restoration, and forestry research (National Forest Accounting Plan 2018). However, this rate has declined over time due to policy, economic, and social shifts, and it has now almost lost its original purpose (Margaletić 2024). Croatia confirmed its commitment to sustainable forest management by signing several international agreements: the Strasbourg ministerial resolutions (1990), the Rio Declaration (1992), and four Helsinki ministerial resolutions (1993) (Martinić 1999, Matić et al. 2015). In 1921, a natural approach to silviculture was promoted, which is still relevant today. It emphasises that artificial regeneration should follow the principles of natural regeneration (Anić et al. 2020).

Since 1990, forest restoration in Croatia—especially in *Q. robur* stands—has mainly involved planting or sowing on small strip- or circle-shaped areas after regeneration cuts (Matić et al. 1994), with the aim of improving forest adaptation and resilience to climate change and disturbances (Anić 2025). Due to soil depletion and disease susceptibility of *Populus × canadensis* Moench clones (Majer 1994), forestry shifted toward converting poplar monocultures into natural stands of native *Populus nigra* L., *Populus alba* L., *Salix alba* L., *F. angustifolia,* and *Q. robur*. In eastern Slavonia and Baranja, afforestation on Danube river islands and floodplains involved planting one- or two-year-old F. angustifolia seedlings, spaced 2 × 2 meters apart, covering 102.68 hectares restored between 1999 and 2007 (Mayer and Hećimović 2008).

Biotic stressors, especially ash dieback caused by the invasive *Hymenoscyphus fraxineus*, have intensified restoration needs, severely affecting *Fraxinus excelsior* L. and *F. angustifolia* stands. Over 5,000 hectares in Croatia are critically affected, with restoration costs over €6.6 million (Vincenc 2019). Current silvicultural responses mostly include monitoring and sanitary and salvage cuts (Gross et al. 2014, Landolt et al. 2016). Large projects like the SUPERB initiative use integrated strategies, restoring about 80 hectares in the Croatian Serbian cross border area by removing non-native *Populus × canadensis* and planting native species such as *Q. robur*, *Prunus* spp., and *Sorbus* spp., supporting biodiversity and climate resilience goals (https://forest-restoration.eu/).

Under Measure M08 of the EU Rural Development Programme (2014–2020), established by Regulation (EU) No 1305/2013, funding is provided for investments in the development of forest areas and the improvement of forest sustainability. Eligible forest landowners and associations must submit approved management plans to receive non-refundable support for forest conversion based on standard costs per hectare. In 2023, the total allocation was €11,764,706.25, with projects covering 2,511 hectares and €15 million approved since the first calls. Restoration cost varies by species and terrain, ranging from about €11,000 to €22,000 per hectare, depending on the method and tree type.

Technological advances since the late 20th century have improved forest growth modelling and restoration planning, exemplified by SIMPLAG software designed to support *Q. robur* forest recovery (Teslak et al. 2012). Remote sensing technology, such as Landsat 8 and RapidEye, now offers better spatial resolution to detect forest damage and monitor restoration, with NDVI remaining the most common vegetation index. For example, after a severe 2013 windstorm damaged about 40,000 m³ of *F. sylvatica* on Medvednica Mountain, restoration involved skid trails and natural regeneration, successfully supported by the species' strong regenerative ability (Matić et al. 2003, Pilaš et al. 2019, Anić et al. 2020).

The growing momentum behind forest restoration initiatives in Croatia and across Europe reflects a response to decades of ecological degradation, much of which stems from anthropogenic pressures (Menéndez-Miguélez et al. 2024). Agricultural expansion, particularly in lowland regions, has historically lacked ecological sensitivity, contributing to habitat fragmentation, soil exhaustion, and the suppression of natural regeneration processes. In Eastern Slavonia, hydrological interventions — including canalisation, drainage, and floodplain regulation — have significantly altered water regimes, accelerating the decline of *Q. robur* forests and reducing their resilience to climate extremes (Matić et al. 1994, Pilaš and Planinšek 2011).

Demining Activities

From 1991 to 1995, the Croatian War of Independence caused widespread damage to forests, many of which were left unmanaged. Large areas were also contaminated with landmines. In 1991, forest governance shifted from Yugoslavia to Croatia, and state forests came under the management of Croatian Forests Ltd., which has since restored and maintained around 50,000 hectares of forest annually (Martinić 1999). However, starting in 1995, Croatia's forest area began to decline due to legal provisions allowing infrastructure development—such as roads, pipelines, and power lines—without compensation or reforestation obligations. For example, 566 hectares of state forest were lost during the construction of the A1 motorway (Matić et al. 2015). Since 1991, major efforts have been made to clear mines from forested areas. Between 1998 and 2021, continuous demining and survey operations reduced mine-suspected areas (MSA) by 1,631 km². Over 7 billion kuna—approximately 930 million euros—were invested in these efforts. Funding came from the national budget, public enterprises, donations, the World Bank, and EU funds, with 15% national co-financing. A detailed summary of funding sources and their contributions is provided in Table 2.

Between 2015 and 2023, the Naturavita project, and between 2019 and 2023, the Karlovac Karst project, made significant contributions to forest ecosystem restoration in Croatia, especially by reforesting stands of non-native tree species. A key component of both projects was demining mine-suspected areas. The Naturavita project received €49,971,620 in funding, 85% of which was co-financed by the EU Cohesion Fund. It focused on demining and restoring forests and forestland in protected areas and Natura 2000

Table 2. Funding sources for demining in the Republic of Croatia from 1998 to 2021.

Funding source	Approximate amount (EUR)	Share of total funding (%)
National budget	~544,500,000	58.6
European Union	~159,300,000	17.1
Public enterprises	~121,400,000	13.1
Donations	~71,300,000	7.7
World Bank loan	~34,250,000	3.7
Total	~930,000,000	100

Source: Hrvatski sabor 1998–2021. Note: Euro amounts were calculated from the provided data using the exchange rate on 21 September 2025.

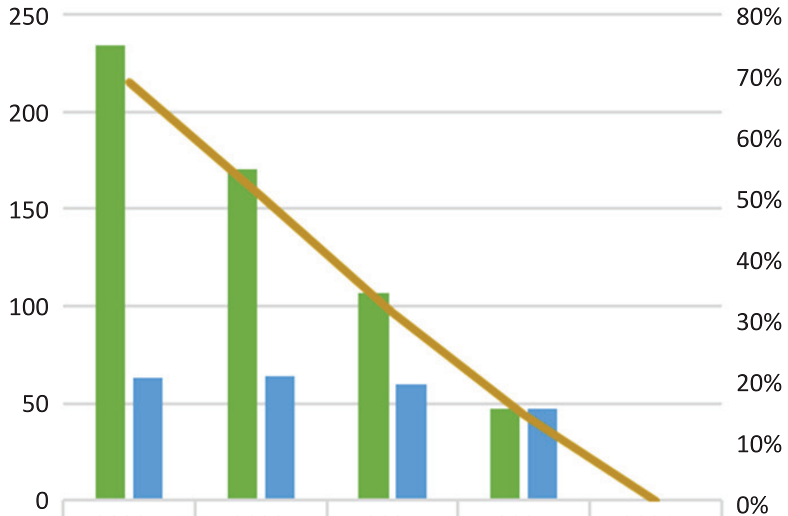
sites within the Danube-Drava region (Herceg et al. 2019). The Karlovac Karst project was funded with 189.5 million Croatian kuna, of which 188.3 million kuna was allocated for demining activities. Thanks to these sustained efforts and significant investments, Croatia is on track to clear all mine-contaminated areas by 2026 (Figure 4).

Contemporary Post-disturbance Restoration

Disturbances in forests in Croatia are numerous, and almost no economically important tree species are completely resilient to them. Therefore, only a few prominent examples are mentioned, which have strongly affected forestry in the country and emphasise the need for active restoration.

Over the past thirty years, forest fires in Croatia have become more frequent due to climate change (Vučetić 2014), human negligence, and growing tourism. In 2017 alone, Dalmatia had 220 wildfires that burned 34,508.73 hectares of forest, causing damages worth 779.5 million kuna, according to Croatian Forests Ltd. (Anić 2019). The Mediterranean region—especially Dalmatia and Istria—is the most vulnerable because of its hot, dry climate. Between 1992 and 2007, over 4,851 forest fires burned 251,901 hectares (Jurjević et al. 2009). From 2007 to 2015, 2,137 fires affected 75,572 hectares (National Forest Management Plan 2016–2025). The Croatian Fire Board reports that between 2016 and 2021, 19,966 fires burned 179,244 hectares. Data from 2016–2025 show that bushes and thickets make up 39% of the total burned area, while forest cultures represent only 2%. Fires in karst areas cause severe soil erosion, which makes restoration harder and more expensive. Restoration is regulated by the Forest Law and carried out by Croatian Forests Ltd., which replants about 300 hectares annually in Dalmatia, using over 500,000 seedlings. However, with around 1,500 hectares burning each year, reforestation efforts are not enough. Afforestation usually involves special machines that dig holes 40 cm deep in rows 3 meters apart. Seedlings are planted every 2 meters, resulting in about 2,000 plants per hectare. To reduce fire risk, Croatian Forests Ltd. has installed 98 surveillance cameras in 48 locations, supported by four regional operation centres. Public education campaigns are also part of their strategy. Several initiatives have helped with post-fire restoration in Croatia. The "Boranka" campaign, launched in 2017, mobilised over 8,500 volunteers who planted 100,000 trees on 50 hectares in Dalmatia. In 2018, the Feniks project restored 10.24 hectares near Split using native plants to focus on ecological recovery. Since 2019, the national campaign "Plant a tree. Don't be a stump." has given away free seedlings to citizens, leading to more than 150,000 trees being planted across the country.

F. angustifolia is one of the most common trees in Croatia's lowland floodplain forests, covering about 72,690 hectares. It ranks second in area and volume after *Q. robur* (Čavlović 2010). This species has high ecological and economic value and is well-suited for climate change adaptation and restoration due to its drought and heat tolerance (Schmidt 2007; Fuchs et al. 2021, 2024). In eastern Slavonia, it acts as a pioneer species, forming forest edges near wetlands and providing important benefits (Vukelić and Rauš 1998, Prpić et al. 2005). Ash was traditionally used for restoring floodplain forests, but its use has declined due to seedling susceptibility to *Hymenoscyphus fraxineus*. Recent problems with disease and dieback in *F. angustifolia* have affected nursery production; since 2022, no seedlings have been produced. This fungal pathogen, introduced from Asia in the 1990s, causes dieback by damaging the entire tree, including roots (Gross et al. 2014, Kranjec Orlović et al. 2020). In



	2022.	2023.	2024.	2025.	2026.
🟩 Remaining MSA (km^2)	233,9	170,6	106,9	47	0
🟦 Annual reduction of MSA	63.3	63,7	59,9	47	0
▬ Remaining MSA (%)	69%	505	31%	14%	0%

Figure 4. Decrease in total mine suspected area (MSA) in Croatia until 2026 (Source: Hrvatski sabor 2023).

2019, about 5,000 hectares of ash stands urgently needed restoration, with costs over €6.6 million. Since no effective treatment exists, efforts focus on monitoring and removing infected trees. Between 2017 and 2022, Croatian Forests Ltd. spent around €13.1 million restoring areas affected by various disturbances (Annual Report 2017–2024).

In 2013, *Corythucha arcuata* was first found in the Spačva basin and has since spread rapidly through *Q. robur* stands. In early 2014, an ice storm damaged 56,021 hectares of forest in Gorski Kotar, leading to a mass outbreak of *Ips typographus*. This prompted authorities to classify it as a natural disaster in 2016. By 2017, windthrow affected about 500,000 m³ of timber in the area (Anić et al. 2020). Meanwhile, *Thaumetopoea pityocampa,* usually found on Pinus species in the Mediterranean, has expanded inland due to climate change. Its range now includes the Lika region of Croatia and the Neretva Valley in Bosnia and Herzegovina, with a notable population increase (Anić 2019, Zorić et al. 2024, Hartmann et al. 2025).

Since the 1990s, the impacts of climate change have steadily intensified, resulting in more frequent extreme weather events—including windstorms, heavy storms, and forest fires—as well as widespread forest degradation and fragmentation. These effects include tree dieback caused by drought, pests, and diseases, a rising occurrence of invasive species, erosion, and excessive homogeneity (Anić et al. 2020).

The analysis raises questions about the current state of forests, forest ecosystems, and the forestry sector today, and especially about the investments that will be required for future restoration, considering increasing risks and challenges. Societal perceptions, the legislative framework, and daily decision-making in practical forestry differ from historical understandings and efforts, but the question remains whether our current knowledge is sufficient to face what lies ahead for foresters and the forestry sector. The role and interplay of newly developed ideas, such as prestoration, nature-based solutions, circular bioeconomy, assisted migration, protection of genetic resources, and other contemporary topics recognised by the scientific and expert forestry community, are yet to be fully determined. Nevertheless, some points can clearly be drawn—for example, the conclusion that current forest cover, species composition, management types, and associated benefits, such as biodiversity and ecosystem services, are highly dependent on historical social and management contexts. Thus, understanding the principles of natural species spread under a changing climate cannot be considered without accounting for the complex historical and anthropogenic legacies, still visible today, through current social, economic, political, and environmental barriers.

CONCLUSIONS AND LESSONS LEARNED

Forest restoration in Croatia has evolved over time in response to changing environmental, social, political, and economic conditions (Erdozain et al. 2024).

Prior to 1940, efforts focused on establishing legal frameworks and reversing deforestation from industrial expansion, agriculture, and grazing, with afforestation on karst terrains and natural regeneration in lowland *Q. robur* forests, while hydrotechnical disruptions in Slavonia destabilised oak stands. Between 1940 and 1990, large-scale reforestation responded to overexploitation and post-war degradation, supported by mechanisation, stricter legislation, and international support, with a continued focus on karst regions and a gradual shift toward artificial regeneration of *Q. robur*. Since the 1990s, restoration has focused on demining and addressing climate-related stressors, including droughts, storms, and invasive pests, which have caused severe diebacks, erosion, and flooding. *F. angustifolia* has suffered severe dieback due to *H. fraxineus*, which has led to a shift toward native mixed-species planting, increased tree diversity, monitoring, and research.

Author Contributions

SP secured the research funding, MĐ and LŽ wrote the manuscript. EM and SdM assisted with editing and revising the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Comparative Effects of NAA and IBA on the Rooting Success of Apical and Lateral Stem Cuttings of *Kerria japonica* (L.) DC

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ABSTRACT

Kerria japonica (L.) DC is a deciduous shrub that is often used in green areas in horticulture and landscape architecture due to its decorative, yellow-colored, multilayered flowers, as well as its high tolerance to drought, low temperatures, and the dust-holding capacity of its leaves. Given the growing demand for this species and the difficulty of generative propagation by seeds, it is necessary to optimise vegetative propagation protocols. This research aimed to examine the influence of phytohormones on the rooting of two types of softwood cuttings (apical and lateral stem cuttings) of *Kerria japonica* in a greenhouse with a misting system. Cuttings were rooted in 3 treatments: INCIT 8 (commercial phytohormone based on α-Naphthalene Acetic Acid (NAA) in the concentration of 0.8%) and IBA (Indole-3-Butyric Acid in the concentration of 0.6%), as well as a control treatment without hormones. After 30 days of the experiment, the height of the plant, the number of newly formed leaves, the length and the number of roots, the percentage of survival, and rooting were analysed. Apical cuttings consistently exhibited greater plant height, longer roots, and a higher number of roots compared to lateral cuttings, highlighting the importance of cutting position in vegetative propagation. Both auxin treatments (NAA and IBA) resulted in 100% rooting, whereas the control treatment achieved 80%. The results demonstrate that auxin application significantly enhances the rooting quality and growth of *Kerria japonica* softwood cuttings. These results demonstrate that phytohormone application significantly enhances the rooting and growth of *Kerria japonica* softwood cuttings. Apical cuttings are preferable for efficient vegetative propagation, but lateral cuttings could be used if needed, providing a basis for the establishment of routine propagation protocols for this species.

Keywords: auxins; adventitious roots; cutting position; propagation techniques; vegetative multiplication; ornamental shrubs

INTRODUCTION

The successful propagation of plant species through vegetative propagation using softwood cuttings is crucial for horticultural practices, nursery production, and plant breeding programs, ensuring genetic uniformity, rapid multiplication, and preservation of desirable traits. *Kerria japonica* (L.) DC, commonly known as Japanese kerria, is a deciduous *Rosaceae* shrub valued for its vibrant yellow flowers, ornamental foliage, frost resistance, and high dust-holding capacity that suits urban green spaces (Sabina and Cornelia 2011, Huo et al. 2019, Zhang et al. 2023). Widely used in landscape architecture for shrub borders, erosion control, and biodiversity enhancement, its generative propagation is challenging due to seed dormancy and low germination rates, making softwood cuttings the preferred method for commercial production (Gümüş and Solmaz 2022).

Its generative propagation through seeds is challenging due to seed dormancy and low germination rates, making vegetative propagation the preferred method for large-scale production while maintaining the desirable traits of parent plants. The success of propagation via stem cuttings is influenced by multiple factors, including genetic predisposition for root development, physiological status of the donor plant, position of the cutting at the plant (topophysis), juvenility, hormone balance, environmental conditions, moisture levels, and temperature. Additionally, optimising propagation techniques, such as the application of rooting hormones, can significantly enhance the success rate of cutting establishment and improve overall plant quality. Improving propagation efficiency directly supports sustainable planting design by enabling the production of robust, uniform, and stress-tolerant shrubs capable of withstanding urban environmental pressures, including soil compaction, pollution, and fluctuating moisture regimes.

Among these techniques, the application of exogenous plant growth regulators, particularly auxins, has been widely recognised for enhancing rooting efficiency (Pacholczak and Nowakowska 2020). Auxins such as Indole-3-Butyric Acid (IBA) and Indole-3-Acetic Acid (IAA) play a pivotal role in stimulating root initiation and improving overall rooting success (Nasri et al. 2015). Numerous studies have demonstrated the positive effects of IBA on root formation across different species. For example, in *Punica granatum* L. 'Ganesh,' IBA application at 100 ppm (slow dip) and 2000 ppm (quick dip) significantly enhanced rooting percentage, root number, and shoot growth (Singh et al. 2011). Similarly, Pruski et al. (2005) observed improved root induction in *Prunus* species treated with 2 $mg \cdot L^{-1}$ IBA *in vivo*. Furthermore, IBA at concentrations of 4.92 and 7.38 µM resulted in 100% rooting in *Prunus persica × P. davidiana* (Zhou et al. 2010), while in olive (*Olea europaea* L.), Kurd et al. (2010) reported the highest rooting percentage (60%) at 3000 ppm IBA, with the maximum root number and root length at 4000 ppm. Comparable findings were recorded in *Rosa* sp., where IBA at 500 ppm yielded the highest survival rate (91%), and 1000 ppm IBA induced the greatest number and length of roots (Susaj et al. 2012). Additionally, Gad et al. (2018) demonstrated that *Olea europaea* 'Picual' cuttings treated with 4000 ppm IBA exhibited superior root development compared to natural auxin alternatives.

Similarly, the influence of α-Naphthalene Acetic Acid (NAA) on root formation has been documented in various species. In *Rosa* spp., Susaj et al. (2012) demonstrated that NAA significantly enhanced rooting percentage and root development in stem cuttings, supporting its efficacy in Rosaceae species. In *Bougainvillea* sp., Hajano et al. (2015) observed that stem cuttings treated with 4000 $mg \cdot L^{-1}$ NAA exhibited the highest rooting percentage, root number, and fresh root weight. The application of NAA significantly improved sprouting and root formation, particularly under covered conditions, resulting in higher leaf numbers and reduced mortality. However, little is known about the comparative effects of both IBA and NAA on the rooting and growth performance of *Kerria japonica*.

Given the limited research on auxin-mediated propagation of *Kerria japonica*, this study aims to evaluate the influence of IBA and NAA on the rooting success of its stem cuttings. Specifically, this study assesses their effects on key morphological parameters, including survival rate, rooting percentage, plant height, the number of newly formed leaves, root number, and root length. Additionally, we examine the interaction between hormone treatment and cutting type (apical vs lateral) to determine potential variations in response. This simultaneous evaluation of cutting position and auxin treatment addresses both physiological optimisation and practical nursery challenges. These findings will provide commercial propagators with actionable protocols to maximise rooting efficiency, plant vigour, and production uniformity of Kerria japonica for scalable nursery production and resilient landscape applications. By elucidating the role of auxins in the green cutting rooting dynamics of *Kerria japonica*, this research seeks to contribute to the development of efficient propagation techniques, supporting sustainable nursery production. The findings of this study are relevant not only for nursery production but also for landscape architectural practice, where high-quality planting material is a prerequisite for resilient, aesthetically consistent, and sustainable shrub-based design solutions.

MATERIALS AND METHODS

Plant Material

This research was conducted in a greenhouse with a misting system at the experimental field of the Faculty of Agriculture, University of Novi Sad in Rimski Šančevi (45.3373°N, 19.8442°E; EPSG: 4326), located in the northern part of Serbia. *Kerria japonica* softwood cuttings, taken from mother plants from the Botanical Garden collection of the Faculty of Agriculture, University of Novi Sad, were selected for the experiment. The selection of mother plants was based on their general growth, phenology, resistance to diseases and pests, and ornamental value. Two types of softwood cuttings, apical and lateral stem cuttings, were collected in June, following the method of Grbić (2004), and prepared to a uniform length of 6 cm. The cuttings were taken from the ends of axillary shoots and cut at a 45° angle using sterile instruments. To prevent dehydration, the cuttings were immediately placed in a moist environment at 22-24°C and 85-90% relative humidity and kept under these controlled conditions for up to 2 hours before treatment, with periodic misting to maintain turgor. Before treatment, each cutting was prepared by retaining two leaves, while the others were removed to reduce moisture loss.

Phytohormone Treatment

The choice of auxin concentrations was based on literature data, ensuring optimal conditions for rooting. The cuttings were treated with three different treatments: (C) a control group without hormone application, (T1) INCIT 8, a commercial phytohormone containing 0.8% α-Naphthalene Acetic Acid (NAA) with no additional components, and (T2) Indole-3-Butyric Acid (IBA) at a concentration of 0.6% combined with 50 µM Co^{2+} as a specially formulated powder, to inhibit ethylene biosynthesis via ACC oxidase blockade, thereby enhancing

IBA-induced adventitious rooting (Kovačević et al. 2014). The basal 1-2 cm portion of each cutting was dipped into the phytohormone treatment powder (drop treatment), and excess powder was removed by tapping.

Growth Conditions

After auxin application, the cuttings were planted in pots (67 mm diameter × 67 mm height) filled with commercial propagation substrate (Steckmedium), consisting of a fine white sphagnum peat mixture with 25% perlite (1–7.5 mm), enriched with water-soluble fertilizer, microelements, and the wetting agent Hydro S (0.5 g·L^{-1}, pH 6). The experiment utilised 60 softwood cuttings of Kerria japonica in a 2×3 factorial design (2 cutting types × 3 treatments), with 10 replicates per treatment/cutting type combination (total n = 60; each cutting = 1 replicate; see Table 1). This design follows standard horticultural propagation protocols. The greenhouse environment was maintained under an intermittent misting system, ensuring relative humidity between 70–90% and temperatures ranging from 25 to 28°C during daytime and 22–24 °C at night. The rooting experiment lasted for 30 days, during which the cuttings were regularly irrigated by misting. Cuttings were misted for 7 minutes every 2 hours from 9:00 AM to 7:00 PM daily to prevent desiccation without causing waterlogging.

Measured Parameters and Statistical Analysis

At the end of the experiment, plant height (PH [cm]), the number of newly formed leaves (NL), the number of roots (NR), and the length of the longest root (LR [cm]) were recorded. Survival and rooting percentages (SP and RP) were determined by evaluating all cuttings within each treatment of interaction Phytohormone treatment × Cutting type. Rooting and survival percentages were determined by recording the number of cuttings that developed roots and the number of cuttings that produced both roots and shoots, respectively, relative to the total number of cuttings planted in each treatment combination.

The data were analysed using a two-way factorial analysis of variance (ANOVA), and differences between treatments were assessed using Fisher’s Least Significant Difference (LSD) test at p = 0.05. The relationships among measured parameters were examined using the Pearson correlation coefficient at the level of interaction Phytohormone treatment × Cutting type and principal component analysis (PCA), based on the correlation matrix.

Statistical analysis was performed using STATISTICA 13 software (TIBCO Software Inc. 2020), while data visualisation was conducted using the R packages ggplot2 (Wickham 2016) and corrplot (Wei and Simko, 2021). To meet the normality assumptions required for statistical tests, the number of newly formed leaves and roots was transformed using the square root transformation (√x + 1).

RESULTS

Analysis of variance showed that phytohormone treatment significantly affected plant height, the number of newly formed leaves, the number of roots, and the length of the longest root. Cutting type was significant for plant height and the number of roots and length of the longest root, while the interaction between phytohormone and cutting type was significant only for plant height (Table 1).

Effects of Phyohormone Treatments on Survival and Rooting Percentage

The highest survival and rooting percentage (100%) was achieved in treatments with the use of phytohormones (T1 and T2), whereas the survival and rooting percentage in the control treatment was 80%. Based on the obtained results, it can be observed that the type of cutting had no major influence on rooting, as there was no difference in the rooting percentage between the examined cutting types. There was no difference in either the control or the treatments (Figure 1).

Table 1. Results of the F-test on examined characters.

Trait	Effect	F	Significance
Plant height (PH, cm)	Phytohormone treatment	12.30	**
	Cutting type	23.26	**
	Phytohormone treatment × Cutting type	7.48	**
Number of newly formed leaves (NL)	Phytohormone treatment	12.27	**
	Cutting type	1.05	ns
	Phytohormone treatment × Cutting type	0.41	ns
Number of roots (NR)	Phytohormone treatment	70.80	**
	Cutting type	5.25	*
	Phytohormone treatment × Cutting type	0.51	ns
Length of the longest root (LR, cm)	Phytohormone treatment	4.83	*
	Cutting type	5.17	*
	Phytohormone treatment × Cutting type	0.85	ns

* -: significant at p<0.05; **: significant at p<0.01; ns - not significant

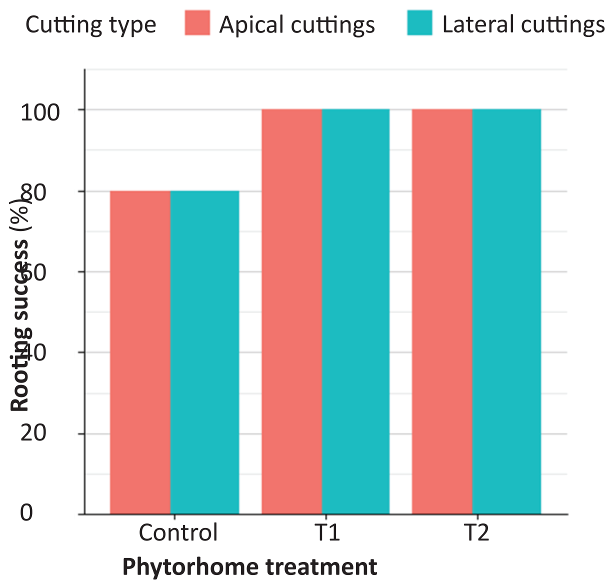

Figure 1. Rooting success of *Kerria japonica* L.

Effects of Phytohormone Treatments and Cutting Type on Plant Height

The greatest shoot height was recorded in apical cuttings under T2 treatment (14.65 cm), while the lowest value was observed in the control treatment for lateral cuttings, at 6.95 cm (Figure 2). Overall, rooted apical cuttings exhibited better growth compared to rooted lateral cuttings. Specifically, apical cuttings in the T1 treatment (NAA) achieved an 11% greater height than those in the control, whereas in the T2 treatment (IBA), the same type of cutting reached a 66% greater height compared to the control. Considering the average values of phytohormone treatments, plant height in the T1 (NAA) treatment was 19% higher than in the control, while in the T2 treatment (IBA), it was 47% higher relative to the control. However, this difference was statistically significant only in the T2 treatment. Furthermore, significant superiority over the corresponding control was observed solely in apical cuttings treated with T2.

Effects of Phytohormone Treatments on the Number of Newly Formed Leaves

A statistically significant increase in the number of newly formed leaves was observed in the T2 treatment (IBA) compared to both the control and T1 (NAA). Specifically, the number of newly formed leaves in T2 was 99% higher than that in the control and 104% higher than in T1. Overall, the type of cutting did not have a statistically significant effect on the number of newly formed leaves (Figure 3).

Effects of Phytohormone Treatments and Cutting Type on Longest Root Length

On average, the shortest length of the longest root (LR) was measured in the control treatment (10.19 cm), while the longest LR was observed in the IBA treatment (T2) at 46.26 cm. Both cutting types exhibited significantly greater longest root length (LR) in T2 compared to the control, with apical cuttings showing 4× and lateral cuttings 6× increases over their respective controls (Figure 4). The main effect of phytohormone treatment was significant (Table 1), while no interaction with cutting type was detected. In the NAA treatment (T1), apical cuttings developed the longest roots that were three times longer, while lateral cuttings had LR five times longer than those in the control. Within each treatment, apical cuttings consistently produced longer roots than lateral cuttings. Specifically, in T1, apical cuttings had 10% longer LR compared to lateral cuttings, while in T2, the LR of apical cuttings was 14% greater than that of lateral cuttings.

Effect of Phytohormones and Cutting Type on Root Number

Overall, a significantly higher number of roots were observed in treatments with phytohormones compared to the control treatment without hormones. As shown in Figure 5, the lowest number of roots was recorded in the control treatment (6.65), while the highest number was observed in the IBA treatment (8.68). Interestingly, this was the only parameter for which there was no statistically significant difference between the NAA (T1) and IBA (T2) treatments. Considering average values, apical cuttings developed a significantly higher number of roots (8.28) compared to lateral cuttings (6.43). However, within both the control and the NAA treatment, there was no significant difference between cutting types. In contrast, in the IBA treatment, apical cuttings produced 48% more roots than lateral cuttings. The strong effect of phytohormone application on rooting success is evident in Figure 5, where it can be observed that the number of roots, one of the key indicators of successful rooting, increased by 40% with the application of the NAA (T1) and by 53% with IBA (T2) compared to the control.

Correlation Analysis

Based on the results of the correlation analysis, a strong positive correlation was observed between the length of the longest root and plant height (Figure 6). As expected, the number of roots was positively correlated with both

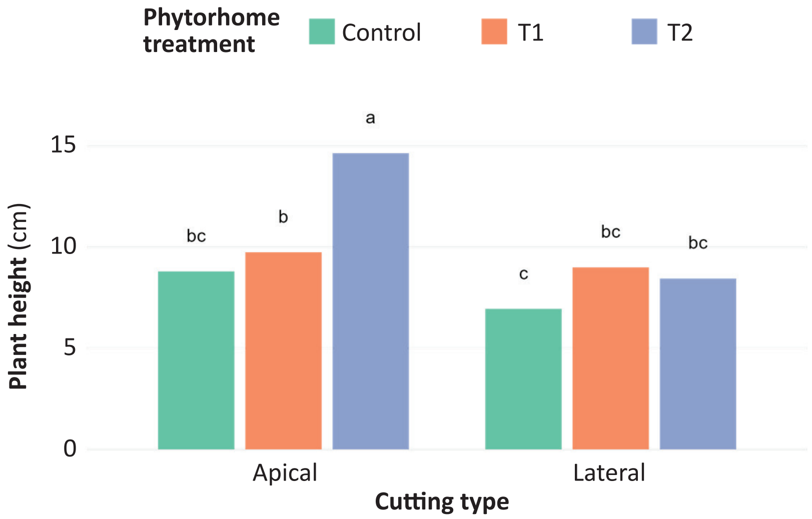

Figure 2. Effect of phytohormones on plant height. Differences between values of the same trait that are labelled with the same letter are not statistically significant (p<0.05).

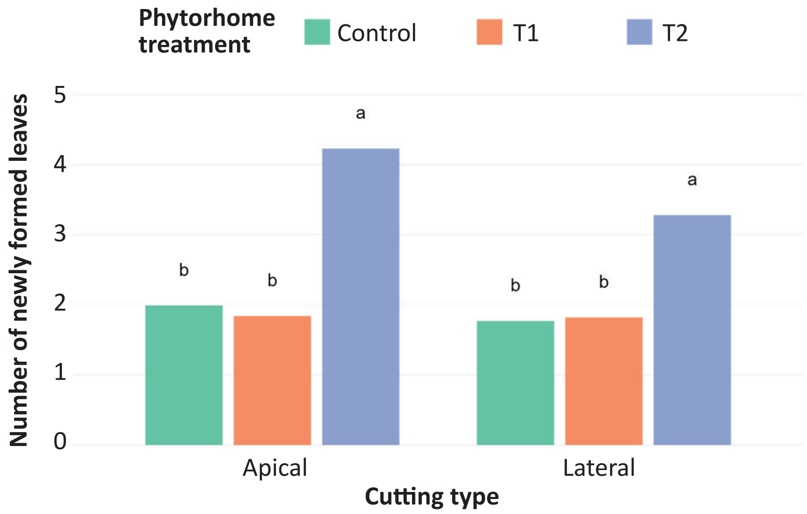

Figure 3. Effect of phytohormones on leaf formation. Differences between values of the same trait that are labelled with the same letter are not statistically significant (p<0.05).

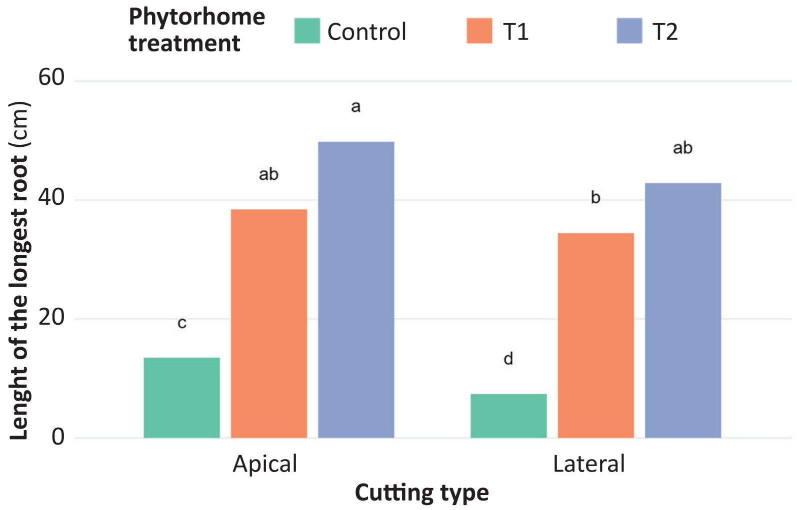

Figure 4. Effect of phytohormones on root length. Differences between values of the same trait that are labelled with the same letter are not statistically significant (p<0.05).

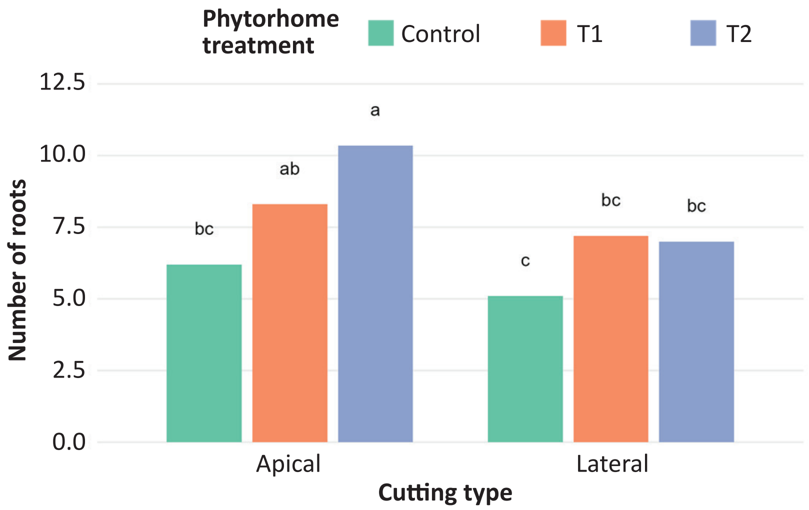

Figure 5. Effect of phytohormones on root number. Differences between values of the same trait that are labelled with the same letter are not statistically significant (p<0.05).

the survival rate and rooting percentage. Additionally, a positive correlation was found between the number of roots and the length of the longest root. A perfect positive correlation (r = 1.000) was observed between the survival rate and rooting percentage. Interestingly, the number of newly formed leaves did not exhibit a statistically significant correlation with any of the examined parameters. Notably, no negative correlations were detected among the investigated parameters.

Principal Components

According to the loadings with the first two principal components, which explain 94.5% of the total variance, the analysed parameters were grouped into two groups (Figure 7). The first cluster included the number of roots, survival rate, and rooting percentage, while the second cluster comprised the length of the longest root, the number of newly formed leaves, and plant height. The correlation between parameters of different clusters appeared to be weak, as confirmed by the correlation analysis results.

Principal component analysis (PCA) revealed a clear separation of treatments along the first two principal components (Figure 8), with PC1 explaining 78.09% and PC2 accounting for 16.41% of the total variance. Control treatments (KA and KL) were positioned on the positive side of PC1, although separated along the PC2 axis, indicating moderate differences between control variants. In contrast, treatments T1A, T1L, and T2L clustered closely together in the negative region of PC1 and the positive region of PC2, suggesting a similar response pattern with respect to the analysed parameters. The T2A treatment was clearly separated from both the control group and other treatments, being located in the negative region of both PC1 and PC2, which indicates a distinct physiological or biochemical response. Overall, the PCA demonstrates that PC1 was the primary axis discriminating control and treated samples, while PC2 contributed to the differentiation among individual treatments, confirming that the applied treatments substantially influenced the variability of the measured traits.

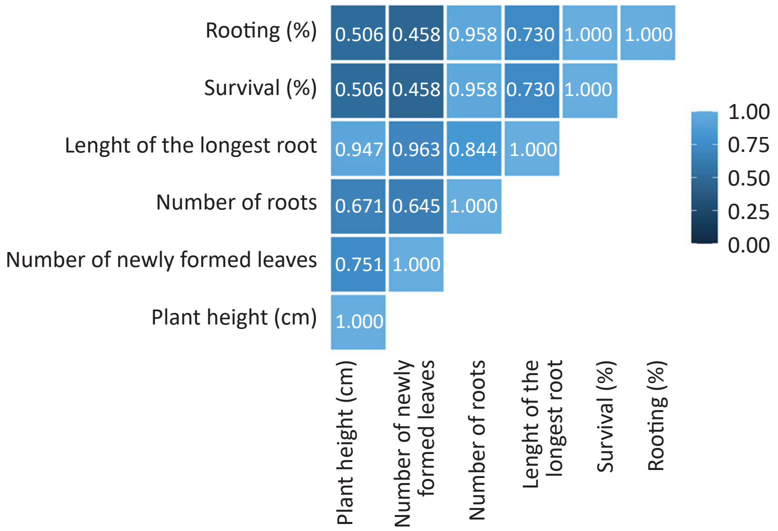

Figure 6. Pearson's correlation matrix of analysed parameters.

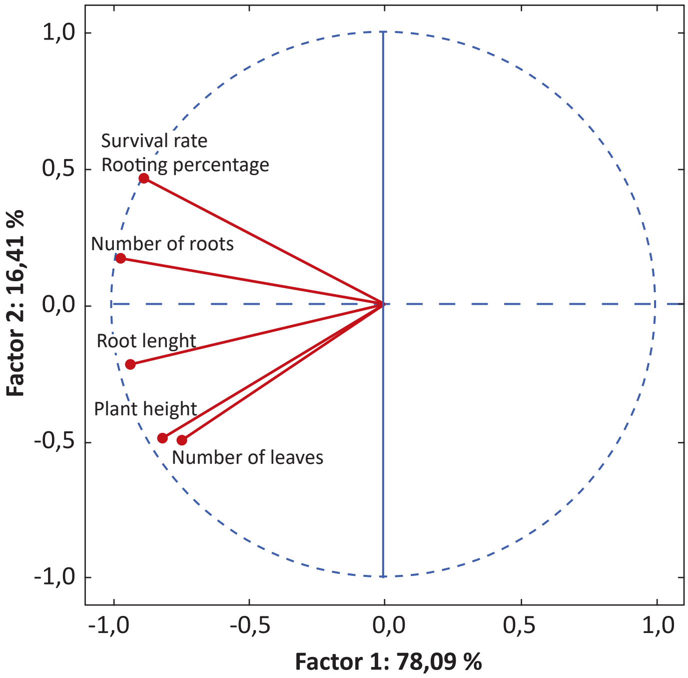

Figure 7. Loadings of the first two principal components for the examined parameters.

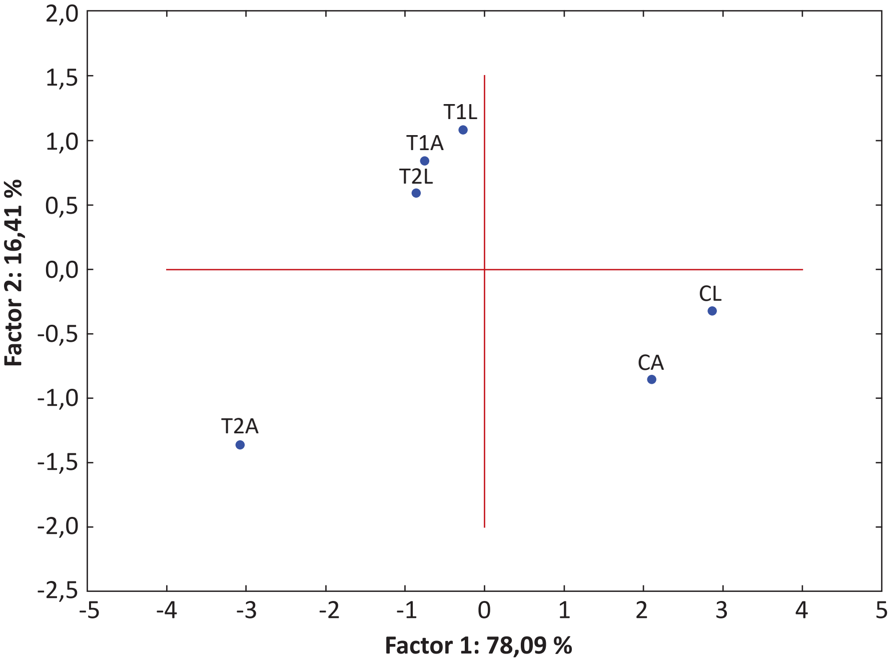

Figure 8. Loadings of the first two principal components for the examined treatments. (The abbreviations C, T1, and T2 stand for phytohormone treatments, and the last letter indicates apical (A) or lateral (L) cuttings).

DISCUSSION

The results of this study provide valuable insights into the rooting and survival of Kerria japonica cuttings, particularly in relation to the application of phytohormones. It was observed that the use of auxin-type plant growth regulators, specifically IBA (Indole-3-Butyric Acid) and NAA (α-Naphthalene Acetic Acid), significantly enhanced the rooting and survival of the softwood cuttings compared to the control group. This finding aligns with previous studies that investigated the effects of various growth regulators, including IBA, NAA, triadimefon (TRF), and paclobutrazol (PBZ), on rooting in Thymus vulgaris L. (Prasad et al. 2000). They found that IBA significantly outperformed other growth regulators in terms of root formation, thus confirming the positive impact of IBA on rooting success. Examining the effect of exogenous application of phytohormones IBA (1000, 2000, 3000, 4000 ppm) and NAA 0.5% on the success of rooting of mature fig cuttings, Popović et al. (2017) concluded that a better effect was achieved with the use of IBA compared to NAA. While the superior rooting performance of T2 was primarily driven by IBA, the inclusion of 50 µM Co^{2+} must be acknowledged as a key synergistic component. Cobalt ions inhibit ethylene biosynthesis by blocking ACC oxidase activity, thereby alleviating ethylene-mediated suppression of auxin-induced adventitious rooting—a formulation established in prior studies on hardwood cutting propagation (Kovačević et al. 2014). This IBA + Co^{2+} combination represents a commonly used preparation for enhancing IBA efficacy in recalcitrant species, allowing direct comparison with the NAA-only commercial product (T1). Jurčević et al. (2018), examining the effect of hormones IAA and NAA on the rooting of Abies alba cuttings, achieved the greatest success with the application of hormone IBA at a concentration of 2500 ppm.

The application of IBA to cuttings increases sprouting by promoting the production of certain materials in the roots responsible for sprouting, as noted by Rehana et al. (2020). Additionally, the better utilisation of stored carbohydrates and other factors with the aid of growth regulators can lead to earlier sprouting and increased sprout length (Chandramouli 2001). The basipetal translocation of auxins from leaves may be reduced, diverting more auxins toward shoot growth, as outlined by Rani et al. (2018) in guava. This enhanced shoot growth is consistent with our findings that IBA promotes superior shoot elongation and leaf production in Kerria japonica.

In this study, the height of the rooted Kerria japonica cuttings was significantly influenced by the application of IBA, with apical cuttings in the T2 treatment (IBA) achieving a remarkable 66% greater height compared to the control group. Kovačević et al. (2014) also got a positive effect on the stem height of white poplar rooted cuttings at the end of the first growing season, after the hardwood stem cuttings were treated with the same formulation as in the T2 treatment.

The results showed that IBA increased both root length and root number, consistent with findings by Gehlot et al. (2014) in Azadirachta indica and Shahab et al. (2013) in Chrysanthemum, who reported improved rooting and enhanced shoot development following IBA application. Enhanced root growth likely improved water and nutrient uptake, contributing to greater leaf formation, as also noted by Shahab et al. (2013). In our study, IBA treatment increased the number of newly formed leaves by 99% compared with the control and 104% compared with NAA, supporting results reported by Jurčević et al. (2018) in Abies alba. Moreover, IBA-treated cuttings developed significantly longer roots than NAA-treated or untreated cuttings, with apical cuttings exhibiting roots approximately twofold longer than the control, in agreement with Frick and Strader (2018), who highlighted the role of IBA in promoting root elongation and cell division. The increased root length observed in IBA-treated cuttings may also enhance the plant's ability to withstand abiotic stress conditions, particularly drought, as root development is directly correlated with water and nutrient uptake in plants. Interestingly, the number of roots was significantly higher in the phytohormone treatments compared to the control, with IBA showing the highest root number, significantly differing from both the NAA treatment and the control. This indicates that while both hormones were effective at promoting root formation, IBA may be more beneficial for improving other growth parameters, such as shoot elongation and leaf development.

The age and type of cutting material, as well as the season, can significantly influence rooting success, a concept supported by Swamy et al. (2002). Their research on Robinia pseudoacacia and Grewia optiva showed that juvenile cuttings generally rooted better than mature hardwood cuttings, and the optimal hormone treatment varied with species and season.

The results of this study indicate that cutting position had a clear influence on the growth and root development of Kerria japonica, even though it did not significantly affect the overall rooting percentage. Apical cuttings consistently showed greater plant height, longer roots, and a higher number of roots compared to lateral cuttings. This trend is in line with the concept of topophysis, where the physiological state and endogenous growth regulators of a cutting vary depending on its original position on the mother plant.

Similar findings have been reported in other species. Aini et al. (2010) observed that top-position cuttings of Gonystylus bancanus exhibited higher survival and rooting percentages, and generally produced a greater number and vigour of roots compared to middle and bottom cuttings. In Stevia rebaudiana, apical cuttings achieved higher rooting percentages, longer roots, and greater root mass than medium and basal cuttings, highlighting the importance of the apical position for vegetative propagation (Bortoloso Pigatto et al. 2018). Furthermore, Otende et al. (2017) demonstrated that apical cuttings of rose rootstocks had higher shoot height, root number, and total root length, correlating with higher endogenous sucrose and mineral nutrient contents, emphasising the physiological advantage of apical tissue for root formation.

In the present study, the superior performance of apical cuttings, particularly under IBA treatment, supports these observations. Apical cuttings developed 10–14% longer roots and a higher number of roots compared to lateral cuttings across treatments. These results suggest that the apical region retains greater meristematic activity and responsiveness to exogenous auxins, which enhances root elongation and overall vegetative growth. Therefore, consideration of cutting position is critical in propagation protocols, as the use of apical cuttings may result in more vigorous and uniform plants, improving both quality and efficiency of commercial production.

In summary, the results of this study highlight the positive influence of IBA and NAA on the rooting and growth of Kerria japonica cuttings. The application of IBA in particular led to superior growth in terms of root length, plant height, and leaf production. Furthermore, apical cuttings consistently outperformed lateral cuttings, exhibiting greater plant height, longer roots, and a higher number of roots, which emphasises the importance of cutting position in vegetative propagation. These findings support the use of IBA and the selection of apical cuttings as an effective means of promoting rooting and enhancing the growth of Kerria japonica in commercial production.

This research investigated the influence of the phytohormones NAA (in the form of the commercial preparation INCIT 8) and IBA on the rooting of Kerria japonica cuttings. The results demonstrated that the application of both NAA and IBA significantly enhances rooting compared to the control group, with both hormone treatments achieving 100% rooting success. While both hormones had a positive effect, IBA proved superior in stimulating the growth of new leaves, root number, and root length. It was also observed that cutting position had a notable influence on growth, with apical cuttings generally exhibiting greater plant height, longer roots, and a higher number of roots compared to lateral cuttings. Specifically, apical cuttings in the IBA treatment (T2) achieved a 66% greater height compared to the control, highlighting the combined efficacy of IBA application and apical cutting selection in promoting robust growth. However, these results also support the use of lateral softwood cuttings in the propagation of Kerria japonica if the supply of apical cuttings is not sufficient, suggesting a positive effect of the use of IBA in this case.

These findings underscore the importance of using phytohormones, particularly IBA, for the successful vegetative propagation of Kerria japonica. The application of IBA, together with the use of apical cuttings, can significantly increase the efficiency and quality of plant production, which is crucial for meeting the growing demand for this ornamental species in horticulture and landscape architecture. Furthermore, these results provide a basis for establishing routine propagation protocols, emphasising the optimisation of hormone concentrations and the selection of appropriate cutting types. Future research could focus on the long-term effects of hormone treatments on plant development and evaluate the influence of environmental factors on rooting success. Given the strong positive correlation observed between root length and plant height, further studies could also explore the potential use of these parameters in selecting drought-tolerant Kerria japonica genotypes, thereby enhancing resilience under abiotic stress conditions.

CONCLUSIONS

This study demonstrates that the application of auxin-based plant growth regulators significantly improves the vegetative propagation efficiency of Kerria japonica. Both IBA and NAA enhanced rooting compared to the untreated control, achieving 100% rooting success; however, IBA exhibited superior performance in promoting root length, root number, shoot elongation, and leaf production. The enhanced effect observed in treatment T2 can be attributed to the synergistic interaction between IBA and Co^{2+}, which likely contributed to improved adventitious rooting and overall vegetative growth. Cutting position also played a decisive role in propagation success. Although it did not significantly influence rooting percentage, apical cuttings consistently produced taller plants with longer and more numerous roots compared to lateral cuttings. From a practical perspective, these findings provide a reliable propagation protocol for commercial production of Kerria japonica, recommending the use of IBA-preferably in combination with apical softwood cuttings—to maximise plant quality and production efficiency. Nevertheless, lateral cuttings treated with IBA also represent a viable alternative when apical material is limited. Overall, the results contribute to the optimisation of nursery practices for this increasingly important ornamental species and provide a foundation for further research on long-term performance and stress resilience of propagated plants.

Author Contributions

Conceptualization, L.P., V.V., M.O., and B.K.; methodology, V.V., L.P., and B.K.; software, L.P.; formal analysis, V.V.; data curation, L.P., V.V., and M.O.; writing—original draft preparation, L.P. and V.V.; writing-review and editing, V.V., L.P., B.K., O.K., and S.O.; visualization, L.P.; supervision, S.O.; funding acquisition, S.O. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Physical Properties and Germination Capacity of Black Alder Seeds: Informing Reforestation Efforts for a Threatened Population near Lake Prespa, North Macedonia

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ABSTRACT

Widely spread across Europe, black alder (*Alnus glutinosa* (L.) Gaertn.) is a keystone species in riparian ecosystems. In North Macedonia, the black alder population present in the proximity of Lake Prespa is under severe threat due to the combined impact of anthropogenic factors, climate change, and the age and state of the mature trees. Recent reforestation activities where locally produced seedlings were planted have been conducted; however, significant knowledge gaps regarding the species biology and current state of the reproductive material may decrease the output, i.e., seedling survival. Therefore, in the present study, we analyse the physical and physiological properties of seed lots collected in 2023 and 2024. Furthermore, we also analyse the results from five pre-sowing treatments (cold stratification, ethanol treatment, hydrogen peroxide treatment, water soak, and hydrothermal treatment) selected based on the *A. glutinosa* seed properties. The results indicate a lower viability in the seed lot from 2023, which could be due to the longer storage as well as a potentially low-seed year. The lower viability is reflected in the lower thousand seeds weight (1.165 g in 2023, 1.725 g in 2024), lower germination capacity (20.7% in 2023, 33.4% in 2024), lower germination energy (19.9% in 2023, 31.9% in 2024), and lower viability (18.5% of healthy seeds in 2023, 36.24% in 2024). The comparison between the pre-sowing treatments showed cold stratification as superior to all other treatments, for the seeds lots from both years. Furthermore, with ethanol treatment and water soak, no seeds germinated, indicating a deep dormancy of black alder seeds that needs to be overcome by more intensive physical and/or chemical stimulation. To the best of our knowledge, this is the first study conducted on black alder seeds' germination capacity in North Macedonia. As such, it provides valuable insight into the overall state of the reproductive material as well as practical data on the pre-sowing treatments' effect. With conservation in mind, future research analysing the results of direct sowing as compared to planting, as well as the improvement of nursery production with higher seeding rates (due to the natural low viability of black alder seeds) and the implementation of arbuscular ectomycorrhiza could be of interest.

Keywords: riparian; forest; nursery; plant production; seed characteristics; reforestation; germination

INTRODUCTION

The combined effects of climate change and land-use intensification pose a severe threat for numerous tree species worldwide (Iralu et al. 2019). Reforestation (or revegetation) is a commonly used active restoration approach that relies on direct seeding on field or planting nursery-grown plant material (saplings) of the target species (Greet et al. 2020). Both methods have positive and negative sides. Direct seeding is less costly but constricted by seed predation, germination, and early seedling survival. On the other hand, while it surpasses these obstacles, the planting of saplings also requires more inputs in terms of facilities, labour, and management (Moore et al. 2011, Grossnickle and Ivetić 2017, Willoughby et al. 2019). Since seeds are required for the propagation of most tree species, seed germination studies can be an important tool for species conservation, providing insight into the species' plasticity *in situ* and the requirements for germplasm conservation *ex situ* (Iralu et al. 2019). A combined effect of numerous factors conditions the germination outcome, but most relevant are seed coat properties and general morphological seed parameters as internal factors, and temperature, humidity, and water availability as external factors (Kumar et al. 2024). Thus, pre-sowing (or germination) treatments, during which the seed coat can be impacted and ecological factors manipulated, have an important role for the germination success (Chowdhury et al. 2024). Treatment selection is based on the species biology, and commonly involves soaking, stratification, scarification, manipulation of humidity, light, temperature and growing media, and application of plant growth regulators, i.e., plant hormones such as abscisic acid, gibberellins, auxins, etc. (Iralu et al. 2019). Germination success is also impacted by the genetic predispositions and the continuous environmental conditions that preceded the seed production period which need to be considered, especially during the mother plant selection and seed collection phase.

Commonly present in riparian ecosystems, black alder (*Alnus glutinosa* (L.) Gaertn.) is a widespread tree species across Europe (Verbylaitė et al. 2023). It has been found to be of particular value since it can aid water purification from excessive nutrient concentration, provide soil stabilization and erosion control as a pioneer species, has a nitrogen-fixing ability, and can serve as food sources for wildlife during the winter months (Peterjohn and Correll 1984, Mingeot et al. 2016, Willoughby et al. 2019, Sanglyne et al. 2021). The seeds of such riparian plant species are commonly dispersed via anemochory (by wind), hydrochory (by water), and zoochory (by animals) (Fraaije et al. 2017). Since the dispersal mechanisms are crucial for the species survival strategy, and as such have co-evolved with the preferred environmental conditions of the species, they have also impacted the morphophysiological seed characteristics (Levin and Muller-Landau 2000). Please change this sentence to:

In the case of A. glutinosa, the seeds -actually winded fruits or achenes containing a single seed without endosperm and surrounded by a pericarp (de Atrip et al. 2007) - are relatively small (2 - 3.5 mm), with low seed specific weight and high buoyancy due to the cork-like seed coat and a waxy surface. Seed production begins when the individuals are 20–30 years old, and every two to three years high seed production occurs, although a certain percentage of viable seeds can be present even in the low seeding years (Göktürk and Güner 2024). Black alder seeds exhibit an orthodox storage behaviour, meaning that when stored in proper conditions (low moisture content and low temperature), they can maintain their viability (Koutsovoulou et al. 2025). Storage conditions and duration can impact germination, and in the case of black alder, studies have shown that seed storage in proper conditions enables the conservation of the seed quality (Chmielarz 2010, Tylkowski 2014, Koutsovoulou et al. 2025)

In North Macedonia, in the Prespa region located in the south-west part of the country, *A. glutinosa* dominates several types of alluvial riparian forests, i.e., *Alno-Padion*, *Alnion incanae*, and *Salicion albae* (Fotiadis et al. 2018). However, these stands, fragmented and consisting mainly of older individuals, are further threatened by the expansion of orchards in the area, the increasing population of the invasive alien species *Amorpha fruticosa* L., and climate change (Fotiadis et al. 2018). Since this is a priority habitat type (Council Directive 92/43/EEC 1992, Fotiadis et al. 2018), attempts for its conservation also include reforestation efforts since the aforementioned threats have also diminished the natural regeneration potential.

Since an important aspect of species conservation is identifying and providing optimal conditions for seed and seedling survival and establishment (Ehardt-Kistenmacher et al. 2019), practical knowledge regarding seed quality and germination is extremely relevant for improving plant production and the success of the seedlings after their outplanting (Chowdhury et al. 2024). With species such as *A. glutinosa*, whose seeds undergo natural cold stratification in the soil during the winter months, this could be of even higher relevance considering the climate change extremes during which temperatures might fluctuate differently (Twardosz et al. 2021). Therefore, the present study aimed to compare the seed viability and germination capacity of two consecutive sampling years and test how five different pre-sowing treatments impact seeds from *A. glutinosa*. We hypothesized that:

1. the seed quality, reflected in the physical and physiological seed properties will not differ between the seeds collected in 2023 and the seeds collected in 2024;
2. cold stratification, as a pre-sowing treatment, would be most appropriate for black alder seeds.

MATERIALS AND METHODS

Study Area and Seed Collection

The study was conducted in the Prespa region, in the south-west part of North Macedonia, characterized by the presence of Lake Prespa (Figure 1). The infructescences from *A. glutinosa* (cone-like strobiles) were manually gathered from five known mother trees which had been selected due to desirable phenotypic characteristics: overall tree vigour, tree health, vital branches with leaves, relatively rich crowns, absence of mechanical damage, and observed reproductive capacity as indicated by strobile formation. Notably, they were all located in the proximity to rivers. The infructescences were then processed by manual pressing and sieving until the seeds (samaras) were extracted. Both the seeds gathered in October 2023 and October 2024 were conserved in paper bags at 4°C and estimated relative humidity of around 20%, until the seed tests were performed in January 2025. A total of 0.5 kg and 0.5 kg of seeds were gathered in 2023 and 2024, respectively.

Figure 1. Study area in Prespa (North Macedonia) where the cones and seeds from black alder (*Alnus glutinosa* (L.) Gaertn.) were collected (Al. gl. 1–5).

Seed Properties Analysis

The two seed lots, collected in 2023 and 2024, were processed in the same manner but analysed separately for the purpose of the study. From each seed lot, a manually obtained primary sample, which served as a submitted sample, was randomly obtained, in the quantity of 10.99 g (2023) and 12.04 g (2024). In the laboratory, these submitted samples were used as working samples since their weight corresponded with the minimal submitted sample rules indicated by ISTA (ISTA 2025). From each working sample, by randomly taking ten separate scoops with continuous mixing in between, smaller working samples were formed for further analysis. The samples were weighed and cleaned by sieves and the manual removal of non-seed material. This material was first used to measure seed purity (SP) as a physical property used to assess seed quality by comparison of seed weight before and after cleaning calculated as:

$$\text{SP [\%]} = \frac{\text{Wps}}{\text{TW}} \cdot 100 \tag{1}$$

where, Wps is the weight of the cleaned sample, and TW is the total weight of the working sample (Rajendra Prasad 2023, ISTA 2026a).

Then, from these samples, 24 × 100 smaller samples were separated and weighted for both sampling years (2023 and 2024). Each of them was weighted and used to measure the thousand seed weight (TSW) as a physical property correlated with germination vigour and field performance, and calculated as:

$$\text{TSW [g]} = \left(\frac{X_1+X_2+\ldots+X_n}{n}\right) \cdot 10 \tag{2}$$

where X1, X2, and Xn indicate the individual weight of working samples of 100 seeds, and n is the number of working samples used (ISTA 2026b).

Regarding the physiological seed properties, we calculated the standard germination capacity (GC), germination energy (GE), and practical seed value (PCV). GC is used for measuring the maximum germination capacity and it is executed by placing four samples of 100 seeds under optimal conditions after exposure to pre-germination treatments. In the present study, this was achieved by placing the seeds on constantly moistened gauze with an adapted growing tray, and maintaining them in constant conditions at room temperature (22°C ± 2°C) and high humidity (80%–90%) (Qi et al. 2019). All seeds were observed every 2–3 days, over the period of 28 days, and, if present, germinated seeds were counted and removed from the germination tray. The GC was calculated as:

$$\text{GC [\%]} = \frac{\text{Ngs}}{\text{TNs}} \cdot 100 \tag{3}$$

where Ngs is the number of germinated seeds, and TNs is the total number of tested seeds (Rajendra Prasad 2023).

GE was calculated to evaluate seed vigour, i.e., the speed and uniformity of germination during the first seven days (after transferring to the germination tray) calculated as:

$$\text{GE [\%]} = \frac{\text{Ne}}{\text{TNs}} \cdot 100 \tag{4}$$

where Ne is the number of germinated seeds in the first 7 days, and TNs is the total number of tested seeds (Si et al. 2018).

PCV is a derived metric calculated based on seed purity and standard germination capacity used to determine the actual planting value of a seed lot calculated as:

$$\text{PCV [\%]} = \frac{\text{SP} \cdot \text{GC}}{100} \tag{5}$$

where SP is seed purity, and GC is germination capacity.

Furthermore, a seed viability test (VT) was performed to evaluate seed viability and vigour, and to examine the internal structures (embryo, endosperm, cotyledons) without full germination. In the case of *A. glutinosa*, an adapted VT was performed by manually cutting 400 seeds from each sampling year, longitudinally, and noting the state of the seeds (Frischie et al. 2020). The cut seeds were classified in one of the four categories – healthy seeds, empty seeds, rotten seeds, and suspicious seeds. After cutting, the VT was calculated as:

$$VT\ [\%] = \frac{Nhs}{Ts} \cdot 100 \qquad (6)$$

where Nhs is the number of healthy seeds, and TS is the total number of cut seeds.

Pre-sowing Treatments

Based on the literature revision and the known seed properties of *A. glutinosa,* five pre-sowing seed treatments were selected for the purpose of the experiment. A total of 400 seeds (4 × 100) were used for each of the treatments, as described in detail in Table 1.

Statistical Analysis

For the physical properties (SP and TSW) only descriptive analysis of the relative differences were performed, since single measurements were obtained per year. For the physiological properties (GC, GE, and PCV), the treatment effects were assessed with a two-way ANOVA with interaction terms (Treatment × Year) for each response variable. When significant effects were detected, Tukey's HSD tests for pairwise comparisons was used. For GE, the data contained zero-inflated values for treatments T3 and T5; therefore, a Kruskal-Wallis non-parametric test was applied instead of ANOVA. For VT, the percentage of each seed category (healthy, empty, rotten, and suspicious) was calculated relative to the total number of cut seeds per year. To assess the seed viability distribution between the two

Table 1. Applied pre-sowing treatments for black alder seeds (*Alnus glutinosa* (L.) Gaertn.).

Treatment code	Treatment name	Treatment description
T1	Cold stratification	The cold stratification treatment for the present study was developed considering literature revision and previous studies (de Atrip and O'Reily 2007, O'Reilly and de Atrip 2007, de Atrip et al. 2007, Gosling et al. 2009). In detail, the cold stratification was done by placing the seeds in plastic containers with aeration holes, in sterilized moist sand, with no standing water, at 4°C and relative humidity of 100% (in the refrigerator). This aimed to mimic the natural winter conditions and serves as preparation for the spring sowing. The cold stratification lasted for 49 days. Afterwards, the seeds were transferred to the germination tray. Considering it is the most used pre-sowing treatment, in the present study it was used as the control.
T2	Ethanol (C_2H_5OH) treatment	An adapted treatment based on literature revision included soaking the seeds in 35% ethanol solution, in plastic containers covered with aluminium foil for 24 hours, at room temperature (20–25°C), since ethanol can help in removing or softening the waxy cover of the seed that might be a germination inhibitor (Vishwanath et al. 2013, Shahin et al. 2015, Mensah and Ekeke 2016). Afterwards, they were transferred to the germination tray.
T3	Hydrogen peroxide (H_2O_2) treatment	Adapted method of soaking the seeds in 1% hydrogen peroxide for seven days, at room temperature (20–25°C), in the dark (Ivetić 2002). The method includes three solution changes: i) the seeds are soaked in hydrogen peroxide for 24 hours; ii) the hydrogen peroxide solution is replaced with a freshly prepared batch, and the seeds are soaked for 72 hours; iii) the hydrogen peroxide solution is replaced with a freshly prepared batch, and the seeds are soaked for another 72 hours. Afterwards, they were transferred to the germination tray.
T4	Water soak	During soaking the seeds in water, at room temperature, the seeds swell, which eases the germination (McVean 1955). In the present study, the seeds were soaked in water at room temperature for 35 days, and the water was changed every two days. The prolonged period of hydration was chosen due to the waxy, impermeable seed coat of black alder seeds, and the principle that prolonged hydration may promote greater dormancy release (Gallagher et al. 2004, Bolingue et al. 2010). Afterwards, the seeds were transferred to the germination tray. If seeds germinated during the treatments, they were counted and removed.
T5	Hydrothermal treatment	The hydrothermal treatment, i.e., soaking the seeds in boiling water, could soften the seed coat, and accelerate germination (Alvarado and Bradford 2005, O'Reilly and de Atrip 2007, Burrows et al. 2009). In the present experiment, the seeds were exposed to boiling water (100°C) for 10 seconds, and then left at room temperature water for 24 hours. Afterwards, they were transferred to the germination tray.

years, a Fisher's exact test was applied, and a two-sample proportion test was used to compare the prevalence of healthy seeds between the years. Statistical significance was assessed at $p < 0.05$. All analyses were conducted in R version 4.3.0 (R Core Team 2021).

RESULTS

The studied samples from both sampling years corresponded with the indicated criteria in the International Seed Testing Association (ISTA) guidelines for *A. glutinosa*, i.e., the maximum weight of lot (in kg) was 1000 kg and the minimum submitted sample (in g) was 8 g (ISTA 2025).

Physical Seed Properties

The results from the physical seed properties indicate an overall increase as all parameters exhibited higher values in the second sampling year. Both SP and TSW were higher in 2024 (Table 2, Figure 2a, 2b).

Physiological Seed Properties

The analysis excluded the seeds subjected to the ethanol treatment (T2) and the water soak treatment (T4). In the case of T2, the seeds from both collecting years were excluded from the analysis since no germination took place. In the case of T4, for samples from 2023, 19 seeds germinated during the water soaking stage, while none germinated once transferred to the germination tray; and for samples from 2024, no seeds germinated during the water soaking stage, and only one seed germination on the germination tray. For the other three treatments (T1, T3, and T5), the two-way ANOVA revealed highly significant treatment effects on all measured parameters ($p < 0.001$) (Table 3). Regarding GC, T1 resulted in superior performance (27.1% mean germination) compared to T5 (9.5%) and T3 (1.8%), with all pairwise differences being statistically significant (Tukey's HSD, $p < 0.05$). This pattern was further observed in the GE comparison, as T1 exhibited substantial early germination (19.9% in 2023; 31.9% in 2024) while T3 and T5 had complete germination absence at the 7-day evaluation point. Consequently, PCV mirrored these trends, with T1 producing significantly higher values (12.4% in 2023, 24.7% in 2024) than other two treatments ($p < 0.001$) (Table 3). A significant Treatment × Year interaction indicated that treatment effects varied between years. Notably, with the samples from 2024, T1 showed a germination increase of 12.7% and PCV by 12.3%, compared to 2023 values, while T3 and T5 remained consistently low for both sampling years. In summary, the most substantial treatment differences were observed in the 2024 PCV measurements, as T1 (24.7%) exceeded T3 (0.9%) by 23.8 percentage points, demonstrating the combined impact of treatment efficacy and seed purity on overall seed quality (Table 3, Figure 2c, 2d, 2e).

Regarding the VT, the proportion of healthy seeds increased from 18.5% in 2023 to 36.25% in 2024, while the empty seeds decreased from 81.5% to 56.25% (Table 4). No rotten or suspicious seeds were recorded in either year. Fisher's exact test indicated a significant difference in the distribution of seed viability categories between the two years. The two-sample proportion test, which compared the number of healthy seeds, also showed a significant increase, from 11.44% in 2023 to 24.06% in 2024, with a 95% confidence interval.

Table 2. Physical seed properties of black alder (*Alnus glutinosa* (L.) Gaertn.) seeds from 2023 and 2024.

Year	Purity (%)	TSW (g)
2023	59.51	1.165
2024	74	1.725

Table 3. The effects of seed treatment and sampling year on physiological seed properties of black alder (*Alnus glutinosa* (L.) Gaertn.).

Parameter	Treatment	2023 Mean ± SD	2024 Mean ± SD	Statistical significance	Post-hoc comparison
Germination capacity (GC) [%]	T1	20.7 ± 7.7	33.4 ± 3.2	< 0.001*	T1 > T5 > T3
	T3	2.2 ± 1.5	1.3 ± 1.5	0.017†	
	T5	11.8 ± 9.2	7.3 ± 5.7		
Germination energy (GE) [%]	T1	19.8 ± 0.9	31.8 ± 3.0	< 0.001‡	T1 > (T3=T5)
	T3	0.0 ± 0.0	0.0 ± 0.0		
	T5	0.0 ± 0.0	0.0 ± 0.0		
Practical seed value (PCV) [%]	T1	12.4 ± 4.6	24.7 ± 2.4	< 0.001*	T1 > T5 > T3
	T3	1.3 ± 0.9	0.9 ± 1.1	0.019†	
	T5	7.0 ± 5.5	5.4 ± 4.2		

*Two-way ANOVA treatment effect ($F_{2,18}$ = 42.0 for final germination; $F_{2,18}$ = 41.2 for PCV); †Significant Treatment × Year interaction; ‡Kruskal-Wallis test (χ^2 = 18.9) due to zero-inflation in T3/T5; T1, cold stratification treatment; T3, hydrogen peroxide (H_2O_2) treatment; T5, hydrothermal treatment

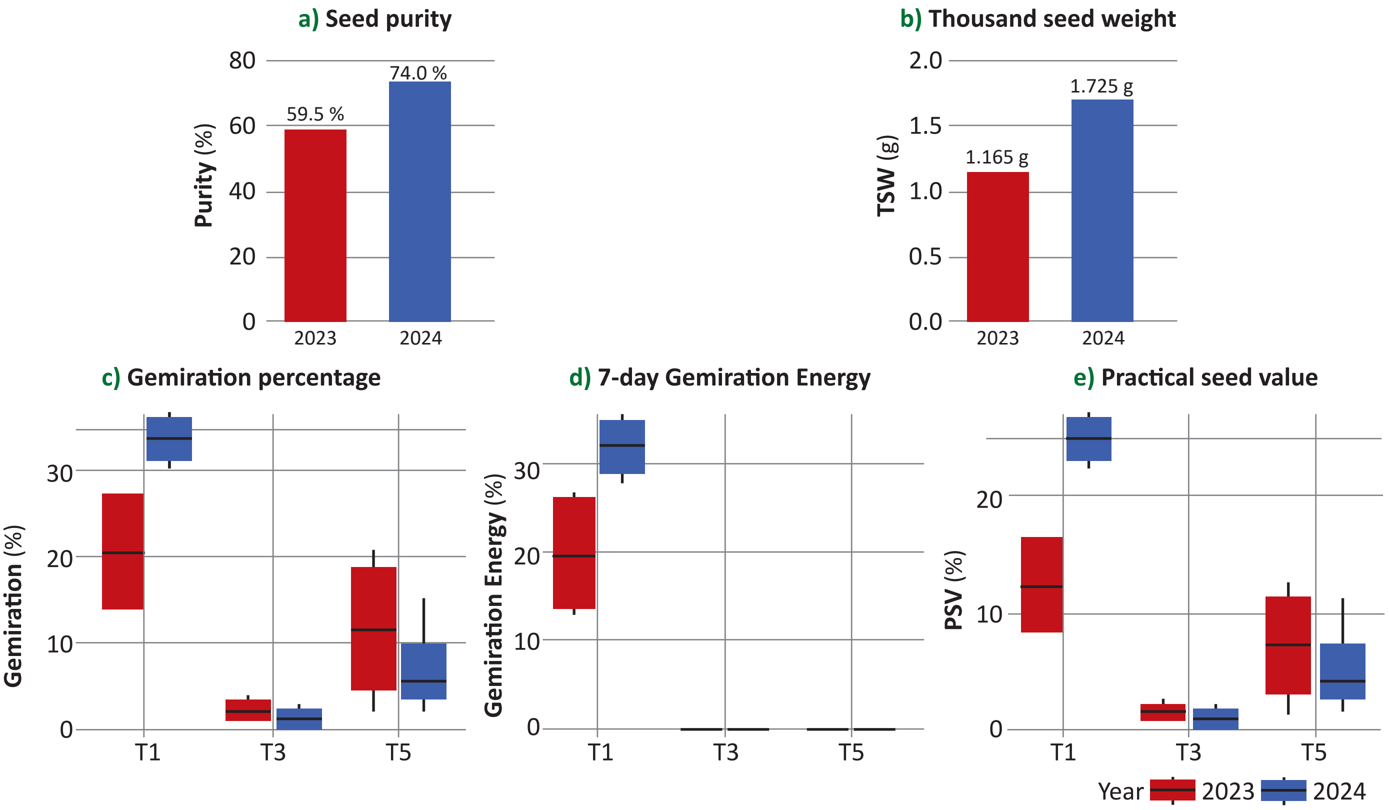

Figure 2. Seed quality analysis of physical and physiological characteristics: **a)** – seed purity; **b)** – thousand seed weight; **c)** – germination percentage; **d)** – 7-day germination energy; **e)** – practical seed value; T1 – cold stratification treatment; T3 – hydrogen peroxide (H_2O_2) treatment; T5 – hydrothermal treatment.

Table 4. The number of healthy, empty, rotten, and suspicious seeds obtained through the seed viability test for black alder (*Alnus glutinosa* (L.) Gaertn.) seeds collected in 2023 and 2024.

Year	Healthy (N)	Rotten (N)	Empty (N)	Suspicious (N)	Total (N)
2023	74	0	326	0	400
2024	145	0	225	0	400

DISCUSSION

The present study provides an investigation of physical characteristics and germination analysis on *A. glutinosa* seeds sampled in two consecutive years (2023 and 2024) in a riparian ecosystem in the Prespa region (in the south-west of North Macedonia) subjected to five different pre-sowing treatments. The results show a difference of the seed properties between the two sampling years, i.e., higher weight and germination rate in the 2024 seed batch. Therefore, we have to reject the first hypothesis as the seeds collected in 2023 show lower quality than the seeds collected in 2024. While it is expected for seeds to lose moisture (i.e., weight) overtime, conservation at lower temperatures has shown to be able to preserve viability in *A. glutinosa* seeds (Hall and Nyong'o 1989, Harrington et al. 2008, Koutsovoulou et al. 2025). However, other studies have shown that *A. glutinosa* seeds stored for two years at 4°C also report a lower germination rate with cold-stratification as a pre-sowing treatment in the experimental design, or potentially, an insufficient sowing rate (Willoughby et al. 2019). Regardless of the sampling year, we have observed an overall low seed viability and germination capacity, which would further reflect as a low number of seedlings (produced in nursery or obtained by direct seeding in open field). Although species with smaller and lighter seeds usually produce them in higher numbers than those with larger and heavier seeds (Leyer and Pross 2009), the germination rates of *A. glutinosa* are known to be low (de Atrip et al. 2007, de Atrip and O'Reilly 2007, Morales et al. 2012). Self-fertilization can also contribute to seeds with aborted ovules (empty seeds) and such occurrences have been reported in *A. glutinosa* and other alder species (Harrington et al. 2008), which could also explain the overall large number of empty seeds in both sampling years in our case. A high proportion of empty seeds (> 60%) has also been noted in another study in neighbouring Greece (Koutsovoulou et al. 2025). Numerous other factors can contribute to the low seed viability (Sanglyne et al. 2021) and they are not mutually exclusive, but rather act conjointly. The reduced capacity of seed production could be due to self-fertilization or senescence. Further phenological observations focusing on pollen production and fruit formation need to be conducted for concrete conclusions. However, frequent external disturbances can reduce the seed regeneration capacity in woody plants (Ehardt-Kistenmacher et al. 2019). Climate change, i.e., reduced water availability and prolonged periods of high temperature in crucial parts of the year when the seeds are forming and maturing are a probable scenario, especially since *A. glutinosa* has a higher need for moisture and abrupt precipitation changes are likely to impact seed characteristics (Göktürk and Güner 2024)**.**

The low seed viability is further reflected in the results from the pre-sowing treatments, although they were selected to address seed dormancy, notably present in *A. glutinosa* seeds*.* Most common inhibitors cause physical dormancy, where the seed coat impermeability can keep the seeds dormant from few months to up to 5 years (Iralu et al. 2019). Indeed, the complete germination absence with ethanol and water soak treatments suggests that dormancy breaking for *A. glutinosa* required physical or chemical stimulation, e.g., complementary treatment combinations such as scarification and soaking in water or specific environmental cues (i.e., temperature and humidity fluctuations) for breaking the dormancy. Seed dormancy and germination characteristics may be due to niche adaptation of a species for securing regeneration (Stromberg et al. 2011, Kanazashi et al. 2015). Considering the ecological niche of riparian species, this is highly likely for *A. glutinosa* and corresponds with the pronounced superiority of the cold stratification across all parameters since the treatment effectively overcame seed dormancy barriers without causing seed damage, likely through enhanced imbibition and the activation of metabolic processes. These results confirm our second hypothesis since the cold stratification showed to be the most effective pre-sowing treatment. The hydrothermal treatment was proven the next most effective after the cold stratification, although not as significantly more as the hydrogen treatment. The hydrothermal treatment involves exposing the seeds to temperature extremes and the hydrogen treatment involves soaking for an extended period in dark conditions. Due to the seed characteristics, future research could explore if combined treatments could be used to provide both physical and chemical stimulation for breaking the dormancy. Dormant seeds can cycle between sensitive (latent soft seeds) or insensitive (hard seeds) stages to dormancy breaking treatments (Taylor 2005). Thus, considering that cold stratification requires more time and in order to ensure higher germination percentages of the viable seeds, further studies that combine it with hydrothermal treatment may be of value. As noted in another study, there is a difference if *A. glutinosa* seeds have been sown in the autumn directly in the soil (mimicking the time of natural seed fall and exposure to the period of moist winter chilling and spring emergence when spring temperatures rise sufficiently) or if they have been artificially pre-chilled and sown in the spring (which notably provides for later emergence and potentially negative impact of dry and hot conditions during April and May) (Willoughby et al. 2019). Since seed types such as *A. glutinosa* are less likely to succumb to predation, autumn sowing might be better but in the case of spring sowing, pre-chilling of at least 20% of the total seed batch is recommended (Willoughby et al. 2019). Indeed, since temperature has been identified as the most crucial factor for germination, seedbeds in nurseries often provide a suboptimal temperature, especially for the spring sowing since temperature is more difficult to manage than moisture (de Atrip et al. 2007).

Although *A. glutinosa* has a wide natural distribution, from the Mediterranean to mid Scandinavia, it is more adapted to moderate and cooler climate with continuous water availability, which might be an issue if the seasonal characteristics change (i.e., warmer autumns, shorter winters, and dried springs and summers) (Gosling et al. 2009, Nave et al. 2021). Furthermore, small population of mature individuals and continuously reduced seed production can be a characteristic of a threatened species (Iralu et al. 2019). Considering this, to ensure the conservation of *A. glutinosa* in the Prespa region, several restoration activities could be attempted. Considering the high benefit from cold stratification, a potential strategy could be direct seeding in the autumn, directly after collection. In this case, the highest risk would be from low temperatures in the spring, but higher seeding rates that follow recommendations from a seed quality analysis could be a potential mitigation strategy. While it has been noted that an optimal germination temperature for *A. glutinosa* and green alder (*Alnus viridis* subsp. *crispa* (Aiton) Turrill) is between 22 and 26°C, these results are based on studies conducted in the United Kingdom and northwestern Ontario where the climatic conditions differ (Farmer Jr. et al. 1985, de Atrip et al. 2007). In the Mediterranean climate, germination occurs as soon as proper moisture is available in the soils, which is why climate change may impact the outcome from known pre-sowing treatments more severely due to reduced rainfall and higher temperatures (Luna et al. 2023). Therefore, it would be of value to monitor how the local climatic conditions impact the on-field germination as well as potential adaptations of the nursery practices.

Additional threats to the populations of *A. glutinosa* in Prespa also need to be anticipated based on relevant research outcomes from other countries. For instance, in Central Europe the host-specific pathogen *Phytophtora alni* species complex is widely present and adapted for water survival (Nave et al. 2021). Since many years can pass from the first symptoms onset (bleeding cankers), and anthropogenic activity is an important transmission factor (22), regional studies in Prespa would be of interest. The risk of invasive species in riparian forests should also be considered. In a study in native floodplain forests in Central Spain that have undergone significant degradation, the exotic *Ailanthus altissima* (Mill.) Swingle*, Ulmus pumila* L., and *Robinia pseudoacacia* L. have exhibited high germination rates, long-lasting seed banks and the capacity for successful germination under heterogeneous conditions that suppress the high proportion of empty seeds and low germinability in the native *Ulmus minor* Mill. and *Fraxinus angustifolia* Vahl (Cabra-Rivas and Castro-Díez 2016). Considering the invasive species *Amorpha fruticosa* has been noted in the study region (Fotiadis et al. 2018), monitoring of its expansion and proper measures for both *in situ* and *ex situ* conservation of *A. glutinosa* is needed. In the nurseries, cold stratification seems to be the most suitable pre-sowing treatment, and it should be adopted as a regular practice. However, the seeding rate needs to be adapted based on the results from germination rates, as we observe a variability between the two sampling years. Some alternative treatments have been proven effective, e.g., priming seeds of Caucasian alder (*Alnus subcordata* C.A.Mey.) with multi-walled carbon nanotubes which improved the germination under drought stress (Rahimi et al. 2016), or the use of symbiotic relationships between *A. glutinosa* roots with arbuscular ectomycorrhiza and nitrogen fixing bacteria from the genus *Frankia* (Wheeler et al. 1991, Ehardt-Kistenmacher et al. 2019). However, such practices are often costly and require a specific infrastructure, which might be a major obstacle. Further nursery and field-based studies exploring this interaction might also support the seeding establishment.

CONCLUSIONS

Riparian ecosystems provide numerous benefits and, as such, are of a particular conservation interest. In the Prespa region, in south-west North Macedonia, black alder, one of the main species in the riparian ecosystem, is under threat due to combined effect of naturally occurring and anthropogenic factors. Reforestation efforts currently rely on seedling production from locally collected seeds. However, no information is available on the state of the mother trees, and thus the seed quality. Furthermore, since black alder seeds require pre-sowing treatment, it is of great practical value to understand how different treatments impact the seeds and subsequently select the most suitable one. The present study addresses both issues, by analysing seeds collected in two consecutive years (2023 and 2024) and a comparative analysis of five pre-sowing treatments, selected based on the black alder seeds' properties. The results show that the seeds collected in 2024 are of higher quality. This can be linked to the negative impact of seed storage on the seed viability. Regarding the pre-sowing treatments, cold stratification has shown to be the most effective pre-sowing treatment, followed by hydrothermal treatment and hydrogen peroxide treatment, clearly indicating the need for physical and/or chemical stimulation to remove the dormancy. On the contrary, the ethanol treatment and the water soak had no effect on the seed germination. These results emphasize the need for continuous monitoring of the seed quality and quantity of *A. glutinosa* from the region, as well the analysis of the state of the mother trees and their potential diversification during the collection. Notably, the current study is limited due to the relatively small sample size and the results should be carefully considered. However, considering the significant knowledge gap for the black alder, especially in terms of seed quality and processing, it might provide insights and bases for future research. For future conservation efforts, on-field implementation of direct seeding in test plots could be useful to observe how the seeds will behave in natural conditions. At the same time, nursery seedling production must include cold stratification to ensure germination of the viable seeds. According to the conditions and availability, combining cold stratification with hydrothermal treatment and testing the effect of arbuscular ectomycorrhiza and nitrogen fixing bacteria from the genus *Frankia* could also be implemented.

Author Contributions

AD, SP, and DDK conceived and designed the research, SP carried out the field sampling, AD and DDK performed laboratory analysis, AD, OO, and IM prepared the figures, AD processed the data and wrote the draft version of the manuscript, AD, SP, OO, IM and DDK revised and edited the manuscript. AD dealt with the revision process.

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Conflicts of Interest

The authors declare no conflict of interest.

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The Influence of Genotype and Morphological Characteristics of Rowan (*Sorbus aucuparia*) Leaves on the Development of *Gymnosporangium cornutum*

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ABSTRACT

Rust caused by *Gymnosporangium cornutum* Arthur ex F. Kern is one of the most common diseases of rowan (*Sorbus aucuparia* L.). Currently, there is little information on the development of this disease in forestry literature. In order to eliminate this deficiency, the examination of the influence of *Sorbus aucuparia* genotype and leaf dimensions on the occurrence and abundance of aecia of *Gymnosporangium cornutum* was carried out. The genotype had a statistically significant influence on aecia occurrence, while leaf length, width, circumference, and area had a statistically significant influence on aecia abundance. About 10% of *Sorbus aucuparia* genotypes showed greater tolerance to *Gymnosporangium cornutum*, with a nearly 4-fold lower probability of aecia occurring on leaves. Larger leaves of *Sorbus aucuparia* sensitive genotype had a greater *Gymnosporangium cornutum* aecia abundance compared to smaller leaves. The results obtained for the first time indicate the possibility of selecting and producing reproductive material of *Sorbus aucuparia* tolerant to *Gymnosporangium cornutum*.

Keywords: aecial stage; plant breeding; gene pool; protection measures

INTRODUCTION

Rowan or mountain ash (*Sorbus aucuparia* L.) is one of the most widespread tree species in Europe, except for the southernmost parts and large islands (Räty et al. 2016). This tree species has significant ecological importance in forest ecosystems (Cvjetićanin et al. 2016). Moreover, it represents a source of antioxidants, tocopherols, and pigments (Šavikin et al. 2017).

So far, the variability of characteristics of *Sorbus aucuparia* has been determined. First of all, there is a difference in the morphological parameters of different *Sorbus aucuparia* half-sib families (Sæbø and Johnsen 2000), while the size of anatomical elements differed between certain genotypes (Umarov et al. 2024). There was a difference in time and degree of fruit bearing between different Sorbus aucuparia genotypes (Munilla and Guitián 2014), and seeds from different *Sorbus aucuparia* populations showed differences in germination (Raeisi et al. 2021). In addition, the fruits of *Sorbus aucuparia* had different colours, pH values, antioxidant activities, and contents of vitamin C, organic acids, sugars, and phenolic components depending on the genotype of the trees (Bozhuyuk 2021).

Genus *Gymnosporangium* consists of significant fungal parasites of trees and shrubs, whereby there is a small number of scientific publications related to this genus and its species (Lâce 2017). Rust caused by *Gymnosporangium cornutum* Arthur ex F. Kern causes great damage to Sorbus aucuparia (Karadžić and Milijašević 2003). The pathogen is a heteroecious rust fungus with an incomplete life cycle: spermagonia and aecia are formed on *Sorbus aucuparia*, whereas the primary host is juniper (*Juniperus* spp.), on which telia and basidia are produced (Ellis and Ellis 1985, Karadžić and Milijašević 2003). The characteristic of rust is pronounced yellow-orange aecia of relatively large dimensions on *Sorbus aucuparia* leaves (Karadžić and Milijašević 2003).

When controlling rust on forest species, there are many challenges, including insufficient efficacy in removing the alternative host and the consequences of applying chemical measures (Hamelin 2013). The genetic control of rust pathogens was recognized more than a hundred years ago, and recently this method of protection has been primarily strived for (Oliver 2024).

In accordance with the above, the objective of this research was to examine whether genotypic and morphological variability of *Sorbus aucuparia* leaves influences the development of rust caused by *Gymnosporangium cornutum*. The tested null hypotheses were: I) *Sorbus aucuparia* genotype does not influence the occurrence of *Gymnosporangium cornutum* aecia; II) *Sorbus aucuparia* leaf dimensions do not affect the occurrence of *Gymnosporangium cornutum* aecia; III) In the case of the sensitive genotype of *Sorbus aucuparia*, there is no difference in abundance of *Gymnosporangium cornutum* aecia on leaves of different dimensions.

MATERIAL AND METHOD

Plant Material

Ten rowan (*Sorbus aucuparia*) trees, 7-8 years old, were sampled on the site Bukovica, Montenegro. The distance between the trees was 20 m. The presence of rust caused by *Gymnosporangium cornutum* was confirmed by the presence of aecia. The aeciospores were 20-30 × 18-25 µm, and the aecia were 4-5 mm, within standard dimensions (Figure 1, Figure 2), matching the description of Ellis and Ellis (1985).

For the analysis of the occurrence of aecia, from each tree, 35 leaves were collected at breast height from the middle of the crown by completely random selection. From the tree with a visually pronounced occurrence of aecia, an additional 100 leaves were collected for the analysis of the abundance of aecia. The analysis of aecia abundance was conducted on leaves collected from a single selected tree with pronounced infection in order to assess within-genotype relationships between leaf morphology and the number of aecia. Moreover, leaves were collected from a single tree to avoid potential genotype effects on leaf abundance. Due to a large number of variables, the genotype was excluded from the analysis of aecia abundance.

The leaves were packed in plastic bags and transported to the Institute of Forestry's laboratory in Belgrade. Until the moment of measurement, they were stored in the refrigerator for 24 hours.

Aecia were counted on the underside of leaves. Immediately afterwards, the leaves were scanned, and

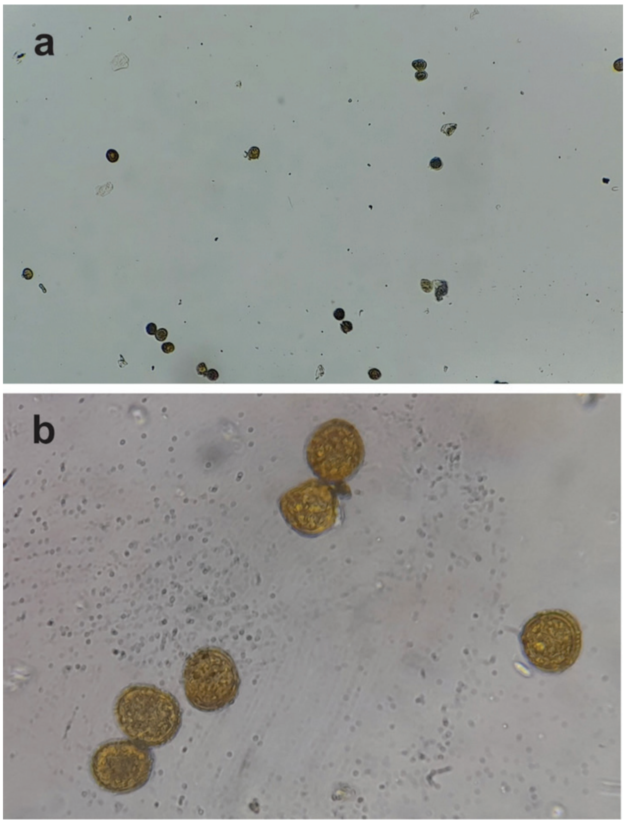

Figure 1. Aeciospores of *Gymnosporangium cornutum*: **(a)** remote representation, 100×; **(b)** close representation, 400×.

leaf blade length, width, leaf circumference, and area were calculated using LAMINA software (Umea University) (Table 1).

Statistical Methods

Binary logistic regression was used for testing the influence of genotype, leaf blade length, leaf blade width, leaf circumference, and leaf area of *Sorbus aucuparia* on the occurrence of *Gymnosporangium cornutum* aecia. The exponential parameter estimates were used to calculate the probability of aecia occurrence.

A general linear model (GLM) was used to test differences in the average *Gymnosporangium cornutum* aecia values across *Sorbus aucuparia* leaf dimensions. Pearson's correlation was used for the analysis of a link between the *Sorbus aucuparia* leaf dimension and the number of *Gymnosporangium cornutum* aecia.

RESULTS

Occurrence of *Gymnosporangium cornutum* Aecia

Binary logistic regression showed statistically significant influence of genotype (Table 2, Figure 2, Figure 3) on the occurrence of *Gymnosporangium cornutum* aecia on *Sorbus aucuparia* leaves. Genotype 1 had the highest probability of occurrence of *Gymnosporangium cornutum* aecia (Exp(B) = 20.503, Figure 2). Genotype 7 had the highest probability of absence of *Gymnosporangium cornutum* aecia (Exp(B) = 3.713, Figure 3). The remaining 80% of genotypes were at the transition between the lowest and highest tolerance to *Gymnosporangium cornutum* (Figure 2).

Abundance of *Gymnosporangium cornutum* Aecia

The general linear model showed that, within the analysed genotype, the number of *Gymnosporangium cornutum* aecia on leaves was influenced by leaf length, width, circumference, and area (Table 2, Figure 2). Pearson's correlation showed statistically significant and positive correlation between leaf length and number of aecia (r = 0.229, p = 0.022), leaf width and number of aecia (r = 0.478, p < 0.001), leaf circumference and number of aecia (r = 0.313, p = 0.002), and leaf area and number of aecia (0.402, p < 0.001) within the analyzed *Sorbus aucuparia* genotype. The number of aecia ranged between 1 and 21 per leaf.

Table 1. Dimensions of leaves of tested rowan (*Sorbus aucuparia*) genotypes.

Genotype	Leaflet length (mm)	Leaflet width (mm)	Circumference (mm)	Area (mm^2)
1	53.41 ± 9.61	18.54 ± 2.14	122.39 ± 19.66	786.20 ± 196.97
2	48.94 ± 9.37	16.68 ± 2.14	114.34 ± 19.60	648.10 ± 191.74
3	43.57 ± 8.87	17.52 ± 2.75	99.46 ± 21.18	576.31 ± 223.80
4	40.11 ± 7.62	16.68 ± 2.84	90.79 ± 18.61	504.67 ± 182.10
5	42.88 ± 7.42	15.52 ± 1.59	95.43 ± 16.37	508.67 ± 130.44
6	44.42 ± 8.02	16.47 ± 2.49	99.57 ± 18.14	569.90 ± 180.08
7	37.11 ± 8.62	14.45 ± 2.64	83.78 ± 19.97	417.59 ± 169.99
8	44.49 ± 5.66	18.24 ± 2.31	99.93 ± 13.01	620.42 ± 146.37
9	36.73 ± 6.10	14.94 ± 2.02	84.50 ± 12.55	427.45 ± 108.54
10	36.54 ± 8.58	14.71 ± 2.03	84.08 ± 20.60	418.06 ± 143.21

Table 2. The influence of genotype and morphology of rowan (*Sorbus aucuparia*) leaves on the occurrence of *Gymnosporangium cornutum*.

Source	Wald-Chi Square	df	Significance
Genotype	30.752	9	<0.001
Leaflet length	0.216	1	0.642
Leaflet width	1.048	1	0.306
Circumference	2.071	1	0.150
Area	2.641	1	0.104

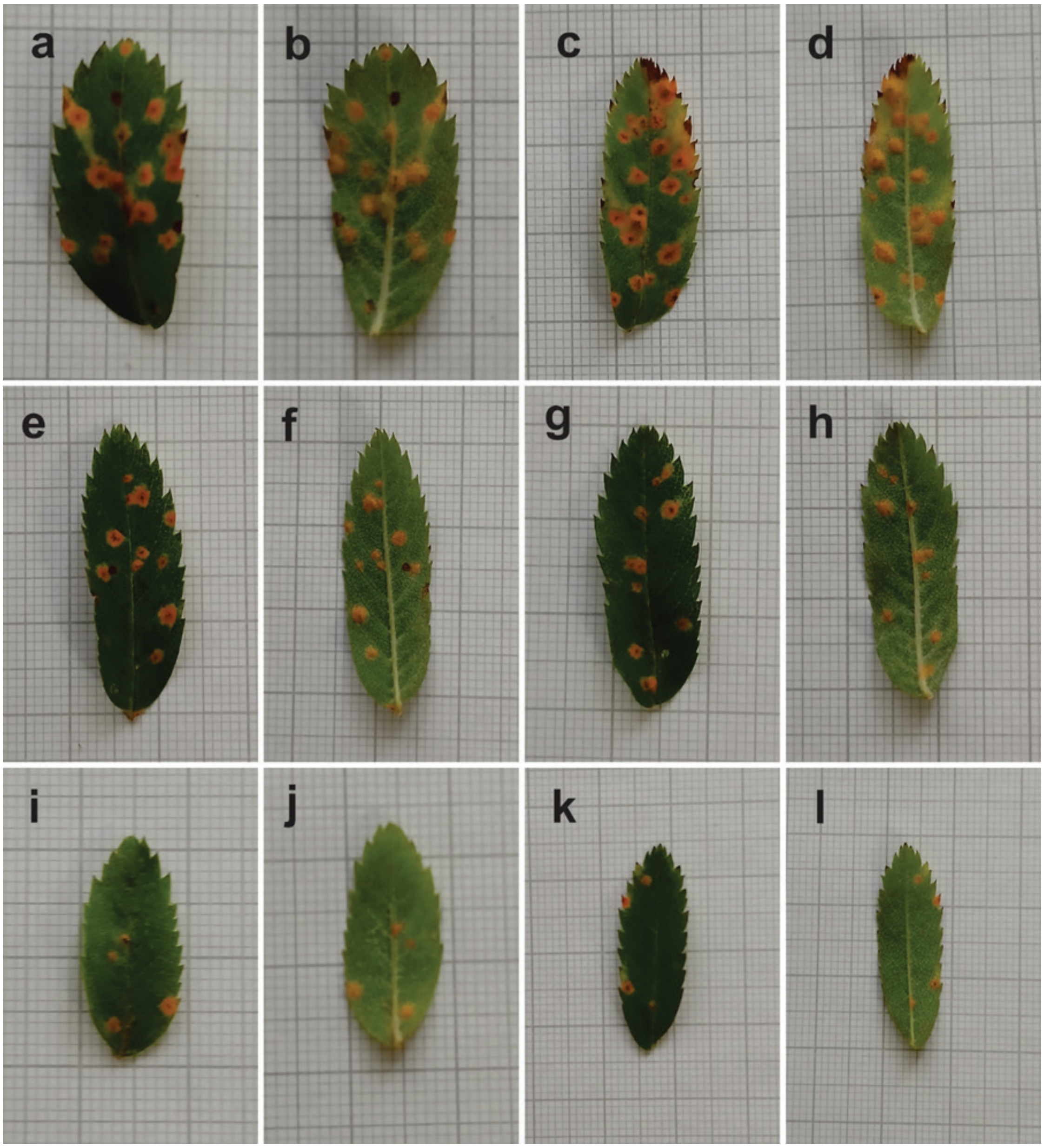

Figure 2. The abundance of *Gymnosporangium cornutum* aecia on rowan (*Sorbus aucuparia*) leaves within the analyzed genotype: **(a, c)** symptoms on the front (adaxial) side of sensitive leaves; **(b, d)** aecia on the back (abaxial) side of sensitive leaves; **(e, g)** symptoms on the front (adaxial) side of moderately tolerant leaves; **(f, h)** aecia on the back (abaxial) side of moderately tolerant leaves; **(i, k)** symptoms on the front (adaxial) side of tolerant leaves; **(j, l)** aecia on the back (abaxial) side of tolerant leaves.

Table 3. The influence of the morphology of rowan (*Sorbus aucuparia*) leaves on the abundance of *Gymnosporangium cornutum* aecia within the analyzed genotype.

Source	Type III Sum of Squares	df	Mean Square	F	Significance
Leaflet length	131.636	1	131.636	5.438	0.022
Leaflet width	571.983	1	571.983	29.013	<0.001
Circumference	245.373	1	245.373	10.646	0.002
Area	403.979	1	403.979	18.852	<0.001

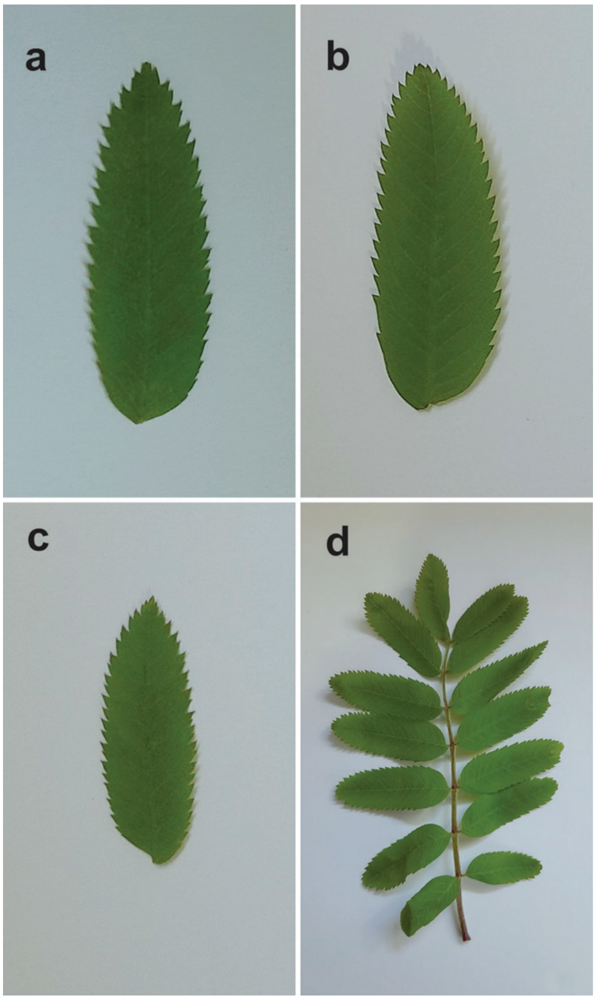

Figure 3. Leaves of *Sorbus aucuparia* tolerant on the occurrence of *Gymnosporangium cornutum* aecia.

DISCUSSION

The genotype of *Sorbus aucuparia* showed great significance, while the dimensions of leaves did not show an influence on the occurrence of *Gymnosporangium cornutum* symptoms. The first null hypothesis is rejected. The alternative hypothesis is accepted that the *Sorbus aucuparia* genotype influences the occurrence of *Gymnosporangium cornutum* aecia. On the other hand, the second null hypothesis that *Sorbus aucuparia* leaf dimensions do not affect the occurrence of *Gymnosporangium cornutum* aecia was accepted. A limitation of this study is that the analysis of aecia abundance in relation to leaf morphology was conducted on a single *Sorbus aucuparia* genotype. Consequently, the observed relationships describe within-genotype variation and should not be generalised to the species level without further multi-genotype validation. Monitoring the health of trees is necessary to ensure long-term resistance to rust, prevent or limit pathogen spread, and reduce the risk of pathogen evolution and the breakdown of host resistance (Hamelin 2022). Genotypes were not genetically verified. In this sense, it would be interesting in the future to monitor, over several years, the influence of micro-locality conditions and ontogenetic factors on the health status of *Sorbus aucuparia* seedlings that exhibited less pronounced symptoms caused by *Gymnosporangium cornutum*. Moreover, the proximity of both hosts required for the spread of *Gymnosporangium cornutum*, primarily common juniper (*Juniperus communis* L.), may interact strongly with the aforementioned factors under increased infection pressure. Therefore, these phenomena should be studied jointly. In this sense, we consider that increasing *Sorbus aucuparia's tolerance* (i.e., mitigating the harmful effects of infection) based on the results of this study provides a stable approach to controlling *Gymnosporangium cornutum*.

For the increase in the representation of *Sorbus aucuparia-tolerant* trees, it is necessary to adjust the methods of regeneration of this species. Examples of the introduction of wild service tree (*Sorbus torminalis* (L.) Crantz), a species belonging to the same genus, showed a positive effect on biodiversity, resistance, and ecosystem functions (Višnjić et al. 2025). Fruit bearing of *Sorbus aucuparia* trees is stimulated by tree diameter and light availability, while competition has a negative effect (Kondrat et al. 2024). The mentioned factors must be considered during natural regeneration. The largest production of

Sorbus aucuparia seeds happens just before the die-back of trees (Pesendorfer et al. 2019). Therefore, in the case of artificial regeneration, it is recommended to wait for an abundant yield of trees with weak rust symptoms to increase the amount of collected high-quality seed. To determine which part of the *Sorbus aucuparia* crown is most susceptible to *Gymnosporangium cornutum*, we recommend the application and analysis of mixed-effects models. Furthermore, to improve the use of tolerant seedlings across different sites, it is necessary to consider the microclimatic characteristics of habitats, which may be more favorable to this type of reproductive material.

Within the sensitive genotype of *Sorbus aucuparia*, a larger number of *Gymnosporangium cornutum* aecia was recorded on larger leaves. Thus, the third null hypothesis was rejected. The alternative hypothesis is accepted: in the case of a sensitive genotype of *Sorbus aucuparia*, there is a difference in the abundance of *Gymnosporangium cornutum* aecia on leaves of different sizes. However, we consider that it is not useful to favour the development of the smaller leaves of *Sorbus aucuparia*. Especially because the effect of leaf size was examined within the genotype susceptible to *Gymnosporangium cornutum*. Larger leaves have several advantages, among which better survival in the shade, greater photosynthesis, more efficient use of water and nutrients, faster acclimatisation, and larger ecosystem productivity stand out (Murphu et al. 2012, Milla and Matesanz 2017, Wright et al. 2017, Song et al. 2018, Lusk et al. 2019, Li et al. 2020). Therefore, the obtained results should primarily be used as a recommendation that *Sorbus aucuparia* trees with small leaves, which are usually a consequence of phenotype or unfavourable habitat conditions, should not be removed from stands affected by *Gymnosporangium cornutum.*

To preserve *Sorbus aucuparia* populations at the edges of the distribution area, it is necessary to connect trees through habitat restoration or the introduction of genetic material (Yousefzadeh et al. 2021). We consider that both approaches can be used for the protection of *Sorbus aucuparia* trees on the edges of the distribution area from *Gymnosporangium cornutum*. During habitat restoration, the focus should be on encouraging the development of trees with less pronounced symptoms, while during the introduction of genetic material, preference should be given to reproductive material obtained from such trees.

Individual mother trees are more important than the population for progeny quality (Sæbø and Johnsen 2000). Therefore, *Sorbus aucuparia's* high tolerance to *Gymnosporangium cornutum*, determined at the individual level, enables an easier initial material selection process with greater flexibility in selecting trees.

CONCLUSIONS

This study demonstrated the significance of genotype and leaf morphology of *Sorbus aucuparia* for the aecial stage of the development of rust caused by *Gymnosporangium cornutum* fungus. The tree genotype influenced the occurrence of *Gymnosporangium cornutum* aecia on *Sorbus aucuparia* leaves, whereas leaf dimensions influenced aecia abundance. For the first time, this study provides a basis for the selection of *Sorbus aucuparia* mother trees with reduced susceptibility to *Gymnosporangium cornutum*, which may contribute to slowing pathogen spread. Future research should focus on progeny tests to obtain the most tolerant reproductive material, which will also be suitable for other forestry needs.

Author Contributions

AV and VP conceived the idea and designed the study, conducted fieldwork, and performed statistical analyses. SL, AL, and LJR performed laboratory analyses. VP, MV, and MĆ performed data curation and visualisation of results. AV, AL, and VP wrote the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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Ectomycorrhizal Diversity of Two Economically Most Important Riparian Forest Tree Species in Serbia – A Case Study of Gornje Podunavlje Special Nature Reserve

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ABSTRACT

Gornje Podunavlje Special Nature Reserve in Serbia is recognized by its specific, unique and diverse flora and fauna. Pedunculate oak (*Quercus robur* L.) and Euramerican poplar (*Populus × euramericana* (Dode) Guinier) are the most widespread and economically important tree species grown in this area. The aim of our study was to analyze for the first time the diversity of ectomycorrhizal (ECM) fungi on these two tree species in Gornje Podunavlje. ECM fungi were identified combining morphological and anatomical characterization with molecular analysis based on PCR amplification of the ITS region of fungal nuclear ribosomal DNA. The number of different categories of fine roots were counted, diversity indices were calculated, and ECM fungi were classified into exploration types. Some soil physical and chemical properties were analyzed as well. Twenty-eight ECM fungal taxa were found in total at two sites. Sixteen ECM fungi were identified to the species level, eight to the genus level, and three fungi remained unidentified. A total of 17 ECM fungal taxa were identified in the pedunculate oak stand and 15 in the Euramerican poplar plantation. Short-distance exploration type dominated on both tree species, but higher abundance of long-distance exploration type was recorded in the oak stand, which is likely related to the higher humus content measured at this site.

Keywords: ectomycorrhiza; pedunculate oak; Euramerican poplar; molecular identification; exploration types

INTRODUCTION

Study area is situated in Gornje Podunavlje Special Nature Reserve (SNR), which is part of much bigger protected area – the UNESCO Biosphere Reserve Amazon of Europe – a transboundary biosphere reserve along the Drava, Mura and Danube Rivers that provide an important tool in learning about floodplain management (UNESCO 2025). Gornje Podunavlje SNR is located in northwestern part of Serbia, stretching over 19,648 ha along the left bank of the Danube River and including different ecosystems such as meadows, marshes, and forests (Stojanović et al. 2021a). It is recognized by its well-preserved characteristics of the floodplain along the Danube River, and it is one of the rarely conserved natural sceneries in predominantly agrarian surrounding of northern Serbia. The flora and fauna of Gornje Podunavlje SNR are specific and unique but also diverse and abundant with many rare plant and animal species which have national and international importance (Stojanović et al. 2021a). Economically most important forest tree species in forest ecosystems of this SNR are pedunculate oak and Euramerican poplar.

Ectomycorrhizal (ECM) fungi are important symbionts of forest trees which have a great influence on the functioning and productivity of the forest ecosystems (Smith and Read 2008). They provide their host with hardly accessible water and nutrients from the soil in exchange for photosynthetically produced carbohydrates (Smith and Read 2008). A common mycelial network, formed by ECM fungi, links forest trees of the same and different species with belowground components of forest ecosystems and increases seedlings' survival and growth, as seedlings can receive carbon, nutrients, and water from the older trees (Selosse et al. 2006). Due to the common mycelial network, ECM fungi have an especially important role in forest regeneration, succession, and resistance against different stress factors (Selosse et al. 2006, Milović et al. 2021a). Furthermore, mycorrhizas play an essential role in the stability of forest ecosystems, which is of special importance under environmental stress (Milović et al. 2021a, Stojanović et al. 2021b). Since functional compatibility of ectomycorrhizae depends on both partners, information on the diversity of ECM fungi can provide valuable insight into the physiology of forest ecosystems and their adoptability and resilience (Kraigher and Al Sayegh-Petkovšek 2011).

Riparian forest tree species, pedunculate oak and black poplars, are of great importance for forestry in Serbia. Nowadays, the diversity of ECM fungi on oaks and poplars is being investigated worldwide (Courty et al. 2008, Karlinski et al. 2013, Suz et al. 2014, Szuba 2015, Bzdyk et al. 2019, Frymark-Szymkowiak et al. 2024), and some research has already been done in Serbia (Katanić et al. 2015a, Milović et al. 2021b, Milović et al. 2022, Milović et al. 2023). Since Gornje Podunavlje SNR is a riparian ecosystem with specific traits, rich in plant and animal species, our aim was to study for the first time the diversity of ECM fungi on the most widespread and economically important tree species grown in this area.

MATERIALS AND METHODS

Sampling Site and Procedures

The sampling was conducted at two sites: 120-year-old pedunculate oak (*Quercus robur* L.) stand and 40-year-old plantation of Euramerican poplar (*Populus × euramericana* (Dode) Guinier) which is the most cultivated poplar in Serbia and represents an interspecies hybrid between Eastern cottonwood (*Populus deltoides* Bartr. Ex Marsh) and European black poplar (*Populus nigra* L.) (Đilas et al. 2023, Pilipović et al. 2023). Pedunculate oak trees were of good vitality and quality, while poplar plantation was degraded, consisted of overmatured Euramerican poplar trees and situated in an area which was planned for future restoration with autochthonous tree species. Both sites were located in the protected part of the alluvial plain of the Danube River, which is under regular management of Public Enterprise "Vojvodinašume" (Table 1). Habitat conditions are not optimal for either species due to limited groundwater supply. The poplar plantation was part of the chronosequence within the Horizon Superb project whose goal is to transform hybrid poplar plantation with low vitality into a vital forest where the dominant tree species would be the autochthonous pedunculate oak.

According to meteorological records obtained from the nearby station in Sombor over the period 1991–2020, the mean annual temperature in the area is 11.7 °C and the average annual rainfall is 636.0 mm. Examination of the average monthly sum of precipitation for this area showed that the months with the most and the least precipitations were June and March, respectively (RHMZ 2025).

Soil physical and chemical properties were determined in the surface layer of the soil (upper 30 cm). The following soil characteristics were analyzed: particle-size distribution (%) by the international B-pipette method with preparation in sodium pyrophosphate; determination of soil textural classes based on particle-size distribution using Atteberg classification; $CaCO_3$ percentage (%) measured volumetrically by using Scheibler's calcimeter; and pH in H_2O determined with electrometric method using a combined electrode on Radiometer pH meter. Carbon and nitrogen content were determined with CHN element analyzer (Vario® EL III, Elementar Analysensysteme, Germany), while the content of humus was measured by the method of Turin. All analyses were performed in the Laboratory of Soil Science at the Institute of Lowland Forestry and Environment in Novi Sad, applying the methodology described by Galić et al. (2018).

At both sites, soil samples were taken on 9th November 2022 using a soil corer (total volume 274 ml and length 18 cm) at about 1 m from the target tree trunk. The total number of soil samples per site was eight and they were stored in a refrigerator at 4 °C. Before analyses each sample was submerged overnight in tap water to loosen the soil structure. All fine roots were carefully washed from the soil, placed in a petri dish above a grid and evenly distributed.

Table 1. Site characteristics of the studied pedunculate oak stand and Euramerican poplar plantation from Gornje Podunavlje Special Nature Reserve, Serbia.

Site	Pedunculate oak stand	Poplar plantation
Coordinates	N 45°54´47˝ E 18°53´02˝	N 45°54´35˝ E 18°52´44˝
Altitude	85.1 m	84.8 m
Climate	Moderate continental	Moderate continental
Management type	Regularly managed	Regularly managed
Soil type	fluvisol	fluvisol
Species	*Quercus robur* L. mixed with *Carpinus betulus* L. and *Acer* spp.	*Populus × euramericana* (Dode) Guinier.

One square color from the grid was randomly chosen and within this square 500 fine roots were counted. The total number of fine roots analyzed for both species was 4000. All obtained roots were divided into vital ECM root tips, or old, non-turgescent, and non-mycorrhizal roots using a dissecting stereomicroscope Olympus SZX10® (Olympus Corp., Tokyo, Japan) with magnifications 10-63× (light source: Olympus Highlight 3100, daylight filter). Vital ECM root tips were categorized into different morphotypes of ectomycorrhizas based on their morphological and anatomical characteristics using a dissecting microscope and a microscope Olympus BX53® (Olympus Corp., Tokyo, Japan) with magnifications 100-1000×.

Morphotypes of ectomycorrhizae were described following the methodology given by Agerer (1991) and Kraigher (1996). When it was possible, a fungal partner was determined by comparison of the obtained descriptions with published descriptions in Agerer (2008) or Agerer and Rambold (2025). Morphotypes of ectomycorrhizae were also classified into the exploration types (ETs) as proposed by Agerer (2001). All categories of fine root tips were quantified by counting under the dissecting microscope.

Molecular Identification of Ectomycorrhizal Fungi

Fungi from ECM root tips were identified with molecular tools based on PCR amplification of internal transcribed spacer (ITS) region of fungal nuclear rDNA. Genomic DNA was extracted from 2-5 ECM root tips of every ECM morphotype using a DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany). Amplification reactions were performed using ITS 1F (Gardes and Bruns 1993) and ITS 4-primer pair (White et al. 1990) in Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany). Detailed procedure was given by Milović et al. (2023). Amplified DNA fragments were sent for sequencing to Macrogen Europe BV (Amsterdam, Netherlands) after purification with the QIAquick PCR® purification kit (Qiagen, Valencia, CA, USA). The ECM fungi were determined at the level of species, genus, or family by comparing the obtained sequences to those deposited in GenBank (NCBI 2021) and UNITE (Nilsson et al. 2018) database. The threshold value applied to differentiate the different operational taxonomic units (OTUs) based on ITS sequence similarity was 97%.

Data Analysis

Diversity indices were calculated as average value per sample and as total value per site (i.e., by pooling the ECM community data) following the formulas given by Atlas and Bartha (1981) and Taylor et al. (2000): (i) species richness index (d) = $(S-1)/\log_{10}N$, where S is the number of ECM fungal taxa and N is the number of all mycorrhizal tips; (ii) Shannon-Weaver's diversity index (H) = $C/N\ (N \log N - \Sigma\, n_i \log n_i)$, where C = 2.3, N is the number of all mycorrhizal tips and n_i is the number of mycorrhizal tips of an individual ECM fungal taxon; (iii) Evenness (e) = H/logS, where H is the Shannon-Weaver's diversity index and S is the number of ECM fungal taxa; (iv) Equitability (J) = H/H_{max}, where H is the Shannon-Weaver's diversity index and H_{max} is the theoretical maximum H assuming that each ECM fungal taxon was equally abundant; (v) Berger-Parker's evenness index (BP) = $1 - (N_{max}/N)$, where N_{max} is the number of mycorrhizal tips of the most frequent ECM fungal taxon and N is the number of all mycorrhizal tips.

In order to fit the normal distribution, percentage values were transformed by arcsine transformation using the Bliss formula (Snedecor and Cochran 1976). Tukey test was used to determine the significance of the differences between stands in an abundance of ETs. All statistical analyses were performed using the package STATISTICA® ver. 12 (StatSoft Inc., Tulsa, OK, USA).

RESULTS

The physico-chemical properties of the soil from pedunculate oak stand and Euramerican poplar plantation were in some traits quite similar: both soils have loam texture class, similar values for the content of $CaCO_3$ and C/N ratio, and their pH is close to neutral. However, humus, carbon and nitrogen content were considerably higher in soil samples from pedunculate oak stand, suggesting higher soil fertility at this site (Table 2).

In the pedunculate oak stand and Euramerican poplar plantation, 17 and 15 ECM fungal taxa were recorded, respectively. The total number of analysed vital ECM roots was 1463, 810 on oak and 653 on poplar. The average number of ECM taxa per sample was 4.4 in the oak stand and 4.8 in the poplar plantation. The average value of species richness index was higher in Euramerican poplar plantation (1.98 vs. 1.7), while the Shannon-Weaver index was higher in the oak stand (1.24 vs. 1.01). Furthermore, evenness, equitability and Berger-Parker index on average were higher in the oak stand while in total these values were higher in the poplar plantation (Table 3).

In Gornje Podunavlje Special Nature Reserve, 28 ECM fungal taxa were found in total. Sixteen ECM fungi were identified to the species level, eight to the genus level, and three ECM fungi remained unidentified. At both sites a few species dominated the ECM community, while others were rare. Only two ECM fungi colonized more than a half of all ECM roots at both sites. In the poplar plantation the most abundant were *Cenococcum geophilum* and *Tomentella* sp. 2, while in the pedunculate oak stand *Humaria*

Table 2. Physico-chemical properties of the soil in pedunculate oak (*Quercus robur*) stand and Euramerican poplar plantation (*Populus × euramericana*) plantation in Gornje Podunavlje Special Nature Reserve, Serbia.

Site	Total sand (%)	Total silt (%)	Total clay (%)	Texture class	pH	Humus content (%)	$CaCO_3$ content (%)	Carbon content (%)	Nitrogen content (%)	C/N ratio
Pedunculate oak	37.8	40.0	22.2	Loam	7.49	9.54	8.85	5.15	0.24	21.6
Poplar	44.6	37.2	18.2	Loam	7.60	6.58	8.03	2.95	0.15	19.4

Table 3. Total and average values (± standard error) of the number of ectomycorrhizal fungal taxa, vital ectomycorrhizal root tips, old, non-turgescent and non-mycorrhizal roots, total fine roots, % of vital ectomycorrhizal roots and diversity indices in the pedunculate oak (*Quercus robur*) stand and Euramerican poplar (*Populus × euramericana*) plantations in Gornje Podunavlje Special Nature Reserve, Serbia.

Parameter	Pedunculate oak		Euramerican poplar	
	Total value per site	Average value per sample	Total value per site	Average value per sample
Number of ectomycorrhizal fungal taxa	17	4.37±0.4	15	4.75±0.5
Number of vital ectomycorrhizal root tips	810	101.3±16.2	653	81.6±12.3
Number of old, non-turgescent and non-mycorrhizal root tips	3190	398.8±16.2	3347	418.4±12.3
Total number of fine roots	4000	500	4000	500
% of vital ectomycorrhizal root tips	20	20±3	16	16±0.02
Species richness index	5.50	1.73±0.2	4.97	1.98±0.2
Shannon-Weaver index	2.14	1.24±0.1	2.14	1.01±0.1
Evenness	1.74	1.96±0.1	1.82	1.55±0.2
Equitability	0.76	0.85±0.0	0.79	0.67±0.1
Berger-Parker index	0.66	0.56±0.1	0.67	0.43±0.1

hemisphaerica and *Hortiboletus engii* colonized more than 55% of all roots (Figure 1, Supplement Table 1). ECM taxa *H. hemisphaerica*, *Sebacina incrustans*, *Scleroderma bovista*, *Tuber brumale*, and *C. geophilum* were common for both sites/species. In the pedunculate oak stand the most species rich genus was *Inocybe* with four ECM taxa, but in the Euramerican poplar plantation no members of this genus were observed. On the other hand, genus *Tomentella* was the richest in the poplar plantation with three taxa, but it had only one representative in the oak stand (Figure 1).

In the pedunculate oak stand, families Pyronemataceae, Boletaceae and Inocybaceae made association with 77% of all root tips, while in the Euramerican poplar plantation only two families (Thelephoraceae and Gloniaceae) colonized 75% of all ECM root tips (Figure 2). The most species-rich family in the pedunculate oak stand was Inocybaceae with 5 members, while in the Euramerican poplar plantation it was Thelephoraceae with also 5 members (Figure 3).

At both sites Basidiomycota fungal group had more ECM taxa and colonized more root tips compared to Ascomycota (Table 4). However, in the oak stand a bit more ECM taxa and ECM roots belonging to Ascomycota were observed compared to the poplar plantation.

In the pedunculate oak stand short distance ET dominated, followed by long-distance ET, while medium distance ET was the least abundant. On the other hand, in the poplar plantation the most abundant was short-distance ET, then medium-distance ET, while other ETs were relatively rare. Tukey's test showed significantly higher abundance of long-distance ET in the oak stand than in the poplar plantation (Table 5).

Table 4. Ratio of the number of ECM taxa and the number of roots belonging to Ascomycota/Basidiomycota (%) in the pedunculate oak (*Quercus robur*) stand and Euramerican poplar (*Populus × euramericana*) plantation in Gornje Podunavlje Special Nature Reserve, Serbia.

	Ascomycota/Basidiomycota ratio for the number of ECM taxa (%)	Ascomycota/Basidiomycota ratio for the number of ECM roots (%)
Pedunculate oak	6:11	43:57
Euramerican poplar	4:11	39:61

Table 5. Relative abundance (± standard error %) of ectomycorrhiza exploration types in the pedunculate oak (*Quercus robur*) stand and Euramerican poplar (*Populus × euramericana*) plantation from Gornje Podunavlje Special Nature Reserve, Serbia, and the significance of Tukey test for the effect of species (sites).

Exploration type	Pedunculate oak	Euramerican poplar	p value
Contact	0 a	5.2±5.2 a	0.334
Short distance	60.8±9.6 a	72.0±7.3 a	0.440
Medium distance (smooth subtype)	11.3±5.8 a	17.8±7.3 a	0.366
Long distance	27.9±7.5 a	5.0±4.7 b	0.016

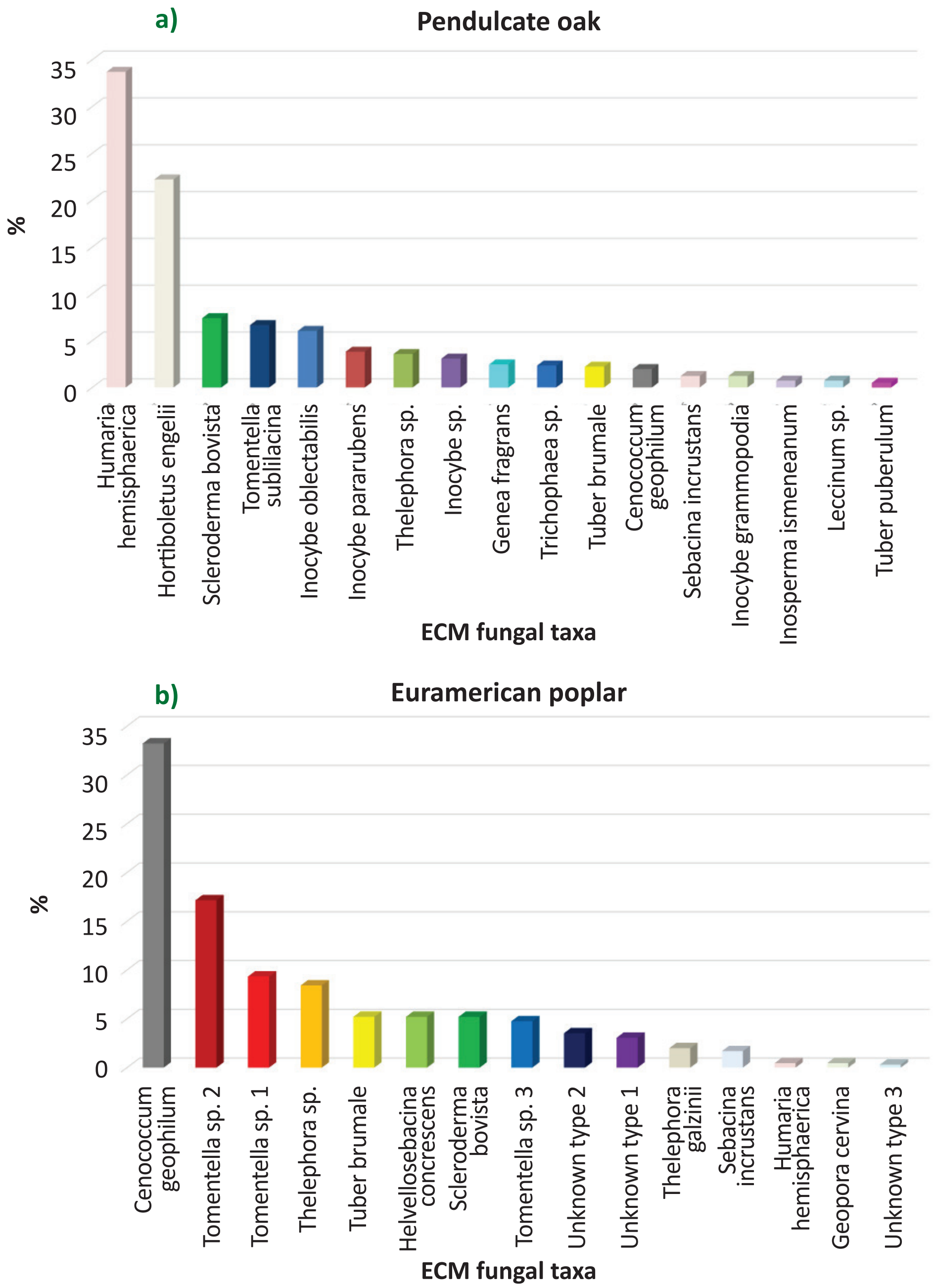

Figure 1. Relative abundance of ectomycorrhizal fungal taxa (%) (based on the number of ectomycorrhizal root tips belonging to the particular ectomycorrhizal fungal taxon in relation to all ectomycorrhizal root tips) in the: **(a)** pedunculate oak (Quercus robur) stand, and **(b)** and Euramerican poplar (Populus × euramericana) plantation in Gornje Podunavlje Special Nature Reserve, Serbia.

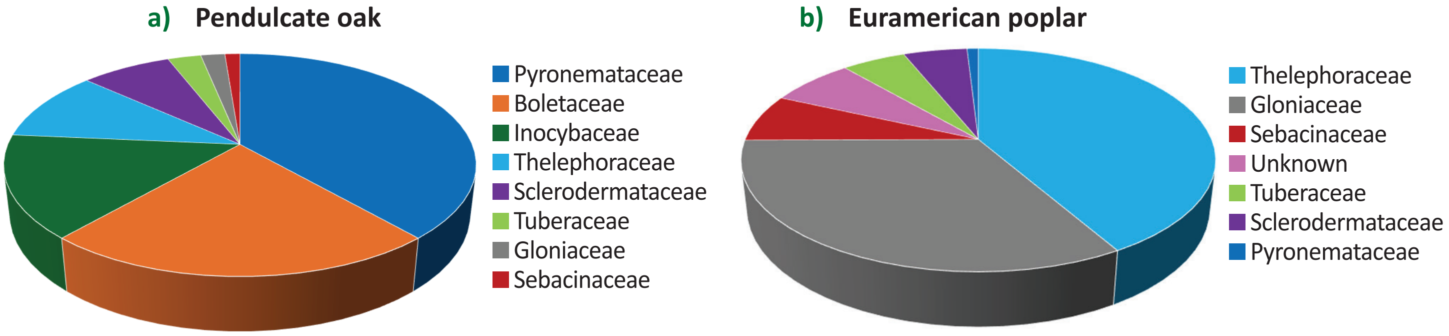

Figure 2. Relative abundance of taxonomic families of ectomycorrhizal fungi based on the number of ectomycorrhizal root tips belonging to a particular family in relation to all ectomycorrhizal root tips in the: **(a)** pedunculate oak (Quercus robur) stand, and **(b)** and Euramerican poplar (Populus × euramericana) plantation in Gornje Podunavlje Special Nature Reserve, Serbia.

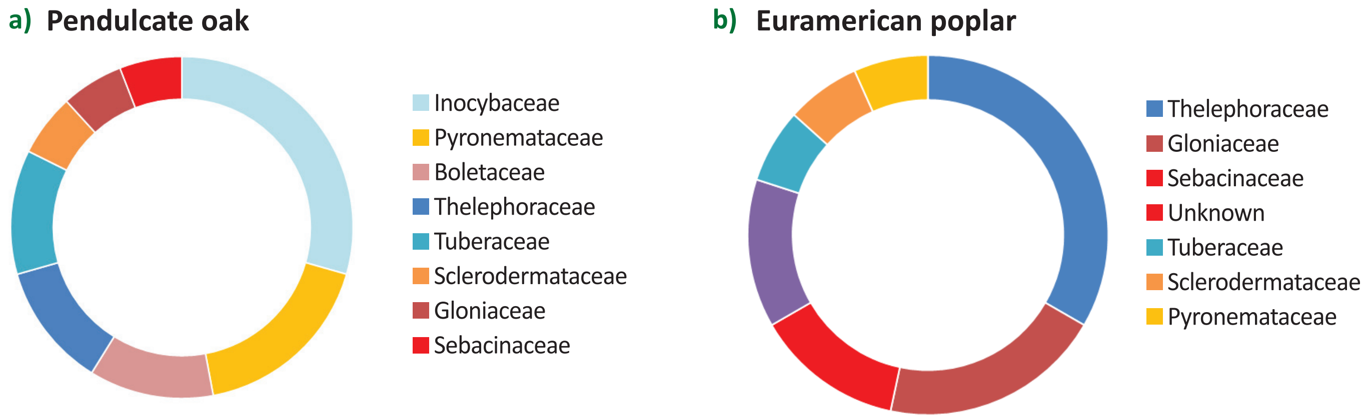

Figure 3. Species richness of taxonomic families of ectomycorrhizal fungi based on the number of ectomycorrhizal taxa belonging to a particular family in relation to all ectomycorrhizal taxa in the pedunculate oak (*Quercus robur*) stand **(a)** and Euramerican poplar (*Populus × euramericana*) plantation **(b)** in Gornje Podunavlje Special Nature Reserve, Serbia.

DISCUSSION

In Gornje Podunavlje Special Nature Reserve situated in the northwestern part of Serbia, 17 ECM fungal taxa were found in the pedunculate oak stand. The obtained results are comparable with the results recorded in some other oak stands in Serbia. According to Milović et al. (2022), 18 ECM fungal taxa were recorded in the young pedunculate oak stand near Morović. In the mature pedunculate oak stand situated near the previous site, 21 ECM fungal taxa were observed (Milović et al. 2023), from which 19 in spring and 13 in autumn. Moreover, in two sessile oak stands from Fruška Gora National Park, 17 and 12 ECM taxa were found (Milović at al. 2021c).

In Europe, 12–14 ECM taxa were recorded in a 150-year-old pedunculate oak forest in north-eastern part of Poland (Olchowik et al. 2019), 18 ECM fungal taxa were recorded at the Taurus mountains in Central Germany (Schirkonyer et al. 2013), while 21 ECM taxa were determined on pedunculate and sessile oaks from Ireland (O'Hanlon and Harrington 2012). However, the average number of ECM fungi in oak plots across Europe during one season was 55, ranging from 24 fungal taxa in the Netherlands to 83 in Romania (Suz et al. 2014). In our study, the average number of ECM taxa per soil sample in the pedunculate oak stand was 4.4. Similar results were obtained in the mature pedunculate oak stand in Morović in spring (4.5) but in autumn this number was lower (2.9) (Milović et al. 2023). However, in young pedunculate oak higher average number of ECM taxa (5.6) was observed (Milović et al. 2022).

In our study, 15 ECM fungal taxa were observed in the Euramerican poplar plantation. The obtained results are comparable to the data recorded on poplars in other sites in Serbia. In an autochthonous white poplar (*Populus alba* L.) stand in the Kovilj-Petrovaradin marshes in Serbia, Katanić et al. (2015a) observed 20 ECM fungal taxa in total, from which 16 in spring and 15 in autumn. Furthermore, in a mature white poplar plantation during four consecutive seasons, 30 taxa of ECM fungi were recorded in total (Milović et al. 2021b). The highest number of ECM taxa was recorded in winter (22) followed by spring (19), while the lowest number was counted in autumn and summer (17). However, at pyrite tailings contaminated site near the Timok River Katanić et al. (2015b) recorded only 4 ECM taxa.

The results of our study are comparable to the results of Visser et al. (1998), who recorded 22 ECM types in a mixed forest dominated by American aspen (*Populus tremuloides*) in Canada, and Kaldorf et al. (2004), who observed 23 morphotypes in a five-year-old experimental aspen plantation in Germany. In addition, at three experimental sites with poplars in Poland, Karlinski et al. (2013) found in total 27 ECM fungal taxa. On the other hand, on an individual *Populus tremula* tree in an old-growth mixed forest from Estonia, Bahram et al. (2011) found 122 ECM fungal species. Jakucs (2002) recorded 70 ECM types at two sites with white poplars in Hungary during three years, while Krpata et al. (2008) found 54 ECM taxa in association with *Populus tremula* at a heavy metal polluted site in Austria. The average number of ECM taxa per soil sample in the poplar plantation was 4.8. In white poplar (*Populus alba*) from the Kovilj-Petrovaradin marshes, about 5 ECM taxa were observed per sample (Katanić et al. 2015a), while in the white poplar plantation this number ranged from 4 (in summer) to 5.8 (winter).

The percentage of vital ECM roots in the oak stand and poplar plantation was 20 and 16, respectively. Those values are comparable with the previous results obtained in Serbia in a young pedunculate oak stand (Milović et al. 2022) and a white poplar stand in Kovilj-Petrovaradin marshes (Katanić et al. 2015a).

If we compare diversity indices obtained in our study with previously published data from similar sites, we can state that they are comparable. Values of species richness index, Shannon Weaver index, evenness, equitability and Berger Parker index calculated for pedunculate oak stand from Gornje Podunavlje in autumn are similar to the same indices recorded in a mature pedunculate oak in spring (Milović et al. 2023) and a young pedunculate oak in summer (Milović et al. 2022), while values of species richness index and Shannon Weaver index were higher in comparison with sessile oak (Milović et al. 2021b). If we analyse diversity indices obtained for poplar plantation in

Gornje Podunavlje and compare them to the ones from similar sites in Serbia, we can see that species richness index at our site was the highest. The value of Shannon Weaver index at our site was mostly similar to the one recorded in Kovilj-Petrovaradin marshes in spring (Katanić et al. 2015a), while evenness, equitability and Berger Parker index had values very similar to the ones obtained in a mature white poplar plantation in summer (Milović et al. 2021b).

In Gornje Podunavlje Special Nature Reserve, 17 ECM fungal taxa were found in the pedunculate oak stand where fungi *Humaria hemisphaerica*, *Hortiboletus engii* and *Scleroderma bovista* colonized the majority of fine roots. In the Euramerican poplar plantation, 15 taxa were recorded and among them the most abundant were *Cenococcum geophilum*, *Tomentella* sp. 2, *Tomentella* sp. 1 and *Thelephora* sp. ECM fungal community made of few abundant and many rare fungal taxa is a common phenomenon in other forest ecosystems (Suz et al. 2014, Katanić et al. 2015, Milović et al. 2021b, Milović et al. 2023). ECM fungi *Humaria hemisphaerica*, *Sebacina incrustans*, *Scleroderma bovista*, *Tuber brumale*, and *C. geophilum* were recorded on both species in our study, so they can be regarded as generalists.

In the pedunculate oak stand the most species-rich genus was *Inocybe* with four ECM taxa, while families Pyronemataceae, Boletaceae and Inocybaceae made association with most of the root tips. Studying ECM communities in temperate oak forests across nine European countries, Suz et al. (2014) discovered that overall, *Lactarius quietus*, *C. geophilum* and *Russula ochroleuca* were the most abundant taxa. Similarly, in two soil horizons of a temperate oak forest for 15 months, the most abundant species were: *Lactarius quietus*, *Tomentella sublilacina*, *Cenococcum geophilum*, and *Russula* sp. 1 (Courty et al. 2008). Previous work on the ECM community of a mature pedunculate oak stand in Serbia (Milović et al. 2023) also revealed *Lactarius* and *Russula* as a dominant fungal group. However, in our study, members of *Russula* and *Lactarius* genera were not observed in the pedunculate oak stand at all. This absence could be explained by differences in soil type, physico-chemical soil properties, previous land use and management practice between these sites.

In the poplar plantation in Gornje Podunavlje Special Nature Reserve the most abundant ECM taxa were: *C. geophilum*, *Tomentella* sp. 2 and *Tomentella* sp. 1, while genus *Tomentella* was the richest. *Tomentella* group was well represented in previous studies on white poplars in Serbia (Katanić et al. 2015a, Milović et al. 2021b), as well as genus *Inocybe*. However, no member of genus *Inocybe* was observed in the poplar plantation in our study. In the mature white poplar plantation from Sebia the most abundant ECM fungi were: *Inocybe griseovelata*, *I. splendens*, *Tuber rufum*, and *Tomentella* sp. 2, which together represented up to 50% of all ECM root tips (Milović et al. 2021b). In Kovilj Petrovaradin marsh the most abundant ECM fungi were: *Entoloma* sp., *Tuber maculatum*, *C. geophilum*, *Tuber rufum* and *Peziza* sp. (Katanić et al. 2015a).

At both analyzed sites, Basidiomycota dominated the ECM fungal community, which confirm numerous studies on poplars and oaks (Krpata et al. 2008, Milović et al. 2015a, Milović et al. 2021b, Milović et al. 2021c, Milović et al. 2022, Milović et al. 2023) where Basidiomycota was more abundant and had more members compared to Ascomycota group.

In our study, at both sites, short-distance ET dominated and made a large part of all ECM root tips. It was followed by long-distance ET in the pedunculate oak stand and by medium-distance smooth subtype ET in the Euramerican poplar plantation. Also, the difference in the abundance of long-distance ET between the pedunculate oak stand and the Euramerican poplar plantation was found. Similarly, in two white poplar stands in Serbia, the most abundant ET was short-distance ET. In the autochthonous white poplar stand whose soil was with 8% carbon, 0.3% nitrogen and C/N ratio 27.8, dominant short-distance ET was followed by medium-distance ET, while long-distance ET was not found (Katanić et al. 2015a). In the mature white poplar plantation, in soil with carbon content of 7.5%, nitrogen content of 0.13% and C/N content of 58.3, after dominant short distance ET, contanct ET was present to a lesser extent and long-distance ET was rare (Milović et al. 2021b). In the pedunculate oak stand from Morović, in soil with 3.1 % humus, 2.3% C, 0.13% N, and 18% C/N, Milović et al. (2023) noted that contact ET dominated the ECM community, while short-distance ET was less abundant and long-distance ET was rare. Investigating ECM diversity, community composition, and ETs in beech, pine, and spruce forests across Europe was noted that contact, and short-distance ETs had higher mean abundance compared to medium-distance and long-distance ETs (Rosinger et al. 2018).

ECM fungi have two main strategies for growth and nitrogen (N) acquisition. One focuses on the uptake of labile N forms (for example, amino acids, ammonium, and nitrate), and the other focuses on insoluble, complex organic resources of N (Hobbie and Agerer 2010). Mycorrhizas with contact, short- and medium-distance smooth ETs use labile, mainly inorganic nitrogen, while medium-distance fringe and mat, and long-distance ETs have access to insoluble and nonlabile substances due to hydrolytic exoenzymes. The latest ETs are rich in extraradical mycelium and have medium and long-distance hydrophobic rhizomorphs, but their demands for carbon are bigger (Hobbie and Agerer 2010, Lilleskova et al. 2011).

At both sites in Gornje Podunavlje Special Nature Reserve, the majority of ECM root tips belong to short-distance ET, which might indicate that soils contained sufficient labile and easily available N forms. The humus content in the Euramerican poplar plantation soil was lower, suggesting poorer conditions for the development of ECM fungi with long-distance ET, which was probably the cause of lower partition of long-distance ET at this site. On the other hand, higher abundance of long-distance ET recorded in the pedunculate oak stand is likely related to the higher humus content measured at this site. Therefore, ECM fungal community in the pedunculate oak stand consisted of ECM fungi with short-distance ET, which focuses on the use of labile N forms and long-distance ET which can degrade insoluble and non-labile substances from humus.

CONCLUSIONS

This is the first time that diversity of ectomycorrhizal fungi was studied in Gornje Podunavlje Special Nature Reserve in Serbia. In total 28 ECM fungal taxa were found, from which 17 ECM taxa in the pedunculate oak stand and 15 ECM fungal taxa in the Euramerican poplar plantation. The average value of species richness index was higher in the Euramerican poplar plantation, while Shannon-Weaver index, evenness, equitability and Berger-Parker index were higher in the pedunculate oak stand. At both sites a few species dominated the ECM community, while others were rare. The most abundant ECM taxa in the Euramerican poplar plantation were *Cenococcum geophilum* and *Tomentella* sp. 2, and in the pedunculate oak stand *Humaria hemisphaerica* and *Hortiboletus engii.* The most species-rich family in the pedunculate oak stand was Inocybaceae, while in the Euramerican poplar plantation it was Thelephoraceae. At both sites, the majority of ECM root tips belong to short-distance ET, which indicates that soils likely contain sufficient quantity of labile and easily available N forms. Higher abundance of long-distance ET recorded in the pedunculate oak stand is probably related to the higher humus content measured at this site.

Author Contributions

MM, ZG and SO conceived and designed the research, ZG and ZN carried out the field measurements, MM and ZG performed laboratory analysis, MM and BK processed the data and performed the statistical analysis, SO secured the research funding and supervised the research, MM, MD, SP, ZG and BK helped to draft the manuscript, MM, BK and ZG wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Community Structure, Diversity, and Population Dynamics of Black Poplar Leaf Miners and Galling Aphids in the Urban Environment

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ABSTRACT

Trees in the urban environment, such as the black poplar (*Populus nigra* L.), are strongly affected by environmental changes, which can even lead to their decline. Since the development of large cities on the river banks, black poplar is losing its habitat, but is also threatened by factors such as pollution, climate change, and altered habitat characteristics. These changes affect the insects feeding on black poplar, especially the endophagous species, which are in close contact with the host through their entire development, such as the leaf miners (LMs) and galling aphids (GAs). That is why we examined the community structure, diversity, and population dynamics of black poplar LMs and GAs. The study was conducted in Belgrade, Serbia, on five urban sites differing in vegetation characteristics. Sampling was conducted once per month in the period May-September, in 2016 and 2017. Thirteen species of LMs and seven GAs were identified on the studied trees. The seasonal and site differences affected the LM and GA communities, showing their different adaptation strategies for coexistence on the same host, while the effect of the study year was modest. Greater GAs species richness and LMs diversity were recorded on sites with higher participation of canopy and green area cover. The results highlight the importance of urban green structure for sustaining specialized herbivorous insects and suggest that LMs and GAs on black poplar can serve as sensitive indicators of ecological quality in urban environments.

Keywords: abundance; green areas; canopy cover; *Populus nigra* L.; seasonal dynamics; species richness

INTRODUCTION

With increasing urbanization, many tree species are disappearing from the cities or are being suppressed to the city edges (Helen et al. 2019, Korányi and Markó 2022, Łukaszkiewicz et al. 2024, Xie et al. 2024). One such species is the black poplar (*Populus nigra* L.), a key tree species of floodplain and riverbank ecosystems in the basins of major European rivers (Lefèvre et al. 2001, de Rigo et al. 2016, Łukaszkiewicz et al. 2024). Black poplars are ecologically significant as they stabilize and remedy the soil in sensitive habitats, sustain great biodiversity, and improve air quality due to their rapid growth and large leaf area (Gilman and Watson 1994, Lefèvre et al. 2001, Šiler et al. 2014, de Rigo et al. 2016, Levei et al. 2021, Łukaszkiewicz et al. 2024). However, several of its traits are considered a drawback for urban environments: its brittle branches break easily and can pose safety risks, its strong roots can damage infrastructure, and its abundant pollen and fluffy seeds can cause discomfort to people (Gilman and Watson 1994, Costello et al. 1997, Minić et al. 2020, Costache et al. 2021, Shams et al. 2021, Łukaszkiewicz et al. 2024).

Besides the direct effect of urbanization on the decline of black poplar from the urban environment, the trees in the cities can be affected in many ways (Rickman and Connor 2003, Dobrosavljević et al. 2020, 2023, Korányi and Markó 2022, Percival 2023, Franceschi et al. 2023, Gao et al. 2023). Elevated temperatures and air pollution in the urban environment can cause significant stress, which is further increased by altered soil properties and groundwater regimes (Percival 2023, Franceschi et al. 2023, Gao et al. 2023). These, and other environmental disturbances, negatively affect organisms trophically connected to the black poplar trees (Southwood 1961, Schowalter 1995, Dobrosavljević et al. 2023). Among these organisms, insects are the most numerous and significant (Southwood 1961, Lefèvre et al. 2001, Vanden Broeck 2003). The majority of them are herbivorous species, which are highly sensitive to environmental change (Raupp et al. 2010, Valdés-Correcher et al. 2022, Dobrosavljević et al. 2023). They are affected both directly and indirectly, through the host plants (Schowalter 2006, Jones and Leather 2012, Sallé et al. 2021). The most sensitive are the species that cannot migrate far from their hosts, and those that develop in close contact with their hosts, such as the leaf miners and gall-inducing insects (Moir et al. 2014, Dobrosavljević et al. 2020, 2023, Sallé et al. 2021, Valdés-Correcher et al. 2022). They respond to environmental changes by altering their population spatial distribution and seasonal dynamics (Sunil et al. 2023, Boggs 2024, Ojija et al. 2025). LMs and GAs are diverse insect groups with numerous species that are taxonomically well studied in the study area and globally distributed across a wide range of habitats (Hałaj and Osiadacz 2013, Dobrosavljević et al. 2017, Ellis 2020, Dobrosavljević and Marković 2024).

As many European capitals are located near big rivers, the black poplar is becoming increasingly endangered (Lefèvre et al. 2001, de Rigo et al. 2016). Its status is further threatened by the practice of conversion of wetlands to agricultural land, which is now largely prohibited across Europe (Ramsar Convention Secretariat 2016). To determine how the insect community inhabiting black poplar behaves in an urban setting, we investigated the community structure, diversity, and population dynamics of LMs and GAs. The study aimed to determine the effect of (1) interannual variation, (2) seasonal dynamics, and (3) site characteristics on the LMs and GAs abundance, species richness, and diversity in the urban environment.

MATERIALS AND METHODS

Study Design and Sampling

The study was conducted in 2016 and 2017 on five sites in Belgrade: Ada Ciganlija (44°47'09"N, 20°24'41"E), Dorćol (44°49'49"N, 20°27'29"E), New Belgrade block 69 (44°48'11"N, 20°25'14"E), New Belgrade Studenjak (44°49'32"N, 20°24'00"E), and Rakovica (44°46'13"N, 20°26'23"E). On each site, five trees measuring at least 30 cm in diameter and at least 10 m in height were selected. Once per month in the period May-September, five 50-100 cm long lateral branches with leaves were collected from each tree to a height of about 4 m (5 branches · 5 trees · 5 sites = 125 branches per session or 25 branches per site).

All samples were brought to the Entomological laboratory of the Faculty of Forestry, University of Belgrade, where the total number of leaves was counted. Leaves with mines and galls were separated and individually analyzed. The presence of live larvae or pupae within mines and galls was recorded. However, not all mines and galls contained live individuals due to factors such as prior emergence, mortality, or parasitism.

Species identification was primarily based on the characteristics of mines and galls. If immature stages (eggs, larvae, pupae, larval cases, and cocoons) were present in the analyzed material, they were examined and used for morphological identification. To increase the reliability of the identification and to confirm the species' identity, adults were reared from a part of the collected larvae and pupae (Hering 1957, Blackman and Eastop 1994, Hałaj and Osiadacz 2013, Doorenweerd et al. 2014, Haarder and Liston 2018, Laštůvka et al. 2018, Langmaid 2019, Lepiforum 2019, Liston et al. 2019, Ellis 2020, Babichev and Kirichenko 2020).

Site Characteristics

Belgrade, the capital of Serbia, was selected as the study area because it represents a large urban centre situated at the confluence of two major rivers, the Sava and the Danube. This geographic setting, combined with diverse levels of urbanization and green space distribution, makes Belgrade an ideal environment for studying how urban conditions and proximity to riverbanks influence insect communities associated with black poplar. The trees at the sampling sites were all distributed in groups of up to 10 trees. All of the studied sites were located in urban areas, New Belgrade and Dorćol in heavily urbanized areas, while Ada Ciganlija and Rakovica in areas with slightly less intense urbanization. Sites New Belgrade block 69, Dorćol, and Ada Ciganlija were located in the immediate vicinity of the Sava and Danube rivers. At the same time, sites New Belgrade, Studenjak, and Rakovica were more than 500 meters from the large river banks. At the sampling sites, 200 m diameter buffer zones were selected. In each buffer zone, the green spaces (grasslands, parks, dirt trails, etc.), spaces with no greenery (impervious areas, bodies of water, railroad tracks, etc.), as well as the total area covered by tree canopies were measured. The aerial images used for the analysis were obtained from Google Earth Pro 7.3.6.10201, from the images made in 2018 (Google Earth Pro (Desktop), 2025). ImageJ 1.53e (Schneider et al. 2012) was used for all the area measurements and creation of the buffer zone circles.

Characterisation of the Leaf Miner (LM) and Galling Aphid (GA) Community Analyzed

LM and GA communities were quantified by the following parameters at the level of a single tree (Dobrosavljević et al. 2020, 2023):

- The abundance of individual species (A_i) is calculated as the number of mines per 100 leaves:

$$A_i = \frac{N_i}{l_i} \cdot 100 \tag{1}$$

where n_i is the number of mines of one particular species found in one sample, and l_i is the number of leaves per sample.

- The total abundance of (Ab) is calculated as the sum of all individual species' abundance.

Figure 1. Buffer zones (200 m) around the sampled trees in each of the studied sites: **(a)** Ada Ciganlija; **(b)** Dorćol; **(c)** New Belgrade Block 69; **(d)** Rakovica; **(e)** New Belgrade Studenjak.

- Species richness (S) (number of species identified).
- Shannon's index of diversity (H'), calculated as:

$$H' = -\sum_{i=1}^{S}(p_i \cdot \ln(p_i)) \quad (2)$$

where S is the Species richness and p_i is the proportion of individuals belonging to the i-th species in the dataset (Shannon 1948).

Dominant species were defined as the subset of the most abundant species that cumulatively accounted for 90% of the total LMs or GAs abundance in a given year. The 90% threshold was selected to capture the core community while excluding rare species (Magurran 2004).

Statistical Analysis

As the Kolmogorov–Smirnov test indicated that the data distribution does not fit any of the standardized distributions, nonparametric methods were used for the analysis. The differences in the LM and GA abundance, species richness, and diversity between the months in the vegetative season and locations were determined by the Kruskal–Wallis test, while the differences between the studied years were determined by the Mann–Whitney U test. Only dominant species were included in the individual species analysis. When the Kruskal–Wallis test highlighted significance, the Mann-Whitney U test was used as a post hoc test to conduct pairwise analysis. Linear regression was used to determine how the analyzed parameters relate to the green spaces and canopy cover of the investigated sites. Data were analyzed at a significance level of $p < 0.05$. The diversity index was calculated in PAST 5.2.2. (Hammer et al. 2001). All of the statistical analyses were conducted in Statistica 12.0 (StatSoft 2014).

RESULTS

Thirteen species of LMs and seven species of GAs were identified on the leaves of the studied trees (Table 1). The most abundant LM species were *Phyllocnistis unipunctella* (Stephens, 1834), *Ph. extrematrix* Martynova, 1955, and *Stigmella trimaculella* (Haworth, 1828), while the most abundant GAs were *Pemphigus spyrothecae* Passerini, 1860 and *Thecabius lysimachiae* Börner, 1916.

Dominant LM species in 2016 were by their participation in the total abundance *Ph. unipunctella* (27.5%), *Ph. extrematrix* (27.1%), *S. trimaculella* (19.9%), and *A. populi* (13.1%), while in 2017 the community was dominated by *Ph. unipunctella* (40.2%), *L. sinuella* (20.2%), *P. populifoliella* (10.8%), *S. trimaculella* (7.9%), *Ph. extrematrix* (5.4%), and *F. hortulana* (4.1%). Dominant GA species in 2016 were, by their participation in the total, *P. spyrothecae* (50.7%), *T. lysimachiae* (19.9%), *T. affinis* (13.4%), and *P. populinigrae* (5.7%), while in 2017 the community was dominated by T. *lysimachiae* (46.7%), *P. spyrothecae* (21%), *T. affinis* (12.7%), and *P. populinigrae* (7.9%).

LM abundance differed significantly between the investigated years for all the dominant species except *Ph. extrematrix* (Table 1). Only half of the dominant GA species were affected by the interannual variation (*T. affinis* and *T. lysimachiae*) (Table 1). The dominant LM species were affected by the differences between the months in the vegetative season, both in 2016 and 2017 (Figure 2a,b, Table A1), while the GAs species showed a different pattern, as only one species was significantly affected by the seasonality, only in 2016 (Figure 2c,d, Table A1). Site characteristics significantly affected all the dominant LM and GA species in both of the analyzed years (Table 2, Table A1).

The total LM and GA abundance did not differ significantly between the investigated years ($Z = -1.258$, $p = 0.208$; $Z = 0.839$, $p = 0.401$). LM abundance was highest in August, while GA abundance remained relatively consistent throughout the season, with the highest values noted in May (Figure 3a,c). Month of the vegetative season as well as site characteristics affected both LMs ($H = 108.542$, $p < 0.01$; $H = 22.764$, $p < 0.01$) and GAs abundance ($H = 241.001$, $p < 0.01$; $H = 12.140$, $p = 0.016$) (Figure 3, Table A2, Table A3).

LM species richness and diversity did not differ significantly between the two years ($Z = -1.097$, $p = 0.265$; $Z = 0.868$, $p = 0.385$). GA species richness differed significantly between the two years ($Z = 2.002$, $p = 0.045$), while the diversity did not ($Z = 1.551$, $p = 0.060$). Further down, the characteristics of the community that did not vary between years were analyzed using pooled data, whereas those that showed yearly variation were analyzed separately for each year.

The total LM species richness was affected by both the study site and seasonality ($H = 13.923$, $p = 0.008$; $H = 76.930$, $p < 0.001$) (Figure 4, Table A4), while the diversity was affected only by seasonality (H = 20.745, p

Table 1. Abundance of LMs and GAs species in 2016 and 2017 (± sd), and the influence of the interannual variation on the dominant species assessed by the Mann–Whitney U test.

Taxonomic information	2016	2017	Z	P
LEAF MINERS (LMs)				
Diptera				
Agromyzidae				
Agromyza albitarsis Meigen, 1830	0.180 ± 0.702	0.050 ± 0.366		
***Aulagromyza populi* (Kaltenbach, 1864)**	**4.753 ± 10.618**	**1.149 ± 3.244**	**5.242**	**0.000**
A. populicola (Walker, 1853)	0.205 ± 0.638	0.009 ± 0.104		
Hymenoptera				
Tenthredinidae				
***Fenusella hortulana* (Klug, 1818)**	**0.344 ± 1.179**	**1.243 ± 3.045**	**-2.172**	**0.030**
Heterarthrus ochropoda (Klug, 1818)	0.694 ± 1.759	1.276 ± 2.799		
Zeugophora flavicollis (Marsha, 1802)	0.659 ± 2.077	0.688 ± 1.255		
Lepidoptera				
Nepticulidae				
***Stigmella trimaculella* (Haworth, 1828)**	**5.934 ± 7.602**	**2.567 ± 3.293**	**3.471**	**0.001**
Gracillariidae				
Caloptilia stigmatella (Fabricius, 1781)	0.137 ± 0.533	0.162 ± 0.644		
Phyllocnistis extrematrix Martynova, 1955	9.958 ± 20.341	1.275 ± 2.753	1.062	0.288
***Ph. unipunctella* (Stephens, 1834)**	**10.238 ± 19.901**	**17.156 ± 26.696**	**-2.433**	**0.015**
Phyllonorycter pastorella (Zeller, 1846)	0.086 ± 0.438	0.388 ± 1.187		
***P. populifoliella* (Treitschke, 1833)**	**0.867 ± 1.643**	**3.405 ± 6.341**	**-3.307**	**0.001**
Lyonetiidae				
***Leucoptera sinuella* Reutti, 1853**	**0.693 ± 2.096**	**7.211 ± 17.638**	**-3.517**	**0.000**
GALLING APHIDS (GAs)				
Hemiptera				
Aphididae				
Pemphigus bursarius (Linnaeus, 1758)	0.226 ± 0.934	0.415 ± 1.338		
P. populi Courchet, 1881	0.307 ± 1.039	0.658 ± 1.830		
P. populinigrae (Schrank, 1801)	0.331 ± 0.932	0.978 ± 2.420	-1.152	0.249
P. protospirae Lichtenstein, 1885	0.220 ± 1.200	0.367 ± 1.638		
P. spyrothecae Passerini, 1860	3.149 ± 8.051	2.519 ± 7.258	0.527	0.599
***Thecabius affinis* (Kaltenbach, 1843)**	**0.786 ± 2.447**	**0.311 ± 1.994**	**2.260**	**0.020**
***T. lysimachiae* Börner, 1916**	**1.469 ± 3.493**	**1.149 ± 3.425**	**2.184**	**0.030**

NOTE: Statistically significant differences in abundance of LMs and GAs species between 2016 and 2017 are highlighted in bold

= 0.004) (Figure 5a,b, Table A5). GA species richness was affected by the locality both in 2016 and 2017 ($H = 28.381$, $p < 0.001$; $H = 75.649$, $p < 0.001$) (Figure 6b,d, Table A4), while it was not affected by seasonality in neither in 2016, nor in 2017 ($H = 1.783$, $p = 0.776$; $H = 5.467$, $p = 0.243$) (Figure 6a,c, Table A4). The total GA diversity was only affected by the study site ($H = 86.775$, $p < 0.001$) (Figure 5c,d, Table A5).

Green areas in the 200 m radius buffer zone comprised, on average, 53.4 ± 14.26% of the total area, while the tree canopies covered, on average, 29.4 ± 13.31% of the total area. The site with the greatest green area participastion was Rakovica (79%), while Dorćol had the lowest (35%), mainly because the water area comprised a large part of the buffer zone (31%). Tree canopy cover was greatest at NBG Studenjak (45%), and lowest at NBG block 69 (9%) (Figure 1, Table 3).

Although the number of investigated sites was not great enough for a detailed insight into the effect of their characteristics on the studied insect communities, we determined that greater green area and canopy cover positively affected GA abundance and species richness, LM diversity, while the green area on the studied sites affected GA species richness and LM diversity (Table 4).

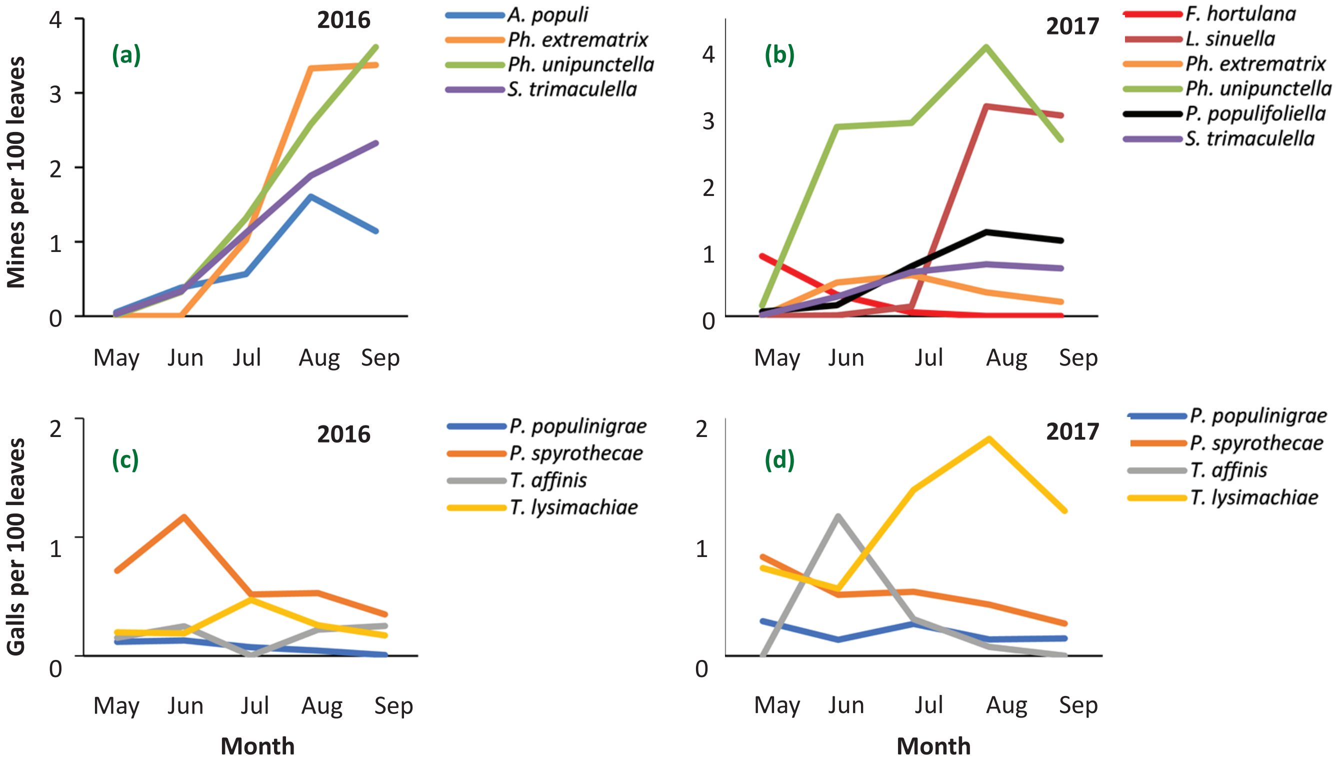

Figure 2. Seasonal variation of the dominant **(a, b)** leaf miner (LM) and **(c, d)** galling aphid (GA) species' abundance.

Table 2. Site and interannual variation of the dominant leaf miner (LM) and galling aphid (GA) species' abundance.

Year	Species	Site				
		Ada	Dorćl.	Bl. 69	Stud.	Rak.
2016	*A. populi*	0.050	0.384	0.564	1.606	1.141
	Ph. extrematrix	n/a	n/a	1.022	3.328	3.375
	Ph. unipunctella	0.012	0.321	1.312	2.578	3.614
	S. trimaculella	0.027	0.331	1.120	1.886	2.321
	P. populinigrae	0.117	0.129	0.071	0.042	0.007
	P. spyrothecae	0.716	1.169	0.517	0.528	0.348
	T. affinis	0.152	0.248	n/a	0.219	0.250
	T. lysimachiae	0.198	0.187	0.472	0.257	0.172
2017	*F. hortulana*	0.905	0.324	0.057	n/a	n/a
	L. sinuella	n/a	0.009	0.145	3.170	3.033
	Ph. extrematrix	n/a	0.507	0.620	0.357	0.215
	Ph. unipunctella	0.157	2.859	2.921	4.065	2.664
	P. populifoliella	0.067	0.163	0.752	1.269	1.142
	S. trimaculella	0.017	0.290	0.666	0.785	0.723
	P. populinigrae	0.292	0.135	0.268	0.138	0.145
	P. spyrothecae	0.832	0.514	0.538	0.431	0.271
	T. affinis	0.000	1.175	0.310	0.074	n/a
	T. lysimachiae	0.740	0.564	1.398	1.828	1.219

NOTE: Abbreviation n/a stands for not applicable; abbreviations that refer to the studied sites: Ada – Ada Ciganlija, Dorć. – Dorćol, Bl. 69 – New Belgrade Block 69, Stud. – New Belgrade Studenjak, Rak. – Rakovica

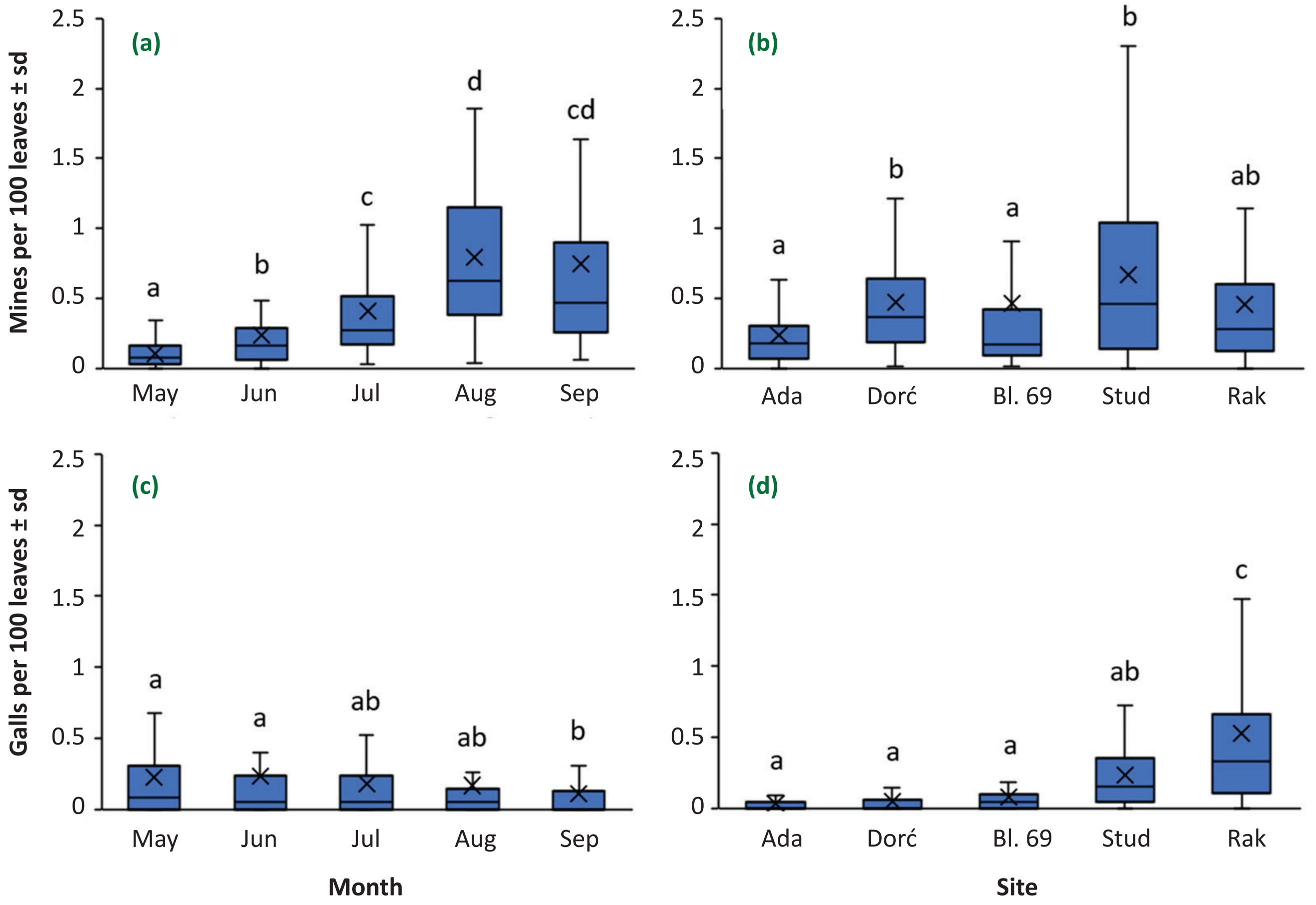

Figure 3. Seasonal variation of **(a)** leaf miner (LM) and **(c)** galling aphid (GA) abundance, and site variation of **(b)** LM and **(d)** GA abundance (x – mean, line – median, variation – ± standard deviation). Abbreviations that refer to the studied sites: Ada – Ada Ciganlija, Dorć. – Dorćol, Bl. 69 – New Belgrade Block 69, Stud. – New Belgrade Studenjak, Rak. – Rakovica. Different lowercase letters next to data points indicate a statistically significant difference ($p < 0.05$) in abundance between different months and sites for mines and galls.

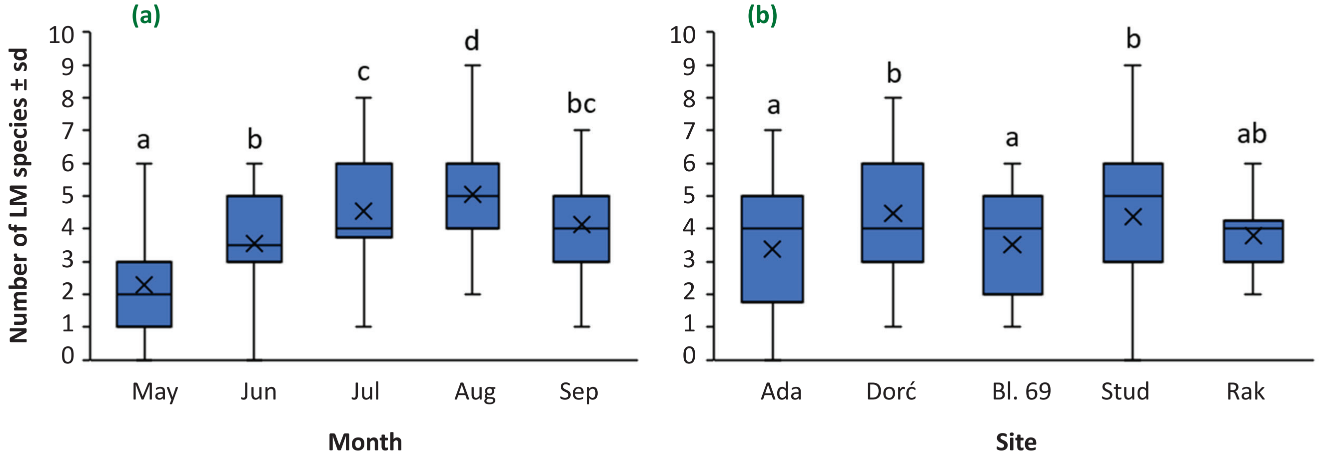

Figure 4. (a) Seasonal and **(b)** site variation of leaf miner (LM) species richness (x – mean, line – median, variation – ± standard deviation). Abbreviations that refer to the studied sites: Ada – Ada Ciganlija, Dorć. – Dorćol, Bl. 69 – New Belgrade Block 69, Stud. – New Belgrade Studenjak, Rak. – Rakovica. Different lowercase letters next to data points indicate a statistically significant difference ($p < 0.05$) in number of LM species between different months and sites.

Table 3. Analyzed site characteristics and characteristics of the leaf miner (LM) and galling aphid (GA) community.

Analyzed characteristic	Study site				
	Ada Ciganlija	Dorćol	NBG Block 69	NBG Studenjak	Rakovica
Green area (%)	53	35	50	50	79
Tree canopy cover (%)	36	19	9	45	38
LM abundance	0.237	0.471	0.468	0.673	0.457
GA abundance	0.031	0.044	0.079	0.235	0.525
LM species richness	3.380	4.480	3.520	4.380	3.780
GA species richness	0.580	0.480	0.920	1.340	3.200
LM diversity	1.346	1.512	1.432	1.519	1.445
GA diversity	0.186	0.146	0.257	0.318	1.280

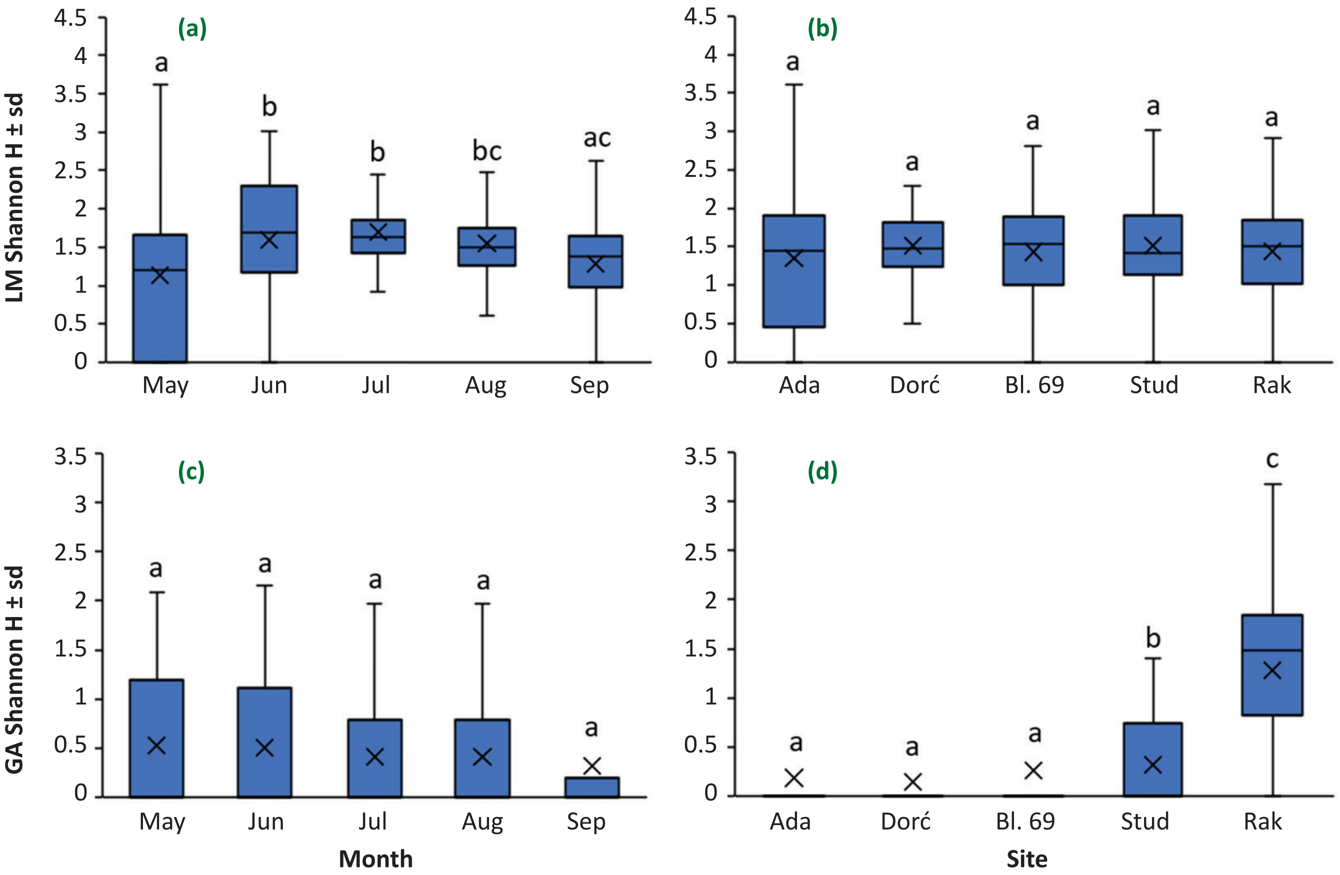

Figure 5. Seasonal variation of **(a)** (leaf miner) LM and **(c)** (galling aphid) GA diversity, and site variation of **(b)** LM and **(d)** GA diversity (x – mean, line – median, variation – ± standard deviation). Abbreviations that refer to the studied sites: Ada – Ada Ciganlija, Dorć. – Dorćol, Bl. 69 – New Belgrade Block 69, Stud. – New Belgrade Studenjak, Rak. – Rakovica. Different lowercase letters next to data points indicate a statistically significant difference ($p < 0.05$) in Shannon between different months and sites for leaf miner and gallid aphids.

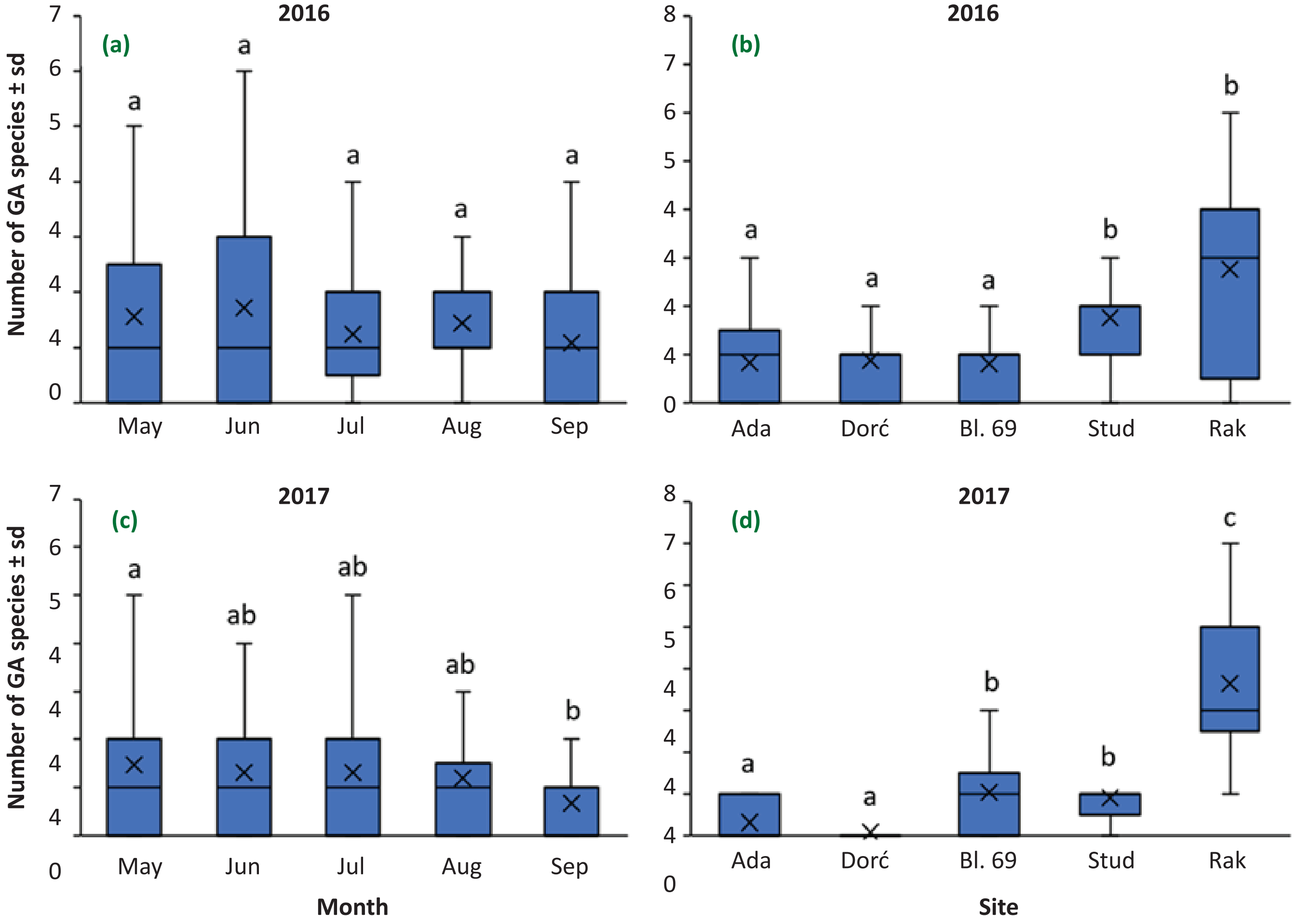

Figure 6. Seasonal **(a, c)**, site **(b, d)**, and interannual variation of galling aphid (GA) species richness (x – mean, line – median, variation – ± standard deviation). Abbreviations that refer to the studied sites: Ada – Ada Ciganlija, Dorć. – Dorćol, Bl. 69 – New Belgrade Block 69, Stud. – New Belgrade Studenjak, Rak. – Rakovica. Different lowercase letters next to data points indicate a statistically significant difference ($p < 0.05$) in the number of GA between different months and sites.

Table 4. Regression results of the influence of site characteristics on the leaf miners (LM) and gallid aphids (GA) community characteristics.

Site characteristics	Insect community characteristics	Regression results			
		R	R^2	F	p
Green area (%)	LM abundance	0.025	0.001	0.153	0.696
	GA abundance	**0.457**	**0.208**	**65.329**	**0.000**
	LM species richness	0.060	0.004	0.888	0.347
	GA species richness	**0.141**	**0.020**	**5.004**	**0.026**
	LM diversity	**0.158**	**0.025**	**6.331**	**0.012**
	GA diversity	0.081	0.007	1.637	0.202
Canopy cover (%)	LM abundance	0.043	0.002	0.461	0.498
	GA abundance	**0.268**	**0.072**	**19.148**	**0.000**
	LM species richness	0.001	0.000	0.000	0.983
	GA species richness	0.038	0.001	0.354	0.552
	LM diversity	**0.179**	**0.032**	**8.178**	**0.005**
	GA diversity	0.021	0.000	0.113	0.737

NOTE: Statistically significant differences are highlighted in bold

DISCUSSION

The results of this study highlight that different group of black poplar endophagous insects - leaf miners (LMs) and galling aphids (GAs) - respond differently to changes in environmental and temporal factors in the urban environment. Among the analyzed drivers, interannual variation had the lowest influence on the overall community patterns, whereas microclimatic and habitat-related factors played a more prominent role, consistent with previous studies on urban insect assemblages (Korányi and Markó 2022, Dobrosavljević et al. 2023).

Interannual differences significantly affected GA species richness, but their impact on community-level abundance remained limited. However, the differences between the studied years had a significant effect on the abundance of most of the dominant LM species, such as *S. trimaculella* and *Ph. unipunctella*. These species were abundant in both years, but the environmental differences between the two studied years affected them in opposite ways. While the abundance of *S. trimaculella* declined, *Ph. unipunctella* increased its abundance significantly, which can be a consequence of utilizing the increased available resources, such as leaf availability and improved leaf quality, according to Balaji et al. 2024. This highlights the importance of individual species biology for adaptation to even subtle differences in environmental conditions, and stressors such as temperature or humidity, as well as diversity of surrounding plants, and other factors (Connor and Taverner 1997, Rickman and Connor 2003, Fernandes et al. 2004, Rodrıguez-Castañeda et al. 2017, Barantal et al. 2019, Dobrosavljević et al. 2023, Chen et al. 2023). These differences show that even though the individual species were affected by the interannual variation, the overall community abundance remained stable, suggesting a compensatory response among dominant species that maintained overall community stability. On the other side, the interannual effect was not that significant for the GA community, as only half of the dominant species were affected. P. spyrothecae, the most abundant GA species in this study, likely maintained its population levels due to biological traits like the protective features of the gall, as noted in previous studies (Blackman and Eastop 1994, Alton 1999, Hałaj and Osiadacz 2013, Kot and Kmieć 2020). The effect of interannual variation on the LM and GA communities is likely to become more pronounced under climate change, as growing variability in temperature, humidity, and precipitation can directly influence insect development, survival, and phenology, as well as indirectly alter host plant quality and defenses (Bale et al., 2002, Parmesan, 2006, Pincebourde et al. 2012, Robinet and Roques, 2010). Additionally, the increasing frequency of extreme climatic events may further amplify year-to-year fluctuations in insect populations, particularly in urban environments where microclimatic conditions are already highly heterogeneous (Oke 1982, Meineke et al. 2013, Korányi and Markó 2022). The importance of the interannual variation, due to climate change, as many environmental factors, such as the temperature, humidity, and precipitation patterns, will change. That is why long-term monitoring of the insect-plant interactions, especially in the urban environment, is necessary.

The LM and GA communities reacted differently to seasonal variation. The LMs' abundance, species richness, and diversity peaked in the middle of the studied period (July and August), similar to previous studies conducted in both rural and urban environments (Southwood et al. 2004, Dobrosavljević et al. 2020, Marković et al. 2021b). This trend also occurs in other insect species such as coccinellids and LM communities on other host plants (Southwood et al. 2004, Marković et al. 2018). This is a consequence of the better leaf quality as food for the LMs and interguild competition in the earlier part of the year (Connor and Taverner 1997, Southwood et al. 2004). Another reason why the LM community peaked in the middle of the studied period is that most of the dominant LM species are multivoltine, and their second generation, which feeds during July and August, is more abundant than the first generation (Hering 1951, Ellis 2020). The only dominant species that did not peak during this period was *Fenusella hortulana*, as it develops only one generation in early spring (Hering 1951, Ellis 2020, Dobrosavljević et al. 2023). In contrast, the GA community characteristics peaked much earlier in the vegetative season, in the period May-June. One of the reasons for this lies in the behaviour of the most abundant dominant species, *P. spyrothecae*, which tends to form galls on the petioles of young, developing leaves (Blackman and Eastop 1994, Alton 1999, Hałaj and Osiadacz 2013, Kot and Kmieć 2020). Their contrasting seasonal dynamics suggest temporal partitioning between the two endophagous herbivore guilds, which may facilitate their coexistence on the same host trees by reducing potential overlap in resource use. Besides the vegetative season, the studied guilds, especially the GA community, which was less affected by the seasonal variation, were affected by habitat characteristics. We determined that the analyzed habitat characteristics (percentage of green area and canopy cover on the 200 m buffer zones) had a significant effect on some of the investigated insect community characteristics, although not with a strong correlation coefficient (GA abundance and species richness, and LM diversity), similar to other studies (Knuff et al. 2020, Fenoglio et al. 2023, Matilda Collins et al. 2024, Abrego and Medianero 2025, Sinclair et al. 2025). This highlights the importance of urban greenery, especially of the urban trees, for diversity conservation. Even though the investigated factors influenced the LM and GA communities, there are also many other factors such as landscape, microclimate, host trees characteristics, predator and parasitoid community characteristics, etc. which can also affect insect communities in the urban environment (Rickman and Connor 2003, Baraniak et al. 2004, Fenoglio et al. 2009, Bairstow et al. 2010, Peralta et al. 2011, Jones and Leather 2012, Cebeci et al. 2018, Milanović et al. 2020, Dobrosavljević et al. 2020, 2023, 2025, Marković et al. 2021a, Valdés-Correcher et al. 2022, Matilda Collins et al. 2024, Horák et al. 2024). That is why more detailed studies, which can give insights into the influence of other individual environmental factors on the herbivorous insect behaviour in urban environments, need to be conducted. All this suggests that habitat characteristics and seasonal variation have a stronger effect on the investigated insect guilds than interannual variation, similar to studies on plant-insect interactions in similar climate conditions (Southwood et al. 2004, Korányi and Markó 2022).

Although the LMs and GAs behave similarly (both feed endophagously on the same host plant), they are differently affected by the environmental differences. This is even more pronounced on the level of individual species, where, i.e. the abundance of the miner *P. unipunctella* varies significantly between years and sites, reflecting its sensitivity to microhabitat conditions, while the dominant aphid species *P. spyrothecae* shows a more stable dynamics due to the differences in their biology (Hering 1951, Alton 1999, Ellis 2020, Kot and Kmieć 2020). Although LMs and GAs comprise a significant component of black poplar insect diversity, the influence of environmental changes caused by urbanization and climate change on other insects needs to be addressed, as black poplar is a host for more than 100 insect species (Southwood 1961). Conversely, insects can cause significant damage to trees, especially when they are young and sensitive (Marković et al. 2024), which is why the significance of these insect species as pests should be reviewed. Even after identifying significant pests, their management in the urban environment is challenging, as chemical insecticides, which could be used in their natural habitat, can pose risks for the already fragile environment, and even for people in the cities (Md Meftaul et al. 2020). This highlights the importance of constant monitoring of insect populations in the cities, and their integrated pest management (IPM) approaches (Dreistadt and Flint 1995, Dhang 2014, Tabassum et al. 2024, Milanović et al. 2025). Integrating such strategies into urban forestry planning can help balance tree protection, biodiversity conservation, and ecosystem health, thereby supporting the black poplar and its insect communities.

CONCLUSIONS

This study showed that leaf miners (LMs) and galling aphids (GAs) on black poplar in urban habitats react differently to temporal and environmental changes. The interannual variation had a modest influence, affecting only GA species richness and a few dominant LM species. Seasonal changes and habitat characteristics influenced their abundance and diversity much more significantly. While GAs abundance remained relatively consistent throughout the season, with the highest values noted in May, LMs' notably peaked mid-summer, reflecting the differences in their life cycles and survival strategies. Such differences may facilitate their coexistence on the same host trees by reducing overlap in resource use, rather than implying direct competitive interactions. Habitat characteristics, particularly the proportion of green areas and canopy cover, were important drivers of insect community structure, highlighting the role of urban greenery in supporting biodiversity. At the species level, responses were highly variable, emphasizing the importance of species-specific traits in shaping population dynamics under urban conditions. Given the ecological importance and ongoing decline of black poplar in cities, these findings underline the need for its conservation and for the implementation of sustainable, ecologically based pest management strategies. Overall, the results contribute to a better understanding of plant–insect interactions in urban ecosystems and provide a basis for integrating biodiversity conservation with urban forestry planning.

Author Contributions

JD, ČM and MM conceived and designed the research, JD, ČM and MM carried out the field measurements, JD performed laboratory analysis, JD processed the data and performed the statistical analysis, JD and ČM secured the research funding, supervised the research and helped to draft the manuscript, JD and ČM wrote the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Appendix A

Table A1. Influence of the seasonality and study site on dominant leaf miner (LM) and galling aphid (GA) species' abundance assessed by the Kruskal-Wallis test.

Factor		Seasonality				Site			
Year		2016		2017		2016		2017	
Species		H	p	H	p	H	p	H	p
LM	*F. hortulana*	n/a	n/a	**58.203**	**0.000**	n/a	n/a	**11.076**	**0.026**
	L. sinuella	n/a	n/a	**72.809**	**0.000**	n/a	n/a	**12.752**	**0.013**
	A. populi	**32.465**	**0.000**	n/a	n/a	**14.438**	**0.006**	n/a	n/a
	Ph. extrematrix	**59.999**	**0.000**	**33.877**	**0.000**	**16.557**	**0.002**	**11.619**	**0.020**
	Ph. unipunctella	**48.582**	**0.000**	**24.765**	**0.000**	**16.790**	**0.002**	**51.956**	**0.000**
	P. populifoliella	n/a	n/a	**24.163**	**0.000**	n/a	n/a	**45.979**	**0.000**
	S. trimaculella	**78.868**	**0.000**	**43.109**	**0.000**	**12.309**	**0.015**	**12.273**	**0.015**
GA	*P. populinigrae*	6.146	0.189	2.873	0.579	**16.723**	**0.002**	**66.288**	**0.000**
	P. spyrothecae	3.673	0.452	6.934	0.139	**49.833**	**0.000**	**45.673**	**0.000**
	T. affinis	**12.513**	**0.014**	8.422	0.077	**17.084**	**0.002**	**14.766**	**0.005**
	T. lysimachiae	7.289	0.121	8.536	0.074	**25.006**	**0.000**	**35.573**	**0.000**

Table A2. Influence of the study site on LM (leaf miner) and GA (galling aphid) abundance assessed by Mann–Whitney U test.

M-W U TEST		LM		GA	
f1	f2	Z	p	Z	p
Ada Ciganlija	NBG Block 69	-0.842	0.400	-1.230	0.219
Ada Ciganlija	Dorćol	**-3.243**	**0.001**	0.277	0.782
Ada Ciganlija	Rakovica	-1.263	0.207	**-8.965**	**0.000**
Ada Ciganlija	NBG Studenjak	**-2.956**	**0.003**	**-2.662**	**0.008**
NBG Block 69	Dorćol	**-2.268**	**0.023**	1.459	0.145
NBG Block 69	Rakovica	-0.424	0.672	**-7.661**	**0.000**
NBG Block 69	NBG Studenjak	**-2.026**	**0.043**	-1.469	0.142
Dorćol	Rakovica	1.907	0.056	**-9.019**	**0.000**
Dorćol	NBG Studenjak	0.226	0.821	**-2.872**	**0.004**
Rakovica	NBG Studenjak	-1.661	0.097	**6.004**	**0.000**

Table A3. Influence of the seasonality on LM (leaf miner) and GA (galling aphid) abundance assessed by Mann–Whitney U test.

M-W U TEST		LM		GA	
f1	f2	Z	p	Z	p
May	June	**-3.145**	**0.002**	0.087	0.930
May	July	**-5.995**	**0.000**	0.781	0.435
May	August	**-7.553**	**0.000**	0.761	0.447
May	September	**-5.369**	**0.000**	**2.081**	**0.037**
June	July	**-3.064**	**0.002**	0.677	0.498
June	August	**-4.809**	**0.000**	0.664	0.507
June	September	**-2.671**	**0.008**	**2.020**	**0.043**
July	August	**-1.966**	**0.049**	-0.006	0.995
July	September	0.010	0.992	1.292	0.196
August	September	1.868	0.062	1.343	0.179

Table A4. The influence of study site and seasonality on LM (leaf miner) and GA (galling aphid) species richness, assessed by Mann–Whitney U test.

M-W U TEST		LM		GA			
				2016		2017	
f1	f2	Z	p	Z	p	Z	p
Ada Ciganlija	NBG Block 69	-0.345	0.730	0.058	0.954	**-2.639**	**0.004**
Ada Ciganlija	Dorćol	**-2.609**	**0.009**	-0.233	0.816	1.446	0.148
Ada Ciganlija	Rakovica	-0.838	0.402	**-3.337**	**0.001**	**-5.821**	**0.000**
Ada Ciganlija	NBG Studenjak	**-2.468**	**0.014**	**-3.085**	**0.002**	**-2.969**	**0.003**
NBG Block 69	Dorćol	**-2.554**	**0.011**	-0.330	0.742	**3.745**	**0.000**
NBG Block 69	Rakovica	-0.641	0.521	**-3.347**	**0.001**	**-4.928**	**0.000**
NBG Block 69	NBG Studenjak	**-2.389**	**0.017**	**-3.347**	**0.001**	0.029	0.977
Dorćol	Rakovica	1.923	0.054	**-3.240**	**0.001**	**-5.995**	**0.000**
Dorćol	NBG Studenjak	0.283	0.777	**-3.056**	**0.002**	**-4.191**	**0.000**
Rakovica	NBG Studenjak	-1.830	0.067	1.940	0.052	**5.268**	**0.000**
May	June	**-4.226**	**0.000**	-0.223	0.823	0.175	0.861
May	July	**-6.549**	**0.000**	0.223	0.823	0.689	0.491
May	August	**-7.228**	**0.000**	-0.136	0.892	0.407	0.684
May	September	**-5.388**	**0.000**	0.844	0.399	**1.979**	**0.048**
June	July	**-3.054**	**0.002**	0.475	0.635	0.475	0.635
June	August	**-4.505**	**0.000**	0.087	0.930	0.223	0.823
June	September	-1.834	0.067	1.048	0.295	1.717	0.086
July	August	**-1.703**	**0.089**	-0.398	0.691	-0.310	0.756
July	September	1.086	0.278	0.699	0.485	1.154	0.248
August	September	**2.637**	**0.008**	1.096	0.273	1.610	0.107

Table A5. The influence of study site and seasonality on LM (leaf miner) and GA (galling aphid) diversity, assessed by Mann–Whitney U test.

M-W U TEST		LM		GA	
f1	f2	Z	p	Z	p
Ada Ciganlija	NBG Block 69	-0.548	0.584	-0.562	0.574
Ada Ciganlija	Dorćol	-0.658	0.510	0.190	0.850
Ada Ciganlija	Rakovica	-0.438	0.662	**-5.925**	**0.000**
Ada Ciganlija	NBG Studenjak	-0.565	0.572	-1.741	0.082
NBG Block 69	Dorćol	-0.093	0.926	0.782	0.434
NBG Block 69	Rakovica	0.176	0.860	**-5.808**	**0.000**
NBG Block 69	NBG Studenjak	-0.014	0.989	-0.989	0.323
Dorćol	Rakovica	0.252	0.801	**-6.173**	**0.000**
Dorćol	NBG Studenjak	0.196	0.844	**-1.989**	**0.047**
Rakovica	NBG Studenjak	-0.179	0.858	**5.622**	**0.000**
May	June	**-2.699**	**0.007**	0.014	0.989
May	July	**-3.595**	**0.000**	0.455	0.649
May	August	**-2.809**	**0.005**	0.489	0.625
May	September	-1.110	0.267	1.127	0.260
June	July	-0.172	0.863	0.555	0.579
June	August	0.924	0.356	0.669	0.504
June	September	**2.272**	**0.023**	1.293	0.196
July	August	1.565	0.118	0.107	0.915
July	September	**3.261**	**0.001**	0.700	0.484
August	September	1.958	0.050	0.527	0.598

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Pathogenic Micromycete *Neocatenulostroma germanicum* in Pine Plantations of the Republic of Armenia

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ABSTRACT

From the needles of the Scots pine (*Pinus sylvestris* L.) collected in 2022 and 2023 in Northeastern and Central regions of the Republic of Armenia the causative agent of pine needles browning *Neocatenulostroma germanicum* (Crous & U. Braun) Quaedvlieg & Crous was identified. Both morphological and molecular approaches along with phylogenetic analysis were applied. This is the first report of *N. germanicum* identified in the territory of Armenia.

Keywords: ascomycetous fungus; pine needles browning; *Pinus sylvestris*; morphological examination; internally transcribed spacer (ITS) region; phylogenetic analysis

INTRODUCTION

Armenia has relatively limited forest resources. The total forest area in the country is approximately 11% of the total land area (Mkrtchyan and Grigoryan 2014, Bondarev 2020). Among these, pine plantations constitute the smallest share among the main forest-forming species, accounting for 5.3% of Armenia's total forest area (Alaverdyan 2002).

Despite the relatively small area of pine forests in Armenia, they play an important economic and nature conservation role. Pine planting (*Pinus* spp.) in Armenia started in the mid-20th century primarily to replenish the forest fund in areas initially treeless or bare as a result of logging, in areas where pine was the most acceptable species for the existing soil and climatic conditions and where reforestation using other species was considered inappropriate. Currently, pine forests play an anti-erosion role on mountain slopes. They also have a certain protective value for agricultural lands, and contribute to the creation of favorable microclimatic conditions, particularly in areas of traditional recreation.

Drying of pine plantations, mainly artificially planted *Pinus sylvestris*, in Armenia was first observed in 2018 in the northern provinces. In the following years, this phenomenon spread widely to the central and southern regions of the country as well. To elucidate the underlying causes of this phenomenon, comprehensive surveys were carried out, including studies of insect pests (Karagyan et al. 2024) and nematodes (Arbuzova et al. 2025). Preliminary data on the fungal community were also obtained (Shchukovskaya et al. 2024).

In the frame of the reconnaissance surveys of fungal communities' symptoms of lesion (browning and dying of the ends of needles), symptoms similar in appearance to those caused by *Neocatenulostroma germanicum* were found on needles of *Pinus sylvestris* L.

The genus *Neocatenulostroma* includes both plant-pathogenic species and those occurring on various substrates (Quaedvlieg et al. 2014). Within the genus, a distinct complex has formed, consisting of three morphologically similar and phylogenetically closely related species: *N. abietis* (Butin & Pehl) Quaedvlieg & Crous, *N.*

germanicum (Crous & U. Braun) Quaedvlieg & Crous, and *N. microsporum* (Joanne E. Taylor & Crous) Quaedvlieg & Crous (Quaedvlieg et al. 2014, Markovskaja et al. 2016).

Recently, the identification of pathogenic micromycetes has increasingly relied on a combination of morphological and molecular diagnostic methods, which were also used in our study. This is the first record of this pathogenic species in the mycoflora of the country.

MATERIALS AND METHODS

Sample Collection

During 2022 and 2023, the surveys of drying pine plantations were carried out in some provinces of Armenia (Kotayk, Gegharkunik, Tavush, Lori and Aragatsotn) (Figure 1, Table 1). Samples were collected from 5 to 10 trees in each location, with the number of pine needles analyzed from each tree being approximately 15. Needles showing obvious signs of damage (such as discoloration, formation of spore-bearing structures, necrosis, and wilting) were collected. Wood fragments were collected for further mycological analyses as well.

Fungi Isolation, Microscopy and Morphometry

Needle fragments showing signs of necrosis were sterilized in 70% ethanol for 10 seconds, then rinsed with sterile water 3–5 times, dried, placed in a moist chamber, and incubated for 1–2 weeks at 20–22°C (Stamets and Chilton 1983). The culture of the fungus was obtained by subculturing well-developed sporodochia that had formed on infected needles onto a nutrient medium potato dextrose agar (PDA). PDA medium was prepared from dry powder using the commercial “HiMedia Laboratories Pvt. Limited” (India) according to the manufacturer’s instruction. Fragments of mycelium for subculturing were taken from the periphery of actively growing colonies to

Table 1. Sample collection data in Armenia.

Locality	Dates of collection	GPS	Habitats
env. Tsilkar	28.06.2022 07.07.2023	N40.73039 E44.19521 El. 2168 m	artificial pine forest
env. Gyulagarak, “Sochut” dendropark	28.06.2022 06.07.2023	N40.93709 E44.48176 El. 1481 m	mix of artificially grown trees
env. Dilijan	29.06.2022 04.07.2023	N40.75897 E44.89333 El. 1180 m	artificial pine forest
env. Semyonovka	29.06.2022 04.07.2023	N40.67862 E44.88021 El. 1898 m	artificial pine forest mixed with deciduous trees
env. Hankavan	30.06.2022 08.07.2023	N40.63037 E44.47231 El. 2016 m	artificial pine forest
env. Tsaghkadzor	30.06.2022 08.07.2023	N40.53125 E44.73662 El. 1796 m	artificial pine forest
env. Tsovagyugh	01.07.2022 04.07.2023	N40.61208 E44.95750 El. 1929 m	artificial pine forest on the former bottom of the lake Sevan
env. Tsapatagh	01.07.2022	N40.40041 E45.47052 El. 1905 m	artificial pine forest on the former bottom of the lake Sevan
env. Jil	01.07.2022	N40.47126 E45.40908 El. 1914 m	artificial pine forest on the former bottom of the lake Sevan
env. Yeranos	03.07.2023	N40.25537 E45.21249 El. 1916 m	artificial pine forest on the former bottom of the lake Sevan
env. Nerkin Getashen	03.07.2023	N40.16468 E45.26379 El. 1918 m	artificial pine forest on the former bottom of the lake Sevan
env. Tsovak	03.07.2023	N40.20168 E45.64730 El. 1923 m	artificial pine forest on the former bottom of the lake Sevan
env. Koghb, “Zikatar” Environmental Center	05.07.2023	N41.12210 E44.92322 El. 1309 m	beech forest with artificially grown plots of pine trees
env. Jrvezh, Forest Park	10.07.2023	N40.17968 E44.61874 El. 1559 m	artificial mixed forest with plots of pine trees

avoid contamination by bacteria and other fungi. Isolation was also carried out on a growth medium (PDA), incubated for 3 to 6 weeks at the temperature of 20–22°C. For molecular analysis, a pure fungal culture was obtained through multiple subcultures on PDA.

The formed mycelium was examined under a light microscope Olympus BX53 Evident equipped with Olympus DP74 digital camera for revealing morphological structures. The sporodochia formed on the surface of the needles were photographed using a Zeiss SteReo Discovery V12 stereomicroscope equipped with a Canon EOS 5D MkIII camera. Initial species identification was carried out by morphological features using reference literature (Crous et al. 2007, Quaedvlieg et al. 2014, Markovskaja et al. 2016).

DNA Extraction, Polymerase Chain Reaction (PCR) Amplification and Sequencing

Genomic DNA was isolated from sporulating cultures using the commercial "PhytoSorb" kit (Syntol, Russia), based on purification with magnetic particles, in accordance with the manufacturer's instructions. In the two studied isolates, the amplification of the nuclear genome region internal transcribed spacer (ITS) was performed using the following pair of universal primers (White et al. 1990):

- ITS-5- 5'- GGAAGTAAAAGTCGTAACAAG G -3',
- ITS-4 - 5'- TCCTCCGCTTATTGATATGC -3'.

The PCR mixture (25 µl) contained 5 µl 5× PCR buffer Mas^{OR} TaqMix-2025 ("Dialat Ltd", Russia), 0.5 µl of each primer, 2 µl of the respective genomic DNA extract and 17 µl sterile water. PCR conditions were as follows: initial denaturation for 3 min at 95°C, followed by 40 cycles of 30 s at 95°C, 30 s at 52°C and 30 s at 72°C, with final extension for 7 min at 72°C.

Visualization of PCR products was performed by gel electrophoresis in 1% agarose gel stained with ethidium bromide. PCR products intended for Sanger sequencing were purified using the GeneJET PCR Purification Kit (Thermo Fisher, USA) according to the manufacturer's instructions.

The sequencing reaction was performed using BigDye Terminator v3.1 Cycle Sequencing Kit reagents (Applied Biosystems, USA) according to the manufacturer's instructions, followed by separation of fragments on a 3500 genetic analyzer (Applied Biosystems, USA).

Two original sequences of *N. germanicum* specimens from Hankavan village (accession number PX219661) and Dilijan town (PX219657) obtained for the 572 bp fragment, and one sequence of *Diplodia sapinea* from Tsilkar village (PX219648) obtained for the 600 bp fragment of the ITS region were submitted to GenBank.

Samples and Sequence Alignment, Phylogenetic Tree Construction

Nucleotide sequences obtained in this study were edited and aligned using the Clustal W algorithm in the BioEdit software (Hall 1999). Comparative analysis of the obtained nucleotide sequences with the sequences deposited in GenBank was carried out using the BLAST program on the NCBI website (https://www.ncbi.nlm.nih.gov). Phylogenetic analysis was performed in MEGA 11.0 program (MEGA software development team, USA) using Maximum Likelihood (ML) method and Kimura 2-parameter model. The standard bootstrap (1000 replicates) was used to evaluate the statistical nodal support of the tree (Kimura 1980, Kumar et al. 2016).

For phylogenetic analysis the ITS region sequences of 27 samples, both original and those obtained from GenBank were used, namely: 12 samples of *Neocatenulostroma germanicum*, 3 samples of *N. microsporum,* 4 samples of *N. abietis*, 1 sample of *N. castaneae* Phukhams., Bhunjun & K.D. Hyde, 6 species belonging to the other genera of order Mycosphaerellales and *Diplodia sapinea* (Fr.) Fuckel (Botryosphaeriales, as out group) (Table 2).

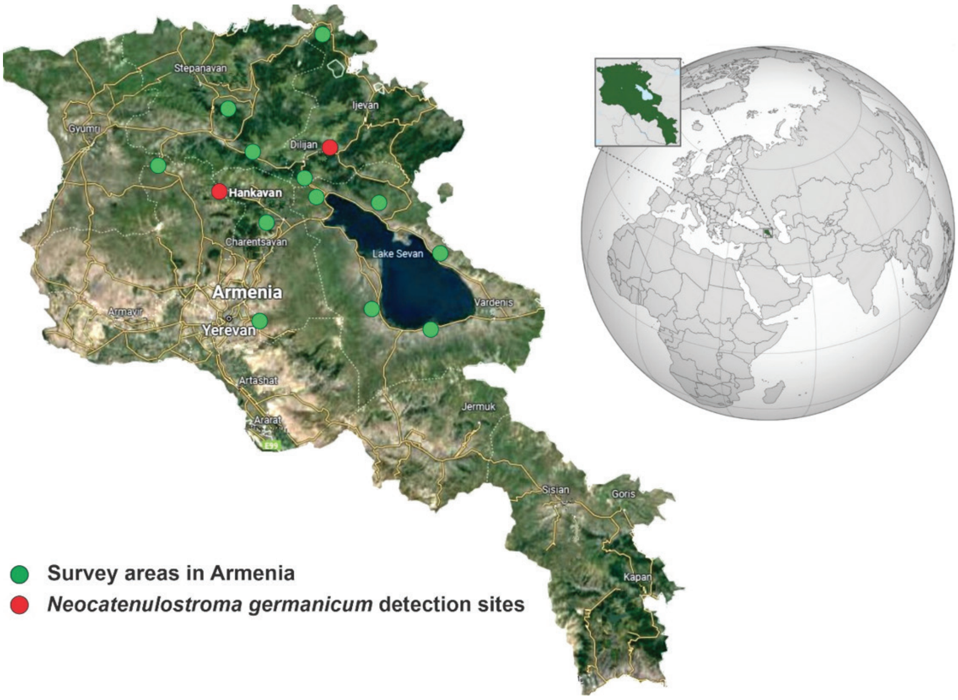

Figure 1. Map with sampling sites and localities of the record of *Neocatenulostroma germanicum* in Armenia.

Table 2. Species of Mycosphaerellales and *Diplodia sapinea* (Botryosphaeriales) taken as out group used for phylogenetic analysis (ITS region).

Species	Country	Isolation source	GenBank Accession No	Reference
Neocatenulostroma germanicum	Armenia	*Pinus sylvestris*	PX219661	This study
			PX219657	
N. germanicum	Lithuania	*P. mugo*	KR995100	Markovskaja et al. 2016
			KR995103	
		P. sylvestris	KR995101	
			KR995102	
N. germanicum	Ukraine	*P. nigra* subsp. *pallasiana*	KR995104	
			KR995105	
			KR995106	
N. germanicum	Germany	Stone (CBS 539.88)	EU019253.2	Crous et al. 2007
N. germanicum	Germany	Holotype of *Catenulostroma germanicum* Crous & U. Braun (CBS 539.88)	MH862143.1	Vu et al. 2019
N. germanicum	Belarus	*Pinus nigra*	MK622897.1	Golovchenko et al. 2021
N. microsporum	South Africa	*Protea cynaroides*	KF901499.1	Quaedvlieg et al. 2014
N. microsporum	South Africa	Culture from holotype of *Trimmastostroma microsporum* (CBS 101951)	EU167572.1	Simon et al. 2009
N. microsporum	South Africa	*Protea cynaroides*	NR_145114.1	Quaedvlieg et al. 2014
N. abietis	USA	Soil	MW471927.1	Bazzicalupo et al. 2022
N. abietis	Germany	Type of *Trimmatostroma abietis* Butin & Pehl (CBS 459.93)	NR_145103.1	Crous and Groenewald 2005
N. abietis	Mallorca	Limestone	AY559362.1	Ruibal et al. 2005
N. abietis	Germany	Type of *Trimmatostroma abietis* Butin & Pehl (CBS 459.93)	MH862431.1	Vu et al. 2019
N. castaneae	Italy	*Castanea sativa*	MZ519072.1	Phukhamsakda et al. 2022
Teratosphaeria verrucosa	South Africa	*Eucalyptus cladocalyx*	KF901645.1	Quaedvlieg et al. 2014
T. cryptica	Australia	*E. globulus*	KF901573.1	Quaedvlieg et al. 2014
Catenulostroma protearum	South Africa	*Hakea sericea*	GU214628.1	Crous et al. 2009
C. hermanusense	South Africa	*Phaenocoma prolifera*	JF499833.1	Crous and Groenewald 2011
Mycosphaerella parva	Australia	*Austroafricana parva*	AY509780.1	Maxwell et al. 2005
Neophaeothecoidea proteae	South Africa	*Protea repens*	NR_157417	Crous et al. 2008
Diplodia sapinea	Armenia	*P. sylvestris*	PX219648	This study

RESULTS AND DISCUSSION

Cultural and Morphological Characteristics of *Neocatenulostroma Germanicum*

Laboratory studies on the surface of the necrotized part of the needles collected in the vicinity of Dilijan town (Tavush Province) and Hankavan village (Kotayk Province) revealed the formed conidial sporulation of the fungus – black stromatic beds, as well as sporodochia exiting through the stomata (Figures 2 a, b).

Conidia are thick-walled, dark brown, ellipsoid, with one septum, combined into short chains, 9.4–25.2 × 4.9–6.4 µm (Figure 2 c).

Colonies are slow-growing, on the 25th day at 20°C the average diameter was 11–12 mm, their shape is rounded, with smooth edges (Figure 2 d). At the initial stage of growth, the colony appears gray with an olive tinge; as it grows, it takes on a rich dark gray (steel-like) color with a faint olive-green tint. The colony reverse is uniformly black in color.

According to morphological characteristics, the isolated fungus was identified as *Neocatenulostroma germanicum* (Crous et al. 2007, Markovskaja et al. 2016).

Comparison of the Sequences and Phylogenetic Analysis

According to Maximum Likelihood (ML) phylogenetic analysis of the original sequences obtained in this study and deposited in GenBank, some sequences showed that they belong to the *Neocatenulostroma abietis/germanicum/microsporum* species complex (Figure 3). As expected, these species form a single clade with high support (99%) of the bootstrap test, whereas the newly described species *N. castaneae* from Italy (MZ519072.1) is not included in this clade. It should be noted that the target isolates from Armenia are 99.6–99.8% identical to *N. germanicum* isolates from Germany (EU019253.2), Latvia (KR995101), and Ukraine (KR995104, KR995105, KR995106).

Among morphologically similar species of the group, which include *Neocatenulostroma abietis*, *N. germanicum* and *N. microsporum*, one of the most potentially dangerous and poorly studied pathogenic micromycetes is *N. germanicum*, the causal agent of needle browning. The species is distributed in several European countries, including Germany, Poland, Lithuania, Montenegro, Belarus, and some regions of Russia (Markovskaja et al. 2016, Lasarević and Menkis 2020, Golovchenko et al. 2021, Bulgakov 2022). *N. germanicum* can behave not only as a saprotroph developing on various substrates, but also as a serious pathogen, massively affecting the needles of young pines in spring and summer, sometimes together with fungi of the genus *Dothiostroma* sp. (Markovskaja et al. 2016). Therefore, the application of both microscopic and morphometric methods, as well as molecular analysis (using a part of ITS region) confirmed the presence of *N. germanicum* in two localities of the artificial pine plantations in the vicinity of the Dilijan town and the Hankavan village, in the Republic of Armenia. In both localities, artificially grown pine forests planted on fairly steep, south-facing slopes are present. Despite different altitudes, the habitats are characterized by similar ecological conditions.

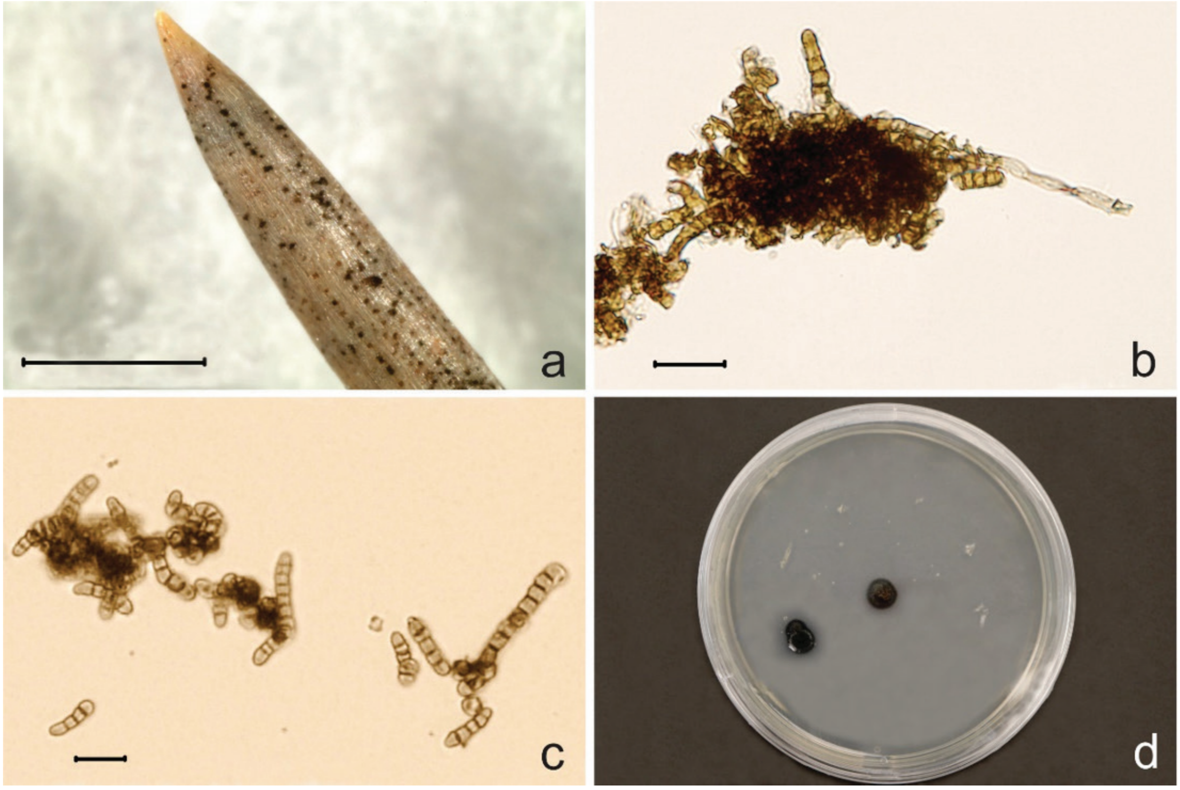

Figure 2. *Neocatenulostroma germanicum.* **(a)** Dark sporodochia on the necrotic part of pine needles. Scale bar = 3.2 mm; **(b)** Sporodochia with conidia chains. Scale bar = 100 µm; **(c)** Chains of conidia. Scale bar = 200 µm; **(d)** Colonies on PDA after 25 days.

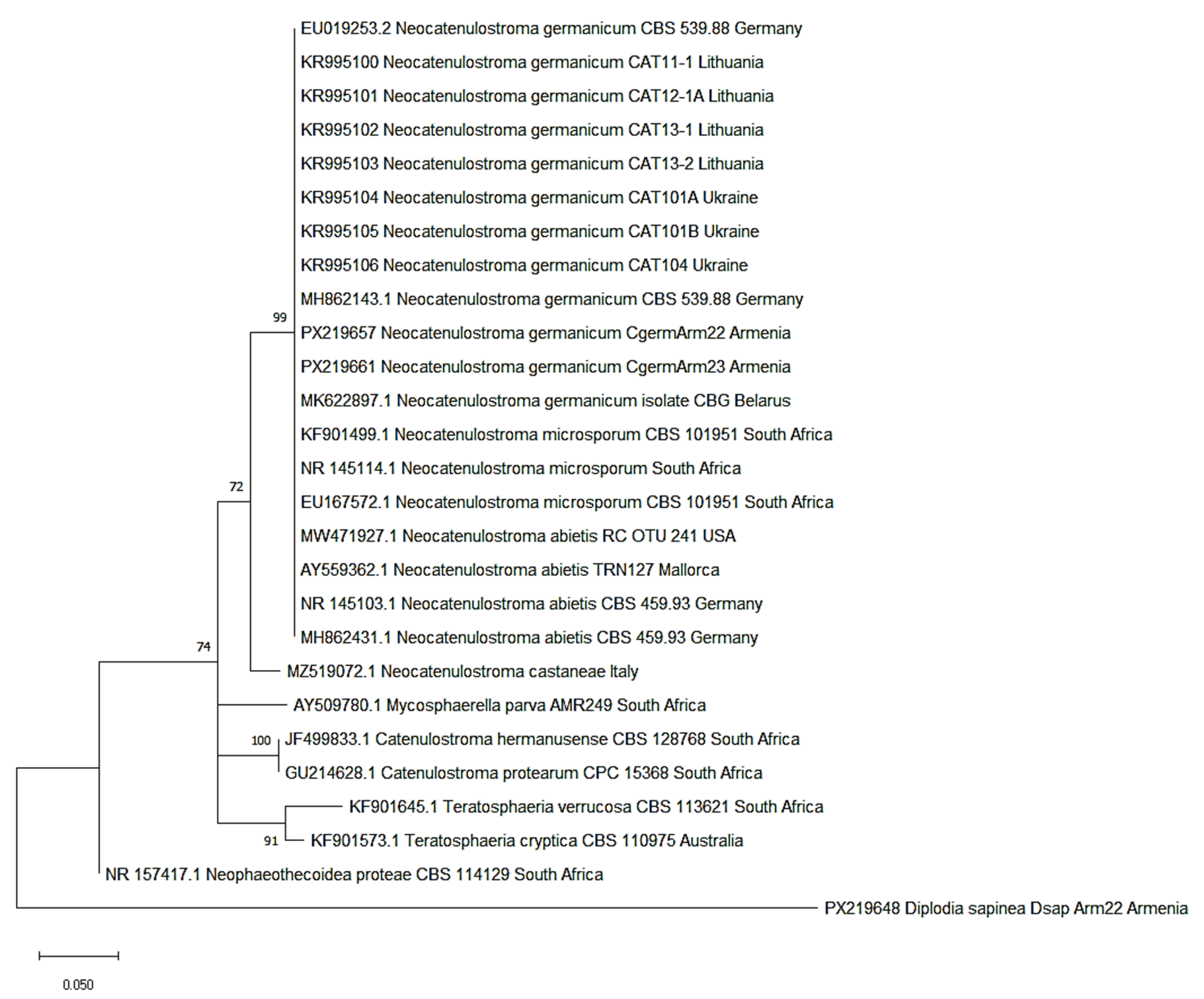

Figure 3. Maximum Likelihood (ML) phylogenetic tree based on sequences of ITS genome fragment of 12 specimens of *Neocatenulostroma germanicum*, some representatives of the order Mycosphaerellales and *Diplodia sapinea* as out group. The robustness determined by bootstrap analysis (1000 alternative dendrograms) is indicated as percentage at nodes. The GenBank accession number and country of origin are listed for every sequence.

CONCLUSION

The results of our research allow us to draw a definite conclusion that in the north-eastern and central regions of the Republic of Armenia (Tavush and Kotayk provinces) on the needles of Scots pine (*Pinus sylvestris* L.) a pathogenic micromycete *Neocatenulostroma germanicum* has been revealed, whose species identity is confirmed by the results of studies based on morphological, microscopic and molecular diagnostic methods. This is the first report of the discovery of the species on the territory of the Republic of Armenia.

It is important to note that further molecular genetic studies are planned, during which more variable and informative regions of the nuclear genome will be tested to enable more precise species differentiation within the genus *Neocatenulostroma*.

Author Contributions

KG, GT – conceived and designed the research, KG, AK, GT, SA, AE, EI, SI – conducted field research and collected material, SA, KN, – performed laboratory studies, SA, KN, KG – processed the data, KG - secured the field research funding; KG, KM – assisted with the preparation of the drafted manuscript, SA – wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Influence of Geological Substrate on the Structure and Diversity of Dinaric Mixed Beech–Fir–Spruce Forests: A Case Study from Mt. Konjuh

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ABSTRACT

This study explored the relationships between geological substrate and the structural and compositional attributes of mixed beech (*Fagus sylvatica* L.), fir (*Abies alba* Mill.), and spruce (*Picea abies* [L.] Karst.) forests on Mt. Konjuh in northeastern Bosnia and Herzegovina. Research was conducted on 81 experimental plots established across three dominant substrates: limestone, peridotite, and chert. Stand structure, diversity, and spatial organization were assessed using the Shannon diversity index, Pretzsch's species profile index, Gini coefficient, and the Clark–Evans and Füldner indices. The analyses revealed consistent differences among substrates, suggesting that geological conditions influence forest structure and diversity. Higher diversity and vertical heterogeneity were generally associated with limestone, while stands on peridotite and chert exhibited simpler but more balanced structures. All forest types displayed a reverse J-shaped diameter distribution, indicating uneven-aged composition and ongoing natural regeneration. Spatial patterns showed a tendency toward clustering of beech and spruce and higher species mingling on limestone. Overall, mixed beech–fir–spruce forests on Mt. Konjuh appear to be stable ecosystems whose structure and diversity are shaped by an interplay of geological, edaphic, and ecological factors. The results highlight the relevance of site-specific and adaptive silvicultural approaches that account for local variability in substrate and stand conditions.

Keywords: *mixed forests; geological substrate; structural diversity; spatial structure; Mt. Konjuh*

INTRODUCTION

Mixed forests of beech (*Fagus sylvatica* L.), silver fir (*Abies alba* Mill.), and Norway spruce (*Picea abies* [L.] Karst.) represent one of the most important forest types within the temperate zone of Europe. They cover more than 10 million hectares across an altitudinal range of 600–1,400 m a.s.l., predominantly in mountainous regions characterized by a moderately humid climate (Brus et al. 2012, EUFORGEN 2017). In Bosnia and Herzegovina, these forests occupy approximately 562.500 ha, accounting for about 22% of the country's total forest area (Matić et al. 1971). Thus, they represent its most significant forest resource—both economically and ecologically. Their distribution follows the Dinaric Mountain range from northwest to southeast (Stefanović et al. 1983), occurring on a broad spectrum of geological substrates and soil types. Based on taxonomic and ecological characteristics, eight principal types of mixed beech–fir–spruce forests have been identified (Ćirić et al. 1971). Owing to the wide range of edaphic and climatic conditions, these forests exhibit pronounced differentiation in both floristic composition and structural organization, with edaphic factors—particularly soil chemistry and water regime—playing a decisive role in their development (Beus 2011).

A defining feature of these forests is their capacity for natural regeneration (Višnjić and Prljača 2016), which results in predominantly uneven-aged structures where trees of various sizes and ages coexist within small areas. The maintenance of such stands relies on the selection system, a silvicultural approach designed to sustain continuous growth and preserve natural stand structure (Bončina et al. 2014). Originating in Central Europe during the 19th century as an alternative to clear-cutting (Schütz 2001), the selection cutting system gradually became the dominant management approach for uneven-aged forests in both Alpine and Dinaric regions (Diaci 2006). In Bosnia and Herzegovina, it has been practiced for more than a century (Čilaš et al. 2023), and for a long time served as the only management system for mixed forests, irrespective of their species composition. However, due to local variability in site conditions and management intensity, outcomes have differed widely—from stable, productive stands to cases of degradation (Pintarić and Izetbegović 1980).

Forest structure is a central concept in understanding forest ecosystem dynamics, encompassing the spatial arrangement of trees, species mixture, and dimensional distribution (Gadow and Hui 2002). It reflects the combined effects of natural processes and silvicultural interventions and, in turn, governs many ecological, productive, and social functions of forests (Pretzsch 2019). Continuous processes of regeneration, growth, and mortality constantly reshape stand structure, making it a dynamic indicator of ecosystem state and development (Gadow et al. 2012).

Studies of mixed beech–fir–spruce forests across Europe have shown that, despite regional differences, they often display similar structural patterns: a reverse J-shaped diameter distribution, high tree density, and large growing stock, frequently exceeding 350 $m^3 \cdot ha^{-1}$ (Pretzsch et al. 2015, Hilmers et al. 2018, Torresan et al. 2020). Nonetheless, variation in species composition and horizontal distribution substantially influences both diversity levels and structural complexity among stands.

Diversity, a fundamental component of biodiversity, encompasses not only the number of species but also their relative abundance and interactions (Konnert 1992, Hattemer 1994, Višnjić et al. 2013, 2017, Bartsch et al. 2020). It is shaped by a multitude of factors, including energy availability, habitat complexity, disturbance regime, stress conditions, ecosystem stability, and habitat area (Hansen and Rotella 1999). Among these, geological substrate plays a particularly influential role because it determines the chemical and physical properties of the soil (Kruckeberg 2004), such as texture, nutrient content, and water-holding capacity (Troeh and Thompson 2005, Vojniković 2021). This heterogeneity enhances the number of ecological niches and supports the coexistence of a wider range of plant species (Levine and HilleRisLambers 2009).

Floristic and vegetation studies of beech and fir forests on different substrates in Serbia and Montenegro (e.g., granodiorites, serpentinites, and eruptives) by Novaković-Vuković et al. (2016, 2022) revealed clear differences in floristic composition and diversity indices among the examined stands, emphasizing the importance of edaphic variation in shaping forest diversity.

Given the complex interactions among structural characteristics, site factors, and regeneration processes, mixed beech–fir–spruce forests are intricate ecological systems of exceptional significance. Their understanding requires an integrated approach that links structural indicators with ecological and edaphic variables. Therefore, the objective of this study is to analyze the structural characteristics of mixed beech–fir–spruce forests on different geological substrates and to assess how structural variation influences the diversity of trees and ground vegetation.

MATERIALS AND METHODS

Study Area

The study area is located in northeastern Bosnia and Herzegovina, on Mount Konjuh (44°18′32″ N, 18°30′57″ E), within the Forest Management Unit “Gornja Drinjača” of the Forest Management Area “Konjuh” (Figure 1). It lies within the Central Bosnian ophiolite zone and features hilly to mountainous terrain. The geology is diverse, dominated by serpentinitized peridotites, eruptive rocks, and cherts, with a smaller proportion of limestone. Soils are mainly eutric and dystric cambisols, locally accompanied by pseudogleys and pockets of calcomelanosols and calcocambisols. This heterogeneous substrate and complex relief create a pronounced vegetation mosaic (Stefanović 1983): pine and sessile oak forests prevail on warmer slopes, while cooler, more humid sites support beech–fir and beech–fir–spruce forests. In our study, all study plots are located in uneven-aged mixed stands of beech, fir, and spruce, representative of the prevailing stand structure in the study area. The moderately mountainous climate, with a mean annual precipitation of 1,219 mm and an average temperature of 7.4°C (Višnjić 2006), provides favorable conditions for the development of these mixed forests.

Field Measurements

Data collection was conducted on 81 circular sample plots (radius 12.62 m; area 500 m²), evenly distributed across three geological substrates—peridotite, limestone, and chert (27 plots each)—and spaced 100 m apart in a regular square grid arrangement to ensure uniform coverage. A complete inventory was carried out on each plot, recording tree species, diameter at breast height (DBH > 5 cm), and total height. For spatial structure analysis, an additional square plot of 100 × 100 m (1 ha) was established per substrate, within which all trees with a DBH > 5 cm were mapped using the orthogonal method. For each tree, diameter at breast height, total height, and height to the live crown (HLC) were measured. Tree spatial arrangement was quantified using the Clark–Evans aggregation index (Clark and Evans 1954), calculated from nearest-neighbor distances. Since the Clark–Evans index is a distance-based method, no angle threshold or angle-based sampling design was applied. Simultaneously, vascular plant diversity was surveyed on 400 m² plots (27 per substrate) using the Braun–Blanquet cover–abundance scale.

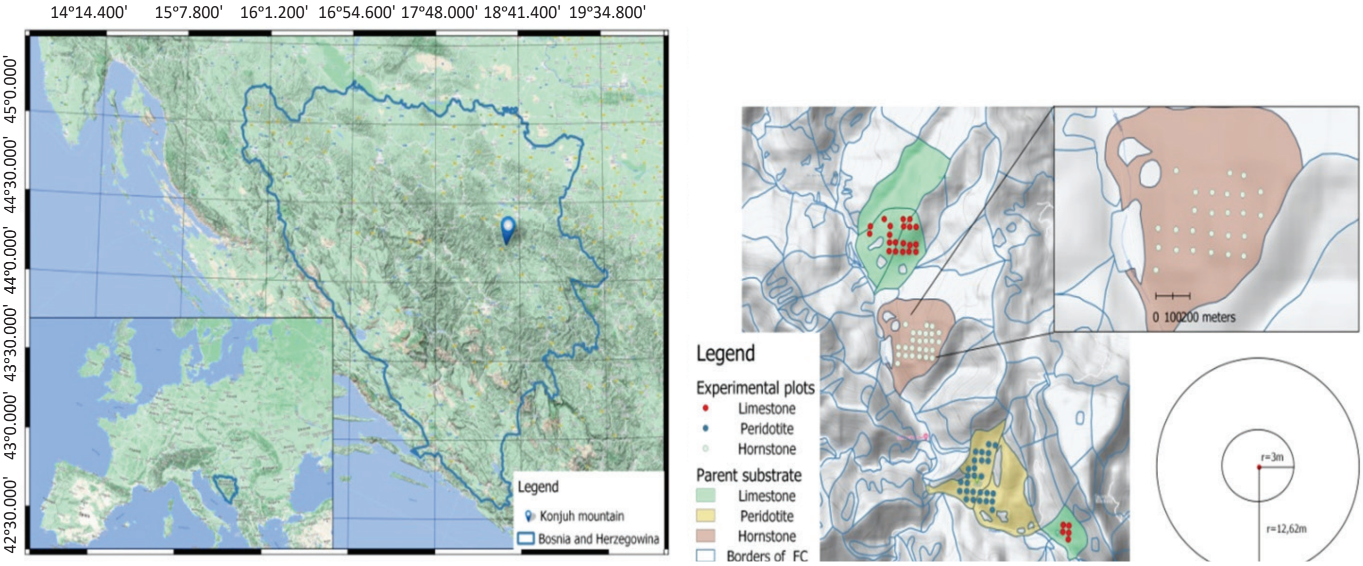

Figure 1. Location of the study area on Mount Konjuh in northeastern Bosnia and Herzegovina, showing the distribution of sample plots across the three geological substrates: peridotite, limestone, and chert.

Data Analysis

Phytosociological data were processed in Turboveg 2.38 (Hennekens and Schaminee 2001), while structural and ecological indices were calculated in R (R Core Team 2024) using the packages vegan (Oksanen et al. 2022), spatstat (Baddeley et al. 2015), ineq (Zeileis 2014), and dplyr (Wickham et al. 2023). Species diversity was quantified using the Shannon index (Shannon 1948), computed with diversity() from vegan based on both stem number and basal area, with evenness expressed as the ratio between observed and maximum diversity. Floristic similarity among plots was assessed using the Sørensen index implemented in vegdist(). Horizontal structural diversity of tree diameters was expressed by the Gini coefficient (Gini(), ineq), while species mingling was evaluated using Füldner's mingling index (1996), based on the four nearest neighboring trees. Spatial tree patterns were analyzed in spatstat, and vertical structure was quantified using the species profile index (Pretzsch 2019), combining Shannon's formulation with species distribution across height layers (≤ 50%, 50–80%, > 80% of maximum stand height). The Clark–Evans aggregation index (Clark and Evans 1954) was applied to characterize tree spatial arrangement, comparing observed to expected nearest-neighbor distances ($R < 1$ = aggregated, $R \approx 1$ = random, $R > 1$ = regular; clarkevans.test(), spatstat). Regeneration counts from 3 m radius subplots were converted to individuals per hectare, and understory vegetation diversity was analyzed using the same set of indices as for trees.

RESULTS AND DISCUSSION

Structural Characteristics

The structural characteristics of the studied mixed beech–fir–spruce stands varied markedly across geological substrates. To quantify these differences, key stand indicators—tree number (N), quadratic mean diameter (Dg), mean height (H), basal area (G), and growing stock volume (V)—were analyzed by species and substrate (Table 1).

Analysis of these indicators revealed pronounced variations in stand characteristics depending on the underlying geology. The total number of trees was highest on peridotite (601 trees·ha^{-1}) and lowest on limestone (428 trees·ha^{-1}). The largest average dimensions occurred on chert (Dg = 37.9 cm; H = 25.9 m), while the smallest were recorded on peridotite. Total basal area and volume were also highest on chert (G = 35.8 m^2·ha^{-1}; V = 485 m^3·ha^{-1}). Across all substrates, silver fir dominated both in stem number and growing stock, whereas spruce and beech were less frequent but attained larger dimensions. The variation among substrates may reflect differences in site conditions mediated by geological and edaphic factors, as well as by past management. Bedrock influences soil development and the physical and chemical environment of roots, thereby shaping water and nutrient availability, although these effects are often indirect and context-dependent (Vestin et al. 2013, Richter and Billings 2015). Previous studies have shown higher forest growth on more weatherable, or nutrient-rich substrates such as limestone or shale compared to granitic or sandstone bedrock (Jiang et al. 2020, Basnet et al. 2024). In addition to these geochemical effects, differences in historical use and management intensity may have modified stand composition and structure, potentially amplifying or masking substrate-related trends. The observed structural variation therefore likely results from the combined influence of geological substrate, soil formation, climatic factors, and long-term management history.

Across all geological substrates, the diameter structure follows a negatively exponential curve, indicating an uneven-aged stand structure (Schütz 2001). On chert and peridotite, fir predominates, while beech occurs across all diameter classes, particularly the intermediate ones. Spruce is more abundant on limestone and peridotite, where it reaches larger diameter classes. On limestone,

Table 1. Stand-level indicators describing the structure of mixed beech–fir–spruce forests across three geological substrates (limestone, peridotite, and chert) on Mt. Konjuh where N is tree number per ha, Dg quadratic mean diameter (cm), H is the mean height (m), G is basal area (m^2·ha^{-1}), and V is the growing stock (m^3·ha^{-1}).

Supstrate	N (trees·ha^{-1})	Dg (cm)	H (m)	G (m^2·ha^{-1})	V (m^3·ha^{-1})
Limestone					
Beech	96 ± 139	33.9 ± 14.1	22.7 ± 9.0	7.8 ± 12.4	113.0 ± 197.0
Silver fir	183 ± 188	34.3 ± 14.2	21.1 ± 8.4	10.1 ± 10.3	119.0 ± 139.0
Spruce	85 ± 105	41.9 ± 16.0	27.2 ± 8.0	9.8 ± 10.8	135.0 ± 166.0
Other sp.	64 ± 15	26.9 ± 15.8	20.3 ± 7.4	3.5 ± 1.1	42.5 ± 15.7
Total	428 ± 200	34.3 ± 15.7	22.9 ± 8.5	31.2 ± 11.2	410.0 ± 188.0
Peridotite					
Beech	38 ± 42	21.9 ± 14.9	18.6 ± 9.0	1.7 ± 3.3	24.7 ± 55.7
Silver fir	425 ± 216	3.1 ± 9.6	25.6 ± 5.4	24.1 ± 12.8	291.0 ± 198.0
Spruce	104 ± 112	30.1 ± 15.5	22.4 ± 10.1	6.0 ± 6.7	76.3 ± 90.9
Other sp.	34 ± 13	34.0 ± 15.0	20.3 ± 8.0	3.4 ± 1.4	37.6 ± 17.1
Total	601 ± 219	31.4 ± 14.5	22.2 ± 8.5	35.3 ± 8.9	430.0 ± 173.0
Chert					
Beech	113 ± 103	41.0 ± 11.7	27.3 ± 6.1	9.6 ± 7.3	139.0 ±114.0
Silver fir	344 ± 182	37.8 ± 5.5	26.1 ± 3.3	21.9 ± 12.1	290.0 ± 176.0
Spruce	34 ± 53	41.4 ± 15.1	27.7 ± 8.1	3.9 ± 7.4	53.0 ± 101.0
Other sp.	17 ± 8	21.7 ± 10.2	18.0 ± 6.5	0.4 ± 0.2	3.0 ± 1.4
Total	508 ± 196	37.9 ± 11.7	25.9 ± 6.2	35.8 ± 8.9	485.0 ± 138.0

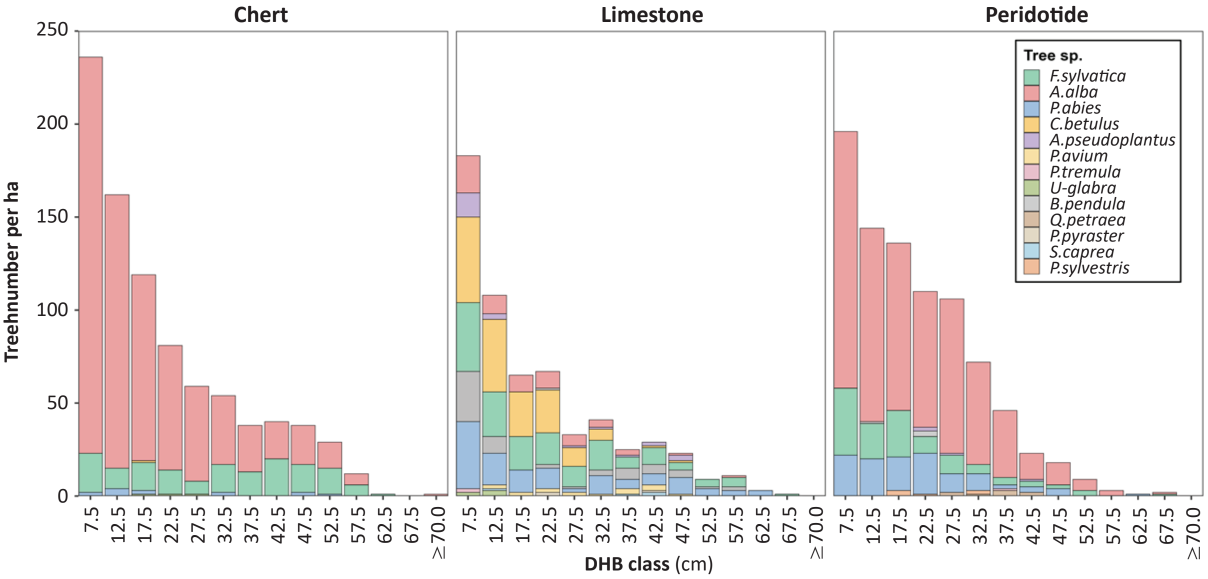

Figure 2. Diameter distribution of trees by species and geological substrate.

a higher proportion of other broadleaved species, such as sycamore maple and hornbeam, indicates a more heterogeneous species composition.

Species Diversity

The analysis of variance (one-way ANOVA) revealed statistically significant differences in tree-layer diversity among the geological substrates (Table 2). Species richness was highest on limestone (3.80 ± 1.37) and significantly higher than on chert (2.50 ± 1.22; $p < 0.001$). The Shannon index based on tree number likewise reached its highest mean value on limestone (0.94 ± 0.41), followed by peridotite (0.69 ± 0.27) and chert (0.61 ± 0.38). In contrast, diversity indices calculated from basal area and the evenness index showed no significant differences among substrates ($p > 0.05$), suggesting a comparable proportional distribution of species within the stands.

In the vascular flora layer, differences among geological substrates were not statistically significant ($p > 0.1$). Nonetheless, species richness was highest on limestone (37.14 ± 13.9), followed by peridotite (35.66 ± 9.94), and lowest on chert (30.96 ± 8.69). Comparable results were

Table 2. Analysis of variance for diversity indicators by different substrates with LSD test, where I refers to the mean value of the observed indicator and SD is the standard deviation.

Indicator	Substrate	I	SD	ANOVA	LSD test	
Tree layer						
Number of tree species	Limestone	3.80	1.37	$p < 0.05$	*	
	Peridotite	3.30	0.83		*	
	Chert	2.50	1.22			*
Shannon’s index (based on tree number)	Limestone	0.94	0.41	$p < 0.05$	*	
	Peridotite	0.69	0.27			*
	Chert	0.61	0.38			*
Shannon’s index (based on basal area)	Limestone	0.74	0.37	$p = 0.47$	*	
	Peridotite	0.64	1.34		*	
	Chert	0.63	0.34		*	
Evenness (based on tree number)	Limestone	69.12	23.29	$p = 0.21$	*	
	Chert	64.02	29.66		*	
	Peridotite	57.88	14.66		*	
Evenness (based on basal area)	Chert	66.68	31.85	$p = 0.23$	*	
	Limestone	55.40	25.51		*	
	Peridotite	55.10	26.51		*	
Vascular plants						
Number of species	Limestone	37.14	13.94	$p = 0.11$	*	
	Peridotite	35.66	9.94		*	*
	Chert	30.96	8.69			*
Shannon’s index	Limestone	2.84	0.61	$p = 0.18$	*	
	Peridotite	2.80	0.47		*	
	Chert	2.58	0.54		*	
Evenness	Limestone	75.90	8.85	$p = 0.20$	*	
	Chert	74.40	9.77		*	
	Peridotite	712.00	10.96		*	

reported by Beus and Vojniković (2010), who found greater species richness on limestone than on basic and ultrabasic substrates. Beus (1979) likewise noted that beech–fir–spruce forests on limestone are richer in species in both the tree and ground layers, particularly with respect to the presence of noble broadleaves. In contrast, forests on peridotitic–serpentinitic soils exhibit a more specific floristic composition, characterized by a lower frequency of typical mesophilous species. Therefore, based on the analysis of site factors, floristic composition, and stand structure, Beus (1979) proposed that these communities represent a distinct syntaxonomic unit: *Abieti-Fagetum serpentinicum*. The Shannon and evenness indices followed a similar pattern, with slightly higher values on limestone, indicating greater floristic diversity and a more balanced community structure on calcium-rich substrates.

Structural Diversity and Spatial Pattern of Trees Distribution

The analysis of structural diversity revealed that stand complexity varied with the underlying geological substrate. Pretzsch’s species profile index (A) reached its highest values on limestone (1.80), indicating greater vertical and horizontal heterogeneity and a more complex stratification pattern than on peridotite (1.50) or chert (1.45). The wider interquartile range on limestone further reflects higher structural variability among plots (Table 3). The elevated values of the species profile index (A) align with observations from studies of primary and near-natural mixed forests, where greater structural heterogeneity is commonly linked to enhanced ecological stability and resilience to disturbance (Pretzsch 2009). Such complexity is often associated with sites exhibiting pronounced topographic or edaphic variability, conditions typical of carbonate (limestone) substrates.

In contrast, the profile evenness index (A_{rel}) was highest on chert (0.75), suggesting a more balanced distribution of individuals across height layers. The analysis of the Gini coefficient showed that diameter inequality ranged between 0.29 and 0.33, with peridotite exhibiting slightly greater variability in tree dimensions, and limestone the lowest. No statistically significant differences were observed among substrates ($p > 0.05$). Nevertheless, the observed range (0.29–0.33) is typical for mixed stands (Motta et al. 2015, Keren et al. 2020, Szmyt et al. 2020, Trifković and Ficko 2025). The somewhat higher inequality on peridotite (0.33) may reflect greater variation in tree dimensions, often associated with slow growth and long tree lifespans under nutrient-poor and ecologically demanding site conditions.

The spatial analysis based on the Clark–Evans aggregation index (R) and Füldner's mingling index (M) revealed clear differences in tree distribution among geological substrates. Beech and spruce exhibited aggregated spatial patterns on all substrates ($R < 1$; $p < 0.05$), whereas silver fir showed aggregation only on limestone ($R = 0.75$) and a random or slightly uniform arrangement on peridotite and chert ($R \approx 1$). High mingling values for beech ($M = 0.8$) indicate pronounced interspecific mixing, typical of Dinaric mixed mountain forests where species coexist closely within small spatial scales, which is an important feature contributing to structural complexity and ecological stability (Čilaš and Višnjić 2024). At the stand level, forests on limestone displayed a clustered but moderately mixed structure ($R = 0.83$; $M = 0.65$), while those on peridotite and chert showed patterns approaching randomness ($R \approx 1$; $M = 0.40$). Comparable spatial configurations have been described across various European mixed forests, where both managed and near-natural stands frequently exhibit random tree distributions at the hectare scale (Parobeková et al. 2018, Dukić et al. 2021, Keren et al. 2024). Management system plays a key role in these patterns: fine-scale selection cutting tends to produce random or uniform spatial arrangements, whereas group-selection interventions enhance local clustering (Lafond et al. 2014). The low mingling values for fir and spruce on peridotite and chert ($M < 0.4$) likely reflect reduced neighborhood diversity and greater spatial segregation, consistent with strong intraspecific competition or adaptation to microsite-specific conditions. To visualize these spatial patterns more clearly, Figure 3 illustrates the horizontal distribution of trees by species and diameter classes on the 1 ha plots established on limestone, peridotite, and chert.

Table 3. Values of Pretzsch's species profile index (A), profile evenness index (A_{rel}), and the Gini coefficient of tree diameter across different geological substrates.

Substrate	Species profile index (A)	Profile evenness (A_{rel})	Gini coefficient
Limestone	1.80	0.67	0.29
Peridotite	1.50	0.63	0.33
Chert	1.45	0.75	0.30

Table 4. Clark–Evans aggregation index (R) and Füldner's mingling index (M) for spatial structure analysis of trees on the 1 ha plot.

Tree species	Limestone		Peridotite		Chert	
	R	M	R	M	R	M
Beech	0.82	0.59	0.79	0.81	0.89	0.83
Silver fir	0.75	0.79	1.00	0.25	1.00	0.20
Spruce	0.82	0.61	0.73	0.68	1.00	0.96
Stand level	0.83	0.65	0.97	0.40	0.99	0.32

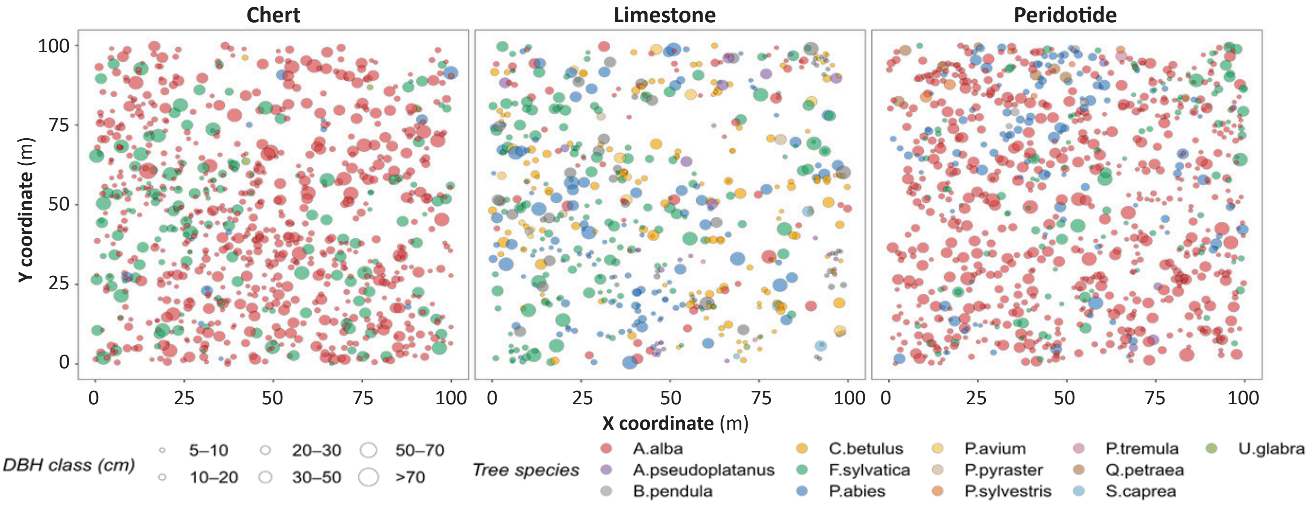

Figure 3. Spatial distribution of trees by species and diameter class on limestone, peridotite, and chert substrates.

CONSCLUSIONS

The study demonstrated that geological substrate plays a key role in shaping the diversity and structure of mixed beech–fir–spruce forests on Mt. Konjuh. Stands on limestone showed the highest species richness, vertical heterogeneity, and spatial mingling, indicating greater structural complexity and ecological stability. In contrast, forests on peridotite and chert were simpler in structure, with lower diversity and weaker interspecific mixing, although chert supported a more balanced vertical profile. Diameter distributions followed a reverse J-shaped pattern across all substrates, confirming stable uneven-aged stand structures. Higher values of Pretzsch's species profile and mingling indices on limestone reflected greater spatial and vertical differentiation, whereas the range of the Gini coefficients indicated moderate inequality and well-developed structural diversity throughout. From a management perspective, these results suggest that silvicultural strategies should account for the substrate-related site potential. On limestone, where conditions favor species coexistence and complex canopy layering, selection systems that maintain multi-layered structures are most appropriate. On nutrient-poor peridotite and chert, management should focus on promoting stand stability and regeneration through lower-intensity interventions and the gradual enrichment of underrepresented species. Integrating geological and edaphic variability into forest planning will enhance both productivity and resilience under changing climatic conditions.

Author Contributions

BB handled conceptualization, methodology development, field investigation, original draft preparation, and project administration. ĆV provided supervision, contributed to methodological refinement, validated results, and assisted with review and editing. MČ performed formal data analysis, curated datasets, prepared visualizations, and contributed to review and editing. SV and MLJ supported field investigation, supplied necessary resources, contributed to visualization, and assisted with manuscript review and editing. All authors approved the final manuscript.

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Conflicts of Interest

The authors declare no conflict of interest

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Assessing Wildfire Susceptibility in Mediterranean Forest Ecosystems: A Spatial Ensemble Machine Learning Approach in Portugal

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ABSTRACT

Wildfires constitute a significant ecological disturbance in Mediterranean ecosystems, exerting profound effects on forest dynamics, biodiversity, and land management practices. The development of precise susceptibility mapping is essential for informing prevention strategies, optimizing resource allocation, and promoting sustainable forest management under increasing fire pressure. This study employed and compared four machine learning classifiers—Random Forest, Classification and Regression Trees (CART), Gradient Boosting, and Extreme Gradient Boosting (XGBoost)—to model wildfire susceptibility across Portugal. Six environmental and anthropogenic predictors were utilized: vegetation indices (NDVI), land use/land cover, slope, land surface temperature (LST), wind speed, and distance to human settlements. The results indicated that vegetation-related variables, particularly NDVI and land cover, were the most significant determinants of fire occurrence, followed by slope and wind speed, thus underscoring the role of biophysical conditions in shaping the fire regimes. A spatial block cross-validation strategy was implemented to rigorously account for spatial autocorrelation. Under this evaluation, XGBoost demonstrated the highest predictive performance (overall accuracy = 90.03%, AUC = 0.951), surpassing or equaling that of the other ensemble methods. The resulting susceptibility maps, generated utilizing simple Kriging interpolation to translate discrete model predictions into continuous surfaces, identified the northern and central interior regions as the most fire-prone, consistent with historical fire records. Quantitatively, the optimal model classified 19.08% of the national territory as having a very high fire susceptibility. Our findings underscore the efficacy of ensemble machine learning techniques in capturing complex fire–environment interactions and provide spatially explicit information that can enhance fire prevention planning, support conservation priorities, and guide adaptive forest management in Mediterranean regions.

Keywords: wildfire susceptibility; forest fire risk assessment; Mediterranean forest ecosystems; forest management; spatial block cross-validation; biophysical drivers; fire ecology

INTRODUCTION

Portugal, distinguished by its Mediterranean climate and dynamic land use and land cover (LULC) changes, has witnessed evolving wildfire trends that present significant environmental and socio-economic challenges (Parente et al. 2018, Oliveira and Zêzere 2020). The Mediterranean region is particularly susceptible to wildfires because of its hot, dry summers, frequent droughts, and vegetation that is prone to ignition. In recent years, extreme heatwaves with temperatures surpassing 48°C have precipitated devastating wildfires across Mediterranean countries, resulting in loss of life, extensive property damage, and environmental degradation (Turco et al. 2019).

For instance, in Portugal, over 7,032 rural fires consumed 25,429.5 hectares of land from January to August 2025, which was more than three times the area burned during the same period in 2024 (ICNF 2025).

The accumulation of dry vegetation and human activities in proximity to forested areas increases the risk of uncontrollable mega-fires that often exceed firefighting capacities. In terms of specific causes, the overwhelming majority of wildfire ignitions in Portugal are anthropogenic. These are primarily driven by the negligent use of fire in agricultural and forestry practices (such as traditional brush burning), accidental ignitions from machinery, and intentional acts, whereas natural causes, such as lightning strikes, account for a small minority of events. In Portugal, climate change coupled with trends in rural land abandonment and land-use changes has led to increased fuel loads and wildfire incidents within vulnerable forest ecosystems (Eurostat 2025, Thies 2025).

These fires pose threats to lives, ecosystems, and economic assets, and significantly contribute to carbon emissions. For instance, wildfires across the European Union in 2025, in the period up to late August, released an estimated 38.37 million tonnes of CO_2, reaching a record for that time period (European Commission 2025).

Despite the recognized dangers, wildfire management in the Mediterranean region typically emphasizes full suppression, which can paradoxically elevate the risk of larger and more intense fires by permitting fuel accumulation (Fernandes and Botelho 2003). To move toward proactive management, recent advancements have increasingly utilized machine learning to assess regional fire risk. For example, Thies (2025) applied machine learning to map wildfire susceptibility across Germany, while Durlević et al. (2025) demonstrated the efficacy of multi-sensor satellite data fusion for predicting fires in Serbia. Similarly, Sapkota et al. (2025) utilized comparable algorithms to advance wildfire prediction in Nepal. Within the Mediterranean context, foundational studies such as Tonini et al. (2020) have mapped wildfire susceptibility using machine learning techniques. Despite these advancements in wildfire susceptibility modelling, critical gaps related to spatial bias and overly optimistic model validation persist, potentially limiting its accuracy and practical applicability (Parente et al. 2018, Jaafari and Pourghasemi 2019).

Specifically, many existing regional assessments overlook the impact of spatial autocorrelation, leading to inflated accuracy. Addressing these issues is crucial; therefore, this study represents a substantial methodological improvement that was achieved by implementing a rigorous spatial block cross-validation strategy to ensure the ecological validity of the predictions.

This study addresses these gaps by pursuing two primary objectives: first, to systematically compare the performance of four ensemble machine learning models—Random Forest, CART, Gradient Boosting, and XGBoost—for wildfire susceptibility mapping in Portugal under strict spatial validation; and second, to identify and evaluate the most influential environmental and anthropogenic predictors driving fire occurrence in these forest ecosystems. These findings provide a robust framework for wildfire risk assessment, offering spatially explicit insights to inform forest management and policy decisions.

By integrating the understanding of wildfire dangers specific to Mediterranean environments and addressing modelling limitations, this study aims to contribute to more accurate, reliable, and spatially explicit wildfire risk assessment in Portugal. This will aid in adopting proactive fire management strategies that balance suppression and ecosystem resilience, thereby helping to mitigate the impacts of wildfires in a changing climate.

MATERIALS AND METHODS

Study Area

Portugal, situated at approximately 39.4° N, 8.22° W on the Iberian Peninsula, has a total area of 92,212 km^2 and a population of approximately 10.2 million (Central Intelligence Agency 2025). The country is characterized by a Mediterranean climate with hot, dry summers and mild and wet winters (Parente et al. 2018, Oliveira and Zêzere 2020). Combined with the varied topography and extensive forest cover, this climate makes Portugal highly susceptible to wildfires during the summer months.

The landscape is a mosaic of forests, shrublands, agricultural land, and settlements. Forests cover approximately 36% of the territory (Eurostat 2025), with fire-prone regions concentrated in the northern and central inland districts, such as Vila Real, Braga, Aveiro, and Coimbra. Mountainous terrain with steep slopes and dense vegetation further increases the wildfire risk due to fuel accumulation and enhanced fire spread potential.

Wildfires have intensified in recent years. In the first seven months of 2025, over 7,032 rural fires were recorded, burning approximately 254,295 hectares across the country (Figure 2) (ICNF 2025). These events affected regions nationwide, causing widespread damage and evacuations.

Land use and land cover (LULC) changes also play critical roles. Rural depopulation and land abandonment contribute to higher fuel loads, whereas flammable eucalyptus and maritime pine plantations exacerbate fire intensity under drought conditions aggravated by climate change.

This combination of environmental, climatic, and human pressures makes Portugal a challenging study area for wildfire prediction. Understanding the spatial and temporal fire susceptibility patterns of this species is essential for advancing prevention strategies and risk mitigation.

Historical Forest Fires

Historical forest fire data for this study were sourced from the Institute for Nature Conservation and Forests (ICNF) of Portugal, the principal national authority responsible for forest and wildfire monitoring. The ICNF database contains detailed records of fire incidents, including annual fire counts and total burned areas, offering a comprehensive overview of wildfire trends in Portugal from 2006 to 2025 (ICNF 2025).

As illustrated in Figure 2, from 2006 to 2013, annual fire counts exceeded 25,000 events, peaking at nearly 30,000 fires around 2013. Since 2020, fewer than 10,000 fires annually have been reported, indicating a significant decline in fire frequency.

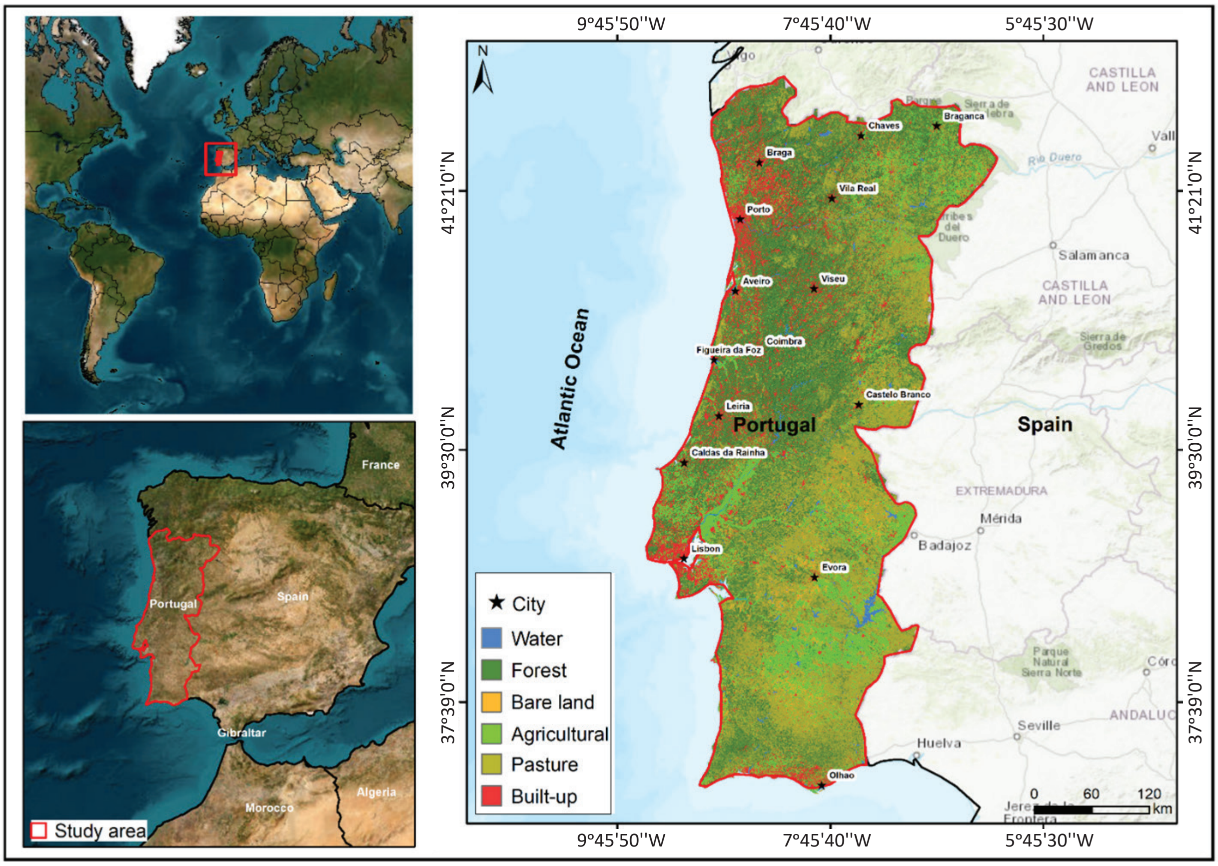


Figure 1. Study area location.

The patterns of burned areas exhibit greater interannual variability (Figure 2). Most years between 2006 and 2024 experienced burned areas ranging from 50,000 to 200,000 hectares. However, 2017 is notable as an extreme fire season with over 500,000 hectares burned (Turco et al. 2019). Following this peak, annual burned areas generally remained lower, although recent increases in 2024 and 2025 suggest a potential resurgence of large-scale fire activity. In 2025 (January to August), the burned area exceeded 254,000 hectares.

These data reflect the evolving fire regime in Portugal and underscore the importance of the long-term monitoring capabilities maintained by ICNF. The detailed ICNF records serve as both a historical context and a crucial validation reference for this study's analysis and modelling of wildfire susceptibility (Almeida et al. 2024).

Overall, the historical analysis demonstrates that while the number of fires has decreased over time, the magnitude of burned areas remains highly variable, reflecting the strong influence of climatic extremes, vegetation conditions, and human factors. These findings highlight the need for robust susceptibility modelling and spatial planning to mitigate the risks of severe fire events in the region.

Data Collection and Sample Generation

Wildfire susceptibility modelling in this study was based on a binary dependent variable: fire presence or absence. Fire presence data were obtained from the MODIS Active Fire product (MCD14ML, FIRMS) for the period 2015–2024 (Giglio et al. 2016). The dataset was subjected to a cleaning process to ensure its ecological relevance and accuracy. Specifically, fire detections located within built-up areas were removed to restrict the analysis to forest and shrubland fires, and spurious detections, such as single isolated pixels and cloud-related anomalies, were also excluded.

Although the initial database contained over 200,000 recorded fire pixels, utilizing the entire dataset in machine learning classifiers introduces severe spatial autocorrelation and data redundancy. Because large wildfire events span thousands of adjacent pixels with nearly identical environmental characteristics, using all available points leads to severe model overfitting, in which the algorithm memorizes specific fire shapes rather than learning the underlying biophysical drivers. To mitigate this and ensure statistical independence, a spatial thinning approach was used.

From this cleaned dataset, 1,000 spatially independent fire points were randomly selected as positive samples. To create a balanced dataset, an equal number of no-fire points (1,000) were randomly generated in areas that showed no active fire detection during the same period (Tonini et al. 2020). Maintaining a strict 1:1 ratio of presence to absence points is critical for tree-based ensemble models, which are highly sensitive to class imbalance and may otherwise develop a predictive bias toward the majority class.

A minimum buffer distance was applied to ensure no spatial overlap with fire pixels and to further reduce the spatial dependency between samples. A final balanced

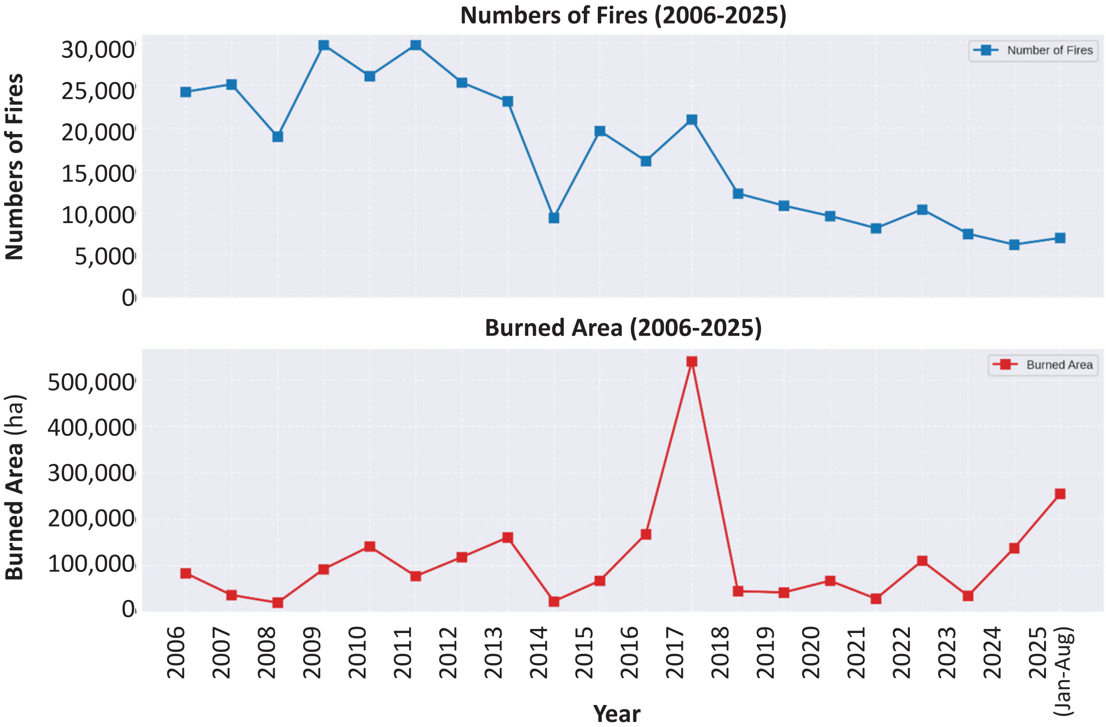


Figure 2. The number of forest fires (top) and burned area in hectares (bottom) in Portugal from 2006 to August 2025, based on ICNF historical wildfire records.

dataset of 2,000 samples was used for model training and validation, providing a robust, computationally efficient, and ecologically representative foundation for spatial algorithms.

Explanatory Variables

Six explanatory variables were downloaded and processed using Google Earth Engine (GEE), which provides convenient access to extensive satellite imagery and geospatial datasets with planetary-scale computational capabilities. Although wildfire occurrence is influenced by a vast array of factors, these six specific predictors were deliberately selected based on established Mediterranean fire ecology principles (Jaafari and Pourghasemi 2019, Oliveira and Zêzere 2020). They represent the core biophysical and anthropogenic drivers of fire regimes: fuel availability and continuity (NDVI, LULC), topographic spread capability (slope), chronic climatic preconditioning (LST, wind speed), and human ignition pressure (distance to settlements). Although high-temporal-resolution meteorological data (e.g., daily humidity or precipitation) are highly effective for short-term active fire forecasting, the chosen long-term averaged and static variables are the most appropriate predictors for mapping chronic, long-term baseline wildfire susceptibility at the national scale.

The use of GEE allowed for the efficient extraction of variable values at 2,000 sample points directly at their native spatial resolutions, facilitating consistent and reproducible data preparation for model training with minimal need for broad resampling procedures.

- Vegetation: NDVI (2015–2024 average) derived from Landsat 8 and 9 surface reflectance images (Vermote et al. 2016).
- Topography: Slope derived from the 30 m SRTM DEM (Farr et al. 2007).
- Human Pressure: Distance to settlements calculated from the GHSL Built-Up product (2024) using a Euclidean distance transform (European Commission Joint Research Centre 2024, Verde and Zêzere 2010).
- Land Cover: LULC extracted from the Dynamic World product (Sentinel-2, 10 m) for 2024 (Brown et al. 2022).
- Climate: Land surface temperature (LST) from MODIS Terra (MOD11A2.061) and Aqua (MYD11A2.061), averaged for 2015–2024 (1 km) (Wan et al. 2021). Wind speed was extracted from the ERA5-Land reanalysis; although its native spatial resolution is 10 km, it was resampled to 1 km using bilinear interpolation to ensure perfect spatial alignment with the other environmental (Muñoz-Sabater et al. 2021).

Methodology Workflow

The methodology of this study followed a structured workflow for predicting wildfire susceptibility, as illustrated in Figure 3. The process began with data preparation and continued with machine learning model development, validation, and final generation of the susceptibility maps.

Data Preparation

The final dataset for modelling consisted of 2,000 balanced samples (1,000 fire and 1,000 no-fire) derived from the methods described in Section 2.3. The explanatory variables included vegetation (NDVI), topography (slope), land surface temperature (LST), wind speed,

Table 1. Summary of variables used in wildfire susceptibility modelling.

Variable	Description	Source	Spatial Resolution	Temporal Coverage	Unit
Fire presence	Active fire detections	MODIS Active Fire Product (MCD14ML, FIRMS)	~1 km	2015–2024	Binary (presence/absence)
NDVI	Proxy for vegetation greenness and fuel availability	Landsat 8 and 9 SR (USGS/NASA)	30 m	2015–2024 (average)	Index (−1 to +1)
Slope	Topographic steepness derived from DEM	SRTM DEM (NASA/USGS)	30 m	Static	Degrees (°)
Distance to settlements	Euclidean distance from built-up areas	GHSL Built-Up (EC–JRC, 2024)	100 m	2024	Meters (m)
Land cover (LULC)	Land cover classes (forest, cropland, shrubland, etc.)	Dynamic World (Sentinel-2)	10 m	2024	Categorical
LST	Land surface temperature	MODIS Terra (MOD11A2.061) and Aqua (MYD11A2.061)	1 km	2015–2024 (average)	°C
Wind speed	Surface wind speed	ERA5-Land reanalysis (ECMWF)	10 km	2015–2024 (average)	m/s

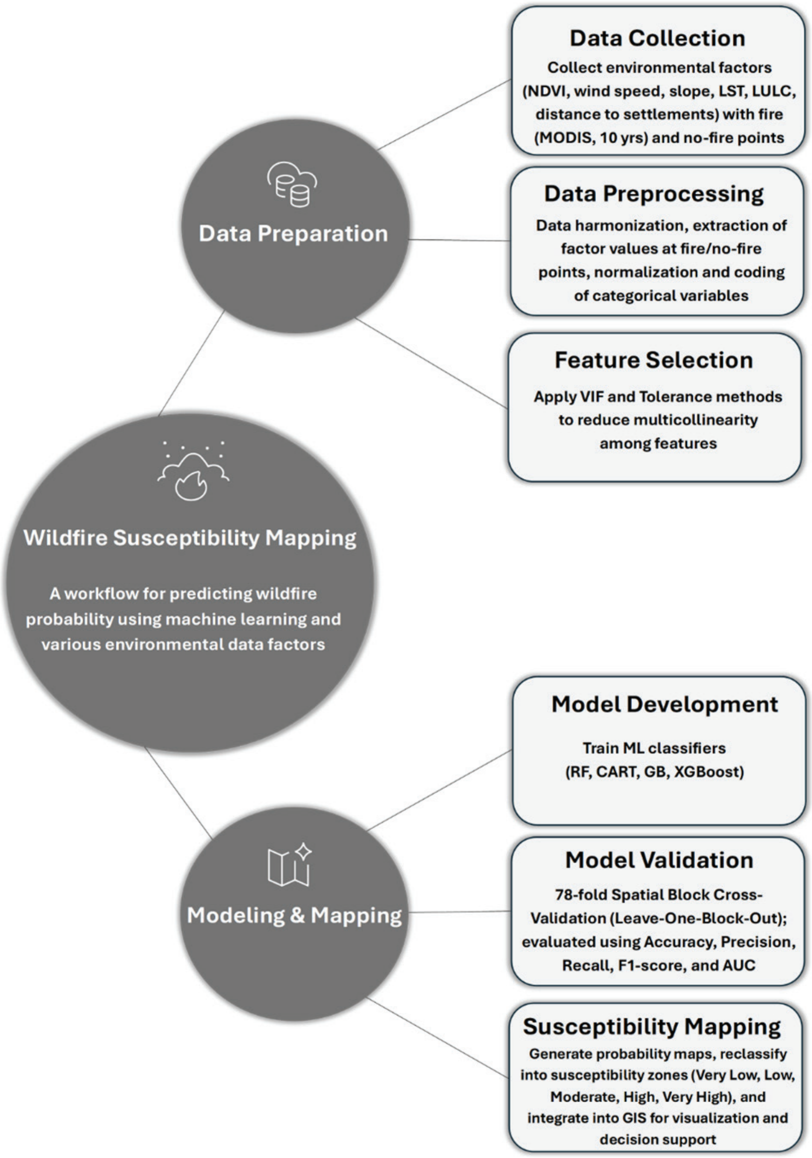


Figure 3. Workflow of wildfire susceptibility mapping in Portugal, integrating data preparation, machine learning model development, validation, and mapping.

land cover (LULC), and distance to settlements (Section 2.4). Using a point-based sampling approach, values for each of these variables were extracted directly from their native-resolution raster layers at the 2,000 sample locations, ensuring that multi-resolution datasets could be consistently integrated into a single feature table for model training (Tang et al. 2022).

Following the machine learning predictions at these sample locations, the discrete probability outputs were interpolated into a continuous spatial surface using the kriging method. Kriging was specifically selected because it is a robust geostatistical technique that accounts for spatial autocorrelation, providing statistically optimal predictions across unsampled areas (Diggle et al. 2003). This process yielded a final national wildfire susceptibility map with a uniform spatial resolution of 1 km, effectively harmonizing the varied resolutions of the initial predictor variables.

Before model training, the dataset was pre-processed. Categorical and continuous predictors were standardized into a compatible format, and anomalous records were removed to improve the data quality. To address the issue of multicollinearity among the explanatory variables, the Variance Inflation Factor (VIF) and tolerance methods were applied (Ahmed et al. 2024). All six variables were found to have VIF values below the conventional threshold of 10, indicating no significant multicollinearity issues and confirming their suitability for inclusion in the model (El Mazi et al. 2024).

Machine Learning Models

Four distinct machine learning algorithms were employed to model wildfire susceptibility: Random Forest (RF), Classification and Regression Tree (CART), Gradient Boosting (GB), and Extreme Gradient Boosting (XGBoost). These specific tree-based and ensemble algorithms were selected over traditional parametric models because wildfire ignition and spread are governed by highly complex nonlinear interactions between climate, topography, and human activity. Tree-based models are highly robust to outliers, do not require assumptions of data normality, and effectively manage the multidimensionality of spatial environmental datasets (Hastie et al. 2009). These models were selected because of their proven performance in spatial predictions and their ability to capture both linear and nonlinear relationships among predictors.

- Random Forest (RF): An ensemble learning method that builds a forest of K=200 decision trees $h(x, \Theta k)$, where Θk represents randomness in the tree-building process. The final classification is made by aggregating all tree votes via majority voting to improve prediction stability and reduce overfitting:

  $$\hat{f}(x) = \mathrm{mode}\{h(x, \Theta k), k=1, ...200\} \qquad (1)$$

  The choice of 200 trees balances model accuracy with computational efficiency (Breiman 2001).
- Classification and Regression Tree (CART): CART recursively partitions the feature space into distinct regions by selecting splits that maximize the reduction in impurity, which is typically measured by the Gini index for classification:

  $$\mathrm{Gini}(t) = 1-\sum_{i=1}^{j} p_i^2 \qquad (2)$$

  where p_i is the proportion of class i observations in node t. The result is a tree of decision rules that creates homogeneous groups with respect to wildfire occurrence (Breiman et al. 2017).
- Gradient Boosting (GB): GB builds an additive model in a forward stage-wise manner by minimizing a differentiable loss function L(y, F(x)) using weak learners $h_m(x)$ added sequentially:

  $$F_m(x) = F_{m-1}(x) + v \cdot h_m(x) \qquad (3)$$

  where v is the learning rate, and each h_m is trained on the residuals of the previous model F_{m-1} (Friedman 2001).
- Extreme Gradient Boosting (XGBoost): An optimized version of GB that includes regularization terms to control the model complexity. The objective function includes the loss function and a regularization term $\Omega(f)$:

  $$\mathrm{Obj} = \sum_j L(yi, \hat{y}) + \sum_k \Omega(f_k) \qquad (4)$$

  where f_k are the decision trees, and $\Omega(f_k)\ \Upsilon T + \frac{1}{2} \lambda \, ||w||^2$ with T as the number of leaves and w the leaf weight (Chen and Guestrin 2016).

Each model was trained on the training subset with hyperparameters optimized through a grid search, maximizing performance metrics such as accuracy, Area Under the Receiver Operating Characteristic Curve (AUC-ROC), and F1-score. Rather than utilizing a standard random hold-out validation set, which is susceptible to spatial data leakage, the grid search was executed using a nested cross-validation approach strictly within the 78-fold Spatial Block Cross-Validation framework.

Specifically, during each iteration, the hyperparameters were tuned exclusively on the data within the training blocks, ensuring the spatial test block remained completely unseen. The final optimized hyperparameters for each model are listed in Table 2. To ensure the robustness of the models and mitigate overfitting caused by spatial autocorrelation, a strict 78-fold Spatial Block Cross-Validation strategy was employed. Unlike traditional random k-fold cross-validation, which can leak spatially proximate training data into the validation set, this spatial blocking approach forces the models to predict wildfire occurrences in entirely unseen geographic regions, thereby guaranteeing the ecological and spatial validity of the final prediction.

These models complement each other by balancing bias-variance trade-offs and exploiting different strengths in handling variable interactions, missing data, and noise, making them suitable choices for spatial wildfire susceptibility prediction.

Model Validation and Feature Importance

All analyses were performed in Python within the Google Collab environment, using scikit-learn for machine learning and model evaluation, and stats models for multicollinearity diagnostics (Seabold and Perktold

Table 2. Optimized hyperparameters for the machine learning models were obtained via a grid search.

Model	Hyperparameter	Optimized Value
Random Forest	n_estimators	200
	max_depth	10
	max_features	sqrt
	min_samples_split	2
CART	max_depth	20
	min_samples_split	2
	min_samples_leaf	3
Gradient Boosting	n_estimators	200
	learning_rate	0.05
	max_depth	3
XGBoost	n_estimators	200
	learning_rate	0.05
	max_depth	3
	subsample	1.0
	colsample_bytree	0.8

2010, Tonini et al. 2020). A multi-step validation strategy was adopted to ensure the reliability of the wildfire susceptibility models. First, the explanatory variables were examined for multicollinearity using the Variance Inflation Factor (VIF) and tolerance statistics (O'Brien 2007).

The VIF for the ith predictor is defined as:

$$\mathrm{VIFi} = \frac{1}{1-R_i^2} \tag{5}$$

where R_i^2 is the coefficient of determination of the regression of the ith predictor against all other predictors. All six predictors (NDVI, slope, LST, wind speed, LULC, and distance to settlements) showed VIF values well below the threshold of 10, confirming the absence of significant multicollinearity and ensuring their suitability for the model.

To explicitly address spatial autocorrelation and provide rigorous algorithmic validation, a 78-fold Spatial Block Cross-Validation procedure was implemented. Unlike traditional random sampling, spatial blocking ensures that the training and validation points are geographically separated, forcing the model to predict entirely unseen spatial regions. Each model was evaluated using five key metrics (Area Under the Curve (AUC), accuracy, precision, recall, and F1-score) to comprehensively assess the overall correctness, the ability to detect fire occurrences, sensitivity to actual fires, and balance between precision and recall (Sokolova and Lapalme 2009). The performances were averaged across 78 spatial blocks to yield robust estimates, thereby mitigating spatial overfitting.

Instead of a traditional independent hold-out split (e.g., a random 80/20 split), which is prone to spatial data leakage, this spatial blocking effectively functions as a rigorous leave-one-block-out (LOBO) evaluation. By iteratively hiding an entire geographic region during training and evaluating solely on that unseen block, this method definitively confirms the predictive power of the models and their ability to generalize to new, unmapped territories.

To evaluate the influence of the predictors, permutation importance was utilized (Altmann et al. 2010). Unlike built-in impurity-based metrics that can be biased toward continuous variables, this model-agnostic technique quantifies feature importance by randomly shuffling the values of a single predictor and measuring the resulting decrease in the model performance score (e.g., AUC). A larger drop indicates that the model relies heavily on that feature. This robust technique allowed us to accurately quantify the relative contribution of each environmental and anthropogenic predictor to the wildfire susceptibility.

Finally, to validate the geostatistical interpolation of the model outputs, a cross-validation of the simple kriging method was performed in ArcGIS Pro (Esri 2025). Measured values were compared with predicted values at withheld locations, and error statistics such as the Root Mean Square Error (RMSE) and Mean Error (ME) were calculated (Cressie 1993).

These metrics are defined as follows:

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(Z(s_i) - \hat{Z}(s_i)\right)^2} \tag{6}$$

$$\mathrm{ME} = \frac{1}{n}\sum_{i=1}^{n}\left(Z(s_i) - \hat{Z}(s_i)\right)^2 \tag{7}$$

where $Z(s_i)$ and $\hat{Z}(s_i)$ are the observed and predicted values at location s_i, Z and $\hat{Z}$ are their respective means, and n is the number of validation points.

This additional step ensured that the interpolated susceptibility surface preserved the spatial structure of the predictions, while maintaining acceptable accuracy.

This combined validation framework, including multicollinearity analysis, spatial block cross-validation, and permutation importance diagnostics, ensured both the statistical robustness and ecological interpretability of the wildfire susceptibility models.

Susceptibility Mapping

The final wildfire susceptibility map was generated by applying the best-performing machine learning model to the predictor variables across the entire study area, producing continuous wildfire probability values between 0 and 1. To account for spatial dependencies, these outputs were interpolated using the geostatistical method of simple kriging (O'Sullivan and Unwin 2010).

While kriging is conventionally applied to continuous physical variables, its application to machine learning probability outputs was deliberately chosen for this study. It effectively smooths discrete, pixel-based probabilities into a continuous regional susceptibility surface, mitigating localized noise.

This geostatistical smoothing accounts for the inherent spatial autocorrelation of fire spread, providing decision-makers with generalized regional "hot-spots" that are more practical for landscape-scale management than fragmented pixel predictions. This method incorporates spatial autocorrelation to produce a smooth and realistic susceptibility surface compared to deterministic approaches such as Inverse Distance Weighting (IDW).

Simple kriging estimates the value at an unsampled location s_0 as a weighted sum of the observed values:

$$\hat{Z}(s_0) = \sum_{i=1}^{n} \lambda_i Z(s_i) \tag{8}$$

where $Z(s_i)$ are the known values at locations s_i , and weights λ_i are calculated based on the spatial covariance structure between the points to minimize estimation variance and ensure unbiasedness (Cressie 1993).

The interpolated susceptibility surface was reclassified into five risk levels: very low (0.00–0.20), low (0.2–0.40), medium (0.40–0.60), high (0.60–0.80), and very high (0.80–1.00) (Thies 2025). These thresholds were defined based on exploratory data analysis and expert consultation to support meaningful interpretation for fire risk management. The choice of a 1 km grid size for the final output ensured that the map remained computationally efficient for national-scale planning while matching the spatial grain of the primary input data.

The uncertainty of the kriging interpolation was examined through cross-validation in ArcGIS Pro (Geostatistical Wizard), where predicted values were compared with the observed samples. The full validation metrics are presented in the Results section.

The integration of machine learning predictions with geostatistical interpolation produced a wildfire susceptibility map that is both statistically robust and spatially coherent, offering valuable insights to guide targeted fire prevention and mitigation strategies.

RESULTS

Variable Analysis and Multicollinearity

Before developing the wildfire susceptibility model, it was essential to evaluate the predictor variables to ensure their relevance to wildfire occurrence and to confirm statistical independence. Multicollinearity among explanatory variables can distort model estimates and reduce predictive reliability, especially in machine learning techniques such as Random Forest (Breiman 2001, Dormann et al. 2013). To address this, six environmental and anthropogenic variables were examined—Land Use/Land Cover (LULC), Normalized Difference Vegetation Index (NDVI), Land Surface Temperature (LST), Slope, Wind Speed, and Distance to Settlements—and their spatial distribution was analysed alongside a statistical multicollinearity assessment using Variance Inflation Factor (VIF) and tolerance values.

The spatial distributions of these predictor variables are illustrated in Figure 4, with each subfigure highlighting a specific environmental factor. Land use and land cover (Figure 4a) reflect the spatial extent of forests, agriculture, bare soil, and built-up areas, where forest-dominated zones, particularly in mountainous regions, correspond to areas of high fuel accumulation, and thus increase wildfire susceptibility (Vanderhoof and Hawbaker 2018). Normalized difference vegetation index (Figure 4b) measures vegetation density, where high NDVI values indicate dense vegetation that serves as potential fuel for aiding fire ignition and spreading. Land surface temperature (Figure 4c) captures thermal variation, with elevated temperatures in exposed lowlands and urban regions coinciding with higher ignition probabilities (Vermote et al. 2016). Terrain slope (Figure 4d) has a dual influence on wildfire dynamics; steeper slopes accelerate fire spread by promoting the preheating of upslope vegetation, whereas flatter and more accessible terrains are frequently associated with higher rates of anthropogenic fire ignition. Wind speed (Figure 4e) influences fire dynamics, increasing spread rates, and determining direction, with higher wind speeds correlating with elevated wildfire hazards (Stephens 2001). Finally, distance to settlements (Figure 4f) reflects anthropogenic ignition pressure, with areas closer to urban clusters exhibiting elevated ignition potential (Grala and D'Agata 2024).

Multicollinearity was further assessed through VIF and tolerance metrics for each predictor (Figure 5). These statistics demonstrate the degree of correlation between each variable. All six predictors exhibited VIF values between 1.45 and 3.9, well within acceptable limits (threshold < 10), and tolerance values ranged from 0.26 to 0.69, exceeding the common cutoff of 0.1 (O'Brien 2007).

For instance, NDVI (VIF = 2.54; tolerance = 0.39) and LST (VIF = 3.28; tolerance = 0.30) showed moderate correlations, as expected owing to biophysical links, but remained statistically independent. The highest degree of multicollinearity was observed in LULC (VIF = 3.81; tolerance = 0.26), whereas the lowest was found in Distance to Settlements (VIF = 1.45; tolerance = 0.69), confirming that no problematic redundancy exists within the feature set.

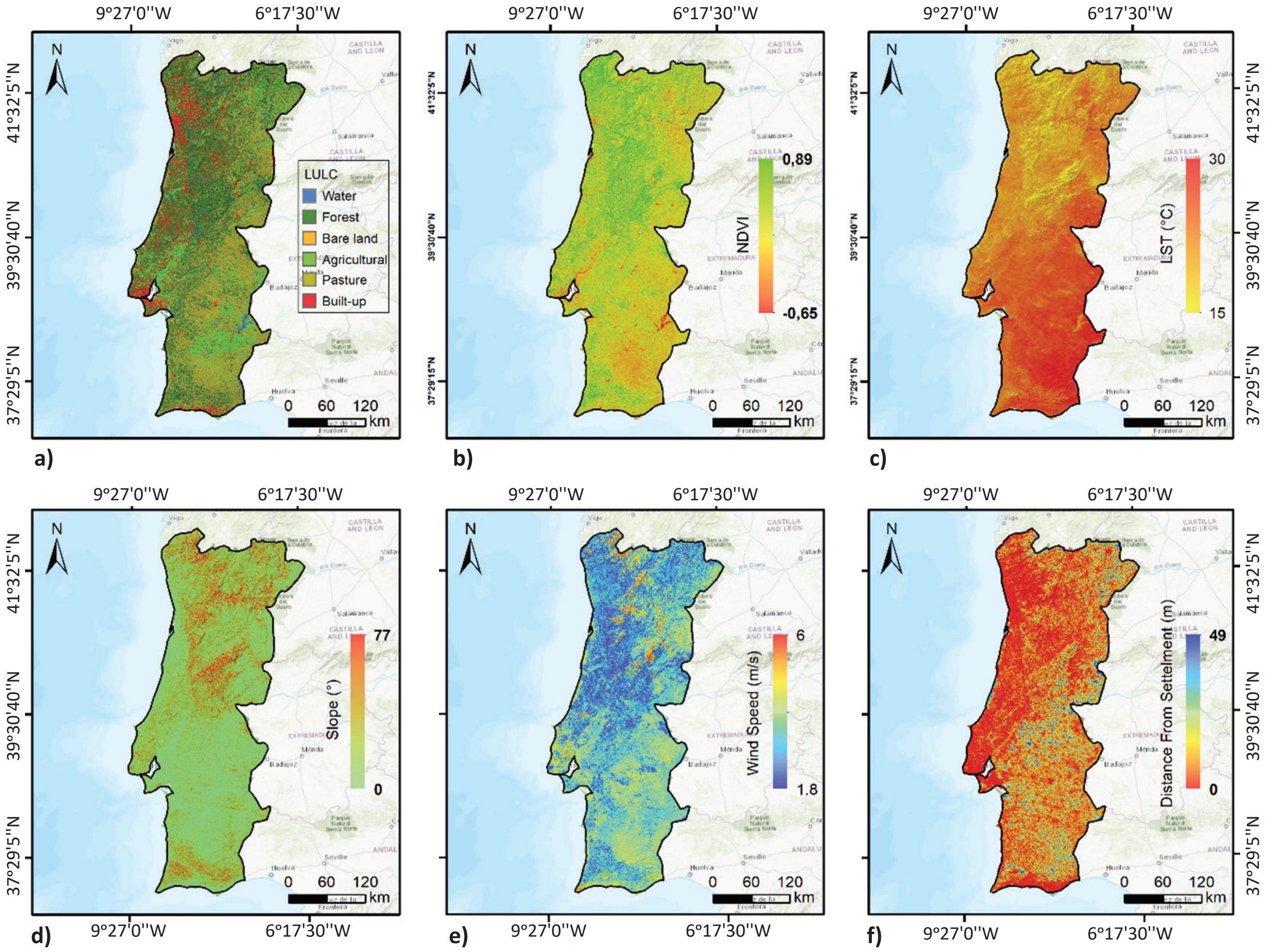


Figure 4. Spatial distribution of wildfire predictor variables: **(a)** LULC, **(b)** NDVI, **(c)** LST, **(d)** Slope, **(e)** Wind Speed, and **(f)** Distance to Settlements.

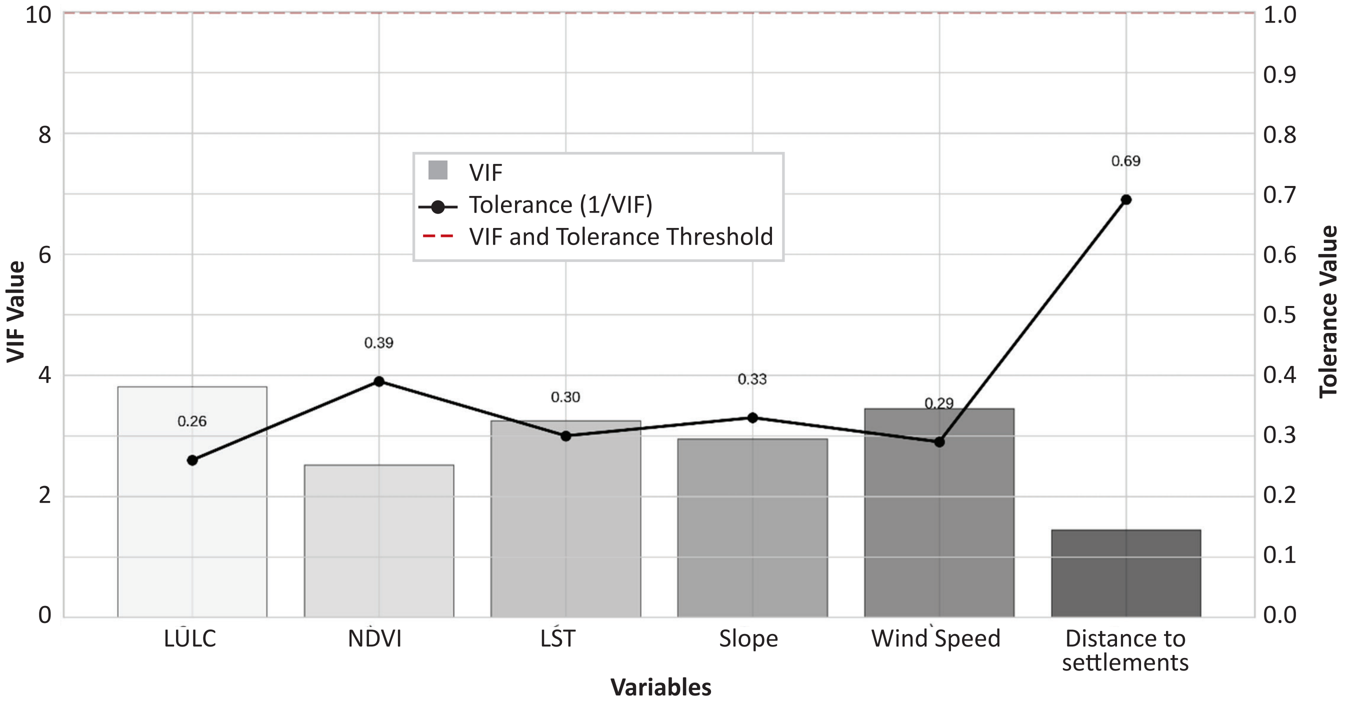


Figure 5. Variance Inflation Factor (VIF) and tolerance values for wildfire predictor variables.

The combined spatial and statistical analyses confirm that the selected variables are ecologically meaningful and statistically independent. This supports their integration within the Random Forest modelling framework, ensuring stability and reliability of wildfire susceptibility predictions. Hence, the six variables—LULC, NDVI, LST, Slope, Wind Speed, and Distance to Settlements—are retained for subsequent modelling stages.

Model Performance and Cross-Validation

After validating the selected variables, the next step was to evaluate the performance of the machine learning models used in this study. To provide a clear comparison, Figure 6 summarizes the key performance metrics obtained for each model—Random Forest (RF), Classification and Regression Trees (CART), Gradient Boosting (GB), and Extreme Gradient Boosting (XGBoost)—evaluated rigorously using the 78-fold Spatial Block Cross-Validation procedure, which ensures spatial independence between training and testing data (Chen and Guestrin 2016).

Figure 6 presents both the visual bar charts and the averaged quantitative values for Accuracy, Precision, Recall, F1-Score, and the Area Under the Curve (AUC) across the 78 unseen geographic blocks. These metrics provide a comprehensive understanding of each model's ability to correctly classify instances while accounting for both false positives and false negatives in spatially independent test regions (Sokolova and Lapalme 2009).

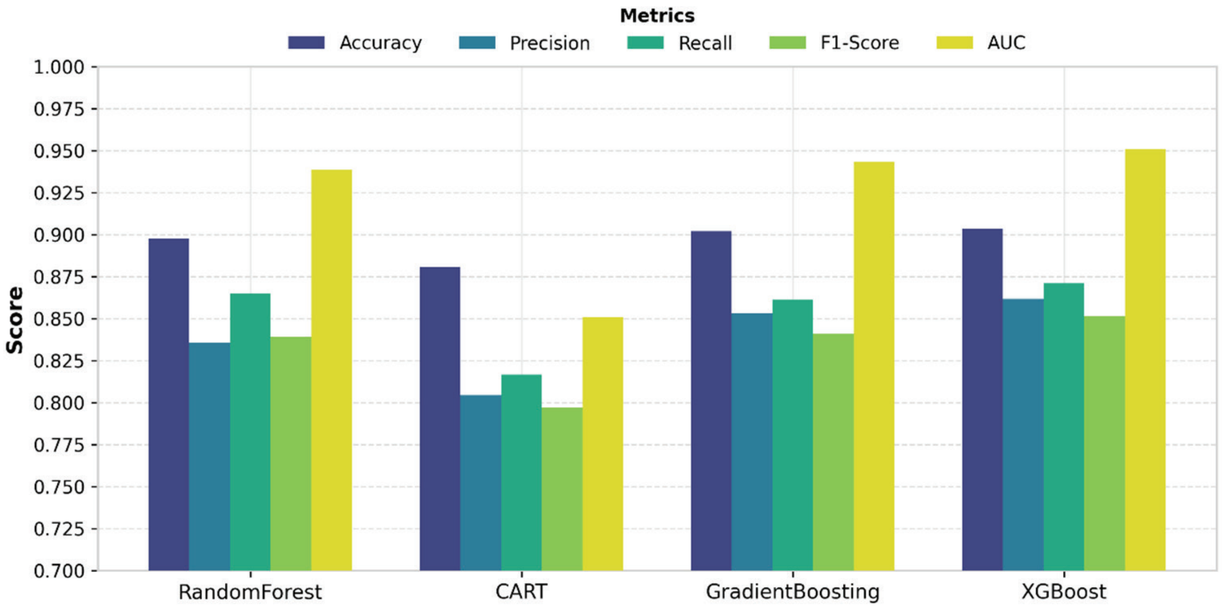
As detailed in Figure 6, XGBoost (Chen and Guestrin 2016) achieved the highest overall performance, with an accuracy of 90.03%, precision of 86.17%, recall of 87.10%, F1-score of 85.12%, and an outstanding spatial AUC of 0.9510. It is closely followed by Gradient Boosting and Random Forest (Breiman 2001) with CART lagging notably behind in all metrics.

Overall, based on the spatial block cross-validation results, XGBoost was identified as the best-performing model. Its superior metrics and proven ability to generalize to unseen geographic regions justify its selection for subsequent analysis and final predictions.

Wildfire Susceptibility Maps and Area Statistics

The spatial distribution of wildfire susceptibility across the study area was modelled using four machine learning approaches: Random Forest (RF) (Breiman 2001), Classification and Regression Trees (CART) (Breiman et al. 2017), Gradient Boosting (GB) (Friedman 2001), and Extreme Gradient Boosting (XGBoost) (Chen and Guestrin 2016). The resulting susceptibility maps (Figure 7) classified the territory into five fire risk categories: Very Low, Low, Medium, High, and Very High risk.

The visual examination of the wildfire susceptibility maps (Figure 7) revealed a coherent spatial distribution across all four machine learning models. Predominantly, the central and northern regions exhibit extensive areas classified as having high and very high susceptibility,



	Accuracy	Precision	Recall	F1-Score	AUC
RandomForest	0.8977	0.8357	0.8651	0.8389	0.9386
CART	0.8806	0.8045	0.8166	0.7972	0.8506
GradientBoosting	0.9019	0.8531	0.8612	0.8412	0.9435
XGBoost	0.9033	0.8617	0.8710	0.8512	0.9510

Figure 6. Comparison of Spatial Cross-Validation metrics (Accuracy, Precision, Recall, F1-Score, and AUC) across the RF, CART, GB, and XGBoost models.

corresponding closely to forested and mountainous terrains known for elevated fuel loads and complex topography (Catry et al. 2010). Importantly, isolated pockets of very high susceptibility were also evident in the southern part of the study area, underscoring localized conditions that may facilitate fire ignition and spread despite generally sparser vegetation. While the broad spatial patterns remained consistent, subtle differences in the extent and delineation of high-risk zones were apparent among the models, reflecting inherent variations in the handling of predictor interactions and algorithmic sensitivity.

To quantitatively assess these variations, the classified maps were overlaid with the national boundary, and the areal extent of each susceptibility class was calculated (Table 3). Across the models, the Very Low and Low susceptibility categories consistently covered the largest portion of the territory, jointly accounting for approximately 42%–48% of the study area. The Medium susceptibility class remained highly stable across all algorithms, representing approximately 20%–21% of the region. The high-risk zones (High and Very High combined) constituted between 30% and 36% of the landscape. Notably, XGBoost and CART allocated the highest proportion to the Very High class (19.08%), which aligns with their sensitivity to capturing extreme spatial variations in fire risk and XGBoost superior performance in the cross-validation analysis.

To synthesize these findings and reduce the uncertainty inherent in any single algorithm, an integrated wildfire susceptibility map was generated (Figure 8). This consensus map was created by aggregating and averaging the continuous probability outputs of the four machine learning models prior to the final risk classification. By

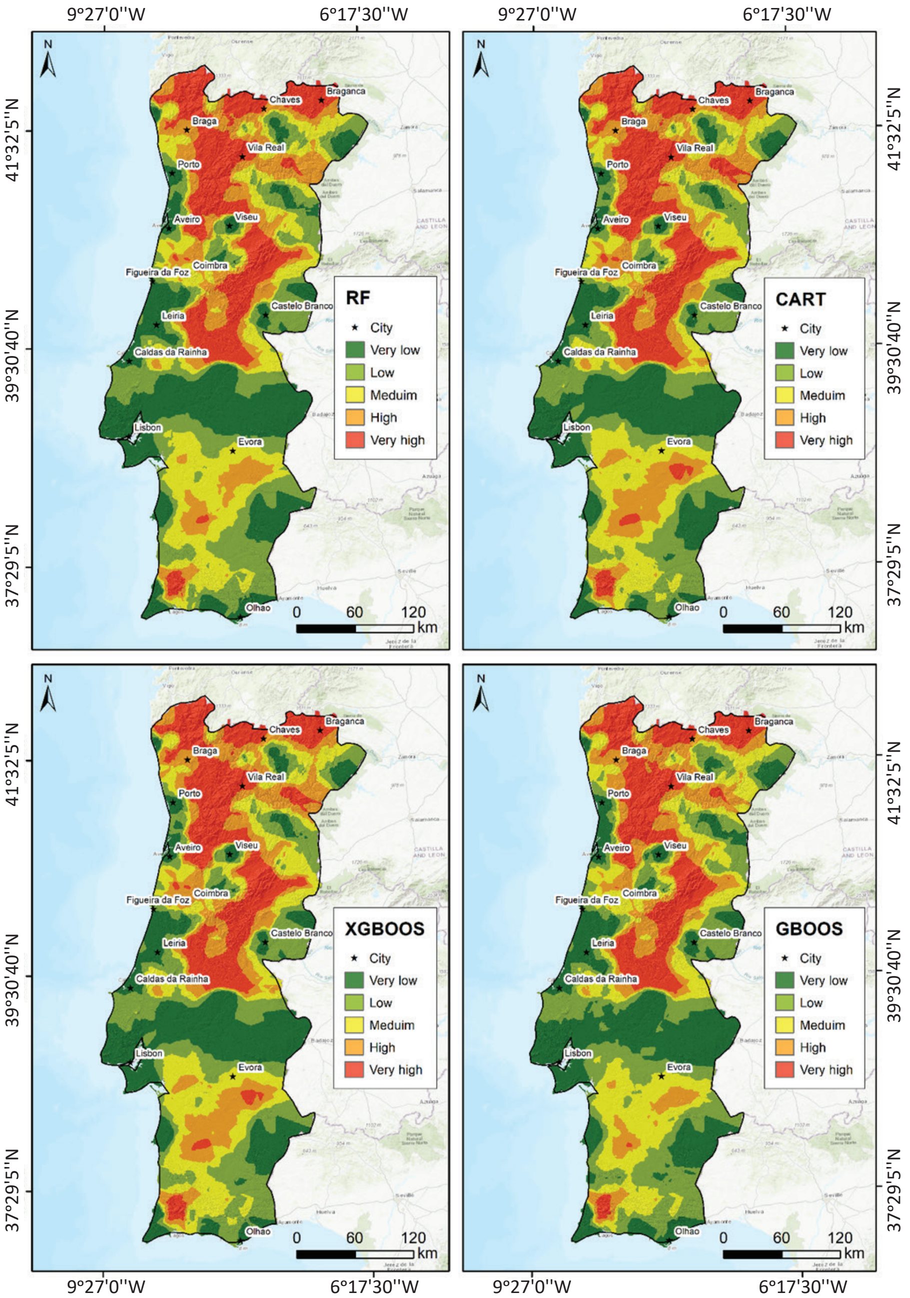


Figure 7. Wildfire susceptibility maps derived from RF, CART, GB, and XGBoost models.

Table 3. Area distribution of wildfire susceptibility classes (Very Low, Low, Medium, High, Very High) across the RF, CART, GB, and XGBoost models.

Model	Distribution	Susceptibility Classe				
		Very low	**Low**	**Medium**	**High**	**Very high**
Random Forest	Area (km²)	20,351.66	20,766.60	18,335.25	13,534.84	15,703.38
	Percentage (%)	22.94	23.41	20.67	15.26	17.70
CART	Area (km²)	19,405.70	18,418.99	18,541.25	15,397.12	16,928.43
	Percentage (%)	21.88	20.76	20.90	17.36	19.08
Gradient Boosting	Area (km²)	21,615.00	20,851.81	18,784.53	12,382.65	15,057,51
	Percentage (%)	24.37	23.51	21.17	13.96	16.97
Extreme Gradient Boosting	Area (km²)	19,420.52	19,110.56	17,873.16	15,358.84	16,928.43
	Percentage (%)	21.89	21.54	20.15	17.31	19.08

combining the predictive strengths of the individual algorithms, the integrated map effectively mitigates model-specific spatial biases and provides a more robust and high-confidence delineation of wildfire-prone areas. The resulting ensemble surface reinforces the critical high-risk zones identified in the central and northern regions, establishing a highly reliable baseline for national fire management and resource allocation planning.

The probabilistic model outputs were interpolated into continuous susceptibility surfaces using the simple kriging method (Diggle et al. 2003). Cross-validation of the kriging interpolation resulted in moderate accuracy, with a Root Mean Square Error (RMSE) of 0.1462 (normalized between 0 and 1) (Chai and Draxler 2014), indicating that average prediction errors account for approximately 15% of the data range. The Mean Error (ME) was very close to zero (+0.00044), confirming the unbiased nature of the interpolated predictions. This suggests that while the interpolation effectively captures broad spatial trends, finer local variations may be less accurately represented.

Together, the wildfire susceptibility maps and area statistics highlight the pronounced spatial heterogeneity of fire risk in the region. Although all models broadly agree on the identification of northern and central hotspots, the creation of an integrated consensus map provides the most actionable insight for prioritizing fire-prevention strategies. Among the individually tested algorithms, XGBoost emerged as the most robust, whereas the integrated ensemble approach offered the highest degree of spatial reliability.

Permutation Feature Importance

To better understand the contribution of each explanatory factor in shaping wildfire susceptibility, a feature importance analysis was performed using the model-agnostic permutation importance technique (Altmann et al. 2010). This approach quantifies the strength of influence of each variable by measuring the decrease in model performance (AUC) when a specific predictor is randomly shuffled, thereby providing unbiased insights into the ecological and environmental drivers of wildfire risks without the biases inherent to traditional tree-based impurity scores.

The results summarized in Figure 9 show the relative importance of the six predictor variables across the four classifiers (Random Forest, CART, Gradient Boosting, and XGBoost). Two consistent patterns can be observed:

- NDVI (vegetation greenness) was the most influential variable across nearly all models, highlighting the central role of vegetation density and fuel availability in the occurrence of wildfires (Chuvieco et al. 2008).
- Slope and LULC were ranked as secondary but critical predictors, indicating that topography and land cover structure also strongly modulate susceptibility patterns and fire behaviour.
- LST and wind speed contributed moderately, reflecting their roles as climatic and micro-environmental conditions that influence ignition probability and spread rates.
- The distance to settlements generally showed the lowest influence, although it was still non-negligible. This suggests that while anthropogenic proximity is a factor, it is less dominant than biophysical and topographic conditions in determining overall susceptibility in this region.

The exclusive use of permutation importance strengthens the robustness of these results. Because it measures the actual predictive loss on unseen data when a variable's spatial structure is broken, it provides a highly reliable metric of variable influence (Tonini et al. 2020). Together, these analyses confirm that vegetation condition (NDVI), terrain (slope), and land cover type (LULC) are the most decisive factors driving wildfire susceptibility in the study area. This provides valuable ecological insight into both the spatial drivers of fire risks and the interpretability of machine learning predictions.

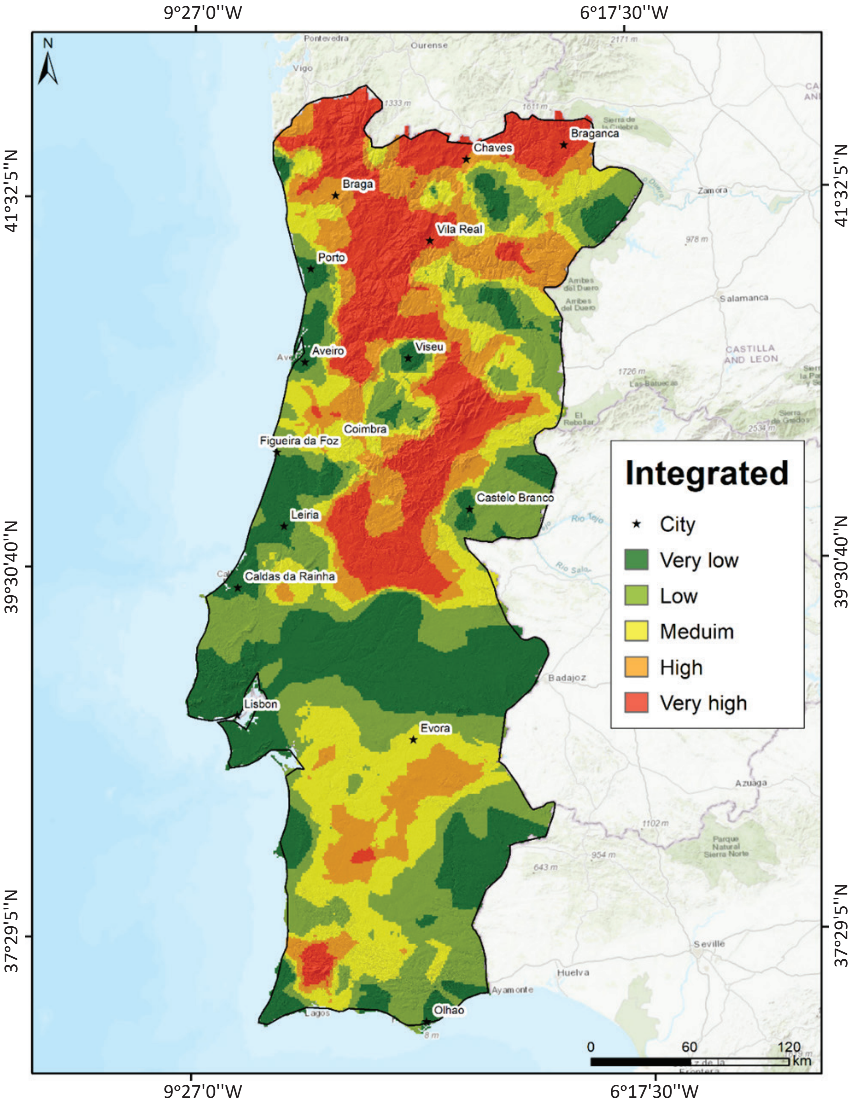


Figure 8. Integrated wildfire susceptibility map combining the probabilistic outputs of the four machine learning models.

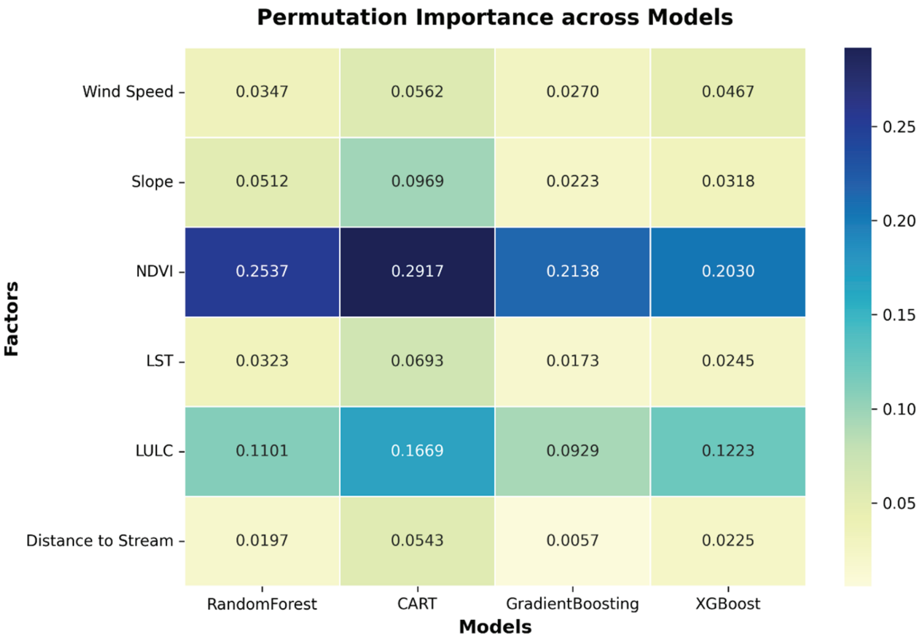


Figure 9. Permutation feature importance scores for the wildfire predictors across the RF, CART, GB, and XGBoost models.

DISCUSSION

The findings of this study offer significant insights into the spatial patterns and primary determinants of wildfire susceptibility in Portugal. In contrast to numerous prior studies that predominantly utilized a single machine learning classifier, this study systematically compared four algorithms—Random Forest, Classification and CART, Gradient Boosting, and XGBoost—and synthesized them into an integrated ensemble map. This comparative framework enhances model reliability and contributes to the expanding body of literature that assesses the strengths and limitations of ensemble learning approaches for wildfire prediction (Bjånes et al. 2021, Bhowmik et al. 2023).

The analysis of variable importance, evaluated using robust permutation techniques, indicated that vegetation-related factors, particularly NDVI and LULC, were the most influential predictors of wildfire susceptibility in the study area. These findings align with ecological studies that highlight the role of fuel availability and type in influencing ignition likelihood and fire spread (Fernandes and Botelho 2003, Parente et al. 2018). Topographic variables such as slope and climatic variables, including wind speed, were also significant, corroborating their well-established influence on fire behaviour (Parisien et al. 2016). The relatively lower weight of anthropogenic variables, such as distance to settlements, suggests that biophysical conditions remain the dominant drivers of susceptibility in Portugal.

Model performance evaluation confirmed the superior predictive capability of the ensemble techniques, with XGBoost achieving the highest accuracy (90.03%), followed by Spatial AUC (0.9510). These values are comparable to, and in some cases exceed, those reported in recent wildfire susceptibility studies across the Mediterranean and other fire-prone landscapes (Chuvieco et al. 2019, Rodrigues et al. 2021). The high predictive skill demonstrates the ability of XGBoost to capture complex, nonlinear relationships between environmental conditions and fire occurrence.

These results are highly consistent with recent advancements in wildfire susceptibility mapping that utilize similar machine learning frameworks across diverse geographic contexts. For instance, Thies (2025) achieved high predictive accuracies using machine learning models in Germany, highlighting the broad robustness of these algorithms. Similarly, Durlević et al. (2025) demonstrated that the fusion of multi-sensor satellite data with machine learning models yielded exceptional susceptibility maps in Serbia, reinforcing the value of integrating high-resolution topographic and climatic variables. Furthermore, Sapkota et al. (2025) successfully advanced wildfire prediction in Nepal using machine learning, echoing our findings that ensemble methods, such as XGBoost, which are highly adept at capturing nonlinear environmental drivers across complex terrains.

Given the exceptionally high predictive performance (AUC > 0.95), it is important to note that such metrics can sometimes raise concerns regarding model overfitting. However, the strict implementation of the 78-fold Spatial Block Cross-Validation in this study explicitly prevented spatial data leakage, ensuring that the model's accuracy reflected true predictive power rather than geographic memorization. Therefore, the high score is likely driven by the stark biophysical contrasts inherent to the Portuguese landscape, specifically to the sharp ecological dichotomy between the highly flammable, densely forested northern topographies and the more fuel-limited southern plains. This clear geographic separation makes the binary classification of fire presence/absence highly distinct for advanced tree-based algorithms such as XGBoost.

The final susceptibility maps consistently identified the northern and central interior regions as the most fire-prone, which is in line with historical fire records and prior research on Portuguese fire regimes (Calheiros et al. 2022, European Commission Joint Research Centre 2023). Quantitative area statistics further underscored the dominance of the Very High susceptibility category in these zones. These outputs not only validate the modelling framework but also offer actionable insights into fire management, land-use planning, and resource prioritization.

Nevertheless, certain limitations must be acknowledged. The uniform 1 km spatial resolution of certain variables, such as LST and wind speed, while appropriate for national-scale kriging interpolation, may not capture critical micro-scale heterogeneity in topography and fuel types. Furthermore, the fire occurrence dataset may be subject to sampling bias and under-reporting. Finally, although wildfire dynamics are inherently temporal, the explanatory variables used in this study represent static snapshots.

This static approach, which does not account for seasonal droughts or shifting human activity over time, represents a simplification of real-world complexity and could influence the model’s predictive precision at the local scale. Future research should integrate higher-resolution environmental datasets, adopt multi-temporal variables to account for seasonal variability, and test hybrid approaches that combine remote sensing with socio-economic drivers to build a more comprehensive and robust model.

CONCLUSIONS

This study demonstrated the efficacy of machine learning, with a particular focus on XGBoost, in mapping wildfire susceptibility in Portugal. Through a systematic comparison of four classifiers, rigorous spatial block cross-validation, and the creation of an integrated consensus map, this study confirmed that vegetation (NDVI, LULC) and topography (slope) are the primary determinants of fire occurrences. A key contribution of this research is the methodological shift from traditional random cross-validation toward a strict spatial blocking approach, which effectively eliminates spatial data leakage and provides a more honest, ecologically valid assessment of model generalization.

The practical applicability of these findings is highly relevant to national and regional decision-makers. The resulting susceptibility maps, which clearly identify northern and central Portugal as the most critical risk zones, provide a data-driven baseline for institutions such as the

Portuguese National Authority for Emergency and Civil Protection (ANEPC), the Institute for Nature Conservation and Forests (ICNF), and local municipal planning boards. By utilizing the integrated ensemble map, these agencies can optimize the allocation of limited firefighting resources during the peak summer fire season.

Furthermore, the identification of specific environmental drivers directly informs the development of targeted wildfire protection measures. As vegetation density (NDVI) and land cover (LULC) were identified as the most dominant risk factors, land managers can implement proactive mitigation strategies. These include targeted fuel reduction treatments, the establishment of controlled prescribed burns, and the creation of strict buffer zones (firebreaks) around wildland-urban interfaces (WUI) in the Very High susceptibility zones. Additionally, early warning systems and localized monitoring can be enhanced in areas where steep slopes and high wind speeds intersect with dense-forest cover.

Despite certain limitations related to the variable spatial resolution and static nature of the environmental datasets, the framework provides highly reliable and transferable tools for supporting fire management, land-use planning, and risk reduction. Future research should incorporate finer-scale, multi-temporal environmental, and socioeconomic data to further enhance predictive performance and capture the dynamic nature of fire regimes.

Author Contributions

MAL conceived and designed the study, performed data collection and processing, implemented the machine learning models, conducted the analysis, and wrote the manuscript. MA contributed to maps preparation, supervised the research, and revised the manuscript. BA supervised the research and contributed to manuscript revision. All authors have read and approved the final version of the manuscript.

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Competing Interests

The authors declare no competing interests.

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Climatic Drivers of Vegetation Activity and Radial Growth in Mediterranean *Pinus sylvestris* from Central Albania

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ABSTRACT

Sustainable management of *Pinus sylvestris* L. plantations under changing climatic conditions requires a comprehensive understanding of how climate variability and drought influence tree growth. This study investigates vegetation activity assessed via the Normalised Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI), and radial growth variability, (EW – earlywood width, LW – latewood width, TRW – tree-ring width, and BAI – basal area increment), in a *P. sylvestris* plantation in central Albania. This is the first such study conducted in Albania, addressing a significant geographical gap in the southernmost natural range of this species in the Balkans. Climate-growth relationships were explored using Pearson correlation analysis with temperature, precipitation, and drought indices (SPI – Standardised Precipitation Index and SPEI – Standardised Precipitation Evapotranspiration Index). August NDVI and EVI correlated positively with LW and BAI. Both vegetation indices were positively associated with spring and autumn temperatures, while EVI with June and September precipitation. NDVI was most responsive to the 7-month SPI and SPEI in April, whereas EVI was sensitive to the 7-month SPI in September and the 4-month SPEI in June. Summer temperatures negatively impacted LW and BAI. July precipitation strongly enhanced LW, TRW, and BAI. EW showed the highest positive correlation with the 4-month SPI in August but was negatively correlated at scales longer than 14 months, in winter and spring. LW and TRW demonstrated remarkable positive associations with SPI and SPEI in July (1-month). BAI was most drought-sensitive in August (4-month for SPEI and 6-month for SPI). These findings provide critical insights into the climate sensitivity of *P. sylvestris* and offer a scientific basis for adaptive forest management in Mediterranean regions increasingly affected by drought and warming trends.

Keywords: vegetation indices; tree-ring; temperature; precipitation; drought

INTRODUCTION

Mediterranean forests represent ecosystems of exceptional ecological and socio-economic significance, delivering a range of crucial services such as carbon sequestration, biodiversity conservation, hydrological regulation, and soil stabilisation (Schueler et al. 2014, Fyllas et al. 2017, Güner and Makineci 2017, Sidor et al. 2018). However, their functional integrity is increasingly undermined by the multifaceted impacts of climate change, particularly rising temperatures and prolonged drought episodes (Lévesque et al. 2014). These climatic stressors pose substantial threats to forest health, growth and productivity, especially within the southern and eastern sectors of the Mediterranean basin, where ecosystems are already operating near the limits of their ecological tolerance (Herguido et al. 2016, Sidor et al. 2018, Herrero et al. 2023). Among these climatically vulnerable areas, the Balkans occupy a distinctive position due to the climatic heterogeneity and transitional ecological nature, situated at the confluence of continental and Mediterranean climate regimes (MedECC 2020, IPCC 2023).

The broader Mediterranean region is recognised as a global climate change hotspot, with models projecting pronounced temperature increases (currently approximately 1.5°C above pre-industrial levels, potentially reaching 3.8°C by 2100) and substantial reductions in precipitation (10–15% currently, with projections of up to 30% decline by 2100) (MedECC 2020, IPCC 2023). These shifts are anticipated to exacerbate drought frequency and severity, elevate wildfire risk, and prolong dry spells, thereby imposing mounting hydrological stress on forest ecosystems with direct consequences for tree growth, regeneration, and ecosystem resilience (Sánchez-Salguero et al. 2015, Senf et al. 2018). Mountainous and orographically complex landscapes, such as those characteristics of the Balkans, are particularly susceptible to such climatic perturbations, which are predicted to substantially alter forest functioning (Sánchez-Salguero et al. 2015, Herguido et al. 2016, Fyllas et al. 2017). Tree species at the limits of their distribution are especially vulnerable due to heightened exposure to microclimatic extremes, including increased radiation, elevated temperatures, and reduced humidity relative to forest interiors (Herguido et al. 2016, Herrero et al. 2023). These edge effects intensify physiological stress, inducing earlier stomatal closure, reduced photosynthetic capacity, and, in some cases, long-term carbon depletion (Sánchez-Salguero et al. 2015, Römer et al. 2025).

Within this context, elucidating the sensitivity and resilience of key forest species such as *Pinus sylvestris L.* is essential for anticipating climate change impacts and informing adaptive management strategies (Taeger et al. 2013, Lévesque et al. 2014). *P. sylvestris* ranks among the most extensively distributed and ecologically plastic coniferous species in the Northern Hemisphere (Camarero et al. 2021, Waszak et al. 2021, Römer et al. 2025). Within Europe, its distribution spans a wide climatic gradient, from boreal forests in Scandinavia to arid mountainous regions in the Mediterranean basin (Houston Durrant et al. 2016; see also Figure 1). In the Balkans and Eastern Europe, *P. sylvestris* predominantly occupies upland and mountainous habitats, frequently forming pure or mixed stands with broadleaf species (Passayiannes et al. 1998, Gurcay et al. 2025). These forests often occur on steep slopes, dry ridges, and nutrient-poor acidic soils, where the species' pioneer character, drought tolerance, and low nutrient requirements facilitate persistence in environmentally extreme habitats (Sidor et al. 2018). However, its productivity and vitality are increasingly affected by reduced precipitation and rising temperatures, with greater vulnerability to heatwaves and soil moisture deficits leading to lower growth rates (Candel-Pérez et al. 2012, Sánchez-Salguero et al. 2015, Herguido et al. 2016, Sidor et al. 2018, Brichta et al. 2024).

Remote sensing techniques offer powerful means to monitor forest productivity and health over broad spatial and temporal scales (Pasho et al. 2011b, Vicente-Serrano et al. 2012). Vegetation indices such as the Normalised Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI) provide robust proxies for canopy greenness and primary production, correlating closely with chlorophyll content and photosynthetic activity (Alla et al. 2017, 2025, Buras et al. 2023, Mašek et al. 2023). While NDVI reliably captures seasonal and inter-annual vegetation dynamics, EVI enhances sensitivity within dense canopies by mitigating atmospheric and background interference, thereby facilitating a more comprehensive characterisation of photosynthetic dynamics (Huete et al. 2002, Alla et al. 2025). Complementing remote sensing, dendroecology enables detailed reconstruction of tree growth and vitality through annual tree-ring analysis (Lévesque et al. 2014, Römer et al. 2025). Dissecting tree-ring width (TRW) into earlywood (EW) and latewood (LW) components affords seasonal resolution of growth responses to climate, with EW formation reflecting spring moisture availability and LW growth correlating with summer climatic conditions (Tabakova et al. 2020). This differentiation illuminates physiological mechanisms, such as the reliance of EW on carbohydrate reserves from the previous year and LW dependence on current photosynthetic carbon assimilation (Camarero et al. 2021).

Although the integration of dendrochronology and remote sensing is well recognised, research on *P. sylvestris* in the Balkans remains limited (Passayiannes et al. 1998, Fyllas et al. 2017), with no research conducted to date in Albania, its southernmost natural range. While a recent multi-species dendroecological study across central and eastern Europe combined tree-ring and remotely sensed vegetation data to map annual forest growth (Jevšenak et al. 2024), Albanian populations of *P. sylvestris* were not included, leaving the southern part of the species' European range underrepresented. This knowledge gap is of particular concern given the species' ecological relevance and increased sensitivity at its southern range margins, where climate stress responses often emerge first (Misi and Náfrádi 2017, Herrero et al. 2023, Gurcay et al. 2025, Römer et al. 2025). Findings from southern Iberia show that *P. sylvestris* forests are increasingly impacted by drought and warming, leading to defoliation, reduced radial growth, and higher mortality rates (Herguido et al. 2016, Royo-Navascues et al. 2021, Herrero et al. 2023). Although local adaptation may offer partial resilience, the species' overall ability to cope with increasingly severe hot droughts remains uncertain (Taeger et al. 2013, Matías et al. 2017, Nechita et al. 2022). These threats jeopardise both forest sustainability and the ecological integrity of vulnerable Mediterranean ecosystems (Matías et al. 2017, Shestakova et al. 2017).

Addressing this critical research gap, the present study aims to characterise the sensitivity and growth response of *P. sylvestris* to climatic variability and drought stress within the Mediterranean region of Albania. The study objectives were to: (i) assess temporal variability in canopy-level vegetation activity (NDVI and EVI) and radial growth (EW, LW, TRW, BAI) of *P. sylvestris* in central Albania and examine their interrelations; and (ii) evaluate the impacts of climatic variables (temperature, precipitation) and drought indices (SPI, SPEI) on primary (NDVI, EVI) and secondary (EW, LW, TRW, BAI) growth dynamics. By integrating high-resolution dendrochronological analyses with satellite-derived vegetation monitoring, this work seeks to deepen the understanding of *P. sylvestris* growth dynamics and its adaptive capacity under climate perturbations within a climatically sensitive Mediterranean environment (MedECC 2020, IPCC 2023).

MATERIALS AND METHODS

Study Area and Species

The study area is situated in central Albania, within the Tirana municipality (Figure 1).

Topographically, the study site is positioned at geographical coordinates 41°20'14"N, 20°07'35"E, at a mean elevation of 1270 metres above sea level, facing west and with a mean slope of 27%. The climate is characterised as pre-mountainous Mediterranean, featuring dry summers and cold winters (IHM 1975), with a mean annual temperature of 10.4°C and total annual precipitation of 931 mm (Harris et al. 2020). Vegetation is predominantly composed of pure *P. sylvestris* plantations. In this region, *P. sylvestris* was established in the late 1970s as part of soil conservation efforts to mitigate high erosion risks.

Field Sampling, Variables Measurement and Dendrochronological Methods

In mid-spring 2018, radial wood cores were extracted at breast height (1.3 m) from opposite sides of each sampled tree using a Pressler increment borer. Sample preparation followed standard dendrochronological procedures, including air-drying, mounting, and progressive sanding to enhance ring boundary visibility (Fritts 1976). Each core was visually cross-dated and measured to 0.001 mm precision using a stereomicroscope coupled with a LINTAB measuring system and TSAP software (Rinntech, Heidelberg, Germany). EW and LW were distinguished based on density and colour contrast. The accuracy of visual cross-dating was assessed with the COFECHA program (Holmes 1983). To remove long-term growth trends unrelated to climate, ring-width series were detrended using a double-detrending approach (Cook and Peters 1981, 1997). Each ring-width series was first detrended using either a negative exponential or a linear function, depending on the form of the age-related growth trend. In a second step, a cubic smoothing spline with a 50% frequency response was applied to remove the remaining medium- and low-frequency variability not directly related to climate. Standardisation involved transforming raw values into dimensionless indices, facilitating inter-series comparison. Following detrending, autoregressive modelling was applied to eliminate first-

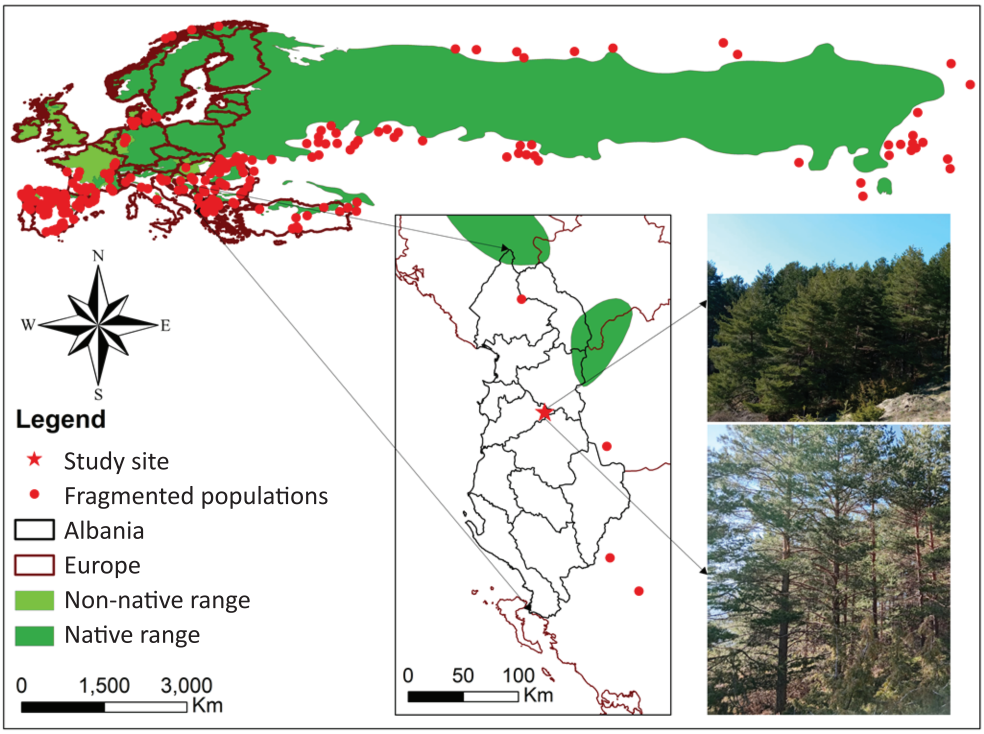

Figure 1. Distribution of *P. sylvestris* and location of the study site in central Albania. The species distribution map is adapted from Caudullo et al. (2026).

Table 1. Dendrochronological statistics of radial growth chronologies (EW – earlywood width, LW – latewood width, TRW – tree-ring width).

Variables	Trees (radii)	Period	MW (mm)	SD (mm)	AC1	Rbar	EPS
EW	21 (41)	1982 – 2017	2.40	1.00	0.60	0.37	0.89
LW	21 (41)	1982 – 2017	1.72	0.92	0.34	0.77	0.99
TRW	21 (41)	1982 – 2017	4.20	1.69	0.56	0.61	0.98

MW – mean tree-ring width; SD – standard deviation; AC1 – first-order autocorrelation coefficient; Rbar – mean correlation between trees; EPS – expressed population signal.

order autocorrelation, and the resulting indexed residuals were averaged using a biweight robust mean to produce site-level chronologies for EW, LW, and total ring width (TRW). These analyses were carried out using the ARSTAN software (Cook 1985). Chronology quality was assessed via dendrochronological statistics (Briffa and Jones 1990), including mean ring width, standard deviation (SD), first-order autocorrelation (AC1), expressed population signal (EPS), and mean inter-series correlation (Rbar) (Table 1). Only periods with EPS values above the 0.85 threshold, typically from 1985 to 2016, were included in further analyses to ensure statistical reliability and adequate sample replication (Wigley et al. 1984).

To reduce the influence of age and stem geometry on tree-ring width data, raw series were converted to basal area increment (BAI), which more accurately represents radial growth, climatic sensitivity, and tree responses to environmental factors, particularly drought (Gea-Izquierdo et al. 2015). This transformation assumes a concentric distribution of tree rings and was conducted from the pith towards the bark using the equation (1):

$$BAI_n = \pi(R_n^2 - R_{n-1}^2) \tag{1}$$

where R_n and R_{n-1} represent the radius at breast height (1.3 m) at the end and the beginning of the annual tree-ring, respectively. For cores lacking a visible pith, a concentric circle model with known radii was applied to the innermost rings to estimate the missing pith distance and infer the number of absent rings (Norton et al. 1987). Following transformation, BAI series were pre-whitened using first-order autoregressive modelling to remove temporal autocorrelation and normalise variance (Gea-Izquierdo et al. 2015). This procedure preserves growth variability attributable to climatic drivers while filtering out non-climatic, age-related trends.

Remote Sensing Data

Vegetation dynamics were evaluated using time series of the Normalised Difference Vegetation Index (NDVI) and the Enhanced Vegetation Index (EVI) over the period 2000–2016. These indices were derived from LANDSAT 7 and 8 satellite imagery obtained via the United States Geological Survey (USGS) Earth Explorer platform (https://earthexplorer.usgs.gov/). Only scenes with low cloud cover (<10%) were selected to ensure data quality and temporal consistency. To minimize the influence of atmospheric artefacts, cloud contamination and snow, the quality assurance bands provided with Landsat surface reflectance products were used to mask clouds, cloud shadows, and snow pixels prior to calculating NDVI and EVI. The resulting time series were then visually inspected, and any anomalous values were removed before computing monthly averages. NDVI was calculated as (NIR–RED)/(NIR+RED), while EVI was computed using the formula 2.5*(NIR–RED)/(NIR+6RED–7.5BLUE+1), following the approach of Huete et al. (2002). Both indices were computed on a bimonthly basis and subsequently averaged to produce 12 continuous monthly values for each year, resulting in a 17-year time series characterising intra-annual variability in vegetation activity within the *P. sylvestris* plantation.

Climatic Data and Drought Indices Calculation

Climatic data were obtained from the global Climatic Research Unit (CRU) TS3.24.01 dataset (Harris et al. 2020) via the KNMI Climate Explorer platform (http://climexp.knmi.nl/). This dataset provided monthly series of mean temperature and total precipitation for the period 1985–2016, at a spatial resolution of 0.5° latitude/longitude (41.0–41.5°N, 20.0–20.5°E), encompassing the study site. These climatic variables were used to compute two multi-scalar drought indices: Standardised Precipitation Index (SPI) and Standardised Precipitation Evapotranspiration Index (SPEI), both widely employed to assess drought conditions. SPI is based solely on monthly precipitation data, which are aggregated over varying temporal scales (e.g. 1–24 months) and fitted to a Pearson Type III distribution to calculate cumulative probabilities. The resulting values are then standardised to yield SPI values with a mean of 0 and a standard deviation of 1, facilitating temporal and spatial comparisons (McKee et al. 1993). SPEI, by contrast, integrates both precipitation and temperature effects through the calculation of a monthly climatic water balance. This parameter is also aggregated over multiple timescales and standardised using a three-parameter log-logistic distribution, which is well-suited for representing the statistical properties of such data, including negative values (Vicente-Serrano et al. 2010). Both indices were computed over cumulative timescales up to 24 months, recognising that tree growth responses are modulated by the interplay between water input and atmospheric demand (Pasho et al. 2011a, Alla et al. 2025).

Statistical Analysis

Prior to correlation analysis, the distributions of the analyzed variables were visually assessed using histograms and Q-Q plots to evaluate approximate normality and to detect potential outliers. Additionally, scatterplots were inspected to confirm that the relationships between variables were approximately linear, ensuring the appropriateness of Pearson correlation. For the period 2000–2016, Pearson correlation analyses were conducted to assess the strength and direction of associations between vegetation indices (NDVI and EVI) and radial growth variables (EW, LW, TRW, and BAI), climate (temperature and precipitation), and drought indices (SPI and SPEI). Pearson correlation analysis were also applied to examine the relationships between radial growth variables, climatic factors and drought indices. Residual, standardised chronologies of EW, LW, TRW, and BAI were correlated with monthly, seasonal (winter: previous December – February; spring: March – May; summer: June – August; autumn: September – November), and annual mean temperature and total precipitation from 1985 to 2016, using a 12-month temporal window spanning January to December of the growth year. Furthermore, the relationships between radial growth variables and drought conditions were assessed using detrended monthly SPI and SPEI, calculated at 1- to 24-month-long scales, for the same time interval.

RESULTS

Variability of Vegetation Activity and Radial Growth Variables

Throughout the growing season, vegetation activity increased from spring to summer, reaching peak values in July, before declining towards autumn, with NDVI consistently exhibiting higher values than EVI (Figure 2). Seasonal variability was pronounced, with NDVI ranging from 0.36 in March to 0.67 in July, and EVI ranging from 0.24 to 0.41.

Radial growth variables of P. sylvestris revealed distinct temporal dynamics (Figure 3). EW and TRW exhibited marked increases during the late 1980s, followed by a gradual decline and subsequent stabilisation after 2010. LW displayed greater inter-annual variability than EW, whereas BAI increased steadily until the early 2000s, before levelling off. Although LW displayed moderate synchrony with TRW, it exhibited lower amplitude.

The radial growth chronology of *P. sylvestris* trees extended back to 1982, encompassing a 35-year period (Table 1). On average, EW exhibited greater width than LW.

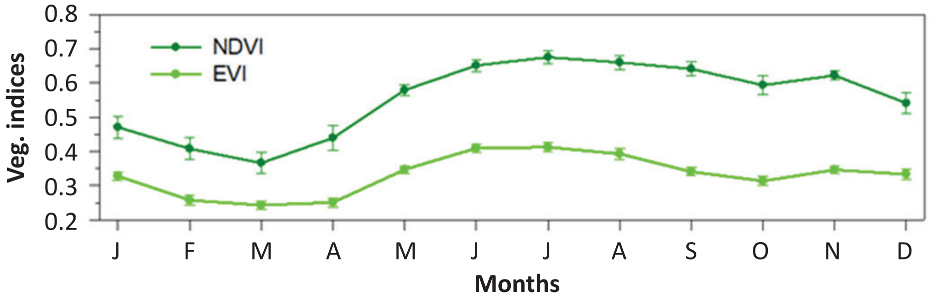

Figure 2. Mean (± SE) of vegetation indices (NDVI – Normalized Difference Vegetation Index, and EVI – Enhanced Vegetation Index) for the period 2000–2016.

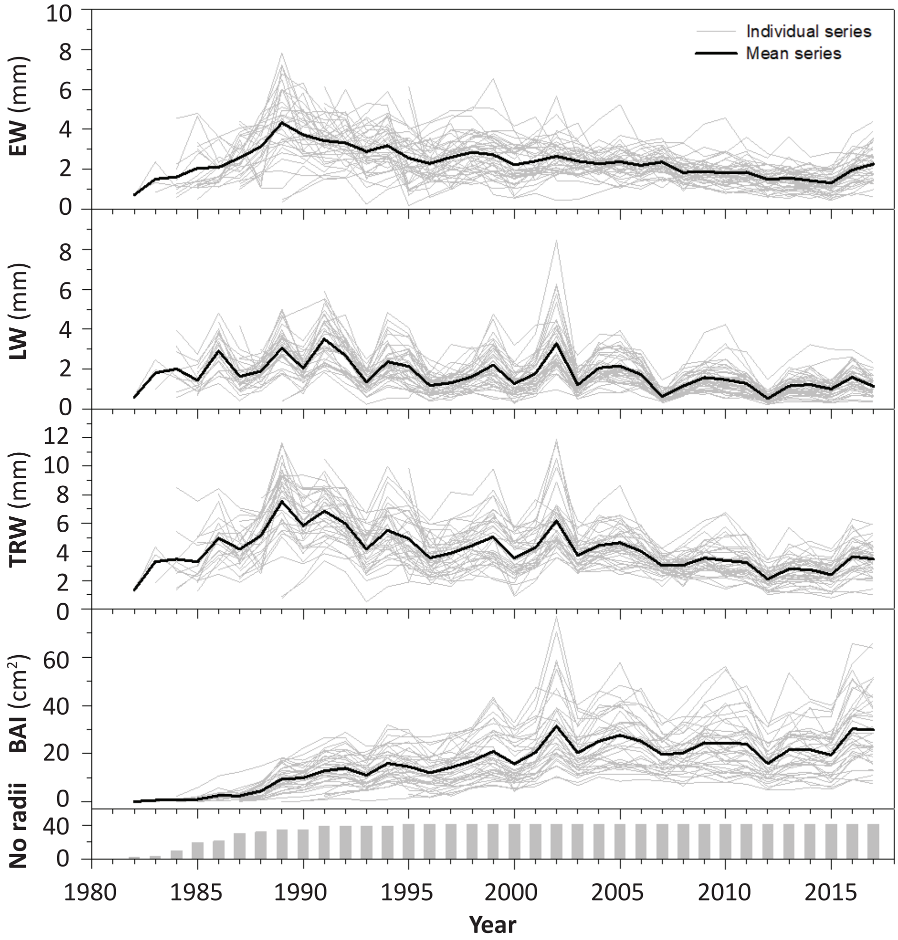

Figure 3. Radial growth (EW – earlywood, LW – latewood, TRW – tree-ring width, and BAI – basal area increment) raw chronologies. The individual series of radial growth variables as well as their overall mean are shown with grey and black lines, respectively. The columns in the lower graph represent the annual sample size (number of radii analysed).

AC1 values were highest for EW and lowest for LW, whereas Rbar was greater for LW than for EW. In all instances, the EPS exceeded the threshold value of 0.85, indicating robust chronology quality.

Growth–Climate (Drought) Relationships

Radial growth dynamics showed clear associations with vegetation activity and climatic variables. A strong positive relationship was identified between August vegetation indices and radial growth variables, particularly for the LW and BAI. Specifically, significant correlations were observed for NDVI–LW ($r = 0.52$, $p = 0.032$), NDVI–BAI ($r = 0.52$, $p = 0.033$), and EVI–LW ($r = 0.51$, $p = 0.037$). Years marked by high and low values of both vegetation indices showed strong synchrony with corresponding fluctuations, peaks and troughs, in LW and BAI (Figure 4).

Climatic variables exerted a highly significant influence on vegetation dynamics at the study site (Figure 5). Overall, statistically significant positive correlations were observed between vegetation indices and temperature. NDVI exhibited a more consistent and robust response to temperature variation in February ($r = 0.56$, $p = 0.020$) and September ($r = 0.48$, $p = 0.048$), whereas EVI showed greater sensitivity in April ($r = 0.55$, $p = 0.023$) and October ($r = 0.49$, $p = 0.045$). Additionally, EVI showed significant positive correlations with precipitation in June ($r = 0.56$, $p = 0.020$) and September ($r = 0.50$, $p = 0.039$), but a significant negative correlation with January precipitation ($r = -0.62$, $p = 0.008$).

Tree growth responses to climatic variability differed among the radial growth variables (Figure 6). Temperature exerted a temporally variable yet statistically significant influence on radial growth, shifting from a positive effect in February (EW: $r = 0.41$, $p = 0.021$; BAI: $r = 0.50$, $p = 0.003$) and March (LW: $r = 0.37$, $p = 0.039$; TRW: $r = 0.44$, $p = 0.012$) to a negative impact during summer (LW: $r = -0.39$, $p = 0.028$; BAI: $r = -0.35$, $p = 0.049$). In contrast, summer precipitation (LW: $r = 0.70$, $p < 0.001$; TRW: $r = 0.63$, $p < 0.001$; BAI: $r = 0.43$, $p = 0.013$), particularly in July (LW: $r = 0.79$, $p < 0.001$; TRW: $r = 0.72$, $p < 0.001$; BAI: $r = 0.44$, $p = 0.013$), was consistently and positively associated with radial growth. Years characterised by high and low July precipitation coincided with marked increases and decreases, respectively, in LW, TRW, and BAI (Figure 7). Meanwhile, negative correlations were observed in November (LW: $r = -0.46$, $p = 0.009$; TRW: $r = -0.42$, $p = 0.016$) and during winter (EW: $r = -0.42$, $p = 0.015$).

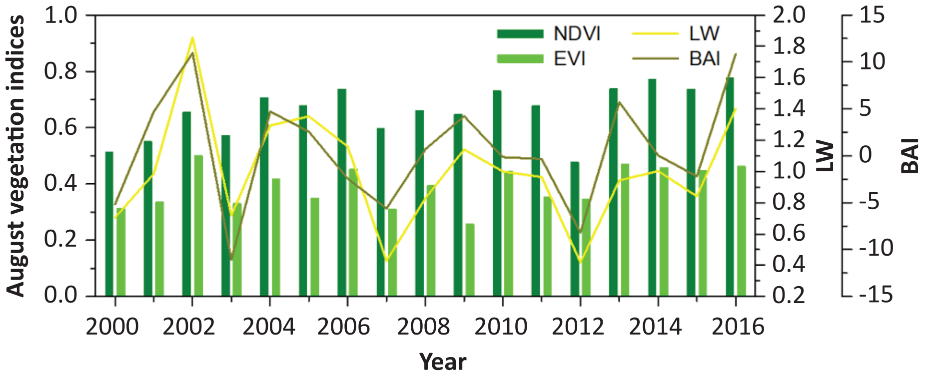

Figure 4. Interannual variability in August vegetation indices (NDVI – Normalized Difference Vegetation Index, and EVI – Enhanced Vegetation Index) and radial growth indices (LW – latewood, and BAI – basal area increment).

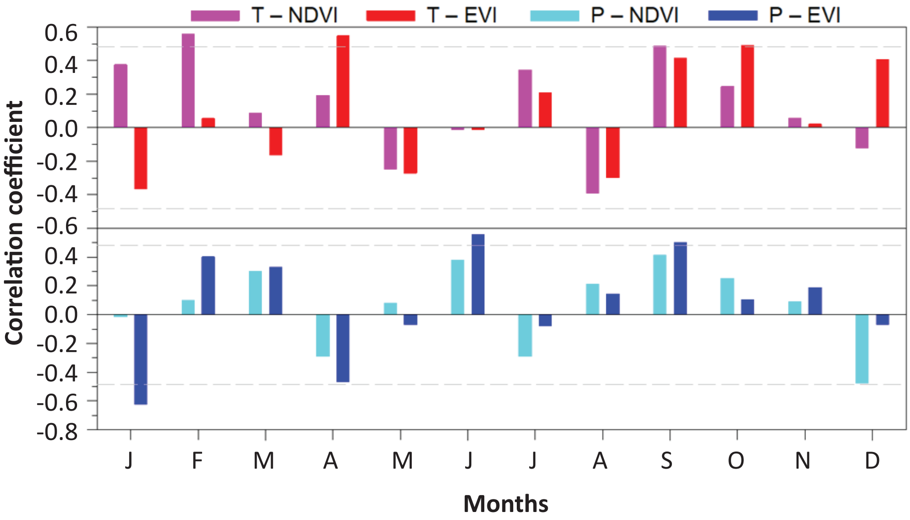

Figure 5. Correlation coefficients calculated between monthly climatic data (mean temperature – pink and red bars; total precipitation – cyan and blue bars) and vegetation indices (NDVI – Normalized Difference Vegetation Index, and EVI – Enhanced Vegetation Index). The horizontal grey dashed lines correspond to the significance ($p \leq 0.05$) thresholds.

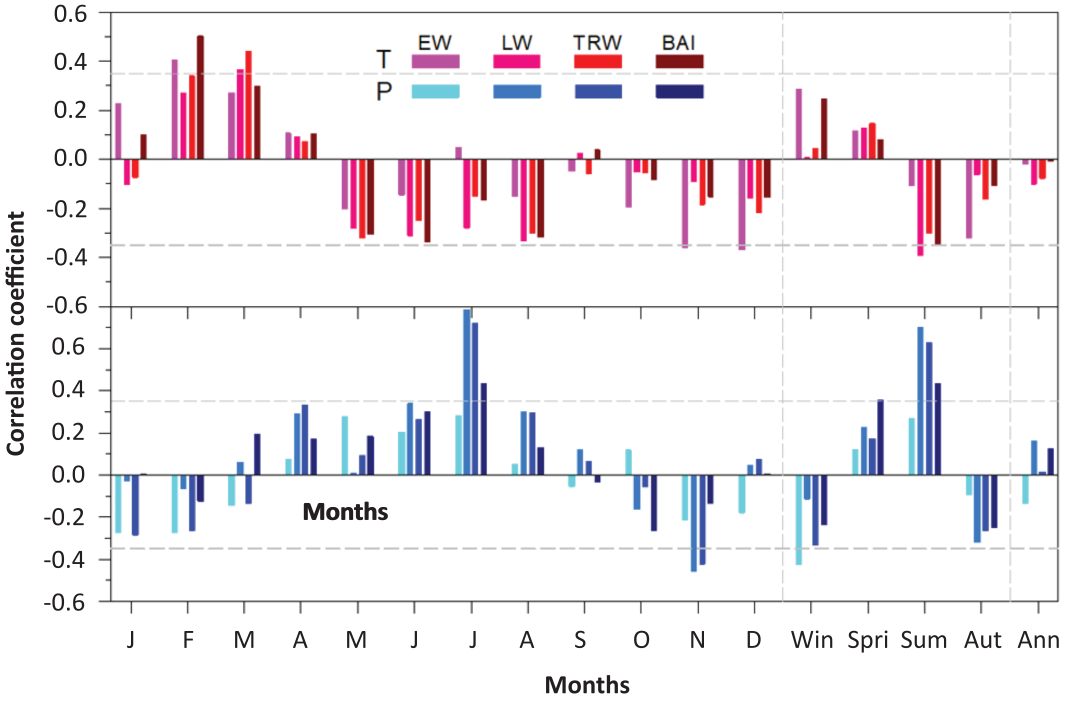

Figure 6. Correlation coefficients calculated between monthly, seasonal, and annual climatic data (mean temperature – pink, purple, red, and brown bars; total precipitation – cyan, light blue, blue, and dark blue bars) and radial growth indices (EW – earlywood width, LW – latewood width, TRW – tree-ring width, and BAI – basal area increment). The horizontal grey dashed lines correspond to the significance (p ≤ 0.05) thresholds.

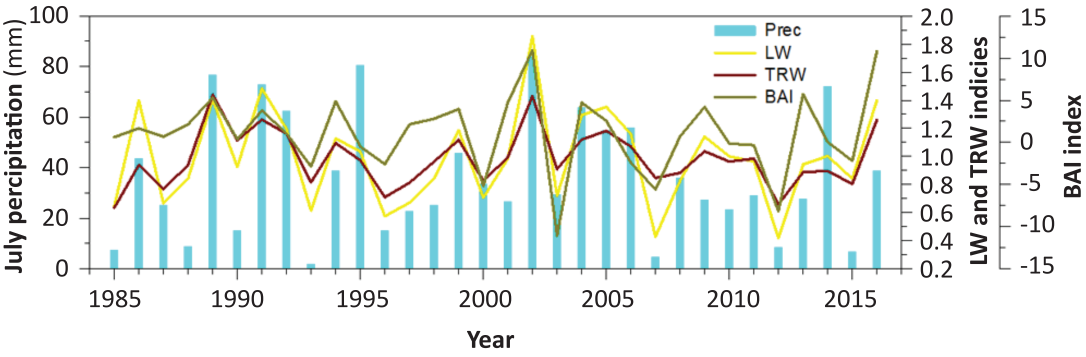

Figure 7. Interannual variability in July precipitation and radial growth indices (LW – latewood, TRW – tree-ring width, and BAI – basal area increment).Drought indices showed statistically significant (p < 0.05) positive correlations with both NDVI and EVI across multiple temporal scales, particularly from April to September, coinciding with key phases of vegetative development (Figure 8). NDVI in April exhibited the strongest correlation with the SPI at a 7-month aggregation scale (r = 0.71, p = 0.001), whereas EVI displayed its highest association with SPI in September at a 9-month scale (r = 0.57, p = 0.016). Regarding SPEI, NDVI exhibited a pattern similar to that observed for SPI, with the strongest correlation in April at a 7-month aggregation scale (r = 0.69, p = 0.002). Conversely, EVI presented its highest correlation with SPEI in June at a 4-month aggregation scale (r = 0.60, p = 0.011). Overall, NDVI showed greater sensitivity to both drought indices compared to EVI, particularly to SPI, while EVI responded more strongly to variations in SPEI.

Radial growth variables at the study site exhibited clear temporal and magnitude-related responses to drought indices (Figure 9). EW showed a significant positive correlation with the SPI only in August at a 4-month timescale (r = 0.36, p = 0.049), but negative association with both SPI and SPEI over longer timescales (up to 14 and 18 months, respectively), particularly during winter and spring. LW correlated positively with SPI and SPEI at timescales up to 14 months, especially from June to August, peaking in July at the 1-month scale (SPI: r = 0.78, p < 0.001; SPEI: r = 0.69, p < 0.001), and showed negative correlations in November and December at ≤3-month timescales. TRW presented significant positive correlations with both indices from June to November at ≤8-months timescales, and negative relationships during winter–spring at ≥10-months timescales. The strongest positive TRW-drought correlation occurred in July at the 1-month timescale (SPI: r = 0.69, p < 0.001; SPEI: r = 0.60, p < 0.001). BAI exhibited its highest response to drought in August, with maximum correlations at the 6-month timescale for SPI (r = 0.53, p = 0.002) and the 4-month timescale for SPEI (r = 0.47, p = 0.008).

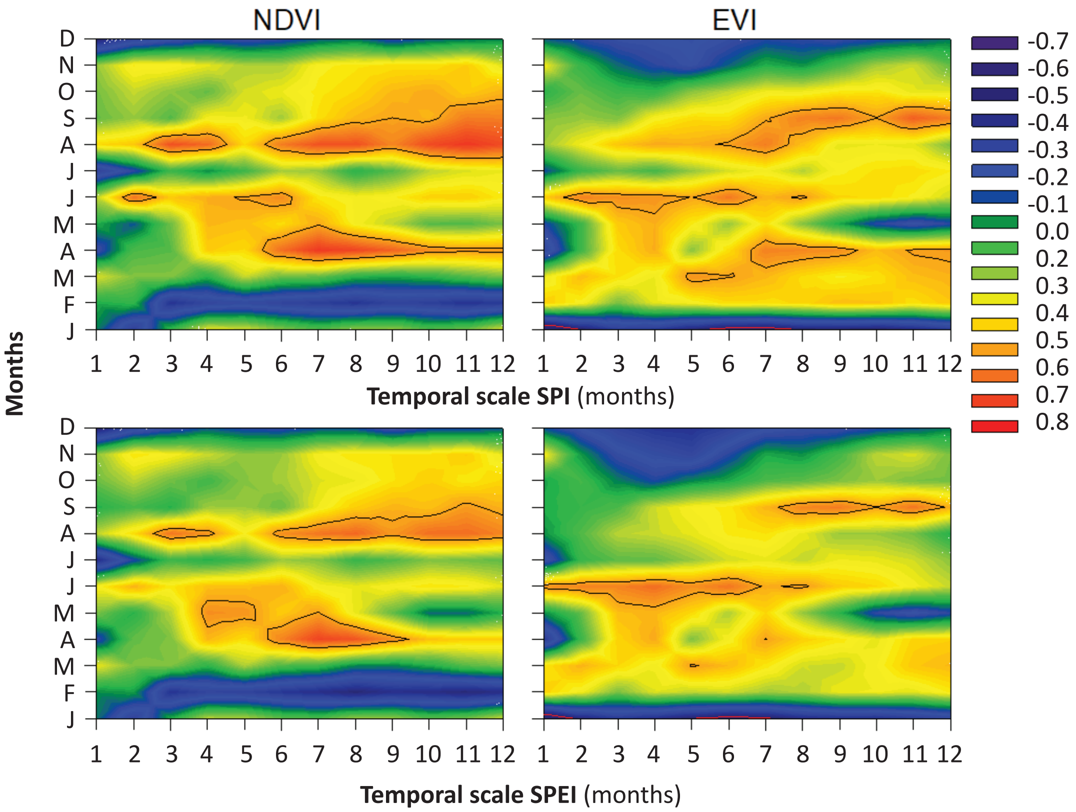

Figure 8. Correlations between monthly vegetation (NDVI – Normalized Difference Vegetation Index, and EVI – Enhanced Vegetation Index) and drought (SPI – Standardized Precipitation Index, and SPEI – Standardized Precipitation Evapotranspiration Index) indices at different timescales. Bold lines frame significant positive (black) and negative (red) correlations ($p < 0.05$).

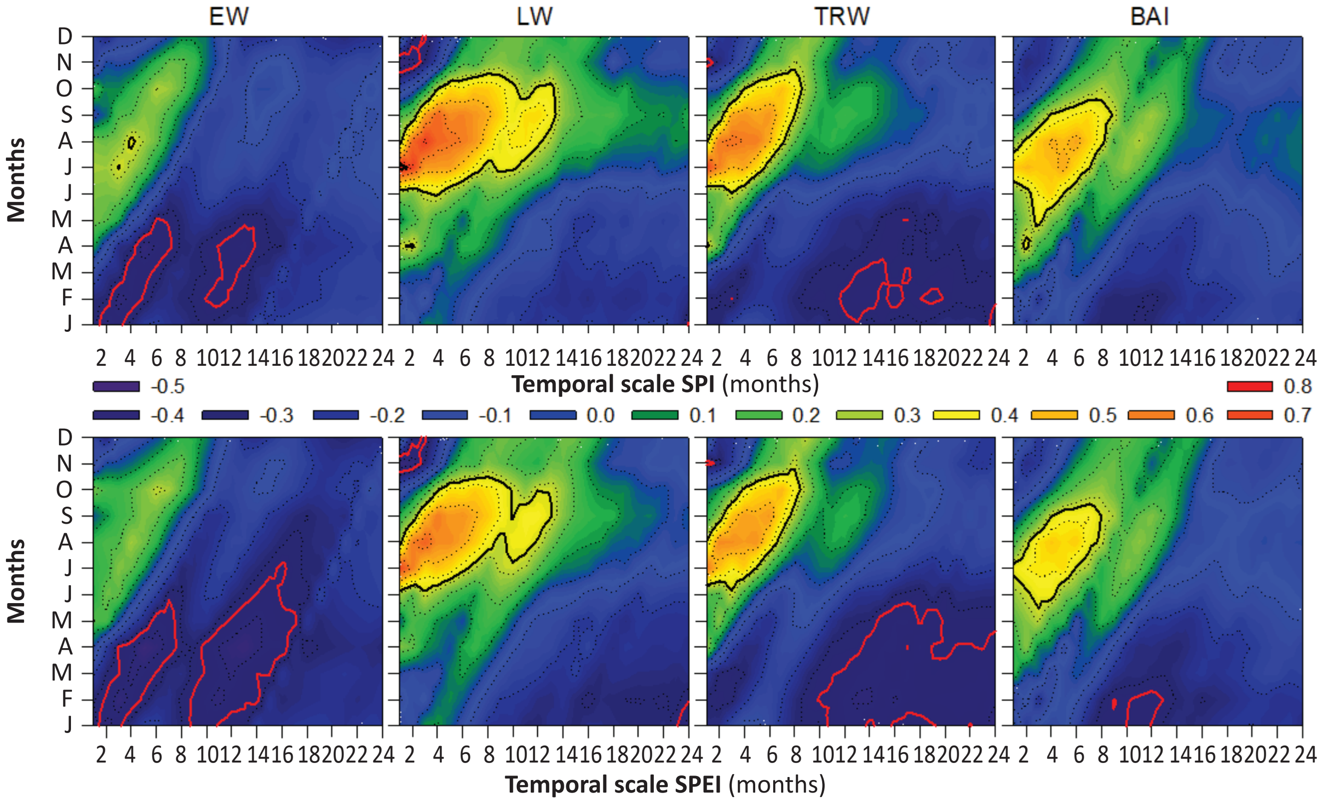

Figure 9. Correlations between radial growth residual chronologies (EW – earlywood, LW – latewood, TRW – tree-ring width, and BAI – basal area increment) and monthly drought indices (SPI – Standardized Precipitation Index, and SPEI – Standardized Precipitation Evapotranspiration Index) at different timescales. Bold lines frame significant positive (black) and negative (red) correlations ($p < 0.05$).

DISCUSSION

The results provide a comprehensive assessment of the interactions between climatic variables, vegetation indices, and radial growth dynamics of *P. sylvestris* in central Albania. Vegetation indices effectively captured seasonal fluctuations in canopy activity, with peak values in spring and summer corresponding to optimal photosynthetic conditions, and a decline in autumn indicating reduced physiological activity as evergreen trees approach dormancy (Alla et al. 2025, Alla and Pasho 2026). Throughout the growing season, NDVI consistently exhibited higher values than EVI, suggesting greater sensitivity to overall canopy greenness. Previous studies have shown that NDVI tends to saturate under conditions of high canopy density, which may enhance its responsiveness to peak greenness (Mašek et al. 2023, Alla et al. 2025). Temporal trends in radial growth revealed distinct developmental phases in *P. sylvestris*. The increase in both EW and TRW during the late 1980s likely reflects a period of favourable climatic and site conditions, while the decline and subsequent stabilisation observed after 2000 may be associated with increasing stand competition, recurrent summer droughts, and rising evaporative demand (Candel-Pérez et al. 2012, Misi and Náfrádi 2017). The high year-to-year variability of LW reflects its strong sensitivity to late-season moisture availability, while its higher synchrony with TRW, compared to EW, indicates that LW contributes more consistently to interannual variability in total ring width (Schweingruber 1996, Homfeld et al. 2025). The BAI growth pattern suggests a potential plateau, possibly driven by site-specific resource limitations or increasing climatic stress (Taeger et al. 2013, Herguido et al. 2016). This radial growth pattern corresponds with findings reported for other Mediterranean and continental populations of *P. sylvestris* (Taeger et al. 2013, Camarero et al. 2021). The 35-year radial growth chronology (1982–2017) revealed distinct structural and statistical differences among growth components. The consistently wider EW than LW reflects its formation under more favourable spring conditions, whereas the higher AC1 observed in EW indicates stronger temporal persistence and memory effects (Sánchez-Salguero et al. 2015). Moreover, higher LW Rbar values indicate a stronger response to year-to-year climatic variability (Camarero et al. 2021). The consistently high EPS values across all chronologies confirm the robustness and support their suitability for climate-growth analyses (Wigley et al. 1984).

The study revealed a high degree of synchrony between August vegetation activity and radial growth (particularly LW and BAI), highlighting the close coupling between photosynthetic activity and cambial processes in *P. sylvestris* (Mašek et al. 2023). These findings imply that carbohydrate supply plays a key role in LW growth, with climatic conditions indirectly shaping this supply by influencing photosynthetic activity and canopy dynamics (Cartenì et al. 2018, Alla et al. 2025). This confirms the significance of remote sensing-based vegetation indices as effective proxies for monitoring tree growth in Mediterranean forest ecosystems, thereby linking satellite-derived canopy signals with tree-ring measurements (Vicente-Serrano et al. 2012, Pasho and Alla 2015, Alla et al. 2025). In this context, a comparable integration of tree-ring and remotely sensed data was applied by Jevšenak et al. (2024) at a continental scale across central and eastern Europe. Their results similarly demonstrated a strong coupling between canopy activity and radial growth in multiple species, including *P. sylvestris*. However, as their dataset did not include sites from Albania, the present study extends these findings to the southern margin of the species' distribution, where climatic constraints and growth responses may differ from those observed in more central populations.

NDVI and EVI exhibited marked sensitivity to temperature and water availability, thereby emphasising their effectiveness in capturing intra-annual climatic influences on canopy function. Temperature emerged as the primary driver of canopy greenness in February, April, and the transitional months of September and October, suggesting that milder temperatures likely enhance early and late-season photosynthetic activity and reserve mobilization (Alla et al. 2017, 2025). The strong response of EVI to April and October temperatures is consistent with its sensitivity to changes in canopy structure and leaf area, thereby enabling it to capture seasonal phenological dynamics more effectively than NDVI (Huete et al. 2002, Pasho and Alla 2015, Alla et al. 2025). Conversely, precipitation exerted a more pronounced influence in June, and to a lesser extent in September, indicating a seasonal asymmetry in hydric control, whereby early- and late-season moisture availability enhances canopy vigour (Alla et al. 2017, 2025). The dual climatic influence is further elucidated through drought indices, as both SPI and SPEI significantly affected April–September NDVI and EVI values, highlighting the critical role of water balance in regulating vegetative activity throughout the growing season (Alla et al. 2017, 2025). The highest sensitivity of NDVI to SPI and SPEI on a 7-month timescale in April underscores the importance of antecedent moisture conditions in facilitating early canopy development, cambial reactivation, and the onset of EW growth (Pasho and Alla 2015, Alla et al. 2025, Römer et al. 2025). In contrast, the strongest correlations of EVI with SPI and SPEI in September and June indicate a closer relationship with mid- to late-season moisture availability, an essential determinant of sustained canopy function and LW growth (Pasho and Alla 2015, Alla et al. 2017, 2025, Camarero et al. 2021). These results demonstrate the responsiveness of vegetation indices to both thermal and hydric conditions, and further reinforce the value of remotely sensed data in providing temporally continuous insights into fine-scale climate–vegetation interactions. (Vicente-Serrano et al. 2012).

Radial growth of *P. sylvestris* was strongly influenced by both temperature and precipitation throughout the growing season. Positive correlations between February–March temperatures and radial growth variables align with previous studies linking early-season warming to the reactivation of cambial activity and EW formation (Matías et al. 2017, Waszak et al. 2021, Gurcay et al. 2025, Römer et al. 2025, Alla and Pasho 2026). In contrast, elevated summer temperatures reduced growth, reflecting the combined effects of increased evapotranspiration, soil moisture depletion, and hydraulic stress (Matías et al. 2017, Herrero et al. 2023, Gurcay et al. 2025). Populations of *P. sylvestris* in Mediterranean regions exhibit pronounced sensitivity to elevated summer temperatures (Sánchez-Salguero et al. 2015, Shestakova et al. 2017, Herrero et al. 2023). This vulnerability is likely attributable to the species' isohydric water-use strategy, which limits carbon assimilation under drought through early stomatal closure (Taeger et al. 2013, Buras et al. 2023).

Summer precipitation emerged as a growth trigger for LW, TRW and BAI, suggesting that adequate moisture during mid-season appears to mitigate drought-induced growth constraints (Matías et al. 2017, Camarero et al. 2021, Gurcay et al. 2025, Alla and Pasho 2026). These findings are consistent with broader trends observed across southern and central Europe, where *P. sylvestris* growth is predominantly regulated by summer moisture availability, and where the potential benefits of warming are largely offset by the overriding influence of drought (Candel-Pérez et al. 2012, Tabakova et al. 2020, Camarero et al. 2021). Summer precipitation directly supports photosynthesis and carbon assimilation processes, which are essential for sustaining radial growth during periods of high evaporative demand (Sánchez-Salguero et al. 2015, Sidor et al. 2018). Conversely, negative correlations between November precipitation and both LW and TRW suggest potential frost-related growth inhibition, likely resulting from reduced light availability, lower temperatures, and excess moisture interfering with dormancy and carbon storage (Taeger et al. 2013, Matías et al. 2017, Römer et al. 2025).

Drought indices further clarified the temporal dynamics of moisture and temperature sensitivity in *P. sylvestris* growth. Positive associations between EW growth and short-term SPI and SPEI values in August highlight the importance of immediate soil moisture availability (Pasho et al. 2014). This indicates that EW growth depends heavily on current-year soil moisture availability, which directly influence cell division and expansion (Buras et al. 2023). However, negative relationships with longer-term drought conditions during winter and spring suggest that insufficient soil moisture recharge or depleted carbohydrate reserves may delay the onset of early-season growth (Waszak et al. 2021, Alla et al. 2025). The strong positive relationships between LW and short-term drought indices in July reveal heightened sensitivity to concurrent summer moisture conditions. This supports earlier findings that LW formation, occurring later in the season when evaporative demand peaks, is constrained by both immediate water availability and the depletion of stored resources (Candel-Pérez et al. 2012, Tabakova et al. 2020). The results also suggest that even short-term summer rainfall events can substantially enhance LW growth (Pasho et al. 2014, Pasho and Alla 2015, Alla and Pasho 2026). The bimodal response of TRW to drought indices reflects both immediate and long-term effects (Lévesque et al. 2014, Misi and Náfrádi 2017). The strongest positive TRW–drought correlation in July (1-month scale) underscores mid-summer moisture's crucial role in *P. sylvestris* radial growth across both Mediterranean and continental environments (Sánchez-Salguero et al. 2015, Misi and Náfrádi 2017, Shestakova et al. 2017, Camarero et al. 2021). Similarly, BAI responses to drought indices, particularly in August at 4- to 6-month timescales, suggest a cumulative impact of seasonal moisture deficits (Taeger et al. 2013, Herguido et al. 2016, Alla et al. 2025). This highlights the prolonged influence of hydrological stress on wood growth and underscores the species' vulnerability to increasingly frequent and severe summer droughts (Gruber et al. 2010, Candel-Pérez et al. 2012, Sánchez-Salguero et al. 2015).

Despite providing valuable insights into the climate–growth relationships of *P. sylvestris* at its southernmost range in central Albania, our study has some limitations. The analysis relies on a single study site, which may constrain the generalizability of the results across the broader regional distribution of the species. Moreover, the use of gridded climate data with relatively coarse spatial resolution may not fully capture local microclimatic variability, particularly in complex mountainous terrain where topography strongly affects temperature and moisture conditions. These factors should be taken into account when interpreting the magnitude and spatial representativeness of the observed climate–growth relationships.

CONCLUSIONS

This study offers an in-depth assessment of how climate and drought conditions affect vegetation dynamics and radial growth of *P. sylvestris* at its southern range limit in Albania. The results highlight the ecological responsiveness of *P. sylvestris* to increased early-season temperatures, sufficient mid-season precipitation, and the synergistic impact of elevated summer heat and drought stress, particularly during critical phenological periods. High correlations between vegetation indices and radial growth variables indicate a common response to climatic stress, highlighting the tight coupling between canopy activity and radial growth. The findings indicate that the species' marginal position within its climatic range heightens its vulnerability to intensifying summer droughts and future warming. The implementation of adaptive forest management strategies will be essential to mitigate declines in *P. sylvestris* forest structure and function, strengthen ecosystem resilience, and maintain key ecosystem services. In the context of climate change, integrative and proactive management approaches are essential for promoting evidence-based conservation and enhancing resilience in Mediterranean forest ecosystems. Future research incorporating multiple sites along environmental gradients, as well as higher-resolution climate and remote sensing datasets, would help refine our understanding of spatial variability in growth responses and improve projections of forest dynamics under changing climatic conditions.

Author Contributions

AQA and EP conceived and designed the research; conducted field sampling and laboratory analyses; performed data curation and statistical analysis; and wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Impact of Protective Forest Belts (PFBs) on Wind Reduction, Microclimate, and Soil Properties

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ABSTRACT

This study examines the impact of Protective Forest Belts (PFBs) on wind speed, air temperature, evapotranspiration, and soil properties in South Banat, Serbia. Six PFBs, composed of black walnut (*Juglans nigra*) and Siberian elm (*Ulmus pumila*), were analyzed for their effectiveness in improving environmental conditions in a highly susceptible to wind erosion Vojvodina region. The research involved measuring wind speed and air temperature windward of the PFBs (at 50 m) and leeward at distances of 30 m, 60 m, and 90 m within the protection zone. Composite soil samples were collected in the PFBs, as well as leeward at 30 m and 90 m within the protection zone. The results demonstrate a significant reduction in wind speed within the protected zone, with a 50% decrease recorded at 30 meters from the PFBs during the growing season. This wind reduction contributes to lower evapotranspiration, enhancing soil moisture retention. Additionally, air temperature increased immediately behind the PFBs before gradually declining with distance. The soil within the PFBs exhibited physical and chemical properties compared to adjacent agricultural land. Notably, PFBs-protected areas contained higher levels of organic matter, nitrogen, and phosphorus, along with improved moisture retention and reduced clay content. These benefits were most pronounced near the belts and diminished with increasing distance. A statistically significant correlation was also found between changes in wind speed and temperature on one hand, and the optical porosity of the belt on the other. The study underscores the multifunctional role of PFBs in enhancing microclimatic conditions and soil quality, positioning them as a crucial agroforestry strategy for sustainable agriculture. Given the significant threats of wind erosion in Vojvodina's intensively farmed landscapes, the findings advocate for the broader implementation of PFBs.

Keywords: windbreaks; shelterbelts; agroforestry; ecosystem services; Republic of Serbia; Vojvodina

INTRODUCTION

Wind erosion is a significant type of soil degradation, primarily occurring in arid and semiarid areas (Zhibao et al. 2000, Zhang et al. 2019). However, it also presents a risk to certain regions of Europe (Borrelli et al. 2014). According to an estimate by the Joint Research Centre (Panagos et al. 2012), up to 42 million hectares of agricultural land in Europe are threatened by this process.

Protective Forest Belts (PFBs) are one of the most widely accepted and commonly used practices to protect soil from wind erosion, also known as windbreaks or shelterbelts. In addition to their primary function, wind speed reduction and consequently protection from wind erosion (Lukić and Dožić 2006, Rehacek et al. 2017), they also provide a wide range of other direct and indirect benefits for humans and the environment (Mize et al. 2008). PFBs influence the change in air temperature (Baker et al. 2021), which, together with the reduction of the incoming wind speed in the protected zone, contributes to evapotranspiration change and consequently to water resource management improvement (Thevs et al. 2017). PFBs designed with lower permeability structural design are an effective solution for controlling snowdrifts (AAFC 2009).

Furthermore, the change in evapotranspiration can improve soil moisture and create more favourable conditions for agricultural production. PFBs also maintain the inherent soil characteristics within their area, compared to the soil in adjacent areas used for agricultural production (Wu et al. 2018). In addition, PFBs contribute to a higher content of organic matter and better physical and chemical properties of the soil in the protected zone (Kong et al. 2022).

In the Republic of Serbia, the region of Vojvodina faces significant threats from wind erosion (Baumgertel et al. 2019). Furthermore, climate change is expected to intensify wind erosion in the future (Baumgertel et al. 2022) and also threaten agricultural production (Baumgertel et al. 2024). This is particularly significant because AP Vojvodina is one of the key economic regions of Serbia, as reflected in its contribution to the regional Gross Domestic Product (GDP). It accounts for 61.5% to 88.3% of the total production of major agricultural commodities in the country (Milošević et al. 2015). At the same time, this area is characterized by an extremely low density of forest with only 7.97% (Glavonjić et al. 2024).

One of the ways to introduce a woody component in such dominantly oriented agricultural systems is through the application of agroforestry practices. Agroforestry is the integration of trees and crops in interactive systems and is widely recognized as a sustainable land-use approach (Nair et al. 2021), where PFBs are one of the most frequently applied practices in the temperate zone and specific conditions (Suratman and Brandle 2024). The application of PFBs would increase the forest cover of this area, while at the same time contributing positively in many ways in terms of ecosystem services (Lukić et al. 2018, Belanović Simić et al. 2024). Although the theory highlights the multifunctional benefits of PFBs, there is limited research quantifying the specific functions they provide. The objectives of this research are: (1) to analyze the potential of PFBs to influence microclimate conditions in terms of wind speed reduction, change of air temperature and consequently change of evapotranspiration, (2) to analyze the relationship between the optical porosity of the belt and its function in reducing wind speed and altering air temperature, and (3) to evaluate the impact of PFBs on the physical and chemical properties of the soil within the belts compared to surrounding agricultural areas.

MATERIALS AND METHODS

Research Area

The research was conducted in the South Banat region in Vojvodina, near the city of Pančevo. According to MeteoBlue (2025) and based on weather models using historical data from 1985 and a continuous 30-year global historical dataset with hourly meteorological data, the research area is characterized by a continental climate with a maximum temperature of up to 30 °C (July–August) and a minimum temperature of around −1 °C during the winter period (Figure 1), with maximum rainfall recorded in May and June at approximately 75 mm.

The prevailing wind direction is SE with the highest speed, while most often the wind speed is in the range of 10 to 20 km·h^{-1} (Figure 2). This area is characterized by the occurrence of strong southeast wind (Košava), where the average monthly wind speed is around 6.3 m·s^{-1} (WEB).

Among the six representative PFBs (Figure 3) in this research, three (PFBs 1, 2, and 3) were established with black walnut (*Juglans nigra* L.), while the remaining three (PFBs 4, 5, and 6) were composed of Siberian elm (*Ulmus pumila* L.) (Table 1). The study area is characterized by high-quality soil, specifically carbonate Chernozem and Phaeozems.

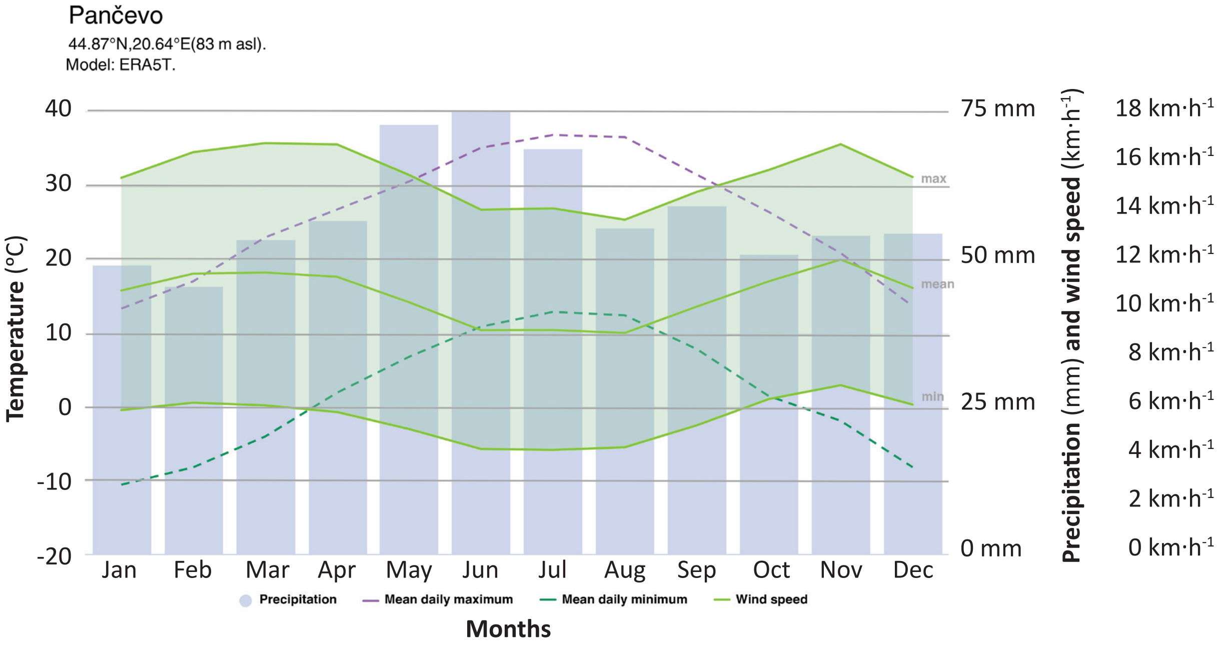


Figure 1. The climate data of the research area (precipitation, mean minimum and maximum temperatures, and wind speed) (MeteoBlue 2025).

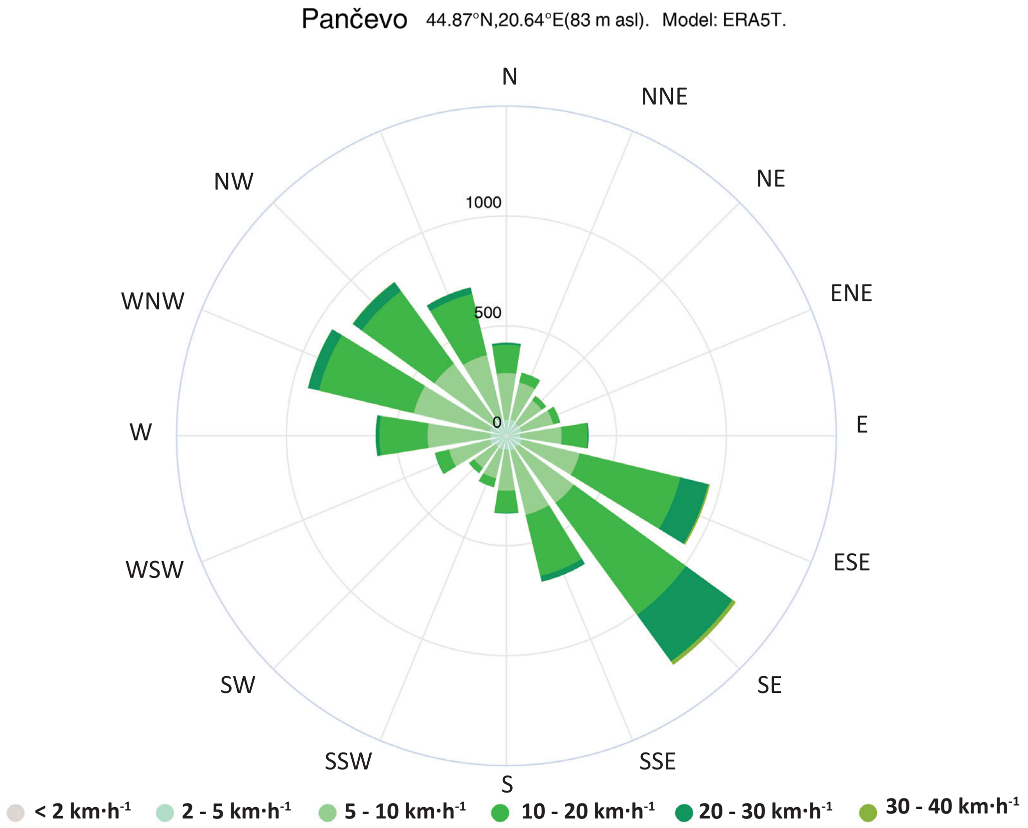
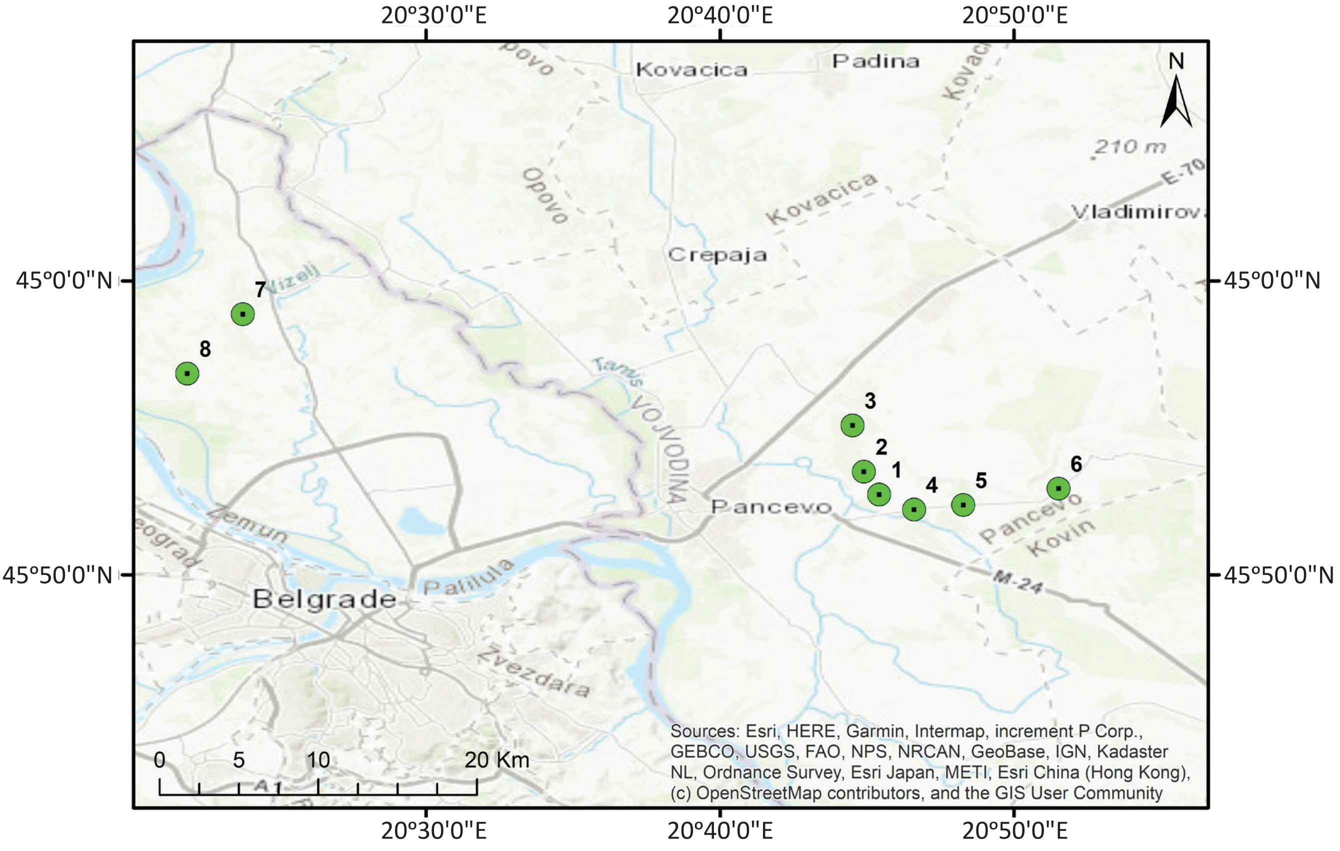


Figure 2. The wind rose for the research area (MeteoBlue 2025).



Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

Figure 3. Research area and the locations of the protective forest belts (PFBs).

Table 1. The information about the investigated protective forest belts (PFBs).

PFB	Tree species	Coordinates		Height (m)	Width (m)	Direction
		X	Y			
P1	*Juglans nigra*	20.756808	44.878963	13.50	14.0	W-E
P2	*Juglans nigra*	20.748095	44.891951	12.07	14.0	W-E
P3	*Juglans nigra*	20.741681	44.917998	12.60	14.0	SW-NE
P4	*Ulmus pumila*	20.776533	44.870506	8.08	5.0	W-E
P5	*Ulmus pumila*	20.804224	44.873153	8.57	14.5	W-E
P6	*Ulmus pumila*	20.858535	44.882195	9.71	8.0	SW-NE

Method

Optical Porosity

Optical porosity was determined using the high-contrast photo analysis method (Středa et al. 2008). This method includes perpendicular photographing to the PFB and determining the ratio of pixels representing vegetation silhouettes in relation to the number of pixels representing the "background" (sky, surfaces behind the belt, etc.). The images were captured using a triple Leica camera, ensuring high resolution and sufficient contrast for accurate image processing. Optical porosity was assessed using photos (Figure 4) taken across all six study PFBs during both the growing and non-growing seasons.

Wind Speed Reduction and Temperature Change

The wind speed reduction and air temperature change were measured using a mobile anemometer type YK 2005-AM with a logger (Lutron electronic). This type of anemometer records air temperature with an accuracy of ± 0.8 °C and wind speed of ± 0.2 $m \cdot s^{-1}$. Anemometers were placed at predetermined measurement points in relation to the PFBs (Figure 5) and were positioned at an elevation of 1.67 m to ensure consistency in measurements. The first anemometer (A1) is placed at 50 m in front of the PFBs on the windward side in relation to the dominant wind. Another three anemometers (A2, A3 and A4) were placed at measuring points at 30 m, 60 m and 90 m behind the PFBs in the protected zone (on the leeward side) (Figure 5). The measurement points could be defined based on the height of the belt (e.g., 1H at a distance equal to one belt height, 5H at a distance equal to five belt heights) (Rehacek et al. 2017). However, in this study, as in Středa et al. (2008) or Aili et al. (2024), fixed distances between measurement points were used (30, 60, 90 m). Since the average height of the belt in this study is about 10 m (Table 1), the measurement points at 30 m correspond to 3h, 60 m corresponds to 6h, and 90 m corresponds to a distance of 9h from the belt.

Wind speed measurements per zone were performed in a time interval of 30 minutes, which means that approximately 900 incoming changes in wind speed and air temperature were detected on each anemometer during each series of measurements. Then, the wind speed was averaged on each anemometer, thus obtaining the average incoming wind speed at each measurement point. As part of the field work on measuring wind speed and air temperature, 18 measurements were performed on six studied zones in the period from June 2022 to August 2023.

The wind speed reduction and temperature change are expressed as a percentage of the wind speed/temperature in the protected zone (at measuring points A2 (30 m), A3 (60 m) and A4 (90 m)) in relation to the incoming wind speed/temperature outside the protected zone (at measuring point A1 – 50 m) in percentage expressed in decimal notation.

Figure 4. Photo of protective forest belts (PFB) 1 during the growing and non-growing periods, used to determine the optical porosity.

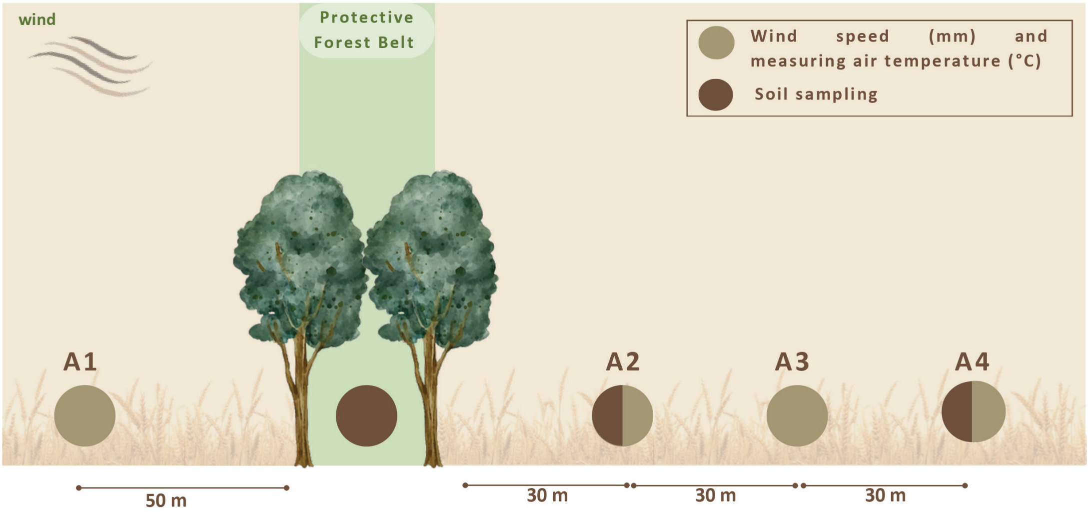


Figure 5. Spatial arrangement of anemometers (A1 - anemometer on the windward side, A2 – 30 m, A3 – 60 m and A4 – 90 m - anemometers in the protected zone of the protective forest belt (PFBs)), with the soil sampling.

Soil Properties

Soil samples were taken based on a composite sample, which consisted of five subsamples, at depths of 0 to 10 cm in each of the PFBs collected at 30 m and 90 m from the PFBs (Figure 5).

The table (Table 2) shows the physical and chemical analyses performed in this research, as well as the used methodology and standards (according to ISO).

Reference Evapotranspiration

Reference evapotranspiration was calculated at the same measurement points where the wind speed reduction and air temperature change were measured. The calculation of evapotranspiration was performed using the FAO Penman Monteith equation (FAO 2025). This method, according to Sentelhas et al. (2010) is considered a universal and reference method for calculating evapotranspiration. According to the FAO Penman Monteith equation, the calculation of reference evapotranspiration is enabled based on the data on air temperature, relative air humidity, wind speed and insolation. Relative humidity data were obtained from the Republic Hydrometeorological Institute (RHI) yearbook for the Pančevo meteorological station, covering a 30-year period (1990-2020) for both growing and non-growing periods.

Statistical Analysis

The Two Independent Samples t-test was used to assess the statistical significance of the difference in the mean values of the potential of the PFBs regarding wind speed reduction and air temperature change between the growing and non-growing periods. To evaluate the statistical significance of the difference in the mean values of the physical and chemical properties of the soil at the measurement points (30 m and 90 m) within the PFBs' protective zone, the Two-Samples Paired t-test was used. To assess the relationship and the strength of the association between the reduction in wind speed and air temperature, on the one hand, and optical porosity, on the other, a One-Factor Simple Regression analysis was applied. Statistical analysis was performed using Statgraphics v.16.1.11 software.

Table 2. Methods used for physical and chemical analyses of oil samples.

Analysis		Method	Standard
Physical analysis	Granulometric composition	Combined sieve and sedimentation test (Hydorimeter method)	SRPS ISO 17892-4:2017
	Soil moisture	Drying method	SRPS ISO 17892-1:2015
	Volumetric masses	Cutting ring of known volume	SRPS ISO 17892-2:2015
Chemical analysis	pH in water and in $CaCl_2$	Electrometric determination	SRPS ISO 10390:2023
	$CaCO_3$	Volumetric determination - Scheibler's calcimeter	SRPS ISO 10693:2005
	Organic matter	Bichromatic method according to Tyurin	SRPS ISO 10694:2005
	C	Calculated	
	N	Modified method according to Kjeldahl, with dry combustion	SRPS ISO 11261:2005 SRPS ISO 13878:2005
	Readily available phosphorus	AL-method according to Egner Riehm	SRPS ISO 11263:2016 SRPS ISO 14870:2005

RESULTS

Wind Speed Reduction

Based on the measurement, the results show a reduction of the incoming wind speed under the influence of PFBs in both observed periods (growing and non-growing periods) (Table 3).

Wind speed reduction at 30 m leeward of PFBs is statistically significantly ($p < 0.05$) greater in the growing season compared to the non-growing season, accounting 48% and 22% of the initial wind speed at 50 m upwind of PFBs, respectively (Table 3).

Temperature Change

Based on the measurement of air temperature at the measurement point outside the protected zone of the PFBs (on the windward side of the belt - A1) and in the protected zone (A2 – 30 m, A3 – 60 m, and A4 – 90 m), the results show that in both observed periods (growing and non-growing period), there is an increase in air temperature immediately behind the PFB (A2 – 30 m). With distance from the PFB, at measuring points A3 – 60 m and A4 – 90 m, there is a decrease in temperature (Table 4).

The Relationship between the Optical Porosity of the Belt and the Reduction in Wind Speed and Changes in Air Temperature

The results indicate a statistically significant ($p < 0.05$) strong positive correlation ($R = 0.71$) between the reduction in wind speed at 30 m in the impact zone and the optical porosity of the belt (Figure 6). With increasing optical porosity, the belt reduces wind speed on the leeward side to a lesser extent. The results also indicate a statistically significant ($p < 0.05$) negative correlation ($R = -0.51$) between the increase in air temperature at 30 m in the impact zone and the optical porosity of the belt (Figure 6). The cooling effect of the belt increases with increasing optical porosity.

Evapotranspiration Change

PFBs in the protected zone (at measuring points A2 – 30 m, A3 – 60 m and A4 – 90 m) significantly affect the reduction of wind speed and increase in air temperature. A change in these two indicators leads to a change in the reference evapotranspiration (ETo) in the protected zone. The graphs (Figure 7) show the change in reference

Table 3. Mean wind speeds (mean ± SD) at measuring points in front of the belt on A1 (50 m) and the protected zone (A2 - 30 m, A3 - 60 m and A4 - 90 m) in the growing and non-growing periods, for the whole year, as well as the mean optical porosity of the protective forest belts (PFBs).

Period	Wind speed in front of the belt on A1 (50 m) ($m \cdot s^{-1}$)	Wind speed reduction (%)		Mean optical porosity
Growing	3.45 ± 0.96	A2 (30 m)	0.52 ± 0.10*	19.37
		A3 (60 m)	0.60 ± 0.19	
		A4 (90 m)	0.71 ± 0.26	
Non-growing	4.83 ± 1.41	A2 (30 m)	0.73 ± 0.15*	59.75
		A3 (60 m)	0.72 ± 0.19	
		A4 (90 m)	0.83 ± 0.17	
Whole year	4.22 ± 1.43	A2 (30 m)	0.64 ± 0.15	39.56
		A3 (60 m)	0.67 ± 0.18	
		A4 (90 m)	0.77 ± 0.19	

* The difference between the mean values of wind speed in the growing and non-growing periods detected at the first measuring point in the influence zone (A2), with statistical significance ($p < 0.05$)

Table 4. Temperature increases at measuring points in front of the belt on A1 (50 m) and in the zone of influence (A2 – 30 m, A3 – 60 m and A4 – 90 m) in the vegetation and non-vegetation period, for the whole year, as well as the mean optical porosity of the belt.

Period	Air temperature in front of the belt at A1 (50 m) (°C)	Air temperature change (%)		Mean optical porosity
Growing	27.03 ± 1.30	A2 (30 m)	1.08 ± 0.04	19.37
		A3 (60 m)	1.05 ± 0.05	
		A4 (90 m)	1.03 ± 0.05	
Non-growing	14.00 ± 1.17	A2 (30 m)	1.04 ± 0.04	59.75
		A3 (60 m)	1.03 ± 0.01	
		A4 (90 m)	1.01 ± 0.01	
Whole year	19.36 ± 6.40	A2 (30 m)	1.06 ± 0.04	39.56
		A3 (60 m)	1.03 ± 0.03	
		A4 (90 m)	1.02 ± 0.03	

evapotranspiration (ETo) in the protected zone of the studied PFBs in the growing and non-growing periods.

Soil Properties Change

All soil samples in the PFBs compared to soil samples on the agricultural fields (90 m) are characterized by higher sand content, lower clay content and higher moisture content. Regarding chemical analysis, a higher content of humus, nitrogen and phosphorus is observed in the soils sampled in the PFBs compared to the soils sampled in agricultural fields (Table 5).

Based on the analysis of soil samples, a statistically significant difference in clay content was observed between 30 and 90 m from the PFBs (Table 6). Higher clay content was recorded at 90 m (19.41%) compared to 30 m, where it was 15.83%.

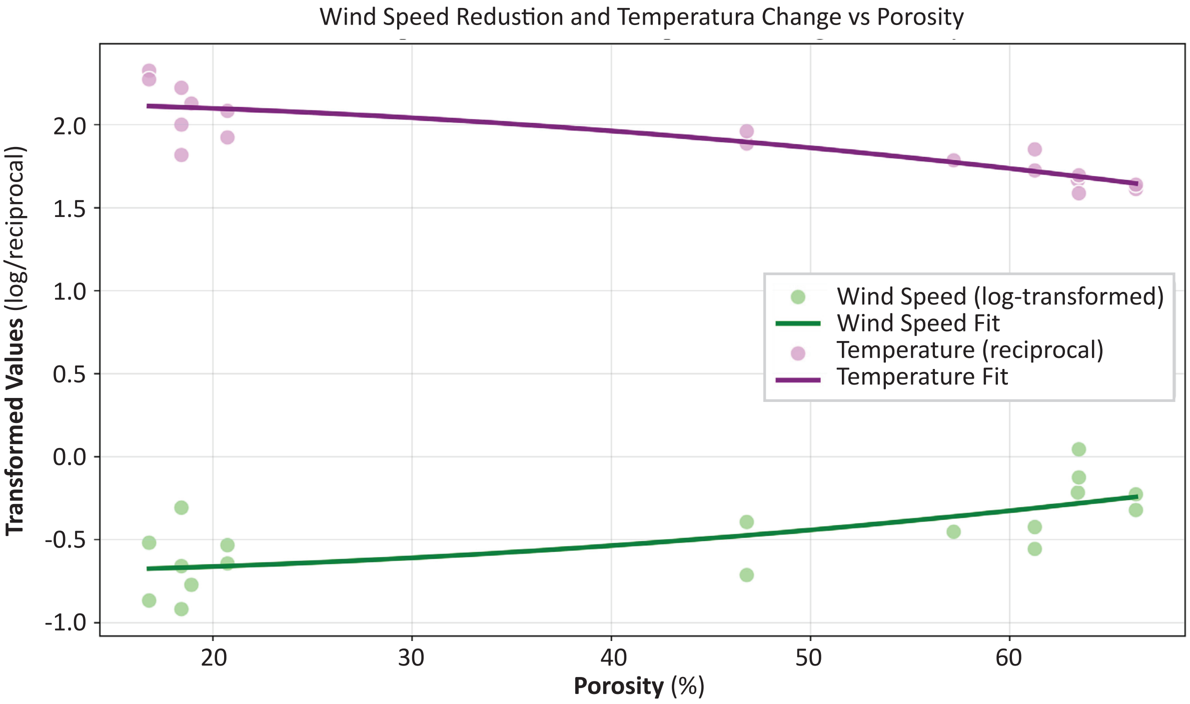


Figure 6. The relationship between the optical porosity of protective forest belts (PFBs) and the reduction of wind speed and changes in air temperature.

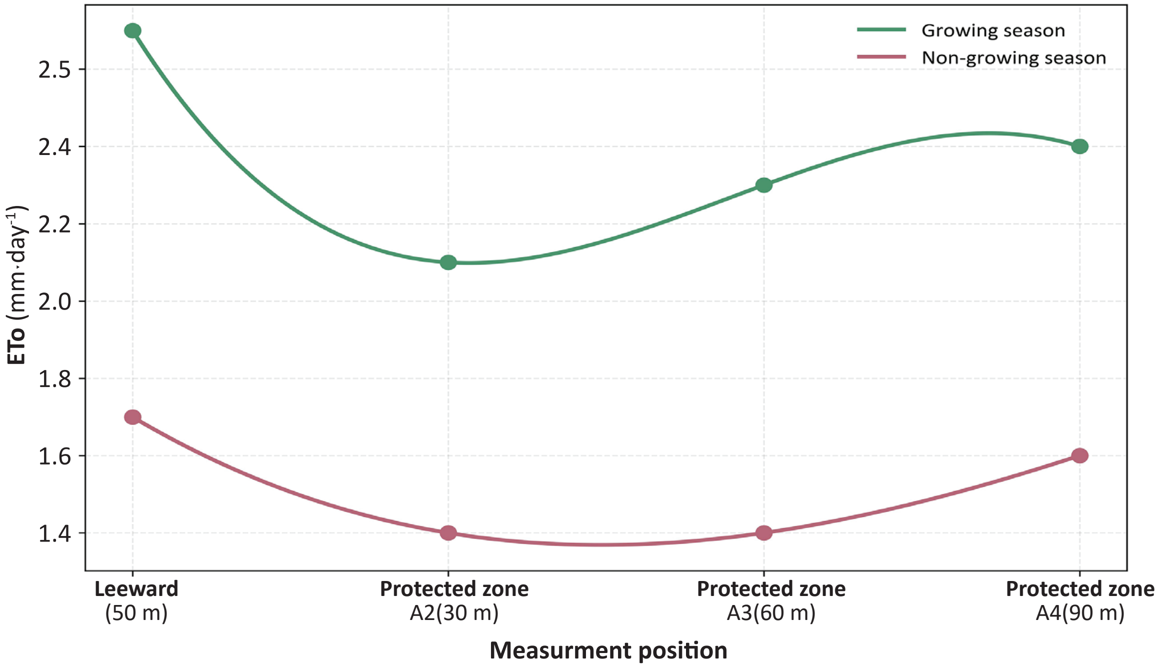


Figure 7. Change in reference evapotranspiration (ETo) in the protected zone (A2 – 30 m, A3 – 60 m and A4 – 90 m).

Table 5. The analysis of the physical and chemical properties of soil in protective forest belts (PFBs) and agricultural areas.

Indicator	PFB (mean ± SD)	Agriculture areas (mean ± SD)
Clay (%)	14.00 ± 4.03	19.42 ± 3.04 *
Sand (%)	30.73 ± 4.40 *	21.37 ± 3.13
Moisture (%)	24.48 ± 2.97 *	19.91 ± 1.68
Volumetric mass ($Mg \cdot m^{-3}$)	1.35 ± 0.08	1.50 ± 0.12
$CaCO_3$ (%)	5.30 ± 3.38	5.28 ± 3.01
C (%)	3.17 ± 0.89*	1.92 ± 0.15
Nitrogen (%)	0.30 ± 0.08*	0.17 ± 0.02
Phosphorus ($mg \cdot 100g^{-1}$)	29.24 ± 0.06	26.10 ± 5.94

*Statistically significant difference ($p < 0.05$) between the mean values within the PFBs compared to agricultural land areas

Table 6. The analysis of the physical and chemical properties of soil at distances of 30 and 90 m from the protective forest belts (PFBs).

Indicator	30 m (mean ± SD)	90 m (mean ± SD)
Clay (%)	15.83 ± 2.69	19.42 ± 3.04 *
Sand (%)	23.91 ± 3.54	21.37 ± 3.13
Moisture (%)	20.48 ± 2.96	19.91 ± 1.68
Volumetric mass ($Mg \cdot m^{-3}$)	1.61 ± 0.09	1.50 ± 0.12
$CaCO_3$ (%)	5.36 ± 2.48	5.28 ± 3.01
C (%)	1.93 ± 0.20	1.92 ± 0.15
Nitrogen (%)	0.19 ±0.04	0.17 ± 0.02
Phosphorus ($mg \cdot 100g^{-1}$)	31.0 ± 5.04	26.10 ± 5.94

*Statistically significant difference ($p < 0.05$) between the mean values at 30 and 90 m from the PFBs

DISCUSSION

PFBs significantly contribute to reducing wind speed within their protected zone. However, the literature presents various statements regarding the distance within the protected zone where the belt's influence on wind speed reduction is observed. Based on the research conducted in the United States of America, Gloyne (1984) shows that at 20H (for a belt height of 10 m, this distance is 200 m) in the protected zone, there is a reduction in wind speed of about 40%. Heisler and Dewalle (1988) report that the wind speed reduction effect of shelterbelts extends to a distance of approximately 50H (for a belt height of 10 m, this distance is 500 m). However, most studies indicate a smaller distance from the belt where the reduction of the incoming wind speed occurs and that it is greatest in the zone from 3H to 5H (30 to 50 m). For example, Vacek et al. (2018), similarly to this study as well, show that at the first measuring point at 3H, which is approximately 30 m for a belt with an average height of 10 m, the incoming wind speed is reduced by about 60%, while at the farthest measuring point 12H, (approximately 120 m), the wind speed is reduced by only 20%. Similar results were obtained by Středa et al. (2008), Van Thuyet et al. (2014), and Rahacek et al. (2017). Based on the results of this research, at the third measurement point (A4), i.e. at 90m (approximately 9H) from the belt, in both observed periods, there is a reduction in wind speed of 23%.

When analyzing the influence of the PFBs in the growing and non-growing periods separately, there is a statistically significant difference in the reduction of wind speed at the first measurement point (A2), that is, at 30 m (about 3H) from the PFB. Similar results were reached by Rahcek et al. (2017), which show the greater potential of belts for reducing wind speed when the optical porosity is lower, because when the porosity of a belt is higher, there is physically greater airflow passing through the belt, i.e. when it is the growing period. Lukić and Dožić (2007) show that belts in the growing season have a greater potential for reducing wind speed and wind erosion control. Greater reduction of the incoming wind speed in the growing period is a consequence of lower belt porosity, i.e. optical porosity values closer to the optimum, in contrast to the non-growing period when the optical porosity is higher. To achieve optimal optical porosity even during the non-growing season, the use of coniferous species for PFBs could also be considered, as PFBs containing conifers exhibit higher optical porosity in comparison to broadleaf PFBs during the winter season.

Although numerous research has mentioned the influence of PFB on the change in wind speed reduction, a limited number of studies quantify the impact of the belts on the change in air temperature in the zone of influence. Nevertheless, Campi et al. (2009) reported an increase in temperature with distance from the PFBs. That research shows that air temperatures are higher near the PFB (in the protected zone) compared to the open field (outside the protected zone). Similar results were obtained in the research of Lampartová et al. (2015), where the mean maximum temperature in front of the PFB was 2°C lower compared to the area behind the PFB. Also, in the research of Skidmore et al. (1972), results indicate higher air temperatures recorded in the protected zone immediately behind the belt in relation to the temperature on the windward side. The aforementioned studies are in agreement with the results of this study, which indicate lower air temperatures in the open field where the influence of the PFBs is not recorded (in front of the PFB on the windward side) and increased air temperature in the protected zone, with a recorded increase in temperature with greater distance from the PFB. The impact of the PFB on temperature change can be explained by their effect on wind speed reduction (Baker et al. 2021), as in the protected zone, decreased wind speed and wind-driven cooling effect, which consequently leads to higher air temperatures.

The findings of this study align with the meta-analysis performed by Helfer et al. (2009) and showed that under the influence of PFBs, evapotranspiration is reduced by up to 35% on the windward side. In the same research, it is stated that the greatest reduction in evapotranspiration, up to 50%, is in the protected zone from 4H to 7H (around 40 to 70 m) on the leeward side of the PFBs. Similar results were obtained in the study by Campi et al. (2009), who show that there are three zones of PFBs' influence on the reduction of evapotranspiration. They show that the greatest decrease in reference evapotranspiration occurs in the protected zone at 5H from the PFBs. Further in the next zone, there is a gradual increase in evapotranspiration (from 5 to 17H), while in the third zone, evapotranspiration is the same as outside the zone of influence (beyond 17H). Also, in this research, as in Campi et al. (2009), it is observed that with distance from the PFB, there is an increase in evapotranspiration (Figure 7). Such positive effects of PFBs, including reduced wind speed and lower evapotranspiration, can significantly enhance agricultural production by increasing crop yields (Enescu et al. 2025).

Some authors state the positive influence of the forest on the physical characteristics of the soil in terms of better mechanical composition (optimal content of sand and clay particles), moisture, bulk density, as well as chemical composition (more organic matter, nutrients (NPK)) (Wu et al. 2018, Wu et al. 2023). Regarding the impact of PFBs on soil conditions, the findings are consistent with previously reported results, confirming their positive effects (Wu et al. 2018). The results of this study are compared with findings from previous research in which soil conditions within PFBs and in adjacent agricultural and pasture areas were analyzed, with a consensus indicating improved soil properties within the PFBs. This refers to non-capillary porosity (Yu et al. 2018) and higher soil moisture within shelterbelts, which can be directly attributed to the presence of trees and forest floor litter that enhance moisture retention and reduce water loss. Additionally, a higher organic matter content has been observed within PFBs compared to surrounding agricultural areas, which is a result of the positive influence of forest vegetation, such as the decomposition of organic material. Such a positive impact on soil conditions can create more favourable conditions for agricultural production.

When observing the impact of PFBs in the protected zone and analysing the physical and chemical properties at the measured points from PFB, there is a lack of scientifically supported literature on this topic. However, some studies (Qiao et al. 2016, Kong et al. 2022) show that with distance from the PFBs, the influence of the PFBs in terms of increasing erodibility and nutrient content (organic matter and nitrogen) decreases. This research shows a statistically significant increase in clay content with increasing distance from the PFB, with higher values observed at 90 m (approximately 9H) compared to 30 m (approximately 3H). This pattern can be explained by the influence of intensive agricultural production and the application of mechanization, which results in soil compaction and higher content clay (Mileusnić et al. 2022) Additionally, higher concentrations of nutrients (carbon, nitrogen, phosphorus), although not statistically significant, observed near the PFB (at 30 m) may indicate a positive effect of the belt, which could further contribute to increased yields from agricultural land.

CONCLUSIONS

This study demonstrates the significant impact of Protective Forest Belts (PFBs) on wind speed reduction, improving microclimatic conditions, and enhancing soil properties in South Banat, Serbia. Results showed that wind speeds were reduced by up to 50% at 30 m from the PFB during the growing season, contributing to a decrease in evapotranspiration and higher soil moisture retention. These factors are crucial for mitigating soil erosion and sustaining agricultural productivity. PFBs also had a positive effect on some soil properties, with areas near the belts exhibiting higher organic matter, nitrogen, and phosphorus levels, alongside better moisture retention and lower clay content. These benefits were most pronounced closer to the PFBs and gradually diminished with increasing distance, underlining the need for careful planning in agroforestry systems. Additionally, air temperatures immediately behind the PFBs were slightly higher due to the reduction in wind speed, further contributing to favourable growing conditions. The study highlights the multifunctionality of PFBs, not only as a tool for controlling wind erosion but also for enhancing soil health, regulating temperature, and improving the overall microclimate. In regions like Vojvodina, where wind erosion poses a significant threat to agriculture, expanding the use of PFBs could be a sustainable solution. This research supports the wider adoption of PFBs in agricultural landscapes, advocating for their role in increasing ecosystem services and improving the resilience of agro-ecosystems against climate change. Their wider application in agricultural production systems is important due to their positive impact on crop yields. The findings contribute valuable data to the growing body of research on agroforestry practices, emphasizing the importance of PFBs in sustainable land management.

Author Contributions

AB, SL conceived and designed the research, PM, MCM, SM, NŽ carried out the field measurements, NŽ, AB performed laboratory analysis, AB, PM, and MCM processed the data and performed the statistical analysis, SBS and SL secured the research funding and supervised the research and helped to draft the manuscript, AB, SL, PM and MCM wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Analysis of Wood Defects in Norway spruce (*Picea abies* (L.) Karsten) from Mixed Uneven-Aged Forest Stand

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ABSTRACT

Norway spruce (*Picea abies* (L.) Karsten) is one of the most economically important conifer species in Europe. Efficient utilisation and processing of its wood require detailed knowledge of its technical properties, as well as the most common wood defects that substantially affect both properties and utilisation. Given the crucial role of wood defects in the roundwood classification system, the primary objective of this study was to identify defects in Norway spruce and to analyse the influence of forest assortment characteristics (diameter and position along the stem) and tree attributes (diameter at breast height and position within the stand) on the size of wood defects. The research was conducted in Bosnia and Herzegovina, within a forest compartment of an uneven-aged, mixed beech and silver fir stand with spruce. Trees were felled and processed into assortments using a chainsaw, predominantly applying the cut-to-length method. After measuring the assortment dimensions, the occurrence of defects was assessed, and their sizes were determined. The analysis showed that, following knots, the most common wood defect was rot, followed by pith eccentricity, compression wood, scars, mechanical damage, and resin pockets. Statistically significant differences were found in the size of knots, ellipticity, and taper among different diameter classes of assortments ($p<0.05$), as well as assortment positions along the stem ($p=0.0000$). Also, a statistically significant difference was observed in the size of the knots and ellipticity in relation to both diameter at the breast height and tree position within the stand ($p<0.05$). Overall, the findings align with previous studies, confirming the higher quality of the lower stem section, as reflected in smaller defect sizes critical for roundwood quality classification, such as knots, rot, ellipticity, and taper.

Keywords: quality classification; roundwood; wood defect; knots; rot

INTRODUCTION

Norway spruce (*Picea abies* (L.) Karsten) is one of the most economically significant conifer species in Europe. Its high productivity and wood quality across diverse site conditions distinguish it from other species (Skrøppa 2003). Owing to its favourable mechanical properties and low density, Norway spruce wood is widely used in construction and the furniture industry (Šoškić et al. 2008). The proper use and processing of wood require knowledge of its technical properties, as well as of the most common wood defects that significantly affect its performance and utilisation. The quality structure of forest wood assortments depends on numerous factors, primarily the dimensions of the assortment and the size of external defects (knots, etc.), and to a lesser extent internal defects (rot, etc.), as well as damage incurred during tree felling and processing (Krajnc et al. 2023). The external appearance of a tree and its properties, such as stem form, branching, bark features, anatomical structure, and wood colour, are determined by a range of characteristics that develop during its normal life cycle. Environmental factors, including temperature fluctuations, precipitation, snow, wind, and light, also affect tree growth. Biological influences, such as attacks by fungi, insects, animals, parasitic plants, and human activities, likewise play a significant role (Richter 2015).

Wood defects thus result from the combined effects of biotic, abiotic, and anthropogenic factors, and can occur on the stem, branches, and roots.

A particular characteristic of a tree resulting from growth under varying conditions may sometimes be regarded as a defect, and at other times not. When viewed from the perspective of tree growth, only those features that shorten the expected lifespan of the tree, such as rot or stem breakage, are considered defects. Irregular stem shape and knots, for example, are not regarded as defects from a biological standpoint. However, from the perspective of wood utilisation, defects are characteristics that make the use of wood for a specific purpose difficult or impossible (Richter 2015). In forestry practice of Bosnia and Herzegovina, wood defects are commonly defined according to Gurda and Music (2015) as deviations from the normal structure, form, colour, or properties of wood, particularly those that reduce its usability. The quality classification of wood, according to the applicable standards in Bosnia and Herzegovina (Yugoslav standards), is based on the presence, size, and frequency of wood defects, as well as the dimensions of the wood assortments (diameter and length).

Knots, rot, scars, curvature, compression wood, spiral grain, hazel wood, sunburn bark, resin pockets, burls, ring shakes, mechanical damage and damage caused by insects are cited as the most common wood defects in Norway spruce (Ugrenović 1950, Karahasanović 1992, Barszcz and Michalec 2007). Previous studies of Norway spruce identify knots and rot as the wood defects with the greatest negative impact on wood quality (Barszcz et al. 2010a, 2010b, Michalec et al. 2013, Kadunc 2013, Vlad et al. 2018, Noordermeer et al. 2023, Lara et al. 2024).

Spruce wood contains resin pockets, which can reduce its usability and commercial value. Resin pockets directly affect the aesthetic quality of wood products. The leakage of resin produces a sticky layer on contact with objects or people, which poses a greater disadvantage for carpentry applications than for construction wood. These negative effects have led to the number and size of resin pockets being regulated in national standards for wood classification (Gjerdrum and Bernabei 2006). Gryc and Horáček (2007) found that the density of Norway spruce wood is higher when compression wood is present. In cases where compression wood accounts for 80% of the stem, the wood density is 1.5 times greater compared to wood without this defect. Due to the higher density, working with compression wood results in increased energy consumption. Additionally, products containing compression wood exhibit a different colour, which may be considered an undesirable trait unless otherwise specified. Gryc and Vavrčík (2009) reported that the compressive strength parallel to the grain of Norway spruce compression wood is slightly higher than that of wood without the defect, reaching a value of 50 MPa.

The effects of bark beetle infestation on the tensile and compressive strength of Norway spruce wood were studied by Löwe et al. (2022). They reported that the longer trees remain in the stand after infestation, the greater is the reduction in these strength properties. In trees infected three years prior to analysis, tensile strength decreased by 14% compared to healthy trees (from 93.185 MPa to 80.709 MPa), while compressive strength declined by 25.6% (from 46.144 MPa to 34.318 MPa).

Lojo et al. (2021) found that the proportion of Norway spruce logs increases significantly with tree diameter. They also highlighted the influence of tree height on assortment volume, noting that taller trees produce a greater volume of high-quality assortments within the same diameter class.

Considering the previously emphasized importance of wood defects in the classification system of roundwood, the primary aim of this study was to identify wood defects in Norway spruce and to analyse the influence of forest wood assortment characteristics (diameter and position along the stem), as well as tree attributes (diameter at breast height and position within the stand), on the size of specific wood defects.

MATERIALS AND METHODS

The research was conducted in Bosnia and Herzegovina, in a forest compartment within an uneven-aged, mixed beech and silver fir forest with spruce, growing on shallow cambisol, leptosol and their complexes on limestone and dolomite, and phaeozems on saccharoid limestone and dolomites. The study area is located at 43°45'16'' N and 18°14'47'' E. Average elevation in forest compartment is 1,310 m, with terrain slope ranging from 10° to 25°. In 2023, trees were selected for felling according to the group selection system principles, with a total of 2,766 m^3 of merchantable wood (not smaller than 7 cm over-bark) marked. Field research focused on wood defect analysis was carried out in November 2024. A total of 30 Norway spruce trees were included in the study. To minimise sampling bias, trees were analysed consecutively along the felling lines, regardless of their external characteristics. Before tree felling, the diameter at breast height (DBH) and position within the stand were recorded for each analysed tree. DBH (measured at 1.3 m above ground level) was determined using a caliper. Tree position within the stand was classified according to Kraft's classification (1884), assigning each tree to one of five categories: predominant, dominant, co-dominant, suppressed, or overtopped.

The trees were felled and processed into forest wood assortments using a chainsaw. The cut-to-length processing method was predominantly applied, while the half-tree length method was used to a lesser extent. Length and diameter of all processed assortments were measured immediately after felling. Length was measured at the shortest point of each assortment using a measuring tape. Diameters were measured with a caliper at designated points crosswise (maximum and minimum), with values rounded down to the nearest centimeter. The reduction from over-bark to under-bark diameter was performed by subtracting double bark thickness in the amount of 1 cm for forest wood assortments with diameters up to 35 cm at the middle of length, and 2 cm for those with diameters exceeding 35 cm, in accordance with the internal regulations of the enterprise managing the forests in the study area. Diameters were recorded at three positions along each wood assortment: the thicker end, the middle of the length, and the thinner end. For the first assortment taken from the base of the tree, two crosswise diameters were measured at the root collar end, located at a maximum distance of 1 m from the thicker end instead of measuring diameter at the thicker end. The volume of forest wood assortments was determined using Huber's formula (Equation 1) cited in Banković and Pantić (2006).

$$V = g_{½} \cdot L \qquad (1)$$

where V is volume of wood assortment (m^3), $g_{½}$ is basal area at the middle of length (m^2), and L is length of wood assortment (m).

After measuring the assortment dimensions, the presence of wood defects was analysed and their sizes were determined. Defects were measured according to the national Yugoslav standard from 1969 (JUS D.A0.101 - Wood Defects: Terminology, Definitions, and Measurement). The formulas used to calculate the size of individual defects are presented in Table 1.

For some defects (split tree stem, compression wood, resin pockets, ingrown bark, and insect damage), only their presence or absence was determined.

Knots were classified according to the following criteria: mutual position (individual knots; knots in a whorl), degree of their tightness with the surrounding wood (tight or intergrown; partially intergrown; encased or loose knots), health status (sound; partially rotten; rotten), and size (pin knots <6 mm; small knots 7–20 mm; medium knots 21–40 mm; large knots >41 mm). A similar classification of knots can be found in Barszcz et al. (2010a, 2010b). For knot size classification, the smallest diameter of an individual knot was recorded, whereas for knots in a whorl, the smallest diameter of the largest knot was measured.

Statistical data processing was performed using the Statgraphics Centurion XVII software package. After testing for data normality (Shapiro-Wilk W-test) and homogeneity of variance (Levene's test), the ANOVA or Kruskal-Wallis non-parametric test was applied to analyse the influence of forest wood assortment and tree characteristics on the size of wood defects. $\chi 2$ test was used to determine compression wood occurrence depending on wood assortment and tree characteristics.

RESULTS AND DISCUSSION

The research included 30 Norway spruce trees with an average DBH of 71.17 cm (±15.39 cm), ranging from 22 to 94 cm. The total gross volume of all analysed trees was 141.289 m^3, corresponding to an average of 4.710 m^3 per tree. From these trees, 133 wood assortments were processed (Table 2). Sawlogs accounted for 76.58% of the total volume of processed forest assortments, followed by pulpwood with 21.18% and round pit timber with 2.24%.

Table 1. Formulas for calculating wood defect sizes.

Wood defect	Formula
Ellipticity (%)	((LD–SD)/LD) · 100
Taper (%)	(((TKD-THD)/L)/TKD) · 100
Double heart (%)	(DP/D) · 100
Pith eccentricity (%)	(DC/D) · 100
Curvature (%)	(HC/L) · 100
Rot (%)	(DR/D) · 100
Scars and damage caused by physical and mechanical factors (mm^2)	Scar/damage length (mm) · Scar/damage width (mm)

LD – larger diameter; SD – smaller diameter; TKD – thicker end diameter; THD – thinner end diameter; L – length; DP – distance between piths; D – diameter; DC – distance from centre; HC – height of curvature arc; DR – diameter or depth of rot

Table 2. Features of processed wood assortments.

Feature	Number of data	Minimum	Average	Maximum	Standard deviation
N	30	2.00	4.43	6.00	±1.28
V_t (m^3)	30	0.20	4.45	8.10	±1.91
L (m)	133	1.10	6.05	11.10	±1.51
D_m (cm)	133	11.00	45.90	86.00	±18.20
D (cm)	103	19.00	47.47	83.00	±14.09
D_r (cm)	30	26.00	80.77	99.00	±15.62
d (cm)	133	10.00	40.65	83.00	±17.89
V_i (m^3)	133	0.03	1.00	2.87	±0.73

N – number of assortments per tree; V_t – volume of all assortments per tree; L – assortment length; D_m – diameter at the midlle of length; D – diameter at the thicker end; D_r – diameter at the root collar end; d – diameter at the thinner end; V_i – Volume of individual assortment

Analysis of the processed forest assortments revealed that after knots, the most prevalent defect was rot, observed in 56.67% of the analysed trees. Pith eccentricity and compression wood were each recorded in 50% of the trees. Scars were observed in 26.67% of trees, whereas damage from physical and mechanical factors and resin pockets were present in 20% of cases each. Other recorded wood defects (double heart, curvature, split tree stem, ingrown bark, and insect damage) were present in 10% or fewer of the analysed trees (Figure 1). The most frequent wood defects are presented in Figure 2.

Some of the commonly reported wood defects in Norway spruce trees (Ugrenović 1950, Karahasanović 1992, Barszcz and Michalec 2007, Sandak et al. 2020) were also observed in this study, including compression wood, resin pockets, curvature, rot, scars, mechanical damage, and insect damage. Other defects frequently cited in the literature, such as spiral grain, hazel wood, sunburn bark, burls, and ring shakes, were not observed in the analysed trees.

Knots were predominantly in whorls (80.76%), tight (87.09%) and sound (92.87%) (Figure 3). Barszcz et al.

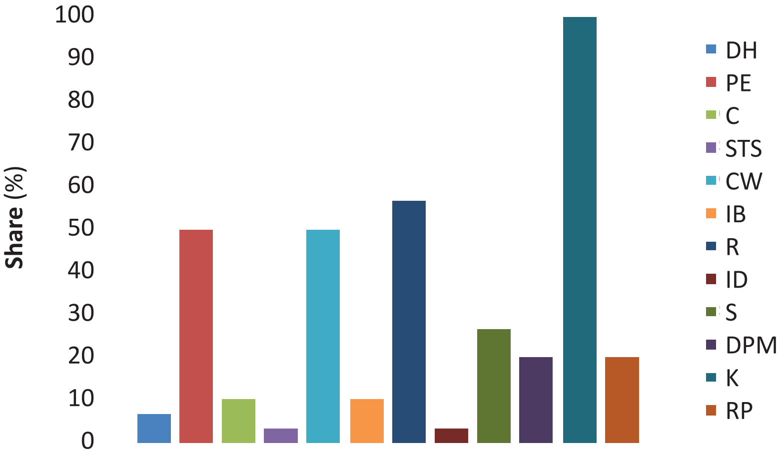

Figure 1. Proportion of wood defects in the analysed trees. DH – double heart; PE – pith eccentricity; C – curvature; STS – split tree stem; CW – compression wood; IB – ingrown bark; R – rot; ID – insect damage; S – scars; DPM – damage caused by physical and mechanical factors; K – knots; RP – resin pockets.

Figure 2. Some of the recorded wood defects in Norway spruce. **(a)** knots; **(b)** rot; **(c)** pith eccentricity and compression wood; **(d)** scar; **(e)** damage caused by mechanical factor; **(f)** resin pockets.

(2010a) also found that sound-tight knots prevailed (60%) in mature 150-year-old Norway spruce stands situated at an altitude of 1,450-1,740 m above sea level in northern Italy. Medium-sized knots (21–40 mm) and large knots (>41 mm) were the most frequently found. Small knots (7–20 mm) comprising 15.28% of the total. Based on measurements of 1,263 individual knots and knots in a whorl, it was determined that its size ranged from 10 mm to 75 mm, with an average of 33.81 mm. The average knot diameter was higher than 20.3 mm reported by Barszcz et al. (2010a) in 150-year-old Norway stands situated at an altitude of 1,450–1,740 m above sea level in northern Italy.

Rot was measured at both the thicker and thinner ends of the logs. The average extent of rot at the thicker end of the assortments was 51.65%, ranging from 10.19% to 100%. At the thinner end, the average extent of rot was slightly higher, 67.63%, ranging from 10.19% to 94.34%.

The average scar size was 561.55 cm², with the majority of scars (81.82%) observed on the first and second assortments, moving upward from the base of the tree toward the crown. The average size of damage caused by physical and mechanical impacts was 598.11 cm². The highest incidence of damage caused by physical and mechanical impacts was also recorded in the lower part of the stem, specifically on the first assortment (44.44%) and second assortment (44.44%). These findings support the well-established fact that the lower stem section is the most susceptible to injuries during timber extraction by cable skidders, which are commonly used in forest harvesting in Bosnia and Herzegovina. Kadunc (2013) notes that mechanical damage is a significant factor influencing the occurrence of wood rot in Norway spruce. Also, Michalec et al. (2013) stated that Norway spruce is highly susceptible to mechanical stem damage, which can rapidly lead to rot. Several researchers have investigated fungi affecting Norway spruce trees. Lara et al. (2024) stated that the presence of fungi from genera *Heterobasidion* and *Armillaria* results in financial losses and a reduction in the usable wood volume. Also, Noordermeer et al. (2023) report that butt end rot is the most significant defect in Norway spruce, causing substantial financial losses. In a study of 140,000 m³ of felled spruce wood in Norway, 16% of trees showed rot, with a higher incidence in larger-diameter trees. When root rot is present, the volume of sawlogs is reduced by 48%. The same authors also reported that the presence of wood rot reduces timber revenue by 7%, corresponding to approximately 18.5 million euros in Norway. According to Kadunc (2013) wood rot in Norway spruce can reduce timber value by up to 19 €·m^{-3}, with the most significant losses observed in the 50–70 cm diameter class. Therefore, careful consideration is required when conducting harvesting operations in spruce stands. In this study, injuries (damage caused by physical and mechanical impacts and/or scars) were observed in 41.18% of trees in which rot was recorded.

Compression wood was found in 23.31% of wood assortments. The χ^2 test was used to analyse the occurrence of compression wood in relation to the characteristics of processed wood assortments and the tree (Table A1, Table A2, Table A3, and Table A4). A statistically significant difference was found in the proportions of assortments with and without compression wood across different stem sections (p=0.0206). For the other analysed factors (assortment diameter, DBH, and position within the stand), no statistically significant effects were observed (p>0.05). This wood defect was most frequently observed in the first assortment (45.16%), while in the upper parts of the stem, in the fourth and fifth assortments, it was recorded in only 19.63% of cases. No defects were observed in the sixth assortment. The distribution of compression wood along the stem is consistent with the findings of Warensjö (2003), who reported that this wood defect is typically

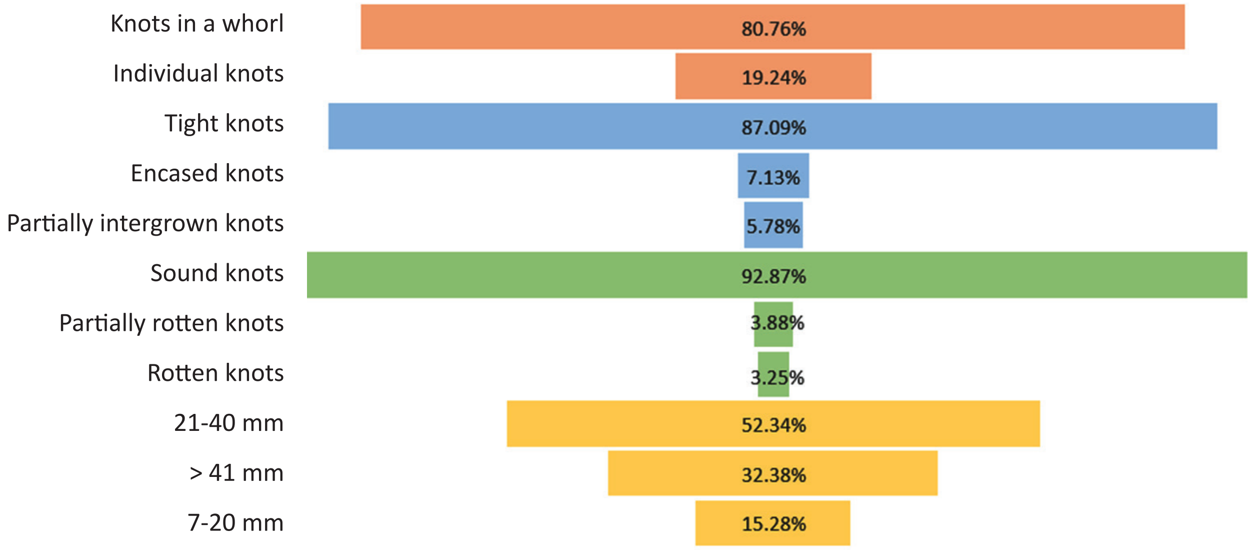

Figure 3. Distribution of knots by mutual position, degree of their tightness with the surrounding wood, health status, and size.

most pronounced near the ground and decreases toward the upper parts of the stem.

The pith eccentricity varied from 6.77% to 24.47% at the thicker end, and from 6.77% to 24.14% at the thinner end. The lower number of defects recorded at the thinner end (16) compared to the thicker end (29) supports the observation that these defects tend to disappear at a certain height of the tree.

Resin pockets were found in 5.26% of the wood assortments. The defect was recorded at tree heights ranging from 6.54 m to 25.11 m, with an average height of 14.99 m. These results are consistent with the findings of Gjerdrum and Bernabei (2006), Temnerud (1997), and Seifert et al. (2010), which report an increased occurrence of resin pockets with increasing height in Norway spruce trees.

The ellipticity at different positions (thicker end, root-collar end, mid-length, and thinner end), as well as the taper for each wood assortment, were determined. Ellipticity ranged from 0% to 9.09%. The applicable JUS standard defines a section as regular if its ellipticity is less than 10%. Analysis of ellipticity measured along different lengths of the wood assortments showed that this defect did not exceed the threshold value in any case. Taper ranged from 0.96% to 19.41%, with a mean of 5.01%.

The relationship between the wood defect size and the diameter of processed forest assortments, as well as their position along the stem, was analysed. The analysis focused on the most frequent wood defects identified in this study (knots, rot, and pith eccentricity) and on ellipticity and taper, which were determined for each wood assortment. The diameter at mid-length of the forest wood assortment was considered in the analysis. The position along the stem was defined by the assortment's ordinal number (1–6), with assortment 1 corresponding to the lowest position on the stem, closest to the ground.

A statistically significant difference (Table 3 and Table 4) was found in the size of knots, ellipticity and taper for different diameter classes of processed forest wood assortments ($p< 0.05$), as well as for different assortment positions along the stem ($p=0.0000$). Larger knots were observed in thinner assortments originating from the upper part of the stem. The smallest knot sizes were recorded within assortments with diameters from 81 cm to 90 cm, as well as the first assortment in the stem, near the ground. Similar results regarding the size of the knots along the stem were obtained by Barszcz et al. (2010a). Ellipticity decreases with increasing assortment diameter. On the other hand, with respect to position within the stem, the relationship is inverse: ellipticity increases from the root collar toward the crown. Exceptions are 71–80 cm the 81–90 cm diameter classes, as these assortments belong to the first stem section of old trees, where the root collar deformation caused higher ellipticity. Taper decreases from thinner to thicker assortments and increases from the root collar toward the crown. The deviation from trend is expressed in the 81-90 cm diameter class, and the first assortment from the stem, similar to ellipticity.

The relationship between the size of wood defects and tree characteristics (DBH and position within the stand) was also analysed (Table 5 and Table 6). A statistically significant difference was observed in the size of the knots and ellipticity in relation to both DBH and tree position within the stand ($p<0.05$). The largest knots were found in trees with DBH >81 cm and in "predominant trees". The largest size of ellipticity was observed in trees with DBH from 21 cm to 30 cm, and in "co-dominant trees". The result of the previous study (Noordermeer et al. 2023) showed that root collar rot in Norway spruce is more common in trees with larger DBH. Also, Chomicz (2013) emphasised the relationship between tree age and the occurrence of butt end rot in Norway spruce under comparable stand and site conditions, reporting that the probability of rot increases with tree age. However, in this case, no statistically significant effect of DBH on rot occurrence was observed.

Table 3. Analysis of the influence of assortment diameter on the wood defect size.

Diameter of wood assortment (cm)	**K** (mm)	**RTKD** (%)	**RTHD** (%)	**PETKD** (%)	**PETHD** (%)	**E** (%)	**T** (%)
11-20	35.54a	86.21		14.26	16.67	4.58a	7.87b
21-30	32.21a	75.86	93.33	15.51		3.69a,b	6.63b,c
31-40	35.84a	64.16	68.97	13.17	14.58	3.54a,b,c	5.31c,d
41-50	33.63a	53.66	68.29	11.13	12.74	2.53b,c,d	3.62d
51-60	32.74a	47.59	58.36	14.57	12.09	2.41c,d	3.82d
61-70	30.80a	45.03	75	20.35	19.05	1.77d	4.06d
71-80	32.93a	53.42		17.91	14.65	1.90d	3.97d
81-90	26.00b	22.32		16.67		2.34c,d	10.34a
p value	K-W 0.0003*	K-W 0.3743	A 0.9110	A 0.4384	A 0.7370	K-W 0.0000*	K-W 0.0000*
Total	33.81	51.65	67.62	15.49	13.83	2.92	5.01

K – knots size; RTKD – rot at the thicker end; RTHD – rot at the thinner end; PETKD – pith eccentricity at the thicker end; PETHD – pith eccentricity at the thinner end; E – ellipticity; T – taper; SD – standard deviation; * – statistically significant difference at the 95% confidence level; K-W – Kruskal-Wallis p value; A – ANOVA p value; different letters show significant differences among diameter classes according to the performed statistical test

Table 4. Analysis of the influence of assortment position on the wood defect size.

Assortment position along the stem	**K** (mm)	**RTKD** (%)	**RTHD** (%)	**PETKD** (%)	**PETHD** (%)	**E** (%)	**T** (%)
1	27.41b	44.97	53.52	18.23	15.30	2.49c	5.82b
2	33.05a	53.52	76.98	14.27	12.31	2.29c	3.04d
3	35.45a	69.50	72.53	12.25	13.64	2.59c	3.68c,d
4	35.67a	51.72	86.21	13.64	13.69	3.33b,c	5.42b,c
5	34.26a	86.21		11.89		4.08a,b	7.16a,b
6	34.75a					4.85a	8.18a
p value	K-W 0.0000*	K-W 0.3411	A 0.6749	A 0.1185	A 0.8136	K-W 0.0000*	K-W 0.0000*
Total	33.81	51.65	67.62	15.49	13.83	2.92	5.01

K – knots size; RTKD – rot at the thicker end; RTHD – rot at the thinner end; PETKD – pith eccentricity at the thicker end; PETHD – pith eccentricity at the thinner end; E – ellipticity; T – taper; SD – standard deviation; * – statistically significant difference at the 95% confidence level; K-W – Kruskal-Wallis p value; A – ANOVA p value; different letters show significant differences among positions according to the performed statistical test

Table 5. Analysis of the influence of DBH on the wood defect size.

DBH (cm)	**K** (mm)	**RTKD** (%)	**PETKD** (%)	**PETHD** (%)	**E** (%)	**T** (%)
21-30	33.25a,b		17.31	16.67	6.23a	7.54
31-50	29.44a,b	79.55			5.90a	6.59
51-80	32.91b	51.69	15.08	13.64	2.86b	4.96
>81	35.77a	42.05	17.61		2.76b	4.92
p value	K-W 0.0050*	K-W 0.4356	K-W 0.6526	K-W 0.2325	K-W 0.0183*	K-W 0.2648
Total	33.81	51.65	15.49	13.83	2.92	5.01

K – knots size; RTKD – rot at the thicker end; PETKD – pit eccentricity at the thicker end; PETHD – pit eccentricity at the thinner end; E – ellipticity; T – taper; SD – standard deviation; * – statistically significant difference at the 95% confidence level; K-W – Kruskal-Wallis p value; different letters show significant differences among DBH classes according to the performed statistical test.

Table 6. Analysis of the influence of tree position within stand on the wood defect size.

Position within the stand	**K** (mm)	**RTKD** (%)	**PETKD** (%)	**PETHD** (%)	**E** (%)	**T** (%)
Predominant	37.19a	22.32	15.10	15.29	2.88b	4.98
Dominant	32.50b	52.93	15.45	13.04	2.81b	4.93
Co-dominant	31.88b	79.55	17.31	16.67	6.06a	7.07
p value	K-W 0.0000*	K-W 0.1173	K-W 0.9616	K-W 0.2320	K-W 0.0094*	K-W 0.1384
Total	33.81	51.65	15.49	13.83	2.92	5.01

K – knots size; RTKD – rot at the thicker end; PEKD – pit eccentricity at the thicker end; PETHD – pit eccentricity at the thinner end; E – ellipticity; T – taper; SD – standard deviation; * – statistically significant difference at the 95% confidence level; K-W – Kruskal-Wallis p value; different letters show significant differences among positions according to the performed statistical test.

CONCLUSIONS

The research results on the identified wood defects are consistent with previous findings of higher quality in the lower stem, as reflected in smaller defect sizes relevant to roundwood quality classification, such as knots, rot, ellipticity, and taper. Exceptions are pith eccentricity and compression wood, which are more frequent in the lower part of the stem. Additionally, the highest number of scars and damage from physical and mechanical factors was observed in the lower part of the stem, particularly in the first and second assortments from the ground. Given that scars and/or damage from physical and mechanical factors, most often caused by skidders during the wood extraction phase, were confirmed in a large number of trees affected by rot, the necessity of implementing protective measures to preserve the residual trees in the stand during forest harvesting is emphasised. The use of harvesters in uneven-aged mixed stands in Bosnia and Herzegovina requires mathematical models of individual tree species' morphology, as well as the occurrence and size of the most common wood defects, to enable accurate automatic bucking. Consequently, continued research is needed to expand the database of wood defects and to develop reliable mathematical models. Also, future research is planned to focus on assessing the importance of the analysed factors through their influence on the value structure.

Author Contributions

JK and AH conceived and designed the research and carried out the field measurements; JK, AH, EP and AK processed the data and performed the statistical analysis; JK and JM supervised the research and helped to draft the manuscript; JK, JM and VH wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Apendix

Table A1. Analysis of the dependence of compression wood occurrence on diameter of wood assortment.

Diameter of wood assortment (cm)	Number of wood assortments		
	Compression wood present	Compression wood not present	Total
11-20	3	15	18
21-30	3	7	10
31-40	4	20	24
41-50	3	22	25
51-60	8	22	30
61-70	3	9	12
71-80	6	5	11
81-90	1	2	3
Total	31	102	133
χ^2 p value	0.2215		

Table A2. Analysis of the dependence of compression wood occurrence on assortment position along the stem.

Assortment position along the stem	Number of wood assortments		
	Compression wood present	Compression wood not present	Total
1	14	16	30
2	5	25	30
3	5	21	26
4	5	19	24
5	2	15	17
6	0	6	6
Total	31	102	133
χ^2 p value	0.0206*		

(*) – statistically significant difference at the 95% confidence level.

Table A3. Analysis of the dependence of compression wood occurrence on DBH.

DBH (cm)	**Number of wood assortments**		
	Compression wood present	**Compression wood not present**	**Total**
21-30	1	0	1
31-50	0	1	1
51-80	11	9	20
>81	3	5	8
Total	15	15	30
χ^2 p value	0.4402		

Table A4. Analysis of the dependence of compression wood occurrence on tree position within the stand.

Tree position within the stand	**Number of wood assortments**		
	Compression wood present	**Compression wood not present**	**Total**
Predominant	3	4	7
Dominant	11	10	21
Co-dominant	1	1	2
Total	15	15	30
χ^2 p value	0.9092		

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Physiological Workload of Chainsaw Felling and Processing Workers – Case Study

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ABSTRACT

Chainsaw felling and processing work is conducted in various natural conditions and requires significant physical effort from the workers, movement in severe weather and environmental conditions, and has a high risk of injury. The aim of this study was to determine the physiological workload of chainsaw operators through continuous heart rate measurement during the entire working day. The research was carried out during the summer of 2024, encompassing different parts of the Federation of Bosnia and Herzegovina. Heart rate was measured using a Polar H10 Heart Rate Monitor Chest Strap with continuous data logging and storage of heart rate readings. A time study was performed based on recordings conducted simultaneously with the recording of heart rate, with the aim of determining the duration of individual work operations and identifying the work operation with the highest negative impact on the worker. The average working heart rate during productive work time for subject 1 was 104 bpm, 83 bpm for subject 2, 109 bpm for subject 3, 94 bpm for subject 4 and 129 bpm for subject 5. The results of the Kruskal-Wallis test showed a statistically significant difference in average heart rate in relation to the time study element. The heart rate reserve (%HRR) for the whole study time was estimated at 41.05 % for subject 1; 22.69% for subject 2; 44.50% for subject 3; 24.04% for subject 4, and 45.78% for subject 5. The results of the study showed that the %HRR of chainsaw operators during felling and processing exceeded the value of 40% for 3 out of 5 subjects, which corresponds to hard work and may have negative consequences for operators´ health.

Keywords: ergonomy; Federation of Bosnia and Herzegovina; forest harvesting; heart rate reserve; chainsaw operator

INTRODUCTION

Based on data from the Federal Ministry of Agriculture, Water Management and Forestry (2021), the Federation of Bosnia and Herzegovina´s (FBiH) total forest and forest land area is 1,518,466 hectares, of which 1,241,336.1 hectares are state-owned. According to the Information on Forest Management in the FBiH in 2021, 40% of the state forests of the FBiH are high forests with natural regeneration, which constitute the basis of forest production. The forests of Bosnia and Herzegovina are identified as a major ecological and economic resources (Musić et al. 2013).

Logging in Bosnia and Herzegovina is mainly conducted through a combination of manual and mechanised work, felling and production of forest wood assortments are done motor-manually with chainsaws and extraction is mostly handled by tractors (Sokolović and Musić 2009, Halilović et al. 2015, Knežević et al. 2017). Work operations within the tree felling and wood processing phase are conducted in various terrain and stand conditions and require significant physical effort from the workers. The workload of workers in forest exploitation represents a complex combination of physical effort, movement in severe weather and environmental conditions, and a high risk of injury. Consequently, the profession of forestry workers is defined as very dangerous, with a high rate of injuries and work-related diseases during their working life (Çalışkan and Çağlar 2010, Šporčić et al. 2015, Bačić et al.

2020, Halilović et al. 2021, Masci et al. 2022, Ljuboš et al. 2023, Landekić et al. 2023, Staněk and Mergl 2024).

The physical workload of forestry workers can be determined based on heart rate measurements (Grzywiński et al. 2017). Heart rate during work represents the average pulse value for the entire working day, including breaks (Kirk and Sullman 2001). The maximum heart rate serves as a standard indicator of the highest acceptable individual effort in work physiology (Masci et al. 2021, Sammito et al. 2024). Variations in heart rate during work are directly proportional to the intensity and duration of the task (Martinić et al. 2006). Determining physical workload based on heart rate is a commonly used method for research in forestry because of its practicality (Melemez and Tunay 2010a, Eroglu et al. 2015, Grzywiński et al. 2017, Tsioras et al. 2022, Poje et al. 2024, Abramuszkinová Pavlíková et al. 2024, Okuda et al. 2025).

Melemez and Tunay (2010b) found that the average physiological workload of loading machine operators during forestry work was 49%, which corresponds to medium-heavy work. Cheţa et al. (2018) evaluated the ergonomic working conditions in flatland poplar forests, and the results indicate a heavy load during the productive time. Halilović et al. (2021) cited that the heart rate reserve (%HRR) of chainsaw operators during tree felling and processing exceeded the value of 40%, which is classified as heavy work. Masci et al. (2022) determined that the most demanding work task was tree felling, which requires a higher level of cardiac load and longer periods where the worker is in an awkward working posture. Grzywiński et al. (2022) examined physiological workload during logging operations depending on the season (summer-winter), and concluded that heart rate indices and energy expenditure values were higher in winter than in summer. Pavliková et al. (2024) in their study were focused on analysing the impact of the weight of the work tool, and the results proved that using a lightweight work tool contributes to the decrease in heart rate and reduces the time a worker spends in a non-ergonomic working position. Poje et al. (2024) found that the physiological workload was lower when using a battery-powered chainsaw than a petrol-powered chainsaw. Okuda et al. (2025) concluded that the workload in forestry operations cannot be interpreted comprehensively due to the diversity of work operations, insufficient information about measurement conditions, and limited sample sizes. Consequently, the authors suggest a more detailed analysis focusing on workers' environment, working conditions, measurement and work periods, and break times.

The complexity and significance of forest felling and processing, alongside a domestic research gap, highlights the need for further studies. This study aims to determine the physiological workload of chainsaw operators through continuous heart rate measurement during the entire working day, identifying the work operation with the highest negative impact on the worker.

MATERIALS AND METHODS

The research was carried out during the summer of 2024, encompassing different parts of the Federation of Bosnia and Herzegovina, including Una-Sana Canton (Cantonal Public Enterprise "Unsko-sanske šume" Ltd. Bosanska Krupa), Tuzla Canton (Cantonal Public Enterprise "Šume Tuzlanskog kantona" Ltd. Kladanj), Bosnian-Podrinje Canton (Cantonal Public Enterprise "Bosansko-podrinjske šume" Ltd. Goražde) and Zenica-Doboj Canton (Cantonal Public Enterprise "Šume Zeničko-dobojskog kantona" Ltd. Zavidovićima) (Figure 1).

Figure 1. The location of the study area.

The workers were selected based on their consistent productivity over a prolonged period. The focus was on those demonstrating average or standard levels of effort, deliberately excluding both outliers with extreme output and those with suboptimal results. The study was conducted with the prior authorisation and full cooperation of each forestry enterprise involved. Additionally, each worker provided their explicit consent to be part of the study, following a clear explanation of the research objectives. A total of 5 chainsaw workers were sampled in different areas (Table 1). The trees were felled and processed into wood assortments with Husqvarna 372 XP and Stihl MS 382 chainsaws. Anthropometric characteristics of the chainsaw workers are shown in Table 2.

Heart rate was measured using a Polar H10 Heart Rate Monitor Chest Strap with continuous data logging and storage of heart rate readings (Figure 2). The measurements were carried out during productive work time and delays. Simultaneously with the pulse recording of the chainsaw operator, a work and time study was conducted with the aim of determining the duration of individual work operations during tree felling and processing. Recorded heart rate data was downloaded via Polar Beat App and sorted out in MS Excel 2021. Statistical analyses were carried out using the Statgraphics Centurion XVI software. Productive work time was divided into work operations, as shown in Table 3.

Table 1. Terrain characteristics of the study area.

Compartment	Forest enterprise	Average elevation (m)	Slope (°)	Terrain topography	Exposure
1	FTK	320-630	15-25	valley and basin	N; NW
2	FTK	400-650	18-20	valley and basin	NE-N; NW-W
3	BPF	770-1120	10-30	steep downhill	N; NW
4	BPF	770-1121	10-30	steep downhill	N; NW
5	USF	800-950	10	slightly downhill	E

Table 2. Anthropometric characteristics of the chainsaw workers.

Subject	Age (years)	Weight (kg)	Height (cm)	Work experience (years)	HRrest (bpm)	HRmax (bpm)	BMI (kg·m^{-2})
1	47	102	185	16	63	173	29.80
2	46	85	184	15	53	174	25.11
3	53	80	189	20	74	167	22.40
4	52	72	168	14	61	168	25.51
5	28	110	184	10	77	192	32.49

Table 3. Time study elements.

Time study element		Descriptions of effective work time and delays
Productive time	Preparatory-final time	Taking of work orders, chainsaw preparation, cleaning and service maintenance
	Moving to felling site	Walking from landing site to felling site
	Moving	Walking from felled and processed tree to another marked tree
	Felling	Preparing of workplace, choosing of felling direction and tree felling
	Delimbing and processing	Cutting of branches from stem and processing of wood assortment by chainsaw horizontal cut
	Bucking the fuelwood	Cutting log into shorter lengths (fuelwood or other purposes)
	Moving to landing site	Walking from felling site to landing site
Delays		Personal delay (meal time, resting)
		Equipment servicing (service of chainsaw and chainsaw bar by operator)
		Technical delay (malfunctions and their removal)
		Organizational delay (planning, field survey)
		Delay due to adverse weather conditions
		Other delays

Relative heart rate at work was obtained using following formula:

$$\%HRR=\frac{(HR_w - HR_r)}{(HR_{max} - HR_r)} \times 100$$

where HR_w is the average working heart rate, HR_r is the resting heart rate, and HR_{max} is the maximum heart rate (Vitalis 1987).

The maximum heart rate is different between workers and decreases with ageing. The most common formula for its estimation is:

$$HR_{max}=220-\text{age (Rodahl 1989).}$$

The minimum measured heart rate value for the whole working day was taken as the resting heart rate (Sammito et al. 2024). Classification of the physiological workload was done by following the scale in Table 4.

Table 4. Grading the physiological workload (Apud et al. 1989).

Physiological workload	Average heart rate (bpm)
Very low	<75
Low	75 - 100
Moderate	100 - 125
High	125 - 150
Very high	150 - 175
Extremely high	>175

Figure 2. Polar H10 Heart Rate Monitor Chest Strap.

RESULTS

The operational data and harvesting statistics are summarised in Table 5. A total of 102 trees were harvested over a period of 15 working days. The species composition of the harvested timber primarily consisted of European Beech and other broadleaf species. Initial analysis of the harvesting data indicates a total wood volume of 156.17 m3, with a daily output that varied across the different forest compartments.

The average working heart rates, during productive work time, that reached the highest value are for subject 3 (121-140 bpm) and subject 5 (95-123 bpm). The minimum average working heart rates is for subject 2 (62-92 bpm). A detailed overview of the statistical results and workload indicators is provided in Table A1. The minimum heart rate for the whole study was recorded during the preparatory-final time, 62 bpm (subject 2), and the maximum heart rate recorded during equipment servicing, 146 bpm (subject 3). Time study elements, such as dealing with hung-up trees, bucking the fuelwood, equipment servicing and moving to the landing and felling site, were among those with the highest value of heart rate reserve (%HRR). The minimum %HRR was during the preparatory-final time (7.24%). %HRR for the whole study time was 41.05 % for subject 1, 22.69% for subject 2; 44.50% for subject 3; 24.04% for subject 4 and 45.78% for subject 5. According to Apud at al. (1989) physiological workload for subject 1 was low to moderate depending on time study element, for subject 2 every time study element was low, for subject 3 most of the time study element was moderate except equipment servicing (146 bpm), for subject 4 every time study element was low except personal delay (71 bpm) and for subject 5 most of time study elements was high (Figure 3).

Different letters show significant differences among time study elements according to post hock Tukey´s test

The non-parametric Kruskal-Wallis test was used to determine the differences between the average heart rates for various time study elements (Table 6)**.** Post hoc cross-comparisons between average heart rates were performed using Tukey´s test. Results showed a statistically significant difference in average heart rate in relation to the time study element (p=0.0000).

DISCUSSION

The average working heart rates for productive time for subjects 2 and 4 are 83 and 94 bpm, respectively. This discrepancy for subject 2 can be because of easier working conditions despite high productivity, whereas for subject 4, the lower values were likely the results of a reduced workload, which mitigated the physiological impact of the more demanding working conditions. Poje et al. (2024) in a study on the effects of using petrol and battery-powered chainsaws reported higher values, 90.4 bpm and 86.9 bpm. The average working heart rates determined for subjects 1 and 3 (104 and 109 bpm) are similar to the range (108-116 bpm) cited by Martinić (1995). The average working heart rate for subject 5 is 129 bpm, which is similar to the winter-time results determined by Grzywinski (2022) and Masci et

Table 5. Timber harvesting and operational data.

Subject	Volume of processed wood assortments (m^3)	Work duration (days)	Number of felled trees	Tree species
1	36.57	5	32	European Beech and other broadleaf species
2	44.30	3	23	European Beech and other broadleaf species
3	37.98	3	22	European Beech and other broadleaf species
4	17.55	2	13	European Beech and other broadleaf species
5	15.97	2	10	European Beech
	3.80		2	Silver Fir

Table 6. Results of Kruskal-Wallis test.

Time study element	Average heart rate (bpm)				
	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5
Preparatory-final time	96h	62g	95f	81e	139a
Moving to felling site	100f,g	68f	95f	-	-
Moving from tree to another tree	109e	87b	111e	89c	122e
Felling	114c,d	86b,c	111e	92b	121e
Delimbing and processing	115c,d	86c	111e	97a	130c
Bucking the fuelwood	118b	92a	113d	92b	129c
Moving to landing site	93j	88b	123c	98a	140a
Personal delay	94i	82d	127b	71g	133b
Equipment servicing	100f,g	72e	146a	75f	126d
Technical delay	100f,g	72e	-	85d	122d,e
Organizational delay	81k	82d	124b,c	-	136a,b
Other delay	121a	89a,b	-	-	-
Rest time	63l	-	-	-	-
Kruskal-Wallis p value	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*

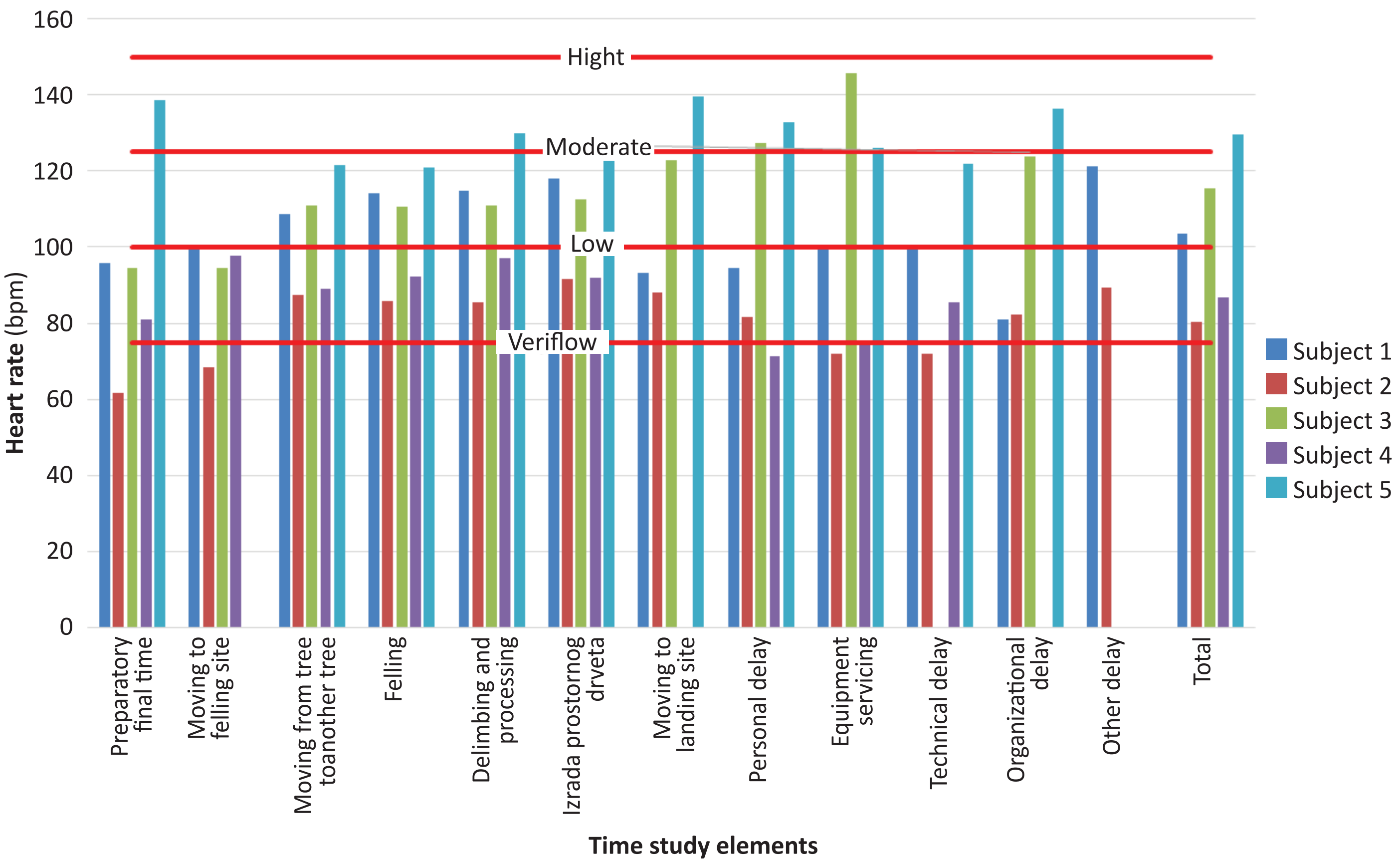
*Denotes statistically significant difference according to Kruskal-Wallis test

al. (2022). Çalişkan & Çağlar (2010) found a mean working heart rate of 122.8 bpm, which is in line with findings of this study and specifically those of subject 5. Arman et al. (2021) determined that the average working heart rate was 116.08 bpm, while Melemez and Tunay (2010) reported that heart rates while working were 115±7 bpm. Heart rates are influenced by numerous modifiable and non-modifiable factors, including the test subjects´ living habits, resulting consequences and external conditions (Sammito et al. 2024).

The maximum heart rate values were measured during other delays, which included dealing with hung-up trees (121 bpm), bucking the fuelwood (92 bpm), equipment servicing (146 bpm), moving to the felling site (98 bpm) and moving to the landing site (140 bpm). Masci et al. (2022) found that the mean heart rate during felling was 136.7 bpm, 143.5 bpm during delimbing and 135.63 bpm during bucking. The resting heart rates for all subjects range from 53 to 77 bpm, which aligns with the selected and presented studies in the scoping review by Okuda et al. (2025).

The average physical workloads (%HRR) for subjects 2 and 4 are lower than those presented by other authors. The result for subject 1 is similar to that shown by Memelez and Tunay (2010). The average physical workloads for subjects 3 and 5 are higher than those of others and similar to Çağlar (2021) for debarking, Çalışkan and Çağlar (2010) for forest workers in felling operations, Cheţa et al. (2018) during motor-manual tree felling and processing in poplar clear cuts and Arman et al. (2021) for clearcutting

Figure 3. Grading the physiological workload.



operations. Potočnik and Poje (2017) described that the permissible value of heart rate reserve should not exceed value of 40% for all work tasks of effective time and delays.

The highest physiological workload in this study was recorded during equipment servicing (76.97%), dealing with a hung-up tree (56.26%), delimbing and processing (50.65%) and moving to the landing site (54.45%). The lowest physiological workload was measured during the organisational delay (22.05%) for subject 1, preparatory-final time (7.24%) for subject 2, preparatory-final time and moving to felling site (both 22.10%) for subject 3, personal delay (9.63%) for subject 4 and felling (38.15%) for subject 5. Cheţa et al. (2018) found that the lowest physiological workload was during work preparation (21.76%) and the highest was during technical delay (57.49%).

CONCLUSIONS

Motor-manual felling and processing is difficult and dangerous work that puts heavy loads on the operators. The primary objective of this study was to analyse the workload of foresters during the felling and processing phase. The research faced certain limitations, including a small sample size and a lack of interest among some workers. The results of the study showed that the %HRR of chainsaw operators during felling and processing exceeded the value of 40% for 3 out of 5 subjects, which corresponds to hard work and may have negative consequences for operators´ health. The other two operators had the lowest HRR%. This variability could be caused by working conditions, organisational structures, anthropometry and the living habits of the operators. In order to reduce the %HRR and improve productivity, it is necessary to consider measures such as operator training, changes in work organisation, proactive health monitoring and optimised recovery protocols. Future research should be conducted in collaboration with occupational medicine specialists to ensure more precise measurement and interpretation of results. Furthermore, incorporating advanced methods, such as Holter monitoring, would provide deeper insights into the physiological strain on workers.

Author Contributions

VH, JM, JK, AA conceived and designed the research and carried out the field measurements, JK, AK and EP processed the data and performed the statistical analysis, VH supervised the research and helped to draft the manuscript, JK and EP wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Appendix A

Table A1. Descriptive statistics of heart rate per chainsaw worker.

Time study element	Heart rate (bpm)																								
	Subject 1					Subject 2					Subject 3					Subject 4					Subject 5				
	min	max	mean	SD	HRR%	min	max	mean	SD	HRR%	min	max	mean	SD	HRR%	min	max	mean	SD	HRR%	min	max	mean	SD	HRR%
Preparatory-final time	75	132	96	12.12	34.66	55	79	62	4.16	7.24	77	125	95	9.03	22.10	72	104	81	6.22	18.69	120	151	139	8.98	53.56
Moving to felling site	72	151	100	21.09	38.07	53	108	68	12.34	12.63	74	150	95	17.67	22.10	71	138	98	18.96	34.23					
Moving from tree to another tree	82	140	109	13.88	45.37	62	126	87	18.54	28.49	81	166	111	19.80	39.83	63	116	89	13.02	26.06	86	154	122	20.52	38.70
Felling	84	140	114	11.03	50.17	60	123	86	14.11	27.05	79	169	111	17.15	39.39	69	133	92	15.90	29.36	81	161	121	20.25	38.15
Delimbing and processing	86	136	115	9.09	50.65	56	124	86	16.11	26.98	78	177	111	15.64	39.78	65	136	97	13.40	33.64	79	162	130	20.83	45.97
Bucking the fuelwood	91	141	118	10.57	53.30	56	119	92	15.38	31.99	79	178	113	20.78	41.55	65	136	92	13.60	28.97	77	158	129	19.55	45.13
Moving to landing site	75	139	93	12.14	32.32	76	107	88	7.29	28.89	90	158	123	13.69	52.44						113	166	140	8.94	54.45
Productive time	72	151	104	17.62	42.53	53	126	83	16.70	24.79	74	178	109	18.29	37.63	63	138	94	14.52	30.84	77	166	129	19.88	45.22
Personal delay	75	150	94	13.98	33.47	56	129	82	16.71	23.62	87	167	127	21.38	57.32	61	123	71	10.75	9.63	82	159	133	18.66	48.51
Equipment servicing	79	136	100	11.13	38.01	57	117	72	13.36	15.55	125	162	146	9.77	76.97	62	134	75	10.82	12.93	84	165	126	18.29	42.61
Technical delay	82	130	100	13.13	38.21	60	82	72	6.64	15.59						73	131	85	13.62	22.84	88	149	122	18.28	39.10
Organizational delay	63	121	81	8.04	22.05	63	112	82	13.73	24.32	112	132	124	5.06	53.55						98	154	136	18.19	51.61
Other delay	115	129	121	3.08	56.26	79	105	89	7.06	29.94															
Rest time	55	98	63	6.72																					
Total	63	151	103		41.05	53	129	80		22.69	74	178	115		44.50	61	138	87		24.04	77	166	130		45.78

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The Roe Deer (*Capreolus capreolus*) as the Bioindicator of the Presence of Heavy Metals (Cd and Pb) in Three Hunting Grounds of Central Serbia

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ABSTRACT

The increasing human impact on the environment, which contributes to its contamination, has imposed the need for active monitoring of such elements through certain bioindicators. The roe deer, as the most numerous species of game among the autochthonous wild ungulates, which is widespread throughout the territory of the Republic of Serbia, was used as a bioindicator. The concentrations of cadmium and lead (Cd and Pb) were measured using atomic absorption spectrophotometry in the liver (59) and kidney (68) of roe deer harvested during the regular hunting season in three hunting grounds in Central Serbia. The mean value of Cd in liver samples from all hunting grounds was 0.223 $mg \cdot kg^{-1}$wet weight with a range of 0.046-0.247 $mg \cdot kg^{-1}$, while in kidney was 0.474 $mg \cdot kg^{-1}$ and ranged from 0.356-0.541 $mg \cdot kg^{-1}$. However, the mean value of Pb in the liver was 0.551 mg/kg^{-1} with a range of 0.470-0.686 $mg \cdot kg^{-1}$, while in the kidney it was 0.395 $mg \cdot kg^{-1}$ with a range of 0.292-0.640 $mg \cdot kg^{-1}$. With respect to mean Cd concentrations in the liver and kidneys, statistically significant differences were observed for both the locality and tissue factors, whereas for Pb, significant differences were detected only in relation to the locality factor. Cadmium (Cd) concentrations were within permissible limits in both liver and kidney tissues (0.5 and 1.0 $mg \cdot kg^{-1}$). In contrast, the mean lead (Pb) concentration in the liver exceeded the allowed limit (0.5 $mg \cdot kg^{-1}$), while kidney Pb concentrations showed occasional exceedances but remained within permissible limits on average (0.5 $mg \cdot kg^{-1}$).

Keywords: game species; liver; kidney; cadmium; lead; biomonitoring

INTRODUCTION

Over the last two decades, one of the main focuses of researchers has been serious environmental threats, particularly the presence of heavy metals. Regardless of their origin, which can be from natural sources, it is most often the result of human activities (agriculture, industry), and they can cause environmental contamination with consequences for the entire biodiversity, including humans (Okorondu et al. 2022). Some heavy metals such as cadmium (Cd) and lead (Pb) are bioaccumulative and do not break down easily in the environment, which is the main reason for their accumulation in water, soil, plants and transmission to herbivorous wild mammals, game, livestock and ultimately humans (Bilandžić et al. 2010, Ackova 2018). Acute or chronic exposure to Pb and Cd may have serious consequences for the mentioned living organisms because these heavy metals accumulate in almost all tissues, but mostly in targeted tissues such as the liver and kidney (Wieczorek-Dąbrowska et al. 2013, Cokoski et al. 2023). The deposition of heavy metals and their movement through the environment depend on many physical and biological processes and factors (Briffa et al. 2020). Therefore, it is necessary to monitor their environmental levels and changes in ecosystems through appropriate biomonitors. Biomonitoring can be defined as a process in which plant and animal organisms or their fragments provide continuous analytical information that can be used to determine the degree of environmental contamination (Gadzała-Kopciuch et al. 2004).

Something that was not the case in the past, and has changed in the last twenty years, is the increasing number of studies where different species of game are used as bioindicators for environmental pollution (Bilandzić et al. 2010, Srebočan et al. 2012, Cokoski et al. 2023, Lénárt et al. 2023). The roe deer (*Capreolus capreolus* L.), along with the wild boar (*Sus scrofa* L.), the red deer (*Cervus elaphus* L.), the fallow deer (*Dama dama* L.), the chamois (*Rupicapra rupicapra* L.) and the hare (*Lepus europaeus* Pallas), are often used species of game in such biomonitoring studies, as a result of their bioecological characteristics and the relatively simple sampling procedure during the regular hunting season (Wieczorek-Dąbrowska et al. 2013, Durkalec et al. 2015, Maľová et al. 2019, Beuković et al. 2022, Cokoski et al. 2024a, Cokoski et al. 2024b). The identification of heavy metal concentrations in the organs of free-living animals can provide a clear picture of the presence and distribution of heavy metals in the environment, whether at a local level or across a wider area. Such data make it possible to detect potential sources of pollution, assess the degree of exposure of animals to these elements in a given region, and offer an early warning of possible adverse environmental consequences.

In the Republic of Serbia, the number of studies where game has been used as a bioindicator has been limited so far, and the most common species of game used has been the hare (Petrović et al. 2013, Beuković et al. 2022). On the other hand, the roe deer is the most numerous autochthonous species of wild ungulates and is distributed almost throughout the entire territory of the country, including forest and agricultural areas (more than 90% of the total hunting area of Serbia) (Popović et al. 2007, Popović et al. 2023). Also, hunting this type of game is quite attractive and popular in this country, which is confirmed by the number of harvested individuals (around 10,671 in 2023) (Statistical Office of the Republic of Serbia, 2023a). As an herbivorous game species with distinctly territorial characteristics and a relatively long lifespan (8-10 years in the wild) (Maletić 2024), it will be the subject of research in a biomonitoring study conducted in a specific region of the Republic of Serbia.

MATERIALS AND METHODS

Study Area

The collection of roe deer samples was carried out in three hunting grounds in central Serbia.

Hunting ground "Barajevska reka" (44°29′56″–44°38′57″N; 20°16′20″–20°30′02″E) with a total area of 21,312 ha, is characterised by the dominance of agricultural land, primarily arable fields, while forest ecosystems are less represented and mostly degraded. Within the forest communities, species such as oak, beech, acacia, poplar, maple, and willow are present. The floristic composition of meadows is favourable, with grass-legume communities where species such as Kentucky bluegrass (*Poa pratensis L.*), meadow foxtail (*Alopecurus pratensis L.*), and white clover (*Trifolium repens L.*) dominate. Pastures are characterised by a higher presence of poor and lower-quality grasses, low legume content, and a higher presence of herbaceous plants. The elevation of the hunting ground ranges from 100 m to 408 m, and the terrain is moderately rugged, classifying this area as hilly. The area is marked by a well-developed hydrographic network with permanent and intermittent watercourses, including the Barajevska River and its tributaries. The climate of this area is moderately continental, with pronounced seasonal variations in temperature and precipitation.

Hunting ground "Takovo" (43°52′48″–44°10′48″N; 20°12′00″–20°39′00″E), with a total area of 74,292 ha, is characterised by the dominance of meadows and pastures, while forest ecosystems and arable land are also significantly represented. Forest communities include species such as beech, oak, hornbeam, ash, linden, and elm, along with conifers like pine, spruce, and fir. Meadows are composed of grass-legume communities dominated by species such as Kentucky bluegrass (*Poa pratensis*), fescue (*Festuca* spp.), and white clover (*Trifolium repens*). Pastures are characterised by a higher presence of medium-quality grasses, lower legume content, and increased occurrence of herbaceous plants. Among arable crops, wheat, oats, maize, and barley are the most common. Based on terrain configuration and elevation, the hunting ground Takovo is classified as hilly. The area has a well-developed hydrographic network with numerous watercourses belonging to the basins of the West Morava and Sava rivers. The climate of this area is moderately continental.

Hunting ground "Resava" (44°03′18″–44°19′12″N; 21°03′00″–21°19′12″E), with a total area of 22,471 ha, is characterized by a dominance of arable land, primarily fields, which are intensively used and covered with various agricultural crops (wheat, maize, oats, barley, sunflower, soybean, etc.), while forest ecosystems, meadows, and pastures are less represented. In the riparian parts of watercourses, hydrophytic communities are present, dominated by species such as willow, poplar, and ash, while in hillier parts, smaller forest complexes occur with species such as oak, hornbeam, and ash, as well as shrub species like hawthorn, hazel, and cornelian cherry. Based on altitude and terrain configuration, this hunting ground is classified as a lowland type. The area features a developed hydrographic network, with the Velika Morava River flowing along the western boundary, while the Resava River and its tributaries extend through the centre of the hunting ground. The climate of this area is temperate-continental, with pronounced seasonal variations.

Sampling

A total of 59 liver and 68 kidney samples were obtained from roe deer harvested at three hunting grounds in the Republic of Serbia. Sampling was conducted during the regular hunting seasons (2022). The sample distribution across the study sites was not uniform. A total of 15 liver and 24 kidney samples were collected from locality 1 (Hunting ground Barajevska reka), 40 liver and 40 kidney samples from locality 2 (Hunting ground Takovo), and 4 kidney and 4 liver samples from locality 3 (Hunting ground Resava). The samples were collected from adult roe deer. During sampling, special care was taken to avoid damaged

Figure 1. Geographical locations of the hunting grounds "Barajevska Reka", "Takovo" and "Resava".

parts of the bullet path. Tissues damaged by the bullet pathway were not included in the analysis (Danieli et al. 2012). The samples were individually packed in polyester bags, appropriately labelled, and transported in small refrigerators to the laboratory where they were kept at -20°C until analysis.

Laboratory Analysis

Our previously optimised method was used for the quantification of cadmium and lead in roe deer liver and kidney samples (Beuković et al. 2015). About 1 g of sample was weighed on an analytical balance EL 204 – IC (Mettler Toledo, Switzerland) and placed in a tube where it was digested by the wet ashing technique, by adding 4 ml of the concentrated nitric and hydrochloric acid mixture (3:1, v/v). The mixture was prepared with trace analysis-grade nitric acid (Fisher Scientific, USA) and hydrochloric acid (Carl Roth, Germany). The tube was then heated in the thermal block ReactiThermTM TS-18820 (Fisher Scientific, USA) at 120°C for 2 hours. The content of lead was determined by the electrothermal atomic absorption spectrometer, model PinAAcle 900T (Perkin Elmer, Waltham, MA, USA). The instrument was calibrated using analytical standards for lead and cadmium (Fluka Analytical, USA), both 1000 mg·l^{-1} in 2% nitric acid. Cadmium working solutions ranged from 0.2 to 2.0 μg·ml^{-1}, while lead calibration points were from 2.0 to 20.0 μg·ml^{-1}. For cadmium, the limit of detection (LOD) was 2.32 μg·kg^{-1}, while the limit of quantification (LOQ) was 7.04 μg·kg^{-1}. For lead, LOD was 4.26 μg·kg^{-1} and LOQ was 12.9 μg·kg^{-1}. All results are expressed on the fresh weight (fw) basis.

Statistical Analysis

Statistical analysis was performed using the TIBCO Statistica software version 14 (StatSoFT STATISTICA Software) for descriptive analysis and for analysis of variance. Data were grouped according to element, tissue and sampling localities. Concentrations were expressed as mean ± standard error, median, minimum and maximum values. To examine differences between sampling localities, we used the two-way analysis of variance (ANOVA) test. All statements of significance were based on the 0.05 level of probability ($p \leq 0.05$).

RESULTS

The results of Cd and Pb concentrations in the liver and kidney of roe deer are presented in Table 1, Table 2 and Table 3.

Cadmium (Cd)

The analysis confirmed the presence of cadmium in 98.3% of liver samples and in 100% of the kidney samples, in other words, only a single liver sample fell below the detection limit for this heavy metal. The mean Cd concentrations in liver samples from the hunting grounds investigated in this study were observed in the following order: the lowest concentration of 0.046 mg·kg^{-1} was recorded in the Resava hunting ground, followed by 0.-205 mg·kg^{-1} in the Barajevska Reka hunting ground, and the highest concentration of 0.247 mg·kg^{-1} was observed in the Takovo hunting ground, with an overall mean of 0.223 mg·kg^{-1}. The mean cadmium concentrations in the kidney followed the same order as the concentrations in the liver, with the lowest value recorded in Resava (0.356 mg·kg^{-1}), followed by Barajevska Reka (0.382 mg·kg^{-1}), and the highest in Takovo (0.541 mg·kg^{-1}), resulting in an overall mean of 0.474 mg·kg^{-1}.

Lead (Pb)

Out of the total number of samples collected from the three hunting grounds, lead was not detected in 16.9% of the liver samples and in 29.7% of the kidney samples (Table 2). In contrast to the cadmium results, the lowest mean Pb concentrations in the liver, 0.47 mg·kg^{-1}, were recorded in the Takovo hunting ground, followed by 0.649 mg·kg^{-1} in Barajevska Reka, and the highest, 0.686 mg·kg^{-1}, in the Resava hunting ground (Table 2). The mean Pb concentrations in the kidney did not follow the same distribution across hunting grounds. The lowest mean Pb concentration in the kidney was recorded in the Takovo hunting ground (0.292 mg·kg^{-1}), followed by Resava (0.435 mg·kg^{-1}), and the highest in Barajevska reka hunting ground (0.649 mg·kg^{-1}). In this case, clear differences were observed with higher Pb concentrations in the liver of roe deer compared to the concentrations in the kidneys in the Takovo and Resava hunting grounds, which is different from the findings for the presence of Cd in the same examined tissues. However, similar concentrations of this heavy metal were observed in the kidney and liver in the Barajevska Reka hunting ground.

Table 1. Cadmium (Cd) concentrations (in mg·kg^{-1} wet weight) in the livers and kidneys of roe deer from three hunting grounds in the Republic of Serbia.

Hunting grounds	Tissue	N	N < LODs %	Mean	Median	Min	Max	N > MPC (%)
Barajevska reka	liver	15	0 (0)	0.205	0.174	0.042	0.745	1 (1.7)
	kidney	24	0 (0)	0.382	0.369	0.048	0.699	0 (0)
Takovo	liver	40	0 (0)	0.247	0.227	0.025	0.564	5 (8.5)
	kidney	40	0 (0)	0.541	0.530	0.234	0.926	0 (0)
Resava	liver	4	1 (1.7)	0.046	0.047	0.008	0.083	0 (0)
	kidney	4	0 (0)	0,356	0.347	0.086	0.643	0 (0)
All	liver	59	1 (1.7)	0.223	0.182	0.008	0.745	6 (10.7)
	kidney	68	0 (0)	0.474	0.456	0.048	0.962	0 (0)

N – number of samples; N < LODs % - number of samples bellow the limit of detection (%); N > MPC % - number of samples exceeding the maximum permitted concentration (%).

Table 2. Lead (Pb) concentrations (in mg·kg^{-1} wet weight) in the livers and kidneys of roe deer from three hunting grounds in the Republic of Serbia.

Hunting grounds	Tissue	N	N < LODs %	Mean	Median	Min	Max	N > MPC (%)
Barajevska reka	liver	15	0 (0)	0.649	0.454	0.046	1.470	1 (1.7)
	kidney	24	11 (16.2)	0.640	0.559	0.036	1.570	0 (0)
Takovo	liver	40	10 (16.9)	0.470	0.321	0.036	1.990	4 (6.8)
	kidney	40	8 (11.7)	0.292	0.221	0,016	1.040	19 (28)
Resava	liver	4	0 (0)	0.686	0.357	0.200	1.820	1 (1.7)
	kidney	4	0 (0)	0.435	0.452	0.240	0.590	1 (1.7)
All	liver	59	10 (16.9)	0.551	0.428	0.036	1.990	6 (10.2)
	kidney	68	19 (27.9)	0.396	0.274	0,016	1.570	20 (29.4)

N – number of samples; N < LODs % - number of samples bellow the limit of detection (%); N > MPC % - number of samples exceeding the maximum permitted concentration (%).

DISCUSSION

The mean concentrations of Cd in the liver and kidney showed statistically significant differences between localities and among tissue types, indicating that both factors influence Cd accumulation (Table 3). In the Takovo hunting ground, the average values of Cd in the liver and kidney are markedly higher compared to the other two localities. However, the Cd concentration in the liver at this hunting ground was higher than in the Barajevska Reka hunting ground, the difference was not statistically significant. The lowest mean concentration of cadmium in the liver was determined in the Resava hunting ground, which is significantly lower than the other two localities, but this concentration may be the result of the small number of samples at this locality compared to the others. Also, regarding the tissue factor, statistically significantly higher concentrations of Cd in the kidney were determined in all three examined localities compared to concentrations in the liver. This confirms the well-known finding of the high affinity of Cd for the kidneys, where it is deposited directly from the bloodstream and indirectly from the liver, followed by a low excretion rate, which has been confirmed in several studies where different species of game (omnivores and herbivores) were the subject of biomonitoring (Bilandzić et al. 2009, Srebočan et al. 2012, Durkalec et al. 2015).

We compared our results with several studies from other European countries in which roe deer were used as bioindicators of environmental pollution. Therefore, in a study conducted in northwestern Poland, Wieczorek-Dąbrowska et al. (2013) determined higher Cd concentrations in both tissues (0.887 and 0.245 mg·kg^{-1} in liver) (9.117 and 2.054 mg·kg^{-1} in kidney). Additionally, in Poland, a study was conducted where wild boar and roe deer were used as biomonitors of heavy metal contamination at two industrial sites and one control site. The results obtained confirm higher concentrations of Cd in roe deer liver (6.435 and 0.492 mg·kg^{-1}) at both industrial sites compared to the results of our study, and drastically higher concentrations in kidney at the same sites (39.6 and 4.653 mg·kg^{-1}), including the control site (1.582 mg·kg^{-1}) where it was stated that there was no potential pollutant present (Durkalec et al. 2015). Also, significantly higher cadmium concentrations compared to our results were observed in neighbouring Bulgaria, where mean concentrations in the liver were 3.15 mg·kg^{-1} and in the kidney 20.4 mg·kg^{-1} (Markov and Ahmed 2019).

In their study in Croatia, Srebočan et al. (2011) observed Cd concentrations of 0.293 mg·kg^{-1} in the liver and 3.331 mg·kg^{-1} in the kidneys of middle-aged roe deer, indicating slightly higher accumulation in the liver and much higher in the kidneys compared with our results. In the same context as the previous study, similar or lower values for Cd concentrations in roe deer liver and higher concentrations in kidney were also observed in a study conducted in Poland, covering the period from 1987 to 1991 (Falandysz 1994). Overall, it can be emphasised that the lower and in only a few cases similar results to those shown by research in various European countries indicate a lower level of contamination in the examined hunting grounds with Cd in the Republic of Serbia. This is most likely a result of differences in the choice of locality for such research and the presence or proximity of some anthropogenic contaminant, as is the case with the research conducted in Poland by Durkalec et al. (2015). The investigated hunting areas are situated at a considerable distance from major pollution sources and, although agricultural activities are present, they are of low intensity.

Regulations establishing maximum levels of contaminants in foodstuffs do not cover game animals; the maximum levels of toxic metals are specified only for slaughtered livestock. In the Republic of Serbia, according to the regulations on the permissible levels of pesticides, metals and metalloids, and other toxic substances, as well as chemotherapeutics, anabolic agents, and other substances that may be present in food, the maximum allowed concentrations of cadmium are 0.5 mg·kg^{-1} in liver and 1.0 mg·kg^{-1} in kidney (Official Gazette of the Republic of Serbia 2011). The regulations and standards for maximum permitted concentrations of specific contaminants are fully aligned with European legislation (European Commission 2006). Accordingly, the laboratory analyses confirmed that Cd concentrations in liver exceeded the maximum allowed limits in 6 samples or 10.7% of the samples, while for Cd concentrations in the kidney, those values were undetectable, and it was confirmed that even the maximum individual values for the concentrations of this contaminant in a given tissue did not exceed the legally permitted concentration. Regardless of the fact that individual liver samples were detected with cadmium concentrations higher than the permissible limit at each locality in the study area, it is important to note that the average concentrations are far below the permissible limit. These findings indicate that cadmium is present in these hunting grounds, but the situation is far from concerning.

Table 3. Test of significance ($p<0.05$) for cadmium (Cd) and lead (Pb) concentration depending on location and tissue.

	p	
	Cd	**Pb**
Location	0.000513	0.013588
Tissue	0.000000	0.190191
Location*Tissue	0.182667	0.587828

The observed differences in Pb concentrations among the examined tissues of roe deer across locations suggest that local environmental factors may influence lead accumulation. Namely, the mean values for Pb concentrations in both tissues were statistically significantly higher in the Barajevska Reka hunting ground compared to the Takovo hunting ground. The results confirm that the presence of Pb dominates in the first hunting ground, and the presence of the contaminant Cd in the second. The most acceptable explanation for the observed locational differences in heavy metal concentrations is the presence of potential pollution sources (such as landfills, industrial facilities, or chemicals used in agriculture) near these hunting grounds. Therefore, further research is needed to confirm the presence of these sources and to determine their relationship with the bioindicators used in such studies. The results showed that there were no statistically significant differences in Pb concentrations among the examined tissues of roe deer (Table 3), although in two hunting grounds (Takovo and Resava), slightly higher Pb concentrations were observed in the liver than in the kidney. Similar findings were found in studies conducted by Srebočan et al. (2011) and Wieczorek-Dąbrowska et al. (2013), where slightly higher Pb concentrations were recorded in the liver than in the kidney of roe deer.

Despite the limited number of studies where roe deer have been used as a bioindicator for determining the presence of heavy metals, we have compared our results on Pb concentrations with the small number of similar studies conducted in some European countries. In a study conducted in Poland at two industrial sites and one control site, Durkalec et al. (2015) reported lower or similar Pb concentration in the liver (0.303; 0.559; 0.058 mg·kg^{-1}). Kidney Pb levels were also lower at two sites (0.099; 0.102 mg·kg^{-1}), except at the industrial site (Upper Silesia), where concentrations (0.906 mg·kg^{-1}) were higher than those observed in our study. In the northwestern part of the same country, which is known for the presence of anthropogenic pollutants, the results show mixed findings: both higher and lower lead concentrations in the liver (0.946 and 0.271 mg·kg^{-1}) and in the kidneys (0.610 and 0.134 mg·kg^{-1}) were reported compared with the results of our study (Wieczorek-Dąbrowska et al. 2013). In Bulgaria, similarly to the findings for Cd, significantly higher Pb concentrations were reported in roe deer tissues. Pb concentrations reached 3.5 mg·kg^{-1} in the liver and 3.3 mg·kg^{-1} in the kidneys, values that were more than five times higher than those observed at the three hunting grounds in our study (Markov and Ahmed 2019). Only in Croatia, in the study conducted by Srebočan et al. (2011), lower lead concentrations were observed in both the liver and kidneys of roe deer across three age categories. Liver concentrations were 0.049, 0.055, and 0.018 mg·kg^{-1} in young, middle-aged, and old individuals, respectively, while kidney concentrations were 0.058, 0.033, and 0.024 mg·kg^{-1}. In general, comparison of our findings with those reported in the literature indicates mixed opinions, with a somewhat higher presence of Pb in our study areas (hunting grounds) than in several other European countries. Of course, there were exceptions, such as the study conducted in Bulgaria and certain localities in Poland, where higher lead concentrations were reported than those observed in the present study.

According to the previously mentioned regulation on the maximum permitted concentrations of certain contaminants in foodstuffs, which is also used and accepted for game species, the maximum permitted concentration of Pb is 0.5 mg·kg^{-1} in both tissue (liver and kidney) (Official Gazette of the Republic of Serbia 2011). Therefore, the results obtained indicate that in the Barajevska reka hunting ground, slightly higher Pb concentrations than the maximum permitted were noted in both examined tissues, namely the mean concentrations in the liver were 0.649 mg·kg^{-1}, and in the kidney 0.64 mg·kg^{-1}. Also, in the Rasava hunting ground, but only in liver tissue, slightly higher mean Pb concentrations were determined than the maximum permitted values (0.686 mg·kg^{-1}). Particular attention should be given to hunting grounds where mean values exceed the legally permitted concentrations of this heavy metal, and the possibility of investigating potential exposure pathways and sources of lead contamination. The results also show individual samples exceeding the maximum permitted concentrations for this contaminant in all three hunting grounds, with 29.4% of the kidney samples and 10.2% of the liver samples (Table 2). Furthermore, it should be emphasised that 95% of the kidney samples and 67% of the liver samples with Pb concentrations above the permitted limits were collected from the Takovo hunting ground. In this case, where the average values for lead are below the maximum permitted concentrations, and a high percentage of individual samples (especially in the kidney) exceed the legally prescribed limits, it leads to the conclusion that the presence of lead is still more noticeable than the presence of cadmium in the Takovo hunting ground. The high percentage of individual samples indicates that the game still comes into frequent contact with this contaminant, for which the potential source, which is most likely of an anthropogenic nature, needs to be determined.

CONCLUSIONS

The presence of the investigated heavy metals was confirmed in the territory of Central Serbia. Roe deer, as a representative game species, showed variable exposure to these contaminants, reflecting the overall state of the environment. At each locality, the results for Cd based on both mean and maximum values confirmed a low level of contamination, with concentrations generally lower and only in a few cases equal to the results of most studies from countries in Europe. The levels of Pb were confirmed with higher concentrations than the previous contaminant. This is confirmed by the average values in some of the hunting grounds where the concentrations were slightly above the legally permitted maximum concentrations for this heavy metal, followed by a somewhat higher presence of Pb than in several other European countries. Although the results are generally positive, with only minor exceptions at certain localities, further research is needed to identify potential sources of pollution.

Author Contributions

All authors listed under the title made substantial contributions to the conception, design, execution, and interpretation of the study. DB, ZP, KC, and MPH conceived and designed the research. DB, ZP, KC and NM conducted the fieldwork and were responsible for the collection of roe deer samples. MPH, NM and MV carried out the laboratory procedures and performed the statistical analyses. DB, KC, and MPH wrote the manuscript, except the part of method of laboratory analysing which was written by MPH and NM.

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Conflicts of Interest

The authors declare no conflict of interest.

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Effect of Initial Moisture Content of *Prosopis laevigata* Firewood on Charcoal Yield and Quality

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ABSTRACT

Charcoal yield and quality are influenced by both process parameters and raw material characteristics. In industrial settings, temperature, heating rate, and residence time are typically controlled. However, in rural and traditional production systems, raw material properties—particularly moisture content—become critical for improving efficiency. This study evaluated the effect of initial moisture content (MC) in *Prosopis laevigata* firewood on charcoal yield and quality. Firewood samples (20×20×20 mm) were conditioned to MC levels of 0%, 20%, 40%, and 60%, and subjected to pyrolysis under laboratory conditions at 450°C for 30 minutes. Firewood and charcoal characteristics were analyzed using one-way ANOVA, Pearson correlation, and linear regression ($p<0.05$). Results showed that the highest charcoal yield (28%) and density (0.28 g·cm^3) were obtained at 0% MC. Ash content decreased significantly with lower MC, reaching the lowest value (1.58%) at 0% MC. Strong correlations were found between firewood MC and basic density ($R^2=0.81$), charcoal yield ($R^2=0.87$), and energy efficiency ($R^2=0.65$). These findings suggest that reducing firewood moisture content is a simple and effective strategy to improve charcoal yield and quality in traditional production systems.

Keywords: biomass; pyrolysis; charcoal yield; moisture content; energy efficiency; *Prosopis laevigata*

INTRODUCTION

Charcoal has been produced and used globally for thousands of years. Today, its most common uses include domestic cooking, residential heating, and various industrial applications (Jahirul et al. 2012). Today, it remains a key resource for cooking, household heating, and various industrial processes (Antal and Grønli 2003, Dias Júnior et al. 2015, Hu and Gholizadeh 2019, Dias Junior et al. 2020, Oke et al. 2022).

More than one-third of the global population relies on firewood and charcoal as primary energy sources, particularly in regions where access to fossil fuels is limited (Dam 2017). The demand for charcoal has been increasing, driven by the rise of gourmet cooking and the lack of affordable alternative energy sources in developing countries. In 2023, global charcoal production was estimated at 61,228 million t, with Brazil (11.67%), Ethiopia (8.28%), Nigeria (8.15%), the Democratic Republic of the Congo (5%), India (4.70%), and China (3.98%) being the leading producers (FAO, 2024). However, inefficient charcoal production significantly contributes to deforestation and carbon emissions, highlighting the need to optimize production methods (Surup et al. 2020).

Charcoal production technologies range from traditional methods to industrial-scale processes (Mencarelli et al. 2023). Advanced techniques, such as batch-type kilns, Brazilian hives, metal kilns, and Adam retorts, provide some level of control over pressure, gas flow, temperature, and pyrolysis time, achieving efficiency rates of 35%–40% (Bustamante-García et al. 2013, Rodrigues and Braghini 2019, García-Quezada et al. 2021). However, in many rural areas of developing countries, traditional technologies like earth-mound kilns remain prevalent, often yielding less than 25% (Arias-Chalico 2018, Rodrigues and Braghini 2019).

Charcoal yield and quality are determined by multiple variables, including the composition of the raw material, heating rate, peak pyrolysis temperature, pressure, gas flow, and the presence of natural or synthetic catalysts (Antal and Grønli 2003, de Jesus et al. 2019). In small-scale charcoal production, temperature and moisture content (MC) are the most easily controlled parameters (Sangsuk et al. 2020). Therefore, optimizing production efficiency by regulating initial moisture content could be a key strategy to minimize biomass losses and improve product quality. However, one of the most critical factors affecting charcoal production efficiency and quality is the initial moisture content of the raw material. High moisture levels in firewood reduce process efficiency, increase charcoal friability, and lead to higher pollutant emissions (Sikarwar et al. 2017, Singh et al. 2017, Canal et al. 2020, da Silva et al. 2023). Some studies have determined the effect of moisture content on some quality aspects of charcoal, but without controlling production conditions. The missing gap is the lack of comparative data across species under standardized carbonization parameters. This study addresses this by evaluating moisture effects under controlled conditions, allowing clearer attribution of its impact on yield and quality.

This study aims to determine the effects of the initial moisture content of *Prosopis laevigata* firewood on the yield and quality of charcoal produced under controlled laboratory conditions. The findings of this research could contribute to the development of strategies to optimize charcoal production, particularly in traditional production settings where efficiency is low and environmental degradation is a growing concern.

MATERIALS AND METHODS

Tree Sampling

Three mature *Prosopis laevigata* trees were randomly selected from the Ejido La Reforma in Linares, Nuevo León, Mexico. Logs approximately 1 meter long were harvested from each tree, leaving a 0.3-meter stump. Each log was sawn, and 40 firewood samples with dimensions of 20×20×20 mm were obtained per log.

Samples Conditioning

Ten samples were selected from each log. Five samples were used to determine the initial MC using the gravimetric method, while the remaining five were subjected to carbonization. The heating process was manually controlled, with temperature increments of 50°C every 30 minutes, corresponding to a heating rate of 1.67°C·min^{-1}, until reaching a final temperature of 450°C, which was maintained for 30 minutes (Meneiros et al. 2012, Briseño-Uribe et al. 2015, Fialho et al. 2022, Gomes et al. 2024, Teixeira et al. 2024). The charcoal produced under these conditions served as the control treatment (CT).

In contrast, the remaining samples (30 per log) were conditioned in an electric chamber (SHC28) at 20°C and exposed to different relative humidities (RH) to progressively reach moisture contents of 20, 40, and 60. To obtain 0% MC, the samples were oven-dried at 103±5°C until a constant weight was achieved. Once the samples stabilized at each RH level, ten were selected: five for experimental moisture content determination and five for charcoal production.

Firewood Characterization

Green, dry, and basic density of firewood were determined with Equations (1) and (2), respectively:

$$FW_{d(g,d)}=\frac{m_{(g,d)}}{v_{(g,d)}} \tag{1}$$

$$FW_{bd}=\frac{m_d}{v_g} \tag{2}$$

where $FW_{d(g,d)}$ is the firewood density at green or oven dry condition (g·cm^{-3}), FW_{bd} is the firewood basic density (g·cm^{-3}), $m_{(g,d)}$ is the firewood mass at green or oven dry condition (g) and $v_{(g,d)}$ is the firewood volume at green or oven dry condition (cm^3), these were determined by Archimedes' principle (Borrego-Núñez et al. 2025), in this case the samples were immerses in mercury contained in a graduated cylinder, the displacement of the mercury was the volume of the firewood sample.

Charcoal Yield and Proximate Analysis

Charcoal yields (ychar) (%) from samples of each moisture content were determined from the relationship between weight at the beginning and the end of the process, according to Somerville and Jahanshahi (Somerville and Jahanshahi 2015). The charcoal volume was also determined using Archimedes' principle.

Proximate analysis, including moisture content (MC; %), volatile material (VM; %), ash (ASH; %), and fixed carbon (FC; %) , was determined according to the international standard ASTM D-1762-84 (2021) with Equations (3), (4), (5), and (6), respectively:

$$MC=\left(\frac{A-B}{A}\right)\cdot 100 \tag{3}$$

$$VM=\left(\frac{B-C}{B}\right)\cdot 100 \tag{4}$$

$$ASH=\left(\frac{D}{B}\right)\cdot 100 \tag{5}$$

$$FC=100-MC-VM-ASH \tag{6}$$

where A is the grams of air-dry sample used, B is the grams of sample after increasing the temperature to 105°C, C is the grams of sample after increasing the temperature to 950°C, and D is the mass of residues (g).

Higher Heating Value

The higher heating value (HHV; KJ·kg^{-1}) was determined according to Equation (7) using the method developed by Cordero et al. (2001):

$$HHV = 354.3FC + 170.8VM \tag{7}$$

Gravimetric Yield, Energy Efficacy of Carbonization and Energetic Density

These characteristics were calculated using Equations (8), (9), and (10) , respectively (Matali et al. 2016):

$$GY=\left(\frac{m_{ch}}{m_f}\right)\cdot 100 \quad (8)$$

$$EY=GY\cdot\frac{HHV_{ch}}{HHV_f} \quad (9)$$

$$EY=FW_{bd}\cdot HHV_f \quad (10)$$

where GY is the gravimetric yield (%), m_{ch} is the dry mass of charcoal (g), m_f is the dry mass of firewood (g), EY is the energy yield (%), HHV_{ch} is the charcoal higher heating value ($KJ\cdot kg^{-1}$), HHV_f is the firewood higher heating value ($KJ\cdot kg^{-1}$) and ED is the energetic density ($KJ\cdot m^{-3}$).

Energy efficiency (EE; $KJ\cdot kg^{-1}$) was determined by the relationship between net heating value and mass of wet firewood using Equation (11) (Shah et al. 1992):

$$EE=\frac{ED\cdot V_g}{M_g} \quad (11)$$

where ED is the energetic density ($KJ\cdot m^{-3}$), V_g is the green volume without bark (m^3) of the tree, and M_g is the green mass (kg).

Statistical Analysis

Means and standard errors were calculated for firewood and charcoal properties at each MC level. One-way ANOVA was used for a completely randomised design with 15 replicates. Moisture content was the independent variable, while yield and charcoal quality parameters were the dependent variables Steel and Torrie (1960). Percentage data were transformed using the arcsine square root method before analysis. Tukey's HSD test ($p<0.05$) was used for pairwise comparisons. Pearson correlation and linear regression were used to assess relationships between moisture content and other variables. All statistical analyses were performed using R software, version 3.2.2.

RESULTS AND DISCUSSION

Firewood Properties

The average MC measured immediately after logging of *Prosopis laevigata* trees was 64%, which is lower than the 100% reported by Carrillo-Parra et al. (2013). Green, oven-dry, and basic densities of the firewood under different laboratory relative humidity conditions are presented in Table 1. The three density types respond differently to moisture because each one incorporates specific moisture conditions in its calculation. Green density increased markedly with moisture content (0.67–1.03 $g\cdot cm^{-3}$), as expected, since it includes both solids and water while the firewood volume remains relatively stable. This resulted in statistically significant differences among treatments. In contrast, oven-dry density showed only minor variation (0.65–0.67 $g\cdot cm^{-3}$) because it depends solely on dry mass and final dry volume, both of which tend to remain constant within a species; therefore, most treatments did not differ statistically. Basic density (0.63–0.66 $g\cdot cm^{-3}$) exhibited a similar pattern, as the small volumetric changes associated with moisture-induced swelling were insufficient to alter the relationship between oven-dry mass and green volume.

Basic density values at 0, 20, 40, and 60% MC were lower than the 0.79 $g\cdot cm^{-3}$ reported by Carrillo-Parra et al. (2011). This difference may be attributed to variations in tree age, season of sampling, site conditions, or sampling methodology.

Overall, green density showed statistically significant differences among moisture levels, whereas oven-dry and basic densities did not. This behavior reflects the nature of each density calculation: green density incorporates both solids and moisture, making it highly sensitive to water content, while oven-dry and basic densities rely on dry mass and relatively stable volumes, resulting in non-significant differences across moisture levels.

Different letters in Green density represent statistically significant difference among firewood MC by Tukey test ($p\leq0.05$).

Charcoal Density and Mass Yield

The charcoal density showed statistical differences ($p<0.05$) at different firewood MC (Figure 1a), the highest charcoal density value (0.28 $g\cdot cm^{-3}$) was produced from firewood at 0% MC, followed by values of 0.26 $g\cdot cm^{-3}$ produced at 40 and 60% MC, the lowest charcoal density (0.25 $g\cdot cm^{-3}$) was at 20% MC.

The charcoal yield showed statistical differences ($p<0.001$) between different firewood moisture content (Figure 1b). The highest charcoal yield (28%) was obtained at the lowest firewood samples at MC 0%, while the lowest

Table 1. Mean and standard error of green, oven dry and basic density of *Prosopis laevigata* firewood from an experimental plantation, conditioned at different moisture content.

Moisture Content (%)	Green density ($g\cdot cm^{-3}$)		Oven dry density ($g\cdot cm^{-3}$)		Basic density ($g\cdot cm^{-3}$)	
	Mean	Std. error	Mean	Std. error	Mean	Std. error
0	0.67 (d)	0.01	0.66	0.01	0.66	0.01
20	0.73 (c)	0.01	0.67	0.01	0.63	0.01
40	0.89 (b)	0.02	0.65	0.04	0.64	0.01
60	1.03 (a)	0.01	0.66	0.01	0.63	0.01

yield (18%) was at 60%. Baghel et al. (2022) reported a yield of 63.40, 44.5, 36.65 and 30.50% for temperatures of 300, 400, 500 and 600°C in *Prosopis juliflora* species, this difference is related to the use of inert gas in carbonization, on the other hand, Chandrasekaran et al. (2021) obtained a yield of 36.18% at 400°C with a moisture content of less than 12%, while Nigatu et al. (2012) obtained a higher yield with *Prosopis juliflora* (33.1%) at a firewood moisture content of 21.2%. Missio et al. (2014) reported a similar relationship between yields and moisture content on charcoal of *Eucalyptus benthamii* firewood produced at high pressure. Yields of 36.34%, 34.79%, and 30.30% were obtained at moisture content of 0%, 30%, and 50%, respectively. On the other hand, Darmstadt et al. (2000) reported no differences in yield, regarding moisture content, on *Acer saccharum* bark particles pyrolyzed under vacuum conditions. The reduction of charcoal yield after increasing the moisture content of the raw material is attributed to extending the period of devolatilization produced by steam. High levels of moisture content also contribute to reducing the heating rate and to standardizing and delaying the process, the lower charcoal yield at higher moisture contents is due to the longer steam-driven degradation during pyrolysis and the extra firewood consumed to generate the heat needed to remove water, leaving less material available for charcoal formation (de Diego et al. 2003, Missio et al. 2014, Di Blasi et al. 2016).

Proximate Analysis

Results of proximate analysis of charcoal produced from different moisture content of firewood samples showed that only ash content was statistically different (p<0.001) (Figure 2). The highest and lowest values (3.43% and 1.68%) were obtained from firewood at 60% and 0% MC, respectively. Contrary to these findings, Missio et al. (2014) described a non-linear relationship between

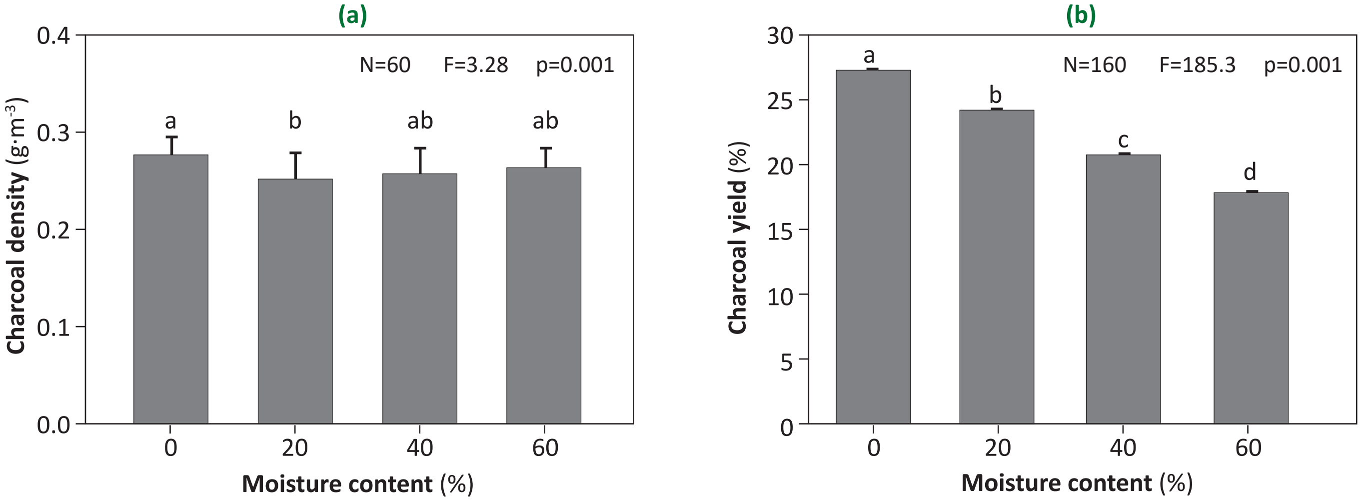

Figure 1. Mean and standard error of charcoal density and yield produced from firewood at four initial moisture contents: **(a)** Charcoal density (g·cm^{-3}); **(b)** Charcoal yield (%).

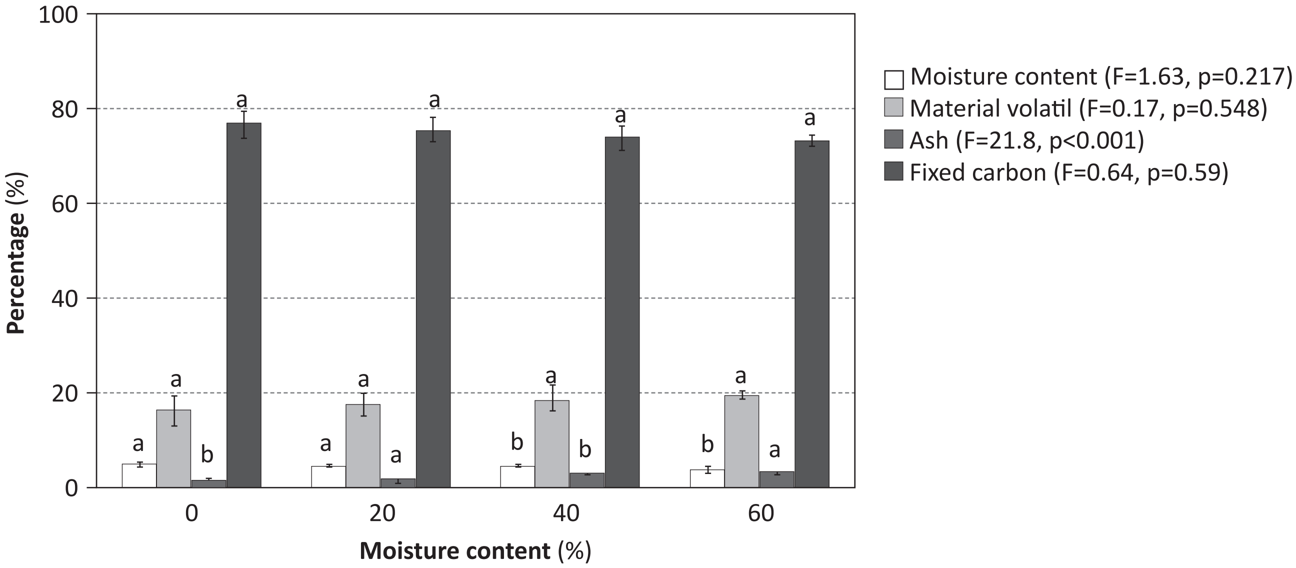

Figure 2. Proximate analysis of charcoal produced from firewood at four initial moisture contents, n=60, line bars represent the standard error.

reductions of moisture content of raw material, from 50%, 30%, and 0%, to the percentage reduction of ashes; the difference in the patterns is attributed to residence times. In this research, the residence time may not be enough to leach the firewood, reducing the amount of demineralization by water. Regarding charcoal quality, high ash content is a negative characteristic (Sangsuk et al. 2020). According to Antal and Grønli (2003), good charcoal quality should present ash values between 0.5 and 5%. Charcoal moisture content was the lowest at 60% and highest at 0%. There were no statistically significant differences in charcoal moisture content, nor were there any significant differences in volatile material nor in fixed carbon (Figure 2). Similar results were reported by Antal and Grønli (2003). However, Shah et al. (1992) found differences in fixed carbon at different moisture contents of the firewood used for charcoal production in a pilot kiln.

Higher Heating Values, Energetic Density, and Energy Efficiency of Carbonisation

Higher heating values of charcoal showed no statistical differences at different values of firewood moisture content

Table 2. Correlation analysis between moisture content with respect to yields and characteristics of charcoal.

Variable	Equation	R2	F	P
FWd*	FWd = 0.666+0.00565(MC)	0.8130	257.6	<0.0001**
CHd	CHd = 0.265+ 0.000105(MC)	-0.0053	0.68	0.412
CHy (m/m)	CHy(m/m) = 27.465 - 0.148(MC)	0.8680	389.2	<0.0001
CHy (v/m)	CHy(v/m) = 5.401 - 0.0009(MC)	-0.0130	0.231	0.633
CHMC	CHMC = 4.961 - 0.017(MC)	0.0330	2.99	0.089
CHVM	CHVM = 16.398 - 0.057(MC)	0.0056	1.33	0.253
CHAsh	CHAsh = 1.701 - 0.027(MC)	0.4510	50.28	<0.0001
CHFC	CHFC = 76.856 - 0.063(MC)	0.0094	1.563	0.216
CHHHV	CHHHV = 30031 -12.347(MC)	0.0107	1.638	0.206
CHED	CHED = 7964 -6.533(MC)	0.0211	2.274	0.137
CHEE	CHEE = 5503.2 - 33.742(MC)	0.6450	108.1	<0.0001

FWd= Firewood basic density, CHd= Charcoal density, CHy= Charcoal yield (mass/mass), CHy (v/m) = Charcoal yield (volume/mass), CHMC= Charcoal moisture content, CHVM = Charcoal volatile material, CHAsh = Charcoal ash, CHFC= Charcoal fixed carbon, CHHHV = Charcoal higher heating values, CHED = Charcoal energy density, CHEE = Charcoal energy efficacy. **Values of p<0.001 are bold.

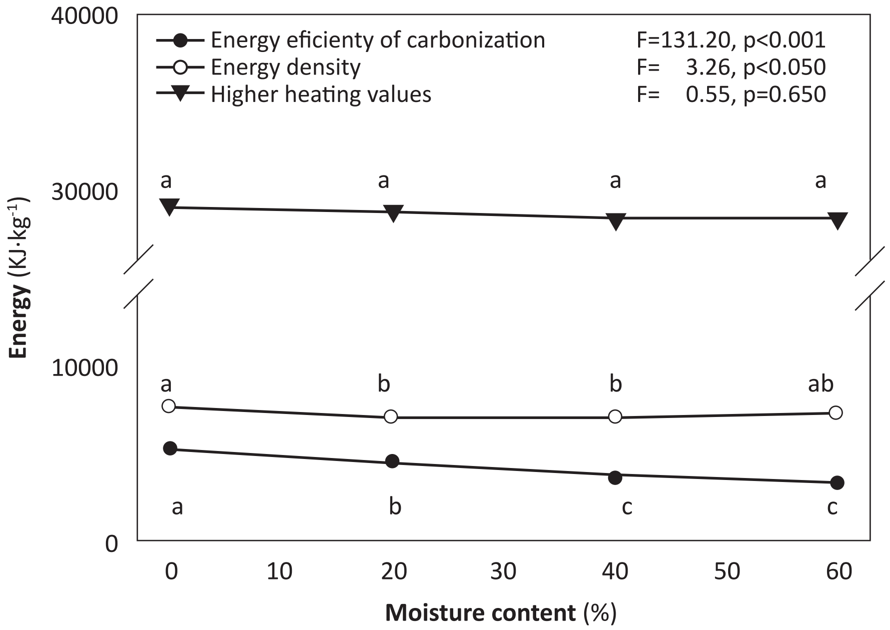

Figure 3. Higher heating values, energy density and energy efficiency of carbonization from firewood at four initial moisture contents, n=60.

(Figure 3). However, the values obtained (29,354 KJ·kg^{-1} – 30,079 KJ·kg^{-1}) are within the requirements of international standards. Energy density, which involves calculating the higher heating values and the moisture content (Equation 10) of firewood, was statistically different ($p<0.05$) for different moisture contents. The highest energy density value (8,294) was obtained from firewood at 0% MC, and the lowest (7,739) from firewood at 60% MC (Figure 3). Energy efficiency of carbonisation also showed statistical differences between moisture contents of the firewood; the values decreased when increasing moisture content and showed a similar pattern to that reported by Demirbas (2016).

Relationship Between Firewood Moisture Content, Charcoal Yield, and Charcoal Characteristics

High determination coefficient values (0.81, 0.86, 0.41, 0.64) were found between the moisture content of firewood and firewood density of charcoal yields (%), charcoal ash content, and energy efficiency of carbonization, as shown in Table 2. Figure 4 presents the relationship among variables. According to Adamu et al. (2018) and Baqir et al. (2019), wood moisture content affects combustion efficiency because part of the energy from wet wood is expended on evaporating its internal water. Consequently, increasing firewood moisture content reduces energy output, prolongs thermochemical conversion time, and leads to higher emissions, lower yields, and greater friability (Dias Júnior et al. 2015, Assis et al. 2016, Özyuğuran and Yaman 2017).

The reduction in charcoal energy associated with firewood with higher moisture content is reflected in the negative correlations observed (Figure 4). Since the moisture content of charcoal is typically kept below 10%, this promotes efficient energy release during combustion without significant energy losses due to water evaporation. Although some authors, such as Canal et al. (2020), reported no significant differences in volatile matter, fixed carbon, ash content, bulk density, or gross calorific value of charcoal. However, they note that pyrolysis removes part of the firewood as volatile compounds from hemicelluloses and extractives, increasing the concentration of fixed carbon and ash. This behavior is also evident in these results (Figure 4, Table 2).

It has been observed that the ideal moisture content for a carbonization process is between 0 and 20%. In the industry, it is difficult to reach 0% moisture, but it is possible to reach 20% and thus achieve a correct production of charcoal.

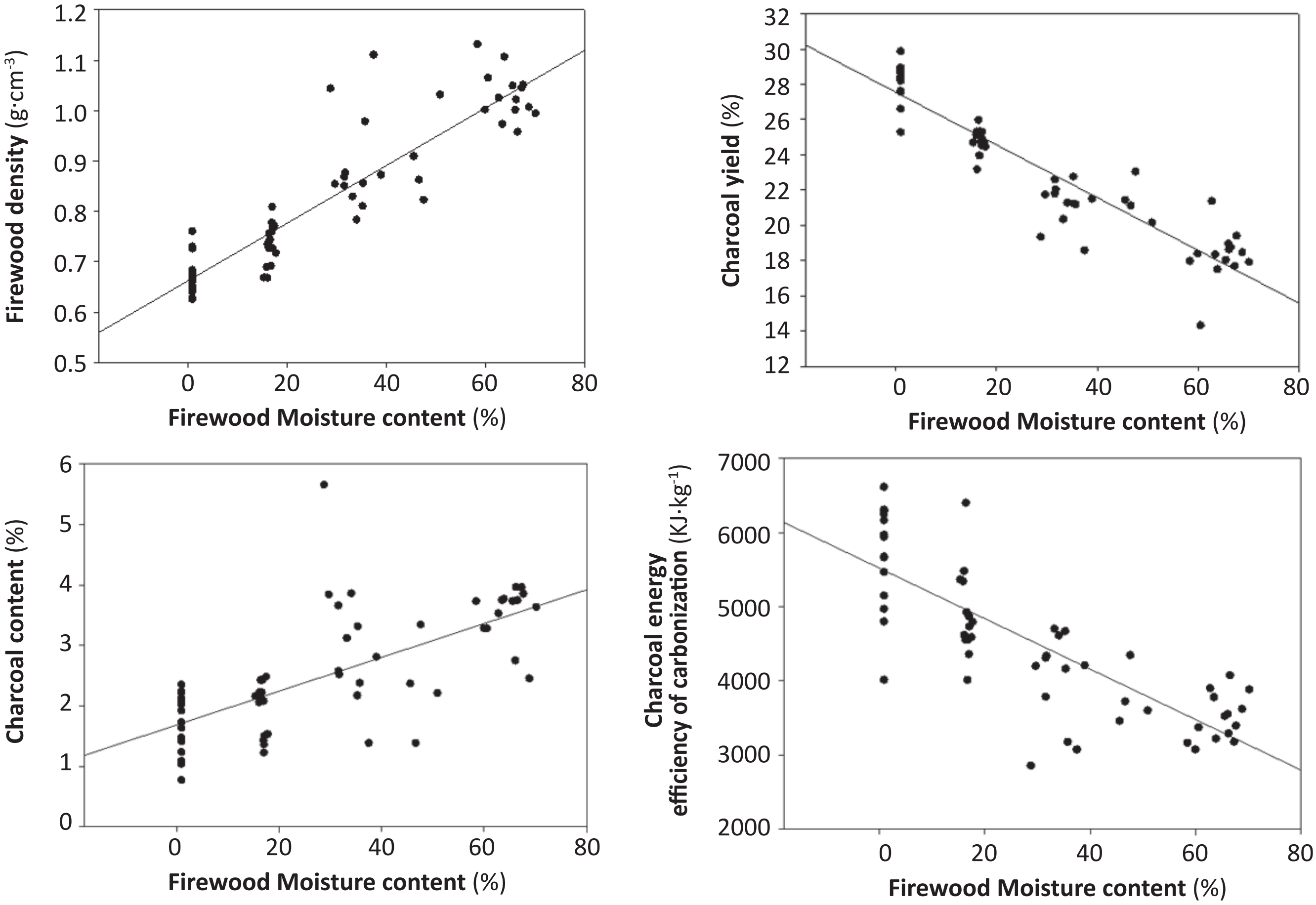

Figure 4. Regressions between moisture content of firewood with respect to: **(a)** firewood density (g·cm^{-3}); **(b)** charcoal yields (%); **(c)** charcoal ash content (%) and **(d)** charcoal energy efficiency of carbonization (KJ·kg^{-1}).

CONCLUSION

The yield, quality, and some other quality parameters of *Prosopis laevigata* charcoal increased when it was produced with firewood at low moisture content. The most important charcoal parameter for the producers is yield, and it was observed that by seasoning the firewood up to 20% and 0% of MC, the yields were higher than 25%.

The strong linear correlations between firewood moisture content and variables such as yield, ash content, and energy efficiency suggest that moisture control is not only a key driver of quality but also a practical, quantifiable lever for optimization. The predictive models generated in this research provide a valuable reference for improving traditional and semi-industrial charcoal practices, especially in rural or resource-limited settings.

Since moisture content is a very easily modifiable characteristic of the firewood used in the traditional charcoal production process, producers in normal environmental conditions should pay attention to reducing the firewood moisture content as low as possible, in order to increase the yield and quality of charcoal.

Author Contributions

Conceptualization: AC-P and JG-Q; Methodology: AC-P; Software: AC-P and JG-Q; Validation: AC-P and JG-Q; Formal analysis: AC-P; Resources: AC-P; Data curation: AC-P and JG-Q; Writing-original draft preparation: AC-P; Writing-review and editing: JG-Q and AC-P; Visualization: AC-P and JG-Q; Supervision: AC-P and JG-Q; Project administration: AC-P; Funding acquisition: AC-P. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Understory Vegetation Structure in Remnant Natural Forests and *Acacia* Plantations on Coastal Sand Dunes in North Central Vietnam

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ABSTRACT

In the coastal sand dune forests of North Central Vietnam, vegetation has been seriously damaged by war and overexploitation. To recover ecosystem functions, including sand stabilisation under harsh environments, exotic species like *Acacia* spp. have been planted as a monoculture. However, the long-term sustainability of this practice remains unclear. To assess the long-term effectiveness of revegetation with *Acacia* spp., this study aims to understand the differences and similarities in ecological characteristics of remnant natural forests and *Acacia* plantations on the coastal sand dune of North Central Vietnam by comparing understory vegetation structure and environmental conditions. We investigated the understory vegetation (height < 130 cm) in a total of 54 quadrants (1 m × 1 m), including nine natural forests and nine *Acacia* plantations. We compared diversity indices by mixed ANOVA and examined the differences in the understory vegetation structure between the two forest types through PERMANOVA. We also determined some abiotic environmental factors (e.g. light and soil water availability, and soil pH). We identified 951 individuals, with 792 found in natural forests and 159 in plantations. The species found in natural forests were well-distributed among Liana phanerophytes (Lp), Microphanerophytes (Mi), Mega-Mesophanerophytes (MM), and Cryptophytes (Cr). In contrast, species found in plantations were predominantly Cr, Hemicryptophytes (Hm), and MM. All diversity indices were significantly higher in natural forests ($P < 0.05$), and the NMDS analysis confirmed significant differences in the understory vegetation structure between natural forests and plantations. Only soil pH was significantly lower in natural forests ($P < 0.05$), while none of the environmental factors had a statistically significant impact on the variations in understory vegetation structure. Our results indicate that succession by native tree species does not seem to occur naturally in *Acacia* plantations. Hence, to restore and sustainably develop coastal sand dune forests in North Central Vietnam, it is essential to establish a scientifically based strategy for managing and protecting the remaining natural remnant forest areas.

Keywords: natural forest; *Acacia* plantation; coastal sand dunes forest; diversity; understory vegetation; life forms; environmental factor

INTRODUCTION

Coastal sand dune forests are crucial ecosystems that serve both ecological and socio-economic functions. They stabilise sand dunes, mitigate the impacts of natural disasters such as storms and floods, and provide vital ecosystem services that support biodiversity and local livelihoods (Acosta et al. 2009, Tanaka et al. 2009, Arens et al. 2013). Despite their importance, these forests are increasingly threatened by anthropogenic pressures, including habitat degradation, overexploitation, and pollution, which have led to significant biodiversity loss and declining ecosystem resilience (O'Shea and Kirkpatrick 2000, Dolan and Walker 2006, Lotze et al. 2006, Lu et al. 2018).

Vegetation in coastal sand dune environments must adapt to harsh abiotic conditions such as sand burial, strong winds, salt spray, nutrient-poor soils, and high temperatures. These challenges have led to the evolution of specialised morphological and physiological traits in native dune plant species that enhance their survival and contribute to ecosystem stability and complexity (Maun 2009, Torca et al. 2019, Tordoni et al. 2018, 2019, 2021). Environmental heterogeneity, particularly in soil moisture, salinity, and microclimatic conditions, further shapes local species composition, resulting in diverse plant communities adapted to dynamic and stressful environments (Hesp 1991, Spanò et al. 2013, Bonari et al. 2017, Ciccarelli et al. 2023).

Out of the members of coastal sand dune systems, understory vegetation plays a key role in maintaining ecological functions, facilitating tree regeneration, stabilising soils, cycling nutrients, and supporting biodiversity (MacLean and Wein, 1977, Allen et al. 2002, Kerns et al. 2006, Griffiths et al. 2007). The composition and diversity of understory communities often reflect the structure of the overstory and the surrounding environmental conditions (Barbier et al. 2008, Hu et al. 2013, De Lombaerde et al. 2021). Understanding these relationships is particularly important in restoration contexts.

In North Central Vietnam, coastal sand dune forests have been severely degraded over the past decades due to the combined effects of war, deforestation, and unsustainable land use (Cam 2011, Hoang and Thao 2015, Sterling et al. 2006, Wittmann et al. 2019). Restoration efforts have historically relied on fast-growing exotic species, such as *Acacia crassicarpa* and *Casuarina equisetifolia*, which were introduced to stabilise dunes and reestablish vegetation cover (Nambiar et al. 2015, Pasieczni and McDonald 2016, Wittmann et al. 2019). While these species have proven effective in the short term due to their rapid growth, ability to stabilise degraded soils, and economic value, they may not support the long-term recovery of native biodiversity or complex ecosystem processes (Avis 1989, Peperkorn et al. 2005, Buffa et al. 2021).

Recently, there has been growing interest in using native plant species to improve ecological outcomes in coastal forest restoration (Thao 2016, Hien et al. 2022, Pistorius et al. 2023). However, knowledge gaps remain regarding the comparative ecological characteristics of natural forests and exotic plantations in these systems. This study aims to assess and compare the species composition, understory diversity, and environmental conditions between natural coastal sand dune forests and *Acacia* plantations in North Central Vietnam. The findings are intended to inform future restoration strategies that prioritise both ecological functionality and biodiversity conservation.

MATERIALS AND METHODS

Study Site

The study was conducted in inland, fixed coastal sand dune areas located in three provinces of North Central Vietnam: Thua Thien Hue, Quang Tri, and Quang Binh. These inland dune forests are characterised by dry, less-flooded conditions. The average distance is approximately 4 km from the coastline (Figure 1, Table 1). In the natural forest (NF) sites, vegetation is composed of scattered patches of woody species interspersed with open sand areas, forming what is locally referred to as secondary shrub communities on coastal sand (Figure 2a). The dominant species belong to families Myrtaceae, Lauraceae, Clusiaceae, Dipteracapaceae and Euphorbiaceae (Thao 2016, Wittmann et al. 2019, Thao 2021). These forests have a relatively simple structure, with a canopy height ranging from 5 to 12 meters (Thao 2016, Thao 2021). The *Acacia* plantation (AP) sites were located adjacent to the natural forest areas and included both state-managed coastal protection forests and privately owned plantations (Figure 2b). The plantation stands ranged from 9 to 17 years in age and were dominated by two exotic species: *Acacia crassicarpa* and *Acacia* hybrid (*A. auriculiformis* × *A. mangium*), both commonly used in reforestation programs in Vietnam. (Table 1).

The climate in this area is predominantly tropical monsoon, characterised by two distinct seasons: a rainy season from September to December and a dry season from January to September. The mean annual temperature is approximately 25°C, but the temperature at the ground surface in sandy areas can reach over 60°C (Hoa et al. 2010, Thao 2016). This extreme heat presents a critical factor influencing the germination, growth and development of vegetation (Hoang and Thao 2015, Thao 2016). Mean annual precipitation is approximately 3,000 mm, and about 40% of all annual precipitation falls between October and December (Bach and Hien 2004, Thao 2016, Canh et al. 2019). The topography of the region is relatively flat, with an altitude ranging from 7 to 31 meters above sea level (Table 1).

Disturbance history differed between forest types. Natural forests are relatively protected with limited recent disturbance, whereas plantation forests, established on degraded lands, are actively managed through thinning and understory clearing (Nambiar et al. 2015, Thao 2021) (Figure 3 a,b).

Understory Vegetation Census and Environmental Condition

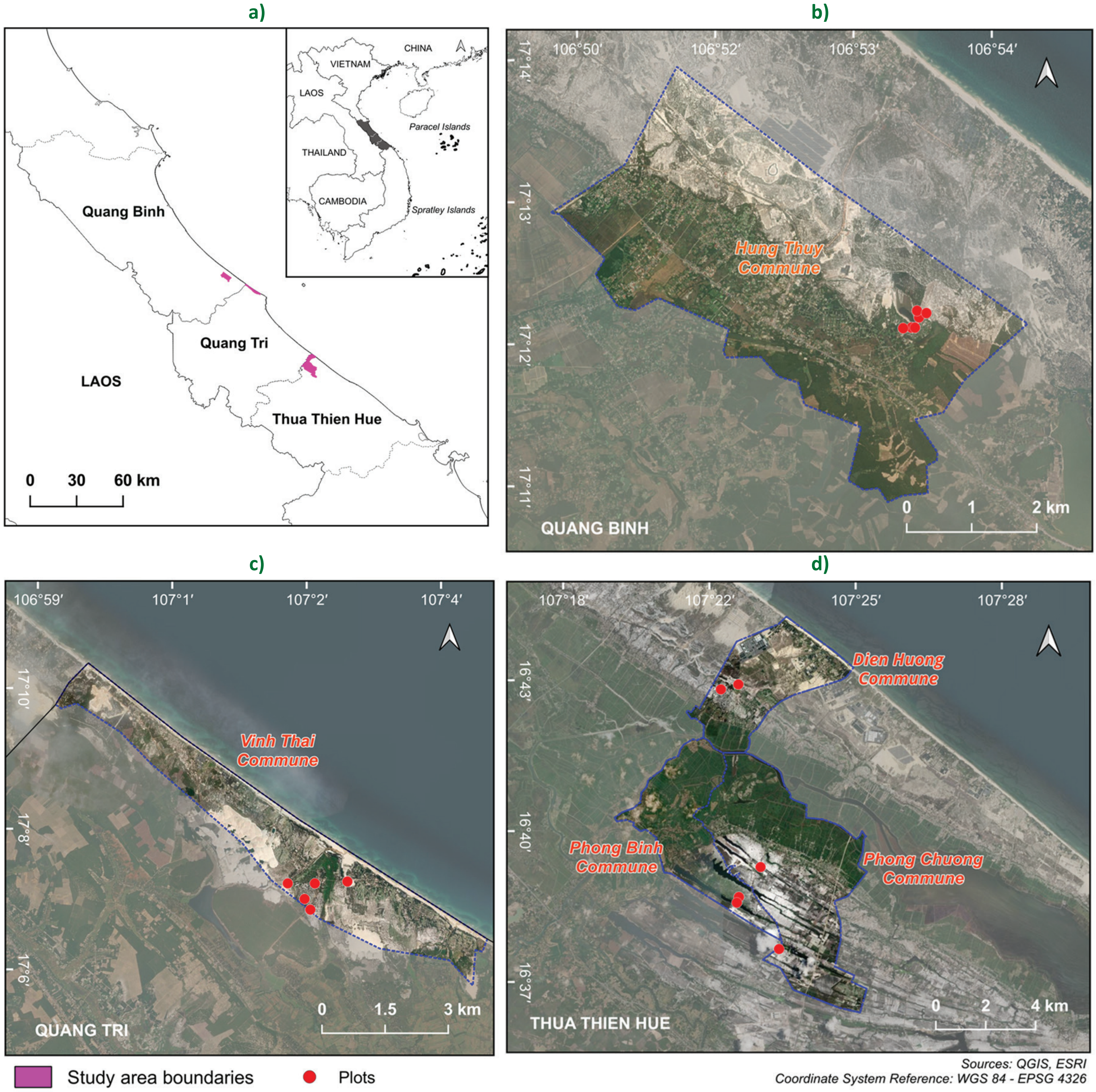
In three provinces of Central Vietnam, a total of 18 plots (15 × 15 m) were established, including nine plots in natural forests (NF) and nine in *Acacia* plantations (AP) (Table 1). In each plot, we established 3 subplots (1 × 1 m), resulting in a total of 54 subplots (27 in NF and 27 in AP) (Figure 4a,b). In NF, subplots were located directly beneath the canopy of dominant native tree species, selected to represent typical closed-canopy conditions within the plot (Table 1). Similarly, in AP, the subplots were established under the canopy of *Acacia spp*. Subplots were placed at least

1 meter away from large gaps, treefalls, or forest edges to ensure representation of typical understory conditions under canopy cover. All understory plant individuals (height < 130 cm) within each subplot were identified and counted during the field survey.

To address environmental factors that affect understory vegetation, we also determined some abiotic environmental factors. First, we measured the thickness of the organic soil layer and the soil volumetric water content of the mineral soil layer by using a portable sensor (ML3,

Table 1. General information of the study plots in natural forests and *Acacia* plantations.

Plot			Location	Distance from coastline (km)	Altitude (m)	Main overstory species	Stand type (NF) or Age (AP)
Natural forest (NF)	Quang Binh	**1**	17°12’8.46"N 106°53’18.28"E	3.3	21	*Vatica mangachapoi* *Syzygium corticosum* *Lithocarpus concetricus*	Secondary forest
		2	17°12’8.42"N 106°53’20.04"E	3.2	19	*Lithocarpus concetricus* *Vatica mangachapoi*	Secondary forest
		3	17°12’8.12" 106°53’13.89"	3.3	31	*Lithocarpus concetricus* *Vatica mangachapoi*	Secondary forest
	Quang Tri	**1**	17°6’45.81"N 107°2’26.92"E	1.6	14	*Lithocarpus concetricus* *Syzygium corticosum* *Myrsine linearis*	Secondary forest
		2	17°6’54.06" N 107°2’22.27" E	1.4	12	*Syzygium corticosu* *Myrsine linearis*	Secondary forest
		3	17°6' 58.52"N 107°2' 20.64"E	1.4	10	*Syzygium corticosu* *Myrsine linearis*	Secondary forest
	Thua Thien Hue	**1**	16°38’27.49" N 107°22’16.02" E	9.8	11	*Syzygium corticosu* *Myrsine linearis* *Vatica mangachapoi*	Secondary forest
		2	16°38’20.08" N 107°22’13.26" E	10.1	7	*Syzygium corticosu* *Lithocarpus concetricus*	Secondary forest
		3	16°43’0" N 107°21’51.63" E	3.8	10	*Syzygium zeylanicum* *Syzygium corticosu* *Myrsine linearis* *Syzygium corticosu*	Secondary forest
***Acacia* plantation (AP)**	Quang Binh	**1**	17°12’13.6" N 106°53’22.17" E	3.1	18	*Acacia* hybrid	10
		2	17°7’5.93"N 107°2’30.53"E	3.0	15	*Acacia* hybrid	9
		3	17°7’7.39"N 107°2’56.68"E	2.9	22	*Acacia* hybrid	10
	Quang Tri	**1**	17°6’45.81"N 107°2’26.92"E	1.3	15	*Acacia* hybrid	9
		2	17°6’54.06" N 107°2’22.27" E	1.0	8	*Acacia* hybrid	10
		3	17°6' 58.52"N 107°2' 20.64"E	0.5	10	*Acacia* hybrid	14
	Thua Thien Hue	**1**	16°38’27.49" N 107°22’16.02" E	10.5	10	*Acacia crassicarpa*	17
		2	16°38’20.08" N 107°22’13.26" E	8.4	8	*Acacia crassicarpa*	14
		3	16°43’0" N 107°21’51.63" E	3.2	10	*Acacia crassicarpa*	12



Sources: QGIS, ESRI
Coordinate System Reference: WGS 84 - EPSG 4326

Figure 1. The study areas are located in **(a)** three provinces: **(b)** Quang Tri, **(c)** Quang Binh, and **(d)** Thua Thien Hue in central Vietnam.

Delta-T Devices, Cambridge, UK), at the centre and four corners of each subplot. Understory light availability was quantified by estimating canopy cover using hemispherical photographs taken at 1 m height at the centre of each subplot. We measured the pH (KCl) of surface mineral soil (0–10cm) using electrodes (sensION+ PH3 GLP, Hach, USA) in a 1:5 soil-water suspension. We also determined the distance from each plot to the nearest coastline using aerial imagery. Distances were calculated from the GPS coordinates of the plots to a digitised shoreline derived from high-resolution aerial photographs (Google Earth, 2023) within QGIS. The shortest Euclidean distance (in meters) was measured after reprojecting the data to the UTM coordinate system. These field surveys were conducted from July to August 2023.

Life Forms Classification

The classification of plant life forms was based on the Raunkiaer (1934): Phanerophytes (Ph) and Mega-Mesophanerophytes (MM), comprising medium- to large-sized woody species (>8 m in height); Microphanerophytes (Mi), consisting of small trees (2–8 m); Nanophanerophytes (Na), including shrubs and small trees (25 cm–8 m); Liana phanerophytes (Lp), Chamaephytes (Ch), Hemicryptophytes (Hm), and Cryptophytes (Cr).

Diversity Indices

For each subplot, we calculated four diversity indices: Species richness, Shannon, evenness, and Menhinick (Shannon 1948, Pielou 1975, Magurran 2004, Morris et al. 2014), using the formulas below:

Species richness (S) = Number of species

Shannon index $(H') = -\sum p_i \ln p_i$

Evenness index $= H'/\ln(S)$

Mehinick index $= S/\sqrt{n}$

where p_i is the proportion of individuals belonging to species i, and n is the total number of individuals in a subplot.

Statistical Analyses

All analyses were conducted using R version 4.3.3 (R Core Team, 2023), and statistical significance was set at $P < 0.05$.

Species richness in relation to sampling effort was assessed using sample-based rarefaction and species accumulation curves (packages vegan and rareNMtests) (Chao et al. 2014, Oksanen et al. 2022, Cayuela and Gotelli 2014). Asymptotic species richness (Total Estimated Species, TES) was estimated using both non-parametric methods (specpool) and nonlinear Michaelis–Menten models fitted to accumulation curves with random method and 100 permutations (specaccum) (Soberón and Llorente, 1993, Gotelli and Colwell, 2001, Colwell et al. 2012, Zou et al. 2023). The statistical significances of TES and B were tested using t values in the Wald test.

Differences in diversity indices and environmental variables between forest types were tested using mixed one-way ANOVA (aov), with subplots nested within plots as random effects, and differences in life-form composition were assessed using chi-squared tests (chisq.test) (package stats).

Differences in community composition among groups were analysed using PERMANOVA (adonis) based on Bray–Curtis and Jaccard dissimilarity matrices, after confirming the homogeneity of understory vegetation composition data between forest types by PERMDISP (betadisper) (package vegan). The NMDS analysis (metaMDS) was used for the visualisation (package vegan).

Figure 2. Study sites: **(a)** natural forest and **(b)** *Acacia* plantation.

Figure 3. Disturbance caused by human activities: **(a)** constructions built in natural forest (NF); **(b)** harvesting of *Acacia* plantation (AP) after 7–10 years.

Figure 4. Understory vegetation in 1 m × 1 m subplots: **(a)** natural forest (NF); **(b)** *Acacia* plantation (AP).

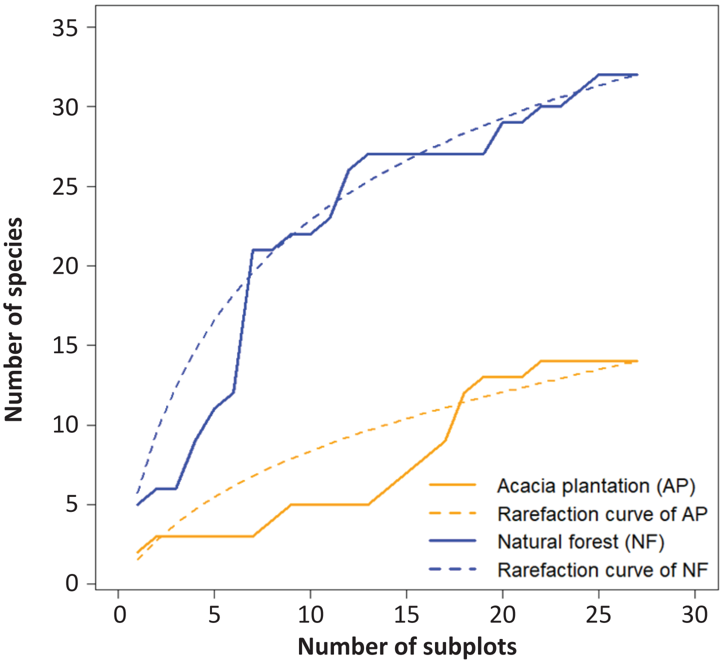


Figure 5. Species accumulation and rarefaction curves of understory vegetation in natural forest (NF) and Acacia plantation (AP).

RESULTS

We recorded a total of 951 plant individuals across 54 subplots, comprising various life forms such as Liana phanerophytes (Lp), Microphanerophytes (Mi), Mega-Mesophanerophytes (MM), Cryptophytes (Cr), Nanophanerophytes (Na), Chamaephytes (Ch), and Hemicryptophytes (Hm). These individuals represent a total of 39 species, 35 genera, and 26 families (Table 2).

The species accumulation and rarefaction curves showed that the observed species richness increased steadily with the number of subplots, and reached 32 species in NF, but that in AP plateaued much earlier and stabilised at 14 species (Figure 5). The total estimated species (TES) values were 38.8 in NF and 21.5 in AP, and the shape parameters (B) were 6.5 in NF and 15.2 in AP, with relatively low standard errors (Table 3).

Species abundance and life form composition differed significantly between forest types. The natural forest (NF) contained 792 individuals (83.27% of the total), while only 159 individuals (16.73%) were found in the *Acacia* plantation (AP). In NF, the dominant life form was Lp, accounting for 49.21% (468 individuals), followed by Mi (17.42%), MM (10.73%), Cr (7.2%), Na (4.04%), Ch (1.26%), and Hm (0.25%). In contrast, AP showed a different composition, with Cr being the most abundant (36.48%), followed by Hm (28.93%), MM (21.38%), Lp (5.04%), Na (4.4%), Ch (2.52%) and Mi (1.26%) (Figure 6).

The mean values for species richness, Shannon, evenness, and Menhinick indices in NF were 5.70, 1.17, 0.32 and 1.24, respectively. In AP, they were 1.63, 0.26, 0.09 and 0.75, respectively. These indices were significantly lower in AP compared to NF ($P < 0.05$) (Figure 7a-d).

Table 2. Species and number of individuals of understory vegetation in two forest types.

Family	Species	Life form	AP			NF		
			QB	QT	TTH	QB	QT	TTH
Ferns Pteridaceae	*Acrostichum aureum*	Cr	0	0	0	0	2	0
Magnoliids Annonaceae	*Fissistigma poilanei*	Mi	0	0	0	0	0	1
Annonaceae	*Polyathia cerasoides*	Mi	0	0	0	2	0	0
Lauraceae	*Lindera caudata*	Mi	0	0	0	0	2	24
Monocots Asparagaceae	*Ophiopogon japonicus*	Cr	0	0	0	6	1	0
Asphodelaceae	*Dianella ensifolia*	Cr	0	0	0	15	13	5
Comelinaceae	*Cyanotis burmanniana*	Ch	0	4	0	7	1	2
Cyperaceae	*Cyperus radians*	Hm	0	38	8	0	1	1
Pandanaceae	*Pandamus tectorius*	Na	1	0	0	0	0	0
Poaceae	*Eriachne chinensis*	Cr	3	10	19	2	5	5
Poaceae	*Paspalum viginatum*	Cr	0	15	0	0	0	0
Poaceae	*Spinifex littoreus*	Cr	10	1	0	0	0	0
Smilacaceae	*Smilax glabra*	Lp	0	0	0	0	0	2
Eudicots Apocynaceae	*Alyxia pseudosinensis*	Lp	0	0	2	32	85	311
Apocynaceae	*Strophanthus divaricatus*	Na	0	0	0	7	0	0
Dilleniaceae	*Tetracera scandens*	Lp	3	0	0	3	16	11
Dipterocarpaceae	*Vatica mangachapoi*	Mi	0	0	0	2	0	0
Euphorbiaceae	*Croton touramensis*	Mi	0	0	0	20	60	0
Fabaceae	*Acacia crassicarpa*	MM	0	0	33	0	0	0
Fabaceae	*Acacia mangium*	MM	0	1	0	0	0	0
Fagaceae	*Castonopsis indica*	MM	0	0	0	1	0	0
Fagaceae	*Lithocarpus concentricus*	MM	0	0	0	0	0	2
Myrtaceae	*Syzygium corticosum*	MM	0	0	0	12	12	32
Myrtaceae	*Syzygium cumini*	Mi	0	0	0	0	1	2
Myrtaceae	*Syzygium czeylanticum*	MM	0	0	0	0	12	8
Oleaceae	*Jasminum subtriplinerve*	Lp	0	0	0	0	2	3
Phyllanthaceae	*Breynia fruticosa*	Na	0	1	0	0	0	0
Phyllanthaceae	*Cleistanthus concinnus*	Mi	0	0	0	0	1	0
Phyllanthaceae	*Phyllanthus fasciculatus*	Na	0	0	0	7	1	0
Phyllanthaceae	*Phyllanthus thaii*	Na	0	0	0	0	0	3
Primulaceae	*Rapanea linearis*	Mi	0	0	2	5	2	3
Rhizophoraceae	*Carallia brachiata*	MM	0	0	0	1	0	1
Rubiaceae	*Ixora coccinea*	Na	0	0	0	10	0	4
Rubiaceae	*Morinda parvifolia*	Lp	2	1	0	3	0	0
Rubiaceae	*Psychotria montana*	Cr	0	0	0	0	1	2
Rutaceae	*Severinia monophylla*	Na	3	2	0	0	0	0
Salicaceae	*Scolopia spinoda*	Mi	0	0	0	0	0	12
Sapindaceae	*Lepisanthes tetraphylla*	MM	0	0	0	0	3	1
Simaroubaceae	*Eurycoma longifolia*	Mi	0	0	0	0	0	1
	Total		22	73	64	135	221	436

Table 3. Total estimated species (TES) and model parameters for understory plant communities in NF and AP.

Forest Type	TES (SE)	B (SE)	Residual SE	t value of TES	t value of B
NF	38.8 (0.4)	6.5 (0.2)	0.5	98.3^{***}	31.3^{***}
AP	21.5 (0.3)	15.2 (0.4)	0.2	73.2^{***}	35.1^{***}

letters on the t values indicate the result of Wald test (^{ns}, not significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001)

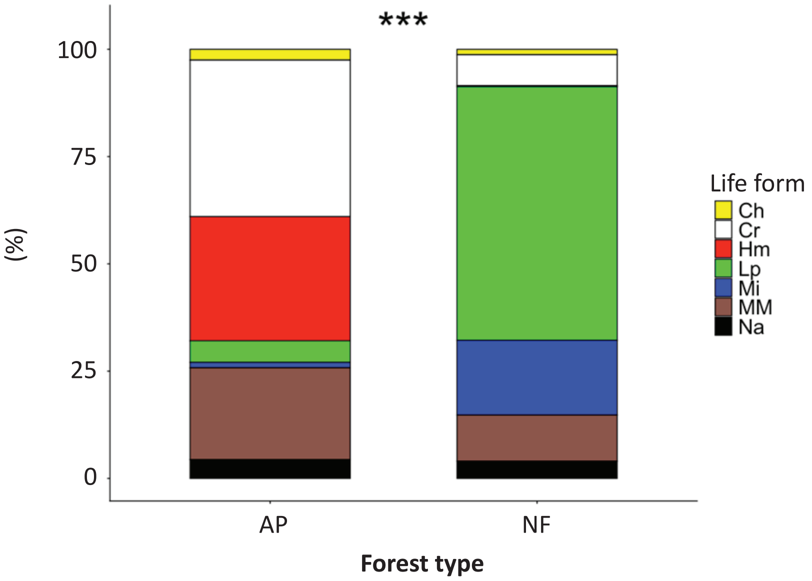


Figure 6. The proportion of each life form of understory vegetation in the natural forests and *Acacia* plantations. Letters in the figure indicate the result of χ^2 test (^{ns}, not significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001).

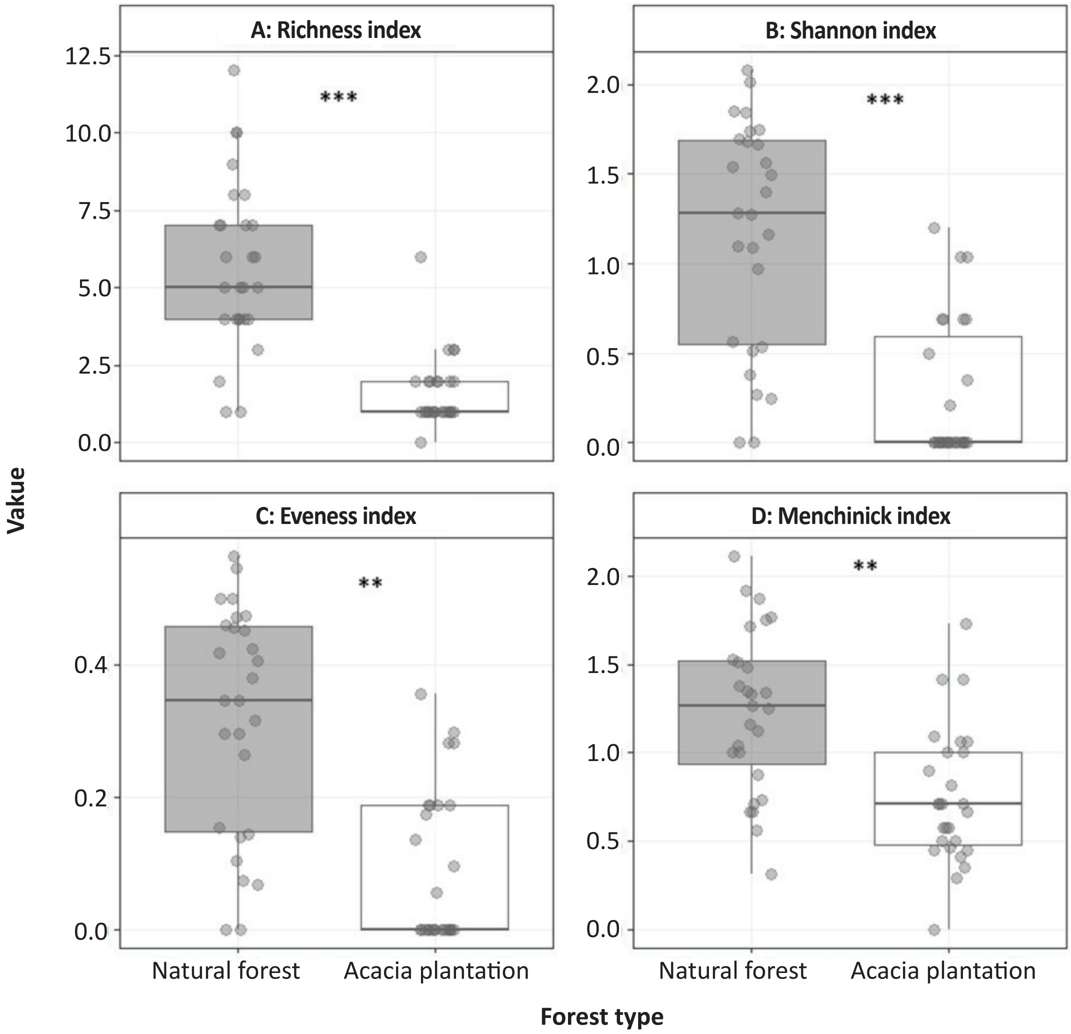


Figure 7. Diversity indices of understory vegetation in the natural forests and Acacia plantations: **(a)** Richness index; **(b)** Shannon index: **(c)** Evenness index; and **(d)** Menhinick index. Letters in the figure indicate the results of Mixed one-way ANOVA (^{ns}, not significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001).

The mean values for canopy coverage (%), soil volumetric water content (%), thickness of organic soil layer (cm) and distance from the coastline (km) in NF were 86.5, 3.3, 3.2, and 3.9 respectively, and those in AP were 76.1, 4.1, 2.6, and 3.9 in AP respectively (Figure 8a-d). These environmental factors did not show a significant difference between NF and AP ($P > 0.05$). By contrast, pH (KCl) was significantly lower in NF (3.0) than in AP (3.3) (Figure 8e) ($P < 0.05$).

The Two-dimensional NMDS analysis was successful with a stress value of 0.08. The NMDS diagram showed a clear spatial separation of the understory vegetation composition between NF and AP (Figure 9). The results of PERMDISP indicated no significant differences in dispersion between groups for either Bray–Curtis ($P = 0.07$) or Jaccard distances ($P = 0.09$), and the PERMANOVA analysis revealed significant differences in understory species composition between forest types based on both Bray–Curtis ($R^2 = 0.29$, $F = 6.46$, $P = 0.001$) and Jaccard distances ($R^2 = 0.21$, $F = 4.22$, $P = 0.001$), however, no environmental factor was found to affect the differences in understory vegetation composition on the two-dimensional NMDS diagram.

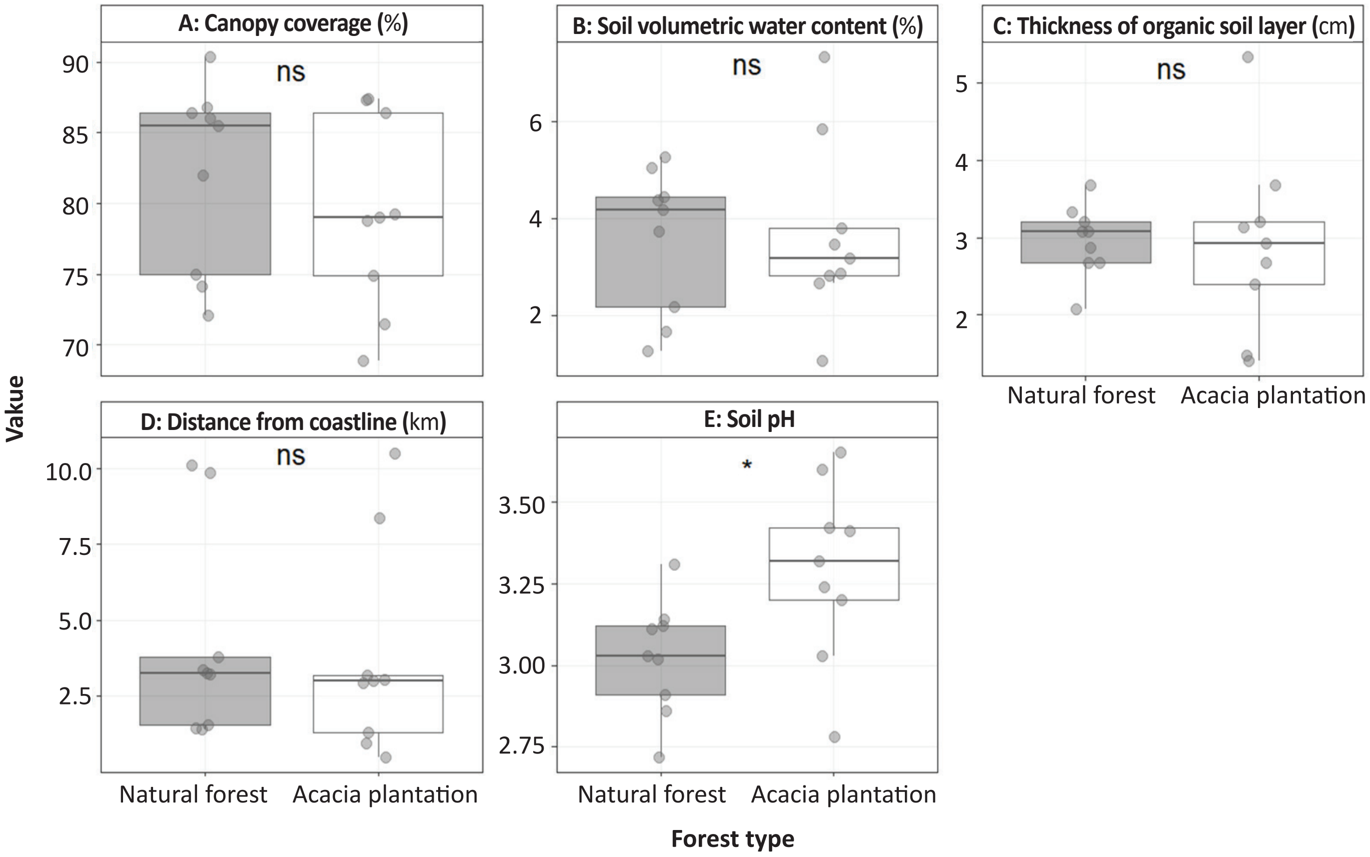


Figure 8. Environmental factors in the natural forests and *Acacia* plantations: **(a)** Canopy coverage; **(b)** surface mineral soil water content; **(c)** thickness of the organic soil layer; **(d)** distance from the coastline; and **(e)** soil pH. Letters in the figure indicate the results of Mixed one-way ANOVA (^{ns}, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

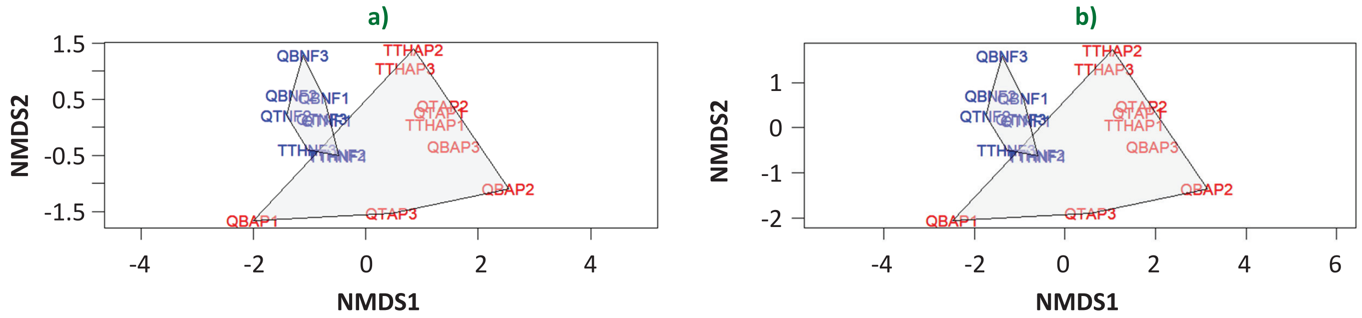


Figure 9. Non-metric Multidimensional Scaling (NMDS) plot showing the understory vegetation structure of the study plots with **(a)** Bray–Curtis distance and with **(b)** Jaccard distance. Letters in the figure indicate the location and forest type of study plots (QB: Quang Binh, QT: Quang Tri, TTH: Thua Thien Hue, NF: natural forest, AP: Acacia plantation).

DISCUSSION

Our study demonstrated that there were significant differences in understory plant community structure, diversity, and composition between natural forests (NF) and *Acacia* plantations (AP) in coastal sand dune ecosystems in North Central Vietnam (Figures 6–9, Tables 2, 4). Although the calculated rarefaction curves in both plots were relatively stabilised at the maximum number of subplots (Figure 5), understory species found in this study accounted for 82.5 % in NF and 66.7% in AP against the total estimated species (Table 3), suggesting that the species diversity at the regional scale may partly be underestimated in our census effort, especially for APs. However, the estimated species diversity from the rarefaction curves critically differed between the two forest types (Table 3), and this tendency can be well-reproduced by various diversity indices (Figure 7). The overall plant diversity in this study (Figure 7, Table 2) was consistent with previous studies in this region (Hoang and Thao 2015, Thao et al. 2015, Thao 2020, 2021, 2022, 2024).

The significantly higher values of species richness, Shannon diversity, evenness, and Menhinick index in NF highlight the superior ecological quality and complexity of the natural forest environment (Figure 7). The mean richness of 5.70 species per subplot in NF, compared to only 1.63 in AP, underscores a dramatic reduction in species coexistence under plantation conditions. Significant differences in the Shannon index (1.17 in NF vs 0.26 in AP) and evenness (0.32 vs 0.09) show that not only is the number of species greater in NF, but also their relative abundances are more balanced (Thao 2016, Thao 2022). In contrast, the low evenness in AP reflects a community dominated by a few species with limited diversity, a pattern often associated with simplified systems such as monocultures (Bremer and Farley 2010, Pawson et al. 2013).

The quantitative difference was accompanied by contrasting life form compositions (Figure 6). The dominance of Lp in NF (49.21%) reflects a structural characteristic commonly observed in tropical coastal sand dune forests where lianas represent an important life form contributing substantially to overall plant community composition (Black et al. 2010, Castanho et al. 2012, Chun & Choi 2012, White et al. 2024). The presence of a diverse spectrum of life forms, including Mi, MM, Cr, and Na, further highlights the structural complexity and ecological heterogeneity of NF. Studies of sand dune forests in other regions also reported that natural forests supported greater species diversity due to their structural complexity and ecological characteristics (Hesp 1991, Maun 2009, Acosta et al. 2009, Tordoni et al. 2021). In contrast, abundant life forms in AP (Cr and Hm) (Figure 6) were indicative of disturbance and early successional dynamics (Zou et al., 2023) and reflected the simplified structure and reduced diversity typical of afforested plantations (Brockerhoff et al. 2008, Kayes et al. 2010). Our findings support research that has shown that monoculture plantations often fail to replicate the natural heterogeneity and biodiversity of native forests (Provoost et al. 2011).

These differences in plant community structure between NF and AP were clearly reflected in the results of the NMDS analysis (Figure 9) and PERMANOVA, particularly in terms of species composition and abundance. These findings are consistent with previous studies on understory vegetation in coastal sand dune forests dominated by *Acacia* species, which reported negative impacts of *Acacia* on the diversity and abundance of understory species (Lorenzo et al. 2010, Rascher et al. 2011a, Fuentes et al. 2011, Le Maitre et al. 2011, González et al. 2012). Moreover, the PERMDISP test indicated that the differences observed were not due to heterogeneity in within-group dispersion ($P > 0.05$), thereby validating that the differences detected by PERMANOVA reflect genuine shifts in community centroids rather than differences in variance among plots (Ecklu-Mensah et al. 2023). This distinction is critical in ensuring that the observed patterns are driven by ecological differences between forest types, rather than by variability within them.

Although significant differences in vegetation composition and diversity were observed between two forest types, most environmental variables (thickness of the organic soil layer, surface mineral soil water content, canopy coverage, and distance from the coastline) did not differ significantly between forest types (Figure 8). Consequently, no environmental factor explains the variation in plant community structures (Figure 8). This suggests that abiotic conditions at the regional scale are relatively homogeneous across the study area, and that the observed differences in plant community structure are primarily driven by forest type and associated biotic processes rather than environmental gradients (Vandvik et al. 2020). On the other hand, the vegetative organs of *Acacia* trees (leaf, bark, and twigs) are known to release chemical compounds that inhibit the germination and growth of other plant species (Lorenzo et al. 2011, Ismail and Metali, 2014). Such allelopathic effects might be one of the possible factors that explain why vegetation under *Acacia* plantations tends to be less diverse compared to other forest types (Le Maitre et al. 2011, Lorenzo et al. 2011, González et al. 2012). Only soil pH (KCl) was significantly lower in NF (3.0) than in AP (3.3) (Figure 8). Isermann (2005) reported that the species richness of sand dune vegetation in northern Germany showed a peak at the site with intermediate soil pH when they compared species diversity between areas with soil pH ranging from 3.97 to 7.75. Therefore, the significantly higher soil pH in the *Acacia* plantation can potentially promote plant species diversity. The soil pH values in this study were relatively low and strongly acidic ($pH < 4.0$) in both forest types, as compared to the previous works in the sand dune forest of the North Central Coast of Vietnam (Hoa et al. 2004, Son et al. 2023). Even though the difference in pH values is extremely small, it may affect plant species diversity in the strongly acidic soil conditions. In addition, the lower species diversity observed in *Acacia* plantations may be attributed to differences in seed sources, along with intensive management and simplified canopy structure, which can limit understory species establishment (Lorenzo et al. 2011, Ismail and Metali 2014, Nambiar et al. 2015).

Currently, several research projects and investment initiatives are focused on rehabilitating and enhancing the coastal sand dune forests of North Central Vietnam using native species instead of *Acacia* (Wittmann et al. 2019, Hien et al. 2022, Pistorius et al. 2023). Globally, many coastal sand regions have successfully implemented restoration efforts using native tree species, highlighting the effectiveness of this approach in promoting biodiversity and ecosystem resilience (Page 2005, Griffiths et al. 2007, Williams 2007, Emily 2023). Besides, to enhance biodiversity and ecological functionality in plantations, management practices should prioritise mixed-species planting and promote natural regeneration (Anbarashan et al. 2017, Gao et al. 2018). Our results indicate that succession by native tree species does not seem to occur naturally in *Acacia* plantations. Hence, to restore and sustainably develop coastal sand forests in North Central Vietnam, it is essential to establish a scientifically based strategy for managing and protecting the remaining natural remnant forest areas. A long-term plan should focus on reforestation activities, prioritising the use of native tree species to conserve biodiversity and maintain ecosystem stability.

CONCLUSIONS

This study elucidated the fundamental differences in the understory vegetation structure between natural and plantation forests in the coastal sand dunes forest in Central Vietnam. These differences were clearly reflected in the species composition, species richness, and life forms, with distinct variations highlighted by biodiversity indices and NMDS modelling. The environmental factors did not directly influence the structural differences between the two forest types; allelopathic effects from the leaves of certain acacia species have been shown to negatively impact the germination capacity of understory species. The results of this study affirm the significant ecological value disparity between natural forests (NFs) and Acacia plantations (APs). NFs play a crucial role in maintaining and developing a stable and sustainable understory ecosystem, whereas APs tend to have negative effects on understory diversity and ecosystem stability. These findings provide a critical foundation for developing and implementing strategies for the restoration and enhancement of ecosystems in natural forest areas.

Author Contributions

MH and TKM conceived the research idea and the experimental design of the study; TQD, TKM, TTD, HTL, THN and MH contributed to the data collection and identified the species; TQD, TKM, TTD and MH performed the statistical analyses and wrote the paper; NHM and HTDH, provided critical revisions and approved the final version. All authors read, revised and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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